Compound Specific Isotope Analyses of Harp Seal Teeth:

Tools for Trophic Ecology Reconstruction

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Abstract

As sentinels of ecosystem health, high trophic level predators integrate information through all levels of the food web. Their tissues can be used to investigate spatiotemporal variability in foraging behaviour, and with the appropriate analytical methods and tools, archived samples can be used to reconstruct past trophic interactions. Harp seal (*Pagophilus groenlandicus*) teeth collected in the 1990s from the Northwest Atlantic were analysed for bulk stable carbon and nitrogen isotopes (δ"C<sub>bulk</sub> and δ"N<sub>bulk</sub>), and compound specific stable nitrogen isotopes of amino acids (δ"N<sub>AA</sub>) for the first time. We developed a fine-scale, annual growth layer group (GLG) dentine sub-sampling method corresponding to their second and third year of life. In accordance with previous diet studies, while there was individual variability in δ"N<sub>bulk</sub>, δ"C<sub>bulk</sub>, and δ"N<sub>AA</sub> measurements, we did not detect significant differences in isotopic niche widths between males and females, or between GLGs. Relative trophic position was calculated as the baseline corrected δ"N<sub>AA</sub> values using trophic (glutamic acid) and source (phenylalanine and glycine) amino acids. Variability was measured between individuals in their relative trophic position, but within individual variability was low, suggesting that they fed at the same trophic level over these two years of life. These novel δ"N<sub>AA</sub> data may therefore suggest individual, specialist harp seal foraging behaviour in sub-adults. Our findings show that compound specific stable isotope signatures of archived, inert predator tissues can be used as tools for the retrospective reconstruction of trophic interactions on broad spatiotemporal scales.

Key Words

Phocid seals, foraging specialisation, isotopic niche, trophic position, diet, dentine, inert tissues
1. Introduction

Wide-spread changes have been documented in the Arctic over the last three decades in terms of ocean warming and the resulting loss of sea ice (e.g. Greene et al., 2008; Stroeve et al., 2008; Strong, 2012). To protect this unique polar environment, it is becoming increasingly important to quantify and understand long-term responses of the Arctic ecosystem to environmental change. However, detecting changes in the marine Arctic environment is difficult due to the logistical limitations of the extent to which these areas can be practically sampled both spatially and temporally. Developing novel methods to sample and quantify these changes and better understand how the ecosystem is being altered is a vital component of conservation-oriented management programs aiming to protect these ecosystems from additional anthropogenic stressors. One solution to overcome limited sampling opportunities is to use high trophic level predator tissue samples as they integrate information from the base to the top of the food chain (Bossart, 2011; Hazen et al., 2019; Moore and Huntington, 2008).

Harp seals (Pagophilus groenlandicus) are important Arctic ecosystem sentinel species (Stenson et al. 2020). Harp seals are a migratory phocid, and in the Northwest Atlantic specifically, they are currently the most abundant pinniped with an estimated population size of approximately 7.6 million (Hammill et al., 2020). This population migrates annually from the Gulf of St Lawrence, Newfoundland and Labrador, northwards to Baffin Bay and the eastern Canadian Arctic to feed during the summer on a variety of pelagic invertebrates, fish, and sea ice associated amphipods (Stenson et al., 2020). The seals also feed during their southward migration in the late autumn and early winter to prepare for whelping, mating, and moulting that take place in sub-Arctic regions (Sergeant, 1991). Therefore, as a species that forages over an extensive area of the North Atlantic during their annual life cycle, they are excellent monitors of ecosystem variability.

Through a combination of commercial hunts since the 1800s, subsistence hunts and the scientific sampling of catches, historical archives of harp seal canine teeth have been collected by several research institutions in Arctic countries, including the Department of Fisheries and Oceans, Canada. These teeth archives present valuable opportunities to investigate harp seal diet, and by extension, the environmental variability experienced by the seals through their lifetimes. In order to exploit these opportunities, novel methods are required for the application of various biochemical analyses using archived teeth. Teeth contain mineralised and soft-tissue components that can preserve a timeline of their chemical composition during growth, and
therefore allow retrospective studies of diet and contaminant exposure of individuals (e.g. Hirons et al. 2001, Zhao and Schell 2004, Ferreira et al. 2011, Carroll et al. 2013, Matthews and Ferguson 2015). Teeth have therefore been used as quantitative monitoring tools to detect and investigate long-term ecological changes and anthropogenic threats to the environment (Boyd and Roberts 1993; Outridge, et al. 2009; Hanson, et al. 2017). In pinnipeds, teeth are also routinely used to age individuals by counting annual growth layer groups (GLGs) in the dentine and/or cementum (Bowen et al. 1983; Frie et al., 2011; Hall et al., 2019; Hanson et al., 2017). With the appropriate methods, analysing biomarkers in these metabolically inert, annual growth layers of dentine in known-age animals therefore provides great potential to study temporal ecological changes (Hobson and Sease 1998).

Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are commonly used as tracers to reconstruct food webs to investigate ecological change. $\delta^{13}C$ of bulk tissue generally exhibits minimal fractionation (< 1.5‰ with each trophic level) (Fry et al. 1984) and can be used to determine the origin of food sources in terms of inshore/offshore gradients, the identification of areas of higher productivity (Ceia, et al. 2018), and the identification of marine, ice or terrestrially derived matter (Søreide, et al. 2013; Boutton 1991; Keeley and Sandquist 1992). $\delta^{15}N$ of bulk tissue ($\delta^{15}N_{\text{bulk}}$) increases by 2-5 ‰ at each trophic level, providing a continuous measure of trophic position in predators (Post, 2002). However, $\delta^{15}N_{\text{bulk}}$ is influenced by changes in $\delta^{15}N$ in nutrient inputs at the base of the food web, or “baseline” (Chikaraishi et al., 2009). Compound specific isotope analysis (CSIA) of nitrogen of amino acids (AA) ($\delta^{15}N_{\text{AA}}$) in predator tissue is being increasingly applied to disentangle baseline and trophic level effects. The $\delta^{15}N$ of “source” amino acids experiences negligible fractionation during trophic transfer and conservatively traces the $\delta^{15}N$ baseline, whereas significant fractionation of “trophic” amino acids results in $^{15}N$ enrichment between each trophic transfer (McMahon and McCarthy, 2016). This is important when determining variability in diet as an indicator of environmental change because it can distinguish between true changes in trophic position of a predator, or an overall change in the $^{15}N$ of the environmental baseline (McMahon and McCarthy, 2016). This, in turn, allows for more precise estimates of food chain length (Chikaraishi et al., 2009).

