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Hyperbenthic Food-Web Structure in Kongsfjord:
A Two-Season Comparison using Stable Isotopes and Fatty Acids.

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Abstract

Current knowledge of the Arctic marine ecosystem is based primarily on studies performed during the polar day on the pelagic and benthic realms. Both the polar night and the hyperbenthic layer remain substantial knowledge gaps in the understanding of the marine system at high latitudes. To help address these knowledge gaps, this project investigates the hyperbenthic food web structure in Kongsfjord, a high-latitude, ice-free fjord, in September and January. The hyperbenthic food web was analyzed using a multi-biomarker approach including carbon and nitrogen stable isotopic signatures as well as fatty acid profiles of a variety of hyperbenthic taxa. Results suggest no difference in biomarker composition between September and January, although fatty acid profiles reveal a division in the community between pelagic and benthic consumers. Suggestions for seasonal similarities include slow turnover of stable isotopes and fatty acids in consumer tissue, as well as an increase in dependence on microbial-detrital food webs during the polar night.

Keywords: Kongsfjord, hyperbenthos, stable isotopes, fatty acids, polar night, food web.

1. Introduction

1.1 The Arctic Ecosystem

The Arctic is a seasonal environment with extreme variations in sunlight and ice cover. The light regime of the Arctic is most simply described in terms of day length. North of the polar circle, the sun remains above the horizon for at least 24 hours during the summer (polar day) and below the horizon for at least 24 hours during the winter (polar night). With increased latitude, the length of the polar day and polar night increase, and reach a maximum at the poles with only one sunrise and one sunset over the entire year. Hence, the Arctic light regime is far from homogenous, with vast differences in light availability with latitude (Berge *et al.,* 2015). Ice cover is also highly variable across Arctic regions, ranging from thick multi-year ice, to first-year ice, to open ocean. Sea-ice cover varies seasonally due to changes in solar radiation and temperature. It reaches its maximum extent at the end of the winter cold season in March and its minimum extent in September (Maslanik *et al.,* 2011).

The extreme cycles in solar radiation and sea-ice cover in the Arctic greatly influence the cycle of primary production and subsequent flux of organic matter to the benthos. With the return of the sun in early spring, temperatures increase and the sea-ice begins to melt. Eventually, sunlight is able penetrate to the water below where ice-associated microalgae and pelagic phytoplankton can perform photosynthesis. Where ice is present and light penetration is sufficient, an early spring ice-algal bloom (Leu *et al.,* 2011) preceeds a later spring/summer phytoplankton bloom (Falk-Petersen *et al.,* 1998; Wassmann *et al.,* 2006). While ice-algae is a dominant source of carbon in many Arctic regions (Nozais *et al.,* 2001), phytoplankton production is especially important in marginal ice zones. Ice margins and polynyas are highly episodic and productive ecosystems (Wassmann *et al.,* 1996) due to several abiotic factors. Winter mixing brings abundant nutrients to the surface waters while freshwater input from melting sea-ice creates a density-stratification. These physical characteristics provide ideal conditions for phytoplankton growth, resulting in large blooms of phytoplankton every spring. The flux of this organic matter to the benthos is controlled by the relative amounts of primary production and primary consumers. Due to the episodic nature of phytoplankton production at high latitudes, the spring bloom often provides more organic carbon than herbivorous zooplankton can consume, and many phytoplankton cells sink to the benthos where they maintain a flourishing benthic community (Morata and Renaud, 2008). The significant transfer

of organic matter and its efficient assimilation by the benthic community (Grebmeier and Barry, 1991; Ambrose and Renaud, 1995) suggest a tight pelagic-benthic coupling in Arctic ecosystems. Pelagic-benthic coupling has several implications for carbon cycling. Upon reaching the benthos, organic matter may be stored as benthic biomass, used for growth and reproduction, respired and remineralized into CO_2 and dissolved organic carbon, or buried in the sediment and sequestered for millions of years.

Climate warming has accelerated over the past 30 years, with the Arctic recording the most extreme changes. While climate warming has no effect on the seasonal cycle in sunlight, it has greatly impacted sea-ice extent and thickness. Models predict an 80% loss of sea-ice in summer and a 20% loss of sea-ice in winter by 2100 (Johannessen *et al.,* 2004). With these dramatic changes, ice-dominated coastal systems are likely to see substantial changes in carbon inputs as well as pelagic-benthic coupling. The disappearance of sea-ice from coastal areas will eliminate sea-ice microalgae as a carbon source. The lack of stratification from sea-ice melt may also diminish the intensity of spring phytoplankton blooms, resulting in a closer match between levels of production and consumption. Efficient grazing of phytoplankton would subsequently lead to a decrease in the flux of organic material to the benthos. We may conclude, therefore, that food-web structure in Arctic coastal areas is going to change as global temperatures increase (Kortsch *et al.*, 2012). Changes in food-web structure could have implications for the biological pump and the sequestration of carbon from the atmosphere (Nishino *et al.,* 2011). However, the pattern of seasonality with regard to light availability will remain. It is of interest, therefore, to investigate the food-web structure and carbon sources of high latitude systems influenced by warming temperatures.

1.2 Food-Web Studies

Knowledge of food-web structure, including the length, connectivity, and primary sources of trophic pathways, is important for understanding the ecosystem's function. Food webs can help distinguish pathways of biogeochemical and contaminant cycling, as well as elucidate the relationships between biodiversity and ecosystem functions. Knowledge of energy flow can also provide insight into how the community may be impacted by future biotic and abiotic change such as species introductions, altered patterns in productivity, warming temperatures and other environmental and anthropogenic changes (Renaud *et al.*, 2011). In the marine system,

direct observation of feeding is usually impractical, so ecologists must depend on indirect methods for examining diet. Basic approaches, such as dissecting gut contents or fecal pellets, can be useful, but these can also be misleading, as they tend to underestimate the importance of soft and highly digestible food items and overestimate that of recently consumed items (Kelly and Scheibling, 2012). To provide a time-integrated measure of food source and trophic position, ecologists use biochemical tracer methods such as stable isotope analysis and fatty acid biomarker analysis. In the Arctic, food-web studies using these methods have demonstrated the importance of alternative carbon sources and pelagic-benthic coupling, and have been able to illuminate regional differences in carbon source for benthic communities (Renaud *et al.*, 2011, 2015; Kedra *et al.*, 2012). The importance of different potential food sources and the pathways of energy flow remain poorly understood. Coastal areas of the Arctic are predicted to be the first to experience the impacts of climate change, and understanding current food-web processes provides the necessary baseline for predicting Arctic ecosystem function in the coming years (Renaud *et al.*, 2015).

1.3 Polar Night Research

Much of what we know about Arctic marine systems is based on data collected during the polar day. Since the early expeditions to the Arctic (Nansen, 1902), there have been very few 'winter' biological studies in this region, largely due to the logistical difficulties of performing fieldwork under Arctic winter conditions. However, it is likely also due to the particular interest in the spring bloom period due to its importance with respect to primary production, accompanied by the general assumption that there is limited biological activity during the winter months (Smetacek and Nicol, 2005; Piepenburg, 2005). During the last few years, however, polar night biology has become a focus for researchers based out of the University of Tromsø. Since January 2013, researchers aboard the Helmer Hanssen have spent the month of January investigating various biological aspects of the polar night in and around the fjords of Svalbard (Lønne *et al.*, 2015).

Historically, the few studies conducted during the polar night have indicated that processes simply slow down, as organisms enter 'survival mode' after having stored enough energy during the productive summer. Weslawski *et al.* (1991) found a ten percent decrease in zooplankton biomass in Hornsund, as well as decreased growth rates among pelagic and

hyperbenthic species during the polar night. At the time, these findings seemed adequate to explain how organisms in such an extreme environment could survive the long period of limited resources that follows only a short productive period at high latitudes. It was concluded that organisms must develop a strategy where they can use the productive period for growth and reproduction and simply wait out the long winter (Weslawski *et al.*, 1991).

Recent studies, however, indicate that the polar night is a time of unexpected biological activity. Findings of both diel vertical migration (DVM) of zooplankton (Berge *et al.*, 2009) and bioluminescence (Berge *et al.*, 2012), suggest that we still have much to learn about the dark portion of the year. Studies also indicate that, rather than entering 'survival mode,' species may actually capitalize on periods of low primary production and reduced predation pressure by using these months for reproduction (Berge *et al.*, 2015). Nevertheless, the polar night represents a period of time when levels of primary production are close to zero. Understanding what fuels the ecosystem during these dark months and how this impacts trophic structure is essential for understanding the ecosystem as a whole, and how it could be impacted by future climatic changes. Few studies have investigated seasonal variability in food-web structure in Arctic fjords, and those that have took place between late spring and early autumn (Kedra *et al.*, 2012; Renaud *et al.*, 2011), omitting the dark winter months. It is uncertain how representative the polar day is of how the ecosystem operates over the entire year, and it is therefore essential to collect data during the polar night as well. Furthermore, studies investigating food-web structure and carbon flow have focused on pelagic and benthic realms, leaving out an important layer connecting the two: the hyperbenthos.

1.4 The Hyperbenthic Layer

The term *hyperbenthos* refers to the group of small marine organisms that dominate the layer of water just above the seafloor. Mainly crustaceans of pelagic and benthic origin, these organisms may be either part-time or permanent residents of this near-bottom water layer, which can range from just a few centimeters off the seafloor to several tens of meters into the water column (Koulouri *et al.*, 2013). Unfortunately, the hyperbenthos has had a variety of other names including suprabenthos, nektobenthos, hypoplankton, BBL (benthic boundary layer) zooplankton, semiplankton, demersal zooplankton, and benthopelagic plankton, which has resulted in a lack of coherence within the literature (Dauvin and Vallet, 2006).

Our knowledge of the hyperbenthos is relatively limited, due in part to the greater interest and focus on the pelagic and benthic regions, which are easier to sample. The techniques used to sample the pelagic and benthic realms, including trawls, dredges and grabs, are not ideal for sampling the hyperbenthos. In the early 1900s, samples of the water column indicated an increase in biomass just above the seafloor, leading scientists to experiment with a variety of new nets and creative sampling techniques in an attempt to find and describe new species in this layer (Elmhirst, 1932; Russel, 1928; Watkin, 1939). However, the epi-benthic sled, perhaps the most successful of these new tools, wasn't developed until the 1950s. Scientists have since used many versions of the epi-benthic sled with a variety of nets sizes and closing devices. Typically, this sled rides along the bottom of the seafloor, with one or two nets fastened above it that are dragged parallel to, yet just above, the bottom of the ocean. The use of this sampling technique has led to the discovery of new species and more complete descriptions of community structure and biodiversity within the hyperbenthos (Mees and Jones, 1997).

Hyperbenthic species include a variety of mysids, amphipods, copepods, cumaceans, decapod larvae, chaetognaths, isopods, euphausiids and polychaete larvae. Late-stage larval fish are also known to be abundant in some areas. The composition of the hyperbenthic community in an area is dependent upon many abiotic and biotic factors. The sediment type can be especially important, with amphipods tending to dominate over sand, and cumaceans and polychaetes over mud. Depth is another important factor, with isopods and fish larvae dominating in shallow areas and mysids in deeper areas. Community structure has also been known to change seasonally and with the time of day, as a variety of taxonomic groups, including amphipods, isopods, cumaceans, copepods, decapods, crab larvae and polychaetes, have been reported to migrate vertically into the water column (Mees and Jones, 1997). How the variability in community structure influences trophic ecology, however, is complex and poorly understood.

The hyperbenthic community plays a key role in several ecosystem functions, including carbon and nutrient cycling. Swimming activities of hyperbenthic groups such as mysids and amphipods can contribute to re-suspension of organic material from the seafloor, while those swimming higher up may aid in the fragmentation of organic matter in the water column. In this way, members of the hyperbenthos have been shown to influence the flux of organic matter to and from the seafloor (Graf and Rosenberg, 1997). Hyperbenthic organisms are largely generalists, feeding on meiobenthos and plankton, but also scavenging for debris, thereby

facilitating the degradation of organic matter (Koulouri *et al.*, 2013) before and after it reaches the seafloor.

Many hyperbenthic species are known to migrate daily out of the hyperbenthos into the pelagic (Rudstam *et al.*, 1986). These species may contribute to the export of carbon when they feed on resources from the pelagic and respire and produce fecal pellets at depth (Darnis and Fortier, 2012). This migration also strengthens an essential link in the food chain between pelagic producers and benthic consumers. A study in Antarctic waters found a thick hyperbenthic layer dominated by a population of shrimp feeding on phytoplankton sinking out of the water column. By vertically migrating into the pelagic to feed during the night, and being consumed at depth, these species brought benthic carbon to the pelagic and pelagic carbon to the benthos, facilitating carbon flux in both directions (Mees and Jones, 1997). Hyperbenthic taxa are also an essential food source for demersal fish and adult shrimp species, as documented in shallow and coastal areas (Hostens and Mees, 1999). A study on the feeding habits of cod in 1982 in Balsfjorden in northern Norway found that of the 72 animal taxa found in cod stomachs, only 11 were considered 'primary prey items.' *Pandalus borealis* was one of the most common prey species while others including *Arrhis phyllonyx* and *Halirages fulvocinctus* were of secondary importance. The 11 most important species were all inhabitants of the hyperbenthic zone (Klemetsen, 1982).

Despite their role in carbon and nutrient cycling, and their importance as a food source for key commercial fish species, few studies have focused on the chemical composition or feeding ecology of hyperbenthic species (Mees and Jones, 1997), particularly in the Arctic.

1.5 This Study

This study aims to describe the food web structure of the hyperbenthic community in Kongsfjord, a high-latitude and ice-free fjord, in September and January. The hyperbenthic food web is analyzed using a multi-biomarker approach including fatty-acid trophic markers as well as carbon and nitrogen isotopic signatures of a variety of hyperbenthic taxa. Stomach contents of several fish were analyzed as well. A combination of pelagic production, macroalgae, detritus, and terrestrial inputs are expected to contribute to the majority of the carbon sources. Results from this study will illuminate similarities and differences between the polar night and polar day

in regard to carbon inputs and trophic levels of consumers, with implications for both the benthic and pelagic systems.

2. Methods

Sample collection took place in Kongsfjord (figure 1) onboard the R/V Helmer Hanssen on two cruises. The first cruise took place as part of the UNIS course AB320 in September, 2014 and sample collection took place between 23/09/2014 and 26/09/2014. The second cruise was part of the Marine Night cruise in January, 2015 with samples collected from 12/01/2015 to 15/01/2015. Samples were analyzed for stable isotope composition at the Institute of Oceanology and Polish Academy of Sciences (IOPAS) in Sopot, Poland. Fatty-acid biomarker analysis was performed at Unilab at Akvaplan-niva in Tromsø. Statistics and plots were run and produced in R.

2.1 Background: Kongsfjord

Kongsfjord (figure 1) is an Arctic fjord on the west coast of Spitzbergen. At 79°N, it experiences the light regime of the high Arctic. The sun does not set (polar day) from mid-April until late-August and does not rise (polar night) from late-October until mid-February.

Kongsfjord is an open fjord with no sill. The main water masses include Surface Waters from glacier meltwater, Transformed Atlantic Waters originating on the Spitsbergen shelf, locally produced fjord waters, and Winter Bottom Waters formed by a process of deep convection in the winter (Wlodarska-Kowalczuk *et al.*, 2005). The proportion of each water mass changes seasonally. During the winter, Atlantic Water cannot enter Kongsfjord due to a density front at the fjord mouth. Slow modification of the fjord water during spring reduces the effectiveness of the density barrier, and by midsummer, Atlantic Water is able to rush into the fjord. This warm, saline water-mass quickly changes the fjord character, switching it from being Arctic dominant to Atlantic dominant. Atlantic Water continues to flow into Kongsfjord throughout the summer and by September, the fjord reaches a ‘steady state’ condition where the fjord adopts a ‘cold’ or ‘warm’ mode according to the degree of Atlantic Water occupation (Cottier *et al.*, 2005). In recent years, this Atlantic Water intrusion has intensified, and strong Atlantic influence can be seen even in CTD profiles in January (personal observation). This strong Atlantic influence has caused the disappearance of sea ice, and a subsequent regime shift

in community structure and a 'borealization' of Kongsfjord (Kortsch *et al.*, 2012).

Kongsfjord has three tidewater glaciers that terminate in the fjord waters: Kongsbreen, Conwaybreen, and Blomstrandbreen. Small icebergs are found throughout the fjord year-round, and large icebergs, up to 10 m high, circulate or stay anchored in the inner basin (Wlodarska-Kowalczuk *et al.*, 2005). The glaciers provide the major source of fresh water. Other sources come from snowmelt, precipitation, run-off and groundwater discharge. The mean annual flux of fresh water from all of these sources is estimated to constitute about 5% of the mass balance in the fjord (Cottier *et al.*, 2005) and supplies the fjord with both organic and inorganic terrestrial carbon. The degree of glacial effects on the physical and biological characteristics diminishes towards the outer fjord, where oceanographic influences begin to dominate local conditions (Wlodarska-Kowalczuk *et al.*, 2005).

These physical characteristics control the biotic processes in Kongsfjord, which vary seasonally within the fjord. The depth of the euphotic layer ranges from 30 m at the fjord mouth to 0.3 m in the glacier-influenced inner fjord. Production is dependent on light, stratification and mixing, and the start of the phytoplankton growth season has been detected in March, with the presence of diatoms in the water column. The spring bloom, however, it not in full swing until April, and some years not until June. The bloom is limited by light in the beginning and by zooplankton grazers towards the end. Grazers also play a role in determining the amount of material that is able to sink to the bottom of the fjord. Sinking phytoplankton represents an important source of organic matter to the benthos (Hop *et al.*, 2002).

The physical and biological characteristics of Kongsfjord have the potential to influence the food sources available to pelagic, and benthic consumers at different times of year, and at different locations within the fjord. The lack of sea ice eliminates ice algae as a carbon source, but other possible carbon sources for primary consumers include pelagic primary production from the spring and summer blooms, macroalgae and microphytobenthos, terrestrial material, bacteria, zooplankton carcasses, detritus of each of these sources, fecal pellets produced by grazers, and material brought by the West Spitzbergen Current.

Figure 1. Map of Spitzbergen. Kongsfjord is noted in red.

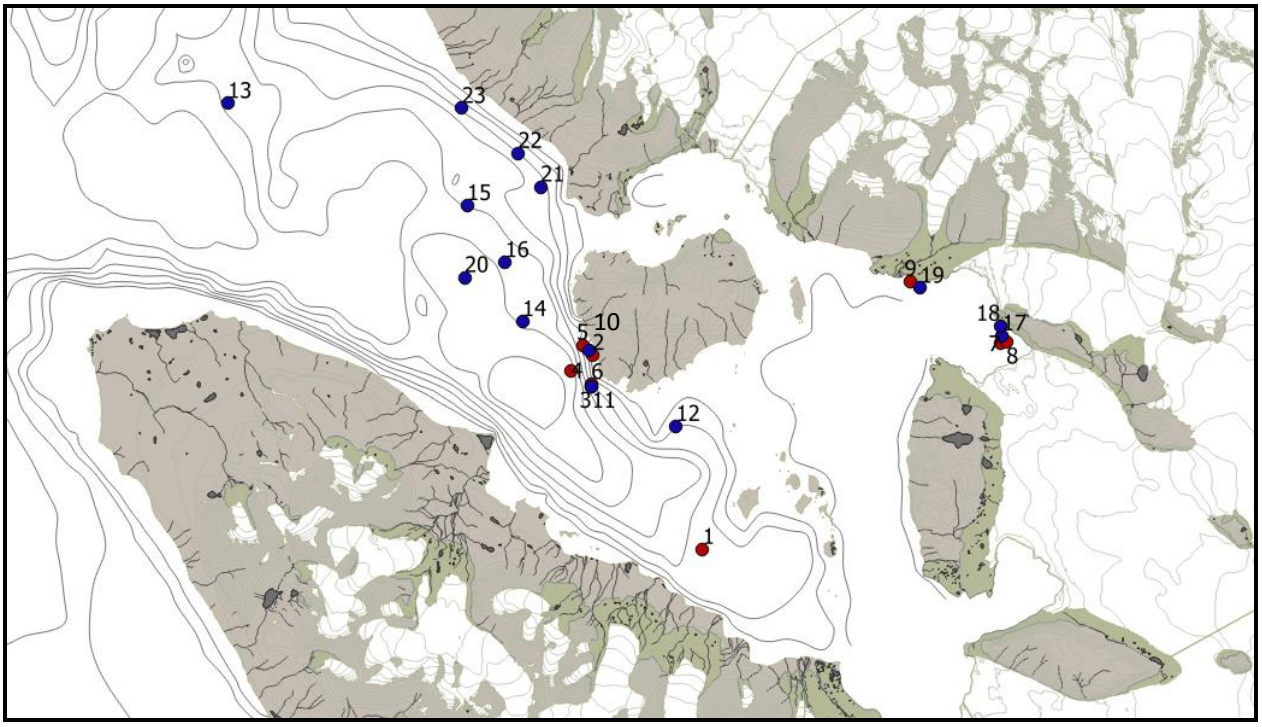

Figure 2. Map of Stations in Kongsfjord. Red = September 2014, Blue = January 2015.

2.2 Sampling Techniques

Samples were collected from a variety of locations within Kongsfjord (figure 2) using several different sampling gears (table 1). In September, 2014 and January, 2015, an epi-benthic sled (EBS) was used to collect hyperbenthic organisms just above the sea floor at depths ranging from 53 m to 338 m. The EBS was composed of 2 stacked nets, each 100 cm wide and 33 cm high, with a 500 um mesh size. The EBS was slowly lowered to the seafloor, where it was towed for 10 minutes at speeds ranging from 0.1 to 3.1 kn, before being brought back to the surface. The nets were hung above the deck and rinsed with a hose into the cod-end. Each sample was rinsed from the cod-end, sieved if necessary, and poured into white trays for organism selection. All hand-picked organisms (table 2) were packed in aluminum foil and frozen for stable isotope analysis (at -20°C) and for fatty-acid marker analysis (at -80°C). Sample sizes (n) ranged from 1 to 5 replicates. Muscle tissue was collected for fish and large crustaceans, while whole organisms were used for smaller organisms. Samples of *Pandalus borealis* were split into two size classes. Individuals with a total length greater than 5 cm were considered 'large' and individuals smaller than 5 cm were considered 'small.' The number of individuals in each sample ranged from 1 for large decapods and fish samples, to 15 or 30 for small amphipods, chaetognaths, and copepods. On return to the lab, samples for stable isotope analysis were dried in an oven at 60°C.

Unfortunately, in January, the EBS at station 150 was lost in inner Kongsfjord, an area known to have very soft sediments due to glacial deposits. Therefore, no samples were taken from EBS 150, and the last three EBS samples (172, 178,179), were taken with a different EBS, which only had one net and was full of sediments and foraminifera every time it was used. These samples were sieved and organisms were hand-picked for analysis. Fish and several large invertebrates were collected using a bottom trawl (BT) at 102 m and 285 m. Lengths and weights of individuals were taken, and stomachs were placed in 70% alcohol for identification. Within three days of the trawl, gut contents were sorted and identified. Muscle tissue was collected from each organism immediately after trawling and frozen for stable isotope (-20°C) and fatty acid (-80°C) analysis. Bottom water was collected by Niskin bottles on a CTD rosette 10-15 m above the bottom at the location of each EBS. The water was filtered (2-3 L per filter) onto 0.7 μm GF/F glass microfiber filters. Three replicates were taken for fatty acid and three for stable

isotope analysis. Filters used for stable isotope analysis were burned (at 450°C for 12 hrs) beforehand to remove carbon or nitrogen residues that could influence results.

Table 1. Station Information.

