BIOGEOCHEMISTRY OF DISSOLVED FREE AMINO ACIDS IN MARINE SEDIMENTS

by

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Abstract

Dissolved free amino acids (DFAA) were measured in interstitial water samples squeezed from sediments collected in a variety of depositional environments. These sediments were further characterized by measurements of total organic carbon, total nitrogen, dissolved organic carbon, total hydrolyzable amino acids, and pore water-dissolved remineralization products.

Surface sediments from the oxygen minimum zone of the Peru Upwelling Region, which consisted of a filamentous bacterial mat, were sampled at three locations. DFAA concentrations within the mat ranged from 5 to 220 μM, with the highest concentrations found in the upper 4 cm at two stations on the landward and seaward edges of the zone, and lower concentrations at a station in the middle of the oxygen minimum zone. Within cores, lower concentrations were found at depths below the mat; and below 30 cm depth concentrations were between 0.7 and 3 μM. Two short cores of offshore sediments had concentrations between 14 and 40 μM (1400 m depth) and between 3 and 8 μM (5200 m). Glutamic acid was the predominant amino acid in nearly all surface sediment samples, making up 30 to 70 mole %. In sediments below 15 cm depth, β-aminoglutaric acid was often more abundant than glutamic acid and other amino acids were virtually absent.

Glutamic acid, both from several analyses performed during this work and from data available in the literature, is a major DFAA of bacterial pools, and bacteria are a likely source for the high concentrations seen in interstitial water samples. DFAA may be extracted from living cells by the squeezing process, or may be excreted by the bacteria under natural conditions. β-Aminoglutaric acid is a non-protein amino acid isomer of glutamic acid which has not been previously reported as a natural product. However, this work has shown it to be a constituent of the free amino acid pools of some bacteria at about 5 mole %. Its much larger relative abundance in sediments could stem from organisms which biosynthesize greater amounts than those analyzed, or from relatively slow biodegradation.

Buzzards Bay, Massachusetts surface sediments (17 m water depth) also contained high DFAA concentrations, near 50 μM, which decreased gradually with depth to about 5 μM at 30 cm. Glutamic acid and β-aminoglutaric acid were the major components, with β-aminoglutaric acid

becoming relatively more abundant with depth in core. Repeated sampling of this station was carried out, and both the concentration and compostion of DFAA in replicate samples was very similar. Sediments from the Pettaquamscutt River Estuary, Rhode Island (an anoxic basin), had low DFAA concentrations ranging from 2 to 6 μM. Glutamic and β-aminoglutaric acids made up 30 to 50 % of the total.

Three cores of Gulf of Maine basin sediments had DFAA concentrations and compositions which were similar to each other and to Buzzards Bay sediments, except that glycine was a major constituent of some of the samples. Its distribution was irregular over the less than 30 cm depth intervals sampled. Glycine is the major DFAA in the pools of many benthic invertebrates. Its presence in these cores is consistent with independent evidence that Gulf of Maine basin sediments are extensively bioturbated.

Two cores of carbonate-rich sediments from the continental rise to the east of the Gulf of Maine and from the Bermuda rise had surface sediment DFAA concentrations of 33 and 0.9 μM, respectively. Despite the large difference in concentration, compositions were very similar, with glycine and glutamic acid the major constituents. The very low concentrations in the Bermuda Rise sediments may be related to very low metabolizable organic carbon concentrations. Two nonprotein amino acids, γ-aminobutyric acid and β-alanine, were major constituents of the total hydrolyzable amino acids in the Bermuda Rise sediments.

Biological processes, specifically microbial, appear to be responsible for the major features of DFAA concentration and composition in the sediments studied. The concentrations of DFAA measured could be of significance to the nutrition of benthic organisms via transepidermal uptake or to the formation of humic substances in sediments, if these levels are found outside cells. However, as a sink for DFAA in sediments, the latter two processes are slow relative to microbial uptake.

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Dr. Nelson Frew obtained the amino acid mass spectra. Dr. Zofia Mlodzinska performed most of the interstitial water ammonia, nitrate, and nitrite analyses. Phil Clarner measured the total organic carbon and nitrogen in sediment samples. Dr. Roy Carpenter and Robert Anderson performed the Pb-210 analyses on Peru sediment samples. Dr. James Quinn allowed me to use his DOC analyzer, and Gary Mills provided instruction in its operation. Nancy Hayward and Carolyn Taylor provided conscientious assistance in the laboratory. Hovey Clifford, Dr. Arthur Gaines, Bruce Tripp, Jack Livramento, Dave Mason, and Dr. Stuart Wakeham assisted at various times with sampling. The officers and crew of the R/V Knorr and the R/V Oceanus, and Dick Colburn of the R/V Asterias, also contributed to the success of sampling operations.

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CHAPTER 1: GENERAL INTRODUCTION

Near-surface sediments are a zone of transition and interaction between the water column and the permanent deposit. The non-equilibrium association of organic matter, mineral particles, and seawater results in early diagenetic reactions, which are often biologically mediated (Berner, 1976a). Briefly, organic matter deposited to the sediment-water interface is utilized as an energy source by benthic organisms. Organisms can remineralize the organic matter, use it to synthesize new cell components, or excrete it in solid or soluble form. The incorporated and excreted organic matter may be ingested repeatedly; ultimately only a small proportion of the total supplied to the sediment-water interface is buried (Rhoads, 1974). The soluble products may return to the water column via diffusion, precipitate in various mineral phases, or interact with detrital minerals via adsorption or ion exchange. Further geochemical reactions result from changes in acidity and redox potential in sediments due to the oxidation of organic matter (Price, 1976).

Study of the decomposition and transformation of organic matter in near-surface sediments is thus central to understanding the process of early diagenesis. However, the extreme complexity and heterogeneity of marine organic matter (Blumer, 1975) has hampered investigations of processes affecting it in sediments. Measurements of total organic content give only the net result of a variety of reactions occurring at different rates and influenced by different properties of the sedimentary environ-

ment. Measurements of individual organic compounds or compound classes can provide specific information on processes affecting a small proportion of the organic matter, and can be used to trace certain reactions affecting the bulk of organic matter (Andersen, 1977; Degens and Mopper, 1976). A complication, though, is suggested by the work of Johnson (1974) who found at least 18 types of organic-mineral particle associations in an estuarine sediment. Each of the particle types could represent one or more microenvironments with different conditions for the transformation of organic matter. For example, Thompson and Eglinton (1978) have found different concentrations and compositions of fatty acids and hydrocarbons in different particle-size fractions of a Recent sediment. It has been shown, using several extraction methods to release sterols and fatty acids from sediments, that differences in chemical accessibility can be related to differences in diagenetic behavior (Lee et al., 1977; Farrington et al., 1977a). Amino acid racemization rates in sediments depend on physiochemical parameters such as temperature, catalysis by certain metal ions, whether the amino acid is free or peptide bound, and on the structure of the particular compound (Schroeder and Bada, 1976).

This thesis describes a study of dissolved free amino acids in Recent sediments. This study was undertaken in order to improve our understanding of the diagnenesis of organic matter in near-surface sediments, a process which includes remineralization and transformation of biosynthesized organic compounds to the relatively complex, poorly-defined organic matter in sediments. Amino acids have been chosen as the primary

subject of this study because, in peptide-bound form, they are major constituents of both organisms and sediment organic matter; the range of structure within the amino acid compound class provides individual compounds with potential as source markers and a range of chemical and biochemical activity; and the literature on the geochemistry of amino acids indicates that they undergo significant diagenesis in near-surface sediments (Schroeder and Bada, 1976; Kvenvolden, 1975).

Interstitial water was chosen as a sediment subenvironment of particular interest, since variations in pore water composition are very sensitive indicators of chemical and biological reactions in sediment. Analyses of O_2, NO_3^-, NO_2^-, NH_4^+, SO_4^{2-}, S^{2-}, HCO_3^-, CH_4, Ca^{2+}, Fe^{2+}, Mn^{2+}, and PO_4^{3-}, for example, have shown large concentration gradients between bottom water and interstitial water which are directly or indirectly the result of the microbial decomposition of organic matter (Berner, 1976a). Soluble organic compounds are also released during bacterial decomposition of organic matter (Doelle, 1975); and, by analogy to the above biologically active inorganic substances, inputs of organic compounds to pore waters should be present. However, there have been only a few previous measurements of organic substances dissolved in interstitial water.

Dissolved Organic Carbon in Interstitial Water

Starikova (1970) measured dissolved organic carbon (DOC) in interstitial water of marine sediments from the Pacific and Indian Oceans, the Black Sea, and the Sea of Azov. Interstitial water of surface sediments

from these regions, including even Pacific red clays and carbonate oozes containing only 0.1 to 0.3 % total organic carbon (TOC), were enriched ten times in DOC with respect to the overlying seawater, with measured concentrations in the range 5 to 10 mgC/L. No consistent trend with depth was observed in oxidizing sediments. However, in reducing sediments DOC increased with depth to concentrations of 20 to 60 mgC/L at 3 to 6 m.

Krom and Sholkovitz (1977) measured DOC and molecular weight fractions in pore water of organic-rich sediments from Loch Duich, Scotland, a fjord-type estuary. In two cores of oxidizing sediments, 40 to 60 cm in length, DOC remained fairly constant with depth at 8 to 16 mgC/L, about twice the concentration in overlying seawater. DOC in three cores of anoxic sediment increased linearly with depth from an average of 14 mgC/L at the surface to 56 to 71 mgC/L at 80 cm. The low molecular weight (<1000 a.m.u.) fraction was approximately constant with depth at 10 mgC/L in both oxidizing and reducing sediments. The high molecular weight fraction increased with depth only in the anoxic sediments.

Nissenbaum et al. (1972) measured DOC in the pore waters of sediment from Saanich Inlet, an anoxic fjord on the coast of Vancouver Island. They found very high concentrations, ranging from 50 to 150 mgC/L. From 5 to 30 % of this material consisted of a high molecular weight polymer. DOC concentrations tended to increase with depth. Martens and Goldhaber (1978) found DOC concentrations ranging from 50 to 150 mgC/L in interstitial water from sediments of the White Oak River Estuary, North Carolina. There were no consistent trends in DOC concentration with pore

water salinity, total sediment organic content, or depth in core.

DOC in sediment pore waters from the Florida Everglades and Mobile Bay was measured by Lindberg and Harriss (1974). The seven cores of Everglades sediment were extremely high in TOM (20 to 80 %), with DOC ranging from 20 to 70 mgC/L. DOC was essentially constant with depth. Mobile Bay sediments had lower TOM (5 to 20 %) and DOC (6 to 10 mgC/L), and again no trend with depth was observed. About 60 % of the DOC in the Everglades samples had a molecular weight of less than 500, but in Mobile Bay 70 % of the pore water DOC had a molecular weight of greater than 100,000.

Other than those by Starikova (1970), there have been few measurements of DOC in deep-sea sediments. Karl, et al. (1976) found 2 mgC/L in surface sediments from the Nares Abyssal Plain, decreasing to 0.7 mgC/L at 16 cm. Suess (1976) found that DOC increased with depth from 2 to 6 mgC/L in an 80 cm core from the central Pacific.

These data indicate that DOC is substantially enriched in interstitial water with respect to overlying seawater. Thus some process(es) in sediments must be supplying soluble organic compounds to pore waters. The data also indicate that rates and/or processes of supply and removal vary in different sedimentary enviroments, resulting in different concentrations, depth distributions, and molecular weights of organic matter. However, measurement of a complex, heterogeneous mixture such as DOC can provide only limited information on the specific processes involved.

Specific Organic Compounds in Interstitial Water

A few studies of specific organic compounds or compound classes in interstitial waters have been done. Dissolved free amino acids in pore waters of Georgia salt marsh sediments were measured by Gardner and Hanson (1979). Concentrations ranged from less than 100 nM up to 7500 nM in vegetative sediments made up largely of Spartina alterniflora roots and detritus. Pore water amino acid concentrations in non-vegetative surface sediments (from a mud flat and creek bank) were 1014 and 442 nM respectively. Glutamic acid, alanine, and an unknown ninhydrin-positive compound were the most abundant amino acids.

Jorgensen et al. (in preparation) found dissolved free amino acid concentrations ranging from 2 to 20 μM in four intertidal sediments from Limfjorden, Denmark. Glutamic acid (especially in anoxic sediments), serine, glycine, and leucine were the most abundant amino acids. Close resemblance of the amino acid composition of interstitial water and overlying seawater at some locations suggested diffusion from sediments was a source of free amino acids to the water column.

Starikova and Korzhikova (1972) analyzed amino acids in pore waters of reducing surface sediments from the Black Sea. Using paper chromatography, they found from 1290 to 6000 μg total hydrolyzable amino acids/L, an average of 12 times the concentration in Black Sea water. The amino acid compostion was similar to that found in sediment.

Nissenbaum et al. (1972) investigated the compostion of high molecular weight material dissolved in interstitial water from Saanich Inlet. The polymer contained large quantities of amino acids (more than 35 %)

which were released by 6N HCl hydrolysis. The presence of non-protein amino acids suggested that at least some of the material was of bacterial origin. Non-protein amino acids were also found in interstitial water from Lake Ontario sediments by Kemp and Mudrochova (1973). They made up only a few per cent of the total dissolved amino acids, however, which were present at concentrations of 7 µg/g (free) and 36 µg/g (hydrolyzable).

Jorgensen (1979) found 16 to 56 µM dissolved free primary amines (by fluorescamine assay) in sediments from Kysing Fjord (Jutland). Concentrations varied with depth in sediment and seasonally, with concentration maxima associated with the redox discontinuity layer. Stephens (1975) and Stephens et al. (1978) also measured dissolved free primary amines in pore waters and found concentrations in the range of 10 to 100 µM. Ho and Lane (1973) found 3 to 10 mgN/L dissolved free α-amino acid nitrogen in organic-rich, highly reducing sediments from the Barataria Bay (Louisiana) system.

Specific organic compounds other than amino acids have been even less frequently measured in interstitial water. Lyons et al. (1979) found 0.2 to 10 mg/L dissolved carbohydrate and from less than 1 to 9 mg/L dissolved humic substances in pore waters of nearshore Bermuda carbonate sediments, where DOC ranged from 4 to 19 mgC/L. A decrease in carbohydrate concentrations with depth was attributed to microbial utilization. Romankevich and Urbanovich (1971) measured dissolved carbohydrates in interstitial water from the Peru Upwelling Region using the phenol-sulfuric acid method. They found concentrations ranging from less

than 1000 to more than 4000 µg/L. Interstitial water carbohydrate concentrations decreased with overlying water column depth.

Aliphatic hydrocarbons and fatty acids were enriched 2 to 160 times in interstitial waters of surface sediments from the Cariaco Trench, Demerara plain, and Amazon River cone relative to seawater (Saliot and Tissier, 1978). Barcelona et al. (1980) measured 150 µM C_1 to C_5 volatile organic acids in a surface sediment pore water sample from the Newport Canyon (California).

Like the available information on DOC in interstitial waters, the data on specific organic compounds suggest that biogeochemical processes in near-surface sediments are a net source of soluble organic substances. However, the data are limited both in number of analyses and geographic coverage. Because of varied sampling and analytical methods, it is difficult to compare results of different workers. Further, the distribution of dissolved organic compounds in seawater results from complex interactions between biological sources and sinks (Andersen, 1977). Thus, an understanding of the distribution of dissolved organic compounds in interstitial water probably requires knowledge of the chemical, biological, and physical characteristics of the sedimentary environment. Although some efforts have been made in this direction (e.g. Krom and Sholkovitz, 1977), the available dissolved organic concentration data are not usually part of a systematic study of the sedimentary enviroment.

Sources and Sinks

Five areas of research suggesting processes which might act as sources or sinks for dissolved organic compounds in sediments are:

(1) Biological cycling of organic matter.

(2) Transepidermal uptake of dissolved organic compounds by soft-bodied benthic organisms.

(3) Sorption of organic compounds by clay mineral and other sediment components.

(4) Melanoidin formation or the "humification" reaction.

(5) Physical transport via diffusion and bioturbation.

These processes will be discussed below.

Sources of dissolved organic compounds in seawater include extracellular release of photosynthetic products by plankton, decomposition of detritus by bacteria, and excretion by zooplankton (Whittle, 1977). The dissolved compounds may be taken up by heterotrophic bacteria and decomposed to inorganic nutrients or used to synthesize cellular material which provides food for higher heterotrophs (Gagosian and Lee, 1980). Several studies (Crawford et al., 1974; Andrews and Williams, 1971; Lee and Bada, 1977; Wright, 1978) provide evidence that turnover rates for simple monomers such as amino acids and glucose are very rapid in estuarine and open ocean surface waters, in the range of 10 to 100 % per day. A parallel for the seawater cycle, involving decomposition of organic detritus by bacteria and benthos to release soluble organics, uptake and decomposition or incorporation by bacteria, and ingestion of bacterial cells by benthos, can be hypothesized. The actual importance

and rates of these processes in sediments is not well-known. Some attempts have been made to measure the turnover rates of free amino acids in salt marsh, estuarine, and lake sediments using radiolabelled substrates. A range of turnover times, from less than one hour (Harrison et al., 1971; Christensen and Blackburn, in press; Henrichs et al., in preparation) to several days (Hanson and Gardner, 1978) have been measured. Both differences in methodology and differences in the sediments studied probably contribute to the range of values. The observed uptake of radiolabelled amino acids has been largely attributed to microbial activity, although other processes may have been significant.

One potentially important sink for dissolved organic compounds in pore water which has received considerable attention is trans-epidermal uptake by benthic invertebrates. Using radiolabelled substrates, uptake of amino acids (primary amines), glucose, and/or fatty acids has been demonstrated in polychaete worms, echinoderms, and pogonophores (Ferguson, 1971; Southward and Southward, 1972; Stephens, 1975). Because the concentrations of these compounds in natural habitats is not well-known and because the uptake has most often been studied on isolated organisms in aquaria, the rate of uptake in sediments and the significance of this process to the overall nutrition of the organisms is uncertain.

Adsorption by clays and other sediment minerals could also affect dissolved organic distributions. Free amino acids and sugars are adsorbed by kaolinite and montmorillonite clays to the extent of only a few per cent of the total in solution at seawater concentrations ranging

from 10^{-3} to 10^{4} mg/L (Hedges, 1977). Fatty acids, however, are strongly adsorbed from saline solutions by a variety of clay minerals, although the presence of other dissolved organic compounds decreased adsorption (Meyers and Quinn, 1973). Carter (1978) found that quartz and calcite were weak adsorbers of fulvic acids, taking up a few per cent or less from distilled water solutions at concentrations of 5 to 500 mgC/L. Clay-mineral adsorption of melanoidin-type polymers (formed by reacting free amino acids and sugars) was strongly dependent on their composition (Hedges, 1978). Basic polymers (formed from lysine and glucose) were quantitatively sorbed by montmorillonite from 10 to 1000 mg/L solutions, while neutral (valine) and acidic (glutamic acid) polymers were about 10 % and 2 % adsorbed, respectively. These results, although they apply strictly only to artificial systems, suggest that both solubility and specific organic-mineral and organic-organic interactions may be important to determining the amount of adsorption in natural sediments.

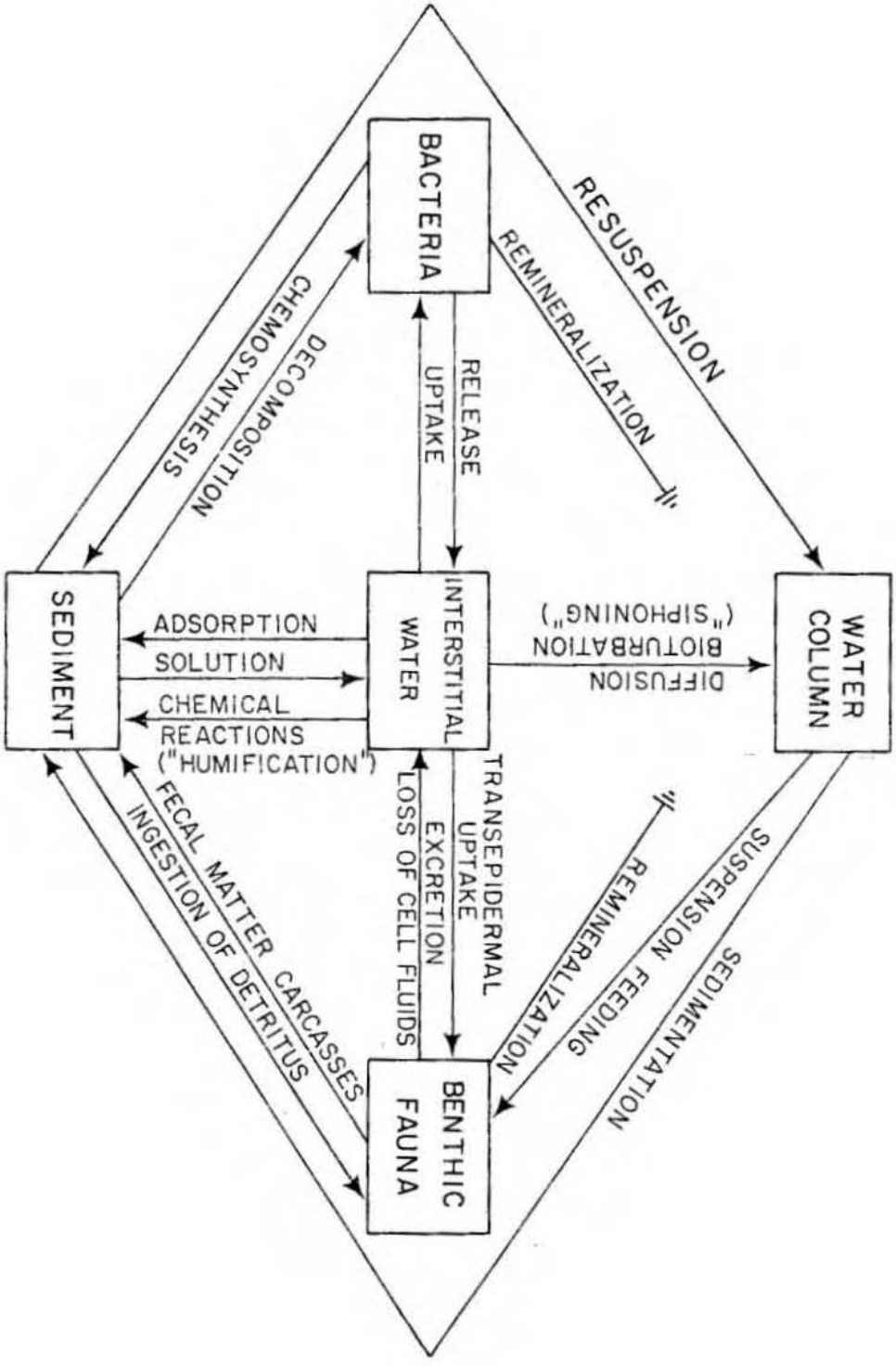
Humic substances, organic polymers of complex, ill-defined structure, make up much of the organic matter in seawater and marine sediments. Nissenbaum (1974) has proposed a pathway for the formation of humic substances which involves a Maillard-type condensation of amino acids with reducing sugars to give soluble products. These polymers undergo further condensation, dehydration, cyclization, elimination of labile functional groups, and aromatization to form insoluble, macromolecular products. This process could be a sink for labile dissolved organic compounds in sediments. Laboratory studies support the potential significance of this reaction. Hedges (1978) reacted various combinations of

0.01 M solutions of glucose, lysine, valine, and glutamic acid at 80°C for 20 hours, and found that the melanoidin polymers formed strongly resembled natural humic substances. The basic amino acid lysine reacted with glucose at a much greater rate than glutamic acid or the neutral amino acid valine. Abelson and Hare (1971) found that marine kerogens and humic acids took up amino acids when reacted with dilute solutions (0.1 mM). Again, dibasic amino acids were the most reactive, with 90 to 100 % uptake by most of the macromolecular substances investigated.

Transport along concentration gradients via diffusion and bioturbation has been shown to be important to the distribution of solutes in interstitial water, especially near the sediment-water interface. The concentration profiles and fluxes of substances dissolved in interstitial water have been modelled (eg. Lasaga and Holland, 1976; Berner, 1976b; Schinck and Guinasso, 1977). In thoroughly studied environments, these models can be used to quantify specific sources and sinks, and have been applied to calculations of remineralization rates from pore water alkalinity, sulfate, and ammonia profiles (Murray et al., 1978; Goldhaber et al., 1977).

Figure 1-1 summarizes the discussion of processes which may influence the distribution of dissolved organic substances in sediments. Although the complexity of this picture is cautionary, it also indicates the potential of careful study of dissolved organic substances in sediments for improving our understanding of several aspects of the sedimentary carbon cycle.

Figure 1-1: Schematic of sources and sinks for organic substances in interstitial water.


BACTERIA
RESUSPENSION
CHEMOSYNTHESIS
DECOMPOSITION
UPTAKE
RELEASE
REMINERALIZATION
SEDIMENT
INTERSTITIAL WATER
WATER COLUMN
ADSORPTION
SOLUTION
CHEMICAL REACTIONS ("HUMIFICATION")
DIFFUSION
BIOTURBATION ("SIPHONING")
FECAL MATTER CARCASSES
INGESTION OF DETRITUS
EXCRETION
LOSS OF CELL FLUIDS
TRANSEPIDERMAL UPTAKE
REMINERALIZATION
SUSPENSION FEEDING
SEDIMENTATION
BENTHIC FAUNA

Organization of the Thesis

Chapter 1 has been a general introduction to the subject of this thesis and a review of previous work in the area of dissolved organic matter in interstitial water. Chapter 2 will discuss sampling and analytical methods used in the collection of data for this thesis. Particular attention will be given to methods of pore water extraction and their effects on dissolved free amino acid reservoirs in sediments.

Chapters 4, 5, and 6 will present data on the distribution of dissolved free amino acids (DFAA) in sediments from the Peru Upwelling Region, Buzzards Bay, the Pettaquamscutt River Estuary, the Gulf of Maine, and the Northwestern Atlantic. These sediments represent a wide range of environments, from water column depths of 10 to 5000 m, total organic carbon contents of 0.1 % to 20 %, and oxidizing to reducing conditions. These environments have been characterized by collection of data on DOC, TOC, TN (total nitrogen), THAA (total hydrolyzable amino acids), and pore-water dissolved remineralization products. Variations of DFAA concentration and composition with depth in sediment and sedimentary environment, and specific relationships between DFAA and certain environmental characteristics, will be used to identify probable sources and sinks.

Chapter 7 will summarize the most important results of this research. Data used in the preparation of figures will be given in an Appendix.

CHAPTER 2: METHODS

Sampling

Coring:

No one corer was available or suitable for sampling at all locations. The coring devices used in this work are listed below:

(1) Gulf of Maine, Knorr 69-1: Sandia-Hessler Type MK3 (Oceanic Instruments, San Diego, CA), 0.25 m^2 X 30 cm box corer.

(2) Peru Upwelling Region, Knorr 73-2: Soutar box corer, 0.1 m^2 X 1 m, Stations 4, 5A, and 6; Sandia-Hessler Type MK3, Stations 8 and 2A.

(3) Buzzards Bay, Station P, 6/26/79: Sphincter corer (Burke, 1968), 0.03 m^2 X 1 m; van Veen-type grab sampler.

(4) Pettaquamscutt River Estuary: Hand-driven plastic core liner, 40 cm^2 X 1 m.

(5) Bermuda Rise, Oceanus 74: Gravity corer, 40 cm^2 X 1.5 m.

(6) Buzzards Bay Station P, 12/5/79: Soutar corer, van Veen grab.

(7) Buzzards Bay, Station BBBC: Diver-emplaced BEB-corer, 0.02 m^2 X 30 cm.

(8) Great Harbor, Woods Hole: Diver-emplaced BEB-corer.

The length dimensions given are the maximum which could be obtained with a particular corer.

The Sandia-Hessler Type MK3 corer is a slow-entry type which recovered a relatively undisturbed sample of surface sediments. The large

volume of sediment allowed sectioning at 2 cm intervals. The Soutar box corer also retrieved an intact surface sediment sample. Sectioning intervals were controlled by the width of removable plates along the box. The sphincter corer produced dome-shaped distortion of unconsolidated surface sediments. The small-cross section Pettaquamscutt River Estuary and Bermuda Rise cores required 6-cm sectioning intervals to obtain sufficient sample for analysis. The BEB-corer is a diver-operated device which recovered an undisturbed surface sediment sample.

All cores were sectioned immediately after recovery with the exception of the Pettaquamscutt River core which was stored overnight at 10°C prior to sectioning. Sediment samples were placed in clean glass jars and stored at 2°C until squeezed (usually within 24 hours).

Squeezing:

Squeezing of sediments for organic analysis was carried out in a hydraulically-powered, stainless steel squeezer at about 2000 p.s.i. The portions of this apparatus which contacted the sample were carefully cleaned to eliminate possible contamination. The pore water sample passed through two internal Reeve Angel(R) glass fiber filters (precombusted at 450°C for 24 hours to eliminate organic matter) and then passed through an external precombusted Gelman Type A(R) glass fiber filter. Filtered pore water was collected in clean glass bottles and refrigerated at 2°C prior to cation exchange chromatography.

Pore water for inorganic analysis was obtained using a hydraulically-powered squeezer constructed of Delrin and polycarbonate. The

pore water passed through internal Whatman #42 or #541 ashless cellulose filters and was refiltered through 0.4 μ Millipore or Gelman membrane filters.

Amino Acid Analysis

Glass capillary gas chromatography (GCGC) was used to determine the amino acid concentrations and compositions of the samples analyzed for this thesis. This method involves three steps:

(1) Separation of amino acids from interfering organic and inorganic substances in the sample by cation-exchange chromatography.

(2) Derivatization of the free amino acids to form the volatile (N,O)-heptafluorobutyryl n-butyl esters.

(3) GCGC of the amino acid derivatives.

Gas chromatography has seen some application in the measurement of amino acids in geological materials, especially for the determination of enantiomer ratios (Kvenvolden, 1975). But ion-exchange or reverse phase high performance liquid chromatography, with post-column formation of ninhydrin or fluorescent derivatives for detection, has been more frequently used than GCGC in recent studies of amino acids in seawater and sediments (e.g. Lee and Bada, 1977; Schroeder and Bada, 1976; Dawson and Pritchard, 1978; Garrasi et al., 1979). A recently developed method employing pre-column formation of fluorescent derivatives and high performance liquid chromatography for the separation of amino acids offers the advantage that step (1) can be eliminated, since seawater samples can be used

directly (Lindroth and Mopper, 1979). However, GCGC can be readily interfaced with mass spectrometry, allowing the identification of unknown chromatographic peaks which are all-too-frequently present in environmental samples. It will be shown that the necessary clean-up and derivatization procedures do not compromise the GCGC analytical results.

Cation Exchange Chromatography:

Free amino acids were isolated from the sample matrix (interstitial water, sediment hydrolyzate, etc.) via cation-exchange chromatography on a 15 to 20 cm^3 BioRad$^{(R)}$ AG 50W-X8 (50 to 100 mesh) resin column (procedure similar to Degens and Reuter, 1963). The resin was brought to the H^+ form by eluting with 20 ml of 6N HCl and washing out the excess acid with water. Typically, a 10 to 25 ml sample was spiked with an internal standard (usually norleucine) and applied to the column. The column was then eluted with 20 ml H_2O and about 20 ml of 1.5 to 2.0 N NH_4OH, until the base front (identified by a warm zone) just reached the bottom of the column. The first 70 ml of basic eluate, containing the amino acids, were collected, evaporated to dryness on a Buchi all-glass rotary evaporator at 40°C, and the residue redissolved in 0.1 N HCl to a final concentration in the range of 0.01 to 1 µM.

Recoveries of amino acids from the cation exchange chromatography were checked by dissolving known amounts of 25 protein and nonprotein amino acids in 10 ml seawater with very low indigenous amino acid content. Total amino acid concentrations used were 16, 32, 80, and 160 µM, with individual components at about 0.6, 1.3, 3.2, and 6.4 µM. Recoveries of nearly all amino acids was good (greater than 80 %) and repro-

TABLE 2-1

Recoveries of Amino Acids from Cation Exchange Chromatography

Amino Acid	% Recovery from Seawater	% Recovery from Distilled Water
ala[a]	114 (15)[b]	106 (5)
gly	98 (20)	95 (3)
α-aba	101 (3)	104 (6)
val	106 (12)	105 (5)
thr	97 (7)	100 (7)
ser	98 (6)	91 (2)
leu	92 (10)	96 (0.5)
ile	94 (10)	97 (2)
norleu	89 (9)	---[c]
γ-aba	100 (14)	92 (7)
pro	99 (10)	92 (3)
hypro	111 (9)	89 (2)
daba	75 (3)	82 (5)
met	73 (31)	93 (4)
asp	104 (12)	104(11)
phe	83 (11)	81 (3)
orn	81 (13)	82 (7)
glu	106 (12)	92 (9)
β-glu	108 (18)	88 (6)
lys	59 (17)	80 (6)[e]
tyr	0[d]	80 (6)[e]
α-aaa	100 (5)	87 (10)
tryp	0	11 (8)
dapa	116 (15)	87 (10)
cys	23 (17)	78 (10)

[a]See Appendix I for explanation of amino acid abbreviations.
[b]Standard deviation of four runs: see text.
[c]Internal standard.
[d]Very small peak, not resolved from lysine.
[e]Lysine and tyrosine were not resolved. Recovery reported is for sum of two peaks (i.e. the average recovery).

ducible (Table 2-1, col. 1). Recovery of basic amino acids (diaminobutyric acid. ornithine, lysine) was slightly lower than average. Sulfur-containing amino acids (methionine and cystine) were poorly recovered at 0.6 μM, but their recoveries increased with concentration (from 36 to 98 % and from 0 to 48 %, respectively). Tyrosine recovery was low and variable, and tryptophan was not recovered. However, a similar experiment (in quadruplicate) with a 20 μM amino acid solution in distilled water gave good recoveries of tyrosine, methionine, and cystine (Table 2-1, col. 2). Cation exchange recoveries for samples were monitored by comparing internal standards (generally norleucine and norvaline) added before and after column chromatography.

Derivatization:

Prior to gas chromatography, free amino acids must be converted into less polar, more volatile compounds by derivatization of the carboxylic acid and amino functions. A wide variety of derivatization methods have been developed for this purpose (Husek and Macek, 1975). Esterification of the carboxylic acid and subsequent acylation of the amino (and any hydroxyl) groups has been extensively investigated by Gehrke and co-workers (Roach and Gehrke, 1969). This appears to be the most satisfactory method available in terms of stability and chromatographic characteristics of the derivative. In this work, the (N,O)-heptafluorobutyryl n-butyl esters (HFBBE) have been used. These compounds are more stable with respect to hydrolysis, less volatile (which minimizes evaporative losses during workup), and less polar than the corresponding (N,O)-trifluoroacetyl n-butyl esters commonly used.

The following derivatization procedure has been optimized to give maximum and reproducible yields:

(1) An aliquot of the 0.1 N HCl solution of amino acids recovered from cation exchange chromatography, containing approximately 0.01 to 1.0 μmole total amino acids, is placed in a 1 ml Reactivial$^{(R)}$ (Pierce) and the solution evaporated to dryness at 100°C under an N_2 stream. CH_2Cl_2 (0.2 ml) is added and evaporated to azeotrope any remaining water.

(2) After the vial has cooled to room temperature, 0.2 ml 3N HCl in n-butanol (Regis) and 0.05 ml CH_2Cl_2 are added. The vial is sealed with a Teflon-lined screw cap, sonicated for 15 minutes to aid solution of the amino acids, and then heated to 110°C for 30 minutes. Excess reagent is evaporated at 60 to 70°C under an N_2 stream until about 0.01 ml remains, 0.2 ml of CH_2Cl_2 is added, and the solution is evaporated to dryness at room temperature under an N_2 stream.

(3) 0.1 ml of 20 % v/v heptafluorobutyric anhydride (Pierce or Regis) in acetonitrile is added, and the solution heated at 110°C for 15 minutes.

(4) After the vials have cooled to room temperature, excess reagent is evaporated under an N_2 stream. When dry the vials are immediately sealed with septum screw caps. The HFBBE derivatives are dissolved in CH_2Cl_2 for GCGC analysis. Samples are withdrawn through the septum to prevent exposure to atmospheric moisture and oxygen.

(5) Reagent-grade CH_2Cl_2 and acetonitrile must be redistilled from anhydrous $CaCl_2$ to remove water and stored in a desiccator. Heptafluorobutyric anhydride and 3 N HCl in n-butanol are used as received from the suppliers noted. N_2 was prepurified grade.

(6) HFBBE derivatives are stable for about a month except for those of serine, tyrosine, and methionine which decompose in about one week.

Glass Capillary Gas Chromatography

Gas chromatographic analyses were carried out on a 32 m X 0.3 mm i.d. SE-54 or a 20 m X 0.3 mm i.d. SE-52 glass capillary column (Grob et al., 1978; Grob et al., 1979) installed in an HP 5840 gas chromatograph equipped with a splitless injector and a flame ionization detector. Run parameters were varied to optimize resolution. Typical values were: injector temperature 250°C; initial column temperature 40°C; temperature program 30°C/min to 70°C and then at between 2°C/min and 4°C/min to 250°C; He flow 1 to 3 ml/min; FID temperature 250°C. Under these GCGC conditions and using the derivatization procedure described above, cysteine and histidine gave no peak on the chromatogram, and the arginine peak was highly variable. These amino acids were not measured in this study. Aspargine and glutamine are esterified during the derivatization procedure and thus were not distinguished from aspartic and glutamic acids respectively. The remaining protein amino acids. and several nonprotein amino acids, can be measured with good accuracy and precision. ß-Alanine/valine and

lysine/tyrosine were not always resolved, and are reported together in some cases. A typical GCGC of a mixed amino acid standard is shown in Figure 2-1. When resolution of amino acid enantiomers was desired, a Chirasil-Val$^{(R)}$ (N-propyl L-valine t-butylamide polysiloxane) coated, 25 m X 0.3 mm i.d. glass capillary column (Applied Science Laboratories) was used.

Table 2-2 shows typical relative molar response factors (RMRF) for 25 amino acids relative to norleucine (col. 1). Relative standard deviations (col. 2) are for derivatization and GCGC analysis of 4 subsamples (1.6 μmoles each) of a mixed amino acid standard solution and average about 5 %. The relative error due to GCGC alone (including automatic peak integration) determined from four replicate injections of a single derivatization, is 1 to 5 % (col. 3). As expected, the RMRF are primarily a function of the number of carbon atoms in the molecule (but with negligible contribution from flourinated carbon). It was found that a "hot needle" injection technique (Grob and Grob, 1979) increased relative response and precision for amino acids eluting after ornithine. GCGC RMRF were checked periodically, but varied only slightly (10% to 20%), mostly for the less stable derivatives of serine, methionine, and tyrosine.

The linearity of the derivatization and GCGC analysis over the concentration range encountered was also checked. Derivatization of 0.16, 1.6, 8.0, and 16 μmoles total amino acids (approximately 8, 80, 400, and 800 nmoles/component) was carried out. The mean and relative standard deviation of the RMRF are given in columns 4 and 5 of Table 2-2. No

Figure 2-1: Glass capillary gas chromatogram of the (N,O)-heptaflurobutyryl n-butyl ester derivatives of a mixed amino acid standard. In order, from the arrow, the major peaks are: alanine, glycine, α-aminobutyric acid, valine, threonine, norvaline, serine, leucine, isoleucine, norleucine, γ-aminobutyric acid, proline, hydroxyproline, diaminobutyric acid, methionine, aspartic acid, phenylalanine, ornithine, glutamic acid, β-aminoglutaric acid, lysine, tyrosine, α-aminoadipic acid, tryptophan, diaminopimelic acid, and cystine. Conditions are given in the text.

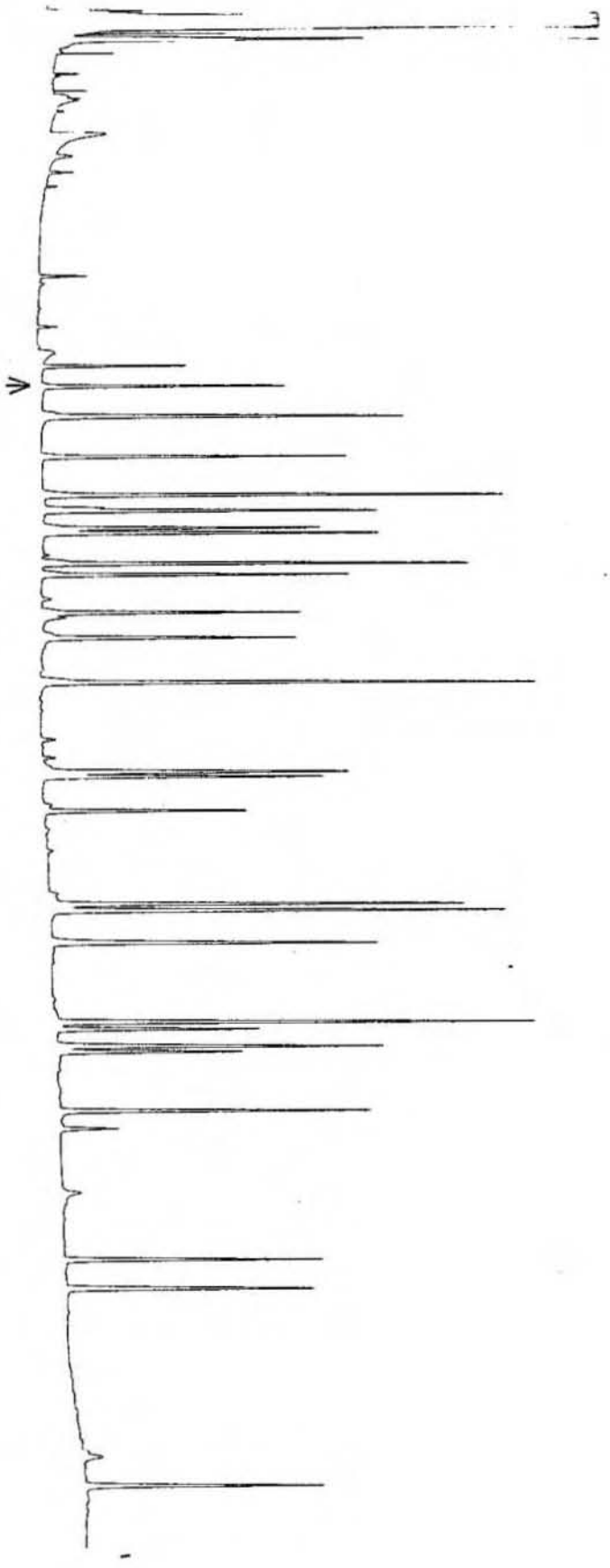


TABLE 2-2

Relative Molar Response Factors
for (N,O)-Heptafluorobutyryl n-butyl esters[a]

Amino Acid	RMRF[b]	RSD (%)[c]	RSD (%)[d]	RMRF[e]	RSD (%)
ala	0.705	7.0	1.4	0.635	10.0
gly	0.623	5.5	1.1	0.571	3.7
α-aba	0.850	5.2	0.96	0.811	7.9
val	0.952	4.5	0.85	0.914	8.3
thr	0.959	5.0	0.80	0.923	4.6
norval	1.01	5.2	2.0	1.04[f]	11.5
ser	0.867	4.8	1.1	0.693	4.5
leu	1.10	3.8	1.2	1.06	2.0
ile	1.09	5.0	1.0	1.06	3.4
norleu	1.00	---	---	1.00	---
γ-aba	0.735	4.4	4.7	0.714	11.2
pro	0.963	3.4	1.2	0.894	4.6
hypro	1.08	4.6	0.60	1.02	9.9
daba	0.813	4.7	2.4	0.784	10.5
met	0.492	7.4	2.0	0.606	13.7
asp	1.10	4.1	1.5	1.12	5.5
phe	1.41	4.4	1.1	1.22	9.8
orn	0.986	6.1	3.6	0.956	8.4
glu	1.21	4.3	3.5	1.13	6.2
β-glu	1.23	4.9	6.0	1.07	7.9
lys	1.09	5.2	4.1	1.08	8.6
tyr	1.19	4.5	4.2	1.00	8.6
α-aaa	1.20	4.9	3.1	1.11	7.7
tryp	1.03	4.4	3.8	0.743	4.2
dapa	0.581	6.9	5.8	0.549	4.5
cys	0.757	10.1	9.2	0.750	3.7

[a]Relative to norleucine.
[b]Average of four derivatizations of 1.6 μmoles mixed standard solution.
[c]Relative standard deviation, derivatization + GCGC.
[d]Relative standard deviation, GCGC only (four runs).
[e]Relative molar response factor for derivatizations of 0.16, 1.6, 8.0, and 16 μmoles mixed amino acid standard.
[f]Spiked at a constant 9.1 nmoles.

trend with concentration was observed. Also, solutions containing 7.9 nmoles of norleucine and 2.2, 22, 220, and 2200 nmoles of glutamic acid (the amino acid with the largest concentration range in pore water samples) were derivatized. The GCGC peak area ratios (glutamic acid/norleucine) were 0.301, 2.87, 33.8, and 321, which give an average RMRF of 1.15 (s = 0.08).

Peak identification in samples was by comparison of GCGC retention times and electron-impact ionization (and in some cases chemical ionization) mass spectra to those of authentic standards (Sigma). Electron-impact ionization mass spectra were obtained using an SE-52 glass capillary column installed in a Varian Aerograph 1400 gas chromatograph interfaced with a Finnigan 1015C quadrupole mass spectrometer. Mass spectral fragmentation patterns of (N,O)-trifluoroacetyl n-butyl esters have been discussed by Leimer et al. (1977). Those of the HFBBE are virtually identical except that m/e of all fragments containing the heptafluorobutyryl group is increased by 100 a.m.u.

Blanks.

At the low amino acid concentrations found in geological samples, contamination from sources such a human fingers, impure reagents, and unclean glassware can invalidate results (Oro and Skewes, 1965; Lee, 1975). The following precautions were taken in this study to reduce analytical blanks to low levels:

(1) All glassware was Chromerged overnight, rinsed with tap water, 3X with 3N HCl, 5X with distilled water, and 5X with double-distilled water.

TABLE 2-3

Blanks (Oceanus 74) (nmoles)[a]

Amino Acid	Blank Number					
	1	2	3	4	5	6
ala	0.28	0.07	0.10	---	0.10	0.23
gly	1.1	0.36	0.10	0.38	0.38	0.51
val	0.22	0.31	0.16	0.29	---	0.17
thr	0.14	---[b]	---	---	---	---
pro	0.08	---	---	---	---	---
asp	0.11	0.04	---	---	0.07	0.07
glu	0.11	0.04	---	---	0.07	0.07
TOTAL	2.0	0.8	0.4	0.7	0.6	1.1
d.l.[c]	0.05	0.04	0.08	0.08	0.07	0.07

[a]Per 70 mL cation exchange column eluate.
[b]Below detection limit.
[c]Detection limit.

(2) Cation exchange resin was repeatedly washed with 6N HCl, water, 7N NH_4OH, and water until the eluates were colorless before use. The same resin was used repeatedly throughout this work, and resin blanks decreased with time. Resin was always rinsed with at least one cycle (HCl, H_2O, NH_4OH, H_2O) before use and between samples.

(3) Water used for column chromatography and final glassware rinsing was prepared by distilling twice, the second time from potassium permanganate solution in an all-glass still. Aqueous NH_4OH (1.5 to 2.0 N) was prepared by bubbling anhydrous ammonia through double-distilled water. Two successive azeotropic distillations in an all-glass still were used to prepare purified 6N HCl for column chromatography and hydrolyses (Peltzer, 1979).

(4) Glassware and resin used for low-level samples (porewater and seawater) was kept separate from that used for high-level samples (sediments and organisms).

(5) Care was taken at all times to prevent sample contact with fingers, air-borne contaminants, and other sources of extraneous amino acids.

Blanks for column chromatography were run on shipboard during Knorr 73-2 and Oceanus 74. Table 2-3 gives the results of the six blanks from Oceanus 74, which are representative of all blanks run. The average total amino acid blank was 0.9 (s = 0.6) nmoles, made up mostly of glycine. This would be equivalent to a concentration of 0.05 μM for a sample size of 20 ml. Blanks were only rarely significant with respect to sample concentrations.

Other Methods

Total Hydrolyzable Amino Acids:

Approximately 1 to 2 g wet sediment was placed in a 5 ml Reactivial along with 2 ml twice-distilled 6N HCl and an internal standard (norleucine). The vial was flushed with N_2 and sealed with a Teflon-lined screw cap. The vial was shaken to thoroughly disperse the sediment and then sonicated for 30 minutes. Hydrolysis was carried out at 110°C for 24 hours. Rehydrolysis of sediments yielded less than 10 % of the original extract, except for a slightly higher amount of sterically hindered residues (valine and isoleucine) in the organic-rich Pettaquamscutt River sediments and of γ-aminobutyric acid in the Bermuda Rise sediment (Table 2-4). The hydrolyzate was filtered through a precombusted glass fiber filter to remove the sediment, which was then washed with approximately 25 ml of double-distilled water. The filtrate was evaporated to near-dryness, redissolved in double-distilled water, and re-evaporated to remove acid. Analysis of the free amino acids in the hydrolyzate was carried out as described earlier.

Total Carbon, Total Organic Carbon, Total Nitrogen, and Dissolved Organic Carbon:

For total carbon (TC), total organic carbon (TOC), and total nitrogen (TN) analyses, sediment subsamples were dried overnight at 110°C and then ground in a mortar and pestle. The sample was split in two and one part was treated with 2M twice-distilled HCl to pH 2 to destroy carbonate. The sediments were redried, and the carbon and nitrogen contents

TABLE 2-4

Amino Acid Concentrations in Re-hydrolyzed Sediment
(% of first hydrolysis)

Amino Acid	PRE 24-30 cm	PRE 30-36 cm	BBP12/5 0-2 cm	BBP12/5 2-5 cm	BR3 6-12 cm
ala	5.9	6.6	2.4	3.5	---
gly	3.5	3.8	1.2	1.7	---
val+β-ala	13	14	4.6	5.9	5.1
thr	4.3	4.8	1.5	2.5	---
ser	4.0	4.8	2.3	3.2	---
leu	11	11	4.3	6.3	---
ile	17	18	7.0	9.2	---
γ-aba	---	---	---	---	22
pro	5.0	5.2	1.2	2.0	---
hypro	3.6	4.0	---	---	---
asp	3.5	4.2	1.2	2.0	---
phe	8.3	8.3	3.5	5.6	---
glu	4.6	5.5	1.6	2.5	---
lys+tyr	6.4	7.5	2.4	3.5	---
TOTAL	5.7	6.3	2.8	3.0	1.5

measured on a Perkin-Elmer CHN Analyzer.

