

To the Graduate Council:

I am submitting herewith a thesis written by Tabbatha Anne Cavendish entitled “Amino Acid Analysis of Marine Sediments, Hauraki Gulf, New Zealand.” I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Geology.

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**AMINO ACID ANALYSIS OF MARINE SEDIMENTS, HAURAKI GULF,
NEW ZEALAND**

A Thesis

Presented for the

Masters of Science

Degree

The University of Tennessee, Knoxville

Tabbatha Anne Cavendish

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DEDICATION

I dedicate my determination and perseverance through my college career and the completion of this thesis to my late uncle, Daryl Cavendish, without whom, I may not have ever had the chance to attempt. As a child growing up in a devastated home, my uncle took so much time out of his life to take me out of the negative environment I was in and to show me that my life didn't have to end up the same way. He put food on my table and clothes on my back. He enlightened me with trips to museums, antique shows, and the great outdoors. He funded my school fieldtrips and he helped me with science and history projects. He shared with me his passions for exotic plants, photography, architecture and travel. He took me on holiday to the Yucatan Peninsula, when I was in 5th grade, to learn about the Mayan culture; he sent me to England in 6th grade with my aunt, Kim, and cousin, Lauren, to learn about the vast interesting history there, including my family's; he took me to Yosemite, NP when I was in 7th grade, creating within me, a lifelong love affair with the outdoors; he even paid for my crazy idea to attend geology field camp in Italy, an experience that was so amazing. He had a wonderful sense of humor, a great smile, and a creative mind. He led by example. He always supported my ideas and always believed in my success.

This world is definitely at a loss without him. I love him and I know that he will always be a part of my life because he is my hero.

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ABSTRACT

Amino acid compositions, bulk organic carbon, total nitrogen, and total organic matter were measured for eight sediment samples from the Hauraki Gulf, New Zealand, in order to assess the source and fate of organic matter in a shallow marine environment. Samples were collected from near-shore and off-shore shore sites, as well as from a site receiving freshwater input from multiple rivers. A comparison of carbon, nitrogen, and amino acid compositions of samples between 1 cm depth (Uhle 2004) and 5 cm depth was used to assess changes in organic matter composition through time. Amino acid analyses in particular should be effective in deciphering organic decomposition through both the presence and abundance of amino acids.

C:N values, which range from 6.5-9.3, are typical for marine sediments and indicate that the primary source for organic matter is marine despite potential terrigenous input from the surrounding environment. This interpretation is supported by carbon isotopes (-20‰ and -22‰ for most sites) which is typical for marine-derived organic materials. C:N ratios do not appreciably vary from 1-5 cm depth, suggesting that there has not been significant depletion of organic matter in these young deposits. Furthermore, both D- and L-amino acids are present at 5 cm depth, supporting minimal organic matter decomposition. However, concentrations of amino acid decreases with depth, and the presence of D-alanine, D-aspartic acid, D-glutamic acid, glycine, and non-protein amino acids, at 5 cm depth, suggests at least a small contribution to the organic carbon source from bacteria. A decrease in $\delta^{15}\text{N}$ with depth further suggests the possibility of bacterial

reworking under anoxic conditions. Combined, these results support hypotheses of microbial reworking of organic matter through time, but are not clearly diagnostic of this process.

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INTRODUCTION

Definition of the parameters that are involved in the formation, composition and preservation of organic matter are critical to understanding biogeochemical cycling and the distribution and genesis of hydrocarbon reservoirs and other economically important organic deposits (Summons 1993). Geochemical characterization of organic deposits requires an understanding of everything from macromolecular scales where genetic information can be extrapolated; to molecular scales where sources, diagenetic information and thermal history can be discerned; to atomic scale, where details of mass transport and pathways are resolved. Characterization of organic matter from modern systems is also critical to understand deposition and early diagenetic processes and to help distinguish between natural processes that would have also affected ancient deposits, and processes that have been anthropogenically influenced.

Organic matter in marine systems is of particular interest since it represents the majority of organic matter that will be preserved within the sedimentary record. Organic matter within marine sediment typically derives from a combination of marine phytoplankton, marine zooplankton, terrigenous vascular plants, and dissolved soil organic matter (Keil and Fogel 2001, Uhle 2004). Early diagenesis of this organic material involves not only the bacterial degradation of labile organic compounds, but also the bacterial synthesis of new organic compounds, termed the microbial biomass component. Recent studies have shown that microbial biomass could represent a significant source of organic matter in marine sediments (Keil and Fogel 2000, Uhle 2004). Unfortunately, it is not yet clear to what extent microbial

reworking, or the bacterial degradation and production of microbial biomass, impacts the fate of organic matter in shallow marine surface sediments. Biomarkers, however, may be a helpful tool for extrapolating bacterial influence on organic matter. Biomarkers are organic molecules that can be structurally related to a specific biological source. For example, particular fatty acids can indicate what types of bacteria and archaea inhabited an environment. Also, the presence of long-chain n-alkanes (C27, C29, and C31) and n-alkanoic acids (C24, C26, and C28) are indicative of input from land-plant waxes, whereas short chain n-alkanoic acids (C12, C14, and C16) are dominant components of algal-derived lipids (Howard 2003). Reactive biochemicals, such as phospholipids can be used to quantify living biomass since they only persist for a short period of time after the death of the source organism (Hedges and Prahl 1993 and references therein). Long-chain alkenone concentrations in marine sediment profiles can be used to reconstruct the productivity history at any given oceanic site (Farrington et al. 1988, cited in Hedges and Prahl 1993). In sum, because the occurrence and relative abundance of certain biomarkers can be used to distinguish organic sources, they provide a critical analytical pathway for the characterization and understanding of this dynamic system (Mayer 1993).

Amino Acids

Amino acids (Fig. 1) are organic compounds that comprise peptides and proteins, which are the building blocks of all organic matter. Proteins comprise more than 50 percent of the dry weight of most organic tissues (Mitterer 1993). As such, amino acids are ubiquitous and can potentially be used as a marker for the presence, abundance, and

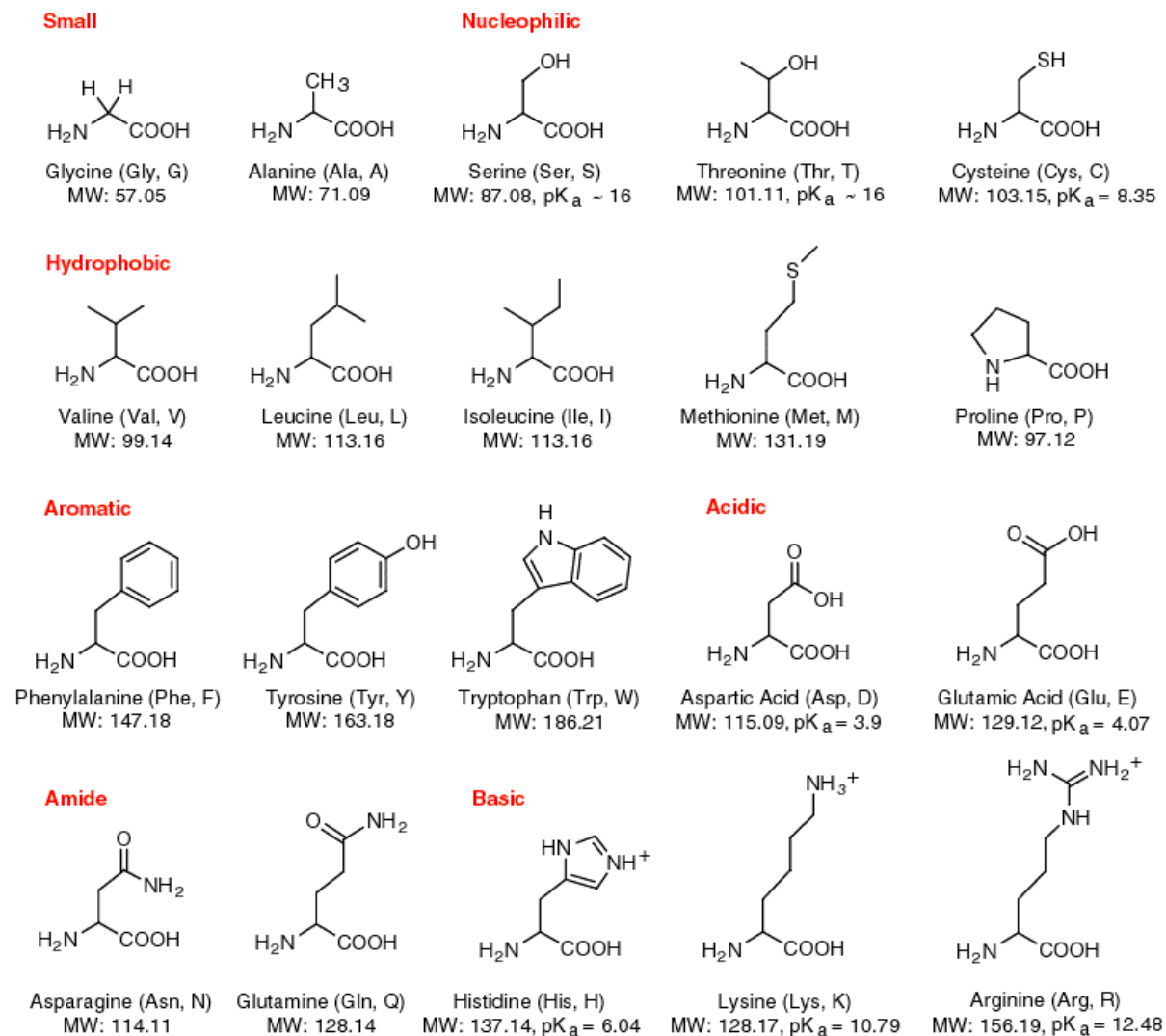


Figure 1. 20 naturally occurring protein-building amino acids with their structures and molecular weights, obtained from BioLabs Inc. Amino acids consist of a central α carbon atom to which is always attached a $-\text{COOH}$ group, an $-\text{NH}_2$ group and a hydrogen atom. The fourth position is the only variable part of the molecule, it is known as the R-group (alkyl group). This R-group, or functional group, is responsible for the differing properties of amino acids. All amino acids except Glycine have a chiral center and occur as either D- or L- isomers.

characterization of organic matter. Amino acids are abundant in both modern and ancient sediments (Cowie and Hedges 1992, Mayer 1993, Suthhof 2000). They account for a significant portion of the organic carbon and nitrogen in surface sediments (Lomstein 1998). Previous work on surface sediments in Dabob Bay, Washington (Cowie and Hedges 1992) revealed that amino acids accounted for 13-37% of the total organic carbon and 30-81% of the total nitrogen, and on average, accounted for 10 and 37% of sedimentary C and N, respectively. Amino acids have been used to determine age and paleothermometry of strata, to explore the sources and fate of organic matter (Macko et al. 1993, Hedges 1993) and to investigate the potential for extraterrestrial origins of organic matter (Engle and Macko 2001). Amino acids, however, as well as lipids and alkane biomarkers, are generally subject to alteration and degradation in surface sediments. Although alteration of amino acids can hinder interpretation of more ancient organic carbon, variation in amino acids concentration and composition in modern sediment can be a powerful tool in understanding depositional and early diagenetic processes that affect sedimentary organic material.

There are twenty protein-building amino acids: Histidine (His), Isoleucine (Ile), Leucine (Leu), Lysine (Lys), Methionine (Met), Phenylalanine (Phe), Threonine (Thr), Tryptophan (Trp), Valine (Val), Alanine (Ala), Arginine (Arg), Asparagine (Asp), Aspartic Acid (Asx or Asp), Glutamine (Gln), Glycine (Gly), Proline (Pro), Serine (Ser), Tyrosine (Tyr), Cysteine (Cys) and Glutamic Acid (Glx or Glu). All these amino acids, except Glycine, are diastereomers and occur as either L- or D-stereoisomers (Fig. 2). Most

living organisms produce predominately L-stereoisomers, and formation of D-amino acids results from either amino acid racemization or bacterial reworking. Amino acid racemization is the process by which L-isomers convert to D-isomers after an organism dies. As a decomposition process, rates of racemization depend primarily on time and temperature. As a time-function, racemization affects the ratio of D/L isomers present in marine organic matter until equilibrium is achieved (D/L = 1). At 0 °C, racemization will take anywhere from 10^4 to 10^6 years, with rates increasing with increasing temperature (Bada 1982). The broad range of time required for racemization reflects the differences in racemization rates among individual amino acids. The presence of D-amino acids in the sediment, therefore, is a function of both the age of the sediment and the temperature of the sediment-water interface at the time that the sediments were deposited. In addition to racemization, D-amino acids also originate through the production of bacterial

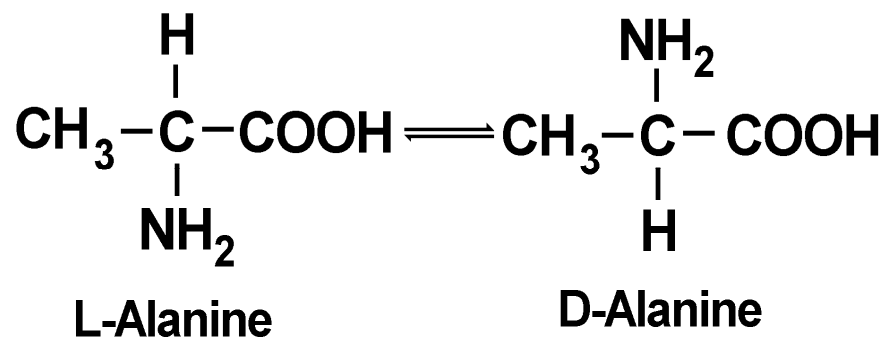


Figure 2. An example of a racemization reaction for the amino acid, alanine. The L- and D- forms of these distereomers have the same chemical properties but differing physical properties. These molecules are mirror images of each other and at an organism's death, one changes to the other until equilibrium is achieved, or the ratio is 1:1. Racemization rates reflect a time-temperature dependent reaction, as well as the original D/L ratio of the preserved organic matter.

peptidoglycan (cell walls) (Schleifer and Kandler 1972, McCarthy 1998, Pedersen 2001, Grutters 2002). The common amino acids produced by bacteria include glycine, D-aspartic acid, D-glutamic acid, D-serine, and D-alanine (Schleifer and Kandler 1972, McCarthy 1998, Grutters 2002, Perez 2003, Lomstein 2006), with D-alanine and D-aspartic acid being the most common. Other D- and L-amino acids, such as D-valine are also produced in peptidoglycan (Perez 2003). The specific amino acids produced vary between bacterial strains. Deciphering microbial contribution to marine organic matter therefore requires knowledge of amino acids present and their relative abundances.

