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Penguin tissue as a proxy for relative krill abundance in East Antarctica during the Holocene

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Antarctic krill (*Euphausia superba*) is a key component of the Southern Ocean food web. It supports a large number of upper trophic-level predators, and is also a major fishery resource. Understanding changes in krill abundance has long been a priority for research and conservation in the Southern Ocean. In this study, we performed stable isotope analyses on ancient Adélie penguin tissues and inferred relative krill abundance during the Holocene epoch from paleodiets of Adélie penguin (*Pygoscelis adeliae*), using inverse of $\delta^{15}\text{N}$ (ratio of $^{15}\text{N}/^{14}\text{N}$) value as a proxy. We find that variations in krill abundance during the Holocene are in accord with episodes of regional climate changes, showing greater krill abundance in cold periods. Moreover, the low $\delta^{15}\text{N}$ values found in modern Adélie penguins indicate relatively high krill availability, which supports the hypothesis of krill surplus in modern ages due to recent hunt for krill-eating seals and whales by humans.

The Southern Ocean is biologically the world's most productive ocean. At the hub of the Antarctic marine food web, krill is the primary consumer of diatoms, the major prey for many species of fishes, penguins, seals and whales^{1–3}, and a substantial commercial fishery resource¹. The abundance of krill is very sensitive to climate change and has significant impacts on high trophic-level predators in the Southern Ocean ecosystems^{4,5}. Recent Antarctic krill populations have been significantly influenced by rapid climate change, human removal of krill-eating predators and intense commercial fishing^{1,6,7}. Therefore, the records of krill population change, especially those pre-dating the onset of human harvesting in Antarctic, are crucial for understanding and predicting responses of krill population to natural climate changes.

Modern krill abundance can be obtained directly from acoustic and net surveys. Krill population data from scientific trawls are available for the past ~30 years⁶. For historical krill abundance, only about 100 years of data have been inferred from Antarctic fur seals⁸. The long-term historical krill abundance prior to human intervenes remains unknown. Similar to Antarctic fur seal, Adélie penguin is an important land-based krill predator, which has been chosen as an indicator of changes in krill availability and abundance by the commission for the conservation of Antarctic marine living resources⁹. Furthermore, Adélie penguin is a circum-Antarctic distributed species; continuous and long time-series remains of Adélie penguin could be preserved in various lake sediments¹⁰. Thus, Adélie penguin is an ideal indicator for tracking krill availability and abundance over a long period of time.

Stable isotope analysis of animal tissues is a powerful tool for examining wildlife foraging habitats, diet and migration patterns^{11–13}, especially in historical periods^{14,15}. Keratinous tissues such as toe nails, feathers and hair, and bone collagen can preserve dietary information for long periods of time¹⁶, particularly in the cold and dry Antarctic environment. They are ideal for investigating paleodiets of krill predators⁸. Hairs and faeces in lake sediments have been used successfully to infer past population dynamics of seals and penguins in Antarctica^{17–19}. Similar stable isotopes and biomarkers have also been used extensively in northern high-latitude or Arctic regions to study the effects of climate change, seabird colonisation and past whaling activities on lake ecosystems^{20–22}.

Here, we analyzed stable nitrogen isotope ratios ($^{15}\text{N}/^{14}\text{N}$, expressed as $\delta^{15}\text{N}$) of modern and ancient Adélie penguin bones and feathers from the Vestfold Hills, East Antarctica (Figure 1^{23,24} and Figure 2²⁵), with the main aim of inferring the relative krill abundance over a long historical time. Several observations follow. First, $\delta^{15}\text{N}$ values in penguin tissues show an enrichment of ~3–5‰ from prey to predator in marine ecosystems¹¹. Second, as a diet of penguins, krill are much lower in $\delta^{15}\text{N}$ values than fishes are¹⁵. Third, Adélie penguins feed preferentially on krill species^{26–28} of *Euphausia superba* in the Antarctic Peninsula and of *Euphausia crystallorophias* along

