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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 ($\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$), and compound-specific stable nitrogen isotopes of amino acids ($\delta^{15}N_{AA}$) for the first time. We developed a fine-scale, annual growth layer group (GLG) dentine sub-sampling method corresponding to the second and third year of life. In accordance with previous diet studies, while there was individual variability in $\delta^{15}N_{bulk}$, $\delta^{13}C_{bulk}$ and $\delta^{15}N_{AA}$ 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 $\delta^{15}N_{AA}$ 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 2 yr of life. These novel $\delta^{15}N_{AA}$ 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 3 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

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and quantify these changes and better understand how the ecosystem is being altered is a vital component of conservation-oriented management programmes 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 (Moore & Huntington 2008, Bossart 2011, Hazen et al. 2019).

Harp seals *Pagophilus groenlandicus* are an important Arctic ecosystem sentinel species (Stenson et al. 2020). Harp seals are migratory phocids, and in the Northwest Atlantic specifically, they are currently the most abundant pinniped with an estimated population size of ~7.6 million (Hammill et al. 2021). 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, given that harp seals forage 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 tooth archives present valuable opportunities to investigate harp seal diet, and by extension, the environmental variability experienced by the seals though 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 et al. 2004, Aubail et al. 2010, Juarez-Rodriguez et al. 2020). Teeth have therefore been used as quantitative monitoring tools to detect and investigate long-term ecological changes and anthropogenic threats to the environment (Boyd & Roberts 1993, Newsome et al. 2007, Outridge et al. 2009, Hanson et al. 2018). 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, Hanson et al. 2018, Hall et al. 2019). 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 & 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 1988) 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 (Keeley & Sandquist 1992, Søreide et al. 2013). $\delta^{15}N$ of bulk tissue ($\delta^{15}N_{bulk}$) increases by 2–5‰ at each trophic level, providing a continuous measure of trophic position in predators (Post 2002). However, $\delta^{15}N_{bulk}$ 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 (AAs) ($\delta^{15}N_{AA}$) in predator tissue is being increasingly applied to disentangle baseline and trophic level effects. The $\delta^{15}N$ of 'source' AAs experiences negligible fractionation during trophic transfer and conservatively traces the $\delta^{15}N$ baseline, whereas significant fractionation of 'trophic' AAs results in ^{15}N enrichment between each trophic transfer (McMahon & McCarthy 2016). This discrepancy 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 & McCarthy 2016), which, in turn, allows for more precise estimates of food chain length (Chikaraishi et al. 2009).

$\delta^{15}N_{AA}$ 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 analysis and CSIA. Extracting dentine samples from individual tooth GLGs provides the opportunity for longitudinal, fine temporal scale sampling to investigate both within- and between-individual vari-

ability. 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 2 GLGs, corresponding to the second and third years of life of individual seals. Previous studies of stomach content analyses and bulk stable isotopes suggest that there are no differences in diet between males and females, or between 2 and 3 yr old sub-adult seals (Beck et al. 1993, Lawson & Stenson 1995). Thus, to confirm that our fine temporal scale dentine extraction and stable isotope 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. MATERIALS AND METHODS

2.1. Canine tooth 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 and early 1995 were used for analysis. All individuals were 5 yr old (see ageing methods in Section 2.2.1) and were born in March 1990. Ten of the samples were collected during the winter prior to pupping in March 1995, while the other 7 were collected in the spring following the 1995 moulting period (Fig. A1 in the Appendix). Upon collection, lower jaws were collected and boiled in water for 1 h to facilitate extraction of teeth.

2.2. Tooth sectioning and sub-sampling

In order to maximise the ecological information that can be gained from a single tooth, teeth were sectioned along 2 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 CSIAs (Fig. 1).

