Growth rates of vibrissae of harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*)

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Abstract: Growth rates of vibrissae (whiskers), which act as a temporal record of feeding in harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*), were estimated using ^{13}C- and ^{15}N-labeled glycine followed by stable-isotope analysis. The labeled glycine was incorporated into keratin and served as a temporal marker for growth-rate calculation. One captive harbor seal received two doses 147 days apart, while a second seal received one dose; vibrissae were analyzed after 86 and 154 days. The peak positions indicated that growth began in the fall, continued into spring, but ceased in June, with active growth rates of 0.33 mm/day. Two adult captive Steller sea lions each received two labeled doses during a 308-day period. After 427 days vibrissae in both sea lions showed two peaks corresponding to the markers; growth rates were calculated as 0.05–0.07 mm/day. Growth rates in captive juvenile and wild adult Steller sea lions, 0.10–0.17 mm/day, supported the assumption that major isotopic oscillations in vibrissae of wild sea lions were annual. The multiyear records imply that Steller sea lions retain their vibrissae; harbor seal vibrissae, in contrast, have periods of rapid growth and appear to be shed, at least in part, annually.

Résumé : Les taux de croissance des vibrisses, organes qui peuvent être utilisés pour l'enregistrement temporel des activités alimentaires des Phoques communs (*Phoca vitulina*) et des Otaries de Steller (*Eumetopias jubatus*), ont été estimés à l'aide de glycine marquée au ^{13}C et au ^{15}N; une analyse des isotopes stables a suivi. La glycine marquée s'incorpore à la kératine et sert de marqueur temporel dans le calcul du taux de croissance. Un Phoque commun gardé en captivité a reçu deux doses à 147 jours d'intervalle, alors qu'un autre phoque n'en a reçu qu'une; leurs vibrisses ont été analysées après 86 et 154 jours. La position des pics indique que la croissance commence à l'automne, se poursuit jusqu'au printemps et cesse en juin; le taux de croissance active a été évalué à 0,33 mm/jour. Deux Otaries de Steller adultes, gardées en captivité, ont reçu chacune deux doses marquées au cours d'une période de 308 jours. Après 427 jours, deux pics ont été enregistrés dans les vibrisses des deux otaries, correspondant à l'utilisation des marqueurs; les taux de croissance ont été estimés a 0,05–0,07 mm/jour. Les taux de croissance des otaries juvéniles en captivité et des adultes libres en nature, 0,10–0,17 mm/jour, appuient l'hypothèse selon laquelle les oscillations importantes des isotopes dans les vibrisses des otaries en nature sont annuelles. Les données échelonnées sur plusieurs années indiquent que les Otaries de Steller gardent leurs vibrisses, alors que les vibrisses des Phoques communs ont des périodes de croissance rapide, mais semblent être rejetées chaque année, au moins en partie.

[Traduit par la Rédaction]

Introduction

Vibrissae (whiskers) are keratinous, hairlike structures but they differ considerably from pelage (hair). The structure of vibrissa follicles is similar to that of pelage follicles; however, vibrissae are larger overall, are highly innervated, have large blood sinuses, and are controlled by voluntary muscles. The whiskers of pinnipeds occur in the musculature on the muzzle and above the eyes; most of these muscles control the positioning of the vibrissae (Ling 1977). Dehnhardt and Kaminski (1995) described how the vibrissae of harbor seals (*Phoca vitulina*) are capable of complex movement and could

discriminate differences in diameter among disks by touching them with their mystacial (muzzle) vibrissae. The vibrissae of harbor seals and Steller sea lions (*Eumetopias jubatus*) show some anatomical differences from each other. In the otariids, eared seals, including Steller sea lions, the vibrissa shafts are outwardly smooth and taper from the base to the tip, while those of harbor seals and other phocids have a wavy surface and a more uniform diameter along their length. Otariids tend to have shorter anterior and longer posterior mystacial vibrissae but phocid vibrissae are more uniform in length. No known information exists regarding the significance of these characteristics in different species, but differences in vibrissa structure may be associated with slightly different functions.

Ling (1966) determined that vibrissae of the southern elephant seal (*Mirounga leonina*) were not shed during their annual pelage molt but were shed periodically only after the seals were older than 2 years. However, the vibrissae of the marsupial *Tricosurus vulpecula*, as noted by Lyne et al. (1974), had prolonged but variable growth cycles compared with its pelage. The pelage in pinnipeds is molted annually and replaced during this time. No additional growth in their fur is

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Table 1. Chronology of an experiment on growth rates of vibrissae of captive harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*) from 9 January 1996 through 9 July 1998.

	Seal HS-N	Seal HS-P	Seal SSL-L	Seal SSL-S
Glycine label	$2 \times {}^{13}\mathrm{C}$ and ${}^{15}\mathrm{N}$	$1 \times {}^{13}\mathrm{C}$ and ${}^{15}\mathrm{N}$	$1 \times {}^{13}\mathrm{C}$ and ${}^{15}\mathrm{N}$; $1 \times {}^{15}\mathrm{N}$	$2 \times {}^{15}\mathrm{N}$
Delivery date	9 Jan. 1996; 4 June 1996	4 June 1996	18 June 1996; 22 Apr. 1997	20 Aug. 1996; 22 Apr. 1997
Sampling date(s) (vibrissae)	29 Aug. 1996; 5 Nov. 1996; 9 July 1998	5 Nov. 1996; 9 July 1998	23 June 1998	17 Nov. 1996; 23 June 1998

	Day No. (relative to 1 January 1996)										
	1	100	200	300	400	500	600	700	800	900	1000
Seal HS-N	CN		CN	V V						V	
Seal HS-P			CN	V						V	
Seal SSL-L			CN			N				V	
Seal SSL-S				N		N				V	

Note: CN, carbon and nitrogen label; N, nitrogen-only label; V, vibrissae collected.

observed throughout the year (Scheffer 1962; Ling 1970). Because vibrissae appear to function as sensory structures, periodic replacement or renewal necessitated by physical damage could be selectively more advantageous than total seasonal replacement. Based on this information we hypothesized that these two species of pinnipeds would maintain their vibrissae from year to year and that they would grow continually, but the uncertainty concerning their seasonal growth prompted this study. Understanding the relationship between growth and isotope ratios in the vibrissae will facilitate interpretation of the isotope data as a temporal record of food consumption by these animals (Fry and Sherr 1984; Schell and Saupe 1993; Michener and Schell 1994). This paper describes measurements of the growth of vibrissae of captive harbor seals and Steller sea lions and the interpretation of data from vibrissae of wild individuals as they relate to a larger study on the impact of trophic changes on these populations.

