ABSTRACT

PIRTLE-LEVY, REBECCA SUNSHINE. Lipid-Based Investigation of Food Web Dynamics for the Benthic Community on the West Antarctic Peninsula. (Under the direction of Dr. Carrie J. Thomas).

The western Antarctic Peninsula (WAP) is experiencing significant rates of regional climate warming, both the atmosphere (~5°C in last 50 years; Vaughan et al., 2003) and surface ocean (~1°C in the last 50-60 years; Meredith and King, 2005). Subsequent reduction of the extent and duration of winter sea-ice has led to shifts in physical processes that are causing changes at all trophic levels of the pelagic food web, from phytoplankton to penguins. The benthic community is dependent on pelagic production for organic matter (OM) input as a food source. Currently, the benthic community on the WAP appears to feed throughout the year, even during ice-covered winter months with little to no pelagic production input, due to the presence of a sediment food bank. Changes in the structure of the pelagic ecosystem will soon thereafter reach the benthos, possibly affecting quality and quantity of OM flux. As part of the FOODBANCS2 Project, the WAP continental shelf benthic community was sampled from 63°S - 68°S, a latitudinal transect spanning annual sea-ice duration from 2 to >8 months. In this study, lipids (i.e. fatty acids and sterols) were used to elucidate the benthic food web of this region at 4 stations during winter 2008 and summer 2009 in order to better understand current feeding habits of benthic deposit feeders along the sea-ice gradient.

In the first part of this study, fatty acids (FAs) and sterols (STs) were used to investigate food sources (i.e. sediment trap and surface sediment samples) and diets of two species of deposit feeding holothurians, *Protelpidia murrayi* (surface deposit feeder) and *Molpadia musculus* (subsurface deposit feeder). Polyunsaturated FAs (PUFAs) were low in

both types of food sources and elevated in the holothurian species, in particular 20:4ω6 and 20:5ω3. FA and ST compositions indicated phytoplankton derived OM was present in sediment trap and surface sediment samples. Diatom FA markers, flagellate FA markers, and phytosterols were prevalent in sediment trap samples. Flagellate FA markers were present at very low levels in surface sediment and both species of holothurians. Diatom FA markers, bacterial FA markers, and phytosterols were abundant in surface sediments. Variations in lipid composition between holothurian tissues indicated access to OM of varying lability. *Protelpidia murrayi* appeared to assimilate primarily phytoplankton derived OM as indicated by high levels of diatom FA markers and phytosterols. *Molpadia musculus* had increased levels of bacterial FA markers and 5α(H)-stanols indicative of feeding on reworked sediment at depth but also appeared to utilize phytoplankton-derived OM as suggested by low levels of diatom FA markers and phytosterols. Prevalence of diatom FA markers coupled with low levels of flagellate FA markers in sediments and holothurian tissues implies the importance of diatoms from pelagic production as a food source for the WAP benthic community.

Lipid compositions of surface sediment, *P. murrayi*, and *M. musculus* were compared during winter 2008 and summer 2009 to investigate spatial or temporal variation on the north-south sea-ice gradient. Surface sediment FA and ST compositions of the WAP region exhibited no latitudinal or seasonal variations and appeared to supply the benthic community with a constant food source throughout the year. A latitudinal difference in species-specific lipid compositions of the holothurians was evident but can be attributed to small variations in specific FAs and STs. These differences were quite variable and could not be easily attributed to variability in OM input. Instead the latitudinal differences in winter and summer are likely the cause of natural variability attributed to reproduction driven lipid accumulation,

assimilation variability in response to fresh input of OM, or differing conversion efficiencies of STs from dietary sources. Long term sampling of the benthic community of the WAP is necessary to determine benthic responses to climate induced changes in pelagic production.

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Lipid-Based Investigation of Food Web Dynamics for the Benthic Community on the west Antarctic Peninsula

by
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A dissertation submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

Marine, Earth and Atmospheric Sciences

Raleigh, North Carolina

2016

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DEDICATION

My family's support was incredibly helpful in the completion of this project, and I dedicate this dissertation to the most important people in my life, my husband, Ben, and my son, Samuel.

BIOGRAPHY

Rebecca Pirtle-Levy spent her childhood in the Appalachian Mountains of eastern Tennessee. She was always curious about nature and how the world around her interacted. Summer vacations at the ocean sparked this curiosity even further and eventually led to a career in marine science. Though the road to get there winded in unexpected directions, she eventually found her passion through a part time laboratory job during her undergraduate college years at the University of Tennessee in Knoxville, TN. At the urging of her academic advisor, she knocked on the door of one of the two oceanographers at UT, Dr. Jackie Grebmeier, and asked for a job. After a semester of sorting Arctic benthic macrofauna in her lab, Dr. Grebmeier asked if she would like to participate in a research cruise. Having no idea what to expect, she enthusiastically said YES! Dr. Grebmeier and her husband, Dr. Lee Cooper, study the invertebrate communities in the western Arctic and how they are responding to climate change. During that first cruise, she realized how much she enjoyed the work, the long hours, the breathtaking scenery, and the comradery.

Upon finishing her undergraduate degree in Ecology and Evolutionary Biology, Rebecca began a Master's program under the direction of Dr. Grebmeier. She participated in many Arctic research cruises in the Bering, Chukchi, and Beaufort Seas, and her own research during this time focused on preservation of food sources in seafloor sediments and their implications for the benthic community. Rebecca completed her Masters and needed a break from academia. She remained in Dr. Grebmeier and Dr. Cooper's lab as their research coordinator for a year while searching for a Ph.D. program that felt right. She finally found a good fit at North Carolina State University and embarked on research that gave her a chance to visit Antarctica and explore a benthic community from a new perspective. North Carolina

quickly felt like home, and Rebecca enjoyed the challenges of her work and living in the community of Raleigh. She is looking forward to the next chapter of her career and the adventures that lie ahead.

ACKNOWLEDGMENTS

This dissertation could not have been completed without the support of my friends and family. I am extremely grateful to my husband, Benjamin Axmann, who supports me in everything and without whom I could not have found the time necessary to complete a project of this size. I thank my mother, Dianne Levy, who has always supported me in my endeavors and never faltered in her positive outlook, kindness, and loving words. I am the person I am because of her. I am also thankful for my in-laws, Thomas and Christine Axmann, who provided many hours of babysitting and moral support so I could focus on writing. I had many encouraging friends along the way that helped in any way they could, and though I cannot list everyone here I thank them with all my heart.

I have been fortunate to work with many professors that provided their guidance and expertise. I will be forever grateful to Dr. Carrie Thomas for taking me under wing. She has provided me with her trust, patience, resources, and the knowledge to push me when I needed it most. Thank you for your support at every turn. I also got to work closely with Dr. Dave DeMaster whose positive outlook and support during field work and in the lab were invaluable.

My committee member, Dr. Chris Osburn paved the way to collaboration with Dr. Rudolf Jaffe, thus enabling the analysis of my samples. This work could not have been completed without his help. I thank my committee member Dr. Dave Eggleston, who agreed to step in and provide his knowledge and support during the final chapter of this endeavor.

I would like to thank Dr. Rudolf Jaffe for generously opening his lab to me and providing resources to run all of my samples and the expertise to help me analyze them. He

also allowed his postdoctoral fellow, my dear friend Dr. Laura Belicka, to teach me and provide guidance in all aspects of lipid chemistry. Without them I would have no project.

I thank Dr. Craig Smith, who is no longer on my committee, but helped move my project forward during field work and whose insights and knowledge of the study region helped improve this research.

I would like to thank the captains and crews of the RV *Lawrence M. Gould* and RV *Nathaniel B. Palmer*, which provided the platform and support necessary for gathering samples in the harsh Antarctic environment. Also, a big thanks to the Raytheon Polar Services science technicians onboard for making our sampling efforts a success and the fellow scientists of the three FOODBANCS2 cruises for help with sample collection.

This work would not have been possible without funding and financial support from NSF Polar Programs (grant ANT-0636773).

Finally I would like to thank my son, Samuel, who made my focus shift for a while but in the best way possible. You might have turned my world upside down, but there is nothing more enjoyable than seeing the world through your eyes.

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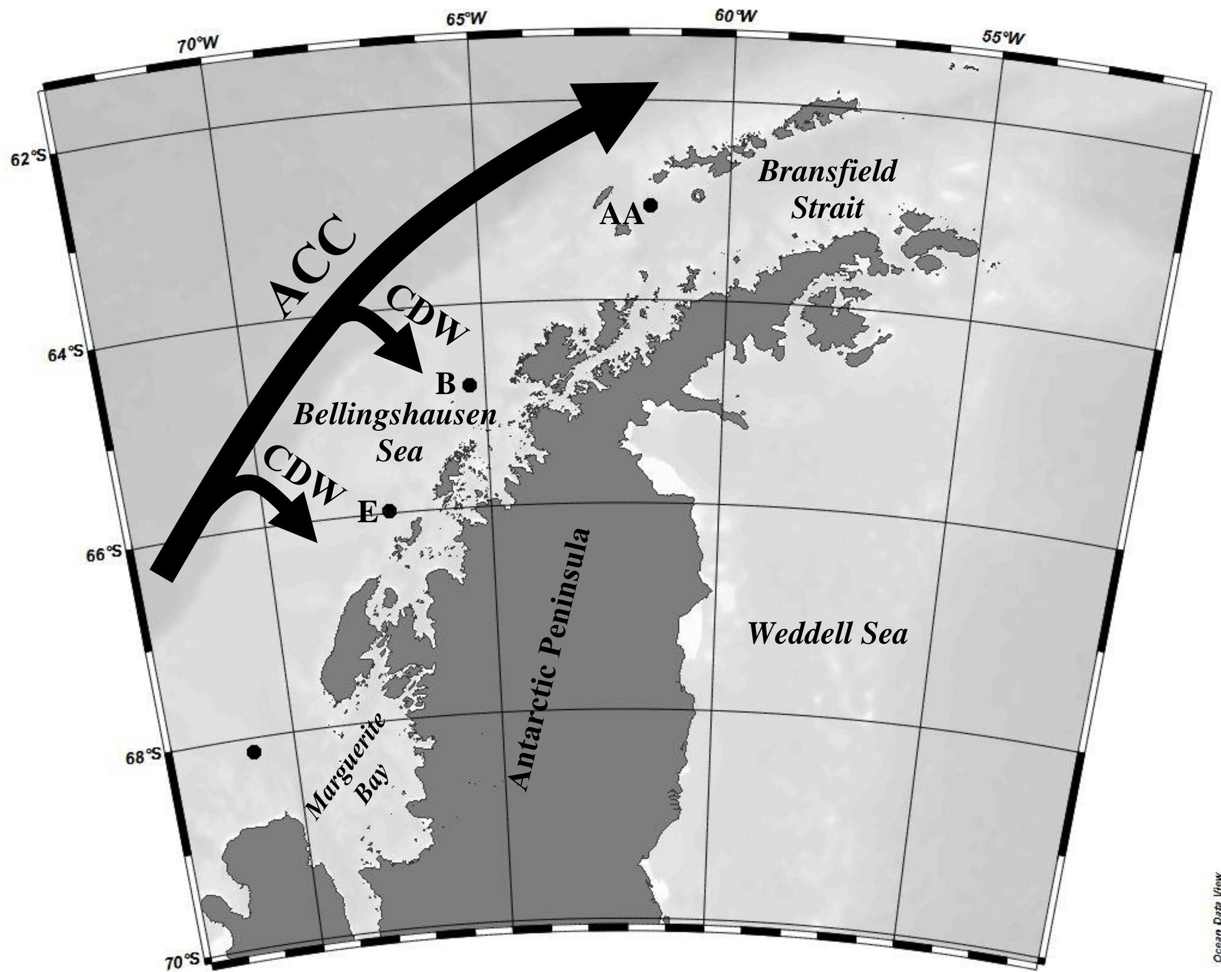
CHAPTER 1

Introduction

General Circulation of the west Antarctic Peninsula

The Antarctic Peninsula is the northernmost extension of the Antarctic continent into the Southern Ocean and is bordered on the west by the Bellingshausen Sea, to the east by the Weddell Sea, and to the north by the Bransfield Strait. This region of the Antarctic has characteristics of deep-sea regions and the Arctic. Similar to the deep sea and the Arctic, temperatures are cold and nutrient input to the benthic community comes from seasonal pulses of primary production (Arntz et al., 1994, Ginger et al., 2000, Ginger et al., 2001). The continental shelves of the Antarctic differ from the Arctic by being unusually deep and lacking input from rivers and estuaries (Clarke, 1996). The west Antarctic Peninsula (WAP) is characterized by a continental shelf with a mean width of 200km and mean depth of 500m (Prezelin et al., 2000, Hansom and Gordon, 2013).

The hydrographic structure of the WAP is complex and has a seasonal cycle associated with formation and break up of sea ice. The WAP is bordered along the shelf break by the Antarctic Circumpolar Current (ACC; Martinson and McKee, 2012), which flows northeast and contributes to the oceanic character of water masses on the shelf (Fig. 1; Hofmann et al., 1996, Smith et al., 1999). The surface layer is made of Antarctic Surface Water (AASW), a fresh, cold water mass, which is ubiquitous around the Antarctic continent (Fig. 2; Moffat et al., 2009). During fall and winter, cooling surface waters and brine rejection from sea ice formation deepen the surface layer to 100m (Moffat et al., 2009). In spring, air temperatures increase, sea ice breaks up, and meltwater from coastal



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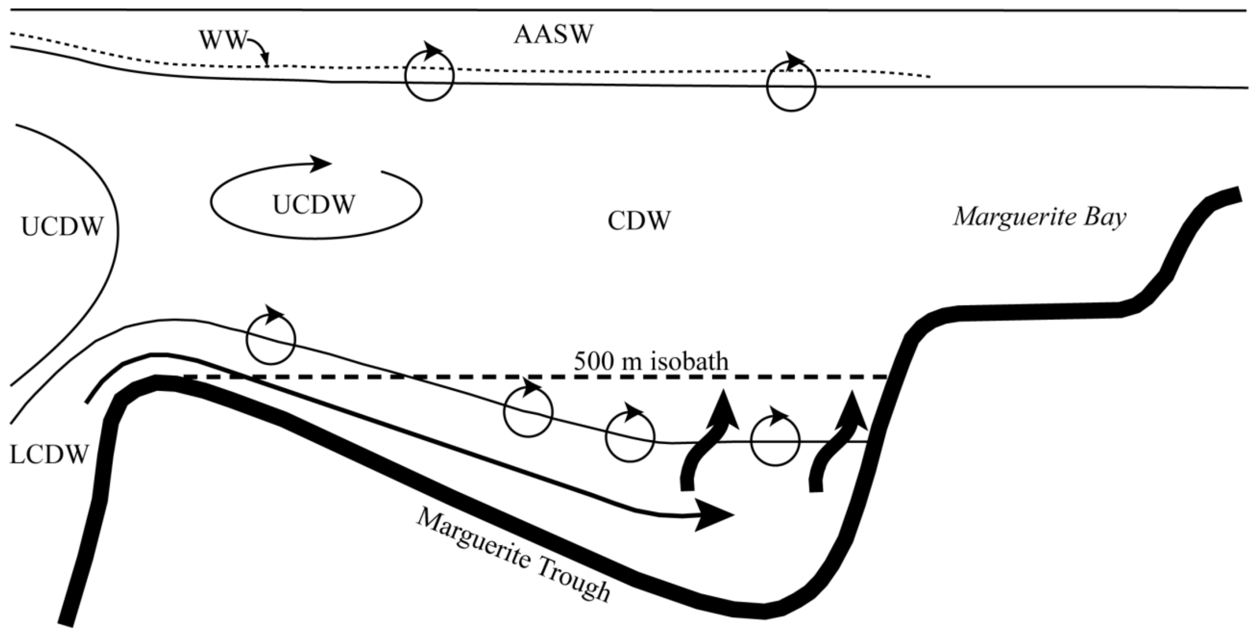

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origins lead to a stratification of the surface layer (Moffat et al., 2009). Winter Water (WW), present at 100m, is a remnant of the cold, winter layer. Below WW is the warmer and more saline modified Circumpolar Deep Water (CDW; Moffat et al., 2009), which occupies the shelf in the Bellingshausen Sea (Smith et al. 1999). The CDW originates from the ACC offshore of the shelf and has temperature maximums of 1.6 – 2.0°C (Fig. 1; Holland et al., 2010, Dinniman et al., 2012). The CDW consists of the Upper Circumpolar Deep Water (UCDW) and Lower Circumpolar Deep Water (LCDW). Warm water from the UCDW is delivered onto the WAP by flow through shelf crossing canyons (Moffat et al., 2009) and eddies shed from the ACC (Martinson and McKee, 2012).

UCDW intrusions are typically found at ~150m and supply heat and nutrients to the WAP shelf (Prezelin et al., 2000, Martinson and McKee, 2012). These intrusions play an important role in marine ecosystems of the region (Ducklow et al., 2012) by providing nutrients important for diatom community abundance (Prezelin et al., 2000). In areas where the UCDW is not a dominant influence, such as near Gerlache Strait and in glacial melt zones near shore, other phytoplankton taxa dominate (Prezelin et al. 2000). Therefore, it is evident that there is a coupling of physical environmental processes with nutrient distributions, effecting phytoplankton assemblages in the WAP (Prezelin et al. 2000).

Pelagic and Benthic Communities of the WAP

Primary production along the WAP is strongly seasonal with marine ecosystem dynamics of the WAP strongly influenced by duration and extent of seasonal sea ice (McClintock et al., 2008, Montes-Hugo et al., 2009, Smith et al., 2012, Ducklow et al., 2013). Many species' life cycles are adapted to this seasonality (Ducklow et al., 2012,

Ducklow et al., 2013). Macronutrients are abundant in the WAP (Prezelin et al., 2000, Ducklow et al., 2012), however, micronutrients, such as iron, appear to limit primary productivity in offshore and slope areas (Prezelin et al., 2000, Garibotti et al., 2003, Prezelin et al., 2004). Phytoplankton blooms begin in spring as winds decrease and sea ice and glacial melting contribute to surface water stratification (Vernet et al., 2008, Montes-Hugo et al., 2009, Ducklow et al., 2008, Ducklow et al., 2012). There is high diversity of phytoplankton assemblages along the shelf with small-celled phytoflagellates and cryptophytes dominating phytoplankton biomass in the north and large-celled diatoms dominating in the south (Montes-Hugo et al., 2009, Ducklow et al., 2012). Zooplankton taxa in the region include krill, copepods, salps, and pteropods (Loeb et al., 1997, Ashjian et al., 2004, Ross et al., 2008, Ducklow et al., 2012). Distribution and abundance appear to correlate with seasonal sea ice dynamics (Ashjian et al., 2004, Ross et al., 2008) with krill appearing at ice edges (Ross et al., 2008) and the range of salps increasing southward coincident with decreasing sea ice (Ashjian et al., 2004). Cross-shelf gradients also exist in the WAP with populations of krill dominating inshore areas and salps being more abundant in offshore, outer slope areas (Ross et al., 2008, Ducklow et al., 2012).

Pelagic productivity plays a critical role for the benthic community in the WAP. Benthic invertebrates living beneath the euphotic zone receive most of their food via flux of primary production from surface waters. The benthic community on the continental shelf of the WAP usually receives an intense pulse of organic matter (OM) during austral spring-summer due to decreased sea ice and increased primary production (Smith et al., 2012). Benthic biomass in this region can be high (Arntz et al., 1994) suggesting efficient transfer of surface primary production to the seafloor (Smith et al., 2006).

Deposit feeding echinoderms, including holothurians (*Peniagone vignoni, Protelpidia murrayi, Pseudostichopus* sp., and *Molpadia musculus*) and echinoids (*Amphpineustes lorioli, Sterechinus antarcticus,* and *Ctenodicaris perrieri*), are prevalent in the megafaunal benthic community of the WAP continental shelf (Sumida et al., 2008, Wigham et al., 2008) and are suggested to play key roles in benthic trophic dynamics and biogeochemical processing within the region (Smith et al., 2006, 2008). The feeding behavior and digestive capacities of deposit feeding holothurians are adapted to maximize the use of OM, optimizing growth and survival (Roberts et al. 2000). They process large volumes of benthic sediments from which they assimilate bacterial and detrital OM (Deming and Colwell, 1982, Lopez and Levinton, 1987, Billett, 1991, Amaro et al., 2009). Cycling of OM by benthic organisms is of global significance (Karlson et al., 2010) because the seabed is the ultimate sink for pelagic production, making benthic communities long-term integrators of overlying water processes.

Predictions and Impacts of Climate Change

The WAP is experiencing some of the most rapid regional climate warming rates in the world, where atmospheric temperatures have increased ~1°C per decade over the last 60 years (Vaughan et al., 2003). The surface ocean of the region has warmed more than 1°C since the mid-1950s (Meredith and King, 2005). Similar impacts of warming have been noted in the Arctic, where atmospheric warming has increased ocean temperatures and resulted in decreased extent of sea ice (Comiso, 2003, Stroeve et al., 2007, Kwok and Rothrock, 2009, Polyakov et al., 2010) by as much as 10% per decade (Comiso et al., 2008). Waters of the Antarctic Peninsula are predicted to change only slightly, based on global

climate models (Clarke et al., 2012), but recent *in situ* measurements by the Palmer-LTER program suggest more frequent intrusions of warm UCDW waters onto the shelf contribute to increased heat input (Martinson et al., 2008, Martinson and McKee, 2012). This process could be associated with an increase in wind forcing over the ACC and subsequent strengthening of upwelling at the shelf break (Thompson and Solomon, 2002, Thoma et al., 2008). Increased heat at the surface ocean and at depth has contributed to declining sea ice (Moffat et al., 2009, Holland et al., 2010) as well as the retreat of glaciers (Cook et al. 2005). Variability of estimated atmospheric climate warming in the WAP region from models and projections indicates the degree of uncertainty in our ability to predict future scenarios for the region.

It is likely that sea ice will continue to decline, enhancing changes to the pelagic and possibly benthic ecosystem (Smith et al., 1996, Stammerjohn et al., 2008). Warming in the WAP has caused shifts in the function and structure of all trophic levels of the pelagic food web, from phytoplankton composition to penguin communities (Montes-Hugo et al., 2008, Ross et al., 2008, Ducklow et al., 2007, Ducklow et al., 2012, Ruck et al., 2014). The magnitude of phytoplankton blooms in the WAP are coupled with seasonal sea ice, with increased biomass occurring during high sea ice years, stratified surface waters, and increased light availability (Ducklow et al., 2013). Since the 1970s, primary production in the northern WAP has declined (Montes-Hugo et al., 2009). Physical factors influencing the decrease of phytoplankton biomass in the northern WAP include increased surface wind during ice-free conditions which causes the surface mixed layer of the ocean to deepen (Mitchell and Holm-Hansen, 1991, Montes-Hugo et al., 2009) exposing phytoplankton cells to less light for photosynthesis. As phytoplankton biomass has decreased in the northern

WAP, the southern WAP has experienced increased phytoplankton biomass due to more ice-free days in areas previously ice covered most of the year (Montes-Hugo et al., 2009). These conditions have been accompanied by a shift in phytoplankton and zooplankton community compositions.

The northern WAP has exhibited declines in phytoplankton biomass while the southern WAP has seen a significant increase (Montes-Hugo et al., 2009). These regional trends in phytoplankton biomass have likely contributed to changes in higher trophic levels, such as declining krill (Atkinson et al., 2004) and Adélie peguin (Ducklow et al., 2012) populations. Zooplankton community composition has shifted from being krill dominated throughout the region to salp populations dominating in the northern WAP and krill populations dominating in the southern WAP (Ross et al., 2008). Shifting the zooplankton community structure from krill to salp dominated could affect biogeochemical cycling in the WAP (Ducklow et al., 2012). Changing phytoplankton and zooplankton compositions have the potential to affect benthic communities by impacting the quality and quantity of OM flux to the seafloor.

The fauna of the continental shelf of Antarctica have been isolated from the rest of the world by temperature, the ACC, and an expanse of deep ocean (Clarke et al., 2012). Compared to lower latitudes, Antarctic benthic species are characterized by poor capacities to change their physiological state in response to climate change (Peck, 2005). Climate induced shifts in the pelagic ecosystem may affect benthic organisms causing shifts in the benthic ecosystem that could affect carbon burial (Smith et al., 2012). Warm water intrusions onto the continental shelf could also alter the benthic community by increasing microbial remineralization efficiencies (Arnosti and Jorgensen, 2003) and particulate organic carbon

mineralization rates in the sediment (Robador et al., 2010), effectively reducing the food bank (Mincks et al., 2005, Smith et al., 2012). These warm water intrusions could also lead to invasions by predators (e.g. crabs) formerly kept out by temperature constraints, which have the potential to greatly reduce benthic biodiversity and cause drastic shifts to the ecosystem (Aronson et al., 2007, Smith et al., 2011, Aronson et al., 2014, Aronson et al., 2015). In the Bering Sea region of the Arctic, benthic biomass has been declining in response to the warming climate (Grebmeier et al., 2006). Climate-induced responses in Antarctic benthos have been detected in only a few species, including bryozoans (Barnes et al., 2014, Moon et al., 2015) and ascidians (Sahade et al., 2015). In nearshore areas, the effects of warming temperatures, including increased sedimentation due to glacial retreat, are causing shifts in coastal benthic communities (Sahade et al., 2015). It is unknown how climate change will impact the benthic community of the WAP, and effects at the community level are unknown because there are few baseline studies to assess change against (Sahade et al., 2015).

Improved knowledge of the functional roles of marine benthic invertebrates is required to understand energy flow through food webs in order to better predict benthic climate related changes (Hughes et al., 2011) on the WAP. The complexity of interactions between the physical environment and biological processes of the region make it nearly impossible to predict the outcome of varied changes occurring due to climate change (Clarke et al., 2012). Even though predictions of ecosystem responses to climate change should be made with care, there are some predictions for which there is much agreement. This includes a predicted increase in primary productivity due to enhanced water column stability from increased freshwater input (Clarke et al., 2012). However, the decrease in sea ice will allow a

deepening of the mixed layer which might decrease production with a shift in phytoplankton community from diatoms to smaller flagellates (Walsh et al., 2001).

Benthic Holothurians of the WAP

Marine organisms living at low temperatures, such as those in the Antarctic, often exhibit lower physiological performance than similar warm water species (Clarke 1983, Clarke 2003, Peck 2002, Peck 2005). Growth (Everson, 1977, Peck et al., 2000, Brockington, 2001, Peck, 2005, Barnes et al., 2007), developmental (Bosch et al., 1987, Klages, 1993, Stanwell-Smith and Peck, 1998, Powell, 2001, Peck, 2005), metabolic (Peck et al., 2004, Peck, 2005), and activity (Peck et al., 2004, Peck, 2005, Peck et al., 2006) rates are all slower compared to similar temperate species. Holothurians are the dominant benthic invertebrate megafauna in abundance and biomass in deep-sea regions (Billett, 1991). The diversity and abundance of holothurians in the Antarctic is comparable to the deep sea (Gage and Tyler, 1991). Diversity of holothurians in the Antarctic region is great, with 187 species described, representing about 4% of total documented Antarctic marine organisms (O'Loughlin et al., 2011). They are also prevalent on the continental shelf of the WAP (Sumida et al., 2008). Life spans are extended and sexual maturity is deferred in Antarctic benthic fauna (Clarke, 1987, Peck, 2005), and slower metabolism is an advantage because less energy is required to survive during periods of low food availability in winter (Peck, 2005).

Quality and quantity of food availability has been the focus of many studies of benthic communities. Abundance of benthic macro- and megafauna was strongly correlated with food supply in the Bering and Chukchi Seas (Grebmeier et al., 1988), equatorial Pacific (Smith et al., 1997), northeast Pacific (Ruhl and Smith, 2004), and northeast Atlantic (Bett et

al., 2001). Mobility and feeding strategies are important for resource partitioning in the benthic community and can influence community composition. This has been documented in the Porcupine Abyssal Plain (Bett et al., 2001, Billett et al., 2001, Ginger et al., 2001), Arabian Sea (Jeffreys et al., 2009), WAP (Mincks et al., 2005, Purinton et al., 2008), and north Pacific (Kaufmann and Smith, 1997). In order to gain more insight into food utilization of the benthic community of the WAP, the two holothurians focused on in this study were the surface deposit-feeder, *Protelpidia murrayi*, and the subsurface deposit feeder, *Molpadia musculus*. They were chosen because they exhibited different feeding strategies and were prevalent throughout the study region.

P. murrayi is a member of the order Elasipodida, which live on the surface of the seabed and in deep-sea environments (Hansen, 1975, Billett 1991). About 30 species of elasipodid holothurians have been reported from the Southern Ocean deep sea (Brandt et al., 2012). They are characterized by soft, gelatinous bodies (Billett, 1991) with tentacles surrounding a ventrally positioned mouth (Hansen, 1975, Roberts and Moore, 1997). *P. murrayi* is mobile (Billett, 1991) and able to feed on the freshest fraction of OM present on the uppermost layer of the seabed (Yingst, 1982). In the WAP, they consumed bulk sediments and selectively assimilated optimal nutritional components during digestion (Mincks et al., 2008, Purinton et al., 2008). Enriched δC^{13} values (Mincks et al., 2005) could be the result of utilizing bacteria and their exudates as a food source (Lovvorn et al., 2005, Jeffreys, 2006). *P. murrayi* has exhibited seasonal isotopic variability similar to the isotopic composition of bulk sediment on which it feeds (Mincks et al., 2008). It exhibited seasonal spawning in the WAP, which may be linked to seasonal food availability (Galley et al., 2005). *P. murrayi* were dominant in video surveys of the WAP and exhibited interannual

population differences between spring and summer seasons (Sumida et al., 2008). Population differences were likely due to pelagic larval stages and winter recruitment of this species (Galley et al., 2008). Similar species have been studied in deep-sea regions including the Japan Trench (Sibuet et al., 1988), Weddell Sea (Gutt, 1991), Ross Sea (O'Loughlin et al., 2011), Santa Catalina Basin (Smith and Hamilton, 1983, Plante and Jumars, 1992, Miller et al., 2000), and the NE Pacific (Kaufmann and Smith, 1997, Ruhl and Smith, 2004).