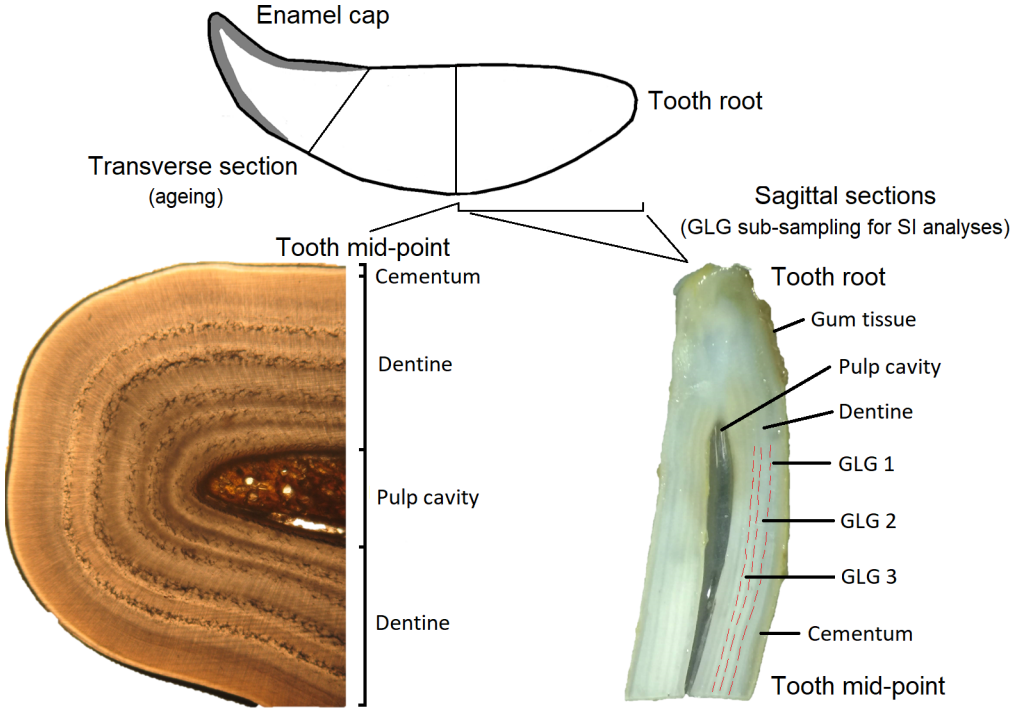

Fig. 1. Individual tooth sectioning schematic from a 5 yr old harp seal harvested in Newfoundland, Canada. Top: Each whole canine tooth was sectioned along 2 planes: transverse and sagittal. Bottom left: Transverse cross section of the tooth mid-point showing the outer cementum layer, the incremental 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

2.2.1. 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 generally consists of 3 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 mineralisation 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 and 250 µm thick were cut just below the enamel cap (Fig. 1) and examined under transmitted polarized light (6 × 50 binocular microscope). When estimating age from transverse sections, it can be difficult to define the extent of first-year 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 in Section 2.2.2. Previous work by Chua et al. (2020) has demonstrated the lack of preservation effects on CSIA results.

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 × 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, CSIAs require larger tissue masses than conventional $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ 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 µm section thickness was chosen following a number of trials at varying thicknesses, as it provided enough mass of tooth dentine for the bulk analysis and the CSIA of individual GLGs (~weighing between 3 and 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 µm thick sections were immersed in 0.25 M HCl for between 12 and 24 h. Needle-shaped crystals (presumably calcium chloride salts) formed on the surface of the sections, but they 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 at 20× magnification (2× objective and 10× 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 first year of life, was not used for analysis, as the stable isotope signature in this GLG is expected to be affected by the isotopic signature of the mother 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 stable isotope analysis. For this reason, they were not included in this study. Future studies that do not require such fine temporal scale reso-

lution in GLG subsampling could use these narrower GLGs combined for bulk and CSIA investigations.

2.3. Stable isotope analyses

2.3.1. Bulk analyses — $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$

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.

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

$$\delta^{a}X\,(‰) = [({}^{a}X/{}^{b}X)_{sample} / ({}^{a}X/{}^{b}X)_{standard} - 1] \times 1000 \quad (1)$$

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 characterised $\delta^{13}C$ 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. AA-specific analyses — $\delta^{15}N_{AA}$

GLG samples (weighing between 3 and 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^{-1}). Samples were then transferred into clean micro-reaction vessels and frozen at –80°C prior to lyophilisation. The AAs 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, reagents were evaporated under a gentle stream of N_2, and DCM was added (3 × 0.25 ml) and evaporated to remove excess reagents. AA 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_2 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 of 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 AAs were dissolved in DCM and stored at –20°C until 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 AAs was achieved using an HP Innowax capillary column (30 m × 0.25 mm i.d. × 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 $\delta^{15}N$ values of each AA peak were automatically computed (Isodat version 3.0; Thermo Fisher) by comparison with a standard reference N_2 gas, which was repeatedly measured (4×) 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 2 different dilutions. The first run was used to separate the following AAs: 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' program, 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 AA standard prepared from 8 AAs with known $\delta^{15}N$ values (University of Indiana, and SI Science Japan). The mixed standard was analysed 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 AAs 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:

$$\delta^{15}N_{\text{sample reported}} = \text{avg } \delta^{15}N_{\text{sample measured}} - \delta^{15}N_{\text{standard measured}} - \delta^{15}N_{\text{known}} \quad (2)$$

where avg $\delta^{15}N_{\text{sample measured}}$ is the average $\delta^{15}N$ for an AA in a sample (n = 2), $\delta^{15}N_{\text{standard measured}}$ is the $\delta^{15}N$ for the AA in the nearest mixed standard, and $\delta^{15}N_{\text{known}}$ is the known elemental analysed offline value for the same standard.

2.4. Statistical analyses

2.4.1. $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$

To determine which factors best explained the variation in the $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ data, we specified linear mixed effect models ('glmer' function in the 'lme4' package v.1.1-21, Bates et al. 2015; R v.3.6.2, R Core Team 2019), with individual as a random effect to take into account the repeat sampling of 2 GLGs for each animal. The covariates used in the model were sex, GLG (2 or 3) and the $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ values for the $\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$ models, respectively. The global $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ models, separately, included all covariates, and backwards model selection using the 'dredge' function ('MuMIn' package v0.12.2/r18, Barton et al. 2009) 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 ('car' package v3.0-6, Fox & Weisberg 2019) were used to assess the relationship between the $\delta^{15}N_{bulk}$ values in GLGs 2 and 3, and between the $\delta^{13}C_{bulk}$ values in GLGs 2 and 3. Statistical significance for all models was considered at $p < 0.05$.