Severe declines in harbor seal and Steller sea lion populations in the Bering Sea and Gulf of Alaska have been recorded for more than two decades (Pitcher 1990; Strick et al. 1997). No cause-and-effect relationships have yet been established; however, the concurrent increase in commercial fishing pressure may be requiring pinnipeds to compete for many of the same resources. Food limitation has been hypothesized as the likely cause of the declines in pinniped populations resulting from decreases in prey populations and (or) alteration of the prey base (Alaska Sea Grant 1993; Alverson 1991; Anderson et al. 1997; Merrick 1995; Merrick et al. 1997). Trophic changes resulting from diet switching would be recorded in the stable-isotope ratios in the animals' vibrissae.

Vibrissae consist of growing tissue and are found in all pinniped species. Vibrissae from seals and sea lions contain a timeline of stable-isotope ratios derived from prey items (Hirons 2001). By comparing the isotope ratios along the length of a vibrissa with those of suspected prey items, changes in food sources and habitat can be surmised for the temporal span represented by the growth of the whisker. As part of this larger study we attempted to determine how growth-rate patterns in the vibrissae of harbor seals and Steller sea lions

changed throughout the year in order to interpret the isotope ratios.

Materials and methods

Dosing experiment

Two adult male harbor seals and two adult female Steller sea lions were maintained for 2–8 years in an outdoor exhibit at Mystic Aquarium in Connecticut. All were born in captivity; the sea lions were transferred from another facility. They were fed a daily ration of vitamin-supplemented fish (herring, capelin, mackerel, smelt) and squid at a rate that sustained appropriate body mass. Glycine enriched with ${}^{15}\mathrm{N}$ or both ${}^{13}\mathrm{C}$ and ${}^{15}\mathrm{N}$ isotopes (98%) (Cambridge Isotope Laboratories, Andover, Mass.) was employed to mark vibrissae because of the high mole percentage (8.6%) of glycine found in vibrissa keratin. It was administered intravenously as a solution of 100 mg/mL in sterile physiological saline at a dosage of 5 mg glycine/kg of body mass. All procedures were approved by the Institutional Animal Care and Use Committees of both the Mystic Aquarium and the University of Alaska Fairbanks and were carried out in accordance with guidelines established by the Canadian Council on Animal Care.

Table 1 details the sequence of label additions and whisker-clipping. The glycine was metabolically incorporated into the keratin during growth of the whiskers. Whole-blood samples (1–2 mL) were collected prior to dosing and at 24-h intervals for 48–72 h after dosing to monitor clearance of the label. Whiskers were allowed to grow for a minimum of 5 months before a second dose of glycine was administered. The second peak was desired to establish two known dated markers in order to calculate the growth rate. After a minimum of 5 more months, a whisker was cut from each animal as close to the skin as possible and analyzed for stable-isotope ratios at close intervals along its length to locate the markers (Table 1).

Natural abundance

A second type of growth-rate study using endogenous markers was conducted simultaneously at the Vancouver Aquarium in British Columbia, Canada, on one male and one female subadult Steller sea lion. All the animals were born in the wild but were found abandoned as pups of 2 months of age or less and taken to the Aquarium. Vibrissae were clipped from the muzzle of each animal periodically during a 3-year period. The vibrissae of animals fed a mass-sustaining diet (herring and pollock) were analyzed for inherent variability in stable-isotope ratios, and all the whiskers from an

animal were plotted together along a timeline. Overlap in growth from one vibrissa to the next was measured from an inflection point that was obvious on at least two separate segments. The date of each clipping was known and the growth rate calculated.

Whisker growth in wild harbor seals and Steller sea lions

An adult male harbor seal was captured by the Alaska Department of Fish and Game in southeastern Alaska in September 1994, and again 7 months later, in April 1995 (ADFG 1996). A vibrissa was removed on each occasion and the patterns in the isotope ratios were compared in an effort to determine the average growth rate during the elapsed time period. A second harbor seal was recaptured and a whisker removed for isotope analysis in 1997, 2 years after a whisker had been initially collected and sampled. A third harbor seal, a yearling, was also recaptured 1 year after it had been initially sampled as a pup.

One or two vibrissae were sampled from 30 subadult and adult sea lions. These animals were sampled on the Pribilof Islands in the Bering Sea and Chirikof Island in the western Gulf of Alaska by researchers from the National Marine Fisheries Service and the University of Alaska. Their vibrissae were analyzed for carbon- and nitrogen-isotope ratios and data were used for comparison with those from the captive animals. All 10 of the sea lions sampled in the Gulf of Alaska were adult females, while 65% of the sea lions from the Pribilof Islands were less than 5 years of age and almost exclusively male.

All the mystacial vibrissae were from an adult female harbor seals harvested at Sitka, Alaska, and an adult female Steller sea lion harvested at St. Paul, Pribilof Islands, Alaska, by Alaskan native subsistence hunters. The vibrissae were pulled and analyzed for carbon- and nitrogen-isotope ratios. The patterns of isotope ratios in the vibrissae of each animal were compared, particularly in the anterior versus the posterior whiskers, to determine if growth rates varied among the seal vibrissae and the sea lion vibrissae.

Laboratory procedures

Vibrissae were scrubbed with steel wool to remove any debris and segmented at 1.5-mm intervals from the base to the tip. Blood samples were dried for several days at 60°C and then ground for homogeneity. Stable-isotope ratios were determined using a Europa 20/20 continuous-flow isotope-ratio mass spectrometer. Results are reported in the standard $\delta^{13}C$ and $\delta^{15}N$ notation:

$$\delta X \ (\text{‰}) = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000$$

where X is ^{13}C or ^{15}N and R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. R_{standard} for ^{13}C is Pee Dee Belemnite and for ^{15}N it is atmospheric N_2 (air). Peptone was utilized as a reference check for machine drift for every 10 samples. Analytical error for samples was approximately ±0.1‰ for both carbon and nitrogen.

