Characterizing the diet and habitat niches of coastal fish populations in the Beaufort Sea Tarium Niryutait Marine Protected Area

by

Jasmine D. Brewster

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Abstract

To evaluate the niche of coastal fish populations in the Beaufort Sea, stable isotopes (SI) and fatty acids (FA) were used to characterize species-specific niches, niche overlaps and resource partitioning (nicheROVER) of the Shingle Point fish populations. Fishes were grouped into three isotopic groups: marine, coastal, and freshwater (Ward's clustering analysis), and five dietary groupings (using FA), where benthic feeding strategies were prevalent (correspondence analysis). Niche metrics were used to evaluate if total mercury (THg) could contribute complementary trophic information (residual permutation procedure (RPP)). Three THg groups (high, intermediate, low) were identified (boxplot analysis). High THg was identified in high trophic and benthic feeders, high THg ranges were observed in species with large niche sizes, high trophic feeding, and freshwater influences (RPP). The bioavailability of freshwater introduced THg to marine biota was assessed, however further research needs to be performed. Combining dietary indicators SI, FA, and THg, allowed for the characterization of the diet and habitat use of coastal fish populations, better understanding of the niches of these species, and developed baseline information for future monitoring in an MPA, as climate change continues to effect the Beaufort coastal environment.

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> *"Land of the silver birch. Home of the beaver. Where still the mighty moose Wanders at will.*
> *Blue lake and rocky shore, I will return once more…"*

Thesis Format and Manuscript Claims and Accomplishments

The thesis is presented and prepared in a manuscript format, and is comprised of five chapters. Chapters Three and Four are written in manuscript style. Chapter One introduces background information on the study area, dietary biotracers used to characterize niches of coastal fish populations, and objectives of this research. Chapter Two focuses on the research rationales, qualitative and quantitates approaches to assess the species niches, as well as the importance of incorporating traditional ecological knowledge (TEK) to characterize species ecologies. Chapter Five synthesizes and concludes major findings. Appendix identifies TEK results from interviews conducted on the fish populations and environmental changes.

Chapter Three: Brewster JD, Giraldo C, Swanson H, Walkusz W, Loewen TN, Reist JD, Stern GA, Loseto LL (2016) Ecological niche of coastal Beaufort Sea fishes defined by stable isotopes and fatty acids. Marine Ecological Progress Series. doi 10.3354/meps11887. J. Brewster analyzed the data, made inferences on results, and was the corresponding author of the manuscript. Co-authors contributed statistical assistance, and guidance in writing the manuscript. The manuscript was accepted to Marine Ecological Progress Series.

Chapter Four: Brewster JD, Stern GA, Osterag S, Loseto LL (2016) Using mercury, stable isotopes and fatty acids to identify niches and the importance of freshwater influences on coastal fish species in the Canadian Beaufort Sea (in progress). The data was processed and analyzed by the corresponding author J. Brewster. Co-authors contributed support with statistical analyses, and guidance in writing the manuscript. The manuscript will be submitted to Science of the Total Environment or Environment and Pollution.

Appendix: Brewster JD, Neumann D, Ostertag SK, Loseto LL (2016) Traditional Ecological Knowledge (TEK) at Shingle Point, YT: Observations on Changes in the Environment and Fish Populations. Can. Tech. Rep. Fish. Aquat. Sci. 3174: v + 23p. The questionnaire and interviews were prepared, conducted, and analyzed by J. Brewster. Co-authors provided support and guidance in writing the report.

In addition to research presented in this thesis, J. Brewster was lead on a manuscript that was submitted to Polar Biology on June 2016: Brewster JD, Giraldo C, Choy ES, Lynn B, Hoover C, McPhee S, McNicholl DG, Majewski A, Rosenberg B, Power M, Reist JD, Loseto LL . Feeding patterns in Beaufort Sea Gadidae assessed by stable isotopes and fatty acids. Polar Biology (in review)

J. Brewster was a co-author on a manuscript with the tentative title: Trophic linkages in the Beaufort Sea ecosystem: a comparison of SI and ecosystem model approaches (in progress)

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Chapter One: Introduction

1.1 Background and Study Area

Defining Arctic marine ecosystems including predator-prey interactions and individual species habitat needs are necessary for evaluating effects of climate change. Variations in sea ice distribution, extent, and early melting in the Arctic has lead to possible shifts in primary energy sources in the ecosystem (Serreze et al. 2008, Stroeve et al. 2012, Arrigo et al. 2008). Primary producers such as ice algae, and phytoplankton supply energy to the base of food chains, with the sea-ice extent changing in the Arctic, the effects this will have on the transfer of organic matter to higher trophic levels are less known (Arrigo et al. 2008; Connely et al. 2014; Lavoie et al. 2009; Serreze et al. 2008; Stroeve et al. 2012). Furthermore, the direct importance of sea ice for refuge, habitat, reproduction, and foraging to Beaufort marine mammals, fishes and invertebrates (Serreze et al. 2008, Stroeve et al. 2012, Arrigo et al. 2008, Gradinger & Bluhm 2004, Loseto et al. 2009, Harwood et al. 2014), indicates that understanding marine food chains is crucial in identifying the implications that the decrease in sea ice will have on the trophic interactions and energy flow in the Beaufort marine ecosystem.

The coastal habitats of the southern Beaufort Sea are unique as they experience influences from both the cooler marine water inputs of the Beaufort Sea, as well as warmer freshwater inputs from large rivers (Craig 1984). A diverse range of freshwater, marine, and anadromous fishes migrate to the brackish environment to feed and grow before returning to their respective freshwater and marine habitats (Carmack & Macdonald 2002,Coad & Reist 2004). The freshwater inputs into the near shore environments also introduce carbon sources, facilitate nutrient mixing important to coastal habitats productivity and can influence the fish community in the area (Von Biela et al. 2013, Dunton et al. 2006). With the increase in water temperatures and changes in oceanography, the Arctic is opening up, enabling range extension of southern species distributions (Logerwell et al. 2015, Parmesan & Yohe 2003), creating the possibility of competition for the native Arctic species. Understanding the trophic interactions among- and within- fish species is crucial in monitoring and sustaining the population and habitat that supports them. Numerous studies have been underway in the Beaufort Sea and surrounding regions due to the climate change, increase pressure for industrial development in these areas, and the realization that there are gaps in knowledge of the Beaufort marine ecosystem (Cobbs et al. 2008, Loseto et al. 2009, Coad & Reist 2004).

Marine protected areas (MPAs) are delineated as coastal and offshore waters that are legally restricted from some industrial development and human activities, in efforts to sustain and monitor natural resources in the area (Kelleher & Kenchington 1992). For an area to be designated as a MPA there must be endangered or sensitive organisms and habitat that warrant protection. Currently in Canada only 0.9% of the marine waters are recognized as MPAs. The recent development of the "2020 Biodiversity Goals and Targets for Canada" sets the objective of increasing MPAs to 10% by the year 2020 (Environment and Climate Change Canada 2016). The successful methodology and indicators used to monitor and sustain current MPAs are suspected to influence the template for monitoring future MPAs. The first Arctic MPA, Tarium Niryutait Marine Protected Area (TNMPA) was established in 2010 in the Mackenzie River Estuary. This MPA was established to protect beluga, other marine species (fishes, seabirds, and waterfowl), and their supporting habitats (Loseto et al. 2010). The TNPMA is divided into three sub regions: Niqunnaq (Shallow Bay), Okeevik (part of Beluga Bay), and Kittigaryuit (Kugmallit Bay) (Fig. 1.1). Shingle Point, Yukon is a traditional and modern day fishing and hunting camp, which makes up part of the Niqunnaq region of the TNMPA. The cultural importance and continuous use of this region, dynamic coastal habitat, and being part of an established MPA, makes monitoring the Shingle Point fish populations essential in sustaining ecosystem health of the region.

1.2 Rationale and Study Context

The Mackenzie River Plume contributes to the majority of freshwater influences into Niqunnaq, contributing to the brackish and highly productive habitat. (Craig 1984). Sixteen species of fish (see Chapter 3) with diverse feeding strategies and life histories frequent coastal and estuarine habitats in the southern Beaufort Sea (Coad & Reist 2004, Carmack & Macdonald 2002). Many species captured at Shingle Point are culturally important and subsistence food for the Inuvialuit and Gwich'in peoples; these species include: dolly varden char, broad whitefish, and Arctic cisco. Also these fishes have been identified as important prey to top marine predators (i.e. beluga, bearded and ringed seals), therefore, developing baseline information is crucial in understanding the health of these species (Reist & Bond, 1988, Reist 1989, Papik et al. 2003, Loseto et al. 2010). Currently, large knowledge gaps still exist in regards to trophic ecology of marine and coastal fishes (Coad & Reist 2004, Chiperzak et al. 2003, Reist & Bond 1988).

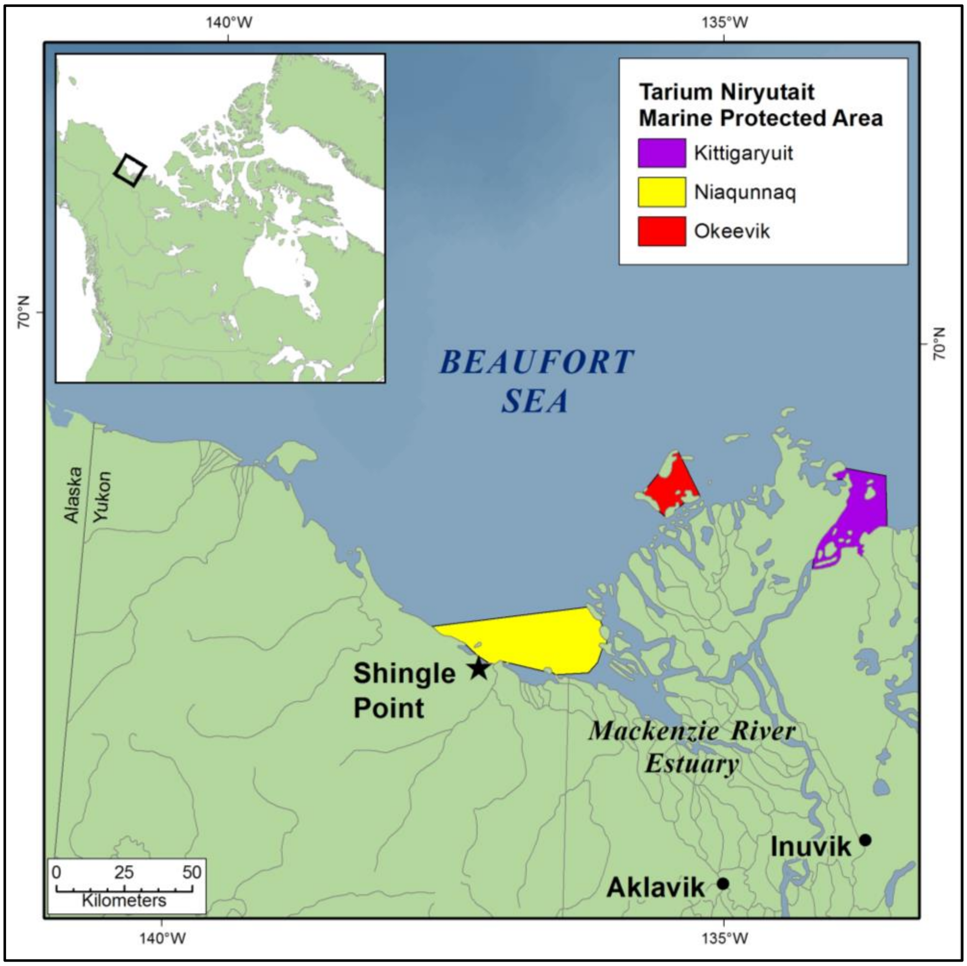

Figure 1.1 Map of the Tarium Niryuitat Marine Protected Area sub regions: Niaqunnaq (including Shingle Point), Okeevik, Kittigaryuit. Community members from Aklavik and Inuvik, NT and Alaska return to Shingle Point each summer

1.3 Dietary Biotracers

The introduction of biotracers primarily through diet, and the predictable transfer in which they propagate up through trophic levels (Hall et al. 1997, Post 2002, Iverson et al. 2004), make these organic compounds a crucial tool in identifying the ecology and trophic interactions of organisms within an ecosystem. Dietary biotracers such as stable isotopes (SI), fatty acids (FA) and mercury can serve as tools in identifying the transfer of organic matter through aquatic and terrestrial food webs through predator prey interactions (Hobson et al. 1996, Dahl et al.

2003, Connelly et al. 2014). In particular, numerous studies have used SI and FA as biotracers to characterize the diet, habitat, and trophic ecology of aquatic organisms (Budge et al. 2006, Budge et al. 2007, Cherel et al. 2011). Incorporating mercury, as an additional trophic indicator, can provide further understanding of habitat use and diet of marine, freshwater and coastal fish species as they co-occur in coastal environments. The trophic transfer of mercury through foodwebs, and spatial and temporal variations present among and within species of aquatic environments (Depew et al. 2013, Chen et al. 2005), similar to SI and FA, suggest this biotracer could provide additional trophic information.

1.3.1 Stable isotopes

Stable isotopes have been used successfully as indicators of trophic ecology of organisms in both terrestrial and aquatic systems (Peterson & Fry, 1987). Specifically, stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopes have been used as indicators of trophic feeding and habitat use of freshwater, coastal, and marine fish species (Post 2002). Due to the predictable fractionation of $\delta^{15}N$, consumer's tissues will be ^{15}N-enriched by 2-4‰ relative to its prey, providing a measure for trophic level feeding in an ecosystem (Peterson & Fry, 1987; Post, 2002). Variations in $\delta^{13}C$ serve as an indicator of primary production and the origin of energy that is cycled within a food web (Jardine et al. 2003). Here, diets have been discriminated based on variations in photosynthetic pathways of plants (e.g. C_3 versus C_4, Peterson & Fry 1987). Specifically, at higher trophic levels $\delta^{13}C$ can be used to identify habitat use in marine ecosystems since more depleted ^{13}C are observed in tissues of species in offshore systems compared to coastal systems; this can be explained by the decrease in $\delta^{13}C$ (by 0.8-1‰) in particulate organic matter as you move more offshore (Hill et al. 2006, Cherel et al. 2011). Also, $\delta^{13}C$ can be used to identify benthic feeding (more enriched ^{13}C) or pelagic feeding (more depleted) (e.g. Peterson & Fry 1987, Jardine, et al. 2003, Cherel et al. 2011). Large variations in SI signatures among and within species can exist. These large variations are dependent on the tissue-type, growth, and life stage of that consumer (Matley et al. 2013); the use of additional biotracers can help to explain these variations. Due to a longer turnover rate (e.g. months), SI is an indicator of spatial and temporal distributions of consumers and can help analyze complex marine food webs (Hobson et al. 1996, Post 2002). This provides an advantage over other short-term dietary methods (i.e. stomach contents).

1.3.2 Fatty acids

Complimentary to SI, fatty acids (FA) are another biotracer used to define the diet of consumers. Fatty acids are a type of nutrient that makes up a large component of lipids, and can be characterized into two groups: polar lipids (structural FA synthesized by the consumer) and neutral lipids (triacylglycerol FA composed from diet) (Iverson et al. 2004). Since FA are not readily synthesized or significantly modified when consumed, but are instead released from the lipids as long chains of carbon (i.e. 14- carbon chains) they are assimilated into a consumer's adipose tissues (mostly as triglycerol) or are used as energy (Iverson et al. 2004). This allows dietary FA profiles from prey to be stored in consumer's tissues in a predictable way (Thiemann et al. 2008). The analysis of FA is commonly explained as "*you are what you eat*", where FA profiles of a consumer are reflective of their prey, thus can be used to identify specific prey and infer spatial information as well as characteristics of the population (i.e. trophic positioning and feeding behaviours) (Iverson et al. 2004). Furthermore, variability in FA present in a single species can provide information on the temporal and spatial patterns of a consumer's diet, similar to SI (Budge et al. 2008; Thiemann et al. 2008). Differences within and among species can affect the assimilation of FA into tissues (i.e. turnover rate, reproduction, migration, growth rate and tissue type), thus understanding these variations and normalizing data, as well as using multiple indicators for habitat use and diet, allow for comparisons to be made with a population (Iverson et al. 2004; Budge et al. 2008).

1.3.3 Mercury

Monomethyl mercury (MeHg) is the organic form of total mercury (THg) and a neurotoxin to vertebrate organisms. The occurrence of MeHg in marine organisms is uncommon compare to other contaminants in that THg exposure is a byproduct of not only human activities, but natural sources as well. The bioaccumaulation and biomagnification characteristics of MeHg as it is trophically transferred to top predators is a concern to Western Arctic indigenous peoples, who rely on marine and coastal fish species, and marine mammals for subsistence (Papik et al. 2003, Loseto et al. 2008). MeHg will make up the majority of the THg present in top trophic level predators and their prey (i.e. marine and coastal fishes) (Atwell et al. 1998, Loseto et al. 2008). Variable levels of THg have been found within and among fishes (i.e. Arctic charr, Arctic cod); this variability is influenced by somatic growth rate, tissue type, prey, trophic level and habitat (AMAP, 2005, Stern & Macdonald 2005, van Der Velden et al. 2013). Climate change and the amplified effects it has on the Arctic marine ecosystem (i.e. rising temperatures, decrease in sea-ice extent) is suspected to promote the re-emission of THg, as well as increase human induced sources with the opening of the Arctic (AMAP 2011). Moreover, an increase in methylation of THg and the assimilation of MeHg by marine organisms has been linked with increases in water temperature, nutrients and productivity (Ulrich et al. 2001, St. Louis et al. 2005, Stern et al. 2012, van Der Veldon et al. 2013). Thus, MeHg has the potential to serve as an indicator for these changes.

Although information is lacking on the behavior of THg as a biotracer and trophic indicator in the marine environment; studies have indicated that over the past 150 years there has been a significant increase of MeHg in higher trophic levels of top predators (Chen et al. 2014). While many factors influence how THg is introduced to marine species, the main source of MeHg to fishes and top predators such as marine mammals, is through their diet (Hall et al. 1997, AMAP 2011, Stern et al. 2012). The concentration of MeHg present at the higher trophic levels are dependent on: the concentrations of THg found at the base of the food web, the structure and number of trophic levels present in the food web; the rate that bioaccumulation, biomagnification, and the growth dilution occurs within each species of the food web (AMAP 2011). It has been speculated that seasonal foraging differences between the offshore and coastal fishes might influence the bioaccumulation of THg (Loseto et al. 2008, Macdonald & Loseto 2010).

Sediments of estuarine benthic zones serve as a sink for mercury originating from both natural sources and human activities (Lavoie et al. 2010), and strong positive correlations between concentrations of MeHg in the water column and pelagic fish in estuaries have been reported (i.e. Chen et al. 2014). The positive relationship between THg and trophic level ($\delta^{15}N$) suggest THg could provide additional trophic information of marine organisms (i.e. fish species) (Bank et al. 2007), particularly in species with enriched ^{15}N associated with prevalent benthic feeding strategies (Peterson & Fry 1987, Dunton et al. 2006). Additionally, the analyses of SI, FA and THg could be used to assess the effects of diet preference of species and THg concentration within a food web (Jardine et al. 2006).

1.4 Thesis Objectives

The primary objective of my thesis was to: 1) characterize the niches of coastal fish populations, 2) identify ecologically similar species defined by niches, to 3) examine niche overlaps, resource partitioning, and niche shifts. These niche characterizations would provide baseline information on the ecology of coastal fishes, thus provide recommendations for future monitoring of this MPA. To achieve theses objectives dietary indicators SI and FA were used to assess aspects of the ecological niche (i.e. habitat and diet) of the anadromous, coastal, freshwater, and marine fishes collected at Shingle Point over three summers (i.e. 2011-2013). Isotopic signatures ($\delta^{15}N$, $\delta^{13}C$) and FA profiles were used to define the trophic interactions and niche overlaps among these species. Additionally, THg was assessed as a biotracer alongside SI and FA, to better understand the accumulation of this contaminant in the coastal system, and how it can provide complimentary habitat and dietary information of the fish populations.

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Chapter Two: Techniques to Measure Fish Niches

2.1 Rationale

With the increase in water temperature, decrease in sea-ice extent and early spring melt, the distribution and migration of many fishes are changing; the effects this will have on higher trophic levels is still not fully understood (Arrigo et al. 2008, Barber et al. 2012). Additionally, environmental changes have also been reported in the terrestrial ecosystem, where the degradation of land and increases in freshwater inputs (i.e. ground runoff) is expected to affect the coastal ecosystem (i.e. introducing nutrients) (Carmack & Macdonald 2002). In efforts to successfully monitor the fish populations occurring in the TNMPA, trophic energy sources and inter and intra-specific interactions must also be monitored. Understanding resource partitioning within and among species is needed to evaluate the adaptability and plasticity of species to environmental stressors (Hutchinson 1957, Swanson et al. 2015). The role of a species within an ecosystem can be defined as the ecological niche (e.g. Leibold 1995, Layman et al. 2007, Peterson et al. 2011); aspects of this niche include trophic interactions, habitat use and diet, which can be evaluated through multiple methodologies including quantitative and qualitative analyses. Previously, fish niches have been characterized using dietary biotracers (Layman et al. 2007, Swanson et al. 2015), and long-term observational data of species (Reist & Bond 1988, Coad & Reist 2004).

2.2 Quantitative Niche Metrics

Niche overlap among species has been a subject of interest, when concerned with resource partitioning and competition among species (Hutchinson 1957). The use of SI (i.e. $\delta^{15}N$, $\delta\ ^{13}C$) to quantify species-specific niches and niche overlap has lead to additional metrics of quantifying and defining temporal and spatial niche variation, aspects of niche dimensionality and niche breadth (e.g. Layman et al. 2007, Jackson et al. 2011), and the inclusion of other biotracers (i.e. FA) to further define a species niche (Swanson et al. 2015). Recent advances have moved passed the conventional two-dimensional analyses of SI (i.e. isotope biplots) and FA, where these two biotracers have been applied separately to each dataset, and inferences made by qualitatively considering results of each. A new probabilistic approach in quantifying trophic (or other) niches beyond two dimensions has been developed in the statistical program "nicheROVER" (Newsome et al. 2007, Swanson et al. 2015). This method can be used to quantify niche region using any continuous data the investigator has used to define the niche, and can be used to quantify probability of overlap within or among species. Although SI analyses can provide information on trophic positioning, niche shifts, and variability in general diet within a species (Post 2002), the incorporation of continuous variables indicating diet (i.e. FA) is a unique aspect of this analysis (Swanson et al. 2015), that allows for the evaluation of both the trophic niche and diet niche together.

