

# Intrinsic and extrinsic forcing in life histories: patterns of growth and stable isotopes in male Antarctic fur seal teeth

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**ABSTRACT:** Life-time records of the trophic sources of carbon, nitrogen and of growth rate can be generated from biogenic structures that show accretionary growth, including fish scales, whale baleen and the teeth of some animals. Records generated from individual teeth can also be combined to provide longer time series elucidating changes in environmental conditions encountered by a population. Both intrinsic (i.e. ontogenetic) and extrinsic (i.e. environmental) factors are important in modulating variation in growth and the apparent dietary sources of C and N. We used the canine teeth of a large marine predator, the male Antarctic fur seal *Arctocephalus gazella* from South Georgia, to investigate both intrinsic and extrinsic sources of variation. Substantial ontogenetic shifts occurred in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in individual teeth, indicating a change in the trophic sources of C and N as individual animals age. Over the 40 yr period from 1964 to 2005, and after statistical reduction of ontogenetic variation, we also detected long-term declines in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, indicating that the population has become more dependent on energy from a lower trophic level. A concurrent decline in annular tooth growth may be a consequence of rapid population growth during this period. The time series of  $\delta^{13}\text{C}$  values was also inversely correlated with sea surface temperatures in the region, although isolating a causal relationship remains elusive. Our analyses suggest that both intrinsic and extrinsic sources of variation, and their interaction, must be considered from such time series data; failure to do so could result in a biased interpretation.

**KEY WORDS:** Annulus · Dietary reconstruction · Stable isotope · Time series analysis · Southern Ocean

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## INTRODUCTION

Apportioning biological variation between intrinsic (i.e. ontogenetic) and extrinsic (i.e. environmental) variables amongst free-ranging vertebrates is a central challenge in understanding how environmental factors influence populations, with particular relevance for predicting the effects of future environmental change. The present study examines this question in a species

of seal that is exposed to considerable interannual and long-term variation in its food supply.

The biological response of top predators may be influenced by direct factors, such as changes in habitat availability, as well as indirect factors, such as prey abundance and distribution, that result in changes to competitive dynamics between species sharing resources (Croxall et al. 1992, Constable 2006). Long-term monitoring of the diet of top predators can pro-

vide information concerning trophic dynamics at the ecosystem level as well as population dynamics of both predators and their prey (Croxall 2006, Reid et al. 2006). In some circumstances, such as in the Southern Ocean, characterising and understanding the relationships between top predators, their prey and environmental variability represents an important step towards ecosystem-based management of populations of commercially exploited species (Constable 2006).

While approaches to trophic studies have included stomach and scat analysis, radio-labelling and, more recently, quantitative fatty acid analysis, stable isotopes of carbon and nitrogen are increasingly used to better inform ecologists of trophic relationships. Predators are typically enriched in  $^{15}\text{N}$  relative to their prey, with an average increase of  $3.3 \pm 0.26\%$  between trophic levels (McCutchan et al. 2003), and therefore,  $\delta^{15}\text{N}$  values can be used to resolve trophic positions (Peterson & Fry 1987). In contrast,  $\delta^{13}\text{C}$  values vary less ( $\sim 1\%$ ) between trophic levels but are often used to indicate the geographic source of prey items due to well-documented variation in  $\delta^{13}\text{C}$  with different photosynthetic processes (Hobson 1999). In the marine environment, phytoplankton  $\delta^{13}\text{C}$  values can vary in relation to cell physiology, morphology, growth and the source of inorganic carbon (Michener & Schell 1994, Burkhardt et al. 1999). Variations in the  $\delta^{13}\text{C}$  values of some animals can be linked to benthic versus pelagic or nearshore versus offshore feeding habitats (Hobson et al. 1994, Michener & Schell 1994, Hobson 1999, Cherel & Hobson 2007).

Pinnipeds show incremental growth in their teeth throughout their lives. Thus, the life-history of individual animals may be reflected in the incremental pattern of growth, represented as both the rate of change in tooth growth and changes in the chemical composition of each growth layer (Boyd & Roberts 1993, Newsome et al. 2006, Newsome et al. 2007). Like the teeth of many seal species, the teeth of Antarctic fur seals *Arctocephalus gazella* are formed from annular growth layers of dentin. These layers form a series of stacked cones, the outer edges of which are visible on the exterior of the tooth and have been used to determine the age and relative growth rate of individuals (Boyd & Roberts 1993).

These layers, called 'annuli', are presumed to be metabolically inert after their formation. Thus, the isotopic compositions of annuli are thought to encode a temporal record that can be used to examine changing diet and trophic status throughout the life of an individual animal (Hobson & Sease 1998, Balasse et al. 2001, Newsome et al. 2006, 2007, Knoff et al. 2008). Additionally, variations in the width of each annulus may be related to variations in the growth of the animal (Boyd & Roberts 1993), serving as a proxy mea-

surement of growth rate that may correlate with the total food available to individuals at different stages of their lives. However, there is a natural and constant decline in annulus width with the age of the animal that must be taken into account before relating this information to food availability or growth (Boyd & Roberts 1993). The teeth of Antarctic fur seals have the potential to offer an insight into their trophic life-history and, potentially, into broader environmental variability.

