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Advances in Amino Acid Analysis for Marine Related Matrices and Its Application to Coastal Shelf Settings in The Canadian Arctic

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ADVANCES IN AMINO ACID ANALYSIS FOR MARINE RELATED MATRICES AND ITS APPLICATION TO COASTAL SHELF SETTINGS IN THE CANADIAN ARCTIC

by

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ABSTRACT

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Rachel M. McMahon
Old Dominion University, 2018
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Amino acids comprise up to 50% of organic matter in cellular material and are a major fraction of oceanic organic carbon. Amino acids are also considered highly labile during organic matter recycling, making them useful proxies for organic carbon cycling. Nevertheless, analysis of individual amino acids has been burdened by lengthy derivatization and complex analysis since the 1950s. In this thesis, I describe the modification of advanced analytical techniques, developed in the biomedical field, for analysis of marine matrices which allow the determination of at least 40 amino acids without the need for lengthy sample preparation and derivatization, twice the number of most common biosynthetic amino acids. Combining ion pairing separation and mass selection software with liquid chromatography tandem mass spectrometry (LC/MS/MS) allows detection of amino acids that were previously difficult to measure due to coelution and incomplete derivatization. The method was validated by examining a suite of marine matrices of increasing complexity including free amino acids (FAA) in seagrasses, total hydrolyzable amino acids (THAA) in mixed diatom-bacteria cultures, and sediment THAA. This method was then combined with additional measures and applied to examine the carbon cycling and conditions of the Arctic Ocean Mackenzie shelf system. Analysis of organic carbon in particles and sediments, fatty acid and sterol biomarkers, and the developed amino acids method, together with sediment grain size and meiofaunal community structure, allowed characterization of organic carbon in the Mackenzie River shelf system during the fall of 2016. Results show the coastal shelf system near the Mackenzie River delta experience significant inputs of both in-situ marine production and terrestrial organic matter to the sediments. Overall

results suggest that mid-shelf waters receive relatively higher contributions of labile marine carbon than shallow or deep waters along the eastern Beaufort Sea shelf and over time these inputs are reflected by increased meiofaunal diversity and abundance. Despite the large flux of terrestrial organic material exiting the delta, higher meiofaunal abundance appears to be the result of localized input of marine primary production rather than terrestrial carbon carried through the Mackenzie outflow.

This thesis is dedicated to my forever supportive family, without their endless years of help and guidance, I would not be where I am today.

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CHAPTER I

INTRODUCTION AND OVERVIEW

Amino acids are best known as the basic constituent of proteins and have a variety of functional groups including acidic, polar, neutral and chiral. Some 240 amino acids occur in nature, while over 500 are known to exist (Wagner and Musso 1983). The twenty most abundant amino acids are associated specifically with proteins, protein synthesis, and the genetic code (Table I.1**)**. In marine systems, amino acids are involved in many intracellular and intercellular in addition to protein synthesis, including diatom stress signaling, planktonic larvae recruitment, and the ammonium or urea cycle present in many animals and bacteria (Eschenbrenner and Jorns 1999; Lange 1963; Forster and Goldstein 1979). Amino acids account for the major fraction of organic nitrogen present in cells, and thus comprise 1% to 40% of the total organic matter identified in marine particulate organic matter (POC) and sediments (Hecky et al. 1973; Burdige and Martens 1988; Dauwe et al. 1999; Keil et al. 2000). This also makes amino acids one of the most abundant fractions of organic carbon found in the marine environment, and important for the sources and biogeochemical cycling of organic matter (Harvey et al. 1995). Amino acids have been used to monitor the degradation of marine particles and organic matter in sediments (Hedges et al. 1994; Wakeham et al. 1997; Horiuchi et al. 2004) as well as a wide variety of other processes from intracellular cycling to cellular signaling. (Touchette and Burkholder 2000; Jonasson et al. 2010; Shelp et al. 2017). Despite the relative importance of these molecules, identification and quantification of individual amino acids historically was a lengthy and complicated process that did not always yield complete results. Early methods of analysis required lengthy extraction and derivatization steps with less than complete yields, resulting in either less than full compositional information or co-elution with other compounds during analysis. Despite these limitations, the fundamental importance of amino acids as a major contributor to organic matter and as markers of important biogeochemical processes have led to their broad analysis.

Table I-1 Main functional groups of the 20 most abundant biosynthetic amino acids and their corresponding abbreviation.

Group	Characteristics	Most abundant amino acids	abbreviations
Neutral	Hydrophobic	Glycine, Alanine, Valine, Methonine, Leucine, Isoleucine	Gly, Ala, Val, Met, Leu, Ileu
Polar Acidic	Positively charged Hydrophilic	Aspartic Acid, Glutamic Acid	Asp, Glu
Polar Basic	Negatively charged	Lysine, Arginine, Histidine, Asparagine, Glutamine	Lys, Arg, His, Asn, Gln
Polar Uncharged	---	Serine, Threonine, Cysteine	Ser, Thr, Cys
Chiral	Chiral and hydrophobic	Proline, Phenylalanine, Tryptophan, Tyrosine	Pro, Phe, Trp, Tyr

The goal of this thesis research was twofold. The first goal was to utilize analytical advances in tandem mass spectrometry to address some of the prior difficulties of analyzing and quantifying amino acids, including those of well-known biosynthetic origin and those with a broader array of structures. This was done to allow quantitative measures of a broad suite of amino acids across multiple marine matrices without the potential drawbacks of derivatization. The second goal was to apply this improved approach to a transect of particles and sediments across the complex setting of the Arctic continental shelf to examine the use of a broader suite of amino acids as markers for carbon cycling and possible links to meiobenthic communities.

Chapter 2 of this thesis describes the development of methods to create a streamlined protocol for analyzing amino acids. The priority was to avoid a lengthy derivatization process and reduce time without sacrificing precision and accuracy. This was accomplished by employing a combination of electron spray ionization mass spectrometry (ESI-MS), liquid chromatography (LC), and ion-pairing reagents, along with advanced mass selection software. Selection of the analysis process was achieved through a series of trials with amino acid standards and marine related matrices that varied in organic and amino acid complexity.

Chapter 2 describes the development of this method and assesses its effectiveness for use in marine-related systems.

To test the effectiveness of the method developed and address the second goal, a series of marine related matrices were selected based on their increasing complexity. Starting with a suite of 40 amino acid standards as the least complex matrix, structural masses and secondary daughter products were identified for each amino acid using tandem mass spectrometry based on literature values and in-lab trial runs. Paired with 5 deuterated standards, selected to represent the five major functional groups of amino acids, calibration curves were constructed over a broad concentration range. Mixtures of cultured diatoms and cultured bacteria were then used as a more complex matrix, with varied ratios of diatoms to bacteria used to alter the protein signal. Previous analysis of total hydrolyzable amino acids (THAA) conducted in parallel using derivatization and gas chromatography-mass spectrometric flame ionization detection (GC-MS/FID) allowed for a direct comparison of the developed method with anestablished method.

Samples of free amino acids sourced from seagrasses as a complex sample matrix with no cleanup phase were evaluated using the developed method as well. Free amino acids often represent a pool of non-proteinaceous amino acids sourced from intercellular cycling and intracellular interactions, and are often difficult to measure using standard methods. The last selected type of matrix was gathered from Arctic Ocean coastal marine sediments. Located off the Canadian Mackenzie River delta, the marine sediments represent a complex matrix for environmental samples, and the large terrestrial input contributed to humic and refractory materials in the sediment not easily removed without time-consuming cleanup processes. In the development of LC-MS/MS analysis of amino acids, these sediments were analyzed without a clean-up step to test the effectiveness of the method for complex matrices.

Chapter 3 of this thesis combines the application of this new method with other types of organic analysis to examine cross-shelf organic carbon processes. Results of these multiple organic analyses are used to assess carbon cycling across the Mackenzie continental shelf. Additional data on meiobenthic abundance and diversity structure provided by collaborators from parallel samples allowed the inclusion of meiofaunal community analysis. The Mackenzie coastal shelf was chosen as an area of highly variable conditions where intense mixing of marine and terrestrial organic matter drive a complex interplay of carbon cycling (Macdonald et al. 1998; Yunker et al. 2005). In winter the area is under constant ice coverage that breaks up in early May and begins to refreeze in mid-October (O'Brien et al. 2006; Richerol et al. 2008). During the time of spring break up, a freshening of the water column occurs, referred to as the spring freshet, large fluxes of POC from the Mackenzie River move across the shelf and are then consumed and recycled or buried in the shelf sediments (Macdonald et al. 1998; Belicka et al. 2002). The organic material that reaches the shelf sediments can then be grazed upon by the benthic community; meiofauna in this region is specifically dependent on the flux of organic matter from the surface to the seafloor as their major source of sustenance (Stein et al. 2014). By addressing the link between benthic communities and carbon cycling, we can address the final two goals of this thesis, to examine the sources and fluxes of carbon in the Mackenzie shelf, and determine how the newly developed amino acid analysis can be employed in such environmental studies.

In October of 2016, aboard the CCGS Laurier, four stations along the Mackenzie River shelf in the Beaufort Sea were occupied to study the variability in conditions seen along increasing water depth and distance from the mouth of the Mackenzie Delta. The year 2016 was the lowest Arctic summer sea ice extent recorded in recent history; it is also the first recorded year in which there was no multi-year ice remaining after the spring and summer melt. Along with the selection of the Mackenzie shelf site, one other site was sampled, as a comparative pelagic site in the Western Chukchi Sea along the Chukchi Plateau where river runoff, sea ice, and other land influences are minor compared to the Mackenzie shelf.

Stations along the Mackenzie shelf were sampled at water depths as shallow as 40 meters (M1) and as deep as 300 meters (M4). Collected particles and surface sediments were analyzed for five parameters;

total organic carbon (TOC), sediment grain size, meiofaunal community structure and abundances, amino acids, and fatty acid-sterol biomarkers. Particles were analyzed for POC and amino acids specifically. Total organic carbon was used as a general metric of organic carbon at these stations, while grain size was used to evaluate sediment sorting across the shelf. Meiofaunal community information is compared to organic carbon analysis, amino acids, and biomarkers to evaluate carbon sources and recycling at each of four shelf stations. These analyses from the water column and the sediment samples were then used together to discuss the movement of the Mackenzie Shelf terrestrial and marine organic carbon during the fall of 2016.

CHAPTER II

APLICATION OF LC/MS/MS AMINO ACIDS ANALYSIS FOR MARINE SYSTEMS

Abstract

Amino acids, the primary building blocks of proteins, comprise a large fraction of marine organic matter in both living and detrital forms. Measuring the broad suite of amino acids present requires multiple approaches to capture these varied structures, the most common problems occurring because derivatization can be selective, and multiple methods are tedious to capture the range of functional groups both bound and free forms. The use of electron spray ionization liquid chromatography tandem mass spectrometry (ESI-LC-MS/MS) together with an ion-pairing reagent was developed as an alternative approach to methods that employ derivatization and gas chromatography for measuring a broad suite of amino acids across a diversity of marine related matrices. Evaluation included calibration to allow quantitative measures of at least 45 amino acids with no derivatization and detection limits of 50 pg. The method was applied on a varied set of marine matrices including pure cultures of bacteria and diatoms, particulate organic matter sediments, and eelgrasses. Findings not possible by other methods of amino acid analysis included significant amounts of arginine and gamma-aminobutyric acid (GABA), and lower limits of detection across all amino acids. This method allows smaller and complex marine organic matter samples to be analyzed for amino acids while avoiding the constraints of multiple derivatization steps.

Introduction

Amino acids are the basic constituents of all proteins and compromise the largest fraction of organic nitrogen in cellular material (Hecky et al. 1973). In the marine environment, the most abundant amino acids are the twenty biosynthetic forms that constitute protein-bound amino acids (Wakeham et al. 1997; Dauwe et al. 1999). In most living organisms, the percentage of total nitrogen represented by amino acids is greater than 38% and can be as high as 84%, and it has been argued that plankton and microbes tend to

have higher percentages of organic carbon as total hydrolyzable amino acids (THAA) than vascular plants (Cowie and Hedges 1992). Amino acids typically contribute between 0.7% and 28% of diatom cells by weight, and vary across cellular compartments. Glycine and hydroxyl amino acids can be enriched in the cell walls while glutamic and aspartic acid is higher in the cellular contents (Hecky et al. 1973). In other planktonic organisms such as foraminifera, King and Hare (1972) found that amino acids only account for 0.02 to 0.04 % by weight, but the distribution of amino acids could be used to separate different foraminifera species. This was then used to study POM fluxes of sinking foraminifera and to establish paleo-records of past oceanic foraminifera species.

In addition to their role in cellular architecture, amino acids are also fundamental intermediates in a host of metabolic pathways. For example, sarcosine is an intermediate and a by-product of glycine synthesis and degradation (Eschenbrenner and Jorns 1999). Arginine is both a proteinaceous amino acid as well as a key intracellular component of many cellular processes such as the urea cycle and cell division (Cunin et al. 1986). Studies have also shown amino acids to be important circulating osmoregulators in bivalves (Lange 1963; Forster and Goldstein 1979). In higher plants such as seagrasses, the production and presence of free glutamine and glutamic acid have been suggested as important for sequestering and storing nitrogen (Touchette and Burkholder 2000).

In past studies of marine organic matter, non-protein amino acids were generally considered to be of diagenetic origin (Lee and Cronin 1982), but this group of amino acids also appears to play several roles in intercellular and intracellular processes. Examples include gamma-aminobutyric acid (GABA), glutamine (Gln), and beta-methylamino-L-alanine (BMAA), all important non-protein bound amino acids that have historically escaped standard methods of detection. BMAA is an unusual amino acid and well-known cyanobacterial toxin that may also have implications for human health (Cox et al. 2005; Ince and Codd 2005). GABA is a neurotransmitter present in most mammalian species (Olsen 2002), but it has also been found in significant concentrations in diatom and marine bacteria, and may have a role related to stress signaling (Allen et al. 2006; Loper et al. 2012).

Reflecting primary production in the water column, amino acids represent a significant fraction of the organic carbon (OC) in water column particles (POM) and underlying sediments. As a major fraction of oceanic organic material, amino acids have also been widely used as markers of organic matter degradation (Dauwe et al. 1999; Carr et al. 2016). Although the fraction of particulate organic matter (POM) in the surface ocean that can be identified at the molecular level varies with degradative state, typically up to 50% is present as amino acids (Henrichs and Williams 1985). With increasing depth, the amount of identifiable organic matter in particles decreases exponentially (Harvey et al. 1995), and its bioavailability also declines (Harvey et al. 1995; Dauwe et al. 1999).

In the past, understanding the source and specific chemical makeup of marine organic matter was limited by analytical capacities to identify and measure these compounds, often at very different concentrations. Early successful amino acid measurements relied on liquid chromatography (LC) combined with the use of fluorescent tags for quantification, including the use of o-phtalaldehyde (OPA) derivative (Lindroth and Mopper 1979) and 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate derivative (AccQ-Waters Tag) (Cohen and Michaud 1993; Cohen and Antonis 1994). Both OPA and ACQ take advantage of the separation capabilities of liquid chromatography and derivatization reagents that allowed for extensive separation of each amino acid. These methods were capable of measuring most of the twenty common amino acids, but was unable to resolve some coeluting peaks that were often affected by the sample matrix. Also important was the need for extensive standards to confirm the structures seen as chromatographic peaks via fluorescence, making assignments difficult and coelution an issue. Subsequent approaches used capillary gas chromatography-mass spectrometry (GC-MS) which also allowed structural analysis (Mawhinney et al. 1986; Neves and Vasconcelos 1987; Stabler et al. 1987). While widely applied in marine systems, the use of a specific derivative remains selective and often does not measure non-alpha-amino acids, and some methods also require relatively large sample sizes (European Pharmacopoeia 2005). Limits of detection can also be poor with derivatives of amino acids, especially with the use of GC-MS over LC-MS (Piraud et al. 2003, 2005a; b). Even with newer methods of chemical derivatization, there are still some limitations, including labor-intensive sample preparation (upwards of 3 hours), coeluting

peaks, variable yields for final products, and larger sample sizes required (Table II-1). With the introduction of highly sensitive, high mass accuracy mass spectrometry, the fraction of identified organic matter has expanded along with methods that allow for lower limits of detection, streamlined sample preparation, and increased structural resolution (Grebe and Singh 2011). Amino acid quantification has seen similar advances, for samples that previously required extensive or even multiple derivatization steps in which the amino acids must be modified to be identified and quantified. Advances in mass spectrometry now present the potential to expand the analysis of amino acids to include a broader range of of amino acids beyond the major twenty amino acids. Combined with further advances in liquid chromatography using an ion-pairing reagent, this presents an opportunity to revisit the analytical suite of amino acids that can be investigated in environmental and oceanographic settings.

Table II-1. The four most common methods of amino acid analysis and their summary properties. IEX stands for ion exchange chromatography, RP for reverse phase, IC for ion chromatography, ECD for electrochemical detection and GC/MS-FID for gas chromatography tandem mass spectrometry or flame ionization detection. Resolution refers to how efficiently two co-eluting peaks can be separated.*

	IEX with post column derivatization	**RP with pre-column derivatization**	**IC with ECD detection**	**GC/MS-FID with pre-column derivatization**
Sample preparation	Difficult protein and salt removal process	Desalting	Removal of salts and detergents	Solid phase extraction
Derivatization	Ninhydrin-limited sensitivity or OPA – no proline	Salts and proteins affect reaction	No derivatization	Limited by amino acid functional group
Analysis time	60 -120 minutes	40-70 minutes	75 minutes	10-20 minutes
Identifiable amino acids	10	10-20	10-20	20-30
Resolution	Poor resolution of critical amino acids	Poor resolution of critical amino acids	Poor peak capacity	Some so-eluting peaks
Accuracy/ Reproducibility	N/A	Contaminants co-elute with some amino acids, *OPA derivatives decompose slightly on column	Salts cause retention time shifts	Not affected by composition
Quantitation limits	Proline/ Hydroxyproline not detected using OPA (1 µg of protein sample required) §	10-50 µmol/L lower limit of detection **	Large inject volumes required of 25 µL or more Ɨ	~1-10 µmol/L limit of detection, no arginine

* Table modified from phenomenex website method bulletin (http://www.phenomenex.co.kr/skin/page/00000000130/EZfaast.pdf)
** (Barret and Elmore 1998)
§ (Henderson Jr and Brook 2010; Steed 2010)
Ɨ (European Pharmacopoeia 2005)

Amino acids have a variety of functional groups, often characterized by their polarity at physiological pH, including neutral, basic, acidic and sometimes highly polar moieties that can create challenges for separation and identification by standard methods. Electrospray ionization mass spectroscopy (ESI-MS) is well suited to study molecules under approximately 1000 Dasuch as amino acids because it is relatively simple to separate compounds based on known masses. Similarly, ion-pair reverse phase chromatography has been developed to measure underivatized amino acids at high sensitives and low limits of detection (LOD) (Qu et al. 2002a; b). Developed by Gordon Schill in 1973, ion-pairing chromatography provides a useful option when used with ESI-MS (Varvara et al. 2009). Ion-pairing reagents have both a charged end and a hydrophobic region, the charged end carries the opposite charge of the analyte to encourage the analyte to elute with the reagent while the hydrophobic end interacts with the stationary phase, or column packing, causing the ion pairing reagent to stick to the column. Stationary phases chosen for ion-pairing include neutral resins such as bonded silica, for example the hydrophobic octodecyl - C18 phase coating is commonly chosen as an effective column packing for ion pairing chromatography. For the present study, heptafluoric butyric acid (HFBA) was chosen as an ion-pairing reagent that is compatible with common C18 columns as well as LC-MS. HFBA as an amendment allows for the separation of difficult amino acids, including those with the same mass but different molecular properties, such as alanine and sarcosine, diaminobutyric acid (DAB) and BMAA, GABA and Beta amino-isobutyric acid (B-AiB) (Eckstein et al. 2008). The use of ESI-LC-MS/MS and an ion pairing reagent was tested as an alternative option to methods that employ derivatization and GC-MS for measuring a broad suite of amino acids in marine related matrices.

To assess the effectiveness of LC-MS and an ion pairing reagent to measure a broad suite of amino acids, a set of varied sample matrices were selected. These matrices were chosen to reflect a diversity in marine types of materials and as a test of method effectiveness across different sample matrix complexities.

Materials and Procedures

Selection of Samples

The sample matrices, in order from least to most complex, included: a suite of 40 amino acids and 5 deuterated internal standards; POM from cultures of both marine bacteria and marine diatoms as well as samples of POM that were from bacteria and diatoms mixtures; plant tissue from the eelgrass *Zostera marina L.*; POM from the water column in the Arctic Mackenzie self-basin; and surface sediments gathered from the Mackenzie shelf-basin.

Dilution series of mixed phytoplankton/bacterial cultures

The marine diatom *Thalassiosira pseudonana* (Thaps, CCMP1335) was grown in f/2 media (Guillard 1975) with autoclaved and filtered artificial seawater (salinity 30) at ambient room temperature (18-22°C) under a 13:11 hour light:dark schedule. Cell counts and cellular health were checked throughout the growth cycle with a hemocytometer on an Olympus optical epifluorescence microscope. Diatom growth was monitored by absorbance measurements at 550 nm as a proxy based on cell density and turbidity (Spectronic Educator, Flinn Scientific, Batavia, IL). The culture was harvested during exponential growth.

The marine heterotrophic bacterium *Ruegeria pomeroyi* (Rpom, NCMA B3) was reconstituted in autoclaved and filtered 0.5 YTSS media (Gonzales et al. 1996) and slowly transitioned into a low carbon (as 0.625 mM glucose) medium over multiple generations. Cultures were grown under axenic conditions at room temperature and bacterial growth tracked by absorbance measurements at 600 nm, a measure of turbidity due to culture density. The culture was harvested during the early stationary phase.

To mimic oceanic POM samples, mixtures of Rpom and Thaps were created using different cellular ratios of bacteria:phytoplankton based on previous publications of bacteria and phytoplankton counts in 1) mesocosm experiments (Smith et al. 1995; Riemann et al. 2000) 2) before, during, and after phytoplankton blooms (Suzuki et al. 2011; Teeling et al. 2012; Bunse et al. 2016), and 3) as a function of depth (Li et al.