Results

Following injection of the labeled glycine, isotopic analyses of blood samples in both captive seals and sea lions showed a rapid increase in both $\delta^{13}C$ and $\delta^{15}N$. The changes in the nitrogen-isotope ratio were the most pronounced, reflecting the higher ratio of label to ^{15}N in the body composition. Decreases in blood $\delta^{13}C$ and $\delta^{15}N$ over time indicated loss of the isotope through respiration (carbon) or excretion and incorporation into body proteins (DeNiro and Epstein 1978, 1981; Boutton 1991).

Fig. 1. Stable-isotope plots from vibrissae of a captive adult harbor seal (*Phoca vitulina*), HS-N. The doubly labeled ($\delta^{13}C$ and $\delta^{15}N$) glycine peak is visible in the vibrissae that were cut in August 1996 (A). The vibrissae that were cut in November 1996 reveal the same peak in approximately the same location (B). No peak is evident from a vibrissa cut in July 1998 (C). Plots show the most recent growth at the base of the vibrissae (0 cm).
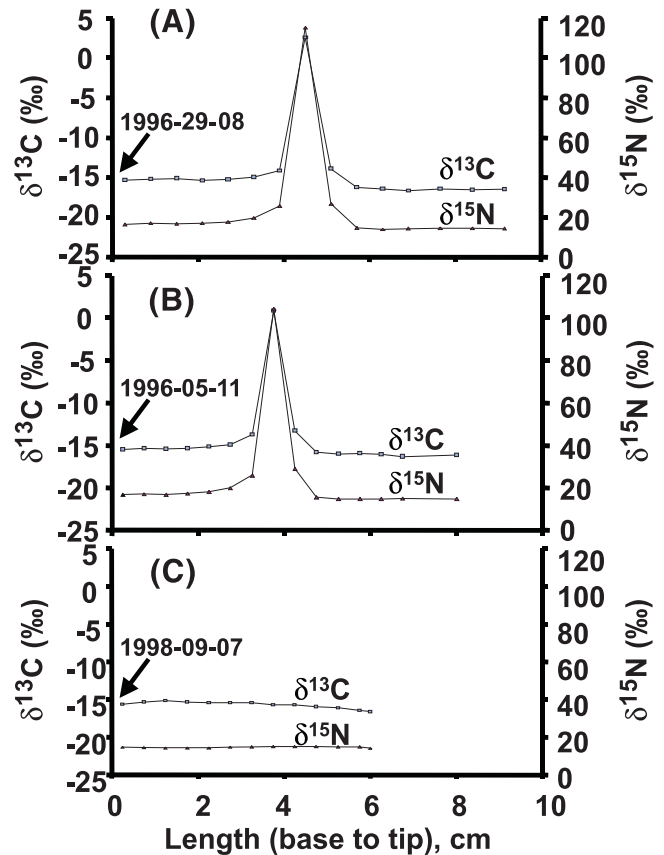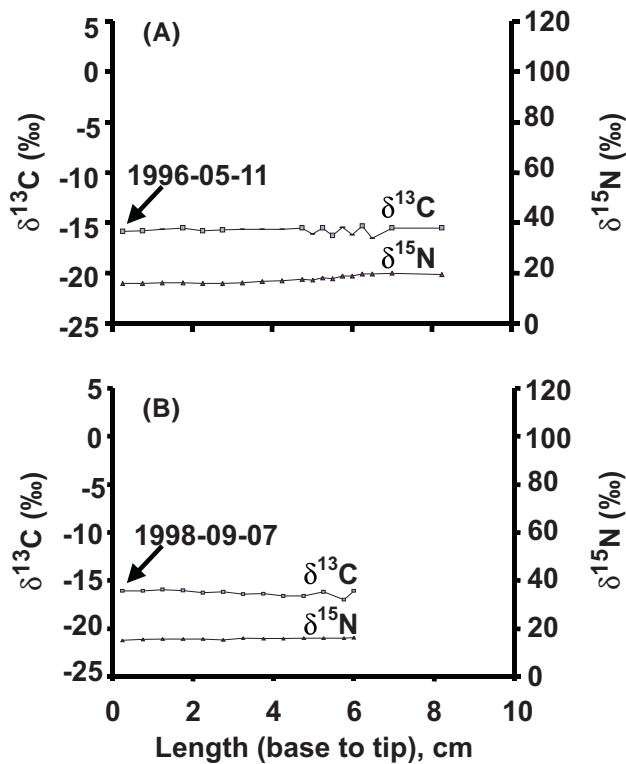

Harbor seals

The vibrissae of the first harbor seal sampled in August 1996, HS-N, showed only one peak despite administration of two doses of labeled glycine 84 and 233 days earlier. An identical peak occurred in approximately the same location in a vibrissa collected after an additional 68 days (November 1996) (Fig. 1). The label was administered to the second harbor seal (HS-P) in June 1996 and a whisker was cut in November, after 155 days. No marker was evident in HS-P's whisker (Fig. 2) in spite of high concentrations of the label in the blood samples confirming the availability of the labeled amino acid (Fig. 3). No marker was evident in either seal between the time the last label was administered (June 1996) and the last cutting of the whiskers 765 days later (July 1998). HS-N showed a significant increase in keratin $\delta^{15}N$ and $\delta^{13}C$, by 3 and 1.1‰, respectively, once the isotopic peak diminished and isotopic values returned to constant levels.
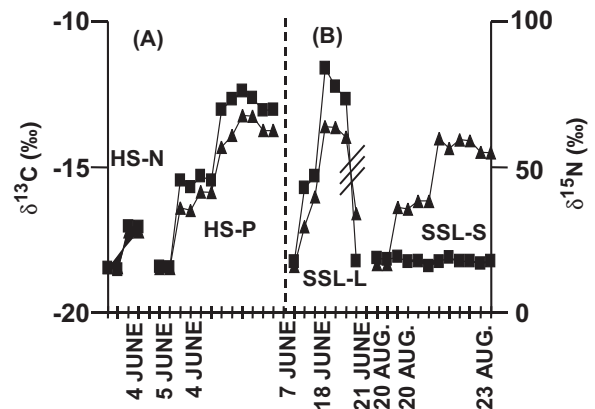
The decrease in blood-serum isotope ratios shown in Fig. 3 (SSL-L) are evidence of the carbon-isotope ratios returning to pre-injection levels 64 days later, while the nitrogen-isotope ratios in the serum showed a large enrichment equivalent to