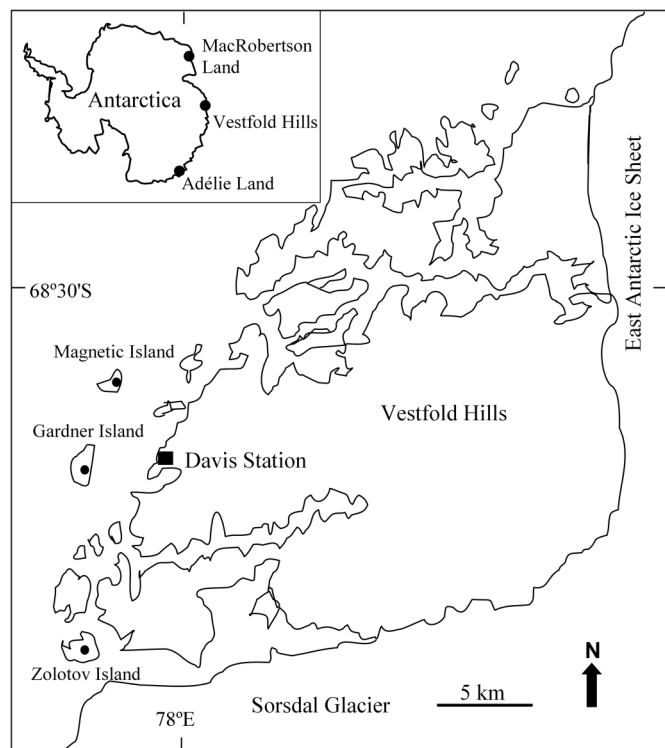


Figure 1 | Map of the Vestfold Hills including the sampling site in this study and two sites in previous studies^{23,24} in East Antarctica. (The map was drawn using Microsoft Excel 2010 and then converted to tiff format using Microsoft Office Visio 2007).

the East Antarctic coasts. Lower $\delta^{15}\text{N}$ values in Adélie penguins indicate diets based primarily on krill, while higher values indicate diets richer in fishes and other species of potentially higher trophic level (e.g., squid). The variations in $\delta^{15}\text{N}$ values of penguin tissues

indicate the changes in the proportion of krill in penguin diets and thus the krill availability and abundance in the foraging area²⁹. The $\delta^{15}\text{N}$ values in penguin tissues through time could serve as a proxy for krill availability and abundance in foraging areas.

Results

The $\delta^{15}\text{N}$ values of penguin bones and feathers at different depths of a sediment core DG4, as well as modern samples from Magnetic, Zolotov, and Gardner Island at Vestfold Hills, are given in Table 1. The $\delta^{15}\text{N}$ values of main preys for Adélie penguin in East Antarctica^{30,31} are plotted in Figure 3. Sample sizes (n) reported below are the numbers of the distinct depths in the DG4 sediment at which samples were collected, as shown in Table 1. Modern Adélie penguins at Vestfold Hills have $\delta^{15}\text{N}$ value (mean \pm standard error of mean) of $10.1 \pm 0.3\text{‰}$ for bones ($n = 6$) and $10.0 \pm 0.04\text{‰}$ for feathers ($n = 6$), which are very close to those reported in Adélie Land ($9.4 \pm 0.09\text{‰}$, $n = 20$) and MacRobertson Land ($9.4 \pm 0.2\text{‰}$, $n = 31$), East Antarctica^{23,24} (Figure 3). The $\delta^{15}\text{N}$ values of ancient Adélie penguins are much higher. They ranged from 12.5‰ to 18.1‰ in the bones with a mean of $15.3 \pm 0.6\text{‰}$ ($n = 10$), and ranged from 11.3‰ to 15.5‰ with a mean of $13.6 \pm 0.4\text{‰}$ ($n = 11$) in the feathers (Table 1 and Figure 4).

The $\delta^{15}\text{N}$ values in modern Adélie penguin bones and feathers at Vestfold Hills are similar (Wilcoxon rank sum test statistic $T = 45$, $p = 0.35$). For the same type of tissues, $\delta^{15}\text{N}$ in modern and ancient samples are significantly different (for bones: $T = 115$, $p = 0.0002$, and for feathers: $T = 132$, $p = 0.0002$), with higher $\delta^{15}\text{N}$ found in ancient penguins.