When the teeth of seals dying of natural causes are collected over time and in sufficient numbers, it is possible to build a time-series of changes in chemical composition and layer deposition. These patterns can be used in 2 main ways: (1) to examine life histories of individuals and (2) to examine changes in relation to the calendar year of deposition. In the present study, we use contrasting insights from these records to examine the hypotheses that dietary variation, reflected in dietary C and N, and annulus width are affected at least as strongly by intrinsic factors, such as ontogenetic development or longevity, as they are by extrinsic factors, such as environmental conditions. We also use time series of stable isotope variation to investigate broad-scale perturbations and long-term changes in marine ecosystems that may be commonly reflected in the biological responses of top predators (Forcada et al. 2005, Croxall 2006, Murphy et al. 2007). We reason that since fur seals that breed on South Georgia range widely over a region that is characterised by highly dynamic oceanographic conditions (Boyd et al. 1998, 2002), any residual signal contained within fur seal teeth, once intrinsic life-history effects are partitioned out, is likely to reflect general ecological changes at a regional scale.

## MATERIALS AND METHODS

### Tooth preparation and stable isotope analysis.

Antarctic fur seal teeth were collected from males that died of natural causes at breeding beaches on Bird Island, South Georgia ( $54^{\circ}00' \text{S}$ ,  $38^{\circ}03' \text{W}$ ) during December to January. Left and right upper canines were extracted after the carcass had decayed sufficiently to allow extraction using tooth pliers. We randomly selected a sub-sample of these teeth, archived at the British Antarctic Survey (Cambridge, UK) and the Sea Mammal Research Unit (St. Andrews, UK) for analysis.

An average of 10 teeth were selected from a series of collection years for which teeth were available: 1971, '76, '78, '79, '87, '91, '92, '93, '94, '95, '98, '99, '01, '04 and '06 (collection years are referred to by the second year of the season as in Forcada et al. 2005). Consider-

ing that up to 10 dentinal annuli were sampled from each tooth, this meant that samples were available for annuli laid down in all years from 1961 to 2005. Each whole tooth was sawn in half using a diamond-burred circular saw and one half soaked in 10% formic acid overnight to etch the surface of the tooth and facilitate visual discrimination between individual annuli. The age of the animal was estimated by counting individual growth layer groups (GLGs) determined as one depressed, etched section and one raised, un-etched section. The number of GLGs was counted 3 times by a single observer. If 2 of the 3 estimates were the same, that age became the best estimate for that tooth. If all estimates differed by no more than a year, then the mean of these estimates was used. If estimates differed by more than 1 yr, the section was re-examined. Each layer width was measured where growth layers were parallel in the center of the dentin and in a step-wise manner so as to account for all layers (as in Boyd & Roberts 1993) using digital callipers (Fig. 1). The standard deviation of measurements was estimated to be 0.09 mm based on re-analysis of 20% of canine teeth. Each annulus was then assigned to a calendar year by back-counting from the calendar year of collection using the age at death. A total of 141 teeth were cut, aged and measured.

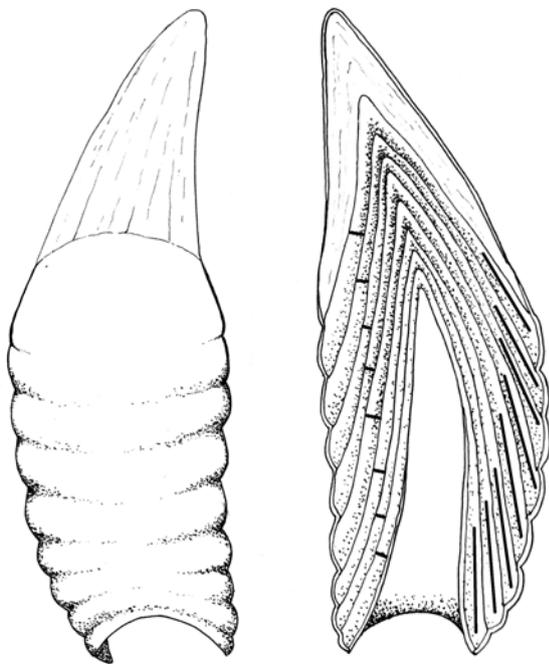


Fig. 1. *Arctocephalus gazella*. Diagram of the outer surface and a longitudinal cut through a 7-yr old male Antarctic fur seal canine tooth. Growth layer groups (GLGs) were visually identified and measured on the etched surface of the tooth. Black horizontal band: area where measurements were taken. Vertical black lines: where powdered dentin samples were extracted for analysis

Approximately 2 to 3 mg of dentin was extracted for isotope analysis from individual growth layers using an electric drill with a 900  $\mu\text{m}$  diamond-tipped dental burr (FG801LS-009 Medium Grit). Material from the outer surfaces was not used in order to prevent any confounding isotopic effect of the formic acid treatment on the external surface of the tooth, and dentin was drilled to a depth of no more than 500  $\mu\text{m}$  to reduce contamination from other layers. Material was only taken from annuli large enough to reliably drill within the band (mainly the outer annuli deposited in the first 5 yr of life) and, where possible, consecutive samples were taken from each successive annual growth band.

