Exxon Valdez Oil Spill
Restoration Project Final Report

Isotope Ratio Studies of Marine Mammals in Prince William Sound

Restoration Project 98170
Final Report

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Study History: This project originated as part of the Sound Ecosystem Assessment program conducted by the University of Alaska Fairbanks and the Prince William Sound Science Center. In cooperation with K. Frost of the Alaska Department of Fish and Game, we began a stable isotope study of harbor seals and potential prey species in Prince William Sound. T. Kline, then of the University of Alaska, was a co-investigator but upon his taking a position with the Prince William Sound Science Center, the project was split into two parts, with Kline collecting data on lower trophic levels and this project focusing on harbor seals and prey species. Since FY 96, this project has remained separate, although we have been responsible for all of the stable isotope analyses run for the Prince William Sound Science Center, for the University of Alaska Fairbanks, and for other investigators using isotopic data. This project has expanded upon the scope of data in a journal article recently published (Schell et al. 1998. Carbon and nitrogen isotope ratios in zooplankton of the Bering, Chukchi and Beaufort seas. Marine Ecology Progress Series 162:11–23). Two additional manuscripts nearing publication and included in this report have resulted from this project as well.

Abstract: Archived and recent harbor seal tissues have been used to determine food web structure and trophic dynamics of seals within Prince William Sound (PWS) and the adjacent Gulf of Alaska. Within the sound, isotope ratios confirm that most harbor seals are at the top of food chains that are based on in situ primary and secondary productivity and not on allochthonous production from outside the Sound. Carbon isotope ratios also indicate that benthic prey are a large component of harbor seal diets. Isotope ratios along wild seal whiskers indicate, however, that some individuals migrate into areas (presumably in the Gulf) wherein food web structures are different and isotope ratios of prey are considerably lower than within the sound. Experiments with captive seals to determine whisker growth rates showed that vibrissal growth is highly seasonal and occurs primarily in early spring. Sea lions and fur seals have relatively constant vibrissal growth. Data on isotope ratios of potential prey species from PWS and from other sites in the Gulf of Alaska indicate that a geographic isotopic gradient in both carbon and nitrogen exists between onshelf and deep pelagic waters. The detailed patterns of these isotopic regimes have not yet been fully defined.

Key Words: *Exxon Valdez* oil spill, food webs, harbor seals, $\delta^{13}C$, $\delta^{15}N$, isotope ratios, *Phoca vitulina*, Prince William Sound.

Project Data: Data consist of carbon and nitrogen stable isotope ratios of zooplankton, forage fishes and harbor seals from Prince William Sound and selected areas of the Gulf of Alaska. The data are in spreadsheets and tabular format in Corel QuattroPro and Microsoft Excel and will be included in refereed publications and a graduate dissertation. The project PI will maintain these data files and can be contacted as follows:

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EXECUTIVE SUMMARY

This study of the food webs supporting harbor seals in Prince William Sound was based on the natural stable isotope abundances of carbon and nitrogen as tracers of energy and nutrient transfers. The goal of the study was to determine if declining populations of harbor seals resulted from oil-spill-related effects or if ecosystem variables were shifting in response to external forcing.

Three major findings emerged from this study: First, analysis of isotope ratios in archived and modern pinniped tissues revealed that carbon isotope ratios in the animals, and by extension, the primary producers, have declined over the past 50 years, whereas nitrogen isotope ratios have remained constant. We had hypothesized that isotope ratios in primary producers reflect phytoplankton growth rates and that in a highly seasonal productivity regime, this would translate into an estimate of energy available for secondary production. Since nitrogen supply to the euphotic zone is limiting, any changes in carbon isotope ratios would not be mirrored in nitrogen isotope ratios. We observed that although no temporal pattern was evident in average nitrogen isotope ratios in both whale baleen and pinnipeds, the carbon isotope ratios showed a marked decline in the past 30+ years. This decline in $\delta^{13}C$ is evident in both the Gulf of Alaska and the Bering Sea indicating that it is not a function of an onshore-offshore geographic gradient or a local phenomenon. No apparent trophic shifts have occurred over time but the seasonal carrying capacity of the marine system has declined. This strongly suggests, but does not prove, that the decline in marine mammals and other top consumers may arise from food stress imparted by overall lower abundances of prey.

Second, the presence of a geographic gradient in carbon and nitrogen isotope ratios declining with distance offshore has been shown to exist along the Gulf of Alaska coast. Although data are not available for the central Gulf of Alaska, indications are that both nitrogen and carbon isotope ratios reach minima within some distance offshore. This information will be essential for separating geographic and trophic effects on consumer isotope ratios.

Third, captive animal studies on the growth rates of vibrissae revealed a major difference between harbor seals and Steller sea lions and fur seals. Harbor seals grow their vibrissae over a few months' time, whereas the latter two species grow their whiskers continuously. The short time span represented in harbor seal whiskers restricts their usefulness as a temporal record of isotope ratios in these seals' diet.

During the past three years, vibrissae (whiskers) and other tissues were collected from harbor seals within Prince William Sound and the surrounding Gulf of Alaska. Samples were obtained from modern animals and from specimens archived at the Alaska Department of Fish and Game, the University of Alaska Museum, and the National Marine Mammal Laboratory. One or two long vibrissae were cut or pulled from live animals, while harvested or dead animals had all vibrissae removed for analysis. We have analyzed tissues from over 150 seals, and the data indicate that each whisker has a temporal record of several months to a year. This allows comparisons of changes in feeding and trophic position over the temporal span represented. When possible, samples from different organ tissues, e.g., muscle and blubber, were also taken. A variety of tissues from individual animals were analyzed to determine isotopic fractionation among the tissues. This has allowed normalization of isotope data to a single tissue type when samples of only one tissue type were available.

Carbon isotope ratios were also used as conservative tracers of energy supply between trophic levels (phytoplankton to zooplankton to fishes to top consumers). To establish the required baseline information, we collected potential prey species of fishes and other organisms

from within Prince William Sound and the adjacent Gulf of Alaska and compared the isotope ratios with those from the seals. Stable isotope ratios within most harbor seal vibrissae do not appear to fluctuate greatly or with any regular periodicity, although some individuals show large changes between enriched and depleted values, indicating longer-range movements. More often there are minor fluctuations in the $\delta^{13}C$ with somewhat larger fluctuations in the $\delta^{15}N$. These shifts in the nitrogen isotope ratios probably reflect seasonal changes in prey availability within a small region.

Samples of zooplankton collected by cooperating investigators revealed that primary production is much lower in offshore waters, as indicated by depletions in both $\delta^{13}C$ and $\delta^{15}N$. These low values provide a distinctive geographic indicator visible in vibrissae of seals that feed in pelagic regions or on prey that have emigrated from offshore areas. Samples of fatty acids from the seals have been analyzed in a collaborating study (K. Frost, Alaska Department of Fish and Game) and have been found to be very different among regions, supporting the hypothesis that seals tend to reside in relatively small ranges with distinct food web structures in Prince William Sound.

To enable estimation of the time represented by the growth of a whisker, captive seals and sea lions held at the Mystic MarineLife Aquarium were infused with ^{13}C- and ^{15}N -labeled glycine in 1996 and 1997. The added label was detectable in the analyzed whisker and allowed estimation of the vibrissae growth rate. These calibration data were essential to the interpretation of temporal changes in vibrissae taken from wild seals. Remodeling of the Mystic facility and the moving of the two labeled seals to the new Alaska Sealife Center in Seward, Alaska, however, interrupted this study, and obtaining the vibrissae was delayed until the end of summer 1998. Further calibration came from one wild seal tagged in fall 1994 and recaptured in spring 1995. Whiskers from both time points were analyzed and the results compared with captive-animal data. This recapture, and isotopic labeling data on the captive animals, indicated that vibrissal growth is episodic and tied to the spring breeding season. We conclude that only part of the annual feeding cycle is represented in the whisker keratin. Vibrissae from a a dead seal showed that they all grow at approximately the same rate. Further experiments will be continued at the Seward facility.

Archived tissue samples from harbor seals were analyzed to determine if the trophic structures of the food webs changed between the period prior to the decline in population and current years. Our data show that seals taken in 1995 had a similar range in $\delta^{13}C$ but were split into two clusters of $\delta^{15}N$ values, suggesting multiple trophic status within the population. The values for one group of animals remained close to those collected 6 and 20 years previously, whereas the other group had higher $\delta^{15}N$ values, implying feeding at a higher trophic level. Because respiration reduces biomass approximately 80-90% in going up each trophic level, the seals with higher $\delta^{15}N$ values may be nearing food-limited conditions. In contrast, seals from southeastern Alaska showed no apparent change in isotope ratios over the period 1975-1995.

