LONGITUDINAL DIET STUDIES OF ARCTIC WHALES

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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

DOCTOR OF PHILOSOPHY

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ABSTRACT

*An animal's foraging ecology can vary over a range of temporal scales, mirroring seasonal and longer term changes in prey availability, as well as ontogenetic shifts in diet and distribution. Obtaining individual-based, longitudinal diet information through direct observation, however, is logistically challenging for marine mammals that pursue and consume prey underwater, and are often widely distributed. Isotopic profiling along continuously growing tissues like teeth and baleen, which archive dietary inputs at the time of growth in their stable isotope composition, allows for chronological dietary reconstructions over multi-year timespans. This thesis reports longitudinal diet studies of three Arctic whale species, killer whales (*Orcinus orca*), bowhead whales (*Balaena mysticetus*) and beluga whales (*Delphinapterus leucas*), derived from serial isotopic measurements along teeth and baleen. Study objectives varied by species, but general goals were to characterize seasonal, ontogenetic, and/or individual diet variation.*

Results revealed similar trophic-level diet, but regional spatial separation, among eastern Canadian Arctic/Northwest Atlantic killer whales. However, isotope and tooth wear differences between two individuals and the rest of the sampled whales suggested potential specialisation on sharks, while the other whales likely had diets comprising marine mammals. Small within-tooth isotopic variation indicated individual killer whale diets were stable over periods up to 25 yrs.

Cyclic isotopic variation along Eastern Canada-West Greenland bowhead whale baleen was consistent with year-round foraging, although at a reduced rate during winter. Resting zooplankton could be an important food resource outside of periods of peak productivity, and accessibility likely drives winter habitat selection. Isotopic cycling did not differ between female *and male bowheads, or among age classes, indicating similar seasonal foraging patterns despite reported spatial segregation throughout their summer range.*

Individual beluga whales from three eastern Canadian Arctic populations varied in timing of ontogenetic diet shifts (i.e. weaning age), as well as overall trophic position, which could reflect size-specific energetic requirements and foraging capabilities (with larger whales able to dive deeper to access larger, more energetically profitable prey). As with killer whales, low within-tooth isotopic variation indicated long-term individual diet stability, although diet shifts at sexual maturity in males were consistent with dispersal from natal groups. Population-specific beluga whale diet trends over a period of several decades likely reflected climate-related expansions of southern forage fish.

Collectively, findings of seasonal, ontogenetic, and/or individual diet variation contribute a greater understanding of intrapopulation variation in foraging ecology of these species, and of large-scale structuring of Arctic marine ecosystems.

ACKNOWLEDGEMENTS

My academic advisor, Steve Ferguson, supported my research throughout, always making time to discuss research questions and results, finding funding to explore new ideas, and providing opportunities to attend conferences and workshops. Steve promptly reviewed each chapter of this thesis. Norm Halden was a particularly involved committee member who supervised a large component of my Ph.D. research measuring trace element profiles in teeth. This work was ultimately not included in this thesis, but we generated a lot of data that I hope we can revisit. I would also like to thank committee member Gail Davoren for her time and commitment, which always reminded me to consider where my research fit within a broader ecology context. Rob Stewart (DFO, Winnipeg) was an honorary committee member of sorts whose input in the planning stages of my Ph.D. is greatly appreciated. Rob also reviewed Chapter 5.

Thank you to the many Inuit hunters from the eastern Canadian Arctic communities of Arviat, Cape Dorset, Coral Harbor, Grise Fiord, Igloolik, Hall Beach, Iqaluit, Kangiqsujuak, Kugaaruk, Pangnirtung, Pond Inlet, Rankin Inlet, Repulse Bay, and Resolute who collected teeth and baleen specimens in community-based monitoring programs. Blair Dunn (DFO, Winnipeg) arranged shipments and assisted with locating specimens, along with Sheila Atchison and Tara Bortoluzzi (DFO, Winnipeg). Killer whale teeth were generously provided by the Canadian Museum of Nature, the Nova Scotia Museum, the Manitoba Museum, the Royal BC Museum, John Ford (DFO, Nanaimo), Jack Lawson (DFO, St. John’s), and Wayne Ledwell (Portugal Cove, NL).

Many people generously shared their lab space and equipment: Rob Stewart and Blair Dunn (wet saw and sectioning equipment); Al Turnock (University of Manitoba) and Rick Wastle (DFO, Winnipeg) (polishing wheel and sonicator); R. Wastle and Ravinder Sidhu (University of Manitoba) (imaging equipment); and Misuk Yun (University of Manitoba) always found time for me to use the micromill and microbalance. Dave Yurkowski, Brent Young, and Ricardo Ibarra assisted with baleen sample collection, Nick Sader assisted with beluga teeth extraction and cleaning, and Randi Anderson assisted with beluga teeth polishing.

Paul Middlestead, Wendy Abdi, and Patricia Wickham (G.G. Hatch Stable Isotope Laboratory, University of Ottawa) analysed all samples for nitrogen and carbon isotopic composition, and shared their expertise and hospitality during several lab visits. Brian Popp and Natalie Wallsgrove (Stable Isotope Biogeochemistry Laboratory, University of Hawai'i) performed amino acid specific isotope analysis of killer whale samples, and reviewed Chapter 2. Richard Heemskerk and William Mark (Environmental Isotope Laboratory, University of Waterloo) performed sulfur isotopic analysis on baleen samples. I would especially like to thank Panseok Yang of the LA-ICP-MS Laboratory at the University of Manitoba for his mentorship spanning two years of trace element data collection, and R. Sidhu for her assistance mapping calcium distribution in beluga teeth using electron microprobe analysis.

I was lucky to have one of the world's beluga ageing experts, Barb Stewart, conveniently located just down the hall, which made for many *impromptu* visits to her lab to discuss interpretation of beluga dentine growth layers. Barb confirmed age estimates of several specimens, and reviewed Chapter 5. I owe much to the bodies of work by Keith Hobson (Environment Canada, Saskatoon) and Seth Newsome (University of New Mexico), which I referred to often. Rebecca Killick (Lancaster University), Theo Koulis (University of Manitoba), and Marianne Marcoux

(Université du Québec à Rimouski) advised on data analysis and R code. Andy Foote (Uppsala University) reviewed Chapter 2, and Bernard LeBlanc (DFO, Winnipeg) and Nadine Lysiak (Woods Hole Oceanographic Institution) reviewed Chapter 3. Thank you to my labmates for discussions about Arctic ecology and research, and to Cortney Watt in particular, whose love-hate relationship with stable isotope data often fueled my own.

Research funding was provided by the ArcticNet Network of Centres of Excellence of Canada, the Nunavut Wildlife Management Board, and various DFO Canada programs, including Canada’s Species at Risk Act and the Nunavut Implementation Fund. Conference funding was provided by the Stu Innes Award, ArcticNet, the Arctic Research Consortium of the United States, the Society for Marine Mammalogy, and the University of Manitoba’s Departments of Biological Sciences and Graduate Studies and Faculty of Science. Generous scholarship funding was gratefully received from the Duff Roblin Fellowship and the E. Scherer Memorial Scholarship at the University of Manitoba, and the Garfield Weston Foundation.

Finally, thank you to my roster of dogsitters: Dale Bially, Elly Chmelnitsky, Kyle Green, Carie Hoover, Marilyn Kullman, Chantal Sawatsky, Brent Young, and Dave Yurkowski. Only once was I ever truly concerned, when Kyle phoned during the first day of a conference to ask which end of the leash goes around the dog’s head. Oh, and that time Elly nervously phoned me in the field about a ‘huge’ vet bill that turned out to be just $146 (Elly now has her own dog … enough said). Your commitment, especially Dale and Elly (who each took care of Maddy for a cumulative four months), allowed me to spend late nights at the lab, attend conferences, conduct Arctic fieldwork, and enjoy the odd visit home.

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THESIS FORMAT AND MANUSCRIPT CLAIMS

This thesis is presented in manuscript format, with chapters 2, 3, 4, 5, and 6 written with Abstract, Introduction, Materials and Methods, Results, Discussion, and Literature Cited sections. Chapter 1 introduces the overall theme and rationale for the thesis, while chapter 7 summarises its major findings. Student contributions to each chapter are provided below.

CHAPTER 2

Matthews C.J.D. and Ferguson S.H. 2014. Spatial segregation and similar trophic-level diet among eastern Canadian Arctic/north-west Atlantic killer whales inferred from bulk and compound specific isotopic analysis. Journal of the Marine Biological Association of the United Kingdom 94:1343-1355.

C. Matthews formulated the question, wrote funding proposals, designed and performed the sampling, arranged and participated in isotopic analysis, analysed the data, and wrote the manuscript. S. Ferguson provided advice and input at each stage, allocated funding, and reviewed the manuscript during submission and revision stages.

CHAPTER 3

Matthews C.J.D. and Ferguson S.H. Seasonal foraging behaviour of Eastern Canada-West Greenland bowhead whales: an assessment of isotopic cycles along baleen. Marine Ecology Progress Series. In Press.

C. Matthews formulated the question, wrote funding proposals, designed and performed the sampling, arranged isotopic analysis, analysed the data, and wrote the manuscript. S. Ferguson provided advice and input at each stage, allocated funding, and reviewed the manuscript prior to submission.

CHAPTER 4

Matthews C.J.D. and Ferguson S.H. 2014. Validation of dentine deposition rates in beluga whales by interspecies cross dating of temporal $\delta^{13}C$ trends in teeth. NAMMCO Scientific Publications. doi:10.7557/3.3196.

C. Matthews formulated the question, wrote funding proposals, designed and performed the sampling, arranged and participated in isotopic analysis, analysed the data, and wrote the manuscript. S. Ferguson provided advice and input at each stage, allocated funding, and reviewed the manuscript prior to submission.

CHAPTER 5

Matthews C.J.D. and Ferguson S.H. Weaning age variation in beluga whales (*Delphinapterus leucas*). Journal of Mammalogy. Accepted.

C. Matthews formulated the question, wrote funding proposals, designed and performed the sampling, arranged and participated in isotopic analysis, analysed the data, and wrote the manuscript. S. Ferguson provided advice and input at each stage, allocated funding, and reviewed the manuscript prior to submission.

CHAPTER 6

Matthews C.J.D. and Ferguson S.H. Individual specialisation in resource use in a generalist marine predator, the beluga whale. Ecology. In Preparation.

C. Matthews formulated the question, wrote funding proposals, designed and performed the sampling, arranged and participated in isotopic analysis, analysed the data, and wrote the manuscript. S. Ferguson provided advice and input at each stage, allocated funding, and has reviewed the manuscript.

Chapter 1. Temporal diet variation of Arctic whales: longitudinal foraging patterns inferred from isotopic profiles of teeth and baleen

An animal's foraging ecology typically varies over a range of temporal scales, reflecting both extrinsic (e.g. prey availability) and intrinsic (e.g. growth and development) factors. Short- and long-term diet variation is, in turn, linked to other aspects of individual and population biology. For example, many animals undertake long migrations to access seasonally available food resources, and time reproductive events such that maximum energy requirements are synchronized with maximum food availability (Lockyer 1986). Energetic requirements and foraging ability can also change with growth and development, reflected in ontogenetic diet shifts between age classes. Such intraspecific niche partitioning also occurs between sexes and among individuals with distinct dietary preferences (Bolnick et al. 2003), with important consequences for reproductive success (Pierotti and Annett 1987), community structure and food web connectivity (Quevedo et al. 2009), and evolutionary processes such as speciation (Foote et al. 2009, Riesch et al. 2012).

Marine mammals occupy high trophic positions, and can exert considerable influence on prey populations and, consequently, ecosystem structure and function (Williams et al. 2004). Baleen whales, which occupy low trophic positions, can also alter energy flow through food webs by consuming massive amounts of zooplankton and forage fish (Laws 1985). At the same time, foraging behavior is dependent on prey abundance and distribution, which exert bottom-up influence on marine mammal populations (Trillmich and Limberger 1985). Understanding short- (e.g. seasonal) and long-term (e.g. ontogenetic) variation in foraging ecology is therefore essential for understanding ecological roles of marine mammals in their communities. Acquiring longitudinal data required to assess temporal diet variation in marine mammals over extended timeframes, however, is logistically challenging (see Ford et al. 1998, Estes et al. 2003). Underwater pursuit and consumption of prey cannot be observed directly, and extensive seasonal migrations leave large gaps in accessibility. Therefore, seasonal or multi-year diet records for individual animals cannot realistically be compiled through direct observation.

Stable nitrogen and carbon isotope ratios ($\delta^{15}N$ and $\delta^{13}C$, respectively) of consumer tissues offer an indirect means of characterizing diet and foraging behaviour (Newsome et al. 2010). Abiotic and biotic processes in the marine environment, like vertical mixing and phytoplankton growth rates, interact to produce baseline variation in primary producer $\delta^{15}N$ and $\delta^{13}C$ values (e.g. Pancost et al. 1997). Baseline $\delta^{15}N$ and $\delta^{13}C$ values are modified, in turn, with each trophic step due to isotopic fractionation during metabolic reactions. Urea is a ^{15}N depleted by-product of amino acid deamination whose excretion from the body leaves the organism enriched in ^{15}N relative to its diet (DeNiro and Epstein 1981, Macko et al. 1986). Diet-tissue ^{15}N discrimination is relatively large (Minagawa and Wada 1984, McCutchan et al. 2003), making $\delta^{15}N$ values a useful proxy for consumer trophic position. Animal tissues are also enriched in ^{13}C, on average, relative to diet owing to ^{13}C depletion of CO_2 produced during cellular respiration (DeNiro and Epstein 1978). However, diet-tissue $\delta^{13}C$ discrimination is typically much smaller than $\delta^{13}C$ differences among carbon sources within food webs, and consumer $\delta^{13}C$ values are most often used to identify foraging along pelagic vs. benthic (France 1995) or near shore vs. offshore (Walker et al. 1999) gradients. Large-scale variation in both $\delta^{15}N$ and $\delta^{13}C$ along latitudinal gradients (Rau 1982, Graham et al. 2010) also provides spatial context with which marine mammal migration and distribution can be assessed (e.g. Mendes et al. 2007a).

Increasingly, long-term foraging patterns of marine mammals are being inferred from serial isotopic measurements along continuously growing tissues such as vibrissae (Newsome et al. 2009a), claws (Ferreira et al. 2011), baleen (Schell et al. 1989, Hobson and Schell 1998), and teeth (Hobson and Sease 1998, Mendes et al. 2007a, Newsome et al. 2009b). These tissues are metabolically inert once formed, and serial measurements along the axis of growth produce a chronological isotopic profile that can be used in longitudinal foraging reconstructions. Baleen plates of right whales (*Eubalaena* spp.) and bowhead whales (*Balaena mysticetus*) can exceed 3 or 4 m in length, and with an annual growth rate approaching 25 cm (Schell et al. 1989), archive up to 20 years of growth (continual abrasion by the tongue removes growth beyond that period). Baleen is easily sampled at spatial scales corresponding to monthly resolution, and is therefore amenable to studies of seasonal diet variation and migration. The pulp cavity of teeth, on the other hand, progressively fills with dentine, providing a lifelong foraging archive for pinnipeds and toothed whales (although apical tooth wear can result in considerable loss of early growth, and compressed growth layers in later years can be difficult to sample). Dentine annuli, which are typically several hundred microns in width, are commonly sampled whole, producing multi-year profiles of annually-resolved diet suitable to longer term diet studies.

Retrospective characterisation of marine mammal diet from isotopic profiles of teeth and baleen has increased in popularity over the past several years, and at least three broad ecological themes are emerging from the growing body of research using this approach: 1) seasonal diet variation and migration, 2) ontogenetic diet and distribution shifts, and 3) intrapopulation variation in resource use. Most seasonality studies have focused on baleen whales because, in addition to the ease with which baleen can be sampled at sub-seasonal scales, most baleen whales undertake extensive seasonal migrations between regions with different baseline isotopic composition. Further, baleen whales endure prolonged fasts after intensive foraging on seasonally abundant food resources (Lockyer 1981), adding a trophic influence on baleen isotope composition (see Aguilar et al. 2014). Oscillations in $\delta^{15}N$ and $\delta^{13}C$ values along baleen have accordingly been linked with seasonal migrations and fasting-foraging cycles. Schell et al. (1989) first linked regular oscillations in $\delta^{15}N$ and $\delta^{13}C$ values along bowhead whale baleen with seasonal migrations between wintering and summering grounds in the Bering and Beaufort Seas, respectively. Isotopic profiles along baleen have since been used to infer migrations and foraging patterns of a number of species (e.g. Best and Schell 1996, Hobson and Schell 1998, Hobson et al. 2004, Caraveo-Patiño et al. 2007, Lysiak 2009).

Ontogenetic diet and distribution shifts are typically inferred from isotopic profiles of annually-deposited dentine growth layers. Weaning is denoted by a pronounced decline in $\delta^{15}N$ values as offspring transition from a diet of milk, which is maternally-derived and therefore has a higher $\delta^{15}N$ value than the trophic position at which the mother feeds, to one comprising solid food (Jenkins et al. 2001). Researchers have taken advantage of this reliable nursing proxy to characterize weaning age of several pinniped (e.g. Hobson and Sease 1998, York et al. 2008) and odontocete (Newsome et al. 2009b) species, and to compare nursing duration among species with differing maternal investment strategies (Newsome et al. 2006). Ontogenetic shifts in isotopic composition have also been associated with diet and distribution differences between juveniles and adults. For example, Newsome et al. (2009b) attributed $\delta^{15}N$ increases in maturing male killer whales (*Orcinus orca*) to an ontogenetic increase in trophic position, while Mendes et al. (2007b) correlated $\delta^{13}C$ decreases in teeth of male sperm whales (*Physeter macrocephalus*) at sexual maturity with male segregation from natal groups to high-latitude foraging areas. Similar ontogenetic patterns in dentine of female and male elephant seals (*Mirounga leonina*) revealed dispersal to sub-Antarctic or Antarctic regions after juvenile stages, and pronounced trophic position increases in adult males relative to females (Martin et al. 2011).

In addition to short- and long-term *shifts* in diet and distribution, longitudinal foraging records derived from isotopic profiling of continuously growing tissues also allow for assessment of individual diet *consistency*. Because consumer $\delta^{15}N$ and $\delta^{13}C$ values integrate both diet and habitat use, a consumer's 'isotopic niche', or breadth of its isotopic composition, is analogous to its ecological niche (Newsome et al. 2007). Relatively constant $\delta^{15}N$ and $\delta^{13}C$ values over time therefore indicate little variation in resource use (and vice versa). Further, comparison of within- vs. among-individual isotopic variation reveals the degree of intrapopulation variation in resource use, with isotopic separation among individuals reflecting the extent to which their foraging preferences differ. Newsome et al. (2009a) showed the utility of this approach by matching differences in isotopic composition among serially sampled vibrissae of California sea otters (*Enhydra lutris nereis*) with individual foraging specialisations confirmed through years of direct observation. Isotopic profiles have also revealed individual foraging specialisations among other marine consumers, including pinnipeds (Cherel et al. 2009, Hückstädt et al. 2012) and odontocetes (Newsome et al. 2009b).

This thesis is a compilation of longitudinal diet studies of three eastern Canadian Arctic (ECA) whale species, killer whales (*O. orca*), bowhead whales (*B. mysticetus*), and beluga whales (*Delphinapterus leucas*), derived from isotopic profiles of teeth and baleen. Over the past few decades, Arctic sea ice cover has diminished in extent (Comiso 2006), thickness (Rothrock et al. 1999), and seasonal duration (Smith 1998), and these trends are expected to continue at an accelerated pace into the current century (Cavalieri and Parkinson 2003). Sea-ice loss will likely induce changes in timing, distribution, and abundance of primary and secondary production, disrupting tight trophic interactions between prey and higher trophic positions (Laidre et al. 2008). Top-down regulation may also occur as consumers expand their Arctic distributions, reaching areas that were previously inaccessible due to sea ice. Bowhead whales, which are zooplankton specialists (Pomerleau et al. 2012), and belugas, which forage on invertebrates and fish (Kelley et al. 2010, Marcoux et al. 2012), are ice-associated species that may be vulnerable to climate-induced changes in zooplankton and fish community composition. An increased killer whale presence with declining sea ice, on the other hand, may re-shape Arctic food web dynamics from the top down. Understanding the foraging ecology of these species will improve our understanding of current and potential future structuring of Arctic marine ecosystems.

Killer whales are seasonal inhabitants of the ECA, and the regular, possibly annual, visits made by killer whales into ECA waters each summer appear to be associated with seasonal aggregations of other marine mammals (Reeves and Mitchell 1988, Higdon et al. 2011). Increases in killer whale sightings accompanying sea ice declines in some regions of the ECA (Higdon and Ferguson 2009) have led to questions about the ecological impacts of a potential increased killer whale presence in the Arctic, given their implication in population declines of prey species elsewhere (Estes et al. 1998). Although such assessments are controversial (see Kuker and Barrett-Lennard 2010), the role of apex predators in structuring ecosystems is well-recognized (e.g. Myers et al. 2007). A better understanding of killer whale foraging ecology and distribution is therefore required to assess their potential influence on ECA marine ecosystems.

Chapter 2 summarizes research on ECA/northwest Atlantic killer whale diet and distribution using stable isotope ratios in dentine collagen. The general objective was to characterise the degree of dietary specialisation, if any, which occurs within the population(s). Varying degrees of diet specialisation occur in other killer whale populations, ranging from strict specialisation on a narrow prey base (Ford et al. 1998) to more generalist foraging on a variety of marine mammals and fishes (Baird et al. 2006). Although isotopic profiles of teeth have proven an invaluable tool in diet reconstructions, a limitation of this retrospective approach is trophic and spatial (i.e. baseline) isotopic variation cannot be disaggregated using bulk tissue isotope measurements (Popp et al. 2007). This is problematic for marine mammals whose vast regional distributions typically encompass baseline $\delta^{15}N$ and $\delta^{13}C$ variation that can confound trophic interpretations. Amino acid specific $\delta^{15}N$ analysis, which is a relatively new isotopic application (certainly in studies of cetacean foraging ecology), was used to tease apart diet and distribution influences on bulk tissue (dentine collagen) isotope patterns through characterization of 'trophic' and 'source' amino acid $\delta^{15}N$ values (the theory and rationale behind this approach are described in detail in Chapter 2).

In an interesting contrast to killer whales, bowhead whales occupy a year-round distribution in the ECA, where they forage exclusively on zooplankton. Seasonal sea ice and light availability constrain productivity to short pulses during the open water season, when bowhead whales are thought to gain most of their annual energy intake during intensive feeding bouts on grazing zooplankton. Studies from western Greenland (Laidre et al. 2007) and the Bering-Chukchi-Beaufort bowhead population (Citta et al. 2014), however, indicate at least some feeding likely occurs outside of the productive summer months. Bowhead whales are vulnerable to changes in zooplankton phenology and/or community composition, primarily through mismatches between prey availability and timing of migrations and reproductive events, as well as lower energetic value of southern zooplankton species relative to lipid-rich Arctic species (Laidre et al. 2008). A better understanding of their seasonal diet variation is therefore essential for understanding individual energy budgets (i.e. when most of their nutrition needs are met).

Chapter 3 outlines research on seasonal foraging patterns of Eastern Canada-West Greenland (EC-WG) bowhead whales inferred from serial isotopic measurements along baleen plates. $\delta^{15}N$ and $\delta^{13}C$ values are interpreted relative to predictions of isotope dynamics in fasting mysticetes (e.g. Aguilar et al. 2014) and regional baseline isotope variation spanning the EC-WG bowhead range (e.g. Pomerleau et al. 2012). Additionally, stable sulfur isotope ratios ($\delta^{34}S$) were measured to help resolve ambiguous interpretation of $\delta^{15}N$ and $\delta^{13}C$ values due to confounding influences of seasonal migrations between summer and winter ranges. Although rarely measured in marine mammal diet studies (and to our knowledge, not previously measured in baleen), negligible fractionation of ^{34}S with trophic position (McCutchan et al. 2003) and the large isotopic difference between seawater sulphate and sulfides produced in marine sediments (Canfield 2001), can allow discrimination between trophic and spatial sources of consumer $\delta^{34}S$ variation (Connolly et al. 2004). Results from recent studies linking $\delta^{34}S$ variation with protein intake (e.g. Florin et al. 2011) were also considered in evaluations of seasonal foraging scenarios.

Beluga whales, the most abundant cetacean species in the ECA, migrate seasonally between deep offshore waters with heavy ice cover in winter, to shallow coastal areas in summer. Beluga populations exhibit considerable habitat segregation by sex, age class, and reproductive status during the open water season, with females and young located primarily in estuaries, and males occurring offshore (Loseto et al. 2006). Belugas are generalist foragers on a wide range of fish and invertebrate prey, which is thought to make them among the most resilient of Arctic species to climate-induced changes in their prey base (Laidre et al. 2008). However, noted diet differences among spatially segregated components of the population, such as females and males, or different age classes (Loseto et al. 2008), indicate that population segments may respond differently to ecosystem changes.

Chapters 4, 5, and 6 outline studies of long-term isotopic variation in teeth of beluga whales from three ECA populations. Chapter 4 outlines a dendrochronology approach whereby multi-decade isotopic chronologies were constructed at the population level for both belugas and killer whales in the ECA. Long-term trends in δ^{13}C values of both species, thought to reflect the oceanic ^{13}C Suess effect, were compared to provide support for annual GLG deposition in beluga whales. Chapter 5 focuses on isotopic variation over the first five years of dentine deposition to characterise individual variation in weaning age. Chapter 6 addresses further ontogenetic foraging shifts at sexual maturity, but focuses primarily on individual foraging differences spanning periods up to 20+ years.

Chapter 7 is a synthesis of compiled study results, with references to current research questions on the foraging ecology of each species, as well as Arctic ecosystem structure and function.

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Chapter 2. Spatial segregation and similar trophic-level diet among eastern Canadian Arctic/Northwest Atlantic killer whales inferred from bulk and compound specific isotopic analysis

Killer whales in the Eastern Canadian Arctic (ECA) prey on narwhal, beluga, bowhead whales, and seals, while farther south in the Northwest Atlantic (NWA), killer whales off the coast of Newfoundland and Labrador prey on both marine mammals and fish. Bulk and amino acid (AA) specific isotopic composition of dentinal collagen in teeth of 13 ECA/NWA killer whales were analysed to assess the degree, if any, of dietary specialization of killer whales across the region. Dentine was sampled from within annual growth layer groups (GLGs) to construct chronological profiles of stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotopic compositions for individual whales spanning three to 25 years. Interannual isotopic variation across GLGs was less than that among individuals, and median bulk $\delta^{15}N$ values differed by up to 5 ‰ among individuals. Significant correlation between bulk $\delta^{15}N$ values and baseline (source AA) $\delta^{15}N$ values indicates much of the observed isotopic variation among individuals reflected foraging within isotopically distinct food webs, rather than diet differences. This interpretation is supported by consistent differences in bulk $\delta^{13}C$ values between the two individuals with lowest source AA $\delta^{15}N$ values and the remaining whales. After accounting for baseline isotopic variation, comparable $\delta^{15}N$ values among individuals indicates similar trophic-level diet, although uncertainties in relative trophic ^{15}N enrichment of individual AAs currently limits trophic position estimates for top consumers. Future research is required to clarify seasonal movement patterns and possible diet shifts of ECA/NWA killer whales to better define their role in marine ecosystems across the region.

KEYWORDS: *Orcinus orca*, dentine, growth layer groups (GLGs), stable isotope analysis, nitrogen, carbon, amino acids, marine mammals, distribution

INTRODUCTION

Killer whales (*Orcinus orca*, Linnaeus 1758) occupy high trophic levels in marine ecosystems (Jefferson et al. 1991), preying on a diverse range of species including fish, cephalopods, sea turtles, seabirds, and marine mammals (Forney and Wade 2006). While this broad prey range is reflected in generalist diets of some killer whales, for example in waters off Hawaii (Baird et al. 2006), New Zealand (Visser 2000), and South Africa (Best et al. 2010), several killer whale populations worldwide comprise distinct ecotypes or forms with more restricted prey preferences (Ford et al. 1998, Saulitis et al. 2000, Pitman and Ensor 2003, Burdin et al. 2004; Foote et al. 2009, Ford et al. 2011). As apex predators, killer whales can potentially exert important top-down regulation on prey populations (e.g. Estes et al. 1998, but see Kuker and Barrett-Lennard 2010), and conversely, highly specialized foraging leading to dependence on a narrow range of prey can introduce bottom-up control on killer whale population dynamics (Ford et al., 2010). Understanding killer whale foraging behavior is therefore essential to understanding structure and function of marine ecosystems in which they occur.

In the Eastern Canadian Arctic (ECA), where killer whales occur seasonally during the open-water period from July to September, killer whales have been documented feeding on narwhal (*Monodon monoceros*), beluga (*Delphinapterus leucas*), and bowhead (*Balaena mysticetus*) whales, as well as several seal species (Reeves and Mitchell 1988, Higdon et al. 2011). Although marine mammals have been the only confirmed prey of killer whales in the ECA (Higdon et al. 2011, Ferguson et al. 2012a), evidence suggests killer whales in Davis Strait off western Greenland also forage on fish (Heide-Jorgensen 1988, Laidre et al. 2006). In adjacent regions of the Northwest Atlantic (NWA), killer whales off the coast of Newfoundland and Labrador have been documented pursuing or feeding on both marine mammals and fish, including humpback (*Megaptera novaeangliae*) and minke whales (*Balaenoptera acutorostrata*), beluga, white-beaked dolphins (*Lagenorhynchus albirostris*), seals, seabirds, and herring and tuna (Sergeant and Fisher 1957, Whitehead and Glass 1985, Lawson et al. 2008).

Although the broad prey range taken by whales across the ECA and NWA suggests they may be generalist predators, assessment of the degree of individual diet specialization within this population(s) is impeded by a lack of individual foraging records over time. Increasingly, stable isotope (SI) analysis of dentine layers in marine mammal teeth is being used to reconstruct chronological diet records over periods not possible through direct observation. Dentine is laid down in discrete annual growth layer groups (GLGs) consisting of inorganic (hydroxyapatite) and organic (mainly collagen) components derived from diet (Hobson and Sease 1998, Walker and Macko 1999). Post-depositional alteration of dentine does not occur (Bloom and Fawcett 1975), so dentine GLGs represent a lifelong chronological archive along the axis of tooth growth.

Isotopic analysis of sequentially sampled dentine GLGs in marine mammal teeth has revealed ontogenetic distribution and diet patterns (e.g. Hobson and Sease 1998, Mendes et al. 2007a,b, Martin et al. 2011, Riofrío-Lazo et al. 2012), as well as individual diet preferences (e.g. Newsome et al. 2009). Trophic position can be inferred from nitrogen isotope composition ($\delta^{15}N$), owing to metabolic fractionation causing consistent ^{15}N enrichment in consumer tissue relative to prey (DeNiro and Epstein 1981). Incorporation of dietary carbon into consumer tissue occurs with less isotopic fractionation (DeNiro and Epstein 1978), such that more conservative carbon isotope composition ($\delta^{13}C$) across trophic levels primarily reflects underlying biogeochemical processes at the base of the food web. Foraging patterns of marine predators over several spatial scales have been inferred from tissue $\delta^{13}C$ values (Cherel and Hobson 2007, Mendes et al. 2007a), which can reflect differences between benthic vs. pelagic (France 1995) and coastal vs. offshore (Walker et al. 1999) environments, as well as latitudinal $\delta^{13}C$ gradients (e.g. Rau et al. 1982).

Isotopic composition across dentine GLGs of wide-ranging marine mammals also integrates $\delta^{15}N$ variation at the base of the food web during movements among regions with isotopically distinct source nitrogen, which can confound trophic interpretations of $\delta^{15}N$ values. Bulk tissue $\delta^{15}N$ measurements cannot differentiate between baseline $\delta^{15}N$ variation, which can exceed 5–10 ‰ across ocean basins (Montoya et al. 2002, Graham et al. 2010), and that due to trophic ^{15}N enrichment, which typically ranges from 3–5 ‰ with each trophic transfer (Minagawa and Wada 1984, McCutchan et al. 2003). While the two sources of tissue $\delta^{15}N$ variation can be decoupled by characterizing primary producer or consumer $\delta^{15}N$ values, baseline isotopic variation cannot be independently and retroactively resolved across the considerable spatial and temporal scales over which dentine deposition occurs in highly mobile, long-lived species.

Recent studies have shown that compound specific isotope analysis of individual amino acids (AA-CSIA) can be used to constrain baseline influences on bulk tissue $\delta^{15}N$ values. Certain amino acids ('source' AAs, *sensu* Popp et al. 2007) undergo little consumer modification and retain the isotopic value of source nitrogen, while kinetic isotope fractionation during transamination and deamination reactions causes consistent ^{15}N enrichment in other AAs (‘trophic’ AAs, *sensu* Popp et al. 2007) (Gaebler et al. 1966, McClelland and Montoya 2002, Chikaraishi et al. 2007, 2009). AA-CSIA can therefore be used to constrain baseline δ^{15}N variation of a consumer’s foraging habitat via source AA δ^{15}N, and subsequent comparison against trophic AA δ^{15}N allows for internal calibration of trophic position (e.g. McClelland and Montoya 2002, Popp et al. 2007, Chikaraishi et al. 2009). AA-CSIA has been applied in foraging studies of a growing number of marine consumer taxa, including invertebrates (Schmidt et al. 2004, Hannides et al. 2009, O’Malley et al. 2012), teleost fish and elasmobranchs (Popp et al., 2007, Dale et al. 2011, Choy et al. 2012), sea turtles (Seminoff et al. 2012), and seabirds (Lorrain et al. 2009), but has not been rigorously validated for marine mammals (e.g. Germain et al. 2013).

Increases in killer whale sightings in both the ECA and NWA over the past several decades (Lawson and Stevens 2013, Higdon and Ferguson 2009), along with anticipated range expansions accompanying reductions in sea ice extent (Higdon and Ferguson 2009), have underscored the need for a better understanding of killer whale predation in these regions (e.g. Ferguson et al. 2012b). To that end, we measured bulk dentine isotopic composition across GLGs of ECA/NWA killer whales (n = 13), followed by AA-CSIA to constrain potential baseline isotope influences on bulk δ^{15}N values. Our combined bulk and AA-specific isotope analyses allowed us to assess individual isotopic profiles over periods up to 25 years, and indicated killer whales in our sample foraged generally at the same trophic level, but with consistent large scale spatial separation at the individual and regional levels.

MATERIALS AND METHODS

Killer whale tooth specimens

Teeth were collected opportunistically from stranded killer whales across the ECA (n = 6) and NWA (n = 7) over several decades (1948 to 2011), including Cumberland Sound, Hudson Bay/Foxe Basin, Admiralty Inlet, southwestern and southeastern Newfoundland, and Sable Island, Nova Scotia (Figure 2.1). When possible, large teeth with minimal occlusal wear were selected to maximize the number of GLGs available for sampling. All teeth had been stored dry since collection. Although sex and body size measures were available for some individuals, relevant biological information was not available for all (Table 2.1). Killer whale population structure across the ECA and NWA remains unknown, and reference to individuals from either area solely reflects collection location without inference of broad population structure across the region.

Tooth sectioning and ageing

Teeth were sectioned longitudinally to remove an approximately 2-mm thick section following the midline of each tooth. Sections were polished using 30 and 9 µm AlOx lapping film, then placed in 10 % formic acid for 12 h to etch the polished surface and accentuate GLG definition. Sections were rinsed thoroughly with distilled water for several hours following formic acid treatment and air-dried. Acid etching at the tooth’s surface has been assumed not to influence isotope values of underlying dentinal collagen (Hobson and Sease 1998, Newsome et al. 2009).

Annual dentine GLG deposition has been confirmed in killer whales through calibration of tetracycline labelled teeth with treatment history of captive individuals (Myrick et al. 1988), as

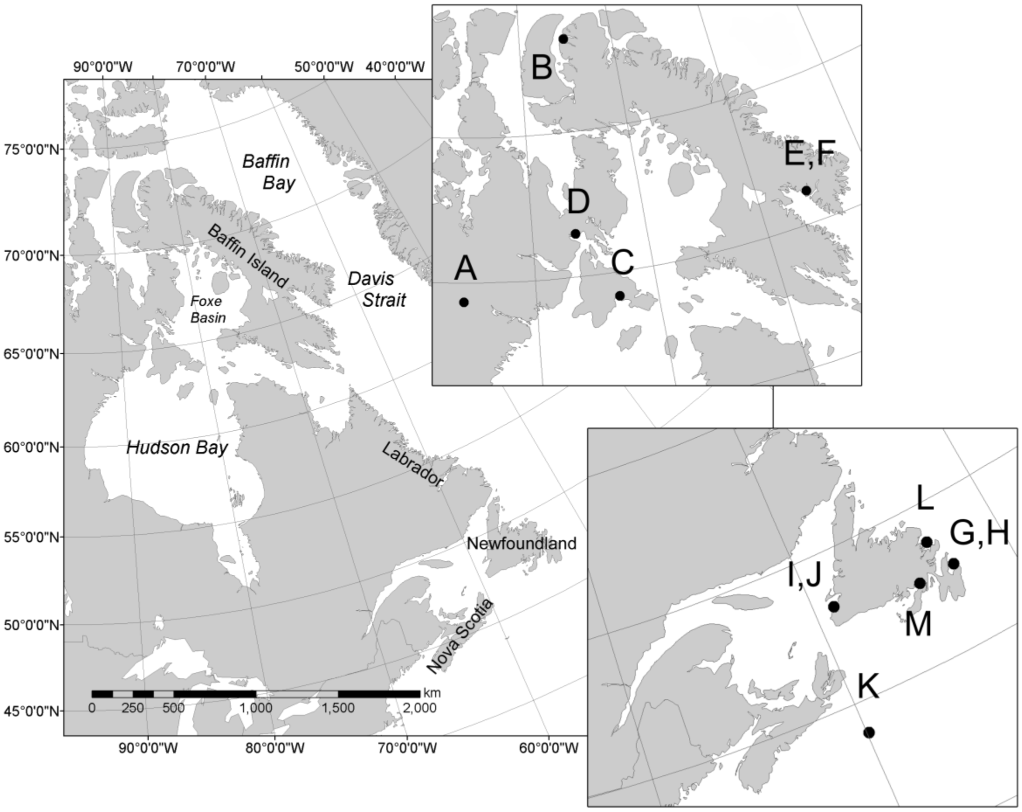

Figure 2.1. Teeth used in this study were collected from killer whale specimens in various locations throughout the eastern Canadian Arctic (ECA) and Northwest Atlantic (NWA) off the coasts of Newfoundland and Nova Scotia. Letters correspond to specimens listed in Table 2.1.

Table 2.1. Estimated age and number of growth layer groups (GLGs) sampled for each Eastern Canadian Arctic (ECA) and Northwest Atlantic (NWA) killer whale tooth, along with location and year sampled.

