Compound Specific Isotope Analyses of Harp Seal Teeth: Tools for Trophic Ecology Reconstruction

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29 **Abstract**

30 As sentinels of ecosystem health, high trophic level predators integrate information through all
31 levels of the food web. Their tissues can be used to investigate spatiotemporal variability in
32 foraging behaviour, and with the appropriate analytical methods and tools, archived samples
33 can be used to reconstruct past trophic interactions. Harp seal (*Pagophilus groenlandicus*) teeth
34 collected in the 1990s from the Northwest Atlantic were analysed for bulk stable carbon and
35 nitrogen isotopes ($\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$), and compound specific stable nitrogen isotopes of
36 amino acids ($\delta^{15}N_{AA}$) for the first time. We developed a fine-scale, annual growth layer group
37 (GLG) dentine sub-sampling method corresponding to their second and third year of life. In
38 accordance with previous diet studies, while there was individual variability in $\delta^{15}N_{bulk}$,
39 $\delta^{13}C_{bulk}$, and $\delta^{15}N_{AA}$ measurements, we did not detect significant differences in isotopic niche
40 widths between males and females, or between GLGs. Relative trophic position was calculated
41 as the baseline corrected $\delta^{15}N_{AA}$ values using trophic (glutamic acid) and source (phenylalanine
42 and glycine) amino acids. Variability was measured *between* individuals in their relative
43 trophic position, but *within* individual variability was low, suggesting that they fed at the same
44 trophic level over these two years of life. These novel $\delta^{15}N_{AA}$ data may therefore suggest
45 individual, specialist harp seal foraging behaviour in sub-adults. Our findings show that
46 compound specific stable isotope signatures of archived, inert predator tissues can be used as
47 tools for the retrospective reconstruction of trophic interactions on broad spatiotemporal scales.

48

49 **Key Words**

50 Phocid seals, foraging specialisation, isotopic niche, trophic position, diet, dentine, inert
51 tissues

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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 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

90 therefore allow retrospective studies of diet and contaminant exposure of individuals (e.g.
91 Hirons et al. 2001, Zhao and Schell 2004, Ferreira et al. 2011, Carroll et al. 2013, Matthews
92 and Ferguson 2015). Teeth have therefore been used as quantitative monitoring tools to detect
93 and investigate long-term ecological changes and anthropogenic threats to the environment
94 (Boyd and Roberts 1993; Outridge, et al. 2009; Hanson, et al. 2017). In pinnipeds, teeth are
95 also routinely used to age individuals by counting annual growth layer groups (GLGs) in the
96 dentine and/or cementum (Bowen et al. 1983; Frie et al., 2011; Hall et al., 2019; Hanson et al.,
97 2017). With the appropriate methods, analysing biomarkers in these metabolically inert, annual
98 growth layers of dentine in known-age animals therefore provides great potential to study
99 temporal ecological changes (Hobson and Sease 1998).

100 Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes are commonly used as tracers to reconstruct
101 food webs to investigate ecological change. $\delta^{13}C$ of bulk tissue generally exhibits minimal
102 fractionation (< 1.5‰ with each trophic level) (Fry et al. 1984) and can be used to determine
103 the origin of food sources in terms of inshore/offshore gradients, the identification of areas of
104 higher productivity (Ceia, et al. 2018), and the identification of marine, ice or terrestrially
105 derived matter (Søreide, et al. 2013; Boutton 1991; Keeley and Sandquist 1992). $\delta^{15}N$ of bulk
106 tissue ($\delta^{15}N_{bulk}$) increases by 2-5 ‰ at each trophic level, providing a continuous measure of
107 trophic position in predators (Post, 2002). However, $\delta^{15}N_{bulk}$ is influenced by changes in $\delta^{15}N$
108 in nutrient inputs at the base of the food web, or “baseline” (Chikaraishi et al., 2009).
109 Compound specific isotope analysis (CSIA) of nitrogen of amino acids (AA) ($\delta^{15}N_{AA}$) in
110 predator tissue is being increasingly applied to disentangle baseline and trophic level effects.
111 The $\delta^{15}N$ of “source” amino acids experiences negligible fractionation during trophic transfer
112 and conservatively traces the $\delta^{15}N$ baseline, whereas significant fractionation of “trophic”
113 amino acids results in ^{15}N enrichment between each trophic transfer (McMahon and McCarthy,
114 2016). This is important when determining variability in diet as an indicator of environmental
115 change because it can distinguish between true changes in trophic position of a predator, or an
116 overall change in the ^{15}N of the environmental baseline (McMahon and McCarthy, 2016). This,
117 in turn, allows for more precise estimates of food chain length (Chikaraishi et al., 2009).

118 $\delta^{15}N_{AA}$ is a powerful technique for quantifying changes in food webs, but relies on access to
119 larger quantities of tissue/material than the more widely used bulk isotope analyses. Here, we
120 provide a proof-of-concept study extending previous CSIA methods applied to marine mammal
121 teeth to an Arctic phocid for the first time. Despite the small size of the canines in this species,
122 we were able to extract individual GLGs to provide sufficient material for both bulk and CSIA

123 analysis. Extracting dentine samples from individual tooth GLGs provides the opportunity for
124 longitudinal, fine temporal scale sampling to investigate both within and between individual
125 variability. Thus, we aimed to establish a method that can be extended in future studies to
126 maximise the ecological information available from teeth as a potential 'archive' of data used
127 to monitor and interpret change in Arctic and subarctic ecosystems. Such studies could
128 ultimately subsample teeth spanning multiple decades, as well as multiple GLGs to cover the
129 full life-span of individual seals. These methods are therefore of interest for ongoing efforts to
130 investigate past environmental conditions and thus characterise long-term changes in marine
131 ecosystems.

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

142 ## 2. Methods

143 ### 2.1 Canine Teeth Collection

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

151 ### 2.2 Teeth Sectioning and Sub-Sampling

152 In order to maximise the ecological information that can be gained from a single tooth, they
153 were sectioned along two planes: transverse and sagittal (Fig. 1). The teeth were cross sectioned

154 using a precision low speed diamond saw (Buehler, Isomet^{TM}). Transverse sections were used
155 for ageing (Bowen et al., 1983) and sagittal sections were used for dentine GLG sub-sampling
156 for bulk and compound-specific stable isotope analyses (Fig. 1).

