Vol. 305: 249–259, 2005 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published December 23

Stable carbon and nitrogen isotope analysis reveals seasonal variation in the diet of leopard seals

Sophie A. Hall-Aspland[1, 2, *], Tracey L. Rogers[2], Rhondda B. Canfield[1]

[1]Faculty of Veterinary Science, JD Steward Building B01, University of Sydney, Sydney, New South Wales 2006, Australia
[2]Australian Marine Mammal Research Centre, Zoological Parks Board NSW/Faculty of Veterinary Science, University of Sydney, PO Box 20, Mosman, Sydney, New South Wales 2088, Australia

ABSTRACT: In order to assess seasonal and spatial changes in diet, the $\delta^{15}N$ and $\delta^{13}C$ signatures of vibrissae from leopard seals *Hydrurga leptonyx* obtained from Prydz Bay, Eastern Antarctica, were compared with those of a captive seal on a known diet. Using the isotopic signatures of known prey, and those revealed by the assimilation rates of vibrissae, we constructed trophic models to estimate diet composition. Assuming that current diet was reflected only in the actively growing portion of the vibrissae, the latter were sectioned. Each section was then analysed independently. Two methods of analysis of the vibrissae isotopic data were compared in order to ascertain the best analytical approach to these data. A simple linear model and a von Bertalanffy growth model were used to estimate section age and vibrissae growth rates. The age predictions of the von Bertalanffy growth model allowed the existence of repeated seasonal oscillations in both $\delta^{15}N$ and $\delta^{13}C$ values. Temporal variations in stable isotope ratios consistent with changes in source of feeding (inshore vs. offshore) and prey types were identified in the Antarctic leopard seals, but not in the captive seal. This preliminary study has possible implications for the use of vibrissae to track dietary changes over time and may serve as a tool for investigating foraging preferences of highly mobile or migratory pinniped species.

KEY WORDS: Leopard seal · Stable isotope · Vibrissae · von Bertalanffy · Antarctic · Prey switching

INTRODUCTION

Stable isotope analysis has been used extensively to determine the trophic ecology of birds and mammals. The isotope ratios in a consumer's tissue reflect assimilated and not just ingested food and can provide more information than standard dietary analysis. Inshore, benthic food webs are often more enriched in ^{13}C than offshore, pelagic food webs (Fry 1981, France 1995) and ^{15}N enrichment is caused by changes in trophic level (Fry 1988, Hobson & Welch 1992).

Leopard seals are top-order predators in the Antarctic ecosystem. Diets of opportunistic predators are difficult to examine due to their complex food webs and wide range of possible prey types. Scat and stomach contents analysis suggests that Adelie penguins *Pygoscelis adeliae* are eaten throughout the year, but are also the main prey item in the Prydz Bay region during the austral summer (Rogers & Bryden 1995, Hall-Aspland & Rogers 2004). Crabeater seals *Lobodon carcinophagus* become important in the diet between November and February when newly weaned pups are available as easy prey targets (Siniff & Bengtson 1977, Siniff et al. 1979, Bengtson 1982, Siniff & Stone 1985). Krill and fish are preyed on throughout the summer from September to March whereas Antarctic silverfish *Pleuragramma antarcticum* predominated during winter (Green & Williams 1986).

The metabolic activity of different tissue types can provide dietary information over a range of time scales (Tieszen et al. 1983, Hobson & Clarke 1992) from a few days (plasma and liver) to months (red blood cells, muscle and fur) depending on the tissues analysed (Tieszen et al. 1983, Hobson & Clark 1993, Hildebrand et al. 1996, Darimont & Reimchen 2002). Tooth annuli of marine mammals have been used to identify interyear variation in diet (Hobson & Sease 1998, Hobson et

*Email: s_hallasp@hotmail.com

al. 2004). Changes along the length of whale baleen have provided an isotopic record of seasonal variation in the location of feeding (Best & Schell 1996, Hobson & Schell 1998, Lee et al. 2005) and diet (Schell et al. 1989, Hobson & Schell 1998). Vibrissae may be a useful tissue for isotopic analysis and the study of long-term and seasonal dietary changes. Vibrissae lengths may be transformed into measurements of time, which can then be related to isotopic ratios.

Growth rates have been determined for a number of pinniped species using dated vibrissae. Harbour seal vibrissae growth rates were irregular (Hirons et al. 2001) or variable (Zhao & Schell 2004), while growth rates in the case of grey seals *Halichoerus grypus* varied according to the length and age of the vibrissae and involved asynchronous shedding and discontinuous replacement (Greaves et al. 2004). Conversely, Steller sea lions *Eumetobias jubatus* displayed more consistent growth and annual retention of their vibrissae (Hirons et al. 2001). Greaves et al. (2004) suggested that vibrissae growth follows a von Bertalanffy growth curve.

This study aims to transform vibrissae lengths into time lines using both a simple linear and von Bertalanffy growth model. With information gained through stable carbon and nitrogen isotope analysis of sequentially segmented vibrissae, their potential as indicators of seasonal change in the leopard seal diet can be assessed.

MATERIALS AND METHODS

Study area and sampling. Following the methodology outlined in Hall-Aspland & Rogers (2004), aerial, boat, and land surveys using all-terrain vehicles were conducted in the Prydz Bay region to locate leopard seals between 68° 20′ S, 78° 36′ E and 68° 40′ S, 77° 48′ E (Fig. 1). Surveys were conducted during the austral summers between November and March in 1999/2000, 2000/01 and 2001/02. Leopard seals were sedated following the procedure outlined in Higgins et al. (2002). Sample collection protocols were reviewed and approved by the Animal Care and Ethics Committee (ACEC) of the Australian Antarctic Division (ASAC 1144).

Five vibrissae, either plucked or cut from the face, were collected from Antarctic leopard seals (seal A, n = 1, seal B, n = 2 and seal C, n = 2) for stable carbon and nitrogen isotope analysis. The vibrissa from seal A was collected during 2001/02. Seals B and C each had 1 vibrissa collected during 1999/00 (B_1 and C_1) and again during 2000/01 (B_2 and C_2). Muscle samples from potential prey items were collected from the Prydz Bay region: Adelie penguins, (n = 7) were collected opportunistically; icefish *Chiondraco hamatus* (n = 7) were collected using hand lines deployed from Zodiacs, and Antarctic krill *Euphausia superba* (n = 2) were collected by trawling. Facial masks incorporating all vibrissae and facial muscle were collected opportunistically from 2 leopard seals (1 male adult, 1 female sub-adult) to determine the location, length and number of vibrissae. All samples were frozen at –20°C for storage and transportation.

Captive animal collections. During March 2000, 2 vibrissae were collected from a captive female leopard seal (D) held at the Zoological Parks Board of NSW under protocols approved by their ACEC. This seal was held in captivity from July 1997 and fed a variable fish diet including mullet *Mugil cephalus*; mackerel *Scomber australasicus*; herring *Arripis georgianus*; whiting *Sillago flindersi* and pilchards *Sardinops neopilchardus*. Muscle samples were collected from each fish prey item for stable carbon and nitrogen isotopic analysis.

