MOVEMENT AND DIET OF HUMPBACK WHALES (*MEGAPTERA NOVAEANGLIAE*) IN RELATION TO CAPELIN *(MALLOTUS VILLOSUS*) OFF THE EAST COAST OF NEWFOUNDLAND

By

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A thesis submitted to the Faculty of Graduate Studies of

The University of Manitoba

In partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

Department of Biological Sciences

University of Manitoba

Abstract

Knowledge of critical foraging areas in time and space, of large marine predators are important to inform management plans for shipping lanes and conservation programs such as marine protected areas. An important foraging ground for humpback whales (*Megaptera novaeangliae*) is coastal Newfoundland. The goal of this study was to investigate the foraging movements and site fidelity of humpback whales at varying spatial scales, as well as diet in relation to their primary prey, capelin (*Mallotus villosus*), on their summer foraging grounds off the east coast of Newfoundland. In this study, I determined that humpback whale movement patterns within their Newfoundland foraging grounds were associated with the availability of capelin. At the regional scale, humpback whales were consistently abundant within bays when capelin was present. At the bay scale, humpback whale presence was influenced by the timing of spawning, rather than specific capelin shoal characteristics, and individual humpback whales returned to a small area (10 km^2) centered on a cluster of capelin deep-water spawning sites. Using stable isotope analysis, I found minimal dietary niche overlap between years (9%). These differences, however, were driven by inter-annual variation in prey $\delta^{13}C$ values and, thus, diet reconstruction resulted in capelin/herring (*Clupea harengus harengus*) comprising > 90% of humpback whale diet in both years. Together, our findings suggest that persistent capelin deep-water spawning sites may be important foraging areas for humpback whales in coastal Newfoundland.

Acknowledgements

Firstly, I would like to extend the greatest thank you to my advisor, Dr. Gail Davoren, who provided guidance and support since I began in her lab. Your enthusiasm and thoroughness in research is contagious. Thank you for providing me with all the opportunities to succeed in this degree and in life. I will miss the late nights of drinking apple cider and talking about life at the Auk house.

I would also like to thank my committee Dr. Alejandro Costamagna, Dr. Dave Yurkowski and Dr. Jim Roth for your insightful input and encouragement. Thank you to Jim for the use of his lab and stable isotope analysis guidance.

The biggest thank you to all of my lab mates throughout the years, you guys are all incredible and have such unique amazing skills. Thank you to Paloma Carvalho, Alison Leopkky, Julia Gulka, Edward Jenkins, Muriel Magnaye, Laurie Maynard, Mikala Epp, Marissa Berard, Wesley Ogloff, Kevin Scharffenberg, Laura Bliss and Ashley Tripp for support in the field as well as in the lab. Thank you to the Stable Isotope in Ecology group for the great discussions and feedback. Special thank you to the crew of the *Lady Easton*, the best people to work with. I am indebted to the whale crew, Larry Easton and Jeffery Chaulk for all of your patients and enthusiasm.

Thank you to my family, especially my parents, Gail-Ann Breese and Curtis Johnson, for supporting me in everything I do and encouraging me to follow my passion. Thank you to my partner in crime, Michael Gross, for putting up with my shenanigans.

Principal funding was provided by Natural Science and Engineering Research Council of Canada Discovery and Ship Time Grants (GKD), the University of Manitoba Faculty of Science Field Work Support Program Grants (GKD) and Graduate Enhancement of Tri-Council Stipends funding. Additional funding for stable isotope analysis was provided by World Wildlife Fund-Canada (GKD).

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General Introduction

Foraging theory predicts that predators will attempt to maximize their net energy gain while foraging, by maximizing energy consumed and minimizing energy lost searching for and handling (i.e., pursue, subdue and ingestion) prey, to ultimately maximize fitness (Krebs and McCleery 1984). Therefore, the selection of prey types and prey patches is highly influenced by the movement and abundance of profitable prey types. Indeed, the diet model predicts that the profitability of an encountered prey type is a trade-off between the energy gained and energy lost during searching and handling (Krebs and McCleery 1984). Therefore, the variety of prey types consumed is determined by this trade-off, which is influenced by the abundance of each prey type in the area (Krebs *et al.* 1977; Krebs and McCleery 1984). Similarly, predators are predicted to be associated with larger and more dense prey patches in an area (Hazen *et al.* 2009; Burrows *et al.* 2016) and spend most of their time in patches with the highest abundance of prey (Smith and Dawkins 1971). Predators, however, face resource depression within a patch, whereby prey density decreases due to consumption by predators or evasive maneuvers by prey (Charnov *et al.* 1976). The Marginal Value Theorem predicts that if patches are variable in prey density, a predator should leave a patch once energy intake is equal to the average that is available in the environment (Stephens and Krebs 1986). Therefore, predator movement among available prey patches is driven by the abundance and density of prey patches.

Not all individuals within a population consume the same prey types or the same proportion of prey types. Trophic niche breadth of a population can be determined by quantifying the number of prey species/types consumed, proportion of each prey species consumed, number of trophic levels consumed and geographic foraging area of multiple individuals within a population (Bearhop *et al.* 2004). A population may have a narrow dietary

breadth, with one or a few prey types consumed, or a broad dietary breadth, with many prey types consumed. Diet models predict that when abundance of the most-profitable prey is high, predators will have a narrower dietary niche breadth, as they do not have to search for the most profitable prey types. Alternately, predators will have a broader dietary niche breadth under lower prey abundance conditions, as they will consume all prey types encountered to reduce energy expenditure searching for the most profitable prey types. The most profitable prey types, however, may differ among individuals within a population often resulting in different diets among age classes, sex or with morphological variation (i.e. individual specialization; Bolnick *et al.* 2003).

Stable Isotopes

Tissue samples can be used to determine isotopic niche breadth, as well as reconstruct dietary composition using stable isotope ratios (Bearhop *et al.* 2004; Boecklen *et al.* 2011; Stock and Semmens 2013). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are common proxies used to reconstruct diet (Bearhop *et al.* 2004). In marine environments, variation in $\delta^{13}C$ is observed between pelagic and benthic (France 1995) as well as inshore and offshore prey types (Hobson *et al.* 1994), while $\delta^{15}N$ typically increases 3-5‰ between each trophic level (Peterson and Fry 1987). When using stable isotopes for dietary reconstruction, there are many considerations to ensure appropriate interpretation. First, tissue lipid content is important and often overlooked. Lipids are depleted in ^{13}C compared to proteins and carbohydrates and, thus, have lower $\delta^{13}C$ values (DeNiro and Epstein 1977). As lipid concentrations vary among species, as well as tissues within a species due to reproductive status, age, life stage, life history or feeding strategies (Bowen *et al.* 1987; Fagan *et al.* 2011), lipid extraction is necessary to standardize comparisons

and minimize inter- and intra-individual variation in $\delta^{13}C$ (Post *et al.* 2007). The general rule is to extract lipids when the C:N ratio is ≥3.5 (Post *et al.* 2007); however, others have cautioned against this rule, because they have observed a significant increase in $\delta^{13}C$ even after lipid extraction of presumed lean tissue (≤3.5 C:N; Lesage *et al.* 2010; Yurkowski *et al.* 2015). Two common methods used for standardizing lipids are mathematical normalization and chemical lipid extraction. Mathematical normalization uses the C:N ratio to normalize $\delta^{13}C$ and many equations have been generated that attempt to generalize C:N ratios across a wide range of species and tissue types (McConnaughey and McRoy 1979; Post *et al.* 2007; Logan *et al.* 2008). The general equations, however, are not as good predictors for lipid-free $\delta^{13}C$ relative to more species- and tissue-specific equations (Yurkowski *et al.* 2015). Due to insufficient species- and tissue-specific equations, chemical lipid extraction is often used to standardizing $\delta^{13}C$ (Dobush *et al.* 1985; Sardenne *et al.* 2015; Yurkowski *et al.* 2015).

Second, turnover rates of the targeted tissue must be known to determine the period over which the consumer's isotopic ratios reflect the averaged ratios of prey consumed (Bearhop *et al.* 2002). Tissues that are more metabolically active have a shorter turnover rate and reflect the averaged diet over a shorter period (Tieszen *et al.* 1983). Finally, isotopic ratios of prey must be known to accurately reconstruct diets, but prey isotopic ratios are typically modified during incorporation into the consumer tissue. Therefore, a diet-tissue discrimination factor, which is may be species- and tissue-specific (Browning *et al.* 2014; Hobson and Clark 1992; Tieszen *et al.* 1983), is needed to reconstruct dietary proportions in consumers based on stable isotope ratios.

Study Species

Humpback whales (*Megaptera novaeangliae*) are found in all oceans in both the northern and southern hemispheres (Winn and Reichley 1985). They occupy subtropical and tropical waters to breed during winter months and during polar summers can migrate over 8000 km toward the poles to forage on high density aggregations of prey (Dawbin 1966; Stone *et al.* 1990). Individuals within a breeding population may migrate to different feeding grounds where they may converge with other populations (Katona and Beard 1990), but often show high site fidelity to breeding and foraging grounds (Katona and Beard 1990; Clapham *et al.* 1993; Stevick *et al.* 2006; Acevedo and Mora 2014). Determining site fidelity is possible due to distinct individual-level morphological traits (e.g., fluke pigmentation, dorsal fin shape and peduncle knobs), which have been photographed and used to identify returning individuals to an area (Blackmer *et al.* 2000).

Humpback whales are a long-lived species (>48 years; Chittleborough 1965), that delay sexual maturity for four-eight years (Clapham 1992), give birth to a single calf every two-five years (Clapham and Mayo 1990), have long gestation periods (e.g., 11-12 months) and once born, there is a high level of parental care, with the calf remaining with their mother for 10-12 months (Chittleborough 1958). All populations were hunted until 1956, when all commercial whaling was banned in the North Atlantic due to depletion of populations (Winn and Reichley 1985). The population has increased from ~6,000 in the 1980s (Winn and Reichley 1985) to 10,600-10,752 in the late 1990s (Smith *et al.* 1999).

As humpback whales forage underwater, it is difficult to determine the prey composition of their diet. Dietary studies on highly mobile marine species, including humpback whales, are especially challenging, because they occupy large regions as foraging grounds. Owing to these difficulties, a variety of techniques have been developed to investigate the diets of cetaceans.

Initially, researchers focused on dead whales and examined stomach contents (Fitch and Brownell 1968). More recently, researchers have used biopsies of skin and blubber to determine trophic niche and breadth, as well as reconstruct dietary composition using stable isotope ratios (Witteveen *et al.* 2011). Indeed, there are a few large-scale studies that investigated stable isotopes of humpback whales for entire ocean basins, such as SPLASH (Structure of Populations, Levels of Abundance, and Status of Humpback whales) in the North Pacific basin (Calambokidis *et al.* 2008) and YONAH (Years of the North Atlantic Humpback) in the North Atlantic basin (Smith *et al*. 1999).

Study site

Newfoundland and Labrador represents an important foraging ground for humpback whales in the North Atlantic (Katona and Beard 1990). The primary prey species in Newfoundland during the summer is capelin (*Mallotus villosus*), which arrive in coastal areas of Newfoundland to spawn at deep-water spawning sites (15-40 m) and beach spawning sites (Davoren *et al.* 2006, 2008). Pre-spawning shoals of capelin tend to be smaller, ephemeral and evasive under predatory attacks (Davoren *et al.* 2006). Once capelin arrive at spawning sites, they form larger, dense spawning aggregations that are highly stationary and have minimal responses to predatory attacks (Davoren *et al.* 2006; Penton *et al.* 2012). High densities of marine predators including birds, fish and baleen whales aggregate at these spawning sites of capelin in shallow water (15-40 m) on the northeast Newfoundland coast (Notre Dame Bay; Davoren 2007; 2013). Capelin are thought to be the main prey of humpback whales in coastal Newfoundland, as evidenced by the association of humpback whales with capelin aggregations (Whitehead *et al.* 1980). Indeed, the number of humpback whales increases as the abundance and

size of capelin shoals increase in coastal areas (Piatt *et al.* 1989; Whitehead 1983; Piatt and Methven 1992). In coastal Newfoundland, humpback whales exhibit a northward movement along the coast throughout the summer (Whitehead *et al.* 1982). Foraging behaviour studies on humpback whales in coastal Newfoundland, however, have not been conducted since the capelin population collapsed in the early 1990s (Buren *et al.* 2014), which was associated with 3-4 week delays in the timing of spawning (Carscadden *et al.* 2001). The impact of these changes on foraging movements of humpback whales within this foraging ground is unclear.

Objectives

The overall objective of this thesis is to study the influence of capelin availability on the foraging ecology of humpback whales in coastal Newfoundland during their summer foraging season. My first objective was to investigate the foraging movements and site fidelity of humpback whales within their North Atlantic foraging grounds in relation to their preferred prey fish, capelin (Whitehead *et al.* 1980; Piatt *et al.* 1989; Piatt and Methven 1992) at both the regional scale on the east Newfoundland coast, and the bay scale, within Notre Dame Bay (Chapter 1). To do this research, I identified individuals from fluke photographs to examine population- and individual-level movement along the east coast, and I used at-sea surveys coupled with fluke photographs at a biological hotspot to explore the importance of capelin spawning sites for humpback whales. My second objective was to investigate the diet of humpback whales on their summer foraging grounds off the northeast Newfoundland coast during July-August, 2016 and 2017 using stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in whale skin, as well as to explore methods in tissue processing for stable isotope ratios of humpback whale skin (Chapter 2). To do this research, I collected tissue samples from humpback whales and potential

prey types during summers of 2016 and 2017. Lipid extracted and non-lipid extracted humpback whale skin samples were used to produce a lipid normalization equation for humpback whale skin. This study provides new insights on the importance of spawning capelin for foraging humpback whales in Newfoundland.