In addition, the $\delta^{15}\text{N}$ values in ancient penguin feathers and bones between warm and cold climate conditions are also significantly different. Here, we determined the episodic warm periods (7500–6300 yr BP and 4800–2200 yr BP) and cold periods (8500–7600 yr BP and 6300–5700 yr BP) according to previously reconstructed climate change during the Holocene in East Antarctic based on ice cores³² and marine sediment cores^{33,34}. Using information of warm/cold periods provided in Table 1, we were able to compare $\delta^{15}\text{N}$

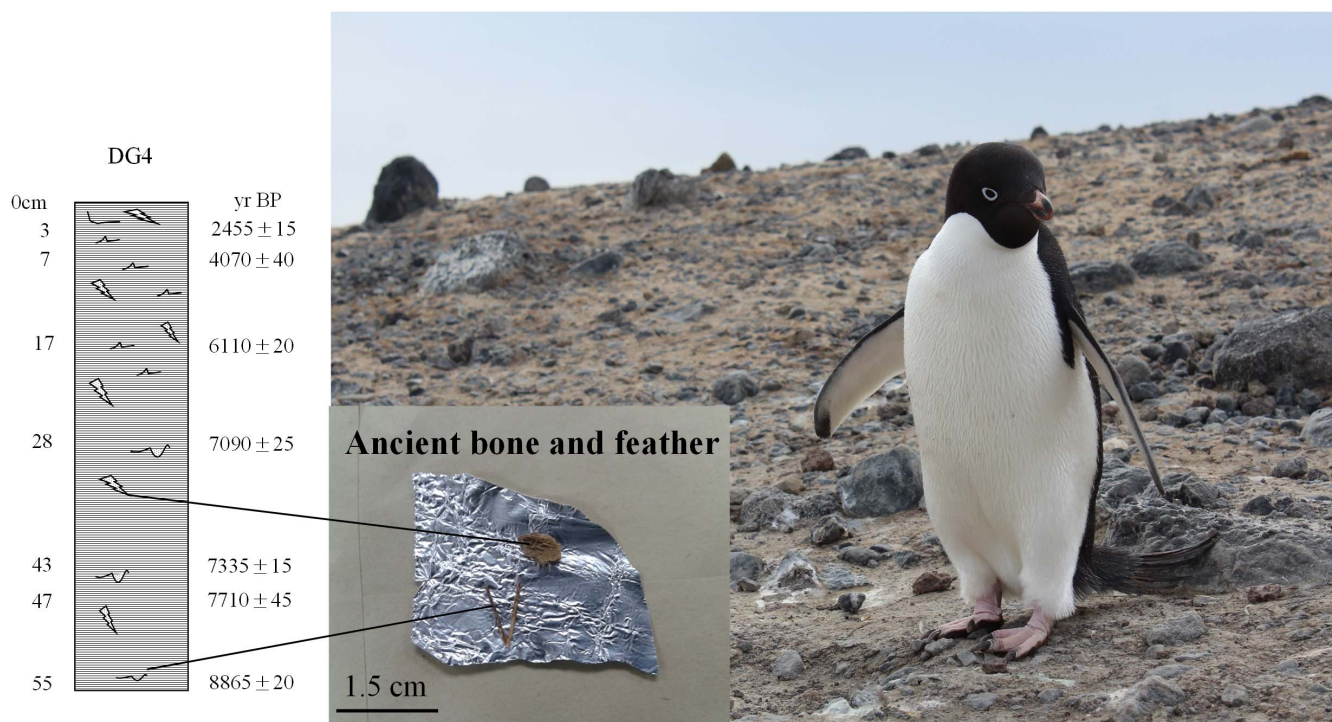


Figure 2 | Adélie penguin in the Vestfold Hills and the bones and feathers from the sediment core DG4 profile with conventional AMS ^{14}C dates²⁵ (yr BP: years before present). Photo credit: T. Huang.



Table 1 | Stable nitrogen isotope ratios (expressed as $\delta^{15}\text{N}$) of modern and ancient penguin tissues collected from Vesfold Hills, East Antarctica. The interpolated radiocarbon age of ancient penguin tissues were calculated based on AMS ^{14}C data from a previous study²⁵. Also indicated are reconstructed warm/cold climate periods^{32–34} to which the sample at each depth corresponds (slash = neither warm nor cold)