Pore water samples for DOC analysis were diluted from 5 to 40 times with double distilled water to give solutions containing from 1 to 10 mgC/L. Seawater samples were analyzed without dilution. The samples were acidified with 3 % H_3PO_4 and purged with N_2 to remove inorganic carbon. Three 10 ml aliquots of each sample were placed in precombusted glass ampules, 0.2 g of potassium persulfate was added, and the ampule purged with N_2 and sealed. Oxidation was carried out at 100° to 110°C for three hours. The CO_2 evolved was measured on an Oceanography International Model 0524 Carbon Analyzer. Calibration was relative to sucrose solutions of known concentration treated as for samples. Analytical precision was 10 %.

Inorganic Pore Water Analyses:

Nitrate, nitrite, and ammonia were measured using a Technicon Autoanalyzer according to the methods described in Technicon Industrial Systems Method No. 168-71W (1972) and Adamski (1976). During the Gulf of Maine cruise (Knorr 69-1), ammonia was measured using the method of Solorzano (1969).

Total carbon dioxide was measured using a head-space gas chromatographic method. From 0.5 to 1 ml of pore water was placed in a 20 ml Vacutainer(R), 0.05 ml of 2 M H_3PO_4 was added, and a subsample of the evolved CO_2 was withdrawn in a gas-tight syringe. The CO_2 was measured gas chromatographically using a Porpak(R) QS column and a thermal conductivity detector. Precision was 5%, but accuracy may have been affected in some cases by sampling problems due to the precipitation

of calcium carbonate from supersaturated pore waters.

Sulfide was measured by the method of Gilboa-Garber (1971). The pH of pore water samples was measured using a Beckman #39102 combination electrode. Total water content of sediment was determined by the difference between wet weight and weight after drying at 110°C to constant weight.

Reproducibility and Sample Storage

During the course of this research, replicate squeezing and analyses have been conducted on selected sediment samples to find the magnitude of variability in amino acid concentration and composition due to sampling. This data is summarized in Table 2-5. Total concentrations of replicate squeezings differ by an average of 18 %, about 2 to 3 times the strictly analytical error but still in quite good agreement. The concentration differences are largely due to glycine, which is the major free amino acid in several benthic organisms (see Chapter 3). Thus some of the variability may be due to heterogeneous distribution of these organisms. The number in parentheses next to the second of each pair of analyses is the time in hours of storage at 2°C between squeezings. There are no consistent trends in concentration or composition with time, indicating that storage of sediment for reasonably short times has little effect. DOC in replicated samples differed by an average of 20 % and again showed no consistent direction of change with storage.

It was also necessary to store samples after squeezing, usually for no more than 24 hours at 2°C, before cation exchange chromatography

TABLE 2-5

Reproducibility of Squeezing

Amino Acid	Composition (mole %)							
	BBP6/79G		BBP6/79 22-26 cm		PRE 0-30 cm		PRE 52-82 cm	
	A	B(6)[a]	A	B(14)	A	B(27)	A	B(27)
ala	16	13	4.3	4.5	8.6	8.2	9.0	9.4
gly	4.4	4.3	7.0	6.4	5.6	5.0	13	11
val	1.4	2.0	1.9	1.4	2.0	2.7	5.7	6.3
thr	1.2	1.5	1.9	1.4	2.3	2.2	3.8	5.2
ser	1.2	1.4	5.0	3.3	3.6	3.4	7.4	7.2
leu	0.8	1.9	1.5	2.0	1.2	1.4	5.2	5 6
ile	0.5	1.2	0.91	0.99	0.5	0.7	1.7	3.1
pro	1.9	2.9	---[b]	---	6.5	3.2	2.8	3.0
asp	7.5	8.0	5.0	4.7	7.2	6.7	9.2	8.7
phe	0.5	1.0	---	---	0.4	0 5	1.5	1.7
glu	46	46	32	33	38	40	20	23
β-glu	13	12	39	40	14	15	13	10
lys	1.3	1.1	---	---	3.3[b]	3.1[b]	5.5[b]	5.0[b]
tyr	0.8	1.2	---	---	---	---	---	---
TOTAL (μM)	57	59	7.6	7.1	7.1	9.2	6 5	10.0
DOC (mgC/L)	33	23	17	26	50	51	65	66

Amino Acid	Composition (mole %)					
	BBP12/79G-1		BBP12/79G-2		BBP12/79G-3	
	A	B(48)	A	B(48)	A	B(48)
ala	10	12	14	15	13	15
gly	17	8.9	23	11	11	18
val	2.3	1.3	1.2	1.3	1.3	1.3
thr	0.9	1.1	0.9	1.1	1.5	1.7
ser	2.4	2.8	2.5	3.1	3.1	2.5
leu	0.5	0.4	0.5	0.5	0.5	0.7
ile	0.3	0.3	0.4	0.4	0.3	0.5
pro	1.1	1.1	1.4	1.3	1.7	1.9
asp	4.7	5.3	4.4	5.2	5.2	5.2
phe	0.3	0.3	0.3	0.3	0.3	0.3
orn	1.2	1.3	1.4	1.5	2.3	1.8
glu	42	49	34	44	43	39
β-glu	15	14	15	13	14	11
lys+tyr	1.1	1.3	1.0	1.4	1.6	1.4
TOTAL (μM)	55	45	61	51	53	50
DOC (mgC/L)	12	13	13	12	12	15

[a]Hours of refrigerated storage between squeezing of subsamples A and B.
[b]None detected.

could be completed. The glass fiber filters used did not completely exclude bacteria (nominal pore size of glass fiber filters is 0.3 to 1 μ). Table 2-6 shows replicate analyses of four Buzzards Bay (BBBC) surface sediment pore water samples. Sample A in each case was acidified to pH 2 immediately after squeezing to halt bacterial activity and then subjected to cation exchange chromatography. Sample B was stored for 24 hours at 2° before cation exchange chromatography. There are no significant differences between the two analyses. Samples kept for excessive periods without acidification do, however, deteriorate as seen in Table 2-7. Seven days of storage resulted in losses of all amino acids except β-aminoglutaric acid in two out of three samples. Higher bacterial cell counts (provided by J. E. Hobbie) were present in the more decomposed samples. Similar decomposition was observed after two years of frozen storage for some samples from Knorr 73-2, which were not acidified prior to freezing, as compared to duplicate samples which had been subjected to on-board column chromatography.

Comparison of Squeezing with Other Interstitial Water Sampling Methods

Water in sediments may be present as:

(1) Free water in interstices or "pores" between sediment grains. Such water may interact with a portion of the solid phase having a particular composition and/or be in limited diffusive contact with other "pores", resulting in a unique composition.

(2) "Bound" water which is adsorbed to clays or present as water of hydration in iron oxides or other minerals, and has a charac-

TABLE 2-6

Effect of Sample Storage after Squeezing

Amino Acid	Composition (mole %)							
	0-2 cm		2-4 cm		4-6 cm		6-8 cm	
	A[a]	B[b]	A	B	A	B	A	B
ala	22	21	12	13	7.6	6.7	10	8.6
gly	15	14	3.5	3.1	4.8	3.0	3.7	3.3
val	1.4	1.5	1.2	1.3	0.8	0.6	0.8	0.8
thr	1.2	0.9	1.1	0.9	1.1	0.7	1.0	0.9
ser	2.4	1.9	1.7	1.4	1.6	1.2	1.8	1.7
leu	0.6	0.6	0.6	0.6	0.5	0.5	0.5	0.5
ile	0.5	0.6	0.4	0.4	0.4	0.3	0.4	0.4
pro	---[c]	---	---	---	---	---	---	---
asp	8.1	8.0	6.0	6.0	4.4	4.2	3.3	3.2
phe	0.4	0.4	0.4	0.4	0.4	0.2	0.4	0.4
orn	1.2	1.9	1.7	2.0	3.8	2.0	3.7	2.6
glu	37	38	48	48	46	50	42	44
β-glu	7.8	8.1	20	21	27	30	31	33
lys+tyr	3.4	3.2	2.7	2.0	1.7	0.8	1.4	1.3
TOTAL (μM)	83	75	55	60	50	40	30	27

[a]Acidified immediately after squeezing.
[b]Stored for 24 hours at 2°C unacidified before cation exchange chromatography.
[c]Proline peak in these samples was obscured by a contaminant.

TABLE 2-7

Effect of Prolonged Sample Storage after Squeezing

Amino Acid	Composition (mole %)					
	BBP12/5G-1A		BBP12/5G-2A		BBP12/5G-3A	
	1[a]	2[b]	1	2	1	2
ala	10	13	14	0.6	13	1.5
gly	17	14	23	---	11	11
val	2.3	1.1	1.2	2.4	1.3	1.2
thr	0.9	1.1	0.9	0.4	1.5	1.0
ser	2.4	2.4	2.5	---	3.1	---
leu	0.5	0.5	0.5	0.1	0.5	---
ile	0.3	0.4	0.4	---	0.3	---
pro	1.1	1.0	1.4	---	1.7	---
asp	4.7	5.0	4.4	2.5	5.2	1.7
phe	0.3	0.4	0.3	---	0.3	0.2
orn	1.2	1.0	1.4	---	2.3	2.7
glu	42	45	34	26	43	56
β-glu	15	13	15	65	14	23
lys+tyr	1.1	1.2	1.0	3.4	1.6	1.7
TOTAL (μM)	55	51	61	13	53	28
Bacteria (10^6 cells/mL)		0.37		6.3		7.2

[a]Cation exchange chromatography within 24 hours after squeezing (from Table 4).
[b]Stored refrigerated for 1 week prior to cation exchange chromatography.

teristic rate of exchange with the bulk solution.

(3) Cell fluids of benthic organisms and bacteria.

Thus interstitial water or pore water cannot a priori be assumed to have uniform composition. Further, the composition of water extracted from sediments may vary according to the sampling method used.

Most of the interstitial water samples analyzed for organic compounds in this thesis work were extracted using a stainless steel, hydraulically-powered squeezer. This squeezer was operated at a pressure of about 2000 p.s.i., and extracted approximately 50 % (depending on sediment type and water content) of the total water in the sediment. The squeezing process took about 15 minutes. Possible effects of this extraction method include:

(1) Changes in adsorption, solubility, ion-exchange, or other equilibria due to changes in temperature and pressure from in situ to laboratory conditions. Temperature-of-squeezing effects have been shown to be important for some ions, especially K^+, in pore water extracted from deep-sea sediments (Manglesdorf et al., 1969). Pressure effects are mainly important for gasses, e.g. CH_4 (Manheim, 1974). However, adsorption of amino acids by Buzzards Bay sediments has been investigated (see discussion later in this section and also in Chapter 5) and, while not negligible, is probably not a major determinant of the free amino acid distributions.

(2) Changes in composition during the time interval between coring and squeezing due to biological activity or chemical reaction. Stability of the DFAA composition of stored sediment samples

has been discussed earlier.

(3) In addition to sampling "free" water, cell fluids of organisms may be extracted, either by rupturing cell membranes or by stress-induced excretion. This effect could be of particular importance in the case of organic compounds, where intracellular fluids may have very different composition from extracellular.

To evaluate squeezing as a pore water extraction method for DFAA, pore water obtained by several other methods was analyzed for amino acids. The sampling methods included water extractions of core subsamples, separation of pore water from sediment by centrifugation, and *in situ* sampling with "peepers" (diffussion samplers, described in more detail below).

Water Extractions:

Data from extractions of several sediment samples with various aqueous solutions is summarized in Table 2-8. The Buzzards Bay (BBP) and Pettaquamscutt River Estuary (PRE) sediments are described in Chapter 5. The Great Sippewisset Marsh (GSM) sediment consisted largely of living and dead roots and rhizomes of *Spartina alterniflora*, and was about 15 % organic carbon by weight. Work on the marsh sediment was carried out in collaboration with Drs. J. Hobbie, R. Howarth, and P. Kilham of the Marine Biological Laboratory, Woods Hole (Henrichs *et al.* in preparation).

Extractions were carried out by mixing the water and sediment in the weight/weight ratio given in parentheses in Tables 2-8 and 2-9 for five minutes, and then filtering the slurry through a precombusted Gelman Type A glass fiber filter with aspirator suction. Sargasso Sea surface water

TABLE 2-8

Comparison of Water Extractions with Squeezing

	Concentration (μM)[a]								
		BBP12/5G-1				BBP12/5G-2		BBP12/5G-3	
Amino Acid	Squeeze[c]	SSSW[a] (3:1)[d]	DW:SSSW[a] (3:1)	DW[a] (3:1)	BDW[a] (3:1)	SSSW (3:1)	BDW (3:1)	SSSW (3:1)	BDW (3:1)
ala	6.9	0.5	3.9	8.6	53	0.5	41	0.4	44
gly	6.0	---	1.4	4.4	36	0.7	43	1.0	31
val	0.8	---	0.5	1.4	11	0.2	14	---	12
thr	0.6	---	0.4	1.5	11	---	12	---	13
ser	1.4	---	---	3.0	14	---	20	---	19
leu	0.3	---	---	0.4	17	---	15	---	16
ile	0.2	---	---	0.3	5.8	---	4.5	---	6.0
pro	0.7	---	0.2	0.9	6.8	---	4.7	---	5.9
asp	2.6	0.2	1.5	5.1	28	0.3	26	0.2	22
phe	0.2	---	---	0.4	8.3	---	8.5	---	9.1
orn	0.8	---	---	---	6.1	---	4.0	---	4.1
glu	22	0.3	13	83	220	---	180	0.2	131
β-glu	7.2	---	4.4	26	50	---	38	---	26
lys+tyr	---	---	0.2	1.3	12	---	14	---	13
TOTAL	53	1.0	26	140	470	1.7	430	1.7	350
DOC[e]	13	10	21	60	530	N.D.[f]	520	9.2	570

	BBP12/5				PRE				GSM	
	11-14 cm		29-32 cm		0-30 cm		52 82 cm			
Amino Acid	Squeeze	BDW	Squeeze	BDW	Squeeze	DW	Squeeze	DW	Squeeze	$NaNO_3$ Solution
ala	0.8	11	0.2	1.5	0.7	2.0	0.8	0.7	13	13
gly	0.7	12	---	2.9	0.4	1.2	1.0	0.7	2.7	7.6
val	0.2	5.5	0.2	1.5	0.2	0.9	0.5	0.7	4.8	6.3
thr	0.1	6.3	0.2	1.1	0.2	0.7	0.4	0.4	5.3	7.0
ser	---	7.4	---	---	0.3	0.9	0.6	0.7	7.6	11
leu	0.09	8.8	0.06	0.8	0.1	0.4	0.5	0.5	1.8	2.8
ile	0.06	2.4	---	0.2	0.05	0.3	0.2	0.2	1.9	2.3
pro	0.1	1.6	---	---	0.4	0.9	0.2	0.2	58	49
asp	0.7	8.7	0.2	1.2	0.6	1.5	0.7	0.8	27	20
phe	0.05	4.6	---	0.4	0.04	0.2	0.1	0.2	0.9	1.7
glu	11	49	1.8	4.0	3.2	15	1.8	2.2	50	42
β-glu	8.3	18	2.3	3.7	1.2	5.5	0.9	1.6	7.8	3.5
lys+tyr	---	---	---	---	0.3	0.6	0.4	---	2.3	2.1
TOTAL	21	140	5.2	20	8.2	31	8.3	9.1	190	180
DOC[e]	17	510	21	400	51	120	66	100	N.D.	N.D.

[a]Concentrations in water extracts corrected for dilution.
[b]SSSW = Sargasso Sea surface water; DW = distilled water; BDW = boiling DW
[c]Average of data in Table 4.
[d]Water:sediment weight:weight ratio for extraction mixture.
[e]Units of mgC/L.
[f]Not determined.

(SSSW) extractions of three Buzzards Bay surface sediment samples gave negligible free amino acid concentrations (the values given are upper limits). A 1:1 mixture of SSSW and distilled water (DW), with a salinity of about 14°/oo, gave free amino acid concentrations which were about 50 % of the concentration obtained by squeezing. DW extraction of the same sediment gave about three times the concentration obtained by squeezing (Table 2-8). The DFAA composition of the squeezed, 1:1 SSSW:DW, and DW extracts were all similar, with glutamic acid, β-aminoglutaric acid, aspartic acid, glycine, and alanine predominating.

Three Buzzards Bay surface sediment samples and two deeper sections were extracted with boiling distilled water. Extracts were cooled to room temperature before filtering. Concentrations of free amino acids in these extracts were very high, eight to ten times squeezed concentrations for the surface samples, and seven and four times the squeezed concentrations, respectively, for the 11-14 cm and 29-32 cm sections. Boiling DW and squeezed extracts were very similar in composition for the surface sections. For the 11-14 cm and 29-32 cm sections, glutamate and β-aminoglutarate were slightly less abundant relative to other amino acids in the boiling water extract, but were the most abundant amino acids in both squeezed and boiling water extracts.

Pettaquamscutt River Estuary sediments from 0-30 cm and 52-82 cm depth were extracted with room-temperature DW. The surface sediments gave three to four times the squeezed concentration (Table 2-8), while the DW-extract and squeezed concentrations were not significantly different in the deeper sediment sample. Compositions of squeezed and DW

extracts were again similar.

Tne vegetative sediments from Great Sippewisset Marsh were extracted by percolating a 21°/oo $NaNO_3$ solution (ionic strength equal to pore water) through the core section, assisted by gentle aspirator suction. The extract was then refiltered to remove fine particles. The amino acid compositions and concentrations obtained by this method were virtually identical to those obtained by squeezing (Henrichs et al., in preparation).

DOC concentrations in the SSSW extracts (when corrected for dilution) were very close to squeezed concentrations at a water/sediment ratio of 3:1, but were somewhat greater at ratios of 10:1 and 30:1 (Table 2-8). DW extracts had substantially greater concentrations than SSSW or squeezer extracts, while boiling DW-extract DOC concentrations were 15 to 50 times greater.

Three non-protein amino acids, α-aminoadipic acid, diaminobutyric acid, and norleucine (which have net negative, positive, and zero charges at pH 7, respectively) were added to the solutions used to extract Buzzards Bay sediments. The % recoveries in the filtrate are given in Table 2-9. Recoveries were higher at higher concentrations of spike and higher water:sediment ratios. Recoveries were also greater for 14°/oo water and DW relative to SSSW, and greater still for boiling DW extractions. In general, norleucine (neutral) and α-aminoadipic acid (acidic) were recovered to about the same extent, while recoveries of diaminobutyric acid (basic) were significantly lower.

The results of the water extraction experiments appear to be due to a

TABLE 2-9

Recovery of Spikes from Water Extracts (%)

Sample, Extract	Norleucine	α-Aminoadipic Acid	Diaminobutyric Acid	DOC (mgC/L)
BBP12/5G-1				
SSSW(3:1)(1X)[a]	38	24	12	10
SSSW:DW(3:1)(1X)	51	70	13	21
DW(3:1)(1X)	62	118	25	60
BDW(3:1)(1X)	91	150[b]	38	530
BBP12/5G-2				
SSSW(3:1)(1X)	43	39	14	N.D.
SSSW(3:1)(10X)	78	75	43	N.D.
SSSW(3:1)(100X)	84	89	63	N.D.
BDW(3:1)(1X)	100	146	36	520
BBP12/5G-3				
SSSW(3:1)(1X)	44	40	12	9.2
SSSW(10:1)(1X)	67	44	37	18
SSSW(30:1)(1X)	74	48	36	40
BDW(3:1)(1X)	71	99	42	570

[a](Water:sediment ratio)(relative spike concentration). The concentrations in the 1X-spiked solutions were 0.79 μM norleucine, 0.93 μM α-aminoadipic acid, and 2.77 μM diaminobutyric acid.
[b]Recoveries greater than 100 % are due to a co-eluting substance extracted from the sediment.

combination of chemical (adsorption) and biological effects. Increased extraction of DOC and recovery of amino acid spikes at higher water/sediment ratios and decreased S^{o}/oo could be reasonably explained by adsorption of both DOC and amino acids by some sediment component(s). The lower recoveries of diaminobutyric acid relative to α-aminoadipic acid and norleucine are consistent with the results of Hedges (1978) and Abelson and Hare (1971), who found greater uptake (in an irreversible process) of basic amino acids by melanoidin and marine humic-type polymers. Similar substances make up a substantial proportion of total sediment organic matter (Borodovskiy, 1965).

However, the lack of DFAA (other than the spikes) in the SSSW extracts and the extremely high DOC and free amino acid concentrations in boiling DW extracts are probably due to the presence of living organisms in the sediment. The boiling water extracts likely represent virtually all of the free amino acid pool in the Buzzards Bay sediments, as this treatment should have extracted intracellular as well as extracellular amino acids. The larger DOC concentrations and spike recoveries might also be due to the destruction of cells, if much of the "adsorptive" loss of the spikes was in fact due to uptake by organisms. (The use of non-protein amino acids was intended to minimize this but may not have been completely effective.) Alternatively, if loss of the spikes was due to non-biological adsorption by sediment organic matter, perhaps "denaturation" or changes in organic matter structure at boiling water temperatures was responsible for decreased sorption of both DOC and free amino acids. Further implications of the adsorption experiment will be

discussed in Chapter 5.

The very low DFAA concentration of free amino acids in the SSSW extracts may imply that nearly all of the free amino acids extracted by the squeezer are extracted from cells or other sites which were inaccessible to SSSW extraction. Osmotic shock due to dilution with distilled water might cause release or excretion of amino acids (Griffiths et al., 1974) and be responsible for the higher concentration in DW extracts. (If this explanation is correct, the excellent agreement between the $NaNO_3$ solution extract and squeezed extract of the vegetative marsh sediment is puzzling). However, Wood (1970) found that turnover times of C-14 labelled amino acids in sediments which had been mixed with water were very short, on the order of a few seconds. If a similar effect were present in these samples, then water extractions cannot be relied on to give accurate concentration data.

Centrifugation:

Results of centrifugation extractions of various sediments are given in Table 2-10. Centrifugation was carried out for 15 to 30 minutes at 1500 rpm in an IEC Model HN-SII centrifuge. At this speed, bacteria and fine sediment particles were not effectively removed from the pore water, and the samples were subsequently filtered through Gelman Type A glass fiber filters. Bacteria counts of the Buzzards Bay surface sediment pore water samples (provided by J. E. Hobbie) showed that centrifuge-extracted pore water contained 3×10^6 to 3×10^7 cells/mL even after filtration.

Concentrations of free amino acids relative to those in squeezed

TABLE 2-10

Comparison of Centrifugation with Squeezing

Amino Acid	Concentration (μM) BBP12/79 Squeeze[a]	G-1 Centr.	G-2 Centr.	G-3 Centr.	BBBC 0-2 cm Squeeze	0-2 cm Centr.	2-4 cm Squeeze	2-4 cm Centr.
ala	6.9	0.04	0.05	0.05	17	0.10	7.5	---
gly	6.0	0.3	0.1	0.2	12	---	1.9	---
asp	2.6	0.02	0.02	0.02	6.4	---	3.4	---
glu	22	0.03	0.05	0.07	30	0.2	28	0.4
β-glu	7.2	---	---	0.02	6.3	0.04	12	0.02
TOTAL[b]	53	0.4	0.3	0.4	79	0.4	58	0.4
DOC[c]	13	9.7	11	14	38	5.3	21	11

Amino Acid	Concentration (μM) PRE 0-30 cm Squeeze	Centr.	PRE 52-82 cm Squeeze	Centr.	GSM Squeeze	Centr.
ala	0.7	0.3	0.8	0.4	13	2.4
gly	0.4	0.5	1.0	1.0	2.7	0.9
asp	0.6	0.4	0.7	0.4	27	2.5
glu	3.2	1.2	1.8	1.0	50	10
β-glu	1.2	0.2	0.9	0.5	7.8	2.6
TOTAL[b]	8.2	3.7	8.3	5.4	190	36
DOC[c]	51	230	66	160	N.D.	N.D.

Amino Acid	Concentration (μM) GH Squeeze	Centr.	Squeeze	Centr.	Squeeze	Centr.
ala	21	0.9	14	1.5	4.8	8.7
gly	25	2.4	23	5.1	19	24
asp	6.4	0.1	4.8	0.2	2.6	1.4
glu	44	0.3	36	0.6	18	5.7
β-glu	8.1	0.1	9.1	0.3	6.0	1.9
TOTAL[b]	110	4.6	93	8.7	55	49
DOC[c]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

[a]Squeezed concentrations are averages of data given in Tables 4 and 5.
[b]Includes some amino acids not shown.
[c]Units of mgC/L.

samples were highly variable, from essentially 0 % to 100 % (Compare Table 2-10 with Table 2-8). Compositions were similar when concentrations were comparable, although glutamic and β-aminoglutaric acids were in general relatively less abundant in centrifuge extracts. DOC concentrations were likewise quite variable when measured, ranging from 0.2 to 4 times the concentrations in squeezed pore waters.

Jorgensen et al. (in preparation) investigated the effect of centrifugation times on the concentrations of DFAA in pore waters extracted from estuarine sediments. They found that concentrations decreased with increasing time up to 12 minutes (the longest time investigated). Composition also changed significantly, although not consistently in all samples. Their apparatus and samples differed from those used in this study, so quantitative extrapolation of their results is not possible. However, it seems likely that 15 to 30 minute centrifuging times may have contributed to the low amino acid recoveries in some samples. Bacterial activity seems the most likely cause for this effect.

In situ Sampling:

In situ sampling was carried out at two different shallow-water stations using "peepers" (Hesslein, 1976). The "peeper" apparatus used consisted of a series of 0.8 cm depth-interval, 4 ml-volume cavities in a plexiglas slab. These cavities were filled with sterile SSSW containing non-protein amino acids (norleucine, or a mixture of norleucine, α-aminoadipic acid, and diaminobutyric acid) as equilibration monitors. The cavities were covered with a 0.2 μ Nucleopore membrane filter, a glass fiber filter, Nitex mesh, and a perforated plexiglas plate to

secure the filters. The assembled "peeper" was sterilized by boiling in a bath of filling solution for 15 minutes.

In situ samplers were placed in sediments at two stations, BBBC (located close to Buzzards Bay station P, with a similar sediment type) and GH, a sandy, anoxic sediment at about 15 m depth in Great Harbor, Woods Hole, Massachusetts. Divers inserted the "peepers" by hand, one at BBBC and two, located about 1 m apart, at GH. The BBBC deployment was not completely successful, as the sampler was left in place for three weeks (4/30/80 to 5/20/80) and at some time was disturbed by the mooring line of the marker bouy. The GH deployment was for a period of 1 week (6/10/80 to 6/17/80). In all three samplers, loss of the nonprotein amino acid spikes indicated equilibration with sediment pore water had taken place.

The concentrations of DFAA measured in the "peeper" cavities after recovery are given in Table 2-11. Comparing BBBC "peeper" data with Table 2-5, and GH "peeper" data with Table 2-10, it can be seen that only a small fraction of the amino acid concentration sampled by squeezing was found in the "peepers". This data could tend to confirm the hypothesis that most of the free amino acids measured in squeezed extracts were extracted from cells, and thus are not free to diffuse into the "peeper". However, another interpretation is possible. Counts of the bacteria in the filling solution of the GH-1 "peeper" (provided by F. Valois) after recovery were quite high, from 2 to 6 x 10^6 cells/mL. The cells were mostly more than 0.2 μ in diameter, but may have grown after passing through the membrane or have penetrated it actively (C.

TABLE 2-11

In situ Sampler

Total DFAA Concentration (μM)[a]					
GH-1		GH-2		BBBC	
Depth (cm)	DFAA	Depth (cm)	DFAA	Depth (cm)	DFAA
0-2	0.9	1.3	0.9	0-2	0.6
2-4	1.7	2-4	0.7	2-4	0.3
4-6	0.7	4-6	0.5	4-6	0.3
6-10	0.3	6-10	0.3	6-8	0.08
10-14	0.6	10-14	0.7	8-10	0.4
14-17	0.7	14-18	0.3	10-12	0.3

[a]Glutamic acid and glycine were the major components in most cases. Aspartic acid, alanine, serine, and β-aminoglutaric acid were also present in some samples.

Taylor, pers. comm.). These bacteria alone probably could not have metabolized all of the DFAA which would have diffused into the "peeper" from an exterior pool of 50 to 100 μM, judging from the rates of bacterial uptake of amino acids in estuarine enviroments with bacterial populations on the order of 10^6/mL, about 0.1 to 1 μM/day (Crawford et al., 1974; Dawson and Gocke, 1978). However, additional bacterial growth may have taken place on the chamber walls or on the filter membrane sur face itself, acting as a "biological filter" to metabolize amino acids before they could diffuse into the "peeper" cavities (C. Taylor, pers. comm.).

Summary and Conclusions

Analytical error (including cation exchange chromatography, derivatization, and GCGC) was about 10 % for most of the amino acid concentrations reported in this thesis. Measurement of methionine, tyrosine, and to some extent lysine was less accurate. These amino acids were minor constituents of pore water samples, however. The detection limits, determined by analytical blanks, were about 0.05 μM for most amino acids. However, sampling rather than analytical error is the most important consideration in valid comparison and interpretation of DFAA data. Analyses of replicate squeezings agreed within about 20 % for total concentrations, and compositions of replicates were very similar except for the relative abundance of glycine in some samples. In the following discussions, concentration differences of less than 20 % will not be considered significant.

It is not clear from the extraction experiments described in this chapter which, if any, of the investigated sampling methods best recovers the in situ dissolved free amino acid distribution in interstitial water. It is apparent that the effects of biological processes during sampling and pore water extraction can be important to measured free amino acid distributions and that the ideal sampling strategy would produce minimum disturbance to the sediment. Features of an ideal sampling apparatus would include: in situ operation; relatively short deployment time; extraction of interstitial water with minimum shear and pressure stress, but with effective exclusion of bacteria from the sample; and design to minimize organic and biological contamination by the sampler.

Squeezing falls short of this ideal. Samples must be removed from in situ conditions, and the pressure applied may extract free amino acids from living cells. However, squeezing does give reproducible results for a given sediment sample; for Buzzards Bay sediments, concentrations and compositions recovered by squeezing were consistently related to the total free amino acid pools which could be extracted with boiling distilled water; and it will be shown in the remaining chapters of this thesis that the results from squeezed samples form an internally consistent body of data in which DFAA concentration and composition are related to aspects of the sedimentary environment.

CHAPTER 3

AMINO ACID COMPOSITION OF SEVERAL MARINE ORGANISMS AND BACTERIA

Introduction

Data on the composition of dissolved free amino acids and insoluble protein amino acids from several marine organisms and bacteria as determined using GCGC will be presented and discussed in this chapter. Considerable information on the free amino acid composition of marine invertebrates (e.g. Awapara, 1962) and bacteria (e.g. Brown and Stanley, 1972) is available in the literature. However, a surprising finding of this thesis work is that β-aminoglutaric acid ($HOOCCH_2CH(NH_2)CH_2COOH$) is a major component of the dissolved free amino acids in most marine sediment samples analyzed (See chapters 4, 5, and 6). A thorough search of the biochemical and chemical literature has failed to find any reference to this compound as a natural product. This suggested the possibility that β-aminoglutaric acid was formed as the product of a geochemical rather than a biochemical diagenetic process. However, there was no apparent biological source of a suitably reactive precursor molecule.

Another alternative is that some marine organisms or bacteria can biosynthesize β-aminoglutaric acid. Its occurrence may be restricted to a group of organisms which have not been previously examined, or the analytical techniques used in previous studies may not have resolved or permitted identification of β-aminoglutaric acid. Therefore, GCGC analysis of the amino acids of several biological samples was under-

taken. The number of specimens analyzed by no means constitutes an exhaustive survey of potential biological sources of β-aminoglutaric acid. The objective was to determine if this compound is a fairly common constituent of marine organisms or bacteria which had been overlooked in previous studies.

The study of bacterial amino acid composition benefitted greatly from the efforts of Russell Cuhel (Woods Hole - M.I.T. Joint Program in Biological Oceanography), who donated the Chromobacterium sp. and Pseudomonas halodurans cultures, performed the selective enrichment of fermenting bacteria from the Pettaquamscutt River Estuary (PRE) sediment, and provided much useful information and discussion. Dr. C. Taylor and F. Valois/Dr. S. Watson also donated culture material for analysis.

Samples and Methods

Sediments containing Nepthys incisa were collected at three stations in the New York Bight and wet-sieved to separate infaunal organisms. The Nepthys were frozen whole in clean glass jars and stored frozen until analyzed. The tissues were homogenized in a Tekmar Tissumizer$^{(R)}$, and an aliquot was removed for amino acid analysis. Free amino acids were extracted by adding distilled water and mixing for 5 minutes with the Tissumizer. The solution was then filtered to remove particulates and analyzed for DFAA as described in Chapter 2. The particulate matter was hydrolyzed in 6 N HCl for 24 hours and analyzed for amino acids. A portion of the water extract was also hydrolyzed to determine soluble protein composition. However, soluble protein composition was virtually

identical to that of the particulate proteins and will not be reported separately. An Ophioroid (about 5 cm in diameter) captured on the top of a core taken in the Georges Basin of the Gulf of Maine (GM8) was analyzed in the same manner.

The Chromobacterium sp. (The bacterium identified as Chromobacterium may in fact be another purple-pigmented type, Alteromonas luteoviolaceus, described by Gauthier (1976); R. Cuhel, pers. comm.) and P. halodurans were grown in an artificial seawater medium (ASW) (Lyman and Fleming, 1940), with added 20 mM D-glucose, 1 mM NH_4^+, 80 µM PO_4^{3-}, 1 mM SO_4^{2-}, and trace metals. Exponentially growing cells were harvested by centrifuging at 5000g for 5 minutes, then resuspended in ASW and innoculated into the above medium at 0.05/250 v/v. After 51 hours (P. halodurans) or 95 hours (Chromobacterium) of growth at 22°C, the cells were harvested by centrifuging at 5000g for 20 minutes and the pellet rinsed twice with ASW (R. Cuhel, pers. comm.). The pellets were extracted with boiling distilled water and the resulting solutions filtered to remove particulates. DFAA in the filtrates were analyzed as described in Chapter 2. Insoluble protein from the Chromobacterium was hydrolyzed in 6 N HCl for 24 hours and analyzed for freed amino acids.

Desulfovibrio salexigens was cultured as described by Taylor et al. (in preparation) in an anaerobic ASW medium with added Tris-base (1.21 g/L), NH_4Cl (0.020 g/L), L-calcium lactate (10 mM), and a variety of trace constituents. Nitrosococcus oceanus was grown in batch culture in the medium described by Watson (1965), with the addition of phenol red (1 mL/L 0.5 % solution). The pH was adjusted as needed by adding 0.1 M

K_2CO_3, and the culture was grown to a density of 10^8 cells/mL. The cells in both cases were harvested by centrifugation and extracted as described above.

A selective enrichment of fermenting cells from PRE surface sediments (see Chapter 5) was carried out in the following manner (R. Cuhel, pers. comm.). A sediment sample which had been stored frozen for approximately four months was thawed and 0.25 mL was suspended in 10 mL Sargasso Sea water, with added NH_4^+ (500 μM), PO_4^{3-} (40 μM), trace metals, and 5 mM of one of the following: glucose, fructose, glycerol, sucrose, galactose, mannitol, δ-gluconolactone, or mannose. The eight innoculated media were sparged for 10 minutes and capped. After five days the cultures were spun down at 5000g for 10 minutes and then resuspended in 5 mL ASW. They were innoculated at 0.25 mL/25 mL into the above media, except substrate concentrations were increased to 20 mM. After 24 days the cells were harvested; the glucose, fructose, galactose, and mannitol substrates yielded enough material for analysis. The cells were extracted as described above and analyzed for DFAA.

Results and Discussion

The results of the free amino acid analyses of Nepthys (a polychaete worm) and the Ophioroid (brittle star) are given in Table 3-1. Glycine is the most abundant amino acid in both organisms analyzed, with alanine, aspartic acid, and glutamic acid each at about 1/3 the glycine concentration. The Nepthys samples from the three different stations had virtual-

TABLE 3-1

Dissolved Free Amino Acid and Protein Composition of Two Benthic Invertebrates

	Composition (mole %)					
	Free				Protein	
Amino Acid	Nepthys STA LL	Nepthys STA H	Nepthys STA M	Ophioroid (GM8)	Nepthys (avg. of 3)	Ophioroid (GM8)
ala	9.5	12	11	8.1	8.6	8.1
gly	29	26	35	29	16	16
val	3.7	5.0	3.3	4.7	5.5	4.4
β-ala	---[a]	---	---	8.5	---	---
thr	3.3	4.2	3.6	3.4	5.3	5.5
ser	3.1	3.7	3.8	5.6	3.0	4.9
leu	7.2	7.8	7.4	4.2	6.6	6.2
ile	4.0	4.8	4.1	3.7	4.8	3.9
pro	9.4	6.4	6.9	2.8	5.7	7.5
hypro	---	---	---	---	0.7	1.9
met	2.3	2.5	0.8	0.7	0.6	---
asp	7.3	8.6	7.0	8.5	17	15
phe	3.2	3.0	3.2	1.6	3.1	3.5
glu	7.5	9.3	7.9	9.5	13	14
β-glu	---	---	---	---	---	---
lys	7.5	4.7	5.5	6.6	6.7	6.1
tyr	2.9	1.6	2.9	2.9	3.4	2.4

[a]Below detection limit of 0.1 mole %.

ly identical compositions. The Ophioroid DFAA were quite similar, except that a relatively large amount of a nonprotein amino acid, β-alanine, was present. These compositions are similar to those reported for many other benthic invertebrates (Awapara, 1962). Alanine and/or taurine (an amino sulfonic acid not measured in this study) are often more abundant than glycine in molluscs (Awapara, 1962; de Zwaan, 1977). Serine has been reported as the major constituent of the ceolomic fluid of a sea urchin (Giordano et al., 1950). The amino acid compositions of the insoluble proteins are similar to the free amino acid compositions (Table 3-1), except that glycine is reduced by about a factor of 0.5 in relative abundance and aspartic and glutamic acids are correspondingly increased. β-Aminoglutaric acid was not detected in either the protein or free amino acid fractions (detection limit about 0.1 mole %).

The dissolved free amino acid compositions of the five pure bacterial cultures analyzed are shown in Table 3-2. The bacteria represent several nutritional types: the obligately-aerobic heterotrophs Chromobacterium and P. halodurans; the sulfate-reducing heterotroph Desulfovibrio; and the autotrophic ammonia oxidizer Nitrosococcus oceanus. However, all have glutamic acid as the major dissolved free amino acid. In Desulfovibrio, glutamic acid makes up 95 % of the dissolved free amino acid pool. This may be because Desufovibrio lacks α-ketoglutarate dehydrogenase, which truncates the TCA cycle at α-ketoglutarate, the immediate biosynthetic precursor of glutamic acid (Lewis and Miller, 1977). Nitrosococcus oceanus possesses all TCA cycle enzymes, although succinate dehydrogenase is in very low abundance (le B. Williams and

TABLE 3-2

Free Amino Acid Pool Composition of Some Marine Bacteria

Amino Acid	Composition (mole %)				
	Chromobacterium[a]				
	A	B	P. halodurans	N. oceanus	Desulfovibrio
ala	5.9	9.6	4.8	11	1.4
gly	3.9	4.5	12	15	0.9
val	1.9	3.8	2.7	4.8	0.4
β-ala	---	---	3.3[b]	---	---
thr	1.8	1.4	1.7	2.9	0.3
ser	3.5	1.8	1.9	5.1	---
leu	2.5	2.3	0.6	6.3	---
ile	1.0	1.2	0.8	3.3	---
γ-aba	---	---	11[b]	---	---
pro	0.6	0.9	0.5	3.0	0.5
asp	6.5	1.9	1.5	4.6	0.9
phe	1.8	1.0	0.5	4.9	---
glu	62	63	47	30	95
β-glu	6.2	5.3	---	---	---
lys	0.7	2.4[c]	3.0[c]	8.5[c]	---
tyr	1.3	---	---	---	---
TOTAL[d]	N.D.	2.4	0.23	2.6	0.63

[a]May be Alteromonas luteoviolaceus.
[b]Presence confirmed by high performance liquid chromatography (C. Lee, pers. comm.).
[c]Sum of lysine and tyrosine.
[d]Units of μmoles/10^{10} cells

Watson, 1968), and glutamic acid makes up only 30 mole % of its DFAA pool.

Brown and Stanley (1972) and Stanley and Brown (1976) have also found glutamic acid (or glutamine, which the GCGC method does not distinguish from glutamate) to be the major constituent of free amino acid pools of most of the marine bacteria studied. In some cases proline (which is derived biosynthetically from glutamic acid) was a major constituent of the pools. They also found alanine and aspartic acid in trace amounts.

The DFAA pool of Chromobacterium contained β-aminoglutaric acid at about one-tenth of the abundance of glutamic acid. β-Aminoglutaric acid was not detected in the other species analyzed. The identity of the β-aminoglutaric acid peak in the GCGC of Chromobacterium DFAA was confirmed by retention time and by GC/MS (Figure 3-1). The mass spectrum is identical to that of the compound found in marine sediments (see Figure 5-10) and to that of authentic β-aminoglutaric acid. The occurrence of β-aminoglutaric acid in Chromobacterium was further confirmed by a second analysis of culture material. The bacteria were maintained in the laboratory of R. Cuhel for 1 year between analyses. The major features of DFAA composition, including the ratio of β-aminoglutaric acid to total free amino acids, were not significantly different in the two cultures.

Because bacteria include several non-protein amino acids in their cell walls (e.g. diaminopimelic acid, D-alanine, and D-glutamic acid; Salton, 1961), the insoluble protein residues of the water extractions of Chromobacterium and Desulfovibrio were checked for the occurrence of β-aminoglutaric acid (Table 3-3). The protein compositions of the two bacteria were very similar, with alanine, glycine, valine, serine, aspar-

Figure 3-1: Mass spectrum of β-aminoglutaric acid from Chromobacterium. (SE-52 glass capillary column interfaced with a Finnigan GC/MS 3200, ionization voltage 70 eV).

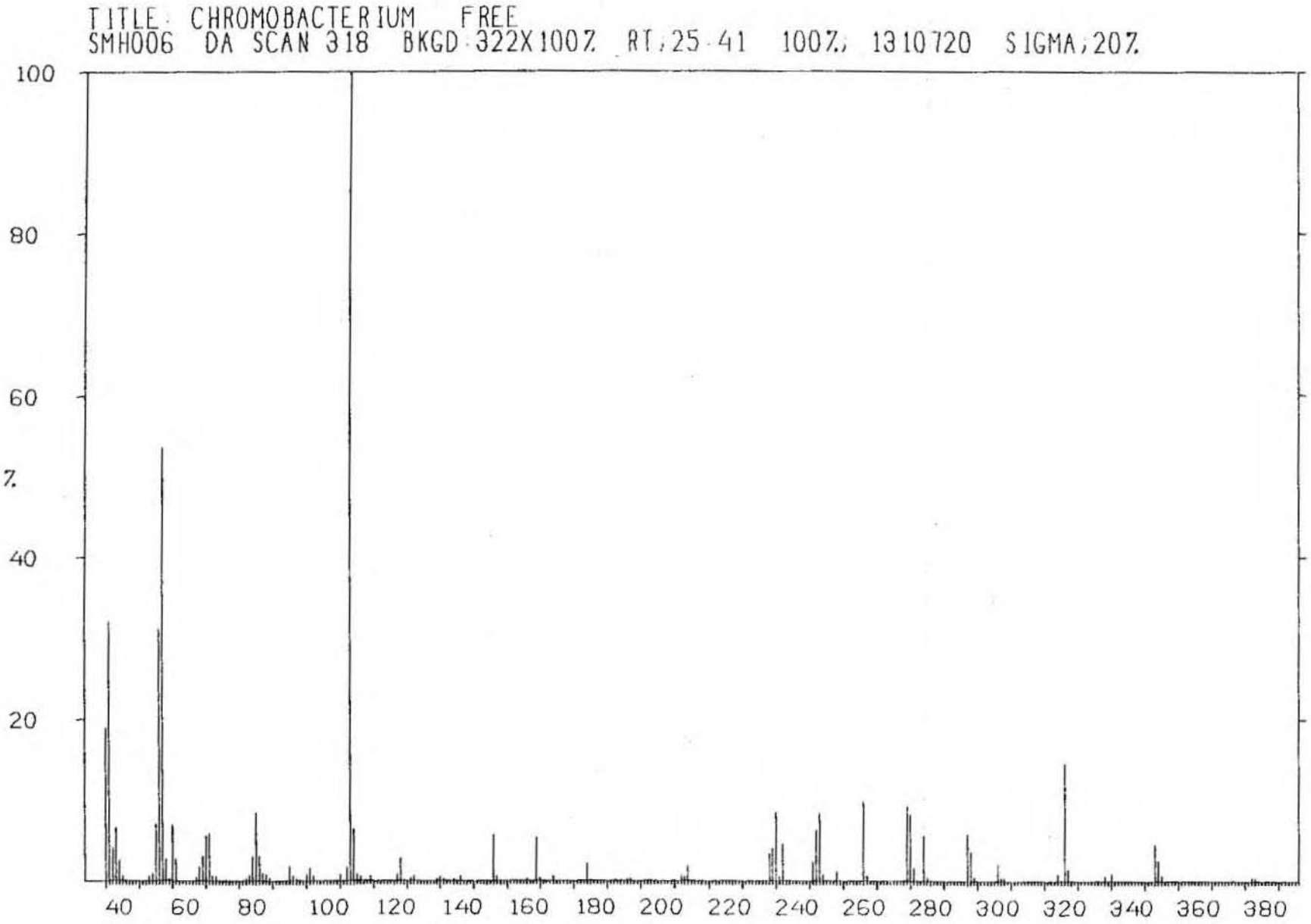

TITLE: CHROMOBACTERIUM FREE
SMH006 DA SCAN 318 BKGD 322X100% RT;25-41 100%; 1310720 SIGMA;20%
100
80
60
%
40
20
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360 380

TABLE 3-3

Composition of Insoluble Bacterial Protein[a]

Amino Acid	Composition (mole %)	
	Chromobacterium[b]	Desulfovibrio
ala	12	12
gly	11	13
val	9.0	7.8
thr	6.7	6.1
ser	9.5	9.4
leu	6.1	5.8
ile	3.6	4.4
pro	3.5	4.4
met	0.4	0.8
asp	8.3	8.7
phe	3.4	3.7
glu	11	9.9
β-glu	---	---
lys	8.0	9.0
tyr	2.6	2.4

[a]Includes but is not limited to cell membrane/wall.
[b]May be Alteromonas luteoviolaceus.

TABLE 3-4

Free amino Acid Pool Composition of Fermenting Bacteria from Pettaquamscutt River Estuary Sediment

Amino Acid	Composition (mole %) Substrate			
	Mannitol	Fructose	Galactose	Glucose
ala	11	17	20	9.6
gly	7.2	13	9.3	12
val	7.9	13	9.8	10
thr	3.5	8	---	3.1
ser	---	---	---	---
leu	4.8	11	6.3	7.0
ile	5.5	12	6.8	10
pro	3.8	---	3.9	---
asp	5.4	7.8	2.9	2.1
phe	2.1	4.8	2.0	3.5
glu	46	13	36	35
β-glu	---	---	4.1	---
lys+tyr	3.8	---	---	7.0
TOTAL[a]	27	16	19	21

[a]Cell number unknown: total (nmoles)/number of cells present at end of growth.

tic acid, glutamic acid, and lysine all present at about 10 mole %. No β-aminoglutaric acid was detected.

In order to determine whether bacteria actually present in marine sediments synthesize β-aminoglutaric acid, mixed cultures of fermenting bacteria were enriched from anoxic PRE sediments. Four substrates gave enough cell material for amino acid analysis (Table 3-4). Three cultures had compositions similar to those of the pure cultures, with glutamic acid as the major component. Alanine had the highest relative concentration in cells grown on fructose. Only the galactose-substrate grown cells contained dissolved free β-aminoglutaric acid, at about one-tenth of the glutamate concentration. The different compositions of the cells grown on the four substrates are likely due to different species assemblages being selected during the enrichment process.

Conclusions

These results show that β-aminoglutaric acid can be biosynthesized by some bacteria. Its occurrence is not universal, but its presence in one of four pure cultures analyzed by GCGC suggests fairly widespread distribution (or enormous luck). The Chromobacterium is an obligate aerobe, but β-aminoglutaric acid was also found in a mixed culture of fermenters enriched from PRE sediments. The ratio of β-aminoglutaric acid/glutamic acid was about 0.1 in both cases, however, which is substantially less than the ratio in DFAA of most sediment samples (see Chapters 4, 5, and 6). It is possible that some types of bacteria not analyzed synthesize relatively more of this compound, or that conditions in culture relative to conditions in sediments discouraged its production or accumulation.

CHAPTER 4: RESULTS FROM THE PERU UPWELLING REGION

Introduction

The coastal waters of Peru are highly productive because of upwelling in the Peru Current system, which forms the eastern boundary of the South Pacific Ocean. Two centers of consistent upwelling are identified by Zuta et al. (1975), located between 4°and 6°S and between 14° and 16°S. Upwelling originates from depths of no more than 100 m in the Peru coastal region, and the strongest upwelling is associated with maximum wind speeds during southern-hemisphere winter (Wyrtki, 1963).

Primary production rates during upwelling typically range from 1 to 3 gC/m^2-day (Zuta and Guillen, 1970; Gagosian et al., 1980), and may exceed 10 gC/m^2-day (Ryther, et al., 1971). These rates are about an order of magnitude greater than the average daily rates reported for open ocean surface water, 0.1 to 0.2 gC/m^2-day (Menzel and Ryther, 1960). The high rates of primary production result in high rates of supply of detrital organic matter to the sediments off Peru. During the 1978 upwelling Staresinic (1978) measured POC fluxes (using free-drifting sediment traps) of 500 mgC/m^2-day below the euphotic zone (10 to 15 m depth), an average of 19 % of the daily primary production. The material consisted largely of anchoveta fecal casts, euphausid molts, and unidentified fecal pellets. Fluxes through 50 m depth averaged 250 mgC/m^2-day, or 9 % of the daily primary production. Fluxes through 50 m depth measured at 7 stations during the 1977 upwelling averaged 230

mgC/m^2-day, and consisted of unidentified fecal material or diatoms.

Oxygen is consumed during remineralization of this organic matter, resulting in an oxygen-minimum zone which impinges on the continental slope at depths between 100 and 500 m (Wyrtki, 1967). Sulfate reduction in the water column has been reported to occur intermittently in this zone (Dugdale et al., 1977). Gallardo (1976, 1978) describes a benthic community including Thioploca-like sulfur bacteria which form a microbial mat at the sediment surface in the low-oxygen region. Because of oxygen stress, benthic metazoans (mostly Polychaeta) are in relatively low abundance.