157 **2.2.1 Transverse Section for Ageing**

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

166 The 17 canine teeth were aged upon collection following methods developed by Bowen *et al.*
167 (1983) by a single experienced reader. Briefly, transverse sections between 200 – 250 µm thick
168 were cut just below the enamel cap (Fig. 1) and examined under transmitted polarized light (6
169 x 50 binocular microscope). When estimating age from transverse sections, it can be difficult
170 to define the extent of the first year's growth in the dentine as often there are accessory opaque
171 incremental growth layers in the translucent dentine which could be wrongly counted. For this
172 reason, the neonatal line is an important reference when determining the extent of the first
173 annual GLG as it acts as a marker for where to start counting (Bowen et al. 1983), and was
174 used here to aid in the ageing process. The remaining part of each tooth was stored in a solution
175 of equal parts of water, 70 % ethanol, and glycerine before they were removed, manually dried,
176 and processed for further sectioning and analyses in 2019 described below. Previous work by
177 Chua and colleagues has demonstrated the lack of preservation effects on CSIA results (Chua
178 et al., 2020).

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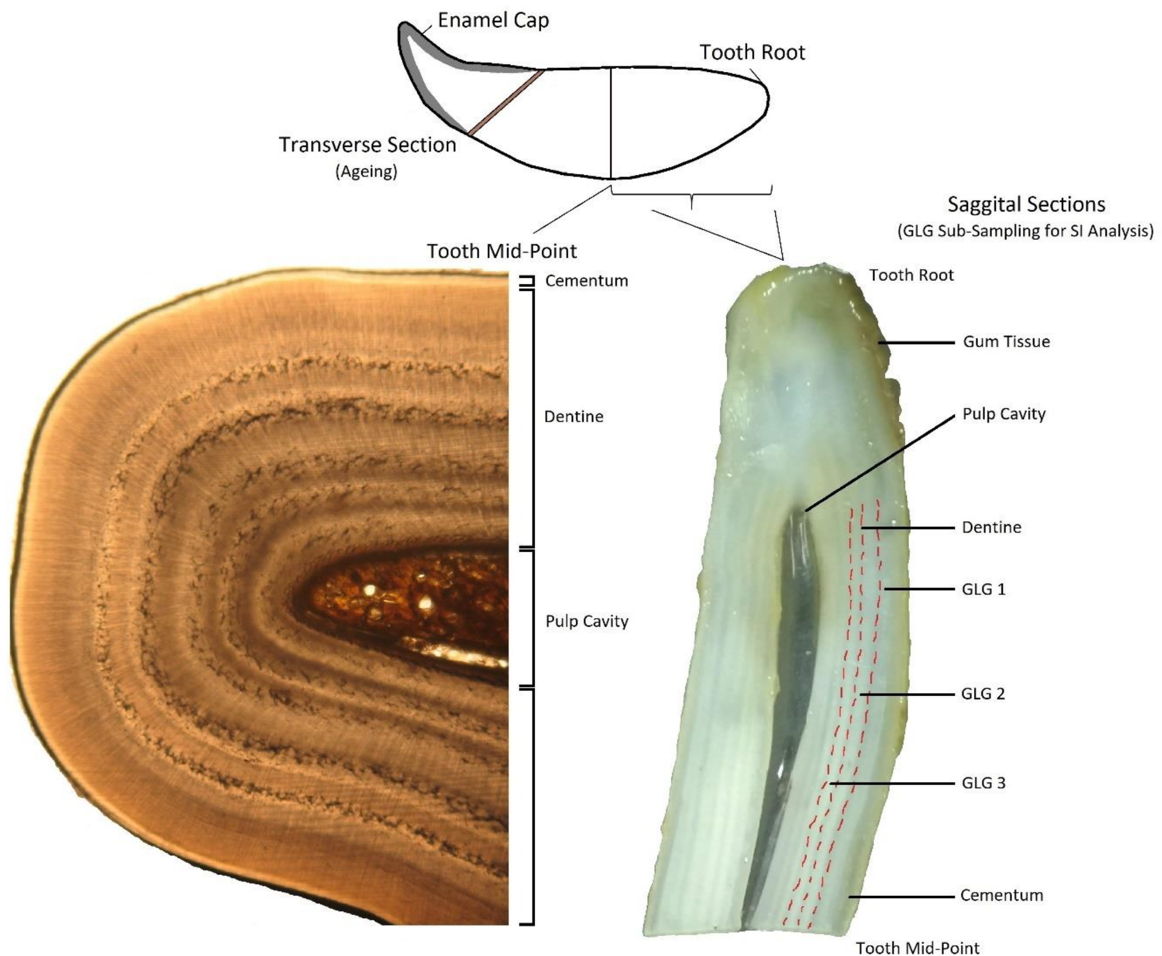


180 **Fig. 1.** Individual tooth sectioning schematic from a 5 year old harp seal harvested in
181 Newfoundland, Canada. **Top:** Each whole canine tooth was sectioned along two planes;
182 transverse and sagittal. **Bottom Left:** Transverse cross section of the tooth mid-point
183 showing the outer cementum layer, the incremental dentine growth layer groups (GLGs)
184 and the pulp cavity. **Bottom Right:** Sagittal sections (rotated 90^0 here for demonstration
185 purposes) from the mid-point of the tooth to the root were used to subsample individual
186 dentine GLGs indicated by the dashed red lines.

187

188 **2.2.2 Sagittal Sections and GLG Subsampling**

189 A second transverse cut was made along the maximum circumference at the mid-point of the
190 tooth (Fig. 1). The point of maximum circumference was measured using callipers, and marked
191 on the tooth for alignment with the saw for sectioning. The remaining part of the tooth including
192 the root was mounted onto a ~2 cm x 2 cm piece of plexiglass with superglue and left to dry
193 overnight. Two 700 µm thick sagittal sections were then cut as close as possible to the central

194 plane of the tooth (Fig. 1). Typically, compound specific stable isotope analyses require larger 195 tissue masses than conventional $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ protocols. For this reason, when aiming 196 to subsample the teeth at such a fine, annual scale to separate individual GLGs, it was important 197 to find the balance between large enough sample masses and precision in defining the GLG 198 boundaries for accurate sub-sampling. The 700 µm section thickness was chosen following a 199 number of trials at varying thicknesses as it provided enough mass of tooth dentine for the bulk 200 and the CSIA of individual GLGs (~weighing between 3 – 10 mg), but was not so thick that 201 the delineations of each GLG were obscured under transmitted light microscopy.

202 In order to accurately sub-sample the individual GLGs, the sections were de-mineralised to 203 remove bioapatite from the tooth matrix, causing them to soften and allow sampling. The 700 204 µm thick sections were immersed in 0.25 M HCl for between 12 and 24 hours. Neoformed, 205 needle-shaped crystals (presumably calcium chloride salts) formed on the surface of the 206 sections but were easily removed by rinsing thoroughly with de-ionised water. This procedure 207 allowed the sections to become soft enough to sub-sample with a scalpel, but did not cause 208 them to lose so much rigidity and/or structural integrity that the GLGs were no longer 209 distinguishable when viewed under transmitted light x 20 magnification (2x objective and 10x 210 oculars). Once softened, any remaining gum tissue and cementum was cut away from the outer 211 edge of the tooth (Fig. 1).