Other factors that can affect amino acids during early diagenesis include hydrolysis and defunctionalism (Mitterer 1993). Hydrolysis is the chemical reaction by which proteins break down into peptides and eventually into free amino acids. This process initiates immediately upon the death of an organism and can result in the leaching of free amino acids from the organic matter deposited, causing a net loss in the concentration of amino acids. Additionally, this early transport of amino acids can result in contamination from adjacent sources. In contrast to hydrolysis, defunctionalism, which includes a set of possible chemical reactions such as decarboxylation, deamination, and other reactions, results in the creation of organic acids, amines, low molecular weight hydrocarbons, and in some cases, amino acids. These new amino acids may be both non-protein amino acids such as α -aminobutyric acid, or the more common protein-building amino acids such as alanine and glycine (Mitterer 1993). These new amino acids add to the total

concentration of amino acids in the sediment and must be distinguished from amino acids produced by bacterial reworking of sedimentary organic material.

Clearly, with the wide variety of amino acids, and their variable forms and functions, there are multiple amino acid fractions that can be studied in the marine environment. These include the dissolved combined amino acids (DCAA), the dissolved free amino acids (DFAA), and total hydrolysable amino acids (THAA). The THAA fraction is the most significant in sediments and includes both the free amino acids and those incorporated within peptides and proteins. This study will focus on the THAA fraction. The THAA fraction includes both DCAA and DFAA, which deposit from the water column or are contained within pore water in the surface sediments, and amino acids produced *in situ* by communities at the sediment-water interface or adsorbed to mineral surfaces.

Origin of D- Amino Acids in Marine Sediments

Because D-amino acids result both from the time-dependent racemization process and from bacterial production and reworking, their distribution should vary depending on the environment of deposition. In deep marine sediments, organic matter is sourced from planktic rainout from the water column. By the time this organic matter has settled to the sediment-water interface, it has undergone extensive degradation. As a result, the organic carbon deposited is relatively nutrient-poor and is not as likely to undergo continued microbial reworking. Also, low deposition rates in deep ocean environments result in a

greater average age of surface sediment (as compared to the shallow marine environment), this greater age is reflected in advancement of the racemization process and the presence of both D-amino acids and non-protein amino acids (Goodfriend 2000).

In contrast to deep sea environments, shallow marine environments are characterized by input of organic carbon from planktonic rain, deposition of terrigenous allochthonous materials, and the *in situ* production of organic carbon from microbial reworking. Although relatively high depositional rates in shallow marine environments result in a generally young age for surface sediments, many recent studies have shown the presence of D-amino acids (Pollock and Kvenvolden 1978, Pedersen 2001, Keil and Fogel 2001, Grutters 2002, Bada 2002, Uhle 2004, Lomstein 2006). The young age of shallow marine sediments suggests that amino acid racemization is not a likely source for these D- amino acids. Rather, several lines of evidence suggest a predominantly bacterial source for D-amino acids found in shallow marine sediments. First, although amino acids are labile and easily altered or removed during early organic diagenesis, bacterial peptidoglycan, the main component of bacteria cell walls, is refractory and can survive in sediment for thousands of years (Jorgenson 1990, McCarthy 1998, Pedersen 2001). The structure of these cell walls contributes to their ability to withstand degradation (McCarthy 1998). Most bacteria are of the Gram positive or negative type. For Gram positive (G+) bacteria, cell walls are thick and contain peptide interbridges that are composed of L-amino acids and D-amino acids, other than D-alanine, and glutamic acid (Pedersen 2001). Most peptidoglycan found in marine sediments, however, is derived from Gram negative

bacteria (G-), which show predominance over Gram positive bacteria in marine sediments under both oxic and anoxic conditions (Pedersen 2001).

Several studies support the idea that bacterially produced peptidoglycan is primarily responsible for the presence of D-amino acids in shallow marine environments. Pollock and Kvenvolden (1978) examined shallow marine sediments at depths of 210 to 230 m, between Tanner and Cortes banks, California, and found a significantly higher concentration of D-aspartic acid, D-alanine and D-glutamic acid with respect to other amino acids. Observed concentrations were comparable to those found in modern soil samples (Salton 1964, cited in Pollock and Kvenvolden 1978). The higher concentration of these specific D-amino acids strongly suggests a bacterial source. In near-shore sediments from Aarhus Bay, Denmark (< 2,300 years old at 4.6 m), the concentration of D-amino acids was also observed to increase with sediment depth, with the highest concentration of D-amino acids occurring at 10 cm depth (Pedersen 2001). This depth correlates with an earlier study at the same location where a maximum bacterial density has been observed (Jorgensen 1990) and suggests that the D-amino acids present are from the bacteria cell walls produced by this population (Pedersen 2001). In a study from Grutters (2002), young sediments and sediments as old as 18 ka from the Atlantic continental margin were analyzed. The D-amino acid concentrations in these sediments also increased with increasing depth. A transport-racemization-degradation model was used to investigate the likelihood that these D-amino acids resulted from racemization. In this model, a two layer diagenetic scenario incorporates factors such as sedimentation,

sediment mixing, degradation, and racemization. Model results suggest that, in this case, racemization was not likely to be a significant source for D-amino acids, even for the older sediments. The increase in D-amino acids with depth was therefore attributed to a bacterial source. In work done on coastal Chilean sediments (~288 yr) from Lomstein (2006), the bacteria-derived D-amino acids were found to increase with increasing water depth, as well as increasing sediment depth. These bacterial D-amino acids accounted for >18% of the THAA in all samples. Concentrations of these specific amino acids increased up to >26% in surface sediments with increasing water depth and up to 59% with increasing sediment depth. They concluded that the large fraction of these D-amino acids originated from their preservation in refractory peptidoglycan residues rather than from racemization based on the concentrations of the D-amino acids, independent estimates of D-amino acid concentrations in selected laboratory bacterial strains, and bacterial counts (Lomstein 2006).

Carbon isotopes of individual amino acids have also been used to infer a bacterial contribution of D-amino acids to organic matter in the shallow marine environment. In work done by Keil and Fogel (2001), the $\delta^{13}\text{C}$ of nine individual amino acids were examined from phytoplankton and zooplankton, estuarine plankton, terrigenous material, marine fecal material, and clay mineral isolates collected along the Washington coast. The isotopic values for glycine and valine were reported to be outside of the range of the sampled materials. This deviation implies a bacterial reworking during early diagenesis (Keil and Fogel 2001). In addition, recent analysis on core-top sediment from Hauraki

Gulf, New Zealand, (Uhle 2004), found D-amino acids to be present in all samples and, since racemization is not expected to be significant at 1 cm depth, bacteria were interpreted to be the likely source. Additionally, the Uhle (2004) study takes the work of Keil and Fogel (2001) a step further by considering the $\delta^{13}\text{C}$ of individual D- and L-amino acids. D-amino acids that originate from racemization should have the same carbon isotopic value as the L-amino acids from which they originate, since there is no fractionation that results from the racemization process (Uhle 2004). By contrast, microbial reworking should be associated with isotopic enrichment (Keil and Fogel 2001). In the Uhle (2001) study, carbon isotope compositions for the D- and L-amino acids analyzed were plotted on a two end-member diagram using marine and terrigenous values from the work of Keil and Fogel (2001). Glycine and leucine at all sites were found to be significantly depleted in ^{13}C relative to typical values for marine and terrestrial sources. The extent of isotopic depletion could not be resolved from mixing of the two end-members, suggesting, instead, a microbial source (Uhle 2004). In addition, the presence of specific non-protein amino acids such as β -alanine is associated with microbial reworking of the organic matter. The concentration of β -alanine found in the Uhle (2004) study is consistent with the concentrations commonly found in coastal marine environments that have experienced a significant amount of microbial diagenesis (Mayer 1993, Uhle 2004). Combined, these studies suggest that bacterial peptidoglycan is commonly preserved in sediments, and that in relatively young (<15,000 years), near-surface marine sediments, amino acid racemization is not likely to be a large contributor of D-amino acids.

The present study represents an expansion of Uhle (2004), which utilized D- and L-amino acids and their carbon isotopes of core-top strata to explore the sources and fate of organic matter in the Haruaki Gulf. Here, samples from a depth of 5 cm are examined to explore changes in microbial contribution with depth (as a proxy for time). The presence of specific amino acids (D-ala, D-asx, D-glx, D-ser, β -ala) and their relative abundances were used to infer bacterial contributions, as well as any differences in bacterial contribution that might reflect differential sources of organic matter (i.e. marine plankton versus terrigenous organic carbon). Specifically, the amino acids and their D/L ratios yielded information about the sediment source and provided information about the influence of bacteria on this sediment. Additionally, this study utilized bulk organic carbon and nitrogen data. The C and N content and their isotopic ratios can help determine sources of the organic material. This data can also yield information about diagenetic activity when compared at different sediment depths (Muller and Suess 1979, cited in Macko et al. 1993). Combined, these analyses provide a detailed look at microbial reworking as a function of location to organic matter source, sediment depth, sediment-water interface temperature, and sedimentation rate. Sample sites within the Haruaki Gulf were chosen to reflect potential differences in environmental or oceanographic situation such as high versus low sedimentation rate, proximity to a land source, and proximity to major channels.

LOCATION OF STUDY & SITE SELECTION

Study Area

Hauraki Gulf is a shallow, partially enclosed sea on the northeast coast of the North Island of New Zealand (Fig. 3). It is bounded to the south and west by the mainland, and partially enclosed to the east by the Coromandel Peninsula and Great Barrier Island (Fig. 3). Waters enter and exit the gulf through channels between the mainland and Little Barrier Island (Jellicoe Channel), between Little and Great Barrier Islands (Cradock Channel) and between Great Barrier Island and the northern tip of Coromandel Peninsula (Colville Channel). The continental shelf along the northeast North Island is typically narrow (<30 km), but widens to ~80 km in the Hauraki region. Sea floor relief is characteristically subdued, except for small areas of basement outcrop and isolated rock pinnacles. Gradients in the central Gulf are as low as 1:2000, steepening in the outer Gulf and reaching 1:400 towards the shelf break, which occurs at about 150-350 m water depth. The Firth of Thames, at the southern end of the Gulf, shoals gradually from 35 m to swampy, estuarine mudflats at a gradient of 1:1500 (Manighetti 1999).

Site Selection

Sample sites within the Hauraki Gulf were chosen with the intention of representing different morphologic and physical environments within the gulf, as well as areas that potentially receive organic matter from different sources. A total of 23 sites were chosen that reflect near-shore and shelf environments, differing positions with respect to organic sources (terrigenous, algal, and marine), and different positions along major

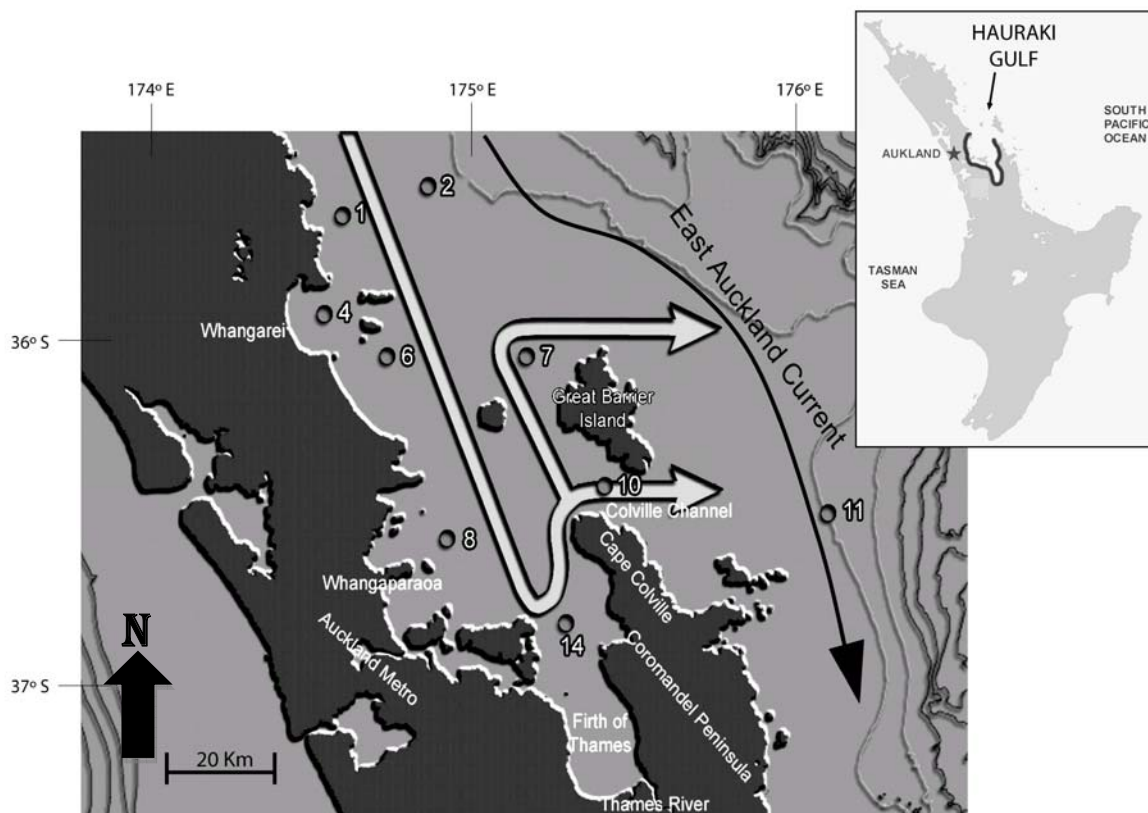


Figure 3. General map of New Zealand and a regional map of the Hauraki Gulf, New Zealand, modified from Uhle (2004). The primary currents, driven by residual tidal flows and augmented by the East Auckland Current, are shown with arrows. Sample sites are indicated by numbers. Sample sites were chosen to represent the variety of nutrient conditions that are present in the gulf. Sites near the mainland are expected to receive nutrients primarily from terrigenous sources, while those farther from the mainland are expected to receive nutrients primarily from marine phytoplankton and zooplankton. Site 14, which sits at the mouth of the Firth of Thames receives a large amount of sediment, and previous work from Uhle (2004) found there to be a terrestrial algal source from $\delta^{13}\text{C}$ analysis. Sites 4 and 6 are considered to have similar nutrient sources and have been used interchangeably in previous work (Uhle 2004).

currents (Fig. 3). This study focuses on eight sites (1, 2, 4, 7, 8, 10, 11 and 14) that were chosen to represent the most diverse environments throughout the gulf. Two sites (2, 11) are located along the shelf break and are expected to contain the greatest proportion of marine-derived organic matter. Because these sites are located farther from the mainland, where sedimentation rates are expected to be lower, these sites are also expected to have the oldest sediment age. Three sites (1, 4, 8) are located close to the mainland, and are expected to receive a nutrient input primarily from both phytoplankton and proximal terrigenous sources. Proximity to terrigenous environments and are also expected to result in higher sedimentation rates and therefore younger sediment ages. Two sites (7, 10) are located near an island and a peninsula, respectively, and may also potentially receive organic matter from both marine and terrigenous sources. These sites may also have a higher sedimentation rate since they are both located near a land source. In addition, sites 1, 4, 8, 7, and 10 are located along major currents in the gulf. Although the sedimentologic response of the currents is unknown, they likely contribute to nutrient input from differing sources. The final site, site 14, is located at the mouth of the Firth of Thames. The Firth of Thames is bound to the west by the mainland and to the east by the Coromandel Peninsula. Two rivers, the Waiohau and the Piako, empty into the Firth at the southern end. The high sediment load of these rivers is expected to provide site 14 with the highest sedimentation rate of all the sites. This site is also expected to have an increased input of terrigenous organic material, such as land plants and dissolved soil organic matter.

