NARWHAL (*MONODON MONOCEROS*) DIET AND DIVE BEHAVIOUR AS AN ASSESSMENT OF FORAGING ADAPTABILITY WITH CHANGING CLIMATE

By

Cortney A. Watt

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Department of Biological Sciences
University of Manitoba
Winnipeg, MB

Abstract

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Narwhals (*Monodon monoceros*) are sentinel species in the Arctic environment and are a vital component for Inuit culture and subsistence. The Arctic is undergoing rapid changes in temperature and sea ice cover and relatively little is known about how this has and will change narwhal foraging behaviour. There are three narwhal populations in the world, the Baffin Bay (BB), Northern Hudson Bay (NHB), and East Greenland (EG) populations; however, foraging behaviour, in terms of dive behaviour and primary dietary components, has really only been investigated in the BB population. Using a combination of stable isotopes, fatty acids, genetic techniques, and satellite tracking technologies I evaluated foraging behaviour in all three of the world's narwhal populations. I also investigated social structure in the BB population to determine how adaptable narwhals are to a changing and dynamic Arctic environment. Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and fatty acids are chemical signatures in the tissues of an organism that can provide long-term information on their diet over varying temporal scales depending upon the tissue. Stable isotope analysis in the three narwhal populations found they forage on different primary prey, suggesting narwhal are adaptable in their preferred prey and that there is potential for them to adjust foraging behavior in the face of changing climate. Dietary changes were also assessed over three decades to determine how sea ice changes have

affected narwhal foraging for the NHB and BB populations. Dietary changes were evident and can be attributed to changes in sea ice patterns and an altered migratory pathway for narwhals. An understanding of narwhal social structure is also needed to determine how behaviourally flexible narwhal are in diet and site fidelity. Genetic relatedness and dietary signatures from fatty acids were assessed for an entrapped group to determine if individuals that are closely related forage together, which would support a matrilineally driven social structure where females teach their young foraging strategies, and/or travel and forage together. I found no evidence that narwhals form a matrilineal social group, but they may display a fission-fusion structure, which may be an adaptation to patchy prey distribution in the Arctic. Finally, narwhal dive behaviour in all three populations was investigated to determine if dive behaviour could be used to predict diet. Dive differences among populations did correspond with differences in diet, suggesting that narwhals employ specialized foraging strategies. This has repercussions for their ability to adapt to ecosystem changes. Overall, narwhals may be more flexible in terms of their foraging behaviour than previously believed. However, an increased resilience to changing food webs will not be the only predictor of how narwhals will fare in the face of a changing climate; how they respond to increased industrial activities in their preferred habitats, increased predation from southern predators, and increased competition from southern cetaceans and humans alike, will play an equally large role in how they cope with the future.

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In dedication to my father, who is gone but not forgotten,

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Chapter One

General Introduction

Foraging ecology, a branch of behavioural ecology, focuses on an organism's foraging behaviour in response to its environment. Optimal foraging theory, which began with research by Emlen (1966) and MacArthur and Pianka (1966), is one of the most heavily cited and studied theories on foraging ecology. The theory suggests natural selection optimizes an animal's foraging fitness (Pyke et al. 1977). Optimal foraging theory has many facets, but in general makes predictions about an organism's decision to have an optimal diet, patch choice, time allocation, and patterns and speed of movements (Pyke et al. 1977). An animals foraging ecology is limited by 1) food availability, in some cases, optimal food may not be available at all times, 2) morphology, they may not be able to forage on the predicted optimal prey because of limitations such as gape size, 3) behavioural and cultural traits, foraging behaviour shows heritable variation, and in addition, may be a learned behaviour, passed down through generations, and 4) predator avoidance (ie. they may not be able to monopolize on a patch if they are evading predators) (Mangel and Clark 1986). An animal's procedure for behaving optimally is interpreted as having three parts: choosing the prey, establishing constraints, and solving for the optimum by determining the diet that maximizes energy intake while considering the various constraints, including minimizing energy expenditure for capturing and metabolizing prey (Schoener 1971). Schoener (1971) argued that if an animal's time is limited, in marine mammals for example that need to dive to forage and can only hold their breath for a fixed amount of time, fitness is maximized when animals capitalize on their food intake within the minimal time frame. To understand whether an organism is foraging optimally, one must first understand the basic

elements of the animal's diet and biology. Only through an understanding of an animal's preferred prey, foraging habitat, and limitations on inherited behavioural foraging tactics, can one determine whether an animal is foraging optimally (Schoener 1971).

Humans are having drastic impacts on the environment, and climate change is evident at the Earth's poles. Sea ice is in decline all across the Arctic (Tivy et al. 2011) and this is having a profound impact on both the top-down and bottom-up processes in the Arctic food web (Zweng and Münchow 2006). Facing uncertain environments and shifts in the timing of seasons, organisms are tasked with the job of adjusting their behaviours, habitat, and prey to meet their needs. How animals will adjust foraging behaviour to meet their energetic requirements is uncertain; however, foraging theory can be used to generate predictions about how animals may adjust to changes in their habitat and food web. Changes in the types of resources they will be able to exploit, and therefore their foraging efficiency, will undoubtedly be impacted by climate change, and predictions about how these resources have and will continue to shift are vital for understanding their chance of survival. Their breadth of diet may also increase or decrease with climate change, which may have positive or negative impacts on the organisms of interest. For example, it has been observed that polar bears (*Ursus maritimus*) consume a greater proportion of terrestrial prey, including snow geese (*Chen caerulescens caerulescens*), in the ice free period, even though foraging theory suggests a diet including snow geese is not optimal (Iles et al. 2013). Thus, in some instances, a greater diet breadth may not maximize energy efficiency.

The habitats animals exploit may also vary with changing climate, and may be evident in changes in home range size or in migratory pathways. A northern shift in distribution is a common prediction for animals trying to evade climate change (Simmonds and Isaac 2007); however, changes in migration routes may also be evident as a result of sea ice changes,

particularly for populations that already live at the southern edge of their species' range. Understanding social organization in an organism can give insight into how flexible they are in terms of altering behaviour. For instance, animals that inherit their home range and migratory pathways from their mothers often display a matrilineal social structure (Kappeler et al. 2002). If foraging is a leaned behaviour and prey is shared among the social group, animals may be less flexible in adjusting their behaviour in the face of rapidly changing environmental conditions.

Narwhals (*Monodon monoceros*) are part of the Monodontidae family, literally meaning "one tooth." Male narwhals have a long spiral tusk, which erupts out of the left upper jaw. The purpose of the tusk has been hotly debated, but it is generally considered a sexually selected trait that attracts females (Kelley et al. 2014). Narwhals live in upwards of 95% pack ice and in darkness over the winter months, travelling in large herds and using leads and cracks in the ice for breathing (Laidre and Heide-Jørgensen 2011). As a result of the harsh conditions faced by these organisms, studying their ecology is costly and logistically difficult. There are three populations of narwhals in the world, the Baffin Bay (BB), Northern Hudson Bay (NHB), and East Greenland (EG) populations. These populations are geographically and genetically distinct (Petersen et al. 2011). Research has primarily focused on the most numerous BB narwhal population with little attention focused on the other populations; however, how animals will fare in the face of changing climate may be specific to foraging adaptations by specific populations.

Current knowledge about narwhal diet comes primarily from stomach content analysis of hunted narwhals from BB. No stomach contents have been assessed since 2004 (Laidre and Heide-Jørgensen 2005). Given that the Arctic is currently undergoing changes in climate and ice conditions (Stroeve et al. 2005), which are also impacting the predator prey dynamics, and subsequently, the Arctic food web (Higdon and Ferguson 2009, Wassmann et al. 2011), an

understanding of diet in all populations and in all seasons across multiple years, is required to evaluate how changes in the food web are impacting these sentinel species, which the Inuit rely on. Specific objectives of my thesis include: 1) identifying and comparing diet in the world's three narwhal populations, 2) investigating temporal changes, both seasonally and annually over the last three decades, in foraging behaviour in the BB and NHB narwhal populations, 3) investigating social structure in a sample of entrapped BB narwhals, and 4) to investigate dive behaviour in all three populations.

I used stable isotope, fatty acid and genetic analyses, and satellite-tracking technologies to address these objectives. Stable isotopes are variations of an element with a different number of neutrons, and therefore a different atomic mass. There is natural variation in the abundance of the isotopes in the environment, as the isotopes behave differently depending upon their mass. In predator tissues, the stable isotope ratios of nitrogen (^{15}N:^{14}N or commonly expressed as $\delta^{15}N$) and carbon (^{13}C:^{12}C or $\delta^{13}C$) are directly related to the ratios found in their prey (Peterson and Fry 1987). Stable isotopes can give detailed information regarding the integrated dietary and geographic information of a species and since tissues turn over at different rates, different tissues can provide dietary information over varying temporal and spatial scales (Tieszen et al. 1983). Nitrogen ratios are often used as indicators of the relative trophic position of an organism, while $\delta^{13}C$ is used to evaluate the source of carbon such as benthic versus pelagic (Post 2002). If stable isotope values of potential prey are also known $\delta^{13}C$ and $\delta^{15}N$ can be used to model diet and determine primary prey components (Crawford et al. 2008). Stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) of skin tissues can be used to evaluate dietary differences among the world's three narwhal populations and to identify important prey. Through identification of primary prey for all three of

the world's narwhal populations I can determine how behaviourally flexible narwhals are to switching primary prey in the face of a changing food web.

Fatty acids are the main constituents of lipids and are long chain carbon molecules that are incorporated, relatively unmodified, from prey into predator tissues (Budge et al. 2002). Much like stable isotopes, these lipid molecules can be used to evaluate diet and investigate long-term changes in diet. Analysis of fatty acids has been conducted in a number of marine mammals to investigate diet, including minke whales (*Balaenoptera acutorostrata*) (Olsen and Grahl-Nielsen 2003), bottlenose dolphins (*Tursiops truncates*) (Samuel and Worthy 2004), polar bears (*Ursus maritimus*) (Grahl-Nielsen et al. 2003, Thiemann et al. 2008), bottlenose whales (*Hyperoodon ampullatus*) (Hooker et al. 2001), grey seals (*Halichoerus grypus*) (Grahl-Nielsen et al. 2000) and a number of other seals (Thiemann et al. 2008), beluga whales (*Delphinapterus leucas*) (Dahl et al. 2000, Thiemann et al. 2008), and on a small samples of narwhals (Thiemann et al. 2008). Individually, analyses of stable isotopes and fatty acids have limitations; however, when used in conjunction they can provide a comprehensive understanding of diet (Hooker et al. 2001, Loseto et al. 2008, Tucker et al. 2008, Loseto et al. 2009). Fatty acid analysis of blubber and stable isotope analysis of skin and muscle can be used in conjunction to evaluate long-term trends in diet in two of these three populations over a 30-year time period. Unlike stomach contents, which only provide information on the most recent meal, these chemical techniques can provide long-term information on diet in seasons when sampling may not be possible. This is particularly important for narwhals that live in heavy ice cover and darkness throughout the winter, at which time observations and sampling are not feasible. Fatty acid and stable isotope analysis is also beneficial because through a collection of different tissues (blubber, skin, and muscle) with different turnover rates, an evaluation of seasonal changes in diet is possible and

can be used to determine how unpredictable sea ice changes may be altering seasonal prey availability.

Little is currently known about narwhal social structure; however, to determine how they may adapt to a changing Arctic, it is important to have an understanding of their social organization. Previous knowledge on narwhal social structure comes from observations in the summer (Marcoux et al. 2009), but with herds of narwhals numbering up to 1000, it is difficult to observe close connections among individuals within the larger group. Studies evaluating social structure in pilot whales (*Globicephala melas*) and river otters (*Lontra Canadensis*) have found significant differences in dietary signatures of individual social groups (Blundell et al. 2002, de Stephanis et al. 2008). Cetaceans displaying matrilineal social structure have closely related family members travelling and socializing together (Whitehead 1998) and within matrilineal societies prey are often shared among members of this familial group (Ford and Ellis 2006). For a herd of narwhals trapped in the ice, if diet differs among distinct genetic groups there is indirect evidence that these genetic clusters form social groups or that foraging is a matrilineally driven learned behaviour. In this case narwhals may not be behaviourally flexible even if they are able to forage on varying prey, as they may not be willing or able to diversify their summer and winter home ranges. Thus, genetic relatedness in conjunction with fatty acid analysis can be used to evaluate narwhal social structure.

Previous satellite tracking of narwhals has provided information regarding the home-range size in summer and winter locales (Heide-Jørgensen et al. 2002), migratory pathways (Dietz et al. 2001, Heide-Jørgensen et al. 2002, Heide-Jørgensen et al. 2003), diving habits (Martin et al. 1994, Heide-Jørgensen and Dietz 1995, Laidre et al. 2002, Laidre et al. 2003, Laidre et al. 2004a, Lydersen et al. 2006), evasion behaviour (Laidre et al. 2006), stock

assignment (Dietz and Heide-Jørgensen 1995, Dietz et al. 2001, Heide-Jørgensen et al. 2002), and even to infer tusk growth (Heide-Jørgensen et al. 2008). Dive behaviour can also provide insight into foraging behaviour and potential prey (Laidre et al. 2003). No study has investigated dive behaviour in the EG narwhal population, and investigations of the NHB population are limited to analysis of time spent at the surface for calibrating survey abundance estimates (Westdal et al. 2013). Although there has been no investigation of foraging behaviour among all three narwhal populations there is evidence that narwhals that winter in different areas display varied foraging behaviours and may preferentially feed on different prey (Laidre et al. 2003). By considering environmental factors, such as bottom bathymetry, benthic versus pelagic foraging can be inferred, and previous knowledge suggests bottom bathymetry is correlated with dive behaviour in narwhals (Laidre et al. 2004b). I compare foraging behaviour in all three of the world's narwhal populations in their summer and winter grounds to determine important seasons and depths for foraging.

Prelude

Each of the subsequent chapters evaluates one of the objectives of the thesis, and together, creates a framework for understanding narwhal foraging behaviour in the face of changing climate. Chapters are designed as publications and can stand-alone. As such, some information has been repeated in the introductions of different chapters to guide the reader.

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Chapter Two

How adaptable are narwhal: a comparison of foraging patterns among the world's three narwhal populations

Watt, C. A.[1], M. P. Heide-Jørgensen[2], and S. H. Ferguson[1,3]

[1]Department of Biological Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
[2]Greenland Institute of Natural Resources, 3900 Nuuk, Greenland
[3]Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada

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Contribution

Dr. Heide-Jørgensen shipped skin samples from narwhals from the East Greenland population and provided minor editorial comments on a final version of the manuscript.

Dr. Ferguson provided funding for stable isotope analysis and provided guidance and comments throughout the writing process.

Cortney Watt prepared all samples for stable isotope analysis (which were then shipped to University of Windsor for analysis), wrote the manuscript in its entirety, conducted all statistical analysis and created all accompanying figures and tables.

Abstract

How organisms will fare in the face of climate change depends on their behavioral adaptability to changing conditions. Adaptability in foraging behavior will be particularly critical as food web changes are already occurring in Arctic regions. Stomach contents from narwhals (*Monodon monoceros*) in the Baffin Bay (BB) population have suggested that narwhals are dietary specialists with little behavioral flexibility, but there are two other narwhal populations in the world, the Northern Hudson Bay (NHB) and East Greenland (EG) populations, of which very little is known about diet. We investigated whether plasticity in foraging behaviors existed among the world's narwhal populations and between sexes by comparing their stable isotope values and niches, and running stable isotope mixing models to determine primary prey. Stable isotope analysis was conducted on skin collected by Inuit hunters during their subsistent narwhal hunt in Canada and Greenland. Stable isotope analysis on carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) revealed the three populations have distinct stable isotope values that are not expected based on geographic differences and that males in all populations had significantly higher $\delta^{13}C$. Stable isotope mixing models revealed narwhals in EG forage more on pelagic prey, particularly capelin, while those in NHB typically forage in the benthos. Males, probably because of their size and enhanced diving ability, likely feed more intensively on benthic organisms, resulting in their higher $\delta^{13}C$. Stable isotopic niches were similar between males and females in each population, and between NHB and BB, but EG narwhals had a significantly larger niche, suggesting they either forage across a larger geographical expanse, or whales within the population employ individual dietary specialization. This is the first study to use stable isotopes to evaluate and compare diet in all three narwhal populations, which is vital for understanding how they will fare in the face of changing climate. We discuss how narwhal are adaptable in

their preferred prey and how there is potential for narwhal to adjust foraging behavior in the face of the dramatic ecosystem shifts occurring with climate warming.

Keywords: adaptability; Arctic; climate change; diet; isotopic niche; mixing models; *Monodon monoceros*; stable isotopes.

Introduction

Historically, rapid climate change has been implicated as a cause of adaptation, range shift, and evolution for mammals (Bruyn et al. 2009). Phenotypic plasticity, which is the capacity for one genotype to produce alternate morphological, physiological or behavioral forms in response to variable conditions (West-Eberhard 1989), increases a species chance of survival in a changing environment. Plasticity, in terms of foraging behavior, within a species can result from spatial segregation resulting in individuals experiencing different environmental conditions (MacArthur and Wilson 1967). In these allopatric populations, evolutionary change in foraging behavior with no changes in physiology or morphology can account for differences in preferred prey (Futuyma and Moreno 1988). In some cases, behavioral modifications across groups of organisms precede genetic differentiation into populations (Price et al. 2003).

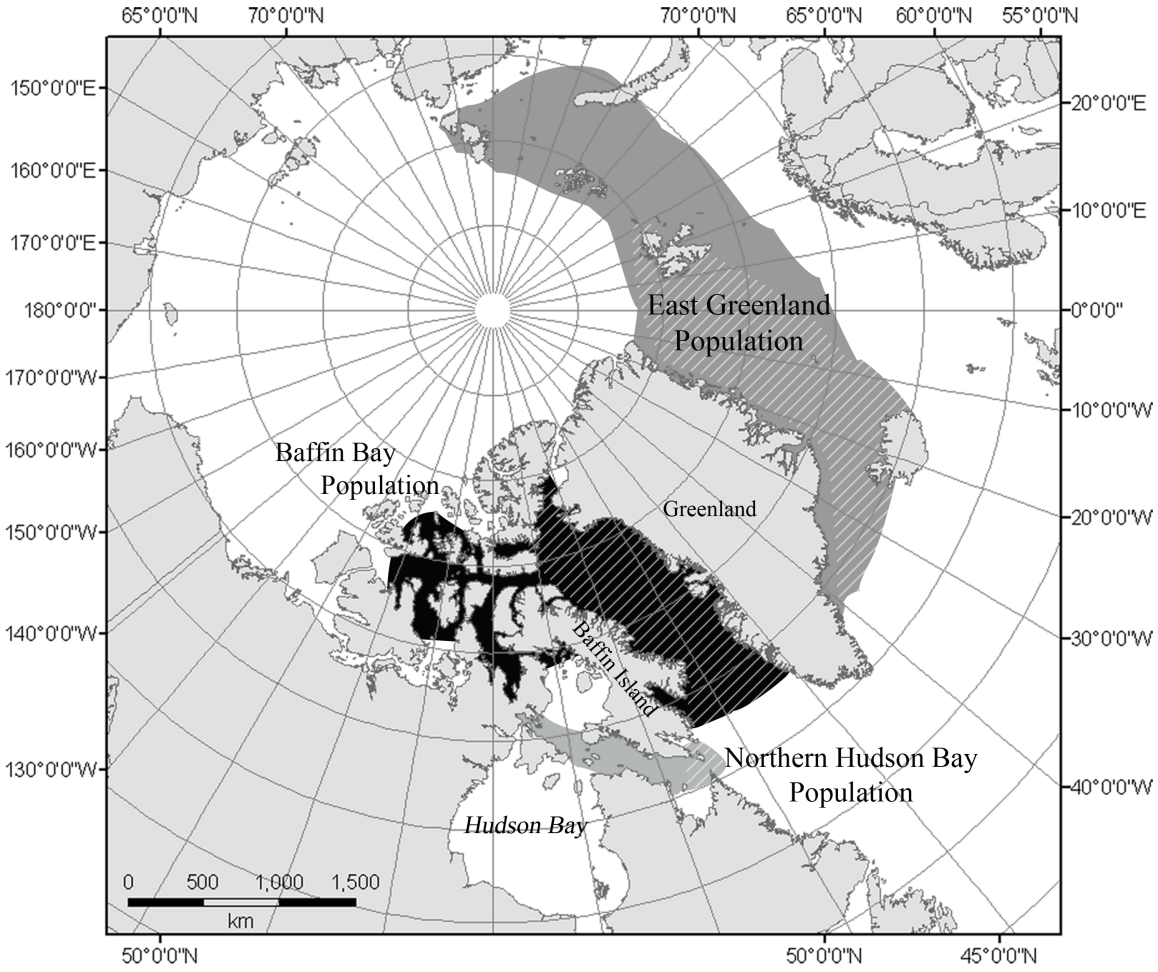
Narwhals (*Monodon monoceros* L.) are medium sized, toothed whales that live exclusively in Arctic waters. The species underwent a significant range contraction during the little ice age that accompanied changes in ice conditions (Hay and Mansfield 1989, Laidre et al. 2008). As a species, narwhals have extremely low genetic diversity (Palsbøll et al. 1997, Petersen et al. 2011), which may reduce their ability to evolve in response to environmental changes. In addition, narwhals are believed to be extremely sensitive to environmental changes as a result of their limited distribution, selective diet, and reduced capability for behavioral modification (Laidre et al. 2008). However, the sensitivity of the species as a whole is based exclusively on information from only one of the three narwhal populations and different populations within a species can demonstrate different adaptations, particularly if these populations are spatially segregated and encounter different environmental conditions (Price et al. 2003). We investigated whether plasticity in foraging behaviors exists among the world's

narwhal populations to understand if they may be able to modify their foraging behaviour in a changing Arctic environment.

There are currently three spatially and genetically segregated narwhal populations recognized in the world (Petersen et al. 2011) (Fig. 2.1). The East Greenland (EG) population inhabits the eastern shores of Greenland and the Greenland Sea with current estimates of about 6,000 individuals (Heide-Jørgensen et al. 2010). The Baffin Bay (BB) population overwinters in the Davis Strait and spends summer in the fiords and inlets of northern Canada and western Greenland and is estimated to be larger than 60,000 individuals (Richard et al. 2010), and the Northern Hudson Bay (NHB) population spends winter in the Hudson Strait and summers in northern Hudson Bay (Richard 1991) and is estimated at 12,500 individuals (Asselin et al. 2012). Narwhals are a culturally and economically important species to the Inuit (Hoover et al. 2013) and the reported annual subsistent harvest of narwhals is approximately 110 for the EG population, 800 for the BB population, and 100 for the NHB population (Heide-Jørgensen et al. 2010, DFO 2012). Narwhals are considered to be one of the most sensitive Arctic marine mammals to climate change and the narwhal's limited distribution (only occurring in the Atlantic region of the Arctic), small population size (ca. 100,000), and dietary specialization are all contributing factors for their high sensitivity (Laidre et al. 2008).

Current knowledge about narwhal diet comes primarily from stomach content analysis on hunted narwhals from the BB population, with no studies being conducted on the NHB or EG narwhals. Stomach contents from BB narwhals have identified the primary prey as Greenland halibut (*Reinhardtius hippoglossoides*), Arctic cod (*Boreogadus saida*), polar cod (*Arctogadus glacialis*), and squid (*Gonatus fabricii*) (Finley and Gibb 1982, Laidre and Heide-Jørgensen 2005a). Finley and Gibb (1982) identified a few other species, such as snailfishes (*Liparis* spp.),

Figure 2.1. Approximate summer (solid) and winter range (hashed) for narwhals from the three populations: Baffin Bay (black), East Greenland (dark grey), and Northern Hudson Bay (light grey).



redfish (*Sebastes marinus*), sculpins (Cottidae), eelpout (*Lycodes* spp.), and skate egg sacs (*Raja* spp.), and Hay and Mansfield (1989) identified some shrimps (*Pasiphaea tarda* and *Hymenodora glacialis*) in narwhal stomachs, albeit in small amounts. Laidre and Heide-Jørgensen (2005a) also identified the shrimp *Pandalus borealis* as being abundant in narwhal stomachs, and capelin (*Mallotus villosus*), skate egg sacs, and wolffish (*Anahichas lupus* and *A. minor*) as minor prey items.

A sexual size dimorphism exists between male and female narwhals (Garde 2011) and males have an erupted left tooth that results in a spiraled tusk. Few studies have been able to compare diet between male and female narwhals because of low sample size of females (Laidre and Heide-Jørgensen 2005a), and the only study that was able to compare did not find evidence of dietary segregation between narwhal sexes based on stomach contents, although they also had a relatively small female sample (n = 29) (Finley and Gibb 1982). Although no dietary differences have been noted, a sexual size dimorphism does exist in all populations, and typically hunter collected samples are biased towards males, limiting the ability to detect differences in diet between males and females. Diving capacity is greater for larger animals (Schreer and Kovacs 1997) and male narwhals are likely capable of making deeper dives and potentially foraging more in the benthos in deep waters. In addition, males, because of their larger size, may be able to manipulate larger prey than females.

Stomach content analysis of the BB population, although insightful to give a first understanding of narwhal diet, can result in biased estimates as a result of varying digestion rates, only provides information on what was consumed and not what was assimilated into tissues, and only provides information on the organism's last meal. This is particularly a problem in Arctic marine mammals that experience seasonal changes in food availability and diet (Laidre

and Heide-Jørgensen 2005a, Bluhm and Gradinger 2008) and have high-energy requirements (Laidre et al. 2004a) because stomach contents may not be representative of typical feeding events. More recent studies investigating diet in marine mammals have used stable isotope analysis to determine differences in trophic level, pelagic versus benthic feeding, and to determine proportions of prey consumed (Newsome et al. 2010)

Stable isotope analysis is a powerful tool for studying animal feeding ecology because predator tissues are directly related to the ratios found in their prey with a progressive enrichment factor. The stable nitrogen isotope ratio (^{15}N:^{14}N, expressed as $\delta^{15}N$) provides information on an organism's trophic level and the stable carbon ratio (^{13}C:^{12}C, expressed as $\delta^{13}C$) reflects its spatial foraging distribution (pelagic versus benthic, or offshore versus coastal) (Peterson and Fry 1987, Newsome et al. 2010). Previous studies investigating stable isotope ratios in narwhals have determined their trophic position (Hobson and Welch 1992, Hobson et al. 2002) and found differences in $\delta^{15}N$ and $\delta^{13}C$ between narwhals from two different locations in West Greenland (Dietz et al. 2004), but no study has conducted a large-scale comparison of narwhal stable isotope values across the three populations. Stable isotope ratios vary with geography as a result of variable $\delta^{15}N$ and $\delta^{13}C$ at the base of the food web, and there is known to be a large gradient in $\delta^{13}C$ across the narwhal's geographic span (Graham et al. 2010). An organism's isotopic niche, or the extent of stable isotope values for all the resources used by an organism, can provide insight into an animals' dietary niche (Bearhop et al. 2004, Jackson et al. 2011). In general, animals with very small isotopic niches are often dietary specialists that consume only one or a few types of resources and therefore have relatively low variability in their isotopic values, whereas dietary generalists that forage on a variety of organisms have a much larger isotopic niche. This general rule, however, has caveats and depends on the isotopic

variability of the environment in which the organisms live and the prey sources they consume and usually stable isotope niche width is not directly comparable to an animal's trophic niche (Cummings et al. 2012). Despite the limitations of stable isotope niche analysis, if isotopic variation in prey is taken into account, niche analysis can provide information on dietary variation at the population level (Newsome et al. 2012)

In this study we investigated three hypotheses. Our first was that narwhals display phenotypic plasticity, in terms of their foraging behavior, and this would result in populations having different $\delta^{13}C$ and $\delta^{15}N$ values. We expected $\delta^{13}C$ and $\delta^{15}N$ would differ for the three populations because stable isotope ratios at the base of the food web would likely differ across such a wide geographic expanse; however, if the three populations also have distinct foraging behavior which results in different preferred prey, the magnitude of the differences in $\delta^{13}C$ and $\delta^{15}N$ should be greater than would be expected by geography alone. Second, we hypothesized that dietary niche sizes for the three populations would differ. We predicted the isotopic niche of the BB population would be larger than the NHB and EG populations because they are part of a much larger population, which may increase competition for resources and result in individuals or groups of individuals specializing on specific prey to limit competition with other groups for their preferred prey. Alternatively, narwhals in East Greenland have a larger range compared to the other two populations, which means they may encounter different geographic regions with different baseline $\delta^{13}C$ and $\delta^{15}N$ values, which would result in this population having a larger isotopic niche. Third, we hypothesized that males, having a larger body size, would be able to exploit a greater range of resources than females, which would result in them having different $\delta^{13}C$ and $\delta^{15}N$, as well as a larger isotopic niche. In particular, we expected they might be able dive deeper and spend greater time at depth compared to females, which would result in their

skin tissue expressing a higher $\delta^{13}C$ value, and that they could forage on larger prey, which may increase the $\delta^{15}N$ value of their tissues. Finally, we wanted to estimate the importance of primary prey components for each of the three populations and discuss the potential for narwhal to adjust foraging behavior in the face of the dramatic ecosystem shifts occurring with climate warming.

Methods

Inuit hunters and researchers collected narwhal skin samples from narwhals in Pond Inlet, Nunavut, Canada (representative of the BB population), Repulse Bay, Nunavut, Canada, (representative of the NHB population), and Ittoqqortormiut, Greenland (representative of narwhals from EG). Since all samples are collected opportunistically, this resulted in a varying number of male and female samples, which spanned various temporal scales; however, we limited our analyses to include only samples collected in the summer months, which we defined as June through September following Finley and Gibb (1982). Unfortunately, samples from other seasons were rare across populations and we were unable to compare seasonal diet.

Narwhal skin tissue was sub-sectioned and a 0.5 g piece of skin was diced, freeze-dried for 48 hours, homogenized with a glass mortar and pestle, and lipid extracted using a 2:1 chloroform: methanol solution. A continuous flow isotope ratio mass spectrometer (IRMS, Finnigan MAT Deltaplus, Thermo Finnigan, San Jose, CA, USA) was used to determine $\delta^{13}C$, $\delta^{15}N$ and % C and N of 400-600 μg of tissue. The standard reference material was Vienna Pee Dee Belemnite carbonate for CO_2 and atmospheric nitrogen N_2. Every 12^{th} sample was run as a triplicate to assess precision; the mean standard deviation of these samples was 0.1 ‰ for $\delta^{13}C$ and 0.4 ‰ for $\delta^{15}N$. Internal lab and National Institute of Standards and Technology (NIST) standards were analyzed after every 12 samples for quantification of samples and to assess analytical precision. The analytical precision (standard deviation) for NIST standard 8414

(bovine muscle, n = 152) and an internal lab standard (tilapia muscle, n = 152) for $\delta^{13}C$ was 0.07 ‰ and 0.09 ‰, and for $\delta^{15}N$ was 0.15 ‰ and 0.19 ‰. To assess accuracy, certified NIST standards were analyzed during sample analysis. For $\delta^{13}C$, the mean value for NIST 8542 (-10.48 ± 0.03 ‰; n = 10) was within 0.01 of the certified value of -10.47 and for NIST 8573 (-26.26 ± 0.04 ‰; n = 10) was within 0.13 of the certified value of -26.39 ‰. For $\delta^{15}N$, the mean value for NIST 8542 (4.58 ± 0.11 ‰; n = 10) was within 0.12 of the certified value of 4.70 ‰ and for NIST 8548 (20.11 ± 0.38 ‰; n = 9) was within 0.30 of the certified value of 20.41 ‰. All stable isotope analyses were conducted at the University of Windsor, Great Lakes Institute for Environmental Research. $\delta^{13}C$ values were corrected for the "Suess effect" by applying a correction of 0.02 ‰ (an average of values reported by Körtzinger and Quay (2003) and Sonnerup et al. (1999) from the North Atlantic Ocean), per year beyond 1982 (the oldest sample included in the data set).