Estimates of vibrissae age. In a simple linear model, segments of equal length represent a similar time interval anywhere along the vibrissa (Hirons et al. 2001). In the simple von Bertalanffy model, the relationship between length (L) and time (T) is given by:

$$L = L_a (1-\exp[-KT]) \quad (1)$$

where L_a is the asymptotic length (von Bertalanffy 1938) and the vibrissa has zero length at time $T = 0$. K is known as the growth coefficient, a measure of the

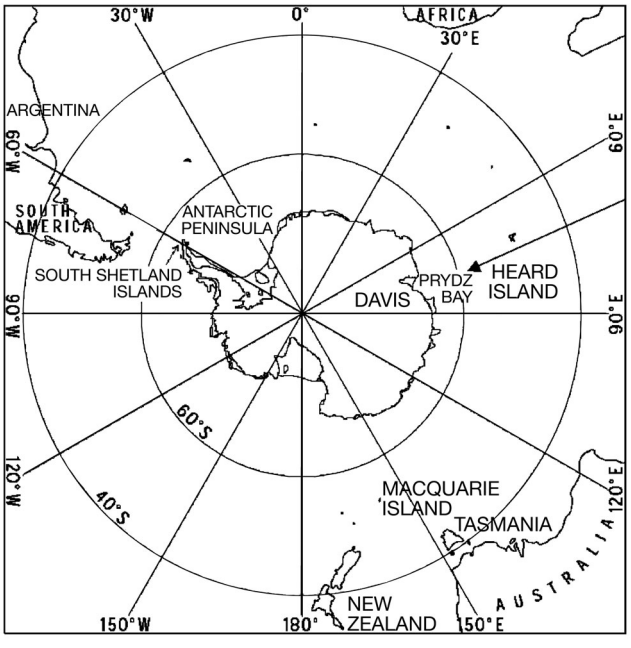

Fig. 1. Antarctica showing Prydz Bay where the sampling occurred

rate at which the growth rate declines, and is the rate at which L_a is achieved. A high value of K is generally associated with fast early growth. It is the consequence of the model that growth at the earlier stage (tip) will be more rapid than in the later stages (root), where the length of the vibrissa is then close to the asymptotic length L_a.

To obtain an estimate of the growth coefficient K, some measure of a time interval along a known length of the vibrissa is required. In the continuous von Bertalanffy representation, natural growth includes an exponential relationship between an existing length L and the age T, at that instant. The location of the sampling time is therefore difficult to establish accurately within the distribution. Other pitfalls include: (1) the possibility of 'resting' phases during the growth cycle, (2) the time delay before the isotopic signature produces changes in the vibrissae and (3) the collection method of the vibrissae, where cutting rather than plucking of samples may miss a segment of vibrissae making it difficult to ascertain the starting point of the diet history.

Discrete von Bertalanffy analysis. A discrete step-by-step von Bertalanffy approach is more controlled than the continuous von Bertalanffy growth analysis. Times can be accumulated from vibrissa segments where a finite time interval is produced for each. The discrete von Bertalanffy relationship follows from:

> Growth rate, at any given time T is assumed to be in some proportion K to the difference between the length $L(T)$, at the age T, and the expected maximum length L_a (von Bertalanffy 1938).

In the discrete representation this is interpreted as: growth rate $\delta L/\delta T = K(L_a - L_{p-1})$ for the general segment p−1 to p.

Replacing $\delta L = (L_p - L_{p-1})$ and $\delta T = (T_p - T_{p-1})$ allows time interval

$$(T_p - T_{p-1}) = (L_p - L_{p-1})/[K(L_a - L_{p-1})] \quad (2)$$

for the number of days associated with the segment of width $(L_p - L_{p-1})$. The difference between the length L (T) at the age T and the expected maximum length L_a is $(L_a - L_{p-1})$, consequently the time interval for the final segment $(L_a - L_{a-1})$ is $1/K$. Here $T_0 = 0$ and $L_0 = 0$ and the first interval of time T_1 is associated with the segment (L_0 to L_1). The process proceeds from tip to root and the estimated age at any stage is obtained by summing the set of times for the individual segments involved.

Stable isotope analysis. All seal vibrissae and prey muscle samples were cleaned before analysis. Debris was removed from the vibrissae using an ultrasonic cleaner; they were washed twice in diethyl ether, left to air dry and stored in vacuum-sealed bags. Muscle and whole krill were homogenised and treated following procedures outlined by Blight & Dyer (1959). Muscle and krill samples were dried at 60°C for 48 h, and then reground prior to analysis. Vibrissae were usually cut into 2.5 mm segments, though there were some segments near the tip which were closer to 5 mm in length. Each section was analysed separately. Four mg of each tissue was loaded into tin capsules for stable carbon and nitrogen isotope analysis. All samples were analysed, apart from the vibrissae, in duplicate. A Finnegan Mat 252 isotope-ratio mass spectrometer was used coupled with a Finnegan control interface and Europa preparation element analyser. Samples were flash combusted at approximately 1700°C, followed by on-line chromatographic separation of sample N_2 and CO_2. Enrichment of a particular isotope was reported as the deviation from standards in parts per thousand (‰). Samples analysed for $^{13}C/^{12}C$ and $^{15}N/^{14}N$ were standardized using Vienna PeeDee Belemite (VPDB) for $\delta^{13}C$ and atmospheric nitrogen (AIR) for $\delta^{15}N$. International standards (USG24 and NBS22 for C and IAEA-N-1 and IAEA-N-2 for N) were used for calibration. The mass spectrometer is calibrated against NBS19 ($\delta^{13}C = 1.95$, $\delta^{18}O = -2.20$) and IAEA-N-1 ($\delta^{15}N = 0.4$) and IAES-N-2 ($\delta^{15}N = 20.3$). A laboratory working standard was run every 24 samples during analysis. Based on laboratory standards, the analytical precision was estimated to be ±0.2‰ for $\delta^{13}C$ and ±0.3 for $\delta^{15}N$.

Data analysis. The simple linear and discrete von Bertalanffy growth models were compared for all Antarctic vibrissae. Estimates of growth rates of vibrissae were made based on timed intervals of length.

A 4-prey mixing model incorporating a Moore-Penrose pseudoinverse analysis (Hall-Aspland et al. 2005) was applied to vibrissae $\delta^{15}N$ values from Antarctic seals A, B_1, B_2 and C_1 to determine the proportions of prey consumed. The method is applicable to the case of a single isotope, in this case nitrogen N, and a set of mixing fractions ($f_1, f_2, ..., f_k, ..., f_n$) associated with a set of prey isotopic signatures ($\delta^{15}N_1, \delta^{15}N_2, ..., \delta^{15}N_k, ..., \delta^{15}N_n$), which are assumed to produce a mixture $\delta^{15}N_{Mix}$ for the prey. It can be shown that by applying the method of 'least squares' to the set of equations $f_k = \alpha\delta^{15}N_K + \beta$, where α and β are variational parameters and including the linear mixing model constraints $\Sigma_k f_k = 1$ and $\Sigma_k f_k \delta^{15}N_k = \delta^{15}N_{Mix}$ the fraction f_k, for the k-th prey source contribution, is given by $f_k = \boldsymbol{\delta N_k}[(\mathrm{inv}(\mathbf{D}\,\mathbf{D^T})]\,\boldsymbol{\delta N_M}^T$. Here the 2 × n matrix $\mathbf{D}$ has the simple form $\mathbf{D} = (\delta^{15}N_1, \delta^{15}N_2, ..., \delta^{15}N_k, .., \delta^{15}N_n; 1,1,..,1, ...1)$. $\boldsymbol{\delta N_k} = (\delta^{15}N_k, 1)$ is the vector for the k-th prey contribution and $\boldsymbol{\delta N_M} = (\delta^{15}N_{Mix}, 1)$ is the isotopic vector of the predator. The solution always includes the inverse of a 2 × 2 matrix inv $(\mathbf{D}\,\mathbf{D^T})$.