$\delta^{15}N_{\text{AA}}$ is a powerful technique for quantifying changes in food webs, but relies on access to larger quantities of tissue/material than the more widely used bulk isotope analyses. Here, we provide a proof-of-concept study extending previous CSIA methods applied to marine mammal teeth to an Arctic phocid for the first time. Despite the small size of the canines in this species, we were able to extract individual GLGs to provide sufficient material for both bulk and CSIA.
analysis. Extracting dentine samples from individual tooth GLGs provides the opportunity for longitudinal, fine temporal scale sampling to investigate both within and between individual variability. Thus, we aimed to establish a method that can be extended in future studies to maximise the ecological information available from teeth as a potential ‘archive’ of data used to monitor and interpret change in Arctic and subarctic ecosystems. Such studies could ultimately subsample teeth spanning multiple decades, as well as multiple GLGs to cover the full life-span of individual seals. These methods are therefore of interest for ongoing efforts to investigate past environmental conditions and thus characterise long-term changes in marine ecosystems.

Here, we document a new method to extract specific growth layers of harp seal canine teeth collected from Newfoundland, Canada, in 1994/1995. Specifically, we extracted dentine from two GLGs, corresponding to the second and third years of life of individual seals. Previous studies of stomach contents analyses and bulk stable isotopes suggest that there are no differences in diet between males and females, or between two and three year old sub-adult seals (Beck, Hammill and Smith, 1993; Lawson and Stenson 1995). Thus, to confirm that our fine temporal scale dentine extraction, and SI analysis methods are representative of harp seal foraging behaviour, we hypothesised that there were no significant difference in stable isotope signatures between male and female seals, or between the GLGs representing the foraging habits in their second and third years of life.

2. Methods

2.1 Canine Teeth Collection

A total of 17 archived (9 males and 8 females) harp seal canine teeth, collected by licenced commercial hunters along the coast of northern Newfoundland or southern Labrador, Canada, in late 1994/early 1995 were used for analysis. All individuals were 5 years old (see ageing methods below) and were born in March 1990. Ten of the samples were collected during the winter prior to pupping in March 1995, while the other seven were collected in the spring following the 1995 moultng period (Supplementary Figure 1). Upon collection, lower jaws were collected and boiled in water for 1 hour to facilitate extraction of teeth.

2.2 Teeth Sectioning and Sub-Sampling

In order to maximise the ecological information that can be gained from a single tooth, they were sectioned along two planes: transverse and sagittal (Fig. 1). The teeth were cross sectioned
using a precision low speed diamond saw (Buehler, Isomet™). Transverse sections were used for ageing (Bowen et al., 1983) and sagittal sections were used for dentine GLG sub-sampling for bulk and compound-specific stable isotope analyses (Fig. 1).

2.2.1 Transverse Section for Ageing

Age-estimation using the dentine or cementum is species dependent, and in harp seal canines, counting the dentine GLGs is considered more reliable as only a thin layer of cementum is deposited throughout the lifetime of an animal (Bowen et al., 1983; Frie et al., 2011). Thus, in the dentine of harp seal teeth, each annual GLG consists generally of three incremental growth layers of different optical properties visible under transmitted light, thought to be due to the seasonal variation in foraging through their annual cycle (Bowen et al., 1983; Frie et al., 2011). These distinct growth and mineralization patterns deposited in the dentine remain unchanged over time (Bowen et al., 1983).

The 17 canine teeth were aged upon collection following methods developed by Bowen et al. (1983) by a single experienced reader. Briefly, transverse sections between 200–250 µm thick were cut just below the enamel cap (Fig. 1) and examined under transmitted polarized light (6 x 50 binocular microscope). When estimating age from transverse sections, it can be difficult to define the extent of the first year’s growth in the dentine as often there are accessory opaque incremental growth layers in the translucent dentine which could be wrongly counted. For this reason, the neonatal line is an important reference when determining the extent of the first annual GLG as it acts as a marker for where to start counting (Bowen et al. 1983), and was used here to aid in the ageing process. The remaining part of each tooth was stored in a solution of equal parts of water, 70% ethanol, and glycerine before they were removed, manually dried, and processed for further sectioning and analyses in 2019 described below. Previous work by Chua and colleagues has demonstrated the lack of preservation effects on CSIA results (Chua et al., 2020).
Fig. 1. Individual tooth sectioning schematic from a 5 year old harp seal harvested in Newfoundland, Canada. Top: Each whole canine tooth was sectioned along two planes; transverse and sagittal. Bottom Left: Transverse cross section of the tooth mid-point showing the outer cementum layer, the incremental dentine growth layer groups (GLGs) and the pulp cavity. Bottom Right: Sagittal sections (rotated 90° here for demonstration purposes) from the mid-point of the tooth to the root were used to subsample individual dentine GLGs indicated by the dashed red lines.

2.2.2 Sagittal Sections and GLG Subsampling

A second transverse cut was made along the maximum circumference at the mid-point of the tooth (Fig. 1). The point of maximum circumference was measured using callipers, and marked on the tooth for alignment with the saw for sectioning. The remaining part of the tooth including the root was mounted onto a ~2 cm x 2 cm piece of plexiglass with superglue and left to dry overnight. Two 700 μm thick sagittal sections were then cut as close as possible to the central
plane of the tooth (Fig. 1). Typically, compound specific stable isotope analyses require larger
tissue masses than conventional $\delta^{15}N_{\text{bulk}}$ and $\delta^{13}C_{\text{bulk}}$ protocols. For this reason, when aiming
to subsample the teeth at such a fine, annual scale to separate individual GLGs, it was important
to find the balance between large enough sample masses and precision in defining the GLG
boundaries for accurate sub-sampling. The 700 $\mu$m section thickness was chosen following a
number of trials at varying thicknesses as it provided enough mass of tooth dentine for the bulk
and the CSIA of individual GLGs (~weighing between 3 – 10 mg), but was not so thick that
the delineations of each GLG were obscured under transmitted light microscopy.

