Stable isotope composition of Antarctic marine mammals collected at the turn of the 20th century: assessing the baseline for long-term changes in the marine ecosystem.

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Abstract

Great Antarctic expeditions, and subsequently developed seal hunting and whaling industries left an enormous legacy in natural history museum collections. Stable isotopes of carbon $\delta^{13}C$ and nitrogen $\delta^{15}N$ from specimens preserved in the collections can provide valuable information on long-term changes in foraging ecology of species and specify the baselines for the future comparisons with modern samples. To provide the basis for analysing the impact of whaling on marine ecosystem structuring, we conducted the bulk isotope analysis from the specimens of baleen whales (*Balaenoptera musculus* and *B. physalus*), beaked whale *(Berardius arnuxii)*, and seals *(Arctocephalus australis* and *Hydrurga leptonyx*) collected between 1843 and 1951, preserved in the collection of Natural History Museum, London. Having controlled for the Suess effect, $\delta^{13}C$ values in *B. musculus*, *B. physalus* and *H. leptonyx* were different before and after the onset of industrial whaling (1904). Bone collagen $\delta^{15}N$ values and corresponding trophic position consistently changed in *B. musculus* and *A. australis,* suggesting potential temporal shifts in their diets. This study highlights the use of museum specimens for tracing the historical trends that could be associated with changes in the population structure and distribution of species and indicate long-term variability in their foraging ecology.

Keywords

Antarctic, cetacean, pinniped, bone collagen, museum collections, carbon, nitrogen, stable isotopes, Suess effect, trophic position, foraging ecology

Introduction

The Southern Ocean is highly productive (1), and the waters surrounding the Antarctic Peninsula characterized by high krill abundance are usually considered one of the most

productive areas of the Antarctic (2,3). High productivity is also typical of the Subantarctic islands including South Georgia located beyond the northern extent of sea ice (4), as well as the Patagonian shelf and southern tip of South America due to northward branches of the high nutrient Antarctic Circumpolar Current (5). High densities of krill, *Euphasia superba* in the offshore areas and *E. crystallorophias* in the coastal waters around Antarctica attract huge numbers of seabirds (penguins and Procellariiformes) and marine mammals, including pinnipeds and cetaceans (6,7).

Antarctic marine ecosystems are currently experiencing rapid change, especially around the Antarctic Peninsula, identified as one of the most rapidly warming regions of the Southern Hemisphere (8,9). The Antarctic seas were effectively pristine prior to discovery of the continent in the eighteenth century followed by the development of seal hunting and subsequently whaling which resulted in a severe decline in commercially important species. Intensive seal hunting brought Antarctic fur seals *Arctocephalus gazella* almost to extinction by the late 1700-early 1800s in South Georgia, and the whaling industry peaked in the early 1900s when the permanent whaling station was opened at Grytviken (4,10). Following international moratoria on industrial whaling activities, whale populations have been recovering over recent decades after a long period of decline, but recent census studies indicate that their abundance has still not attained pre-whaling numbers (11,12).

The significant decline in the abundance of marine mammals in the late 19th – early 20th centuries caused by industrial-scale exploitation may well have had a significant impact on the marine ecosystem of Antarctica, as well as on the structure and distribution of populations of the species affected. Intensive whaling and seal hunting in Antarctica coincided with the period known as “Heroic Age of Antarctic exploration”, a series of naval and land expeditions between the late 19th century and 1920s. This period left an enormous legacy in natural history museum collections around the world, including the specimens of species targeted by industrial whaling and sealing. Museum specimens are increasingly important for gaining ecological and biological data, and as carriers of geochemical and molecular information, that are important for studying rare, elusive and even extinct species. Historical museum “specimens of opportunity” are often challenging to use due to uncertainties in time and location of collections, and in the quality of post-collection curation, but similarly to the subfossil specimens from excavations of archaeological or paleontological sites can provide snapshots of past genetic diversity, spatial origin and diet (13–15). Stable isotope analysis, most commonly the $\delta^{13}C$ carbon and $\delta^{15}N$ nitrogen isotopes of marine species, can indicate latitudinal range shifts, migrations between nearshore and offshore environments as well as changes in trophic structure (16).

Historical specimens of Antarctic whales from various collections have become increasingly important in isotopic and genetic studies, being used to describe niche partitioning between fin (*Balaenoptera physalus)* and sei (*B. borealis*) whales (17), and changes in mitochondrial DNA diversity in blue (*B. musculus)*, humpback (*Megaptera novaeangliae*) and fin whales (18). Isotopic analyses of collections representing Antarctic fur seal (*A. gazella*) in Antarctica (19) and South American fur seal (*A. australis*) in Patagonia (20) indicate long-term latitudinal and longitudinal shifts in their prey distributions. In this study, we measured $\delta^{13}C$ and $\delta^{15}N$ compositions of the oldest Antarctic and Subantarctic marine mammal bone specimens of opportunity preserved in the Natural History Museum in London (NHMUK). These specimens represent the species most targeted by sealing and whaling activity and thus collected during the expeditions prior to and during the development of industrial scale harvesting. Following

the timeline and southward trend of exploration of Subantarctic and Antarctic waters, the oldest specimens in the dataset correspond to the period of survey of the Strait of Magellan by the HMS *Alert* Expedition (1876–1884) and the James Clark Ross Expedition onboard HMS *Erebus* and HMS *Terror* (1838-1843). The most recent specimens deposited in the NHM were collected by the *Southern Harvester* in the 1960s, the last UK whaling vessel operating in Antarctic and Subantarctic waters; these provide a snapshot from the later phases of 20th century whaling activity. By focusing primarily on well-studied whale species, particularly blue whale *B. musculus* and fin whale *B. physalus*, as well as two species of seals – leopard seal *Hydrurga leptonyx* and South American fur seal *A. australis* - we explore the following research questions: (i) How have $\delta^{13}C$ and $\delta^{15}N$ stable isotopes varied over the period of intensive whaling; (ii) Do $\delta^{13}C$ values show any historical trends that could be associated with changes in the distribution of species; and (iii) Can the observed trends in $\delta^{15}N$ indicate long-term changes in trophic position of the species. We also analysed $\delta^{13}C$ and $\delta^{15}N$ bulk isotopic signatures of several specimens of Arnoux's beaked whale *Berardius arnuxii* from the same period. While this species has rarely been exploited, this study provides a trophic snapshot of early 20th century populations of this elusive Antarctic species.

Materials and methods

Sampling

Samples were collected at the mammal collection of Natural History Museum (London) using handheld cutting tools and a Dremel tool with either dental bur or carbide cutting disk. Before sampling, the surface of the target fragment of the bone was cleaned using Dremel with the bristle polishing brush bit. In total, 42 samples of bone fragments were analysed (Table 1).