212 Using a scalpel, cutting from the middle part of the tooth towards the root, the sections 213 were cut along the opaque layers that separate the first, second and third years of life of 214 the individual (Fig. 1). Care was taken while sub-sampling not to desiccate the sections as 215 they become translucent and the GLGs are not as easily distinguishable. Dentine samples 216 representing the individual GLGs for the second (GLG 2, deposited through 1991) and 217 third (GLG 3, deposited through 1992) years of life were lyophilised and stored in plastic 218 vials until stable isotope analysis. The first GLG, representing the individual's first year 219 of life, was not used for analysis as the stable isotope signature in this GLG is expected to 220 be affected by the mother's isotopic signature transferred to the pup through both gestation 221 and lactation. Due to the narrowing of GLGs with increasing age (Fig. 1), the fourth and 222 fifth GLGs could not be precisely separated whilst also maintaining the minimum sample 223 mass required for SI analysis. For this reason, they were not included in this study. Future 224 studies that do not require such fine temporal scale resolution in GLG subsampling could 225 use these narrower GLGs combined for bulk and CSIA investigations.

226 **2.3 Stable Isotope Analyses**

227 **2.3.1 Bulk Analyses - $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$**

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

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

233 Eq. 1: $\delta^{a}X$ (‰) = $(({}^{a}X/{}^{b}X)_{sample} / ({}^{a}X/{}^{b}X)_{standard} - 1) \times 1000$

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

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

240 **2.3.2 Amino Acid Specific Analyses - $\delta^{15}N_{AA}$**

241

242 **Sample Preparation:** GLG samples (weighing between 3 – 10 mg) were hydrolyzed in
243 reaction vessels (6M, 1 mL, 100°C for 22 h). L-Norleucine (Sigma-Aldrich) was added to each
244 sample as an internal standard (80 µl of 5 mg/mL). Samples were then transferred into clean
245 micro-reaction vessels and were frozen at -80°C prior to lyophilization. The amino acids were
246 propylated in 0.25 mL of acidified isopropanol solution (prepared by addition of acetyl chloride
247 to anhydrous isopropanol (1:4 v/v) in an ice bath) at 100°C for 1 h. The reaction was quenched
248 in a freezer and reagents were evaporated under a gentle stream of N_2, DCM was added (3 x
249 0.25 mL) and evaporated to remove excess reagents. Amino acid methyl esters were then
250 treated with 1 mL of a mixture of acetone:trimethylamine:acetic anhydride (5:2:1, v/v) to each
251 sample and heated at 60°C for 10 min. Following acetylation, the reagents were evaporated
252 under a gentle stream of N_2 and were dissolved in 2 mL of ethyl acetate, to which 1 mL of
253 saturated NaCl solution was added. Phase separation was enabled via mixing and the organic
254 phase was collected; separation was repeated 3 times with addition of 2 mL ethyl acetate.

255 Residual water was removed from the combined organic phases by passing through a glass
256 wool plugged glass Pasteur pipette filled with $MgSO_4$. Finally, samples were evaporated under
257 N_2 and the derivatized amino acids were dissolved in DCM and stored at -20°C until analysis.
258

259 **Instrumental Analysis:** $\delta^{15}N_{AA}$ values were determined using a Trace Ultra gas
260 chromatograph (GC) coupled to a Delta V Advantage IRMS with a ConFlo IV interface (Cu/Ni
261 combustion reactor held at 1000°C, Thermo Fisher). A liquid nitrogen trap was added after the
262 reduction oven to remove CO_2 from the sample stream. The separation of amino acids was
263 achieved using an HP Innowax capillary column (30 m x 0.25 mm i.d. x 0.5 µm film thickness,
264 Agilent). The sample was introduced to the column using a split/splitless injector set at 260°C.
265 The GC was programmed as follows: held at 50°C for 2 min, 10°C min^{-1} to 180°C and 3°C
266 min^{-1} to 260°C, and held for 8 min. The carrier gas was ultra-high purity helium (flow 1.1
267 $mL.min^{-1}$). The ion intensities of m/z 28, 29 and 30 were monitored and the $\delta^{15}N$ of each amino
268 acid peak were automatically computed (Isodat version 3.0; Themo fisher) by comparison with
269 a standard reference N_2 gas, which was repeatedly measured (x4) at the beginning and the end
270 of each sample analysis. All results are reported in per mil (‰) relative to N_2.

271 Each sample was run in duplicate using two different dilutions. The first run was used to
272 separate the following AA: Alanine (Ala), Valine (Val), Leucine (Leu), Glycine (Gly), Aspartic
273 acid (Asp) and Glutamic acid (Glu). Phenylalanine (Phe) was often below the limits of
274 detection and so all samples were concentrated and run again using a different 'time events'
275 programme, to isolate the Phe peak with an optimal peak size of 500 to 1200 mV. A triplicate
276 measurement was made if the mean $\delta^{15}N_{AA}$ values fell outside the expected measurement error
277 (<1.0‰). Precision and accuracy were determined using a mixed amino acid standard prepared
278 from eight amino acids with known $\delta^{15}N$ values (University of Indiana, USA and SI Science
279 Japan). The mixed standard was analyzed every 4 injections. Typical precisions and accuracies
280 were ± 0.9 ‰ and ± 0.2 ‰ (1σ, n = 48), respectively.

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

285 Eq. 2: $\delta^{15}N_{sample\ reported} = avg\ \delta^{15}N_{sample\ measured} - \delta^{15}N_{standard\ measured} - \delta^{15}N_{known})$.

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

289 **2.4 Statistical Analyses**

290 **2.4.1 $\delta^{15}N_{bulk}$ and $\delta^{13}C_{Bulk}$**

291 To determine which factors best explained the variation in the $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ data, we
292 specified linear mixed effect models (*glmer* function in *lme4* package, R version 3.6.2, 2019),
293 with individual as a random effect to take into account the repeat sampling of two GLGs for
294 each animal. The covariates used in the model were sex, GLG (2 or 3) and the $\delta^{15}N_{bulk}$ and
295 $\delta^{13}C_{bulk}$ values for the $\delta^{13}C_{bulk}$ and $\delta^{15}N_{bulk}$ models, respectively. The global $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$
296 models, separately, included all covariates, and backwards model selection using the *dredge*
297 function (*MuMIn* library) was used to identify the covariates that best explain the variation in
298 the data. The best-fitting model for each dataset was selected using the smallest Akaike's
299 information criterion corrected for small sample sizes (AICc), which provides a relative
300 measure of the goodness-of-fit of the models. Linear regression models (*car* package) were
301 used to assess the relationship between the $\delta^{15}N_{bulk}$ values in GLGs 2 and 3, and between the
302 $\delta^{13}C_{bulk}$ values in GLGs 2 and 3. Statistical significance for all models was considered as $p <$
303 0.05.