1993). Aliquots of diatom and bacteria cultures at concentrations of 10^8 cell ml^{-1} (Rpom) and 10^5 cell ml^{-1} (Thaps), determined by calibrating cell counts using DAPI stain microscopy to absorbance, were mixed to yield desired Rpom:Thaps ratios and subsequently collected on 47 mm, 0.2 μm Nuclepore polycarbonate filters and frozen until analysis.

Amino acids of intracellular material and cell wall proteins

To compare amino acids present in soluble cellular protein versus residual cellular material, diatom/bacterial mixtures on filters were extracted prior to amino acids analysis. Proteins were extracted from filters by shaking the filters suspended in 500 μl of 6M urea in a bead beater with no beads (repeat 3 times:1 min shaking; ice 5 minutes). After removing the filters from the liquid, cells were lysed using a sonicating probe (Nunn et al., 2015). Samples of urea extracted liquid from bacteria (*Ruegeria pomeroyi*) and diatoms (*Thalassiosira pseudonana*) were then hydrolyzed in 6M hydrochloric acid for 16 hours at 110 °C. After the urea extracted fraction was hydrolyzed, the residual filter pieces were also hydrolyzed in the same manner to obtain a total hydrolyzable amino acids estimate. Once at room temperature, samples were then spiked with the internal standard norvaline. Norvaline is a common internal standard for GC-MS analysis, allowing direct comparison of GC-MS to LC-MS results. Samples were hydrolyzed and first run via GC-MS methods following the EZ:Faast derivatization and sample perperation kit (Phenonomex), they were then analyzed by LC-MS using the method outlined in this chapter.

Eelgrass samples

Plant shoots of eelgrass *Zostera marina* L. were obtained from plants isolated and grown in a series of tanks at different pH conditions to study the effects of simulated ocean acidification (see Zimmerman et al. 2017). Multiple experimental tanks were used with eelgrass grown over more than two years (June 2013 to November 2014). Individual seagrasses were sampled in August of 2013 at pH 6 and 8 and plant leaves and rhizomes analyzed for intracellular free amino acids involved in cellular processes. Eelgrasses

tissues were then scraped of all surface epiphytes, cleaned, frozen with liquid N_2, then ground with a mortar and pestle before amino acid extraction. Samples were then extracted using boiling 80% ethanol to extract the free amino acids (FAA), the ethanol was evaporated (Zimmerman 1989), and the sample re-dissolved in ultrapure water and spiked with the set of stable isotope internal standards as discussed below.

Arctic organic matter

To examine the amino acid composition in a complex environmental matrix, a transect of 4 stations across the continental shelf of the Mackenzie Delta, Canada to the Beaufort Sea were occupied for collection of water column particles by filtration and surface sediments by box core. Samples were also collected from an additional site collected on the western flank of the Beaufort Sea (designated AIM) to represent a pelagic marine site. Particles were filtered onto combusted GF/F filters from water collected by CTD rosette at the chlorophyll maximum and near-bottom water of each station. This chapter examines only one of the five stations; chapter 3 describes the detailed examination of all five stations. The particle samples were hydrolyzed similarly to the mixed bacterial diatom cultures by using 6 N HCl on a heating block set to 110 °C for 16 hrs. Surficial sediments only were examined in this study, these sediments were hydrolyzed, and the extracted amino acid supernatant removed and dried under N_2 gas to prevent oxidation of amino acids. After drying, both the particles and sediments were spiked with the set of deuterated internal standards.

Evaluation of method stability across amino acid suite

To evaluate the effectiveness and consistency of the developed method across all amino acids measured, a series of comparisons with literature values and calibration spikes were performed. A standard of Bovine Serum Albumin (BSA) was hydrolyzed with the same methodology outlined for THAA, spiked with the deuterated internal standards and then analyzed with the outlined LC-MS/MS method, Results were compared with reported literature values.

Amino acid yields were also evaluated through spiking experiments. Known concentrations of amino acid standards were added to both water and sediments and then hydrolyzed to evaluate the effect of hydrolysis on loss of amino acids. Standards were also spiked into water and sediments after hydrolysis to compare and evaluate the yields of amino acids without acid treatment. All spikes were analyzed with LC-MS/MS and quantified using external calibration curves and internal standard additions.

Reagents and standards

All reagents were of high purity LC-MS grade. Heptafluorobutyric acid (HFBA) and hydrochloric acid were supplied by Thermo Fisher Scientific (Rockford, IL USA). Methanol (MeOH) and acetonitrile (ACN) were supplied by J. T. Baker (USA) and LC-MS grade water was supplied by Millipore (St. Louis, MO USA). L-leucine, L-isoleucine, DL-norvaline, L-cysteine, L-asparagine, L-glutamine, L-arginine and tryptophan standards, for conformation of retention times and mass spectrum identifiers, were purchased individually at grades of >98% purity from Sigma Aldrich (St. Louis, MO USA). A suite of 40 amino acids for calibration curves was supplied by Phenomenex (Torrance, CA USA). The internal standards DL-Cystine-2,2′,3,3,3′,3′-d6, DL-Lysine-4,4,5,5-d4 2HCl, L-Aspartic-2,3,3-d3 Acid were purchased from C/D/N isotopes laboratories (Pointe-Claire, Quebec Canada). L-leucine (5,5,5-d3, 99%), l-phenylalanine (ring-d5, 98%) internal standards were supplied by Cambridge isotopes laboratory (Tewksbury, MA USA).

Liquid chromatography tandem electron spray ionization mass spectroscopy

An Agilent 1290 infinity, binary pump LC was interfaced to a bonded silica C18 Dionex Acclaim Polar advantage rapid separation liquid chromatography (RSLC) column (2.2 µm, 120 A X 150 mm) for chromatographic separation. A guard column was added in line having the same column packing as the analytical column but with a 5 µm particle size. Samples were loaded onto the column via an Agilent auto sampler, at 10 µl per injection. The mobile phase flow rate was set at 0.3 mL min^{-1}. The gradient used for mobile phase A included the ion-pairing reagent HFBA (0.4% of phase by volume), formic acid (0.02% of

phase) and LC-MS grade water. Mobile phase B included formic acid (0.1% of phase) in acetonitrile. The gradient program, modified from Piraud et al. 2005, utilized 100% mobile phase A from 0 to 1 minutes, 85% A from 1-6 minutes, 85% to 75% between 6 to 9 minutes, and 75% mobile phase A from 9-15 minutes, then from 15-16 minutes the gradient was set to an isocratic state of 100% mobile phase A, finally from 16-31 minutes 100% mobile phase A was maintained. All amino acids were observed over the first 16 minutes of each sample run, from 16-31 minutes of a sample run flushing of the column with a 100% mobile phase A occurred to remove impurities and restore the ion pairing reagent to the analytical column before analysis of the next sample.

The LC was integrated with a Thermo Scientific Orbitrap XL mass spectrometer via an ESI interface for structural analysis. Data was acquired and processed in Xcalibur software (Thermo Scientific, Rockford, IL) at a scan range of m/z 50-300 using positive ion mode. The collision energy was kept constant through the entire run at 35 V with CID activation in the primary scan and a scan cycle (resolution) of 30000. The ESI source parameters included: capillary temperature of 275 °C, spray voltage 3.5 V, and capillary voltage 2.5 V. All ESI source parameters were tuned and maximized before sample runs. The ESI source utilized nitrogen as the carrier gas.

Calibration and standards

Amino acid concentrations were calibrated using an internal standard/external standard paired set to provide coverage over the major functional groups of amino acids being determined. An individual calibration curve was constructed for each amino acid and normalized to one of the five chosen internal standards based on their functional groups. Most amino acids were calibrated from a commercial set of 30 known amino acids (Phenomenex), and normalized to peak areas of the internal standards which include L-aspartic-2,3,3,d_3 acid, L-cystine-2,2',3,3,3',3'-d_6, DL-lysine-4,4,5,5-d_4, L-leucine-5,5,5-d_3, L-phenylalanine ring –d_5. The final sample concentrations were determined based on the ratio of amino acid peak area to the internal standard peak area, using the following formulas. In total, 40 amino acids and

five deuterated standards were calibrated and identified with mass values based on retention times and published mass spectrums for each amino acid.

$$(1) \qquad PA\left(\frac{amino\ acid}{ITSD}\right) = m * \left(\frac{concentration\ of\ Amino\ acid}{Concentration\ of\ ITSD}\right) + b$$

$$(2) \qquad AAconc. = \frac{(PA - b)}{m} * concentration\ of\ ITSD$$

PA stands for the peak area of the selected amino acid divided by the peak area of the corresponding internal standard; *m* is the slope of the linear curve, while *b* is the *y*-intercept of the linear calibration curve. *AAconc* is defined as the final concentration of an amino acid in each sample.

Mass selection software

All samples were scanned for both the full mass spectrum, from 50 m/z to 500, and the daughter products (MS^2 spectrum) using Xcalibur software. Peaks in samples were identified based on their MS^1 retention times and MS^2 primary daughter products. Primary ion identifiers of each amino acid were identified using values of M+H or molecular weight plus one for a hydrogen ion. This information was obtained from a series of injected standards, as well as literature values for MS and MS^2 (Piraud et al. 2003, 2005a; Fonteh et al. 2006). Amino acid quantification was based on mass abundances, as peak area, of each identified amino acid compared to the internal standard selected for that amino acid.

Results

Instrument calibration and internal standards

Analysis of 40 amino acids and five deuterated internal standards in the standard matrix were used to determine limits of detection (LOD) and quantification (LOQ) for amino acids following RP-LC separation. LOQ was defined as a signal to noise ratio (S/N) of 5 to 1. Calibration of the 40 amino acids

and five deuterated amino acid standards resulted in a calibration curve that showed ionization responses varied with the amino acid functional group but showed a minimum limit of quantification (LOQ) around 50 pg per injection (Table II-2). The calibration also showed an upper linear limit of detection of up to 10,000 pg per injection, depending upon the amino acid functional group. All amino acids were observed in positive mode over the 16-minute window of analysis (Figure II-1). Repetitive runs of the standard suite on the same day yielded error margins of less than ±10% for all amino acids. Repeated standard runs were seen to drift over multiple days as seen in slight changes in integrated peak area, but remained consistent in having less than 10% calculated variation in concentration.

Table II-2. A comparison of methods for amino acid analysis and amino acids structures identified. Information about ortho Phthalaldehyde (OPA) and fluorenylmethoxy chloroformate (FMOC) derivatization methods is from Agilent technologies (Henderson Jr and Brook 2010; Steed 2010) . Information about gas chromatography tandem mass spectrometry (GC/MS) methods gathered from Phenomenex Ez:faast derivatization user's manual and liquid chromatography tandem mass spectrometry (LC/MS/MS) information gathered from this experiment. Check marks indicate detection of the listed amino acids. Limit of quantification (LOQ) reported for only the method developed here. LOQ for internal standards are not included in this table and any amino acids not calculated for a LOQ are designated NR (not reported) in the table.

Chemical Name	Abbreviation	HPLC-OPA/FMOCC[1]	GC/MS[2,3]	LC-MS/MS[4]	LOQ (ng per injection)
Glycine	Gly	✓	✓	✓	75
Asparagine	Asn	✓	✓	✓	132
Serine	Ser	✓	✓*	✓	132
Sarcosine	Sar	✓	✓	✓	52.5
Hydroxyproline	Hyp	✓	✓	✓	65
Glutamine	Gln	✓	✓	✓	73
Aspartic Acid	Asp	✓	✓	✓	66.5
Alanine	Ala	✓	✓	✓	44.5
Threonine	Thr	✓	✓	✓	59.5
Glutamic Acid	Glu	✓	✓	✓	73.5
Proline	Pro	✓	✓	✓	57.5
Cysteine	C-C	✓	✓	✓	120
Valine	Val	✓	✓	✓	58.5
Lysine	Lys	✓	✓	✓	73
Norvaline	Nor	✓	✓	✓	-
Methionine	Met	✓	✓	✓	149

Table II-2 Continued

Chemical Name	Abbreviation	HPLC-OPA/ FMOCC[1]	GC/MS[2,3]	LC-MS/MS[4]	LOQ (ng per injection)
Tyrosine	Tyr	✓	✓	✓	181
iso-Leucine	Ile	✓	✓	✓	65.5
Leucine	Leu	✓	✓	✓	65.5
Phenylalanine	Phe	✓	✓	✓	165
Tryptophan	Trp	✓	✓	✓	204
Proline-Hydroxyproline	Php		✓	✓	228
y-Amino-n-Butyric acid	GABA		✓*	✓	51.5
Cysteine	Cys		✓	✓	NR
Thioproline	Tpr		✓	✓	66.5
β-Methylamino-L-alanine	BMAA		✓**	✓	59
α-Amino-butyric acid	ABA		✓***	✓	51.5
β-Aminoisobuytric acid	BAiB		✓***	✓	51.5
Glycl-Proline	GPr		✓	✓	172
Diamino-Buytric Acid	DAB		✓**	✓	59
Hydroxylysine	Hlys		✓	✓	162
Ornithine	Orn		✓	✓	132
Cystanthionine	Cth		✓	✓	111
α-aminopimelic acid	APA		✓	✓	87.5
α-aminoadipic acid	AAA			✓	80.5
Amino ethylglycine	AGly			✓	NR
Arginine	Arg			✓	176
1-methyl-Histidine	1MHis			✓	169
3-methyl-Histidine	3MHis			✓	169
L-aspartic-2,3,3,d_3 acid	Asp-D3			✓	-
L-cystine-2,2',3,3,3',3'-d_6	Cys-D6			✓	-
DL-lysine-4,4,5,5-d_4	Lys-D4			✓	-
L-leucine-5,5,5-d_3	Leu-D3			✓	-
L-phenylalanine ring –d_5	Phe-D5			✓	-

* Serine and GABA coelute with GC/MS methods and are often quantified as one peak
** DAB and BMAA coelute and are often quantified as one peak
*** BAiB and ABA also coelute and are often quantified as one peak

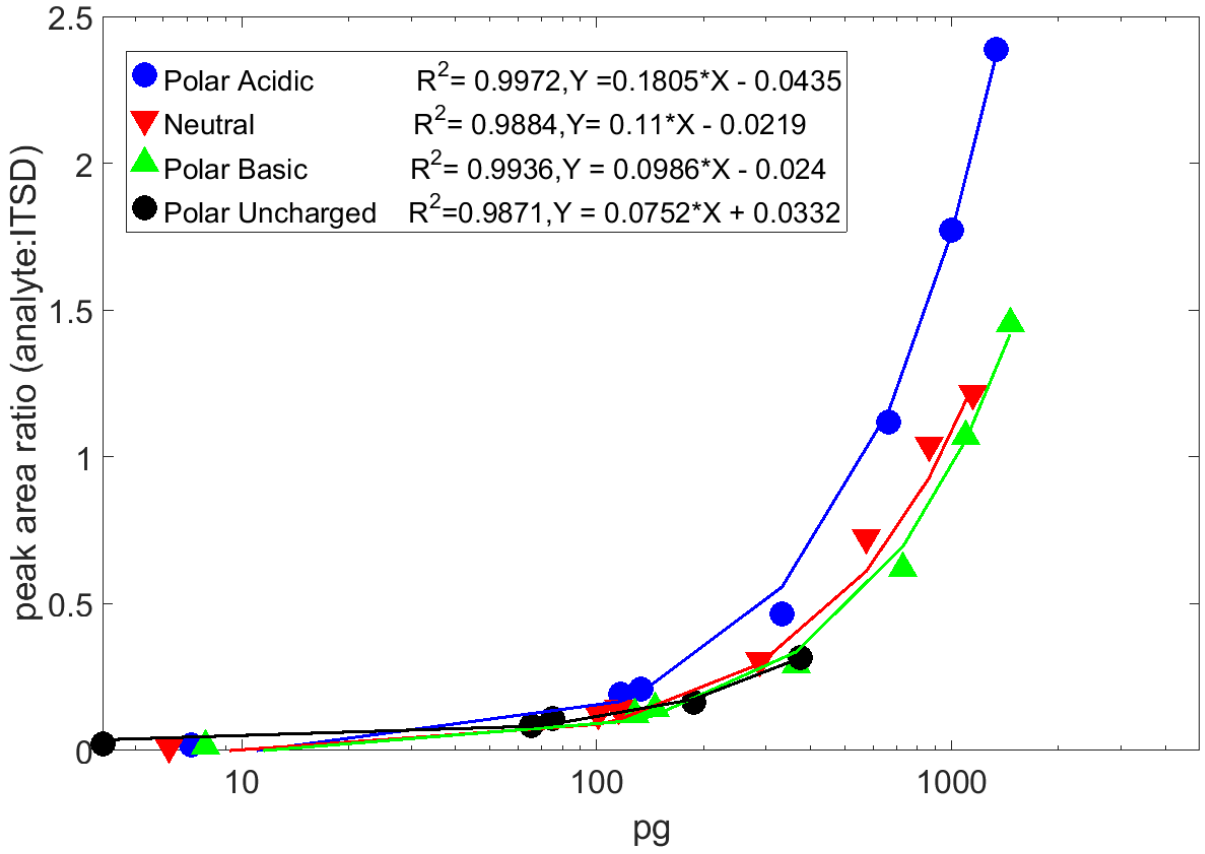

Figure II-1. LC-MS/MS calibration curves of four representative amino acids spanning the major amino acid functional groups. Polar Acidic, Neutral, Polar Basic, and Polar Uncharged amino acids are represented by aspartic acid, proline, lysine, and glycine, respectively. The y axis describes the peak area ratio as a function of the amino acid's peak area (analyte) over the peak area of the internal standard (analyte:ITSD) and its response over three orders of magnitude. The x axis is measured as picograms amino acid per injection onto the column.

Environmental samples

The mixed bacteria/diatom samples yielded varied amounts of each amino acid dependent on the volume of diatoms filtered. GABA cannot be typically analyzed concurrently with other amino acids, but was observed in amounts corresponding to increases in the proportion of diatoms [Figure II-2]. There was no distinct bacterial amino acids observed, but small amounts of unexpected non-alpha-amino acids including thio-proline, sarcosine and threonine expanded the number of amino acids quantified compared to previous methods. Arginine is also highly concentrated in these samples which was notable as it is not

captured by parallel GC-MS analysis. A comparison of the total amino acid concentration in samples by LC-MS with those analyzed as derivatives by GC-MS showed similar changes with the level of the dilution. LC-MS analysis also always observed higher concentrations of total hydrolyzable amino acids even when peaks not identified were removed from the summed total of amino acids [Figure II-3].

Comparison of GC/MS, LC/MS/MS and OPA/FMOCC (Brown 1991) methods, show variability in diatom amino acid distributions depending on the choice of method analysis. Brown 1990 observed higher levels of glycine, alanine and aspartic acid using OPA/FMOCC than the other two methods, but much lower levels of lysine than both LC/MS/MS and GC/MS [Figure II-4]. Notably, glycine is not observed using LC/MS/MS while it is seen in the other two analyses methods. Most amino acids were measured with the three methods, but only OPA/FMOCC and LC/MS/MS were able to capture arginine, and only LC/MS/MS was able to observe considerable amounts of GABA.

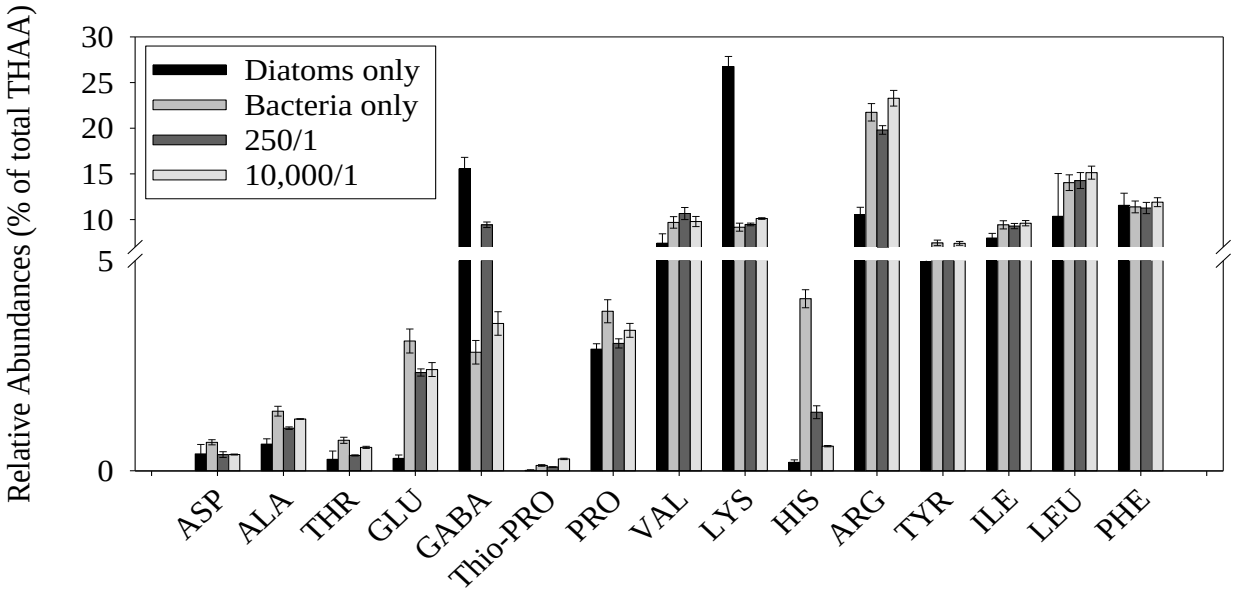

Figure II-2. The relative abundance of total hydrolyzed amino acids (THAA) in a series of individual and mixed cellular ratios of bacterial and diatom cultures. Some amino acids were dependent on the mix of bacteria to diatoms while others were common to both bacteria and diatoms. The error bars represent the standard deviation (n=3) of analytical replicates of the samples. Ratios reported as 250/1 and 10,000/1 represent cell concentration ratios of bacteria to diatoms.