In order to accurately sub-sample the individual GLGs, the sections were de-mineralised to
remove bioapatite from the tooth matrix, causing them to soften and allow sampling. The 700
$\mu$m thick sections were immersed in 0.25 M HCl for between 12 and 24 hours. Neoformed,
needle-shaped crystals (presumably calcium chloride salts) formed on the surface of the
sections but were easily removed by rinsing thoroughly with de-ionised water. This procedure
allowed the sections to become soft enough to sub-sample with a scalpel, but did not cause
them to lose so much rigidity and/or structural integrity that the GLGs were no longer
distinguishable when viewed under transmitted light x 20 magnification (2x objective and 10x
oculars). Once softened, any remaining gum tissue and cementum was cut away from the outer
edge of the tooth (Fig. 1).

Using a scalpel, cutting from the middle part of the tooth towards the root, the sections
were cut along the opaque layers that separate the first, second and third years of life of
the individual (Fig. 1). Care was taken while sub-sampling not to desiccate the sections as
they become translucent and the GLGs are not as easily distinguishable. Dentine samples
representing the individual GLGs for the second (GLG 2, deposited through 1991) and
third (GLG 3, deposited through 1992) years of life were lyophilised and stored in plastic
vials until stable isotope analysis. The first GLG, representing the individual’s first year
of life, was not used for analysis as the stable isotope signature in this GLG is expected to
be affected by the mother’s isotopic signature transferred to the pup through both gestation
and lactation. Due to the narrowing of GLGs with increasing age (Fig. 1), the fourth and
fifth GLGs could not be precisely separated whilst also maintaining the minimum sample
mass required for SI analysis. For this reason, they were not included in this study. Future
studies that do not require such fine temporal scale resolution in GLG subsampling could
use these narrower GLGs combined for bulk and CSIA investigations.
2.3 Stable Isotope Analyses

2.3.1 Bulk Analyses - $\delta^{15}$N<sub>bulk</sub> and $\delta^{13}$C<sub>bulk</sub>

**Sample Preparation:** Approximately 0.5 mg of each of the GLG 2 and GLG 3 samples were precisely weighed (± 1 µg) and sealed in a tin capsule.

**Instrumental Analysis:** Samples were analysed using an elemental analyser (Costech) coupled to Delta V isotope ratio mass spectrometer (IRMS; Thermo-Scientific). Stable isotope values are reported in standard δ-notation (‰) (Eq. 1):

$$\text{Eq. 1: } \delta^X (\text{‰}) = \left( \frac{aX/bX}_{\text{sample}} / \frac{aX/bX}_{\text{standard}} - 1 \right) \times 1000$$

where (a) is the heavier, and (b) the lighter isotope of element X.

To determine precision and for calibration, international reference standards, USGS40 and USGS41a, were analysed at the beginning, middle and end of each run. Precision was typically better than 0.1 ‰. An internal standard of ground prawn (*Penaeus vannamei*) with well characterized $\delta^{13}$C and $\delta^{15}$N values (-22.6 ‰ and 6.8 ‰, respectively) was analysed every 10 samples to monitor precision, which was <0.2 ‰ for both $\delta^{13}$C and $\delta^{15}$N.

2.3.2 Amino Acid Specific Analyses - $\delta^{15}$N<sub>AA</sub>

**Sample Preparation:** GLG samples (weighing between 3 – 10 mg) were hydrolyzed in reaction vessels (6M, 1 mL, 100°C for 22 h). L-Norleucine (Sigma-Aldrich) was added to each sample as an internal standard (80 µl of 5 mg/mL). Samples were then transferred into clean micro-reaction vessels and were frozen at -80°C prior to lyophilization. The amino acids were propylated in 0.25 mL of acidified isopropanol solution (prepared by addition of acetyl chloride to anhydrous isopropanol (1:4 v/v) in an ice bath) at 100°C for 1 h. The reaction was quenched in a freezer and reagents were evaporated under a gentle stream of N<sub>2</sub>, DCM was added (3 x 0.25 mL) and evaporated to remove excess reagents. Amino acid methyl esters were then treated with 1 mL of a mixture of acetone:trimethylamine:acetic anhydride (5:2:1, v/v) to each sample and heated at 60°C for 10 min. Following acetylation, the reagents were evaporated under a gentle stream of N<sub>2</sub> and were dissolved in 2 mL of ethyl acetate, to which 1 mL of saturated NaCl solution was added. Phase separation was enabled via mixing and the organic phase was collected; separation was repeated 3 times with addition of 2 mL ethyl acetate.
Residual water was removed from the combined organic phases by passing through a glass wool plugged glass Pasteur pipette filled with MgSO$_4$. Finally, samples were evaporated under N$_2$ and the derivatized amino acids were dissolved in DCM and stored at -20°C until analysis.

**Instrumental Analysis:** $\delta^{15}$N$_{AA}$ values were determined using a Trace Ultra gas chromatograph (GC) coupled to a Delta V Advantage IRMS with a ConFlo IV interface (Cu/Ni combustion reactor held at 1000°C, Thermo Fisher). A liquid nitrogen trap was added after the reduction oven to remove CO$_2$ from the sample stream. The separation of amino acids was achieved using an HP Innowax capillary column (30 m x 0.25 mm i.d. x 0.5 µm film thickness, Agilent). The sample was introduced to the column using a split/splitless injector set at 260°C. The GC was programmed as follows: held at 50°C for 2 min, 10°C min$^{-1}$ to 180°C and 3°C min$^{-1}$ to 260°C, and held for 8 min. The carrier gas was ultra-high purity helium (flow 1.1 mL.min$^{-1}$). The ion intensities of m/z 28, 29 and 30 were monitored and the $\delta^{15}$N of each amino acid peak were automatically computed (Isodat version 3.0; Thermo fisher) by comparison with a standard reference N$_2$ gas, which was repeatedly measured (x4) at the beginning and the end of each sample analysis. All results are reported in per mil (‰) relative to N$_2$.

Each sample was run in duplicate using two different dilutions. The first run was used to separate the following AA: Alanine (Ala), Valine (Val), Leucine (Leu), Glycine (Gly), Aspartic acid (Asp) and Glutamic acid (Glu). Phenylalanine (Phe) was often below the limits of detection and so all samples were concentrated and run again using a different ‘time events’ programme, to isolate the Phe peak with an optimal peak size of 500 to 1200 mV. A triplicate measurement was made if the mean $\delta^{15}$N$_{AA}$ values fell outside the expected measurement error (<1.0‰). Precision and accuracy were determined using a mixed amino acid standard prepared from eight amino acids with known $\delta^{15}$N values (University of Indiana, USA and SI Science Japan). The mixed standard was analyzed every 4 injections. Typical precisions and accuracies were ± 0.9 ‰ and ± 0.2 ‰ (1σ, n = 48), respectively.