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Chapter One: Movement patterns of humpback whales (*Megaptera novaeangliae*) in relation to capelin (*Mallotus villosus*) along the Newfoundland East Coast

Abstract

Animals typically aggregate in areas of high prey densities to maximize foraging efficiency, resulting in temporally and spatially important foraging areas for populations. An important foraging ground for humpback whales (*Megaptera novaeangliae*) is coastal Newfoundland. The objective of this study was to investigate the foraging movements and site fidelity of humpback whales in relation to their primary prey fish, capelin (*Mallotus villosus*) at both the regional scale on the eastern Newfoundland coast, and the bay scale, within Notre Dame Bay. To conduct this research, I combined scientific cruise data with citizen science reports of humpback whale presence/abundance and availability of spawning capelin in four bays along the east coast of Newfoundland, along with fluke photographs for individual identification. I found population- and individual-level movement northward along the east coast, with speeds ranging from 2.1-38.4 km/day. At the regional scale, humpback whales were consistently abundant within bays when spawning capelin were available, but whales often arrived later than capelin in northerly bays. Within Notre Dame Bay, humpback whale individuals returned annually (22% return rate of 1679 ± 155 individuals) to a known fine-scale foraging site associated with a cluster of capelin deep-water spawning sites. Annually, whale presence within this foraging site was not influenced by capelin shoal characteristics (e.g., number of shoals, shoal area, shoal density and average density/survey), unlike results of other studies, but instead was influenced by date of capelin spawning. Together, our findings suggest that persistent capelin deep-water spawning sites may be important foraging areas for humpback whales in coastal Newfoundland.

Introduction

Movement of populations and individuals are driven by numerous external and internal factors (Nathan *et al.* 2008). External factors may include biotic factors, such as predation and prey availability, and/or abiotic factors, such as temperature, while internal factors may include amount of energy stored or breeding status (Madsen and Shine 1996; Corkeron and Connor 1999; Avgar *et al.* 2014; Somveille *et al.* 2015). A common animal movement pattern often involves large-scale travel from breeding areas to highly productive foraging grounds during the non-breeding season (Urquhart and Urquhart 1978; Stutchbury *et al.* 2009; Hart *et al.* 2012; Hedd *et al.* 2012). Although such long-distance movements may be energetically costly, high foraging efficiency resulting from high prey density on foraging grounds outweighs the costs, allowing replenishment of energy reserves after breeding (Lockyer 1986; Draulans 1987; Niaess *et al.* 1998; Tynan 1998). Within foraging grounds, smaller-scale movements are most likely driven by regional and local prey distribution and density. Indeed, foraging theory predicts that predators should select prey patches that maximize net energy gain (Stephens and Krebs 1986) and, thus, should leave a patch once the net energy intake is equal to the average of the environment (Stephens and Krebs 1986). Prey density in a patch may become depleted due to exploitation by other predators or movement of prey out of the patch, either evasive or otherwise (Charnov *et al.* 1976).

A marine mammal species that undertakes a large-scale movement to foraging grounds is the humpback whale (*Megaptera novaeangliae*), typically breeding in warmer waters of low latitudes (e.g., West Indies, Brazil, Hawaii, Australia) and travelling to cooler, productive high-latitude foraging grounds (e.g., Antarctic, Alaska, Newfoundland; Dawbin 1966; Kennedy *et al.* 2014). As little foraging occurs during the energetically costly breeding season (Christiansen *et*

al. 2016), foraging grounds are vital areas for humpback whales to replenish energy stores between reproductive events. Humpback whales are known to have high annual fidelity to regional foraging grounds (Katona and Beard 1990; Clapham *et al.* 1993; Stevick *et al.* 2006). Many studies have used photo-identification or satellite tags to document regional-scale movement of individual humpback whales in foraging grounds, but none have coupled movement patterns with prey availability (Whitehead *et al.* 1982; Dalla Rosa *et al.* 2008; Kennedy *et al.* 2014; Curtice *et al.* 2015). Humpback whale aggregations, however, have been associated with regionally large aggregations of prey, including forage fish in the Northwest Atlantic (Piatt *et al.* 1989; Piatt and Methven 1992; Friedlaender *et al.* 2009; Hazen *et al.* 2009) and Northeast Atlantic (Volkenandt *et al.* 1005), along with forage fish and krill in Northeastern Pacific (Witteveen *et al.* 2008; Witteveen *et al.* 2015; Burrows *et al.* 2016) and krill in Antarctica (Nowacek *et al.* 2011; Curtrice *et al.* 2015; Friedlaender *et al.* 2016). Within a region, foraging humpback whales have also been associated with certain characteristics of prey shoals, such as near-surface, dispersed krill patches at night in the Antarctic (Friedlaender *et al.* 2016), larger sandlance shoals (i.e., shoal height and shoal area) during the day in the Gulf of Maine (Hazen *et al.* 2009, Friedlaender *et al.* 2009) and high density krill patches in Alaska (Burrows *et al.* 2016), presumably because these shoal types require less energy to either search for or catch and, thus, maximize foraging efficiency.

Newfoundland and Labrador represents an important foraging ground for humpback whales in the North Atlantic (Katona and Beard 1990). The primary prey species in Newfoundland during the summer is capelin (*Mallotus villosus*; Chapter 2), which arrive in coastal areas of Newfoundland to spawn at deep-water (15-40 m) and beach spawning sites (Davoren *et al.* 2006, 2008). Upon arrival of capelin in coastal Newfoundland, they are present

in small ephemeral pre-spawning shoals that are evasive to predatory attacks and move northward along the coast toward suitable spawning habitats (Davoren *et al.* 2006). Once these shoals reach a suitable spawning site, they form larger, dense shoals that are highly stationary and have minimal responses to predatory attacks (Davoren *et al.* 2006; Penton *et al.* 2012). High densities of marine predators including birds, fish and baleen whales aggregate at these capelin spawning sites in shallow water (15-40 m) on the northeast Newfoundland coast (Notre Dame Bay; Davoren 2007; 2013). Humpback whales are often associated with capelin aggregations in coastal Newfoundland (Whitehead *et al.* 1980; Whitehead and Carscadden 1985). Indeed, the number of humpback whales increases as the number and size of capelin shoals increase in coastal areas (Whitehead 1983; Piatt *et al.* 1989; Piatt and Methven 1992). In Newfoundland, humpback whales exhibit a northward movement along the coast throughout the summer, which was suggested to be due to high abundances of capelin shoals becoming available in northern regions later in the season (Whitehead *et al.* 1982; Whitehead *et al.* 1980). Foraging behaviour studies on humpback whales in coastal Newfoundland, however, have not been conducted since the capelin population collapsed in the early 1990s (Buren *et al.* 2014), which was associated with 3-4 week delays in the timing of spawning (Carscadden *et al.* 2000). The impact of these changes on foraging movements of humpback whales within this foraging ground is unclear.

The goal of this study is to explore the foraging movements and site fidelity of humpback whales within their North Atlantic foraging grounds in relation to their preferred prey, capelin (Whitehead *et al.* 1980; Piatt *et al.* 1989; Piatt and Methven 1992; Chapter 2) at both a broader regional scale on the eastern Newfoundland coast and at a finer bay scale within Notre Dame Bay. As similar studies were conducted in the late 1970s and early 1980s (Whitehead *et al.* 1980, 1982; Piatt and Methven 1992), my secondary goal was to compare our results with these

previous studies, given recent major changes in capelin biology (Carscadden *et al.* 2000; Buren *et al.* 2014). I hypothesized that humpback whale presence in certain bays and movement along the east coast are associated with the timing of inshore presence of spawning capelin within each bay (H1). At both the regional- and bay-scales, I predicted that the presence of humpback whales will coincide with the inshore presence of spawning capelin. I also hypothesized that capelin shoal characteristics (e.g., number, density, area (height x width) within bays influenced the presence of whales (H2) and predicted that the number of shoals in Notre Dame Bay coincided with whale presence. Finally, I hypothesized that humpback whales will exhibit site fidelity to known foraging areas within bays (H3) and predict individual humpback whales will return annually to persistent capelin deep-water spawning sites in Notre Dame Bay.

Methods

To examine regional-scale timing of presence of humpback whales (H1), I used Facebook posts from whale tour companies to estimate the date ranges when humpback whales were consistently abundant within Witless Bay and northern Trinity Bay along the east coast of Newfoundland, which were combined with regular monitoring of whale presence within northern Bonavista Bay and Notre Dame Bay (Fig. 1). The dates of consistent abundance were linked to the timing of inshore presence of spawning capelin within each bay, based on Twitter and citizen science posts on *eCapelin.ca*, as well as regular monitoring of capelin presence inshore within northern Bonavista Bay and Notre Dame Bay. Additionally, to examine movement patterns of individual whales along the coast (H1), I combined a photo-identification catalogue from July-August, 2016 and 2017 within Notre Dame Bay with pictures posted by whale tour operators in Witless Bay and Trinity Bay. To examine prey characteristics (e.g., number of prey shoals, shoal

height and width (shoal area)) associated with humpback whale presence within Notre Dame Bay (H2), fine-scale surveys (15-25 km) were repeatedly conducted throughout July-August, 2009, 2010, 2012, 2014-2017 typically every 2-7 days (n = 3-7 surveys/yr). These surveys were centered over a cluster of four annually persistent deep-water (15-40 m) spawning sites of capelin (Penton and Davoren 2012), which is a known biological hotspot, where high abundances of marine predators aggregate to forage on spawning capelin, including humpback whales and many breeding and non-breeding seabirds (Davoren 2007; 2013). To test whether humpback whales exhibit site fidelity to this hotspot in Notre Dame Bay (H3), I used a photo-identification catalogue of individuals compiled from 2003-2017.

Regional scale

To quantify the date ranges when humpback whales were consistently abundant within Witless Bay and Trinity Bay, I used Facebook posts from March-September, 2016 and 2017 from four Newfoundland whale tour companies: three located near Witless Bay (Gatherall's Puffin Whale Watch, O'Brien's Whale and Bird Tours, Molly Bawn Whale and Puffin Tours) and one within Trinity Bay (Sea of Whales Adventure). Observations of these tour operators were used due to their consistent effort (i.e., daily tours from March-September) and consistent reporting of humpback whale sightings (i.e., 1-3 Facebook posts per day). From Facebook posts, I defined the timing when humpback whales were consistently abundant in each bay as the dates when ≥ 10 humpback whales were first observed for at least 3 days in a row to the date when they were no longer observed in high numbers (≥ 10 individuals). Consistent abundance was used to ensure the population of humpback whales arrived in the bay as opposed to one or two individuals being present. Facebook posts by tour companies were used as proxies for presence

in the bay they are located. In Bonavista Bay and Notre Dame Bay, the date ranges when humpback whales were consistently abundant during July-August were determined during the repeated fine-scale surveys within the biological hotspot (see details below). Survey data were supplemented with observations of whale presence collected every 1-2 days during other boat-based research near the survey area as well as land-based observations.

The range of dates when spawning capelin were available inshore within Witless Bay, Conception Bay, Trinity Bay and Bonavista Bay was determined by following #capelinroll and other variations (e.g., #caplinroll, #capelinroll2016, #capelinroll2017) on Twitter (2016, 2017) as well as uploaded observations to a citizen science tool to identify the spatial extent of capelin spawning beaches along the Newfoundland coast (*eCapelin.ca*; 2016, 2017). Again, both Twitter and *eCapelin.ca* were used due to the consistent effort of reporting from citizens about the presence of spawning capelin at beaches (Fig. 1). Although population density of citizens varied throughout bays, most capelin spawning beaches are reliably visited by locals. Within each bay, I defined the date range that spawning capelin were reported at beaches, based on pictures or specific descriptions (i.e., "capelin beach spawning today") within posts. This date range was used as a proxy of the inshore presence of spawning capelin. In Notre Dame Bay, the inshore presence of capelin, along with timing of spawning was determined through monitoring known beach spawning sites daily and deep-water spawning sites at 3-5 day intervals, following methodology in Crook *et al.* (2017).

To quantify movement patterns of individual humpback whales along the Newfoundland coast (H1), I used photographs of the underside of flukes from individual whales (Katona *et al.* 1979). Photographs were collected opportunistically within and nearby the biological hotspot in Notre Dame Bay from 2003-2010, with more rigorous and targeted photograph collection during

2011-2017. Photographs within two other bays were obtained from posts to Facebook and Flickr accounts by two tour companies throughout May-September, 2016 and 2017: Witless Bay (Molly Bawn Whale and Puffin Tours) and Trinity Bay (Sea of Whales Adventures). I focused on these two tour companies because they conducted daily tours and collected multiple fluke photographs per day when possible, which were made available to the public. Fluke photographs within each bay were first ranked from 1-5 based on the percentage of black on the underside of the fluke (1 = 0%, 2 = 25%, 3 = 50%, 4 = 75%, 5 = 100%; Allen *et al.* 1994), and then were further sorted by patterns of fluke pigmentation to more efficiently identify individuals within each bay. Photographs were then compared among bays within each year (i.e., 2016, 2017) to examine individual-level movement patterns. Individual-level speed of movement was estimated by measuring the distance (± 1 km) between each sighting of an individual using ArcGIS (version 10.3, ESRI), as the most direct route between sightings without going over land, and dividing by the number of days between sightings of that individual.