In order to remove inorganic material,  $\sim 1.5 \pm 0.1$  mg of powdered dentin was weighed out into silver capsules then washed with 100  $\mu\text{l}$  0.5 M HCl and left to dry for 24 to 36 h at 20°C.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , and carbon and nitrogen weight percent (%C and %N, respectively) were determined using a Costech Elemental Analyzer fitted with a zero-blank auto-sampler coupled via a ConFloIII to a ThermoFinnigan DeltaPlus<sup>XL</sup> using continuous-flow isotope ratio mass spectrometry (CF-IRMS) at the University of St. Andrews Facility for Earth and Environmental Analysis. Stable isotope results are reported as per mil (‰) deviations from the Vienna PeeDee Belemnite (VPDB) and atmospheric  $\text{N}_2$  reference standard scale for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively. Precisions (SD) on internal standards were better than  $\pm 0.1$  and 0.2‰ for carbon and nitrogen, respectively.

**Data analysis.** Two main sources of potential variation were present in the dataset and had to be statistically disaggregated.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in teeth have previously been shown to vary throughout an individual seal's life history (Hobson & Sease 1998, Newsome et al. 2006) and when life history profiles are aggregated, there is also the possibility of multi-decadal and inter-annual variation in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values arising as a function of this aggregation over the 41 yr time period for which data were available. In addition, there was the possibility that differences in stable isotope values may be related to the longevity of an individual animal. To partition the variance in the data between these different variables, and to investigate data in relation to each variable individually, a series of multiple linear regressions (mixed effects models) including all combinations of independent variables was performed to model the data. We tabulated the weighted deviance of all models and compared them using a chi-squared test for significance (Boyd 1996). Akaike's information criterion (AIC) score (Akaike 1976) was also used when determining the model of best fit.

The effects of individual variables were investigated using the partial regression coefficients derived from the best-fitting model. This reduced the effect of the other variables and helped to reconstruct the effect of

each individual explanatory variable. Simple bivariate linear regressions were then used on data corrected for other effects to investigate trends in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in relation to each variable (annulus number, age-at-death and calendar year) individually. Linear regression was also applied to the annulus width time-series with the effect of annulus number removed to highlight variations in tooth growth over time.

Yearly averaged stable isotope time-series (corrected for age-at-death and annulus number) were de-trended by subtracting the linear trend, and correlated with 3 similarly de-trended climate indices previously correlated with biological indices of marine animals in the Southern Ocean—including Antarctic fur seals and local krill stocks (Forcada et al. 2005, Murphy et al. 2007). We reasoned that as Antarctic fur seal diet is highly dependent on the availability of krill in a given year, proxy measurements of their diet could be related to the same environmental forcing as krill. We therefore used annual de-trended grid averages of sea surface temperature (ERSSTv2) (Smith & Reynolds 2003) and sea ice cover (HadISST1) (Rayner et al. 2003) from 45 to 80° S and 20 to 100° W, a large region encompassing both the breeding and potential feeding grounds of male Antarctic fur seals, and an observation-based Southern Annular Mode (SAM) index (Marshall 2003). We used cross correlation analyses to explore the relationship between the climate indices and experimental data lagged up to 2 yr. Statistical computations were performed in R version 2.4.1 (R Development Core Team 2005) and time series data was extracted using the Royal Netherlands Meteorological Institute Climate Explorer ([climexp.knmi.nl](http://climexp.knmi.nl)).

We explored the use of cross-dating accuracy checks commonly used in dendrochronology to ensure the most accurate assignment of growth layers to calendar years. Individual uncorrected width time series were correlated to the mean temporal pattern of all other series from individual animals using the computer program COFECHA version 6.06 (Holmes 1983). First order differencing was used during this process to reduce the intrinsic linear patterns present within tooth measurements. The series intercorrelation provides a measure of the strength of a common signal in the time series and the program highlights individual 'problem' series that may be misdated, suggesting how series should be shifted in order to improve the overall temporal signal. In order to improve the chronology, these series were moved a maximum of 2 yr back in time because the time of death was known and assignment errors were most likely due to inaccurate identification of the neonatal line. A re-evaluated chronology was then related to the same 3 climate indices as the original chronology to test for any differences due to the adjustments made.

## RESULTS

### Statistical models

Statistical modelling of the effects of the set of independent variables on  $\delta^{13}\text{C}$  values (see Table 1) provided an AIC score that was lowest for the model including an interaction between all 3 explanatory variables (Model 11): calendar year, annulus number and age-at-death, while Model 12 (Year + Age  $\times$  Annulus) provided a marginally lower AIC for  $\delta^{15}\text{N}$  values. Further inspection of Models 8 to 14 showed that there were consistent interactions between terms and that calendar year was especially important. These results suggest that the response variables depend on a complex interaction between calendar year, age-at-death and annulus number and that it would be insufficient to consider one variable without consideration of the other two. We have attempted to do this by accounting for the linear effects of other variables in order to highlight the effect of the variable of interest. The lowest AIC for annulus width included an interaction between calendar year and annulus number although there was a significant interaction between all 3 variables. The partial regression coefficients used to extract the relationships for each of the independent variables in the regression models are provided in Table 2 along with the descriptive statistics for each resulting dataset. The linear relationship between raw (uncorrected) data and each independent variable is shown in Fig. 2.