Raw $\delta^{15}$N$_{AA}$ sample values were corrected following the methods of McCarthy et al. (2013). This method takes into consideration the response of individual amino acids to the stationary phase of the column and is based on the offset between the measured $\delta^{15}$N$_{AA}$ values in the nearest mixed standard and their known $\delta^{15}$N$_{AA}$ values (Eq. 2).

**Eq. 2:** $\delta^{15}$N$_{sample}$ reported = avg $\delta^{15}$N$_{sample}$ measured - $\delta^{15}$N$_{standard}$ measured - $\delta^{15}$N$_{known}$.
Where $\text{avg} \delta^{15}\text{N}_{\text{sample measured}}$ is the average $\delta^{15}\text{N}$ for an amino acid in a sample ($n = 2$), $\delta^{15}\text{N}_{\text{standard measured}}$ is the $\delta^{15}\text{N}$ for the AA in the nearest mixed standard and $\delta^{15}\text{N}_{\text{known}}$ is the known elemental analysed offline value for the same standard.

2.4 Statistical Analyses

2.4.1 $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{13}\text{C}_{\text{bulk}}$

To determine which factors best explained the variation in the $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ data, we specified linear mixed effect models ($\text{glmer}$ function in $\text{lme4}$ package, R version 3.6.2, 2019), with individual as a random effect to take into account the repeat sampling of two GLGs for each animal. The covariates used in the model were sex, GLG (2 or 3) and the $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ values for the $\delta^{13}\text{C}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{bulk}}$ models, respectively. The global $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{13}\text{C}_{\text{bulk}}$ models, separately, included all covariates, and backwards model selection using the $\text{dredge}$ function ($\text{MuMIn}$ library) was used to identify the covariates that best explain the variation in the data. The best-fitting model for each dataset was selected using the smallest Akaike's information criterion corrected for small sample sizes (AICc), which provides a relative measure of the goodness-of-fit of the models. Linear regression models ($\text{car}$ package) were used to assess the relationship between the $\delta^{15}\text{N}_{\text{bulk}}$ values in GLGs 2 and 3, and between the $\delta^{13}\text{C}_{\text{bulk}}$ values in GLGs 2 and 3. Statistical significance for all models was considered as $p < 0.05$.

2.4.2 $\delta^{15}\text{N}_{\text{AA}}$ data

Two-way ANOVA tests were used to evaluate possible differences in $\delta^{15}\text{N}_{\text{AA}}$ isotopic values among seals simultaneously in terms of sex and GLG using the $\text{aov}$ function ($\text{car}$ library) for each amino acid individually. As above, statistical significance was considered as $p < 0.05$.

2.4.3 Relative Trophic Position Determination

Several source amino acids can be used to trace the baseline $\delta^{15}\text{N}$. Phenylalanine is typically used in most studies to reconstruct the baseline and estimate trophic position (TP). Glycine has also been considered as a source AA, but its $\delta^{15}\text{N}$ can be strongly affected by microbial degradation (McMahon and McCarthy 2016) and so should be treated with caution depending on the tissue type and sample storage conditions caution (Nielsen et al. 2015). Here, we used the $\delta^{15}\text{N}$ of phenylalanine ($\delta^{15}\text{N}_{\text{Phe}}$) as a source amino acid, and tested the potential use of
glycine ($\delta^{15}$N$_{Gly}$) as a source amino acid in harp seal dentine. A linear regression model (car package) was used to assess the relationship between $\delta^{15}$N$_{Phe}$ and $\delta^{15}$N$_{Gly}$ values in the two GLGs.

We used $\delta^{15}$N of glutamic acid ($\delta^{15}$N$_{Glu}$) to estimate TP. Glutamic acid is the most abundant amino acid in a consumer’s tissue, and is considered as the canonical trophic amino acid while all of the other trophic amino acids are related to the central nitrogen pool via glutamic acid (McMahon and McCarthy, 2016). Glutamic acid has therefore widely been used to estimate the TP of aquatic organisms (Chikaraishi et al., 2007; Germain et al., 2013; Nielsen et al., 2015). However, the uncertainty regarding trophic fractionation factors between “source” and “trophic” amino acids across taxa in entire food webs prevents accurate estimation of an organism’s absolute TP (Nielsen et al., 2015). To compare the two baseline AAs (Phe and Gly) we calculated relative TP ($\text{TP}_{rel}$) by independently subtracting $\delta^{15}$N$_{Phe}$ and $\delta^{15}$N$_{Gly}$ values from $\delta^{15}$N$_{Glu}$ to obtain baseline-corrected $\delta^{15}$N$_{Glu}$ values. Linear regression models (car package) were used to assess the relationship between GLGs 2 and 3 for both $\delta^{15}$N$_{Phe}$ and $\delta^{15}$N$_{Gly}$ values, and $\text{TP}_{rel}$ values to determine if values in the second year of life were related to those in the third. To compare $\text{TP}_{rel}$ between individual seals, we used a one-way ANOVA with seal as a factor variable and the mean $\text{TP}_{rel}$ as the explanatory variable. Mean $\text{TP}_{rel}$ for each seal was calculated as the mean of $\delta^{15}$N$_{Glu}$ - $\delta^{15}$N$_{Phe}$ and $\delta^{15}$N$_{Glu}$ - $\delta^{15}$N$_{Gly}$ of GLG2 and GLG3 ($n = 4$). Statistical significance for all analyses was considered as $p < 0.05$.

### 2.4.4 Trophic Niche Estimation

We determined isotopic niche spaces for each sex and in each GLG using Stable Isotope Bayesian Ellipses in R (SIBER package in R, Jackson & Parnell, 2015). Standard Bayesian ellipses were calculated from $\delta^{15}$N$_{bulk}$ and $\delta^{13}$C$_{bulk}$ values, $\delta^{15}$N$_{Glu-Phe}$ and $\delta^{13}$C$_{bulk}$ values, and $\delta^{15}$N$_{Glu-Gly}$ and $\delta^{13}$C$_{bulk}$ values. Standard ellipse area was corrected for small sample sizes (SEAc, Jackson et al., 2011) and ellipse overlap (95%) was calculated.