The sediments of the Peru continental margin are compositionally heterogeneous. Diatomaceous muds, sometimes containing abundant fish bones and scales, are common (Saidova, 1971; Gallardo, 1978). Carbonates (especially near shore, as shell fragments), clay minerals, volcanic glass, and authigenic phosphorites are also present (Rosato et al., 1975; Veeh and Burnett, 1973). The organic content of the Peru margin sediments is high, but variable. Organic carbon contents of from 3 % to more than 5 % are found in sediments underlying centers of upwelling (Rosato et al., 1975). Romankevich and Urbanovich (1971) measured total carbohydrate concentrations in Peru sediments by the phenol-sulfuric acid method and found between 0.5 and 10 mg/gdw in near-shore sediments. Concentrations of dissolved carbohydrate in pore water ranged from 1000 to 7000 µg/L.

Sediment and interstitial water samples described in this chapter were obtained during February and March, 1978 from the Peru Upwelling

Figure 4-1: R/V Knorr 73-2 station locations.

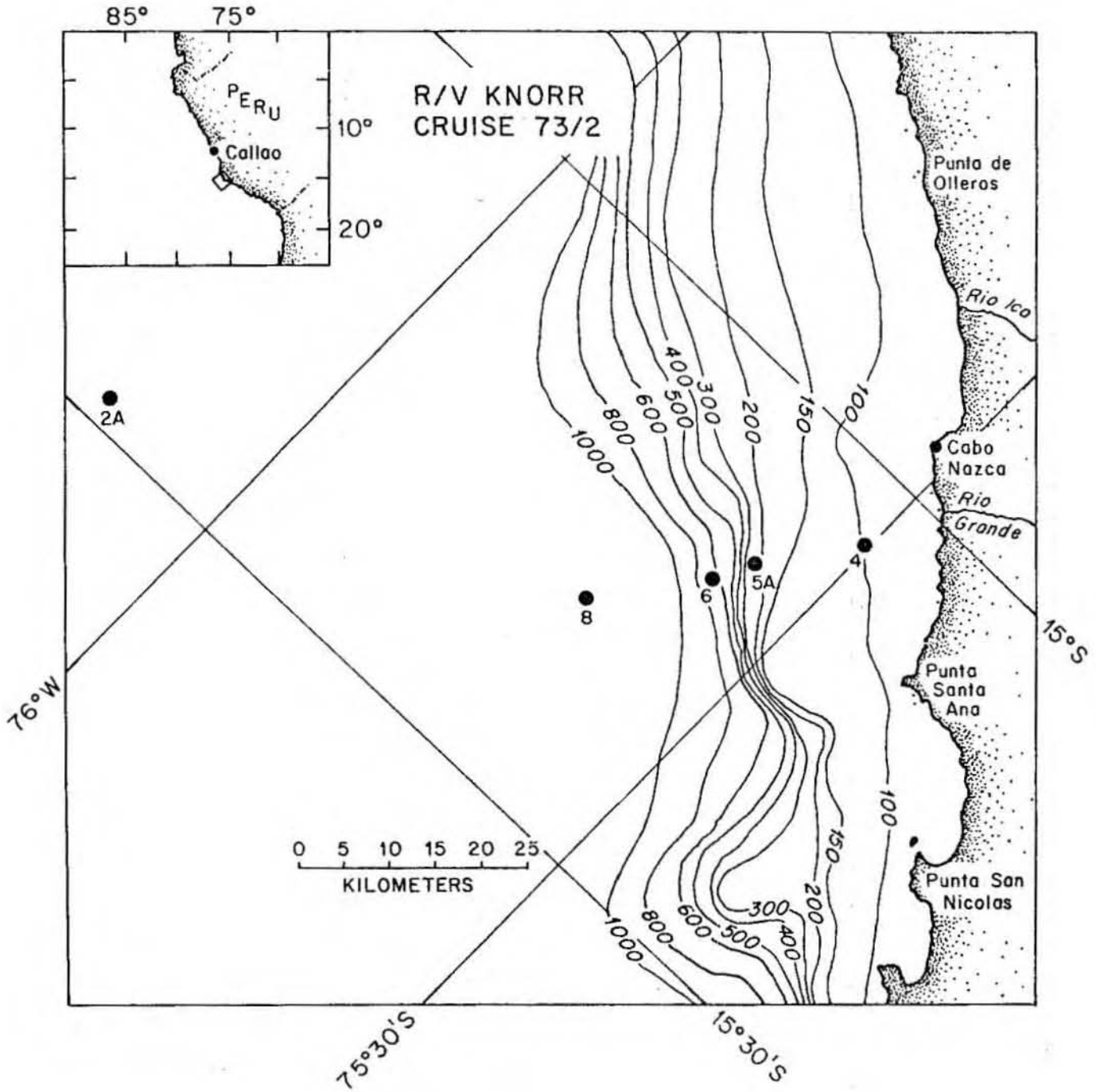
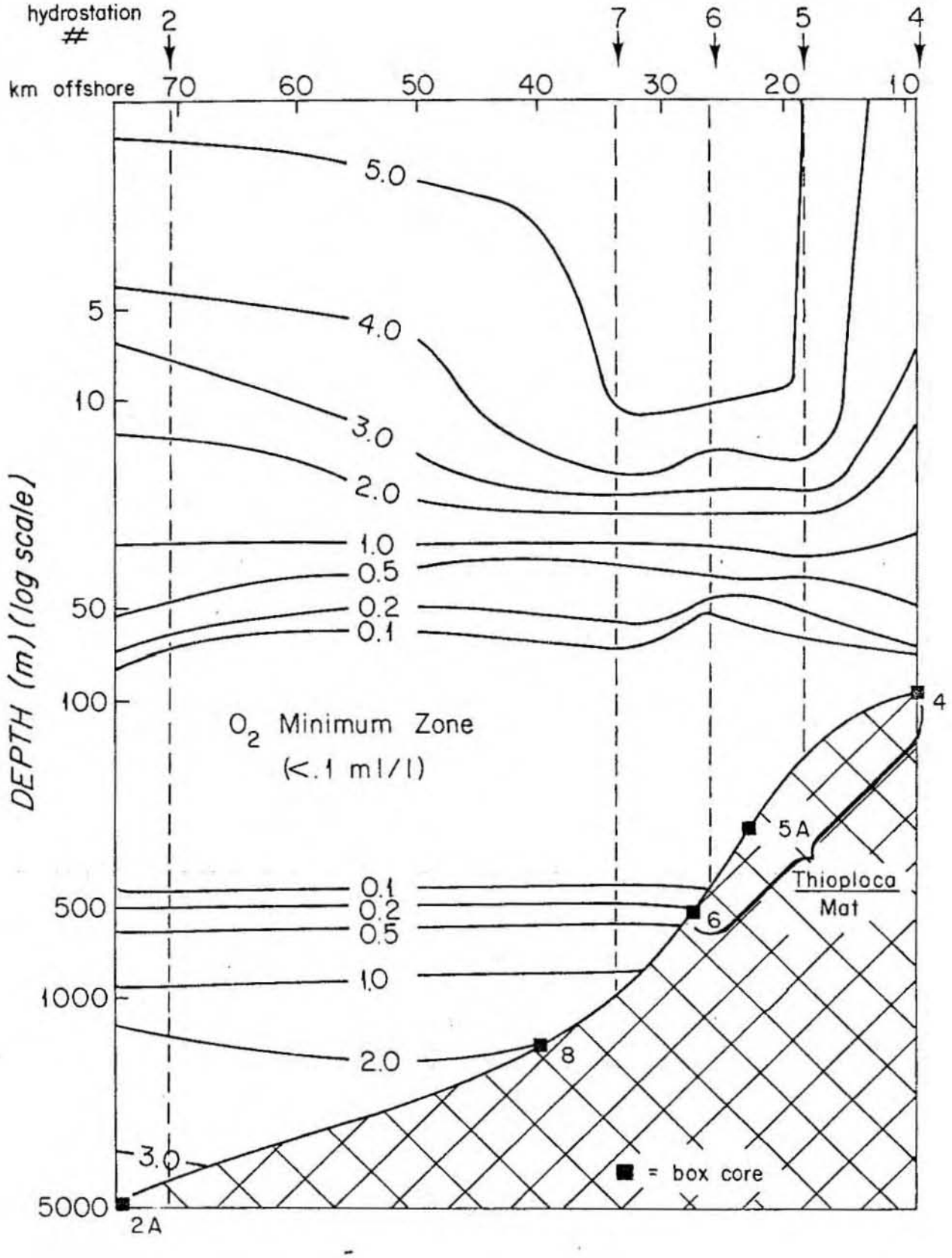

85°
75°
PERU
Callao
10°
20°
R/V KNORR
CRUISE 73/2
Punta de Olleros
Rio Ico
Cabo Nazca
Rio Grande
Punta Santa Ana
Punta San Nicolas
2A
6
5A
8
4
100
150
200
300
400
500
600
800
1000
0 5 10 15 20 25
KILOMETERS
76°W
75°30'S
15°S
15°30'S

Figure 4-2: Dissolved oxygen section running west from the Peru coast near 15°S. Oxygen data are from Gagosian et al. (1980).


hydrostation #
2
7
6
5
4
km offshore
70
60
50
40
30
20
10
5.0
4.0
3.0
2.0
1.0
0.5
0.2
0.1
5
10
50
100
500
1000
5000
DEPTH (m) (log scale)
O2 Minimum Zone
(<.1 ml/l)
0.1
0.2
0.5
1.0
2.0
3.0
4
5A
Thioploca
Mat
6
8
2A
= box core

Region near 15° S aboard R/V Knorr Cruise 73, Leg 2. Station locations are shown in Figure 4-1. Five sediment cores were obtained over a depth range of 5000 m along a 70 km transect perpendicular to the coast. Three cores were located in the oxygen minimum zone: Station 4 at 92 m, Station 5A at 268 m, and Station 6 at 506 m (See Figure 4-2; Oxygen data are from Gagosian et al., 1980). All of the sediments sampled were diatomaceous oozes.

Squeezing of the sediment samples to extract the interstitial water was completed as soon as possible after core recovery (within 24 hours). Cation exchange chromatography to isolate free amino acids was carried out on board, and the NH_4OH eluates were frozen and returned to Woods Hole for amino acid analysis, as described in Chapter 2. Ammonia, nitrate, nitrite, total carbon dioxide, and pH were measured on board. Sulfide in pore water samples was precipitated as ZnS, frozen under N_2, and returned to Woods Hole. Frozen sediment and interstitial water samples were also shipped to Woods Hole for TOC, TN, $\%H_2O$, THAA, and DOC analyses. Pb-210 measurements on selected sediment samples were kindly performed by Dr. R. Carpenter (University of Washington) and by R. F. Anderson (Woods Hole Oceanographic Institution).

Results

TOC, TN, THAA, TN/TOC, THAA/TN, DOC, $\%H_2O$, and Pb-210 data are given in Table 3-1. TOC, TN, and THAA are high at all five stations, reflecting the high rate of supply of organic matter from surface waters. Surface sediment TOC, TN, and THAA are greatest at Stations 5A

TABLE 4-1

Sediment Composition Data for Samples Collected during Knorr 73-2

Station No.	Depth in core(cm)	TOC[a]	TN[b]	THAA[c]	$\frac{TN}{TOC}$[d]	$\frac{THAA}{TN}$[e]	DOC (mgC/L)	$\%H_2O$	Pb-210 (dpm/gdw)
4	0-2	32	4.0	130	0.125	0.46	48	79.6	26.6
	2-4	23	2.5	74	0.109	0.41	19	73.1	17.2
	4-7	23	2.7	130	0.117	0.67	24	69.4	14.9
	7-10	27	3.0	90	0.111	0.42	27	69.9	17.1
	10-13	20	1.9	61	0.095	0.45	16	67.0	8.5
	13-16	14	1.6	30	0.114	0.26	16	65.9	3.9
	19-22	27	3.0	81	0.111	0.38	15	68.2	N.D.
	25-28	16	1.8	40	0.113	0.31	20	61.5	N.D.
	31-34	22	2.6	59	0.118	0.32	43	68.8	8.6
	40-46	8.0	1.0	19	0.125	0.27	34	57.2	N.D.
	52-58	15	1.6	35	0.107	0.31	12	70.7	N.D.
	64-70	23	2.6	54	0.113	0.29	23	66.9	2.9
5A	0-3	68	8.2	310	0.121	0.53	11	91.2	50.1
	3-6	62	7.3	290	0.118	0.56	21	90.0	46.6
	6-9	76	8.7	390	0.114	0.63	11	89.7	44.7
	9-12	72	8.2	290	0.114	0.50	18	89.1	31.8
	12-15	68	7.9	310	0.116	0.55	30	89.0	29.4
	15-18	74	8.5	330	0.115	0.54	17	88.7	29.3
	18-21	81	9.4	300	0.116	0.45	(82)[f]	87.3	25.0
	24-27	87	10.3	270	0.118	0.37	32	85.3	20.9
	30-33	57	6.3	180	0.111	0.40	31	88.7	5.9
	33-36	42	5.1	95	0.121	0.26	35	88.6	N.D.
	42-45	67	8.2	150	0.122	0.26	38	84.8	4.8
	48-51	62	7.5	150	0.121	0.26	41	75.1	2.2
6	0-2	75	9.5	470	0.127	0.69	37	92.1	65.0
	2-4	89	10.8	430	0.121	0.56	54	89.3	92.2
	4-6	85	10.1	280	0.119	0.39	20	86.3	44.4
	6-9	95	11.1	310	0.115	0.39	12	84.9	59.2
	9-12	91	10.5	280	0.116	0.37	11	84.3	N.D.
	12-15	85	9.9	260	0.116	0.37	15	84.3	N.D.
	15-18	68	7.9	110	0.112	0.19	8	84.9	N.D.
	18-21	49	5.5	110	0.110	0.28	8	87.0	N.D.
	21-24	39	4.3	94	0.110	0.30	11	88.8	N.D.
	24-30	35	3.8	80	0.109	0.29	7	89.0	N.D.
	30-36	42	4.8	110	0.114	0.32	7	87.0	N.D.

TABLE 4-1 (cont.)

8	0-2	56	6.5	97	0.116	0.21	11	76.2	N.D.
	2-4	50	5.8	100	0.116	0.24	16	71.4	N.D.
	4-6	51	5.9	93	0.116	0.22	20	70.9	N.D.
	6-8	44	5.0	70	0.114	0.20	21	69.0	N.D.
	8-10	48	5.4	70	0.113	0.19	22	66.7	N.D.
	10-12	42	4.8	87	0.114	0.25	33	65.2	N.D.
2A	0-2	39	4.1	62	0.105	0.21	24	78.3	N.D.
	2-4	37	4.8	69	0.130	0.20	25	75.5	N.D.
	4-6	38	5.0	66	0.132	0.18	20	74.4	N.D.
	6-8	39	3.5	66	0.090	0.26	48	74.0	N.D.
	8-10	44	5.5	86	0.125	0.22	42	74.3	N.D.
	10-14	37	3.4	59	0.092	0.24	42	73.0	N.D.

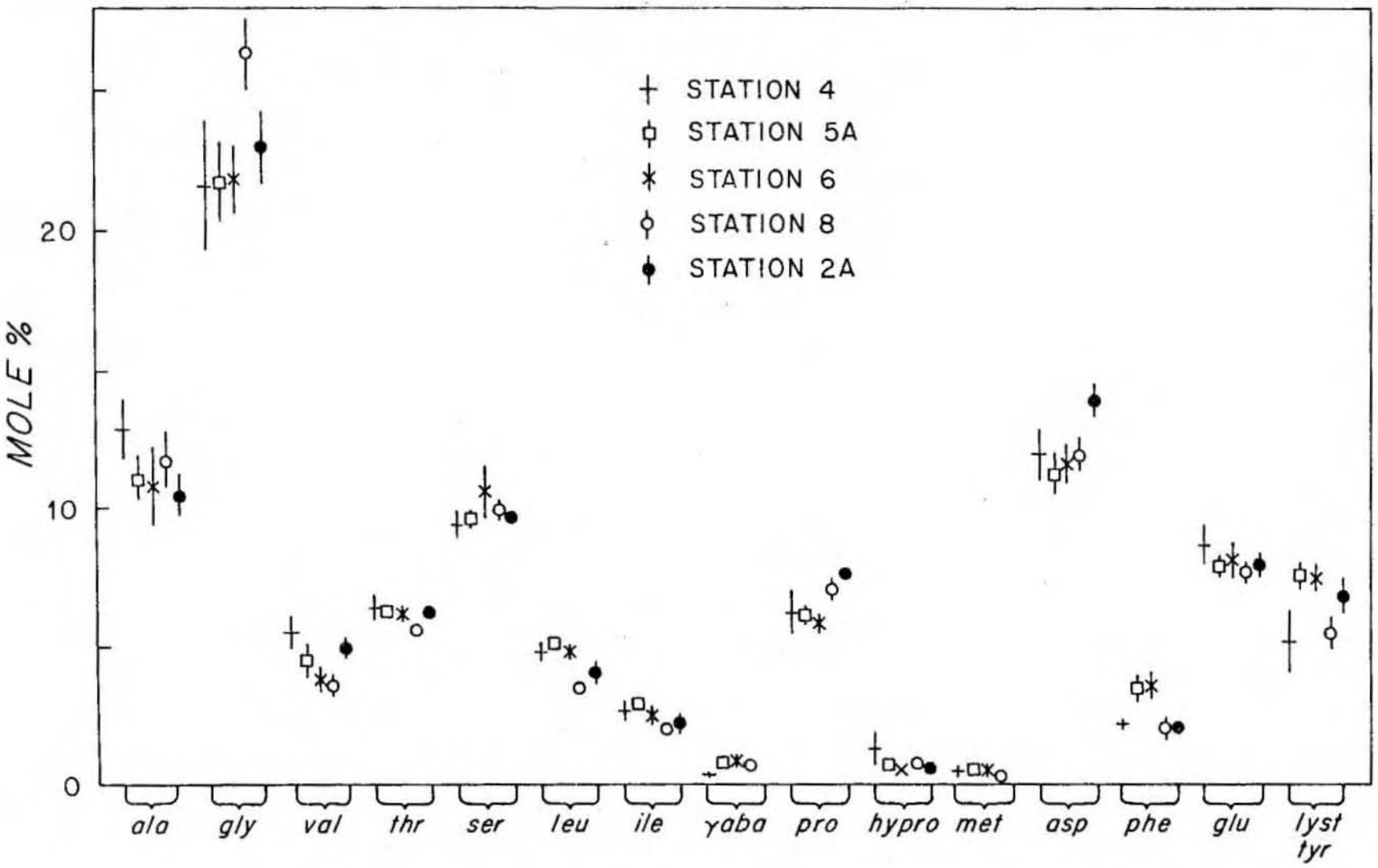
[a]Units of mgC/gdw.
[b]Units of mgN/gdw.
[c]Units of μmoles/gdw.
[d]Weight:weight ratio.
[e]Weight N:weight N ratio.
[f]Value seems unreasonably high; sample may have been contaminated.

and 6 in the oxygen minimum zone. Station 4 was located less than 10 km from the coast, and sediments from this station contain relatively more terrigenous detritus and relatively fewer diatom frustules and less organic matter than sediments further offshore.

Station 4 sediments show a marked variability in TOC, TN, and THAA with depth, with minima in the 13-16, 25-28, and 40-46 cm core sections. These minima correspond to visually apparent lithological changes in the core which were noted during sectioning. The lower TOC, TN, and THAA contents at Station 5A in the 30-33 cm section and below, and at Station 6 in the 18-21 cm section and below, are also found in sediments with different color/texture than the nearer-surface sediments. The short cores at Stations 8 and 2A sampled sediments of relatively uniform composition.

Because of the apparently non-steady-state sedimentation in the sampling area, it is difficult to discern any trends in TOC, TN, and THAA which might be due to remineralization of organic matter. The TN/TOC ratio (col. 4) shows no consistent trends with depth in cores between the five stations. The THAA/TN ratio does, however, decrease fairly steadily with depth in core and is significantly lower in surface sediments from Stations 2A and 8 than in surface sediments from the oxygen minimum zone stations. This trend suggests that in these sediments the proteinaceous material is being remineralized more rapidly than the bulk of organic carbon and nitrogen. However, there were no trends in the composition of the THAA between stations or with depth in sediment. The composition data is summarized in Figure 4-3. The length of the bars represents one

Figure 4-3: Composition of total hydrolyzable amino acids in Peru Upwelling Region sediments. The length of the bar represents one standard deviation around the mean mole % for all core sections at each station.


STATION 4
STATION 5A
STATION 6
STATION 8
STATION 2A
MOLE %
20
10
0
ala
gly
val
thr
ser
leu
ile
γaba
pro
hypro
met
asp
phe
glu
lyst
tyr

standard deviation on either side of the mean of all core sections analyzed at a particular station. The variance is only slightly larger than that which would be expected from analytical error.

DOC was measured on frozen samples which were stored for about 2 years prior to analysis. During storage, precipitates formed in samples from Stations 4 and 5A. These precipitates were removed by filtration before DOC analysis, and may have contained some organic carbon. The DOC results for interstitial water samples are given in Table 4-1. Bottom water DOC was also measured (Table 4-2). The interstitial water DOC concentrations at Station 4 are quite erratic, but are generally substantially higher than bottom water concentrations. Station 5A DOC concentrations increase with depth, as do those at Stations 8 and 2A. Station 6 shows a marked opposite trend with DOC concentrations decreasing five-fold over the depth range of the core. Pore water DOC is consistently greater than bottom water DOC, and thus sediments may be a source of DOC to the water column.

Pb-210 activities were measured on selected samples from Stations 4, 5A, and 6. Interpretation of total activities in terms of sedimentation rates is complicated by the fact that the supported Pb-210 levels are high due to the high concentration of radium in siliceous sediments (Koide et al, 1976). Variable amounts of silica and/or variable Ra/Si ratios as well as sedimentation and decay rates of Pb-210 may thus contribute to variations in total Pb-210 activity, since the Ra content of these samples was not measured. Also, the abrupt decrease in Pb-210 activity between the 24-27 cm and the 30-33 cm sections in core 5A sug-

gests a hiatus in sedimentation. With these problems in mind, sedimentation rates for Stations 4 and 5A were calculated as follows: The Pb-210 activities were corrected for supported Pb-210 using the activity of the 64-70 cm section for Station 4 and the average of the 30-33, 42-45, and 48-51 cm core sections for Station 5A. Dry weight activities were corrected for changes in porosity using water content data, and the sedimentation rates were calculated using the equation $A_z = A_o \exp(-\lambda z/s)$, where A_z is the excess Pb-210 activity at depth z, A_o is the excess Pb-210 activity in surface sediment, λ is the decay constant for Pb-210, and s is the average sedimentation rate. The sedimentation rates calculated for Stations 4 and 5A were 1.1 and 1.2 cm/yr, respectively. Surface sediments at these stations are anoxic and contain few macrofauna (Gallardo, 1976); thus these sedimentation rates should not be much affected by bioturbation (Koide et al., 1976). These rates are, however, substantially higher than those found by DeMaster (1979) using Pb-210 for oxygen minimum zone sediments further north on the Peru coast (0.05 cm/yr for 370 m depth at $13^{\circ}37'S$; 0.16 cm/yr for 186 m depth, $11^{\circ}15'S$). DeMaster's stations were located outside the centers of upwelling activity identified by Zuta et al. (1975) which may account for the disagreement. Station 6 Pb-210 results were too erratic to be used to calculate a sedimentation rate.

Ammonia, nitrate, nitrite, total carbon dioxide, and sulfide data for the five Knorr 73-2 stations is summarized in Figures 4-4a to 4-4e. Sulfide was present in surface sediments from the three oxygen minimum zone stations, but was absent at depth in sediments from Stations 4 and 6 on

Figure 4-4a: Ammonia, total carbon dioxide, nitrate, nitrite, and sulfide concentrations in Station 4 interstitial water.

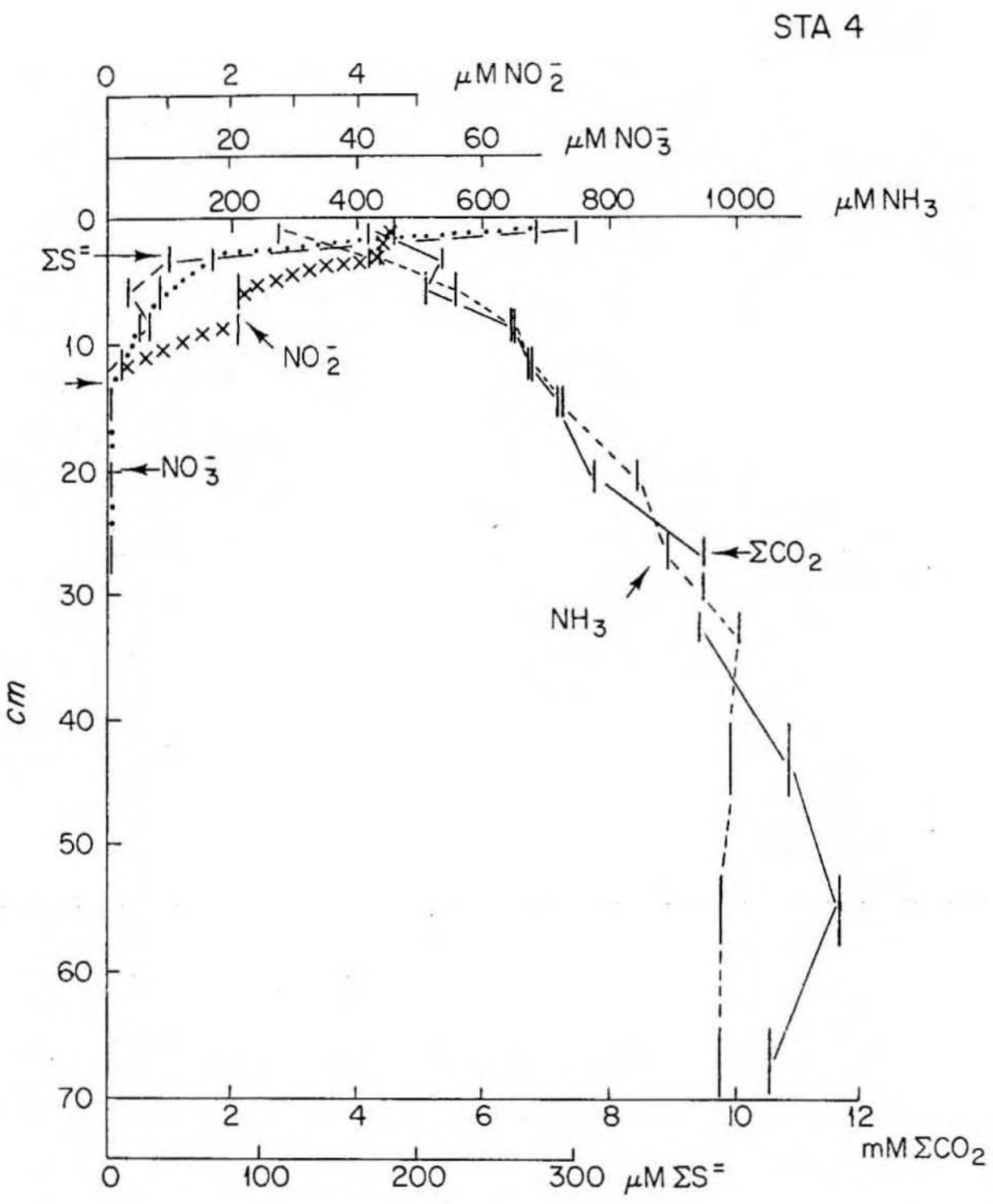

STA 4
0
2
4
μM NO2-
20
40
60
μM NO3-
200
400
600
800
1000
μM NH3
0
ΣS=
10
NO2-
20
NO3-
30
ΣCO2
NH3
40
cm
50
60
70
2
4
6
8
10
12
mM ΣCO2
0
100
200
300
μM ΣS=

Figure 4-4b: Ammonia, total carbon dioxide, nitrate, nitrite, and sulfide concentrations in Station 5A interstitial water.

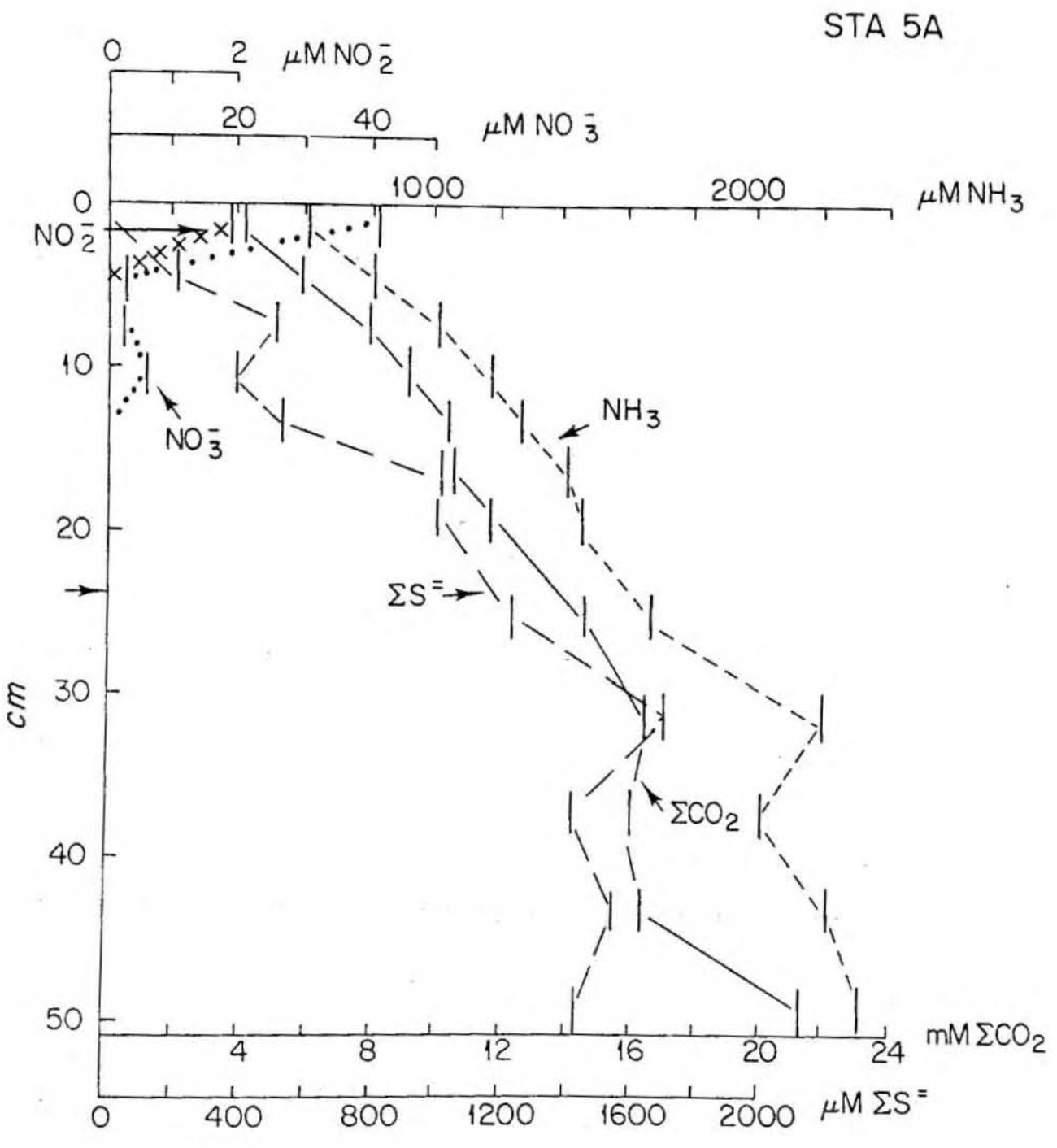
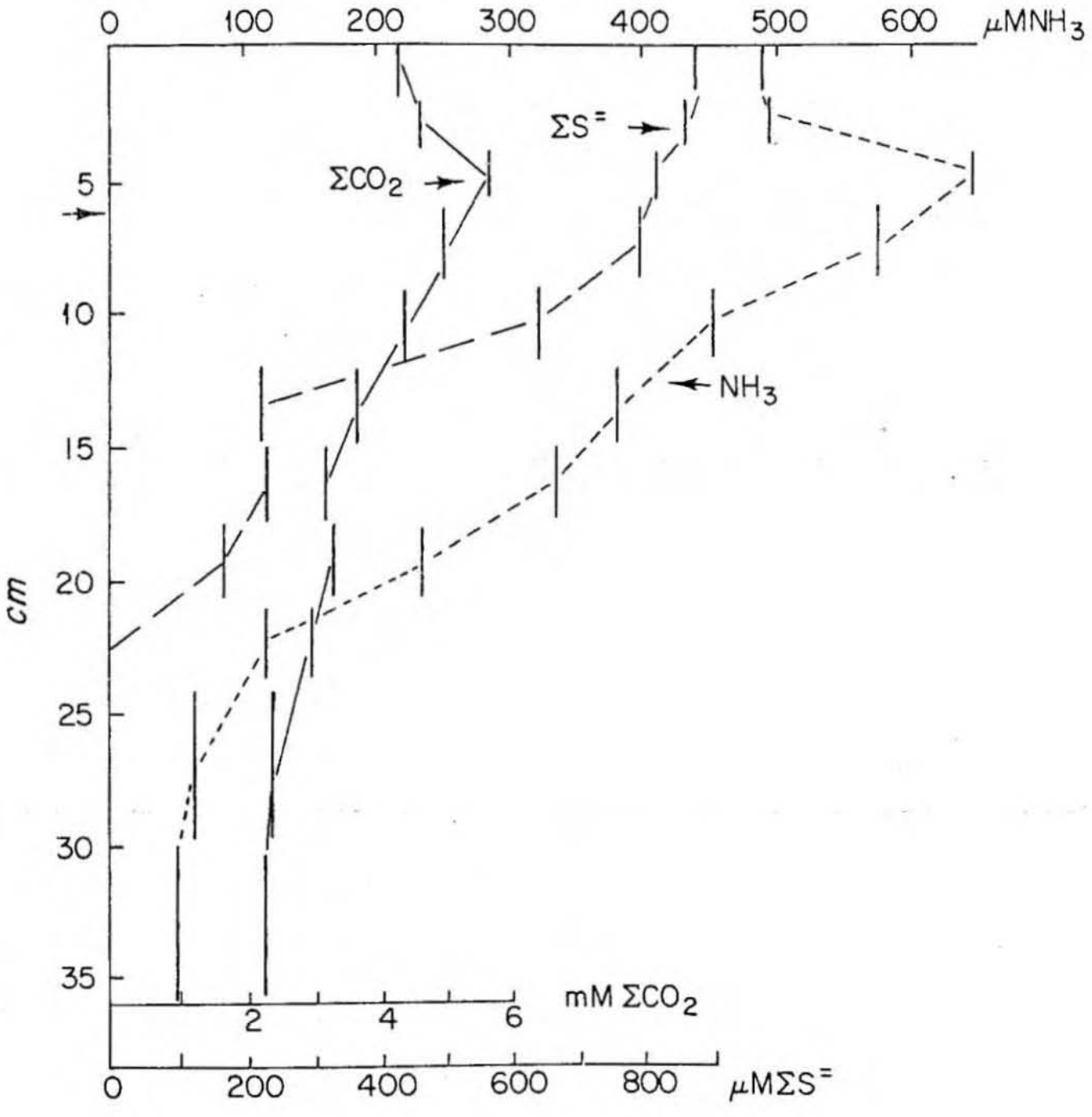

STA 5A
0
2
μM NO2-
20
40
μM NO3-
1000
2000
μM NH3
NO2-
NO3-
NH3
ΣS=
ΣCO2
cm
10
20
30
40
50
4
8
12
16
20
24
mM ΣCO2
0
400
800
1200
1600
2000
μM ΣS=

Figure 4-4c: Ammonia, total carbon dioxide, and sulfide concentrations in Station 6 interstitial water.

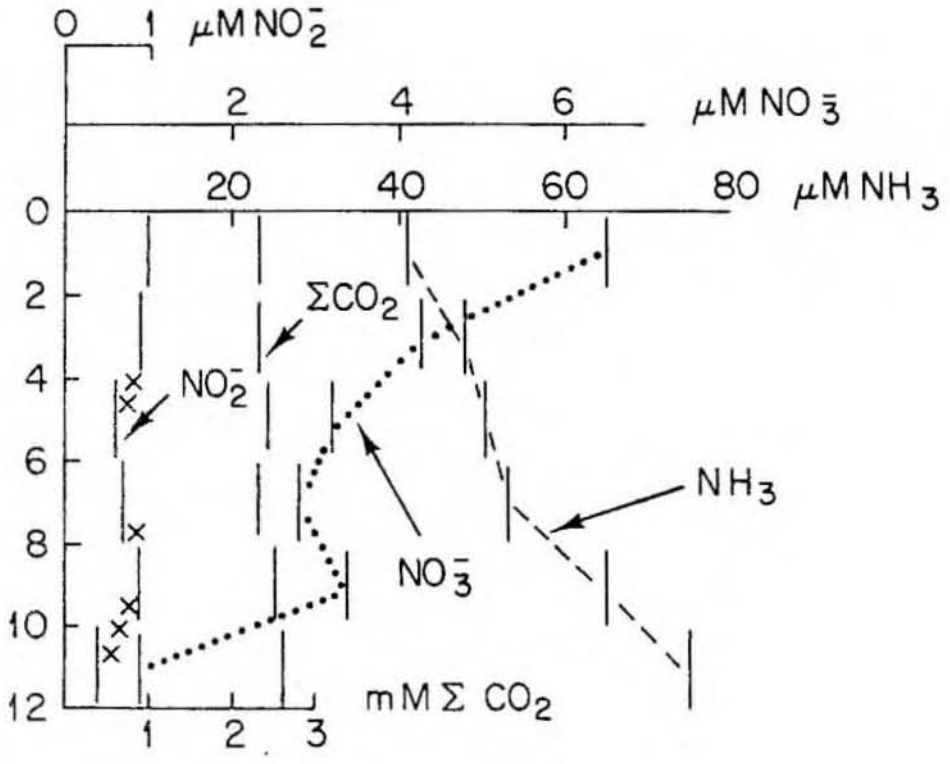
STA 6


0
100
200
300
400
500
600
μMNH3
ΣS=
ΣCO2
5
10
15
20
25
30
35
cm
NH3
mM ΣCO2
2
4
6
0
200
400
600
800
μMΣS=

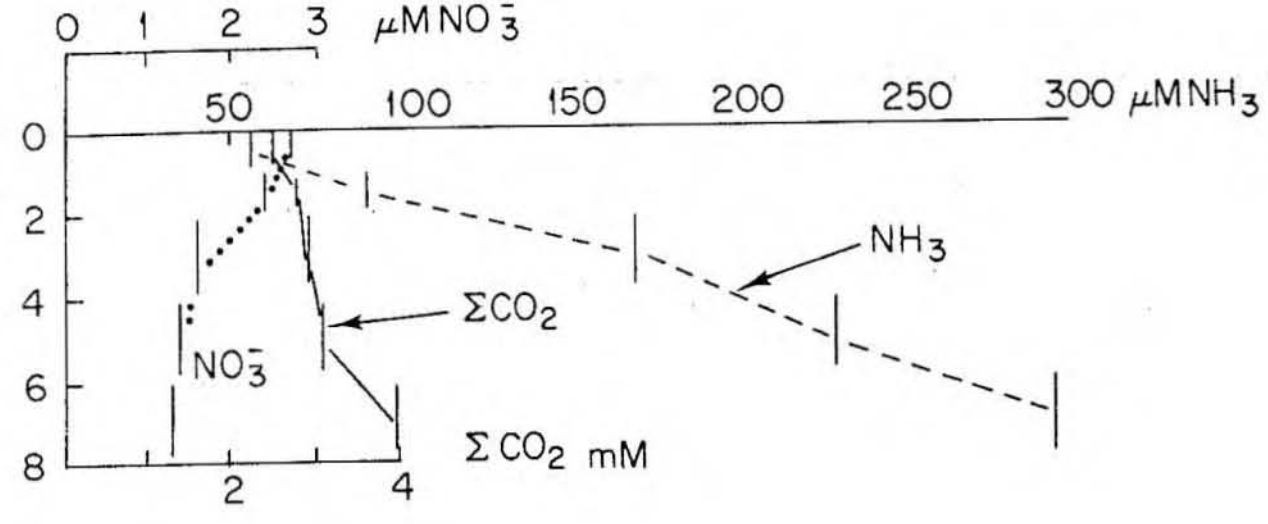
(Top) Figure 4-4d: Ammonia, total carbon dioxide, nitrate, and nitrite concentrations in interstitial water from Station 8. No sulfide was detected.

(Bottom) Figure 4-4e: Ammonia, total carbon dioxide, and nitrate concentrations in interstitial water from Station 2A. No nitrite or sulfide was detected.

STA 8


0 1 μM NO2-
2 4 6 μM NO3-
20 40 60 80 μM NH3
0
2
4
6
8
10
12
ΣCO2
NO2-
NH3
NO3-
mM Σ CO2
1 2 3

STA 2A


0 1 2 3 μM NO3-
50 100 150 200 250 300 μM NH3
0
2
4
6
8
NH3
ΣCO2
NO3-
Σ CO2 mM
2 4

the edges of the zone. However, appreciable concentrations of nitrate and nitrite were also present in surface sediment pore waters from Stations 4 and 5A. (Nitrate and nitrite could not be measured in Station 6 interstitial water because of the very high sulfide levels). Ammonia and total carbon dioxide concentrations increase with depth to very high levels at Stations 4 and 5A. Gradients are high in surface sediments and tend to decrease with depth, especially below 30 cm. At Station 6 total carbon dioxide and ammonia are substantially greater than bottom water concentrations in surface sediment interstitial waters and increase to 6 cm depth, but their concentrations decrease with depth below 6 cm.

The position of the arrow in the left margin of Figures 4-4a to c marks the depth of transition from a brownish-black, spongy sediment containing abundant white filaments to a more compact greenish sediment. This transition is interpreted to be the base of the Thioploca mat community, from the description given by Gallardo (1978).

No sulfide was present in sediments from Stations 8 and 2A. Small amounts of nitrate and nitrite were present at Station 8, and a few μM nitrate were found in surface sediments from Station 2A. Ammonia increased with depth at both stations, but the increase was four times greater at Station 2A. Total carbon dioxide was almost constant with depth at Station 8, but increased by almost a factor of two at Station 2A.

Very steep gradients were present in ammonia, sulfide, and total carbon dioxide at the sediment-water interface at the oxygen-minimum zone stations. (Compare Table 4-2 to Figures 4-4a to c). This was particularly

TABLE 4-2

Bottom Water Composition

Station No.	Water Depth (m)	Total CO_2 (mM)	NH_3 (μM)	NO_3^- (μM)	NO_2^- (μM)	H_2S (μM)	DOC (mgC/L)
4	82	2.3	0.5	35	0.0	0.0	5.5
5A	250	2.4	0.4	39	0.2	0.0	2.7
6	485	2.2	0.8	45	0.0	0.0	2.0
7[a]	1000	2.4	0.9	47	0.2	0.0	0.9
2A	4800	2.3	0.0	20	0.0	0.0	6.5[b]

[a]Hydrostation 7 was located slightly east of coring station 8.
[b]Measured DOC concentration seems unreasonably high for Station 2A.

TABLE 4-3

Calculated Remineralization Rates

Station No.	Ammonia (k/w) (cm^{-1})	r[a]	Total Carbon Dioxide (k/w) (cm^{-1})	r
4	0.095 (0.079-0.11)[b]	0.88	0.062 (0.051-0.085)	0.92
5A	0.059 (0.041-0.069)	0.92	0.039 (0.030-0.048)	0.95
8	0.079 (0.035-0.093)	0.87	0.084 (0.050-0.13)	0.71[c]
2A	0.20 (0.18-0.22)	0.98	0.055 (0.036-0.12)	0.86

[a]Linear correlation coefficient for data fit to model.
[b]Given as: best value (range).
[c]Total carbon dioxide gradient was very small at Station 8.

Figure 4-5a: Dissolved free amino acids in Station 4 sediments.

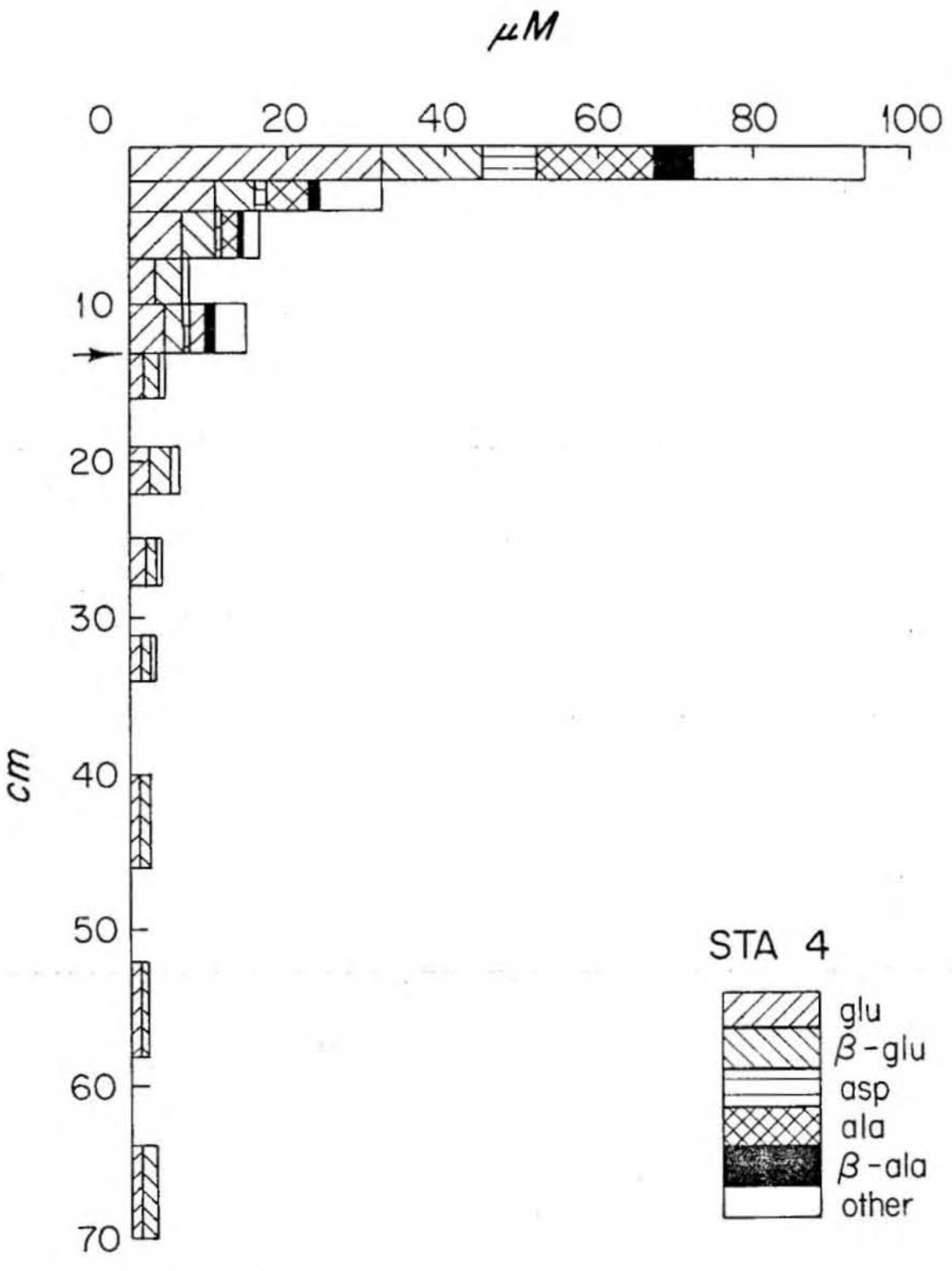

μM
0
20
40
60
80
100
10
20
30
40
50
60
70
cm
STA 4
glu
β-glu
asp
ala
β-ala
other

Figure 4-5b: Dissolved free amino acids in Station 5A sediments.