M. musculus is a member of the order Molpadida, which are subsurface deposit feeders and mound builders (Billett, 1991, Amaro et al., 2010). Molpadia typically live buried in muddy or silty sediments from shallow to abyssal depths (Rhoads and Young, 1971, Pawson et al., 2001). Molpadiid species have been found in deep-sea regions including Santa Catalina Basin (Millet et al., 2000), the Norwegian Sea (Hoisaeter, 1990), and the NE Atlantic (Iken et al., 2001, Amaro et al., 2009), as well as the shallow depths of Cape Cod Bay (Rhoads and Young, 1971). They have simple tentacles, no tube feet, and a smooth body wall that is soft and pliant (Pawson, 1977, Billett, 1991). They have been found to non-selectively ingest sediment feeding head down while keeping their tail in contact with the sediment surface (Pawson et al., 2001) causing an upward transport of particles (Rhoads, 1974). In the WAP region, *M. musculus* likely feed deeper than the sediment surface and thus not directly on the phytodetrital layer (Wigham et al., 2008).

Using Lipid Biomarkers in Food Web Studies

Although many investigations focusing on nutrition of deposit-feeding holothurians have been done (Deming and Colwell, 1982, Sibuet et al., 1981, Billett et al., 1988, Roberts et al., 1991, Roberts et al., 2000, Ginger et al., 2001, Witbaard et al., 2001, Hudson et al.,

2005), the biochemical composition of OM ingested and assimilated by holothurians is still largely unknown. Lipid biomarkers have been used to gain insight into available food sources and preferential utilization of these food sources by benthic invertebrates. Lipids are useful biomarkers because they are less prone to rapid degradation than proteins or carbohydrates and are more structurally diverse (Jeffreys, 2006). Biomarkers such as these are especially useful in environments where direct observation poses significant challenges, such as the WAP. Lipids are essential biochemical components found in all organisms and many cannot be synthesized by benthic invertebrates *de novo* and must be obtained through their diet (Brett and Mueller-Navarra, 1997, Ginger et al., 2001).

Dietary sources of lipids, specifically fatty acids (FAs) and sterols (STs) differ in structure and lability. FAs appear to be more reactive and readily assimilated than STs (Wakeham and Canuel, 1986, Volkman et al., 2008), but the diversity in ST structure and stability in an ecosystem also make them useful biomarkers for surface production (Parrish et al., 2000). Coupling the two lipids together can help verify source of OM in an ecosystem. These compounds are transferred through the food web with little to no modification (Dalsgaard et al., 2003, Drazen et al., 2008) and can be used to track food sources, identify feeding niches, and explore feeding modes (Ginger et al., 2000, Hudson et al., 2004). Certain FAs and STs can be used as markers for specific types of phytoplankton (Volkman, 1986, Parrish et al., 2000, Dalsgaard et al., 2003, Volkman, 2006), bacteria (Howell et al., 2003, Parrish et al., 2005) or to trace the origin/source of particulate OM to the seafloor (Boon and Duienvald, 1996, Wakeham et al., 1997, Suhr et al., 2003).

Benthic organisms are long term integrators of OM flux to the seafloor. Lipids are ideal biomarkers to study in this context because there is no rapid turnover in tissues (Iverson

et al., 2004). FAs are incorporated into consumer tissue and are either used for energy or stored, thus FAs deposited into tissues provide an integrated record of an organisms diet (Iverson et al., 2004). A number of recent studies have pursued baseline research for biochemical composition of benthic communities in the world's oceans. Lipids have been used to investigate inputs, quality, and cycling of OM in a variety of environments including continental shelves of Greenland, Spitsbergen, and the Barents Sea (Graeve et al., 1997), Prydz Bay, Antarctica (Skerratt et al., 1995), Porcupine Abyssal Plain (Ginger et al., 2000, Hudson et al., 2004, Neto et al., 2006), the WAP (Suhr et al., 2003), the northeast Atlantic (Kiriakoulakis et al., 2004), the Bering and Chukchi Seas (Budge et al., 2007), the Florida Everglades (Belicka et al. 2012), the Southern Ocean (Giulini et al, 2013), and the Coral and Tasmanian Seas (Parrish et al., 2015).

Graeve et al. (1997) measured fatty acid compositions of various benthic organisms located on the continental shelves of Greenland, Spitsbergen, and the western Barents Sea and found that polyunsaturated FAs (PUFAs) were prevalent and the FA composition was influenced by food source. An investigation of the lipids of holothurians of the Porcupine Abyssal Plain (PAP) in the north east Atlantic showed seasonal and reproductive differences in fatty acid composition (Hudson et al., 2004). FA composition of foraminifera from the continental shelf of the western Antarctic Peninsula, indicated the foraminifera fed selectivity on specific components of deposited OM (Suhr et al., 2003). Analysis of lipids determined that microbial and algal food sources were of great importance to the freshwater ecosystem of the Everglades (Belicka et al., 2012). Kiriakoulakis et al. (2004) used lipids to assess that the quality of OM as a food source was high at the Darwin Mounds in the Rockwall Trough north of the United Kingdom due to the high concentrations of PUFAs. Seasonal and

interannual changes in plankton biomass in the eastern Antarctic were detected using lipids (Skerratt et al., 1995).

FAs are carboxylic acids with a long hydrocarbon chain. FAs are named here as A:BωC, where A refers to the number of carbon atoms in the molecule, B refers to the number of double bonds, and C, when confirmed, indicates the position of the first double bond counted from the terminal methyl group (Parrish et al., 2000). All additional double bonds are assumed to be separated by a single methylene group (i.e., $-CH_2$). Variation in FA structure occurs in length of hydrocarbon chain, number of double bonds (i.e. degree of unsaturation), and the position of the double bonds in the chain (Parrish et al., 2000, Gurr et al., 2002).

STs constitute a large, diverse group of compounds, and are characterized by a four-ring skeleton with the carbons numbered as in Figure 3 (Martin-Creuzberg and von Elert, 2009). They usually have 27 to 30 carbon atoms, a hydroxyl atom at the C_3 position, and a side chain of variable length at the C_{17} position (Heupel, 1989). STs are defined by number and position of double bonds, presence of methyl groups on ring structure, and length of side chain (Heupel, 1989, Martin-Creuzberg and von Elert, 2009).

Importance and Objectives of Study

A key issue in our efforts to document ecological impacts of climate change in polar regions is the lack of reliable baseline information from which change can be identified (Wassmann et al., 2011). Animal distributions are likely to shift, although the scale and nature of such shifts, and implications for ecological relationships, reproduction, and survival

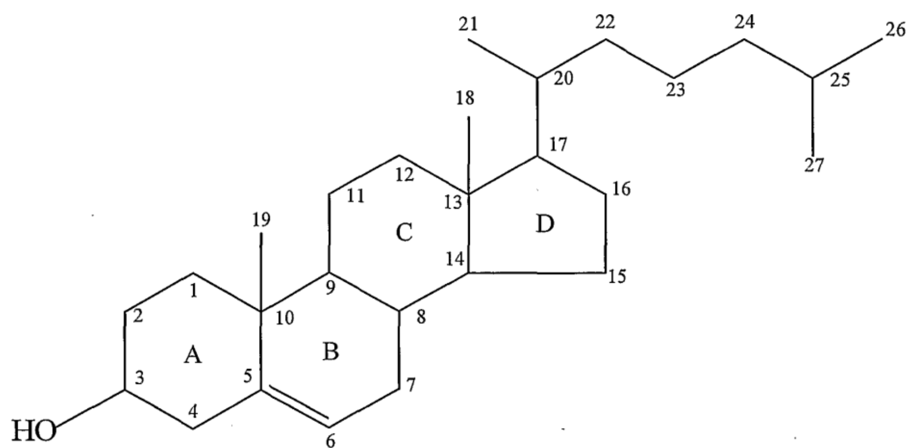

Figure 3. Example of carbon numbering system and ring structure of sterols. This example illustrates the sterol cholesterol.

are poorly understood (Clarke and Harris, 2003). The research discussed in the following chapters was accomplished as part of the FOODBANCS2 Project, the purpose of which was to observe and document changes in the benthic environment along a north-south transect of the WAP (Smith et al., 2008). This transect exhibited a gradient in seasonal sea ice coverage and provided an opportunity to study benthic ecology and biogeochemical cycling in response to differing pelagic regimes (i.e. mostly ice free in the north and mostly ice covered in the south).

The objectives of this research are:

1) To use lipid biomarkers to assess the biochemical makeup of food sources and benthic consumers. Establish a baseline which can be used as a reference to monitor changes in the region
2) To investigate latitudinal variation in lipid composition along the WAP
3) To investigate seasonal variation in lipid composition along the WAP

In chapter 2, the food web of the WAP was investigated by characterizing FA and ST compositions of food resources, namely phytodetritus, and benthic consumers with different feeding strategies, *P. murrayi* and *M. musculus*. Chapter 3 focused on latitudinal and seasonal differences of FA and ST compositions on the north-south transect of the WAP in surface sediments, *P. murrayi*, and *M. musculus* to determine if pelagic climate-induced changes could be observed in the benthos. Through these chapters a baseline of biochemical composition of food sources and benthic consumers was established which can be used as a reference to monitor changes in the region.

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CHAPTER 2

Feeding ecology based on fatty acid and sterol composition of holothurians and their food sources from the western Antarctic Peninsula

Introduction

The deep sea covers about 60% of the Earth's surface and is usually a food-limited environment, where the seafloor acts as a sink for organic matter (OM) derived from primary production in surface waters. Holothurians dominate benthic invertebrate megafaunal populations in both abundance and biomass in many deep-sea regions (Billett, 1991, Billett et al., 2001, Smith et al., 2009, De Leo et al., 2010). Many of these animals consume and utilize the freshest particles derived from phytodetritus and OM present in surface sediments, whereas others feed on more refractory OM at subsurface depths (Billett, 1991). Benthic deposit feeders can respond quickly to the arrival of OM to the seafloor (Billett, 1991, Hudson et al., 2004) and through their feeding and movement, can rework the OM in the upper layers of the seabed (Ginger et al., 2000, Bett et al., 2001), contributing to the consumption and redistribution of nutrients and cycling of carbon and nitrogen within the sediments (Lauerman et al,. 1996).

The deep continental shelf of the Western Antarctic Peninsula (WAP; 500-800m) is characterized by strong seasonality of primary production, with low production levels during dark, ice-covered winter months and higher production rates during ice-free spring and early summer months (Clarke, 1988, Laureillard et al., 1997, Ducklow et al., 2006, Vernet et al., 2008). Transfer of bloom-derived OM to the seafloor occurs through rapidly sinking fecal pellets of herbivorous zooplankton, phytoplankton aggregates, and slowly sinking intact

phytoplankton (Suzuki et al., 2001) that can lead to high accumulation rates of OM on the shelf floor (Gutt, 1998, Smith et al., 2006, 2008), sustaining detritivores living in the benthic community. This pattern of extensive deposition of phytodetritus after massive seasonal blooms has been observed in the Arctic (Grebmeier, 1988, Grebmeier and Barry, 1991), Antarctic (Grebmeier and Barry, 1991, Gutt et al., 1998, Smith et al., 2006) and many deep-sea environments (Billett et al., 1983, Hobson et al., 1995, Bett et al., 2001, Witte et al., 2003). However, climate-induced changes in the WAP region could impact the way OM is produced and utilized in the pelagic zone before it reaches the seafloor.

The WAP is experiencing some of the most rapid regional climate warming rates in the world. Winter temperatures have increased ~1°C per decade over the last 60 years (Vaughan et al., 2003), causing water temperatures to increase (Martinson et al., 2008, Meredith et al., 2008) and the duration and extent of sea ice to decline (Ducklow et al., 2007, Martinson et al., 2008, Stammerjohn et al., 2008). Warming temperatures on the WAP are causing a north-south gradient in climate that is warmer and more moist in the north, i.e. more subpolar, and colder and more dry in the south, i.e. typical polar climate (Ducklow et al., 2007). As physical characteristics of the WAP region shift, biological processes have been changing too, including increases in bacterial activity, shifts in phytoplankton composition (i.e. cryptophytes and prymnesiophytes replacing diatoms), zooplankton composition (i.e. salps replacing krill), and decreases in ice-dependent penguin populations (Atkinson et al., 2004, Montes-Hugo et al., 2008, Ross et al., 2008, Ducklow et al., 2007, Ducklow et al., 2012). Changes to the autotroph community structure resulting from changes in sea ice coverage would likely propagate to higher trophic levels in the water column.

Furthermore, changes in the quality and quantity of OM flux to the benthos will also likely impact the structure of the benthic community.

Deposit feeding echinoderms, including holothurians (*Peniagone vignoni, Protelpidia murrayi, Pseudostichopus* sp., and *Molpadia musculus*) and echinoids (*Amphpineustes lorioli, Sterechinus antarcticus,* and *Ctenodicaris perrieri*) are prevalent in the megafaunal benthic community of the WAP continental shelf (Sumida et al., 2008, Wigham et al., 2008) and are suggested to play key roles in benthic trophic dynamics and biogeochemical processing of the region (Smith et al., 2006, 2008). Different feeding strategies, including tentacle morphology (Hansen, 1975) and species mobility (Miller et al., 2000), allow deposit feeders to selectively process OM in sediments. Deposit feeders have the ability to ingest large quantities of sediment by extending their tentacles and capturing food particles, which coupled with low metabolism, make them suited for deep-sea life (Hansen, 1975). Surface deposit feeders are prominent in deep-sea regions and are able to feed on the freshest fraction of OM on the top layers of seafloor sediment (Hansen, 1975, Yingst, 1982). Their mouths are positioned ventrally, allowing direct contact of tentacles with phytodetritus collected by shoveling or raking, mechanical trapping, or adhesion (Lawrence, 1987).

Elasipods, such as *Protelpidia murrayi*, are mobile, surface deposit feeders and have shield-shaped tentacles used for shoveling sediment into the mouth and may be selective feeders on the basis of particle size or OM content (Roberts, 1979, Billett et al., 1988, Billett, 1991). The subsurface deposit feeder *Molpadia musculus* typically lives buried in muddy or silty substrates from shallow to abyssal depths (Rhoads and Young, 1971, Pawson et al., 2001). This infaunal deposit feeder has no tube feet and simple tentacles are used to non-selectively ingest sediment (Pawson et al., 2001). By ingesting sediment at depth and

excreting at the sediment surface, *M. musculus* transports particles upward, building mounds during the process (Billett, 1991, Pawson et al., 2001, Amaro et al., 2010).

Improved knowledge of the functional roles of marine benthic invertebrates is required to understand energy flow through food webs to better predict climate-related changes (Hughes et al., 2011) on the WAP. Soft bottom ecosystems, such as those found in our study area, are particularly important on a global scale due to storage and cycling of nutrients (Snelgrove et al., 1997, Covich et al., 2004). Cycling of OM by benthic organisms is of global significance (Karlson et al., 2010) because the seabed is the ultimate sink for pelagic production, and OM buried deep within the sediment can remain a valuable food source for weeks to months (van de Bund et al., 2001). Benthic communities are often described as long-term integrators of overlying water processes (Grebmeier et al., 1988, Mincks et al., 2008). Feeding and bioturbation rates of benthic organisms may result in altered oxygen conditions in the seabed, which, in turn, may affect mineralization rate of phytodetritus (Fenchel et al., 1998).

While both types of deposit feeders consume bulk sediment, surface deposit feeders feed upon the freshest fraction of OM on the sediment surface (Yingst, 1982) and may be selective feeders on the basis of particle size (Roberts, 1979) or quality of OM deposits (Billett, 1991), whereas subsurface deposit feeders are exposed to and ingest more refractory material. Recent studies in the region using ^{14}C and ^{234}Th tracers suggest that megafaunal deposit feeders of the WAP ingest and assimilate OM throughout the year (Purinton et al., 2008, McClintic et al., 2008). In addition the ^{14}C age of the ingested organic matter (younger typically being fresher) in *M. musculus* was nearly identical to that of the bulk organic matter ^{14}C age in the near-surface seabed (Purinton et al., 2008). In contrast, the sediment ingested

by *P. murrayi* was typically younger (fresher) than that of *Molpadia musculus* (Purinton et al., 2008).

In this study, lipid biomarkers, specifically fatty acids (FAs) and sterols (STs), were used to track OM through the food web of the WAP. Biomarkers such as these are especially useful in environments where direct observation poses significant challenges. Lipids are essential biochemical components found in all organisms. Benthic invertebrates are unable to synthesize many required lipids *de novo* and must obtain them through their diet (Brett and Mueller-Navarra, 1997, Ginger et al., 2001). FAs and STs can be used as markers for types of phytoplankton (i.e., diatoms or dinoflagellates; Volkman, 1986, Parrish et al., 2000, Dalsgaard et al., 2003, Volkman, 2006), bacteria (Howell et al., 2003, Parrish et al., 2005), or to trace the origin of OM to the seafloor (Boon and Duienvald, 1996, Wakeham et al., 1997, Suhr et al., 2003). FAs and STs are transferred through the food web with little to no modification (Dalsgaard et al., 2003, Drazen et al., 2008) and can be used to identify food sources, feeding niches, and explore feeding modes (Ginger et al., 2000, Hudson et al., 2004).

The goal of this study was to characterize FA and ST compositions of food resources (i.e., OM in sediments) and benthic consumers (i.e., *P. murrayi* (surface deposit feeder) and *M. musculus* (subsurface deposit feeder) to investigate OM cycling through the benthic food web of the WAP. Additionally, we investigated the selectivity of holothurian feeding and the importance of bacteria in the food web.

Materials and Methods

Study area and sampling

Samples were collected at 4 stations on a north to south latitudinal gradient (63°S - 68°S) along the WAP during cruises in winter 2008 (15 July – 1 August; FOODBANC2-2) and summer 2009 (22 February – 5 March; FOODBANC2-3) (Fig.1). Station depths ranged from 516 – 685 m and each site had muddy sediments on the seafloor. Sampling sites were selected based on latitudinal temperature gradients and differences in sea ice extent (Smith et al. 2012).

A PARFLUX Mark 78H sediment trap was moored 150m above the seafloor at stations B and G. The instruments contained 21 cups to collect settling particles every 17 days from March 2008 – March 2009. Cups were filled with a supersaline formalin mixture. The sediment trap at station B malfunctioned and only one bottle, collected in spring 2008 (October), was used. Five bottles, collected from spring 2008 – early summer 2009, were sampled from the trap at station G. Sediment trap samples were filtered onto combusted GF/F 25mm filters and immediately frozen at -20°C. The samples were thawed prior to lipid extraction. Sediment cores (10cm diameter) were collected at each sampling site using a Bowers & Connelly Megacorer. Sediment cores (10cm diameter) were extruded to 1 cm, sliced, trimmed, and homogenized. The 0-1cm layer was immediately frozen at -80°C shipboard. Prior to analysis, the sediment samples were freeze-dried and homogenized.

Benthic megafauna were collected during both seasons using a 5.5 m semi-balloon otter trawl (2cm mesh). Two types of holothurians were collected for analysis. *P. murrayi,* the surface deposit feeder was present at stations B, E, and G, whereas *M. musculus,* the

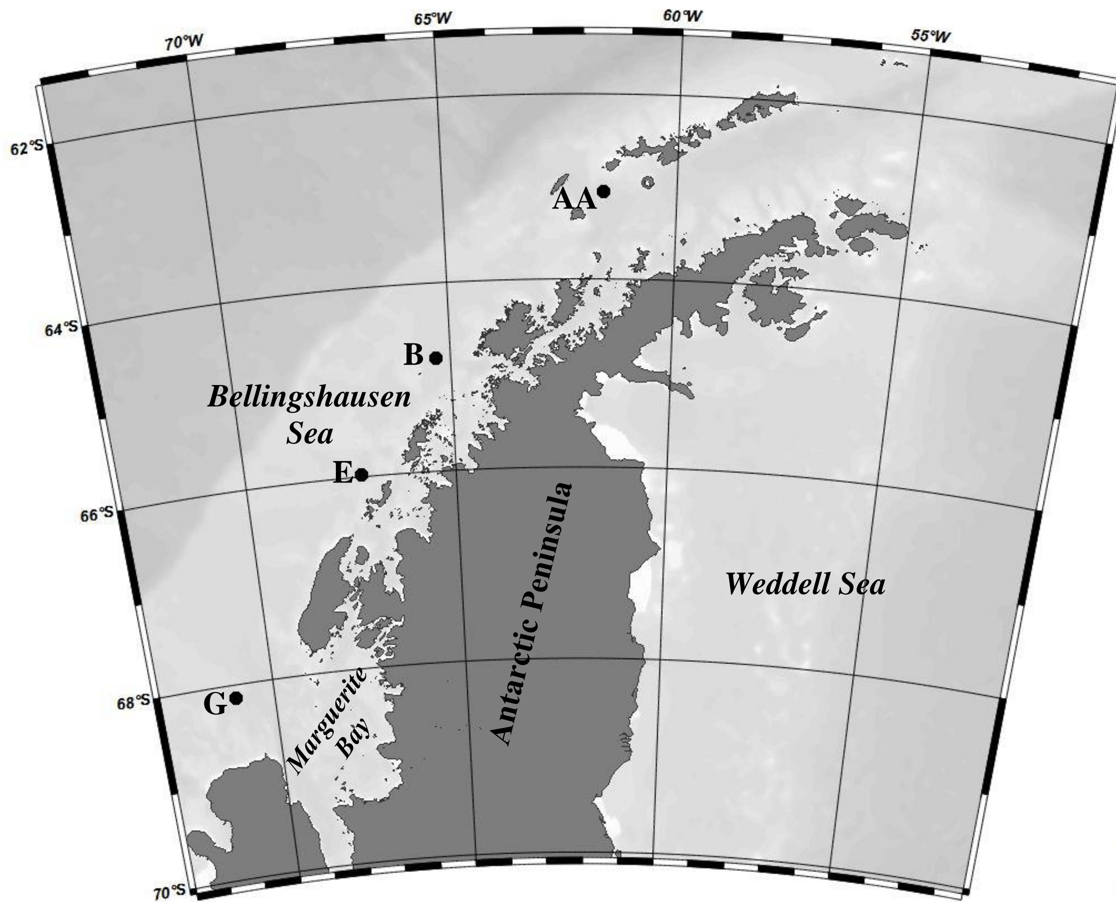

Figure 1. Sampling sites for the FOODBANCS2 Project. The sites from north to south were AA (63°04.727'S, 61°36.120'W), B (64°48.002'S, 65°21.453'W), E (65°58.949'S, 67°16.930'W), and G (68°08.547'S, 71°01.441'S).

subsurface deposit feeder, was collected at stations AA, B, and E. The two holothurians were chosen due to their abundance at most sampling sites and their contrasting feeding mechanisms. Up to 5 individuals of each species were dissected shipboard, with tissue samples stored in plastic bags, and frozen at -80°C. Upon moving them to the land-based laboratory, the samples were stored at -20°C until analysis could be performed. Body wall samples from the deposit feeders were thawed prior to lipid analysis. Body wall samples were chosen because gut contents are often contaminated with lipid from gut lining due to changes in pressure and temperature during recovery from the seafloor (Ginger et al., 2001, Hudson et al., 2005).

Lipid extraction and analysis

Lipids were extracted from surface sediment and freshly thawed holothurian body tissue following the method of Folch et al. (1957) with the modifications of Budge et al. (2006). Two modifications were made to this methodology. First, the antioxidant BHT was not used due to interferences with shorter-chain fatty acids and neutral lipid markers, and second, chloroform ($CHCl_3$) was replaced with similar, but less toxic, methylene chloride (CH_2Cl_2). Methylene chloride has been shown to be a suitable replacement for chloroform in lipid extractions (Cequier-Sanchez et al., 2008), with no significant changes in lipid distribution or lipid class analysis between the two extraction solvents.

Total lipids were extracted from homogenized samples ultrasonically with a 2:1 mixture (vol/vol) of methylene chloride:methanol (CH_2Cl_2:MeOH). Ultrapure (milli-Q) water was added to achieve a final ratio of 2:1:0.7 CH_2Cl_2:MeOH:H_2O, the samples were strongly agitated, and the lower organic phase was removed into a rotary evaporation flask. Fresh organic solvent was added to the remaining sample in the test tubes and the extraction

was repeated two more times. The three extracts were combined in the evaporation flask and excess solvent was removed by rotary evaporation. Total lipid extracts were flushed with N_2 gas and stored in CH_2Cl_2 at -20°C.

Total lipid extracts were saponified with 0.5N methanolic potassium hydroxide (KOH) at 70°C for 60 minutes (Ju et al., 2009). Neutral lipid fractions were partitioned three times with a mixture of hexane:diethyl ether (9:1), dried under N_2 gas, and treated with 50μl bis(trimethylsilyl) trifluoroacetamide (BSTFA) with 30% pyridine to convert free alcohols to their corresponding trimethylsilyl (TMS) esters. Polar lipid fractions were acidified to pH <2 with HCl, and free FAs were partitioned into 9:1 hexane:diethyl ether and combined. Solvent was evaporated from FA fraction under N_2 gas and FAs were methylated to corresponding methyl esters (FAMEs) with freshly-distilled diazomethane. Internal standards (5α-cholestane for neutral and squalane for polar) were added to each fraction prior to analysis with gas chromatography-mass spectrometry (GC/MS). A known quantity of internal standard was added to each extract to permit fatty acid methyl ester (FAME) quantification.

STs and FAs were identified and relative abundances determined using GC/MS with an Agilent 6890 gas chromatograph (GC) coupled to an Agilent 5973 mass spectrometer (MS) operating in electron ionization mode at 70 eV. The GC was equipped with a Restek Rtx®-5MS capillary column (5% diphenyl/95% dimethyl polysiloxane stationary phase, 30m length X 0.25mm i.d. X 0.25μm film thickness). Samples were injected in splitless mode with an initial oven temperature of 60°C. After a 2 minute hold at the intial temperature, the oven temperature was raised to 300°C at a rate of 6°C min^{-1}, followed by a 15 minute hold at 300°C. Peaks were visualized and integrated using ChemStation software and identification of STs and FAs were based on comparison of retention times with authentic standards

(Supelco PUFA No. 3 and C_4-C_{24} FAME mix) and mass spectra of standard and previously reported compounds. Following quantification of the FAMEs, double bond positions in some predominant unsaturated FAs were further confirmed through analysis of picolinyl esters following the method of Dubois et al. (2006). Drying organisms before lipid extraction to determine dry weight can influence lipid composition (Connelly et al., 2014).

A total of 35 FAs and 34 STs were identified in surface sediment and holothurian samples (Appendix A and B, respectively). The relative abundances of each were determined using peak areas and were expressed as a percentage of the total identified FAs and STs, respectively. FAs and STs were grouped based on source assignments (i.e., bacterial, diatom, and flagellate for FAs and phytosterols and 5α(H)-stanols for STs), similar to that described by Lebreton et al. (2011) and Belicka et al. (2012), to examine contributions of each (as defined in Table 1). High levels of Δ^5-sterols, such as those in this study, are often found in eukaryotes (Wakeham et al., 1997). Bacteria can modify sterol structures through biohydrogenation of Δ^5-stenols to 5α(H)-stanols (Rosenfel and Hellman, 1971, Eyssen et al., 1973, Volkman et al., 1990). Thus, an increase in the ratio of 5α(H)-stanols/Δ^5-stenols can be used to indicate bacterial alteration of OM (Li et al., 1995, Wakeham et al., 1997, Jeffreys, 2006).

Multivariate statistical analyses

Non-parametric, multi-dimensional scaling (nMDS) plots of FA and ST compositions, based on a Bray-Curtis similarity matrix, were used to explore relationships among the samples. Principal component analysis (PCA) was used to compare FA and ST compositions among the samples and identify FAs and STs contributing most to their

Table 1. Fatty acids grouped together to define sources of bacterial, diatom, and flagellate OM as well as phytosterols and stanols within the WAP samples based on the literature.

Carbon Source	References
Bacterial	
15:0i, 15:0a, 15:0, 17:0i, 17:0a, 17:0, 18:1ω7	Sargent et al., 1987
	Volkman et al., 1990
	Bowman et al., 1991
Diatom	
16:1ω7, 20:5ω3	Sargent et al., 1987
	Volkman et al., 1989
	Viso and Marty, 1993
	Graeve et al., 1997
	Parrish et al., 2000
	Dalsgaard et al., 2003
Flagellate	
18:1ω9, 22:6ω3	Sargent et al., 1987
	Volkman et al., 1989
	Graeve et al., 1997
	Parrish et al., 2000
Phytosterols	
cholest-5-en-3β-ol	Nichols et al., 1991
cholesta-5,22-dien-3β-ol	Barrett et al., 1995
24-methylcholesta-5,22-dien-3β-ol	Volkman et al., 1998
24-methylcholesta-5,24(28)-dien-3β-ol	Muhlebach and Weber, 1998
24-ethylcholesta-5,22-dien-3β-ol	Volkman, 2003
23,24-dimethylcholest-5-en-3β-ol	Dahl et al., 2004
24-ethylcholest-5-en-3β-ol	Drazen et al., 2008
4α,23,24-trimethylcholest-22-en-3β-ol	Wisnieski et al., 2014
cholest-7-en-3β-ol	
cholesta-7,22-dien-3β-ol	
24-methylcholesta-7,22-dien-3β-ol	
24-ethylcholesta-7,22-dien-3β-ol	
24-ethylcholest-7-en-3β-ol	
5α(H)-stanols	
5α-cholestan-3β-ol	Hudson et al., 2001
24-ethylcholestan-3β-ol	Jeffreys, 2006
24-methylcholest-22-en-3β-ol	Wisnieski et al., 2014
24-ethylcholestan-3β-ol	

separation. Only the first two principal components (PC1 and PC2) are presented. To investigate differences of lipid availability to each species along the latitudinal gradient, analysis of similarity (ANOSIM) was used to examine differences in FA and ST signatures among samples. Similar to Pethybridge et al. (2011), ANOSIM R-values were used to determine the extent to which the sample groups differed in FA and ST compositions ($R>0.75$: well separated groups; $R = 0.5-0.75$: separated but overlapping groups: $R = 0.25-0.50$: separated but strongly overlapping groups; $R<0.25$: barely separated groups). Differences in the FA and ST signatures between these stations were further explored using similarity percentages (SIMPER) to determine specific FAs and STs that contributed most to differences noted with ANOSIM. Data were left untransformed (Howell et al. 2003) so artificial weight would not be given to FAs and STs with trace contributions to FA and ST profiles (Hall et al. 2006). Multivariate analyses of FA and ST profiles were performed using the PAST statistical package (Hammer et al. 2001).