### Variation due to annulus number and age-at-death

Model deviances did not detect any significant effect of age-at-death on either  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  when  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were adjusted to account for the linear effects of the calendar year in which the dentin annulus was deposited and the annulus number ( $\delta^{13}\text{C}$ ,  $\chi^2 = 11.62$ ,  $df = 6$ ,  $p > 0.05$ ;  $\delta^{15}\text{N}$ ,  $\chi^2 = 11.77$ ,  $df = 6$ ,  $p > 0.05$ ). However, the developmental stage of the animal had a significant effect on diet as inferred from both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. This was because stable carbon isotope values decreased linearly with annulus number ( $b = -0.165 \pm 0.0411$ ), although this accounted for only 5% of the variation in the data. But stable nitrogen isotope values increased with annulus number ( $b = 0.256 \pm 0.0297$ ) and accounted for nearly 23% of the variation in the data.

### Temporal variation

A simple least squares linear regression model fitted to 41 yr of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  data, excluding the effects of tooth annulus number and age-at-death, showed there

Table 1. Results of multiple regression analyses relating stable carbon and nitrogen isotope values and annuli width measurements to calendar year, annulus number and age-at-death (degrees of freedom are given in parentheses). \* $p < 0.05$ ; \*\* $p < 0.01$ ; \*\*\* $p < 0.001$ ; ns: not significant. Model deviances,  $R^2$  and AIC values are also given

Model	k	Year (40)	Annulus (6)	Age (6)	Annulus $\times$ Age	Year $\times$ Age	Year $\times$ Annulus	Year $\times$ $\times$ Age	$R^2$ (%)	Deviance	AIC	
<b><math>\delta^{13}\text{C}</math></b>												
11	9	–	–	–	–	–	–	***	39.40	29.03	756.50	
13	6	–	–	ns	–	–	***	–	37.80	38.79	760.26	
14	6	–	ns	–	–	***	–	–	37.10	41.28	762.75	
12	6	***	–	–	***	–	–	–	36.30	44.46	765.93	
10	5	–	–	–	–	–	***	–	35.10	29.03	769.64	
7	5	***	*	ns	–	–	–	–	35.40	48.50	770.84	
9	5	–	–	–	–	***	–	–	33.20	50.17	777.09	
4	4	***	*	–	–	–	–	–	32.80	59.30	779.61	
5	4	**	–	ns	–	–	–	–	31.60	63.70	784.00	
1	3	***	–	–	–	–	–	–	28.50	76.10	794.44	
8	5	–	–	–	***	–	–	–	12.50	124.98	844.45	
6	4	–	ns	***	–	–	–	–	11.10	129.60	849.95	
3	3	–	–	***	–	–	–	–	9.70	134.50	852.82	
2	3	–	ns	–	–	–	–	–	1.80	155.60	873.91	
<b><math>\delta^{15}\text{N}</math></b>												
12	6	***	–	–	***	–	–	–	52.40	–133.29	588.18	
11	9	–	–	–	–	–	–	***	52.80	–138.49	588.98	
13	6	–	–	ns	–	–	***	–	50.20	–122.06	599.41	
7	5	***	***	ns	–	–	–	–	48.80	–114.06	605.41	
14	6	–	***	–	–	***	–	–	48.80	–114.86	606.61	
10	5	–	–	–	–	–	***	–	48.10	–110.45	609.02	
4	4	***	***	–	–	–	–	–	46.70	–103.20	614.31	
5	4	***	–	ns	–	–	–	–	34.00	–49.68	667.79	
9	5	–	–	–	–	***	–	–	33.90	–50.07	669.40	
1	3	***	–	–	–	–	–	–	33.00	–44.60	670.83	
8	5	–	–	–	***	–	–	–	31.50	–41.30	678.17	
6	4	–	***	***	–	–	–	–	26.30	–24.31	693.16	
2	3	–	***	–	–	–	–	–	19.10	2.30	717.80	
3	3	–	–	**	–	–	–	–	6.40	38.80	754.27	
<b>Annulus width</b>												
10	5	–	–	–	–	–	***	–	27.30	–2932.84	–67.93	
11	9	–	–	–	–	–	–	***	27.60	–2940.68	–67.77	
13	6	–	–	ns	–	–	***	–	27.30	–2932.87	–65.96	
4	4	ns	***	–	–	–	–	–	26.90	–2926.31	–63.41	
7	5	ns	***	ns	–	–	–	–	26.90	–2926.37	–61.47	
14	6	–	ns	–	–	***	–	–	26.90	–2928.10	–61.20	
12	6	ns	–	–	***	–	–	–	26.80	–2926.39	–59.49	
6	4	–	***	ns	–	–	–	–	23.80	–2884.77	–21.86	
2	3	–	***	–	–	–	–	–	23.70	–2882.12	–21.22	
8	5	–	–	–	***	–	–	–	23.80	–2885.00	–20.10	
5	4	***	–	ns	–	–	–	–	11.10	–2729.18	133.73	
9	5	–	–	–	–	***	–	–	11.10	–2730.22	134.69	
1	3	***	–	–	–	–	–	–	10.10	–2717.35	143.56	
3	3	–	–	***	–	–	–	–	3.40	–2645.36	215.54	