Normal quantile plots for $\delta^{13}C$ and $\delta^{15}N$ were normally distributed across populations and between sexes. Variances were also homogenous for the three populations across both sexes for $\delta^{13}C$ (Levene's test: $F_{5, 211} = 0.38$, $p = 0.86$) and $\delta^{15}N$ (Levene's test: $F_{5, 211} = 1.31$, $p = 0.26$), thus no data transformations were required. A generalized linear model, which included population, sex and the interaction between the two factors, was used to assess if $\delta^{13}C$ and $\delta^{15}N$ values differed among populations and between sexes. When significant differences were detected in the full model, Tukey's HSD tests were used to determine which populations differed. Niche widths were calculated and statistically compared using a Bayesian framework, which uses a multivariate ellipse-based metric that is useful for comparing populations of different sizes (Jackson et al. 2011). Ellipse standard areas, which are the bivariate equivalent to standard deviations, were calculated using the SIBER package within SIAR in R (Parnell et al. 2010).

Next, we compared predator and prey stable isotope values to understand what prey may be contributing to the differences in stable isotope ratios among populations. Potential prey was identified based on stomach content analyses of hunted narwhals from the BB population conducted by Finley and Gibb (1982) and Laidre and Heide-Jørgensen (2005a) and included Arctic cod (*B. saida*), polar cod (*A. glacialis*), Greenland halibut (*R. hippoglossoides*), shrimp (*P. borealis*), squid (*Gonatus* spp.), and capelin (*M. villosus*). Discrimination factors of 2.6 ‰ for $\delta^{15}N$ and 1.9 ‰ for $\delta^{13}C$ were added to the potential prey. These values were calculated by taking an average of fractionation factors reported by the only two studies that have investigated trophic enrichment in the skin of odontocetes (studies were conducted on killer whales (*Orcinus orca*) and bottlenose dolphins (*Tursiops truncatus*) (Caut et al. 2011, Fernández et al. 2011)). Since prey stable isotope values may vary with geography, we attempted to obtain prey from the specific regions, unfortunately this was not always possible, and in these cases we relied on values reported in the literature (Table 2.1). SIAR was used to assess the contribution of each prey to the diet of narwhals for the three populations (Parnell et al. 2010).

Results

The three populations had significantly different $\delta^{13}C$ ($F_{2, 211} = 319.88$, $P < 0.0001$) and $\delta^{15}N$ ($F_{2, 211} = 201.10$, $P < 0.0001$) (Fig. 2.2). NHB had the highest mean $\delta^{13}C$ (-17.0 ‰) and BB had the highest mean $\delta^{15}N$ (16.6 ‰); EG had the lowest mean $\delta^{13}C$ (-19.1 ‰) and $\delta^{15}N$ (14.6 ‰). Males and females differed in $\delta^{13}C$ ($F_{1, 211} = 9.72$, $P < 0.01$) with males having significantly higher $\delta^{13}C$ (-17.9 ‰) than females (-18.1 ‰) (Fig. 2.2). Sexes did not differ significantly in their $\delta^{15}N$ values ($F_{1, 211} = 1.28$, $P = 0.26$) and there was no significant interaction between sex and

Table 2.1. Collection location, date, and stable isotope values (mean ± SD) for potential narwhal prey used in isotope mixing models for each population.

Population	Prey	*Genus species*	Location	n	Year	$\delta^{13}C \pm SD$ (‰)	$\delta^{15}N \pm SD$ (‰)	Source
BB	Polar cod[†]	*A. glacialis*	Davis Strait	8	2004	-20.5 ± 0.3	13.5 ± 0.5	
	Arctic cod[†]	*B. saida*	Davis Strait	15	2004-2011	-20.5 ± 0.4	14.0 ± 1.0	
	Squid[‡]	*G. fabricii*	Davis Strait	15	1999-2011	-20.3 ± 1.3	11.9 ± 1.9	
	Capelin[‡]	*M. villosus*	Cumberland Sound	7	2008	-19.3 ± 0.1	12.9 ± 0.1	1
	Shrimp	*P. borealis*	Davis Strait	10	1999	-18.7 ± 0.2	14.5 ± 0.5	
	Greenland halibut	*R. hippoglossoides*	Davis Strait	20	1996-2011	-20.0 ± 0.8	15.0 ± 1.7	
NHB	Arctic cod	*B. saida*	Hudson Strait	5	2011	-19.9 ± 1.3	13.5 ± 0.5	
	Squid[‡]	*G. fabricii*	Hudson Strait	11	2004-2011	-19.1 ± 1.3	12.0 ± 1.8	2
	Capelin[‡]	*M. villosus*	Hudson Strait	5	2011	-19.0 ± 0.5	12.1 ± 0.3	
	Shrimp	*P. borealis*	Northeast Newfoundland Offshore	10	1995	-17.9 ± 0.3	11.3 ± 0.2	3
	Greenland halibut	*R. hippoglossoides*	Hudson Strait	5	2011	-19.4 ± 0.7	14.2 ± 0.5	
EG	Polar cod	*A. glacialis*	Northeast Greenland fiords	60	2003	-21.1 ± 0.4	14.6 ± 0.6	4
	Arctic cod	*B. saida*	Northeast Greenland fiords	60	2003	-21.4 ± 0.3	13.9 ± 0.6	4
	Squid	*G. fabricii*	West	26	2003	-18.8 ± 0.5	13.1 ± 1.7	5

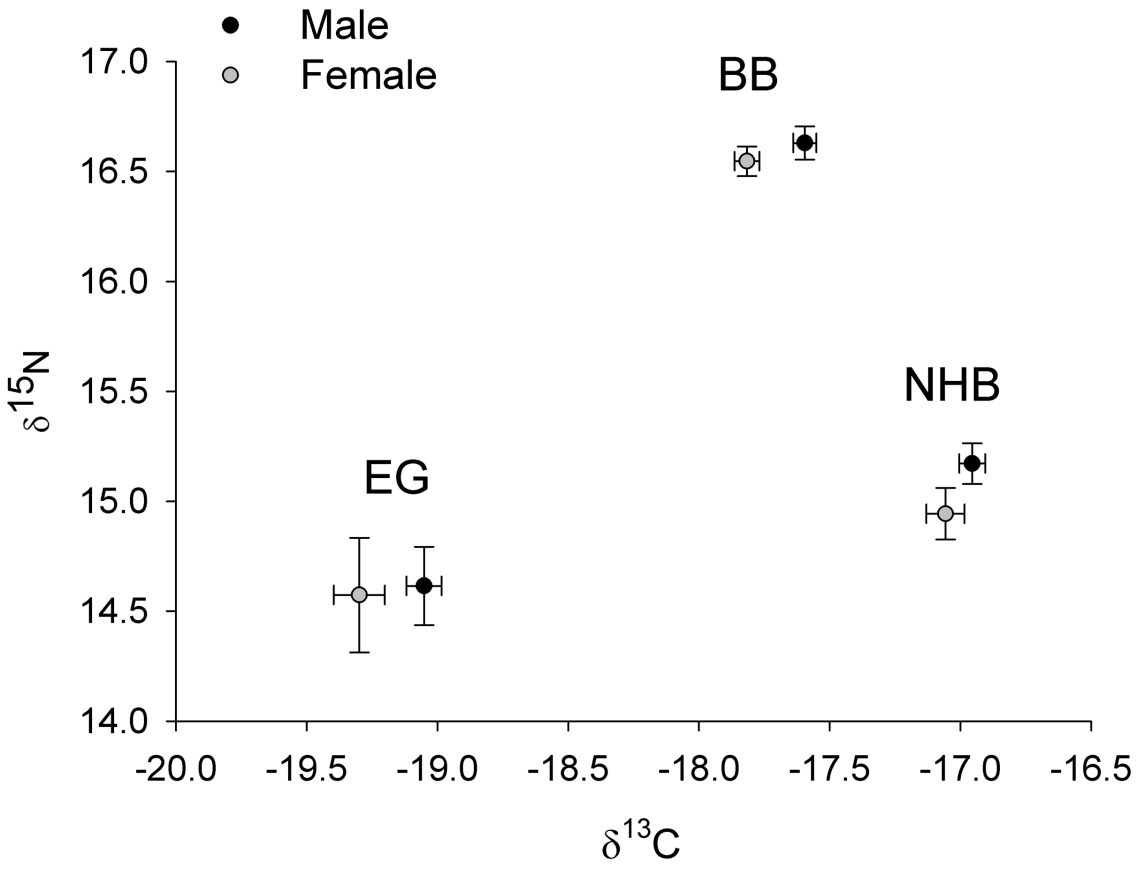
		Greenland					
Capelin	*M. villosus*	Northern Iceland	16	1994-1995	-21.3 ± 0.3	10.8 ± 0.1	6
Shrimp	*P. borealis*	Northern Iceland	8	1994-1995	-19.4 ± 0.1	11.0 ± 0.1	6
Greenland halibut	*R. hippoglossoides*	West Greenland	13	2003	-18.4 ± 0.9	13.9 ± 1.0	5

Note: Sources are: 1, Dennard et al. (2009); 2, Chambellant, Elliott and Ferguson unpublished data and this study; 3, Lawson and Hobson (2000); 4, Christiansen et al. (2012); 5, Møller (2006); 6, Thompson et al. (1999). All other values came from this study.

[†]Arctic and polar cod stable isotope values were not distinct and were combined to define an individual cod value.

[‡]Squid and capelin values were not distinct and were combined.

Figure 2.2. $\delta^{13}C$ and $\delta^{15}N$ (mean ± SE) for males and females in the BB (n = 69 and 58), NHB (n = 41 and 24), and EG (n = 17 and 8) narwhal populations.



population for $\delta^{13}C$ ($F_{2, 211} = 0.69$, $P = 0.50$) or $\delta^{15}N$ ($F_{2, 211} = 0.37$, $P = 0.69$) (Fig. 2.2). Standard ellipse area of the EG population ($n = 25$) was larger than the ellipse for the BB population ($n = 127$), although this result was not quite significant ($P = 0.06$), and significantly larger than the NHB ellipse (n = 65; $P = 0.04$). There was no significant difference between ellipse area for narwhals from NHB and BB ($P = 0.66$) (Fig. 2.3). Male and female narwhals did not differ in their isotopic niche size in the BB ($n = 69$ and 58, respectively; $P = 0.15$), EG ($n = 17$ and 8, respectively; $P = 0.78$) or NHB ($n = 41$ and 24, respectively; $P = 0.90$) populations (Fig. 2.3). Cod (*A. glacialis* and/or *B. saida*), Greenland halibut (*R. hippoglossoides*), shrimp (*P. borealis*), squid (*Gonatus* spp.), and capelin (*M. villosus*) were all considered potential prey for narwhals in each population (Table 2.1, Fig. 2.4A). Results from the stable isotope mixing models revealed that narwhals from EG consume significantly more capelin than other populations, and less shrimp (Fig. 2.4A). Narwhals from BB consumed slightly more Arctic and polar cod (the two could not be distinguished for this population) than NHB narwhals, and NHB narwhals consumed more Greenland halibut (Fig. 2.4A). Male and female narwhals typically had similar diets within a population, but in BB, males appeared to consume more shrimp than females, while females ate more cod, and in NHB males ate more halibut and less capelin and squid compared to females (Fig. 2.4A). Males and females in EG were difficult to distinguish based on their prey proportions (Fig. 2.4A). When prey were assigned to their respective habitats (pelagic or benthic), and mean proportion of prey was assessed it was evident that male and female narwhals from EG feed in the pelagic zone to a greater extent, while narwhals in NHB forage more in the benthos (Fig. 2.4B). Males in BB spend more time foraging in a benthic food web, while females in this population forage similarly in a benthic and pelagic food web (Fig. 2.4B).

Figure 2.3. A) $\delta^{13}C$ and $\delta^{15}N$ biplot with all male and female narwhals from the BB (n = 69 and 58), EG (n= 17 and 8), and NHB (n = 41 and 24) populations. Thin dashed lines indicate convex hulls of total niche width (Layman et al. 2007). Solid lines (males) and thick dashed lines (females) indicate standard ellipse area, representative of isotopic niches (Jackson et al. 2011) and B) boxplot indicating the area (per mil^2) of the isotopic niche for male and female narwhals in each population. Boxes indicate 50, 75, and 95 % credibility intervals.

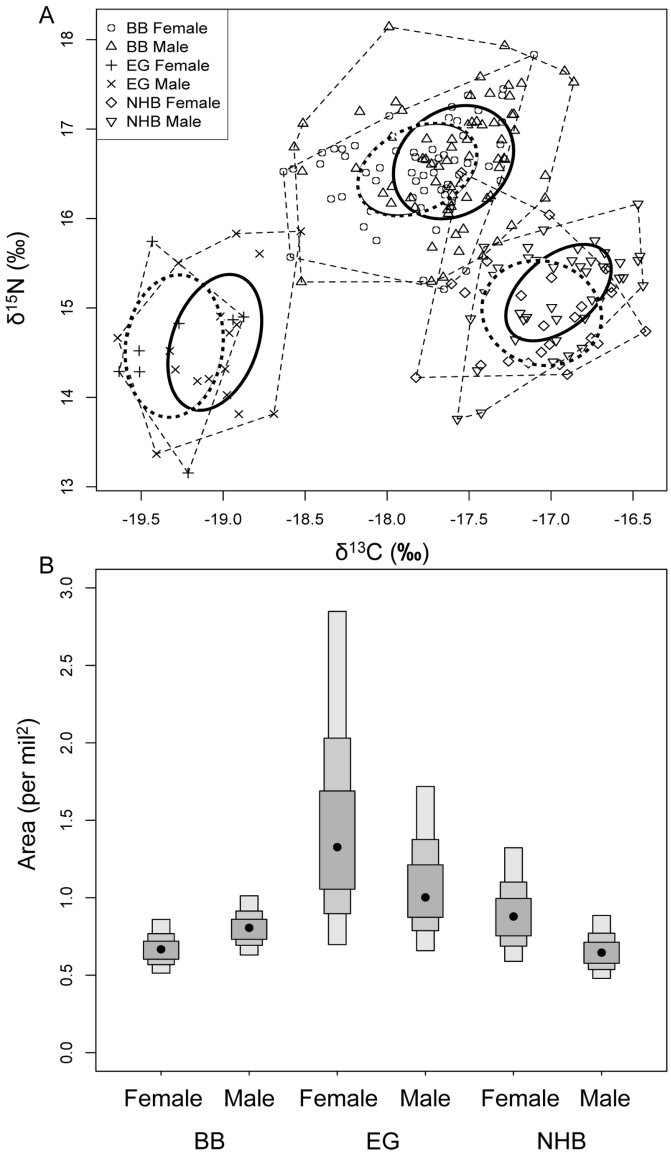
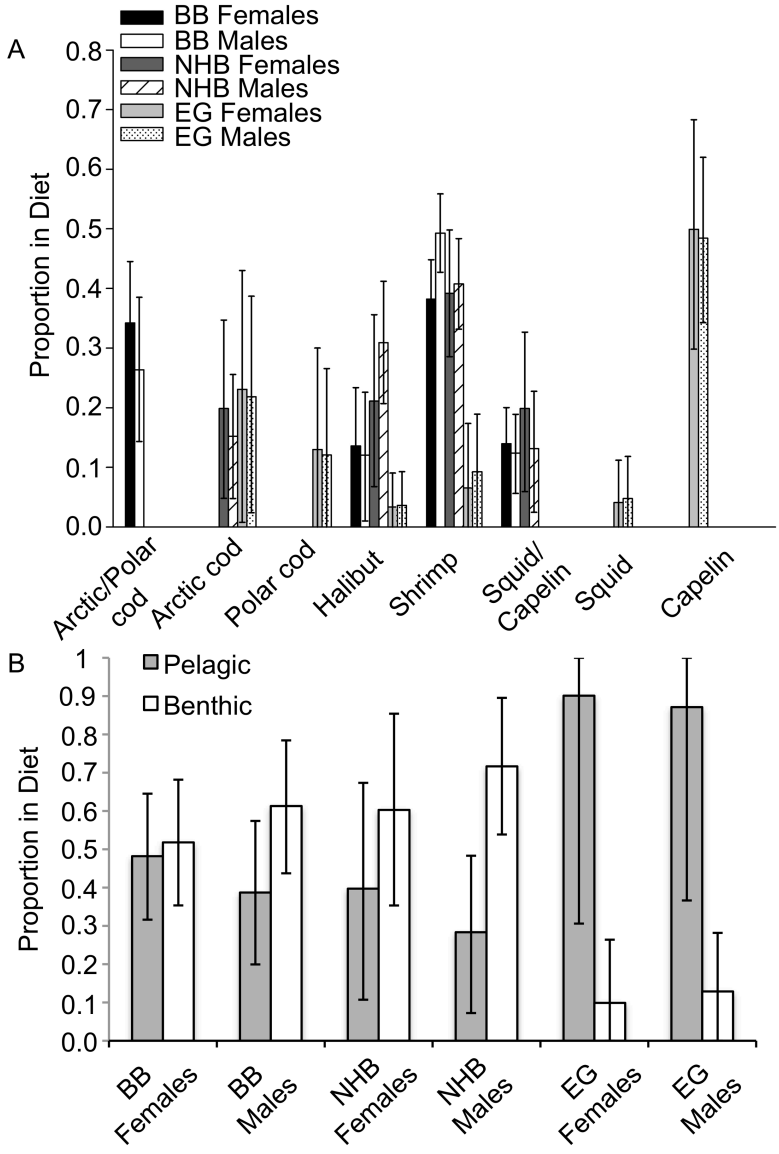


Figure 2.4. The proportion each prey (A) and prey type (B) contributes to the diet of male and female narwhals from the BB (n = 69 and 58), NHB (n = 41 and 24) and EG (n = 17 and 8) populations. Error bars indicate 95 % upper and lower probability values. A) Arctic and polar cod, and capelin and squid could not be distinguished within the mixing models for some populations, and in this case they are listed together on the y-axis. B) Pelagic prey included cod, capelin, and squid, and Greenland halibut and shrimp contributed to the proportion of benthic prey consumed.



Discussion

The world's three narwhal populations have very different $\delta^{13}C$ and $\delta^{15}N$ values suggesting they have different preferred prey and thus, may be more flexible in their foraging behaviour than previously thought. Narwhal flexibility in preferred prey may help them face changing food web structure and prey distribution that are accompanying climate change (Tynan and DeMaster 1997). $\delta^{13}C$ values change as you move from benthic or inshore food webs to pelagic food webs, with organisms in the benthic environment displaying a higher $\delta^{13}C$ value (France 1995). Mean $\delta^{13}C$ was lowest in the EG population suggesting narwhals in this region feed within a more pelagic food web compared to the NHB and BB populations and this was confirmed by the stable isotope mixing model results that showed EG narwhals preferentially feed on capelin. The NHB population had the highest $\delta^{13}C$ values suggesting they feed in a more benthic food web, which is consistent with them inhabiting a much shallower ecosystem compared to BB and EG. NHB narwhals consumed a large proportion of shrimp, while pelagic prey such as capelin and squid played a minor role in their diet. The NHB food web has been changing in the last 30 years as a result of climatic change in the region, which has resulted in an increase in capelin and sandlance (*Ammodytes* sp.) and a decrease in Arctic cod and some benthic species (Gaston et al. 2003). It is possible that, if necessary, narwhals may be able to switch primary prey and monopolize on the increase in capelin abundance, which may mitigate the negative impacts of reduced cod and benthic species. BB narwhals had intermediate $\delta^{13}C$ values and the highest $\delta^{15}N$ values indicating they feed at a higher trophic level than the other two populations. These values are consistent with narwhals in this population consuming high proportions of Greenland halibut (Laidre and Heide-Jørgensen 2005a); however, the mixing models did not show that Greenland halibut is the primary prey. This may be indicative of the timing of sample collection (restricted

to summer months) and the time frame that this tissue represents in the diet. Stable isotope turnover rates are unknown in this species; however, St. Aubin et al. (1990) found that complete tissue turnover in the skin of beluga whales (*Delphinapterus leucas*), the narwhal's closest relative, was approximately 2-3 months. In this case, the stable isotope values in narwhal skin in BB would not necessarily reflect a large proportion of Greenland halibut, which is primarily consumed in the winter (Laidre and Heide-Jørgensen 2005a), because it would only reveal foraging in the late spring and summer. Alternatively, much longer turn over rates (~1 year) have been determined in large mammals (Sponheimer et al. 2006), which may indicate that narwhals in BB have a more variable diet than stomach content studies have revealed and Greenland halibut is not the major prey item. A more variable diet than stomach samples from one area/season is likely given the opportunities narwhal have to vary diet over seasons and the large space afforded by migrations; however, in our assessment we have included all high trophic level potential prey species, such as halibut, that could be resulting in BB narwhals having a high $\delta^{15}N$ value.

Stable isotope mixing models provide best estimates of diet for the three narwhal populations; however, there is uncertainty surrounding the discrimination factor used and these models are known to be sensitive to the specified discrimination factor (Bond and Diamond 2011). Discrimination factors are known to be species and tissue specific (Hobson and Clark 1992) and there is currently no study evaluating tissue specific discrimination factors for narwhal, or for beluga. We used an average of published discrimination factors for odontocete skin for whales that consume higher trophic level prey (Caut et al. 2011, Fernández et al. 2011), which is currently the best estimate available. Stable isotope mixing models are also sensitive to the number of prey included, and it is possible that some potential prey were excluded from the

models, especially for the EG and NHB populations where no studies investigating stomach contents have been conducted. We did investigate many other prey stable isotope values for the two populations (including sandlance (Ferguson unpublished data), Atlantic cod (*Gadus morhua*) (Thompson et al. 1999, Sarà et al. 2009), the shrimps *H. glacialis* and *P. multidentata* (Rau et al. 1989), and Atlantic halibut (*Hippoglossus hippoglossus*) (Sarà et al. 2009); however, none of these prey had stable isotope values within the range seen for narwhals in the two populations and thus they were excluded from the stable isotope mixing models. Overall the model results are insightful but should be interpreted with caution.

The size of a species isotopic niche can provide insight into the extent of their dietary diversity (Newsome et al. 2012). However, the isotopic niche, although related to the ecological niche, is not directly comparable and to interpret the variance seen within narwhal populations, we have to consider the variability of the stable isotope values within the prey. We corrected $\delta^{13}C$ values using the mean and range of $\delta^{13}C$ for the prey from each region (Olsson et al. 2009) and $\delta^{15}N$ to trophic level using baseline $\delta^{15}N$ from Graham et al. (2010). We present uncorrected data for comparative purposes and because the baselines presented by Graham et al. (2010) are only roughly estimated; however, relative niche area differences among the regions did not qualitatively change for corrected data (results on file). The EG narwhal population had the largest niche width, which is consistent with these whales having the greatest geographical range; however, our EG samples came from a restricted area where narwhals are hunted, near the community of Ittoqqortoormiit, Greenland. This particular subpopulation of narwhals has been equipped with satellite transmitters and data suggests these whales move very little throughout the year (M.P. Heide-Jørgensen unpublished data). Although it is generally assumed that a larger isotopic niche can be interpreted as larger trophic niche where organisms are typically generalists

that feed on array of prey, it has been shown that populations confined to one site may display greater isotopic variances within their population due to individual specialization, whereby individuals exploit different aspects of the niche to reduce intraspecific competition (Cummings et al. 2012). This may be the case in EG, however, further evidence, such as that from fatty acids or stomach contents, is needed to confirm this hypothesis.

The BB population had a relatively small isotopic niche despite its population being vastly larger than the other two, which suggests that there is little intraspecific competition to result in individual specialists; however, there may be strong interspecific competition resulting in specialization at the population level. From stomach content analysis, the BB population is assumed to gain much of their energy reserves over the winter when they feed heavily on Greenland halibut (Laidre and Heide-Jørgensen 2005a) and, as a population, appears to specialize on this species. The supply of Greenland halibut in the winter must be substantial enough to sustain the current population in this region; however, an expanding halibut fishery in the Arctic has the potential to compete with narwhal feeding (Dennard et al. 2010).

There is sexual segregation in diet for all populations, which may be related to the diving ability of males and females. Studies investigating dive behavior in narwhal sexes have been limited by sample size and perhaps, as a result, have documented conflicting results. It has been shown that female narwhals have significantly lower dive rates than males (Heide-Jørgensen and Dietz 1995) and they typically make dives <400 m (Laidre et al. 2004b), but another study reported no difference in diving performance between female narwhals and their male counterparts (Laidre et al. 2003). Further evidence is required to support differences in dive behavior between males and females, but given the sexual size dimorphism (Garde et al. 2007), males are likely capable of making deeper dives and therefore capable of foraging in the benthos

even in deep waters. It has also been suggested the supinate swimming behavior of narwhals may be related to foraging. By swimming upside-down, males can orientate their tusk toward the bottom to guide benthic prey towards their mouth (Dietz et al. 2007). Increased benthic foraging for males would explain their increased $\delta^{13}C$ value (France 1995). We predicted males would have higher $\delta^{15}N$ values and a larger isotopic niche than females, however results indicated no differences. Males may be as specialized as females in their preferred prey and suction feeding may limit the size of prey that both male and female narwhals can manipulate, resulting in them displaying a similar trophic level.

Beluga whales are considered to eat a much more diverse range of prey than narwhals (Laidre et al. 2008). Polar and Arctic cod were found to contribute more than any other item to the diet of beluga in Greenland, the Canadian high Arctic, Russia, Svalbard and the Beaufort Sea (Heide-Jørgensen and Teilmann 1994, Dahl et al. 2000, Boltunov and Belikov 2002, Loseto et al. 2009, Marcoux et al. 2012). These beluga populations are geographically isolated and inhabit very different environments, yet in all cases the dominant prey across populations is cod. Overall belugas consume a greater range of prey than narwhal, but based on our results, narwhal may also be flexible in their preferred prey. Although there is some range overlap between narwhals and belugas, typically the species have different preferred habitats (Reeves et al. 1994), which has reduced competition for food. However, our study and a study conducted by Thiemann et al. (2008) suggest there is substantial overlap in preferred prey, and competition may become more intense when both prey and the predators shift their distribution with changing climate.

Stable isotope ratios fluctuate with geography as a result of variable $\delta^{15}N$ and $\delta^{13}C$ at the base of the food web (Graham et al. 2010) and therefore we have to consider that differences among populations may be a result of the isotopic differences across the large geographical span.

Although there are only crude estimates for baseline $\delta^{15}N$ and $\delta^{13}C$ in the ocean, generally $\delta^{15}N$ is greater in NHB compared to BB and EG, and $\delta^{13}C$ is higher in EG compared to BB and NHB (Graham et al. 2010). In this case, correcting for baseline differences in $\delta^{15}N$ and $\delta^{13}C$ would result in NHB narwhals to have slightly lower mean $\delta^{15}N$ and EG narwhals to have lower mean $\delta^{13}C$. If these differences are taken into account there is even greater separation in stable isotope ratios among the populations. In addition, dietary differences determined by stable isotope mixing models incorporated prey from each of the three respective regions, suggesting that narwhals in each of the populations do have different preferred prey and this is not a result of underlying spatial patterns in stable isotopes.

We restricted our comparisons to narwhal samples collected in summer months, which eliminated any confounding that may be caused by seasonal changes in diet, but annual changes in diet could also have impacted differences among the populations. To assess this possibility we compared samples for the EG population from 1994 and 1995 with samples from 1994 and 1995 for the BB population (unfortunately there were no samples for the NHB population from these exact years) and still found significant differences between the populations for $\delta^{13}C$ and $\delta^{15}N$, indicating these results are not a result of annual changes in diet within a population (data on file).

Fuller et al. (2010) identified four possible outcomes for a species that is experiencing climate change: the species can shift distribution, genetically adapt, go extinct, or employ phenotypic plasticity. For narwhals, their distribution is already relatively restricted (Laidre et al. 2008) and although some populations may be able to move further north, range shift will only be a temporary solution for dealing with climate change. Genetic adaptation takes many generations, and given the long lifespan and generation time for narwhals (Garde et al. 2007,

Garde 2011) and the speed of current climate changes (IPCC 2007), it is unlikely they will be able to genetically adapt to an ice-free life style. Narwhals are thought to be quite rigid in terms of their behavior, particularly given their high site fidelity (Laidre and Heide-Jørgensen 2005b); however, they may be more adaptable in terms of their foraging behavior than previously thought. We showed that stable isotope values differ among the populations and this difference is related to differences in preferred prey and foraging behavior among the populations. Future studies should monitor changes in preferred prey of narwhals in NHB, the most southerly narwhal population, that is currently experiencing documented food web modifications as a result of changes in climate (Gaston et al. 2003). In addition, investigations of stomach contents and fatty acids from narwhals in the EG and NHB populations would provide a clearer picture of how flexible narwhal are in their foraging behavior.

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Chapter Three

Fatty acids and stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) reveal temporal changes in narwhal (*Monodon monoceros*) diet linked to migration patterns

Watt, C. A.[1], and S. H. Ferguson[1,2]

[1]Department of Biological Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
[2]Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada

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Contribution

Dr. Ferguson provided funding for stable isotope and fatty acid analyses and provided guidance and comments throughout the writing process.

Cortney Watt prepared all samples for stable isotope analysis (which were then shipped to University of Windsor for analysis), ran tissue extraction protocols and conducted fatty acid analyses, wrote the manuscript in its entirety, conducted all statistical analysis and created all accompanying figures and tables.

Abstract

Narwhals (*Monodon monoceros*) are sentinel species in the Arctic and to investigate marine food web changes from 1982-2011 we examined diet using fatty acids, $\delta^{15}N$, and $\delta^{13}C$, in narwhals from Baffin Bay (BB) and northern Hudson Bay (NHB). We predicted temporal changes would be greater in NHB due to a significant reduction in summer ice cover. In NHB, $\delta^{15}N$ significantly increased, $\delta^{13}C$ displayed a parabolic trend, and fatty acids gradually shifted, albeit not significantly, over time. $\delta^{15}N$ was stable, $\delta^{13}C$ decreased, and fatty acids significantly changed over time in BB. Stable isotope mixing models indicated a dietary reduction in capelin and increase in Greenland halibut from 1994-2000 to 2006-2011 in BB, while capelin was an important dietary component for narwhals in NHB in recent years (2006-2011). These dietary changes may be attributed to changes in sea ice and narwhal migration. Seasonal dietary changes, as evidenced by changes in blubber fatty acids and skin and muscle stable isotopes, were not as apparent in the NHB population, which may be indicative of a reduced migratory distance. Long-term monitoring of narwhal diet and migratory patterns associated with reduced sea ice provides invaluable information about how the marine ecosystem will redistribute with global warming.