The 4 prey were Adelie penguins, icefish, crabeater seals and Antarctic krill. The mean (±SE) stable nitro-

gen and carbon isotopic values for krill are: 2.6 ± 0.1‰, –27.50 ± 0.2‰ (n = 2); for icefish: 12.6 ± 0.3‰ (range 10.4 to 13.9‰), –22.5 ± 0.3‰ (range –24.2 to –21.4‰), (n = 8); for Adelie penguins: 9.3 ± 0.2‰ (range 8.2 to 10.3‰), –23.4 ± 0.1‰ (range –24.0 to –22.9‰), (n = 8) Hall-Aspland et al. (2005); and for crabeater seals: 7.7‰, –24.7‰ (Zhao et al. 2004). Data for prey were added or removed from the model depending on season and prey availability. Fish and krill were included in the mixing model during autumn and winter, penguins and crabeater seals during late spring and all 4 prey items throughout the summer. The relative significance of each type of prey was determined through calculation of prey percentages. Comparison of predicted dates with actual $\delta^{15}N$ values and the percentages of possible prey occurring in the diet for each season allowed testing of the validity of each model.

RESULTS

Vibrissae distribution and correction factor

Five rows of vibrissae were observed on the face, with longer and coarser vibrissae located on the bottom row close to the chin. The mean length (±SE) of leopard seal vibrissae (n = 55) was 43.4 ± 1.7 mm, range 10 to 63 mm. Fifteen mm of vibrissae were found between the surface of the skin and the root bulb. No correction factor was required for vibrissae from Antarctic seal A and captive seal D, which were both plucked from the face. The vibrissae from Antarctic seals B and C were collected by cutting, therefore an increase in L_a of 15 mm was required in order to compensate.

K-values

The cycle observed in the $\delta^{15}N$ values collected from seal A (Fig. 2a) was used to calculate a *K*-value of 0.003168 d^{-1}. This was applied, using the discrete von Bertalanffy growth model, to the vibrissae L_a = 72 mm. Since the length of D_1 was similar to that of A, we used the same *K*-value of 0.003168 d^{-1} for this captive seal vibrissa.

An overlap in isotopic values between the 2 vibrissae collected from Antarctic seal B, (B_1 = 50 mm and B_2 = 53 mm) is displayed in Fig. 2b. From the overlap, a *K*-value of 0.0027 d^{-1} was calculated. No overlap was observed between the 2 vibrissae collected from Antarctic seal C; therefore a timeline was calculated for vibrissae C_1 only, using a *K*-value of 0.0032 d^{-1}. The compensating factor of 15 mm was included for B_1, B_2 and C_1.

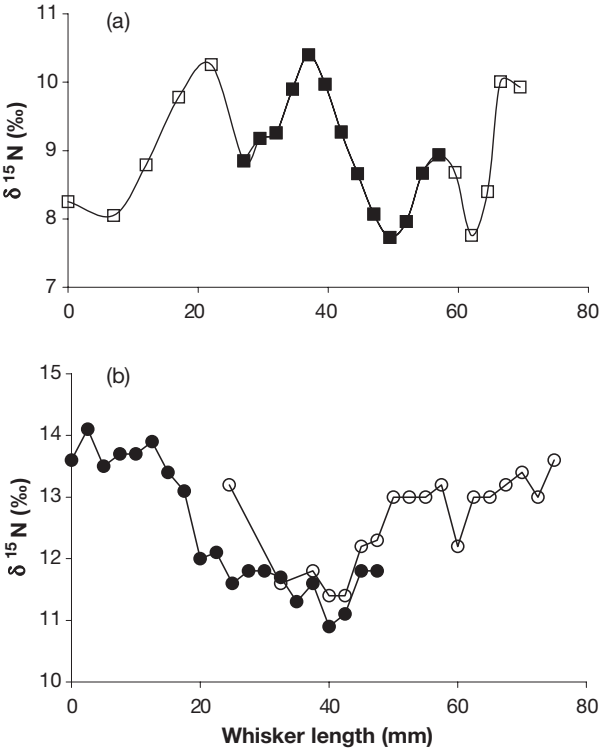

Fig. 2. *Hydrurga leptonyx*. (a) Change in $\delta^{15}N$ values of seal A over time displaying changes in trophic position, (■,●) Cycle used to calculate the *K*-value. (b) Overlap in $\delta^{15}N$ values of 2 vibrissae collected from seal B

Temporal variation

Periodic annual fluctuations in both $\delta^{15}N$ and $\delta^{13}C$ occurred along the length of each Antarctic seal vibrissae for the von Bertalanffy growth model only. The simple linear method produced a vibrissa growth timeline for Antarctic seal A between 15 October 1999 and the date of collection, 16 December 2001, a period of 874 d. Vibrissae lengths were transformed using Eq. (2). The discrete von Bertalanffy method produced a commencement of growth data from 14 September 1998 with a time interval of 1219 d. Comparison of the 2 methods showed a time difference of approximately 1 yr between them (Fig. 3a,b). The simple linear growth rate was 0.08 ± 0.01 mm d^{-1}, while the mean (±SE) overall and time-segmental von Bertalanffy growth rates were 0.10 ± 0.01 mm d^{-1} and 0.09 ± 0.01 mm d^{-1}, respectively. Mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ values were 8.9 ± 0.2‰ and –23.0 ± 0.1‰. Krill was the main prey item during spring and summer 1999/00 with some crabeater seals and Adelie penguins, but very few fish occurring. The proportion of krill increased to 80% during winter, the remainder being fish. This cycle was repeated again during 2000/01 (Fig. 3b). In Fig. 3b, the variations in fish percentages

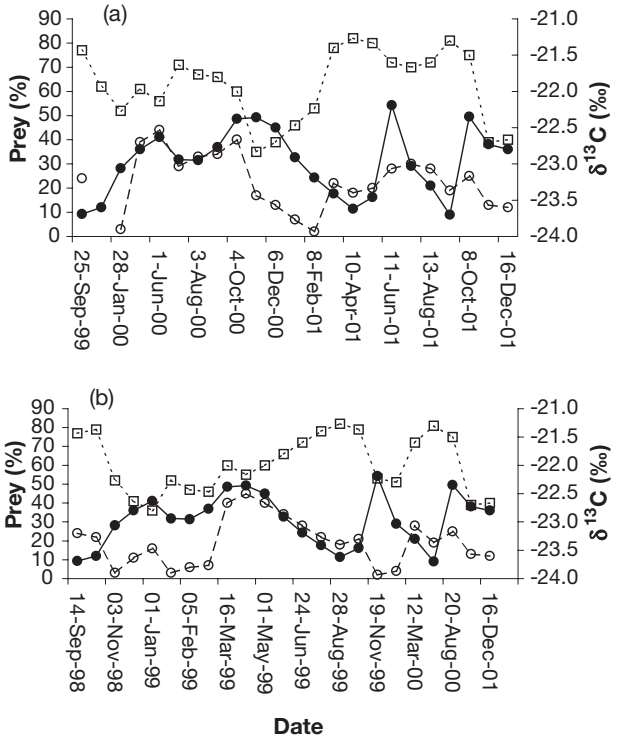

Fig. 3. *Hydrurga leptonyx.* Seasonal variation in percentage of fish (○), krill (□), and $\delta^{13}C$ values (●) for seal A over time using (a) simple linear and (b) discrete von Bertalanffy growth models

and the $\delta^{13}C$ values generally follow one another, whereas the percentages of krill are out of phase with this behaviour. These effects were more evident in the discrete von Bertalanffy growth analysis than in the linear model.

