

# Stable isotope analysis of ancient and modern gentoo penguin egg membrane and the krill surplus hypothesis in Antarctica

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**Abstract:** The ‘krill surplus’ hypothesis in Antarctica posits that the historic depletion of krill-eating whales and seals in the 18–20th centuries provided a surplus of krill in the Southern Ocean that benefited penguins. A previous study which examined stable isotopes in ancient and modern tissues of Adélie penguin (*Pygoscelis adeliae*) provides support for this hypothesis. Specifically, a significant decrease in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values occurred in modern versus ancient tissues from an apparent dietary shift from fish to krill associated with the purported krill surplus. Here, we present new data on gentoo penguin (*Pygoscelis papua*) tissues from active and abandoned colonies at three locations in the Antarctic Peninsula. We found an overall, but weak, decrease in modern versus fossil  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values of gentoo penguin egg membrane with considerable variation across three breeding sites. Dietary mixing models suggest that shifts between fish and krill in gentoo penguins were likely not as strong as those previously observed in Adélie penguins. This weaker signal probably results from the greater reliance on fish in their diets, past and present, though we cannot rule out declines in primary productivity or other ecosystem shifts which also could account for declines in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values.

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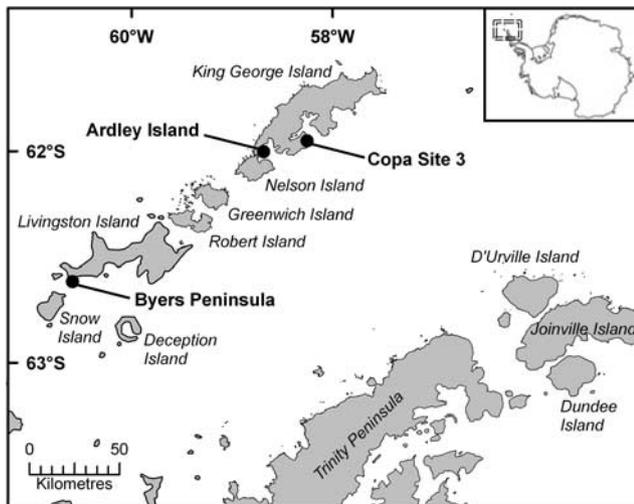
## Introduction

The ‘krill surplus’ hypothesis was first proposed by Sladen (1964) and expanded upon by Laws (1977, 1985) to explain increases in Antarctica penguin populations in the 1960s as a result of historic depletion of baleen whales and fur seals in the Southern Ocean. The severe depletion or near extinction of numerous krill-eating species such as the southern right whale (*Eubalaena australis* Gray), blue whale (*Balaenoptera musculus* (L.)), humpback whale (*Megaptera novaeangliae* (Borowski)), and southern fur seal (*Arctocephalus gazella* (Peters)) in particular left tons of unconsumed krill (*Euphausia* spp.) for penguins to exploit (Laws 1977). Chinstrap (*Pygoscelis antarctica* Forster) and Adélie penguins (*Pygoscelis adeliae* (Hombron & Jacquinot)) in particular seem to have benefited the most from this surplus with populations increasing in the Antarctic Peninsula through the 1970s. By the late 1970s and 1980s, however, a decline in Adélie penguins was observed while chinstrap penguins continued to increase. This discrepancy was attributed to a decline in sea ice in the Antarctic Peninsula that impacted the Adélie penguin more so than the chinstrap (Fraser *et al.* 1992).

Since the mid-1980s, however, climate warming, a continued decline in sea ice and therefore krill productivity, and a drop in ocean productivity in the western Antarctic Peninsula have severely impacted populations of chinstrap and Adélie penguins (Atkinson *et al.* 2004,

Montes-Hugo *et al.* 2009, Trivelpiece *et al.* 2011). Thus, any krill surplus that may have existed from a decline in baleen whales and fur seals was no longer evident. Emslie & Patterson (2007) provided the first direct evidence in support of the krill surplus hypothesis with stable isotope analysis ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of Adélie penguin eggshell from active and abandoned colonies, the latter record extending to over 38 000 years before present (BP). They documented an abrupt shift in penguin diet within the past 200–300 years to lower trophic prey, or a krill-based diet, in correlation with the removal of baleen whales and krill-consuming seals in the Southern Ocean. Additional evidence for this dietary shift also has been documented by Huang *et al.* (2010) in  $\delta^{15}\text{N}$  in modern to 8000 year old Adélie penguin bone and feather from Vestfold Hills, Antarctica.