Keywords: *Monodon monoceros*, diet, stable isotopes, fatty acids, temporal trends, long-term data, migration patterns

Introduction

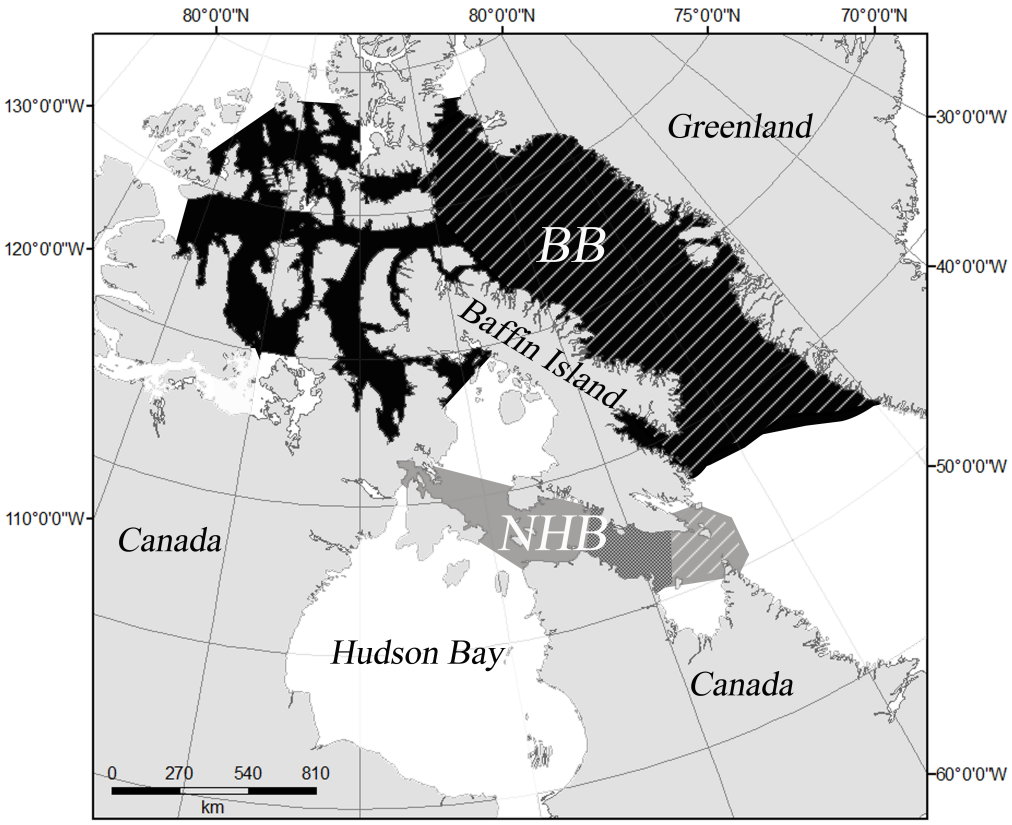
Climate has been changing in recent years and the effects are most drastically evident at the earth's poles. Annual summer sea ice is approximately one third smaller than average ice cover from 1979-2000 (AMAP 2011) and summer ice extent has declined by 7.7% per decade from 1979-2004 (Stroeve et al. 2005). Loss of Arctic sea ice has the potential to have large global impacts on climate (Francis et al. 2009). Predictions regarding future Arctic sea ice extent are not promising a recovery in the ice. Using the 2007 Arctic ice minimums as a starting point, Wang and Overland (2012) have predicted the Arctic may experience an ice-free summer by the 2030s. Altering ice conditions also has biological impacts on organisms, with those living in the Arctic being affected the most dramatically. Both bottom-up and top-down processes are changing with ice conditions, resulting in altered trophic dynamics. Freshwater influx, increased river discharge, unstable salinity, and changing physical landscape have all altered plankton communities (Greene et al. 2008). Temperature changes are impacting fish and mammal communities by altering their density and geographic distribution (Wassmann et al. 2011, Eriksen et al. 2012). Southern predators are also moving into and becoming more frequent visitors of Arctic regions (Higdon and Ferguson 2009), which can have cascading top-down impacts on food webs.

In Canada, summer sea ice cover in Hudson Bay has declined by 11.3% ± 2.6% per decade from 1968-2008 and the region is currently experiencing changes in trophic dynamics (Tivy et al. 2011). Arctic cod (*Boreogadus saida*), a keystone species in Arctic environments, has decreased in abundance while more southern species, such as capelin (*Mallotus villosus*), have increased (Gaston et al. 2003, Provencher et al. 2012). Polar

bear (*Ursus maritimus*) body condition has declined in western Hudson Bay and this is related to an earlier spring ice break up date (Stirling and Derocher 2012). Ringed seals (*Pusa hispida*) have experienced dietary changes in Hudson Bay (Chambellant et al. 2013, Young and Ferguson 2013) and killer whale (*Orcinus orca*) sightings have increased in this region (Higdon and Ferguson 2009). Baffin Bay has also seen a decrease in summer sea ice cover, albeit to a reduced extent (8.9% ± 3.1% per decade from 1968-2008 (Tivy et al. 2011)). Although there have been documented changes, including a decrease in salinity and a general warming trend in this region (Zweng and Münchow 2006), changes in trophic dynamics have not been widely reported in Baffin Bay. Biological populations of narwhals (*Monodon monoceros*) inhabit each of these Arctic regions (Fig. 3.1), and we compared their diet from 1982-2011 to see if food web modifications are changing diet, with particular interest in the whales inhabiting the Hudson Bay region, as they have experienced the most drastic ice cover declines and documented food web changes.

Narwhals are ice-adapted cetaceans that live exclusively in the Arctic environment, surviving winter periods in up to 90% pack ice (Laidre and Heide-Jørgensen 2011). In Canada there are two genetically and spatially isolated narwhal populations inhabiting northern Hudson Bay and Baffin Bay (Petersen et al. 2011). The northern Hudson Bay (NHB) population has ~12,500 individuals (Asselin et al. 2012). Narwhals in this population summer in northern Hudson Bay from approximately June until late October, when they travel ~1,250 km to their wintering grounds, just outside (Richard 1991, Heide-Jørgensen et al. 2013), or more recently, in the Hudson Strait (Elliott et al. 2013) (Fig. 3.1). The Baffin Bay (BB) population numbers greater than

Figure 3.1. The estimated (from Petersen et al. 2011) summer (solid) and winter (hashed) range for narwhals from the Baffin Bay population (BB) are indicated in black. The Northern Hudson Bay population's (NHB) summer (solid), historical winter (hashed; Richard 1991), and more recent winter (checkered; Elliot et al. 2013) distributions are indicated in gray.



60,000 individuals and spends winter in northern Davis Strait and southern Baffin Bay and summer in the northwestern fiords and shores of Greenland and the fiords and inlets in northeastern Canada (Richard et al. 2010) (Fig. 3.1). This population typically begins their autumn migration in September and travels ~1,700 km to their wintering area in the northern Davis Strait and southern Baffin Bay where they remain until approximately April when they begin their spring migration (Heide-Jørgensen et al. 2013).

Studies investigating diet in *M. monoceros* have primarily relied on stomach content analyses and found Arctic cod, polar cod (*Arctogadus glacialis*), Greenland halibut (*Reinharditius hippoglossoides*), boreoatlantic armhook squid (*Gonatus fabricii*), and shrimp (*Pandalus borealis*) as being common in the diet, while capelin was identified as a minor prey item (Finley and Gibb 1982). Stomach content studies provide only a snapshot of diet (typically the previous meal) and can be under representative of some prey items because of varying digestion rates (da Silva and Neilson 1985). More recent investigations of diet have used stable isotopes and fatty acids. These chemical techniques are useful to look at long-term changes in diet, since different tissues reflect different time frames (Newsome et al. 2010). To determine if narwhals have experienced temporal shifts in diet we used fatty acid and stable isotope analyses to investigate changes in diet over the last 30 years in the BB and NHB populations. A dietary analysis for narwhals has not been conducted since 2005, and no dietary studies have been done on the NHB population (although see *Watt et al.* 2013). This is also the first study to evaluate long-term changes in narwhal diet across seasons and over decades.

Isotope analysis of stable carbon (^{13}C:^{12}C, expressed as $\delta^{13}C$) and nitrogen (^{15}N:^{14}N, expressed as $\delta^{15}N$) can give detailed information on the location of feeding and

the trophic level of a predator. In odontocete skin, $\delta^{13}C$ is enriched ~2‰ per trophic level, while $\delta^{15}N$ is enriched ~2.6‰ per trophic level (an average of values reported by Caut et al. (2011) and Fernández et al. (2011)) and by intergrating these trophic level changes we can identify the source of $\delta^{13}C$ at the base of the food web and estimate the trophic position of an organism (Newsome et al. 2010). In addition, if $\delta^{13}C$ and $\delta^{15}N$ of potential prey species are also known, quantitative estimates of prey proportions can be calculated. Much like stable isotopes, fatty acids can also provide information on an organism's diet. Fatty acids are relatively unmodified from prey to predator tissues and if fatty acid profiles of potential prey are known, qualitative inferences can be made regarding primary prey (Iverson et al. 2004, Budge et al. 2006).

Concurrent analysis of stable isotopes and fatty acids can provide a comprehensive understanding of marine mammal foraging. For instance, together the two techniques identified Arctic cod as the dominant prey item for beluga whales (*Delphinapterus leucas*) (Loseto et al. 2008, Loseto et al. 2009) and boreoatlantic armhook squid as the main component of bottlenose whale (*Hyperoodon ampullatus*) diet (Hooker et al. 2001). Similar diet composition estimates have also been found for gray seals (*Halichoerus grypus*) using both techniques (Tucker et al. 2008). Together the two methods can also provide temporal integration of diets since tissues turn over at different rates (Tieszen et al. 1983). Fatty acid turnover in blubber occurs on the order of weeks to months (Kirsch et al. 2000, Iverson et al. 2004, Budge et al. 2006), while skin turnover in beluga whales suggests stable isotope turnover on the order of 2-3 mo (St. Aubin et al. 1990), and muscle is a tissue with low to medium metabolic turnover, representing diet over the previous several months to a year (Sponheimer et al. 2006, Newsome et al.

2010). By analyzing different tissues we can evaluate seasonal changes in diet, and through an investigation of long-term temporal trends in tissues we gain an understanding of how diet has changed over decades.

Trophic niche analysis was introduced as a method for comparing and quantifying trophic diversity and determining whether organisms are specialists or generalists (Layman et al. 2007a). When the range of prey consumed and their isotopic variability is taken into consideration, comparisons of isotopic variability among populations can provide information on their diet (Layman et al. 2007a, Newsome et al. 2012). How trophic niche widths vary in response to seasonal and annual changes in prey has important implications for competition among narwhals and between narwhals and other species, and can have important ramifications for trophic linkages within the Arctic ecosystem as a whole. Although there are complications comparing trophic niche size across populations (see Cummings et al. (2012)), within a population, if the isotopic variability of the environment is minimal, it can be a useful measure to describe dietary isotopic variability both intra- and inter-annually.

The objectives of our study were 1) to investigate narwhal diet, 2) to investigate seasonal and annual changes in diet for narwhals from the BB and NHB populations, and 3) to compare these changes in light of the currently documented ice cover and food web changes in Hudson Bay relative to those changes seen in Baffin Bay. We predicted changes in diet would be more evident in narwhals from NHB as summer ice cover has reduced substantially in this region and trophic changes are already well documented. We investigated isotopic niche size over time, since niche contraction or expansion may have occurred as a result of changes in species composition with changing climate. We

expected narwhals from both regions would display seasonal changes in diet, as this has been documented from stomach contents (Laidre and Heide-Jørgensen 2005) and is typical for Arctic marine mammals (Bluhm and Gradinger 2008). We suspected that narwhal diet is more specialized in the winter when they are known to feed intensively on Greenland halibut (Laidre and Heide-Jørgensen 2005) and predicted that isotopic niche analysis would reveal a niche contraction during this time.

Methods

General

Inuit hunters from Repulse Bay, Nunavut, Canada collected skin, muscle, and blubber tissues from NHB narwhals from 1993-2009, and hunters from Pond Inlet, Nunavut, Canada collected tissues from BB narwhals from 1982-2011 (Table 3.S1). All samples were immediately frozen in -20°C freezers.

Stable isotope analysis

For stable isotope analysis an approximately 0.5 g section of skin was cut from each sample, rinsed with water, cut into small pieces, and placed in the freeze-drier for a minimum of 48 h. Muscle tissue was subsectioned, freeze-dried for 48 h and ground and homogenized with a mortar and pestle. All samples were shipped to the stable isotope lab at Windsor University. Skin samples were lipid extracted using a 1:1 chloroform/methanol agitation protocol prior to analysis (Bligh and Dyer 1959). Analytical precision for the NIST standard bovine muscle (n = 152) and an internal lab standard of tilapia muscle (n = 152) was 0.15‰ and 0.19‰ for $\delta^{15}N$ and 0.07‰ and 0.09‰ for $\delta^{13}C$. Sample precision, calculated as the standard deviation for 43 samples that were run in triplicate, was 0.10‰ for $\delta^{15}N$ and 0.13‰ for $\delta^{13}C$. Since narwhal diet is

Table 3.S1. Table indicating the narwhal samples used for each analysis.

Statistical test	Population	Narwhals	Tissue	Time Period	*n*
Seasonal dietary changes					
Stable isotope analysis					
Matched paired *t*-test	BB	Males	Skin	1994-2000	28
			Muscle	1994-2000	28
		Females	Skin	1994-2000	26
			Muscle	1994-2000	26
	NHB	Males	Skin	1993-2009	30
			Muscle	1993-2009	30
		Females	Skin	1993-2009	16
			Muscle	1993-2009	16
SIAR - skin vs muscle	NHB	Males	Skin	2006-2011	17
			Muscle	2006-2011	17
	BB	All	Skin	1994-2000	54
			Muscle	1994-2000	54
Fatty acid analysis					
PCA - FA & prey	NHB	All	Blubber	2006-2011	20
	BB	All	Blubber	1994-2000	54
		All	Blubber	2006-2011	22
Annual dietary changes					
Stable isotope analysis					
ANOVA – $\delta^{13}C$ & $\delta^{15}N$	NHB	All	Skin	1982-1993	4
				1994-2000	28
				2001-2005	9
				2006-2011	25
	BB	All	Skin	1982-1993	36
				1994-2000	58
				2001-2005	12
				2006-2011	24
SIAR - across time periods	BB	Male	Skin	1994-2000	30
				2006-2011	9
		Female	Skin	1994-2000	28
				2006-2011	15
Fatty acid analysis					
MANOVA - FA	NHB	All	Blubber	1982-1993	4
				1994-2000	28
				2001-2005	9
				2006-2011	20
	BB	All	Blubber	1982-1993	48
				1994-2000	54
				2001-2005	13
				2006-2011	23

known to change seasonally (Laidre and Heide-Jørgensen 2005) we limited all of our stable isotope analyses to only adult narwhal samples that were collected in summer months (June through September as defined by Finley and Gibb (1982), with the majority collected in August). Added CO_2 in the atmosphere has caused a decrease in $\delta^{13}C$ because fossil fuels are depleted in ^{13}C, known as the Suess effect (Sonnerup et al. 1999). We corrected our $\delta^{13}C$ values for the oceanic ^{13}C Suess effect in the north Atlantic by applying a correction value of 0.02‰ per year (an average of mean values reported by Körtzinger and Quay (2003) and Sonnerup et al. (1999)).

Fatty acid analysis

For fatty acid analysis, lipids were extracted using the Folch procedure (Folch et al. 1957) with modifications recommended by Budge et al. (2006). Lipids were extracted from a 0.5 g subsection of blubber, which was isolated from the larger tissue sample. Blubber extractions were conducted as deep as the sample allowed; however, this was often within 1 cm from the skin. Lipids were extracted with 2:1 chloroform-methanol containing 0.01% butylated hydroxytoluene. Blubber was squished and removed from the solution and 3.5 mL of sodium chloride was added. Production of fatty acid methyl esters (FAME) was conducted using a dichloromethane/0.01% butylated hydroxytoluene and Hilditch (a mixture of sulfuric acid and dry methanol) solution that was heated for one hour at 100°C. Hexane, distilled water, and sodium sulfate were added in series and the FAME and hexane were placed under an evaporative nitrogen stream. FAME samples were analyzed using an Agilent Technologies 7890A GC system. Supelco (37 component FAME mix) and Nucheck (54 component mix GLC-463) standards were used for fatty acid analysis, and FAME were identified via retention time and known standard mixtures.

Not all fatty acids arise from diet; many are biosynthesized within the predator and therefore should not be considered when using fatty acids to elucidate diet (Iverson et al. 2004). As a result, only those fatty acids known to transfer from prey to predator were used in analysis (Iverson et al. 2004). There were 32 dietary fatty acids identified in this study. An arbitrary small number (0.00001) was added to individuals who were interpreted as having 0% of a given fatty acid (Kenkel 2006), as this is often a result of the detection limit of the GC system. Percent fatty acid values were divided by the percentage of the reference FA C18:0 and log transformed (Budge et al. 2006). Twenty-one samples were run in duplicate to calculate precision and the average standard deviation across the 32 dietary fatty acids was 0.23%.

Seasonal dietary changes within populations

Stable isotope analysis

To investigate whether narwhal diet changed with seasons, we compared $\delta^{15}N$ and $\delta^{13}C$ values in muscle and skin tissues collected from male and female narwhals in each of the populations (Table 3.S1) using a matched paired *t*-test to avoid violating assumptions of independence (Sokal and Rohlf 1995). An ANOVA on muscle and skin $\delta^{15}N$ and $\delta^{13}C$ was used to investigate is there was a difference between males and females in each population. A Bayesian framework, which uses a multivariate ellipse-based metric that is useful for comparing populations of different sizes (Jackson et al. 2011), was used to calculate and compare niche widths between tissues and sexes within each population. Standard ellipse area, which is the bivariate equivalent to standard deviations, were calculated using the SIBER package within SIAR, the stable isotope mixing model package in R (Parnell et al. 2010).

SIAR was used to identify which prey items contributed to the observed differences between narwhal muscle and skin in each population (Parnell et al. 2010). Potential prey included species identified in previous stomach contents, including Arctic cod, Greenland halibut, and boreoatlantic armhook squid. Capelin was also included given its increasing abundance in Arctic ecosystems. All prey were collected in the Davis Strait and Hudson Strait by Fisheries and Oceans Canada. We did not collect any capelin in Baffin Bay and instead used stable isotope values presented in Dennard et al. (2009) from Cumberland Sound, a Bay on Baffin Island which opens into Baffin Bay, and in Møller (2006) from the west coast of Greenland. We modeled skin and muscle values from 2006-2011 for males in the NHB population and prey used to model diet for these narwhals all came from 2011 (Table 3.1A and 3.S1); females were excluded as the sample size for this period was too small ($n = 2$). All muscle samples for the BB population came from 1994-2000 (Table 3.S1) and we attempted to obtain prey from this time period, however, capelin values came from 2003 (Møller 2006), and Arctic cod values from 1990 (Table 3.1B). Discrimination factors of 1.9 ± 0.5‰ for $\delta^{13}C$ and 2.6 ± 0.7‰ for $\delta^{15}N$ (an average of values reported for odontocete skin by Caut et al. (2011) and Fernández et al. (2011)) were added to potential prey. Significance was determined using 95% confidence intervals, where overlap indicated no significant difference.

Fatty acid analysis

We investigated seasonal diet using fatty acid analysis in blubber, which represented the shortest time frame of the three tissues. Principal component analysis (PCA), a qualitative analysis that reduces the dimensionality of the data to only a few uncorrelated dimensions that explain the majority of the variability in the data (Jolliffe

Table 3.1. Prey used in stable isotope mixing models and principal component analyses for fatty acids from A) NHB and B) BB. We were unable to include capelin from BB in PCAs for fatty acids, but used published values for stable isotope mixing models.

Population	Prey	*Genus species*	Location	*n*	Month/Year	$\delta^{13}C \pm SD$ (‰)	$\delta^{15}N \pm SD$ (‰)	Source
A) NHB								
2006-2011	Arctic cod	*Boreogadus saida*	Hudson Strait	5	Sept/2011	-19.9 ± 1.3	13.5 ± 0.5	This study
	Boreoatlantic armhook squid	*Gonatus fabricii*	Hudson Strait	5	Sept/2011	-20.0 ± 0.2	10.8 ± 0.4	This study
	Capelin	*Mallotus villosus*	Hudson Strait	5	Oct/2011	-19.0 ± 0.5	12.1 ± 0.3	This study
	Greenland halibut	*Reinhardtius hippoglossoides*	Hudson Strait	5	Oct/2011	-19.4 ± 0.7	14.2 ± 0.5	This study
B) BB								
1994-2000	Arctic cod	*Boreogadus saida*	Davis Strait	2	Aug/1990	-19.5 ± 0.1	15.8 ± 0.3	This study
	Boreoatlantic armhook squid	*Gonatus fabricii*	Davis Strait	2	Oct/1999	-20.1 ± 0.7	16.0 ± 0.4	This study
	Capelin	*Mallotus villosus*	West Greenland	12	July/2003	-19.0 ± 0.6	11.6 ± 1.2	Møller (2008)
	Greenland halibut	*Reinhardtius hippoglossoides*	Davis Strait	8	1996	-19.9 ± 0.6	16.6 ± 0.4	This study
2006-2011	Arctic cod	*Boreogadus saida*	Davis Strait	5	Oct/2011	-20.8 ± 0.2	13.0 ± 0.7	This study
	Boreoatlantic armhook squid	*Gonatus fabricii*	Davis Strait	5	Sept/2011	-19.8 ± 0.6	11.2 ± 1.3	This study
	Capelin	*Mallotus villosus*	Cumberland Sound	7	Aug/2008	-19.3 ± 0.1	12.9 ± 0.1	Dennard *et al.* (2006)
	Greenland halibut	*Reinhardtius hippoglossoides*	Davis Strait	2	Sept/2011	-19.6 ± 0.1	15.3 ± 0.6	This study

2002), of narwhal with potential prey from each region was used to make qualitative inferences about diet (Table 3.1 and 3.S1). Unfortunately we had no fatty acid values for capelin for the BB population and therefore we were unable to include it in the PCA.

Annual dietary changes between populations and sexes

Stable isotope analysis

An ANOVA on $\delta^{13}C$ and $\delta^{15}N$ with time period, population, sex, and all possible interactions among the factors was used to examine whether stable isotopes varied among populations, sexes, and years. Normal quantile plots revealed data were distributed approximately normal, and Levene's test across all possible interactions revealed variances were homogenous for $\delta^{13}C$ ($F_{15, 180} = 0.70$, $P = 0.78$), but not for $\delta^{15}N$ ($F_{15, 180} = 1.91$, $P = 0.02$). As a result, $\delta^{15}N$ values were transformed using the inverse tan function to meet the homogeneity of variance assumption (Levene's test: $F_{15, 180} = 1.62$, $P = 0.07$). Although transformed values for $\delta^{15}N$ were used in all statistical tests, graphical representations include only untransformed values. When significant differences were found within the main effects models, *t*-tests for sex and population, and Tukey's HSD tests for time period were used to determine which groups significantly differed. When significant interactions were identified within the main effects model, simple effects models were constructed to evaluate the factors. Finally, to investigate whether dietary niche size changed over time in either population we used the SIBER package within SIAR, described previously (Parnell et al. 2010).

With the software SIAR (Parnell et al. 2010) we modeled and compared narwhal diet in the BB population from 1994-2000 with 2006-2011 using prey from each time period (Table 3.1B and 3.S1). We did not have prey from 1983-1993 or from 2000-2005,

which is why prey estimates were not calculated for these time periods. Unfortunately we did not have prey stable isotope values from a previous time frame from NHB and were unable to compare prey proportions over time for this population.

Fatty acid analysis

A multiple analysis of variance (MANOVA) was used to investigate whether fatty acids differed between populations, sexes, and time periods. To reduce the number of variables and to meet MANOVA assumptions, a PCA was conducted on the correlation matrix. The first three PCA scores were input into a MANOVA model that included population, sex, time period, and all possible interactions among the factors. *Pillai's trace* was used to determine significance for each of the factors because it is considered the most powerful and robust MANOVA statistic (Olson 1974). When significant differences were found in the main effects model, Tukey's HSD tests were used to determine what time periods significantly differed within each population. A canonical plot with scores obtained from a discriminant analysis on the principal components was used to graphically display the multivariate fatty acid data. All multivariate statistics were calculated using JMP 9.0 software.

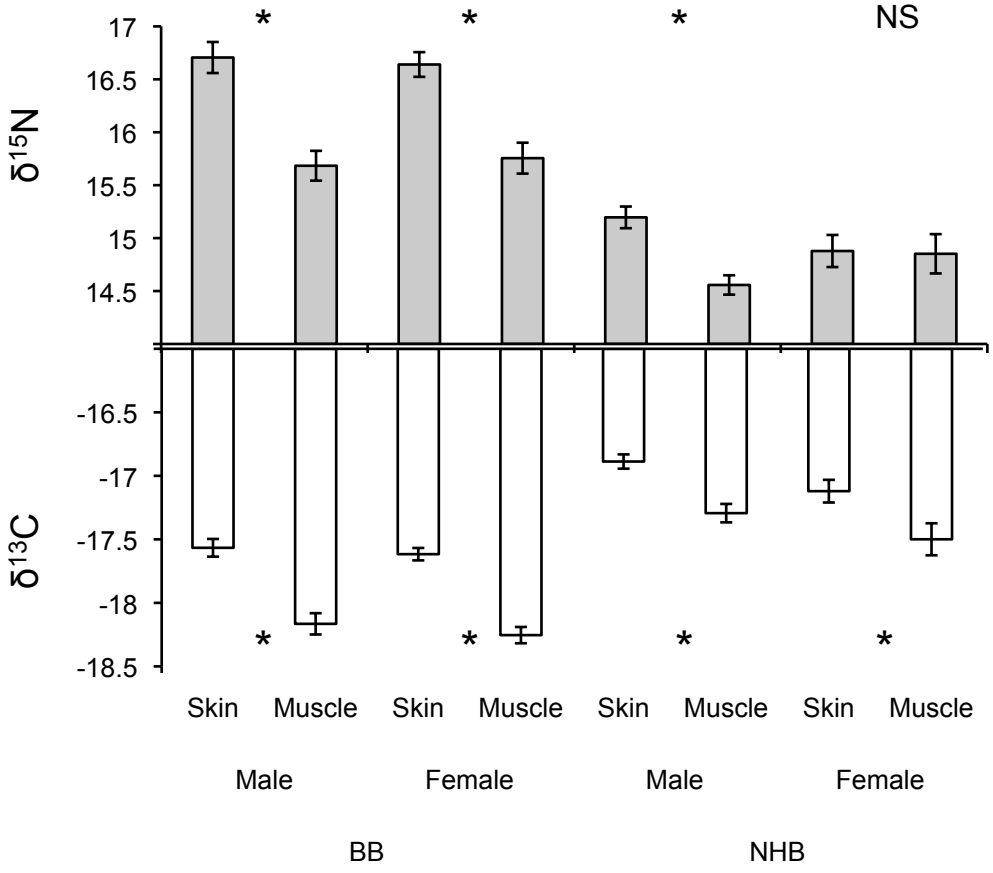
Results

Seasonal dietary changes within populations

Stable isotope analysis

ANOVA comparing male and female $\delta^{15}N$ and $\delta^{13}C$ in NHB found no significant difference ($P > 0.05$; Fig. 3.2). In NHB, skin and muscle $\delta^{15}N$ values differed significantly for male narwhals ($t_{29} = 5.04$, $P < 0.0001$), but not for females ($t_{15} = 0.16$, $P = 0.44$). There were significant differences in $\delta^{13}C$ values for skin and muscle for male

Figure 3.2. $\delta^{13}C$ and $\delta^{15}N$ (± SE) in skin and muscle tissue for male and female narwhals from the BB (*n* = 28 and 26 for males and females respectively) and NHB (*n* = 30 and 16 for males and females respectively) populations. Asterisks (*) indicate significant differences between tissues for the sexes, while NS indicates a nonsignificant difference.



($t_{29} = 5.02$, $P < 0.0001$) and female ($t_{15} = 3.83$, $P < 0.001$) narwhals in NHB (Fig. 3.2). There was no significant difference between sexes for skin niche size in NHB ($P = 0.10$), but the trophic niche for female muscle was significantly larger than that for males ($P = 0.02$; Fig. 3.S1). The isotopic niche size for muscle and skin within male ($P = 0.37$) and female ($P = 0.16$) narwhals from NHB was also not significantly different (Fig. 3.S1). Within NHB, muscle and skin isotopic niches overlapped substantially for females (overlap area = 0.46) and less so for males (overlap area = 0.05) (Fig. 3.3). Stable isotope mixing models on skin and muscle in NHB indicated capelin as the primary prey component for both tissues; however, muscle indicated a significantly greater proportion of boreoatlantic armhook squid in the diet compared to skin ($P < 0.05$), while skin tissues expressed a greater proportion of Greenland halibut in the diet, although this result was not significant ($P > 0.05$, Fig. 3.4A).

ANOVA found there was no significant difference between male and female $\delta^{15}N$ or $\delta^{13}C$ in BB ($P > 0.05$; Fig. 3.2). However, matched paired *t*-tests found there were significant differences in $\delta^{15}N$ values for skin and muscle within males ($t_{27} = 10.17$, $P < 0.0001$) and females ($t_{25} = 6.62$, $P < 0.0001$). There were also significant differences in $\delta^{13}C$ values for skin and muscle for male ($t_{27} = 6.17$, $P < 0.0001$) and female ($t_{25} = 9.22$, $P < 0.0001$) narwhals in BB. Male and female isotopic niche size for skin ($P = 0.07$) and muscle ($P = 0.73$) were not significantly different in BB (Fig. 3.S1). Muscle and skin isotopic niche size did not differ significantly within males ($P = 0.35$) or females ($P = 0.11$) from BB (Fig. 3.S1). There was no overlap in the isotopic niche for muscle and skin in female narwhals (overlap area = 0.00) and little overlap for males (overlap area = 0.05) in BB (Fig. 3.3). Stable isotope mixing models were unable to distinguish among male

Figure 3.S1. Standard ellipse area (per mil^2) for skin and muscle isotopic niches for male and female narwhals from BB (n = 28 and 26 for males and females respectively) and NHB (n = 30 and 16 for males and females respectively). Boxes indicate 95, 75, and 25 % credibility intervals and the asterisk indicates the significant difference ($P < 0.05$) between niches for male and female muscle in NHB.