Using the discrete model, 2 vibrissae (B_1 and B_2) collected from Antarctic seal B provided a diet history from 4 July 1996 to 9 October 1997, and from 19 June 1997 to 13 October 1998 (Fig. 4). The simple linear growth rate of the vibrissae was 0.07 ± 0.01 mm d^{-1}, while the mean overall and time-segmental von Bertalanffy growth rates were 0.09 ± 0.01 mm d^{-1} and 0.08 ± 0.01 mm d^{-1}, respectively. Mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ values for B_1 were 12.4 ± 0.2‰ and –20.8 ± 0.1‰. Data from vibrissa B_1 indicated that during winter and early spring 1996, fish contributed approximately 78% to the diet, and krill 22%. During late spring and summer 1996, the diet included slightly more fish, but fairly equal proportions of Adelie penguins, crabeater seals and krill. During autumn and winter 1997, there was an overlap in the proportions of fish and krill. $\delta^{13}C$ values were high initially in winter 1996, decreasing to –21.3‰ in summer 1996/97 and variable throughout the remainder of the year (Fig. 4b). Mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ values for B_2 were 12.6 ± 0.2‰ and –21.6 ±

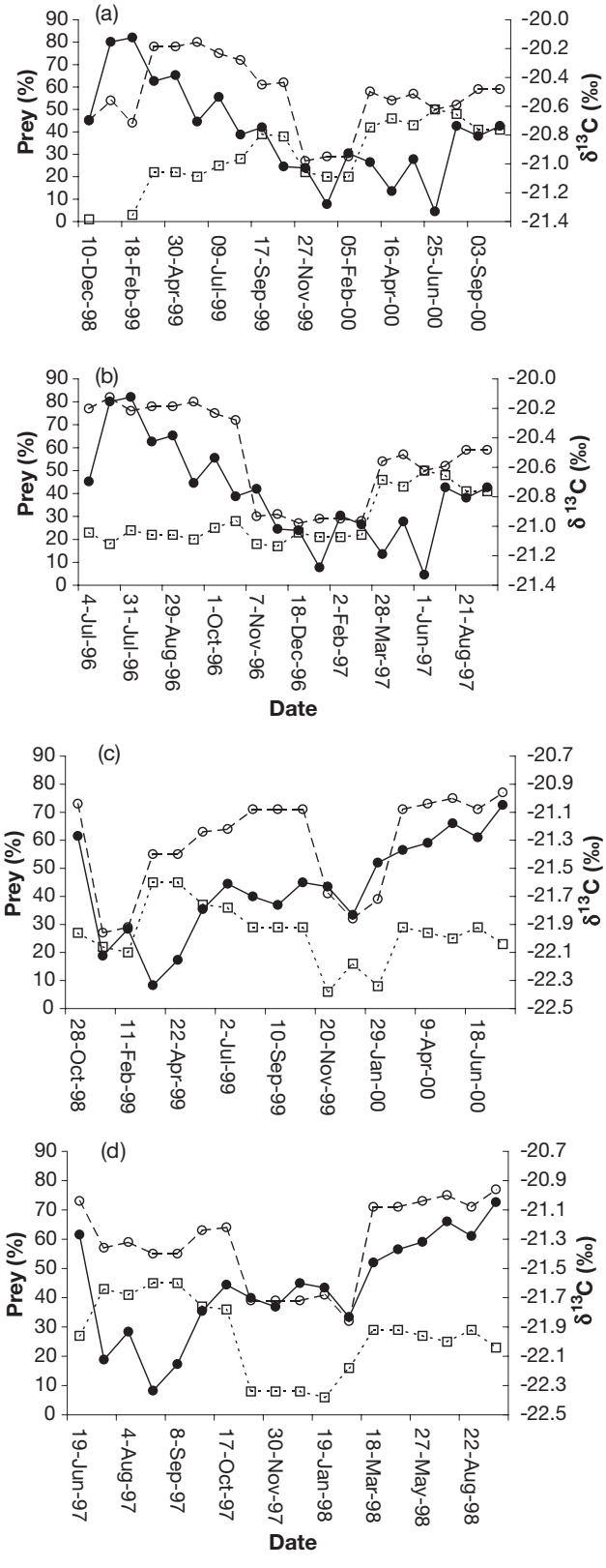

Fig. 4. *Hydrurga leptonyx.* Seasonal variation in percentage of fish (○), krill (□), and $\delta^{13}C$ values (●) for seal B_1 and B_2 using (a,c) simple linear and (b,d) discrete von Bertalanffy growth models

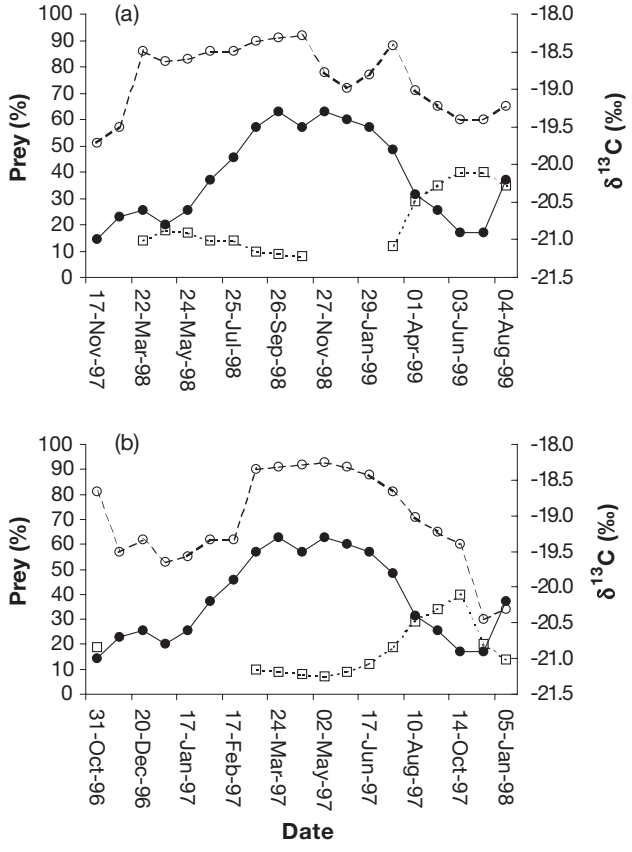

Fig. 5. *Hydrurga leptonyx.* Seasonal variation in percentage of fish (○), krill (□), and $\delta^{13}C$ values (●) for seal C_1 using (a) simple linear and (b) discrete von Bertalanffy growth models

0.1‰. Vibrissa B_2 indicated approximately 60% fish and 40% krill in the diet during winter 1997. In late spring and summer, penguins and crabeater seals contributed approximately 25% each to the diet, whereas the proportion of krill decreased to 8%. During winter 1998, the quantity of fish increased to 73%, and the proportion of krill was only 27%. $\delta^{13}C$ values were variable during winter and spring 1997, constant at –21.7‰ during summer, and increased by 0.5‰ during winter 1998 (Fig. 4d).