Additionally, niche dimensionality has been further evaluated through the characterization of $\delta^{15}N$ and $\delta^{13}C$ dispersion within a bivariate space (Layman et al. 2007). Six metrics were proposed by Layman et al. (2007) to evaluate dispersion of individuals within an isotopic niche (or bivariate space generated from SI), where Residual Permutation Procedure (RPP) and linear models were used to develop a hypothesis–tested framework to further evaluate variations between these niche metrics within and among species (Turner et al. 2010). Five of the niche metrics pertain to this thesis and include: 1) $\delta^{15}N$ Range, defined as the distance between the most depleted and enriched ^{15}N within a niche; 2) $\delta^{13}C$ Range, defined as the distance between the most depleted and enriched ^{13}C within a niche; 3) Total area, or niche size (generated by $\delta^{15}N$ and $\delta^{13}C$); 4) Mean Distance to Centroid (MD), calculated from the mean

Euclidean distance between the niche centroids (mean $\delta^{15}N$ and $\delta^{13}C$) of two species; 5) Mean Nearest Neighbor Distance/ species packing (MNN), the mean Euclidean distance of individuals to their nearest neighbor within a niche (Layman et al. 2007,Turner et al. 2010).

2.3 Qualitative Niche Observations: Traditional Ecological Knowledge

Traditional Ecological Knowledge (TEK) has been defined as the collection of beliefs and techniques of indigenous peoples, and has provided an alternative way of knowing the organisms and their supporting environments (Johannes 1989). From the indigenous prospective TEK is described as learning how to live on the land from experiences of family, the community, and stories passed on through generations (ICC 2015). The western scientific perspective has utilized TEK for collecting observational and experience-based explanations of environmental components. TEK has been used to identify long-term observational data of populations and environmental changes, as well as to develop ecosystem baseline information for natural resource management and industrial development (Johannes 1989).

As part of the TNMPA, Shingle Point continues to be a current fishing and hunting camp, where Inuvialuit and Gwich'in families return to their traditional lifestyle, and partake in hunting, fishing, berry picking, and traditional games. In particular, residents from Aklavik, NT, Inuvik, NT, and Alaska return during the summer months (June-August) to capture fish for subsistence. At this time important cultural fishes of dolly varden char and Arctic cisco are migrating from the offshore marine systems to freshwater streams to overwinter (Reist & Bond, 1988, Reist 1989, Reist & Sawatzky 2010). The migration of these species along the Beaufort coast attracts returning families to Shingle Point (Brewster et al. 2016). Additionally, other important marine, freshwater, and coastal fish species (i.e. whitefish, inconnu) are captured (Jarvela & Thorsteinson 1999, Papik et al. 2003). In the summer months beluga have been reported to aggregate in the Mackenzie estuary and coastal environments, possibly for foraging, molting, calving, and as a refuge in a protected habitat (Harwood et al. 1996, Harwood & Smith 2002). The annual harvesting of beluga in these coastal and estuarine habitats is culturally important, and still serves as traditional food for the Inuvialuit of the Western Arctic (Fraker & Fraker 1979, McGhee 1988).

Since data generated from science-based monitoring have only been collected since 2010, the incorporation of TEK is essential in understanding the long-term observational changes of the environment and species over time. To assess the long-term monitoring of the Shingle Point fish populations, and in response to requests made by the Aklavik HTC and FJMC, a TEK study was conducted at Shingle Point during the 2015 field season. These interviews allowed for a further understanding of changes in fish occurrence, fish health, and the environment through documenting observations made by local indigenous peoples. In the summer of 2016 the TEK interviews were published as a DFO technical report (see Appendix), and were presented to the Shingle Point community members.

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Chapter Three: Ecological niche of coastal Beaufort Sea fishes defined by stable isotopes and fatty acids

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Abstract

Little is known about the trophic ecology of freshwater, coastal, and marine fish species that utilize coastal environments in the Beaufort Sea. In this study we used stable isotopes (SI) and fatty acid (FA) profiles to 1) characterize habitat and diet components of the ecological niche for 16 co-occurring fish species, 2) quantify niche overlap among these species and groups of species, and 3) identify resource partitioning and niche shift indicators for future monitoring. Ward's cluster analysis of SI ($\delta^{13}C$, $\delta^{15}N$) results identified 3 representative isotopic groups that were consistent with known life-history groups: marine, freshwater-rearing and coastal fishes. Correspondence and Ward's clustering analyses on FA profiles resulted in five FA groups that indicated feeding preferences and included: pelagic marine-feeding, benthic and pelagic brackish (both freshwater and marine)-feeding, benthic freshwater-feeding, benthic marine-feeding, and benthic brackish feeding groups. Isotopic niche size and feeding preferences (FA) indicated generalist and specialist strategies that could be used as indicators for resource partitioning and niche shifts. Understanding the habitat use, diet, and trophic interactions among fish species is important in monitoring the Tarium Niryutait Marine Protected Area. Combining SI and FA tracers to quantify probability of niche overlap is a unique aspect of understanding species-specific niche interactions within the Beaufort Sea coastal environment, and our results contribute to understanding how these biotracers can contribute to current and future monitoring and management of this remote MPA.

3.1 Introduction

Direct and indirect effects of climate variability and change on the marine environment and organisms of the Beaufort Sea are substantive and anticipated to continue (Cobb et al. 2008, Serreze & Barry 2011, Barber et al. 2012, Stroeve et al. 2012). Many climate-induced modifications of marine food webs will manifest through changes in the productivities and trophic structures of affected ecosystems (e.g. see Wrona et al. 2006, Arrigo et al. 2008). Therefore, a baseline understanding of the feeding and habitat ecology of animals in the Beaufort Sea ecosystem is needed to understand and predict the full effects of future climate change. Coastal habitats of the southern Beaufort Sea are unique as they integrate the cooler marine water inputs of the Beaufort Sea and the warmer freshwater inputs from large rivers (Craig 1984, Wrona et al. 2006). The Mackenzie River and estuary serve as important transitional habitats between fresh and saltwater and, coupled with the stable buoyancy boundary current of brackish water along the Yukon coastal environments, serve as migration corridors for anadromous fishes in the summer months (Craig 1984, Carmack & Macdonald 2002). Summer freshwater inputs that mix with seawater also provide complex coastal habitats for eurythermal and euryhaline marine fishes. Accordingly, coastal habitat use by anadromous and marine fishes, and use of freshened marine waters by freshwater species, is complex (Carmack & MacDonald 2002). Documenting such usages through biological tracers (biotracers) such as stable carbon and nitrogen isotopes and fatty acid signatures can provide basic understanding of trophic structure and patterns in this important coastal fish community. Moreover, baselines established under present circumstances allow for follow-on studies monitoring changes and potentially documenting causation resulting from changes induced by climate shifts and other stressors.

Stable isotope (SI) ratios of carbon and nitrogen ($\delta^{13}C$ and $\delta^{15}N$) have successfully been used in many investigations of feeding ecology, trophic interactions, and habitat use in fish populations (e.g. Hobson et al. 2002, Cherel et al. 2011, Radabaugh et al. 2013). $\delta^{13}C$ values are often used to differentiate between benthic and pelagic, terrestrial and marine prey sources, whereas $\delta^{15}N$ values are widely used as indicators of trophic level (e.g. see Post 2002, Boecklen et al. 2011). Fatty acids (FAs) are also commonly used chemical tracers that reflect trophic interactions and predator diets (e.g. Iversom et al. 1997, 2004, Kolts et al. 2013). More recently, SI ratios and FA compositions have been used together to better characterize and understand long-term (3 to 4 months; Post 2002, Iverson et al. 2004) feeding ecology and habitat use (Dahl et al. 2003, Wan et al. 2010, Connelly et al. 2014).

Recent advances in quantitative and statistical analyses of both SI and FA data (e.g. multi-dimensional plots and combined qualitative assessments) have enabled stronger analytical outcomes than those achieved through results of each individual marker type (Newsome et al. 2007, Swanson et al. 2015). A new approach in quantifying trophic (or other) niches beyond 2 dimensions has been developed in the statistical program ‘nicheROVER’ (Newsome et al. 2007, Swanson et al. 2015). This method can quantify niche region and the probabilities of overlap within or among species and may help better assess species-specific resource partitioning and plasticity.

Several locations along the Beaufort Sea coast have been designated as marine protected areas (MPAs). The Tarium Niryuitait MPA consists of three regions, Niaqunnaq (including Shingle Point), Okeevik, and Kittigaryuit, and was established to protect Beaufort marine species and their supporting habitats (Gazette 2010). Efforts to conserve and protect the biota in this MPA require the establishment of effective monitoring. These monitoring programs must be founded on existing bodies of baseline research. This is particularly important for Shingle Point, where the ecology (diets/habitats) of the many fish species that use the area in summer months is poorly understood.

In this study, we used SI and FA data in conjunction with Bayesian and multivariate techniques to 1) characterize the niches of 16 co-occurring fish species from the coastal area of Shingle Point, 2) identify and quantify niche overlap among these species and group species based on their niche similarities, and 3) identify indicators for resource partitioning and niche shifts. Increased understanding of the Beaufort Sea coastal ecosystems can then be used to select key species and parameters relevant to the assessment of long-term ecosystem health of this MPA.

3.2 Materials and Methods

3.2.1 Sample Collection

Fish samples from 16 species (see Table 1) were collected under the auspices of the Arctic Coastal Ecosystem Study program (ACES, Department of Fisheries and Oceans) from coastal habitats at Shingle Point, Yukon Territory, Canada (Fig. 3.1), in each year between 2011 and 2013. The study species represent seasonally anadromous forms of salmonids that are important in subsistence fisheries as well as coastal (mixed low-salinity) and marine (high-salinity) species (Coad & Reist 2004).

Fish were collected in the brackish coastal environment of Shingle Point by local fisheries monitors and harvesters. Gill and seine nets allowed for the capture of a large range of fish sizes. The samples for this study were opportunistic, and collected in the months of July and August (2011 to 2013). Most fishes were vacuum-sealed and kept frozen (–18°C) on site before they were shipped to the Freshwater Institute (DFO, Winnipeg, Manitoba, Canada), for processing. Dolly varden char *Salvelinus malma* are an important traditional food for the Inuvialuit and Gwich'in peoples (Jarvela & Thorsteinson 1999). As such, the harvesters will normally prepare and consume the entire fish. Thus, muscle tissue for SI analysis was only taken from this species if local harvesters allowed us to sample their catches. FA analysis was not run for dolly varden char.

Table 3.1 Biological data of the 16 fish species captured at Shingle Point, YK. Sample size (n), (mean±SE) fork length (FL), carbon nitrogen ratio (C:N), % total lipid content (%TL), and stable isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) for each species. Are shown as well as ^{13}C and ^{15}N ranges. Species are grouped by family. N/A: information not available.

Species common name (code) *Scientific name*	n	FL (mm)	C:N	%TL	$\delta^{13}C$	$\delta^{13}C$ Range	$\delta^{15}N$	$\delta^{15}N$ Range	Habitat
Catostomidae									
Longnose sucker (LNSK) *Catostomus catostomus*	11	160.09 ± 22.60	3.33 ± 0.039	16.12 ± 2.21	-26.15 ± 0.46	3.79	9.64 ± 0.34	3.51	Freshwater/ brackish[a]
Clupeidae									
Pacific herring (PCHR) *Clupea pallasii*	18	277.50 ± 3.70	4.63 ± 0.30	37.42 ± 3.00	-23.79 ± 0.14	2.58	13.00 ± 0.11	2.09	Marine/ brackish[b]
Cottidae									
Fourhorn sculpin (FHSC) *Myoxocephalus quadricornis*	105	247.64 ± 5.27	3.42 ± 0.02	16.43 ± 1.00	-23.62 ± 0.08	6.7	15.18 ± 0.07	5.53	Marine[b]
Esocidae									
Northern pike (NRPK) *Esox Lucius*	9	426.33 ± 53.19	3.18 ± 0.06	9.94 ± 1.63	-26.84 ± 0.17	1.46	10.26 ± 0.20	1.72	Freshwater/ brackish[a]
Gadidae									
Saffron cod (SFCD) *Eleginus gracilis*	129	442.26 ± 2.38	3.33 ± 0.02	11.94± 0.56	-22.87 ± 0.03	1.99	15.47 ± 0.05	3.31	Brackish[b]

Burbot (BRBT) *Lota lota*	9	452.56 ± 87.12	3.42 ± 0.03	7.25 ± 1.20	-25.95 ± 0.51	4.39	10.26 ± 0.36	3.45	Freshwater/ Brackish[c]
Osmeridae									
Rainbow smelt (RBSM) *Osmerus mordax*	25	225.08 ± 5.96	3.43 ± 0.04	16.26± 1.92	-24.15 ± 0.09	1.85	13.96 ± 0.07	1.27	Anadromous[d]
Pleuronectidae									
Arctic flounder (ARFL) *Liopsetta glacialis*	97	192.89 ± 3.68	3.32 ± 0.01	18.40± 1.34	-24.01 ± 0.12	9.84	11.55 ± 0.07	4.45	Brackish/ marine/ freshwater [b]
Starry flounder (STFL) *Platichthys stellatus*	75	275.09 ± 4.60	3.31 ± 0.02	14.1 ± 1.22	-24.85 ± 0.13	5.94	12.42 ± 0.09	4.06	Brackish/ marine/ freshwater [b]
Salmonidae									
Arctic cisco (ARCS) *Coregonus autumnalis*	74	327.82 ± 7.33	3.65 ± 0.06	28.68± 2.38	-23.64 ± 0.17	8.25	12.35 ± 0.10	3.82	Anadromous[d]
Broad whitefish (BDWF) *C. nasus*	117	352.22 ± 10.38	3.45 ± 0.04	19.91± 1.27	-27.47 ± 0.25	13.57	9.10 ± 0.13	8.79	Anadromous[d]
Lake whitefish (LKWF) *C. clupeaformis*	113	251.65 ± 8.04	3.31 ± 0.03	13.77± 0.88	-25.94 ± 0.17	9.06	10.90 ± 0.10	5.47	Anadromous[d]
Least cisco (LSCS) *C. sardinella*	70	257.24 ± 3.86	3.23 ± 0.02	12.12 ± 2.68	-26.13 ± 0.11	10.96	11.84 ± 0.06	4.87	Anadromous[b]

Inconnu (INCN) *Stenodus leucichthys*	86	497.23 ± 10.65	3.28 ± 0.03	10.71 ± 0.88	-26.29 ± 0.09	4.78	12.78 ± 0.08	4.4	Anadromous[d]
Dolly varden char (DVCH) *Salvelinus malma*	37	448.62 ± 14.05	6.17 ± 0.31	N/A	-23.42 ± 0.27	9.48	13.88 ± 0.13	3.92	Anadromous[d]
Round whitefish (RDWF) *Prosopium cylindraceum*	73	131.59 ± 4.73	3.21 ± 0.02	11.04 ± 0.57	-25.79 ± 0.25	11.04	9.95 ± 0.11	4.86	Freshwater/ brackish[d]

[a] Scott and Crossman (1973); [b] Coad & Reist (2004); [c]Kottelat & Freyhof (2007); [d] Riede (2004)

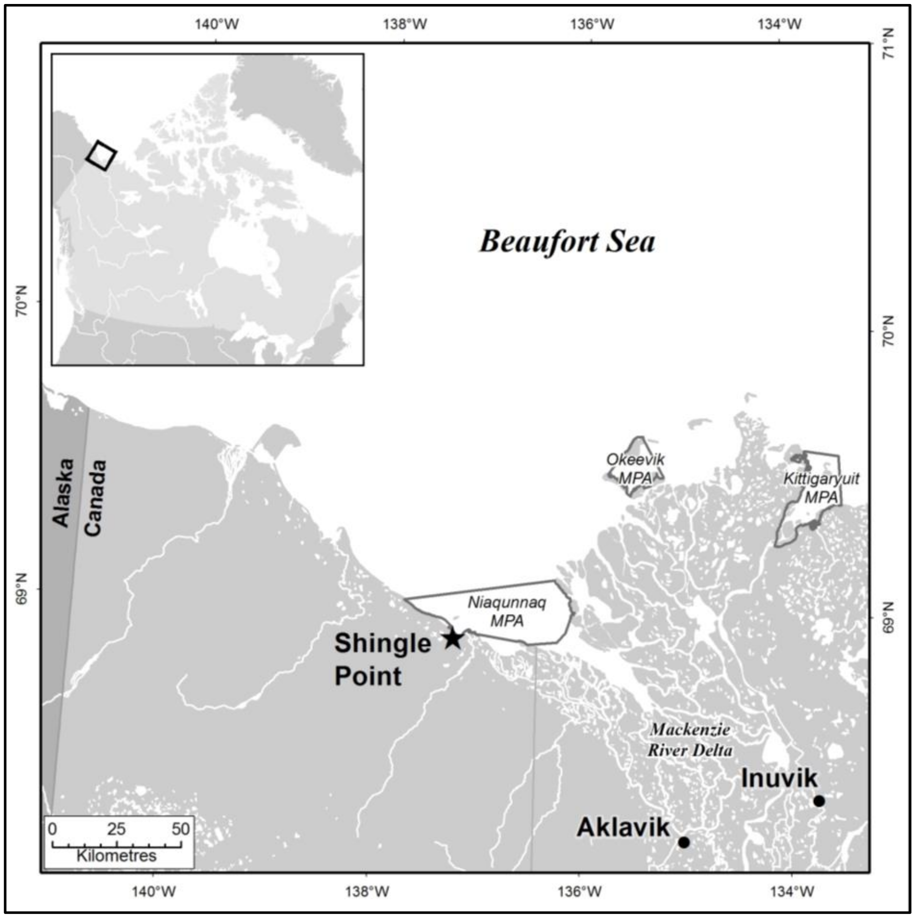

Figure 3.1 Map of the Tarium Niryutait Marine Protected Area including the Kittigaryuit, Niaqunnaq, and Okeevik regions. The study area Shingle Point is part of the Niaqunnaq area

3.2.2 Stable Isotope Analysis

Dorsal muscle tissue was removed from individuals of the 16 species of fish to be processed for SI analyses. Each sample was freeze-dried and ground into fine powder using a mortar and pestle. Samples were sent to the Environment Isotope Laboratory at the University of Waterloo, Ontario, Canada, where standard isotopic methods analyzed each sample for carbon (C) and nitrogen (N) stable isotope ratios on a Thermo-Finnigan Delta Plus continuous flow isotope mass spectrometer (Thermo Finnigan) equipped with a Carlo Erba Elemental Analyzer (CHNS-O EA1108, Carlo Erba). Stable nitrogen ($^{15}N/^{14}N$) and carbon ($^{13}C/^{12}C$) isotopic ratios relative to the international standards of atmospheric nitrogen (Mariotti 1983) and Vienna Pee Dee Belemnite (Craig 1957) for N and C, respectively, were expressed in standard notation (δ) measured as per mil (‰). Internal laboratory standards included a standard of cellulose for $\delta^{13}C$ (–25.5‰) and two standards of $(NH_4)_2SO_4$ for $\delta^{15}N$ (0.77 and 20.2‰). Repeatability of sample material for $\delta^{13}C$ and $\delta^{15}N$ was 0.1 and 0.2‰, respectfully, based on repeat analysis of duplicates (duplicates run every n = 10). Repeat measurements of laboratory standards cross-calibrated against the International Atomic Energy Agency standards CH_6 for C and N1 and N2 for N validated the analytical precision since error did not exceed 0.2 and 0.3‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively.

3.2.3 Fatty Acid Analysis

The FA samples comprised the lateral half of each fish cut lengthwise along the sagittal plane and excluded the gastrointestinal tract. Each sample was homogenized and stored in a –80°C freezer for a minimum of 24 h before being freeze-dried. The freeze-dried homogenate was sub-sampled and aliquots weighed (0.25 g), and a standard lipid extraction method using 2:1 chloroform-methanol containing 0.01% butylated hydroxytoluene (BHT) (v/v/w) was performed

(Folch et al. 1957). The lipid phase of each sample was obtained by using anhydrous sodium sulphate to wash, collect and dry each sample, and then the sample was evaporated under N_2. The lipid phase was then weighed and recorded for lipid weight before being treated with Hilditch reagent (0.5 N H_2SO_4 in methanol) resulting in transesterfication and FA methyl esters (FAMES) (Morrison & Smith 1964). Samples were heated at 100°C for 1 h. The techniques and processes in identifying FA peaks are outlined in more detail in Giraldo et al. (2015). Briefly, gas chromatography (on an Agilent Technologies 7890N GC equipped with a 30 m J&W DB-23 column) combined with a Flame Ionization Detector (FID) (running at 350°C) was used to identify FA compounds. Hydrogen was used as the carrier gas flowing at 1.25 ml min^{-1} for 14 min and ramped to 2.5 ml min^{-1} for 5 min. The split/splitless injector was heated to 260°C and run in splitless mode. The oven program was as follows: 60°C for 0.66 min, increase of 22.82°C min^{-1} to 165°C with a 1.97 min hold, 4.56°C min^{-1} to 174°C and 7.61°C min^{-1} to 200°C with a 6 min hold (Giraldo et al. 2015). Percent FAs were used for statistical analysis. A total of 73 FAs were identified, 25 of which accounted for 90% or more of the total. These 25 FA profiles (FA signatures) are expressed using the notation A:BnX, reported as percent of total FA (%), and were used to identify the general diet of the fishes.

3.2.4 Study Design and Statistical Analysis

Trophic level ($\delta^{15}N$) and habitat use/carbon source ($\delta^{13}C$) often vary with fish size and life stage (e.g. Peterson & Fry 1987, Bosley et al. 2002, Romanuk et al. 2011). As such, SI and FA analyses were thus limited to adult, non-spawning individuals. There was no significant relationship between $\delta^{13}C$ and fish size (fork length) observed ($r^2 < 0.3$, for all species) for adult, mature individuals (determined by visual inspection of gonads). In order to compare $\delta^{15}N$ among multiple fishes, $\delta^{15}N$ was normalized for each species separately. Species-specific residuals of the relationship between $\delta^{15}N$ and fork length for each individual were calculated and added to the mean $\delta^{15}N$ of the respective species, with analyses of $\delta^{15}N$ data performed on these size-corrected data (Swanson & Kidd 2010).

Lipid content can significantly affect $\delta^{13}C$ values and interpretation (Post et al. 2007). Since lipids were not chemically extracted from the dorsal muscle prior to SI analysis, a mathematical approach that incorporates the C to N ratio (C:N) within tissues was used (Post et al. 2007):

$$\delta^{13}C_{normalized=}\ \delta^{13}C_{untreated} - 3.32 + 0.99 * C:N \qquad (1)$$

All statistical analyses were performed in the R v.3.1.0 (R Core Team 2012). Ward's hierarchical clustering analysis with Euclidean distance generated groups from mean $\delta^{13}C$ and $\delta^{15}N$ values for each species using the package 'ade4'. Two-dimensional ($\delta^{13}C$ and $\delta^{15}N$) probabilistic (95%) regions for each of the groups identified from the Ward's clustering were developed using a Bayesian framework in the package 'nicheROVER' (Swanson et al. 2015). We also used nicheROVER to estimate probability of isotopic niche overlap (95% region) among species. Results for niche region size generated with the 40% probabilistic niche region are presented in Table S1 in the Supplement at www.int-res.com/articles/suppl/mXXXpXXX_supp.pdf. Here we define high niche overlap as a median overlap greater than 50% between two species. The default 'non-informative' prior was used in nicheROVER. Differences in niche centroid (defined by mean $\delta^{13}C$ and $\delta^{15}N$) among species within the three isotopic groups (A, B, and C) were investigated using the residual permutation procedure (RPP) metric mean distance (MD). Variations in species-specific isotopic niche positions were identified if Euclidean distance between niche centroids was significantly greater then zero (Turner et al. 2010).