ID number	Date	Gear Type	Station number	Latitude	Longitude	Depth (m)
1	23/09/14	BT	684	7855.09° N	1208.98° E	102
2	24/09/14	EBS	694	7857.64° N	1159.56° E	88
3	24/09/14	EBS	695	7857.35° N	1159.60° E	203
4	24/09/14	CTD	625	7857.49° N	1157.75° E	336
5	26/09/14	EBS	717	7857.73° N	1159.05° E	109
6	26/09/14	EBS	718	7857.37° N	1159.58° E	201
7	25/09/14	CTD	626	7858.33° N	1233.01° E	137
8	25/09/14	EBS	702	7858.34° N	1232.89° E	135
9	25/09/14	EBS	703	7858.87° N	1225.29° E	53
10	12/01/15	EBS	95	7857.69° N	1159.35° E	101
11	12/01/15	EBS	96	7856.95° N	1200.01° E	207
12	12/01/15	CTD	97	7856.62° N	1206.60° E	232
13	12/01/15	BT	98	7901.39° N	1128.68° E	285
14	13/01/15	EBS	112	7857.92° N	1154.06° E	338
15	13/01/15	EBS	113	7900.17° N	1149.06° E	205
16	13/01/15	CTD	114	7859.26° N	1151.87° E	258
17	14/01/15	CTD	148	7858.40° N	1232.68° E	136
18	14/01/15	EBS	149	7858.49° N	1232.56° E	139
19	14/01/15	EBS	150	7858.81° N	1225.78° E	n/a
20	15/01/15	EBS	172	7858.70° N	1149.13° E	295
21	15/01/15	EBS	178	7900.38° N	1155.00° E	122
22	15/01/15	EBS	179	7901.08° N	1153.02° E	154
23	15/01/15	CTD	180	7901.47° N	1148.15° E	169

ID number = Station number referred to in figure 1. Date = September 2014 and January 2015. Gear type= BT bottom trawl, CTD Niskin bottles on CTD rosette collected at depth, EBS epi-benthic sled. Station number = station number given by the Helmer Hanssen.

Table 2. Species collected from Kongsfjord with the type of sample taken during each sampling period.

TG	Species	Sep-14	Jan-15	Label
A	*Ancanthostepheia malmgreni*	SI	SI	Am
A	*Andaniexis lupus*	SI, FA	SI	Al
A	*Arrhis phyllonyx*	SI, FA	SI, FA	Ap
A	*Halirages fulvocinctus*	SI, FA	SI, FA	Hf
A	*Syrrhoe crenulata*	SI	SI	Sc
M	*Erythrops erythropthalma*	SI	SI	Ee
E	*Thysanoessa inermis*	SI	SI	Ti
D	*Pandalus borealis* (large)	FA, SI	FA, SI	Pbl
D	*Pandalus borealis* (small)	FA, SI	FA, SI	Pbs
D	*Sabinea septemcarinata*	SI, FA	SI, FA	Ss
D	*Lebbeus polaris*		SI	Lp
Ch	*Parasagitta elegans*	SI, FA	SI, FA	Pe
F	*Hippoglossoides platessoides*	SI, FA	SI, FA	Hp
F	*Gadus morhua*	SI, FA		Gm
F	*Melanogrammus aeglefinus*	SI, FA	SI, FA	Ma
F	*Boreogadus saida*		SI	Bs
F	*Leptoclinus maculatus*		SI	Lm
C	*Calanus* spp.	FA		Cs
MA	*Laminaria digitata*	FA		Ld
MA	*Desmarestia aculeata*	FA		Da
MA	*Rhodomela confervoides*	FA		Rc
POM	Bottom Water POM	SI, FA	SI, FA	POM

SI = Stable isotopes, FA = Fatty acid profiles. TG=Taxonomic group: A Amphipoda, M Mysida, E Euphausiida, D Decapoda, Ch Chaetognatha, F Fish, C Copepoda, MA Macroalgae, POM Bottom water. Label = species label used in figures.

2.3 Background: Stable Isotopes

Stable isotopes of carbon and nitrogen are useful tools for studying food webs as they track assimilated food over long time-scales. The $\delta^{15}N$ values are useful for estimating the trophic level of a consumer, as its tissues are predictably enriched in $\delta^{15}N$ by 3–4‰ relative to its diet due to urinary loss of $\delta^{15}N$ depleted ammonium and urea (Peterson and Fry, 1987). In comparison, the $\delta^{13}C$ values vary little (1%) as carbon moves through marine food webs. The $\delta^{13}C$ values can

therefore provide information about an organism's major carbon sources, as long as the available sources have distinct $\delta^{13}C$ signatures (Søreide *et al.,* 2006a). Mechanisms controlling $\delta^{13}C$ fractionation between inorganic substrates and marine algae are not well understood. However, isotope fractionation associated with carbon fixation may vary between primary producers due to local differences in the availability of CO_2 for photosynthesis. Differences might also be due to isotopic discrimination of algae. In aquatic plants, isotopic discrimination is related to the thickness of diffusive boundary layers that ultimately determine the rate of CO_2, or $HC0_3^-$ diffusion and availability (Hobson *et al.,* 1995). Such boundary layers have been shown to differ between species as well as position in the water column and proximity to shore (France, 1995). Differences in $\delta^{13}C$ fractionation among primary producers, as well as the small and predictable changes in $\delta^{13}C$ with trophic level, are what enable scientists to detect carbon sources within consumer tissues.

Studies performed in offshore polar regions have shown that in these systems, only two primary carbon sources exist: the relatively ^{13}C -enriched ice algae growing underneath and inside sea ice and the relatively ^{13}C -depleted phytoplankton in pelagic waters (Tamlander *et al.,* 2006a; Søreide and Nygård, 2012). While it is possible that a significant input of terrestrial matter, depleted in ^{13}C, can be incorporated in sea ice and transported offshore, these sources are considered to be of minor importance compared to phytoplankton and ice algae (Søreide *et al.,* 2006a). In these studies, $\delta^{13}C$ is very useful in determining carbon source, especially due to the relative ease of obtaining pure samples of each primary producer (Søreide and Nygård, 2012). In shallow coastal areas, however, the process is not as straightforward. With river-runoff and macroalgal growth contributing to the carbon pool, the variety of carbon sources available to consumers is much greater. Not only are there overlaps in $\delta^{13}C$ signatures, but it becomes challenging to obtain pure samples of each possible carbon source. This challenge is then exacerbated as the seasons progress following the spring phytoplankton bloom. As levels of fresh algal material decline moving into autumn and winter, their uneaten cells live on in degraded clumps of detritus in the water column and on the seafloor. It can be especially difficult to determine both the role of detritus and its origins using stable isotopes, as detritus can come from many sources, and may be enriched or depleted in $\delta^{13}C$, which potentially makes the $\delta^{13}C$ values in detritus-feeding organisms highly variable (Post, 2002).

Stable isotope analysis performed on small crustaceans is faced with further challenges concerning trophic level estimation. Most studies using stable isotopes, including this one, use a 'bulk method' approach. In order to have enough material to measure isotopic ratios, whole organisms, and in some cases many individuals, are taken for each sample. Due to the small sizes of these organisms, entire individuals are necessary for the analysis. However, the internal tissues have very different signatures from the external skeletons, and combining the two can lead to a miscalculation of trophic level (Søreide and Nygård, 2012). Internal tissues vary considerably because different tissues fractionate isotopes in various ways. For example, lipids are usually depleted in ^{13}C relative to proteins and carbohydrates. At the same time, chitin, a major component of exoskeletons, can be strongly depleted in ^{15}N compared to the rest of the body. Therefore, analyses of chitin-rich samples may bias, and underestimate, trophic levels calculated from stable isotopes. However, the differences in $\delta^{13}C$ values among exoskeleton, soft tissue and whole individuals are small, which suggest that the bias is only an issue for $\delta^{15}N$ and trophic level estimation.

To avoid these biases, stable isotope measurements should be performed on the soft tissue of chitin-rich organisms. If this is not possible, compound specific stable isotope analysis should be used, which analyzes the stable isotope composition of distinct amino acids and fatty acids (Søreide and Nygård, 2012). However, it is not always feasible to remove exoskeletons from small crustaceans, and compound specific stable isotope analysis is expensive and not always an option. Therefore, another route is to combine the use of stable isotopes with other methods in order to cross reference results and identify sources at a finer resolution.

Despite these challenges, stable isotopes have been used successfully in Svalbard fjords and elsewhere to elucidate food web processes in the benthic realms. Marine phytoplankton are identified using $\delta^{13}C$ values higher than -25.5‰ and terrestrial material using signatures lower than -25.5‰ (Divine *et al.*, 2015). A study performed in Isfjord demonstrated the importance of macroalgae as an additional source of carbon to the benthos in fjord systems (Renaud *et al.*, 2015). Stable isotope analysis performed on macroalgae determined $\delta^{13}C$ values to fall between -14.6 and -25.2, although some red algae can be considerably more depleted. While carbon isotopic ratios were enough to distinguish between macroalgae and other groups of primary producers, they were unable to differentiate between several types of macroalgae. Highlighting the importance of a

multi-method approach to food web studies, fatty acid trophic markers were used in addition to stable isotopes in order to distinguish between red and brown macroalgae (Hanson *et al.*, 2010). This stresses the need to combine multiple approaches when investigating food webs. In this study, stable isotopes are combined with fatty acid biomarker analysis and stomach content analysis.

2.4 Stable Isotope Analysis

Stable isotope analysis was performed at IOPAS in Sopot, Poland. Analyses was done in bulk, meaning that entire individuals, and in the case of small crustaceans, many individuals, were pooled for analysis. The dried samples were ground to fine powder using a glass mortar and pestle, after which lipids and inorganic carbonate were removed. When there was too little matieral for analysis, samples were combined, reducing the number of original replicates. This was only an issue for some small crustaceans and chaetognaths, and were usually combined with samples taken from the same EBS. There were two occasions when samples were combined from multiple locations within the fjord, and these are noted in the results.

Stable isotope analysis prioritized protein rich tissues with relatively slow turnover, such as muscle tissue, where possible. Lipids, which constitute the major compound for energy storage in polar regions, have a higher turnover (Graeve *et al.*, 1994) and are strongly depleted in $\delta^{13}C$ and $\delta^{15}N$ compared to other compounds in the body. Despite their importance as an energy pathway through the Arctic ecosystem (Falk-Peterson *et al.*, 1990), lipids are usually removed prior to stable isotope analysis in order to diminish the variability of carbon isotopic ratios due to seasonal fluctuation as well as the dramatic variability in lipid concentration within and among species (Hobson *et al.*, 1995). Inorganic carbon was also removed prior to analysis because inorganic carbon has a heavier $\delta^{13}C$ signature than organic carbon, and thus also skews the $\delta^{13}C$ results. This is especially important when making comparisons within and among different taxonomic groups with different amounts of exoskeleton.

However, the carbonate and lipid removal techniques can influence the $\delta^{15}N$ measurements (Søreide *et al.*, 2006b). Therefore, the samples were run through the mass spectrometer twice, with values of $\delta^{15}N$ and $\delta^{13}C$ calculated on both runs. Values of $\delta^{15}N$ were taken for data analysis after the first run, as those are unaffected by lipid removal techniques.

Values of $\delta^{13}C$ were taken from the second run, as these represent the organic carbon in the tissue without the inorganic and lipid components.

Powdered samples were dried in an oven at 60°C. A subsample was then taken and packed in tin capsules, weighed to the nearest 0.000001 g and analyzed for $\delta^{15}N$. A second subsample was taken for lipid and calcite removal. Lipids were removed first and then acidified by addition of 2 M HCl. Lipids were removed by extracting them from the samples in 2:1 (by volume) chloroform-methanol solution (2:1 CM) overnight at room temperature. After extraction, samples were quickly rinsed in new 2:1 CM and then air dried under a fume hood at room temperature. Following lipid removal, samples underwent acidification for removal of calcium carbonate. Dried samples were wetted in 2M HCl and then dried at 60°C four times. These samples were then dried, packed in capsules, weighed, and analyzed for $\delta^{13}C$ measurements. The analyses were performed in an Elemental Analyzer Flash EA 1112 Series combined with a Delta V Advantage Isotopic Ratio Mass Spectrometer (Thermo Electron Corp., Germany). Isotopic ratios of $\delta^{13}C$ and $\delta^{15}N$ were calculated using pure laboratory reference gases CO_2 and N_2 calibrated against IAEA standards CO-8 and USGS40 for $\delta^{13}C$ and N-1 and USGS40 for $\delta^{15}N$.

The ratio of carbon and nitrogen isotopes, ^{13}C:^{12}C, and ^{15}N:^{14}N, are the most commonly used isotopic ratios for food-web studies. The 'δ' is a widely used notation representing each ratio as the deviation from the reference material in parts per thousand (‰). A more positive (less negative for carbon) isotopic value is said to be isotopically *enriched*, meaning that the sample contains proportionally more of the heavy stable isotope (^{13}C or^{15}N). The reference material, or 'standard' is Pee Dee Belemite limestone for carbon and atmospheric N_2 for nitrogen.

$\delta^{13}C$ and $\delta^{15}N$ are calculated as follows:

$$\delta X = [Rsample/Rstandard) - 1] \times 1000$$

X=Carbon or Nitrogen, R= the appropriate ratio, so ^{13}C:^{12}C or ^{15}N:^{14}N, of the sample and of the standards.

Trophic levels (TL) for each consumer were calculated from their δ15N value using the following equation:

$$TL_{consumer} = [(\delta^{15}N(consumer) - \delta^{15}N(\textit{Thysanoessa inermis})/\delta^{15}N(\text{fractionation constant})] + 2.6$$

$\delta^{15}N$ for *Thysanoessa inermis* was used as the baseline for January (8.23) and September (8.75). This assumes that all taxa analyzed are supported by the same base food source as *Thysanoessa inermis*. This appears to be true for some, but not all of the species sampled here. However, POM samples, which are typically used as a baseline, do not represent the carbon source for this community (see discussion below). *Thysanoessa inermis* is a well-studied lower-trophic level consumer. While previously believed to be a true herbivore (Falk-Petersen *et al.*, 2000), recent studies have found that it is likely omnivorous–carnivorous with trophic levels ranging from 2.5-2.7 (Søreide *et al.*, 2006a; Tamelander *et al.*, 2006b; Petursdottir *et al.*, 2012). Based on these studies, a trophic level of 2.6 was assumed. A value of 3.4‰ was used for the trophic level fractionation constant in $\delta^{15}N$ (Post, 2002; Søreide *et al.*, 2006a).

2.5 Background: Fatty Acids

Lipids are one of the major classes of biological macromolecules. They are high-energy molecules used by all living organisms for energy storage and as structural components of cell membranes. Fatty acids are one of the fundamental categories of biological lipids, and are commonly used as building-blocks of more structurally complex lipids. Fatty acids are also the most important lipid biomarkers for trophic studies, as they tend to remain intact within tissues (Dalsgaard *et al.*, 2003) and can accumulate over time, representing dietary intake over longer time scales. Fatty acids are synthesized in characteristic ways by different groups of organisms, and their tendency to remain intact through the food chain allows scientists to identify prey items from predator tissues (Budge *et al.*, 2008).

Fatty acids are made of a hydrocarbon chain that terminates with a carboxylic acid group, creating a molecule with a polar, hydrophilic end, and a nonpolar, hydrophobic end that is insoluble in water. The carbon chain, typically between four and twenty-four carbons long, may be saturated or unsaturated, and may be attached to functional groups containing oxygen, halogens, nitrogen, or sulfur. Saturated fatty acids (SFA) are hydrocarbon chains with no double bonds, while unsaturated fatty acids (UFA) can have one, mono-unsaturated (MUFA), or many,

polyunsaturated (PUFA), double bonds. The nomenclature used for fatty acids is in the form of "A:B ω-X", where A represents the number of carbon atoms, B the number of double bonds, and X the position of the double bond closest to the terminal methyl group (Parrish *et al.*, 2000).

Fatty-acid biomarkers in consumer tissue can provide insight into the type of producer that was consumed (table 3). The use of biomarkers is based upon the conservative transfer of dietary fatty acids into the neutral lipid fraction of the consumer. The neutral lipids serve as an energy source for organisms and reflect, to a large extent, the fatty acid composition of their diet (Graeve *et al.*, 1994). The fatty acid composition of polar lipids, meanwhile, is largely determined genetically, and is therefore species specific for the consumer (Christie, 1982). In particular, many polyunsaturated fatty acids (PUFA), essential for animals, are selectively retained (Dalsgaard *et al.*, 2003). Fatty acid composition of algae is mostly determined by taxonomy, however, factors such as temperature and light conditions, nutrient availability and growth phase can also influence their fatty acids composition (Leu *et al.*, 2006, 2010).

Typically, marine primary producers synthesize fatty acids ranging from 14 to 24 carbon atoms. Polyunsaturated fatty acids (PUFA) are highly labile, so high levels of PUFA in deeper waters, often indicate fresh, high-quality algal matter (Dalsgaard *et al.*, 2003). This would suggest a bloom event where the phytoplankton production is greater than the grazing rate. If low levels are present in deep waters, this suggests that the algal matter has been degraded, and most likely consumed by zooplankton or degraded by bacteria on its way down. Marine algae are the only known organisms that can produce the long-chain PUFA 20:5ω-3 and 22:6ω-3 de novo. For higher trophic levels, these PUFA are known as the essential fatty acids (EFA), because they must be supplied by the diet (Tocher, 2003), and they serve an important nutritional role for marine consumers. PUFA are especially important in polar regions because they may help to maintain membrane fluidity at low temperatures (Hall *et al.*, 2002). It is generally recognized that (18:2ω-6), (18:3ω-3), (20:4ω-6), (20:5ω-3) and (22:6ω-3) are the most important long-chain PUFA in some invertebrates, mammals and fishes, because they are required for normal somatic growth, neural development, reproduction, survival, and pigmentation (Parrish, 2009). The fatty acids 20:5ω-3 and 22:6ω-3 are the dominant fatty acids in phospholipids, and together with 16:0 compose up to 80% of total phospholipid fatty acids. Phospholipids compose cell membranes, so these structural components are essential at all trophic levels, and are typically dominant in all fatty acid profiles. However, the proportions of each have been shown to vary seasonally.

Diatoms are rich in 20:5ω-3 while dinoflagellates are rich in 22:6ω-3, so in the Arctic, levels of 20:5ω-3 are greater during the spring bloom, when diatoms dominate the phytoplankton biomass. As summer approaches, the standing stock of phytoplankton decreases and there is a higher proportion of dinoflagellates, and therefore 22:6ω-3, available for secondary production (Kattner and Hagen, 1995).

It is a well-established that diatoms and dinoflagellates strongly differ in their fatty acid composition. Diatoms are characterized by high proportions of 20:5ω-3, C16 PUFA, and 16:1ω-7, while flagellates such as *Phaeocystis pouchetii* and dinoflagellates are characterized by elevated C18 PUFA (principally 18:4ω-3) and C22 PUFA (especially 22:6ω-3) (Søreide *et al.*, 2013). Greave *et al.* (1994) found that after switching from feeding *Calanus finmarchicus* with dinoflagellate to diatoms, the dinoflagellate faty acid pattern was entirely replaced by the characteristic diatom fatty acids within 6 weeks.

Bacteria and microbial food webs are essential to the functioning of the marine system. Bacteria are identified by odd-numbered, iso- and anteiso-branched SFA and MUFA, cyclopropyl FA and 18:1ω-7 (Dalsgaard *et al.*, 2003). Meanwhile, terrestrial matter is primarily important in coastal and estuarine ecosystems, and FA biomarkers for terrestrial matter, such as 24:0 + 22:0, have been used to detect the entrainment of terrestrial organic matter into coastal food webs (Dalsgaard *et al.*, 2003).

Arctic zooplankton, such as *Calanus* spp., are well known for their ability to store large reserves of lipids, which are slowly depleted while they overwinter at depth. Wax esters serve as long-term metabolic reserves while trycyloglycerols are utilized for short term demands. *Calanus* spp. produce massive amounts of wax esters de novo with long-chained C20 and C22 monounsaturated FA and fatty alcohols (Sargent and Falk-Petersen, 1988; Kattner and Hagen, 1995). These fatty acids are useful biomarkers for illuminating predator-prey relationships at higher trophic levels (Falk-Petersen *et al.*, 2009).

In addition to differentiating carbon source, fatty acids have been used to provide information on trophic level. The essential fatty acid 22:6ω-3 has been known to bio-accumulate, and therefore may be higher in higher trophic levels. The same has been noted for the prevalence of 20- and 22- monounsaturates, as they seem to increase in proportion with trophic level as well (Dalsgaard *et al.*, 2003). However, due to the variety of sources in the diet, and varying rates of biosynthesis, fatty acids may not be the best indicators of trophic level. However, fatty acids can

provide a qualitative assessment of trophic level. High proportions of PUFA generally indicate herbivorous feeding, while elevated proportions of 18:1ω-9 relative to 18:1ω-7 can be used as a marker for carnivory (Falk-Petersen *et al.*, 1990).

FA profiles are useful for food-web analysis because they provide time-integrated information about assimilated food particles and can help determine both food source as well as trophic position. However, there are some limitations with this method that can hinder the identification of the correct food source. Some higher trophic-level organisms can synthesize fatty acids de novo or can make modifications to their consumed fatty acids through saturation or elongation. These considerations can be frustrating when attempting to interpret biomarker datasets, as they greatly reduce the usefulness of specific biomarkers in certain predator species (Bec *et al.*, 2011). The best solution to this problem is to perform feeding experiments in the laboratory and investigate biosynthesis of certain FA in the target species. However, these experiments require time and resources. The next best solution is to use a multi- method approach when analyzing food webs in order to interpret the results from a variety of perspectives. In this study, fatty-acid trophic-marker analysis is combined with stable isotopes and stomach content analysis.

Table 3. Summary of FA used as dietary tracers in the Arctic ecosystem.

	Dietary FA Tracer	References
Flagellates	High 16:0/16:1ω-7 ratio 18:4ω-3, 22:6ω-3, 16:0, C18, C18PUFA + 22:6ω-3	Nelson *et al.* (2001) Sargent *et al.* (1987) Reuss and Poulsen (2002) Falk-Petersen *et al.* (1998)
Dinoflagellates	C18PUFA + 22:6ω-3 22:6ω-3 + 18:1 ω-9	Falk-Petersen *et al.* (1998) Kelly and Scheibling (2012)
Diatoms	16:1ω-7 + C16PUFA + 20:5ω-3 High 20:5ω-3/22:6ω-3 ratio High 16:1ω-7/16:0 ratio	Dalsgaard *et al.* (2003) Nelson *et al.* (2001) Reuss and Poulsen (2002)

Ice diatoms	16:1ω-7	Søreide *et al.* (2008)
Melosira arctica association	16:1ω-7 + C16PUFA + 20:5ω-3	Falk-Petersen *et al.* (1998)
Nitzschia frigida association	16:1ω-7	Falk-Petersen *et al.* (1998)
Macroalgae	N6 PUFA (particularly 20:4ω-6)	Dalsgaard *et al.* (2003)
Rhodophyta	20:5ω-3, 16:0, 16:1ω-7, 20:4ω-6, 18:1ω-7	Kelly and Scheibling (2012), Graeve *et al.* (2002),Wessels *et al.* (2012)
Phaeophyta	18:4ω-3, 20:5ω-3, 20:4ω-6, 16:0, 18:1ω-9	Kelly and Scheibling (2012), Graeve *et al.* (2002),Wessels *et al.* (2012)
Chlorophyta	18:3ω-3, 18:2ω-6, 16:0, 18:4ω-3, 18:1ω-7	Kelly and Scheibling (2012), Graeve *et al.* (2002),Wessels *et al.* (2012)
Bacteria	Odd-numbered, iso- and anteiso-branched SFA and MUFA, cyclopropyl FA, 18:1ω-7 Branched and/or odd-numbered FA + 18:1ω-7	Sargent *et al.* (1987), Dalsgaard *et al.* (2003) Volkman *et al.* (1980)
Aerobes/facultative aerobes	16:1ω-7, 16:1ω-7t, 18:1ω-7	Guckert *et al.* (1985)
Anaerobes	15:0, 17:0, iso- andanteiso-branched C15 and C17	Guckert *et al.* (1985)
Sulphate reducing bacteria	Cy 17:0, cy 19:0, 17:1ω-7, 10Me 16:0, 17:1ω-6	Sargent *et al.* (1987) Findlay *et al.* (1990)
Detritus	SFA (particularly 18:0) 18:0 + 18:1ω-9	Mayzaud *et al.* (2013b) Søreide *et al.* (2008)
Foraminifera	20:4ω-6	Suhr *et al.* (2003)
Carnivorous feeding	18:1ω-9 High 18:1ω-9/18:1ω-7 ratio	Sargent and Falk-Petersen (1981) Graeve *et al.* (1997)

Calanus copepods	20:1ω-9 + 22:1ω-11	Sargent and Falk-Petersen (1988)
Terrestrial vegetation	18:2ω-6 + 18:3ω-3 > 2.5 22:0 + 24:0	Budge and Parrish (1998)

Adapted from (Kelly and Scheibling, 2012) and (Legezynska *et al.*, 2014).

