

A stable isotopic investigation into the causes of decline in a sub-Antarctic predator, the rockhopper penguin *Eudyptes chrysocome*

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Abstract

The rockhopper penguin (*Eudyptes chrysocome*) is a conspicuous apex marine predator that has experienced marked population declines throughout most of its circumpolar breeding distribution. The cause(s) for the declines remain elusive, but the relatively large spatio-temporal scale over which population decreases have occurred implies that ecosystem-scale, at-sea factors are likely to be involved. We employ stable isotope analyses of carbon ($^{13}\text{C}/^{12}\text{C}$, expressed as $\delta^{13}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$, $\delta^{15}\text{N}$) in time-series of rockhopper penguin feather samples, dating back to 1861, in order to reconstruct the species' ecological history. Specifically, we examine whether rockhopper penguin population decline has been associated with a shift towards lower primary productivity in the ecosystem in which they feed, or with a shift to a diet of lower trophic status and lower quality, and we use long-term temperature records to evaluate whether shifts in isotope ratios are associated with annual variations in sea surface temperature. Having controlled temporally for the Suess Effect and for increases in CO_2 concentrations in seawater, we found that overall, $\delta^{13}\text{C}$ signatures decreased significantly over time in rockhopper penguins from seven breeding sites, supporting the hypothesis that decreases in primary productivity, and hence, carrying capacity, for which $\delta^{13}\text{C}$ signature is a proxy, have been associated with the decline of penguin populations. There was some evidence of a long-term decline in $\delta^{15}\text{N}$ at some sites, and strong evidence that $\delta^{15}\text{N}$ signatures were negatively related to sea surface temperatures across sites, indicative of a shift in diet to prey of lower trophic status over time and in warm years. However, a site-by-site analysis revealed divergent isotopic trends among sites: five of seven sites exhibited significant temporal or temperature-related trends in isotope signatures. This study highlights the utility of stable isotope analyses when applied over relatively long timescales to apex predators.

Keywords: climate change, primary productivity, sub-Antarctic, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$

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Introduction

Anthropogenic climate change is perceived to be a major threat to the world's biodiversity (Thomas *et al.*, 2004), yet among birds, perhaps the best-known taxon, only 25 out of 1215 globally threatened species are

currently listed as threatened by climate change (Bird-Life International, 2004). Six of these 25 threatened species are penguins belonging to the genus *Eudyptes*, including the rockhopper penguin (*Eudyptes chrysocome*), an abundant predator in south temperate and sub-Antarctic oceans. It has a circumpolar distribution and breeds at sites lying largely in the productive frontal waters between the Subtropical Front and the Antarctic Polar Front (Williams, 1995; Fig. 1). It is

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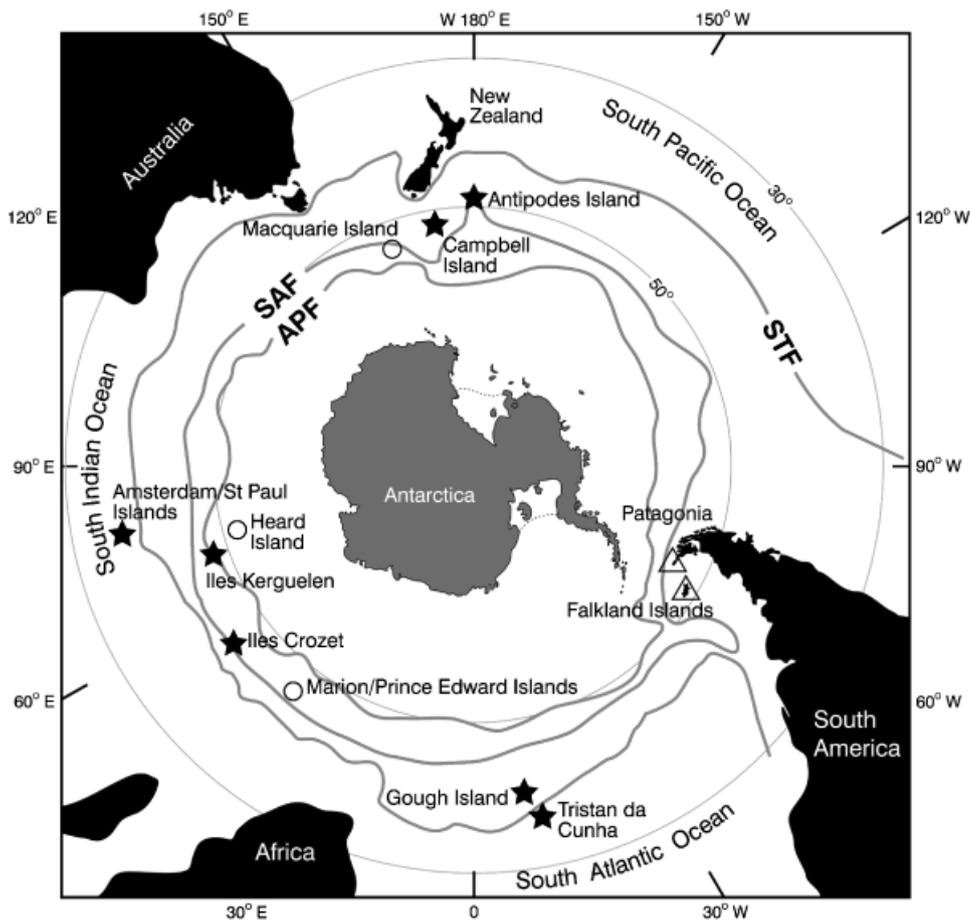


Fig. 1 Map showing the locations of rockhopper penguin breeding sites: ★, sites included in the analysis; ○, sites excluded from analysis because of small sample size; △, sites excluded from analysis because of strongly bi-modal patterns in contemporary isotopic data. All contemporary samples from Patagonia were from Staten Island (54°50'S 64°40'W). STF, Subtropical Front; SAF, Sub-Antarctic Front; APF, Antarctic Polar Front.

classified as globally 'vulnerable', because of rapid declines at almost all breeding sites throughout its range for which data are available (BirdLife International, 2004). Although the world population is estimated at ca. 1.5 million pairs (BirdLife International, 2004), it seems probable that since the early part of the 20th century the population has decreased by several million pairs (Moors, 1986; Ellis *et al.*, 1998; Crawford *et al.*, 2003; Pütz *et al.*, 2003; BirdLife International, 2004). Unless there have been concurrent increases among other members of the rockhopper penguin's feeding guild, there must have been a major decrease in prey consumption in this ecosystem – the species is probably one of the major seabird consumers within its biome (Brooke, 2004).

The cause(s) of the decline of rockhopper penguin populations is largely unknown, but its wide temporal and spatial scale suggests that ecosystem-scale at-sea factors may be involved. Two recent papers ascribe site-

specific declines to sea temperature changes, though the evidence is largely circumstantial, being based on temporal correlations between declining populations and changing temperature (Cunningham & Moors, 1994; Guinard *et al.*, 1998). Nevertheless, there is much current concern about 'system shifts', resulting from climate change, in southern hemisphere marine systems (e.g. Reid & Croxall, 2001; Croxall *et al.*, 2002; Fraser & Hofmann, 2003; Jenouvrier *et al.*, 2003; Weimerskirch *et al.*, 2003).