Individual	**Museum ID**	**Location Collected**	**Year Collected**	**Age**	**Sex***	**# GLGs sampled**
A. ECA-BL-1978	5754-26	Baker Lake, NU	1978	17	M	13
B. ECA-AB-1948	MM406	Arctic Bay, NU	1948	31	U	15
C. ECA-RB-2009		Repulse Bay, NU	2009	28	F	16
D. ECA-CH-2011		Coral Harbor, NU	2011	35	M	23
E. ECA-CS-1997-1		Cumberland Sound, NU	1977	4	U	4
F. ECA-CS-1977-2		Cumberland Sound, NU	1977	18	U	13
G. NWA-CB-1971-1	D119-71	Conception Bay, NF	1971	31	M	15
H. NWA-CB-1971-2	D118-71	Conception Bay, NF	1971	29	F	25
I. NWA-SC-1975-1	VMK5	Ship Cove, NF	1975	20	M	19
J. NWA-SC-1975-2	VMK6	Ship Cove, NF	1975	23	M	12
K. NWA-SI-1977	10783	Sable Island, NS	1977	13	U	12
L. NWA-BB-2002		Bonavista Bay, NF	2002	3	M	3
M. NWA-BP-1998		Burin Peninsula, NF	1998	5	F	5

*U= Sex undetermined

well as comparisons of GLG counts with corpora counts in sexually mature females (Amano et al. 2011) and GLG count with estimated age of a well-known wild killer whale (Mitchell and Baker 1980). Growth layers were observed under reflected light and counted three times by one reader over several weeks to estimate the age of each specimen. Successive readings typically differed by 1 to 3 GLGs, and the median of these measurements was recorded as the age (Table 2.1). Calendar year of GLG deposition was calculated from whale age and year of death to examine longitudinal isotopic trends.

Dentine collection and preparation

A high-resolution micromill (Merchantek) was used to collect dentine from within individual GLGs for bulk isotopic analysis. GLGs were milled using a 500-µm diameter carbide dental drill bit at a depth of 400 to 500 µm to prevent drilling into adjacent layers. Sampling started at the first visible GLG adjacent to the enamel/dentine interface and continued until GLG definition became uncertain or layers adjacent to the pulp cavity became too thin to mill (i.e. < 500 µm wide), resulting in 3 to 25 GLGs sampled per tooth (Table 2.1). Myrick et al. (1988) measured relatively constant dentinal deposition across all months in teeth of captive killer whales, so each sampled GLG is assumed to represent diet integrated over each year of the animal's life from birth year (first GLG sampled) to the final sampled GLG.

Given considerable spatial heterogeneity in $\delta^{15}N$ values across the North Atlantic Ocean basin (Waser et al. 2000, Graham et al. 2010) and the potentially large range of ECA/NWA killer whales (e.g. Matthews et al. 2011), AA-CSIA was performed to constrain baseline isotope influences on bulk $\delta^{15}N$ values. Consistent bulk collagen $\delta^{15}N$ values across GLGs within individuals (see ‘Results’) allowed us to collect representative ‘whole-tooth’ dentine samples to provide sufficient material for AA-CSIA. Dentine was milled perpendicular to the axis of GLG growth using a 1-mm diameter drill bit, encompassing all GLGs except for the youngest three, which showed isotopic variation related to weaning (see ‘Results’).

Collagen was isolated from powdered dentine samples using repeated rinses (12 h each) of 0.25 N HCl at 4 ^{o}C. Dentine was demineralized after two to three acid rinses, and remaining collagen was washed using successive rinses of deionized water. Samples were centrifuged between each rinse to minimize sample loss, and freeze-dried for 48 h after the final rinse. Collagen atomic C:N (mean ± sd = 2.9 ± 0.1; range = 2.8 to 3.3) was within the range of unaltered collagen (DeNiro 1985), indicating adequate removal of inorganic carbon during acid rinses.

Stable isotope analysis

BULK STABLE ISOTOPE ANALYSIS

GLG collagen samples (~0.5 mg) were weighed into tin cups for isotopic analysis on a Vario EL III elemental analyser (Elementar, Germany) interfaced with a DELTAplus XP isotope ratio mass spectrometer (Thermo, Germany) at the G.G. Hatch Stable Isotope Laboratory, University of Ottawa. Isotope ratios are reported in delta notation (δ; units are per mil, ‰), defined as δ^{15}N or δ^{13}C = (R_{sample} - $R_{standard}$) / $R_{standard}$) * 1000, where R is ^{15}N/^{14}N or ^{13}C/^{12}C. All isotope values are normalized to international standards (atmospheric N_2 for δ^{15}N and Vienna Pee-Dee Belemnite limestone for δ^{13}C) calibrated through repeated measures of laboratory reference materials. Analytical precision based on repeated measures of reference material not used in calibrations was 0.05 ‰ for δ^{15}N and 0.06 ‰ for δ^{13}C, and that based on duplicate measures of ~15 % of samples was 0.11 and 0.07 ‰ for δ^{15}N and δ^{13}C, respectively.

AMINO ACID COMPOUND SPECIFIC ISOTOPE ANALYSIS

Approximately 2.5-5.5 mg of each whole-tooth collagen sample was acid hydrolysed and derivitised to produce trifluoroacetic AA esters following procedures described in Dale et al. (2011). δ^{15}N values of derivitised AAs were measured on a Thermo Scientific Delta V Plus mass spectrometer interfaced with Thermo Finnigan Trace GC gas chromatograph via a Thermo Finnigan GC-C III combustion/reduction system at the Stable Isotope Biogeochemistry Laboratory, University of Hawai’i. All samples were analysed in triplicate and normalised relative to co-injected reference compounds of known isotopic composition (L-2-aminoadipic acid and L-(+)-norleucine). Co-elution and interference prevented measurement of some AAs, but reliable measurements were obtained for 13 individual AAs. Mean analytical precision based on repeated measures of the two reference compounds was 0.46 ‰, while that based on triplicate measures of each sample was 0.32 ‰ (range: 0.02-1.21 ‰). AA-specific δ^{15}N values are reported relative to isotopic composition of atmospheric N_2.

Data analysis

TEMPORAL ISOTOPIC TRENDS ACROSS GLGs

Potential sources of temporal SI variation across dentine GLGs include whale age, in terms of ontogenetic diet shifts (e.g. Newsome et al. 2009) and growth-related variation in diet-tissue SI discrimination (e.g. Trueman et al. 2005), as well as variation in baseline SI over the broad timeframe represented by sampled GLGs. Generalized linear mixed effects models with random intercepts were used to assess bulk δ^{15}N and δ^{13}C profiles across GLGs with respect to these variables. The variable ‘Decade’ (1920s to 2000s) was constructed from calendar year of GLG formation and treated as a fixed effect, along with GLG (age). Whale identity was included as a random effect, allowing models to account for correlation of repeated measures within individuals. Models were run using the maximum likelihood method, and the best-fit model was selected based on ANOVA results comparing full and reduced models. Statistical significance was assessed at $p < 0.05$, and analyses were performed using the nlme package (Pinheiro et al., 2012) available for R software (R Core Team 2012).

BULK ISOTOPIC VARIATION AMONG INDIVIDUALS

Differences in bulk $\delta^{15}N$ and $\delta^{13}C$ among individuals were assessed using one-way repeated measures ANOVA on rank-transformed data (Conover and Iman 1981) using the nlme package in R (Pinheiro et al. 2012), followed by Tukeys HSD *post-hoc* tests using the multcomp package in R (Hothorn et al. 2008). The systematic decrease in $\delta^{15}N$ values over the first three GLGs (see ‘Results’) was interpreted as a weaning signal (e.g. Newsome et al. 2009) and removed from each profile before further analysis to ensure comparison of diet after completion of nursing. This decision resulted in two individuals (ECA-CS-1977-1 and NWA-BB-2002) being dropped from the dataset due to their young age. The number of remaining GLGs for a third individual (NWA-BP-1998) was insufficient for statistical comparisons. Although killer whales show other ontogenetic SI patterns across dentine GLGs which may not be entirely diet-related (Newsome et al. 2009), we did not observe any clear age-related patterns beyond the first three GLGs. Comparisons among individuals therefore included entire profiles beyond the third GLG. We applied a *post-hoc* correction of 0.019 ‰ yr^{-1} to $\delta^{13}C$ values ($\delta^{13}C_{cor}$) to account for the oceanic ^{13}C Suess effect in the North Atlantic (Quay et al. 2003) over the period of GLG deposition.

AA-SPECIFIC δ^{15}N – BASELINE δ^{15}N AND TROPHIC POSITION INDICES

Because bulk and AA-specific isotopic measurements were conducted at different labs on differently sampled material (i.e. individual GLGs vs. whole-tooth), we compared bulk collagen δ^{15}N values with those calculated from individual AA δ^{15}N values using a mass balance approach. The δ^{15}N value of each individual AA was multiplied by its percent contribution to total dentinal collagen nitrogen, which was determined from AA % composition of dentinal collagen (Eastoe 1963) multiplied by weight % N of each individual AA. Contributions of each individual AA, which together represented ~80.4 % of total collagen N, were then summed. Correlation between calculated and measured bulk collagen δ^{15}N values was determined using linear regression.

Source AAs phenylalanine, glycine, and serine, and trophic AAs alanine, leucine, proline, aspartic acid, and glutamic acid were used to assess baseline and trophic contributions to bulk collagen δ^{15}N patterns. To clarify baseline SI influences on bulk δ^{15}N values, linear regression of mean bulk GLG δ^{15}N values against mean source AA δ^{15}N values was performed. Generalized linear mixed effects models with random intercepts and slopes were also fit to rank-transformed source AA δ^{15}N values (dependent variable) along groupings identified in Figure 2.3 (fixed effect), with whale identity set as a random effect to account for multiple AA measures from each individual. Tukeys HSD *post-hoc* tests were performed to identify pairwise differences. AA-CSIA also provided an additional check on temporal baseline δ^{15}N variation over the timeframe of the study through linear regression of mean source AA δ^{15}N values against calendar year. Regression analyses were performed using the R Stats Package (R Core Team 2012).

Differences in trophic AA δ^{15}N values among individuals were investigated using ANOVA as per source AA. Trophic position of marine consumers has been estimated using the difference in δ^{15}N values of glutamic acid ($\delta^{15}N_{Glu}$) and phenylalanine ($\delta^{15}N_{Phe}$) using

$$\text{Trophic Position} = \frac{(\delta^{15}\mathrm{N}_{\mathrm{Glu}} - \delta^{15}\mathrm{N}_{\mathrm{Phe}}) - 3.4}{7.6} + 1 \qquad (2.1)$$

to account for the isotopic difference between the two AAs in primary producers (β, 3.4 ‰) and trophic enrichment ($TEF_{Glu\text{-}Phe}$, 7.6 ‰) (Chikaraishi et al. 2009). This calculation was derived largely from experimental studies on invertebrates and fish, and recent studies have suggested that a $TEF_{Glu\text{-}Phe}$ of 7.6 ‰ results in lower than anticipated trophic position estimates of higher marine consumers (Lorrain et al. 2009, Dale et al. 2011), including marine mammals (Germain et al. 2013). Given equation (2.1) also results in trophic position estimates too low for killer whales (see 'Results'), we used simpler 'trophic indices' to compare relative trophic level differences among individual killer whales. We estimated relative trophic position using the difference in δ^{15}N values of the primary trophic (glutamic acid) and source (phenylalanine) AAs ($\Delta\delta^{15}N_{Glu\text{-}Phe}$) (McLelland and Montoya 2002, Chikaraishi et al. 2009), as well the difference in mean δ^{15}N values of multiple trophic and source AAs ($\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$) (e.g. Popp et al. 2007, McCarthy et al. 2007, Hannides et al. 2009, Seminoff et al. 2012), which can compensate for uncertainty in any single AA measurement.

RESULTS

Temporal isotopic trends across GLGs

Decade of GLG formation was not retained as a significant predictor of δ^{15}N values in generalized linear mixed effects models ($p > 0.1$), and there was no temporal trend in source AA δ^{15}N values over the study period (linear regression, adj R^2 –0.030, $p > 0.4$). δ^{13}C values, on the other hand, showed a clear linear decline over the same period (data not shown), and decade of GLG formation was a significant predictor of δ^{13}C values ($p < 0.001$). However, models did not retain decade as a significant explanatory variable of $\delta^{13}C_{cor}$ ($p > 0.2$).

Best-fit generalized linear mixed effects models indicated GLG (age) was a significant predictor of δ^{15}N values ($p < 0.005$), but not $\delta^{13}C_{cor}$ values ($p > 0.6$). Teeth of most individuals showed a decrease in δ^{15}N values over the first 1 to 3 GLGs of ~1 to 2 ‰ (Figure 2.2). Although adjacent GLGs differed by up to 2.1 ‰, no clear δ^{15}N patterns were observed beyond the first three GLGs in most teeth. There were no discernible ontogenetic $\delta^{13}C_{cor}$ patterns, although values differed by up to 1.1 ‰ between adjacent GLGs (Figure 2.2).

Bulk isotopic variation among individuals

Bulk δ^{15}N values differed among individuals ($F = 29.91$, $p < 0.0001$, df = 9). Tukeys *post-hoc* comparisons indicated significant differences among whales within and between collection regions (Figure 2.3). Four ECA individuals had significantly higher δ^{15}N values than all other individuals (median δ^{15}N range: 17.7 to 18.4 ‰, adj $p < 0.01$), but did not differ among each other (adj $p > 0.7$). δ^{15}N values were similar among five individuals collected in the NWA (median δ^{15}N range: 16.3 to 17.1 ‰) (adj $p > 0.2$). δ^{15}N values of one ECA individual (median:

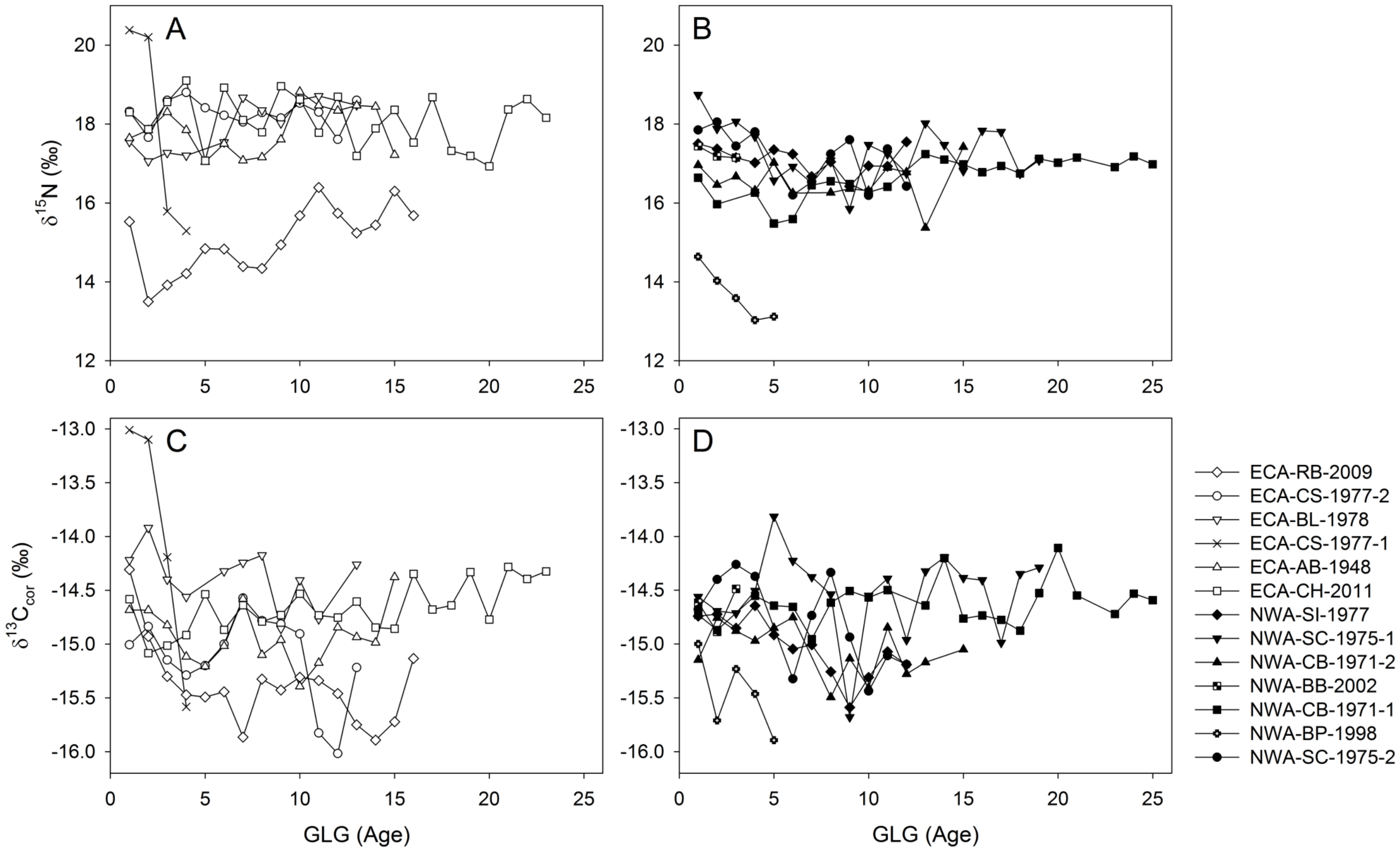

Figure 2.2. δ^{15}N values of individual dentinal growth layer groups (GLGs) in teeth of eastern Canadian Arctic (ECA) (A) and Northwest Atlantic (NWA) (B) killer whales show consistent long-term separation of individuals. Similar profiles for $\delta^{13}C_{cor}$ values (*post-hoc* adjustment of 0.019 ‰ yr^{-1} to account for oceanic Suess effect; panels C and D) show less distinction among individuals.

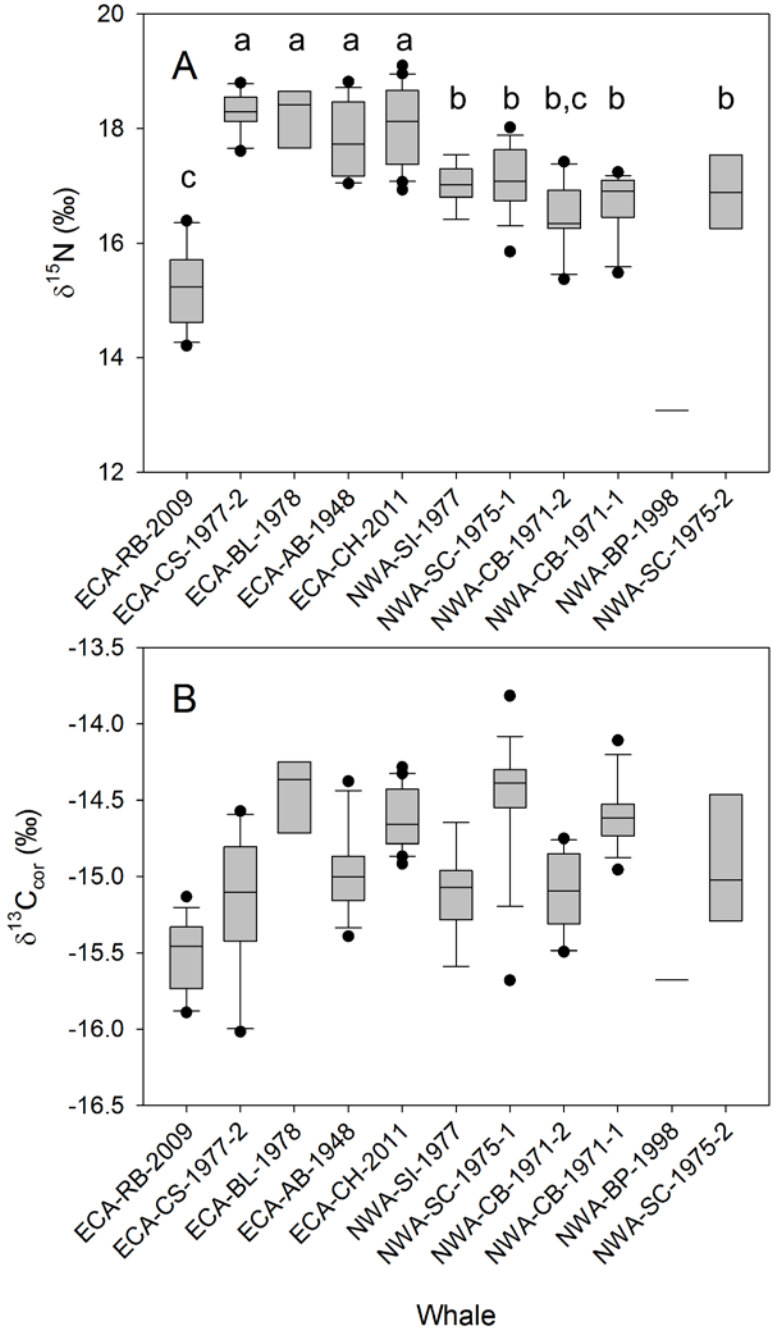

Figure 2.3. Box-and-whisker plots of median dentinal GLG $\delta^{15}N$ (A) and $\delta^{13}C_{cor}$ values (B) (first three GLGs not included) in teeth of eastern Canadian Artic (ECA) and Northwest Atlantic (NWA) killer whales. Horizontal lines represent the median, box width represents the interquartile range (IQR), and whiskers mark the 10th and 90th percentiles. Distinct grouping of individuals noted with $\delta^{15}N$ values (indicated by lower case letters) were not reflected in $\delta^{13}C_{cor}$ values.

15.2 ‰) were significantly less than all but one of the individuals of the previous 'groups' (adj $p < 0.01$) (Figure 2.3). The remaining NWA individual (NWA-BP-1998) had lower $\delta^{15}N$ (13.1 ‰) than all other individuals (Figure 2.3).

While $\delta^{13}C_{cor}$ values differed among individual whales ($F = 15.62$, $p < 0.0001$, df = 9), *post-hoc* analyses indicated this result was driven primarily by individual ECA-RB-2009. This whale had significantly lower $\delta^{13}C_{cor}$ values than all individuals (adj $p < 0.01$) except ECA-CS-1977-2 (adj $p > 0.3$) and NWA-SI-1977 (adj $p > 0.5$). While significant differences in $\delta^{13}C_{cor}$ values occurred between several of the remaining whales, for the most part, similar $\delta^{13}C_{cor}$ values among most individuals (adj $p > 0.25$) did not reflect the clear distinctions among individuals and between regions noted for $\delta^{15}N$ (Figure 2.3). $\delta^{13}C_{cor}$ of whale NWA-BP-1998 was lower than that of most other whales, as it was for $\delta^{15}N$.

Source AA and trophic position indices

Individual AA $\delta^{15}N$ values spanned a range of ~25 ‰ (Table 2.2). Trophic AAs (alanine, leucine, proline, aspartic acid, and glutamic acid) had higher $\delta^{15}N$ values (mean 23.92 ± 2.0, range 17.80 to 27.46 ‰) than source AAs phenylalanine, glycine, and serine (mean 11.13 ± 0.98, range 3.00 to 15.59 ‰) (Table 2.2, Figure 2.4). Bulk collagen $\delta^{15}N$ values calculated from mass balance of individual AA $\delta^{15}N$ values correlated strongly with mean bulk collagen $\delta^{15}N$ values measured across GLGs (adj R^2 0.89, $p < 0.001$), although with a consistent offset of –1 to –2 ‰.

Table 2.2. δ^{15}N values ± SD (‰) of trophic and source amino acids (AA) in dentinal collagen of Eastern Canadian Arctic (ECA) and Northwest Atlantic (NWA) killer whales.

		Eastern Canadian Arctic (ECA)					**Northwest Atlantic (NWA)**					
		ECA-RB-2009	ECA-CS-1977-2	ECA-BL-1978	ECA-AB-1948	ECA-CH-2011	NWA-SI-1977	NWA SC-1975-1	NWA CB-1971-2	NWA CB-1971-1	NWA BP-1998	NWA SC-1975-2
Trophic AA	Alanine	23.97±0.12	25.17±0.03	24.14±0.07	24.32±0.54	22.99±0.24	23.86±0.04	23.60±0.48	24.92±0.10	23.99±0.30	20.51±0.60	23.94±0.17
	Leucine	23.56±0.25	24.24±0.54	23.84±0.30	24.19±0.23	22.98±0.06	22.71±0.64	22.73±0.65	22.85±0.13	23.57±0.14	20.02±0.18	23.00±0.05
	Proline	25.73±0.32	26.41±0.07	25.85±0.25	27.36±0.19	27.44±0.08	24.76±0.06	27.05±0.15	26.48±0.25	25.47±0.13	21.07±0.27	27.01±0.07
	Aspartic acid	21.11±0.24	21.02±0.33	20.26±0.18	21.76±0.55	20.98±0.16	20.63±0.18	20.33±0.42	20.44±0.12	20.67±0.47	17.80±0.35	20.44±0.28
	Glutamic acid	25.64±0.18	25.94±0.25	24.47±0.14	26.88±0.27	25.25±0.37	25.07±0.18	26.19±0.16	24.12±0.36	25.71±0.02	21.80±0.15	26.34±0.23
	Mean Trophic AA	24.00±0.52	24.56±0.72	23.71±0.57	24.90±1.07	23.93±0.69	23.41±0.89	23.98±0.96	23.76±0.49	23.88±0.60	20.24±1.21	24.15±1.28
Source AA	Phenylalanine	8.05±0.22	13.85±0.25	11.12±0.35	12.31±0.45	13.89±0.15	12.77±0.40	8.57±0.53	10.02±0.48	11.06±0.30	3.00±0.21	7.44±0.36
	Glycine	8.43±0.23	11.89±0.12	11.10±0.41	12.92±0.20	12.70±0.03	9.81±0.14	12.53±0.09	12.64±0.35	11.63±0.29	6.09±0.37	12.03±0.12
	Serine	11.45±0.30	13.89±0.21	13.03±0.57		15.59±0.16	12.56±0.46	13.68±0.13	12.18±0.56	12.74±0.28	7.16±0.82	14.59±0.31
	Mean Source AA	9.31±0.64	13.21±0.53	11.75±0.93	12.62±0.49	14.06±0.43	11.71±0.80	11.59±0.65	11.61±0.96	11.81±0.67	5.422±1.00	11.35±0.67
Trophic Index	$\Delta\delta^{15}N_{Glu\text{-}Phe}$	17.59±0.28	12.09±0.35	13.35±0.38	14.57±0.52	11.37±0.40	12.30±0.44	17.63±0.56	14.10±0.61	14.65±0.30	18.80±0.26	18.91±0.43
	$\Delta\delta^{15}N_{\Sigma trophicAA\text{-}\Sigma sourceAA}$	14.69±0.82	11.35±0.90	11.96±1.09	12.28±1.18	9.87±0.81	11.69±1.20	12.39±1.16	12.15±1.07	12.07±0.90	14.82±1.57	12.79±1.44

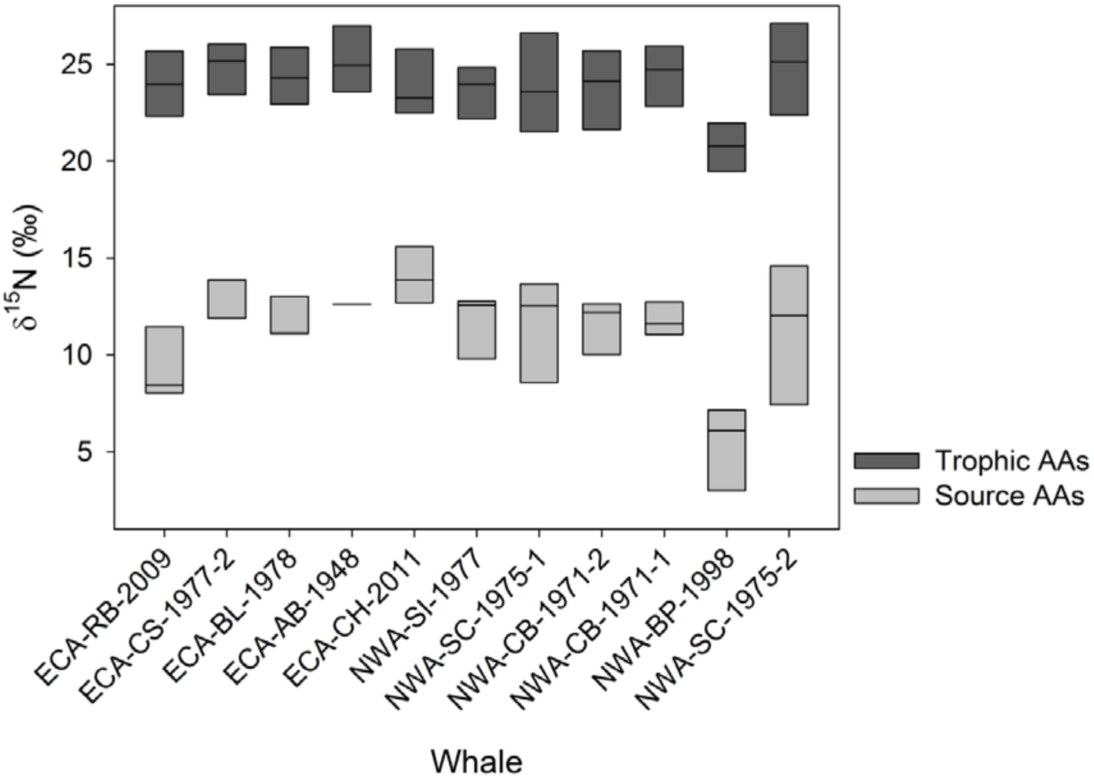

Figure 2.4. Box plots of median trophic (alanine, leucine, proline, aspartic acid, and glutamic acid) and source (phenylalanine, glycine, and serine) amino acid (AA) $\delta^{15}N$ values from 'whole-tooth' dentinal collagen of eastern Canadian Arctic (ECA) and Northwest Atlantic (NWA) killer whales show typical separation between the two types of AAs, reflecting ^{15}N enrichment of trophic relative to source AAs.

Mean bulk GLG and mean source AA $\delta^{15}N$ values were significantly correlated (linear regression, adj R^2 0.91, $p < 0.001$, unstandardized regression coefficient [B] 0.64 ± 0.06 [s.e.]) (Figure 2.5). This relationship remained significant (adj R^2 0.45, $p < 0.05$, B 0.54 ± 0.19) after removal of the two whales with the lowest bulk and source AA $\delta^{15}N$ values (Figure 2.5). Groupings identified by differences in bulk $\delta^{15}N$ values (Figure 2.3) differed similarly in their source AA $\delta^{15}N$ values (ANOVA, F = 11.07, $p < 0.005$, df = 3). ECA individuals with high bulk $\delta^{15}N$ values (group 'a', Figure 2.3) had significantly higher source AA $\delta^{15}N$ values than NWA whales with high bulk $\delta^{15}N$ values (group 'b') ($p < 0.05$), as well as whales ECA-RB-2009 ('c') ($p < 0.001$) and NWA-BP-1998 ($p < 0.001$). Whales ECA-RB-2009 and NWA-BP-1998 had lower source AA $\delta^{15}N$ values than NWA whales (group 'b') ($p = 0.05$ and 0.004, respectively), but did not differ from each other ($p > 0.8$).

Trophic AA $\delta^{15}N$ values differed among individuals (ANOVA, F = 3.35, $p < 0.05$, df = 3), although *post-hoc* tests indicated this was entirely driven by significant differences between whale NWA-BP-1998 and each of groups 'a', 'b', and 'c' identified in Figure 2.3 ($p < 0.001$). Trophic AA of the remaining groups did not differ ($p > 0.2$). Variation in trophic indices ($\Delta\delta^{15}N_{\text{Glu-Phe}}$ and $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$) among individuals (Table 2.2) was therefore driven by source AA $\delta^{15}N$ rather than trophic AA $\delta^{15}N$. Among ECA whales, ECA-RB-2009 had the greatest $\Delta\delta^{15}N_{\text{Glu-Phe}}$ (17.59 ‰). $\Delta\delta^{15}N_{\text{Glu-Phe}}$ of the remaining ECA whales ranged from 11.37 to 14.57 ‰ (Table 2.2). Similar variation was observed among NWA whales, with whales NWA-SC-1975-2, NWA-BP-1998, and NWA-SC-1975-1 having greater $\Delta\delta^{15}N_{\text{Glu-Phe}}$ (18.91, 18.80, and 17.63 ‰, respectively) than the other NWA whales (range 12.30 to 14.65 ‰) (Table 2.2).

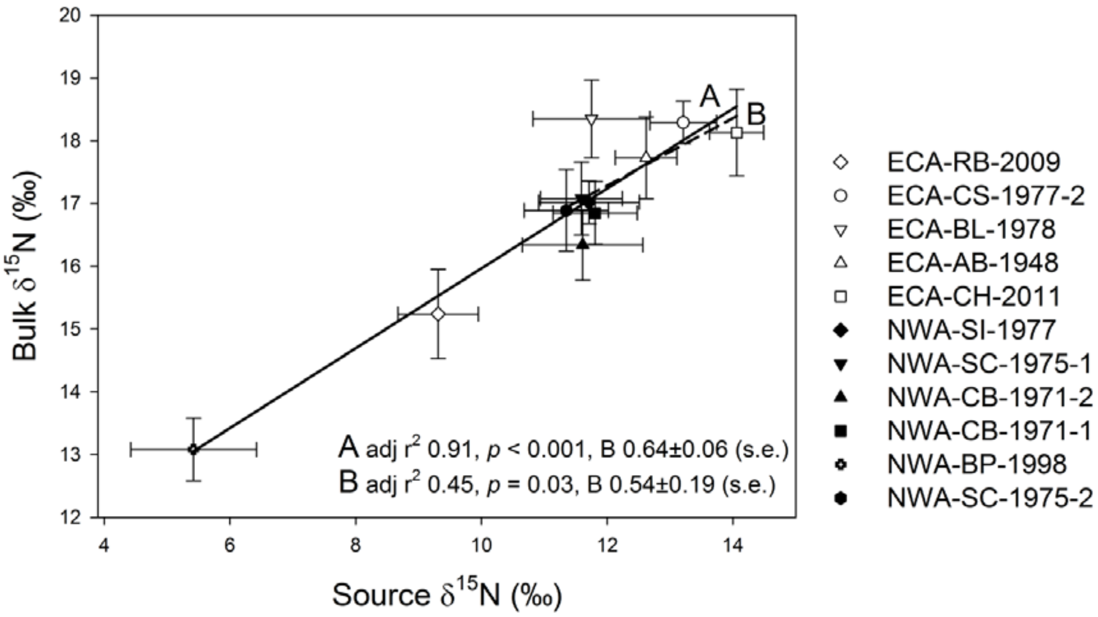

Figure 2.5. Simple linear regression of mean bulk GLG vs. mean source AA δ^{15}N values shows up to 91% of variation in bulk δ^{15}N is explained by isotopic variation in source AA among individuals (A, solid line). The relationship holds when whales ECA-RB-2009 and NWA-BP-1998 are not included in the regression (B, dashed line). Regression coefficients (slopes) are similar for both.

Trophic positions calculated using equation (2.1) ranged from 2.1 ± 0.1 to 3.0 ± 0.1 (data not shown).

$\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ values were more consistent among individuals than $\Delta\delta^{15}N_{\text{Glu-Phe}}$ values (Table 2.2). Among ECA whales, ECA-RB-2009 had a greater $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ (14.69‰) and ECA-CH-2011 had a lower $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ (9.87 ‰) relative to the other ECA whales (11.35 to 12.28 ‰; Table 2.2). Among NWA whales, NWA-BP-1998 had a greater $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ (14.82 ‰) than the remaining whales (11.69 to 12.79 ‰; Table 2.2).

DISCUSSION

Longitudinal $\delta^{15}N$ and $\delta^{13}C$ profiles across dentinal GLGs indicated consistent isotopic differences among ECA and NWA killer whales at individual and regional levels. While we recognize complex ecosystem-level processes can influence baseline isotope values over the period of GLG deposition (1917 to 2000), the lack of temporal patterns in bulk GLG and source AA $\delta^{15}N$ values over the 80-year study period indicated isotopic differences among individuals were not related to temporal isotopic variation, and that observed for $\delta^{13}C$ values was attributed to the oceanic ^{13}C Suess effect. Analysis of AA-specific $\delta^{15}N$ values, however, showed as much as 91 % of the bulk $\delta^{15}N$ variation among individuals was due to baseline (source AA) $\delta^{15}N$ variation, rather than diet differences. Results therefore indicate killer whales included in our sample foraged consistently at similar trophic levels, but within food webs with distinct baseline isotopic values.

Our assessment of the degree to which bulk isotopic differences among individuals reflect baseline vs. trophic-level diet differences depends on interpretations of AA-specific $\delta^{15}N$ values. While its application to marine mammal foraging studies has not been rigorously validated, AA-CSIA has been used successfully to decouple baseline vs. trophic influences on bulk $\delta^{15}N$ in a variety of other marine consumers. AA-specific $\delta^{15}N$ patterns in this study generally follow that of previous studies on marine birds and mammals. Lorrain et al. (2009) reported a $\delta^{15}N$ difference between mean trophic and source AA in penguin blood of ~13 ‰, while that between trophic and source AAs in harbour seal serum reported in Germain et al.'s (2013) study is ~11-12 ‰ (calculated from values presented in Table 2.1 using the same trophic and source AAs measured in this study). Relative differences between trophic and source AA $\delta^{15}N$ values in this study are also similar to $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ of bone collagen of an unidentified whale and two Cape fur seals (*Arctocephalus pusillis*) (~13 ‰; Styring et al. 2010). We therefore proceed with interpretation of AA-specific $\delta^{15}N$ results while recognizing this method has not yet been validated in marine mammals, and that further research, especially in terms of trophic position estimation, is required (see below).

While all AA can undergo deamination leading to ^{15}N enrichment of the remaining AA pool (Macko et al. 1986), the negligible trophic fractionation of ~0.4 ‰ between diet and zooplankton $\delta^{15}N_{Phe}$ values (McClelland and Montoya 2002) appears to be conserved in higher marine consumers (e.g. Styring et al. 2010, Naito et al. 2010). In the only controlled feeding study on AA-specific trophic ^{15}N enrichment in marine mammals, Germain et al. (2013) reported similar $\delta^{15}N_{Phe}$ values between captive harbor seals (*Phoca vitulina*) ($\delta^{15}N_{Phe}$ range: 9.1±1.0 to 12.7±0.9 ‰) and their diet comprising Atlantic herring (*Clupea harengus*) ($\delta^{15}N_{Phe}$ 11.3±4.4 ‰). The strong linear relationship between bulk and source AA $\delta^{15}N$ values in killer whale dentine therefore most likely reflects foraging within regions with distinct baseline δ^{15}N values (e.g. Hannides et al. 2009). Measured source and trophic AAs account for similar amounts of total dentinal collagen nitrogen (Eastoe 1963), so the correlation between bulk and source AA δ^{15}N is not simply a reflection of greater contribution of the latter to total collagen nitrogen. While generally poor resolution of baseline isotopic variation across the NWA limits interpretations within a spatial context, Graham et al. (2010) reported a gradual decrease in δ^{15}N values in upper water column plankton from the high Arctic to more southern latitudes in the North Atlantic. The low δ^{15}N values of ECA-RB-2009 and NWA-BP-1998 relative to the other whales could therefore reflect consistent foraging within food webs at lower latitudes in the North Atlantic Ocean, a plausible scenario given the track of a killer whale recently satellite-tagged in the ECA (Matthews et al. 2011) spanned a gradient in δ^{15}N values similar to the range in source AA δ^{15}N values among individuals measured in this study.