METHODS

Sampling Procedures

Sediment samples were recovered from the Hauraki Gulf, New Zealand during a cruise aboard the HMS Tangaroa in December 1999 (summer), using an Ocean Instruments MC-800 multi-corer. The multicore uses up to 12 plastic tubes that are ~60 cm in length and from 5-10 cm in diameter. The tubes collect sediments down to 30 cm and only disturb the first few millimeters (Schulz 2000) making this technique useful for geochemical work on the centimeter scale. After collection, cores were subdivided into centimeter intervals, placed into clean sample jars, and frozen until laboratory analysis (Uhle 2004). For this study, I chose samples representing sediments from 5 cm depth. However, two sites (2 and 11) did not have “5 cm” depths available. These samples may have been used prior to this work. For these sites, the available depth nearest 5 cm was used. For site 2, the sample depth of 6-7 cm was used and for site 11, the sample depth of 5-7 cm was used.

Contamination Protocols

Because amino acids are ubiquitous in nature, extreme care was taken to avoid contamination during sample processing. A lab coat, gloves and protective eyewear were worn during all stages of lab work to aid in the protection of the samples. Lab work areas were cleaned regularly. All glassware was washed with a non-organic detergent (Alconox), then triple-rinsed with tap water, triple-rinsed with ultrapure (Milli-Q Gradient System) water, triple-rinsed with HPLC-Resolve acetone and methanol, triple-

rinsed with ultrapure water again, and then ashed in a furnace at 550 °C for a minimum of 2.5 hours. All disposable glassware was ashed at 550 °C for a minimum of 2.5 hours before use. Ashed glassware was stored sealed with ashed foil to reduce the risk of particulate matter settling into openings. All solvents and acids used were at least HPLC-grade or GC-Resolve grade. The hydrochloric acid used in contact with these samples was ultrapure grade.

Analytical Methods

The sediment samples were first analyzed for amino acid content and relative abundance following the steps outlined in Figure 4 (see description below) which include acid hydrolysis, used to free bound amino acids and derivitization, used to turn the non-volatile amino acids into their TFAA isopropyl ester derivative in preparation for analysis on a Gas Chromatographic Mass Spectrometer (GC/MS). In addition, bulk organic carbon and nitrogen isotopes and bulk organic carbon and nitrogen weight percentages were determined for these samples.

Amino Acid Enantiomer Extractions

Prior to amino acid extraction, sediment samples were freeze-dried (sites 4, 8, 10) or put in an oven (sites 1, 2, 7, 11, 14) at 60 °C for 24 hours to drive off water. A portion of the samples (about half of the available sediment, ~17 to 25 grams) was weighed and powdered for use in this study. Amino acids were extracted from sediment samples by hot acid hydrolysis, as reported in (Uhle 2004). Acid hydrolysis is the most common

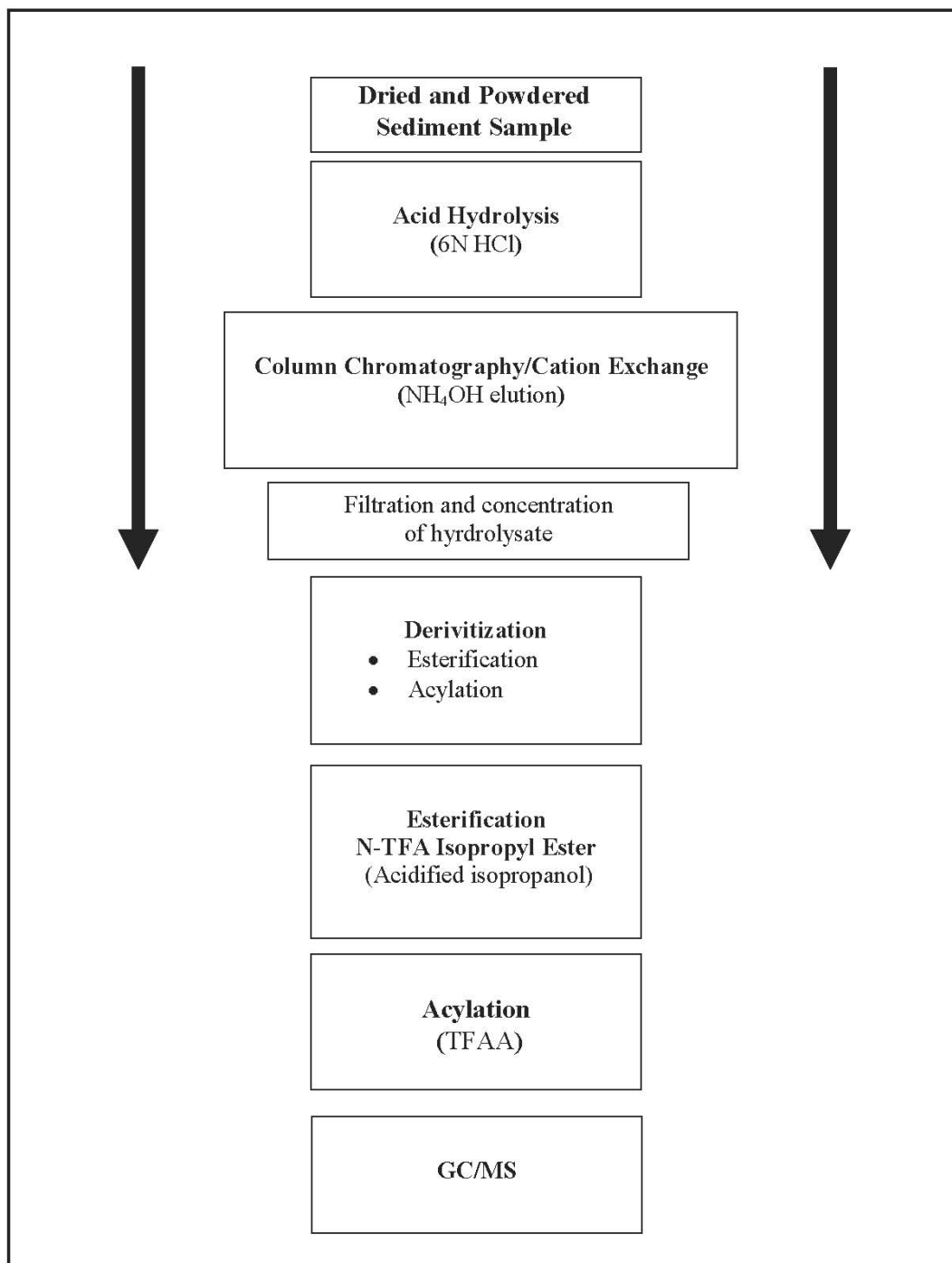


Figure 4. Schematic of methods for amino acid extraction, isolation, and analysis (see text for full explanation).

method for extracting amino acids from sediments and is found to be the best method (Nunn and Keil 2005). First, an excess of 6 M HCl was poured over powdered sediment samples in Kinmax 35 bottles. These bottles were then sealed with a Teflon-lined cap and put into an oven at 110 °C for 24 h to facilitate the breaking of bonds in peptides and proteins to release free amino acids in to the acid hydrolyzate. The hydrolyzate was then vacuum-filtered to remove supernatant, which was dried down using a Rotavap and then re-suspended in 0.1 M HCl. Sample aliquots (typically ~10 mL) underwent removal of anions and non-polar organics (such as oils and waxes) via column chromatography using ammonium hydroxide passed through activated columns. The resulting eluent was then evaporated to dryness using a Rotavap, re-suspended using 0.1 M HCl, and evaporated to dryness again under N₂ at 40 °C. The aliquots were then derivatized to their N-TFA isopropyl ester equivalents (Fig. 5) according to the procedure described by Silfer et al. (1991) to facilitate analysis by Gas Chromatographic/Mass Spectrometry (GC/MS).

Derivitization: esterification and acylation

The hydrolysates were esterified (Fig. 5) with acidified 2-propanol for 1 h at 110 °C and, after quenching the reaction, the solvent was removed by evaporation under a gentle stream of N₂ at 25 °C. Two successive aliquots of dichloromethane were added to each sample and subsequently evaporated to remove excess 2-propanol and water. The amino acid isopropyl esters were then acylated (Fig. 5) with trifluoroacetic anhydride (TFAA) and dichloromethane for 10 min at 110 °C. The reaction was quenched by being placed in

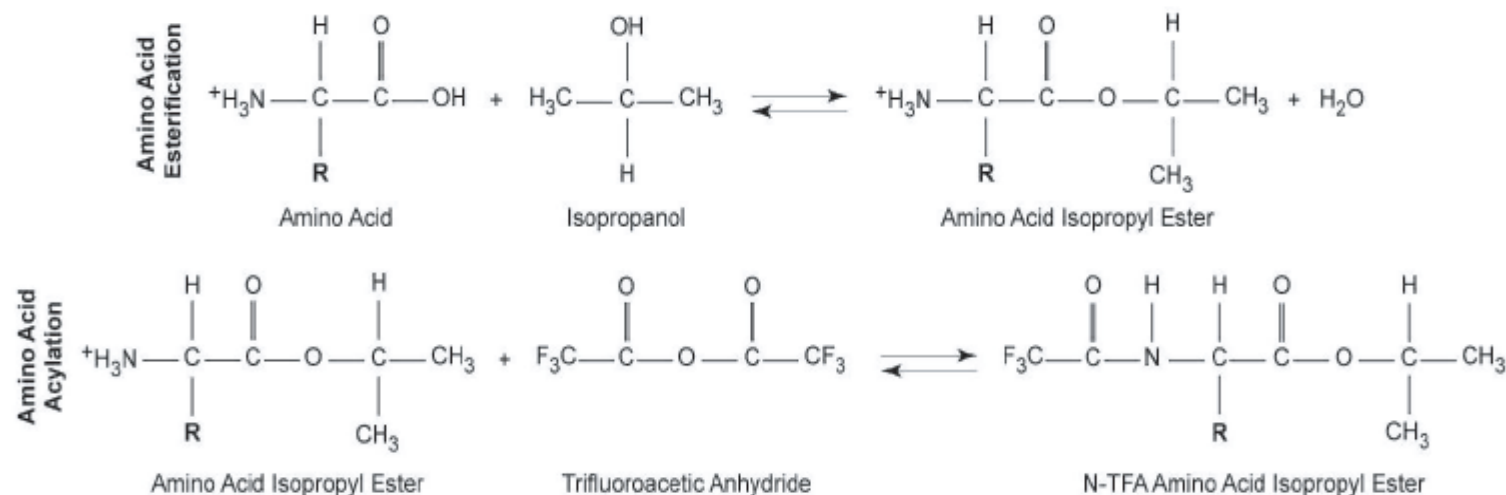


Figure 5. Schematic representation of amino acid esterification and acylation, from Silfer (1991). Formation of the amino acid isopropyl ester results in the addition of three carbon atoms to each carboxyl moiety present. Formation of the N-TFA amino acid isopropyl ester results in the addition of two carbon atoms to each nitrogen atoms in the molecule. These steps aid making the amino acid molecule become volatile in preparation for analysis on the GC/MS.

a freezer for 30 min and then transferred into ampoules. Excess TFAA and dichloromethane was removed by evaporation under N₂ in an ice bath. Formation of the amino acid isopropyl ester results in the addition of three carbon atoms to each carboxyl moiety present; formation of the N-TFA amino acid isopropyl ester results in the addition of two carbon atoms to each nitrogen atoms in the molecule. These steps are necessary for the amino acid molecule to become more volatile in preparation for analysis on the GC/MS. Dried samples were sealed with a Teflon cap and stored in the freezer until preparation for analysis on the GC/MS. Prior to being loaded onto the GC/MS, samples were re-dissolved in 1.0 mL dichloromethane and 100 µL were transferred into an auto sample vial. Each sample was run, in duplicate, on a 6890 GC coupled to a 5973 Quadrupole mass spectrometer to identify and quantify amino acids. The remaining re-dissolved sample was fire sealed in an ampoule and stored in the freezer.

Bulk Carbon and Nitrogen Isotope Analysis

Bulk carbon (¹³C/¹²C) and nitrogen (¹⁵N/¹⁴N) compositions were obtained for the eight Hauraki Gulf samples by S.A. Macko at the University of Virginia using a Fisons NA 1500-R Series 2 elemental analyzer equipped with an auto-sampler and Waters-Isochrom diluter coupled to a Waters Optima isotope ratio mass spectrometer (EA/IRMS). Sediment samples were initially freeze-dried (sites 4, 8, 10) or roasted in an oven at 60 °C for 24 hours (sites 1, 2, 7, 11, 14) to drive off pore water. Samples were then ground in a mortar and pestle to a powder for bulk carbon and nitrogen isotope analysis. Prior to δ¹³C analysis, calcium carbon was removed from the sediment samples by drop-wise addition

of HCl. Samples were then loaded into a combustion reactor where flash combustion occurred at 1020 °C for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses. Combustion products were carried by a stream of ultra pure helium to an oxidation furnace, maintained at 1020 °C, and then to a reduction furnace, maintained at 650 °C. Sample combustion products were then passed through a perchlorate water trap and introduced to a packed column (Porapak QS 50-80 mesh) for separation of N_2 and CO_2 . After column separation, a thermal conductivity detector (TCD) measured concentration of N_2 and CO_2 , and the mass spectrometer measured isotopic composition. All values are reported in δ notation, where $\delta = [(R_{\text{spl}}/R_{\text{st}}) - 1] \times 10^3$, where R_{spl} = isotope ratio of sample and R_{st} = isotope ratio of the standard, in ‰ using the standards PeeDee Belemnite for carbon and atmospheric air for nitrogen.