One other penguin species also depends on krill for part or most of its diet, the gentoo penguin (*Pygoscelis papua* Forster), and thus, should also exhibit a dietary shift associated with the krill surplus. This species has been increasing in the Antarctic Peninsula, and expanding farther to the south, since the 1980s despite a decline in krill throughout this region (Smith *et al.* 1999, Zhu *et al.* 2005, Ducklow *et al.* 2007). Gentoo penguins also feed relatively more on fish than Adélie and chinstrap penguins and this, among other reasons, may explain why a decline in sea ice and krill has not impacted this species as much as the other two (Trivelpiece *et al.* 1987, 2011). Unfortunately, the past record for gentoo penguin in Antarctica is more limited



**Fig. 1.** Map of the Antarctic Peninsula (inset) with details of boxed area showing the location of sampling sites on Livingston, Ardley, and King George islands.

than that for the Adélie penguin and only a few ancient deposits have been located and sampled.

Emslie *et al.* (2003) reported excavations of abandoned gentoo penguin colonies at King George Island, Antarctic Peninsula. Although the material recovered was relatively young in age, dating to the late Holocene or 500–600 BP after correcting and calibrating for the marine carbon reservoir effect, it was still older than when the purported ‘krill surplus’ began at about 150–200 years ago. A recent paper by Emslie *et al.* (2011) reported excavations of gentoo penguin colonies at Byers Peninsula, Livingston Island. This record also is relatively young, dating to the past 1000 years, with ancient DNA extracted from penguin eggshell membranes to verify the identification of the penguin species. Finally, one other gentoo penguin deposit reported herein was excavated in 2011 at an active colony

on Ardley Island, King George Island, where penguin occupation has existed for at least the past 2400 years (Wang *et al.* 2007). Our excavations produced additional penguin tissues that predate the krill surplus. Here, we present stable isotope analysis of ancient and modern gentoo penguin eggshell membrane from all three of these localities to determine if this species also underwent an historic dietary shift in accordance with the krill surplus hypothesis. We focused our analyses on egg membrane (the inner lining of the eggshell) because it has preserved better than other tissues in the ancient gentoo penguin deposits that we investigated.

## Methods

### *Study sites and sample collection*

Eggshell membrane was recovered during excavations of abandoned gentoo penguin colonies at two locations in the Antarctic Peninsula: Copa Site 3 at King George Island (Emslie *et al.* 2003) and from Mound 3 at Devils Point, Byers Peninsula, Livingston Island (Fig. 1; Emslie *et al.* 2011). The egg membrane samples from Copa Site 3 were recovered from levels 2 ( $n = 5$ ) and 3 ( $n = 5$ ); those from Byers Mound 3 were recovered from levels 2, 4, 5, 7, and 8 ( $n = 6$  samples per level). Identification of gentoo penguin tissue from these sites was confirmed with ancient DNA analyses. Modern eggshell membrane samples were collected near active gentoo penguin colonies at Byers Peninsula ( $n = 22$ ) and Admiralty Bay, King George Island ( $n = 20$ ), in 2008/09 and at Ardley Island ( $n = 20$ ) in 2010/11. These samples were collected by gathering recently hatched eggs in and near the colonies or recently depredated eggs near skua (*Stercorarius* spp.) nests near the colonies.