Fig. 2. Stable-isotope plots from vibrissae of a captive adult harbor seal, HS-P. No ¹⁵N peak was visible in the vibrissae cut in November 1996 (A) or July 1998 (B). Plots show the most recent growth at the base of the vibrissae (0 cm).
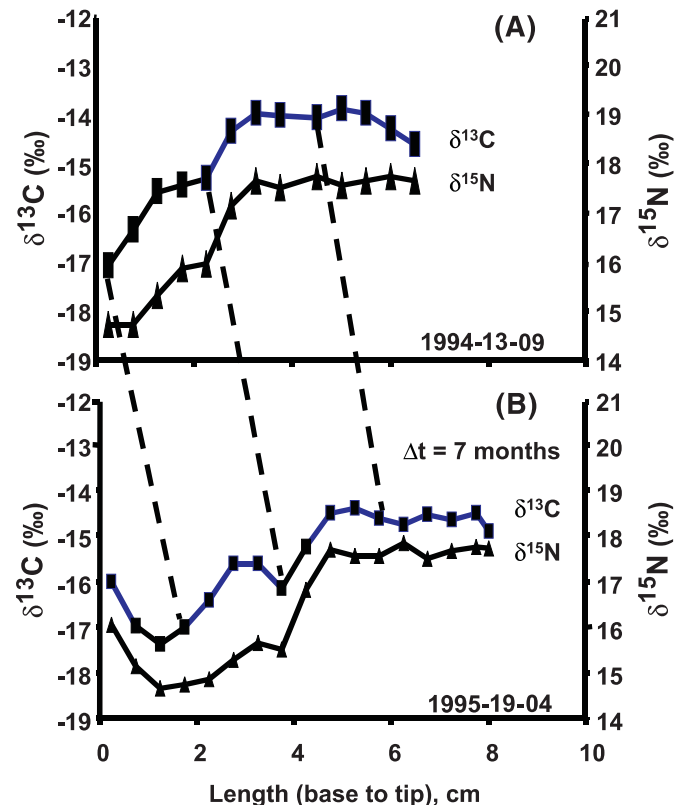

Fig. 3. Stable-isotope ratios in blood serum following injections of δ^{13}C- and δ^{15}N-labeled glycine into harbor seals HS-N and HS-P (A) and Steller sea lions (*Eumetopias jubatus*) SSL-L and SSL-S (B). Note the time-scale break with SSL-L. SSL-S received only the ¹⁵N label. (■, ¹³C; ▲, ¹⁵N).


Fig. 4. Stable-isotope plots of vibrissae from an adult male harbor seal recaptured in southeastern Alaska. A vibrissa sampled in September 1994 (A) is contrasted with a vibrissa taken from the same seal 7 months later (B). Plots show the most recent growth at the base of the vibrissae (0 cm).

approximately 100% of the initial increase. It was determined that this 64-day period was the overall time elapsed between the initial increase and subsequent decrease in blood-serum isotope ratios exhibited in SSL-L after administration of the labeled glycine (refer to Fig. 3). In the absence of harbor seal data, information on blood-serum clearance from the Steller sea lion was used. Owing to the availability of the label, the period from the start of the increase in isotope ratios in HS-N's vibrissae until the point when the ratios returned to constant levels was presumed to be 64 days. The enriched portion of the whisker measured approximately 2.1 cm and if the label was available for as long as 64 days, from mid-January to mid-March, the growth rate would be 0.33 mm/day. The distance before and after the marker represented growth of HS-N's whiskers from late September 1995 to potentially mid-June 1996, assuming a constant growth rate. Growth had not resumed as of early November, when the second whisker was analyzed, as indicated by the same relative position of the marker in the vibrissae. If the isotope peak represented the label administered in January 1996, then the growth rate from the beginning of the peak until the time the vibrissa was cut in August 1996 would be 0.37 mm/day. However, if the peak represented the label administered in June 1996, then the growth rate through August would have been 0.60 mm/day. The vibrissa growth rate based on the January injection date is nearly identical with the rate calculated from the rise and fall of the δ values from the marker in the blood. These data indicate that the peak resulted from the January injection of the label but that growth had ceased

some time in June, before the second label could be administered and incorporated. A third whisker removed from HS-N 20 months later showed no evidence of any carbon and nitrogen enrichment.

An adult harbor seal from southeastern Alaska that was originally tagged by the Alaska Department of Fish and

Fig. 5. Stable-isotope plots from vibrissae of an adult female harbor seal killed in Prince William Sound, Alaska. Anterior (A and B) and posterior (C and D) vibrissae and vibrissae from the left (A and C) and right side of the muzzle (B and D) are contrasted. Plots show the most recent growth at the base of the vibrissae (0 cm).
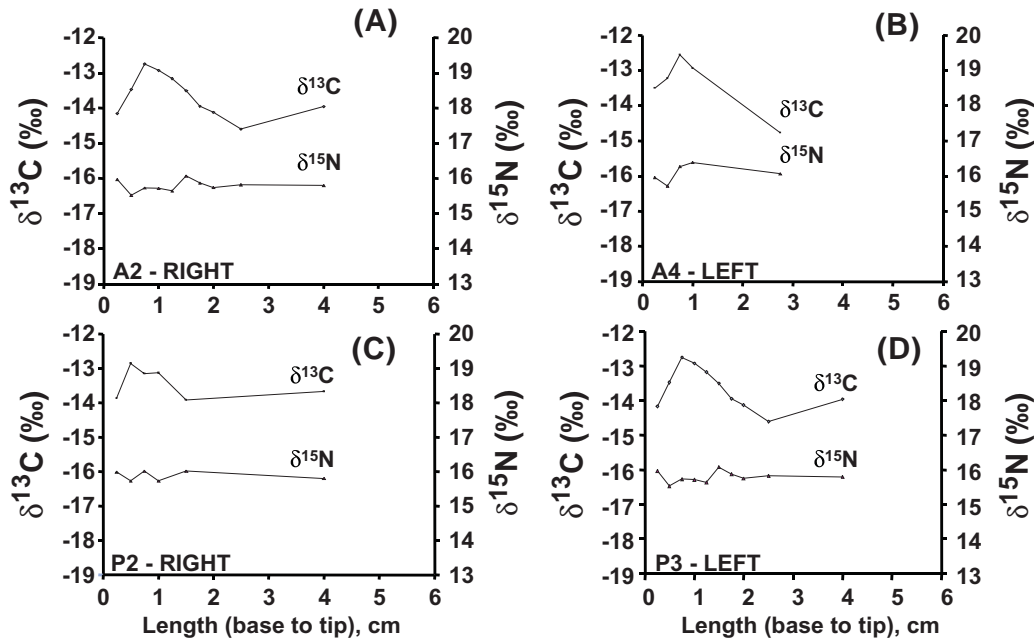
Game and sampled in September 1994 was recaptured in April 1995. Whiskers that had been collected at both times were analyzed for their stable-isotope ratios. During the 7 months, the whiskers had an average growth rate of 0.08 mm/day (Fig. 4). Neither the second nor the third recaptured harbor seal showed any similarity or overlap between their two vibrissae.