2.4.2. $\delta^{15}N_{AA}$ data

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

2.4.3. Relative trophic position determination

Several source AAs can be used to trace the baseline $\delta^{15}N$. Phe is typically used in most studies to reconstruct the baseline and estimate trophic position (TP). Gly has also been considered as a source AA, but its $\delta^{15}N$ can be strongly affected by microbial degradation (McMahon & McCarthy 2016) and so should be treated with caution depending on the tissue type and sample storage conditions (Nielsen et al. 2015). Here, we used the $\delta^{15}N$ of Phe ($\delta^{15}N_{Phe}$) as a source AA, and tested the potential use of Gly ($\delta^{15}N_{Gly}$) as a source AA 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 2 GLGs.

We used $\delta^{15}N$ of Glu ($\delta^{15}N_{Glu}$) to estimate TP. Glu is the most abundant AA in a consumer's tissue, and is considered as the canonical trophic AA while all of the other trophic AAs are related to the central nitrogen pool via Glu (McMahon & McCarthy 2016). Glu 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' AAs across taxa in entire food webs prevents accurate estimation of the absolute TP of an organism (Nielsen et al. 2015). To compare the 2 baseline AAs (Phe and Gly), we calculated relative TP (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 TP_{rel} values to determine if values in the second year of life were related to those in the third. To compare TP_{rel} between individual seals, we used a 1-way ANOVA with seal as a factor variable and the mean TP_{rel} as the explanatory variable. Mean 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). All analyses were considered statistically significant at $p < 0.05$.

2.4.4. Trophic niche estimation

We determined isotopic niche spaces for each sex and in each GLG using the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011). Standard Bayesian ellipses were calculated from $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ values, $\delta^{15}N_{Glu\text{-}Phe}$ and $\delta^{13}C_{bulk}$ values, and $\delta^{15}N_{Glu\text{-}Gly}$ 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 $\delta^{13}C_{bulk}$ 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 $\delta^{15}N_{bulk}$ or the $\delta^{13}C_{bulk}$ 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 $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ values between males and females or between GLGs 2 and 3 (Fig. 2). There was no significant relationship between the $\delta^{15}N_{bulk}$ and the $\delta^{13}C_{bulk}$ data (linear regression model; $p > 0.5$). There was also no relationship between the $\delta^{15}N_{bulk}$ values in GLGs 2 and 3, or between the $\delta^{13}C_{bulk}$ values in GLGs 2 and 3 (linear regression models; all $p > 0.5$).

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

Average values of $\delta^{15}N$ were between ~18.5 and ~25.0‰ for trophic AAs (Val, Ala, Glu, Asp, Leu), and

Table 1. Published $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ values (mean ± SD unless otherwise stated) measured in harp seals and other Arctic phocids

Species	Location	Tissue	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)	Source
Harp seal *Pagophilus groenlandicus*	Gulf of St Lawrence, Canada	Muscle	13.5 ± 0.7	−18.0 ± 0.5	Hammill et al. (2005)
	Svalbard, Norway	Muscle	12.1 ± 0.8	−21.1 ± 0.4	Haug et al. (2021)
	Newfoundland, Canada[a]	Muscle	14.1 ± 1.0	−17.8 ± 0.5	Lawson & Hobson (2000)
	Southern Barents Sea, Russia	Muscle	15.07 ± 0.6	−19.37 ± 0.3	Haug et al. (2017)
	Newfoundland and Labrador, Canada	Teeth	14.9 ± 1.1	−15.2 ± 0.3	This study
Ringed seal *Phoca hispida*	Central West Greenland	Teeth	16.35 ± 1.0	−16.04 ± 0.5	Aubail et al. (2010)
	Central East Greenland	Teeth	14.90 ± 1.1	−17.23 ± 0.5	Aubail et al. (2010)
	Beaufort Sea, Alaska, USA	Muscle	16.9 ± 0.6	−18.5 ± 0.8	Hoekstra et al. (2002), Dehn et al. (2007)
	Northwest Territories, Canada	Muscle	17.2 ± 0.7	−20.4 ± 0.4	Dehn et al. (2007)
	Central West Greenland	Muscle	17.0 ± 0.1	−19.4 ± 0.1	Hobson et al. (2002)
	Nunavut, Canada	Muscle	17.3 ± 1.1	−17.3 ± 0.7	Hobson & Welch (1992)
	Hudson Bay, Canada	Muscle	13.9 ± 1.4	−19.7 ± 0.9	Muir et al. (1995)
	Beaufort Sea, Canada	Claws	17.6 ± 1.0	−17.9 ± 0.6	Boucher et al. (2020)
	Bering and Chukchi Seas, Alaska, USA	Claws	15.0–19.4 (range)	−21.1 to −14.6 (range)	Carroll et al. (2013)
	Bering and Chukchi Seas, Alaska, USA (1953–1968)	Claws	17.5 ± 0.6	−15.6 ± 0.5[b]	Crain et al. (2021)
	Bering and Chukchi Seas, Alaska, USA (1998–2014)	Claws	17.1 ± 0.8	−17.2 ± 1.2[b]	Crain et al. (2021)
Bearded seal *Erignathus barbatus*	Beaufort Sea, Alaska, USA	Muscle	16.8 ± 0.9	−17.1 ± 0.5	Hoekstra et al. (2002), Dehn et al. (2007)
	Northwest Territories, Canada	Muscle	16.8 ± 0.1	−16.6 ± 0.3	Hobson et al. (2002)
	Bering and Chukchi Seas, Alaska, USA	Claws	14.6–18.2 (range)	−18.3 to −13.7 (range)	Carroll et al. (2013)
	Bering and Chukchi Seas, Alaska, USA (1953–1968)	Claws	15.4 ± 0.6	−14.7 ± 0.5[b]	Crain et al. (2021)
	Bering and Chukchi Seas, Alaska, USA (1998–2014)	Claws	15.9 ± 0.7	−15.5 ± 0.7[b]	Crain et al. (2021)
Spotted seal *Phoca largha*	Bering and Chukchi Seas, Alaska, USA	Muscle	17.8 ± 1.0	−18.5 ± 0.9	Dehn et al. (2007)
Ribbon seal *Phoca fasciata*	Bering and Chukchi Seas, Alaska, USA	Muscle	16.0 ± 1.2	−18.7 ± 0.1	Dehn et al. (2007)