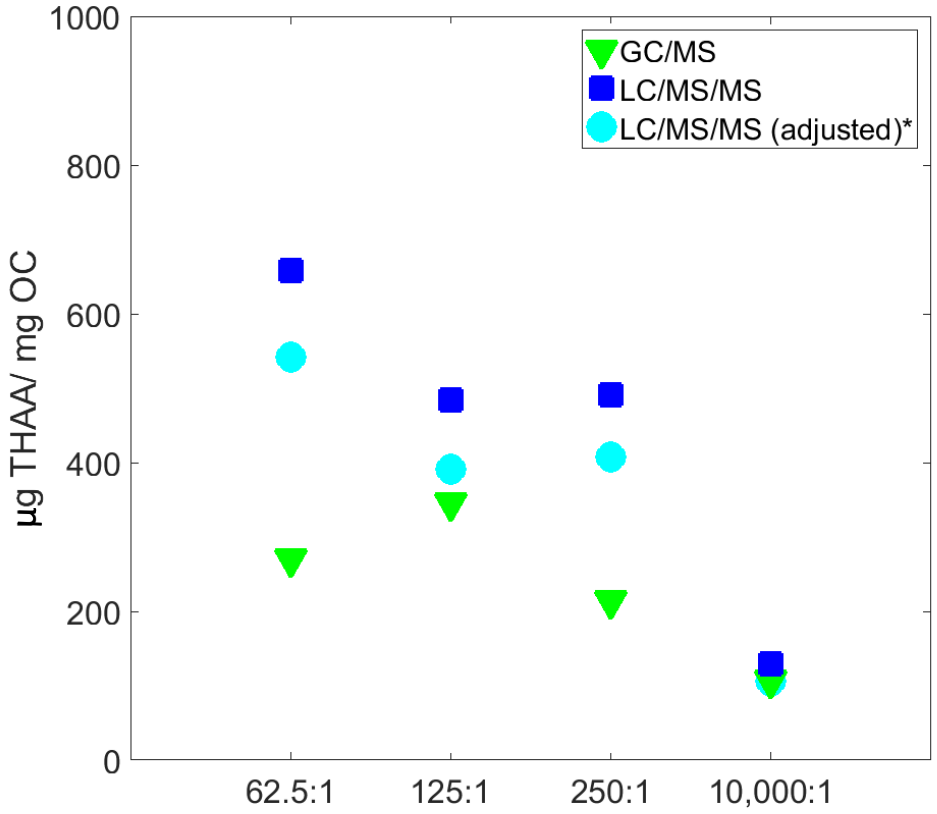

Figure II-3. The sum of soluble protein THAA from mixed diatom/bacteria cultures. Comparison of methods of measuring total hydrolysable amino acids (THAA) from soluble proteins, both GC/MS and LC/MS/MS were used to analyze the same dilution experiment samples and normalized to organic carbon (µg THAA/ mg OC). The GC/MS samples were derivatized using the phenomenex Ez:faast kit The x-axis describes the the ratio of bacteria (*Ruegeria pomeroyi)* to diatoms (*Thalassiosira pseudonana*) in the sample. Error bars not included due to the summed nature of the presented data.
* Amino acids that were only seen by using LC/MS/MS, like arginine and GABA, were removed from the total measured THAA in a sample.

The extracted free amino acids (FAA) of seagrass showed a very different distribution compared to the marine diatoms and bacterial samples ([Figure III-5]). Glutamine and GABA were the most concentrated amino acids, followed closely by asparagine instead of aspartic acid, proline, arginine, and leucine that are often high in hydrolyzed samples. Identification of glutamine and asparagine in previous GC-MS approaches, was complicated by the deamination of glutamine to glutamic acid and asparagine to aspartate durring analysis. Other free amino acids were also complicated using the GC/MS approach due

to coelution of similar amino acids. With the use of LC-MS and mass selection for parent and daughter product confirmation and ion pairing separation of amino acids, this LC-MS/MS approach is able to separate and quantify these peaks effectively. The most abundant amino acids varied depending on the part of the seagrass plant and the pH of the water that the sample was grown in. In the leaves of the seagrasses, GABA and glutamine dominated the majority of FAA in samples grown at pH of 6 and 8. Between the two pH treatments, eelgrass leaves expressed little changes in free amino acids, but in rhizomes, asparagine accounted for almost double the fraction in the pH 8 treatment versus the pH 6 treatment while also having half the GABA observed in the pH 6 treatment. In the pH 8 treatment, proline was present in high amounts of the rhizome FAA fraction, while the pH 6 treatment had no measurable proline.

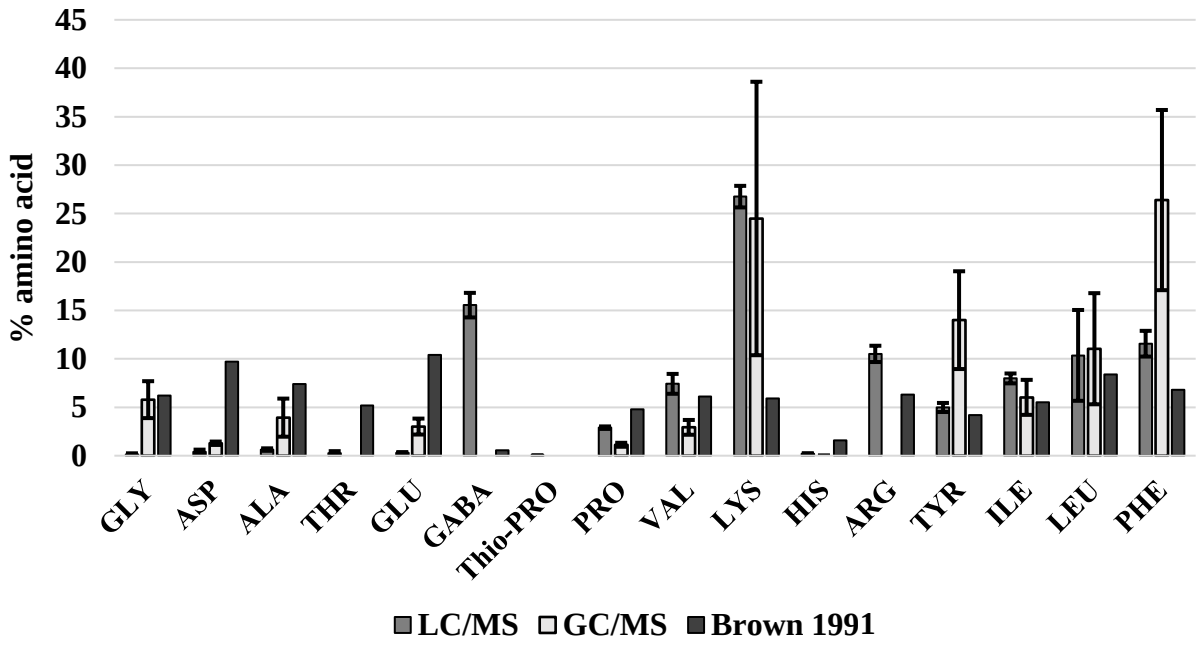

Figure II-4. Comparison of amino acid distributions in the diatoms *Thalassiosira pseudonana* by three methods. Relative abundances of each measured amino acid as a percentage of the sum of all amino acids. Three distributions are reported as three diferent methodologies of measuring diatom amino acids. LC/MS and GC/MS values are reported from the same diatom culture grown in this experiment. Brown 1990 reports values of amino acids from the same species using OPA/FMOCC derivatization for amino acids. Error bars for LC/MS represent standard deviation of analytical replicates, error bars for GC/MS are calculated based on the standard deviation of procedural replicates. Brown 1990 did not report analytical error.

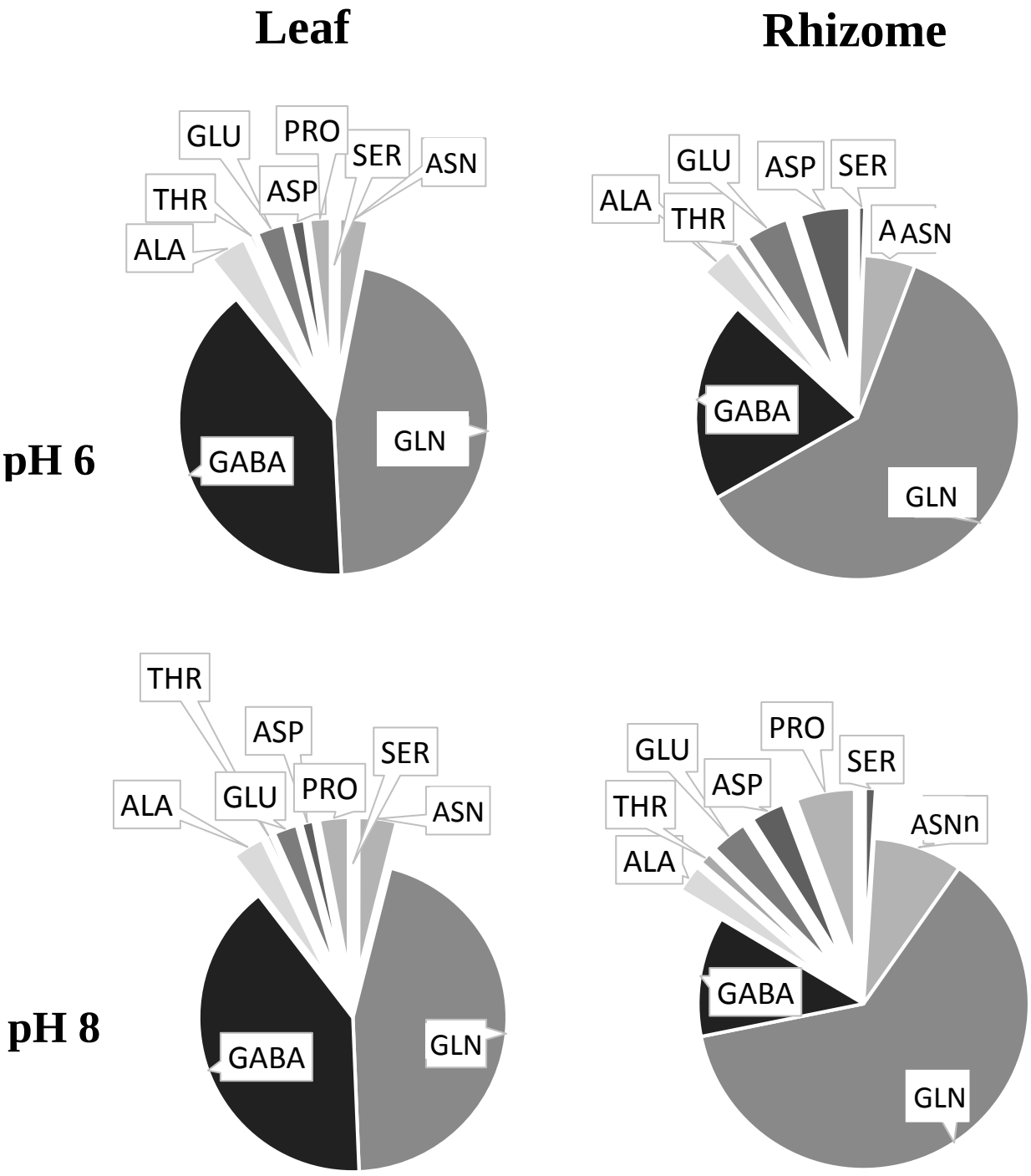

Figure II-5. Free amino acid distributions in leaf and rhizomes of the Eelgrass (*Zostera marina* L.). Eel grass roots and rhizomes grown at pH 6 and 8 contain gamma-aminobutyric acid (GABA), glutamine (Gln) and asparagine (Asn), the largest fraction of free amino acids observed and rarely quantifiable using standard analytical techniques. The percent contribution of each individual amino acid is calculated based on mass of each amino acid measured out of the mass of total free amino acids.

Sediments and particle samples from station M2 had notably different total concentrations of THAA as well as different amino acid distributions [Figure II-6]. The surface sediment was higher in glycine and serine than the particle samples. Both water column particle samples had significant amounts of GABA, arginine, and proline; these three amino acids are less commonly measured or often fall below detection limits in other methods. Glutamic acid was the most dominant amino acid through the water column and in the surface sediments, accounting for over 30% of the total amino acids; glycl-proline was also found in larger amounts in the water column, but not in the sediment [Figure II-6]. In surface waters and surface sediments, lysine accounted for 5-10% of the total hydrolysable amino acids, but was absent in bottom water particles The concentration of amino acids per mg of organic carbon decreased with depth through the water column, ranging from 20 µg THAA/mg OC to less than 1 µg THAA/mg OC. The surface sediments contained ten times less carbon and amino acids than that seen in the water column with values around 1 µg THAA/ mg OC or less [Figure II-7].

Bovine serum albumin (BSA) was hydrolyzed to examine the amino acid distributions verses the original method based on ninhydrin separation (Spahr et al., 1967). Comparison with literature values were consistent with previous methods of amino acid analysis. Only those amino acids lost during hydrolysis were not seen in the LC/MS/MS results; individual amino acid contributions to the relative distribution were also consistent between the literature values and the results from this study [see Appendix II-1].

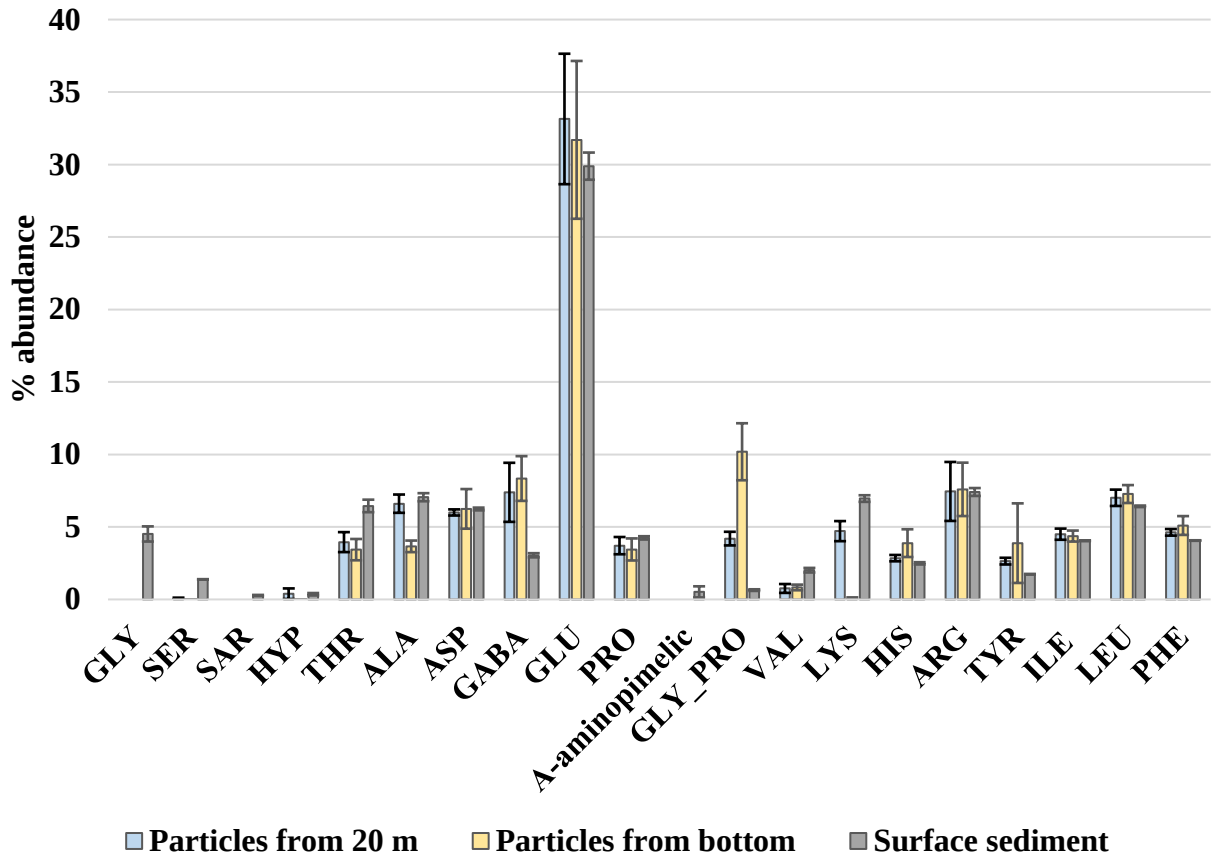

Figure II-6. The relative abundance of THAA in water column particles and surface sediments from the Arctic Mackenzie shelf.
Particles from 20 m represents the chlorophyll maximum of this station while bottom particles were collected 2 m above the seafloor. All values are reported in relative abundances, calculated as ng of individual amino acids divided by the sum of all observed amino acids and normalized to organic carbon. The error bars represent analytical standard deviations (n=3).

Standards spiked into water and into sediments resulted in varied individual amino acid yields. Amino acid yields were more consistent and stable across the full set of standards when spiked into water for both pre and post hydrolysis [see Appendix II-2]. Sediments showed more variability in individual amino acids, but consistently summed to 100% for THAA [see Appendix II-3]. One notably low yield from spiked water and sediments was glycine; this amino acid was determined to have little to no yields when hydrolyzed and still low yield when spiked post hydrolysis in both matrices.

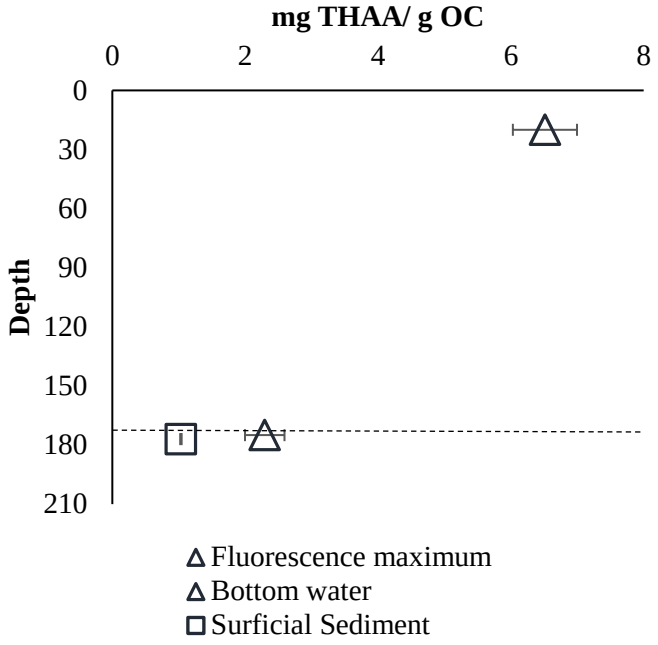

Figure II-7. Total hydrolyzable amino acids in Mackenzie shelf waters and sediments. Sum of hydrolyzable amino acids seen at station M2 normalized to organic carbon (mg THAA/ g OC). M2 at 20 m represents the chlorophyll maximum while M2 bottom water represents particles at the near the bottom of the water column. Surficial sediment was defined as the first 1 cm of the sediment column. Error bars represent analytical standard deviation (n=3) of repeated runs of the sample.

Discussion

The method described covers a large range of concentrations and can accurately determine 40 amino acids, 20 more than the most commonly abundant and measured biosynthetic amino acids. With limits of quantification for most of the amino acids of 50 to 100 pg per injection, this method has the advantage of measuring smaller quantities of amino acids than is possible by derivatization style analysis (Lindroth and Mopper 1979).

Past studies of amino acids in marine related matrices have made important contributions to our understanding of organic matter cycling in the ocean. These measures, however, did not encompass the full suite of amino acids that might be present, or those at very low abundances (Sommerville and Preston 2011). In previous studies of such marine matrices, amino acids including arginine, GABA, and proline

were not captured because they were below detection, coeluted with other peaks, or could not be derivatized (Cowie and Hedges 1992; Waldhier et al. 2010). Amino acid distributions of the cultures of bacteria and diatoms determined by GC-MS (Figure II-4) did not observe either arginine or GABA, yet this was seen by LC-MS/MS in significant quantities. This can be problematic when discussing the different distributions between the two species; the diatoms had observably higher GABA than the bacteria, even when the cultures were mixed, while the bacteria had observably higher amounts of arginine than the diatoms. One recent study theorized that GABA can be used as a stress signal in diatoms, explaining why it is higher in the diatoms than in the bacteria (Allen et al. 2006). The use of LC-MS/MS showed the sum of THAA concentrations to be consistently higher than that measured by GC-MS. At least two factors are likely responsible for this difference. The first is that several amino acids not detectable using GC-MS were quantified by LC-MS/MS. This includes those in high concentrations such as arginine and GABA. Secondly, GC-MS measurements employ and assume a singular and linear relationship between all amino acids and the internal standard (typically norvaline), which may not reflect actual responses across the range of functionalities. Analysis by LC-MS does not assume this, and in fact, calibration curves show significant variability across functional groups (Figure II-1). This suggests that LC-MS provides a more comprehensive and accurate reflection of the suite of amino acids present and their concentrations in marine plankton. LC-MS/MS also resulted in a much lower limit of detection than that of GC-MS (50 pg per injection for LC-MS/MS and 10 ng for GC-MS); this also may contribute to the significant differences in total amino acids measured in these samples (Fountoulakis and Lahm 1998).

The ability to measure small amounts of intracellular free amino acids holds particular promise in the case of amino acids such as GABA since it is typically outside the analytical window for standard methods, but could provide important insight into metabolic processes and nitrogen cycling. Several studies discuss the role of GABA in plants as a metabolic pathway during periods of root anoxia, but only a few studies have examined this idea in seagrasses or eelgrasses (Pérez et al. 2007; Pregnall et al. 1984; Sousa and Sodek 2002). Perez et al. (2007) observed different concentrations of GABA and alanine, depending on the conditions of the seagrass and which part of the plant was being measured. In the samples measured

in this study, significant quantities of GABA were measured and the concentrations vary depending on which part of the plant is analyzed and what growth conditions the plant experienced.

Similar to that seen for samples of mixed bacteria and diatoms, the Arctic marine samples would be underrepresented in total amino acids measured by previous methods. In these sediments and particles, amino acids such as GABA, threonine, arginine, and proline would not have been quantified by methods that employ derivatization. These four amino acids account for approximately 30% of the total amino acids present in the samples tested. The particles were low in concentration, using other methods, suggesting many of the amino acids could have fallen below the limit of detection. For example, valine was as low as 3 ng in the particle samples at the chlorophyll maximum and near the seafloor. Serine would also have been missed, as GC-MS amino acid analysis have limits of detection as high as 16 µM (16 nmol/ml or about 100 ng) while both the sediments and particles had values below 100 ng of serine. Glycine was measured in the sediment but not in the water column, whereas the water column does have glycl-proline while the sediments do not. This difference could be due to the availability of proteins to hydrolyze into each individual amino acid, meaning the proteins in the water column that contained glycine and proline were more refractory than those in the sediments. This could reflect factors such as more humic materials in the water column and/or fresher or more easily hydrolyzable organic carbon in the surface sediments.