We also sampled modern and older sediment at four locations (Sites 1–4) within an active gentoo penguin

**Table 1.** Radiocarbon dates with calibrated  $2\sigma$  ranges on gentoo penguin egg membrane from Copa Site 3 and Ardley Island Sites 1 and 4, Antarctic Peninsula. All dates were completed at the University of California-Irvine AMS Facility and the University of Georgia Accelerator Mass Spectrometry facility and are catalogued with UCIAMS and UGAMS numbers, respectively. The conventional radiocarbon ages were calibrated using the Calib 6.0 program and a  $\Delta R = 700 \pm 50$  years to produce  $2\sigma$  ranges in calendar years BP. The mid-point of these ranges was used to assign ages to associated membranes by level; an asterisk (\*) indicates mid-points calculated using the minimum and maximum of multiple dates from the same stratigraphic level.

Lab #	Sample #	Location	Radiocarbon age	Calibrated $2\sigma$ range	Mid-point
UCIAMS 75543	2	Copa Site 3 Lev 2	1310 $\pm$ 20	405–95	250
UCIAMS 75544	7	Copa Site 3 Lev 3	1330 $\pm$ 20	415–130	295*
UGAMS 8853	60B	Ardley Site 1 Lev 3	1450 $\pm$ 25	485–285	385
UGAMS 8852	41A	Ardley Site 1 Lev 4	1250 $\pm$ 25	285–45	165
UGAMS 8685	21A	Ardley Site 1 Lev 6	1450 $\pm$ 25	485–285	385
UGAMS 8684	11A	Ardley Site 1 Lev 7	1560 $\pm$ 25	600–370	485
UGAMS 8682	1A	Ardley Site 1 Lev 8	2050 $\pm$ 25	1025–760	765*
UGAMS 8683	2A	Ardley Site 1 Lev 8	1690 $\pm$ 25	660–500	765*
UGAMS 8687	42A	Ardley Site 4 bottom	1530 $\pm$ 25	535–320	465*
UGAMS 8688	43A	Ardley Site 4 bottom	1570 $\pm$ 25	610–390	465*

colony on the east end of Ardley Island, King George Island, in January 2011 (Fig. 1; Table I). These areas were excavated in 5–10 cm levels to the bottom of ornithogenic deposits to a depth of 60 cm below surface at each site using methodology similar to excavations at Copa and Byers. All excavated sediment were screen-washed and sorted using the methods of Emslie *et al.* (2003, 2011). Two of these locations, Sites 1 and 4 (62°13'00.6"S, 58°55'13.0"W and 62°12'43.7"S, 58°55'06.6"W, respectively) produced enough egg membrane for use in stable isotope analyses here. At Site 1, egg membrane was recovered and analyzed from levels 3 ( $n = 10$ ), 4 ( $n = 11$ ), 6 ( $n = 10$ ), 7 ( $n = 10$ ), and 8 ( $n = 10$ ), including samples that were split in half and used for radiocarbon dating (Table I). Site 4 was a 60 cm deep deposit below an active penguin mound. The bottom 5 cm of this deposit was sampled to recover ten egg membrane samples used here.