Applying the discrete von Bertalanffy model, vibrissa C_1 provided a diet history between 31 October 1996 and 5 January 1998 (Fig. 5b). The simple linear growth rate was 0.08 mm d^{-1}, while the mean overall Bertalanffy growth rate was 0.10 mm d^{-1}. Mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ values for C_1 were 13.9 ± 0.2‰ and –20.2 ± 0.1‰. During late spring and summer 1996/97, the diet comprised 60% fish, 30% penguins and 10% crabeater seals, but no krill. During autumn 1997, the diet comprised 91% fish and 9% krill, the proportion of krill increasing to 40% by spring. All 4 prey items occurred in the diet during summer 1997/98 with 34% fish, 28% penguins, 24% crabeater seals and 14% krill. Isotopic carbon was approximately –21.0‰ in the austral spring/summer of 1996/97 and 1997/98, increasing to –19.5‰ during winter 1997.

The vibrissa D_1 from the captive seal provided a diet history between 20 December 1996 and 18 March 2000. There was little deviation from the mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ values, 13.7 ± 0.1‰ and –14.2 ± 0.1‰ until July 1997, when a decrease in isotopic carbon values occurred (Fig. 6). Mean (±SE) $\delta^{15}N$ and $\delta^{13}C$ prey muscle values were 12.4 ± 2.3‰, range 8.7 to 21.3‰ and –17.8 ± 1.2‰, range –12.9‰ to –19.8‰. Following the results for seal A, the simple linear growth rate for D_1 was 0.08 mm d^{-1}, and the mean overall von Bertalanffy growth rate was 0.10 mm d^{-1}.

Captive versus wild seals

Stable isotope values of vibrissae for leopard seals displayed isotopic variation in $\delta^{13}C$ values between Antarctic and captive seals with some overlap in $\delta^{15}N$ values (Table 1, Fig. 7). There was a positive correlation between stable nitrogen and carbon isotope values ($r^2 = 0.88$, $p < 0.01$) for all Antarctic seals. For captive seals, mean $\delta^{15}N$ and $\delta^{13}C$ values (±SE) were 13.7 ± 0.1‰ and –14.3 ± 0.1‰; for Antarctic seals they were 11.9 ± 0.2‰ and –21.5 ± 0.1‰. Vibrissae collected from leopard seals in 1999/00 were more enriched in $\delta^{15}N$ (13.2 ± 0.2‰) and $\delta^{13}C$ (–20.5 ± 0.1‰) than in other years.

DISCUSSION

Comparison of growth models

This study has presumed that vibrissae may provide a continuous time line of diet history, extending beyond the limited time frame obtained using other val-

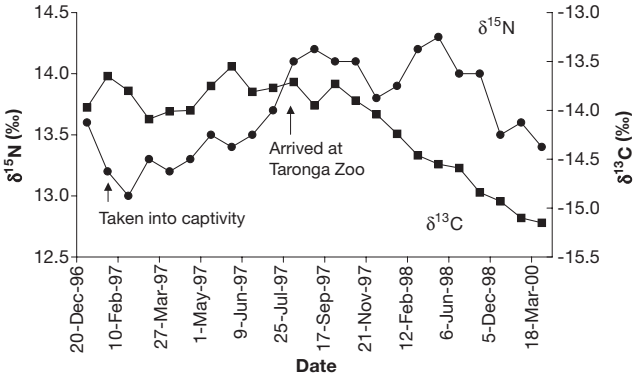

Fig. 6. *Hydrurga leptonyx.* $\delta^{13}C$ values and $\delta^{15}N$ values of vibrissa collected from captive seal D_1

Table 1. *Hydrurga leptonyx.* Number of sections taken, dates of diet history and mean (±SE) stable nitrogen and carbon isotope values of leopard seal vibrissae

Seal	No. of sections	Mean $\delta^{15}N$ (‰)	Range	Mean $\delta^{13}C$ (‰)	Range
A	22	8.9 ± 0.2	7.7 to 10.4	−23.0 ± 0.1	−23.7 to −22.2
B_1	19	12.4 ± 0.2	10.9 to 14.1	−20.8 ± 0.1	−21.3 to −20.1
B_2	17	12.6 ± 0.2	11.4 to 13.6	−21.6 ± 0.1	−22.3 to −21.0
C_1	18	13.9 ± 0.2	11.9 to 15.2	−20.2 ± 0.1	−21.0 to −19.2
C_2	22	13.7 ± 0.1	13.0 to 14.3	−14.2 ± 0.1	−15.4 to −13.6

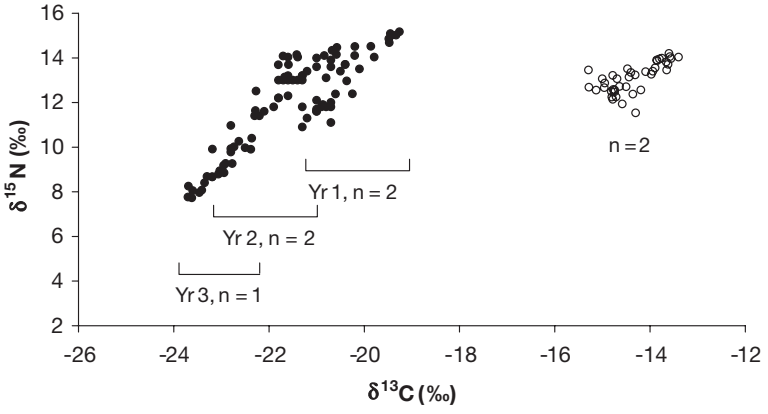

Fig. 7. *Hydrurga leptonyx.* $\delta^{13}C$ values and $\delta^{15}N$ values of Antarctic (●) and captive (○) leopard seal vibrissae. Yr: year

ues from tissues such as blood, muscle and fur. Using 2 models of growth, the discrete von Bertalanffy growth model and a simple linear model, methods of transforming vibrissae length measurements into time units were explored, providing up to 39 mo of diet history.

The relationship between vibrissa length and age for the discrete von Bertalanffy and the simple linear growth models is displayed in Fig. 8. The 'time segment' is a length of the vibrissa identified with an assumed time interval, which is used to establish a time scale and allow the calculation of a value for the growth coefficient *K*. In the discrete von Bertalanffy model the rate of growth is changing throughout the process; the rate of growth is maximum at the tip and minimum at the root. An overall mean was calculated from an average of the set of growth rates obtained from the individual segments of the vibrissa. A time segmental mean was similarly obtained from the set of segments for the selected time interval to allow comparison with the linear value. The linear model allowed a single constant value for a growth rate to be obtained from the length of the time segment in mm and the assumed time interval in days.