Recovery measures of amino acid spike standards into water and sediments suggest that responses are consistent although are shown to differ for individual amino acids. Most important was the poor response of glycine at low concentrations, therefore this method may not be effective in measuring this low molecular weight amino acid. Glycine's small size causes it to elute very early in the analytical retention time, before the organic mobile phase is added to the analysis gradient. The organic mobile phase B enhances the signal of each amino acid to be more consistent throughout the run; the standard gradient program shown may not be effective for glycine. Responses for some amino acids were also affected by increases in background noise after long analysis windows. To improve such signals, Praud et al. (2003) recommend flushing the mass spectrometer and LC column every 25-40 sample runs for 16 hours at the flow rate established for the method.

Conclusions

Across multiple sample types including bacteria, diatoms, sediments, marine particles and seagrasses, LC/MSMS with ion pairing was able to quantify free and bound amino acids at low concentrations (50 pg per injection) without the need for derivatization, and within 30 minutes. Amino acids that commonly co-elute in previous methods of analysis were separated with the new method, including GABA and B-AiB, alanine and sarcosine, all of which were observed to be of importance in discussing marine samples. When using the degradation index created by Dauwe and Middleburg (1998), beta-alanine along with GABA is often referenced as an amino acid useful in predicting the degradation state of a marine organic matter. This method has yet to be tested in its ability to measure beta-alanine but based on the results, beta-alanine would be a good addition to the total 40 amino acids already quantified within this method. Improvements to the analysis method can be made by expanding the amino acids measurable to include beta-alanine and others as well as resolving low recoveries of glycine by adjusting gradients and employing increasing system cleaning pre and post analysis.

CHAPTER III

LINKING ORGANIC CARBON SOURCES AND TRANSFORMATION WITH MIEOBENTHIC DIVERSITY ACROSS THE COASTAL SHELF OF THE MACKENZIE DELTA

Abstract

During the Arctic spring freshet, the Mackenzie River delivers large amounts of dissolved and particulate terrestrial organic carbon and nutrients to the Canadian Beaufort Sea shelf. This pulse of nutrients fuels phytoplankton blooms in late spring and summer, and together with under ice production, contributes marine derived organic carbon to both the water column and the sediments. Regional carbon budgets suggest that both organic sources may contribute to benthic production. As a component of the Marine Arctic Ecosystem Study (MARES), samples were collected across the western Mackenzie River coastal shelf in fall 2016, to examine the relative contribution of marine and terrestrial carbon to marine sediments. With contributions by collaborators, the relationship of organic carbon sources with meiobenthic diversity and abundance was also examined. Organic biomarker analysis via tandem mass spectrometry of specific lipid proxies was used to constrain the amount and type of organic sources, and total hydrolyzable amino acids (THAA) were employed as markers of organic matter lability and transformation. Organic carbon biomarkers (lipids and amino acids) were then compared to meiofaunal abundance and diversity across the same surface sediments. Across the shelf transect, both organic proxies and meiofauna abundance showed enrichment at mid-shelf stations, with abundances and diversity of meiofauna greatest in the upper part of sediments, with dominant taxa seen as foraminifera, polychaeta, crustacea, and nematoda. Insufficient organic carbon input may have limited benthic production in the deepest location, while predominately terrestrial inputs at the shallowest location may have prevented greater mieofaunal populations. Results of lipid biomarker analysis show significant amounts of algal-derived carbon in the sediments remained even in late fall with contributions by both diatom and dinoflagellates to sediments, seen as diagnostic sterols and highly unsaturated fatty acids. Total

hydrolyzable amino acids showed increased breadth of structures and contributions to total organic carbon in sediments and the water column at the mid-shelf station, in parallel with greater benthic abundance. Diatom frustules were also abundant at the two mid-shelf locations, but absent or low in abundance and diversity in the shallowest and deep stations. Overall results suggest that mid-shelf waters receive higher contributions of labile marine organic carbon than shallow or deep waters along the eastern Beaufort Sea shelf, and over time these inputs are reflected by increased meiofauna diversity and abundance. Despite the large input of terrestrial organic material exiting the delta, higher meiofaunal abundance appears to be the result of localized amounts of marine primary production rather than terrestrial carbon carried through the Mackenzie River outflow.

Introduction

The Mackenzie River continental shelf spans the northern part of the Canadian Arctic Ocean between the Amundsen Gulf on the east and the Mackenzie Trough to the west (Richerol et al. 2008a). Sea ice typically begins to form on the shelf in mid-October and the Mackenzie River delta and nearshore areas stay ice covered until the spring break up in mid-May and the ensuing spring freshet (O'Brien et al. 2006). About 70% of the Mackenzie River freshwater discharge occurs between May and September period, with 90% of the deltaic particulate flux occurring in June, July and August (Yunker et al. 1991). Across the Mackenzie shelf and into the adjacent Beaufort Sea, the Mackenzie River dominates the supply of particulate organic carbon (POC), with estimated annual inputs of 2.1 Mt POC, the largest sediment load of any river in the Arctic Ocean (Macdonald et al. 1998). Autochthonous primary production in the delta and over the shelf is estimated to add an additional 3.3 Mt a^{-1} of POC to the carbon budget (Macdonald et al. 1998). MacDonald et al. 1998 argue that of the organic carbon produced, about 97% of the production is recycled and never reaches the underlying sediments while 60% of the POC from the terrestrial dominated Mackenzie River outflow is preserved in the sediments. Primary production is dependent on seasonal light, ice and Mackenzie River outflow in a heavily light limited community, making the late spring and summer the dominant time of high primary production (Yunker et al. 1995). Sinking particulate fluxes are highest

early in the summer June, July and August, due to the breaking up of the ice in early to mid-May (Juul-Pedersen et al. 2010; O'Brien et al. 2006). When the ice begins to first break up along the land fast ice near the Mackenzie River Delta, blooms begin and by late May and early June, the freshwater from the Mackenzie River, elicits high export production. Sediments in the northern Beaufort Sea appear to be dominated by a mixture of refractory marine and terrigenous organic carbon (Magen et al. 2010). On the west side of the Beaufort Sea shelf, the sinking carbon flux is highest in the summer because of the influence of the Mackenzie River meltwater and nutrients and an increase in solar irradiance (O' Brien et al. 2006). The vertical flux is influenced by eddies that play an important role in moving particulate organics in the Beaufort Sea Shelf, mixing old and newer materials at the shelf edge dominated by biogenic organic matter (O'Brien et al. 2013). The complicated physical transport paired with other system properties, including wind-driven upwelling and transport, make the sources of POC eventually deposited, and the corresponding effect of the meiofaunal community structure along the Mackenzie River shelf complex.

The characterization of organic material and its cycling in the past has often relied on molecular markers which can provide both source and degradative state information. A number of studies have utilized amino acids as an indicator of organic matter degradation in marine sediments (Dauwe and Middelburg 1998). Cape Lookout Bight sediments were found to contain 10-15% of the total organic carbon as amino acids, and 30-40% of the nitrogen as amino acids, making amino acids one of the most abundant fractions of organic matter found in marine sediments (Burdige and Martens 1988). Dauwe and Middlelburg proposed amino acids as an indicator of degradation in both marine particles and in sediments, using total amino acids and individual amino acid characterization. Other studies have used specific amino acid characterization to examine the source of amino acids to the sediment, glycine (GLY) and threonine (THR) are found to be enriched in sediments from diatom source material, due to selective preservation of sinking particles while gamma-aminobutyric acid (GABA) has been associated with intracellular processes (Hecky et al. 1973; Keil et al. 2000).

Lipid and fatty acid biomarkers have been commonly used in marine systems to track the major sources of organic matter in sediments, and study the transformation and sequestration of carbon in both

the water column and sediments (Yunker et al. 2005; Belicka et al. 2004; Belicka and Harvey 2009). Specific sterols and neutral lipids can be employed to identify organic matter that is sourced from marine allocthonous production, as well as terrestrial organic matter sources as well. The following table outlines the specific compounds commonly employed for biomarker purposes.

These specific biomarkers employed on the Mackenzie River shelf can help to identify and better understand the dynamics of sinking organic carbon and organic matter sources. Coupled with meiofaunal diversity, biomarkers can provide information on carbon cycling across this dynamic shelf. Meiofauna in the Arctic Ocean is mainly comprised of benthic grazers that rely on the vertical flux of particulate organic matter as their main source of sustenance (Stein et al. 2004). Soltwedel et al. 2013 concluded that areas of lower organic carbon fluxes also had lower concentrations of meiofauna. Due to this close relationship between sinking carbon fluxes and meiofaunal communities, much of the literature has concluded that benthic abundance and likely diversity is dependent on the flux of material from the overlying water column. Klages et al. (2004) estimates a third of the pool of marine sedimentary organic carbon is stored in the form of benthic grazers. Because of the grazing of this meiofaunal community, understanding carbon cycling is directly linked to the remineralization of organic matter by the benthic community, and the fraction left to be buried in the sediments.

Table III-1. Sterols, fatty acids and amino acids used as organic source markers. Specific sterols, fatty acids and amino acids used to identify the source of organic matter. The carbon structure, dominant source and the source of information on the compound is included.

Biomarker	Carbon Range	Dominant source	Reference
n-alkanes	C_{15}, C_{17}, C_{19}	Algae	Han et al. (1968)
	C_{20}–C_{36} with odd predominance	Vascular plant	
	C_{20}–C_{35} with no odd/even predominance	Petroleum	Volkman et al. (1997)
n-alcohols	C_{14}–C_{22} saturated and Monounsaturated	Zooplankton	Sargent et al. (1981)
	C_{22}–C_{32} with even predominance	Vascular plant	
n-alkanoic acids	C_{12}–C_{18} saturated, even	Ubiquitous	
	C_{13}–C_{17} branched	Bacteria	Perry et al. (1979)
	C_{20}–C_{30} saturated, even predominance	Vascular plant	Volkman et al. (1989)
	C_{18}–C_{22} polyunsaturated	Marine algae, zooplankton	Dalsgaard et al. (2003)
	C_{22+} dicarboxylic	Vascular plants	Wakeham (1999)
Sterols	24-methylcholesta-5,22E-dien-3β-ol ($C_{28}\Delta^{5,22}$)	Diatoms, haptophytes, cryptophytes	Volkman et al. (1997)
	24-methylcholesta-5,24(28)-dien-3β-ol ($C_{28}\Delta^{5,24(28)}$)	Centric diatoms	Barrett et al. (1995)
	4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol (dinosterol)	Select dinoflagellates, minor contributions from diatoms	Volkman et al. (1993)
	24-ethylcholesta-5,22E-dien-3β-ol ($C_{29}\Delta^{5,22}$)	Higher plants, some microalgae contribution	Volkman et al. (1997)
	Terpenoids α-amyrin, β-amyrin, taraxerol	Higher plants	Volkman (2006)
	24-ethylcholest-5-en-3β-ol ($C_{29}\Delta^{5}$)	Higher plants, some microalgae contribution	Volkman (1986)
Amino Acids	Enriched relative abundance of glycine, and threonine	Diatoms	Hecky et al. (1973), Keil (2002)
	Arginine	Urea cycling and intracellular waste management	Cunin et al. 1986; Allen et al. (2006)
	Glutamic acid : GABA	GLU>GABA = Young material GABA>GLU= older material	Keil et al. (2000b)
	Enrichment in GABA	Diatom stress signal, highly degraded organic matter	Burdige (2006); Roberts (2007); Carr et al. (2016); Shelp et al. (2017)

** *Table modified from Belicka et. al. 2009 to include amino acids*

Organic carbon on the Mackenzie River Shelf is under the influence of multiple sources and physical drivers. The goal of this study was to investigate the linkages between organic matter fluxes, how they affect the benthic community, and how they transition across the broad Mackenzie River shelf. To accomplish this, samples of both water column particles and sediments were analyzed for a suite of organic biomarkers, together with the identification of meiofaunal community structure. The analysis of amino acids, total organic carbon, and fatty acid and sterol biomarkers were performed on a series of stations transecting the Mackenzie shelf. At each station, amino acids and total organic carbon analysis was completed on particle samples and surficial sediments, while fatty acids and sterols were analyzed in sediments only. Four stations were selected and sampled, starting at the 20 meter isobath and extended below the 400 m isobath [Figure III-1]. Along with the stations sampled along the Mackenzie shelf, an opportunistic site used as a pelagic monitoring station at the edge of the Chukchi Plateau, referred to as the Arctic ice monitoring site (AIM), was included as an open water Arctic Ocean station. Of these stations, samples along the Mackenzie shelf were analyzed for meiofaunal benthic abundances.

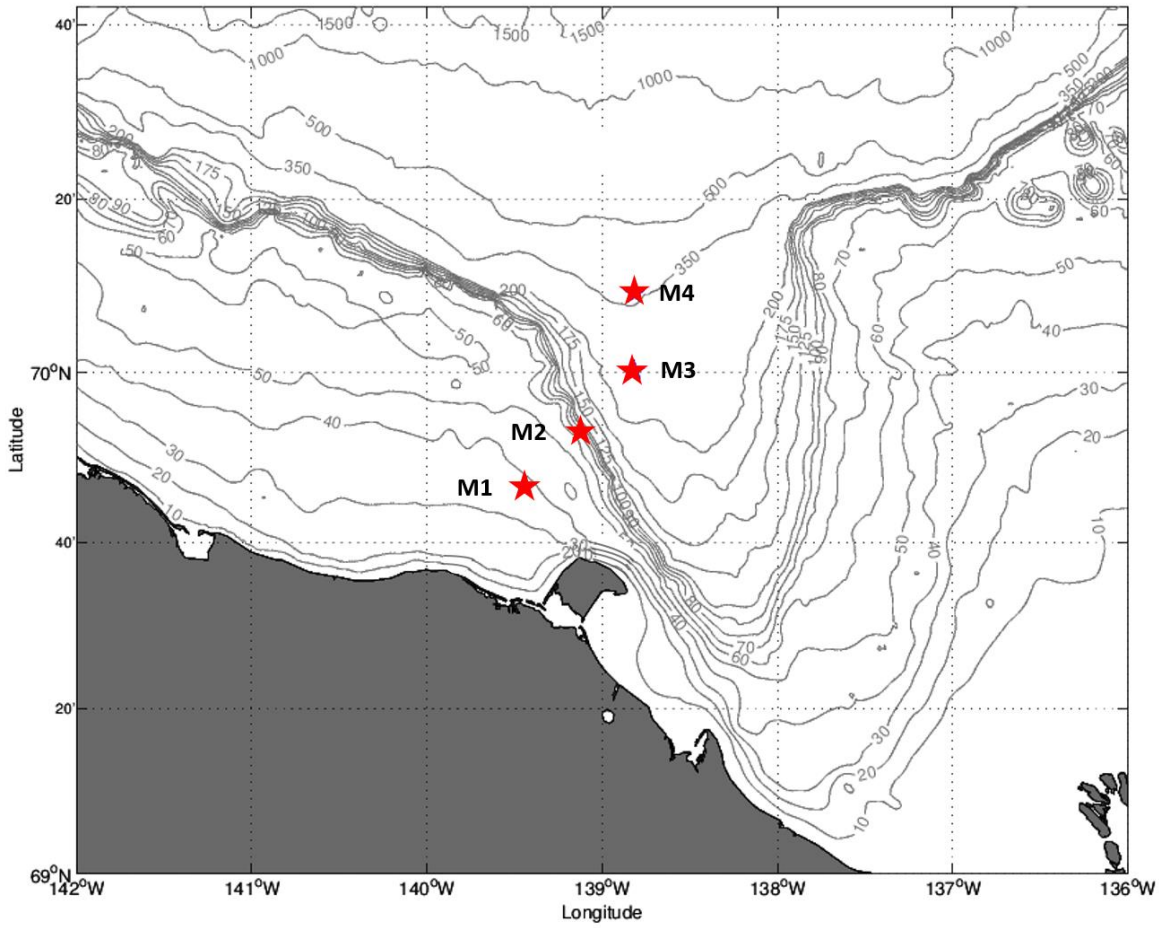

Figure III-1. Transect stations across the Mackenzie River shelf.
Map of the 4 mooring stations locations where sediments and particles were collected across the Canadian Mackenzie River shelf of the Arctic Ocean. Depth contours show depths with M1 mooring station closest to shore (map provided by MARES program).

Methods

Sampling locations and procedures

All field sampling was conducted on the Canadian coast guard ship CCGS *Sir Wilfrid Laurier* in the fall (early-October) of 2016, as part of the Marine Arctic Ecosystem Study (MARES) [Table III-1]. Particles were collected from the water column using a conductivity, temperature, and density (CTD) rosette with Niskin bottles. As the Niskin bottles were brought up from the bottom of the water column, water samples were taken at about 1 m off the seafloor, and at the chlorophyll maximum for each station.

Results of the CTD cast including physical parameters and fluorescence levels were recorded *in situ*. The particle fraction of the samples was collected using filtration through a pre-combusted Whatman glass fiber filters (GF/F) (Maidstone, UK) with an average pore size of 0.7 μm and were immediately frozen at -20 ° C. For long-term storage all particles were kept at -70 °C.

Table III-2. Location of surface sediments The Mackenzie River shelf (M1-M4) samples and their corresponding location, depth, and distance from the Mackenzie River delta. In addition, a pelagic site at the edge of the Canadian Basin titled AIM site was sampled.

Site Name	Latitude	Longitude	Depth (m)	Distance from delta(km)
M1	69 46.24 N	139 15.26 W	40 m	144.8
M2	69 54.43 N	138 59.81 W	177 m	147.2
M3	70 02.92 N	138 47.62 W	301 m	153.3
M4	70 15.09 N	138 47.65 W	440 m	171.1
AIM	75 05.79 N	168 03.54 W	161 m	1260.0

Sediments were sampled using a stainless steel 0.25 m^2 box core (KC Denmark). The box core containing sediment was sliced upon retrieval into 1 cm increments to the 10 cm depth horizon using a slicing table after removal of undisturbed interface water. Samples were sliced in a closed van, and protected from smoke stack contamination, grease drips from winches and wires, and any other ship contamination sources. After homogenization, parallel separate samples were taken for meiofaunal abundance and biomass along with organic matter characterization using pre-cleaned I-Chem jars with Teflon lined lids. Meiofaunal samples were preserved with the addition of 0.5% v/v buffered formalin in seawater. Sediment samples collected for chemical characterization were immediately frozen and stored until analysis in the shore based laboratory. In this study we examined the surficial 0-1 cm layer of stations M1 through M4, and at the pelagic AIM site the surface 0-0.5 cm layer was analyzed. Prior to organic matter analysis, sediments at each depth increment were lyophilized. Samples for organic analysis were

taken from freeze-dried sediments only, while meiofaunal counts were done with the water in the sediment as a separate sample.

Sediment grain size

Samples of lyophilized and homogenized sediments from all five stations were analyzed for grain size using standard laser diffraction methods. Addition of sodium hexametaphosphate to the sediment, which was allowed to sit overnight, helped prevent clays from forming aggregates and being miscalculated as larger particles during laser diffraction analysis. The samples were then sonicated and the sediments analyzed using a Malvern Mastersizer 3000 following standard methods (Sperazza et al. 2004).

Organic Carbon

TOC/PN was determined using standard combustion methods after removal of carbonates using HCl dissolution drop wise in a clean desiccator and evaporated in a clean 60 °C oven (United States Environmental Protection Agency 1997). After particulate filters were dried completely, samples were repackaged in foil for analysis. Sediments were similarly treated to remove carbonates in pre-combusted 4 ml vials using 1 ml of 1 N HCl and stirred using a combusted glass rod. Sediments were then left uncapped, at room temperature for two hours to allow evaporation of CO_2. Acidified sediments were then placed overnight in a drying oven set to 60° C overnight to dry.

Meiofaunal populations

Samples in 1 cm intervals through the first 5 cm of the sediment column were stored in samples sizes of 250 ml of sediment or one Ichem jar. Sediments were then analyzed for meiofaunal biomass and abundance at stations M1 through M4 but not site AIM post cruise. Sediment samples were individually diluted in water, sorted over 45 μm screens, and separated into major classes of meiofaunal organisms and intact diatom frustules.

Amino acids

Both particles and sediments were hydrolyzed at 110 °C for 16 hours in 6N HCl. After hydrolysis the liquid extracts were decanted from the sediments, leaving only the supernatant for analysis. The particle filters were decanted as well leaving behind only the extracted liquid for analysis. All samples hydrolyzed for amino acids were then dried down under ultrapure liquid nitrogen to prevent oxidation and then redissolved three times and dried with nanopure water to remove all excess HCl. The samples were then spiked with a set of deuterated internal standards representative of each functional group present in measured amino acids. All analysis was done using liquid chromatography-mass spectrometry (LC/MS/MS), with no derivatization step, using ion pairing mobile phases and retention time based mass selection and quantification. For analysis an Agilent 1290 infinity LC was paired with a Thermo Scientific Orbitrap-mass spectrometer. The choice in mobile phases and gradient elutions were modified from previous studies (Priaud et al. 2003, 2005; Crafts et al. 2012); details of the gradient program and instrumentation are discussed in chapter 2. All samples were calibrated to an external-internal calibration curve based on the quantity of internal deuterated standard added to each sample. Primary and secondary mass values were selected based on previous work (Priaud et al. 2003), available MSDS information, and standards run individually in house.

Lipid Biomarkers

Sediments were weighed and extracted via microwave-assisted solvent extraction (MASE; CEM MARS-5 system) with 2:1 dichloromethane: methanol (DCM:MeOH) (Belicka and Harvey 2009; Taylor and Harvey 2011). Extracted sediments were then filtered through combusted, solvent-rinsed glass wool to remove any particulates, evaporated to dryness, and redissolved in the original 2:1 DCM: MeOH solvent mixture. Following base extraction for total lipids, neutral lipids were partitioned with 9:1 hexane:diethylether. Following acidification with concentrated hydrochloric acid (aq), polar fatty acids were similarly partitioned. The sedimentation neutral fraction was derivatized using BSTFA to form their trimethylsilyl (TMS) products, and fatty acids were converted into their corresponding methyl esters using

boron trifluoride (10% in methanol). Both fatty acids and neutral lipids were analyzed using a Agilent 6890N gas chromatograph with flame ionization detector (GC-FID) and identified with a coupled Agilent 5975 mass spectrometer (GC–MS) using a 60 m DB5-MS column. Chromatographic details are described in detail in Belicka et al. (2002), with an inlet temperature of 250 °C. Neutral lipids were analyzed for individual structure; however, most individual components were categorized and summed as totals of each category.