Mid-latitude foraging in the North Atlantic by these two whales is also supported by their relatively low $\delta^{13}C_{cor}$ values, which indicate they foraged primarily within a region(s) characterized by distinctly lower baseline δ^{13}C values. Although δ^{13}C values in the North Atlantic generally increase with decreasing latitude, a large region of relatively lower δ^{13}C values at ~30-40 ^{o}N (Graham et al. 2010) also coincides with locations of the satellite-tagged killer whale (Matthews et al. 2011). Other possibilities that could potentially account for relative differences in $\delta^{13}C_{cor}$ values (but may be difficult to reconcile with concurrent δ^{15}N patterns) include foraging along a coastal-offshore gradient (e.g. Walker et al. 1999), or in Northeast Atlantic waters off eastern Greenland, Iceland, and the British Isles, where zooplankton δ^{13}C values are lower than off Newfoundland and Nova Scotia (but not relative to the ECA) (Graham et al. 2010). Although impossible to narrow potential distributions with certainty, we speculate whales ECA-RB-2009 and NWA-BP-1998 had a different over-wintering range than the other killer whales, or could have been infrequent visitors to the ECA or coastal Newfoundland.

Significant bulk $\delta^{15}N$ differences between remaining ECA and NWA whales (groups ‘a’ and ‘b’ in Figure 2.3) due largely to baseline isotopic variation provides support for separate, largely non-overlapping populations of killer whales within the ECA and NWA, at least during some portion of the year. Currently little is known about seasonal movements of ECA and NWA killer whales, and whether they move regularly between the two areas or share a common winter range. Higdon (2007) found little evidence for large-scale migrations from southern latitudes into the ECA during summer months given killer whale sightings occur along a range of latitudes throughout the ECA and NWA over that period, and to date, no re-sightings of photo-identified killer whales have occurred between the ECA and NWA (Young et al. 2011). Considerable ^{15}N enrichment in ECA marine mammals such as narwhal, beluga, and ringed seal ($\delta^{15}N$ ~16–18 ‰; Hobson and Welch 1992) relative to those occupying similar trophic position along the coast of Newfoundland such as common dolphin (*Delphinus delphis*) and harp seals (*Pagophilus groenlandicus*) ($\delta^{15}N$ ~14–15 ‰; Ostrom et al. 1993, Lawson and Hobson 2000) suggests baseline $\delta^{15}N$ values are higher in the ECA. Regular seasonal foraging trips into Arctic waters by ECA killer whales would have allowed them to prey on marine mammals with relatively higher $\delta^{15}N$ values (but not higher trophic position), which could account for the observed differences in $\delta^{15}N$ values if residency time within the ECA and foraging intensity was of sufficient duration to be recorded by dentine growth. Energy intake of killer whales can be greater during seasonal periods of prey availability (Baird and Dill 1995), and this is likely the case for killer whales foraging on seasonally predictable aggregations of marine mammals in the ECA (Ferguson et al. 2012b).

Baseline $\delta^{15}N$ differences could also reflect spatial segregation throughout the year, given each micromilled GLG sample represents diet integrated over an entire year of GLG deposition. Killer whales are not regularly sighted in the ECA during winter months (Higdon et al. 2011), and Reeves and Mitchell (1988) hypothesised their winter range could include the Labrador Sea, the open North Atlantic, and the North American coast as far south as the Caribbean, while winter sightings of killer whales along the west coast of Greenland (Heide-Jørgensen 1988) suggest it as a possible winter range of ECA killer whales. Lawson and Stevens (2013) reported similar sightings patterns around Newfoundland between summer and winter, and suggested certain locations are important throughout the year. However, it remains to be determined whether winter sightings off Newfoundland comprise individuals that summer in the ECA. Considerable overlap in dentinal $\delta^{13}C_{cor}$ values among ECA and NWA killer whales provides little additional indication of geographical separation. However, since $\delta^{13}C$ values of potential prey show little distinction across these regions (Hobson and Welch 1992, Ostrom et al. 1993, Lawson and Hobson 2000, Hobson et al. 2002), foraging within distinct areas across the broader ECA/NWA would not be expected to produce considerable differences in $\delta^{13}C_{cor}$ values. Nonetheless, consistent isotopic values across GLGs within individuals appears to be largely related to spatial foraging patterns, suggesting potential long-term site fidelity and separate killer whale groups/populations across the greater Northwest Atlantic. Order of magnitude differences in several classes of persistent organic pollutants (D. Muir, Environment Canada, Burlington, ON, unpublished data) and correlations between genetic and isotopic differences among some of the whales included in this study (Morin et al. 2010, A. Foote, Uppsala University, Uppsala, Sweden, personal communication) support this assertion.

Absolute trophic position estimates derived from AA-specific δ^{15}N values and equation (2.1) were too low for ECA/NWA killer whales known to feed at least to some extent on other marine mammals (expected trophic position of 4.5–4.6; Pauly et al. 1998). Recent studies (Lorrain et al. 2009, Dale et al. 2011, Germain et al. 2013) have also produced unexpectedly low trophic level estimates for higher marine consumers, suggesting $TEF_{Glu\text{-}Phe}$ in higher marine consumers is less than the 7.6 ‰ determined for zooplankton and fish (but see Naito et al., 2010, who obtained reasonable trophic level estimates for sea lions [*Zalophus calitorianus japonicus*] and porpoises [Phocoenidae] calculated with $TEF_{Glu\text{-}Phe}$ = 7.6 ‰). Germain et al. (2013) performed a controlled feeding study to investigate nitrogen isotope fractionation in amino acids in harbor seals, measuring $TEF_{Glu\text{-}Phe}$ of ~4.3 ‰. The authors propose using a multi-TEF calculation of marine mammal trophic position to account for differences in nitrogen cycling between consumers excreting ammonia (e.g. zooplankton and teleost fish) vs. uric acid or urea (e.g. elasmobranchs, marine birds and mammals) (Germain et al. 2013, see also Dale et al. 2011). Recalculation of ECA/NWA killer whale trophic position estimates using just a single $TEF_{Glu\text{-}Phe}$ of 4.3 ‰ (i.e. provides higher trophic position estimates than a dual-TEF approach since the lower TEF is applied to all trophic transfers) and equation (2.1) increases trophic position estimates to only 2.9–4.6. While the upper end of this range is plausible for killer whales with diets comprising approximately equal amounts of fish and marine mammals, the lower end is less than that of baleen whales foraging exclusively on large zooplankton (trophic position range: 3.2-3.6; Pauly et al. 1998).

Although further research is required before AA-specific δ^{15}N values can be used to calculate absolute trophic position of marine mammals, we assume differences between trophic and source AA are diagnostic in terms of relative diet comparisons. Differences between $\Delta\delta^{15}N_{\text{Glu-Phe}}$ and $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ in their relative placement of individuals may reflect uncertainty in phenylalanine isotopic measurements, which can be influenced by co-eluting compounds (N. Wallsgrove, University of Hawai'i, Honolulu, HI, personal communication). We therefore interpret $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ rather than $\Delta\delta^{15}N_{\text{Glu-Phe}}$. Comparable $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ values among most whales (certainly when error estimates are considered) suggest similar trophic-level diet. Although observational data forming the basis of our understanding of killer whale diet can be limited to conspicuous predation occurring at the surface, and is temporally biased due to seasonal variation in killer whale abundance and observer effort in both the ECA (Reeves and Mitchell 1988, Higdon et al. 2011) and NWA (Lien et al. 1988, Lawson and Stevens 2013), observations of killer whale predation in both regions suggest isotope patterns reflect a broad range of marine mammal prey. Monodontids (narwhal and beluga) are the most frequently reported prey of killer whales observed in the ECA (51% of predation records), followed by bowhead whales (32%) and seals (12%) (Higdon et al. 2011). Reinhart et al. (2013) identified rake marks left by killer whale teeth on flukes of ~10 % of photographed bowhead whales from five regions across the ECA. These observations in the ECA correspond with historical and recent observations of killer whale predation off Newfoundland and Labrador. Whalers off southern Labrador in the 1950s encountered killer whales attacking blue (*Balaenoptera musculus*) and fin whales (*Balaenoptera physalus*) (Mitchell and Reeves 1988), and sealing and whaling literature from the area describes killer whales tearing at carcasses of harvested whales (Mitchell and Reeves 1988), and preying on harp seals (*Phoca groenlandica*) within ice fields

(Sargeant and Fisher 1957). Killer whales have recently been observed within nearshore ice fields near breeding harp seals off northern Newfoundland (Lawson et al. 2008), and photo-identified killer whale groups have been recorded regularly killing minke whales (Lawson and Stevens 2013).

An exception to similar $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ among individuals are the larger $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ values (by ~ 2-3 ‰) of whales ECA-RB-2009 and NWA-BP-1998, suggesting the diets of these whales comprised a greater proportion of higher trophic level prey. This pattern is difficult to explain given the strong relationship between bulk and source $\delta^{15}N$ values (one would expect bulk $\delta^{15}N$ values of these two whales to be higher than values predicted by the linear fit if they foraged at a higher trophic level relative to the other whales). Differences in apical tooth wear patterns, which have been associated with foraging differences among killer whale ecotypes (Ford et al. 2011) or ecologically divergent groups (Foote et al. 2009), provide some independent support for diet differences between these two and the remaining individuals. General inspection of the lower mandibles of whale ECA-RB-2009 and ECA-CH-2011 indicated whale ECA-RB-2009 had considerably more tooth wear, despite being an estimated 7 years younger. NWA-BP-1998 had particularly extensive apical tooth wear exposing the pulp cavity, even though this whale, estimated to have been just five years old when it died, was considerably younger than other whales with intact teeth. Comparable tooth wear has been linked with diets of shark in other killer whale populations (Ford et al. 2011), which would be consistent with the greater $\Delta\delta^{15}N_{\Sigma\text{trophic AA-}\Sigma\text{source AA}}$ of whales ECA-RB-2009 and NWA-BP-1998, given sharks are generally tertiary consumers occupying high trophic positions in marine food webs (trophic position ~4; Cortés 1999, Estrada et al. 2003). Further research into the relative rates of trophic

^{15}N enrichment of individual AAs will likely help elucidate patterns, and eventually clarify trophic position estimates of higher consumers such as killer whales based on AA-specific δ^{15}N.

Evidence of significant baseline isotopic variation among ECA and NWA killer whales suggests consistent, long-term isotopic variation recorded in dentine GLGs reflected spatial segregation, rather than individual diet specialisation. Consistent isotope profiles across GLGs spanning periods up to 25 years suggest both distribution and diet of sampled individuals were fairly stable over the long-term. Future research efforts on ECA and NWA killer whales should focus on microspatial chemical analysis of teeth or tissues with fast turnover rates (e.g. metabolically active blubber) that can allow diet and habitat reconstructions over narrow periods of time, given GLGs sampled with annual resolution limit the scope for investigating seasonal distribution and diet patterns. This would provide a clearer idea of seasonal variation in killer whale diet in these regions, and could help determine, for example, whether killer whales show diet plasticity over the short-term. Diet variation could be especially pronounced in the ECA, where seasonal aggregations of marine mammals offer a predictable food resource, and recent studies (e.g. Higdon et al. 2011, Reinhart et al. 2013) have identified possible spatial and temporal patterns in predation too fine to be detected using diet indices integrated over the entire year. Our results add to a growing literature on global killer whale predation patterns, and provide critical trophic information necessary for ecosystem management and conservation at broad spatial scales.

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Chapter 3. Seasonal foraging behaviour of Eastern Canada-West Greenland bowhead whales: an assessment of isotopic cycles along baleen

*Eastern Canada-West Greenland (EC-WG) bowhead whales (*Balaena mysticetus*) migrate seasonally between northwestern Hudson Bay/Foxe Basin and Gulf of Boothia in summer and Hudson and Davis Straits in winter. Despite recent advances in knowledge of summer diet composition, determining seasonal variation in foraging behaviour of EC-WG bowhead whales remains a priority for understanding how annual metabolic requirements are met, as well as identifying factors driving seasonal habitat selection. We measured stable nitrogen, carbon, and sulfur isotope composition (*$\delta^{15}N$*, *$\delta^{13}C$*, and *$\delta^{34}S$*) along continuously growing baleen plates (n = 14) to assess alternative seasonal foraging hypotheses, namely winter fasting vs. year-round foraging. Synchronous *$\delta^{15}N$* and *$\delta^{13}C$* cycles, with periods of *^{15}N* enrichment corresponding to foraging on the summer grounds, were inconsistent with standard fasting predictions, although *$\delta^{15}N$* cycles could reflect changes in diet-tissue *$\delta^{15}N$* discrimination between periods of intense foraging throughout the open-water season and supplemental protein intake during winter/spring. Correlations between *$\delta^{15}N$* and *$\delta^{34}S$* values, potentially meditated through amino acid metabolism, support this interpretation. Reasonable agreement between baleen isotope oscillations and regional baseline *$\delta^{15}N$* and *$\delta^{13}C$* variation also indicated foraging occurs within isotopically distinct food webs across the summer and winter ranges. We conclude that EC-WG bowhead whales forage throughout their distribution, and conservatively interpret *$\delta^{15}N$* and *$\delta^{34}S$* cycles to reflect reduced food consumption during winter. Foraging outside of periods of peak productivity likely contributes to annual metabolic requirements and winter habitat selection.*

KEY WORDS**:** Arctic, *Balaena mysticetus*, diet, fasting, isoscape, marine mammal, stable isotopes, sulfur, time series

INTRODUCTION

Predictable changes in food abundance have shaped the morphological, behavioural, and physiological adaptations of animals that routinely experience periodic food limitation (Young 1976, Lindstedt and Boyce 1985, Fryxell and Sinclair 1988, Millar and Hickling 1990). Large body size and an extensive blubber layer allow migratory baleen whales to withstand prolonged seasonal fasting after amassing lipid stores on productive summer foraging grounds (Lockyer 1981), with only opportunistic foraging on food resource-poor wintering grounds (e.g. Baraff et al. 1991). Prey abundance during the relatively brief foraging season has been correlated with annual variation in blubber thickness and reproductive success of several baleen whale species (Lockyer 1986, Víkingsson 1990, Miller et al. 2011), highlighting the energetic constraints imposed on fasting animals that must partition endogenous reserves among competing interests such as growth, maintenance, and reproduction. How an animal meets annual metabolic requirements in seasonally variable environments therefore links individual energetics and population dynamics (e.g. Regehr et al. 2007, Rode et al. 2010).

Bowhead whales (*Balaena mysticetus* Linnaeus 1758) are resident within Arctic and sub-Arctic waters year-round (Reeves et al. 1983, Moore and Reeves 1993), where distinct seasonal pulses of primary productivity during the ice algae and phytoplankton blooms support large biomasses of grazing zooplankton (Longhurst et al. 1984, Runge and Ingram 1988). Bowhead whales, which are zooplankton specialists, enjoy optimal foraging conditions on pelagic copepods (primarily *Calanus* spp.) and euphausiids, as well as epibenthic invertebrates (Lowry 1993, Finley 2001, Lowry et al. 2004, Moore et al. 2010, Pomerleau et al. 2011a, b), over several months into late summer and fall, when zooplankton descend to overwintering depths that can exceed 1000 m (Longhurst et al. 1984, Hirche 1991). While there has been general consensus that winter feeding contributes little to overall bowhead nutrition (Lowry 1993, Finley 2001), studies have provided mixed results on the relative importance of foraging across different seasons (Schell and Saupe 1993, Hoekstra et al. 2002, Lee et al. 2005, Noongwook et al. 2007, Citta et al. 2014).

In the eastern Canadian Arctic, Eastern Canada-West Greenland (EC-WG) bowhead whales track receding sea ice into summer foraging grounds in northwestern Hudson Bay/Foxe Basin and Gulf of Boothia (Reeves and Mitchell 1990, NWMB 2000; Figure 3.1). Spring migrations coincide with early season ice algal production (ca. April-June), when calanoid copepods and other zooplankton rise from overwintering depths to feed on ice-associated diatoms and their detritus (Conover and Huntley 1991). Finley (2001) thought spring and early summer zooplankton densities to be too low to be energetically profitable to EC-WG bowhead whales, but Pomerleau et al. (2012) provided evidence of limited foraging on ice-associated zooplankton. Intense feeding occurs during the open-water season (Finley 2001, Pomerleau et al. 2011a, b, 2012), and high levels of fatty acids unique to calanoid copepods in EC-WG bowhead blubber indicates they are a major prey item (Pomerleau et al. 2014). Finley et al. (1993) suggested the bulk of EC-WG bowhead whale annual energy requirements are met during fall foraging on high densities of mature stage *Calanus* copepods, followed by fasting while overwintering within the unconsolidated pack ice in Hudson and Davis Straits. A portion of the population comprising primarily mature females at Disko Bay off western Greenland, however, engage in late winter and early spring (February-May) feeding on high densities of pre-ascension stage calanoid copepods at depths ranging from 100-400 m (Laidre et al. 2007, Heide-Jørgensen et al. 2010,

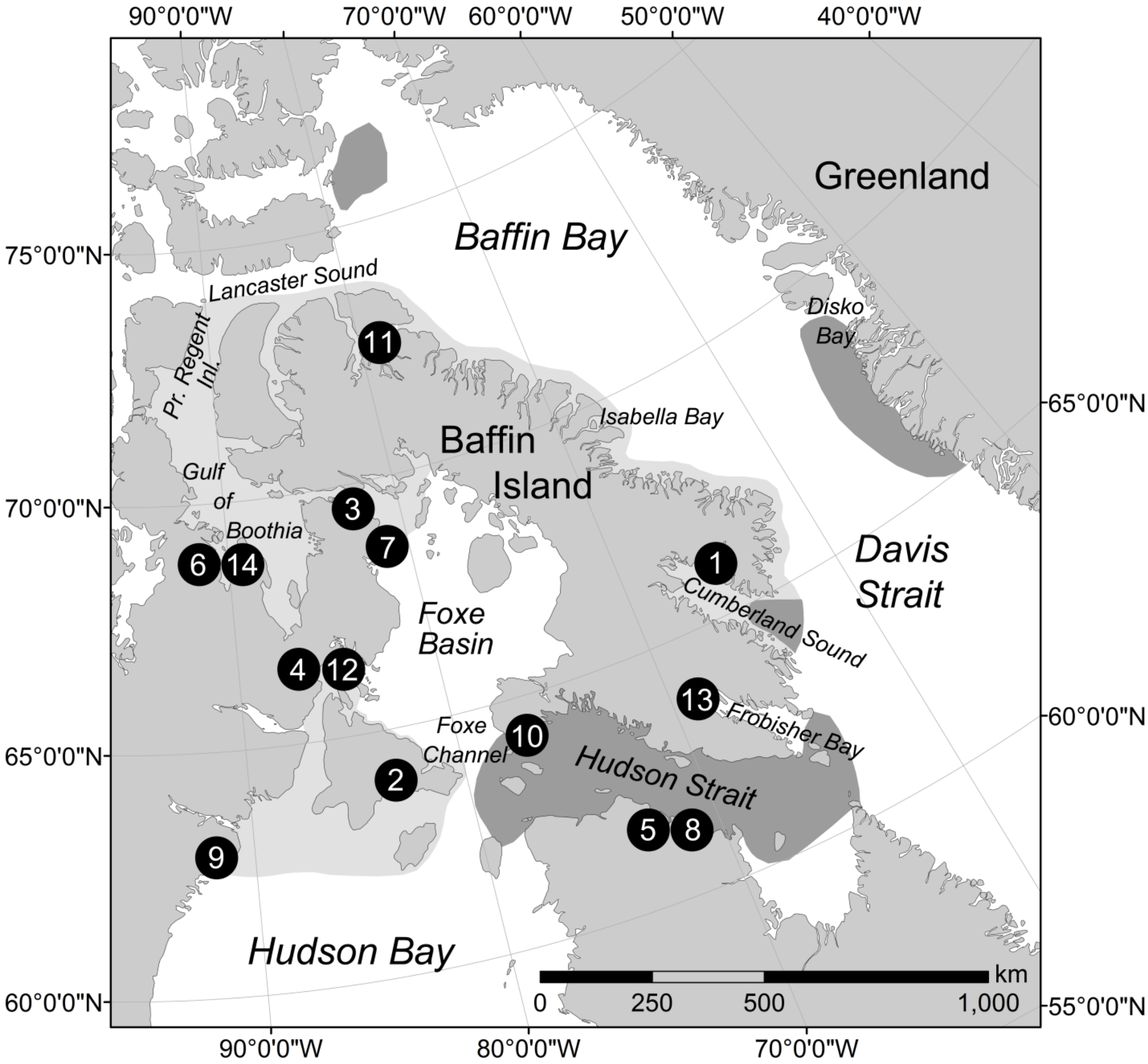

Figure 3.1. Baleen was collected from Eastern Canada-West Greenland (EC-WG) bowhead whales (*Balaena mysticetus*) throughout the Eastern Canadian Arctic during July to September, 1998 to 2011. Numbers on map correspond to animals listed in Table 3.1. Winter distribution (shown in dark grey) and areas of summer aggregations (light gray) were reproduced from COSEWIC (2005), with modifications after Laidre et al. (2007) and Ferguson et al. (2010).

2013). Estimates of high zooplankton consumption during their 4-month residency at Disko Bay (Laidre et al. 2007) suggest resting zooplankton could be accessible at sufficient densities to be an energetically feasible food resource during periods of minimal productivity.

The incremental measurement of stable nitrogen (δ^{15}N) and carbon (δ^{13}C) isotope composition of baleen to resolve seasonal foraging patterns of baleen whales was developed by Schell *et al.* (1989a), who matched δ^{13}C oscillations along the length of baleen plates with geographic variation in zooplankton δ^{13}C values to infer when and where Bering-Chukchi-Beaufort (BCB) bowhead whales foraged across their range. Regional baseline isotope values, which vary due to underlying biogeochemical processes, are assimilated by primary producers and propagated more or less predictably into consumer tissues via trophic transfer (Minagawa and Wada 1984, McCutchan et al. 2003). Continuously growing keratinous baleen forms from amino acids in the bloodstream, and its stable isotope composition is presumed to rapidly reflect that of diet (Schell et al. 1989a). Because baleen is metabolically inert after formation, isotopic profiling along its length can provide past foraging information with well-constrained temporal resolution if baleen growth rate is known. When coupled with knowledge of regional baseline isotopic variation across a species' range, this approach has proven useful for linking temporal and spatial foraging patterns of migratory baleen whales (e.g. Schell et al. 1989a, Best and Schell 1996, Caraveo-Patiño and Soto 2005).

While baleen δ^{13}C variation has invariably been attributed to movements among (and foraging within) isotopically distinct food webs (e.g. Schell et al. 1989a, Caraveo-Patiño and Soto 2005), δ^{15}N cycles along baleen have also been provisionally attributed to seasonal foraging/fasting cycles characteristic of baleen whales (e.g. Best and Schell 1996, Hobson et al. 2004, Summers et al. 2006). Isotopic fractionation during amino acid metabolism causes preferential retention of the heavier nitrogen isotope (^{15}N) in body tissues, which elevates consumer $\delta^{15}N$ values relative to diet (DeNiro and Epstein 1981). Tissues formed during periods of negative nitrogen balance, when amino acids are sourced from catabolised body protein pools, undergo further ^{15}N enrichment (Hobson et al. 1993, Cherel et al. 2005, Lee et al. 2012). This effect has allowed researchers to consider seasonal fasting as a potential driver of baleen $\delta^{15}N$ cycles, assuming peak values correspond to periods of fasting or negative nitrogen balance (e.g. Best and Schell 1996). However, recent studies have linked ^{15}N enrichment in baleen of several whale species with summer foraging, rather than winter fasting (Hobson and Schell 1998, Mitani et al. 2006, Aguilar et al. 2014). Aguilar et al. (2014) suggest fasting mysticetes reduce or prevent catabolism of body protein during the seasonal fast through metabolic adaptations and limited foraging, and that baleen $\delta^{15}N$ cycles reflect shifts in trophic ^{15}N discrimination in response to seasonal shifts in food consumption.

Hobson and Schell (1998) measured long-term isotopic records along baleen of three EC-WG bowhead whales, and suggested $\delta^{15}N$ cycles in the absence of $\delta^{13}C$ cycles indicates they 1) migrated annually between regions differing in food web $\delta^{15}N$, but not $\delta^{13}C$, values, 2) shifted diet annually by about one third of a trophic level (seasonal $\delta^{15}N$ oscillations were ~1 ‰, or approximately one third the increase in $\delta^{15}N$ values due to trophic ^{15}N enrichment [McCutchan et al. 2003]), or 3) underwent seasonal fasting, assuming catabolism of endogenous protein to supply continuous baleen growth would have led to ^{15}N enrichment of baleen (Hobson et al. 1993). Here we interpret isotopic variation along baleen from an additional 14 EC-WG bowhead whales acquired since Hobson and Schell's (1998) study to assess alternative seasonal foraging hypotheses, namely seasonal (winter) fasting and year-round foraging. Regional variation in zooplankton stable isotope composition encompassing the EC-WG bowhead range (e.g. Pazerniuk 2007, Pomerleau et al. 2011c) provides spatial context for assessment of baleen $\delta^{15}N$ and $\delta^{13}C$ profiles. Stable sulfur isotope composition ($\delta^{34}S$) was also measured along a subset of plates. Although rarely applied in studies of cetacean foraging behaviour (e.g. Hoekstra et al. 2002, Niňo-Torres et al. 2006), negligible ^{34}S discrimination with trophic level (McCutchan et al. 2003) and considerable spatial variation in $\delta^{34}S$ values along benthic-pelagic (Peterson et al. 1985) and nearshore-offshore (Barros et al. 2010) gradients make $\delta^{34}S$ values potentially useful for discerning between trophic and spatial influences that can lead to ambiguous interpretation of $\delta^{15}N$ and $\delta^{13}C$ values (e.g. Connolly et al. 2004). Recent studies linking trophic ^{15}N and ^{34}S discrimination with dietary protein content (e.g. McCutchan et al. 2003, Florin et al. 2011) also suggest correlated $\delta^{15}N$ and $\delta^{34}S$ values may reflect periods of restricted food consumption.

Clarifying seasonal foraging behaviour of EC-WG bowhead whales remains a priority for understanding individual energy budgets and seasonal habitat selection (Lockyer 1981, Moore et al. 2000, Ferguson et al. 2010), which are relevant for predicting how potential sea ice-driven changes in zooplankton phenology and community composition may impact bowhead whale populations under future climate scenarios (Tynan and DeMaster 1997, Laidre et al. 2008). We predicted fasting or food-restricted whales would show distinct $\delta^{15}N$ cycles (perhaps correlated with $\delta^{34}S$ values), but do not necessarily assume that ^{15}N enrichment would occur during the winter fast (see Aguilar et al. 2014). On the other hand, we predicted foraging within isotopically distinct food webs across the summer and winter ranges would lead to synchronous $\delta^{15}N$ and

$\delta^{13}C$ oscillations reflecting regional differences in zooplankton isotope composition. Further, we predicted seasonal shifts in foraging depth (Heide-Jørgensen et al. 2013) may lead to inversely correlated $\delta^{13}C$ and $\delta^{34}S$ cycles, as benthic systems are ^{13}C enriched and ^{34}S depleted relative to pelagic systems (Peterson et al. 1985, Hobson and Welch 1992).

MATERIALS AND METHODS

Baleen Samples

Baleen plates were collected from bowhead whales (n = 14) harvested in Inuit subsistence hunts throughout the eastern Canadian Arctic from 1998-2011. Whales were taken during the open-water season (July-September) from Hudson Strait, Hudson Bay, Foxe Basin, Gulf of Boothia, and along northern and eastern Baffin Island (Figure 3.1). Several of the longest baleen plates were excised from within the gum (i.e. included the most recent growth) from 10 whales, while those from the remaining four whales were cut at the gum and included only erupted baleen (Table 3.1). All baleen and biological and morphological data (Table 3.1) were obtained within 24-48 hours of death, and baleen was frozen at -25 ^{o}C.

Sample Preparation and Stable Isotope Analysis

The longest complete baleen plate from each whale was cleaned of algae and other adhered material using water and scrubbing pads, then scraped with a scalpel blade to remove surface baleen. Starting at the base, the entire length of each plate was sampled at 2-cm increments along the outside edge using a hand-held rotary tool fitted with a 1/16 inch drill bit. With an annual baleen growth rate of 16-25 cm yr^{-1} (Schell et al. 1989a), sample increments represent sub-seasonal to monthly temporal resolution. We assume each baleen plate provided an accurate

Table 3.1. Location and biological data for Eastern Canadian Arctic-West Greenland (EC-WG) bowhead whales (*Balaena mysticetus*) from which baleen was collected.

Whale Sample ID	**Location**	**Date (mo/yr)**	**Sex**	**Total Length (m)**	**Baleen Length (cm)**	**Stable Isotopes Measured**
1. NSA-BM-98-01	Cumberland Sound, Pangnirtung, NU	07/1998	M	12.75	266*	δ^{15}N, δ^{13}C
2. BM-CH-2000-001	Hudson Bay, Coral Harbor, NU	08/2000	M	11.65	270^{+}	δ^{15}N, δ^{13}C
3. BM-IG-HB-2002-001	Foxe Basin, Igloolik, NU	08/2002	F	14.19	263^{+}	δ^{15}N, δ^{13}C, δ^{34}S
4. BM-RB-2005-001	Foxe Basin, Repulse Bay, NU	08/2005	F	16.40	267^{+}	δ^{15}N, δ^{13}C
5. BM-01-2008	Hudson Strait, Kangiqsujuak, QC	08/2008	M	14.88	319*	δ^{15}N, δ^{13}C, δ^{34}S
6. BM-NSA-2008-001	Gulf of Boothia, Kugaaruk, NU	09/2008	M	10.51	181*	δ^{15}N, δ^{13}C, δ^{34}S
7. BM-NSA-2008-002	Foxe Basin, Hall Beach, NU	08/2008	M	13.43	235*	δ^{15}N, δ^{13}C
8. BM-01-2009	Hudson Strait, Kangiqsujuak, QC	08/2009	F	17.29	338*	δ^{15}N, δ^{13}C
9. BM-NSA-2009-02	Hudson Strait, Rankin Inlet, NU	08/2009	F	16.15	232^{+}	δ^{15}N, δ^{13}C
10. BM-NSA-2009-03	Hudson Strait, Cape Dorset, NU	09/2009	M	15.77	330*	δ^{15}N, δ^{13}C
11. BM-NSA-2010-01	Eclipse Sound, Pond Inlet, NU	08/2010	M	12.80	230*	δ^{15}N, δ^{13}C
12. BM-NSA-2010-02	Foxe Basin, Repulse Bay, NU	08/2010	F	14.32	296*	δ^{15}N, δ^{13}C
13. BM-NSA-2011-01	Frobisher Bay, Iqaluit, NU	08/2011	M	14.33	298*	δ^{15}N, δ^{13}C
14. BM-NSA-2011-03	Gulf of Boothia, Kugaaruk, NU	08/2011	F	9.04	176*	δ^{15}N, δ^{13}C

*total length (embedded + erupted)
$^{+}$erupted length

isotopic history for each whale, based on nearly identical isotope profiles in adjacent baleen plates from a gray whale (*Eschrichtius robustus*; Caraveo-Patiño and Soto 2005), and those from opposite sides of the mouth of a bowhead whale (Schell et al. 1989a).

Baleen samples were weighed (~1 mg) directly into tin cups for analysis on a Vario EL III elemental analyser (Elementar, Germany) interfaced with a DELTAplus XP isotope ratio mass spectrometer (Thermo, Germany) at the G.G. Hatch Stable Isotope Laboratory, University of Ottawa (Ottawa, ON). Stable isotope ratios are reported in delta notation (δ) as parts per thousand (‰) deviation from the isotope ratios of international standards atmospheric N_2 and Vienna Pee-Dee Belemnite limestone (for $\delta^{15}N$ and $\delta^{13}C$, respectively), defined as $\delta^{15}N$ or $\delta^{13}C$ = $(R_{sample} - R_{standard}) / R_{standard}) * 1000$, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Values were normalised using reference materials whose isotopic composition spanned the sample range (nicotinamide, ammonium sulfate + sucrose, and caffeine; $\delta^{15}N$ -16.61 to 16.58 ‰, $\delta^{13}C$ -34.46 to -11.94 ‰), calibrated to international standards IAEA-N1, IAEA-N2, USGS-40 and USGS-41 for $\delta^{15}N$, and IAEA-CH6, NBS-22, USGS-40, and USGS-41 for $\delta^{13}C$. Analytical precision based on repeated measures of a laboratory reference material not used in calibrations (glutamic acid) was 0.11 ‰ for $\delta^{15}N$ and 0.05 ‰ for $\delta^{13}C$, and that based on duplicate measures of ~5 % of samples was 0.09 and 0.06 ‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively.

Stable sulfur isotopic composition was measured along a subset (n = 3) of the 14 plates (Table 3.1). Baleen sulfur content is relatively high owing to large amounts of the amino acid cysteine (Block 1937, Gillespie and Inglis 1964), making it amenable to $\delta^{34}S$ analysis. Samples were weighed (~0.6 mg) into tin cups and analysed on an Isochrom continuous flow stable isotope ratio mass spectrometer (GV Instruments/Micromass-UK) coupled to a Costech elemental analyzer (CNSO 4010UK) at the Environmental Isotope Laboratory, University of Waterloo (Waterloo, ON). $\delta^{34}S$ values are reported in delta notation (R = $^{34}S/^{32}S$) relative to Canyon Diablo Meteorite calibrated through repeated measures of organic sulfur materials (NIST bovine liver and muscle). Analytical precision based on repeated measures of a homogenous baleen reference material, as well as duplicate measures of ~10 % of samples, was 0.24 ‰.

Data analysis

Methods for exploring seasonality in stationary time series, primarily, autoregressive (AR) models, spectral analysis, and sample cross correlation functions (CCF), were used to characterize variation in $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ profiles. These methods are appropriate for analysis of spatially collected data given an assumed linear growth rate (Shao et al. 2004). Baleen grows at a uniform rate in bowhead whales >12 m body length (Schell et al. 1989a, Schell and Saupe 1993). Given our sample largely comprised whales near or >12 m (Table 3.1), we assume baleen growth rates were linear over the lengths sampled for most whales, with the exception of whales BM-NSA-2011-03 (9.04 m) and BM-NSA-2008-001 (10.51 m).

Each isotopic series was first detrended using a Gaussian low pass filter to remove long-term trends (e.g. Park and Gambéroni 1995, Klvana et al. 2004). Attempts to characterize cycle parameters such as period, amplitude, and phase shift by fitting a nonlinear model with a cosine term to the seasonal component of each detrended series (e.g. Shao et al. 2004) generally provided poor fits to data (results not shown). Parametric spectral estimation (Parzen 1983, Cowpertwait and Metcalfe 2009, Shumway and Stoffer 2011) was therefore used to estimate series periodicity. High-order autoregressive ($AR_{(p)}$) models, with model order p selected based on minimum AIC, were fit to each detrended series. The spectral peak frequency for each modelled time series was converted to samples per period (1/peak frequency), then multiplied by sample increment (2 cm) to estimate period length.

The magnitude of each cycle (i.e. peak-to-peak amplitude) was calculated as the difference between maximum and minimum values within each oscillation of detrended series with significant periodicity (indicated by AR models). To assess whether $\delta^{15}N$ and $\delta^{13}C$ magnitudes were similar among whales whose baleen growth overlapped temporally, calendar year of growth was assigned to each oscillation by assuming the most recent complete oscillation represented the previous year's growth, and that each oscillation thereafter was annual in nature (Schell et al. 1989b).

Correlations between isotopic series within a given plate were assessed using CCF calculated for each combination of elements ($\delta^{15}N$ vs. $\delta^{13}C$, $\delta^{15}N$ vs. $\delta^{34}S$, and $\delta^{13}C$ vs. $\delta^{34}S$). All analyses were performed using the TSA package (Chan and Ripley 2012) available for R software (R Core Team 2012).

RESULTS

Considerable variation in $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ values occurred along all baleen plates for which they were measured, although the degree to which such variation constituted cycles differed for each element, within individuals, and among individuals (Figure 3.2). Atomic C:N of baleen

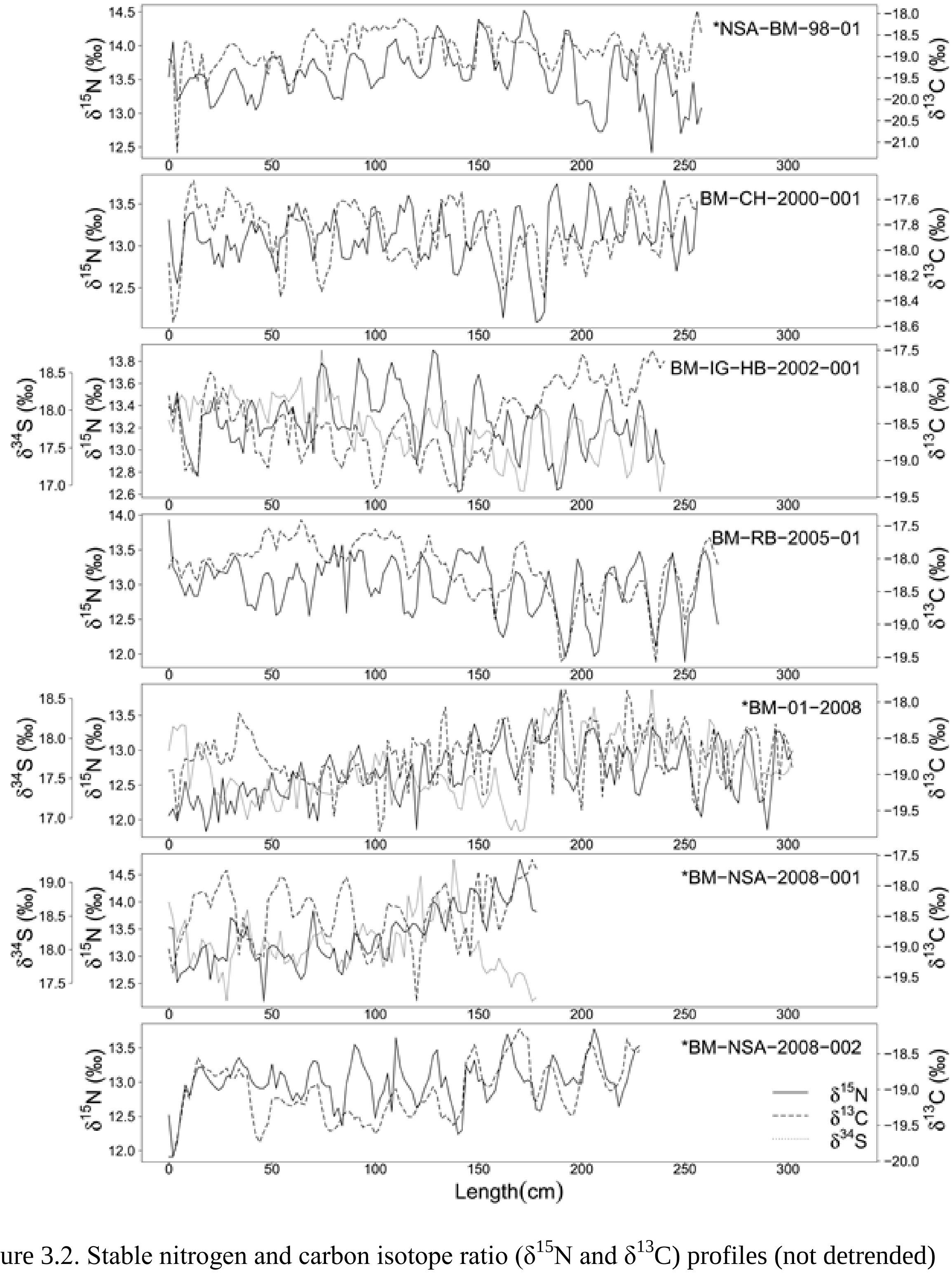

Figure 3.2. Stable nitrogen and carbon isotope ratio (δ^{15}N and δ^{13}C) profiles (not detrended) along baleen plates of 14 Eastern Canada-West Greenland (EC-WG) bowhead whales (*Balaena mysticetus*). Stable sulfur isotope ratios (δ^{34}S) were measured for a subset of the whales (n = 3). Sample 0 cm was taken at the base (proximal end), and represents the most recent growth in whales from which total baleen was collected (*). y-axes not drawn at same scale.