Results

FAs and STs were analyzed for all samples to decipher food source availability and food source preferences or preferential feeding/assimilation mechanisms of benthic organisms in this region. STs in holothurians have high levels of Δ^7 saturation (Ginger et al. 2000, 2001), which were evident in this study's samples. Phytosterols with Δ^5 saturation were prevalent in sediment trap and surface sediment samples, whereas their Δ^7 derivatives were prevalent in both holothurians. Thus, Δ^5 phytosterols that were present in holothurian tissues as Δ^7 STs will be interpreted as phytosterols in this study (Table 1). Cholest-5-en-3β-ol (cholesterol) is often present in zooplankton samples (Phleger et al., 2000, Volkman, 2005,

Wisnieski et al., 2014), and also can be derived from phytoplankton (Wisnieski et al., 2014), specifically diatoms (Volkman, 1986, Nichols et al., 1990, Barrett et al., 1995). Cholest-7-en-3β-ol can be produced by *de novo* biosynthesis (Goad, 1981), and abyssal holothurians appear to biotransform Δ^5 (i.e. cholest-5-en-3β-ol) to Δ^7 STs (i.e. cholest-7-en-3β-ol) (Voogt and Over, 1973, Smith and Goad, 1975, Goad, 1981, Ginger et al., 2000). Cholest-7-en-3β-ol has also been found in diatoms (Volkman, 1986, Barrett et al., 1995, Dahl et al., 2004). The prevalence of cholest-7-en-3β-ol in holothurian samples indicated that the biotransformation of cholesterol is likely a source of this ST since the presence of cholest-7-en-3β-ol in sediment trap and surface sediment samples is limited. Cholesterol and cholest-7-en-3β-ol were both prevalent in phytoplankton samples all along the latitudinal gradient of the WAP (Pirtle-Levy, Appendix B) and were considered to be phytosterols in this region.

Fatty acid and sterol compositions

Sediment traps

Sediment traps collected OM originating from the most prevalent phytoplankton in the WAP (i.e., diatoms and flagellates), and there was little evidence of degraded material or bacterial input indicating fresh OM flux from the surface ocean. All sediment trap samples were collected in summer 2009. Only 1 bottle was sampled from station B due to trap malfunction, and five bottles were sampled from station G. Sediment trap samples from stations B and G were similar in their FA composition (Fig. 2). Saturated FAs (SFAs) and monounsaturated FAs (MUFAs) were dominant, while polyunsaturated FAs (PUFAs) were present at <10% of total FAs (Fig. 2a). Diatom (9 – 23% of total FAs) and flagellate (8 – 37% of total FAs) markers were prevalent (Fig. 2b). Bacterial markers were present at <10%

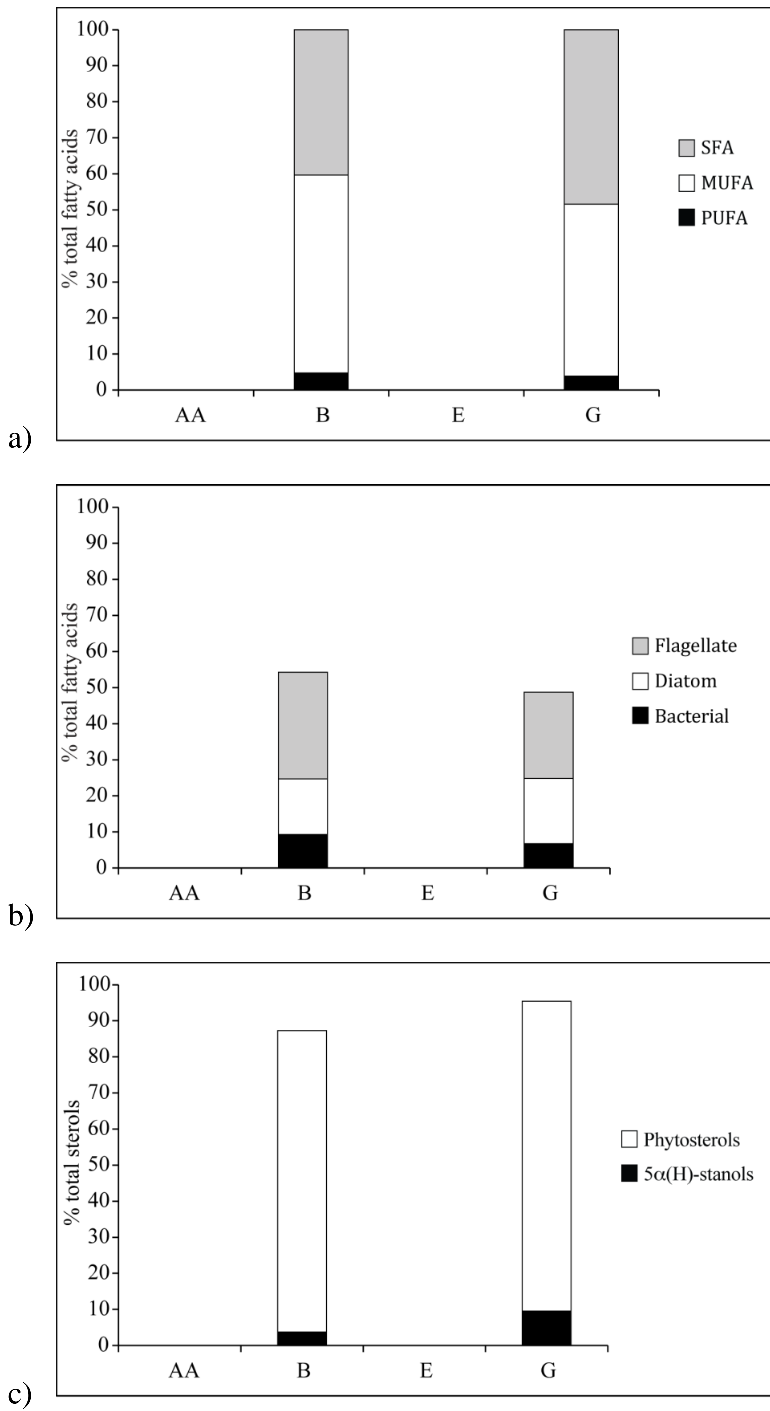

Figure 2. Composition of FAs and STs in sediment traps at stations B and G during summer a) SFA, MUFA, and PUFA as % of total FAs, b) FA biomarkers as % of total FAs, c) Phytosterols and 5α(H)-stanols as % total STs.

of total FAs (Fig. 2b). The FAs 16:0n and 18:0n were the most prevalent SFAs. The major MUFAs were 16:1ω7 and 18:1ω9 (Appendix A) indicating diatom and flagellate input, respectively (Sargent et al., 1987, Volkman et al., 1989, Parrish et al., 2000). Phytosterols made up >70% of total STs present in the trap samples (Fig. 2c). Cholest-5-en-3β-ol, 24-methylcholesta-5,22-dien-3β-ol, cholest-7-en-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol, and 24-ethylcholest-5-en-3β-ol were most prevalent. The presence of diatoms were indicated by the occurrence of cholest-5-en-3β-ol (Nichols et al., 1993, Volkman, 2003, Rampen et al., 2010), cholest-7-en-3β-ol (Volkman, 1986, Barrett et al., 1995, Dahl et al., 2004), 24-methylcholesta-5,24(28)-dien-3β-ol (Barrett et al., 1995, Drazen et al., 2008, Rampen et al., 2010), 24-methylcholesta-5,22-dien-3β-ol (Volkman, 2003, Drazen et al., 2008), and 24-ethylcholest-5-en-3β-ol (Nichols et al., 1993, Volkman, 2003). In addition to diatoms, 24-methylcholesta-5,22-dien-3β-ol could indicate the presence of cryptophytes (Dunstan et al., 2005, Volkman, 2006) and 24-ethylcholest-5-en-3β-ol could indicate presence of prymnesiophytes (Volkman, 1986). 5α(H)-stanols, as described by Hudson et al. (2001) and Wisnieski et al. (2014), accounted for <14% of total STs in these samples (Fig 2c).

Surface sediments

Phytoplankton sourced OM was evident in surface sediments indicating pelagic-benthic coupling. Bacterial inputs were more prevalent compared to sediment trap samples indicating degradation processes were occurring. Surface sediments were collected during winter 2008 and summer 2009. There were no differences in FA and ST composition on the latitudinal gradient (Fig. 3). Sediment FAs were dominated by SFAs and MUFAs which made up >94% of total FAs. PUFAs contributed ≤ 5% of total FAs consistent with

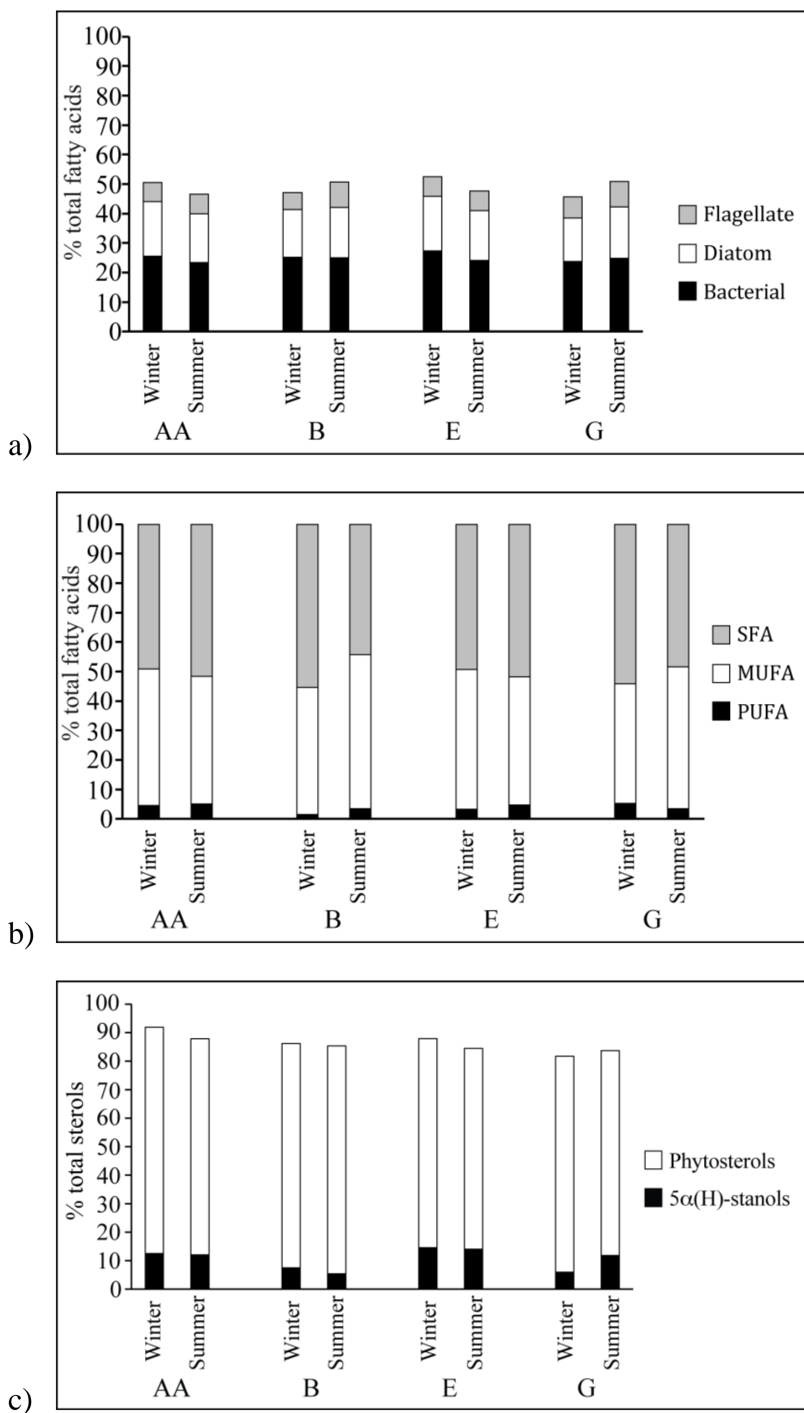

Figure 3. Composition of FAs and STs in surface sediment at stations AA, B, E, and G during winter and summer a) FA biomarkers as % of total FAs, b) SFA, MUFA, and PUFA as % of total FAs, c) Phytosterols and 5α(H)-stanols as % total STs.

distributions found in deep-sea sediments (Nichols, 2003). The FAs 16:0n and 18:0n were the most prevalent SFAs and 16:1ω7 and 18:1ω7 were the dominant MUFAs. 16:0n is present in many phytoplankton (Sargent et al., 1987) but this FA, in addition to 18:0n, are also considered to be products of degradation of long-chain, unsaturated FAs (Suhr et al., 2003). 16:1ω7 indicated the presence of diatoms (Graeve et al., 1997, Parrish et al., 2000) and 18:1ω7 the presence of bacteria (Bowman et al., 1991, Pearson et al., 2001, Hudson et al., 2004). Bacterial biomarkers accounted for 23 - 27% and diatom markers were 15 – 19% of total FAs. Flagellate markers made up <9% of total FAs in sediment. Since there was no difference in surface sediment FA composition on the latitudinal gradient during winter or summer, the samples were pooled together by season to determine if there was a seasonal difference within the study area. Based on ANOSIM ($R=-0.1$, $p > 0.05$), there was no significant seasonal difference and SIMPER showed the pooled seasonal samples to have 90% similarity.

Phytosterols accounted for ≥70% of total STs in surface sediment samples. The most prevalent STs were cholesterol, 24-methylcholesta-5,22-dien-3β-ol, and 24-ethylcholest-5-en-3β-ol. 5α(H)-stanols ranged from 5 – 15% of total STs in sediment samples. Based on ANOSIM ($R=0.11$, $p > 0.05$), there was no significant seasonal separation in ST composition and SIMPER showed the pooled seasonal samples to have 84% similarity.

Benthic deposit feeders

The two deposit feeding holothurians examined from the WAP in winter 2008 and summer 2009, *P. murrayi* and *M. musculus*, have different feeding mechanisms; *P. murrayi* is a surface deposit feeder and *M. musculus* is a subsurface deposit feeder. The feeding mechanisms and mobility of these holothurians enables them to access different sediment

horizons. *P. murrayi* is mobile and can access the freshest phytodetrital material at the sediment surface, while *M. musculus* feeds vertically or obliquely within the substrate of the seafloor and ingests sediment at depth, giving it access to older, more refractory OM (Wigham et al., 2008).

Protelpidia murrayi

P. murrayi fed at the sediment surface and had tissue with high levels of phytoplankton-derived lipids, predominately diatoms, indicating they are an important food source. SFAs were present at low levels and ranged from 16 – 22% of total FAs. MUFAs and PUFAs dominated the FA composition of the surface deposit feeder. MUFAs accounted for 17 – 38 % of total FAs. PUFAs were present at higher levels of 43 – 50% of total FAs. The SFAs were dominated by 18:0n. The FAs 22:1ω9 and 23:1ω9 were the prevalent MUFAs and are produced *de novo* by holothurian FA biosynthesis (Ginger et al., 2000). 20:4ω6 and 20:5ω3 were the prevalent PUFAs. 20:4ω6 is prevalent in phospholipids in marine animals (Suhr and Pond, 2006) and plays a role in cell maintenance and cold adaptation in deep-sea organisms (Harwood and Vigh, 2006), and 20:5ω3 indicated diatom input (Sargent et al., 1987, Viso and Marty, 1993, Parrish et al., 2000, Dalsgaard et al., 2003). Diatom FA markers were high, ranging from 20 – 24% of total FAs. Bacterial and flagellate markers were low, each making up <13% of total FAs. There was no seasonal difference of FA composition in *P. murrayi* tissues at stations B (ANOSIM, $R = 0.276$, $p > 0.05$) and G (ANOSIM, $R = 0.01$, $p > 0.05$). At station E, based on ANOSIM, FA compositions were separated but still had strong overlap ($R = 0.44$, $p < 0.05$).

STs in *P. murrayi* tissues had high levels of Δ^7 saturation, which has been found in many echinoderms (Ginger et al. 2000). The most prevalent Δ^7 STs were cholest-7-en-3β-ol

and 24-ethylcholest-7-en-3β-ol, which indicated the presence of diatoms (Volkman, 1986, Barrett et al., 1995, Volkman, 2003, Dahl et al., 2004). Phytosterols ranged from 49 – 77% of total STs with cholest-7-en-3β-ol being dominant. 5α(H)-stanols were significantly different between winter and summer at stations B (ANOSIM, $R = 1$, $p < 0.05$), E (ANOSIM, $R = 0.72$, $p < 0.05$), and G (ANOSIM, $R = 0.94$, $p < 0.05$). The differences were due to higher 5α(H)-stanol levels in summer compared to winter due to an increase of 24-ethylcholest-22-en-3β-ol at B, E, and G. 5α(H)-stanols ranged from 2 – 10% of total STs in winter and 9 – 22% of total STs in summer.

Molpadia musculus

M. musculus feeds at depth in seafloor sediment and had tissues with a mixture of phytoplankton-derived and bacterial lipids indicating a mixed diet of fresh and refractory OM. SFAs accounted for 9 – 17% of total FAs. Similar to *P. murrayi*, MUFAs and PUFAs dominated the FA composition of this subsurface deposit feeder. MUFAs accounted for 37 – 49 % of total FAs. PUFAs accounted for 34 – 49% of total FAs. The FAs 18:1ω7 (bacteria; (Bowman et al., 1991, Pearson et al., 2001, Hudson et al., 2004), 22:1ω9 (*de novo* produced; Ginger et al., 2000), and 23:1ω9 (*de novo* produced; Ginger et al., 2000) dominated the MUFAs and 20:4ω6 (cell maintenance; Harwood and Vigh, 2006) was the prevalent PUFA. Bacterial FA markers were prevalent at AA, B, and E during both winter and summer making up 9 – 15% of total FAs. Since holothurian body wall tissue samples were analyzed instead of whole organism or gut samples, it is evident the bacterial component reflected dietary input and not gut associated microbes. Diatom markers made up < 12% of total FAs, and flagellate markers accounted for <5% of total FAs. At stations AA (ANOSIM, $R = 0.53$,

$p > 0.05$), B (ANOSIM, $R = -0.5$, $p > 0.05$), and E (ANOSIM, $R = 0.56$, $p > 0.05$) FA composition in the samples from winter and summer were separated but still had overlap.

Similar to *P. murrayi*, ST composition in *M. musculus* was high in Δ^7 sterols making up 27 – 43% of total STs, with the exception of station E during winter (Δ^7 made up 12% of total STs). The most prevalent Δ^7 STs were 24-methylcholesta-7,22-dien-3β-ol (diatoms and cryptophytes; Volkman, 2003, Dunstan et al., 2005, Volkman, 2006, Drazen et al., 2008) and 24-ethylcholest-7-en-3β-ol (diatoms and prymnesiophytes; Volkman, 1986, Nichols et al., 1993, Volkman, 2003). Phytosterols (Table 1) ranged from 9 – 53% of total STs with 24-ethylcholest-7-en-3β-ol being prevalent. 5α(H)-stanols were high, ranging from 15 – 57% of total STs in *M. musculus* tissue. 24-methylcholest-22-en-3β-ol and 5α-cholestan-3β-ol were most prevalent. There were no seasonal differences in total ST composition, phytosterols, or 5α(H)-stanols in this species (i.e. ANOSIM, $R < 0.33$ at stations AA, B and E).

Multivariate Analysis

nMDS plots were used to examine relationships between FA and ST composition of all the sample types (Fig. 4a & b). The “stress” (i.e., 0.09 for FAs and 0.14 for STs) of these plots explains the degree to which the two dimensional graphical representation of data is distorted. Values below 0.05 provide an “excellent representation,” while values 0.1 – 0.2 provide “a potentially useful” representation (Clarke and Warwick, 2001). The stress of the FA (i.e. 0.09) and ST (i.e. 0.14) nMDS plots suggest they are useful representations of the data. Both plots separated the samples into 4 distinct clusters, however, there was a difference in the clusters produced. The FA nMDS plot separated into distinct clusters of sediment trap, surface sediment (both seasons), *P. murrayi* (both seasons), and *M. musculus* (both seasons) samples (Fig. 4a). The ST nMDS plot (Fig. 4b) separated into sediment

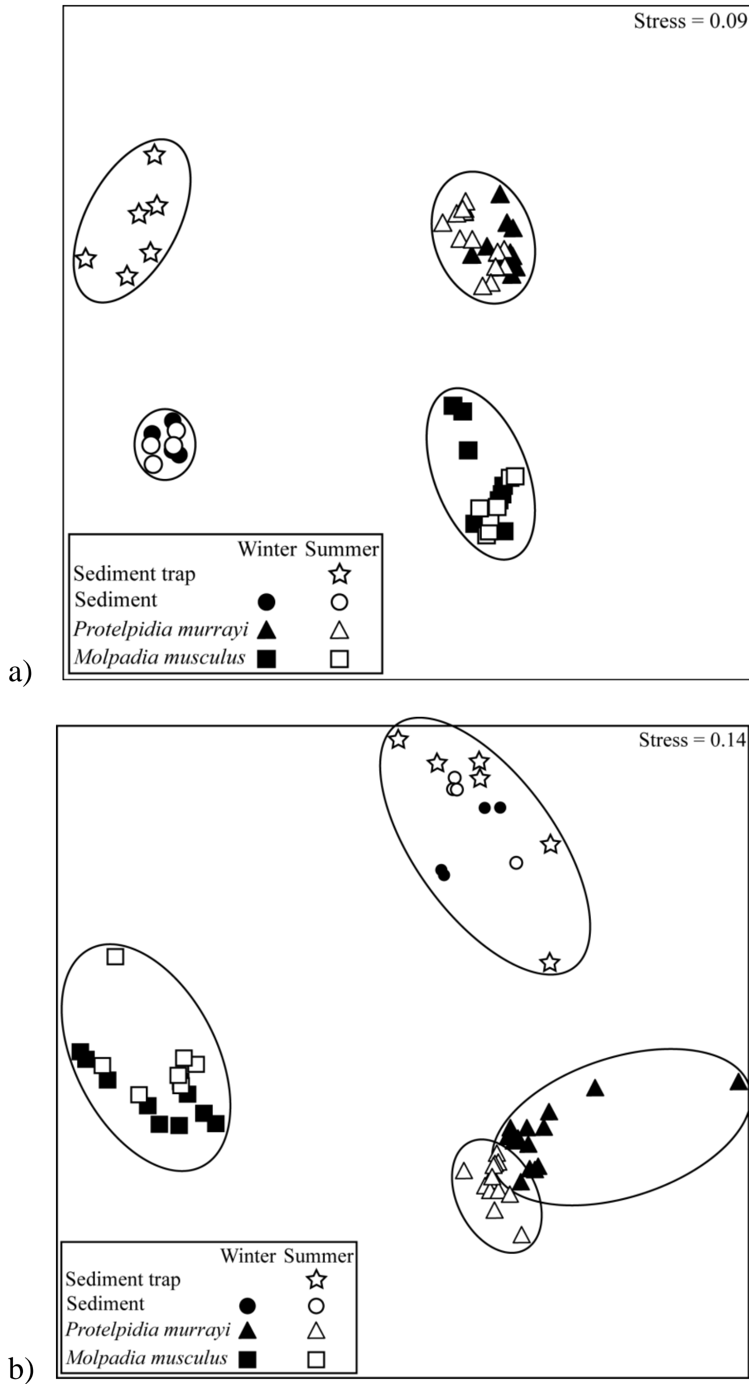

Figure 4. nMDS plots of untransformed a) FA (stress = 0.09) and b) ST (stress = 0.14) compositions in sediment trap, surface sediment, *P. murrayi*, and *M. musculus* samples. The different sample types clustered into defined groups in both plots.

trap/surface sediment (both seasons), *M. musculus* (both seasons), winter *P. murrayi*, and summer *P. murrayi* clusters. The separation of *P. murrayi*'s ST composition into distinct seasonal clusters is consistent with ANOSIM results. These results indicate that FA compositions of food sources, i.e. sediment traps and surface sediments, differ from each other and from the consumers, the holothurians. ST compositions of food sources grouped together indicating similarity between the sediment traps and surface sediments. The holothurian ST compositions differed from the food sources. In both the FA and ST nMDS plots the separation of holothurians from each other indicates these animals are assimilating different FA and ST from their food sources.

PCA plots of FA and ST compositions separated the samples into 3 similar clusters (Fig. 5a & b). In the FA PCA plot (Fig. 5a), most separation was along PC1 which explained 67% of the variance and separated food sources (sediment trap and surface sediment) from consumers (*P. murrayi* and *M. musculus*). The FAs that contributed most to PC1 separation were 16:0n, 16:1ω7, 20:4ω6, 22:1ω9, and 23:1ω9. PC2 explained 19% of the variance and separated the holothurian species with 20:5ω3, 20:4ω6, 18:1ω7, and 22:6ω3 contributing most to the separation along this component. In the ST PCA plot (Fig. 5b), the separation along PC1 explained 38% of the variance and separated *P. murrayi* from *M. musculus*, surface sediment, and sediment trap samples. The STs 24-methylcholesta-5,22-dien-3β-ol, 24-methylcholest-22-en-3β-ol, cholest-7-en-3β-ol, and C_{29} unknown contributed most to the separation along PC1. PC2 accounted for 31% of the variance and separated *M. musculus* from all other samples. Cholesterol, 24-methylcholesta-5,22-dien-3β-ol, and 24-methylcholest-22-en-3β-ol contributed most to the separation. The FA PCA results indicate that phytoplankton-derived OM in the food sources and MUFAs from *de novo* synthesis and

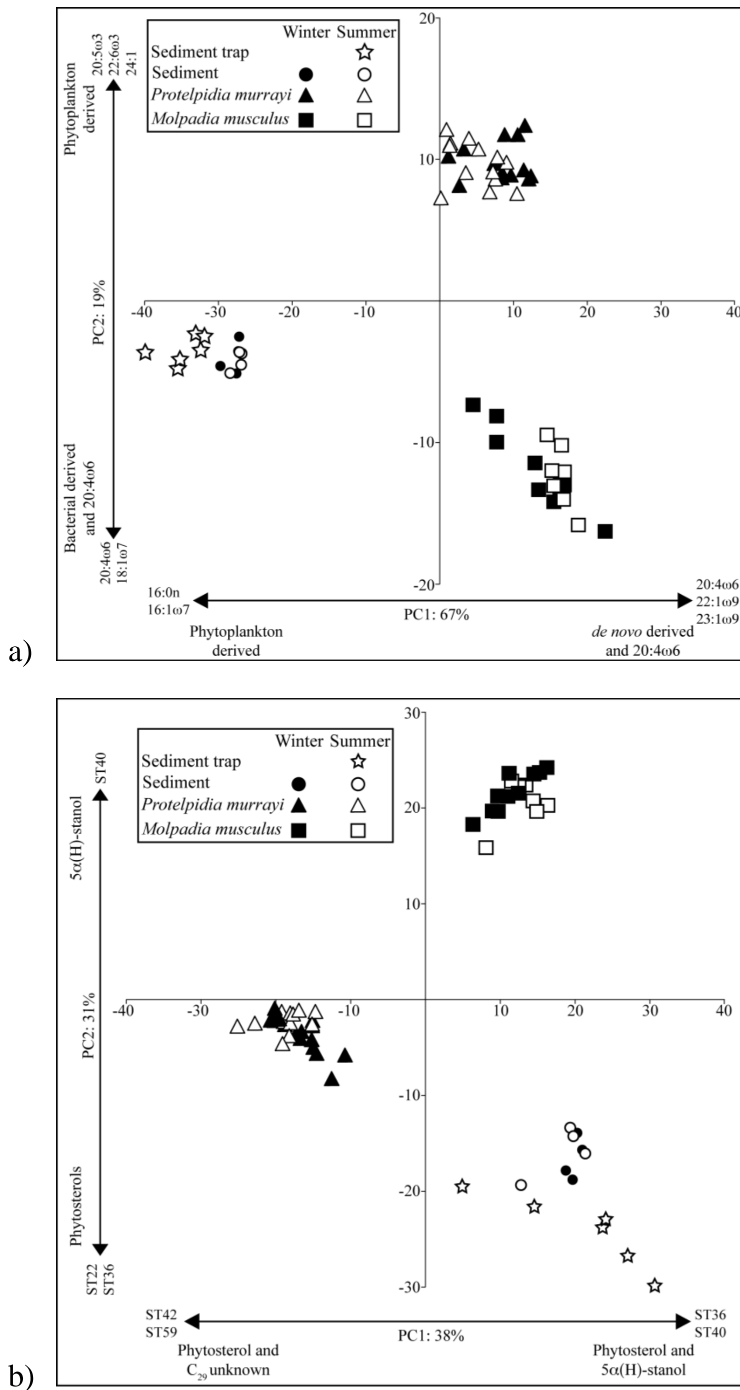

Figure 5. PCA plots of a) FA and b) ST composition in sediment trap, surface sediment, *P. murrayi*, and *M. musculus* samples collected along the WAP. Sediment trap samples were collected only in summer 2009.

the PUFA, 20:4ω6, in the holothurians contribute most to the separation on PC1 (Fig. 5a). Along PC2, phytoplankton-derived OM in the *P. murrayi* and bacterial-derived OM and 20:4ω6 in *M. musculus* and the food sources contribute most to separation of the samples (Fig. 5a). The ST PCA results indicate that phytoplankton-derived phytosterols and a species specific C_{29} ST in *P. murrayi* and a mixture of phytosterols and stanols in *M. musculus* and the food sources contribute most to the separation along PC1 (Fig. 5b). A stanol in *M. musculus* and phytosterols in *P. murrayi* and the food sources contribute most to the separation along PC2 (Fig. 5b).

