Patterns of variability in the fatty acid and stable isotope profiles of ice algae, phytoplankton, and zooplankton during early spring in the Canadian High Arctic

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A thesis submitted to The Faculty of Graduate Studies of
York University
in partial fulfillment of the requirements of the degree

MASTER OF SCIENCE

GRADUATE PROGRAM IN BIOLOGY
YORK UNIVERSITY
TORONTO, ONTARIO

July 2013

Abstract

Sea ice-associated primary producers are a major source of energy within Arctic marine ecosystems, particularly when pelagic primary growth is temporally and spatially limited. Using samples and data collected in spring 2011 and 2012, the variation in the fatty acid composition and stable isotopes of ice-based primary producers and primary consumers were investigated over several spatial scales in the Canadian Arctic Archipelago. Snow and ice thickness significantly affected ice algae fatty acid composition. Broad scale year-to-year variation in snow and ice conditions indirectly affected the fatty acid compositions, particularly the levels of polyunsaturated fatty acids, of a keystone zooplankton species. Environmental influence on fatty acid composition decreased as trophic level increased. Despite the presence of high quality pelagic phytoplankton under the sea ice, the data suggest herbivores rely mainly on ice algae.

Acknowledgements

I would like to thank my supervisor Dr. Gregory Thiemann for his unrelenting support and mentorship and for granting me the freedom to pursue this project in my own way. A big thank you to Dr. Christine Michel as well for all of her advice and for supporting the project. I would like to thank Dr. Suzanne Budge and my supervisory committee member Dr. Norman Yan for their time and helpful advice. Thanks you to Dr. Roberto Quinlan for letting me use and borrow lab and field equipment. Thanks to Dr. Sapna Sharma for statistical help and to all the members of the Polar Bear Lab: Brandon for his help in the field, and Mel and Lou for their help and support. Thank you to every one of my family and friends who always stood by me, helped proof read and were there for stress relief, particularly the Free Radicals. Last but not least, thank you to Nikki for all of your love, patience, and support.

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Chapter 1

Introduction

Polar environments experience extremely low temperatures and variable photoperiods, ranging from 24 hours of daylight in the summer to complete darkness during winter months. The defining characteristic of the Arctic landscape is the sea-ice, which can cover up to 14 million km^2 and last for more than 9 months of the year (Spindler 1994). Arctic marine ecosystems experience short but intense periods of productivity as darkness and sea ice cover combine to restrict primary growth during the winter (Søreide et al., 2010). The sea ice also acts as habitat for primary producers and invertebrates that subsist within and under the ice. Adapted for growth under extremely low irradiance, ice algae initiates the Arctic spring bloom and accounts for 25-30% of the total annual productivity in Arctic seas (Legendre et al., 1992). The energy from primary producers is transferred to higher trophic levels primarily by calanoid copepods, zooplankton which have adapted to the variation in food availability by making and storing large amounts of lipid (Falk-Petersen et al., 2009).

Arctic marine food webs are highly dependent on the production and transfer of lipids to maintain their structure and biodiversity (Darnis et al., 2012), making fatty acids a useful tool for studying the ecology of this system.
This thesis sought to provide a baseline of the fatty acid signatures of primary producers and consumers in sea-ice ecosystems. The aim of the work was to examine what environmental features had the most influence on fatty acids and

investigate what spatial patterns arise in fatty acid composition as a result of environmental variability. Arctic marine food webs rely on the production and transfer of lipids, and the study of fatty acid dynamics therefore provides unique insight into how energy flow is dictated within the system. I specifically focused on omega-3 and omega-6 polyunsaturated fatty acids due to their higher values as dietary components (Sargent et al., 1995). In conjunction with fatty acids, I also examined $\delta^{13}C$ and $\delta^{15}N$ stable isotopes to help determine carbon sources and trophic positions. Other studies investigating the influence of environmental factors on fatty acid composition and stable isotope profiles have been undertaken, but few have examined sea-ice communities, and none have encompassed a large spatial area during the spring bloom. Record lows in ice cover and earlier break up of the sea ice highlights the need for increased understanding of the early spring lipid dynamics of Arctic food webs.

Fieldwork for this study was carried out in May of 2011 and 2012 near Cornwallis Island, Nunavut in the Canadian Arctic Archipelago. The specific objectives of this thesis were to: 1) characterize fatty acid patterns of ice algae over a large area, 2) determine what variance in primary producer fatty acid profiles could be attributed to environmental drivers, 3) examine how primary producer fatty acid compositions and environmental variables influence zooplankton fatty acid dynamics, and 4) characterize the fatty acids of a phytoplankton bloom I found occurring under the sea-ice.

1.1 Fatty acids

Fatty acid molecules are usually straight, even numbered chains of carbon between 14-24 atoms long with 0-6 double bonds. One end is a methyl terminus while the other is a carboxyl, making it an acid. Although fatty acids shorter than 12 or longer than 24 carbons are present in marine food webs, they usually comprise less than 0.1% of the total of all fatty acids (Budge et al., 2006). Fatty acids can be separated into three categories based on the presence and number of double bonds: saturated (SAFA), monounsaturated (MUFA) or polyunsaturated (PUFA). The nomenclature used for describing fatty acids is *A:B*n-*X*, where *A* is the number of carbon atoms, *B* is the number of double bonds, and *X* is the position of the first double bond relative to the methyl terminus. Biosynthesis of fatty acids begins with the addition of carbon chains to acetyl-CoA to produce a saturated fatty acid, often 16:0. Other fatty acids are then synthesized from elongation and desaturation of 16:0. Although most fatty acids exist as straight chains, some are branched. These are indicated in relation to the methyl end by prefacing the name with an *i*, for a branch at the second carbon or *ai* for a branch occurring at the third carbon.

Cellular lipids are constructed from specific combinations of fatty acids. Phosholipids (PL) are the structural components of cell membranes and are made from two fatty acids esterified to a glycerol molecule that also contains a polar derivative of phosphatidic acid. However, because of their functional nature, fatty acids in cell membranes are relatively insensitive to dietary changes, unlike storage lipids, which are energy reserves (Sargent et al., 1995). The most common storage molecule is a triacylglycerol (TAG), which is three fatty acids esterified to a glycerol

backbone. Wax esters are another common form of storage lipid, particularily in Arctic zooplankton (Lee et al., 2006). These consist of one fatty acid esterified to a fatty alcohol. These fatty alcohols are generally saturated or monounsaturated but the fatty acid portion can be from any of the fatty acid categories.

When ingested, the ester bonds of TAG and PL are hydrolyzed, releasing free fatty acids, which are then absorbed by the body (Budge et al., 2006). Short chain fatty acids are completely broken down while fatty acids longer than 13 carbons are utilized in several different ways (Budge et al., 2006). They can be metabolized if energy is required immediately or they may be re-esterified into TAG for storage. In copepods, fatty acids may be incorporated into a secondary type of storage in the form of wax esters, where a MUFA may be reduced to form the fatty alcohol component (Dalsgaard, et al., 2003). PUFAs in particular may be incorporated into structural tissues or used as precursors for hormone production.

Minimal, or at least predictable, modifications are made to fatty acids ingested by consumers (Budge et al., 2006). While no fatty acid is unique to any one species, it is possible to use similarities in fatty acid compositions to trace energy flow through the food web (Iverson et al., 1997). Quantitative methods for estimation of dietary composition also exist, however they require complete knowledge of all prey fatty acid compositions (Iverson et al., 2004). Qualitative estimates can be made using specific combinations of fatty acids, such as 16:1n-7/16:0 for diatoms (Dalsgaard et al., 2003), to determine the source of the consumer's lipid intake.

PUFAs 20:5n-3 and 22:6n-3 are particularly useful, as they are only produced by algae, yet are necessary for homeostasis in animals. Termed essential fatty acids (EFA), these PUFAs maintain cell membrane fluidity and associated enzyme functions as well as act as precursors for certain hormones (Sargent et al., 1995). There is poor genetic control of PUFA levels in cell membrane synthesis and therefore dietary deficiencies of EFAs will quickly lead to general health problems in animals (Sargent et al., 1995). Despite all PUFAs having lower energy per unit than saturated fatty acids of the same length, EFAs are exceptionally nutritious: higher levels increase zooplankton egg production, and the subsequent viability of their eggs (Arendt et al. 2005, Søreide et al., 2010). The success of copepods in Arctic ecosystems has hinged adapting their life cycles to take advantage of available EFAs (Søreide et al., 2010).

1.2 Stable isotopes

The ratios of $\delta^{13}C$ and $\delta^{15}N$ stable isotopes provide measures of trophic positions and carbon sources in a food web (Post 2002). During energy transfer between trophic levels, heavier isotopes are enriched in a predictable manner (Hobson et al., 1995). For $\delta^{15}N$, this fractionation is about 3-4‰ greater in the consumer relative to its diet (Hobson and Welch, 1992). The enrichment of $\delta^{13}C$ between trophic levels is very low, less than 1‰ (Post 2002). Therefore, if primary producers are isotopically distinct, mixing models can be used to determine the relative contributions of each carbon source to consumers (Post 2002).

1.3 Ice algae

Ice algae thrive under extremely low light conditions, can grow at temperatures as low as -5.5°C and salinities as high as 95 psu (Spindler 1994). These adaptations allow ice algae to initiate growth extremely early in the year, at a time when pelagic primary growth is temporally and spatially limited (Gosselin et al. 1997; Carmack and Wassmann 2006). Ice algae communities are an assemblage of species and can include dinoflagellates, prasinophytes, cryptophytes, chlorophytes, and euglenophytes, however pennate diatoms are usually dominant (Cota et al., 1991; Niemi et al., 2011). Ice algae can form thick mats on the lower surface of sea ice and even grow in the interstitial spaces in the sea ice that are the result of brine formation (Cota et al. 1991). Blooms can occur with almost any type of ice and have been found in multi-year as well as frazil ice (Cota et al. 1991). The spring bloom of ice algae is the first source of food for herbivorous copepods in the season, and has the added benefit of being rich in the EFA 20:5n-3 (Søreide et al., 2010).

1.4 Copepods

Calanoid copepods make up a large proportion of the total zooplankton biomass in Arctic seas (Tremblay et al., 2006). They act as the energetic bridge between primary production and higher consumers and are regarded as a keystone for Arctic marine food webs (Tremblay et al., 2006; Søreide et al., 2010). During the summer, calanoids take advantage of algal blooms to develop large amounts of lipids, which can be up to 70% of their dry mass (Falk-Petersen et al., 2009). Then during autumn, they descend to great depths and significantly decrease their

metabolic rate, entering a state called diapause until the spring (Auel et al., 2003). Female copepods spawn just prior to or during the ice algal bloom, allowing utilization of these nutrients for the reproductive effort (Søreide et al., 2010). Once eggs hatch, juveniles feed on the summer phytoplankton and grow through 6 nauplii, and 4 or 5 copepodite stages before overwintering at depth (Falk-Petersen et al., 2009).

1.5 Thesis organization

This thesis is organized as two, stand-alone manuscripts and an overall introduction and conclusion. Chapter 1 is an introduction to fatty acids, their role in Arctic marine ecosystems, and their utility as trophic biomarkers.

Chapter 2 investigates environmental drivers of spatial variation in low trophic level fatty acid compositions and the influence of this variation on food web dynamics. I utilized a combination of fatty acid analysis and stable isotopes to determine energy flow.

In Chapter 3, I focused on a pelagic diatom, *Coscinodiscus centralis,* which was found under the sea ice in the both years of sampling. The fatty acid and stable isotope composition of *C. centralis* is described and compared to ice algae. I used the clustering of lipid compositions and isotopic values to determine the origins of *C. centralis.*

In Chapter 4, I provide an overview of my main findings and conclusions, and suggest some directions for future research.

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Chapter 2.

Living off the fat of the sea: environmental drivers and spatial patterns of fatty acids at the base of an Arctic marine food web

Abstract

Arctic marine food webs are highly dependent on the production and transfer of lipids to maintain their structure and biodiversity. Ice algae provide a pulse of nutritious polyunsaturated fatty acids (PUFAs) early in the spring before open water production is prevalent. Little is known about what drives the spatial distribution of ice-associated PUFAs and what impact this has on lipid flux to higher trophic levels. This study investigated the variation in fatty acid composition and bulk carbon and nitrogen stable isotope ratios of ice-based primary producers and zooplankton over multiple spatial scales in the Canadian Arctic Archipelago during the spring of 2011 and 2012. No spatial patterns were evident in ice algae fatty acid compositions, but they were significantly affected by snow and ice cover. Snow depth was also linked to annual PUFA levels in *Calanus* spp. Pelagic phytoplankton and zooplankton fatty acids were primarily dictated by current, although the strength of that influence decreased with increasing trophic level. Some zooplankton fatty acid profiles were also significantly affected by daily light levels; increased levels of 22:6n-3 on brighter days were likely caused by dinoflagellates released from shade inhibition. Based on these findings, global warming-associated changes in sea-ice and snowfall will not only directly affect habitat, but will indirectly alter the nutritional dynamics of Arctic marine ecosystems.

2.1 Introduction

Each winter, Arctic ecosystems undergo a period of several months where the water column is too dark to sustain photosynthesis. When light levels increase in the spring, communities of microorganisms begin to grow on the underside of the sea ice and are the first available source of primary production (Welch and Bergmann, 1989). These communities are generally dominated by diatoms and are adapted for growth under very low light levels (Cota 1985). As photosynthetically active radiation (PAR; 400-700 nm) levels increase, polyunsaturated fatty acid (PUFA) proportions in algae drop, which decreases food quality for herbivores (Leu et al., 2010). Conversely, excess UV radiation (280-400 nm) has no significant affect on PUFA levels, but causes significant decreases in biomass due to physiological damage (Leu et al., 2006). The total contribution of ice algae to yearly production varies widely across the Arctic, and accounts for about 10% in the Canadian Arctic Archipelago (Michel et al., 1996). However, the importance of ice algae to local food webs comes not from its biomass, but from the production of high quality PUFAs so early in the spring, which is a biologically important time for calanoid copepods (Michel et al., 1996; Leu et al., 2010).

The nutritional benefits of foods high in PUFA, especially long chain essential fatty acids (EFAs) 20:5n-3 and 22:6n-3, are evident at every stage of copepod life cycles. At all stages, Arctic copepods require high levels of algal PUFAs in phospholipids to maintain cell membrane fluidity (Lee et al., 2006). The early pulse of PUFAs from ice algae is used by female *Calanus* to finish their development and reproduce (Wold et al., 2011). Higher amounts of PUFAs, particularly the EFA 20:5n-

3, increase egg production and hatching survival rates (Arendt et al., 2005). Nauplii that feed on higher quality food have lower mortality rates compared to individuals that consume diets poor in PUFAs (Daase et al., 2011). Adult copepods use excess fatty acids to accumulate large amounts of wax esters, a fatty alcohol esterified to a fatty acid, which are used as slow release energy reserves during diapause (Lee et al., 2006). Additionally, by reproducing early in the season, offspring have time for maturation and are then able to take advantage of the pelagic bloom that occurs after the sea ice breaks up (Søreide et al., 2010). Therefore, because copepod population dynamics control the transfer of energy from primary producers to higher trophic levels (Welch et al., 1992), the nature of this initial pulse of high quality food has implications for the entire food web.

The Arctic is currently experiencing unprecedented losses in total sea ice cover and with this, progressively earlier dates of sea ice break up (Comiso et al., 2008). Additionally, Arctic marine systems can vary significantly from year-to-year (Michel et al., 2006). Environmental conditions such as nutrient availability, temperature, and light significantly influence fatty acid composition in plants and animals (Dalsgaard et al., 2003, Leu et al., 2006). However, broad-scale drivers of Arctic fatty acid compositions and subsequent food web dynamics are poorly understood (Søreide et al., 2010). Most studies that have investigated environmental influences on fatty acid composition have done so on small spatial scales, or during cruises that occur after the initial ice algae bloom (Leu et al., 2010; Scott et al., 2002). An understanding of the trophic dynamics of primary producers

and consumers is necessary for predicting the effects of further ice loss on food webs, and for sustainable management of changing Arctic ecosystems.

In this study I tested the hypothesis that environmental conditions drive large-scale variability in ice algae fatty acid profiles and that these patterns will be evident in their zooplankton grazers. I conducted a multi-year study on ice algae and zooplankton from a large area around Resolute Bay, Nunavut. Utilizing a combination of stable isotope and fatty acid analysis, my specific objectives were to: 1) to characterize fatty acid patterns of ice algae over a large area, 2) determine what variance in primary producer fatty acid profiles could be attributed to environmental drivers, and 3) examine how primary productivity and environmental variables influence zooplankton fatty acid dynamics. In addition to these goals, I hoped to establish baseline fatty acid and stable isotope data for use in future food web studies in the Canadian Arctic Archipelago.

2.2 Methods

2.2.1 Study area

In 2011 and 2012, I collected ice algae, phytoplankton and zooplankton samples from landfast sea ice near Cornwallis Island, Nunavut. Ice formation in this area begins mid to late September and lasts until breakup around the end of June, but ice can also last until the beginning of August (Brown and Cote, 1992). The majority of sea ice in the area is predominately first year, although some patches of multiyear ice are present (Welch and Bergmann, 1989). Average sea ice thickness reaches a maximum of 2 m in mid April (Brown and Cote, 1992). Snow depth can

reach a maximum average thickness of 22 cm although the patterns of accumulation are dictated by prevailing winds (Brown and Cote, 1992).

The dominant ocean current around Cornwallis Island flows from west to east and averages 6.16 cm/s in the spring, with speeds increasing up to 60 cm/s during the tidal flux (Prinsenberg and Bennett, 1987; Cota et al., 1987). The three main inflows into Barrow Strait are from Viscount Melville Sound in the west, Penny Strait to the north, which flows around both sides of Cornwallis Island, and from Peel Sound in the south (Fig. 2.1; Prinsenberg and Bennett, 1987). With a sill of ~125m depth, Barrow Strait is the narrowest and shallowest point of the Northwest Passage (Cota et al, 1990; Michel et al, 2006).

2.2.2 Field observations

Ice thickness and snow depth as well as meteorological observations such as air temperature and wind direction were recorded at each station. Measurements of photosynthetically active radiation (PAR, 400 – 700 nm) were taken of downwelling light, upwelling light (reflectance) and underwater light using a LICOR quantum sensor. Measurements under the sea ice were obtained by mounting the sensor on a hinged aluminum arm. All PAR readings were conducted under or over undisturbed snow cover. Water column salinity and temperature measurements were collected both years using a Sea-bird conductivity-temperature-depth (CTD) probe, however only CTD data from 2011 were available. The values for chlorophyll A (Chl A) and particulate organic carbon (POC) were collected by the Department of Fisheries and Oceans.

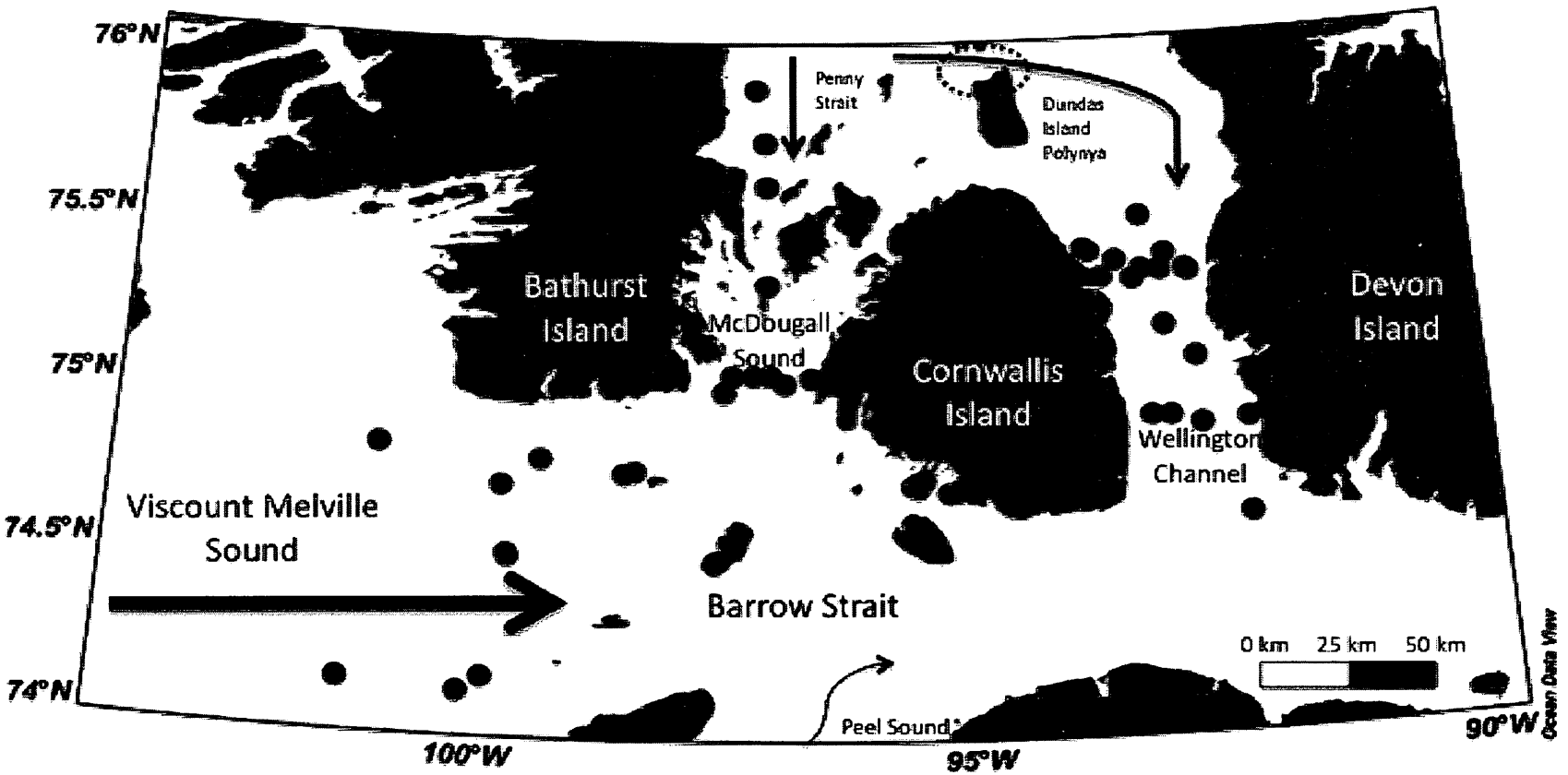

Figure 2.1. Stations sampled over 2011-2012 field seasons near Cornwallis Island, NU. Arrows indicate direction and relative strength of currents; solid circles are the locations of sampling stations.

2.2.3 Sample collection

I sampled a total of 47 stations during the springs of 2011 and 2012 for zooplankton, phytoplankton and ice algae (Fig. 2.1). In 2011 sampling occurred May 4-18 at a total of 23 locations. In 2012, samples were collected May 1-17 at 24 separate stations. A manual ice corer (Mark II coring system, 9 cm internal diameter, Kovacs Enterprises) was used to collect the ice algae. The bottom 3 cm of each core was cut off and melted overnight in 500 ml of Millipore micropore (0.2 μm) filtered seawater (FSW) to avoid osmotic stress (Garrison and Buck, 1986). Samples were then measured for total volume, filtered onto pre-combusted (500°C for 4 hours) glass fiber filters (GF/F) and treated with 10 ml boiling FSW to deactivate lipolytic enzymes (Budge and Parrish 1999). In 2011 most filters were then placed into chloroform and stored at -20°C. Several duplicates were not placed in chloroform and were stored in cryovials at -80°C. Similar to Falk-Petersen et al. (1998), no differences were found in the fatty acids of ice algae stored in chloroform vs. those stored at -80°C (Two-sample Hotelling Test, $p = 0.36$). In 2012, all ice algae samples were stored in cryovials at -80°C until analysis.

Zooplankton was collected using a 20 cm mouth diameter net 160 cm long, (mesh size 153 μm, Aquatic Sampling Company, Buffalo, New York) which was fitted with a flowmeter (General Oceanics model 2030RC, one way clutch) in 2012. Three vertical hauls were taken from each station from 5 m above bottom to a maximum of 150 m. Hauls were pooled and separated by size using 500 μm and 250 μm sieves. The remaining filtrate was collected using a 20 μm mesh sieve in 2011 and onto GF/F filters in 2012. Calanoid copepods and *Gammarus setosus*, ice associated

amphipods, were collected by picking large individuals out of the 500 μm size fraction. In 2012, I used a dissecting microscope to separate the 500 μm size fraction into copepods larger than 500 μm (referred to as the 500 μm size category), gelatinous zooplankton, *Themisto libullela (*a pelagic amphipod), *Clione limacina (a pelagic pteropod),* and *Sagitta elegans* (a chaetognath). A larval fish, some mysids, the ctenophore *Mertensia ovum,* and another species of pteropod, *Limacina helicina,* were also isolated and included in investigations of differences between sample types, but because of insufficient sample sizes they were excluded from individual redundancy analysis of fatty acids. All zooplankton samples were immediately placed into chloroform (Omnisolv grade, VWR) and stored at -20°C until analysis.

Additionally in 2012, the diatom *Coscinodiscus centralis* was isolated from the 250 μm size fraction using 3 rounds of settlement and then by hand-picking any remaining zooplankton from the sample using a pasteur pipette and a dissecting microscope. The purified samples were then filtered onto pre-combusted GF/F filters and immediately placed into chloroform and stored at -20°C.

2.2.4 Lipid extraction

Lipids were extracted using 2 different methods. Zooplankton and *C. centralis* lipids were extracted using a modified Folch et al. (1957) procedure by sonicating the filters for 5 minutes in 10.5 ml of 2:1 chloroform and methanol solution; 2.6 ml of 0.7% saltwater was then mixed in before centrifuging and extracting the bottom phase. The upper phase, filter, and all remaining tissues were kept for stable isotope analysis. Lipid extracts were dried over anhydrous sodium sulfate before being

transesterified to produce fatty acid methyl esters (FAME) using sulfuric acid (Hilditch et al. 1964) as a catalyst.

Most lipids in *Calanus* copepods are stored as wax esters (Falk-Petersen et al., 2009). Therefore, thin layer chromatography was used to separate FAME from fatty alcohols that were also generated during the transesterification process. Samples were streaked on to silica gel plates along with standards of fatty alcohols and triacylglycerols. Plates were placed in toluene (Omnisolv grade, VWR) until the solvent front reached 3 cm from the top and then dried in a fume hood before being sprayed with a 0.2% dichloroflourocine in ethanol mixture. FAME were identified under UV light, collected from the plates and re-extracted using a 1:1 chloroform and hexane mixture (Omnisolv grade, VWR). Alcohols were extracted using chloroform and archived at -20°C.

