Combining δ^{13}C and δ^{15}N from bone and dentine in marine mammal palaeoecological research: insights from toothed whales

Short title (up to 70 characters)

δ^{13}C and δ^{15}N from bone and dentine: insights from toothed whales

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Abstract

Rationale

Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope compositions of bone and dentine collagen extracted from subfossil specimens of extinct and extant mammalian species have been widely used to study the paleoecology of past populations. Due to possible systematic differences in stable isotope values between bone and dentine, dentine values can be transformed into bone-collagen equivalent using a correction factor. This approach has been applied to terrestrial species, but correction factors specifically for marine mammals are lacking. Here, we provide correction factors to transform dentine δ^{13}C and δ^{15}N values into bone-collagen equivalent for two toothed whale sister species: narwhal and beluga.

Methods

We sampled bone and tooth dentine from the skulls of 11 narwhals and 26 belugas. In narwhals, dentine was sampled from tusk and embedded tooth; in beluga, dentine was sampled from tooth. δ^{13}C and δ^{15}N were measured using an elemental analyzer coupled to a continuous flow isotope ratio mass spectrometer. Intraindividual bone and dentine isotopic compositions were used to calculate correction factors for each species, and to translate dentine isotopic values into bone-collagen equivalent.

Results

Our analysis revealed differences in δ^{13}C and δ^{15}N between bone and dentine. In narwhals, we found (i) lower average δ^{13}C in bone compared with dentine from tusk and embedded tooth; (ii) no difference in dentine δ^{13}C between tusk and embedded tooth; (iii) lower average δ^{15}N in bone compared with dentine, with the highest values found in

embedded tooth. For belugas, we also detected lower δ^{13}C and δ^{15}N in bone compared with tooth dentine.

Conclusions

Based on our analysis, we provide bone/dentine correction factors for narwhals (both at species and population level), and for belugas. The correction factors, when applied to dentine δ^{13}C and δ^{15}N values, enable the combined analysis of stable isotope data from bone and dentine.

Keywords

Beluga whale, marine mammals, narwhal, paleoecology, stable isotopes

Introduction

Stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotope compositions of bone and dentine collagen extracted from subfossil specimens have been used to study the paleoecology of past populations and extinct species of mammals. Most of these studies have focused on terrestrial taxa, including northern hemisphere species such as brown bear (*Ursus arctos*), hyena (*Crocuta crocuta*), musk ox (*Ovibos moschatus*), saiga (*Saiga tatarica*), and woolly rhinoceros (*Coelodonta antiquitatis*) [1-5], and South American species including camelids, Equids, and Gomphotheres (extinct elephant-like proboscideans) [6,7].

Although to a lesser extent, stable isotope studies have also been applied to fossil bone and dentine material of marine mammal species, including pinnipeds and cetaceans [e.g., 8-12]. Stable isotope analysis of osseous materials has also been applied to contemporary marine mammal populations to elucidate foraging ecology and dietary niche of e.g., narwhals (*Monodon monoceros*) and belugas (*Delphinapterus leucas*) [13,14].

As a palaeoecological marker, carbon provides information on the photosynthetic pathways and environmental parameters of the producers at the base of the food web. In terrestrial environments, δ^{13}C represents plants [15], while in marine environments, δ^{13}C primarily reflects phytoplankton and algae composition [12]. In both terrestrial and marine ecosystems, δ^{15}N reflects the trophic position of an individual or species [16].

Combined, δ^{13}C and δ^{15}N from bone collagen provide information on the average diet of an individual over multiple years [16,17]. In contrast with bone, which is continually remodelled, dentine grows incrementally, and therefore the isotopic composition of dentine corresponds to different years of an individual's life, depending on the specific tooth/tusk development of the target species [18]. Thus, despite bone and dentine collagen being the same substrate, they incorporate species' life histories differently. In narwhals, for example, where males have one (and rarely two) long erupted tusks, stable isotope values from the various dentine layers of the tusk represent an individual's diet in different years [19]. In mammal species, where young nurse on their mother's milk until they are weaned, collagen extracted from dentine of the first set of teeth in calves/juveniles, or from the roots of the permanent teeth, usually have higher δ^{15}N values, reflecting the suckling period [2,20, 21].