2.6 Fatty Acid Analysis

FA profiles were run at Unilab at Akvaplan-niva in Tromsø, Norway. Total lipids were extracted according to Folch *et al.* (1957). A known amount of 21:0 was added to the samples of extracted total lipid as an internal standard. An acid-catalysed transesterification was carried out with 1% sulphuric acid in methanol. The total lipid extract of each sample was cleaned on a silica column (Christie, 1982). FA were analyzed with an Agilent 6890 N gas chromatograph, equipped with a fused silica, wall-coated capillary column (50 m×0.25 mm i.d., Varian Select FAME), and an Agilent 7683 injector and flame ionization detection. Hydrogen was used as the carrier gas. Samples were injected at 280 °C, and the thermal gradient was raised from 60 to 150 °C at 30 °C min^{-1}, and then to a final temperature of 230 °C at 1.5 °C min−1, and kept there for 2 minutes. Individual methyl esters of FA were identified by comparison with three known standards (GLC-96 and GLC-68D, quantitative standards from NU-CHEK Prep. Inc., USA, and MARINOL qualitative standard obtained from University of Stirling, ref. J. Henderson), and were quantified using HPChemStation software (Hewlett-Packard) (Søreide *et al.*, 2008).

2.7 Background: Statistical Analysis

Data sets of fatty acid profiles can be challenging to analyze because they are compositional, with each of the over 40 fatty acids representing a percentage of the whole, summing to 100%. Furthermore, some FA are hard to detect, so datasets are often full of ‘n.d’, or ‘not enough data’, which are used as zeros in the analysis. There are several multivariate techniques that can be useful for FA dataset analysis. This study makes use of some of these techniques

Correspondence analysis (CA) is a statistical technique applied to multivariate data. It is used to construct an ordination from a contingency table, thereby collapsing a large matrix of variables onto a two-dimensional plane in order to facilitate interpretation. The distances between points in the ordination represent the 'chi-squared distances' calculated between the samples based on the percentages of the 43 fatty acids in its profile. CA is often used by ecologists to visualize abundances, biomasses, or other positive variables associated with a number of species from a variety of sampling locations. CA analyzes row or column profiles, and because it is interested in the *relative* differences between these profiles, it creates a 'compositional' data set in its calculations. This is done by dividing each value by the sum of its row or column profile, making it possible to compare, for example, relative species abundance across stations, with each species weighted in proportion to its total abundance. As fatty-acid data sets are inherently compositional, this part of the CA calculation is already done. The chi-squared distances used in CA are also beneficial with the fatty-acid data set, as they help account for the large disparity in variances found in such datasets (Greenacre and Primicerio, 2013).

In this study, CA was used to visualize the community based on the fatty acid profiles of its components within each season, as well as between seasons in two-dimensional space. An especially useful technique performed was the 'contribution biplot.' Based on the CA ordination, this visualization technique highlights the most important variables (in this case specific fatty acids) that are contributing to the output of the CA. Highly relevant fatty acids are presented in enlarged fonts while less-significantly contributing fatty acids are pulled to the center and marked with smaller font. This is done in order to easily pinpoint the important fatty acids. In order to facilitate interpretation, all low-contributing lipids were removed from the plot. While they are still incorporated in the calculation of the ordination, they are not visible on the plot. Only the fatty acids that had a greater than average contribution to the CA are shown. By combining the use of both the normal CA and the contribution biplot, it was possible to both visualize the similarities and differences between the taxa and the seasons (CA plot), and to determine the main fatty acids responsible for these similarities and differences (contribution biplot) on the community level.

There are several drawbacks to this technique. Fatty acids that are found in high concentrations are emphasized while differences in low-concentration, or 'rare' fatty acids are hidden. Another drawback is that some fatty acid biomarkers are described in ratios. For

example, in Arctic regions, carnivory can be indicated when the ratio of 18:1 ω-9 to 18:1 ω-7 is greater than 1 (Graeve *et al.*, 1997). The contribution biplot, while it can pick out individual fatty acid biomarkers, does not do an adequate job of dealing with ratios. When using this technique, it is important to keep in mind that further investigations are needed to assess the importance of ratios and rare fatty acids. For this study, the interpretation of the CA plots is limited to pinpointing the highest contributing fatty acids in each plot, and using these results to track seasonal differences on the basis of these fatty acids (Greenacre and Primicerio, 2013). However, a second technique is needed to statistically quantify the difference between the two seasons. Procrustes analysis is used for this purpose.

In Greek mythology, Procrustes, whose name means 'he who stretches,' was a host with a special iron bed. If the guest proved too tall or wide to fit the bed, Procrustes would rotate, stretch, and chop their bodies in order to make them fit. In statistics, the Procrustes Rotation is a useful technique, and an analogue of the ancient myth. This technique statistically compares two ordinations by rotating a configuration to maximum similarity with another configuration. In this study, the two configurations rotated to maximum similarity with one another were the September and January CA ordinations. In the Procrustes plot, segments are drawn between corresponding species from each ordination. The relative length of segments represents greater dissimilarity in the ordinations, and therefore greater differences between the two seasons based on fatty acid profiles. This technique analyzes the data on the species level, revealing which species are responsible for the greatest difference in fatty acid profiles between seasons. However, this also limits the data that can be used for the analysis, as only species that have been collected in both seasons at the same location can be included. In this study, only nine taxa met these criteria and were included in the ordinations. Another useful aspect of Procrustes is the 'protest' function, which tests the non-randomness (i.e. significance) in the difference between the two configurations. Procrustes allows us to investigate differences in fatty acid profiles at the species level and the significance of these differences, with a p-value (Oksanen *et al.*, 2015).

In statistics, the term 'canonical' describes methods which relate two sets of data. Canonical, or constrained CA (CCA), is often used by ecologists to relate biological and environmental data sets pertaining to the same set of samples. With basic CA, biological variables are displayed in an ordination with the distances between samples representing approximate chi-squared distances amongst only their fatty acid profiles. In CCA, environmental

variables are accounted for as well. Instead of simply overlaying the environmental variables on top of an existing ordination, CCA accounts for these variables within the formulation of the ordination itself (Greenacre and Primicerio, 2013). In this case, CCA was used to account for the variation due to the physical characteristics of where and when the samples were collected. In this study, CCA was performed with the constraining variables of depth, taxa, and season, which was noted for each sample along with the 43 fatty acids. Following this was an analysis of variance (ANOVA) performed on the CCA output to look for the statistical significance of each of these constraining variables.

2.8 Statistical Analysis

Plots and statistics were done in R. Procrustes analysis and Correspondence Analysis (CA) were computed using the *vegan* package. Contribution biplots were constructed according to R coding by Michael Greenacre used for Exhibit 14.11 in "Multivariate Analysis of Ecological Data" (Greenacre and Primicerio, 2013). CCA was also computed in *vegan*, with depth, taxa, and season as constraining variables. An ANOVA was used to determine which factors (depth, taxa, and season) were significant to the outcome of the plot.

3. **Results**

3.1 Stable Isotopes

Stable isotope analysis was performed on 16 different species as well as near-bottom water POM. In September, mean $\delta^{15}N$ values ranged from 7.1‰ in *Halirages fulvocinctus* to 13.1‰ for *Melanogrammus aeglefinus*. In January, mean $\delta^{15}N$ values ranged from 8.2‰ for *Thysanoessa inermis* to 12.1‰ for *Andaniexis lupus* (figure 3). In September, $\delta^{13}C$ values ranged from -19.8‰ for *Arrhis phyllonyx* to -23.4‰ for *Parasagitta elegans*. A similar range of values was found in January, ranging from -19.3‰ for *Sabinea septemcarinata* to -24.4‰ for *Syrrhoe crenulata* (figure 3). In September, the mean value of $\delta^{15}N$ for POM samples taken from two locations within the fjord was 5.9‰ while in January the mean taken from three locations was much lower, at 1.9‰ (figure 4). Meanwhile, the $\delta^{13}C$ values for POM ranged from a mean of -22.9‰ in September to 24.0‰ in January (figure 5).

All of the amphipods were more enriched in $\delta^{15}N$ in January relative to September. *Halirages fulvocinctus* had the lowest levels of $\delta^{15}N$, with 7.1‰ in September and 8.6‰ in January. *Syrrhoe crenulata* had levels of 8.6‰ in September and 9.2‰ in January. *Arrhis phyllonyx* and *Ancanthostepheia malmgreni* were more enriched with 9.8‰ in September and 10.4‰ in January, and 10.1‰ in September and 11.2‰ in January, respectively. *Andaniexis lupus* had the highest levels with 11.4‰ in September and 12.1‰ in January (figure 4). The amphipods had a wide range of $\delta^{13}C$ values. *Syrrhoe crenulata* had the lowest levels of $\delta^{13}C$, with -23.2‰ in September and -24.4‰ in January. *Halirages fulvocinctus* was slightly more enriched in September, with $\delta^{13}C$ values of -20.4‰ relative to January's value of -21.5‰. *Andaniexis lupus* had similar levels of $\delta^{13}C$ between seasons, with -21.0‰ in September and in January. Levels of $\delta^{13}C$ for *Ancanthostepheia malmgreni* were also similar, varying between -20.4‰ in September and -20.5‰ in January. Of the amphipods, *Arrhis phyllonyx* was the most enriched in $\delta^{13}C$ with levels of -19.8‰ in September and -19.4‰ in January (figure 5).

Levels of $\delta^{15}N$ in the decapod *Sabinea septemcarinata* were 10.0‰ in September and 11‰ in January. *Lebbeus polaris* was also more enriched in January, with 7.6‰ in September and 10.1‰ in January. The small *Pandalus borealis* size fraction was slightly less enriched in $\delta^{15}N$ relative to the large size fraction in both seasons. In September, the small size fraction had a mean of 9.2‰ and in January, 10.1‰ while the large size fraction had a mean of 10.1‰ in September and 10.7‰ in January (figure 4). *Sabinea septemcarinata* was slightly more depleted in $\delta^{13}C$ in September, at -21.7‰, relative to January, when the mean was -20.1‰. *Lebbeus polaris* had similar values between seasons with -20.2‰ in September and -20.4‰ in January. The small size fraction of *Pandalus borealis* was more enriched in $\delta^{13}C$ relative to the large size fraction in both seasons. The small size fraction had mean $\delta^{13}C$ values of -21.4‰ in both September and January while the large size fraction had mean values of -20.7‰ in September and -20.1‰ in January (figure 5).

The mysid *Erythrops erythropthalma* had similar values of $\delta^{15}N$ between seasons with 10.2‰ in September and a mean of 10.6‰ in January (figure 4). $\delta^{13}C$ levels were also similar with -21.4‰ in September and -21.2‰ in January (figure 5). The same trend was found for the euphausiid *Thysanoessa inermis*, with $\delta^{15}N$ values of 8.8‰ in September and 8.2‰ in January (figure 4) and $\delta^{13}C$ values of -22.9‰ in September and -22.6‰ in January (figure 5). Stable

isotope values for the chaetognath *Parasagitta elegans* ranged widely among and within samples, with high standard deviations seen in table 4. $\delta^{15}N$ ranged from 12.0‰ in September to 11.1‰ in January and $\delta^{13}C$ from -22.1‰ in September to -22.7‰ in January.

As for the fish, *Gadus morhua* had the highest $\delta^{15}N$ levels at 13.0‰ in September, along with *Melanogrammus aeglefinus* with 13.1‰ in September and 12.1‰ in January. Meanwhile, *Hippoglossoides platessoides* had levels of 12.0‰ in September and 10.3‰ in January (figure 4). *Hippoglossoides platessoides* was the most enriched in $\delta^{13}C$ with values of -19.9‰ in September and -21.0‰ in January. *Melanogrammus aeglefinus* had similar values with -20.6‰ in September and -21.4‰ in January. *Gadus morhua,* collected in September, had $\delta^{13}C$ levels of -21.4‰ (figure 5).

Boreogadus saida, which was only collected in January, had very different values at different locations within the fjord. At the outer-fjord station (station 13), $\delta^{15}N$ values were 11.2‰ while at the inner station (station 18), they were 12.0‰. Meanwhile, $\delta^{13}C$ values were -23.8‰ at station 13 and -20.7‰ at station 18 (map: figure 2, values: tables 4 and 5). The same trend was found for *Leptoclinus maculatus*. Collected only in January, values of $\delta^{15}N$ ranged from 10.0‰ in the middle of Kongsfjord (station 11) to 10.5‰ in the inner fjord (station 18). Values of $\delta^{13}C$ ranged from -22.7‰ in the middle of the fjord to -21.9‰ at the inner fjord (Map: figure 2, Values; tables 4 and 5).

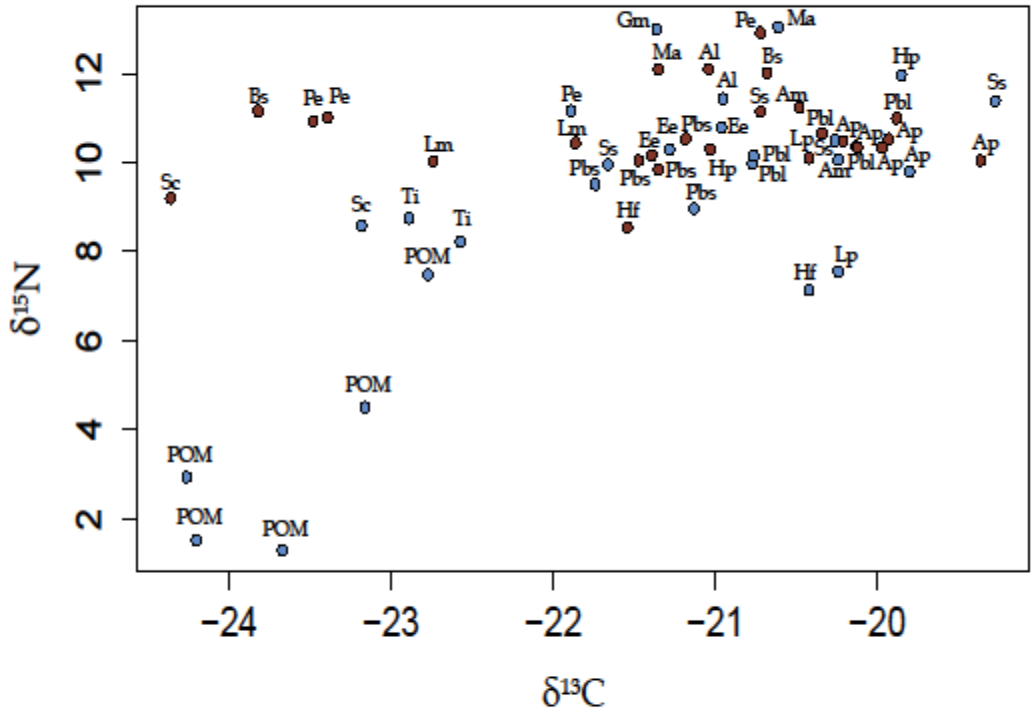

Figure 3. $\delta^{13}C$ vs $\delta^{15}N$ for all samples. Red= September 2014, Blue= January, 2015. Species abbreviations are found under 'Label' in Table 2.

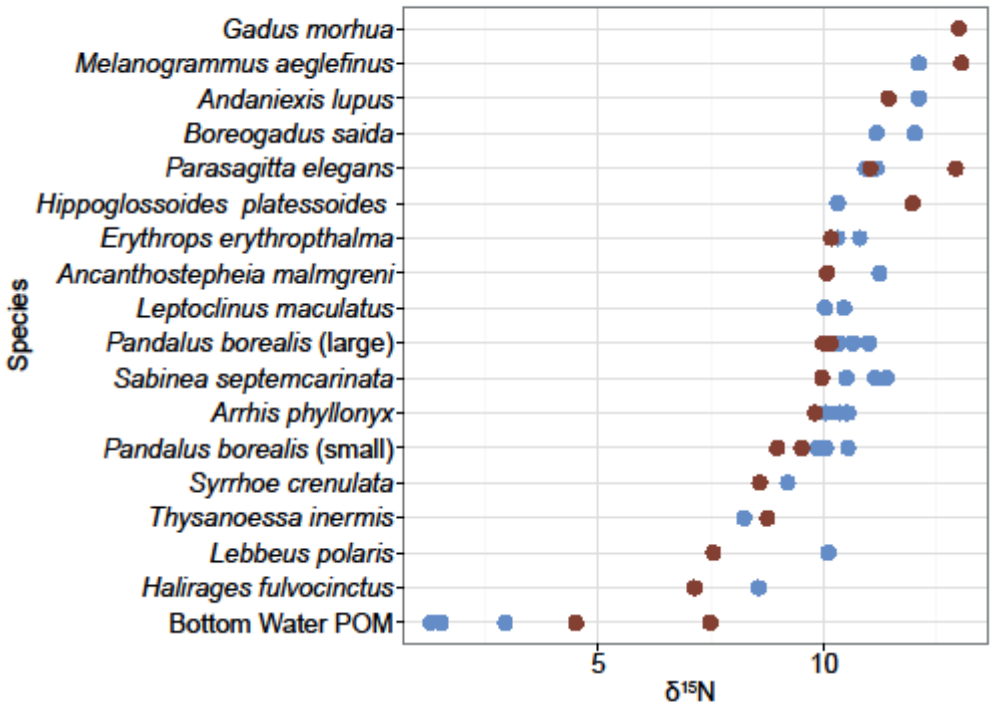

Figure 4. $\delta^{15}N$ values of tissue collected from the above species in September 2014 (in red) and January 2015 (in blue). Values for each replicate are shown to indicate the variability.

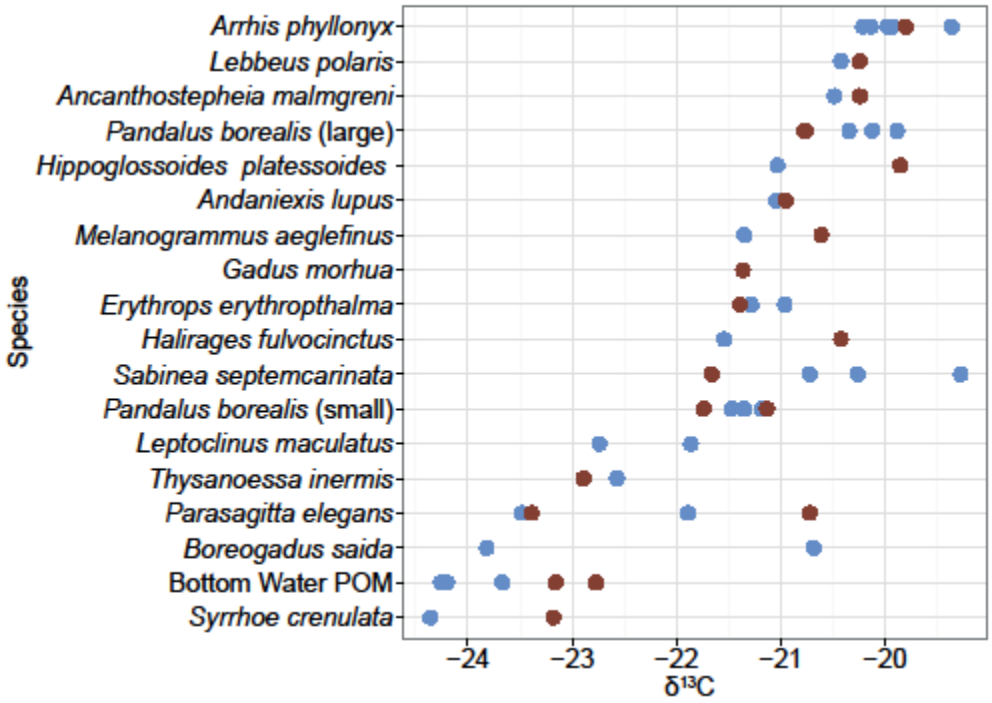

Figure 5. $\delta^{13}C$ values of tissue taken from the above species in September 2014 (in red) and January 2015 (in blue). Values for each replicate are shown to indicate the variability.

Table 4. September results of stable isotopes and dominant fatty acids as well as calculated trophic levels with standard deviations. Fatty acid levels are shown as percent of total fatty acid.

Species	Map #	n-SI	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]	TL	n-FA	16:0	22:6 ω-3	20:5 ω-3	18:1 ω-9	16:1 ω-7	18:0	18:1 ω-7	20:4 ω-6
Ancanthostepheia malmgreni	9	1	10.1	-20.2	3									
Andaniexis lupus	6	2	11.4 (0.2)	-21.0 (0.3)	3.3	2	14.8 (0.5)	15.2 (1.7)	16.8 (0.8)	14.8 (0.6)	5.7 (1.3)	1.2 (0.1)	4.6 (0.3)	1.5 (0.3)
Arrhis phyllonyx	6	5	9.8 (0.4)	-19.8 (0.3)	2.8	3	12.5 (0.4)	7.3 (0.2)	16.5 (1.6)	8.4 (0.1)	8.8 (0.9)	1.8 (0.1)	10.0 (0.7)	12.4 (1.1)
Calanus spp.	5					3	16.1 (1.6)	11.5 (1.7)	10.2 (0.4)	4.3 (0.1)	6.2 (0.9)	5.9 (1.3)	0.8 (0.0)	0.1 (0.2)
Desmarestia aculeata	6					1	26	1.4	8.3	9.8	2.1	1.7	0.8	14.9
Erythrops erythropthalma	2	2	10.2 (0.6)	-21.4 (1.4)	3									
Gadus morhua	1	5	13.0 (0.6)	-21.4 (0.3)	3.8	3	19.9 (0.6)	42.5 (1.7)	13.1 (1.0)	6 (0.6)	1.2 (0.2)	3.9 (0.1)	3.0 (0.5)	1.4 (0.1)
Halirages fulvocinctus	6	3	7.1 (0.3)	-20.4 (0.4)	0.9	3	13.5 (0.6)	11.9 (2.1)	14.4 (2.2)	10.7 (1.2)	5.3 (1.0)	1.1 (0.2)	2.8 (0.4)	1.7 (0.4)
Hippoglossoides platessoides	1	3	12.0 (0.7)	-19.9 (0.6)	3.4	3	20.6 (2.0)	28.3 (0.8)	14.4 (1.5)	8.3 (1.8)	4 (1.2)	4.4 (0.5)	3.7 (0.6)	3.1 (1.1)
Laminaria digitata	6					3	37.9 (9.2)	0.4 (0.4)	2.3 (1.2)	20.1 (14.7)	5.8 (2.4)	1.6 (0.5)	0.8 (0.7)	2.0 (1.6)
Lebbeus polaris	6	3	7.6 (1.1)	-20.2 (1.2)	2.1									
Melanogrammus aeglefinus	1	5	13.1 (0.8)	-20.6 (0.5)	3.9	3	18.9 (0.8)	30.5 (1.4)	19.6 (0.3)	6.6 (1.0)	1.6 (0.2)	3.9 (0.6)	3.8 (0.7)	2.4 (0.2)
Pandalus borealis (large)	6	3	10.0 (0.9)	-20.8 (0.3)	3	3	19.2 (1.8)	17.1 (1.9)	21.4 (2.1)	10.2 (0.4)	5.5 (1.2)	3.0 (0.3)	8.8 (0.2)	2.4 (0.2)
Pandalus borealis (large)	8	3	10.2 (0.4)	-20.8 (0.4)	3									
Pandalus borealis (small)	6	3	9.0 (1.1)	-21.1 (0.4)	2.6	3	17.9 (0.1)	20.2 (0.0)	19.6 (0.5)	7.8 (0.7)	4.1 (0.5)	3.2 (0.1)	6.9 (0.3)	1.7 (0.4)
Pandalus borealis (small)	8	2	9.5 (0.6)	-21.7 (0.6)	2.7									
Parasagitta elegans	5					3	13.8 (0.2)	21.4 (0.6)	13.4 (0.2)	8.2 (0.5)	6.3 (0.5)	1.6 (0.2)	1.3 (0.1)	0.4 (0.0)
Parasagitta elegans	3	1	11.0	-23.4	3									
Parasagitta elegans	6	1	12.9	-20.7	3.5									
Rhodomela confervoides	6					1	20.6	3.5	21.2	3.9	18	1.1	2.7	2.1
Sabinea septemcarinata	3	3	10.0 (1)	-21.7 (1.1)	2.9	3	13.8 (0.6)	10.5 (2.8)	20.1 (2.0)	7.1 (1.3)	7.4 (3.2)	4.4 (0.6)	8.1 (0.9)	3.5 (0.5)
Syrrhoe crenulata	6	2	8.6 (0.1)	-23.2 (1.2)	2.2									
Thysanoessa inermis	3	1	8.8	-22.9	2.6									
Bottom Water POM	4	3	4.5 (1.2)	-23.2 (0.2)	3.9	3	23.5 (2.9)	1 (0.9)	1.5 (1.4)	15.6 (7.3)	5.0 (1.3)	23.2 (3.0)	1.7 (0.6)	0.0 (0.0)
Bottom Water POM	7	3	7.5 (1.2)	-22.8 (0.1)	1.4									

Table 5. January results of stable isotopes and dominant fatty acids (with standard deviation) and calculated trophic level. Fatty acid levels are shown as percent of total fatty acid. Combined samples are noted by multiple stations listed under 'Map #.'