To extract ice algae lipids, I used an *in situ* BCl_3 catalyzed transesterification method similar to Park and Goins (1994). The *in situ* method yields equivalent results for algae when compared to the combination of Folch and Hilditch methods and is more efficient for very small samples (Hall MSc 2012).

I identified 78 individual FAME using gas chromatography and quantified using 5-α cholestane as an internal standard. Every sample was analyzed using two injections, which were averaged to get the final values. A combination gas chromatograph/mass spectrometer was used to identify fatty acids. Fatty acid data are presented as mean % ± 1 SD of total mass of fatty acids.

2.2.5 Stable isotope analysis

Zooplankton and *C. centralis* tissues were removed after lipid extraction in 2:1 chloroform (Søreide et al., 2006) and methanol and freeze-dried at -40°C for 48 hours before being sent to the Great Lakes Institute for Environmental Research (University of Windsor, Windsor Ontario) for $\delta^{13}C$ and $\delta^{15}N$ stable isotope quantification. Isotopic signatures are expressed as a deviation ($\delta X = [(\mathbf{R}_{sample} / \mathbf{R}_{standard}) - 1] \times 1{,}000$) from international standards calibrated against Vienna-PeeDee Belemnite (VPDB), for carbon, and atmospheric N_2 (AIR) for nitrogen. Ice algae stable isotopes were prepared and analyzed at the Freshwater Institute (Department of Fisheries and Oceans) in Winnipeg, Manitoba.

Stable isotope fractionation values of 3.4‰ for $\delta^{15}N$ and 0.6‰ for $\delta^{13}C$ were selected based on the trophic enrichment rates of similar ecosystems (Søreide et al., 2006). Single source approximations of trophic levels were calculated using:

Equation 1.

$$TL_{consumer} = 1 + (\delta^{15}N_{consumer} + \delta^{15}N_{primaryproducer})/3.4$$

Where 1 represents the trophic position of the primary producers and 3.4 is the amount $\delta^{15}N$ is enriched at each trophic transfer (Post 2002). To estimate trophic levels while accounting for multiple nitrogen sources a two-source model was calculated from Post (2002):

Equation 2.

$$TL_{consumer} = 1 + (\delta^{15}N_{consumer} - [\delta^{15}N_{primaryproducer1} \times \alpha + \delta^{15}N_{primaryproducer2} \times (1-\alpha)])/3.4$$

Where α represents the proportion of nitrogen in the consumer derived from primary producer 1.

Equation 3.

$$\alpha = (\delta^{13}C_{consumer} - \delta^{13}C_{primaryproducer2}) / (\delta^{13}C_{primaryproducer1} - \delta^{13}C_{primaryproducer2})$$

2.2.6 Statistical analysis

Statistical analyses were done using R statistical software (R Core Team, 2012). Differences between sample categories were tested using a permutational MANOVA so further analysis to test the effects of environmental variables could be conducted separately for each group. Any fatty acid that accounted for less than 1% of the total mass of fatty acids in every sample was not included in analysis. Selected fatty acids were calculated separately for samples that were isolated in 2012. Where possible, permutation tests were used to avoid transforming data, however fatty acid proportional data were arcsin square-root transformed before using parametric multivariate analyses such as principal component analysis (PCA) or hierarchical clustering on the principal component axes (HCPC) (Sokal and Rohlf, 1995). Redundancy analysis (RDA) using R package vegan was used to calculate the influence of environmental variables on fatty acid signatures (Oksanen et al., 2013). RDA analysis tests the significance and explanatory power of environmental variables using Monte Carlo permutations, selecting the best model using the Akaike Information Criterion. Lastly, the response variables (fatty acids) were constrained linearly to the predictive environmental variables and the model was tested again using Monte Carlo permutations. Only fatty acid profiles that had complete sets of

environmental variables were used in redundancy analysis. Hierarchical clustering analysis and HCPC were used to find any internal groupings within sample categories (R package FactoMineR (Husson et al., 2013)). Principal component analysis was calculated to determine the importance of individual fatty acids for any internal clusters found (R package vegan).

2.3 Results

2.3.1 Field observations:

Average snow depth was higher in 2011 (mean = 8.3 cm, range = 1.1 to 20.6 cm) compared to 2012 (mean = 5.9 cm, range = 1.7 to 13.9 cm). Ice thickness showed the reverse trend and was thicker in 2012 (mean = 162.7 cm, range = 121.4 to 235.0 cm) than in 2011 (mean = 133.0 cm, range = 96.2 to 167.6 cm). Areas of open water were visible at the end of the sampling period in 2011 but in 2012, 100% ice cover remained throughout field sampling. Light levels in both years were above the minimum value required for photosynthesis (2–9 $\mu E\ m^{-2}\ s^{-1}$; Horner and Schrader, 1982), but were lower and less variable in 2011 (mean = 4.90 ± 3.03 $\mu E\ m^{-2}s^{-1}$) than 2012 (mean = 9.36 ± 8.76 $\mu E\ m^{-2}s^{-1}$). CTD data were only available for 2011. Casts showed water column temperature and salinity were effectively homogenous throughout most of the study area.

2.3.2 Trophic interactions

Fatty acid profiles were significantly different between sample types (one-way PerMANOVA, $p < 0.001$). RDA was applied to investigate similarities between

sample types and explained 66.3% of the total variability (Fig. 2.2). The first two axes explained 46.4% of the total unconstrained variance in fatty acid compositions.

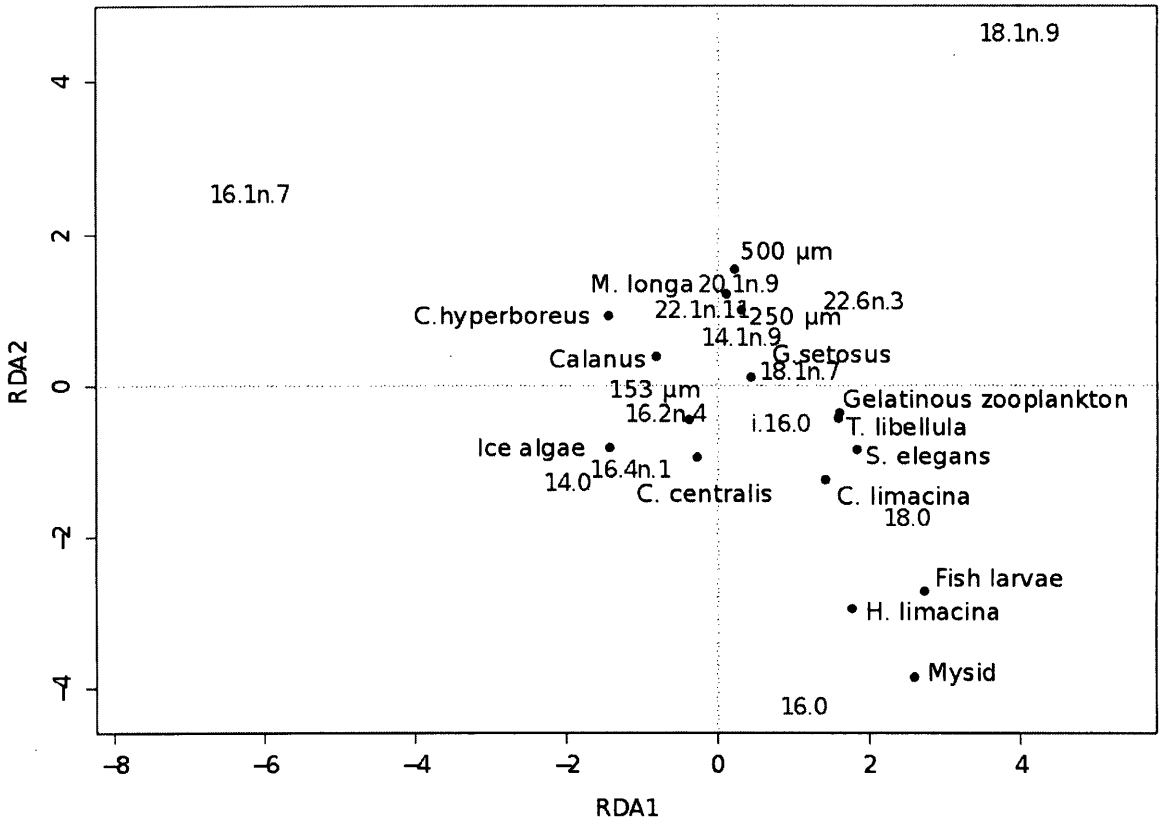

Figure 2.2. Redundancy analysis plot based on fatty acid composition of all individual samples. Blue circles indicate mean values of each sample type. Sample types were assigned roles as dummy variables with fatty acids that were present >1% as the response matrix. The variance in fatty acid compositions explained is 26.2% and 20.2% for axes 1 and 2 respectively.

Ice algae had the highest and most variable values of $\delta^{13}C$ of all sample types (Fig. 2.3). While having similar $\delta^{13}C$ values to other sample types (Fig. 2.3), *C.*

centralis fatty acid profiles were isolated during clustering, and it is therefore unclear if they are a viable food source for local zooplankton (Fig. 2.4). When *C. centralis* and ice algae $\delta^{13}C$ values were input into the 2 source mixing model, only chaetognaths had a trophic level higher than 1. Ice algae fatty acids were more similar to herbivores than *C. centralis* (Fig. 2.4), therefore trophic positions were recalculated using ice algae as the base of a single source model (Table 2.1).

Table 2.1. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope values and trophic levels of primary producers and zooplankton. Trophic levels were calculated from a single source food web model using ice algae as the base.

Sample type	n	$\delta^{13}C$	$\delta^{15}N$	Trophic level
153 μm	25	-18.2 ± 1.9	8.4 ± 1.8	1.6
250 μm	20	-19.6 ± 1.3	10.8 ± 1.0	2.3
500 μm	19	-19.8 ± 1.3	11.1 ± 0.7	2.4
G. setosus	1	-16.0	8.3	1.6
Calanus	9	-20.1 ± 0.8	10.4 ± 0.5	2.2
C. centralis	24	-19.0 ± 0.7	4.8 ± 1.4	0.6
S. elegans	2	-20.5 ± 1.1	13.1 ± 0.9	3.0
T. libellula	1	-19.5	10.9	2.3
Gelatinous zooplankton	16	-20.7 ± 0.9	11.8 ± 0.4	2.6
Ice algae	21	-12.8 ± 2.6	6.3 ± 0.6	1.0

When calculated using only ice algae as a carbon source, *S. elegans* had the highest trophic level, followed by gelatinous zooplankton (Table 2.1). These two groups were also the most depleted out of all sample types for $\delta^{13}C$ (Fig. 2.3). The fatty acid profiles of known predators grouped together, although *Clione limacina* had slightly more distinctive fatty acid compositions than *Sagitta elegans*, gelatinous zooplankton and *Themisto libellula* (Fig. 2.2, Fig. 2.4). Consisting mainly of copepods,

500 μm and 250 μm had similar trophic levels to the amphipod *T. libellula* (Table 2.1). A fatty acid indicator of carnivory, 18:1n-9, increased with sample body size (Fig. 2.2).

Calanus fatty acid profiles were more similar to ice algae than were 250 μm or 500 μm categories (Fig. 2.2). Size categories 250 μm and 500 μm were similar and closely related to the cluster including ice amphipods and 153 μm size category (Fig. 2.4). Fatty acids of samples with only a single copepod species were all more similar to one another than the cluster of mixed species samples (Fig. 2.4). The 153 μm size category was well separated from other groups isotopically (Fig. 2.3) but had a similar trophic level to that of ice amphipods (Table 2.1). Mysids, fish and *Helicina limacina* fatty acid profiles were distinct compared to the rest of the sample categories (Fig. 2.2, 2.4).

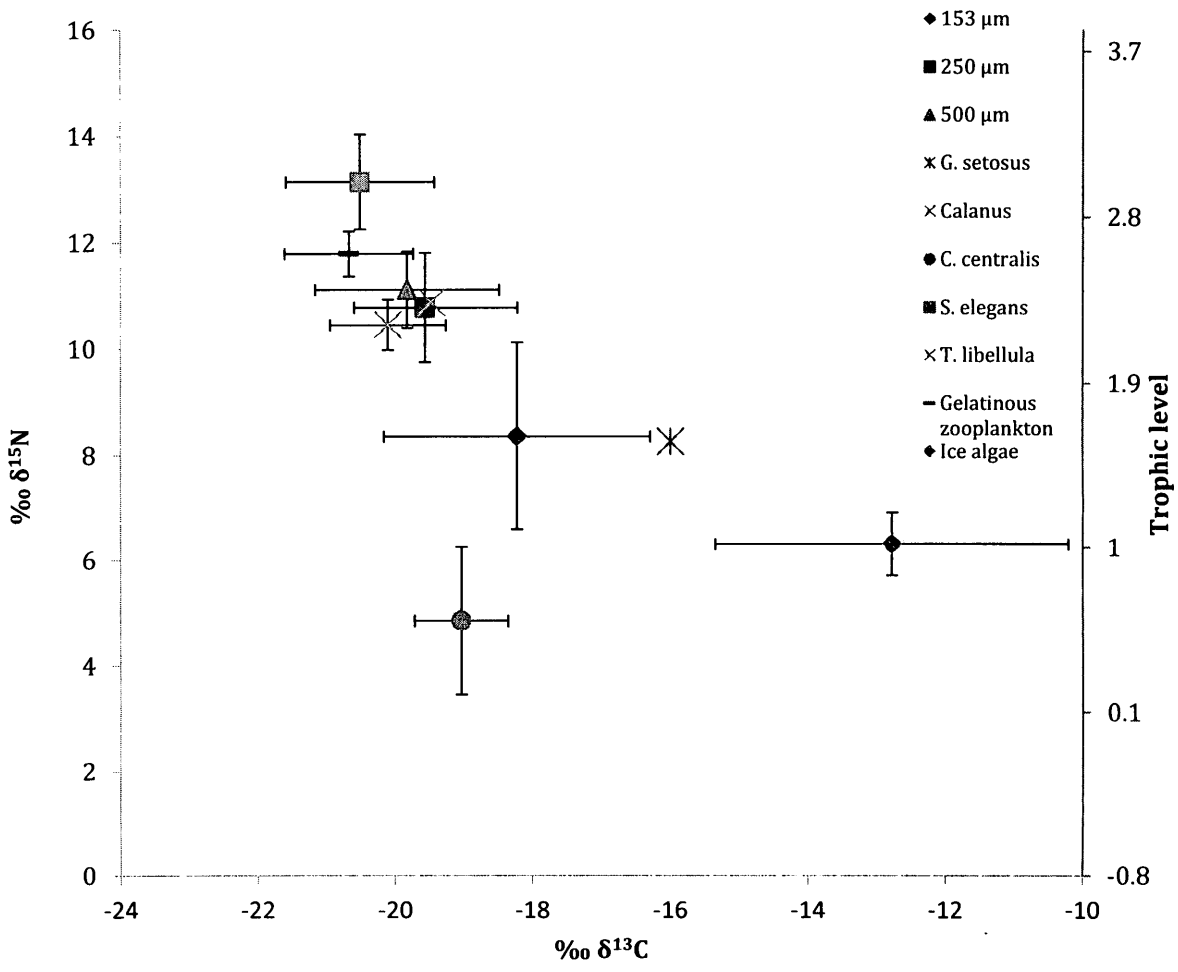

Figure 2.3. Stable isotope values (mean ± SD) of carbon and nitrogen for all sample types collected in 2012. Trophic levels calculated from the single source model are on the right side.

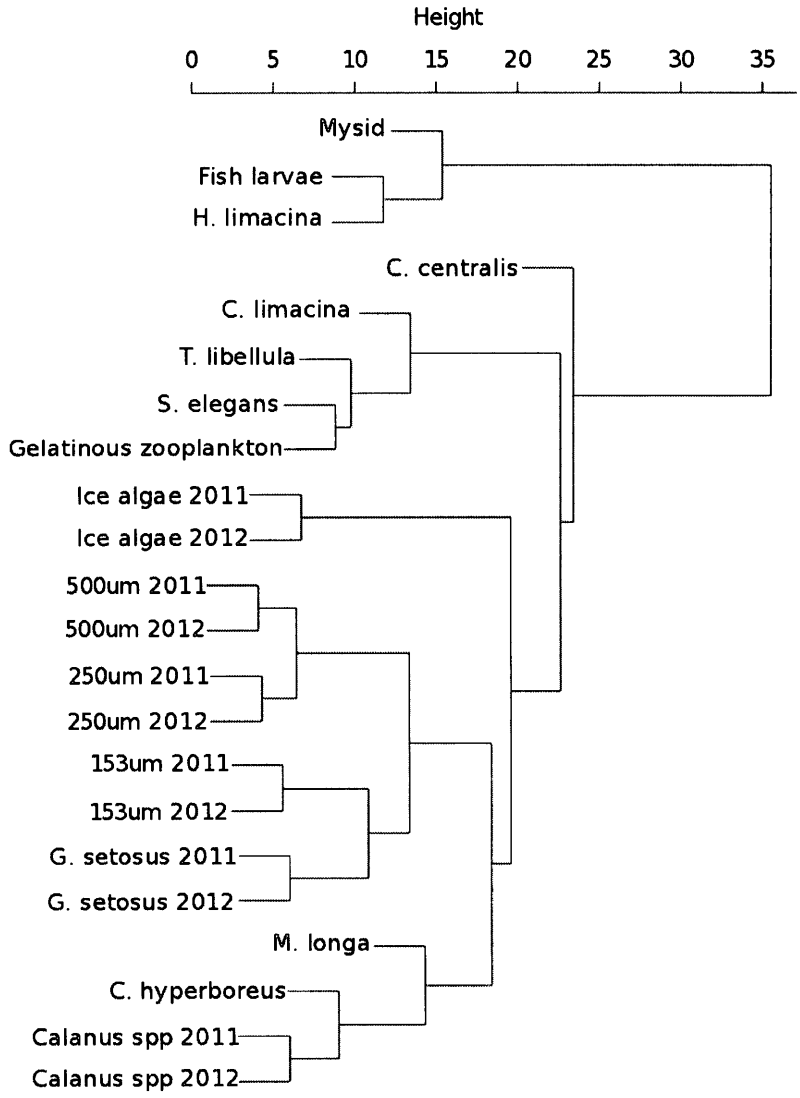

Figure 2.4. Hierarchical cluster analysis of average fatty acid composition of all sample types that were isolated during 2011 and 2012. Clusters were found using Euclidean distance of all fatty acids and between-groups linkage method.

Table 2.2 Fatty acid composition (mass % of total FA ±SD) of sample types that were collected in both years. *PUFA* polyunsaturated fatty acids, *MUFA* monounsaturated fatty acids, *SAFA* saturated fatty acids.

	Ice algae		153 µm		250 µm		500 µm		*Calanus*		*G.setosus*	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
	n = 23	n = 22	n = 17	n = 25	n = 18	n = 24	n = 17	n = 25	n = 18	n = 16	n = 3	n = 4
14:0	6.6 ± 1.5	8.9 ± 1.8	5.3 ± 1.6	7.3 ± 1.8	2.9 ± 1.6	2.7 ± 1.7	2.5 ± 1.1	2.2 ± 1.1	6.6 ± 2.1	8.4 ± 2.2	3.5 ± 0.6	3.6 ± 0.5
14:1n-9	0.1 ± 0.2	0 ± 0	0.4 ± 0.5	0.2 ± 0.2	1.6 ± 0.7	1.9 ± 0.4	1.6 ± 0.7	2 ± 0.8	0.5 ± 0.2	0.6 ± 0.2	0.1 ± 0	0.1 ± 0
16:0	16.3 ± 5.2	16.8 ± 3.4	14.4 ± 4	16.5 ± 2.2	8.4 ± 1.8	9 ± 2.7	6.3 ± 0.8	6.6 ± 1.5	7.5 ± 4.2	8.1 ± 2.1	14.7 ± 0.7	16.2 ± 0.4
16:1n-11	1.1 ± 0.9	0.4 ± 0.9	0.2 ± 0.1	1.1 ± 0.5	0.2 ± 0.2	0.2 ± 0.1	0.2 ± 0	0.1 ± 0	0.3 ± 0.1	0.2 ± 0	0.4 ± 0.1	0.3 ± 0
16:1n-9	0.9 ± 0.5	2.8 ± 2.5	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.3	0.1 ± 0	0.1 ± 0	0 ± 0	0.1 ± 0	0 ± 0	0.1 ± 0	0 ± 0
16:1n-7	29.8 ± 11.4	35.2 ± 7.2	24.9 ± 6.5	26.2 ± 6.6	23.1 ± 2.8	26.1 ± 2.9	25 ± 1.9	27.6 ± 2.4	26.5 ± 2.3	30.2 ± 4.7	20.6 ± 0.9	22.2 ± 5.5
16:2n-4	2.5 ± 0.8	2.9 ± 0.8	1.2 ± 0.4	1.9 ± 0.7	1.3 ± 0.5	1.1 ± 0.2	0.9 ± 0.2	1.1 ± 0.1	1.5 ± 0.2	1.8 ± 0.3	1.2 ± 0.1	1.2 ± 0.3
16:3n-4	1.6 ± 1	1.4 ± 0.9	0.5 ± 0.4	0.9 ± 0.4	0.7 ± 0.4	0.6 ± 0.2	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	0.7 ± 0.2	0.5 ± 0.2
16:4n-1	4.6 ± 2.3	4.6 ± 2.2	0.4 ± 0.6	1.9 ± 1.1	0.7 ± 0.8	0.5 ± 0.3	0.2 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.2 ± 0.1	0.9 ± 0.2	1 ± 0.8
18:0	0.8 ± 0.8	0.3 ± 0.1	1.9 ± 0.8	1.4 ± 0.6	1.4 ± 1.6	1 ± 0.5	0.7 ± 0.2	0.6 ± 0.6	1.2 ± 1.3	0.9 ± 0.5	0.9 ± 0.2	1.2 ± 0.4
18:1n-11	0.1 ± 0.2	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	1 ± 0.8	1.3 ± 0.4
18:1n-9	1.1 ± 0.5	0.6 ± 0.4	6.8 ± 3.2	4 ± 1.9	14.2 ± 3.3	16.6 ± 3.8	15.9 ± 3.7	18.1 ± 3.7	3.1 ± 0.6	3.2 ± 0.4	11.3 ± 3.4	14.5 ± 3.3
18:1n-7	0.5 ± 0.1	0.3 ± 0.1	2.8 ± 1.1	2.2 ± 0.9	2.9 ± 0.8	2.6 ± 0.7	2.4 ± 0.3	2.3 ± 0.4	1.7 ± 0.2	1.7 ± 0.3	3.5 ± 1.3	2.6 ± 0.4
18:1n-5	0.4 ± 0.6	0 ± 0.1	0.9 ± 0.3	0.7 ± 0.3	1.3 ± 0.5	1.1 ± 0.3	1 ± 0.1	0.9 ± 0.2	1 ± 0.1	1 ± 0.1	0.6 ± 0.2	0.5 ± 0.2
18:2n-6	0.7 ± 0.3	0.4 ± 0	1.5 ± 0.6	1.5 ± 0.7	1.4 ± 0.6	1.4 ± 0.6	0.7 ± 0.1	1 ± 0.2	0.7 ± 0.2	0.8 ± 0.1	0.8 ± 0.2	1 ± 0.1
18:3n-6	1.2 ± 0.5	1.2 ± 0.4	0.5 ± 0.2	0.6 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0	0.5 ± 0.1	0.6 ± 0.2
18:3n-3	1.2 ± 0.9	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0	0.3 ± 0	0.3 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.2 ± 0	0.1 ± 0
18:4n-3	2.3 ± 0.9	2 ± 0.7	0.7 ± 0.4	1.2 ± 0.4	1 ± 0.4	1 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	1.4 ± 0.3	1.3 ± 0.6
20:1n-11	0.2 ± 0.3	0 ± 0	0.3 ± 0.1	0.4 ± 1.5	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.1	0.6 ± 1.5	0.6 ± 0.2	0.3 ± 0.1	0.9 ± 0.7	2.7 ± 3.5
20:1n-9	0.3 ± 0.8	0 ± 0	4 ± 2.5	2.9 ± 1.7	2 ± 1.3	1.3 ± 0.5	5.2 ± 2.2	4.9 ± 3.8	9.8 ± 2.7	11.1 ± 1.6	4.6 ± 3.2	4.7 ± 2.7
20:1n-7	0.3 ± 0.2	0 ± 0	1.7 ± 0.7	1 ± 0.6	0.8 ± 0.3	0.5 ± 0.1	0.9 ± 0.2	0.5 ± 0.3	1.3 ± 0.4	0.9 ± 0.6	1.4 ± 0.1	0.6 ± 0.4
20:5n-3	12.4 ± 4.4	13.6 ± 5	11.5 ± 3.6	12.9 ± 2.5	16.2 ± 2.9	15.8 ± 2.7	14.6 ± 2	13.7 ± 2.5	11.4 ± 2.7	7.8 ± 1.6	17.2 ± 5.1	13.8 ± 2.3
22:1n-11	0.3 ± 0.6	0.1 ± 0.1	2.5 ± 1.6	1.5 ± 1.2	1.5 ± 1.3	0.7 ± 0.5	3.7 ± 1.7	3 ± 2.7	8 ± 2.4	6.9 ± 1.6	1.6 ± 1.1	1.2 ± 0.9
22:1n-9	0.1 ± 0.1	0 ± 0	1.4 ± 0.5	2.1 ± 1.7	0.5 ± 0.3	0.2 ± 0.1	0.8 ± 0.3	0.6 ± 0.8	2.2 ± 0.8	1.7 ± 0.6	0.7 ± 0.3	0.9 ± 0.8
22:6n-3	1.3 ± 0.7	1.7 ± 0.6	5.1 ± 1.4	3.9 ± 1.8	6.9 ± 1.2	6.7 ± 2.1	7.7 ± 1.3	6.2 ± 1.3	5.2 ± 1.2	5 ± 1.4	4.2 ± 1.6	2.4 ± 0.5
Σ PUFA	34.3 ± 12.1	30.6 ± 10.1	26 ± 6.3	27.6 ± 4.9	32.9 ± 4.4	31 ± 4.2	28.9 ± 2.9	26.4 ± 3.5	24.2 ± 3.7	19.3 ± 2.5	30.6 ± 8.6	24 ± 4.2
Σ MUFA	38.3 ± 8.2	41.4 ± 6.5	49.8 ± 5.5	44.8 ± 5.6	52.2 ± 5.8	53.9 ± 4.3	60 ± 3.2	62.6 ± 4.5	58.5 ± 5.1	60.6 ± 4.5	48.7 ± 7.8	54 ± 4.2
Σ SAFA	27.2 ± 4.6	27.9 ± 4.3	24 ± 5.3	27.4 ± 2.4	14.7 ± 4.2	14.9 ± 4.6	10.9 ± 1.5	10.8 ± 2.9	17.2 ± 6.6	20 ± 4.6	20.5 ± 0.8	21.8 ± 0.9

2.3.3 Ice algae

Monounsaturated fatty acids (MUFAs) were the dominant class of fatty acid of ice algae in both years, and PUFAs were the second most abundant. The individual fatty acid present in the highest amount was 16:1n7, followed by 16:0. Two common diatom fatty acid biomarkers, 16:4n1 and 20:5n3, were also present in high amounts both years (Table 2.2). Once the effect of snow cover was taken into account, there were no significant differences in fatty acid profiles between years (Fig. 2.5). Ice and snow thickness explained 28.27% of total variance in fatty acid profiles (R^2=0.28, AIC = 167, step= 999, p=0.001). Snow depth had the strongest influence on fatty acids, responsible for 17% of the total variance. Ice algae fatty acid profiles separate into three distinct clusters (Fig. 2.6), however no environmental or spatial explanations could be found for their cause. These clusters were separated by inverse relationships of 20:5n3 and 16:4n1 with 16:0 and 16:1n7 fatty acids (Fig. 2.7).