A conceptual model of harbor seal feeding has been constructed based on the known isotope ratios in lower trophic levels and fishes, primarily capelin, herring, and pollock. Predicted isotope ratios in seals using these food sources matched observed $\delta^{15}N$ values closely, but the measured $\delta^{13}C$ values were higher than predicted. We suggest that benthos, which are usually enriched relative to pelagic species at a given site, are important in the food supply of these seals.

INTRODUCTION

This report describes results of a study of food webs that support harbor seals in Prince William Sound (PWS). This project also contributed to the Sound Ecosystem Assessment (SEA) program being conducted to describe the food chains supporting important commercial fish species that were injured by the *Exxon Valdez* Oil Spill (EVOS). In addition, it contributes to studies by Alaska Department of Fish and Game (ADFG) personnel to determine reasons for the decline of harbor seal and Steller sea lion populations in Prince William Sound. The integrating methodology for this wide range of tasks is the use of stable isotope ratios as natural tracers of carbon and nitrogen transfers through the food webs.

Carbon isotope ratios ($^{13}C/^{12}C$) serve as conservative tracers of energy supply among trophic levels (phytoplankton to zooplankton to fishes to top consumers). Seals, cetaceans, birds, etc. acquire the isotope ratios in proportion to the amount of food derived from each differing source. This, in turn, is reflected in the composition of body tissues and in keratinous tissues (claws, feathers, baleen, and whiskers) as a temporal record when multiple sources of food are consumed over time and space. This allows us to discern important habitats and food resources in animals that seasonally migrate or undergo periods of hyper- and hypotrophy.

Nitrogen isotope ratios ($^{15}N/^{14}N$) reflect both the food sources and the trophic status of the consumer. As nitrogen in food is consumed and assimilated by an animal, the heavy isotope is enriched by approximately 3‰ with accompanying loss of the lighter isotope through excretion. The enrichment occurs with each trophic step and thus allows the construction of conceptual models and food webs and the assignment of trophic status to species for which dietary data are sparse. The data obtained from these measurements are unique in that they trace materials actually assimilated and can thus be used for more accurate ecosystem modeling.

It can be postulated that the natural stable isotope abundances of PWS biota will shift because of changes in trophic level, food web structure, and primary productivity in the environment, thus providing an independent tool to verify, quantify, and model ecosystem processes. The tracer nature of the approach enables the integration of ecosystem components.

The project comprised three elements:

1. A research component on marine mammals, focusing on the trophic energetics and ecosystem dynamics of harbor seals, was conducted by Dr. Schell, PI, in cooperation with ADFG personnel working as part of the marine mammal program. An additional effort, using captive animals to calibrate responses to changing isotopic composition in diet and to determine vibrissae growth rates, was also conducted.

2. A research effort focusing on lower trophic levels having direct application to the testing of hypotheses regarding fisheries resources was conducted by Dr. T. Kline of the Prince William Sound Science Center (PWSSC) in cooperation with the marine mammal component. Isotopic data from this study were used to assist in describing the food resources available to seals, but the primary results have been published elsewhere as part of the Sound Ecosystem Assessment.

3. As the major isotope ratio analysis facility, we have provided analytical services for obtaining carbon and nitrogen isotope ratios for other PIs involved with EVOS studies,

and have assisted with the interpretation of the acquired data. This task has required approximately 20-30% of the analytical and research effort.

OBJECTIVES

The objectives of this isotope study included:

1. Collect and analyze samples of harbor seal vibrissae through cooperative work with the Alaska Department of Fish and Game in Prince William Sound.

2. Collect and analyze samples of harbor seal prey species including forage fishes, salmon, and herring in the vicinity of major haulouts and high population densities. Samples of seal tissues were collected from animals killed by Native hunters. These samples were obtained with the assistance of ADFG personnel who were monitoring harvests, and through the efforts of T. Kline.

3. Perform stable isotope ratio analyses on tissues and organisms collected during the sampling program. Through the use of carbon isotope data on taxa collected over geographical regions, the presence/absence of isotopic gradients useful in sorting out habitat dependencies were determined.

4. Assist other research programs in the Prince William Sound ecosystem study by conducting stable isotope ratio analyses on samples provided, and aid in the interpretation of results. We have provided isotope ratio analysis for several studies sponsored by EVOS.

5. Through the use of nitrogen isotope ratios in collected taxa, assign trophic status to species in each region. These trophic states were then compared with those from predictive models based on conceptual food webs.

6. Determine temporal changes in harbor seal trophic status and food dependencies by comparing isotope ratios along the lengths of vibrissae with isotope ratios from available prey. Through the use of captive animals fed labeled diets or by direct infusion of labeled amino acids, establish the relationships between vibrissae growth rate and temporal changes and the fractionation factors between the $\delta^{13}C$ and $\delta^{15}N$ values of diet and consumer.

METHODS

Sampling of tissues for stable isotope analysis has been described for both bulk tissues (muscle, blubber) and temporally variable tissues (whiskers, claws, etc.) (Schell et al. 1989, Michener and Schell 1994). This report includes only the pertinent sampling protocols and a synopsis of the analytical methods.

Forage Fishes

Lower trophic level organisms within Prince William Sound were obtained by T. Kline and analyzed within the scope of this project. Stable isotope ratios for these species were used to construct food webs for harbor seals foraging within PWS. Samples of a few additional forage fishes from areas of harbor seal haulouts have been provided by ADFG personnel and combined with other lower trophic level organisms to assist in assigning trophic status. Pelagic and benthic species were sampled during shellfish surveys conducted by ADFG personnel in the western Gulf of Alaska. These prey were used as indicators of regional isotopic differences. Regional differences in prey were used to help locate areas of foraging for seals traveling outside Prince William Sound. The National Marine Fisheries Service triennial survey of the entire Gulf of Alaska provided prey from areas for which data were previously lacking.

A few grams of muscle tissue were extracted from several samples of each species at a sampling site. The tissues were frozen in a standard -10°C freezer and transported to the stable isotope facility for analysis. Subsamples of the frozen muscle tissues were dried at 60°C, ground for homogeneity and prepared for mass spectroscopy.

Pinnipeds

Harbor seal tissues were collected with the assistance of the Alaska Department of Fish and Game and native subsistence hunters. Multiple tissue types were collected from each animal to identify the isotope fractionation that occurs among differing tissues as a result of variations in biochemical metabolism. Biochemical components of tissues are isotopically different from each other; therefore, various proportions of these components in different tissues may affect the tissues' isotopic compositions.

During the past three years, vibrissae from harbor seals were collected within Prince William Sound and from the surrounding Gulf of Alaska. One to two long vibrissae were cut or pulled from live animals, and harvested or dead animals had all their vibrissae removed for analysis. When possible, samples from different organ tissues, e.g., muscle and blubber, were taken for analysis. Alaska Department of Fish and Game personnel working as part of the marine mammal monitoring effort provided tissues from Prince William Sound, Southeast Alaska, and Kodiak harbor seals.

ADFG researchers have provided archived harbor seal tissues, dating from the mid-1970s, for stable isotope comparisons. These comparisons were useful in determining if a dietary shift in harbor seals had occurred during the past two decades. The University of Alaska Museum and the Kodiak Historical Society provided bone tissue for collagen extraction from harbor seals, Steller sea lions, and northern fur seals from various regions of the Gulf of Alaska from the 1950s to the present. The stable isotope ratios of these tissues were used for comparison with the stable isotope ratios of modern samples. Assessing the isotopic ratios of seal tissues from multiple regions prior to the population decline (pre-1970) allowed any significant changes in these ratios to be used as indications of change in ecosystem productivity over the past several decades.

Vibrissae and tissues from 219 harbor seals were analyzed for stable isotope ratios. Vibrissae were scrubbed with steel wool to remove any debris and segmented from base to tip in

2.5mm segments. Every other segment was analyzed for carbon and nitrogen isotope ratios and the reserved segments were archived for future reference. Collagen was extracted from bone samples using the technique of Matheus (1997). Tissues were dried at 60°C, ground for homogeneity and prepared for mass spectroscopy.