Data Analysis

Data collected from the GC/MS (Fig. 6, 7) is expressed as a series of peaks, where the x-axis represent elution time, the y-axis represents the magnitude of response, and the area under the curve represents the relative abundance of each amino acid enantiomer. Concentration of each amino acid was determined through comparison of data with a calibration curve created using amino acid standards of known concentration. Ranges of concentration used for the calibration set were determined by preliminary sample runs and previous work on these sediment cores (Uhle 2004). Concentrations determined from the GC/MS represent the concentration of amino acids in solution, in mg/L. Concentrations used for the calibration set (in mg/L) were: 1×10^4 , 7.5×10^3 , 5×10^3 , 2.5×10^3 , 1×10^3 , 750, 500, 250, 100, and 50. Concentrations of amino acids obtained from

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erator   : Tabby
quired   : 27 Aug 2006  23:23      using AcqMethod AMINO27
strument : GC-MS
mple Name: Site1/5cm
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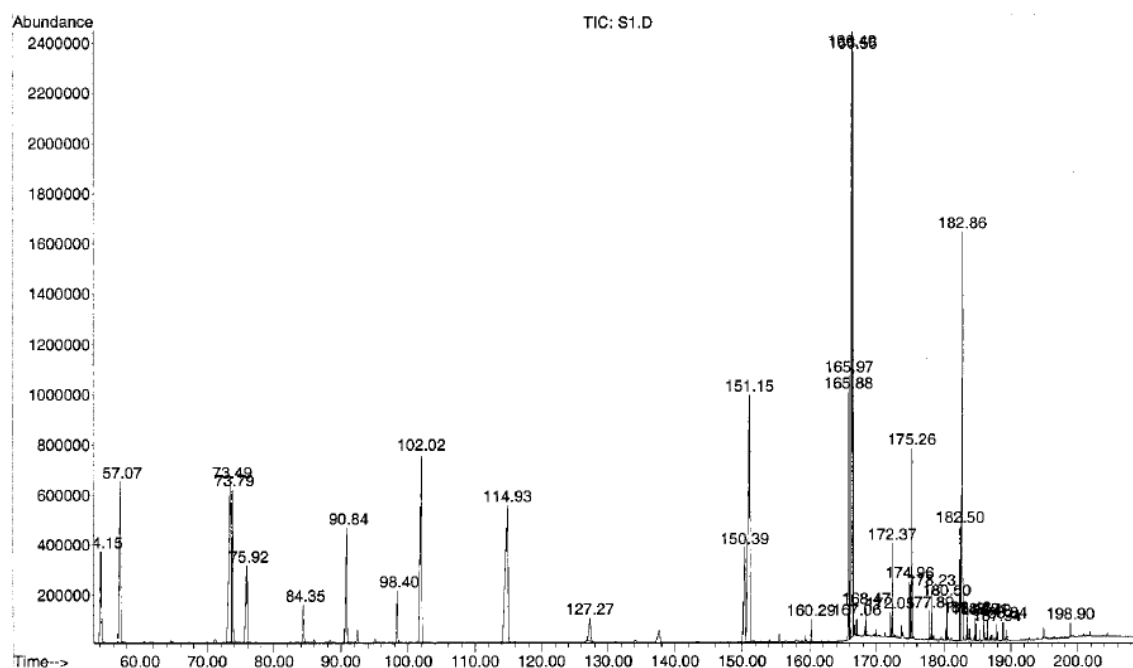


Figure 6. Typical chromatogram of a single run for a Hauraki Gulf sediment sample on the GC/MS. The peaks represent individual D- and L- amino acids. Each amino acid has an expected order of elution that results from the chemical structure of the amino acid. The temperature program is optimized by adjusting temperatures, ramping rates and hold times to achieve well separated peaks. Once the program is optimized, the elution times for each amino acid are determined.

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 Misc :

Vial: 16
 Operator: Tabby
 Inst : GC-MS
 Multiplr: 1.00
 Sample Amount: 0.00

MS Integration Params: autoint1.e
 Quant Time: Aug 30 14:31:50 2006

Quant Results File: AMINO27.RES

Quant Method : C:\MSDCHEM\1\METHODS\AMINO27.M (Chemstation Integrator)
 Title : D & L Amino Acids
 Last Update : Wed Aug 30 14:29:03 2006
 Response via : Initial Calibration
 DataAcq Meth : AMINO27

Internal Standards	R.T.	QIon	Response	Conc	Units	Dev(Min)

Target Compounds						Qvalue
1) D-Alanine, N-(trifluoroace	54.15	140	17364169	2967.88	mg/L	97
2) L-Alanine TFA isopropyl es	57.07	140	20201990	Below Cal	#	35
3) D-2-Aminobutyric Acid	67.26	154	84046	194.07	mg/L #	45
4) D-Valine, N-(trifluoroacet	71.14	168	299540	197.53	mg/L #	64
5) L-Valine, N-(trifluoroacet	73.43	168	10424271	547.00	mg/L #	51
6) Glycine	73.79	126	25009244	9156.04	mg/L #	49
7) .beta.-Alanine, N-(trifluo	0.00	168	0	N.D.		
8) D-Serine	90.84	TIC	59192651	13998.28	mg/L	100
9) L-Serine	0.00	TIC	0	N.D.		
10) D-Leucine, N-(trifluoroace	98.40	69	2174495	1130.50	mg/L	89
11) L-Leucine TFA Isopropyl es	0.00	69	0	N.D.		
12) D-Aspartic Acid	150.38	43	6678386	2758.35	mg/L #	87
13) L-Aspartic Acid	151.15	43	21418189	12319.56	mg/L #	71
14) D-Glutamic Acid	165.97	198	9400647	3137.06	mg/L	96
15) L-Glutamic Acid TFA Isopro	166.55	198	34197772	11221.47	mg/L #	87

(#) = qualifier out of range (m) = manual integration (+) = signals summed
 S1.D AMINO27.M Wed Aug 30 14:31:54 2006

Figure 7. Typical data file corresponding to the chromatogram in Figure 6. “R.T.” stands for retention time and is used to identify which amino acid the peak represents. “Conc” is the raw concentration the GC/MS obtains in mg/L.

the GC/MS were then back calculated to concentration measured in milligrams per gram dry weight sediment (mg gdw^{-1}) for data reporting. Samples in this study were not analyzed for all known amino acids. Amino acids that were considered include D- and L-alanine (ala), D-2-aminobutyric acid (D-2-aba), beta-alanine (b-ala), D- and L-valine (val), glycine (gly), D- and L-serine (ser), D- and L-leucine (leu), D- and L-aspartic acid (asx), and D- and L-glutmaic acid (glx). These selected amino acids were chosen as the most representative for identifying bacterial sources (Bada 1982, Pedersen 2002, Keil and Fogel 2001, and Uhle 2004).

The elution order for all D- and L-amino acids is known, and is used when running standards to obtain an actual elution time for each amino acid for the temperature program used on a specific GC/MS. Once the elution times are determined, the peaks can properly be identified (Table 1). In addition, it is necessary to optimize the GC/MS temperature program in order to obtain peaks that are well separated in order for the GC/MS to be able to accurately identify the peaks. For this study, the final temperature program started with an oven temperature of 35 °C and ramped up to 90 °C at 1 °C per 0.5 minutes. After holding at 90 °C for 30 minutes, the temperature was ramped up to 210 °C at a rate of 1 °C every 2.0 minutes and was then held at 210 °C for 10 minutes. The inlet was set to 185 °C and 12.8 psi. Each sample had a run-time of 210 min with this temperature program. The 1998 National Institute of Standards and Tests (NIST) library of amino acids was loaded onto the GC/MS and was used for identification of

Table 1. The elution times for each amino acid enantiomer was determined by running a standard set of the D- and L-amino acids to be used in this study. The standards were run 4 times and an average taken of the precise time each amino acid eluted. These times were then used to identify amino acids from Hauraki Gulf sediment samples.

Amino Acid stds	STDmix 07.10.06 run1	STDmix 07.10.06 run2	STDmix 07.10.06 run3	STDmix 07.10.06 run4	AVG elution time
D-ala	54.22	54.26	54.26	54.24	54.25
L-ala	57.25	57.27	57.27	57.24	57.26
D-2-aba	67.44	67.48	67.48	67.47	67.47
D-val	71.38	71.39	71.38	71.38	71.38
L-val	73.43	73.45	73.45	73.47	73.45
gly	73.72	73.75	73.75	73.75	73.74
β-ala	84.58	84.61	84.61	84.61	84.60
D-ser	90.91	90.96	90.99	90.97	90.96
L-ser	92.69	92.718	92.746	92.718	92.72
D-leu	98.59	98.6	98.62	98.61	98.61
L-leu	102.25	102.28	102.27	102.29	102.27
D-asf	150.45	150.46	150.47	150.48	150.47
L-asf	150.99	151.02	151.01	151.04	151.02
D-glu	165.97	165.98	165.98	165.99	165.98
L-glu	166.46	166.47	166.47	166.48	166.47

peaks. A second library was created from the laboratory standards, which was also used to aid in peak identification.

Occasionally, unidentified peaks occurred that did not correspond to an expected elution time of an amino acid. Such peaks are considered to represent contamination of the sample, most likely by volatiles residing in the GC/MS from a previous sample run. The contamination problem was reduced by running a temperature program designed to burn off any materials on the column or in the injection site. This clean run used dichloromethane at high temperature (220 °C) to burn off any residue. Then, a blank sample was run, using dichloromethane run at the temperature profile used for the samples, to ensure absence of any substance in the column the injection site.

RESULTS

Data presented in this study included amino acid concentrations (in mg gdw⁻¹) for 8 Hauraki Gulf sites at depths of 1 and 5-7 cm, bulk organic carbon and total nitrogen concentration (in weight %), bulk carbon and total nitrogen isotopic compositions (in ‰), and total organic matter (TOM); (in weight %). Amino acid concentrations at 1 cm were obtained from Uhle (2004). Total organic matter for depths and carbon and nitrogen data at 1 cm was obtained from Dr. Scott Nodder at the National Institute of Water and Atmospheric Research. Amino acid concentrations at 5 cm were obtained for the present study at the University of Tennessee. Bulk organic carbon and nitrogen data at 5 cm depth was determined for the present study by S.A. Macko at the University of Virginia. For each result, a prefix is used with each site number to indicate if the location of the site is near-shore (NS), off-shore (OS), or in the Firth of Thames (FOT).

Amino acid concentrations

Amino acid concentrations were normalized to TOM for each corresponding depth and are reported in Table 2. The concentrations of amino acids at 1 cm are compared to those at 5 cm and are illustrated in Figure 8. D/L ratios for 1 and 5 cm depth are reported in Table 3 and illustrated in Figure 9. Hauraki Gulf samples contain both L- and D-amino acids at all sites and all depths examined. At 1 cm, the average concentration of all

Table 2. D-and L-amino acid concentrations measured in mg/gdw for all sites evaluated at 1 and 5* cm. Dashes indicate amino acids that were not evaluated at that that depth.

*Sites 2 and 11 did not have sediment samples have sediment samples available for the depth of 5 cm. Therefore, for sties 2, a sample was used from 6-7 cm depth, and for site 11, a sample was used from 5-7 cm depth.

Location Type	New Name	Site #	Depth (cm)	D-Ala	L-Ala	γ -Aba	D-Val	L-Val	Gly	β -Ala	D-Ser	L-Ser	D-Leu	L-Leu	D-Asx	L-Asx	D-Glx	L-Glx
MARINE	OS-2	2	1cm	0.78	1.89	-	0.00	1.04	3.03	0.51	-	-	0.00	1.27	1.04	1.78	0.91	1.63
			6-7cm	0.14	0.67	0.05	0.06	0.71	0.97	0.19	0.89	0.25	0.08	0.59	0.25	2.47	0.16	0.94
	OS-11	11	1cm	0.65	0.91	-	0.00	0.71	2.31	0.42	-	-	0.00	0.63	0.95	1.77	0.69	1.41
			5-7cm	0.31	0.73	0.07	0.07	0.65	1.16	0.19	0.96	0.31	0.16	0.66	0.57	2.10	0.32	1.24
TERRIGENOUS	NS-1	1	1cm	0.98	2.22	-	0.00	1.26	4.03	0.59	-	-	0.00	1.28	1.31	2.48	0.70	1.61
			5cm	0.60	1.20	0.06	0.08	1.42	1.51	0.26	1.99	0.34	0.30	1.34	0.55	4.02	0.55	1.83
	NS-7	7	1cm	0.44	1.01	-	0.00	0.73	3.12	0.31	-	-	0.00	0.31	1.17	2.22	0.60	2.07
			5cm	0.00	0.08	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.10	0.10
	NS-10	10	1cm	0.77	1.06	-	0.00	0.91	2.02	0.25	-	-	0.00	0.96	0.78	1.42	0.56	1.10
			5cm	0.05	0.15	0.00	0.00	0.16	0.22	0.06	0.27	0.16	0.00	0.12	0.08	0.54	0.20	0.21
	NS-4	4	1cm	0.82	2.14	-	0.00	1.51	3.59	0.43	-	-	0.00	1.61	1.47	2.51	1.06	1.90
			5cm	1.03	2.22	0.07	0.06	0.58	0.00	0.50	2.78	0.55	0.44	1.89	0.76	5.41	0.85	3.29
FOT	NS-8	8	1cm	0.50	1.78	-	0.00	1.43	2.96	0.36	-	-	0.00	1.30	0.99	1.91	0.89	1.43
			5cm	0.10	0.55	0.00	0.05	0.51	0.86	0.21	0.63	0.24	0.07	0.38	0.18	1.78	0.13	0.68
	FOT-14	14	1cm	0.72	2.37	-	0.00	1.99	4.02	0.66	-	-	0.00	2.03	1.36	2.92	1.18	2.73
			5cm	0.29	0.81	0.05	0.00	0.92	1.17	0.16	0.99	0.30	0.13	0.68	0.21	1.77	0.24	1.12