[a]Sub-adults specifically; [b]Suess-corrected to account for increased fractionation of carbon due to increased use of fossil fuels from 1850 to present day

Table 2. Result of linear mixed effects (LME) model selection for $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ showing the 3 best-fitting models with the lowest values of second-order Akaike's information criterion corrected for small sample size (AICc). GLG: growth layer group

LME model	Model covariates	df	AICc	ΔAICc	Weight
1	$\delta^{15}N_{bulk}$ ~	3	112.3	0.0	0.27
2	$\delta^{15}N_{bulk}$ ~ Sex	4	114.3	2.07	0.10
3	$\delta^{15}N_{bulk}$ ~ Sex + GLG	5	114.6	2.39	0.08
1	$\delta^{13}C_{bulk}$ ~	2	24.0	0.0	0.38
2	$\delta^{13}C_{bulk}$ ~ Sex	3	26.3	2.34	0.12
3	$\delta^{13}C_{bulk}$ ~ $\delta^{15}N_{bulk}$	3	26.4	2.41	0.11

between ~10.5 and ~11.5‰ for the 2 source AAs (Phe and Gly; Fig. 3). $\delta^{15}N_{Gly}$ values were similar to $\delta^{15}N_{Phe}$ (ANOVA; $F_{1,32}$ = 2.7, p = 0.11), demonstrating that Gly can potentially be considered as an alternative to Phe, and used as a source AA in inert harp seal tooth tissues. There were no significant differences in the $\delta^{15}N_{AA}$ values between the 2 GLGs (all ANOVA p > 0.1) (Fig. 3), or between the sexes (all ANOVA p > 0.1). There was considerable variability in the $\delta^{15}N$ of Val and Leu both within a single GLG and between GLGs compared to the other AAs (Fig. 3). Leu and Val are both non-polar AAs, 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.

3.3. $\delta^{15}N$ baseline and TP_{rel}

There was considerable variability in $\delta^{15}N$ of the 2 source AAs, representing the $\delta^{15}N$ at the base of the food web; $\delta^{15}N_{Phe}$ ranged from 8.05 to 14.7‰ and $\delta^{15}N_{Gly}$ ranged from 5.9 to 15.1‰, with a weakly significant relationship between them (linear regression model; adjusted R^2 = 0.1, p = 0.05) (Fig. 4a). There was a strong positive relationship between GLG 2 and GLG 3 for both $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Gly}$ (linear regression models; both p < 0.01) (Fig. 4b,c). The relationship between GLGs was more variable for $\delta^{15}N_{Gly}$ compared to $\delta^{15}N_{Phe}$ (Fig. 4b,c).

TP_{rel} estimated using Phe and Gly ($\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu} - \delta^{15}N_{Gly}$) showed a positive relationship between GLGs (Fig. 5a,b). A large range in TP_{rel} values were calculated between individuals ($\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ min = 7.18‰, max = 14.0‰, and $\delta^{15}N_{Glu} - \delta^{15}N_{Gly}$ min = 6.23‰, max = 15.75‰). The mean TP_{rel} varied significantly between individuals (ANOVA: $F_{1,15}$ = 3.008, p = 0.001; Fig. 5c). There was no evidence for a difference in TP_{rel} between these sub-adult males and females either for $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ (ANOVA $F_{1,15}$ = 0.003, p = 0.96) or for $\delta^{15}N_{Glu} - \delta^{15}N_{Gly}$ (ANOVA $F_{1,15}$ = 0.568, p = 0.46).