Determination of vibrissae growth rates was done using stable carbon and nitrogen isotope-labeled glycine in adult seals and sea lions. The amino acids were injected intravenously over one- to two-day periods. Following the infusion, blood samples were taken to verify the appearance of a label in the animal. The large amount of labeled-isotopes created large peaks in the vibrissae; these acted as temporal markers. After several months had passed, vibrissae were clipped as close to the skin as possible. Vibrissae were analyzed for isotope ratios and the distance between isotopic peaks was measured. Growth rates were calculated by dividing the distance by the number of days between the markers.

A second type of growth rate experiment was conducted at the Vancouver Aquarium in British Columbia, Canada, on subadult Steller sea lions. Vibrissae had been cut from the muzzle of each animal periodically during the three-year period. The vibrissae were then analyzed for their stable isotopes and all the whiskers from an animal plotted together. Overlap in growth from one vibrissae to the next was measured from an inflection point conspicuous on at least two separate segments. The date of each cutting was known, and from these data the growth rate was calculated.

Analytical Techniques

The samples were dried and powdered for homogeneity, and the isotope ratios of carbon and nitrogen were determined with a Europa 20/20 continuous flow isotope ratio mass spectrometer. The samples were combusted at high temperature and the nitrogen and carbon dioxide gases separated and purified by gas chromatography. The gases were subsequently led into the mass spectrometer by capillary action and the isotope ratios determined. All samples were analyzed in duplicate. Results are reported in the standard $\delta^{13}C$ and $\delta^{15}N$ notation relative to Pee Dee Belemnite and air standards for carbon and nitrogen, respectively. Standard replicates were analyzed for every twelve samples. If the difference between replicates was greater than 0.5‰, samples were re-analyzed. A difference of 0.2‰ was considered acceptable. Analytical error for samples was approximately ±0.1‰ for both carbon and nitrogen.

Statistical Analysis

Hotelling's T-test was used to distinguish if regional differences existed among harbor seals based on their stable isotope ratios. Multiple analysis of variance (MANOVA) and Wilk's Lambda were used to investigate isotopic differences based on the sex and age of the seals and the region of Prince William Sound and years samples were gathered. ANOVA and Bonferroni correction tests were run for the entire data set of harbor seal tissues to establish significant differences among the tissues. Fractionation differences in harbor seal tissues were calculated using least square means and standard error equations. Multiple analysis of variance tests and linear regression analyses were conducted on the 47-year data set of both $\delta^{13}C$ and $\delta^{15}N$ (SYSTAT for Windows 1992).

SUMMARY OF RESULTS AND DISCUSSION

The major findings of this project are included in detail as manuscripts in the Appendices. We summarize the different elements of the study and the important findings. Readers are encouraged to seek the appropriate manuscript for details.

Isotope Ratio Gradients Between Offshore and Nearshore Environments

The isotope ratio gradients first identified in the waters of the Beaufort and Chukchi seas (Saupe et al. 1989) were further defined for the Bering Sea, detailed in work sponsored by the U.S. Minerals Management Service, and published in 1998 (Schell et al. 1998). The pronounced isotope ratio gradients observed in the Bering Sea led to the belief that similar gradients might be present in the Gulf of Alaska and extending into Prince William Sound. Knowledge of the magnitude and position of these gradients was essential for the interpretation of observed shifts in isotope ratios in seal vibrissae. Unfortunately, no detailed or extended offshore sampling was carried out in the EVOS-sponsored programs. This led to the acquisition of samples collected by Canadian researchers who had undertaken cruises across the Gulf of Alaska as part of high seas salmon research. We are especially indebted to Dr. David Welch of the Pacific Biological Station, Nanaimo, B.C., for access to samples collected in 1996 and 1997. These samples were sorted for calanoid copepods and euphausiids and the samples run for isotope ratios. The $\delta^{13}C$ and $\delta^{15}N$ data are presented in Figures 1 and 2. A detailed description of temporal and spatial variability within PWS has been presented by Kline (in press 1999).

Harp Seal Study

Through cooperation with Keith Hobson of the Canadian Wildlife Service, we were able to acquire vibrissae from two harp seals that had been held in captivity and fed known diets of herring. The whiskers from these animals were analyzed along their lengths and were compared with the isotopic composition of the diets. Results indicated that the seals closely reflect the diet, remaining within 1.5‰ in carbon and within approximately the same range in $\delta^{15}N$ but showing the expected 3‰ trophic enrichment. Data from this study provided the preliminary analysis techniques necessary to analyze the harbor seal vibrissae and interpret the trophic dynamics in wild populations. The data were compiled and published (Hobson et al. 1996).

Isotope Ratios in Prey Species

The isotope ratios of prey species important to harbor seals were defined within and outside Prince William Sound. Based on the natural history of harbor seals, including information from stomach content analyses, pollock, herring, squid, octopus, salmon, and capelin were evident most often in seal stomachs from Prince William Sound (Pitcher 1980). Imler and Sarber (1947) found the remains of pollock and octopus most abundant from the stomachs of harbor seals in Prince William Sound. The pleuronectid, yellowfin sole, had been observed being taken by seals in

an area west of Montague Island. A few samples of these, as well as high-lipid eulachon, were collected and added to the plot. The PWS prey plots (Figs. 3 and 4) were created using $\delta^{13}C$ and $\delta^{15}N$ values for nine potential prey species for harbor seals. *Neocalanus* spp. were included in the food web as first-order consumers within the sound. The most enriched isotope ratios along the seals' vibrissae were defined as "max" and the most depleted values were defined as "min" for use within the prey plots. For the sake of clarity, only a random sampling of harbor seals was added to the plot. These plots are not meant to represent the absolute prey variety in the diet, but more as likely sources of prey for seals foraging within the Sound.

Based on historical information, harbor seals appear to forage on one to two preferred prey but will also feed on seasonally available species such as salmon. The $\delta^{15}N$ in harbor seals having the more enriched stable isotopes ("max") (mean $\delta^{15}N = 17.2$) was isotopically similar to that in pollock, yellowfin sole, octopus, and silver salmon from PWS, based on a 3‰ trophic level enrichment in marine food webs (Schoeninger and DeNiro 1984, Hobson et al. 1994) (Fig. 3). However, the $\delta^{13}C$ in the seals (mean $\delta^{13}C = -15.4$) was even generally more enriched than the expected 1‰ trophic increase for any of the prey species sampled either in PWS or in the Gulf (Fig. 4). The source of these enriched values may be due to the consumption of demersal or benthic organisms for which samples have not been readily available for isotopic analyses. Benthic environments tend to have more enriched values due to recycling of nutrients and the presence of bacterial food webs (Coffin et al. 1994, France 1995). Both yellowfin sole and octopus are benthic feeders, which would result in these organisms having more enriched $\delta^{13}C$. Overlap in the range of $\delta^{13}C$ between these benthic feeders and the seals' "max" values does have the expected 1‰ increase. Seals feeding on these animals would exhibit those enriched values (Wells 1978). Harbor seals generally have a mixed diet that results in them digesting prey of different isotope ratios so the resulting isotope ratios they exhibit in their vibrissae often do not have an exact 1‰ and 3‰ enrichment in their carbon and nitrogen isotope ratios, respectively (Hobson et al. 1997). The $\delta^{15}N$ values in harbor seals having the more depleted stable isotopes ("min") (mean $\delta^{15}N = 14.7$) were isotopically similar to that of capelin and pollock from the Gulf of Alaska south of PWS, and capelin, herring, and squid in PWS (Fig. 3). The $\delta^{13}C$ values in these seals (mean $\delta^{13}C = -17.6$) were most similar to those from pollock from the Gulf and herring and squid from PWS (Fig. 4). Hobson et al. (1997) reported harbor seals from the Copper River Delta (CRD) in Alaska having mean $\delta^{15}N = 18.6$ and mean $\delta^{13}C = -17.6$. The nitrogen values are more enriched than any found in seals residing in PWS. As Hobson et al. pointed out, the seals from the CRD were likely sampled at a time when they were foraging on enriched coho (silver) salmon, which could account for the high nitrogen values. The carbon values for the CRD seals are very similar to the "min" values for PWS seals and provide additional evidence supporting the hypothesis that the depleted $\delta^{13}C$ values in some PWS seals resulted from foraging on prey outside the sound. Most harbor seals do not migrate extensively, but some have been tracked over many kilometers out into the Gulf of Alaska (Frost and Lowry 1997).

Similar to work done by Schell et al. (1998) in the Bering Sea, areas of the Gulf of Alaska are being refined into smaller isotopic regions to better define feeding areas for traveling phocids or prey transport into Prince William Sound (Figs. 1 and 2). Stable isotope values for Prince William Sound prey species have been provided and reported by Kline (in press 1999) as part of the SEA program conducted by the Prince William Sound Science Center.