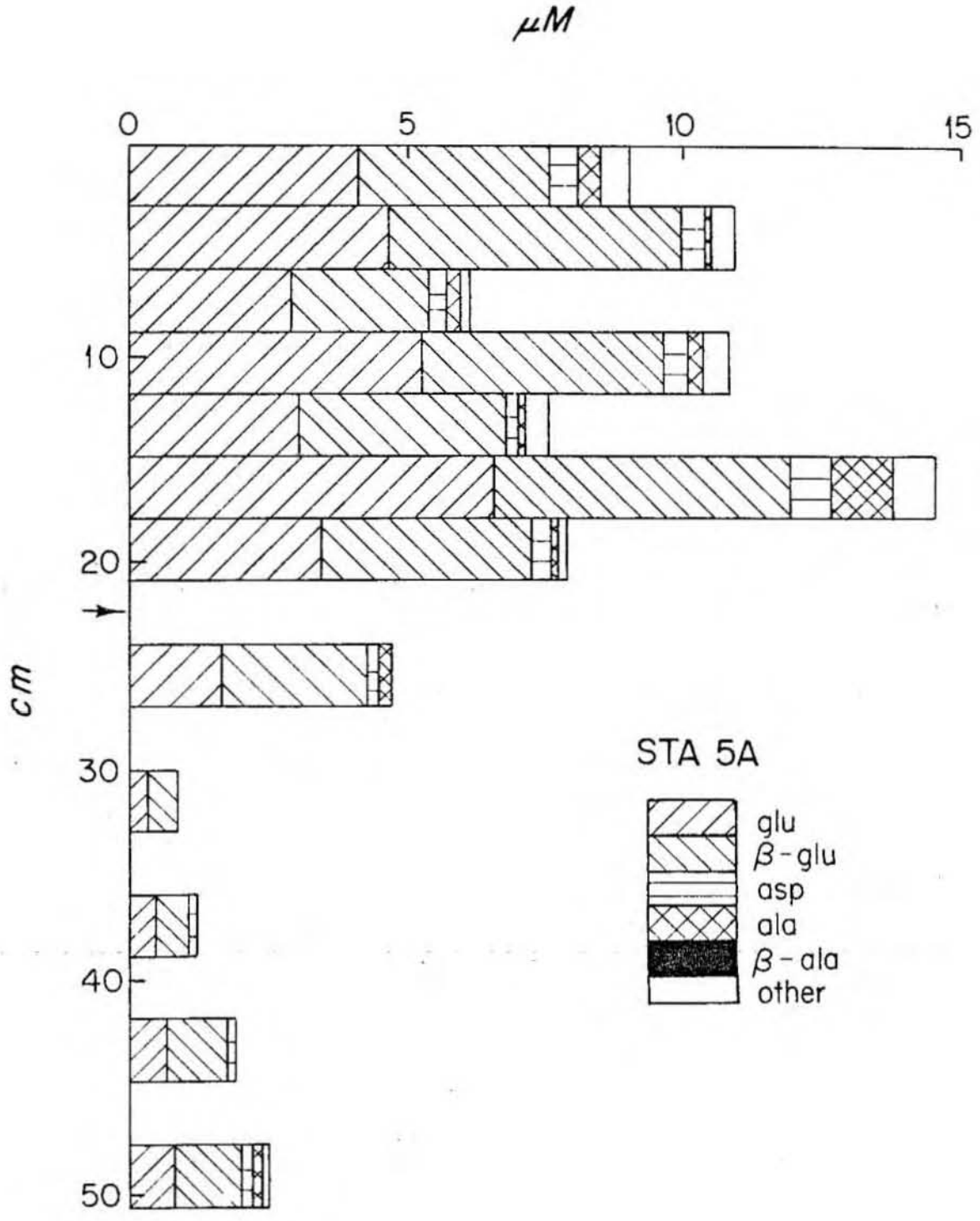
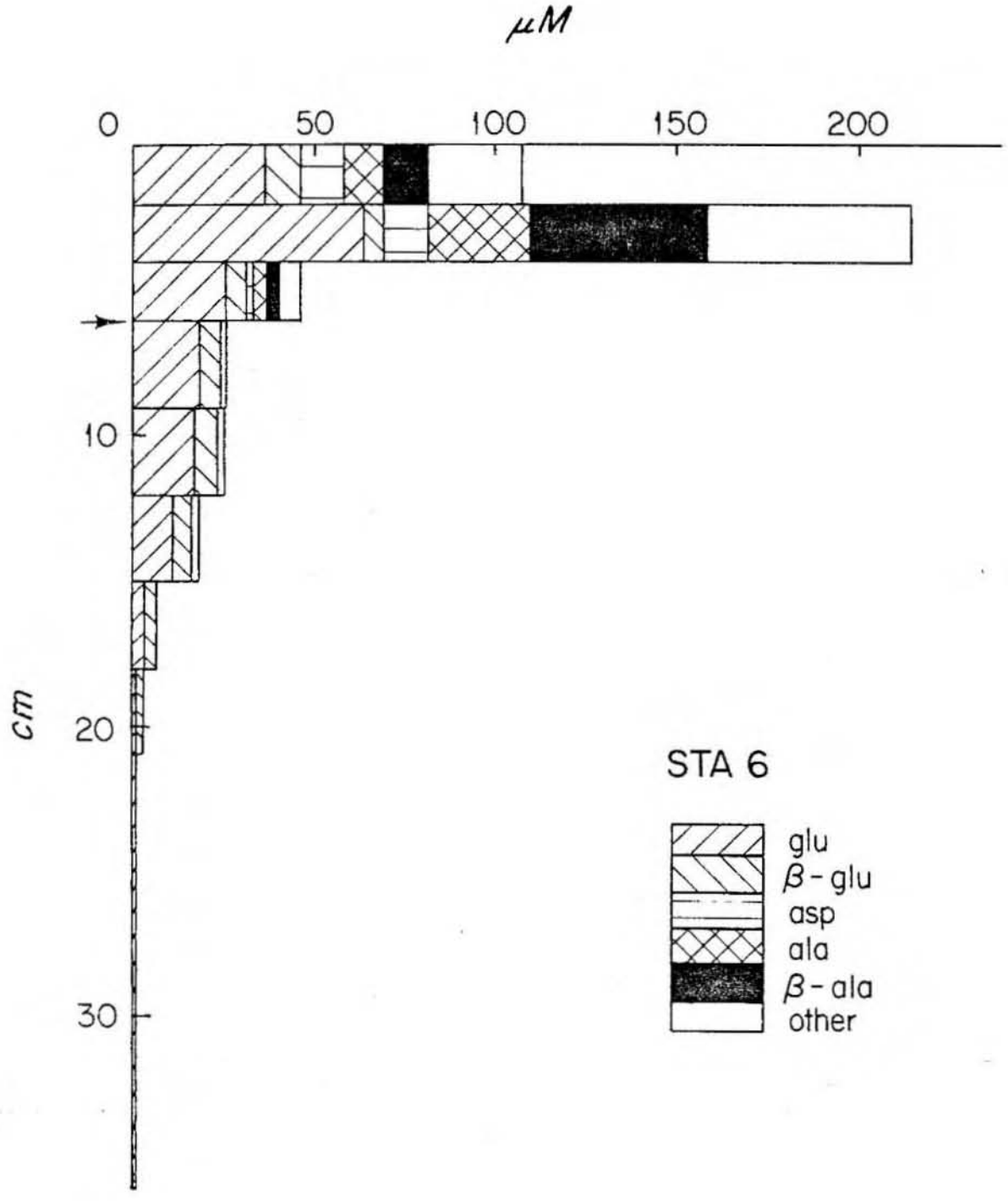
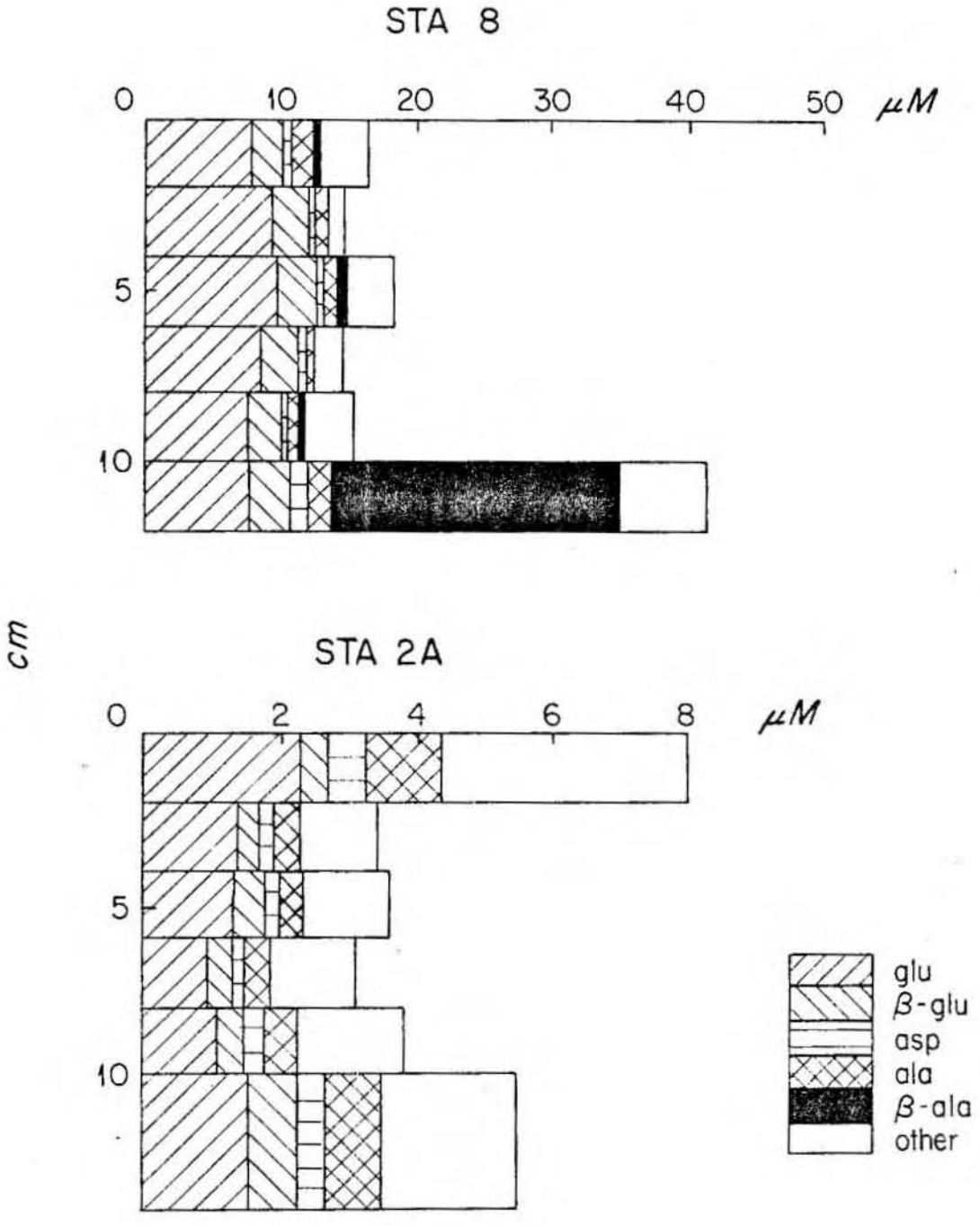

μM
0
5
10
15
10
20
30
40
50
cm
STA 5A
glu
β-glu
asp
ala
β-ala
other

Figure 4-5c: Dissolved free amino acids in Station 6 sediments.


μM
0
50
100
150
200
10
20
30
cm
STA 6
glu
β - glu
asp
ala
β - ala
other

(Top) Figure 4-5d: Dissolved free amino acids in Station 8 sediments.

(Bottom) Figure 4-5e: Dissolved free amino acids in Station 2A sediments.


STA 8
0
10
20
30
40
50
μM
5
10
cm
STA 2A
0
2
4
6
8
μM
5
10
glu
β-glu
asp
ala
β-ala
other

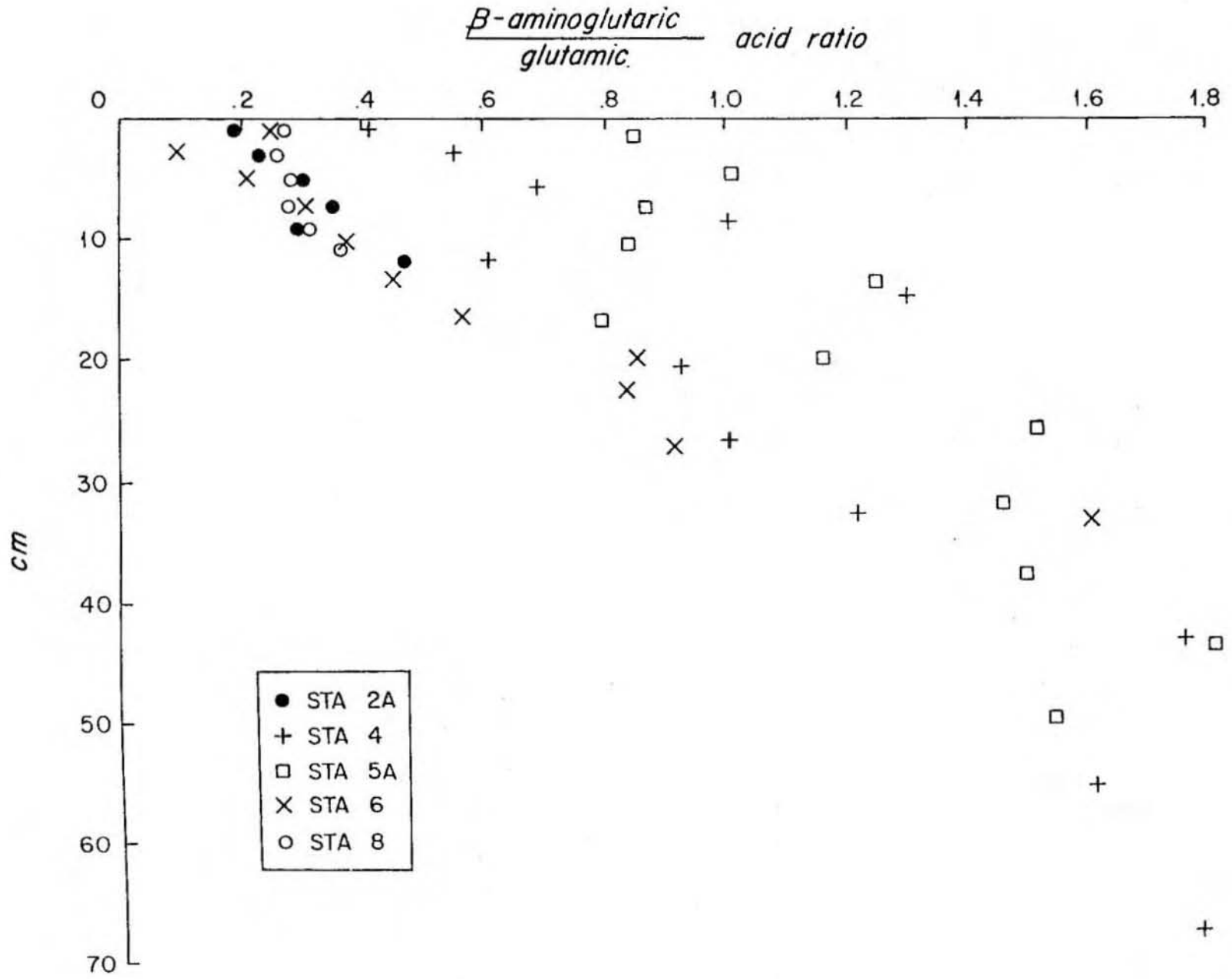
true for ammonia, which was several hundred times more concentrated in surface sediments than bottom water, and for sulfide at Stations 4 and 6.

Figures 4-5a through 4-5e illustrate data for dissolved free amino acids in interstitial water. The bars, representing total dissolved free amino acid (DFAA) concentration, are divided according to the relative abundance of glutamic acid, β-aminoglutaric acid, alanine, aspartic acid, and β-alanine. "Other" includes glycine, valine, threonine, serine, leucine, isoleucine, proline, lysine, and tyrosine, and may also include alanine, aspartic acid, and β-alanine if their concentrations are too low to be shown separately. The marginal arrows again indicate the inferred base of the Thioploca mat community.

Both Station 4 and Station 6, on the landward and seaward edges of the oxygen minimum zone, respectively, had very high near-surface DFAA concentrations. At Station 4, concentrations decrease roughly exponentially with depth from the nearly 100 μM surface concentration maximum. At Station 6 the maximum (220 μM) occurs in the 2-4 cm depth interval, with concentrations decreasing rapidly to only 1 μM below 21 cm. Station 5A, located in the middle of the oxygen minimum zone, had no surface concentration maximum. DFAA were relatively constant at 10 ± 4 M in the upper 21 cm, and decreased to levels between 1 and 5 μM in deeper sections.

Station 8 DFAA concentrations were essentially constant at 15 to 20 μM between 0 and 10 cm, but increased to 40 μM in the 10-12 cm section, mostly due to a large amount of β-alanine. Station 2A DFAA concentrations show a very slight surface maximum, but have only a small concen-

Figure 4-6: Dissolved β-aminoglutaric acid/glutamic acid ratio vs. depth in core in Peru Upwelling Region sediments.


β-aminoglutaric acid ratio
glutamic
0
.2
.4
.6
.8
1.0
1.2
1.4
1.6
1.8
10
20
30
40
50
60
70
cm
STA 2A
STA 4
STA 5A
STA 6
STA 8

tration range, from 3 to 8 μM.

Glutamic acid and β-aminoglutaric acid are important constituents of the DFAA at all stations. β-Aminoglutaric acid is a non-protein amino acid isomer of glutamic acid. Its identification in pore water samples will be discussed in Chapter 5. The presence of β-aminoglutaric acid has been confirmed by GC/MS of selected samples from the Peru Upwelling Region. The relative abundance of β-aminoglutaric acid and glutamic acid, with respect to other amino acids, is greatest at Station 5A and at Stations 4 and 6 below the bacterial mat. The β-aminoglutaric acid/glutamic acid ratio also shows an interesting pattern (Figure 4). The ratio increases with depth at all five stations. At a given depth, Stations 6, 8, and 2A have lower ratios than Stations 4 and 5A. Within cores, high ratios are associated with low DFAA concentrations, low abundance of amino acids other than glutamic and β-aminoglutaric acids, and to some extent with lower TOC and TN, but these correlations do not hold between cores.

DFAA other than glutamic acid and β-aminoglutaric acid are relatively abundant in sediments within the Thioploca mat zone from Stations 4 and 6 and at all depths from Stations 8 and 2A. β-Alanine, a non-protein amino acid isomer of alanine, is present in relatively high concentrations in surface sediments from Stations 4 and 6 and in the 10-12 cm section from Station 8. β-Alanine co-elutes with valine on the GCGC column used for these analyses, but its presence was confirmed by GC/MS where it was a major constituent. Alanine, aspartic acid, and sometimes glycine were other amino acids present at relatively high concentrations.

Selected samples were subjected to GCGC on a Chirasil-Val$^{(R)}$ column, which resolves optical isomers. No significant quantities (more than 5 % of the total for major peaks) of D-amino acids were found.

Discussion

Accumulation and Remineralization of Organic Matter in Sediments:

The TOC, TN, and interstitial water ammonia, total carbon dioxide, and sulfide data for Stations 4 and 6 strongly suggest that variations in sediment composition, source, and/or sedimentation rate have occurred over the approximately 100-year time span sampled by the cores. At Station 6, there is a pronounced (roughly twofold) decrease in the organic content of the sediment with depth. This decrease could be attributed to remineralization, except that the concentrations change rather abruptly at about 20 cm instead of continuously with depth. Also, the total carbon dioxide, ammonia, and sulfide distributions in interstitial water are wholly inconsistent with this explanation. Below 6 cm these remineralization products actually decrease with depth; sulfide disappears entirely below 21-24 cm and total carbon dioxide and ammonia concentrations level off at values similar to those at Station 8. TOC, TN, and THAA in the bottom of the Station 6 core are also similar to those at Station 8. This evidence suggests that in the past sedimentation rates, rates of supply of organic carbon to the sediment, and remineralization rates at Stations 6 and 8 were similar, while at present Station 6 more closely resembles Station 5A.

This, in turn, suggests that a change in the extent of the oxygen

minimum zone and/or a change in the locus of upwelling has occurred. At present, the northward-flowing Peru Coastal Current turns away from the coast at about 15°S. North of 15°S a shallow northward wind drift is underlain by the southward-flowing, relatively high-salinity, low-oxygen water of the Peru Undercurrent (Wyrtki, 1967). At R/V Knorr 73-2 Stations 6 and 7, the salinity maximum was centered at 100 to 200 m, with decreasing salinities to the base of the oxygen minimum zone at 400 to 500 m (Gagosian et al., 1980). Comparison of February-March 1978 temperature and salinity data to temperature and salinity sections running westward from Pta. San Juan during June, 1931 (Gunther, 1936) shows no discernable difference in the circulation pattern. However, the location of Station 6 at both an east-west and a north-south water mass boundary supports the idea that oceanographic conditions there are not stable over time.

At Station 4 there is no obvious trend in organic content with time. After pronounced minima at 13-16 cm, 25-28 cm, and 40-46 cm, TOC and TN return to levels found near the sediment surface. The interstitial water ammonia and total carbon dioxide profiles show fairly smooth increases with depth due to remineralization of organic matter. Apparently variations in sedimentation and/or remineralization rates over time were not large enough to generate non-steady-state profiles (Lasaga and Holland, 1976). The sections of the Station 4 core with lower organic content also showed a lower content of diatom frustules and a higher content of amorphous material (probably clay) when examined under a dissecting microscope relative to other sections. This could result

either from a constant detrital input with superimposed productivity fluctuations, or from variations in the rate of supply of terrigenous detritus from the nearby coast.

Remineralization rates: After Murray et al. (1978) and Goldhaber et al. (1977), the following equation has been fit to the interstitial water ammonia and total carbon dioxide data:

$$C_z = (C_o - C_\infty)\exp(-kz/w) + C_\infty, \qquad (4\text{-}1)$$

where z is the depth in sediment; C_z, C_o, and C_∞ are the concentrations of ammonia or total carbon dioxide at z, the sediment surface, and as dC/dz approaches 0; w is the sedimentation rate; and k is the remineralization rate of organic matter.

Equation 4-1 is the solution of the following differential equation:

$$D_s \frac{d^2C}{dz^2} - w(1 + S\rho K)\frac{dC}{dz} + BkM_m \qquad (4\text{-}2)$$

Equation 4-2 is a simplified version of the general diagenetic equation given by Berner (1976b), and requires these assumptions:

(1) No change in concentration (C) with time.

(2) The sedimentation rate w is constant.

(3) Diffusion of solutes is Fickian, and the diffusion coefficient in sediment (D_s) is equal to $\phi^2 D_o$, where D_o is the bulk solution coefficient and ϕ is the porosity.

(4) ϕ is constant with depth.

(5) Any adsorption of solutes by the solid phase is described by

$\Gamma = KC$, where Γ is the adsorbed concentration per unit area of solid and K is the adsorption coefficient, which is constant with depth. In equation 4-2, S is the surface area per unit weight of sediment and ρ is the dry sediment density.

(6) The rate of remineralization of organic matter is proportional to the concentration of metabolizable organic matter in the solid phase, i.e. $\partial M_m/\partial t = -kM_m$, and the remineralization constant k does not vary with z. B in equation 4-2 is a constant to convert the units of M_m to pore water concentration units.

These assumptions are not strictly valid for the sediments discussed here. However, by using average values for varying parameters, the model may give an approximate fit to the data and allow calculation of an estimate of the average remineralization rate constant. It will also be of interest to compare the model results to actual profiles, where poor fit may indicate that the above assumptions are particularly invalid.

Table 4-3 summarizes the results of the calculations of k/w by least squares fit of equation 4-1 to ammonia and total carbon dioxide data from Stations 4, 5A, 8, and 2A. Station 6 was omitted because its profiles are totally inconsistent with model predictions. In each case, a best value and a range (in parentheses) are given. The range represents uncertainty in the estimated k/w due to scatter of the data around the calculated profile. To a first approximation, k/w is similar for all four cores. This probably results from the remineralization rate being approximately proportional to the sedimentation rate, rather than from lack of variability in the individual terms. For the two cores where w

was measured k_N (k calculated from the ammonia profile) is 0.1/yr and 0.07/yr for Stations 4 and 5A, respectively. Christensen and Packard (1977) found quite comparable rates of 17 to 200 mgC/m^2-day in surface (0-10 cm) sediments from depths of 65 to 480 m in the Northwest African Upwelling system. Conversion of the Peru rates to the same units gives maximum values of 45 mgC/m^2-day for Station 4 and 110 mgC/m^2-day for Station 5A.

A possibility which should be considered, however, is that the sedimentation rates for Stations 4 and 5A were grossly overestimated because the supported Pb-210 in surface sediments was much greater than was estimated from the activities in the bottom of the cores. This possibility can be discounted because:

$$C_o - C_\infty = \frac{-w^2 M_m^o b\rho(1-\phi)/\phi}{D_s k + w^2(1 + S\rho K)} \quad \text{and } S\rho K \geq 0. \tag{4-3}$$

Using $\rho = 2.2$; $\phi = 0.88$ for Station 4 and $\phi = 0.95$ for Station 5A; $D_s = \phi^2 D_o = \phi^2 \times 13 \times 10^{-6}$ cm^2/sec (Li and Gregory, 1974); $k_N/w = 0.01$ for Station 4 and 0.06 for Station 5A; and $b = 10^6 \times$ N/C ratio of sediment organic matter x 1/12,

$$w \geq \frac{\phi D_s k(C_\infty - C_o)/w}{M_m^o b\rho(1-\phi) - (C_\infty - C_o)\phi} \geq \frac{7.1 \times 10^{-4}}{M_m^o(2200)-700} \quad \text{for Station 4,}$$

and

$$w \geq \frac{1.3 \times 10^{-3}}{M_m^o(920)-1900} \quad \text{for Station 5A.}$$

Since TOC shows no consistent trends with depth at these stations, M_m^o is chosen to be the maximum value such that remineralization of that amount of carbon would be concealed by the variability in the TOC data, 15 mgC/gdw at Station 4 and 40 mgC/gdw at Station 5A. The calculated minimums for the sedimentation rate are then 0.7 cm/yr and 1.2 cm/yr, respectively, which are not substantially different from the Pb-210 rates. Using the Pb-210 rates, the same argument gives minimum values for M_m^o of 9.6 mgC/gdw and 39 mgC/gdw. Rosenfeld (1979) measured ammonia adsorption in Long Island Sound sediments, and found adsorbed/dissolved ratios near 1. This amount of adsorption would be consistent with Station 4 and Station 5A data.

Calculation of the sedimentation rates at Station 8 and 2A using equation 4-3 was atempted. For M_m^o = 20 mgC/gdw, k_N/w = 0.08 for Station 8 and 0.2 for Station 2A, ϕ = 0.90, and other parameters as before, the resulting sedimentation rates were 0.06 cm/yr and 0.6 cm/yr for Stations 8 and 2A, respectively. The calculated w for Station 2A seems unreasonably high, but the gradients in ammonia and total carbon dioxide were also unexpectedly large. Another core was taken in the Peru-Chile Trench during Knorr 73-2 less than 1 km from Station 2A. This core had interstitial water ammonia and total carbon dioxide profiles which were essentially identical to those from Station 8 (other analyses were not performed). Thus the sedimentation rates in the trench may be highly variable horizontally, perhaps due to slumping. In any case, the calculated sedimentation rates should be regarded as order-of-magnitude estimates only.

Carbonate may be removed from interstitial water by precipitation of mineral phases, e.g. calcite, siderite, or carbonato-fluoroapatite. Since only simple linear adsorption is included in the remineralization model, authigenic mineral formation could affect the calculated k/w. Solution of detrital carbonates could also affect total carbon dioxide concentrations, but calcium carbonate input to the Peru Upwelling sediments is probably quite low. However, despite the possible sink for carbonate in these cores, ranges of k/w calculated using total carbon dioxide data overlap those calculated using ammonia data, except at Station 2A.

The calculated profiles, if C_o is set at measured bottom water concentrations, fit the data quite poorly in the upper 10 cm of Station 4 and Station 5A cores, especially in the case of ammonia. These near-surface points can be fit to the model by assuming C_o's substantially greater than bottom water concentrations, 200 μM and 400 μM for Stations 4 and 5A respectively. This is equivalent to assuming that 2 mgC/gdw (7mgC/gdw) were remineralized in the upper 1 cm (1.5 cm) at Station 4 (Station 5A) in addition to that predicted by the model, if all constants remain as before. One possible explanation for this anomaly is that a relatively labile fraction of the organic matter is remineralized very close to the sediment-water interface, while more resistant organic matter is being decomposed deeper in the core. The very high microbial biomass in near-surface sediments (about 1 kg/m^2 according to Gallardo, 1978) is probably also a factor.

The accumulation rates of organic carbon in surface sediments of

Stations 4 and 5A (calculated from the organic carbon content of surface sediments and the Pb-210 sedimentation rates) are nearly equal at about 90 gC/m^2-yr. Multiplication of the daily POC flux through 50 m, 250 mgC/m^2-day (Staresinic, 1978) during the 1977 and 1978 upwelling, by 365 gives an annual flux of 91 gC/m^2-yr. Surface sediments represent only two or three years of deposition, but a potential source of error in comparison of the POC flux and the sediment organic carbon accumulation rate is seasonal variability in the POC flux. The primary productivity measured at these stations during the POC flux measurements was in the same range as the average annual primary productivity for the Pta. San Juan - Cabo Nazca area reported by Zuta and Guillen (1970), 2 to 3 g C/m^2-day, indicating that the sediment trap studies may give a reasonable value for the average flux. Still, since the annual POC fluxes have been extrapolated from measurements over two two-week periods, the very close agreement with the sediment accumulation rate may be fortuitous.

The N/C ratio in surface sediments is very close to the average N/C of the sedimenting material, 0.12 ± 0.01 (Staresinic, 1978). Since the N/C ratio of organic matter tends to decrease with remineralization (Price, 1976), the similarity of the ratios supports the conclusion that the bulk of organic matter leaving the euphotic zone accumulates in surface sediments. However, the N/C ratio is not very sensitive to relatively small proportional amounts of remineralization (less than about 30 %). Despite the high rates of remineralization of organic matter calculated earlier in this section for Station 4 and 5A sediments, N/C varies little with depth in core. But because of the very high sedi-

mentation rates, remineralization of a fairly small proportion of the total sedimented organic matter can explain the observed accumulations of dissolved carbon dioxide and ammonia. Using the range of remineralization rates and M_m^o calculated earlier, 30 to 40 gC/m^2-yr would be remineralized over the upper 50 cm of these cores, which is about 30 to 50 % of the total annual carbon flux.

THAA and THAA/TN do decrease with depth in sediment at Stations 4 and 5A, by about 0.08 mmoles/gdw (or 4 mgC/gdw) and 0.15 mmoles/gdw (or 8 mgC/gdw) respectively. Thus, more than 25 % of the organic carbon being remineralized in these sediments is proteinaceous material. However, THAA composition does not change appreciably despite the twofold decrease in concentration. This suggests that either most types of proteinaceous material in sediment are remineralized in proportion to their rates of supply or that the bulk of proteinaceous material in sediment has roughly the same composition.

Dissolved Free Amino Acids:

Concentration: The highest concentrations of dissolved free amino acids are found in surface sediments at all stations except Station 8. At Stations 4, 5A, and 6 a decrease in DFAA concentration is present below the transition which probably represents the base of the microbial mat, although at Stations 4 and 6 there are large (up to ten-fold) changes in concentration within the mat zone as well. The near-surface concentration maxima at Stations 4 and 6 may be due to increased inputs from either higher organisms or bacteria. However, Gallardo (1978) found that the biomass of metazoan infauna larger than 0.25 mm^2 was only 10 %

of the microbial biomass in a Thioploca mat community sampled at a water depth of 60 m off Concepcion, Chile.

Bacterial biomass (or activity) in sediments, as measured by a variety of methods, is usually highest near the sediment-water interface. ZoBell (1964), using culture-enumeration methods, found an exponential decrease in cell numbers with depth in oxidizing sediments sampled at depths of 430 and 950 m off California. Numbers decreased more than 10 times from the surface to 10 cm depth in the sediment. Karl (1978) and Karl et al. (1976) found maxima in ATP concentration (a measure of microbial biomass) within the upper 6 cm of Black Sea sediments for 450 and 2200 m depth and North Atlantic Abyssal Plain sediments. Christensen and Packard (1977) measured metabolism (probably mostly bacterial but including meiofauna) using respiratory electron transport system (ETS) activity in sediments from the Northwest African upwelling system (27 to 1820 m depth). Maxima in ETS activity occurred most often in the upper 2 cm, but subsurface maxima (4-6 cm) were observed for water depths less than 140 m.

In all cases these measures of microbial biomass (activity) showed large increases between bottom water and surface sediments (three orders of magnitude or more). Free amino acid concentrations in seawater, even in biologically active surface waters, seldom exceed 100 to 500 nM (e.g. Dawson and Pritchard, 1978). Free amino acid concentration measurements performed on bottom water samples collected during Knorr 73-2 indicate that concentrations were 100 nM at most. Thus, surface sediment DFAA concentrations were at least two or three orders of magnitude greater

than those in bottom water, corresponding, although not strictly proportional, to the increase in microbial biomass.

Maxima in bacterial activity may also be associated with redox potential discontinuities. Maxima in numbers of bacteria (Sorokin, 1964) and ATP concentration (Karl, 1978) have been observed at the O_2-H_2S interface in the Black Sea. Karl et al. (1977) found a similar maximum in ATP concentration at the oxic-anoxic interface in the Cariaco Trench. This is of interest because Stations 4 and 6, where large near-surface DFAA concentration maxima are present, are located on the landward and seaward fringes of the oxygen minimum zone, and the overlying water column contains slightly higher O_2 concentrations than at Station 5A where surface DFAA concentrations are much lower. However, the interstitial water chemistry of these three sediments with respect to the distribution of oxidized and reduced species is peculiar and not completely consistent with the above interpretation. Stations 4 and 6 show very high sulfide concentrations near the sediment-water interface and decreasing concentrations with depth, while Station 5A had no sulfide in the 0-3 cm depth interval but rapidly increasing concentrations with depth. Both nitrate and nitrite were present along with sulfide in the upper 10 cm of sediments from Stations 4 and 5A. (They were not measured at Station 6.) This indicates a distinctly non-equilibium situation with respect to redox potential, but does not support the conclusion that Station 4 and Station 6 surface sediments are more oxidizing than those at Station 5A. The possibility of sampling artifacts cannot be excluded, since while samples were squeezed in a closed system there was some

exposure to air during transfers from core to squeezer.

The distribution of sulfide in interstitial water is also at variance with expectations with respect to the extent of the microbial mat. The growth and physiology of Thioploca have not been studied, but may be similar to another group of sulfur bacteria, Beggiatoa. Since Beggiatoa oxidize sulfide while growing autotrophically (Kowallik and Pringsheim, 1966), sulfide concentrations would be expected to decrease toward the sediment-water interface (even without nonbiological oxidation of sulfide). Further, these organisms require oxygen, or at least a relatively oxidized species such as nitrate, in order to grow autotrophically on sulfide and flourish at interfaces where both oxygen and sulfide are available. The presence of a fairly uniform mat-like structure to depths of 10 to 20 cm where there is no available oxygen may be explained by the fact that Beggiatoa can also grow heterotrophically (Pringsheim, 1964), or the organisms may be inactive deeper in sediments. Another interesting attribute of these bacteria is that they are mobile within their filaments, perhaps over distances as large as several cm (Strohl and Larkin, 1978), which could allow them to move through a redox-potential discontintuity in sediment. Therefore, it is unclear how the metabolic activity of the Thioploca is distributed witnin the mat zone and how it might relate to the distribution of DFAA.

If DFAA concentrations are related to metabolic activity, then they would be expected to decrease with water column depth, based on a variety of studies of benthic metabolism from the literature. Smith (1978) measured in situ benthic community respiration rates from depths of 40 to

5200 m along the Gay Head to Bermuda transect and found a decrease of 3 orders of magnitude with increasing depth. The results of Christensen and Packard (1977) from the Northwest African Upwelling area show a 100-fold decrease in surface sediment ETS activity from 27 to 1820 m depth. Karl (1978) found that ATP concentrations in California coastal sediments were about 100 times those from 6000 m depth in the Atlantic. Jannasch et al. (1976) incubated undecompressed microbial populations collected from 1600 to 3100 m depth. Metabolism of glutamate and Casamino acids was reduced from 5 to 60 times over 1 atm controls, indicating that reduced rates of microbial degradation in the deep sea (Jannasch and Wirsen, 1973) can be attributed at least in part to hydrostatic pressure.

However, surface sediment DFAA do not decrease smoothly with increasing overlying water column depth. DFAA concentrations in surface sediments at Stations 4 and 6 are five to ten times higher than those at Stations 8 and 2A, but concentrations at Station 5A are no higher than at Station 8. The remineralization rate constants or rates (kM_m) calculated in the previous section likewise show no particular correlation with water column depth, however. The lack of the expected trend may be due to the fact that sediments from Stations 8 and 2A are very dissimilar to sediments from 1400 or 5000 m examined in the benthic metabolism studies. For example, surface sediment organic carbon content is 56 and 39 mgC/gdw, as compared to 6 to 10 mgC/gdw for sediments at these depths along the Gay Head to Bermuda transect. Surface water productivity is five times or more that of the Sargasso Sea, and sedimentation rates may

be one or two orders of magnitude greater.

Remineralization rate constants or rates (kM_m) are not correlated with surface sediment DFAA concentrations. This could stem from the fact that the model used to calculate k does not accurately describe near surface sediments. Therefore an attempt was made to relate DFAA concentration directly to the ammonia profiles. Since DFAA are an intermediate rather than an end-product in the remineralization process, their concentration should be related to the ammonia gradient rather than to the ammonia concentration. However, plots of DFAA vs. ammonia gradient showed good correlations only for Stations 4 and 2A ($r = 0.93$ and 0.90, respectively). Thus, while there appears to be some relation between microbial activity and DFAA concentration in Peru Upwelling Region sediments, other factors must be important as well. The rate of ammonia production in, for example, Station 4 surface sediments is about 0.6 nmoles/sec as calculated from the remineralization rate constant k_N. For a turnover time of 1 hour (see Chapter 1), the rate of amino acid input to give a concentration of 100 μM is 0.06 nmoles/sec, or only 10 % of the ammonia production. Therefore a direct coupling of the DFAA and ammonia distributions would not necessarily be expected, as the bulk of ammonia may be produced via an intermediate other than pore water DFAA.

Composition: The composition of DFAA from these sediments is characterized by a very high relative abundance of glutamic acid and β-aminoglutaric acid. As discussed in Chapter 3, glutamic acid is also the major constituent of the free amino acid pools of most bacteria. The free amino acid composition of benthic metazoans is more variable. In

most cases glycine and/or alanine are relatively more abundant that glutamate (see discussion in Chapter 3). Disablement of the TCA cycle during anaerobic metabolism (de Zwaan, 1977) might result in the accumulation of glutamate by some metazoans under some circumstances. But the large relative abundance of glutamate in nearly all pore water samples from both oxidizing and reducing sediments suggests that bacteria are a major source.

However, the large relative abundance of β-aminoglutaric acid in these samples is not characteristic of the free amino acid composition of the few types of bacteria which have been analyzed by GCGC. β-Aminoglutaric acid was present in two cultures of marine bacteria (Chromobacterium and a mixed culture of fermenters from the Pettaquamscutt River Estuary), but only at about 10 % of the glutamic acid concentration. β-Aminoglutaric acid was not present in the free or bound amino acids of several benthic macrofauna or in bacterial proteins analyzed by GCGC, nor has this compound been reported as a natural product in the literature. The very small concentrations found in sediment hydrolyzates from the Peru Upwelling region are close to those expected from the dissolved pool alone, and thus β-aminoglutaric acid is not present to a significant extent in the proteinaceous matter in sediment. Bacteria are thus the only known biological source for β-aminoglutaric acid, but data are very limited.

The trends in dissolved free amino acid composition with depth in core and between different sedimentary environments which were described in the Results section of this chapter could be due to:

(1) Differences in free amino acid pool compositions of the bacteria (or other organism) species which are dominant in different sediment samples.

(2) Variations in composition due to environmental effects within species of bacteria (or other organisms).

Both differences in species dominance or metabolic processes (leading to differences in composition) could be related to changes in redox potential, water column depth (temperature, pressure), characteristics of the organic matter being metabolized, or pore water composition, among other aspects of the sedimentary environment.

(3) The compositional trends might also be the result of differential sinks for particular amino acids, which will be discussed in a later section.

Sources of DFAA: Hypothesis (1) cannot be fully tested because of the lack of information on the DFAA composition of a wide variety of marine species (particularly with respect to β-aminoglutaric acid), and the lack of detailed knowledge of the particular species present in Peru Upwelling Region sediments. The literature and results discussed in Chapter 3 indicate that there is some variability in the amino acid composition of different kinds of bacteria cultured under similar conditions. The composition of the aerobic cultures of bacteria analyzed by GCGC was generally similar to that in surface and oxidizing sediments, with a high relative abundance of glutamic acid and smaller, but significant, amounts of other amino acids such as alanine and aspartic acid. The very large abundance of glutamate in Desulfovibrio (more than 95 %),

which is probably related to the truncation of the TCA cycle at the level of α-ketoglutarate dehydrogenase in these organisms (Lewis and Miller, 1977), is consistent with the higher relative abundance of glutamic acid in anoxic sediments, but does not explain the abundance of β-aminoglutaric acid. The work of Smith et al. (1967) is also of interest with respect to the Peru sediment results. These authors examined the incorporation of exogenous organic compounds (pyruvate, acetate, and glutamate) by obligate autotrophs including blue-green algae and Thiobacilli (sulfide oxidizers). The restricted incorporation of carbon from these substrates suggested that the TCA cycle was blocked at the level of α-ketoglutarate dehydrogenase. Carbon incorporated from C-14 acetate was predominantly found in glutamate (about 40 %) and amino acids derived biosynthetically from glutamate.

The source of the large relative abundance of β-alanine in a few of the Peru interstitial water samples is unclear. This non-protein amino acid is a constituent molecule in coenzyme A and is also a product of spermidine metabolism. Spermidine is a polyamine which is biosynthesized from arginine, which in turn is derived from glutamate via ornithine and the urea cycle (Metzler, 1977). However, there are no reports of β-alanine being a major constituent of free amino acid pools in organisms. It was a minor constituent of the DFAA of P. halodurans and an Ophioroid analyzed by GCGC (see Chapter 3).

There is also some evidence for hypothesis (2). Amarasingham and Davis (1965) measured levels of α-ketoglutarate dehydrogenase in E. coli grown aerobically and anaerobically. The enzyme could not be detected in

cells grown anaerobically, although high levels were found in aerobic cells. (As above, levels of this enzyme may affect glutamate accumulation, since α-ketoglutarate is the immediate biosynthetic precursor of glutamate.) Stanley and Brown (1976) and Brown and Stanley (1972) found that salinity, inorganic nitrogen source, growth rate, and substrate limitation affect the composition and/or concentration of free amino acid pools in bacteria. Their data, however, appears to have little specific application to sediment DFAA data because culture conditions differed substantially from those in the marine environment.

Compositional similarity between free amino acid pools of bacteria and pore water DFAA would not necessarily follow from the above discussion, even if bacteria are the source of most interstitial water amino acids, since bacteria would not necessarily excrete amino acids in proportion to their intracellular abundance. There is no information available on the excretion of amino acids by bacteria under natural conditions. Brown and Stanley (1972) found that a gram-negative, psychrophilic marine bacillus (designated PL-1 but otherwise unidentified) excreted large amounts of glutamine and glutamate when grown on glucose with ammonia as the nitrogen source. Several other strains showed little or no amino acid excretion, however.

There is an extensive body of literature on amino acid excretion by bacteria relative to the commercial production of amino acids (chiefly glutamate for monosodium glutamate). Nearly all of the primary literature is in Japanese, but fortunately it has been summarized in a series of reviews edited by Yamada et al. (1972). Although bacteria which

produce commercial quantities of glutamate (yields may be up to 50 % of consumed substrate) mainly fall into only a few genera (Brevibacterium and Bacillus being the most likely to occur in the marine environment), a wide variety of organisms excrete small amounts (as compared to the above 50 % yields) of amino acids into their media. About 20 % of 650 strains screened showed significant glutamate production (Kinoshita et al., 1972). Of the other amino acids, aspartic acid, alanine, glycine, serine, valine, and leucine were excreted at lower but significant levels. These results are suggestive in terms of the observed amino acid compositions of Peru DFAA, although the specific organisms and growth conditions were different from those present in the marine environment.

With this caveat in mind, however, some of the specific culture conditions employed in glutamate production are of interest. The largest yields are from facultatively anaerobic bacteria grown under low-oxygen (0.01 to 0.02 atm), but not anoxic conditions (Hirose, 1972). A wide variety of carbon sources, including acetate, monosaccharides, and ethanol can be used. Large amounts of ammonia are required. Fe^{2+} (1 to 10 ppm), K^{+} (0.1 to 0.6 ppt) and Mn^{2+} (1 to 10 ppm) ions are important for high glutamate production. The optimum pH range is 7 to 8 (Kinoshita et al., 1972). These conditions are quite similar to those in interstitial waters, especially near the sediment-water interface of anoxic sediments.

One common characteristic of commercial glutamate-producing bacteria is a requirement for biotin. Its role is apparently in controlling the permeability of the cell membrane via its role in fatty acid synthesis.

Low levels (2.5 mg/L) enhance excretion of glutamate, but excessive amounts result in very low yields (Fukui and Ishida, 1972; Aida, 1972). Other compounds (e.g. oleic acid) may partially or completely replace the biotin requirement. Biotin levels in coastal seawater are very low (on the order of 1 ng/L; le B. Williams, 1975), but concentrations in sediments are unknown. It is unclear how this relates to interstitial water data, except to indicate that other organic compounds dissolved in interstitial water may have an important effect on DFAA distributions.

Excess glutamate production in commercial organisms results from their weak or absent ability to oxidize α-ketoglutarate. In some organisms, the cell membrane is also poorly permeable to TCA cycle acids and NADPH oxidase (necessary for the reduction of citrate) is absent. Thus glutamate synthesis and excretion may prevent excess acid accumulation during fermentation (Kinoshita et al., 1972). The absence of α-ketoglutarate oxidase in several other types of bacteria was noted in earlier discussion.

Alanine, which was found in relatively high concentrations in some samples, is produced in commercial yields by some organisms including members of Pseudomonas, Bacillus, and Aerobacter. One pathway for alanine formation is via transamination or reductive amination of pyruvate, proposed as an overflow mechanism to remedy excessive accumulation of pyruvate (Kitai, 1972).

As discussed in Chapter 2, it is not certain whether the amino acids measured in squeezed interstitial water samples have been excreted by organisms in sediment or are extracted from living cells by the squeezing

process. The DFAA composition of pore water samples is consistent with a bacterial source in either case, since the composition of intracellular pools and excreted amino acids appears to be very similar. This question, however, is important to the following discussion of sinks for DFAA in sediments. Therefore, potential sinks will be discussed in terms of both possibilities.

Sinks: The few available measurements of turnover rates for dissolved free amino acids in sediments were reviewed in Chapter 1. They suggest that turnover times are very short, from less than one hour to about one day in the estuarine environments studied. It therefore seems likely that the observed DFAA distributions in Peru sediments are the result of a dynamic equilibrium between sources and sinks. The net changes in distribution are the result of changes in the position of the equilibrium, while turnover rates are the sum of the rates of supply or removal.

Some of the trends in amino acid concentration and composition seen in Peru sediments may stem from changes in the relative rates of supply and decomposition by organisms (hypothesis 3). Both accumulation of intracellular pools and excretion seem to be related to high rates of metabolic activity, as found in surface sediments. Deeper in sediments, decomposition is relatively greater than supply, resulting in lower concentrations. This would apply to both extracellular and intracellular pools, although the actual rates of decomposition would be expected to differ. The increase in β-aminoglutaric acid/glutamic acid ratio with depth in core and with decreasing DFAA concentration may be due to

glutamate and other protein amino acids being more readily decomposed by bacteria than β-aminoglutaric acid. The relative increase in β-aminoglutarate abundance and the decrease in absolute concentration of other amino acids in unacidified stored samples (see Chapter 2) supports this suggestion.

Diffusion tends to smooth fluctuations in pore water compostition which may result from, for example, variations in sedimentation rate or sediment composition (Lasaga and Holland, 1976). Thus abrupt changes in DFAA concentration and composition observed in some of the Peru sediment cores could indicate that DFAA are not free to diffuse, i.e. are contained by cell membranes. However, if interstitial water amino acid turnover rates were sufficiently rapid, such gradients could be maintained despite diffusion. As an example, the most abrupt maximum in concentration, found for glutamate in the 0-2 cm core section from Station 4, will be considered. The gradient at the sediment-water interface (assuming a bottom water concentration of 0 and calculating from the midpoint of the depth interval) is 30 μM/cm, and the gradient at the bottom of the interval is 10 μM/cm. Assuming Fickian diffusion; constant, linear gradients; and $D_s = 1 \times 10^{-5}$ cm^2/sec (as for ammonia):

$$Flux_o = 3 \times 10^{-4} \text{ nmoles/cm}^2\text{-sec}$$

$$Flux_2 = 1 \times 10^{-4} \text{ nmoles/cm}^2\text{-sec.}$$

However, if the turnover time for amino acids in this interval is one hour, then tne input rate must be 30 μM glutamate/hr x 0.002 L/cm^2 = 1.6×10^{-2} $nmoles/cm^2$-sec, much greater than the loss via diffusion. A turnover time of 1 day gives an input rate of 6×10^{-4} $nmoles/cm^2$-sec,

close to the rate of diffusive loss. For this turnover rate, the near-surface maximum could be maintained only in the absence of other major sinks, i.e. the rate of metabolism could be only about 1/3 the rate of excretion. It is of interest to note, however, that an even larger gradient in ammonia concentration is maintained in this depth interval by biological activity.

DFAA may be adsorbed by either the mineral phases or organic matter in sediments. This potential sink will be discussed in more detail for Buzzards Bay sediments (see Chapter 5) for which some measurements of amino acid adsorption have been done. While this process may be of some significance to the net DFAA concentrations observed in Peru sediments, it seems unlikely that adsorption is primarily responsible for the concentration and composition trends observed, because both the mineralogy and organic content of the sediments changes relatively little with depth in cores and between stations.

The humification reaction, involving condensation of labile dissolved organic compounds in interstitial waters to form macromolecular organic compounds, has been described in Chapter 1. This reaction is a potential sink for DFAA in pore waters, but not for free amino acid pools in living organisms. Because the polymers formed by Maillard-type condensation reactions are relatively resistant to breakdown by organisms, this reaction has been proposed as a mechanism for the accumulation of DOC with depth in anoxic sediments by Krom and Sholkovitz (1977). It is apparent that the DOC profiles in Peru sediments are distinctly different from the DFAA profiles, suggesting that the bulk of DOC is not made up of

compounds which are turned over as rapidly as DFAA. DFAA themselves make up a maximum of 20 % of the DOC in surface sediments, and a much smaller proportion below.

At Stations 4 and 6 there is a slight maximum in DOC in surface sediments associated with the DFAA maximum, but it is much smaller in relative intensity. DOC concentrations show a net increase with depth at Stations 5, 8, and 2A, a net decrease at Station 6 (as does TOC), and random variation over about a factor of 2 at Station 4. Since gradients within sediments are small, diffusive fluxes of DOC are small except at the sediment-water interface. Again taking Station 4 as an example, and assuming $D_s = 1 \times 10^{-6}$ for polymeric organic matter, the production rate required to balance diffusion would be about 5×10^{-5} $\mu gC/cm^2$-sec for the 0-2 cm depth interval. If all of the DOC were made up of a melanoidin-type polymer, and 50 % of the carbon in the polymer was derived from amino acids, then melanoidin formation would be a sink for 5×10^{-4} nmoles amino acids/cm^2-sec, roughly equivalent to the sink due to diffusion. If a large proportion of the melanoidins formed were rapidly adsorbed (Hedges, 1978), humification could be a relatively more important sink, especially deeper in sediments where rates of biological turover are lower.

Summary of Conclusions

(1) Remineralization rates in Peru oxygen minimum zone sediments are very high, approximately 30 to 40 gC/m^2-yr over 50 cm depth at Stations 4 and 5A.

(2) This remineralization amounts to about 5 % of the total annual primary production, and about 30 to 50 % of the total annual POC flux through 50 m. Thus a major proportion of the organic carbon supplied to sediments survives to be buried below 50 cm.

(3) High concentrations of DFAA (10 to more than 100 μM) were measured in surface sediments. The composition of the DFAA suggests that bacteria are a major source.

(4) Concentrations of DFAA are not strongly related to remineralization rates, apparently because other aspects of the sedimentary environment also influence their accumulation and/or excretion by organisms.

(5) The major control on DFAA concentration and composition appears to be biological. Other processes considered (adsorption, diffusion, humification) appear to have a relatively minor influence, either because biological turnover rates are rapid or because the bulk of DFAA measured in interstitial water samples are extracted from living cells.

CHAPTER 5

RESULTS FROM BUZZARDS BAY AND THE PETTAQUAMSCUTT RIVER ESTUARY

Introduction

Results from two near-shore, shallow water sedimentary environments will be discussed in this chapter. The purposes of this study of estuarine sediments were twofold: to examine short-distance and seasonal variability in sediment DFAA at Buzzards Bay, Station P; and to compare and contrast these results to measurements from a very different environment, the anoxic Pettaquamscutt River Estuary.