If differences in stable isotope values between bone and dentine are systematic, dentine isotopic values could be translated into the bone-collagen equivalent using a correction factor. This would enable the direct comparison between δ^{13}C and δ^{15}N derived

from these distinct skeletal elements. The correction factor is an estimate of the average difference between bone and dentine $\delta^{13}C$ and $\delta^{15}N$ from the same individual, and across conspecific samples. This approach has been used to estimate the average difference in $\delta^{15}N$ retrieved from bone and tooth in terrestrial mammals; e.g., wolves and hyenas, which across samples resulted in a correction factor of 1.92 ± 0.19‰ and 1.08 ± 0.55‰, respectively [2]. However, it is important to keep in mind the difference in growth pattern between marine and terrestrial mammals. Marine mammals' tooth growth pattern is characterized by the layered deposition of dentine, which happens yearly in most marine mammal species [22].

Museum collections housing faunal remains are a crucial resource for studying changes in populations and species across time and space. However, when analysing specimens of the same species from collections or from the field, it is not always possible to access the same skeletal element. Targeting only a specific skeletal element (e.g., cranial bone or tooth) for analysis may drastically reduce the number of available specimens, affecting the robustness and reliability of results. Here, we present a systematic approach to circumvent this issue in two toothed whale species, narwhal and beluga (Monodontidae).

To calculate a correction factor for $\delta^{13}C$ and $\delta^{15}N$ in narwhal and beluga, we obtained collagen $\delta^{13}C$ and $\delta^{15}N$ from bone and dentine collected from voucher specimens housed at the Natural History Museum of Denmark, University of Copenhagen, and from the Greenland Institute of Natural Resources. The various samples were used to estimate isotopic differences between bone and dentine collagen within individuals, and average differences among individuals within each species. Average differences were used to calculate a correction factor, which can be applied to transform dentine collagen isotopic values into bone-collagen equivalent.

Materials and Methods

1. Samples

The sampled material comprised skulls from 11 narwhals and 26 belugas. We sampled skulls for both bone and dentine at the Natural History Museum of Denmark, University of Copenhagen, and from the Greenland Institute of Natural Resources (see detailed sample information in Supplementary Table 1). Five narwhal skulls were from West Greenland (WG) and six from East Greenland (EG). All belugas were sampled in West Greenland, as the species is not present in East Greenland. The skulls were collected in the field between 1990 and 2018.

Narwhals have a unique dentition; they lack teeth in the lower jaw, and in the upper jaw males have one long erupted tusk and one embedded tooth, while females have two embedded teeth [23]. Hence for narwhals, we determined the isotopic compositions of three skeletal elements: tusk, embedded tooth, and bone. For belugas, which have a row of homodont teeth in both the upper and lower jaw, we sampled tooth and bone.

All individuals sampled for this study were adults, to avoid issues of high $\delta^{15}N$ values in the bone and dentine of nursing calves/juveniles. Sex information for the samples was based on the presence/absence of a tusk in narwhals, and/or the inspection of sexual organs in both narwhals and belugas.

2. Stable isotope $\delta^{13}C$ and $\delta^{15}N$ analysis

We drilled 200-300 mg of each skeletal element. For narwhals, we drilled (i) bone, (ii) tusk, and (iii) embedded tooth. For one of the narwhal samples (ID: 956), we did not have access to the tusk, and therefore only sampled bone and embedded tooth. For belugas, we drilled tooth dentine from each skull; $\delta^{13}C$ and $\delta^{15}N$ data from the same specimens have been published previously in [13], and re-analyzed by [14]. See detailed sample information in Supplementary Table 1.

Stable carbon and nitrogen isotope compositions were determined using a continuous flow isotope ratio mass spectrometer at the Water Quality Centre, Trent University, Canada, following the protocol described in. Full details are available in Supplementary Text.

Differences in $\delta^{13}C$ in samples collected at different time points may reflect changes in $\delta^{13}C$ of atmospheric CO_2, reflecting the industrial revolution and the use of fossil fuels [24]. Therefore, to avoid erroneous interpretations, $\delta^{13}C$ from samples collected across time require correction. We corrected for this change, termed the Suess effect, following the formula from [25].

To contextualise the data generated in our study, we combined our findings with published $\delta^{13}C$ and $\delta^{15}N$ bone records (also from skulls) from 40 WG narwhals, 39 EG narwhals, and one additional beluga [14].