304 **2.4.2 $\delta^{15}N_{AA}$ data**

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

308

309 **2.4.3 Relative Trophic Position Determination**

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

316 glycine ($\delta^{15}N_{Gly}$) as a source amino acid in harp seal dentine. A linear regression model (*car*
317 package) was used to assess the relationship between $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Gly}$ values in the two
318 GLGs.

319 We used $\delta^{15}N$ of glutamic acid ($\delta^{15}N_{Glu}$) to estimate TP. Glutamic acid is the most abundant
320 amino acid in a consumer's tissue, and is considered as the canonical trophic amino acid while
321 all of the other trophic amino acids are related to the central nitrogen pool via glutamic acid
322 (McMahon and McCarthy, 2016). Glutamic acid has therefore widely been used to estimate
323 the TP of aquatic organisms (Chikaraishi et al., 2007; Germain et al., 2013; Nielsen et al.,
324 2015). However, the uncertainty regarding trophic fractionation factors between "source" and
325 "trophic" amino acids across taxa in entire food webs prevents accurate estimation of an
326 organism's absolute TP (Nielsen et al., 2015). To compare the two baseline AAs (Phe and Gly)
327 we calculated relative TP (TP_{rel}) by independently subtracting $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Gly}$ values from
328 $\delta^{15}N_{Glu}$ to obtain baseline-corrected $\delta^{15}N_{Glu}$ values. Linear regression models (*car* package)
329 were used to assess the relationship between GLGs 2 and 3 for both $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Gly}$ values,
330 and TP_{rel} values to determine if values in the second year of life were related to those in the
331 third. To compare TP_{rel} between individual seals, we used a one-way ANOVA with seal as a
332 factor variable and the mean TP_{rel} as the explanatory variable. Mean TP_{rel} for each seal was
333 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 ($n = 4$).
334 Statistical significance for all analyses was considered as $p < 0.05$.

335 **2.4.4 Trophic Niche Estimation**

336 We determined isotopic niche spaces for each sex and in each GLG using Stable Isotope
337 Bayesian Ellipses in R (SIBER package in R, Jackson & Parnell, 2015). Standard Bayesian
338 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
339 $\delta^{15}N_{Glu-Gly}$ and $\delta^{13}C_{bulk}$ values. Standard ellipse area was corrected for small sample sizes
340 (SEAc, Jackson et al., 2011) and ellipse overlap (95%) was calculated.

341

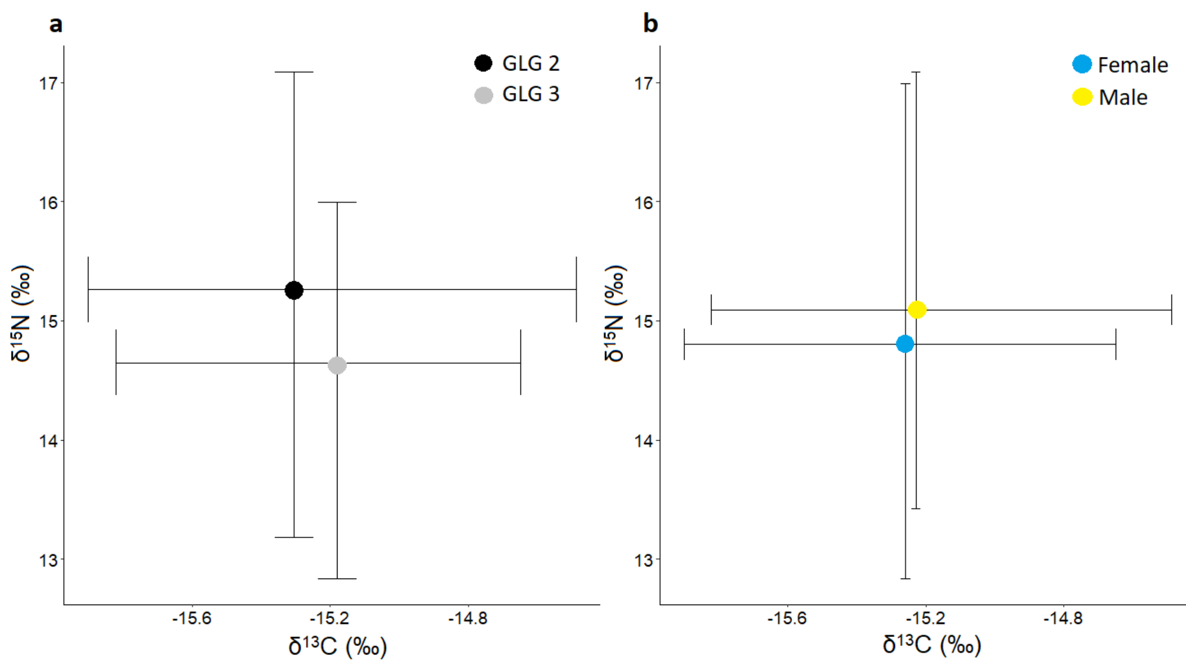
342 **3. Results**

343

344 **3.1 $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ data**

345 The $\delta^{15}N_{bulk}$ values ranged between 12.8 and 17.1 ‰, and $\delta^{13}C_{bulk}$ values ranged between -15.9
346 and -14.5 ‰, and were both normally distributed (Shapiro-Wilk test, $p > 0.05$). Our $\delta^{15}N_{bulk}$

347 and $\delta^{13}C_{bulk}$ values were within the ranges of those already published from a range of tissue
348 types in Arctic phocids (Table 1). Backwards model selection revealed that no covariates were
349 retained as important factors to explain the variability in the $\delta^{15}N_{bulk}$ or the $\delta^{13}C_{bulk}$ data (best
350 fitting models with the lowest AICc were 2 units smaller than the next, best-fitting model;
351 Table 2). There were therefore no significant differences in measured $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$
352 values between males and females or between GLGs 2 and 3 (Fig. 2). There was no significant
353 relationship between the $\delta^{15}N_{bulk}$ and the $\delta^{13}C_{bulk}$ data (linear regression model; $p > 0.5$). There
354 was also no relationship between the $\delta^{15}N_{bulk}$ values in GLGs 2 and 3, or between the $\delta^{13}C_{bulk}$
355 values in GLGs 2 and 3 (linear regression models; p-values all > 0.5).



356

357 **Fig. 2. a)** Mean, maximum and minimum $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ values measured for each growth
358 layer group (GLG). There was no overall difference between the GLGs 2 and 3 representing
359 the second and third year of life of the harp seals ($n = 17$). **b)** Mean, maximum and minimum
360 $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ values measured by sex. There was no overall difference between these
361 male and female sub-adult harp seals ($n = 17$).