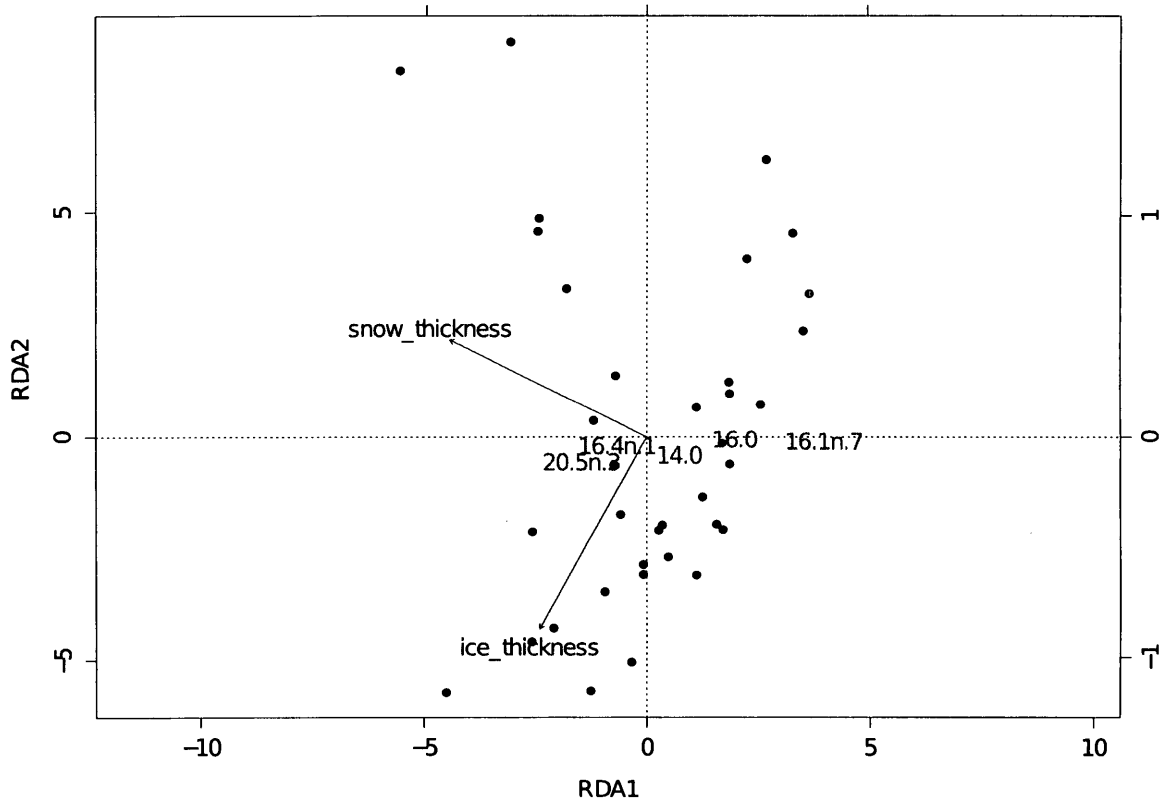

Figure 2.5. Redundancy plot of the fatty acid composition of ice algae. The first and second axes explained 31.61% and 0.75%, respectively, of the variability in ice algae fatty acids.

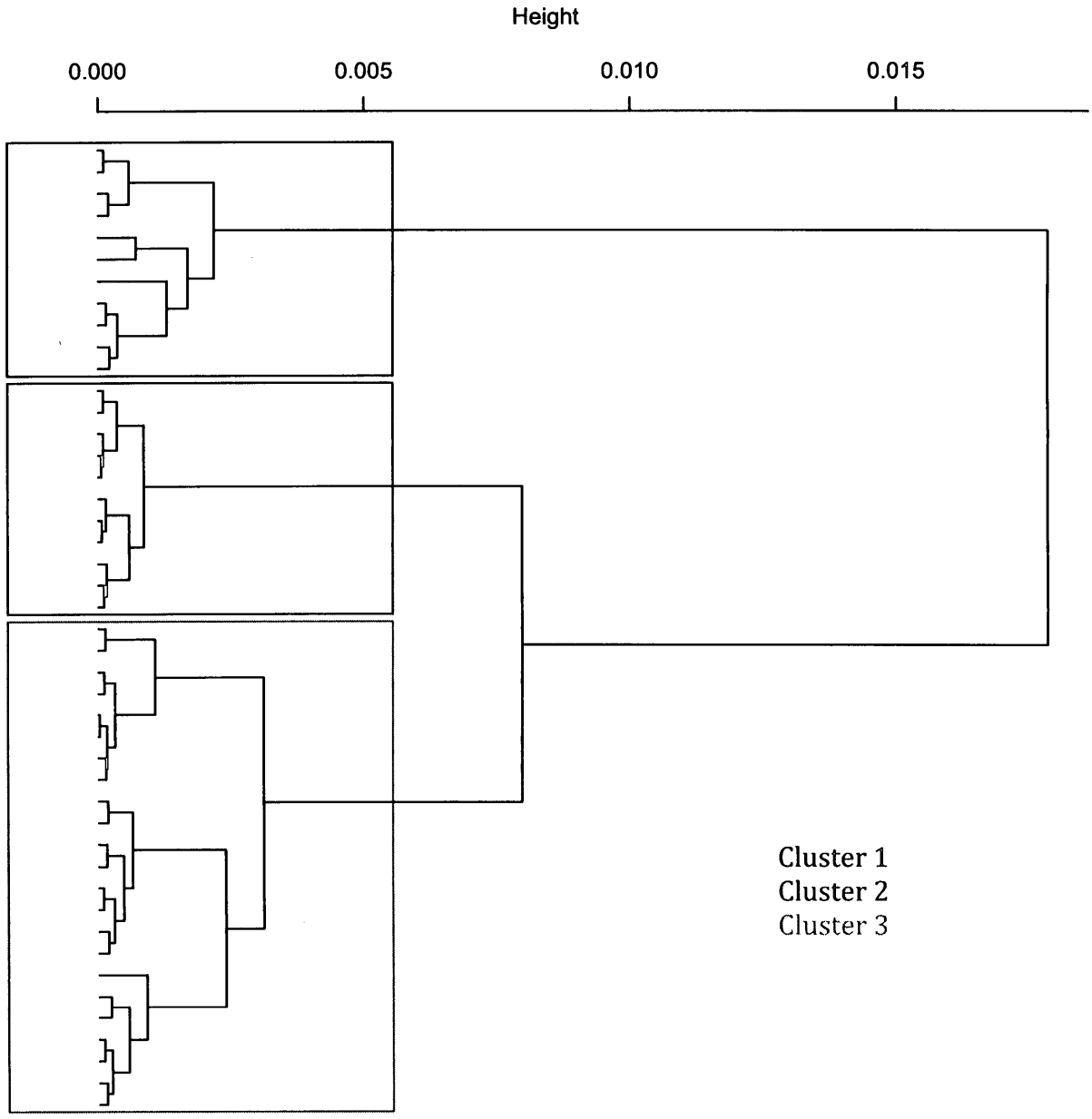

Figure 2.6. Hierarchical cluster analysis of arcsin square-root transformed ice algae fatty acids sorted according to the first principal component. Clusters were selected at the point of maximum between-group variances.

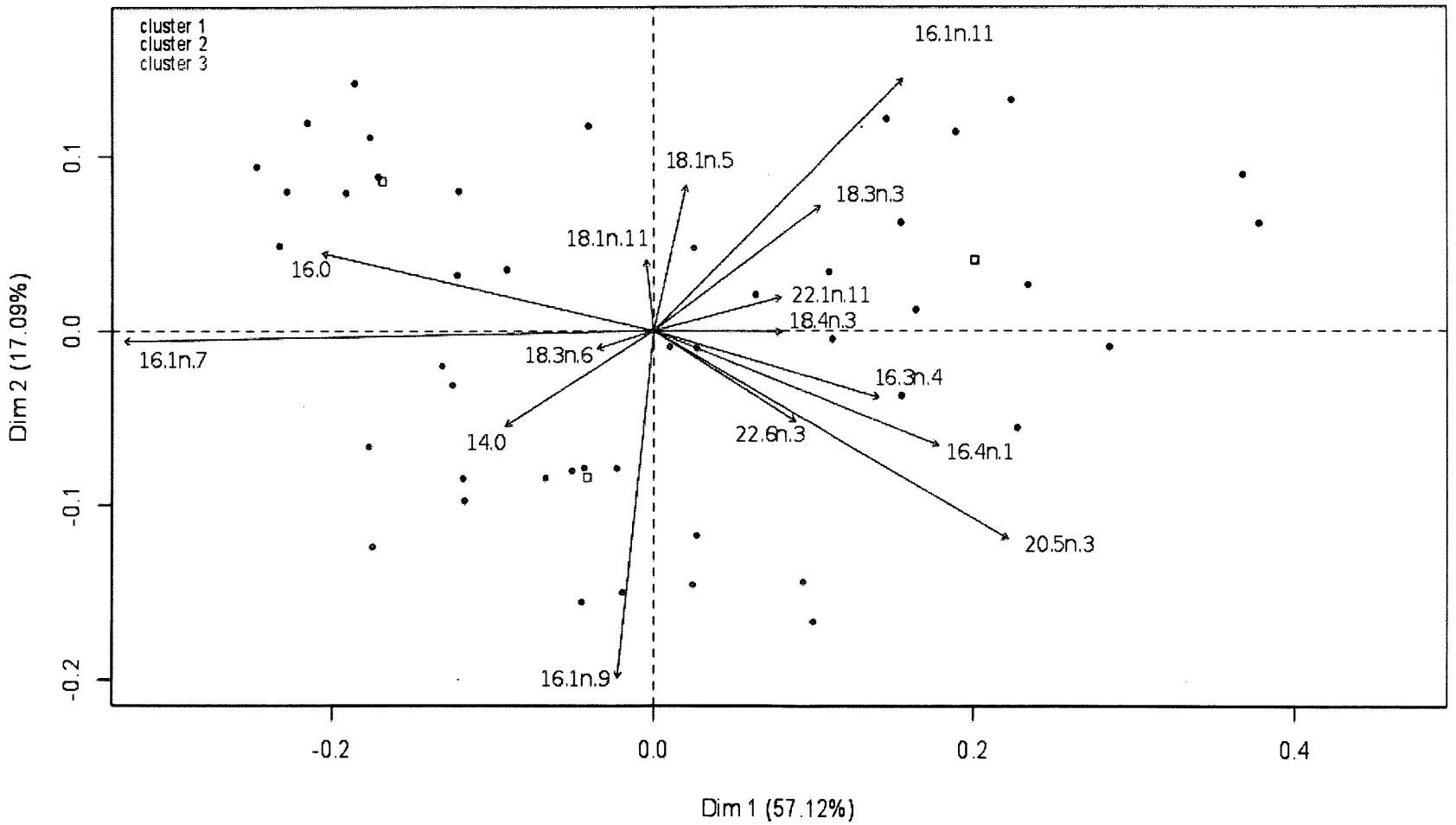

Figure 2.7. Cluster map of principal component analysis using arcsin square-root transformed ice algae fatty acids. Boxes indicate cluster means.

2.3.4 Coscinodiscus centralis

C. centralis samples had high proportions of PUFAs, which were present in higher amounts than in any other sample type (Table 2.2, 2.3). MUFAs and saturated fatty acids (SAFAs) accounted for 26.4% and 23.3% of total fatty acids, respectively. *Coscinodiscus* fatty acids were comprised mainly of 14:0, 16:0, 16:1n-7, and 20:5n-3; although 16:4n-1 was also present in high amounts (Table 2.3). Environmental variables had no significant explanatory effects on *C. centralis* fatty acid profiles, however cluster analysis indicated two distinct groups existed that were related to known inflows. *C. centralis* samples collected in Barrow Strait had higher levels of 20:5n-3 and 16:4n-1 while sites downstream of Penny Strait had greater amounts of 16:1n-7, 18:2n-6 and 18:1n-9.

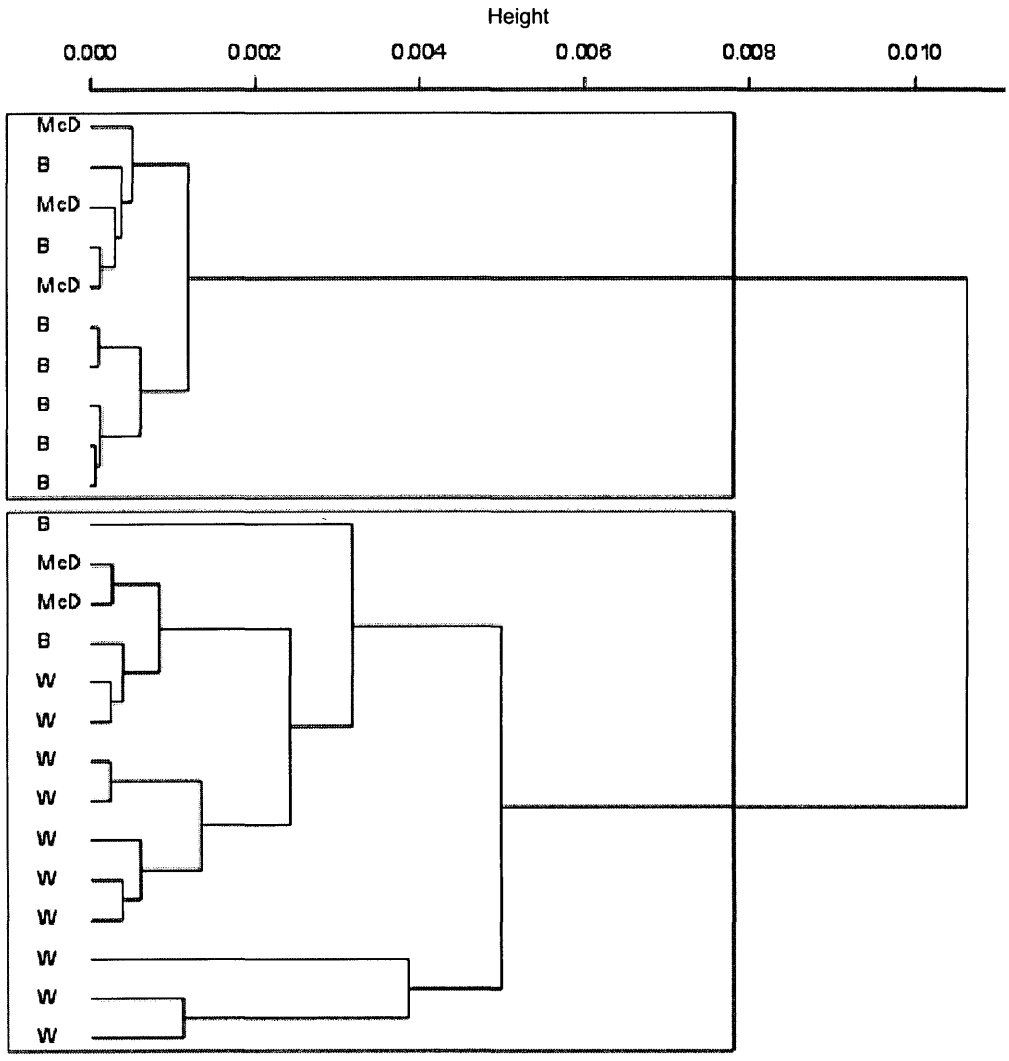

Figure 2.8. Hierarchical cluster analysis of arcsin square-root transformed *C. centralis* fatty acids. McD =McDougall Sound; B=Barrow Strait; W=Wellington Channel.

2.3.5 153 μm size category

In both years, the most abundant fatty acid class was MUFA while PUFAs and SAFAs were present in similar amounts year-to-year (Table 2.2). The fatty acid present in the highest proportions in both years was 16:1n-7, with 20:5n-3 being the second most common. Compared to ice algae and *C. centralis*, 153 μm samples had greater amounts of fatty acids that are not associated with diatoms: 18-carbon MUFAs, 20:1n-9, and 22:6n-3. Snow thickness, latitude and year sampled all had significant effects on 153 μm fatty acid signatures (R^2 = 0.21, AIC = 136.9, step = 999, p = 0.004) (Fig. 2.9). Clustering analysis of 153 μm fatty acid profiles showed 3 groups (Fig. 2.10). Cluster 1 was characterized by diatom fatty acid trophic markers 16:1n-7, 16:2n-4 and 16:4n-1. Cluster 2 was differentiated by 18-carbon MUFAs and dinoflagellate biomarker 22:6n-3, and cluster 3 had higher levels of copepod fatty acid biomarkers 20:1n-7, 20:1n-9 and 22:1n-11. Diatom fatty acids were more prevalent in 2012 compared to more dinoflagellate and copepod signatures in 2011. Non-diatom fatty acid trophic markers were also linked to the northern inflow (Fig. 2.9).

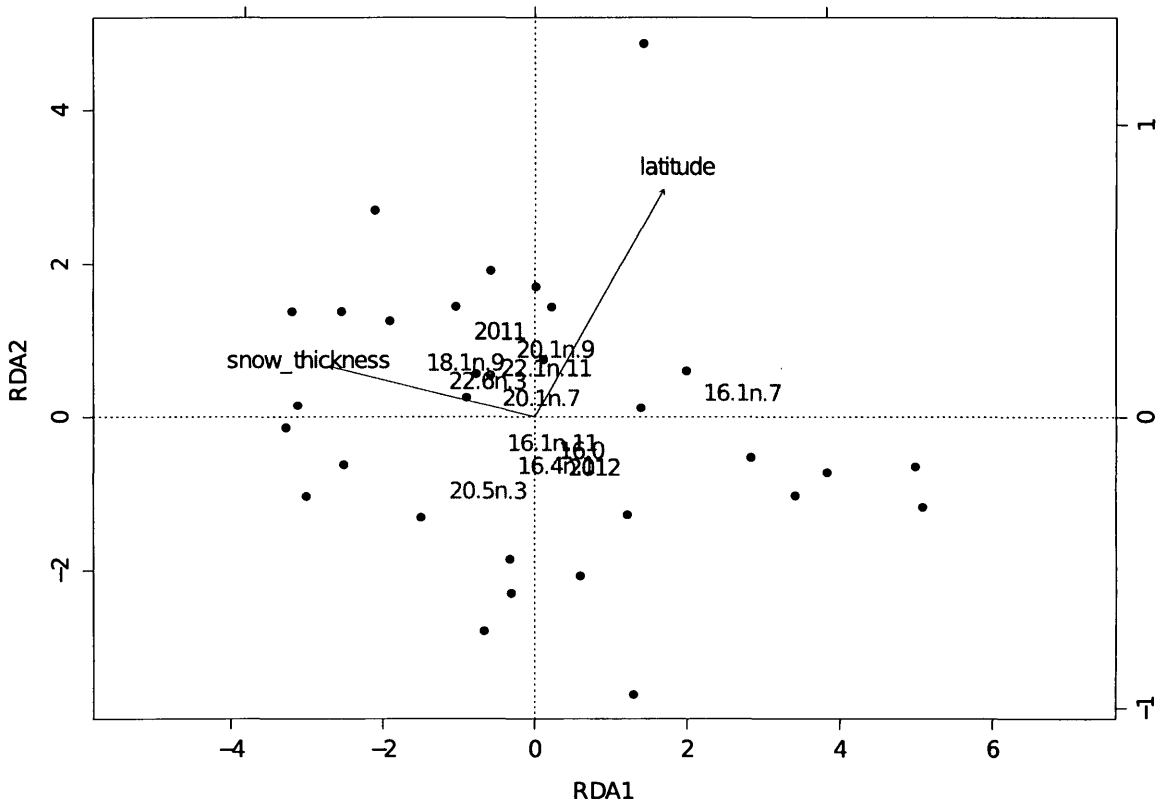

Figure 2.9. Redundancy analysis of 153 μm size category fatty acid profiles. The variance in 153 μm fatty acids explained is 18.22% and 8.36% by the first and second axes, respectively.

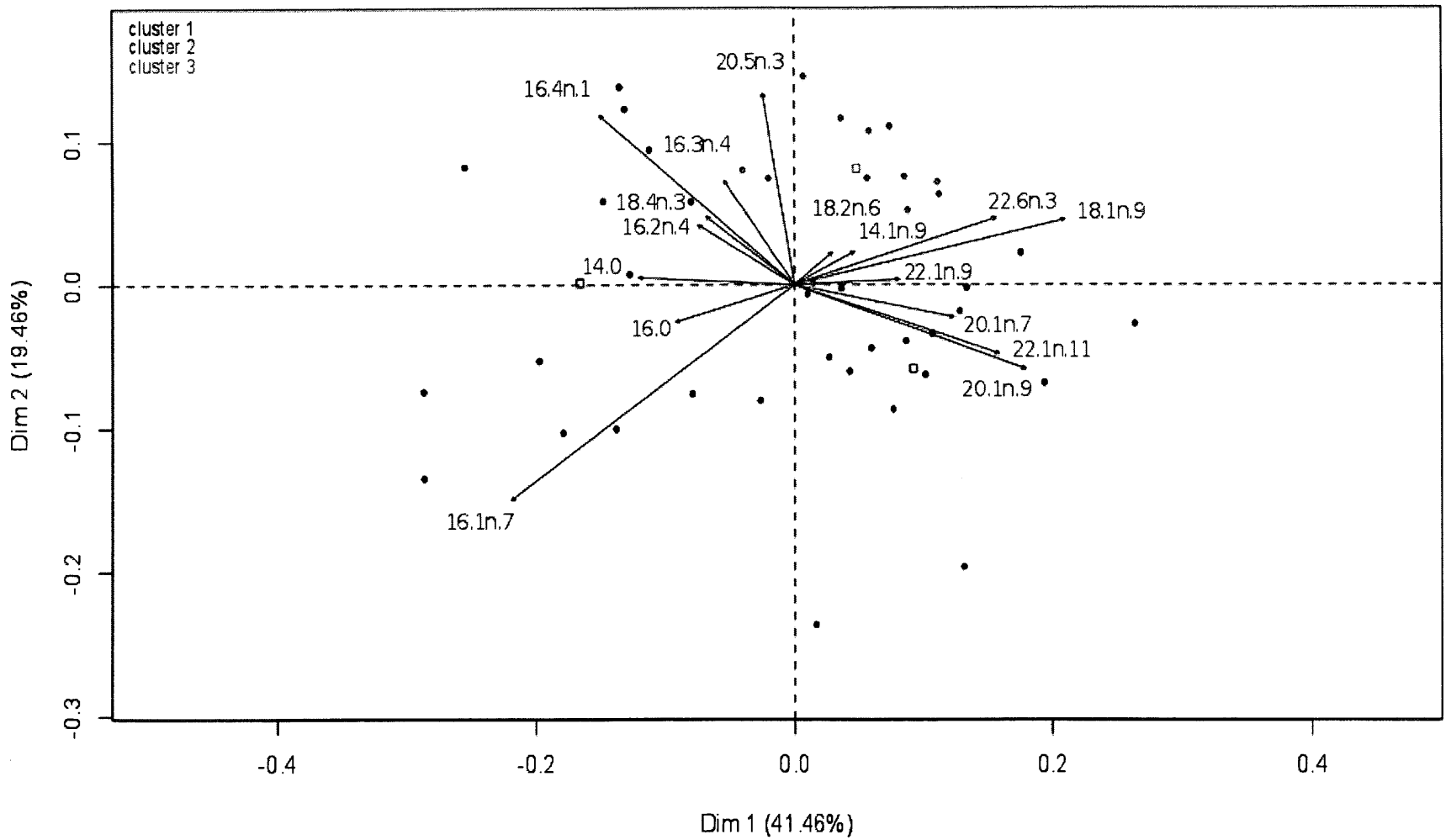

Figure 2.10. Principal component analysis of arcsin square-root transformed 153 μm fatty acid profiles with cluster membership. Boxes indicate cluster means.

2.3.6 250 μm size category

Fatty acid profiles for 250 μm differed significantly between years ($p > 0.001$). Because of the removal of *C. centralis* from the 250 μm size category in the second season, 2011 and 2012 were analyzed separately.

2011

The most dominant fatty acid class was MUFA followed in decreasing order by PUFA and SAFA (Table 2.2). An indicator fatty acid of diatoms, 16:1n-7, was present in high amounts, as was 20:5n-3, although 16:4n-1 was present in only trace amounts. Dinoflagellate biomarker 22:6n-3 and 18:1n-9 accounted for significant proportions of 250 μm fatty acid signatures in 2011 (Table 2.2). Fatty acid compositions were primarily affected by inflow and then by chlorophyll A concentrations in the sea ice ($R^2 = 0.29$, AIC = 46.4, step = 999, p =0 .031) (Fig. 2.11).

2012

MUFAs made up the bulk of 250 μm fatty acids in 2012, followed by PUFAs and then by SAFAs. The major fatty acids were the same as those in 2011 and were present in similar amounts (Table 2.2). Fatty acids were most significantly affected by inflow, although in 2012 this was determined by their proximity to the west inflow of Barrow Strait. Downwelling light also had a significant effect (R^2=0.25, AIC = 71.0, step = 999, p = 0.007) (Fig. 2.12).