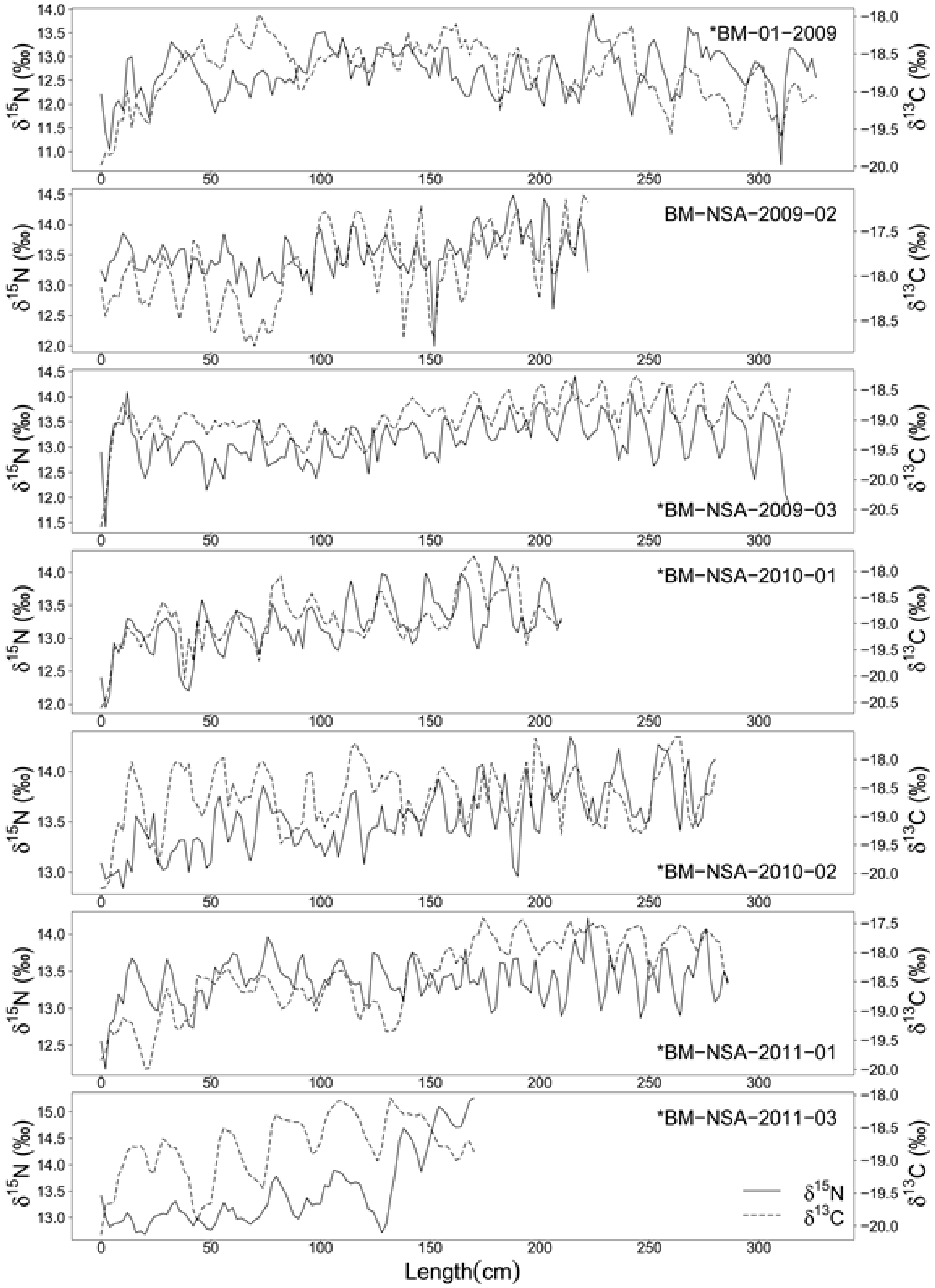

Figure 3.2. continued.

samples (3.3 ± 0.1; mean ± sd) was within the range of measured atomic C:N of baleen (Lysiak 2009, Bentaleb et al. 2011) and hair and nail keratin (O'Connell and Hedges 2001).

High order AR models were successfully fit to 13 of the 14 detrended δ^{15}N profiles, and estimated periods from peak spectral frequencies of modelled δ^{15}N oscillations ranged from 14.4 to 26.3 cm (Table 3.2). Average magnitudes of δ^{15}N cycles were similar among whales, ranging from 0.63 to 1.10 ‰ (Table 3.3). Individual δ^{15}N oscillations ranged from 0.16 to 2.70 ‰ (Table 3.3), and were not synchronous among whales within any given calendar year (data not shown). In baleen for which the total length was collected, which allowed isotope patterns to be temporally anchored to the month of death, δ^{15}N values decreased gradually through winter and early spring, reaching minimum values in late spring before increasing in summer-grown baleen (samples 0-2 cm of embedded baleen; Figure 3.2).

AR models were successfully fit to 10 of the 14 detrended δ^{13}C series, and periods estimated from peak spectral frequencies were similar to those for δ^{15}N oscillations, ranging from 14.2 to 28.2 cm (Table 3.2). Average magnitudes of δ^{13}C cycles within plates ranged from 0.63 to 1.25 ‰, while individual oscillations ranged from 0.07 to 2.12 ‰ (Table 3.3). Like δ^{15}N oscillations, δ^{13}C oscillations differed in magnitude among whales within a given calendar year (data not shown). δ^{13}C values in most recently grown baleen followed a similar pattern as δ^{15}N values, with minimal δ^{13}C values occurring in winter and spring, followed by δ^{13}C increases in summer (Figure 3.2). CCF indicated δ^{15}N and δ^{13}C patterns were largely synchronous within plates, with significant positive lag 0 correlations between the two measures in all but three of the 14 plates,

Table 3.2. Periods of $\delta^{15}N$, $\delta^{13}C$, and $\delta^{34}S$ cycles along baleen plates of Eastern Canadian Arctic-West Greenland (EC-WG) bowhead whales (*Balaena mysticetus*) estimated from spectra of autoregressive models fit to detrended data.

Whale Sample ID	**$\delta^{15}N$ period (cm)**	**Model order (*p*)**	**$\delta^{13}C$ period (cm)**	**Model order (*p*)**	**$\delta^{34}S$ period (cm)**	**Model order (*p*)**
1. NSA-BM-98-01	20.6	AR(13)	/	/		
2. BM-CH-2000-001	17.9	AR(10)	/	/		
3. BM-IG-HB-2002-001	20.2	AR(5)	18.0	AR(10)	17.3	AR(7)
4. BM-RB-2005-001	15.0	AR(10)	15.4	AR(10)		
5. BM-01-2008	14.6	AR(9)	/	/	/	/
6. BM-NSA-2008-001	15.5	AR(2)	23.3	AR(5)	/	/
7. BM-NSA-2008-002	20.8	AR(5)	26.0	AR(3)		
8. BM-01-2009	25.6	AR(4)	/	/		
9. BM-NSA-2009-02	14.4	AR(10)	14.2	AR(9)		
10. BM-NSA-2009-03	14.9	AR(10)	16.8	AR(3)		
11. BM-NSA-2010-01	17.5	AR(4)	19.8	AR(7)		
12. BM-NSA-2010-02	26.3	AR(7)	20.4	AR(11)		
13. BM-NSA-2011-01	17.1	AR(10)	17.3	AR(6)		
14. BM-NSA-2011-03	/	/	28.2	AR(4)		

Table 3.3. Peak-to-peak amplitudes and mean peak and trough $\delta^{15}N$ and $\delta^{13}C$ values in baleen plates of Eastern Canadian Arctic-West Greenland (EC-WG) bowhead whales (*Balaena mysticetus*) with significant $\delta^{15}N$ and $\delta^{13}C$ cycles.

Whale Sample ID	**$\delta^{15}N$**			**$\delta^{13}C$**		
	Peak-to-peak amplitude (‰) mean (range)	**Peak (‰) mean±sd**	**Trough (‰) mean±sd**	**Peak-to-peak amplitude (‰) mean (range)**	**Peak (‰) mean±sd**	**Trough (‰) mean±sd**
1. NSA-BM-98-01	0.86 (0.4-1.56)	14.02 ± 0.29	13.13 ± 0.33	/	/	/
2. BM-CH-2000-001	0.78 (0.34-1.62)	13.51 ± 0.18	12.72 ± 0.29	/	/	/
3. BM-IG-HB-2002-001	0.64 (0.32-1.06)	13.58 ± 0.33	12.94 ± 0.20	0.72 (0.32-1.34)	-18.38 ± 0.30	-19.04 ± 0.33
4. BM-RB-2005-001	0.90 (0.34-1.60)	13.38 ± 0.13	12.49 ± 0.35	0.59 (0.07-1.61)	-17.89 ± 0.31	-18.47 ± 0.57
5. BM-01-2008	0.73 (0.33-1.41)	13.09 ± 0.35	12.34 ± 0.34	/	/	/
6. BM-NSA-2008-001	0.77 (0.22-1.24)	13.57 ± 0.29	12.78 ± 0.44	1.05 (0.36-1.90)	-18.03 ± 0.26	-19.09 ± 0.46
7. BM-NSA-2008-002	0.86 (0.35-1.27)	13.46 ± 0.19	12.59 ± 0.31	0.70 (0.36-1.06)	-18.54 ± 0.34	-19.29 ± 0.25
8. BM-01-2009	1.10 (0.20-2.47)	13.18 ± 0.31	12.06 ± 0.58	/	/	/
9. BM-NSA-2009-02	0.80 (0.35-1.75)	13.89 ± 0.30	13.08 ± 0.36	0.76 (0.24-1.47)	-17.60 ± 0.36	-18.38 ± 0.29
10. BM-NSA-2009-03	0.99 (0.42-2.70)	13.67 ± 0.37	12.67 ± 0.41	0.65 (0.44-0.89)	-18.39 ± 0.33	-19.06 ± 0.15
11. BM-NSA-2010-01	0.93 (0.55-1.39)	13.72 ± 0.32	12.80 ± 0.37	0.85 (0.26-1.62)	-18.45 ± 0.41	-19.29 ± 0.38
12. BM-NSA-2010-02	0.63 (0.16-1.05)	13.88 ± 0.32	13.24 ± 0.24	1.25 (0.36-2.12)	-18.01 ± 0.23	-19.28 ± 0.43
13. BM-NSA-2011-01	0.71 (0.47-1.44)	13.69 ± 0.19	12.96 ± 0.33	0.63 (0.23-1.30)	-18.01 ± 0.48	-18.66 ± 0.59
14. BM-NSA-2011-03	/	/	/	0.91 (0.46-1.33)	-18.33 ± 0.48	-18.71 ± 0.58

and cycled thereafter over repeated lags similar to periods estimated using parametric spectral estimation (Figure 3.3).

Of the three plates analysed for sulfur isotope ratios, $\delta^{34}S$ cycles were identified in just one plate (BM-IG-HB-2002-001). Period length estimated from the peak spectral frequency of an AR model fitted to the detrended series (17.3 cm) was similar to those of $\delta^{15}N$ and $\delta^{13}C$ cycles along that plate (Table 3.2). Significant positive lag 0 correlations between $\delta^{34}S$ and $\delta^{15}N$ values occurred in two of the plates (BM-01-2008, BM-IG-HB-2002-001), but not between $\delta^{34}S$ and $\delta^{13}C$ values (Figure 3.4).

DISCUSSION

Estimated periods of isotopic cycles are consistent with annual bowhead whale baleen growth rates (Schell et al. 1989a), and are assumed to reflect seasonal variation in foraging. The most obvious pattern in our data are synchronous $\delta^{15}N$ and $\delta^{13}C$ cycles, with peaks in both values occurring on the summer foraging grounds. This pattern contradicts standard assumptions that fasting induces ^{15}N enrichment in newly grown tissues, although $\delta^{15}N$ declines during the purported winter fasting period may reflect metabolic pathways and limited food consumption that prevent catabolism of body protein reserves (Aguilar et al. 2014). Baleen $\delta^{13}C$ cycles, however, have invariably been linked with migrations between isotopically distinct food webs (e.g. Schell et al. 1989), rather than fasting physiology. Reasonable agreement between baleen $\delta^{15}N$ and $\delta^{13}C$ values and geographic variation in zooplankton isotopic composition across the EC-WG bowhead range indicates foraging on both the summer and wintering grounds is a plausible explanation of baleen isotope cycles. While baleen $\delta^{34}S$ values could also reflect

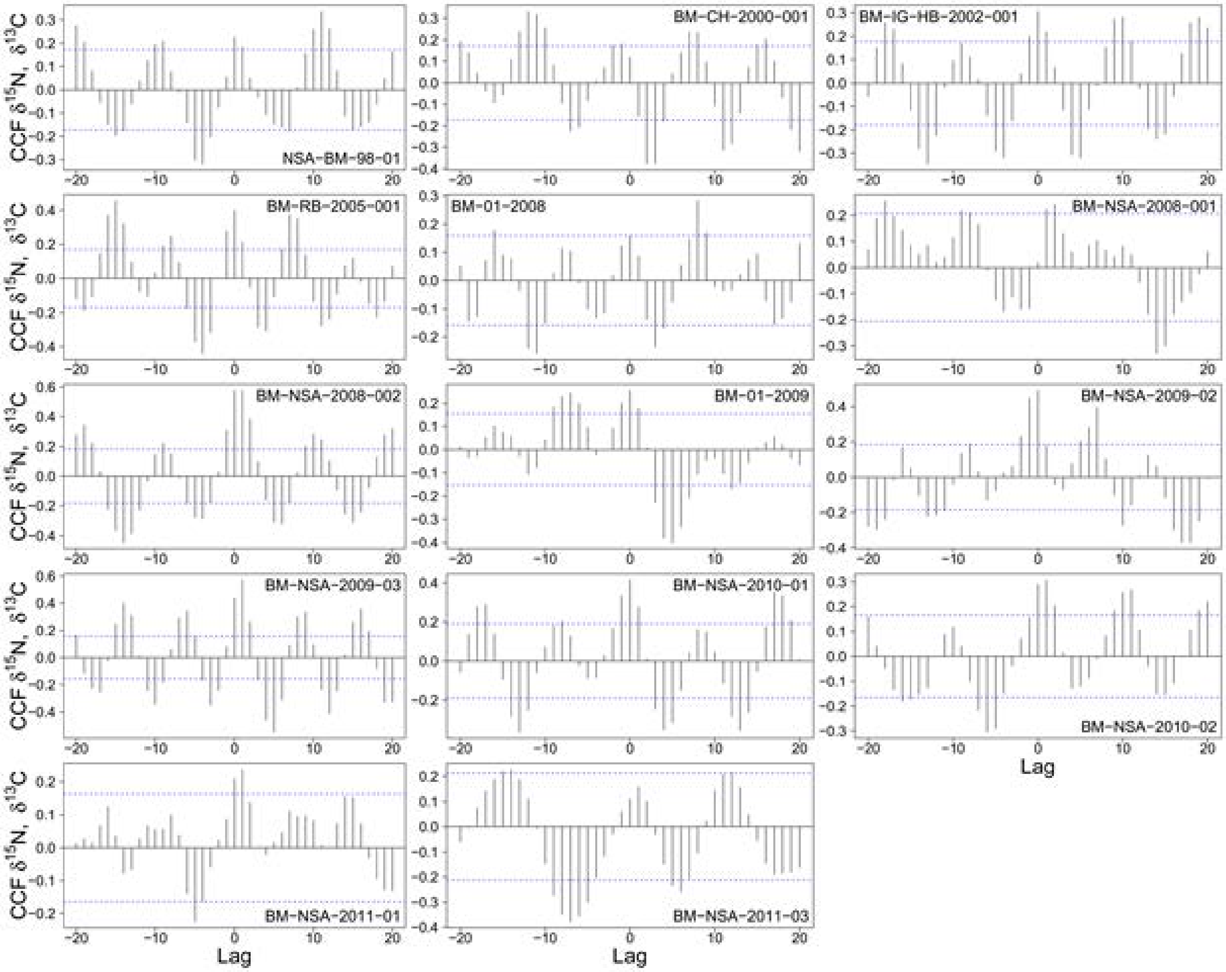

Figure 3.3. Sample cross correlation functions (CCF) show evidence of significant, in-phase correlations between $\delta^{15}N$ and $\delta^{13}C$ values along eastern Canada-West Greenland (EC-WG) bowhead whale (*Balaena mysticetus*) baleen. Values falling outside the dotted lines (drawn at 1/n ± 2/√n, where n = series length) provide evidence of statistical significance at the 5% level. Lag unit is the sample interval, 2 cm. y-axes not drawn at same scale.

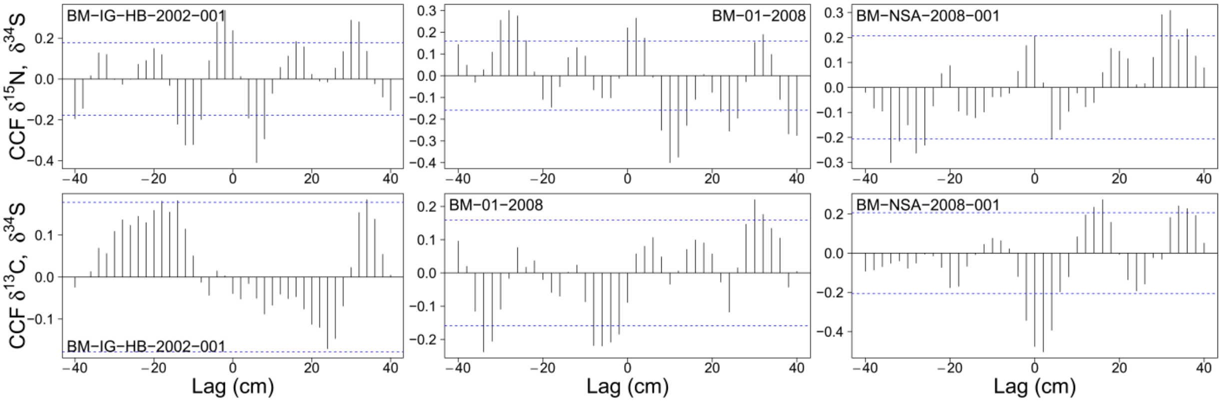

Figure 3.4. Sample cross correlation functions (CCF) of δ^{15}N and δ^{13}C against δ^{34}S values (top and bottom, respectively) along eastern Canada-West Greenland (EC-WG) bowhead whale (*Balaena mysticetus*) baleen plates show significant, in-phase correlations between δ^{15}N and δ^{34}S values in two of the three plates (BM-IG-HB-2002-001 and BM-01-2008), but not between δ^{13}C and δ^{34}S values. Values falling outside the dotted lines (drawn at $1/n \pm 2/\sqrt{n}$, where n = series length) provide evidence of statistical significance at the 5% level. Lag unit is the sample interval, 2 cm. y-axes not drawn at same scale.

regional baseline variation (see Strauss 2004), we also consider whether correlated δ^{15}N and δ^{34}S values, mediated through changes in amino acid metabolism during periods of restricted protein intake (Florin et al. 2011), could reflect reduced foraging during winter, when zooplankton accessibility is reduced.

Previous interpretations of baleen δ^{15}N cycles have assumed that catabolism of endogenous protein reserves, which are ^{15}N enriched relative to diet (DeNiro and Epstein 1981), under fasting conditions leads to additional ^{15}N enrichment of newly formed tissues (see Cherel et al. 2005, Lee et al. 2012). The timing of δ^{15}N oscillations observed here do not fit this assumption, as ^{15}N enrichment occurred on the summer foraging grounds rather than during the purported winter fast (Finley 1993), a point also noted by Hobson and Schell (1998) regarding ^{15}N enrichment peaks along baleen of two other EC-WG bowhead whales. We assume growth of the basal portion of embedded baleen occurred on the summer foraging grounds when baleen was collected, and that baleen isotope values reflect diet with little delay. Both assumptions are supported by results of several studies linking isotopic composition of newly grown baleen with recent foraging movements among regions of well-characterized isotopic composition (Schell et al. 1989a, Best and Schell 1996, Caraveo-Patiño and Soto 2005). Aguilar et al. (2014) attributed similar patterns of ^{15}N enrichment in summer-grown fin whale (*Balaenoptera physalus*) baleen to migrations between different marine isoscapes (with higher baseline δ^{15}N values occurring on the summer foraging grounds), as well as maintenance of positive nitrogen balance during winter through protein-sparing and limited foraging, thereby meeting protein demands while avoiding catabolism of endogenous protein reserves that normally elicits ^{15}N enrichment associated with fasting. Resumption of intense seasonal foraging, with a concomitant increase in diet-tissue δ^{15}N discrimination as protein catabolism and urea excretion are ramped up (Martínez del Rio and Wolf 2005), would contribute to relative baleen ^{15}N enrichment on the summer foraging grounds (additive to any baseline isotopic variation between the summer foraging and wintering grounds; Aguilar et al. 2014).

It is unclear if baleen whales require limited foraging to avoid substantial endogenous protein catabolism while fasting, as some fasting-adapted mammals endure prolonged fasting with minimal loss of protein reserves (Castellini and Rea 1992, McCue 2010), and if they do not, could relative ^{15}N depletion over the winter fast occur as a result of efficient protein sparing? Polischuk et al. (2001) reported concurrent δ^{15}N and δ^{13}C decreases of 1 ‰ and 0.5 ‰, respectively, in plasma of female polar bears (*Ursus maritimus*) that had been fasting for 3-4 months, which was attributed to preferential catabolism of body fat. This was supported by the fact that ^{15}N and ^{13}C depletion occurred in bears that had a relatively high percentage of body fat, and continuing to fast for 7-8 months caused plasma ^{15}N and ^{13}C enrichment, presumably as the animals turned to catabolism of endogenous proteins (Polischuk et al. 2001). Baleen whales similarly rely on catabolism of extensive lipid reserves in blubber to fuel energetic needs while fasting, when food consumption is below maintenance levels (Lockyer 1981). Incorporation of lipid-derived carbon from mobilized blubber stores into amino acids during the fasting period, as observed in black bears (*Ursus americanus*; Ahlquist et al. 1984), could conceivably lead to ^{13}C depletion of baleen, since lipids are ^{13}C depleted relative to dietary protein (DeNiro and Epstein 1978). To our knowledge, however, a fasting effect on δ^{13}C values has not been reported in mysticetes, when periods of fasting inferred from baleen δ^{15}N cycles and, more importantly, declines in lipid reserves (Aguilar and Borrell 1990), have been accompanied by relatively constant baleen $\delta^{13}C$ values (A. Aguilar, University of Barcelona, Barcelona, Spain, pers. comm.).

Baleen $\delta^{13}C$ cycles, rather, have invariably been attributed to source $\delta^{13}C$ variation as migratory whales forage within isotopically distinct food webs across their distribution (e.g. Schell et al. 1989a, Best and Schell 1996, Lee et al. 2005, Caraveo-Patiño and Soto 2005). Compiled records of isotopic compositions of particulate organic matter (POM) and zooplankton communities spanning the summer and winter distributions of EC-WG bowhead whales (Table 3.4) show reasonable agreement between baleen isotope patterns and regional isotopic variation across the eastern Canadian Arctic, although the latter is based on few studies. Both $\delta^{15}N$ and $\delta^{13}C$ values were higher, on average, in POM and zooplankton in Gulf of Boothia and Baffin Bay, where EC-WG whales forage during the ice-free season, than in Hudson and Davis Straits, where EC-WG bowheads overwinter, which is consistent with $\delta^{15}N$ and $\delta^{13}C$ increases in basal baleen grown on the summer foraging grounds. Individual baleen $\delta^{15}N$ and $\delta^{13}C$ oscillations ranging up to 2.7 and 2.1 ‰, respectively (Table 3.3), encompass regional differences in POM and zooplankton $\delta^{15}N$ and $\delta^{13}C$ values (1.3-1.9 and 0.8-2.1 ‰, respectively; Table 3.4), although smaller oscillations, or the altogether absence of regular $\delta^{13}C$ cycles in four of the plates, are not consistent with regional baseline variation. Inconsistencies may be due to incomplete understanding of spatial and temporal isotopic variation of regional food webs across the eastern Canadian Arctic and western Greenland, given only a small percentage of the EC-WB bowhead range has been surveyed for zooplankton isotopic composition over several discontinuous years. For example, recent efforts to characterize isotopic composition of zooplankton throughout the Eastern Canadian Arctic (e.g. Pomerleau et al. 2011c) have excluded Hudson Strait, where a large

Table 3.4. Stable isotope composition (δ^{15}N and δ^{13}C) of particulate organic matter (POM) and zooplankton sampled throughout the range of Eastern Canadian Arctic-West Greenland (EC-WG) bowhead whales (*Balaena mysticetus*)

Range	**Region**	**Taxonomic group/species**	**N**	**δ^{15}N (‰)**	**δ^{13}C (‰)**	**Reference**
summer/ fall	Lancaster Sound	POM	5*	5.4 ± 0.8	-21.6 ± 0.3	Hobson and Welch 1992
	Baffin Bay	POM	38*	6.8 ± 0.3	-22.3 ± 0.2	Hobson et al. 2002
	mean$_{POM}$			**6.1**	**-22.0**	
	Gulf of Boothia	*Calanus glacialus*	–	10.1 ± 1.0	-21.1 ± 1.2	Pomerleau et al. 2011c
	Gulf of Boothia	*C. hyperboreus*	–	9.6 ± 0.7	-21.0 ± 0.6	Pomerleau et al. 2011c
	Arctic archipelago	*C. glacialus*	–	8.1 ± 0.3	-21.2 ± 0.1	Pomerleau et al. 2011c
	Arctic archipelago	*C. hyperboreus*	–	8.6 ± 1.0	-21.5 ± 0.7	Pomerleau et al. 2011c
	Lancaster Sound	*C. hyperboreus*	6*	9.2 ± 0.5	-20.4 ± 0.4	Hobson and Welch 1992
	Baffin Bay	*C. glacialus*	–	8.7 ± 0.4	-21.0 ± 0.3	Pomerleau et al. 2011c
	Baffin Bay	*C. glacialus*	2*	9.1 ± 0.4	-20.6 ± 0.8	Hobson et al. 2002
	Baffin Bay	*C. hyperboreus*	–	8.2 ± 0.4	-21.5 ± 0.3	Pomerleau et al. 2011c
	Baffin Bay	*C. hyperboreus*	80*	7.9 ± 0.1	-21.1 ± 0.1	Hobson et al. 2002
	mean$_{zooplankton}$			**8.8**	**-21.0**	
winter/ spring	Hudson Strait	POM	–	4.69	-25.52	Kuzyk et al. 2010
	Hudson Strait	POM	–	4.56	-21.50	Kuzyk et al. 2010
	Hudson Strait	POM	–	5.22	-24.54	Kuzyk et al. 2010
	Hudson Strait	POM	–	5.19	-22.52	Kuzyk et al. 2010
	Hudson Strait	POM	–	4.65	-24.66	Kuzyk et al. 2010
	Hudson Strait	POM	–	4.47	-25.92	Kuzyk et al. 2010
	mean$_{POM}$			**4.8**	**-24.1**	
	Hudson Strait	*Calanus* sp.	6	7.1 ± 0.3	-22.1 ± 0.8	Pazerniuk 2007
	Foxe Channel	*Calanus* sp.	4	6.7 ± 0.5	-21.5 ± 0.5	Pazerniuk 2007
	E. Hudson Bay	*Calanus* sp.	5	6.2 ± 0.4	-23.6 ± 0.4	Pazerniuk 2007
	Davis Strait	*C. glacialus*	–	7.4 ± 1.1	-20.4 ± 1.7	Pomerleau et al. 2011c
	Davis Strait	*C. hyperboreus*	–	6.9 ± 0.4	-21.2 ± 0.6	Pomerleau et al. 2011c
	mean$_{zooplankton}$			**6.9**	**-21.8**	
Summer - winter range (POM)				**1.3**	**2.1**	
Summer - winter range (zooplankton)				**1.9**	**0.8**	

*grouped samples comprising several individuals portion of the EC-WG bowhead whale population overwinter (Reeves and Mitchell 1990), and the few existing measurements from summer (Pazerniuk 2007, Kuzyk et al. 2010) may differ from winter values we are assuming they represent.

Seasonal migrations within the open ocean would not be expected to produce $\delta^{34}S$ variation in baleen, given the relative uniformity of ocean water sulfate $\delta^{34}S$ values (Rees et al. 1978). However, sulfate $\delta^{34}S$ values in marginal seas similar to Hudson Bay are, on average, about 0.5-1 ‰ less than sulfate values in the open ocean due to the greater influence of relatively ^{34}S depleted riverine inputs in restricted basins (Strauss 2004). Hudson Bay receives considerable terrestrial freshwater inputs from river run-off (Jones and Anderson 1994), potentially lowering food web $\delta^{34}S$ values on the EC-WG bowhead whale wintering grounds in Hudson Strait. Baseline data required to assess this hypothesis are unavailable, but a comparison of $\delta^{34}S$ values in muscle of EC-WG bowhead whales (18.35-18.66 ‰; SHF, unpublished data) and beluga whales from Hudson Bay and Hudson Strait (17.25-17.50 ‰; Kelley 2014) suggests Hudson Bay/Hudson Strait food webs may be relatively depleted in ^{34}S (assuming beluga muscle integrates a $\delta^{34}S$ value that primarily reflects Hudson Bay/Hudson Strait, and bowhead muscle integrates a greater open ocean signal). This admittedly crude comparison does not take into account potential diet- or habitat-related differences (e.g. the summer estuarine habitat of belugas), although similar $\delta^{34}S$ values in muscle of bowheads (17-17.6 ‰) and belugas (17.7 ‰) off northern Alaska (Hoekstra et al. 2002) suggest little influence of these potentially confounding factors. Lower baleen $\delta^{34}S$ values in winter vs. summer would be consistent with a regional $\delta^{34}S$ gradient between Hudson Strait and the rest of the eastern Canadian Arctic, and

$\delta^{34}S$ oscillations on the order of 0.5-1 ‰ are similar to sulfate $\delta^{34}S$ differences between other marginal seas and the open ocean (Strauss 2004).

Wide differences between seawater sulfate and sedimentary sulfide $\delta^{34}S$ values can also lead to considerable isotopic separation of marine consumers foraging in near vs. offshore regions (Barros et al. 2010). Seasonal shifts in foraging depth of EC-WG bowhead whales in response to vertical zooplankton distribution have been observed at Disko Bay, where whales dove close to the seabed to feed on copepods throughout March, and switched to shallower depths when copepods ascended to surface waters in April and May (Heide-Jørgensen et al. 2013). Descent to winter depths alone would not alter the sulfur isotopic composition of pelagic zooplankton, but seasonal shifts in foraging depth accompanied by consumption, either intentional or incidental, of benthic and epibenthic species (e.g. Pomerleau et al. 2011a) could lead to lower $\delta^{34}S$ values in winter-grown baleen because benthic food webs with sedimentary sulfur inputs are ^{34}S depleted relative to pelagic systems (Peterson et al. 1985). However, higher $\delta^{13}C$ values would be expected with consumption of more benthic prey, since benthic components of Arctic marine food webs are ^{13}C enriched (Hobson and Welch 1992; Hobson et al. 2002). The lack of inverse correlation between $\delta^{34}S$ and $\delta^{13}C$ values, with ^{13}C enrichment during summer rather than winter, therefore does not fit this scenario.

While $\delta^{34}S$ variation in animal tissues has conventionally been attributed to spatial variation, a handful of recent studies suggest synchronous $\delta^{15}N$ and $\delta^{34}S$ cycles may reflect a common trophic influence, perhaps mediated through changes in amino acid metabolism during periods of limited food consumption. ^{34}S enrichment in keratinous tissues over organic dietary sulfur by 2-5

‰ (Tanz and Schmidt 2010) indicates metabolism of sulfur amino acids (methionine and cysteine) has an associated isotopic fractionation. Methionine is the metabolic precursor of cysteine (Stipanuk 1986, 2004, Griffith 1987), which is the most common sulfur amino acid in keratins like baleen (Block 1937, Gillespie and Inglis 1964). Conversion of methionine to cysteine via several intermediates during the transsulfuration pathway involves transfer of the thiol group (Aitken et al. 2011), which introduces potential for kinetic ^{34}S fractionation. Cysteine, in turn, is the direct or indirect precursor of other sulfur-containing organic molecules, and the relative ^{34}S depletion of its oxidation product (taurine) indicates ^{34}S fractionation occurs with cysteine catabolism (Tanz and Schmidt 2010). Methionine and cysteine catabolism is restricted at low intakes in rats and humans, when both sulfur amino acids are channelled to protein synthesis rather than transsulfuration and oxidation (Storch et al. 1988, 1990, Stipanuk et al. 1992). Direct incorporation of sulfur amino acids into proteins by mysticetes during periods of limited protein intake would presumably reduce diet-keratin $\delta^{34}S$ discrimination and lead to lower baleen $\delta^{34}S$ values than when methionine and cysteine are in adequate supply and readily catabolised.

A limited number of studies have linked diet-tissue $\delta^{15}N$ and $\delta^{34}S$ discrimination ($\Delta^{15}N$ and $\Delta^{34}S$) with dietary protein quantity and quality, although none have focused on fasting-adapted animals under natural fasting conditions. McCutchan et al. (2003) found $\Delta^{34}S$ was significantly greater among consumers of high protein (+1.9 ± 0.51 ‰) than low protein (-0.5 ± 0.65 ‰) diets, although the authors could not isolate the role of protein quantity from among several potentially confounding variables. However, positive correlation between $\Delta^{15}N$ and $\Delta^{34}S$, which were high when dietary nitrogen exceeded growth requirements, indicates $\Delta^{34}S$ is affected by diet protein content. Similarly, Florin et al. (2011) found Δ^{34}S was positively correlated with various measures of total dietary sulfur amino acid content fed to rats. Results from these studies are consistent with a trophic, rather than spatial, explanation of concurrent baleen δ^{15}N and δ^{34}S cycles. Further, mediation of baleen δ^{15}N and δ^{34}S values through changes in amino acid metabolism during restricted protein consumption could explain why observed correlations between δ^{15}N and δ^{34}S values were not stronger, since δ^{34}S values would reflect metabolism of only sulfur amino acids, while δ^{15}N values would reflect the bulk of amino acid metabolism (see Florin et al. 2011). We also acknowledge that the composition and activity of gut microbial communities can vary with nutrition state in foregut fermenters like bowhead whales (Herwig et al. 1984), with likely influences on amino acid catabolism and synthesis not considered here.

By necessity, we have focused on generalized isotopic trends to assess potential foraging scenarios, but it is worth addressing potential causes of variation in isotopic cycling observed within and among whales. Periodic δ^{15}N variation in the absence of strong δ^{13}C cycles, which, despite overall correlations between δ^{15}N and δ^{13}C values, occurred at some point in most plates, could mark years when winter foraging was more limited, such that baleen retained δ^{13}C values acquired on the summer foraging grounds. Three of the four whales lacking δ^{13}C cycles were mature males, which may be more likely to forego winter foraging when preoccupied with mating behaviour. Variable residency and migration patterns could also lead to isotopic variation within and among individuals. Although a majority of EC-WG bowhead whales summer and winter in Prince Regent Inlet/Gulf of Boothia and Hudson Strait, respectively, seasonal aggregations also occur along the east coast of Baffin Island in areas such as Cumberland Sound and Isabella Bay, as well as western Greenland (Reeves et al. 1983, Finley 1990, Dueck and

Ferguson 2008, Ferguson et al. 2010). Migration routes also vary, with whales traveling across Davis Strait, along eastern Baffin Island, or between Foxe Basin and Gulf of Boothia (Reeves et al. 1983, Dueck and Ferguson 2008). Females may alter feeding behaviour spatially and temporally with reproductive cycles, possibly feeding earlier in the year at Disko Bay when mating or pregnant, then switching to feeding in Foxe Basin later in summer when with a calf or juvenile (see Cosens and Blouw 2003, Laidre et al. 2007). Different residency and migration patterns could also account for the lack of synchronous isotopic oscillations among whales in any given calendar year, since underlying regional isotopic variation would be synchronously recorded only in baleen of whales foraging within the same region. Interestingly, all whales lacking clear $\delta^{13}C$ cycles were harvested in northern Hudson Bay, Hudson Strait, or Cumberland Sound. If seasonal migrations of these animals were shorter than animals harvested in Foxe Basin/Gulf of Boothia (i.e. between Hudson Strait and Hudson Bay, or within Cumberland Sound), then the absence of $\delta^{13}C$ cycles could reflect their smaller range.