was a significant decline in both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values in relation to calendar year ( $\delta^{13}\text{C}$ ,  $b = -0.0678 \pm 0.00665$ ;  $\delta^{15}\text{N}$ ,  $b = -0.0521 \pm 0.00623$ ). Additionally, both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  appeared to oscillate over time (Fig. 3). After accounting for the effect of decreasing annulus width with age, there was also a significant linear decline in annulus width from 1961 to 2005 ( $b = -0.005 \pm 0.001$ ). According to this regression, there was

an average 0.225 mm decrease in annulus width over the 41 yr data series.

In addition to these long-term declines,  $\delta^{13}\text{C}$  values were negatively correlated with sea surface temperature (SST) ( $^{\circ}\text{C}$ ) values in the same year (Pearson product-moment correlation,  $p = 0.0128$ ,  $df = 39$ ,  $r = -0.385$ ). No other significant patterns appeared from the cross-correlation analysis.

Table 2. Descriptive statistics for the original datasets and each subsequent dataset calculated from the partial regression coefficients (*b*) for Year, Annulus and Age

Variable	Corrected for:	Mean	SE	Minimum	Maximum	Units	n	<i>b</i> (Year)	<i>b</i> (Annulus)	<i>b</i> (Age)
$\delta^{13}\text{C}$	Uncorrected	-18.88	0.09	-22.48	-14.82	‰	250	-0.068	-0.163	-0.158
$\delta^{13}\text{C}$	Year, Age-at-death	-21.52	0.01	-26.05	-21.52	‰	250			
$\delta^{13}\text{C}$	Annulus, Age-at-death	-20.61	0.01	-24.10	-16.74	‰	250			
$\delta^{13}\text{C}$	Year, Annulus	-17.07	0.07	-20.01	-13.37	‰	250			
$\delta^{15}\text{N}$	Uncorrected	9.87	0.07	7.59	14.13	‰	250	-0.052	0.256	-0.115
$\delta^{15}\text{N}$	Year, Age-at-death	7.89	0.01	5.15	12.80	‰	250			
$\delta^{15}\text{N}$	Annulus, Age-at-death	9.76	0.01	7.06	14.76	‰	250			
$\delta^{15}\text{N}$	Year, Annulus	10.17	0.07	8.45	13.36	‰	250			
Annulus width	Uncorrected	0.81	0.01	0.30	2.10	mm	1006	-0.0052	-0.0494	
Annulus width	Year	0.95	0.01	0.41	1.08	mm	1006			
Annulus width	Annulus	1.03	0.01	0.50	2.20	mm	1006			

### Chronology validation

We found that 36.8% of individual annulus width series and 35.6% of isotope series were more closely aligned with the total time series mean pattern once shifted by 1 to 2 yr. However, these shifts did not significantly improve the standard error of the mean time series except for  $\delta^{13}\text{C}$  (2-tailed t-test,  $df = 37$ ,  $p < 0.05$ ), suggesting that, overall, tooth chronologies were adequately determined in the first instance. The re-evaluated series were not significantly cross-correlated with any environmental time series, although the relationship between  $\delta^{13}\text{C}$  and SST remained strong ( $p = 0.065$ ,  $df = 39$ ,  $r = -0.29$ ).

## DISCUSSION

### Intrinsic effects

We found evidence for consistent changes in stable isotope ratios over the first 7 yr of the lives of male Antarctic fur seals once the variation due to the calendar year of deposition and the age-at-death of the animal was removed. Considering that relatively few male seals live more than 10 yr (Boyd & Roberts 1993), this suggests that the trophic level at which male Antarctic fur seals forage, and their source of carbon, changes systematically during the period of rapid growth included in this analysis (Payne 1977). Other studies of pinniped teeth (Hobson & Sease 1998, Newsome et al. 2006) have observed high  $\delta^{15}\text{N}$  and low  $\delta^{13}\text{C}$  values in the first 1 to 2 yr of life presumably due to the metabolic routing of milk protein and lipids during nursing. However, Newsome et al. (2006) found this pattern lacking among male Northern fur seals, presumably due to the short (~4 mo) lactation period, a characteristic shared with Antarctic fur seals. Both studies note that there is a large amount of variation in ontogenetic patterns among individuals.

In the present study we have used larger sample sizes and have described the linear change in  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values into the 7th year of life, when animals are physically and sexually mature, thus creating a more complete view of ontogenetic variation than previous studies. While not statistically different, when  $\delta^{15}\text{N}$  values were averaged for each annulus there was a peak at Year 2 followed by a decrease of nearly 1‰ in the third year. Estimates of isotopic equilibration of dentin after a switch in diet have been suggested to be between 1 to 4 mo (Balasse et al. 2001). As weaning occurs after approximately 4 mo in these animals, it is plausible that a milk-derived  $\delta^{15}\text{N}$  signal could influence the isotopic profile throughout the first ~8 mo of dentin growth. Little information is available about post-weaning diet; Warren et al. (2006) tagged 5 male pups after weaning and found that they moved progressively offshore to more oceanic conditions throughout the first winter, potentially following the availability of prey items. This change in foraging location could mask any  $\delta^{15}\text{N}$  signal due to the short lactation period and contribute to a decrease in  $\delta^{15}\text{N}$  values in the third year if pups begin to forage predominately on krill at this time. However, it is difficult to draw firm conclusions without more detailed information on juvenile diet.