No long-term studies of rockhopper penguin population biology have been conducted, largely because of the difficulty of permanently marking penguins (Jackson & Wilson, 2002). As a result, the links between demographic, ecological and environmental variables have never been elucidated in this species, and so existing data cannot be used to diagnose the cause(s) of the declines. However, stable isotope analysis of a long-term series of feather samples (see Thompson

et al., 1995) should enable us to reconstruct the ecological history of rockhopper penguins. In doing so, this information should provide clues as to the cause of population decreases, and the hypothesized link to climate change. This approach is based on fundamental properties of the ratios of stable isotopes of carbon and nitrogen, and how they change in response to variation in ecological processes.

Phytoplankton carbon fractionation is a function of, among other factors, cell growth rate (e.g. Laws *et al.*, 1995). Hence, as phytoplankton grows more rapidly, its $\delta^{13}\text{C}$ value increases. Because of this phenomenon, $\delta^{13}\text{C}$ declines in aquatic ecosystems have recently been used as indicators of declining primary productivity – hence, carrying capacity of the ecosystem (Schell, 2000; Hirons *et al.*, 2001; O'Reilly *et al.*, 2003). We, therefore, tested the hypothesis that the decline of rockhopper penguin populations has been associated with a shift towards lower primary productivity in the ecosystem in which they feed, as indicated by the carbon isotope ratios in the penguins' feathers.

Forero *et al.* (2002a, b) suggested that for Magellanic penguins (*Spheniscus magellanicus*), shoaling fatty fish were an optimal diet, as opposed to squid and crustaceans, which were the main alternative prey groups. This is because fish are more energy dense, and more rapidly digested than squid and crustaceans (Jackson *et al.*, 1987; Hilton *et al.*, 1998). They showed that because fish occupy a higher trophic level, they have higher $\delta^{15}\text{N}$ values than squid and crustaceans, and so proposed and demonstrated that for Magellanic penguins there is a positive relationship between diet quality and tissue $\delta^{15}\text{N}$ values. Rockhopper penguins choose between different proportions of the same three broad food types (Williams, 1995), and are assumed to favour a diet that is energy dense and digestible; we therefore tested a second hypothesis that the decline of rockhopper penguin populations has been associated with decreasing $\delta^{15}\text{N}$ ratios in their feathers.

There is some evidence that system shifts in southern hemisphere marine environments have been driven by recent climate warming (e.g. Weimerskirch *et al.*, 2003), and that warming of water bodies can lead to reductions in phytoplankton productivity (e.g. O'Reilly *et al.*, 2003). We, therefore, use long-term temperature records to evaluate whether shifts in isotope ratios are associated with annual variations in sea surface temperature.

This paper combines several long-term data sets (isotopic signatures, sea temperature) to explore, for the first time, the relative importance of contributory factors towards the widespread and profound population declines in a conspicuous, apex marine predator over most of its circumpolar distribution.

Materials and methods

Feather samples

By measuring stable isotope signatures in the feathers of rockhopper penguins we get a direct measurement of the ecosystem in which they feed: any isotopic changes in feathers indicate that the penguins must be experiencing variations in the ecosystem, via their diet. Following breeding, rockhopper penguins depart the breeding colony and spend several weeks at sea, before returning to undergo a complete feather moult on land, without feeding, for 3–4 weeks. The premoult exodus is a time of intensive feeding, to obtain the energy reserves required for feather growth while fasting (Cherel *et al.*, 1993; Williams, 1995). Isotope signatures of feathers reflect the bird's diet during the period of feather growth (Hobson & Clark, 1992; Bearhop *et al.*, 2002; Pearson *et al.*, 2003), but because rockhopper penguins do not feed during feather growth, feather isotope signatures therefore are very likely to reflect the diet of the birds during the restricted summer premoult feeding period. The peculiar moult strategy of penguins makes this period a critical energetic bottleneck and there is some evidence to suggest that the premoult and moult periods are crucial for the species' demography. For example, in the Falkland Islands, where the rockhopper penguin population has declined since the 1930s (Pütz *et al.*, 2003), a substantial population decrease took place during the 1987 moult period, when there was mass starvation of adult birds. It appeared that they had been unable to lay down sufficient reserves during the premoult exodus (Keymer *et al.*, 2001).

Stable isotopes of carbon and nitrogen were measured in body feathers of 535 rockhopper penguins. The penguins comprised contemporary samples, obtained from living individuals during 2000–2003, and historical samples, obtained from museum skins, and dating from 1837 to 1985. Contemporary feather samples were obtained from adult birds at the following locations, austral summer breeding seasons in parentheses: Campbell Island (2000/2001 and 2002/2003), Antipodes Island (2000/2001, 2001/2002 and 2002/2003), Kerguelen Island (2000/2001), Crozet Island (2000/2001), Amsterdam Island (2000/2001), Marion Island and Prince Edward Island (2001/2002), Gough Island (2000/2001 and 2001/2002), Staten Island (2000/2001) and the Falkland Islands (2000/2001; Fig. 1). Historical feather samples were obtained from study skins held in ornithological collections of museums. Feathers were taken only when complete capture information (at least date and location) was present with a particular study skin. See Acknowledgements for a complete list of museums from which feather samples were obtained.

For each individual sampled, three to 10 body feathers were cut from random positions on the bird's body, and placed in a polythene bag to await isotope analysis.

Stable isotope analysis

To remove surface contamination before analysis, all feathers were washed firstly with distilled water and then acetone, and then rinsed repeatedly with distilled water. Washed feathers were dried in an oven at 50 °C for 24 h and then allowed to equilibrate to ambient laboratory temperature (approximately 20 °C). Clean feathers were cut into very small pieces using stainless-steel scissors and approximately 0.5 mg weighed accurately into a thin capsule. Stable isotopes of carbon ($^{13}\text{C}/^{12}\text{C}$) and nitrogen ($^{15}\text{N}/^{14}\text{N}$) were determined in all samples by continuous-flow isotope-ratio mass spectrometry using a Finnigan MAT Delta^{Plus} mass spectrometer coupled to a Carlo-Erba elemental analyzer at the National Institute for Water and Atmospheric Research's stable isotope laboratory in Wellington, New Zealand. Results are presented in conventional δ notation in units of parts per thousand (‰, per thousand), and are expressed relative to international standards Pee Dee Belemnite and atmospheric nitrogen for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, respectively, according to the following equation: $\delta = \{(R_{\text{sample}} - R_{\text{standard}}) / R_{\text{standard}}\} \times 1000$ where R_{sample} is the isotopic ratio of the sample and R_{standard} is the isotopic ratio of the relevant international standard. Sample analyses included in-house standards (urea) every 10 samples. Based on hundreds of analyses of the in-house urea standard, analytical error for $\delta^{13}\text{C}$ is $\pm 0.2\text{‰}$, and for $\delta^{15}\text{N}$ is $\pm 0.3\text{‰}$.