Table 1. Bone material and number of specimens used in the study

Species	n	Years of collections	Bone fragments sampled
Balaenoptera musculus	11	1879 - 1951	Periotic; ear bone; lumbar vertebra; shoulder blade; pelvic
Balaenoptera physalis	4	1912 - 1951	Periotic; ear bone; pelvic
Berardius arnuxii	5	1922 - 1931	Rib; sternum; skull
Arctocephalus australis	10	1876 - 1951	Skull
Hydrurga leptonyx	12	1839 - 1963	Skull

The initial weight of the bone samples was about 500 mg. Collagen was extracted and prepared for stable isotope analysis at the BioArCh laboratories in the Department of Archaeology, University of York (UK). The bone samples (approx. 200 to 300 mg) were demineralized using 0.6 M HCl at 4°C for several weeks, then rinsed with ultrapure H_2O (milli-Q) and gelatinized with 0.001 M HCl at 80°C for 48 h. The supernatant containing the collagen was filtered using polyethylene Ezee filters (Elkay Laboratories, 9 ml, pore size 60–90 µm) and then 30 kDa Amicon Ultra-4 centrifugal filter units (Millipore, MA, USA). Samples were then frozen for 24–48 hours at −20°C, lyophilized, and weighed into tin capsules (0.5 mg per duplicate) for stable isotope analysis.

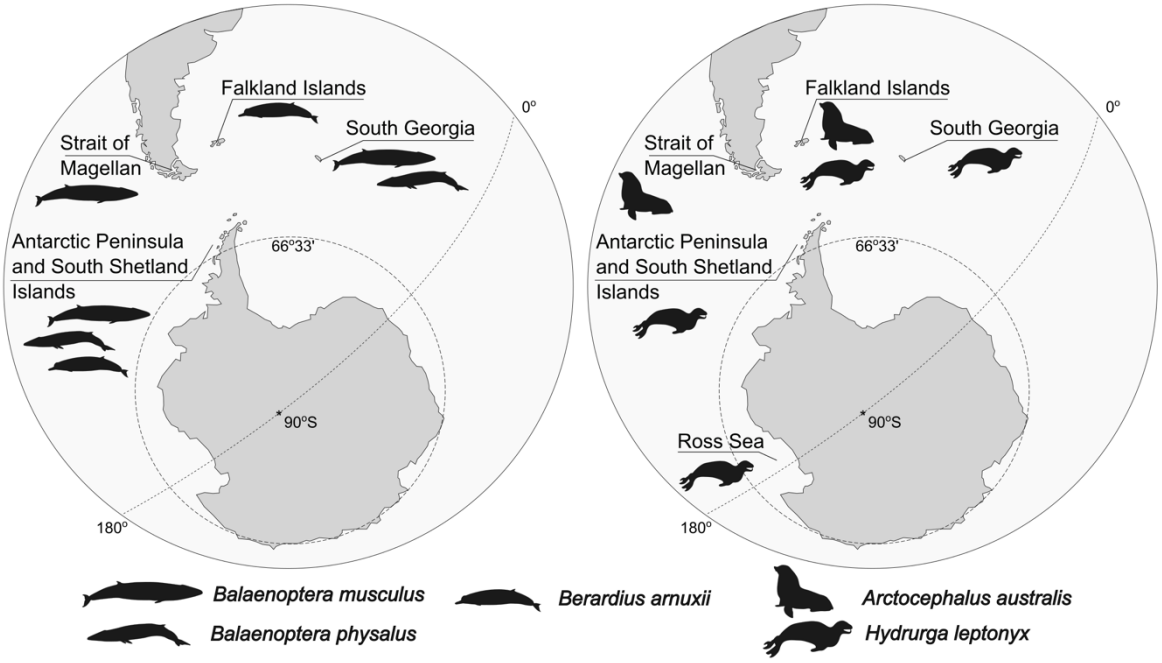

Figure 1. Locations of samples of marine mammals analysed in the study. Map redrawn from Google Earth. Detailed description of locations and NHM accession numbers are available in Table 1 Supplementary material 2.

Bulk EA-IRMS analysis

The BioArCh laboratories at the University of York's Department of Archaeology conducted duplicate measurements of stable carbon and nitrogen isotope ratios. Using a Sercon EA-GSL elemental analyzer linked to a Sercon 20-22 continuous flow isotope ratio mass spectrometer (Sercon, Crewe, UK), the ratios of ^{13}C:^{12}C and ^{15}N:^{14}N were assessed relative to standards (V-PDB for carbon and AIR for nitrogen) and reported in delta notation (δ) in parts per mil (‰). Stable carbon and nitrogen isotope ratios were measured using a Sercon HS2022 continuous flow isotope ratio mass spectrometer at BioArCh, University of York (UK).

Stable carbon and nitrogen isotope ratios were calibrated relative to VPDB and AIR scales using the standard reference materials IsoAnalytical Cane ($\delta^{13}C$ = -11.64‰ ± 0.03), Sigma Methionine ($\delta^{13}C$ = -35.83‰ ± 0.03, $\delta^{15}N$ = -0.76‰ ± 0.05) and IAEA N2 ($\delta^{15}N$ = 20.41‰ ± 0.12). Uncertainty was monitored using the standard reference materials Sigma fish gel (δ13C = -15.27‰ ± 0.04, δ15N = 15.21‰ ± 0.13), IsoAnalytical Alanine ($\delta^{13}C$ = -23.33‰ ± 0.10, $\delta^{15}N$ = -5.56‰ ± 0.14) and IsoAnalytical Soy ($\delta^{13}C$ = -25.22‰ ± 0.03, $\delta^{15}N$ = 0.99‰ ± 0.07). Precision (u(Rw)) was determined to be ± 0.165‰ for $\delta^{13}C$ and ± 0.124‰ for $\delta^{15}N$ based on repeated measurement of calibration standards, check standards and sample replicates. According to calibration and check standards (Table 1 Supplementary material 1), measurement precision (ssrm, pooled measured standard deviation of calibration and check standards) was ± 0.151‰ for $\delta^{13}C$ (df = 28) and ± 0.124‰ for $\delta^{15}N$ (df = 24). All the samples (42/42) were analysed in duplicate (Table 2 Supplementary material 1). The measurement precision specific to samples (srep, pooled standard deviation of sample replicates) was ± 0.094‰ for $\delta^{13}C$ (df = 47) and ± 0.050‰ for $\delta^{15}N$ (df = 47). Accuracy (u(bias)) was determined to be ± 0.091‰ for $\delta^{13}C$ and ± 0.159‰ for $\delta^{15}N$ based on the difference between observed and reported δ values (RMSbias) and long-term standard deviations (u(Cref)) of check standards. Total analytical standard uncertainty (uc) was estimated to be ± 0.188‰ for $\delta^{13}C$ and ± 0.204‰ for $\delta^{15}N$ (21).

Data analysis

Basic descriptive statistics to explore variation for both $\delta^{13}C$ and $\delta^{15}N$ within the bone samples were performed using R (R Core Team, 2024) with RStudio (RStudio Team, 2024). Raw $\delta^{13}C$ values were corrected to consider the oceanic Suess effect – the depletion in the $\delta^{13}C$ isotopic composition of dissolved inorganic carbon (DIC) in oceans due to the increase in anthropogenic CO_2 released into the atmosphere since the Industrial Revolution (Keeling, 1979; Misarti et al., 2009; Keeling et al., 2017). Calculations were performed using the following equation introduced by Hilton et al. (2006), modified by Misarti et al. (2009) and later presented within the SuessR package (22):

Suess effect correction factor = $\boldsymbol{a} * \exp(\boldsymbol{b}*0.027)$

Where $\boldsymbol{a}$ is a constant reflecting the maximum observed rate of $\delta^{13}C$ decline in surface waters DIC in a specific region, $\boldsymbol{b}$ is the year of sample collection minus 1850 (taken as the environmental onset of the Industrial Revolution) and 0.027 is the parameter value obtained by Hilton et al. (2006) after fitting an exponential curve to the global ocean $\delta^{13}C$ data from 1945 to 1997 published by Gruber et al. (1999). The values of total accumulation of Suess effect for the constant $\boldsymbol{a}$ were obtained from Eide et al. (2017) for the surface waters (0 meters depth layer in a database available under the doi:10.1594/PANGAEA.872004). These values were obtained and averaged for five regions (Table 2). Per-year ratios of $\boldsymbol{a}$ were obtained by dividing these values by 140, i.e., the number of years between the year of 1850 (Industrial Revolution) and 1990 (start of the main decade of collecting data in Eide et al.,2017).