3. Skeletal element comparisons

To estimate a correction factor, we calculated pairwise average isotopic differences between skeletal elements for each sampled individual (data for narwhal: Supplementary Table 2; data for beluga: Supplementary Table 3). For narwhal, these estimates were also calculated for each population, West Greenland and East Greenland; results are summarised in Table 1. Correction factors were estimated as the average of the pairwise average differences for each species, and for each population in narwhal.

Results

1. Correction factor in narwhals

In narwhals, $\delta^{13}C$ values from bone collagen were lower than in dentine; this pattern was consistent in both WG and EG narwhals (Supplementary Figure 1). In WG narwhals, differences in $\delta^{13}C$ between tusk and bone (0.81 ± 0.13‰) were larger than differences between embedded tooth and bone (0.37 ± 0.45‰). In EG narwhals, we observed the opposite, and differences between tusk and bone (0.31 ± 0.25‰) were smaller than differences between embedded tooth and bone (0.51 ± 0.35‰) (Table 1, Figure 1).

In both narwhal populations, $\delta^{15}N$ values from bone collagen were lower than in dentine (Supplementary Figure 1). Both WG and EG narwhals showed smaller differences between tusk and bone (0.49 ± 0.60‰ and 0.48 ± 0.37‰, respectively), than between embedded tooth and bone (1.65 ± 0.69‰ and 1.45 ± 0.57‰, respectively) (Table 1, Figure 1).

2. Correction factor in belugas

In belugas, δ^{13}C values from bone collagen were lower than in dentine (Supplementary Figure 1). The average difference between tooth and bone was 0.37 ± 0.26‰ (Table 1, Figure 1). For δ^{15}N, bone collagen values were lower than dentine (0.59 ± 0.69‰).

Average differences among skeletal elements for each species and population (for narwhal) were used as a correction factor to translate dentine collagen isotopic values to bone-collagen equivalent (Table 1). Differences in δ^{13}C and δ^{15}N before and after correcting the data are visualised in Figure 1. Absolute comparisons can be found in Supplementary Figure 1 and full data can be found in Supplementary Table 4.

We also summarized average δ^{13}C and δ^{15}N before and after correcting the data derived from each dentine element (tusk, embedded tooth, tooth); we report these average values for each species, and for the two geographic populations for narwhals in Table 2. Overall, we detected a reduction in the difference between skeletal element average values after application of the correction factor (Table 2, Figure 1). Dentine corrected values fall within the bone average of a combined panel of newly generated and published data for these species (Supplementary Figure 1, bone_reference).

Discussion and Conclusions

We measured δ^{13}C and δ^{15}N from bone and dentine collagen derived from narwhal (n = 11) and beluga (n = 26). The bone and dentine were sampled from whale skulls housed in museum collections. We performed a systematic comparison of isotopic composition of various skeletal elements from the same individuals, and across individuals within each species and/or population (for narwhal). These values were successfully used as a correction factor, allowing the direct comparison of data from different skeletal elements, and from different populations.

For both narwhal and beluga, we detected lower values of δ^{13}C and δ^{15}N in bone collagen than in dentine collagen. For narwhal, the highest δ^{15}N values were found in embedded tooth. Due to the smaller size of the embedded tooth compared to the tusk, its isotopic composition could still present higher δ^{15}N due to the suckling effect [18,20]. Differences in isotopic composition across tusk layers have been reported [19]; however, our sampling strategy did not target a specific layer, but rather sampled a chunk of tusk comprising several layers, and hence reflected average δ^{13}C and δ^{15}N across several years. Because of this sampling strategy, the correction factors generated in this study are applicable to samples collected in a similar manner.

Using δ^{13}C and δ^{15}N from bone collagen as dietary indicators, population differences in the foraging ecology of narwhals in West Greenland and in East Greenland have been reported [14]. Our narwhal bone and dentine data also showed systematic differences in δ^{13}C and δ^{15}N based on populations. Thus, for narwhal, we estimated a species-wide correction factor, as well as a correction factor specifically for each population (Table 1, Figure 1). When we applied the species-wide correction factor, the average differences between tusk and bone, or embedded tooth and bone, were reduced to values approaching zero (Figure 1B, Figure 1C). Thus, we show that in the absence of *a priori* information on population differences due to life history in isotopic values within a species, it is feasible to apply a species-wide correction factor to combine bone and dentine data. However, if possible, one

should consider life history differences among populations of a species when estimating correction factors, as it might bias our results.