Data analysis

In analyses, we omitted sites for which we had samples in <6 years. These were Marion/Prince Edward Islands, Heard Island and Macquarie Island (Fig. 1). Contemporary isotopic data for both Patagonia (from Staten Island) and Falkland Islands (Fig. 1) showed strongly bi-modal patterns, which suggests that populations in these areas have two alternative foraging regions – probably on the Patagonian Shelf and in oceanic frontal waters (see Pütz *et al.*, 2002). Long-term patterns in isotope ratios at these two sites cannot be determined, as it was not possible to assign a particular historical sample to a specific sampling colony within the archipelagos. Consequently, unknown and potentially differential sampling of birds between the different foraging regions confounds isotopic trends from these two sites. Hence, in the analysis of global trends in stable isotope ratios, we omitted data for these two sites. Data for the geographically close islands of

Table 1 Details of sites from which samples were obtained and used in global analyses

Site	Location	Date range of feather samples	Sample size*
Tristan da Cunha	37°S, 12°W	1873–1973	9
Gough Island	40°S, 10°W	1921–2001	6
Crozet Island	46°S, 52°E	1900–2000	9
Kerguelen Island	49°S, 70°E	1873–2000	9
Amsterdam/ St Paul Islands	38°S, 78°E	1875–2000	11
Campbell Island	52°S, 169°E	1890–2002	11
Antipodes Island	50°S, 179°E	1861–2002	14

*Number of unique *site* \times *year* combinations.

Amsterdam and St Paul were combined to give a single site, as they were statistically indistinguishable isotopically and the two islands lie in the same (subtropical) zone. Summary information about contemporary sites sampled and included in the global analyses is provided in Table 1.

Generalized linear models (GLMs), with isotope ratio as response variable, were performed using Proc GENMOD in SAS v.8 to test for temporal trends in isotope values and to examine whether variation in isotope values is associated with variation in sea surface temperature. Initial examination of data from contemporary samples indicated that there was substantial between-site variation in isotope values. We, therefore, controlled for site effects in all GLMs by inclusion of a categorical *site* effect as an explanatory variable. *Year* was a continuous explanatory variable, denoting the calendar year in which the sampled feathers were grown. *Temperature* was a continuous explanatory variable denoting the mean of monthly sea-surface temperature anomalies for the calendar year before feather growth, in the grid cell in which the site is located. We tested for *site* \times *covariate* interactions in all models. We also included *year* \times *temperature* interactions; this allowed us to test for 'regime shifts' (i.e. temporal changes in the relationship between environmental variables and bird ecology (Durant *et al.*, 2004)). We tested both quadratic and linear fits to the data, to allow accelerating or decelerating curves to be fitted. Non-significant interactions, quadratic and linear terms were sequentially deleted to produce a minimum adequate model.

To avoid pseudoreplication, we pooled all $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values obtained from the same site in the same year, and used the mean value of each unique *site* \times *year* combination as an observation in analyses. All GLMs were weighted by the square root of the number of

samples for each site–year combination (it was not possible to weight by variance, as for some *site* × *year* combinations, there was only a single sample). The weighting procedure gives greater weight to those *site* × *year* combinations that have larger sample sizes, and hence, tend to be more precise estimates of the population's isotope ratio.

For most sites, the timing of onset of declines in rockhopper penguin populations is not clear. Furthermore, if oceanic ecosystem changes are implicated, one would not necessarily expect a linear response throughout the time period under study. Therefore, we developed generalized additive models (GAMs), using Proc GAM in SAS v.8, with *site* as a fixed factor and *year* as a nonparametric smoothed spline, to examine nonlinear temporal trends in isotope ratios. Generalized cross-validation was used to determine the optimal degrees of freedom objectively. These analyses allowed us to assess whether there were notable 'turning points' in the overall temporal trends, and whether there were marked oscillations around the overall trend.

Subsequently, we repeated the GLM analyses for each site separately, including both linear and quadratic *year* and *temperature* terms in an initial full model, without interactions, and making stepwise deletions of nonsignificant variables to give a minimum adequate model.

Controlling for anthropogenic changes to $\delta^{13}\text{C}$ values

We aimed to examine temporal trends in $\delta^{13}\text{C}$ in rockhopper penguins. However, human fossil fuel burning is known to have caused an exponentially accelerating decrease in $\delta^{13}\text{C}$ in the biosphere since the industrial revolution: this is the 'Suess Effect', caused by the fact that carbon introduced into the biosphere by the burning of fossil fuels has a lower $\delta^{13}\text{C}$ than background carbon (Keeling, 1979). In addition, the increase in atmospheric CO_2 that results from fossil fuel burning has increased the concentration of aqueous CO_2 ($[\text{CO}_2]_{\text{aq}}$) in the ocean (Louanchi & Hoppema, 2000). Phytoplankton $\delta^{13}\text{C}$ ratios are a positive function of $[\text{CO}_2]_{\text{aq}}$ (Rau *et al.*, 1989, 1992).

Therefore, we adjusted each of our raw $\delta^{13}\text{C}$ values to take into account these two effects, before data analysis (Appendix A). We reviewed published literature on the oceanic Suess Effect and the phytoplankton fractionation effect and derived models that predict the magnitude of the two effects for any given *site* × *year* combination, and added the values to the raw $\delta^{13}\text{C}$ data. Both the Suess Effect and the phytoplankton fractionation effect differ between sites, because of differences in biogeochemistry between oceanic water bodies (Louanchi & Hoppema, 2000; Sonnerup *et al.*, 2000; McNeil *et al.*, 2001). The effect on the apparent

temporal trends in $\delta^{13}\text{C}$ of making these adjustments was examined by comparing the curves obtained from raw data values with those obtained by using adjusted values.

Sea-surface temperature data

Monthly sea surface temperature anomaly data and absolute sea surface temperature data for the period were obtained from UK Meteorological Office – HadISST 1.1 Global Sea Ice Coverage and Sea Surface Temperature data set (1870–present) (<http://badc.nerc.ac.uk/data/hadisst/>). These data were converted into annual mean sea-surface temperature anomaly values for each site–year combination for which we had isotope data. For each study site, we used temperature data for the 5° latitude–longitude cell in which it falls. If a site was within 1.5° of the edge of a cell, we used data from the two closest cells combined. Where particular *site* × *year* combinations were missing from the temperature data set, we used data from the geographically nearest available grid cell.

Results

Global trends in rockhopper penguin feather isotope ratios

Sixty-nine unique *site* × *year* combinations, from seven sites encompassing most of the range of the rockhopper penguin (Fig. 1), were used in analyses. No data from the large south-west Atlantic populations (Falkland Islands and Patagonia) were used in analyses (see Materials and methods; Table 1).

Adjusted $\delta^{13}\text{C}$ decreases significantly over time in rockhopper penguin feathers, when between-site differences in carbon isotope ratios are controlled for (Fig. 2a; Table 2). The slope of the relationship (−0.0042) implies a decline in adjusted $\delta^{13}\text{C}$ of 0.67‰ over the 160 years for which we have samples. There is no evidence that this downward trend differs between sites (*site* × *year* interaction $\chi^2_{6,59} = 6.58$, $P = 0.36$).

There is no convincing evidence of a linear temporal decrease in $\delta^{15}\text{N}$ across all sites (Fig. 2b; Table 2). However, the *site* × *year* interaction term approaches significance ($\chi^2_{6,59} = 12.2$, $P = 0.058$), indicating that $\delta^{15}\text{N}$ trends may differ between sites. When this marginally nonsignificant interaction term is retained in the model, the *year* main-effect shows a significant linear downward trend over time (*year* $\chi^2_{1,59} = 4.47$, $P = 0.034$, slope = −0.0085, SE = 0.0070), suggesting that there may have been an overall trend for $\delta^{15}\text{N}$ to decrease over time, but with important variation between sites.