Bay scale

To examine the prey characteristics associated with humpback whale presence within bays (H2), a fine-scale survey (~25 km) was repeatedly conducted typically every 2-7 days from July-August, 2009, 2010, 2012, 2014-2017 (n = 3-7 surveys/yr) within the biological hotspot in Notre Dame Bay (Davoren 2007, 2013; Fig 1). During surveys, vessel speed was maintained between 9 and 11 $km \cdot h^{-1}$, while continuous counts of whales were made from the bridge of the ship, conforming to a line-transect method. Although counting methods for marine mammals generally involve higher vantage points (Moulton and Mactavish 2004), consistent methods resulted in systematic biases over all surveys. Conditions influencing animal detection, including

sea state (Beaufort wind force scale), cloud cover, visibility (e.g., fog, rain), glare and approximate wind speed and direction were recorded at the start of the survey and as conditions changed. To conduct surveys conditions had to be good, therefor no surveys were eliminated due to low detection. Counts and species name were entered directly into a laptop (Birds and Beasty Counter Software, 1998; D. Senciall, Fisheries and Oceans Canada, version 1.0), which was connected to the GPS system on the ship for the software to append a latitude, longitude and time to each count entered.

During the survey, continuous measurements of capelin (prey) density were collected simultaneously along the survey, using a Biosonics DTX 6000 scientific hydroacoustic system (BioSonics Inc., Seattle, Washington). The sounder was operated through a 70 kHz split-beam transducer (5.5° full narrow beam width, 15° full wide beam width) calibrated with a tungsten carbide sphere and mounted in a towed body. The transducer was towed on the starboard side of the vessel 1 m below the surface, and thus, acoustic signals were not reliable until 3 m. The sounder was operated at 1 ping s^{-1} and pulse duration of 0.4 ms. Raw high resolution hydroacoustic data (s_v, volume backscattering coefficients) were continuously acquired above a threshold of -90 dB. Echoview software (version 4, SonarData, Myriax Software Pty. Ltd., Hobart, Tasmania) was used to analyze the hydroacoustic data. Prior to integration, acoustic signals within 0.5 m of the seabed were omitted if the seafloor could not be distinguished from marine organisms (e.g., side-lobing; Simmonds and MacLennan 2005). To quantify acoustic biomass (area backscattering coefficient, or s_a, $m^2 \cdot m^{-2}$; MacLennan *et al.* 2002), I integrated acoustic signals using a minimum s_v threshold of -80 dB in 100 m segments along the survey. As acoustic signals are primarily due to capelin nearby the spawning sites (Davoren *et al.* 2006), this threshold allowed the detection of single capelin targets in this shallow area (< 50 m), while

filtering out most other noise. I used a published target strength-length relationship for capelin (Rose 1998) along with the average length and mass of capelin captured at the spawning sites over all years (Davoren 2013) to convert s_a into number of fish/m^2 and then to capelin biomass (g/m^2). For each survey, I determined the presence or absence of whales, given the limitations of counting methods, and calculated the average capelin density (g/m^2) for the survey by averaging the capelin density over all 100 m segments.

For each survey, capelin shoals were identified by visually assessing each echogram in Echoview software. Based on capelin-likely s_v thresholds at different depths, along with the distinctive shape of capelin shoals (Davoren *et al.* 2006), I identified capelin shoals within each survey. Shoals were not included if they were near the ocean surface and if there was evidence of diving seabirds to avoid overestimating capelin density. Three characteristics of each capelin shoal were estimated similar to Davoren *et al.* (2006), including: the maximum horizontal length (width, m) and maximum vertical length (height, m) of shoal, along with the acoustic biomass (area backscattering coefficient, or s_a, $m^2 \cdot m^{-2}$; MacLennan *et al.* 2002) of the shoal, which was determined by integrating acoustic signals using a minimum s_v threshold of -70 dB. A higher threshold was used to better define the boundaries of capelin shoals. An estimate of the area of each shoal was calculated by multiplying the height by the width of the shoal. Shoals identified as capelin comprised > 95% of all shoals identified throughout all surveys, suggesting that few other fish prey were available.

Our photo-identification catalogue for Notre Dame Bay (2003-2017) was also used to examine annual return rates and presence of humpback whales at the biological hotspot (H3). This annual return rate is the number of individuals that were observed in one year that were also observed in the previous year (Clapham *et al.* 1993). I also calculated the overall return rate, or

the number of individuals re-sighted in one year from any previous year. Residency was also examined for individuals, which is the number of days between the first and last sighting of an individual within each summer (July-August). A population estimate within Notre Dame Bay was also calculated.

Data analysis

To test H2, the variables quantified for each fine-scale survey included: average capelin density (g/m^2) per survey, total number of capelin shoals, average area (m^2) of capelin shoals, average capelin density (g/m^2) of shoals and humpback whale presence/absence. All prey characteristics were then standardized by subtracting the mean and dividing by the standard deviation for that year to control for variation among years. A generalized linear model with a binomial distribution and a logit link was used to determine which prey characteristics influenced the presence of humpback whales. Prior to running the model, multicollinearity among all fixed effects was first tested. Fixed effects included in the model were the number of days before or after capelin spawned at deep-water sites (date of spawning), number of shoals, shoal density, shoal area and survey density and no random effects were included. Date of spawning was included because changes in prey characteristics are influenced by the timing of capelin spawning (Davoren unpubl. data). Statistical analyses were run in JMP 13.2.0, and all means are reported as mean ± standard error and tested with an α value of 0.05.

The Lincoln-Peterson Index was used to produce a rough estimate of the number of individuals within Notre Dame Bay in a given year (Krebs 1989):

$$N=(n/r)*M$$

Where N is the annual population estimate, n is the total number of individuals photo-identified in the given year, r is the number of individuals re-sighted in that year from previous years and M is the total number of individuals photo-identified prior to the given year.

Results

Humpback whales were consistently abundant first in Witless Bay, on the southeast Newfoundland coast, during both years (2016, 2017). Whales were then consistently abundant in Trinity Bay, on the east coast, and then later in bays farther north during both years (Fig. 2). Whales were consistently abundant at similar dates in Witless Bay in both years, but were consistently abundant for a greater number of days in both Witless Bay and Trinity Bay during 2017 relative to 2016. Humpback whales became consistently abundant later in the more northern Witless Bay, Bonavista Bay and Notre Dame Bay during 2017 compared to 2016 (Fig. 2). The first date of inshore presence of spawning capelin was similar among all bays in both years, with some northern bays reporting earlier presence of capelin relative to more southerly bays. Capelin tended to be available later in the summer, however, in the more northern bays (Fig. 2).

The number of posted fluke pictures of high enough quality to identify humpback whale individuals was 171 in Witless Bay (41 in 2016, 130 in 2017) and 283 in Trinity Bay (233 in 2016, 50 in 2017). These were combined with 136 pictures in Notre Dame Bay (84 in 2016, 58 in 2017). Out of these photographs, 17 individuals were identified in at least two different bays within a year (2016: 15 individuals; 2017: 2 individuals). Individuals were always re-sighted in a more northern bay, indicating that all individuals moved northward over both summers. Given the high variation in the number of days (5-50 days) and distance (39.8-243.9 km) traveled

between sightings of individuals, estimated speeds also varied from 2.1-38.4 km/day (Table 1), with the highest speeds being > 30 km/day.

When examining whether capelin shoal characteristics within Notre Dame Bay influenced whale presence (H2), all fixed effects were included in the generalized linear model as none were correlated. The model including all fixed effects significantly influenced humpback whale presence (χ_5^2 = 21.50, n = 35, p = 0.0007). The presence of humpback whales, however, was not influenced by the number of capelin shoals (χ_1^2 = 1.24, p = 0.26), capelin shoal area (χ_1^2 = 0.49, p = 0.48), capelin density within shoals (χ_1^2 = 0.04, p = 0.84), or average capelin density per survey (χ_1^2 = 0.32, p = 0.57), but was influenced by the date of capelin spawning (χ_1^2 = 13.75, p = 0.0002; Fig. 3).

The annual estimate of the number of humpback whales in the study area, based on fluke photos from Notre Dame Bay, ranged between 1003-2305 individuals with an average of 1679 ± 155 per year (Table 2). Annual return rates, or the number individuals observed in one year that returned in the next year, ranged from 0-7% (Table 3), while overall return rates, or the number of individuals re-sighted in one year from any previous year, reached 22% by the end of the study period (Table 3). Many individuals were identified over multiple years within a small area, whereby 31 individuals were re-sighted in two years, three individuals re-sighted in three years and one individual re-sighted in four years. Individuals were also sighted more than once within a year (2-8 individuals/year) and residency ranged from 1-21 days (7.4 ± 1.0; Table 2).

Discussion

Humpback whales were photographed first in southeastern Newfoundland bays and then more northern bays later in the season, suggesting humpback whales typically move northward

along the east coast of Newfoundland throughout the summer, similar to previous studies in the 1970s (Whitehead *et al.* 1980; Whitehead *et al.* 1982). The timing of consistent abundance of humpback whales (i.e. ≥ 10 whales for ≥ 3 consecutive days) within eastern bays either coincided with or was delayed relative to the inshore presence of spawning capelin at regional scale, supporting humpback whale movement within this foraging ground is influenced by prey availability as previously suggested in other studies (Whitehead *et al.* 1980; Whitehead and Carscadden 1985; Piatt *et al.* 1989; Piatt and Methven 1992). Although capelin shoal size, age class and the number of shoals have been shown to influence humpback whale abundance within bays on the east coast of Newfoundland (Whitehead 1983; Whitehead and Carscadden 1985; Piatt and Methven 1992), capelin shoal characteristics did not influence the presence of humpback whales at a known fine-scale foraging site associated with a cluster of deep-water spawning sites of capelin within Notre Dame Bay. Instead the timing of capelin spawning influenced humpback whale presence at this foraging site and individuals returned to this site over multiple years, indicating that predictable sites where capelin spawning in deep water are likely high quality prey patches for humpback whales.

Many studies have examined regional-scale movement patterns of humpback whales within foraging grounds (Whitehead *et al.* 1982; Dalla Rosa *et al.* 2008; Kennedy *et al.* 2014; Curtice *et al.* 2015), and our maximum movement speeds (32.4-38.4 km/day) were similar or slower than those observed on other foraging grounds (32 km/day; Dalla Rosa *et al.* 2008, 46 km/day; Kennedy *et al*. 2014). Our documented slower speeds, however, may simply indicate that whales remain in certain regions of coastal Newfoundland for longer periods than on other foraging grounds, which is supported by similarly slow speeds previously documented on the east coast of Newfoundland (3.7 km/day; Whitehead *et al.* 1982). Despite a number of studies

examining regional-scale movement patterns of humpback whales on foraging grounds (Whitehead *et al.* 1982; Dalla Rosa *et al.* 2008; Kennedy *et al.* 2014; Curtice *et al.* 2015), none have linked these movements to prey availability. At the population level, the date ranges during which humpback whales were consistently abundant in bays overlapped with the date ranges spawning capelin were available inshore, but whale consistent abundance in northern bays was delayed by 8-20 days relative to the first reports of capelin spawning. Humpback whales were originally thought to follow migrating capelin northward along the coast (Whitehead *et al.* 1980; Whitehead *et al.* 1982). Indeed, tagged spawning capelin were historically shown to arrive inshore in southern bays first and move into northern bays later in the summer (Nakashima 1992) and humpback whales similarly aggregated in southern bays earlier in the season and moved to northern bays throughout the summer (Whitehead *et al.* 1982). Our findings suggest, however, that spawning capelin arrive and become available inshore at similar dates in most bays within a year and, thus, there is no northward movement of spawning capelin for whales to follow. Therefore, humpback whales arriving on the foraging grounds in southerly bays may remain there until the net energy gain is equal to that of the environment, as predicted by prey patch selection theory (Stephens and Krebs 1986), after which they move north later in the season regardless of the earlier inshore availability of capelin.

At the bay scale, humpback whale presence within a known fine-scale foraging site in Notre Dame Bay associated with an annually predictable cluster of capelin deep-water spawning sites (Davoren 2007, 2013) was not influenced by the number of capelin shoals or shoal characteristics. Humpback whale presence, however, was influenced by the initiation of capelin spawning, and the number of capelin shoals was higher and shoals were more dense when humpback whales were present. These findings suggest that whales aggregate during capelin

spawning, which coincides with a greater amount of capelin in the area. Similarly, Whitehead *et al.* (1980) found that humpback whales become abundant once capelin begin spawning in coastal Newfoundland. Additionally, Piatt and Methven (1992) found a threshold response of humpback whales to the number of capelin shoals, whereby whales aggregated in a southern Newfoundland bay when there were ≥ 5 capelin shoals per km. During the summer, capelin behaviour in coastal regions shifts from pelagic, ephemeral, pre-spawning schools to dense, persistent spawning shoals associated with the seabed in < 50 m that are non-evasive to predators (Davoren et al. 2006). Spawning shoals in shallow water represent predictable, high abundance prey patches where many whales aggregate along with other predators (Davoren 2007, 2013), likely resulting in low search effort for prey patches. The possible use of memory to minimize energetically costly search activities is supported by individual whales returning to this fine-scale foraging site across years. Additionally, capture of capelin within spawning shoals likely results in reduced handling times, owing to their non-evasive manner (Davoren *et al.* 2006). Similarly, humpback whales appear to maximize net energy gain in other regions by feeding on prey patches that require less energy expenditure to capture prey and appear to modify their foraging behaviour based on prey behaviour (Witteveen *et al.* 2008; Friedlaender *et al.* 2009; Hazen *et al.* 2009; Friedlaender *et al.* 2016). Overall, prey density within shoals and the larger surveyed area, along with the number of shoals, may have less of an influence on whale presence relative to the presence of predictably located, non-evasive spawning shoals in shallow water.