#### Sample preparation and stable isotope analysis

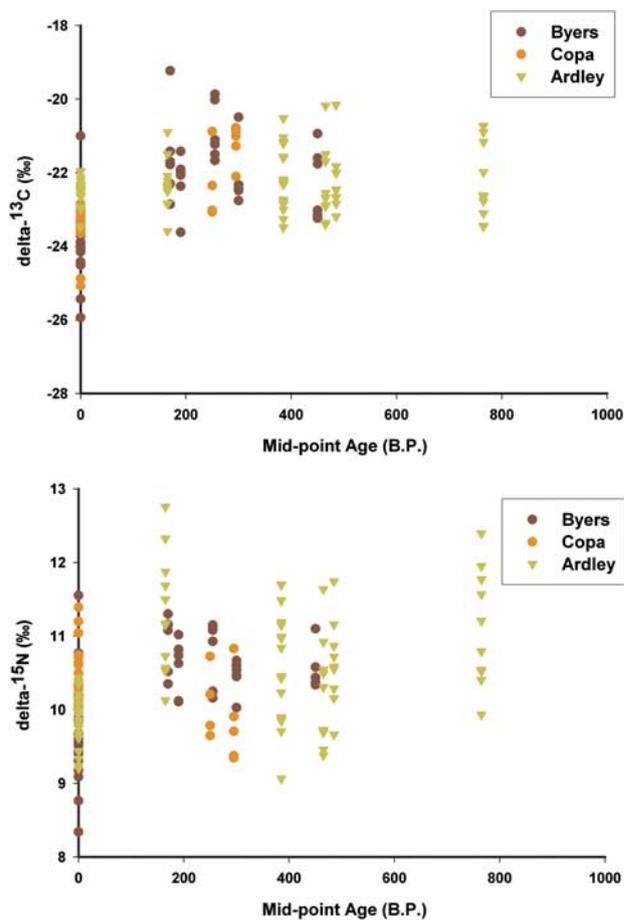
All egg membranes recovered from excavated deposits were rinsed with distilled water and if necessary cleaned with a small brush to remove any adhering dirt and other matter. Egg membranes from modern egg samples were extracted from the inner shell surface, cleaned with a small brush and rinsed in distilled water to remove any organic material adhering to the membrane. Approximately 0.5 mg of clean, dry egg membrane was loaded into tin cups, flash-combusted (Thermo-Finnigan and Costech ECS4010 elemental analyzers) and analysed for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  through Con-Flo III devices interfaced with Thermo Finnigan Delta Plus XL and Delta V Plus continuous-flow stable isotope ratio mass spectrometers. Raw  $\delta$  values were normalized using IAEA NO-3, IAEA CH-6, USGS-40, USGS-41 and internal standards. Precision was better than  $\pm 0.2\text{‰}$  for both carbon and nitrogen isotope values. Stable isotope abundances are expressed in  $\delta$  notation in per mil (‰), according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \cdot 1000$$

Where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the corresponding ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$ . The  $R_{\text{standard}}$  values relative to the Vienna PeeDee Belemnite (VPDB) standard for  $^{13}\text{C}$  and atmospheric  $\text{N}_2$  (AIR) standard for  $^{15}\text{N}$ .

#### Radiocarbon dating

Radiocarbon dates were obtained from penguin egg membrane, eggshell, feather, or bone at all sites investigated here. Most dates have been published elsewhere for Copa Site 3 (Emslie *et al.* 2003) and for the abandoned mounds at Byers Peninsula (Emslie *et al.* 2011). Here, two additional dates were completed on egg membrane from Copa Site 3. Radiocarbon analyses were also conducted on eight egg membrane samples from



**Fig. 2.** Gentoo penguin  $\delta^{13}\text{C}$  (top) and  $\delta^{15}\text{N}$  (bottom) values of ancient and modern egg membrane from three sites in the Antarctic Peninsula: Byers Peninsula, Livingston Island, Mound 3 (diamonds), Copa Site 3, King George Island (squares), and Ardley Island Sites 1 and 4 (triangles). Plotted ages are mid-points of calibrated  $2\sigma$  ranges in calendar years BP from dates obtained on samples from each stratigraphic level.

excavated sediment at Ardley Island Sites 1 and 4 (Table I). All membrane samples were split in half so that one-half could be used for stable isotope analysis and the other half for radiocarbon analysis. This method provides an absolute date for stable isotope results reported here.

All dates were corrected and calibrated for the marine-carbon reservoir effect using Calib 4.2 to 6.0.2 version software (Stuiver & Reimer 1993; <http://calib.qub.ac.uk/calib>) and a  $\Delta R = 700 \pm 50$  BP to obtain a  $2\sigma$  calibrated range in calendar years BP. The minimum and maximum ages of these ranges were rounded to the nearest five years. Egg membranes used for stable isotope but not radiocarbon analysis were assigned ages in accordance with dated membranes and other tissues from the same stratigraphic level. To simplify analysis and data plots, the date corresponding to the mid-point of each  $2\sigma$  calibrated

**Table II.** Mean  $\pm$  SD of eggshell membrane  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values and the estimated percent krill (*Euphausia superba*) and fish (*Lepidonotothen squamifrons*) in the diets of ancient and modern gentoo penguins from Copa Site 3 and Ardley Sites 1 and 4, King George Island, and Devils Point, Byers Peninsula, Livingston Island. The C:N ratio also is given for each sample group. Diet estimates are provided with 95% confidence intervals (in parentheses) and were calculated using a two-source Bayesian mixing model (Parnell *et al.* 2010). Modern samples were collected at Byers Peninsula and King George Island in 2008/09, and at Ardley Island in 2010/11. Superscript letters for ancient versus modern stable isotope values within each locality indicate significant differences if letters differ (Tukey Kramer post-hoc tests,  $P < 0.05$ ).