Results

Conditions across the shelf

All four Mackenzie River shelf stations display a fresher surface layer with increased salinity at depth [Figures III-2, III-3, III-4, III-5]. The near-shore location, station M1, had the shallowest thermocline and halocline at 7 meters below the surface, and similar near surface fluorescence maximum occurring close to the halocline [Figure III-2]. The other three stations in the transect all had similar halocline and thermocline depths around 20-30 meters. Overall fluorescence corresponded closely to the halocline depth, decreasing rather rapidly below the halocline. Nevertheless, the fluorescence maximums for these stations did vary, station M2 had fluorescence peaks at both 20 meters and 40 meters while both station M3 and M4 had fluorescence maximums near the halocline that were twice as high as station M1 and M2. Compared to the Mackenzie shelf transect stations, the pelagic AIM site had lower fluorescence values that never peaked above 0.4 RFU (relative fluorescence), and a much colder surface layer [Figure III-6]. The surface waters at the time of the CTD cast at the AIM station were below 0 °C and never peaked above 1 °C at 20-40 meters. Salinity at the AIM station was comparable to the Mackenzie shelf stations with a 30 meter halocline and an increase in fluorescence at the halocline.

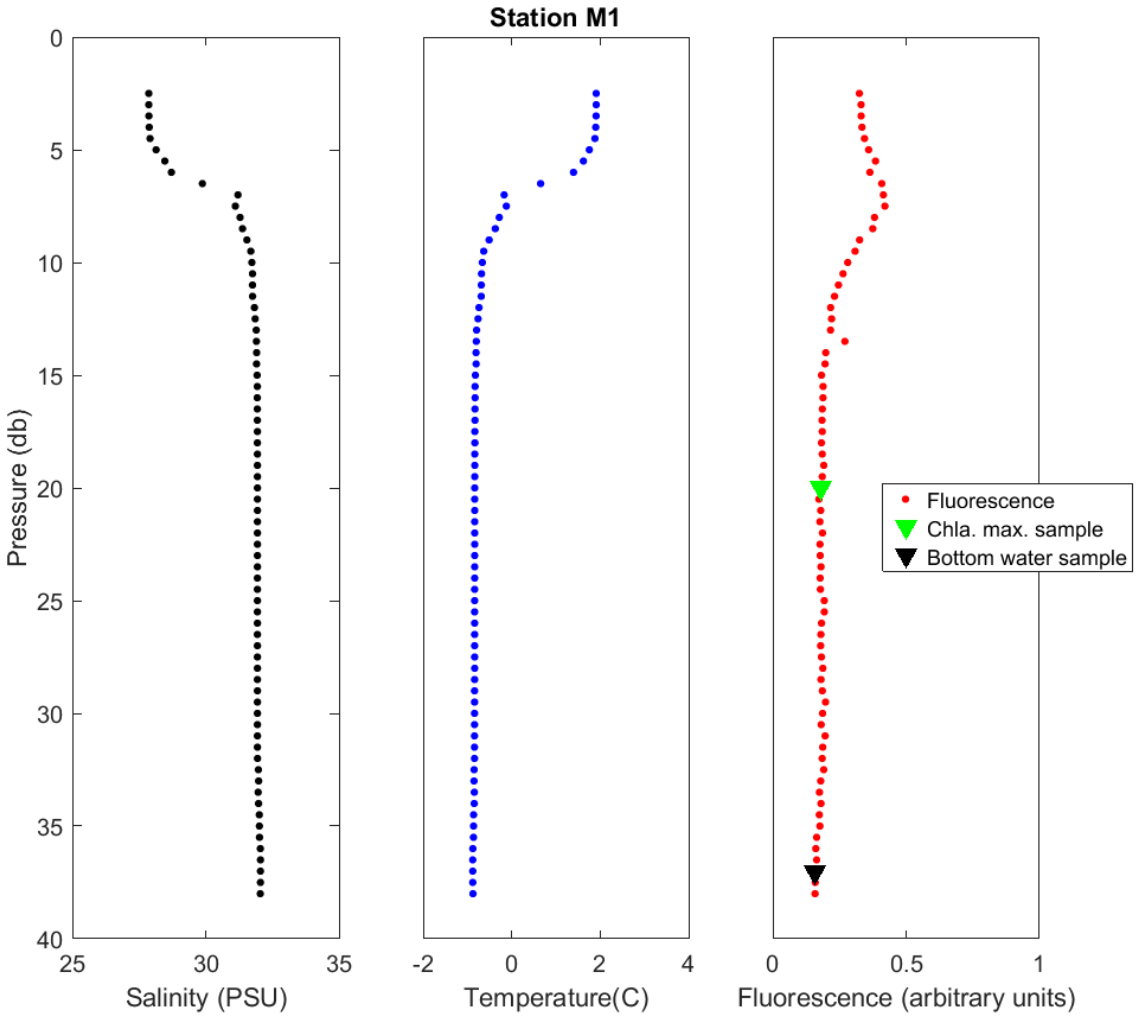

Figure III-2. Water column physical and fluorescence parameters at the time of sampling M1. Salinity, temperature, and fluorescence are shown through the water column. Sampling depths for particles at the approximate chlorophyll maximum (based on fluorescence) and bottom of water column are represented by the green and black triangles, respectively. At this station, sampling of the chlorophyll maximum was done below the maximum to avoid river export at the shallow depth and avoid the 3 meter draft of the coast guard vessel.

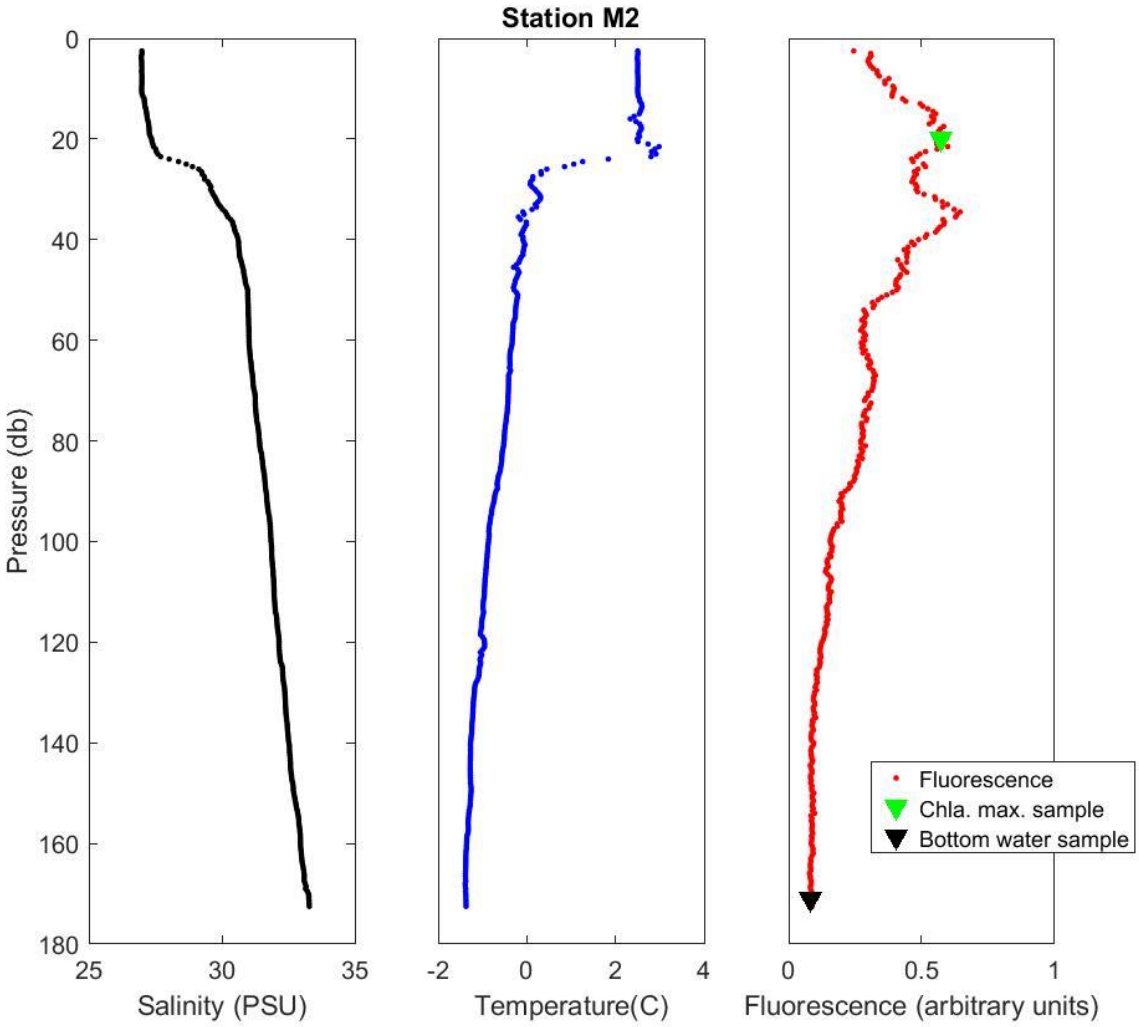

Figure III-3. Water column physical and fluorescence parameters at the time of sampling M2. Salinity, temperature, and fluorescence are shown through the water column. Sampling depths for particles at the approximate chlorophyll maximum (based on fluorescence) and bottom of water column are represented by the green and black triangles, respectively.

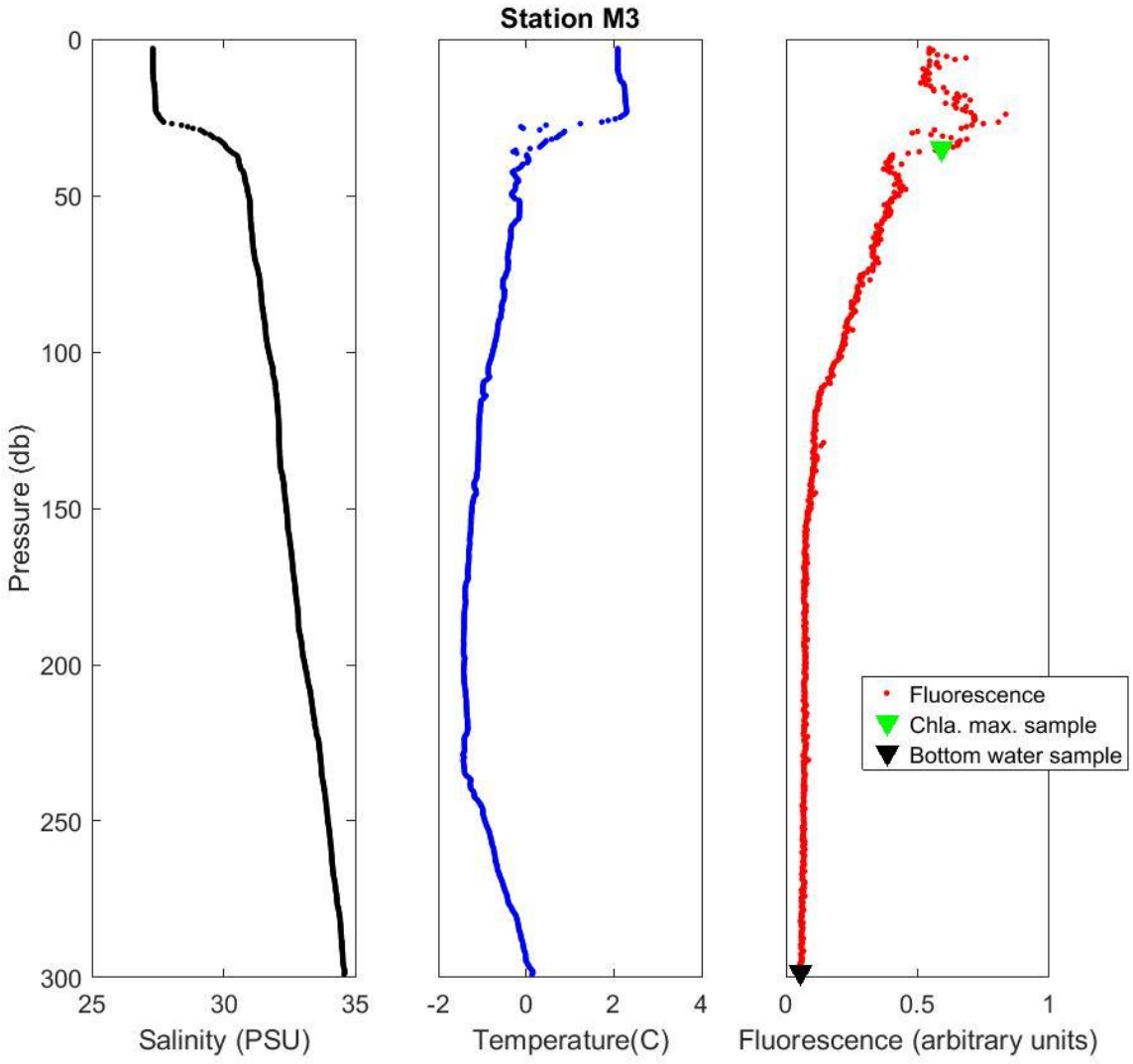

Figure III-4. Water column physical and fluorescence parameters at the time of sampling M3. Salinity, temperature, and fluorescence are shown through the water column. Sampling depths for particles at the approximate chlorophyll maximum (based on fluorescence) and bottom of water column are represented by the green and black triangles, respectively.

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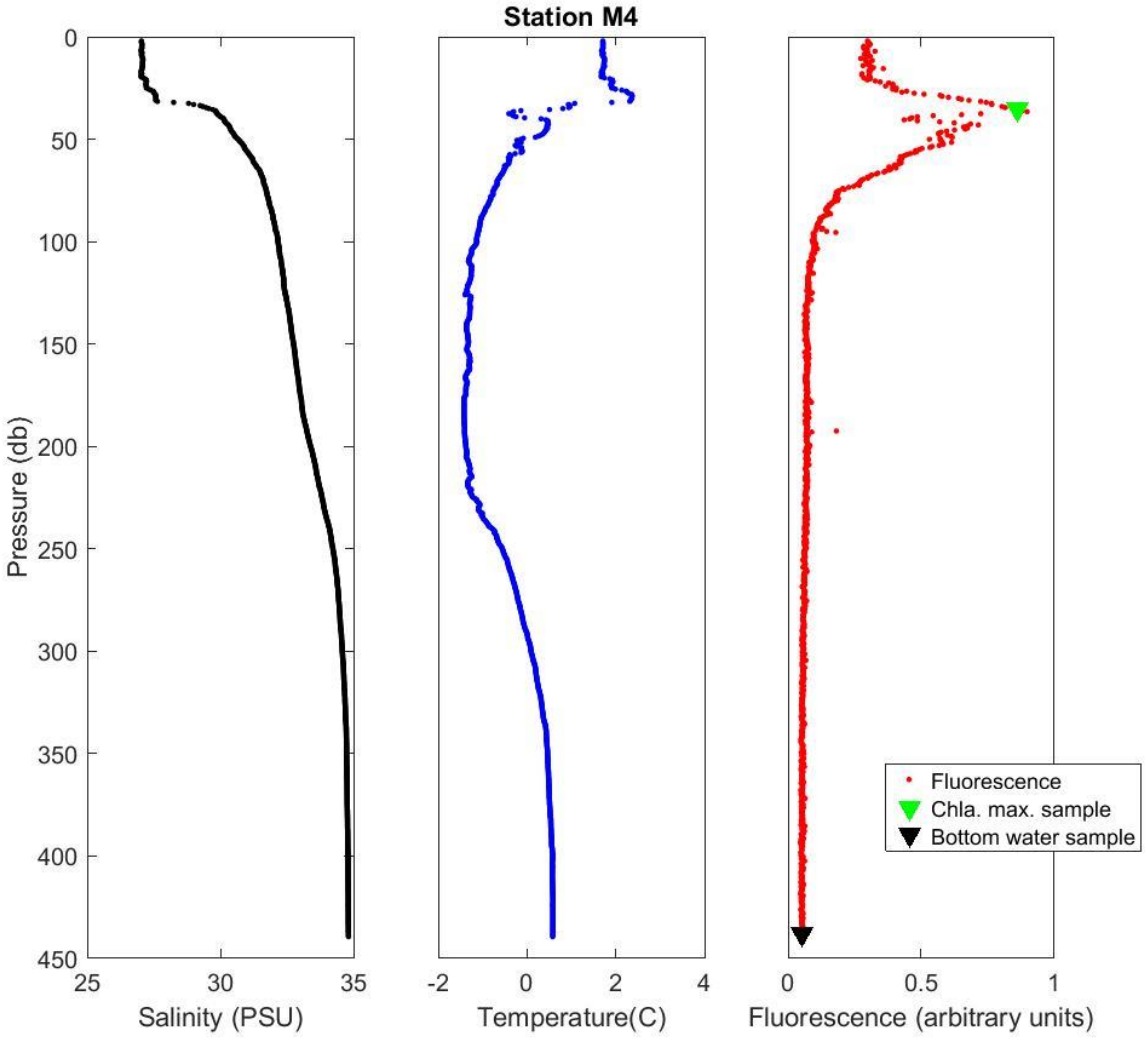

Figure III-5. Water column physical and fluorescence parameters at the time of sampling M4. Salinity, temperature, and fluorescence are shown through the water column. Sampling depths for particles at the approximate chlorophyll maximum (based on fluorescence) and bottom of water column are represented by the green and black triangles, respectively.

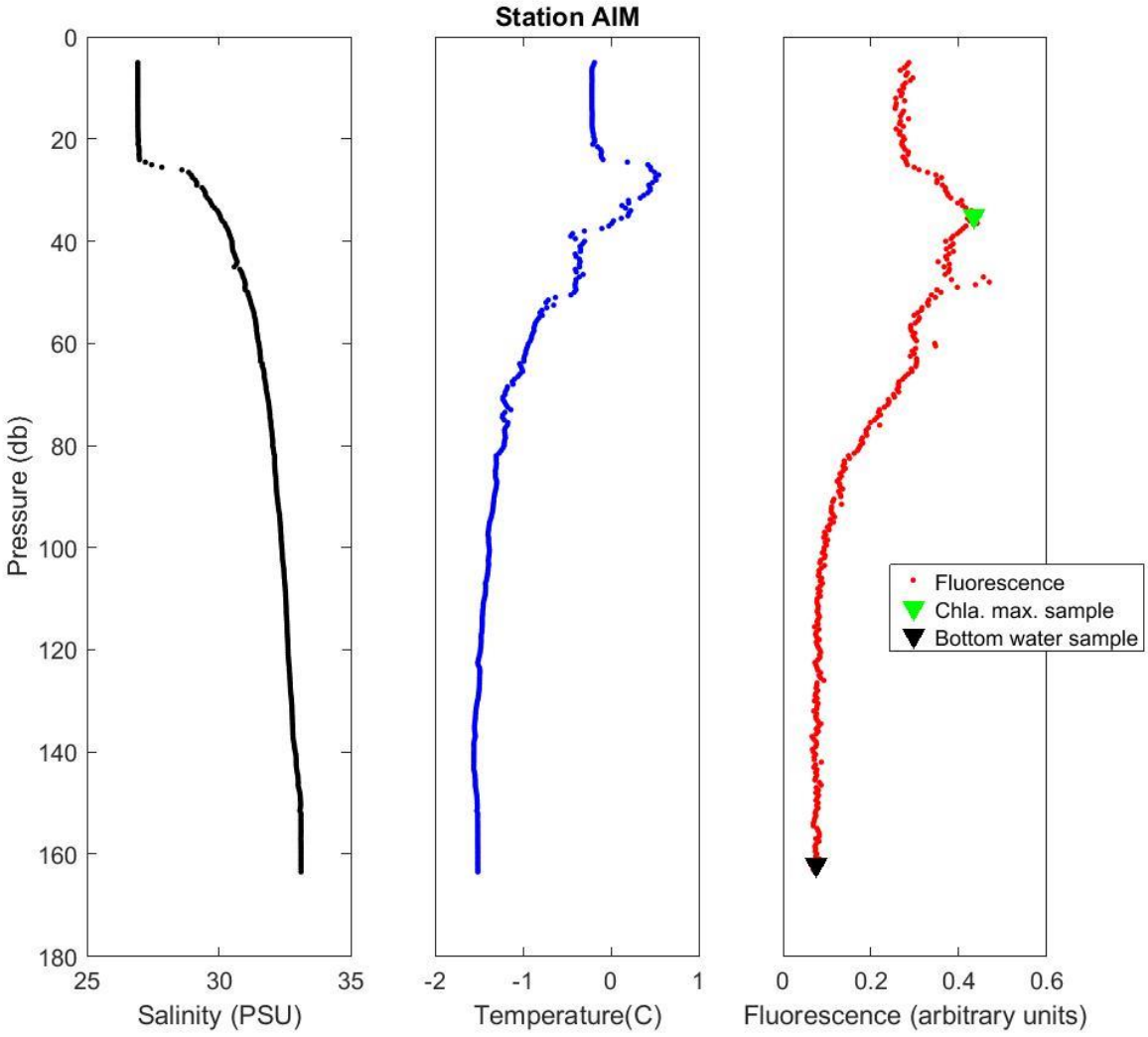

Figure III-6. Physical and biological parameters at the time of sampling station AIM
Salinity, temperature, and fluorescence are shown through the water column. Sampling depths for particles at the approximate chlorophyll maximum (based on fluorescence) and bottom of water column are represented by the green and black triangles, respectively.

In the sediments, uniform grain sizes were seen across all samples, including the more pelagic AIM site [Figure III-7]. The majority of these sediments are in the fine silt to clay grain size category with the majority of sediment falling between 0.6 μm and 20 μm. Levels of organic carbon stayed below 2% of the dry weight at all stations with station M2 having the highest value at 1.6 % organic carbon and the AIM site the lowest with 0.86% organic carbon [Table III-3]. In the water column at the chlorophyll maximums, station M4 had the lowest value of carbon at 36 μg OC/L while the highest values were seen at station M2 with 62 μg OC/L. The AIM site and station M3 were also high compared to station M4 at almost double station M4 with 56 and 53 μg OC/L respectively [Table III-3].