### 3. Results

#### 3.1 $\delta^{15}$N$_{bulk}$ and $\delta^{13}$C$_{bulk}$ data

The $\delta^{15}$N$_{bulk}$ values ranged between 12.8 and 17.1 ‰, and $\delta^{13}$C$_{bulk}$ values ranged between -15.9 and -14.5 ‰, and were both normally distributed (Shapiro-Wilk test, $p > 0.05$). Our $\delta^{15}$N$_{bulk}$
and δ^{13}C_{bulk} values were within the ranges of those already published from a range of tissue types in Arctic phocids (Table 1). Backwards model selection revealed that no covariates were retained as important factors to explain the variability in the δ^{15}N_{bulk} or the δ^{13}C_{bulk} data (best fitting models with the lowest AICc were 2 units smaller than the next, best-fitting model; Table 2). There were therefore no significant differences in measured δ^{15}N_{bulk} and δ^{13}C_{bulk} values between males and females or between GLGs 2 and 3 (Fig. 2). There was no significant relationship between the δ^{15}N_{bulk} and the δ^{13}C_{bulk} data (linear regression model; p > 0.5). There was also no relationship between the δ^{15}N_{bulk} values in GLGs 2 and 3, or between the δ^{13}C_{bulk} values in GLGs 2 and 3 (linear regression models; p-values all > 0.5).

![Figure 2](image)

**Fig. 2.** a) Mean, maximum and minimum δ^{15}N_{bulk} and δ^{13}C_{bulk} values measured for each growth layer group (GLG). There was no overall difference between the GLGs 2 and 3 representing the second and third year of life of the harp seals (n = 17). b) Mean, maximum and minimum δ^{15}N_{bulk} and δ^{13}C_{bulk} values measured by sex. There was no overall difference between these male and female sub-adult harp seals (n = 17).

**Table 1.** Published δ^{15}N_{bulk} (%) and δ^{13}C_{bulk} (%) values measured in harp seals and other Arctic phocids. Values are mean ± SD unless otherwise stated.

*Subadults specifically

**Suess corrected to account for increased fractionation of carbon due to increased use of fossil fuels from 1850 to present day.

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<td>-17.8 ± 0.5</td>
<td>Lawson and Hobson, 2000</td>
<td></td>
</tr>
<tr>
<td><strong>Southern Barents Sea, Russia</strong></td>
<td>Muscle</td>
<td>15.07 ± 0.6</td>
<td>-19.37 ± 0.3</td>
<td>Haug et al. 2017</td>
<td></td>
</tr>
<tr>
<td><strong>Newfoundland and Labrador, Canada</strong></td>
<td>Teeth</td>
<td>14.9 ± 1.1</td>
<td>-15.2 ± 0.3</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td><strong>Ringed seal</strong></td>
<td><strong>Central West Greenland</strong></td>
<td>Teeth</td>
<td>16.35 ± 1.0</td>
<td>Aubail et al. 2010</td>
<td></td>
</tr>
<tr>
<td><strong>Central East Greenland</strong></td>
<td>Teeth</td>
<td>14.9 ± 1.1</td>
<td>-17.23 ± 0.5</td>
<td>Aubail et al. 2010</td>
<td></td>
</tr>
<tr>
<td><strong>Beaufort Sea, Alaska, USA</strong></td>
<td>Muscle</td>
<td>16.9 ± 0.6</td>
<td>-18.5 ± 0.8</td>
<td>Hoekstra et al. 2002, Dehn et al. 2007</td>
<td></td>
</tr>
<tr>
<td><strong>Northwest Territories, Canada</strong></td>
<td>Muscle</td>
<td>17.2 ± 0.7</td>
<td>-20.4 ± 0.4</td>
<td>Dehn et al. 2007</td>
<td></td>
</tr>
<tr>
<td><strong>Central West Greenland</strong></td>
<td>Muscle</td>
<td>17.0 ± 0.1</td>
<td>-19.4 ± 0.1</td>
<td>Hobson et al. 2002</td>
<td></td>
</tr>
<tr>
<td><strong>Nunavut, Canada</strong></td>
<td>Muscle</td>
<td>17.3 ± 1.1</td>
<td>-17.3 ± 0.7</td>
<td>Hobson and Welch, 1992</td>
<td></td>
</tr>
<tr>
<td><strong>Hudson Bay, Canada</strong></td>
<td>Muscle</td>
<td>13.9 ± 1.4</td>
<td>-19.7 ± 0.9</td>
<td>Muir et al. 1995</td>
<td></td>
</tr>
<tr>
<td><strong>Beaufort Sea, Canada</strong></td>
<td>Claws</td>
<td>17.6 ± 1.0</td>
<td>-17.9 ± 0.6</td>
<td>Boucher et al. 2020</td>
<td></td>
</tr>
<tr>
<td><strong>Bering and Chukchi seas, Alaska, USA</strong></td>
<td>Claws</td>
<td>15.0 - 19.4 (range)</td>
<td>-21.1 to -14.6% (range)</td>
<td>Carroll et al. 2013</td>
<td></td>
</tr>
<tr>
<td><strong>Bering and Chukchi seas, Alaska, USA (1953–1968)</strong></td>
<td>Claws</td>
<td>17.5 ± 0.6</td>
<td>-15.6 ± 0.5**</td>
<td>Crain et al. 2021</td>
<td></td>
</tr>
<tr>
<td><strong>Bering and Chukchi seas, Alaska, USA (1998–2014)</strong></td>
<td>Claws</td>
<td>17.1 ± 0.8</td>
<td>-17.2 ± 1.2**</td>
<td>Crain et al. 2021</td>
<td></td>
</tr>
<tr>
<td><strong>Bearded Seal</strong></td>
<td><strong>Beaufort Sea, Alaska, USA</strong></td>
<td>Muscle</td>
<td>16.8 ± 0.9</td>
<td>Hoekstra et al. 2002, Dehn et al. 2007</td>
<td></td>
</tr>
<tr>
<td><strong>Northwest Territories, Canada</strong></td>
<td>Muscle</td>
<td>16.8 ± 0.1</td>
<td>-16.6 ± 0.3</td>
<td>Hobson et al. 2002</td>
<td></td>
</tr>
<tr>
<td><strong>Bering and Chukchi seas, Alaska, USA</strong></td>
<td>Claws</td>
<td>14.6 - 18.2 (range)</td>
<td>-18.3‰ to -13.7 (range)</td>
<td>Carroll et al. 2013</td>
<td></td>
</tr>
<tr>
<td><strong>Bering and Chukchi seas, Alaska, USA (1953–1968)</strong></td>
<td>Claws</td>
<td>15.4 ± 0.6</td>
<td>-14.7 ± 0.5**</td>
<td>Crain et al. 2021</td>
<td></td>
</tr>
<tr>
<td><strong>Bering and Chukchi seas, Alaska, USA (1998–2014)</strong></td>
<td>Claws</td>
<td>15.9 ± 0.7</td>
<td>-15.5 ± 0.7**</td>
<td>Crain et al. 2021</td>
<td></td>
</tr>
<tr>
<td><strong>Spotted Seal</strong></td>
<td><strong>Bering and Chukchi seas, Alaska, USA</strong></td>
<td>Muscle</td>
<td>17.8 ± 1.0</td>
<td>Dehn et al. 2007</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Result of Linear Mixed Effects (LME) Model selection for $\delta^{15}N_{\text{bulk}}$ and $\delta^{13}C_{\text{bulk}}$ showing the three best-fitting models with the lowest second-order Akaike information criterion (AICc) values.