Individual humpback whales show high site fidelity to foraging grounds within the North Atlantic, with low exchange of individuals among foraging grounds (Katona and Beard 1990; Stevick *et al.* 2006). Indeed, a long-term photo-identification study (10 years) by Clapham et al. (1993) in the Gulf of Maine found a high annual return rate of 73.4%, with high effort within

years (April-October, 704.5 ± 103.1 trips/year, 4 hours/trip). Although our annual return rate was much lower, our effort was also much lower (5-12 days/year, 2-6 hours/day) and there appears to be a higher number of whales returning to our study area (mean ± 95% CI; 2310 ± 580 individuals; Katona and Beard 1990; 1678 ± 304; our study) relative to the Gulf of Maine (240 ± 93 individuals; Katona and Beard 1990). Humpback whale overall return rates to other foraging grounds are more similar to our study, including 0-43% in a three-year study along the western coast of North America (California, British Columbia and Alaska; Calambokidis *et al.* 2001) and 5-56% in a 35-year study in the Western North Atlantic (Iceland, Greenland, Newfoundland, Gulf of St. Lawrence and Gulf of Maine; Katona and Beard 1990). Specifically, our overall return rates were similar to previous estimates in coastal Newfoundland of 15% (Katona and Beard 1990). The 328 individual humpback whales photographed near the Notre Dame Bay hotspot appear to represent ~20% of our current estimated number of humpback whales within this bay. Residency, or the days between sightings within a bay, was similar (1-12 d) to previous findings on the Bay de Verde Peninsula on the east coast of Newfoundland (< 3 d; Whitehead *et al.* 1980), but much lower than the Gulf of Maine (62.0-111.9 days), which again likely results from higher effort in the latter study. Residency may explain the slower speeds of movement by individuals in our study relative to other areas, as they suggest that humpback whales remain in bays longer. Overall, the return rates and residency durations within the small (10 km^2) foraging area in Notre Dame Bay suggest that deep-water spawning sites of capelin represent important, high quality prey patches for humpback whales.

Similar to previous studies, the inshore presence and spawning of capelin influenced the movement and consistent abundance of humpback whales within bays along the east coast of Newfoundland. Annual site fidelity of humpback whales to annually predictable deep-water

spawning sites of capelin reinforce the importance of these sites as high quality prey patches for humpback whales along with multiple predator species (Davoren 2007, 2013). This annually predictable high abundance food source likely minimizes energy expended searching for prey patches and capturing prey, resulting in high foraging efficiency for these whales. Although net energy gain of humpback whales is likely maximized at these sites, further studies on underwater movements and prey capture behaviour of foraging humpback whales on capelin are necessary to confirm high foraging efficiency.

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Tables and Figures

Table 1. Individual humpback whales that were photo-identified during June-August, 2016 and 2017, indicating the first and second dates each individual was photographed, estimated minimum distance travelled between sightings and estimated speed per day.

Individual ID	First Date	Second Date	Distance (km)	Speed (km/day)
16NL_17	21-Jul-16	27-Jul-16	230.2	38.4
16NL_16	17-Jun-16	27-Jul-16	98.4	2.1
16NL_18	05-Jul-16	27-Jul-16	229.8	10.4
16NL_26	04-Aug-16	15-Aug-16	63.3	5.8
16NL_6a	30-May-16	09-Jul-16	88.1	2.2
16NL_6b	09-Jul-16	21-Jul-16	52.5	4.4
Aug15PI_1	04-Aug-16	15-Aug-16	111.1	10.1
Aug15PI_3	04-Aug-16	15-Aug-16	109.1	9.9
Aug15PI_4	26-Jun-16	15-Aug-16	127.2	2.5
Aug15PI_5	26-Jul-16	15-Aug-16	126.5	6.3
Aug15PI_8	04-Aug-16	15-Aug-16	135.3	12.3
HWC7266	15-Jul-16	04-Aug-16	163.9	8.2
Jul21PI_2	27-Jun-16	21-Jul-16	60.5	2.5
Jul28PI_6	20-Jul-16	28-Jul-16	39.8	5.0
Jul27PI_4a	14-Jul-16	20-Jul-16	224.7	37.4
Jul27PI_4b	20-Jul-16	27-Jul-16	40.9	5.8
Jul31PI_1	20-Jul-16	31-Jul-16	113.5	10.3
HWC1704	23-Jul-17	27-Jul-17	161.9	32.4
Aug4/17PI_3	20-Jul-17	04-Aug-17	243.9	16.3

Table 2. Summary of the number of individuals identified from fluke photographs taken during July-August, 2003-2017 within Notre Dame Bay. Indicating the number of unique individuals identified per year, number of unique individuals identified prior to the given year (*M* in the Lincoln-Peterson estimate), number of re-sighted individuals in each year from all previous years (percent of individuals re-sighted from all previous years in brackets), residency (i.e. the range in the number of days between the first and last sighting of each individual within a year), along with sampling effort (date ranges of sampling and the number of boat days, with 2-6 hours per boat day) and the estimated number of individuals within the study area for each year.

Year	2003–2010	2011	2012	2013	2014	2015	2016	2017	Total
No. of unique individuals identified	37	51	65	56	19	19	75	39	361
No. of unique individuals previously identified	-	37	88	153	209	228	247	322	
No. of re-sights within years	0	2	8	5	3	0	4	4	26
No. of re-sights from previous years	0 (-)	1 (2%)	4 (6%)	5 (8%)	5 (21%)	3 (14%)	9 (11%)	11 (22%)	38
Residency (d)	-	2–3	1–14	1–21	1–10	-	1–9	5–13	
Sampling date range	-	Jul 11–23	Jul 11–28	Jul 5–29	Jul 17–28	Jul 19–Aug 11	Jul 21–Aug 15	Jul 31–Aug 16	
No. of sampling days	23	8	11	12	5	7	8	9	
No. of individuals in the area	-	1924	1518	1866	1003	1672	2305	1463	

Table 3. The percentage of individuals from the base year (under year) that were re-sighted the following years (e.g. 5% of the individuals sighted in 2012 were re-sighted in 2014). N is the total number of unique individuals from the base year.

Year	N	2011	2012	2013	2014	2015	2016	2017
2003-2010	37	3%	3%	8%	0%	0%	0%	3%
2011	51		6%	0%	2%	0%	4%	0%
2012	65			3%	5%	0%	5%	6%
2013	56				2%	4%	5%	2%
2014	19					5%	0%	5%
2015	19						5%	0%
2016	75							7%
2017	39							n/a

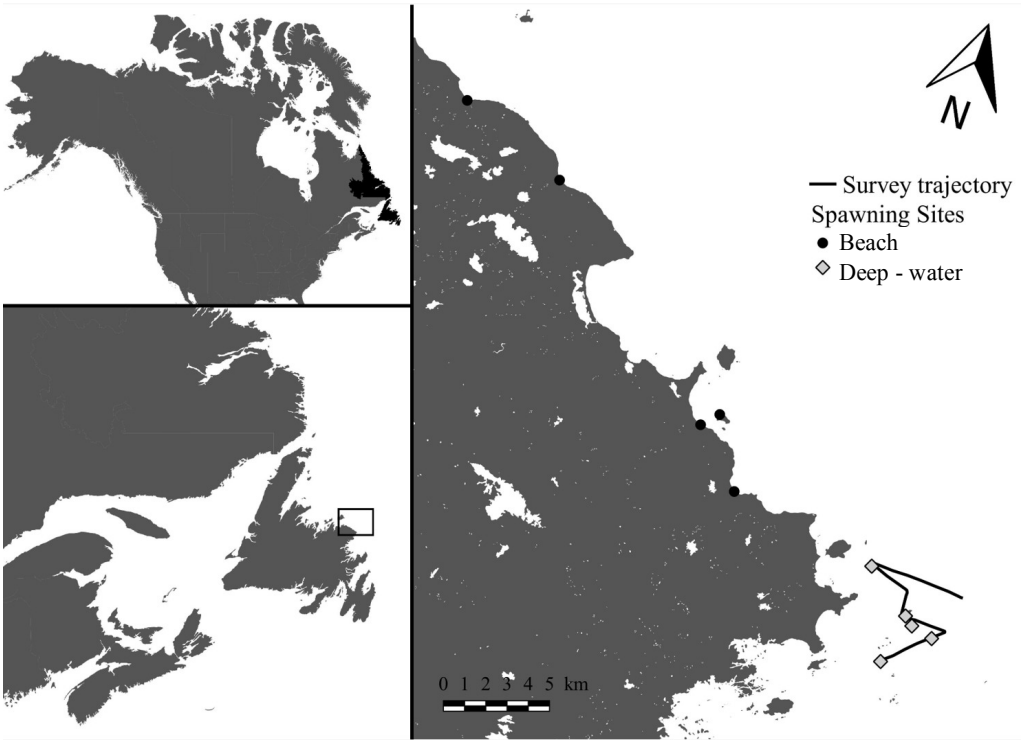
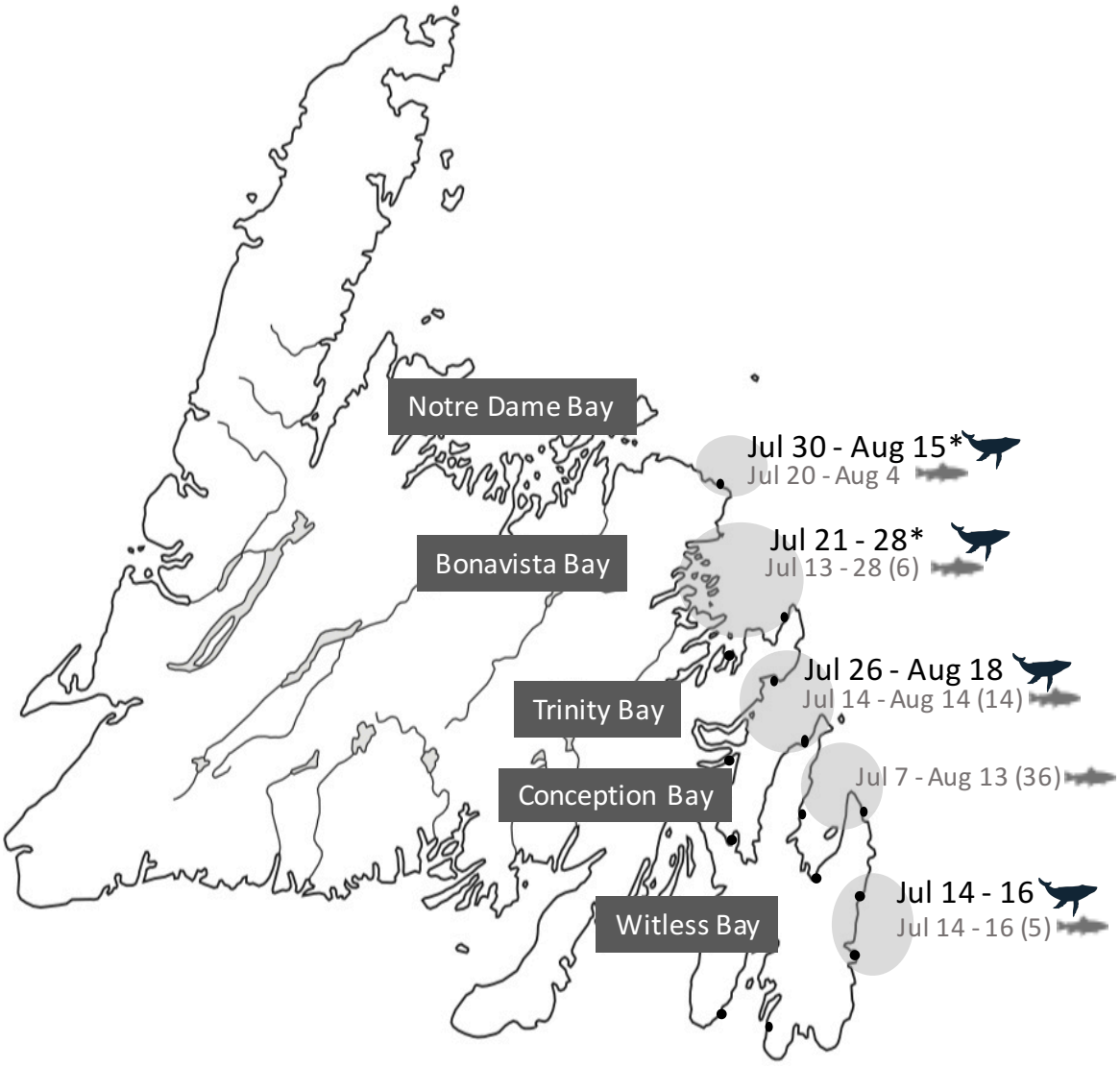


Figure 1. Location of survey in our study site along the northeast coast of Newfoundland. With persistent deep-water (diamond) and beach (circle) spawning sites of capelin indicated.