Table III-3. Organic Carbon properties of stations M1 through M4 and AIM site.
Organic carbon content of all samples from the water column and surface sediments. For particle samples organic carbon is reported as particulate organic carbon (POC) in μg/L or μg carbon per liter of water filtered. Surface sediment organic carbon reported as % carbon, or % carbon in grams per dry weight of the sediment in grams.

Location	Depth	POC (μg/L)
M1	10 m	46
M2	20 m	62
M2	175 m	58
M3	35 m	53
M4	35 m	36
AIM	35 m	56
AIM	160 m	43

sample site	depth in core (cm)	organic carbon (%)
M1	0-1	1.109
M2	0-1	1.656
M3	0-1	1.355
M4	0-1	1.297
AIM	0-0.05	0.858

Samples of particles and sediments along the Mackenzie River shelf basin yielded varied concentrations of hydrolyzable amino acids with differing distributions. The range of amino acids contributing to the particulate organic carbon ranged from 0.02% amino acids (bottom water POM station

M2) and a maximum of 3.5% (reported as µg amino acid/ g OC at M4 chlorophyll maximum POM) [Figure III-8]. Among the sediments, M1 has the lowest levels of THAA in organic carbon of the four stations, station M1 is also one of the lowest particulate THAA normalized to carbon. Station M2 had the highest amino acids per gram of organic carbon in the sediments although in the chlorophyll maximum station M4 had the highest sum of amino acids. M3 and M4 fall in the mid-range of values observed across the transect [Figure III-8]. Levels of amino acids (THAA) remained elevated compared to the rest of the transect at station M3, making station M3 the second highest THAA sediment sample while the observed THAA levels in the water column at M3 were also relatively high compared to the two stations closest to the Mackenzie River Delta.

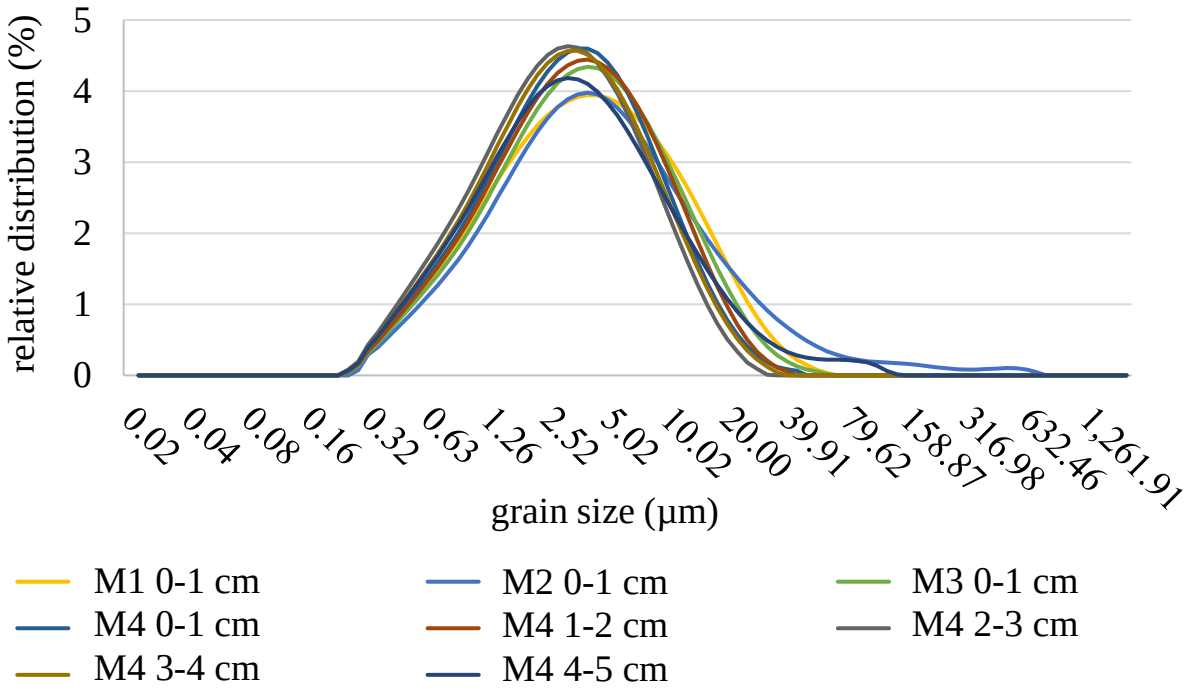

Figure III-7. Sediment grain Size distribution of the Mackenzie transect
Grain size distribution of Mackenzie shelf basin sediments. All sediments showed a similar distribution with maximum near 5µm and generally uniform grain size across the continental shelf. Depth in sediment horizon is indicated in centimeters from 0-1 cm to 4-5 cm. Only station M4 sediments are reported below the surficial sediment layer. The x axis indicates grain size in microns (µm) while the y axis indicates relative percent of sediment sample as the given grain size.

Across the stations, glutamic acid and lysine were the most abundant amino acids at all 5 stations in the sediments while glycine was observed at all four stations and the AIM site in the sediments but was not seen in the water column [Figure III-9]. Station M4 had more hydroxyproline than M1, and M1 contained tyrosine while M4 did not. The sediment samples also had higher threonine and alanine across all stations than seen in the particles, two amino acids thought to be primarily sourced from diatom organic matter (Hecky et al. 1973). In the particulate matter, glutamic acid and GABA were the most abundant amino acids. At the beginning of the transect (M2), glutamic acid contributed more to the sum of amino acids than at the station furthest from shore (M4). In contrast, GABA was highest in the particles furthest from shore [Figure III-11].

Fatty acids and sterol biomarkers

Sediment fatty acids, phytol, and sterols showed an increase in marine related biomarkers at mid-shelf stations, with terrestrial related markers highest at station M1. The sum of all fatty acids was highest at station M2 at 2 mg/g dry weight, while the second highest, at almost half the quantity, was 1 mg/g dry weight at station M1. Fatty acids were grouped based on their structure and are shown in Figure III-12(a). Polyunsaturated fatty acids were higher at stations M2 and M4 while long chain fatty acids were highest at station M1. Among the sterols, dinosterol was observed in significant amounts across all samples but was highest at station M1. 24-methyl cholest-5,22-dien-3β-ol (brassicasterol), a diatom-specific sterol (Volkman et al. 1998), was seen only at stations M2 and M4, but not station M1, and in smaller amounts at the AIM site [Figure III-12(b)]. Fatty acid and sterol biomarkers show increases in algal related markers further from shore. Long chain fatty acids, 24-ethylcholestan-3b-ol, and isofucosterol, all indicators of higher plants, decreased offshore, while increases in marine diatom markers including brassicasterol, 24-methylcholest-22-en-3β-ol and polyunsaturated fatty acids (PUFAs) occur at mid-shelf and offshore stations [Figure III-13].

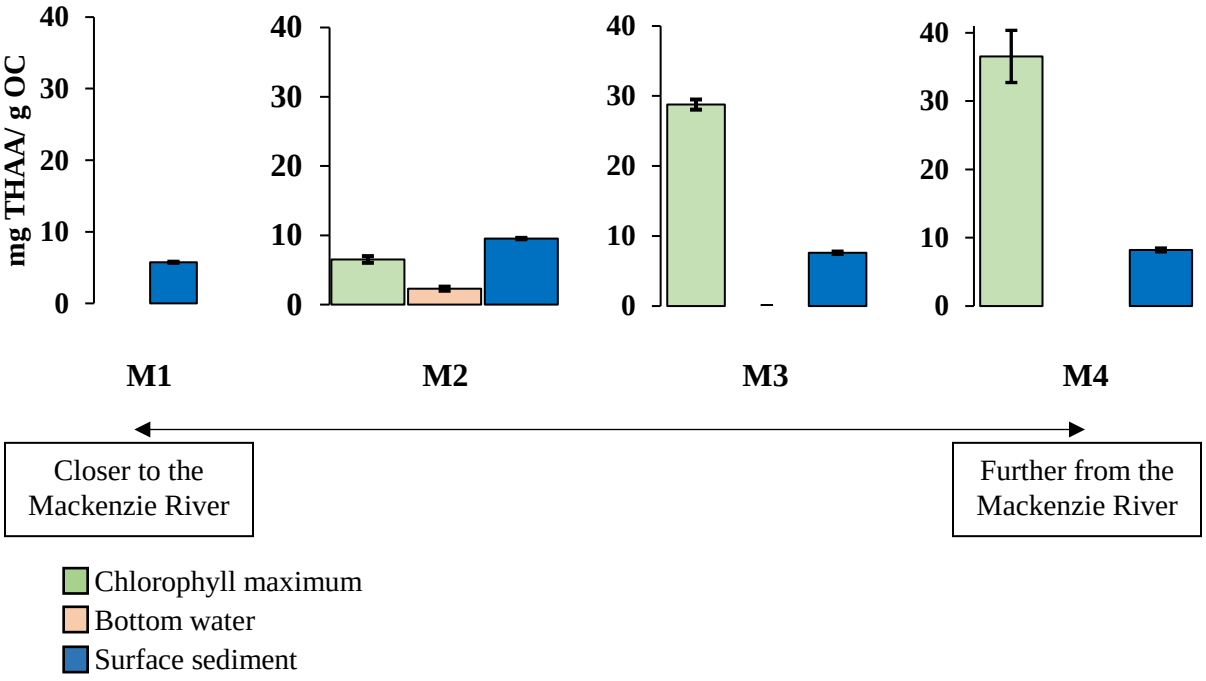

Figure III-8. Total hydrolysable amino acids (THAA) normalized to organic carbon (mg THAA/ g OC) in particles and sediments across the Mackenzie shelf.
Samples depths for the chlorophyll maximum and bottom particles (POM) are shown in figures [III-2]-[III-6]. Measure of THAA in surface sediment is from the 0-1cm horizon. Bottom waters at stations M1, M3 and M4 not measured for THAA and values of THAA for the chlorophyll maximum at M1 lost during analysis. Error bars represent standard deviation in analytical replicates (n=3).

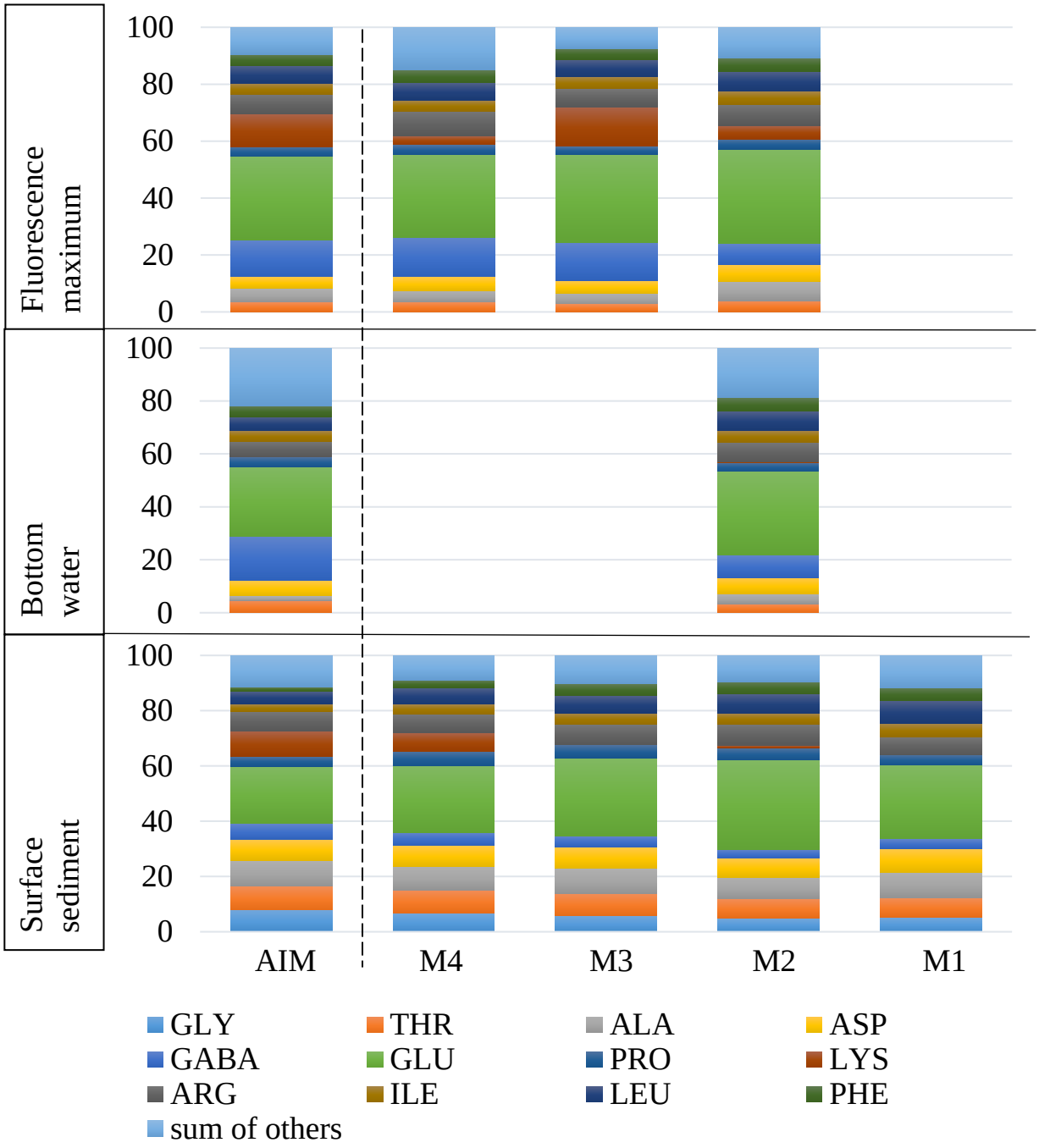

Figure III-9. Individual amino acids present in surface sediments and water column particles. Distributions of individual amino acids at each site represented as percent of total amino acids. Samples from the AIM site are separated to the left with a black dashed line, this sample is separate from the main transect of the Mackenzie self-basin. Particles samples taken from the fluorescence maximum and above the sea floor are represented in the upper two plots while surface sediments (0-1 cm) are shown in the bottom plot. Amino acids not displayed in the top most abundant 12 amino acids were summed together in the category of *sum of others*. Bottom water particles were only measured for THAA at station M2 and the AIM site. THAA data for M1 at the fluorescence maximum was lost during analysis.

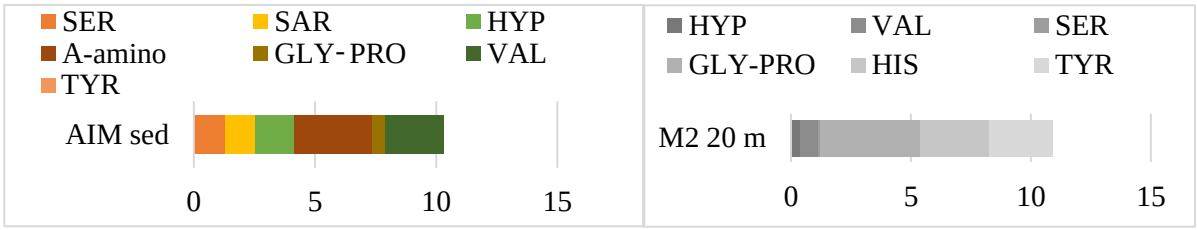

Figure III-10. Low abundance amino acids found in sediments and particles.
Individual amino acids that contributed a small percentages of the total amino acids. These low abundance amino acids were represented by the *sum of others* category in Figure III-9. Sediments and particles had different amino acids in this low category as represented by the examples of AIM sed (surface sediments at the AIM site) and M2 20 (the particle sample taken at the fluorescence maximum).

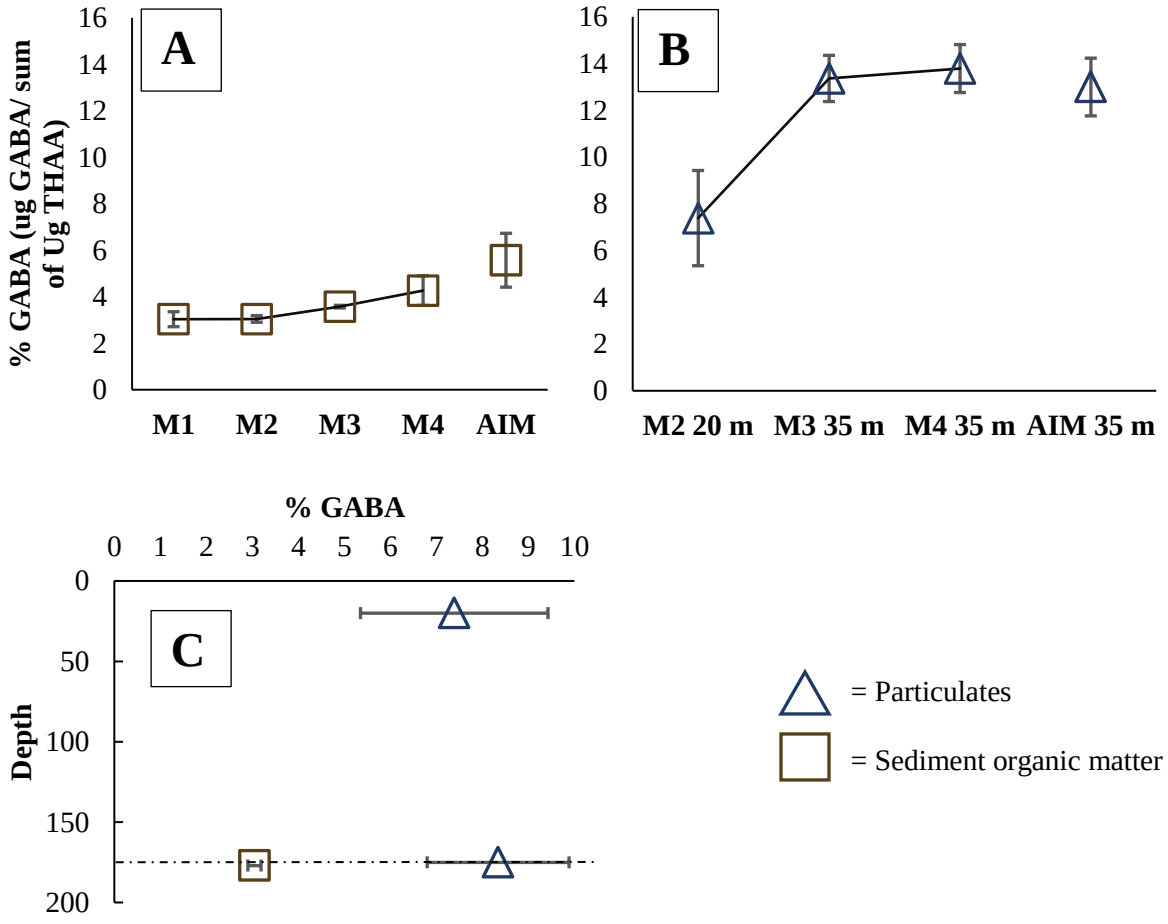

Figure III-11. Fraction of GABA seen in THAA particulates and surficial sediments.
A) The % GABA as fraction of total amino acids in surface sediments of the MARES stations and AIM site. B) The % GABA as fraction of amino acids in particles collected at the chlorophyll maximum at four of the five sites. M1 was lost during analysis. C) The % GABA through the water column at station M2. The dashed line represents the interface between the water column and sediments. Error bars in this plot represent the standard deviation of analytical replicates (n=3).

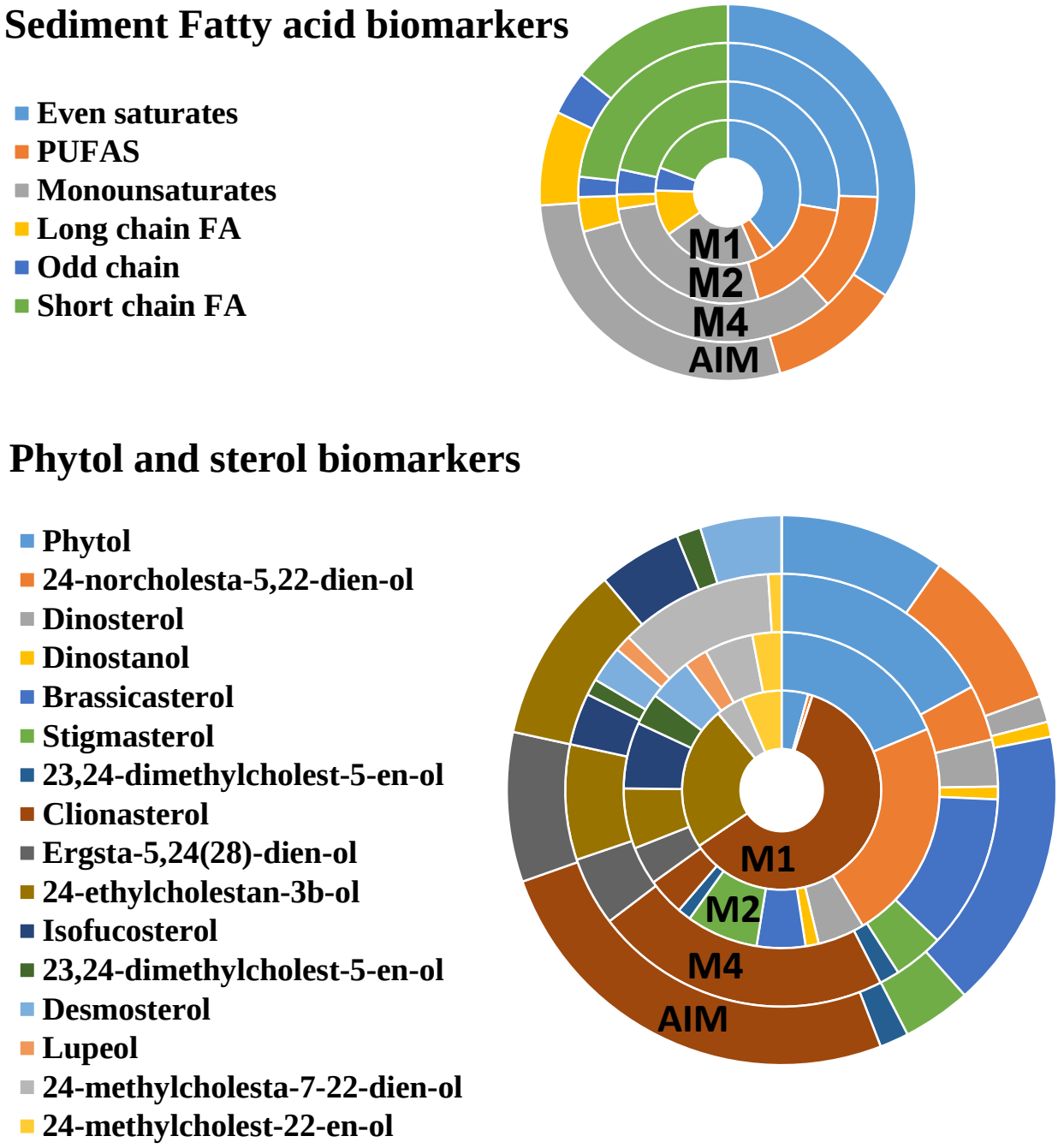

Figure III-12. Lipid and fatty acid biomarkers present in Canadian Basin Arctic sediments
Fatty acid and sterol biomarkers measured in the sediments of the Mackenzie River shelf and the AIM site shown as fractional contribution to the total observed. Concentrations are represented in this figure as relative abundance. Station M3 is not shown in this figure due to sample loss during analysis.