<table>
<thead>
<tr>
<th>LME Model</th>
<th>Model Covariates</th>
<th>df</th>
<th>AICc</th>
<th>$\Delta$ AICc</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$\delta^{15}N_{\text{bulk}} \sim$</td>
<td>3</td>
<td>112.3</td>
<td>0.0</td>
<td>0.27</td>
</tr>
<tr>
<td>2</td>
<td>$\delta^{15}N_{\text{bulk}} \sim \text{Sex}$</td>
<td>4</td>
<td>114.3</td>
<td>2.07</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>$\delta^{15}N_{\text{bulk}} \sim \text{Sex + GLG}$</td>
<td>5</td>
<td>114.6</td>
<td>2.39</td>
<td>0.08</td>
</tr>
<tr>
<td>1</td>
<td>$\delta^{13}C_{\text{bulk}} \sim$</td>
<td>2</td>
<td>24.0</td>
<td>0.0</td>
<td>0.38</td>
</tr>
<tr>
<td>2</td>
<td>$\delta^{13}C_{\text{bulk}} \sim \text{Sex}$</td>
<td>3</td>
<td>26.3</td>
<td>2.34</td>
<td>0.12</td>
</tr>
<tr>
<td>3</td>
<td>$\delta^{13}C_{\text{bulk}} \sim \delta^{15}N_{\text{bulk}}$</td>
<td>3</td>
<td>26.4</td>
<td>2.41</td>
<td>0.11</td>
</tr>
</tbody>
</table>

3.2 $\delta^{15}N_{\text{AA}}$ data

Average values of $\delta^{15}N$ were between ~18.5 ‰ and ~25.0 ‰ for trophic AAs (valine, alanine, glutamic acid, aspartic acid, leucine), and between ~10.5 ‰ and ~11.5 ‰ for the two source amino acids (phenylalanine and glycine; Fig. 3). $\delta^{15}N_{\text{Gly}}$ values were similar to $\delta^{15}N_{\text{Phe}}$ (ANOVA; $F_{1,32} = 2.7$, $p = 0.11$), demonstrating that glycine can potentially be considered as an alternative to phenylalanine, and used as a source amino acid in inert harp seal teeth tissues.

There were no significant differences in the $\delta^{15}N_{\text{AA}}$ values between the two GLGs (all ANOVA $p$-values > 0.1) (Fig. 3), or between the sexes (all ANOVA $p$-values > 0.1). There was considerable variability in the $\delta^{15}N$ of valine and leucine both within a single GLG and between GLGs compared to the other amino acids (Fig. 3). Leucine and valine are both non-polar amino acids and the variability in $\delta^{15}N$ is likely, at least partly, to be a result of a mismatch with the highly polar stationary phase of the GC column used.
Fig. 3. δ¹⁵N ‰ in GLGs 2 (second year of life; grey boxes) and 3 (third year of life; white boxes) of harp seal teeth for δ¹⁵N<sub>bulk</sub> and δ¹⁵N<sub>AA</sub> measurements. The boxplots indicate the median and the first and third quartiles (the 25th and 75th percentiles), the whiskers extend to the most extreme data points which are no more than 1.5 x the interquartile range, and outliers are shown as individual data points. There were no significant differences measured between the two GLGs for either the δ¹⁵N<sub>bulk</sub> measurements, or any of the seven δ¹⁵N<sub>AA</sub> analysed. Val = valine, Ala = alanine Glu = glutamic acid, Asp = aspartic acid, Leu = leucine, Phe = phenylalanine and Gly = glycine.

### 3.3 δ¹⁵N Baseline and Relative Trophic Position

There was considerable variability in δ¹⁵N of the two source amino acids, representing the δ¹⁵N at the base of the food web; δ¹⁵N<sub>Phe</sub> ranged from 8.05 to 14.7 ‰ and δ¹⁵N<sub>Gly</sub> ranged from 5.9 to 15.1 ‰, with a weakly significant relationship between the two (linear regression model; adjusted R² = 0.1, p = 0.05) (Fig. 4a). There was a strong positive relationship between GLG 2 and GLG 3 for both δ¹⁵N<sub>Phe</sub> and δ¹⁵N<sub>Gly</sub> (linear regression models; p values both < 0.01) (Fig. 4b and c). The relationship between GLGs was more variable for δ¹⁵N<sub>Gly</sub> compared to δ¹⁵N<sub>Phe</sub> (Fig. 4b and c).
Fig. 4. a) Weakly significant relationship between the two source amino acids, δ\textsuperscript{15}N\textsubscript{Phe}‰ and δ\textsuperscript{15}N\textsubscript{Gly}‰ measured in harp seal teeth GLGs with associated 95% CIs (linear regression model; adjusted $R^2 = 0.1$, $p = 0.05$) b) Significant positive relationship between δ\textsuperscript{15}N\textsubscript{Phe}‰ measured in GLGs 2 and 3 (second and third years of life) with associated 95% CIs (linear regression model: Adjusted $R^2 = 0.9$, $p < 0.001$). c) Significant positive relationship between δ\textsuperscript{15}N\textsubscript{Gly}‰ measured in GLGs 2 and 3 (second and third years of life) with associated 95% CIs (linear regression model: Adjusted $R^2 = 0.5$, $p = 0.007$).