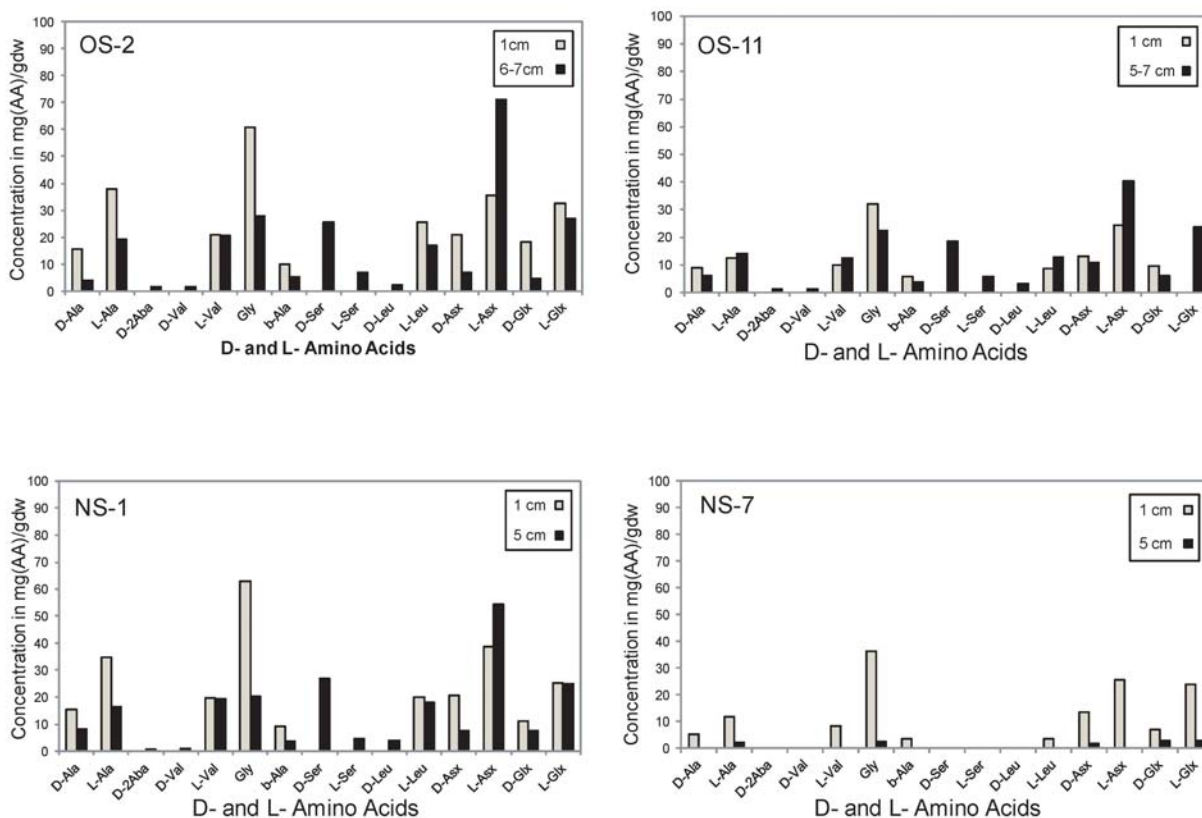


Figure 8. Concentration of D- and L-amino acids at Site 2, 11, 1, and 7 at 1 cm and 5 cm. Sites 2 and 11 are both off-shore samples. Sites 1 and 7 are near-shore samples but may have a mixture of terrigenous and marine sources. Most amino acids concentrations decrease with depth from 1 cm to 5 cm. D-2-aminobutyric and Serine were not evaluated at 1 cm and therefore show no values at 1 cm.

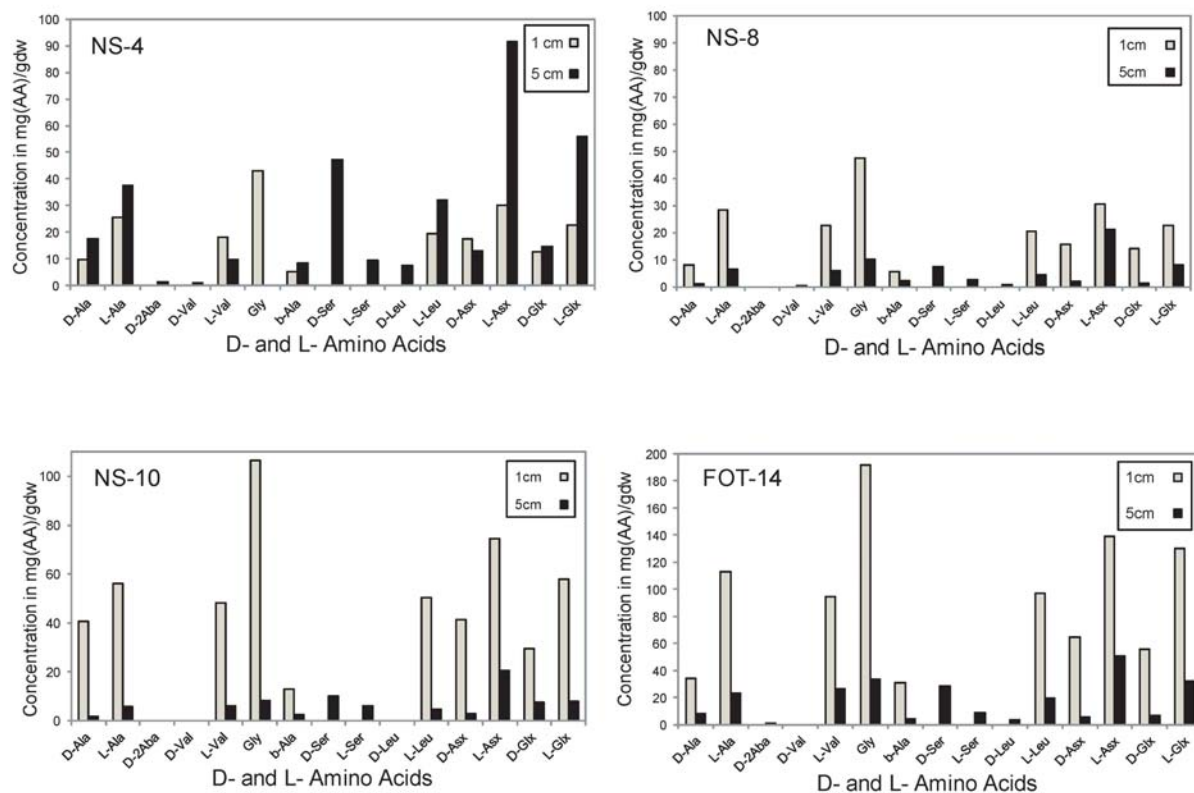


Figure 8. (continued). Concentration of D- and L-amino acids at Site 4, 8, 10 and 14 at 1 cm and 5 cm. Sites 4, 8, and 10 are near-shore samples. Site 10 is located in the Coville Channel and may receive nutrients from surrounding sources. Site 14 is located at the mouth of the Firth of Thames and may receive fresh water organic matter. Most amino acids concentrations decrease with depth from 1 cm to 5 cm. D-2-aminobutyric and Serine were not evaluated at 1 cm and therefore show no values at 1 cm.

amino acids examined is 28.21 mg gdw⁻¹, and decreases to 11.79 mg gdw⁻¹ at 5 cm. At 1 cm, the average concentration of all L-amino acids is 38.52 mg gdw⁻¹ and the average concentration of D-amino acids is 12.58 mg gdw⁻¹. At 5 cm, the average concentration of all L-amino acids is 21.66 mg gdw⁻¹ and the average concentration of D-amino acids is 4.42 mg gdw⁻¹. Glycine is the most abundant amino acid present at all sites at 1 cm depth and L-aspartic acid is the dominant amino acid present at all sites, except NS-7, at 5 cm depth. The average concentration of glycine is 72.54 mg gdw⁻¹ at 1 cm and 15.65 mg gdw⁻¹ at 5 cm. The average concentration of L-aspartic acid is 40.00 mg gdw⁻¹ at 1 cm and 15.60 mg gdw⁻¹ at 5 cm. For the non-protein amino acid, β -alanine, the average concentration is 10.46 mg gdw⁻¹ at 1 cm and 3.84 mg gdw⁻¹ at 5 cm. NS-7 has the lowest overall amino acid concentrations and FOT-14 has the highest overall concentrations. In most cases, amino acid concentrations decrease from 1 to 5 cm depth, where NS-10 having the largest overall decrease. However, NS-4 shows a significant increase in most amino acids analyzed with depth, including the non-protein amino acid β -alanine, and OS-11 also shows an increase with depth. Amino acid data at 5 cm are not statistically significant since they were only run in duplicate.

D/L ratios

D/L ratios were determined for alanine, valine, aspartic acid, glutamic acid, and leucine. Serine was not analyzed at 1 cm and is therefore not included in the ratio analysis. D-2-aminobutyric acid and β -alanine are also not used here since they are non-protein amino acids and do not have D- and L- forms. The D/L ratio for alanine increases from 1 to 5

cm in sites 1, 4, and 14 (Table 3, Fig. 9) and decreases in sites 2, 7, 8, 10, and 11 (Table 3). The D/L ratio for valine increases from 1 to 5 cm in sites 1, 2, 4, 8 and 11 (Table 3, Fig. 9) and doesn't change in sites 7, 10, and 14 (Table 3). The D/L ratio for leucine increases from 1 to 5 cm in sites 1, 2, 4, 8, 11, and 14 (Table 3, Fig. 9) and doesn't change in sites 7 and 10 (Table 3). The D/L ratio for aspartic acid increases from 1 to 5 cm in site 7 (Table 3, Fig. 9) and decreases in sites 1, 2, 4, 8, 10, 11, and 14 (Table 3). The D/L ratio for glutamic acid increases from 1 to 5 cm in sites 7 and 10 (Table 3, Fig. 9) and decreases in sites 1, 2, 4, 8, 11, and 14 (Table 3).

In both off-shore sites (2 and 11), as well as near-shore site 8, the D/L ratios follow the same pattern, an increase for valine and leucine and a decrease for alanine, aspartic acid, and glutamic acid. In near-shore sites, sites 1 and 4 follow the same pattern, an increase in alanine, valine, and leucine and a decrease in aspartic acid and glutamic acid. There are no other correlations observed between off-shore sites, near-shore sites, or the Firth of Thames.

Bulk Organic Carbon and Total Nitrogen

Bulk carbon and nitrogen data for the 8 Hauraki Gulf sites at 1 and 5 cm are reported in Table 4 and illustrated in Figures 10-17. There is a positive correlation between weight % carbon and nitrogen at both 1 and 5 cm (Fig. 10) and their values plot on a line similar to the Redfield Ratio (Rullkötter 2000, and references therein). NS-10 has the lowest % C and N values for both depths (Table 4). There does not appear to be any significant correlation between site

Table 3. D/L amino acid ratios for each site at 1 and 5 cm. Arrows indicate an increase or decrease of the D/L ratio with depth from 1 to 5 cm. An “X” indicates no change.

Off-Shore								
S-2	1cm	6-7cm	ΔD/L		S-11	1cm	5-7cm	ΔD/L
Ala	0.4138	0.2091	↓		Ala	0.7162	0.4245	↓
Val	0.0000	0.0845	↑		Val	0.0000	0.1145	↑
Leu	0.0000	0.1377	↑		Leu	0.0000	0.2400	↑
Asx	0.5872	0.1001	↓		Asx	0.5347	0.2714	↓
Glx	0.5600	0.1758	↓		Glx	0.4870	0.2551	↓
Near-Shore								
S-1	1cm	5cm	ΔD/L		S-7	1cm	5cm	ΔD/L
Ala	0.4417	0.4970	↑		Ala	0.4348	0.0000	↓
Val	0.0000	0.0563	↑		Val	0.0000	0.0000	X
Leu	0.0000	0.2253	↑		Leu	0.0000	0.0000	X
Asx	0.5299	0.1355	↓		Asx	0.5263	#DIV/0!	↑
Glx	0.4368	0.2981	↓		Glx	0.2887	1.0232	↑
S-8	1cm	5cm	ΔD/L		S-4	1cm	5cm	ΔD/L
Ala	0.2818	0.1851	↓		Ala	0.3810	0.4625	↑
Val	0.0000	0.0998	↑		Val	0.0000	0.0979	↑
Leu	0.0000	0.1755	↑		Leu	0.0000	0.2330	↑
Asx	0.5169	0.1026	↓		Asx	0.5854	0.1398	↓
Glx	0.6250	0.1909	↓		Glx	0.5591	0.2584	↓
S-10	1cm	5cm	ΔD/L	Firth of Thames	S-14	1cm	5cm	ΔD/L
Ala	0.7253	0.3268	↓		Ala	0.3048	0.3599	↑
Val	0.0000	0.0000	X		Val	0.0000	0.0000	X
Leu	0.0000	0.0000	X		Leu	0.0000	0.1991	↑
Asx	0.5537	0.1418	↓		Asx	0.4651	0.1159	↓
Glx	0.5106	0.9547	↑		Glx	0.4298	0.2136	↓

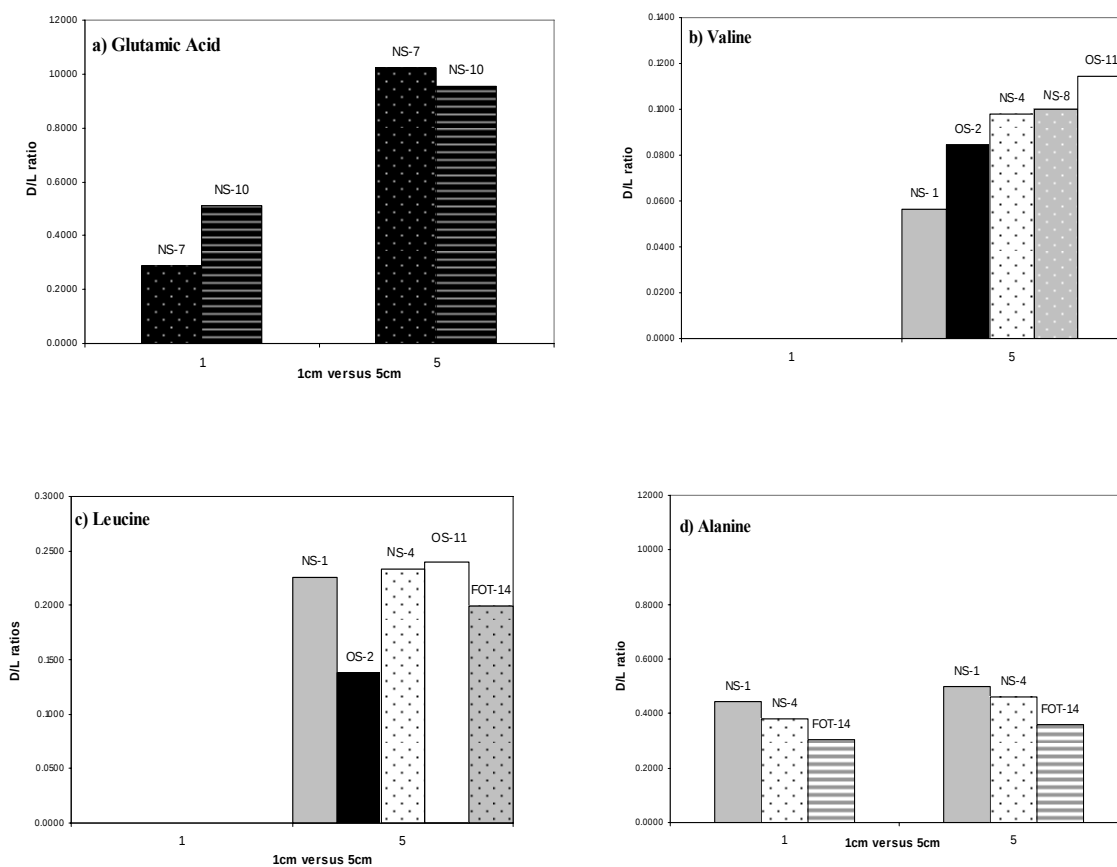


Figure 9 (a, b, c, d). Bar graph showing the D/L ratios for a) glutamic acid b) alanine c) leucine and d) valine. The sites and amino acids illustrated here are all that show an increase in D/L ratios from 1 to 5 cm.