We repeated the above global models, removing the *year* explanatory variable, and replacing it with *temperature*.

These models suggest that there is no global effect of sea-surface temperature anomaly on carbon isotope ratios, and that the observed temporal trend in $\delta^{13}\text{C}$ is

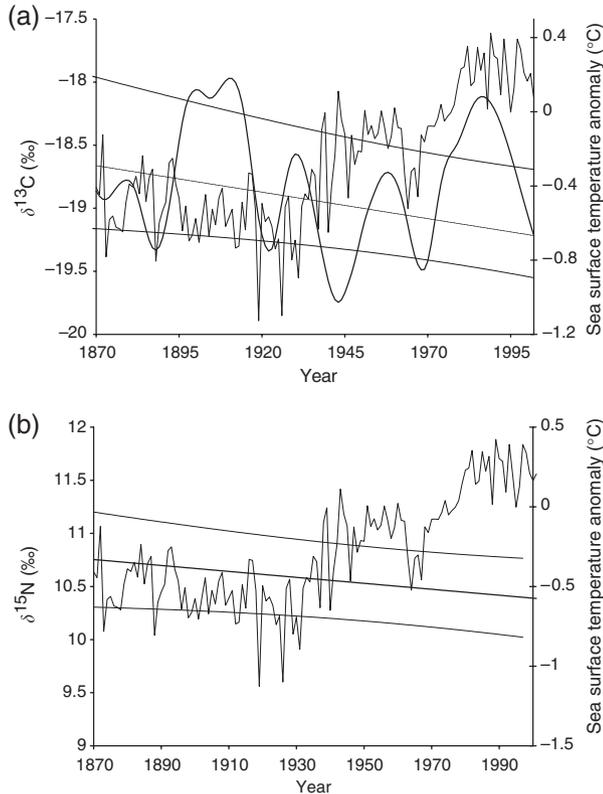


Fig. 2 Global temporal trends in rockhopper penguin feather stable isotope ratios. The fitted global linear trend, based on the generalized linear model (Table 2) is shown (dashed-line) with 95% confidence limits. Fitted annual values from the general additive model (GAM) (thick solid line), and annual mean sea-surface temperature anomaly (mean value across sites) (thin solid line); (a) Adjusted $\delta^{13}\text{C}$, (b) $\delta^{15}\text{N}$. The fitted values from the GAM are not shown, because the model had very low significance, and was effectively linear.

Table 2 Relationships between stable isotope values and site, year, and sea surface temperature for rockhopper penguins across study sites

Response variable	Term	Wald χ^2 (df)	<i>P</i>	Estimate (SE)
$\delta^{13}\text{C}$ (adjusted)	Site	157 (6,65)	<0.0001	—
	Year	4.77 (1,65)	0.029	-0.0042 (0.0021)
$\delta^{15}\text{N}$	Site	345 (6,65)	<0.0001	—
	Year	3.09 (1,65)	0.079	-0.0028 (0.0016)
$\delta^{13}\text{C}$ (adjusted)	Site	162 (6,64)	<0.0001	—
	Temperature	0.29 (1,64)	0.59	-0.0012 (0.0022)
$\delta^{15}\text{N}$	Site	409 (6,64)	<0.0001	—
	Temperature	7.33 (1,64)	0.0068	-0.0046 (0.0017)
$\delta^{15}\text{N}$	Site	13.4 (5,57)	0.020	-0.0061 (0.0066)
	Year	0.28 (1,57)	0.60	-0.0051 (0.0021)
	Temperature	5.64 (1,57)	0.018	—
	Site \times Year	13.2 (6,57)	0.040	—

therefore not directly driven by the rising temperatures observed in the period 1920–present (Table 2). There is weak evidence for variations in $\delta^{13}\text{C}$ response to temperature among sites, because the *site* \times *temperature* term approaches significance ($\chi^2_{6,58} = 12.4$, $P = 0.054$), but the *year* main effect is not significant in the model retaining the interaction term ($\chi^2_{1,58} = 2.08$, $P = 0.15$).

There is a significant linear effect of *temperature* on $\delta^{15}\text{N}$: in relatively warm years, $\delta^{15}\text{N}$ values tend to be lower (Table 2). This relationship implies that the typical warming observed at our study sites (≈ 1.0 – 1.3°C between ca. 1915 and 2000) would result in a decrease in $\delta^{15}\text{N}$ of about 0.4–0.6‰ over the same period. Our study sites show short-term between-year variations (independent of the long-term trend) in annual mean temperature of 2.0– 3.7°C , which would lead to between-year variation in $\delta^{15}\text{N}$ of 0.8–1.2‰. The *site* \times *temperature* interaction is not significant ($\chi^2_{6,58} = 10.8$, $P = 0.095$).

All quadratic models for both *site* and *temperature* as explanatory variables, and both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as response variables are nonsignificant.

Finally, we developed models incorporating both year and temperature (as well as site) as explanatory variables, with all possible two-way interactions between them.

Stepwise deletion of nonsignificant interactions, and then main effects, gives the same final model for adjusted $\delta^{13}\text{C}$ as that presented in Table 2, including only *site* and *year* terms. Hence, there is no evidence that sea-surface temperature explains variation in carbon isotope ratios additional to the temporal trend, or that temperature effects on carbon isotope ratios have varied over time.

For $\delta^{15}\text{N}$, a similar stepwise deletion process yields a final model that again indicates a negative relationship between nitrogen isotope ratios and sea-surface temperatures, but with additional variation explained by

Table 3 General additive model* of temporal trends in adjusted $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

	Smoothing parameter [†]	df [†]	χ^2 [‡]	<i>P</i> [‡]
$\delta^{13}\text{C}$ (adjusted)	0.675	15.7	47.7	<0.0001
$\delta^{15}\text{N}$	1.00	1.00	0.0001	0.99

*Site was included as a parametric factor (seven levels), with the *year* covariate fit as a nonparametric spline function.

[†]Degrees of freedom selected by generalized cross validation, which provides an objective means of selecting df by minimizing the sum of residuals of the smoothing parameter.

[‡]Estimated from an analysis of deviance model, which compares the deviance of the full model with the deviance of the model after omitting each variable

Table 4 Response of adjusted $\delta^{13}\text{C}$ to site, year and temperature, for the period 1940–2002

Variable	χ^2 (df)	<i>P</i>	Estimate (SE)
Site	163 (6,37)	<0.0001	
Year	2,87 (1,37)	0.090	0.0069 (0.0040)
Site	159 (6,37)	<0.0001	—
Temperature	4.00 (1,37)	0.046	0.0058 (0.0029)

Models incorporate *site* as a seven-level factor, and either *year* or *temperature* as covariates. Adjusted $\delta^{13}\text{C}$ was the response variable.

between-site variations in temporal trends (a significant *site* \times *year* term), and no overall temporal trend (Table 2).