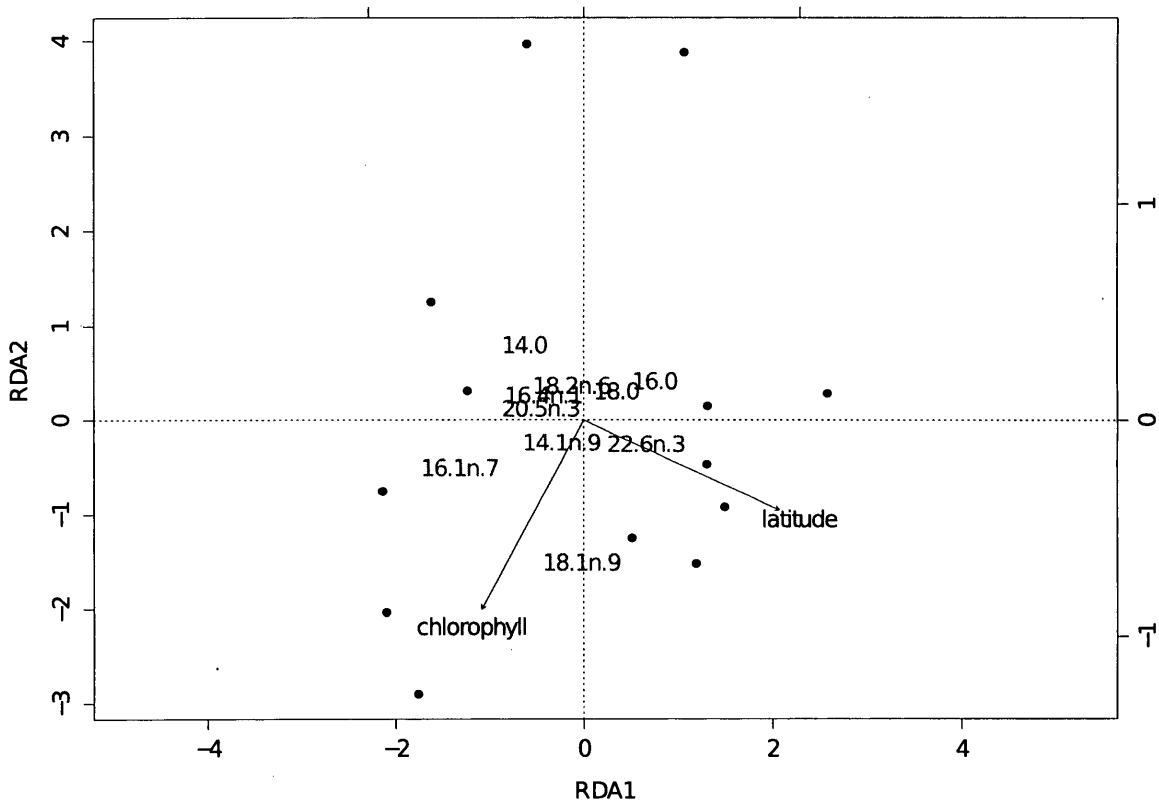

Figure 2.11. Redundancy analysis plot of 250 μm size category samples from 2011. The first axis accounts for 23.01% of the variance in 250 μm fatty acids, while the second explains 17.97%.

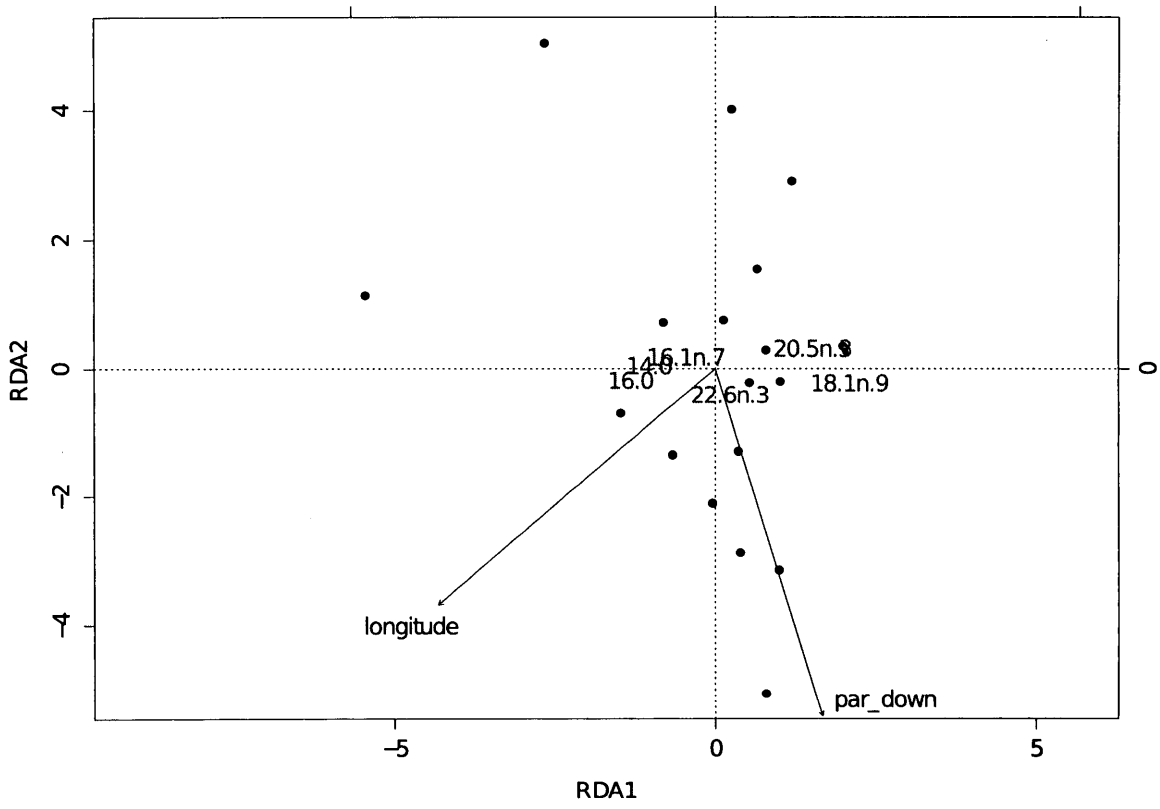

Figure 2.12. Redundancy analysis plot of 250 μm size category samples from 2012 under the reduced model. The variance in 250 μm fatty acids explained by the axes is 31.87% and 1.43%, respectively.

2.3.7 500 μm size category

Fatty acids of zooplankton in the 500 μm size category were mostly MUFAs followed by PUFAs. SAFAs were present in the least abundance in both years (Table 2.2). The most abundant individual fatty acid was 16:1n-7 in both 2011 and in 2012. The second most prevalent was 18:1n-9, followed closely by 20:5n-3. A calanoid copepod trophic marker, 20:1n-9, was present in nearly the same amounts as 16:0 (Table 2.2). Fatty acids were not significantly affected by any of the environmental

variables, had no spatial patterns or differed between years. Clustering resulted in 3 groups, the main differences being driven by *Calanus* biomarkers 20:1n-9 and 22:1n-11 (Fig. 2.13).

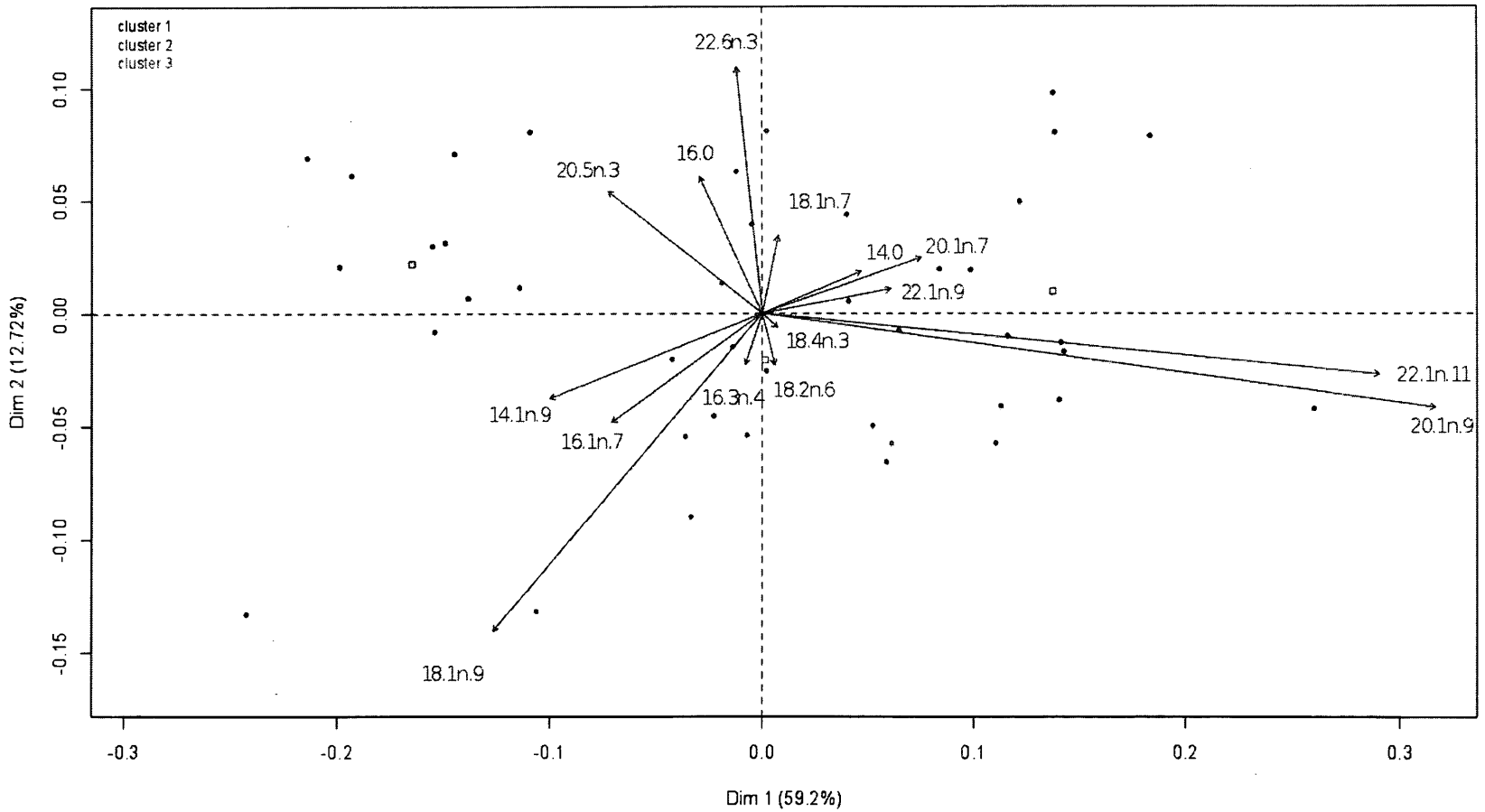

Figure 2.13. Hierarchical clusters of 500 μm size category fatty acids overlaid on the first two principal component axes. Boxes indicate cluster centroids. Fatty acids were arcsin square-root transformed.

2.3.8 Calanus spp.

Calanoid fatty acids were dominated by MUFAs in both years (Table 2.2). In 2011 PUFAs were more abundant than SAFAs. In 2012 however, PUFAs and SAFAs were present in nearly equal amounts. PUFA levels were significantly higher in 2011 compared to 2012 ($p < 0.001$) (Fig. 2.14). The most abundant individual fatty acids in 2011 were 16:1n-7 and 20:5n-3 (Table 2.2). The relative proportions of 14:0, 16:0, and 22:6n-3 were similar in 2011 and 2012 while 16:1n-7 and 20:1n-9 increased in 2012. Not surprisingly, the *Calanus* biomarkers 20:1n-9 and 22:1n-11 were present in higher amounts in *Calanus* samples than any other sample type (Table 2.2, 2.3). *Calanus* fatty acid profiles differed significantly between years and inflows ($R^2 = 0.29$, AIC = 92.8, step = 999, $p = 0.001$). Samples from 2011 had higher amounts of 20:5n-3 and 16:1n-7 drives the separation of 2012 (Fig. 2.15).

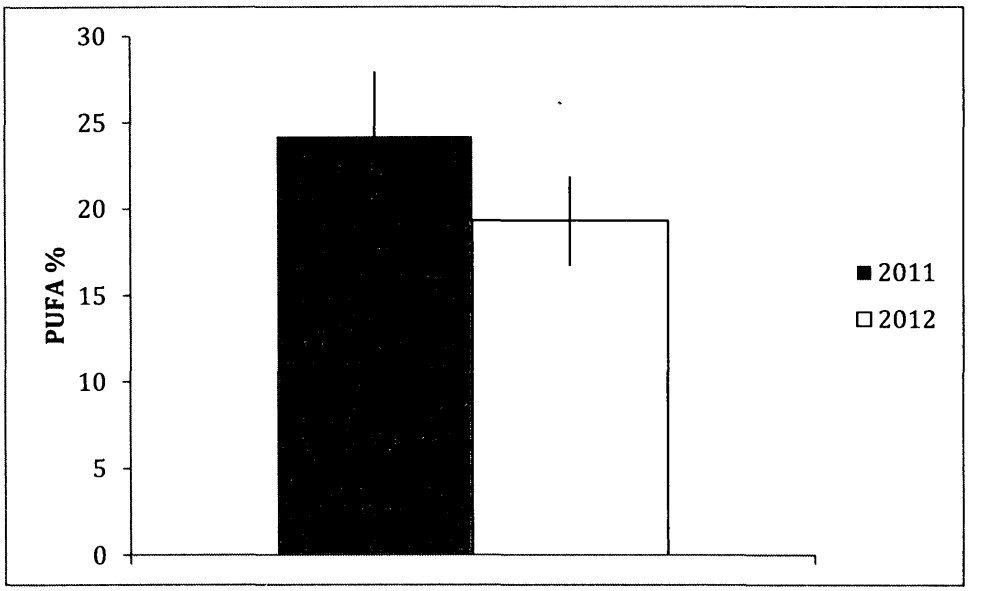

Figure 2.14. Difference of mean PUFA levels in calanoid copepods between 2011 and 2012. Lines indicate one standard deviation.

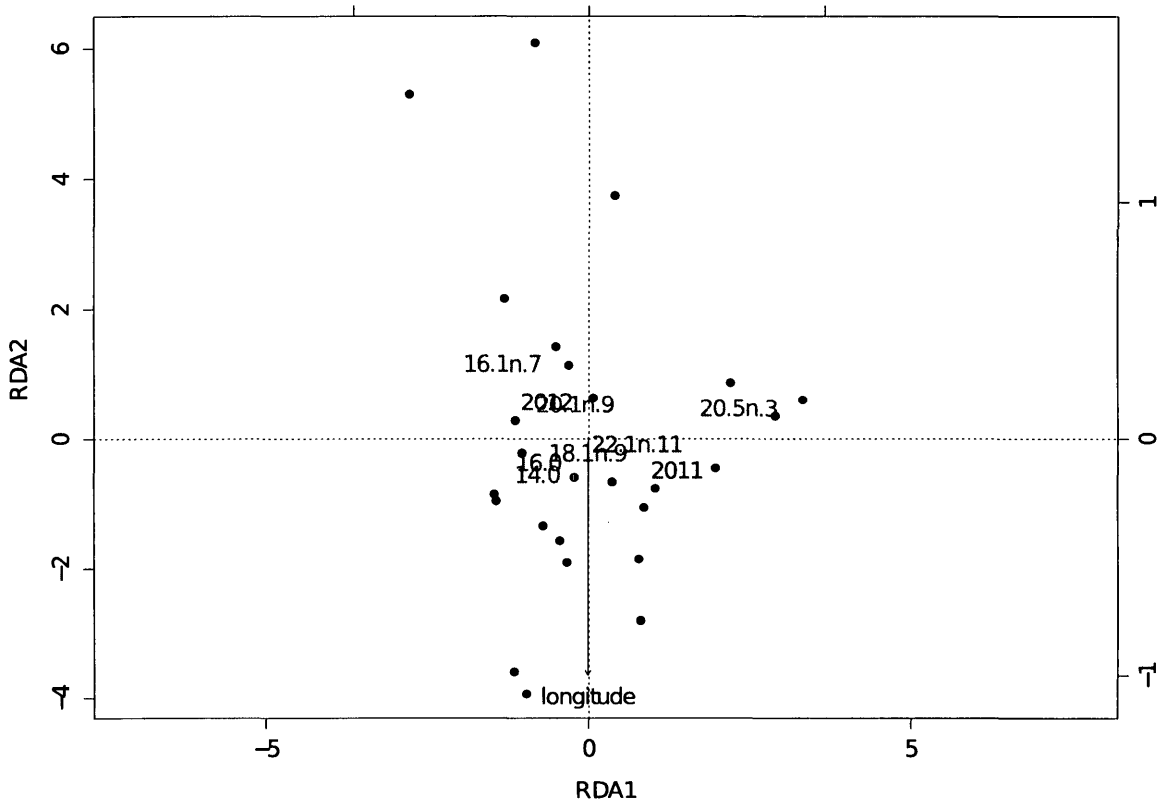

Figure 2.15. Redundancy analysis of *Calanus* copepods. The first and second axes account for 27.76% and 7.18% of the variance in *Calanus* fatty acids.

2.3.9 Ice associated Amphipod (Gammarus setosus)

Amphipod fatty acid profiles were dominated by MUFAs in both years. PUFA levels decreased in 2012 while SAFA levels were similar in both years (Table 2.2). Amphipod fatty acid profiles consisted of relatively few fatty acids, 16:0, 16:1n-7, 18:1n-9, and 20:5n-3, that were present in high amounts (Table 2.2). No other fatty acid was present over 5%. Variation in fatty acids was significantly affected by ice thickness, although the inclusion of a non-significant variable, particulate organic

carbon (POC) in sea ice (p = 0.08), increased the fit of the model from $R^2 = 0.22$ to $R^2 = 0.40$ (AIC = 27.8, step = 10000, p = 0.027) (Fig. 2.16).

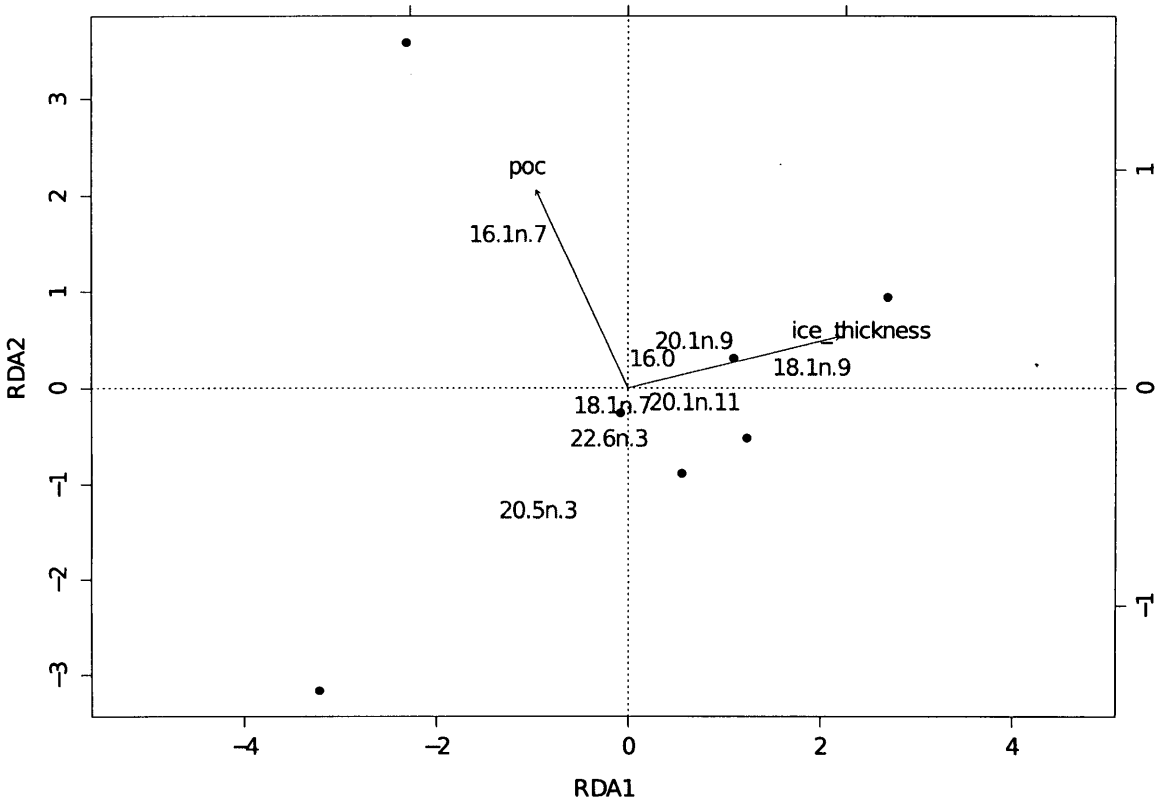

Figure 2.16. Redundancy analysis of ice amphipod fatty acids. The non-significant variable POC is included in the plot. The variance in ice amphipod fatty acids accounted by the axes is 35.4% and 24.72% respectively.

Table 2.3 Fatty acid composition (mass % of total FA ±SD) of sample types collected only in 2012. *PUFA* polyunsaturated fatty acids, *MUFA* monounsaturated fatty acids, *SAFA* saturated fatty acids.

	C.centralis n = 24	*S. elegans* n = 8	Gelatinous zooplankton n = 20	*T. libellula* n = 14	*C. limacina* n = 6
14:0	10.2 ± 2.2	2.1 ± 0.7	3 ± 0.9	1.9 ± 0.6	1.7 ± 0.4
15:0	0.2 ± 0.3	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	2.6 ± 0.2
15:1n-6	0.1 ± 0.1	0.2 ± 0.5	1.7 ± 7.4	0 ± 0.1	0 ± 0.1
i-16:0	0.2 ± 0.4	2.5 ± 2.5	2.3 ± 3.1	3.1 ± 6.7	4.4 ± 5.1
16:0	8.9 ± 2.6	20 ± 5.4	14.4 ± 3.8	16.6 ± 5.7	16.6 ± 3.2
16:1n-11	2.5 ± 0.7	0.1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	0.6 ± 0
16:1n-7	17.4 ± 5.1	11.7 ± 4.1	11.8 ± 4.6	12.6 ± 3.3	10.6 ± 1.9
16:1n-5	0.2 ± 0	1.7 ± 1	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1
17:1(a)	0 ± 0	0 ± 0	0 ± 0.1	0 ± 0	0.4 ± 0.9
ai-17:0	0 ± 0	0 ± 0.1	0.1 ± 0	0 ± 0	1.2 ± 0.2
16:2n-4	4.7 ± 1.3	0.1 ± 0.1	0.4 ± 0.2	0.2 ± 0.1	0 ± 0.1
17:0	0 ± 0	0.3 ± 0.2	0.3 ± 0.1	0.2 ± 0.2	2.8 ± 0.4
16:3n-4	2.7 ± 1	0 ± 0	0.1 ± 0.1	0 ± 0	0 ± 0
17:1	0 ± 0	0.1 ± 0.1	0.1 ± 0	0 ± 0	7.7 ± 1.6
16:4n-3	0 ± 0.1	0.1 ± 0.2	0 ± 0	0 ± 0.1	0.4 ± 0.9
16:4n-1	7.1 ± 2.5	0 ± 0	0 ± 0.2	0 ± 0	0 ± 0
18:0	2 ± 1.2	8.8 ± 7.1	6.6 ± 2.8	6.5 ± 5.7	5.8 ± 3.5
18:1n-9	2.3 ± 1.4	12.8 ± 4.8	11.9 ± 3.5	12.2 ± 2.8	4.7 ± 0.8
18:1n-7	0.9 ± 1.1	3.2 ± 1	2.9 ± 0.8	4.7 ± 1.1	4 ± 0.3
18:1n-5	0.1 ± 0.2	3.8 ± 1.2	0.7 ± 0.2	1.2 ± 0.4	0.4 ± 0.2
18:2n-6	3.5 ± 1.1	1.6 ± 0.5	1.3 ± 0.4	2.1 ± 0.4	1.2 ± 0.5
18:4n-3	2.6 ± 0.4	0.1 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	0.4 ± 0.2
20:0	0 ± 0	0.4 ± 0.5	0.3 ± 0.4	0.4 ± 0.4	0.3 ± 0.2
20:1n-11	0.1 ± 0.2	0.5 ± 0.7	0.1 ± 0.1	2.8 ± 1.8	0.8 ± 0.4
20:1n-9	0.4 ± 0.4	1.2 ± 0.6	3.5 ± 1.6	7.9 ± 4.4	2.2 ± 0.6
20:1n-7	0.4 ± 0.9	1 ± 0.9	1 ± 0.3	2.9 ± 1	3 ± 0.2
20:5n-3	24.4 ± 5	10.1 ± 2.9	13.9 ± 4.8	8.8 ± 3.7	10.1 ± 1.4
22:0	0 ± 0	0.5 ± 0.5	0.3 ± 0.2	0.3 ± 0.3	0.2 ± 0.2
22:1n-11	0.1 ± 0.4	1.1 ± 1.5	1.5 ± 0.9	2 ± 1.2	0.9 ± 1.4
22:6n-3	2.6 ± 0.9	8.2 ± 2.5	9.8 ± 4.8	5.3 ± 2.6	7.7 ± 1.7
Σ PUFA	50.3 ± 8.9	23.6 ± 6.7	30.2 ± 9.4	19.3 ± 6.6	24.4 ± 5.6
Σ MUFA	26.3 ± 6.3	39.4 ± 11.2	39.3 ± 6.3	49.9 ± 13	37.7 ± 4.3
Σ SAFA	23.3 ± 3.9	36.8 ± 13.7	30.4 ± 8.9	30.7 ± 16.5	37.7 ± 7.3

2.3.10 Gelatinous zooplankton (Hydrozoa: Euphysa aurata, Eumedusae birulai, Botrynema brucei, Aglantha digitale)

The most abundant fatty acid class in the gelatinous zooplankton samples was MUFAs, with PUFAs and SAFAs present in similar amounts (Table 2.3). Gelatinous zooplankton had higher amounts of fatty acids from dinoflagellate origins (16:1n-7/16:0 <1) than from diatoms (Dalsgaard et al., 2003). Individually, 16:0 and 18:1n-9 were the most abundant individual fatty acids after 20:5n-3. There were also small amounts of *Calanus* biomarker 20:1n-9 present. I found no observable spatial patterns, and environmental variables had no significant effect on gelatinous zooplankton fatty acid profiles.

2.3.11 Themisto libellula

Themisto libellula fatty acids consisted mainly of MUFAs and then, in decreasing amounts, SAFAs and PUFAs (Table 2.3). The most abundant fatty acid was 16:0. The ratio of 16:1n-7/16:0 was less than one, indicating that dinoflagellates are an important dietary source for *T. libellula;* however the 20:5n-3/22:6n-3 ratio was 1.7, characteristic of a food web supported by ice algae (Auel et al., 2002). The third most abundant fatty acid in *T. libellula* was 18:1n-9. *Calanus* biomarkers 22:1n-11 and 20:1n-9 also made up a significant proportion of *T. libellula* fatty acids (Table 2.3). There was an indication that inflow source may have some impact on *Themisto* fatty acid profiles, but the effect was not significant ($p = 0.099$).