Location/age	<i>n</i>	C/N	$\delta^{13}\text{C}$ (‰)	$\delta^{15}\text{N}$ (‰)	% krill	% fish
Byers Peninsula, Livingston Island						
Modern	22	3.2 $\pm$ 0.2	-23.7 $\pm$ 0.9 <sup>a</sup>	9.7 $\pm$ 0.7 <sup>a</sup>	70.8 (66.9–74.6)	29.2 (25.4–33.1)
Ancient	30	3.2 $\pm$ 0.1	-21.8 $\pm$ 1.0 <sup>b</sup>	10.7 $\pm$ 0.4 <sup>b</sup>	53.7 (50.7–56.6)	46.3 (43.4–49.3)
Copa Site 3, King George Island						
Modern	20	3.2 $\pm$ 0.1	-23.4 $\pm$ 0.6 <sup>a</sup>	10.5 $\pm$ 0.4 <sup>a</sup>	59.1 (55.4–62.9)	40.9 (37.1–44.6)
Ancient	10	3.3 $\pm$ 0.0	-21.8 $\pm$ 0.9 <sup>b</sup>	9.9 $\pm$ 0.5 <sup>b</sup>	64.1 (58.3–69.7)	35.9 (30.3–41.7)
Ardley Island, King George Island						
Modern	20	3.1 $\pm$ 0.1	-22.5 $\pm$ 0.4 <sup>a</sup>	9.9 $\pm$ 0.4 <sup>a</sup>	64.9 (61.3–68.5)	35.1 (31.5–38.7)
Ancient	61	3.3 $\pm$ 0.1	-22.3 $\pm$ 0.8 <sup>a</sup>	10.7 $\pm$ 0.8 <sup>b</sup>	52.6 (50.0–55.2)	47.4 (44.8–50.0)
All Locations						
Modern	62	3.2 $\pm$ 0.2	-23.2 $\pm$ 0.9 <sup>a</sup>	10.0 $\pm$ 0.6 <sup>a</sup>	65.7 (63.6–67.8)	34.3 (32.2–36.4)
Ancient	101	3.3 $\pm$ 0.1	-22.1 $\pm$ 0.9 <sup>b</sup>	10.6 $\pm$ 0.7 <sup>b</sup>	54.0 (52.2–55.8)	46.0 (44.2–47.8)

range was used as an estimated age for those samples associated with that date. When multiple dates were available from the same stratigraphic level, the minimum and maximum of all calibrated ranges collectively was used to calculate the mid-point. For example, three calibrated ranges for one date from level 7 Byers Mound 3 were 455–225, 210–200, and 160–150 BP with the minimum and maximum range as 455–150 yielding a midpoint of approximately 305 BP.

### Statistical analysis

Dietary shifts were first assessed by comparing modern and ancient eggshell membrane  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values across and within each site. As the mid-point date of all calibrated ranges predated the purported initiation of the krill surplus in Antarctica, all ancient samples from each locality were used to calculate mean isotope values for those localities for comparison to means from modern egg membrane samples. A two-way ANOVA with Tukey-Kramer multiple comparison tests was then used to assess differences in mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values with age (ancient versus modern) and locality (Copa, Byers, and Ardley) as independent variables. Significance was assumed at  $P < 0.05$ .