Table 2. Oceanic Suess effect correction values extracted from doi:10.1594/PANGAEA.872004 (see Eide et al., 2017 for details). N – number of point estimates of total accumulated Suess effect in surface (0 meter) water layer. Total CSE – mean value of total Oceanic Suess effect for each region. The a-value – expected per-year $\delta^{13}C$ decline (‰).

	Latitude range		Longitude range		n	Total CSE	a-value
Patagonia / Strait of Magellan	-49	-55	-77	-67	19	-0.91	-0.0065
Falkland Islands	-50.8	-52.5	-62	-57.4	2	-0.93	-0.0066
South Georgia	-53	-56	-38.7	-33.9	13	-0.73	-0.0052
South Shetlands	-61	-63	-63	-57	9	-0.75	-0.0054
Ross Sea	-71	-77	171	-160	173	-0.71	-0.0051

Bone $\delta^{15}N$ signatures were converted to trophic position (TP) using the following equation (23,24):

$$TP = 2 + (\delta^{15}N_{sample} - \delta^{15}N_{POM})/TEF,$$

where Dm is $\delta^{15}N$ value in marine mammal bone, POM is $\delta^{15}N$ value of marine particulate organic matter, and TEF is trophic enrichment factor in $\delta^{15}N$ for marine mammals (23,25,26). Data on POM were obtained from previously published modern datasets (8,27–30).

Consumer and source isotope data were analysed using the MixSIAR package in R (31–33), where source data were composed of mean and standard deviation (SD) from potential prey sources. The consumer data per blue whale and South American fur seal were divided into

three consecutive datasets, each representing a period of collection, and each dataset was treated as having no covariates, i.e. including all individuals without separation by their IDs.

Table 3. Stable isotope signatures of potential prey items for the blue whale and South American fur seal, mined from the literature (see text for references) and used for the MixSIAR modeling.

Prey group / Species	Foraging area	n	δ13C		δ15N	
			mean	SE	mean	SE
Euphasia superba	South Shetland Islands	24	-26.87	0.94	3.63	0.38
Euphasia superba	South Georgia	26	-23.87	1.22	4.27	0.54
Thysanoessa spp./ Euphasia frigida	Polar front	7	-23.25	1.05	5.10	0.20
Euphasia superba	Antarctic shelf waters	43	-31.20	0.10	2.85	0.75
Euphasia lucens	Subantarctic (SE Patagonian shelf waters)	7	-19.80	0.70	7.30	0.80
Squid (*Doryteuthis gahi*)		10	-19.00	0.60	13.60	0.70
Demersal fish (*Patagonotothen spp.)*		5	-19.30	0.60	14.80	1.00
Small planktonic fish (*Sprattus fuegensis*)		25	-20.20	1.00	13.10	0.90
Piscivorous fish (Gadidae)		6	-14.90	0.30	17.20	0.40

For both species, source isotopic data were obtained based on recent dietary studies of the species concerned and spatial variability of isotopic signatures of potential prey items (34–37) (Table 3). The models ran three Markov Chain Monte Carlo (MCMC) chains with 50000 iterations, a burn in of 25000, and were thinned by 25 ('short' MCMC run parameters). MixSIAR reports results as mean, SD and credible intervals for each posterior density distribution per prey source.

Results

Stable isotope data

The raw $\delta^{13}C$ and $\delta^{15}N$ values varied significantly in two of three whale species studied (Figure 2, Table 4). The $\delta^{13}C$ values varied significantly in blue whale specimens between -23.67 to -13.23, and similarly $\delta^{15}N$ values varied between 5.26 and 17.05. In fin whale, $\delta^{13}C$ values ranged between -21.66 to -15.43, and $\delta^{15}N$ values ranged between 6.13 to 11.64. In Arnoux's beaked whale $\delta^{13}C$ values were less variable, with a narrow range between -19.62 to -18.88, and $\delta^{15}N$ values between 11.55 and 13.94.

The raw $\delta^{13}C$ values in South American fur seal demonstrated a narrow range between -12.97‰ to -11.42‰, while raw $\delta^{15}N$ values varied between 16.73‰ to 23.56‰. In leopard seal, $\delta^{13}C$ values showed more pronounced variation (-22.02 to -15.8), compared to variation in $\delta^{15}N$ between 7.45 and 11.27. The Suess-corrected $\delta^{13}C$ values demonstrated the same trends and did not deviate more than 0.12 ‰ from raw values (Figure 2, Table 4).

The complete set of individual $\delta^{13}C$ and $\delta^{15}N$ values, and detailed information about the origin of each specimen analysed in this study is presented in Table 1 Supplementary material 2.

Table 4. Mean and range stable isotope values from the bone collagen values of marine mammals collected in Subantarctic waters between the 1880s and 1960s.

species	n	Raw $\delta^{13}C \pm SE$	Suess-corrected $\delta^{13}C$	$\delta^{15}N \pm SE$	C:N mass ratio
Balaenoptera musculus blue whale	11	-19.86 ± 1.00	-19.88 ± 1.00	7.73 ± 1.07	3.58 ± 0.11
Balaenoptera physalus fin whale	4	-19.01 ± 0.70	-19.13 ± 1.32	8.36 ± 0.58	3.55 ± 0.10
Berardius arnuxii Arnoux's beaked whale	5	-19.29 ± 0.15	-19.34 ± 0.15	12.46 ± 0.53	3.33 ± 0.03
Arctocephalus australis South American fur seal	10	- 12.31 ± 0.22	-12.33 ± 0.15	19.37 ± 0.49	3.33 ± 0.02
Hydrurga leptonyx leopard seal	12	-19.74 ± 0.50	- 19.76 ± 0.50	10.12 ± 0.30	3.44 ± 0.04

Carbon to nitrogen (C:N) atomic ratio ranged from 3.24 to 4.3 in various species. So, while most of the samples fell within the expected range (from 2.9 to 3.6) for unaltered bone samples (DeNiro, 1985), several specimens demonstrated C:N values outside this range, particularly three blue whale specimens collected in 1914 near South Georgia (3.72, 4.17, 4.3), one fin whale also collected in 1914 near South Georgia (3.85), and one leopard seal collected near the Falklands before 1885 (3.84) (Table 1 Supplementary Material 2).