Species	Map #	n-SI	$\delta^{15}N$ [‰]	$\delta^{13}C$ [‰]	TL	n-FA	16:0	22:6 ω-3	20:5 ω-3	18:1 ω-9	16:1 ω-7	18:0	18:1 ω-7	20:4 ω-6
Ancanthostepheia malmgreni	20	2	11.2 (0.2)	-20.5 (0.8)	3.5									
Andaniexis lupus	14	1	12.1	-21.0	3.7									
Arrhis phyllonyx	10	3	10.1 (0.2)	-19.4 (0.5)	3.1	3	11.6 (0.1)	9.5 (0.8)	13.9 (2.1)	8.8 (0.3)	5.3 (0.3)	1.9 (0.1)	11.1 (0.7)	13.9 (0.8)
Arrhis phyllonyx	14	3	10.4 (0.5)	-20.0 (0.5)	3.2									
Arrhis phyllonyx	22	3	10.5 (0.1)	-20.2 (0.1)	3.3									
Arrhis phyllonyx	21	3	10.5 (0.1)	-19.9 (0.6)	3.3									
Arrhis phyllonyx	20	3	10.4 (0.3)	-20.1 (1.3)	3.2									
Boreogadus saida	13	5	11.2 (0.4)	-23.8 (0.6)	3.5									
Boreogadus saida	18	3	12.0 (0.5)	-20.7 (0.2)	3.7									
Erythrops erythropthalma	14	4	10.3 (0.2)	-21.0 (0.7)	3.2									
Erythrops erythropthalma	14,20	2	10.8 (0.2)	-21.0 (0.8)	3.4									
Halirages fulvocinctus	14, 21,22	3	8.6 (0.0)	-21.5 (0.7)	2.7	1	13.1	9.6	12.6	20.4	4.9	0.8	2.8	0.8
Hippoglossoides platessoides	13	2	10.3 (0.0)	-21.3 (0.1)	3.2	3	15.1 (1.1)	13.0 (3.1)	12.9 (2.4)	8.3 (1.9)	7.4 (4.5)	3.4 (0.8)	5.5 (2.0)	2.0 (1.1)
Lebbeus polaris	22	1	10.1	-20.4	3.2									
Leptoclinus maculatus	11	2	10.0 (0.5)	-22.7 (0.3)	3.1									
Leptoclinus maculatus	18	3	10.5 (0.1)	-21.9 (0.4)	3.3									
Melanogrammus aeglefinus	13	4	12.1 (0.4)	-21.4 (1.4)	3.7	3	18.9 (2.1)	32.6 (0.8)	17 (2.3)	7.5 (2.1)	1.6 (0.4)	4.4 (0.4)	4.4 (0.4)	2.4 (0.4)
Pandalus borealis (large)	14	4	10.3 (0.3)	-20.1 (0.5)	3.2									
Pandalus borealis (large)	18	4	10.7 (0.6)	-20.3 (0.4)	3.3									
Pandalus borealis (large)	10	4	11.0 (0.6)	-19.9 (0.3)	3.4	3	20.4 (0.1)	17.9 (1.7)	22.3 (0.8)	12.9 (2.0)	4.9 (0.5)	2.2 (0.1)	8.6 (0.4)	1.7 (0.2)
Pandalus borealis (small)	14	4	9.9 (0.5)	-21.4 (0.4)	3.1									
Pandalus borealis (small)	10	4	10.0 (0.5)	-21.5 (0.3)	3.1	3	14.8 (1.0)	18.8 (1.3)	19.4 (0.6)	10.5 (1.0)	5.1 (0.7)	2.1 (0.3)	6.1 (0.4)	2.0 (0.4)
Pandalus borealis (small)	18	4	10.5 (0.2)	-21.2 (0.2)	3.3									
Parasagitta elegans	11	3	10.9 (0.6)	-23.5 (2.4)	3.4									
Parasagitta elegans	10	3	11.2 (0.8)	-21.9 (1.2)	3.5	3	10.6 (0.2)	18.5 (0.8)	11.9 (0.4)	6.7 (0.3)	8.4 (0.6)	1.2 (0.3)	2.1 (0.6)	0.4 (0.1)
Sabinea septemcarinata	14	3	10.5 (0.4)	-20.3 (0.8)	3.3	3	16.7 (3.1)	16.3 (6.1)	24.8 (2.0)	6.8 (1.6)	4.1 (1.7)	4.2 (0.5)	6.1 (4.2)	4.1 (0.4)
Sabinea septemcarinata	13	3	11.1 (0.3)	-20.7 (1.0)	3.5									

Sabinea septemcarinata	21	3	11.4 (1.0)	-19.3 (1.2)	3.5									
Syrrhoe crenulata	14	1	9.2	-24.4	2.9									
Thysanoessa inermis	10	2	8.2 (0.3)	-22.6 (1.6)	2.6									
Bottom Water POM	12	3	1.3 (1.2)	-23.7 (0.3)	0.6	3	15.1 (0.5)	1.1 (0.5)	1.1 (0.7)	16.9 (12.8)	4.7 (1.6)	16.8 (2.8)	3 (0.6)	0.3 (0.2)
Bottom Water POM	17	3	1.5 (1.2)	-24.2 (0.7)	0.6									
Bottom Water POM	23	3	2.9 (0.3)	-24.3 (2.0)										

3.2 Fatty acids

The most abundant fatty acids across all taxa were 16:0, 22:6ω-3, 20:5ω-3 and 18:1ω-9 (tables 4 and 5). To simplify the interpretation of the Procrustes plot, residuals between configurations after optimal fit are represented by straight lines connecting each species between the September and January data sets (figure 6). Samples such as the small size-fraction of *Pandalus borealis* and the bottom water POM have small residuals indicating a close match between September and January feeding strategies. In contrast, species having larger residuals, such as *Sabinea septemcarinata*, have greater differences in fatty acid profiles between the two seasons. Furthermore, results of the PROTEST function reveal highly significant concordance between the two seasons ($m12 = 0.046$, $p<0.001$), which were highly correlated (r-squared=0.977; table 6). For more about the PROTEST function, see (Peres-Neto and Jackson, 2001). The CCA ordination (figure 7) shows very little difference in species position between the two seasons. The ANOVA results of the CCA ordination indicate that there is no significant trend in fatty acid profiles with depth ($F_{1,11} = 0.85$; $p > 0.05$) or with season ($F_{1,11} = 0.79$; $p > 0.05$), but rather the taxonomic group is best at explaining the variances in fatty acid composition ($F_{4,11} = 4.46$; $p = 0.001$; table 7).

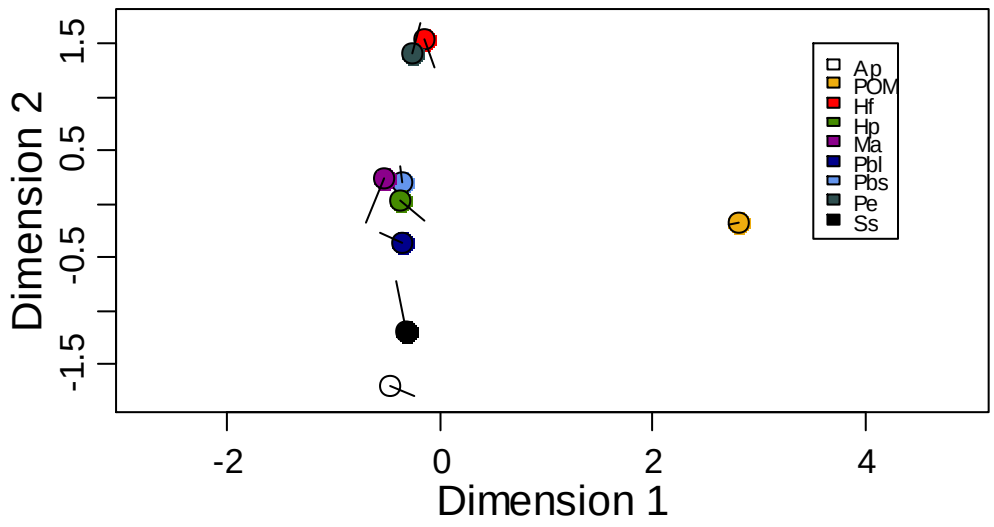

Figure 6. Procrustes rotation of January CA on top of September CA, using 9 taxa that were collected in both September and January in similar locations and depths within Kongsfjord. The circles represent the mean September sample value and the lines are drawn towards the mean January sample value. Longer lines therefore indicate greater residual variation between the positions of each sample within each ordination.

Table 6. PROTEST (Procrustes significance test) of the Kongsfjord community comparing the September to January CA plots based on 999 free permutations.

Procrustes Sum of Squares (m12 squared)	0.046
Significance (P-value)	0.001*
Correlation (r-squared)	0.977

The Procrustes sum of squares (m12) represents the sum of the residual variations. A value of zero would indicate that they are exactly the same. The significance value is based on the permutations of the m12 statistic and is indicated by (*); 0.001 is highly significant. The r-squared value indicates the level of correlation between the two ordination plots.

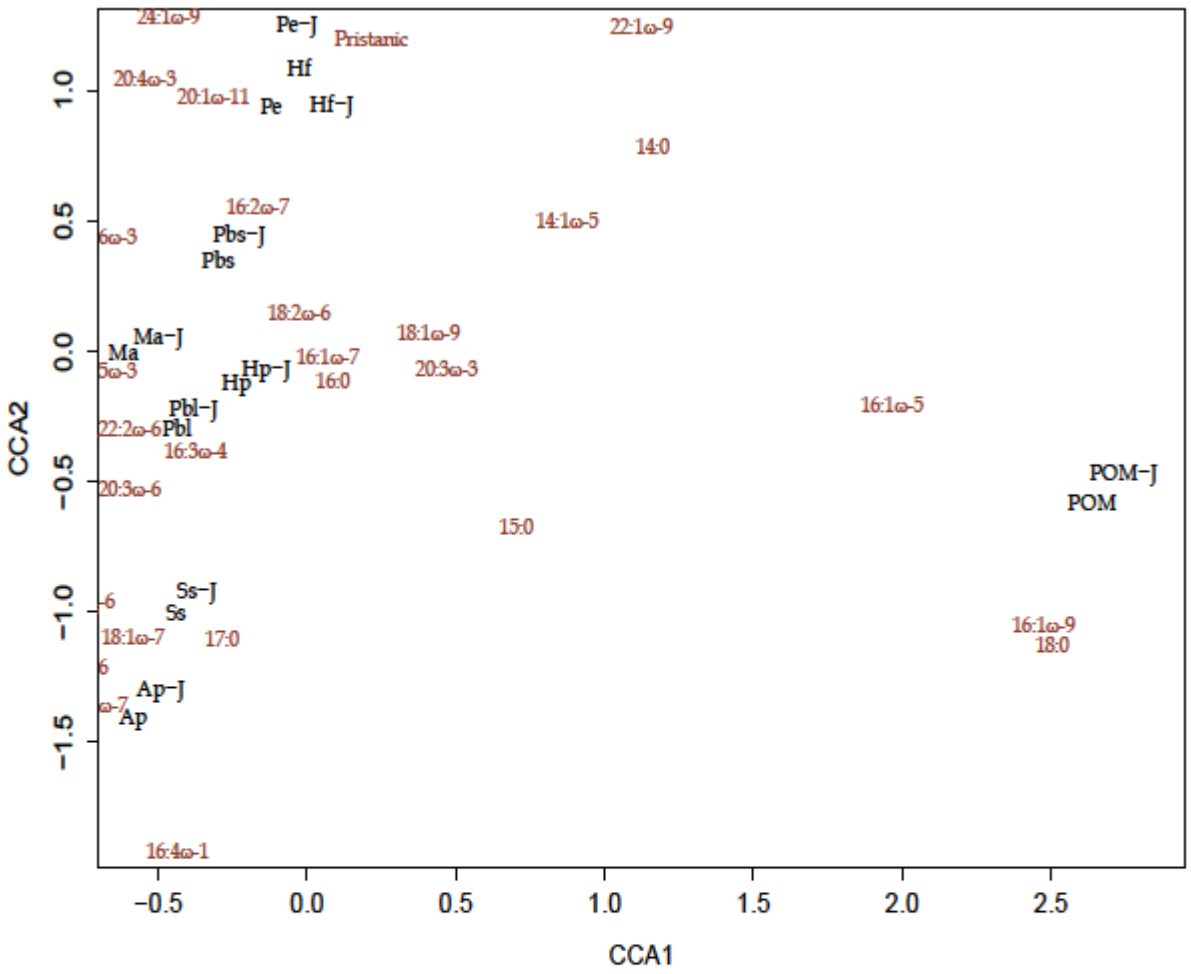

Figure 7. CCA comparing all data. Constraining variables are taxa, depth, and date.

Table 7. ANOVA results of the CCA (fatty acids as a function of taxa, depth, and date) based on 999 free permutations.

Constraining Variable	Df	F-statistic	P-value
Taxa	4	4.45	0.001*
Depth	1	0.84	0.517
Date	1	0.79	0.690
Residual	11		

The bottom water POM was similar between seasons based on fatty acid profiles (figure 6). According to the September CA contribution biplot (figure 8), the highest contributing fatty acids were 18:0, 16:1ω-5, 16:1ω-9, 18:3ω-6, which are biomarkers for detrital material and phytoplankton. In January, the detritus biomarker 18:0 is still the highest contributing fatty acid to the position of the POM sample, with the terrestrial biomarkers 22:0 and 24:0 contributing as well (figure 9).

The contribution biplot with both seasons (figure 10) was useful for distinguishing differences in carbon sources among groups of taxa. The highest contributing fatty acids were 22:6ω-3 and 20.5ω-3 for *Melanogrammus aeglefinus, Pandalus borealis,* and *Hippoglossoides platessoides. Parasagitta elegans* and *Halirages fulvocinctus,* which are strongly correlated with the *Calanus* biomarkers 20:1ω-9 and 22:1ω-11 as well as several phytoplankton biomarkers. In the September biplot (figure 8), the *Calanus* spp. sample is also found close-by. *Arrhis phyllonyx* and *Sabinea septemcarinata* were most strongly correlated with 20:4ω-6, a biomarker for a variety of sources including macroalgae and foraminifera. The MUFA 18:1ω-7, a biomarker for bacteria was also a highly contributing fatty acid (figure 8).

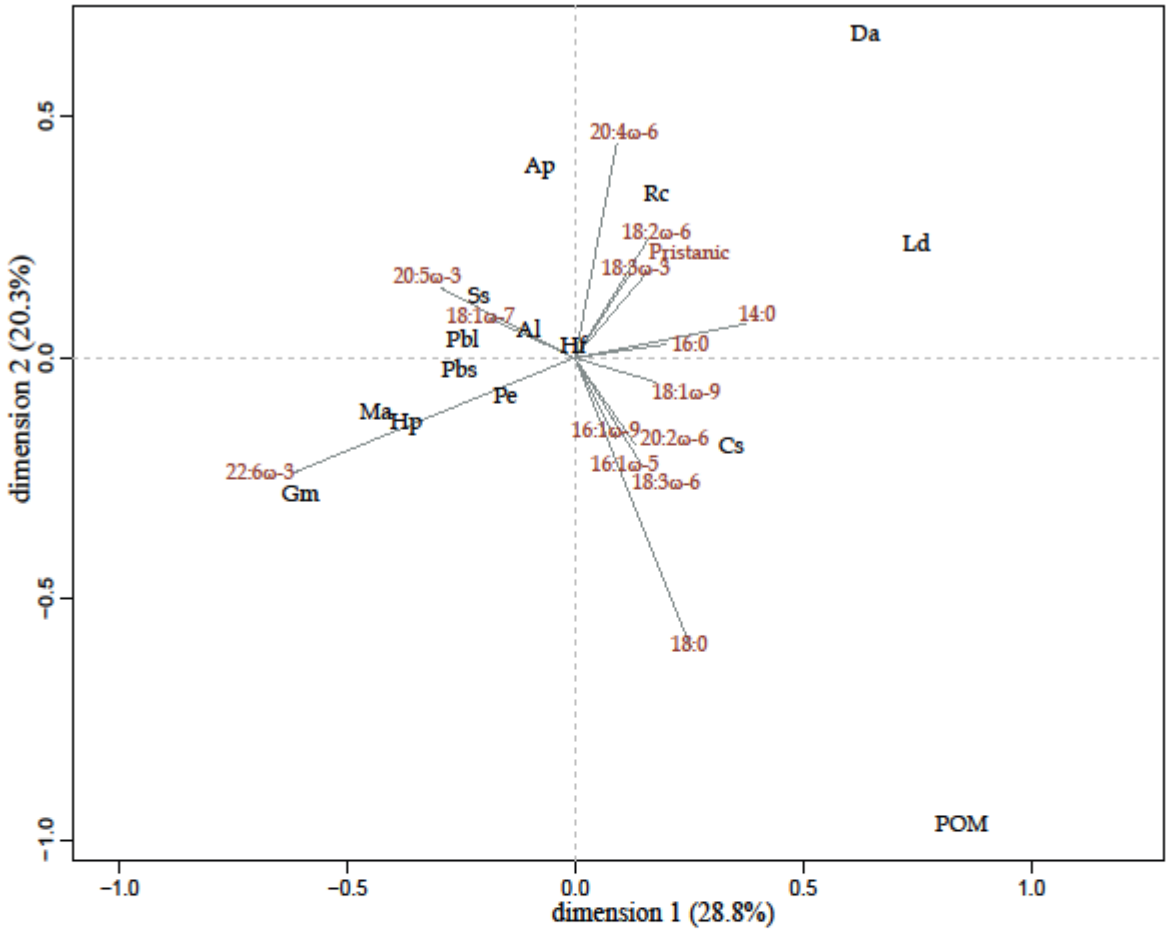

Figure 8. Contribution CA biplot of September data based on all 43 compositional fatty acids. Only the highest contributing fatty acids are shown. Longer segements represent a greater contribution to the ordination. Species abbreviations represent samples taken in January. Specie's identities are found under 'Label' in Table 2. Axis labels indicate the percent variance explained by each axis.

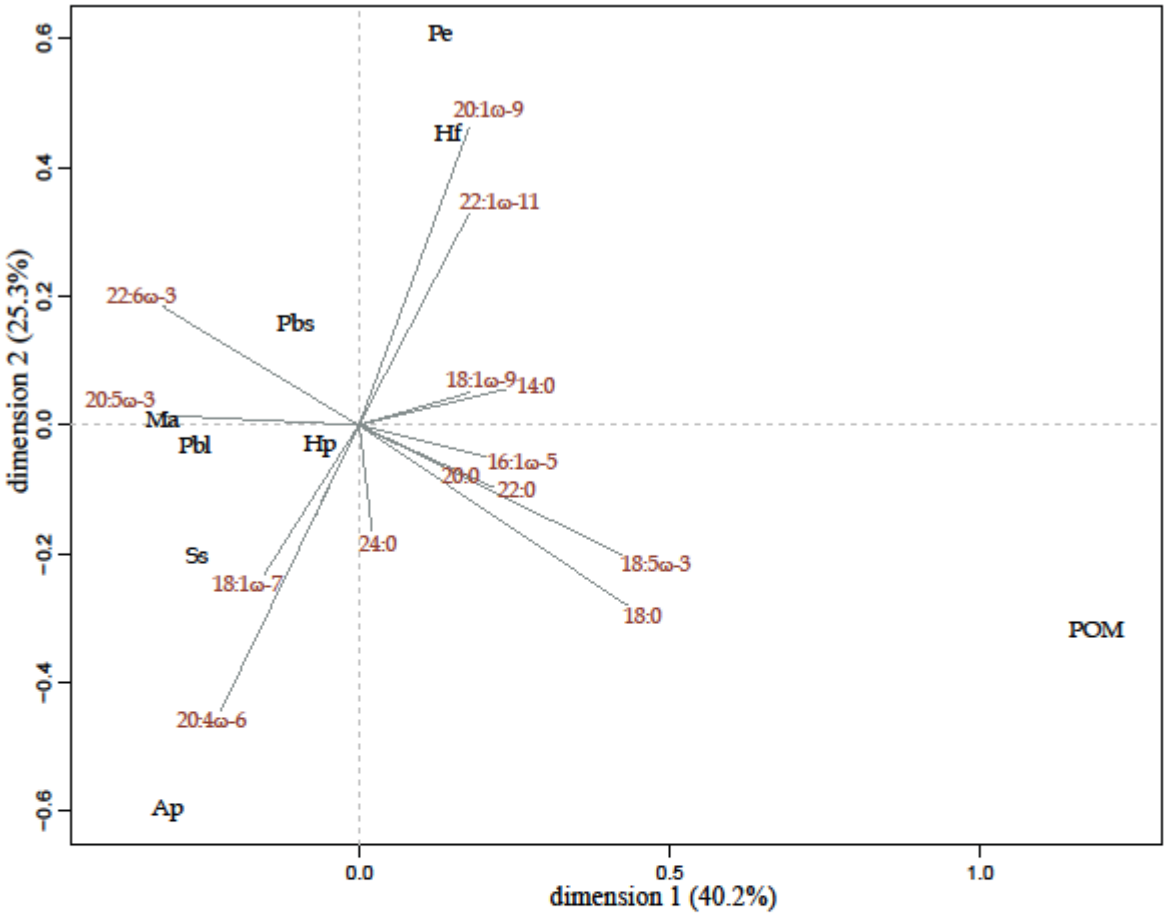

Figure 9. Contribution CA biplot of January data based on all 43 compositional fatty acids. Only the highest contributing fatty acids are shown. Longer segements represent a greater contribution to the ordination. Species abbreviations represent samples taken in January. Specie's identities are found under 'Label' in Table 2. Axis labels indicate the percent variance explained by each axis.