2.3.12 Chaetognath (Sagitta elegans)

MUFAs and SAFAs were present in similar amounts while PUFA levels were lower (Table 2.3). The most abundant individual fatty acid was 16:0, followed by 16:1n-7 and 18:1n-9. Chaetognaths had the highest relative % of 18:0 among all samples (Table 2.2, 2.3). Diatom- and dinoflagellate-derived n-3 fatty acids were present in similar amounts in *S. elegans* samples (Table 2.3). Fatty acid profiles were significantly influenced by the reflectivity of the surface (upwelling PAR) ($R^2 = 0.56$, AIC = 25.0, step = 9999, p = 0.036). Increasing reflectivity was positively linked with fatty acids 18:1n-9 and 16:1n-7 and negatively linked with 18:0 (Fig. 2.17).

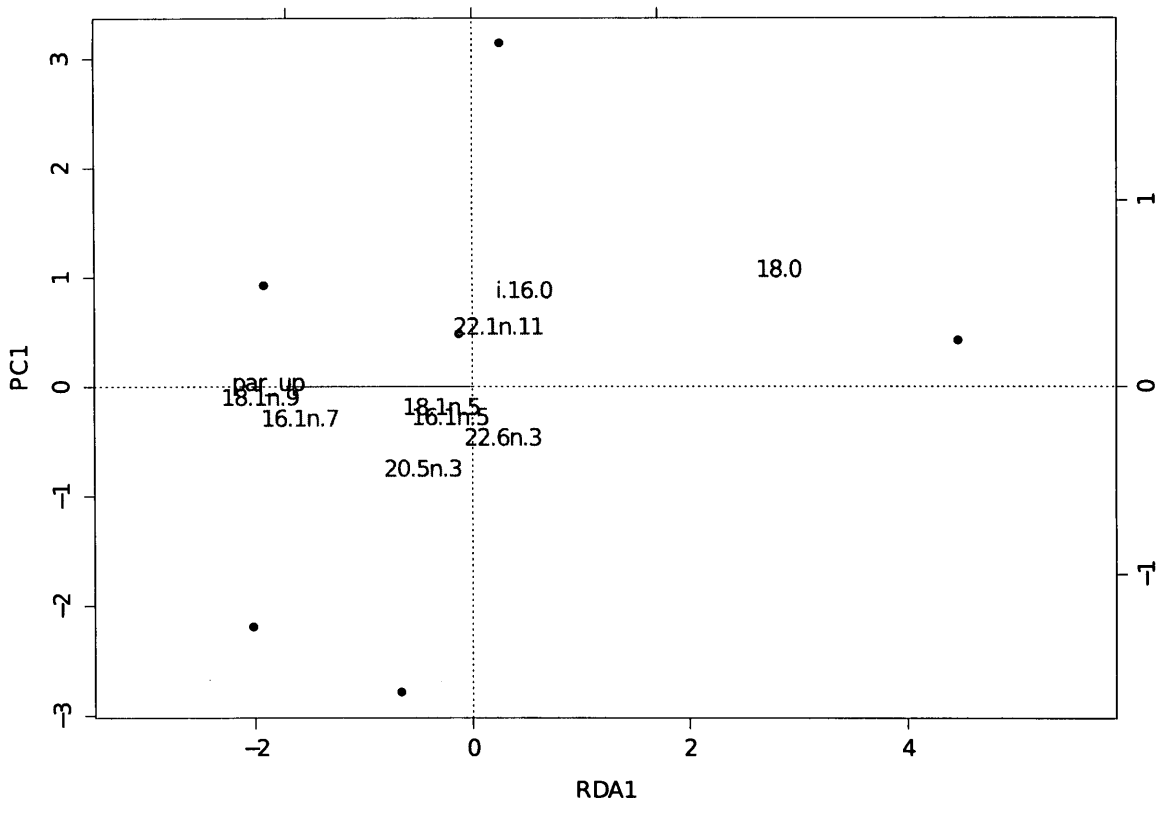

Figure 2.17. Redundancy analysis of chaetognath fatty acids sampled in 2012. The variance in chaetognath fatty acids accounted by the first RDA axis is 39.6%.

2.3.13 Clione limacina

Similar to *S. elegans*, SAFA and MUFA levels in *C. limacina* were similar with PUFAs making up the remaining 24.5%. The most abundant individual fatty acid was 16:0, with diatom biomarkers 16:1n-7 and 20:5n-3 being second most. Dinflagellate markers were also present, although in lower amounts (Table 2.3). Lastly, *C. limacina* samples had significant amounts of i-16:0 and 17:1, fatty acids that were rare in all of the other sample types. There was a significant influence on fatty acids by inflow, with i-16:0 being positively linked to sampling sites closer to Barrow Strait (R^2 = 0.15, AIC = 21.0, step = 9999, p = 0.033) (Fig. 2.18).

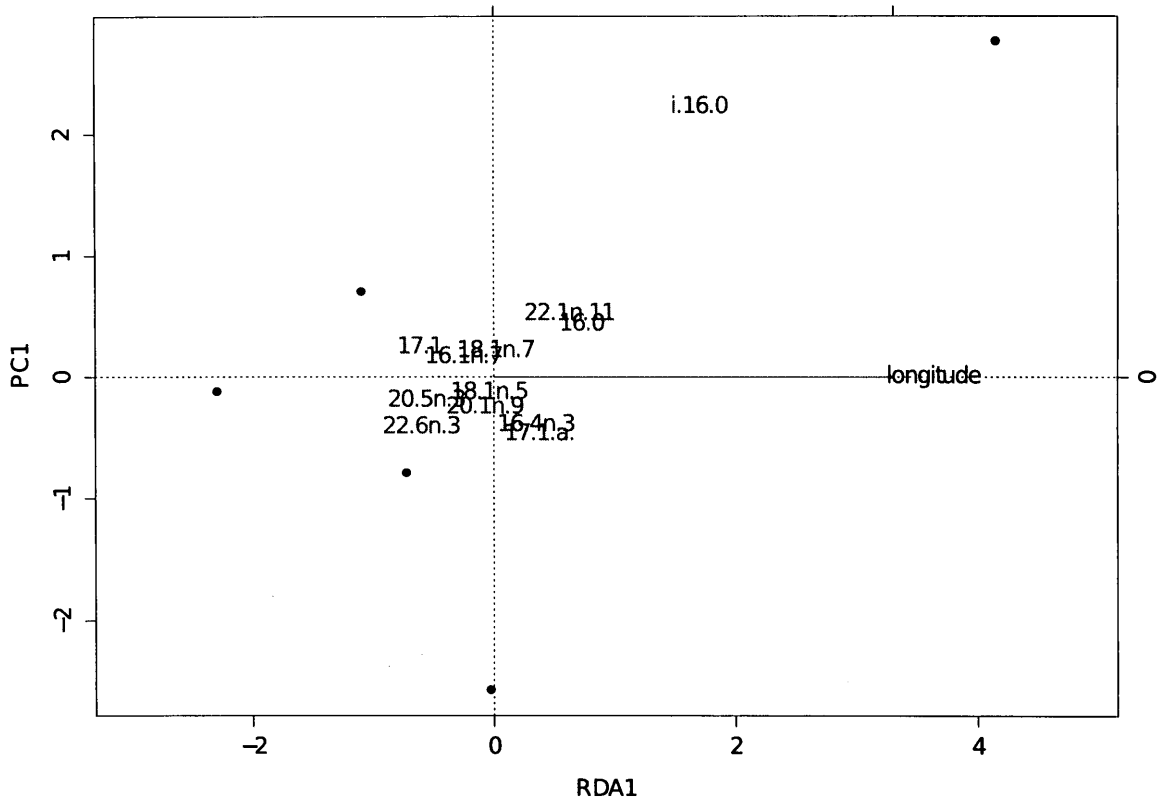

Figure 2.18. Redundancy analysis of pteropod *C. limacina* fatty acids sampled in 2012. The variance in fatty acids accounted by the first RDA axis is 37.8%.

2.4 Discussion

Ice algae communities function as the initial nutritional basis of Arctic marine food webs, and thus influence the nature of energy transfer throughout the whole system (Michel et al., 1996; Leu et al., 2011). Contrary to my initial hypothesis, no spatial patterns were observable in ice algae fatty acids. However, I did find that zooplankton rely heavily on ice algae (Fig. 2.4), and I identified broad scale spatial influences on zooplankton fatty acid levels (Fig. 2.9, 2.11, 2.12, 2.15). Therefore, it is possible that spatial patterns do exist in ice algae, but on a larger scale than my study examined. In addition to spatial patterns, my data indicate that some trophic levels exhibit day–to–day responses in their fatty acid profiles to environmental changes (Fig. 2.12). Furthermore, this study found that environmental effects predictably varied with plankton size and functional group. My results demonstrate how snow and sea ice conditions can affect the nutritional content of marine plankton and contribute to an improved understanding of the potential effects of climate change on Arctic food web dynamics.

2.4.1 Trophic interactions

My analysis indicated that, based on the similarity of their fatty acid compositions, ice algae is an important food source for 153 μm, 250 μm, and 500 μm size categories as well as for ice amphipods and *Calanus* (Fig. 2.2, 2.4). Carnivory increased with size in the mixed species sample types based on 18:1n-9, an indicator of carnivory in zooplankton (Fig. 2.2) (Falk-Petersen et al., 1990). This is most likely due to larger omnivorous copepods being more able to prey on smaller copepods.

Values for trophic levels calculated by the two-source food web model (Eq. 2) were unusable. While the fractionation of $\delta^{13}C$ is small (0-1‰), higher trophic levels will still be enriched relative to the primary producers forming the base of the food web. The $\delta^{13}C$ values I found were slightly higher than other studies in regards to zooplankton (~1‰), however $\delta^{13}C$ values in primary producers were much higher (Forest et al., 2011; Søreide et al., 2013). Forest et al. (2011) reported ice algae from the southwestern part of the Canadian Arctic Archipelago had maximums of ca. -19 ‰ and pelagic particulate organic matter ca. -26 ‰. In contrast, my values were -12.8 ‰ for ice algae and -19.0 ‰ for pelagic phytoplankton. Lighter isotopes are stoicheometrically preferred for use in metabolism, but during bloom conditions bulk carbon values for Arctic ecosystems are known to increase dramatically as carbon supply diminishes (Post 2002; Tamelander et al., 2009). My study took place in an area where ice cover lasts until July, and therefore high $\delta^{13}C$ values may be due to the system being carbon limited for a longer time. The $\delta^{13}C$ values of ice algae are also known to be dependent on the makeup of the protist community, and so differences in local species composition may also have an influence (Pineault et al., 2012).

Secondary calculations of trophic levels using a single source equation (Eq. 1) resulted in a trophic structure similar to what has been previously reported for under-ice systems (Falk-Petersen et al., 1987; Søreide et al., 2006). One exception however, was the low level of $\delta^{15}N$ found in *T. libellula.* The trophic level of 2.3 was inconsistent with its known position as a second order consumer, similar to *S. elegans* (Hop et al., 2002). Other studies have noted *T. libellula* is initially

herbivorous at small sizes before transitioning to a more carnivorous diet once they are larger (Tamelander et al., 2006).

2.4.2 Ice algae

PUFA levels of ice algae (30-34%) were relatively high compared to other reports of algal spring fatty acids (Budge et al., 2008; Wold et al., 2011), although levels exceeding 50% are known to occur (Falk-Petersen et al., 1998). PUFAs are at their highest during periods of exponential cell division (Sargent et al., 1985; Parrish et al., 2005); the mid-range levels I found, coupled with very high $\delta^{13}C$ values, may therefore be an indication that ice algae communities in the area were approaching their maximum biomass. Snow and ice cover largely dictated individual ice algae fatty acids levels (Fig. 2.5), similar to the findings of Leu et al. (2010). Increasing light levels cause desaturation of long chain fatty acids in the thylakoid membranes of single celled organisms as chloroplasts are activated for photosynthesis (Klyachko-Gurvich 1999, Mock and Kroon, 2002). Conversely, I found no link with the percentage of light transmittance (downwelling PAR/under ice PAR). Compared with the study by Leu et al. (2010), which investigated the effects of high and low light on fatty acid composition at only three sites, I found that snow cover explained a similar amount of the total variance even when assessed over a large spatial scale. However, this still left 67.7% of the total variance unexplained and did not account for the three distinct groups I found in ice algae fatty acid profiles (Fig. 2.6). This grouping may have several causes including differences in the concentrations of nutrients such as silica (Smith et al., 1993), diatom community species compositions

or even grazing pressures (Falk-Petersen et al., 1998). There was a gradient from high 20:5n-3 to high 16:1n-7, ratios of which have been used as an indicator of growth activity, but the reasons for the dissimilarity between clusters remains unclear (Fig. 2.7).

2.4.3 Coscinodiscus centralis

Pelagic primary producers exhibit a very different life strategy compared to ice algae and so it was no surprise that the factors determining their fatty acid compositions were also different. *C. centralis* fatty acid composition had the highest amounts of PUFA of any size category or species (Table 2.2, 2.3). Fatty acid profiles showed that clusters of stations located in Barrow Strait and McDougall Sound were more similar than stations sampled in Wellington Channel (Fig. 2.8) although none of the environmental variables I measured had significant effects on fatty acid composition. Leu et al., (2010) state that despite being lower quality food than open-water species, the early timing of the ice algae bloom is what makes it such an important resource. *C. centralis* nutritional quality was higher than ice algae, had more consistent broad scale patterns, and was present under ice cover. Despite this, there were no obvious signs that zooplankton were utilizing diatoms as a food source (Fig. 2.4). Increasing reports of phytoplankton blooms under sea ice are challenging the traditional view of the dynamics of Arctic primary production during ice cover (Arrigo et al., 2012). The presence and fatty acid characteristics of *C. centralis* make it a potentially valuable food source for Arctic herbivores in a time when other sources of pelagic production may be limited.

2.4.4 153 μm size category

The 153 μm size category occupied a mid range trophic level between the primary producers and obligate consumers and was much more variable than other consumer trophic levels (Fig. 2.3). Fatty acids were significantly related to snow cover, year and inflow (Fig. 2.9). This wide range of signatures and environmental influence is most likely caused by a mixture of settling aggregations of ice algae that have sloughed off of the ice, and microzooplankton grazing on these aggregations. Based on the similarity to ice algae (Fig. 2.2) and that snow cover had the strongest influence, a large component of the 153 μm size component was most likely ice algae that had sloughed off of the sea ice. The remaining portion of the 153 μm size category was made up of small pelagic consumers, the fatty acids of which were linked to inflow. The year-to-year variation was probably driven by changes in community composition, as higher levels of dinoflagellate (22:6n-3) and copepod (20:1n-9) fatty acid biomarkers were present in 2011 as compared to a stronger diatom signature for 2012 (Table 2.2). The wide range of $\delta^{13}C$ values, all of which were much lower than for ice algae by itself, and the larger proportion of 18-carbon PUFAs relative to ice algae is an indication that a portion of the pelagic dinoflagellate primary producer community was collected in this size category.

2.4.5 250 μm size category

2011

PUFA levels for 250 μm samples were 2% higher in 2011, however it is unclear if this is an effect of the presence of *C. centralis.* Dinoflagellate biomarker

22:6n-3 was linked with the northern inflow, and characteristic diatom fatty acids were consistent with more *C. centralis* coming from the west into Barrow Strait (Fig. 2.11). Chl A was the only other significant environmental driver, but there doesn't appear to be much impact on any specific fatty acid. Higher Chl A levels have been linked to increased zooplankton feeding rates in the past (Campbell et al., 2009).

2012

Once *C. centralis* were removed, the 250 μm category consisted mainly of *Calanus* and *M. longa* copepodites and nauplii, along with low numbers of various other species of zooplankton. This mixture of species is most likely responsible for the slightly higher trophic level of 250 μm compared to the pure *Calanus* samples. PUFA levels were relatively high, and comparable to what is present in ice algae (Table 2.2). While inflow is known to influence zooplankton community compositions (Matsuno et al., 2012) I also found that absolute light levels had a measurable effect on 250 μm fatty acid profiles. To my knowledge, this is the first evidence that day-to-day environmental variations may be able to cause shifts in community level fatty acid dynamics in Arctic ecosystems. Brighter days had a trend towards increased levels of 22:6n-3, a dinoflagellate biomarker (Fig. 2.12). The implications are that dinoflagellates in the water column are light limited, but bright days are sufficient to activate photosynthesis under the sea-ice. The change in 250 μm fatty acid profiles may have several explanations. Dinoflagellates from the genus *Ceratium* were observed in low numbers in 250 μm samples and this increase could be caused by an increase in their populations. Alternatively it could be due to zooplankton grazing on increased numbers of smaller dinoflagellates and the

subsequent concentration of the phytoplankton in their guts. The link between feeding behaviour and increased Chl A would also help to augment the effect of light on zooplankton fatty acid profiles (Campbell et al., 2009). This signal would not be evident in the 500 μm or the *Calanus* samples as juvenile copepodites and nauplii have low levels of lipid reserves compared to the well-established lipid sacs of larger animals, and smaller changes in proportions would be more noticeable (Falk-Petersen et al., 2009). My results suggesting community level fatty acid profiles could react suddenly to environmental stimuli are novel for Arctic systems. Under-ice light environments are expected to change with warming trends (Matsuoka et al., 2009). If Arctic systems are sensitive to day-to-day variation early in the season, large scale, and chronic deviations to light levels could lead to changes in fatty acid dynamics and even food web structures.

2.4.6 500 μm size category

The 500 μm size category was mainly copepods, of which *Calanus spp.*, *M. longa* and *Pseudocalanus acuspes* make up the vast majority in this area (Michel et al., 2006). For the *Calanus* samples, which were isolated from the large bodied 500 μm category, inflow had a significant effect on fatty acid profiles. However, the remaining mixed species composition loses this effect. The 500 μm size category had a higher trophic level than calanoids, and had elevated levels of 18:1n-9, which is another indicator of carnivorous feeding (Graeve et al., 1997) (Fig. 2.13). *Metridia* are known omnivores, and while some debate exists for *Calanus*, most evidence suggests that they are predominantly herbivorous (Falk-Petersen et al., 2009). Like

other omnivores, *M. longa* are active throughout the year (Seuthe et al., 2007) whereas calanoids stop feeding and descend into deep water where they undergo diapause, a period of decreased metabolic activity, during winter months (Falk-Petersen et al., 2009). Fatty acid signatures are known to "blur" in regards to sources as trophic level increases (Dalsgaard et al., 2003), and neither 500 μm, nor any of my other sample types with elevated $\delta^{15}N$ values were related to inflow.

Three groups could be distinguished within the 500 μm category based on fatty acid profiles (Fig. 2.13). The first cluster is the most tightly grouped and characterized by high amounts of diatom biomarkers 16:1n-7 and 20:5n-3. Interestingly, 14:1n-9 was found in abundances >1% in only 250μm and 500 μm size categories. This fatty acid is not usually found above trace amounts in *Calanus*, *Metridia*, or *Psuedocalanus* spp. and its origins in the 500 μm samples are unclear (Falk-Petersen et al., 1987, Peters et al., 2006). The relative rarity of 14:1n-9 in other groups may make it useful as a potential biomarker if its source can be identified. The second cluster is strongly linked with *Calanus* biomarkers 20:1n-9 and 22:1n-9. The last cluster was situated centrally and is most likely caused by an intermediate mixture of herbivorous and omnivorous species. Unlike 153 μm, which also had internal groupings characterized by different fatty acid biomarkers, there is no significant year-to-year variation, indicating that species abundances in the 500 μm size range are relatively stable between years.

2.4.7 Calanus

Calanus samples had the third lowest trophic level of all zooplankton sample types, supporting other findings that large calanoids are primarily herbivorous (Falk-Petersen et al., 2009). Because *Calanus* react rapidly to changing levels of primary production and feed at high rates early in the spring, I would expect fatty acids in *Calanus* to reflect locally available food sources despite their large lipid stores (Campbell et al., 2009; Forest et al., 2011). The majority of fatty acids in *Calanus* samples were derived from diatom sources, with high levels of 16:1n-7 and 20:5n-3. Despite *C. centralis* having higher relative PUFA content than ice algae, the dissimilarity between pelagic phytoplankton and *Calanus* fatty acid compositions imply that copepods are not heavily grazing on these large diatoms (Fig. 2.4). The $\delta^{13}C$ ‰ of *Calanus* samples were more depleted than either of the primary producers sampled, which may indicate that there is a significant pelagic component to their diets (Hobson et al., 1995). As Arctic zooplankton subsist on a combination of pelagic and ice associated primary production during the course of the year, $\delta^{13}C$ values are never as enriched as late bloom ice algae (Søreide et al., 2013). However, I found higher $\delta^{13}C$ values than other studies of calanoids under sea-ice, which is likely caused by the longer duration of ice cover in the High Arctic, and therefore, an increased reliance on ice algae (Forest et al., 2011, Wold et al., 2011).

Inflow had a significant effect on calanoid fatty acid compositions, indicating that two potential source populations of copepods exist. More importantly however, broad scale year-to-year variation in ice and snow conditions appears to have an important influence in the fatty acid composition of large copepods (Fig. 2.15).

Higher PUFA levels in copepods for 2011 correspond with higher PUFA levels in ice algae due to thicker snow cover (Fig. 2.14). As far as I am aware, this is the first evidence of inter-annual variation in environmental conditions directly influencing the nutritional quality of copepods. The amplified effect of snow and ice (mediated by primary producers) on the nutritive quality of a keystone zooplankton species means changes in ice conditions will not only affect habitat, but the amount of high quality food available to all animals in Arctic marine food webs.

2.4.8 Ice associated amphipod (Gammarus setosus)

The range of *Gammarus setosus* extends throughout the Arctic, and although they are more commonly found benthically (Arndt and Swadling, 2006), they were collected near the ice/water interface. The fatty acid 18:1n-9 was linked with ice thickness (Fig. 2.16). High levels of this fatty acid have been associated with increased amounts of carnivory (Falk-Petersen et al., 2000) or reliance on detritus (Dalsgaard et al., 2003). The diatom biomarker 16:1n-7 was positively linked with the non-significant variable POC. Because of the low trophic position indicated by stable isotopes (Fig. 2.3) and the close similarity to the fatty acid profiles of 153 μm size class (Fig. 2.2, Fig. 2.4), it is likely that individuals residing in areas of thicker ice cover feed more on decaying matter. In shallow areas with seasonal ice that may not be accessible to obligate ice species such as *Gammarus wiltzikii*, benthic species may fill an important trophic niche.

2.4.9 Gelatinous zooplankton (Hydrozoa: Euphysa aurata, Eumedusae birulai, Botrynema brucei, Aglantha digitale)

The sum of individual fatty acids in gelatinous zooplankton that were present in amounts >1% was 90.9%, a lower total than any other category and is indicative of a diverse diet. Based on the ratio of 16:1n-7/16:0, dinoflagellates were the dominant origin of jellyfish fatty acids (Budge and Parrish, 1998). Only a small proportion of copepod fatty acid biomarkers were present, probably because the hydrozoans sampled were too small to effectively prey on calanoids. As there are over 200 identified species of cnidarians in the Arctic Ocean, I expected to see groupings of fatty acid profiles as a result of differing species abundances, similar to the 500 μm size category (Fig. 2.13). However, as no clustering was observed, all of the species collected appear to occupy the same trophic niche at small body sizes. Gelatinous zooplankton were the only sample group that were not influenced by any of the environmental variables nor had any other discernible patterns in their fatty acid profiles. This homogeneous spatial pattern is probably caused by gelatinous zooplankton's carnivorous, non-selective method of feeding.

2.4.10 Themisto libellula

The levels of PUFA I found in *Themisto* samples were much lower than has been reported for *T. libellula* in other regions of the Arctic (Auel et al., 2002). In particular, 16:0 levels were nearly double to previous reports while 20:5n-3 levels were approximately half compared to the Fram Strait (Auel et al., 2002). Levels of 18:1n-9 were higher than of the Central Arctic Basin, but levels of *Calanus*

biomarkers 20:1n-9 and 22:1n-11, were lower (Auel et al., 2002). This may be due to smaller individuals of *T. libellula* relying more on detritus and only preying on copepods once reaching a sufficient body size (Tamelander et al., 2006). The lack of influence by current is most likely due to the small number of samples, but may also relate to the year-round activity of *T. libellula* and their omnivorous diet (Kraft et al., 2013).

2.4.11 Chaetognath (Sagitta elegans)

Despite seemingly low levels of characteristic calanoid fatty acids 20:1n-9 and 22:1n-11 in chaetognath fatty acid profiles, *S. elegans* is known to prey primarily on copepods and nauplii (Øresland 1987). This is due to *S. elegans* only maintaining modest amounts of triacylglycerols for energy storage (Falk-Petersen et al., 1987). Chaetognaths occupied the highest trophic level of all sample types (Table 2.1), and while 18:1n-9 levels indicate *S. elegans* were carnivorous, they were nearly 10% lower than reported by Falk-Petersen et al. (1987).

S. elegans fatty acid profiles were significantly affected by surface reflectance, although the internal variation and small sample size made it difficult to see any patterns in the data. A fatty acid indicator of carnivory, 18:1n-9 was positively linked to upwelling PAR (Fig. 2.17). This may relate more to overall light levels as Falkenhaug (1991) found chaetognath feeding activity increased with brightness. Alternatively, this influence could be due to the physical properties ice and snow. The albedo (reflectance) of melting snow decreases (Meinander et al., 2010), potentially chaetognath prey choice could be influenced by melt conditions.

Chaetognaths have been estimated to consume ~3% of secondary production and it is unclear how changes in ice and snow cover might alter these predation dynamics (Falkenhaug, 1991).

2.4.12 Clione limacina

C. limacina are known to produce many of their fatty acids *de novo*, and branched or odd numbered fatty acids are common (Kattner et al., 1998). Compared to *C. limacina* that were sampled in June to August, I found 9.3%-13.8% less 22:6n-3 in early spring (Kattner et al., 1998; Böer et al., 2006). This is most likely a reflection of the low abundance of dinoflagellates in the water column during periods of ice cover, as diatom derived 20:5n-3 levels were similar between spring and summer (Böer et al., 2006). A branched chain fatty acid, i-16:0, made up 4.4% of total fatty acids and was only reported in trace amounts in other studies (Kattner et al., 1998; Böer et al., 2006). Interestingly, *C. limacina* was the only predatory sample type to be significantly affected by inflow (Fig. 2.18). This is likely because of *C. limacina's* extremely specialized diet of only *Limacina helicina* (Kattner et al., 1998). A single dietary source would reduce the "blur" of fatty acid transfer in higher trophic levels and better retain environmental signals from *C. limacina's* prey fatty acid profiles.