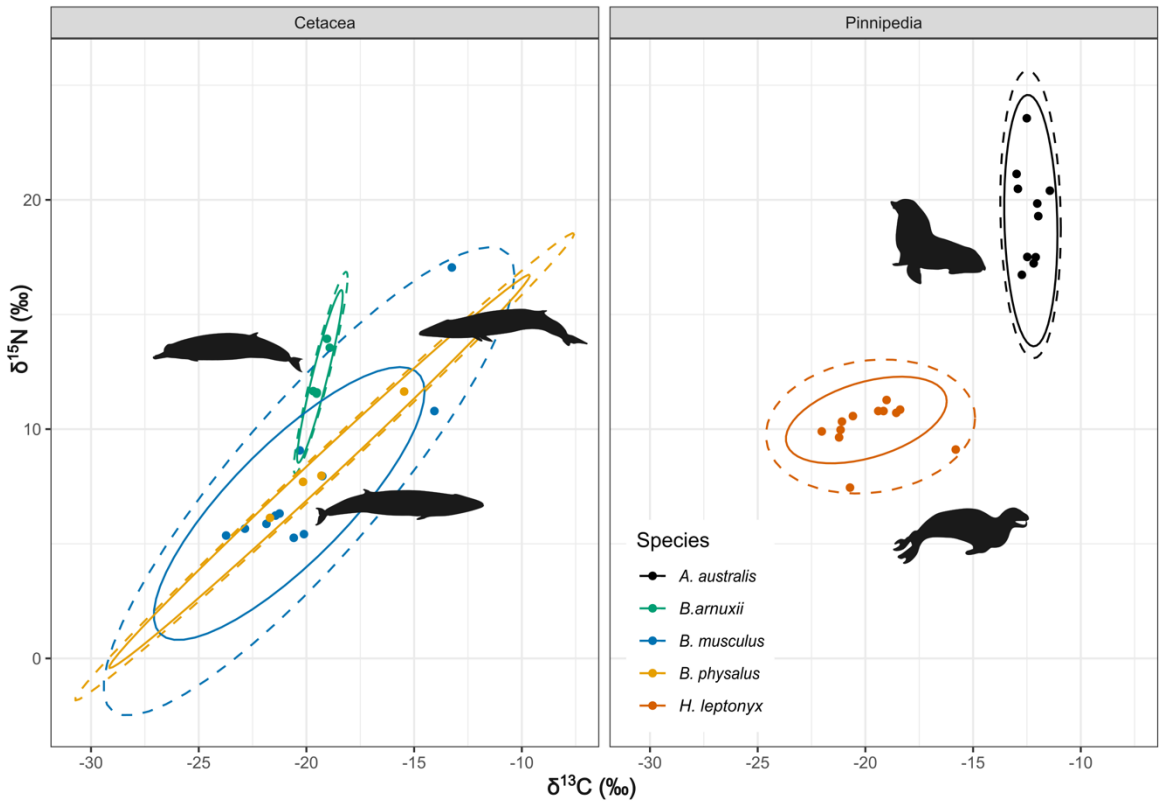

Figure 2. $\delta^{13}C$ (Suess corrected) and $\delta^{15}N$ biplot in the bone of marine mammals sampled in Subantarctic waters between the 1880s and 1960s. Solid line ellipses correspond to a multivariate t-distribution, dashed line ellipses correspond to a multivariate normal distribution.

pre- and post-1904 stable isotopic values

In total, 20 specimens pre-dating the opening of the whaling station at South Georgia were analyzed, including three specimens of blue whale, six specimens of South American fur seal, and 10 specimens of leopard seal (Table 5). The mean raw $\delta^{13}C$ value in blue whale over this

period was -15.86 ± 2.2, and mean $\delta^{15}N$ value 12.3 ± 2.42 (range 9.07 – 17.05), South American fur seal $\delta^{13}C$ -12.26 ± 0.21 and $\delta^{15}N$ 20.32 ± 0.7, and leopard seal -19.71 ± 0.59 and $\delta^{15}N$ value 10.32 ± 0.21. Suess corrected $\delta^{13}C$ values deviated by 0.003‰ and 0.11‰ with a mean value of 0.029‰ across all samples and species studied (Table 5).

Table 5. Stable isotope ratios in bone collagen of NHM specimens pre-dating the opening of whaling station at South Georgia.

NHMUK ID	Species	Location	Year*	Raw δ13C	Suess corrected δ13C	δ15N	C:N mass ratio
ZD.1880.7.28.19	*B.musculus*	Strait of Magellan*	March 1879	-20.29	-20.31	9.07	3.58
ZD.1879.8.21.9	*B.musculus*	Strait of Magellan	March 1879	-14.04	-14.06	10.79	3.49
ZD.1879.8.21.8	*B.musculus*	Strait of Magellan	February 1879	-13.23	-13.23	17.05	3.45
ZD.1852.3.11.5	*A.australis*	Falkland Islands	before 1850	-12.97	-12.98	21.13	3.34
ZD.1879.8.21.5	*A.australis*	Strait of Magellan	1878 to 1880	-12.08	-12.09	17.50	3.31
ZD.1880.7.28.12	*A.australis*	Strait of Magellan	1878 to 1880	-11.96	-11.97	19.29	3.35
ZD.1880.7.28.13	*A.australis*	Strait of Magellan	1878 to 1880	-11.42	-11.43	20.40	3.29
ZD.1880.7.28.14	*A.australis*	Patagonian west coast	1878 to 1880	-12.49	-12.51	23.56	3.34
ZE.1950.11.14.3	*A.australis*	Patagonian west coast	04/01/1876	-12.01	-12.02	19.84	3.30
ZE.1950.11.14.4	*A.australis*	Patagonian west coast	04/01/1876	-12.91	-12.92	20.48	3.48
ZD.1843.1.8.4	*H.leptonyx*	Antarctic Seas	1843	-22.02	-22.02	9.90	3.36
ZD.1846.4.15.23	*H.leptonyx*	Antarctic Seas	1843	-18.38	-18.39	10.85	3.34
ZD.1846.4.15.24	*H.leptonyx*	Antarctic Seas	1843	-21.08	-21.09	10.33	3.39
ZD.1876.1.28.81	*H.leptonyx*	Kerguelen	13/10/1874	-15.80	-15.80	9.11	3.32
ZD.1880.7.28.5	*H.leptonyx*	Falkland Islands	1878 to 1880	-19.00	-19.02	11.27	3.51
ZD.1885.10.20.1	*H.leptonyx*	Falkland Islands	before 1885	-18.55	-18.57	10.71	3.84
ZD.1893.9.14.1	*H.leptonyx*	South Georgia	before 1893	-19.39	-19.40	10.79	3.35
ZD.1908.2.20.54	*H.leptonyx*	Ross Sea	1901 to 1904	-21.20	-21.22	9.64	3.51
ZD.1908.2.20.56	*H.leptonyx*	Ross Sea	1901 to 1904	-20.56	-20.58	10.57	3.38
GERM.325.e	*H.leptonyx*	Antarctic Seas	1839 to 1843	-21.15	-21.15	9.97	3.42

*'Strait of Magellan' dataset consists of material from the Strait itself and surrounding archipelagoes along the Chilean Pacific coastline / West coast of Patagonia (see Table 1 Supplementary material 2 for details).