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

364 *subadults specifically

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

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*	Muscle	14.1 ± 1.0	-17.8 ± 0.5	Lawson and 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 and 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**	Crain et al. 2021
	Bering and Chukchi seas, Alaska, USA (1998–2014)	Claws	17.1 ± 0.8	-17.2 ± 1.2**	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**	Crain et al. 2021
	Bering and Chukchi seas, Alaska, USA (1998–2014)	Claws	15.9 ± 0.7	-15.5 ± 0.7**	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

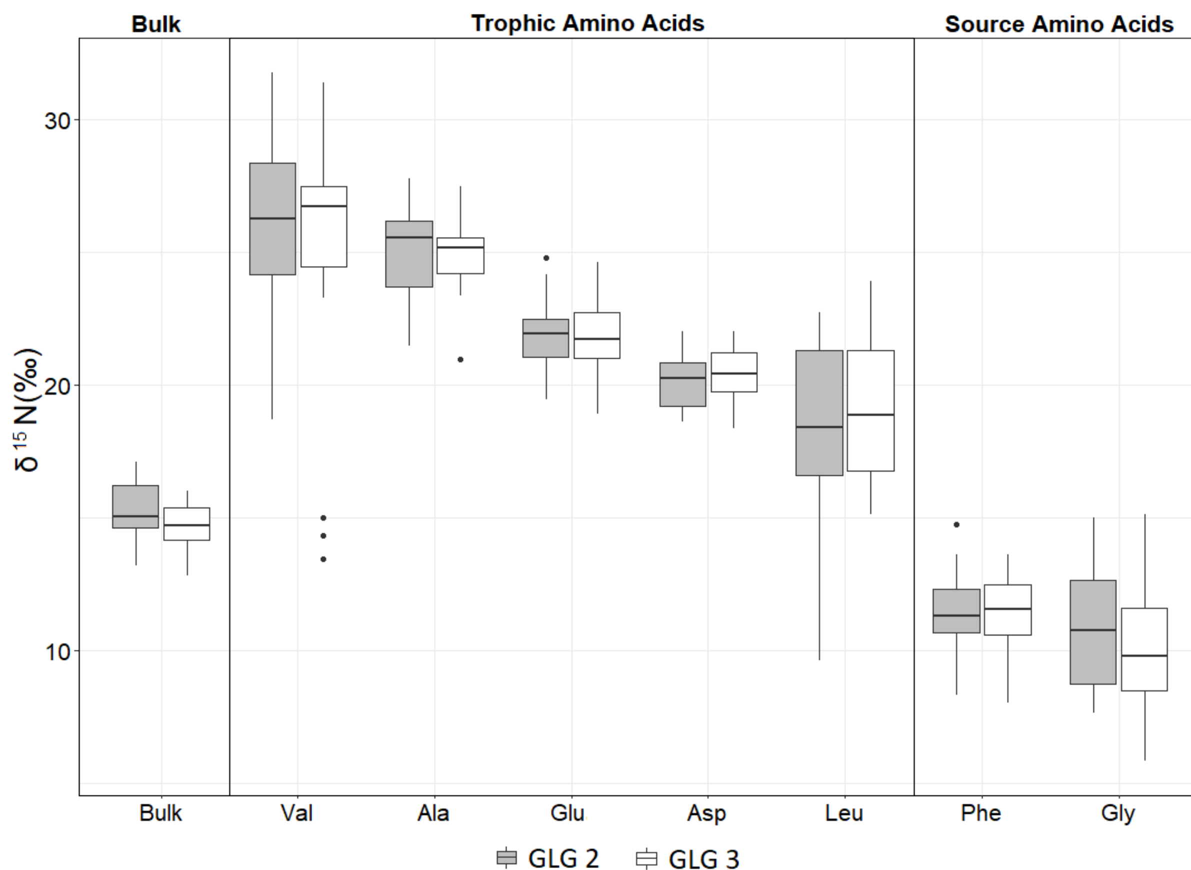
367 **Table 2**. Result of Linear Mixed Effects (LME) Model selection for $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$
368 showing the three best-fitting models with the lowest second-order Akaike information
369 criterion (AICc) values.

LME Model	Model Covariates	df	AICc	Δ AICc	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

370

371 **3.2 $\delta^{15}N_{AA}$ data**

372 Average values of $\delta^{15}N$ were between ~18.5 ‰ and ~25.0 ‰ for trophic AAs (valine, alanine,
373 glutamic acid, aspartic acid, leucine), and between ~10.5 ‰ and ~11.5 ‰ for the two source
374 amino acids (phenylalanine and glycine; Fig. 3). $\delta^{15}N_{Gly}$ values were similar to $\delta^{15}N_{Phe}$
375 (ANOVA; $F_{1,32} = 2.7$, $p = 0.11$), demonstrating that glycine can potentially be considered as
376 an alternative to phenylalanine, and used as a source amino acid in inert harp seal teeth tissues.
377 There were no significant differences in the $\delta^{15}N_{AA}$ values between the two GLGs (all ANOVA
378 p-values > 0.1) (Fig. 3), or between the sexes (all ANOVA p-values > 0.1). There was
379 considerable variability in the $\delta^{15}N$ of valine and leucine both within a single GLG and between
380 GLGs compared to the other amino acids (Fig. 3). Leucine and valine are both non-polar amino
381 acids and the variability in $\delta^{15}N$ is likely, at least partly, to be a result of a mismatch with the
382 highly polar stationary phase of the GC column used.