A GAM suggests that $\delta^{13}\text{C}$ has shown complex, nonlinear temporal variation: the best fit smoothing parameter had nearly 16 df, and analysis of deviance suggests that the nonparametric year parameter is highly significant ($P < 0.0001$; Table 3). The GAM suggests that there are no major turning points in the temporal decline in $\delta^{13}\text{C}$, but that the decline has been accompanied by decadal oscillations that are large relative to the underlying downward linear trend (Fig. 2a). Peaks (relative to the downward linear trend) in $\delta^{13}\text{C}$ appear during ca. 1890–1915, and during 1970–2000, with small peaks around 1930 and in the 1950s.

A GAM also suggests that there is little evidence of nonlinear variations in $\delta^{15}\text{N}$ over time. Generalized cross-validation gives df = 1.00, with $P = 0.99$, hence a weak linear downward trend in $\delta^{15}\text{N}$ (Fig. 2b; Table 3).

Visual examination of the fitted nonlinear $\delta^{13}\text{C}$ curve from the GAM (Fig. 2a) shows a curious pattern, which we investigated further. From ca. 1940 onwards, there appears to be a strong positive relationship between *temperature* and adjusted $\delta^{13}\text{C}$, with both variables showing general increases over time, but with lower

values during the 1960s. However, in the period before 1940, no such carbon–temperature relationship is apparent.

We, therefore, extracted observations for years later than 1940, and use a GLM to examine linear trends in adjusted $\delta^{13}\text{C}$ for this period only. For the period post-1940, there is a strong positive relationship between adjusted $\delta^{13}\text{C}$ and *temperature*, with the *year* effect being positive, but marginally nonsignificant (Table 4). Hence, although over the full time-period of our data we detect a modest temporal decrease in $\delta^{13}\text{C}$, with no *temperature* effect, over these recent decades we find a strong positive relationship between $\delta^{13}\text{C}$ and *temperature*.

Site-by-site analysis

Despite the evidence for a global temporal decrease in $\delta^{13}\text{C}$, and a negative global relationship between *temperature* and $\delta^{15}\text{N}$, with weak *site* \times *effect* interactions, site-by-site analysis indicates rather divergent trends among sites (Fig. 3). Overall, for five out of seven sites, there are significant temporal or temperature-related trends in isotope ratios (Tables 5 and 6).

At Antipodes Island there is a linear temporal decrease in $\delta^{13}\text{C}$, whereas at Crozet Island, there appears to be a decline in $\delta^{13}\text{C}$ until the 1950s, with a subsequent slight increase. At Gough Island there is a positive temporal trend in $\delta^{13}\text{C}$, and a largely positive relationship between $\delta^{13}\text{C}$ and *temperature*, resulting in a general upward trend in fitted values of $\delta^{13}\text{C}$ over the whole time-period.

At Crozet Island there is a linear temporal decrease in $\delta^{15}\text{N}$. At Kerguelen Island and Tristan da Cunha there is a linear negative relationship between $\delta^{15}\text{N}$ and *temperature*, which tends to result in a decreasing temporal trend in predicted values since the warming period began in the 1920s. There are no significant isotopic trends at Amsterdam/St Paul Islands and at Campbell Island.

Effect of controlling for the Suess Effect and phytoplankton fractionation on the apparent global $\delta^{13}\text{C}$ trend

Controlling for the Suess Effect and the phytoplankton fractionation effect makes a very large difference to the apparent global $\delta^{13}\text{C}$ trend. The apparent decrease in $\delta^{13}\text{C}$ would be far steeper and more significant if we had not attempted to control for these effects. The temporal trend in raw $\delta^{13}\text{C}$ values has a slope of -0.0121 (SE 0.0019, $\chi^2_{1,65} = 39.9$, $P < 0.0001$), which would give a decrease of 1.94‰ in $\delta^{13}\text{C}$ between 1840 and 2000. Adjusting the raw $\delta^{13}\text{C}$ values for our estimates of the Suess Effect reduces the slope of the

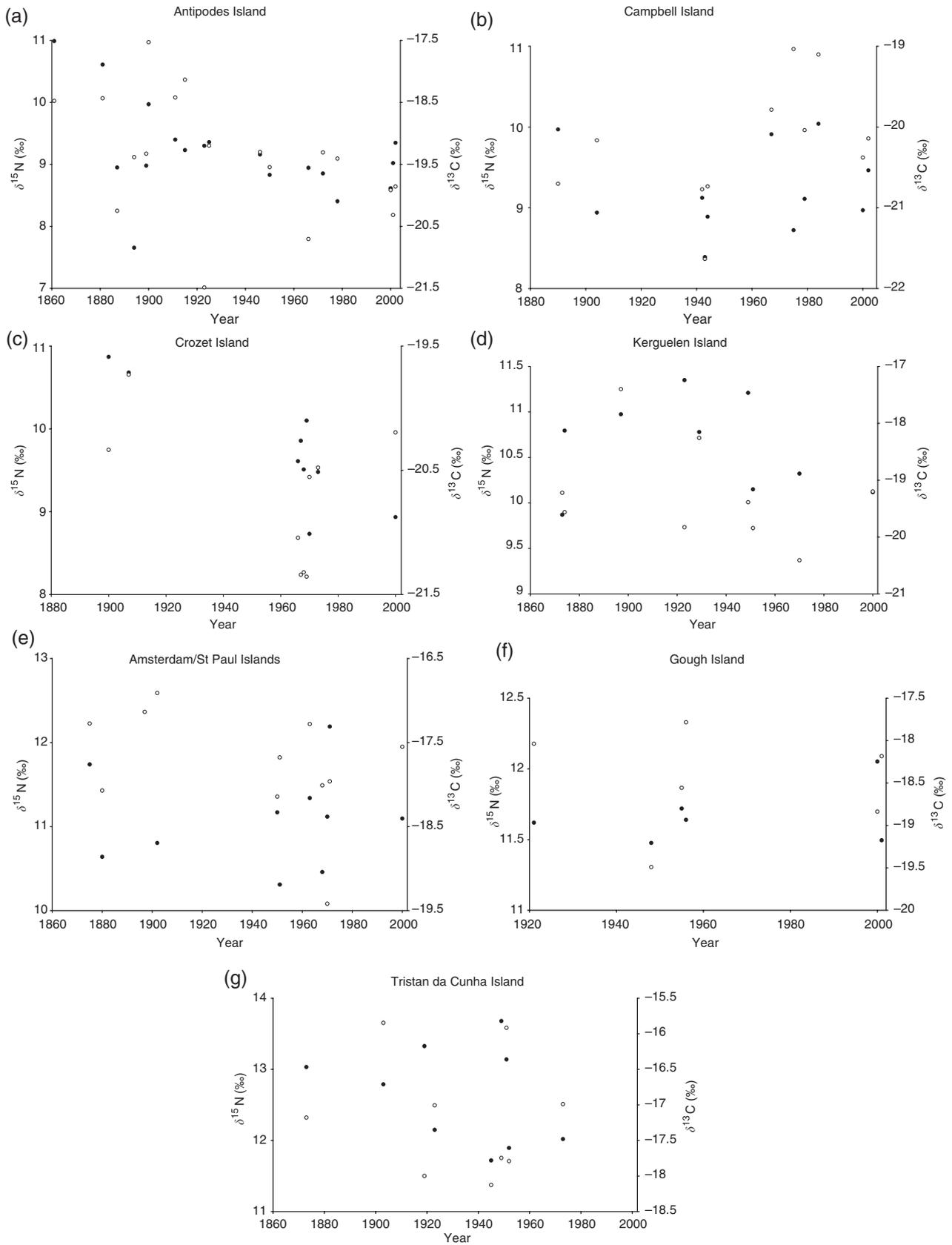


Fig. 3 Site-by-site time series of $\delta^{15}\text{N}$ (●) and adjusted $\delta^{13}\text{C}$ (○).