In addition, we used a two-source Bayesian mixing model (Parnell *et al.* 2010) in the R environment (R Development Core Team 2007) to quantify shifts between fish and krill consumption between modern and ancient time periods across and within each site. We used published stable isotope values ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) of Antarctic krill (*Euphausia superba* Dana) and a common prey fish species (*Lepidonotothen squamifrons* (Günther)) collected from the northern Antarctic Peninsula and South Shetland Islands from 2005/06 to 2008/09 as our two prey sources (Polito *et al.* 2011). For this model we incorporated gentoo

penguin egg membrane discrimination factors ( $\delta^{13}\text{C}$ :  $2.8 \pm 0.5$ ;  $\delta^{15}\text{N}$ :  $4.4 \pm 0.5$ ; Polito *et al.* 2009, M. Polito, unpublished data) and ran 1 million iterations, thinned by 15, with an initial discard of the first 40 000 resulting in 64 000 posterior draws. While this mixing model approach may be informative for examining diet shifts over time, it does not account for variation in krill and fish stable isotope values between modern and ancient time periods. As in other studies, detailed historical stable isotope data from krill and fish prey species, that are currently unavailable, are required to conclusively validate comparisons between modern and ancient times (Bond & Jones 2009).

### Results

Mid-points of calibrated radiocarbon dates from dates reported here (Table I) and in Emslie *et al.* (2003, 2011) indicate that all egg membrane samples from ancient deposits are at least 165 years old or older.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of these ancient membranes were plotted with modern egg membranes collected at the same localities (Fig. 2). After averaging all ancient sample  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  results regardless of age (Table II) and comparing these means to those of the modern egg membranes, we found a significant decline in mean values of both  $\delta^{13}\text{C}$  ( $-1.1\%$ ;  $F_{1, 162} = 68.90$ ,  $P < 0.0001$ ) and  $\delta^{15}\text{N}$  values ( $-0.6\%$ ;  $F_{1, 162} = 14.35$ ,  $P < 0.0002$ ). Similarly, mixing model results suggest an overall 11.7% increase in the proportion of krill in modern gentoo penguin diets across all sites, relative to ancient samples (Table II). While there were no differences in egg membrane  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values across sites independent of age ( $F_{2, 162} < 2.54$ ,  $P > 0.0820$ ), a significant interaction occurred between time period and site for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $F_{2, 162} > 12.58$ ,  $P < 0.0001$ ). This interaction occurred because Copa had a 0.6% increase in  $\delta^{15}\text{N}$  from ancient to modern, opposite of

Ardley and Byers Peninsula which exhibited a 0.8–1.0‰ decrease. In addition, Ardley showed only a slight decrease of 0.2‰ in  $\delta^{13}\text{C}$  from ancient to modern in contrast to a more significant decrease of 1.9‰ at Byers Peninsula and 1.6‰ at Copa (Table II). Our isotopic mixing model suggests a 12.3% and 17.1% increase in the proportion of krill in modern gentoo penguin diets at Byers Peninsula and Ardley Island, respectively, relative to ancient samples (Table II). In contrast, there was a 5% decrease in the proportion of krill in modern gentoo penguin diets at Copa relative to ancient samples, though 95% confidence intervals overlapped between these two time periods.

## Discussion

The significant decrease in both  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in ancient to modern egg membrane at Ardley Island and Byers Peninsula implies that a dietary shift occurred in gentoo penguins in accordance with the krill surplus hypothesis. However, shifts in gentoo penguin stable isotope values (all  $< 2\%$ ) were not as large as those found in Adélie penguins over the same time periods ( $\sim 4\text{--}6\%$  decrease in  $\delta^{15}\text{N}$  and  $\sim 8\text{--}10\%$  decrease in  $\delta^{13}\text{C}$ ; Emslie & Patterson 2007). Furthermore, our isotopic mixing model suggests a maximal 17.1% increase in the proportion of krill in modern relative to ancient diets. These small shifts in gentoo penguin diets during the krill surplus may be indicative of their generally higher propensity to consume fish relative to other *Pygoscelis* penguin prey species. For example, the diets of breeding Adélie and chinstrap penguins in the South Shetland Islands are dominated by krill and often contain little fish (Trivelpiece *et al.* 1987). In contrast, breeding gentoo penguins can feed from up to 21 to 37% on fish with males relying on this prey source more than females (Miller *et al.* 2010). Outside of the breeding season, non-krill prey such as fish and squid can comprise up to 58% of gentoo penguin diets (Jablonksi 1985, Williams 1995, Polito *et al.* 2011).