The 25 FA signatures for all species studied, except dolly vaden char, were used to infer the general diet of each species (Table S2 in the Supplement). A Kruskal-Wallis test was used to analyze differences in percent total lipid content (%TL) among species. The relationship between fork length (mm) with FA profiles and %TL for each species was assessed using linear regression analysis. For statistical and modeling analyses some FAs were combined. The FA markers for *Calanus* copepods 20:1n9, 20:1n11 and 22:1n9, 22:1n11 were combined as FA *Calanus* (e.g. Falk-Petersen et al. 2002). The 16 PUFAs (polyunsaturated fatty acids) included: 16:2n6, 16:2n4, 16:3n4, 16:4n3, and 16:4n1. The 18PUFAs included: 18:2n7, 18:2n6, 18:2n4, 18:3n6, 18:3n4, 18:3n3, 18:3n1, 18:4n3, and 18:4n1. Essential FAs found in freshwater fish: 18:3n3 and 18:2n6 were separated from the 18PUFAs (Tocher 2010). Finally, non-methylene interrupted (NMI) FAs, indicative of bivalves or gastropods (Budge et al. 2006) included: 20:2n, 20:3n, 22:2n, and 22:3n.

Correspondence analysis (CA) was used to compare FA signatures among species using the packages ‘Factoshiny’ and ‘FactoMineR’. Greatest explained variance was indicated in the CA using the 25 FAs. To group individuals based on similar FA compositions, a Ward’s cluster analysis was performed using mean FA signatures and two significant CA axes. This was done in the package ‘hclust’. The FA groups generated from the Ward’s cluster analysis were characterized by significant high and low proportions of FA signatures indicated by a v-test. The probability of niche overlap within groups (identified by the isotopic Ward’s cluster analysis) was re-evaluated using both the mean SI signatures and mean dietary FA profiles identified by the v-test. The FA signatures included as variables in the probability of niche overlap calculation were: 20:3n6, 20:2n6, 21:5n3, 18:2n6, *Calanus* FA and 22:6n3.

3.3 Results

3.3.1 Stable Isotopes

Relatively wide variability in $\delta^{13}C$ and $\delta^{15}N$ was observed among the 16 species of fish (n = 1048) (Table 1). The $\delta^{13}C$ ranges among all species was (mean ± SE, 4.6 ± 0.22‰). The species most depleted in ^{13}C was broad whitefish *Coregonus nasus* (–27.47 ± 0.25‰), and the species most enriched in ^{13}C was saffron cod *Eleginus gracilis* (–22.87 ± 0.03‰) (Table 3.1). Our results suggest a span of approximately two trophic levels identified among all species with a $\delta^{15}N$ range of 6.37‰; the lowest values were observed in broad whitefish (9.10 ± 0.13‰) and highest in saffron cod (15.47 ± 0.05‰) (Table 3.1).

3.3.2 Stable Isotope Determined Groups

Based on the SI values (Fig. 3.2), Ward's cluster analysis identified 'isotopic groups' among the 16 species (Table 3.1, Fig. 3.3). The species in Group A were characterized by more enriched ^{13}C and ^{15}N, and included: fourhorn sculpin *Myoxocephalus quadricornis*, saffron cod, Pacific herring *Clupea pallasii*, dolly varden char, and rainbow smelt *Osmerus mordax*. Group B was characterized by depleted ^{13}C and lower $\delta^{15}N$ ratios and included: broad whitefish, longnose sucker *Catostomus catostomus*, burbot *Lota lota*, round whitefish *Prosopium cylindraceum*, lake whitefish *Coregonus clupeaformis*, and northern pike *Esox lucius*. The species in group C had intermediate $\delta^{13}C$ and $\delta^{15}N$ compared to the other groups, and included: inconnu *Stenodus leucichthys*, least cisco *Coregonus sardinella*, Arctic flounder *Liopsetta glacialis*, Arctic cisco *Coregonus autumnalis* and starry flounder *Platichthys stellatus* (Fig. 3.4).

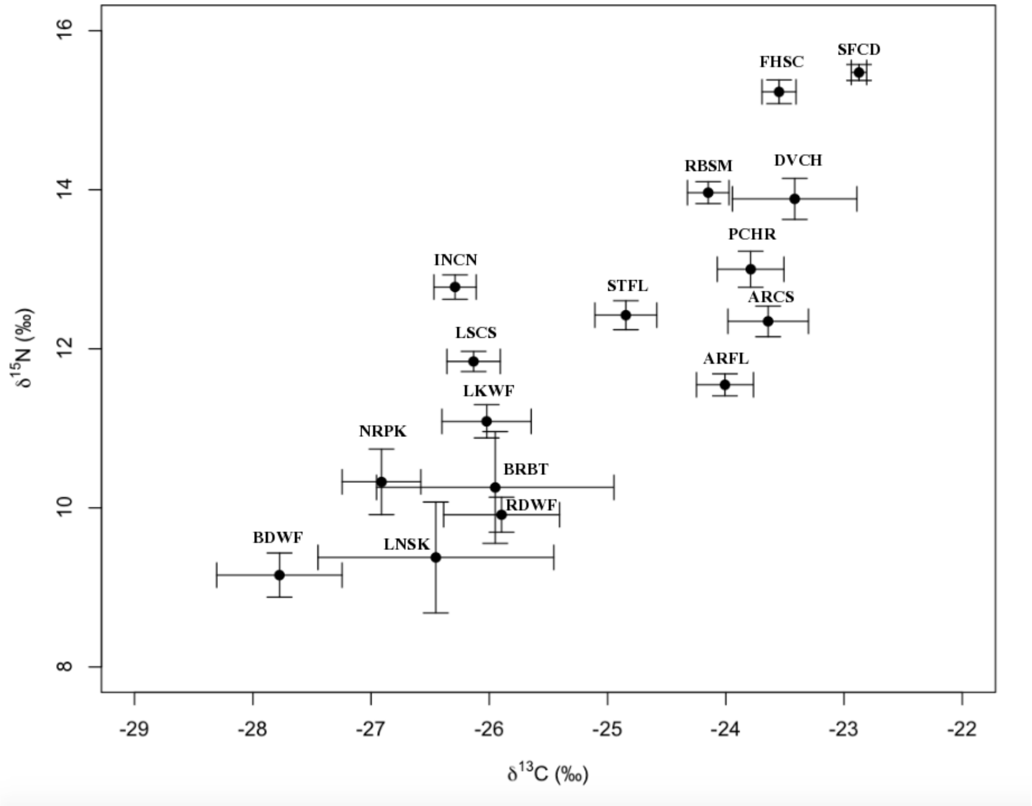

Figure 3.2 Stable isotope biplot indicating the mean $\delta^{13}C$ and $\delta^{15}N$ position of the 16 species captured at Shingle Point. See Table 3.1 for species names and codes. The SE is indicated by error bars.

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3.3.3 Isotopic Niche Assessment

Among the three isotopically defined groups (Fig. 3.3), the greatest niche overlap was observed between Groups B and C (Table 3.2, Fig. 3.4). Probabilistic niche regions (95% level of inclusion) ranged from the narrow niche of rainbow smelt (2.93) to the broad niche of broad whitefish (73.04), (Table 3.2). The species with the broadest probabilistic 2-dimensional niches were dolly varden char, broad whitefish, and Arctic cisco (Fig. 3.4).

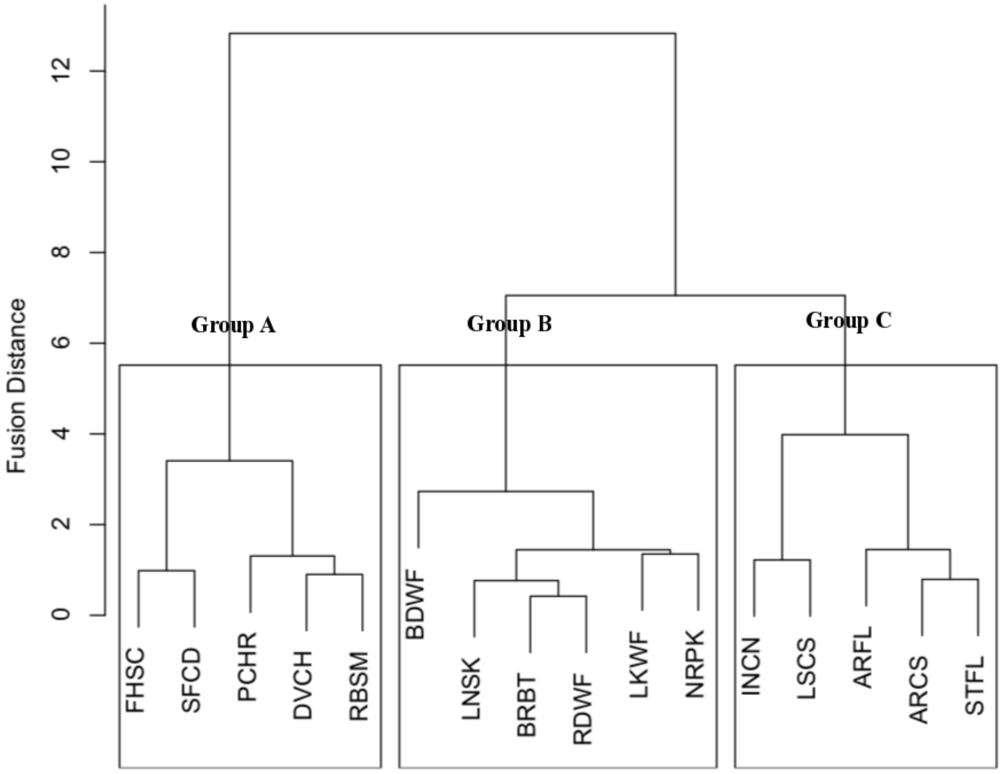

Figure 3.3 Dendrogram of the 16 species of fish captured at Shingle Point, produced from Ward's cluster analysis using the mean isotopic signatures ($\boldsymbol{\delta}^{13}$C and $\boldsymbol{\delta}^{15}$N) as variables. See Table 3.1 for species names and codes.

Table 3.2 Results of the probabilities of niche overlap among the fishes in Groups A, B, and C (see Fig. 3.2 for the corresponding species and Table 3.1 for abbreviations). The mean probability indicates the probability of Species A niche being found within the niche of Species B. The probability of overlap is indicated by mean and credible intervals (2.5%, 97.5%). The niche overlaps were calculated using alpha=0.95. 95% isotopic ellipses or niche region size (alpha=0.95) of each species was calculated using isotopic signatures (δ^{13}C and δ^{15}N) and recorded (‰). N/A: information not available.

Species B (95% niche region size)	Species A	SI Mean Probability [Credible Interval]	FA and SI Mean Probability [Credible Interval]
Group A			
DVCH (24.86)	FHSC	67.49 [45.15, 88.89]	N/A
	PCHR	95.36 [80.75, 99.97]	N/A
	RBSM	99.93 [99.36, 100.00]	N/A
	SFCD	64.42 [37.05, 91.02]	N/A
FHSC (11.45)	DVCH	41.05 [26.87,56.45]	N/A
	PCHR	15.08 [2.47,37.33]	16.53 [2.80, 39.00]
	RBSM	92.02 [75.68, 99.39]	92.13 [76.19, 99.60]
	SFCD	98.48 [95.86, 99.76]	98.82 [96.00, 100.00]
PCHR (5.61)	DVCH	32.29 [18.12,51.22]	N/A
	FHSC	5.43 [1.21,15.86]	6.11 [1.29, 17.40]
	RBSM	45.05 [12.37, 87.65]	47.68 [13.30, 90.70]
	SFCD	1.45 [0.08, 7.20]	0.95 [0.00, 5.50]
RBSM (2.93)	DVCH	26.68 [16.83, 39.12]	N/A
	FHSC	20.32 [11.74, 32.57]	20.36 [12.10, 32.40]
	PCHR	22.33 [6.45, 47.37]	23.70 [6.90, 46.32]
	SFCD	3.63 [0.65, 11.29]	3.23 [0.40, 9.90]
SFCD (4.11)	DVCH	12.72 [7.07, 19.77]	N/A
	FHSC	46.73 [38.03, 55.88]	48.19 [37.79, 58.30]
	PCHR	0.88 [0.01, 4.38]	0.52 [0.00, 2.91]
	RBSM	5.40 [0.68, 15.55]	4.71 [0.40, 16.31]
Group B			
BDWF (73.04)	BRBT	95.94 [81.95, 99.94]	41.04 [21.48, 61.50]
	LKWF	90.84 [80.97, 97.25]	46.42 [33.76, 59.01]
	LNSK	98.06 [89.92, 99.99]	71.77 [42.38, 93.94]
	NRPK	99.86 [98.66, 100.00]	37.32 [18.37, 58.59]
	RDWF	97.15 [92.44, 99.50]	68.61 [56.19, 80.06]
BRBT (31.23)	BDWF	48.51 [28.46, 73.80]	0.05 [0.00, 0.13]
	LKWF	77.06 [55.81, 94.38]	0.08 [0.01, 0.20]
	LNSK	79.29 [51.44, 98.04]	0.00 [0.00, 0.00]
	NRPK	98.06 [85.56, 100.00]	0.56 [0.00, 2.06]
	RDWF	77.26 [57.45, 94.52]	0.08 [0.01, 0.21]
LKWF (35.49)	BDWF	51.34 [40.80,62.67]	36.51 [25.51, 48.15]
	BRBT	91.59 [72.15,99.49]	69.71 [40.67, 90.66]

	LNSK	82.07 [59.27,96.51]	81.07 [52.93, 96.99]
	NRPK	99.50 [95.62,100.00]	77.44 [52.31, 96.06]
	RDWF	85.75 [75.78,93.43]	80.45 [69.74, 89.19]
LNSK (31.97)	BDWF	58.46 [39.06,80.42]	0.01 [0.00, 0.04]
	BRBT	85.00 [57.30,99.25]	0.00 [0.00, 0.00]
	LKWF	72.20 [47.08,93.54]	0.02 [0.00, 0.07]
	NRPK	97.77 [83.31,100.00]	0.00 [0.00,0.02]
	RDWF	81.47 [63.27,95.88]	0.06 [0.00, 0.15]
NRPK (5.57)	BDWF	13.34 [6.98, 24.30]	0.00 [0.00, 0.02]
	BRBT	31.70 [13.23, 58.29]	0.03 [0.00, 0.19]
	LKWF	25.65 [14.43, 43.05]	0.01 [0.00, 0.04]
	LNSK	27.69 [11.39, 51.88]	0.00 [0.00, 0.01]
	RDWF	24.68 [14.17, 40.65]	0.01 [0.00, 0.04]
RDWF (36.30)	BDWF	65.58 [54.37, 76.99]	38.51 [28.11, 49.39]
	BRBT	91.88 [72.90, 99.56]	46.69 [24.01, 69.84]
	LKWF	83.36 [72.23, 92.63]	55.66 [43.87, 67.40]
	LNSK	90.67 [72.44, 99.19]	90.78 [68.74, 99.48]
	NRPK	99.26 [94.01, 100.00]	43.01 [21.82, 67.40]
Group C			
ARCS (23.89)	ARFL	92.74 [83.69, 98.20]	93.64 [85.69, 98.60]
	INCN	72.82 [47.99, 92.10]	73.81 [50.09, 92.60]
	LSCS	80.39 [60.10, 94.40]	80.28 [61.69, 95.50]
	STFL	93.25 [85.60, 98.30]	94.94 [87.60, 99.20]
ARFL (15.71)	ARCS	73.43 [61.50, 84.30]	67.01 [55.40, 79.30]
	INCN	27.87 [11.19, 50.91]	23.52 [8.90, 44.10]
	LSCS	72.63 [52.80, 88.40]	70.99 [51.89, 88.01]
	STFL	73.18 [60.00, 84.71]	72.34 [56.80, 85.60]
INCN (11.62)	ARCS	19.00 [10.80, 29.00]	19.17 [11.40, 28.80]
	ARFL	10.57 [5.20, 18.00]	10.19 [4.80, 17.80]
	LSCS	72.20 [56.70, 86.21]	72.23 [55.39, 85.20]
	STFL	45.54 [32.70, 58.70]	50.06 [36.19, 64.00]
LSCS (9.82)	ARCS	30.10 [19.30, 43.30]	29.71 [19.09, 42.71]
	ARFL	32.67 [21.10, 45.50]	33.16 [21.90, 46.40]
	INCN	55.24 [40.39, 71.00]	54.68 [40.40, 70.50]
	STFL	58.08 [43.08, 71.21]	63.45 [47.60, 77.41]
STFL (17.62)	ARCS	76.34 [63.40, 87.40]	70.14 [57.20, 83.71]
	ARFL	75.56 [61.00, 87.90]	71.61 [53.70, 87.50]
	INCN	85.07 [69.40, 95.30]	81.76 [65.50, 93.90]
	LSCS	89.77 [78.30, 97.80]	81.37 [66.00, 94.20]

We expected that probability of overlap would be high among all species within each group; however, considerable overlap (i.e. > 50%) was only observed within Group A. All species in Group A overlapped with the large niche region of dolly varden char (24.86), whereas rainbow smelt and saffron cod overlapped considerably with fourhorn sculpin (Table 3.2, Fig. 3.4a). The niche region size of Pacific herring (5.61; Table 3.2) was similar to saffron cod (4.11) and rainbow smelt (2.93). The lower probability of isotopic niche overlap of other Group A species onto the isotopic niches of Pacific herring, rainbow smelt, and saffron cod can be attributed to the narrow $\delta^{13}C$ range and $\delta^{15}N$ range that was observed for each of these species (Table 3.1 & 2). Additionally, the lower $\delta^{15}N$ of Pacific herring separated this species from others in Group A (Fig. 3.4a). The narrow $\delta^{13}C$ range for saffron cod and rainbow smelt fell within the larger niche of fourhorn sculpin, which resulted in a 98.48% and 92.02% probability of overlap of saffron cod and rainbow smelt onto fourhorn sculpin, respectively (Table 3.2). Niche centroids differed among all species (MD > 0.74, $p < 0.03$ for all).

Within a group, the highest probabilities of niche overlap (>50%) were observed among Group B fishes; this was due to similar mean isotopic ratios and similar niche region sizes (Table 3.2). Probabilistic niche region sizes were similar among burbot (31.23), lake whitefish (35.49), longnose sucker (31.97), and round whitefish (36.30). Broad whitefish and northern pike had the largest and smallest niche region size 73.04 and 5.57, respectively (Table 3.2). Although the isotopic niche for northern pike is located at the center of all species in Group B, the small isotopic niche of northern pike (5.57) resulted in relatively low probability of other Group B species overlapping onto the northern pike niche (Fig. 3.4b, Table 3.2). Differences among Group B niche centroids only existed between broad whitefish and both lake whitefish (MD = 2.49, $p = 0.00$) and round whitefish (MD = 1.70, $p = 0.00$), and between lake whitefish and longnose sucker (MD = 1.66, $p = 0.00$), northern pike (MD = 1.32, $p < 0.01$), and round whitefish (MD = 1.01, $p = 0.00$).

High probability of niche overlap was observed among most fishes in Group C with the exception of inconnu (see Group C in Table 3.2). Inconnu were characterized by a relatively small niche region size (11.62), narrow $\delta^{13}C$ range and depleted ^{13}C (Table 3.1), and higher $\delta^{15}N$ relative to other fishes in this group (Fig. 3.4c). Arctic cisco (23.89), starry flounder (17.62), and Arctic flounder (15.71) had the largest niche regions compared to other Group C species and showed similar $\delta^{13}C$ and $\delta^{15}N$ ranges (Table 3.2, Fig. 3.4c). In turn, this resulted in high probability of finding other Group C species within these isotopic niches. Niche centroids differed among all species (MD < 0.71, $p > 0.02$ for all).

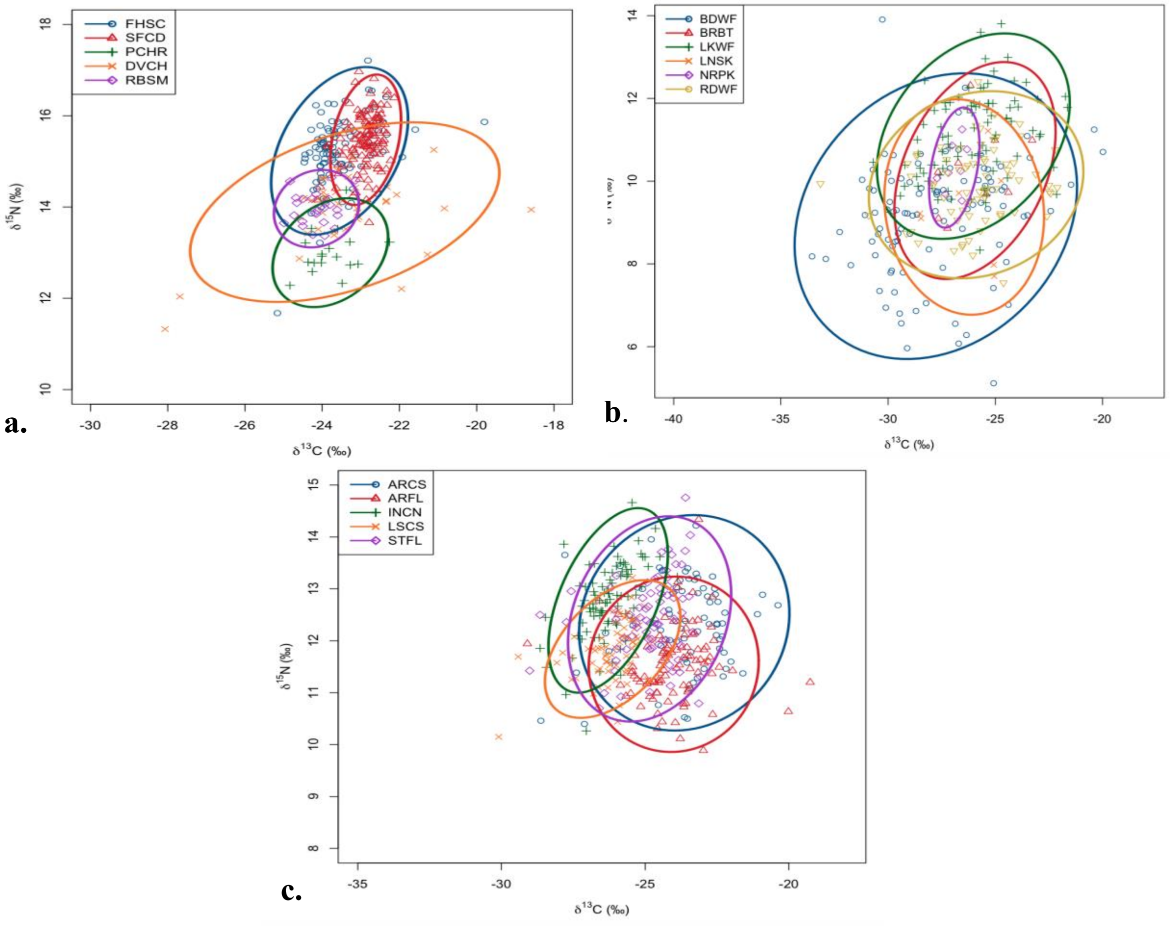

Figure 3.4 The 95% isotopic ellipses of the 16 species of fish captured at Shingle Point, seperated into three groups (generated from the Ward's cluster analysis). See Fig. 3. 3. (a) Group A; (b) Group B; (c) Group C. See Table 3.1 for species names and codes.