2.5 Conclusion

Ice algae fatty acid profiles were primarily driven by ice and snow thickness. The importance of light on the nutritional value of ice-associated producers was confirmed but concluded that small-scale studies may underestimate the natural variability of fatty acids in sea ice communities. Lower snow depth in 2012 yielded lower PUFAs in ice algae, which caused significantly lower PUFA levels in *Calanus* compared to 2011. Zooplankton herbivore fatty acid profiles were largely dictated by inflow, implying that while not visible on the scale of my study, broad scale patterns in ice algae fatty acids exist. Zooplankton fatty acid profiles showed decreasing sensitivity to environmental variables with increased trophic level. By demonstrating that broad scale environmental variations have a significant effect on the food quality of a keystone species, I am able to provide not only a baseline, but show that changes in ice and snow conditions will have a large impact on how these systems function.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council (NSERC), Fisheries and Oceans Canada, Natural Resources Canada (Program of Energy Research and Development and Polar Continental Shelf Program), Environment Canada, Kenneth Molson Foundation, Churchill Northern Studies Centre, Northern Scientific Training Program (NSTP) and the Faculty of Graduate Studies at York University. Thanks to Anne Timmins, Carrie Greene, July Hall, LeeAnn Fishback, Carley Basler, Brandon Laforest, Nathalie Morata, and Duane Jordan for help in the field and lab.

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Chapter 3.

Green giants in the frozen food section: the lipid composition and origins of a large pelagic diatom found under sea ice.

Abstract

Primary production in Arctic seas has traditionally been viewed as having two temporally and spatially distinct components, ice algal communities which grow within and on the bottom of sea ice, and phytoplankton blooms that occur in open water after sea-ice breakup. There is growing evidence however, that under-ice blooms of pelagic phytoplankton may be a regular occurrence. In 2011 I found substantial numbers of a large centric diatom, *Coscinodiscus centralis*, under the sea ice near Cornwallis Island, Nunavut. During the springs of 2011 and 2012, I documented the abundance patterns of *C. centralis* and investigated their fatty acid and stable isotope profiles to determine where they originated, what were the growth conditions, and what influence this alternate source of primary production might have on local food webs. Higher numbers of pelagic diatoms were observed in Barrow Strait than in more northerly channels. Based on their fatty acid profiles, *C. centralis* represent a significantly different, and potentially more nutritious food source for local herbivores than ice algae. The pelagic diatoms had significantly higher levels of polyunsaturated fatty acids (PUFA) (mean ± SD: 50.3 ± 8.9%) compared to ice-associated producers (30.6 ± 10.3%). Spatial patterns of fatty acid profiles and stable isotopes indicated there were two source populations for *C. centralis*: a western origin with low light conditions and high nutrients, and a northern origin with lower nutrient levels but brighter conditions. In a captive zooplankton feeding experiment, no changes in copepod fatty acids were observed and chlorophyll levels of *C. centralis* actually increased in the presence of

zooplankton. I conclude that *C. centralis* is able to initiate growth well before the open-water bloom and can do so in a variety of nutrient and light conditions.

3.1 Introduction

Arctic marine food webs are dependent on the production and transfer of lipids to maintain their structure and biodiversity. These food webs are classically viewed as being supported by two spatially and temporally distinct types of primary production: ice associated algae and pelagic open water algae (Gosselin et al., 1997; Carmack and Wassmann 2006; Brown et al., 2010). It has generally been accepted that under light-limiting ice cover, the water column is unable to support significant numbers of pelagic autotrophs and therefore ice algae are the first and only available source of food for zooplankton grazers in the early spring (Søreide et al., 2010).

Specifically, ice algae were viewed as the first significant pulse of polyunsaturated fatty acids (PUFAs), upon which reproducing zooplankton are reliant (Michel et al., 1996; Søreide et al., 2010). Higher PUFA levels increase zooplankton egg production and the subsequent viability of the eggs (Arendt *et al.* 2005; Søreide *et al.* 2010). PUFAs are important in cold environments for maintaining cell membrane fluidity and associated enzyme functions (Sargent et al. 1993). There is poor genetic control of PUFA levels in cell membrane synthesis so dietary deficiencies quickly lead to general health problems in animals (Sargent et al. 1993). Copepods take advantage of early availability of high quality food by foraging at much higher rates in the spring compared to summer, despite higher levels of primary production later on in the year (Campbell et al., 2009). The timing of peak

ice algae PUFA availability is therefore important to secondary consumers and there is concern that early melting of sea ice could cause a mismatch between food availability and copepod spring hatch (Søreide et al., 2010). Subsequent population declines in copepods would have immediate and far-reaching effects on higher trophic levels (Moline et al., 2008).

Contrary to the classical view of a water column devoid of producers, there is mounting evidence that pelagic blooms occur under sea ice throughout the Arctic, sometimes hundreds of kilometers from open water (Strass and Nöthig, 1996; Mundy et al., 2009; Arrigo et al., 2012). Arrigo et al. (2012) found that failing to account for this pelagic component meant underestimating the net annual primary production of the Chukchi sea continental shelf by an order of magnitude. The intense blooms of *Chaetocerus* and *Thalassiosira* that follow breakup are thought to require several processes, including release from light limitation and the stratification of the water column that concentrates nutrients in the upper euphotic zone (Strass and Nöthig, 1996; Michel et al., 2006; Leu et al., 2010). Blooms occurring under sea ice would not follow similar dynamics to blooms commonly observed in the marginal ice zone, and could potentially provide a buffer for food webs during early ice melt.

One species commonly found in early phytoplankton assemblages is *Coscinodiscus centralis*, a large centric diatom averaging between 180-200 μm in diameter with individuals as large as 300 μm (Hasle and Lange 1992). The general cell shape is tympaniform with a gentle central depression in the valve (Hasle and

Lange, 1992; Hasle and Syvertsen, 1997). They are free-living and contain very high amounts of chlorophyll per cell (Atkins and Park, 1951).

C. centralis is a cosmopolitan species, found in every marine biogeographic region (Hasle and Lange, 1992). Reports of its presence in the Arctic date back to the late 1800s and it is a dominant species early in the spring bloom of the North Water Polynya (Cleve 1883; Lovejoy et al., 2002). Although *C. centralis* has been found under the ice at the North Pole in the late summer and early fall, most reports are from ice-free areas or marginal ice zones (Budge et al., 2008; Katsuki et al., 2009).

C. centralis peak abundances generally occur in high salinity water (>32.5‰), but they have been found to tolerate levels as fresh as 24‰ (Hasle and Lange, 1992). *Coscinodiscus* are often situated deeper in the water column at the base of the euphotic zone, and there is some evidence that suggests they may be adapted to very low light levels (Kemp et al., 2000). Large *Coscinodiscus spp.* may be able to alter their buoyancy to sink below established haloclines, allowing them to take advantage of deeper, nutrient-rich water (Kemp et al., 2000). Despite being present in all of the world's oceans, *C. centralis* is poorly studied and little is known about its growth requirements or general population dynamics (Hasle and Lange, 1992; Kemp et al., 2000).

During zooplankton sampling in the Canadian Arctic, I identified *C. centralis* in the water column under complete ice cover. The aim of this study was to investigate the presence of *C. centralis* under the sea ice and its potential role in under-ice food webs. I believe this is the first report of growth being initiated under sea ice (Hasle and Lange, 1992; Ratkova and Wassmann, 2005). I describe the

environmental conditions associated with *C. centralis* and examine its stable isotope ($\delta^{13}C$ and $\delta^{15}N$) and fatty acid composition.

The combination of stable isotope and fatty acid analysis can provide potentially powerful insights into trophic relationships. The ratio of δ^{13} to δ^{12} carbon isotopes is modified by a very small amount during trophic transfers and can therefore be used to determine the importance of isotopically distinct primary producers to higher trophic levels (Post, 2002). The amount of $\delta^{15}N$ can initially be variable in producers, depending on growth conditions, but is enriched by a constant amount with each successive trophic level (Tamelander et al., 2009). Fatty acids are the primary components of most lipids. Biochemical limitations on the synthesis of fatty acids in animals result in predictable, quantitative relationships between the fatty acids present in a consumer and those present in its constituent foods (Dalsgaard et al., 2003). Together, these analyses can provide insights into ecosystem structure and functioning (Budge et al., 2008) and thus help reveal the origin and importance of under-ice blooms of *C. centralis* in Arctic marine food webs.

3.2 Methods

3.2.1 Study area

Sampling was done in 2011 and 2012 on landfast sea ice near Cornwallis Island, Nunavut (Fig. 3.1). Ice formation begins in mid- to late September and lasts until breakup around the end of June, although breakup can be delayed until the beginning of August (Brown and Cote, 1992). The majority of sea ice in the area is predominately first year, although some patches of multiyear ice are present. Ice

thickness reaches an average maximum of 2 m in mid-April (Welch and Bergmann, 1989; Brown and Cote, 1992). Snow depth can reach a maximum average thickness of 22 cm although the patterns of accumulation are significantly affected by prevailing winds (Brown and Cote, 1992).

The main ocean current direction is from west to east and averages 6.16 cm/s in the spring with speeds increasing up to 60 cm/s during the tidal flux (Prinsenberg and Bennett, 1987; Cota et al., 1987). The three main inflows into Barrow Strait are from Viscount Melville Sound in the west, Penny Strait to the north, which flows around both sides of Cornwallis Island, and from Peel Sound in the south (Prinsenberg and Bennett, 1987). Most of the inflow water is Arctic surface water, although the ratio of Pacific/Atlantic water may have a profound effect on primary production (Prinsenberg and Bennett, 1987; Michel et al. 2006). With a sill of approximately 125 m, Barrow strait is the narrowest and shallowest point of the Northwest Passage (Michel et al., 2006).

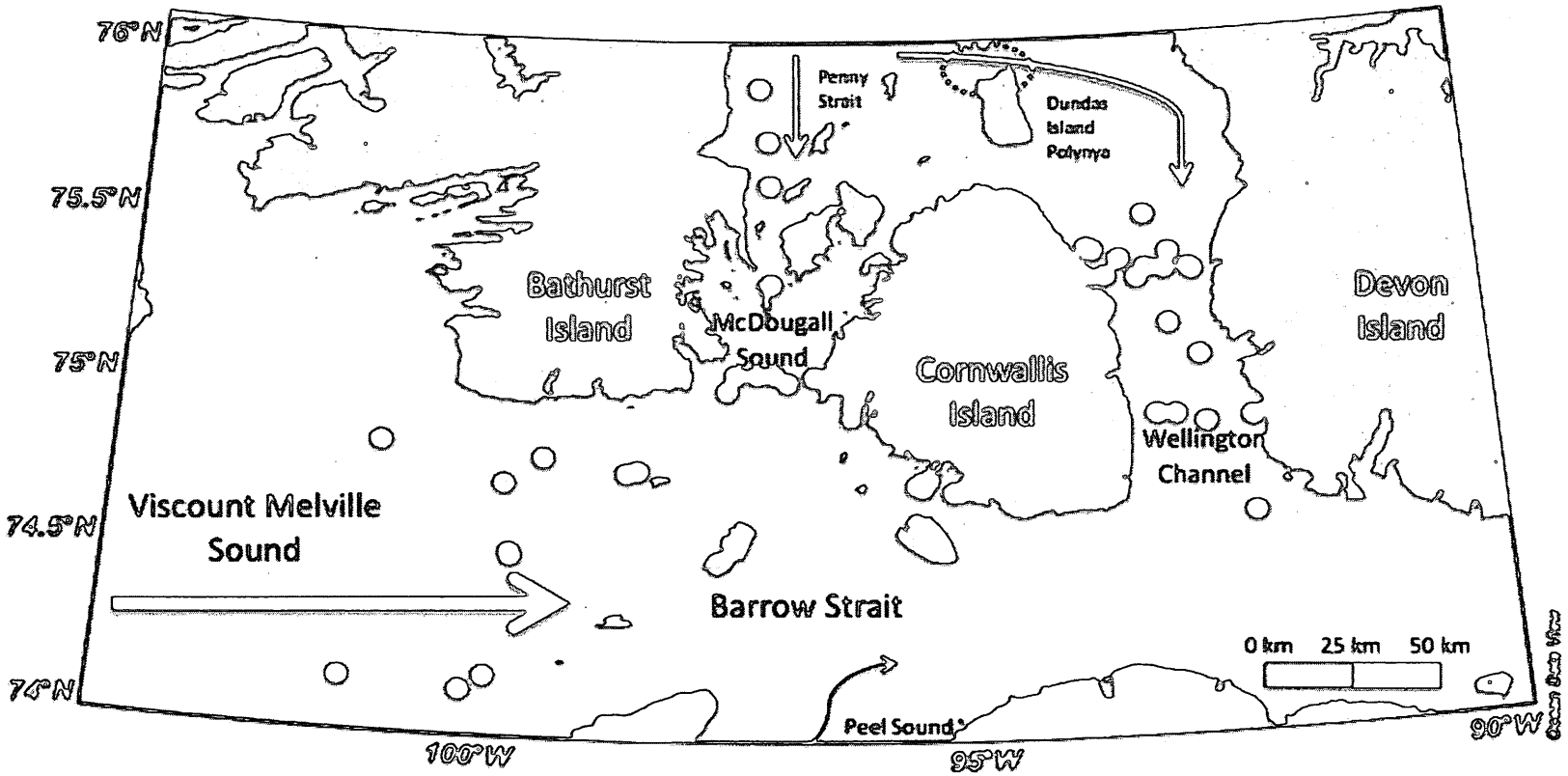

Figure 3.1: Stations sampled over 2011-2012 field seasons near Cornwallis Island, NU.

3.2.2 Field observations

Ice thickness, snow depth, and freeboard (the distance from the surface of the water to the top of the ice), were recorded at each station. Measurements of photosynthetically active radiation (PAR, 400 – 700 nm) were taken under the sea ice using a LICOR quantum sensor mounted on a hinged aluminum arm. All PAR readings were conducted under undisturbed snow cover. Water column salinity and temperature measurements were collected in 2011 using a Sea-bird conductivity-temperature-depth (CTD) probe.

3.2.3 Sample collection

A total of 46 stations were sampled May 1-18 in 2011 and 2012 (Fig. 3.1). No isolated samples of *C. discus* could be obtained for fatty acid analysis in 2011 but qualitative estimates of abundance on a 0-3 scale were made. A value of 3 meant that if shaken in a scintillation vial, it was impossible to see through, 2 was that the vial darkens when shaken but the other side is clearly visible, 1 meant that few individuals present, with 0 indicating no diatoms were present.

In 2012, phytoplankton and ice algae samples were collected at 24 separate stations. A manual ice corer (Mark II coring system, 9 cm internal diameter, Kovacs Enterprises) was used to collect the ice algae. The bottom 3 cm of each core was cut off and melted overnight in 500 ml of Millipore micropore (0.2 μm) filtered seawater (FSW) to avoid osmotic stress (Garrison and Buck, 1986). Samples were then measured for total volume, filtered onto pre-combusted (500°C for 4 hours) glass fiber filters (GF/F) and treated with 10 ml boiling FSW to deactivate lipolytic enzymes (Budge and Parrish 1999). Filters were then stored in cryovials at -80°C.

Samples of *C. centralis* were collected using a zooplankton net (25 cm mouth diameter, mesh size 153 μm) fitted with a flowmeter (General Oceanics model 2030RC, one way clutch). Three vertical hauls were taken from each station from 5 m above bottom to a maximum of 150 m. Hauls were pooled and separated by size using 500 μm and 250 μm sieves. *C. centralis* were isolated from the 250 μm size fraction using 3 rounds of settlement and then by hand-picking any remaining zooplankton from the sample using a pasteur pipette and a dissecting microscope (Fig. 3.2). The purified samples were then filtered onto pre-combusted GF/F filters and immediately placed into chloroform (Omnisolv grade, VWR) and stored at -20°C.

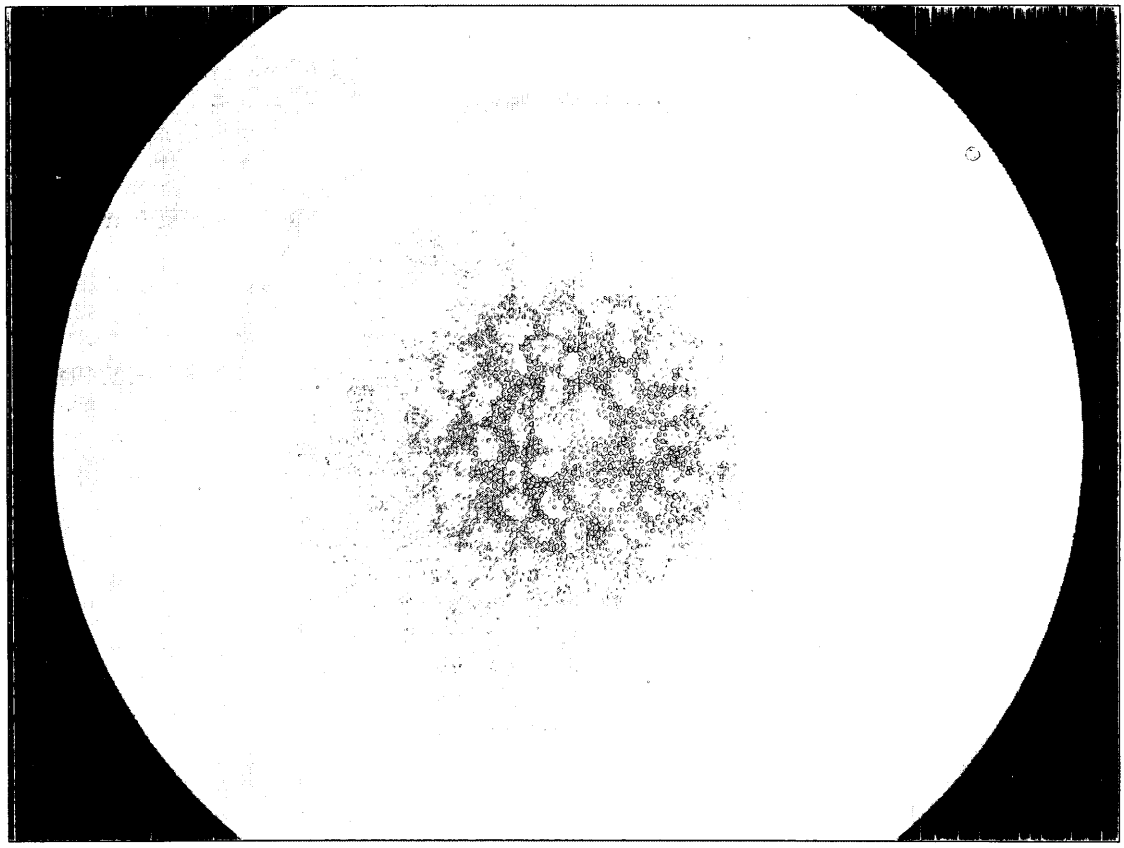

Figure 3.2. Photo of *Coscinodiscus centralis* collected in 2012. Cell diameter is >250 μm and the large green patches are chloroplasts. (Photo credit: M. Poulin, Canadian Museum of Nature).

3.2.4 Feeding Experiment

To test whether *C. centralis* are eaten by copepods, I collected zooplankton from Resolute Passage on May 14th 2011. Calanoid copepods greater than 500 µm in length were retained from pooled vertical hauls taken from 30 m depth to the surface. The copepods were placed into bottles containing 450 ml of FSW, placed into a seawater bath at 0°C with light levels of 2.5-3.5 µE $m^{-2}s^{-1}$ and starved for 24 hours. The bottles were randomized into two treatments, and fed either ice algae or *C. centralis.* One bottle of ice diatoms and one of *C. centralis* were incubated with no copepods to serve as controls. The food added consisted of 4 cores, 9 cm in length, diluted into 2 L of FSW (2750 ml total) for ice algae, and a 500 ml solution of *C. centralis* that was isolated from the vertical hauls. Duplicate 150 ml subsamples of the ice algae solution were preserved for FA analysis while only a single 150 ml sample was taken from the *C. centralis* solution. Additionally two subsamples were taken for determination of chlorophyll A (Chl A) levels (100 ml for ice algae and 50 ml for *C. centralis*) as well as 125 ml from each for taxonomic verification and cell counts. On May 19th 2011 each bottle was sieved through 500 µm mesh and copepods were retained for FA analysis. The filtrate was then collected for Chl A analysis. Samples were prepared with 90% acetone and incubated in darkness at 4°C over night. 10% HCl was used for acidification before being analyzed in a fluorometer (Turner Designs 10AU).

3.2.5 Fatty acid analysis

Lipids were extracted using 2 different methods. *C. centralis* lipids were extracted using a modified Folch et al. (1957) procedure by sonicating the filters for 5 minutes in 10.5 ml of 2:1 chloroform and methanol solution; 2.6 ml of 0.7% saltwater was then added before centrifuging and extracting the bottom layer. The filter, top layer, and all remaining tissues were kept for stable isotope analysis. Lipid extracts were dried over anhydrous sodium sulfate before being transesterified to produce fatty acid methyl esters (FAMEs) using sulfuric acid (Hilditch et al. 1964) as a catalyst.

For ice algae lipids, an in situ BCl_3 catalyzed transesterification method similar to Park and Goins (1994) was used. This was chosen instead of the 2-step process due to concerns that the limited amounts we were able to collect may only contain trace amounts of lipids. This method has been found to yield equivalent results for algae when compared to the combination of Folch and Hilditch methods and is more efficient for very small-quantity samples (Hall 2012). 78 individual FAMES were identified using gas chromatography and quantified using 5-α cholestane as an internal standard. In cases where the identification of a peak was in doubt, a combination gas chromatograph/mass spectrometer was used to cross-verify the identity of the fatty acid.

3.2.6 Stable isotope analysis

C. centralis tissue was removed after lipid extraction in 2:1 chloroform and methanol (Søreide et al., 2006) and freeze-dried at -40°C for 48 hours before $\delta^{13}C$ and $\delta^{15}N$ stable isotope analysis at the Great Lakes Institute for Environmental

Research (University of Windsor, Windsor Ontario). Isotopic signatures are expressed as a deviation ($\delta X = [(\mathbf{R}_{sample} / \mathbf{R}_{standard}) - 1] \times 1{,}000$) from international standards calibrated against Vienna-PeeDee Belemnite (VPDB), for carbon, and atmospheric N_2 (AIR) for nitrogen. Ice algae stable isotopes were prepared and analyzed at the Freshwater Institute (Department of Fisheries and Oceans) in Winnipeg, Manitoba.

3.2.7 Statistical analysis

Statistical analysis was done using R statistical software (R Core Team, 2012). Wilcoxon sign rank tests were used to compare untransformed fatty acid proportions of ice algae and diatoms. Fatty acid proportional data were arcsin square-root transformed before using multivariate analyses (Sokal and Rohlf, 1995). A two-sample Hotellings T test was used to compare multivariate means of ice algae and *C. centralis* based on 16 fatty acids (R package: rrcov (Valentin and Filzmoser, 2009)). These 16 fatty acids were chosen based on known trophic markers in addition to the most common present in samples (>1%). Principal component analysis (PCA) was used to assess the importance of the 16 fatty acids on the separation of ice algae and *C. centralis* (R package: vegan (Oksanen et al., 2013)). Significance of PCA axes were calculated based on Equiprobability = (1/#var)* 100 = 1/16*100=6.25% (Helaouët and Beaugrand 2007). Internal groupings within *Coscinodiscus* samples were found using hierarchical clustering analysis and hierarchical clustering analysis based on principal components (HCPC) (R package: FactoMineR (Husson et al., 2013)). Hierarchical clustering was applied to the principal component axes, using the PCA as an initial preprocessing step to de-noise

the data (Husson *et al.*, 2010). The number of clusters (Q) was chosen to maximize between-group variance while minimizing within-group variance: $\Delta Q/\Delta(Q+1)$ (Husson *et al.*, 2010). A two-sample t-test assuming equal variance (variances were tested using an F test) was used to measure the difference in $\delta^{15}N$ between clusters. Regressions were used to assess relationships between stable isotopes, environmental variables and lipid abundances. Interpolation of lipid masses between stations was done using DIVA (Data-Interpolating Variational Analysis) gridding in the program Ocean Data View 4 (Schlitzer, 2013). Unless otherwise noted, all fatty acids are reported as mean ± one standard deviation.

3.3 Results

3.3.1 Field observations

Mean ice thickness in 2011 was 133.0 ± 19.5 cm (range = 167.6 to 96.2 cm). Near the end of the expedition there were signs of open water east of Cornwallis Island and several polynyas opened up in Wellington Channel. In 2012, ice was thicker, (mean 163 ± 35 cm; range = 235 to 121.4 cm) and we saw no signs of melting or open water. Mean snow depth was 8.3 ± 4.3 cm (range 20.6 to 1.1 cm) in 2011 and 5.9 ± 2.9 cm in 2012 (range 13.9 to 1.7 cm). Snow values cannot be treated as representative of average regional values as specific depths were chosen at sampling locations. Underwater PAR was lower and less variable in 2011 (mean = 4.90 ± 3.03 μE $m^{-2}s^{-1}$) than 2012 (mean =9.36 ± 8.76 μE $m^{-2}s^{-1}$), but both were high enough to support photosynthesis (2–9 μE $m^{-2}s^{-1}$; Horner and Schrader, 1982). CTD casts at each station showed that temperature and salinity were usually homogeneous throughout the water column (Fig 3.3). Three stations in the

southwest portion of our sampling range showed fresher values for surface water and an increase in salinity and temperature around 125 m.