Because the common prey species of Antarctic fur seals are known to encompass at least 3 different trophic levels (e.g. krill, krill-eating fish, and piscivorous fish and sea-birds) our results showing an ontogenetic increase in  $\delta^{15}\text{N}$  values indicate that male Antarctic fur seals tend to forage on higher trophic level prey as they age. Antarctic krill sampled around the Antarctic Peninsula and the Lasarev Sea had  $\delta^{15}\text{N}$  values of 3.6‰, whereas fish species sampled around the Falkland Islands and the Antarctic Peninsula had values ranging from 8.4 to 11‰ (Dunton 2001, Cherel et al. 2002, Schmidt et al. 2003). Thus, the ontogenetic increase in  $\delta^{15}\text{N}$  values in Antarctic fur seals dentin indicates that they are including more krill-eating and/or piscivorous fish in their diets rather than directly feed-

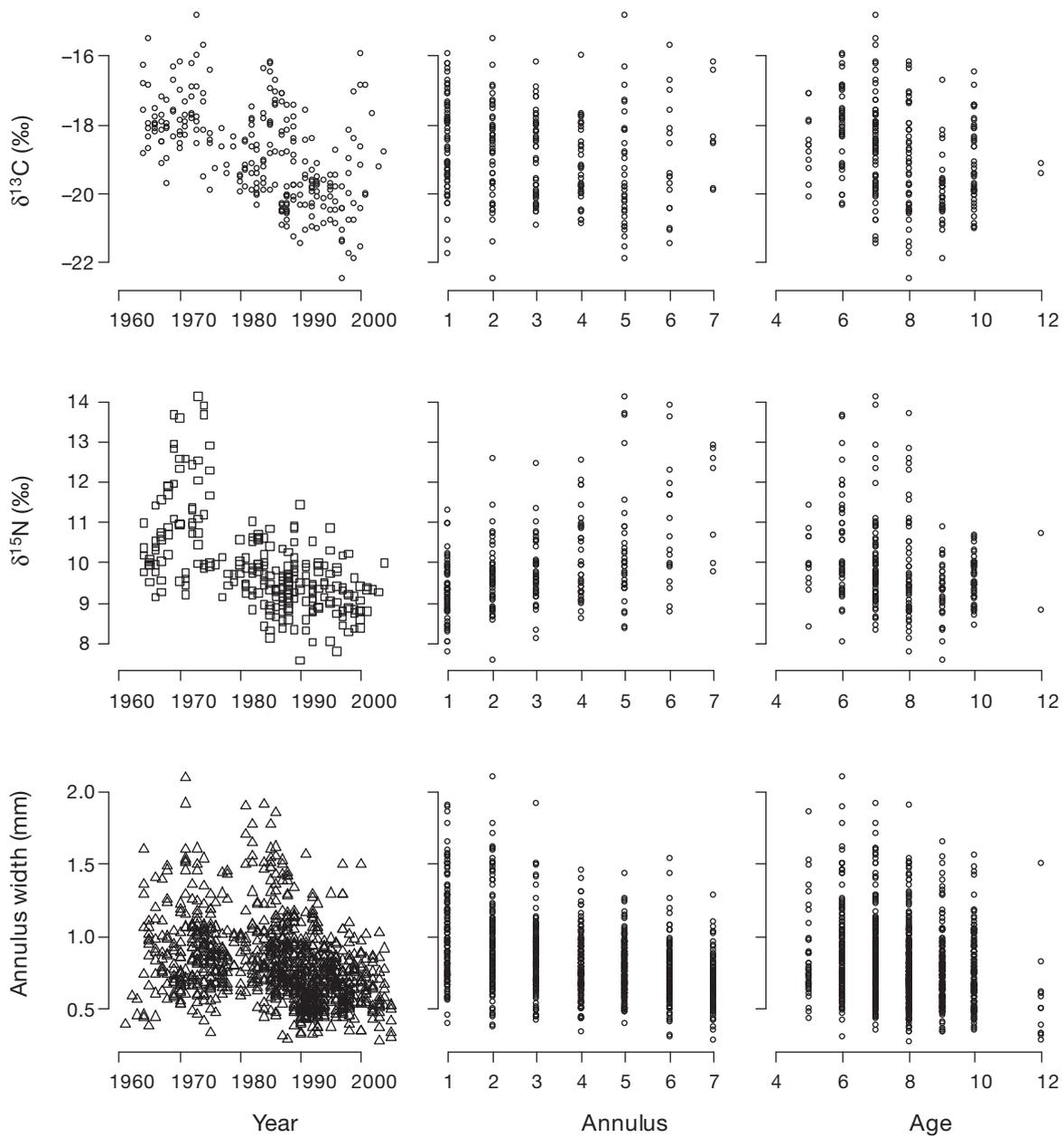


Fig. 2. *Arctocephalus gazella*. Relationships between uncorrected datasets and calendar year, annulus number and age-at-death. Stable isotope values are per mil (‰) deviations from the Vienna PeeDee Belemnite (VPDB) and atmospheric  $\text{N}_2$  reference standard scale for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, respectively. Year: calendar year of annulus deposition; Annulus: layer number; Age: age-at-death of the animals