Assuming baleen $\delta^{13}C$ variation reflects regional baseline influences, a conservative interpretation of baleen isotope cycles is EC-WG bowhead whales forage throughout their distribution, although at a reduced rate during winter, given $\delta^{15}N$ and $\delta^{34}S$ cycles may reflect trophic influences to some extent. Late winter/spring foraging on resting calanoid copepods at overwintering depths is well-documented in bowhead whales at Disko Bay, off west Greenland (Laidre et al. 2007, Heide-Jørgensen et al. 2010, 2013). Bowheads overwintering in Hudson Strait in March have also been observed deep diving, which is consistent with foraging well below the surface (W. Koski, LGL Limited, King City, ON, pers. comm.). This interpretation is supported by recent dive data from satellite tagged bowheads in Hudson Strait that regularly dove (>60 % of dives) deeper than 250 m during January and February (B. LeBlanc, Fisheries and Oceans Canada, Winnipeg, MB, pers. comm.), likely to reach mature zooplankton near the seafloor. There is also limited evidence that EC-WG bowhead whales take advantage of early season production during the ice algae bloom, when large aggregations of adult calanoid copepods graze near the ice-water interface throughout the eastern and central Canadian Arctic from March-June (Bradstreet and Cross 1982, Conover et al. 1986, Runge and Ingram 1988). Finley (2001) suggested zooplankton are too dispersed over this period to be energetically valuable to bowhead whales, but acknowledged some feeding along ice edges and within pack ice likely occurs. Pomerleau et al. (2012) found the isotopic composition of EC-WG bowhead whale skin reflected limited foraging on ice-associated zooplankton, while fatty acid profiles indicated reliance on diatom-derived food webs (although sympagic and planktonic species could not be differentiated; Pomerleau et al. 2014).

Year-round foraging by EC-WG bowhead whales inferred from baleen isotope cycles is also consistent with foraging behavior of conspecifics from the Bering-Chukchi-Beaufort (BCB) population. BCB bowheads feed occasionally, if not commonly, during spring migrations along the north Alaska coast ca. April-June (Carroll et al. 1986, Lowry et al. 2004, Asselin et al. 2012). While there has been general consensus that winter feeding contributes little to overall nutrition of BCB bowhead whales (Lowry 1993), Schell et al. (1989a) and Lee et al. (2005) concluded the carbon isotopic composition of bowhead muscle was largely derived from fall and winter foraging in the Bering and Chukchi Seas. As with EC-WG bowheads, direct observations of winter foraging by BCB bowheads are limited, although Noongwook et al. (2007) report consistent winter foraging in at least one area of the Bering Sea. Citta et al. (2014) provide supporting evidence from dive profiles that BCB bowhead whales forage throughout the year at core-use areas where oceanographic processes produce high zooplankton densities. Whales overwintered (November-April) in two core-use areas in the Bering Sea, spending almost half their time diving near the seafloor and presumably feeding along a subsurface front between a cold water current and warmer shelf waters (Citta et al. 2014). Lydersen et al. (2012) and Falk-Petersen et al. (2014) provide evidence that bowhead whales from the Spitsbergen population off eastern Greenland overwinter at high-latitude locations, where upwelling of Atlantic water along the shelf break may allow foraging on *Calanus glacialis* as early as mid-January. Similar localized oceanographic features and upwelling events that aggregate zooplankton within bowhead diving range (Finley 1990, Okkonen et al. 2011, Walkusz et al. 2012) may also determine winter habitat selection by EC-WG bowhead whales, since the bulk of pelagic zooplankton important in their diet have descended to overwintering depths by late summer (Longhurst et al. 1984, Head and Harris 1985). Such oceanographic features may also explain the relatively restricted winter distribution of EC-WG bowhead whales compared with the more expansive summer range, when high densities of grazing zooplankton are widely accessible.

Bowhead whale numbers in the eastern Canadian Arctic are currently recovering from commercial whaling prior to the 20th century (Heide-Jørgensen et al. 2007, Higdon 2010), and understanding seasonal foraging ecology improves our ability to identify and protect critical habitat. Our conclusion that EC-WG bowhead whales engage in *limited* foraging over winter and spring is conservative, and hinges on whether $\delta^{15}N$ (and $\delta^{34}S$) variation is caused primarily by trophic or spatial influences. Given links between individual energy budgets and population dynamics, as well as between seasonal food availability and habitat selection, further research into the degree of winter foraging by EC-WG bowhead whales is warranted. Better characterization of spatial and temporal zooplankton isotope values across the eastern Canadian Arctic/western Greenland could be used to retrospectively assess existing baleen isotope data, while advances in stable isotope applications to marine mammal diet studies such as amino acid specific isotope analysis (e.g. Matthews and Ferguson 2014) could help clarify trophic and source influences on isotopic variation in baleen with greater specificity.

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Chapter 4. Validation of dentine deposition rates in beluga whales by interspecies cross dating of temporal $\delta^{13}C$ trends in teeth

*Isotopic time series from sequentially sampled growth layer groups (GLGs) in marine mammal teeth can be combined to build chronologies allowing assessment of isotopic variation in marine ecosystems. Synchronous recording of baseline isotopic variation across dentinal GLGs of species with temporal and spatial overlap in foraging offers a unique opportunity for validation of marine mammal age estimation procedures through calibration of GLG deposition rates in one species against another whose GLG deposition has been independently determined. In this study, we compare trends in stable carbon isotope ratios ($\delta^{13}C$) across dentinal GLGs of three eastern Canadian Arctic (ECA) beluga (*Delphinapterus leucas*) populations through the 1960s-2000s with a $\delta^{13}C$ time series measured across dentinal GLGs of ECA/Northwest Atlantic killer whales (*Orcinus orca*) from 1944-1999. We use confirmed annual GLG deposition in killer whales as a means to assess beluga GLG deposition, and show linear $\delta^{13}C$ declines across chronologies of both species were statistically indistinguishable when based on annual GLG deposition in beluga whales, but differed when based on biannual deposition. We suggest $\delta^{13}C$ declines reflect the oceanic ^{13}C Suess effect, and provide additional support for annual GLG deposition in beluga whales by comparing rates of $\delta^{13}C$ declines across beluga GLGs with published annual $\delta^{13}C$ declines attributed to the oceanic ^{13}C Suess effect in the North Atlantic.*

KEYWORDS: *Delphinapterus leucas*, ageing, growth layer groups (GLGs), oceanic ^{13}C Suess effect, North Atlantic, Arctic, time series, sclerochronology

INTRODUCTION

Physical characteristics of growth layer groups (GLGs) in teeth have had important applications in marine mammal ecological studies, from counting GLGs as a method of age estimation (Perrin and Myrick 1980) to linking GLG width and layering patterns with sexual maturation (Klevezal and Stewart 1994), parturition (Klevezal and Myrick Jr 1984, von Biela et al. 2008, Medill et al. 2010), and environmental variation (Manzanilla 1989). More recently, advances in analytical techniques allowing chemical characterization of small amounts of bulk sample, as with continuous flow isotopic ratio mass spectrometry (CF-IRMS) of micromilled material, or *in situ* micro-spatial analysis using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) or secondary ion mass spectrometry (SIMS), have opened a new area of ecological study focused on GLG microchemistry (e.g. Hobson and Sease 1998, Stern et al. 1999).

Chemical diet proxies such as stable isotopes (SI) are incorporated into teeth via diet and water (Walker and Macko 1999), which in turn reflects underlying biogeochemical processes within the ecosystem in which an animal fed. Chemical profiling of sequentially sampled GLGs therefore allows for chronological diet and habitat reconstructions spanning periods over which GLG deposition occurred, providing access to long-term ecological information that is difficult to obtain through direct observation. Recent studies characterizing SI ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) in GLGs of marine mammal teeth have uncovered patterns consistent with ontogenetic diet and distribution shifts (Hobson and Sease 1998, Mendes et al. 2007), while approaches combining individual SI profiles to build longer chronologies (in a manner analogous to crossdating techniques employed in dendrochronology) have revealed baseline temporal isotopic variation related to climate oscillations (Newsome et al. 2007, Hanson et al. 2009), primary productivity (e.g. Schell 2000, Schell 2001, Hirons et al. 2001; but see also Cullen et al. 2001), and anthropogenic carbon emissions (Newsome et al. 2007, Lysiak 2009).

One of the primary means through which anthropogenic carbon emissions influence baseline marine $\delta^{13}C$ values is through the oceanic ^{13}C Suess effect, which is the serial decrease of marine dissolved inorganic carbon (DIC) $\delta^{13}C$ values caused by the dilution of atmospheric $^{13}C/^{12}C$ by isotopically light CO_2 emissions from burning of fossil fuels (Quay et al. 1992). Variation in baseline food web isotope dynamics due to this and other processes, such as changes in primary productivity (e.g. Laws et al. 1995, Popp et al. 1998), are expected to be synchronously recorded across the teeth of marine mammals with distributional overlap. The extent of spatial variation depends on whether the process is regional in scope, such as primary productivity (e.g. Pancost et al. 1997), or larger, such as the oceanic ^{13}C Suess effect, which has influence over ocean-basin and global scales (Quay et al. 1992). Although not commonly documented in high trophic level marine mammals, Newsome et al. (2007) attributed $\delta^{13}C$ declines in teeth of northern fur seals (*Callorhinus ursinus)* in the North Pacific over 1948-2000 to anthropogenic carbon inputs consistent with the oceanic ^{13}C Suess effect.

Comparison of synchronous isotopic variation across dentinal GLGs of species with similar distributions offers a unique opportunity for validation of marine mammal age estimation procedures through calibration of GLG deposition rates in one species against another whose GLG deposition has been independently determined. Validation of age estimation procedures in free-ranging animals has proven difficult, and such an approach could prove useful for species for which age validation has not previously been possible or has been met with contention, such as beluga whales (*Delphinapterus leucas*) (e.g. Lockyer et al. 2007). Although beluga ages have routinely been estimated from dentinal GLG counts, only recently has the prevailing assumption of biannual dentinal GLG deposition (Goren et al. 1987, Brodie et al. 1990, Heide-Jørgensen et al. 1994) been rejected after studies examining radiocarbon patterns in beluga teeth (Stewart et al. 2006) and allometric relationships between female age and length at maturity among various delphinoid cetaceans (Luque et al. 2007) have indicated annual GLG deposition. The ecological implications of this shift in age estimation have not been trivial; the doubling of beluga lifespan has had significant management consequences for estimating life history parameters such as growth and age at maturity, as well as reproductive rates and population growth projections (Stewart et al. 2006).

Here we provide further support for annual GLG deposition in beluga whales by comparing temporal δ^{13}C declines across dentinal GLGs of beluga from three eastern Canadian Arctic (ECA) populations over the 1960s-2000s with δ^{13}C declines observed across GLGs of ECA/northwest Atlantic (NWA) killer whales (*Orcinus orca*) deposited over 1944-1999. We assume the spatial extent of baseline δ^{13}C variation was sufficient to cause synchronous δ^{13}C patterns across the GLGs of both species, and use confirmed annual GLG deposition in killer whales (Mitchell and Baker 1980, Myrick et al. 1988, Amano et al. 2011) as a reference from which to calculate annual δ^{13}C declines and assess GLG deposition in beluga whales. δ^{13}C trends in teeth of both species were statistically indistinguishable when based on annual GLG deposition in beluga teeth, but differed when biannual deposition was assumed. While we recognize various processes affecting marine δ^{13}C dynamics introduce uncertainty in our interpretations, we also found $\delta^{13}C$ declines observed across GLGs of both species match the oceanic ^{13}C Suess effect, thereby providing additional support for annual GLG deposition in beluga whales using published oceanic ^{13}C Suess effect rates in the North Atlantic as a reference point.

MATERIALS AND METHODS

Tooth collection and micromilling

BELUGA WHALES

Teeth were obtained from whales harvested in subsistence hunts near several Nunavut communities (Arviat, Pangnirtung, and Grise Fiord) during the 1980s-2000s, representing three ECA beluga whale populations, respectively: Western Hudson Bay (WHB; n = 21), Cumberland Sound (CS; n = 28), and Eastern High Arctic-Baffin Bay (EHA-BB; n = 20) (Figure 4.1). Mandibles were collected from each whale and sent to Fisheries and Oceans Canada, Winnipeg, where they were frozen until sampled. Following beluga ageing protocol (Stewart 2012), teeth were selected from the standard positions 2 and 5, except when noticeably less wear occurred in adjacent teeth.

Teeth were air-dried and sectioned longitudinally using a water-cooled diamond-edged blade to expose the midline, and polished using 30 and 9 µm aluminum oxide (AlOx) lapping film to accentuate GLG definition. Powdered dentine was collected from within dentinal GLGs using a high-resolution micromill (Merchantek) fitted with a 300 µm-diameter carbide dental drill bit at a depth of 150 µm to prevent collection of material from underlying layers. Only GLGs of sufficient clarity and width to ensure discrete sample collection were micromilled, resulting in approximately 5-15 sampled GLGs from each individual.

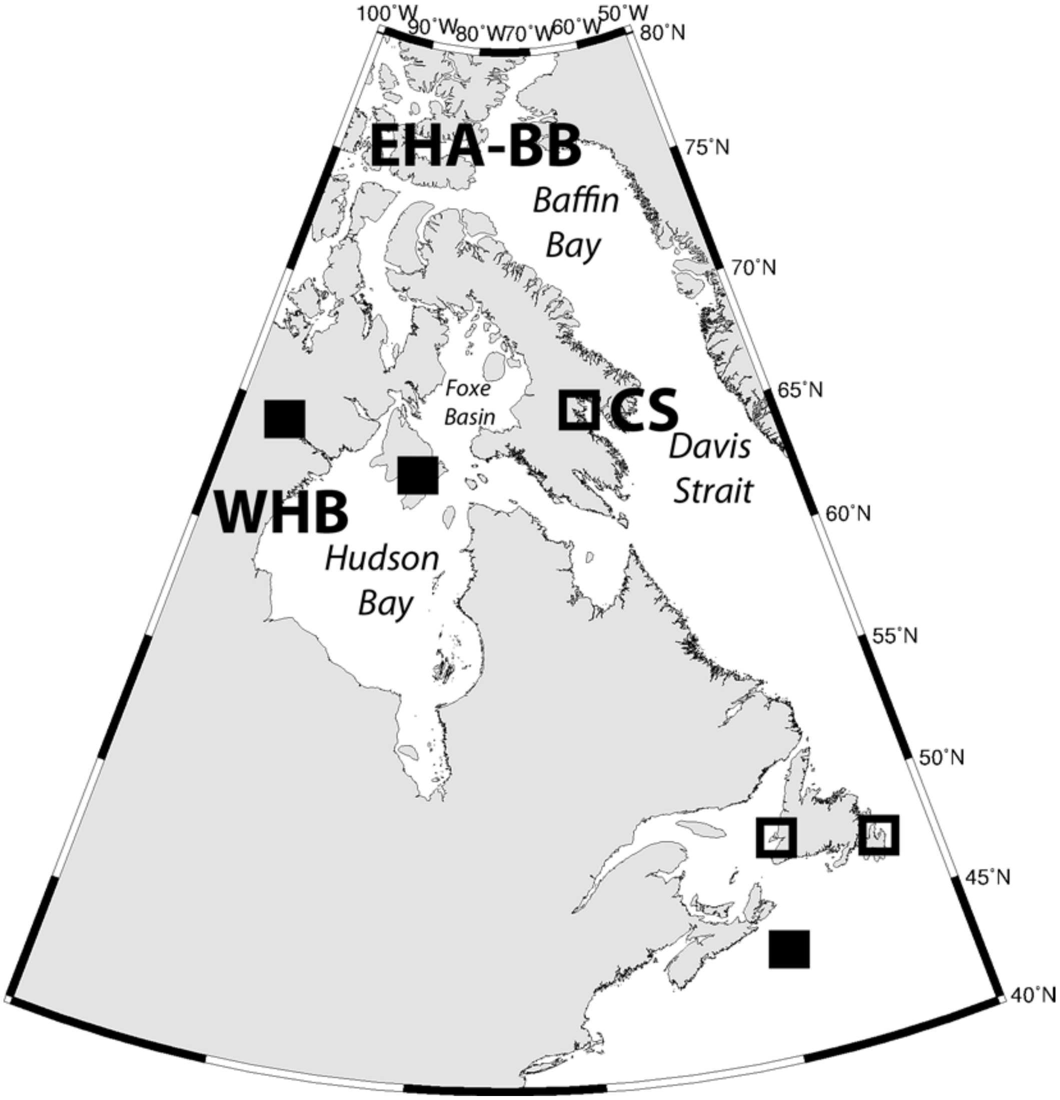

Figure 4.1. Teeth were collected from belugas harvested in Arviat, NU (Western Hudson Bay population; **WHB**), Pangnirtung, NU (Cumberland Sound population; **CS**), and Grise Fiord (Eastern High Arctic-Baffin Bay population; **EHA-BB**) through the 1980s-2000s. Locations where killer whale specimens were collected throughout the eastern Canadian Arctic, Newfoundland, and Nova Scotia are indicated by closed (single specimen) and open (two specimens) squares.

KILLER WHALES

Teeth collected from killer whales (n = 9) from locations throughout the ECA and along the coasts of Newfoundland and Nova Scotia (Figure 4.1) during the 1970s-2000s were acquired from museum and government collections. When possible, the largest tooth with minimal wear was chosen from each specimen. All teeth had been stored dry since collection.

Longitudinal sections approximately 2-mm thick following the midline of the tooth were polished using lapping film, then placed in 10 % formic acid for 12 h to etch the polished surface and accentuate GLG definition. Sections were air-dried for several weeks following thorough rinsing with distilled water. GLGs were micromilled using a 500 µm-diameter drill bit at a drilling depth of 400-500 µm to accommodate the larger size of dentinal GLGs in killer whale teeth. Up to 25 GLGs were sampled from larger teeth, while a minimum 5 GLGs were sampled from the youngest whale.

Stable isotope analysis

Studies of carbon isotopic composition of dentine typically measure δ^{13}C values of either collagen (Newsome et al. 2009) or the inorganic mineral matrix (Hobson and Sease 1998), since metabolic routing of dietary proteins to collagen, and metabolites from all classes of biomolecules to structural carbonate, leads to isotopic differences between the organic and mineral components (Ambrose and Norr 1993). Small sample amounts collected from beluga GLGs made removal of inorganic carbon using acidification prior to analysis impractical, so bulk powdered dentine was analysed. Killer whale samples, on the other hand, were acidified using repeated rinses of 0.25 N HCl at 4 ^{o}C, and isolated collagen was rinsed using deionized water and freeze-dried. In a comparison of δ^{13}C values between untreated and acidified portions of 15 beluga dentine samples, untreated samples had significantly higher mean $\delta^{13}C$ values (-14.2 ± 0.2 ‰) than acidified samples (-14.4 ± 0.2 ‰) ($p < 0.001$, paired samples t-test, df = 14, t = -5.365), and differences between untreated and acidified pairs were consistent across all samples (Matthews and Ferguson, unpubl. data). Therefore, although different treatments of beluga and killer whale dentine complicates direct comparison of absolute $\delta^{13}C$ values between species, $\delta^{13}C$ *trends* (i.e. relative changes over time) within a species are comparable.

Approximately 1 mg of powdered dentine (beluga) or ~0.5 mg of collagen (killer whale) was weighed into tin cups for isotopic analysis on a Vario EL III elemental analyser (Elementar, Germany) interfaced with a DELTAplus XP isotope ratio mass spectrometer (Thermo, Germany). Stable carbon isotope ratios are reported in delta notation (δ) as parts per thousand (‰) deviation from the isotope ratio of Vienna Pee-Dee Belemnite limestone (V-PDB), defined as $\delta^{13}C = (R_{sample} - R_{V\text{-}PDB}) / R_{V\text{-}PDB}) * 1000$, where R is $^{13}C/^{12}C$. Analytical precision based on repeated measures of laboratory reference materials not used in calibrations was 0.06 ‰, as was that based on duplicate measures of ~10 % of samples. Atomic C:N of beluga dentine (2.98 ± 0.06; mean ± sd) and killer whale dentinal collagen (2.91 ± 0.1) were within the range of unaltered collagen (DeNiro 1985).

Data analysis

Each GLG was aged based on counts from the first GLG adjacent to prenatal dentine (beluga) or enamel (killer whales), and assigned a calendar year of deposition using the final GLG deposited during the year of death as a reference. Beluga GLG age and calendar year of formation were assigned assuming both annual and biannual GLG deposition. Isotope values from the first three

GLGs were removed from all individual beluga and killer whale series, since significant ontogenetic trends associated with weaning were observed across those ages in both species (Matthews and Ferguson 2014, unpubl. data). Chronologies constructed from individual tooth $\delta^{13}C$ profiles spanned ~50 years (1960s-2000s) for each beluga population, and from 1944-1999 for killer whales (Table 4.1, Figure 4.2).

Generalized linear mixed effects models with random intercepts were used to compare temporal $\delta^{13}C$ trends in each population. Visual inspection indicated trends differed among populations, so models were run separately for each. Calendar year was treated as a fixed effect, along with sex and age class to account for variation in $\delta^{13}C$ values due to those factors. Age class included the categories ‘immature’ and ‘adult’, which comprised GLGs 4-11 and 12-27, respectively (see Marcoux et al. 2012). Whale identity was included as a random effect to account for correlation of repeated measures within individuals. Models were run twice for each beluga population to estimate annual rates of $\delta^{13}C$ declines assuming both annual and biannual GLG deposition. To compare rates of $\delta^{13}C$ declines among populations, a model comprising all data was run with all fixed effects, including the interaction term calendar year*population to test equality of slopes among beluga and killer whale populations. All models were run using the maximum likelihood method, and the optimal model among full, reduced, and null models was selected based on AIC. Analyses were performed using the nlme package (Pinheiro et al. 2012) available for R software (R Core Team 2012). Matthews and Ferguson (2014) have shown previously that $\delta^{13}C$ values did not differ with age in ECA/NWA killer whales.

Table 4.1. Rates of $\delta^{13}C$ declines across dentinal GLGs of killer whales (1944-1999) and beluga (1960s-2000s) in the eastern Canadian Arctic/North Atlantic. 95 % confidence intervals in parentheses.

Species	**Population**	**Annual GLG Deposition**		**Biannual GLG Deposition**	
		$\delta^{13}C$ (‰ yr^{-1})	**Years**	**$\delta^{13}C$ (‰ yr^{-1})**	**Years**
Killer whale (*O. orca*)	Eastern Canadian Arctic/ Northwest Atlantic (ECA/NWA)	–0.0184 (–0.00781, –0.0291) ($p = 0.001$)	1944-1999	–	–
Beluga whale (*D. leucas*)	Western Hudson Bay (WHB)	–0.0186 (–0.00689, –0.0304) ($p < 0.01$)	1963-2008	–0.0362 (–0.0153, –0.0570) ($p < 0.001$)	1980-2008
	Cumberland Sound (CS)	–0.00138 (0.00468, –0.00744) ($p > 0.5$)	1966-2007	–0.00231 (0.00569, –0.0103,) ($p > 0.5$)	1977-2007
	Eastern High Arctic-Baffin Bay (EHA-BB)	–0.0285 (–0.0195, –0.0375) ($p < 0.001$)	1976-2001	–0.0442 (–0.0283, –0.0600) ($p < 0.001$)	1981-2001

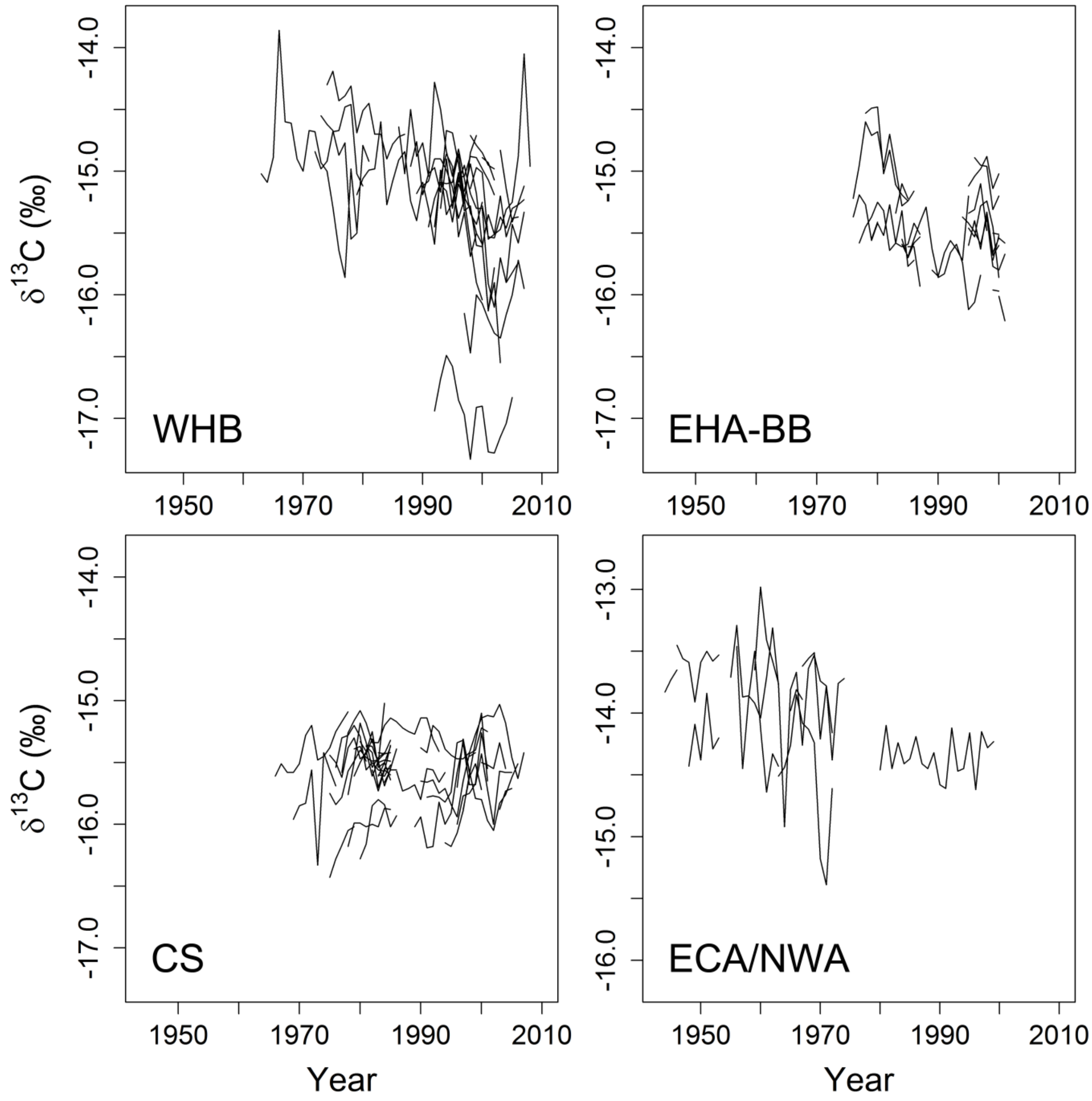

Figure 4.2. Significant $\delta^{13}C$ declines across dentinal GLGs of –0.0186 ± 0.00602 ‰ yr^{-1} and –0.0285 ± 0.00458 ‰ yr^{-1} in both the Western Hudson Bay (WHB) and Eastern High Arctic-Baffin Bay (EHA-BB) beluga populations, respectively, were statistically indistinguishable from $\delta^{13}C$ declines across dentinal GLGs of Eastern Canadian Arctic/Northwest Atlantic killer whales (–0.0184 ± 0.00540 ‰ yr^{-1}; ECA/NWA) over a similar timeframe when rates were based on annual GLG deposition in beluga whales. In contrast, $\delta^{13}C$ values in the Cumberland Sound (CS) population were relatively constant over the same timeframe.

Approximate 95 % confidence intervals around estimated rates of $\delta^{13}C$ declines in each population were compared with published oceanic ^{13}C Suess effect rates in the North Atlantic to assess whether it was a plausible cause of observed trends.

RESULTS

Best-fit models indicated population-specific $\delta^{13}C$ trends occurred over the period of GLG deposition (calendar year*population interaction term, $p < 0.001$). Significant linear $\delta^{13}C$ declines occurred in the ECA/NWA killer whale and WHB and EHA-BB beluga populations, while no $\delta^{13}C$ trend occurred in the CS beluga population (Figure 4.2, Table 4.1). The interaction term sex*age class remained in the final model of WHB beluga $\delta^{13}C$ values, and age class was a significant predictor of $\delta^{13}C$ values in the Cumberland Sound population. $\delta^{13}C$ variation was unrelated to sex or age class in the EHA-BB population ($p > 0.5$).

Rates of $\delta^{13}C$ declines in the ECA/NWA killer whale (–0.0184 ± 0.0054 ‰ yr^{-1}) and WHB and EHA-BB beluga populations (–0.0186 ± 0.0060 ‰ yr^{-1} and –0.0285 ± 0.0046 ‰ yr^{-1}, respectively) (Figure 4.2, Table 4.1) were statistically indistinguishable when rates were based on annual GLG deposition in beluga whales ($p > 0.65$), but differed between the killer whale and beluga populations when biannual GLG deposition was assumed in beluga teeth (–0.0362 ± 0.011 ‰ yr^{-1} and –0.0442 ± 0.0081 ‰ yr^{-1} for the WHB and EHA-BB populations, respectively) ($p < 0.001$).

Approximate 95 % confidence intervals around slope estimates showed $\delta^{13}C$ declines based on annual GLG deposition in both species encompassed published oceanic ^{13}C Suess effect rates in the North Atlantic Ocean. When $\delta^{13}C$ declines were based on biannual deposition, Suess effect rates were outside the 95 % C.I. of estimates for the EHA-BB population, and largely so for the WHB population (Tables 4.1 and 4.2).

DISCUSSION

Annual dentinal GLG deposition in killer whales has been confirmed through comparisons of GLG counts with estimated age of a well-recognized wild killer whale (Mitchell and Baker 1980) and corpora counts in sexually mature wild females (Amano et al. 2011), along with assessment of tetracycline labels in teeth of captive individuals with known treatment history (Myrick et al. 1988). We contend statistically indistinguishable rates of $\delta^{13}C$ decline across dentinal GLGs of killer whales and beluga whales based on deposition of one GLG per year provides support for annual GLG deposition in beluga whales, as has been recently shown by other studies (Stewart et al. 2006, Luque et al. 2007). Our approach implicitly assumes that regional baseline $\delta^{13}C$ variation caused synchronous $\delta^{13}C$ patterns across the GLGs of both species, although we acknowledge other factors, such as shifts in foraging behavior and processes affecting marine $\delta^{13}C$ dynamics at more localised scales (e.g. Pancost et al. 1997), introduce a degree of uncertainty in our interpretations.

Gradual changes in diet composition over time would confound our assumption that observed $\delta^{13}C$ trends reflected only baseline isotopic variation. Gradual inclusion of a higher proportion of ^{13}C depleted prey, such as lower trophic level (McCutchan et al. 2003) or pelagic (France 1995) fishes, over time could hypothetically account for observed $\delta^{13}C$ decreases. $\delta^{15}N$ values measured across dentinal GLGs offer an internal check on diet shifts, since concurrent (and more pronounced) declines in $\delta^{15}N$ values are expected to accompany $\delta^{13}C$ decreases associated with trophic-level diet variation (McCutchan et al. 2003). The lack of linear $\delta^{15}N$ trends across GLGs of ECA/NWA killer whales or WHB and EHA-BB belugas (Matthews and Ferguson 2014; unpubl. data) indicates trophic-level dietary shifts are an unlikely explanation for observed $\delta^{13}C$ declines in any of these populations, but does not rule out potential incorporation of more pelagic prey over the timeframe of the study.

There is, however, credible evidence of a pronounced dietary shift within the CS beluga population over the timeframe of our study, which could account for different $\delta^{13}C$ patterns in their teeth. Marcoux et al. (2012) attributed declines in CS beluga skin and muscle $\delta^{15}N$ values over 1982-2009 to a dietary shift precipitated by recent invasion of capelin (*Mallotus villosus*) into the Cumberland Sound ecosystem. Diet comprising a greater proportion of capelin may have offset $\delta^{13}C$ declines observed in the other populations, given that capelin are enriched in ^{13}C by ~1 ‰ relative to Arctic cod (*Boreogadus saida*) (Marcoux et al. 2012, Watt et al. 2013), the primary prey of CS beluga (Kelley et al. 2010, Marcoux et al. 2012). Evidence of recent diet shifts among CS beluga offers a reasonable explanation for the absence of $\delta^{13}C$ declines observed across the GLGs of the other beluga and killer whale populations.

Decreasing $\delta^{13}C$ values in the absence of $\delta^{15}N$ trends suggests isotope patterns across GLGs were driven by variation in baseline carbon isotope composition. Processes influencing marine $\delta^{13}C$ dynamics can vary over local, regional, and larger scales, which, depending on species distributions, could lead to decoupling of isotopic patterns between different populations (e.g. Hirons et al. 2001). Although beluga and killer whale populations included in our study occur throughout the eastern Canadian Arctic during the open water season (Reeves and Mitchell 1988, Higdon et al. 2012), ECA/NWA killer whale movements in the North Atlantic at other times of the year (Matthews et al. 2011) span a gradient in surface zooplankton δ^{13}C values of several per mil (Graham et al. 2010). Matthews and Ferguson (2014) examined distributional differences of ECA/NWA killer whales using amino acid specific δ^{15}N analysis of dentine, and two whales with δ^{13}C values related to spatial variation were excluded from population-level δ^{13}C declines we assess here. Distributional differences also exist among the three Eastern Canadian Arctic beluga populations. CS whales have a restricted distribution relative to the more expansive seasonal migrations undertaken by the WHB and EHA-BB populations (Smith and Martin 1994, Richard et al. 1998, 2001, Richard and Stewart 2008). CS belugas may therefore be more influenced by local processes in Cumberland Sound and less influenced by region-scale processes, which may have contributed to population-specific δ^{13}C differences.

Sea-ice reductions in the Arctic marine ecosystem spanning the duration of GLG deposition could have caused changes in primary production or algal community composition, which in turn may have altered baseline δ^{13}C values (e.g. Laws et al. 1995, Bidigare 1997, Pancost et al. 1997, Popp et al. 1998, Burkhardt et al. 1999). WHB beluga summer in Hudson Bay, where the length of the open water season has increased by approximately 3 days decade^{-1} from the 1970s to 2000s (Gough et al. 2004). Farther north on the summering grounds of EHA-BB beluga in the Canadian Arctic archipelago, ice-free conditions have increased by 7 days decade^{-1} over the same period (Howell et al. 2009). Primary production, chlorophyll, and nutrient concentrations measured in the Canadian Arctic archipelago during the 1980s-2000s (Michel et al. 2006) however, are similar in magnitude and interannual variability to those measured in the 1950s and

60s (Apollonio and Matria 2011), suggesting observed $\delta^{13}C$ trends are not a reflection of changing levels of Arctic primary production in response to changing sea ice dynamics. Another possibility with earlier ice melt and later ice formation is reduced contribution of sympagic (ice-associated) algal production to overall ecosystem primary production. Due to well-defined boundary layers that limit diffusion of DIC, reduced isotopic discrimination during photosynthesis leads to higher $\delta^{13}C$ values of ice algae than phytoplankton (Hobson et al. 1995). A gradual decrease in ice algae production related to loss of Arctic sea ice could therefore have led to lower baseline $\delta^{13}C$ values, which presumably would have influenced belugas feeding entirely within Arctic food webs more so than killer whales, which are seasonal inhabitants.

Although confounding factors outlined above prevent dismissal of $\delta^{13}C$ declines based on biannual deposition with certainty (i.e. we cannot rule out that the populations did not experience population-specific processes leading to different long-term $\delta^{13}C$ trends), we propose similar $\delta^{13}C$ declines among two of the three beluga populations, when based on annual GLG deposition, and ECA/NWA killer whales reflect regional variation in baseline carbon isotope composition. The most parsimonious explanation for synchronous $\delta^{13}C$ trends across the GLGs of marine mammals ranging over such a large area is the oceanic ^{13}C Suess effect, which influences baseline marine $\delta^{13}C$ values at ocean basin scales (Quay et al. 1992). Several studies have provided estimates of oceanic ^{13}C Suess effect rates ranging from –0.018 to –0.026 ‰ yr^{-1} in the North Atlantic over the past several decades (Table 4.2). These independent estimates agree with rates of $\delta^{13}C$ decline based on annual GLG deposition in killer whale and beluga teeth, but fall largely or entirely outside estimates based on biannual GLG deposition. Although the oceanic ^{13}C Suess effect varies among ocean basins and at different depths (Quay et al. 1992),

Table 4.2. Oceanic ^{13}C Suess effect rates for the North Atlantic Ocean basin spanning the timeframe represented by sampled killer whale and beluga GLGs. Estimates are restricted to depths < 1000 m.

Depth (m)	**Latitude**	**Years**	**Suess Effect (‰ yr^{-1})**	**Source**
600-800	25°N-37°N	1969-1979	–0.018 ± 0.002*	Sonnerup et al 1999
800-1000	45°N-60°N	1973-1990	–0.023 ± 0.006*	Sonnerup et al 1999
<1000	5°S-65° N	1993	-0.026 ± 0.002	Körtzinger and Quay 2003
Surface	32°N	1981-1983	-0.025 ± 0.002	Gruber et al 1999
Surface	tropical to polar	1981-1983; 1992-1995	-0.019*	Quay et al 2003

*published as ‰ $decade^{-1}$

nowhere could we find rates approaching –0.036 to –0.044 ‰ yr^{-1} to account for biannual GLG deposition in beluga whales. While dentinal GLGs integrate various factors affecting marine $\delta^{13}C$ values, good agreement between $\delta^{13}C$ declines across dentinal GLGs and independently assessed oceanic ^{13}C Suess effect rates provides additional support for annual GLG deposition in beluga teeth.

In this study, we assume variation in baseline isotope dynamics occurred over a sufficient spatial scale to led to synchronous $\delta^{13}C$ declines across dentinal GLGs of ECA beluga and ECA/NWA killer whale populations, which we use to calibrate GLG deposition rates in beluga against annually-deposited GLGs in killer whales. We outline several factors that introduce limited uncertainty in our interpretations, but contend that agreement between $\delta^{13}C$ declines across beluga and killer whale GLGs and published annual oceanic ^{13}C Suess effect rates in the North Atlantic provides support for annual GLG deposition in beluga whales. The lack of similar declines in a third beluga population (CS) is most likely related to pronounced and well-documented dietary shifts over the past several decades, which likely masked baseline $\delta^{13}C$ trends recorded in GLGs of the other populations. Results from this novel approach using isotopic chronologies derived from teeth to assess dentinal deposition rates in beluga whales add to the considerable evidence of recent studies confirming one GLG is deposited per year in this species.