Vibrissae growth rates

The simple linear and the von Bertalanffy growth models both provided similar overall mean vibrissae growth estimates of approximately 0.10 mm d^{-1} for each leopard seal. The maximum growth rate at the tip was estimated to be 0.23 mm d^{-1} for seal A and 0.18 mm d^{-1} for seal B_2, whereas the growth rate at the root was 0.01 mm d^{-1} for both seals, A and B_2.

By comparison, captive harbour seals have displayed very high vibrissae growth rates during summer and autumn at the early stage of growth, with a reduced rate throughout winter and early spring (Zhao & Schell 2004). The mean growth rate of 0.33 mm d^{-1} (Hirons et al. 2001) and the initial growth rate of 0.78 mm d^{-1} compared with the lower growth rate of 0.07 mm d^{-1} at the later growth stage (Zhao & Schell 2004) are consistent with a von Bertalanffy model of annual growth of a harbour seal vibrissa where L_a is 100 mm and coefficient K is 0.008 d^{-1}.

Mean growth rates were 0.24 mm d^{-1} for grey seals (Greaves et al. 2004) and 0.11 mm d^{-1} for Steller sea lions (Hirons et al. 2001). Information concerning the length, growth rate and distribution of leopard seal vibrissae is limited. It is predicted by this study that leopard seals have lower vibrissae growth rates than harbour seals, which replace their vibrissae on an annual basis. Leopard seals may therefore retain their vibrissae for a number of years.

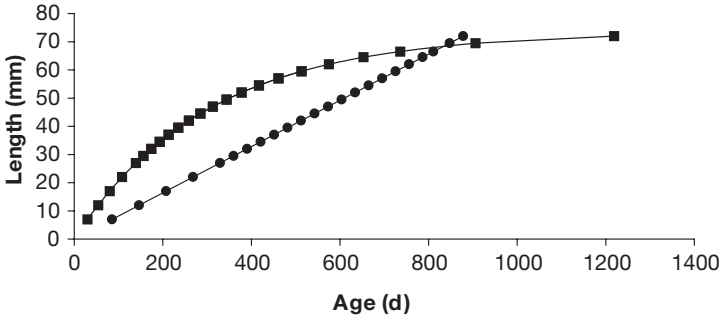

Fig. 8. Relationship between vibrissae length and age for discrete von Bertalanffy (●) and simple linear (■) growth models

It should be noted that the season predicted by the model for a particular segment of vibrissa determined which prey were to be included in the mixing model for the calculation of percentages. The magnitude of the leopard seal $\delta^{15}N_{Mix}$ value determined these percentages. The calculated percentages of prey for the chosen season finally determined whether the outcome was reasonable. The simple linear model did not display any repeat seasonal changes in diet but the von Bertalanffy model did, and was taken to be the most successful in a first attempt to transform vibrissae lengths into time lines in order to extract a diet history.

Diet and trophic level interpreted from $\delta^{15}N$ values

Seal A was consuming prey with a mean $\delta^{15}N$ value of 8.9‰, over the 3 yr period between spring 1998 and summer 2001. A fluctuation in amplitude of +2.5‰ occurred between winter and summer; this represents nearly one trophic shift. The main prey item throughout the year was krill, although young crabeater seals, Adelie penguins and some fish were incorporated into the diet during late spring and summer, increasing the $\delta^{15}N$ signature. Seal A may have been consuming both coastal krill *Euphausia crystallorophias* and the more pelagic Antarctic krill *E. superba* depending on the feeding location. Adelie penguins are distributed throughout the Prydz Bay region in numerous coastal breeding colonies, dispersing into the pack-ice during early March and returning in late October (Woehler 1993). Crabeater seals are distributed throughout the Antarctic pack-ice and feed primarily on Antarctic krill (Laws 1984). Newly weaned pups are available as leopard seal prey items from November onwards (Siniff & Bengtson 1977). The $\delta^{15}N$ signature was highest during winter and the diet consisted mainly of fish. The icefish is a benthic species, abundant in the shallow Prydz Bay region (Williams & McEldowney 1990).

Seal B was consuming prey with a mean $\delta^{15}N$ value of 12.4‰ between winter 1996 and 1998, with a fluctuation in amplitude of –2.1‰ between winter and summer. Equal proportions of Adelie penguins and crabeater seals, occupying a lower trophic level than fish, were predicted to have occurred in the diet during summer 1996/97, and during 1997/98 less krill were predicted. The variation in $\delta^{15}N$ values represented nearly one trophic shift, and could result from variations in the proportion of fish occurring in the diet between summer and winter months.

Seal C provided 2 yr of diet history between spring 1996 and summer 1998, and was consuming prey with a relatively high mean $\delta^{15}N$ value of 13.9‰. A fluctuation in amplitude of –3.3‰ in $\delta^{15}N$ occurred between autumn and spring, representing one trophic shift. The main prey item during autumn was fish, but other prey items, including penguins, were incorporated into the diet during spring, when the proportion of fish in the diet decreased.

Captive versus wild seals

The stable nitrogen isotopic composition of the vibrissa of captive seal D remained relatively constant along its length, consistent with the regular fish diet compared to the regular oscillations observed along the Antarctic leopard seal vibrissae (seals A, B and C). Leopard seal D arrived in captivity in August 1996 and was kept in a facility in Queensland, Australia for a period of approximately 1 yr, following which time she was transferred to another captive facility 850 km south, where she remained until March 2000. The $\delta^{15}N$ values were indicative of a diet consisting of fish or other prey with a high $\delta^{15}N$ signature. The differing proportion of fish species eaten, may explain the variation in $\delta^{15}N$ values with time. The decrease in $\delta^{13}C$ values occurred following her transfer between captive facilities in July 1997, indicative of geographic variation in the collection location of the prey species fed. Like seal D, captive harp seals *Pagophilus groelandicus* fed a constant herring diet displayed little variation in isotopic composition along the vibrissa length (Hobson et al. 1996). When captive leopard seals and Antarctic leopard seals were eating fish or prey with a high $\delta^{15}N$ value there was some overlap in the trophic position.

The difference in $\delta^{13}C$ values between captive and Antarctic seals was consistent with the geographic variability of prey items between the Southern Ocean and temperate waters.

Variation in $\delta^{13}C$ values

All Antarctic seal vibrissae displayed periodic oscillations in $\delta^{13}C$ values along their length. Using the age predictions of the von Bertalanffy model, mean $\delta^{13}C$ values fluctuated between summer and winter by –1.0‰ for seal A, +1.2‰ for seal B and +1.6‰ for seal C.

These oscillations could be a result of a number of factors, including seasonal shelf – offshelf movements by leopard seals or their prey items between foodwebs with different isotopic nitrogen and carbon signatures (Michener & Schell 1994, Schell et al. 1998). Owing to the higher phytoplankton productivity in neritic waters of the Antarctic continental shelf (Rau et al. 1991),

these waters are enriched in $\delta^{13}C$ compared to pelagic, offshore waters. The seasonal variation in $\delta^{13}C$ values for seal A indicated that the seal or the prey species moved into pelagic, offshelf waters to feed during winter. Recent satellite telemetry studies have shown that female leopard seals in the Prydz Bay region tend to be sedentary and so do not show significant seasonal movement (Rogers et al. 2005). Seasonal migration of prey species such as Adelie penguins could be one possible explanation.