3.3.4 Fatty Acid Analysis

Differences in %TL existed among species (Kruskal-Wallis chi-squared = 189.21, df = 14, $p < 0.05$), and ranged from (mean ± SE) 7.25 ± 1.20% for burbot to 37.42 ± 3.00% for Pacific herring (Table 3.1). Within-species no significant relationship was observed between fork length and %TL ($r^2 < 0.48$, $p < 0.97$ for all) and between fork length and most FA profiles. Northern pike did indicate some relationship between fork length and 20:4n6 ($r^2 = 0.54$, $p = 0.02$), however, this relationship was not strong.

To assess how FAs defined species groups, a CA was performed on 15 species of fish (n = 1011) processed for FAs. The total variance of the first and second dimensions of the CA was 57.45%, where the first dimension explained 38.67% of the variance and the second dimension explained 18.78% of the variance. Dimension 1 illustrated a marine pelagic–freshwater gradient, with the greatest influence on the positive side (+) for *Calanus*-markers (pelagic), and docosahexaenoic acid (DHA, 22:6n3) and palmitoleic acid (16:1n7) on the negative side (–) (Fig. 3.5a) Pacific herring and Arctic cisco were most associated with the positive dimension 1. This was consistent with the high content of *Calanus*-markers in these species (~12–23% of total FAs for Arctic cisco and Pacific herring, respectively). *Calanus*-markers were also found in inconnu, least cisco, and round whitefish tissues, but to a lesser extent (~1–7%, Table S2 in the Supplement) (Fig. 3.5b).

The second dimension of the CA was characterized by DHA, *Calanus*-markers, oleic acid (18:1n9), linoleic acid (18:2n6), and alpha-linoleic acid (18:3n3) (Fig. 3.5a). Lake whitefish, followed by least cisco and Arctic cisco, were most associated with the second dimension (5b). Results indicate that 1) Arctic flounder and starry flounder were highly associated with the benthic marker NMI 2) burbot was highly associated with the FA arachidonic acid (20:4n6) 3) broad whitefish, lake whitefish, and round whitefish had relatively greater proportions of 18:2n6 and 18:3n3 (Table S2 in the Supplement, Fig. 3.5a), and 4) Pacific herring and starry flounder had the greatest proportions of 16:1n7 (Table S2) and Pacific herring had the lowest proportions of DHA.

3.3.5 Groups Inferred from Fatty Acid Analysis

A hierarchical analysis on the first two dimensions of the CA identified groups of species based on similarities of FA signatures. A total of five groups were identified (Fig. 6) using the 25 FA profiles described earlier for each species. The FA composition of the one fish species (Pacific herring) in Group 1 was characterized by high proportions of *Calanus*-markers and low proportions of the essential FA DHA (Table 3.3) Group 2 (also 1 species; burbot) was characterized by high proportions of 20:3n6 and 20:2n6, followed by 20:4n6, and low proportions of 16:1n7 (Table 3.3). The proportions of FA were not distinct enough to describe diets of fish within Group 5 (rainbow smelt, northern pike, least cisco, inconnu, saffron cod, and Arctic cisco), however higher levels of *Calanus*-markers (compared to Groups 2, 3, and 4) and combined benthic markers found in these fishes (Table S2) suggest feeding in both pelagic and benthic zones. Fish species in Group 3 (broad whitefish, round whitefish, lake whitefish, and longnose sucker) were characterized by high proportions of heneicosapentaenoic acid (21:5n3) and 18:2n6 and low proportions of *Calanus*-markers (Table 3.3). High proportions of 21:5n3 and low proportions of DHA characterized the FA composition of Group 4 (fourhorn sculpin, Arctic flounder, starry flounder; Table 3.3).

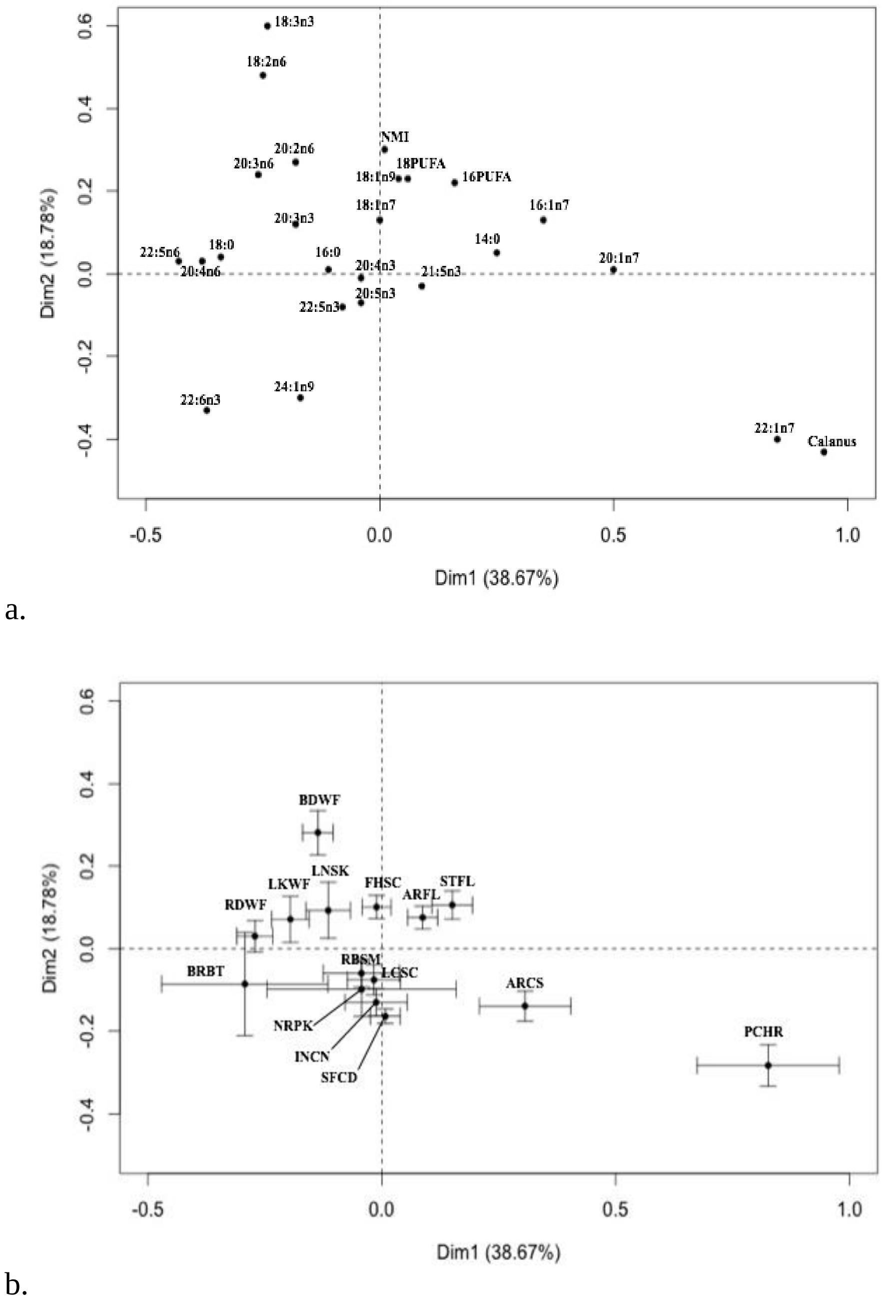

Figure 3.5 Correspondence analysis on the (a) 25 fatty acid signatures of (b) 15 species of fish captured at Shingle Point. Bars represent the SE for each species and were included to represent the spread of individuals within each species. See Table 3.1 for species names and codes

3.3.6 Fatty Acid and Stable isotope Group Niche Assessment

Probabilities of niche overlap within the three isotopic groups previously identified by the cluster analysis (Fig. 3.3) were re-analyzed with SI and FA data combined. The dietary FA signatures that characterized the five FA groups: 20:3n6, 20:2n6, 18:2n6, 21:5n3, 22:6n3, and *Calanus*-markers (Table 3.3) were combined with the isotopic data, and probabilities of niche overlap were re-calculated using a total of 8 dimensions of data. The probabilities of niche overlap using SI and FA data together only marginally differed from the results of the overlap in Groups A and C previously determined using only SI data (Table 3.2). For Group B, the probability of niche overlap decreased considerably when key dietary FA profiles were combined with SI data (Table 3.2). The SI data alone indicated that Group B had the greatest niche overlap among species, but when FA data were included, high (>50%) niche overlap was restricted to various combinations of longnose sucker, burbot, northern pike, and the three species of whitefish (Table 3.2).

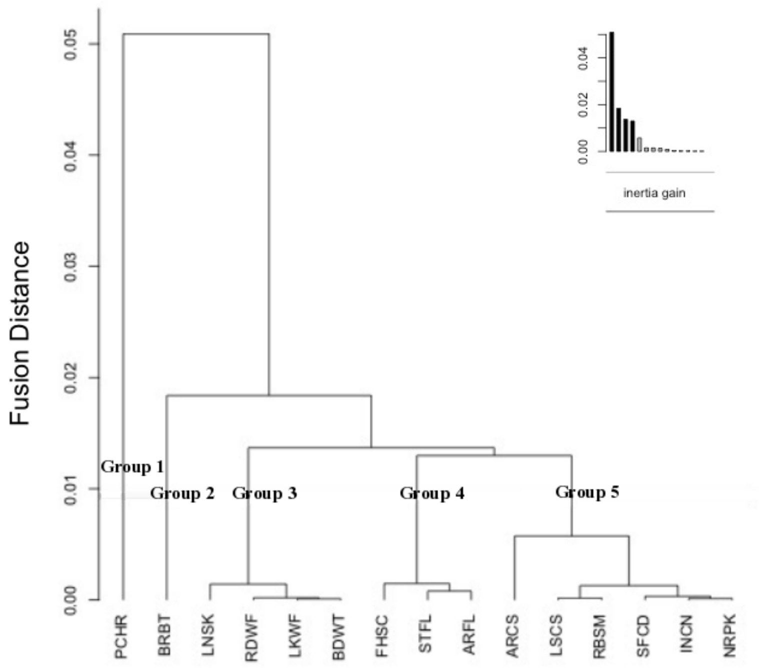

Figure 3.6 Hierarchical Ward's cluster analysis performed on a correspondence analysis of 25 fatty acid profiles. The 15 species of fish were grouped based on similarities using the mean fatty acid compositions. The five groups were characterized by high fatty acid (+) and low fatty acid (-) proportions indicated by the v.test (see Table 3.3). See Table 3.1 for species names and codes

Table 3.3 Results from the Ward's cluster and correspondence analysis on fatty acids (FA) profiles. The 15 species of fish were used and grouped based on similar FA signatures. Groups 1, 2, 3, 4, and 5 are characterized by the highest to lowest proportions of FA, indicated by the highest to lowest (-) v.test values, respectively. Infinite values indicated by Inf. Null indicates FAs were unable to characterize the group.

FA cluster groups	Species	FA	V-test	P-value
Group 1	Pacific herring	Calanus	5.86	0
		22:6n3	-2.81	0
Group 2	burbot	20:3n6	Inf	0
		20:2n6	Inf	0
		20:4n6	3.19	0
		16:1n7	-2.44	0.01
Group 3	broad whitefish, round whitefish, lake whitefish, longnose sucker,	21:5n3	Inf	0
		18:2n6	2.05	0
		Calanus	-4.71	0
Group 4	fourhorn sculpin, Arctic flounder, starry flounder	21:5n3	Inf	0
		22:6n3	-2.40	0.02
Group5	rainbow smelt, northern pike, least cisco, inconnu, saffron cod, Arctic cisco	Null	Null	Null

3.4 Discussion

Characterizing diet and habitat ecology of co-occurring freshwater, coastal, and anadromous fishes in summer Beaufort Sea coastal environments will further our understanding of prey sources and partitioning of resources in the study species and environment (Post 2003, Layman et al. 2007, Swanson et al. 2015). These results may be used to indicate future shifts in species-specific niches, understand implications of climate change on important habitat features (Carmack & Macdonald 2002, Kelly & Scheibling 2012), develop monitoring programs, and inform testable hypotheses for competitive interactions among species.

3.4.1 Habitat and diet niche overlap

A total of three distinct isotopic groups and five FA-based dietary groups identified the general habitat and feeding ecologies of the 16 fish species studied. The $\delta^{13}C$ ranges and $\delta^{15}N$ ranges found here suggest that these species are feeding across a range of salinities, in both pelagic and benthic habitats, and that the species assemblage examined represents approximately two trophic levels (Post 2002, Hill et al. 2006, Cherel et al. 2011). The FA analyses further demonstrated the importance and heavy reliance on benthic prey sources. we recognize potential problems when interpreting dietary FAs from species with low %TL (<10%); however, since FA interpretations are supported by SI results and previous literature-based stomach analyses, and samples were limited to adult non-spawning individuals, we are confident FA interpretations are representative of the species diet.

Group A had enriched isotopic ratios and was isotopically delineated as marine fishes. High variability among species in $\delta^{15}N$ along with narrow species niches resulted in the lowest niche overlap among species. The relatively ^{13}C-enriched muscle tissue in dolly varden char compared to other species reinforces the fact that, although dolly varden char move to freshwater habitats for spawning and overwintering (Reist 1989, Reist & Sawatzky 2010), the majority of feeding is occurring in the marine environment (Morita et al. 2009, Courtney et al. 2016). Some consumption of fresh- or brackish-water prey by dolly varden char likely explains the larger $\delta^{13}C$ range observed for this species relative to other Group A species. The small isotopic niche region and pelagic marine feeding ecology with a diet likely comprised primarily of *Calanus*, or *Calanus* consumers (Group 1) is consistent with previous findings (Dahl et al. 2000, Coad & Reist 2004), and resulted in Pacific herring having lower overlap with other Group A species. The isotopic niche of rainbow smelt was narrow, indicating low within-species diet variability. It is likely that fish and crustaceans are a resource shared among rainbow smelt, fourhorn sculpin, and saffron cod (Lacho 1991, Coad & Reist 2004), as indicated by benthic feeding inferred from FAs for Group 5 species.

Group B species were characterized as freshwater-rearing fish, and had depleted ^{13}C and benthic and nearshore FA indicators. Similar δ^{13}C ranges and δ^{15}N ranges among species within Group B indicate shared habitat use and foraging strategies (Hesslein et al. 1991, Coad & Reist 2004, Gallagher & Dick 2015) and resulted in high isotopic overlap. A large δ^{13}C range and the most depleted ^{13}C suggest that broad whitefish is feeding upon a variety of prey in multiple habitats. The freshwater input of the Mackenzie River introduces terrestrial carbon into the Mackenzie estuary and coastal environments (Saupe et al. 1989, Schell et al. 1989, Dunton et al. 2012); thus, the nearshore and benthic FA signatures 18:2n6 and 18:3n3 (found in green algae and vascular plants, Kelly & Scheibling 2012) are reflected in FA in tissues of Group 3 species (broad whitefish, round whitefish, lake whitefish, and longnose sucker) (Steffens 1997, Carmack & Macdonald 2002). These freshwater influences and benthic feeding strategies are consistent with known diets for these fishes, i.e. amphipods, crustaceans and bivalves (Ackman 1967, Graeve et al. 1997, Kharlamenko et al. 2008, Legezynska et al. 2014). Low proportions of *Calanus*-markers (Falk-Petersen et al. 2002, Hill et al. 2006) in Group 3 fishes further supports benthic feeding for this group.

Group C isotopically defined species were characterized as ‘coastal’ fish, and were characterized further as benthic feeders as indicated by Group 4 (i.e. Arctic flounder and starry flounder) and both pelagic and benthic feeders by Group 5 (i.e. inconnu, least cisco, Arctic cisco). These species are known benthic and shallow-water foraging fish that feed at higher trophic levels on fishes and crustaceans (Hesslein et al. 1991, Jarvela & Thorsteinson 1999, Coad

& Reist 2004, Logerwell et al. 2015); similar feeding strategies coincide with the large overlap among Group C fishes. Arctic cisco had the broadest niche region in this group, and had high proportions of benthic and pelagic FAs and high δ^{13}C ranges, indicating feeding both in the benthic and pelagic zones. Arctic cisco also appeared to feed at both higher (i.e. crustaceans and small fishes), and lower (i.e. *Calanus*) trophic levels (Coad & Reist 2004, Dunton et al. 2006, Von Biela et al. 2013). This is in contrast to starry flounder, a species that shares a similar isotopic niche size but narrow feeding strategy observed in Group 4 species. Group 4 species characterized as benthic feeders had high proportions of 21:5n3 and FA profiles with an origin linked to marine algae and also the diatom species *Skeletonema costatum* (Mayzaud & Ackman 1978). These FA profiles, along with greater NMI markers (indicative of feeding on bivalves and gastropods, Budge et al. 2006), suggest feeding on the benthos.

3.4.2 Partitioning of resources

The ability to partition resources through habitat and trophic segregation is an important feature in ecologically similar species and serves as a measure of interspecific competition and plasticity (Schoener 1974). Variation in δ^{13}C observed across species likely indicates variation in habitat use of benthic vs. pelagic prey and differences in use of marine, brackish, and freshwater environments over the last several months (e.g. Fry & Sherr 1984, Peterson & Fry 1987, Cherel et al. 2011), since at the time of collection (July–August) these species shared the same coastal environment. This variation also reflects known differences in life history among species that are known to be anadromous (i.e. dolly varden char, Arctic cisco, Coad & Reist 2004, Morita et al. 2009), those that obviously migrated to the coastal environment but are usually characterized as freshwater species (i.e. northern pike, broad whitefish), and those that are coastal residents species (i.e. saffron cod, flounders) (Hesslein 1993, Coad & Reist 2004).

Estuarine and coastal environments at higher latitudes remain relatively unstudied in regard to understanding structure of the benthic communities, importance of terrestrial carbon and how it is assimilated, overall trophic structure, and resource partitioning among co-occurring freshwater, anadromous and marine fish species (Reist et al. 2006, Dunton et al. 2006, 2012). The high probability of overlap and large reliance on benthic resources within FA groupings (excluding Pacific herring [Group 1] and Arctic cisco [Group 5]) likely indicates that benthic prey are more abundant/available then pelagic prey, as indicated by previous studies (Kharlamenko et al. 2008, Dunton et al. 2012, Kelly & Scheibling 2012).

Known generalist feeding strategies of some Beaufort Sea fishes (e.g. broad whitefish, Arctic cisco) is optimal in Arctic marine ecosystems where productivity is generally low (Hesslein et al. 1993), McLaughlin & Carmack 2010, Varela et al. 2013). Similar isotopic ratios and large niche regions within Group B were observed for burbot, longnose sucker, lake whitefish, and round whitefish. Broad niche regions observed in both Group B and C species (e.g. broad whitefish, Arctic cisco) can act as an indicator for plasticity in habitat and diet resource partitioning (Layman et al. 2007, Svanback & Bolnick 2007).

We expected to find a smaller probability of niche overlap when combing both SI and key dietary FAs (identified by the CA). Results indicated that the addition of FAs did not alter the probabilities of niche overlap for Groups A and C, but that these data did in general lower probabilities of niche overlap among species in Group B. This likely indicates that, although species in Group B may share habitat and trophic levels, specific prey sources differ. For all groups, FAs better defined the diets of each species and highlighted the importance of benthic prey resources for the fish populations at Shingle Point.

3.4.3 Use of niche metrics in monitoring (MPAs and elsewhere)

Studies of the direct effects of climate change on Arctic marine ecosystems (e.g. warming temperatures and change in productivity) can inform our understanding of the resilience and resistance in different Arctic fish species, and these investigations can be relatively straightforward. The secondary effects of climate change, such as changes in larger ecosystem structure and drivers (i.e. top-down and bottom-up control) are less known and more intractable to strong inference studies (Reist et al. 2006, Baber et al. 2012), making it difficult to predict species responses. Further understanding of basic habitat and diet ecology, and inter- and intra-specific competition and resource partitioning will aid in developing effective and efficient monitoring programs for fish populations in MPAs. Presently, whether benthic prey will be a limiting factor is unknown; however, shifts in primary production to pelagic sources are expected to have an effect on primary consumers in Beaufort Sea shelf habitats (Carmack et al. 2004, Arrigo et al. 2008). Species with more narrow niches may be more susceptible to environmental changes (Layman et al. 2007, Svanbäck & Bolnick 2007, Turner et al. 2010). The susceptibility of species with narrow niches to environmental changes (e.g. reduced prey availability) has been observed in species including European perch *Perca fluviatilis* (Svanbäck & Persson 2004) and sea birds (e.g. *Calonectris diomedea*, Ceia et al. 2014). Narrow niches can also reflect specialist feeding strategies in piscivores (e.g. northern pike). Since the signatures of prey are reflected in consumer's tissues, the high habitat and diet overlap observed for these predatory fish with other co-occurring fish species can reflect significant changes in prey species (McCann et al. 2005, Guzzo et al. 2016), and act as potential indicators to niche shifts in prey availability and/or predator choice.

3.5 Conclusion and Future Directions

3.5.1 Future Shingle Point Sampling

Identifying and understanding how fish species partition resources in the coastal Canadian Beaufort Sea is important for understanding trophic interactions in this highly dynamic and sensitive environment. Both marine and anadromous fishes in the Beaufort Sea are culturally important and serve as a direct food source to the Inuvialuit people (Papik et al. 2003); they also play an important role as the main prey of beluga whales *Delphinapterus leucas* and seal species (Loseto et al. 2008, 2009, Harwood et al. 2014). Knowledge gaps remain, however, regarding trophic interactions, habitat use, and ecological niches occupied by marine and anadromous fishes (Reist & Bond 1988, Chiperzak et al. 2003, Coad & Reist 2004) in the nearshore Beaufort Sea. This makes it difficult to predict effects of climate variability and change. Delineating trophic groups of fishes using SI and FA data will inform development of management and monitoring indicators. Together with parallel monitoring of both drivers (e.g. oceanographic parameters) and stressors (e.g. climate change driven shifts in habitats, species complements, functional relationships), the findings outlined herein will inform our continuing assessment of the integrity and health of this northern coastal ecosystem.