Among the more recent specimens, the $\delta^{13}C$ mean value for blue whale was -20.33 ± 0.51, and mean $\delta^{15}N$ value was 6.01 ± 0.31 (N = 8). In South American fur seal, the mean $\delta^{13}C$ value

was -12.42 ± 0.16 and $\delta^{15}N$ mean value 17.16 ± 0.23 (N=3). In leopard seal, the mean $\delta^{13}C$ value -was 19.85 ± 0.8 and $\delta^{15}N$ 9.12 ± 1.67 (N = 2).

Differences were most pronounced in $\delta^{15}N$ values for South American fur seal (PERMANOVA F=14.61, p = 0.041) (Figure 3), yet no difference in $\delta^{13}C$ values for this species was found between the end of the 19th and early 20th centuries. Despite the large variability in $\delta^{13}C$ values in general, no significant changes in either $\delta^{13}C$ or $\delta^{15}N$ in leopard seals were found. Changes in stable isotope signatures after 1904 in blue whale were significant for both carbon and nitrogen with more pronounced changes in $\delta^{15}N$ (PERMANOVA F=38.94, p=0.0011); differences in $\delta^{13}C$ were also significant (PERMANOVA F=12.73, p=0.0046).

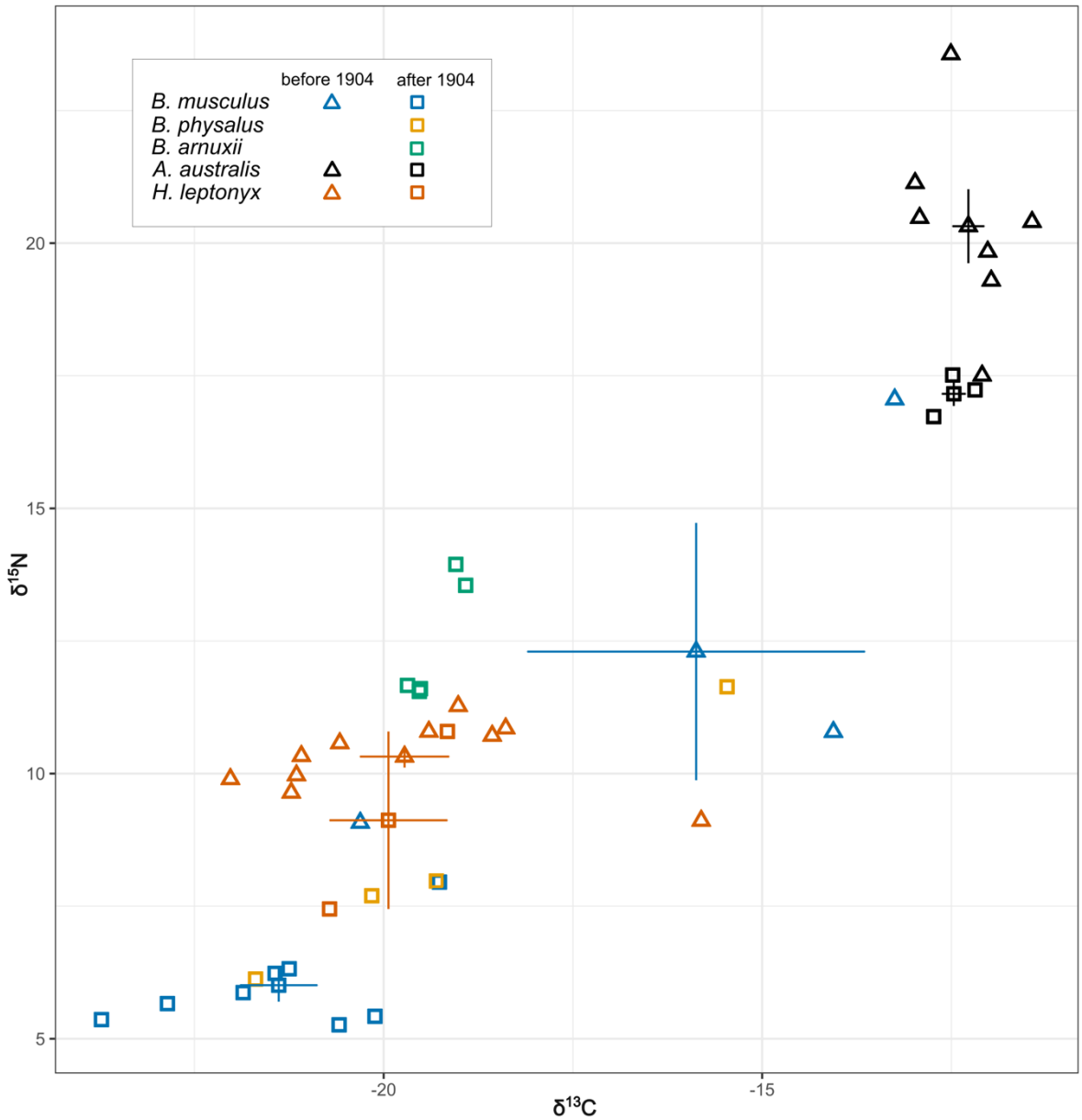

Figure 3. Suess corrected $\delta^{13}C$ and $\delta^{15}N$ biplot summarizing within-species variability of samples collected before (triangles) and after (squares) the opening of whaling station at South Georgia in 1904.

Estimation of trophic positions using $\delta^{15}N$ values

Among the baleen whale individuals, the calculated trophic position (TP) varied between 3.1 and 5.3 in fin whale and between 2.4 and 5.7 in individual blue whales, but averaged TP was 3.4±0.3 across all specimens (Figure 4A). In both fin and blue whale specimens pronounced spatial heterogeneity was found showing that TP was higher at the South Shetland Isles compared to South Georgia. In Arnoux's beaked whale TP varied between 2.9 to 7.3 with higher values at the South Shetlands and lower values at the Falklands.

South American fur seal TP varied from 5.1 to 8.4 with higher values in the Straits of Magellan and lower values in the Falkland Islands. Leopard seal TP varied between 2.6 and 6.3. Highest TP values in leopard seal were found in the Ross Sea, lowest values in specimens from the Falklands.