Relative trophic position ($T_{rel}$) estimated using Phe and Gly (δ\textsuperscript{15}N\textsubscript{Glu} - δ\textsuperscript{15}N\textsubscript{Phe} and δ\textsuperscript{15}N\textsubscript{Glu} - δ\textsuperscript{15}N\textsubscript{Gly}) showed a positive relationship between GLGs (Fig. 5a and b). A large range in $T_{rel}$ values were calculated between individuals (δ\textsuperscript{15}N\textsubscript{Glu} - δ\textsuperscript{15}N\textsubscript{Phe} min = 7.18 ‰, max = 14.0 ‰, and δ\textsuperscript{15}N\textsubscript{Glu} - δ\textsuperscript{15}N\textsubscript{Gly} min = 6.23 ‰, max = 15.75 ‰). The mean $T_{rel}$ varied significantly between individuals (ANOVA: $F_{1,15} = 3.008$, $p = 0.001$; Fig. 5c). There was no evidence for a difference in relative trophic position between these sub adult males and females neither for δ\textsuperscript{15}N\textsubscript{Glu} - δ\textsuperscript{15}N\textsubscript{Phe} (ANOVA $F_{1,15} = 0.003$, $p = 0.96$), nor for δ\textsuperscript{15}N\textsubscript{Glu} - δ\textsuperscript{15}N\textsubscript{Gly} (ANOVA $F_{1,15} = 0.568$, $p = 0.46$).
Fig. 5. a) Significant positive relationship between the relative trophic position calculated as baseline corrected $\delta^{15}N$‰ ($\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$) measured in GLGs 2 and 3 (second and third years of life) of harp seals with associated 95% CIs (linear regression model: Adjusted $R^2 = 0.9$, $p < 0.001$). b) Significant positive relationship between the relative trophic position calculated as baseline corrected $\delta^{15}N$‰ ($\delta^{15}N_{Glu} - \delta^{15}N_{Gly}$) measured in GLGs 2 and 3 (second and third years of life) of harp seals with associated 95% CIs (linear regression model: Adjusted $R^2 = 0.3$, $p = 0.01$). c) Mean relative trophic position calculated as the average baseline-
corrected $\delta^{15}$N$_{\text{Glu}}$ values ($\delta^{15}$N$_{\text{Glu}} - \delta^{15}$N$_{\text{Phe}}$ and $\delta^{15}$N$_{\text{Glu}} - \delta^{15}$N$_{\text{Gly}}$) from GLG2 and GLG3 ($n = 4$) for each seal showing significant variability between individuals.

3.4 Isotopic Niche Width

Considerable overlap in the standard Bayesian ellipses calculated for the two GLGs and the sexes using both the bulk and the two baseline-corrected $\delta^{15}$N values indicated no differences in isotopic niche width (Fig. 6). Niche width across both GLGs and sexes appears to be slightly smaller using the $\delta^{15}$N$_{\text{bulk}}$ data compared to the baseline corrected, $\delta^{15}$N$_{\text{Glu}} - \delta^{15}$N$_{\text{Phe}}$ data (Fig. 6). There were no differences between the estimated niche width calculations using the two baseline-corrected $\delta^{15}$N values.

**Fig. 6.** Representation of trophic niche variation between sexes and growth layer groups (GLGs) measured by SEAc. Ellipses are drawn for each group independently and contain approximately 95% of the data for a) $\delta^{15}$N$_{\text{bulk}}$ and $\delta^{13}$C$_{\text{bulk}}$, b) baseline corrected $\delta^{15}$N ($\delta^{15}$N$_{\text{Glu}} - \delta^{15}$N$_{\text{Phe}}$) and $\delta^{13}$C$_{\text{bulk}}$, c) baseline corrected $\delta^{15}$N ($\delta^{15}$N$_{\text{Glu}} - \delta^{15}$N$_{\text{Gly}}$) and $\delta^{13}$C$_{\text{bulk}}$.

4. Discussion

4.1 Development of teeth demineralisation and subsampling methods

Here we demonstrate that the microsampling of demineralised teeth provides bulk SI data in accordance with previous studies investigating harp seal diet (Hammill et al. 2005). Considerable intra- and inter-individual variation was seen in the $\delta^{15}$N$_{\text{bulk}}$ and $\delta^{13}$C$_{\text{bulk}}$ measurements, which are likely the result of differences in diet, baseline isotope signatures, and metabolic processes. While a small sample size of just 17 seals was used for these preliminary method development investigations, the variation between individuals observed in
both bulk stable carbon and nitrogen isotopes values indicated wide isotopic niches for the population as a whole. From stomach content analyses, harp seals are known to feed on a variety of species, but the bulk of their diet is thought to be comprised of relatively few species, such as capelin (Mallotus villosus) polar cod (Boreogadus saida), herring (Clupea harengus), krill (Thysanoessa spp.) and pelagic hyperiid amphipods (e.g. Themisto libellula) (Stenson et al., 2020). We have demonstrated that our SI data from dentine GLGs are consistent with the aforementioned evidence that harp seals have wide isotopic niches irrespective of sex and age as sub-adults (Beck, Hammill and Smith, 1993; Haug et al., 2017; Lawson and Stenson 1995).

Here, we apply CSIA to phocid teeth for the first time. The $\delta^{15}$N values of both source and trophic amino acids were similar to previously published values in muscle of phocid seals ($\delta^{15}$N_Phe range 9.1 - 12.7‰; $\delta^{15}$N_Gly range 9.6 - 18.9‰; $\delta^{15}$N_Glu range 19.7 - 25.8‰ measured in harbour seals in Germain et al., 2013). Furthermore, there were no differences in $\delta^{15}$N AA between males and females, or between the second and third years of life. These results therefore support the use of teeth demineralisation and subsampling methods for CSIA. These findings are in line with a previous study on sperm whale (Physeter microcephalus) teeth (Brault et al., 2014), which demonstrated that decalcification prior to CSIA analyses of dentine avoided significant matrix-effects, and did not alter the amino acid molar composition or isotopic values of the tissue.

4.2 Use of $\delta^{15}$N_Gly as a source amino acid in harp seal dentine

Compound specific isotope analyses of amino acids are increasingly applied to studies investigating trophic structure as this method can provide increased understanding of complex ecosystems by taking into account variation in the isotopic value of the environmental baseline. $\delta^{15}$N_Phe is frequently used to represent the $\delta^{15}$N at the base of the food web. The similar range in values and the correlation between $\delta^{15}$N_Phe and $\delta^{15}$N_Gly here suggest that in harp seal dentine, $\delta^{15}$N_Gly can also be used as a reliable source amino acid, representative of the environmental baseline. This is further supported by the similar isotopic niche size estimated using the two baseline-corrected $\delta^{15}$N values, and because both $\delta^{15}$N_Phe and $\delta^{15}$N_Gly captured the variation between individuals.