Because gentoo penguins feed relatively more on fish than the other pygoscelid penguins, it can be expected that their  $\delta^{15}\text{N}$  values will be higher and more variable between years and colonies. For example, our results suggest that modern diets of gentoo penguins at each of our three sites differed to varying degrees. This variation is also apparent in a recent study of modern Adélie and gentoo penguin eggshell isotopes from numerous colonies throughout the Antarctic Peninsula as well as the South Orkney and South Shetland Islands (Polito *et al.* 2011). These authors found mean  $\delta^{15}\text{N}$  values to be up to 0.8‰ higher in gentoo penguins compared to Adélie penguins at the same location;  $\delta^{13}\text{C}$  values also were from 0.3 to 1.0‰ higher in gentoo penguins at these sites. The more eclectic diet in gentoo penguins has probably characterized this species in the past as well as today as suggested by the variation in  $\delta^{15}\text{N}$  that we observed among the three sites and time

periods, with one site (Copa Site 3) even showing a slight increase in  $\delta^{15}\text{N}$  values in modern samples (Table II, Fig. 2).

Lorenzini *et al.* (2009) suggest that high nitrogen values in ornithogenic sediment due to ammonia volatilization (Mizutani & Wada 1988) would explain high  $\delta^{15}\text{N}$  values in ancient eggshell reported by Emslie & Patterson (2007). However, the high values related to bacterially mediated volatilization occur only in the soil and in plants growing in that soil that utilize ammonia as their nitrogen source. Detrital remains that are allochthonous to the soil, including penguin bone, feather, and eggshell are not influenced by this process. This absence of an effect on eggshell is further evinced by the relatively low  $\delta^{15}\text{N}$  values 10–16‰ in penguin samples reported by Emslie & Patterson (2007) versus the very high values (31–35‰) in soil and cyanobacteria reported by Mizutani & Wada (1988). In addition,  $\delta^{15}\text{N}$  values on modern versus ancient gentoo penguin egg membranes reported here display opposite trends, i.e. decreases at two sites and an increase at one (Copa Site 3). Such trends would not occur if volatilization was impacting these remains. Thus, volatilization does not appear to influence ancient penguin remains in ornithogenic sediment and the argument presented by Lorenzini *et al.* (2009) is not valid.

Anthropogenic carbon may also influence  $\delta^{13}\text{C}$  values in marine and terrestrial ecosystems via the Suess effect (McNeil *et al.* 2001). These authors calculated a decrease in  $\delta^{13}\text{C}$  of  $-0.005 \pm 0.003\%$  per year, or a negligible amount of  $-0.1\%$  every 20 years. We believe the Suess effect has had only a slight influence on our results for penguin egg membranes because old carbon is consistently upwelling in the Antarctic Peninsula, especially in areas near many penguin colonies. Moreover, the gentoo penguin is known to forage primarily inshore (Trivelpiece *et al.* 1986, 1987), where upwelling would be most intense, though this behaviour can vary throughout their range (Lescroël & Bost 2005). However, Hilton *et al.* (2006) attributed a historic to modern decrease in  $\delta^{13}\text{C}$  in rockhopper penguin (*Eudyptes chrysocome*) feathers to changes in ocean productivity, even after correcting for a Suess effect of 0.34–0.75‰ in  $\delta^{13}\text{C}$  between 1840 and 2000. The Suess effect corrections of Hilton *et al.* (2006) are for the sub-Antarctic and thus may be too high for the Antarctic. However, even an effect of half the magnitude they apply ( $\sim 0.4\%$ ) could account for a portion of the declines in  $\delta^{13}\text{C}$  in penguin egg membranes observed here and might act to bias mixing model estimates of penguin diet.

Our data indicate that gentoo penguins responded only slightly to the krill surplus, with variable responses depending on colony location. This response is not surprising given the large variation in dietary signals present in modern gentoo penguins at numerous locations throughout the Antarctic Peninsula (Polito *et al.* 2011). Additional ancient sites are needed from other regions of

the Antarctic and sub-Antarctic to fully assess dietary shifts in this species with the krill surplus hypothesis.

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