3.4. Isotopic niche width

Considerable overlap in the standard Bayesian ellipses calculated for the 2 GLGs and the sexes

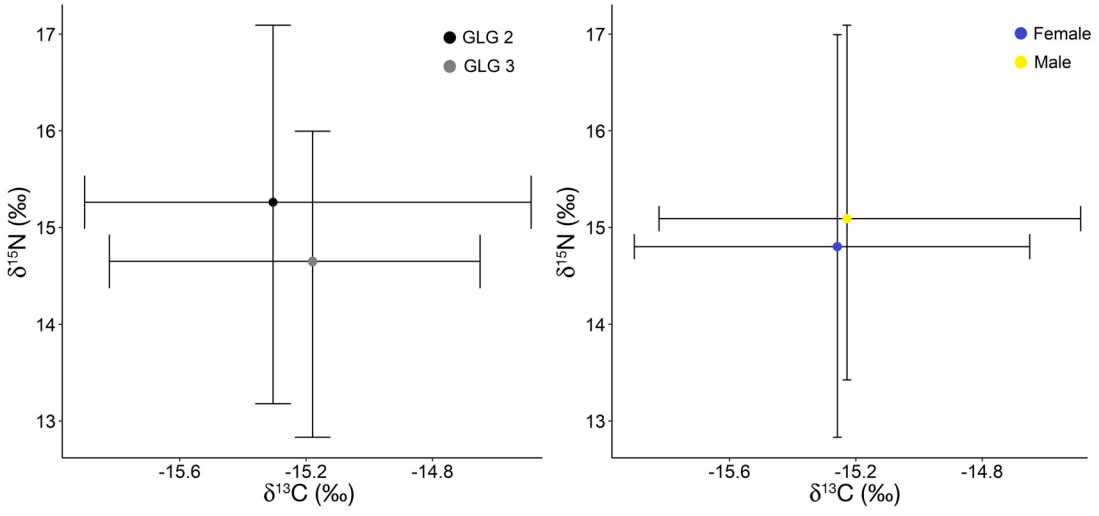

Fig. 2. (a) Mean, maximum and minimum $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ values measured for each growth layer group (GLG). There was no overall difference between GLGs 2 and 3, representing the second and third year of life of the harp seals (n = 17). (b) Mean, maximum and minimum $\delta^{15}N_{bulk}$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

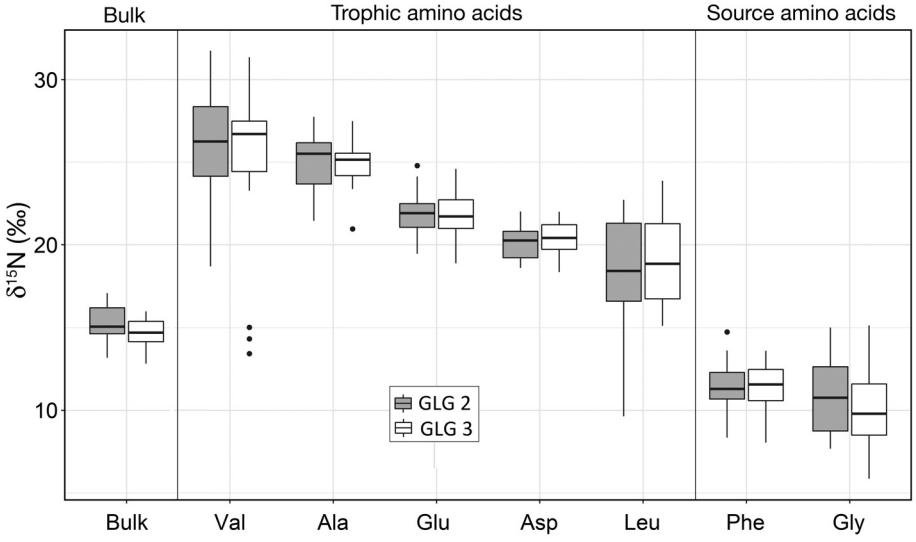

Fig. 3. $\delta^{15}N$ (‰) in growth layer groups (GLGs) 2 (second year of life; grey boxes) and 3 (third year of life; white boxes) of harp seal teeth for $\delta^{15}N_{bulk}$ and $\delta^{15}N_{AA}$ measurements. The boxplots indicate the median and the first and third quartiles (25th and 75th percentiles), the whiskers extend to the most extreme data points which are no more than 1.5× the interquartile range, and outliers are shown as individual data points. There were no significant differences measured between the 2 GLGs for either the $\delta^{15}N_{bulk}$ measurements, or any of the 7 $\delta^{15}N_{AA}$ analysed. Val: valine; Ala: alanine; Glu: glutamic acid; Asp: aspartic acid; Leu: leucine; Phe: phenylalanine; Gly: glycine

using both the bulk and the 2 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 $\delta^{15}N_{bulk}$ data compared to the baseline corrected, $\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ data (Fig. 6). There were no differences between the estimated niche width calculations using the 2 baseline-corrected $\delta^{15}N$ values.