Table 4. Water depth, coordinates, general environmental characteristics, average weight percent of total organic matter, bulk organic carbon and total nitrogen isotopic data, and molar percents for carbon and nitrogen of Hauraki Gulf samples are presented. Bulk C and N data at 5 cm was obtained for the present study by Dr. S.A. Macko at the University of Virginia; total organic matter for all depths and bulk C and N at 1 cm was obtained from Dr. Scott Nodder at the National Institute of Water and Atmospheric Research.

Site Name	Site #	Water Depth (m)	Lat.	Long.	Location Characteristics	Avg. TOM (%)	$\delta^{13}\text{C}$ (1 cm)	$\delta^{13}\text{C}$ (5 cm)	$\delta^{15}\text{N}$ (1 cm)	$\delta^{15}\text{N}$ (5 cm)	%C (1 cm)	%C (5 cm)	%N (1 cm)	%N (5 cm)	C:N (1 cm)	C:N (5 cm)
OS-2	2	153	35° 37.01'	174° 54.48'	off-shore	4.09	-20.98	-21.37	8.15	3.26	0.71	0.54	0.08	0.09	8.74	6.00
OS-11	11	335	36° 24.90'	176° 07.00'	off-shore	5.04	-20.65	-21.53	9.41	4.36	0.79	0.52	0.11	0.08	7.4	6.50
NS-1	1	82	35° 41.87'	174° 39.03'	near-shore	6.14	-20.56	-21.89	7.38	2.97	1.25	0.76	0.17	0.11	7.53	6.91
NS-7	7	85	36° 00.01'	175° 13.25'	near-shore	6.49	-19.74	-21.32	7.81	2.55	1.49	1.09	0.2	0.16	7.39	6.81
NS-4	4	40	-	-	near-shore	7.15	-20.2	-20.16	5.36	5.07	0.52	1.24	0.07	0.17	7.46	7.29
NS-8	8	28	36° 33.06'	174° 53.29'	near-shore	8.92	-20.36	-20.32	9.45	6.73	1.72	1.44	0.24	0.21	7.14	6.86
NS-10	10	41	36° 23.52'	175° 25.02'	near-shore	2.03	-15.52	-19.36	10.66	2.71	0.32	0.28	0.04	0.03	8.93	9.33
FOT-14	14	32	36° 45.83'	175° 17.61'	near-shore/river	2.75	-21.83	-21.56	5.07	5.30	0.67	1.56	0.08	0.21	7.9	7.43

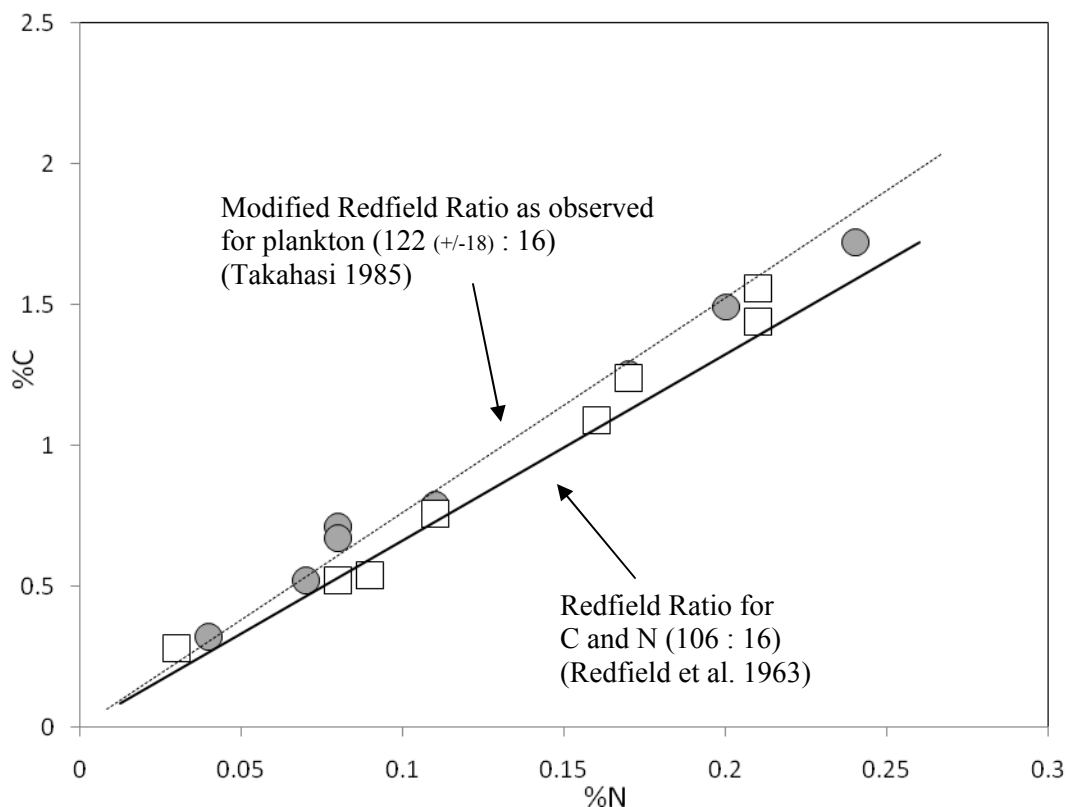


Figure 10. %C versus %N. Filled circles are samples from 1 cm depth (Uhle 2004) and open squares are samples from 5 cm depth. Carbon and nitrogen content are positively correlated and fall on line that is between the Redfield Ratio and the modified Redfield Ratio that has been observed for marine plankton, suggesting marine organic matter is the primary source for preserved organic matter. There are no observable trends among site locations or between depths within a site.

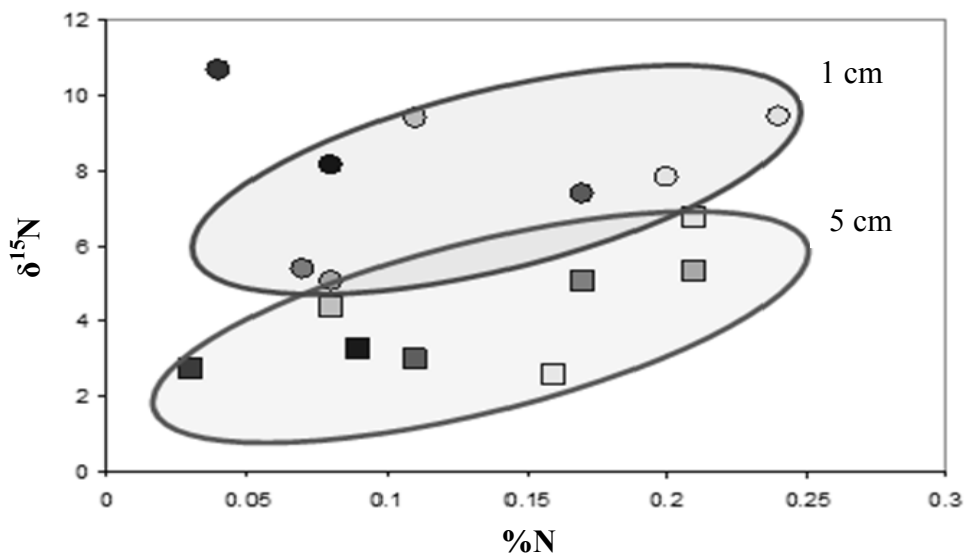


Figure 11. $\delta^{15}\text{N}$ versus the weight % N. In general, the samples at 1 cm (circles) are more enriched in ^{15}N than those at 5 cm depth (squares), which may suggest bacterial reworking.

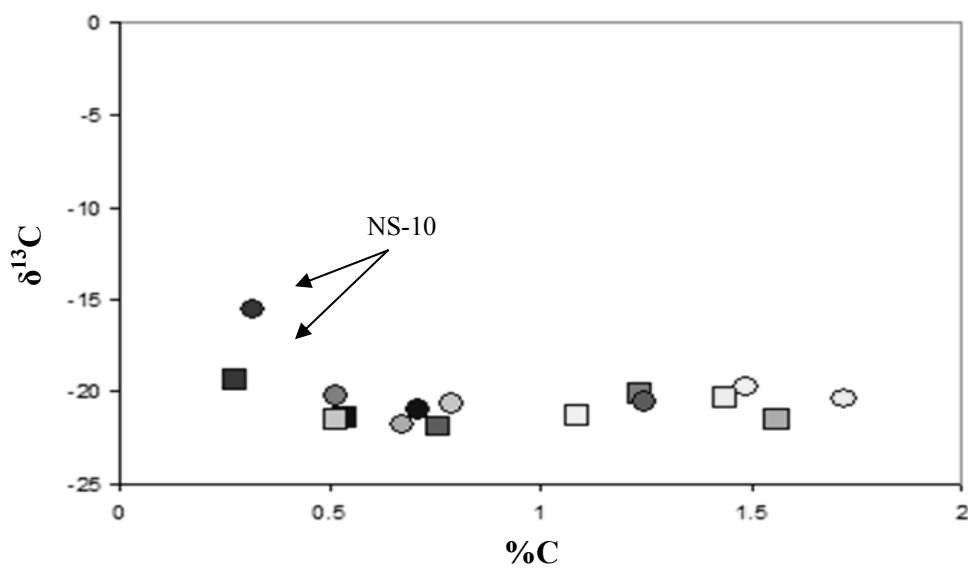


Figure 12. $\delta^{13}\text{C}$ versus weight % C. Most samples range between -20 ‰ and -22 ‰, which is consistent for marine plankton and surface sediments (Rullkötter 2000). NS-10, however, is more enriched in ^{13}C suggesting input from a terrigenous source.

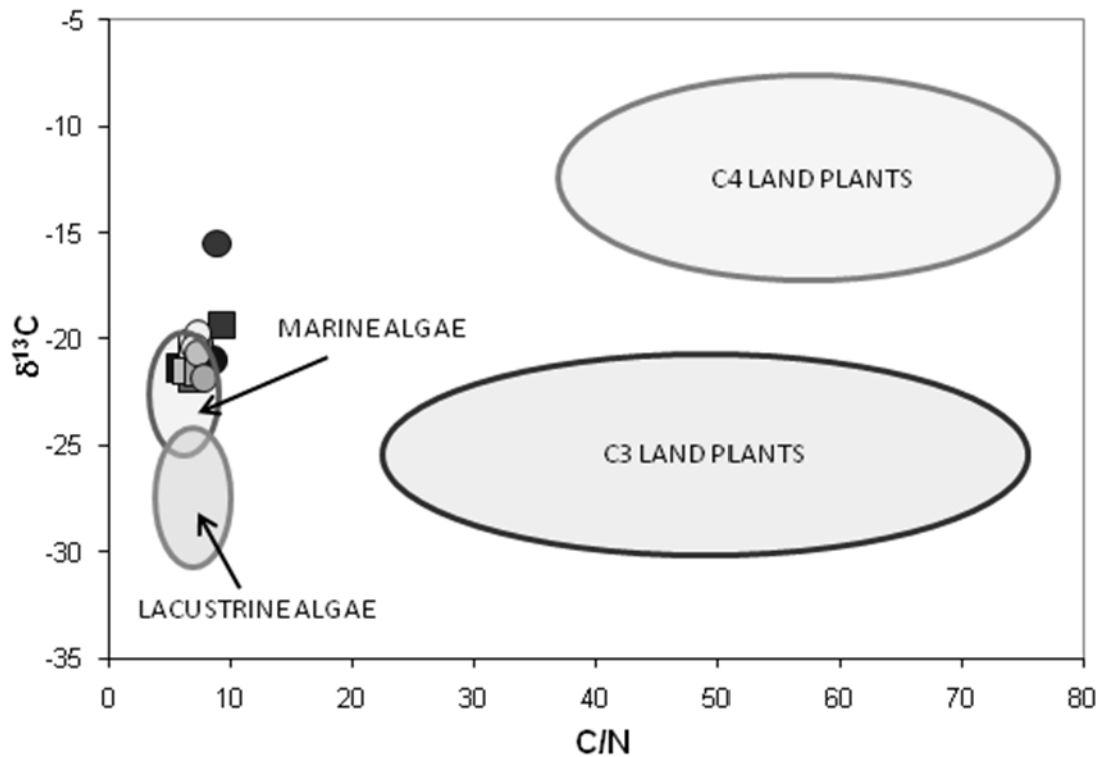


Figure 13. $\delta^{13}\text{C}$ versus C/N ratio with identified organic matter source fields, modified from Meyer (1994). Most Hauraki Gulf samples plot within the marine algae field. Those plotting outside the field tend toward the C4 Land Plant field. Circles represent measurements made at 1 cm; squares represent measurement from 5 cm depth.

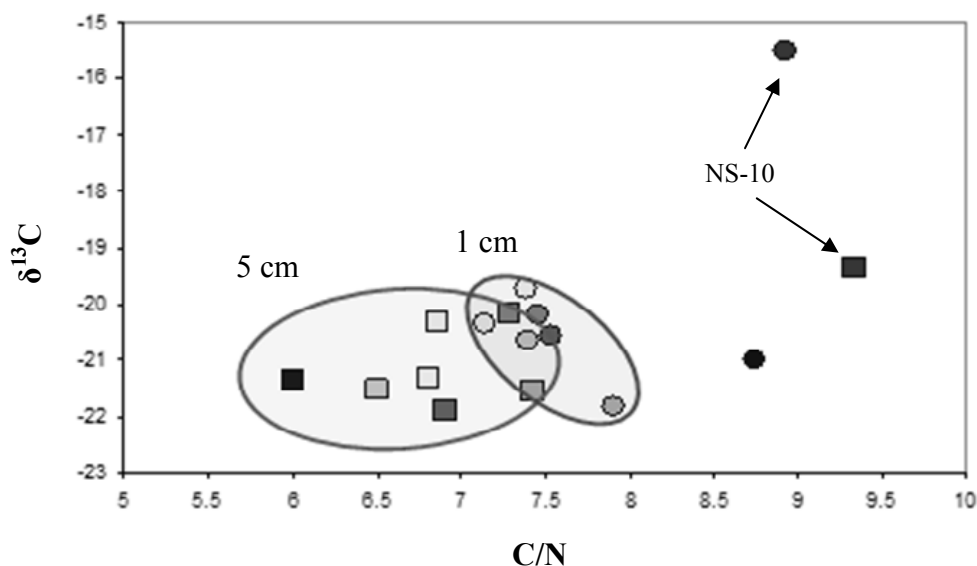


Figure 14. $\delta^{13}\text{C}$ versus C/N ratio. Samples at 1 cm depth (circles) have generally higher C/N values than samples at 5 cm (squares). However, NS-10 at both depths and OS-2 at 1 cm have relatively higher C/N values than all other samples. Typical marine values range from 6-9. (See discussion in text).