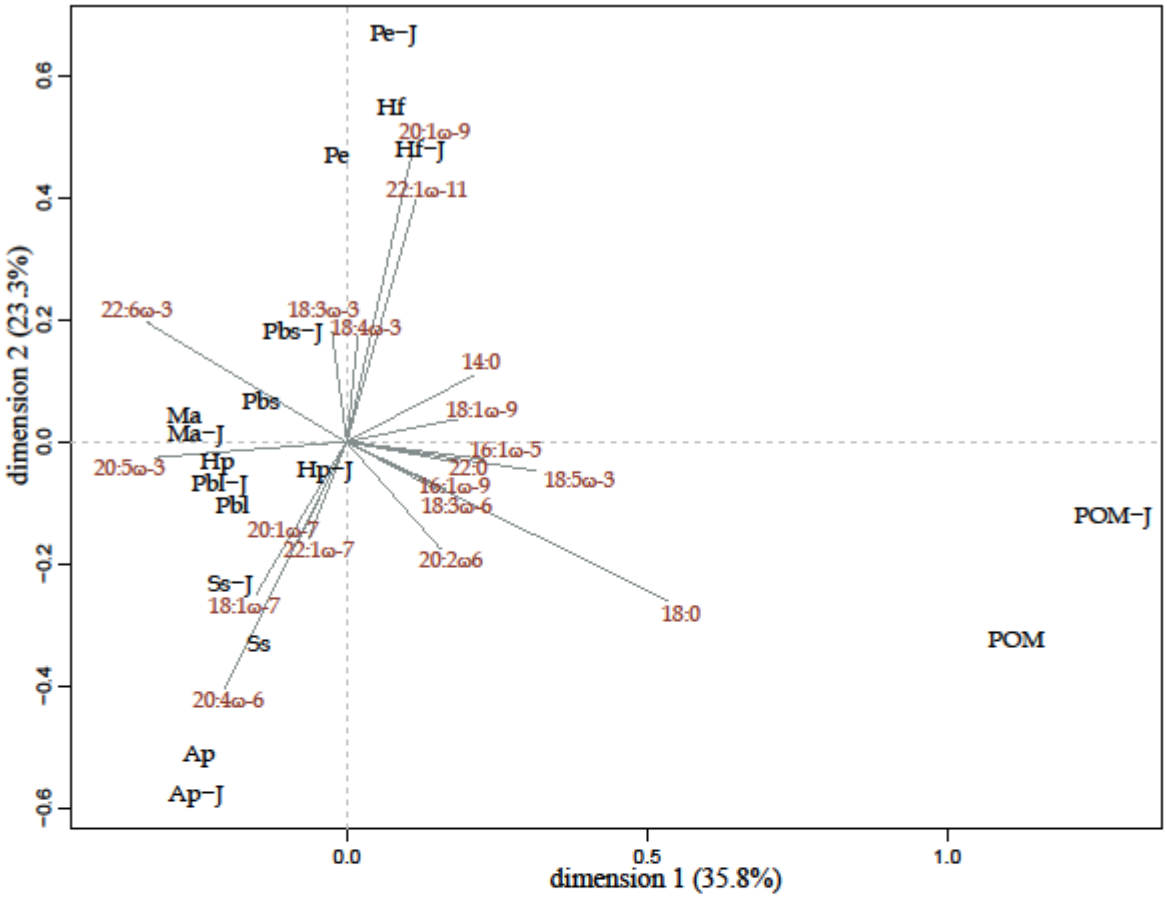

Figure 10. Contribution CA biplot based on 43 compositional fatty acids. Only species collected in both seasons are included. January samples are indicated with a J following the species abbreviation, which are found under 'Label' in Table 2. Fatty acids with a greater than average contribution to the outcome of the CA ordination are shown, with segment lengths signifying the relative degree of contribution. Axis labels indicate the percent variance explained by each axis.

Table 8. Stomach contents of fish species collected with the bottom trawl

Species	Taxa found in Stomach	Number of Individuals	
Gadus morhua		**September**	**January**
	Pandalus borealis	1	1
	Thysanoessa spp.	8	9
	Themisto abyssorum	105	
	Small fish		1
	Polychaeta		2
Melanogrammus aeglefinus			
	Strongylocentrotus sp.	1	
	Fish (12cm and 14cm)	2	
	Polychaeta	3	
	Arrhis phyllonyx	3	
	Pandalus borealis	4	
	Cumacea	11	
	Amphipoda	37	
	Bivalvia	176	
	Thysanoessa spp.		51
	Clione limacina		15
	Meganyctiphanes norvegica.		1

Stomach contents represent totals from five replicates for each species. Individuals were identified to the closest possible taxonomic level. For September samples, individuals of *Gadus morhua* ranged from 39.5-50 cm and 0.79 to 0.93 kg. Individuals of *Melanogrammus aeglefinus* ranged from 42-53cm and 0.71-1.68 kg. January data was collected by Marine Cusa. In January, individuals of *Gadus morhua* ranged from 19.5-25cm and 0.05 to 0.10 kg. Individuals of *Melanogrammus aeglefinus* ranged from 14.6-24.0 cm and 0.02-0.12 kg.

Analysis of stomach contents of *Melanogrammus aeglefinus* and *Gadus morhua* demonstrate the importance of the euphausiid *Thysanoessa* spp. for both of these fish species. A variety of other taxa were identified including the pelagic amphipod *Themisto abyssorum*, which filled several stomachs of *Gadus morhua* in September, as well as a variety of benthic species and amphipods for *Melanogrammus aeglefinus* (table 8).

Levels of the fatty acid 22:6ω-3 were particularly high in the fish samples, representing 43% of total fatty acids in *Gadus morhua*. Meanwhile, levels were quite low in bottom water

POM samples at 1% of total fatty acids in both season (figure 11). The fatty acid 20:5ω-3, known to be in high concentrations in diatoms (Dalsgaard *et al.*, 2003; Nelson *et al.*, 2001) was high in all biological samples, constituting 10-25% of total fatty acids. Levels were quite low in bottom water POM samples in both seasons (2% of total fatty acids in September and 1% in January). Levels were highest in *Sabinea septemcarinata* at 20% and 25% in September and January as well as *Pandalus borealis,* at 21% and 22% in September and January (figure 12).

The ratio of 22:6ω-3 to 20:5ω-3, an indicator of fish health, was highest in fish samples at 3.2 for *Gadus morhua* in September, and 1.6 and 1.9 for *Melanogrammus aeglefinus* in September and January, respectively (figure 13). Flagellates, including dinoflagellates, are known to contain high levels of 22:6ω-3 (Sargent *et al.*, 1987; Falk-Petersen *et al.*, 1998; Kelley and Scheibling, 2012). However, this omega-three fatty acid was also highly correlated with $\delta^{15}N$ levels, explaining 60% and 70% of the variance in $\delta^{15}N$ in January and September respectively (figure 14). Therefore, it may accumulate through the food chain, and the high percentages observed in the fish samples may be because fish feed at higher trophic levels. Analysis of other dinoflagellate and flagellate biomarkers, however, reveals a similar trend. Falk-Petersen *et al.* (1998) found high levels of C18 PUFA in addition to 22:6ω-3 to indicate dinoflagellate and or flagellate consumption. *Gadus morhua* once again, had the highest levels of these fatty acids while bottom water POM and *Arrhis phyllonyx* had the lowest levels (figure 15).

Levels of the diatom biomarker (∑ 16:1ω-7, C16 PUFA, 20:5ω-3; Dalsgaard *et al.*, 2003) were highest in *Sabinea septemcarinata,* totaling almost 30% of total fatty acids in both seasons. This biomarker was lowest in the bottom water POM sample and in *Gadus morhua* (figure 16). Meanwhile, the ratio of 20:5ω-3 to 22:6ω-3, another diatom biomarker (Nelson *et al.*, 2001) was also highest in *Sabinea septemcarinata* as well as *Arrhis phyllonyx*, which both had higher ratios in September than in January (figure 17).

Interestingly, the copepod biomarker ∑20:1 + 22:1 was higher in several species than in the *Calanus* spp. sample, for which is made up 10% of total fatty acids. This biomarker was clearly highest in *Halirages fulvocinctus* and *Parasagitta elegans,* with values as high as 23 % of total fatty acids. It was lowest in *Gadus morhua* and the large size fraction of *Pandalus borealis,* for which it only contributed to about 1.5% of total fatty acids (figure 18). *Parasagitta elegans* and *Halirages fulvocinctus* both had high levels of the carnivory biomarker 18:1ω-9/18:1ω-7. *Parasagitta elegans* had a ratio of 6.3 in September and *Halirages fulvocinctus* had a ratio of 7.3

in January. *Andaniexis lupus* also had high a high ratio of 3.2 in September. The *Calanus* spp. sample had a ratio of 5.4 in September. Interestingly, the bottom water POM had a ratio of 9.2 in September and 5.6 in January (figure 19).

Levels of 20:4ω-6 were three times higher in *Arrhis phyllonyx* than other taxa (figure 20). *Arrhis phyllonyx* was also highest in sum (ω-6 PUFA) / sum (PUFA), a biomarker for macroalgae (figure 21), but this may be due to its high levels of 20:4ω-6 relative to other taxa.

Gadus morhua and *Melanogrammus aeglefinus* had very high levels of PUFA at 62%, and 59% respectively. PUFA levels constituted greater than 20% of total fatty acids in all biological samples and ranged from 11% to 13% in September and January for the bottom water POM samples (figure 22). Levels of ω-3 PUFA, an indicator for high quality food (Dalsgaard *et al.*, 2003) were highest in the fish species in September and were lowest in the bottom water POM in both seasons (figure 23).

The bacteria biomarker, which consists of all odd-numbered, iso- and anteiso-branched SFA and MUFA as well as 18:1ω-7 (Sargent *et al.*, 1987; Dalsgaard *et al.*, 2003), was highest in *Sabinea septemcarinata* and *Arrhis phyllonyx*. Percent of total fatty acids totaled 3.4% in September and 2.9% in January, and 2.6% in September and 2.5% in January for these species, respectively. The bottom water POM sample also had high levels of this biomarker in January at 2.2% of total fatty acids and the flatfish *Hippoglossoides platessoides* also had higher levels in January at 2.8% of total fatty acids (figure 24). A biomarker for aerobic bacteria (16:1ω-7 + 18:1ω-7; Guckert *et al.*, 1985) was highest in *Arrhis phyllonyx*, contributing almost 20% to the total fatty acids. Interestingly, many of the samples have a higher level of this biomarker in January than in September (figure 25). A biomarker for anaerobic bacteria (C15 + C17; Guckert *et al.*, 1985) was highest in *Sabinea septemcarinata*, *Arrhis phyllonyx*, bottom water POM, and the January sample of *Hippoglossoides platessoides* (figure 26).

The detritus biomarker 18:0 + 18:1ω-9 was highest in the bottom water POM samples representing 38.8% of total fatty acids in September and 33.7 % in January (figure 27). The terrestrial vegetation biomarker was highest in the bottom water POM sample and in *Arrhis phyllonyx* (figure 28).

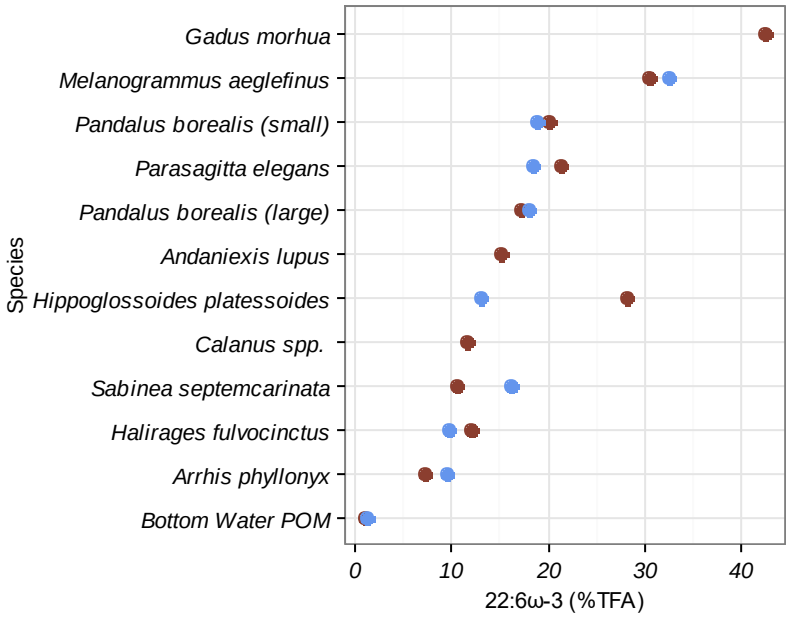

Figure 11. Levels of the fatty acid 22:6ω-3 in each species as percent total fatty acid. September samples are in red and January samples are in blue.

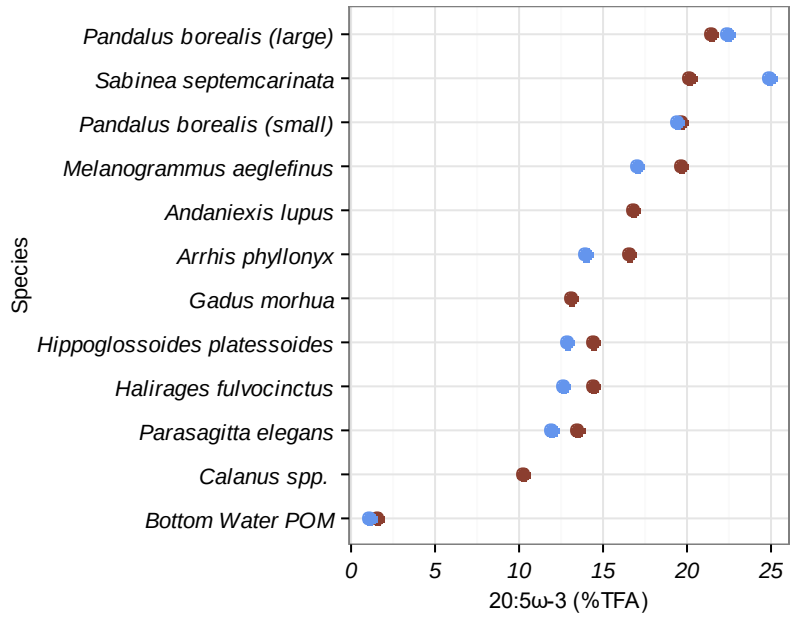

Figure 12. Levels of the fatty acid 20:5ω-3 in each species as percent total fatty acid. September samples are in red and January samples are in blue.

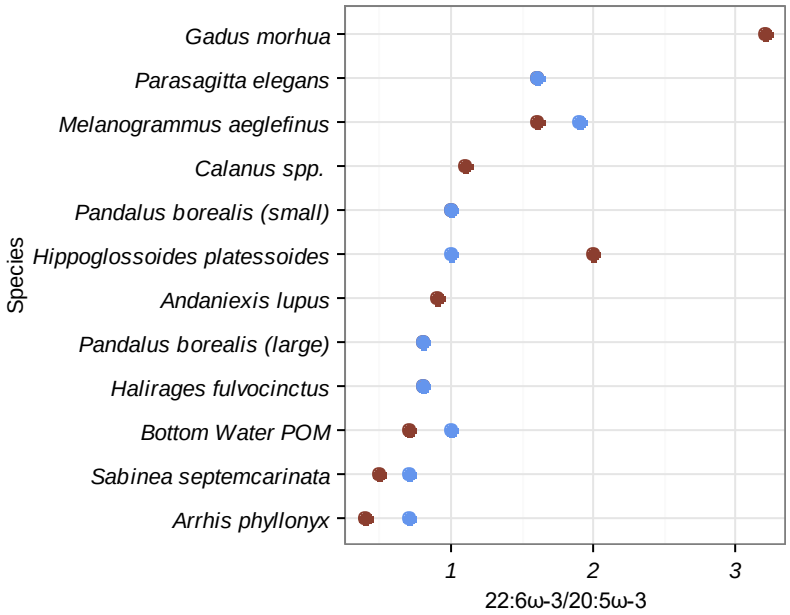

Figure 13. Levels of the ratio of the two omega three fatty acids 22:6ω-3 and 20:5ω-3 in each species. September samples are in red and January samples are in blue.

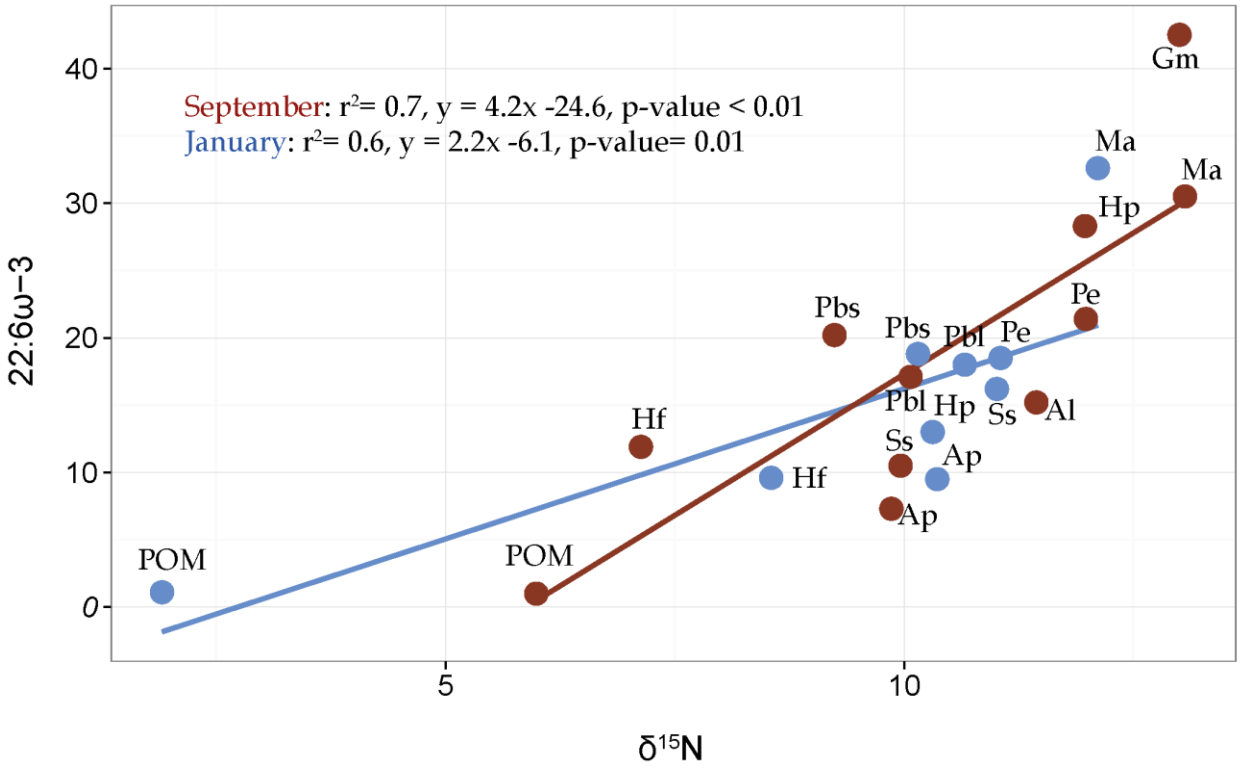

Figure 14. The relationship between 22:6ω-3 and $\delta^{15}N$. Red= September, Blue= January.

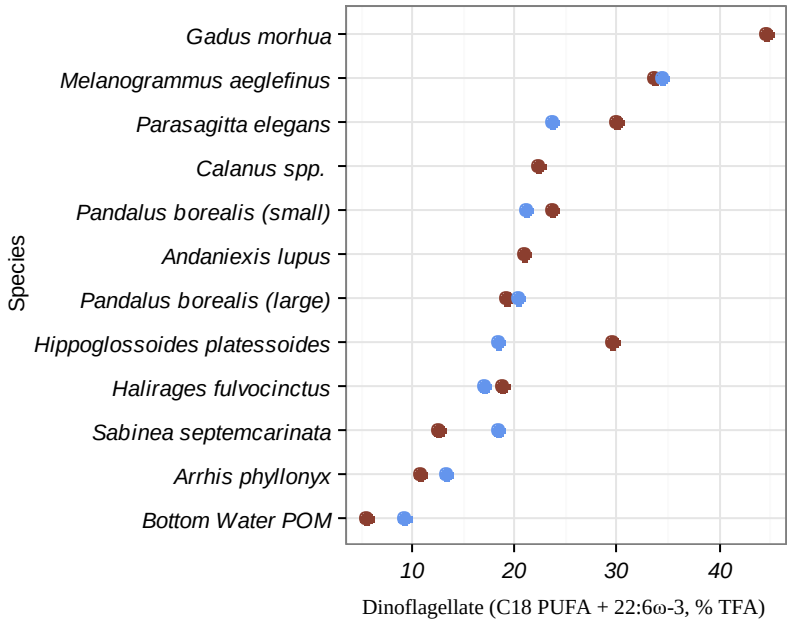

Figure 15. Levels of dinoflagellate biomarker (sum of all C18 PUFA and 22:6ω-3) as percent total fatty acid for each species. September samples are in red and January samples are in blue.

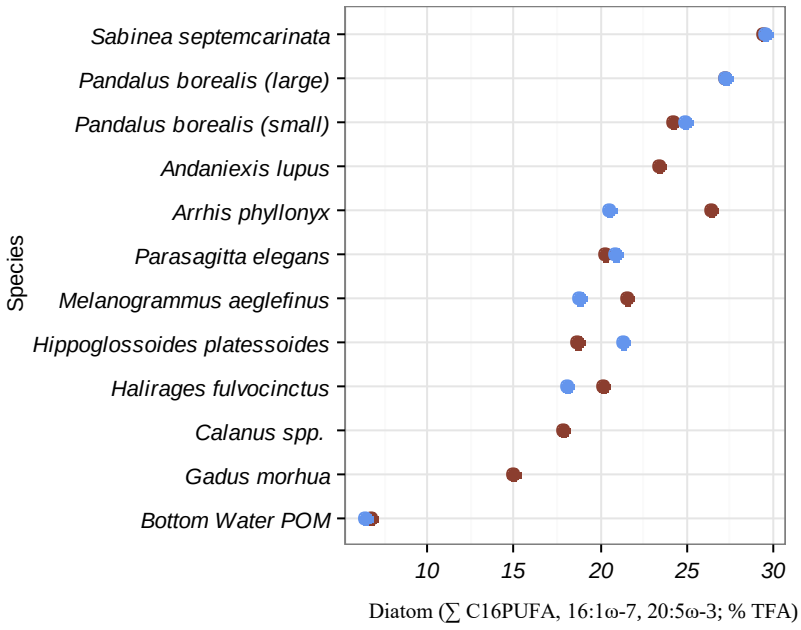

Figure 16. Levels of the diatom biomarker (∑ 16:1ω-7, 16:2ω-7, 16:3ω-4, 16:4ω-1, 20:5ω-3) as percent total fatty acid in each species. September samples are in red and January samples are in blue.