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Chapter 5. Weaning age variation in beluga whales (*Delphinapterus leucas*)

*Beluga whales (*Delphinapterus leucas*) have a protracted nursing period estimated to last from 6-32 months, although current estimates of beluga nursing duration are derived using approaches subject to capture bias. Recent studies have shown stable isotope (SI) profiles of dentine growth layer groups (GLGs) in marine mammal teeth serve as a reliable nursing proxy, and can be used to assess individual weaning patterns. We measured stable isotope ratios of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) of dentine GLGs in teeth from eastern Canadian Arctic belugas to estimate weaning age and assess relative contributions of milk and solid food during the nursing period. $\delta^{15}N$ declines of ~1 ‰ over the first 3 GLGs of most individuals was interpreted as evidence of weaning. Individual $\delta^{15}N$ profiles indicated 15 of 27 whales were completely weaned by the end of their second year, although a number of whales were weaned by the end of their first or third year (9 and 3, respectively). Intermediate GLG2 $\delta^{15}N$ values relative to GLGs 1 and 3 indicated most whales consumed a mixture of milk and solid food during their second year, consistent with gradual weaning. Contrary to predictions based on parental care theory, nursing duration was not related to relative GLG width (used as a proxy for somatic growth), and did not differ for females and males, or among populations. $\delta^{13}C$ variation was not a reliable indicator of nursing duration, as approximately half of the whales showed no ontogenetic $\delta^{13}C$ patterns across GLGs deposited over the nursing period. This study provides novel life history information which may inform beluga conservation and management decisions, and indicates belugas share prolonged nursing duration marked by individual variation observed in other odontocetes.*

KEYWORDS: dentine, growth layer groups (GLGs), marine mammal, nursing, stable isotopes, teeth, Arctic

INTRODUCTION

Parental investment is defined by Trivers (1972) as expenditure of parental resources to increase an offspring's chances of survival that diminishes ability to invest in other offspring. One of the defining mammalian characteristics is the provision of milk to dependent young, when transfer of maternal nutrients and energy supports offspring until they can forage independently (Pond 1977). Lactation is the most energetically costly stage of female mammalian reproductive effort (Gittleman and Thompson 1988), when allocation of maternal resources to offspring comes at the expense of self-maintenance and future reproductive success of the mother (Clutton-Brock et al. 1989). Maternal provisioning strategies therefore balance investment in offspring growth and survival against condition and reproductive costs incurred by mothers (Lee et al. 1991, Fairbanks and McGuire 1995, Rogowitz 1996).

Cetacean calves are weaned when they reach a threshold length relative to maternal size (Huang et al. 2009), similar to other large-bodied mammals (Lee et al. 1991). Capital breeding mysticetes have brief, but intensive, nursing periods during which more than 30 % of maternal body fat is converted into high fat milks until calves are weaned at 6 months to 1 year (Tyack 1986, Oftedal 1997, 2000). Odontocetes, by comparison, are income breeders that nurse their calves over prolonged periods ranging up to several years (Tyack 1986, Oftedal 1997). Continual food consumption throughout the nursing period, along with lower rates of energy transfer (odontocete milks have less fat than mysticete milks), allow energetic demands of lactation to be distributed over a longer period (Oftedal 1997). Nevertheless, odontocete mothers must increase their daily food consumption by 32-63 % over non-lactating requirements to fuel milk production (Lockyer 1981). These energetic costs translate into fitness returns, as the extended nursing period provides time for development of sensorimotor systems used to pursue highly mobile prey (as opposed to grazing by baleen whales; Brodie 1969, Hayssen 1993). Prolonged periods of maternal dependence may also facilitate social learning in complex groups characteristic of odontocetes (Brodie 1969, Mann and Smuts 1999, Colbeck et al. 2013).

Beluga whales (*Delpinapterus leucas*, Pallas 1776) are a mid-sized odontocete that occupy a discontinuous circumpolar distribution in Arctic and sub-Arctic waters (Stewart and Stewart 1989). Mating occurs seasonally in late winter to early spring (Brodie 1971, Burns and Seaman 1985), and reproductive tract morphologies suggest belugas have a promiscuous rather than polygynous mating system (Kelley et al. 2014). Females give birth to a single calf following a gestation of 12-14.5 months (Brodie 1971, Sergeant 1973, Seaman and Burns 1981, Doidge 1990). Calving varies geographically, but generally peaks from June to mid-August in the eastern Canadian Arctic (Brodie 1971, Sergeant 1973, Cosens and Dueck 1990, Kilabuk 1998). The calving interval is largely accepted to be 3 years (Sergeant 1973, Doidge 1990, Heide-Jørgensen and Teilmann 1994), although a biennial breeding cycle is suggested in some females (Seaman and Burns 1981), and some observers believe belugas give birth annually (Kleinenberg et al. 1964, Kilabuk, 1998, Stewart 2001). Female belugas are reproductively active throughout their adult life, but birth rate declines gradually with age (Burns and Seaman 1985).

Beluga whales have a protracted nursing period typical of odontocetes, although estimates of nursing duration vary considerably. Brodie (1971) captured 2 year old calves alongside lactating females in Cumberland Sound, Baffin Island, and concluded beluga calves are nursed for at least 2 years. Sergeant (1973) calculated a lactation period of 21 months in western Hudson Bay belugas based on the ratio of lactating to pregnant animals captured in nets, multiplied by a gestation period that was based on fetus length and calf length at birth. Using the same ‘ratio method’, lactation duration estimates were about 32 months for beluga whales off northern Quebec (Doidge 1990) and 6-12 months for belugas off northwest Alaska (Burns and Seaman 1985), while Kleinenberg et al. (1964) cite a lactation period of just 5-6 months for belugas in the Sea of Okhotsk. Milk is considered the only form of nutrition during the first year (Brodie 1971), but studies reporting a mixture of milk, fish, and squid in stomachs of 2 year old calves (Heide-Jørgensen and Teilmann 1994) indicate diet is supplemented with solid food in the second year.

Current estimates of beluga nursing duration based on the ratio and cow-calf methods are subject to harvest or capture bias (Perrin and Reilly 1984). For example, slowed movements of lactating females accompanied by calves may make them more susceptible to harvest than pregnant females, or to capture in nets if they return to assist an entangled calf (Doidge 1990). Stomach contents can be similarly misleading for species that consume solid food before deriving much nutritional value from it (Lee 1997, see Heyning 1988), or engage in non-nutritive suckling (e.g. Best et al. 1984). Isotopic analysis has shown $\delta^{15}N$ values in tissues of suckling mammals are higher than those of their mothers (Jenkins et al. 2001, Polischuk et al. 2001), which has been attributed to consumption of milk, a maternally-derived tissue enriched in ^{15}N relative to the trophic level at which the mother feeds (Jenkins et al. 2001). Nursing has also been associated with lower stable carbon isotope ratios ($\delta^{13}C$), which presumably reflect incorporation of carbon derived from relatively ^{13}C depleted milk lipids (Hobson and Sease 1998). However, this nursing effect on offspring $\delta^{13}C$ values is not consistently observed (Jenkins et al. 2001; Habran et al.

2010). Newsome et al. (2006) suggest tissue $\delta^{13}C$ patterns over the nursing period reflect the degree of preferential routing of carbon from milk lipids vs. proteins to tissue synthesis.

Isotopic profiling of dentine growth layers in teeth spanning the nursing period has documented chronological $\delta^{15}N$ declines that are consistent with weaning in several marine mammal species (Hobson and Sease 1998, Hobson et al. 2004, Newsome et al. 2006, Knoff et al. 2008, Newsome et al. 2009, Matthews and Ferguson 2014a). Conversely, weaning-associated ontogenetic $\delta^{15}N$ shifts are absent or dampened in teeth of pinnipeds that abruptly wean their pups after a brief nursing period because the bulk of the first year spent foraging attenuates any nursing signal (e.g. Newsome et al. 2006). $\delta^{15}N$ patterns in teeth can therefore be used as a nursing proxy, offering an alternative to other methods that have been used to estimate lactation duration in cetaceans (e.g. Brodie 1971, Sergeant 1973). This approach is ideal for belugas because they are born with a full set of teeth that are retained throughout their lifetime (Stewart 2012). The pulp cavity of each tooth gradually fills with dentine that undergoes little post-depositional modification (Bloom and Fawcett 1975). A neonatal line formed just after birth marks the beginning of post-natal dentine deposition (Stewart 2012), and alternating broad and narrow bands define annually deposited growth layer groups (GLGs) from then on (Stewart et al. 2006, Luque et al. 2007, Campana and Stewart 2014, Matthews and Ferguson 2014b).

Here, we use isotopic profiles across dentine GLGs of beluga whales from the eastern Canadian Arctic to determine whether belugas have prolonged nursing periods typical of toothed whales. Further, we examine individual isotope profiles to infer whether beluga whales have variable nursing periods, which has been reported in other odontocetes (e.g. Mann et al. 2000). In accordance with parental care theory, which posits that mothers should invest in their offspring in a manner that maximizes their own fitness (Clutton-Brock 1991), we predicted nursing duration may vary 1) with relative GLG width, which we considered a proxy for growth, since faster growing individuals attain size required for independence quicker and should be weaned earlier (see Lee et al. 1991, Huang et al. 2009); 2) by sex, since beluga are sexually dimorphic (Sergeant and Brodie 1969) and male calves may have greater lactational requirements than females, and investment in males potentially confers greater fitness to the mother if larger males are more likely to successfully reproduce (e.g. Clutton-Brock et al. 1985); and 3) by population, since whales at different latitudes with varying migration patterns may experience different environmentally-imposed energetics constraints on nursing (e.g. Trillmich 1990, Luque and Ferguson 2010). We also consider the influence of additional factors (e.g. maternal age; Clutton-Brock 1984) on beluga nursing duration.

MATERIALS AND METHODS

Tooth collection and micromilling

Teeth were extracted from mandibles of beluga whales (n = 30) harvested in subsistence hunts throughout the eastern Canadian Arctic during the 1980s-2000s. Our sample included equal numbers of females and males from three geographically separated and genetically distinct populations (Brown Gladden et al. 1997, Brennin et al. 1997): Western Hudson Bay (WHB; n = 7), Cumberland Sound (CS; n = 11), and Eastern High Arctic-Baffin Bay (EHA-BB; n = 12) (Figure 5.1). Teeth were extracted from the standard ageing positions 2 and 5 (Stewart 2012), except when noticeably less wear occurred in adjacent teeth.

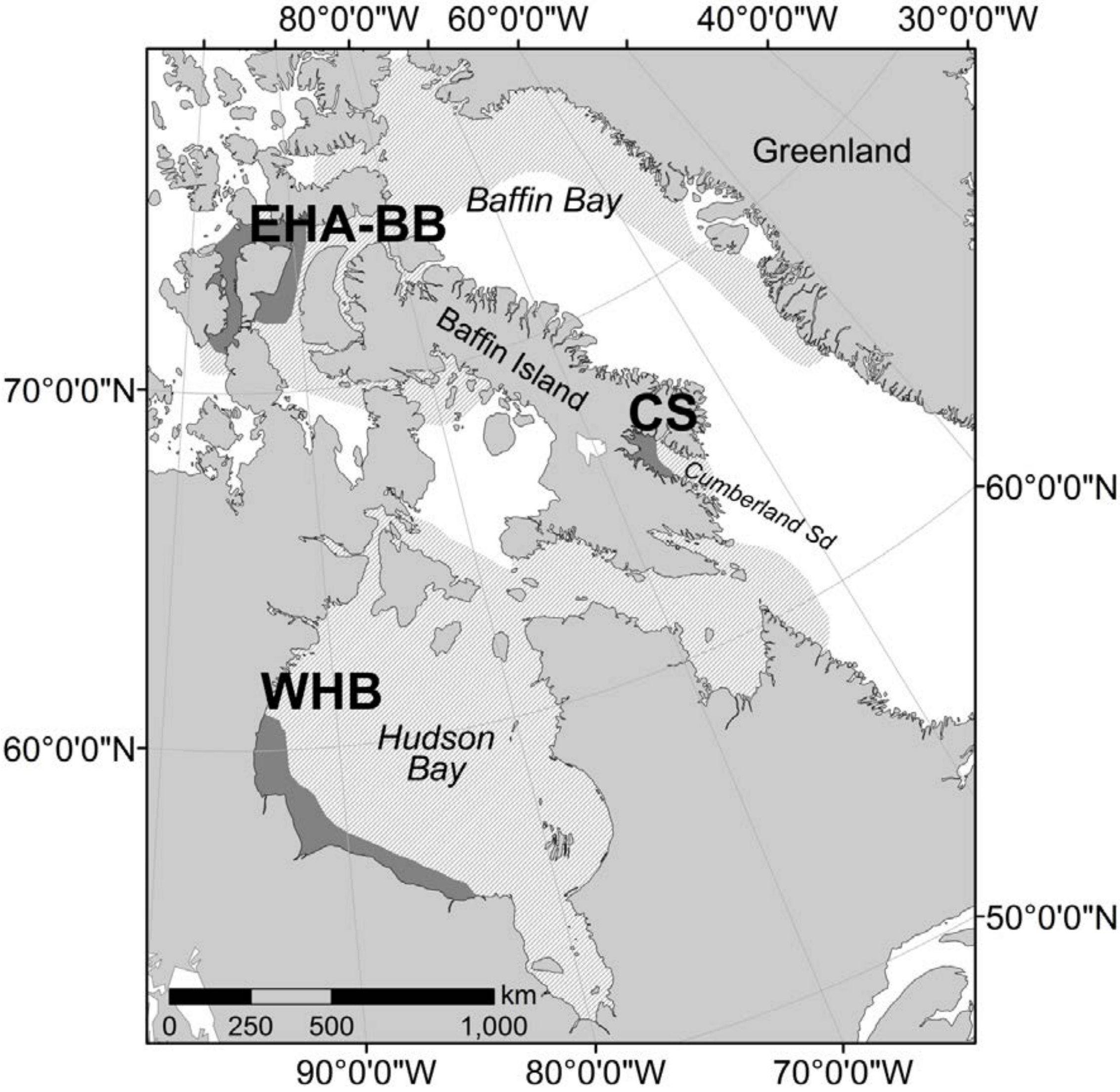

Figure 5.1. Teeth were collected from 3 beluga populations in the eastern Canadian Arctic: the Western Hudson Bay (WHB) population, the Cumberland Sound (CS) population, and the Eastern High Arctic-Baffin Bay (EHA-BB) population. Distributions (grey hatch lines) and areas of summer occupancy (dark grey) of each population were reproduced from COSEWIC (2004), with modifications to WHB distribution after Smith et al. (2007).

Teeth were air-dried and sectioned longitudinally along the midline using a water-cooled diamond encrusted saw blade. Beluga dentine is deposited in a series of stacked cones which appear in longitudinal section as GLGs comprising 1 thick and 1 thin annulus (Stewart 2012). Tooth sections were polished using 30 and 9 µm aluminum oxide (AlOx) lapping film to accentuate GLG definition. GLGs were counted under reflected light and aged assuming annual deposition, using the neonatal line separating pre- and post-natal dentine as a reference (Stewart 2012). GLG widths (µm) were measured from digital micrographs using the image analysis software Image-Pro® Plus (©2011 Media Cybernetics). Although the relationship between dentinal GLG width and somatic growth has not been studied in detail in belugas, Sergeant (1973) speculated the width of growth bands was related to skeletal growth because thicker GLGs in males corresponded to body size differences between the sexes. Dentine deposition varies with overall somatic growth in other species (Klevezal 1980, York et al. 2008); GLG width was therefore assumed to reflect growth during the nursing period.

Dentine GLGs were sampled using a high-resolution micromill (Merchantek) fitted with a 300 µm-diameter drill bit at a depth of 150 µm to minimize sampling of adjacent layers. Only teeth with a very clear neonatal line demarcating the first GLG from prenatal dentine were chosen for analysis, and only GLGs of sufficient clarity and width to allow discrete sample collection were micromilled.

Stable isotope analysis

The organic, mainly collagen, component of dentine is typically isolated from its mineral matrix prior to isotopic analysis (e.g. Hobson and Sease 1998), since both fractions contain carbon (in protein and carbonates, respectively) routed from biomolecules with differing $\delta^{13}C$ values (Ambrose and Norr 1993). Removal of inorganic carbon from dentine using acidification prior to analysis was not feasible due to small sample amounts, so bulk dentine was analysed instead. Recent studies (e.g. Martin et al. 2011, Matthews and Ferguson 2014b) have shown little difference between $\delta^{13}C$ values of bulk vs. acidified dentine.

Powdered dentine was weighed (~1 mg) into tin cups for isotopic analysis on a Vario EL III elemental analyser (Elementar, Germany) connected to a DELTAplus XP isotope ratio mass spectrometer (Thermo, Germany) at the G.G. Hatch Stable Isotope Laboratory, University of Ottawa (Ottawa, ON). Isotope ratios are reported as per mil (‰) deviation from the isotope ratios of atmospheric N_2 and Vienna Pee-Dee Belemnite limestone (V-PDB) for nitrogen and carbon, respectively, defined as $\delta^{15}N$ or $\delta^{13}C = (R_{sample} – R_{standard}) / R_{standard}) * 1000$, where R is $^{15}N/^{14}N$ or $^{13}C/^{12}C$. Values were normalised using reference materials whose isotopic composition spanned the sample range (nicotinamide, ammonium sulfate + sucrose, and caffeine; $\delta^{15}N$ -16.61 to 16.58 ‰, $\delta^{13}C$ -34.46 to -11.94 ‰), calibrated to international standards IAEA-N1, IAEA-N2, USGS-40 and USGS-41 for $\delta^{15}N$, and IAEA-CH6, NBS-22, USGS-40, and USGS-41 for $\delta^{13}C$. Analytical precision based on repeated measures of laboratory reference materials not used in calibrations was 0.09 and 0.06 ‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively, and that based on duplicate measures of ~10 % of samples was 0.14 and 0.06 ‰. Atomic C:N of bulk dentine (2.98 ± 0.06; mean ± sd) was within the range of unaltered collagen (DeNiro, 1985).

Data Analysis

Individual $\delta^{15}N$ and $\delta^{13}C$ profiles were restricted to the first 5 GLGs, which encompassed previous estimates of lactation duration in beluga whales. As a proxy for *complete* weaning, the point at which $\delta^{15}N$ values stopped decreasing relative to GLG1 was determined visually for each individual. Visual assessment was followed with single detection changepoint analysis on individual profiles using the ‘changepoint’ package (Killick and Haynes 2014) available for R software (R Core Team 2014). Single detection changepoint analysis detects changes in mean (or variance) along a measurement series by comparing the difference between maximum log likelihoods of the null (one mean provides best fit to all data points) and alternative (single changepoint) models against a threshold penalty value, which we set to be sensitive to changes in mean $\delta^{15}N$ values of ~1 ‰ (a signal consistent with weaning; see Jenkins et al. 2001). Changepoints were used to classify intermediate $\delta^{15}N$ values during the nursing period as being more similar to GLG1 (i.e. representative of milk-derived $\delta^{15}N$ values) vs. subsequent GLGs in each profile, which were assumed to reflect the post-weaning diet. This process was repeated on $\delta^{13}C$ profiles.

Assessments of weaning differences between sexes, among populations, and against GLG width were based on $\delta^{15}N$ patterns ($\delta^{13}C$ patterns varied considerably among individuals and could not be used to infer weaning; see Results). ‘Weaning age’ was summarized by sex and population in contingency tables. To increase cell counts, weaning ages were grouped as GLG1 or GLG2+ to create a binary variable. Weaning differences between females and males and among populations were assessed using Fisher’s exact tests recommended for small sample sizes (Agresti 1992). A logistic regression model implementing Firth’s penalized likelihood approach to deal with small sample size (Firth 1993) was fit to pooled data (there was no evidence of sex or population differences in weaning age; see Results) to examine how weaning age varied with relative GLG1 width. Relative GLG1 width used as a measure of a whale's growth in its first vs. subsequent years, and was calculated by dividing GLG1 width by the total width of the first five GLGs. The model was fit using the R package 'logistf' (Heinze et al. 2013).

RESULTS

Complete isotopic profiles (GLGs 1-5) were available for 25 of the 30 whales. GLGs 4 and 5 were too thin or obscure to be milled in the remaining five whales, resulting in profiles from GLGs 1-4 for three whales, and GLGs 1-3 of two whales (Figures 5.2 and 5.3). δ^{15}N declines of 0.4 to 2.79 ‰ (mean 1.26 ‰) were obvious over the first several GLGs of all whales, except whale ARGFxx1042 (Figure 5.2). δ^{15}N values appeared to decline up to GLGs 4 or 5 in whales B95-24, B95-547, and ARGFxx1024, so entire available profiles (up to GLGs 7-10) of these whales were examined to derive weaning age estimates (Figure 5.2). Although δ^{15}N values declined in teeth with profiles comprising just three GLGs (ARGF87-11 and ARPG86-16) (Figure 5.2), they are not considered in further analyses because weaning ages could not be confirmed. Changepoints were detected along all but one of the 28 δ^{15}N profiles comprising four or more GLGs (Figure 5.2).

δ^{15}N values declined between GLGs 1-2 in nine whales, and changepoint detections for six of these individuals also occurred at GLG1. Changepoint detections in the three whales with increasing δ^{15}N values beyond GLG2 (ARARxx1057, ARGFxx1035, and ARPGxx 1281) are not considered representative of weaning since they were influenced by high δ^{15}N values in older GLGs (Figure 5.2, Table 5.1). These nine whales were therefore interpreted as having been weaned in their first year (Table 5.1).

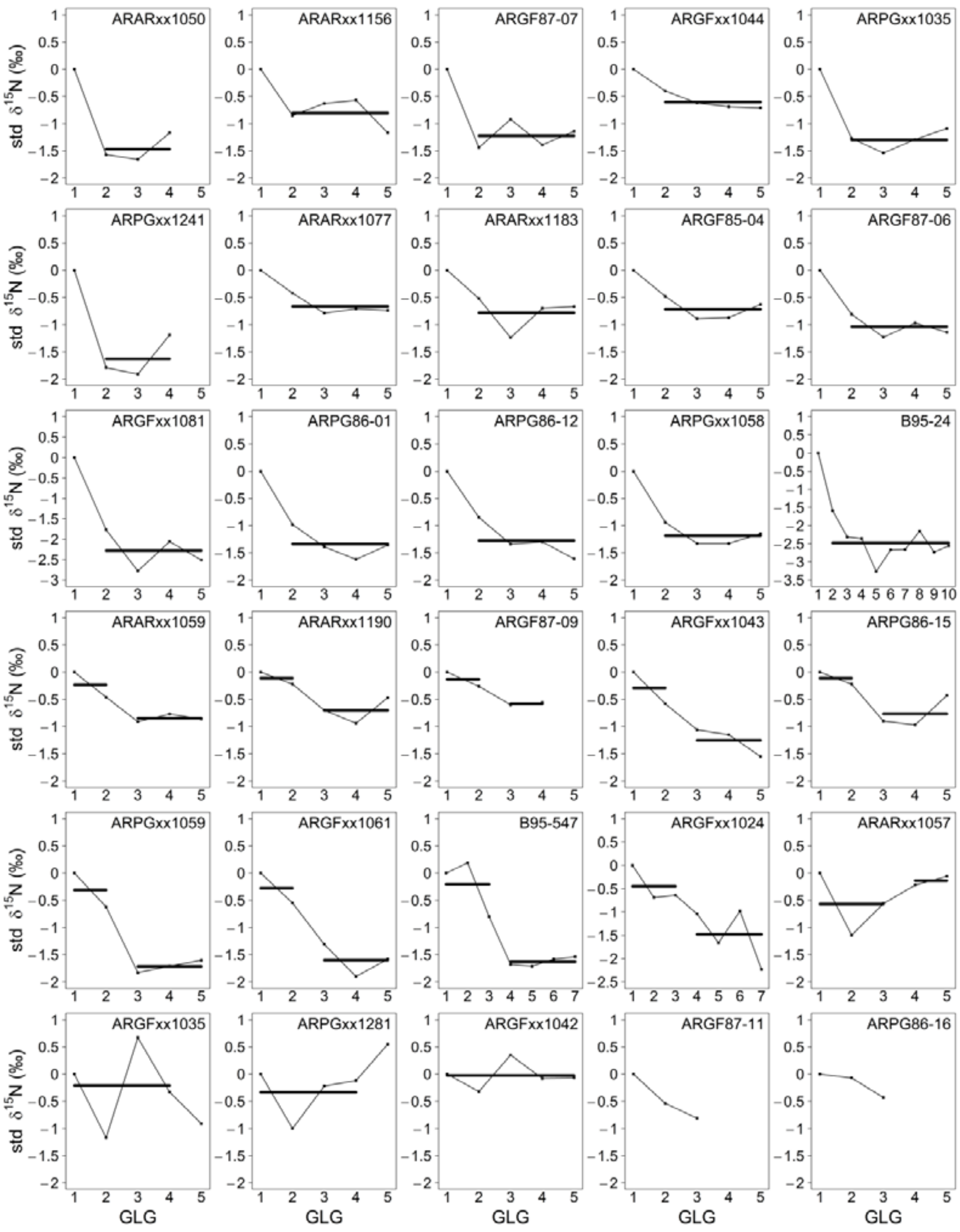

Figure 5.2. Standardized δ^{15}N values across the first 5 GLGs of eastern Canadian Arctic beluga. Most individuals show δ^{15}N declines over GLGs 1-4 that are consistent with weaning. Changepoints are indicated by breaks in black horizontal lines, which represent the mean value of that segment. δ^{15}N values in each series were standardized by subtracting GLG1 δ^{15}N values from each GLG within a given tooth to control for potential extrinsic factors that influence offspring isotope values such as maternal trophic level and regional and temporal baseline isotopic variation. The 5 whales with incomplete profiles were aged 5 years or greater, but thin or obscure GLGs prevented milling of GLGs 4 and/or 5. Note different scale of y axes for whales ARGFxx1081, B95-24, and ARGFxx1024.

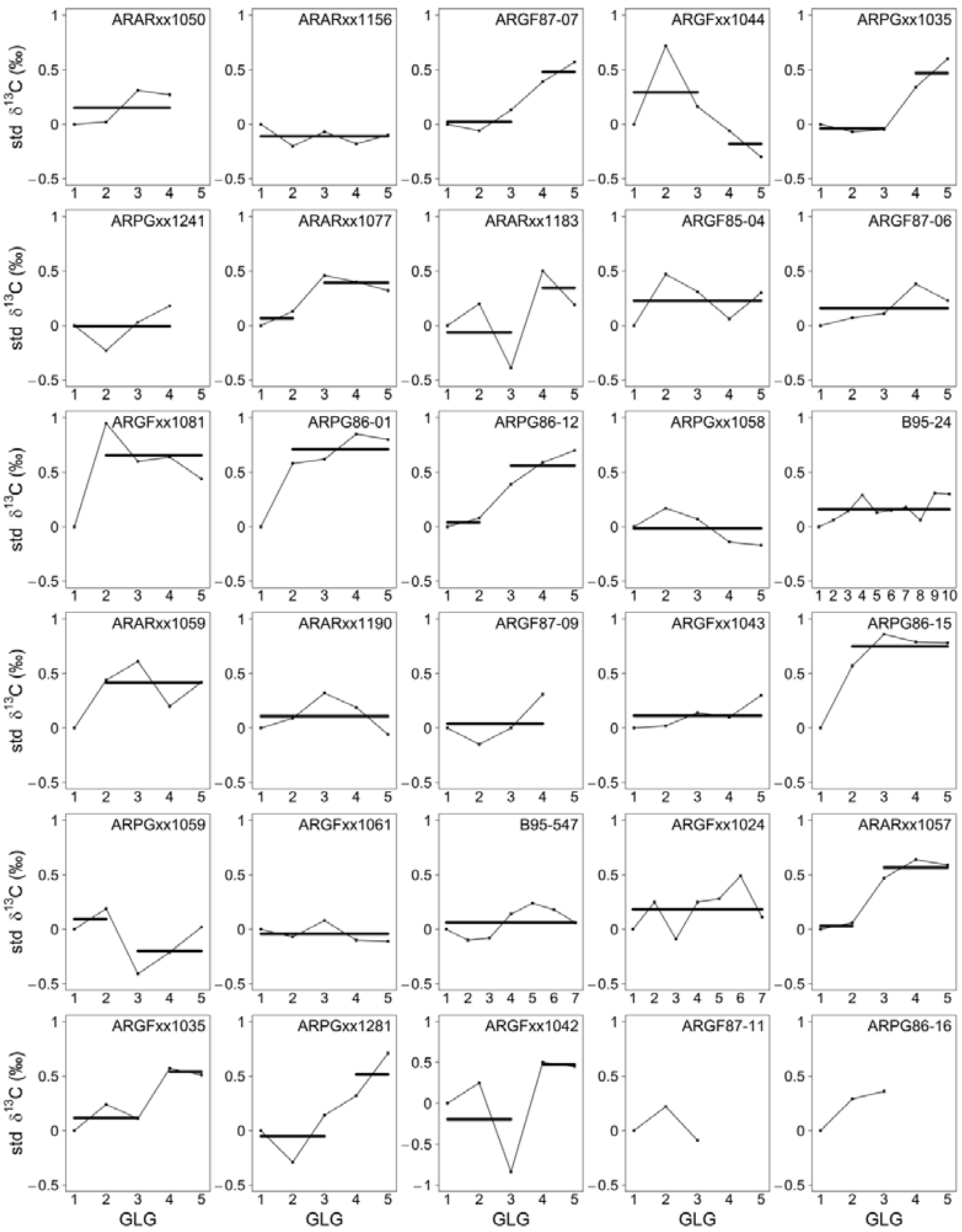

Figure 5.3. Unlike δ^{15}N profiles, standardized δ^{13}C values showed no consistent patterns across the first 5 GLGs of eastern Canadian Arctic beluga. δ^{13}C increases relative to GLG1 values occurred in less than half the whales, while many individuals had relatively constant δ^{13}C values. Changepoints are indicated by breaks in black horizontal lines, which represent the mean value of that segment. δ^{13}C values in each series were standardized as per δ^{15}N values (see Figure 5.2). The 5 whales with incomplete profiles were aged 5 years or greater, but thin or obscure GLGs prevented milling of GLGs 4 and/or 5. Note different scale of y axes for whale ARGFxx1042.

Table 5.1. Weaning interpretations of δ^{15}N profiles in teeth of 30 eastern Canadian Arctic beluga from the Western Hudson Bay (WHB), Cumberland Sound (CS), and Eastern High Arctic-Baffin Bay (EHA-BB) populations.

Whale	**Population**	**Sex**	**Relative GLG1 width**	**Visual**	**Changepoint**	**Interpretation**
ARARxx1050	WHB	F	0.229	GLG1	GLG1	weaned in 1st year
ARARxx1156	WHB	M	0.226	GLG1	GLG1	
ARGF87-07	EHA-BB	M	0.213	GLG1	GLG1	
ARGFxx1044	EHA-BB	F	0.182	GLG1	GLG1	
ARPGxx1035	CS	F	0.195	GLG1	GLG1	
ARPGxx1241	CS	M	0.178	GLG1	GLG1	
ARARxx1077	WHB	F	0.240	GLG2	GLG1	weaned in 2nd year; solid food > milk in 2nd yr
ARARxx1183	WHB	F	0.195	GLG2	GLG1	
ARGF85-04	EHA-BB	F	0.275	GLG2	GLG1	
ARGF87-06	EHA-BB	M	0.225	GLG2	GLG1	
ARGFxx1081	EHA-BB	M	0.219	GLG2	GLG1	
ARPG86-01	CS	M	0.190	GLG2	GLG1	
ARPG86-12	CS	M	0.179	GLG2	GLG1	
ARPGxx1058	CS	F	0.203	GLG2	GLG1	
B95-24	CS	F	0.204	GLG2	GLG1	
ARARxx1059	WHB	F	0.222	GLG2	GLG2	weaned in 2nd year; milk ≈ solid food in 2nd yr
ARARxx1190	WHB	M	0.208	GLG2	GLG2	
ARGF87-09	EHA-BB	M	0.275	GLG2	GLG2	
ARGFxx1043	EHA-BB	F	0.166	GLG2	GLG2	
ARPG86-15	CS	M	0.144	GLG2	GLG2	
ARPGxx1059	CS	F	0.224	GLG2	GLG2	
ARGFxx1061	EHA-BB	F	0.261	GLG3	GLG2	weaned in 3rd year; solid food > milk in 2nd yr
B95-547	CS	F	0.152	GLG3	GLG2	
ARGFxx1024	EHA-BB	M	0.187	GLG4	GLG3	weaned in 4th year; solid food > milk in 3rd yr
ARARxx1057	WHB	F	0.204	GLG1	-	weaned in 1st year; possible trophic or baseline δ^{15}N variation post-weaning
ARGFxx1035	EHA-BB	F	0.219	GLG1	-	
ARPGxx1281	CS	M	0.188	GLG1	-	
ARGFxx1042	EHA-BB	M	0.214	none	none	no nursing or weaning signal detected
ARGF87-11	EHA-BB	F	-	-	-	not assessed; profile consisted of 3 GLGs
ARPG86-16	CS	F	-	-	-	

δ^{15}N values declined across the first 2 GLGs of 15 whales (Figure 5.2), indicating they were weaned during their second year. Of these 15 whales, GLG2 δ^{15}N values were grouped with GLG1 in 6 whales and with the remaining GLGs in 9 whales (Figure 5.2, Table 5.1), indicating solid food was more important than milk in the second year diet of most animals. δ^{15}N values declined over the first 3 GLGs of 2 whales and over the first 4 GLGs of one whale, with were judged to have been weaned in their third and fourth years, respectively (Figure 5.2, Table 5.1). As with the whales that were nursed for two years, changepoint detections in these 3 whales indicated solid food contributed more to the diet than milk during the final year of nursing.

Inferred weaning age did not differ between females and males or among populations (Fisher's exact p = 1 for all tests), nor with relative GLG1 width (logistic regression; $\chi^2 = 0.15$, df = 1, $p > 0.6$) (Table 5.1).

Visual inspection and changepoint analysis of δ^{13}C profiles revealed no consistent patterns in δ^{13}C values across GLGs 1 through 5 (Figure 5.3).

DISCUSSION

δ^{15}N profiles and beluga nursing duration

δ^{15}N profiles over the first several GLGs in the majority of teeth are consistent with weaning, when the transition from milk to solid prey was predicted to cause a commensurate decline in dentine δ^{15}N values. δ^{15}N declines are similar to δ^{15}N differences between tissues of nursing offspring-mother pairs across a range of mammal species (1-3 ‰; Hobson et al. 2000, Jenkins et al. 2001, Polischuk et al. 2001, Ducatez 2008, Habran et al. 2010), and chronological δ^{15}N declines (1-3 ‰) in dentine spanning the nursing period of pinnipeds (Hobson and Sease 1998, Hobson et al. 2004, Newsome et al. 2006, Riofrío-Lazo et al. 2012), bottlenose dolphins (*Tursiops truncatus*; Knoff et al. 2008), and killer whales (*Orcinus orca*; Newsome et al. 2009, Matthews and Ferguson 2014a).

δ^{15}N-derived estimates of beluga nursing duration are similar to previous estimates of weaning age and lactation duration for eastern Canadian Arctic beluga using the cow-calf or ratio approaches (21-24 months; Brodie 1971, Sergeant 1973), but are shorter (in the majority of whales) than the 32 month estimate for belugas off northern Quebec (Doidge 1990). Some discrepancy between the approaches is expected (harvest bias of the cow-calf and ratio methods notwithstanding) because isotope-derived estimates of nursing duration are based on whales that survived to weaning age, while estimation of lactation duration using the ratio method takes into account differential calf mortality (the deaths of calves prior to weaning, which decreases the proportion of lactating females in the population; see Perrin and Reilly 1984). Additional discrepancy can be expected since the majority of our sample comprised whales aged 5+ years, and isotope-derived estimates could be biased toward the high side if a longer nursing period conveys increased survival, or, conversely, on the low side if early weaning reflects good foraging conditions conducive to higher survival (York et al. 2008). We also acknowledge selection of teeth with clearly defined neonatal lines and annuli could bias estimates if these factors are related to health or growth.

Accuracy of isotope-derived estimates of nursing duration is also limited by several factors, including: 1) uncertainty in the timing of formation of the broad and narrow bands of individual

GLGs, 2) the spatial scale, and therefore temporal resolution, of GLG sampling, and 3) delays in isotopic equilibration between diet and dentine. Alternating broad and narrow bands in marine mammal teeth are assumed to reflect physiological responses to seasonality, mediated perhaps through seasonal food availability or endocrine responses to environmental variation (Myrick 1980, Klevezal 1980). In a meta-analysis of the timing of dentine growth layer formation in marine mammals, Klevezal (1996) found the broad band formed during spring-fall in the majority of species examined, while the narrow band corresponded to fall-early spring. As the broad band constitutes the bulk of each sampled GLG, $\delta^{15}N$ values of individual GLGs are weighted towards diet integrated over the period of its formation. While seasonality of dentine growth layers in beluga teeth has not been studied in detail, the majority of whales included in this study had been forming a broad band at the time of death (June-September; data not shown), indicating the broad band of each GLG forms during summer (but does not inform the period over which the narrow band forms).

More complete understanding of growth layer seasonality coupled with subsampling GLGs over a finer spatial scale to increase temporal resolution would improve accuracy of estimates currently constrained to yearly integers. However, delayed isotopic equilibration of dentine with diet of~2-3 months (Zazzo et al. 2006), coupled with a 2-3 month birthing period, such that the first GLG represents a different amount of time for whales born earlier vs. later, ultimately restricts accuracy of isotope-derived estimates. Owing to these limitations, it cannot be ruled out that whales weaned by the end of GLG1 were not weaned as early as 5-6 mo, as suggested by Kleinenberg et al. (1964) or Burns and Seaman (1985). However, while population differences in weaning age among beluga populations in Canada (this study) and Russia (Kleinenberg et al.

1964) or Alaska (Burns and Seaman 1985) cannot be discounted (see Lee 1997 for examples with primates), the range in previous estimates of beluga lactation using the ratio method likely reflects biases affecting the ratio of lactating to pregnant females, such as the season of capture or differential capture success of females accompanied by young (Perrin and Reilly 1984, Doidge 1990). The low weaning ages cited by Kleinenberg et al. (1964) in particular appear to stem from unreliable age assignments to calves based on body size. The authors estimated 280 cm belugas to be 5-6 months old, while published growth curves for several beluga populations indicate they would have been considerably older (Stewart 1994, Heide-Jørgensen and Teilmann 1994, Suydam 2009).

Intermediate GLG2 $\delta^{15}N$ values in whales that were nursed beyond their first year likely reflect diets comprising milk and solid prey, indicating weaning was gradual. This is consistent with previous reports of 2 year old beluga calves with a mixture of milk, fish, and invertebrates in their stomachs (Brodie 1971, Sergeant 1973, Heide-Jørgensen and Teilmann 1994), and yearling calves suckling throughout their second summer (Smith et al. 1992). Smith et al. (1994) observed calves judged to be 2+ years old based on body size in suckling position, but could not verify that nursing had occurred. GLG2 $\delta^{15}N$ values were more similar to those of GLGs 3-5 (assumed to represent the post-weaning diet) in a majority of whales, indicating either that milk supplemented solid prey over the second half of the nursing period, or that those whales were weaned relatively early during their second year compared to whales with GLG2 $\delta^{15}N$ values that were more similar to GLG1 values (i.e. reflecting milk diet). The former interpretation is more consistent with the gradual weaning typical of odontocetes, when milk becomes a nutritional supplement to prey after the first year (Kasuya and Marsh 1984, Lockyer 1993).