Our bone/dentine correction factor was estimated based on cranial bone and dentine derived from three dentine elements: tusk and embedded tooth in narwhals, and tooth in belugas. As only one bone element was included (skull), our correction factor may not be fully representative of the intraskeletal bone isotopic variation in either species. δ^{13}C and δ^{15}N values in bone tissue are dependent on bone remodeling and biochemical turnover rates [17]. In humans, higher bone turnover rates are significantly and negatively correlated with lower δ^{15}N [26]. Smith et al. 2020 [27] compiled published and new data on intraskeletal bone variation in δ^{13}C and δ^{15}N across marine mammals. The data showed variation in intraskeletal bone δ^{13}C ranging from 0.1‰ in walrus (*Odobenus rosmarus*) (between cranium and mandible [28]) to 1.4‰ in dusky dolphin (*Lagenorhynchus obscurus*) (among atlas, humerus and basioccipital [29]). Variation in intraskeletal bone δ^{15}N ranged from 0.3‰ in walrus and dusky dolphin [28, 29] to 1.3‰ in South American sea lion (*Otaria flavescens*) (among atlas, humerus and basioccipital [29]). However, other studies could not detect intraskeletal variation when performing similar analysis; e.g., [30] did not detect differences in isotopic composition between cortical and trabecular bone in harp seals (*Pagophilus groenlandicus*).

Intraskeletal bone variation in δ^{13}C and δ^{15}N has also been investigated in terrestrial mammals (e.g., in humans [26]; in cow (*Bos taurus*), pig (*Sus domesticus*), long-tailed weasel (*Mustela frenata)*, and raccoon (*Procyon lotor)* [30]). Using an isotopic distance value estimated from δ^{13}C and δ^{15}N bivariate plots, Hyland et al. 2021 [30] detected an average isotopic distance value of bone sampled within individuals of 0.52‰. These data support the existence of intraskeletal variation in isotopic composition; however, there are no clear patterns, and variation appears to be species-specific. Our results showed intraskeletal variation between bone and various dentine elements. In narwhal, we found a species-wide variation in δ^{13}C of 0.51 ± 0.33‰ between tusk and bone, and 0.45 ± 0.38‰ between embedded tooth and bone. For δ^{15}N, these values were 0.49 ± 0.44‰ and 1.54 ± 0.57‰, respectively. In beluga, the δ^{13}C average difference between tooth and bone was 0.37 ± 0.26‰; for δ^{15}N, it was 0.59 ± 0.69‰.

Paleoecological inference from museum specimens is crucial for elucidating changes in populations and species across time and space. However, the same skeletal element is often not available across specimens. Bone and dentine collagen are known to show systematic differences in δ^{13}C and δ^{15}N [31]. Thus, analyzing them together without applying a correction factor may influence biological inferences. Here, we estimate and apply a correction factor to our data from narwhal and beluga, and transform dentine values to bone-collagen equivalent. Our approach assumes that one correction factor can be broadly applied to transform dentine collagen to bone-collagen equivalent, and that data from different bone skeletal elements can be analyzed in unison. Application of our correction factors allow the direct comparison of δ^{13}C and δ^{15}N retrieved from these different skeletal elements (Figure 1 and Table 2). We acknowledge that our approach may not capture the full variation of skeletal stable isotopes within an individual. However, using the correction factors when analysing stable isotope data retrieved from bone and dentine reduces a large fraction of the intraskeletal variation. This approach has been successfully applied to terrestrial mammals (e.g., [2] reviewed the topic for several species). Here, we show its applicability in two marine mammal species: narwhals and beluga. When dentine values were transformed to

bone-collagen equivalents using our approach, they fall within the range of known bone $\delta^{13}C$ and $\delta^{15}N$ values for these species demonstrating their applicability (Figure 1).

Data Accessibility:

Electronic Supplementary Table 1.

Author Contributions:

Research idea and study design: ARI, PS, EDL; data acquisition: ARI, MK, EG, MPHJ, EDL; data generation: TW, JR, PS; data analysis/interpretation: ARI, TW, JR; manuscript drafting: ARI, EDL with input from all the co-authors.