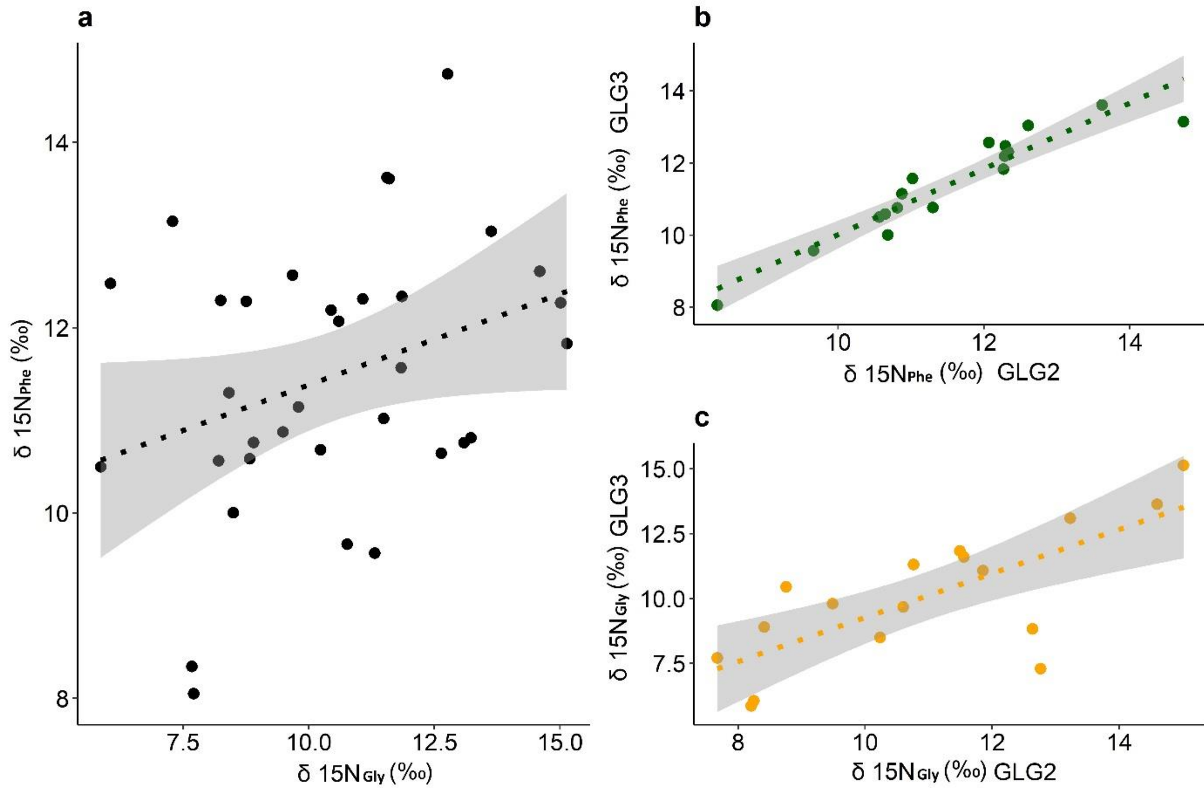


383

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

392 **3.3 δ^{15}N Baseline and Relative Trophic Position**

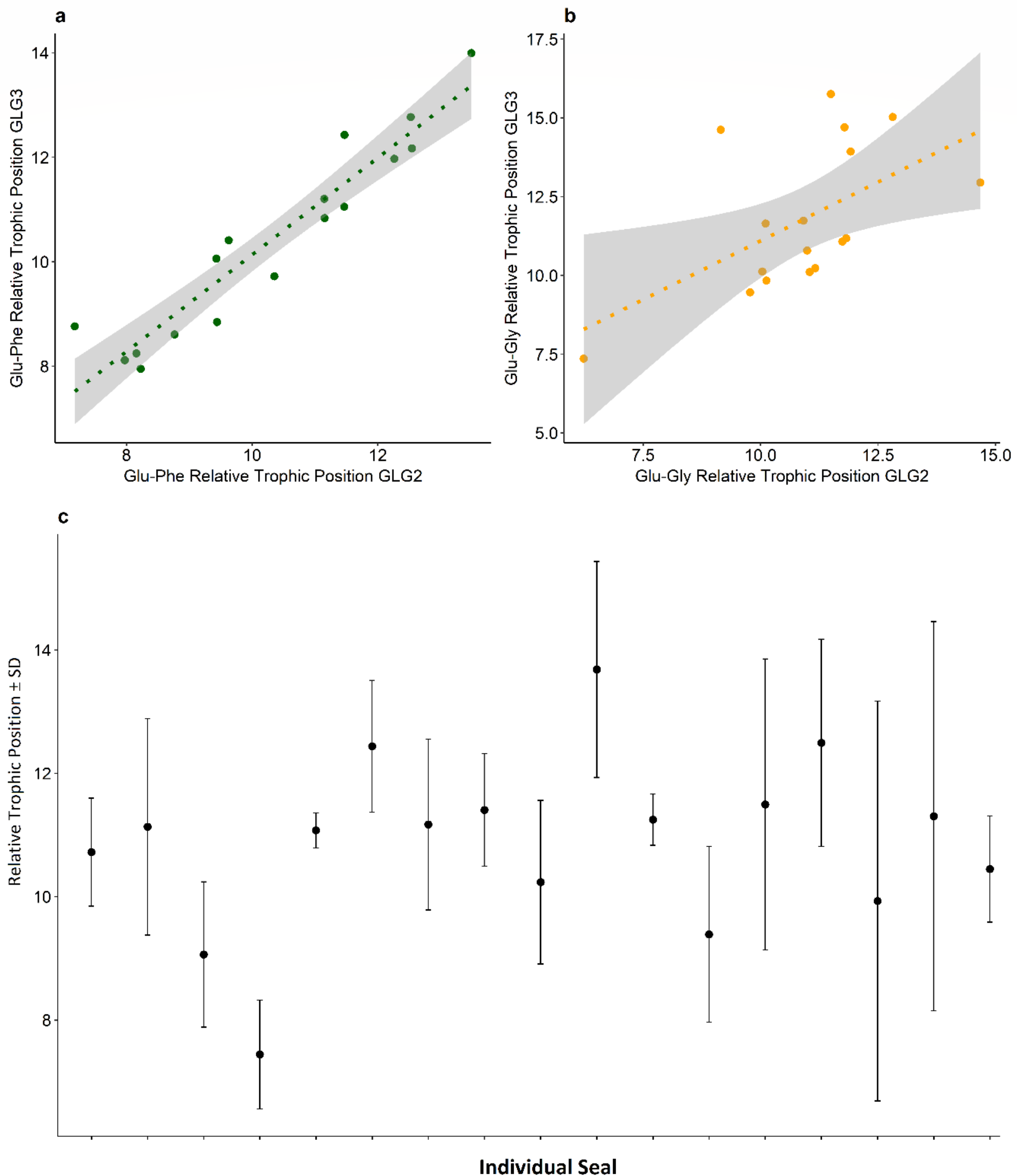
393 There was considerable variability in δ^{15}N of the two source amino acids, representing the δ^{15}N
394 at the base of the food web; $\delta^{15}N_{Phe}$ ranged from 8.05 to 14.7 ‰ and $\delta^{15}N_{Gly}$ ranged from 5.9
395 to 15.1 ‰, with a weakly significant relationship between the two (linear regression model;
396 adjusted $R^2 = 0.1$, $p = 0.05$) (Fig. 4a). There was a strong positive relationship between GLG 2
397 and GLG 3 for both $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Gly}$ (linear regression models; p values both < 0.01) (Fig.
398 4b and c). The relationship between GLGs was more variable for $\delta^{15}N_{Gly}$ compared to $\delta^{15}N_{Phe}$
399 (Fig. 4b and c).



400

401 **Fig. 4. a)** Weakly significant relationship between the two source amino acids, $\delta^{15}N_{Phe}$ ‰ and
402 $\delta^{15}N_{Gly}$ ‰ measured in harp seal teeth GLGs with associated 95% CIs (linear regression model;
403 adjusted $R^2 = 0.1$, $p = 0.05$) **b)** Significant positive relationship between $\delta^{15}N_{Phe}$ ‰ measured
404 in GLGs 2 and 3 (second and third years of life) with associated 95% CIs (linear regression
405 model: Adjusted $R^2 = 0.9$, $p < 0.001$). **c)** Significant positive relationship between $\delta^{15}N_{Gly}$ ‰
406 measured in GLGs 2 and 3 (second and third years of life) with associated 95% CIs (linear
407 regression model: Adjusted $R^2 = 0.5$, $p = 0.007$).