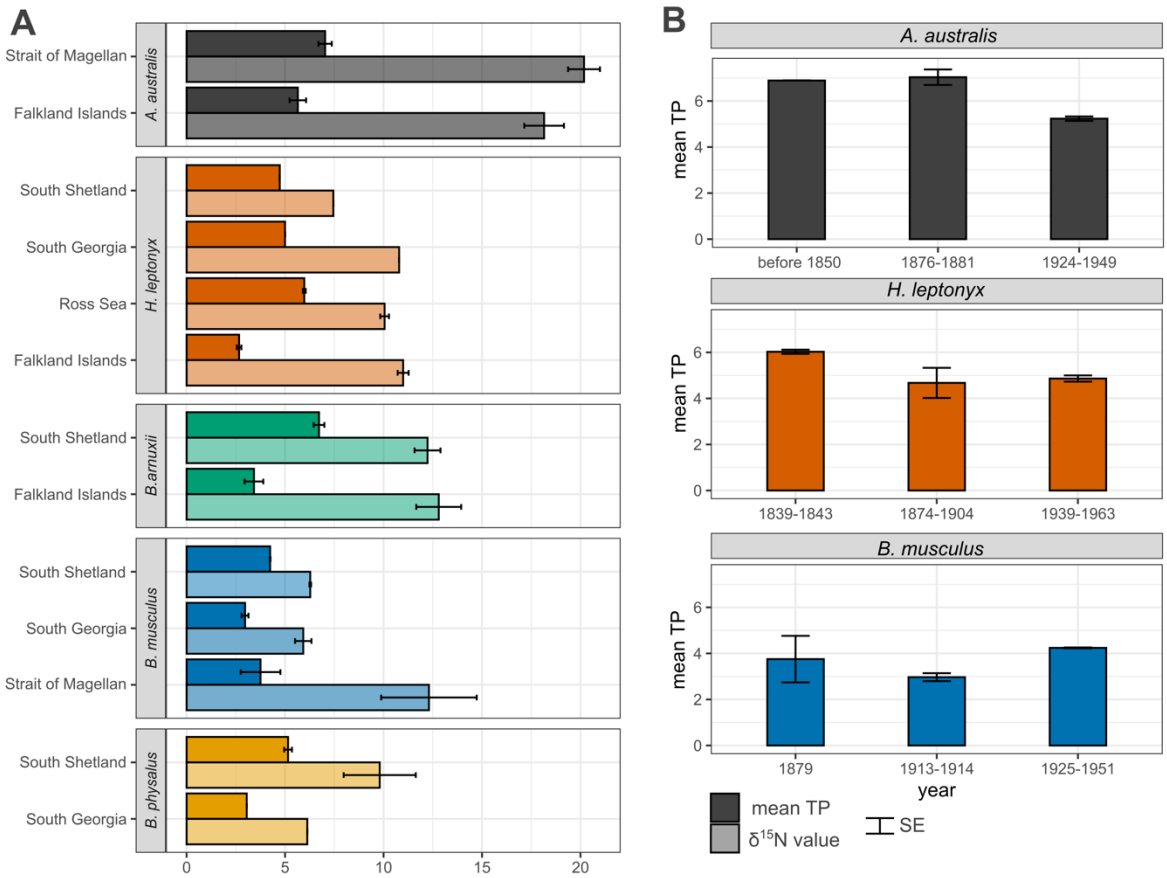

Figure 4. Averaged trophic position (**A,** solid-filled bars) and corresponding averaged bone collage $\delta^{15}N$ values (**A,** semi-transparent bars) of five marine mammal species in the various regions studied. Changes of trophic position estimates (**B**) for South American fur seal (*A. australis*), leopard seal (*H. leptonyx*), and blue whale (*B. musculus*) between the 1850s and 1960s.

Comparison of TP in different time periods (Figure 4B) of sampling using PERMANOVA analysis indicates significant TP differences in blue whale between the material from the 1913-1914 and 1925-1951 (PERMANOVA F=1.91, p=0.0367). Due to high within-sample variability, no differences between the 1879 samples and the subsequently collected material were revealed using PERMANOVA. The South American fur seal material from the 1870-1880s is significantly different from material collected during 1925-1951 (PERMANOVA F=8.7, p=0.0549). Contrasting with these two species, no significant temporal changes in TP of leopard seal between 1839 and 1963 is revealed (PERMANOVA F=1.37, p=0.0663).

Source prediction by MixSIAR

The Bayesian mixing modelling approach was applied to the datasets on blue whale and South American fur seal, because these species demonstrated significant changes in trophic position over the period between 1850s and 1950s. Based on stable isotope data of four species of krill from contrasting locations around study area, MixSIAR estimated that the major source of food of blue whales collected in the Magellan Strait in the late 19th-century originated in Subantarctic waters, matching the geographic origin of the bone specimens (Figure 5A). MixSIAR model predictions for specimens representing the two following periods (Figure 5B,C) were non-identical. While all these samples were collected in waters surrounding South Georgia, MixSIAR indicated primary foraging grounds matching the geographic origin of the specimens dated 1913-1914, and a mix of foraging areas in 1925-1951, including the Polar Front region.

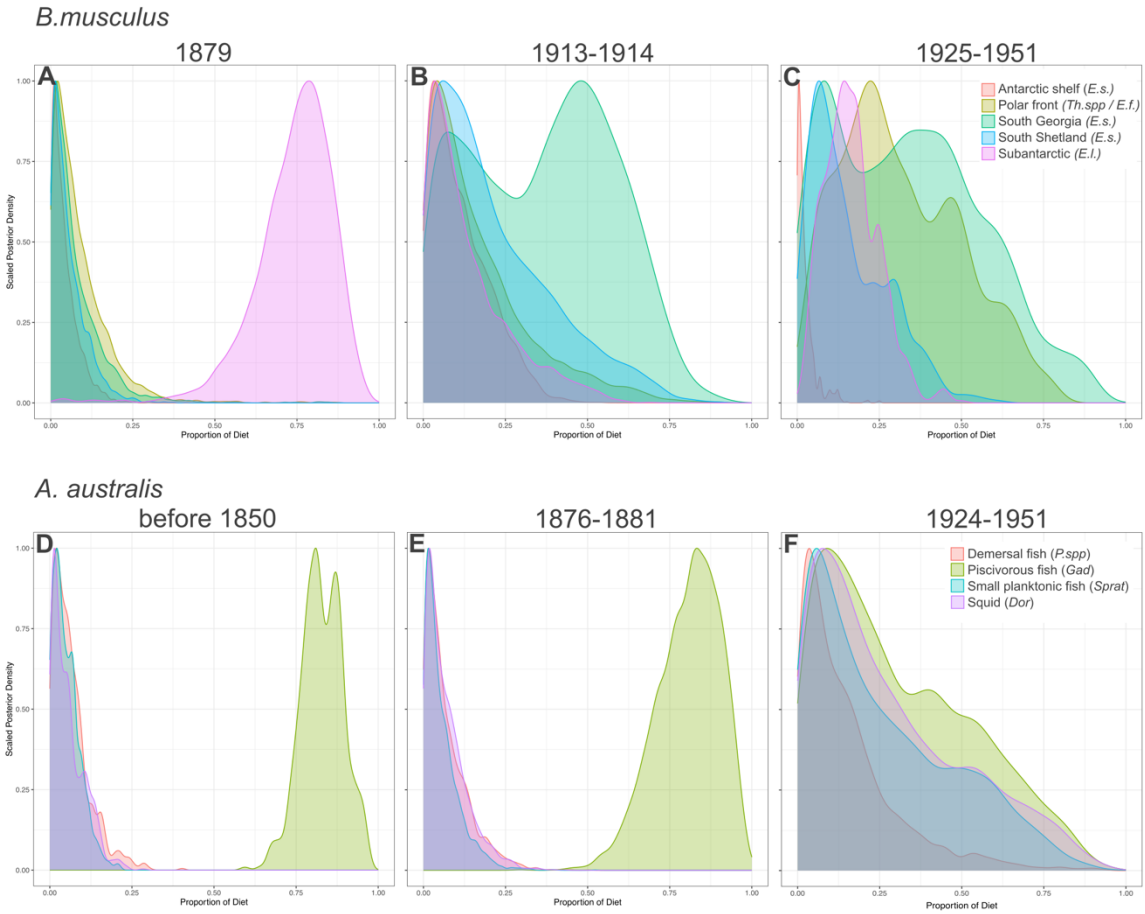

Figure 5. Comparison of MixSIAR-estimated dietary source contributions (as Bayesian credibility intervals and posterior densities) for the blue whale *B. musculus* (A to C) and South American fur seal *A. australis* (D to F). Abbreviations indicate potential prey items: *E.s. – Euphasia superba, Th. spp – Thysanoessa spp., E.f. – Euphasia frigida, E.l. – Euphasia lucens, P.spp - Patagonotothen spp., Gad – Gadidae, Sprat - Sprattus fuegensis, Dor – Doryteuthis (Loligo) gahi.*

MixSIAR model predictions for South American fur seal considered only one foraging area, Subantarctic waters. All specimens from the 19th century indicated similar dominant prey sources – piscivorous fish belonging to Gadidae family (Figure 5D,E). Specimens dated

between 1924 and 1951 demonstrated a changed pattern of mixed dietary sources in which each species included in the analysis contributed to diet nearly equally.