ing on krill. The concomitant decline in  $\delta^{13}\text{C}$  values could represent this shift to a new diet if fish were relatively depleted in  $^{13}\text{C}$  compared to krill, but as the opposite is true (Dunton 2001, Cherel et al. 2002, Schmidt et al. 2003) this explanation seems unlikely. The  $\delta^{13}\text{C}$  values of particulate organic carbon decrease with increasing latitude (Goericke & Fry 1994), a

phenomenon also reflected in the isotopic values of Antarctic krill (Schmidt et al. 2003); therefore, the ontogenetic decline in  $\delta^{13}\text{C}$  values may reflect the movement of males to more southerly foraging grounds (Boyd et al. 1998). It is likely that these developmental shifts in diet reflect the changing energetic needs and foraging capabilities of male fur seals as they grow.

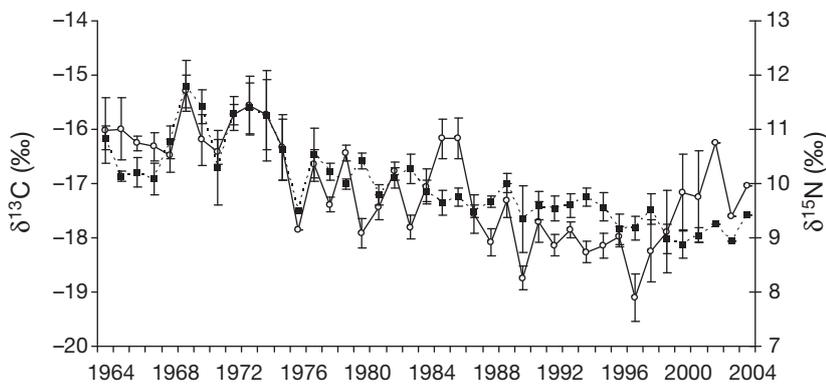


Fig. 3. *Arctocephalus gazella*. Averaged pattern of stable carbon (○) and nitrogen (■) isotope values ( $\pm$  standard error) in the organic fraction of tooth dentin from 1964 to 2004

### Extrinsic effects

The computer program COFECHA is a widely used dendrochronological tool that has been useful for cross-dating other biological proxy series such as those obtained from tree rings, mollusc shells and coral skeletons. The series obtained from Antarctic fur seal teeth, however, are much shorter than those commonly used with this program ( $\sim 7$  yr compared to decades) and this is probably the most limiting factor to its application to tooth time series analysis. Any interpretation of results based on time series that are re-evaluated using COFECHA must consider the limitation of short time series. We explored the use of this tool to improve the dating of our chronology but did not find sufficient evidence to warrant changing it. Such resources from other disciplines could be useful to future studies of this kind, however, and we recommend their consideration.

The apparent changes in stable isotope composition of teeth during the lifetimes of male Antarctic fur seals discussed in the previous section have taken place against a background of a temporal trend in trophic level and carbon source. Male fur seals at South Georgia have experienced a  $\sim 2\%$  decline in dentin  $\delta^{15}\text{N}$  values from 1964 to 2004, representing a substantial drop in trophic level. This result is consistent with Antarctic fur seals becoming more dependent on krill in their diet in recent years. Similarly, a decline in  $\delta^{13}\text{C}$  values, indicative of a more pelagic/offshore feeding environment (Hobson et al. 1994, Cherel & Hobson 2007) supports this interpretation. It is possible that a proportion of the annual decline in  $\delta^{13}\text{C}$  values could be attributed to the Suess effect: the decline in oceanic  $\delta^{13}\text{C}$  values of dissolved organic carbon due to anthropogenic input of atmospheric  $\text{CO}_2$ . However, this decline has been estimated by McNeil et al. (2001) to be  $0.015 \pm 0.003\% \text{ yr}^{-1}$  in the Sub-Antarctic Zone and

we observed a  $0.068\% \text{ yr}^{-1}$  decline in  $\delta^{13}\text{C}$  over the time period. The decline in  $\delta^{13}\text{C}$  could also be related to large-scale processes governing primary productivity in the Southern Ocean. It is possible that the trend in  $\delta^{13}\text{C}$  values of Antarctic fur seal dentin reflects a global decline in primary productivity (Gregg et al. 2003), especially pronounced in high latitudes, because of the inverse relationship between algal cell growth rates and  $\delta^{13}\text{C}$  (Hofmann et al. 2000, Schell 2000).