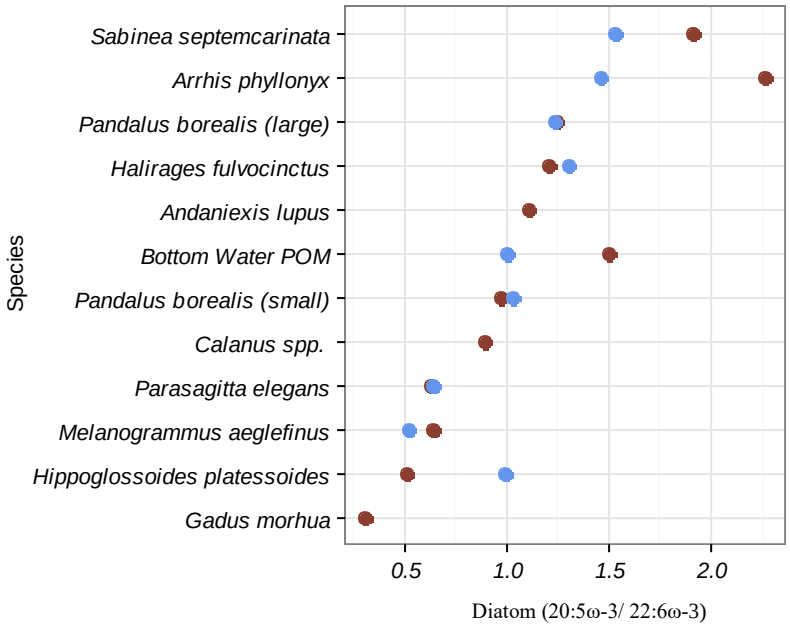

Figure 17. Levels of the diatom biomarker (20:5ω-3/ 22:6ω-3) in each species. September samples are in red and January samples are in blue.

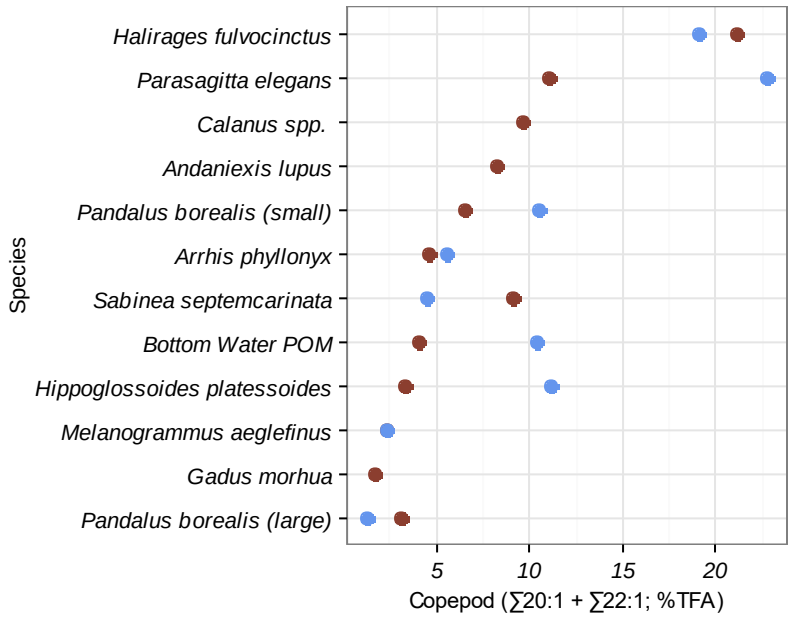

Figure 18. Levels of the copepod biomarker (sum 20:1 + sum 22:1) in each species. September samples are in red and January samples are in blue.

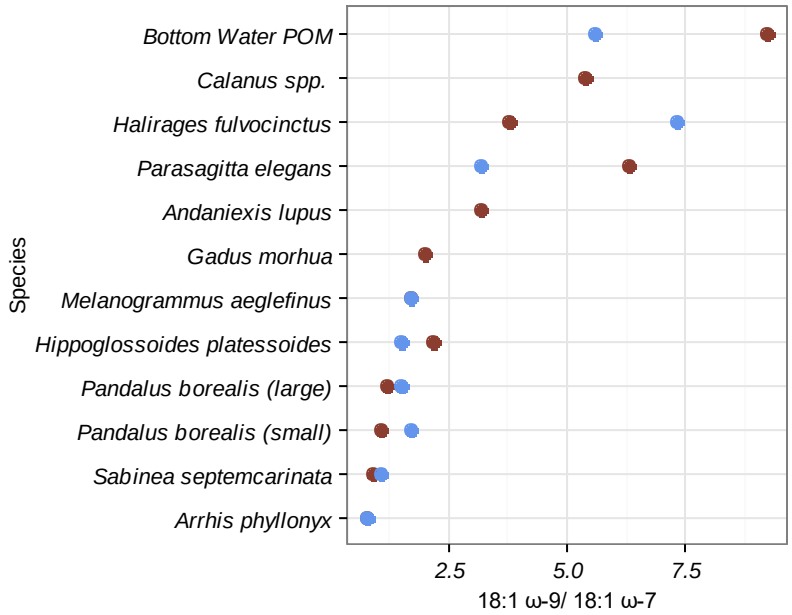

Figure 19. Levels of the biomarker for carnivorous feeding (ratio of 18:1 ω-9/ 18:1 ω-7) in each species. September samples are in red and January samples are in blue.

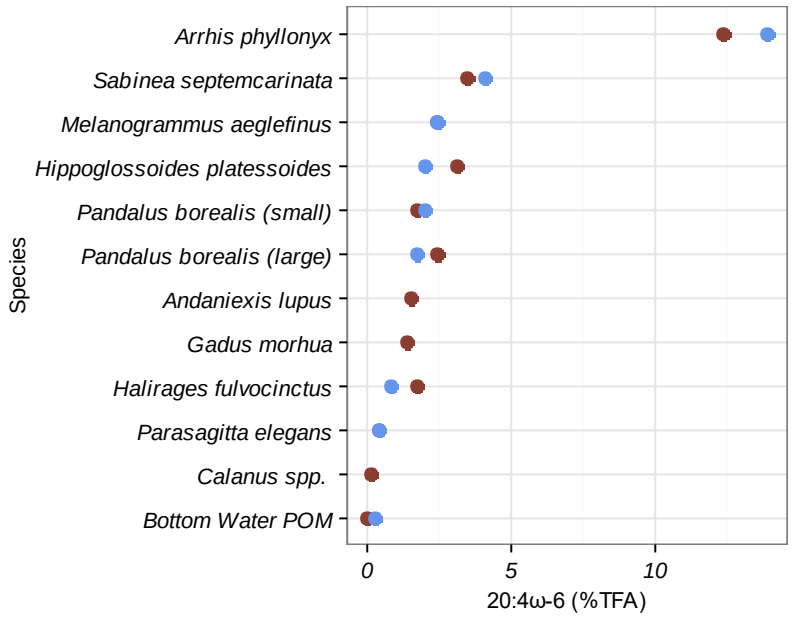

Figure 20. Levels of the macroalgal biomarker (20:4ω-6) for each species in percent total fatty acid. September samples are in red and January samples are in blue.

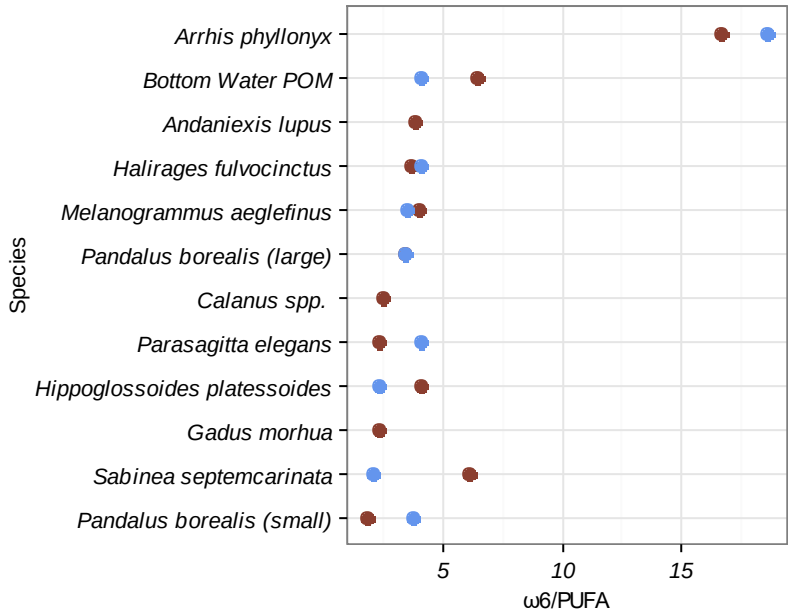

Figure 21. Levels of the macroalgal biomarker (ratio of Omega-6 fatty acids to total PUFA) in each species. September samples are in red and January samples are in blue.

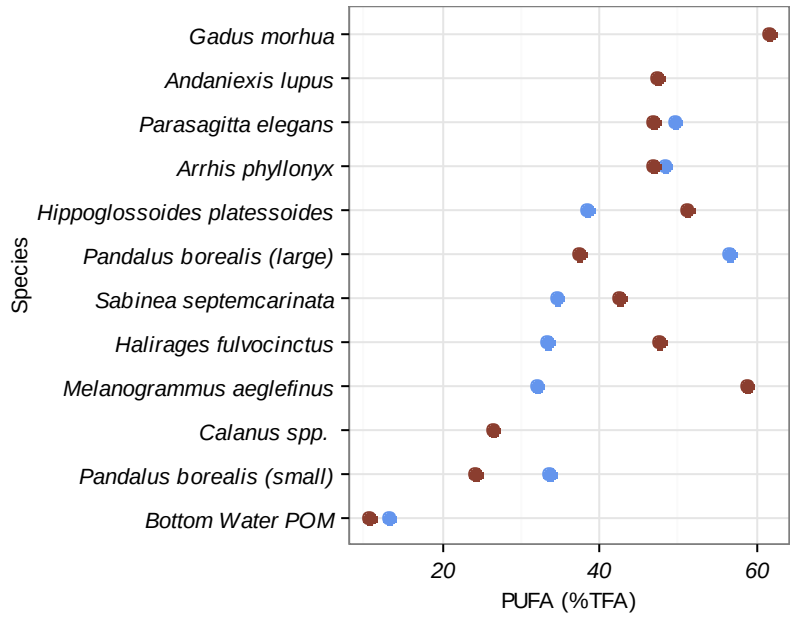

Figure 22. Levels of total PUFA in all samples in percent of total fatty acid. September samples are in red and January samples are in blue.

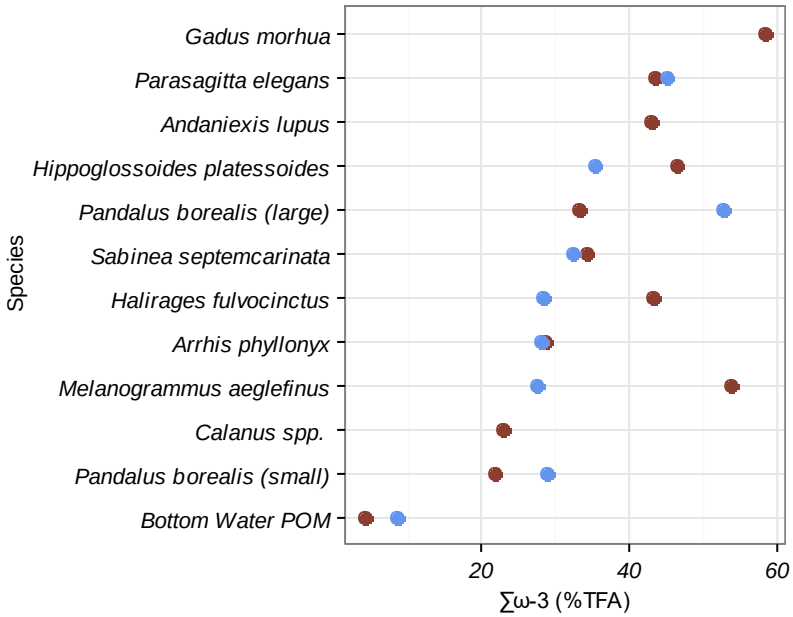

Figure 23. Levels of the food-quality indicator (Omega-3) in each species in percent total fatty acid. September samples are in red and January samples are in blue.

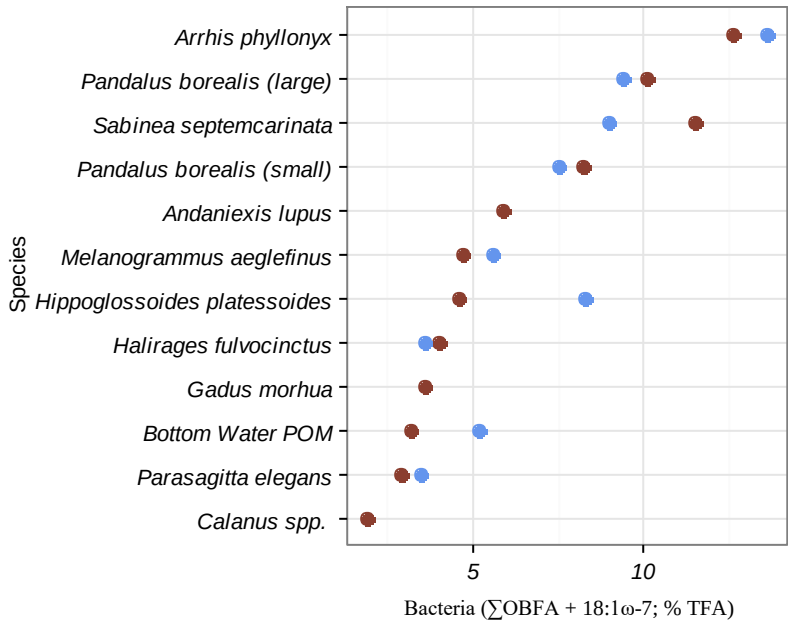

Figure 24. Levels of the bacteria biomarker (∑OBFA + 18:1ω-7) as percent total fatty acid in each species. September samples are in red and January samples are in blue.

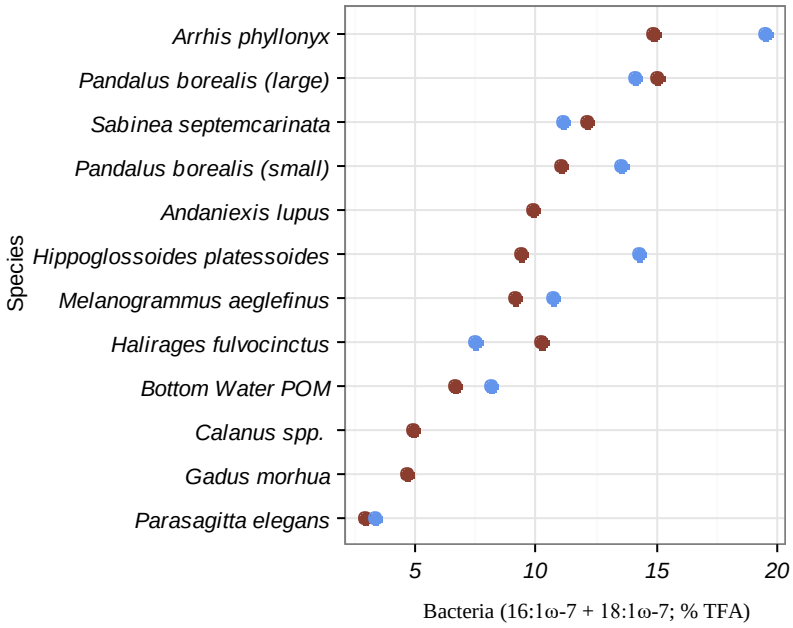

Figure 25. Levels of the biomarker for bacteria (16:1ω-7 + 18:1ω-7) as percent total fatty acid in each species. September samples are in red and January samples are in blue.

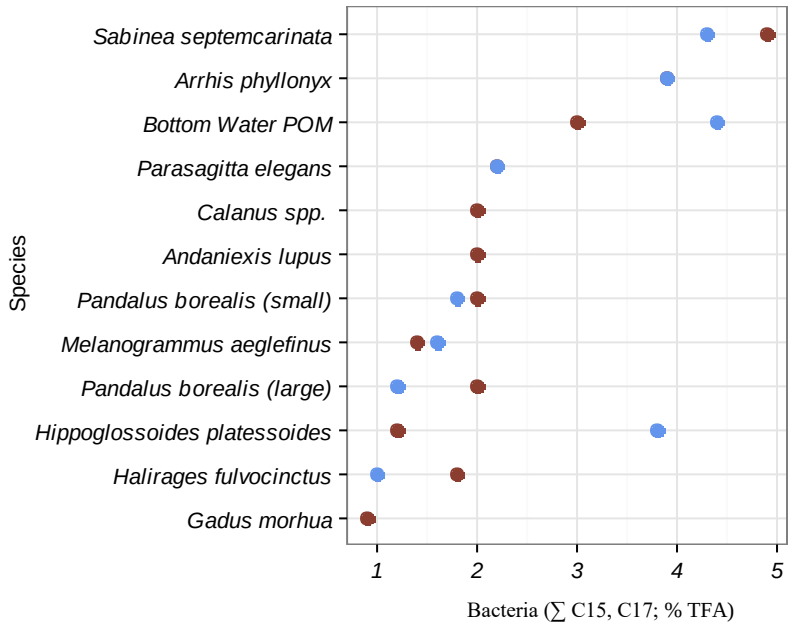

Figure 26. Levels of the biomarker for bacteria (C15 + C17) as percent total fatty acid in each species. September samples are in red and January samples are in blue.

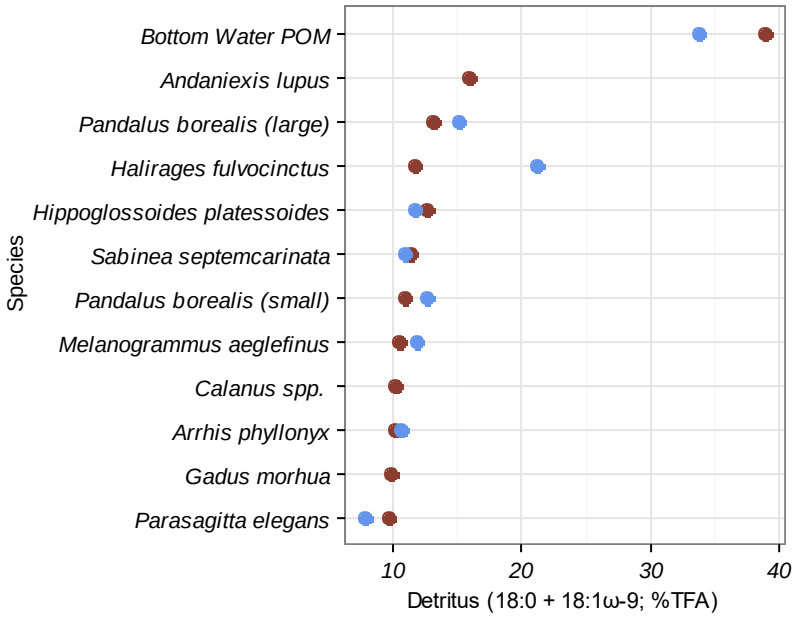

Figure 27. Levels of the detritus biomarker (18:0 + 18:1ω-9) as percent of total fatty acid in each species. September samples are in red and January samples are in blue.

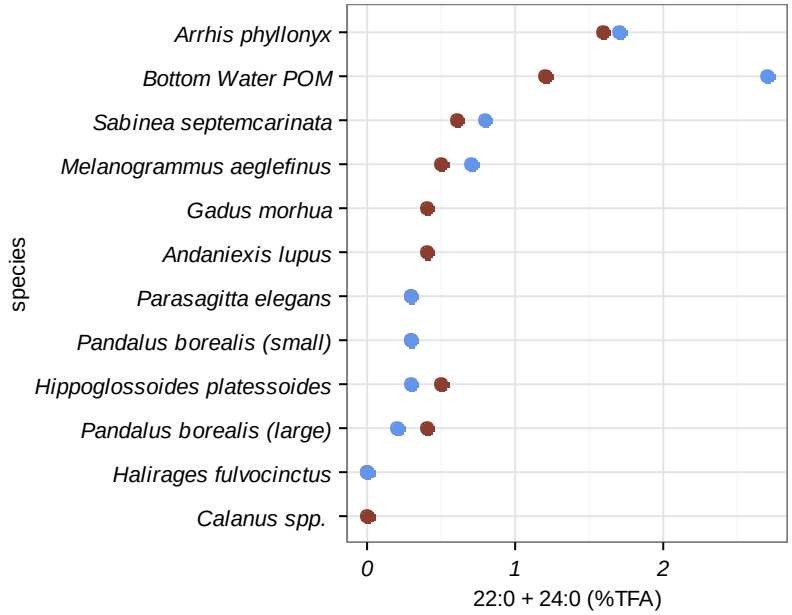

Figure 28. Levels of the terrestrial material biomarker (22:0 +24:0) as percent of total fatty acid in each species. September samples are in red and January samples are in blue.

4. Discussion

4.1 Stable Isotopes

4.1.1 $\delta^{15}N$

The $\delta^{15}N$ signatures are useful for estimating the trophic level of consumers, as tissues are predictably enriched in $\delta^{15}N$ by 3–4‰ relative to diet (Peterson and Fry, 1987). In order to compare trophic levels and carbon flow among the hyperbenthic community, the isotopic values for nitrogen and carbon at the base of the food web are needed. In this study, bottom water POM was the only sample collected that could represent the base of the food chain. In September, the mean value of $\delta^{15}N$ for POM was 5.9‰ while in January it was 1.9‰ (figure 4). Meanwhile, the $\delta^{13}C$ values for POM changed from a mean of -22.9‰ in September to 24.0‰ in January (figure 5). The combination of the two stable isotopes for POM in relation to the biological samples (figure 3) demonstrates that POM does not adequately represent the source of carbon for the majority of the species, especially in January. This is further supported by the outcome of the CA contribution biplots, where POM samples are opposed to all biological samples on at least one axis (figure 10). Therefore, rather than use the isotopic values of POM for the trophic level calculation, I used the values for *Thysanoessa inermis* as the base. This assumes that all other species are supported by the same base food source as *Thysanoessa inermis*, which is pelagic phytoplankton. While this assumption may not hold true for *Sabinea septemcarinata*, *Hipploglossoides platessoides*, or *Arrhis phyllonyx (*discussed below), most of the species collected do appear to rely on pelagic production for both seasons.

Thysanoessa inermis is a well-studied euphausiid (Einarsson, 1945; Dalpadado and Skjoldal, 1996; Falk-Petersen and Hopkins, 1981; Falk-Petersen *et al.*, 1981). Formerly, based on lipids, *Thysanoessa inermis* was believed to be a true herbivore (Falk-Petersen *et al.*, 2000). However, recent studies using stable isotopes have determined that it also feeds on copepods and is an omnivore with a trophic level ranging from 2.5-2.7 (Petursdottir *et al.*, 2012; Søreide *et al.*, 2006a; Tamelander *et al.*, 2006b). Primary consumers, such as *Calanus glacialis*, have been used in other studies for trophic level calculation. They are known to be generally less variable in $\delta^{13}C$ and $\delta^{15}N$ than primary producers and can represent a temporally and spatially integrated signal of the primary producers in the system (Post, 2002; Iken *et al.*, 2010; McMeans *et al.*, 2013; Renaud

et al., 2015). While *Thysanoessa inermis* is not a true herbivore, and may therefore not be the most reliable source, the $\delta^{15}N$ values found are consistent between seasons and align with other studies (Petursdottir *et al.*, 2012; Søreide *et al.*, 2006a; Tamelander *et al.*, 2006b). This decision is further supported by the high abundances of *Thysanoessa* spp. in stomach contents analyzed in this study.

Based on these calculations, in September, trophic levels ranged from 0.9 for *Halirages fulvocinctus* to 3.9 for *Melanogrammus aeglefinus* (table 4). In January, they ranged from 2.7 for *Halirages fulvocinctus* to 3.7 for *Melanogrammus aeglefinus* (table 5). This suggests that the hyperbenthic community in Kongsfjord feeds within a relatively narrow range of trophic levels, representing more of a 'trophic continuum' (Pahl-Wostl, 1997), than a classic linear food chain.