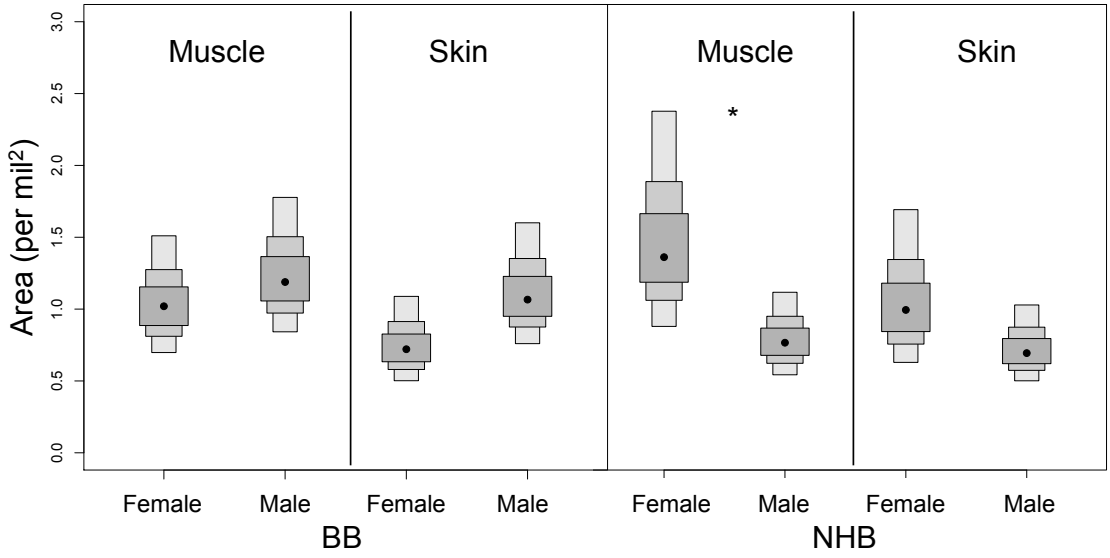


Figure 3.3. $\delta^{13}C$ and $\delta^{15}N$ biplot for muscle and skin samples from narwhals representative of the BB (n = 28 and 26 for males and females respectively) and NHB (n = 30 and 16 for males and females respectively) populations. Thin dashed lines indicate convex hulls of total niche width (Dennard et al. 2009). Standard ellipse area for muscle (solid lines) and skin (dashed lines) are indicated by gray (male) and black (female) lines (Layman et al. 2007b).

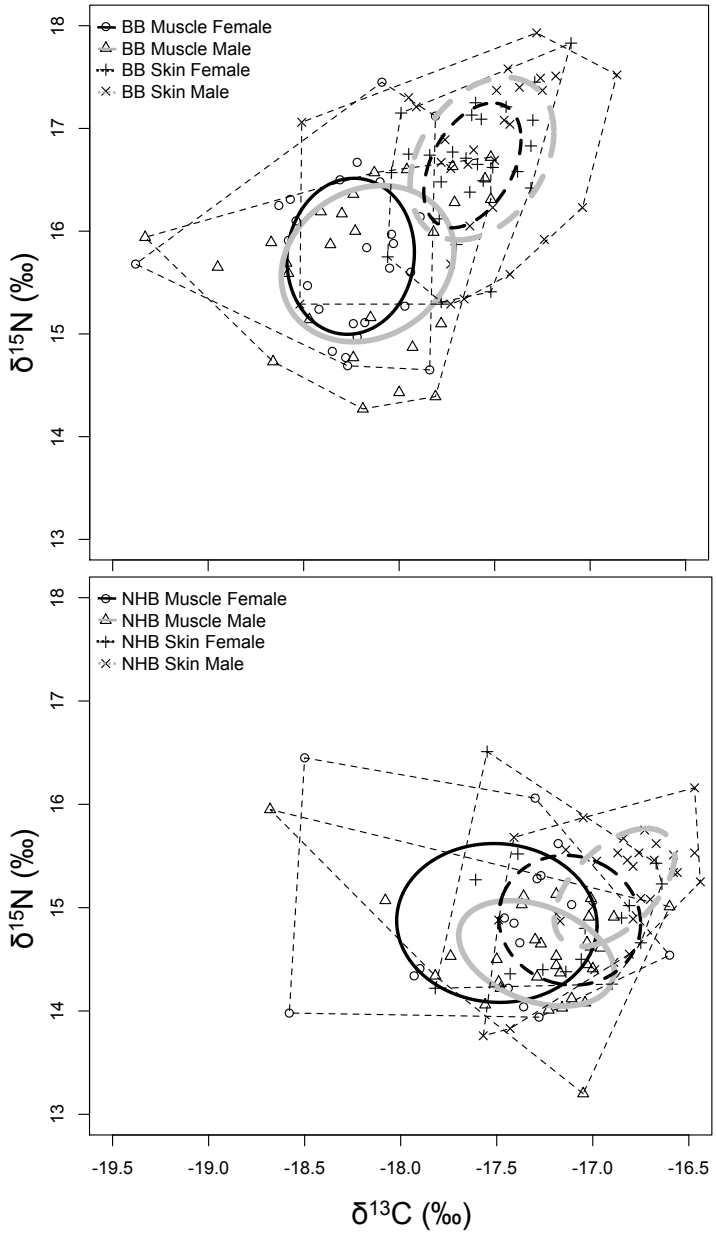
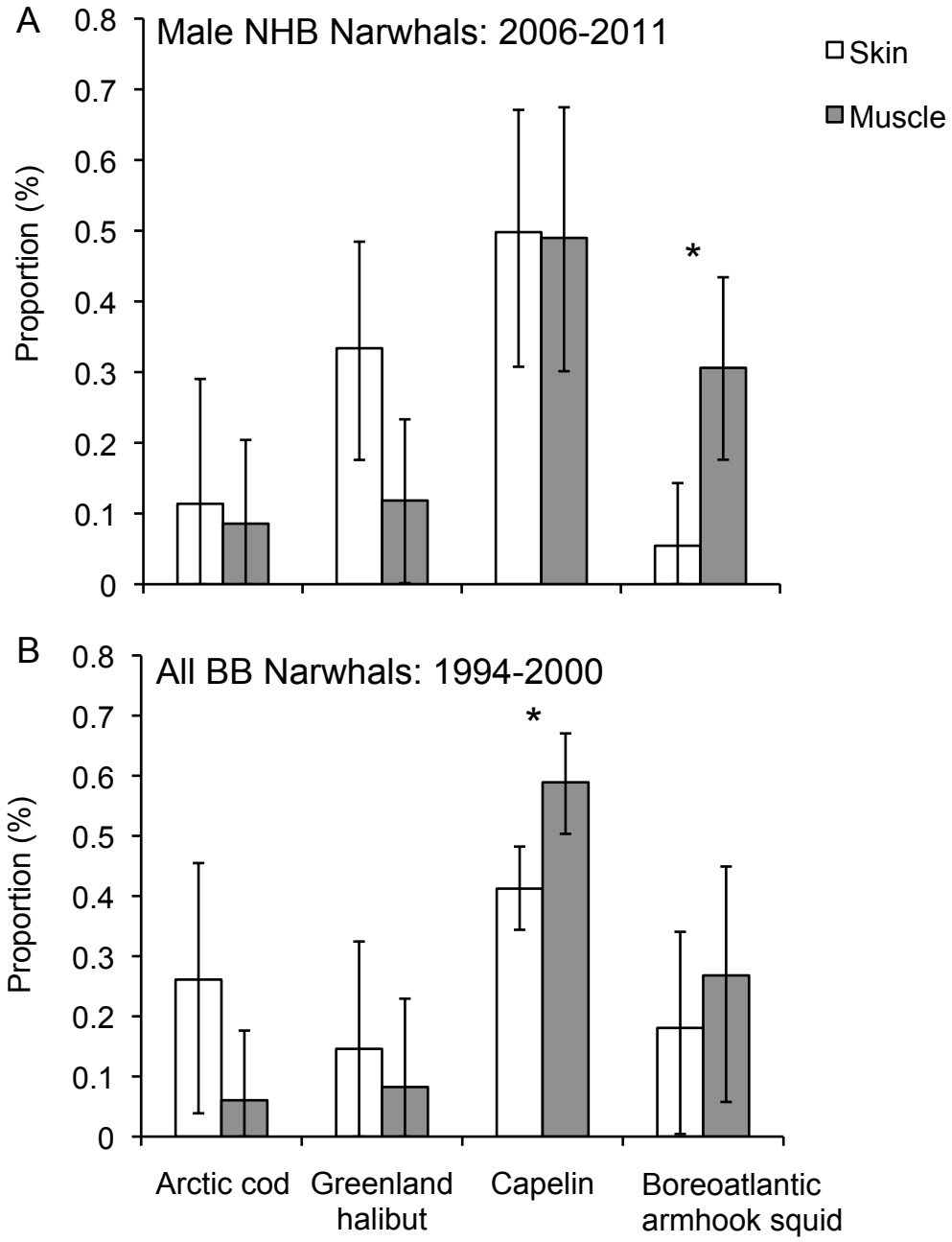


Figure 3.4. The proportion each prey contributes to $\delta^{13}C$ and $\delta^{15}N$ values in skin and muscle in A) male narwhals in NHB from 2006-2011 (n = 17) using prey from 2011 (Table 3.1A) and in B) BB for all narwhals (n = 54) from 1994-2000 using prey from 1990-2003 (Table 3.1B). Error bars indicate 95% upper and lower probability values and asterisks indicate significant differences between skin and muscle.



and female tissues and showed that capelin dominated both skin and muscle (Fig. 3.4B); however, muscle tissues indicated a significantly greater proportion of capelin ($P < 0.05$) and a reduction in Arctic cod compared to skin, albeit not significantly ($P > 0.05$, Fig. 3.4B).

Fatty acid analysis

Fatty acids in blubber indicated narwhal in NHB in 2006-2011 did not group tightly with any of the potential prey, but were closest to capelin and boreoatlantic armhook squid from the region (Fig. 3.5A), while most narwhals from BB from 2006-2011 grouped closely with Greenland halibut (Fig. 3.5C).

Annual dietary changes within populations and sexes

Stable isotope analysis

ANOVA on transformed $\delta^{15}N$ values found population ($F_{1, 180} = 179.76$, $P < 0.0001$) and time period ($F_{3, 180} = 5.45$, $P = 0.0015$) were the only significant factors and there was a significant interaction between population and time period ($F_{3, 180} = 8.48$, $P < 0.0001$) (Fig. 3.6). Simple effects on time period within each population revealed that for $\delta^{15}N$ there were no significant differences among time periods in BB ($F_{3, 126} = 0.77$, $P = 0.51$), but time period was significantly different in NHB ($F_{3, 62} = 11.55$, $P < 0.0001$). Tukey's HSD tests revealed $\delta^{15}N$ was significantly lower from 1982-1993 and 1994-2000 than in 2001-2005 and 2006-2011 ($P < 0.05$), but there was no significant difference between 1982-1993 and 1994-2000 ($P > 0.05$) or 2001-2005 and 2006-2011 ($P > 0.05$) in NHB (Fig. 3.6). ANOVA on $\delta^{13}C$ found population ($F_{1, 180} = 89.99$, $P < 0.0001$), sex ($F_{1, 180} = 4.89$, $P = 0.03$), where males had significantly lower $\delta^{13}C$, and time period ($F_{3, 180} = 8.14$, $P < 0.0001$) were all significant factors (Fig. 3.6). No interactions among the factors

Figure 3.5. Principal component analysis of narwhal fatty acids with potential prey from A) NHB narwhals (*n* = 20) from 2006-2011, B) BB narwhals (*n* = 54) from 1994-2000, and C) BB narwhals (*n* = 22) from 2006-2011. Prey fatty acid values were collected in this study with capelin absent from the PCAs for BB (Table 3.1).

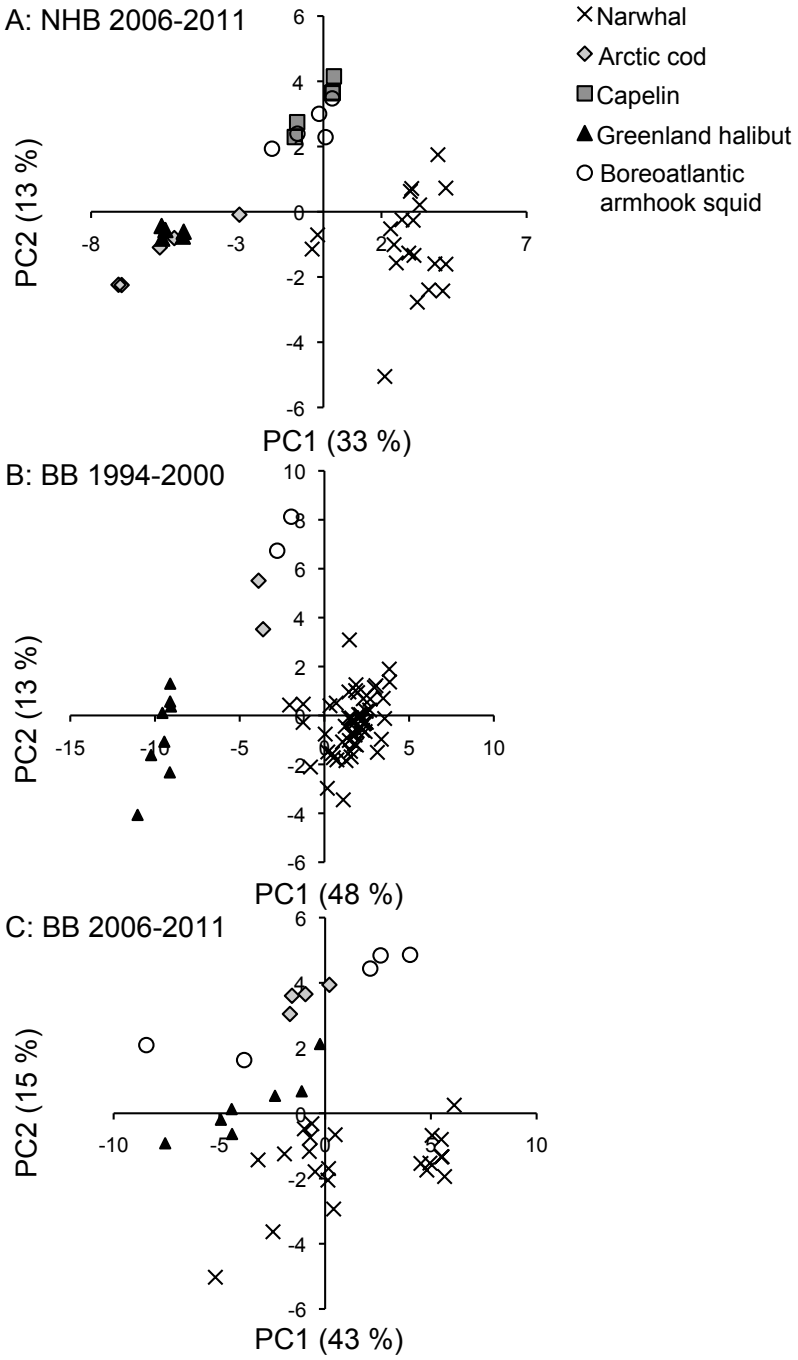
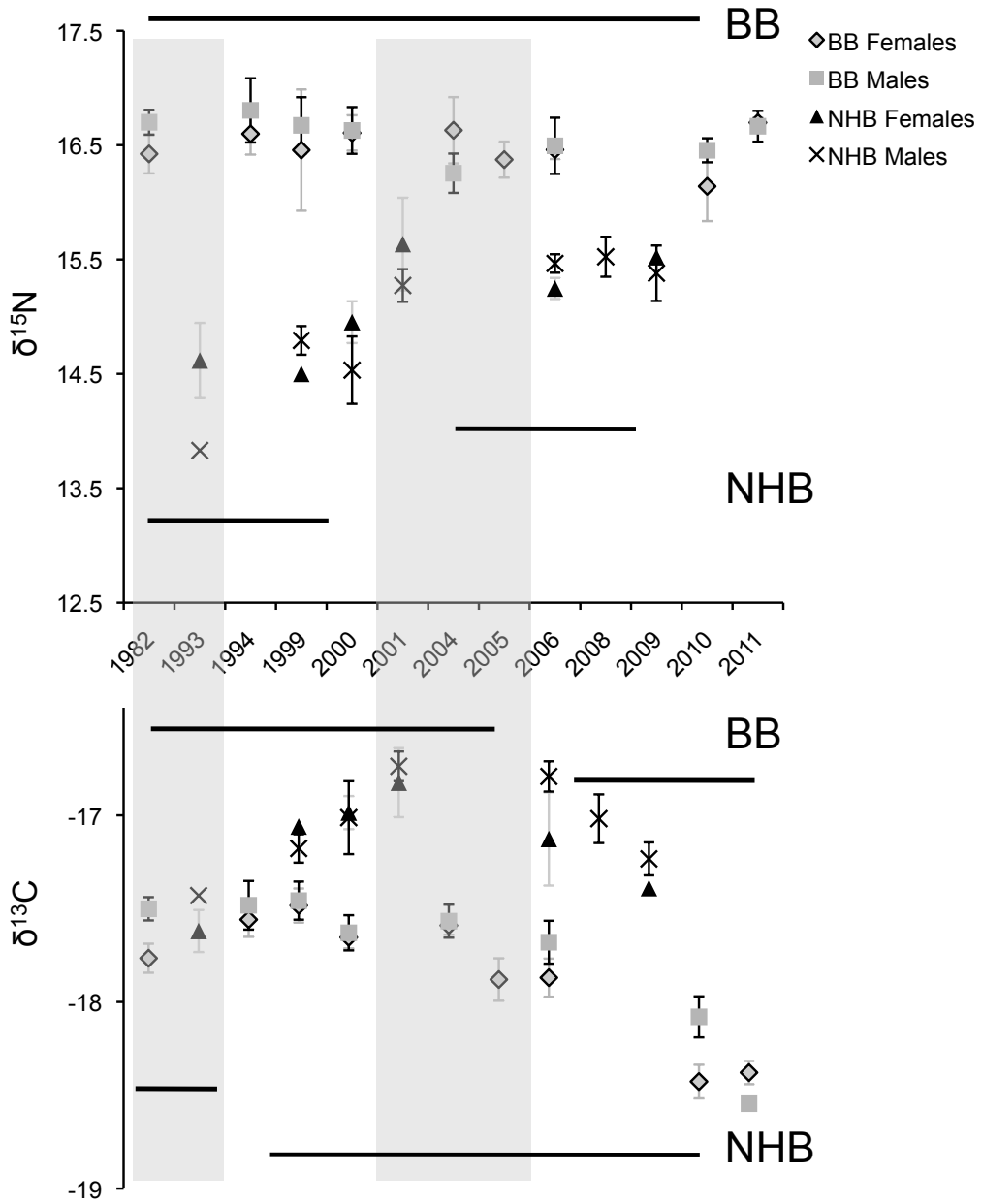


Figure 3.6. Mean $\delta^{13}C$ and $\delta^{15}N$ (± SE for males (black) and females (gray)) for each year for BB males (n = 70) and females (n = 60), and NHB males (n = 41) and females (n = 25). Shadows denote the four time periods analyzed. When time periods are connected by lines on the top this indicates no significant difference among these periods in BB, while those on the bottom indicate no difference in NHB. When lines are disconnected and unaligned, this indicates a significant difference among the time periods.



were significant ($P > 0.05$), except population and time period ($F_{3, 180} = 9.13$, $P < 0.0001$). Simple effects on time period within each population for $\delta^{13}C$ revealed that in BB $\delta^{13}C$ was significantly lower in 2006-2011 than in all other time periods ($F_{3, 126} = 18.34$, $P < 0.0001$) and in NHB $\delta^{13}C$ was significantly lower in 1982-1993 compared to all other time periods ($F_{3, 62} = 7.03$, $P = 0.0004$, Fig. 3.6). Trophic niche analysis for the BB population across time periods revealed that niche size in 2001-2005 was significantly larger than niches in 1982-1993 ($P = 0.01$) and 1994-2000 ($P < 0.0001$), but not significantly different from 2006-2011 ($P = 0.15$) (Fig. 3.S2). Niche size in BB in 2006-2011 was significantly larger than in 1994-2000 ($P = 0.001$) but not different from 1982-1993 ($P = 0.08$) (Fig. 3.S2). In NHB, niche size was significantly greater in 1982-1993 compared to 1994-2000 and 2006-2011 ($P < 0.0001$), but not significantly different from 2001-2005 ($P = 0.10$); however, confidence limits were large as there were few samples for this time period ($n = 4$) (Fig. 3.S3). Niche size in 2001-2005 was also significantly greater than in 1994-2000 ($P = 0.01$) and in 2006-2011 ($P = 0.03$) (Fig. 3.S3).

We only had enough prey information to model narwhal diet across time frames for the BB population. Comparisons of diet in 1996-2000 to that in 2006-2011, using SIAR and 95% confidence intervals to determine significance, revealed there was a significant decrease in capelin and an increase in Greenland halibut consumption for female narwhals ($P < 0.05$, Fig. 3.7). There were no significant changes in male narwhal diet between the two time periods ($P > 0.05$, Fig. 3.7).

Figure 3.S2. A) $\delta^{13}C$ and $\delta^{15}N$ biplot for narwhal skin from BB over four time periods (*n* = 36, 58, 12, and 24 respectively), where thin dashed lines indicate convex hulls of total niche width and standard ellipse area is indicated by thick dashed (gray = 1982-1993 and black = 2006-2011), and solid (black = 1994-2000, and gray = 2001-2005) lines. B) box plot indicating the area (per mil^2) of the isotopic niche for each time period. Boxes indicate 95, 75, and 25 % credibility intervals.

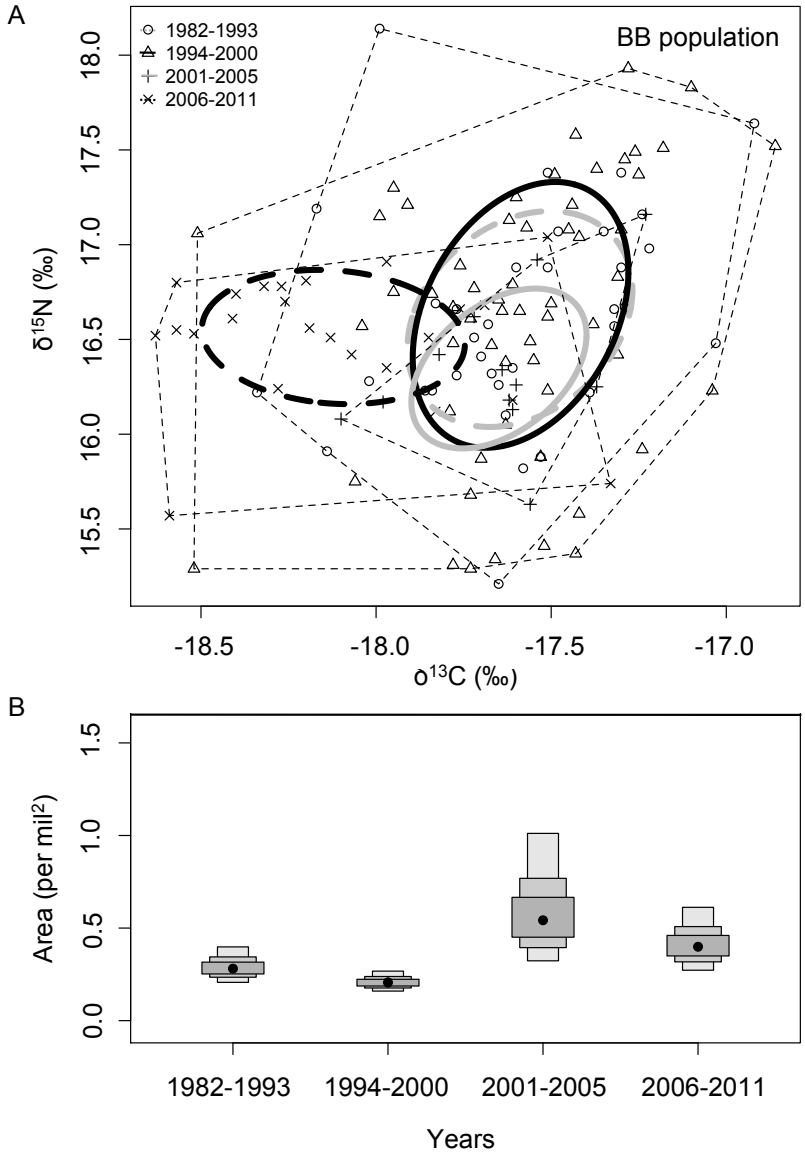


Figure 3.S3. A) $\delta^{13}C$ and $\delta^{15}N$ biplot for narwhal skin from NHB over four time periods (n = 4, 28, 9, and 25 respectively), where thin dashed lines indicate convex hulls of total niche width and standard ellipse area is indicated by thick dashed (gray = 1982-1993 and black = 2006-2011), and solid (black = 1994-2000, and gray = 2001-2005) lines. Arrows connect the centroids for progressive time periods. B) box plot indicating the area (per mil^2) of the isotopic niche for each time period. Boxes indicate 95, 75, and 25 % credibility intervals.

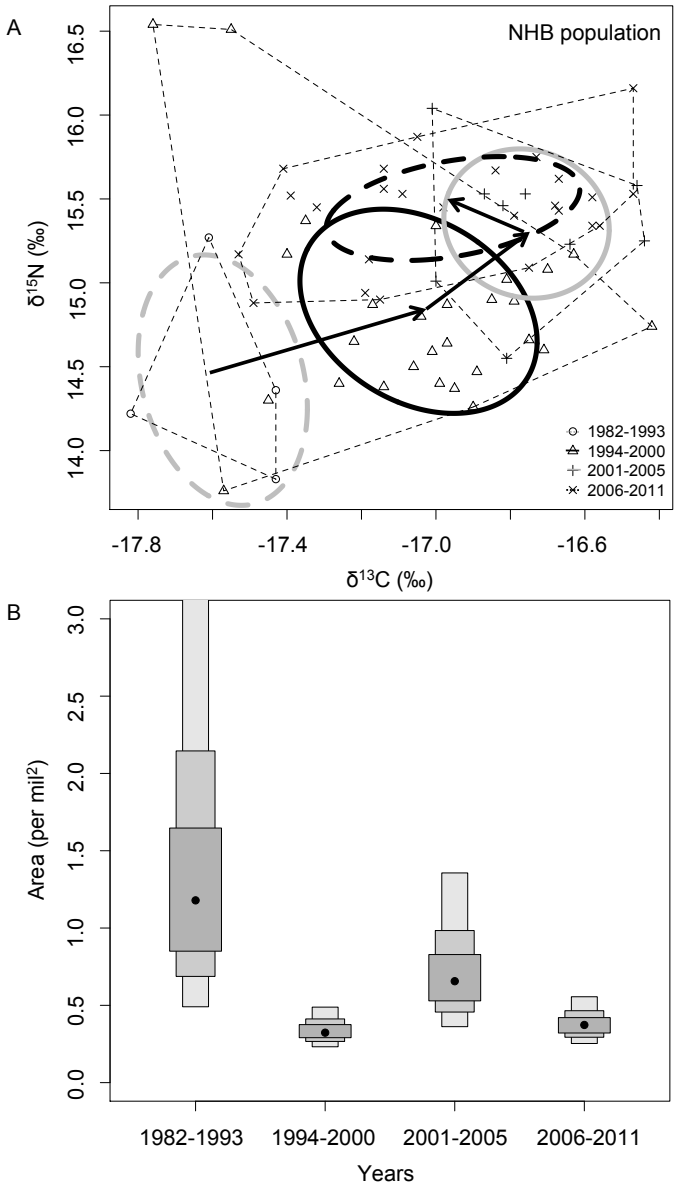
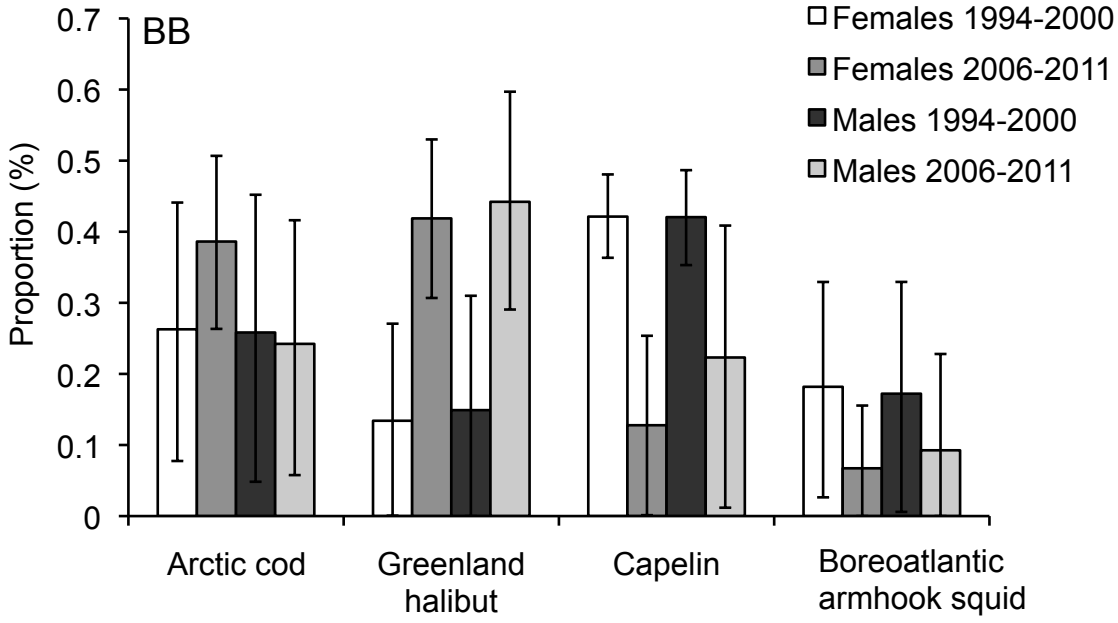


Figure 3.7. The proportion each prey contributes to $\delta^{13}C$ and $\delta^{15}N$ values in skin tissue from BB for 1994-2000 and 2006-2011 for male (n = 30 and 9) and female (n = 28 and 15) narwhals. Models for each time period were run with prey from the respective time frames (Table 3.1B). Error bars indicate 95% upper and lower probability values.