Food sources

ANOSIM was used to determine if there was similarity between the sample types. SIMPER was used to quantify differences in lipid composition and specify the FAs or STs responsible for the difference. Sediment trap samples were collected in summer 2009 from stations B and G, however, only 1 bottle was recovered from B. These samples were pooled and compared to pooled surface sediment samples from summer to get a regional picture of phytodetrital deposition to the seafloor. Sediment trap samples and surface sediment samples had significantly different FA compositions (ANOSIM $R=0.73$, $p < 0.05$). Based on SIMPER analysis, these differences were mostly due to 18:1ω9, 16:0n, and 18:1ω7. There was no significant difference in ST composition (ANOSIM $R=0.24$, $p > 0.05$) between the same samples.

Consumers

A comparison of sediment traps and *P. murrayi* collected from station G was done to determine similarities between lipid composition of phytodetritus in the water column and the surface deposit feeder. This comparison was only made at station G with *P. murrayi* (*M.*

musculus not found here) because of limited sediment trap samples from station B (n=1). FA composition of sediment trap samples differed significantly with *P. murrayi* tissue samples collected from station G in summer 2009 (ANOSIM, R=1, ($p < 0.05$). Based on SIMPER, the FAs that differed most between these samples were 16:0n, 18:1ω9, 20:5ω3, and 20:4ω6. ST composition of the samples also differed significantly (ANOSIM, R=1, $p < 0.05$) with cholesterol, 24-methylcholesta-5,22-dien-3β-ol, cholest-7-en-3β-ol, and C_{29} unknown contributing to the difference, based on SIMPER.

Sediment samples from winter and summer were pooled into seasonal groups to compare food source with *P. murrayi* and *M. musculus* collected during winter and summer. FA composition of surface sediment and *P. murrayi* tissue (from stations B, E, and G) during both seasons was significantly different (ANOSIM, R=1, $p < 0.05$) at all stations where *P. murrayi* was collected. The FAs that contributed most to these differences were the same at stations B, E, and G; 20:5ω3, 20:4ω6, 16:0n, and 16:1ω7, based on SIMPER. ST composition of surface sediment and *P. murrayi* during both seasons also differed significantly at stations B, E, and G (ANOSIM, R=1, $p < 0.05$). Based on SIMPER, the ST that contributed most to the differences were C_{29} unknown, 24-methylcholesta-5,22-dien-3β-ol and cholest-7-en-3β-ol.

FA comparison of surface sediment and *M. musculus* (from stations AA, B, and E) were significantly different at most stations during both seasons (ANOSIM, R=1, $p < 0.05$). The exceptions were at stations B and E during summer where the difference was not significant (ANOSIM, R=1, $p > 0.05$ for both). Based on SIMPER, the FAs contributing most to the differences were 20:4ω6, 16:0n, and 16:1ω7 at all stations during both seasons except at station E in winter when the differences were from 20:4ω6, 16:0n, and 18:0n. ST

composition was significantly different at all stations during both seasons (ANOSIM, R=1, $p < 0.05$), with the exception of stations B and E during summer (ANOSIM, R=1, $p > 0.05$). Based on SIMPER the STs contributing most to the differences were different at every station; station AA during summer 24-methylcholesta-5,22-dien-3β-ol, 24-methylcholesta-7,22-dien-3β-ol, and cholesterol; station AA during winter 24-methylcholesta-5,22-dien-3β-ol, 24-methylcholest-22-en-3β-ol, and cholesterol; station B during winter 24-methylcholesta-5,22-dien-3β-ol, 24-methylcholest-22-en-3β-ol, and 24-ethylcholest-5-en-3β-ol; station E during winter 24-methylcholesta-5,22-dien-3β-ol, 24-methylcholest-22-en-3β-ol, and 5α-cholestan-3β-ol.

Comparison of consumer lipid compositions

The extent of *P. murrayi* and *M. musculus* samples overlapped at stations B and E on the latitudinal gradient of the WAP. Their different feeding mechanisms give them access to different horizons of sediment, and therefore, different freshness of OM. FA and ST compositions were compared at stations B and E during winter and summer to determine food preferences and/or FA and ST needs. FA composition of the holothurians differed significantly at station B in winter and summer (ANOSIM, R=1, $p < 0.05$) and at station E during winter (ANOSIM, R=1, $p < 0.05$). The FAs contributing most to differences in FA composition at station B (in winter and summer) were 20:4ω6 and 20:5ω3 and at station E (winter) 20:5ω3 and 16:1ω7. ST compositions differed significantly at station B in winter and summer (ANOSIM, R=1, $p < 0.05$) and station E during winter (ANOSIM, R=1, $p < 0.05$). The STs contributing most to differences at station B (winter) were 24-methylcholest-22-en-3β-ol , cholest-7-en-3β-ol , and C_{29} unknown, at station B (summer) were C_{29}

unknown, cholesta-7,22-dien-3β-ol, and cholest-7-en-3β-ol, and at station E (winter) were 24-methylcholest-22-en-3β-ol, cholest-7-en-3β-ol and 5α-cholestan-3β-ol.

Discussion

Food sources

Primary production in marine environments is considered the key component in most aquatic trophic studies (Claustre et al., 1989, Cotonnec et al., 2001, Richoux et al., 2005). In general, the quality and quantity of OM settling to the benthic region depends on primary production in the water column and how it is altered as it descends (Wakeham and Lee, 1989). Utilization and degradation of FAs by organisms in the water column occurs as phytodetrital material settles to the seafloor (Goutx and Saliot, 1980), lowering the unsaturation degree of PUFAs, which decrease with degradation of OM (Derieux et al. 1998). As OM sinks, the more reactive FAs, especially PUFAs, are selectively removed in favor of the less reactive SFAs, MUFAs, and STs (Wakeham and Canuel, 1986, Wakeham and Lee, 1989). PUFAs were present at low levels (< 10% of total FAs) in sediment traps suggesting degradation processes were dominant; however, FA flagellate (18:1ω9; Kelly and Scheibling, 2012, Legezynska et al., 2014) and diatom (16:1ω7; Graeve et al., 1997, Dalsgaard et al., 2003) markers were prevalent in sediment trap samples from spring and early summer 2008-2009, indicating flux of fresh phytoplankton-derived OM from surface waters. Degradation of OM in sediment trap samples was likely not due to microbial processes in the water column as bacterial FA markers were low (< 10%), and trap cylinders were spiked with formalin, so little microbial activity was expected. However, changes in OM in traps could have been due to alterations of lipids during zooplankton feeding and

digestion in the water column (Prahl et al., 1984, Cowie and Hedges, 1996) prior to settlement in trap cylinders. Phytosterol levels in the sediment traps, present at > 70% of total STs, provide additional evidence of fresh phytoplankton-derived OM from surface waters.

Surface sediments were abundant in SFAs and MUFAs, with PUFAs present at very low concentrations (Appendix A), consistent with studies of other deep-sea locations in which reactive PUFAs were preferentially removed, leaving the less reactive SFAs and MUFAs behind (Wakeham and Lee, 1989, Nichols, 2003). Low levels of PUFAs have been found in the surface sediments of many locations including Fram Strait (Birgel et al., 2004), the NE Atlantic (Mejanelle and Laureillard, 2008), and the Indian Ocean (Laureillard et al., 1997). In sedimentary environments, preferential loss of PUFAs and increase in SFAs could also be due to utilization by heterotrophs in the water column (Wakeham and Lee, 1989), preferential uptake and metabolism of benthic fauna (Wakeham and Canuel, 1986, Suhr et al., 2003), or microbial processes (Wakeham and Canuel, 1986). Often SFAs, such as 16:0 and 18:0, are recognized as products of degradation of unsaturated and longer-chained FAs (Suhr et al., 2003) and were the most prevalent SFAs in surface sediment samples of the WAP. It should be noted that 16:0n was prevalent in phytoplankton samples in the region during summer 2009 (Pirtle-Levy, Appendix A) and is often present in many algal species (Sargent et al., 1987). Therefore, presence of 16:0n could be due to an influx of surface production instead of degradation processes. Even though PUFAs were low, there is still evidence of diatom and flagellate markers in the FAs (i.e. 16:1ω7 and 18:1ω9 respectively) with diatoms being more prevalent than flagellates in surface sediments. Diatoms have been found to play an important role in the transfer of lipids to the seabed, even when they constitute less biomass than flagellates in surface water blooms (Rossi et al., 2013), and are

prevalent in sediments around the Antarctic continent such as the Maud Rise in the Lazarev Sea (Brandt et al., 2011), the Ross Sea (Villinski et al., 2008), and the Larsen B continental shelf (Sane et al, 2013). Phytosterols were also present at high levels, ≥ 69% of total STs, providing additional evidence of phytoplankton-derived OM settling to surface sediments within the region. Sterol composition of surface sediments in the WAP were similar to the Beaufort Sea, where cholest-5-en-3β-ol and 24-methylcholesta-5,22-dien-3β-ol also dominate (Belicka et al., 2004, Rontani et al., 2012).

Bacteria are a major component of the microbial food web in Antarctic marine environments (Skerratt et al., 1995) and can be estimated by the concentration of odd-numbered, iso- and anteiso- branched chain fatty acids and 18:1ω7 (Sargent et al., 1987, Bowman et al., 1991, Saliot et al., 1991, Dalsgaard et al., 2003). These FA bacterial markers (Table 1) were found in higher levels in surface sediments compared to sediment trap samples, indicating bacterial processes were more prevalent in surface sediments. Evidence of bacterial biomass in sediments has been documented by FA biomarkers in other deep-sea environments such as the Barbados Trench (Guezennec and Fiala-Medioni, 1996), the northern Atlantic Ocean (Gooday et al., 1990), and the fjord-like Trinity Bay in Newfoundland (Budge and Parrish, 1998). Increased levels of bacterial markers in WAP sediment samples suggest bacteria were available as a potential food source for the benthic community, especially during winter months when primary production in surface waters is minimal and the flux of OM is reduced.

Based on nMDS plots of FA composition, the sediment trap and surface sediment samples had no overlap and formed distinct clusters. FA differences indicated the phytoplankton-derived FA biomarkers were more prevalent in sediment traps and bacterial

FA biomarkers were prevalent in surface sediment. This suggested degradation processes and bacterial abundance were enhanced in surface sediments. There was no significant difference in ST composition between trap and sediment samples possibly due to the stability of STs as they sink through the water column and settle on the seafloor (Wakeham and Canuel, 1986, Volkman et al., 2008).

Comparison of lipid composition in food sources and consumers

Benthic megafauna respond to environmental forcing, such as increase/decrease in food supply (Gage and Tyler, 1991), and their distribution and structure are expected to integrate these variations on long time scales (Piepenburg, 2005). FA and ST compositions provide a time-integrated view of an organism's assimilated feeding history (Parrish et al., 2000). FAs provide information on the type and quality of resources assimilated by animals over ecologically meaningful time periods (Dalsgaard et al., 2003) and STs are excellent biomarkers due to their stability and structural diversity (Parrish, 2013). STs are important structural components of membranes (Drazen et al., 2008) and are precursors to specific enzymes (Parrish, 2013), necessary for hormone production (Vinci et al., 2008) and regulation (Hannich et al., 2009). Lipids, particularly the PUFA components, have vital structural and functional roles in membranes and are required for growth and reproduction (Arts, 1999). FAs that are required for proper biological functioning but cannot be synthesized *de novo* by animals, called essential FAs (EFAs), include the PUFAs 20:5ω3, 22:6ω3, and occasionally 20:4ω6 (Olsen, 1999). The EFAs are obtained in the diet, highly conserved in aquatic systems, and considered fundamental drivers of ecosystem fitness and stability (Arts et al., 2001, Parrish et al., 2013).

During winter 2008 and summer 2009, the FA and ST compositions of the holothurian species body tissues differed from each other as well as from food sources. These differences were evident in the nMDS plots which formed distinct clusters of each holothurian species, sediment trap, and surface sediment samples indicating similar FA and ST compositions within each cluster (Fig. 3a and b). Differences between FA compositions of *P. murrayi* and sediment traps at station G during early spring/summer reflected higher flagellate content in sediment traps (i.e. 18:1ω9) and higher PUFA content in *P. murrayi* (i.e., 20:5ω3 and 20:4ω6). This indicated that flagellate phytoplankton do not contribute to *P. murrayi* diet but selective ingestion of diatom (20:5ω3; Parrish et al., 2000, Dalsgaard et al., 2003) and possibly foraminifera (20:4ω6; Suhr et al., 2003) were important. ST compositions also differed between sediment traps and *P. murrayi*, with cholesterol and 24-methylcholesta-5,22-dien-3β-ol higher in sediment trap samples and cholest-7-en-3β-ol and C_{29} unknown higher in *P. murrayi*. The C_{29} unknown ST was present only in this holothurian and is suggested to be a marker for *P. murrayi* in this region. Phytosterols were prevalent in both sample types with Δ^5 cholesterol present in sediment traps and Δ^7 cholest-7-en-3β-ol present in *P. murrayi*.

The lipid composition of surface sediment samples differed from the lipid compositions of both holothurians during winter and summer, which was unexpected given this was the main food source for these organisms. Temporal and spatial variability in surface sediments was negligible during both seasons, likely due to the integrative nature of surface sediments. Concentrations of algal pigments and lipids have been found to be much higher in benthic organisms, such as foraminifera (Cedhagen et al., 2014) and holothurians (Hudson et al., 2005), than in the surrounding sediment on which they feed. This pattern was also

evident in both species of holothurians from the WAP. PUFAs were prevalent in the holothurians (i.e. 20:5ω3 in *P. murrayi*, 20:4ω6 in *P. murrayi* and *M. musculus*) and SFAs (i.e. 16:0n and 18:0n) and MUFAs (16:1ω7) were prevalent in surface sediments. Both holothurians are known to be selective during digestion and assimilation (McClintic et al., 2008, Purinton et al., 2008), which was indicated by increased PUFA levels relative to food source content. Although phytoplankton are often considered the source of PUFAs in marine systems (Parrish et al., 2000, Dalgaard et al., 2003), bacteria have also been found to produce PUFAs (Russell and Nichols, 1999, Nichols et al., 2003). Therefore, bacteria in sediments as a source of PUFA for holothurians in the WAP region needs further investigation.

ST composition differed between surface sediments, where Δ^5 phytosterols dominated, and both holothurians. In *P. murrayi*, the difference was attributed to prevalence of Δ^7 ST (i.e. cholest-7-en-3β-ol) and the species-specific C_{29} unknown ST. In *M. musculus*, differences were due to high levels of Δ^7 ST and 5α(H)-stanols. *P. murrayi* appeared to preferentially assimilate and biotransform cholesterol to cholest-7-en-3β-ol (Voogt and Over, 1973, Smith and Goad, 1975, Goad, 1981), whereas *M. musculus* biotransformed the phytoplankton-derived 24-methylcholesta-5,22-dien-3β-ol and 24-ethylcholest-5-en-3β-ol (Nichols et al., 1993, Drazen et al., 2008) to Δ^7 STs.

There are few studies comparing lipids in food sources to consumer assimilation. However, a major shift in the megafaunal community of the Porcupine Abyssal Plain in the northeast Atlantic appeared to be driven by food supply to the region (Billet et al., 2001). A holothurian species, *Amperima rosea*, that had been a minor component of the benthic community during the 1970s – 1980s, increased in abundance by two orders of magnitude during sampling from 1997-2000 (Bett et al., 2001, Billett et al., 2001). At Station M in the

northeast Pacific, interannual variability of megafaunal abundances have also been linked to shifts in food supply (Ruhl and Smith, 2004, Ruhl, 2007) with some species thriving during high flux of food and other thriving during lower food fluxes (Ruhl and Smith, 2004). A similar shift has not been noted in the WAP, possibly due to limited data with which to compare current trends.

Consumers

The MUFAs 22:1ω9 and 23:1ω9 were abundant in both holothurians. Although 22:1 isomers have been found in calanoid copepods (Parrish et al., 2000, Dalsgaard et al., 2003) and used as a marker for carnivorous/omnivorous feeding (Drazen et al., 2008), the lack of these isomers in surface sediments indicates they must be from another source. Ginger et al. (2000, 2001) speculated that 22:1 and 23:1 isomers were *de novo* products of holothurian FA biosynthesis used for maintenance of membrane fluidity at high pressure and low temperatures. It seems likely that 22:1ω9 and 23:1ω9 in *P. murrayi* and *M. musculus* serve a similar purpose and may also be derived through *de novo* FA biosynthesis.

Protelpidia murrayi

P. murrayi is mobile and moves around feeding on the top millimeter of sediment. During periods of high phytodetrital flux, this species can rework the top millimeter of sediment with a turnover rate 7 times faster than during winter months (Sumida et al., 2014). These actions make *P. murrayi* an important species for organic carbon cycling on the deep shelf of the WAP and can control availability of deposited fresh phytodetritus to the broader shelf community (Sumida et al., 2014). Mobility gives this species the ability to search out patches of freshly deposited OM on the seafloor. However, based on other studies, *P. murrayi* does not appear to always selectively feed on the freshest OM available (Purinton et

al. 2008), instead consuming bulk sediments at times and selectively assimilating optimal nutritional components (Mincks et al., 2008). For the data presented here, this behavior is reflected in the strong PUFA signal present in the holothurians but absent in the surface sediments, which suggests that *P. murrayi* can quickly incorporate the nutritional signals from the freshest OM. In the Beaufort Sea, benthic activity and community composition were affected by spatial and seasonal variations in OM flux (Morata et al, 2008), which is similar to seasonal responses of spawning (Galley et al., 2008) and preferential feeding on fresh phytodetrital inputs (Mincks et al., 2008) exhibited by *P. murrayi.*

Molpadia musculus

M. musculus is less mobile and feeds head down in the sediment, keeping its tail in contact with the sediment surface (Gutt, 1991, Pawson et al., 2001). *M. musculus* feeds deeper than the top 0.5cm of sediment, thus not directly on the fresh phytodetrital layer (Wigham et al., 2008). Due to its limited mobility, *M. musculus* must take advantage of the surrounding sediment and likely has access to more refractory OM. Deep-sea holothurians are known to have bacteria in their intestinal lining, usually of a different taxonomic composition to bacteria in surrounding sediments (Bensoussan et al., 1984), that aids in digestion of older OM (Deming and Colwell, 1982, Roberts et al., 2000). There is evidence that *M. musculus* selectively assimilates components of ingested bulk sediment and shows no seasonal differences in this feeding strategy (Purinton et al., 2008). It has been suggested that holothurians can biosynthesize PUFAs (Neto, 2002). Phytoplankton-derived PUFAs, 20:5ω3 and 22:6ω3, were absent from *M. musculus* in previous studies (Neto, 2002), however, they were present at low levels in this study. Bacteria within sediments may be an important food source for *M. musculus* in the WAP, similar to *Molpadia blakei* from the Porcupine Abyssal

Plain (Ginger et al., 2000). Through bioturbation, bioirrigation, and organic matter cycling, *M. musculus* plays a key role in benthic trophic dynamics and biogeochemical processing in Nazare Canyon (Amaro et al., 2010) and could play a similar role in the WAP.

Holothurian feeding selectivity

The two holothurians have different feeding mechanisms as well as mobility on the seafloor, which was reflected in their lipid compositions. PCA of lipid composition suggested *P. murrayi* and *M. musculus* have access to different sediment horizons and utilize different FA and ST components. *P. murrayi* appeared to have a phytoplankton-based diet, with relatively high abundances of FA diatom biomarkers (e.g. 20:5ω3; Parrish et al., 2000, Dalsgaard et al., 2003) and phytosterols (e.g. cholest-7-en-3β-ol in winter and 24-methylcholest-7,22-dien-3β-ol and 24-ethylcholesta-5,22-dien-3β-ol in summer; Volkman, 1986, Barrett et al., 1995, Hudson et al., 2001, Dahl et al., 2004, Drazen et al., 2008). *M. musculus* had a lipid composition reflecting a diet of more reworked OM with higher levels of 5α(H)-stanols and bacterial FA biomarkers. The FA 20:4ω6, a dominant PUFA in both holothurians, is a major component of phospholipids in marine animals (Suhr and Pond, 2006) that plays a possible role in cold adaptation and maintaining cell membranes in deep-sea organisms (Harwood and Vigh, 2006). It has also been attributed to preferential feeding on benthic foraminifera (Legezynska et al., 2014), which have elevated levels of this FA (Suhr et al., 2003) and are known sources of 20:4ω6 in other invertebrates including isopods (Wurzberg et al., 2011) and amphipods (Legezynska et al., 2012). Foraminifera may be an overlapping food source for these two species in the WAP region.

Conclusion

FA and ST analyses have proved to be useful tools in assessing utilization of OM from surface production and identifying main food sources for the holothurians on the seafloor. All samples were rich in phytosterols and had varying levels of phytoplankton-derived FA, such as 16:1ω7, 18:1ω9, and 20:5ω3, which suggested coupling to surface production. Both the phytosterols and phytoplankton-derived FAs indicated a prevalence of diatom input into the system. With only one season of sediment trap data and limited sampling at one of the sampling sites, it was evident that flagellate-based OM was present at depth in the water column but was degraded or utilized before reaching the seafloor. High levels of SFAs and MUFAs coupled with low levels of PUFAs in surface sediments suggested degradation and microbial processes during phytodetrital deposition and within the sediment. Negligible temporal and spatial variability in surface sediments is consistent with the presence of a "food bank" (Mincks et al. 2005), which can sustain the benthic community during periods of low OM flux. Differences in lipid composition of the holothurian species and surface sediment indicated preferential assimilation of OM components within a depleted food source.

The different feeding modes of the holothurians, i.e. surface versus subsurface deposit feeding, enabled *P. murrayi* and *M. musculus* to utilize different lipid components in the sediment. *P. murrayi* had high levels of phytoplankton-derived FAs and STs, especially from diatom, whereas *M. musculus* had high levels of bacterial-derived FAs and 5α(H)-stanols. These differences suggest *P. murrayi* feeds on fresher, diatom-derived phytodetrital material and *M. musculus* feeds on more reworked OM. The only overlap may be their utilization of foraminifera as a food source, which was evident by increased levels of 20:4ω6 in both

holothurians. The C_{29} unknown ST may be a marker for *P. murrayi* in the region and should be further investigated. The characterization of lipids in the food sources and holothurian consumers in this region provided information on preferential utilization of food sources and will assist in future lipid biomarker studies. Climate change is a rapid phenomenon compared to evolutionary time scales, which can take several generations for change to be seen. Given the long life spans of the benthic animals in the WAP (Clarke, 1987, Peck, 2005), they will likely not have time to adapt to environmental changes. It may be the animals that are less selective and adapted to a more diverse food source, e.g. *M. musculus*, which will continue to thrive in changing conditions. The pelagic community is currently exhibiting change in response to warming climates. Changes in phytoplankton and zooplankton community structures may affect OM flux to the benthic community. Gaining a better understanding of this community is important because a shift in the benthic community could result in changes in regional biogeochemical cycling and availability of nutrients to other trophic levels.

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CHAPTER 3

Lipid characterization of benthic parameters on a latitudinal transect of the western Antarctic Peninsula continental shelf

Introduction

The benthic community of the Antarctic continental shelf is diverse and relies on the flux of phytoplankton-produced organic matter (OM) from surface waters as the primary food source (Mincks et al., 2005, Smith et al., 2006, 2008). Determining the contribution of different phytoplankton assemblages to the benthos is important for understanding what sustains heterotrophs in this region and how consumers utilize available food sources. However, dominant phytoplankton communities on the western Antarctic Peninsula (WAP) are likely to change as surface waters warm and physical dynamics drive the system toward a deeper mixed layer. The region is sensitive to climate change, and it is critical to understand how this warming will impact the benthic community. Here, we use biochemical tools to determine how consumers utilize OM substrates to provide necessary baseline data for understanding the impacts of climate change-induced shifts on the quality and quantity of food sources reaching the benthos.

The Antarctic continental shelf is deep compared to other continental shelves due to ice loading of the continent, and this characteristic is reflected in the community structure of the region. An affinity between benthos of the deep sea and benthos of the Antarctic shelves is well known (Brandt et al., 2007, Rogers, 2007) and the diversity and abundance of consumers is comparable (Gage and Tyler, 1991). In particular, benthic deposit feeders are important fauna, with echinoderms often dominating the benthic invertebrate megafauna. Echinoderms are well suited to handle the physiological and ecological challenges of the

harsh Antarctic environment (Clarke and Johnston, 2003). They dominate the megafaunal community on the continental shelf of the WAP (Starmans et al., 1999, Sumida et al., 2008) and are important to benthic trophic dynamics and biogeochemical processing in the region (Smith et al., 2006, 2008). Through feeding mechanisms and movement on and within the seabed, these animals can rework OM and contribute to the consumption and redistribution of nutrients (Smith et al., 1997, Lauerman et al., 1996, Ginger et al., 2000, Bett et al., 2001). Despite the importance of these organisms, the benthic community in the WAP has not been investigated extensively due to remoteness and ice prevalence most of the year (Turner and Owens, 1995, Grotov et al., 1998, Clarke and Johnston, 2003).

The food source for the WAP megafauna derives largely from intense phytoplankton blooms initiated as sea ice retreats in spring (Ducklow et al., 2007). Much of this pulse of OM, primarily diatom aggregates and fecal pellets (Gutt et al., 1998), is transported to the seafloor and can either be sequestered into the seabed, immediately consumed, or microbially degraded. Because little primary production occurs during the ice-covered winter months, the sequestered fraction of this pulse of OM can act as a "food bank" for benthic organisms (Mincks et al., 2005), sustaining them until the following spring.

Phytodetrital deposition on the sea floor has been found to vary on seasonal and interannual timescales in deep sea and continental shelf environments (Danovaro et al., 2000, Bett et al., 2001, Gooday, 2002 and references therein, Smith et al., 2006, 2008, Isla et al. 2011). Climate-induced regional warming has the potential to cause major shifts in the physical and biological ecosystems of the WAP, some of which have already been documented. Warming temperatures on the WAP are causing a north-south gradient in climate that is warmer and more moist in the north, i.e. a more subpolar climate, and colder

and more dry in the south, i.e. typical polar climate (Ducklow et al., 2007). Winter air temperatures have increased ~1°C per decade over the last 60 years (Vaughan et al., 2003) which has caused water temperatures to increase (Martinson et al., 2008, Meredith et al., 2008) coincident with declines in duration and extent of sea ice (Martinson et al., 2008, Stammerjohn et al., 2008, Ducklow et al., 2007). As physical characteristics of the WAP region shift, biology has been changing too, including an increase in bacterial activity, shifts in phytoplankton composition (i.e. cryptophytes and prymnesiophytes replacing diatoms), changes in zooplankton composition (i.e. salps replacing krill), and a decrease in ice-dependent penguin populations (Atkinson et al., 2004, Ducklow et al., 2007, Montes-Hugo et al., 2008, Ross et al., 2008, Ducklow et al., 2012).

Shifts in pelagic communities will likely impact trophic energy transfer and food web dynamics as seawater temperatures increase and duration and extent of sea ice decreases. Pelagic-benthic coupling may be altered affecting food availability for benthic populations. The extent of these changes is difficult to predict, especially in regions where ecosystem function is still being investigated. As part of the FOODBANCS 2 Project, the latitudinal climate gradient present on the western Antarctic Peninsula (i.e. warmer in the north and colder in the south) was sampled to evaluate long-term, climate-induced changes and response within the benthic ecosystem usually noticeable only over extended periods of time.

In many polar studies stable isotopes have been the primary tool for investigating trophic structure (Dunton et al., 2001, Mincks et al., 2008). However, in a region like the WAP where the benthic food source is dominated by primary production from the euphotic zone, stable isotopes cannot easily be used to tease out which portions of this bulk material are utilized. Lipid biomarkers provide insight into the fraction of OM benthic animals

preferentially utilize and have emerged as an important tool for food web studies in many environments, including deep-sea (Ginger et al., 2001, Hudson et al., 2004), polar (Graeve et al., 1997, Budge et al., 2007) and nearshore ecosystems (Budge and Parrish, 1998, Xu and Jaffe, 2007, Belicka et al., 2012). Lipids are important biochemical components utilized for energy storage, hormone regulation, reproduction, buoyancy, and maintenance of cell membranes (Laureillard et al., 1997, Ginger et al., 2000, Ginger et al., 2001, Hudson et al., 2004, Neto et al., 2006, Rozner and Garti, 2006, Jeffreys et al., 2009). Fatty acids (FAs) and sterols (STs) are essential for invertebrate function and many must be obtained from food sources (Ginger et al., 2000). FAs are reactive and utilized quickly (Sun et al., 1997, Danovaro et al., 2000) compared to STs that are comparably stable and persist longer in marine sediments (Volkman, 1986). Certain lipids are source-specific biomarkers, not modified during trophic transfer (Dalsgaard et al., 2003, Jeffreys et al., 2009), that can be used for identification of algal and zooplankton contribution to animal diets (Sargent et al., 1987, Parrish et al., 2000, Ginger et al., 2001). Variation in phytoplankton biomarkers in benthic sediments and deposit feeders can provide spatially integrated views of ecosystem dynamics that may yield insights into climate-driven pelagic changes mirrored in the benthic ecosystem.

FAs and STs in body wall tissues from the surface deposit feeder, *Protelpidia murrayi*, and the subsurface deposit feeder, *Molpadia musculus*, were compared to assess food source utilization within sediments during winter 2008 and summer 2009 along a latitudinal gradient of the WAP. Species of holothurians vary in their effectiveness of exploiting and assimilating OM, and holothurian response to a variable food supply can reflect their feeding mode (Roberts et al., 2001), rate of locomotion (Roberts et al., 2001), gut

anatomy (Penry and Jumars, 1986), tentacle structure (Roberts et al., 2001), as well as specific nutritional requirements (Ginger et al., 2001). The holothurians in this study feed upon different horizons within the sediment column but they both have been found to selectively digest or assimilate fresh, easily digestible OM from sediment within their guts (Purinton et al., 2008). Examination of differences in lipid biomarker composition in surface and subsurface deposit-feeding holothurians should demonstrate differences in feeding mechanisms and food source utilization. It is important to document current biochemical properties within benthic invertebrates to gauge changes in and responses of these animals to their varying ecosystem. The goal of the study was to investigate FA and ST assimilation by the two deposit feeders on a spatial scale (northern versus southern WAP) during winter and summer seasons to distinguish differences in food resource usage.