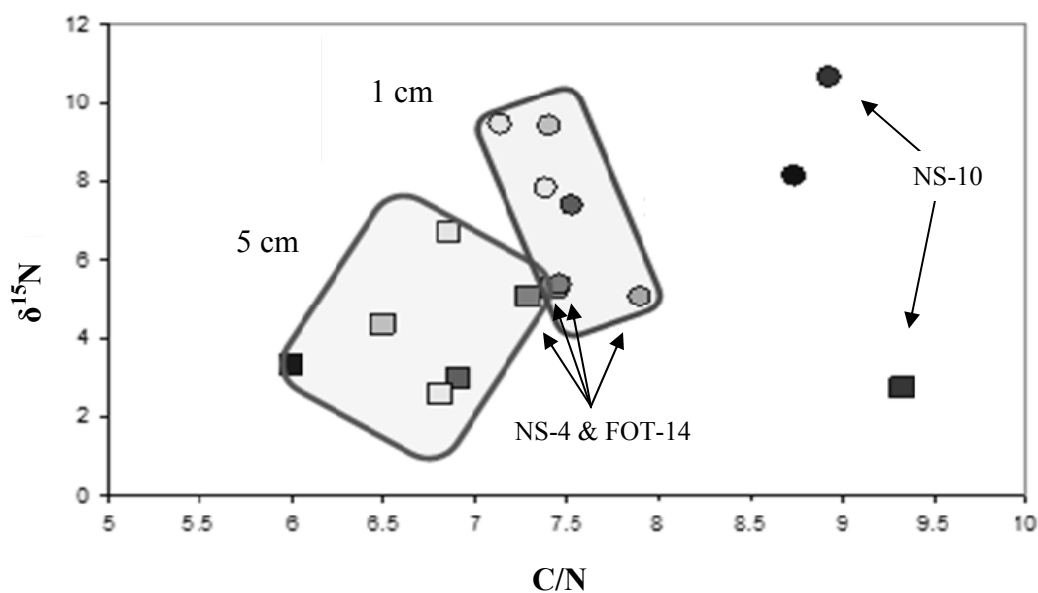


Figure 15. $\delta^{15}\text{N}$ versus C/N ratio. Samples generally show higher C/N values and higher $\delta^{15}\text{N}$ values at 1 cm (circles) than at 5 cm depth (squares). However, NS-10 at both depths and OS-2 at 1 cm depth have relatively higher C/N values than the other samples and the $\delta^{15}\text{N}$ for NS-4 and FOT-14 do not change significantly with depth. (See discussion in text).

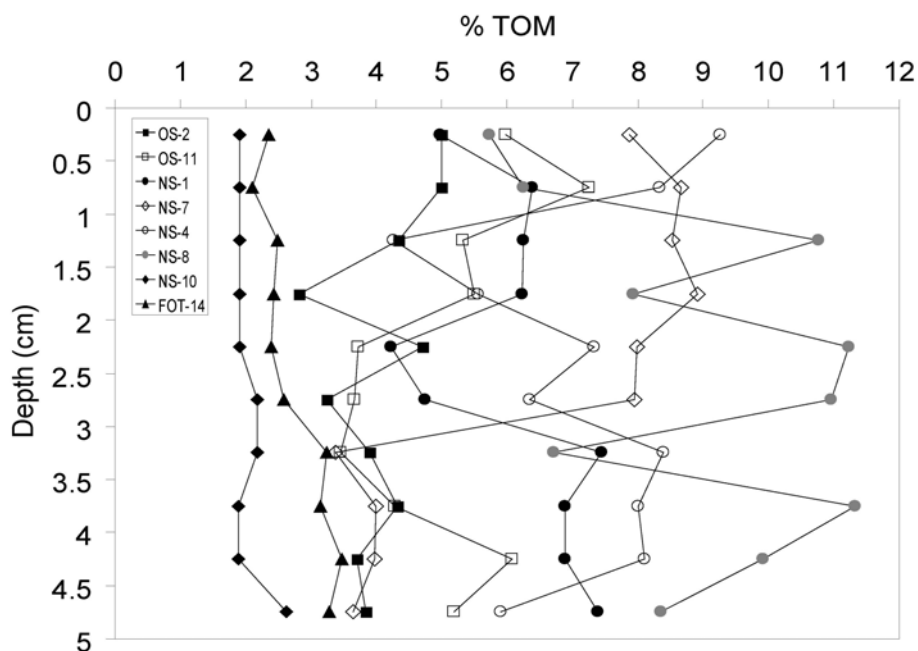


Figure 16. Total organic matter for each site. All sites, except 10, were analyzed at 0.5 cm intervals. TOM for site 10 was sampled every 1 cm and was not analyzed until 1.5 cm. The TOM for site 10 from 0.0 to 1.5 cm is given the value at 1.5 cm. This extrapolation is justified since TOM does not change significantly down-core

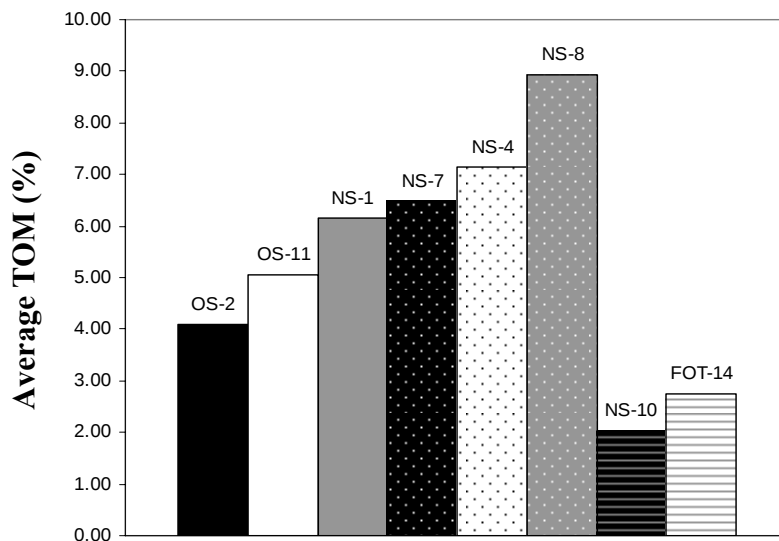


Figure 17. Average TOM for each site. Site 8 has the highest TOM (8.92 %) and site 10 has the lowest TOM (2.03 %)

location or depth for %C and N. Bulk $\delta^{13}\text{C}$ values for each site at 5 cm range from -21.89 ‰ to -19.36 ‰, averaging -20.94 ‰. NS-10 is more enriched in ^{13}C than all other sites at both depths, decreasing with depth from -15.52 ‰ to -19.36 ‰. The $\delta^{13}\text{C}$ values for most sites decrease from 1 to 5 cm (Fig. 12 and 13). NS-4, NS-8 and FOT-14 show a slight increase in $\delta^{13}\text{C}$. Bulk $\delta^{15}\text{N}$ values for each site range from 2.55 ‰ to 6.73 ‰, averaging 4.12 ‰ (Table 4). The $\delta^{15}\text{N}$ values decrease significantly (~40 to 75 %) from 1 to 5 cm at all sites except NS-4 and FOT-14 (Fig. 11 and 14). There is only a small decrease in $\delta^{15}\text{N}$ at NS-4 and a slight increase at FOT-14. The C:N ratios for all sites and both depths range between 6.00 and 9.33, which are consistent values for marine materials, though NS-10 is slightly higher (Fig. 13 and 14).

Total Organic Matter

Total organic matter is reported in Table 4 and is illustrated in Figure 15 and 16. TOM for all sites, except NS-10, were analyzed every 0.5 cm. Site NS-8 has the highest relative TOM with an average of 8.92% followed by NS-4, with an average of 7.15 %. NS-10 has the lowest relative TOM, with an average of 2.03 %, followed by FOT-14 with an average of 2.75 %. OS-2, 11, and NS-1, 7 have TOM values averaging 4.09 % to 6.49 % (Table 4, Fig. 16). TOM for NS-10 was not available from the group sampled for amino acid analysis. TOM for NS-10 was obtained from a sample collected for a separate analysis that was analyzed every 1 cm, and data for this sample was not available for depths above 1.5 cm. For this study, values used for TOM at NS-10, from 0.0 to 1.5 cm, is the same value found at 1.5 cm. This extrapolation is acceptable since TOM doesn't change significantly with depth (Fig. 16).

DISCUSSION

D- and L-Amino Acid Concentrations

Amino acids account for a major portion of the organic carbon and nitrogen in surface sediments (Lomstein 1998, Pantoja and Lee 2003). Previous work utilizing amino acid analysis on sediments from environments similar to the Hauraki Gulf found THAA to decrease with depth, indicating the labile nature of amino acids; found D-amino acids or D/L-amino acid ratios to increase with depth, particularly with those amino acids common to bacteria peptidoglycan; and found non-protein amino acids to increase with the depth as well, which is interpreted as representing microbial degradation of organic matter (Schleifer and Kandler 1972, Pollock and Kvenvolden 1978, McCarthy 1998, Pedersen 2001, Keil and Fogel 2001, Grutters 2002, Bada 2002, Uhle 2004, Lomstein 2006). In the shallow marine sediments of the Hauraki Gulf, New Zealand, it was expected that these same patterns would be found.

THAA

Biomarkers typically experience substantial degradation and alteration in the near-surface sediments during early diagenesis (Henrichs 1993, Macko et al. 1993). Here, a substantial amount of D- and L-amino acids decreases with depth (Fig. 8). This decrease likely reflects the labile property of amino acids (Macko 1993, Keil and Fogel 2001, Uhle 2004). However, both D- and L- amino acid concentrations increase in some samples, particularly at NS-4. An increase in D-amino acids likely represents the preservation of D-amino acids in the refractory bacterial peptidoglycan. Racemization is not a likely

source of these D-amino acids since the sediments analyzed in this work are young in comparison to the amount of time required for racemization to occur. The increase in L-amino acids is more complex. In previous studies, L-amino acids have been shown to be preferentially removed from the THAA pool with depth (Pedersen and Jorgensen 2001). Some L-amino acids, particularly L-aspartic acid, increase at sites analyzed in this study (Fig. 8). An increase in L-amino acids can be attributed to preservation in peptidoglycan since there are L-amino acids that are formed in peptidoglycan, although in lesser abundance than D-amino acids. However, in NS-4, nearly all amino acids increase. It is possible that this site experienced sufficient sediment mixing to erase down-core trends. Given the shallow depth of samples and the potential substantial bottom-water currents experienced by these sites, it is unclear why NS-4 is the only site that shows this trend. For each amino acid analyzed, no correlation is observed among inferred environmental realms (near-shore sites, off-shore sites, or the Firth of Thames site).

Alanine, Aspartic Acid, Glutamic Acid, and Serine

At 5 cm, alanine, aspartic acid, glutamic acid, and serine are all present. The presence of these 4 D-amino acids (plus Glycine, discussed below) are the primary amino acids produced by bacteria in peptidoglycan, and thus support an interpretation of bacterial contribution to the surface sediments. However, only NS-4 shows an increase in concentration of these amino acids with depth. In addition, when comparing the D/L ratios down-core, the increase that has been seen in other surface near-shore sediments, is not seen here. In fact, of these 4 amino acids, there is only a D/L increase seen in alanine

and glutamic acid and only at a few sites (no sites overlapped). The D/L ratio increase for alanine at NS-1, NS-4 and FOT-14, and for glutamic acid at NS-7 and NS-10 is attributed to preservation of these amino acids in peptidoglycan. Despite D-glutamic acid being one of the common amino acids produced in peptidoglycan, glutamic acid in general has been known to be depleted in sediments; a trend that is not well understood (Goodfriend 2000). For serine, since data at 1 cm is not available, it is not possible to comment on any down-core changes. However, even though serine is produced in peptidoglycan, this amino acid is found to be highly reactive and low concentrations at 5 cm may result from chemical reactivity, rather than a lack of initial presence from a source material or from bacterial production.

That these bacterially produced amino acids did not show substantial increases in either concentration or D/L ratio with depth may result from a number of reasons. First, Jorgensen (1990) found a maximum bacterial density at 10 cm and this correlated with an increase in D-amino acids down-core, with high concentrations of D-ala, D-asx, and D-glx found at the same depth (Pedersen 2001). In the present study it is likely that, at 5 cm, the sediment may not have yet reached depths associated with enhanced bacterial population. This scenario would be expected to result in the degradation of all amino acids without an increase in the D/L ratios. If a high bacteria population exists at some depth below 5 cm, analysis of samples down-core should show an increased amount of bacterially produced amino acids or in the D/L compared to shallower depths.

Glycine

Glycine is the dominant amino acid at 1 cm at all the sites, and is the second most abundant amino acid at most sites. Relatively high concentrations of glycine was observed by Dauwe and Middelburg (1998), who analyzed protein amino acids, non-protein amino acids (β -alanine and γ -aminobutyric acid), hexosamines (galactosamine and glucosamine) and bulk parameters (organic carbon, nitrogen, THAA and carbohydrates) to determine the degradation state of sedimentary organic matter. Dauwe and Middelburg (1998), as well as Goodfriend (2000), suggest that glycine concentration remains high relative to the other amino acids analyzed for any of three reasons: first, glycine is abundant in bacterial cell walls and in diatom cell walls; second, glycine also has a comparatively low food value to micro and macroconsumers as a result of its short chain length, and is therefore not readily recycled; and third, glycine can be produced from many other amino acids as an endmember of heterotrophic metabolism. Decreases in glycine concentration between 1 and 5 cm likely results from typical degradation processes during early organic diagenesis.

Leucine and Valine

L-leucine and L-valine are present at all sites at 1 cm depth. However, D-leucine and D-valine were not detected at 1 cm depth (Uhle 2004) but are present at 5 cm depth. The D/L ratios increase at all sites except NS-7, NS-10, FOT-14 (valine only); there was no change with depth for these three sites. This increase in D-leucine and D-valine may be attributed to their production in peptidoglycan. Even though these amino acids are not

known to be the most commonly produced in peptidoglycan, it is possible that certain strains of bacteria produce them. There is not enough information in this study to infer composition of peptidoglycan for different bacterial strains found in the surface sediments of the Hauraki Gulf. Additionally, it may be possible that the observed increases result from amino acid racemization. Racemization, however, is not likely to be the source of these D-amino acids since the age of these sediments are young compared to the time it takes racemization to occur.