Fatty acid analysis

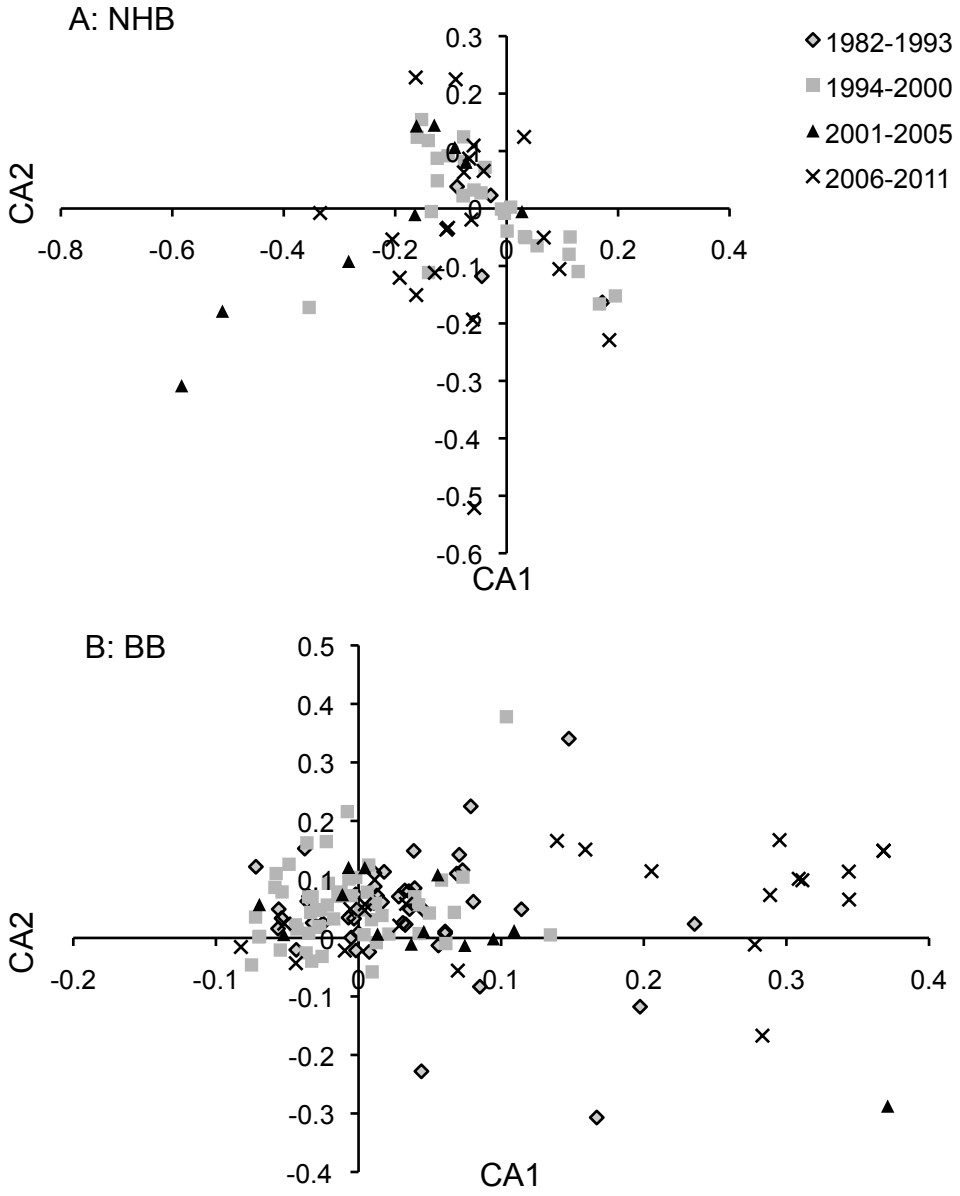
The first three principal components from a PCA on 32 dietary fatty acids, which explained 42% of the variability in the data, were used as variables in the factorial MANOVA. Population ($F_{3, 181} = 10.59$, $P < 0.0001$), time period ($F_{9, 549} = 3.13$, $P = 0.001$), and the interaction between time period and population ($F_{9, 549} = 2.58$, $P = 0.01$) were all significant factors in the MANOVA. Simple effects on time period for NHB showed it was not significant ($F_{9, 171} = 1.59$, $P = 0.12$); however, a canonical centroid plot showed 1982-1993, 1994-2000, and 2006-2011 overlapped substantially, but did not overlap much with 2001-2005 (Fig. 3.8A). In BB, simple effects revealed time period was a significant factor ($F_{9, 402} = 5.52$, $P < 0.0001$) and a canonical centroid plot revealed that 1982-1993 and 2006-2011 were significantly different from one another and 1994-2000 and 2001-2005, which were not significantly different from one another (Fig. 3.8B).

Unfortunately we did not have prey from all time periods to model narwhal diet; however, fatty acids of BB narwhal with their prey from 1994-2000 and 2006-2011 showed that narwhals clustered more closely to Greenland halibut in 2006-2011 than in 1994-2000 and did not cluster tightly to any of the included prey in 1994-2000 but were closest to Arctic cod (Fig. 3.5B and C); however, note that capelin was excluded from the analysis due to lack of samples.

Discussion

Overall there were significant changes in diet both seasonally, and annually in both the BB and NHB narwhal populations. Seasonal dietary changes were more pronounced in the BB population, while annual changes in diet were more apparent in narwhals from

Figure 3.8. Canonical plot of narwhal fatty acids from four time periods for A) NHB (*n* = 4, 28, 9, 20 respectively), and B) BB (*n* = 48, 54, 13, 23 respectively).



NHB. Below we discuss both seasonal and annual dietary changes, in light of concurrent changes in migration behaviour and overwintering range.

Seasonal dietary changes within populations

Stable isotope analysis in conjunction with fatty acid analysis provides long-term information on narwhal foraging habits. The time frame these tissues represent is difficult to interpret as no captive feeding studies on these organisms have been conducted. However, in general, fatty acid turnover in blubber occurs within a few weeks (Kirsch et al. 2000, Iverson et al. 2004, Budge et al. 2006), and skin has a faster turn over rate, integrating 2-3 mo (St. Aubin et al. 1990), than muscle, which integrates several months to a year (Sponheimer et al. 2006). If we assume this is accurate, for our study, fatty acids provide information on the late summer season (a few weeks before samples were collected in August), while stable isotope values in skin tissue integrate diet since the spring/early summer season (approximately June-August) when narwhals are travelling to and arriving at their summering grounds, and muscle indicates a much longer signal that may originate on the wintering grounds.

Northern Hudson Bay

Fatty acid analysis of NHB narwhals and their prey found capelin and boreoatlantic armhook squid had the most similar fatty acid signature even though they did not overlap on the PCA. The lack of overlap is, in part, a result of calibration coefficients not being added to the potential prey since they are unknown for narwhals, but it may also indicate we are missing some potentially important prey. Since no diet investigations have been conducted for this population, prey were chosen based on stomach content studies on BB narwhals and are the best prey currently identified.

Alternatively, Laidre and Heide-Jørgensen (2005) found that over half of the narwhal stomachs analyzed in summer from BB were empty and suggested they may forage less during this time, in which case they may be breaking down fats and mobilizing fatty acids. This could result in narwhal fatty acid profiles being different than any one specific prey. Stable isotope values also differed between spring (skin) and winter (muscle), and mixing models on skin tissues identified capelin as the primary prey component followed by Greenland halibut, while muscle indicated capelin primarily and boreoatlantic armhook squid secondarily. Overall, even though diet changes throughout the season it appears that in recent years capelin is the most important dietary item regardless of season in NHB (Figure 3.4A).

Baffin Bay

In BB, where stomach content studies have been conducted previously, fatty acid analysis identified Arctic cod and Greenland halibut as the most important prey items; however, capelin could not be included in the analysis due to lack of samples. Capelin was indicated as the primary prey from stable isotope mixing models on BB narwhal skin and muscle. In BB, narwhal consumption of capelin was greater in muscle tissue, which suggests capelin may be more important in the winter than spring. This is counter to previous stomach content studies that have found Greenland halibut as the major contributor to diet in the winter months (Laidre and Heide-Jørgensen 2005). Capelin may have been overlooked in stomach contents due to varying digestion rates of their relatively small otoliths. Capelin was also the dominant prey in skin tissues, followed by Arctic cod. In the summer of 1978 and 1979 Finley and Gibb (1982) found Arctic cod in 88% of all narwhal stomachs and this was the most dominant prey item. Arctic cod also

dominated narwhal summer diet in 1984-1985 (Heide-Jørgensen et al. 1994) and from 1999-2004, Laidre and Heide-Jørgensen (2005) found Arctic and polar cod and boreoatlantic armhook squid were the most abundant summer prey; however, less than 15% of stomachs they analyzed contained fresh remains. Our more recent analysis suggests importance of Arctic cod may be decreasing and capelin may be filling this role. Although it is difficult to determine what the long-term effects of this shift in diet may be on the narwhals, capelin's caloric value of 7.54 ± 2.2 kJ/g is comparable to that of Arctic cod (5.89 ± 1.4 kJ/g) (Birkhead and Nettleship 1987), suggesting it may be able to fulfill the same dietary requirements.

Comparison of seasonal diet changes between populations

BB narwhals had a larger seasonal diet shift than NHB narwhals, as the overlap among the skin and muscle isotopic niches was large for NHB, while there was less overlap in niches for BB. The shift in isotope values may be related to geographic variability in $\delta^{13}C$ and $\delta^{15}N$. BB narwhals migrate a greater distance between summer and winter grounds than narwhals from NHB (~500 km more), which may increase the environmental isotopic range they experience and therefore change the stable isotope values for their skin and muscle. This, however, is not supported by current knowledge of latitudinal and longitudinal isotopic variability in the Arctic, which is known to be very little (< 2‰) (Graham et al. 2010). In addition, prey varied relatively little between NHB and BB in their stable isotope values (Table 3.1), suggesting prey within each of the population's range would vary even less. Alternatively, a recent change in migration patterns for narwhals from NHB may reduce the variability in their diet between seasons. In 1981, narwhals congregated in eastern Hudson Strait in the winter season; however, in

2012, narwhals were primarily seen in western Hudson Strait (Koski and Davis 1994) (Fig. 3.1). This shift may be a result of the changing ice regime in the area, which is also supported by Inuit traditional knowledge suggesting that narwhals are now arriving earlier than ever before (Elliott et al. 2013). Changes in the Hudson Bay ice regime and narwhal distribution may limit the narwhal's access to deep waters just outside of Hudson Strait, which in turn would limit access to benthic prey, such as Greenland halibut. This hypothesis seems to be the most realistic since it also explains why narwhals in NHB are consuming a significantly greater proportion of boreoatlantic armhook squid in the winter months and a reduction in Greenland halibut.

Annual dietary changes within populations and sexes

Northern Hudson Bay

Over time, NHB narwhals have experienced a gradual shift in their fatty acid composition, but this change was not significant. This may have been a result of the small sample size for NHB, or alternatively, the short-term diet integrated by fatty acids in the blubber (generally integrating early August diet since samples were collected late August) has not significantly changed over time in NHB. This may be the case because August has always been an ice-free month in NHB, and thus, prey availability may have remained the same over this time period. However, over this same time frame, stable isotope values in NHB have significantly changed, and trophic niche analysis clearly showed there has been a shift in the isotopic niche over time.

Although we were unable to model and compare diet for NHB narwhals from the 1990s to now, due to a lack of historic prey samples, changes in stable isotope values seem to reflect a dietary change in Hudson Bay narwhals. In western Hudson Bay, ringed

seals muscle tissue changed from the 1990s to the 2000s as a result of increased importance of sand lance (*Ammodytes* spp.) in the diet, as well as a reduction in the importance of Arctic cod and an increase in capelin (Elliott et al. 2013). Although Chambellant et al. (2013) could not rule out a baseline shift, their stable isotope results were consistent with concurrent stomach content analysis. Dietary differences were also detected in polar bears (*Ursus maritimus*) in Hudson Bay over the same time period (McKinney et al. 2010), which suggests NHB has not experienced a baseline shift in stable isotopes and the patterns seen in narwhal tissues are a result of dietary changes. $\delta^{15}N$ has progressively increased over time for narwhals in NHB, suggesting they are consuming higher trophic level prey. We were unable to compare previous and current prey proportion estimates for NHB, but capelin, which has a lower trophic level than Greenland halibut, is now an important source of prey for narwhals, suggesting they must be supplementing their diet with other high trophic level prey we have not identified. Similarly, $\delta^{13}C$ has also increased over time, which would indicate an increased importance of benthic prey (France 1995). Greenland halibut was the only benthic prey analyzed and although its $\delta^{13}C$ value did not differ significantly from capelin, the prey were distinguishable through $\delta^{15}N$ and stable isotope mixing models found halibut was less important in narwhal diet than capelin. Overall, there may be important prey we have not included, and stomach content studies are needed to isolate any important prey we may have overlooked.

Baffin Bay

Over the course of the previous two to three decades BB narwhals have seen a significant change in their fatty acid composition suggesting a change in diet over time.

In addition, narwhals from BB have seen a significant decrease in $\delta^{13}C$ from 1982-2011. Marcoux et al. (2012) saw a similar trend in beluga whales in Cumberland Sound, an inlet off of the Davis Strait, and suggested this may be a result of increased capelin in the diet. Increased capelin consumption would explain why BB narwhals have a more pelagic $\delta^{13}C$ value from 2006-2011 than in previous years; however, the stable isotope mixing models comparing 1994-2000 to 2006-2011 in BB actually suggested a decrease in capelin and an increase in Greenland halibut in narwhal diet. This may be an issue with using capelin stable isotope values from Cumberland Sound, which is a shallow sound with a maximum depth of 150 m (Chambellant et al. 2013), rather than the Davis Strait, which reaches depths > 1,500 m (McMeans et al. 2013). $\delta^{13}C$ values for capelin from Cumberland Sound were actually more enriched than the other prey values used (Table 3.1B). Due to the shallow nature of Cumberland Sound, even pelagic prey from this region may have a more benthic isotopic composition when compared to pelagic prey from the Davis Strait. Similarly, capelin used in stable isotope mixing models for BB in 1994-2000 was collected in west Greenland and came from depths of ~200-400 m (Møller 2006), which is much shallower than the Davis Strait. However, fatty acid analysis also showed that narwhals were more similar to Greenland halibut in their composition from 2006-2011 than from 1994-2000. We suggest this may be related to changes in the timing of narwhal migration to their summer grounds.

Comparing narwhals from the BB population tagged in 2001-2002 (Laidre et al. 2002) and those tagged in 2010-2011 (Heide-Jørgensen et al. 2003), narwhals entered their respective summering grounds approximately a month later in recent years, from early July in 2001-2002 to end of July in 2010-2011. In this case, narwhal skin and

blubber samples collected in August in 2010-2011 would look more similar to Greenland halibut than those collected in 2001-2002 because narwhals could still be feeding on halibut in the month of July prior to entering their summering grounds. Changes in migratory timing need to be investigated more fully to gain an understanding of how timing of ice formation and breakup are altering the seasonal diet of narwhals but this may explain the recent increased importance of Greenland halibut in narwhal skin tissues.

In the BB population niche size has increased in recent years, suggesting these whales may be incorporating other prey, such as capelin, into their diet and expanding their isotopic niche. Unfortunately we were unable to include capelin fatty acid values for the BB narwhals due to a lack of samples; however, capelin is known to be one of the most important dietary items for Greenland halibut in Cumberland Sound, comprising up to 99% of their food (Watt et al. 2012). If this is the case in all of Baffin Bay, it would suggest that for fatty acids in this region, Greenland halibut and capelin would be difficult to distinguish from one another even if we had incorporated capelin fatty acid values. Regardless, stable isotope values for Greenland halibut and capelin were distinct and we were able to separate their relative importance using the stable isotope mixing model. Overall, there is no evidence that narwhals in BB are now consuming more capelin; however, it is plausible that narwhals could switch to capelin as their primary prey (Dennard et al. 2009) if access to Greenland halibut is reduced as a result of changes in sea ice or narwhal migration, or if abundance of halibut decreases due to increased fishing effort (Watt et al. 2013).

Sex specific foraging

Generally, diet differences between male and female narwhals were small. Stable isotope values of skin and muscle from 2006-2011 in NHB and 1994-2000 in BB did not differ between sexes, and male and female isotopic niches overlapped substantially. As a result, we did not consider males and females separately in the stable isotope mixing models comparing skin and muscle. Even though narwhal diet varied over time, sex was always a significant variable for $\delta^{13}C$, with males having significantly higher $\delta^{13}C$ in all time periods. This suggests males are foraging more heavily in the benthos and obtaining a more benthic carbon signature (France 1995). This may be a result of their larger body size and therefore increased ability to endure longer dives and forage more efficiently on the bottom, or may be a byproduct of the ability of males to use their tusk to stir up the benthos and guide benthic prey into their mouths more efficiently than females without tusks (Dietz et al. 2007). Despite the difference in $\delta^{13}C$, stable isotope mixing models over time in BB were unable to attribute differences to a specific prey and males and females displayed similar proportional estimates for prey.

Conclusion

Long-term data archives are vital for understanding the impacts of climate change on species and ecosystems as a whole (Dennard et al. 2010). This long-term data set indicated temporal changes in diet, which alluded to changes in distribution and timing of migration for narwhals in BB and NHB, with the greatest stable isotope differences occurring in NHB. The narwhal has been identified as one of the most sensitive Arctic marine mammals to climate change primarily due to their reliance on sea ice (Ferguson et al. 2012) and specialized diet (Laidre and Heide-Jørgensen 2011). However, Watt et al. (2013) found that different narwhal populations foraged differently depending on their

habitat, which suggests they may have more dietary flexibility than previously thought. The long-term data set suggests dietary changes have occurred in NHB and from 2006-2011 capelin acted as a primary prey item for these whales, with a decreased importance of Greenland halibut in the winter months. These changes appear to be linked to changes in sea ice habitat and concurrent migration behavior. There has been relatively little change in narwhal diet in BB, but a progressive depletion of $\delta^{13}C$ may suggest a greater reliance on capelin and again we found evidence of changes in migration behavior for this region. Currently, relatively little is known about the forage fish communities in the Arctic, and no work, to our knowledge, is evaluating changes in forage fish communities with changing climate. Although a direct study of fish distribution is desirable, an understanding of narwhal diet is one way we can potentially evaluate changes in forage fish populations. Narwhals are an essential species in the Arctic ecosystem and play an important cultural role for the Inuit peoples. As a result of their high trophic level, monitoring of their diet and documenting changes in their trophic position provides invaluable information about the ecosystem as a whole.

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Chapter Four

Genetics and fatty acids assist in deciphering narwhal (*Monodon monoceros*) social groupings

Watt, C. A.[1], S. D. Petersen[2], and S. H. Ferguson[1,3]

[1]Department of Biological Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
[2]Conservation and Research Department, Assiniboine Park Zoo, Winnipeg, MB R3R OB8, Canada
[3]Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada

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Contribution

Dr. Ferguson provided funding for fatty acid analysis and provided guidance and comments throughout the writing process.

Dr. Petersen conducted all genetic analyses, and calculated/determined the maximum likelihood relatedness matrix.

Cortney Watt ran tissue extraction protocols and conducted fatty acid analyses, wrote the manuscript in its entirety (with comments provided by Dr. Petersen regarding the genetic analyses), conducted all statistical analysis and created all accompanying figures and tables.

Abstract

Narwhals (*Monodon monoceros*) are an entirely Arctic odontocete, living in upwards of 95% pack ice and complete darkness over the winter. As a result of the harsh conditions they live in, studies of their social structure are currently lacking and logistically difficult; however, it has been hypothesized that narwhals display a matrilineal social structure where pods are centered on the mother. An ice entrapment event near the community of Pond Inlet, NU, Canada, which captured many females and their offspring, offered an opportunity to study social structure among a narwhal herd. Using genetic analyses and fatty acids as a dietary marker, we investigated whether individuals that are closely related forage together, which would support a matrilineally driven social structure where females teach their young foraging strategies, and/or travel and forage together. We found no evidence that genetic relatedness was correlated with the fatty acid biomarkers, which provides some evidence against a matrilineal social structure. The possibility of narwhals displaying a fission-fusion societal structure is discussed, but further observational and experimental techniques are needed to support or refute this hypothesis.

Keywords: narwhal, *Monodon monoceros*, fatty acids, genetics, social structure, sociality

Introduction

Social structure is an integral component of the biology of a species that can impact gene flow (Whitehead 1998; Randall et al. 2009; Andrews et al. 2010), demography (Ferrari et al. 2009), predation risk (Janson 1998), and behaviour (Gowans et al. 2007). Socialization is also the avenue by which cognitive ability and communication develop (Rendell and Whitehead 2001). Benefits to sociality include defense against predators via dilution effects or mutual defense, cooperative care of young, and improved foraging efficiency, while disadvantages include increased competition for resources and mates, and increased disease or parasite transfer (Gygax 2002). Only when the benefits of social grouping outweigh the disadvantages do social groupings evolve in a species (Gygax 2002) and often this occurs in organisms that possess advanced cognitive abilities (Hinde 1976).

Despite the importance of understanding sociality in determining an organism's biology, elucidating social structure in many animal communities is complex. Cetaceans are a particularly difficult group for deciphering social structure because of their entirely aquatic existence; however, due in part to their high cognitive ability, many species have a highly organized social structure and warrant further investigation. Despite the difficulty with evaluating cetacean social structure, some studies have evaluated the social organization of whale, dolphin, and porpoise species (Gowans et al. 2007, Andrews et al. 2010). Structure is often deciphered through direct observation and identification of individuals with the hope that over time, through a mass assemblage of association data, one can determine the social structure of the population (Whitehead 1997). de Stephanis et al. (2008) investigated social structure, using photo identification

techniques, and diet, using stable isotopes of carbon and nitrogen, in long-finned pilot whales (*Globicephala melas*). They found $\delta^{13}C$ values were significantly different between the social groups. Similarly, Blundell et al. (2002) found there were significant differences in $\delta^{13}C$ values between different social groups in river otters (*Lontra canadensis*). In these studies, social structure was pre-determined based on photographic analysis and radio-telemetry; however, some species are not conducive to these techniques. Diet alone, however, may be a useful indicator of social structure because often social groups vary in their diet because individuals preferentially associate with animals that use the same socially learned foraging behaviour (Cantor and Whitehead 2013), resulting in animals that socialize having similar dietary signatures.

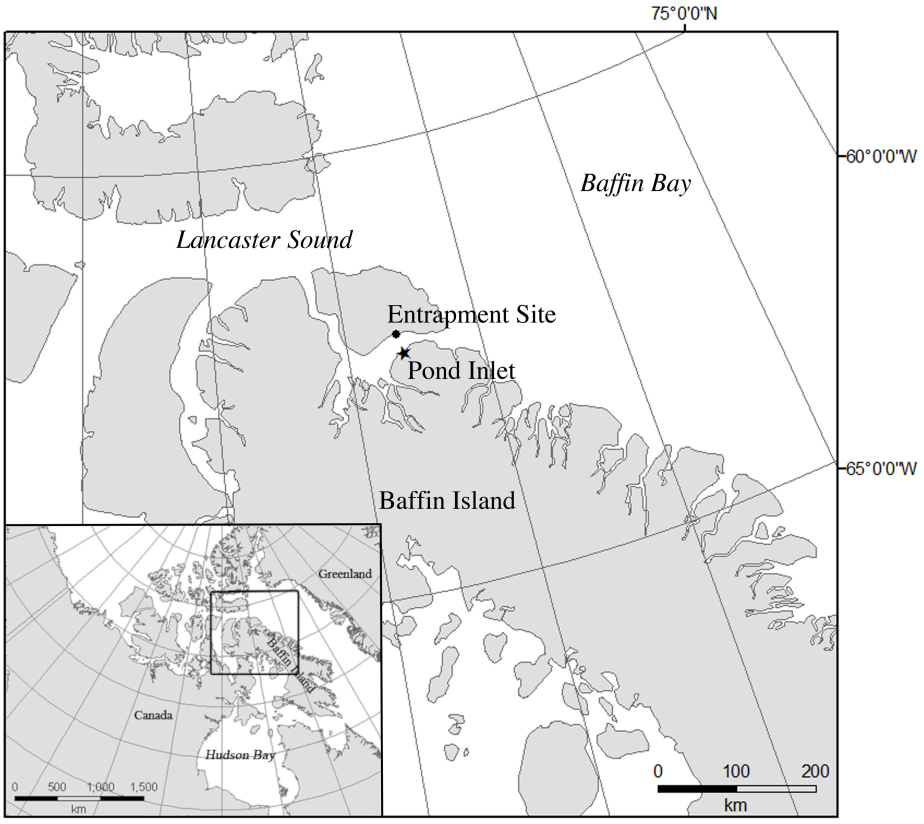
Narwhals (*Monodon monoceros*) live a completely Arctic existence, and have the lowest mitochondrial genetic diversity of any cetacean (Palsbøll et al. 1997; Whitehead 1998). Their social structure has not been well studied, primarily because of the logistic issues associated with observing these animals in their natural environment, particularly during the winter when the Arctic is ice covered and dark. Summer observations of narwhals in Eclipse Sound, northern Baffin Island, have indicated that they tend to travel in large herds, with an average of 78 individuals per herd, divided into many smaller clusters, with an average of 3.5 individuals per cluster (Marcoux et al. 2009). Each herd may have up to 642 clusters with anywhere from 1 – 25 (Marcoux et al. 2009) or 1 - 50 (Cosens and Dueck 1991) individuals in each cluster. Herds usually contain both males and females, but clusters are almost always composed of a single sex (94 % of the time) (Marcoux et al. 2009) or contain mature females with calves and immature males (Palsbøll et al. 1997). Nicks and notches on the narwhal's dorsal ridge have been used for

photo-identification; however, the population is very large and researchers have only ever re-sighted two narwhals (Auger-Méthé et al. 2010). Photo identification takes years of observations, multiple teams of researchers, and can be restricted due to difficulty in sharing data across borders. Given the narwhal's large population and herd size, and the logistics and costs associated with direct observation, alternative methods to direct observation should be sought to reveal their social structure.

It has been suggested that female narwhal clusters have a matrilineal social structure, where pods are centered on the mother (Palsbøll et al. 1997); however, the genetic relatedness of individuals within a herd is not well understood, nor is the genetic structure of the smaller clusters. If narwhals do display a matrilineal social structure (Whitehead 1998), a typical characteristic is that prey is either shared among members of the group (Ford and Ellis 2006), or matrilineal foraging skills are transferred to offspring, which may result in a degree of specialization for each of the distinct matrilineal lineages (Tinker et al. 2009). If diet differs between distinct genetic groups, it provides indirect evidence that these genetic clusters form social groups or at least that foraging is a matrilineally driven learned behaviour. In addition, if closely related individuals travel together in clusters, investigations of genetics and diet together may help untangle the complex nature of narwhal society.

A narwhal ice entrapment event occurred in November 2008 near the community of Pond Inlet, Nunavut, Canada (Fig. 4.1). After locals observed narwhals attempting to travel outside of the inlets and fjords, it became apparent that ice had become too thick and the distance to the offshore open waters too far for escape. Co-management decided

Figure 4.1. Location of the narwhal entrapment site near the community of Pond Inlet, Nunavut, Canada.



to approve a humane harvest of the animals before they starved to death or drowned. This sample provided a unique opportunity to assess social structure in narwhals, which are presumed to have a matrilineal structure (Palsbøll et al. 1997), as it is likely the entrapment captured many of the female clusters and their young that coexisted within the larger herd (Marcoux et al. 2009) and potentially captured entire familial lineages. It is an especially unique sample because of the large number of female narwhals, which are seldom sampled due to a biased hunt of tusked males. Inuit reported that the entrapment was primarily females because only the large males and females were able to make the deep, long dives to escape the entrapment, with juveniles, calves, and the smaller females and females associated with the calves and juveniles, being trapped.

Fatty acids are long chain carbon molecules that are transferred relatively unmodified from prey to predator tissues (Iverson et al. 1995; Lea et al. 2002). Analysis of fatty acids has been conducted in a number of marine mammals to investigate diet, including minke whales (*Balaenoptera acutorostrata*) (Olsen and Grahl-Nielsen 2003), bottlenose dolphins (*Tursiops truncates*) (Samuel and Worthy 2004), grey seals (*Halichoerus grypus*) (Grahl-Nielsen et al. 2000), polar bears (*Ursus maritimus*) (Grahl-Nielsen et al. 2003; Thiemann et al. 2008), bottlenose whales (*Hyperoodon ampullatus*) (Hooker et al. 2001), bearded seals (*Erignathus barbatus*), harbour seals (*Phoca vitulina*), harp seals (*Pagophilus groenlandicus*), hooded seals (*Cystophora cristata*), ringed seals (*Pusa hispida*), walruses (*Odobenus rosmarus*) (Thiemann et al. 2008), beluga whales (*Delphinapterus leucas*) (Dahl et al. 2000), and on narwhals (Watt and Ferguson 2014). Using a combination of fatty acids and genetic signatures we investigated whether closely related individuals also forage similarly, which would provide some support for a

matrilineally driven social structure, where sharing of resources and/or learned foraging behaviours are common (Ford and Ellis 2006; Tinker et al. 2009). To our knowledge, determining social structure through diet, without the use of any photographic database, has never been conducted and thus, if successful, may become a new technique for investigating sociality and would accompany studies already investigating diet in cetaceans.

Methods

General

Local Inuit hunters from Pond Inlet found whales trapped on November 15, 2010. The Mittimatalik (Pond Inlet) Hunters and Trappers' Organization (HTO) then notified Fisheries and Oceans Canada (DFO) and Nunavut Wildlife Management Board (NWMB) who agreed with the community's request to conduct a humane harvest. Department of Fisheries and Oceans Canada sent two employees (B. Dunn and J. Orr) to Pond Inlet to collect samples from the humanely harvested narwhals on December 2-3, 2010. Sample collection was difficult given the number of harvested narwhals, minimal daylight hours, and freezing temperatures; samples were collected with a hatchet and it was not always feasible to determine age class or sample the deepest blubber layers. Samples were frozen at -20 °C, and shipped to the Freshwater Institute in Winnipeg, Manitoba, Canada.

Genetics

Genomic DNA was extracted using the BioSprint platform (Qiagen Inc., Valencia, CA, USA), which uses magnetic particles to isolate nucleic acids and extracts in a 96-well format. Isolated DNA was then quantified and normalized to 10 ng/µl (NanoDrop, Thermo Fisher Scientific Inc., Wilmington, DE, USA). Sex was determined using

primers LGL331 (CAA ATC ATG CAA GGA TAG AC) and LGL335 (AGA CCT GAT TCC AGA CAG TAC CA), which were developed by Shaw *et al.* (2003). The following concentrations of reagents were included in a total volume of 10 µl: 1x PCR buffer (Thermopol: 20 mM Tris-HCl, 10 mM (NH4)2SO4; 10 mM KCl; 2 mM MgSO4; 0.1 % Triton X-100, pH 8.8 @ 25 °C) (New England Biolabs Inc., Ipswich, MA, USA); 0.5 mM MgCl2; 0.2 mM dNTPs; 10 %/V BSA; 0.3 µM each primer; 0.5 U *Taq* polymerase (New England Biolabs Inc., Ipswich, MA, USA); and 20 ng of template DNA. An initial denaturing step of 4 min at 94 °C began the thermocycling, followed by 30 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C. A final extension of 3 min at 72 °C completed the amplification. The QIAxcel system (Qiagen Inc., Valencia, CA, USA) was used to visualize the amplification products. A single amplification product (two X fragments) indicated a female and two fragments indicated a male (X and Y fragments).