Materials and Methods

Study area and sampling

Sediment and holothurian samples were collected at 4 stations on a north to south transect (63°S - 68°S) along the WAP during cruises in winter 2008 (15 July – 1 August; FOODBANC2-2) and summer 2009 (22 February – 5 March; FOODBANC2-3) (Fig. 1). Station depths ranged from 516 – 685m water depth and each site had muddy sediments on the seafloor. Sampling sites were also selected based on latitudinal temperature gradients and sea ice extent (Smith et al., 2012).

Surface sediment samples were collected at each site using a Bowers & Connelly Megacorer. Sediment cores (10cm diameter) were extruded to 1 cm, sliced, trimmed, and

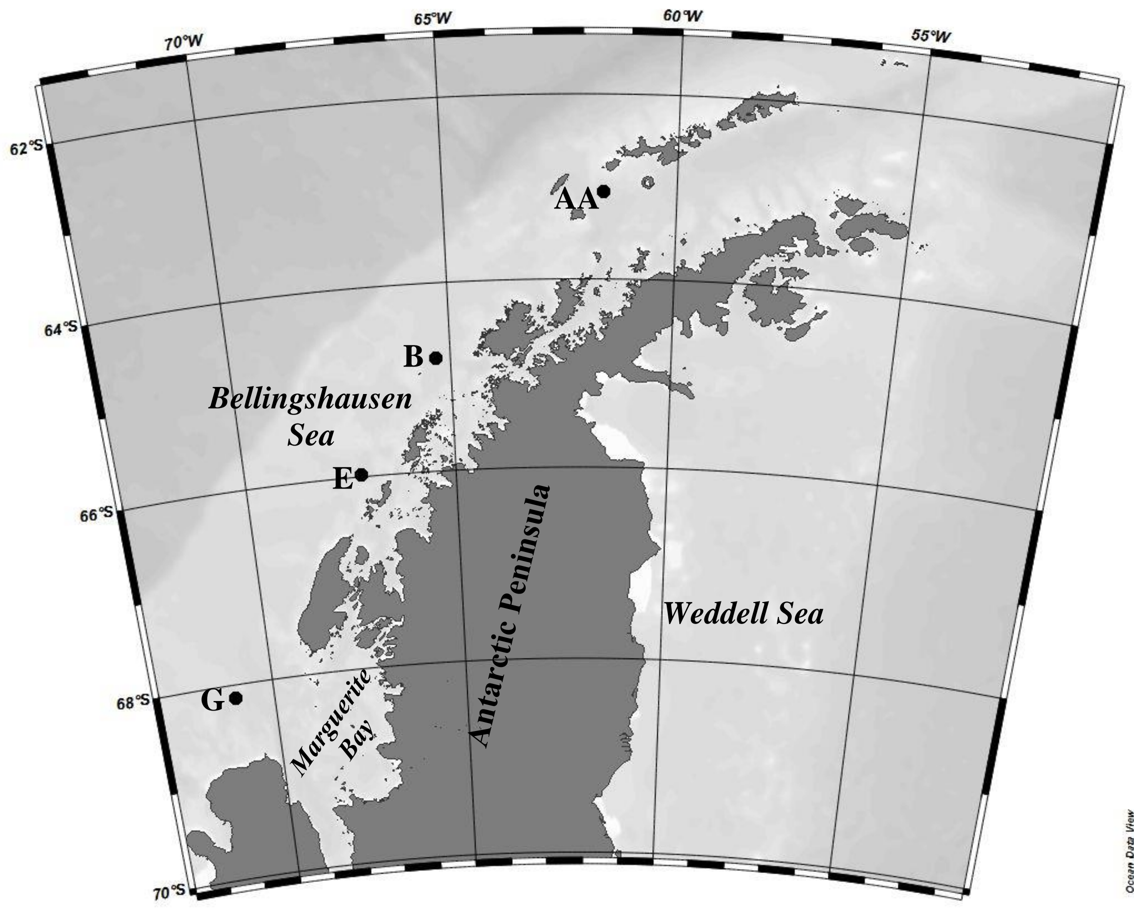

Figure 1. Sampling sites for the FOODBANCS2 Project. The sites from north to south were AA (63°04.727'S, 61°36.120'W), B (64°48.002'S, 65°21.453'W), E (65°58.949'S, 67°16.930'W), and G (68°08.547'S, 71°01.441'S).

homogenized. The 0-1cm layer was immediately frozen at -80°C shipboard. Prior to analysis, the sediment samples were freeze-dried and homogenized.

Benthic megafauna were collected during both seasons using a 5.5 m semi-balloon otter trawl (2cm mesh). Two types of holothurians were collected for analysis. *P. murrayi,* the surface deposit feeder, was present at stations B, E, and G, whereas *M. musculus,* the subsurface deposit feeder, was collected at stations AA, B, and E. The two holothurians were chosen due to their abundance at most sampling sites and their contrasting feeding modes. Up to 5 individuals were dissected shipboard, placed in plastic bags, and frozen at -80°C. Upon moving them to our land-based laboratory, the samples were stored at -20°C until analysis could be performed. Body wall samples from the deposit-feeders remained frozen and were thawed prior to lipid analysis.

Lipid extraction and analysis

Lipids were extracted following a modified method of Folch et al. (1957). Briefly, homogenized samples were ultrasonically extracted with a 2:1 mixture (vol/vol) of methylene chloride:methanol (CH_2Cl_2:MeOH). A saline solution (0.9% NaCl) was added to achieve a final ratio of 2:1:0.7 CH_2Cl_2:MeOH:H_2O, the samples were strongly agitated, and the lower organic phase was removed to an evaporation flask. Fresh organic solvent was added to the remaining sample, and the extraction was repeated two more times. The three extracts were combined, and excess solvent was removed by rotary evaporation. Total lipid extracts were flushed with nitrogen and stored in CH_2Cl_2 at −20°C.

Total lipid extracts were then saponified with 0.5 N methanolic KOH at 70°C for 30 to 60 minutes (Ju et al., 2009). Neutral lipids were partitioned three times with a mixture of hexane:diethyl ether (9:1), dried under N_2 gas, and treated with bis(trimethylsilyl)

trifluoroacetamide (BSTFA) with 30% pyridine to convert free alcohols to their corresponding trimethysilyl (TMS) esters. The polar lipid fraction was then acidified to pH <2 with HCl, and free FAs were partitioned into 9:1 hexane:diethyl ether three times and combined. FAs were methylated to corresponding methyl esters (FAMEs) with freshly-distilled diazomethane. Internal standards (5α-cholestane for neutral and squalane for polar) were added to each fraction prior to analysis with gas chromatography-mass spectrometry (GC/MS).

FAs and STs were identified and relative abundances determined using GC/MS with an Agilent 6890 gas chromatograph (GC) coupled to an Agilent 5973 mass spectrometer (MS) operating in electron ionization mode at 70 eV. Although many studies of quantitative analyses of FAs have been carried out by GC-flame ionization detection (FID), Dodds et al. (2005) have shown that the GC-MS technique used here compares satisfactorily to GC-FID.

The GC was equipped with a Restek Rtx®-5MS capillary column (5% diphenyl/ 95% dimethyl polysiloxane stationary phase, 30m length X 0.25mm i.d. X 0.25µm film thickness). Samples were injected in splitless mode with an initial oven temperature of 60°C. After a 2 minute hold at this initial temperature, the oven temperature was raised to 300°C at a rate of 6°C min^{-1}, followed by a 15 minute hold at 300°C. FA and ST peaks were visualized using ChemStation software; identification of FAs and STs was performed using both comparisons of chromatographic retention times and mass spectral characteristics with authentic fatty acid standards (Supelco PUFA No. 3 from Menhaden oil, Supelco FAME mix, and C_4-C_{24} unsaturates) and previously reported mass spectral data. Following quantification of the FAMEs, double bond positions in some predominant unsaturated FAs

were further confirmed through analysis of picolinyl esters following the method of Dubois et al. (2006).

A total of 35 FAs and 34 STs were identified in surface sediment and holothurian samples (Appendix A and B, respectively). The relative abundances of each were determined using peak areas and were expressed as a percentage of the total identified FAs and STs, respectively.

Statistical analysis

To visualize similarity patterns between holothurian taxa and sediment samples, non-parametric multidimensional scaling (nMDS) was conducted based on a Bray-Curtis similarity matrix. This procedure produces a plot in which samples with similar FA and ST profiles are positioned close together. Principal component analysis (PCA) was used to compare FA and ST profiles among the holothurian species. Loadings of the PCA helped identify FAs and STs that had strong effects on each principal component in the plot (i.e., PC1 and PC2) and thus contribute most to separation between taxa. To investigate differences of lipid availability to each species along the latitudinal gradient, analysis of similarity (ANOSIM) was used to examine differences in FA and ST signatures of sediment among stations. Differences in the FA and ST signatures among these stations was further explored using similarity percentages (SIMPER) to determine specific FAs and STs that contributed most to differences noted with ANOSIM. Data were left untransformed (Howell et al., 2003) so artificial weight would not be given to FAs and STs with trace contributions to lipid profiles (Hall et al., 2006). Significant results from the spatial comparisons were tested using a Mann-Whitney U test with Bonferroni adjustment to determine differences in specific FAs and STs. To examine contributions of food sources within the samples, FAs

were grouped into bacterial-, diatom-, and flagellate-based source assignments and STs were grouped into phytosterols originating from marine algal sources and 5α(H)-stanols indicative of degraded OM (Table 1). Lipid concentrations in surface sediment were compared qualitatively on temporal and spatial scales due to limited sample size. Multivariate analyses of FA and ST profiles were performed using the PAST statistical package (Hammer et al. 2001), and Mann-Whitney U tests were carried out using SPSS 19.0 software.

Results

Comparison of fatty acid and sterol compositions

nMDS was used to visually investigate differences in FA and ST composition between the three sample types: surface sediment and tissues of *P. murrayi* and *M. musculus*. Differences in the FA compositions between the two holothurian species and surface sediment samples were clearly separated into distinct clusters during winter and summer (Fig. 2). Similar results were evident in the ST compositions of the three sample types, which were also separated into winter and summer (Fig. 3).

Fatty acids

Surface sediments

The FAs present in surface sediments indicate a mixture of fresh, phytoplankton-derived OM, degraded OM, and bacterial-derived OM. There was little variation on the latitudinal transect during both seasons indicative of a persistent “food bank” (Mincks et al., 2005). The four dominant FAs in surface sediment samples at all stations during winter and summer sampling were 16:0n (phytoplankton and degradation of FAs; Sargent et al., 1987 and Suhr et al., 2003, respectively), 18:0n (degradation of FAs; Suhr et al., 2003), 16:1ω7

Table 1. Fatty acids and sterols grouped together to define sources of bacterial, diatom, and flagellate OM as well as phytosterols and stanols within the WAP samples based on the literature.

Carbon Source	References
Bacterial	
15:0i, 15:0a, 15:0, 17:0i, 17:0a, 17:0, 18:1ω7	Sargent et al., 1987
	Volkman et al., 1990
	Bowman et al., 1991
Diatom	
16:1ω7, 20:5ω3	Sargent et al., 1987
	Volkman et al., 1989
	Viso and Marty, 1993
	Graeve et al., 1997
	Parrish et al., 2000
	Dalsgaard et al., 2003
Flagellate	
18:1ω9, 22:6ω3	Sargent et al., 1987
	Volkman et al., 1989
	Graeve et al., 1997
	Parrish et al., 2000
Phytosterols	
cholest-5-en-3β-ol	Nichols et al., 1991
cholesta-5,22-dien-3β-ol	Barrett et al., 1995
24-methylcholesta-5,22-dien-3β-ol	Volkman et al., 1998
24-methylcholesta-5,24(28)-dien-3β-ol	Muhlebach and Weber, 1998
24-ethylcholesta-5,22-dien-3β-ol	Volkman, 2003
23,24-dimethylcholest-5-en-3β-ol	Dahl et al., 2004
24-ethylcholest-5-en-3β-ol	Drazen et al., 2008
4α,23,24-trimethylcholest-22-en-3β-ol	Wisnieski et al., 2014
cholest-7-en-3β-ol	
cholesta-7,22-dien-3β-ol	
24-methylcholesta-7,22-dien-3β-ol	
24-ethylcholest-7-en-3β-ol	
5α(H)-stanols	
5α-cholestan-3β-ol	Hudson et al., 2001
24-ethylcholestan-3β-ol	Jeffreys, 2006
24-methylcholest-22-en-3β-ol	Wisnieski et al., 2014
24-ethylcholestan-3β-ol	

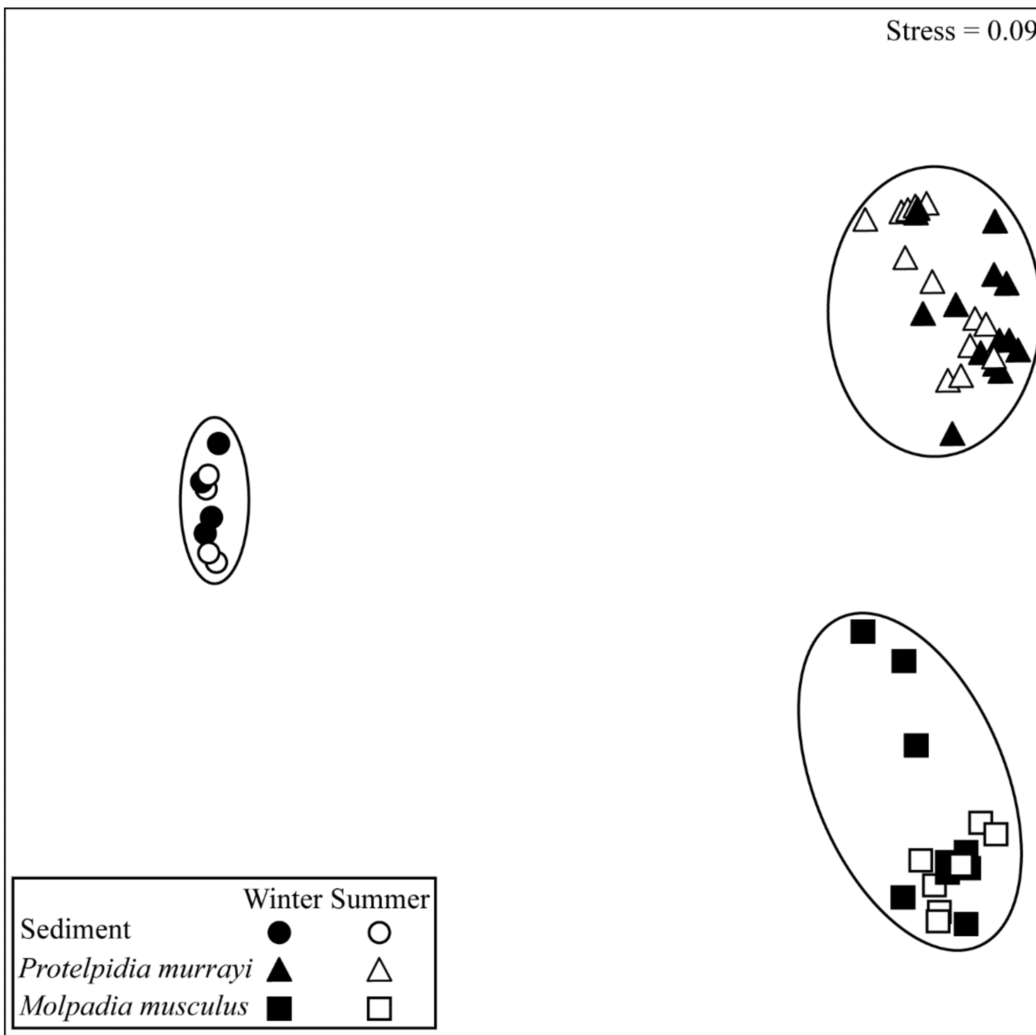

Figure 2. nMDS plot of FA compositions of two holothurian species and surface sediment samples in summer 2008 and winter 2009. Axis scales are arbitrary in nMDS and are therefore omitted.

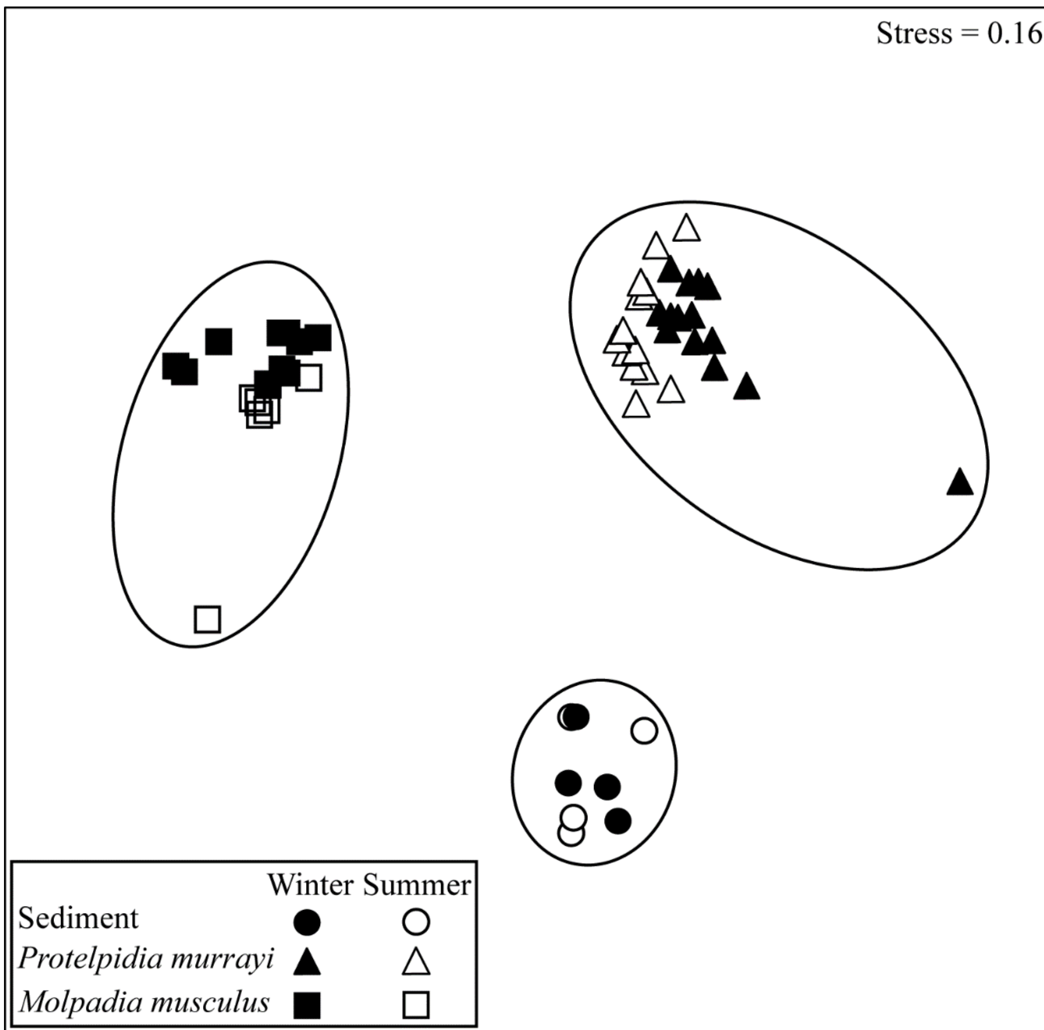

Figure 3. nMDS plot of ST compositions of *P. murrayi*, *M. musculus*, and surface sediment samples in summer 2008 and winter 2009. Axis scales are arbitrary in nMDS and are therefore omitted.

(diatoms; Graeve et al., 1997, Parrish et al., 2000), and 18:1ω7 (bacteria; Bowman et al., 1991, Pearson et al., 2001, Hudson et al., 2004) (Appendix A). These FAs accounted for 57 – 65% of total FAs at each station. 16:0n was the most prevalent FA in sediments at all stations during winter, ranging from 17 – 19% of total FAs, and was present at 15 – 20% of total FAs in summer (Appendix A). 18:0n was present at levels of 9 – 17% during winter and 8 – 16% during summer, 16:1ω7 ranged from 12 – 17% in winter and 14 – 16% during summer, and 18:1ω7 ranged from 12 – 13% in both winter and summer seasons at all stations (Appendix A). 16:0n, 18:0n, 16:1ω7, and 18:ω7 varied little on the latitudinal gradient in winter and summer (Appendix A).

Saturated FAs (SFAs) and monounsaturated FAs (MUFAs) were prevalent at all stations on the transect during both seasons (with totals equaling 44 – 55% and 41 – 52%, respectively), whereas total polyunsaturated FAs (PUFAs) were present at very low levels across the region (2 – 5%) (Appendix A). Lipid markers for zooplankton were negligible in surface sediments of the WAP. FA biomarkers for zooplankton, 20:1 and 22:1 isomers (Parrish et al., 2000, Dalsgaard et al., 2003), contributed ≤ 5% to total FAs at all stations during winter and summer. In both winter and summer, the proportions of SFAs, MUFAs, and PUFAs in surface sediments varied little along the north to south transect. Bacterial markers were dominant at all stations during both seasons ranging from 23 – 27% of total FAs. Diatom markers ranged from 15 – 19% and flagellate markers contributed < 9% of total FAs (Appendix A). FA markers for diatoms, bacteria, and flagellates also exhibited minimal variation along the north-south transect.

Deposit-feeders

Within species by latitude and season: overall fatty acid trends

Both holothurians had high levels of *de novo*-derived FAs (i.e., 22:1ω9 and 23:1ω9; Ginger et al., 2001) and 20:4ω6, which is important for membrane structure and cold adaptation (Harwood and Vigh, 1998). *P. murrayi* had high levels of phytoplankton-derived FAs (i.e., 20:5ω3), while *M. musculus* had a mixture of bacterial- and phytoplankton-derived OM (i.e., 16:1ω7, 18:1ω7 and 20:5ω3). There was little variation on the north to south sampling transect indicating a persistent food source available for the benthic community on the WAP. The dominant FAs present in *P. murrayi* were 20:4ω6, 20:5ω3, 22:1ω9, and 23:1ω9, making up 50-60% of total FAs in winter and 48-57% in summer. During winter, *P. murrayi* FA composition differed between station B and G (ANOSIM, $R = 0.73$, $p < 0.05$) and between E and G (ANOSIM, $R = 0.59$, $p < 0.05$). There were no significant differences between B and E (ANOSIM, $R = 0.16$, $p > 0.05$) during winter. The FAs contributing most to differences between B and G in winter, based on SIMPER, were 20:4ω6 and 20:5ω3 with both FAs in higher abundance at B. FAs contributing most to differences between E and G were 20:4ω6 and 16:1ω7, with 20:4ω6 being higher at station E and 16:1ω7 higher at station G. Summer FA composition in *P. murrayi* tissue differed between stations B and G ($R = 0.38$, $p < 0.05$) with 20:4ω6, 23:1ω9, and 16:1ω7 contributing most. FA composition also differed between stations E and G (ANOSIM, $R = 0.78$, $p < 0.05$) with 20:4ω6, 16:0n, and 23:1ω9 contributing most to the difference, based on SIMPER. No differences in FA composition were detected in *P. murrayi* collected at B and E (ANOSIM, $R = 0.06$, $p > 0.05$).

The dominant FAs in *M. musculus* in winter were 20:4ω6, 18:1ω7, 22:1ω9, and 23:1ω9, making up 54-62% of total FAs. In summer, the dominant FAs were similar, 20:4ω6, 18:1ω7, 22:1ω9, and 23:1ω9 with the addition of 19:1ω12 that together accounted

for 63-68% of total FAs. *M. musculus* winter FA composition differed between station AA and both B (ANOSIM, $R = 0.72$, $p < 0.05$) and E (ANOSIM, $R = 0.81$, $p < 0.05$). There were no significant differences between B and E (ANOSIM, $R = 0.85$, $p > 0.05$). The FAs contributing most to difference in *M. musculus* between AA and B were 20:4ω6, 22:1ω9, and 23:1ω9 (based on SIMPER). Between stations AA and E, the FAs contributing most, based on SIMPER, were 20:4ω6, 23:1ω9, and 16:1ω7. During summer, there were no statistically significant differences between FA compositions of *M. musculus* collected along the sampling transect.

PUFAs were prevalent in both deposit feeders at all stations during winter and summer (Appendix A). In *P. murrayi*, 20:4ω6 and 20:5ω3 contributed 35 – 44% of total FAs in winter and 34 – 39% in summer. PUFAs in *P. murrayi* decreased slightly from the north to the south during winter and were significantly different between stations B and G (t-test, $p = 0.02$) due to decreases in relative abundance of 20:4ω6 (t-test, $p = 0.00$) and 20:5ω3 (Mann-Whitney U, $p = 0.01$) at station G. There was also a significant difference in PUFAs between stations E and G (t-test, $p = 0.04$) due to lower abundance of 20:4ω6 at station G (t-test, $p = 0.01$). There were no differences in PUFA between stations B and E in winter. In summer, *P. murrayi* PUFA was lower at station G compared to E (t-test, $p = 0.01$), due to lower relative abundances of 20:4ω6 (t-test, $p = 0.00$). There were no significant differences in PUFAs at station B compared with E or G in summer. PUFA content in *M. musculus* was mainly due to high relative abundances of 20:4ω6 (range of 26 – 40% in winter and 34 – 40 % in summer; Appendix A). During winter, *M. musculus* PUFAs were higher in abundance at station AA compared to E (t-test, $p = 0.03$) due to a significant difference in 20:4ω6 (t-test, $p = 0.01$). There was a significant difference in 20:4ω6 between stations AA and B (t-test, $p =$

0.04) with this FA higher in abundance at station B. In winter, PUFAs were also present in higher abundances at station B compared to E (t-test, $p = 0.02$) mainly due to a higher relative abundance of 20:4ω6 at station B (t-test, $p = 0.01$). There were no significant differences in PUFA levels of *M. musculus* during summer.

During winter and summer, MUFAs were present at higher relative abundances in *M. musculus* (38 – 50% of total FA) than *P. murrayi* (33 – 38% of total FA). In winter, MUFAs in *P. murrayi* were significantly different between stations B and G (t-test, $p = 0.02$) due to higher relative abundances at station G of 16:1ω5 (t-test, $p = 0.01$), 16:1ω7 (Mann-Whitney U, $p = 0.01$), and 16:1ω9 (Mann-Whitney U, $p = 0.01$). There were no significant differences of MUFAs in *P. murrayi* between station E compared to B or G. During winter, MUFAs in *M. musculus* were significantly higher in relative abundance at station AA compared to B (t-test, $p = 0.00$). This was due to higher abundances of 16:1ω7 (t-test, $p = 0.01$) and 22:1ω9 (Mann-Whitney U, $p = 0.03$) at AA. MUFA abundances were significantly higher at station E compared to B (t-test, $p = 0.00$) due to significantly higher abundances of 16:1ω7 (Mann-Whitney U, $p = 0.05$) and 22:1ω9 (Mann-Whitney U, $p = 0.05$) at station E. There were no significant differences in MUFA content at station E compared with AA during winter. During summer for *P. murrayi* and *M. musculus*, there were no significant differences in relative abundances of MUFAs along the north south transect.

Total SFAs were lower than PUFAs and MUFAs in both species of deposit-feeders (range of 10 – 23% of total FA) during both seasons, with 16:0n and 18:0n being most abundant in both species (Appendix A). During winter, SFA relative abundances in *P. murrayi* were not significantly different between stations B, E, and G. During summer, SFAs in *P. murrayi* tissue were significantly higher at station G compared to B (t-test, $p = 0.001$)

and E (t-test, $p = 0.00$), while there was no significant difference in SFAs between stations B and E. In winter, SFA relative abundances in *M. musculus* were significantly different between stations AA and E (t-test, $p = 0.01$) primarily due to relatively more 16:0n (t-test, $p = 0.00$) at station E. There were no significant differences in SFAs between station B compared with stations AA and E. SFAs in *M. musculus* were not significantly different between stations AA, B, and E during summer.

Diatom FA markers (Table 1) were present at highest relative abundances in *P. murrayi* during both seasons (range of 20 – 23% of total FA). Comparably, *M. musculus* diatom FA marker contributions to total FAs were low (<13% of total FA) during both seasons. There were no significant differences in diatom FA markers in *P. murrayi* between stations B, E, or G during winter 2008. In summer, diatom FA markers in *P. murrayi* were higher at station G than E (t-test, $p = 0.01$). There were no significant differences in diatom FA markers of *P. murrayi* between station B compared to both G and E. There were higher levels of diatom FA markers in *M. musculus* at station E compared to AA during winter (t-test, $p = 0.04$), but no other differences were found. In summer, *M. musculus* diatom FA markers were significantly different between AA and B (t-test, $p = 0.03$). There were no other differences in diatom FA markers in *M. musculus* during summer.

Bacterial FA markers were low in *P. murrayi* (≤12% of total FA) and slightly higher in *M. musculus* (12 – 15% of total FA) during both seasons. In winter and summer, bacterial FA markers increased significantly in *P. murrayi* at station G compared to B (t-test, $p = 0.02$) and E (t-test, $p = 0.04$). There were no differences in bacterial FA markers between stations B and E during either season. *M. musculus* exhibited no significantly different levels of

bacterial FA markers during winter. In summer, bacterial FA markers were higher at station E compared to AA and B.

Flagellate FA markers were present at low levels (< 10% of total FA) in both species during both sampling seasons, although in *P. murrayi*, these markers were significantly higher at station G compared to E during winter (Mann-Whitney U, $p = 0.03$). There were no significant differences in flagellate FA markers of *P. murrayi* between station B compared to G and E in winter, and any stations during summer. *M. musculus* tissue exhibited no significant differences in flagellate FA markers between stations AA, B, and E during both seasons.