The difference between the variation in $\delta^{13}C$ values observed for seal A and those observed for seals B and C implies primary diet sources with higher isotopic carbon ratios during the winter for seals B and C. Isotopic fractionation in marine phytoplankton is still not completely understood; the sources Fof variation in values of $\delta^{13}C$ during the austral winter are not always clear. Possible processes for $\delta^{13}C$ discrimination are known (Francois et al. 1993, Keller & Morel 1999), but those that are significant at any location are still not fully established.

Long-term (50 yr) changes observed in bowhead whale *Balaena mysticetus* stable carbon isotope ratios resulted from an increase in phytoplankton growth rates (Schell et al. 2000). A change in atmospheric source carbon was also suggested to have some impact (Cullen et al. 2001). The short-term seasonal changes observed in leopard seal stable carbon isotope ratios may result from association of seals or their prey with areas of increased primary productivity during winter. Areas of open water, known as polynyas, exist within the pack-ice during winter. Enhanced levels of primary productivity tend to occur there during spring, when the surface waters are subjected to increasing springtime solar radiation (Arrigo & Dijken 2003). Ice particulate organic matter (POM) is enriched in ^{13}C during winter (France et al. 1998); therefore assimilation of such by zooplankton may lead to elevated levels of $\delta^{13}C$ in higher trophic level predators.

The observed oscillations could also reflect seasonal fasting or nutritional stress (Hobson et al. 1993). Seasonal fasting and nutritional stress has been documented previously in marine mammals (Atkinson & Ramsay 1995, Lydersen et al. 1997) although it is suggested to affect $\delta^{15}N$ but not $\delta^{13}C$ values.

CONCLUSIONS

This was a preliminary study to determine whether it is possible to use vibrissae to track changes in foraging location and trophic position over time.

The technique of back calculating dates could be useful in identifying leopard seal diet over winter when accessibility to seals is limited by sea-ice conditions. It is applicable to highly mobile species with definite seasonal migratory patterns.

Examination of leopard seal facial masks revealed that 15 mm of vibrissae were found beneath the skin surface. The length of the vibrissae located beneath the surface of the skin had implications for growth analysis. When the vibrissa is cut and not plucked, a large amount of isotopic data is missed.

In terms of the von Bertalanffy assumption, the interval of 15 mm could represent up to 2 yr of growth. It is estimated from the simple linear model, with a growth rate of 0.08 mm d^{-1}, that 15 mm represents a time interval of 188 d.

There are 2 other main factors to be taken into consideration in the analysis of vibrissae using the von Bertalanffy growth model. Firstly, the period of first uptake of the isotopic signal of the vibrissa is unknown. This refers to the time taken for the respective isotope ratios measured in a particular tissue to shift from those derived from a particular diet to another (Tieszen et al. 1983, Hobson & Clarke 1992). Captive studies with harbour seals involving the injection of a glycine marker led to a half-life of glycine tracer estimated at 47.1 d (Zhao & Schell 2004). An increase in marker began to show in vibrissae after just 4 d. This implied that the marker rapidly incorporates into the protein synthesis of the vibrissae. Hirons et al. (2001) estimated that the start of the increase of the isotope ratios in vibrissae, until the point when the ratios returned to the constant level, was 64 d. Captive studies are required to determine the latency period of leopard seal vibrissae. Secondly, there may be the possibility of a resting phase or phases during growth of the vibrissae. This may be overcome by using the longest vibrissae, which are suggested to indicate the best growth, and no resting phase (Greaves et al. 2004). This suggestion requires further investigation using captive animals. In this present study, the vibrissae analysed were between 50 and 74.5 mm, close to the asymptotic length for leopard seals, and were collected closest to the chin where the longer vibrissae were located.

For future studies,we advise a systematic collection method which involves plucking rather than cutting of vibrissae. If vibrissae are sampled by cutting, the location on the mask should be recorded for next season's sample in order to give a vibrissa segment with a definite time interval. The existing lengths of vibrissae which may be used for sampling during the following season should be recorded.

Captive studies are required to further determine whether leopard seal vibrissae follow a simple linear or a von Bertalanffy growth process. More information on loss and replacement rates of vibrissae should also be obtained.

Acknowledgements. We thank members of ANARE including the leopard seal team R. Gray, D. Higgins, A. Irvine and S. Constable. P. Earnshaw, D. Mole, D. Pullinger and B. Hill provided logistical assistance in the field. J. Barnes, L. Vogelnest, A. Barnes and the Zoological Parks Board of NSW provided samples from captive leopard seals. Thanks to A. Bryce and A. Andrew from the CSIRO, North Ryde for performing the stable isotope analysis. T. Hall provided advice on the von Bertalanffy analysis. Constructive criticism was provided by 4 journal reviewers, which greatly improved the manuscript. The Winifred Scott Foundation, the Sea World Research and Rescue Foundation, the Antarctic Science Advisory Committee and the University of Sydney Postgraduate Research Support Scheme funded this program. This program was reviewed and approved by the Animal Care and Ethics Committee of the Australian Antarctic Division (permit no. ASAC 1144).

LITERATURE CITED

Arrigo KR, van Dijken GL (2003) Phytoplankton dynamics within 37 Antarctica coastal polynya systems. J Geophys Res 108, C8, 271–278

Atkinson SN, Ramsay MA (1995) The effects of prolonged fasting on body composition and reproductive success of female polar bears (*Ursus maritimus*). Funct Ecol 9: 559–567

Bengtson JL (1982) Reproductive ecology of crabeater and leopard seals along the Antarctic Peninsula. Antarct J US 17:185

Best PB, Schell, DM (1996) Stable isotopes in southern right whale (*Eubalaena australis*) baleen as indicators of seasonal movements, feeding and growth. Mar Biol 124: 483–494

Blight EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917

Clarke JR, Kerry K, Fowler C, Lawless R, Eberhard S, Murphy R (2003) Post-fledging and winter migration of Adelie penguins *Pygoscelis adeliae* in the Mawson region of East Antarctica. Mar Ecol Prog Ser 248:267–278

Cullen JT, Rosenthal Y, Falkowski PR (2001) The effect of anthropogenic CO_2 on the carbon isotope composition of marine phytoplankton. Limnol Oceanogr 46(4)996–998

Darimont CT, Reimchen TE (2002) Intra-hair stable isotope analysis implies seasonal shift to salmon in grey wolf diet. Can J Zool 80:1638–1642

France RR (1995) Carbon 13 enrichment compared to planktonic algae: foodweb implications. Mar Ecol Prog Ser 124: 307–312

Francois R, Altabet MA, Goericke R, McCorkle D, Brunet C, Poisson A (1993) Changes in the $\delta^{13}C$ of surface water particulate organic matter across the subtropical convergence in the SW Indian Ocean. Global Biogeochem Cycles 7: 627–744

Fry B (1981) Natural stable carbon isotope tag traces Texas shrimp migrations. Fish Bull US 79:337–345

Fry B (1988) Food web structure on Georges Bank from stable C, N, and S isotopic compositions. Limnol Oceanogr 33: 1182–1190