Interestingly, 10 out of the 11 crustaceans collected were more enriched in $\delta^{15}N$ in January than in September (figure 4), a trend that contributed to increased calculated trophic levels. The increase in $\delta^{15}N$ could be the result of several factors. First of all, it could be due to starvation. Ponsard and Averbuch (1999) found that levels of $\delta^{15}N$ in consumer tissue increased during starvation due to isotopic fractionation during protein catabolism with the excretion of isotopically lighter nitrogen. However, high $\delta^{15}N$ signatures have also been attributed to increased consumption of refractory material (Iken *et al.*, 2010). Increases in $\delta^{15}N$ have been noted in sinking phytoplankton due to microbial degradation of chemical compounds, a process that is enhanced with increased time in the water column (Levinton, 1972; Altabet and McCarthy, 1985; Hansen and Josefson, 2004).

Calculated trophic levels reflect the $\delta^{15}N$ enrichment in January. According to a trophic model devised for the European Arctic, pelagic carnivores fed between TL 2.9 and 3.3 (Søreide *et al.*, 2006a). Based on this model, results of this study suggest that many of these hyperbenthic species may resort to a more carnivorous diet during the winter months. This may make sense biologically, as consumers could shift towards carnivory and detrital-based food webs when fresh phytoplankton is unavailable. Therefore, crustaceans may be relying, to a greater degree, on reworked carbon sources in January relative to September. This would lead to a greater contribution of bacteria to their diet in January, resulting in the enriched $\delta^{15}N$ signatures observed here.

4.1.2 $\delta^{13}C$

The $\delta^{13}C$ signatures change little (0.6 - 1‰) as carbon moves through marine food webs. These isotopic signatures can therefore provide information about an organism's major carbon sources, as long as the available sources have distinct $\delta^{13}C$ signatures (Søreide *et al.*, 2006a). This study revealed similar $\delta^{13}C$ signatures in all taxa in both seasons, ranging from -19.3‰ to -24.4‰. This range of $\delta^{13}C$ is consistent with a general depletion of $\delta^{13}C$ in pelagic food webs with increasing latitude in the northern hemisphere (Dunton and Schell, 1987). However, $\delta^{13}C$ values of hyperbenthic organisms in this study are quite different from the enriched values found for the benthic community in Kongsfjord in previous years (Renaud *et al.*, 2011 found $\delta^{13}C$ values for benthic consumers to range between -20.2‰ and -16.9‰ in October and July). It is difficult to compare values between studies due to the use of different laboratory techniques for stable isotope analysis. However, the differences noted here are quite extreme, and likely not due solely to methodological reasons. In fact, studies on both higher and lower level consumers have observed $\delta^{13}C$ to vary due to differential reliance on pelagic versus benthic prey. A heavy reliance on pelagic primary production by consumers has resulted in less $\delta^{13}C$ enrichment in consumer tissues compared with reliance on detrital-based benthic food sources (Hobson *et al.*, 1995; Hobson *et al.*, 2002).

Findings suggest that the hyperbenthic community sampled here is more tightly linked to pelagic than benthic food sources, especially in September. This is particularly evident for the highly motile fish and invertebrate species that can migrate into the pelagic. Among these, *Syrrhoe crenulata,* and *Thysanoessa inermis,* were only collected for stable isotopes. These species seem to be tightly linked to pelagic production in both seasons. There is reason to propose, however, that part of the hyperbenthic community sampled in this study may be supported to a greater degree by detrital material in both seasons. Organic matter available to the benthos is often recycled by bacterial and meiofaunal groups before ingestion by detritivores. This re-processing has been known to raise carbon isotopic ratios considerably (Hobson *et al.*, 1995; Mincks *et al.*, 2008). *Hippoglossoides platessoides, Arrhis phyllonyx, Ancanthostepheia malmgreni, Lebbeus polaris,* the large size-fraction of *Pandalus borealis,* and *Sabinea septemcarinata*, with more enriched values of $\delta^{13}C$, seem to be more reliant on benthic resources

than the rest of the community in both September and in January. The mysid *Erythrops erythropthalma*, and the amphipod *Andaniexis lupus,* however, seem to fall somewhere in between with a reliance on both pelagic and benthic sources.

There were two species, collected only for stable isotope analyses, which demonstrate a small trend in stable isotope signatures based on position within the fjord. *Boreogadus saida* and *Leptoclinus maculatus* were more enriched in both $\delta^{13}C$ and $\delta^{15}N$ at the inner station. Stomach contents of *Boreogadus saida* in the inner basin of Kongsfjord in January contained high abundances of copepods and fish, especially *Leptoclinus maculatus,* in demersal individuals (Eglund-Newby, 2015). *Leptoclinus maculatus* is known to feed on copepods and other small zooplankton (Murzina *et al.*, 2013). While their immediate prey may not change between seasons, the original carbon source may have (i.e the zooplankton they consume may rely more on flagellates and bacteria in the inner fjord). These trends are likely a result of the water body dynamics within the fjord. All of these samples were collected in January, when primary production is restricted by lack of sunlight. These results may indicate that individuals in the outer and middle fjord may benefit from food brought by the West Spitzbergen current while individuals in the inner fjord, which is more isolated, may have a diet based to a greater degree on microbial/detrital food sources during the dark winter months. However, these suggestions are highly speculative, and there is simply not enough data to draw a confident conclusion on the spatial feeding differences in these two species.

Stable isotope results reflect the importance of pelagic phytoplankton as well as reworked detrital material in fueling the hyperbenthic community in Kongsfjord in September and January. Conclusions about food source, however, are not well supported by isotopic data alone, and fatty-acid signatures are required for a more thorough analysis.

4.2 Fatty acids

A mere 8 fatty acids made up over half of the total fatty acid concentration in all samples in both seasons. These dominant fatty acids included the short-chain saturates 16:0 and 18:0, the monoenes 16:1ω7, 18:1ω9 and 18:1ω7, and the long-chain PUFA 20:4ω6, 20:5ω3 and 22:6ω3 (tables 4 and 5). POM samples were strongly characterized by the biomarker 18:0 (figure 10) in both seasons. High levels of 18:0 and other saturated fatty acids have been found in post-bloom POM samples throughout the water column (Mayzaud *et al.,* 2013b). It is therefore

associated with 'post-bloom' conditions and has been used as a 'detritus' biomarker (Søreide et al., 2008). Since the POM sample is found opposed to all other samples on the primary axis in the CA contribution biplot (figure 10), we conclude that this does not represent a baseline food source for the organisms sampled. However, this does not indicate that detritus was not a source of carbon for these invertebrates, but rather that detrital biomarkers are not very conclusive. 'Detritus' is an ambiguous term used for decaying organic matter, which can come from many different original sources and is difficult to sample directly. However, the presence of bacterial biomarkers, as well as omega-3 PUFA levels may provide another indication of the presence of detritus. High levels of omega 3- PUFA are connected to high quality phytoplankton material and are typically high early in a bloom event and decrease at the end of the bloom (Dalsgaard *et al.*, 2003). High levels of bacterial biomarkers in an individual's tissue, meanwhile, would indicate a reliance on bacteria or detritus. Results of this study show a decrease in PUFA levels in January (figure 22) for at least part of the hyperbenthic community, and an increase in bacterial biomarkers in January for several species (figures 24 and 25). These observations suggest the importance of detritus for several species in January.

4.2.1 Dinoflagellates vs. Diatoms

The three PUFA (22:6ω3, 20:5ω3, and 20:4ω6) are essential nutrients in marine food webs (Alkanani *et al.*, 2005). The fish species *Melanogrammus aeglefinus,* and *Hippoglossoides platessoides,* as well as both size classes of *Pandalus borealis,* are tightly grouped in the CA ordination (figure 10) and had high contributions of these essential fatty acids. Levels of 22:6ω-3, a biomarker for dinoflagellates (Falk-Petersen *et al.,* 1998), were particularly high in the fish samples, representing over 40% of total fatty acids in *Gadus morhua* (figure 11). Levels of 20:5ω3, a biomarker for diatoms (Dalsgaard *et al.*, 2003), made up more than 20% of the total fatty acids in the large size fraction of *Pandalus borealis* and *Sabinea septemcarinata* (figure 12). The ratio of 22:6ω3 to 20:5ω3 is considered to be important in the nutrition of marine fish, with values of 2 or higher being desirable (Copeman *et al.*, 2002; Dwyer *et al.*, 2003). However, this ratio can also be used to distinguish between the relative importance of dinoflagellates and diatoms in the diet. A value greater than one indicates the dominance of dinoflagellates, while a value below one suggests the importance of diatoms (Parrish *et al.*, 2009). This ratio varied

greatly among taxa. *Gadus morhua*, an apparently quite healthy fish in Kongsfjord, had a ratio greater than 3 in September. *Parasagitta elegans*, *Melanogrammus aeglefinus*, *Calanus* spp., and the September sample of *Hippoglossoides platessoides* also had ratios greater than one. The rest of the samples all had ratios less than one, with *Sabinea septemcarinata* and *Arrhis phyllonyx* having the smallest values (figure 13). While this suggests the importance of dinoflagellates for the fish and chaetognath, it is important to note that 22:6ω-3 was also highly correlated with $\delta^{15}N$ levels, explaining 60% and 70% of the variance in $\delta^{15}N$ in January and September respectively (figure 14). This suggests a 'trophic magnification' of 22:6ω-3 within the food web. This trend was also found for the hyperbenthic community in the Beaufort Sea (Connelly *et al.*, 2014) and in Newfoundland's Conception Bay (Parrish *et al.*, 2009).

However, there are other indications of a division within the hyperbenthic community based on differences in dinoflagellate and diatom consumption that may go beyond the differences in trophic level. There are several biomarkers for dinoflagellates and flagellates in the literature (table 3). While it is true that most of them include 22:6ω-3 as an indication of dinoflagellates, C18PUFA, 18:1ω-7, 16:0 and 18:4ω-3 are also important. Falk-Petersen *et al.*, (1998) found that samples taken from the pelagic zone in the Barents Sea, which were rich in flagellates, contained high levels of C18PUFA in addition to 22:6ω-3. A recent study in the Svalbard region found the POM samples rich in *Phaeocystis pouchetii* were also distinguished by high levels of C18PUFA and 22:6ω-3 (Søreide *et al.*, 2013). These fatty acids, therefore, are useful biomarkers for dinoflagellates and flagellates, including *Phaeocystis pouchetii.* In this study, *Gadus morhua*, *Melanogrammus aeglefinus*, and *Parasagitta elegans* had the highest levels of this biomarker, while the bottom water POM, *Arrhis phyllonyx* and *Sabinea septemcarinata* had the lowest levels (figure 15). This trend was reversed in the diatom biomarkers. Levels of the diatom biomarker ($\sum$ 16:1ω-7, C16 PUFA, 20:5ω-3, Dalsgaard *et al.*, 2003) were highest in *Sabinea septemcarinata*, totaling almost 30% of total fatty acids in both seasons. This biomarker was lowest in the bottom water POM sample and in *Gadus morhua* (figure 16). Meanwhile, the ratio of 20:5ω-3 to 22:6ω-3, another diatom biomarker (Nelson *et al.*, 2001) was greater than one in *Sabinea septemcarinata*, *Arrhis phyllonyx*, the large *Pandalus borealis*, *Halirages fulvocinctus*, and *Andaniexis lupus* (figure 17).

To be clear, a high level of dinoflagellate or diatom biomarkers in higher trophic levels does not mean that these species are consuming phytoplankton directly. It is more likely that

these species simply feed from the plankton and benthos, which, in turn consume phytoplankton or heterotrophic individuals. Analysis of *Gadus morhua* and *Melanogrammus aeglefinus* stomachs supports these findings. It should also be noted that these fish were small individuals. While they were caught using a bottom trawl, these species are known to feed more on pelagic material when they are young (Dalpadado *et al.*, 2009), and switch to a more benthic diet at an older age (Lamond *et al*., 1998). Stomachs contained the euphausiid *Thysanoessa* spp. and the pelagic amphipod *Themisto abyssorum.* Analysis of species composition in the hyperbenthic community of Kongsfjord found high densities of *Thysanoessa* spp., which are known to make daily migrations into the pelagic (Hirche *et al.*, 2015). Feeding habits of *Thysanoessa* spp. in Kongsfjord are species specific. Falk-Petersen *et al.*, (2000) demonstrated that the omnivorous *Thysanoessa inermis* and *Thysanoessa raschii* feed mostly on phytoplankton and small copepods, and *Thysanoessa longicaudata* feed more heavily on copepods. *Themisto abyssorum*, meanwhile, is a pelagic amphipod known to feed on both diatoms and dinoflagellates as well as copepods (Noyon *et al.*, 2012; Kraft *et al.*, 2013). To conclude, the phytoplankton biomarkers in the fish species sampled here likely come from these important omnivorous primary and secondary consumers.

4.2.2 Pelagic vs. Benthic Consumers

The identified division in the hyperbenthic community between dinoflagellates and diatoms is an interesting observation. Species with higher levels of dinoflagellate biomarkers were also more depleted in $\delta^{13}C$, suggesting a greater reliance on pelagic phytoplankton. These species, which include *Gadus morhua*, *Melanogrammus aeglefinus*, the small size fraction of *Pandalus borealis*, and *Parasagitta elegans*, are also highly motile species that would be able to take advantage of pelagic resources in the water column. Meanwhile, the species with greater diatom signatures, such as *Arrhis phyllonyx* and *Sabinea septemcarinata*, were more enriched in $\delta^{13}C$, which suggests a greater reliance on benthic sources. These results, therefore, illustrate the importance of diatoms for benthic consumers and the importance of flagellates for pelagic consumers. This is further exemplified by the differences observed between the two size fractions of *Pandalus borealis*.

Pandalus borealis, a vertical migrator, is found in most northern waters and is one of the most important cold-water shrimp species. In the northwest Atlantic, *Pandalus borealis* catches

have been the highest of all Crustacea (Parsons *et al.*, 1998), and the species is important prey for cod off Iceland (Jaworski and Ragnarsson, 2006) and in northern Norway (Parsons *et al.*, 1998). While *Pandalus borealis* is frequently described as a benthic feeder (Shumway *et al.*, 1985), a study in Balfsjord, Norway found distinct differences between size classes, and found the small-sized individuals to be more reliant on the pelagic production. Younger individuals were found with only copepods and krill in their stomachs while stomachs of older individuals also contained mineral particles and occasional remains of benthic polychaetes (Hopkins *et al.*, 1993). Another study in this region found that older, larger individuals of *Pandalus borealis* spend more time on the bottom than the younger, smaller individuals (Nilssen *et al.*, 1986), therefore developing a more benthic diet at an older age. Results of this study indicate that a similar trend can be found for the *Pandalus borealis* community in Kongsfjord. In the contribution biplots for both seasons, the large size fraction is described by large contributions of 20:5ω-3 and is located closer to *Arrhis phyllonyx* and *Sabinea septemcarinata*. The small size fraction has a higher contributions of 22:6ω-3 and is located closer to the *Calanus* spp. biomarkers and samples of *Parasagitta elegans* and *Halirages fulvocinctus* (figure 10). This separation is consistent with the division noted between the more diatom-rich 'benthic' and more flagellate-rich 'pelagic' consumers in this hyperbenthic community.

The importance of diatoms for Arctic benthic systems is not a new idea. Diatoms, especially those under the sea-ice, are known to form large aggregations, enhancing their sinking efficiency and export from the water column (Fernandez-Mendez *et al.*, 2014). Thus, diatoms have been shown to contribute to the majority of high quality phytodetrital material to the benthos in ice-covered Arctic regions (Morata and Renaud, 2008). In the Svalbard region, diatoms and the flagellate *Phaeocystis pouchetii* have been known to make up the bulk of the phytoplankton biomass, with a seasonal succession from diatom-dominance to dinoflagellate and other flagellate (mainly *Phaeocystis pouchetii*) dominance from the early to later bloom (Leu *et al.*, 2006; Wassmann, 2002; Mayzaud *et al.*, 2013a). The importance of these carbon sources has also been noted in seasonal lipid dynamics in herbivorous zooplankton (Søreide *et al.*, 2008, 2013). One expected change in the Arctic system as a result of increased Atlantic water input is the reduced production of diatoms and increased development of flagellates and pelagic protists (Piwosz *et al.*, 2009). Indeed, recent findings in Kongsfjord have demonstrated the growing importance of flagellates in the pelagic system (Mayzaud *et al.*, 2013a; 2013b). Results of this

study suggest that both diatoms and flagellates are an important food source for the hyperbenthic community in Kongsfjord. However, diatoms may be more important for bottom-feeders and flagellates important for the species who can actively feed in the pelagic realm.

4.2.3 The Copepod-Chaetognath Food chain

The chaetognath *Parasagitta elegans* and the amphipod *Halirages fulvocinctus* contain high contributions of the *Calanus* biomarkers (20:1ω-9 and 22:1ω-11; Sargent and Falk-Pettersen, 1988) as well as several phytoplankton biomarkers (figure 10). In the September biplot, the *Calanus* spp. sample is also found close-by (figure 8). This finding suggests that the conventional phytoplankton-copepod-chaetognath food web found in the pelagic, and in the hyperbenthic layer in the Beaufort Sea (Connelly *et al.*, 2014), is also found in the hyperbenthos in Kongsfjord. It also indicates that *Halirages fulvocinctus* is involved in this food web. While these species are mostly isolated by their high levels of copepod biomarkers on the CA plot, they are in close proximity to the dinoflagellate/flagellate biomarkers, and show greater levels of these biomarkers than diatom biomarkers. Although some high latitude copepods are mainly herbivorous suspension-feeders (Kattner and Hagen, 1995), most copepods show flexibility in their diets and show preference for heterotrophic ciliates and flagellates over algae (Mayzaud et al., 2007). Likewise, this phytoplankton-copepod-*Parasagitta elegans* and *Halirages fulvocinctus* food web appears to be based more on flagellates than diatoms.

Studies in Newfoundland have found *Parasagitta elegans* in high concentrations in the hyperbenthic zone (Choe and Deibel, 2000). While a study in Rijpfjord found that a pelagic community of *Parasagitta elegans* demonstrated reduced feeding during the polar night (Grigor *et al.*, 2015), this study found no significant change in fatty acid profiles or stable isotope signatures between seasons. While results of this study cannot be used to comment on the seasonal reduction in total lipid content, another study on hyperbenthic communities has indicated that lipid content variability of this gelatinous carnivore is significantly uncoupled from seasonal cycles in primary production (Parrish *et al.*, 2009). *Parasagitta elegans* is a vertical migrator, and is likely consuming copepods when its travels to the pelagic on daily and seasonal migrations. Copepods also vertically migrate on daily cycles, and enter diapause at depth during the winter months (Hirche *et al.*, 2015), making these prey available to the hyperbenthic

communities of *Parasagitta elegans* throughout the year. It seems the movement of both this species and its prey may be responsible for a lack of seasonal change in its ability to feed.

Oleic acid, 18:1ω-9, is a major fatty acid found in most marine animals. Meanwhile, 18:1ω-7 is derived from the elongation of 16:1ω-7, which likely originates from phytoplankton. This means that the presence of 16:1ω-7 and 18:1ω-7 in animal tissue tends to reflect phytoplankton dietary input, while 18:1ω-9 reflects animal dietary input (Falk-Petersen *et al.*, 2000). Therefore, the ratio 18:1ω-9/18:1ω-7 is often used as general indicator of carnivory in benthic animals when it is greater than 1 (Sargent and Falk-Petersen, 1981; Graeve *et al.*, 1997). However, this ratio should be interpreted very carefully since 18:1ω-9 may be also found in various marine producers, such as brown and red macroalgae and dinoflagellates. The degree to which 18:1ω-9 is synthesized can also vary among taxa and the 18:1ω-9/18:1ω-7 ratio has been known to change during starvation and with fluctuations of the total lipid content (Legezynska *et al.*, 2014). *Parasagitta elegans* and *Halirages fulvocinctus* both had a high ratio of 18:1ω-9/18:1ω-7 (figure 19), but then again, so did *Calanus* spp. and bottom water POM samples, so it is hard to tell just how well these biomarkers represent carnivorous feeding in these samples. The POM samples also contained high levels of the copepod biomarkers, so the combination of high carnivory and copepod biomarkers may reflect the presence of copepods on the GFF filters. While removal of copepods was part of the methods used, it may be assumed that small individuals were missed.

Halirages fulvocinctus had the highest levels of the copepod biomarker (figure 18) of any other species. It is interesting to note, however, that the $\delta^{15}N$ signature of this species was the lowest of all taxa in both seasons, and was quite similar to the values for the euphausiid *Thysanoessa inermis* (figure 4). The calculated trophic levels were 0.9 and 2.7 for September and January, respectively. The September trophic level is quite low compared to other studies, where *Halirages* spp. was found to feed at trophic level 3 (Connelly *et al.*, 2014). The standard deviations, however, are very low, suggesting that it was not an issue with the analysis. It is interesting that the January value is so much higher than the September level. This difference may be due to the presence of lipid-rich diapausing copepods in January. *Calanus* copepods are known to diapause at depth in large densities and in an unaware state during winter, so they may be easy prey for carnivores during the winter months (Darnis *et al.*, 2012). Additonally, studies have shown that the high-energy long-chain monounsaturated 20:1 and 22:1 may be effectively

transferred to the benthic communities with zooplankton fecal pellets (Mayzaud *et al.*, 2007). Fecal pellets, which have relatively fast sinking rates depending on size, are typically enriched in $\delta^{15}N$ relative to the organism's food source due to the preferential loss of the lighter isotope during excretion (Checkley and Entzeroth, 1985). Sometimes, however, copepod fecal pellets can be isotopically depleted compared to the food source. This observation may be related to the individuals' metabolic activity, changing with regard to seasonal feeding activity as well as reproduction and growth (Tamlander et al., 2006b). Therefore, the differences in trophic level for *Halirages fulvocinctus* may be due its reliance on fecal pellets, which have variable $\delta^{15}N$ values. *Halirages fulvocinctus* may also scavenge dead chaetognath debris, which would also provide high levels of the zooplankton biomarkers in its tissues. Several possibilities exist, including copepods, fecal pellets, chaetognath detritus, and phytoplankton, which may explain the high levels of these biomarkers accompanied by large differences in $\delta^{15}N$ signatures in *Halirages fulvocinctus*.

4.2.4 Benthic Consumers

Arrhis phyllonyx and *Sabinea septemcarinata* were tightly grouped in the CA ordination (figure 10). While diatom biomarkers were higher in these species relative to the rest of the community, other biomarkers indicate that these species attain carbon from a variety of sources. *Arrhis phyllonyx,* known as a deposit-feeder/predator (Legezynska *et al.*, 2012), had distinct differences from other taxa in several biomarker levels. The highest contributing fatty acid for this species was arachidonic acid (20:4ω-6), which is a rather ambiguous biomarker, indicating macroalgae (Graeve *et al.*, 2001; Nyssen *et al.*, 2005), diatoms, microbial eukaryotes, terrestrial matter (Sargent *et al.*, 1987; Howell *et al.*, 2003) and foraminifera (Gooday *et al.*, 2002; Suhr *et al.*, 2003). Levels of 20:4ω-6 were three times higher than in other taxa (figure 20). The MUFA 18:1ω-7, a biomarker for bacteria (Sargent *et al.*, 1987; Dalsgaard *et al.*, 2003) was also a highly-contributing fatty acid. Stomach contents of *Arrhis phyllonyx* have suggested a diet of rich in foraminifera supplemented with polychaetes, Halacaroidea (Acari) and sipunculans (Legezynska *et al.*, 2012). However, the close positioning of *Arrhis phyllonyx* and the macroalgae samples on the September contribution biplot above (figure 8), as well as high levels of the bacterial biomarkers (figures 24, 25, 26), suggest that macroalgal detritus may be contributing to its diet as

well. This conclusion may explain the high nitrogen isotope ratios (9.8‰ in September and a range of 10.1‰ to 10.5‰ in January) for this species (figure 4). Detrital lumps tend to be isotopically enriched due to bacterial isotopic fractionation, which would conceivably lead to high $\delta^{15}N$ levels in consumer tissues (Vander Zanden and Rasmussen, 1999; Macko and Estep, 1984). *Sabinea septemcarinata,* meanwhile, appears to be relying on detritus of phytoplankton origin. This species was also enriched in $\delta^{15}N$, and was grouped with *Arrhis phyllonyx* on all contribution biplots (figures 8, 9, 10), most likely due to the high levels of the bacterial biomarker 18:1ω-7 (figure 24). However, levels of 20:4ω-6 were not as high as in *Arrhis phyllonyx* (figure 20). The fatty acid profile of *Sabinea septemcarinata* had a higher percentage of diatom biomarker 20:5n-3 (figure 12) relative to other species. This, combined with high contributions of the bacterial biomarker 18:1ω-7 (figure 24), may reflect direct feeding on bacteria taken with phytodetritus or the consumption of small, bacterivorous organisms (Volkman *et al.*, 1980).