Buzzards Bay is located southwest of Cape Cod, Massachusetts and covers an area of approximately 460 km^2 to an average depth of 13 to 16 m in the central basin (Hough, 1942). Buzzards Bay, Station P (BBP) is located at a depth of 17 m in the southern part of the bay, near open water. Primary productivity shows a large seasonal variation, from 100 to greater than 500 mgC/m^2-day, with the productivity maximum extending from June to November or December (Roman and Tenore, 1978). Sediments at this station have been the subject of several previous organic geochemical studies, dealing with fatty acid (Farrington et al., 1977a), hydrocarbon (Farrington et al., 1977b), and sterol (Lee et al., 1977) distributions and diagenesis. The sediment at BBP is a clayey silt populated primarily by deposit-feeding infauna (greater than 87.5 % by number), which are predominantly two species, Nepthys incisa (a polychaete worm) and Nucula proxima (a bivalve mollusc) (Sanders, 1958). These organisms,

along with numerically less abundant but larger animals such as Yoldia limatula, bioturbate the upper two to three centimeters of sediment extensively; Nepthys may burrow up to 10 cm depth (Rhoads, 1967). The bioturbated surface layer is unstable and can be resuspended by tidal currents.

BBP sediments were sampled three times during the course of this thesis work; a sphincter core taken on 6/28/77 during preliminary methods-development work (BBP6/77); a sphincter core and a grab sample on 6/26/79 (BBP6/79 and BBP6/79 G, respectively); and a Soutar core and three grab samples on 12/5/79 (BBP12/79 and BBP12/79G-1, BBP12/79G-2, and BBP12/79G-3). Samples for organic and inorganic analyses were obtained from the same cores but were squeezed separately.

The Pettaquamscutt River (PRE) is a shallow, drowned-valley type estuary which flows south into Rhode Island Sound. Two basins are located at its northern end: the upper basin which has an area of 0.3 km^2 and a maximum depth of 13.5 m, and the lower basin which has an area of 0.7 km^2 and a maximum depth of 19.5 m. Seawater enters the basins via the relatively shallow river channel (about 1 m deep) and is trapped beneath the fresh water outflow. This saline bottom water stagnates and very high concentrations of sulfide accumulate, up to 4.5 mM (Orr and Gaines, 1974; Gaines and Pilson, 1972). Autumn mixing events partially ventilate the water column at intervals averaging three to four years. The bottom sediments are a highly organic diatomaceous ooze, with very low aluminosilicate mineral content. The PRE has been described in detail by Gaines (1975).

Two cores were obtained from the upper basin at a water depth of 8 m, well within the anoxic zone. The assistance of Dr. Arthur Gaines in the coring operation is gratefully acknowledged. One core was analyzed for DFAA, TOC, TN, THAA, and % H_2O, and the other for pore-water dissolved ammonia, total carbon dioxide, sulfide, and chlorinity (by refractometer). Due to difficulties while bringing the core aboard the research vessel (a rather tippy small boat), the upper few cm of both cores were disturbed. The cores were returned to the laboratory and refrigerated within three hours after sampling, but were not squeezed until the following day.

Results

TOC, TN, THAA, THAA/TOC, THAA/TN, DOC, $\%H_2O$, nitrate, sulfide, and (for PRE only) chlorinity data are given in Table 5-1. TOC in both BBP surface sediment samples is near 20 mgC/gdw. The organic content decreases by 7 to 8 mgC/gdw over the upper 30 cm, but shows little change below 30 cm in BBP6/79. TN is 2 to 3 mgN/gdw in surface sediments, and decreases proportionally to TOC; TN/TOC varies between 0.11 and 0.13, but follows no particular trend with depth. THAA decrease from 50 µmoles/gdw at the surface to about 30 µmoles/gdw at 30 cm, and are fairly constant below that depth. THAA/TN has no consistent depth trend. BBP6/79 and BBP12/79 are extremely similar in all the parameters summarized in Table 5-1. One exception is the slightly higher TN and THAA content of BBP12/79 surface sediment. The sphincter corer produces relatively more disturbance of the sediment surface than the Soutar corer. however, so

TABLE 5-1

Composition of Sediments from Buzzards Bay and the Pettaquamscutt River

Depth in Core (cm)	TOC (mgC/gdw)	TN (mgN/gdw)	THAA[a]	TN/TOC	THAA/TN	DOC (mgC/L)	$\%H_2O$	NO_3 (μM)	S^{2-} (mM)
BBP6/79									
0-2	19	2.2	51	0.118	0.32	12	56	0.9	0.0
2-4	16	1.9	39	0.119	0.29	15	52	0.6	0.0
4-6	16	1.8	39	0.111	0.30	110[b]	53	0.2	0.0
6-10	16	1.8	38	0.111	0.29	41	51	0.2	0.0
10-14	14	1.6	34	0.113	0.30	20	48	0.0	0.0
14-18	14	1.5	31	0.111	0.29	36	44	0.0	0.0
18-22	12	1.5	27	0.127	0.25	19	43	0.0	0.0
22-26	13	1.4	29	0.112	0.29	17	43	0.0	0.0
30-34	13	1.5	29	0.120	0.27	25	46	0.0	0.01
38-42	13	1.5	31	0.117	0.31	25	42	0.0	0.03
46-50	11	1.3	30	0.117	0.32	50	43	0.0	0.60
54-58	13	1.6	34	0.122	0.30	25	42	0.0	0.02
62-66	12	1.4	27	0.114	0.27	26	41	0.0	0.01
BBP12/79									
0-2	20	2.8	76	0.139	0.38	17	57	18	0.0
2-5	18	2.3	53	0.126	0.33	12	51	7	0.0
5-8	17	2.0	39	0.120	0.27	12	47	3	0.0
8-11	16	2.0	47	0.124	0.33	15	47	3	0.0
11-14	16	1.7	41	0.106	0.34	17	44	3	0.01
14-17	16	1.6	35	0.103	0.30	18	41	1	0.01
17-20	11	1.4	21	0.127	0.21	18	40	0.0	0.01
20-23	12	1.6	30	0.133	0.27	62[b]	41	0.0	0.01
23-26	12	1.6	35	0.130	0.31	22	41	0.0	0.01
26-29	13	1.6	27	0.121	0.24	19	42	0.0	0.01
29-32	13	1.6	34	0.125	0.32	21	45	0.0	0.01
PRE								Cl°/oo[c]	
0-6	122	12	570	0.098	0.66	35	92	19.3	0.60
6-12	134	12	490	0.088	0.58	61	90	19.0	0.26
12-18	143	12	450	0.086	0.51	62	89	18.7	0.11
18-24	124	11	420	0.087	0.55	47	88	18.7	0.90
24-30	134	12	400	0.089	0.46	52	87	18.7	1.8
30-36	122	10	330	0.085	0.44	53	86	18.7	1.7
36-42	118	10	330	0.087	0.45	57	85	18.4	2.5
42-48	140	12	340	0.085	0.40	53	84	17.9	0.60
54-60	140	11	310	0.081	0.38	56	85	17.2	0.09
66-72	157	13	340	0.084	0.36	57	86	16.2	0.03
78-84	168	12	280	0.074	0.32	52	87	13.8	0.01

[a]Units of μmoles/gdw.

[b]Value seems unreasonably high (see text).

[c]Composition of PRE pore water is very different from seawater; thus the refractive index Cl°/oo may not be the actual chloride content.

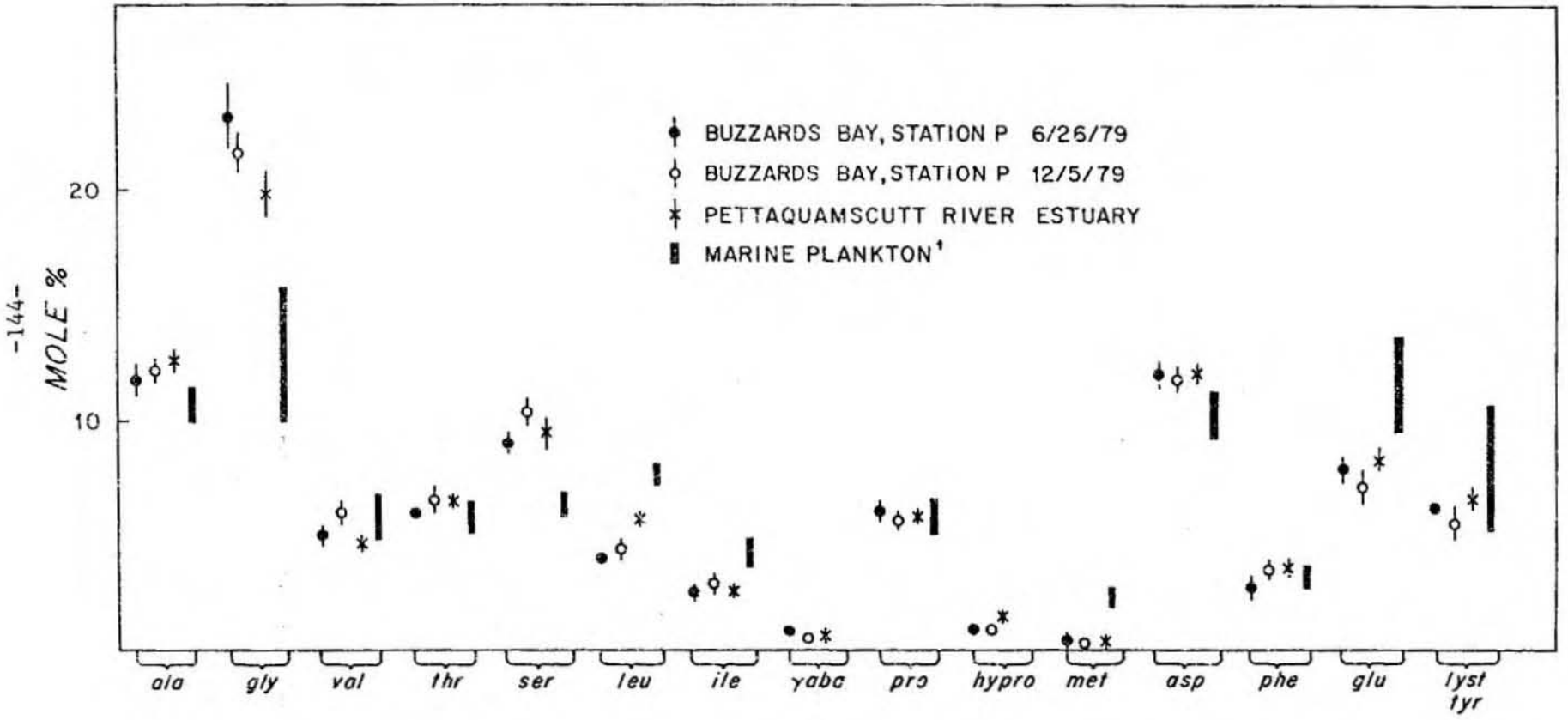
this difference may simply represent loss of a portion of the upper 2 cm of BBP6/79.

PRE sediment TOC is more than six times that of BBP surface sediments, and TN is also much greater although TN/TOC is 20 to 40 % lower. The lower ratio may reflect the relatively large amounts of terrigenous plant detritus in PRE sediments. Well-preserved twigs, leaves, and leaf fragments were present throughout the core. A slight increase in TOC with depth while TN remains constant is responsible for a small decrease in TN/TOC. THAA decrease by about a factor of two over the length of the core but are an order of magnitude greater in concentration in PRE sediments than in BBP sediments. THAA/TN is about twice BBP values in surface sediments, but decreases to similar values at the bottom of the core.

THAA composition of the sediment samples from the three cores is summarized in Figure 5-1. The mean mole percentage of each amino acid in the hydrolyzate from all depths in a core is plotted, with the length of the bar representing ± one standard deviation. The variability in composition with depth is not significantly greater than analytical error. Also, there are no significant differences in THAA composition between the BBP cores and the PRE core, despite the fact that overall concentrations in PRE sediments are an order of magnitude greater. An average of marine plankton protein compositions reported by Mopper and Degens (1972) is given for comparison. Sediment and plankton THAA composition are similar, but the plankton contain relatively less glycine and serine and somewhat more glutamic acid, leucine, and isoleucine.

Figure 5-1: Composition of total hydrolyzable amino acids in Buzzards Bay and Pettaquamscutt River Estuary Sediments.

[1]Mopper and Degens (1972).


BUZZARDS BAY, STATION P 6/26/79
BUZZARDS BAY, STATION P 12/5/79
PETTAQUAMSCUTT RIVER ESTUARY
MARINE PLANKTON¹
MOLE %
20
10
ala
gly
val
thr
ser
leu
ile
yaba
pro
hypro
met
asp
phe
glu
lyst
tyr

DOC concentrations in BBP sediment interstitial water are erratic for unknown reasons, but possibly due to contamination or analytical error. If the outlying values are discarded, there is a small, at most twofold, increase with depth. PRE concentrations are quite constant and about twice the BBP concentrations. The slightly lower surface value might be due to diffusion or the mixing with overlying bottom water during recovery.

Low levels of sulfide were present below 10 cm in BBP12/79 pore waters. A rather large amount of sulfide was present in the 46-50 cm section of BBP6/79, an order of magnitude more than in the nearest sections analyzed above and below this interval (but note that the adjacent 4 cm sections were discarded). This maximum is puzzling and appears to correspond to no other anomalies in the data except a maximum in DOC. A pronounced sulfide odor was noted in the core description for the bottom of the BBP6/79 core, but was not associated with a particular section. Sulfide concentrations in the PRE core, as expected, were high. The profile has a slight surface maximum and a much more pronounced maximum at the 36-42 cm depth interval. Concentrations then decrease to the bottom of the core. A corresponding decrease in chlorinity from $18^{o}/oo$ to $13^{o}/oo$ occurs over the bottom 40 cm of the core.

Data for bottom water samples from the PRE and BBP (6/26/79 only) are given in Table 5-2. BBP bottom water had no detectable sulfide or ammonia and only a trace of nitrate. According to Roman and Tenore (1978) nitrate concentrations are near 0 in the water column during the summer productivity maximum, but greater during the winter months, which may

TABLE 5-2

Bottom Water Composition

	BBP6/79	PRE
NH_3 (μM)	0.3	930
NO_2^-(μM)	0.0	0.0
NO_3^-(μM)	0.6	0.0
S^{2-}(mM)	0.0	3.5[a]
Total CO_2 (mM)	2.2	10.0
DOC (mgC/L)	5.6	5.6
DFAA (nM)		
ala	---	24
gly	44	32
ser	8	24
asp	7	40
glu	8	33
β-glu	---	11
TOTAL	76	200

[a]Lower limit.

Figure 5-2: Buzzards Bay, Station P interstitial water ammonia concentrations. The lines drawn through the points are the concentrations predicted by a remineralization model (see text).

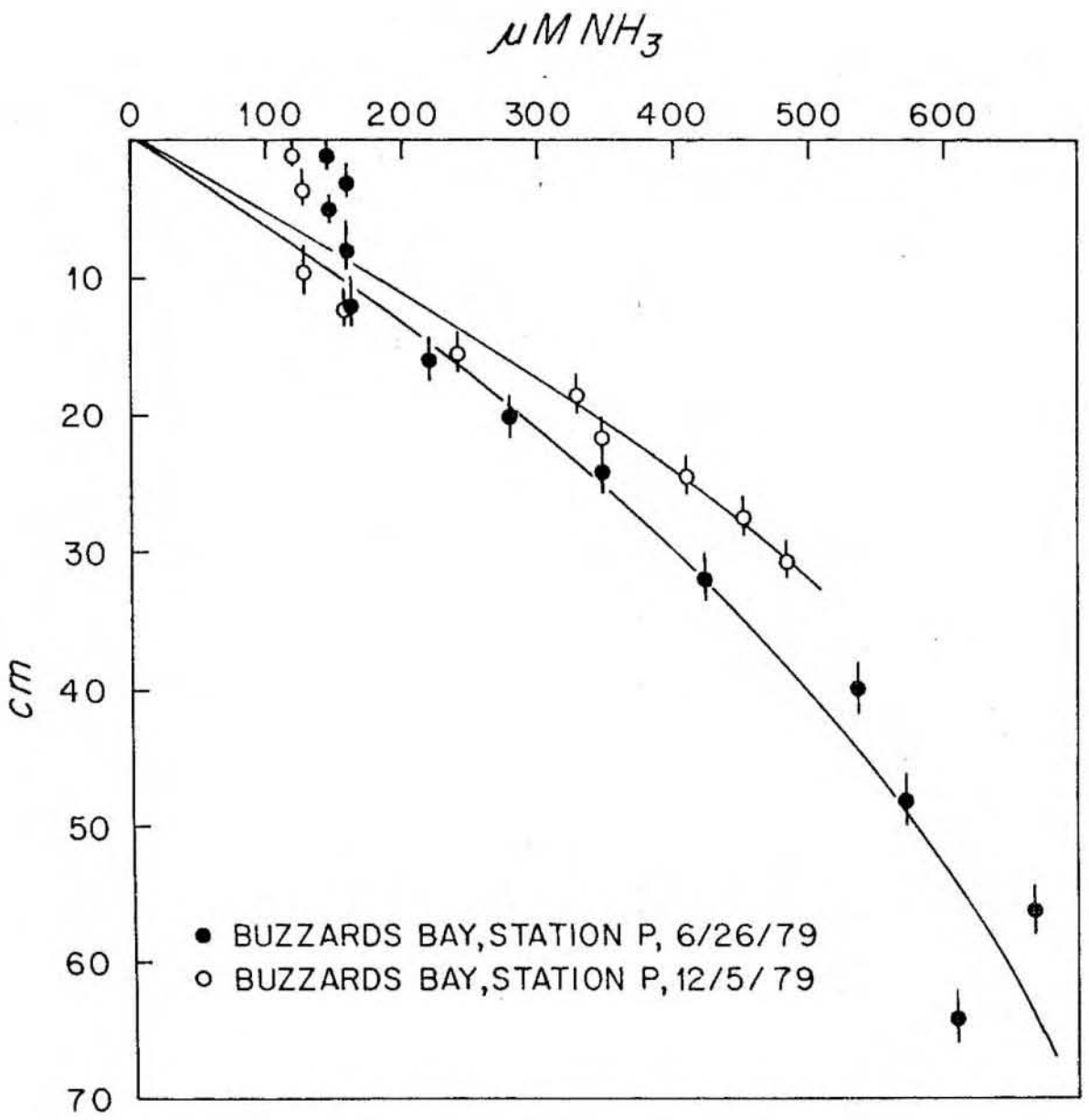
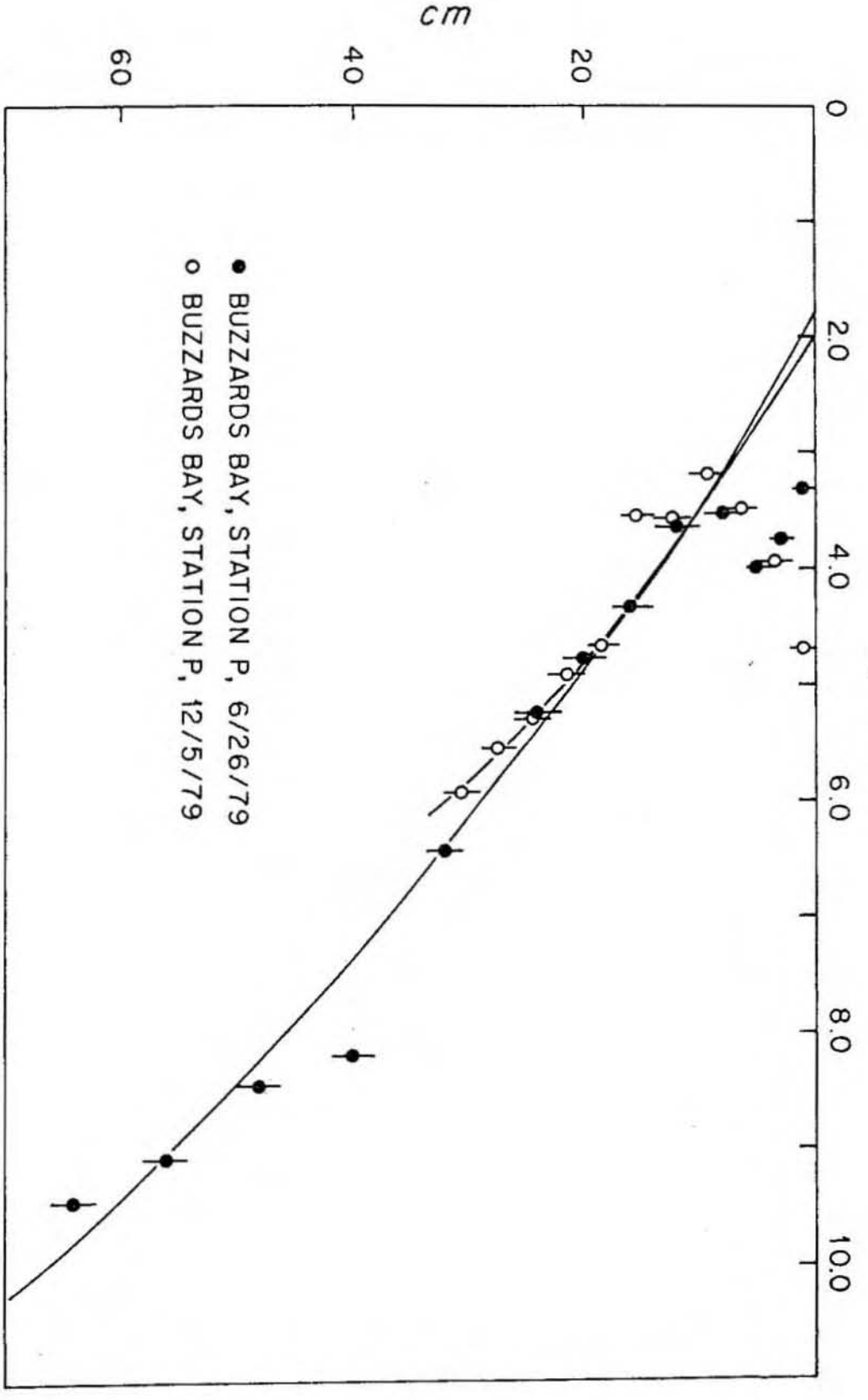

μM NH3
0
100
200
300
400
500
600
10
20
30
40
50
60
70
cm
● BUZZARDS BAY, STATION P, 6/26/79
○ BUZZARDS BAY, STATION P, 12/5/79

Figure 5-3: Buzzards Bay, Station P interstitial water total carbon dioxide concentrations. The lines drawn through the points are the concentrations predicted by a remineralization model (see text).


cm
0
20
40
60
2.0
4.0
6.0
8.0
10.0
mMΣCO2
● BUZZARDS BAY, STATION P, 6/26/79
○ BUZZARDS BAY, STATION P, 12/5/79

account for the higher concentrations seen in BBP12/79 pore water. PRE, in contrast, had more than 900 μM ammonia and very high sulfide concentrations. (Because the sample was improperly diluted, only a lower limit is given). Total carbon dioxide was near normal seawater levels at BBP, but 10 mM in PRE bottom water. DOC concentrations were the same at both locations, slighly more than 5 mgC/L.

Dissolved ammonia and total carbon dioxide in interstitial waters from BBP6/79 and BBP12/79 cores are plotted in Figures 5-2 and 5-3. In general, the BBP6/79 and BBP12/79 profiles agree well for both ammonia and total carbon dioxide. BBP6/79 ammonia concentrations are consistently about 30 μM greater than BBP 12/79 concentrations above 10 cm but tend to be slightly lower than BBP12/79 concentrations deeper in the sediment. Total carbon dioxide concentrations in BBP12/79 interstitial water show a pronounced minimum at 10 cm, while this feature is less evident in the BBP6/79 core.

PRE interstitial water ammonia and total carbon dioxide concentrations are plotted in Figure 5-4. Ammonia concentrations are substantially greater than bottom water but fairly constant in the 0-18 cm depth interval. Below 18 cm ammonia concentrations increase fairly smoothly with depth to very high levels. greater than 2000 μM. Total carbon dioxide concentrations have a similar pattern in surface sediments, but decrease with depth below 50 cm. This trend corresponds to the decreasing sulfide and chloride concentration over the same depth interval.

Dissolved free amino acid concentrations and compositions of sediments from BBP6/79, BBP12/79 and PRE sediments are shown in Figures 5-5,

Figure 5-4: Pettaquamscutt River Estuary ammonia and total carbon dioxide concentrations. The lines drawn through the data are ammonia concentrations predicted by a remineralization model for a range of values of k/w (see text).

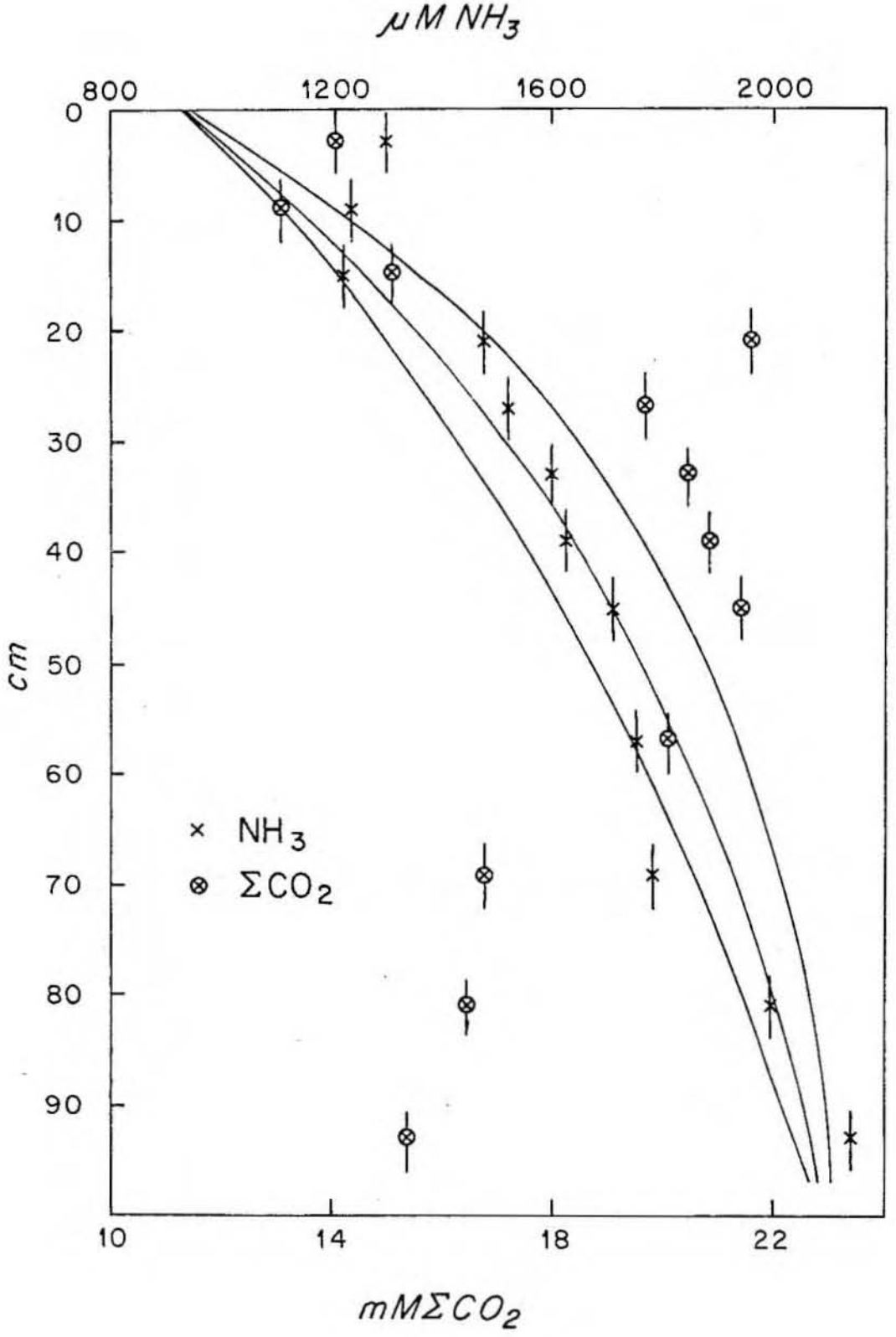

μM NH3
800
1200
1600
2000
0
10
20
30
40
50
60
70
80
90
cm
× NH3
⊗ ΣCO2
10
14
18
22
mMΣCO2

Figure 5-5: Dissolved free amino acid concentrations in Buzzards Bay sediments sampled on 6/26/79.

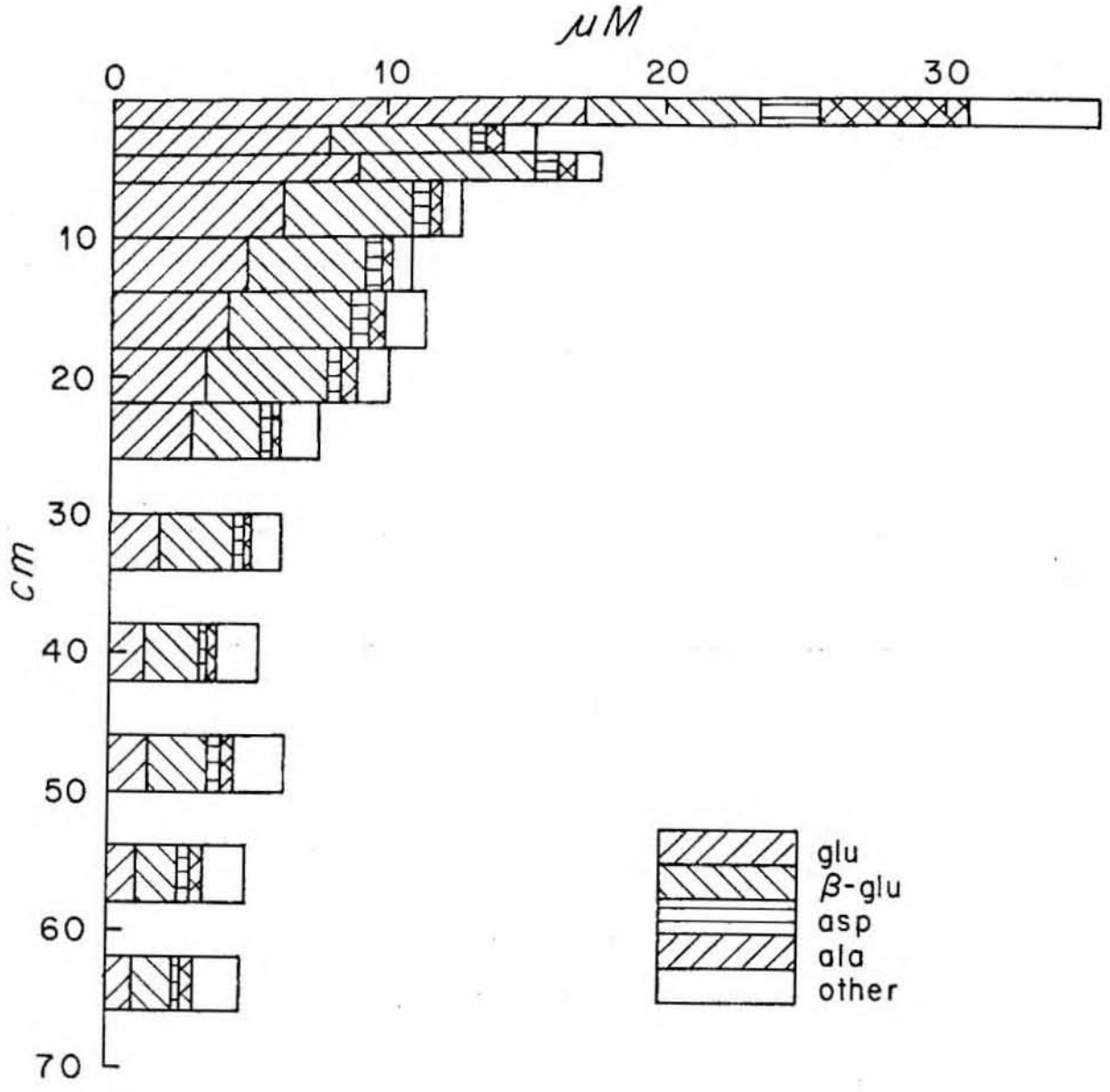

μM
0
10
20
30
cm
10
20
30
40
50
60
70
glu
β-glu
asp
ala
other

Figure 5-6: Dissolved free amino acid concentrations in Buzzards Bay sediments sampled on 12/5/79.

[1]The dotted extension of the bar represents the average DFAA concentration in three grab samples of surface sediment taken on the same date.

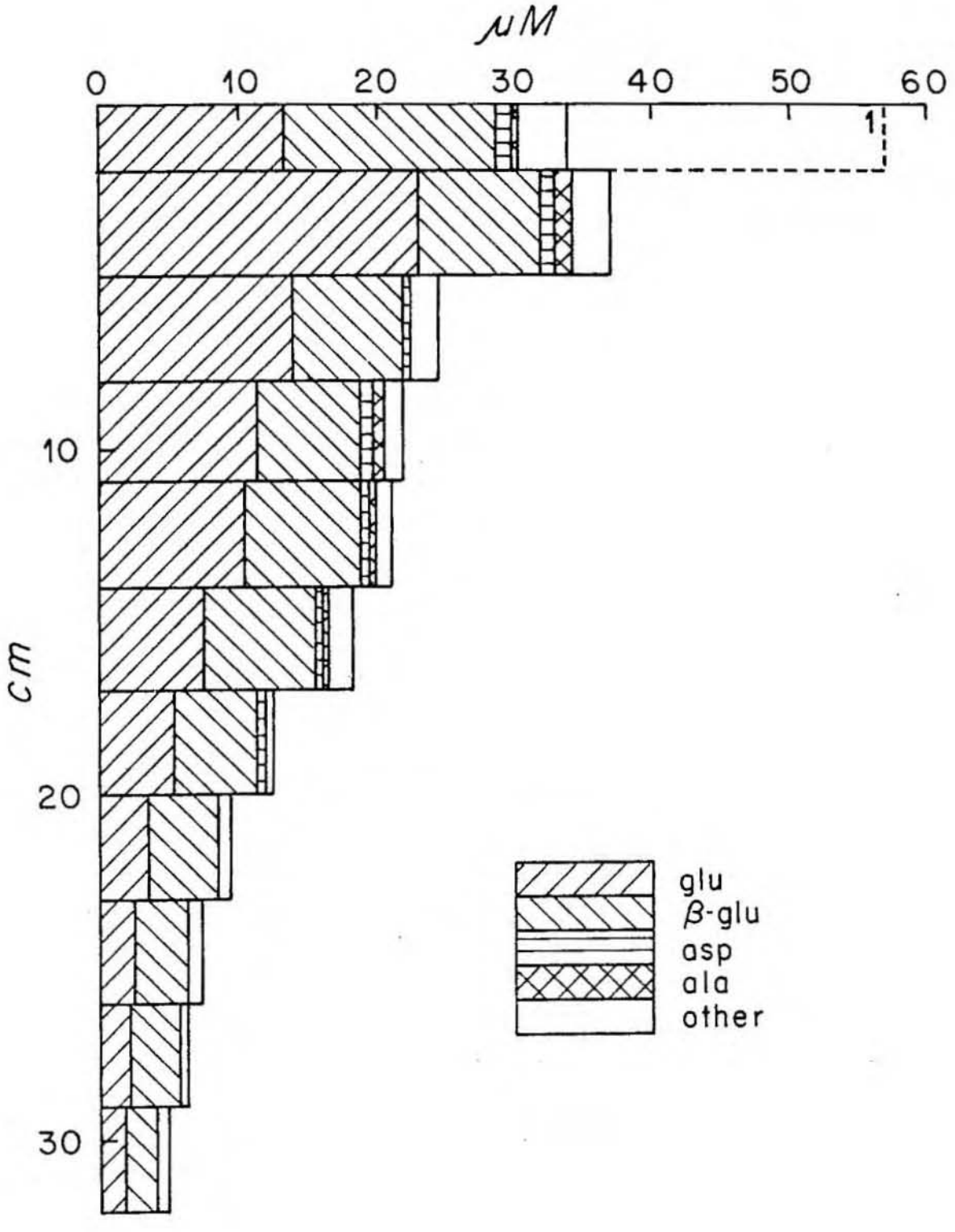
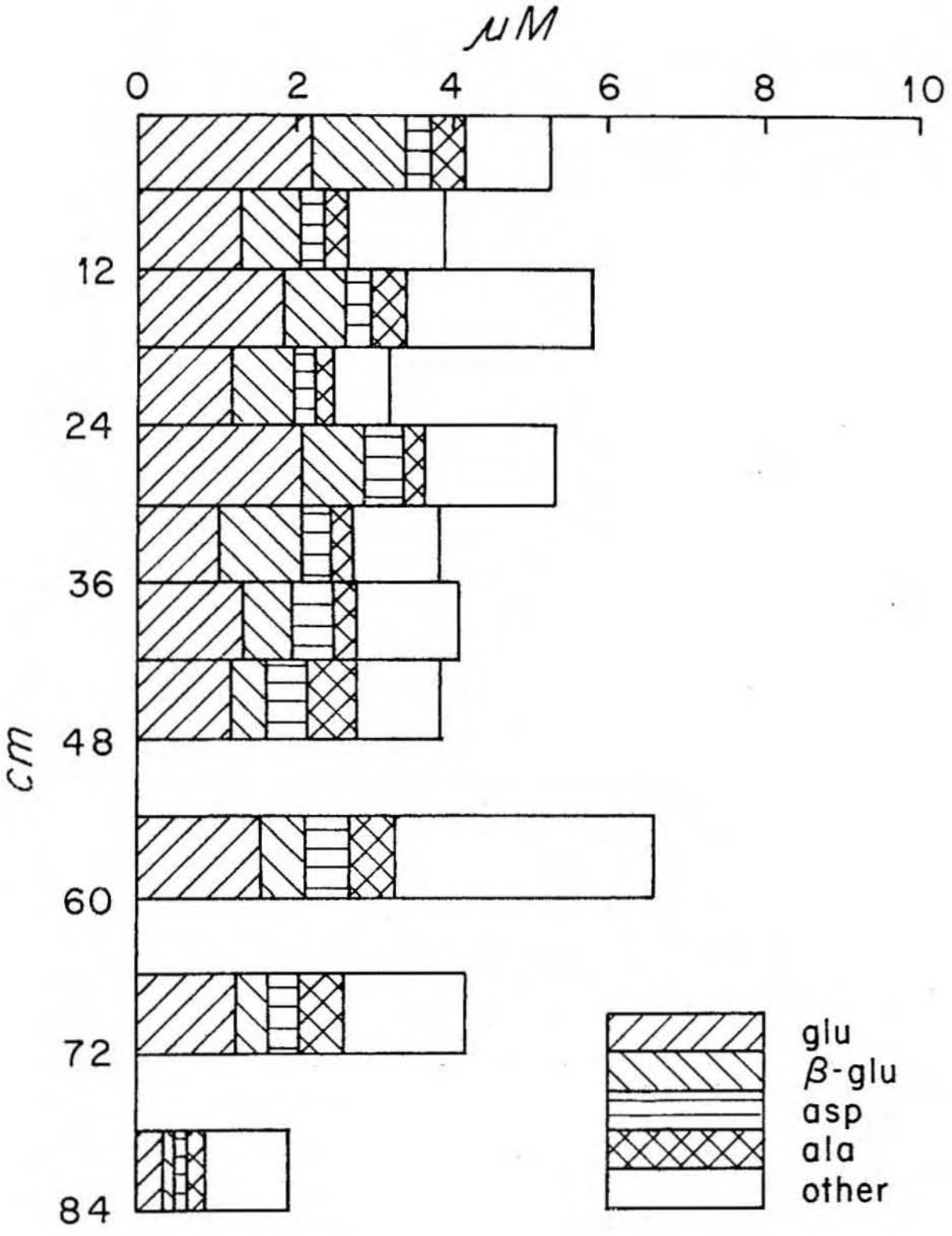

μM
0
10
20
30
40
50
60
1
10
20
30
cm
glu
β-glu
asp
ala
other

Figure 5-7: Dissolved free amino acid concentrations in Pettaquamscutt River Estuary sediments.


μM
0
2
4
6
8
10
cm
12
24
36
48
60
72
84
glu
β-glu
asp
ala
other

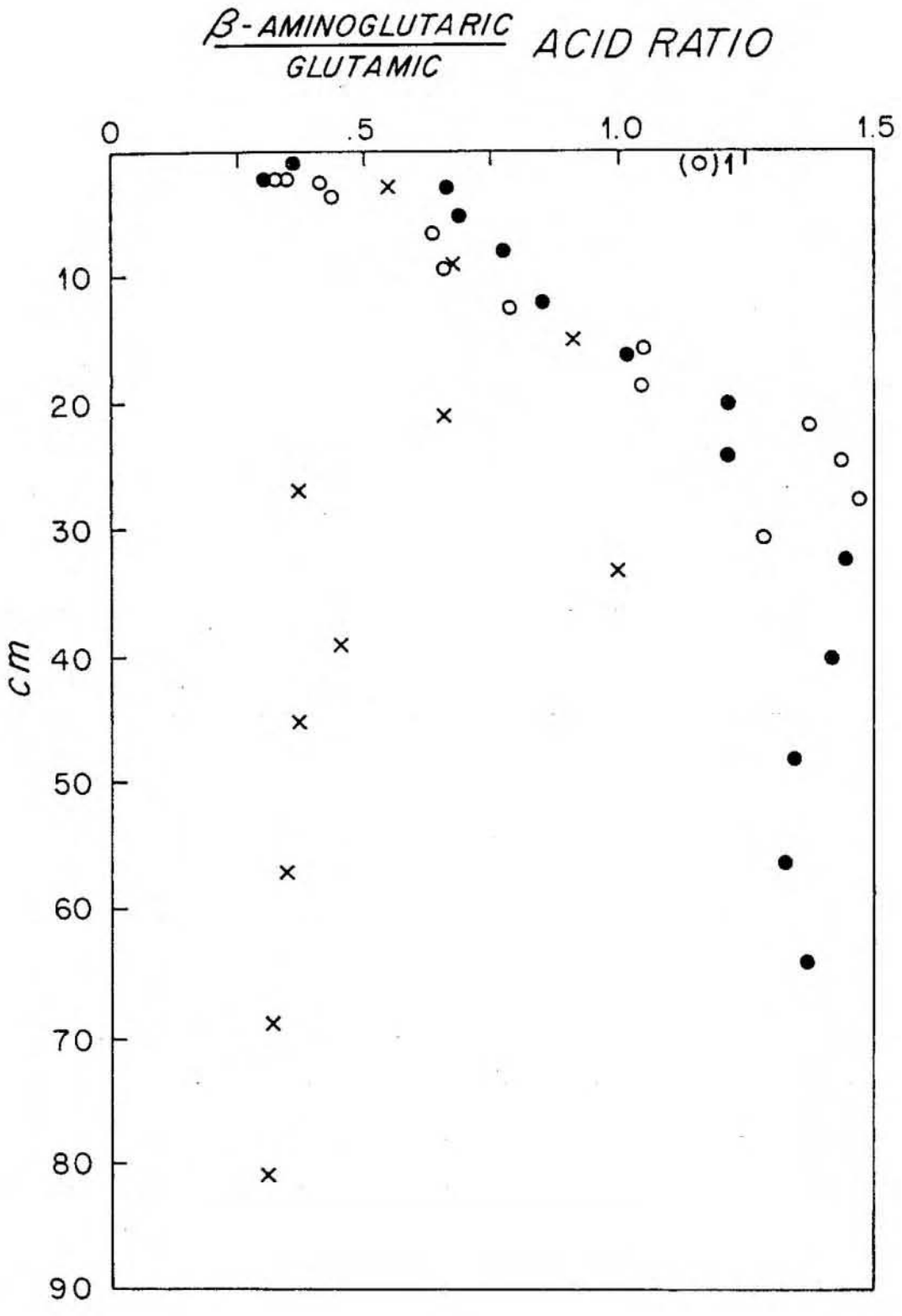
5-6, and 5-7, respectively. The total length of the bars represents total concentration, and the bars are divided according to the relative abundance of specific amino acids as explained in Chapter 4. The 0-2 cm pore water sample for BBP 12/79 was inadvertently stored for four days, refrigerated but unpoisoned, between squeezing and cation-exchange chromatography. The DFAA concentration in this sample is low relative to the three grab samples taken on the same date (see Table 2-4) and the relative abundance of β-aminoglutaric acid is high, indicating that some bacterial consumption of amino acids may have occurred in this sample during storage. The dotted extension of the bar in this depth interval is the average DFAA concentration in the three grab samples of surface sediments taken on 12/5/79.

DFAA concentrations in the BBP6/79 and BBP12/79 cores are very similar, with near-surface concentration maxima and decreasing concentrations with depth. Below 20 cm the concentrations are virtually identical, but above 20 cm BBP12/79 concentrations are consistently greater, by up to a factor of two. With the exception of the BBP12/79 0-2 cm section already discussed, DFAA composition is likewise very similar. Glutamic acid and β-aminoglutaric acid are the major constituents at all depths in both cores. β-Aminoglutaric acid becomes more abundant relative to glutamic acid with depth in sediment to about 30 cm, but their ratio is fairly constant below 30 cm in core BBP6/79 (Figure 5-8).

DFAA concentrations in PRE surface sediments are substantially lower than at BBP. Concentrations are essentially constant over the upper 72 cm of the core, varying by less than a factor of two (between 3 and 6 μM)

Figure 5-8: Dissolved β-aminoglutaric acid/glutamic acid ratio vs. depth in sediment. • = Buzzards Bay. Station P 6/26/79; o = Buzzards Bay, Station P 12/5/79; x = Pettaquamscutt River Estuary.

[1]Sample composition may have changed during storage; see text.


β-AMINOGLUTARIC ACID RATIO
GLUTAMIC
0
.5
1.0
1.5
(o)1
10
20
30
40
50
60
70
80
90
cm

and showing no consistent depth trend. The concentration in the bottom section (78-84 cm) is only slightly lower (2 μM). Glutamic and β-aminoglutaric acids make up 30 % to more than 50 % of the DFAA, but other amino acids are relatively more abundant (although their absolute concentrations are equal or lower) than in Buzzards Bay. In contrast to BBP, the β-aminoglutaric to glutamic acid ratio shows no consistent trend with depth, The ratio is nearly constant at between 0.35 and 0.45 below 36 cm. Above this depth the ratio varies between 0.4 and 1.0, with maxima in the 12-18 and 30-36 cm sections.

DFAA concentrations in bottom water (see Table 5-2) were much lower than in surface sediments, by about 400 times at Station P in Buzzards Bay and by 25 times in the Pettaquamscutt River Estuary. Glycine was the major component in BBP bottom water, while alanine, glycine, serine, aspartic acid, glutamic acid, and β-aminoglutaric acid were all present in significant amounts in PRE bottom water.

Discussion

Remineralization:

The model calculations described in Chapter 4 were repeated for ammonia and total carbon dioxide data from BBP cores and for ammonia data from the PRE core. Because the effects of bioturbation are not included in the model, points above 10 cm were omitted from the calculation. Also, since the asymptotic concentration (C_∞) was not clearly approached at BBP, the fit of the equation was optimized for both C_∞ and k/w. The best-fit calculated profiles are shown for comparison with the actual

TABLE 5-3

Results of Remineralization Rate Calculations

Data Modelled	$k/w(cm^{-1})$	r^a
BBP6/79		
NH_3	0.017 (0.012 - 0.025)[b]	0.97
CO_2	0.011 (0.009 - 0.015)	0.99
TOC	0.045	0.89
THAA	0.041	0.89
BBP12/79		
NH_3	0.012 (0.009 - 0.024)	0.99
CO_2	0.018 (0.016 - 0.022)	0.998
TOC	0.049	0.91
THAA	0.049	0.85
PRE		
NH_3	0.018 (0.013 - 0.028)	0.94
THAA	0.028	0.90

[a]Linear correlation coefficient for fit of data to model.
[b]Given as: best value (range).

data in Figures 5-2, 5-3, and 5-4. The best estimates for k/w, with a range of values consistent with the data, are given in Table 5-3. At BBP, k/w's calculated for total carbon dioxide and ammonia profiles for the two sampling dates agree within the uncertainty of the calculation. The k/w's for the ammonia and total carbon dioxide profiles on each date are also not significantly different, despite the potential for non-adsorptive sinks for carbonate discussed in Chapter 4.

The PRE total carbon dioxide profile is inconsistent with model predictions and also quite different from the ammonia profile. Authigenic mineral formation is one possible explanation for the decreasing carbon dioxide concentrations below 50 cm. However, the decreasing carbon dioxide, sulfide and chlorinity in the same interval suggest alternative explanations. Seawater is believed to have first invaded the upper basin about 1700 ± 300 years B.P. (Orr and Gaines, 1974). For the estimated sedimentation rates for the upper basin (discussed later in this section), this invasion should have occurred before the sediments at the bottom of the PRE core were deposited. Thus the negative sulfide, chlorinity, and total carbon dioxide gradients are probably due to diffusion into an underlying stratum deposited before bottom water stagnation. Another possiblity is intrusion of ground waters into deeper sediments (A. Gaines, pers. comm.). Methanogenesis may also affect carbon dioxide in the PRE core. Sulfate was not measured in the PRE core, but Orr and Gaines (1974) found that sulfate was absent below 5 cm depth in another sediment core from the upper basin.

The sulfide profiles in this core are somewhat at variance with

expectations, however. Sulfide concentrations decrease over the upper 18 cm of the core, but then show a large concentration maximum from 24 to 42 cm. This feature is centered within the total carbon dioxide maximum, but does not correspond to other discernable anomalies in data from this core. Hites et al. (1980) measured $\delta^{13}C$ in PRE basin sediments. They found fairly constant $\delta^{13}C$ (-23.0 to -23.5°/oo) in the upper 20 cm of sediment, but between 20 and 40 cm there were two pronounced maxima in $\delta^{13}C$, -21°/oo and -22°/oo, centered at 24 and 38 cm, respectively. High sulfide concentrations are found in the depth interval between and including the two maxima. It is not clear whether this $\delta^{13}C$ feature is due to changes in the source of organic matter or in diagenetic processes in sediments (Hites et al., 1980).

The profiles calculated from the model fit the BBP data poorly in the upper 10 to 15 cm of sediment, perhaps due in part to the sediment-mixing activities of organisms. This explanation was proposed to account for near-constant sulfate concentrations in surface sediments of Long Island Sound sampled during the summer months (Goldhaber et al., 1977). However, the sediments in the 0-10cm depth interval at BBP are not well-mixed with respect to organic matter content (see Table 5-1). Also, excess Pb-210 activity measured in another core from this station (Farrington et al., 1977a) was uniform over only the first 3 cm and decreased by about a factor of two over the upper 10 cm. One possibility is that water is circulated much more rapidly than sediment, via the siphoning activities of benthic organisms (Aller and Yingst, 1978). The major bioturbation activity at BBP, however, is confined to the upper 3

cm (Rhoads, 1974). Also, PRE sediments show a similar anomaly in the ammonia profile, which could not be due to bioturbation.