408 Relative trophic position (TP_{rel}) estimated using Phe and Gly ($\delta^{15}N_{Glu}$ - $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Glu}$ -
409 $\delta^{15}N_{Gly}$) showed a positive relationship between GLGs (Fig. 5a and b). A large range in TP_{rel}
410 values were calculated between individuals ($\delta^{15}N_{Glu}$ - $\delta^{15}N_{Phe}$ min = 7.18 ‰, max = 14.0 ‰,
411 and $\delta^{15}N_{Glu}$ - $\delta^{15}N_{Gly}$ min = 6.23 ‰, max = 15.75 ‰). The mean TP_{rel} varied significantly
412 between individuals (ANOVA: $F_{1,15} = 3.008$, $p = 0.001$; Fig. 5c). There was no evidence for a
413 difference in relative trophic position between these sub adult males and females neither for
414 $\delta^{15}N_{Glu}$ - $\delta^{15}N_{Phe}$ (ANOVA $F_{1,15} = 0.003$, $p = 0.96$), nor for $\delta^{15}N_{Glu}$ - $\delta^{15}N_{Gly}$ (ANOVA $F_{1,15}$ =
415 0.568, $p = 0.46$).



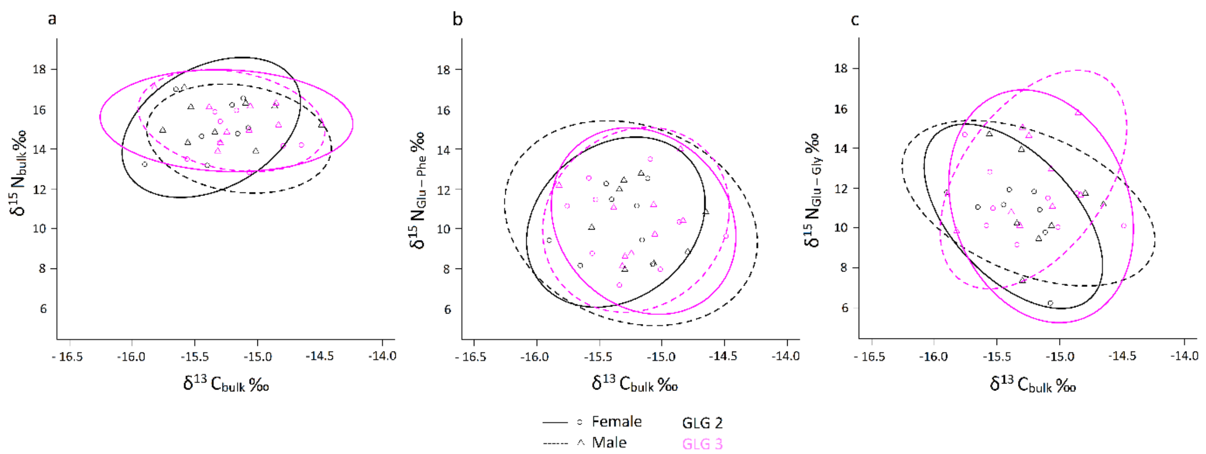
416

417 **Fig. 5. a)** Significant positive relationship between the relative trophic position calculated as
418 baseline corrected $\delta^{15}N$ ‰ ($\delta^{15}N_{Glu}$ - $\delta^{15}N_{Phe}$) measured in GLGs 2 and 3 (second and third
419 years of life) of harp seals with associated 95% CIs (linear regression model: Adjusted R^2 =
420 0.9, $p < 0.001$). **b)** Significant positive relationship between the relative trophic position
421 calculated as baseline corrected $\delta^{15}N$ ‰ ($\delta^{15}N_{Glu}$ - $\delta^{15}N_{Gly}$) measured in GLGs 2 and 3 (second
422 and third years of life) of harp seals with associated 95% CIs (linear regression model: Adjusted
423 R^2 = 0.3, p= 0.01). **c)** Mean relative trophic position calculated as the average baseline-

424 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)
425 for each seal showing significant variability between individuals.

426 **3.4 Isotopic Niche Width**

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



433

434 **Fig. 6.** Representation of trophic niche variation between sexes and growth layer groups
435 (GLGs) measured by SEAc. Ellipses are drawn for each group independently and contain
436 approximately 95% of the data for **a)** $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$ **b)** baseline corrected $\delta^{15}N$ ($\delta^{15}N_{Glu-}$
437 $_{Phe}$) and $\delta^{13}C_{bulk}$ **c)** baseline corrected $\delta^{15}N$ ($\delta^{15}N_{Glu-Gly}$) and $\delta^{13}C_{bulk}$.

438

439 ## 4. Discussion

440 **4.1 Development of teeth demineralisation and subsampling methods**

441 Here we demonstrate that the microsampling of demineralised teeth provides bulk SI data in
442 accordance with previous studies investigating harp seal diet (Hammill et al. 2005).
443 Considerable intra- and inter-individual variation was seen in the $\delta^{15}N_{bulk}$ and $\delta^{13}C_{bulk}$
444 measurements, which are likely the result of differences in diet, baseline isotope signatures,
445 and metabolic processes. While a small sample size of just 17 seals was used for these
446 preliminary method development investigations, the variation between individuals observed in

447 both bulk stable carbon and nitrogen isotopes values indicated wide isotopic niches for the
448 population as a whole. From stomach content analyses, harp seals are known to feed on a
449 variety of species, but the bulk of their diet is thought to be comprised of relatively few species,
450 such as capelin (*Mallotus villosus*) polar cod (*Boreogadus saida*), herring (*Clupea harengus*),
451 krill (*Thysanoessa spp*.) and pelagic hyperiid amphipods (e.g. *Themisto libellula*) (Stenson et
452 al., 2020). We have demonstrated that our SI data from dentine GLGs are consistent with the
453 aforementioned evidence that harp seals have wide isotopic niches irrespective of sex and age
454 as sub-adults (Beck, Hammill and Smith, 1993; Haug et al., 2017; Lawson and Stenson 1995).