Meiofaunal community

Benthic grazers dominated the meiofaunal population across all four station in the M1 to M4 transect (site AIM not sampled). Overall abundances were highest and most diverse at the mid-shelf stations where levels of total abundances reached almost 1000 per sample. Foraminifera was the most abundant phylum in the samples, with increasing abundance along the transect. Copepods and diatoms were the second most abundant taxas with their maximum populations occurring at the mid-shelf stations (Figure III-13). Specifically, station M2 was highest in intact diatom frustules, corresponding well with the biomarkers data, which was highest in algal specific markers at station M2 [Figure III-14]. In contrast to biomarkers and THAA results, which were highest at station M2, the meiofaunal population was highest at station M3 and remained higher than station M2 at M4. Station M1 was dramatically lower than the other stations with one tenth the population of meiofauna and diatoms compared to the other sections of the transect.

Discussion

The analysis of four Mackenzie River shelf stations and comparison with the pelagic AIM site suggest that organic carbon peaks at the mid-shelf stations in the sediments and water column particulates. Total organic carbon, sedimentary biomarkers, particulate/sediment THAA, and meiofaunal abundances all show equal or higher concentrations of carbon at the mid-shelf stations and higher mieofuanal populations. Mid-shelf stations were also higher in marine related organic matter. These results suggest a change in the dynamics of these mid-shelf stations that does not occur inshore and offshore from the Mackenzie River Delta.

Measured physical parameters at the stations at the time of sampling can only provide limited insights into this dynamic system. All four stations along the Mackenzie trough had fresher surface waters that were also warmer than the underlying waters. Fresh Mackenzie River water accounts for this surface layer that was seen across these stations, but provides little insight into the causes of higher mid-shelf

organic carbon. Fluorescence differed amongst the stations with station M1 having the lowest measured fluorescence, but station M4 had the highest fluorescence. While this agrees with lower carbon at M1, it does not provide insight into the presence of increased labile marine carbon at station M2. There was no sea ice at the AIM site, and it is too removed from river runoff to be majorly impacted by river inputs. The surface salinity and temperature structure is then likely a feature of different water masses present in the fall due to stronger winds increasing the flow of Pacific water onto the Chukchi plateau on this relatively shallow shelf (Weingartner et al. 2017). Pacific water is forced through the Chukchi Sea by strong northern winds starting in the fall and continuing into the winter. The observed cold fresh water is likely sourced from the combination of seasonal ice meltwater and runoff, as well as this fresher Pacific waters (Pickart et al. 2010; Linders et al. 2017).

The similarity of grain size distributions among each station suggests that physical mineral size has little effect on the community and organic carbon found at these stations. Organic carbon levels are usually higher at stations closest to the mouth of river deltas as it deposits large amounts of terrestrial organic matter in the form of particles (Ouyang 2003; Ni et al. 2008), yet, this is not the case for this transect. Station M2, a more mid-shelf station, is the highest, albeit only slightly, in organic carbon in both the sediments and at the chlorophyll maximum of the water column, with station M1 the lowest in organic carbon of the four stations. Organic carbon observed at stations M3 and M4 in sediments agrees well with previous literature values in the same area (Forest et al. 2007), although previous data for stations M1 and M2 are not readily available. Sedimentation rates at stations M3 and M4 reported from previous work are estimated to be 0.1 cm $year^{-1}$ (Richerol et al. 2008b). This suggests that the results gathered in this study of the top 1 cm for the sediment is representative of the decadal signal, at a minimum. Richerol et al. (2008) concluded that the first 1 cm of the water column is bioturbated at stations M3 and M4, suggesting that the organic material seen across the Mackenzie River shelf reflects deposition, marine production, and in-situ benthic activity. This makes it likely that the carbon source for these mid-shelf stations could be dominated by a marine sources rather than river deposition.

Although particulate carbon found in the water column is not necessarily analogous to the sedimentary carbon observed at the stations, the fluorescence maximum and bottom water samples of particulates at mid-shelf stations are still highest in organic carbon. Particulate carbon that makes it to the seafloor, as observed in this study, is not largely lower than that seen in the water column. Even at the offshore AIM site, the bottom water has significant particulate organic carbon compared to the chlorophyll maximum. In fact, the resulting organic carbon in water column particles and sediments from the AIM site falls with the same range as the four sites located along the Mackenzie shelf. The shallow water column may allow this making it easier for particulate matter to reach the sediments before being remineralized or consumed.

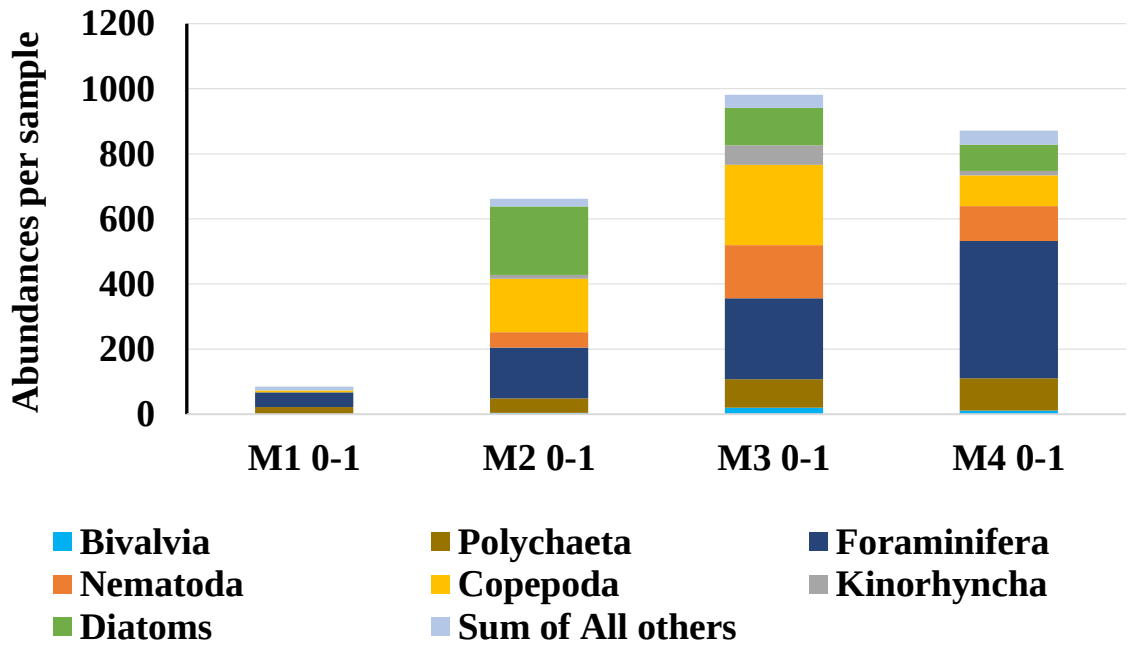

Figure III-13. Meiofaunal and intact diatom frustules abundances across the Mackenzie River shelf transect.
Meiofaunal community reported as abundances per class in 250 ml sediment samples in the surficial 1 cm of sediment. Overall trends across the shelf show larger communities at the mid-shelf stations with corresponding increase in diversity (M2 and M3). Data provided by Pamela Neubert (Stantec).

Beyond total organic carbon, another way to examine the sources and related organic carbon features is through the analysis of amino acids and for sediments and particles, specifically THAA. The range of amino acids contributing to the total organic carbon was in the low range of coastal shelf values (Keil et al. 2000; Lee et al. 2000). Station M1 had the lowest value in the sediments with only 1% THAA; this increased at station M2, and remained higher moving further offshore at stations M3 and M4. In the water column, the highest THAA contributions to organic carbon occured at the chlorophyll maximum of station M4 (3.5%) and the AIM site (2.8%). This increase in THAA in the sediments and water column particles suggests the input of labile material at the mid-shelf stations. Total amino acids per unit organic carbon suggest that the mid-shelf region has the highest deposition of amino acids to the sediments, and the highest water column production further offshore at station M4 and the AIM site. Sampling of particles at the bottom of the water column showed a very low fraction of organic matter present as amino acids at both station M2 and AIM. Amino acids can contribute to upwards of 40% of the available organic carbon (Keil et al. 1998), however in this study, only 1% of the organic carbon was identified to be THAA.

In past studies of amino acids in marine organic matter, the amount of GABA present in a sample in relative abundances (% GABA) was suggested to represent a measure of degradation (Keil et al. 2000). This can allow us to identify where the samples along the transect have the oldest material and what that might reflect towards the materials source. Previous marine sediment studies that have higher % GABA have argued for enhanced degradation of the organic matter in these sediments (Burdige 2006). Figure III-11 represents the % GABA in surface sediments of the sampled 5 stations, as well as particles in the chlorophyll maximum at four of the five sites, particles near the seafloor and the relationship of % GABA with depth. The % GABA suggests more degraded organic matter in the surface sediments occurs further from shore, suggesting slower sedimentation at the offshore stations or material sourced from mid-shelf that is transported offshore by physical mixing. Across all stations in the Mackenzie shelf and at the AIM site, the water column had a higher % GABA contribution in particles than found in the surface sediments. This may suggest the lack of selective preservation for GABA in the sediments at these stations or perhaps mixed sources of amino acids further offshore. Keil et al. (2000) argued that sediment samples low in

organic carbon are often higher in non-protein amino acids like GABA. Keil et al. (2000) also suggests that for sediments and particles with organic carbon less than 10%, non-protein amino acids are produced *in-situ* rather than sourced from deposited particulate matter. The increase in GABA seen in the sediments further offshore together with the decrease in glutamic acid might reflect the *in-situ* decarboxylation of glutamic acid to GABA. Even though the carbon levels of all stations is below 10%, this might still provide a measure of diagenesis in these sediments and particles: the more glutamic acid is converted to GABA, the more degraded the organic matter. Based on the increase in GABA and decrease in GLU seen in the sediments, we can speculate that sediments further from shore are more degraded than those closer to the Mackenzie River delta, or may represent a larger fraction of old terrestrial derived organic material preserved in the sediments.

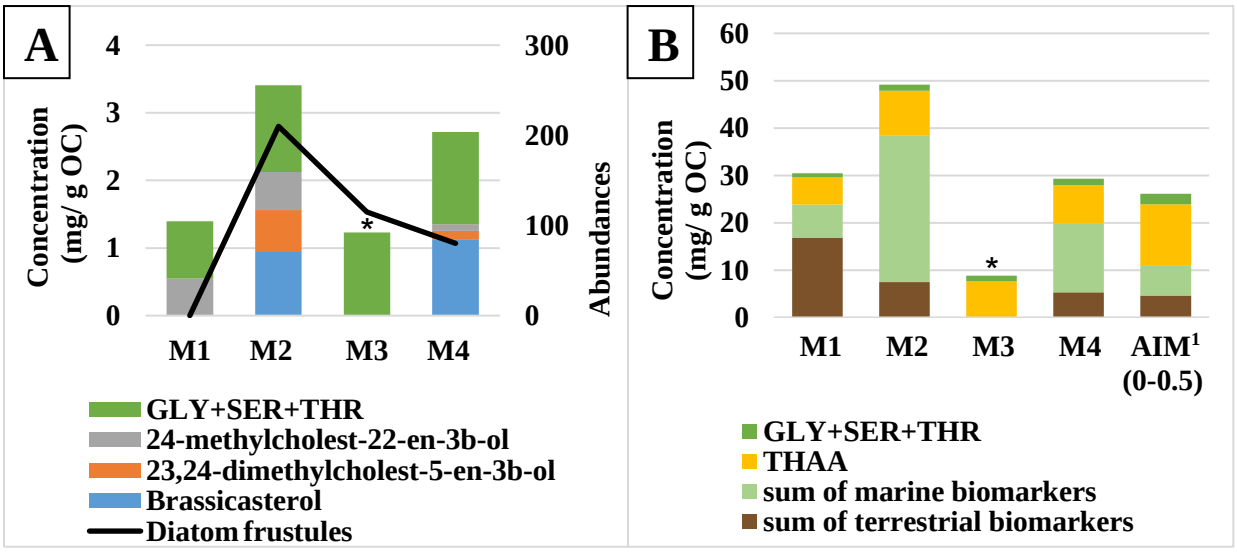

Figure III-14. Major lipid biomarkers assigned to either terrestrial or marine organic matter across the shelf transect.
Plot A) describes diatom specific biomarkers in sediments represented as mg per gram sediment organic carbon. The secondary y-axis describes the abundance of diatom frustules found in the corresponding sediments[1]. Plot B) shows organic matter distributions across the self in the surface sediments compared to the measured total organic carbon. The AIM site sampled organic matter from the first 0.5 cm while the others were done for the first 1 cm due to differences in sedimentation rates of the two locations. Note, both plot A and B are in units of mg/g OC, plot B has a scale 10 times that of plot A.

* All other biomarkers data unavailable at this station
[1] Diatom frustules and meiofaunal data not available at the AIM site.

Other amino acids with potential as markers include glycine, serine, and threonine, all which are well preserved in diatom frustules, allowing us to identify where along the transect marine organic matter is highest (Hecky et al. 1973; Keil et al. 2000). At all stations glycine, serine, and threonine contribute to a larger percentage of the sediment amino acids than those found in the water column particle [Figure III-9]. Glycine is only found in the sediments, which might reflect a source of diatom frustules to the sediment surface not seen in the water column particles sampled. The sum of glycine, serine, and threonine abundances are highest at the mid-shelf stations, again suggesting that marine related material is an important contribution to organic matter in this part of the shelf [Figure III-14].

Beyond amino acids, fatty acid and sterol biomarkers are used to identify sources of organic matter. Previous work in the area of stations M3 and M4 reported a significant population of dinoflagellate cysts in the sediments, agreeing with the large presence of the dinosterol biomarker seen (Richerol et al. 2008a). The presence of other marine related biomarkers such as long chain fatty acids sourced from higher plant matter, show a more significant presence of terrestrial-derived mater closer to shore at stations M1 and M2. The presence of marine related biomarkers is dominant at every station but M1 [Figure III-13]. M1 is unique to the transect in having more than 50% of the measured organic matter that appears to be derived from terrestrial carbon. This is likely because of its proximity to shore and the Mackenzie River delta. Station M2, representing the station with the highest marine biomarkers, also has the highest abundance of diatom frustules and diatom related biomarkers of all the stations [Figure III-14]. Compared to M1, mid-shelf stations appear to have the highest marine organic carbon, based on the biomarkers, diatom frustules, and increases in diatom related amino acids. Therefore, this carbon is heavily sourced from in-situ production instead of transport of Mackenzie delta particulates.

Like organic carbon, benthic meiofauna shows high diversity and abundances at mid-shelf stations. Station M3 has the highest overall abundances and highest diversity of the samples, unlike organic carbon in the sediments, which is highest at station M2. Most of the benthic meiofauna sampled were grazers who

feed off sinking organic fluxes and sedimentary organic carbon (Stein et al. 2004). The mismatch in organic carbon and benthic community could then be sourced from station M3 being quickly grazed over by benthic organisms compared to station M2, as well as station M4 being higher than M2 in benthic life with station M1 being the lowest in all respects (Soltwedel et al. 2013). Larger benthic fauna occurred in higher abundance further from shore; to support a community like this, a larger source of fresh organic matter must reach the sediments from the water column or by other depositional processes.

Results from the meiofaunal population combined with information from amino acids, organic carbon, and lipid biomarkers together indicate that across this transect, highest carbon fluxes and available organics in the sediments occur at mid-shelf stations. Sediments located closest to the opening of a river delta, especially ones with large sediment loads, are known to provide large amounts of terrestrial material to the sediments (Ouyang 2003; Ni et al. 2008). MacDonald et al. 1998 argues 60% of the POC from the terrestrially dominated Mackenzie River outflow is preserved in the sediments. These factors would argue that the majority of the sedimentary carbon should be terrestrially dominated organic matter. However, the resulting conditions along the Mackenzie shelf suggest further factors concerning carbon cycling exist beyond riverine deposition.

These stations, located along the shelf near Herschel Island that were examined have a different dynamic of carbon cycling than traditional riverine costal shelves as discussed in this chapter. Along Hershel Island at about station M1, the stamukhi occurs, the area in which land-fast ice meets and joins the annual and multi-year sea ice (Macdonald and Carmack 1991). During the winter months, this stamukhi ice pack reaches all the way to the seafloor, blocking any interaction of the Mackenzie River runoff with the coastal seawater of the Mackenzie shelf (Carmack and Macdonald 2002). Offshore of this ice every spring, the seasonal sea ice breaks up first before the below water ice at the stamukhi has thawed to release Mackenzie River water. When this break up occurs strong phytoplankton blooms are likely to occur. This bloom leads to large fluxes of carbon to the bottom where the ice opens up during a time when there are no additions of Mackenzie River runoff and particulate fluxes. Carbon fluxes to the sediment then increase as the conditions become more favorable for phytoplankton production after the Mackenzie River runoff can

flow over these mid-shelf areas, increasing nutrients in a time which suffient light is available for primary production (Forest et al. 2007, 2013). This ice formation set up and resulting increase in primary production compared to the other stations along the transect, is likely why the mid-shelf stations show higher amounts of carbon, diatom markers, diatom frustules, and benthic grazer communities than the in-shore or offshore areas.

Conclusions

The broad shelf of the Mackenzie River delta includes organic matter derived from both river flow and *in situ* marine production. Organic carbon, total THAA, and detrital grazer diversity and abundances are highest at the mid-shelf stations (M2, M3), despite a uniform grain size across all stations. Water column THAA is higher than that seen in the sediments, but the water column particles are lower in diatom related amino acids and higher in non-protein amino acids than the surface sediments sampled. Lipid and fatty acid biomarkers also reflect elevated algal inputs to mid-shelf sediments, with major portions appearing as diatoms. The organic analysis results are consistent with benthic meiofaunal observations, with mid-shelf stations the highest in overall abundances, although some phyla such as the foraminifera increase further from shore. The majority of the measured parameters suggest that *in-situ* production in the sediments and water column are more important to regional carbon cycling and fluxes than previously reported. Future analysis of the region may provide more information on the specific conditions that lead to higher mid-shelf organic carbon, water column production, and meiofaunal abundances. If so, scientist will better understand the dynamics in which mid-shelf sediments are highest in organic carbon concentration and how the meiofaunal community responds to those conditions. Some choices to do this include employing further analysis, like sedimentation rates and isotopic sediment analysis, as well as multiyear sample collection.

CHAPTER IV

CONCLUSIONS AND IMPLICATIONS

Trials using LC/MS/MS and ion pairing for amino acid analysis indicate that this method is an effective alternative to the standard methods of derivatization and HPLC or GC/MS analysis. Good calibration linearity was seen across 40 amino acids and 5 deuterated standards and the range of quantifiable concentrations was extended beyond existing standard methods. This method has a limit of quantification of around 5 pg, ten times lower than that of GC/MS or OPA-HPLC methods. Matrix effects on the ability of LC/MS/MS to identify and quantify were not seen to inhibit the determination of amino acids across a range of common environmental matrices. Many amino acids that coeluted in previous chromatographic methods of analysis, including BMAA and DAB, were separated with the new method, allowing more effective identification of a human toxin and its non-toxic analog DAB. Common amino acids observed in the samples studied included arginine, GABA, and proline, which cannot be measured using standard methods. Other uncommon amino acids like thio-proline and sarcosine, that were not measurable in GC/MS analysis of bacteria/diatom mixtures, were quantified using the LC/MS/MS approach. This expansion in quantifiable amino acids could prove useful for future analysis as indicators of many intracellular and intercellular interactions, as well as improving measurements of total hydrolyzable amino acids in marine organic matter from the water column and sediments.

The new LC/MS/MS method was employed to examine carbon cycling in the Canadian Arctic region along the Mackenzie River coastal shelf. Together with the analysis of meiofauna, biomarkers, organic carbon, and grain size, measurements of amino acids provided insights to carbon cycling on this coastal shelf. Common amino acids like threonine and alanine, as well as lipid and fatty acid biomarkers like bassacasterol and colestanol, showed significant amounts of marine related organic matter is preserved in the surface sediments. Of the identified biomarkers and amino acids, more compounds were found to be of marine origin rather than terrestrial river derived matter. Specifically, mid-shelf samples were highest in marine organic matter, total organic carbon parameters, THAA and biomarkers. These results, together

with an observed increase in meiofaunal populations at these mid-shelf stations, suggest a marine related source of organic matter to the sediments that is then grazed upon by meiofauna and stored as meiofaunal biomass.

Advancing amino acid analysis via LC/MS/MS and ion-pairing reagents allows for faster and more sensitive analysis of a broad suite of amino acids, some not previously measured, and this can be applied in future studies in a broad range of settings. By testing the method with environmental samples, future studies can readily determine amino acid related implications such as carbon degradation and cycling, as well as inter and intracellular interactions that may not have been easily discussed before could be examined. Such studies might include free amino acids that are available to seagrasses in the Chesapeake Bay and the role of specific amino acids in intracellular cycling such as cell signaling and stress signals. This new method, together with a suite of other measures to interrogate the conditions of the Mackenzie River shelf, demonstrated the importance of marine and terrestrial organic carbon inputs and their relationship with meiobenthic communities. Future studies could include a further analysis of carbon cycling in this coastal system or other coastal systems as well, cellular stress signals, unusual amino acids toxic to humans like BMAA, and poorly studied amino acids like thioproline (a sulfur containing amino acid) or β-alanine.