Greaves DK, Hammill MO, Eddington JD, Pettipas D, Schreer JF (2004) Growth rate and shedding of vibrissae in the grey seals, *Halichoerus grypus*: a cautionary note for stable isotopic analysis. Mar Mamm Sci 20(2)296–304

Green K, Williams R (1986) Observations on the food remains in faeces of elephant, leopard and crabeater seals. Polar Biol 6(1):43–45

Hall-Aspland SA, Rogers TL (2004) Summer diet of leopard seals (*Hydrurga leptonyx*) in Prydz Bay, Eastern Antarctica. Polar Biol 27(12):729–734

Hall-Aspland SA, Hall AP, Rogers TL (2005) A new solution to the linear mixing model for a single isotope: application to a top order predator. Oecologia 143:143–147

Higgins DP, Rogers TL, Irvine AD, Hall-Aspland SA (2002) Use of midazolam/pethidine and tiletamine/zolazepam combinations for the chemical restraint of leopard seals (*Hydrurga leptonyx*). Mar Mamm Sci 18(2):483–499

Hildebrand GV, Farley SD, Robbins CT, Hanley TA, Titus K, Servheen C (1996) Use of stable isotopes to determine diets of living and extinct bears. Can J Zool 74: 2080–2088

Hirons AC, Schell DM, St. Aubin DJ (2001) Growth rate of vibrissae of harbour seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*). Can J Zool 79:1053–1061

Hobson KA, Clarke RG (1992) Assessing avian diets using stable isotopes. I. Turnover of ^{13}C in tissues. Condor 94: 181–188

Hobson KA, Clarke RG (1993) Turnover of ^{14}C in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. Auk 110:638–641

Hobson KA, Schell DM (1998) Stable carbon and nitrogen isotope patterns in baleen from eastern Arctic bowhead whales. Can J Fish Aquat Sci 55:2601–2607

Hobson KA, Sease JL (1998) Stable isotope analysis of tooth annuli reveal temporal dietary records: an example using Steller sea lions. Mar Mamm Sci 14:116–129

Hobson KA, Welch HE (1992) Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}C$ and $d^{15}N$ analysis. Mar Ecol Prog Ser 84:9–18

Hobson KA, Alisauskas RT, Clarke RG (1993) Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analysis of diet. Condor 95:388–394

Hobson KA, Schell DM, Renouf D, Noseworthy E (1996) Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Can J Fish Aquat Sci 53:528–533

Hobson KA, Sinclair EH, York AE, Thomason JR, Merrick RE (2004) Retrospective isotopic analyses of Steller sea lion tooth annuli and seabird feathers: a cross taxa approach to investigating regime and dietary shifts in the Gulf of Alaska. Mar Mamm Sci 20(3):621–638

Keller K, Morel FM (1999) A model of carbon isotopic fractionation and active carbon uptake in phytoplankton. Mar Ecol Prog Ser 182:295–298

Laws RM (1984) Seals. In: Laws RM (ed) Antarctic ecology. Academic Press, London

Lee SH, Schell DM, McDonald TL, Richardson JW (2005) Regional and seasonal feeding by bowhead whales *Balaena mysticetus* as indicated by stable isotope ratios. Mar Ecol Prog Ser 285:271–287

Lowry L, Testa JW, Calvert W (1988) Winter feeding of crabeater and leopard seals near the Antarctic Peninsula. Polar Biol 8:475–478

Lydersen C, Kovacs KM, Hammill MO (1997) Energetics during nursing and early postweaning fasting in hooded seal (*Cystophora cristata*) pups from the Gulf of St. Lawrence, Canada. J Comp Physiol B Biochem Syst Environ Physiol 167:81–88

Michener RH, Schell DM (1994) Stable isotope ratios as tracers in marine aquatic food webs. In: Lajtha K, Michener RH (eds) Stable isotopes in ecology and environmental science. Blackwell Scientific, London, p 138–157

Oritsland T (1977) Food consumption of seals in the Antarctic pack-ice. In: Llano GA (ed) Adaptations within Antarctic ecosystems. Proc 3rd SCAR Symp Antarctic Biology. Smithsonian Institute, Washington, DC, p 749–768

Rau GH, Hopkins TL, Torres JJ (1991) $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell Sea invertebrates—implications for feeding diversity. Mar Ecol Prog Ser 77(1):1–6

Rau GH, Ainley DG, Bengtson JL, Torres JT, Hopkins TL (1992) $^{15}N/^{14}N$ and $^{13}C/^{12}C$ in Weddell sea birds, seals, and fish: implications for diet and trophic structure. Mar Ecol Prog Ser 84:1–8

Rogers TL, Bryden MM (1995) Predation of Adelie penguins (*Pygoscelis adeliae*) by leopard seals (*Hydruga leptonyx*) in Prydz Bay, Antarctica. Can J Zool 73:1001–1004

Rogers T, Hogg C, Irvine A (2005) Spatial movement of adult leopard seals in Prydz Bay, Eastern Antarctica. Polar Biol 28(6):456–463

Schell DM (2000) Declining carrying capacity in the Bering Sea: Isotopic evidence from whale baleen. Limnol Oceanogr 45(2):459–462

Schell DM, Suape SM, Haubenstock N (1989) Natural isotope abundances in bowhead whale (*Balaena mysticetus*) baleen: markers of aging and habitat use. In: Rundel PW, Ehleringer JR, Nagy KA (eds) Stable isotopes in ecological research. Springer-Verlag, New York, p 261–269

Siniff DB, Bengtson JL (1977) Observations and hypothesis concerning the interactions among crabeater seals, leopard seals and killer whales. J Mammal 58(3):414–415

Siniff DB, Stone S (1985) The role of the leopard seal in the tropho-dynamics of the Antarctic marine ecosystem. In: Siegfried WR, Condy PR, Laws RM (eds) Antarctic nutrient cycles and food webs. Springer-Verlag, Berlin, p 555–559

Siniff DB, Stirling I, Bengtson JL, Reichle RA (1979) Social and reproductive behaviour of crabeater seals (*Lobodon carcinophagus*) during the austral spring. Can J Zool 57: 2243–2255

Tieszen LL, Boutton TW, Tesdahl KG, Slade NA (1983) Fractionation and turnover of stable carbon isotopes in animal tissues: implications for ^{13}C analysis of diet. Oecologia 57: 32–37

von Bertalanffy L (1938) A quantitative theory of organic growth (Inquiries on growth laws, II). Human Biol 10(2): 181–213

Williams R, McEldowney A (1990) A guide to the fish otoliths from waters off the Australian Antarctic Territory, Heard and Macquarie Islands. ANARE Research Notes 75, Australian Antarctic Division, Kingston

Woehler EJ (1993) The distribution and abundance of Antarctic and sub-Antarctic penguins. SCAR, Cambridge

Zhao L, Schell DM (2004) Stable isotope ratios in harbour seal *Phoca vitulina* vibrissae: effects of growth patterns on ecological records. Mar Ecol Prog Ser 281:267–273

Zhao L, Castellini MA, Mau TL, Trumble SJ (2004) Trophic interactions of Antarctic seals as determined by stable isotope signatures. Polar Biol 27:368–373

Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany

Submitted: December 18, 2004; Accepted: July 17, 2005
Proofs received from author(s): November 20, 2005