Notre Dame Bay
Jul 30 - Aug 15*
Jul 20 - Aug 4
Bonavista Bay
Jul 21 - 28*
Jul 13 - 28 (6)
Trinity Bay
Jul 26 - Aug 18
Jul 14 - Aug 14 (14)
Conception Bay
Jul 7 - Aug 13 (36)
Witless Bay
Jul 14 - 16
Jul 14 - 16 (5)

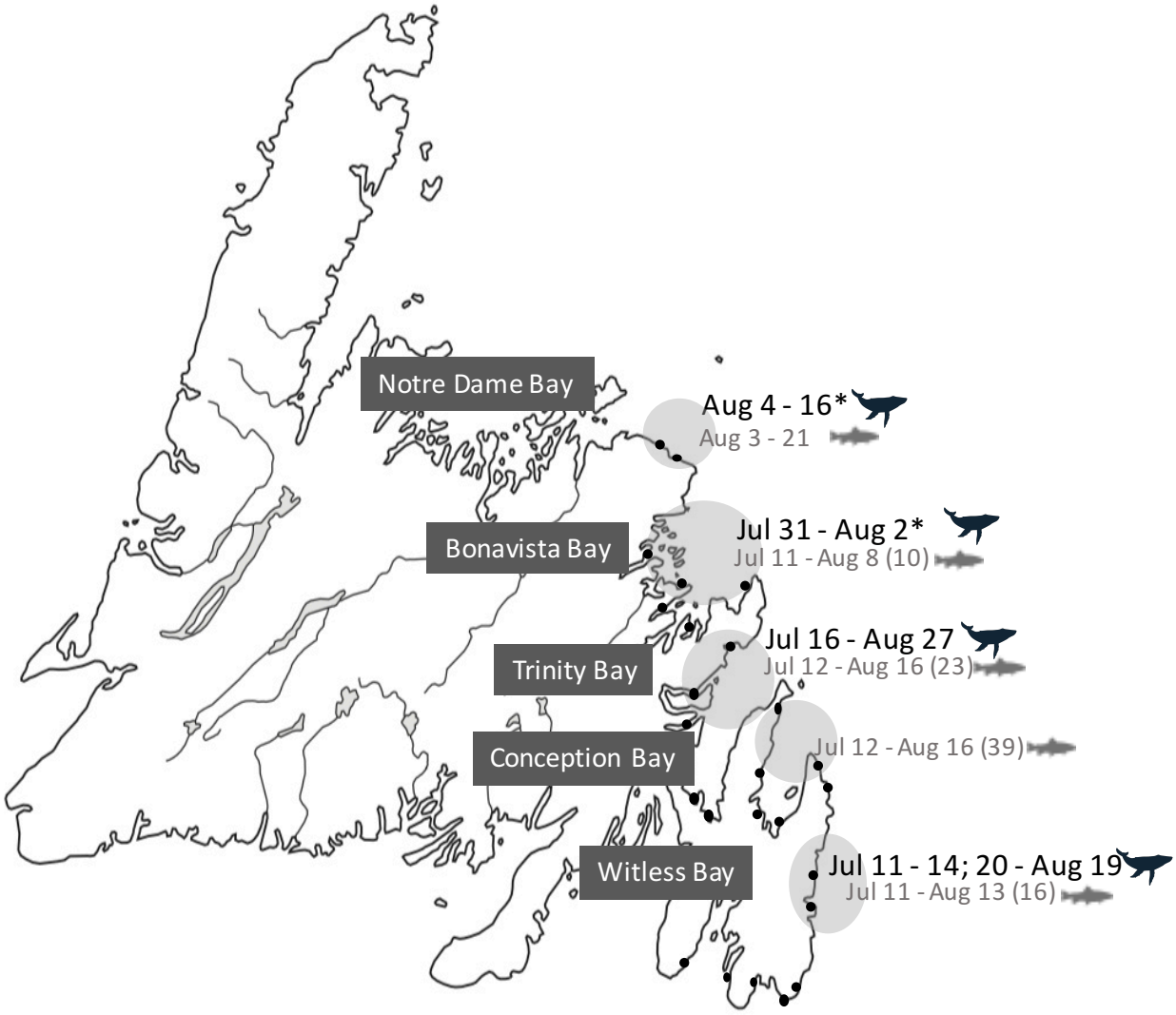


Figure 2. The date ranges (black) that humpback whales were consistently abundant (i.e. 10 ≤ humpback whales present for ≥ 3 days in a row; whale symbol) in bays along the eastern Newfoundland coast (light gray circles) during June-August, 2016 (top) and 2017 (bottom), and date ranges (gray) of the inshore arrival of spawning capelin (i.e. spawning capelin observed at beaches; fish symbol), along with the beach locations spawning capelin were observed (black dots). Note numbers in brackets refer to the number of Tweets and *eCapelin.ca* posts from local citizens regarding spawning capelin observed at beaches. The asterisks indicate the final date of effort for Bonavista Bay and Notre Dame Bay.

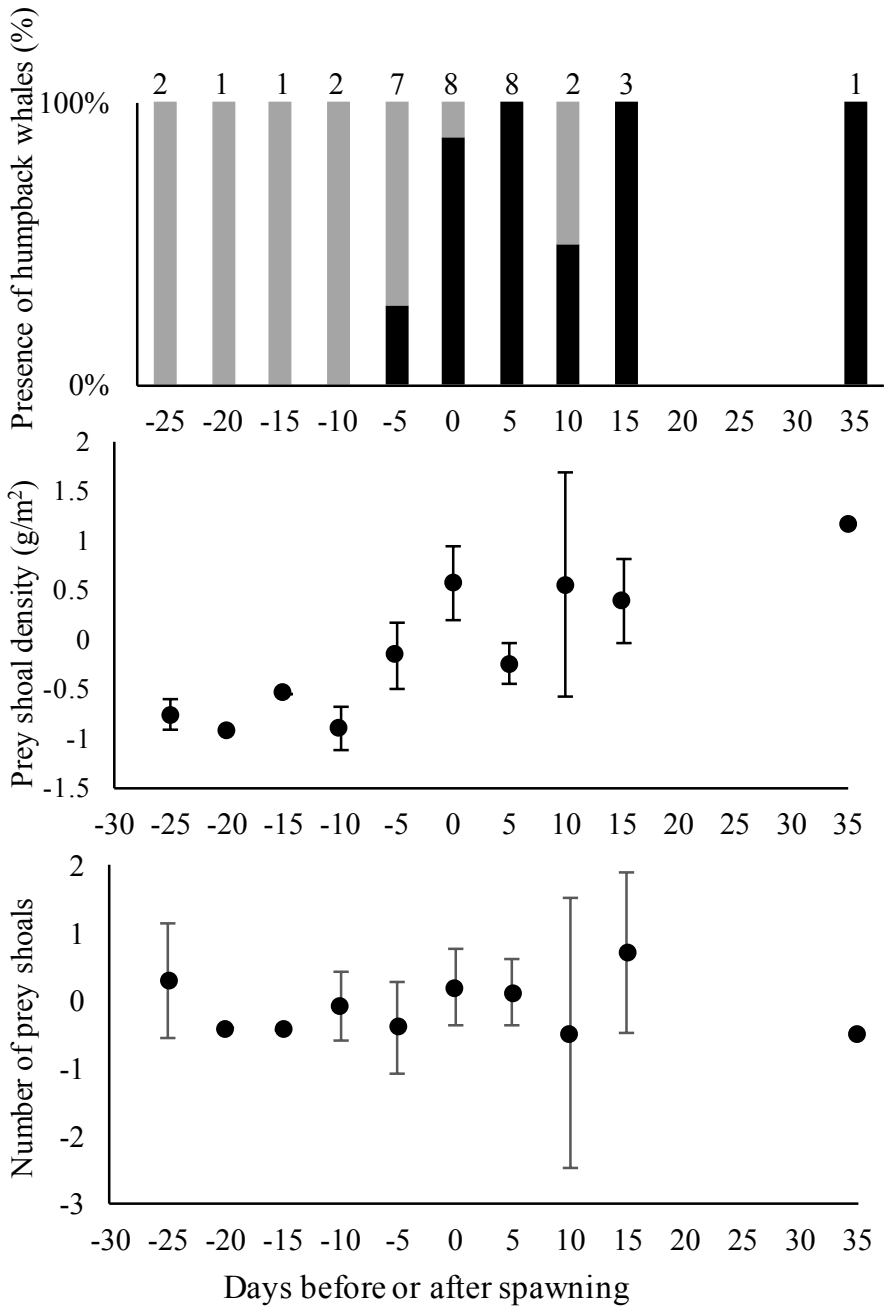


Figure 3. Percentage of surveys when humpback whales were present (black) and absent (grey; top panel), mean (± SE) standardized density of capelin shoals (middle panel), and mean (± SE) standardized number of capelin shoals (bottom panel) in relation to date of capelin spawning determined during repeated surveys within a biological hotspot in Notre Dame Bay during July-August 2009-2010, 2012, 2013-2017. Note the numbers above the bars in the top graph represent the number of surveys conducted across years during each 5-day period.

Chapter Two: Diet and niche breadth of humpback whales (*Megaptera novaeangliae*) on the northeast coast of Newfoundland

Abstract

Determining diet of marine mammals can be challenging due to the difficulties in directly observing foraging events. However, minimally invasive techniques can be used to obtain tissue samples to quantify stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) and the isotopic niche can be calculated as proxy of dietary niche. The primary goal of this study was to investigate the diet of humpback whales on their summer foraging grounds off the northeast Newfoundland coast during July-August, 2016 and 2017 using stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in whale skin. A secondary goal was to explore methods in tissue processing for stable isotope ratios of humpback whale skin. Lipid-extracted and non-lipid-extracted skin samples differed significantly in $\delta^{13}C$, $\delta^{15}N$ and C:N, with a lipid normalization equation for humpback whale skin of $\Delta^{13}C = -3.184 + 1.011(C:N)$. Inner and outer skin layers differed significantly in $\delta^{15}N$ likely due to structural differences, as indicated by differences in the C:N. Minimal dietary niche overlap was observed between years (9%), due to significant differences in $\delta^{13}C$ and $\delta^{15}N$ between years. These differences, however, were driven by inter-annual variation in prey $\delta^{13}C$ values and, thus, diet reconstruction using a Bayesian stable isotope mixing model (*MixSiar*) resulted in capelin/herring (*Mallotus villosus; Clupea harengus harengus*) comprising > 90% of humpback whale diet in both years using both informed and uninformed priors. These findings suggest that humpback whales primarily consume small forage fish in coastal Newfoundland and that reconstructed dietary proportions can be misinterpreted when using stable isotope ratios without lipid-extracting skin tissue and quantifying the isotopic ratios of potential prey types.

Introduction

Foraging theory is used to predict what prey types a predator should include in its diet to maximize net energy gain and, thus, fitness (Krebs and McCleery 1984). The profitability of a prey type is a trade-off between the energy gained by consuming a prey item and the energy expended searching for and handling (i.e. pursue, subdue and ingestion) the prey item (Krebs and McCleery 1984). Dietary niche breadth refers to the variety of prey types consumed by the population (Bearhop *et al.* 2004), whereby a broad niche breadth indicates that the individuals within the population are incorporating different prey types into each of their diets (Van Valen 1965). In contrast, a narrow niche breadth indicates that the population is incorporating one or a few prey types into its diet (Van Valen 1965). Dietary studies on highly mobile marine species, such as large baleen whales, can be difficult, due to our inability to directly observe prey consumption underwater, and because their foraging grounds often range over large regions. Owing to these challenges, a variety of techniques are used to investigate the diets of whales. For instance, researchers examine stomach contents of dead specimens (Fitch and Brownell 1968; Lowry *et al.* 2004) or fecal samples collected from live specimens (Fiedler *et al.* 1998; Gendron *et al.* 2001). However, neither technique is ideal. Sample sizes of stomachs are often small, owing to the need to catch and remove individuals from a population and, thus, many stomach content studies are focused on stranded individuals (Mitchell 1973; Castello 1977; Lydersen *et al.* 1991; Bowen and Iverson 2013). Fecal samples allow for greater sample sizes; however, samples can be biased toward prey species that are not as easily digested (Bowen and Iverson 2013).

More recently, researchers have used non-invasive techniques to collect tissue samples from live, free-ranging large baleen whales (Todd *et al.* 1997, Calambokidis *et al.* 2008, Borrell

et al. 2012, Filatova *et al.* 2013, Haro *et al.* 2016). In particular, skin biopsies can be collected, from which stable isotope ratios are quantified (Newsome *et al.* 2010). Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are the two most commonly quantified stable isotopes when investigating diet (Bearhop *et al.* 2004). Carbon isotope ratios vary based on basal carbon source and, thus, tend to differ along habitat gradients (e.g., inshore – offshore, pelagic – benthic), while nitrogen isotope ratios often increase by 3-5‰ between each trophic level (Peterson and Fry 1987; Hobson *et al.* 1994; Quevedo 2009). Using both isotope ratios, the isotopic niche can be quantified to estimate the trophic or dietary niche, as well as to reconstruct dietary composition using mixing models (Bearhop *et al.* 2004; Witteveen *et al.* 2011). Many considerations, however, must be taken into account to ensure appropriate interpretation of stable isotope ratios. First, lipids are depleted in ^{13}C relative to proteins and carbohydrates and, thus, have lower $\delta^{13}C$ values (DeNiro and Epstein 1977). Therefore, lipid extraction standardizes comparisons and minimizes inter- and intra-individual variation in $\delta^{13}C$ that are not due to dietary differences (Post *et al.* 2007). Chemical lipid extraction is often used to standardize the $\delta^{13}C$ values, whereby a solvent is used to remove the lipids from a tissue (Dobush *et al.* 1985), but mathematical normalization equations are also used (McConnaughey and McRoy 1979; Sweeting *et al.* 2006; Post *et al.* 2007; Lesage *et al.* 2010; Yurkowski *et al.* 2015) and may be preferred if chemical lipid extraction influences $\delta^{15}N$ values (Murry *et al.* 2006; Sweeting *et al.* 2006; Lesage *et al.* 2010; Hussey *et al.* 2012; Elliott *et al.* 2014). Turnover rates of the targeted tissue must also be known to determine the period over which the consumer's isotopic ratios reflect the averaged ratios of prey consumed (Bearhop *et al.* 2002). If tissues become inert once grown, temporal dietary shifts can be determined from sections of the grown tissue (Dalerum and Angerbjörn 2005; Busquets-Vass *et al.* 2017), but only if sections are structurally similar, otherwise variation

in stable isotope ratios may not reflect dietary differences. Finally, isotopic ratios of prey are typically modified during incorporation into the consumer tissue. Therefore, a diet-tissue discrimination factor, which is often species- and tissue-specific (Tieszen *et al.* 1983; Hobson and Clark 1992; Browning *et al.* 2014), is needed to reconstruct dietary proportions in consumers based on stable isotope ratios.