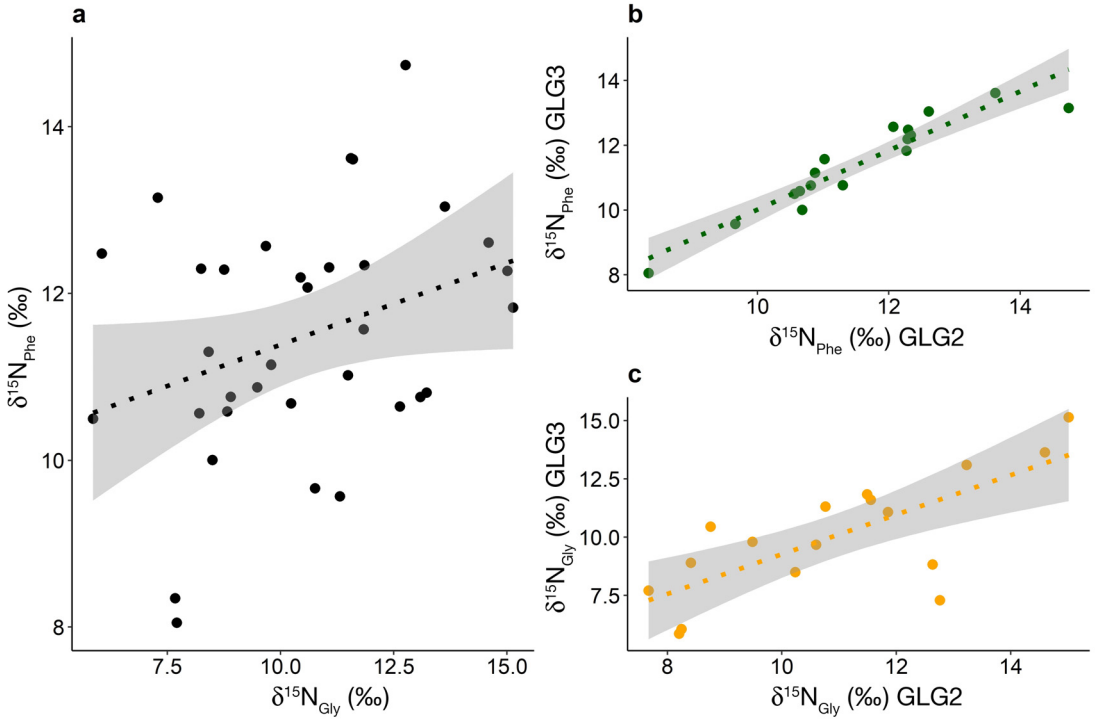

Fig. 4. (a) Weakly significant relationship between the 2 source amino acids, $\delta^{15}N_{Phe}$ ‰ and $\delta^{15}N_{Gly}$ ‰, measured in harp seal teeth growth layer groups (GLGs) with associated 95% CIs (linear regression model; adjusted $R^2 = 0.1$, $p = 0.05$). Phe: phenylalanine; Gly: glycine. (b) Significant positive relationship between $\delta^{15}N_{Phe}$ ‰ 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 $\delta^{15}N_{Gly}$ ‰ measured in GLGs 2 and 3 with associated 95% CIs (linear regression model: adjusted $R^2 = 0.5$, $p = 0.007$)