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Chapter Four: Using mercury, stable isotopes and fatty acids to identify niches and the importance of freshwater influences on coastal fish species in the Canadian Beaufort Sea

Brewster JD, Stern GA, Osterag S, Loseto LL (2016) Using mercury, stable isotopes and fatty acids to identify niches and the importance of freshwater influences on coastal fish species in the Canadian Beaufort Sea (in progress)

Abstract

The Mackenzie River Estuary largely influences Canadian Beaufort Sea coastal habitats by introducing large amounts of freshwater, terrestrial sources of mercury (Hg) and carbon into shelf sediments. With the warming Arctic, there is potential for an increase in Hg sources and /or influence on processes enabling an increase in the bioavailability to the marine biota. The combined use of multiple biotracers allowed us to characterize 7 fishes with different feeding strategies (pelagic and benthic), habitat use (freshwater, coastal, and freshwater), and identify trophic interactions within these populations. In this study we set out to 1) identify habitat use and feeding strategies that influence Hg concentrations in Mackenzie estuarine associated fish species; 2) identify the utility of Hg as a complimentary food web/niche biotracer to stable isotopes (δ^{13}C and δ^{15}N) and fatty acids (FA); and 3) identify the influence and bioavailability of Mackenzie River associated Hg as a source to fishes in the coastal environment. An ANOVA identified three fish groups defined by total mercury (THg) concentrations among the fish species analyzed. The high Hg group included fourhorn sculpin and saffron cod; low Hg group included broad whitefish, Arctic flounder, and Arctic cisco; the intermediate Hg group included rainbow smelt, Pacific herring. Isotopic niches (using δ^{13}C and δ^{15}N) and Hg niches (using δ^{13}C and THg) were generated to compare Hg as a trophic indicator. Residual permutation procedure (RPP) was used to calculate the niche metrics niche size, species packing (MNN), and niche centroids for the isotopic and Hg niches for each species. Large Hg ranges were observed in the high Hg group species (fourhorn sculpin and saffron cod), and the freshwater feeding species (broad whitefish). Incorporating Hg as a biotracer when characterizing fish populations can provide additional information on habitat, feeding and sources of this contaminant, as well as aid in identifying the link between freshwater Hg and marine ecosystems.

4.1 Introduction

Mercury is a contaminant of concern, due to the ability for the organic form, methyl mercury (MeHg), to bioaccumulate and biomagnify up freshwater and marine food chains, as well as its neurotoxic effect on vertebrate organisms (Booth & Zeller 2005, Van Oostdam et al. 2005, Loseto et al. 2008). Because MeHg is the form of Hg that biomagnifies, it makes up the majority (>70%) of total mercury (THg) concentrations found in marine fishes and mammals (Bloom et al. 1992, Morel et al. 1998, Loseto et al. 2008). Together the neurotoxic effects and biomagnification of MeHg within marine foodwebs, are cause for concern in long-lived and higher trophic marine mammals in the Beaufort Sea, such as beluga (*Delphinapterus leucas*), and species of seals, and their prey (e.g anadromous, coastal and marine fishes) (Loseto et al. 2008). Since Hg bioaccumulates and biomagnifies up food webs in marine systems (Hall et al. 1997, AMAP 2011), understanding source and exposure of Hg through diet maybe best defined though the analysis of dietary biotracers stables isotopes (SI) and fatty acids (FAs).

In Arctic marine and aquatic ecosystems methylation of elemental Hg and bio-uptake of MeHg by organisms was found to increase with warming water temperatures, availability of nutrients and increase in productivity (Chetelat & Amyot, 2009, St Louis et al. 2005). As the warming Arctic continues to change, monitoring fish populations and how trophic transfer of MeHg occurs in marine and coastal environments is needed to better define significant sources and accumulation of this contaminant in marine and coastal fish populations and larger marine mammals (Chetelat & Amyot, 2009, St Louis et al. 2005). Stable isotopes $\delta^{13}C$ and $\delta^{15}N$ have been used to characterize the trophic ecology of marine organisms for decades, where ^{15}N - enrichment of consumers' tissues (~2-4‰) relative to their prey can serve as an indicator for trophic position in an ecosystem, and variations in $\delta^{13}C$ (~0.8-1.0‰) at higher trophic levels serves as an indicator for habitat use (i.e. benthic versus pelagic and coastal versus marine; Peterson & Fry, 1987, Post 2002, Jardine et al. 2003). Similar to $\delta^{15}N$, MeHg concentrations tend to increase with trophic position, fish size and age (Atwell et al. 1998, Trudel & Rasmussen 2006, Swanson & Kidd 2010), also prey type and feeding strategies of consumers play a significant role in the enrichment of ^{15}N and bioaccumulation of MeHg in consumer tissues (Atwell et al. 1998, Post 2002, Swanson & Kidd 2010). Similar behaviours between Hg and $\delta^{15}N$ suggest the potential for complimentary trophic tools to inform on marine food chains. Although $\delta^{15}N$ has been readily used as a trophic indicator for consumers, there are some challenges, for example benthic feeders can be overly enriched in ^{15}N, due to the consumption of detritus and detritivores (Peterson & Fry 1987). This can lead to possible misinterpretations of trophic level. There is potential for Hg to assist in the interpretation or dissociation of food web processes and may inform on benthic feeding fish species, complimentary to $\delta^{15}N$ (Cabana and Rasmussen 1994; Chouvelon et al. 2014). Here we propose that the combined use of biotracers SI, FA, and Hg can differentiate between habitat use and diet of species when sharing an environment.

The Arctic has several unique features that require consideration of processes contributing to Hg and MeHg to food webs. Spring atmospheric depletion events have been identified as sources of Hg to the Arctic. In particular, the deposition of elemental Hg into the marine ecosystem (Schroeder et al. 1998, Wang et al. 2012), and how much of the deposited Hg becomes bioavailable for methylation and entry into the marine food web has been studied (St. Louis et al. 2005). The Mackenzie River, a dominant freshwater source to the Beaufort Sea coastal system also represents a pathway of MeHg, THg (Leitch et al. 2007, Macdonald & Loseto 2010) and carbon (Stein & Macdonald 2004). The reactive inorganic form of Hg (Hg

(II)) has been reported to accumulate in the coastal benthos, and water columns of offshore and coastal zones, where it can be converted to MeHg by sulfate-reducing bacteria (Bank et al. 2007, Wang et al. 2012). Although Hg delivered into marine systems have been readily studied, the bioavailability and significant sources of MeHg to marine biota is not fully understood.

Beaufort coastal environments are unique due to the large volume of freshwater and carbon introduced into the area; these large sources of freshwater create brackish transitional environments for a diverse range of anadromous, marine, and coastal fish species (Carmack & Macdonald 2002). The reliance of benthic feeding strategies by many coastal fish species (Brewster et al. 2016a), the prevalence of large estuaries, and the large percentage of coastal shelf along the Arctic Ocean suggest the production of MeHg in benthic zones may be significant (Macdonald & Loseto 2010), and should be monitored. Other sources of Hg to Arctic food webs include terrestrial deposition from ground runoff (i.e. Carmack & Macdonald 2002, Leitch et al. 2007, Bank et al. 2007, Wang et al. 2012), and contributions from permafrost melt and inundate of soils (Macdonald & Loseto 2010). Together these combined sources of Hg may be important determinants of Hg accumulation to coastal fish. Currently, the link between Mackenzie River MeHg sources to coastal systems and the uptake by marine biota have not been evaluated.

Similar to SI, FAs have been identified as food web or predator-prey biotracers used in characterizing the long-term diet and habitat of organisms. Specifically, essential fatty acids (EFA) and polyunsaturated fatty acids (PUFA) have been used in previous studies to provide a more in-depth characterization of diet in Arctic fish species (Iverson 2009, Giraldo et al. 2015), and provide complementary diet information by identifying prey of consumers. Additionally, some FA profiles such as, 18:1n9, docsahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) have been found to increase with $\delta^{15}N$ (i.e. (Koussoroplis et al. 2011, Connely et al. 2014), thus trophically transfer up food webs in freshwater and marine systems (Iverson et al. 2004, Budge et al. 2006).

Previously, the habitat and diet of Beaufort Sea coastal fish species collected in the Mackenzie Estuary (Shingle Point, Yukon) were characterized using similarity metrics in SI and FAs (Brewster et al. 2016a). Habitat groupings defined by SI included freshwater, coastal, and marine foraging strategies; dietary groups (using FAs) differentiated pelagic or benthic feeding, and feeding in more marine, brackish, or freshwater environments. Characterizing species-specific niches (diet and habitat use) led to the question of how Hg as a biotracer could be used to further evaluate the niches of these coastal fish populations. Since the benthos was identified as an important habitat for feeding among these fish populations (Brewster et al. 2016a), and ^{15}N enrichment has been identified in benthic foragers (Peterson & Fry 1987), Hg may be an additional indicator to characterize trophic feeding. Thus, in this study we selected key species to represent niche types or exhibit narrow or broad niche breadth and assessed Hg concentrations in these fish species. Our selected species were: Arctic cisco *(Coregonus autumnalis),* broad whitefish (*C. nasus),* Arctic flounder (*Liopsetta glacialis),* fourhorn sculpin (*Myoxocephalus quadricornis),* Pacific herring *(Clupea pallasii),* rainbow smelt (*Osmerus mordax),* saffron cod (*Eleginus gracilis).* We predicted that a) Benthic feeding fish will have greater levels of THg than pelagic feeding fishes, due to elevated concentrations of Hg in detritus, and methylation processes in shelf sediments b) Higher trophic feeding fish will have greater levels of THg due to biomagnification; c) Highly mobile and opportunistic feeding fish species with a broader niche will have greater variability in THg concentrations; d) Greater

THg will be observed in fish species associated with freshwater influences from the Mackenzie River. Additionally, THg will be assessed as a biotracer, and whether it can provide information on trophic positioning of fish, to complement the use of $\delta^{15}N$, since interpretations on trophic positioning ($\delta^{15}N$) can be exaggerated in benthic feeding fish.

4.2 Materials and Methods

4.2.1 *Study Area*

Shingle Point is a gravel spit approximately 7km long, that extents off the coast of the Yukon Territory (YK), Canada (Fig. 4.1). This coastal area is a traditional and current fishing community for returning Inuvialuit and Gwitch'in families from the Northwest Territories and Alaska. The coastal marine habitat is unique as it experiences both large freshwater influences from the Mackenzie River Plume, and saltwater from the Beaufort Sea (Carmack & Macdonald 2002). This creates a brackish and intermediate environment for a diverse range of fishes with freshwater, marine, and anadromous life histories (Coad & Reist 2004).

4.2.2 *Study Design, Fish Collection & Processing*

Prior to this study, the 16 species of fish consistently captured at Shingle Point were analyzed for SI and FA, where habitat use and diet were identified (Brewster et al. 2016a). Fish collections were conducted in the summer months (July and August) as part of the Arctic Coastal Ecosystem Study (ACES). The 7 species in this study were chosen from the Shingle Point populations (from 2012-2013) to represent diverse feeding ecologies, cultural importance, and possible prey for beluga (Papik et al. 2003, Loseto et al. 2008, Brewster et al. 2016a, Brewster et al. 2016b). Local harvesters and Aklavik Hunters and Trappers Committee (HTC) monitors contributed largely to the collections of these species via gill and seine nets.

Whole fish samples were vacuum-sealed and kept frozen (-18°C), before being sent to Freshwater Institute, Winnipeg, Manitoba (Department of Fisheries and Oceans) for processing. Prior to SI, FA, and THg analysis fish were weighed whole, and fork length (mm) was recorded (Table 4.1). Samples were kept frozen prior to analyses. Estimated age (via otolith analysis) and visual analysis of maturity in the gonads determined only adult individuals were used in this study.

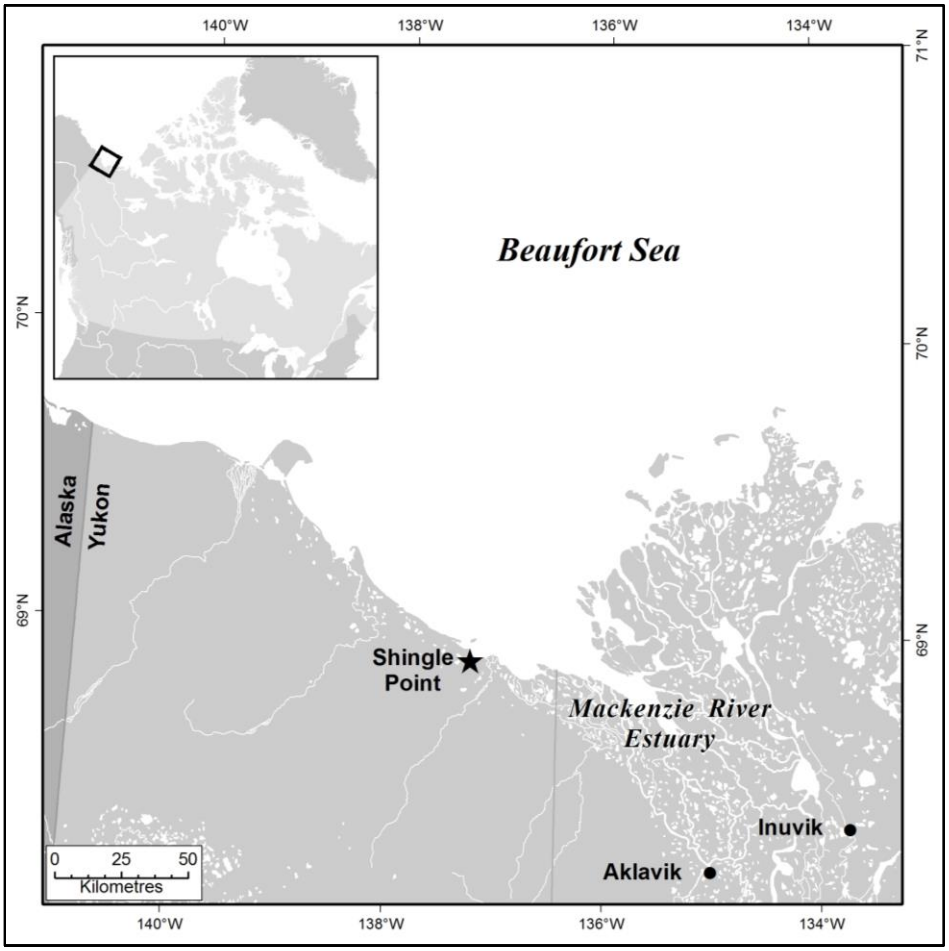

Figure 4.1 Map of coastal habitat of Shingle Point, Yukon. The Beaufort Sea and Mackenzie River Estuary are the major salt water and freshwater influences in the coastal environment.

Table 4.1 Biological information for 7 species of fish including mean carbon and nitrogen isotopic signatures ($\boldsymbol{\delta}^{13}$C and $\boldsymbol{\delta}^{15}$N), and mean total mercury dry weight concentrations. Mean values are reported with standard error (±SE). Sample size (n) for each species is indicated.

Species	n	Fork Length (mm)	δ^{13}C	δ^{13}C Range	δ^{15}N	δ^{15}N Range	THg (ug/g dw)	THg Range
Salmonidae								
Arctic cisco *Coregonus autumnalis*	17	345.59±5.04	-23.31±0.23	4.00	12.75±0.20	2.77	0.18±0.02	0.28
Broad whitefish *C. nasus*	16	394.31±15.00	-27.95±0.59	8.35	9.65±0.25	4.01	0.20±0.03	0.54
Pleuronectidae								
Arctic flounder *Liopsetta glacialis*	21	193.38±6.16	-24.03±0.27	5.49	11.42±0.12	2.03	0.24±0.02	0.30
Cottidae								
Fourhorn sculpin *Myoxocephalus quadricornis*	18	243.94±4.59	-23.73±0.12	2.04	15.38±0.11	1.71	1.33±0.08	1.24
Clupeidae								
Pacific herring *Clupea pallasii*	11	282.36±5.45	-23.82±0.17	1.76	13.17±0.17	2.17	0.25±0.01	0.15
Osmeridae								
Rainbow smelt *Osmerus mordax*	16	222.19±9.00	-24.21±0.13	1.85	14.08±0.09	1.28	0.24±0.01	0.14
Gadidae								
Saffron cod *Eleginus gracilis*	19	467.47±4.55	-22.66±0.06	1.07	15.87±0.08	1.36	1.19±0.05	1.06

Of the 7 species chosen, fishes with marine isotopic signatures were represented by fourhorn sculpin, saffron cod, Pacific herring, and rainbow smelt; the fish representative of freshwater signatures was broad whitefish; coastal signature fishes were represented by Arctic cisco and Arctic flounder (Brewster et al. 2016a). Additionally, feeding strategies were characterized for each species, where benthic foraging was indicated in most species, except for Arctic cisco, which showed both pelagic and benthic feeding strategies, and Pacific herring whose FAs indicated feeding in pelagic zones. Anadromous life histories were also represented in Arctic cisco and broad whitefish with broad $\delta^{13}C$ ranges, and supported by literature on these species (Reist & Bond 1988, Hesslein et al. 1991).

4.2.3 *Mercury Analysis*

Dorsal muscle was subsampled from each fish (n=118) for THg analysis. Muscle tissues (wet weight) were weighed (0.50g). THg of all individuals of the 7 species were quantified with a Hydra II$_c$ automated direct Hg analyzer. The THg analysis was conducted at the Centre for Earth Observation Science (CEOS), University of Manitoba. Combustion Atomic Absorption Spectroscopy (C-AAS) on a Teledyne Leeman HYDRA IIc measured THg. Samples are directly combusted in an oxygen-fed oven, followed by gold trap amalgamation and detection by AAS. Detection limits are 0.04 ng Hg. QA/QC was accomplished using certified reference materials (CRM) from the National Research Council (NRC) Canada (dogfish muscle (DORM-3), dogfish liver (DOLT-3) and lobster hepatopancreas (TORT-2)). Recoveries were 90-110% of established values, and precision was less than 8% relative standard deviation for each of the CRM.

4.2.4 *Stable Isotope Analysis*

Stable isotope analysis was performed on the dorsal muscle tissue of 7 fish species at the Freshwater Institute in Winnipeg, where samples were freeze-dried and ground with a motar and pestle. Samples were then sent to the University of Waterloo Environment Isotope Laboratory to analyze carbon and nitrogen stable isotope ratios. Stable isotope ratios were analyzed using standard procedures on a Thermo-Finnigan Delta Plus continuous flow isotope mass spectrometer (Thermo Finnigan, Bremen, Germany) equipped with a Carlo Erba Elemental Analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy). Carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) ratios were expressed in the standard δ notation and measured as per mil (‰). Results for $\delta^{13}C$ and $\delta^{15}N$ were relative to international standards Vienna Pee Dee Belemnite and atmospheric N_2, respectively (Craig 1957, Mariotti 1983). Two standards of $(NH_4)_2SO_4$ for $\delta^{15}N$ (0.77 and 20.20‰) and one standard of cellulose for $\delta^{13}C$ (-25.50‰) were the internal laboratory standards used. The analytical error did not exceed 0.20 and 0.30‰ for $\delta^{13}C$ and $\delta^{15}N$, respectively. Analysis of duplicates was run (every n=10) indicating that sample material repeatability for $\delta^{13}C$ was 0.1‰ and 0.2‰ for $\delta^{15}N$.

4.2.5 *Fatty Acid Analysis*

Half-fish (excluding the gastrointestinal tract) were homogenized and stored at -80°C for at least 24hrs. Samples were then freeze-dried, and subsampled (0.25g). A standard lipid extraction method using 2:1 chloroform-methanol containing 0.01% butylated hydroxytoluene (BHT) (v/v/w) was performed (Folch et al. 1957). Samples were washed with anhydrous sodium sulphate, and evaporated under nitrogen to obtain the lipid phase for each sample. Prior to being treated with Hilditch reagent (0.5 N H_2SO_4 in methanol) resulting in transesterification and fatty acid methyl esters (FAMES) (Morrison and Smith 1964) the lipid phase was weighed and recorded. Samples were heated for 1 hr (100°C). FA compounds were identified using gas chromatography performed on an Agilent Technologies 7890N GC equipped with a 30 m J&W DB-23 column combined with a Flame Ionization Detector (FID) (running at 350^{o}C). As outlined in Giraldo et al. (2015), the carrier gas used was hydrogen, flowing for 14 min at 1.25 mL/min and ramped to 2.5 mL/min for 5 min. The split/splitless injector was heated (260°C) and run in splitless mode. The oven program was: 60°C for 0.66 min; 22.82°C /min to 165°C with a 1.97min hold; 4.56°C /min to 174°C; and 7.61°C /min to 200°C with a 6 min hold (Giraldo et al. 2015). Seventy-three FA profiles were identified, were 18 EFA and PUFA FA profiles accounted for more then 90% of the total FA, and were analyzed in this study.

4.2.6 *Statistical Analysis*

For comparisons to be made among species, the effect of size on δ^{15}N for multiple species was standardized. Residuals of the relationship between δ^{15}N and fork length was calculated for each species, and added to the mean δ^{15}N (repeated for all species), allowing for analyses to be performed on size corrected δ^{15}N (Swanson & Kidd 2010). This size correction method was also performed on THg for each species. For comparisons in the discussion THg wet weight (wwt) values were converted to dry weight (dw) values using a standard correction of 80% moisture (e.g. Murray & Burt 2001, Bechtel 2003). High variations in lipid content among and within species can lead to misinterpretations of habitat use (Post et al. 2007). To correct for lipid content the approach outlined in Post et al. (2007) was used to correct δ^{13}C.

Isotopic biplot of δ^{15}N and δ^{13}C were generated with mean values and standard error (SE) for each species (Fig. 4.3). To assess if groups exist among species a boxplot analysis (Fig. 4.2) followed by an analysis of variance (ANOVA) was performed on the 7 species using log-transformed THg (logTHg). Isotopic trophic indicator δ^{15}N was compared with logTHg to evaluate if THg could provide trophic information in these fishes. To measure the strength and directional association between $\delta^{15}N$ and logTHg spearman's correlation test (R_s) was performed within species.