Isotope Ratio Variations in Wild Harbor Seals

Tissue samples were collected and analyzed from over 200 harbor seals. Additionally, vibrissae samples were collected from over 100 harbor seals in Prince William Sound. Analyzed vibrissae from harbor seals are listed in Table 1 with the range and mean stable isotope ratios. The isotopic data for each vibrissa collected within Prince William Sound are shown in Appendix 3. These illustrate the $\delta^{13}C$ and $\delta^{15}N$ values at 2.5mm intervals along the lengths of the vibrissae. Vibrissae were collected during ADFG seal surveys in Prince William Sound and body tissues were collected by native subsistence hunters in cooperation with ADFG.

Based on the combined use of [ACH] averaged $\delta^{13}C$ and $\delta^{15}N$ values from vibrissae, harbor seals in Southeast Alaska and Prince William Sound are significantly different by region, $F_{4,98} = 6595.9$, $p = <0.001$. Harbor seals in Southeast Alaska and Kodiak are significantly different by region, $F_{4,62} = 5648.6$, $p = <0.001$. Harbor seals in Prince William Sound and Kodiak are significantly different by region, $F_{4,92} = 12555.5$, $p = <0.001$. Southeast Alaska seals, all from Frederick Sound, had a mean $\delta^{13}C = -18.1 \pm 0.2$ and a mean $\delta^{15}N = 16.2 \pm 0.2$. Prince William Sound seals had a mean $\delta^{13}C = -17.9 \pm 0.2$ and a mean $\delta^{15}N = 17.0 \pm 0.2$. Kodiak seals from the east and west sides of the island had a mean $\delta^{13}C = -16.5 \pm 0.2$ and a mean $\delta^{15}N = 17.3 \pm 0.2$. Both the ^{13}C and ^{15}N isotopes of harbor seals are increasingly enriched from Southeast Alaska westward to Kodiak. This enrichment may be the result of more nutrient-rich water in the western portion of the Gulf, allowing for larger, faster-growing phytoplankton nearshore. The Alaska Coastal Current may transport more nutrients as it travels westward along the Gulf coast of Alaska and the increased amount of nutrients would be available for western phytoplankton communities. These phytoplankton would have more enriched stable isotope ratios and these values would be incorporated and transferred through the food web so all organisms would reflect a greater enrichment (Laws et al. 1995)

Seals from 11 sites have been sampled in Prince William Sound (Fig. 5). The $\delta^{13}C$ and $\delta^{15}N$ values were averaged from the vibrissae for each seal and their values used for statistical analysis. Nine of the 11 sites are in close proximity to one another and were grouped for analysis. The two remaining locations in northeastern Prince William Sound were grouped together for analysis. Adult and subadult harbor seals from the 9 areas in southern Prince William Sound are significantly different by area, $F_{16,1896} = 19.1$, $p = <0.001$; sex $F_{2,955} = 11.1$, $p = <0.001$, and age $F_{4,1908} = 45,953$, $p = <0.001$. The two areas in northeastern Prince William Sound are significantly different from each other $F_{2,86} = 11.8$, $p = <0.001$. There is a significant difference in age $F_{4,170} = 9.4$, $p = <0.001$ but not between sexes. Further analyses were conducted individually for each of the 11 sampling areas. The stable isotope differences observed in seals from different locations appear to agree with some of the location differences defined by the fatty-acid analysis (Iverson et al. 1997). These differences may result from juveniles of a species being eaten in one region of the Sound while adults of the same species are eaten in another region.

Stable isotope ratios within harbor seal vibrissae do not appear to fluctuate greatly or with any regular periodicity, although some seals do show large changes between enriched and depleted values. Harbor seals sampled in 1993 had relatively constant $\delta^{13}C$ values and some fluctuations (<2‰) in $\delta^{15}N$ values that likely correspond to seasonal changes in primary prey type. The periodicity of the fluctuations in the 9 seals does not appear regular. Six of 10 seals sampled from southern PWS in the spring of 1994 had large, synchronous fluctuations, as large as 5.5‰, in $\delta^{13}C$ and $\delta^{15}N$. Two-thirds of the seals sampled in September 1994 had synchronous

fluctuations larger than 1‰ in $\delta^{13}C$ and $\delta^{15}N$ in at least one location along the length of the whisker. Six of the 12 whiskers analyzed in spring of 1995 and 6 out of 7 in fall of 1995 also had synchronous fluctuations larger than 1‰ in $\delta^{13}C$ and $\delta^{15}N$ in at least one location along the length of the whisker. A random sampling of seals in the spring and fall 1996 as well as summer 1997 and 1998 revealed that a majority of the animals had fluctuations greater than 1‰ in $\delta^{13}C$ and $\delta^{15}N$ (Table 1). The temporal patterns appear to depend on the region, season and year in which the seals were sampled.

The cause of these shifts is not currently known, but we hypothesized that prey outside Prince William Sound are more depleted in stable isotopes and that some seals may be foraging on the ^{13}C-depleted prey. Evidence for travel outside the sound is provided by satellite tag data (Frost and Lowry 1996). Prey data, e.g., from herring and pollock, from Dr. Tom Kline have shown very little isotopic variation among locations within the sound. However, Kline has found an isotopic gradient between *Neocalanus cristatus* from the northern Gulf of Alaska just south of Prince William Sound and *N. cristatus* within the Sound. An approximately 4‰ depletion exists in $\delta^{13}C$ of the calanoid copepods outside the sound relative to those within the sound (Kline 1997). Similar isotopic gradients of 2‰ have been identified by Schell et al. (1998) for zooplankton in the Bering Sea and Aleutian Islands, with onshelf waters being more enriched and deep water regions being more depleted in $\delta^{13}C$ and $\delta^{15}N$. Prey data collected south of Prince William Sound during the 1996 NMFS Gulf of Alaska survey revealed some evidence of an isotopic gradient in higher trophic organisms.

The isotope fluctuations in the seal vibrissae were separated into maximum and minimum values based on differences greater than 1‰ in $\delta^{13}C$ and $\delta^{15}N$, respectively. Vibrissae with fluctuations in $\delta^{13}C$ and $\delta^{15}N$ less than 1‰ had their isotope values averaged for the entire whisker. Because depleted isotope values are expected in pelagic food webs, the enriched values along the vibrissae are assumed to correspond to prey from the sound while the depleted values correspond to prey from the Gulf of Alaska. Because harbor seals tend to have strong site fidelity, it is thought that seals with constant isotope ratios forage near their haul-out sites in Prince William Sound (Pitcher and McAllister 1981).

Vibrissae analyzed by Hobson et al. (1996) from captive harp seals showed minor variation in the isotope ratios along their lengths that they suggested may have been due to natural isotopic variation in the animals. Our vibrissae growth rate study on captive harbor seals showed very little variation in the isotope ratios during the time in which they appeared to be growing but the fluctuations in their growth may be associated with metabolic changes in the seals relating to breeding and molting periods. The isotope ratios along the vibrissae continue to reflect assimilated prey but the time periods they reflect are in question. Differences in the vibrissae growth rates and the length of time they are retained are unknown for these two species; however, the harp seal study did provide range estimates for isotopic variation in wild seals feeding on a constant diet. These criteria were used above in order to interpret dietary changes based on isotopic fluctuations.

Table 1. Stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotope ratios in vibrissae of harbor seals from Prince William Sound, Kodiak and southeast Alaska. Age designation refers to adult (A), subadult (SA) , yearling (Y), and pup (P) seals.

