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

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## ABSTRACT

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.

## 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).

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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 cross dating 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 $\delta^{13}$C declines across dentinal GLGs of beluga from three eastern Canadian Arctic (ECA) populations over the 1960s–2000s with $\delta^{13}$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 $\delta^{13}$C variation was sufficient to cause synchronous $\delta^{13}$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 $\delta^{13}$C declines and assess GLG deposition in beluga whales. $\delta^{13}$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 $\delta^{13}$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) (Fig. 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.

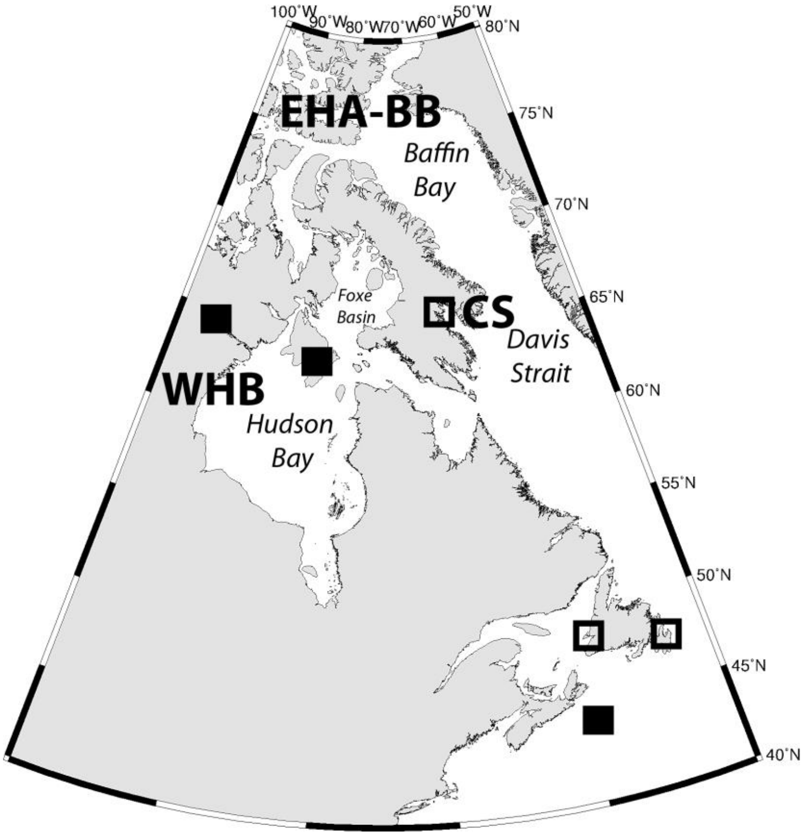

**Fig. 1.** Teeth were collected from belugas harvested in Arviat, NU (Western Hudson Bay population; **WHB**), Pangnirtung, NU (Cumberland Sound population; **CS**), and Grise Fiord (Eastern High Arctic-Baffin Bay population; **EHA-BB**) through the 1980s-2000s. Locations where killer whale specimens were collected throughout the eastern Canadian Arctic, Newfoundland, and Nova Scotia are indicated by closed (single specimen) and open (two specimens) squares.

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.

*Killer whales*

Teeth collected from killer whales (n = 9) from locations throughout the ECA and along the coasts of Newfoundland and Nova Scotia (Fig. 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 $\delta^{13}C$ 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 °C, and isolated collagen was rinsed using deionized water and freeze-dried. In a comparison of $\delta^{13}C$ 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 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, 2015). 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 1, Fig. 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.