Competing interests:

We have no competing interests

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Tables and Figures (with captions)

Figure 1. Boxplots showing the average differences in $\delta^{13}C$ and $\delta^{15}N$ composition between various skeletal elements analyzed from narwhal (tusk:bone, embedded tooth:bone) and beluga (tooth:bone). (A) $\delta^{13}C$ and $\delta^{15}N$ raw composition, with $\delta^{13}C$ corrected for the Suess effect; (B) $\delta^{13}C$ and $\delta^{15}N$ corrected using the correction factor estimated for each species/skeletal element (data for narwhal: Supplementary Table 2; data for beluga: Supplementary Table 3); and (C) $\delta^{13}C$ and $\delta^{15}N$ corrected using the correction factors estimated for each narwhal population, considering known population differences [14]. All data can be found in Supplementary Table 4. Colours represent species and populations. Green palette: West Greenland (WG) narwhal, blue: East Greenland (EG) narwhal, and purple: beluga.

Table 1. Average differences in $\delta^{13}C$ and $\delta^{15}N$ isotopic composition between different skeletal elements in narwhal and beluga. These average differences were used as correction factors to translate dentine values to bone-collagen equivalents.

			$\Delta\delta^{13}C$	**$\Delta\delta^{15}N$**
Species	**Population**	**Skeletal element**	**Average** ± s.d. ‰	**Average** ± s.d. ‰
Narwhal	WG	Tusk/Bone	0.81 ± 0.13	0.49 ± 0.60
Narwhal	WG	Embedded /Bone	0.37 ± 0.45	1.65 ± 0.69
Narwhal	EG	Tusk/Bone	0.31 ± 0.25	0.48 ± 0.60

Narwhal	EG	Embedded /Bone	0.51 ± 0.35	1.45 ± 0.49
Beluga	WG	Tooth/Bone	0.37 ± 0.26	0.59 ± 0.60

Table 2. Average δ^{13}C and δ^{15}N values before and after correcting the data for systematic differences in skeletal element composition.

			Average δ^{13}C		**Average δ^{15}N**	
Species	**Popula-tion**	**Skeletal element**	**Before correcting data (‰)**	**After correcting data (‰)**	**Before correcting data (‰)**	**After correcting data (‰)**
Narwhal	WG	Tusk	-14.12 ± 0.42	-14.93 ± 0.42	17.44 ± 0.98	16.95 ± 0.98
		Embedded tooth	-14.35 ± 0.33	-14.72 ± 0.33	18.59 ± 0.94	16.94 ± 0.94
Narwhal	EG	Tusk	-15.72 ± 0.18	-16.03 ± 0.18	15.95 ± 0.34	15.47 ± 0.34
		Embedded tooth	-15.52 ± 0.56	-16.03 ± 0.56	16.92 ± 0.69	15.47 ± 0.69
Beluga	WG	Tooth	-13.65 ± 0.23	-14.02 ± 0.23	17.92 ± 0.63	17.33 ± 0.63

Supplementary Figures and Tables:

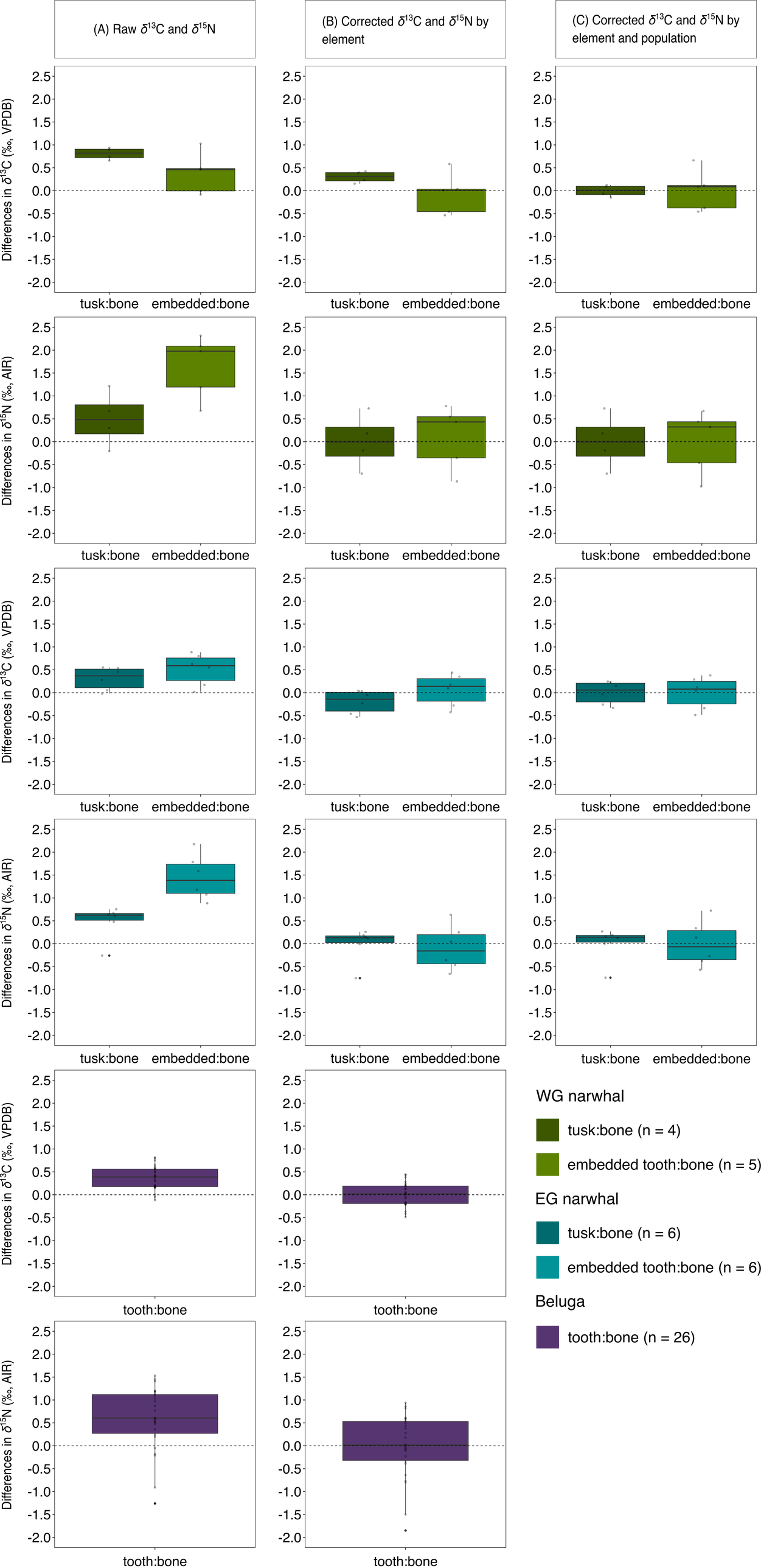
Supplementary Figure 1. Boxplots showing δ^{13}C and δ^{15}N composition of the various skeletal elements analyzed from narwhal and beluga. (A) δ^{13}C and δ^{15}N raw composition, with δ^{13}C corrected for the Suess effect; (B) δ^{13}C and δ^{15}N corrected using the correction factor estimated for each species/skeletal element (data for narwhal: Supplementary Table 2; data for beluga: Supplementary Table 3); and (C) δ^{13}C and δ^{15}N corrected using the correction factors estimated for each narwhal population, considering known population differences [14]. Colours represent species and /populations. Bone_reference represents the combined newly generated (for this study) and published bone data for beluga and narwhal [13,14]. Green palette: West Greenland (WG) narwhal, blue: East Greenland (EG) narwhal, and purple: beluga.

Supplementary Table 1. List of the narwhal and beluga samples used in this study to estimate correction factors.

Supplementary Table 2. Correction factor estimates for the narwhal samples.

Supplementary Table 3. Correction factor estimates for the beluga samples.

Supplementary Table 4. δ^{13}C and δ^{15}N data for the narwhal and beluga samples after applying the correction factor estimates. This table also includes the published data (bone_reference) for narwhal and beluga, which was used in Supplementary Figure 1.


(A) Raw δ13C and δ15N
(B) Corrected δ13C and δ15N by element
(C) Corrected δ13C and δ15N by element and population
Differences in δ13C (‰, VPDB)
Differences in δ15N (‰, AIR)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
tusk:bone
embedded:bone
tooth:bone
WG narwhal
tusk:bone (n = 4)
embedded tooth:bone (n = 5)
EG narwhal
tusk:bone (n = 6)
embedded tooth:bone (n = 6)
Beluga
tooth:bone (n = 26)