Tissue (No.)	Depth (cm)	Age (yr BP)	$\delta^{15}\text{N}$ (‰)	Climate condition	Tissue (No.)	Depth (cm)	Age (yr BP)	$\delta^{15}\text{N}$ (‰)	Climate condition
Feather* (1)	-	Modern	10.0		Bone* (1)	-	Modern	8.7	
Feather* (1)	-	Modern	9.9		Bone* (1)	-	Modern	10.7	
Feather# (1)	-	Modern	9.9		Bone# (1)	-	Modern	10.8	
Feather# (1)	-	Modern	10.0		Bone# (1)	-	Modern	10.7	
Feather ^o (1)	-	Modern	10.0		Bone ^o (1)	-	Modern	10.3	
Feather ^o (1)	-	Modern	10.1		Bone ^o (1)	-	Modern	9.6	
Feather (2)	12	4617	14.9	warm	Bone (1)	6	2756	18.1	warm
Feather (1)	16	5453	13.6	/	Bone (1)	9	3786	17.3	warm
Feather (2)	20	6030	12.2	cold	Bone (2)	11	4361	15.1	warm
Feather (2)	23	6325	12.7	cold	Bone (1)	14	5071	13.9	/
Feather (1)	26	6530	14.4	warm	Bone (2)	21	6140	12.5	cold
Feather (1)	27	6581	15.5	warm	Bone (2)	22	6238	12.7	cold
Feather (1)	34	6805	14.9	warm	Bone (1)	30	6700	16.3	warm
Feather (2)	37	6874	14.0	warm	Bone (2)	36	6850	15.7	warm
Feather (1)	41	6998	13.8	warm	Bone (1)	39	6929	15.3	warm
Feather (1)	47	7725	11.3	cold	Bone (1)	40	6961	16.3	warm
Feather (1)	50	8015	12.8	cold					

Note:

*Magnetic Island,

^oZolotov Island,

[#]Gardner Island, all ancient feathers and bones were sorted from each 1-cm section of the DG4 sediment core from Gardner Island, and the bones or feathers at the depths where two subsamples were present were mixed.

values in cold periods against those in warm periods. It was found that $\delta^{15}\text{N}$ values of ancient Adélie penguin during cold periods are much lower than those during warm periods (Figure 4), in both bones ($T = 3$, $p = 0.05$, $n_{\text{warm}} = 7$, $n_{\text{cold}} = 2$) and feathers ($T = 10$, $p = 0.01$, $n_{\text{warm}} = 6$, $n_{\text{cold}} = 4$). Furthermore, when combining data from bones and feathers to form a single test, we observed even stronger evidence ($T = 21$, $p = 0.0001$, from a tissue-stratified Wilcoxon rank sum test). This implies the relative krill abundance in the cold periods is much higher as compared with that in the warm periods.

Discussion

Different penguin tissues have different metabolic rates, and their stable isotope signatures have different time scales, from days for blood plasma to weeks for feathers, and to months of average turnover-time for collagen of bones¹⁶. Because our samples were collected at a breeding site during a summer season, the stable isotope values of archive feathers in the sediment core DG4 reflect those of the average diets at the time of feather growth. The nitrogen isotope values of penguin bones reflect those of the average diets of Adélie penguins during the last turnover of their bone collagen. Visual inspection