In Chapter 4 it was noted that surface sediment dissolved ammonia and total carbon dioxide concentrations at Stations 4 and 5A were much in excess of those predicted by a model curve which fit points deeper in the core. Because macrofaunal populations in Peru sediments were small, mixing of pore waters high in ammonia and total carbon dioxide from deeper in the sediment toward the surface was not a likely explanation. The alternatives suggested, namely much higher rates of input due to degradation of more labile fractions of organic matter or to higher microbial activity, seem applicable to BBP as well. In addition, metabolic activities of meiofauna and macrofauna may contribute in BBP surface sediments.

An alternative calculation of the remineralization rate was carried out using the following equation:

$$A_T^z = A_m^z + A_i = A_m^o \exp(-kz/w) + A_i \qquad (5\text{-}1)$$

where A_T^z is the total sediment substrate concentration at depth a; A_m^z and A_m^o are the metabolizable substrate concentrations at depth z and the sediment surface; and A_i is the (constant) concentration of inert or unmetabolizable substrate. As was the case for the pore water calculations, this equation involves the assumptions that the remineralization rate is proportional to the metabolizable substrate concentration and that the rate of supply of the substrate to the sediment has been constant over time. The THAA and TOC data from the two BBP cores, and the THAA data from the PRE core, were fit to equation 5-1 and the resulting k/w's are given in Table 5-3. A_i was chosen to be con-

sistent with the lowest concentrations in the cores, and so the A_m^o (calculated from the model fit) plus A_i was approximately equal to the measured surface concentration. The model fit only the upper 30 cm of BBP6/79 well, because TOC and THAA are essentially constant below that depth. The results of fitting only the top 30 cm are given, and agree well with the results of the calculation for BBP12/79. For both BBP cores, k/w calculated from THAA and TOC agree closely, which was expected since the constant THAA/TN and TN/TOC ratios indicate that proteinaceous material, organic nitrogen, and organic carbon are all being remineralized at about the same rate in BBP sediments.

The k/w's calculated from the pore water and sediment data are of the same order, but the sediment k/w's are consistently larger. This is not surprising, since the pore water gradients which fit the model occurred in the deeper parts of the cores while the sediment data fit equation (1) best near the surface. It seems, therefore, that k is not constant with depth at BBP, and in fact is significantly greater in surface sediments. For PRE sediments, the k/w's calculated by the two different methods agree more closely.

Sedimentation rates for both the PRE and BBP have been reported in the literature. Farrington et al. (1977a) used excess Pb-210 activity to calculate a sedimentation rate of 0.30 cm/yr at BBP. Because of fairly intense bioturbation at this station, the actual sedimentation rate is probably somewhat less (Koide et al., 1976). Orr and Gaines (1974) measured C-14 activity in two cores of upper basin PRE sediments and found average sedimentation rates of 0.96 and 1.4 mm/yr over 150 cm.

Goldberg et al. (1977) used excess Pb-210 data to calculate a sedimentation rate of 3 mm/yr for the surface 30 cm of upper basin sediments; however, Pb-210 activities were very erratic in the top 10 cm of the core. The differences between the two rates may represent horizontal differences in sedimentation rate in the upper basin, or increased sedimentation rates in more recent times. The product of k/w and these sedimentation rates gives k's ranging from 0.005/yr to 0.015/year for BBP and 0.002 to 0.008/yr for the PRE. These rate constants are significantly lower than those found for the Peru Upwelling Region (see Chapter 4).

Inserting the calculated k/w for ammonia into equation 4-3 gives some interesting results. Exchangeable adsorbed ammonia in PRE sediments has been measured (Rosenfeld, 1979), and the ratio of adsorbed to dissolved ammonia was found to be 0.8. Thus $(1 + S\rho K) = 1 + 0.8\phi/(1-\phi) = 12.5$, for an average ϕ of 0.94, and for $\rho = 2.2$ g/cm^3, $k/w = 0.018$, $b = 6.1 \times 10^3$, $C_o - C_\infty = 1400$ μM, and $D_s = \phi^2 \times 13 \times 10^{-6}$ cm^2/sec, equation 4-3 reduces to:

$$12.5 = 0.612\ M_m^o - (6.6/w).$$

For w = 0.3 cm/yr (Goldberg et al., 1977), $M_m^o = 56$ mgC/gdw, which would imply the disappearance of 43 mgC/gdw over the length of the core. This is not unreasonable, although TOC actually increases slightly with depth, since the decrease in THAA indicates remineralization of at least 15 mgC/gdw of proteinaceous material. But for a sedimentation rate of 0.1 cm/yr, the calculated M_m^o is 130 mgC/gdw which requires remineralization of 100 mgC/gdw over the length of the core. Thus, the average sedimentation rate for the core examined in this study is probably closer

to 0.3 than 0.1 cm/yr.

Exchangeable ammonia has not been measured for BBP sediments, but for similar silty clays in Long Island Sound Rosenfeld (1979) measured adsorbed/dissolved ratios of 1 to 2. Assuming an intermediate value of 1.5 and an average ϕ of 0.68, $(1 + S\rho K) = 3.2$. For $\rho = 2.5$ g/cm^3, $C_\infty - C_o = 1000$, $k/w = 0.17$, $b = 8.2 \times 10^3$, and $D_s = \phi^2 \times 13 \times 10^{-6}$, equation 4-3 reduces to:

$$3.2 = 9.6\ M_m^o - (3.3/w).$$

For $w = 0.3$ cm/yr, $M_m^o = 1.5$ mgC/gdw, which is much less than the overall decrease in BBP6/79 TOC, but is in reasonable agreement with the 2 to 3 mgC/gdw decrease below 10 cm. If $k/w = 0.045$ calculated from the TOC data is used, M_m^o is calculated to be 3.3 mgC/gdw. This is still lower than the observed TOC decrease, but if the higher remineralization rate applied for the entire core, $C_\infty - C_o$ would also be larger and thus the equation above would not be correct. A sedimentation rate of 0.1 cm/yr still gives a reasonable calculated value of M_m^o, 3.7 mgC/gdw, for the lower part of the core. The BBP data is therefore consistent with sedimentation rates in the range from 0.1 to 0.3 cm/yr.

For the PRE, using a sedimentation rate of 0.3 cm/yr and a surface sediment organic carbon content of 120 mgC/gdw, the rate of accumulation of organic carbon is 47 gC/m^2-yr, as compared to the 8 gC/m^2-yr which is remineralized in the upper 10 cm of sediment. It is interesting to compare these numbers to the rate of oxidation of organic matter in the water column anoxic zone, 110 gC/m^2-yr, calculated by Orr and Gaines (1974) from the rate of sulfate reduction after an overturn event. At

BBP, for 20 mgC/gdw in surface sediments and a sedimentation rate of 0.2 cm/yr, the accumulation rate of organic carbon is quite similar, 32 gC/m^2-yr, as is the remineralization rate for the upper 10 cm of sediments, 8 gC/m^2-yr. Since both accumulation and remineralization rates are similar at BBP and PRE, it seems that the main reason for the striking difference in organic matter concentration in surface sediments is simply that PRE organic matter is diluted less by detrital aluminosilicates, and not that preservation of organic matter (as a whole) is enhanced by anoxic conditions in the PRE.

DFAA:

Higher DFAA concentrations in surface BBP sediments correspond to the higher rates of remineralization found in the upper 10 to 15 cm (as indicated by the excess ammonia and total carbon dioxide). Below 30 cm in core BBP6/79, where remineralization rates are too slow to produce consistent trends in THAA or TOC concentrations, the concentrations of DFAA are also essentially constant. The surface DFAA concentration maximum observed at BBP may be related to high microbial activity in surface sediments, or to redox conditions in sediments as discussed in Chapter 4. It is interesting that BBP DFAA concentrations decrease less sharply with depth than at Peru stations 4 and 6, and that the gradient in redox potential (judging by the distribution of sulfide) is also less steep. Also, like at Peru Station 5A, no surface maximum in DFAA was observed in the PRE core, which is overlain by anoxic bottom water.

Are the concentration differences observed between BBP6/79 and BBP12/79 in the 0 to 20 cm depth interval significant? Replicate squeez-

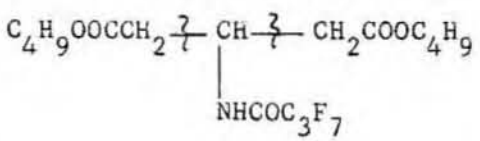
ings of four surface sediment grab samples and a 22-26 cm depth section agreed very closely (average difference 11 %, see Table 2-4), so a two-fold concentration difference is analytically significant. However, it is not certain whether this represents a change in sediment DFAA concentration over time, or short-distance variability in sediment composition. The DFAA content of four separate grab samples, one obtained on 6/26/79 and three on 12/5/79, showed no greater variability than the replicate squeezing (average DFAA concentration 54 ± 4 μM). The core surface sediment DFAA in both cases were somewhat lower, but problems with both these samples were mentioned earlier. Thus surface sediments show very little evidence of either seasonal or short-distance variability. In the other data from these two cores, the only differences in the upper 20 cm were in nitrate concentration (which probably varies on a seasonal basis with water column concentration) and THAA (this difference persisted to only 4 cm), with both being greater in BBP12/79. It is possible the the higher DFAA concentrations are related to these differences, but more intensive sampling and analysis of BBP sediments would be necessary to draw any conclusions.

Bottom water DFAA concentrations were much lower than those in sediments, and thus DFAA should diffuse out of the sediment into the water column (if DFAA in sediments are free to diffuse and not contained in cell membranes). The flux at BBP, neglecting the influence of bioturbation, would be about 0.026 μmoles/cm^2-day, for a 50 μM/cm linear gradient at the sediment-water interface. This gives a turnover time of about 6 days, which is greater than or equal to rates of microbial turn-

over of amino acids in estuaries (Crawford et al., 1974; Lee and Bada, 1977). However, tidal mixing is very strong in this part of Buzzards Bay (Roman and Tenore, 1978) and the water overlying this station probably has a residence time on the order of one day. Thus it is not surprising that the water column composition does not resemble the sediment DFAA composition. PRE bottom waters exchange relatively slowly, but there is no evidence that the microbial activity in the water column is less than in Buzzards Bay. The DFAA gradients at the sediment-water interface are lower, which gives a potential flux (if the DFAA are free to diffuse) of only 0.002 μmoles/cm^2-day. It therefore seems unlikely that the DFAA in PRE bottom water have a sediment source. The similarity in composition probably indicates that bacteria are a source of DFAA in the anoxic water column as well.

The DFAA in BBP sediments are made up primarily of glutamic acid and β-aminoglutaric acids. β-Aminoglutaric acid was first observed during this thesis work as an unknown peak in two pore water samples from the BBP6/77 core. The N-heptafluorobutyryl n-butyl ester derivatives of amino acid mixtures containing the unknown peak isolated from interstitial waters by cation exchange chromatography were subjected to GC/MS. Both chemical ionization (CI-CH_4) and electron-impact ionization (EI) mass spectra were obtained (Figures 5-9 and 5-10). The molecular ion at m/e 455 (confirmed by the presence of M + 1 = 456, M + 29 = 484, and M + 41 = 496) in the CI-CH_4 spectrum indicated that the unknown was an isomer of glutamic acid. The greater intensity of m/e 353 (= M - 102) relative to m/e 354 in the EI spectrum is characteristic of a

β-amino acid rather than an α-amino acid (Lawless and Chadha, 1971). The base peak at m/e 113 probably results from cleavage β to the amide N with loss of hydrogen:



The EI and CI-CH_4 mass spectra and GCGC retention time of authentic β-aminoglutaric acid (which was kindly provided by Drs. Alton Meister and Daniel Purich) were the same as those of the unknown compound. Those of the next most likely isomers of glutamic acid, α-methyl aspartic acid and β-methyl aspartic acid, are markedly different. β-Aminoglutaric acid, to my knowledge, has not been previously reported as a natural product or in geological materials other than interstitial water (Henrichs and Farrington, 1979).

For reasons discussed in detail in Chapter 4, it is probable that bacteria are the major source of dissolved free glutamic acid in sediments, and likely of other amino acids as well. The large relative abundance of β-aminoglutaric acid is still puzzling, however, in view of its apparently limited distribution in organisms. The ratio of β-aminoglutaric acid to glutamic acid increases with depth in sediment to about 30 cm and is very similar in BBP 6/79 and BBP12/79. Below 30 cm in BBP 6/79, there is no further change in the ratio. Remineralization is apparently also very slow below 30 cm relative to rates at the top of the core, and the total DFAA concentration is also constant over this interval. This is consistent with the proposal that the β-aminoglutaric/glutamic acid ratio increases because of relatively slower metabolism of

Figure 5-9: Chemical ionization mass spectrum of β-aminoglutaric acid from an interstitial water sample. Ionization potential, 130 eV; CH_4 reactant gas.

JL2501, SH, CICH4,130EV, SE52,7.5LBS
324 323+324-326-327

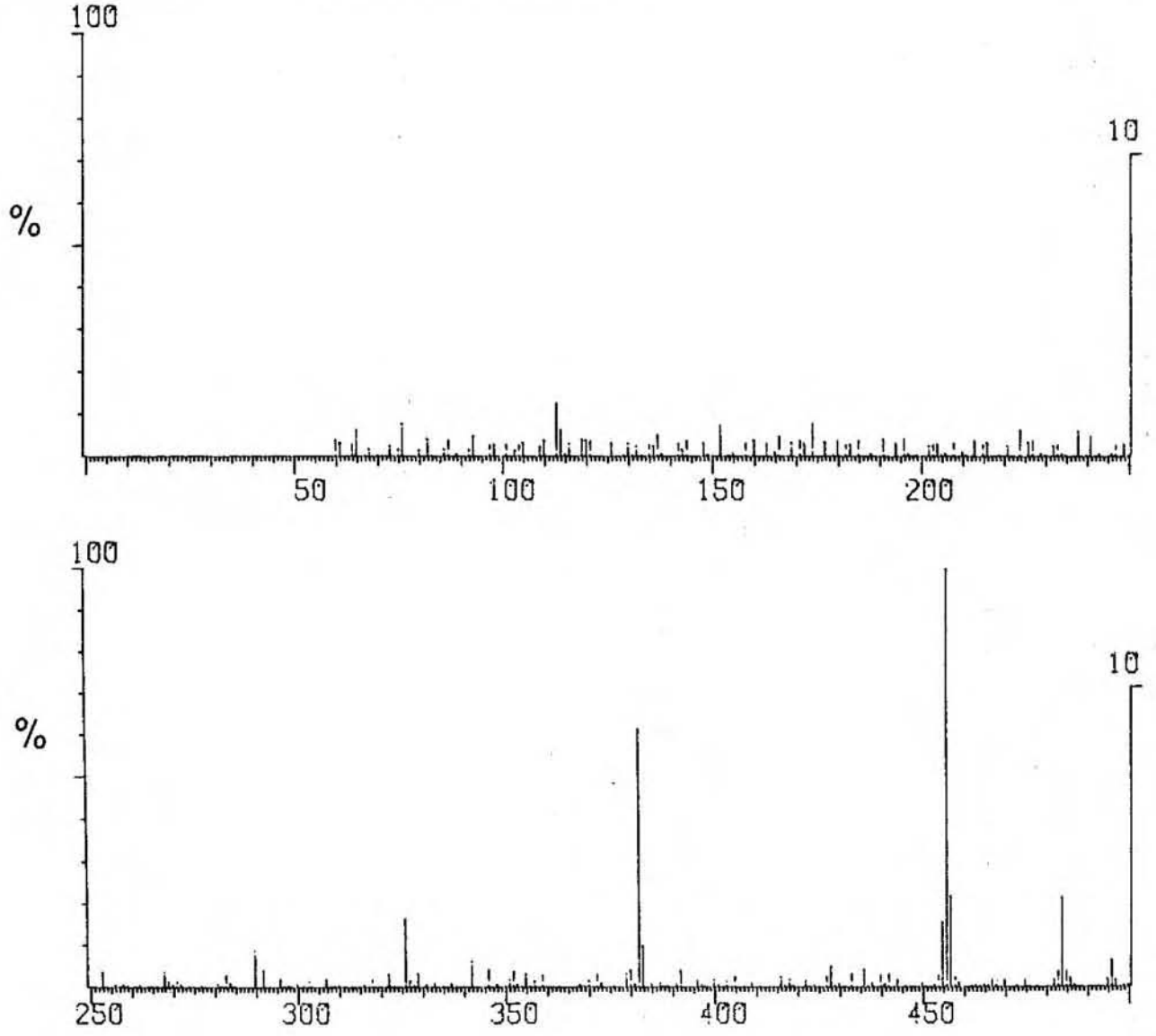
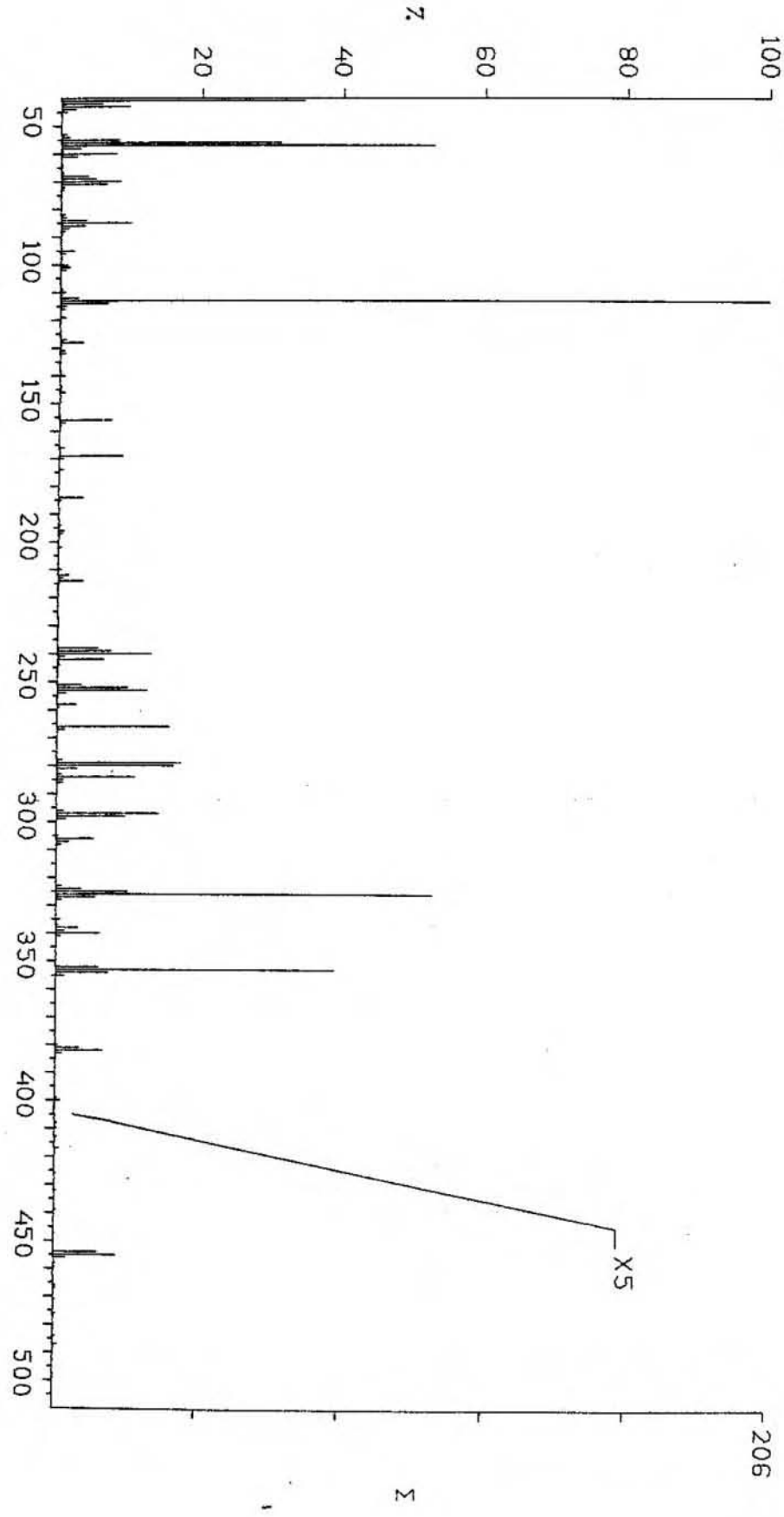


Figure 5-10: Electron - impact ionization mass spectrum of β-aminoglutaric acid from an interstitial water sample. Ionization potential, 70 eV.


SH10
SCAN 413
%
100
80
60
40
20
50
100
150
200
250
300
350
400
450
500
X5
206
M

β-aminoglutaric acid.

DFAA in the PRE again have relatively abundant glutamate and β-aminoglutarate, but in comparison to other highly anoxic sediments from Peru, other amino acids (mostly aspartic acid, alanine, and glycine) are more abundant. Also, in contrast to nearly every other sediment core analyzed for this thesis, the ratio of β-aminoglutaric acid to glutamic acid does not increase with depth. In the upper 15 cm the ratio increases; then there are several abrupt fluctuations before the ratio becomes fairly constant at approximately the surface value below 40 cm. As mentioned earlier in connection with the total carbon dioxide and sulfide profiles, the $\delta^{13}C$ values reported by Hites et al. (1980) also fluctuate in the depth interval between 20 and 48 cm, suggesting that diagenetic processes and/or sources of carbon were variable. The ratios near the bottom of the core are much lower than those at comparable depths from Buzzards Bay. The explanation for this is uncertain, but may be related to different types of bacteria in the two sediments, since all of the available sulfate may have been reduced in the PRE core.

Do the benthic meiofauna and macrofauna influence the DFAA concentrations and composition in BBP surface sediments, either by acting as a source or a sink? Benthic organisms, as for bacteria, could be a source of amino acids measured in pore waters if body fluids were extracted by the squeezing process or if they excreted dissolved free amino acids under normal in situ conditions. Analyses of DFAA in water extracts of tissue homogenates of Nepthys incisa (an abundant benthic organism at BBP) gave glycine as the major component (30 mole %), alanine, glutamic

acid, and aspartic acid at about 10 mole %, and a variety of other amino acids near 5 mole % (see Table 3-1). Awapara (1962) reviews the occurrence of free amino acids in marine invertebrate tissues. Glycine and the amino sulfonic acid taurine are abundant in many cases; alanine, aspartic acid, and glutamic acid are also present but usually in much lower concentrations. Total dissolved free amino acid concentration in the organisms was often in the range of 20 to 50 μmoles/gram tissue, much greater than in related terrestrial or freshwater animals; DFAA make up about one-half of the osmotic pressure in marine invertebrates. For the benthic biomass at Station P (about 12 gdw/m^2; Sanders, 1958), the free amino acid pool in macrofauna would be 0.3 μmole/cm^2, or if the macrofauna are limited to the upper 10 cm and the organism pool is converted to units of pore water concentration, about 40 μM. This is near the concentration measured in squeezed pore water samples, but it is unlikely that squeezing would be 100 % effective in extracting amino acids from organisms. The boiling-water extractions of three BBP surface sediment grab samples (see Table 2-8) gave DFAA concentrations (corrected for dilution) of 350 to 470 μM, and glycine was only 1/4 to 1/6 as abundant as glutamic acid, which probably came predominantly from bacterial cells. Thus it appears that on the order of 10 % of the total free amino acid pool in BBP surface sediments is in macrofauna. The variable amounts of glycine in squeezer extracts of these grab samples (see Table 2-4), ranging from 7 to 14 μM, might have come in part from random sampling of benthic invertebrates such as Nepthys incisa.

There is very little quantitative information on DFAA excretion rates

by benthic invertebrates. DeZwaan (1977) reviews the literature on anaerobic energy metabolism in bivalves. The main amino acid end product of such metabolism is alanine. Some end-products, e.g. acetate and propionate, are excreted when the organisms are returned to oxic conditions, but no significant excretion of alanine was observed. Potts (1967) cites rates from 0.1 to 6 mg amino acid N/100 g organism-day for various molluscs; it is not certain what fraction of this is a net loss to the organisms, since some portion may be recovered by uptake from the medium (see later discussion). Ferguson (1971) measured a net uptake of most amino acids by starfish from a 50 μM solution, but a net excretion of glycine, which is also the major free amino acid in starfish tissues. The rates were variable up to about 20 μmoles/100g organism-day. Johannes et al. (1969) found a net release of free amino acids by a marine turbellarian, 100 μg/g wet weight-day.

The majority of studies of the interaction between soft-bodied benthic organisms and DFAA in their media have shown a net uptake, however. Earlier work using radiotracers has been criticized on the grounds that short exposure times do not allow labelling of the "cold" internal pool, and thus loss of radiotracer from the medium does not necessarily represent net uptake if excretion is also present (Johannes et al., 1969). However, later studies in which the total DFAA content of the medium was monitored have also shown a net uptake in many cases. Phyla in which transepidermal uptake has been observed include Porifera, Coelenterata, Platyhelminthes, Annelida, Mollusca, Echinodermata, Brachiopoda, Pogonophora, and Hemichordata (Stewart, 1979). The uptake

is an active process, occurring against concentration gradients of a factor of 10 or greater.

Stephens (1975) studied uptake by two marine annelids, and found average rates of 0.8 μmoles/g wet weight-hr from an ambient concentration of 50 μM. Similar rates were found for natural porewaters (which had been extracted from sediments by pressure filtration) and solutions of known amino acids, except that one species (Nereis diversicolor) was unable to assimilate glutamate and aspartate to any significant extent, although able to assimilate other amino acids. Ferguson (1971) found uptake rates of 0.01 to 0.1 μmole/gww-hr from 50 μM alanine solutions for several Asteroidea (starfish). Stewart (1979) reports uptake rates of 0.3 to 0.6 μmoles/gww-hr of neutral and basic amino acids for isolated gills of Mya arenaria (most DFAA uptake in molluscs is via the gills) at concentrations near 100 μM. Aspartate, the only acidic amino acid tested, had a much lower uptake rate.

While such rates of uptake would be nutritionally significant to the organisms in question (Stephens, 1975; Southward and Southward, 1972), it is not certain whether these rates apply to animals in sediments, since the experiments have been conducted on isolated organisms in aquaria or isolated tissues in vitro. When experiments are conducted with whole organisms, it is not certain whether DFAA uptake is transepidermal or via the gut, except in organisms lacking a gut (Pogonophores) or in tissues, such as the epidermis, which lack adequate connection with internal sources of nutrition (Ferguson, 1971). This question makes no difference when considering the organisms as a sink for DFAA in pore water. A more

important consideration is whether uptake of DFAA occurs at comparable rates when they are not the sole available source of nutrition, as they are in the experiments described above. Also, if the amino acids measured as DFAA in squeezed samples are extracted from cells, then the rates observed at high dissolved concentrations would not be applicable to sediments. If however, the average uptake rate of about 0.5 μmoles/gww-hr applied to the entire benthic biomass at BBP (about 100 gww/m^2) to a depth of 10 cm, the uptake rate in surface sediments would be 0.7 μmoles/L-hr. For the average concentration in this depth interval of 25 μM, this maximum rate gives a turnover time of 1 to 2 days. While turnover times of dissolved amino acids have not been measured for Buzzards Bay sediments, Christensen and Blackburn (in press) measured turnover times of less than one hour for sediments from Limfjorden, Denmark. If turnover times at BBP are comparable, it appears that some other sink for DFAA, probably microbial uptake, is more important than uptake by benthic invertebrates.

Macrofauna may have an indirect effect on DFAA distributions in sediments, however. Stephens (1975) found that concentrations of dissolved free primary amines (which include DFAA) were increased substantially in artificial cores prepared from sediments which had been screened to remove macrofauna if organisms were reintroduced to the sediment. Surface sediment concentrations increased from 20 to 50 μM. Stephens attributed this change to irrigation of the sediment with overlying seawater by the organisms (annelid worms), since he was able to produce a similar effect by artificial irrigation. Stephens also

proposed that added oxygen supply to subsurface sediments was the important variable. Although the magnitude of this effect in undisturbed sediments is unknown, these results are interesting in terms of the earlier discussion (see Chapter 4) of the effect of oxygen on microbial amino acid production.

Adsorption is a potential sink for DFAA in sediments. Some information on the adsorption of amino acids by Buzzards Bay surface sediments was obtained in the water extraction experiments described in Chapter 2. The data from this experiment have been recalculated on a concentration basis and are given in Table 5-4. As described in Chapter 2, nonprotein amino acids were used in this experiment in an attempt to avoid biological uptake, as fresh, untreated sediments were used in these experiments. However, it is not certain that biological effects were avoided completely. Also, exposure times were short (15 to 30 minutes, including the filtration step), so slow reactions would not be observed.

The data obtained at three different concentrations for adsorption of norleucine, α-aminoadipic acid, and diaminobutyric acid from Sargasso Sea surface water (three parts to one part sediment) were fitted to the Freundlich adsorption equation:

$$\ln C_{adsorbed} = b \ln C_{dissolved} + \ln a$$

$$(\text{or } C_{adsorbed} = aC^{b}_{dissolved})$$

where C is concentration and a and b are constants. This model described the data very well, with r^2 greater than 0.97; the differences between the calculated and measured $C_{adsorbed}$ were close to analytical error (see Table 5-5). An attempt to fit the data to the Langmuir adsorption

TABLE 5-4

Adsorption of Amino Acids by Buzzards Bay Sediments

Sediment Sample	Aqueous Phase	Water/Sediment (wt/wt)	Norleucine Ads.[a]	Norleucine Diss.[b]	Diaminobutyric Acid Ads.	Diaminobutyric Acid Diss.	α-Aminoadipic Acid Ads.	α-Aminoadipic Acid Diss.
BBP12/79G-1								
	SSSW[c]	3:1	0.50	0.26	0.71	0.22	2.4	0.34
	SSSW:DW	3:1	0.39	0.41	0.28	0.63	2.4	0.37
	DW	3:1	0.29	0.49	0.00	1.1	2.0	0.70
	BDW	3:1	0.07	0.72	0.00	1.4	1.7	1.1
BBP12/79G-2								
	SSSW	3:1	0.44	0.34	0.57	0.36	2.4	0.38
	SSSW	3:1	1.7	6.2	2.4	6.9	15	12
	SSSW	3:1	8.8	55	8.4	85	85	180
	BDW	3:1	0.00	0.79	0.00	1.3	1.8	1.0
BBP12/79G-3								
	SSSW	3:1	0.45	0.34	0.56	0.37	2.5	0.33
	SSSW	10:1	0.26	0.53	0.52	0.41	1.7	1.0
	SSSW	30:1	0.21	0.59	0.49	0.44	1.8	1.0
	BDW	3:1	0.22	0.56	0.00	0.93	1.6	1.2
BBP12/79 11-14 cm								
	BDW	3:1	0.00	0.95	0.12	0.82	1.3	1.5
BBP12/79 29-32 cm								
	BDW	3:1	0.17	0.64	0.29	0.65	2.0	0.80
Freundlich Isotherm Coefficients:								
	a		0.80		1.0		4.1	
	b		0.56		0.47		0.57	
	r^2		0.977		0.999		0.997	

[a]Adsorbed concentration, units of μM(solution)/10 gww sediment.
[b]Dissolved concentration, units of μM.

TABLE 5-5

Comparison of Freundlich and Langmuir Isotherms

Relative Initial Concentration	Model	Adsorbed Concentration (μM/10 gww) Norleucine	Diaminobutyric Acid	α-Aminoadipic Acid
1	Data	0.46	2.4	0.61
	Freundlich	0.41	2.3	0.60
	Langmuir	0.15	0.70	0.21
10	Data	1.7	15	2.4
	Freundlich	2.2	17	2.5
	Langmuir	2.4	19	3.0
100	Data	8.8	85	8.4
	Freundlich	7.5	80	8.2
	Langmuir	6.8	78	7.8

model was much less successful (see Table 5-5). The data are limited, but biological uptake might be more likely to behave according to the Langmuir (i.e. Michaelis-Menton) model. The Freundlich isotherm is an empirical model which often fits adsorption data when the adsorption sites have a range of bonding energies with respect to the solute.

The coefficient "a" of the Freundlich model is related to the capacity of the adsorber for the sorbate, while "b" is related to the affinity of the sorbed species for the surface (Adamson, 1967). For all three amino acids, $b = 0.5 \pm 0.1$. But the coefficient "a" was four to five times greater for diaminobutyric acid than for α-aminoadipic acid or norleucine. This result may be related to Hedges (1978) finding that a basic amino acid (lysine) reacted much more rapidly than neutral (valine) or acidic (glutamic acid) amino acids with glucose to form melanoidin-type polymers, and that lysine-containing polymers were adsorbed by clay minerals to a much greater extent than other types. Like lysine, diaminobutyric acid has a net positive charge near pH 7, while α-aminoadipic acid is negatively charged and norleucine is neutral (Metzler, 1977). Positively charged species would interact with the negatively-charged exchange sites provided by clay minerals and humic substances (Parks, 1976; Schnitzer and Khan, 1972) in sediments, whereas adsorption sites for neutral to negative amino acids would be more limited (Hedges, 1978).

Some of the results of this experiment are not completely consistent with the simple adsorption model just discussed, however. All three amino acids were less adsorbed at lower salinities and at greater

water/sediment ratios, and even less in boiling distilled water (cooled before filtration). As discussed in Chapter 2, these results would be reasonable if biological uptake, rather than adsorption, were responsible for the loss of the amino acids from solution. But DOC concentrations (see Tables 2-8 and 2-9) also increased in the same order as adsorption decreased. If the amino acids were interacting primarily with a somewhat soluble fraction of TOC, and if the amino acids could be desorbed during cation exchange chromatography of the extracts, these results could be explained.

The nature of the adsorber was not investigated, but from other studies there is some indication that organic matter may be important. Hedges (1978) work has already been discussed. Abelson and Hare (1971) found slight (10 to 30 %) uptake of acidic and neutral amino acids by kerogens and humic substances from 100 μM solutions, but substantial uptake (70 to 100 %) of basic amino acids. From comparisons of adsorbed ammonia concentrations in sediments with different organic vs. clay contents, Rosenfeld (1979) concluded that much of the adsorbed ammonia might be bound to organic matter. If the adsorption process in fact involves interactions between DFAA and sediment organic matter, then it could represent a first step in the incorporation of DFAA into humic-type polymers.

The quantitative importance of adsorption as a sink for DFAA in Buzzards Bay sediments cannot be assessed precisely from these results because amino acid adsorption decreased with dilution, and thus some extrapolation from the 3:1 water:sediment ratio experiment is necessary.

However, the dilution effect was small for α-aminoadipic acid. If other acidic amino acids (aspartic acid, glutamic acid, and β-aminoglutaric acid) behave similarly (and if the measured concentrations in squeezed pore water are found outside cells in situ) then at 50 μM about 10 % of the total concentration would be adsorbed, while at 1 μM the adsorbed and dissolved concentrations would be equal. Adsorption of neutral amino acids would be comparable, although perhaps a factor of two greater because of the more pronounced decrease in adsorption with dilution. For basic amino acids, however, the adsorbed/dissolved ratio would be 0.8 at 50 μM, and 4 at 1 μM. Only very low concentrations of basic amino acids (lysine and ornithine) were observed in any pore water samples. But basic amino acids are also in very low abundance in bacterial pools, and so the low sediment DFAA concentrations probably also reflect low input.

Summary of Conclusions

(1) Remineralization rates and rates of accumulation of organic carbon are very similar at Buzzards Bay, Station P and in the Pettaquamscutt River Estuary. The large TOC concentrations in PRE sediments result from a lack of dilution by other phases, not from greatly enhanced preservation in the anoxic environment.

(2) Surface sediment DFAA concentrations are much higher at BBP than in the PRE. This may be related to the oxygen supply from the water column, which is lacking in the PRE.

(3) Uptake by macrofauna does not appear to be a major influence on DFAA concentrations at BBP, although it may be of significance to the

organisms.

(4) Adsorption of amino acids is significant relative to their total dissolved concentration, but probably is not responsible for the trends in concentration and composition seen in BBP sediments.

(5) Microbial processes are apparently responsible for most features of DFAA concentration and composition in PRE and BBP sediments.

CHAPTER 6

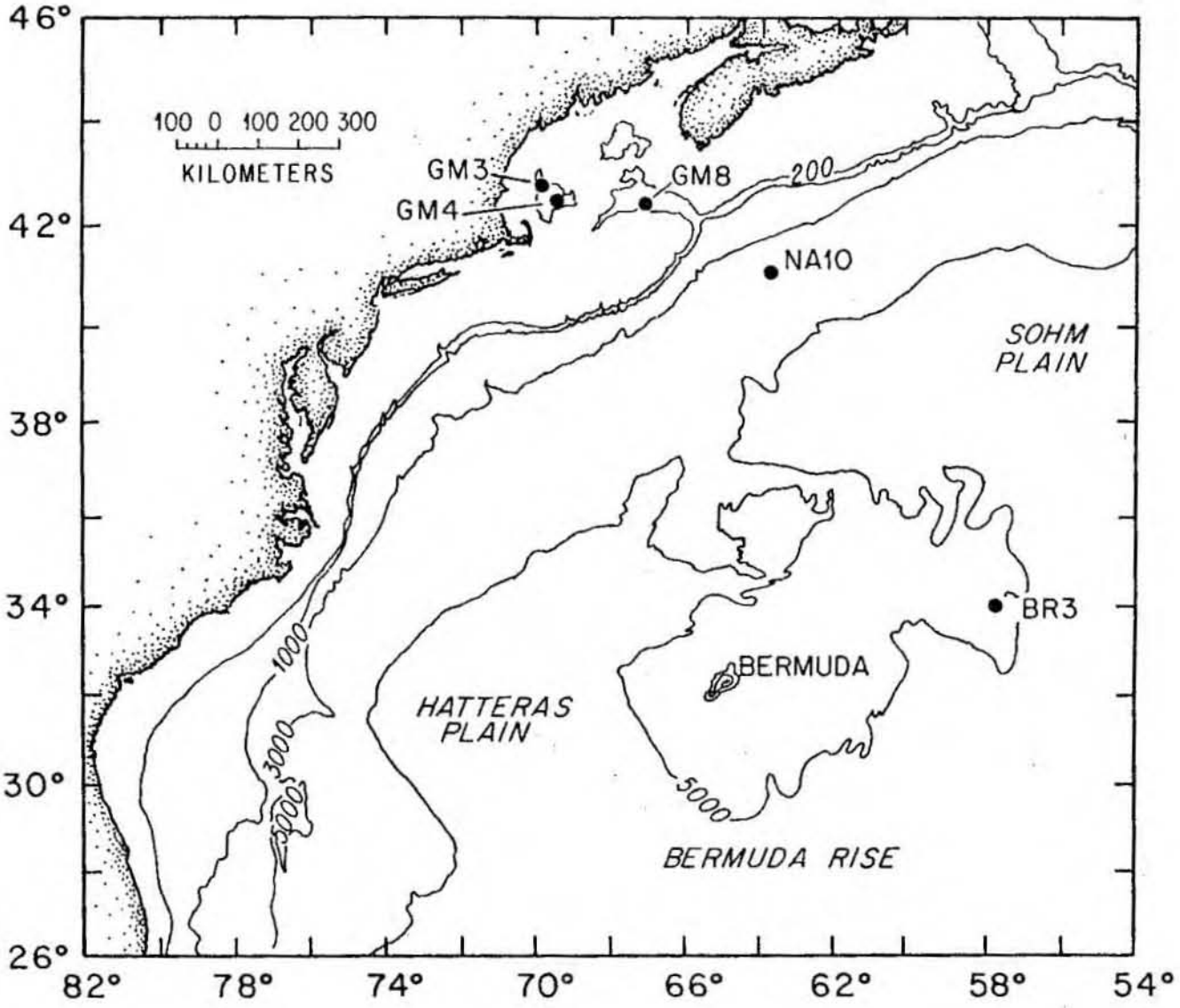
RESULTS FROM THE GULF OF MAINE, NORTHWESTERN ATLANTIC CONTINENTAL RISE, AND BERMUDA RISE

Introduction

The Gulf of Maine is a rectangular depression extending from Massachusetts to Nova Scotia and bounded on its seaward side by Georges Bank and the Scotian shelf. The bottom topography is complex, consisting of irregular, closed basins 64 to 377 m deep, separated by low swells and ridges. This topography is the result of a combination of fluvial erosion and subsequent glacial erosion and deposition during periods of lowered sea level (Uchupi, 1965; Schlee, 1973). Sediments in the basins originate from both pelagic-type inputs and current winnowing from topographic highs. Three basin sediment cores were obtained during September of 1977 on R/V Knorr Cruise 69, Leg 1. Two cores were from depths of 250 m (GM3) and 230 m (GM4) in the Wilkinson Basin. The third core (GM8) was obtained from 390 m depth in the Georges Basin. A fourth core (NA10) was from a depth of 4200 m on the continental rise east of the Gulf of Maine (see Figure 6-1 for station locations).

Cores were sectioned immediately and the subsamples refrigerated until squeezed (within 24 hours). Aliquots of interstitial water were analyzed on board for dissolved ammonia and sulfide. The remainder of the interstitial water samples were frozen and returned to Woods Hole, where they were stored for about 6 months before they were desalted and

Figure 6-1: Station locations, Knorr 69-1 and Oceanus 74.


46°
42°
38°
34°
30°
26°
82°
78°
74°
70°
66°
62°
58°
54°
100 0 100 200 300
KILOMETERS
GM3
GM4
GM8
NA10
BR3
BERMUDA
200
1000
3000
5000
SOHM PLAIN
HATTERAS PLAIN
BERMUDA RISE

analyzed for DFAA. Reanalysis of interstitial water samples from the Peru Upwelling Region which had been stored frozen for two years showed losses of amino acids in some samples compared to the composition of subsamples which were desalted immediately after squeezing. Thus, the composition of Knorr 69-1 samples may have changed during storage. However, the stored Peru samples showed very erratic distributions of DFAA concentration and composition with depth in core; in several samples β-aminoglutaric acid was the only DFAA remaining. The Gulf of Maine DFAA profiles are reasonably smooth and relative abundances of β-aminoglutaric acid are no larger than in comparable samples from other areas which were desalted immediately. Also, they were stored for a relatively short time. Therefore, while some changes may have occurred, they are probably not of major significance.

Two cores of Bermuda Rise sediments (BR3 A and B) were obtained during R/V Oceanus Cruise 74 in December of 1979. The station location is also plotted on Figure 6-1. The water column depth was 4900 m. The BR3 cores sampled a pinkish calcareous ooze, which contained abundant foraminifera. A manganese nodule was found at the sediment surface of BR3A and another in the 96-108 cm section of BR3B. BR3A was analyzed for DFAA, DOC, TOC, TN, THAA, and $\%H_2O$; BR3B was analyzed for dissolved nitrate, nitrite, ammonia, and total carbon dioxide. Cation exchange desalting of pore water for DFAA analysis, nitrate, nitrite, ammonia, and total carbon dioxide analyses were carried out on board. Frozen samples for other analyses were returned to Woods Hole. The BR3 coring location was close to the site of the giant piston core GPC-5 described by Laine

(1977) and Silva et al. (1976). Assuming equal sedimentation rates for the two sampling locations, the BR3 core sampled Holocene sediments; $CaCO_3$ content vs. depth in the two cores corresponds well considering the larger sampling intervals in GPC-5.

Results

TOC, TN, THAA, TN/TOC, THAA/TN, $\%H_2O$, and $\%CaCO_3$ for the GM3, GM4, GM8, and NA10 cores are shown in Table 6-1. TOC, TN, and THAA are remarkably uniform over 28 cm depth at GM3. THAA decrease by about a factor of two over 26 cm at GM4, but TOC and TN are more constant with depth. GM8 TOC, TN, and THAA are about half those at the shallower basin stations. Calcium carbonate content of all three basin sediments is low.

In contrast, the continental rise station, NA10, has very high calcium carbonate in surface sediments, between 30 and 40% in the upper 10 cm. These sediments consisted of abundant foraminifera in a reddish clay. Below 10 cm there was a sharp decrease on carbonate content. THAA decreased gradually with depth in the upper 10 cm, but suddenly by a factor of three between the 6-10 and 10-14 cm sections. TOC and TN were slightly higher in the 10-18 cm depth interval (which is equivalent to a slight decrease on a carbonate-free basis).

Only ammonia, sulfide, and DFAA were measured in pore water from sediments obtained on Knorr 69-1. Sulfide was detected only in the bottom core section at GM3 (a trace amount, less than 0.01 mM). The ammonia data is plotted in Figure 6-2. Ammonia concentration increased with depth at the three basin stations, but at GM4 was nearly constant in

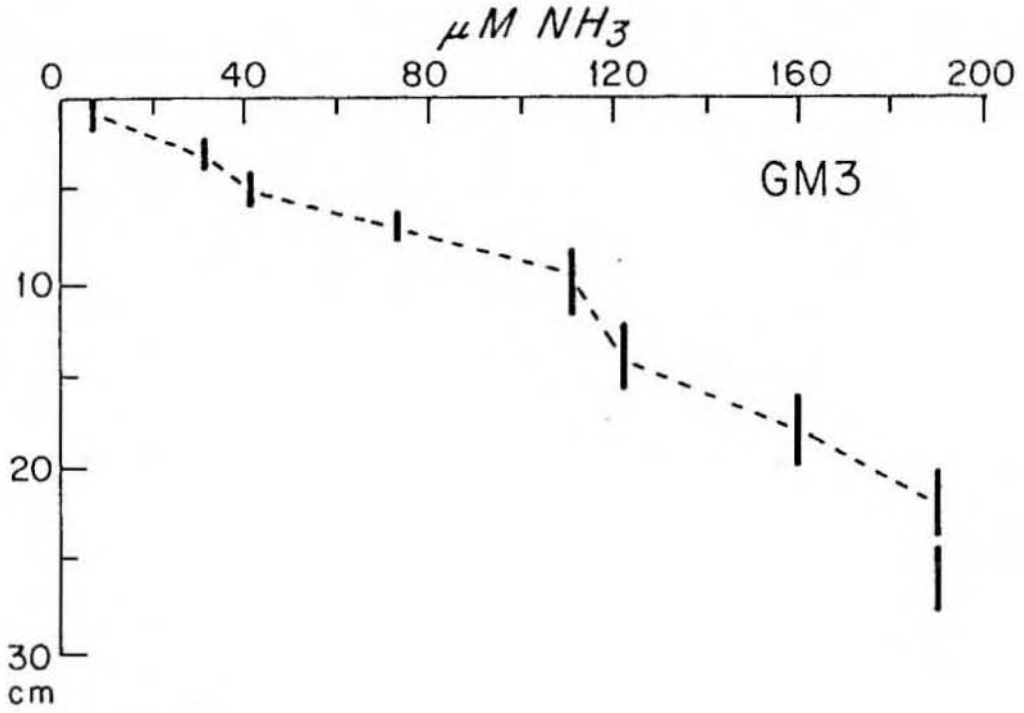
TABLE 6-1

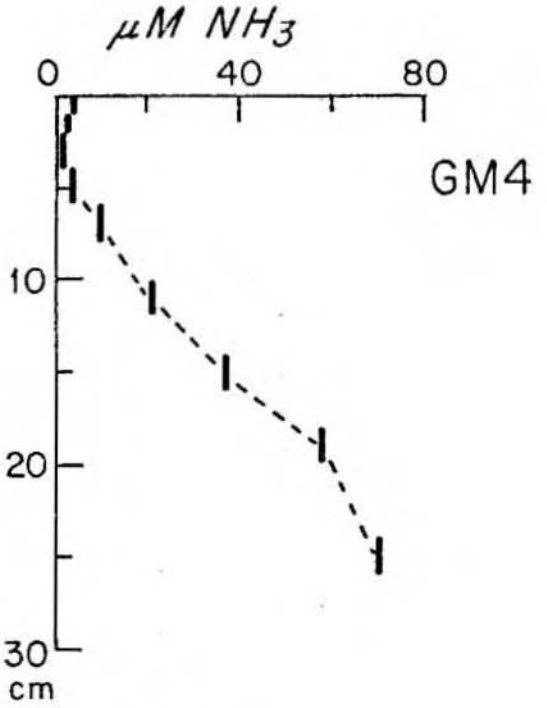
Composition of Sediments from the Gulf of Maine

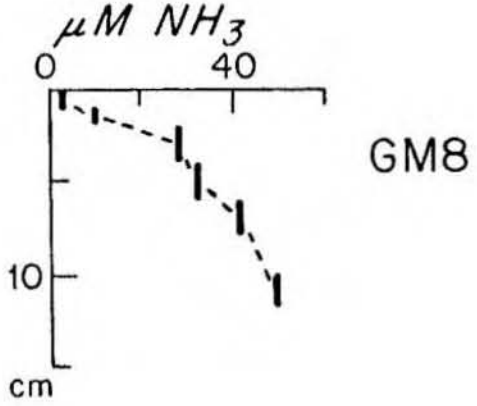
Station & Depth (cm)	TOC[a]	TN[b]	THAA[c]	TN/TOC[d]	THAA/TN[e]	%H_2O	%$CaCO_3$
GM3							
0-2	21	2.7	57	0.129	0.30	68.4	2
2-4	21	2.7	53	0.129	0.27	72.3	1
4-6	21	3.1	51	0.148	0.23	69.0	1
6-8	21	2.8	50	0.133	0.25	66.0	1
8-12	21	2.9	44	0.133	0.21	64.7	1
12-16	21	2.7	48	0.129	0.25	(lost)	2
16-20	21	2.4	43	0.114	0.25	64.6	2
20-24	21	3.0	53	0.142	0.25	64.7	1
24-28	21	2.8	50	0.133	0.25	64.2	1
GM4							
0-2	20	2.8	60	0.140	0.30	80.5	1
2-4	20	2.6	43	0.130	0.23	74.9	1
4-6	20	2.6	36	0.130	0.19	70.3	0
6-10	19	2.6	36	0.137	0.19	65.5	1
10-14	21	2.7	35	0.129	0.18	63.5	0
14-18	20	3.0	42	0.150	0.20	63.4	1
18-22	20	3.0	40	0.150	0.19	62.9	1
22-26	17	2.9	33	0.171	0.16	60.5	1
GM8							
0-2	8.1	1.4	19	0.173	0.19	43.0	2
2-4	8.1	1.2	28	0.148	0.32	41.7	3
4-6	8.7	1.2	18	0.138	0.21	42.4	2
6-10	7.3	1.1	13	0.151	0.17	39.7	2
NA10							
0-2	2.7	0.5	9.4	0.185	0.26	53.0	37
2-4	2.0	0.5	6.1	0.250	0.17	52.4	37
4-6	2.2	0.4	7.2	0.182	0.25	46.2	31
6-10	2.8	0.5	5.8	0.179	0.16	43.7	31
10-14	3.3	0.6	1.9	0.182	0.04	40.3	12
14-18	3.4	0.5	1.3	0.147	0.04	38.6	6

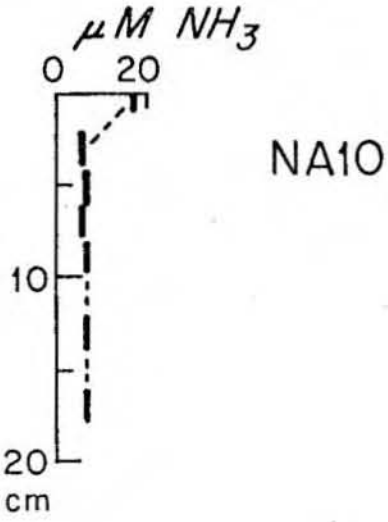
[a]Units of mgC/gdw.
[b]Units of mgN/gdw.
[c]Units of μmoles/gdw.
[d]Weight/weight.
[e]Weight N/weight N

Figure 6-2: Interstitial water ammonia concentrations, Knorr 69-1.