Discussion

The number of studies examining the impacts of industrial whaling during the 20th-century is increasing (11,17,18,27,38). So, it is a priority to accumulate as much as possible data representing the state of their populations prior to the onset of industrial-scale whaling. Despite the several uncertainties in the dataset, particularly the origin and often the unclear state of preservation of several specimens, this study provides a snapshot of stable isotope signatures of common Antarctic and Subantarctic marine mammals during the period of extensive exploration of Antarctic waters in the nineteenth century and at the turn of the 20th-century.

Methodological considerations of stable isotope analysis of specimens of opportunity

Museum specimens represent a unique source of information about past populations and rare species (16), but in some cases can be difficult to handle if their provenance is unclear (39). Our dataset covers the period between the 1840s and 1960s, and while the geographical origin and period of collecting is known, and accurate to at least within a year for several specimens, there are uncertainties associated with the either location or date of collection of others. The highest level of such uncertainty in the dataset is linked to the *Discovery* Investigations programme that ran between 1924 and 1951, including several specimens of blue whale *B. musculus* and South American fur seal *A. australis*, the latter being collected prior to 1949 (Table 1 Supplementary Material 2). For all the specimens with uncertain year of collection, the known time interval of the expedition or research program was used in comparative analyses.

The stable isotope analysis approach is based on the relationship that carbon and nitrogen isotopes in the predator tissues reflect those of its prey in a predictable way (40). Factors such as nutrient resources, composition of primary producers and regional geographic characteristics result in distinct isotopic patterns between different regions (41), allowing the use of stable isotope analysis for investigating large-scale movements of predators (41,42). Stable isotope analysis has been useful in detecting niche overlap and trophic interactions between different trophic levels (43). Implementation of $\delta^{13}C$ and $\delta^{15}N$ isotope analyses, as well as ^{14}C radiocarbon dating of bone fragments, rely on the preservation of collagen in bone samples that can be evaluated using carbon to nitrogen (C:N) atomic ratio (44).

Five specimens in our dataset demonstrated slight deviations from the commonly accepted range of C:N atomic ratio between 2.9 and 3.6. Since these specimens passed the other quality control indicators (Table 1, Supplementary Material 2), namely percentage of carbon higher than 13% and percentage of nitrogen higher than 4% (45) and did not deviate strongly from the other specimens from same locations and periods, they were retained in our dataset.

We used the correction of raw $\delta^{13}C$ values for the Suess effect, which is crucial if modern and historic specimens are included in the analysis, especially if various species in the same region demonstrate different trajectories of long-term changes of $\delta^{13}C$ values. A study of four species of penguins from subantarctic French Southern Ocean and Antarctic territories (46) demonstrated that Suess-corrected $\delta^{13}C$ values did not change over time in Antarctic species from Adelie Land, but decreased in Subantarctic and subtropical species. However, Suess-

corrected $\delta^{13}C$ values of the Weddell seals from the Ross Sea decreased during the 20th century (47), indicating changes in the primary productivity of this sector of the Antarctic. In our dataset, the correction values did not alter the results based on raw $\delta^{13}C$ values but were necessary for comparison of the carbon signatures among specimens from the same species collected at various locations during different periods. The oceanic Suess effect was evaluated to be weak near the Antarctic continent and higher in Subantarctic waters (48). Based on the global database of the full oceanic Suess effect (49), the average correction value for our dataset was 0.027‰, varying between 0.004‰ (for samples dated 1839) and 0.11‰ (for samples dated 1963). When the date of specimen collection was unknown, we used the earliest date within the period indicated.

Evaluation of dietary preferences is conventionally based on $\delta^{15}N$ isotope analysis. By analysing the nitrogen signatures of potential prey items or most common taxa in the ecosystem, it is possible to evaluate the trophic relationships between consumers of different levels, even if stomach content data for the particular population or species are scarce (50–54). Conventional bulk nitrogen isotope analysis is based on assumption that isotopic composition of a consumer reflect the isotopic composition of primary producers at the baseline of the food web (40). Since $\delta^{15}N$ values from bulk stable isotope analysis cannot be compared directly among tissues derived from food webs in different areas (26,55), but the comparisons are possible through calculating trophic position based on $\delta^{15}N$ values of lower trophic levels, or particulate organic matter (POM) in aquatic ecosystems (8,30,56). In recent decade, stable isotope analysis of specific compounds within tissues, such as individual amino acids, became increasingly popular in archaeological, trophic and animal migration studies (57,58), and when possible, comparisons of bulk and CSIA are being implemented (59).

Since specimens representing different periods were often collected at different locations (all species except the South American fur seal), and also because the complexity of food webs in Antarctica varies with latitude (60–62), we calculated the trophic position for all four species in focus based on regional variation of the modern $\delta^{15}N$ values of POM from the corresponding regions (See Materials and Methods for details). Despite the obvious constraints mentioned above, this approach (Figure 4) allowed to provide more accurate geographic comparisons of nitrogen signatures.

In addition, we applied Bayesian mixing models (implemented in MixSIAR package) to evaluate the dietary shifts in two species - blue whale and South American fur seal. Mixing models are extensively used to evaluate the percentage contribution of potential prey items to the diet of consumers (32,63–68). This method is increasingly being used to reconstruct dietary preferences of past populations from museum specimens using modern data on diet and isotopic signatures of major prey items (51,54,69).

The geographic differences in isotopic signatures raise questions about population connectivity and foraging strategies in highly mobile marine species. We discuss below how the observed variation in carbon and nitrogen stable isotope signatures of common marine mammal bone collagen samples can be linked with rapid changes in Antarctic and Subantarctic ecosystems during the period of their acquisition by the NHM.

Spatial and temporal variation in stable isotope values

We found pronounced variation in bone collagen $\delta^{13}C$ and $\delta^{15}N$ values across the two species of baleen whales (*B. musculus* and *B. physalus*) and in leopard seal (*H. leptonyx*). In South

American fur seal (*A. australis)* we observed temporal changes in $\delta^{15}N$ and no variation in $\delta^{13}C$ values. Only a minor variation in isotopic signatures of Arnoux's beaked whale (*B. arnuxii*) was found. The observed intraspecific variation in isotopic signatures mostly corresponded to the geographic origin of specimen collection, but also may potentially indicate temporal variation in foraging strategies and distribution of highly mobile species.