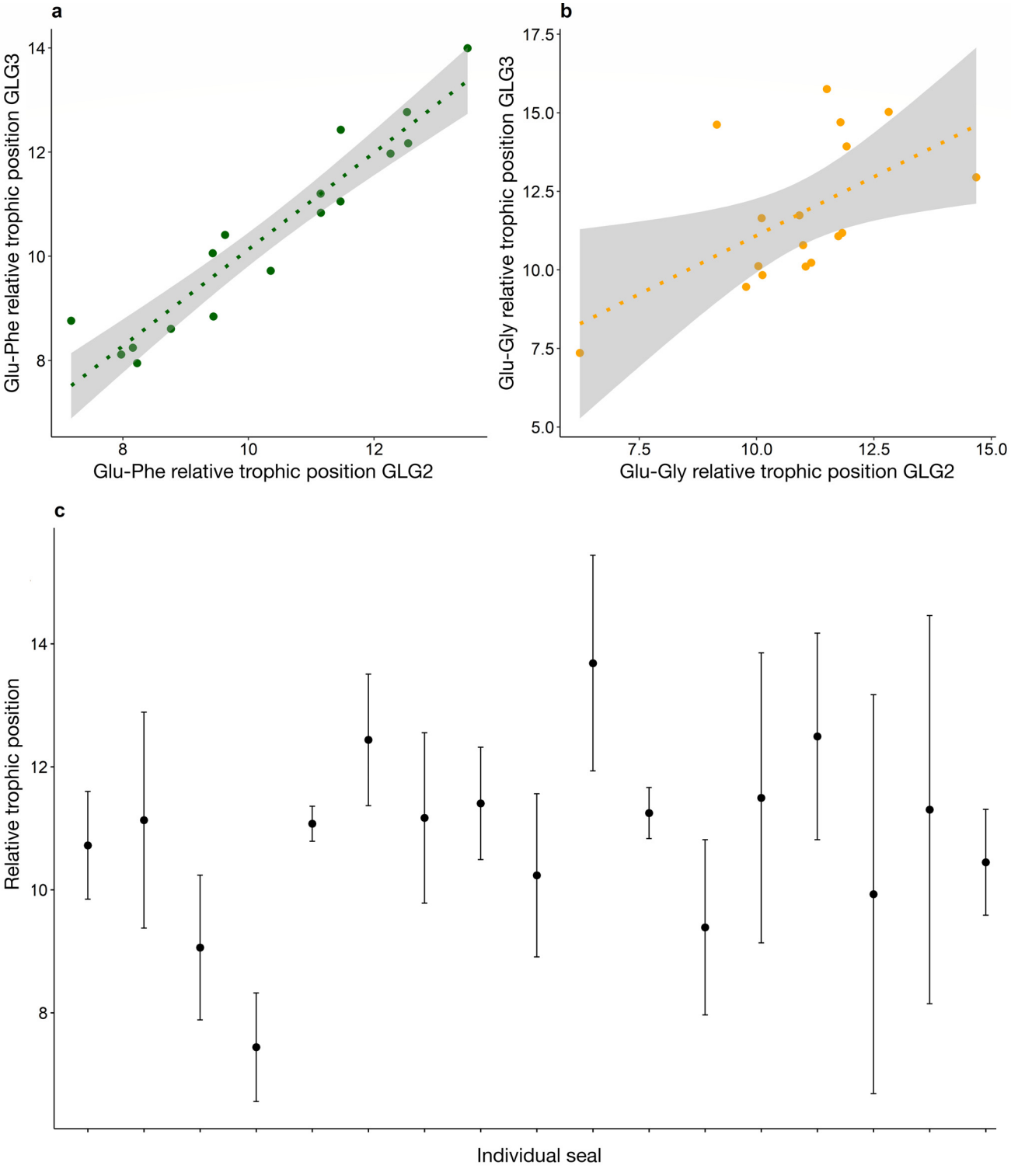

Fig. 5. (a) Significant positive relationship between the relative trophic position calculated as baseline corrected $\delta^{15}N$ ‰ ($\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$) measured in growth layer groups (GLGs) 2 and 3 (second and third years of life) of harp seals with associated 95 % CIs (linear regression model: adjusted $R^2 = 0.9$, $p < 0.001$). Glu: glutamic acid; Phe: phenylalanine. (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 with associated 95 % CIs (linear regression model: adjusted $R^2 = 0.3$, $p = 0.01$). Gly: glycine. (c) Mean ± SD relative trophic position calculated as the average baseline-corrected $\delta^{15}N_{Glu}$ values ($\delta^{15}N_{Glu} - \delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu} - \delta^{15}N_{Gly}$) from GLG2 and GLG3 (n = 4) for each seal showing significant variability between individuals

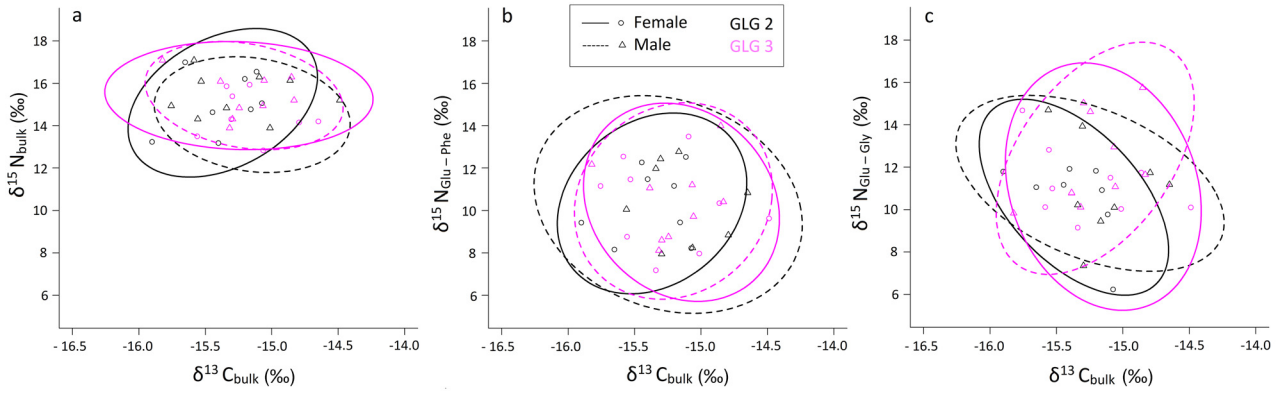

Fig. 6. Trophic niche variation between sexes and growth layer groups (GLGs) measured by standard ellipse area corrected for small sample sizes (SEAc). Ellipses are drawn for each group independently and contain ~95% of the data for (a) $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$, (b) baseline-corrected $\delta^{15}N$ ($\delta^{15}N_{Glu\ -\ Phe}$) and $\delta^{13}C_{bulk}$ and (c) baseline-corrected $\delta^{15}N$ ($\delta^{15}N_{Glu\ -\ Gly}$) and $\delta^{13}C_{bulk}$. Glu: glutamic acid; Phe: phenylalanine; Gly: glycine

4. DISCUSSION

4.1. Development of tooth demineralisation and subsampling methods

Here we demonstrate that the microsampling of demineralised teeth provides bulk stable isotope data in accordance with previous studies investigating harp seal diet (Hammill et al. 2005). Considerable intra- and inter-individual variation was seen in the $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ 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 stable isotope 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 et al. 1993, Lawson & Stenson 1995, Haug et al. 2017).

Here, we applied CSIA to phocid teeth for the first time. The $\delta^{15}N_{AA}$ values of both source and trophic AAs 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 *Phoca vitulina* by 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 tooth 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 of dentine avoided significant matrix effects, and did not alter the AA molar composition or isotopic values of the tissue.