The decline in both stable isotope time series could be attributed to shifts in diet of fur seals if fur seals are feeding more exclusively on krill swarms than in previous years. Myers & Worm (2003) reported a substantial decline in worldwide predatory fish stocks since the onset of commercial exploitation, including an analysis of  $\sim 60$  yr of trawl survey data from South Georgia that contained information on some fish species found in the diet of Antarctic fur seals. Likewise, in their summary of fisheries activity and policy in the Southern Ocean, Kock et al. (2007) noted the over-fishing and subsequent collapse of *Notothenia rossi* and other fish stocks in the 1970s. *N. rossi* was reported as being common in the diet during early analyses of Antarctic fur seal stomach contents (Bonner 1968), but was almost absent from the diet in more recent studies (e.g. Reid 1995). This historical loss of predatory fish in the Southern Ocean could provide a partial explanation for the decrease in average trophic level as indicated by  $\delta^{15}\text{N}$  values. Indeed, the abrupt change in the  $\delta^{15}\text{N}$  time series in the early 1970s coincides almost exactly with the period of greatest catches of *N. rossi* around South Georgia (Kock et al. 2007).

These isotopic trends have been accompanied by a significant decline in annulus width, after accounting for the ontogenetic changes, over the course of the time series. This decline in annulus width could be caused by a density-related response to an increasing fur seal population in the region (Boyd & Roberts 1993). Assuming dentine deposition is indeed an accurate recorder of animal growth, the very high numbers of fur seals at South Georgia may cause competition for food resources between males and a coincident decline in annular growth of individuals. Also, increased competition may mean that a higher proportion of smaller, weaker males have died and been collected at the breeding beach in more recent years.

In addition to these long-term trends, there is evidence that the diet of fur seals has varied on a much shorter time scale. We sought to test for any functional

links between fluctuations in physical indices known to affect krill populations and the sub-decadal variation observed in the Antarctic fur seal teeth time series. These indices represent only a small subset of potential sources of extrinsic variation, but their impact on local environmental conditions, and particularly on krill stocks, is well documented. Relationships between life-history variables in fur seals, some other predators of krill and environmental variability have already been suggested (Forcada et al. 2005, Nicol 2006, Trathan et al. 2006).

A significant proportion of the oscillations in stable carbon isotope values varied inversely with SST in the region. Sea surface temperature could be driving changes in the  $\delta^{13}\text{C}$  values measured in the dentin of male Antarctic fur seals by (1) causing changes in the annual availability or distribution of food sources—mainly krill, (2) causing changes to the baseline  $\delta^{13}\text{C}$  signature of phytoplankton or (3) causing males to travel to more southerly regions when SST is high and to more northerly regions when SST is low. While climatic forcing of the abundance of Antarctic krill has been suggested (Murphy et al. 2007), the process is not instantaneous and lags behind SST anomalies by 2 yr. Although errors in age estimation could cause improper assignment to calendar years, our re-evaluation of the altered time series (as suggested by the COFECHA analysis) did not find a significant correlation at a lag of 2 yr, indicating that changes in krill abundance are not the functional link between our  $\delta^{13}\text{C}$  series and SST. Second, if the  $\delta^{13}\text{C}$  signal present in our time series was directly related to krill availability, we would expect this signal also to be present in the  $\delta^{15}\text{N}$  series as, presumably, males would need to supplement their diets in these years with increased piscivory. Alternatively, SST could be influencing fur seal  $\delta^{13}\text{C}$  values by affecting inter-annual variability in  $\delta^{13}\text{C}$  values of phytoplankton. Popp et al. (1999) found that the decline in  $^{13}\text{C}$  with latitude in Southern Ocean suspended particulate organic matter was at least partially driven by changes in algal assemblages and cell growth rates, and the availability of dissolved  $\text{CO}_2$  in the oceans is considered to be a driver of variation in  $\delta^{13}\text{C}_{\text{POC}}$  (Goericke & Fry 1994, Burkhardt et al. 1999, Popp et al. 1999). Goericke & Fry (1994) showed that  $\text{CO}_2(\text{aq})$  varies inversely with temperature in the global oceans. If these latitudinal differences are partly driven by temperature, then inter-annual changes in SST may also change community structure and growth rates of marine algae between years and provide a link between the  $\delta^{13}\text{C}$  signal present in Antarctic fur seal dentin time series and SST. However, we are not aware of any long-term inter-annual monitoring of  $\delta^{13}\text{C}$  values of particulate organic matter in the Southern Ocean with which to test the above conjecture.

Likewise, there is insufficient data on the distribution of male fur seals outside the breeding season to discard the third hypothesis that inter-annual changes in SST could cause males to travel to widely different foraging locations (and hence  $\delta^{13}\text{C}$  values), perhaps across the Antarctic Polar Front.

In the present study we have shown that the annular deposition of dentin layers in the teeth of pinnipeds can document patterns of ontogenetic and temporal shifts in the availability of stable nitrogen and carbon isotopes. A complex set of intrinsic and extrinsic factors affecting diet will mainly account for this variation in stable isotope composition. Attempting to separate these factors is a necessary first step before identification of potentially important links between diet and environmental conditions. The present study has shown that historical patterns of stable isotope deposition in the teeth of male Antarctic fur seals can provide a measure of long- and short-term environmental variability as seen from a top-down ecological perspective.

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