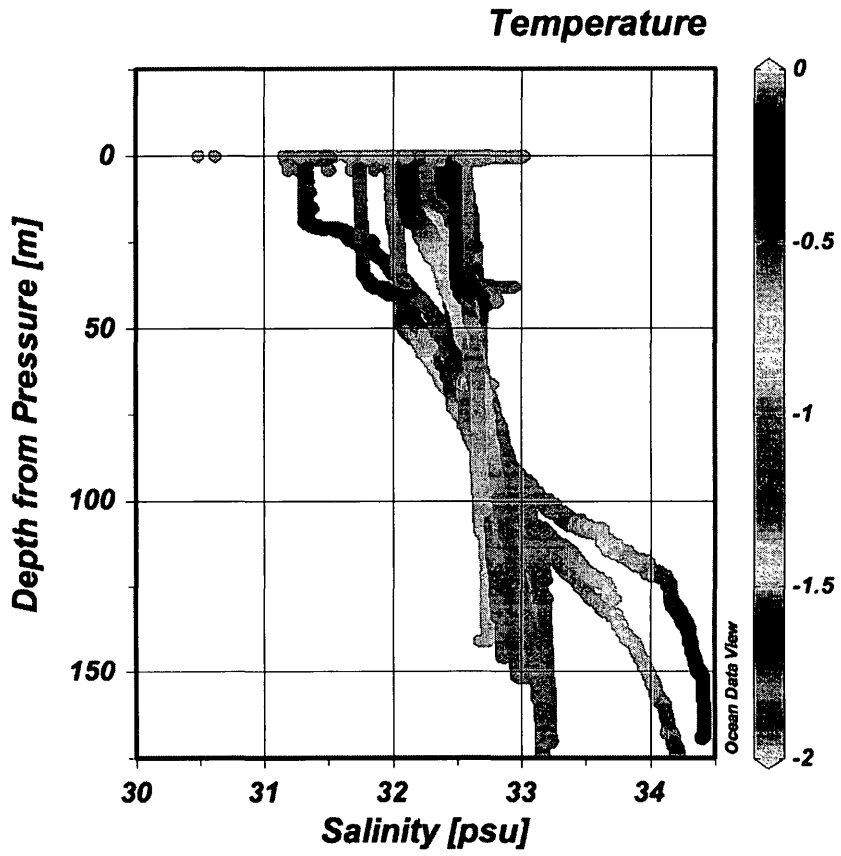

Figure 3.3: CTD profiles of stations from 2011.

3.3.2 Comparisons of stable isotopes and fatty acids in C. centralis and ice algae

Stable isotopes

Pooled *C. centralis* isotopic signatures showed a large range for $\delta^{15}N$ (2.84 to 7.42‰, mean 4.95‰) and were depleted in $\delta^{13}C$ (mean= -19.07 ± 0.67‰) relative to ice-associated values (mean = -12.77 ± 2.57‰). Ice algae samples were generally more enriched and less variable in $\delta^{15}N$ (6.30 ± 0.59‰). $\delta^{13}C$ values had a greater range in ice algae (-17.10 to -8.34‰) compared to *C. centralis* (-20.89 to -18.27‰).

Fatty acids

Ice algae and *C. centralis* had significantly different fatty acid compositions (Hotelling's T-test, $p < 0.001$). In *C. centralis,* 13 of the 78 identified FA were present in amounts ≥ 1%, comprising 91.56% of the total FA. In ice algae 11 FA were present at ≥ 1% for a total of 91.57%. Fatty acids in both ice algae and *C. centralis* were dominated by 16:0, 16:1n-7 and 20:5n-3. Samples from the pelagic diatoms had significantly higher levels of both 20:5n-3 (Wilcoxon rank sum, $p < 0.001$) and total PUFA (Wilcoxon rank sum, $p<0.001$) than ice associated communities (Table 3.1). Ice algae were richer in monounsaturated fatty acids (MUFAs; 41.4%) and both had similar levels of saturated fatty acids (SAFAs; Table 3.1). While *C. centralis* had lower quantities of both 16:1n-7 and 16:0 than ice algae (Table 3.1), the ratios of the two, used as a fatty acid trophic marker for diatoms, were not significantly different (Wilcoxon rank sum, $p = 0.67$). The ratio of 22:5n-3/22:6n-3, another commonly used indicator of diatoms in dietary studies, was significantly different between the two groups (Wilcoxon rank sum, $p < 0.001$). The value for both was greater than 1, indicating fatty acids were predominately produced by diatoms with little input from either dinoflagellate or bacterial sources (Budge and Parrish, 1999; Dalsgaard et al., 2003). *C. centralis* had higher levels of all 18-carbon fatty acids with the exception of 18:3n-6.

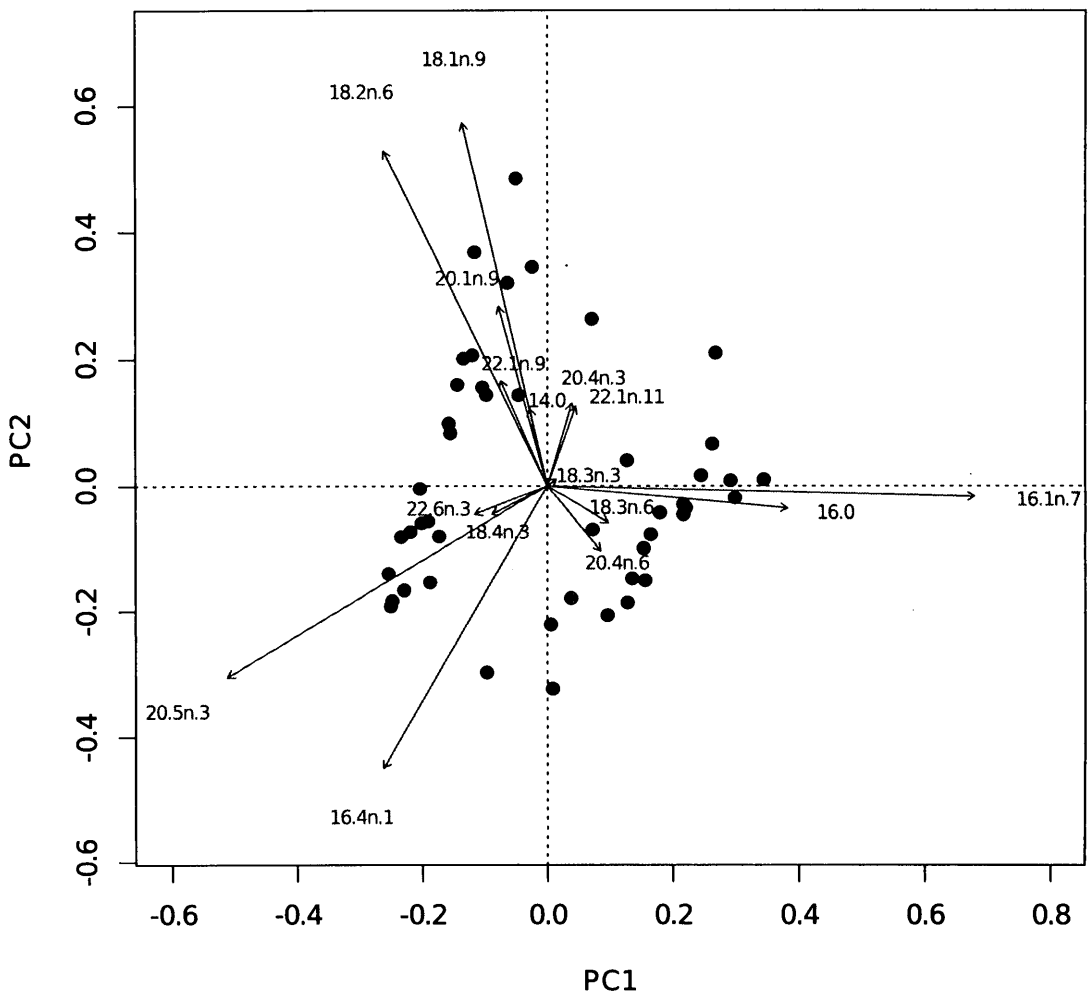

Figure 3.4: Principal component plot of fatty acid proportions (arcsin square root transformed) of *C. centralis* (blue) and ice algae (black). PCA1 explains 72.41% while PCA2 accounts for 9.79% of the variance. Fatty acid vectors are scaled proportional to eigenvalues while sites are unscaled.

PCAs were calculated using 16 fatty acids as variables. The first axis explained 72.41% of the total variance. The 2nd and 3rd component axes explained 9.79% and 5.6% respectively. Equiprobability calculations indicate that only the first two axes are significant, explaining 84.2% of the total variance, and plotted in Figure 3.4.

Fatty acids 16:0, 16:1n-7, 16:4n-1, 18:1n-9, 18:2n-6, and 20:5n-3 had the largest eigenvectors (Fig. 3.4) in relation to the first two principal component axes. Separation of the two groups is driven mostly by the presence of 20:4n-6 in ice algae, and by 18:2n-6 and 18:1n-9 in the pelagic diatoms (Fig. 3.4); despite being present in low amounts (0.24-3.53%) in both sample types (Table 3.1).

3.3.3 Distribution and potential growth sources of C. centralis

Large diatoms (>250 μm in diameter) were present in every zooplankton haul in the 2011 season. Stations in Barrow Strait had the highest amounts while areas in Queen's Channel, Wellington Channel and McDougall Sound had less (Fig. 3.5).

Figure 3.5. Qualitative abundance estimates of diatom abundances in spring, 2011. Circle sizes correspond to abundance estimates (1-3); larger circles indicate a higher estimated number of diatoms compared to smaller circles.

Table 3.1: Mean abundance (±SD) of 16 fatty acids (expressed as mass % of total fatty acids) and characteristic fatty acid ratios in ice algae and *C. centralis* collected in spring 2012. *PUFA* polyunsaturated fatty acids, *MUFA* monounsaturated fatty acids, *SAFA* saturated fatty acids, *EFA* essential fatty acids.

Fatty Acid	Ice algae		*Coscinodiscus centralis*	
14:0	8.94	±1.97	10.23	±2.21
16:0	16.85	±3.57	9.00	±2.67
16:1n-11	0.43	±0.98	2.59	±0.71
16:1n-9	2.81	±2.76	0.12	±0.13
16:1n-7	35.21	±7.86	17.41	±5.16
16:2n-4	2.91	±0.87	4.79	±1.32
16:3n-4	1.42	±0.98	2.78	±1.03
16:4n-1	4.68	±2.24	7.13	±2.59
18:0	0.36	±0.16	2.01	±1.25
18:1n-9	0.63	±0.44	2.35	±1.42
18:2n-6	0.47	±0.11	3.53	±1.18
18:3n-6	1.22	±0.45	0.53	±0.15
18:3n-3	0.34	±0.16	0.27	±0.13
18:4n-3	2.08	±0.76	2.69	±0.45
20:1n-9	0.06	±0.05	0.45	±0.47
20:4n-6	0.24	±0.10	0.03	±0.06
20:4n-3	0.40	±0.27	0.34	±0.67
20:5n-3	13.66	±5.11	24.41	±5.06
22:1n-11	0.15	±0.11	0.18	±0.49
22:1n-9	0.02	±0.03	0.32	±0.33
22:6n-3	1.77	±0.64	2.66	±0.93
Total SFA	27.97	±4.43	23.32	±3.99
Total MUFA	41.41	±7.03	26.38	±6.30
Total PUFA	30.62	±10.33	50.30	±8.90
Total EFA (20:5n-3+22:6n-3)	15.43	±5.70	27.08	±5.43
Diatom ratio (16:1n-7/16:0)	2.12	±0.33	2.03	±0.56
Diatom ratio (20:5n-3/22:6n-3)	7.82	±1.33	9.71	±2.30
Dinoflagellate signal (18:4n-3+ 22:6n-3)	3.85	±1.26	5.35	±0.93
Bacterial signal (sum of odd and branched fatty acids)	1.60	±0.51	2.11	±2.33

Like 2011, *C. centralis* was present at every station in 2012, and for many, accounted for most of the biomass in the net hauls (Fig. 3.6). Total weights of fatty acids of each station were closely related to values standardized by volume (R^2 = 0.78) indicating a uniform distribution of *C. centralis* throughout the water column (Fig. 3.7).

The size range of *C. centralis* collected in the 2012 season ranged in diameter from 153 μm to ~450 μm, with the vast majority being >250 μm. Chloroplasts were clearly visible and appeared healthy in all samples (Fig. 3.2). In samples that had very high amounts of *C. centralis,* some extracellular polymeric substance (EPS) was observed in tendrils dangling from the water surface as well as in some clumping in cells that sank.

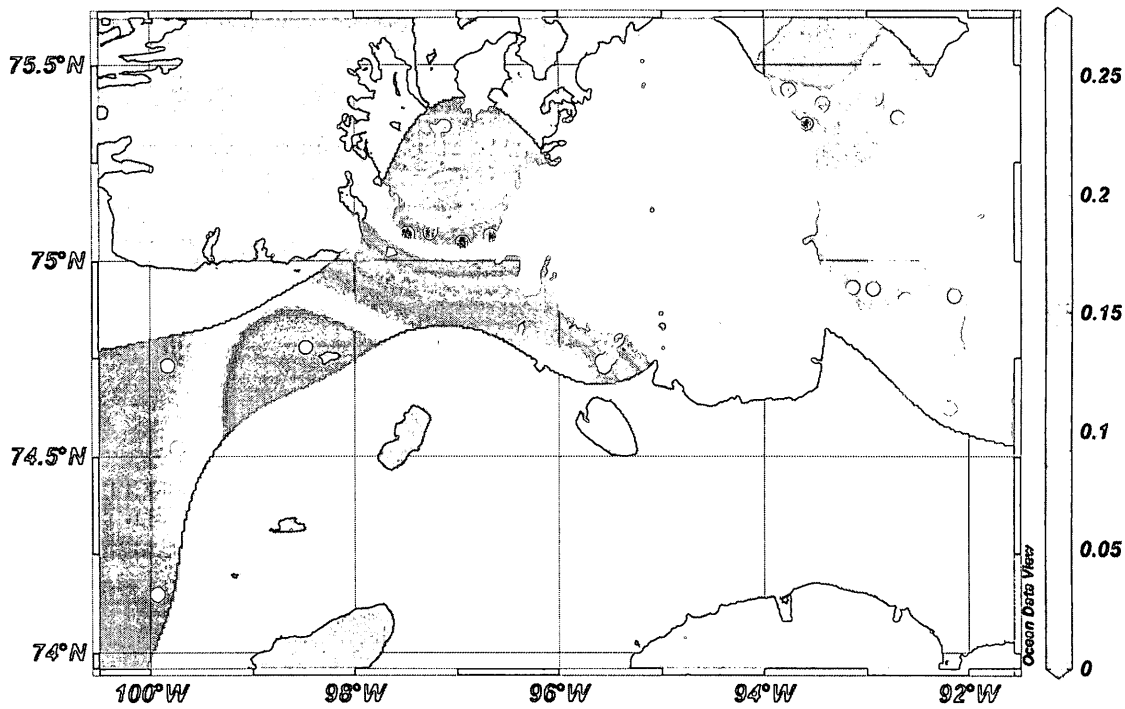

Figure 3.6: Amounts (mg/m^3) of *C. centralis* fatty acids in the water column in spring 2012. Interpolation between stations was done using DIVA gridding.

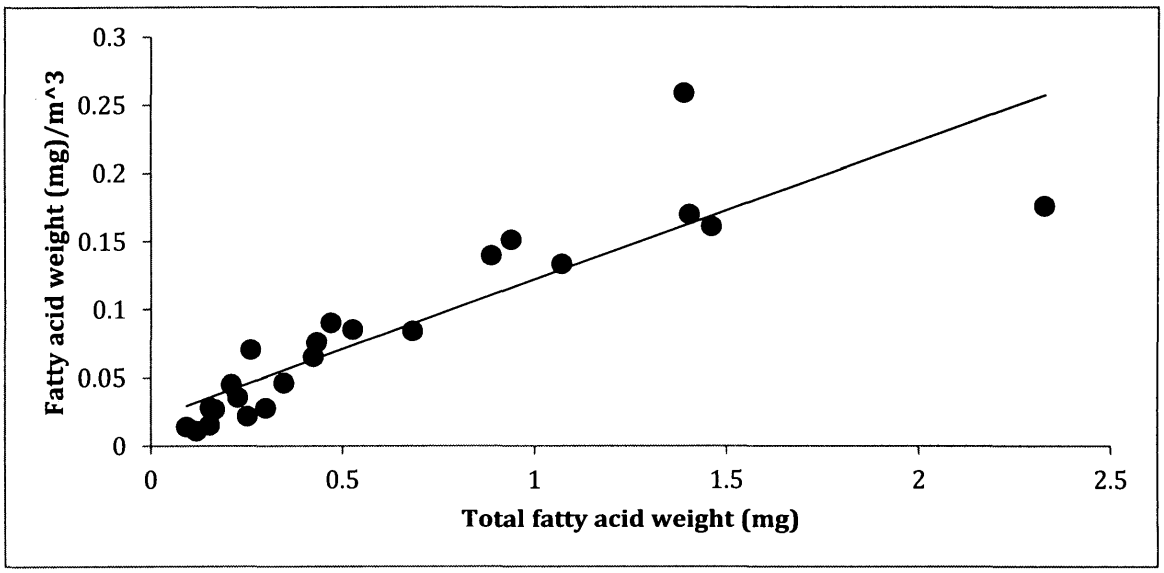

Figure 3.7: Relation of total weight of fatty acids (mg) collected per station to the mg/m^3 of the same stations.

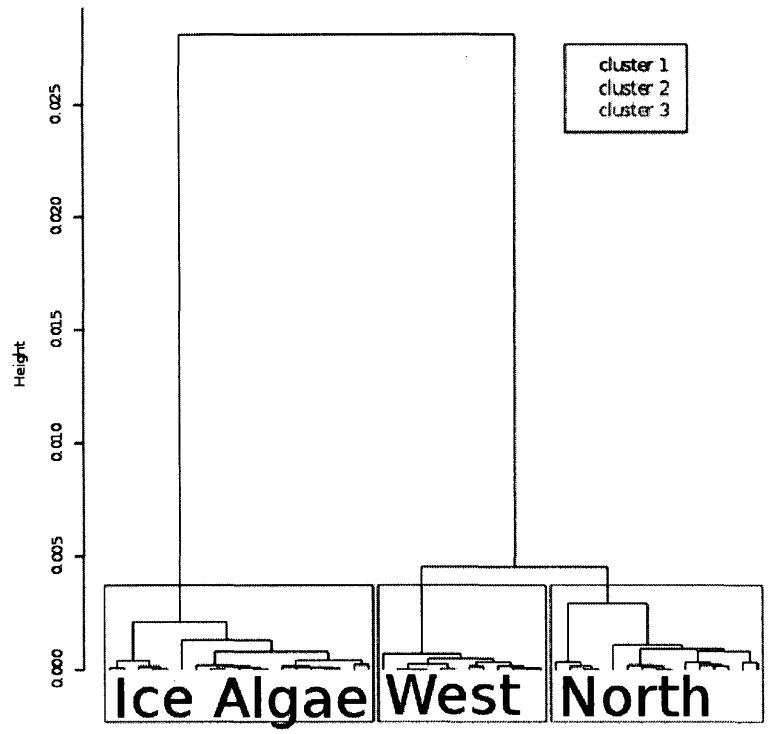

Figure 3.8: Hierarchical clustering dendrogram indicating station locations of arcsin square-root transformed fatty acid proportions. Clusters were selected at the point of maximum between-group variance.

The relative Euclidean distance is largest between ice algae and *C. centralis* but two clusters of *C. centralis* are also clearly visible (Fig. 3.8). Cluster 2 (red) is made up of 11 pelagic and 4 ice algae samples, samples in this cluster are further from each other in Euclidean distance compared to cluster 3 (green) (Fig. 3.9). Nine of the *C. centralis* samples in cluster 2 were taken from Wellington Channel, with the other two being the westernmost stations from the transect across McDougall Sound. Cluster 3 is entirely *C. centralis* and consists of 12 stations: 4 from Resolute Passage, 4 from Barrow Strait, 3 from McDougall Sound and 1 from Wellington channel. Differences between the two clusters are driven by 20:5n-3 and 18:2n6 and 18:1n9 (Fig. 3.4). The *C. centralis* sample taken from station 15 in the south west of Barrow Strait was grouped with the other 18 ice algae samples into cluster 1 despite being closer to cluster 2 on the first two principal component axes (Fig. 3.9).

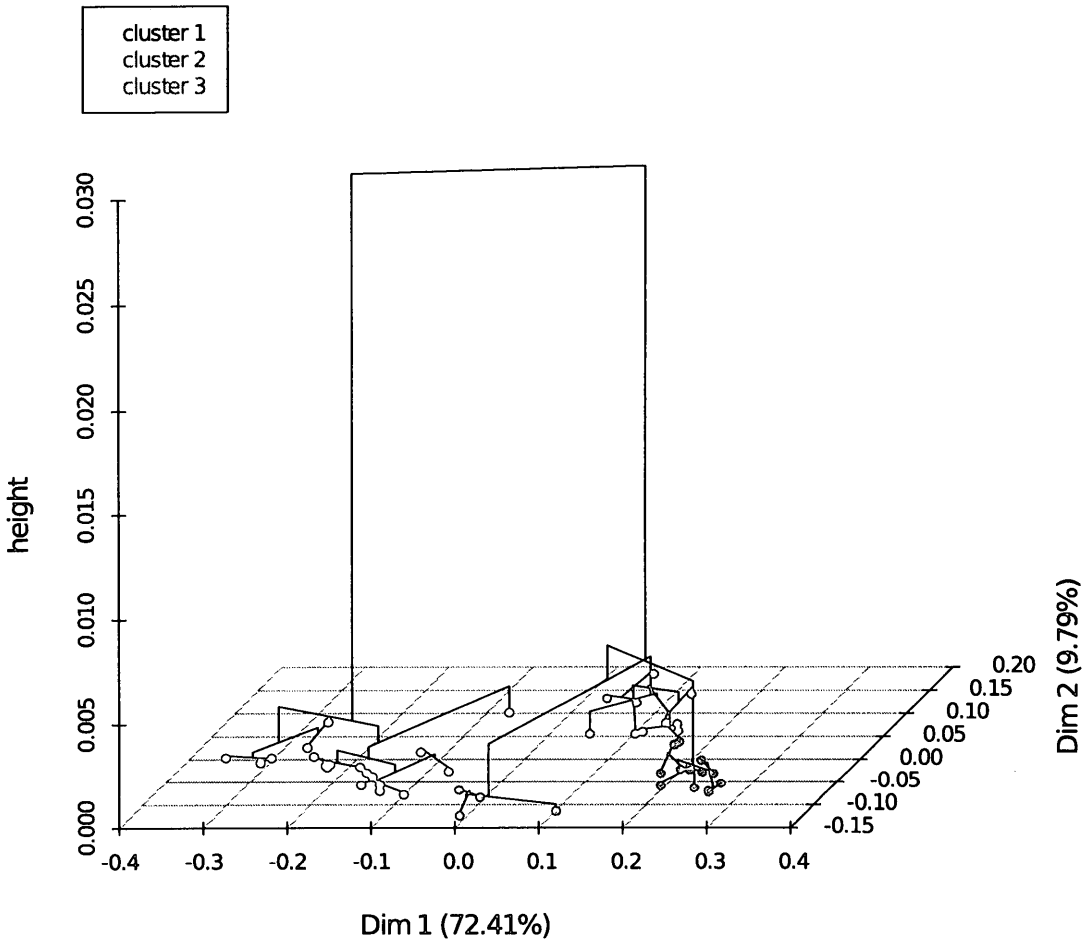

Figure 3.9. Hierarchical clustering dendrogram of arcsin square-root transformed fatty acid percentages overlaid onto the first two principal component axes. Clusters 2 and 3 are mainly *C. centralis* profiles while cluster 1 corresponds to ice algae samples. Height on the y axis indicates Euclidean distances between clusters.

When separated, samples from Barrow Strait (cluster 3, Fig. 3.10) had significantly lower $\delta^{15}N$ values than those from Wellington Channel (cluster 2, Fig. 3.10) ($p=0.004$). Fatty acid weights (mg/m^3 of FA per station) had a negative logarithmic relationship with $\delta^{15}N$ ($y = -0.166\ln x + 0.3372$, $r^2 = 0.50$) (Fig. 3.11).

PUFA were also negatively correlated with $\delta^{15}N$ ($y = -3.7958x + 68.595$, $r^2 = 0.33$) (Fig. 3.12). The 3 outlier stations in cluster 3 with higher $\delta^{15}N$ are stations from Wellington Channel and McDougall Sound (Figs. 3.11, 3.12).

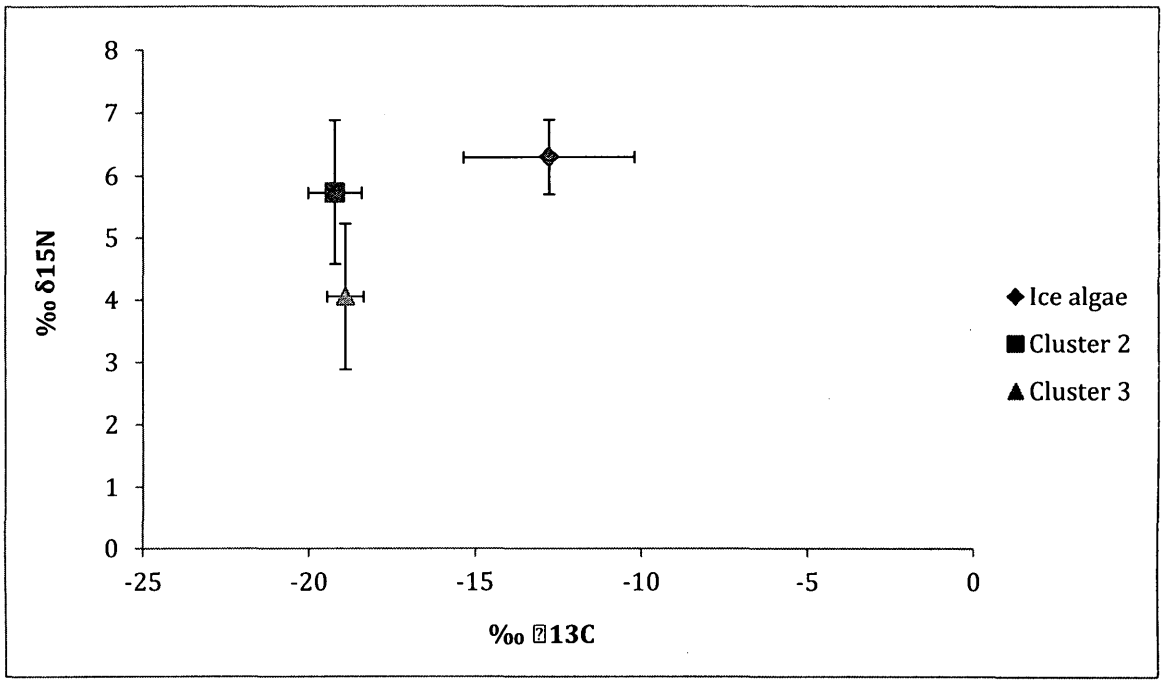

Figure 3.10. The means and standard deviations of stable isotopes for *C. centralis* clusters and ice algae using standard δ notation.