Brodie (1969) and Tyack (1986) hypothesized the extended nursing period of odontocetes allows sufficient time for development of sensorimotor systems used in pursuit of prey, as well as learning communication during coordinated group hunting tactics. Belugas consume a range of fish and invertebrate prey which may vary seasonally (COSEWIC 2004, Kelley 2014), and may require specialized foraging techniques (Matthews and Ferguson, unpublished data) that could be learned during prolonged mother-calf associations. Belugas also undertake long seasonal migrations between offshore regions in winter to shallow coastal areas in summer, to which they exhibit site fidelity (Smith and Hammill 1986, Caron and Smith 1990). Colbeck et al. (2013) found beluga offspring travelled with their mothers, as well as siblings and other relatives, during seasonal migrations, indicating young beluga learn migration routes from their mothers and other family members. Similar social learning has been linked with prolonged nursing and mother-offspring associations in other species that live in complex social groups (e.g. dolphins, Mann and Smuts 1999; primates, Pusey 1983; elephants, Lee and Moss 1986).

Strong seasonality is expected to influence reproductive parameters such as the timing and duration of lactation (Trillmich 1990, Whitehead and Mann 2000). The seasonal environment of belugas may therefore have imposed selective pressures (e.g. seasonal food availability, the calf’s need to accumulate a thick blubber layer) on belugas to evolve traits somewhat similar to the capital breeding strategy of mysticetes. Reduced foraging may be part of the reproductive strategy of beluga mothers while summering in protected estuaries with their calves (Sergeant 1973), although the importance of summer feeding appears to vary with population (Martineau et al. 1987, Lydersen et al. 2001, Loseto et al. 2009). Female, but not male, belugas have seasonal oscillations in blubber thickness that Sergeant and Brodie (1969) linked with reproductive state, with females being particularly fat during late pregnancy and early lactation. The few measurements of beluga milk indicate its fat content (27 %; Lauer and Baker 1969) falls towards the high end of the odontocete range (10-30 %) and approaches the lower end of the mysticete range (30-50 %) (Lockyer 1984, Oftedal and Iverson 1995). However, milk composition varies considerably over the course of lactation (West et al. 2007) and within species (Oftedal 1997), necessitating more measurements on beluga milk for a valid comparison. In an analysis of a range of life history traits (not including lactation duration) in over 80 cetacean species, Ferguson and Higdon (2013) found that belugas were grouped with whales the authors termed ‘bet-hedgers’. Bet-hedgers were characterized by long lifespans and reduced maternal investment, perhaps as an adaptation to environmental variability. Belugas that were weaned by age 1 would be in line with this characterization, although the 2 year nursing period observed in most individuals is on par with average nursing durations of similar-sized odontocetes (Perrin and Reilly 1984, Oftedal 1997, Whitehead and Mann 2000).

$\delta^{13}C$ values over the nursing period

Unlike other studies that have attributed δ^{13}C increases across dentine annuli to weaning (e.g. Hobson and Sease 1998, Hobson et al. 2004), δ^{13}C values across beluga GLGs spanning the nursing period showed no consistent pattern. We assume analysis of bulk dentine, as opposed to isolated collagen, was not a factor, since δ^{13}C measurements of bulk dentine largely reflect that of collagen (Martin et al. 2011, Matthews and Ferguson 2014b). Other studies have found either no δ^{13}C differences between dentine deposited pre- and post-weaning (e.g. Knoff et al. 2008), or no consistent δ^{13}C pattern associated with weaning (e.g. Newsome et al. 2006). Newsome et al.

(2006) suggest that offspring δ^{13}C values over the nursing period vary with the fat content of milk. That lower δ^{13}C values have been noted in nursing otariids (Hobson and Sease 1998, Hobson et al. 2004), which have milk fat content around 30-50 % (Oftedal and Iverson 1995), but not in belugas (this study), bottlenose dolphins (Knoff et al. 2008), or killer whales (Newsome et al. 2009), which collectively have lower fat milks than otariids (10-30 %; Lauer and Baker 1969, Oftedal 1997), offers some support to this hypothesis. However, δ^{13}C values did not vary with weaning in northern elephant seals (*Mirounga angustirostris*, Riofrío-Lazo et al. 2012), despite the higher fat content of their milk than both otariids and odontocetes (Oftedal and Iverson 1995). This was likely unrelated to the abrupt weaning of northern elephant seals, since a clear decline in δ^{15}N values was noted between dentine layers corresponding to pup and adult female age classes. The lack of δ^{13}C signal may have more to do with the fact that the majority of milk fat is routed directly to the blubber layer of phocid seals (Oftedal 2000), such that little lipid-derived carbon is diverted to protein synthesis. Although blubber development in beluga calves has not been quantified, belugas have thick blubber relative to other odontocetes (~40 % body weight; Sergeant and Brodie 1969). As such, we hypothesize that the lack of nursing effect on δ^{13}C values in beluga whales reflects the direct incorporation of dietary lipids into the developing blubber layer of calves.

Variation in nursing duration

δ^{15}N profiles varied among whales, indicating the majority (>50 %) were weaned by the end of their second year, a third by the end of their first year, and about 10 % beyond their second year. Variation in nursing duration has been reported in a variety of other odontocetes. For example, bottlenose dolphins (*Tursiops* sp.) are nursed for 3-6 years, and occasionally for up to 8 years

(Mann et al. 2000). Sperm whales (*Physeter macrocephalus*) and short-finned pilot whales (*Globicephela macrorhynchus*) are reportedly nursed beyond 10 years of age (e.g. Best et al. 1984, Kasuya and Marsh 1984), although the mean nursing duration is just several years in both species (alternative explanations for these extreme reports are provided by Oftedal 1997). Given a gestation period of about 12-14.5 months, nursing duration estimates derived here are consistent with a full female beluga reproductive cycle of about 3 years (i.e. 1 year gestation + 2 years lactation). The smaller proportion of whales weaned during their first year would also be consistent with a 2 year calving interval suggested in some females (Seaman and Burns 1981, Suydam 2009).

Irregular GLG deposition over the nursing period, if it occurred, would lead to variation in $\delta^{15}N$ patterns among individuals. The onset of permanent tooth eruption in belugas ranges from 3-10 years (Stewart 2012), indicating the age at which teeth erupt varies among individuals, or that GLG deposition is irregular in young belugas. Assuming previous estimates of lactation duration in belugas are reasonable, similar $\delta^{15}N$-derived weaning age estimates of 1-2 years allows us to rule out multiple GLG deposition in beluga calves, which would instead draw out $\delta^{15}N$ declines over more GLGs than observed. Interpretation of dentine growth layers, on the other hand, involves a degree of subjectivity due to accessory layers (Hohn et al. 1989, Lockyer 1995), which may be difficult to assess in some beluga teeth (Stewart 2012). For this study, error due to incorrect ageing of GLGs was minimized by selecting only teeth with very clearly defined neonatal lines and GLGs. Thus, while inaccurate age assignments cannot be completely discounted, we do not believe isotopic variation among individuals to be an artefact of age assignment errors.

Lee et al. (1991) found the primary factor determining nursing duration in large-bodied mammals (primates, ungulates, and pinnipeds) was offspring size. Weaning occurs when offspring attain a threshold mass required for nutritional independence, typically 4 times the birth weight. Cetacean calves are weaned when they reach a threshold size relative to that of adult females (Huang et al. 2009). The lack of relationship between relative GLG1 width and inferred weaning age does not support hypotheses linking weaning age and growth, although the constant proportions of dentine deposition represented by each GLG (0.21, 0.19, 0.21, 0.20, and 0.19 for GLGs 1 through 5, respectively), despite a gradual decline in overall growth rate over the same period (Burns and Seaman 1985), suggests beluga GLG deposition rate is independent of overall growth. In contrast, York et al. (2008) showed relative GLG1 width was greater in Steller sea lions (*Eumetopias jubatus*) that were weaned during their first year than in animals that continued to be nursed into their second year, indicating faster growing pups were weaned earlier.

Lee et al. (1991) proposed lactation duration is a function of maternal condition, which influences milk yield/nutrient transfer and concomitant offspring growth rates. Nutritional limitations of poor quality habitat or stressful conditions have been associated with both reduced and extended nursing (e.g. Lee and Moss 1986), while abundant food resources have been linked to early weaning (Ramsay and Stirling 1988, Trillmich 1990). Considerable interannual variation in Arctic marine ecosystems (e.g. sea ice cover; Parkinson and Cavalieri 1989, Parkinson 2000) may have affected beluga nursing duration, especially since birth years spanned several decades. Unfortunately, our restricted sample size did not allow for comparison of nursing duration among calves from specific birth years, which may expose impacts of environmental variation on weaning age. Another factor that probably influences the size attained by a beluga calf over its first year is its birthdate. Although the calving season is generally restricted to June-August (Brodie 1971, Sergeant 1973), newborn belugas have been observed throughout much of the Canadian Arctic from March-September (Braham 1984), and in the EHA-BB population from February-October (Stewart 2001). Belugas born early in the calving season would have more nursing time during their first year relative to calves born later, which could allow them to attain critical weaning size earlier.

Among sexually dimorphic species, the greater growth rate of male offspring is sustained through greater lactational transfers relative to females (Clutton-Brock et al. 1985). Additionally, among polygynous species, sex-biased maternal investment favoring male offspring confers size advantages that provide greater fitness returns later in life (Clutton-Brock et al. 1981, Kovacs and Lavigne 1986). Immature belugas of both sexes have similar growth rates (at least in terms of length) spanning the nursing period, and relative increases in male vs. female size do not occur until age 7-10 years (Sergeant 1973, Heide-Jørgensen and Teilmann 1994). The energetic demands of female and male calves over the nursing period may therefore be similar, and post-weaning growth rates and foraging dynamics may be more relevant to adult male size and condition than maternal investment during lactation. Recent evidence also suggests that, although they are sexually dimorphic, belugas lie more closely to a promiscuous mating system along the promiscuous-polygynous continuum (Kelley et al. 2014). The absence of either a polygynous mating system or maternal effects on male adult size is not conducive to sex-biased maternal investment (Trivers and Willard 1973). That being said, our failure to detect differences in nursing duration between the sexes could be a sample size limitation, which did not allow for proper control of potential confounding variables (e.g. environmental factors), as well as sampling resolution, since measurements integrated timespans that would have obscured differences in weaning age of less than several months. Nursing duration may also not be the best measure of maternal investment, as suckling frequency or milk quality are common manifestations of differential maternal investment (e.g. Anderson and Fedak 1987, Landete-Castillejos et al. 2005).

Nursing duration also varies with maternal age, as older females may compensate for declines in fecundity towards the end of their lifespan with increased reproductive effort in current offspring (Pianka and Parker 1975, Clutton-Brock 1984). Additionally, larger mature mothers can afford to allocate more resources to reproduction while incurring less body condition costs than primiparous mothers, which must often bear the energetic demands of growth and lactation simultaneously (Lockyer 1981, Gomendio 1989). Sperm whale mothers > 20 years old nursed their young for 67 % longer than mothers < 20 years old (Best et al. 1984). Kasuya and Marsh (1984) found associations between older suckling calves and older lactating female short-finned pilot whales, indicating older mothers nurse their calves for longer than younger ones. Similarly, long-finned pilot whale (*Globicephala melas*) mothers over 40 years old lactate about 3 times longer than mothers at the beginning or middle of their reproductive lifespan (Martin and Rothery 1993). Diminishing birth rates with age in belugas (Burns and Seaman 1985) may therefore reflect increasing interbirth intervals caused by increasing reproductive effort by older females. Allonursing (nursing offspring of other females) could also be a factor, especially in the longer nursing durations (> 2 years) inferred for several individuals. Although not reported in wild belugas, allonursing is exhibited by captive belugas (Leung et al. 2010). Given that belugas live in matrilineal groups comprising closely related females (Colbeck et al. 2013), allonursing could be an adaptive behavior via kin selection that promotes calf survival.

Of the components that make up the calving cycle, the lactation period is the most variable, and understanding factors affecting its duration is important for models of reproductive rates in a population (Perrin and Reilly 1984). An isotopic approach was used to derive nursing duration estimates that are similar to previous estimates of lactation duration in eastern Canadian Arctic belugas, but revealed individual variation in weaning age previously unreported in belugas. Future study could assess influence of environment on weaning age by including multiple whales from each of a range of birth years spanning periods of environmental variation (e.g. York et al. 2008), or including belugas from the more southern St. Lawrence Estuary population to examine weaning age along a latitudinal gradient spanning temperate and arctic regions (e.g. Trillmich 1990). Unfortunately, a retrospective isotopic approach provides no direct information about maternal condition and age, important determinants of lactation duration in a range of mammal species. Nevertheless, this study, like previous ones, indicates δ^{15}N profiles of sequentially sampled dentine growth layers deposited over the nursing period can provide estimates of nursing duration, revealing individual variation in weaning age that cannot be detected using other currently available approaches for odontocetes.

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Chapter 6. Individual specialisation in resource use in a generalist marine predator, the beluga whale

*Recent reviews indicate growing evidence of individual diet specialisation across a range of animal taxa. Longitudinal data required to assess the degree of individual diet specialisation within populations is logistically difficult to acquire through direct observation, especially for marine mammals. The measurement of stable isotope composition along continuously growing tissues such as dentine offers an indirect means to infer individual variation in resource use (i.e. diet and habitat) by comparing within- and among-individual isotopic variation over multi-year timespans. Here, we reconstruct longitudinal resource use histories of individual eastern Canadian Arctic beluga whales (*Delphinapterus leucas*), a generalist marine consumer with a broad fish and invertebrate prey base, from profiles of stable nitrogen and carbon isotope ratios (*δ^{15}*N and *δ^{13}*C) measured across annually deposited dentine growth layers. While sex and age class were significant predictors of both *δ^{15}*N and *δ^{13}*C values, as expected from previous studies reporting ecological sexual dimorphism and ontogenetic niche segregation in this species, considerable isotopic variation within populations was attributed to variation among individuals. Qualitative comparisons of beluga isotope values with published prey values, along with a positive correlation between *δ^{15}*N values and body length among adult males, suggests diving capacity allows larger animals to access deep water or benthic fishes like Greenland halibut (*Reinhardtius hippoglossoides), *whose larger size may be more energetically profitable for whales large enough to efficiently capture and consume them. Our study adds to growing documentation of resource specialisation within sex and age class among marine consumers, and is relevant for modelling of beluga-prey dynamics and Arctic marine ecosystem structure.*

KEYWORDS: *Delphinapterus leucas*, diet, trophic level, stable isotopes, teeth, dentine, growth layer groups (GLGs), annuli, marine mammal, Arctic

INTRODUCTION

Resource use can differ within populations along sex, age class, and morphological divisions, as well as among individuals with unique foraging preferences. Females and males of sexually dimorphic species have different energetic requirements and foraging abilities (particularly among air-breathing marine vertebrates, whose dive capacity is related to body size; Schreer and Kovacs 1997), and can differ so greatly in their diet composition and habitat use that they are considered ecologically distinct (e.g. Le Boeuf et al. 2000, Lewis et al. 2006). Ontogenetic niche segregation can be similarly pronounced, as age classes may consume different sizes and types of prey related to growth and other developmental factors (Polis 1984, Werner and Gilliam 1984). A third source of intraspecific variation in resource use, resource polymorphism, ascribes distinct diet and habitat use among morphs within species to morphological variation, particularly in feeding apparatus (e.g. number of gill rakers in fish; mouth size, number of teeth, and jaw musculature in amphibians; and bill size and shape in birds; Smith and Skúlason 1996).

Individual resource specialization, the consistent use of a subset of a population’s resource base that cannot be attributed to sex, age, or morphological differences, also occurs among a range of animal taxa (Bolnick et al. 2003, Araújo et al. 2011). There is presently growing emphasis on documenting the incidence and degree of individual specialisation within populations (e.g. Araújo et al. 2010), and understanding its ecological causes (Estes et al. 2003, Svanbäck and Bolnick 2005, 2007, Araújo et al. 2011, Tinker et al. 2008, 2012) and consequences (Okuyama 2008, Schreiber et al. 2011, Bolnick et al. 2011, Violle et al. 2011). For example, Cantor et al. (2013) found individual dietary differences among frugivorous opossums (*Didelphis albiventris*) had ramifications for seed dispersal and plant community composition in a tropical forest, while

Quevedo et al. (2009) found habitat and dietary partitioning between littoral vs. pelagic subpopulations of perch (*Perca fluviatilis*) limited food web connectivity in lakes.

Indices of individual diet specialisation partition a population's total niche width (TNW), which encompasses all consumed prey resources, into within- and between-individual components (WIC and BIC; Bolnick et al. 2002, 2003). Individual specialists have narrow niche width (small WIC) relative to the TNW, due either to (largely) non-overlapping diets, or restricted prey preferences nested within a broader range consumed by generalist individuals (Sargeant 2007, Araújo et al. 2010). Longitudinal diet sampling, such as repeated observations of predation events or sampling of gut contents from same individuals over an extended period, is required for assessment of individual specialisation because cross-sectional data can provide misleading estimates of WIC/TNW due to spatial and temporal variation in prey consumption (Bolnick et al. 2003). Acquiring sufficient long-term diet data through direct observation, however, is logistically difficult for many species, especially for marine mammals that pursue and consume prey underwater (but see Ford et al. 1998).

Ratios of naturally occurring stable isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) vary more or less predictably along environmental and trophic gradients, and offer a proven, indirect means of characterizing diet and foraging habitat (Newsome et al. 2010). Underlying biogeochemical processes determine baseline food web $\delta^{15}N$ and $\delta^{13}C$ values (Pancost et al. 1997, McClelland et al. 2003). Subsequent modification with each trophic transfer via isotopic fractionation during metabolic processing (e.g. Macko et al. 1986) results in the progressive enrichment of the heavier isotope of both elements (^{15}N and ^{13}C) along food chains (Minagawa and Wada 1984, McCutchan et al. 2003). Relatively large diet-tissue ^{15}N fractionation makes consumer δ^{15}N values an ideal proxy of trophic level within local food webs, while smaller diet-tissue ^{13}C fractionation, coupled with relatively large δ^{13}C variation of primary producers along benthic-pelagic or nearshore-offshore gradients (France 1995, Walker et al. 1999), makes δ^{13}C values a useful tracer of baseline carbon sources. Together, δ^{15}N and δ^{13}C values provide information on diet and habitat use, such that a consumer's 'isotopic niche space' (i.e. breadth of δ^{15}N and δ^{13}C values) is analogous to its ecological niche space (Bearhop et al. 2004, Newsome et al. 2007).

Stable isotopes can provide longitudinal foraging information via two approaches. The first is simultaneous measurement of the isotopic composition of tissues that integrate diet over varying timescales, allowing diet stability to be assessed over periods corresponding to tissue turnover rates (e.g. Quevedo et al. 2009). The second, and increasingly popular, approach is to derive longitudinal foraging histories through measurement of the isotopic composition of continuously growing tissues such as whiskers, baleen, and teeth (e.g. Newsome et al. 2009a, b, Codron et al. 2012). The isotopic composition of these metabolically inert tissues reflects that of diet at the time of their formation, and serial sampling along the axis of growth produces chronological isotopic profiles that can be used in long-term diet and habitat use reconstructions spanning multiple years. Stemming from the isotopic niche concept, isotopic variation within individual profiles can be assessed relative to other sampled individuals to infer individual variation in resource use (see Newsome et al. 2009a, Hückstädt et al. 2012, Vander Zanden et al. 2013).

The beluga whale (*Delphinapterus leucas*, Pallas 1776) is a medium sized toothed whale with a near circumpolar distribution in Arctic and subarctic waters (Stewart and Stewart 1989). Beluga whales are considered generalist consumers that forage opportunistically on a broad fish and invertebrate prey base, including benthic crustaceans, shrimp, and squid (Kleinenburg et al. 1964, Stewart and Stewart 1989). In the eastern Canadian Arctic, important prey include arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*), Greenland halibut (*Reinhardtius hyppoglossoides*), arctic char (*Salvelinus alpinus*), and decapod crustaceans (Sergeant 1973, Kilabuk 1998, Stewart 2001, Kelley et al. 2010, Marcoux et al. 2012). Intraspecific variation in resource use occurs among belugas, which segregate spatially (at least during migration and the open water season) by sex, age class, and reproductive status, with females and calves closely associated with coastal estuaries and adult males more frequently found in deeper, offshore waters (Caron and Smith 1990, Loseto et al. 2006, Colbeck et al. 2013). Biochemical diet proxies indicating both diet and habitat differences among segregated components have been attributed to ecological sexual dimorphism and ontogenetic niche segregation, reflecting size-specific energetic requirements and foraging abilities (Lesage et al. 2001, Loseto et al. 2008, 2009, Marcoux et al. 2012).

Previous cross-sectional studies measuring the isotopic composition of beluga skin and muscle have shown residual variation beyond that which was explained by sex and age class (e.g. Marcoux et al. 2012), and have indicated resource use also varies with individual body size (Loseto et al 2008, 2009). Here, we reconstruct longitudinal foraging records for individual beluga whales from the eastern Canadian Arctic using $\delta^{15}N$ and $\delta^{13}C$ profiles in dentine to test hypotheses of intrapopulation variation in resource use by beluga whales. We predicted females and males would show divergent isotope profiles at sexual maturity (10-12 yr; Sargeant 1973, Robeck et al. 2005), reflecting known sexual and ontogenetic niche segregation in this species.

Further, linear mixed effects models were used to partition isotopic variation unaccounted for by sex and age class into within- and among-individual components, allowing examination of the degree of individual specialisation. To test our hypothesis that residual isotopic differences among individuals unrelated to sex or age class may have been driven by body size, which confers greater diving ability to larger individuals (Schreer and Kovacs 1997; see Weise et al. 2010), isotopic composition of a subset of our sample (adult males) was qualitatively compared with body length and published prey isotope values.

METHODS

Tooth collection, sectioning, and dentine sampling

Teeth were extracted from mandibles of beluga whales (n = 69) harvested in subsistence hunts during the 1980s-2000s from three geographically (Smith and Martin 1994, Richard et al. 1998, 2001, Richard and Stewart 2008, Rioux et al. 2012) and genetically (Brown Gladden et al. 1997, Brennin et al. 1997, Turgeon et al. 2012) distinct populations in the eastern Canadian Arctic. Our sample comprised 21 whales from the Western Hudson Bay (WHB) population, 28 whales from the Cumberland Sound (CS) population, and 20 whales from the Eastern High Arctic-Baffin Bay (EHA-BB) population (Figure 6.1). Total body length was measured in a straight line from the tip of the rostrum to the tail notch, while sex was determined by necropsy and confirmed genetically (D. Tenkula, Fisheries and Oceans Canada, Winnipeg, Canada).

Following standard ageing protocol (Stewart 2012), teeth were extracted from positions 2 or 5, except when less wear occurred in adjacent teeth. Beluga dentine grows by accretion on the pulp cavity surface in a series of annually deposited cones that appear in longitudinal section as

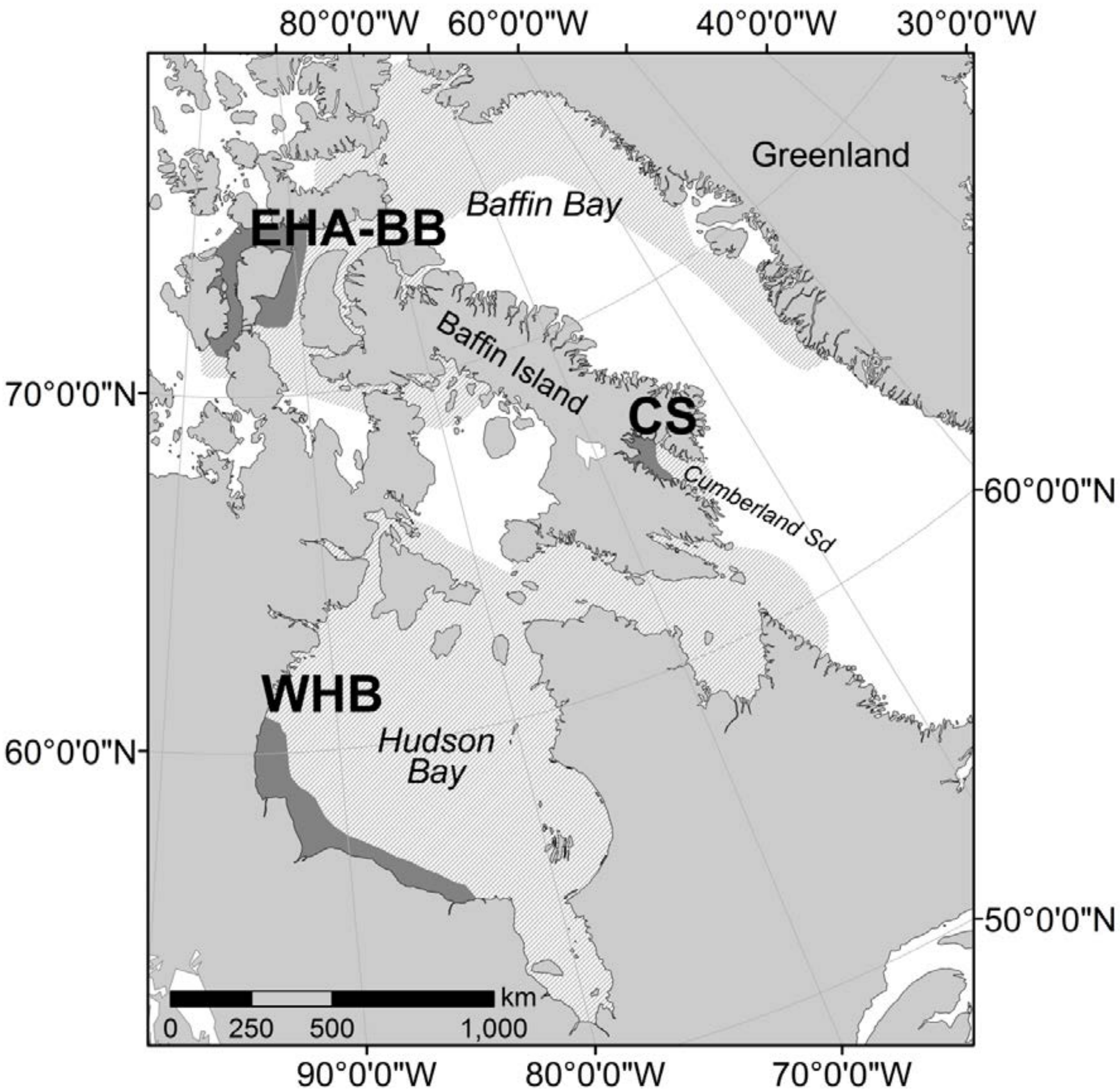

Figure 6.1. Teeth were collected from beluga whales (*D. leucas*) from three eastern Canadian Arctic populations over the 1980s-2000s: the Western Hudson Bay (WHB), Cumberland Sound (CS), and Eastern High Arctic-Baffin Bay (EHA-BB) populations. Winter and summer distributions are shown in light grey hatching and dark grey, respectively, and were reproduced from COSEWIC (2004), with modifications to WHB distribution after Smith et al. (2007).

chevron-shaped growth layer groups (GLGs; Stewart 2012). Teeth were sectioned longitudinally along the midline using a water-cooled diamond saw blade, and polished using 30 and 9 µm aluminum oxide (AlOx) lapping film to enhance GLG definition. GLGs were counted under reflected light starting at the neonatal line that defines the first GLG (Stewart 2012), and aged assuming annual deposition (Stewart et al. 2006, Luque et al. 2007, Campana and Stewart 2014, Matthews and Ferguson 2014a). Calendar year of GLG deposition was assigned using the final GLG, which was usually incomplete and assumed to represent the year of death, as a reference.

Dentine was sampled from each GLG using a high-resolution micromill (Merchantek) fitted with a 300 µm-diameter drill bit at a depth of 150 µm to minimize contamination from adjacent growth layers. GLGs that were slightly thinner than the drill bit (≥ 200 µm) were milled after any remaining material from the previously sampled GLG was sheared off, while thinner GLGs (< 200 µm) were not sampled. Decreasing GLG width with age (Stewart 2012) ultimately limited the number of GLGs that could be discretely sampled while providing enough material for analysis, resulting in ~5-20 sampled GLGs per tooth.

Stable isotope analysis

Dentine is typically acidified prior to isotopic analysis to isolate the collagen fraction from the inorganic matrix (which contains carbonates with different $\delta^{13}C$ values than collagen; Ambrose and Norr 1993); however, repeated rinses and transfers of small amounts of powdered dentine would have resulted in considerable sample loss during the acidification process. We therefore conducted a pairwise comparison of the isotopic composition of 15 dentine samples separated into untreated (bulk dentine) and acidified (primarily collagen) portions. Bulk dentine had slightly, but significantly, higher mean δ^{13}C values (-14.2 ± 0.2 ‰) than acidified samples (-14.4 ± 0.2 ‰) (paired samples t-test, df = 14, t = -5.365). δ^{15}N values followed a similar pattern (19.5 ± 0.4 ‰ vs. 19.2 ± 0.4 ‰ for bulk and acidified samples, respectively; paired samples t-test, df = 14, t = -6.574). Because isotopic differences between untreated and acidified pairs were consistent across samples, and were much smaller than trophic discrimination in consumers of high protein prey (~3.3 and ~1.3 ‰ for δ^{15}N and δ^{13}C, respectively; McCutchan et al. 2003), we proceeded with analysis of bulk dentine (see also Martin et al. 2011).

Approximately 1 mg of powdered bulk dentine from each GLG was weighed into tin cups, and analysed using a Vario EL III elemental analyser (Elementar, Germany) coupled to a DELTAplus XP isotope ratio mass spectrometer (Thermo, Germany). Nitrogen and carbon isotope ratios are reported in delta (δ) notation as per mil (‰) deviation from the isotope ratios of atmospheric N_2 (AIR) and Vienna Pee-Dee Belemnite limestone (V-PDB), respectively, defined as δ^{15}N or δ^{13}C = ($R_{sample} - R_{standard}$) / $R_{standard}$) * 1000, where R is ^{15}N/^{14}N or ^{13}C/^{12}C. Values were normalised using reference materials whose isotopic composition spanned the sample range (nicotinamide, ammonium sulphate + sucrose, and caffeine; δ^{15}N -16.61 to 16.58 ‰, δ^{13}C -34.46 to -11.94 ‰) and had been calibrated to international standards IAEA-N1, IAEA-N2, USGS-40 and USGS-41 for δ^{15}N, and IAEA-CH6, NBS-22, USGS-40, and USGS-41 for δ^{13}C. Analytical precision based on repeated measures of a laboratory reference material not used in calibrations (glutamic acid) was 0.09 and 0.06 ‰ for δ^{15}N and δ^{13}C, respectively, and that based duplicate measures of ~10 % of samples was 0.14 and 0.06 ‰. Atomic C:N of bulk dentine (2.98 ± 0.06; mean ± SD) was within the range of unaltered collagen (DeNiro 1985).

Data analysis

Previous analyses have shown $\delta^{15}N$ and $\delta^{13}C$ values of teeth used in this study were influenced by extrinsic factors that could confound dietary interpretations. Long term $\delta^{13}C$ declines across GLG chronologies spanning the period of deposition (1965-2008) in two of the three beluga populations were consistent with the oceanic ^{13}C Suess effect (Matthews and Ferguson 2014a). We therefore applied a *post-hoc* adjustment of 0.022 ‰ yr^{-1} to $\delta^{13}C$ values ($\delta^{13}C_{cor}$), which is an average of published rates of $\delta^{13}C$ declines in surface waters of the North Atlantic over the 1960s-1990s (Sonnerup et al. 1999, Gruber et al. 1999, Quay et al. 2003, Körtzinger and Quay 2003). All $\delta^{13}C_{cor}$ values are reported in 2008 equivalents, the most recent year of GLG deposition. Ontogenetic $\delta^{15}N$ declines over the first 1-3 GLGs were related to weaning (Matthews and Ferguson 2014b), so the first three GLGs were removed from each isotopic series to ensure only values reflecting independent foraging were included in analyses.

Linear mixed effects models with random intercepts were used to partition variance in $\delta^{15}N$ and $\delta^{13}C_{cor}$ values among fixed (sex, age class, and calendar year) and random (individual whale nested within population) effects. Age class categories were 'immature' and 'adult', which comprised GLGs 4-11 and 12-27, respectively, after designations by Marcoux et al. (2012). The interaction term sex:age class was tested to examine divergent isotope patterns between females and males at age of maturity. Models were fitted separately to $\delta^{15}N$ and $\delta^{13}C_{cor}$ values using maximum likelihood (ML) estimation (package nlme; Pinheiro et al. 2013) in the statistical software R (R Core Team 2014), and model selection was based on likelihood ratio tests (Zuur et al. 2009) produced using the R package AICcmodavg (Mazerolle 2013). Model fits and assumptions were assessed via visual inspection of residual plots for deviations from homoscedasticity and normality.

Optimal models were refitted using the restricted maximum likelihood (REML) method to estimate variance components for random effects, including the residual error variance. Variance associated with the random effect ‘individual’ was a measure of isotopic variation among individuals unaccounted for by fixed effects, while the residual error variance was a measure of mean within-individual isotopic variation (*sensu* the BIC and WIC, respectively). We calculated the degree of individual specialisation as residual error variance/(residual error variance + individual random intercept), which approximated WIC/(WIC + BIC), or WIC/TNW (see Newsome et al. 2009a, Hückstädt et al. 2012, Vander Zanden et al. 2013).

To explore the potential relationship between isotopic variation among individuals and body size, $\delta^{15}N$ and $\delta^{13}C_{cor}$ values of 17 adult males were qualitatively compared with body length. Growth curves for the WHB, CS, and EHA-BB populations indicate male belugas attain ~85 % of adult body length by age 12, and reach asymptotic adult length at around age 20 (Sergeant and Brodie 1969, Doidge 1990, Stewart 1994). We therefore restricted comparisons to whales that were 20+ years old when they were harvested, and used body length at death as a proxy for size spanning the period of GLG formation after age 12. Although we acknowledge the potential problems with this approach, which was necessary because sample size decreased drastically when individual GLG profiles were truncated to 20+ years of age, isotopic composition showed no noticeable ontogenetic trend associated with continued growth beyond GLG 12 (see ‘Results’). Unfortunately, sample sizes were insufficient to allow trends to be examined separately by population, which differ in mean male asymptotic size (Doidge 1990, Stewart 1994). Adult females were excluded altogether due to small sample size.

RESULTS

There was clear separation among individuals based on long-term δ^{15}N values, and to a lesser extent, $\delta^{13}C_{cor}$ values (Figure 6.2). Variation in δ^{15}N values was best described by the full model including all tested fixed effects (sex, age class, their interaction term, and year), and the random effects population and individual (Tables 6.1 and 6.2). Adult males had higher δ^{15}N values in GLGs than immature males, while this pattern was not observed in females (Figure 6.3). Variation in $\delta^{13}C_{cor}$ values (Figure 6.2) was best described by the model that included year as the only fixed effect (model 7), although AICc also supported the full model including all fixed effects (model 1; Tables 6.1 and 6.2). The effect of year is apparent in the CS population, which shows a gradual increase in $\delta^{13}C_{cor}$ values over the timeframe of GLG deposition (Figure 6.2). Given that sex and age class were significant factors in the δ^{15}N model, variance components of random effects were calculated using both $\delta^{13}C_{cor}$ models (7 and 1) to ensure exclusion of the fixed effects sex and age class did not attribute undue variance to the random effect individual.

Variance components analysis of random effects showed the majority of variation in both δ^{15}N and $\delta^{13}C_{cor}$ values unaccounted for by fixed effects was due to variation among individuals (71.8 and 59.5 % for δ^{15}N and $\delta^{13}C_{cor}$, respectively), while a much smaller proportion was attributed to within-individual variation (12.5 and 29.8 % for δ^{15}N and $\delta^{13}C_{cor}$, respectively; Table 6.3). Variation accounted for by ‘population’ was also relatively small (15.7 and 10.8 % for δ^{15}N and $\delta^{13}C_{cor}$, respectively; Table 6.3), consistent with similar ranges in δ^{15}N and $\delta^{13}C_{cor}$ in each

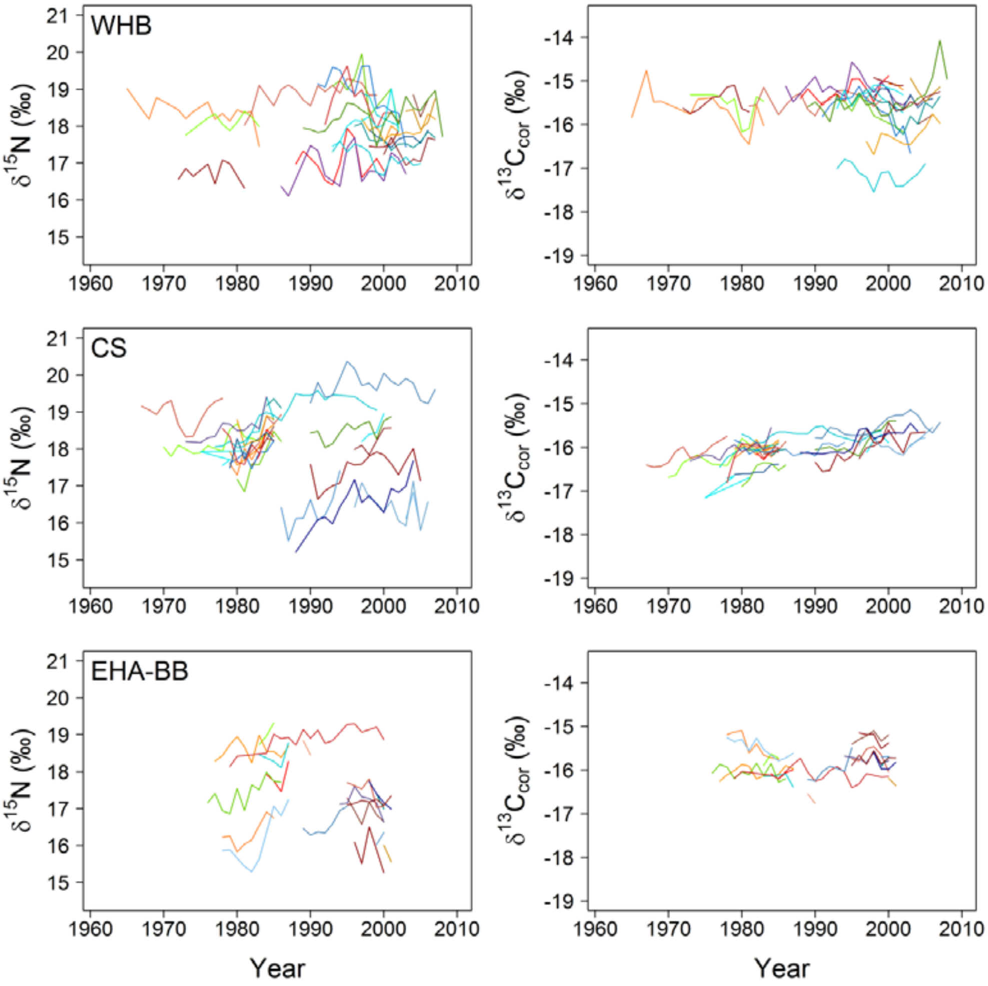

Figure 6.2. Longitudinal δ^{15}N (left) and $\delta^{13}C_{cor}$ (right) profiles across dentine GLGs in teeth of beluga whales (*D. leucas*) from three eastern Canadian Arctic populations: Western Hudson Bay (WHB), Cumberland Sound (CS), and Eastern High Arctic-Baffin Bay (EHA-BB). Little overlap between δ^{15}N values, and to a lesser extent, $\delta^{13}C_{cor}$ values, among individual whales indicate individual partitioning of resource use within each population.