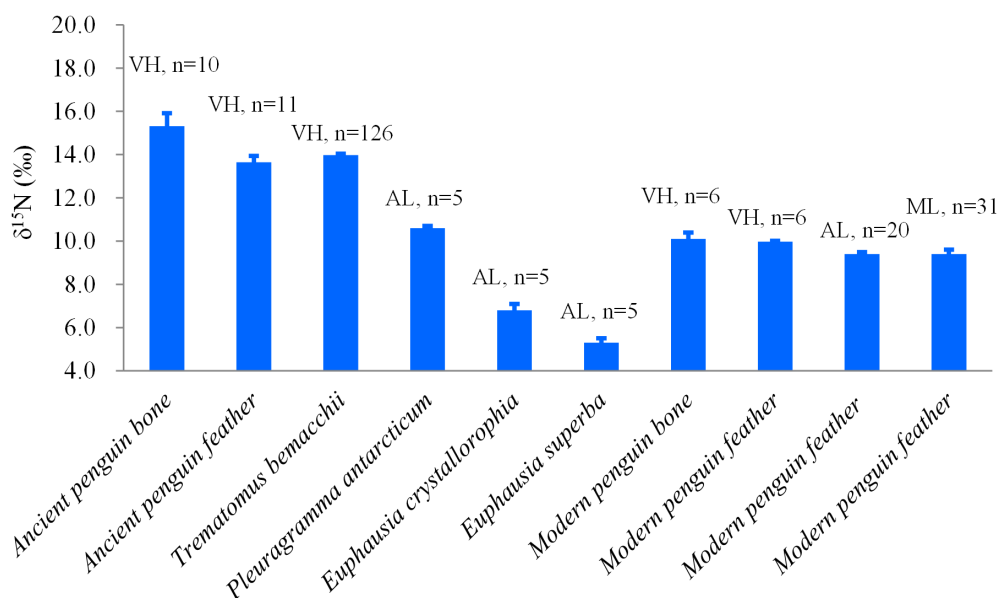


Figure 3 | $\delta^{15}\text{N}$ values (mean \pm standard error of mean) of Adélie penguins in this study and previous studies^{23,24}, and penguin prey $\delta^{15}\text{N}$ values compiled by Cherel et al. (2008)³⁰ for *Euphausia crystallorophias*, *Euphausia superba* and *Pleuragramma antarcticum* and Gillies et al. (2012)³¹ for *Trematomus bemaichii*. (VH: Vestfold Hills, AL: Adélie Land, ML: MacRobertson Land).

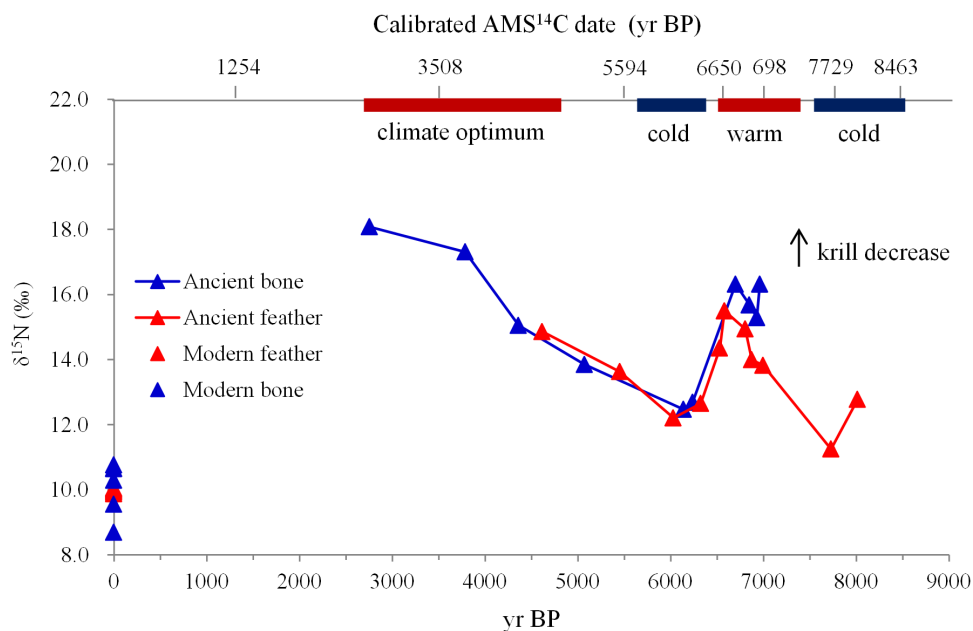


Figure 4 | $\delta^{15}\text{N}$ value fluctuations in Adélie penguin bones and feathers during the Holocene epoch, along with the episodes of climate change as inferred from ice cores and marine sediments in East Antarctica^{32–34}. The instrument error for the isotope analysis is $\leq 0.2\%$. Red bands refer to warm climate periods and blue bands to cold periods and expanded sea ice condition.

showed that the ancient feathers in DG4 were from adult penguins whereas the source was uncertain for the bones (which can be from adults and/or juveniles). Since the trend and magnitude of $\delta^{15}\text{N}$ in the ancient penguin feathers are similar to those in the bones (Figure 4), the possible age difference in the penguin bones seems unlikely to drive the temporal change of $\delta^{15}\text{N}$ during the Holocene epoch.