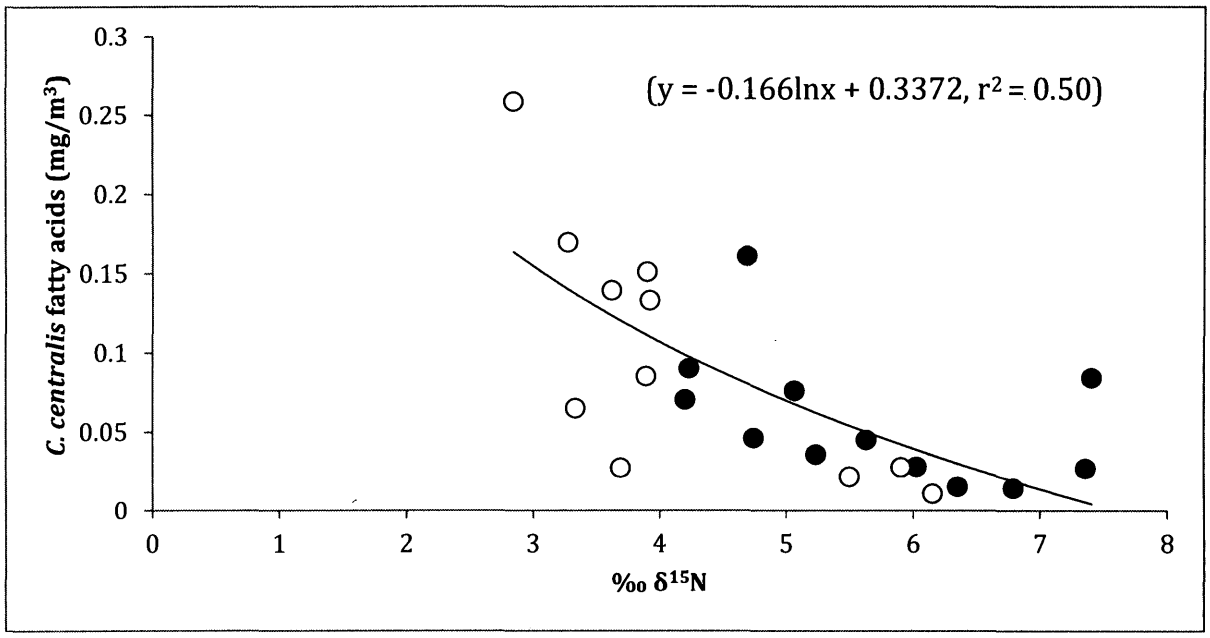

Figure 3.11: Relationship between $\delta^{15}N$ and amounts (mg/m^3) of *C. centralis* fatty acids in the water column. Black circles are individuals from cluster 2, white from cluster 3 and the lone red point is the *C. centralis* sample that was grouped into cluster 1 with ice algae.

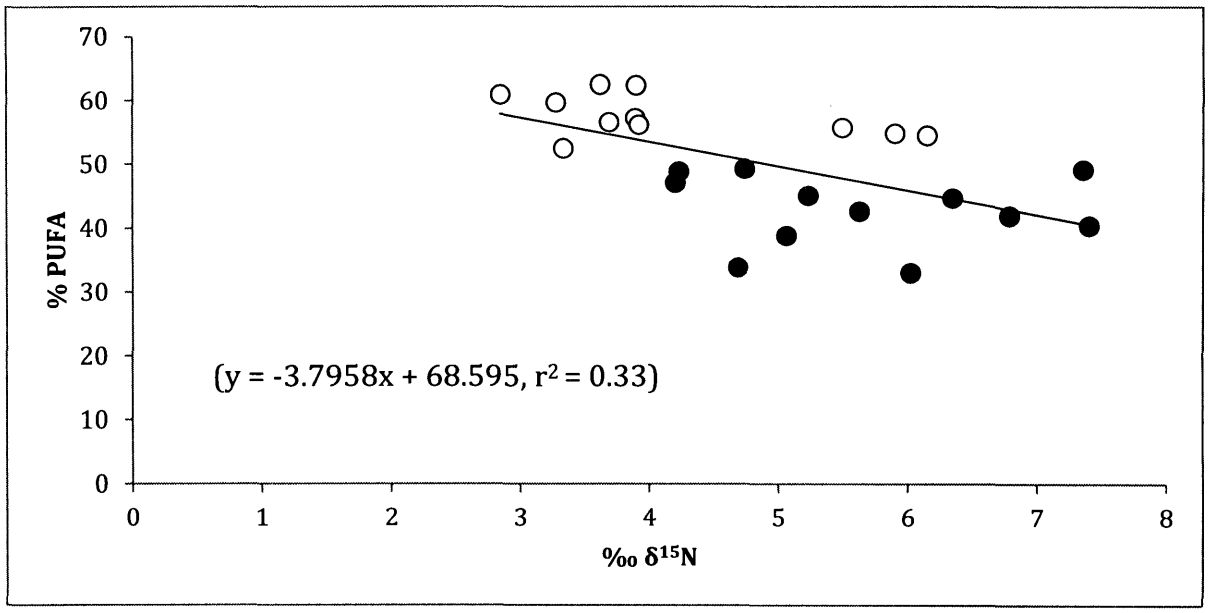

Figure 3.12: The relationship between $\delta^{15}N$ and % PUFA of *C. centralis.* Black circles are samples from cluster 2, white are cluster 3 and the red is the *C. centralis* sample that was grouped into cluster 1 with ice algae.

3.3.4 Feeding experiment

No differences were observed between the fatty acid profiles of copepods fed *C. centralis* compared to those fed ice algae. Only two fatty acids, 16:0, which increased by 2.1% in copepods fed ice algae, and 20:5n-3, which decreased in both *C. centralis* (-3.3%) and ice algae treatments (-4.7%), changed by more than 2% from initial levels.

Chl A levels were higher in bottles that had copepods and *C. centralis* in them compared to the control bottles that contained only *C. centralis*. Conversely, Chl A declined in ice algae replicates that were incubated with copepods relative to copepod-free controls (Fig. 3.13).

When observations were made at the conclusion of the incubation, I found no visible evidence of feeding (i.e. obvious decrease in visible diatoms, empty frustules, or increase in copepod fecal material) in any of the bottles containing *C. centralis* and copepods.

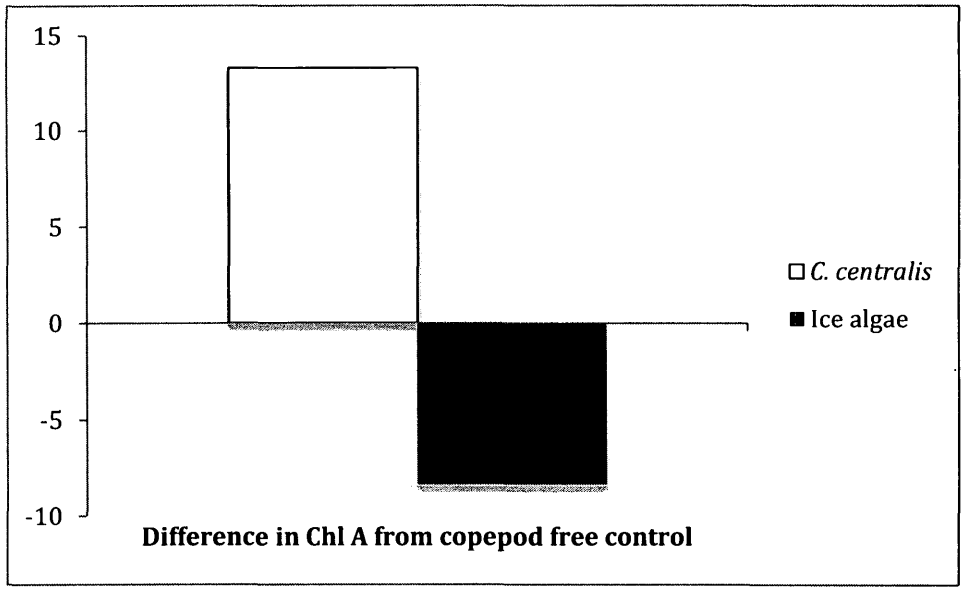

Figure 3.13: Chlorophyll A (ChlA) differences of copepod depredated bottles from diatom only controls at the conclusion of the feeding experiment.

3.4 Discussion

3.4.1 Presence of C. centralis under sea ice

The discovery of *C. centralis* under the sea ice was a surprise to the senior researchers of our expedition, all whom have studied Barrow Strait for decades (C. Michel, M. Poulin, J. Wictor, personal communication). Previously, the earliest reported occurrence of *C. centralis* in the Canadian Arctic Archipelago had also been in May, but in the Northwater Polynya, where open water allows light values to be significantly higher (Lovejoy *et al.*, 2002). Accounts of *Coscinodiscus* sp. occurring as far west as Cornwallis Island are rare, with none indicating the presence of either this particular species, nor the abundance I found (von Quillfeldt, 2000; Riedel *et al.*, 2003). Comprehensive spring sampling campaigns from 1980 to the 1990s had no record of *C. centralis*, despite using almost identical zooplankton sampling methods and locations as our field study (Michel *et al.*, 2006). Nevertheless, it is difficult to tell whether under-ice growth of *C. centralis* has only recently become possible or if this is an annual occurrence that has not been reported. *Coscinodiscus* spp. are often present in low amounts relative to dominant taxa, and may be missed in quantitative phytoplankton sampling routines (Hasle and Lange 1992). This has been posited as a reason for records of it being scarce or irregular in other areas (Hasle and Lange 1992).

The generally large size (250 μm to ~450 μm diameter) of *C. centralis* I found is greater than the average size reported elsewhere (180-200 μm, Hasle and Lange 1992; Hasle and Syvertsen, 1997). The larger size I found may be due to inherent variability of *C. centralis*, which has been found to reach up to 300 μm in diameter (Hasle and Lange, 1992). The large sizes may also be an indication that high rates of

sexual reproduction are occurring. During normal asexual division, frustule sizes linearly decrease, causing the mean population cell size to decrease (Nagai and Imai 1997). Another explanation could be that local herbivores are not exerting significant grazing pressure or alternately, are unable to utilize *C. centralis* as a food source. Herbivorous copepods selectively prey on larger cell sizes; thus, under significant grazing, average cell sizes decrease (Sommer and Lewandowska, 2011; Peter and Sommer, 2012). Lastly, Peter and Sommer (2012) found that increasing temperatures reduced cell size in marine pelagic diatoms; *Coscinodiscus* collections used as the basis for identification were all from water at or above 3°C (Hasle and Lange (1992)). Cold-adapted ecotypes have been found in other cosmopolitan species of phytoplankton; larger than average diameters could be indication that Arctic *C. centralis* represent a new sub-species (Lovejoy *et al.*, 2007).

A large size would be important if these diatoms were to exhibit a completely different life history strategy than the more commonly studied smaller bloom species. Kemp et al. (2000) and Smetacek (2000) theorize that large diatom species have slower growth rates, stay at relatively low numbers, and persist longer than smaller boom and bust species. Due to lower numbers, they are less likely to encounter pathogens and their large size means that they are more heavily armored compared to their smaller counterparts, making them less accessible to consumers (Smetacek 2000). Bigger cell size would also be beneficial for increased buoyancy control, allowing for transfer from nutrient depleted surface waters to deeper strata.

Given the ability of *C. centralis* to grow under a variety of conditions (Hasle and Lange, 1992), its continual and regular presence nearby in the North Water Polynya (Lovejoy et al., 2002) and the lack of significant environmental variation

from the region's normal conditions (Cota et al., 1987; Michel et al., 2006), it is possible that *C. centralis* occurs regularly under sea ice in this area and has avoided detection up to this point. However, given the high numbers of *C. centralis* I found during our sampling, it does not seem likely that these phytoplankton would have been missed by previous expeditions. I suggest that either previous studies sampled before the bloom occurred (most took place in April) or, the bloom was unusually large during our sampling years.

3.4.2 Comparisons of stable isotopes and fatty acids in C. centralis and ice algae

Stable isotopes

Stable $\delta^{13}C$ values for *C. centralis* samples were more depleted and less variable than ice-associated producers (Fig. 3.10), which is characteristic of pelagic production (Hobson *et al.*, 1995). This is due to the high levels of dissolved inorganic carbon available in the water column during growth (Hobson *et al.*, 1995). As carbon is very lightly enriched with successive trophic levels, this may be useful to discern the relative importance of *C. centralis* as a carbon source in higher trophic levels (Post, 2002). Conversely, ice algae had relatively little variation in $\delta^{15}N$ values compared to *C. centralis* (Fig. 3.10), suggesting that nitrate levels are more uniform at the ice/water interface than throughout the water column (Tamelander et al., 2009). The large variation of $\delta^{15}N$ in *C. centralis* may inhibit the future use of $\delta^{15}N$ as an indicator of trophic level (Tamelander et al., 2009).

Fatty acids

C. centralis had significantly higher levels of PUFAs, specifically essential fatty acids (EFAs), compared to ice algae (Fig. 3.4). Although PUFA levels in *C. centralis*

varied depending on growth origin (32-62%) (Fig. 3.12), they were still in the upper ranges of what has been reported previously (Dunstan *et al.*, 1994; Parrish *et al.*, 2005; Leu *et al.* 2006). PUFA levels greater than 30% are indicative of the exponential growth stage of a bloom, where fatty acid deposition is highest in the polar lipids of cell membranes (Parrish *et al.*, 2005; Leu et al., 2006)). The similarities in fatty acids and shared dominance of characteristic diatom PUFAs 20:5n-3 and 16:4n-1 in our samples and spring blooms found in Arctic open water environments (Leu et al., 2006) suggest that *C. centralis* are able to actively grow under sea ice.

C. centralis exhibited similar levels to ice algae of other characteristic diatom fatty acid trophic markers, including ratios of both 16:1n-7/16:0 and 20:5n-3/22:6n-3 (Table 3.1), precluding these indices as identifiers of *C. centralis* in dietary studies (Budge and Parrish 1998; Dalsgaard 2003). I did find larger proportions of 18:0 (2.01 ± 1.25%), 18:1n-9 (2.35 ± 1.42%), 18:2n-6 (3.53 ± 1.18%), 18:4n-3 (2.69 ± 0.45%) and 22:6n-3 (2.66 ± 0.93%) in *C. centralis* compared to ice algae (Fig. 3.4), all of which are more commonly associated with dinoflagellates. However Dunstan *et al.* (1994) also found significant amounts of 18-carbon fatty acids in their laboratory grown culture of *Coscinodiscus spp.* It is unlikely that appreciable numbers of dinoflagellates were present in my samples due to several separation steps, which included rinsing over a 250 μm mesh sieve with FSW and the removal of all organisms and particles that were not *C. centralis* using a dissecting microscope.

In future studies of Arctic food webs, there may be potential to use 16:1n-11 as a dietary indicator of *C. centralis.* I found it in noticeably higher levels (2.59 ±

0.71%) than was present in our ice algae samples (0.43 ± 0.98%; Table 3.1). Furthermore, 16:1n-11 has only been found in trace amounts (<0.8%) in other studies of ice algae (Falk-Petersen *et al.*, 1998; Leu *et al.*, 2010), pelagic diatoms (Budge and Parrish, 1999; Leu *et al.*, 2007), bulk phytoplankton (Lewis, 1969) and other types of primary producers (Kelly and Scheibling, 2012). Even in studies of other *Coscinodiscus* species, 16:1n-11 has not been reported in significant amounts (Dunstan et al., 1994), therefore this fatty acid may be a rare instance of a species-specific biomarker.

3.4.3 Distribution and potential growth sources of C. centralis

In both years of our study, *C. centralis* was found at every station sampled. Estimates from 2011 were similar to the quantified fatty acid weights/m^3 of 2012 indicating that patterns of abundance may be stable from year to year (Figs. 3.5 & 3.6). By examining the relationship between total fatty acid weight (mg/station) to mg/m^3 it is evident that *C. centralis* is uniformly distributed throughout the water column and not concentrated at any specific depth (Fig. 3.7). Barrow Strait had higher weights of fatty acids/m^3 (max 0.32 mg/m^3) and abundance estimates than either McDougall Sound or Wellington Channel (max 0.11 mg/m^3) (Fig. 3.6). Based on current directions, this means that the largest amount of growth occurred to the west, in the region of Viscount Melville Sound, an area that was ice covered during both years. The second growth source was north of Cornwallis, potentially Penny Strait Polynya or Dundas Island Polynya at the top of Wellington Channel, and had much lower associated abundances.

Analysis of stable isotopes and fatty acids confirmed that *C. centralis* were originating from two separate growth sources with significantly different conditions (Fig. 3.8, 3.9). Cluster 2 had significantly higher values of $\delta^{15}N$ than cluster 3 and overlapped with ice algae (Fig. 3.10). The preferential uptake of lighter isotopes by primary producers therefore indicates that the nitrogen availability in Viscount Melville Sound was higher than the growing conditions of the northern source (Needoba and Harrison, 2004; Ganeshram *et al.,* 1995). The bulk nitrogen isotope ratios I found were lower than other Arctic studies for phytoplankton (Tremblay *et al.* 2006) but this may be due to earlier sampling as the NO_3 pool becomes more $\delta^{15}N$ enriched as the bloom progresses (Tamelander *et al.* 2009).

Although I do not have direct measurements of the growth origin conditions, inferences can be made based on the combination of fatty acid and stable isotope values. Samples from Barrow Strait had lower $\delta^{15}N$ values and high PUFA levels (Fig. 3.12), these are indicative of high nutrient levels and lower light values, suggesting that the growth of these diatoms is occurring under the patchy low light environment of pack ice (Tremblay *et al.* 2006; Leu *et al.* 2006). In contrast to lower latitudes, the photosynthetic capability of Arctic phytoplankton significantly increases with nitrogen availability (Matsuoka et al., 2009). Diatoms from the northern source were present in lower abundances, had lower levels of PUFA, and higher $\delta^{15}N$ values (Fig. 3.11, 3.12): consistent with a more depleted nutrient pool with higher light, such as a polynya. These findings follow in the wake of a growing pool of literature that contradicts the traditional view of pelagic production only being possible after ice breakup (Ariggo *et al.,* 2012, Mundy *et al.,* 2009).

3.4.4 Feeding Experiment

Despite *C. centralis* and ice algae having significantly different fatty acid profiles, no difference was observable in copepods fed by the treatments. Based on turnover rates of lipids in calanoids, a dietary switch should have been evident via changes in fatty acids if extensive feeding did take place (Graeve et al., 2005; Kreibich et al., 2011). The decreases in EFA 20:5n-3 in both treatments were consistent with starvation (Sargent et al., 1993), which in the case of ice algae, was probably due to rapid depletion of the added food source. Large cell size of *C. centralis* could preclude nauplii or juvenile copepodites from effectively grazing, which may explain the lack of any observable grazing in our feeding experiment.

Not only were *C. centralis* cells still healthy at the end of the experiment, they actually increased growth in bottles containing zooplankton, implying that bottles with only diatoms were nutrient-limited in comparison (Fig. 3.13). Our feeding study also provides a direct observation of the shade tolerance Kemp et al. (2000) predicted is crucial for a pre-ice breakup strategy to be successful. Based upon our preliminary findings it is conceivable that *C. centralis* may represent another unique form of primary production in addition to the classical ice vs. open water pelagic blooms.

Coscinodiscus can have greater than 10 times the lipid per cell than smaller species (Dunstan et al., 1994). This large amount, coupled with their high levels of PUFA could make these diatoms a nutritious and important food source for copepods, even at relatively low abundances. Alternately, these diatoms could be exported to benthic ecosystems; *C. centralis* has been found in the stomachs of benthic fish on South African coastal shelves (Kemp *et al.,* 2000).

The importance to either pelagic or benthic systems however, will be determined by the palatability of *C. centralis*. I observed indications that some *C. centralis* cells I collected were producing small amounts of extracellular polymeric substance (EPS). EPS has been shown to be a defense mechanism for some diatoms including *Coscinodiscus spp.*, and effectively inhibits copepod grazing (Malej and Harris, 1993). The production of EPS is thought to be inversely related with nutrient levels (Malej and Harris, 1993): a future study looking at differences in EPS production between our two source populations would be worthwhile.

3.5 Conclusion

Unusually large *C. centralis* were present at every station during both years of our study. Regional abundances were similar year to year and diatoms appeared to be uniformly present throughout the water column regardless of depth. *C. centralis* was distinct from ice algae both in its fatty acid and stable isotope profiles, having significantly higher amounts of nutritious EFAs. Despite having greater nutritional value in comparison to ice algae, it does not appear that copepods take advantage of *C. centralis* as a food source. There may be potential to use an unusual fatty acid, 16:1n-11, as a species-specific biomarker for *C. centralis*. I concluded that there are two source populations for *C. centralis,* one to the west growing under the pack ice and one to the north of Cornwallis Island, most likely from a polynya. Lastly, based on the size, historic and present distribution, and chemical signatures of the diatoms I found, there is evidence that the presence of *C. centralis* at our study sites is not a new phenomena but potentially represents an alternative life-history

strategy to the classical view of ice algae transitioning to open water pelagic blooms for primary production in the area.

Acknowledgements

This work was supported by the Natural Sciences and Engineering Research Council (NSERC), Fisheries and Oceans Canada, Natural Resources Canada (Program of Energy Research and Development and Polar Continental Shelf Program), Environment Canada, Kenneth Molson Foundation, Churchill Northern Studies Centre, Northern Scientific Training Program (NSTP) and the Faculty of Graduate Studies at York University.

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Chapter 4.

Conclusion

4.1 Main findings

The main objective of this thesis was to determine if there were environmentally driven, large scale patterns in the lipid composition of ice algae and how this influenced energy transfer to zooplankton. In Chapter 2 I found that ice algae fatty acids did not exhibit any spatial patterns although compositions were significantly affected by the thickness of ice and snow (R^2=0.28, p=0.001). Snow and ice appear to explain similar amounts of variation in ice algae fatty acids regardless of scale (Leu et al., 2010). Ice algae fatty acid compositions grouped into three distinct clusters however I found no spatial or environmental explanations for these groupings. More research is required to explain the remaining variation of ice algae fatty acids.

Calanoid copepods relied heavily on ice algae as a food source (Fig. 2.2, 2.4). Polyunsaturated fatty acids (PUFAs) in *Calanus* samples were significantly lower in 2012 ($p < 0.001$, Fig. 2.14), which I attributed to lower snowfall in 2012 causing a decrease in ice algae PUFA levels (Leu et al., 2010). Aside from *Calanus*, zooplankton trophic levels tended to increase with body size (Fig. 2.3). At the trophic levels of primary consumers, fatty acid composition was primarily dictated by inflow, either from the west or from the north (153 μm: Fig. 2.9; 250 μm: Fig. 2.11, 2.12; *Calanus:* Fig. 2.15). Because of the dietary reliance of low trophic levels on ice algae as a food source (Fig. 2.2, 2.4) (Søreide et al., 2010), the influence of inflow implies that spatial patterns in ice algae exist, just on a larger scale than this study was able to

investigate. Higher trophic levels were less likely to be significantly affected by any environmental variable.

Daily variations in light levels had significant effects on several sample types. Zooplankton of 250 μm body size had increased levels of a fatty acid characteristic of dinoflagellates, 22:6n-3, on brighter days (Fig. 2.12). Light levels also significantly affected the fatty acid compositions of *Sagitta elegans* (Fig. 2.17). This evidence suggesting that community level fatty acid compositions can exhibit day-to-day responses to environmental variation is the first I am aware of for an under-ice food web (Scott et al., 1999; Leu et al., 2006), and a more thorough investigation is needed.

Our interest in ice algae was due to the spatial and temporal discontinuity of Arctic primary production; mainly that ice algae are an important source of food for zooplankton during periods of ice cover when pelagic producers are limited (Michel et al., 1996). Recent findings however, suggest that pelagic producers play an important role in under-ice food webs (Arrigo et al., 2012). In chapter 3, I describe the fatty acids and stable isotopes of a large pelagic diatom, *Coscinodiscus centralis,* I found subsisting under the sea ice. *C. centralis* had significantly higher levels of PUFA compared to ice algae (Wilcoxon rank sum, $p < 0.001$) and more specifically, the essential fatty acids 20:5n-3 (Wilcoxon rank sum, $p < 0.001$). *C. centralis* were present at every station sampled in both years, but were most abundant in Barrow Strait (Fig. 3.5, 3.6). Fatty acid compositions yielded two distinct clusters, indicating *C. centralis* had two source populations (Fig. 3.8, 3.9). One was to the west of Cornwallis Island, likely Viscount Melville Sound, and has fatty acid and stable

isotope characteristics of a nitrogen rich, low light environment (Fig. 3.10). The other population originates to the north of Cornwallis island, and grew under more nutrient limited, higher light environment, probably a polynya. Despite the apparent nutritional benefit, zooplankton did not appear to be utilizing *C. centralis* as a food source.

4.2 Future research

Arctic research is an expensive, logistically challenging endeavour. Because of these constraints, studies are often short and have a very specific scope. This study has served to initiate a time series of low trophic level fatty acids over a large area, and should be maintained. Of particular interest is the relationship between large calanoid copepods, ice algae and snow and ice conditions.

Research in this region should also be expanded to the benthic community, although *C. centralis* does not appear to be important to pelagic grazers, it may be for the benthos. Lastly, a particular isoprenoid fatty acid (IP25) in ice algae has emerged as a paleological indicator of sea ice cover (Brown et al., 2010). In the future I plan on investigating how local environmental conditions influence the production and distribution of this biomarker.

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Appendix:

Table S.1 Summary of fatty acid common names and their uses as trophic markers in Arctic food webs.

Fatty acid	Common name	Trophic marker	Reference
14:0	Myristic acid		
16:0	Palmitic acid		
17:0	Margaric acid		
18:0	Stearic acid		
16:1n-7	Palmitoleic acid		
18:1n-7	Cis-vaccenic acid		
18:1n-9	Oleic acid	Carnivory	Falk-Petersen et al., 1990
18:2n-6	Linoleic acid		
18:3n-6	γ-linoleic acid		
20:4n-6	Arachidonic acid		
22:5n-6	ω6-docosapentaenoic acid		
16:4n-3	Hexadecatetraenoic acid		
18:3n-3	α-linolenic acid		
18:4n-3	Stearidonic acid	Dinoflagellates	Budge and Parrish 1998
20:5n-3	Eicosapentaenoic acid	Diatoms	Budge et al., 2008
22:6n-3	Docosahexaenoic acid	Dinoflagellates	Falk-Petersen et al., 1998
16:1n-7/16:0		Diatoms	Budge and Parrish 1998
20:5n-3/22:6n-3		Diatoms	Dalsgaard et al., 2003
18:4n-3+22:6n-3		Dinoflagellates	Dalsgaard et al., 2003
Σ odd+branched chain FA		Bacteria	Dalsgaard et al., 2003
20:1n-9+22:1n-11		*Calanus*	Falk-Petersen et al., 2009