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 (Fig. 2, Table 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 ‰ $\text{yr}^{-1}$) and WHB and EHA–BB beluga populations (–0.0186 ± 0.0060 ‰ $\text{yr}^{-1}$ and –0.0285 ± 0.0046 ‰ $\text{yr}^{-1}$, respectively) (Fig. 2, Table 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 ‰ $\text{yr}^{-1}$ and –0.0442 ± 0.0081 ‰ $\text{yr}^{-1}$ 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 1 and 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

**Table 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 |

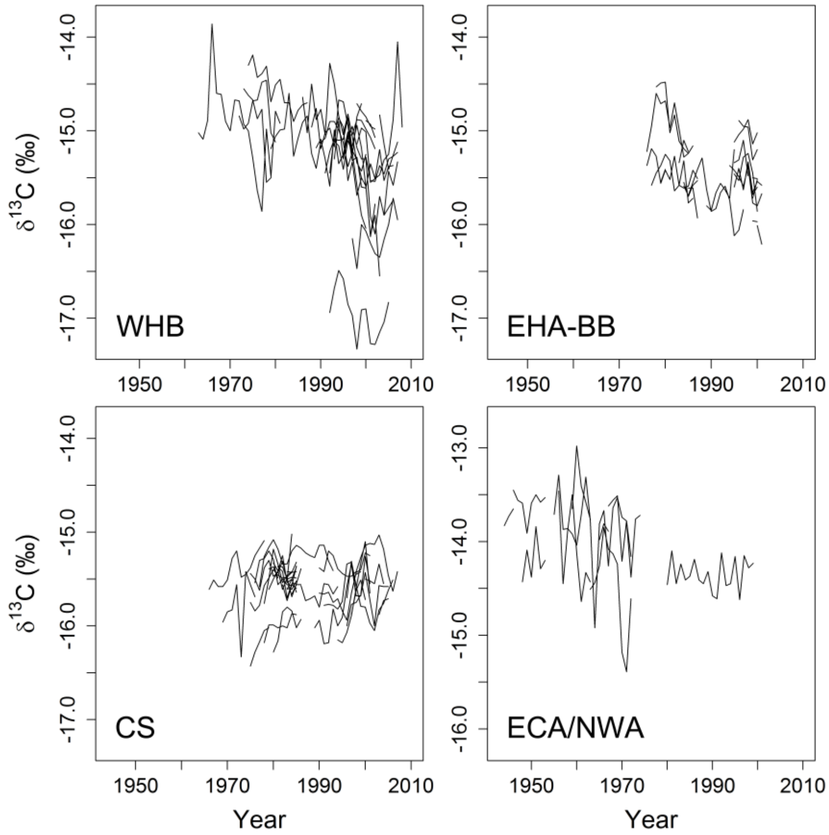

**Fig. 2.** Significant $\delta^{13}C$ declines across dentinal GLGs of –0.0186 ± 0.00602 ‰ $yr^{-1}$ and –0.0285 ± 0.00458 ‰ $yr^{-1}$ in both the Western Hudson Bay (WHB) and Eastern High Arctic–Baffin Bay (EHA–BB) beluga populations, respectively, were statistically indistinguishable from $\delta^{13}C$ 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.

species, although we acknowledge other factors, such as shifts in foraging behavior and processes affecting marine δ$^{13}$C dynamics at more localised scales (e.g. Pancost *et al.* 1997), introduce a degree of uncertainty in our interpretations.

**Table 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$^{-1}$

Gradual changes in diet composition over time would confound our assumption that observed δ$^{13}$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 δ$^{13}$C decreases. δ$^{15}$N values measured across dentinal GLGs offer an internal check on diet shifts, since concurrent (and more pronounced) declines in δ$^{15}$N values are expected to accompany δ$^{13}$C decreases associated with trophic-level diet variation (McCutchan *et al.* 2003). The lack of linear δ$^{15}$N trends across GLGs of ECA/NWA killer whales or WHB and EHA–BB belugas (Matthews and Ferguson 2014; Matthews unpubl. data) indicates trophic-level dietary shifts are an unlikely explanation for observed δ$^{13}$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 $\delta^{13}C$ 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 $\delta^{15}N$ analysis of dentine, and two whales with $\delta^{13}C$ values related to spatial variation were excluded from population-level $\delta^{13}C$ 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 $\delta^{13}C$ 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 $\delta^{13}C$ 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). Further north on the summering grounds of EHA–BB beluga in the Canadian Arctic archipelago, ice-free conditions have increased by approximately 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 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), 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 have 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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