Table 6.1. Likelihood ratio tests of random intercept linear mixed effects models of $\delta^{15}N$ and $\delta^{13}C_{cor}$ values measured across dentine GLGs in teeth of eastern Canadian Arctic beluga (*D. leucas*). Optimal models (bold) have the lowest AIC and AICc values, and highest Akaike weights, wi(AICc).

	logLik	**AICc**	**ΔAICc**	**w_i(AICc)**
$\delta^{15}N$				
Model 1 (full model) **~ sex + ageclass + sex:ageclass + year, random = ~1|population/whale**	**-370.81**	**757.88**	**0.00**	**0.87**
Model 2 ~ sex + ageclass + sex:ageclass, random = ~1|population/whale	-374.00	762.21	4.33	0.10
Model 3 ~ sex + ageclass + year, random = ~1|population/whale	-375.17	764.53	6.65	0.03
Model 4 ~ sex + ageclass, random = ~1|population/whale	-378.98	770.10	12.22	<0.01
Model 5 ~ sex, random = ~1|population/whale	-387.76	770.55	12.67	<0.01
Model 6 ~ ageclass, random = ~1|population/whale	-380.22	785.63	27.75	<0.01
Model 7 (null model) ~ 1, random = ~1|population/whale	-388.86	785.79	27.90	<0.01
$\delta^{13}C_{cor}$				
Model 1 (full model) **~ sex + ageclass + sex:ageclass + year, random = ~1|population/whale**	**-76.64**	**169.54**	**1.32**	**0.30**
Model 2 ~ sex + ageclass + sex:ageclass, random = ~1|population/whale	-81.96	178.12	9.90	<0.01
Model 3 ~ sex + ageclass + year, random = ~1|population/whale	-78.76	171.71	3.49	0.10
Model 4 ~ sex + ageclass, random = ~1|population/whale	-84.66	181.46	13.24	<0.01
Model 5 ~ sex, random = ~1|population/whale	-89.30	188.71	20.49	<0.01
Model 6 ~ ageclass, random = ~1|population/whale	-85.58	181.27	13.06	<0.01
Model 7 **~ year, random = ~1|population/whale**	**-79.06**	**168.22**	**0.00**	**0.59**
Model 8 (null model) ~ 1, random = ~1|population/whale	-90.07	188.22	20.00	<0.01

Table 6.2. Fixed effects estimates from optimal linear mixed effects models fit to $\delta^{15}N$ and $\delta^{13}C_{cor}$. Models were selected based on AICc values; two models fit to $\delta^{13}C_{cor}$ with similar AICc values are presented. p-values for sex and age class were not determined, as their interaction is included in the model.

$\delta^{15}N$	Estimate	SE	t-value	df	P
(model 1)					
Intercept	-12.34	11.34			
sex, male	0.00425	0.27			
age class, immature	0.205	0.11			
Year	0.0151	0.0055	2.66	1	<0.01
sex, male: age class, immature	-0.341	0.12	-2.95	1	<0.005
$\delta^{13}C_{cor}$					
(model 7)					
Intercept	-38.69	4.80			
Year	0.0115	0.0024	4.76		<0.001
(model 1)					
Intercept	-36.18	6.21			
sex, male	0.0487	0.11			
age class, immature	0.109	0.067			
Year	0.0102	0.0031	3.28	1	0.001
sex, male: age class, immature	-0.148	0.072	-2.05	1	0.04

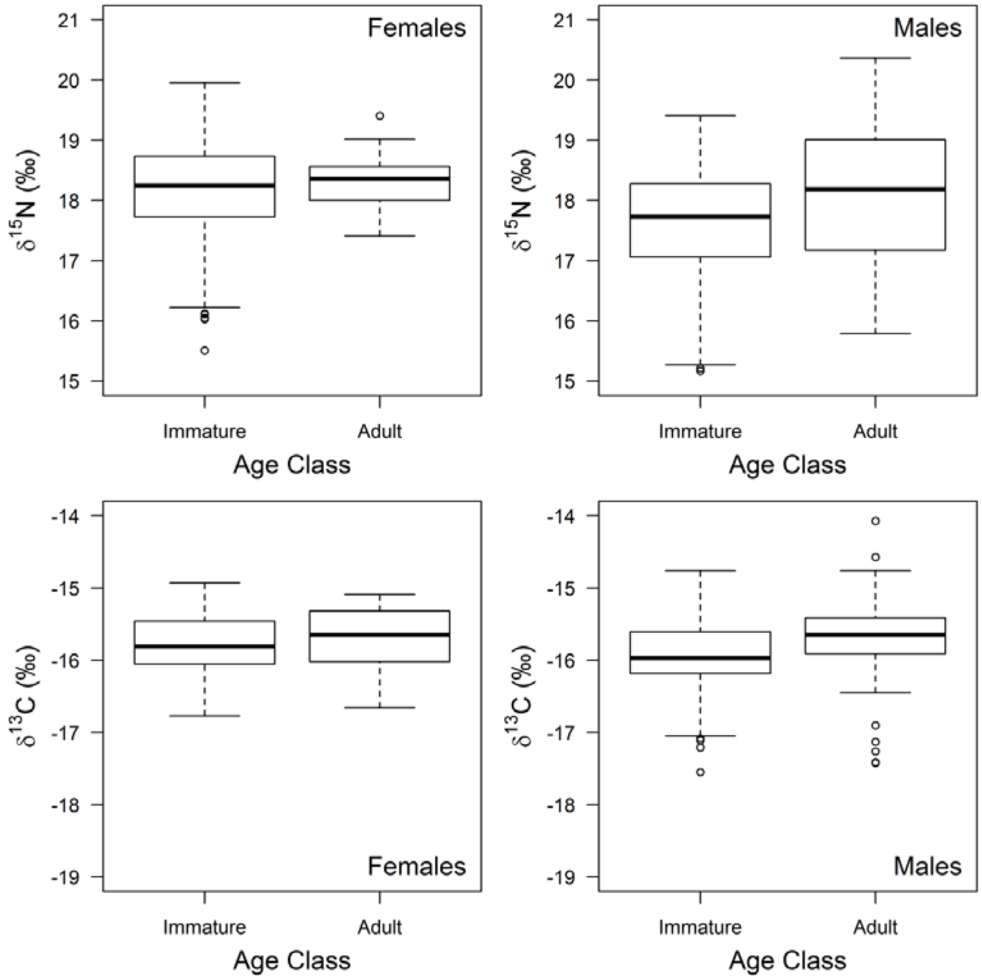

Figure 6.3. δ^{15}N (top) and $\delta^{13}C_{cor}$ (bottom) values of dentine of beluga whales (*D. leucas*) from three eastern Canadian Arctic populations averaged by age class show a significant interaction with sex ($p < 0.005$), with adult males on average having higher δ^{15}N values than immature males. This effect was not observed for females. The same, but only marginally significant ($p = 0.04$), effect was observed with $\delta^{13}C_{cor}$ values.

Table 6.3. Variance and % variance in δ^{15}N and $\delta^{13}C_{cor}$ explained by the nested random effects population, individual (whale), and within-individual (residual). Two models of $\delta^{13}C_{cor}$ that were indistinguishable based on AICc values are included to assess variance attributed to random effects when sex and age class are absent (model 7) and included (model 1) as fixed effects. An index of individual specialization (WIC/TNW) is also presented.

$\delta^{15}N$ (model 1)	**variance**	**% variance**	**$\delta^{13}C_{cor}$ (model 7)**	**variance**	**% variance**
intercept, population	0.174	15.7	intercept, population	0.0201	10.8
intercept, whale	0.798	71.8	intercept, whale	0.110	59.4
Residual	0.139	12.5	Residual	0.0552	29.8
WIC/TNW*	0.15		WIC/TNW*	0.33	
			(model 1)		
			intercept, population	0.0206	0.14
			intercept, whale	0.108	0.33
			Residual	0.0548	0.23
			WIC/TNW*	0.34	

*approximated by residual variance/(residual variance + whale variance) population (Figure 6.2). Variance components of random effects were essentially the same for both models fitted to $\delta^{13}C_{cor}$, indicating inclusion of additional fixed effects (model 1) did not reduce the proportion of overall variance attributed to individuals (Table 6.3). Our approximation of WIC/TNW was 0.15 and 0.33 for $\delta^{15}N$ and $\delta^{13}C_{cor}$, respectively, indicating a high degree of individual specialization.

Mean individual $\delta^{15}N$ values were greater among the 17 adult males with the greatest body length (Figure 6.4). This trend occurred across the sample as a whole, but was also apparent within each population, although small sample sizes preclude any definitive interpretations (Figure 6.4). $\delta^{13}C_{cor}$ values were more consistent across individuals of different body lengths (Figure 6.4). $\delta^{15}N$ differences were consistent across all ages after GLG 12, indicating the relationship with size was independent of age (Figure 6.4).

DISCUSSION

Intrapopulation variation in isotopic composition was expected based on previous studies showing belugas partition their niche space according to sex and age class. The shift in the isotopic composition in GLGs of males (but not females) at around the age of maturity is consistent with previously reported isotopic differences among sex and age classes in several beluga populations, which have been attributed to diet and habitat differences related to ecological sexual dimorphism and ontogenetic niche segregation (Lesage et al. 2001, Loseto et al. 2008, 2009, Marcoux et al. 2012). Adult male belugas segregate in all-male groups at around the age of maturity (Colbeck et al. 2013), spending considerably more time in offshore regions than females (Loseto et al. 2006). Lesage et al. (2001) attributed ^{13}C enrichment in male St.

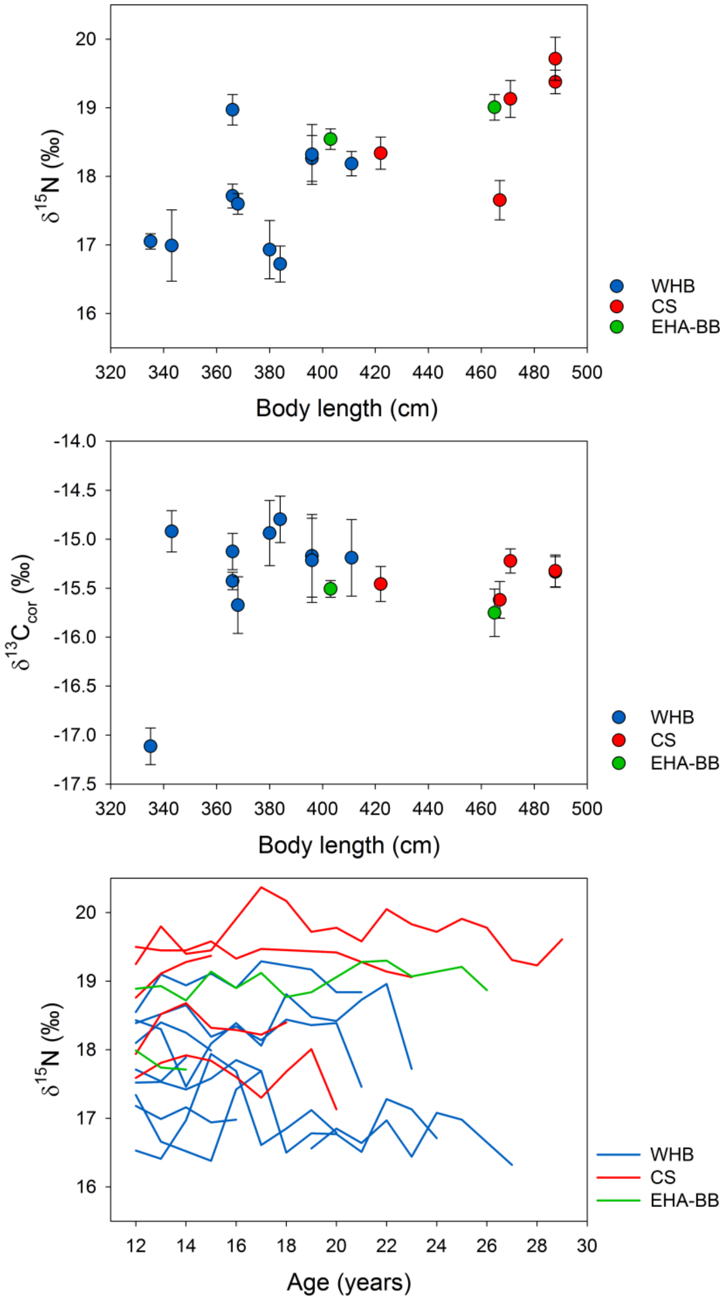

Figure 6.4. Mean individual δ^{15}N values of 17 adult male belugas from three eastern Canadian Arctic populations show an increasing trend with body length (top) that was not observed for $\delta^{13}C_{cor}$ (middle). δ^{15}N differences among whales of the same age, as well as relatively constant δ^{15}N values within individuals over the ages 12-20+ (bottom), indicate isotopic variation among individuals reflected size, and not ontogenetic, differences.

Lawrence Estuary belugas potentially to more benthic feeding, feeding at a higher tropic level, or spatial segregation along upper vs. lower portions of the estuary. Marcoux et al. (2012) offered the same factors as potential explanations of isotopic variation among age classes of CS belugas.

Considerable variation in dentine $\delta^{15}N$ and $\delta^{13}C_{cor}$ values among individuals, however, was not explained by sex or age class. Although calendar year was a significant covariate for both $\delta^{15}N$ and $\delta^{13}C_{cor}$ values, isotopic separation occurred among individuals whose GLG deposition overlapped temporally. Our primary assumption that population ranges are spatially non-overlapping is important because mixing of populations could bias estimates of among-individual variation (i.e. the BIC) if there were regional variation in either prey availability (see Kelley et al. 2010) or baseline isotopic composition (e.g. Popp et al. 2007, Seminoff et al. 2012). Mixing of individuals among populations also has potential to produce spurious relationships between isotopic composition and body length because WHB whales are smaller, on average, than CS and EHA-BB whales (Sergeant and Brodie 1969, Doidge 1990, Stewart 1994).

Satellite telemetry studies and aerial surveys of the WHB, CS, and EHA-BB populations indicate whales from each population occupy distinct summer distributions (Smith and Martin 1994, Richard et al. 1990, 1998, 2001, Richard and Stewart 2008). These findings have recently been corroborated by genetics and chemical tracer studies indicating eastern Canadian Arctic beluga populations occupy discrete summer ranges (Turgeon et al. 2012, Rioux et al. 2012), increasing the likelihood summer-harvested whales belong to the populations assigned based on harvest location. Further, mean isotopic compositions are very similar among eastern Canadian Arctic beluga populations. $\delta^{15}N$ values of WHB beluga skin collected from 1989-2009 were approximately 16.5 to 17 ‰ (Rioux et al. 2012, Kelley 2014), similar to mean skin δ^{15}N values of CS belugas collected from 2000-2009 (16.6 ± 0.5 and 16.8 ± 0.7 ‰ for females and males, respectively; Marcoux et al. 2012), and EHA-BB belugas collected from 1988-1990 (16.6 ± 0.6 ‰; Hobson and Welch 1992) and 1997-1998 (16.0 ± 0.2 ‰; Hobson et al. 2002). Regional variation in beluga isotope values across the eastern Canadian Arctic is therefore insufficient to account for the ~3 ‰ variation in δ^{15}N values within the three populations, not to mention the fact that, if regular movements of individuals among isotopically distinct regions occurred, one would expect within-individual variation to be a greater component of overall variation than was observed.

Our metric approximating WIC/TNW indicates a high degree of resource partitioning by individuals (see Newsome et al. 2009a, Hückstädt et al. 2012, Vander Zanden et al. 2013). Variance components estimates are comparable to those of Newsome et al. (2009a), who found that isotopic variation among specialist sea otters (*Enhydra lutris nereis*) explained 48.1 % of overall variation, while within-individual accounted for 28.2 % (the authors used averages of variance components estimated separately for δ^{15}N and δ^{13}C values measured along vibrissae, which corresponds to values of 65.6 and 21.2 % for among- and within-individual variation in this study; averages of values presented in Table 6.3). Newsome et al. (2009a) were able to confirm isotopic variation reflected individual diet preferences through direct, long-term observation of sea otter foraging bouts. In contrast, Vander Zanden et al. (2013) attributed low WIC/TNW values of <0.015 derived from repeated δ^{15}N and δ^{13}C measurements across scutes of green turtles (*Chelonia mydas*) to consistent foraging within spatially distinct food webs with different baseline isotopic composition.

Low isotopic variation within individual belugas indicates consistent resource exploitation over multiple years. It is unclear, however, whether variation among whales primarily reflects habitat segregation or diet differences. Belugas are philopatric, exhibiting site fidelity to summering areas, and perhaps specific estuaries (Smith and Hammill 1986, Caron and Smith 1990, Colbeck et al. 2012). The summer distribution of WHB belugas extends along much of the western and southwestern coasts of Hudson Bay, where major seasonal aggregations occur at three river estuaries (Richard et al. 1990, Richard 1993), while EHA-BB belugas aggregate at six principle river estuaries mainly around Somerset Island (Smith and Martin 1994). Long-term philopatry to specific summering sites could lead to small within-individual and considerable among-individual isotopic variation if localities differed in prey availability or were isotopically distinct over the long-term. It seems unlikely, however, that site fidelity would lead to the degree of isotopic variation observed among CS whales, which have a restricted range compared to the other two populations (Figure 6.1), and summer primarily in just one estuary/fiord complex (Richard et al. 1990). A small proportion of EHA-BB whales overwinter off western Greenland, rather than Baffin Bay like the majority of the population (Richard et al. 2001, Heide-Jørgensen et al. 2003), which could lead to isotopic separation among EHA-BB whales. However, this would not be expected to produce considerable isotopic variation among CS belugas, which occupy a common winter range in Cumberland Sound (Rioux et al. 2012), or among WHB belugas, which occupy a common winter range in Hudson Strait (Richard et al. 1990).

Diet studies indicate beluga populations feed predominantly on one or two prey species (Loseto et al. 2009, Kelley et al. 2010, Marcoux et al. 2012). Nevertheless, variation in measures of several diet proxies suggests individual dietary differences occur within sex and age classes (e.g. Hobbs et al. 2003, Marcoux et al. 2012). The isotopic ranges of the potential prey base across the range of the WHB and CS populations appear to be great enough to account for the range in beluga values. $\delta^{15}N$ and $\delta^{13}C_{cor}$ values of WHB whales (averaged by individual profile after truncating to calendar years 2000 and greater for better temporal overlap with prey) spanned 2.26 and 2.11 ‰, respectively. This corresponds reasonably well with the isotopic range of potential beluga fish prey, capelin, arctic cod, and Greenland halibut (12.1 ± 0.3 to 14.2 ± 0.5‰ for $\delta^{15}N$, and -19.9 ± 1.3 to -19.0 ± 0.5 ‰ $\delta^{13}C$) in Hudson Strait reported by Watt et al. (2013). There is similar agreement between the isotopic range across CS belugas (3.46 and 1.28 ‰ for $\delta^{15}N$ and $\delta^{13}C_{cor}$, respectively) and that of capelin, arctic char, and Greenland halibut in Cumberland Sound (13.8 ± 0.5 to 16.9 ± 0.7 ‰ for $\delta^{15}N$, and -19.0 ± 0.5 to -18.6 ± 0.6 ‰ $\delta^{13}C$; Marcoux et al. 2012). Inclusion of invertebrate prey would likely increase the potential isotopic range, although recent studies of WHB and CS beluga indicate invertebrate species are not important in the diet (Kelley et al. 2010, Marcoux et al. 2012). Published prey data from across the EHA-BB distribution were unavailable for comparison.

Although small sample sizes prevented rigorous comparisons, there was a relationship between isotopic composition and body length among 17 adult males pooled from all three populations that was independent of age. Although potential population specific isotopic composition and asymptotic body size cannot be ruled out as the primary driver of the observed trend, mean $\delta^{15}N$ and $\delta^{13}C$ values of CS belugas are slightly lower than those of WHB belugas (Rioux et al. 2012); we therefore assume the observed trend in the opposite direction, in which CS whales have considerably higher $\delta^{15}N$ values, is not spurious. We also assume the trend is not due to physiological or metabolic differences related to body size. Sea bass (*Dicentrarchus labrax*) raised on a controlled diet displayed no trend or only slight increases (~0.3 ‰) in δ^{15}N values with size (Sweeting et al. 2007, Barnes et al. 2008). Loseto et al. (2008, 2009) found the relationship between isotopic composition and body size among Beaufort Sea belugas was backed by two other independent dietary correlates, fatty acid profiles and methyl mercury concentrations, that also varied with body length (Loseto et al. 2008, 2009). These findings support interpretations of δ^{15}N variation among different sized whales being diet, rather than physiology, based.

Body size influences which prey a consumer can efficiently capture, handle, and ingest (Griffiths 1980; Mittlebach 1981; Shine 1991). At the same time, larger individuals have greater absolute energy requirements, and may target larger prey to maximize their energy intake (Mittlebach 1981). Diving capacity of air breathing marine vertebrates increases with size because larger animals have greater oxygen storage capacity and lower mass-specific metabolic rates (Schreer and Kovacs 1997), which affects ability to access prey distributed at various depths. Martin and Smith (1999) found time spent foraging at the seafloor by belugas decreased with increasing water depth, and that whales could spend approximately 50% more time foraging in shallow (100 m deep) coastal water than deeper (400 m) offshore waters. Individual foraging specialisations among belugas could therefore be a manifestation of size-specific metabolic requirements and dive capacity, with populations comprising smaller individuals that forage preferentially on smaller pelagic or epipelagic fish species, and larger individuals that are able to exploit larger offshore or deep water species. Fatty acid profiles separated Beaufort Sea belugas into groups of larger whales that foraged predominantly offshore, and smaller whales that fed nearshore (Loseto et al. 2009). Fatty acid profiles of both groups matched those of arctic cod within their respective habitats, indicating larger belugas targeted older/larger arctic cod at depth in offshore waters, and smaller whales foraging on younger/smaller age classes in shallow coastal waters (see Falk-Petersen et al. 1986).

Individual and size-based variation in diving behavior among eastern Canadian Arctic belugas may also be consistent with niche partitioning based on body size. Heide-Jørgensen et al. (1998) found both individual and size-related differences in average dive depths among six EHA-BB belugas affixed with satellite tags. The mean dive depth of one female was 100 m greater than the other individuals, and the averages of the deepest dives of the largest male and females were greater than those of the smaller whales (Heide-Jørgensen et al. 1998). Martin and Smith (1999) found larger EHA-BB belugas also made longer dives, while Kingsley et al. (2001) noted individual differences in dive time and depth among six radio-tagged belugas in eastern Hudson Bay. Relative differences in isotopic variation between CS beluga skin and muscle reported by Marcoux et al. (2012) may also reflect foraging on prey that segregate by depth. Skin δ^{15}N and δ^{13}C values, assumed to represent diet during the open-water season, overlapped with those of capelin and Arctic cod. The isotopic composition of muscle, which integrates diet over a longer period (Tieszen et al. 1983), was more variable, and the δ^{15}N and δ^{13}C values of some individuals more closely resembled those of Greenland halibut (Marcoux et al. 2012). The different degrees of isotopic variation between muscle and skin suggest resource use may be more variable outside of summer months, when the preferred deeper water habitat may provide more opportunity for resource use diversification based on diving capacity.

In addition to potential body size influences, the degree of intrapopulation variation in belugas reported here may reflect a combination of other mechanisms favoring the development and maintenance of individual-based foraging specialisations in predator populations, including 1) learning of complex foraging strategies required to recognize, capture, and handle one prey type occurs at the expense of doing so for another prey type, such that switching between alternative prey is not efficient, coupled with 2) some degree of intraspecific competition that promotes existence of alternative specialists (Estes et al. 2003). There is mounting evidence that the latter is density-dependent, and the degree of individual specialisation is greater when predator density is high, driving down the abundance of preferred prey and pressuring individuals to turn to alternative sources that are suboptimal under lower levels of intraspecific competition (Svanbäck and Bolnick 2005, 2007). Whatever the mechanism, our finding of a high degree of individual diet specialisation among eastern Canadian Arctic belugas has important implications for our understanding of Arctic marine community dynamics (Bolnick et al. 2003). For example, distinct individual foraging preferences could have important consequences for beluga population responses to shifts in prey abundance and distribution, or increased competition from invasive species occupying similar trophic positions, under future climate scenarios.

Hypotheses regarding individual foraging preferences of beluga whales should be readily testable by assessing more specific dietary biomarkers, such as fatty acids, of *individual* belugas relative to that of potential prey, or by examining individual dive profiles from the working hypothesis that a population of specialists that forage on pelagic or benthic prey would comprise individual variation in dive depth distributions (see Lea et al. 2002, Tinker et al. 2007). Advances in understanding of compound specific isotope analysis also offers a means to differentiate spatial from foraging influences on bulk δ^{15}N values (Matthews and Ferguson 2014c), which would help to clarify habitat vs. dietary influences on the isotopic variation among individuals. Understanding mechanisms causing intrapopulation variation in resource use among higher trophic level consumers (e.g. Ford et al. 1998, Estes et al. 2003, Kim et al 2012) is important, given their potential for top-down influence on ecosystem structure and function (Myers et al. 2007, Estes et al. 2011). Considerable intrapopulation variation in resource use within beluga sex and age classes noted here warrants further investigation, particularly regarding the potential relationship between isotopic composition and body length to understand how belugas, and potentially other marine consumers, partition overall niche space.

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Chapter 7. General conclusions

The goal of this thesis was to characterize short- and long-term diet variation of three Arctic whale species. Isotopic profiling of teeth and baleen produced individual-based, longitudinal foraging records, revealing seasonal and ontogenetic diet variation, as well as individual diet consistency over multiple years. Findings contribute to topical issues in foraging ecology of each species, such as assessing the degree of dietary specialisation within killer whale populations (de Bruyn et al. 2013), revealing intra-population variability in weaning age and resource use in beluga whales, and characterising seasonal variation in bowhead whale foraging (e.g. Heide-Jørgensen et al. 2013). Results are also relevant to our understanding of Arctic ecosystem structure and function, especially in light of ongoing and anticipated ecosystem changes expected to impact marine mammal populations across the region (Tynan and DeMaster 1997, Bluhm and Gradinger 2008, Laidre et al. 2008).

Species foraging ecology

Individual diet specialisation has been identified in several marine mammal species (e.g. sea otters, Estes et al. 2003; dolphins, Krützen et al. 2014), but the distinct foraging patterns of killer whales are probably the best known examples of dietary specialisation among marine mammals. Ford et al. (1998) showed killer whales in the eastern North Pacific belong to sympatric ecotypes with distinct dietary preferences, foraging primarily on either fish or marine mammals (a third ecotype with a shark-based diet has since been added to the original two identified; Ford 2011). Accordingly, much of the ensuing research on killer whales has focused on identifying dietary specialisations (e.g. Pitman and Ensor 2003, Pitman and Durban 2011), as well as accompanying behavioural, social, morphological, and genetic variation (Foote et al. 2009).

This thesis examined long-term diet differences among killer whales from a region where they have been little-studied, adding to the growing literature on global killer whale predation patterns. Much of the isotopic variation among individual ECA/NWA killer whales reflected large-scale regional segregation rather than distinct diet differences, evidence that killer whales observed in the ECA occupy a distinct distribution from those in other areas of Northwest Atlantic, at least during some portion of the year. Relatively low within-tooth isotopic variation also indicated individual habitat and diet consistency over decadal periods, and could reflect 1) specialisation on a given prey group or 2) consumption of relatively constant proportions of different types of prey. Although we are in the early stages of understanding AA-specific $\delta^{15}N$ dynamics in marine mammals, and currently cannot use these data to reliably estimate trophic position, marine mammals are likely the preferred prey of ECA killer whales (Higdon et al. 2011). However, the greater range between source and trophic AA $\delta^{15}N$ values of two of the killer whales, along with tooth wear patterns consistent with a shark diet, also suggest dietary specialisations on one of several major prey groups observed in other killer whale populations may also occur among ECA/NWA killer whales.

Although beluga whales have recently been shown to carve up their niche space according to sex, age class, and size (Lesage et al. 2001, Loseto et al. 2008, Marcoux et al. 2012), the high degree of variation in isotopic composition among individuals was unexpected. A growing number of both marine and terrestrial species assumed to be generalists are now understood to comprise individual specialists (Bolnick et al. 2003, Araújo et al. 2011). There is currently recognition among community ecologists that the ‘ecology of individuals’ needs to be incorporated into models of community interactions (Bolnick et al. 2011, Violle et al. 2012), which has driven efforts to understand underlying mechanisms driving individual specialisation in resource use (Svanbäck and Bolnick 2005, 2007). The relationship between adult male size and $\delta^{15}N$ value indicates individual foraging differences reflect, at least in part, greater diving capacity of larger whales, which presumably allows access to larger and more energetically valuable prey for whales large enough to capture and consume them efficiently. Individual-based foraging along a vertical gradient in the water column likely allows belugas to reduce intraspecific competition, which may be important in Arctic food webs with a limited number of suitable prey species. Further research on a larger number of individuals, including female belugas (which also show considerable individual variation in adult size), could help clarify role of body size in individual-based foraging strategies of beluga whales. This could also shed light on the evolutionary drivers of sexual dimorphism in the species, which may be due to resource partitioning rather than sexual selection (see Slatkin 1984, Shine 1989).

The year-round Arctic distribution of EC-WG bowhead whales is a divergence from other baleen whales, whose life histories involve migrations between productive high-latitude foraging areas in summer and low-latitude breeding/calving areas in winter (Clapham 2000). Many migratory baleen whale species endure prolonged seasonal fasting after building up lipid reserves during intense summer feeding bouts, with only opportunistic foraging at food resource-poor calving grounds (e.g. Baraff et al. 1991). Baleen isotope patterns indicate EC-WG bowhead whales forage throughout their distribution, which is in agreement with reports of winter foraging by EC-WG bowheads in Hudson Strait and off western Greenland (e.g. Laidre et al. 2007). Year-round foraging by EC-WG bowheads is also consistent with foraging behaviour of Bering-Chukchi-Beaufort (BCB) bowhead whales that feed at core-use areas that vary seasonally (Citta et al. 2014). Winter foraging may be relatively more important to the annual nutrition budgets of bowhead whales than other baleen whale species that overwinter in nutrient-poor low-productivity tropical regions. The potential role of food resource availability in bowhead whale winter habitat selection also deserves further attention.

Arctic ecosystem structure

The Arctic marine ecosystem comprises several ‘keystone’ zooplankton and fish species that funnel primary production to higher trophic positions. Climate-induced changes in zooplankton and fish distributions, which are tightly linked with sea ice and water temperature, therefore stand to have the most pronounced indirect impact on Arctic marine mammal populations (Tynan and DeMaster 1997, Bluhm and Gradinger 2008, Laidre et al. 2008). Bottom-up impacts stemming from shifts in prey availability, including nutritional stress, reproductive success, and altered migration patterns and geographic range (Tynan and DeMaster 1997), will be particularly important for species like bowhead and beluga whales.

EC-WG bowhead whales are thought to meet the majority of their annual energetic demands during the open water season, when optimal foraging conditions (i.e. dense prey aggregations) persist over several months. Correlations between prey abundance, blubber thickness, and female reproductive success of other baleen whales (e.g. Lockyer 1986) indicate their concentrated seasonal foraging effort makes bowheads vulnerable to changes in zooplankton phenology and community composition through fasting-imposed energetic constraints on female reproductive success. An analogous story is currently unfolding for polar bears in western Hudson Bay, where poor female body condition and declining birth rates have been associated with sea ice declines that have reduced the period bears can hunt seals (Regehr et al. 2007, Rode et al. 2010). Baleen isotope patterns consistent with some degree of foraging on mature-stage zooplankton during periods of low productivity over the winter months could help buffer potential energetic shortfalls during summer foraging. On the other hand, bowhead whales could be vulnerable to changes in zooplankton composition and distribution throughout their range, rather than just during the open-water season.

Beluga whales are thought to be among the most resilient of Arctic marine mammals to ecosystem changes given their opportunistic, generalist diets (Bluhm and Gradinger 2008). Multi-decadal isotopic chronologies in the CS beluga population are consistent with a recent diet shift, perhaps encompassing more capelin (see Marcoux et al. 2012). Capelin are a recent invader in Cumberland Sound that is also prominent in the diets of other consumers in the CS ecosystem (Dennard et al. 2009, Ulrich 2013). Long-term isotopic trends may speak to the degree of diet plasticity of belugas, indicating they are able to adapt quickly to changes in prey availability. However, pronounced individual diet preferences that potentially reflect size-related energetic requirements and foraging abilities suggesting ecological impacts may not be uniform across beluga populations. Energetics budgets of larger animals that forage on larger, energy-rich prey may be disproportionately affected by changes in their prey base. Strong weaning signals in teeth may offer a means to assess reproductive impacts of potential diet shifts because weaning duration is related to female and environmental conditions. Changes in the nursing period could signal ecosystem changes affecting maternal energy allocation.

Recent increases in killer whale sightings across the ECA and range expansions associated with sea ice declines (Higdon and Ferguson 2009) have highlighted a need for better understanding of how killer whales structure Arctic ecosystems through top down forcing. Reeves and Mitchell (1988) speculated generalist killer whales function like a multi-species fishery in the ECA, free from a regulatory response to changes in density of any one prey species, such that they maintain a constant level of predation pressure on all prey populations. Recently, modelling attempts to understand killer whale-multiple prey dynamics in the ECA by Ferguson et al. (2012) indicated killer whales do not show strong prey specialization. Killer whales have been observed hunting most marine mammals that occur in the ECA (Reeves and Mitchell 1988, Hidgon et al. 2011), and low isotopic variation across dentine GLGs would be consistent with specialisation on that prey group. Previous research has shown small numbers of killer whales can impose regulation on prey populations (Williams et al. 2004). Disruptions to prey behavior during and after predation events (Reeves and Mitchell 1988) could also have important impacts on energetics of prey species. Finally, the finding that two killer whales in our sample apparently originated from different regions, along with satellite tracking data showing ECA/NWA killer whales are wide-ranging (Matthews et al. 2011), has implications for potential colonisations of Arctic regions as sea ice declines create favorable killer whale habitat.

Isotopic profiling of teeth and baleen – future research

The serial measurement of isotopic composition along the growth axis of teeth and baleen offers a unique, retrospective means to assess marine mammal diet and distribution, but, like any approach, is not without limitations. For both teeth and baleen, the primary limitation is bulk tissue isotope measurements cannot discern between trophic influences and baseline isotopic variation. This is typically overcome by canvassing all potential prey available to the consumer, sampled preferably at the same time and over a similar spatial scale to control for confounding temporal and spatial influences on food web isotope composition. However, given teeth and baleen grow over decadal timespans, there is considerable potential for both spatial and temporal influences on baseline isotopic variation to confound dietary interpretations.

Limited understanding of spatial and temporal ecosystem isotopic variation, however, should not inhibit future research in this field. Addition of a third isotope, such as our measurement of $\delta^{34}S$ values in bowhead baleen, can improve resolution of diet vs. habitat influences on $\delta^{15}N$ and $\delta^{13}C$ values. Strong correlations between $\delta^{34}S$ and $\delta^{15}N$ values in baleen, for example, provided support for a dietary, rather than spatial, cause of seasonal isotopic variation along bowhead baleen. Stable isotope ratios of oxygen ($\delta^{18}O$) also vary across ocean basins (Yoshida 1991), offering potential discriminatory power between dietary and distributional influences on $\delta^{15}N$ and $\delta^{13}C$ values in wide-ranging animals such as ECA/NWA killer whales (Matthews et al., unpublished data). Compound specific isotopic analysis, however, is likely the way of the future in animal stable isotope ecology. Tracking the isotopic composition of specific compounds committed to different metabolic pathways allows for much more detailed understanding of isotopic routing of nitrogen, carbon, and other elements from diet to tissues. To our knowledge, this thesis represents the first time an amino acid-specific isotopic approach has been used in a directed study of cetacean foraging ecology. Further studies on AA-specific $\delta^{15}N$ dynamics to understand how both source and trophic AA $\delta^{15}N$ values change with trophic position in marine mammals, as well as nutritional state, will be necessary before AA-specific analysis can be used reliably. However, such groundwork will stand to improve the power of retrospective diet characterization immensely because it will allow for the disaggregation of diet and distribution influences, and could be used, for example, to confirm isotopic cycles in bowhead baleen reflect seasonal variation in food consumption.

The second area that would benefit from technical advancement applies to teeth. Dentine growth layer groups are typically just several hundred microns thick, which limits the spatial (and therefore, temporal) scale over which they can be sampled using bulk approaches like micromilling. This currently constrains the type of data that are commonly collected (and hence, the types of research questions that can be addressed) primarily in two ways: 1) growth layers that can be sampled discretely while still providing enough material for isotopic analysis are limited to those of a certain width, which omits compressed GLGs deposited later in life, or thinner GLGs perhaps deposited during periods of poor growth and nutrition; and 2) sampling at smaller scales within GLGs, which would allow for diet variation to be examined over sub-annual periods (Matthews et al., unpublished data), is difficult to do. Further development of *in situ* microspatial sampling techniques like laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) for measurement of trace element concentration and stable isotope ratios will allow for refinement of diet variation studies using teeth, including measurement of biogeochemical dietary and habitat proxies to better link seasonal variation in diet and habitat use. These developments would necessarily be coupled with a greater understanding of seasonal dentine deposition.

Isotopic profiling of continuously growing structures like teeth and baleen is currently one of the only viable approaches for assessing short- and long-term diet variation in marine mammals, especially for Arctic species for which even basic foraging information is lacking. This thesis has provided new information relevant to theoretical and practical considerations in the foraging ecology of three Arctic whale species. For example, individual belugas likely consume unequal proportions of different prey, which should be considered in ecosystem models of trophic interactions, while knowledge of beluga lactation duration, a key parameter in the female mammalian reproductive cycles, is vital for modeling population demography. This thesis has also advanced the field of stable isotope ecology in its application of rare ($\delta^{34}S$) or new (AA-specific $\delta^{15}N$ analysis) approaches. Advancements will ensure isotopic profiling of teeth and baleen, which has increased in popularity over the duration of this thesis, only continues to grow in importance in future studies of animal foraging ecology.

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