Table 5 Site-specific effects of year and sea surface temperature on adjusted $\delta^{13}\text{C}$

Site	Term	Estimate	SE	F	P
Amsterdam/St Paul Islands, N = 11	ns				
Antipodes Island, N = 18	Constant	-1.25	6.71		
	Year	-0.0094	0.0034	7.46	0.015
Campbell Island, N = 11	ns				
Crozet Island, N = 9	Constant	1384	478		
	Year	-1.44	0.490	8.64	0.026
	Year \times Year	0.00037	0.00013	8.64	0.026
Kerguelen Island, N = 9	ns				
Gough Island, N = 6	Constant	-31.7	0.065		
	Year	0.0031	0.00043	54.2	0.018
	Temp	0.088	0.0090	97.2	0.010
	Temperature \times Temperature	-0.00025	0	43.2	0.022
Tristan da Cunha, N = 9	ns				

Linear and quadratic year and temperature terms were fitted, with stepwise deletion of non-significant terms to give a final model in which all terms are significant ($P < 0.05$).
ns, nonsignificant.

Table 6 Site-specific effects of year and sea surface temperature on $\delta^{15}\text{N}$

Site	Term	Estimate	SE	F	P
Amsterdam/St Paul Islands, N = 11	ns				
Antipodes Island, N = 18	ns				
Campbell Island, N = 11	ns				
Crozet Island, N = 9	Constant	46.98	9.71		
	Year	-0.019	0.0049	14.99	0.006
Kerguelen Island, N = 9	Constant	11.30	0.23		
	Temperature	-0.0085	0.0022	14.2	0.007
Gough Island, N = 6	ns				
Tristan da Cunha, N = 9	Constant	13.59	0.42		
	Temperature	-0.0092	0.0039	5.69	0.049

Linear and quadratic year and temperature terms were fitted, with stepwise deletion of non-significant terms to give a final model in which all terms are significant ($P < 0.05$).
ns, nonsignificant.

temporal trend by 0.0053, while adjusting for the phytoplankton fractionation effect reduces it by 0.0026.

Discussion

Carbon isotope ratios

Isotopic data presented here indicate that there have been major changes in the feeding ecology of rock-

hopper penguins during the period ca. 1840–2000. Our results give some support to the hypothesis that decreases in primary productivity, revealed by declining carbon isotope ratios, have been associated with the decline of rockhopper penguin populations. In the Bering Sea, the decline of pinniped populations during the latter half of the 20th century has been attributed to declining primary productivity, as indicated by decreasing tissue $\delta^{13}\text{C}$ ratios (Schell, 2000; Hirons *et al.*, 2001).

Over the last 160 years, $\delta^{13}\text{C}$ is estimated to have decreased in rockhopper penguin diets by ca. 0.7‰. Unadjusted values of $\delta^{13}\text{C}$ in our data give a decrease of ca. 1.9‰ over the same period. Schell (2000) measured a decrease in average $\delta^{13}\text{C}$ of 2.7‰ between 1966 and 1997 in bowhead whales (*Balaena mysticetus*), but did not control for anthropogenic effects on $\delta^{13}\text{C}$ ratios. Hirons *et al.* (2001) reported a decrease of 1.9‰, also in unadjusted $\delta^{13}\text{C}$ signatures, over 50 years in Steller sea lions (*Eumetopias jubatus*) from the Bering Sea. Likewise, unadjusted $\delta^{13}\text{C}$ signatures declined 1.5–2.0‰ in two species of auks from the Gulf of Alaska and the north Pacific Ocean between ca. 1965 and 1985 (Hobson *et al.*, 2004). Finally, decreases of ca. 1‰ in adjusted $\delta^{13}\text{C}$ in Lake Tanganyika since 1950 were preceded by approximately stable values between 1800 and the early 20th century (O'Reilly *et al.*, 2003). The changes observed for rockhopper penguin are therefore of a similar order of magnitude to previous studies, though perhaps occurring at a slower rate.

While there is evidence for global changes in primary productivity, some aspects of our results are equivocal. First, our exploration of the Suess and phytoplankton fractionation effects indicate that these factors influence the overall temporal trend very strongly. Although this is the first study to attempt to quantify and adjust for both these factors, there is considerable uncertainty about the magnitude and spatial variation of both effects. Given this uncertainty, we cannot rule out that the true effects are larger (or, indeed, smaller) than modelled here, and could potentially account for the observed decrease in carbon isotope ratios.

Second, the site-by-site analysis suggests that clear evidence of a linear decrease in carbon isotope ratios is not global, even though there was no evidence of a *site* \times *year* interaction in the global analysis. The only site with strong statistical evidence of a linear decreasing trend in $\delta^{13}\text{C}$ is Antipodes Island, which also has the largest sample size, and so contributes substantially to the global GLM analysis. Perhaps the lack of a consistent isotopic trend across all sites is not that surprising given that rockhopper penguin breeding locations are located within differing water masses, exhibiting a wide range of properties, from subtropical water north of the Subtropical Front, through sub-Antarctic water to Antarctic water south of the Antarctic Polar Front (Fig. 1).

Finally, and perhaps most importantly, the GAM suggests that much of the decline in $\delta^{13}\text{C}$ might have occurred early in the time period of our study (ca. 1840–1940) – a period for which there is no knowledge of population trends. From ca. 1940 onwards, the GAM hints at an increase in $\delta^{13}\text{C}$. A GLM of post-1940 data indicates that as rapid ocean warming commenced,

$\delta^{13}\text{C}$ has overall been positively related to sea-surface temperature, leading to a suggestion of an increase in $\delta^{13}\text{C}$ over this period.

Recently reported declines in other vertebrate marine predators in the sub-Antarctic support the idea of a general decrease in carrying capacity that would be consistent with a decrease in primary productivity. At Campbell Island, there has been an unexplained decline in the grey-headed albatross (*Thalassarche chrysostoma*) population of 82–88% between the 1940s and the 1990s, and concurrent with rockhopper penguin declines of >90% (Cunningham & Moors, 1994; Waugh *et al.*, 1999; Moore, 2004). Additionally, southern elephant seal (*Mirounga leonina*) numbers at Campbell Island declined by ca. 97% between the 1940s and 1980s (Taylor & Taylor, 1989). Similarly, populations of several seabird species and southern elephant seal have decreased at all Indian Ocean islands (Marion, Kerguelen, Macquarie and Amsterdam islands) since the 1960s (Weimerskirch *et al.*, 2003). Interestingly, at South Georgia, the world's largest population of the closely related macaroni penguin (*Eudyptes chrysolophus*), which tends to replace the rockhopper penguin south of the Antarctic Polar Front, is thought to have decreased from ca. 5.4 to 2.7 million pairs between the mid-1970s and mid-1990s (Trathan *et al.*, 1998).