Humpback whales (*Megaptera novaeangliae*) are found in all oceans in both the northern and southern hemispheres (Winn and Reichley 1985). They occupy subtropical and tropical waters to breed during winter months and during polar summers they migrate (Dawbin 1966) over 8000 km toward the poles to forage on high-density aggregations of prey (Stone et al. 1990). A major foraging ground for humpback whales is along the coast of Newfoundland (Katona and Beard 1990). In this region, a key forage fish species is capelin (*Mallotus villosus*), which migrates in high abundances into coastal regions to spawn during the summer (Davoren *et al.* 2006; 2008). It has been assumed that capelin are the primary prey type of humpback whales, based on a study examining stomach contents of humpback whales (n=11) in the late 1960s (Mitchell 1973) that was further corroborated with spatial associations of foraging humpback whales with aggregations of capelin (Whitehead *et al.* 1980; Whitehead 1983; Whitehead and Carscadden 1985; Piatt *et al.* 1989; Piatt and Methven 1992). Another study that quantified the stable isotope ratios of humpback whale skin in coastal Newfoundland showed isotopic change after capelin spawning, suggesting that capelin may be important in the diet and also estimating a quick incorporation rate of prey into skin (7 – 14 days; Todd 1997). A more recent study on blue whales, however, suggested a much longer turnover rate of skin (163 ± 91 days; Busquets-Vass et al. 2017). While a study on captive bottlenose dolphins found a short turnover rate of 11-23 days (Browning *et al.* 2014).

The goal of this study was to investigate the diet of humpback whales on their summer foraging grounds off the northeast Newfoundland coast during July-August, 2016 and 2017 using stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in whale skin. I hypothesized that the high inshore abundance of capelin affects the diet of coastal humpback whales and predicted that capelin comprises a high proportion of the diet of humpback whales in coastal Newfoundland. I also explored methods in tissue processing for stable isotope ratios of humpback whale skin. First, I compared lipid-extracted and nonlipid-extracted skin samples and predicted that lipid-extracted skin would have higher $\delta^{13}C$ and lower C:N ratios, but $\delta^{15}N$ ratios would not be influenced. Second, I compared isotopic ratios of inner and outer skin layers and examined whether structural differences between skin layers, reflected by C:N ratios, may reduce the ability to use these tissue layers to indicate diet during different periods. Overall, this study fills knowledge gaps on stable isotope analysis in cetacean skin, and provides a better understanding of the diet of a large megafauna predator in the North Atlantic.

Methods

Biopsy samples of skin and blubber were obtained from humpback whales foraging near persistent deep-water spawning sites of capelin (17-40 m; 'Gull Island'; Fig. 1; Davoren 2013) as well as a nearby deep-water (~200 m) pre-spawning staging area in Bonavista Bay ('Trench'; Fig. 1; Davoren 2013) during July-August, 2016 (n=30) and 2017 (n=31; Table 1). Generally several individuals of individuals were sampled per day (n = 1-10/d), with 2-15 d between sampling periods.

To collect biopsies, individual humpback whales or groups (up to three) were approached (10-15 m) at a slow speed (5 km/h) from a six-meter fiberglass fishing boat, with the archer

positioned perpendicular to the whale (Brown *et al.* 1995). An Excalibur Matrix Grizzly crossbow was used with a specialized bolt and hollow stainless steel tip (40 x 8 mm) with three small barbs to hold the skin/blubber sample. The tip was fixed onto a specialized bolt with a float to prevent further penetrating the whale and to ensure easy collection from the water surface. Biopsy tips were sterilized just prior to use by immersing in 95% ethanol (Smith *et al.* 1999). Each biopsy sample was removed from the tip using sterilized forceps, ensuring blubber stayed attached to skin. Samples were wrapped in aluminum foil, placed in a labelled plastic tube, and then stored in a small cooler with ice packs until samples could be stored in a freezer. Upon sample collection, the date, time, location (latitude, longitude), visibility, and reaction level (1-4: 1: no reaction, whale continued with normal behaviour; 2: low-level reaction, whale modified behaviour slightly; 3: moderate reaction, whale modified behaviour in a forceful manner but no prolonged evidence of behavioural disturbance; 4: strong reaction, whale modified behaviour in successive forceful actions) were recorded. Whales showed little reaction to biopsy sampling, allowing photographs of the ventral side of the fluke to be taken for 78% of biopsied whales for individual identification.

For dietary reconstruction using stable isotope ratios, potential prey species were collected throughout July-August for stable isotope analysis. Forage fish species, including capelin, sandlance (*Ammodytes sp.*), and herring (*Clupea harengus harengus*), were collected opportunistically during other research (e.g., beach sampling, pelagic seining, seabed bottom grabs) as well as from local fishermen in both 2016 and 2017. For each fish species, a 3 x 1 cm muscle plug, with skin removed, was obtained from the dorsal side between the head and dorsal fin and frozen immediately. Invertebrates (i.e., copepods, euphausiids) were collected during horizontal plankton tows (n = 3) for 15 min with 270 μm mesh net at a depth of 2-3 m. Plankton

tows were conducted early (July 5), mid (August 4) and late (August 16) in the season to account for a seasonal shift in isotopic baseline (Colebrook 1982). Contents were sorted into taxonomic groups, which included euphausiids and other (primarily copepods), which were subsequently frozen separately. Multiple individuals per group were combined to make up samples of each invertebrate group for stable isotope analysis.

Stable isotope analysis

Whale blubber was removed from skin, and each skin sample was then cut in half and separated into outer (surface) and inner (attached to blubber) fragments. In 2016, the inner fragments were homogenized and divided in half to obtain two samples, whereby one was lipid-extracted and the other was not. In 2016 and 2017, the outer skin fragments were lipid-extracted. To prepare whale skin and fish muscle samples for stable isotope analysis, each sample was freeze dried for 72 h (Haro *et al.* 2016; Elliott *et al.* 2017). Samples were then homogenized using a mortar and pestle. For nonlipid-extracted skin, a 0.400-0.600 mg subsample was sealed into a 5 x 9 mm tin capsule. To lipid-extract whale skin, fish muscle and invertebrate samples, each subsample was rolled into a microfiber filter paper and placed into a thimble. Lipids were extracted using a petroleum ether solvent in a Soxhlet for a minimum of 8 h (Dobush *et al.* 1985; Elliott *et al*. 2017). Samples were then oven dried for 24-48 h at 60°C (Witteveen *et al.* 2009; Elliott *et al.* 2017) and a 0.400-0.600 mg subsample was sealed into a 5 x 9 mm tin capsule (Witteveen *et al.* 2009; Haro *et al.* 2016). All capsules were placed in a plastic tray with labelled divides and shipped to the University of Windsor. Bulk invertebrate samples were sent to University of Windsor for lipid extraction and stable isotope analysis. Stable isotopes were

measured using an Isotope Ratio Mass Spectrometer and expressed in delta (δ) notation, calculated using the following equation:

$$\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$$

Where X is either ^{13}C or ^{15}N and the R is the ratio of ^{13}C or ^{15}N in the sample to the standard reference material of ^{12}C (Pee Dee Belemnite) or ^{14}N (atmospheric nitrogen gas; Witteveen *et al.* 2009). The Isotope Ratio Mass Spectrometer analytical precision was ≤ 0.16‰ and ≤ 0.12‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively, and the accuracy was ≤ 0.22‰ for both $\delta^{15}N$ and $\delta^{13}C$.

Data analysis

All statistics were performed in R version 3.3.2. Assumptions underlying parametric statistics were tested on response variables (i.e., $\delta^{13}C$, $\delta^{15}N$, C:N ratios), including normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test) as well as, bivariate normality (multivariate Shapiro-Wilk test). All response variables met underlying assumptions and, thus, univariate parametric tests were used. Bivariate normality, however, was not met in both years and, thus, outliers were removed from each year to meet the assumption of bivariate normality when reconstructing the diet using mixing models. Outliers were removed if they fell outside of the 95% mixing region calculated from the prey sources (Smith *et al.* 2013). From the 2016 samples, $\delta^{13}C$, $\delta^{15}N$ and C:N ratios were compared between inner and outer skin layers as well as nonlipid-extracted and lipid-extracted inner layer samples using paired t-tests. Further, I produced a lipid normalization equation by performing a linear regression of $\Delta^{13}C$ (i.e. $\delta^{13}C_{lipid\text{-}extracted} - \delta^{13}C_{nonlipid\text{-}extracted}$) and C:N ratio by mass of each sample before lipid-extraction. The $\delta^{13}C$, $\delta^{15}N$ and C:N ratios of lipid-extracted outer layer samples were compared between years using a one-factor ANOVA.

Isotopic niche breadth of humpback whales was quantified in each year using the standard ellipse area (SEA), which is defined as a standard deviation around the bivariate mean and encompasses ~40% of the samples. Using the Stable Isotope Bayesian Ellipse in R (*SIBER*) package, the standard ellipse area corrected for small sample sizes (SEA_C) and the Bayesian standard ellipse area (SEA_B) were calculated. When calculating SEA_B, many possible SEA values are provided (posterior distribution), allowing us to quantify the most likely isotopic niche breadth value (i.e., the mode of the distribution) and variation around the most likely SEA_B (i.e., 95% credible intervals; Jackson *et al.* 2011). To calculate SEA_B, the number of iterations was one million, with a burn-in of 50,000, and the posterior distribution was thinned every 10 iterations. The probability that niche breadth in one year was broader than the other was also calculated by doing a pairwise comparison between the two posterior distributions for each year, resulting in a percentage of the number of iterations one year's niche was smaller or larger than the other. To determine isotopic niche overlap between 2016 and 2017, I calculated pairwise comparisons of dietary niche overlap:

$$\% \text{ Overlap} = \text{overlap area} / (SEA_{B\_2016} + SEA_{B\_2017} - \text{overlap area}) * 100$$

Finally, the Bayesian mixing model *MixSiar* was used to determine the probability distribution that each prey type (source) contributed to the diet of the consumer population in each year (Stock and Semmens 2013). A diet tissue discrimination factor of 1.28 ± 0.3‰ for $\delta^{13}C$ and 2.82 ± 0.38‰ for $\delta^{15}N$ obtained from a fin whale (*Balaenoptera physalus*) study was applied to the source isotopic ratios (Borrell *et al.* 2012). The prey types used were capelin, herring, sandlance, copepods, and euphausiids. As forage fish species were collected in each year, annual species-specific isotopic values were used for diet modeling in each year. Due to low number of sandlance collected, those collected in 2015 and 2016 were merged. As

euphausiids and copepods were only collected in 2017, these values were used for diet modeling in both years. Capelin and herring had similar isotope ratios, therefore, I grouped these prey types by calculating weighted means and standard deviations for this merged group (i.e., capelin/herring; Table 3). Models for each year were run with uninformed priors and informed priors, whereby informed priors were based on the stomach contents of eleven of humpback whales caught between May-August of 1969-1971 on the Newfoundland shelf, 10 with capelin, none with copepods or euphausiids and one with sandlance in their stomachs (c(3.63,0.01,0.01,0.35); Mitchell 1973). Yearly models were also run with uninformed priors and outliers removed to meet the assumption of bivariate normality. Each model was run with three chains, a 300,000 chain length with 200,000 burn-ins and thinning at every 100 iterations. Two diagnostics were checked to examine model performance. The Geweke diagnostic uses a two-sided z-test to compare the mean of the first and second part of the chain and is rejected if >5% of the variables are outside of +/- 1.96 (Stock and Semmens 2017). The Gelman-Rubin diagnostic compares the between and within chain variance and is rejected if values are >1.1 (Gelman et al. 2014).

Results

When comparing lipid-extracted versus nonlipid-extracted samples of the inner skin layer during 2016, there was a significant difference in $\delta^{13}C$ ($t_{(29)} = 22.19$, $p < 0.001$), $\delta^{15}N$ ($t_{(29)} = 5.09$, $p = 0.014$) and C:N ratios ($t_{(29)} = -20.88$, $p = 0.002$; Table 1). There was a positive linear relationship between $\Delta^{13}C$ and the C:N ratio by mass of nonlipid-extracted samples ($F_{1,28} = 108.4$, $p < 0.0001$, $r^2 = 0.795$; Fig. 2), with a lipid normalization equation for humpback whale skin of $\Delta^{13}C = – 3.184 + 1.011(C:N)$. When comparing the lipid-extracted outer layer versus the

inner layer of skin, $\delta^{15}N$ differed significantly ($t_{(29)} = -9.31$, $p < 0.001$) along with the C:N ($t_{(29)} = -5.4$, $p = 0.021$), but there was no difference in $\delta^{13}C$ ($t_{(29)} = 0.56$, $p = 0.58$; Table 1).

When comparing prey isotopic ratios between years, capelin differed significantly in $\delta^{13}C$ ($F_{(1,33)}=14.22$, $p<0.001$) but not in $\delta^{15}N$ ($F_{(1,33)} = 1.289$, $P = 0.264$; Table 2). Herring also differed significantly in $\delta^{13}C$ ($F_{(1,24)}=7.253$, $p=0.013$) but not in $\delta^{15}N$ ($F_{(1,24)} = 0.855$, $p = 0.364$; Table 2), whereas sandlance differed significantly in both $\delta^{13}C$ ($F_{(1,18)} = 32.06$, $p < 0.001$) and $\delta^{15}N$ ($F_{(1,17)} = 30.8$, $p < 0.001$; Table 2).