The subsistence-harvested adult seal showed no distinct difference in isotope ratios between anterior and posterior vibrissae or between vibrissae along the left and right sides of the muzzle (Fig. 5). All the vibrissae ranged from 3 to 4 cm in length and eight vibrissae on each side of the muzzle were large enough for isotopic analysis to be conducted.

Steller sea lions

The two captive adult sea lions each received two doses of labeled glycine as shown in Table 1. Whiskers were allowed to grow over a 672- and a 735-day period. The first sea lion, SSL-L, received one dose of doubly labeled amino acid and one dose of ^{15}N-labeled amino acid 308 days apart. The stable-isotope ratios in the vibrissa first showed the presence of both enriched $\delta^{15}N$ and $\delta^{13}C$ as peaks, corresponding to the doubly labeled amino acid, and later only a peak of enriched $\delta^{15}N$ corresponding to the ^{15}N-labeled amino (Fig. 6). SSL-S's vibrissa exhibited two enriched peaks of $\delta^{15}N$ that represented the two doses of ^{15}N-labeled glycine administered 183 days apart (Fig. 7). Growth rates between the two markers ranged from 0.05 to 0.07 mm/day for both sea lions. The vibrissae were retained by the sea lions for more than 735 days, demonstrating retention rates in excess of 2 years.

SSL-L showed a significant increase in keratin $\delta^{15}N$ and $\delta^{13}C$, by 2.4 and 0.6‰, respectively, after the enriched values began to diminish along the vibrissae and isotope values returned to constant levels. No additional change in $\delta^{15}N$ was evident after the second $\delta^{15}N$ peak returned to a constant

level. SSL-S showed an increase in keratin $\delta^{15}N$ by 0.8‰ after the first $\delta^{15}N$ peak returned to a constant level, and also exhibited an additional 0.9‰ increase in $\delta^{15}N$ after the second isotope peak diminished.
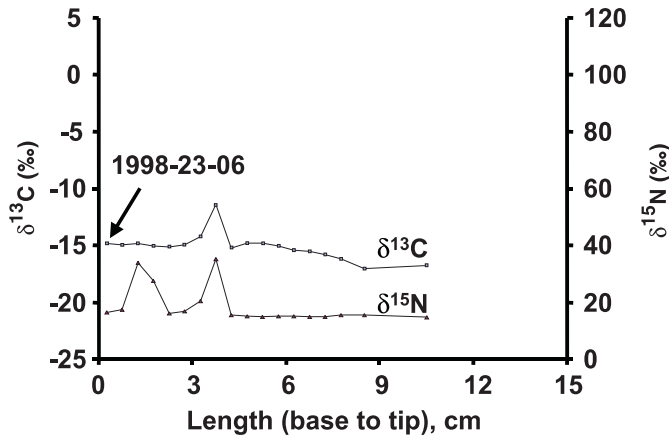
Two subadult Steller sea lions held at the Vancouver Aquarium had their vibrissae clipped annually during a 3-year period. It was possible to match features of the isotopic trends among one sea lion's vibrissae, and the overlap in vibrissae allowed for a growth-rate estimate. The daily growth rate for this animal, averaged over 14 months, was 0.14 mm/day (Fig. 8). A second sea lion had a much shorter overlap of growth in two successively cut whiskers. The daily growth rate for the second animal, averaged over 2 winter months, was 0.17 mm/day.

Subadult and adult sea lions (*n* = 30) sampled from the wild for another study showed consistent isotopic oscillations along their vibrissae, with growth rates ranging from 0.05 to 0.18 mm/day and averaging 0.10–0.14 mm/day, assuming that the major oscillations which were evident were annual (Fig. 9). Oscillation length varied from animal to animal and from year to year. Growth rates averaged over 12 months were 0.11–0.12 mm/day for all sea lions combined. The subsistence-harvested sea lion also showed no distinct variation in isotope ratios between anterior and posterior vibrissae and between vibrissae along the left and right sides of the muzzle (Fig. 10).
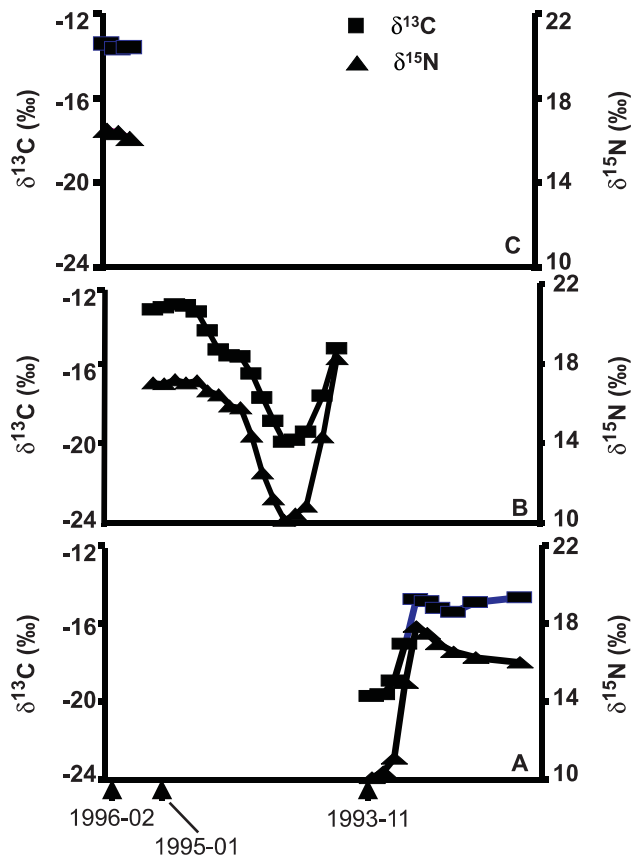
Discussion

These simple marker and observational studies indicate that the growth characteristics of the vibrissae of harbor seals and Steller sea lions are remarkably different. Growth rates in the seals indicated an irregular growth pattern throughout the year and annual loss of vibrissae, while the sea lions

Fig. 6. Stable-isotope plot of a vibrissa from a captive adult Steller sea lion, SSL-L. The peak in $\delta^{13}C$ and the peak in $\delta^{15}N$ farthest to the right represent the doubly labeled glycine administered in June 1996, while the left-hand peak in $\delta^{15}N$ represents the $\delta^{15}N$-labeled glycine administered in April 1997. The vibrissa was cut in June 1998. Plots show the most recent growth at the base of the vibrissa (0 cm).