Harbor Seal	Sample Date	Sex	Age	Range ^{13}C	^{13}C	Max./Min. ^{13}C	Range ^{15}N	^{15}N	Max./Min. ^{15}N
Harbor Seals - southeast Alaska, Gulf of Alaska									
HSA1SE	5 April 1993	F	P	-14.4 to -13.3	-13.8	-13.4 / -14.2	15.6 to 18.2	17.5	18.1 / 17.2
HSA2SE	5 April 1993	M	A	-17.6 to -14.1	-16.8	-14.1 / -17.1	14.4 to 18.5	15.3	16.1 / 14.5
HSA3SE	8 April 1993	M	A	-13.6 to -13.1	-13.3		17.4 to 18.1	17.8	
HSA4SE	9 April 1993	M	SA	-17.0 to -13.4	-14.7	-13.6 / -16.7	14.5 to 17.8	16.6	17.4 / 16.1
HSA5SE	9 April 1993	F	P	-14.6 to -13.9	-14.3		14.0 to 15.7	14.5	
HSA6SE	9 April 1993	F	SA	-14.2 to -13.5	-13.9		14.7 to 16.3	15.5	
HSA7SE	9 April 1993	M	A	-14.1 to -12.4	-13.4	-12.5 / -14.0	16.9 to 17.5	17.1	17.5 / 16.9
HSA8SE	9 April 1993	M	P	-14.1 to -13.0	-13.6	-13.0 / -13.8	14.1 to 17.9	16.9	18.0 / 15.3
HSA9SE	9 April 1993	F	A	-16.9 to -13.5	-15.4	-13.6 / -15.5	13.1 to 17.0	14.6	16.9 / 13.1
HSA10SE	Sept. 1993			-17.8 to -14.5	-16.8	-14.5 / -17.6	14.5 to 17.0	15.6	16.9 / 15.4
HSA11SE	Sept. 1993			-14.1 to -13.5	-13.7		17.8 to 18.6	18.2	
HSA12SE	Sept. 1993			-14.5 to -14.1	-14.3		15.1 to 16.1	15.4	
HSA13SE	Sept. 1993			-14.5 to -14.0	-14.2		15.5 to 17.2	16.1	
HSA14SE	Sept. 1993			-14.4 to -14.1	-14.2		14.9 to 15.4	15.2	
HSA15SE	Sept. 1993	M	A	-16.2 to -13.6	-14.3	-13.7 / -16.2	14.4 to 16.4	15.4	16.4 / 14.4
HSA16SE	Sept. 1993	M	A	-16.8 to -14.8	-16.1	-15.0 / -16.9	14.0 to 15.3	14.5	15.3 / 14.3
HSA17SE	Sept. 1993	M	SA	-17.8 to -14.0	-15.4	-14.1 / -17.6	13.5 to 16.0	14.8	15.8 / 13.7
HSA18SE	Sept. 1993	M	A	-14.3 to -12.9	-13.3	-13.1 / -14.2	16.5 to 17.9	17.2	17.8 / 16.5
HSA19SE	Sept. 1993	F	A	-14.6 to -14.0	-14.2		15.0 to 17.1	15.9	
HSA20SE	Sept. 1993	M	A	-14.5 to -13.5	-13.8	-13.5 / -14.5	14.6 to 16.0	15.3	16.0 / 14.6
HSB1SE	17 Aug. 1994	M	A	-18.0 to -14.6	-16.8	-14.6 / -18.0	14.2 to 16.9	14.9	16.9 / 14.2
HSB2SE	19 Aug. 1994	M	A	-18.1 to -14.8	-17.1	-14.8 / -18.0	13.7 to 16.2	14.5	16.2 / 13.8
HSB3SE	19 Aug. 1994	M	A	-17.7 to -16.0	-17.2	-16.0 / -17.7	13.8 to 15.8	14.6	15.8 / 13.8
HSB4SE	23 Aug. 1994	M	SA	-15.2 to -14.7	-14.8		15.6 to 16.6	16.1	
HSB5SE	23 Aug. 1994	F	A	-15.0 to -14.6	-14.8		15.9 to 17.2	16.4	
HSB6SE	23 Aug. 1994	F	A	-15.7 to -14.4	-14.9	-14.5 / -15.6	14.8 to 16.1	15.4	16.0 / 15.0

HSB7SE	19 Aug. 1994	F	A	-17.9 to -14.2	-17.1	-14.2 / -17.7	13.7 to 15.8	14.4	15.8 / 13.8
HSB8SE	24 Aug. 1994	F	SA	-15.9 to -14.6	-15.1	-14.8 / -15.7	15.0 to 16.2	15.6	16.2 / 15.3
HSB9SE	13 Sept. 1994	M	A	-16.1 to -13.5	-14.3	-13.7 / -16.1	15.3 to 17.3	16.6	17.3 /15.3
HSB10SE	13 Sept. 1994	F	A	-16.2 to -14.0	-14.9	-14.0 / -16.2	14.8 to 16.2	15.7	16.0 / 14.8
HSB11SE	13 Sept. 1994	M	A	-17.6 to -13.8	-15.5	-13.8 / -17.5	14.4 to 17.8	15.9	17.7 / 14.4
HSB12SE	13 Sept. 1994	M	A	-17.5 to -13.9	-15.0	-14.0 / -17.4	14.3 to 16.3	15.5	16.3 / 14.3
HSB13SE	13 Sept. 1994	F	SA	-17.7 to -16.3	-17.0	-16.3 / -17.5	13.8 to 15.6	14.4	15.5 / 13.9
HSB14SE	13 Sept. 1994	F	P	-14.8 to -14.1	-14.4		15.2 to 15.9	15.7	
HSB15SE	13 Sept. 1994	M	A	-17.3 to -14.3	-15.3	-14.4 / -16.9	14.0 to 17.6	15.8	17.6 / 14.0
HSB16SE	13 Sept. 1994	F	A	-17.6 to -15.3	-16.8	-15.3 / -17.6	14.5 to 15.9	15.0	15.9 / 14.5
HSB17SE	13 Sept. 1994	M	A	-17.1 to -13.9	-14.8	-14.0 / -17.1	14.7 to 17.7	16.7	17.7 / 14.7
HSC1SE	19 April 1995	M	A	-15.8 to -13.3	-14.1	-13.3 / -15.8	14.9 to 17.5	16.3	17.5 / 14.9
HSC2SE	19 April 1995	M	A	-17.2 to -14.1	-15.3	-14.1 / -17.2	14.7 to 18.0	16.5	18.0 / 14.7
HSC3SE	19 April 1995	F	A	-16.5 to -13.9	-14.8	-14.1 / -16.5	14.8 to 16.2	15.7	16.2 / 14.9
HSC4SE	19 April 1995	F	SA	-15.6 to -14.1	-14.6	-14.1 / -15.6	14.5 to 15.8	15.1	15.8 / 14.6
HSC5SE	19 April 1995	M	A	-17.7 to -16.4	-17.1	-16.4 / -17.7	14.5 to 16.1	15.2	16.0 / 14.5
HSC6SE	19 April 1995	M	A	-17.8 to -13.9	-16.0	-14.0 / -17.8	14.8 to 17.9	15.9	17.9 / 14.9
HSC7SE	19 April 1995	F	SA	-15.1 to -14.0	-14.3	-14.0 / -15.1	14.7 to 16.3	15.5	16.1 / 15.0
HSC8SE	19 April 1995	M	A	-17.8 to -13.8	-16.7	-13.8 / -17.8	14.3 to 16.6	15.2	16.4 / 14.3
HSC9SE	19 April 1995	F	SA	-15.2 to -13.8	-14.3	-13.8 / -15.2	14.7 to 15.3	15.0	15.3 / 14.7
HSC10SE	19 April 1995	M	A	-17.2 to -13.4	-14.5	-13.4 / -17.2	14.3 to 18.6	16.7	18.6 / 14.3
HSC11SE	20 April 1995	M	A	-17.4 to -14.4	-15.7	-14.6 / -17.4	14.4 to 18.4	16.2	17.8 / 14.9
HSC12SE	20 April 1995	M	A	-14.1 to -13.2	-13.8		17.8 to 19.1	18.2	
HSC13SE	20 April 1995	M	A	-18.1 to -13.9	-15.9	-14.0 / -18.1	14.7 to 17.7	16.3	17.6 / 15.4
HSC14SE	20 April 1995	M	SA	-14.1 to -13.2	-13.6		16.2 to 17.5	16.9	
HSC15SE	21 April 1995	M	A	-17.3 to -13.4	-14.8	-13.4 / -17.3	16.4 to 17.6	17.1	17.3 / 16.4
HSC16SE	21 April 1995	M	A	-17.0 to -13.4	-14.9	-13.4 / -17.0	15.1 to 16.0	15.6	16.0 / 15.1
HSC17SE	21 April 1995	F	A	-18.1 to -13.5	-16.4	-13.5 / -18.0	14.2 to 17.0	15.2	17.0 / 14.2
HSC18SE	21 April 1995	M	A	-14.9 to -14.2	-14.6		17.6 to 18.1	17.8	
HSC19SE	21 April 1995	F	A	-17.9 to -14.3	-15.4	-14.5 / -17.8	14.2 to 15.5	14.7	15.3 / 14.3
HSC20SE	21 Sept. 1995	M	A	-14.9 to -14.0	-14.3		15.5 to 17.1	16.6	
HSC21SE	21 Sept. 1995	M	A	-16.1 to -14.0	-14.6	-14.0 / -16.1	14.9 to 16.6	16.2	16.7 / 14.9