Between species comparison by season

There were differences between FA compositions of the two holothurians during winter at stations B and E. At station B, 20:4ω6 in *M. musculus* and a phytoplankton-derived FA (20:5ω3) in *P. murrayi* contributed most to the differences between species. At station E, a mixture of bacterial (i.e., 18:1ω7) and phytoplankton-derived (16:1ω7) FAs in *M. musculus* and a phytoplankton-derived FA (20:5ω3) in *P. murrayi* separated the two species. During summer at station B, 20:4ω6 and 19:1ω12 in *M. musculus* and a phytoplankton-derived FA (20:5ω3) in *P. murrayi* contributed most to the difference between the species. These results indicate that *P. murrayi* assimilated phytoplankton-derived FAs, mostly from diatoms, while *M. musculus* assimilated a mixture of fresh, phytoplankton- and bacterial-derived FAs.

Between species analysis was restricted to stations where both species were collected, stations B and E. PCA analysis separated FA profiles between the two holothurian species during winter and summer (Figs. 4 & 5). During winter, PC1 explained 75% of the variability, and FAs that contributed most to separation were 20:4ω6 and 20:5ω3 (Fig. 4).

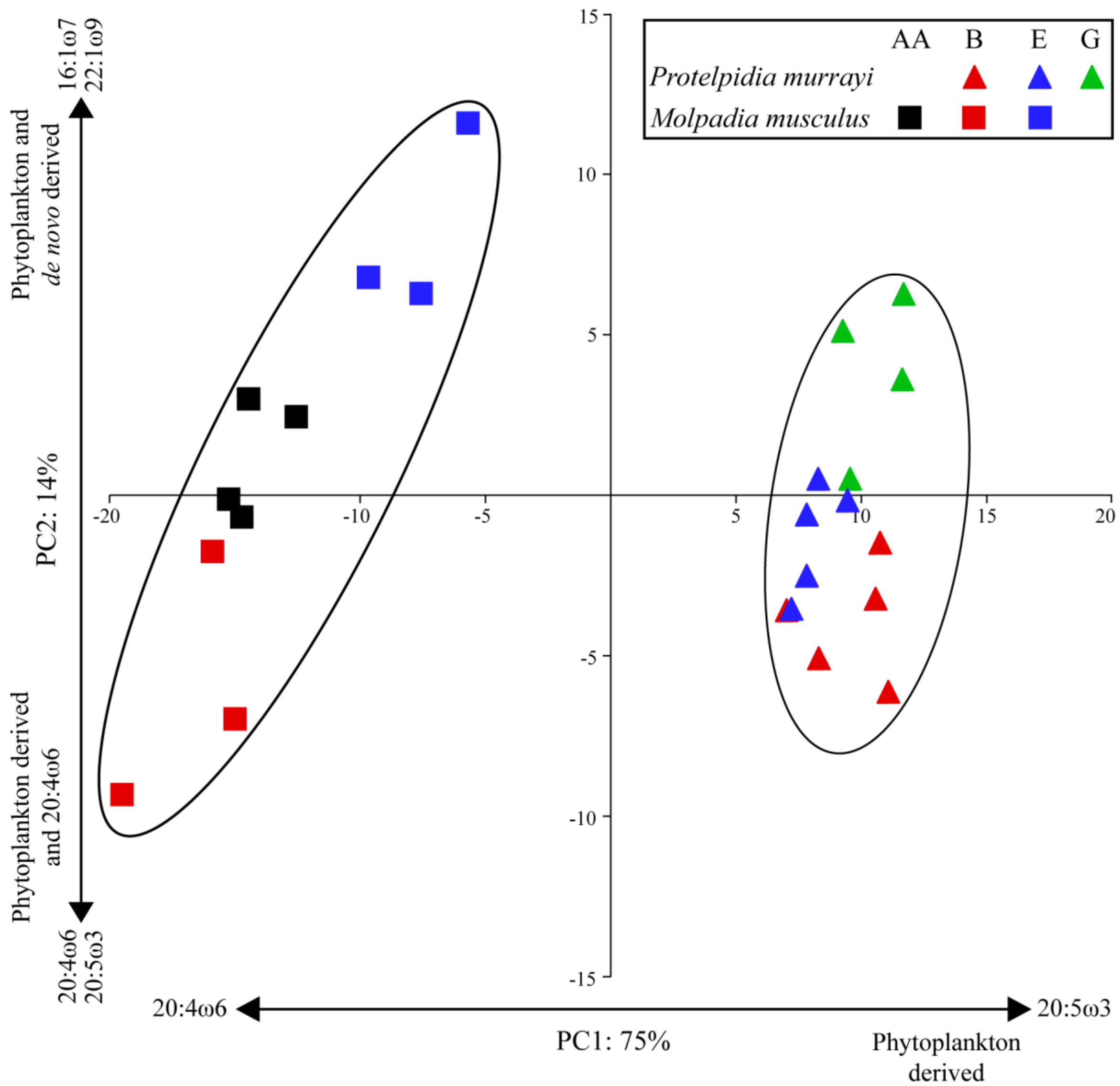

Figure 4. Biplot of first and second principal components derived from fatty acid composition of *Protelpidia murrayi* and *Molpadia musculus* during winter 2009. PC1 explained 75% of the variance between species and PC2 explained 14%. Arrows indicate FA that contributed most to separation along each component.

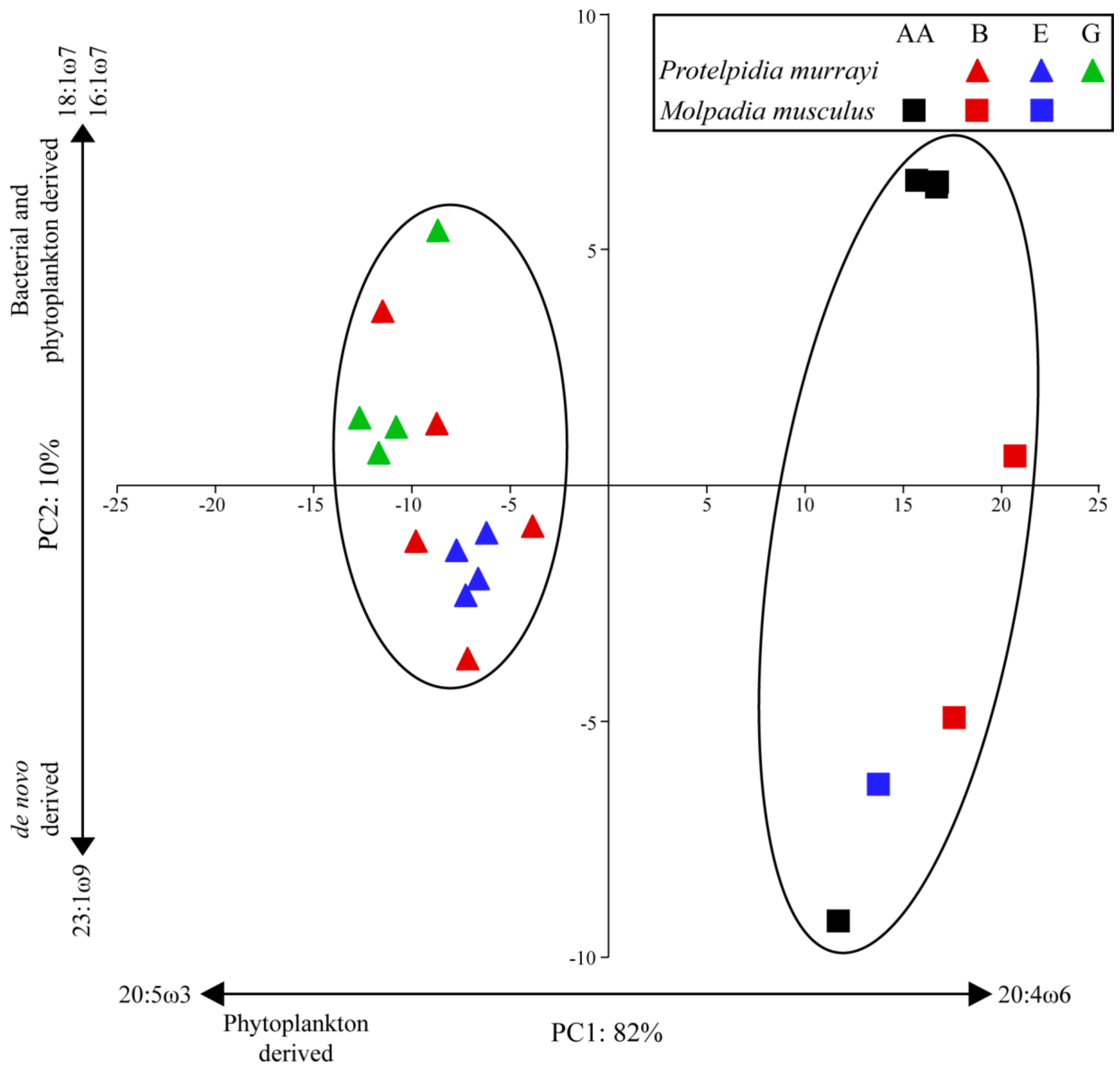

Figure 5. Biplot of first and second principal components derived from fatty acid composition of *Protelpidia murrayi* and *Molpadia musculus* during summer 2008. PC1 explained 82% of the variance between species and PC2 explained 10%. Arrows indicate FA that contributed most to separation along each component.

PC2 explained only 14% of the variance during winter and 16:1ω7, 22:1ω9, 20:4ω6, and 20:5ω3 contributed to separation along the component (Fig. 4). During summer, PC1 explained 82% of the variance (Fig. 5). FAs that contributed most to separation of species in summer were 20:4ω6 and 20:5ω3 (Fig. 5). PC2 explained only 10% of the variance during the summer and 18:1ω7, 16:1ω7, and 23:1ω9 contributed to separation along this component (Fig. 5). Using ANOSIM, a winter comparison of FA compositions of *P. murrayi* and *M. musculus* revealed significant differences at stations B (ANOSIM, $R = 1$, $p < 0.05$) and E (ANOSIM, $R = 1$, $p < 0.05$). SIMPER was used to identify the FAs contributing most to the difference in species composition. During winter at station B, the FAs that differed most between *P. murrayi* and *M. musculus* were 20:4ω6 and 20:5ω3, while at station E the differing FAs were 20:5ω3, 16:1ω7, and 18:1ω7. In summer at station B, there were significant differences in FA composition between the two species at stations B (ANOSIM, $R = 1$, $p < 0.05$) and based on SIMPER, the FAs that contributed most to this difference were 19:1ω12, 20:4ω6, and 20:5ω3. Multivariate statistics could not be run on the samples from station E in summer because there was only 1 sample of *M. musculus* retrieved.

Sterols

Phytosterols are synthesized only by algae and plants (Volkman, 1986, Volkman, 2003, Rozner and Garti, 2006, Drazen et al., 2008) and marine organisms incorporate them through feeding on these sources. Phytosterols produced by plants and algae commonly have Δ^5 saturation (Volkman, 1986, Volkman, 2003, Rozner and Garti, 2006, Drazen et al., 2008). STs in holothurians have high levels of Δ^7 saturation (Stonik et al., 1998, Ginger et al. 2000, 2001, Kanazawa, 2001) which is consistent with holothurian STs in this study's samples. The presence of Δ^7 STs indicates biotransformation of dietary Δ^5 STs into their Δ^7 counterparts

(Ponomarenko et al., 2001). Phytosterols with Δ^5 saturation were prevalent in sediment trap and surface sediment samples while their Δ^7 derivatives were prevalent in both holothurians. Therefore, it was assumed that Δ^7 biotransformed Δ^5 phytosterols summed with Δ^5 phytosterols gave an accurate abundance of phytosterol contribution to total STs in the holothurians. (Table 1).

Cholest-5-en-3β-ol (cholesterol) is often present in zooplankton samples (Phleger et al., 2000, Volkman, 2005, Wisnieski et al., 2014), and can also be derived from phytoplankton (Wisnieski et al., 2014), specifically diatoms (Volkman, 1986, Nichols et al., 1990, Barrett et al., 1995). Abyssal holothurians biotransform Δ^5 (i.e. cholest-5-en-3β-ol) to Δ^7 STs (i.e. cholest-7-en-3β-ol) (Voogt and Over, 1973, Smith and Goad, 1975, Goad, 1981, Ginger et al., 2000). Cholest-7-en-3β-ol has also been found in diatoms (Volkman, 1986, Barrett et al., 1995, Dahl et al., 2004), however, presence of cholest-7-en-3β-ol in sediment trap and surface sediment samples is limited. Thus its prevalence in holothurian samples indicated biotransformation of cholesterol is also a source. Cholesterol and cholest-7-en-3β-ol were both prevalent in phytoplankton samples all along the latitudinal gradient of the WAP (Appendix B) and were considered to be phytosterols in this region. Bacteria can modify sterol structures through biohydrogenation of Δ^5-stenols to 5α(H)-stanols (Rosenfeld and Hellman, 1971, Eyssen et al., 1973, Volkman et al., 1990). Thus, an increase in the ratio of 5α(H)-stanols/Δ^5-stenols can be used to indicate bacterial alteration of OM (Li et al., 1995, Wakeham et al., 1997, Jeffreys, 2006, Carreira et al., 2011, Wisnieski et al., 2014).

Surface sediment

Surface sediments were characterized by a mixture of C_{27} (22 – 38%), C_{28} (28 –

41%), C_{29} (28 – 36%) STs during winter and summer (Appendix B). C_{26} and C_{30} STs were also present but at levels <10% (Appendix B). The predominant ST in surface sediments was 24-methylcholesta-5,22- dien-3β-ol, contributing 22 – 25% of total STs in winter and 19 – 22% in summer (Appendix B). 24-ethylcholest-5-en-3β-ol and cholest-5-en-3β-ol (i.e., cholesterol) were also dominant in surface sediments. These three STs accounted for 42 – 50% of total STs during both seasons. ST levels followed a similar trend to FAs within the surface sediments and the most prevalent STs exhibited little variation along the north-south sampling gradient.

Phytosterols were prevalent in surface sediments at all stations in winter (73 – 79%) and summer (70 – 80%). 5α-stanols ranged from 6 – 15% in winter and 5 – 14% in summer. Phytosterols and 5α-stanols did not show latitudinal variation in winter or summer. The ratio of 5α(H)-stanols/Δ^5 STs was used to evaluate degradation processes or preservation of OM in surface sediments (Hudson et al., 2001). Here ratio values of 0.1 – 0.5 were considered indicative of recently deposited OM. Ratio values > 0.5 indicated degradation of material, implying aging of material within the seabed. All ratios were <0.5, with the exception of 24-ethylcholestan-3β-ol /24-ethylcholest-5-en-3β-ol during winter at station E (value of 0.6). Another indication of recent deposition at these stations was the presence of phytol in surface sediments at all stations during both seasons (Pirtle-Levy, Appendix B), which is derived from degradation of chlorophyll-*a* (Volkman et al., 2008) and has been used as a marker for algal input (Huang et al., 2012).

Deposit feeders

Within species comparison by latitude and season

The surface deposit feeder, *P. murrayi*, had higher levels of phytosterols compared to the subsurface deposit feeder, *M. musculus*, indicating phytoplankton-derived food sources are important for *P. murrayi*. *M. musculus* had higher levels of 5α(H)-stanols than *P. murrayi* but also had moderate levels of phytosterols indicating it fed on a mixed diet of fresh and older OM. *P. murrayi* ST composition exhibited little variation on the latitudinal transect during winter and summer, similar to the surface sediment. *M. musculus* had lower phytosterol levels and higher stanol levels from north to south in its range during both seasons which could indicate less available fresh OM at depth in the sediment for this holothurian to access at station E, its southernmost range.

During winter, phytosterols in *P. murrayi* ranged from 61 – 65% and exhibited little variation on the north to south transect. In *M. musculus*, they ranged from 12 – 44% in winter and decreased from station AA to station E. During summer, phytosterols in *P. murrayi* ranged from 56 – 64%, exhibiting little latitudinal variation. In *M. musculus* they ranged from 27 – 44% in summer and were again lowest at station E. During both seasons, phytosterols were more abundant in *P. murrayi*.

5α(H)-stanols were present in both holothurians, though higher in *M. musculus*. During winter, total 5α(H)-stanol levels in *P. murrayi* were < 8% of total STs and varied little on the north to south transect. *M. musculus* total 5α(H)-stanol levels ranged from 31 – 56% of total STs during winter and increased from station AA to station E. Summer total 5α(H)-stanol levels in *P. murrayi* were a bit higher and ranged from 14 – 17%. *M. musculus* total 5α(H)-stanol levels in summer ranged from 27 – 43% and increased from north to south.

The dominant STs varied between species. At stations B, E, and G during both seasons, *P. murrayi* contained high levels of cholest-7-en-3β-ol, an unknown C_{29} ST, and 24-

ethylcholest-7-en-3β-ol (Appendix B). In winter, *P. murrayi* tissue exhibited negligible latitudinal variation in these STs. In summer, cholest-7-en-3β-ol varied little between stations B and E and increased at station G, whereas the C_{29} unknown also varied little between stations B and E and decreased at station G. The ST 24-methylcholest-22-en-3β-ol was dominant in *M. musculus* tissue at stations AA, B, and E during both seasons. 24-methylcholest-22-en-3β-ol increased along the north to south transect in *M. musculus* tissue during winter, and exhibited little variation along the transect in summer. The STs 5α-cholest-22-en-3β-ol, 24-methylcholest-24(28)-en-3β-ol, 24-methylcholesta-7,22-dien-3β-ol, and cholesta-7,22-dien-3β-ol were also present at high levels. During winter, 5α-cholest-22-en-3β-ol and cholesta-7,22-dien-3β-ol varied little in *M. musculus* tissue on the north to south transect, whereas in summer both STs increased at only station B while levels at AA and E were similar. In winter, 24-methylcholesta-7,22-dien-3β-ol in *M. musculus* tissue was present at similar levels at stations AA and B and decreased at station E. During summer, 24-methylcholesta-7,22-dien-3β-ol in *M. musculus* tissue was present at high levels at station AA and decreased at stations B and E. 24-methylcholest-24(28)-en-3β-ol exhibited little variation in *M. musculus* tissue on the north to south transect during winter and summer.

There were significant differences of ST composition in *P. murrayi* during winter between station B compared to both E (ANOSIM, $R = 0.184$, $p < 0.05$) and G (ANOSIM, $R = 0.35$, $p < 0.05$). Based on SIMPER, the STs contributing most to composition differences between stations B and E were 24-ethycholest-7-en-3β-ol and 24-ethylcholest-5-en-3β-ol and between stations B and G were 24-ethylcholest-5-en-3β-ol and Unknown ST #2. There were no significant differences in ST composition between stations E and G. Summed phytosterol levels did not differ between the stations. However, two phytosterols were significantly less

abundant at station B compare to station G, an isomer of cholesta-7,22-dien-3β-ol (Mann-Whitney U, $p = 0.01$) and 24-methylcholesta-5,22-dien-3β-ol (Mann-Whitney U, $p = 0.01$). 5α(H)-stanols were significantly less abundant at station B compared with E (Mann-Whitney U, $p = 0.02$) and G (Mann-Whitney U, $p = 0.03$). Though summed 5α(H)-stanols exhibited no difference between stations B and G, the 5α(H)-stanol 5α-cholestan-3β-ol was significantly higher at station G (Mann-Whitney U, $p = 0.01$) compared to B. During summer, there were no significant differences in ST composition of *P. murrayi* between stations B, E, and G. The unknown C_{29} was most abundant at station G and significantly different than B (Mann-Whitney U, $p = 0.01$) and E (Mann-Whitney U, $p = 0.01$). 24-ethylcholesta-5,22-dien-3β-ol was least abundant at station G and also significantly different compared to B (Mann-Whitney U, $p = 0.01$) and E (Mann-Whitney U, $p = 0.01$). There was no significant difference in either ST between stations B and E during summer. There was no significant difference in the summed 5α(H)-stanol or phytosterol groups during summer for *P. murrayi*. Though summed 5α(H)-stanols exhibited no difference between stations, the 5α(H)-stanol 5α-cholestan-3β-ol was significantly higher at station G compared to B (Mann-Whitney U, $p = 0.01$) and E (Mann-Whitney U, $p=0.03$). The phytosterol cholest-7-en-3β-ol was significantly different and less abundant at stations B (Mann-Whitney U, $p=0.03$) and E (Mann-Whitney U, $p=0.02$) compared to station G. The phytosterol 24-ethylcholest-7-en-3β-ol was more abundant at station E and significantly different than station G (Mann-Whitney U, $p=0.02$).

During winter, ST composition in *M. musculus* differed only between stations AA and E (ANOSIM, $R=1$, $p < 0.05$). The STs contributing most to this difference, based on SIMPER analysis, were 24-ethylcholestan-3β-ol, 5α-cholestan-3β-ol, 24-methylcholesta-

7,22-dien-3β-ol, and 24-ethylcholest-7-en-3β-ol. The 5α(H)-stanol 5α-cholestan-3β-ol differed significantly between stations AA and E (Mann-Whitney U, $p = 0.03$) and was highest at station E. There was also a significant difference of 5α-cholestan-3β-ol between stations AA and B (Mann-Whitney U, $p = 0.03$) with AA having the lowest levels. No significant difference of this ST was present between stations B and E. Summed phytosterols were highest at station AA and differed significantly from station E (Mann-Whitney U, $p = 0.03$). No significant difference in summed phytosterols occurred during winter between AA and B or between B and E. The level of cholesta-7,22-dien-3β-ol was significantly higher at stations AA (Mann-Whitney U, $p=0.03$) and B (Mann-Whitney U, $p=0.05$) compared to E. The FA cholest-7-en-3β-ol was significantly lower at station E compared to AA (Mann-Whitney U, $p=0.03$) while no differences were found between B and stations AA and E. Both isomers of 24-ethylcholest-7-en-3β-ol were significantly higher at station AA compared to E (Mann-Whitney U, $p=0.03$ for both). The level of 5α(H)-stanols was much higher at station E compared to AA (Mann-Whitney U, $p = 0.03$). No significant difference in 5α(H)-stanols occurred between A and B or B and E during winter. Levels of 24-ethylcholestan-3β-ol were significantly higher at station E compared to AA (Mann-Whitney U, $p=0.03$) and B (Mann-Whitney U, $p=0.05$). 5α-cholestan-3β-ol was significantly lower at station AA compared to stations B (Mann-Whitney U, $p=0.03$) and E (Mann-Whitney U, $p=0.03$). 24-methylcholest-22-en-3β-ol was significantly lower at station AA compared to E ((Mann-Whitney U, $p=0.03$) but no other differences were present. There were no significant differences in ST composition, phytosterols, or 5α(H)-stanols in *M. musculus* between stations AA B during summer. Phytosterols were lower and 5α(H)-stanols were higher at station E compared with AA and B in summer.

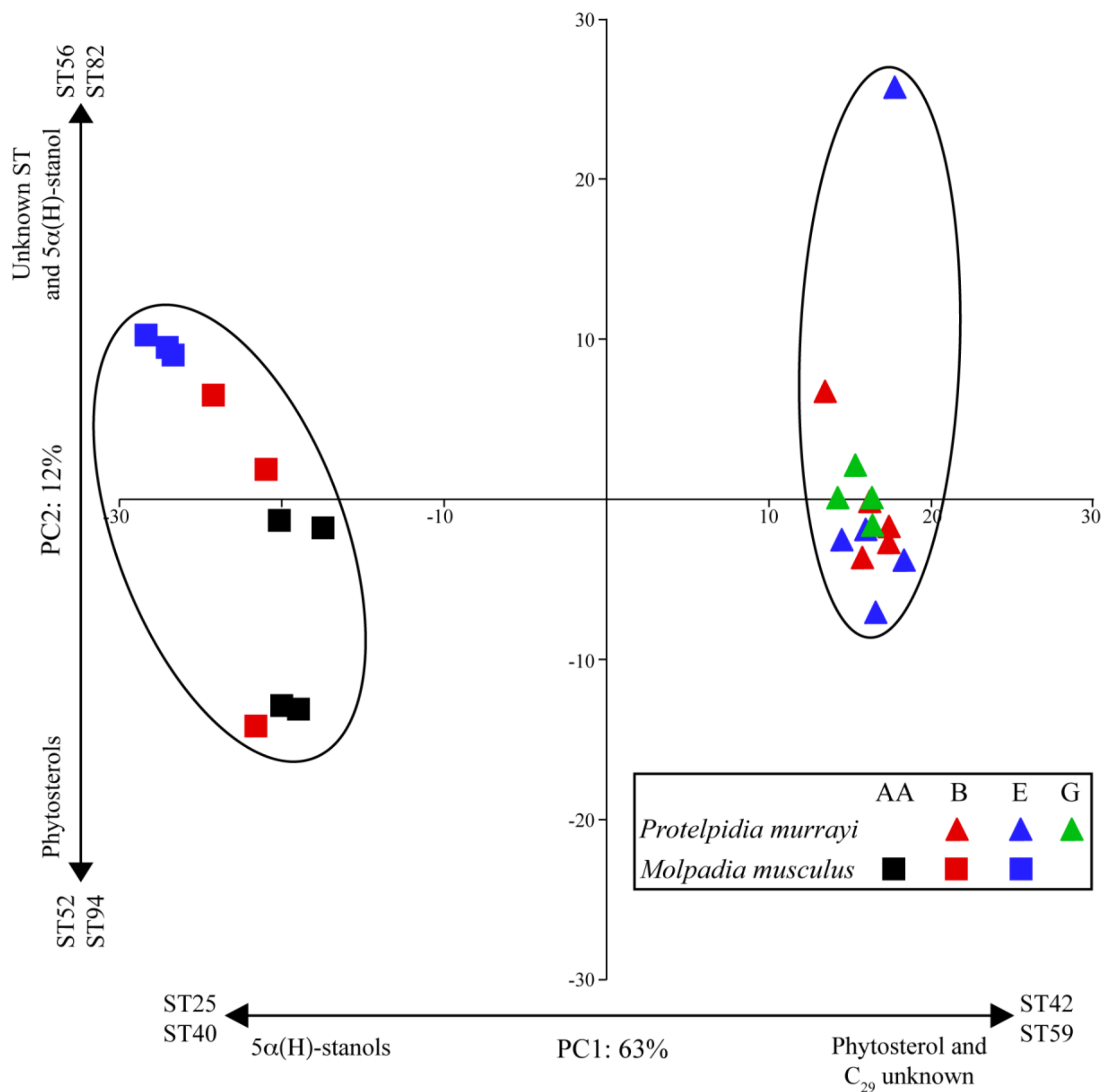

Figure 6. Biplot of first and second principal components derived from sterol composition of *Protelpidia murrayi* and *Molpadia musculus* during winter 2008. PC1 explained 63% of the variance between species and PC2 explained 12%. Arrows indicate ST that contributed most to separation along each component. ST25: 5α-cholestan-3β-ol, ST40: 24-methylcholest-22-en-3β-ol, ST42: cholest-7-en-3β-ol, ST52: 24-methylcholesta-7,22-dien-3β-ol, ST56: Unknown #1, ST59: C_{29} Unknown, ST82: 24-ethylcholestan-3β-ol , ST94: 24-ethylcholest-7-en-3β-ol.

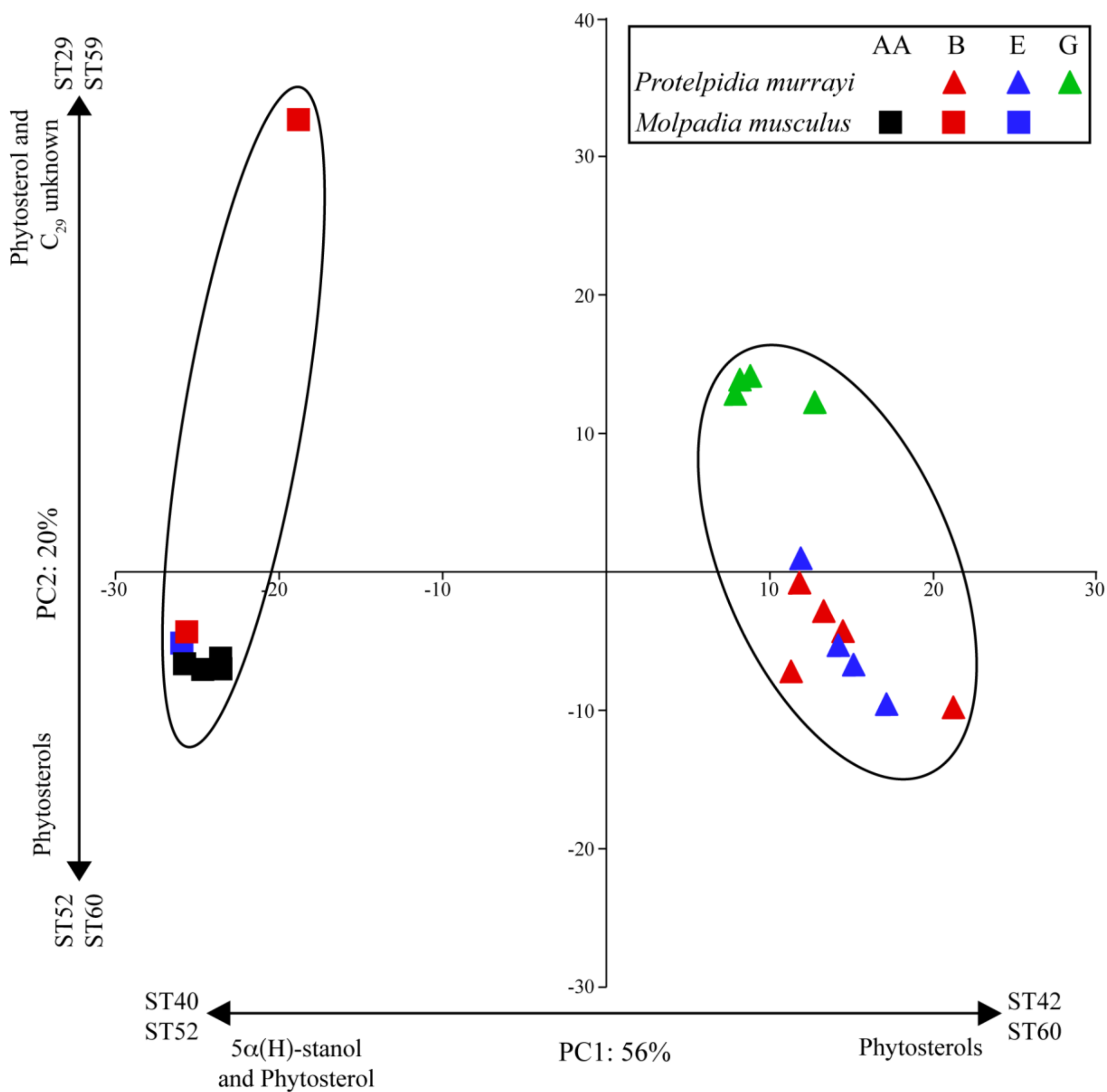

Figure 7. Biplot of first and second principal components derived from ST composition of *Protelpidia murrayi* and *Molpadia musculus* during summer 2009. PC1 explained 56% of the variance between species and PC2 explained 10%. Arrows indicate ST that contributed most to separation along each component. ST29: cholesta-7,22-dien-3β-ol, ST40: 24-methylcholest-22-en-3β-ol, ST42: cholest-7-en-3β-ol, ST52: 24-methylcholesta-7,22-dien-3β-ol, ST:59 C_{29} Unknown, ST60: 24-ethylcholesta-5,22-dien-3β-ol.