Non-protein amino acids

Non-protein amino acids are typically less reactive than the protein amino acids (Cowie and Hedges 1992). The presence of non-protein amino acids such as β -alanine and γ -aminobutyric acid are commonly interpreted as indicative of diagenetic alteration and are usually absent in living organisms (Cowie and Hedges 1992, Duawe and Middelburg 1998, Uhle 2004). The diagenetic origin of these specific amino acids has been inferred by an inverse relationship between the concentration of β -alanine and γ -aminobutyric acid and their parent amino acids, aspartic acid and glutamic acid, respectively. Non-protein amino acids commonly result from decarboxylation of protein amino acids, which would indicate that bacterial reworking of organic matter has occurred (Lee and Cronin 1982). Concentration of these non-protein amino acids are commonly high in deep ocean sediments, where the organic material has been highly degraded through the water column before reaching the sediment. Mass balance calculations suggest, as well, that high concentration of non-protein amino acids may result not only from the

decarboxylation reaction of protein amino acids, but also from *in situ* production of these amino acids by bacteria (Goodfriend 2000). It is thus expected that, with continuing diagenetic alteration, these non-protein amino acids should increase down-core (Duawe and Middelburg 1998). In Hauraki Gulf samples, however, β -alanine only increases at one site (NS-4) and the overall concentrations at 5 cm for both β -alanine and γ -aminobutyric acid are low. It is important to note here that the degradation of aspartic acid and glutamic acid does not always produce β -alanine and γ -aminobutyric acid.

Factors most likely to affect the results in this study include the depth of sample relative to heterotrophic microbial community populations, sediment mixing, and mineral sorption. As mentioned above, it is possible that the depth at which a significant microbial community resides is deeper than 5 cm and result in little down-core change in concentration of bacterially produced D-amino acids. Additionally, because samples analyzed were only 5 cm deep, they may potentially have been homogenized from either biotic or storm/current driven sediment mixing. Bioturbation typically affects the uppermost 10 to 15 cm of the sediment column (Schulz 2000, and references therein). In the Hauraki Gulf, sediment transport is controlled both by tidal influences and by incursions from the East Auckland Current (Manighetti 1999). Bottom water flow velocities can reach 82 cm/sec for the Colville Channel and between 33-44 cm/sec for Jellicoe and Cradock Channels (Manighetti 1999). In the Colville Channel, fine to medium sand modes are mobile for 20 to 60% of the time, with a net eastward movement of fine sand. In the Jellicoe and Cradock Channels, sand is mobile for 33% of the time,

across-shelf, in a southward direction. Incursions from the East Auckland Current and storm events compound the affects of sediment redistribution both in load and direction (Manighetti 1999). Flow speeds in Jellicoe Channel were measured to be 48 cm/sec during cyclone Drena in 1997. This activity created large waves that mixed the bottom sediments down to greater than 100 m water depth. These types of events are likely the dominant means of sediment redistribution in the Hauraki Gulf (Manighetti 1999). Finally, adsorption of organic matter onto mineral surfaces, particularly that of clay minerals, may potentially have a large effect on amino acid concentration. Selective adsorption of amino acids onto clay minerals can be significant and may affect the distribution and reactions of free amino acids (Hedges and Hare 1998). Hedges and Hare (1998) found that positively charged amino acids (e.g. histidine, lysine, and arginine) are strongly adsorbed to clay minerals, up to 15% of neutral amino acids (e.g. alanine, valine, leucine, and glycine) were adsorbed by montmorillonite and a smaller percent by kaolinite, and up to 35% of negatively charged amino acids (e.g. aspartic acid and glutamic acid) were adsorbed primarily by kaolinite. Additionally, Shogenove et al. (1998) found that amino acids could also be strongly adsorbed by chlorites. Carbon to nitrogen ratios (discussed below) suggest that adsorption is occurring at a majority of the sites. And, whereas amino acid extraction through hot acid hydrolysis is the most effective method used, it has been found to only extract 80% of amino acids from the sediments tested (Nunn and Keil 2005). This result may, in part, result from the complex relationships from adsorption of amino acids with varying types of mineral surfaces.

Bulk Carbon and Nitrogen

In order to understand the context of the sediments analyzed, bulk parameters were considered. Bulk organic carbon and nitrogen analysis of sediments can be used to determine sources and fate of organic matter. When compared at different sediment depths, this data can yield information about diagenetic activity (Muller and Suess 1979, cited in Macko 1993). These analyses were used in this study to gain a better understanding of the different sites.

Total Organic Matter and %C & %N

Total organic matter was measured down-core to 5 cm depth (Fig. 16). Since there is not significant change in TOM down to 5 cm, the TOM was averaged for each site (Fig. 17) and used to normalize the amino acid data. NS-10 had the lowest %TOM, followed by FOT-14, even though both of these sites were expected to receive relatively greater sediment input. Sedimentation rates are typically positively correlated with organic matter preservation, resulting from protection of the organic matter by clay minerals and from adsorption onto mineral surfaces (Schultz 2000). It is possible, however, that organic matter preservation for sites NS-10 and FOT-14 is affected by grain size of sediment input. The weight percent carbon and weight percent nitrogen are positively correlated and plot on a line between the Redfield Ratio (Redfield 1963) and the modified Redfield Ratio (Takahasi 1985) (Fig. 10). Modified version of the Redfield Ratio has been demonstrated to be more accurate for planktonic materials (Rullkötter 2000, and references therein; Takahasi 1985).

C:N

Typical C:N ratios (molar) of terrestrial organic matter vary from ~20-400 (Rullkotter 2000). This number decreases during early diagenesis. The range of C:N ratios for phytoplankton is ~6 and that of fresh marine organic matter is ~10 (Rullkotter 2000) and this range remains near constant through diagenesis. At 5 cm, the C:N ratios for the Hauraki Gulf samples range from 6.00-9.33, consistent for shallow marine materials (Rullkotter 2000; Meyers 1997, cited in Uhle 2004) (Table 4, Fig. 13 and 14). The C:N ratio increases at NS-10. Increases in the C:N are typically indicative of preferential loss of nitrogen during early diagenesis (Macko et al. 1993). Amino acids are responsible for a major portion of marine nitrogen in the water column (almost 100%), however, this number drops drastically to only 2-12% in surface sediments from shallow marine environments (Macko et al. 1993). This decrease in nitrogen concentration reflects the primary nature of nitrogen as a nutrient sought after by organisms. In these samples, C:N ratio decreased at off-shore sites 2 and 11 and near-shore sites 1, 7, 8, and 14. It is possible that decreases in C:N occur as a result of adsorption of organic materials or inorganic forms of nitrogen onto silicate surfaces (Macko et al. 1993). NS-4 did not show significant change from 1 to 5 cm depth which suggests that carbon and nitrogen experienced similar mineralization or preservation processes (Macko et al. 1993).

$\delta^{13}C$ and $\delta^{15}N$

Carbon isotopes are useful for source determination of marine organic matter. First, the uptake and intracellular diffusion of CO₂ causes reversible fractionation of ~4‰; and

second, enzymatic carbon fixation results in an irreversible fractionation of up to 40‰ (Bickert 2000). Typical marine values are between -20‰ and -22‰ (Rullkötter 2000). For the Hauraki Gulf samples analyzed in this study, the $\delta^{13}\text{C}$ values for most sites are within the range of typical marine values. Using organic matter source fields from Meyers (1994), the most Hauraki Gulf samples plot within the marine algae field (Fig. 13). A few sites are slightly elevated between -19‰ to -20‰, tending toward the C4 land plants field. This shift may be due to input from terrigenous material. NS-10 is the most enriched in ^{13}C , for both depths, compared to the other sites. At 1 cm, the $\delta^{13}\text{C}$ for NS-10 is -15.52‰ and at 5 cm is -19.36‰. NS-10 is situated between the Great Barrier Island and the Coromandel Peninsula and within the Colville Channel. It is possible that the elevated $\delta^{13}\text{C}$ values can be attributed to input of terrigenous organic matter at this site. It is unclear however, why FOT-14 does not show a similar trend to NS-10, since both sites likely receive terrigenous organic matter from two major rivers.

Nitrogen isotope compositions of marine organic matter and the changes in $\delta^{15}\text{N}$ down-core are difficult to decipher, because there are multiple variables that can affect this value. First, during formation of marine organic matter in the photic zone, nitrogen isotope compositions are driven by the isotopic composition of nitrate and the extent of utilization of this inorganic nitrogen (Bickert 2000). In addition, primary productivity yield $\delta^{15}\text{N}$ values that vary between taxa and growth conditions (Bickert 2000). Fractionation imparted during diagenesis can also further complicate the story. For example, during either deamination or hydrolysis, fractionation occurs, resulting in an

enrichment in ^{15}N of approximately 4‰ and 2-4‰, respectively (Macko and Estep 1984, Silfer et al. 1992). Additionally, Lehmann et al. (2002) conducted experiments under both oxic and anoxic conditions and demonstrated that an enrichment in ^{15}N results from degradation of organic matter under oxic conditions whereas depletion in ^{15}N results under anoxic conditions. This depletion is ascribed to bacterial growth. It is also noted that the differences in fractionation patterns with oxic versus anoxic conditions may be an affect of the types of microbial community present, the timing and degree of microbial activity, and any preferential degradation that may occur. For the Hauraki Gulf samples analyzed in this study, the $\delta^{15}\text{N}$ values decrease substantially with depth at all sites except NS-4 and FOT-14. This depletion in ^{15}N may be attributed to bacterial growth; however this would require microbial communities at 5 cm depth to be utilizing anoxic conditions. The likelihood of mixing to these depths, however, and the absence of abundant bacterially produced amino acids, suggest that anoxic conditions were not significant. At NS-4, $\delta^{15}\text{N}$ decreases slightly and at FOT-14, $\delta^{15}\text{N}$ increases slightly. In experiments conducted by Lehmann (2002), it was found that under oxic conditions $\delta^{15}\text{N}$ increased by ~3‰ and then decreased to values close to the original material, resulting in no net fractionation.

CONCLUSIONS

The Hauraki Gulf, like other shallow marine sediments, contains both D- and L-amino acids. Most amino acid concentrations decrease between the depths of 1 and 5 cm, which is consistent with the lability of these biomarkers. D/L ratios of the amino acid enantiomers were expected to be abundant at all sites, however, D/L ratios increase in only four of the amino acids analyzed. These D/L increases observed in glutamic acid, alanine, valine and leucine, are attributed to bacterial production of peptidoglycan. The presence of β -alanine and γ -aminobutyric acid also supports microbial reworking of sediment. However, these non-protein amino acids were also expected to increase with depth since their presence and concentration are strongly linked to degradation. The only increase seen was at NS-4. For all other sites, a decrease was observed. Factors affecting these results may include: 1) That sediments analyzed from 5 cm may not have been deep enough to intersect high populations of heterotrophic microbial communities; 2) that these relatively shallow sediments may have been substantially disturbed through bioturbation or tidal/storm mixing. Samples from deeper parts of the core, however, may be more helpful for determining contribution of D-amino acids from bacteria versus racemization.

Finally, total organic carbon and bulk carbon and nitrogen data do not appear to shed light on the results of the amino acid data. Most sites were within typical marine values for $\delta^{13}\text{C}$ and for C:N. All sites except NS-4 and FOT-14 showed a significant depletion in ^{15}N with depth. This depletion may result from microbial growth at 5 cm. However, in

order for this to be the case, these communities would have to be sufficient to create a pseudo-anoxic environment at 5 cm. For all parameters, NS-10 was distinct, having the lowest TOM, elevated $\delta^{13}\text{C}$ values at both depths, the greatest change (decrease) in $\delta^{15}\text{N}$ with depth, and the highest C:N ratios (slightly higher than typical marine values). NS-10 also had the highest overall decrease in amino acid concentration with depth. These results suggest that NS-10 may have received a substantial amount of terrigenous organic matter, likely high in C4 plants. The large decrease in $\delta^{15}\text{N}$ and the decrease in amino acid concentration with depth are likely related. It is unclear, however, what would cause this decrease.

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APPENDICES

Appendix 1: *Amino Acid Concentrations*

- Sheet 1. Raw amino acid concentrations for samples at 5 cm depth, given by the GC-MS in mg/L.
- Sheet 2. Back calculations to change the raw concentration in mg/L (from the GC/MS) to the actual concentrations in mg/gdw (sediment).
- Sheet 3. Amino acid concentrations (not normalized) in mg/gdw for samples at 1 cm (Uhle 2004).
- Sheet 4. Amino acid concentrations (not normalized) in mg/gdw for samples at 5 cm.
- Sheet 5. Amino acid bar-graphs of concentrations normalized to TOM for 1 vs 5 cm depths.

Appendix 2: *D/L ratios and related graphs.*

Sheet 1. D/L ratios table showing changes from 1 to 5 cm depth.

Sheet 2. D/L ratio bar graphs showing changes from 1 to 5 cm depth.

Sheet 3. Line plot of D/L ratio change between 1 and 5 cm depth.

Appendix 3: Total Organic Matter, Carbon and Nitrogen Data for 1 cm and 5 cm.

Sheet 1. TOC, TON, C:N from Dr. Scott Nodder from the National Institute of Water and Atmospheric Research.

Sheet 2. TOM for all sites and depths from Dr. Scott Nodder from the National Institute of Water and Atmospheric Research.

Sheet 3. Carbon and Nitrogen data for samples at 1 cm. Original set is taken from Uhle 2004. I was provided with a corrected version from Dr. Scott Nodder.

Sheet 4. Carbon and Nitrogen data for samples at 5 cm determined by Dr. S. A. Macko at the University of Virginia.

Appendix 4: *Data and graphs not used.*

Sheet 1. Total organic carbon was provided by Dr. Scott Nodder from the National Institute of Water and Atmospheric Research. This data was originally used to normalize the amino acids. However, it was later decided to use the total organic matter instead.

Sheet 2. Total organic carbon versus depth from 1 and 5 cm.

Sheet 3. Amino acid loss versus TOC.

VITA

Tabbatha Anne Cavendish was born on March 14, 1976 in Ft. Lauderdale, Florida and was raised by her grandmother, Violet Merle Cavendish. Tabbatha attended Lloyd Estates Elementary School and Lauderdale Lakes Middle School in South Florida, and, in 1995, graduated from Sequoyah High School in Canton, Georgia.

Tabbatha received a Bachelor of Science degree in geology at the University of Tennessee in 2001. She continued at the university to complete a Master of Science degree in Geology in 2008.

The philosophy by which Tabbatha lives is to enjoy life to the fullest every day. She follows this by travelling, going on adventures, trying new things, meeting new people, listening to music, and engaging in laughter.