To quantify the niche of each species and compare among species, niche metrics were evaluated using two-dimensional isotopic regions (using nicheROVER, Swanson et al. 2015). Isotopic niches (using $\delta^{13}C$ and $\delta^{15}N$) indicated the 95% probabilistic ellipse region (isotopic niche size), and Hg niches (using $\delta^{13}C$ and logTHg) indicated the 95% ellipse (Hg niche size) for each species. Variations in niche centroids, defined as mean $\delta^{15}N$ and $\delta^{13}C$ (for isotopic niche), and mean logTHg and $\delta^{13}C$ (for Hg niche) were identified using the mean distance metric (MD) in the residual permutation procedure (RPP). Species packing, calculated as the mean Euclidean distance between individuals within a niche, was used to compare between the isotopic and Hg niches using the RPP metric mean nearest neighbour (MNN). Niche size or 95% ellipse region was calculated using the package "nicheROVER".

To characterize the general diet of each species, 18 FA (EFA and PUFA) associated with diet were analyzed. Then principle component analysis (PCA) with the package "Factoshiny" was used to evaluate differences in species diet. Some FA profiles were combined into one dietary marker including: *Calanus* (20:1n9, 20:1n11, 21:1n9, 21:1n11), 16PUFA (16:2n6, 16:2n4, 16:3n4, 16:4n3, and 16:4n1), and 18PUFA (18:2n7, 18:2n4, 18:3n6, 18:3n4, 18:3n1, 18:4n3, and 18:4n1). From these 18 FA profiles, three FA (DHA, EPA, 18:1n9) previously reported to accumulated with fork length of fishes (Peterson & Fry 1987) were regressed with logTHg to examine possible relationships between THg and FA that are trophically transferred up food webs. All statistical analyses were performed using R ver. 3.1.0 (R Core Team 2012).

4.3 Results

4.3.1 *Mercury Analysis*

Mercury concentrations ranged from 0.18±0.02 ug/g dw in salmonidae Arctic cisco to 1.33±0.08 ug/g dw in fourhorn sculpin. The largest variation in THg concentrations was found in fourhon sculpin (0.89 to 2.13 ug/g dw), whereas Pacific herring and rainbow smelt had the lowest variation in THg concentrations (0.17 to 0.32 ug/g dw, 0.18 to 0.31 ug/g dw, respectively; Table 4.1). Three Hg groups were generated among the 7 species analyzed ($F_{6, 111}$=117.6, $p<0.01$) and are plotted with boxplot analysis on logTHg. Group 1 was classified as low Hg and included broad whitefish, Arctic flounder, and Arctic cisco ($p>0.90$); Group 2 delineated as high Hg included fourhorn sculpin and saffron cod ($p>0.95$); Group 3 delineated as intermediate Hg included Pacific herring, and rainbow smelt ($p>0.95$) (Fig. 4.2). No strong relationships were observed between logTHg with $\delta^{15}N$ within species ($R<0.42$, $p>0.01$, for all).

4.3.2 *Stable Isotopes*

Variability in $\delta^{13}C$ and $\delta^{15}N$ was observed among the 7 fish species (Table 4.1, Fig. 4.3). Broad whitefish had the most depleted ^{13}C with the largest $\delta^{13}C$ range, compared to saffron cod, which had the most enriched ^{13}C and smallest $\delta^{13}C$ range. Saffron cod and fourhorn sculpin had greater $\delta^{15}N$ value and smaller $\delta^{15}N$ ranges, in contrast to broad whitefish that had the smallest $\delta^{15}N$ values and greatest $\delta^{15}N$ range (Table 4.1, Fig. 4.3). Approximately 2-3 trophic positions of feeding were observed among these 7 fish species.

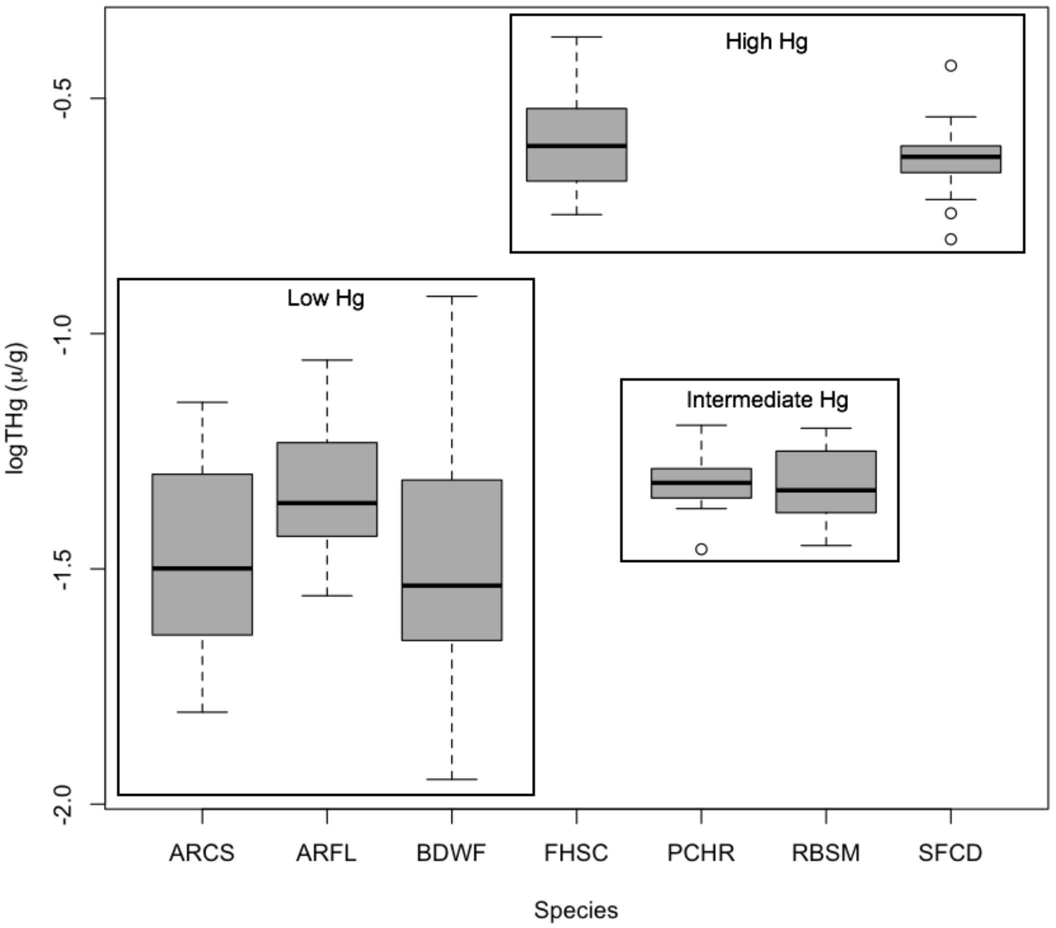

Figure 4.2 Three mercury groups (Low Hg, High Hg, Intermediate Hg) identified by boxplot analysis on size corrected and log-transformed mercury for 7 species of fish. See Table 4.1 for species names and codes.

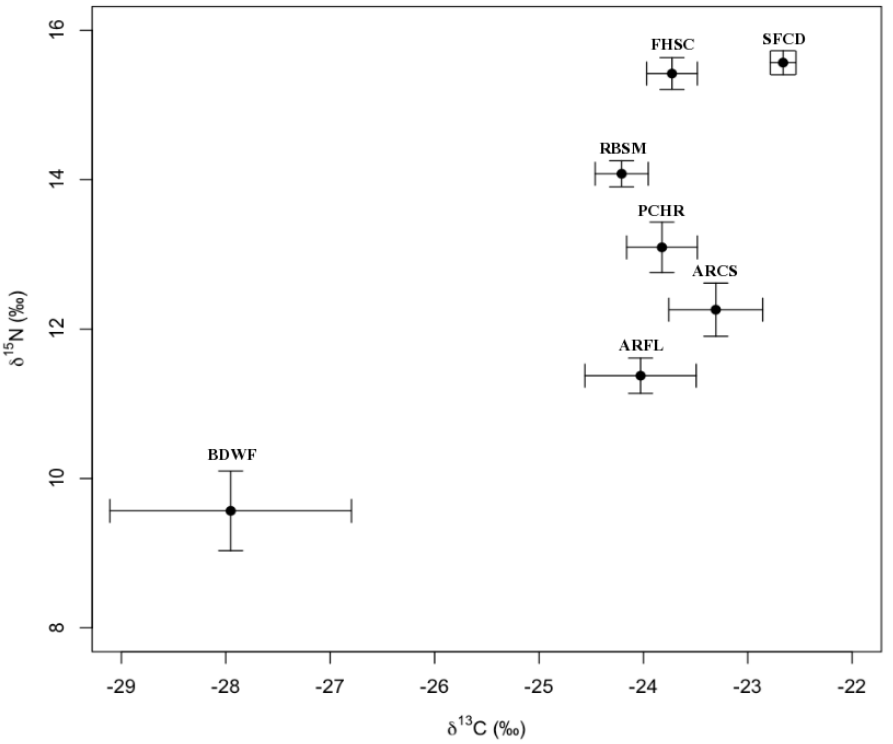

Figure 4.3 Isotopic biplot of mean carbon and nitrogen signatures (**δ**^{13}C and **δ**^{15}N, respectively) for 7 fishes. See Table 4.1 for species names and codes.

4.3.3 *Fatty Acid Analysis*

Fatty acids were used to examine diet; here we used 18 FAs including PUFA and EFA (Table 4.2). Diet analysis using PCA identified the FAs with greatest influence on the fish species analyzed (n=107). The first and second component axes explained 34.53% and 16.37%, respectively, with a combined total of 50.90% (Fig. 4.4). FA profiles 18:0 and 20:4n6 had the largest influence on the positive side (+) of the first component, and *Calanus*-markers had the largest influence on the negative side (-) (Fig. 4.4). On the second component FA profiles 18PUFA, 18:2n6 and 18:3n3 had the largest influence on the negative side and 22:6n3 on the positive side (Fig. 4.4). Broad whitefish was separated from the other species and characterized by the first component axis on the positive side, whereas the negative side characterized rainbow smelt and Arctic cisco the most. The second component axis influenced saffron cod the most on the positive side, and broad whitefish on the negative side (Fig. 4.4).

4.3.4 *Combined Biomarkers: Mercury, Stable Isotope and Fatty Acid Analyses*

Linear regressions were performed for each species to determine relationships between the FA profiles that are transferred up trophic levels 18:1n9, EPA, and DHA and logTHg. No strong relationship was found between logTHg with DHA ($R<0.10$, $p>0.34$) or logTHg with 18:1n9 ($R<0.31$, $p>0.08$) for all species. The FA EPA was negatively correlated to logTHg in Pacific herring ($R=0.44$, $p<0.05$), rainbow smelt ($R=0.75$, $p<0.01$), and saffron cod ($R=0.47$, $p< 0.01$). Greater proportions of the FA profile EPA have been associated with benthic feeding fishes in the Beaufort Sea (Choy, pers.comm).

Table 4.2 Mean proportions (±SE) of 18 fatty acids (FA) analyzed for each species (n=118)**.**

Fatty Acids	Arctic cisco	Arctic flounder	Broad whitefish	Fourhorn sculpin	Pacific herring	Rainbow smelt	Saffron cod
Saturated FA							
14:0	5.01±0.33	4.03±0.25	3.00±0.33	1.94±0.11	7.00±0.90	4.42±0.18	2.32±0.35
16:0	17.20±1.92	13.32±0.55	19.94±1.46	13.64±0.31	22.09±2.24	12.59±0.55	18.60±1.34
18:0	2.22±0.46	2.80±0.20	4.80±0.44	2.27±0.10	2.38±0.45	1.71±0.10	3.00±0.22
Monounsaturated FA							
16:1n7	23.22±1.88	17.86±1.75	16.45±2.14	16.41±0.56	27.21±3.63	17.48±0.79	12.29±1.82
18:1n7	5.22±0.56	5.43±0.38	7.66±0.75	6.63±0.26	6.37±0.86	4.37±0.20	7.34±0.60
18:1n9	12.85±1.64	9.30±0.81	20.21±2.47	15.14±0.85	13.93±1.75	9.71±0.32	12.66±1.10
20:1n7	2.47±0.31	3.78±0.45	1.14±0.17	0.90±0.06	4.17±1.21	1.34±0.22	0.78±0.10
Calanus	19.39±2.59	4.91±1.25	2.66±0.28	3.75±0.55	30.67±4.63	11.35±1.20	7.28±0.89
Polyunsaturated FA							
16 PUFA	2.13±0.27	1.30±0.17	1.69±0.37	0.70±0.09	1.75±0.32	1.06±0.05	0.69±0.24
18 PUFA	2.71±0.25	2.06±0.18	2.22±0.22	1.43±0.12	1.65±0.23	1.36±0.07	1.06±0.25
18:2n6	1.89±0.71	1.76±0.24	6.69±0.87	1.25±0.09	0.94±0.10	0.90±0.05	1.03±0.19
18:3n3	1.53±0.92	1.14±0.13	2.53±0.33	0.58±0.05	0.49±0.08	0.54±0.04	0.54±0.15
20:2n6	0.35±0.07	0.62±0.07	1.05±0.12	0.38±0.02	0.28±0.04	0.22±0.01	0.34±0.06
20:4n3	0.86±0.08	0.50±0.03	0.56±0.05	0.44±0.02	0.44±0.08	0.44±0.03	0.50±0.07
20:4n6	0.94±0.19	2.58±0.24	3.22±0.34	2.38±0.14	0.55±0.14	0.70±0.12	2.42±0.23
20:5n3	12.30±1.02	14.05±0.55	7.82±0.98	13.07±0.55	11.64±1.44	9.70±0.58	13.45±1.47
22:5n3	3.59±0.52	3.96±0.34	2.79±0.34	1.59±0.07	1.55±0.25	2.04±0.27	2.85±0.44
22:6n3	12.85±2.51	9.39±1.11	11.58±1.62	10.69±0.67	10.07±1.83	13.39±1.10	22.25±2.61

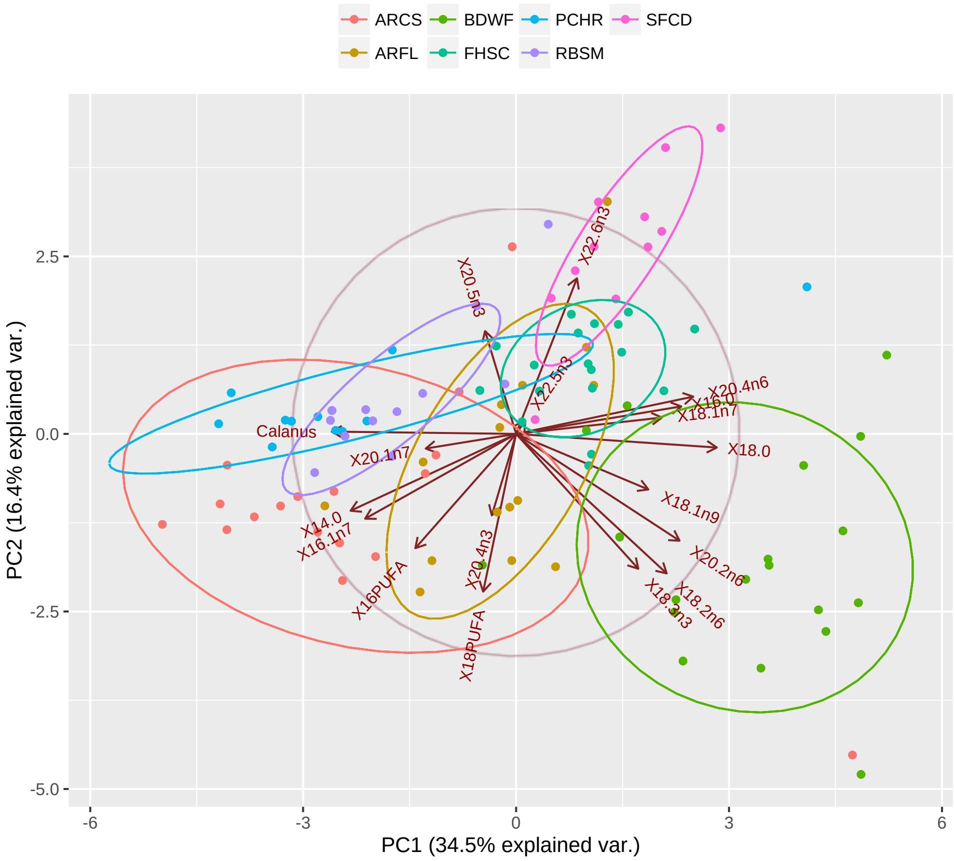

Figure 4.4 Principal component analysis of dietary fatty acids for 7 fish species. See Table 4.1 for species names and codes.

Within species THg was not correlated to δ^{15}N (R_s<0.37, p>0.11, for all). To assess the utility of trophic indicators THg and δ^{15}N, the isotopic niches (using δ^{15}N as the trophic indicator) of the 7 species were compared to their respective Hg niches (using logTHg as the trophic indicator). Broad whitefish and Arctic cisco had the largest isotopic niche sizes (65.32 and 22.46, respectively), and the smallest isotopic niche size was observed in saffron cod (1.84) (Fig. 4.5a, Table 4.3). This was also observed in these species for the Hg niches (Fig. 4.5b, Table 4.3). However, the overall Hg niche sizes of each species decreased compared to sizes observed in the isotopic niche (Fig. 4.5a and b). To identify niche overlaps, niche centroids of both the isotopic and Hg niches were compared. Among species, similar isotopic niche centroids were only shared between Arctic cisco and Pacific herring (MD=0.49, p>0.30, Fig. 4.5a); however, Arctic cisco, Arctic flounder, Pacific herring, and rainbow smelt shared similar niche centroids (MD>0.18, p>0.07, for all) when analyzing the Hg niches (Fig. 4.5b).

Species packing metric values (MNN) were analyzed for the isotopic and Hg niches, where similar trends were observed. Species packing characteristics of broad whitefish (MNN=0.68) indicated that high variation of individuals were present within the respective isotopic niche; only Arctic cisco (MNN=0.44), Arctic flounder (MNN=0.41) and Pacific herring (MNN=0.41) had similar species packing characteristics (p>0.22). The saffron cod isotopic niche had the most densely packed individuals (MNN=0.15), compared to the other species (p<0.04). Similar to the isotopic niches, the Hg niche of broad whitefish had similar species packing characteristics to Arctic cisco, Arctic flounder, and Pacific herring (p>0.23); the Hg niche of saffron cod (0.07) shared similar species packing characteristics with fourhorn sculpin (0.12), Pacific herring (0.15), and rainbow smelt (0.13) (p>0.05; Table 4.3). Individuals of each species were packed more closely together (lower MNN values) within their respective Hg niches, compared to the isotopic niches (Table 4.3).

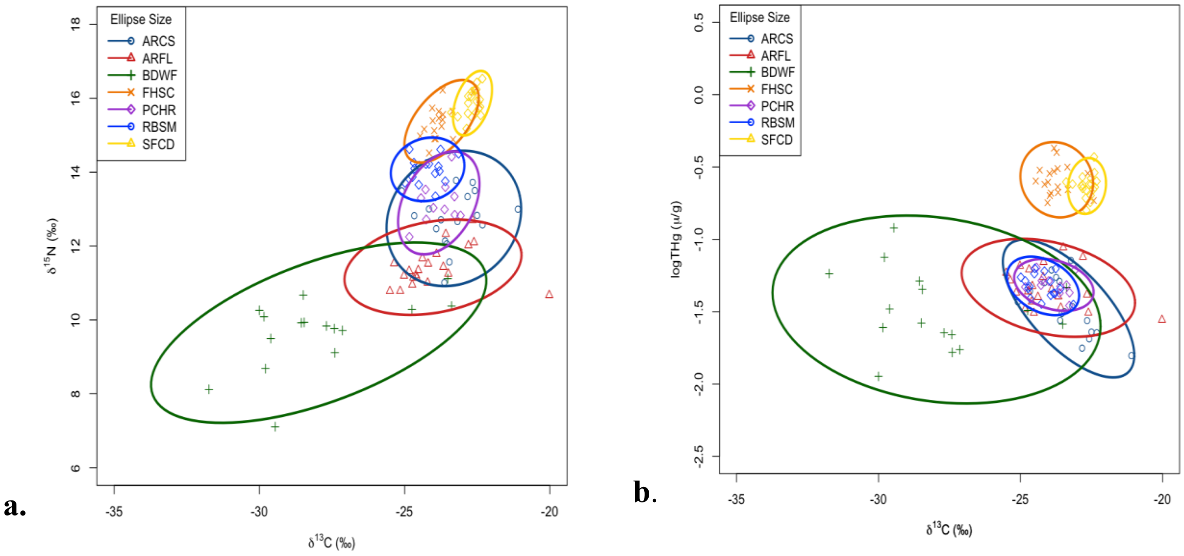

Figure 4.5 Ellipses indicating a) isotopic niches and b) Hg niches generated for 7 fish species. See Table 4.1 for species names and codes.

Table 4.3 Niche metrics of isotopic niche (**δ**^{13}C, **δ**^{15}N) and Hg niche (**δ**^{13}C, logTHg) for 7 species of fish. Mean nearest neighbour (MNN) values for individuals within each niche were used to assess species packing.

Species	Isotopic niche		Mercury niche	
	Niche size	MNN	Niche size	MNN
Arctic cisco	13.36	0.44	3.47	0.26
Broad whitefish	44.30	0.68	11.80	0.41
Arctic flounder	12.37	0.41	3.24	0.28
Fourhorn sculpin	4.50	0.26	1.04	0.12
Pacific herring	6.13	0.41	0.78	0.15
Rainbow smelt	3.42	0.27	0.80	0.13
Saffron cod	1.85	0.15	0.41	0.07

4.4 Discussion

High overlap was observed among the Hg niches of low and intermediate Hg groups (excluding broad whitefish) compared to the isotopic niches. These mercury concentrations are consistent with previously identified values (Loseto et al. 2008). The smaller Hg niche sizes of all species compared to the isotopic niches indicated less variability among individuals within each species-specific Hg niche. Since species are co-occurring in the same habitat at the time of collection, and high reliance on the benthos (excluding Pacific herring and Arctic cisco) has been reported (Carmack & Macdonald 2002, Brewster et al. 2016a) suggests that sources of THg among the 7 species are similar, providing further justification that species are feeding on similar prey.

4.4.1 *Mercury Associated with Benthic Feeding*

As predicted, benthic foraging species (fourhorn sculpin and saffron cod) had the greatest proportions of THg compared to other species analyzed. Concentrations measured were 2.27 times greater in fourhorn sculpin and 3.86 times greater in saffron cod then previously measured concentrations (Loseto et al. 2008), although, small sample size of fourhorn sculpin in Loseto et al. (2008) may account for the difference in THg of this species. Sediments at the bottom of marine systems serve as a sink for THg originating from both natural sources and human activities (Lavoie et al. 2010), and could contribute to the greater levels of THg in these high trophic and benthic feeding fish. Arctic flounder, a bottom associated fish however, did not follow the prediction that benthic feeding fish have higher THg. Variations in feeding strategies could explain the difference in THg accumulation observed in these benthic fish. High δ^{15}N were observed in saffron cod and fourhorn sculpin, and large proportions of DHA in saffron cod indicate higher trophic feeding (Peterson & Fry 1987) compared to the low δ^{15}N observed in

Arctic flounder. The benthos of this coastal system is an important foraging habitat to freshwater, marine and anadromous fishes that co-occur in the summer months (Brewster et al. 2016). The diet of Arctic flounder has been reported to include polychaetes, crustaceans, and molluscs (Coad & Reist 2004), which differs from the piscivorous diet observed in fourhorn sculpin and saffron cod (Scott and Crossman 1973, Coad & Reist 2004). High $\delta^{15}N$ values and THg concentrations observed in saffron cod and fourhorn sculpin relative to the other fish species studied, supports piscivore-feeding strategies, and are consistent with the second prediction, that higher trophic species will have higher levels of THg. Moreover, the saffron cod and fourhorn sculpin FA profiles overlapped with the other species, suggesting feeding on these fishes.