Variation of the inferred relative krill abundance (represented by the inverse of $\delta^{15}\text{N}$ values in penguin tissues) was found to be in accordance with previously reconstructed episodes of climate change during the Holocene in East Antarctica^{32–34}. That is, periods with high krill abundance (lower $\delta^{15}\text{N}$ values) correspond to cold climates^{32,33}, whereas periods with low krill abundance (higher $\delta^{15}\text{N}$ values) correspond to warm climates^{32,34}. Krill is a stenothermal, cold-water planktonic species, and is very sensitive to natural climate changes. Krill abundance has been linked with expanded sea ice cover (extent and duration) and increased latitudinal extent of cool water^{6,35,36}, which are consistent with our findings. Moreover, our results from Adélie penguins are also consistent with those from Antarctic fur seal hairs from a sediment core spanning the past century in the West Antarctic Peninsula⁸. Taken together, these results suggest that the link between krill abundance and climate persists over the past 8,000 years in East Antarctica.

It has been reported³⁷ that $\delta^{15}\text{N}$ values of the primary producer (baseline) and thus penguins' preys may also be influenced by climates. This can in turn give rise to the change of $\delta^{15}\text{N}$ in penguins in response to varied climates. However, we note that the $\delta^{15}\text{N}$ values in the Southern Ocean phytoplankton (*fragilariopsis kerguelensis*, main food of Antarctic krill) are higher during cold climate periods and lower during warm periods³⁸, opposite to the patterns seen in our penguin tissues. Based on these facts, we argue that the temporal variations of $\delta^{15}\text{N}$ in penguin tissues cannot be attributed to the variation of $\delta^{15}\text{N}$ of the krill themselves but primarily to the change of penguin's diet composition.

Modern penguin bones and feathers have much lower $\delta^{15}\text{N}$ values than ancient ones. Similar low $\delta^{15}\text{N}$ values were also observed in modern penguin eggshells by Emslie & Patterson (2007)¹⁵. These differences are most likely caused by substantial dietary changes between modern and ancient Adélie penguins (Figures 3, 4). There are two other possibilities which, however, do not seem to explain the

differences observed in the current study. First, diagenetic processes may change dietary signals in tissues over time. This is unlikely for penguin feathers and bones because of the excellent preservation condition in the dry and cold Antarctic environment³⁹ though the preservation state of ancient tissues has not been directly studied in the present study. Second, ammonia volatilization could cause older tissues to become more enriched in nitrogen-15 than younger or modern tissues⁴⁰. This, however, only applies to sediment, soil and plants growing in the soil. In fact, the ancient penguin bones and feathers in DG4 have much lower $\delta^{15}\text{N}$ values (12–18‰) than those in the sediments of DG4 core (25–39‰, Table S1) and orthonogenic soils (~30‰) (Mizutani & Wada, 1988)⁴⁰. Furthermore, this would not explain the similar patterns of fluctuations in $\delta^{15}\text{N}$ values observed in both feathers and bones if volatilization is a significant factor (see also Emslie et al. 2013⁴¹).

Taken together, the lower $\delta^{15}\text{N}$ values of modern tissues than in ancient tissues indicate higher proportion of krill in modern Adélie penguin diets and thus greater krill abundance in modern ages than in ancient ages, in the East Antarctic. This is consistent with the observations by Emslie & Patterson (2007)¹⁵, which supports the 'krill surplus' hypothesis, i.e., the higher krill abundance is likely due to the hunting of krill-eating seals and whales since as early as the 19th century.

One limitation of the present study is the small sample size for analysis. In the sediment core DG4, each 1-cm section contains only one or two pieces of feathers and bones. To address this issue, we have chosen a nonparametric statistical method to test the differences in $\delta^{15}\text{N}$ values between different conditions. Since Adélie penguins and krill are widely distributed in the circum Antarctic, we expect to find more sampling sites and collect multiple sediments in future field work.

Our results corroborate that climate change has a pronounced effect on krill abundance and hence Adélie penguin diet composition^{26–28}. The reported 8000-year record of the relative krill abundance, as inferred from Adélie penguin paleodiets, provides a unique insight into the change of regional marine food chains in natural status. This will be valuable for assessing impacts of future climate changes on this key species and thus for the conservation and management of Antarctic marine resources.



Methods

Sample collection and chronology. We collected a sediment core DG4 near a large Adélie penguin colony at Gardner Island, Vestfold Hills (Figure 1), East Antarctica, during the 2005–2006 austral summer. There are a large number of penguins in the colony in summer breeding seasons, and penguin remains such as guanos, bones and feathers are deposited into the sediments each year (Figure 2). This sediment thus preserves a record of penguin activities at this colony since it was first formed. The core was sectioned at 1 cm intervals in the lab. Layers from 55 cm to the surface contained numerous penguin bones and feathers, but sample sizes per 1 cm section were quite small. Modern bone and feather samples were collected from dead adult penguins at Gardner, Magnetic and Zolotov Island, Vestfold Hills.