Andaniexis lupus is a small stegocephalid amphipod that was only sampled in September in this study. It is located almost at the center of the September contribution plot (figure 8) and also falls in the middle of the distribution on nearly all of the biomarker plots. Curiously, its fatty acid profile is described by high levels of PUFA (nearly 50 % of total fatty acid, figure 20), but also high levels of the detrital biomarker (more than 15% of total fatty acid, figure 27). *Andaniexis* spp., with incisors well developed for cutting and slicing soft food, are known to graze on cnidarians and soft corrals (Moore *et al.*, 1994). Results of this study suggest that *Andaniexis lupus* has a similar feeding strategy. Reliance on cnidarians and soft corals (pelagic and benthic sources) may explain its curious fatty acid composition as well as why it falls in the middle of the distribution of pelagic to benthic feeders sampled in this study.

4.3 Carbon Sources

Results of both stable isotopes and fatty acid profiles reflect the presence of pelagic production in consumer tissues in both September and January. Fatty acid results reveal contrasting importance of diatoms and flagellates for different parts of the hyperbenthic community. One of the predicted changes in the Svalbard region, due to increased Atlantic Water intrusion, is the reduction of diatoms and subsequent increase in the production of flagellates. A study in Svalbard fjords by Piwosz *et al.* (2009) found that plankton communities varied

compositionally depending on water mass. Comparing Kongsfjord (warm environment) and Hornsund (cold environment), they found that diatoms and dinoflagellates were present in the outer basin of Kongsfjord, while cryptophytes and other nanoflagellates dominated the biomass in the inner basin. Meanwhile, Hornsund showed a dominance of cryo-pelagic diatom species likely supplied by the inflow of Arctic waters. Due to a density barrier at the fjord entrance, Kongsfjord is known to adopt a 'cold' or 'warm' mode according to the degree of Atlantic Water occupation (Cottier *et al.*, 2005). During cold years, Leu *et al.* (2006) recorded a spring bloom dominated by diatoms followed by flagellates, the classic scenario for Arctic regions. During warm years, however, Mayzaud *et al.* (2013a) found a *Phaeocystis pouchetii* bloom accompanied by a general lack of diatoms. Their results, as well as those of Kubiszyn *et al.* (2014), show a dominance of prymnesiophytes, naked ciliates and cryptophytes throughout the summer period, which supports the suggestion by Piwosz *et al.* (2009) that warm years, characterized by greater Atlantic Water intrusion, will sustain a higher standing stock of flagellates and ciliates over diatoms. Results of this study, which reinforce the importance of diatoms for benthic consumers, suggest that these changes in primary producer could negatively impact the benthos. Moran *et al.* (2012) found that, under sea-ice conditions, the spring blooms in the Bering Sea were characterized by a higher proportion of diatoms, less recycling and greater export and, therefore, enhanced pelagic-benthic coupling. In open-water conditions, blooms were characterized by a high proportion of dinoflagellates, accompanied by greater carbon cycling in the water column and lower export to the seafloor. This resulted in reduced pelagic-benthic coupling (Moran *et al.*, 2012). Furthermore, the Hornsund study by Piwosz *et al.* (2009), as well as a study in northern Svalbard (Søreide *et al.*, 2013), suggest that diatoms can be advected to locations where sea-ice was absent. However, with a greater intrusion of warm Atlantic Water and a reduction in sea-ice in the Arctic, pelagic-benthic coupling in the Arctic may be significantly reduced, with harmful consequences for benthic communities.

These results also highlight the importance of macroalgae and macroalgal detritus for several hyperbenthic species. Boreal macroalgae are predicted to expand along the rocky coastlines of the Arctic within the 21st century as a consequence of climate warming (Krause-Jensen *et al.*, 2012). Indeed, a study in Kongsfjord in 2012 supported this prediction. Kortsch *et al.* (2012) attributed the dramatic appearance of erect macroalgae to increases in sea-surface temperature and an increased ice-free season. A recent study in the Isfjord system indicated that

macroalgae potentially play a larger role as a carbon source to benthic communities than is typically acknowledged (Renaud *et al.*, 2015). Results from this study also indicate that macroalgae and its detritus are important carbon sources for several hyperbenthic species.

In spite of three tidewater glaciers draining into Kongsfjord, stable isotope and fatty acid biomarkers indicate that terrestrial carbon is not a significant source of carbon for hyperbenthic organisms. A study in Hornsund and Adventfjord (Koziorowska *et al.*, 2016) found that terrestrial carbon inputs contributed to 80% of the organic carbon in the sediments of Adventfjord. If terrestrial carbon is also an important component of Kongsfjord sediment, the depleted $\delta^{13}C$ values and elevated levels of the terrestrial biomarker 22:0 + 24:0 found in POM samples (figure 26) suggests that carbon of marine origin is more labile than glacially-derived organic carbon in Kongsfjord. One of the predicted consequences of climate change is the increase in terrestrial material entering marine environments. This is to be especially important in the Arctic, where glacial retreat will cause an increase in meltwater outflow accompanied by a flux of inorganic and organic particles to the sea. While studies in the Canadian Arctic have found terrestrial material to be a significant source of carbon to hyperbenthic organisms at the mouth of the Mackenzie river (Connelly *et al.*, 2014; McTigue and Dunton, 2013), it appears that in Kongsfjord, glaciers are more of a source of disturbance to marine communities (Wlodarska-Kowalczuk *et al.*, 2005) than a significant source of functional organic material.

4.4 Seasonality

4.4.1 Two 'Post-Bloom' Seasons

Despite the dramatic seasonality found at high latitudes, this study found very little difference in food-web structure of the hyperbenthos between September and January. In Kongsfjord, seasonality is demonstrated most clearly by light availability and subsequent cycles of primary production and food accessibility. Sampling took place in September and January. In January, the sun does not come above the horizon, characteristic of the true 'polar night.' In contrast, sampling in September occurred within days of the fall equinox, when the sun is above the horizon for twelve hours. Despite the hours of sunlight available for photosynthesis, fluorescence measurements revealed very little standing stock of phytoplankton in the water column (see figure 29 in Appendix 1). Furthermore, 2014 has been described as a very special

year in Kongsfjord. Spring blooms typically come in April or May, followed by a summer bloom in June. However, in 2014, the only observed bloom came in the middle of June, and the rest of the summer phytoplankton biomass was very low (Else Nøst Hegseth, Personal communication). Therefore, it appears that by September, very little fresh phytoplankton was left in the water column, and that sampling in both September and January took place during 'off-bloom' periods in the fjord.

While stable isotope and fatty acid profiles, discussed above, reflect the consumption of pelagic phytoplankton by this hyperbenthic community, it is difficult to know when this feeding took place. Results of laboratory experiments have found that the turnover time of stable isotopes in non-lipid tissue in *Calanus glacialis* was unaffected by prolonged periods of starvation and reflected that of previously consumed food (Tamlander *et al.,* 2006b). If these species are indeed starving, then this may also explain the enriched $\delta^{15}N$ signatures found in January. Even so, recent studies have found that some biological activity does persist through the winter in the Arctic, despite the lack of fresh primary production. In order for this activity to be sustained, these organisms, presumably, must be feeding.

4.4.2 An Active Community

While some species may use their lipid reserves to survive the unproductive dark season, there is no reason to believe that it is the dominant strategy for hyperbenthic species in Kongsfjord. A study on benthic amphipods around Svalbard found that lipid levels were not used as a method of surviving the winter, but rather their fluctuation coincided with reproduction. They also found stomachs full of various food items during the winter months (Legezynska *et al.*, 2012). Similarly, seasonal cycles of total lipid content in benthic shrimp and prawn species have been attributed to gametogenesis, rather than to fluctuations in food availability (Clarke 1977, 1979). Detected levels of bioluminescence (Berge *et al.,* 2012) indicate that at least several zooplankton taxa are active and functioning during the polar night. Hyperbenthic species are highly motile, and many are able to utilize both the benthic and pelagic realms for food acquisition through vertical migration. Diel vertical migration of zooplankton has been noted in Kongsfjord in January (Berge *et al.,* 2009), reiterating that several taxa are feeding and spending energy during this unproductive time of year. The fjord itself is highly advective, with a large volume of Atlantic water entering the fjord from the shelf (Cottier *et al.,* 2005), and likely

bringing some organic material with it. The availability of such resources to the hyperbenthic community would be enhanced by the ability of these species to migrate into the pelagic zone.

While activity levels suggest the necessity of feeding during the dark months, it may be that consumption levels are not high enough to replace isotopic signatures and fatty acids in consumer tissues. Essentially, enough energy is attained for general maintenance, but not enough for storage, meaning that tissues would still reflect the signatures of food consumed during the last period of high production. However, there were subtle differences between September and January results that may indicate that species are feeding and incorporating their food. Results indicate an increase in bacterial biomarkers in January for several taxa, accompanied by a decrease in PUFA levels for some species. Enrichment in $\delta^{15}N$ in January was also observed, which has been attributed to increased consumption of reworked material (Iken *et al.*, 2010). Furthermore, these trends were noted for small taxa, which have more rapid turnover of major lipid components (over 2-3 weeks, Graeve *et al.*, 1994), and not for large taxa, which are subject to slower biochemical turnover rates. These subtle trends in the results may indicate the importance of microbial-detrital carbon pools in 'off-bloom' situations.

4.4.3 Microbial-Detrital Carbon

As photosynthesis shuts down due to light limitation in the Amundesen Gulf, microbial-detrital food webs become increasingly important (Forest *et al.*, 2011). In addition, despite a lack of photosynthetic production, nearly all taxonomic groups of Arctic microbes are sustained. For example, the ecologically important flagellates *Micromonas pusilla* and *Phaeocystis pouchetii* persist in Arctic waters through the winter months, perhaps by resorting to bactivory (Berge *et al.*, 2015). The classic concept of the microbial loop is that protozoa, such as flagellates and ciliates, consume particulate organic matter, and heterotrophic bacteria convert dissolved organic carbon (DOC) into particulate biomass, therefore making these small inaccessible sources of carbon biologically available to higher trophic levels. Likewise, a study in Kongsfjord found that in a bloom situation, the microbial loop acted as a link between small particulate and dissolved production and higher trophic levels. Meanwhile, during the dark, unproductive period of the year, microbes appeared to be in 'regenerative mode,' and were thereby responsible for significant remineralization through the microbial loop in this period of regenerative production (Rokkan-Iversen and Seuthe, 2011). Due to its capacity to perform different functional roles in

periods dominated by new and regenerated production, the microbial loop may enhance the ecological flexibility of marine systems (Rokkan-Iversen and Seuthe, 2011), allowing pelagic systems to sustain a baseline level of heterotrophy for the duration of the polar night. This may explain the lack of seasonal trends observed for *Parasagitta elegans*, the small *Pandalus borealis*, and other small hyperbenthic species with stable isotopes and fatty acids describing a more 'pelagic-carbon' dependence.

Additionally, benthic food-web studies in Kongsfjord comparing March and August (Kedra *et al.*, 2012) and May and July (Renaud *et al.*, 2011) revealed a similar lack of a strong seasonal shift in food-web structure as described above. Using stable isotopes, these studies found that benthic communities in the fjord are primarily sustained by pelagic production, but that the contributions of detrital carbon along with omnivorous feeding strategies diminish the effects of the strong seasonal cycle of primary production (Kedra *et al.*, 2012; Renaud *et al.*, 2011). This study suggests that several of the hyperbenthic organisms sampled, such as *Arrhis phyllonyx, Sabinea septemcarinata*, and especially *Hippoglossoides platessoides*, may invoke similar tactics.

The role of detritus in the structure and function of food webs has long been recognized (Elton, 1927; Lindeman, 1942). Indeed, studies have suggested that system stability is increased (McMeans *et al.*, 2013) through the coupling of multiple energy channels: phytoplankton and detrital food webs. At high latitudes, it is recognized that the vast majority of primary production is not consumed by herbivores in the water column, but rather, is returned to the ecosystem as detritus and assimilated through detritivores (Polis and Strong, 1996). This study indicates that detritus may provide hyperbenthic organisms with a more constant food supply, thereby reducing seasonality of resource availability. The combination of strong seasonality in primary production and the dampening effect of detritus would suggest high levels of omnivory in hyperbenthic species. In an environment with a varying and unpredictable food supply, omnivory would provide for a more responsive and flexible utilization of food sources (Norrko *et al.*, 2007). Studies in Antarctica (Mincks *et al.*, 2005; 2008) have revealed the existence of persistent detrital 'foodbanks' in the sediments which can sustain benthic communities over long time scales. In the Gulf of Maine, the krill species *Meganyctiphanes norvegica* was found with sediment in its gut in February, demonstrating the importance of the sediment detrital pool even for pelagic species during unproductive months (Cleary *et al.*, 2012). This study suggests that

many hyperbenthic organisms also depend on detrital carbon, and that they have the potential to link this persistent source of decomposing phytodetritus with higher trophic levels.

4.5 Implications

The presence of sea-ice in the Arctic exacerbates the intense seasonality in primary production by further controlling the availability of sunlight, as well as influencing stratification and nutrient availability. This results in a system tightly linked to intense pulses of production, with a large contribution of ice-algae as a carbon source. Kongsfjord may represent the future of Arctic fjord systems, when sea-ice has retreated and seawater temperatures have increased. Despite the 'borealization' of Kongsfjord in terms of temperatures and ice cover (Cottier *et al.*, 2007), the fjord continues to experience dramatic seasonal changes in light availability. While ice cover may accentuate this seasonality, and flux to the benthos, the phytoplankton bloom in Kongsfjord may very well provide enough material, with the help of detrital and microbial food webs, to support the ecosystem through the winter months.

Ecosystem-level implications of these results lie in the potential role hyperbenthic organisms play as a connection between the pelagic and benthic realms. Results of this study suggest that hyperbenthic organisms may enhance this connection through benthic-pelagic and pelagic-benthic coupling. Due to their reliance on multiple carbon sources from the pelagic and benthic realms, they can effectively transfer energy from the benthos to the pelagic when they are consumed by fish at depth or on their daily and seasonal migrations to the pelagic. Hyperbenthic species with an appetite for detritus (*Arrhis phyllonyx*, *Sabinea septemcarinata*) and benthic invertebrates (*Pandalus borealis*) would play an especially important role in the transfer of detrital and benthic carbon to the pelagic food web. Indeed, fish stomachs that were analyzed in September and in January contained hyperbenthic invertebrates, demonstrating the importance of their role as a trophic link. As hyperbenthic species were high in essential PUFA, they may also play a role in the nourishment of important fish species. These results indicate that hyperbenthic taxa may also aid in pelagic-benthic coupling. The position of these organisms just above the seafloor, with pelagic carbon in their guts, may facilitate the export of phytoplankton production to depth. This is relevant for both daily and seasonal vertical migrators. For example, copepods that over-winter at depth are within reach of benthic consumers, who may benefit from this additional carbon source during the winter months.

Additionally, these results demonstrate that the zooplankton species collected here are an important part of the pelagic and benthic systems. Neglecting to incorporate them in food web and production studies could lead to serious underestimations and an inaccurate picture of both the pelagic and benthic realms. A recent study in Kongsfjord drew similar conclusions based on the large abundance of pelagic zooplankton observed close to the seafloor (Hirche *et al.*, 2015). By combining a remotely operated vehicle (ROV), an optical zooplankton sensor (MOKI), and Tucker trawl, they demonstrated that with new technology and creative thinking, sampling the hyperbenthos should not be a large concern. Hopefully, the hyperbenthos will receive more attention, leading to greater incorporation of this layer in future studies and ecosystem models.

4.6 Reflections

In hindsight, this study would have benefitted from several key adjustments. When piecing together the results, the most obvious lacking component was the number of carbon sources sampled. While data existed from a January 2014 cruise for sediment, macroalgae, and surface water POM, it did not seem fitting to use these sources for analyzing samples from 2015. Isotopic signatures are known to vary widely both in space and time, so even though it was tempting, these carbon sources were not applied to the 2015 January data. I collected macroalgae in September, but prioritized fatty acid analysis over stable isotope analysis. I should have collected more macroalgae of various types to have enough for both analyses. Pelagic POM, and sediment would have been another good addition as well as larger samples of *Calanu*s spp. These samples could have been used for trophic level calculation, and could have provided a nice visualization of the connection between primary producers and the upper level consumers seen in the contribution biplots. With a greater variety of carbon end-members, I also could have created a mixing model to measure the contribution of each carbon source. Furthermore, the bottom water sample was collected using Niskins on a CTD rosette. This means that the closest it could come to the bottom was about 10-15 m above the seafloor. To get a more accurate sample of 'bottom water,' I should have used a bottom water sampler, which can collect water just above the sediments. As I collected organisms using a sled just above the seafloor, this would have been a better representation of the POM where these species are living.

As mentioned in the methods section, stable isotope analysis has several drawbacks, and the best solution is to use compound-specific stable isotope analysis. While bulk stable-isotope

analysis provided nice results in this study, there is no way of knowing if they are skewed by differences in isotopic signatures in different body tissues. Stable isotope analysis of distinct amino acids or fatty acids, would help avoid isotopic routing, where isotopes are incorporated differently based on the type of tissue in the consumer. Therefore, when relating several taxa, compound specific analysis provides the best means of comparison (Budge *et al.*, 2008). Another issue with stable isotope analysis was the small size of each sample, as I had to combine some samples to have enough content for the analysis. This was especially an issue for small amphipods and chaetognaths. An improvement to the fatty-acid portion of this study would have been to conduct feeding experiments. Feeding experiments along-side the fatty acid data set would have helped support my conclusions in regard to biomarkers and carbon sources.

In this study, a seasonal-comparison was prioritized as the question of interest. Therefore, when picking organisms, I tried to match species found at similar depth and location in both seasons. With a more extensive dataset, it may also have been possible to include a second question regarding spatial differences within the fjord. The environmental conditions vary widely from the glacier front to the mouth of the fjord, and it would be of interest to see how these variations influence hyperbenthic diets. When collecting data for this project, samples were also collected for community analysis and stomach content analysis of invertebrates. Sadly, these samples have not been analyzed in time for this thesis, but would have made a nice addition to the results provided above. While the most abundant organisms were selected from the epi-benthic sled, there is no way of knowing just how representative of the Kongsfjord hyperbenthic community they are. Additionally, stomach contents of invertebrates may provide insights that are masked in the stable isotope and fatty acid datasets.

5. Conclusions

Results reveal very little difference in hyperbenthic food-web structure between September and January in Kongsfjord. While fatty acid profiles and stable isotopes suggest the incorporation of pelagic and benthic carbon sources for the sampled community, fatty acid signatures of POM and fluorescence measurements expose the post-bloom nature of both sampling periods. This study is unable to directly address this conundrum, but learned speculation proposes two possibilities. Either this community fails to assimilate biomarkers in periods of low production,

or microbial-detrital pools are enough to sustain a base-heterotrophic food web, allowing for little change in direct prey items in post-bloom situations. Combining fatty-acid profiles, stable isotopes and stomach-content analysis proved to be very useful for distinguishing carbon sources and assessing seasonal trends. Contribution biplots provided a valuable visualization of the data and helped identify a division in the community between pelagic and benthic consumers. This study highlights the need for the collection of multiple baseline carbon sources when using stable isotopes. The main importance of this study, however, lies in the concentrated effort of collecting and analyzing hyperbenthic organisms, and in doing so during the polar night. Very little is known about the feeding ecology of this part of the water column, and even less is known about these communities during the dark portion of the year. This study demonstrates that the hyperbenthos is an important part of the marine system with implications for benthic-pelagic and pelagic-benthic coupling.

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7. Appendix.

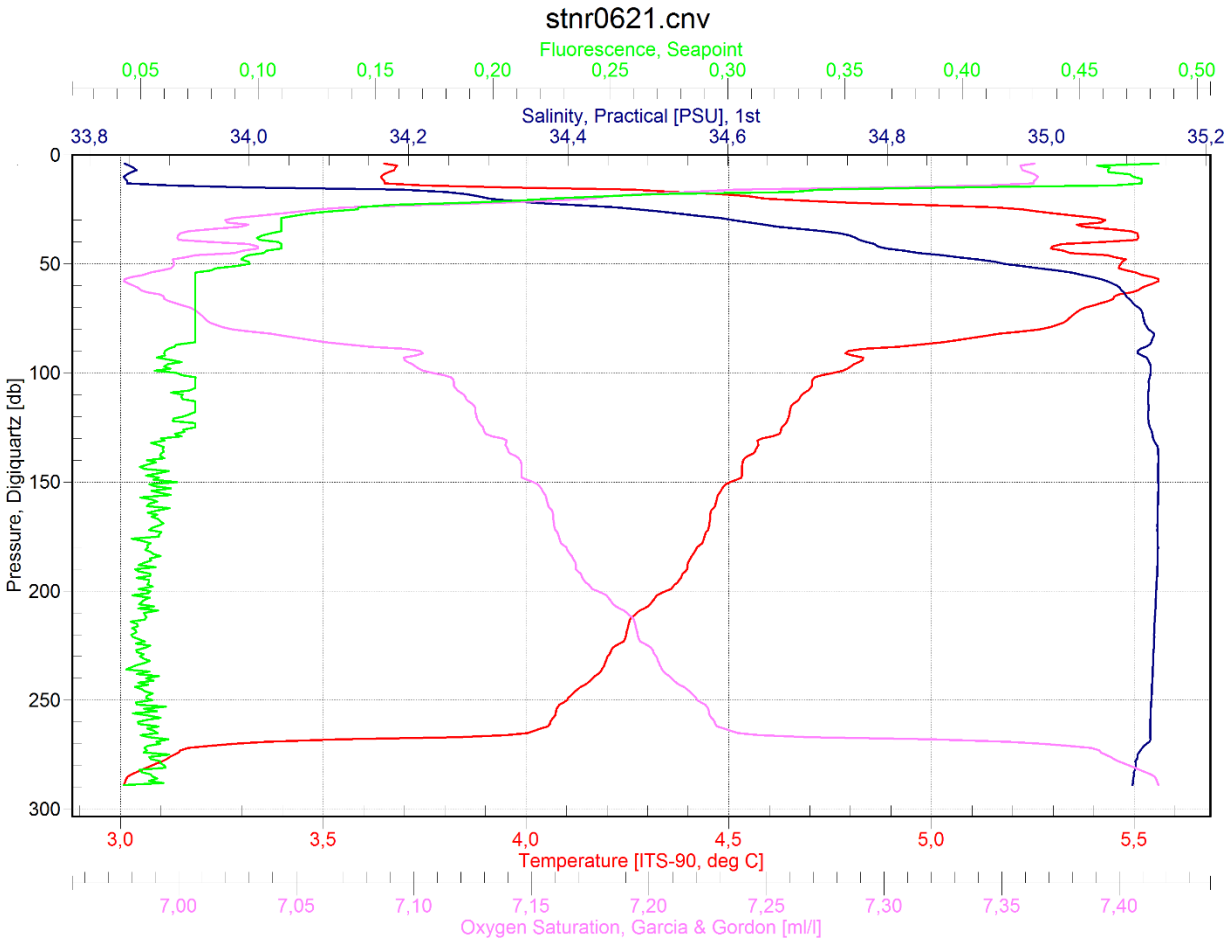

Figure 29. CTD profile from September, 2014 in Kongsfjord (station number 4 in figure 2).