HSC22SE	21 Sept. 1995	F	A	-14.4 to -14.2	-14.3		16.7 to 17.0	16.8	
HSC23SE	21 Sept. 1995	M	A	-16.0 to -14.0	-14.6	-14.1 / -16.0	14.9 to 17.2	16.5	17.2 / 14.9
HSC24SE	22 Sept. 1995	F	SA	-15.7 to -14.4	-14.7	-14.4 / -15.7	15.0 to 15.8	15.3	15.8 / 15.0
HSC25SE	22 Sept. 1995	F	A	-14.6 to -14.1	-14.3		15.7 to 16.9	16.2	
HSC26SE	22 Sept. 1995	M	A	-17.4 to -15.6	-16.6	-15.8 / -17.3	14.3 to 15.5	14.8	15.4 / 14.4
HSC27SE	22 Sept. 1995	M	A	-14.5 to -13.7	-14.0		15.6 to 16.5	16.0	
HSC28SE	22 Sept. 1995	F	P	-15.0 to -13.6	-14.1	-13.7 / -14.9	16.3 to 18.3	17.3	18.3 / 16.3
Harbor Seals - Prince William Sound									
HSA1PWS	7 May 1993	M	A	-14.8 to -13.9	-14.5		18.1 to 19.5	18.8	
HSA2PWS	7 May 1993	F	SA	-16.2 to -14.8	-15.4	-15.0 / -16.2	15.3 to 19.0	17.7	19.0 / 15.3
HSA3PWS	7 May 1993	M	A	-15.8 to -14.8	-15.2		17.3 to 17.9	17.5	
HSA4PWS	7 May 1993	M	A	-16.5 to -15.0	-15.9	-15.0 / -16.4	15.8 to 17.9	16.7	17.8 / 15.9
HSA5PWS	7 May 1993	F	SA	-16.0 to -15.4	-15.8	-17.0 / -17.9	15.8 to 18.8	17.1	15.8 / 14.2
HSA6PWS	8 May 1993	F	SA	-16.4 to -15.0	-15.9		15.4 to 16.7	16.1	
HSA7PWS	8 May 1993	F	A	-16.4 to -15.2	-15.7		15.8 to 16.7	16.2	
HSA8PWS	8 May 1993	M	SA	-15.7 to -15.2	-15.4		15.5 to 17.9	16.4	
HSA9PWS	8 May 1993	M	A	-15.3 to -14.7	-15.0		16.9 to 18.7	17.5	
HSA10PWS	8 May 1993	M	SA	-15.6 to -15.1	-15.3		18.1 to 19.2	18.6	
HSA11PWS	9 May 1993	M	A	-15.1 to -14.7	-14.9		16.3 to 17.9	16.8	
HSA12PWS	9 May 1993	M	SA	-15.2 to -14.2	-14.6		16.1 to 19.3	18.5	
HSA13PWS	9 May 1993	F	SA	-16.3 to -16.0	-16.1		15.8 to 17.1	16.4	
HSB1PWS	26 April 1994	F	SA	-17.1 to -15.7	-16.4		14.7 to 16.7	15.8	
HSB2PWS	27 April 1994	M	SA	-16.6 to -15.7	-16.2		15.2 to 17.3	16.1	
HSB3PWS	27 April 1994	F	A	-16.5 to -12.6	-14.6	-12.6 / -16.3	13.4 to 18.0	16.0	17.8 / 13.8
HSB4PWS	27 April 1994	M	SA	-16.2 to -15.3	-16.1		15.8 to 16.6	16.1	
HSB5PWS	27 April 1994	M	A	-17.9 to -17.0	-17.5		14.0 to 15.9	14.9	
HSB6PWS	28 April 1994	M	A	-17.6 to -15.8	-16.6	-16.0 / -17.5	13.3 to 16.2	15.0	15.8 / 13.5
HSB7PWS	28 April 1994	F	A	-17.8 to -12.5	-15.2	-12.7 / -17.6	13.7 to 17.4	15.6	17.1 / 13.7
HSB8PWS	28 April 1994	M	SA	-17.7 to -15.5	-16.3	-15.6 / -17.6	13.7 to 16.9	15.6	16.7 / 13.9
HSB9PWS	28 April 1994	M	SA	-18.1 to -16.4	-17.1	-16.5 / -18.0	13.6 to 16.8	15.4	16.7 / 13.8
HSB10PWS	28 April 1994	M	A	-17.7 to -14.5	-15.8	-14.8 / -17.7	15.2 to 17.8	16.2	17.4 / 13.8

HSB11PWS	18 Sept. 1994	F	A	-17.9 to -16.3	-17.1	-16.6 / -17.6	14.7 to 17.1	15.4	17.0 / 14.7
HSB12PWS	18 Sept. 1994	F	SA	-16.9 to -15.8	-16.2		16.1 to 17.2	16.7	
HSB13PWS	18 Sept. 1994	M	SA	-16.8 to -15.8	-16.1		15.5 to 16.1	15.8	
HSB14PWS	18 Sept. 1994	M	A	-17.2 to -16.1	-16.6	-16.2 / -17.1	14.8 to 16.1	15.4	16.0 / 14.8
HSB15PWS	18 Sept. 1994	F	SA	-17.0 to -13.2	-15.2	-13.6 / -16.6	15.8 to 18.9	17.7	18.8 / 15.9
HSB17PWS	18 Sept. 1994	M	SA	-16.6 to -15.5	-15.9		15.5 to 16.3	15.9	
HSB18PWS	18 Sept. 1994	M	SA	-16.6 to -16.1	-16.3		15.6 to 17.0	16.2	
HSB19PWS	18 Sept. 1994	M	SA	-16.8 to -16.2	-16.4		15.5 to 16.5	16.0	
HSB20PWS	18 Sept. 1994	F	SA	-16.5 to -15.8	-16.1		16.1 to 17.2	16.7	
HSB21PWS	18 Sept. 1994	M	SA	-17.0 to -14.5	-15.8	-13.5 / -18.2	15.6 to 17.1	16.4	17.1 / 15.6
HSB22PWS	18 Sept. 1994	M	SA	-18.2 to -13.5	-15.1	-13.5 / -18.2	15.4 to 19.2	17.5	19.1 / 15.5
HSB23PWS	18 Sept. 1994	M	A	-17.9 to -16.2	-17.6	-16.4 / -17.8	14.0 to 15.4	14.4	15.3 / 14.3
HSB24PWS	19 Sept. 1994	F	A	-16.1 to -15.6	-15.9		15.6 to 16.8	16.3	
HSB25PWS	19 Sept. 1994	F	P	-17.5 to -14.2	-15.1	-14.3 / -17.3	16.6 to 17.9	17.1	17.3 / 16.2
HSB26PWS	19 Sept. 1994	M	SA	-16.5 to -16.0	-16.3		15.0 to 16.6	15.5	
HSB27PWS	22 Sept. 1994	F	A	-17.3 to -13.9	-15.4	-14.1 / -17.3	14.9 to 17.5	16.4	17.4 / 14.9
HSB28PWS	22 Sept. 1994	M	A	-17.5 to -15.6	-16.4	-15.7 / -17.3	14.6 to 16.4	15.7	16.4 / 14.7
HSB29PWS	22 Sept. 1994	M	P	-16.7 to -15.2	-15.9	-15.2 / -16.5	17.5 to 19.2	18.4	19.0 / 17.5
HSB30PWS	22 Sept. 1994	F	A	-17.8 to -15.2	-16.9	-15.6 / -17.6	14.5 to 16.8	15.2	16.5 / 14.7
HSB31PWS	22 Sept. 1994	F	SA	-17.7 to -16.1	-16.8	-16.1 / -17.6	14.6 to 16.7	15.7	16.5 / 14.5
HSB32PWS	22 Sept. 1994	F	A	-17.8 to -13.8	-16.1	-14.0 / -17.9	14.3 to 17.1	15.5	17.0 / 14.4
HSB33PWS	22 Sept. 1994	F	SA	-17.8 to -14.3	-16.4	-14.3 / -17.5	14.7 to 16.6	15.9	16.4 / 14.8
HSB34PWS	22 Sept. 1994	M	A	-17.2 to -14.4	-15.3	-14.4 / -17.0	14.7 to 17.2	16.0	17.0 / 14.9
HSB35PWS	22 Sept. 1994	F	A	-18.1 to -15.6	-16.8	-15.6 / -18.0	15.0 to 17.4	15.9	17.4 / 15.1
HSB36PWS	22 Sept. 1994	M	A	-17.9 to -16.8	-17.6	-16.9 / -17.9	14.5 to 16.2	15.1	16.2 / 14.5
TAHS1PWS	27 Sept. 1994	F	SA	-18.1 to -16.7	-17.5	-16.8 / -18.0	14.4 to 17.8	15.8	17.8 / 14.9
TAHS3PWS	29 Sept. 1994	F	A	-17.5 to -15.5	-17.0	-15.6 / -17.4	14.3 to 17.1	14.9	17.1 / 14.7
TAHS4PWS	30 Sept. 1994	M	A	-16.4 to -16.1	-15.6		16.1 to 18.7	17.3	
TAHS5PWS	30 Sept. 1994	M	A	-17.9 to -15.7	-16.4	-15.8 / -17.7	14.4 to 16.1	15.6	16.2 / 14.5
TAHS6PWS	1 Oct. 1994	F	P	-17.8 to -16.1	-16.5	-15.5 / -17.7	16.0 to 18.3	16.8	17.5 / 14.5
TAHS7PWS	1 Oct. 1994	M	P	-17.8 to -14.9	-15.7	-15.3 / -17.4	14.3 to 19.8	17.5	19.5 / 14.8
HSC1PWS	9 May 1995	M	SA	-17.3 to -15.5	-16.1	-15.5 / -16.9	15.3 to 17.6	16.3	17.3 / 15.4