The chronology of DG4 has been established by AMS ^{14}C dates on penguin tissues and bulk sediments in the core in a previous study²⁵, and the penguin occupation appears to have been continuous since 8500 year BP to the present²⁵.

Stable isotope analyses. Penguin bones and feathers were picked from each 1-cm section of the sediment core and used for stable isotope analysis. These samples as well as the modern adult penguin tissues were cleaned with Millipore water and 2:1 chloroform:methanol solution, and then dried in an oven at 40°C. The cleaned samples were cut into small pieces and weighed in a tin capsule. Stable nitrogen isotope ratios in whole feathers and bone collagens were determined using isotope ratio mass spectrometer at the G.G. Hatch Isotope Laboratories, Earth Sciences, University of Ottawa, with a precision at $\leq 0.2\%$. Isotope ratio ($^{15}\text{N}/^{14}\text{N}$) in samples are reported in δ notation, and the units are per mil (‰) and defined as $\delta^{15}\text{N} = [(R_{\text{sample}} - R_{\text{standard}})/R_{\text{standard}}]$, where R_{sample} is isotope ratio of the sample, and R_{standard} of the atmosphere (See Supplementary Information for more detail).

$\delta^{15}\text{N}$ as a proxy for krill abundance. We used the inverse of isotopic ratio of nitrogen, $\delta^{15}\text{N}$, in penguin tissues as a qualitative proxy for krill abundance. The $\delta^{15}\text{N}$ in tissues has been used to reconstruct diet composition of marine species^{13–15}. Generally, marine predators are ^{15}N -enriched over their diet, because of the kinetic isotope effects in metabolic pathways involving the bond-breaking or synthesis of biochemical compounds, during which the molecules that contain the lighter nitrogen-14 are preferentially utilized over the same molecules containing the heavier nitrogen-15¹¹. $\delta^{15}\text{N}$ values can therefore be used as an indicator for the dietary change of marine predators.

Information of krill population can be estimated from penguin's diets based on traditional methods such as stomach lavage analyses⁴² which, however, often come with biases associated with sampling of gut contents. The stable nitrogen isotope method as used here can be regarded as a qualitative biochemical method, and reflects the average amount of all the diets rather than specific ones. Since this method assesses paleodiets of wildlife through their ancient tissues⁸, there is no time limitation regarding availability of diets.

Adélie penguins feed primarily on krill and, when krill are scarce, on fishes^{26–28}. Because krill are much more ^{15}N -depleted than fishes¹⁵, $\delta^{15}\text{N}$ in penguin tissues where diet information is well preserved can indicate the changes of the proportion of krill and thus their abundance. Specifically, high $\delta^{15}\text{N}$ indicates low krill abundance and low $\delta^{15}\text{N}$ indicates high krill abundance.

Statistical test for differences in $\delta^{15}\text{N}$ between different conditions. To assess the significance of the difference in penguin $\delta^{15}\text{N}$ isotope values between different time periods (modern vs. ancient), different tissues (feather vs. bone), and different reconstructed climate conditions (warm vs. cold), we adopted the nonparametric Wilcoxon rank sum test. Due to small samples sizes and presence of ties, asymptotic p value resulting from a Normal distribution approximation is invalid. Therefore, we used an exact two-sided p value obtained via a permutation procedure (i.e., randomly reassigning $\delta^{15}\text{N}$ values across groups of samples) for each test as reported in “Results”. The function “wilcox_test” in the R package “coin” was used to perform these tests. The function also implements an extension of the Wilcoxon rank sum test to allow for stratifying on another factor. We've utilized this technique to compare $\delta^{15}\text{N}$ in cold periods vs. $\delta^{15}\text{N}$ in warm periods while accounting for the possible impact of tissues (bone or feather) on $\delta^{15}\text{N}$.

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Author contributions

L.S. and T.H. conceived and designed the study. T.H. performed the experiments, N.L. performed the statistical analysis. L.S., T.H., Y.W., W.H. and N.L. analyzed the data and wrote the paper.

Additional information

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