The higher variability in the $\delta^{15}$N_Gly data is likely driven by the optimal peak amplitude for the GC-IRMS at conditions stated in the methods, which was ~500 to 1200 mV. Gly peaks measured in dentine were in the range of 2000 mV to 4500 mV, and therefore would have benefited from sample dilution. Concomitantly, we targeted Phe as a reliable source amino acid
and concentrated the samples to produce peaks at optimal amplitude. Variability in the relationship between GLGs (adjusted R² value of 0.5 and 0.9 for the Gly and the Phe data, respectively) is most likely a result of method optimisation rather than variability of these amino acids within the seal dentine. Gly is known to be one of the most abundant amino acids in collagen/bone/dentine (Li and Wu, 2018; Yamakoshi et al., 2005), and in this study it was ten times more abundant than Phe. Therefore, the use of Gly instead of Phe has significant implications for future CSIA of teeth samples as smaller masses of material are required for analysis. However, care needs to be taken when targeting both of these source amino acids, in order to achieve optimal measurement conditions and thus generate reliable data.

4.3 Individual consistency in the foraging patterns of sub-adult harp seals

Here, δ¹⁵N_Phe, which represents the δ¹⁵N at the base of the food web, showed variation between individuals, suggesting that there may be variation in where individual harp seals were foraging, as evidenced in a handful of telemetry studies from the Northeast Atlantic (Blanchet et al., 2018; Folkow et al., 2004; Nordøy et al., 2008). Harp seals undergo long-distance migrations, for example between the Labrador Sea and the South of Greenland, two regions which are influenced by water masses having different δ¹⁵N baselines (de la Vega et al. 2020). Specifically, there is an ~2 ‰ difference in δ¹⁵N of nitrate (de la Vega et al., 2021) between the eastern portion of the Labrador Sea, which is influenced by Atlantic water, and Baffin Bay or the Labrador Shelf, which are influenced by Pacific derived water exiting the Arctic through the Canadian Arctic Archipelago (Torres-Valdés et al., 2013). This variation in environmental baseline can explain the variability measured in the seals.

The mean relative trophic position between individual harp seals varied by up to ~ 4‰ which represents approximately 1 absolute TP difference assuming a trophic fractionation of 2.5 ‰ to 4.3 ‰ for trophic amino acids in marine tertiary and higher consumers (Germain et al. 2013, McMahon and McCarthy 2016). Variation in diet with some seals feeding on a higher proportion of zooplanktivorous capelin or amphipods, while others consume more Atlantic cod, for example, could result in the trophic position difference between harp seal individuals seen here.

The positive relationships between the second and third year of life for δ¹⁵N_Phe, δ¹⁵N_Gly and TP_rel suggest year to year consistency in both diet and foraging location in these two and three year olds. This is supported by the similar isotopic niche breadth / width, suggesting that individuals feed on the same functional groups of prey between the second and third year of
These results could reflect either differences in diet between individuals, which would suggest individual specialisation within a generalist population, a phenomenon which has been documented among a number of marine vertebrate species (Martínez del Rio et al., 2009; Hückstädt et al. 2012; Vander Zanden et al., 2010), or differences in migration patterns that could indicate foraging site fidelity and / or habitat selection. Further work should investigate if individual specialisation in terms of diet, or foraging area, persists through adult life in harp seals. Further work should also investigate if individual foraging behaviour is related to prey availability, population density, or physiological characteristics that could potentially affect the diving capacity and / or prey capture and handling ability of individuals (Wathne, et al. 2000; Ogloff, et al. 2019).

4.3 Teeth as tools: Future applications

We have demonstrated the power of using demineralisation and subsampling methods for fine temporal scale bulk isotope and CSIA in harp seal teeth, and show that they are useful tools to investigate both between and within individual variability in foraging patterns. Together, these data highlight the importance of taking into account the isotopic baseline for the correct interpretation of CSIA data that can shed light on individual predator foraging patterns. In addition, combining $\delta^{15}$N$_{Phe}$, representing the $\delta^{15}$N baseline, with $\delta^{13}$C$_{bulk}$ could help to geolocate foraging areas more precisely, if the isoscape for both $\delta^{15}$N and $\delta^{13}$C is spatially (and temporally) constrained. These data also suggest that using $\delta^{15}$N$_{Glu}$ could potentially be used to correct for baseline $\delta^{15}$N instead of $\delta^{15}$N$_{Phe}$ in seal dentine. This finding has important implications for future CSIA of teeth from archives. Specifically, investigations quantifying $\delta^{15}$N$_{Glu}$ and $\delta^{15}$N$_{Gly}$ as the trophic and source amino acids respectively, require smaller sample masses for analysis, thus permitting finer scale work on narrower GLGs from older seals. Combining these CSIA biomarkers with other measurements such as GLG thickness, which can be used as a proxy for individual growth and therefore environmental quality in any given year (Hanson et al., 2009; Knox et al., 2014), would further improve our understanding of responses to environmental and ecological changes.

In addition, these methods offer great potential for analysing biomarkers in archived inert tissues going back in time, as a powerful tool for both modern and historical reconstructions of the marine environment. Moving forward, these methods could be used on larger samples sets of teeth to investigate spatial and temporal changes in Arctic ecosystems. Importantly, using teeth as tools is especially valuable to reconstruct decadal $\delta^{15}$N and $\delta^{13}$C in the environment.
over the last century, which is difficult to achieve from the sedimentary record as a result of biological (bioturbation) and physical (winnowing/slumping) processes (LaRowe et al., 2020; Meysman et al., 2006; Collins and Balson, 2007). Furthermore, shallow sedimentary nitrogen isotope records are potentially compromised by microbial degradation of organic matter (e.g. Freudenthal et al., 2001; Möbius et al., 2010). Teeth archives may therefore provide unique, fine-scale resolution and a ‘true’ isotopic signature. Such reconstructions will allow an improved understanding of how Arctic food-webs, and other environments, have been altered over the last decades, and will directly inform model projections of how ecosystems are predicted to be continually affected by the forecasted environmental changes in the 21st century. Ultimately, using historical changes to inform modern predictions of environmental variation and species foraging ecology can highlight population resilience or susceptibility to environmental change, and help to inform management decisions to mitigate against the cumulative impacts of increased human activity.

Acknowledgements

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