4.4.2 *Mercury Associated with Freshwater Fishes*

Broad whitefish had the largest observed isotopic and Hg niche, and largest species packing metric (MNN) indicating variability in habitat use and possibly intraspecific variation in THg sources. Broad whitefish was delineated to the low THg group, however the large ranges in $\delta^{13}C$ and THg agree with the third prediction that mobile fish will have larger variations in THg. Saffron cod and fourhorn sculpin both had small niche sizes and MNN values, and the highest THg ranges, however this variability may be highly associated with the opportunist feeding strategies of both fourhorn sculpin and saffron cod, and diet consisting of fishes and benthic invertebrates (Scott and Crossman 1973, Coad & Reist 2004). Low trophic feeding of broad whitefish was confirmed through FA analysis, where prey were largely influenced by benthic algae (20:4n6, Connelly et al. 2014), and low $\delta^{15}N$. The anadromous behaviour of broad whitefish as it migrates along a coastal distribution (Hesslein 1993, Coad & Reist 2004) is indicated by the broad niche ($\delta^{13}C$ values). The lower THg levels and trophic feeding, and the highly mobile behaviour of this species in the summer months (Brewster et al. 2016) suggest the larger THg range could be a result of different THg exposures from multiple of environments.

Further studies need to done on the bioavailability of terrestrial THg from freshwater sources on coastal marine biota, and the role these sources take in THg accumulation in marine ecosystems (Macdonald & Loseto 2010). There is evidence to suggest that Hg delivered to coastal systems from freshwater sources is not bioavailable to marine biota. Concentrations of Hg in Arctic cod from the shelf and offshore were compared in Loseto et al. (2008), where Arctic cod captured on the shelf (Mackenzie Estuary) had lower THg then those captured in the offshore (Amundson Gulf). Arctic cod is a pelagic marine forager with known prey consisting of *Calanus hyperboreus* (similar to Arctic cisco in the present study) and *Themisto libellula* (Majewski et al. 2015). Less exposure to freshwater sources of THg and high prevalence of pelagic feeding could account for the lower THg range in Arctic cod and Arctic cisco compared to the benthic and freshwater feeding strategies of broad whitefish.

4.4.3 Benthic Mercury from Freshwater Inputs

The loading of Hg to Arctic shelf sediments (Macdonald & Loseto 2010) may be a significant source of Hg to benthic foragers. The Mackenzie River is an pathway of terrestrial Hg to coastal environments; however the link between the source of Hg deposited to coastal ecosystems (water and sediment) and the bioavailability or uptake by coastal/marine biota remains unknown (Stern & Macdonald 2005, Leitch et al. 2007, Macdonald & Loseto 2010). The large source of Hg found in terrestrial soils (Sunderland & Mason 2008) coupled with the Mackenzie River corridor for Hg into coastal systems (Macdonald & Loseto 2010) highlights the importance of defining the influence of the combined terrestrial and freshwater Hg sources of the Mackenzie River to coastal and marine environments. Of the analyzed fishes the largest THg ranges were observed in benthic foragers fourhorn sculpin, saffron cod, and broad whitefish. The biomagnification of MeHg in higher trophic feeders (Morel et al. 1998) and opportunist feeding strategies of these benthic feeding fishes (Scott and Crossman 1973, Coad & Reist 2004) may explain the high THg concentrations and ranges in saffron cod and fourhorn sculpin. Because broad whitefish is a low trophic feeding fish (low $\delta^{15}N$) the process of biomagnification does not support the large THg range observed. Terrestrial carbon deposited into the Beaufort Sea coastal systems from the Mackenzie River and coastal erosion have reported to be depleted in ^{13}C; this depleted ^{13}C content has been reflected in coastal invertebrate and vertebrate consumers (Reimnitz et al. 1988, Naidu et al. 2000, Dunton et al. 2006). Broad whitefish, a species most influenced by freshwater FA markers (18:3n3, 18:2n6, Tocher 2010) had the most depleted ^{13}C, compared to the other studied species, suggesting this species feeding strategies is largely influenced by freshwater. Known benthic-feeding strategies of this species (Coad & Reist 2004) further indicate that broad whitefish are highly exposed to terrestrial carbon and Hg. As previously stated, the anadromous behaviour of broad whitefish as it migrates along coastal distributions (Hesslein et al. 1991, Hesslein et al. 1993), is reflected in the large $\delta^{13}C$ range. As such broad whitefish feeding in habitats influenced by Mackenzie River water sources may explain the large THg range observed, since large exposures to coastal sediment is expressed in this species through benthic feeding in multiple freshwater influenced environments. Mercury (MeHg and THg) inputs from the Mackenzie River and methylation of THg in shelf sediments have been reported in Arctic coastal environments, and may have influences on the higher THg levels in coastal species (Leitch et al. 2007, Macdonald & Loseto, Wang et al. 2012). These two Hg sources may have made fourhorn sculpin and saffron cod more susceptible to higher exposure to Hg, since these species feed high trophically, and rely heavily on the benthos for foraging. Results allude that contributions of Hg delivered from freshwater sources into coastal habitat sediments influence the Hg concentrations of consumers with specific feeding strategies, since high concentrations of THg were observed in high trophic benthic feeders, as well as large THg ranges in freshwater benthic feeders.

4.4.4 *Conclusion and Future Research*

The combination of SI and FA with THg to characterize diet and habitat provide multiple avenues of defining trophic ecology of a species, as well as identify if co-occurring species experience overlap in exposure to THg. Changes in fish communities in response to environmental changes (e.g. rising temperatures) have been identified using $\delta^{15}N$ (i.e. Jennings et al. 2002, Chouvelon et al. 2014). Although THg alone is unable to identify species trophic ecologies, this indicator provides additional information on the behaviour of THg in a coastal fish community. In addition, the inclusion of THg with SI and FA helps identify how habitat, spatial distributions, and feeding strategies effect the accumulation of this contaminant. Species of sculpin (*Gymnocantheus tricuspis and Myoxocephalus scorpius*) and saffron cod may be possible prey to beluga (Quakenbush et al. 2015). Thus, understanding the sources of Hg in the Arctic coastal habitats, which act as beluga refuges in the summer months, is needed to monitor this contaminant in higher trophic predators. The Mackenzie River has been reported to be a source of THg to coastal environments, however, how this contaminant is taken up by coastal and marine biota is less understood. High Hg concentrations observed in some benthic feeders and large Hg ranges observed in a freshwater benthic associated species suggests that Hg delivered from freshwater sources may be integrated into marine food webs.

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Chapter Five: SYNTHESIS DISCUSSION

5.1 Discussion

Basic knowledge of the ecology of Beaufort Sea fish species is lacking, including species that frequent coastal habitats in the summer months. In Chapter Three, we set out to characterize the habitat use and diet aspects, described as ‘niche’ of 16 fish species that co-occur at a coastal habitat, Shingle Point, YK. These species include anadromous, freshwater, and marine species that have a diverse range of feeding strategies and habitats. Dietary biotracers of stable isotopes (SI) and fatty acids (FA) were used to characterize habitat use and diet of each species. Multivariate analyses with probabilistic and Bayesian statistics were used to group ecologically similar species based on shared diet and habitat use. Niche metrics were then evaluated to identify the ability of resource partitioning and capacity of plasticity to environment stressors. The objective of Chapter Four was to further evaluate the niche of fishes by using THg as a trophic indicator, and to better understand how and where this contaminant accumulates in Arctic coastal environments. Results from these niche characterizations (Chapters Three & Four) were limited to specific life stages (i.e. adult, non-spawning individuals) while fishes were co-occurring in summering habitats. Turnover rates of SI and FA in species tissues, only provided information for a time period of months, thus the niches of these species when in their wintering habitats, and other aspects of their life histories (i.e. spawning, juvenile) still need to be characterized. The results of this research contributed valuable information on coastal fish populations in the southern Beaufort Sea, further informed on the knowledge gaps that currently exist in Beaufort fish populations, combined multiple biotracers to better understand the habitat and diet niches, and used a new approach to evaluate fishes niches by evaluating SI and FA in the same analysis (nicheROVER).

The analysis of SI and FA in Chapter Three identified and grouped the 16 fishes into three isotopic groups including: coastal, freshwater, and marine habitat use; and 5 dietary groups including: pelagic marine feeding, benthic and pelagic brackish (both freshwater and marine) feeding, benthic freshwater feeding, benthic marine feeding, and benthic brackish feeding groups. These groupings identified that benthic zones of the coastal environment are important to feeding in coastal fish populations. Additionally, the niche sizes of anadromous species (i.e. broad whitefish, Arctic cisco, and dolly varden) suggest niche plasticity and ability to partition resources in a changing environment (Layman et al. 2007, Svanbäck & Bolnick 2007). This is in contrast to species with a small niche, limited to small ranges in habitat and prey, that may more effected by environmental stressors (i.e. rainbow smelt, northern pike), and could be used as an indicator to evaluate shifts in species' niches in response to these stressors.

The habitat and diet aspects of fishes niche were further evaluated by analysing THg in 7 of the 16 species, representing a diverse range in habitat and feeding strategies including coastal, freshwater, marine, and anadromous fishes that feed in the benthic and pelagic zones. The Mackenzie River contributes significant freshwater influences, as well as large concentrations of THg and MeHg into estuaries of the Western Arctic, however, the bioavailability of this THg to the marine biota is not understood (Leitch et al. 2007, Macdonald & Loseto 2010). Because the commonly used trophic indicator ^{15}N can be overly enriched in benthic feeding fish, THg was used as an additional trophic indicator. Though THg was not an adequate indicator of trophic position alone, THg did identify habitats and feeding strategies associated with high concentrations of this contaminant. The accumulation of THg in marine food webs is not as well characterized as in freshwater systems (Atwell et al. 1998, Wang et al. 2012). Linking mercury with habitat use and diet, using SI and FA, provides a further understanding on how THg accumulates in marine, freshwater coastal, and anadromous fishes.

Together the chapters (Three & Four) previously discussed have provided a comprehensive analysis of the diet and habitat use of a diverse range of fishes in the Beaufort Sea coastal environment. The combination of SI, FA, and THg has identified the strengths and weaknesses of each biotracer, and how coupling biotracers is important when identifying the niche of species.

5.2 Conclusion & Future Work

Baseline information is needed to identify which species will adapt and which will be more strongly influenced by environmental changes, and increases in human stressors (i.e. industrial development, marine transport, and noise pollution). Identifying and understanding how fish species partition resources at Shingle Point, YK and other MPAs are important for future monitoring. The persistent effects of climate change on the Beaufort Sea marine ecosystem will continue to influence different levels of the trophic structure with unknown consequences. Dietary biotracers can act as tools in monitoring these effected areas. Utilizing SI and FA data has allowed fish to be grouped based on similarities (in diet and habitat use). Here we proposed (Chapter Three) parameters representative fish in each group (coastal, freshwater, marine) that should be monitored, where the selection of key species can follow two approaches: species with a narrow niche that can reflect sudden changes in the environment versus species with a broad niche that can represent fishes with similar niches and therefore reduce the number of species sampled. Utilizing FA and isotopic functional groups are also useful in monitoring how environmental changes may influence or shift the niche of fish species. Rainbow smelt, a representative of more marine fish had the narrowest isotopic niche, indicating narrow habitat range and prey. Thus this species can be an indicator for species more susceptible to environmental stressors and change. Limitations in selecting small niche species are that monitoring the overall health of the habitat is limited to this one niche. In contrast, monitoring a species with a broad niche, such as Arctic cisco, representative of a coastal species feeding in both the pelagic and benthos, can allow for monitoring more of the environment as well as species that highly overlap with this broad niche. As climate change continues, using FA and SI biotracers to characterize and group fish can more efficiently identify: fishes at risk, the health of the pelagic and benthic fish community, and monitor changes in the environment. Furthermore, the utilization of THg as a biotracer in coastal fish populations, can help identify how contaminants accumulate in the marine and coastal fish populations, what environmental factors (i.e. freshwater influences, benthic versus pelagic feeding strategies) play a role in introducing this contaminant in marine food webs. Indigenous peoples have TEK that can provide long-term observational information on trends and changes in environment and marine species. The value of TEK provides year round observations on marine populations, when ice-cover can obscure research in the Arctic, as well as contribute long-term information to better understand the present population and environmental changes. Moreover, TEK can provide insight on variations in species behavior before it is see at an ecosystem level. The incorporation of TEK alongside dietary biotracers as a tool in future monitoring, will provide information on the baseline behaviours of native species, and prove to be beneficial in the long-term monitoring of MPAs.

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Appendix: Traditional Ecological Knowledge (TEK) at Shingle Point, YK: Observations on Changes in the Environment and Fish Populations

Brewster JD, Neumann D, Ostertag SK, and Loseto LL (2016) Traditional Ecological Knowledge (TEK) at Shingle Point, YT: Observations on Changes in the Environment and Fish Populations. Can. Data Rep. Fish. Aquat. Sci. 3174: v + 23 p

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Abstract

Understanding the environmental changes and determining baseline information for Beaufort Sea fishes is a crucial step in sustaining the fish populations and habitats within a changing Arctic climate. Shingle Point, YT is a traditional and modern day fishing and hunting community for Western Arctic indigenous people and is part of a marine protected area and the Inuvialuit Settlement Region (ISR). The incorporation of Traditional Ecological Knowledge (TEK) is important in understanding the changes in this coastal habitat. During the summer of 2015, Shingle Point harvesters and their families participated in TEK interviews. The interviews provided observational information on: 1) fish population changes, 2) environmental changes, 3) concerns, and 4) reasons to return to Shingle Point. Results from the TEK interviews indicate changes have been observed in the environment and coastal fish populations for decades. The utilization of TEK in the form of specific and open-ended interviews/questionnaires can be used to document and understand long-term changes in the environment and marine biota, as well as bolster quantitative research in the future, to better understand the environmental impacts of climate change on the Beaufort marine ecosystems.

Introduction

Located along the coastline of the Yukon Territory in the Canadian Arctic, is a long gravel spit, known as Shingle Point (Fig 1). Shingle Point has long been known as a traditional and modern day fishing and hunting camp for Western Arctic indigenous people. The Inuvialuit and Gwitch'in peoples are the prevalent indigenous groups in the Western Arctic region. Families from the Northwest Territories, Yukon, and northeast Alaska have been returning to Shingle Point for generations. In particular, families from the nearby communities of Aklavik and Inuvik travel to Shingle Point annually for subsistence fishing, whaling and berry picking in the summer and fall, and caribou hunting in the winter. The consistent use of the area by local peoples and the traditional ecological knowledge (TEK) that has been passed on along many generations is of great value for understanding change in the environment over time.

Traditional and current use of Shingle Point for subsistence suggests that this location is a reliable and abundant source of wildlife. Shingle Point is a long narrow coastal spit embedded in estuarine habitat. The mixing of Beaufort Sea marine waters and freshwater from the Mackenzie River creates an intermediate environment for freshwater, anadromous, and marine fish species (Carmack & Macdonald 2002). Many of the anadromous fishes, for example, Arctic cisco (*Coregonus autumnalis*) and dolly varden char (*Salvelinus malma*) are of great cultural importance to the Inuvialuit and Gwich'in. These fishes are also important prey for many of the marine mammals that utilize the area. The months of June, July and August, are the most active time for a number of fish species as they forage in the nutrient rich brackish and marine waters before returning to their respective over-wintering habitats (Reist & Bond 1988; Reist & Sawatzky 2010).

Climate change and increased pressure for industrial development and marine transport continue to impact Arctic marine ecosystems (Cobb et al. 2008; Stroeve et al. 2012). Currently, we still do not fully understand the distribution range for many fish species located in the Beaufort Sea (Reist et al. 2002; Reist et al. 2006). The determination of this type of baseline information is a crucial step in managing and thereby sustaining fish populations and habitats, and the ability to identify the effects of environmental and industrial stressors. For these reasons, understanding the ecosystem and fish use at Shingle Point is important to understanding and sustaining these valuable resources (Usher 2002; Cobb et al. 2008; Loseto et al. 2009).

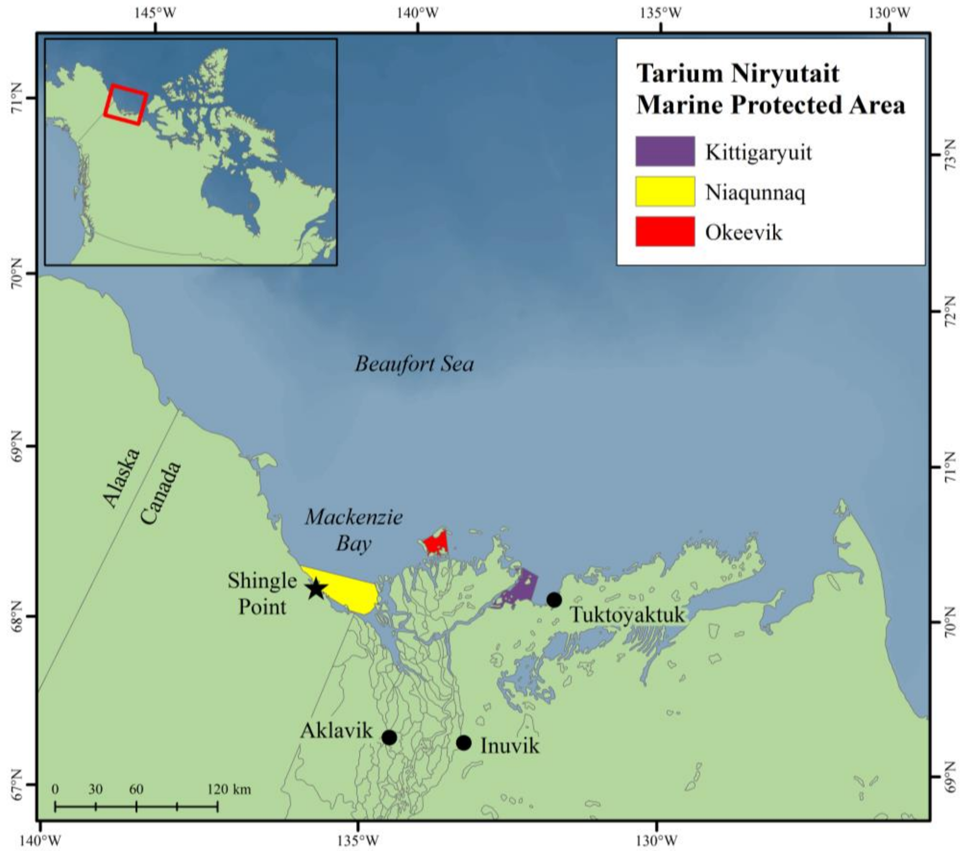

Figure 1. Map of the Tarium Niryutait Marine Protected Area (TNMPA) and place names used in this report.

Fisheries and Oceans Canada (DFO) has established a number of fish monitoring programs from Shingle Point. For example, the Tarium Niryuitait Marine Protected Area (TNMPA) was established in 2010, in effort to protect beluga and their supporting habitat and prey (DFO & FJMC 2013a, 2013b). Three regions of the Mackenzie Estuary make up the TNMPA: Niaqunnaq, Okeevik and Kittigaryuit (Fig 1). Shingle Point is located within the Niaqunnaq region (Fast et al. 2001; Fast et al. 2005, Harwood et al. 2014). As part of the management and monitoring of the TNMPA, fish monitoring is incorporated in the reporting of the conservation objectives for the TNMPA (DFO 2010, DFO 2012). Additionally, the Arctic Coastal Ecosystem Study (ACES) was established at Shingle Point to monitor the fish populations in the nearshore estuarine environment, where in total 16 species of fish (listed in Table 1) have been consistently caught each year (2010-2015). Under the ACES program, researchers and Aklavik Hunters and Trappers Committee (HTC) monitors have been collecting fish samples from Shingle Point since 2010 to present. This is also the case for the ACES and the Dolly Varden Char Harvest Monitoring programs at the Shingle Point location. These monitoring programs are instrumental for reporting under the TNMPA and to help fill a number of knowledge gaps that currently exist at Shingle Point and for a number of Beaufort Sea fishes.

Often long-term data in Arctic ecosystems is difficult to collect, and is limited by season and climatic conditions, but because Shingle Point is regularly visited year round, TEK studies can be conducted in order to understand and monitor the health of the marine ecosystem. The on-going monitoring of fishes at Shingle Point prompts a unique opportunity to merge the generations of traditional observational data with the quantitative biological data collected by researchers. During the 2015 field season TEK questionnaires and interviews were conducted at Shingle Point. In this study TEK is defined as the collection of indigenous knowledge, techniques, and beliefs of how to live on the land; this knowledge is passed on through generations (Johannes 1989). Therefore, participants included elders returning to Shingle Point, as well as younger participants whose knowledge stem from stories being passed down. This study was requested and is supported by the Aklavik HTC and the Fisheries Joint Management Committee (FJMC). The coupling of TEK alongside biological data will help to strengthen the current ecosystem-monitoring programs by focusing the research on the subjects of concern and to better understand the long-term changes in the coastal environment and fish populations.

Materials and Methods

Study Area

Shingle Point is a 7 km gravel spit located on the Yukon coastline (68°57’N, 137°13’W), west of the Northwest Territories border (Fig 1). The spit is connected to tundra on one end and is surrounded by the Mackenzie Bay on the remaining three sides. The north side is exposed to the waters of greater salinity (i.e. Beaufort Sea marine waters). The side opposite of the ocean, referred to by locals as the “bay side”, is less saline, and is known as brackish water. Brackish water is a mixture of marine water and freshwater. The origin of freshwater at this location is due to the outflow of the Mackenzie River (Craig 1984; Carmack & Macdonald 2002). Subsistence fishing takes place on both sides of the spit. Three camps make up the fishing community at Shingle Point (Fig. 2).The “Down the Hill Camp” is located where the spit connects with the tundra (Fig. 2). This camp is the most sheltered from weather and storms coming in from the Beaufort Sea. The “Middle Camp” is located in the center of the gravel spit, and the “Point Camp” is at the far end, at the opening of the bay, and is exposed to the Beaufort Sea on three sides (Fig. 2).