μM NH3
0
40
80
120
160
200
GM3
10
20
30
cm


μM NH3
0
40
80
GM4
10
20
30
cm


μM NH3
0
40
GM8
10
cm


μM NH3
0
20
NA10
10
20
cm

the upper 6 cm. Ammonia concentrations were higher at a given depth at GM3 than at GM4; GM8 ammonia was intermediate between the two shallower stations. The NA10 core had a slight ammonia maximum at the sediment-water interface, with constant 6 to 7 μM concentrations deeper in the core.

BR3 TOC, TN, THAA, DOC, $\%H_2O$, $\%CaCO_3$, and pore-water dissolved nitrate and total carbon dioxide data are summarized in Table 6-3, along with bottom water nitrate, total carbon dioxide, and DOC concentrations. BR3 had lower organic carbon content than any other core analyzed during this thesis work, varying from less than 1 to 4 mgC/gdw. There was no trend in TOC or TN with depth in sediment, but reproducibility of the organic carbon analyses was quite poor (± 50 %) for the samples containing less than 1 mgC/gdw. The material was apparently quite heterogeneous for the 50 mg sample sizes used (P. Clarner, pers. comm.). THAA analyses on larger (1 gram dry weight) samples gave less erratic results. THAA contents were also low; in surface sediments they were similar to concentrations in the 10-18 cm section of NA10. THAA decreased rapidly with depth over the upper 48 cm of the core, by almost a factor of 20. From 40 to 144 cm THAA content fluctuated between 0.13 and 0.35 moles/gdw. These variations in THAA concentration and their relative magnitude are strongly correlated with variations in $CaCO_3$ content in this depth interval. The 20-fold decrease in THAA over the upper 48 cm is accompanied by a 3-fold decrease in $CaCO_3$ content.

Within the analytical precision of the total carbon dioxide data (0.1 mM), there is no net increase with depth but fairly substantial variabil-

TABLE 6-2

Composition of Bermuda Rise Sediments and Interstitial Water

Depth in Core (cm)	TOC[a]	TN[b]	THAA[c]	NO_3^- (μM)	Total CO_2 (mM)	DOC (mgC/L)	$\%H_2O$	$\%CaCO_3$
BR3								
0-6	1.9	0.2	2.8	28	2.6	3.6	42.8	50
6-12	0.7	0.2	1.3	30	1.9	2.9	43.6	33
12-18	3.7	0.2	0.87	30	1.8	2.3	46.0	26
18-24	0.7	0.1	0.48	30	2.4	3.4	45.9	19
24-30	0.4	0.1	0.37	30	2.2	1.7	45.4	16
30-36	0.9	0.1	0.38	32	2.0	1.7	44.9	16
36-42	0.7	0.1	0.29	30	2.3	2.4	44.9	17
42-48	0.9	0.1	0.29	33	2.3	2.9	45.4	28
48-54	0.8	0.2	0.16	31	2.6	2.2	43.2	15
54-60	1.2	0.3	0.35	32	1.8	2.6	44.9	19
60-66	1.9	0.2	0.33	31	2.6	2.6	45.4	21
66-72	0.7	0.4	0.13	32	2.7	2.5	45.5	9.7
72-84	0.9	0.2	0.27	31	2.3	4.4	44.9	25
84-96	1.4	0.1	0.28	33	2.4	4.7	45.5	33
96-108	0.4	0.3	0.16	32	2.8	5.8	48.0	9.9
108-120	1.9	0.2	0.34	36	2.5	2.7	46.3	30
120-132	1.1	0.2	0.26	32	2.5	6.0	45.2	24
132-144	1.1	0.1	0.25	33	2.8	3.9	43.3	25
Bottom Water				21	2.3	0.6		

[a]Units of mgC/gdw.
[b]Units of mgN/gdw.
[c]Units of μmoles/gdw.

ity. This variability suggests that pore water solutions underwent some re-equilibration with calcite due to decreased solubility at sea-surface as opposed to in situ temperature and pressure (Manheim, 1976). The nitrate concentration data has an analytical precision of about 1 μM. Thus, surface sediment interstitial water nitrate concentrations are significantly greater than bottom water concentrations and there is a slight (4 to 5 μM) increase with depth in sediment. Nitrite concentrations were less than 0.2 μM. Ammonia concentrations measured in BR3 pore waters were much greater than expected and also highly variable (from 15 to 150 μM). It was belatedly discovered that the cellulose filter papers used in the squeezer (Whatman #541 hardened ashless) leached significant amounts of ammonia (about 0.2 μmoles/ filter), which for a typical sample size would amount to a concentration of 30 μM. (These filters were not used for any other sets of samples, so previously discussed ammonia data is not affected). Because of variabilty in sample size and probably also in filter composition, the data could not be corrected and will not be included in the discussion. DOC concentrations in BR3 interstitial water are low, between 1 and 6 mgC/L, but greater than the bottom water concentration of 0.6 mgC/L. DOC decreases with depth to 30 to 36 cm but then increases somewhat irregularly to the bottom of the core.

The composition of THAA from Gulf of Maine sediments is summarized in Table 6-3. As before, the mole % compositions of all core sections from each station have been averaged, and the mean and standard deviation are reported. GM3, GM4, and GM8 average compositions are not significantly

TABLE 6-3

Composition of Gulf of Maine Total Hydrolyzable Amino Acids

	Mole %							
	GM3		GM4		GM8		NA10	
Amino Acid	Mean	s	Mean	s	Mean	s	Mean	s
ala	11.6	1.2	12.6	0.6	11.3	1.4	14.9	2.5[a]
gly	21.1	1.4	23.6	2.4	21.8	1.1	20.7	0.5
val	4.7	0.5	4.5	0.7	4.7	0.2	5.9	0.3
β-ala	2.1	0.2	2.4	0.4	1.8	0.2	3.4	1.4[a]
thr	6.6	0.4	5.7	0.7	6.5	0.7	6.4	1.5[b]
ser	11.1	0.7	10.0	0.6	10.8	0.8	9.7	0.5
leu	4.3	0.4	4.2	0.6	4.5	0.3	3.8	0.8[b]
ile	2.3	0.2	2.1	0.3	2.4	0.1	2.3	0.6[b]
γ-aba	0.7	0.2	0.8	0.2	0.5	0.2	1.3	0.6
pro	4.7	0.5	5.3	0.3	5.8	0.5	4.2	0.4
hypro	0.8	0.1	0.9	0.04	1.0	0.3	1.0	0.2
asp	14.3	0.8	13.0	1.1	12.7	1.2	12.9	0.6
phe	2.6	0.3	2.4	0.4	2.4	0.3	2.5	1.0
glu	7.8	0.6	7.4	0.7	8.0	0.5	6.3	1.1
lys	3.0	1.0	3.3	1.2	3.9	2.0	3.7	1.1
tyr	1.4	0.2	1.1	0.3	1.6	0.06	---[c]	

[a]Increased slightly in relative abundance with depth in NA10 core.
[b]Decreased slightly in relative abundance with depth in NA10 core.
[c]Not resolved from lysine.

TABLE 6-4

Total Hydrolyzable Amino Acid Composition of Bermuda Rise Sediments

Depth (cm)	Mole % Composition								
	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48	48-54
Amino Acid:									
ala	11	12	10	9.4	3.8	4.7	6.4	11	5.8
gly	18	21	15	15	9.1	7.6	8.9	13	6.9
val	6.5	---[a]	5.7	---[a]	1.8	3.0	6.2	5.1	2.7
β-ala	17	14	13	29	31	29	28	23	33
thr	4.6	6.9	3.9	3.6	1.4	2.6	4.0	---	---
ser	3.2	5.6	2.8	2.5	---	2.2	---	---	---
leu	2.1	3.4	2.2	1.8	1.4	1.0	1.2	2.1	1.6
ile	1.7	2.2	1.9	1.4	---	0.8	0.9	2.1	---
γ-aba	7.9	3.1	17	23	42	40	35	23	36
pro	4.4	4.4	3.8	1.9	2.0	2.0	2.1	3.2	1.8
hypro	1.3	1.3	1.0	0.4	---	0.6	---	---	---
asp	12	14	12	7.2	5.6	3.4	3.9	10	7.2
phe	1.8	2.2	1.5	---	---	---	---	---	---
glu	6.9	7.3	6.9	3.7	4.3	3.2	3.5	6.8	5.0
lys+tyr	2.8	2.9	3.2	---	---	---	---	---	---
% Surface THAA:									
	100	46	31	17	13	14	10	10	6
% Surface $CaCO_3$:									
	100	66	52	38	32	32	34	56	30

	54-60	60-66	66-72	72-84	84-96	96-108	108-120	120-132	132-144
Amino Acid:									
ala	6.8	7.2	6.3	6.4	10	2.6	9.6	8.1	7.5
gly	16	11	8.5	11	13	11	10	11	8.9
val	---[a]	---[a]	---[a]	---[a]	---[a]	---[a]	5.2	3.8	3.5
β-ala	29	32	39	28	24	35	18	23	24
thr	3.0	2.6	---	---	1.9	---	2.3	---	2.4
ser	3.8	1.8	---	1.9	2.2	---	2.6	1.3	1.4
leu	2.1	1.6	1.2	1.4	2.1	---	2.7	2.2	1.9
ile	1.1	1.1	---	0.9	1.5	---	1.8	1.3	1.0
γ-aba	24	28	30	36	21	39	24	34	37
pro	2.8	2.9	3.2	2.6	3.9	3.2	4.9	3.0	2.8
hypro	1.5	0.8	---	0.7	0.7	---	0.8	0.9	0.7
asp	5.0	4.5	4.3	5.6	11	4.9	8.2	6.2	4.7
phe	---	---	---	---	---	---	0.6	---	---
glu	5.1	4.2	5.4	4.5	7.2	5.0	7.5	4.9	4.7
lys+tyr	---	1.8	2.2	---	0.9	---	0.9	0.5	---
% Surface THAA:									
	13	12	5	10	10	6	12	9	9
% Surface $CaCO_3$:									
	30	42	19	46	66	20	60	48	50

[a]Not resolved from β-alanine.

different from each other or from the compositions of Peru, BBP, and PRE sediments. Likewise, the variability in composition within cores is close to analytical precision and shows no consistent trends. The high-carbonate NA10 sediment has a slightly different THAA composition from the basin sediments. Alanine and β-alanine increase and leucine, isoleucine, and threonine decrease slightly with depth in this core. The THAA composition of the BR3 core (Table 6-4) is different from any other sediment analyzed for this thesis, with very high relative abundances of the non-protein amino acids β-alanine and γ-aminobutyric acid. The identities of these peaks have been confirmed by mass spectrometry (Figures 6-3a and 6-3b).

DFAA concentrations in cores from GM3, GM4, GM8, and NA10 are plotted in Figures 6-4a, 6-4b, and 6-4c. Surface sediment concentrations range from about 15 to 50 μM. Concentrations tend to decrease with depth in all four cores, although not smoothly.

DFAA mole % composition for GM3, GM4, GM8, and NA 10 is given in Table 6-5. Only the major amino acids are shown; small amounts of valine, β-alanine, threonine, leucine, isoleucine, proline, phenylalanine, lysine, and tyrosine were present in some or all of the samples. Serine was a major constituent of DFAA only in the NA10 core, which also had much less β-aminoglutaric acid than the other samples and somewhat less glutamic acid. In the three basin sediments glutamic acid was almost always the most abundant amino acid. Below 10 cm, β-aminoglutaric acid was the second most concentrated, but above 10 cm alanine or glycine concentrations were often greater. β-Aminoglutaric acid

Figure 6-3a: Mass spectrum of β-alanine from BR3, 30-36 cm total hydrolyzable amino acids.

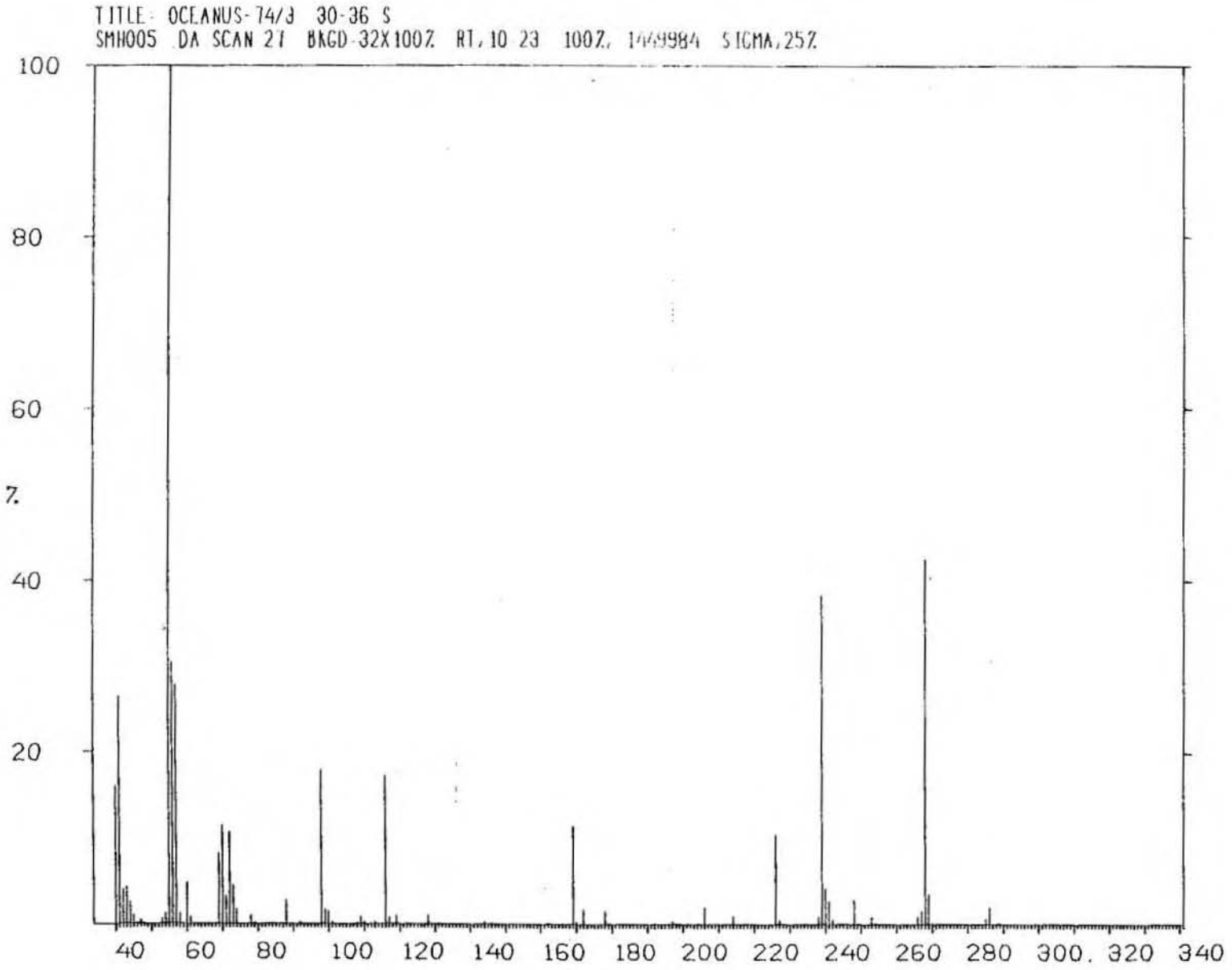

TITLE: OCEANUS-74/3 30-36 S
SMH005 DA SCAN 21 BKGD-32X100% RT, 10 23 100%, 1449984 SIGMA, 25%
100
80
60
%
40
20
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340

Figure 6-3b: Mass spectrum of γ-aminobutyric acid from BR3, 30-36 cm total hydrolyzable amino acids.

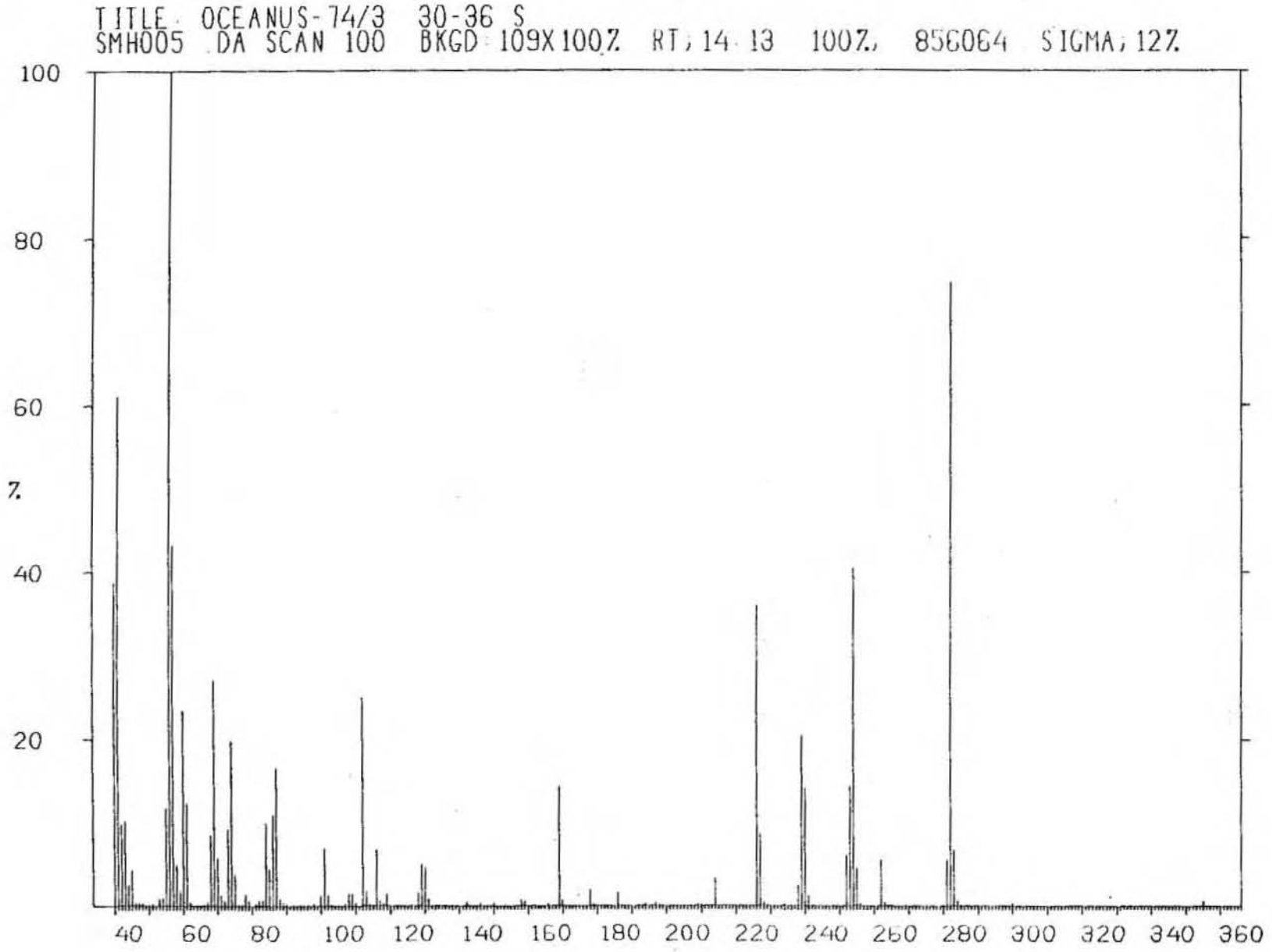

TITLE OCEANUS-74/3 30-36 S
SMH005 DA SCAN 100 BKGD 109X100% RT; 14 13 100%, 856064 SIGMA; 12%
100
80
60
%
40
20
40 60 80 100 120 140 160 180 200 220 240 260 280 300 320 340 360

Figure 6-4*a*: Dissolved free amino acid concentrations in GM3 sediments.

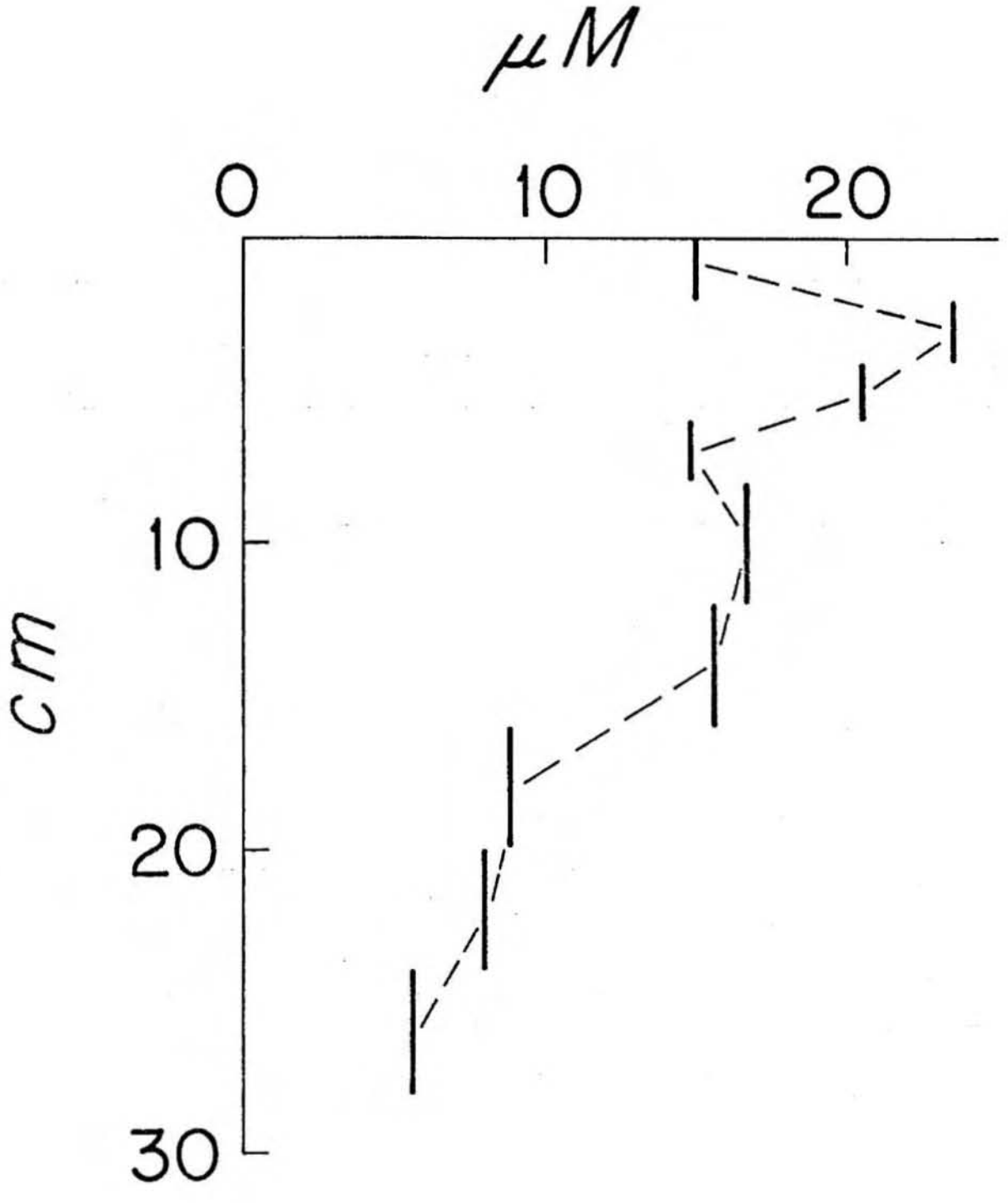

μM
0
10
20
cm
10
20
30

Figure 6-4b: Dissolved free amino acid concentrations in GM4 sediments.

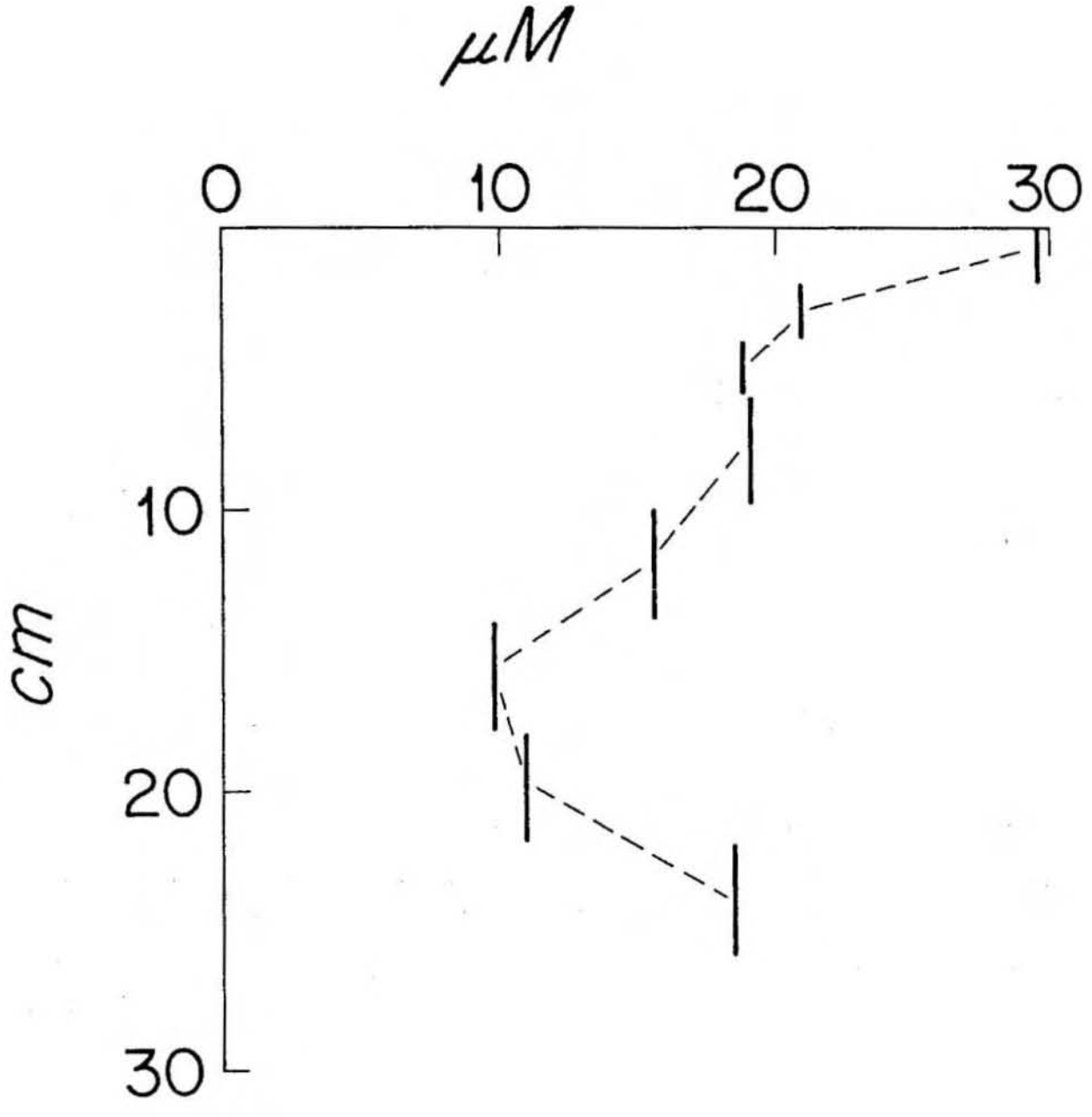
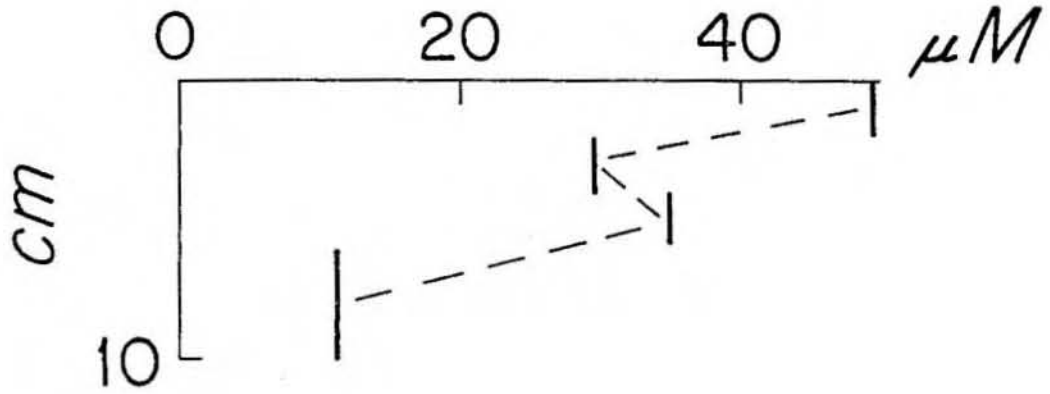

μM
0
10
20
30
cm
10
20
30

Figure 6-4c: (Top) Dissolved free amino acid concentrations in GM8 sediments.

(Bottom) Dissolved free amino acid concentrations in NA10 sediments.


0
20
40
μM
cm
10

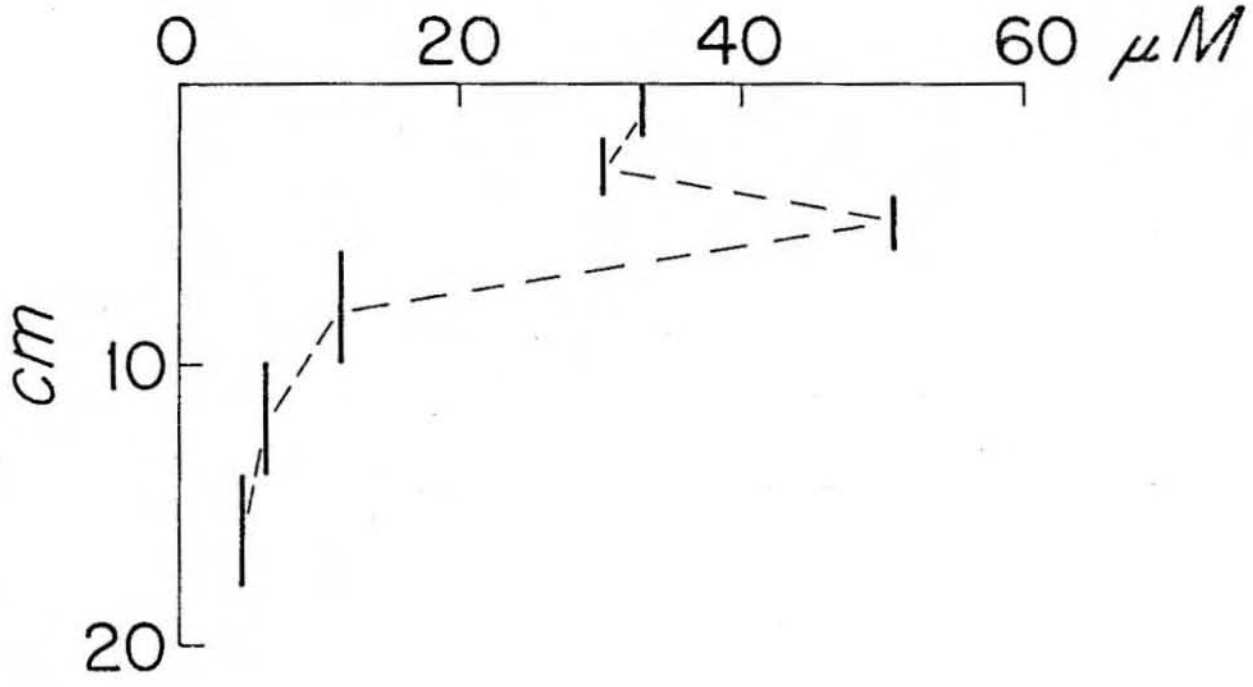

0
20
40
60
μM
cm
10
20

TABLE 6-5

Dissolved Free Amino Acid Composition of Gulf of Maine Sediments

Station & Depth in Core (cm)	Mole %					
	Ala	Gly	Asp	Glu	β-Glu	Ser
GM3						
0-2	4.6	13	5.5	48	17	
2-4	3.7	4.7	3.0	52	25	
4-6	2.9	2.7	2.9	56	27	
6-8	4.0	7.4	3.0	50	24	
8-12	3.3	5.9	3.8	47	26	
12-16	5.4	5.1	5.7	33	21	
16-20	3.4	4.4	3.4	47	31	
20-24	3.8	3.3	4.6	48	32	
24-28	3.0	3.2	3.9	47	37	
GM4						
0-2	6.1	22	5.8	41	13	
2-4	6.2	8.1	5.3	52	17	
4-6	4.0	7.9	4.3	54	24	
6-10	12	20	4.3	36	17	
10-14	4.3	9.6	4.2	47	28	
14-18	3.3	4.5	4.4	46	32	
18-22	5.9	7.3	6.7	42	28	
22-26	6.0	27	3.3	19	12	
GM8						
0-2	7.7	41	3.8	21	6.7	
2-4	6.4	16	4.1	37	17	
4-6	12	39	2.9	23	11	
6-10	5.7	12	3.9	33	26	
NA10						
0-2	16	20	9.7	18	2.5	7.5
2-4	47	7.0	4.6	13	2.0	4.5
4-6	30	20	4.7	11	1.4	4.7
6-10	12	37	4.4	20	3.1	5.0
10-14	11	18	7.3	9.8	1.5	29
14-18	12	20	6.7	4.8	---	37

Figure 6-5: Free β-aminoglutaric acid/glutamic acid ratio vs. depth in Gulf of Maine sediments. o = GM3, • = GM4, Δ = GM8, ▲ = NA10.

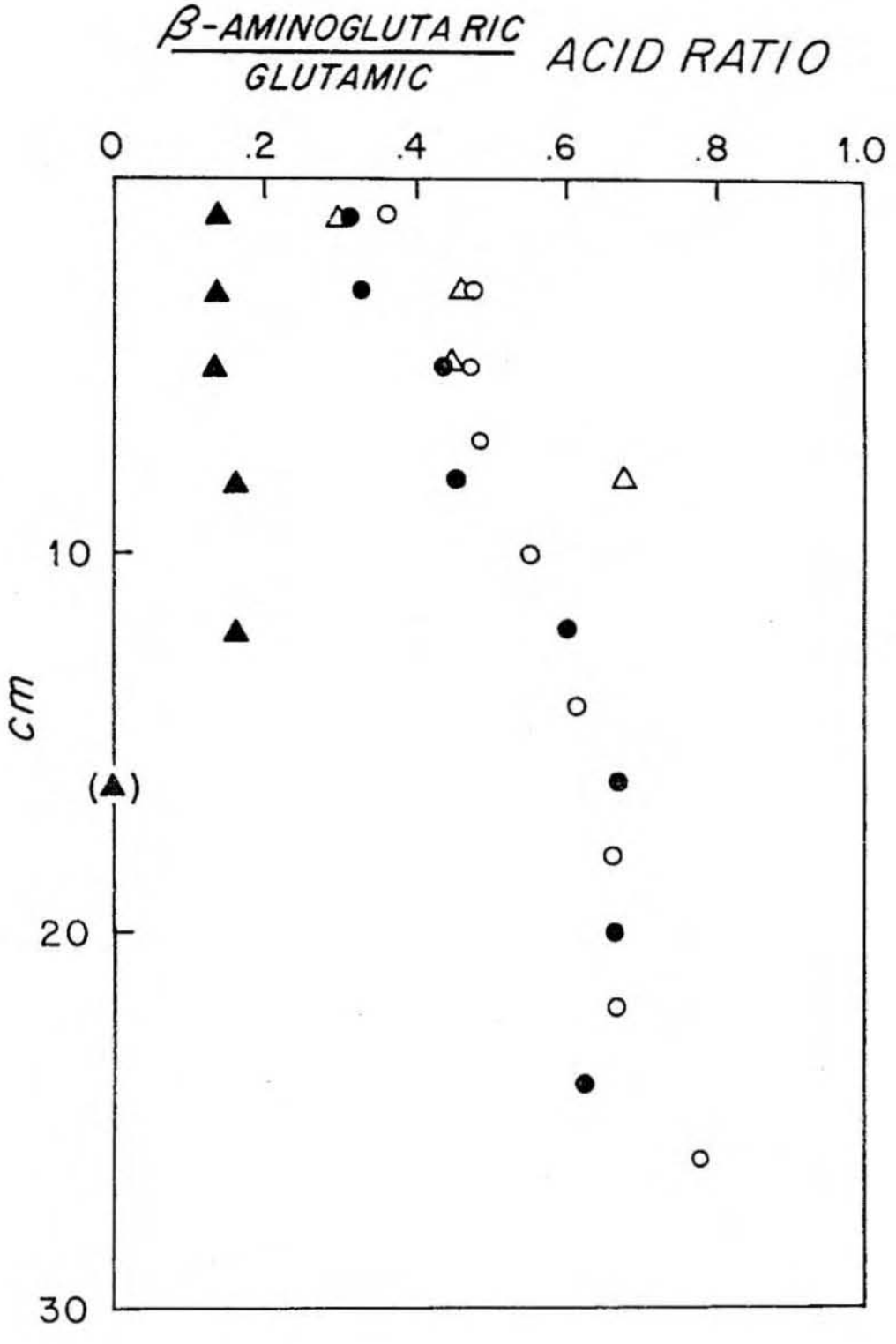

β-AMINOGLUTARIC ACID RATIO
GLUTAMIC
0
.2
.4
.6
.8
1.0
10
20
30
cm
(▲)

TABLE 6-6

Dissolved Free Amino Acids in Bermuda Rise Sediments

Amino Acid	Mole %	
	0-6 cm	6-144 cm[a]
ala	12	---
gly	23	---
val	13	---
thr	4.1	---
ser	---	---
leu	3.2	---
ile	2.6	---
asp	9.6	---
phe	2.1	---
glu	25	---
β-glu	2.0	---
lys + tyr	2.3	---
TOTAL (μM)	0.9	<0.3

[a]For depth intervals see Table 6-2

became more abundant relative to glutamic acid in all cores with depth (Figure 6-5), but the increase at NA10 was small, and no β-aminoglutaric acid could be detected in the 14-18 cm section.

Selected DFAA and THAA samples from the Gulf of Maine were subjected to GCGC on a Chirasil-Val$^{(R)}$ column, which resolves amino acid enantiomers. No significant quantities (more than 5 % of major peaks) of D-amino acids were present.

DFAA could be measured only in the 0-6 cm section of core BR3, which had a total concentration of 0.9 μM (Table 6-6). Glutamic acid, glycine, valine, alanine, and aspartic acid were the major components, with traces of other amino acids. The average concentration, range of concentrations, and compositions of the remaining core sections were very similar to those of cation exchange chromatography blanks run aboard Oceanus 74, and thus total DFAA concentrations below 6 cm were no more than 0.3 μM.

Discussion

Remineralization:

The ammonia data from the GM3 and GM 4 cores were modelled using equation 4-1. GM4 data was fit below 5 cm only. The calculated k/w's were 0.04 and 0.02 cm^{-1} for GM3 and GM4, respectively. From equation 4-3, and setting M_m^o = 10 mgC/gdw (which is equivalent to assuming the remineralization of 3 mgC/gdw at GM3 and 5 mgC/gdw at GM4), the ammonia profiles are consistent with sedimentation rates of 0.05/yr at GM3 and 0.02 cm/yr at station GM4. However, these are minimum rates, calculated under the assumption that the end-product of remineralization of organic

nitrogen compounds is ammonia. At GM4, in particular, ammonia does not begin to accumulate until 4-6 cm depth. The low values in surface sediments could be the result of bioturbation and/or nitrification.

Repeta (unpublished ms.) measured Pb-210 activity in GM4 sediments. There was an initial rapid decrease in Pb-210 activity to 10 cm depth, followed by a slower decrease attributed to the decay of the Ra-226 supporting Pb-210. The sedimentation rates calculated for the two activity vs. depth gradients were 47 and 1 cm/1000 yr, respectively. However, Repeta (after Koide et al., 1976) interpreted the excess Pb-210 distribution above 10 cm depth to be the result of biological mixing of surface sediments, so that 1 cm/1000 yr is the correct sedimentation rate. But w = 1 cm/1000 yr is not consistent with the ammonia profile for any reasonable adjustments of the parameters in equation 4-3. The two data sets could be reconciled if the sedimentation rate at GM4 were in fact more rapid in recent times, or if sedimentation were discontinuous.

THAA in BR3 Sediments:

Only small amounts of nitrate accumulate with depth in the BR3 core, although a pronounced decrease in THAA content occurs in the upper 48 cm. An estimate for k/w of 0.07/cm for the upper 48 cm was calculated from the THAA data using equation 5-1. This value of k/w would be consistent with the low nitrate accumulation (according to equation 4-3) for a sedimentation rate between 2 and 10 cm/1000 yr. This may be an overestimate, however. The positive correlation between calcium carbonate and THAA content suggests that part of the decrease in THAA with depth could be due to a decreasing input rate associated with lower rates of carbon-

ate sedimentation. The correlation could also arise from variations in the degree of dissolution of calcium carbonate in sediments, since proteins in intact foraminiferal tests are protected from microbial attack (Schroeder and Bada, 1976).

The composition as well as the total concentration of THAA in the BR3 core is related to the calcium carbonate content of the sediment. High relative abundances of γ-aminobutyric acid and β-alanine are associated with the calcite and THAA minima (see Table 6-4). Schroeder (1974) proposed that the origin of β-alanine and γ-aminobutyric acid in deep-sea sediment cores was via the decarboxylation of glutamic acid and aspartic acid during diagenesis, probably by bacteria. In core BR3, however, an equally plausible hypothesis is that β-alanine and γ-aminobutyric acid are present in the sedimented material, and increase in relative abundance down core because they are less readily metabolized than protein amino acids, since the absolute concentrations in surface sediments are much higher than those at depth. Also, the concentrations of β-alanine and γ-aminobutyric acid in NA10 surface sediments (0.5 μmoles/gdw, together) are close to the concentration in BR3 surface sediments (0.7 μmoles/gdw), although concentrations of other amino acids are much higher at NA10. Schroeder (1975) observed that β-alanine and γ-aminobutyric acid were not found in cleaned foraminiferal tests from deep-sea sediments, which is consistent with their lower relative abundance in high-carbonate sections of BR3.

DFAA:

In common with their similarity in TOC, TN, and THAA content, the two

Wilkinson Basin cores have nearly identical DFAA concentrations between 2 and 22 cm. DFAA concentrations are somewhat higher in the top and bottommost sections of GM4 than in the corresponding sections of GM3. These two sections have relatively high glycine abundances in the GM4 core, which suggests a contribution from benthic macrofauna or meiofauna (see discussion in Chapter 5). Free glycine is rather heterogeneously distributed in GM4 sediments, but is generally in higher concentration than at GM3, which is consistent with the earlier suggestion that greater bioturbation and/or more oxidizing conditions are responsible for its lower ammonia content. In sections with low glycine relative abundance, the composition of the cores is very similar, with glutamic acid and β-aminoglutaric acid as the major DFAA (together greater than 70 mole %). The probable bacterial source for glutamic acid has been discussed in detail earlier (Chapter 4). Also, bacteria are the only known biological source of β-aminoglutaric acid (see Chapter 3), although its relative abundance in the few types of bacteria analyzed so far is not as large as the high concentrations in sediments would suggest.

Although TOC, TN, and THAA content of the GM8 core is about half that in the Wilkinson Basin cores, DFAA concentrations are as large or larger. The DFAA composition differs from the GM3 and GM4 sediments in that the average mole percentages of glutamic acid and β-aminoglutaric acid are lower, and the percentage of glycine is higher. Repeta (unpublished ms.) found excess Pb-210 to at least 8 cm depth in this core, indicating either a rapid sedimentation rate or, more likely, extensive bioturbation of surface sediment. The fall-out radionuclides Cs-137 and Pu-239,240 are

present to 14 cm depth at this station (H. Livingston et al., unpublished data), which is also evidence for bioturbation of these sediments. A small Ophioroid (brittle star) was captured on the surface of this core. A water extract of its tissues contained large amounts of glycine (see Table 3-1). The brittle star was removed from the sediment before squeezing, so the glycine in the pore water sample may have come either from excretion by this organism (possibly induced by the stress of capture) or from other organisms squeezed with the sediment.

Unexpectedly, the NA10 continental rise sediment sampled at a depth of over 4000 m had very high DFAA concentrations in the upper 10 cm, similar to those at GM8, although THAA, TOC, and TN were only one-fourth to one-half as great. Glycine and/or alanine were the major constituents, with glutamic and β-aminoglutaric acids in substantially lower relative abundance than in basin sediments. Fall-out radionuclides had penetrated to at least 8 cm in NA10 sediment, indicating active bioturbation to that depth (H. Livingston et al., unpublished data).

The surface section of the high-carbonate BR3 core had very similar DFAA composition to the NA10 surface sediment, although the concentration was more than an order of magnitude lower. One possible explanation for the relatively low abundance of glutamic and β-aminoglutaric acids is the strong adsorption of dicarboxylic acids by calcium carbonate (Mitterer, 1972). However, since aspartic acid is not decreased proportionately in NA10 DFAA, this is not likely to be the main process responsible. Nor was the abrupt decrease in calcium carbonate content in NA10 sediment below 10 cm accompanied by a relative increase in acidic amino acids.

Serine is the major component of DFAA in NA10 sediments between 10 and 18 cm. Serine concentrations were substantially greater than in the overlying core section (6-10 cm) or in accompanying blanks, and so are not likely the result of contamination. Serine is also the major dissolved free amino acid in open ocean surface water (Lee and Bada, 1975). Because of the abrupt decrease in THAA and THAA/TN, organisms present in the 10-18 cm depth interval may be metabolizing relatively resistant organic matter, which may account for the change in DFAA concentration and composition. It also seems likely that a hiatus in deposition, or perhaps an abrupt increase in sedimentation rate, occurred near 10 cm depth.

The β-aminoglutaric acid/glutamic acid ratio is substantially lower in NA10 as compared to the basin stations and shows only a slight increase with depth in sediment. In BR3 surface sediments, the ratio is also low, 0.08. One possible explanation is that organisms (probably bacteria) which synthesize this compound do not thrive in strongly oxidizing environments, or that some condition for its synthesis (dissolved ammonia, perhaps, for example) is lacking. Another possibility is that under strongly oxidizing conditions β-aminoglutaric acid can be as readily metabolized as other amino acids and thus does not tend to accumulate.

The five cores discussed in this chapter represent a range of overlying water column depth of nearly 5000 m. If DFAA concentrations were directly related to rates of benthic metabolism, then a difference of two orders of magnitude or more would be expected between the basin and rise sediments (Smith, 1978; see detailed discussion in Chapter 4). Indeed, BR3 DFAA concentrations are 50 times less than those in Gulf of Maine

surface sediments, but surface sediments from NA10 have DFAA concentrations as high as in the basin cores. The critical difference between the BR3 and NA10 sediments in terms of composition appears to be in the organic content, and particularly the THAA concentration. Surface sediment THAA concentrations are about three times greater at NA 10, but the difference is greater when the fact that 25 % of the BR3 THAA are non-protein amino acids is considered. Also the THAA concentration in the BR3 core is close to the average reported for Recent forams, 2.3 μmoles/gdw (King and Hare, 1973 a,b), and thus the proportion of the amino acids accessible to microbial attack may be low.

Summary of Conclusions

(1) Two sediment cores from the Wilkinson Basin, Gulf of Maine were very similar in DFAA concentration and composition. Minor differences in DFAA, as well as differing ammonia profiles, were consistent with more extensive bioturbation of the GM4 core.

(2) Large glycine concentrations in the GM8 core were likewise consistent with independent evidence of extensive bioturbation.

(3) The absolute concentration (as opposed to relative abundance, which varied with glycine content) of glutamic acid and β-aminoglutaric acid, as well as the β-aminoglutaric acid/glutamic acid ratio, was very similar for DFAA at all three basin stations. This could reasonably result from similar bacterial communities in sediments at all three stations.

(4) DFAA concentrations were not directly related to sediment organic

content or to overlying water column depth, but sediments with very low (less than 0.3 μmoles/gdw) THAA content, such as those found at the bottom of the NA 10 core and at BR3, also had low DFAA concentrations.

CHAPTER 7: SUMMARY AND CONCLUDING REMARKS

Dissolved free amino acids, in concentrations ranging from less than 1 μM to greater than 100 μM, have been measured in marine sediments from a variety of depositional environments. DFAA concentrations in surface sediments are two or more orders of magnitude greater than those in overlying seawater. Maximum concentrations were usually found in the upper 6 cm. Concentrations most often decreased with depth in sediment, by as much as 100 times over 30 cm in the Peru Upwelling Region, but more commonly by a factor of five to ten. However, there was no general or simple relationship between DFAA concentration and such variables as overlying water column depth, depth in sediment, sediment organic content, remineralization rates, or redox potential, although all these parameters had some influence. In particular, higher DFAA concentrations were found under oxidizing and especially transitional redox conditions, except in deep-Atlantic sediments where concentrations of metabolizable organic carbon (as indicated by THAA contents of less than 3 μmoles/gdw) were very low. DFAA concentrations were often high near the sediment-water interface where steep gradients in total carbon dioxide and ammonia dissolved in interstitial water indicated that remineralization rates were high, but exceptions, particularly in highly-reducing environments, were found.

Glutamic acid was a major component of DFAA in nearly all samples analyzed, and often was the most abundant amino acid. Bacteria, the

probable source, accumulate glutamic acid in their intracellular free amino acid pools and excrete substantial amounts under certain conditions. An isomer of glutamic acid, β-aminoglutaric acid, was also an important constituent of DFAA. Its abundance relative to glutamic acid and other DFAA nearly always increased with depth in sediments. β-Aminoglutaric acid is an unusual, non-protein amino acid, which, to my knowledge, has not been previously identified as a natural product. However, it was found as a constituent of the free amino acid pools of some bacteria during the course of this thesis work, at about 5 mole % relative abundance. Its larger relative concentration in sediment DFAA may stem from populations of bacteria which synthesize greater amounts or from its relative resistance to biodegradation. Alanine and glycine were found in high concentrations in some relatively oxidizing sediments, but tended to have more irregular distribution than glutamic acid and β-aminoglutaric acid. Benthic invertebrates are a likely source for the major proportion of these amino acids where their concentration is high relative to glutamic acid, but alanine may also be produced in large relative abundance by some bacteria.