HSC2PWS	9 May 1995	M	SA	-17.5 to -13.4	-14.9	-13.5 / -17.5	14.6 to 20.0	17.9	19.9 / 14.6
HSC3PWS	9 May 1995	M	SA	-16.3 to -15.0	-15.4	-15.0 / -15.9	16.4 to 19.5	18.4	19.5 / 16.4
HSC4PWS	9 May 1995	M	SA	-17.5 to -16.1	-16.6	-16.4 / -17.4	14.1 to 17.2	15.8	16.4 / 14.5
HSC5PWS	9 May 1995	M	SA	-17.5 to -15.6	-16.2	-15.6 / -17.5	14.6 to 16.9	15.8	16.9 / 14.8
HSC6PWS	11 May 1995	F	SA	-17.2 to -15.4	-16.2	-15.5 / -17.0	15.3 to 16.8	16.1	16.8 / 15.3
HSC7PWS	11 May 1995	M	SA	-17.6 to -15.1	-16.2	-15.2 / -17.5	14.2 to 16.9	15.7	16.8 / 14.3
HSC8PWS	11 May 1995	F	A	-17.8 to -14.3	-16.4	-14.4 / -17.8	14.1 to 16.8	15.3	16.6 / 14.4
HSC9PWS	11 May 1995	F	SA	-18.0 to -15.1	-15.9	-15.2 / -17.9	16.5 to 18.8	17.8	18.6 / 16.5
HSC10PWS	11 May 1995	M	SA	-17.2 to -12.8	-14.1	-12.9 / -17.1	16.0 to 18.9	17.9	18.7 / 16.1
HSC11PWS	11 May 1995	F	SA	-15.0 to -13.7	-14.3		16.7 to 17.3	17.0	
HSC12PWS	11 May 1995	M	A	-16.5 to -16.0	-16.1		15.6 to 16.9	16.2	
HSC13PWS	12 May 1995	F	SA	-17.1 to -15.0	-16.4	-15.0 / -17.0	15.6 to 17.0	16.1	16.7 / 15.8
HSC14PWS	12 May 1995	M	A	-16.5 to -15.0	-15.5	-15.0 / -16.5	16.7 to 18.1	17.7	18.0 / 16.7
HSC15PWS	12 May 1995	M	A	-17.7 to -15.6	-16.3	-16.0 / -17.7	14.9 to 16.9	16.2	16.8 / 14.9
HSC16PWS	12 May 1995	M	A	-17.4 to -16.0	-16.5	-16.1 / -17.3	14.6 to 16.3	15.7	16.3 / 14.6
HSC17PWS	12 May 1995	F	SA	-17.3 to -16.0	-16.5	-16.3 / -17.3	15.0 to 16.5	15.7	16.3 / 15.1
HSC18PWS	12 May 1995	M	SA	-17.4 to -16.1	-16.5	-16.2 / -17.5	15.0 to 16.7	15.9	16.6 / 15.0
HSC19PWS	12 May 1995	F	SA	-17.0 to -15.4	-15.9	-15.6 / -17.0	15.6 to 16.7	15.9	16.7 / 15.6
HSC20PWS	13 May 1995	F	A	-16.8 to -13.3	-15.1	-13.5 / -16.7	15.5 to 17.3	16.3	17.2 / 15.5
HSC21PWS	13 May 1995	F	SA	-17.5 to -14.8	-16.0	-14.8 / -17.4	15.7 to 18.5	17.5	18.4 / 15.8
HSC22PWS	13 May 1995	M	SA	-17.9 to -15.8	-16.2	-15.8 / -17.9	16.1 to 18.7	17.5	18.7 / 16.2
HSC23PWS	Sept. 1995	F	SA	-17.3 to -16.4	-16.7	-16.4 / -17.3	15.1 to 18.6	16.6	17.7 / 15.8
HSC24PWS	Sept. 1995	F	P	-17.2 to -15.7	-14.9	-15.8 / -16.6	15.9 to 16.9	16.4	16.9 / 16.0
HSC26PWS	Sept. 1995	F	A	-17.3 to -15.4	-16.2	-15.5 / -17.2	13.7 to 16.5	15.3	15.8 / 13.8
HSC27PWS	Sept. 1995	F	A	-17.7 to -16.2	-17.1	-16.5 / -17.3	13.5 to 17.3	15.1	16.7 / 13.6
HSC37PWS	Sept. 1995	M	A	-17.8 to -15.1	-16.7	-15.3 / -17.7	13.6 to 17.9	15.3	17.6 / 13.9
HSC41PWS	Sept. 1995	M	SA	-17.3 to -15.4	-16.2	-15.5 / -17.2	15.6 to 17.7	16.1	17.7 / 15.9
HSC42PWS	Sept.1995	M	SA	-17.6 to -14.9	-15.7	-14.9 / -17.6	14.4 to 18.4	17.3	18.4 / 14.4
HSD2PWS	April 1996	F	SA	-17.6 to -15.7	-16.5	-15.8 / -17.3	14.8 to 15.8	15.3	15.7 / 14.8
HSD3PWS	April 1996	F	SA	-17.7 to -16.0	-16.6	-16.1 / -17.7	15.3 to 16.9	16.0	16.7 / 15.3
HSD4PWS	April 1996	F	Y	-17.7 to -15.3	-16.0	-15.3 / -17.7	15.3 to 19.4	18.0	19.4 / 15.3
HSD5PWS	April 1996	M	A	-16.7 to -14.9	-15.4	-15.0 / -16.7	15.2 to 17.3	16.5	17.3 / 15.3