Samples were genetically profiled using 12 microsatellite loci developed for other cetaceans (Buchanan et al. 1996; Valsecchi and Amos 1996; Hoelzel et al. 1998; Krützen et al. 2001; Caldwell et al. 2002). Detailed information on molecular methodology can be found in Petersen et al. (2011). Loci (Ev14, FCB1, 3, 4, 5, 10, 13, GT39, KWM2, 12, MK8, 9) were amplified individually or in duplexes, in reactions with a total volume of 10 µl and the following reagent concentrations: 1x GenAmp buffer II (10 mM Tris- HCl pH 8.3, 50 mM KCl) (Life Technologies Corporation, Carlsbad, CA, USA); 1.5 mM MgCl2; 0.2 mM dNTPs; a variable amount of each primer (Table 4.1); 0.5 U Ampli*Taq* Gold polymerase (Life Technologies Corporation, Carlsbad, CA, USA); and 20 ng of template DNA. Themocycling conditions varied among reactions. Single step conditions

Table 4.1. Details regarding PCR primers and respective annealing temperatures.

Primers	Source	T_{anneal}
Ev14 -ned	Valsecchi and Amos (1996)	48/55
FCB1	Buchanan et al. (1996)	48/53
FCB3 -ned	Buchanan et al. (1996)	56/51/48
FCB4 -6fam	Buchanan et al. (1996)	63
FCB5 -vic	Buchanan et al. (1996)	63
FCB10 -6fam	Buchanan et al. (1996)	60
FCB13 -vic	Buchanan et al. (1996)	48/53
GT39 -vic	Caldwell et al. (2002)	48/53
KWM2a -vic	Hoelzel et al. (1998)	48/55
KWM12a -6fam	Hoelzel et al. (1998)	56/51/48
MK8 -ned	Krutzen et al. (2001)	48/53
MK9 -6fam	Krutzen et al. (2001)	48/55

began with a denaturing step of 11 min at 94 °C followed by 25 cycles of 45 s at 94 °C, 45 s at annealing temperature, 45 s at 72 °C, and a final extension of 15 min at 72 °C to complete the amplification. One step-up and one step-down program were also used. Amplification products were denatured and run with GeneScan 400HD Rox size standard (Life Technologies Corporation, Carlsbad, CA, USA), using an ABI 3130xl Genetic Analyser (Life Technologies Corporation, Carlsbad, CA, USA). Allele scoring was conducted in GeneMarker 1.90 (SoftGenetics, State College, PA, USA). Although mitochondrial genetic diversity is low for narwhals (Palsbøll et al. 1997), the observed heterozygosity in narwhals (Hz = 0.67; Petersen et al. 2011) is comparable with that found for beluga whales (Hz = 0.65; Buchanan et al. 1996) and we were able to identify parent-offspring, full-sibling and half-sibling pairs. Narwhal tissue samples from the entrapment event also displayed a positive inbreeding coefficient (F_{IS} = 0.02), suggesting the group included some closely related individuals (Petersen et al. 2011).

Fatty acids

The Folch procedure (Folch et al. 1957) with modifications by Budge et al. (2006) was used to extract lipids from freeze dried narwhal blubber samples (0.5 g). A 2:1 chloroform-methanol solution containing 0.01 % butylated hydroxytoluene was used to extract lipids, followed by addition of 3.5 ml of sodium chloride to remove excess water. Fatty acid methyl esters (FAME) were produced using a dichloromethane / 0.01 % butylated hydroxytoluene sulfuric acid and dry methanol solution that was heated for one hour at 100 °C. In series, hexane, distilled water, and sodium sulfate were added to wash the FAME, and finally the FAME and hexane solution were placed under an evaporative nitrogen stream. An Agilent Technologies 7890A GC system was used to analyze the

FAME samples. Nucheck (54 component mix GLC-463) and Supelco (37 component FAME mix) standards were run on the GC every 10 samples. FAME were identified via retention time and these known standard mixtures. Thirty-two dietary fatty acids were identified in this study (Iverson et al. 2004); however, only five fatty acids contributed > 1% to the overall fatty acid profile. These included C20:1n11, C20:1n9, C20:5n3, C22:1n11 and C22:6n3 and were used for all statistical analyses. Twenty-seven samples were run in duplicate to calculate precision and the average standard deviation across all 32 dietary fatty acids was 0.03 %. Percent fatty acid values were divided by the reference fatty acid C18:0 and log transformed prior to analysis (Budge et al. 2006).

Statistics

Relatedness between pairs of entrapped individuals was calculated using the program ML-Relate (Kalinowski et al. 2006) to generate a matrix of maximum likelihood estimates. In addition, ML-Relate also identified relationships among samples by estimating the likelihood that a pair of individuals may be unrelated, half-siblings, full-siblings, or parent and offspring. For information on HWE, LD, and errors rates see Petersen et al. (2011). A ward cluster analysis on the relatedness matrix was calculated to find clusters of individuals that were more related than would occur by random chance (Ward 1963). The five dominant (> 1% composition) fatty acids found in narwhal blubber were input into a principal component analysis (PCA), which reduced the data to two uncorrelated dimensions (Jolliffe 2002). These principal components, which met the assumptions of a MANOVA, were then used to run a MANOVA to determine if genetic groupings had significantly different fatty acid profiles. Finally, to investigate if the genetic structure of the entrapped narwhals was correlated with their fatty acid signatures,

we conducted a Mantel test on the genetic matrix of maximum likelihood relatedness and a Euclidean distance matrix of narwhal's fatty acid profiles. The Mantel test deals with two distance matrices, obtained independently, that describe relationships among the same sampling units (Mantel 1967), and is often used when genetic relationships and some other environmental or ecological trait are measured on the same individuals (Legendre and Fortin 1989; Kameyama et al. 2002; Lloyd 2003; Kitchen et al. 2005). Although the power of the Mantel test and subsequent correlogram has limitations (Legendre et al. 2005; Legendre and Fortin 2010), a recent study suggests the test is both powerful and useful when comparing two distance matrices (Borcard and Legendre 2012). The Mantel test was run using 10,000 replicates in the "ade4" package within the R environment (Dray et al. 2013).

Results

General

The Pond Inlet HTO reported 629 narwhals harvested. Researchers identified age classes based on body size. In total, 68 individuals were identified as calves, 210 as juveniles, and 288 as adults. Samples of blood, skin and blubber were collected from 22 calves, 46 juveniles, 68 adults, and 114 narwhals of unknown age class, for a total of 250 samples; 209 of these samples had reliable fatty acid and genetic markers and were used in analyses.

Genetics and Fatty Acids

Skin samples (n = 209) were genetically analyzed to determine sex; 171 were females and 38 were males. In the full matrix 28,747 individual pairs were evaluated to identify the most likely familial relationship. In total, there were 308 full-sibling relationships and

308 parent offspring pairs found, with 77 parents rearing more than one offspring within the group. Cluster analysis on the matrix of maximum likelihood relatedness revealed nine distinct clusters that were each composed of a mixture of males and females, with no only male or only female clusters being identified (Fig. 4.2).

The first two principal components from a PCA on the five dominant fatty acids, described 89.5% of the variability in the data. MANOVA on the fatty acid principal components found no significant difference between the principal components for the defined genetic groupings identified in the cluster analysis (Pillai's trace; $F_{16, 400} = 1.23$, $p = 0.24$) (Fig. 4.3). The Mantel test on all narwhals revealed no significant correlation between the genetics and fatty acid dissimilarity matrices ($r = -0.033$, $p = 0.99$). Mantel test on only the most closely related individuals (a subset of individuals that comprised a parent-offspring or full-sibling pair) also found no significant correlation between the genetic and fatty acid dissimilarity matrices both when calves were included in the analysis ($r = 0.009$, $p = 0.28$) or excluded ($r = 0.006$, $p = 0.38$).

Discussion

Closely related individuals were no more similar in their fatty acid values than would be expected for unrelated individuals in the group. This provides some evidence that closely related narwhals do not engage in social foraging activities, and therefore likely do not travel as family groups. However, our results suggest that family groups are comprised within the larger herd, as there was a high inbreeding coefficient for the entrapped animals. These whales may also socialize and forage with other members of the herd with whom they are unrelated or distantly related.

Figure 4.2. Cluster dendrogram created from the matrix of maximum likelihood relatedness of narwhal. Lines delineate the 9 identified clusters.

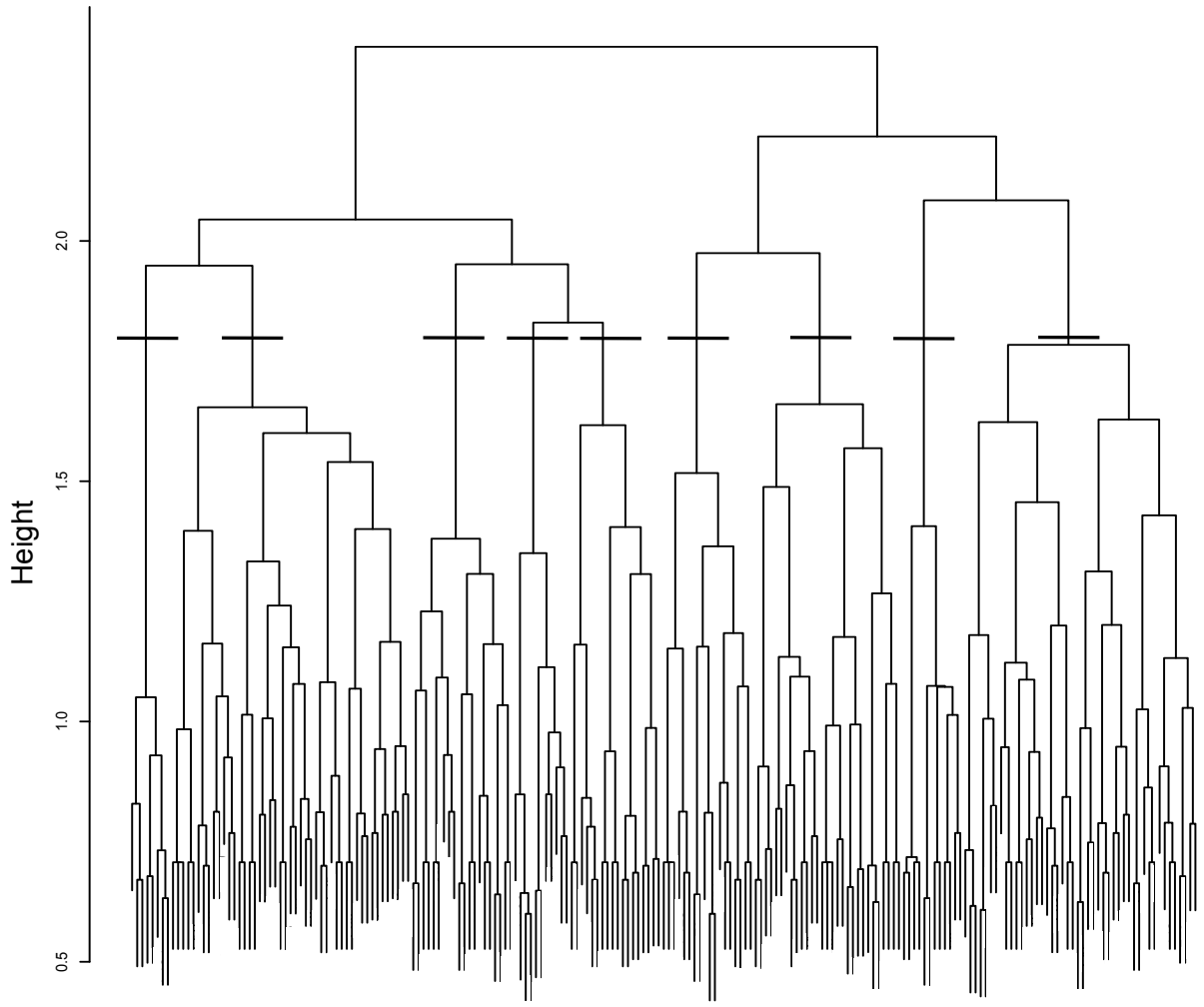
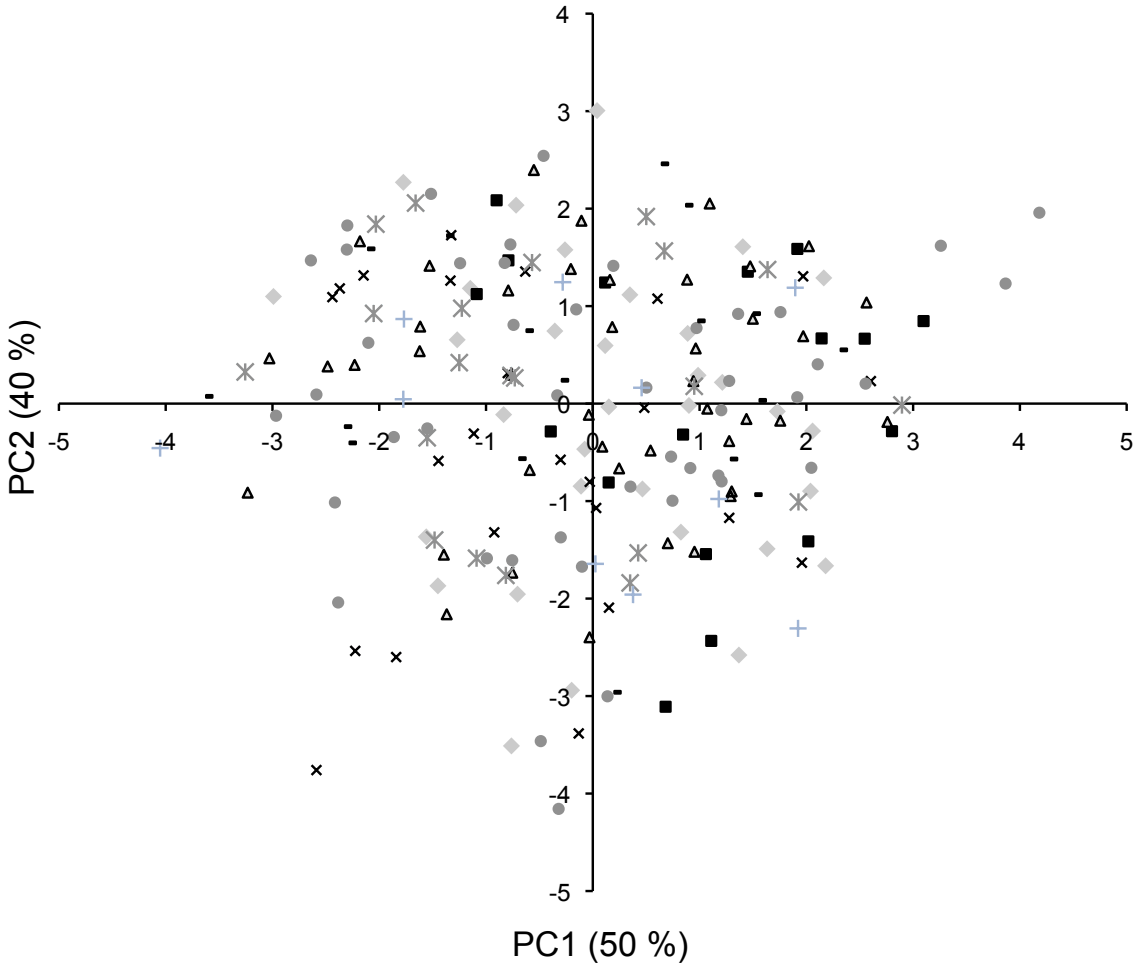


Figure 4.3. PCA of five narwhal fatty acids contributing > 1 % to overall fatty acid composition. Different symbols indicate the 9 genetic groupings identified from cluster analysis.



A fission-fusion social structure, where animals change the size of their groups through fission and fusion of subgroups depending on their activity and resource distribution and availability (Aureli et al. 2008), is common in dolphin populations (Connor et al. 2000; Parra et al. 2011) and may be a useful framework for evaluating narwhal social structure. The degree to which a species displays fission-fusion social dynamics varies from highly cohesive, where members of the group are stable and intact, to highly fluid with flexible subgroup membership (Aureli et al. 2008). Animals with a high degree of fission-fusion structure, where group membership is highly variable, is common for organisms that face a relatively low risk of predation, but have to cope with patchy and temporally varying resource distribution (Wrangham 1980; Symington 1988; Connor et al. 2000). For example, fission-fusion social structure of bottlenose dolphin (*Tursiops truncatus*) populations is interpreted as being an adaptation to the irregularity of prey distribution, with animals spreading out in smaller groups to reduce intraspecific competition when food is limited and aggregating in larger groups when abundant food is available (Connor et al. 2000).

As an Arctic marine mammal, narwhals experience patchy distribution and seasonal changes in prey availability (Laidre and Heide-Jørgensen 2005; Bluhm and Gradinger 2008). In particular, narwhals are known to aggregate in large herds during winter (Laidre and Heide-Jørgensen 2011), and migrate with smaller groups to their summering grounds (Heide-Jørgensen et al. 2003). During the summer, within these groups a small number of individuals will cluster together (Marcoux et al. 2009). We found no evidence to support that these clusters exhibit a matrilineal social structure. Narwhals have the lowest mitochondrial genetic diversity of any odontocete (Palsbøll et

al. 1997) and we did not get samples from the whole herd; 250 individuals were sampled, while over 600 narwhals were harvested. It is likely that many more narwhals drowned or escaped the entrapment. Despite this, we were able to detect parent-offspring and full-sibling relationships among the 250 samples taken, suggesting the herd did consist of some closely related individuals.

It seems likely that narwhals travel in close proximity to closely related individuals within the larger herd, even if they do not form distinct groups that persist over time. In beluga whales (*Delphinapterus leucas*), the narwhal's closest living relative, young belugas remain in close association with their mother, beyond weaning age, and particularly during migration (Colbeck et al. 2012). The fact that belugas display a stronger social connection among mothers and young may relate to the difference in mating systems between the two species. Narwhals have significantly smaller relative testes compared to belugas, suggesting they may have a polygynous mating system, while belugas have a promiscuous one (Kelley et al. 2014). In addition, in narwhals the tusk length is correlated with testes mass, suggesting the tusk acts as an indicator of male fecundity allowing females a choice in their selection of mate (Kelley et al. 2014). Paternal care in promiscuous animals is rare (Huchard et al. 2012), which suggests a longer parental investment from females would be common in belugas. It may be that narwhal clusters display a fission-fusion type social structure with young moving between male and female dominated clusters, but within the larger herd and particularly during migratory periods, closely related individuals travel in close proximity to one another.

Fatty acids can be impacted by stress or starvation (Koopman et al. 2002). Since the samples used in our study came from entrapped narwhals we have to consider that their fatty acid profiles may not be reflective of their diet. In addition, given the harsh sampling conditions, the deepest, and most active blubber layer (Budge et al. 2006) was not always collected and in some cases blubber closer to the skin was sampled. As a result of these limitations, the fatty acid profiles from these narwhals were compared to a healthy sample of narwhals examined by Thiemann et al. (2008). A table comparing the fatty acids in Watt and Ferguson (2011) shows that these narwhals had average fatty values that would be expected based on a healthy sample. As a result, we are confident that the blubber samples collected do reflect a dietary signature and have not been degraded as a result of fasting or stress.

We believe a combination of genetic and dietary signatures within social groups of cetaceans could still be a useful technique for evaluating sociality and may be useful for other Arctic cetaceans that are logistically difficult to study, such as beluga whales (*Delphinapterus leucas*) (O'Corry-Crowe 2008). However, for narwhals, a targeted live biopsy sample collection of all individuals traveling within the smaller clusters may be required to determine if this is a feasible method for allocating individuals to groups. Further investigation of narwhal social structure is needed to support the hypothesis of a fission-fusion structure within the clusters, and familial relationships being apparent in the larger herd.

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Chapter Five

Differences in dive behaviour among the world's three narwhal (*Monodon monoceros*) populations correspond with dietary differences

Watt, C. A.[1], J. R. Orr[2], M. P. Heide-Jørgensen[3], N. H. Nielsen[3], and S. H. Ferguson[1,2]

[1]Department of Biological Sciences, University of Manitoba, Winnipeg, MB R3T 2N2, Canada
[2]Fisheries and Oceans Canada, 501 University Crescent, Winnipeg, MB R3T 2N6, Canada
[3]Greenland Institute of Natural Resources, 3900 Nuuk, Greenland

Chapter Five: Manuscript Summary

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Contribution

J. R. Orr led the field teams that equipped narwhals from the Northern Hudson Bay and Baffin Bay populations with satellite tags.

Dr. Heide-Jørgensen and N. H. Nielsen tagged narwhals from East Greenland and sent the dive information to C. Watt, and Dr. Heide-Jørgensen provided editorial comments on a final version of the manuscript.

Dr. Ferguson provided funding for satellite tagging and guidance and comments throughout the writing process.

Cortney Watt assisted with tagging narwhals from the BB population, wrote the manuscript in its entirety, prepped all data and conducted all statistical analysis, and created all accompanying figures and tables.

Abstract

Satellite tracking of animals can provide information on their foraging behaviour, behavioural flexibility, and potential prey. Differences in diet among narwhals (*Monodon monoceros*) from the Baffin Bay (BB), East Greenland (EG) and Northern Hudson Bay (NHB) populations have been detected using stable isotope analysis. These dietary differences were a result of narwhals consuming different proportions of pelagic versus benthic prey. As a result, differences in diet should correspond to differences in dive behaviour among populations, which we evaluated using satellite-linked tags. Thirty-four narwhals were equipped with transmitters to evaluate the total number of dives and time spent in pre-defined depth categories. Repeated-measures ANOVAs found narwhals from EG made significantly more dives and spent more time in the mid-water column compared to other populations. NHB narwhals made more dives in the deep zone than in the mid-water region. BB narwhals spent time and made most dives within the upper water column and the deep zone, which suggests deep-dwelling prey may contribute substantially to their diet. Within the BB and EG populations there were sex-specific differences in time spent at depth and we identified seasonal changes in diving for all populations. This is the first study to compare dive behaviour in all three of the world's narwhal populations. We found that dive behaviour differences among populations paralleled differences in diet. Results suggest that narwhals employ specialized foraging strategies, which have repercussions for their potential ability to adapt to ecosystem changes that will occur with climate change.

Keywords: narwhal, *Monodon monoceros*, satellite tracking, diving behaviour, time at depth

Introduction

Satellite tracking of animals indirectly provides information on their foraging habits (Thompson et al. 2003, Jonsen et al. 2007, Varo-Cruz et al. 2013), ecological traits (Wade et al. 2006, Andrews et al. 2008), habitat use (Chilvers 2008), and changes in their habitats (McCafferty et al. 1999, Laidre and Heide-Jørgensen 2007). Satellite tracking is particularly useful for monitoring marine mammals, as they are difficult to observe in their natural environment and often make extensive migrations into inaccessible areas. Although the satellite tracking instruments, as well as the process of instrumenting the animals is costly, for some animals and some environments it is the only feasible approach for monitoring individual behaviour. Satellite tracking instruments can also be equipped with sensors for determining dive depth, and satellite-linked time-depth recorders are commonly used to assess diving ability in marine mammals as they provide binned dive data including maximum dive depths and the fraction of time spent in different depth bins (Laidre et al. 2004, Linnenschmidt et al. 2013). These data provide insight into habitat preference, foraging behaviour, and potential prey the animal may be targeting based on presence of prey species at those particular depths.

Narwhal's (*Monodon monoceros*) are Arctic cetaceans, best known for the spiral tusk present in males. The narwhal spends almost half of the year in heavy pack ice and darkness (Laidre and Heide-Jørgensen 2011) and satellite tracking provides a way to monitor their locations and behaviour during this time. Based on previous satellite tracking and genetic analyses, there are three defined populations of narwhals in the world (Dietz et al. 2008, Westdal 2009, Petersen et al. 2011). The Baffin Bay (BB) population numbers greater than 60,000 individuals (Richard et al. 2010) and spends summer (approximately June – September) in the fiords and inlets in the Canadian high

Arctic and the inlets of Northwest Greenland (Dietz et al. 2008). They then begin their migration to Davis Strait, ~1,700 km, where they remain until April, before returning to the summering grounds (Watt et al. 2012). There are approximately 12,500 narwhals in the Northern Hudson Bay (NHB) population (Asselin et al. 2012). They spend summer in northwestern Hudson Bay, and then migrate ~1,250 km to their wintering grounds in the eastern Hudson Strait (Richard 1991). The East Greenland (EG) population is estimated at approximately 6,000 individuals (Heide-Jørgensen et al. 2010). Narwhals from this population have been tracked from their summer residence in fiords and inlets in Scoresby Sound to offshore wintering grounds on the shelf area right outside Scoresby Sound (Heide-Jørgensen et al. unpublished data).

Narwhals from the BB population have been satellite tagged previously (n = 98) (Martin et al. 1994, Dietz and Heide-Jørgensen 1995, Heide-Jørgensen and Dietz 1995, Dietz et al. 2001, Heide-Jørgensen et al. 2002, Laidre et al. 2002, Heide-Jørgensen et al. 2003, Laidre et al. 2003, Laidre et al. 2006, Laidre and Heide-Jørgensen 2007, Dietz et al. 2008, Heide-Jørgensen et al. 2014, Watt et al. 2012). Narwhals in this population have also been tagged with D-tags and instrumented with crittercams, which provided information on dive behaviour (Dietz et al. 2007). However, no information on diving behaviour has been presented for narwhals from the NHB and EG populations. Three juvenile narwhals were tagged near Svalbard, Norway in August 1998 (Lydersen et al. 2006); however, tag duration was maximum 46 days, and therefore more work on narwhals from EG is required. Data on surfacing time for narwhals in NHB, which are useful for correcting visual aerial surveys, have been presented (Westdal 2009, Westdal et al. 2013), but no analysis of dives to all depths has been conducted for these whales.

Narwhals are believed to be among the most sensitive Arctic marine mammals to climate change, in part because of their limited behavioural flexibility and specialized diet (Laidre et al. 2008). In addition, evaluation of narwhal muscle has shown that these animals have morphological and physiological adaptations that allow them to survive the harsh Arctic winters, but which may also limit their behavioural flexibility (Williams et al. 2011). Dive behaviour has only been examined in narwhals from the BB population; thus, no comparisons among populations have been made to determine if populations are flexible in their foraging behaviour, or if different populations have adapted to the varying environmental conditions specific to their region. If differences in narwhal behaviour across populations can be detected it suggests narwhals may have greater behavioural flexibility than previously believed.

Dietary studies of narwhal stomach contents have only focused on the BB population and have found that diet is composed primarily of Greenland halibut (*Reinhardtius hippoglossoides*), Arctic cod (*Boreogadus saida*), polar cod (*Arctogadus glacialis*), and squid (*Gonatus fabricii*) (Finley and Gibb 1982, Laidre and Heide-Jørgensen 2005). An evaluation of diet using stable isotope analysis on narwhals from all three populations suggests significantly different diets (Watt et al. 2013). Stable isotope analysis showed that narwhals from EG fed in a more pelagic food web, while narwhals in NHB fed in a more benthic food web, and those from BB fed in both food webs approximately equally (Watt et al. 2013). Within the BB population, diet has also been shown to vary seasonally (Finley and Gibb 1982), which is common for marine mammals living in these seasonally extreme and dynamic environments (Bluhm and Gradinger 2008). Stable isotopes also indicated a seasonal difference in diet for NHB narwhals

(Watt and Ferguson 2014); however, nothing is known about seasonal changes in diet for the EG population. Seasonal changes in dive parameters have been noted for the BB population, where an increase in deep dives in the winter has been identified (Laidre et al. 2003); however, these deep dives were not matched with the bottom bathymetry at the dive location to investigate whether the narwhal may have been feeding in the deep-water zone. As a result, further investigation of changes in dive behaviour in all populations, and a comparison of dive behaviour among populations is warranted, as differences in dive behaviour would suggest different foraging strategies among the populations.

Differences in foraging behaviour in males and females have been found in a number of marine mammals (Le Boeuf et al. 2000, Beck et al. 2003, Baird et al. 2005). Narwhals display sexual size dimorphism, where adult males are significantly larger than adult females (Garde 2011). Increased size enhances an organisms ability to dive and stay at depth for longer periods of time (Schreer and Kovacs 1997, Irvine et al. 2000, Noren and Williams 2000, Mori 2002). Due to the size differences, males potentially have the ability to dive longer and deeper than females, and may have an increased dive effort if they have greater energy requirements than non-gravid and non-lactating females (Kleiber 1932). Within the BB population, Heide-Jørgensen and Dietz (1995) found female narwhals had lower dive rates than their male counterparts. However, Laidre et al. (2003) found no differences between the sexes, thus warranting further investigation of dive behaviour between males and females.

The specific objectives of our study were to determine if dive behaviour of animals from the three narwhal populations is congruent with what would be expected based on diet. We hypothesized narwhals from NHB would make dives to the deep zone

to feed on prey in this region, those from EG would be mid-water divers given that they forage on pelagic prey, and BB narwhals would make dives to the deep zone as well as some mid-water dive behaviours since they forage in both pelagic and benthic food webs. In addition, based on bottom bathymetry, we expected the narwhals in the three populations to spend a different amount of time diving depending on their geographic location. For instance, narwhals from BB overwinter in Davis Strait, which has depths up to 2000 m, and therefore whales from this population would need to make energetically expensive deep dives and have greater recovery time at the surface, which has been reported for other deep diving animals (Kooyman and Ponganis 1998). The EG region can be as deep as 1000 m, but Watt et al. (2013) found that narwhals in this population forage primarily on pelagic prey; thus, we predicted they would spend more time foraging at mid-depths.

Within each population, we predicted males would make more dives and spend more time at depth because of their greater body size. We also predicted we would identify seasonal changes in dive behaviour that would reflect changes in primary prey and narwhal home range at this time. In BB, a seasonal shift in diet was evident and although the precise prey responsible for this shift were difficult to distinguish, it appeared that narwhals fed more on pelagic prey in the winter and benthic prey in summer, albeit not significantly (Watt and Ferguson 2014). This is counter to Laidre and Heide-Jørgensen 2005 who found benthic prey, such as Greenland halibut (*Reinhardtius hippoglossoides*), are dominant in the winter. Thus, although we expected a change in dive behaviour, whether this would result in more dives to the deep zone in the winter or summer was difficult to predict for BB narwhals. In NHB, seasonal differences in diet

have also been identified, but to a much lesser extent than for narwhals in BB (Watt and Ferguson 2014). As a result, we predicted seasonal shifts in dive behaviour would be less evident for these narwhals. Little is known about seasonal diet or dive behaviour of EG narwhals, but we expected they would also have seasonal differences in dive behaviour related to shifts in diet since they inhabit deep-waters in winter, much like BB narwhals.