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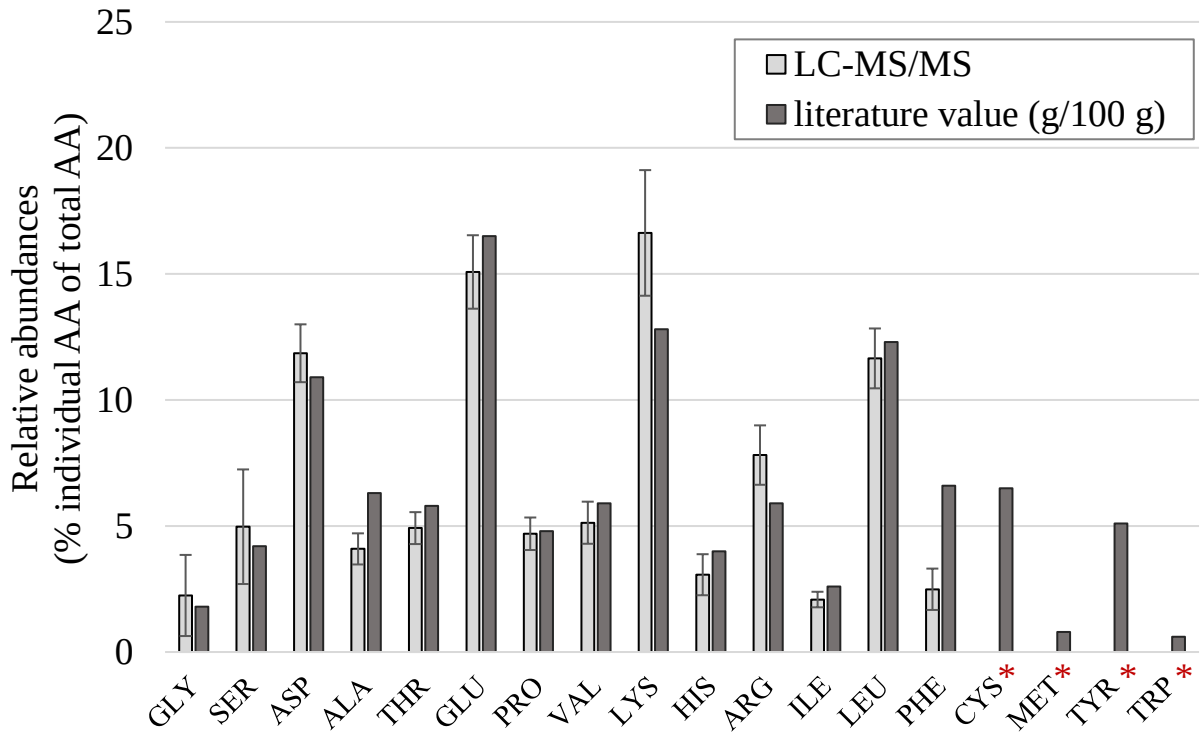
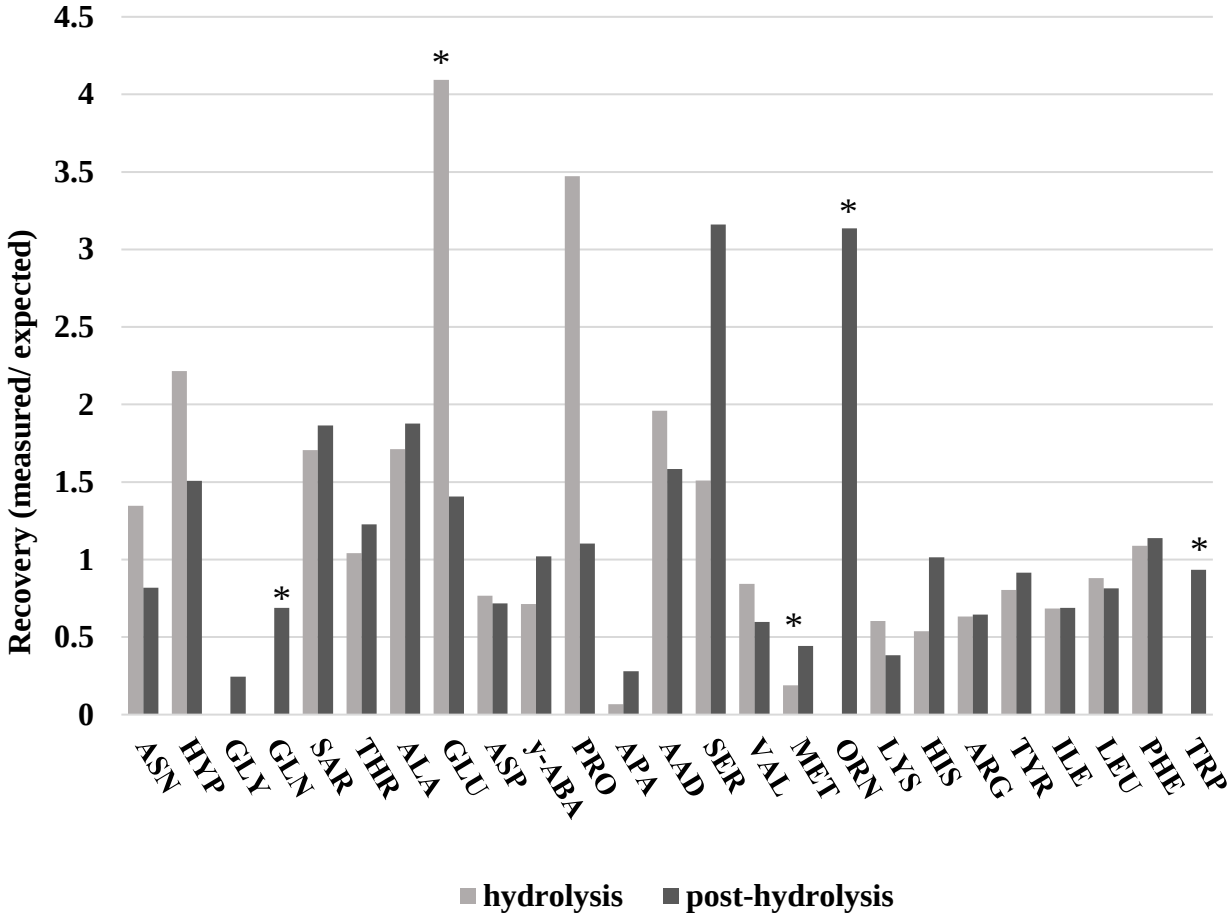
APPENDICES

CHAPTER II APPENDICES



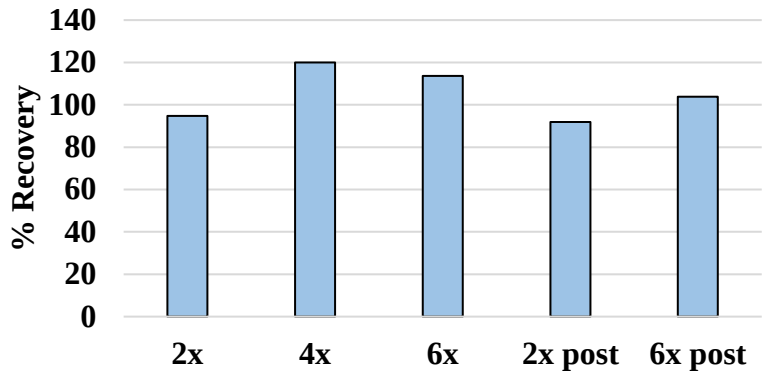
Appendix II-1. A comparison of amino acid distribution of hydrolyzed BSA protein standard by LC-MS/MS and literature values. Relative abundances of individual amino acids found in hydrolyzed bovine serum album (BSA) measured by both the LC-MS/MS method described and by Sphar et al. 1964 using an amino acid analyzer and ninhydrin derivatization in g amino acid per 100 g protein. Error bars describe the standard deviation of analytic repeats (n=3).

* Cysteine, methionine and tryptophan require non-acidic hydrolysis to be analyzed, otherwise these amino acids fall apart during HCl acid hydrolysis. Tyrosine was not measured by LC-MS/MS but was observed in other sample matrices discussed in chapter II.



Appendix II-2. Recovery of individual amino acids spiked in water pre and post hydrolysis using LC-MS/MS. Amino acid recoveries reported as the ratio of measured concentration of each amino acid in ng over the expected concentration of amino acids in ng. Samples with the amino acid standard added before hydrolysis are reported in grey. Samples with amino acids standards spiked post-hydrolysis reported in dark grey.

* The amino acids marked by this star have differing responses depending on hydrolysis. Ornithine and tryptophan fall apart during hydrolysis. Methionine falls partially apart in most acidic conditions. Glutamine is converted completely to glutamic acid during acid hydrolysis, therefore, resulting glutamic acid spiked before hydrolysis is the combination of glutamine and glutamic acid.



Appendix II-3. Recovery of amino acids spiked into Arctic marine sediment. Amino acid standards were spiked into an arctic marine sediment from station M1 of the Mackenzie River shelf transect. The x-axis represents the concentration of standards add to the sediment samples. Sediments were spiked at 3 different levels, 2 times (2x) the concentration of amino acids already found in the sediments was the lowest spike into the sediment and 6 times (6x) the concentration of amino acids was the highest spike. Results labeled *2x post* or *6x post* represent samples that were spiked post hydrolysis with amino acid standards at 2 times the concentration of amino acids found in the sediments and 6 times. Those not labeled with *post* were spiked before hydrolysis.

Appendix II-4. Supplementary data for Figure II-2 individual amino acids in bacteria/diatom cultures. Values for amino acids reported as relative abundances, calculated by summing the concentration of all amino acids together and dividing each amino acid by that sum then finally multiplying that value by 100 to give a reported value in the unit %. Standard deviation was calculated from analytical replicates (n=3).

	diatoms	std dev diatoms	bacteria	std dev bacteria	250/1	std dev 250/1	10000/1	std dev 10000/1
ASP	0.401	0.225	0.677	0.061	0.385	0.069	0.386	0.012
ALA	0.633	0.127	1.418	0.117	1.013	0.031	1.231	0.006
THR	0.272	0.198	0.725	0.073	0.365	0.013	0.556	0.026
GLU	0.295	0.079	3.088	0.286	2.338	0.085	2.408	0.166
GABA	15.543	1.268	2.821	0.281	9.430	0.303	3.506	0.280
Thio-PRO	0.009	0.014	0.126	0.018	0.089	0.005	0.281	0.017
PRO	2.894	0.130	3.797	0.275	3.031	0.109	3.342	0.166
VAL	7.418	1.026	9.690	0.629	10.663	0.661	9.788	0.554
LYS	26.745	1.113	9.168	0.437	9.469	0.149	10.115	0.086
HIS	0.200	0.060	4.095	0.215	1.392	0.153	0.584	0.015
ARG	10.510	0.841	21.740	0.953	19.808	0.470	23.283	0.860
TYR	4.979	0.468	7.450	0.304	6.832	0.160	7.404	0.210
ILE	7.978	0.512	9.426	0.445	9.289	0.292	9.603	0.291
LEU	10.355	4.688	14.041	0.860	14.269	0.873	15.138	0.716
PHE	11.562	1.328	11.386	0.642	11.266	0.617	11.903	0.489

Appendix II-5. Supplementary data for Figure II-3 sum of amino acids in bacteria/diatom cultures. Reported concentrations are in μg/ g organic carbon. The category LC/MS/MS with peaks excluded represents the sum of all amino acids in the sample minus those amino acids not observed by both LC/MS/MS and GC/MS methods.

ratio	GC/MS	LC/MS/MS	LC/MS/MS with peaks excluded
62.5/1	271.17	658.44	541.94
125/1	345.50	484.19	390.77
250/1	214.94	490.74	407.37
10000/1	107.22	129.82	106.35

Appendix II-6. Supplementary data for Figure II-5 free amino acids in eelgrass samples. Amino acid distributions are reported as % abundances calculated from the sum of all amino acids compared to the concentration of that individual amino acid as concentration of amino acid divided by sum of all amino acids times 100.

	pH 6 Leaf	pH 6 Rhizome	pH 8 Leaf	pH 8 Rhizome
SER	0.124	0.624	0.016	0.997
ASN	2.927	5.021	3.922	8.629
GLN	45.748	59.817	45.311	61.164
GABA	39.680	19.607	40.100	11.554
ALA	3.844	3.059	3.317	2.664
THR	0.396	0.848	0.432	1.151
GLU	2.873	0.000	2.432	3.510
ASP	1.427	4.208	1.215	3.235
PRO	2.148	4.881	3.036	5.647
VAL	0.605	0.685	0.218	0.708

CHAPTER III APPENDICES

Appendix III-1. Supplementary data for Figure III-8 sum of THAA in sediments and particles.
THAA values reported in the unit of mg THAA/ g organic carbon (OC). Standard deviation is reported in this table as *std dev* which is calculated from analytical replicates (n=3). Samples from the fluorescence maximum and bottom waters are particle organic matter. Samples marked n/a were either not measured for the following parameter or lost during analysis.

	M1	M2	M3	M4	AIM
Fluorescence Max	n/a	6.514	28.780	36.545	28.127
std dev	-	0.483	0.722	3.818	2.120
Bottom Water	n/a	2.295	n/a	n/a	1.818
std dev	-	0.297	-	-	0.140
Sediment	5.742	9.530	7.610	8.213	12.829
std dev	0.080	0.105	0.181	0.280	0.420

Appendix III-2. Supplementary data for Figure III-9 and Figure III-10 amino acid distributions in sediments and particles.
Values for THAA are reported in %, as a measure of relative abundances based on the total amino acids in the sample. The charts are organized similarly to the figures in chapter III with fluorescence maximum reported first and sediments last. Data from Figure III-10 is reported in the B tables. Columns left blank indicate samples not measured for a specific parameter or a sample lost during analysis.

	AIM	M4	M3	M2	M1
GLY	0.000	0.000	0.000	0.000	
THR	3.457	3.470	2.811	3.954	
ALA	4.779	3.974	3.803	6.604	
ASP	4.132	4.882	4.415	6.000	
GABA	12.997	13.786	13.363	7.387	
GLU	29.359	29.158	30.983	33.144	
PRO	3.388	3.456	2.778	3.710	
LYS	11.465	3.162	13.778	4.711	
ARG	6.848	8.715	6.665	7.448	
ILE	3.826	3.740	3.961	4.501	
LEU	6.266	6.201	6.109	7.011	
PHE	3.923	4.381	3.851	4.628	
sum of others	9.562	15.075	7.483	10.902	

Fluorescence Maximum

Appendix III-2 Continued

Bottom Water

	AIM	M4	M3	M2	M1
GLY	0.000			0.000	
THR	4.698			3.434	
ALA	1.674			3.662	
ASP	5.845			6.246	
GABA	16.808			8.340	
GLU	26.018			31.702	
PRO	3.841			3.448	
LYS	0.000			0.057	
ARG	5.966			7.593	
ILE	3.935			4.371	
LEU	5.309			7.269	
PHE	4.052			5.100	
sum of others	21.855			18.777	

Sediments

	AIM	M4	M3	M2	M1
GLY	7.865	6.814	5.830	4.886	5.313
THR	8.571	8.275	7.988	7.068	7.057
ALA	9.468	8.437	9.047	7.805	9.187
ASP	7.411	7.873	7.772	6.841	8.545
GABA	5.836	4.547	4.022	3.267	3.526
GLU	20.766	24.053	28.189	32.202	26.625
PRO	3.569	5.366	4.779	4.524	3.938
LYS	9.191	6.640	0.000	0.895	0.000
ARG	6.930	6.785	7.408	7.710	6.400
ILE	2.838	3.550	4.145	3.912	4.733
LEU	4.661	6.047	6.245	6.893	8.360
PHE	1.408	2.609	4.227	4.415	4.584
sum of others	11.484	9.004	10.349	9.583	11.732

B Tables

	AIM sed
SER	1.314
SAR	1.215
HYP	1.621
A-amino	3.190
GLY_PRO	0.568
VAL	2.408
HIS	1.169
TYR	0.000

	M2 20 m
HYP	0.394
VAL	0.757
SER	0.065
GLY-PRO	4.196
HIS	2.849
TYR	2.641

Appendix III-3. Supplementary data for Figure III-12 fatty acid and sterol biomarkers in Arctic sediments. Concentrations of each biomarker are reported in units of μg marker per gram dry weight sediment (μg/ g dry wt). Biomarkers for the AIM site are reported for both the surficial 0-0.5 cm of the sediment and the 0.5-1 cm horizon, notated as *AIM 0-0.5* and *AIM 0.5-1*.

Fatty acid Biomarkers

	AIM 0-0.5	AIM 0.5-1	M1	M2	M4
Even saturates	133.184	338.032	633.709	696.637	318.675
PUFAS	44.049	20.931	68.926	452.025	161.322
Monounsaturates	110.583	86.831	352.286	682.585	405.286
Long chain FA	31.573	23.080	165.241	52.941	46.719
Odd chain	14.709	9.880	83.415	92.528	27.336
Short chain FA	55.330	318.246	313.143	544.848	291.358

Phytol and Sterol Biomarkers

	AIM 0-0.5	AIM 0.5-1	M1	M2	M4
Phytol	4.205	3.842	4.046	58.627	21.784
24-norcholesta-5,22-dien-ol	4.178	1.877	0.679	71.311	5.285
Dinosterol	0.666	1.222	0.000	15.087	4.471
Dinostanol	0.382	0.590	0.000	4.234	1.212
Brassicasterol	7.121	4.021	0.000	15.600	14.684
Stigmasterol	1.734	1.198	0.000	23.086	4.771
23,24-dimethylcholest-5-en-ol	0.734	0.000	0.000	4.285	1.915
Clionasterol	10.977	6.965	56.826	11.975	28.299
Ergsta-5,24(28)-dien-ol	3.792	2.334	0.000	12.300	6.559
24-ethylcholestan-3b-ol	4.511	1.761	22.029	19.361	10.987
Isofucosterol	2.119	0.000	0.000	21.256	4.950
23,24-dimethylcholest-5-en-ol	0.616	0.000	0.000	10.234	1.562
Desmosterol	2.064	1.228	0.000	13.916	3.505
Lupeol	0.000	0.000	0.000	7.482	1.560
24-methylcholesta-7-22-dien-ol	0.000	0.000	4.230	15.646	14.684
24-methylcholest-22-en-ol	0.000	0.058	6.088	9.361	1.269

Appendix III-4. Supplementary data for Figure III-13 meiofaunal abundances.
Abundances of major meiofauna and intact diatom frustules counted from a 250 ml sample and reported as a total sediment sample. Meiofauna classes that were lower in abundances than the top 7 classes were summed into the category of *sum of all others*.

	M1	M2	M3	M4
Diatoms	0	210	115	80
Kinorhyncha	0	12	60	14
Copepoda	6	164	247	95
Nematoda	0	47	163	107
Foraminifera	45	157	249	422
Polychaeta	22	44	87	99
Bivalvia	0	4	20	11
sum of all others	12	24	41	43

Appendix III-5. Supplementary data for Figure III-14 diatom biomarkers and organic carbon sources. All parameters but diatoms reported in tables A and B are given in units of mg/ g OC. Diatoms are reports as abundances per samples, from a 250 ml sample of sediment. Data marked n/a was lost during analysis.

A) Diatom specific biomarkers in sediments

	M1	M2	M3	M4
brassicasterol	0.000	0.942	n/a	1.132
23,24-dimethylcholest-5-en-3b-ol	0.000	0.618	n/a	0.120
24-methylcholest-22-en-3b-ol	0.549	0.565	n/a	0.098
GLY+SER+THR	0.848	1.283	1.228	1.363
diatoms	0	210	115	80

B) Source composition of organic carbon in sediments

	M1	M2	M3	M4	AIM 0-0.5
sum terrestrial	16.891	7.495	n/a	5.320	4.656
sum marine	6.949	30.838	n/a	14.424	6.373
THAA	5.742	9.530	7.610	8.213	12.829
GLY+SER+THR	0.848	1.283	1.228	1.363	2.276

VITA

PERSONAL DETAILS

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ACADEMIC RECORD

B.S. **2011-2014** Department of Marine Sciences, Texas A&M Galveston, Supervisor: Karl Kaiser, Varsity Sailor, National Honor Society, Academic Scholarship, Magnum Cum Laude.

M.S. **2014-2018** Department of Ocean, Earth and Atmospheric Sciences, Old Dominion University, Supervisor: H. Rodger Harvey, Topic: Measuring Amino Acids in Marine Matrices by LC tandem MS/MS. GSO President.

KEY TECHNICAL SKILLS

- **Matlab**- Proficient in the language as well as statistical tools like ANOVA, cluster analysis, ANCOVA, Gibbs Seawater tool box, and interpolation.
- **Instrumentation Languages-** Familiar with programs from multiple companies including the Xcalibur Software, LabAdvisor tool box, Chromellion Software.
- **ArcGIS/NOAA SeaDas-** Familiar with the functions and can produce basic maps.

KEY LABRATORY SKILLS

- **Liquid Chromatography-** Measuring Amino acids ,Neutral sugars, and lipids
- **Ion Chromatography-** Silver Chloride ion paired with NaOH buffer solvents.
- **Mass Spectrometry-** Familiar with identifying mostly organic molecules including fatty acids, sterols, some hydrocarbons, amino acids, sugars, lipids and simple molecules like water and carbon dioxide.
- **Basic Lab Skills-** Alkalinity titrations, centrifuges, rotovap/multivap, Microwave extraction, dissolved organic matter (DOM) sensitive laboratory.

PUBLICATIONS

McMahon, R. (2014) *Application of carbohydrates as biomarkers to study dissolved organic matter reservoirs in arctic rivers.* (Undergraduate Thesis, Texas A&M University)

Kaiser, K., Canedo-Oropeza, M., McMahon, R. & Amon, R. M. W. (2017).Origins and transformation of dissolved organic matter in large Arctic rivers. *Scientific Reports* **7(1).**