Fig. 7. Stable-isotope plot of a vibrissa from a captive adult Steller sea lion, SSL-S. The peak in $\delta^{15}N$ farthest to the right represents the $\delta^{15}N$-labeled glycine administered in August 1996, while the left-hand peak represents the $\delta^{15}N$-labeled glycine administered in April 1997. The vibrissa was cut in June 1998. Plots show the most recent growth at the base of the vibrissa (0 cm).

Fig. 8. Stable-isotope plots from vibrissae of a subadult female Steller sea lion in captivity. The first vibrissa (A) was cut in November 1993. The second vibrissa (B) was cut in January 1995 and shows overlap in growth with the first vibrissa. The third vibrissa (C) was cut in February 1996 and was too short to reveal any overlap in growth. Plots show the most recent growth at the base of the vibrissae (0 cm).
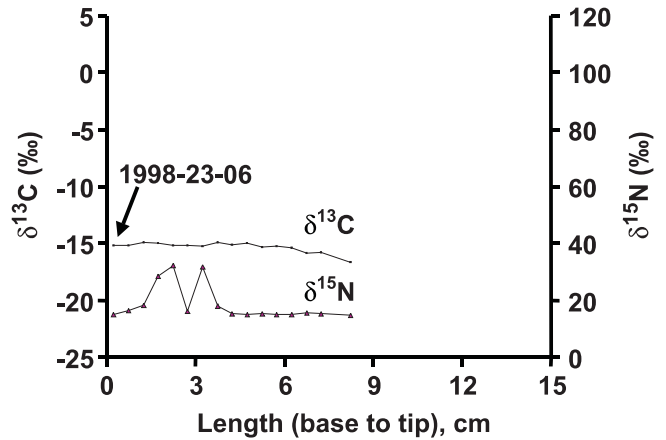
Fig. 9. Stable-isotope plot from a vibrissa of an adult female Steller sea lion, SSLA34SE, collected in southeastern Alaska in June 1993. Each oscillation represents 1 year's growth of the vibrissa. Plots show the most recent growth at the base of the vibrissa (0 cm).
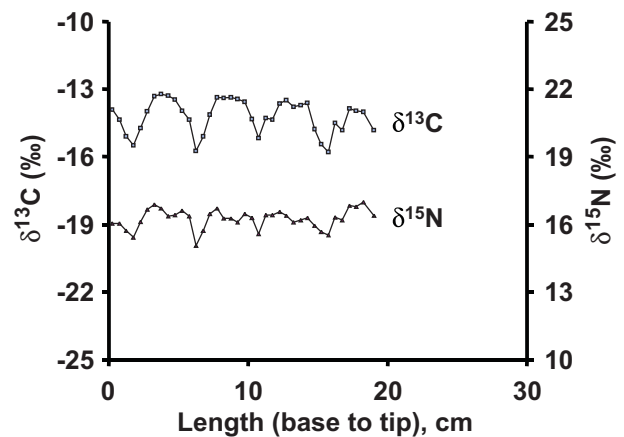
appeared to exhibit more consistent growth and year-to-year retention of their vibrissae (Table 2).

Vibrissae collected from seal HS-N appeared to have grown from the end of September until mid-June. HS-N had one isotope peak after being given one dose of labeled glycine in January, but growth-rate calculations indicate that the second dose, given in June, was not incorporated. The fact that the peak remained in approximately the same location on whiskers sampled from HS-N in August and November lent further support to the conclusion that the isotope peak was the result of the January injection and the assumption that growth decreased to some minimal level or ceased altogether in June. At some time during the next 20 months, however, most or all of the vibrissae appeared to have been lost, and the marker was not evident in the existing vibrissae.

Variations in seasonal metabolic rate may have some connection to the period of rapid growth in the harbor seal

Fig. 10. Stable-isotope plots from vibrissae of a killed adult female Steller sea lion from the Pribilof Islands, Bering Sea, Alaska. Anterior (A and B) and posterior (C and D) vibrissae and vibrissae from the left (A and C) and right side of the muzzle (B and D) are contrasted. The numbers 18 and 15 and 1 and 3 refer to specific anterior and posterior vibrissae, respectively. Plots show the most recent growth at the base of the vibrissae (0 cm).
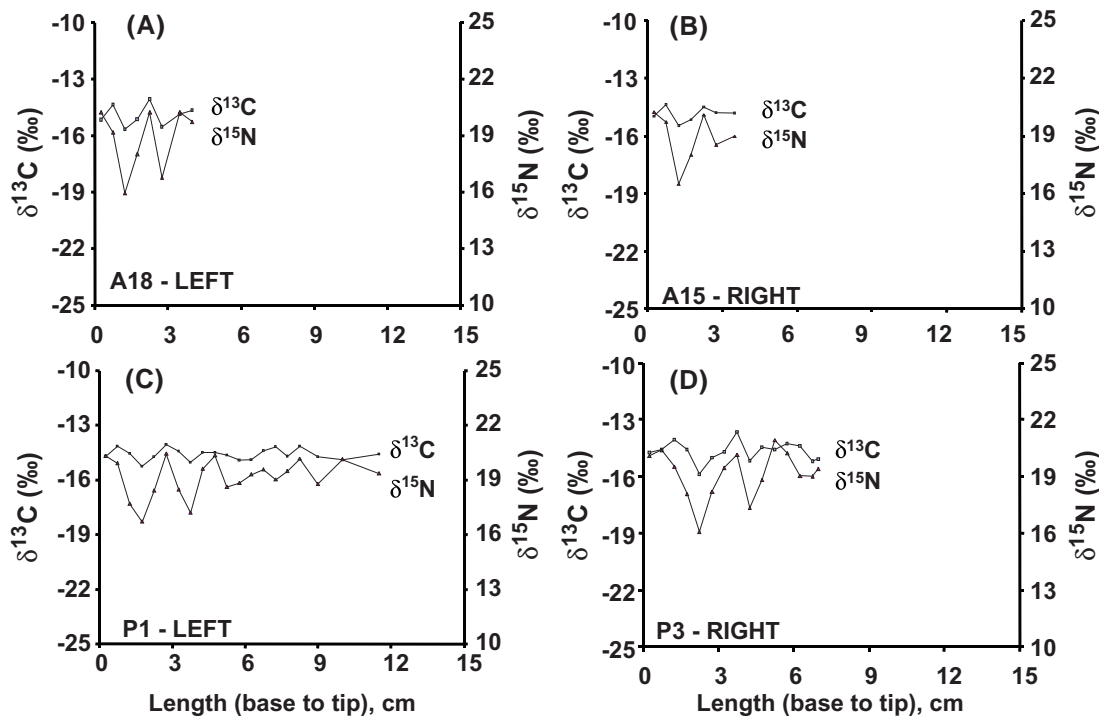
Table 2. Growth rates of vibrissae in harbor seals and Steller sea lions.