The blue whale specimens demonstrated the largest within-species variation in both carbon and nitrogen isotopes in our dataset. Among these specimens, highest variation was observed among the 1870s data from the Magellan Strait. Two of three 1870s specimens demonstrated $\delta^{13}C$ signatures significantly different from the more recent specimens, and all three deviated from the post-1904 specimens in their $\delta^{15}N$ signatures. The averaged trophic position of blue whale was similar in different regions based on the background of the pronounced geographic variation in $\delta^{15}N$ values. The post-1904 isotope data correspond well with 1940s baleen plate data of whales collected near the Ross Sea (56).

The potential explanation for the unexpectedly high individual TP value (5.7) obtained for the specimen dated 1879 from Strait of Magellan could indicate that this whale does not belong to the region where it was collected. We used POM values to calculate TP of the three blue whales from the Strait of Magellan, but this approach could be an oversimplification for the highly migratory animals like blue whales. Studies undertaken between the 1920s and 1950s interpreted the Eastern South Pacific blue whales as Antarctic blue whales regularly migrating between higher and lower latitudes. Following the discovery of nursery and feeding grounds in Southern Chile (70), Eastern South Pacific and Antarctic populations of blue whales were considered as different subspecies, but satellite tracking data indicate that blue whales from Chile migrate as far north as the Galapagos Islands (70). Blue whales in the Southern Hemisphere were hunted nearly to extinction during the 20th century, and the modern population estimates are considered to be less than 1% of the pre-whaling period (71). Thus, the modern within-species structure of blue whales in the Southern Hemisphere is likely to be substantially different to the pre-whaling period. Recent genetic studies reveal that many Antarctic blue whales demonstrate signatures of admixture with populations from higher latitudes (38), providing a plausible basis for the interpretation of past connectivity between these populations.

Krill (Euphasiacea) is of the primary prey sources of baleen whales, of which the Antarctic krill *Euphasia superba* is considered the main prey item of Antarctic blue whales (72,73). Blue whales seasonally forage in productive coastal areas, and separate populations rely on different areas with contrasting oceanographic characteristics and krill species which can be traced using stable isotope analysis (74). As the geographic origin of several of the studied blue whale specimens are unknown, the Bayesian mixing models for the blue whale were applied as source data using several of the most common krill species from various latitudes from the Antarctic shelf to Subantarctic waters around South Patagonia and the Falkland Islands (Figure 5). This approach indicates isotopic signatures matching the origin of specimens for both 1870s specimens from Strait of Magellan and 1913-1914 specimens from South Georgia. In contrast, trophic signatures of blue whales caught in the open sea during the 1925-1950 period were mixed. The latter can either indicate mixed geographic origin of the studied specimens that were later landed at South Georgia, but also can be explained by a shift in the foraging areas of blue whales escaping the whaling regions.

Our dataset included four fin whale specimens collected between 1912 and 1951 at South Georgia, the South Shetlands and in the South Atlantic. These specimens also demonstrated a pronounced variation in carbon and nitrogen isotopes, and while these results can be considered preliminary, they corroborate a recent baleen plate isotopic study concluding that the blue and fin whale in the Southern Ocean occupy a similar trophic level but demonstrate niche differentiation (56).

The $\delta^{13}C$ values of Arnoux's beaked whale did not vary between the specimens from the Falkland Islands and South Shetland Islands. Though there is a minor variation in $\delta^{15}N$ values, this dataset is not sufficient for evaluating diet variation. The isotopic signatures obtained in our study are also similar to the beaked whale from Falkland Islands (37), and indicate a narrow isotopic niche of beaked, at least during 1920s-1930s. While most sightings of this rare species are from the fast-ice edges of the Antarctic seas and the nearshore of South Africa, New Zealand, and Australia (75), our data confirm the previous hypotheses of the association of this species with deep offshore marine areas.

Our data indicate changes in the diet of the South American fur seal from the Falkland Islands against a background of consistent regional $\delta^{13}C$ values. Estimation of trophic position from the $\delta^{15}N$ values reveal a significant change in the trophic preferences of fur seal at the Falkland Islands from the 19th to the 20th century. The diet of South American fur seal includes both coastal and open-sea shelf prey items (76). Currently the dominant prey items of fur seals at the Falklands are Falkland herring *Sprattus fugensis*, and several other taxa including Patagonian squid *Doryteuthis gahi*, and several rock cod species *Patagonotothen spp.* (77). While piscivorous offshore fish species are rarely found in stomachs of fur seals (76,77), these were included among other potential sources in our mixing model (Figure 5), primarily as an outgroup, and because recent studies based on satellite tracking data indicated long-distance foraging migrations in several colonies of South American fur seal (34). Calculation of trophic position and illustration of dietary preferences using the mixing models both indicate that South American fur seals could have had more selective feeding in the 19th-century, and more opportunistic feeding in the 20th-century, possibly due to a decline in preferred prey species. Seals from 1870s were presumably foraging for larger prey of higher trophic levels. As several fish taxa in Patagonian waters have recently experienced overfishing (78–80), our data indicate that observed changes in the diet of South American fur seal represent an adaptation to changes in the pelagic ecosystem.

Leopard seal is a species capable of foraging on taxa at various trophic levels, including other seal species, penguins, fish, and krill (81–83). Huge variation in dietary preferences should affect the TP in different locations, seasons and even particular individuals. The highest TP in leopard seal was typical of specimens from the Ross Sea (5.97 ± 0.19), with samples with the lowest TP from the Falkland Islands (2.66 ± 0.16). TP of leopard seal can be explained by contrasting foraging strategies of this species in these two regions. As with the other species, we divided the TP data into three periods, but no significant differences between seals from these periods were found because differences within each temporal subsets was higher that between the three subsets (Figure 4). According to these data, TP of leopard seal was nearly twice lower compared to the South American fur seal. This finding is remarkable considering that fur seal pups are one of the common prey items for the leopard seal.

CONCLUSIONS

Bulk stable isotope analysis of museum specimens in this study provides a record of Subantarctic and Antarctic marine environments during the period of active exploration and exploitation of marine resources in the region at the turn of the 20th century. Here we show how this dataset can be used to address the questions about the influence of industrial whaling, seal hunting and even development of fishing on the foraging of whales and seals. This study discusses the possible constraints for using this dataset in the standard ecological analyses aimed at understanding the long-term changes in Antarctic marine ecosystem using several keystone species.

Our results suggest that the major foraging grounds of baleen whales could have been different before the extensive whaling started in 1900s from South Georgia and South Shetland Islands with more contacts between the populations from Southern Ocean and South Pacific. South American Fur seal population at the Falkland Islands could have had foraged for a prey of higher trophic levels before the onset of industrial fishing in the region, and the leopard seal demonstrated high degree of adaptation to the local availability of prey objects. Compared to the other species, Arnoux's beaked whale, a rare species that was not targeted by whaling industry, did not demonstrate pronounced variability in stable isotope signatures. These hypotheses, raised on the basis of conventional bulk stable isotope analysis, should be further verified on the basis of larger sample sizes for each species, comparison with modern data from Antarctic and other regions within the species ranges, and using more advanced methods such as compound-specific isotope analysis that can more accurately distinguish environmental and dietary signatures.

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