Figure 2. Photo of the three Shingle Point, YT camps. The Down the Hill camp is connected to the tundra (i.e. mainland) and the Middle and Point camps are located further into the Mackenzie Bay. (Photo courtesy of Dana Neumann).

Monitoring program Fish Collection

As part of the existing DFO monitoring programs that have been ongoing at Shingle Point since 2010, inventories of fish species captured at various sample sites have been collected. These fishes have been collected using various methods over the years, and were collected collaboratively between researchers and the local harvesters (i.e. Aklavik HTC monitors, harvesters from each of the three camps). A total of 16 fish species have been consistently caught every year since the monitoring programs have been operating. This study focuses on those fish species most commonly captured in order to established baseline distributions from the monitoring program with the TEK. Table 1 provides a list of those common species, familiar to the local harvesters, with traditional names and a brief description of their ecology (Table 1).

Traditional Ecological Knowledge (TEK) Questionnaire

A questionnaire was drafted and reviewed by the Aklavik HTC. The HTC provided feedback and requested a local summer student be hired to assist with the interviews. The questionnaire then went through two ethics screening processes to ensure that it, and the interview process, followed the guidelines under the University of Manitoba Fort Garry Campus

Research Ethics Board. The Aklavik HTC and the Ethics committee approved the final draft of the questionnaire in June of 2015 (Appendix 1). Interviews were conducted using the questionnaire questions. Questions included topics on the health and abundance of the 16 common fish species captured at Shingle Point, observational changes in the environment (i.e. land, temperature, water), and other concerns. The results were categorized into four sections: Fish Population Changes, Environmental Changes, Concerns and Why People Return.

TEK interviews were then conducted during and between fish sampling in July 2015 by a field team of DFO, and HTC youth (Appendix 2). In order to encourage collaboration and support, a handout was distributed to the local harvesters and their families prior to the interview. The handout included pictures of the 16 common fish species consistently harvested (Appendix 3) with an extra blank column (information box) for the individual to document any observed changes and interesting observations during their time at Shingle Point.

The Aklavik HTC hired two youth from Aklavik to assist with the TEK interviews. This allowed for a more comfortable and relaxed interview with community members. Interviews were recorded both on a voice recorder and written on each questionnaire. Participants could request to remain anonymous, stop the interview at any time or chose not to be voice recorded. The prepared questions were formatted to be specific and open-ended, to allow for participants to share experiences, observations, opinions and concerns (Huntington, 1998). Guardians of participants under the age of 18 signed a consent form. The HTC summer students were asked to sign forms prior to the interviews to protect the anonymity and privacy of participants. All the interview recordings and results are securely held at the DFO Freshwater Institute, Winnipeg, MB. The qualitative software “NVIVO” was used to analyze word counts and word associations, thereby identifying common trends within each interview.

Results

In total, there were 15 participants (Table 2). Only 12 interviews were conducted, since some participants decided to interview collectively with their respective families (Table 2). Results from the TEK interviews indicated that Shingle Point community members have been observing changes at Shingle Point since the 1960s and 1980s.Participant responses were grouped into four categories: fish population change, increase in air temperature, increase in water temperature and concern (Fig. 3). Overall, most participants were concerned with the observed environmental change (73%; Fig. 3). The most frequent observation was related to potentially higher water temperature at Shingle Point, this lead to concerns on how this would impact fish populations, and their supporting habitat. (Fig. 3).

Table 1. Fish species consistently caught at Shingle Point from 2010-2013. Fishes that migrate from freshwater to marine will stop in the coastal/estuarine environment to adjust to the change in environment. The habitat column uses arrows (→) to indicate fishes migrating to and from different habitats, and function describes why we suspect migration to occur.

Common and Scientific Name	**Traditional Name**	**Habitat**	**Function**	**Feeding Ecology**	**Diet**
Arctic cisco (*Coregonus autumnalis*)	Herring	Freshwater →	- Spawning - Overwinter	- Open water feeding - Bottom feeding	Shellfish, insects, small fishes, worms
		Marine	- Coastal movement - foraging, growth and maturation (summer)		
Arctic flounder (*Liopsetta glacialis*)	Flat fish	Marine	- Non-migratory, but enters coastal waters to feed	- Bottom feeding	Small fishes, shellfish
Broad whitefish (*Coregonus nasus*)	Whitefish	Freshwater →	- Moves upstream to spawn	- Open water feeding (young) - Bottom feeding (adult)	Zooplankton (young), shellfish (adults)
		Coastal/Estuarine (freshened waters)	- Foraging, growth and maturation (summer)		
Burbot (*Lota lota*)	Losh	Freshwater	- Non-migratory, but enters coastal waters to feed	- Open water feeding - Possible Benthic feeding	Insect larvae (young), shellfish (young), fishes (adults)

Common and Scientific Name	**Traditional Name**	**Habitat**	**Function**	**Feeding Ecology**	**Diet**
Dolly varden char (*Salvelinus malma*)	Char	Freshwater →	- Overwintering, growth (young), spawning (adults)	- Open water feeding - Bottom feeding	Insects, shellfish, fish eggs (young), fishes (adults)
		Marine	- Foraging, growth and maturation (summer)		
Fourhorn Sculpin (*Myoxocephalus quadricornis*)	Devil Fish	Marine	- Non-migratory, but enters coastal waters to feed	- Bottom feeding	Shellfish, fishes
Inconnu (*Stenodus leucichthys*)	Conie	Freshwater →	- Spawning - Overwintering	- Open water feeding - Bottom feeding	Insect larvae (young), planktonic shellfish (young), small fishes (adults)
		Marine	- Foraging, growth (summer)		
Lake whitefish (*Coregonus clupeaformis*)	Crooked Back	Freshwater →	- Spawn - Overwinter	- Bottom feeding	Insect larvae, shellfish, fishes, fish eggs (including their own)
		Coastal/Estuarine	- Foraging, growth and maturation (summer)		
Longnose sucker (*Catostomus catostomus*)		Freshwater	- Non-migratory, but enters coastal waters to feed	- Bottom feeding	Shellfish

Common and Scientific Name	**Traditional Name**	**Habitat**	**Function**	**Feeding Ecology**	**Diet**
Least cisco (*Coregonus sardinella*)		Freshwater →	- Spawn - Overwinter	- Open water feeding	Planktonic shellfish, plants
		Marine	- Foraging, growth and maturation (summer)		
Northern pike (*Esox lucius*)	Pike	Freshwater →	- Spawn - Foraging	- Open water feeding	Shellfish (young), crayfish (adults), frogs (adults), fishes including cannibalism (adults)
		Coastal/Estuarine	- Foraging, growth, maturation (summer) - Reduce parasitism (summer)		
Saffron cod (*Eleginus gracilis*)	Tom Cod	Marine →	- Foraging, growth, maturation	- Bottom feeding	Opportunistic (e.g. fish, shellfish)
		Coastal/Estuarine	- Spawn - Overwinter		
Starry flounder (*Platichthys stellatus*)	Flat Fish	Marine	- Non-migratory, but enters coastal waters to feed - May move far offshore in winter	- Bottom feeding	Shellfish, worms, brittle stars, small fishes

Common and Scientific Name	**Traditional Name**	**Habitat**	**Function**	**Feeding Ecology**	**Diet**
Pacific herring (*Clupea pallasii*)		Marine →	- Migratory behavior is not fully understood - Foraging, growth, maturation	- Open water feeding	Shellfish larvae (young), shellfish (adults), small fishes (adults)
		Coastal/Estuarine	- Spawning		
Rainbow smelt (*Osmerus mordax*)	Stink Fish	Freshwater →	- Spawning	- Open water feeding	Shellfish, copepods, small fishes
		Marine	- Foraging, growth, maturation		
Round whitefish (*Prosopium cylindraceum*)	Lake Fish	Freshwater →	- Spawning	- Bottom feeding	Shellfish, fishes, fish eggs
		Coastal/Estuarine	- Foraging, growth, maturation		

Table 2. List of participants and their home community and camp location for the the Shingle Point Traditional Ecological Knowledge (TEK) interview conducted in July 2015. Also includes information about how the interview was conducted (e.g. family, individual).

Interview Number	**Name OR Identification Number**	**Home Community**	**Camp Location**	**Information**
1	Jerry Arey Verna Arey Colton Arey	Aklavik	Down the hill	Family
2	Thomas Gordon	Aklavik	Down the hill	Individual
3	Melinda Cockney Topsy Cockney	Inuvik	No camp; spends time at the Point camp	Mother and Daughter
4	Annie B. Gruben	Aklavik	Point	Individual
5	0001	Anonymous	Point	Individual
6	Gerry Kisoun	Inuvik	Visits friends and family at Down the hill camp	Individual; originally from the Mackenzie Delta
7	0002	Aklavik	Anonymous	Individual; no recording
8	0003	Aklavik	Down the hill	Individual
9	0004	Aklavik	Down the hill	Individual
10	Jordan McLeod	Aklavik	Middle	Individual
11	Tom McLeod	Aklavik, then Inuvik	Point	Individual
12	Denise Arey	Aklavik	Middle Camp	Individual

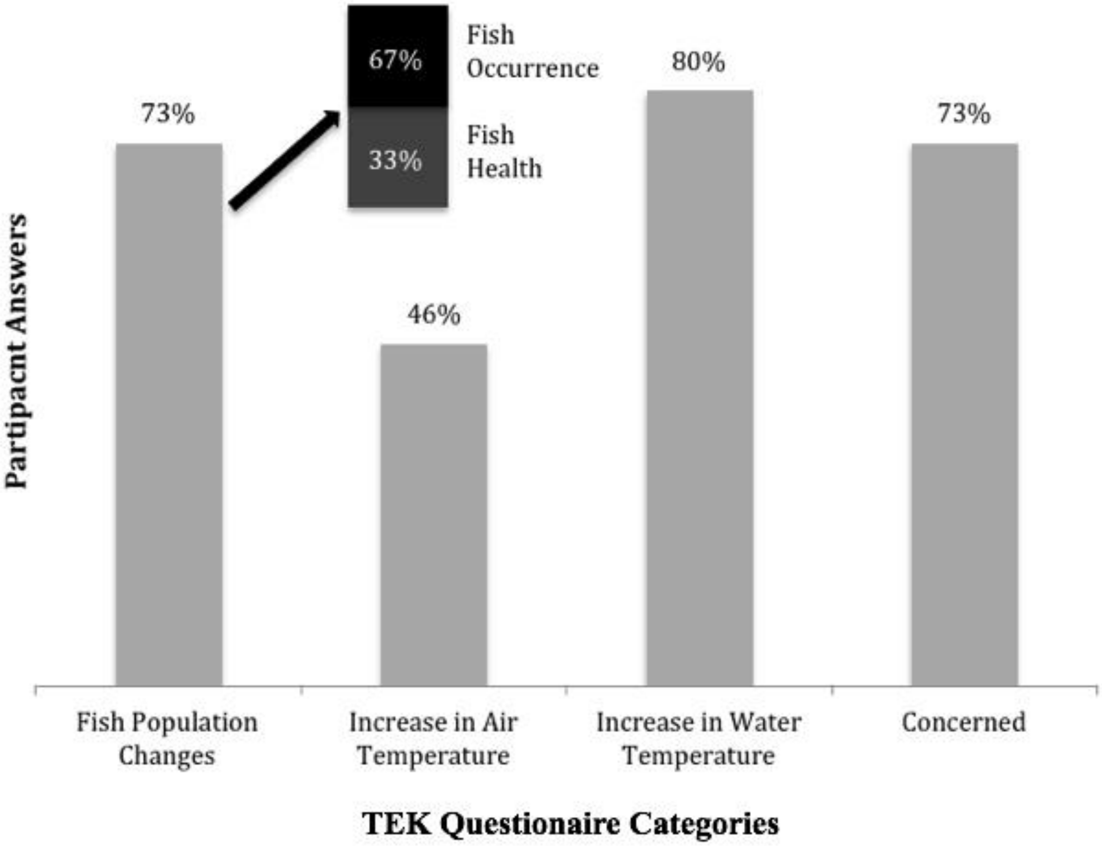

Figure 3. Results (%) of participant answers to the Traditional Ecological Knowledge (TEK) questionnaire from Shingle Point, YT for the 2015 study. The fish population change category was further divided into two sub-categories: fish occurrence and fish health.

Change in Fish Populations

The majority of questions during the interview were related to observed changes in fish occurrence over the years. Of the 15 participants who took part in the TEK questionnaire, 11 (73%) stated that they have noticed changes in fish occurrence (67%) and the general health of fish (33%) in the area (Fig. 3). The greatest change was associated to the time of arrival of dolly varden char, where observations indicate they are migrating earlier (i.e. in July) to the Mackenzie Bay; and at the same time as Arctic cisco. It was speculated that these species prefer cooler waters, and that the increase in water temperature is affecting the time of arrival. Fish population changes were addressed as another concern by participants, where freshwater species were most associated with the fish occurrence sub-category (Fig. 3). Six of the participants noted an observed increase in freshwater species. Specifically, individuals stated an increase in inconnu (*Stenodus leucichthys),* fluctuations in saffron cod (*Eleginus gracilis)* abundance, and a decrease in flounders and fourhorn sculpin (*Myoxocephalus quadricornis)*. Arctic cisco was the species most associated with the fish health category, where 5 participants indicated an increase in the observation of worms present in the muscle tissue.

The highest word count (n) from the interview results indicate that observational changes exist in both occurrence (n=47) and abundance (n=22) of fish. On topics such as, being an important food source, changes in migration and occurrence, abundance changes, and health, Arctic cisco was mentioned the most (n=53), followed by dolly varden char (n=34).

The following quotes are meant to show some common trends in the TEK interviews. An elder from Aklavik, NT, Annie B, uses the walls of her Shingle Point house to document important events. In her interview she pointed to September 1, 1995 and said:

> *" If you are here early sometimes belugas came right in to the bay. They are always on the deep side (ocean side), but in September then come right into the bay. But that was a long time ago, last time was 1995 September 1st." ~Annie B*
>
> *" The timing of fish, char has changed since I started coming to Shingle. They use to come in early July with herring over the years. Last couple years char is later in the season maybe early August late July. Herring when the run is on is the main fish we catch, then conie. We are lucky to get char and whitefish." ~Anonymous*
>
> *"We're not getting as much herring in the mouth east channel around Tuk. This is where the fish are coming from when they come to Shingle Point". ~Gerry Kisoun*

Environmental Changes

The TEK questionnaires indicated that air and water temperatures have increased at Shingle Point over the years (47% and 80%, respectively; Fig. 3). Shingle Point has been a traditional hunting and fishing ground for subsistence for decades. Now community members like Jerry Arey, are saying that it is becoming harder to predict the weather, that this is affecting their hunting season, and that the timing and abundance of caribou herds is also changing. There are more storms in the area and this is affecting when people are able to travel to and from Shingle Point. Other interviews stated that people will not travel to Shingle Point with a warm northwest wind, and that it is becoming harder to tell when it is safe to travel. Environmental changes observed went beyond the marine environment. Common topics included: the degradation and slumping of the hills into the estuary and ocean, the loss of sea ice, and decrease of snow in the hills. Participants stated that there are less caribou, and that there used to be jellyfish on the ocean side, and now there are none.

> *"All kinds of changes too much, you can't keep track of everything. Elders use to look at the sky, look at the water, and they look at the clouds. How all that tells the weather I don't know, but that's how they knew when a big wind was coming, when its going to change the weather. I don't know how they could tell that, but that is how our elders were. Every year it seems to get more hotter, the hills are more different now, all the hills are sliding into the water. Every year is changing." ~Annie B*
>
> *"A lot of changes over the years. I have been here for 20 years around and I have been seeing so much change in such a little amount of time. I always use to see snow, and now*

> *not seeing snow is very unusual. First not seeing ice was unusual now not seeing snow is unusual. Things are changing more rapidly, not slowly, faster and faster." ~Anonymous*

> *"The water temperature is changing. The bay and ocean side had ice and was cold when grandparents were here. Now water is warm, lots of rain and thunder and different winds" ~Anonymous*
>
> *"For hunting there are less caribou, they follow the lichen. Because there is no lichen they follow their food." ~Denise Arey*

Concerns

From the 15 participates 11 (73%) stated that they were concerned with the observed fish population, and environmental changes (Fig. 3). A number of the participants stated that Arctic cisco have more worms in the muscle tissue. They are concerned with the health of this species, and if it is safe to eat when prepared as dry fish. The changes in the environment are evident to the people that frequent Shingle Point; concerns are highly associated with how the physical environmental changes are going to affect the biota. Some examples include:

> *"Why is the ocean moving further and further from my house. When I was eight the ocean was much closer." ~Jerry Arey*
>
> *"The permafrost melting must be causing the slumping and mud slides to increase. What are the implications of this? The mud slights have increased from here to Hershall." ~Melinda Gillis*

“The icebergs have not been here for years. They are part of the habitat for fish. What will happen now?” -Eugene Pascal

Reasons to Return to Shingle Point

Shingle Point is socially and culturally an important destination for the returning families. Interviewed participants shared common views on what Shingle Point is to themselves and their families. Some of the responses as to why they continue to return to the area include: a vacation away from technology, hunting for caribou (August, over winter), berry picking, whaling, fishing, and the annual Shingle Point games. Colton Arey is one of the many youth that return to Shingle Point with his family for fun, and to learn the traditional practices of capturing and preparing fish for food.

“This is Life” ~Denise Arey

Summary

The effects of a changing climate are impacting the Arctic marine environment but the extent and long-term impact are generally unknown (e.g. Arrigo et al. 2008; Lavioe and Denman 2010; Barber et al. 2012). Often the inability to understand change is due to a lack of baseline information and a good understanding of the previous state of the marine environment. Monitoring programs are very useful for identifying change and providing some insight as to the reason for change. The use of TEK and local observations are a useful tool to compliment quantitative monitoring programs to detect long-term change or provide advice to program development and focus monitoring objectives and indicators.

The TEK questionnaires and interviews conducted at Shingle Point in July 2015 was meant to record the TEK and long-term observations of returning Shingle Point families and individuals to discuss fish populations, the environment, and their personal experiences. The results of the TEK questions could be categorized into four sections: observed fish population changes, increases in air and water temperature (environmental changes), and concerns. The majority of participants stated that they did notice changes in the fish population, where fish occurrence, in particular changes in the timing of the dolly varden char migration, and fish health (i.e. worms in Arctic cisco). Arctic cisco, dolly varden char, broad whitefish (*Coregonus nasus*), and inconnu are important subsistence species for the Inuvialuit and Gwich'in people, causing the majority of interviews to focus on these species.

Increases in air and water temperature observed by the majority of participants suggest that environmental changes have been affecting this area for generations. With overwhelming evidence of a warming Arctic, sea-ice extent has decreased affecting the marine ecosystem (e.g. Arrigo et al. 2008; Serreze et al. 2008; Barber et al. 2012), and the Beaufort indigenous peoples who rely on the species for subsistence (Usher 2002). Many of the participants in this study mentioned their concerns about the changes they have observed over the years (e.g. increased mudslides, decrease in ice, change in fish occurrence). Further to this, they all acknowledged the explicit link between the state of the environment (e.g. habitat, water quality) and the marine biota. There is great concern that changes that have been observed will affect the health of the fish populations in the future.

The collaboration between researchers and the Inuvialuit and Gwitch' in during the annual DFO fish monitoring programs at Shingle Point has resulted in an increased understanding of the anadromous, freshwater and marine fishes in nearshore estuaries (e.g. Loseto et al. 2009;

Gallagher et al. 2013; Loewen et al. 2015; Brewster et al. in review). Results from this TEK questionnaire indicated that observational knowledge of TEK holders at Shingle Point contains important long-term monitoring data that can help inform the development of monitoring objectives and indicators, and the assessment of these indicators. Thus, the collection and integration of TEK alongside scientific data should continue to be implemented in monitoring programs of Beaufort coastal habitats. The utilization of TEK in the form of specific and open-ended interviews/questionnaires can be used to document and understand long-term changes in the environment and marine biota, as well as bolster quantitative research in the future, to better understand the environmental impacts of climate change on the Beaufort marine ecosystems.

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APPENDIX 1. Shingle Point, YT fish population questionnaire.

Name:______________________________

Anonymity? Yes No

Hometown:___________________________

1) How many years have you been coming out to Shingle Point? How long has your family?

2) Were there other places that you used to set nets in the past? Has the timing of fish changed since you started coming to Shingle?

3) Have you noticed a change in the weather and water temperature over the years? Can you describe these changes?

4) Did you or your family always come to Shingle Point for fish or were there other reasons to come to Shingle Point in the past (i.e. beluga)?

5) What type of nets do you prefer to fish with, where do you set them (ocean side, or lagoon), and is this species specific?

6) Using the fish guide attached, please provide your observations about changes in the presence and abundance of fish species at Shingle Point.

   a) more abundant b) less abundant c) no change in abundance

7) Which fish do you usually catch?

8) Have you noticed any changes in health of this/these fish over time? Describe the changes that you have noticed for each species that you usually catch:

   Species:____

   a) More fat b) Less fat c) No change

   b) Change in colour:____
   c) Change in muscle texture:____
   d) Other:____

9) How are youth in your family involved in fishing at Shingle point?

10) Do you have concerns about the fish at Shingle Point, or feedback that this questionnaire did not touch on?

APPENDIX 2. Field team for the 2015 Shingle Point Traditional Ecological Knowledge (TEK) interviews.

Name	**Position**	**Organization**
Dana Neumann	Lab and Field Technician	Fisheries and Oceans Canada, Winnipeg, MB
Jasmine Brewster	Graduate Student	University of Manitoba and Fisheries and Oceans Canada, Winnipeg, MB
Kate Snow	Technician	Fisheries and Oceans Canada, Inuvik, NT
Cecilia McLeod	Youth	Aklavik Community Member
Desiree Arey	Youth	Aklavik Community Member

APPENDIX 3. Fish species catalogue for Shingle Point, YT, including common and latin names and pictures for identification.

Species Common and Latin Name	Picture
Arctic cisco **(*Coregonus autumnalis)***	
Arctic flounder ***(Liopsetta glacialis)***	
Broad whitefish ***(Coregonus nasus)***	
Burbot ***(Lota lota)***	
Dolly varden char ***(Salvelinus malma)***	
Fourhorn sculpin ***(Myoxocephalus quadricornis)***	

Species **Common and Latin Name**	**Picture**
Inconnu ***(Stenodus leucichthys)***	
Lake whitefish ***(Coregonus clupeaformis)***	
Longnose sucker ***(Catostomus catostomus)***	
Least cisco ***(Coregonus sardinella)***	
Northern pike ***(Esox lucius)***	
Saffron cod ***(Eleginus gracilis)***	
Starry flounder ***(Platichthys stellatus)***	

Species **Common and Latin Name**	**Picture**
Pacific herring ***(Clupea pallasii)***	
Rainbow smelt ***(Osmerus mordax)***	
Round whitefish ***(Prosopium cylindraceum)***