	Age-class	Mean growth rate (mm/day)
Harbor seals		
Captive (Mystic, Conn.)	Adult	0.33–0.37
Wild (Alaska)	Adult	0.08
Steller sea lions		
Captive (Mystic, Conn.)	Adult	0.05–0.09
Captive (Vancouver, B.C.)	Subadult	0.14–0.17
Wild (Alaska)	Adult	0.10–0.14

vibrissae. Rosen and Renouf (1995) observed an 84% increase in the resting metabolic rate (RMR) of captive adult harbor seals from November through April and a RMR that was higher than the average August estimates for the animals. The rapid growth of HS-N's vibrissae was also observed during the same winter-to-spring period (Lavigne et al. 1982; Davis et al. 1985).

Our first measure of the growth rate of vibrissae in wild seals came from a recaptured adult prior to the beginning of the labeling experiment. During that 7-month period from September to April, vibrissa growth was thought to be constant at a rate one-fourth of that of the captive seal's vibrissae. One or both of these seals may have been impacted by differences in habitat, feeding, and energetics that could have affected their vibrissa growth rates. Further experimentation will be necessary to address these possibilities.

Both captive Steller sea lions that received two doses of singly and (or) doubly labeled glycine showed evidence of the label in their vibrissae. The enriched isotope signals remained in the vibrissae for more than 2 years after the initial doses of glycine were administered, which indicates that vibrissae are retained from year to year. The range of vibrissa growth rates in captive adult sea lions was similar to the estimated rate for each oscillation in wild adult animals. Vibrissae had been collected from the Steller sea lions at the Vancouver Aquarium when the animals ranged in age from 2 to 4 years. Periodic changes in the animals' diets are evident in the shifts in stable-isotope ratios along their vibrissae, as represented by one sea lion in Fig. 8. These changes were confirmed by isotopic analysis of the animals' food. The growth rate in the juvenile sea lions was twice that exhibited by the captive adults. Metabolic observations made by D. Rosen on the captive juvenile sea lions showed a maximum RMR during late fall and a minimum RMR during April and May (personal communication), but no comparable adult levels are available at this time. The oscillations observed in both the carbon- and nitrogen-isotope ratios for the wild sea lion in Fig. 9 are not the result of endogenous rhythms but, rather, of dietary and geographic changes (Hirons 2001). This point is further illustrated by the lack of isotopic oscillations, except those resulting from the labeled glycine, in the sea lion vibrissae represented in Figs. 6 and 7, which did not undergo any dietary changes during the experiment.

Stable-isotope analysis of blood serum revealed that the carbon was cleared faster than the nitrogen. The carbon isotopes showed evidence of respiratory loss, whereas the $\delta^{15}N$ values were additive over the duration of the experiment as a result of transamination and reincorporation into the body tissues. The pronounced changes in nitrogen-isotope ratios

reflected the relative quantities of the element in the body composition of these animals. The nitrogen and carbon isotopes in the vibrissa keratin continued to show enrichment over pre-injection values for a long time following the initial sharp decline in the isotope peaks. The enrichment in HS-N's isotopes was most pronounced and led to a long-term increase of about 2‰ over the duration of the experiment. There were no changes in the diets of the captive harbor seals or Steller sea lions during or after the labeling experiment. The total retention time of detectable label remains unknown, as long-term monitoring of the animals' vibrissae could not be maintained. The residence time of this residual label, particularly in Steller sea lions that retain their vibrissae, should provide valuable information on turnover rates of proteins and could conceivably be used for wild animals when recapture is a possibility.

The carbon and nitrogen isotopes in the vibrissae of wild harbor seals did not show any type of repetitious pattern that might be indicative of an annual cycle. Two wild harbor seals, one recaptured after 1 year and the other after 2 years, also displayed no similarity or overlap in stable-isotope patterns in the vibrissae between years. These data are consistent with the natural histories of the seals. The isotopic oscillations in the vibrissae from wild adult Steller sea lions throughout the Bering Sea and Gulf of Alaska likely resulted from the movement and feeding of the animals throughout various geographic regions (Merrick et al. 1997; Hirons 2001). The similar growth rates in the vibrissae from both wild and captive Steller sea lions, combined with the repetitive isotopic patterns in wild sea lions, are evidence that sea lions retain their vibrissae for several years and likely replace them only when they are broken or worn.

Observations of southern elephant seal vibrissae made by Ling (1966) revealed that they were not shed at the same time as the annual molt occurred but were replaced irregularly. Since vibrissae appear to function as individual sensory organs, any replacement because of loss or damage could confer a greater selective advantage than regular seasonal changes (Ling 1977). D. Bowen (personal communication) observed grey seals in captivity sporadically losing their vibrissae and rapidly regrowing them during the molting period. He has also observed the rapid regrowth of broken vibrissae of grey seals throughout the year. The captive Steller sea lions used in this growth experiment were observed rapidly regrowing cut vibrissae, while the remaining vibrissae showed no apparent change. Morphological differences exist between harbor seal and Steller sea lion vibrissae, but it is not known if these differences have any impact on growth-rate patterns in the vibrissae or vice versa. The wavy, or "beaded," surface of harbor seal vibrissae differs from the smooth veneer of Steller sea lion vibrissae. A harbor seal's vibrissae (~10 cm) tend to be similar in length, while a Steller sea lion's anterior mystacial vibrissae are short (~6 cm) and the posterior vibrissae much longer (>20 cm) (A.C. Hirons, unpublished data).