Similar to comparison of FA compositions, PCA analysis separated ST composition between the two holothurian species during winter and summer (Figs. 6 and 7, respectively). During winter, PC1 explained 63% of the variability and STs that contributed most to separation of species along this component were 24-methylcholest-22-en-3β-ol and cholest-7-en-3β-ol (Fig. 6). Separation of species along PC2, explaining 12% of the variability, was due to 24-methylcholesta-7,22-dien-3β-ol, 24-ethylcholest-7-en-3β-ol, 24-ethylcholestan-3β-ol, and 5α-cholestan-3β-ol (Fig. 6). Similar to PCA from winter, species collected in summer exhibited most separation along PC1, with 56% of the variance explained by this component (Fig. 7), and STs that contributed most to separation of species along PC1 were 24-methylcholest-22-en-3β-ol, 24-methylcholesta-7,22-dien-3β-ol, 24-ethylcholesta-5,22-dien-3β-ol, and cholest-7-en-3β-ol (Fig. 7). PC2 explained 20% of the variance during the summer and cholesta-7,22-dien-3β-ol, C_{29} unknown, 24-methylcholesta-7,22-dien-3β-ol, and 24-ethylcholesta-5,22-dien-3β-ol contributed to separation along this component (Fig. 7).

Between species comparison by season

During winter, a 5α(H)-stanol in *M. musculus* and a phytosterol and an unknown ST in *P. murrayi* contributed to the differences in ST composition of the holothurians at stations B and E. In summer at station B, a mixture of phytosterols contributed to the differences in ST composition of the two holothurians. When the deposit feeders were compared at B and E, there were differences in ST composition at both stations in winter and at B only in summer. In winter, the differences at stations B (ANOSIM, $R = 1$, $p < 0.05$) and E (ANOSIM, $R = 1$, $p < 0.05$), based on SIMPER, were due to the same STs: 24-methylcholest-22-en-3β-ol, cholest-7-en-3β-ol, and C_{29} unknown. During summer, the

difference at station B (ANOSIM, $R = 1$, $p < 0.05$) was due to 24-ethylcholesta-5,22-dien-3β-ol, cholesta-7,22-dien-3β-ol, and cholest-7-en-3β-ol (based on SIMPER).

Discussion

Temporal and spatial variation in sediment lipid composition

The present study indicates that FA and ST compositions within surface sediments varied little along the north-south transect during winter and summer. SFAs, MUFAs, and PUFAs varied little during both seasons along the sampling transect, as did the most prevalent FAs, including 16:0n, 18:0n, 16:1ω7, and 18:1ω7. The presence of phytoplankton-derived FAs, phytosterols, phytol, and low ratios of 5α(H)-stanols/Δ^5 STs indicate fresh OM deposition to the seafloor at our sampling sites. Sedimentation rates in the WAP can be high (4.6 mmol C m^{-2} d^{-1}; Buessler et al., 2010), however, the flux of OM to the seabed exhibits dramatic seasonal variation in the WAP (Smith et al., 1996, Smith et al., 2012). The complexity of seasonal sea ice and phytoplankton bloom dynamics in the region make it difficult to predict the location, timing, and intensity of OM flux to the seabed (Smith et al., 2008). Primary production during some years may result in thick phytodetrital layers on the surface of the seabed, while other years may have low inventories of deposited OM (Smith et al. 2008, Smith et al., 2012). The lack of temporal and spatial trends in the lipid compositions may reflect patchy delivery of fresh OM to the sea floor (Santos et al., 1994) that was missed by low resolution sampling (Neto et al., 2006). However, other seabed parameters measured during the FOODBANCS2 Project also showed little evidence of latitudinal variation during winter and summer including benthic community respiration, sediment microbial biomass, and sediment bioturbation rates based on ^{234}Th excess activities (Smith et al., 2012). Trends

in these benthic parameters, along with lipid biomarkers, may respond to decadal variations in quantity and quality of primary production and OM deposition (Smith et al., 2012).

Spatial and temporal differences in the deposit feeders

The lack of lipid level variation within surface sediments on the north-south latitudinal gradient was not mirrored in the species-specific FA and ST compositions indicating selectivity of fresh, easily digestible components. *M. musculus* collected at the northernmost station (AA) had a different FA/ST signature when compared to individuals collected at the southern stations (stations B and E). Lipid signatures for *P. murrayi* collected at the southernmost station (G) were different from those collected at the 2 more northerly stations (stations B and E). The cause for these for species-specific variations in FA and ST composition on the latitudinal gradient could be the result of lipid accumulation for gamete production (David and MacDonald, 2002, Kuhnz et al., 2014) or rapid response to seasonal bloom production before it is reflected in sediments, occurring at the northernmost station for *M. musculus* and the southernmost station for *P. murrayi*. The mobility of *P. murrayi* may allow them to search for patches of fresh OM, whereas *M. musculus* creates mounds that have been proposed to concentrate OM as it settles (Smith et al., 1986). However, latitudinal differences could also be attributed to assimilation variability rather than reproduction cues or climate-induced changes occurring in the pelagic zone. Selective ingestion and assimilation has been documented in these holothurians (Purinton et al., 2008, McClintic et al., 2008) and assimilation of specific FAs and STs is likely when fresh POM is available.

Temporal differences are more difficult to tease out over this short time scale because this system tends to respond to long-term variability (i.e. decadal scales) in pelagic production (Smith et al., 2006, Mincks et al., 2008). STs are considered to be more stable

compared to FAs (Volkman et al., 2008) and differences in ST composition during winter and summer could be the result of natural variability in holothurian assimilation and biotransformation of these lipids. Variation of FA composition in holothurian tissues may be more indicative of short-term response to seasonal OM input. Diatom FA markers did not vary significantly in winter in *P. murrayi* but were higher at station G in summer which could indicate feeding response to recent deposition of OM. Bacterial FA markers increased significantly for *P. murrayi* at station G from winter to summer. *M. musculus* diatom FA markers increased significantly at station E from summer to winter, which might reflect slower penetration of these FA markers into deeper layers of sediment possibly due to bioturbation. No differences in bacterial markers were found in *M. musculus* in either season. Shifts in MUFAs for both deposit feeders were driven by increases in 16:1ω7, a marker for diatoms (Volkman et al., 1989, Parrish et al., 2000) at station G in *P. murrayi* and station AA in *M. musculus*, indicating higher levels of diatom-based OM at these stations. The PUFA 20:4ω6 decreased in both *P. murrayi* and *M. musculus* at the southernmost stations, station G for *P. murrayi* and station E for *M. musculus*. Without further sampling, it is difficult to determine what caused this variation, however, decreases in 20:4ω6 could affect cell maintenance and membrane fluidity of the holothurians. Seasonal response to OM input has been noted in *P. murrayi* using stable isotopes (Mincks et al., 2008), and there is variability in specific FAs on the north-south gradient. However, FA composition in *P. murrayi* and *M. musculus* had substantial overlap and similarity as seen in the nMDS plot (Fig. 3a).

While the data in the current study are adequate to demonstrate differences in lipid composition between the two holothurian species, it is difficult to strengthen our inferences of spatial and temporal trends because there is a lack of lipid data in the WAP region.

Temporal and spatial trends in the benthic communities of deep-sea regions often become apparent only after many years of sampling (Bett et al., 2001, Billett et al., 2001, Neto et al., 2006, Billett et al., 2010, Glover et al., 2010). Benthic communities are considered long-term integrators of physical and biological processes in the upper water column (Grebmeier et al, 1988, Mincks et al., 2008), and lipid compositions of benthic organisms provide an integrated record of their available food supply (Parrish et al., 2000, Hudson et al., 2004, Iverson et al., 2004, Piepenburg, 2005). Further and continued investigation of lipid compositions in the benthic community of the WAP is necessary to substantiate how benthic invertebrates respond to variations of pelagic production and climate-induced changes in the region.

Conclusion

The surface sediments of the WAP region exhibited no latitudinal or seasonal variation and appeared to supply the benthic community with a consistent food source all year long. The relationship between physical parameters (e.g., warming temperatures and declining sea ice) and lipid content in the seabed and benthic community is complex, making trends difficult to elucidate during short-term sampling (i.e., annual or interannual). Similar to lipid composition of surface sediments in the present study, a number of seabed parameters measured during the FOODBANCS2 Project showed little evidence of latitudinal variation during winter and summer including benthic community respiration, sediment microbial biomass, and sediment bioturbation rates based on ^{234}Th excess activities (Smith et al., 2012). These parameters, including lipid composition of surface sediments and benthic

organisms, may respond to long-term (decadal) variability in primary production and OM deposition (Smith et al., 2012).

Temporal and spatial variations in lipid composition of *P. murrayi* and *M. musculus* did occur, but trends were difficult to tease out and could be due to natural variability and utilization of food sources in the region. Benthic organisms are integrators of overlying production (Grebmeier et al., 1988, Mincks et al., 2008) and lipid compositions of their tissues can provide a time-integrated record of their feeding history on the available food supply (Parrish et al., 2000, Iverson et al., 2004, Piepenburg, 2005). Long-term sampling of the benthic community in the WAP is necessary to determine if variation in lipid composition can be attributed to climate-induced changes in pelagic production.

Lipids utilized by the benthic community are modulated by the flux of surface production to the seafloor. The characterization of the lipid composition of surface sediment and surface and subsurface deposit feeders from the WAP has enhanced our understanding of trophic interactions within the continental shelf benthic community. However, attempting to predict how this community will be affected by continued regional warming is difficult. As smaller-celled flagellate populations continue to overtake diatom prevalence in the region as a result of the warming climate, the future of the benthic community is unknown. Though some benthic parameters appear to be resilient to climate-induced changes, it is too soon to tell how shifts in this region will impact the holothurians.Adaptation is a slow process occurring over multiple generations, especially in long-lived species such as the holothurians in our study. Warming of the regional climate is a comparably fast process. Species better adapted to a stressed environment may become prevalent as the food supply becomes less

predictable. The baseline provided by this study can be used for future comparisons of change in the WAP benthic community.

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CHAPTER 4

Summary and Conclusions

The overarching objectives of this Ph.D. and the FOODBANCS2 Project were to observe and document any changes in the benthic environment along the north-south seasonal sea ice gradient. As seawater and atmospheric temperatures in the WAP continue to warm, it is likely that sea ice extent and duration will continue to decline and influence physical and biological parameters in the region. Changes in phytoplankton and zooplankton community structure may influence the quality and quantity of OM reaching the seafloor. The WAP exhibits a gradient in seasonal sea ice coverage (i.e., mostly ice free in the north and mostly ice covered in the south) that provided the present study an opportunity to sample the benthic community in response to differing pelagic regimes. This study used lipid biomarkers to demonstrate sources of OM to the seafloor and preferential assimilation/utilization of deposited OM by holothurians with different feeding modes. This study also investigated temporal and spatial differences in lipid compositions of food sources and consumers.

In Chapter 2, lipid biomarkers, specifically FAs and STs, were used to investigate cycling of OM through the benthic food web of the WAP. FA and ST characterization of food sources and holothurian consumers proved useful in assessing utilization of OM from surface production in the WAP. Pelagic-benthic coupling was evident based on phytoplankton-derived FAs, such as 16:1ω7, 18:1ω9, and 20:5ω3, and STs present in sediment trap, surface sediment, and holothurian tissue samples. Flagellate-derived OM was present in sediment traps but not evident in surface sediments or holothurians. This indicated

degradation or utilization of flagellate-based phytodetrital OM before reaching the seafloor. High levels of SFAs and MUFAs and low levels of PUFAs in surface sediments suggested degradation and microbial processing occurred during deposition and within the sediment. Diatom-derived FAs (especially 16:1ω7) and STs (cholesta-5,22-dien-3β-ol, 24-methylcholesta-5,22-dien-3β-ol, 24-methylcholesta-5,24(28)-dien-3β-ol) were abundant in surface sediments. Diatom-derived FAs and STs were also prevalent in the surface deposit feeder, *P. murrayi,* and appear to be an important food source. The subsurface deposit feeder, *M. musculus*, fed on a mixed diet of fresh, phytoplankton-derived, older, and bacterial-derived OM. Both holothurians appeared to preferentially assimilate needed components from the surface sediment of the WAP which is consistent with past studies (Mincks et al., 2008). The intention was to establish a baseline of lipid composition to be used as a reference to monitor changes in the WAP.

Chapter 3 focused on temporal and spatial differences of FA and ST compositions of surface sediments, *P. murrayi*, and *M. musculus* to assess if climate-induced changes in the pelagic system could be observed in the benthos. Surface sediments exhibited no latitudinal or seasonal variation and appeared to supply a consistent food source all year long, which is consistent with the concept of a 'food bank' in the region (Mincks et al., 2005). There were temporal and spatial variations in lipid composition of *P. murrayi* and *M. musculus* but definitive trends were difficult to tease out. The variations could be due to natural variability and utilization of regional food sources and more extensive sampling needs to be done to conclusively determine the cause. This is important to understand because these animals are long-lived and it may take multiple generations for adaptations to climate-warming based changes to occur. Long-term sampling of lipid biomarkers in the benthic community is

necessary to determine if the measured temporal and spatial changes can be attributed to climate-induced changes in pelagic production or are the product of natural variability in the region.

Future Work

Deep-sea ecosystems are sensitive to the affects of climate change (Ruhl et al., 2008, Smith et al., 2008, Rogers 2015). Baselines of diversity, abundance, and biomass of benthic communities are needed to understand how changes in parameters such as food supply, temperature, and pH affect community structure and function (Rogers, 2015). Information about the benthic community of the deep continental shelf of the WAP still has gaps due to sampling limitations, such as depth and seasonal ice cover. Continued and consistent monitoring of benthic community in the WAP, similar to yearly sampling of the pelagic system in the region as part of the Palmer Long-Term Ecological Research (LTER) project, would improve our understanding of the benthic system. The Palmer LTER project studies ecological variables in the pelagic system and monitors how they change due to seasonal, interannual, and climate-forced variations in the environment (Ducklow, 2008). Expanding sampling to include the benthos would provide information on how long-term integrators (i.e., sediment and benthic animals) respond to alterations in the pelagic system, giving a more complete picture of how the entire marine system responds to variability, whether "normal" or climate-induced.

Time series analyses should include all the tools available so a part of the story within an ecosystem is not missed. The addition of lipid biomarker analysis in the pelagic and benthic communities of the WAP could provide insight into how the marine system is changing as temperatures warm, sea ice declines, and phytoplankton and zooplankton

communities shift. We know that essential FA availability can influence trophic structures in marine and freshwater ecosystems (Brett and Muller-Navarra, 1997, Kainz et al., 2004, Parrish et al., 2009). Recent studies have proposed that climate change could influence production of essential FAs by phytoplankton (Litzow et al., 2006, Arts et al., 2015) contributing to trophic changes in aquatic food webs, including affects on human health (Arts et al., 2001, Kang, 2011). From the baseline data presented in this study, we can use lipid biomarkers to track future change in the WAP benthic system and assess how warming climates might alter the functions of the ecosystem.

In addition to yearly sampling of lipid biomarkers in the benthic community, the use of sediment traps throughout the study region would enable a greater understanding of the sources of sinking OM and degradation of OM before reaching the seafloor. Sampling sediment from multiple depths in the sediment column would enable a comparison of lipids available at the surface and at depth to consumers. More intensive sediment sampling would also provide a better indication of degradation processes occurring on the seafloor of the WAP. Sampling a wider variety of organisms in the region would provide a more extensive study on lipid assimilation and utilization in the benthic community and may provide a better understanding of available food sources to deposit feeders. Finally, FA and ST compound-specific isotope ratio analyses would further aid our understanding of the lipid biochemistry of OM sources and better constrain feeding/assimilation selectivity of benthic fauna. Compound-specific isotope ratio analyses could also prove useful in determining bacterial utilization and the source of 20:4ω6 in holothurians.

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APPENDICES

Appendix A. Fatty acid compositions of sediment traps, surface sediments, *Protelpidia murrayi*, and *Molpadia musculus*. All samples were collected in winter 2008 and summer 2009. Values are given as mean percentages one standard deviation from the mean of the total fatty acids.

Fatty acid	Sediment trap						Phytoplankton			
	B Summer (n=1)	G Summer (n=1)					AA Summer (n=1)	B Summer (n=1)	E Summer (n=1)	G Summer (n=1)
	B15	B16	B17	B18	B19	B20				
14:0n	6.9	7.6	3.9	8.5	4.0	2.7	16.7	32.9	28.1	20.6
15:0i	0.6	0.4	0.3	0.4	0.3	0.5	0.1	0.4	0.4	0.1
15:0a	0.2	0.2	0.2	0.3	0.4	0.3	-	0.2	0.1	0.1
15:0n	0.5	1.0	0.5	0.8	0.6	0.5	0.5	1.3	1.0	0.6
16:1ω9	0.2	-	-	-	0.1	0.2	-	0.1	0.1	0.2
16:1ω7	13.0	8.9	16.8	20.5	15.9	18.1	26.6	32.6	30.2	26.3
16:1ω5	0.5	0.6	0.5	1.0	0.6	0.8	0.5	1.2	0.8	-
16:0n	19.9	38.8	22.7	27.8	23.6	13.4	19.9	15.9	15.4	9.2
17:0i	0.3	-	-	0.4	0.3	0.3	0.1	0.3	0.2	0.1
17:0a	0.1	0.3	0.2	0.5	0.2	0.2	-	0.1	0.1	-
17:0n	0.3	0.6	0.3	0.3	0.2	0.2	0.1	0.5	0.4	0.1
18:1ω9	27.6	8.4	22.8	19.6	27.1	33.8	3.6	2.5	2.5	1.7
18:1ω7	7.3	4.0	5.8	4.6	3.7	5.2	0.7	1.1	1.0	0.8
18:1ω5	0.7	0.5	0.5	0.5	0.5	0.6	0.2	0.6	0.5	0.4
18:0n	10.8	28.4	18.6	7.0	9.2	12.7	1.9	4.2	5.8	1.1
19:1ω12	-	-	-	-	-	-	-	-	-	-
19:0i	-	-	-	-	-	-	-	-	-	-
19:0a	-	-	-	-	0.1	0.1	-	-	-	-
19:0n	-	-	-	-	0.1	-	-	0.1	0.1	-
20:4ω6	0.1	-	-	0.1	0.6	0.3	0.3	0.1	0.2	0.1
20:5ω3	2.4	-	0.6	2.6	3.4	3.5	23.3	4.6	10.3	35.1
20:1	3.6	-	3.1	1.5	1.4	1.7	0.1	-	-	0.3
20:1	0.9	-	0.9	1.8	1.0	0.4	-	0.1	0.2	-
20:1	-	-	-	-	-	-	0.1	0.1	-	-
20:0n	0.4	-	1.1	-	0.3	-	0.1	0.1	0.2	0.2
21:4	-	-	-	-	-	-	-	-	-	0.2
21:0n	-	-	-	-	0.1	-	-	-	-	0.1
22:6ω3	1.9	-	0.2	0.9	3.2	3.3	4.0	0.5	1.4	1.8
22:5ω3	0.3	-	-	0.2	0.4	0.4	0.3	-	0.1	0.2
22:1	0.7	-	0.5	0.4	1.1	0.3	0.4	0.4	0.8	0.2
22:1ω9	0.1	-	0.1	0.1	0.7	0.1	0.1	0.1	0.1	0.1
22:0n	0.1	-	0.1	0.1	0.2	0.1	0.1	-	0.1	0.1
23:1ω9	-	-	-	-	0.1	-	-	-	-	0.1
24:1	0.3	-	0.2	0.1	0.6	0.2	0.1	-	-	0.1
24:0n	0.1	0.3	-	-	0.1	-	0.1	-	0.1	-
SFA	40.3	77.6	47.9	46.1	39.7	30.8	39.7	56.0	51.9	32.4
MUFA	54.9	22.4	51.3	50.2	52.8	61.6	32.5	38.7	36.2	30.2
PUFA	4.7	0.0	0.8	3.7	7.5	7.5	27.8	5.3	12.0	37.4
Bacterial	9.3	6.5	7.3	7.3	5.6	7.2	1.6	3.8	3.2	1.9
Diatom	15.4	8.9	17.4	23.1	19.3	21.5	49.9	37.2	40.4	61.5
Flagellate	29.5	8.4	23.0	20.5	30.3	37.2	7.6	3.0	4.0	3.5

Appendix A (continued)

Fatty acid	Surface sediment							
	AA Winter (n=1)	B Winter (n=1)	E Winter (n=1)	G Winter (n=1)	AA Summer (n=1)	B Summer (n=1)	E Summer (n=1)	G Summer (n=1)
14:0n	5.5	4.0	4.4	7.1	5.9	3.6	3.8	4.6
15:0i	3.7	4.3	4.5	3.4	3.0	3.8	3.8	4.0
15:0a	4.5	5.2	5.3	3.6	3.6	4.1	4.4	3.9
15:0n	1.0	1.0	1.1	1.3	1.1	1.0	1.0	1.1
16:1ω9	3.2	3.6	3.6	3.2	3.3	4.1	4.1	3.7
16:1ω7	15.9	15.6	17.4	12.0	14.1	15.9	15.2	16.4
16:1ω5	4.1	4.8	4.9	3.1	3.7	4.3	4.6	4.1
16:0n	16.6	18.5	17.6	18.0	17.7	15.5	15.3	20.0
17:0i	1.2	1.0	1.3	1.5	1.3	1.2	1.3	1.5
17:0a	1.0	1.0	1.2	1.1	1.1	0.9	1.0	1.2
17:0n	0.9	0.8	0.8	1.0	1.1	0.7	0.7	0.8
18:1ω9	5.9	5.5	6.2	6.4	6.6	7.7	5.7	7.9
18:1ω7	13.3	12.1	13.3	11.9	12.3	13.4	12.0	12.2
18:1ω5	0.9	0.8	0.9	1.0	1.2	0.8	0.8	1.1
18:0n	12.1	16.6	8.5	14.2	12.6	10.0	15.9	7.9
19:1ω12	-	-	-	-	-	-	-	-
19:0i	0.1	-	0.2	-	0.2	-	0.2	0.2
19:0a	0.2	0.1	0.2	0.3	-	-	-	-
19:0n	0.2	0.2	0.4	0.3	0.3	0.3	0.3	0.2
20:4ω6	1.4	0.8	1.4	1.5	2.2	1.1	1.7	1.4
20:5ω3	2.6	0.5	1.1	2.7	2.4	1.2	1.7	1.0
20:1	1.1	-	-	-	-	3.3	-	-
20:1	0.9	0.6	0.8	1.5	1.5	1.2	0.9	2.3
20:1	0.6	-	-	-	-	0.5	-	-
20:0n	0.4	0.5	0.5	-	0.9	0.7	0.7	0.6
21:4	-	-	-	-	-	-	-	-
21:0n	0.1	0.1	0.3	0.3	0.3	0.4	0.4	0.4
22:6ω3	0.5	0.2	0.6	0.7	-	0.9	1.0	0.7
22:5ω3	0.1	-	0.3	0.3	0.4	0.3	0.4	0.3
22:1	0.2	0.1	0.3	0.5	0.3	0.5	0.3	-
22:1ω9	-	0.1	0.2	0.4	0.3	0.3	-	0.1
22:0n	0.4	0.6	0.9	0.7	1.0	0.7	0.8	0.6
23:1ω9	-	-	-	-	-	-	-	-
24:1	0.3	-	-	0.6	-	0.3	-	0.2
24:0n	1.0	1.5	2.3	1.3	1.5	1.5	2.1	1.1
SFA	49.1	55.4	49.2	54.1	51.5	44.2	51.7	48.4
MUFA	46.4	43.1	47.4	40.7	43.4	52.3	43.5	48.2
PUFA	4.5	1.5	3.4	5.2	5.0	3.5	4.8	3.5
Bacterial	25.6	25.2	27.4	23.8	23.4	25.0	24.2	24.9
Diatom	18.4	16.2	18.5	14.7	16.6	17.1	16.9	17.5
Flagellate	6.4	5.7	6.7	7.1	6.6	8.6	6.7	8.6

Appendix A (continued)

Fatty acid	*Protelpidia murrayi*					
	B Winter (n=5)	E Winter (n=5)	G Winter (n=4)	B Summer (n=5)	E Summer (n=4)	G Summer (n=4)
14:0n	0.1 ± 0.2	0.2 ± 0.1	0.6 ± 0.3	0.2 ± 0.1	0.2 ± 0.1	1.0 ± 0.3
15:0i	0.5 ± 0.2	0.6 ± 0.3	1.3 ± 0.5	0.7 ± 0.2	0.6 ± 0.1	1.7 ± 0.4
15:0a	0.6 ± 0.3	0.9 ± 0.5	1.5 ± 0.3	0.6 ± 0.3	0.7 ± 0.2	1.6 ± 0.3
15:0n	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.3 ± 0.1
16:1ω9	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.2	0.3 ± 0.2	0.3 ± 0.0	0.6 ± 0.2
16:1ω7	1.2 ± 0.4	1.2 ± 0.4	3.6 ± 1.0	4.1 ± 2.4	2.9 ± 0.8	4.5 ± 1.3
16:1ω5	0.4 ± 0.2	0.4 ± 0.2	1.0 ± 0.3	1.0 ± 0.6	0.7 ± 0.1	1.6 ± 0.6
16:0n	2.1 ± 0.7	2.0 ± 0.8	3.8 ± 1.0	2.9 ± 0.9	2.0 ± 0.2	5.0 ± 0.9
17:0i	1.3 ± 0.2	1.3 ± 0.2	1.5 ± 0.2	1.3 ± 0.3	1.2 ± 0.1	1.7 ± 0.3
17:0a	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.9 ± 0.3
17:0n	0.8 ± 0.2	0.9 ± 0.1	1.0 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.2
18:1ω9	2.5 ± 0.4	2.5 ± 0.2	3.4 ± 0.8	3.7 ± 1.6	4.2 ± 1.4	3.7 ± 0.7
18:1ω7	3.6 ± 0.5	3.6 ± 0.9	4.8 ± 0.7	4.6 ± 1.3	3.8 ± 0.6	4.8 ± 0.7
18:1ω5	0.4 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.5 ± 0.2	0.5 ± 0.0	0.5 ± 0.2
18:0n	4.5 ± 0.6	4.4 ± 0.8	3.8 ± 0.2	5.0 ± 0.7	4.0 ± 0.3	3.9 ± 0.6
19:1ω12	0.8 ± 0.2	0.8 ± 0.1	0.7 ± 0.2	0.6 ± 0.4	1.1 ± 0.1	0.6 ± 0.1
19:0i	0.5 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.2	0.7 ± 0.2	0.3 ± 0.1
19:0a	0.8 ± 0.2	1.0 ± 0.2	0.7 ± 0.2	0.7 ± 0.3	1.1 ± 0.3	0.5 ± 0.2
19:0n	1.5 ± 0.1	1.5 ± 0.3	1.2 ± 0.1	1.1 ± 0.2	1.4 ± 0.2	1.2 ± 0.2
20:4ω6	23.6 ± 1.6	23.1 ± 1.7	17.9 ± 2.4	20.4 ± 3.3	20.8 ± 0.2	16.6 ± 1.1
20:5ω3	22.0 ± 2.7	19.1 ± 1.2	18.2 ± 1.1	19.5 ± 1.3	18.2 ± 0.9	19.2 ± 1.7
20:1	0.7 ± 0.2	0.2 ± 0.3	0.4 ± 0.1	0.1 ± 0.3	0.1 ± 0.3	0.4 ± 0.1
20:1	0.8 ± 0.1	0.9 ± 0.2	1.0 ± 0.1	0.6 ± 0.4	0.8 ± 0.1	1.0 ± 0.1
20:1	1.2 ± 0.1	1.2 ± 0.1	1.1 ± 0.0	0.7 ± 0.6	-	-
20:0n	2.1 ± 0.5	1.8 ± 0.2	1.6 ± 0.2	1.8 ± 0.4	1.8 ± 0.2	1.4 ± 0.2
21:4	0.9 ± 0.2	1.4 ± 0.2	1.3 ± 0.3	0.7 ± 0.3	1.3 ± 0.1	1.0 ± 0.1
21:0n	0.6 ± 0.1	0.6 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.6 ± 0.1	0.6 ± 0.1
22:6ω3	3.4 ± 2.2	3.9 ± 2.2	5.0 ± 0.9	4.4 ± 1.2	5.2 ± 0.5	5.4 ± 1.4
22:5ω3	0.2 ± 0.4	0.6 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.6 ± 0.0	0.8 ± 0.2
22:1	0.4 ± 0.4	0.8 ± 0.2	0.8 ± 0.1	0.6 ± 0.5	1.0 ± 0.1	0.4 ± 0.3
22:1ω9	7.3 ± 4.1	9.5 ± 0.9	8.3 ± 0.7	8.4 ± 1.3	9.5 ± 0.6	7.2 ± 0.9
22:0n	0.6 ± 0.1	0.5 ± 0.2	0.4 ± 0.0	0.4 ± 0.2	0.5 ± 0.1	0.4 ± 0.1
23:1ω9	7.9 ± 1.7	7.9 ± 1.5	6.0 ± 0.7	7.2 ± 2.1	8.0 ± 0.6	5.2 ± 1.9
24:1	5.8 ± 1.2	5.4 ± 1.1	5.5 ± 1.0	4.9 ± 0.6	4.5 ± 0.6	4.8 ± 1.5
24:0n	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0
SFA	16.8 ± 1.3	17.0 ± 1.7	19.3 ± 2.6	17.1 ± 0.9	16.3 ± 1.7	21.6 ± 1.5
MUFA	33.0 ± 2.7	35.0 ± 2.1	37.6 ± 1.2	37.3 ± 2.5	37.6 ± 1.8	35.4 ± 1.8
PUFA	50.2 ± 1.7	48.0 ± 1.9	43.1 ± 3.7	45.6 ± 2.9	46.2 ± 1.0	43.0 ± 1.4
Bacterial	7.5 ± 1.4	8.0 ± 1.7	11.0 ± 1.8	8.6 ± 1.5	7.8 ± 1.1	12.1 ± 1.9
Diatom	23.2 ± 2.9	20.3 ± 1.4	21.8 ± 0.8	23.6 ± 3.6	21.1 ± 1.1	23.8 ± 0.8
Flagellate	5.9 ± 2.5	6.4 ± 2.1	8.4 ± 0.7	8.1 ± 0.8	9.4 ± 1.0	9.1 ± 1.4