There is evidence of declining chlorophyll *a* and zooplankton concentrations from the southern Indian Ocean since the 1970s, which suggests a drop in primary productivity (Hunt *et al.*, 2001). This, coupled with the simultaneous decline of seabird and seal populations, led Weimerskirch *et al.* (2003) to suggest that a system shift has taken place in the southern Indian Ocean. In the Bering Sea and Lake Tanganyika, increased temperatures and reduced wind speeds since the 1970s are thought to have reduced mixed layer depths, and hence nutrient supply to the euphotic zone, which in turn reduces primary productivity (Schell, 2000; Hirons *et al.*, 2001; O'Reilly *et al.*, 2003). Conversely, in the short-term, increased temperature *per se* may increase plankton growth rates, until the point at which nutrient availability is adversely affected (Richardson & Schoeman, 2004). Sea-surface temperatures have increased substantially in the range of the rockhopper penguin since ca. 1930 (Fig. 2).

Our data are not consistent with the idea that the decrease we observed in rockhopper penguin $\delta^{13}\text{C}$ is caused by a decrease in primary productivity resulting from rising sea temperatures. First, the overall decreasing $\delta^{13}\text{C}$ trend is linear and appears to be driven largely by changes that predate the rapid ocean warming since ca. 1920. Second, there is no detectable relationship between carbon isotope ratios and annual mean sea-surface temperature in our study.

An alternative explanation for the observed temporal trend in $\delta^{13}\text{C}$ is that a top-down effect in the food chain might have caused a reduction in phytoplankton growth rates, and so reduced $\delta^{13}\text{C}$ values (Schell, 2000). Reduced grazing pressure on phytoplankton results in a larger standing stock; this in turn can lead to lower cell growth rates, with no effect on overall productivity of the system. Such top-down influences have not been examined in the sub-Antarctic, but there have been dramatic changes in predator populations in the sub-Antarctic during the last few centuries. Pinniped populations were hunted to near extinction in much of the rockhopper penguin's range during the 18th and 19th centuries (Croxall, 1992), with subsequent recovery of some populations during the latter half of the 20th century. Similarly, baleen whale populations were devastated during the 19th and 20th centuries, and have yet to recover substantially (Croxall, 1992).

Nitrogen isotope ratios

Our analyses provide some evidence for a long-term decline in $\delta^{15}\text{N}$ at some sites, and strong evidence that $\delta^{15}\text{N}$ is negatively related to annual sea-surface temperature across sites. A decrease in $\delta^{15}\text{N}$ over time and in warm years is consistent with a decrease in trophic level (i.e. probably increased feeding on crustaceans, and less feeding on fish and squid). The global trend implies only a small temporal decrease (ca. 0.6‰) relative to an expected $\delta^{15}\text{N}$ increase of 3–5‰ per trophic level (Mizutani *et al.*, 1992; Michener & Schell, 1994; Cherel *et al.*, 2005), suggesting no major systematic shift in diet. However, the predicted variation in $\delta^{15}\text{N}$ because of between-year temperature fluctuations is relatively large (>1‰) suggesting that diet may be substantially affected, perhaps adversely, during particularly warm years. If sub-Antarctic food chains are affected by annual temperature fluctuations, one would expect the response to be nonlinear, with possible threshold effects. Forero *et al.* (2002a) showed that 42% of variation in the condition of Magellanic penguin chicks could be accounted for by variation of ca. 1.2‰ in $\delta^{15}\text{N}$. Chicks fed on a relatively enriched $\delta^{15}\text{N}$ diet had better body condition, presumably because of their better quality diet. Similarly, adults in colonies with higher breeding success tended to have relatively enriched $\delta^{15}\text{N}$ signatures over a range of ca. 0.6‰ (Forero *et al.*, 2002b). This suggests that the $\delta^{15}\text{N}$ changes observed in rockhopper penguins might be of a magnitude sufficient to affect fitness.

An alternative explanation is that rockhopper penguin diets might have shifted as a consequence of population crashes, rather than a cause, for example by reducing intraspecific competition for favoured food

items. However, this explanation seems unlikely, since our data strongly suggest that sea surface temperature, rather than a temporal trend *per se*, is most strongly associated with $\delta^{15}\text{N}$.

Conclusions

This paper represents a novel attempt to reconstruct a species' ecological history, using stable isotopic information locked in museum skins. Such an approach may be a profitable means of understanding how global environmental change is affecting ecosystems. Rockhopper penguins are a valuable study species in this regard, because they are top predators that temporally and spatially integrate food web changes. Furthermore, their global abundance and massive decline suggest an important role as indicators of environmental change in south temperate and sub-Antarctic seas. We have shown significant shifts in rockhopper penguin isotope values – and hence, in the ecosystem in which they feed. However, no single clear global explanation that accounts for the large spatial and temporal scale of rockhopper penguin population declines emerges from these isotopic trends. The time scale of observed isotopic changes does not agree perfectly with what is known about population declines or shifts in temperature and primary productivity. Furthermore, considerable uncertainty surrounds the scale of adjustments in $\delta^{13}\text{C}$ values needed to account for anthropogenic effects, and this can have a strong effect on the overall modelled trends.

Our analyses are hampered by a relatively small number of *site* × *year* combinations per site, and a lack of systematic sampling across the time period of the study. This makes it difficult to assess nonlinearity, and site-specific variations in the temporal trend. This is coupled to a chronic shortage of information on rockhopper penguin population trends for most sites, and demography for all sites. We strongly suggest that ongoing isotopic monitoring of rockhopper penguins worldwide, coupled to analysis of oceanographic data (chl *a*, SST and sea-surface height) might allow more powerful, controlled analyses of the links between rockhopper penguin demography, ecology and environment. This in turn might elucidate some of the profound ecosystem changes that are evidently occurring in the sub-Antarctic zone.

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Appendix A

Adjustment of raw $\delta^{13}C$ values to account for increases in $[CO_2]_{aq}$ and the Suess Effect: the effect of increasing CO_2 concentrations on phytoplankton fractionation

The extent of fractionation of $\delta^{13}C$ by phytoplankton – ε_p – is affected by factors other than cell growth rate, including cell geometry and cell size (Popp *et al.*, 1998; Burkhardt *et al.*, 1999). Of particular concern in relation to the hypothesis tested here is a well-established relationship between $[CO_2]_{aq}$ and ε_p , such that, as $[CO_2]_{aq}$ increases, phytoplankton fractionation increases, and hence, phytoplankton $\delta^{13}C$ decreases (Mizutani & Wada, 1982; Laws *et al.*, 1995). Oceanic $[CO_2]_{aq}$ has increased as a consequence of anthropogenic carbon emissions (Louanchi & Hoppema, 2000), hence, there is potential for a systematic decrease in rockhopper penguin raw $\delta^{13}C$ signatures to be driven by increasing $[CO_2]_{aq}$, independently of any change in primary productivity. However, sea surface temperature increases have occurred in the 20th century, and these will have caused a reduction in the solubility of CO_2 (Weiss, 1974), which may to some extent have counteracted the effect of increasing anthropogenic CO_2 inputs on $[CO_2]_{aq}$. We modelled the influence of atmospheric CO_2 and sea surface temperature on ε_p , to estimate a $[CO_2]_{aq}$ correction factor for each *site* \times *year* combination.