The lipid-extracted outer layers of humpback whale skin showed little overlap of standard ellipse area between years (9%; Fig 3). There was a significant difference in $\delta^{13}C$ ($F_{(1,58.87)} = 31.11$, $p < 0.0001$), $\delta^{15}N$ ($F_{(1,52.86)} = 7.58$, $p = 0.0081$) and C:N ($F_{(1,45.20)} = 20.17$, $p < 0.0001$; Table 1). Additionally, there was a 91% probability that niche breadth was broader in 2017 ($SEA_B = 0.39$ ‰^{2}) relative to 2016 ($SEA_B = 0.34$ ‰^{2}); however, the broader SEA_B in 2017 appeared to be driven by one outlier. When the outlier was removed, the SEA_B for 2017 decreased ($SEA_B = 0.29$ ‰^{2}), which resulted in 2016 and 2017 having a similar isotopic niche breadth, with a 73% probability that 2016 is broader than 2017.

As stable isotopic values for potential forage fish prey differed between years, year-specific isotopic ratios were used when reconstructing whale diet for each year using mixing models. During each year, the same trends were observed, whereby capelin/herring comprised >90% of the diet, while sandlance contributed <10%, and the contributions of copepods and euphausiids were negligible (Table 3). These trends were similar when uniformed and informed priors were used; however, the informed model failed the Gelman-Rubin diagnostic checks, indicating that model performance was poor. When one outlier was removed in 2016 and three were removed in 2017, the diet shifted slightly with a 1% decrease in capelin/herring in 2017 and

a 2% increase in 2016. The proportions of other prey species did not change except for a 2% decrease in sandlance in 2016.

Discussion

By quantifying the stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of humpback whale skin on their summer foraging grounds off the northeast Newfoundland coast, I found that capelin and/or herring comprised a high proportion of their summer diet in each year (> 90%), as predicted. Although little isotopic niche overlap was observed between years, suggesting dietary differences, primarily due to inter-annual variation in prey isotopic values. This finding highlights the need to sample the prey base for accurate interpretations of stable isotope ratios across years. Lipid-extraction also appears to be important, evidenced by the influence of chemical lipid-extraction on $\delta^{13}C$ ratios, C:N ratios, as well as $\delta^{15}N$ ratios, although the latter was minimal (0-0.76 ‰). Our derived mathematical normalization model had a high predictive value, suggesting it can be used by other researchers to estimate normalized $\delta^{13}C$ values if time and budgetary constraints do not allow chemical lipid extraction. Finally, the isotopic ratios of inner and outer skin layers differed in $\delta^{15}N$ and C:N ratios, likely reflecting structural differences and suggesting that these layers cannot be used to reflect different time periods of averaged diet.

The reconstructed diet of humpback whales indicated that capelin and/or herring primarily comprised the diet. Although capelin and herring were very similar isotopically, which was observed previously (Todd 1997), thus, they could not be distinguished, capelin was likely the primary prey species in the diet. Based on foraging theory, humpback whales were predicted to consume prey types that maximize net energy gain (Krebs and McCleery 1984). When humpback whales are present in coastal Newfoundland, capelin form large, high-density

aggregations that can often be predictably located (Davoren 2013), making capelin the most profitable prey type, owing to low searching and handling time. In support, consistent abundance (see Chapter 1) of humpback whales were present off the south coast of Newfoundland in July-August (Chapter 1), coinciding with the inshore presences of spawning capelin (Davoren *et al.* 2012; Crook *et al.* 2017; Chapter 1). In contrast, spring-spawning (May-June) herring in this region would be declining in late June, when they would be dispersing northward to feed (Wheeler and Winters 1984). A primarily capelin-based diet is further supported by associations of foraging humpback whales and aggregations of capelin in coastal Newfoundland (Whitehead *et al.* 1980; Whitehead 1983; Whitehead and Carscadden 1985; Piatt *et al.* 1989; Piatt and Methven 1992), capelin in stomach contents (Mitchell 1973), and movement of humpback whales northward throughout the summer as spawning capelin are available farther north later in the season (see Chapter 1; Whitehead *et al.* 1982). Depending on the incorporation rate of skin, which may be quick (7-23 days; Todd 1997; Browning *et al.* 2014) or much longer (81-272 days; Busquets-Vass *et al.* 2017), the isotopic ratios of the skin may reflect a diet primarily capelin, as biopsies were collected later in the season (end July-August) or an initial diet of herring early in the season, switching to primarily capelin-based diet throughout the remainder of the summer. A dietary shift, however, would not be reflected in the stable isotope ratios due to similar ratios of these two prey types. Overall, given the abundance of capelin in coastal Newfoundland during the summer (June-August), when herring would not be abundant, I interpret my results to indicate a primary reliance on capelin in coastal Newfoundland during the study period.

Although dietary niche breadth of humpback whales was similar in both years once outliers were removed, the position of the isotopic niche differed with apparently minimal

overlap between years. The difference in isotopic values of outliers may be due to individual variation in diet, with the individuals consuming primarily invertebrates, or difference in arrival time to the Newfoundland foraging ground. Humpback whales have been observed to shift from a primarily invertebrate diet to a primarily piscivore diet within a foraging ground (Fleming *et al*. 2016). The low overlap between years, however, appeared due to inter-annual variation in $\delta^{13}C$ of forage fish. Indeed, using the species-specific isotopic ratios of prey from each year indicated that dietary proportions of prey types were remarkably similar in both years, despite clear differences in stable isotope values. Similarly, other studies have also shown seasonal and annual variation in isotopic niche space of consumers due to baseline isotopic shifts (Grey *et al.* 2004; Solomon *et al.* 2008; Woodland *et al.* 2012). For instance, Nordström *et al.* (2009) monitored within and between year shifts of $\delta^{13}C$ and $\delta^{15}N$ for three trophic levels in a coastal marine food web and found both seasonal and annual changes in isotopic values for all trophic levels, with the greatest variation in primary producers. Other studies have pointed out the importance of incorporating baseline isotopic values when interpreting population or community isotopic niche (Schmidt *et al.* 2007; Hoeinghaus and Zeug 2008). This study highlights the importance of collecting prey samples that represent the diet of the consumer annually or seasonally to ensure appropriate interpretation of stable isotope ratios and the dietary niche.

As expected, chemical lipid-extraction resulted in an increase in $\delta^{13}C$ values and a decrease in C:N ratios, but $\delta^{15}N$ values also increased. Indeed, lipid extraction generally increases the $\delta^{13}C$ by removing the lipids that are depleted in ^{13}C relative to proteins and carbohydrates (Sotiropoulos *et al.* 2004; Hussey *et al.* 2012); however, the effect of lipid extraction on $\delta^{15}N$ is not as consistent (Ryan *et al.* 2012). Indeed, lipid extraction has resulted in $\delta^{15}N$ increasing (Lesage *et al.* 2010, Elliott *et al.* 2014), decreasing (Barrow *et al.* 2008, Ryan *et*

al. 2014) or has resulted in no change (Ryan *et al.* 2014). The inconsistent influence of lipid extraction on $\delta^{15}N$ may result from using different solvents to chemically extract lipids. For instance, non-polar solvents (i.e., petroleum ether) extract only neutral lipids (Dobush *et al.* 1985), while polar solvents (i.e., chloroform-methanol) extract total lipids (i.e., structural and neutral lipids; Sweeting *et al.* 2006) and, thus, extracting with polar solvents may have a greater effect on $\delta^{15}N$. Although I extracted lipids with a non-polar solvent, $\delta^{15}N$ ratios increased significantly, as observed in previous studies using both non-polar (Hussey *et al.* 2012; Elliott *et al.* 2014) and polar solvents (Murry *et al.* 2006; Sweeting *et al.* 2006; Lesage *et al.* 2010). The change in $\delta^{15}N$ was small (0-0.76 ‰), however, suggesting it would not influence the interpretation of the niche breadth and diet of humpback whales in this study. Owing to the variable effect of lipid extraction on $\delta^{15}N$, mathematical normalization of lipids may be preferred (Ryan *et al.* 2014). Indeed, using a normalization model would eliminate changes in $\delta^{15}N$ values from chemical lipid extraction, while also minimizing valuable time and funding required to lipid extract. Taxon-specific normalization equations incorporating multiple species have been developed (McConnaughey and McRoy 1979), but these equations are often poor predictors of lipid-extracted $\delta^{13}C$ compared to more specialized models (Sweeting *et al.* 2006; Post *et al.* 2007; Lesage *et al.* 2010), including species- and tissue-specific models (Yurkowski *et al.* 2015). Therefore, the lipid normalization model generated here offers a reliable way to correct for $\delta^{13}C$ values within lipid-rich humpback whale skin and potentially other baleen whales.

Dietary shifts through time have been monitored using sections of a growing tissue that becomes inert once grown, including whiskers, teeth, claws and baleen (Dalerum and Angerbjörn 2005). Busquets-Vass *et al.* (2017) suggested that layers of cetacean skin may be used to examine dietary shifts, with the inner layer of skin reflecting the most recent diet

compared to the outer layer. Similar to our study, Busquets-Vass *et al.* (2017) found a significantly lower $\delta^{15}N$ in the inner relative to the outer skin layer. Skin layers also differed in C:N, however, suggesting that the variation in $\delta^{15}N$ may be due to structural differences in skin layers rather than reflecting dietary shifts through time. In support, a histological study found layers of southern right whale (*Eubalaena australis*) skin to be composed of three structurally different layers, with the outermost layer being heavily keratinized (Reeb *et al.* 2007). As keratin is a protein, the outermost layer has more protein compared to the other two layers. Therefore, it is cautioned to use whale skin to determine diet shift over time as isotopic differences in layers because differences may be due to structural differences and not dietary differences.

Although humpback whales were widely thought to forage on capelin in their Newfoundland foraging grounds (Mitchell 1973; Whitehead *et al.* 1980; Whitehead 1983; Whitehead and Carscadden 1985; Piatt *et al.* 1989; Piatt and Methven 1992) and skin stable isotope samples have been investigated previously (Todd *et al.* 1997), this is the first study, to my knowledge, to quantify the diet of humpback whales in their Newfoundland foraging ground since the collapse of capelin in the early 1990s (Buren *et al.* 2014). Future studies incorporating different techniques, such as fatty acid analysis, will be needed to tease apart the importance of capelin and herring within summer diet. Multi-sensor tags equipped with cameras could also be used to visually identify the different prey types. Given our findings, further studies using stable isotopes to examine dietary composition of whales should collect potential prey annually owing to inter-annual baseline shifts, which can affect the interpretation of the consumer diet. They should also carefully consider lipid normalization, either using chemical lipid extraction or the normalization equation presented here. Researchers should be careful to use skin layers to interpret dietary shifts, as these layers appear to be structurally different. Furthermore, better

estimates of diet tissue discrimination factors and tissue turnover rates for baleen whales, specifically humpback whales, are needed to better determine diet. Given the collapse of Newfoundland capelin populations (Buren *et al.* 2014) as well as global overfishing of forage fish (Smith *et al.* 2011; Pikitch *et al.* 2012), similar studies will be critical to further elucidate the reliance of whales and other globally-distributed predators on commercially exploited forage fish species to predict population-level impacts.

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Tables and Figures

Table 1. The mean (± SE) of $\delta^{13}C$, $\delta^{15}N$ and C:N from humpback whale skin samples collected on the northeast Newfoundland coast during July-August, 2016 and 2017.

Year/Skin Layer, Method	N	Sample Dates	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	C:N
2016	30	Jul 21, 27 & Aug 3, 15			
Outer skin, lipid-extracted			-18.90 ± 0.03	15.03 ± 0.12	3.40 ± 0.02
Inner skin, lipid-extracted			-18.89 ± 0.03	14.55 ± 0.11	3.32 ± 0.01
Inner skin, nonlipid-extracted			-19.89 ± 0.06	14.35 ± 0.13	4.14 ± 0.04
2017	31	Jul 31 & Aug 2, 4, 6, 8, 10			
Outer skin, lipid-extracted			-19.14 ± 0.03	14.46 ± 0.17	3.32 ± 0.01

Table 2. Potential prey types collected during July-August, 2016 and 2017 on the northeast Newfoundland coast, along with total length range, mean (± SD) of $\delta^{13}C$(‰), $\delta^{15}N$(‰) and sample size. Sandlance was collected and merged for both 2015 and 2016. All prey types listed were used used in mixing models to reconstruct diet.

Species	Length (mm)	$\delta^{13}C$(‰)	$\delta^{15}N$(‰)	n	Length (mm)	$\delta^{13}C$(‰)	$\delta^{15}N$(‰)	n
	2016				2017			
Capelin	123-185	-20.29±0.23	12.28±0.29	20	124-177	-20.63±0.29	12.12±0.54	15
Herring	284-351	-20.20±0.13	12.58±0.24	11	180-340	-20.44±0.27	12.46±0.36	15
Sandlance	99-178	-20.49±0.11	11.09±0.46	10	118-175	-21.19±0.18	10.11±0.10	9
Capelin/Herring	-	-20.26±0.21	12.39±0.30	31	-	-20.53±0.29	12.20±0.48	30
Euphausiids					-	-21.97±0.17	7.67±0.16	5
Copepods					-	-23.01±0.63	7.67±0.61	20

Table 3. The modal proportion with 95% Bayesian credible intervals (in parentheses) of different prey types in the diet of humpback whales during July-August, 2016 and 2017. Prey types included in the mixing models were capelin/herring, sandlance, euphausiids and copepods.