Appendix A (continued)

Fatty acid	*Molpadia musculus*					
	AA Winter (n=4)	B Winter (n=3)	E Winter (n=3)	AA Summer (n=4)	B Summer (n=2)	E Summer (n=1)
14:0n	0.1 ± 0.1	0.0 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.1 ± 0.0	-
15:0i	0.6 ± 0.1	0.3 ± 0.1	0.6 ± 0.2	0.6 ± 0.3	0.5 ± 0.1	0.4
15:0a	0.7 ± 0.1	0.9 ± 0.1	2.0 ± 0.3	0.7 ± 0.2	1.1 ± 0.6	0.7
15:0n	0.1 ± 0.0	-	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	-
16:1ω9	0.1 ± 0.0	0.0 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	-	-
16:1ω7	3.5 ± 0.4	2.1 ± 0.3	7.0 ± 2.1	3.0 ± 0.4	2.8 ± 0.4	2.9
16:1ω5	0.7 ± 0.5	0.8 ± 0.6	1.7 ± 0.5	0.7 ± 0.2	0.7 ± 0.2	0.5
16:0n	0.8 ± 0.6	1.8 ± 0.9	2.9 ± 0.4	1.2 ± 0.4	1.9 ± 0.1	0.7
17:0i	1.8 ± 0.2	1.7 ± 0.5	1.2 ± 0.2	1.9 ± 0.5	1.6 ± 0.2	1.3
17:0a	1.2 ± 0.2	1.3 ± 0.1	1.3 ± 0.1	1.1 ± 0.4	1.4 ± 0.5	1.0
17:0n	0.3 ± 0.0	0.4 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.2
18:1ω9	4.0 ± 0.3	3.9 ± 0.7	4.6 ± 0.7	3.8 ± 0.5	4.4 ± 1.0	4.9
18:1ω7	9.3 ± 1.2	7.8 ± 0.8	9.3 ± 2.6	8.5 ± 1.2	6.4 ± 0.2	5.8
18:1ω5	0.3 ± 0.0	0.3 ± 0.1	0.4 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.2
18:0n	1.2 ± 0.2	2.0 ± 1.0	2.1 ± 0.9	1.3 ± 0.4	2.8 ± 1.3	0.8
19:1ω12	6.2 ± 0.5	4.3 ± 1.6	3.5 ± 1.6	6.7 ± 0.6	5.0 ± 0.8	6.2
19:0i	0.8 ± 0.0	0.7 ± 0.4	0.5 ± 0.1	0.8 ± 0.1	0.7 ± 0.2	0.6
19:0a	1.4 ± 0.1	1.3 ± 0.4	1.0 ± 0.2	1.5 ± 0.1	0.7 ± 0.9	1.4
19:0n	0.5 ± 0.0	0.5 ± 0.0	0.8 ± 0.2	0.6 ± 0.1	0.5 ± 0.1	0.4
20:4ω6	34.3 ± 2.2	41.3 ± 4.8	26.5 ± 2.9	36.1 ± 3.4	40.9 ± 3.4	34.9
20:5ω3	3.6 ± 0.8	4.8 ± 2.2	4.5 ± 1.6	6.1 ± 0.8	3.6 ± 0.4	5.7
20:1	0.7 ± 1.5	2.6 ± 1.4	1.0 ± 1.8	1.5 ± 1.2	1.1 ± 0.3	1.0
20:1	1.1 ± 0.1	1.2 ± 0.5	1.4 ± 0.4	1.0 ± 0.5	0.7 ± 0.0	1.0
20:1	0.8 ± 0.1	0.8 ± 0.2	0.8 ± 0.2	0.7 ± 0.5	0.6 ± 0.0	0.7
20:0n	0.7 ± 0.1	0.8 ± 0.2	1.7 ± 1.0	0.9 ± 0.2	0.8 ± 0.1	0.7
21:4	3.5 ± 0.5	2.9 ± 0.5	2.4 ± 0.7	4.4 ± 0.4	3.7 ± 0.3	2.8
21:0n	0.8 ± 0.1	0.8 ± 0.1	1.0 ± 0.3	1.0 ± 0.1	0.8 ± 0.1	0.8
22:6ω3	-	-	0.1 ± 0.2	-	0.5 ± 0.1	-
22:5ω3	0.4 ± 0.0	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.0	0.5
22:1	0.8 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	1.0 ± 0.2	0.5 ± 0.1	1.1
22:1ω9	7.1 ± 0.8	3.5 ± 3.1	10.0 ± 3.0	8.2 ± 0.9	5.1 ± 2.1	9.1
22:0n	0.5 ± 0.0	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.0	0.5 ± 0.0	0.4
23:1ω9	11.3 ± 0.6	8.7 ± 2.7	7.9 ± 2.9	3.9 ± 7.8	8.9 ± 3.6	12.3
24:1	0.6 ± 0.1	0.7 ± 0.3	1.0 ± 0.1	0.8 ± 0.1	0.7 ± 0.3	0.9
24:0n	0.1 ± 0.0	0.3 ± 0.1	-	0.1 ± 0.0	0.1 ± 0.1	-
SFA	11.5 ± 1.3	13.5 ± 3.6	16.7 ± 2.0	12.9 ± 2.5	13.8 ± 4.1	9.4
MUFA	46.7 ± 2.2	37.1 ± 2.7	49.4 ± 2.4	40.0 ± 6.7	37.1 ± 7.8	46.6
PUFA	41.9 ± 2.9	49.4 ± 6.2	33.9 ± 4.3	47.1 ± 4.4	49.1 ± 3.7	44.0
Bacterial	14.0 ± 1.9	12.5 ± 0.2	15.1 ± 2.1	13.4 ± 1.5	11.5 ± 1.8	9.4
Diatom	7.1 ± 1.2	7.0 ± 2.5	11.5 ± 3.0	9.1 ± 1.1	6.4 ± 0.0	8.6
Flagellate	4.0 ± 0.3	3.9 ± 0.7	4.7 ± 0.9	3.8 ± 0.5	4.9 ± 0.9	4.9

Appendix B. Sterol compositions of sediment traps, surface sediments, *Protelpidia murrayi*, and *Molpadia musculus*. All samples were collected in winter 2008 and summer 2009. Values are given as percentages of the total sterols. * signifies isomers

Sterol	Sediment trap					
	B Summer (n=1)	G Summer (n=1)				
Sediment trap bottle	B15	B16	B17	B18	B19	B20
24-nor-5α-cholest-22-en-3β-ol	-	-	-	0.7	0.6	-
5α-cholest-22-en-3β-ol*	-	-	-	-	-	-
cholesta-5,22-dien-3β-ol	3.0	6.8	9.3	9.4	7.5	7.4
5α-cholest-22-en-3β-ol*	-	-	-	1.0	0.7	-
cholest-5-en-3β-ol (cholesterol)	27.1	24.1	47.2	19.8	30.9	22.1
cholest-7-en-3β-ol*	-	-	-	-	-	-
5α-cholestan-3β-ol	1.3	2.6	5.2	1.8	2.7	1.9
cholesta-7,22-dien-3β-ol*	-	-	-	-	-	-
cholesta-7,22-dien-3β-ol*	-	-	-	-	-	-
cholesta-5,24-dien-3β-ol	-	-	-	-	-	-
24-methylcholesta-5,22-dien-3β-ol	13.0	47.6	17.4	16.5	18.9	23.6
24-methylcholest-22-en-3β-ol	-	6.5	8.2	-	3.6	4.2
cholest-7-en-3β-ol*	16.1	-	-	7.6	-	-
24-methylcholesta-7,22-dien-3β-ol*	8.8	6.0	-	-	-	-
24-methylcholesta-5,24(28)-dien-3β-ol	-	-	5.5	14.8	10.8	10.7
24-methylcholest-5-en-3β-ol	1.1	-	-	-	-	-
24-methylcholesta-7,22-dien-3β-ol*	-	-	-	-	-	-
24-methylcholest-24(28)-en-3β-ol	1.5	-	-	1.6	-	-
Unknown 1	4.9	-	-	-	-	-
C_{29} Unknown	-	-	-	3.1	-	-
24-ethylcholesta-5,22-dien-3β-ol	-	-	-	-	3.0	-
23,24-dimethylcholest-5-en-3β-ol	-	-	-	1.5	-	-
24-ethylcholest-22-en-3β-ol	-	-	-	-	-	-
Unknown 2	-	-	-	-	-	-
24-ethylcholest-5-en-3β-ol	4.1	6.4	7.3	11.0	8.8	13.1
24-ethylcholestan-3β-ol*	2.5	-	-	5.3	2.1	3.8
24-ethylcholestan-3β-ol*	-	-	-	-	-	-
24-ethylcholesta-5,24(28)Z-dien-3β-ol	3.5	-	-	-	5.6	7.5
4α,23,24-trimethylcholest-22-en-3β-ol	2.5	-	-	5.9	2.9	5.8
24-ethylcholest-7-en-3β-ol*	-	-	-	-	-	-
24-ethylcholest-7-en-3β-ol*	8.9	-	-	-	-	-
4α,23,24-trimethylcholest-8(14)-en-3β-ol	1.8	-	-	-	2.0	-
Unknown 3	-	-	-	-	-	-
24-propylcholesta-7,24(28)-dien-3β-ol	-	-	-	-	-	-
ΣC_{26} sterols	-	-	-	0.7	0.6	-
ΣC_{27} sterols	47.4	33.5	61.7	39.5	41.7	31.3
ΣC_{28} sterols	43.4	66.5	38.3	49.2	52.8	62.9
ΣC_{29} sterols	-	-	-	4.6	-	-
ΣC_{30} sterols	4.3	-	-	5.9	4.9	5.8

Appendix B (continued)

Sterol	Phytoplankton			
	AA Summer (n=1)	B Summer (n=1)	E Summer (n=1)	G Summer (n=1)
24-nor-5α-cholest-22-en-3β-ol	0.0	0.0	0.0	0.0
5α-cholest-22-en-3β-ol*	0.0	0.0	0.0	0.0
cholesta-5,22-dien-3β-ol	10.2	15.3	12.5	10.8
5α-cholest-22-en-3β-ol*	0.0	0.0	0.0	0.0
cholest-5-en-3β-ol	23.3	25.7	23.2	35.3
cholest-7-en-3β-ol*	0.0	0.0	0.0	0.0
5α-cholestan-3β-ol	0.0	0.0	2.0	0.0
cholesta-7,22-dien-3β-ol*	0.0	0.0	0.0	0.0
cholesta-7,22-dien-3β-ol*	0.0	0.0	0.0	0.0
cholesta-5,24-dien-3β-ol	13.3	25.9	0.0	15.4
24-methylcholesta-5,22-dien-3β-ol	0.0	0.0	31.4	0.0
24-methylcholest-22-en-3β-ol	0.0	0.0	0.0	0.0
cholest-7-en-3β-ol*	0.0	12.3	8.4	27.2
24-methylcholesta-7,22-dien-3β-ol*	0.0	0.0	0.0	0.0
24-methylcholesta-5,24(28)-dien-3β-ol	33.8	7.3	4.2	5.6
24-methylcholest-5-en-3β-ol	5.6	0.0	2.2	0.0
24-methylcholesta-7,22-dien-3β-ol*	0.0	0.0	0.0	0.0
24-methylcholest-24(28)-en-3β-ol	0.0	0.0	0.0	0.0
Unknown 1	0.0	0.0	0.0	0.0
C_{29} Unknown	0.0	0.0	0.0	0.0
24-ethylcholesta-5,22-dien-3β-ol	0.0	0.0	2.6	0.0
23,24-dimethylcholest-5-en-3β-ol	3.1	0.0	0.0	0.0
24-ethylcholest-22-en-3β-ol	0.0	0.0	0.0	0.0
Unknown 2	0.0	0.0	0.0	0.0
24-ethylcholest-5-en-3β-ol	1.6	3.9	5.0	2.7
24-ethylcholestan-3β-ol*	0.0	0.0	0.0	0.0
24-ethylcholestan-3β-ol*	0.0	0.0	0.0	0.0
24-ethylcholesta-5,24(28)Z-dien-3β-ol	4.6	0.0	0.0	0.0
4α,23,24-trimethylcholest-22-en-3β-ol	0.0	0.0	2.8	0.0
24-ethylcholest-7-en-3β-ol*	0.0	0.0	0.0	0.0
24-ethylcholest-7-en-3β-ol*	4.5	9.6	5.8	2.9
4α,23,24-trimethylcholest-8(14)-en-3β-ol	0.0	0.0	0.0	0.0
Unknown 3	0.0	0.0	0.0	0.0
24-propylcholesta-7,24(28)-dien-3β-ol	0.0	0.0	0.0	0.0
ΣC_{26} sterols	0.0	0.0	0.0	0.0
ΣC_{27} sterols	46.8	79.2	46.1	88.8
ΣC_{28} sterols	39.5	7.3	37.8	5.6
ΣC_{29} sterols	13.8	13.5	13.3	5.6
ΣC_{30} sterols	0.0	0.0	2.8	0.0

Appendix B (continued)

Sterol	Surface sediment							
	AA Winter (n=1)	B Winter (n=1)	E Winter (n=1)	G Winter (n=1)	AA Summer (n=1)	B Summer (n=1)	E Summer (n=1)	G Summer (n=1)
24-nor-5α-cholest-22-en-3β-ol	0.7	0.7	0.6	0.6	0.8	0.7	0.6	0.7
5α-cholest-22-en-3β-ol*	-	-	-	-	0.6	0.6	-	0.6
cholesta-5,22-dien-3β-ol	6.8	6.8	7.3	12.9	5.5	5.1	5.9	6.0
5α-cholest-22-en-3β-ol*	1.4	1.7	1.5	1.6	1.1	1.2	1.0	1.4
cholest-5-en-3β-ol	10.9	12.3	9.7	12.0	13.6	16.9	12.9	11.4
cholest-7-en-3β-ol*	-	-	-	-	-	-	-	-
5α-cholestan-3β-ol	2.7	3.1	2.4	1.9	2.5	2.3	2.1	3.1
cholesta-7,22-dien-3β-ol*	-	-	-	-	-	-	-	-
cholesta-7,22-dien-3β-ol*	-	-	-	-	-	-	-	-
cholesta-5,24-dien-3β-ol	-	-	-	-	-	-	-	-
24-methylcholesta-5,22-dien-3β-ol	23.7	22.2	23.5	21.1	22.1	17.9	18.6	19.6
24-methylcholest-22-en-3β-ol	6.4	-	7.2	-	6.5	-	7.3	5.7
cholest-7-en-3β-ol*	-	-	-	-	-	10.2	-	-
24-methylcholesta-7,22-dien-3β-ol*	-	-	-	-	-	-	-	-
24-methylcholesta-5,24(28)-dien-3β-ol	5.8	6.4	5.8	4.3	6.7	6.4	6.4	7.2
24-methylcholest-5-en-3β-ol	2.2	3.2	2.7	3.6	1.6	2.3	2.3	1.3
24-methylcholesta-7,22-dien-3β-ol*	-	-	-	-	-	-	-	-
24-methylcholest-24(28)-en-3β-ol	-	-	-	-	-	-	-	-
Unknown 1	-	-	4.2	7.0	1.5	3.8	4.9	4.1
C_{29} Unknown	-	-	-	-	-	-	-	-
24-ethylcholesta-5,22-dien-3β-ol	7.5	8.6	7.5	6.4	6.4	5.7	6.4	6.0
23,24-dimethylcholest-5-en-3β-ol	2.3	3.1	2.7	2.1	1.9	2.1	2.5	2.4
24-ethylcholest-22-en-3β-ol	-	-	-	-	-	-	-	-
Unknown 2	-	-	-	-	-	-	-	-
24-ethylcholest-5-en-3β-ol	13.1	10.4	8.7	13.0	12.3	9.1	8.6	11.7
24-ethylcholestan-3β-ol*	3.4	4.4	4.9	4.0	3.0	3.1	4.6	3.0
24-ethylcholestan-3β-ol*	-	-	-	-	-	-	-	-
24-ethylcholesta-5,24(28)Z-dien-3β-ol	-	4.7	-	4.7	4.0	4.0	4.2	5.2
4α,23,24-trimethylcholest-22-en-3β-ol	5.6	4.5	3.9	1.9	5.0	3.3	5.4	4.8
24-ethylcholest-7-en-3β-ol*	-	-	-	-	-	-	-	-
24-ethylcholest-7-en-3β-ol*	3.7	4.4	4.2	2.0	2.4	3.2	3.8	2.8
4α,23,24-trimethylcholest-8(14)-en-3β-ol	3.8	3.5	3.1	0.8	2.5	2.1	2.5	3.0
Unknown 3	-	-	-	-	-	-	-	-
24-propylcholesta-7,24(28)-dien-3β-ol	-	-	-	-	-	-	-	-
Phytol	3.7	6.1	6.5	3.4	4.8	18.2	5.3	1.9
ΣC_{26} sterols	0.7	0.7	0.6	0.6	0.8	0.7	0.6	0.7
ΣC_{27} sterols	21.8	24.0	20.9	28.5	23.4	36.3	21.9	22.5
ΣC_{28} sterols	38.1	31.8	39.2	29.0	36.9	26.6	34.7	33.8
ΣC_{29} sterols	30.0	35.6	28.1	32.3	30.0	27.2	30.1	31.2
ΣC_{30} sterols	9.4	8.0	7.0	2.7	7.5	5.4	7.9	7.9

Appendix B (continued)

Sterol	*Protelpidia murrayi*					
	B Winter (n=5)	E Winter (n=5)	G Winter (n=4)	B Summer (n=5)	E Summer (n=4)	G Summer (n=4)
24-nor-5α-cholest-22-en-3β-ol	-	-	-	-	-	-
5α-cholest-22-en-3β-ol*	-	0.1 ± 0.1	-	0.02 ± 0.1	-	-
cholesta-5,22-dien-3β-ol	0.3 ± 0.2	0.5 ± 0.5	0.6 ± 0.1	0.1 ± 0.2	0.3 ± 0.5	0.4 ± 0.3
5α-cholest-22-en-3β-ol*	0.9 ± 0.1	1.6 ± 0.5	1.7 ± 0.2	0.6 ± 0.4	1.0 ± 0.3	0.9 ± 0.6
cholest-5-en-3β-ol	4.7 ± 2.9	4.4 ± 1.6	4.7 ± 1.0	4.0 ± 1.6	4.0 ± 1.7	5.5 ± 1.7
cholest-7-en-3β-ol*	0.5 ± 0.7	-	-	0.4 ± 0.9	0.3 ± 0.5	-
5α-cholestan-3β-ol	2.3 ± 0.3	2.6 ± 1.7	4.5 ± 1.0	2.1 ± 0.7	2.7 ± 0.7	4.0 ± 0.6
cholesta-7,22-dien-3β-ol*	5.5 ± 0.9	6.9 ± 4.8	7.5 ± 1.2	-	-	-
cholesta-7,22-dien-3β-ol*	-	1.2 ± 2.7	-	6.2 ± 1.7	6.1 ± 1.5	8.4 ± 1.1
cholesta-5,24-dien-3β-ol	0.2 ± 0.5	0.3 ± 0.6	-	0.05 ± 0.1	-	0.4 ± 0.9
24-methylcholesta-5,22-dien-3β-ol	0.1 ± 0.2	0.8 ± 1.2	1.4 ± 0.6	0.7 ± 0.7	1.0 ± 1.1	1.6 ± 1.1
24-methylcholest-22-en-3β-ol	-	-	-	-	-	-
cholest-7-en-3β-ol*	17.6 ± 1.8	20.8 ± 7.0	18.7 ± 1.7	16.5 ± 3.6	16.3 ± 1.1	21.6 ± 2.3
24-methylcholesta-7,22-dien-3β-ol*	7.2 ± 1.5	6.9 ± 3.9	7.6 ± 0.8	5.4 ± 3.1	7.0 ± 1.4	8.7 ± 2.2
24-methylcholesta-5,24(28)-dien-3β-ol	-	0.6 ± 1.3	-	-	-	-
24-methylcholest-5-en-3β-ol	1.3 ± 1.2	-	-	0.6 ± 1.2	-	0.1 ± 0.2
24-methylcholesta-7,22-dien-3β-ol*	-	0.4 ± 0.6	-	1.7 ± 3.9	-	-
24-methylcholest-24(28)-en-3β-ol	0.8 ± 1.1	0.4 ± 0.6	1.3 ± 0.1	1.1 ± 1.0	1.1 ± 0.2	0.8 ± 0.6
Unknown 1	0.2 ± 0.4	5.3 ± 11.0	0.7 ± 0.2	0.3 ± 0.4	0.6 ± 0.4	0.1 ± 0.3
C_{29} Unknown	16.7 ± 4.2	14.7 ± 5.7	15.9 ± 4.7	-	-	15.4 ± 1.9
24-ethylcholesta-5,22-dien-3β-ol	-	-	-	19.3 ± 4.5	21.3 ± 6.9	-
23,24-dimethylcholest-5-en-3β-ol	3.5 ± 0.2	0.5 ± 1.1	-	-	-	-
24-ethylcholest-22-en-3β-ol	-	-	-	13.3 ± 4.5	8.4 ± 2.7	8.7 ± 2.9
Unknown 2	6.4 ± 5.8	5.3 ± 3.1	2.3 ± 3.1	-	-	-
24-ethylcholest-5-en-3β-ol	5.5 ± 7.7	-	2.9 ± 4.5	1.4 ± 3.1	1.0 ± 1.2	-
24-ethylcholestan-3β-ol*	0.5 ± 1.1	3.0 ± 2.2	2.6 ± 2.0	0.7 ± 1.5	3.0 ± 2.1	0.8 ± 1.7
24-ethylcholestan-3β-ol*	-	-	-	0.8 ± 1.8	-	-
24-ethylcholesta-5,24(28)Z-dien-3β-ol	-	-	-	-	-	-
4α,23,24-trimethylcholest-22-en-3β-ol	2.4 ± 0.4	2.0 ± 1.2	3.2 ± 0.6	1.9 ± 2.0	2.2 ± 0.3	1.9 ± 1.3
24-ethylcholest-7-en-3β-ol*	4.9 ± 6.8	1.3 ± 0.7	2.2 ± 0.3	1.8 ± 1.7	1.5 ± 0.5	1.8 ± 1.2
24-ethylcholest-7-en-3β-ol*	12.4 ± 5.5	14.4 ± 8.3	15.3 ± 1.9	16.3 ± 3.1	16.0 ± 1.3	13.8 ± 0.6
4α,23,24-trimethylcholest-8(14)-en-3β-ol	3.1 ± 0.1	2.4 ± 1.4	4.7 ± 0.3	2.4 ± 2.5	3.2 ± 0.7	3.0 ± 2.0
Unknown 3	-	-	-	-	-	-
24-propylcholesta-7,24(28)-dien-3β-ol	2.9 ± 0.4	3.4 ± 0.4	2.2 ± 0.1	2.3 ± 2.1	3.0 ± 0.7	2.0 ± 1.3
ΣC_{26} sterols	-	-	-	-	-	-
ΣC_{27} sterols	32.1	38.4	37.8	30.2	30.6	41.3
ΣC_{28} sterols	9.4	9.2	10.3	9.5	9.1	11.2
ΣC_{29} sterols	43.6	33.9	38.9	53.5	51.3	40.5
ΣC_{30} sterols	8.3	7.9	10.1	6.6	8.4	6.8

Appendix B (continued)

Sterol	*Molpadia musculus*					
	AA Winter (n=4)	B Winter (n=3)	E Winter (n=3)	AA Summer (n=4)	B Summer (n=2)	E Summer (n=1)
24-nor-5α-cholest-22-en-3β-ol	2.0 ± 0.5	1.8 ± 0.5	3.0 ± 0.3	2.3 ± 0.4	1.3 ± 1.3	2.5
5α-cholest-22-en-3β-ol*	2.5 ± 0.7	3.7 ± 2.0	4.9 ± 1.6	2.7 ± 0.4	1.6 ± 1.3	3.1
cholesta-5,22-dien-3β-ol	0.1 ± 0.1	-	-	-	1.9 ± 2.7	0.1
5α-cholest-22-en-3β-ol*	5.5 ± 1.1	7.4 ± 1.5	10.7 ± 1.5	5.7 ± 0.5	16.3 ± 11.7	8.4
cholest-5-en-3β-ol	-	-	-	-	-	-
cholest-7-en-3β-ol*	5.7 ± 0.8	5.5 ± 1.1	3.1 ± 0.4	5.4 ± 0.2	3.6 ± 1.8	4.9
5α-cholestan-3β-ol	7.9 ± 0.9	11.9 ± 2.8	17.7 ± 1.2	7.8 ± 0.4	6.7 ± 4.7	11.0
cholesta-7,22-dien-3β-ol*	-	-	0.9 ± 1.6	-	-	-
cholesta-7,22-dien-3β-ol*	4.1 ± 0.9	5.0 ± 1.6	0.5 ± 0.9	3.4 ± 0.5	23.7 ± 27.1	2.5
cholesta-5,24-dien-3β-ol	0.1 ± 0.1	-	-	0.4 ± 0.1	0.2 ± 0.2	0.2
24-methylcholesta-5,22-dien-3β-ol	-	-	-	-	-	-
24-methylcholest-22-en-3β-ol	18.2 ± 2.1	19.9 ± 2.7	24.6 ± 2.0	17.4 ± 1.8	12.5 ± 8.7	18.2
cholest-7-en-3β-ol*	-	-	-	0.5 ± 0.3	-	-
24-methylcholesta-7,22-dien-3β-ol*	7.8 ± 9.0	7.8 ± 7.2	2.3 ± 2.1	-	-	-
24-methylcholesta-5,24(28)-dien-3β-ol	-	-	-	-	1.0 ± 1.5	-
24-methylcholest-5-en-3β-ol	-	-	-	-	-	-
24-methylcholesta-7,22-dien-3β-ol*	9.5 ± 11.0	7.9 ± 13.7	-	15.5 ± 1.2	7.6 ± 10.7	11.3
24-methylcholest-24(28)-en-3β-ol	8.4 ± 1.0	9.5 ± 0.5	10.0 ± 3.1	8.9 ± 0.5	5.7 ± 3.8	11.2
Unknown 1	-	-	-	0.1 ± 0.2	-	-
C_{29} Unknown	-	-	0.2 ± 0.3	-	-	-
24-ethylcholesta-5,22-dien-3β-ol	-	-	-	-	0.2 ± 0.2	0.3
23,24-dimethylcholest-5-en-3β-ol	0.5 ± 0.6	-	-	0.9 ± 0.2	0.3 ± 0.5	0.5
24-ethylcholest-22-en-3β-ol	0.2 ± 0.4	0.8 ± 0.7	0.3 ± 0.6	-	0.4 ± 0.6	1.0
Unknown 2	0.4 ± 0.8	-	0.8 ± 0.7	-	-	-
24-ethylcholest-5-en-3β-ol	1.2 ± 2.4	-	-	0.7 ± 0.8	-	-
24-ethylcholestan-3β-ol*	3.0 ± 2.1	1.0 ± 1.7	-	3.0 ± 2.1	1.4 ± 2.0	2.8
24-ethylcholestan-3β-ol*	1.9 ± 3.9	3.2 ± 5.6	12.9 ± 1.2	3.3 ± 3.8	6.2 ± 0.8	9.7
24-ethylcholesta-5,24(28)Z-dien-3β-ol	-	-	-	3.4 ± 4.0	-	-
4α,23,24-trimethylcholest-22-en-3β-ol	0.2 ± 0.4	-	-	0.1 ± 0.3	0.2 ± 0.3	-
24-ethylcholest-7-en-3β-ol*	1.1 ± 0.4	0.9 ± 0.3	0.2 ± 0.3	1.1 ± 0.3	0.4 ± 0.6	0.5
24-ethylcholest-7-en-3β-ol*	14.0 ± 1.6	8.9 ± 4.0	5.1 ± 1.2	12.3 ± 1.1	5.2 ± 4.5	7.5
4α,23,24-trimethylcholest-8(14)-en-3β-ol	-	1.6 ± 2.9	0.1 ± 0.2	-	3.4 ± 0.6	-
Unknown 3	5.4 ± 0.6	3.0 ± 2.7	2.6 ± 2.2	4.6 ± 0.4	-	4.3
24-propylcholesta-7,24(28)-dien-3β-ol	0.5 ± 0.1	0.2 ± 0.3	-	0.4 ± 0.1	0.1 ± 0.2	-
ΣC_{26} sterols	2.0	1.8	3.0	2.3	1.3	2.5
ΣC_{27} sterols	25.8	33.4	37.8	25.9	54.0	30.3
ΣC_{28} sterols	43.9	45.2	37.0	41.8	26.8	40.7
ΣC_{29} sterols	21.9	14.7	18.7	24.6	14.2	22.2
ΣC_{30} sterols	0.6	1.8	0.1	0.6	3.7	-