Methods

Study areas

Narwhals from the BB population were tagged in Tremblay Sound (72º 21' 23" N, 81º 6' 24" W) in August 2010 and 2011 (Fig. 5.1). In 2010, three females and two male narwhals, and in 2011, six females and one male were tagged (Table 5.1). Five narwhals, two females and three males, from the NHB population were tagged in Lyon Inlet (66º 30' N, 84º 00' W) near the community of Repulse Bay, in August 2006, and one female and three males were tagged in Repulse Bay (66º 31' 19" N, 86º 14' 06" W) in August 2007 (Fig 5.1; Table 5.1). Thirteen narwhals from the EG population were tagged at Hjørnedal, in Scoresbysund (70 39' 21.59" N, 27 48' 50.38" W); three males and three females in 2010, and six males and one female in 2011 (Fig. 5.1; Table 5.1).

Capturing and instrumenting the narwhals

Methods for whale capture and satellite tagging have been previously described (Orr et al. 2001, Dietz et al. 2008). Briefly, narwhals were caught in nets set perpendicular to the shore in waters with a maximum depth of approximately 60 m. Nets were dark green or black in color with 40 cm x 40 cm mesh and 3.5-5 m deep. Nets were anchored to a large stone on shore on one end and attached to a bag of large rocks at the

Figure 5.1. Map indicating the tagging sites (✱), and closest communities (●) where narwhals were tagged in East Greenland (EG), Northern Hudson Bay (NHB), and Baffin Bay (BB). Tagging locations differed in 2006 and 2007 for the NHB population as indicated.

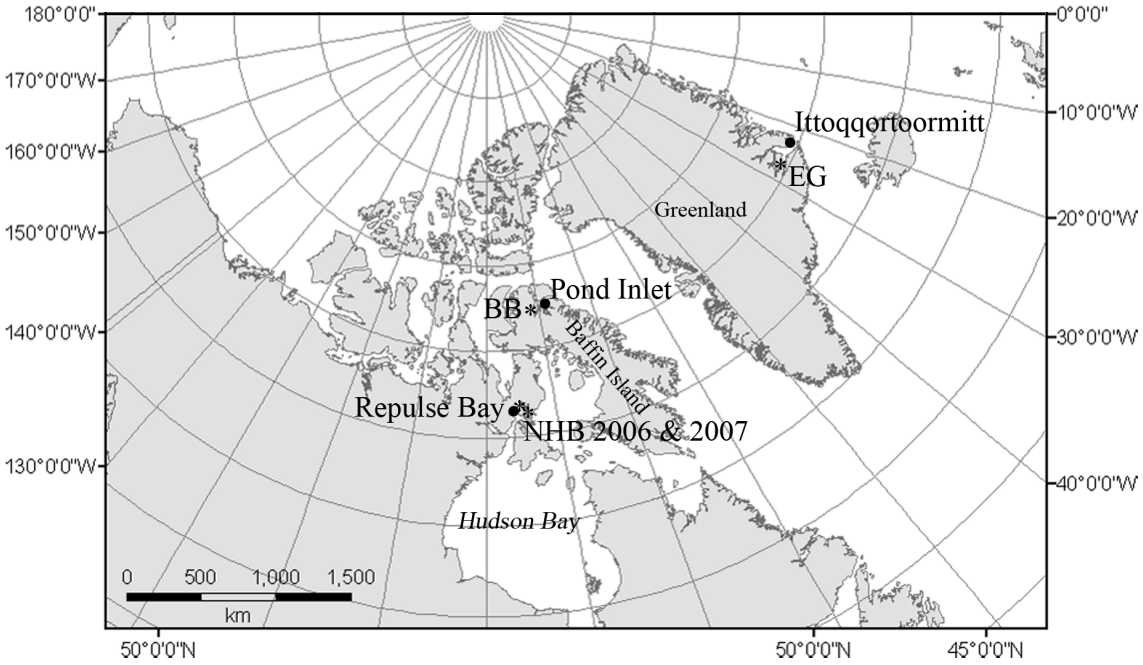


Table 5.1. Deployment date, date of last transmission, and total length of transmission period for narwhals outfitted with satellite-linked transmitters. Gender and morphometric data are also indicated for each narwhal.

Population: Tagging Location	Deployment Date	Last Transmission	Transmit Length (days)	Sex	Tag Number	Length (m)	Fluke Length (m)	Tusk length (m)
NHB: Lyon Inlet	2006-08-11	2007-06-11	305	M	57595	4.37	1.12	1.52
NHB: Lyon Inlet	2006-08-11	2006-12-24	146	M	57597	2.62	0.66	0.27
NHB: Lyon Inlet	2006-08-11	2007-04-20	253	M	57598	3.53	-	0.91
NHB: Lyon Inlet	2006-08-11	2007-04-04	237	F	57599	3.96	0.89	-
NHB: Lyon Inlet	2006-08-11	2007-06-03	297	F	57596	3.96	0.86	-
NHB: Repulse Bay	2007-08-08	2007-11-15	100	F	36641	4.00	0.90	-
NHB: Repulse Bay	2007-08-09	2007-12-09	123	M	40152	3.75	0.89	0.98
NHB: Repulse Bay	2007-08-09	2007-12-17	131	M	37024	3.85	0.92	1.10
NHB: Repulse Bay	2007-08-10	2007-11-22	105	M	40622	3.64	0.91	1.01
BB: Tremblay Sound	2010-08-21	2011-04-28	250	M	51871	4.44	1.07	1.56
BB: Tremblay Sound	2010-08-21	2011-06-06	289	M	51872	4.61	1.03	1.00
BB: Tremblay Sound	2010-08-22	2011-10-10	413	F	51873	4.00	0.90	-
BB: Tremblay Sound	2010-08-22	2011-02-26	187	F	51874	3.90	0.96	-
BB: Tremblay Sound	2010-08-24	2011-01-25	153	F	51875	3.80	0.93	-
BB: Tremblay Sound	2011-08-16	2012-02-23	191	F	51876	3.91	0.85	-
BB: Tremblay Sound	2011-08-16	2011-12-22	129	M	51878	3.10	0.76	0.20

BB: Tremblay Sound	2011-08-16	2012-06-25	314	F	51879	4.01	0.91	-
BB: Tremblay Sound	2011-08-18	2012-03-30	225	F	39314	4.06	0.93	-
BB: Tremblay Sound	2011-08-18	2012-03-07	202	F	39270	3.94	0.97	-
BB: Tremblay Sound	2011-08-19	2011-12-22	126	F	39315	3.89	0.95	-
BB: Tremblay Sound	2011-08-19	2012-01-16	151	F	57590	4.04	1.04	-
EG: Scoresbysund	2010-09-02	2010-12-29	118	M	3960	4.00	1.20	0.90
EG: Scoresbysund	2010-08-22	2011-02-17	179	F	3963	3.95	0.89	-
EG: Scoresbysund	2010-09-02	2011-02-15	166	M	3964	3.85	0.95	1.04
EG: Scoresbysund	2010-09-04	2011-07-12	311	F	6335	3.95	0.95	-
EG: Scoresbysund	2010-09-04	2010-11-13	70	M	93093	2.75	0.69	0.09
EG: Scoresbysund	2010-09-04	2011-06-09	278	F	93094	4.15	0.95	-
EG: Scoresbysund	2011-08-12	2012-03-06	207	F	6336	3.15	0.68	-
EG: Scoresbysund	2011-08-12	2012-01-09	150	M	7926	4.07	1.04	1.00
EG: Scoresbysund	2011-08-12	2012-01-18	159	M	20162	3.92	0.97	0.98
EG: Scoresbysund	2011-08-13	2012-03-03	203	M	93098	2.90	0.72	0.23
EG: Scoresbysund	2011-08-16	2011-10-22	67	M	93095	3.70	0.90	0.83
EG: Scoresbysund	2011-08-19	2012-05-12	267	M	93101	4.53	1.08	1.70
EG: Scoresbysund	2011-08-19	2012-03-18	212	M	10946	3.64	0.80	0.60

off shore end. Nets were kept afloat using 6-8, 30 cm white buoys that were clearly visible from shore. The nets were monitored 24 h and narwhal were easily detected in the net as one or several of the buoys would be completely submerged. When this occurred, one zodiac boat with 3-4 people on board would drive out to where the buoy had gone down and pull the narwhal(s) to the surface. A second zodiac, with 3-4 passengers, would drive to the anchor line and cut the net from the anchor. The shore crew (5-6 people) would then pull the net into shore. Once close to shore, the narwhal was positioned with its tail in the shallowest water, and disentangled from the net. In Canada, a veterinarian monitored heart rate and condition (e.g., blood samples) throughout the tagging process. The satellite tag was attached using two or three 10 mm nylon pins that penetrated through the fat and blubber under the dorsal ridge.

Narwhals in Canada were equipped with Wildlife Computers SPLASH tags that were programmed to transmit daily during the summer from July 1 to September 31 and subsequently on a 3-day duty cycle. Thus, four 6-hour data blocks were programmed to transmit (whether they all transmitted depended on the connection with the tag that day) every day from July-Sept, and every third day from Oct-June for the BB and NHB narwhals. Narwhals in Scoresby Sound were equipped with Mk10 transmitters that were on a 2-day duty cycle, where four 6-hour data blocks were collected every second day. Although daily transmissions provide more detailed movements of narwhals, the duty cycle allows for a longer deployment period. Data on location of all deployed tags were obtained from the ARGOS system (CLS America). ARGOS data files were extracted using WC-DAP 3.0 Build 69 software (Wildlife Computers). Dive information was grouped into defined depth bins, and represented the 6-hour time span directly prior to the

transmission. Due to the differences in the ocean bathymetry for the different populations, tags were programmed to collect data within different dive depth bins. For the BB population, the number of dives to different depths were binned into 6, 8, 10, 12, 15, 20, 100, 200, 400, 800, 1000, 1400, 1800 and >1800 m bins. Percent of time at each depth was binned into 1, 2, 3, 4, 5, 20, 100, 200, 400, 800, 1000, 1400, 1800, and >1800 m bins. Dives from the NHB population were binned in 6, 8, 10, 12, 15, 20, 25, 50, 100, 200, 300, 400, 500, and >500 m depth bins, and time at depth was binned in 1, 2, 3, 4, 6, 8, 10, 12, 20, 36, 50, 100, 200, and >200 m bins. The tags for EG narwhals were programmed to bin dives into 1, 2, 4, 200, 500, 800, 1100, 1400, 1700, 2000, and >2000 m bins, and time at depth was binned in 0, 2, 4, 200, 500, 800, 1100, 1400, 1700, 2000, and >2000 m bins.

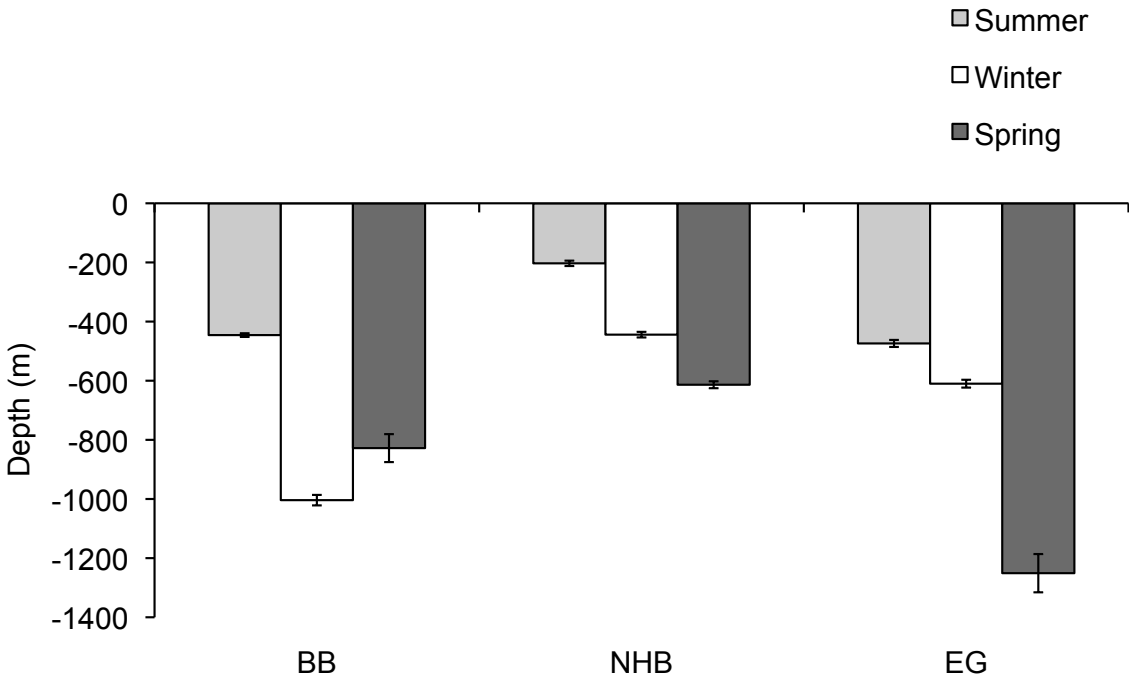
Data Analysis

Dive depth and time at depth bins were associated with a location that occurred within the 6-hour binned time frame on a particular day. Location information from the tags was categorized based on the accuracy of the transmission, varying from class A and B, and 0 to 3. Class A and B provide no location information, Class 0 includes an error range of >1500 m, class 1, 500-1500 m, class 2, 250-500 m and class 3, < 250 m. Shape files of ocean bathymetry were obtained from IBCAO (international bathymetric chart of the Arctic ocean), which provide 500 m^2 grid files (Jakobsson et al. 2012), and used to estimate a depth for each narwhal location through extraction using a North Pole projection in ArcGIS. Only class 2 and 3 locations were used in analysis, as they are both within the error range associated with the IBCAO depths. After bathymetry data for each day was obtained, to compare across populations because different populations live in

areas with vastly different bathymetries (for average depths experienced by each population across seasons see Fig. 5.2), dives to a particular depth bin were converted into a percent of total depth. Dives were then grouped into depth categories, including dives that occurred in the upper water column (surface = 0-25 % of total depth), those that occurred in the upper mid-water region (mid-surface = 26-50 %), in the lower-mid-water region (mid-deep = 51-75 %), and those dives that occurred in the deep zone (deep = 76-100 % of the total depth). Dives were often deeper than the IBCAO depths (15.5 ± 2.5 %), indicating the uncertainty with the bottom bathymetry at these remote locations; in these cases, these dives were included in the 76-100 % category. The same data preparation was done for the time spent at each depth bin to determine how much time was spent at the surface, in the mid-surface and mid-deep regions, or in the deep zone, for the BB and EG populations, but not the NHB population. Bathymetric depths never exceeded the tags programmed depth readings except for the NHB population, where tags were only programmed to >500 m for dive depth and >200 m for time at depth. Dives occurred in the >500 m bin, but this bin was always included in the 75-100% depth category; thus, regardless of whether whales were diving 501 m or 1000 m these dives would have been included in the deep zone. However, for time at depth, a large proportion of time was spent in the >200 m category and 200 m was often not included in the 75-100% depth category. Since we cannot surmise whether these dives should have been included in the mid-surface, mid-deep, or deep zone it was not possible to interpret the time spent at different depths for narwhals from NHB.

We evaluated the total number of dives and time at depth across populations using a repeated-measures full factorial ANOVA, which considered individuals nested within

Figure 5.2. Mean depth (± SE) available to narwhals in each population across seasons according to depth at satellite location (once every 6 hr period). Depth categories were calculated by dividing ocean depth into four equal categories (surface, mid-surface, mid-deep, deep) at each dive location and summing the total number of dives or percent of time spent in each of these depth categories.



each population as a random effect, and population (3 levels: BB, NHB, and EG for dive depth, and 2 levels: BB and EG for time at depth), depth (4 levels: surface, mid-surface, mid-deep, and deep), and the interaction between these factors as main effects (Underwood 1997). Normal probability plots were used to assess normality. The assumption of sphericity was tested using the Mauchly criterion (Mauchly 1940) and in instances where the sphericity assumption was violated, the adjusted Greenhouse-Geisser degrees of freedom were used, which multiplies the calculated epsilon by the original degrees of freedom to create a more conservative test that controls for type I error rates (Greenhouse and Geisser 1959). The nested nature of the design accounted for within-subject variability, while depth was considered a repeated measures factor since number of dives to one depth would impact the number of dives an individual could make to another depth category (ie. they were not independent) (Underwood 1997). When significant factors were identified, post-hoc Tukey's HSD tests were used to determine where significant differences occurred.

To investigate if number of dives and time at depth varied between sexes and across seasons for each population, a full factorial repeated-measures ANOVA which considered individuals nested within each sex as a random effect, and sex (2 levels), season (3 levels: winter, summer, or spring), depth (4 levels: surface, mid-surface, mid-deep, and deep) and all possible interactions among the factors, as main effects; Tukey's tests were used for post-hoc comparisons (Underwood 1997). Diving was defined as occurring in summer (June through September), winter (October through March) or spring (April-May) based on previous satellite tracking (Dietz et al. 2001) and diet (Finley and Gibb 1982) studies. Although there were two years of satellite tagging for

each population, we were unable to include year as a factor in our analysis because the number of males and females tagged per year varied and the length of tag transmission did not always include all seasons, which resulted in insufficient degrees of freedom to evaluate differences between years. All statistics were conducted using JMP 9.0 software.

Results

For number of dives to each depth category there was a significant interaction between population and depth ($F_{4, 15962} = 939.05$, $p < 0.0001$). Tukey's HSD tests revealed that narwhals from BB made significantly more dives within the upper water column (surface) than narwhals from NHB or EG ($p < 0.05$), and narwhals from EG made significantly fewer dives to this zone than NHB or BB narwhals ($p < 0.05$; Fig. 5.3). Narwhals from EG had significantly more dives to the mid-surface and mid-deep depths compared to narwhals from BB and NHB ($p < 0.05$), who made a similar number of dives to these depths ($p > 0.05$; Fig. 5.3). Narwhals from NHB and EG made a similar number of dives to the deep zone ($p > 0.05$), but made significantly more dives to this depth category than narwhals from BB ($p < 0.05$; Fig. 5.3).

For the number of dives, there was a significant interaction between depth and sex for BB ($F_{1, 5682} = 23.64$, $p < 0.0001$) and EG ($F_{3, 5034} = 13.72$, $p < 0.0001$) whales, but not for NHB narwhals ($F_{2, 1314} = 0.45$, $p = 0.64$). Upon further investigation, although there were significant differences in the total number of dives across the depth categories in BB, within each depth category, males and females made a similar number of dives ($p > 0.05$; Fig. 5.4). In EG, male and female narwhals made a similar number of dives in each depth category except in the mid-surface zone where males made significantly more dives than females ($p < 0.05$; Fig. 5.4). There was also a significant interaction between

Figure 5.3. Mean number of dives (± SE) made to the four different depth categories for narwhals from EG (n = 1437), NHB (n = 576), and BB (n = 3540). Asterisks indicate a significant difference, while lines with NS indicate no significant difference among the populations.

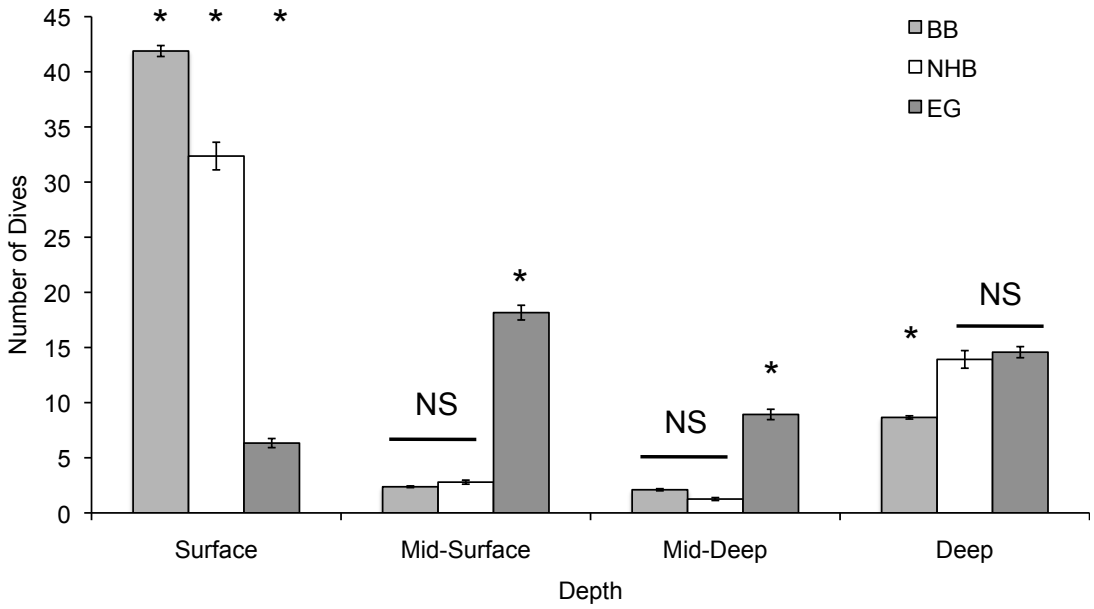
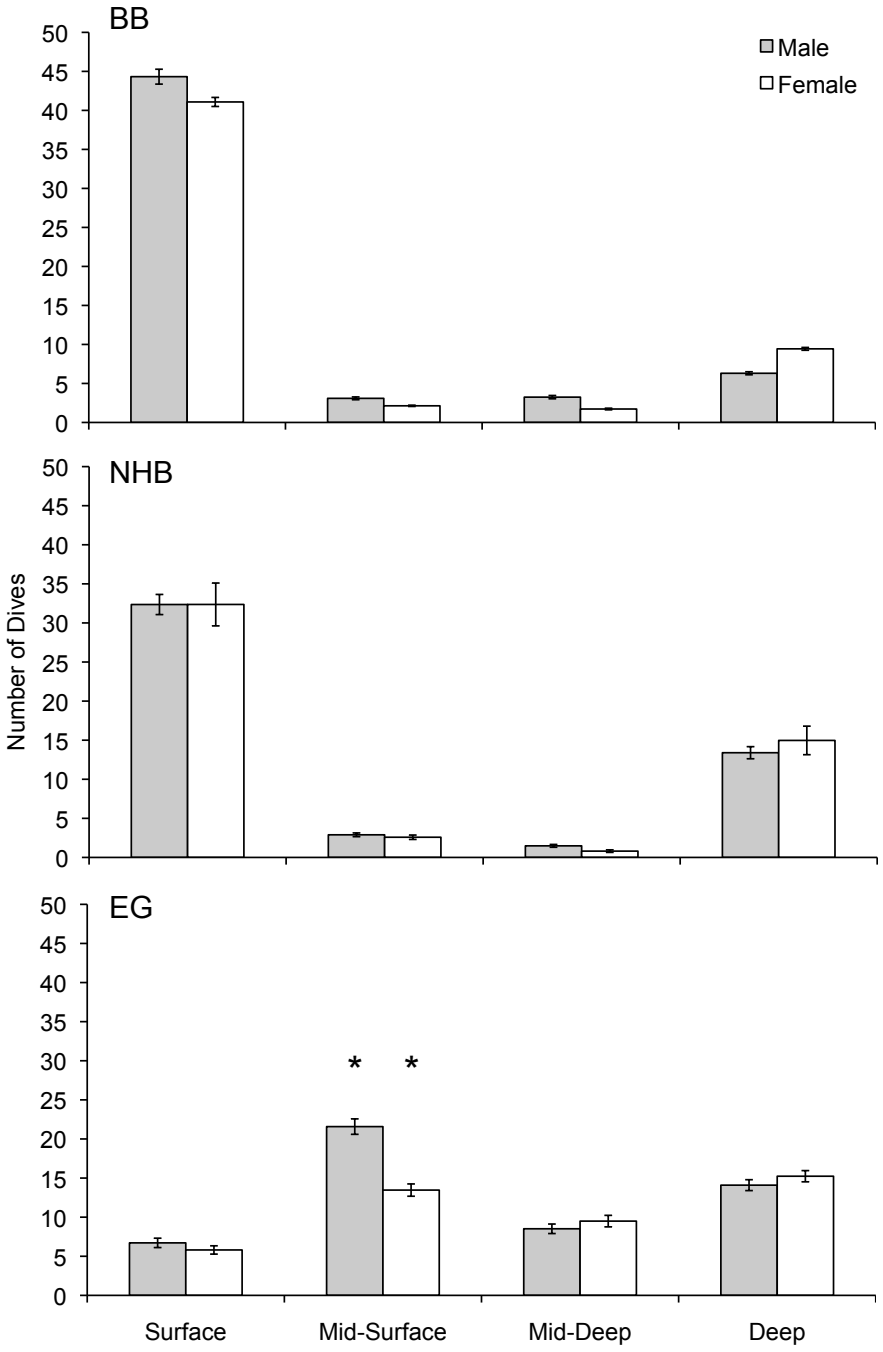


Figure 5.4. Mean number of dives (± SE) made to the four different depth categories for male and female narwhals from BB (n = 877 and 2663 respectively), NHB (n = 384 and 192, respectively), and EG (n = 832 and 605 respectively). Asterisks indicate a significant difference between males and females.



depth and season in the BB ($F_{2, 5682} = 40.94$, $p < 0.0001$), EG ($F_{5, 5034} = 29.69$, $p < 0.0001$) and NHB ($F_{3, 1314} = 4.60$, $p < 0.01$) populations for the number of dives. In BB, narwhals made significantly more dives in the upper water column during the summer, compared to winter and spring ($p < 0.05$; Fig. 5.5). The number of dives made to the mid-water columns did not differ among seasons ($p > 0.05$), while the number of dives to the deep zone was significantly higher in summer compared to winter ($p < 0.05$; Fig. 5.5). In BB, the number of dives targeting the deep zone made in the spring did not differ significantly from winter or summer ($p > 0.05$; Fig. 5.5). In NHB, the number of surface dives was significantly greater in the summer compared to winter or spring ($p < 0.05$); however, there were no significant differences in any other depth category for the different seasons ($p > 0.05$; Fig. 5.5). In EG, narwhals made more dives in the upper water column in spring and the least number of dives in summer ($p < 0.05$; Fig 5.5). Narwhals in EG made significantly more dives to the mid-surface zone in summer ($p < 0.05$), and a similar number of dives to the mid-deep category in all seasons ($p > 0.05$; Fig 5.5). Dives to the deep zone were more frequent in the summer compared to spring or winter ($p < 0.05$; Fig 5.5).

A repeated-measures ANOVA for time spent in each depth category comparing the BB and EG populations revealed a significant interaction between population and depth ($F_{2, 15593} = 1497.52$, $p < 0.0001$). Tukey's HSD tests showed that narwhals from BB spent significantly more time in the surface depth category than narwhals from EG ($p < 0.05$; Fig. 5.6). Narwhals from EG spent significantly more time in the mid-surface, mid-deep, and deep zone compared to narwhals from BB ($p < 0.05$; Fig. 5.6).

Figure 5.5. Mean number of dives (± SE) made to the four different depth categories for narwhals from BB, NHB, and EG during summer (n = 2205, 140, and 450 respectively), winter (n = 1134, 375, and 959 respectively), and spring (n = 201, 61, and 28 respectively). Asterisks indicate a significant difference among seasons, while lines with NS indicate no significant difference among seasons.

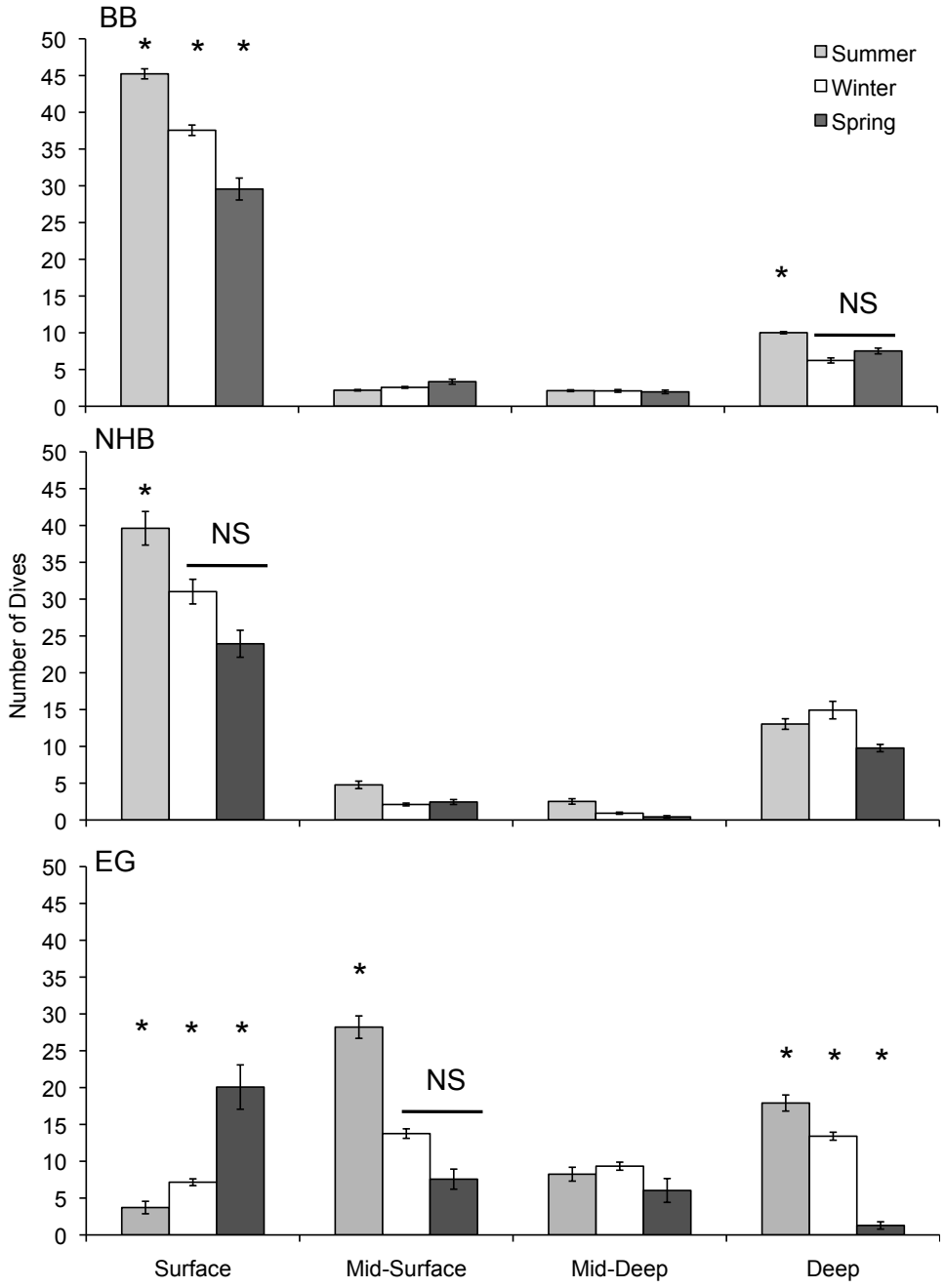
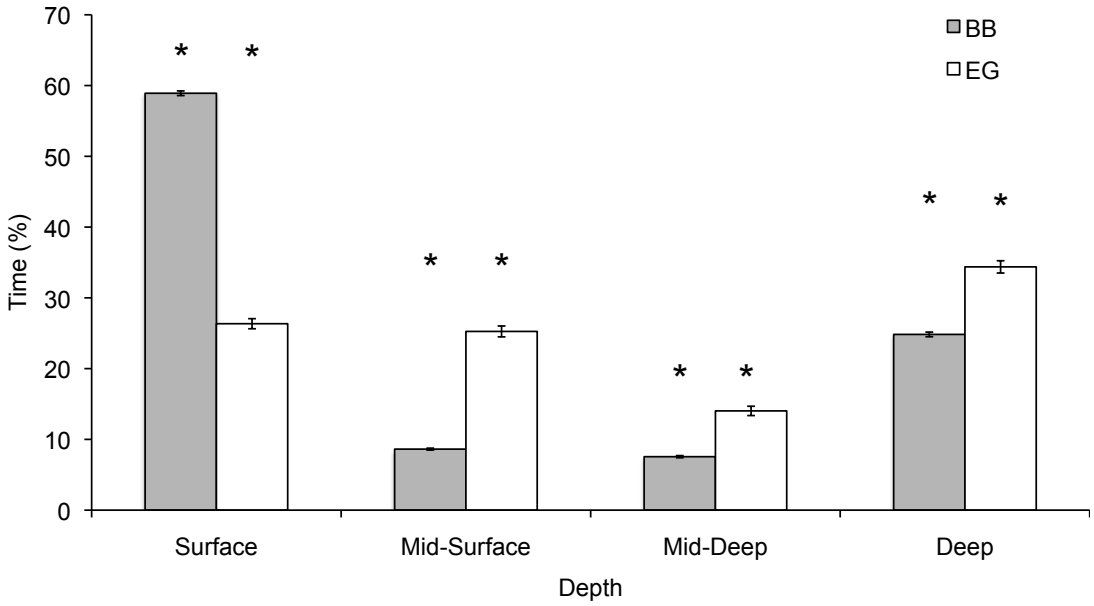


Figure 5.6. Mean percent of time spent (± SE) in the four different depth categories for narwhals from EG (n = 1440), and BB (n = 3542). Asterisks indicate a significant difference.