Model	Capelin/ Herring	Sandlance	Euphausiids	Copepods	n	Capelin/ Herring	Sandlance	Euphausiids	Copepods	n
	2016					2017				
Informed	0.95 (0.81-1.00)	0.05 (0.00-0.19)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	30	0.98 (0.88-1.00)	0.02 (0.00-0.12)	0.00 (0.00-0.00)	0.00 (0.00-0.00)	31
Uninformed	0.91 (0.79-0.97)	0.07 (0.01-0.20)	0.01 (0.00-0.03)	0.01 (0.00-0.02)	30	0.93 (0.86-0.97)	0.04 (0.00-0.12)	0.02 (0.00-0.06)	0.01 (0.00-0.03)	31
No Outlier	0.93 (0.84-0.98)	0.05 (0.01-0.15)	0.01 (0.00-0.03)	0.01 (0.00-0.02)	29	0.92 (0.86-0.96)	0.04 (0.00-0.12)	0.02 (0.00-0.06)	0.01 (0.00-0.03)	28

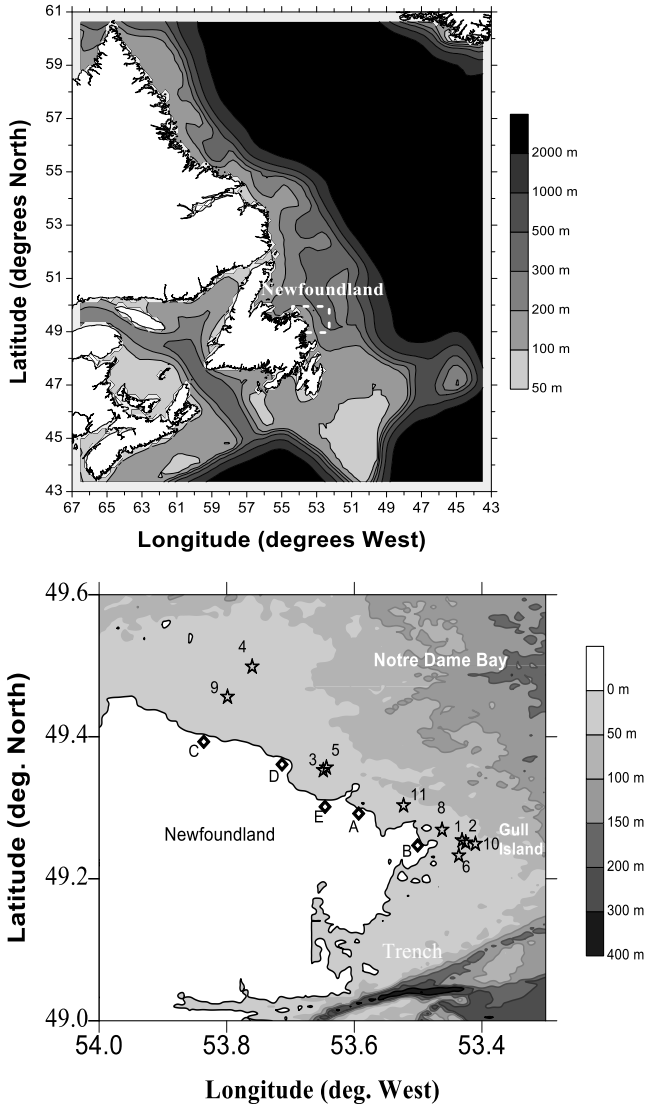

Figure 1. The location of the study area in eastern Canada (above) and the study area (below), indicating the location of beach (diamond) and deep-water (star) spawning sites of capelin off the northeast coast of Newfoundland, Canada.

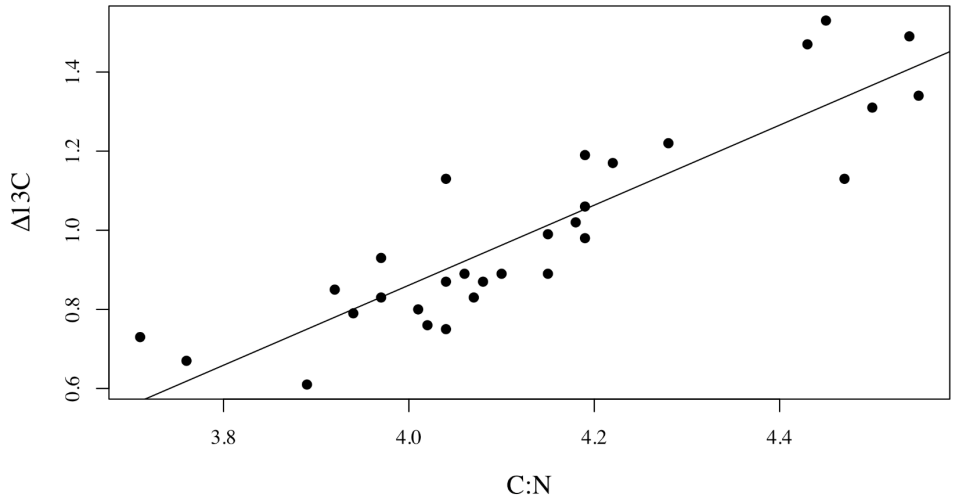

Figure 2. Relationship between $\Delta^{13}C$ ($\delta^{13}C_{lipid\text{-}extracted}$ - $\delta^{13}C_{nonlipid\text{-}extracted}$) and C:N ratio by mass of humpback whale skin collected during July-August, 2016 off the east coast of Newfoundland. The lipid normalization equation for humpback whale skin is $\Delta^{13}C = -3.184 + 1.011(C:N)$ and this relationship was statistically significant ($F_{1,28} = 108.4$, $r^2 = 0.795$, $p < 0.0001$).

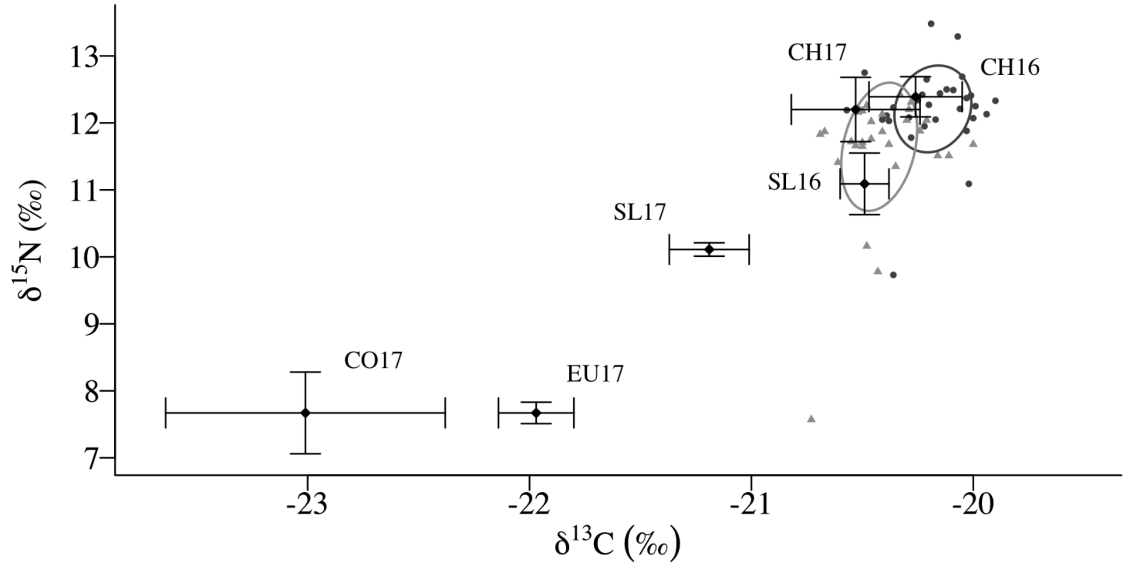

Figure 3. The isotopic niche breadth (SEA_C) of the outer fragment of lipid-extracted humpback whale skin sampled during July-August of 2016 (light grey circles) and 2017 (dark grey triangles) in coastal Newfoundland, along with mean (± SE) $\delta^{13}C$ and $\delta^{15}N$, values for potential prey species collected in the study area (shown in Fig. 1), including merged capelin and herring collected in 2016 (CH16) and 2017 (CH17), sandlance collected in 2016 (SL16) and 2017 (SL17), euphausiids collected in 2017 (EU17) and copepods collected in 2017 (CO17).

General Conclusion

Knowledge of critical foraging areas in time and space of large marine predators, as well as diet, are important to inform management plans for shipping lanes and conservation programs such as marine protected areas. In this study, I found that capelin (*Mallotus* villosus) comprised a large portion of humpback whale diet annually, but different methods are needed to tease apart the relative proportion of capelin and herring (*Clupea harengus harengus*) in the diet. I also determined that humpback whale movement patterns within their Newfoundland foraging grounds were associated with the timing of presence of this important prey species in their diet. Indeed, humpback whales (*Megapter novaeangliae*) were consistently abundant within bays on the east coast when capelin were present within bays. At the bay-scale, humpback whale presence was influenced by the timing of spawning, rather than specific capelin shoal characteristics and individual humpback whales returned to a small area (10 km^2), centered on a cluster of capelin deep-water spawning sites (Davoren 2013), suggesting that these sites are important foraging grounds for humpback whales.

To investigate relationships between presence of spawning capelin and consistently abundant presence of humpback whales in bays along the east coast of Newfoundland, I used citizen science in most bays (Chapter 1). Although citizen science is a great tool, it has some draw backs. First, recordings of capelin presence in bays was limited to near shore sightings. To get a more accurate range of capelin availability and abundance, ship-based surveys must be conducted regularly in each bay. If these data were available, it may have shown a stronger link between humpback whale presence and capelin availability and abundance at the regional-scale. Directly assessing capelin availability, however, would likely be prohibitively costly and time-consuming compared to using citizen science. Second, whale abundance and residency within

bays, and movement among bays were limited to tour operator sightings and uploaded fluke photographs. To more accurately quantify residency and fine-scale movement patterns within bays of these and other large marine predators, many studies have used satellite tags (Dalla Rosa *et al.* 2008; Block *et al.* 2011; Hart *et al.* 2014; Kennedy *et al.* 2014). For instance, Hart *et al.* (2014) used satellite tags to track movements of endangered loggerhead turtles to determine timing of arrival on foraging grounds and foraging hotspots in the Gulf of Mexico. Additionally, they explored human activities that overlap with these critical foraging habitats and found that shrimp trawling and oil rigs have a potential negative impact on these loggerhead turtles (Hart *et al.* 2014). Knowing the spatio-temporal patterns of use of these critical foraging grounds will help inform placement of marine protected areas.

At bay-scale individual humpback whales returned to a previously known foraging area, centered over four deep-water capelin spawning sites (Davoren 2013). However, capelin shoal characteristics (e.g., size, density) and the number of shoals did not influence the presence of humpback whales in this area. Which was surprising as humpback whales are known to aggregate with large capelin shoals and where capelin is abundant (Whitehead *et al*. 1980; Whitehead 1983). In fact, annual variation in humpback whale abundance was correlated with variation in capelin abundance (Piatt *et al.* 1989). In other foraging grounds, humpback whales have been associated with larger and more dense prey shoals (Friedlaender *et al.* 2006; Hazen *et al.* 2009, Friedlaender *et al.* 2009; Burrows *et al.* 2006). However, in this study spawning date of capelin at deep water sites did influence the presence of humpback whales. Capelin pre-spawning and spawning shoals behave differently, which suggests humpback whales may be queuing in on the behaviour of shoals (Davoren *et al.* 2006). Multi-sensor tags equipped with an accelerometer, magnetometer and gyroscope can be deployed to better understand the behaviour

of humpback whales in relation to capelin characteristics (Hazen *et al.* 2009; Friedlaender *et al.* 2009; Friedlaender *et al.* 2013). With these tags we can begin to explore foraging efficiency and effort of humpback whales that forage on capelin. Additionally, these tags embedded with a camera, can be used to visually determine prey being consumed.

When investigating the diet of humpback whales using stable isotopes, I examined both niche breadth and diet composition. Dietary niche breadth in each year showed very little overlap, suggesting an inter-annual shift in diet. Using year- and species-specific stable isotope ratios of prey to reconstruct dietary proportions, however, it became evident that this shift was due to a shift in isotopic ratios of the prey. Indeed, other ecological studies have found variation in the isotopic niche space of the predator due to baseline isotopic shifts (Grey *et al.* 2004; Solomon *et al.* 2008; Nordström *et al.* 2009; Woodland *et al.* 2012). This study further emphasises the importance of collecting potential prey samples that are represented in the annual or seasonal diet of the predator to appropriately interpret stable isotope ratios and dietary niche. Further studies are also needed to determine the relative importance of capelin and herring in the diet of humpback whales in coastal Newfoundland. As capelin and herring did not differ isotopically in the study area, different methods must be used. For instance, fatty acid analysis can be used to distinguish between these prey types by identifying and quantifying different fatty acids within the prey and consumer tissues (Iverson *et al.* 2004). Indeed, many dietary studies of marine mammals have used fatty acid analysis to differentiate fish species including capelin and herring (Iverson *et al.* 2004) and, thus, would provide insight into the importance of these different prey types in coastal Newfoundland.

In conclusion, spawning capelin are an important prey for humpback whales on the east coast of Newfoundland. Supported by the close association of the inshore presence and spawning

of capelin with the movement and presence of humpback whales at both regional- and bay-scale. Furthermore, forage fish make up over 90% of humpback whale diet across years. The annual predictability of a highly abundant food source likely reduces the energy expended searching for prey patches, maximizing long-term net energy gain. However, more studies are needed to tease apart the importance of capelin for humpback whales and the foraging behaviour of humpback whales on capelin. This study can help gain insight on important prey and foraging areas for humpback whales and other endangered baleen whales that use the coast of Newfoundland as foraging grounds.

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