Both the empirical data and the literature seem to support the idea that vibrissa growth rates and retention times vary among pinniped species but the cues, environmental and (or) internal, remain unknown. Further experimentation on captive pinnipeds, supplemented by information from wild seals and sea lions, will be needed to better define these patterns. Stable-isotope-labeled amino acids provide a safe and effective means of applying internal markers for experiments on vibrissa growth rates.

Further studies should expand our understanding of how vibrissa growth may change throughout an animal's lifespan. Vibrissae from seals and sea lions contain a timeline of stable-isotope ratios derived from prey items. By comparing the isotope ratios along the length of vibrissae with those of suspected prey items, changes in food sources and habitat can be surmised for the temporal span represented by the growth of the whisker. The trophic information these tissues provide will enhance our knowledge of the animals' food resources while perhaps providing clues to the causes of their population declines.

References

Alaska Department of Fish and Game (ADFG). 1996. Harbor seal investigations in Alaska. NOAA Grant No. NA57FX0367, Alaska Department of Fish and Game, Anchorage.

Alaska Sea Grant. 1993. Is it food? Addressing marine mammal and seabird declines. Workshop summary, Alaska Sea Grant Report 93-01, University of Alaska, Fairbanks.

Alverson, D.L. 1991. Commercial fisheries and the Steller sea lion (*Eumetopias jubatus*): the conflict arena. Rev. Aquat. Sci. **6**: 203–256.

Anderson, P.J., Blackburn, J.E., and Johnson, B.A. 1997. Declines of forage species in the Gulf of Alaska, 1992–1995, as an indication of regime shift. *In* Forage Fishes in Marine Ecosystems: Proceedings of an International Symposium on the Role of Forage Fishes in Marine Ecosystems, Anchorage, Alaska, 13–16 November 1996. Alaska Sea Grant Report 97–01, University of Alaska, Fairbanks. pp. 531–544.

Boutton, T.W. 1991. Tracer studies with ^{13}C-enriched substrates: human and large animals. *In* Carbon isotope techniques. *Edited by* D.C. Coleman and B. Fry. Academic Press Inc., San Diego. pp. 219–242.

Davis, R.W., Williams, T.M., and Kooyman, G.L. 1985. Swimming metabolism of yearling and adult harbor seals *Phoca vitulina*. Physiol. Zool. **58**: 590–596.

Dehnhardt, G., and Kaminski, A. 1995. Sensitivity of the mystacial vibrissae of harbour seals (*Phoca vitulina*) for size differences of actively touched objects. J. Exp. Biol. **198**: 2317–2323.

DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochim. Cosmochim. Acta, **42**: 495–506.

DeNiro, M.J., and Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochim. Cosmochim. Acta, **45**: 341–351.

Fry, B., and Sherr, E.B. 1984. $\delta^{13}C$ measurements as indicators of carbon flow in marine and freshwater ecosystems. Contrib. Mar. Sci. **27**: 13–47.

Hirons, A.C. 2001. Trophic dynamics of pinniped populations in Alaska using stable carbon and nitrogen isotope ratios. Ph.D. thesis, University of Alaska, Fairbanks.

Lavigne, D.M., Barchard, W., Innes, S., and Øritsland, N.A. 1982. Pinniped bioenergetics. FAO Fish. Ser. 5 No. 4. pp. 191–235.

Ling, J.K. 1966. The skin and hair of the southern elephant seal, *Mirounga leonina* (Linn.) I. The facial vibrissae. Aust. J. Zool. **14**: 855–866.

Ling, J.K. 1970. Pelage and molting in wild mammals with special reference to aquatic forms. Q. Rev. Biol. **45**: 16–54.

Ling, J.K. 1977. Vibrissae of marine mammals. *In* Functional anatomy of marine mammals. *Edited by* R.J. Harrison. Academic Press, London. pp. 387–415.

Lyne, A.G., Downes, A.M., and Chase, H.B. 1974. Growth of vibrissae in the marsupial *Trichosurus vulpecula*. Aust. J. Zool. **22**: 117–129.

Merrick, R.L. 1995. The relationship of the foraging ecology of Steller sea lions (*Eumetopias jubatus*) to their population decline in Alaska. Ph.D. thesis, University of Washington, Seattle.

Merrick, R.L., Chumbley, M.K., and Byrd, G.V. 1997. Diet diversity of Steller sea lions (*Eumetopias jubatus*) and their population decline in Alaska: a potential relationship. Can. J. Fish. Aquat. Sci. **54**: 1342–1348.

Michener, R.H., and Schell, D.M. 1994. Stable isotope ratios as tracers in marine aquatic food webs. *In* Stable isotopes in ecology and environmental science. *Edited by* K. Ljatha and R.H. Michener. Blackwell Scientific Publications, Oxford. pp. 138–157.

Pitcher, K.W. 1990. Major decline in the number of harbor seals, *Phoca vitulina richardsi*, on Tugidak Island, Gulf of Alaska. Mar. Mamm. Sci. **6**: 121–134.

Rosen, D., and Renouf, D. 1995. Variation in the metabolic rates of captive harbour seals. *In* Whales, seals, fish and man. *Edited by* A.S. Blix, L. Walløe, and Ø. Ulltang. Elsevier Science, Amsterdam. pp. 393–399.

Scheffer, V.B. 1962. Pelage and surface topography of the northern fur seal. U.S. Fish Wildl. Serv. N. Am. Fauna No. 64. pp. 34–35.

Schell, D.M., and Saupe, S.M. 1993. Feeding and growth as indicated by stable isotopes. *In* The bowhead whale. *Edited by* J.J. Burns, J.J. Montague, and C.J. Cowles. Allen Press, Lawrence, Kans. pp. 491–506.

Strick, J.M., Fritz, L.W., and Lewis, J.P. 1997. Aerial and ship-based surveys of Steller sea lions (*Eumetopias jubatus*) in southeast Alaska, the Gulf of Alaska, and Aleutian Islands during June and July 1994. NOAA Tech. Mem. NMFS-AFSC-71.