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

465 **4.2 Use of $\delta^{15}N_{Gly}$ as a source amino acid in harp seal dentine**

466 Compound specific isotope analyses of amino acids are increasingly applied to studies
467 investigating trophic structure as this method can provide increased understanding of complex
468 ecosystems by taking into account variation in the isotopic value of the environmental baseline.
469 $\delta^{15}N_{Phe}$ is frequently used to represent the $\delta^{15}N$ at the base of the food web. The similar range
470 in values and the correlation between $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Gly}$ here suggest that in harp seal dentine,
471 $\delta^{15}N_{Gly}$ can also be used as a reliable source amino acid, representative of the environmental
472 baseline. This is further supported by the similar isotopic niche size estimated using the two
473 baseline-corrected $\delta^{15}N$ values, and because both $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Gly}$ captured the variation
474 between individuals.

475 The higher variability in the $\delta^{15}N_{Gly}$ data is likely driven by the optimal peak amplitude for the
476 GC-IRMS at conditions stated in the methods, which was ~500 to 1200 mV. Gly peaks
477 measured in dentine were in the range of 2000 mV to 4500 mV, and therefore would have
478 benefited from sample dilution. Concomitantly, we targeted Phe as a reliable source amino acid

479 and concentrated the samples to produce peaks at optimal amplitude. Variability in the
480 relationship between GLGs (adjusted R^2 value of 0.5 and 0.9 for the Gly and the Phe data,
481 respectively) is most likely a result of method optimisation rather than variability of these
482 amino acids within the seal dentine. Gly is known to be one of the most abundant amino acids
483 in collagen/bone/dentine (Li and Wu, 2018; Yamakoshi et al., 2005), and in this study it was
484 ten times more abundant than Phe. Therefore, the use of Gly instead of Phe has significant
485 implications for future CSIA of teeth samples as smaller masses of material are required for
486 analysis. However, care needs to be taken when targeting both of these source amino acids, in
487 order to achieve optimal measurement conditions and thus generate reliable data.

488 **4.3 Individual consistency in the foraging patterns of sub-adult harp seals**

489 Here, $\delta^{15}N_{Phe}$, which represents the $\delta^{15}N$ at the base of the food web, showed variation between
490 individuals, suggesting that there may be variation in where individual harp seals were
491 foraging, as evidenced in a handful of telemetry studies from the Northeast Atlantic (Blanchet
492 et al., 2018; Folkow et al., 2004; Nordøy et al., 2008). Harp seals undergo long-distance
493 migrations, for example between the Labrador Sea and the South of Greenland, two regions
494 which are influenced by water masses having different $\delta^{15}N$ baselines (de la Vega et al. 2020).
495 Specifically, there is an ~2 ‰ difference in $\delta^{15}N$ of nitrate (de la Vega et al., 2021) between
496 the eastern portion of the Labrador Sea, which is influenced by Atlantic water, and Baffin Bay
497 or the Labrador Shelf, which are influenced by Pacific derived water exiting the Arctic through
498 the Canadian Arctic Archipelago (Torres-Valdés et al., 2013). This variation in environmental
499 baseline can explain the variability measured in the seals.

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

507 The positive relationships between the second and third year of life for $\delta^{15}N_{Phe}$, $\delta^{15}N_{Gly}$ and
508 TP_{rel} suggest year to year consistency in both diet and foraging location in these two and three
509 year olds. This is supported by the similar isotopic niche breadth / width, suggesting that
510 individuals feed on the same functional groups of prey between the second and third year of

511 life. These results could reflect either differences in diet between individuals, which would
512 suggest individual specialisation within a generalist population, a phenomenon which has been
513 documented among a number of marine vertebrate species (Martínez del Rio et al., 2009;
514 Hückstädt et al. 2012; Vander Zanden et al., 2010), or differences in migration patterns that
515 could indicate foraging site fidelity and / or habitat selection. Further work should investigate
516 if individual specialisation in terms of diet, or foraging area, persists through adult life in harp
517 seals. Further work should also investigate if individual foraging behaviour is related to prey
518 availability, population density, or physiological characteristics that could potentially affect the
519 diving capacity and / or prey capture and handling ability of individuals (Wathne, et al. 2000;
520 Ogloff, et al. 2019).

521 **4.3 Teeth as tools: Future applications**

522 We have demonstrated the power of using demineralisation and subsampling methods for fine
523 temporal scale bulk isotope and CSIA in harp seal teeth, and show that they are useful tools to
524 investigate both *between* and *within* individual variability in foraging patterns. Together, these
525 data highlight the importance of taking into account the isotopic baseline for the correct
526 interpretation of CSIA data that can shed light on individual predator foraging patterns. In
527 addition, combining $\delta^{15}N_{Phe}$, representing the $\delta^{15}N$ baseline, with $\delta^{13}C_{bulk}$ could help to
528 geolocate foraging areas more precisely, if the isoscape for both $\delta^{15}N$ and $\delta^{13}C$ is spatially (and
529 temporally) constrained. These data also suggest that using $\delta^{15}N_{Gly}$ could potentially be used
530 to correct for baseline $\delta^{15}N$ instead of $\delta^{15}N_{Phe}$ in seal dentine. This finding has important
531 implications for future CSIA of teeth from archives. Specifically, investigations quantifying
532 $\delta^{15}N_{Glu}$ and $\delta^{15}N_{Gly}$ as the trophic and source amino acids respectively, require smaller sample
533 masses for analysis, thus permitting finer scale work on narrower GLGs from older seals.
534 Combining these CSIA biomarkers with other measurements such as GLG thickness, which
535 can be used as a proxy for individual growth and therefore environmental quality in any given
536 year (Hanson et al., 2009; Knox et al., 2014), would further improve our understanding of
537 responses to environmental and ecological changes.

538 In addition, these methods offer great potential for analysing biomarkers in archived inert
539 tissues going back in time, as a powerful tool for both modern and historical reconstructions of
540 the marine environment. Moving forward, these methods could be used on larger samples sets
541 of teeth to investigate spatial and temporal changes in Arctic ecosystems. Importantly, using
542 teeth as tools is especially valuable to reconstruct decadal $\delta^{15}N$ and $\delta^{13}C$ in the environment

543 over the last century, which is difficult to achieve from the sedimentary record as a result of
544 biological (bioturbation) and physical (winnowing/slumping) processes (LaRowe et al., 2020;
545 Meysman et al., 2006; Collins and Balson, 2007). Furthermore, shallow sedimentary nitrogen
546 isotope records are potentially compromised by microbial degradation of organic matter (e.g.
547 Freudenthal et al., 2001; Möbius et al., 2010). Teeth archives may therefore provide unique,
548 fine-scale resolution and a 'true' isotopic signature. Such reconstructions will allow an
549 improved understanding of how Arctic food-webs, and other environments, have been altered
550 over the last decades, and will directly inform model projections of how ecosystems are
551 predicted to be continually affected by the forecasted environmental changes in the 21st
552 century. Ultimately, using historical changes to inform modern predictions of environmental
553 variation and species foraging ecology can highlight population resilience or susceptibility to
554 environmental change, and help to inform management decisions to mitigate against the
555 cumulative impacts of increased human activity.

556

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561

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