Table A1 Parameters used to model change in phytoplankton fractionation as a function of temporally increasing $[\text{CO}_2]_{\text{aq}}$

Parameter	Description	Measurement units	Value assumed in model
P*	Permeability of plasmalemma to $\text{CO}_2 \times$ surface area of cell	$\text{m s}^{-1} \times \text{m}^2 \text{cell}^{-1}$	1.09^{-9}
C*	Organic carbon content of cell	g C cell^{-1}	4.57^{-10}
ϵ^2	Isotopic discrimination associated with carboxylation	‰	26.5
ϵ_1	Isotopic discrimination associated with import of inorganic carbon into cell through plasmalemma	‰	1
ϵ_{-1}	Isotopic discrimination associated with diffusion of inorganic carbon from cell into surrounding water	‰	1
β	Constant, equal to the ratio of net diffusional loss of CO_2 to carbon fixation		0.2
μ	Growth rate of cells	day^{-1}	0.5
$[\text{CO}_2]_{\text{aq}}$	$\text{CO}_{2\text{aq}}$ concentration in seawater	$\mu\text{mol kg}^{-1}$	Variable, dependent on "Increase in $\text{pCO}_{2\text{seawater}}$ " above, range

*P and C estimated using a cell radius = 1^{-5} μm .

Increase in $\text{pCO}_{2\text{seawater}}$

Atmospheric pCO_2 for years 1744–2000 were downloaded from http://earthtrends.wri.org/searchable_db/index.cfm?theme=3. Louanchi & Hoppema (2000) estimate that the fugacity of CO_2 in surface ocean waters ($f\text{CO}_{2\text{ocean}}$) at 50–59°S in the Southern Ocean (approximately the region in which our study sites are located) has increased at ca. 60% the rate of the atmospheric increase in $f\text{CO}_2$ ($f\text{CO}_{2\text{atmosphere}}$). We, therefore, estimate $f\text{CO}_{2\text{ocean}}$ for each year in our data set as $f\text{CO}_{2\text{ocean}} = f\text{CO}_{2\text{atmosphere}} \times 0.6$.

Oceanic $[\text{CO}_2]_{\text{aq}}$ is a function of $f\text{CO}_{2\text{ocean}}$ and K_o , the solubility of CO_2 , as affected by salinity and temperature (see Weiss, 1974). For each *site* \times *year* combination for which we have isotopic data, we therefore estimated $[\text{CO}_2]_{\text{aq}} = K_o \times f(\text{CO}_2)$. We assume that salinity = 35‰. Sea surface temperature is uniquely estimated for each *site* \times *year* combination (see Materials and methods for the source and processing of long-term site-specific sea-surface temperature data).

The effect of $[\text{CO}_2]_{\text{aq}}$ on phytoplankton $\delta^{13}\text{C}$ fractionation (ϵ_p) can be estimated from Eqn (9) in Laws *et al.* (2002) (see Table A1 for parameters and their values):

$$\epsilon_p = \epsilon_2 + \epsilon_1 - \epsilon_{-1} - \frac{1}{1 + \frac{[\text{CO}_2]_{\text{aq}} P}{\mu C(1+\beta)}} \left(\frac{\epsilon_2 \epsilon_{-1}}{\beta + 1} \right)$$

We vary $[\text{CO}_2]_{\text{aq}}$ in the equation as a function of *year* and *site*, while holding all other parameters constant. The $[\text{CO}_2]_{\text{aq}}$ correction factor for each *site* \times *year* combination is calculated by estimating ϵ_p in that year minus ϵ_p in the first year for which we have temperature data (1874). Table A2 shows that the maximum value of the $[\text{CO}_2]_{\text{aq}}$ correction factor (i.e. that which is estimated for

Table A2 Modelled changes in atmospheric CO_2 , oceanic dissolved CO_2 and phytoplankton fractionation over the course of the study period

Parameter	Initial value	Final value
$f\text{CO}_{2(\text{atmosphere})}$ (μatm)	290	369
$[\text{CO}_2]_{\text{aq}}$ * ($\mu\text{mol kg}^{-1}$)	13.27	15.34
ϵ_p^1 (‰)	6.31	6.47

*Values are calculated using mean temperature for all study sites combined.

year = 2002 and for sites with a small temperature rise is only 0.16‰.

The Oceanic Suess Effect

Anthropogenic CO_2 from fossil fuel burning has a more negative $\delta^{13}\text{C}$ (i.e. is depleted in ^{13}C) than background atmospheric CO_2 (the Suess Effect; Keeling, 1979). Consequently, there has been an exponential decrease in the $\delta^{13}\text{C}$ of oceanic $\text{CO}_{2\text{aq}}$ over the last ~ 150 years (Gruber *et al.*, 1999; Sonnerup *et al.*, 2000; Quay *et al.*, 2003). The magnitude of the effect tends to diminish with increasing latitude (Sonnerup *et al.*, 2000; McNeil *et al.*, 2001), but the trend is not linear; rather it is stepwise, with relatively constant values within water bodies, and sharp changes between them (McNeil *et al.*, 2001). We, therefore, used published data to estimate rates of change of ocean surface $\delta^{13}\text{C}$ separately for each water body with which we are concerned (Table A3). For any given *year* \times *water body* combination, the 'Suess Effect correction factor' is the difference between $\delta^{13}\text{C}$ of oceanic $\text{CO}_{2\text{aq}}$ in 1850 and $\delta^{13}\text{C}$ of oceanic $\text{CO}_{2\text{aq}}$ in that *year* and *water body*.

Table A3 Estimates of the magnitude of the Suess Effect for different study sites

Water body	Maximum annual rate of $\delta^{13}\text{C}$ decrease due to Suess Effect (‰)	Estimated overall magnitude of the Suess Effect, 1850–2002 (‰)	Sites
Subtropical Zone	0.018	0.75	Tristan da Cunha, Amsterdam/St Paul Islands
Sub-Antarctic Zone	0.015	0.62	Gough Island, Antipodes Island, Campbell Island, Crozet Island
Antarctic Polar Front	0.0085	0.35	Kerguelen

We set $\delta^{13}\text{C}$ oceanic $\text{CO}_{2\text{aq}}$ (1850) to zero. To parameterize the exponential curves that describe the Suess Effect from 1850 to 2002 for each of our study sites we used the equation

$$\begin{aligned} \text{Suess Effect correction factor}_{(\text{water body } x \text{ year } y)} \\ = a_{(\text{water body } x)} + \exp(\text{year}_{y-1850} \times y) \end{aligned}$$

To parameterize the equation, we used estimates of the current rate of change in $\delta^{13}\text{C}$ for each water body, from McNeil *et al.* (2001; Table A3). McNeil *et al.* (2001) estimated the rate of decline in $\delta^{13}\text{C}$ for 1978–1998 in the Southern Ocean south of Australia, explicitly showing how the rate varies between different water bodies. The shape of the exponential curve was determined by setting y ($=0.027$) such that it describes the curve presented by Gruber *et al.* (1999) for $\delta^{13}\text{C}_{\text{ocean}}$ over the period 1945–1997.

Our estimates of the overall magnitude of the Suess Effect over the period 1850–2002 (Table A3), derived from these exponential curves, were in accord with published estimates of the magnitude of the effect (e.g. Sonnerup *et al.*, 2000; Quay *et al.*, 2003), and are substantially larger than the estimates for the phytoplankton fractionation effect.

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