We compared the total time spent at different depths across seasons and between sexes within the BB and EG populations and found there was a significant interaction between depth and sex for BB ($F_{2, 7975} = 129.16$, $p < 0.0001$), and EG ($F_{3, 4715} = 27.41$, $p < 0.0001$) narwhals. Tukey's tests revealed that for the BB population, males spent significantly more time at the surface than females ($p < 0.05$; Fig. 5.7), and for both the EG and BB population, females spent significantly more time in the deep zone than males ($p < 0.05$; Fig. 5.7). We also found a significant interaction between season and depth for BB ($F_{3, 7975} = 52.63$, $p < 0.0001$), and EG ($F_{6, 4711} = 15.02$, $p < 0.0001$) narwhals. Narwhals from the BB population spent significantly more time at the surface in winter compared to spring and summer ($p < 0.05$; Fig. 5.8). In spring and winter BB narwhals spent significantly more time at the mid-surface depth than in summer ($p < 0.05$; Fig. 5.8). Time spent in the deep zone was significantly less in winter compared to spring and summer for narwhals in BB ($p < 0.05$; Fig. 5.8). In EG, narwhals spent significantly more time at the surface in spring and the least amount of time in summer ($p < 0.05$; Fig. 5.8). There was no significant difference in the time spent in the mid-surface zone across seasons ($p > 0.05$), but significantly less time was spent in the mid-deep zone in summer compared to the other seasons ($p < 0.05$; Fig. 5.8). Significantly more time was spent in the deep zone in the summer, and the least time was spent at this depth in the spring for narwhals from EG ($p < 0.05$; Fig. 5.8).

Discussion

According to stable isotope biomarkers, narwhals from the three different populations had different diets. In particular, we found evidence that narwhals from EG foraged in a more pelagic food web; narwhals from NHB ate more benthic prey, and those from BB

Figure 5.7. Mean percent of time spent (± SE) in the four different depth categories for male and female narwhals from BB (n = 880 and 2662 respectively), and EG (n = 841 and 599 respectively). Asterisks indicate a significant difference between sexes.

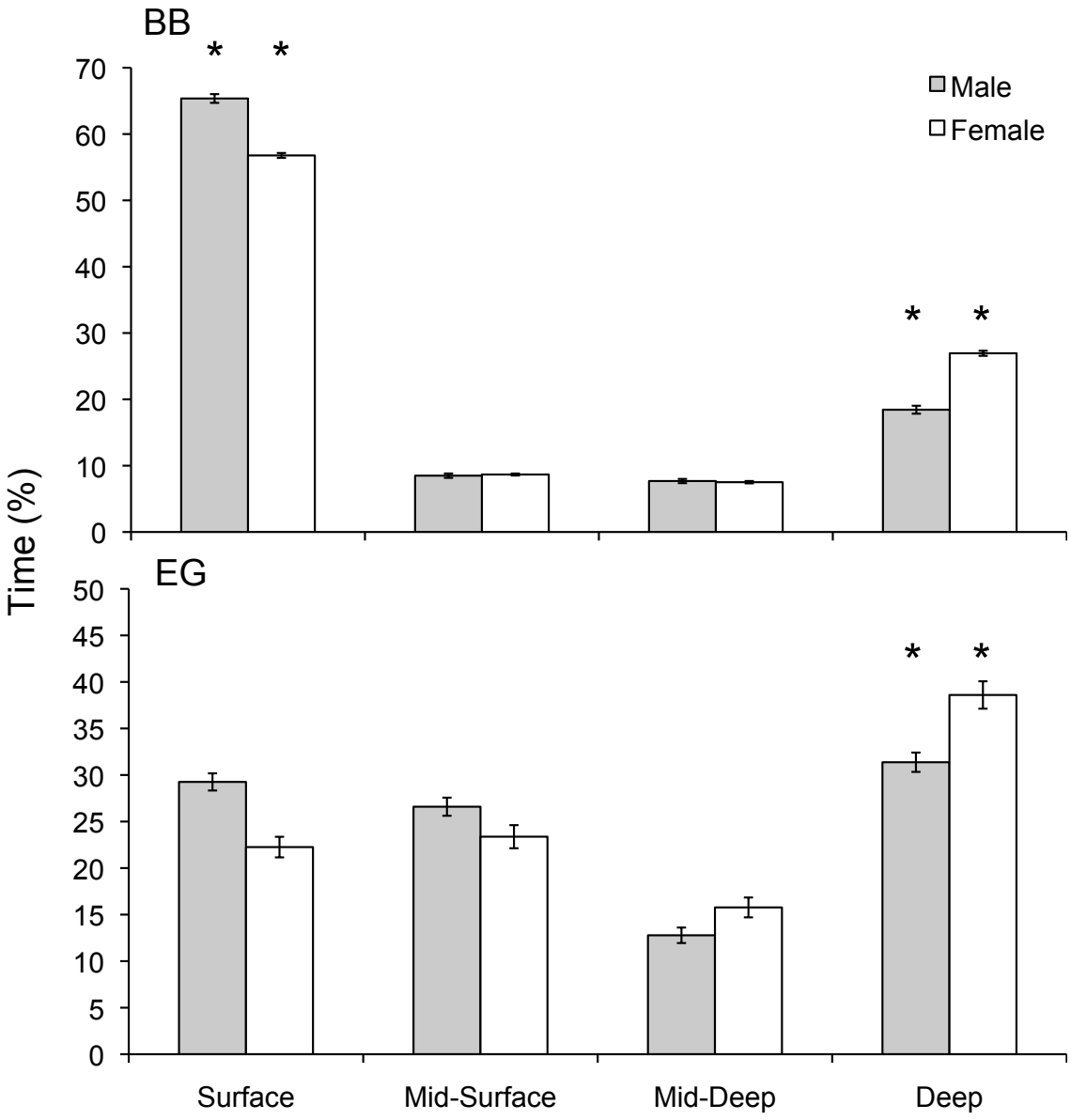
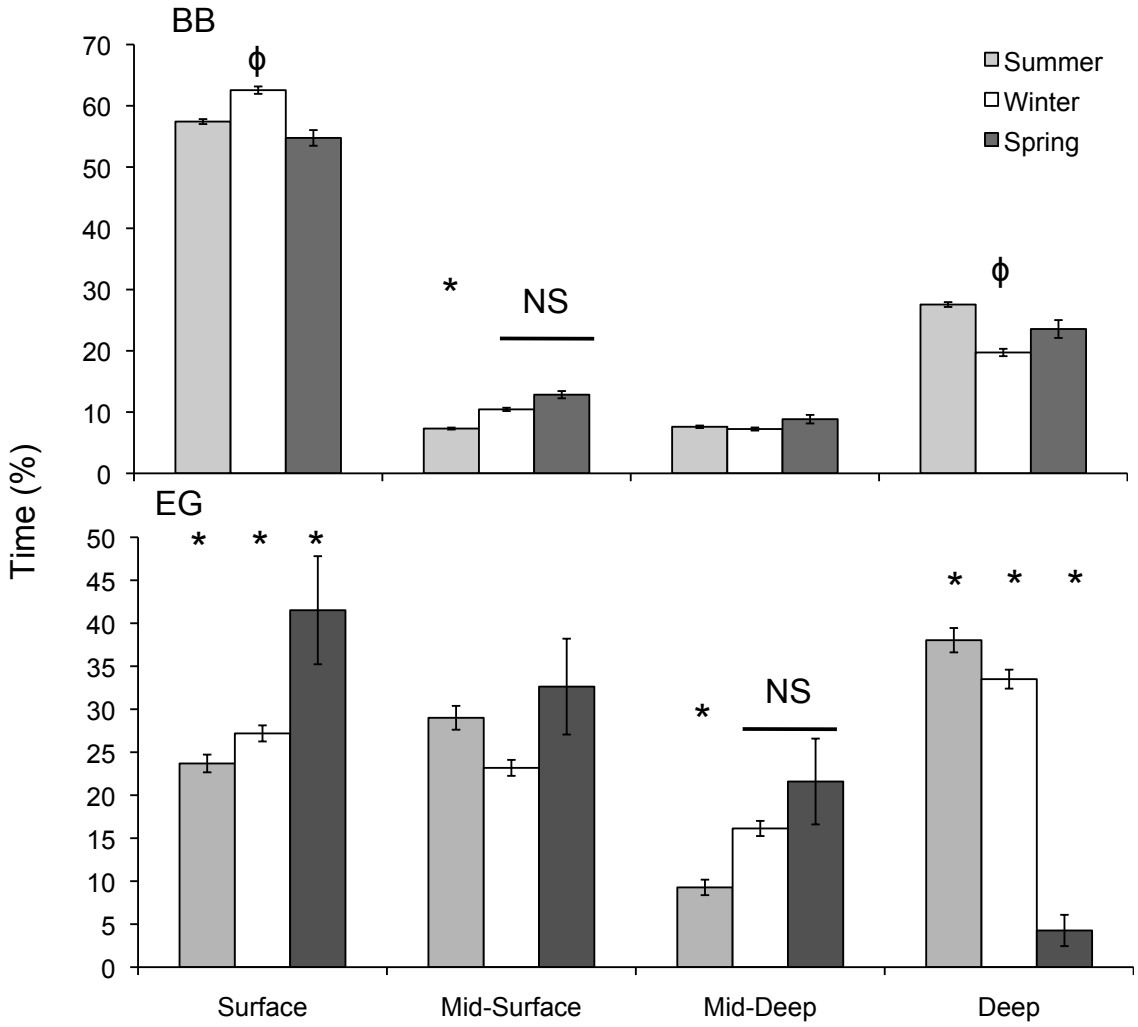


Figure 5.8. Mean percent of time spent (± SE) in the four different depth categories for narwhals from BB, and EG during summer (n = 2212, and 466 respectively), winter (n = 1131, and 945 respectively), and spring (n = 199, and 29 respectively). Asterisks indicate a significant difference among seasons, lines with NS indicate no significant difference among seasons, and phi (ϕ) indicates a significant difference between winter and the other seasons, but no significant difference between summer and spring.



foraged approximately equally in both food webs (Watt et al. 2013). Narwhals from NHB and EG had dive behaviour that reflected what would be expected based on these dietary conclusions. For instance, narwhals from EG made significantly more dives to the mid-water zone than narwhals from the other populations, and spent more time there than narwhals from the BB population. This suggests the mid-water zone is important for foraging for these whales. Narwhals from EG also spent a significant amount of time in the deep zone suggesting they must also be foraging here, although to a lesser extent than in the mid-water column, which their dietary signature reflects. NHB narwhals made more dives to the deep zone than the mid-water regions and made a number of surface dives. Marine mammals are limited in their foraging time because they need access to air. In the Arctic, dives to the deep zone offer greater opportunities for encountering and capturing prey (Bluhm et al. 2011); however, time at the surface to recover increases rapidly with dive length for many species (Kooyman and Ponganis 1998). Unfortunately we were unable to evaluate time at the surface for NHB narwhals, but given that they made a significant number of dives in the upper water column, we would also have expected them to spend ample time at the surface. In addition, NHB narwhals made many dives to the deep zone and we would expect them to spend ample time at the surface since they may need to recover from deep zone dives.

We hypothesized that narwhals from BB who forage in benthic and pelagic food webs approximately equally would spend similar amounts of time in the mid and deep zones, but we actually observed that BB narwhals made more dives to the deep zone and spent the vast majority of their time at the surface. If BB narwhals are diving extremely deep, which has been noted previously (Laidre et al. 2003), they need to spend more time

at the surface recovering from these dives, which may explain why most of their time is spent in this part of the water column. We predicted BB narwhals should also spend a significant proportion of their time in the mid-water regions since dietary studies show they also forage in this zone to a large extent (Watt et al. 2013); however, they spent less time in the mid-water regions (~15 %) compared to the deep zone (~25 %). It is possible some mid-water foraging occurs on the journey to or from the depths, or narwhals could be foraging on prey that are found very deep, but are still pelagic in nature since our deep-water zone included dives that occurred anywhere from 75 %-100 % of total possible depth and they may have been foraging in the upper portion of this range. Pelagic prey, including Arctic cod and capelin (*Mallotus villosus*) are known to make diel migrations (Benoit et al. 2010), and may travel deeper into the water column to evade predators; therefore, narwhals may need to dive to the deep zone to forage on these seasonally pelagic prey. This is also true for some benthic prey, such as Greenland halibut, that are typically found in the benthos but do make vertical migrations (Boje et al. 2014).

We were unable to detect differences in the number of dives made between males and females to the deep zone in any population. Adult males can potentially make more dives to greater depths than females because of their larger body size (Garde et al. 2007), but the males and females we tagged were of similar body size (Table 5.1). Asymptotic body length for female narwhals from the BB population is approximately 4.0 m, while males are estimated at 4.6 m (Garde et al. 2007). The fact that population wide male and female body size was not reflected in our small tagged sample may have limited our ability to detect differences in dive performance between males and females; however,

other studies of narwhal dive behaviour have also been unable to identify differences in total dive number between males and females (Laidre et al. 2003) and previous dietary studies have not found strong differences in diet between the sexes (Watt et al. 2013, Watt and Ferguson 2014).

Despite little difference in the number of dives to different zones, there was a significant difference in the time males and females spent at depth; females actually spent a greater amount of time in the deep zone than males in the BB and EG populations, counter to what we predicted based on body size. This has also been seen in grey seals (*Halichoerus grypus*), where males spend less time at the bottom compared to females (Beck et al. 2003). Beck et al. (2003) suggested this could be a result of larger males being more efficient foragers than females because their larger size may allow them to capture and handle prey more efficiently or they may be less selective foragers and spend less time in search for prey. In addition, smaller animals may be able to dive longer because they rely more heavily on anaerobic respiration (Mori 2002), although there is currently no evidence of anaerobic metabolism in narwhals. In our study, tagged males and females were of similar size, thus size related efficiency does not explain females spending a greater amount of time at depth than males. Another possibility that is specific to male narwhals, may be that the tusk aids in foraging (Dietz et al. 2007) and thus make males more efficient foragers and able to spend less time at depths searching and handling prey. A greater sample size with more equitable representation of both sexes would allow us to better test these hypotheses.

Seasonal differences in the dive behaviour of narwhals from BB have been identified previously (Laidre et al. 2003) and it appears that the other two narwhal

populations also exhibit changes in their dive behaviour across seasons. Laidre et al. (2003) found that for BB narwhals, the number of dives to deep depths and the time spent at depth increased in the winter. In contrast, when we factored in bottom bathymetry and evaluated number of dives and time spent in the deep zone for narwhals tagged in BB, dive rate and time in this zone was actually significantly higher in the summer, when they are in shallow waters, compared to winter when they are in deeper waters. It has been previously suggested that narwhals conduct a minimal amount of foraging in summer and gain most of their energy in the winter months (Laidre and Heide-Jørgensen 2005). However, studies of seasonal changes in body condition of narwhal are limited by sample size and temporal coverage. For example, based on 158 samples collected over three months (July-September), Hay (1984) suggested postnatal narwhals forage in summer because female and male body condition increased throughout the season (Hay 1984), whereas Finley and Gibb (1982) used 73 samples collected over four months (June-September) to suggest condition decreases in females (possibly because of greater demands imposed by lactation), with no change in males throughout the summer. Typically it is assumed that dives to deep depths, which are relatively energetically expensive, are related to foraging. Based on stable isotopes, Watt et al. (2014) found BB narwhals were consuming slightly more benthic prey in summer than winter, which would result in more dives to the deep zone at this time of the year, and is congruent with our results. However, if it is assumed that narwhals gain most of their energy in the winter and are not foraging in the deep zone in summer, there must be another reason for making dives to this depth zone during summer. Narwhals may be evading disturbances on the surface, or they could be using the rough bottom surface as a means for molting

and sloughing off skin. This has been shown for the narwhal's closest relative, the beluga (*Delphinapterus leucas*) (St. Aubin et al. 1990), but has never been documented for narwhals. More research on the potential benefits of diving in the summer, whether it is for molting or foraging, is needed to elucidate the reasons for increased diving to the deep zone during the summer in BB narwhals. In BB, winter and spring diving did not differ across the depth categories except for the surface category, where narwhals made more dives and spent more time at the surface in the winter compared to spring. This may be a result of seasonal changes in sea ice; narwhals may need to spend more time navigating heavy pack ice for leads and cracks in the ice in the winter (Laidre and Heide-Jørgensen 2011), compared to the spring when the ice is breaking up and access to the surface is greater.

Diving behaviour of narwhals in the EG and NHB populations, not previously studied, also differed in dive parameters with season. The NHB narwhals live in a shallow habitat in the summer, and unlike the BB narwhals who migrate to the deep Davis Strait in the winter, NHB whales migrate just outside the Hudson Strait and rarely experience depths greater than 700 m. Likely as a result of this, they showed relatively little variability in the number of dives to different depth categories across seasons. The EG population did display variation in dive behaviour across seasons and showed a similar pattern as the BB population with dives to the deep zone and time in the deep zone being greatest in the summer when in shallower water. This may indicate summer foraging on deep zone prey; however, stomachs analyzed in the summer have been primarily empty (Heide-Jørgensen et al. 2014) and narwhals from EG have a dietary signature significantly influenced by pelagic prey (Watt et al. 2013). If their dietary

signature is not being influenced much by bottom-dwelling prey, it again suggests other advantages to deep-zone diving in the summer months that require further evaluation. Narwhals from EG spend more time in the upper water column in spring and winter, and a similar amount of time in the mid surface region in these seasons, suggesting this may be an important time for foraging since their dietary signature is primarily influenced by pelagic prey (Watt et al. 2013). Since there were almost no dives to the deep-zone in spring, this may also be an important period for migration and horizontal movement with little time for diving and vertical movements.

Satellite tagging has the ability to provide location information as well as information on dive parameters that would otherwise not be possible for some animals. However, there are limitations with this method of data collection. GIS locations can be off by kilometers. In addition, we only have four locations (if conditions are favorable) per day or every three days in some seasons, and the collected dive information represents only a 6-hour window directly before the transmission. Aside from the limitations of the Argos locations, the bottom bathymetry of this region has not been mapped in detail. We used IBCAO to assign a bottom bathymetry to each location. Bathymetries are largely based on ship tracking sounds with interpolation between soundings, and in the Arctic shipping traffic is limited (although it is beginning to increase) and thus many data points are interpolated (Jakobsson et al. 2012). This is currently the most accurate method for assigning depths to locations and it is more useful to compare populations in relation to their habitat rather than to compare absolute values without considering the spatial structuring of their marine environment. These estimates will improve as research in the Arctic progresses.

Our results suggest narwhals from different populations have different dive behaviours, spending variable amounts of time within the depth categories in their respective habitats, and therefore are perhaps more behaviourally flexible than previously believed. Future studies should investigate muscle tissue of narwhals from all populations to determine if this morphological adaptation for living in extreme environments (Williams et al. 2011) is dependent upon the geographic location of the populations and their corresponding dive behaviours. Ours is the first study to evaluate dive behaviour in the NHB and EG narwhal populations, and it is the first to investigate differences in dive behaviour among populations. Results were generally in accordance with what would be predicted based on dietary studies using stable isotopes (Watt et al. 2013) and highlight the behavioural differences in narwhals that are adapted to living in different habitats and foraging on different prey. An increased number of narwhals tagged from all three narwhal populations is required to assess annual or decadal changes in behaviour. Sex differences may also become apparent with a greater proportion of whales tagged, females in particular. Furthermore, differences in dive behaviour between females with calves and those without, may reveal a greater distinction between male and female dive behaviour since females with calves may have to balance limiting their deep zone diving because of the aerobic capabilities of their young, while still foraging enough to support lactation costs. Future satellite tagging studies should continue to monitor changes in dive behaviour that may coincide with changes in foraging behaviour or ice availability and quality. In conclusion, it appears that investigating diet through chemical techniques such as stable isotopes (Watt et al. 2013) can provide congruent information and inform predictions of dive behaviour for marine organisms.

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Chapter Six

General Discussion

The thesis evaluated the diversity in behaviour and diet across the narwhal's geographic distribution, which informs on the adaptive potential of the species. I used a combination of techniques, including stable isotope and fatty acid analyses to investigate narwhal diet, and satellite tracking to assess foraging behaviour. Although stable isotope and fatty acid analysis has been investigated on a small number of narwhals previously (Hobson and Welch 1992, Dietz et al. 2004, Thiemann et al. 2008), they have never been used to determine primary prey components, assess both intra- and inter-annual changes in diet, compare diet of males and females, or compare foraging behaviour among the world's three narwhal populations. Satellite tagging has been conducted on narwhals for the last thirty years (Martin et al. 1994, Dietz and Heide-Jørgensen 1995, Heide-Jørgensen and Dietz 1995, Dietz et al. 2001, Heide-Jørgensen et al. 2002, Laidre et al. 2002, Heide-Jørgensen et al. 2003, Laidre et al. 2003, Laidre et al. 2006, Laidre and Heide-Jørgensen 2007, Dietz et al. 2008, Westdal et al. 2013, Heide-Jørgensen et al. 2014), but very few tagging studies include narwhals from the East Greenland (EG) or Northern Hudson Bay (NHB) populations. An understanding of dive behaviour in all populations was needed to determine how flexible narwhals are in their foraging behaviours.

A difference in diet among the world's three narwhal populations highlighted the importance of evaluating all populations before making assumptions about the species as a whole. Narwhals from each population foraged on similar prey but appeared to have

specific preferences unique to each population, which had never been investigated before. Particularly important was the finding that capelin (*Mallotus villosus*) may be a prey item of narwhals from the EG population who appear to consume a greater proportion of pelagic prey than narwhals from other populations. Capelin are known to be increasing in the Arctic environment (Gaston et al. 2003, Gaston et al. 2012, Provencher et al. 2012), and may be filling a void left by a declining Arctic cod (*Boreogadus saida*) population, whose decline is likely linked to an increase in the length of the open water season. Arctic cod is an important prey for narwhals from the Baffin Bay (BB) population (Finley and Gibb 1982, Laidre and Heide-Jørgensen 2005), but given that their EG counterparts are able to forage and thrive on capelin, there is a chance they may adapt to this change in the food web. From an optimal foraging perspective, capelin and Arctic cod have a similar caloric value and could meet the energetic needs of narwhals (Birkhead and Nettleship 1987); however, overall lipid content of prey is also important, since lipids provide the densest form of energy (Parrish 2013), which is particularly important for Arctic marine mammals that are adapted to seasonal food shortages (Bluhm and Gradinger 2008). Thus, future research may want to compare the lipid content of Arctic cod and capelin and determine the energetic constraints associated with capturing and metabolizing the different prey, as this will effect whether or not capelin is an optimal prey (Schoener 1971). In addition, more information on the overall abundance and distribution of these prey, using fish catch statistics or trawling efforts, may also be important for determining which is optimal, since time allocation and patch choice are also facets of optimal foraging theory (Pyke et al. 1977).

An evaluation of long-term diet trends over multiple decades provided information about narwhal's adaptability to annual changes in ice conditions resulting in an altered food web. Greater changes in diet were evident in the NHB population, whose distribution is the most southern of the world's populations and is experiencing the greatest reduction in ice cover (Tivy et al. 2011). An interesting result of this study was that capelin's importance in narwhal diet may have actually decreased in BB, which is counter to predictions based on an Arctic food web in transition from being dominated by Arctic cod to capelin (Gaston et al. 2003, Gaston et al. 2012, Provencher et al. 2012). However, changes seemed to also be related to a change in migration patterns likely related to ice changes. Changes in distribution and migration patterns have ramifications for the entire food web, and may also alter food availability at certain times of the year. A change in food availability may mean optimal food is not available at all times. Future research could investigate the casual linkages between timing of food availability and the need for lipid-rich food during key life-history stages, such as periods of gestation and lactation for females.

Foraging behaviour shows heritable variation (Pyke et al. 1977) and may also be learned, passed down through generations. I found some evidence that narwhals do not display an obvious matrilineal social structure since there was no correlation between the genetic and fatty acid matrices, even when only the closest relatives were included in the analysis. Alternatively, narwhals may form a fission-fusion social structure, which is common in organisms that display a relatively low risk of predation, and deal with patchy resource distribution (Wrangham 1980, Connor et al. 2000). Although predatory pressures on narwhals have historically been quite low, an Arctic with less sea ice is

allowing greater access for predators such as the orca (*Orcinus orca*) (Higdon and Ferguson 2009, Higdon et al. 2012). An increase in predatory pressure may result in a change in narwhal organization, as smaller groups may attract less attention, or alternatively, large groups may be able to scatter and confuse oncoming predators (Landeau and Terborgh 1986, Inman and Krebs 1987). Either way, a study investigating social structure in all three narwhal populations would be useful, given that whales in the different populations forage differently, experience varying levels of predation, and inhabit different marine ecosystems. Future studies may want to examine dietary markers in social groupings of narwhals in each population by collecting biopsy samples from all members of an individual cluster (Marcoux et al. 2009). This would provide more concrete evidence about the genetic relatedness of the clusters as well as the similarity in diet of members of these tight knit groupings. Furthermore, tagging studies, that specifically tag a few members of the same cluster within each population, could evaluate if clusters remain as a cohesive unit throughout the year. Evaluation of population level differences in narwhal social organization may be useful for monitoring changes in behaviours and predicting behavioural adaptability with changing climate, predatory pressure, and seasonal ice regimes.

A study evaluating differences in dive behaviour among narwhals in all populations had never been conducted and was required to evaluate and corroborate findings from stable isotope analysis that found individuals in the different populations ate different prey. The difference in diet among populations was also evident in individual narwhal's dive behaviour. For instance, narwhals displaying a more pelagic stable isotope value spent a greater proportion of time in the pelagic regions, while others

spent greater time at deep depths likely foraging on bottom-dwelling organisms. Differences in diet among the world's three narwhal populations, as well as distinct differences in the area of the water column that individuals in the different populations use, suggests there may be some specificity in their niches. Historically, had these populations overlapped geographically, they may have specialized in different areas of the habitat to reduce intraspecific competition depending upon the season and food availability. A future climate change scenario, which predicts an increase in southern competitors in the Arctic environment (Moore and Huntington 2008) and a potential competitive interaction between beluga whales (*Delphinapterus leucas*) and narwhals both foraging on capelin (*Mallotus villosus*) (Marcoux et al. 2012, Watt et al. 2013), may require that animals divide up the niche to ameliorate competitive pressures. Narwhals may be able to adapt to these changes better than previously thought since animals within the three populations are already using different aspects of their environment.

Fossil evidence from a closely related species, known as *Bohaskaia monodontoides*, has shown that narwhals and belugas may have adapted to living in an Arctic environment only recently on an evolutionary time-scale (Vélez-Juarbe and Pyenson 2012). The scientists surmise that the northern shift of narwhals may have been related to oceanographic changes that affected the entire marine food chain and resulted in narwhals being displaced further north to either evade competition or follow a preferred food source (Vélez-Juarbe and Pyenson 2012). Thus, it does appear that historically narwhals, or at least their close relatives, were able to adapt to a changing environment. Behavioural changes, which do not necessarily require a change in genetic composition, may occur at a fast rate and complement the quickly changing habitat.

However, genetic changes may take generations. Current changes in climate are occurring at an unprecedented rate and it is predicted that animals would need to speed up their rate of evolution more than 10,000 times to match projected climate changes (Quintero and Wiens 2013).

Narwhals, along with other Arctic marine mammals are facing a number of threats from human induced activities, including climate change, environmental contaminants, offshore oil and gas activities, shipping, hunting, and increases in commercial fishing (Huntington 2009). Climate change, the most complex of these threats, has the largest potential negative impact on Arctic marine mammals and is the most difficult to address because it requires world wide attention (Huntington 2009). Climate change will impact all facets of the narwhal's life cycle and foraging ecology is only one aspect. Narwhals are identified as being the most vulnerable Arctic cetacean to climate change, and diet diversity was only one component of this analysis (Laidre et al. 2008). Narwhals were also highly sensitive to other factors, such as their population size, distribution, site fidelity, and habitat specificity (Laidre et al. 2008). Arctic marine mammals are also negatively impacted by ice entrapments events. These events result from rapid changes in weather causing punctuated changes in ice dynamics and can result in leads and cracks in the ice freezing over and preventing access to air (Siegstad and Heide-Jørgensen 1994, Watt and Ferguson 2011). Although ice entrapment events are natural and likely play a role in population regulation, the incidence of these events may increase with changing climate (Laidre et al. 2012). In 2008, the largest documented narwhal ice entrapment event occurred near the community of Pond Inlet, Nunavut and over 1000 narwhals were believed to have perished as a result (Watt and Ferguson 2011). These large mortality

events have the potential to have major impacts on the overall population size and may affect specific ontogenetic groups more than others. An increased resilience to changing food webs will not be the only predictor of how narwhals will fare in the face of a changing climate; how they respond to other consequences of climate change, increased industrial activities in their preferred habitats, increased predation from southern predators, and increased competition from southern cetaceans and fisheries alike, will play an equally large role in how they cope with the future.

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