4.2. Use of $\delta^{15}N_{Gly}$ as a source AA in harp seal dentine

CSIA of AA is 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 AA, representative of the environmental baseline. This is further supported by the similar isotopic niche size estimated using the 2 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 ~500–1200 mV. Gly peaks measured in dentine were

in the range of 2000–4500 mV, and therefore would have benefited from sample dilution. Concomitantly, we targeted Phe as a reliable source AA and concentrated the samples to produce peaks at optimal amplitude. Variability in the relationship between GLGs (adjusted R^2 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 AAs within the seal dentine. Gly is one of the most abundant AAs in collagen/bone/dentine (Yamakoshi et al. 2005, Li & Wu 2018), and in this study it was 10 times more abundant than Phe. Therefore, the use of Gly instead of Phe has significant implications for future CSIA of tooth samples, as smaller masses of material are required for analysis. However, care needs to be taken when targeting both of these source AAs, 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, $\delta^{15}N_{Phe}$, which represents the $\delta^{15}N$ 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 (Folkow et al. 2004, Nordøy et al. 2008, Blanchet et al. 2018). Harp seals undergo long-distance migrations, for example between the Labrador Sea and the South of Greenland, 2 regions which are influenced by water masses having different $\delta^{15}N$ baselines (de la Vega et al. 2021). Specifically, there is a ~2‰ difference in $\delta^{15}N$ 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 TP_{rel} between individual harp seals varied by up to ~4‰, which represents ~1 absolute TP difference assuming a trophic fractionation of 2.5–4.3‰ for trophic AAs in marine tertiary and higher consumers (Germain et al. 2013, McMahon & 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 TP difference between harp seal individuals seen here.

The positive relationships between the second and third year of life for $\delta^{15}N_{Phe}$, $\delta^{15}N_{Gly}$ and TP_{rel} suggest year to year consistency in both diet and foraging location in these 2 and 3 yr old animals. This supposition 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 life. 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, Vander Zanden et al. 2010, Hückstädt et al. 2012), 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.

4.4. Teeth as tools: future applications

We have demonstrated the power of using demineralisation and subsampling methods for fine temporal scale bulk isotope analysis 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 $\delta^{15}N_{Gly}$ 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 AAs, 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 (Meysman et al. 2006, Collins & Balson 2007, LaRowe et al. 2020). 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). Tooth 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.

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Appendix. Additional data

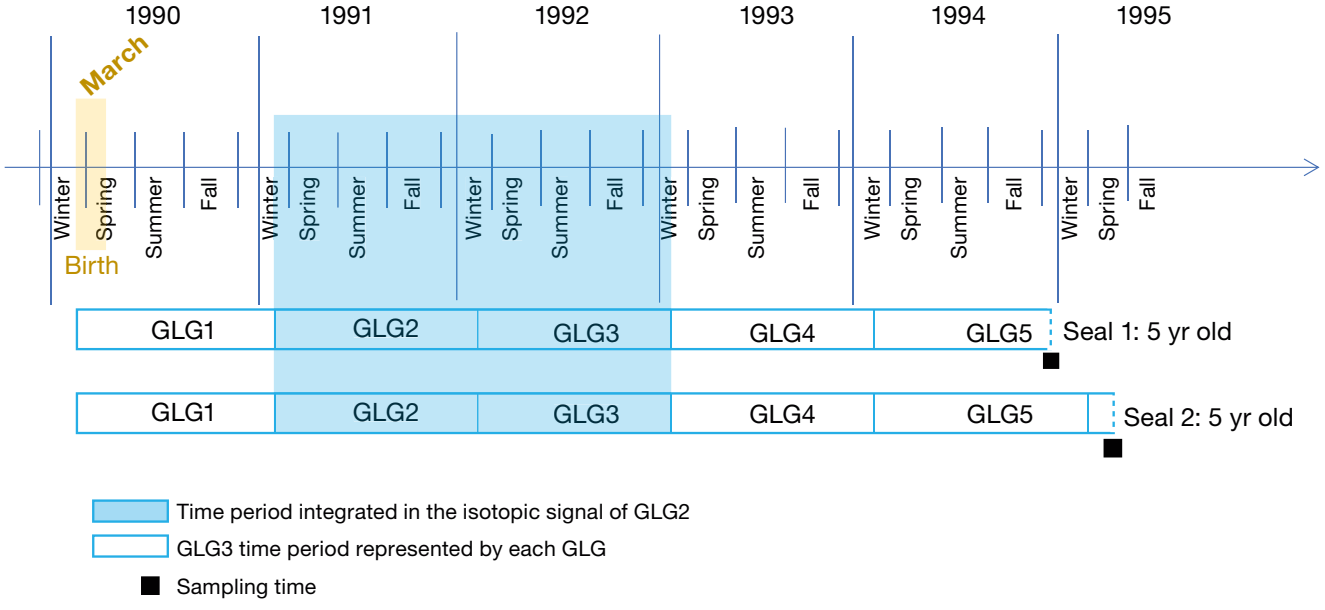

Fig. A1. Harp seal dentine samples, representing the individual growth layer groups (GLGs) for the second (GLG 2, deposited through 1991) and third (GLG 3, deposited trough 1992) years of life

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