Metabolism, specifically microbial metabolism, has been identified as the major sink for DFAA in sediments. Because of extremely steep concentration gradients seen at the sediment-water interface, diffusion must be a relatively minor sink, either because amino acids free in interstitial water are turned over metabolically very rapidly (on time scales of less than a day) or because the bulk of free amino acids measured were confined to intracellular pools <u>in situ</u>. Transepidermal uptake by benthic

invertebrates could turn over the near-surface free amino acid concentrations measured in oxidizing sediments on time scales of several days, provided the bulk of the measured concentration does not represent intracellular pools. Adsorption of free amino acids by sediments does occur (10 % to 50 % of dissolved concentration for acidic and neutral amino acids in Buzzards Bay surface sediment), but is not a major factor in determining the concentration or composition of DFAA measured in pore water samples. Because DOC gradients in the sediments studied are small, condensation reactions of amino acids with other organic compounds to form soluble, humic-type polymers must be slow relative to biological sinks for amino acids, but could be somewhat more important if the polymers formed were rapidly adsorbed. However, reversing the argument, DFAA would be a more than adequate reactant pool for observed DOC concentrations, if the measured DFAA concentrations are found outside of living organisms.

Thus, DFAA in marine sediments represent a dynamic equilibrium between rapid biological processes of supply and removal. An important remaining question is the partitioning of DFAA in sediments between intracellular and extracellular pools; any sampling or extraction method which affects biological processes in the sediment may affect the total DFAA pool and/or the balance between extracellular and intracellular pools. In situ sampling with apparatus designed to minimize such effects will be necessary to answer this question.

A related area which needs further study concerns the rates of biological turnover of amino acid pools in sediments. Rates for intracel-

lular and extracellular pools almost certainly differ, but may be related. Thus knowledge of both total DFAA pools and extracellular DFAA pool size is needed. Because rates of microbial amino acid turnover are so high, measurement of rates of slower processes such as transepidermal uptake and humification in natural systems is probably not possible, but some information can be gained from artificial systems from which microbial activity is eliminated, or possibly by using non-metabolizable analogues of amino acids.

Finally, the occurence of relatively large amounts of β-aminoglutaric acid in sediment DFAA suggests that it may have a previously unsuspected importance in the free amino acid pools of bacteria. The physiological significance and biosynthetic/degradative pathways of this compound are unknown, and offer opportunities for further research.

REFERENCES

Abelson, P. H. and P. E. Hare (1971) Reaction of amino acids with natural and artificial humus and kerogens. Carnegie Inst. Yearbook 69, 327-334.

Adamski, J. M. (1976) Simplified Kjeldahl nitrogen determination for seawater by a semiautomated persulfate digestion method. Anal. Chem. 48. 1194-1197.

Adamson, A. W. (1967) Physical Chemistry of Surfaces, 2nd ed. Wiley-Interscience, New York.

Aida. K. (1972) Control of microbial metabolism. In: The Microbial Production of Amino Acids (K. Yamada, et al., eds.), John Wiley & Sons, New York, pp. 91-122.

Aller, R. C. and J. Y. Yingst (1978) Biogeochemistry of tube-dwellings. A study of the sedentary polychaete Amphitrite ornata (Leidy). J. Mar. Res. 36, 201-254.

Amarasingham, C. R. and B. D. Davis (1965) Regulation of α-ketoglutarate dehydrogenase formation in Escherichia coli. J. Biol. Chem. 240, 3664-3668.

Andersen, N. R., ed. (1977) Concepts in marine organic chemistry. Proc. NATO/ONR Symposium, Edinburgh. Mar. Chem. 5, 303-643.

Andrews, P. and P. J. le B. Williams (1971) Heterotrophic utilization of dissolved organic compounds in the sea III. Measurement of the oxidation rates and concentrations of glucose and amino acids in seawater. J. Mar. Biol. Ass. U. K. 51, 111-125.

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

Barcelona, M. J., H. M. Liljestrand, and J. J. Morgan (1980) Precise and rapid determination of C_1 to C_5 volatile fatty acids in aqueous samples. Anal. Chem. 52, 321-325.

Berner, R. A. (1976a) The benthic boundary layer from the viewpoint of a geochemist. In: The Benthic Boundary Layer (I. N. McCave, ed.). Plenum Press, New York, pp. 33-55.

Berner, R. A. (1976b) Inclusion of adsorption in the modelling of early diagenesis. Earth Planet. Sci. Lett. 29, 333-340.

Blumer, M. (1975) Organic compounds in nature: Limits of our knowledge. Angew. Chem. 14, 507-580.

Borodovskiy, O. K. (1965) Accumulation of organic matter in bottom sediments. Mar. Geol. 3, 33-82.

Brown, C. M. and S. O. Stanley (1972) Environment-mediated changes in the cellular content of the "pool" constituents and their associated changes in cell physiology. J. appl. Chem. Biotechnol. 22, 363-389.

Burke, J. (1968) A sediment coring device of 21 cm diameter with sphincter core retainer. Limnol. Oceanogr. 13, 714-718.

Carter, P. W. (1978) Adsorption of amino acid-containing organic matter by calcite and quartz. Geochim. Cosmochim. Acta 42, 1239-1242.

Christensen, D. and T. H. Blackburn (in press) Turnover of L-(U-^{14}C) alanine in anoxic marine sediments. Mar. Biol.

Christensen, J. P. and T. T. Packard (1977) Sediment metabolism from the Northwest African upwelling system. Deep-Sea Res. 24, 331-343.

Crawford, C. C., J. E. Hobbie, and K. L. Webb (1974) The utilization of dissolved free amino acids by estuarine microorganisms. Ecology 55, 551-563.

Dawson, R. and K. Gocke (1978) Heterotropic activity in comparison to the free amino acid concentrations in Baltic seawater. Oceanol. Acta 1, 45-54.

Dawson, R. and R. G. Pritchard (1978) The determination of α-amino acids in seawater using a fluorimetric analyser. Mar. Chem. 6, 27-40.

Degens, E. T. and K. Mopper (1976) Factors controlling the distribution of early diagenesis of organic material in marine sediments. In: Chemical Oceanography, 2nd edition, V. 6 (J. P. Riley and R. Chester, eds.). Academic Press, London, pp. 59-113.

Degens, E. T. and J. H. Reuter (1963) Analytical techniques in the field of organic geochemistry. In: Advances in Organic Geochemistry, 1964 (U. Colombo and G. Hobson, eds.). MacMillan, New York, pp. 377-402.

DeMaster, D. J. (1979) The marine budgets of silica and ^{32}Si. Ph.D. Thesis, Yale University, 308 pp.

De Zwaan, A. (1977) Anaerobic energy metabolism in bivalve mollusks. Oceanogr. Mar. Biol. Ann. Rev. 15, 103-187.

Doelle, H. W. (1975) Bacterial Metabolism. Academic Press, New York, 738 pp.

Dugdale, R. C., J. J. Goering, R. T. Barber, R. L. Smith, and T. T. Packard (1977) Denitrification and hydrogen sulfide in the Peru upwelling region during 1976. Deep-Sea Res. 24, 601-608.

Farrington, J. W., S. M. Henrichs, and R. Anderson (1977a) Fatty acids and Pb-210 geochronology of a sediment core from Buzzards Bay, Massachusetts. Geochim. Cosmochim. Acta 41, 289-296.

Farrington, J. W., N. M. Frew, P. M. Gschwend, and B. W. Tripp (1977b) Hydrocarbons in cores of Northwestern Atlantic coastal and continental margin sediments. Estuar. Coast. Mar. Sci. 5, 793-808.

Ferguson, J. C. (1971) Uptake and release of free amino acids by starfishes. Biol. Bull. 141, 122-129.

Fukui, S. and M. Ishida (1972) Biomembrane permeability and the accumulation of amino acids. In: The Microbial Production of Amino Acids (K. Yamada et al., eds.). John Wiley & Sons, New York, PP. 123-138.

Gagosian, R. B. and C. Lee (in press) Processes controlling the distribution of biogenic organic compounds in seawater. In: The Organic Chemistry of Seawater (E. K. Duursma and R. Dawson, eds.). Elsevier, Amsterdam.

Gagosian, R. B., T. Loder, G. Nigrelli, Z. Mlodzinska, J. Love, and J. Kogelschatz (1980) Hydrographic and nutrient data from R/V Knorr Cruise 73, Leg 2 -- February to March, 1978 -- off the coast of Peru. WHOI Technical Report 80-1, Woods Hole Oceanographic Institution, Woods Hole, 77 pp.

Gallardo, V. A. (1976) On a benthic sulfide system on the continental shelf of North and Central Chile. International Symposium Coastal Upwelling Proc., 113-118.

Gallardo, V. A. (1978) Large benthic microbial communities in sulphide biota under Peru-Chile subsurface countercurrent. Nature 268, 331-332.

Gaines, A. G. Jr. (1975) Papers on the geomorphology, hydrography, and geochemistry of the Pettaquamscutt River. Ph.D. Thesis, University of Rhode Island.

Gaines, A. G. Jr. and M. E. Q. Pilson (1972) Anoxic water in the Pettaquamscutt River. Limnol. Oceanogr. 17, 42-49.

Gardner, W. S. and R. B. Hanson (1979) Dissolved free amino acids in interstitial water of Georgia salt marsh soils. Estuaries 2, 113-118.

Garrasi, C., E. T. Degens, and K. Mopper (1979) The free amino acid composition of seawater obtained without desalting and preconcentration. Mar. Chem. 8, 71-85.

Gauthier, M. J. (1976) Morphological, physiological, and biochemical characteristics of some violet-pigmented bacteria isolated from seawater. Can. J. Microbiol. 22, 138-149.

Gilboa-Garber, N. (1971) Direct spectrophotometric determination of inorganic sulfide in biological materials and in other complex mixtures. Anal. Biochem. 43, 129-133.

Giordano, M. F., H. A. Harper, and F. P. Filice (1950) The amino acids of a starfish and a sea urchin (Asteroidea and Echinoidea). Wasmann J. Biol. 8, 129-132.

Goldberg, E. D., E. Gamble, J. J. Griffin, and M. Koide (1977) Pollution history of Narragansett Bay as recorded in its sediments. Estuar. Coast. Mar. Sci. 5, 549-561.

Goldhaber, M. B., R. C. Aller, J. K. Cochran, J. K. Rosenfeld, C. S. Martens, and R. A. Berner (1977) Sulfate reduction, diffusion, and bioturbation in Long Island Sound sediments: Report of the FOAM Group. Am. J. Sci. 277, 193-237.

Griffiths, R. P., J. A. Baross, F. J. Hanus, and R. Y. Morita (1974). Some physical and chemical parameters affecting the formation and retention of glutamate pools in a marine psychrophilic bacterium. Z. allg. Mikrobiol. 14, 359-369.

Grob, K. and G. Grob (1979) Practical glass capillary gas chromatography -- A systematic approach. J. High Res. Chromatogr., Chromatogr. Comm. 2, 109-117.

Grob, K., G. Grob, and K. Grob, Jr. (1979) Deactivation of glass capillary columns by persilylation, Part 1: Principles and Basic Techniques. J. High. Res. Chromatogr., Chromatogr. Comm. 2, 31-35.

Grob, K. Jr., G. Grob, and K. Grob (1978) Preparation of apolar glass capillary columns by the barium carbonate procedure. J. High Res. Chromatogr., Chromatogr. Comm. 1, 149-155.

Gunther, E. R. (1936) A report on oceanographical investigations in the Peru coastal current. In: Discovery Reports, V. XIII. Cambridge University Press, London, pp. 109-276.

Hanson, R. B. and W. S. Gardner (1978) Uptake and metabolism of two amino acids by anaerobic microorganisms in four diverse salt marsh soils. Mar. Biol. 46, 101-107.

Harrison, M. J., R. I. Wright, and R. Y. Morita (1971) Method for measuring mineralization in lake sediments. Appl. Microbiol. 21, 698-702.

Hedges, J. I. (1977) The association of organic molecules with clay minerals in aqueous solution. Geochim. Cosmochim. Acta 41, 1119-1123.

Hedges, J. I. (1978) The formation and clay mineral reactions of melanoidins. Geochim. Cosmochim. Acta 42, 69-76.

Henrichs, S. M. and J. W. Farrington (1979) Amino acids in interstitial water of marine sediments. Nature 279, 319-322.

Henrichs, S. M., J. E. Hobbie, R. W. Howarth, and P. Kilham (in preparation) Amino acid cycling in salt marsh sediments.

Hesslein, R. (1976) An in situ sampler for close interval pore water studies. Limnol. Oceanogr. 21, 912-914.

Hirosi, Y. (1972) Oxygen transfer. In: The Microbial Production of Amino Acids (K. Yamada, et al., eds.) John Wiley & Sons, New York, pp. 203-226.

Hites, R. A., R. E. LaFlamme, J. G. Windsor Jr., J. W. Farrington, and W. G. Deuser (1980) Polycyclic aromatic hydrocarbons in an anoxic sediment core from the Pettaquamscutt River (Rhode Island, U. S. A.). Geochim. Cosmochim. Acta 44, 873-878.

Ho, C. L. and J. Lane (1973) Interstitial water composition in Barataria Bay (Louisiana) sediment. Estuar. Coast. Mar. Sci. 1, 125-135.

Hough, J. L. (1940) Sediments of Buzzards Bay, Massachusetts: J. Sed. Petrol. 10, 19-32.

Husek, P. and K. Macek (1975) Gas chromatography of amino acids. J. Chromatogr. 113, 139-230.

Jannasch, H. W. and C. O. Wirsen (1973) Deep-sea microorganisms: An in situ response to nutrient enrichment. Science 180, 641-648.

Jannasch, H. W., C. O. Wirsen, and C. D. Taylor (1976) Undecompressed microbial populations from the deep sea. Appl. Environ. Microbiol. 32, 360-367.

Johannes, R. E., S. J. Coward, and K. L. Webb (1969) Are dissolved amino acids an energy source for marine invertebrates? Comp. Biochem. Physiol. 29, 283-288.

Johnson, R. G. (1974) Particulate matter at the sediment-water interface in coastal environments. J. Mar. Res. 32, 313-330.

Jorgensen, N. O. G. (1979) Annual variation of dissolved free primary amines in estuarine water and sediment. Oecologia 40, 207-217.

Jorgensen, N. O. G., P. Lindroth, and K. Mopper (in preparation) Extraction and distribution of free amino acids and ammonium in sediments and overlying sea waters from the Limfjord, Denmark.

Karl, D. M. (1978) A study of microbial biomass and metabolic activities in marine ecosystems: Development of sensitive techniques and results of selective environmental studies. Ph. D. Thesis, University of California, San Diego, 320 pp.

Karl, D. M. P. A. LaRock, J. W. Morse, and W. Sturges (1976) Adenosine triphosphate in the North Atlantic Ocean and its relationship to the oxygen minimum. Deep-Sea Res. 23, 81-88.

Karl, D. M. P. A. LaRock, and D. J. Schulz (1977) Adenosine triphosphate and organic carbon in the Cariaco Trench. Deep-Sea Res. 24, 105-115.

Kemp, A. L. W. and A. Mudrochova (1973) The distribution and nature of amino acids and other nitrogen-containing compounds in Lake Ontario surface sediments. Geochim. Cosmochim. Acta 37, 2191-2206.

King, K. Jr. and P. E. Hare (1972a) Amino acid composition of planktonic foraminifera: A paleobiochemical approach to evolution. Science 175, 1461-1463.

King, K. Jr. and P. E. Hare (1972b) Amino acid composition of the test as a taxonomic character for living and fossil foraminifera. Micropaleontology 18, 285-293.

Kinoshita, S. and K. Tanaka (1972) Glutamic acid. In: The Microbial Production of Amino Acids. (K. Yamada, et al., eds.) John Wiley & Sons, New York, pp. 263-264.

Kitai, A. (1972) Alanine. In: The Microbial Production of Amino Acids (K. Yamada, et al., eds.). John Wiley & Sons, New York, pp. 325-338.

Koide, M., K. Bruland, and E. D. Goldberg (1976) ^{226}Ra chronology of a coastal marine sediment. Earth Planet. Sci. Lett. 31, 31-36.

Kowallik, U. and E. G. Pringsheim (1966) The oxidation of hydrogen sulfide by Beggiatoa. Am. J. Bot. 53, 801-806.

Krom, M. D. and E. R. Sholkovitz (1977) Nature and reactions of dissolved organic matter in the interstitial waters of marine sediments. Geochim. Cosmochim. Acta 41, 1565-1573.

Kvenvolden, K. A. (1975) Advances in the geochemistry of amino acids. Ann. Rev. Earth Planet. Sci. 3, 183-212.

Laine, E. P. (1978) Geological effects of the Gulf Stream system in the North American basin. Ph.D. Thesis, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program in Oceanography. 164 pp.

Lasaga, A. C. and H. D. Holland (1976) Mathematical aspects of non-steady-state diagenesis. Geochim. Cosmochim. Acta 40, 257-266.

Lawless, J. G. and M. S. Chadha (1971) Mass spectral analysis of C_3 and C_4 aliphatic amino acid derivatives. Anal. Biochem. 44, 473-485.

Lee, C. L. (1975) Biological and geochemical implications of amino acids in seawater, wood, and charcoal. Ph.D. Thesis, University of California, San Diego, 178 pp.

Lee, C. and J. L. Bada (1975) Amino acids in equatorial Pacific Ocean water. Earth Planet. Sci. Lett. 26, 61-68.

Lee, C. and J. L. Bada (1977) Dissolved amino acids in the equatorial Pacific, the Sargasso Sea, and Biscayne Bay. Limnol. Oceanogr. 22, 502-510.

Lee, C. R. B. Gagosian, and J. W. Farrington (1977) Sterol diagenesis in Recent sediments from Buzzards Bay, Massachusetts. Geochem. Cosmochim. Acta 41, 985-992.

Leimer, K. R., R. H. Rice, and C. W. Gehrke (1977) Complete mass spectra of N-trifluoroacetyl n-butyl esters of amino acids. J. Chromatogr. 141, 129-144.

Lewis, A. J. and J. D. A. Miller (1977) The tricarboxylic acid pathway in Desulfovibrio. Can. J. Microbiol. 23, 916-921.

Li, Y. H. and S. Gregory (1974) Diffusion of ions in seawater and in deep-sea sediments. Geochim. Cosmochim. Acta 38, 703-714.

Lindberg, S. E. and R. C. Harriss (1974) Mercury-organic matter associations in estuarine sediments and interstitial water. Environ. Sci. Technol. 8, 459-462.

Lindroth, P. and K. Mopper (1979) High performance liquid chromatographic determination of subpicomole amounts of amino acids by precolumn fluorescence derivatization with o-pthaldialdehyde. Anal. Chem. 51, 1667-1674.

Lyman, J. and R. H. Fleming (1940) Composition of sea water. J. Mar. Res. 3, 134-146.

Lyons, W. B., H. E. Gaudette, and A. D. Hewitt (1979) Dissolved organic matter in pore water of carbonate sediments from Bermuda. Geochim. Cosmochim. Acta 43, 433-437.

Manglesdorf, P. C., T. R. S. Wilson, and E. Daniell (1969) Potassium enrichments in interstitial waters of Recent marine sediments. Science 165, 171-174.

Manheim, F. T. (1974) Comparative studies on extraction of sediment interstitial waters: Discussion and comment on the current state of interstitial water studies. Clays Clay Minerals 22, 337-343.

Manheim, F. T. (1976) Interstitial waters of marine sediments. In: Chemical Oceanography, 2nd ed., V. 6 (J. P. Riley and R. Chester, eds.). Academic Press, London, pp. 115-186.

Martens, C. S. and M. B. Goldhaber (1978) Early diagenesis in transitional sedimentary environments of the White Oak River Estuary, North Carolina. Limnol. Oceanogr. 23, 428-441.

Menzel, D. W., and J. H. Ryther (1960) The annual cycle of primary production in the Sargasso Sea off Bermuda. Deep-Sea Res. 6, 351-367.

Metzler, D. E. (1977) Biochemistry: The Chemical Reactions of Living Cells. Academic Press, London, 1129 pp.

Meyers, P. A. and J. G. Quinn (1973) Factors affecting the association of fatty acids with mineral particles in sea water. Geochim. Cosmochim. Acta 37, 1745-1759.

Mopper, K. and E. T. Degens (1972) Aspects of the biogeochemistry of carbohydrates and proteins in aquatic environments. WHOI Technical Report 72-68, Woods Hole Oceanographic Institution, Woods Hole, 118 pp.

Murray, J. W., V. Grundmanis, and W. M. Smethie Jr. (1978) Interstitial water chemistry in the sediments of Saanich Inlet. Geochim. Cosmochim. Acta 42, 1011-1026.

Nissenbaum, A. (1974) The organic geochemistry of marine and terrrestrial humic substances: Implications of carbon and nitrogen isotope studies. In: Advances in Organic Geochemistry, 1973 (B. Tissot and F. Bienner, eds.). Editions Technip, Paris, pp. 39-52.

Nissenbaum, A., M. J. Baedecker, and I. R. Kaplan (1972) Studies on dissolved organic matter from interstitial water of a reducing marine fjord. In Advances in Organic Geochemistry, 1971 (H. von Gaertner and H. Wehner, eds.). Pergamon Press, London, pp.427-446.

Oro, J. and H. B. Skewes (1965) Free amino acids on human fingers: The question of contamination in microanalysis. Nature 207, 1042-1045.

Orr, W. and A. Gaines (1974) Observations on the rate of sulfate and organic matter oxidation in bottom waters of an estuarine basin: the upper basin of the Pettaquamscutt River (Rhode Island). In: Advances in Organic Geochemistry, 1973 (B. Tissot and F. Bienner, eds.). Editions Technip, Paris, pp. 791-812.

Parks, G. (1975) Adsorption in the marine environment. In: Chemical Oceanography, 2nd ed., V. 1 (J. P. Riley and G. Skirrow, eds.). Academic Press, London, pp. 241-308.

Peltzer, E. T. III (1979) Geochemistry of hydroxy and dicarboxylic acids. Ph.D. Thesis, University of California, San Diego, 209 pp.

Potts, W. T. W. (1967) Excretion in the mollusks. Biol. Rev. 42, 1-41.

Price, N. B. (1976) Chemical diagenesis in sediments. In: Chemical Oceanography, 2nd ed., V. 6 (J. P. Riley and R. Chester, eds.). Academic Press, New York, pp. 1-59

Pringsheim, E. G. (1964) Heterotrophism and species concepts in Beggiatoa. Am. J. Bot. 51, 898-913.

Repeta, D. J. (unpublished ms.) Pb-210 geochronology of Gulf of Maine sediments.

Rhoads, D. C. (1967) Biogenic reworking of intertidal and subtidal sediments in Barnstable Harbor and Buzzards Bay, Massachusetts. J. Geol. 72, 461-476.

Rhoads, D. C. (1974) Organism-sediment relations on the muddy sea floor. Oceanogr. Mar. Biol. Ann. Rev. 12, 263-300.

Roach, D. and C. W. Gehrke (1969) Direct esterification of the protein amino acids: Gas-liquid chromatography of N-TFA n-butyl esters. J. Chromatogr. 44, 269-278.

Roman, M. R. and K. R. Tenore (1978) Tidal resuspension in Buzzards Bay, Massachusetts I.Seasonal changes in the resuspension of organic matter and chlorophyll a. Estuar. Coast. Mar. Sci. 6, 37-53.

Romankevich, E. A. and I. M. Urbanovich (1977) Organic matter (nitrogen, carbohydrates) of the suspended matter, bottom sediments, and interstitial waters of the Peru-Chile region. Trudy Instituta Okeanologii 89, 106-117 (In Russian).

Rosato, J. J., L. D. Kulm, and P. S. Derks (1975) Surface sediments of the Nazca plate. Pacific Science 29, 117-130.

Rosenfeld, J. K. (1979) Ammonium adsorption in nearshore anoxic sediments. Limnol. Oceanogr. 24, 356-364.

Ryther, J. H., D. W. Menzel, E. M. Hulburt, C. J. Lorenzen, and N. Corwin (1971) The production and utilization of organic matter in the Peru Coastal Current. Inv. Pesq. 35, 43-59.

Saidova, H. M. (1971) On the Recent sediments near the Pacific coast of South America. Trudy Instituta Okeanologii 89, 139-145 (In Russian).

Saliot, A and M. J. Tissier (1978) Inventaire et dynamique des lipides a l'interface eau de mer-sediment IV.Hydrocarbures aliphatiques et alicycliques de l'eau de mer et de l'eau interstitielle. In: Geochimie Organique des Sediments Marines Profonds (A. Combaz and R. Pelet, eds.). Centre National de la Recherche Scientifique, Paris, pp. 263-273.

Salton, M. R. J. (1960) Microbial Cell Walls. John Wiley & Sons, New York, 94 pp.

Sanders, H. L. (1958) Benthic studies in Buzzards Bay I.Animal-sediment relationships. Limnol. Oceanogr. 3, 245-258.

Sanders, H. L. (1960) Benthic studies in Buzzards Bay III.The structure of the soft-bottom community. Limnol. Oceanogr. 5, 138-153.

Schink, D. R. and N. L. Guinasso (1977) Effects of bioturbation on sediment-seawater interaction. Mar. Geol. 23, 133-154.

Schlee, J. (1973) Atlantic continental shelf and slope of the United States: Sediment texture of the northeastern part. U.S.G.S. Professional Paper 529-L, 64 pp.

Schnitzer, M. and S. U. Khan (1972) Humic Substances in the Environment. Marcel Dekker, New York, 327 pp.

Schroeder, R. A. (1974) Kinetics, mechanism, and geochemical applications of amino acid racemization in various fossils. Ph.D. Thesis, University of California, San Diego, 275 pp.

Schroeder, R. A. (1975) Absence of β-alanine and γ-aminobutyric acid in cleaned foraminiferal shells: implications for use as a chemical criterion to indicate removal of non-indigenous amino acid contaminants. Earth Planet. Sci. Lett. 25, 274-278.

Schroeder, R. A. and J. L. Bada (1976) A review of the geochemical applications of the amino acid racemization reaction. Earth-Sci. Rev. 12. 347-391.

Silva, A. J., C. D. Hollister, E. P. Laine, and B. E. Beverly (1976) Geotechnical properties of deep-sea sediments: Bermuda Rise. Mar. Geotechnol. 1, 195-232.

Smith, A. J., J. London, and R. Y. Stanier (1967) Biochemical basis of obligate autotrophy in blue-green algae and thiobacilli. J. Bacteriol. 94, 972-983.

Smith, K. L. (1978) Benthic community respiration in the N. W. Atlantic: In situ measurements from 40 to 5200 m. Mar. Biol. 47, 337-347.

Solorzano, L. (1969) Determination of ammonia in natural waters by the phenol-hypochlorite method. Limnol. Oceanogr. 14, 799-801.

Sorokin, J. I. (1964) On the primary production and bacterial activities in the Black Sea. J. Cons., Cons. Perm. Int. Expl. Mer. 29, 41-60.

Southward, A. J. and E. C. Southward (1972) Observations on the role of dissolved organic compounds in the nutrition of benthic invertebrates II.Uptake by other animals living in the same habitat as pogonophores, and by some littoral polychaeta. Sarsia 48, 61-70.

Southward, A. J. and E. C. Southward (1972) Observations on the roles of dissolved organic compounds in the nutrition of benthic invertebrates III.Uptake in relation to organic content of the habitat. Sarsia 50, pp. 29-46.

Stanley, S. O. and C. M. Brown (1976) Inorganic nitrogen metabolism in marine bacteria: the intracellular free amino acid pools of a marine pseudomonad. Mar. Biol. 38, 101-109.

Staresinic, N. (1978) The vertical flux of particulate organic matter in the Peru coastal upwelling as measured with a free-drifting sediment trap. Ph.D. Thesis, Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program, 255 pp.

Starikova, N. D. (1970) Vertical distribution patterns of dissolved organic carbon in seawater and interstitial solutions. Oceanology. 10, 796-807.

Starikova, N. D. and L. I. Korzhikova (1972) Amino acids in suspended matter, sediments, and ooze solutions from the Black Sea. Geokhimiya 2, 230-240.

Stephens, G. C. (1975) Uptake of naturally occurring primary amines by marine annelids. Biol. Bull. 149, 397-407.

Stephens, G. C., M. J. Volk, S. H. Wright, and P. S. Blacklund (1978) Transepidermal uptake of naturally occuring amino acids in the sand dollar Dendraster excentricus. Biol. Bull. 154, 335-347.

Stewart, M. G. (1979) Adsorption of dissolved organic nutrients by marine invertebrates. Oceanogr. Mar. Biol. Ann. Rev. 17, 163-192.

Strohl, W. R. and J. M. Larkin (1978) Enumeration, isolation, and characterization of Beggiatoa from freshwater sediments. Appl. Environ. Microbiol. 36, 755-770.

Suess, E. (1976) Nutrients near the depositional interface. In: The Benthic Boundary Layer (I. N. McCave, ed.). Plenum Press, New York, pp. 57-79.

Taylor, C. D., P. O. Ljungdahl, and J. J. Molongoski (in preparation) Techniques for the simultaneous determination of ^{35}S sulfide and ^{14}C carbon dioxide in anaerobic aqueous samples.

Technicon Industrial Systems (1972) Nitrate and nitrite in water and seawater. Industrial Method No. 168-71 W.

Thompson, S. and G. Eglinton (1978) The fractionation of a Recent sediment for organic geochemical analysis. Geochim. Cosmochim. Acta 42, 199-207.

Uchupi, E. (1965) Basins of the Gulf of Maine. U.S.G.S. Professional Paper 525-D, pp. D175-D177.

Veeh, H. H. and W. C. Burnett (1973) Contemporary phosphorites on the continental margin off Peru. Science 181, 844-845.

Whittle, K. J. (1977) Marine organisms and their contribution to organic matter in the ocean. Mar. Chem. 5, 381-411.

Williams, P. J. le B. (1977) Biological and chemical aspects of dissolved organic material in seawater. In: Chemical Oceanography, 2nd ed., V. 2 (J. P. Riley and G. Skirrow, eds.). Academic Press, London, pp. 301-364.

Williams, P. J. le B. and S. W. Watson (1963) Autotrophy in Nitrocystis oceanus. J. Bacteriol. 96, 1640-1648.

Wood, L. W. (1970) The role of estuarine sediment microorganisms in the uptake of organic carbon under aerobic conditions. Ph.D. Thesis, North Carolina State University, 75 pp.

Wright, R. T. (1978) Measurement and significance of specific activity in the heterotrophic bacteria of natural waters. Appl. Environ. Microbiol. 36, 297-305.

Wyrtki, K. (1963) The horizontal and vertical field of motion in the Peru current. Bulletin, Scripps Institution of Oceanography 8, 313-345.

Wyrtki, K. (1967) Circulation and water masses in the eastern equatorial Pacific Ocean. Int. J. Oceanogr. Limnol. 1, 117-147.

Yamada, K., S. Kinoshita, T. Tsunoda, and K. Aida (1972) The Microbial Production of Amino Acids. John Wiley & Sons, New York, 564 pp.

ZoBell, C. E. (1964) Geochemical aspects of the microbial modification of organic matter. In: Advances in Organic Geochemistry, 1964 (U. Columbo and G. D. Hobson, eds.). MacMillan, New York, pp. 339-356.

Zuta, S. and O. Guillen (1970) Oceanographia de las aguas costeras del Peru. Boletin Instituto del Mar del Peru 2, 157-324.

Zuta, S., T. Rivera, and A. Bustamante (1975) Hydrological aspects of the main upwelling areas off Peru. Third International Symposium on Upwelling Ecosystems, Kiel, 25 pp.

APPENDIX I

Amino Acid Abbreviations Used in this Thesis

ala	alanine
gly	glycine
α-aba	α-aminobutyric acid
val	valine
β-ala	β-alanine
thr	threonine
norval	norvaline
ser	serine
leu	leucine
ile	isoleucine
norleu	norleucine
γ-aba	γ-aminobutyric acid
pro	proline
hypro	hydroxyproline
daba	diaminobutyric acid
met	methionine
asp	aspartic acid
phe	phenylalanine
orn	ornithine
glu	glutamic acid
β-glu	β-aminoglutaric acid
lys	lysine
tyr	tyrosine
α-aaa	α-aminoadipic acid
tryp	tryptophan
dapa	diaminopimelic acid
cys	cystine

APPENDIX II

Data Used in Preparing Figures

A. Gulf of Maine Ammonia Data

Depth (cm)	NH_3(μM)	Depth (cm)	NH_3(μM)
GM3		GM4	
0-2	7	0-1	4
2-4	32	1-2	3
4-6	41	2-4	2
6-8	73	4-6	4
8-12	111	6-8	10
12-16	122	10-12	21
16-20	160	14-16	37
20-24	170	18-20	58
24-28	190	24-26	70
GM8		NA10	
0-1	3	0-1	17
1-2	10	2-4	6
2-4	28	4-6	7
4-6	32	6-8	6
6-8	41	8-10	7
10-12	49	12-14	7
		16-18	7

B. Buzzards Bay, Station P and Pettaquamscutt River Estuary
Ammonia, Total Carbon Dioxide, and pH

	Depth (cm)	NH_3 (μM)	Total CO_2 (mM)	pH
BBP6/79				
	0-2	145	3.4	7.32
	2-4	159	3.7	7.59
	4-6	147	4.0	7.62
	6-10	158	3.5	7.68
	10-14	164	3.6	7.77
	14-18	222	4.3	7.82
	18-22	278	4.8	7.91
	22-26	349	5.3	7.90
	30-34	423	6.5	7.88
	38-42	536	8.2	7.79
	46-50	569	8.5	7.80
	54-58	667	9.1	7.76
	62-66	608	9.5	7.68
BBP12/5				
	0-2	123	4.7	7.19
	2-5	129	3.9	7.23
	5-8	113	3.5	7.47
	8-11	119	3.2	7.45
	11-14	158	3.6	7.59
	14-17	242	3.6	7.76
	17-20	327	4.7	7.80
	20-23	349	4.9	7.76
	23-26	410	5.3	7.77
	26-29	454	5.6	7.85
	29-32	486	5.92	7.63
PRE				
	0-6	1290	14	7.58
	6-12	1240	14	7.70
	12-18	1220	15	7.90
	18-24	1480	22	7.82
	24-30	1520	19	7.44
	30-36	1590	20	7.38
	36-42	1620	21	7.29
	42-48	1710	21	7.44
	54-60	1760	20	7.44
	66-72	1780	17	7.36
	78-84	1990	16	7.32
	90-96	2140	15	7.00

C. Peru Upwelling Region Ammonia, Nitrate, Nitrite, Total Carbon Dioxide, Sulfide, and pH Data

Depth (cm)	NH_3 (μM)	NO_3^- (μM)	NO_2^- (μM)	Total CO_2 (mM)	S^{2-} (μM)	pH
Station 4						
0-2	277	69	4.6	4.2	300	7.80
2-4	437	17	4.2	5.4	50	7.53
4-7	557	8.0	2.1	5.1	19	7.70
7-10	655	4.7	2.1	6.5	35	7.70
10-13	681	2.1	0.0	6.8	0	7.79
13-16	734	0.4	0.0	7.2	0	7.77
19-22	847	0.4	0.0	7.8	0	7.68
25-28	897	0.3	0.0	9.6	0	7.68
31-34	1010	0.1	0.0	9.5	0	7.72
40-46	996	0.0	0.0	10.9	0	7.82
52-58	982	0.0	0.0	11.7	0	7.69
64-70	980	0.0	0.0	10.6	0	7.72
Station 5A						
0-3	622	41	1.9	4.2	0	7.80
3-6	822	2.5	0.0	6.0	210	7.88
6-9	1010	2.3	n.d.	8.0	520	7.90
9-12	1170	5.4	n.d.	9.2	390	8.08
12-15	1270	n.d.	n.d.	10.4	530	7.72
15-18	1410	n.d.	n.d.	10.6	1020	7.77
18-21	1460	n.d.	n.d.	11.7	1010	7.87
24-27	1670	n.d.	n.d.	14.6	1240	7.81
30-33	2200	n.d.	n.d.	16.5	1710	7.85
36-39	2010	n.d.	n.d.	16.0	1420	7.86
42-45	2210	n.d.	n.d.	16.4	1550	7.91
48-51	2310	n.d.	n.d.	21.3	1440	7.77
Station 6						
0-2	492	n.d.	n.d.	4.4	880	7.49
2-4	497	n.d.	n.d.	4.7	870	7.52
4-6	648	n.d.	n.d.	5.7	830	7.56
6-9	579	n.d.	n.d.	5.0	790	7.49
9-12	454	n.d.	n.d.	4.4	650	7.51
12-15	382	n.d.	n.d.	3.7	220	7.54
15-18	334	n.d.	n.d.	3.2	230	7.43
18-21	232	n.d.	n.d.	3.3	170	7.51
21-24	114	0.0	0.0	3.0	0	7.59
24-30	61	0.0	0.0	2.4	0	7.58
30-36	48	0.0	0.0	2.3	0	7.65

Depth (cm)	NH_3 (μM)	NO_3^- (μM)	NO_2^- (μM)	Total CO_2 (mM)	S^{2-} (μM)	pH
Station 8						
0-2	41	6.5	1.0	2.3	0	7.49
2-4	48	4.3	0.9	2.3	0	7.54
4-6	50	3.2	0.6	2.4	0	7.51
6-8	53	2.8	0.7	2.3	0	7.61
8-10	65	3.4	0.9	2.5	0	7.43
10-12	75	0.9	0.4	2.6	0	7.58
Station 2A						
Box Core 4						
0-1	56	2.7	0.0	2.47	0	7.47
1-2	90	2.4	0.0	2.78	0	7.40
2-4	169	1.6	0.0	2.90	0	7.49
4-6	229	1.4	0.0	3.08	0	7.51
6-8	294	1.3	0.0	3.99	0	7.52
Box Core 5						
0-1	40	1.4	0.0	2.37	0	7.38
1-2	43	1.1	0.0	2.60	0	7.32
2-4	56	1.0	0.0	2.87	0	7.31
4-6	67	1.0	0.0	2.77	0	7.40
6-8	83	1.0	0.0	3.23	0	7.42
8-10	96	1.0	0.0	3.14	0	7.41
12-14	72	1.0	0.0	2.94	0	7.50
16-18	106	1.1	0.0	2.87	0	7.49

D. Dissolved Free Amino Acids, Peru Upwelling Region Station 4

Amino Acid	Concentration (μM)											
	0 2	2-4	4-7	7-10	10-13	13-16	19-22	25-28	31-34	40-46	52-58	64-70
ala	15	5.2	1.8	0.2	1.8	0.1	0.2	0.2	0.1	0.07	0.1	0.2
gly	6.1	3.1	1.0	0.1	1.3	0.1	0.2	0.4	0.3	0.3	0.2	0.2
val	4.6	1.3	0.5	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0
thr	1.7	0.6	0.2	0.04	0.4	0.06	0.0	0.0	0.0	0.0	0.0	0.0
ser	0.4	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
leu	2.7	0.8	0.3	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.04
ile	2.2	0.7	0.2	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
pro	2.4	0.8	0.2	0.05	0.5	0.0	0.04	0.0	0.05	0.02	0.03	0.03
asp	6.8	1.6	0.6	0.2	0.8	0.08	0.2	0.1	0.1	0.05	0.04	0.1
phe	1.3	0.4	0.09	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
glu	32	10	6.3	3.2	4.4	1.5	2.6	1.5	1.0	0.6	0.5	0.7
β-glu	13	5.7	4.3	3.3	2.7	2.0	2.5	1.6	1.2	1.0	0.7	1.3
lys+tyr	2.8	0.9	0.4	0.0	0.3	0.2	0.2	0.0	0.0	0.0	0.0	0.0
TOTAL	95	32	16	7.0	15	4.1	5.9	3.7	2.8	2.0	1.6	2.5

E. Dissolved Free Amino Acids, Peru Upwelling Station 5A

Amino Acid*	Concentration (μM)											
	0-3	3 6	6-9	9-12	12-15	15-18	18-21	24-27	30-33	36-39	42-45	48-51
ala	0.4	0.2	0.3	0.3	0.2	1.1	0.1	0.2	0.0	0.0	0.04	0.1
gly	0.09	0.2	0.08	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0	0.1
asp	0.5	0.4	0.3	0.5	0.3	0.9	0.3	0.2	0.06	0.1	0.1	0.2
glu	4.1	4.7	2.9	5.2	3.0	6.6	3.4	1.7	0.3	0.4	0.6	0.8
β-glu	3.4	5.3	2.5	4.4	3.8	5.3	3.9	2.6	0.4	0.6	1.1	1.2
TOTAL	9.0	11	6.2	11	7.5	14	7.9	4.7	0.8	1.1	1.8	2.5

*Other amino acid concentrations less than 0.1 μM.

F. Dissolved Free Amino Acids, Peru Upwelling Station 6

Amino Acid	Concentrations (μM) 0-2	2-4	4-6	6-9	9-12	12-15	15-18	18-21	21-24	24-30	30-36
ala	12	39	3.2	0.3	0.3	0.2	0.04	0.3	0.0	0.0	0.0
gly	5.6	18	4.3	0.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0
β-ala*	11	48	3.3	0.2	0.2	0.2	0.0	0.4	0.0	0.0	0.0
thr	2.3	7.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ser	2.6	3.0	0.3	0.2	0.1	0.2	0.3	0.2	0.0	0.0	0.0
leu	2.3	4.6	0.2	0.06	0.07	0.05	0.0	0.0	0.0	0.0	0.0
ile	1.5	2.6	0.2	0.03	0.0	0.0	0.0	0.0	0.0	0.0	0.0
pro	2.0	3.8	0.3	0.06	0.07	0.07	0.0	0.07	0.0	0.0	0.5
asp	12	11	1.6	0.8	0.8	0.6	0.3	0.2	0.06	0.03	0.04
phe	1.8	3.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
glu	39	64	26	18	17	11	3.8	1.3	0.5	0.5	0.5
β-glu	9.5	6.0	5.3	5.5	6.2	5.1	2.1	1.1	0.4	0.5	0.8
lys+tyr	6.9	6.8	0.7	0.3	0.3	0.2	0.1	0.0	0.0	0.0	0.0
TOTAL	110	220	46	27	25	18	6.6	3.5	0.9	1.0	1.3

*Peak contains small amount of valine.

G. Dissolved Free Amino Acids, Peru Upwelling Region Stations 8 and 2A

Amino Acid	Concentration (μM) Station 8 0-2	2-4	4-6	6-8	8-10	10-12	Station 2A 0-2	2-4	4-6	6-8	8-10	10-12
ala	1.5	0.8	1.3	0.7	0.8	1.9	0.4	0.3	0.3	0.3	0.4	0.5
gly	2.1	0.6	2.2	1.2	2.6	3.0	1.1	0.3	0.3	0.4	0.5	0.8
val	*	*	*	*	*	*	0.4	0.2	0.2	0.2	0.1	0.3
β-ala	0.4	0.0	0.3	0.1	0.2	21	**	**	**	**	**	**
thr	0.2	0.08	0.1	0.07	0.2	0.6	0.2	0.08	0.1	0.09	0.1	0.1
ser	0.4	0.2	0.3	0.4	0.4	0.6	0.7	0.4	0.4	0.4	0.6	0.4
leu	0.2	0.1	0.2	0.08	0.2	0.5	0.6	0.1	0.2	0.1	0.1	0.2
ile	0.2	0.07	0.1	0.05	0.1	0.3	0.4	0.09	0.09	0.08	0.1	0.1
pro	0.1	0.04	0.1	0.1	0.09	0.3	0.4	0.09	0.09	0.08	0.05	0.02
asp	0.6	0.4	0.6	0.5	0.5	1.1	0.5	0.2	0.2	0.2	0.3	0.4
phe	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.04
glu	8.1	9.3	9.7	8.3	7.7	7.8	2.3	1.4	1.4	1.0	1.1	1.6
β-glu	2.2	2.4	2.8	2.3	2.3	2.9	0.4	0.3	0.4	0.3	0.3	0.7
lys+tyr	0.3	0.09	0.3	0.0	0.08	0.9	0.5	0.05	0.0	0.0	0.03	0.2
TOTAL	16	14	18	14	15	41	8.0	3.4	3.6	3.1	3.8	5.3

*Included in β-alanine.
**Included in valine.

H. Dissolved Free Amino Acids, Buzzards Bay Station P

Amino Acid	Concentration (μM) BBP6/79 0-2	2-4	4-6	6-10	10-14	14-18	18-22	22-26	30-34	38-42	46-50	54-58
ala	5.4	0.6	0.6	0.5	0.4	0.6	0.5	0.3	0.3	0.4	0.5	0.4
gly	1.7	0.5	0.4	0.4	0.4	0.6	0.4	0.5	0.5	0.7	0.8	0.6
val	0.4	0.1	0.2	0.06	0.0	0.1	0.2	0.2	0.3	0.3	0.3	0.3
thr	0.4	0.0	0.08	0.0	0.0	0.1	0.1	0.1	0.09	0.08	0.1	0.1
ser	0.5	0.0	0.3	0.2	0.2	0.4	0.4	0.4	0.0	0.3	0.4	0.3
leu	0.2	0.08	0.08	0.0	0.07	0.3	0.1	0.1	0.1	0.1	0.1	0.2
ile	0.1	0.0	0.0	0.0	0.0	0.0	0.06	0.07	0.05	0.05	0.06	0.1
pro	0.6	0.0	0.06	0.08	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
asp	2.2	0.7	0.9	0.6	0.5	0.6	0.5	0.4	0.4	0.3	0.4	0.4
phe	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
glu	17	7.8	9.0	6.2	5.0	4.3	3.6	2.5	1.9	1.4	1.7	1.2
β-glu	6.2	5.2	6.2	4.8	4.2	4.4	4.3	3.0	2.7	1.9	2.2	1.5
lys+tyr	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
TOTAL	37	15	18	13	11	11	10	7.6	6.3	5.6	6.5	5.1

Amino Acid	Concentration (μM) BBP6/79 62-66	BBP12/79 0-2	2-5	5-8	8-11	11-14	14-17	17-20	20-23	23-26	26-29	29-32
ala	0.4	0.6	1.3	0.2	0.8	0.4	0.4	0.3	0.2	0.3	0.1	0.1
gly	0.8	0.9	1.0	0.6	0.7	0.5	0.5	0.0	0.0	0.0	0.0	0.0
val	0.4	0.8	0.3	0.0	0.2	0.2	0.3	0.3	0.2	0.2	0.2	0.2
thr	0.1	0.0	0.0	0.0	0.1	0.2	0.2	0.0	0.04	0.07	0.0	0.2
ser	0.3	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0
leu	0.1	0.1	0.0	0.0	0.09	0.07	0.08	0.07	0.06	0.09	0.05	0.06
ile	0.07	0.06	0.0	0.0	0.06	0.0	0.05	0.04	0.05	0.0	0.02	0.0
pro	0.0	0.2	0.08	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
asp	0.3	1.1	1.1	0.5	0.7	0.7	0.5	0.4	0.3	0.2	0.2	0.2
phe	0.0	0.09	0.0	0.0	0.06	0.05	0.0	0.0	0.03	0.0	0.0	0.0
glu	1.1	13	23	14	11	11	7.6	5.5	3.6	2.7	2.3	1.8
β-glu	1.5	15	9 8	9.0	7.5	8.3	8.1	5.8	4.9	3.8	3.4	2.3
lys+tyr	0.0	0.07	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.04
TOTAL	5.0	34	37	25	22	21	18	13	9.4	7.5	6.4	5.2

I. Dissolved Free Amino Acids, Pettaquamscutt River Estuary

Amino Acid	Concentration (μM)										
	0-6	6-12	12-18	18-24	24-30	30-36	36-42	42-48	54-60	66-72	72-84
ala	0.4	0.3	0.4	0.2	0.3	0.3	0.3	0.6	0.6	0.5	0.2
gly	0.2	0.2	0.8	0.4	0.8	0.3	0.2	0.3	0.8	0.6	0.5
val	0.1	0.2	0.2	0.1	0.1	0.3	0.3	0.3	0.4	0.3	0.2
thr	0.08	0.06	0.09	0.04	0.07	0.09	0.1	0.2	0.2	0.1	0.05
ser	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.2
leu	0.09	0.07	0.09	0.08	0.04	0.07	0.1	0.1	0.2	0.1	0.7
ile	0.0	0.0	0.04	0.08	0.0	0.03	0.06	0.07	0.09	0.08	0.04
pro	0.08	0.4	0.1	0.0	0.0	0.0	0.3	0.0	0.2	0.0	0.0
asp	0.4	0.3	0.3	0.2	0.5	0.4	0.5	0.5	0.6	0.4	0.1
phe	0.0	0.0	0.0	0.0	0.0	0.0	0.05	0.06	0.07	0.09	0.02
glu	2.2	1.3	1.8	1.2	2.1	1.1	1.4	1.2	1.6	1.3	0.4
β-glu	1.2	0.9	1.7	0.8	0.8	1.1	0.6	0.5	0.6	0.4	0.1
lys+tyr	0.1	0.1	0.2	0.06	0.09	0.1	0.2	0.1	0.3	0.3	0.0
TOTAL	5.3	3.9	5.8	3.2	4.7	3.8	4.1	3.9	6.6	4.2	2.0

BIOGRAPHICAL NOTE

The author was born on November 23, 1952 in Anchorage, Alaska and lived there until graduation from West Anchorage High School in June 1971. She attended the University of Washington, from which she received a Bachelor of Science degree (magna cum laude) in Chemistry and Chemical Oceanography in 1975. Since 1975 she has been enrolled in the Massachusetts Institute of Technology/Woods Hole Oceanographic Institution Joint Program in Oceanography. She is a member of Phi Beta Kappa, Sigma Xi, AAAS, and the American Chemical Society.

Publications:

Farrington, J. W., S. M. Henrichs, and R. Anderson (1977) Fatty acids and Pb-210 geochronology of a sediment core from Buzzards Bay, Massachusetts. Geochim. Cosmochim. Acta 41, 289-296.

Henrichs, S. M. and J. W. Farrington (1979) Amino acids in interstitial waters of marine sediments. Nature 279, 319-322.

Henrichs, S. M. and J. W. Farrington (in press) Amino acids in interstitial waters of marine sediments: A comparison of results from varied sedimentary enviroments. In: Advances in Organic Geochemistry, 1979 (A. Douglas and J. Maxwell, eds.).

Henrichs, S. M., J. E. Hobbie, R. W. Howarth, and P. Kilham (in preparation) Amino acid cycling in salt marsh sediments.