HSD12PWS	April 1996	F	A	-17.7 to -12.3	-15.5	-12.3 / -17.7	13.7 to 16.9	15.1	16.9 / 13.7
HSD13PWS	April 1996	M	A	-16.9 to -15.1	-15.8	-15.2 / -16.9	14.7 to 17.7	16.3	17.5 / 14.7
HSD21PWS	May 1996	M	SA	-18.1 to -15.4	-16.2	-15.5 / -17.1	15.4 to 19.8	18.1	19.5 / 15.4
HSD22PWS	May 1996	M	A	-17.6 to -15.6	-16.2	-16.0 / -17.6	14.2 to 17.0	15.9	16.9 / 14.2
HSD29PWS	Sept. 1996	M	SA	-16.5 to -15.0	-15.6	-15.0 / -15.8	15.3 to 17.5	16.3	17.3 / 15.4
HSD30PWS	Sept. 1996	F	A	-16.8 to -15.7	-16.2	-15.8 / -16.6	14.3 to 16.6	14.4	16.5 / 14.5
HSD35PWS	Sept. 1996	F	SA	-15.9 to -14.9	-15.3		15.6 to 16.2	15.9	
HSD36PWS	Sept. 1996	M	SA	-16.4 to -15.3	-15.8		15.5 to 17.4	16.2	
HSE1PWS	June 1997	F	P	-16.4 to -15.0	-15.8	-15.0 / -16.2	16.4 to 18.8	17.6	18.8 / 16.4
HSE15PWS	June 1997	M	A	-16.3 to -15.2	-15.9		15.1 to 16.2	15.6	
HSE16PWS	June 1997	F	SA	-15.4 to -13.6	-14.7	-13.8 / -15.3	15.6 to 17.7	17.0	17.5 / 15.6
HSE25PWS	June 1997	F	A	-17.0 to -12.3	-15.9	-12.3 / -16.9	14.1 to 16.8	15.0	16.8 / 14.1
HSE27PWS	June 1997	F	SA	-15.9 to -14.5	-15.3	-14.5 / -15.9	15.9 to 16.9	16.3	16.9 / 15.9
HSE32PWS	June 1997	F	P	-15.5 to -14.2	-15.0	-14.3 / -15.4	17.1 to 18.7	17.7	18.7 / 17.2
HSE33PWS	June 1997	F	A	-17.1 to -13.2	-15.6	-13.2 / -17.1	14.7 to 17.4	15.9	17.4 / 14.8
HSE49PWS	July 1997	F	A	-15.6 to -14.0	-15.0	-14.0 / -15.3	15.6 to 16.8	16.1	16.8 / 15.6
HSE50PWS	July 1997	M	A	-15.9 to -15.2	-15.5		15.3 to 16.1	15.6	
HSF1PWS	June 1998	F	Y	-16.9 to -15.9	-16.4		15.7 to 16.8	15.9	
HSF3PWS	June 1998	F	Y	-16.0 to -15.0	-15.6		17.3 to 18.2	17.8	
HSF5PWS	June 1998	F	A	-17.0 to -15.2	-16.2	-15.2 / -17.0	15.5 to 17.2	16.4	17.1 / 15.5
HSF21PWS	June 1998	M	Y	-16.7 to -15.7	-16.0		15.9 to 16.5	16.3	
HSF22PWS	June 1998	F	A	-16.9 to -15.4	-16.3	-15.4 / -16.9	14.4 to 17.3	15.7	17.3 / 14.4
HSF27PWS	June 1998	F	A	-17.6 to -14.7	-16.4	-14.7 / -17.5	14.3 to 16.5	15.3	16.5 / 14.4
HSF28PWS	June 1998	F	P	-16.2 to -15.3	-15.5		16.9 to 18.4	17.4	
HSF33PWS	June 1998	F	P	-15.5 to -13.7	-14.9	-13.7 / -15.5	16.8 to 19.3	17.7	19.3 / 16.8
HSF34PWS	June 1998	F	A	-16.4 to -14.4	-15.5	-14.4 / -16.4	15.9 to 17.1	16.4	17.0 / 15.9
HSF39PWS	June 1998	F	Y	-16.9 to -15.9	-16.4		15.7 to 16.8	15.9	
Harbor Seals - Kodiak, Gulf of Alaska									
HSA1KO	22 April 1993	F	A	-14.4 to -13.3	-13.8		15.6 to 16.2	16.0	
HSA2KO	24 April 1993	F	A	-14.6 to -13.3	-14.0		16.7 to 17.2	16.9	
HSA4KO	26 April 1993	F	SA	-15.0 to -14.0	-14.5		16.1 to 17.4	17.0	

HSA5KO	2 Oct. 1993	F	A	-14.3 to -13.8	-14.0		16.3 to 17.3	16.9	
HSB1KO	5 Oct. 1994	M	A	-15.1 to -13.5	-14.2		16.9 to 18.3	17.8	
HSB2KO	5 Oct. 1994	M	A	-15.5 to -14.1	-14.7		16.7 to 18.0	17.3	
HSB3KO	5 Oct. 1994	M	A	-14.4 to -12.8	-13.8		18.0 to 19.1	18.4	
HSB4KO	6 Oct. 1994	F	SA	-15.3 to -14.3	-14.8		15.5 to 18.8	17.2	
HSB5KO	6 Oct. 1994	F	P	-14.2 to -13.6	-13.7		17.7 to 18.9	18.3	
HSB6KO	6 Oct. 1994	M	SA	-17.9 to -13.8	-14.7	-13.8 / -15.9	15.4 to 18.1	17.6	18.1 / 16.2
HSB7KO	7 Oct. 1994	M	A	-15.6 to -14.3	-14.7		16.5 to 17.7	17.4	
HSB8KO	8 Oct. 1994	M	SA	-17.5 to -15.3	-16.0	-15.5 / -17.2	14.5 to 16.9	15.8	16.7 / 14.7
HSB9KO	8 Oct. 1994	F	SA	-16.5 to -15.7	-16.0		15.5 to 17.4	16.4	
HSB10KO	8 Oct. 1994	M	SA	-14.1 to -13.0	-13.7	-13.0 / -14.1	17.1 to 18.9	18.0	18.8 / 17.1
HSC1KO	29 Mar. 1995	M	A	-15.9 to -14.7	-15.2		15.7 to 16.6	16.1	
HSC2KO	29 Mar. 1995	F	SA	-15.7 to -15.0	-15.3		15.5 to 17.4	16.1	
HSC3KO	29 Mar. 1995	F	A	-17.3 to -14.9	-15.8	-15.1 / -16.6	14.3 to 16.5	15.5	16.2 / 14.9
HSC4KO	29 Mar. 1995	M	SA	-16.3 to -13.1	-13.9	-13.4 / -16.3	15.7 to 18.5	17.4	17.6 / 15.7
HSC5KO	29 Mar. 1995	F	SA	-16.4 to -15.4	-15.8	-15.5 / -16.5	15.0 to 16.5	15.7	15.9 / 15.0
HSC6KO	29 Mar. 1995	M	SA	-17.2 to -16.7	-17.0		15.6 to 16.2	15.8	
HSC9KO	9 Oct. 1995	F	SA	-16.4 to -14.6	-15.1	-14.7 / -16.2	14.9 to 16.6	15.6	16.5 / 14.9
HSC10KO	9 Oct. 1995	M	P	-15.3 to -13.8	-14.8	-13.8 / -15.3	16.5 to 20.0	18.1	20.0 / 16.5
HSC11KO	9 Oct. 1995	M	A	-17.1 to -13.9	-15.3	-13.9 / -17.1	14.6 to 17.9	16.3	17.9 / 14.6
HSC12KO	9 Oct. 1995	F	A	-15.5 to -14.0	-14.7	-14.0 / -15.3	15.5 to 17.2	16.4	17.1 / 15.6
HSC16KO	10 Oct. 1995	F	A	-14.6 to -13.6	-14.0		16.6 to 17.3	17.0	
HSC17KO	10 Oct. 1995	M	A	-15.7 to -13.9	-14.5	-14.0 / -15.6	16.1 to 18.9	18.0	18.9 / 16.1

Isotopic Fractionation in Harbor Seal Tissues

Harbor seal tissues were collected with the assistance of the Alaska Department of Fish and Game and Native subsistence hunters. Multiple tissues were collected from each animal to identify the isotope fractionation that occurs among different tissues during assimilation of food and tissue synthesis. Stable isotope values for muscles tend to most accurately reflect the average stable isotope ratios for the whole animal (DeNiro and Epstein 1978). Tissue samples, which had to include muscle, were taken from 60 harbor seals killed by subsistence hunters in Ketchikan, Sitka and Prince William Sound for more than two years. The $\delta^{13}C$ and $\delta^{15}N$ fractionation values were calculated as the difference in isotope ratios of each tissue from muscle in the same animal (Figures 6 and 7). Table 2 lists the fractionation values relative to muscle tissue for 11 tissues collected from harbor seals. No significant differences could be found in $\delta^{15}N$ and that may be the result of multiple prey sources/multiple trophic levels in an individual diet. The $\delta^{13}C$ of blubber and vibrissae significantly differed from muscle. The fractionation differences for $\delta^{13}C$ range with increasing enrichment with blubber < brain < skin < collagen, kidney, liver, heart, blood < fur < vibrissae. The sample size for lung tissue was too small to test for significance.

These fractionation values are similar to those obtained in previous studies on gerbils (Tiezsen et al. 1983) and another study we conducted on captive harp seals (Hobson et al. 1996). The harp seal study was conducted using captive seals held on a consistent diet of herring and vitamin supplements for at least two years whereas this current study dealt with wild seals undoubtedly having a mixed diet. Vibrissae and fur from the seals have a similar biochemical composition and show more enrichment in $\delta^{13}C$ than the more metabolically active tissues much like Hobson et al. (1996) observed in the captive seals and Tiezsen and Boutton (1988) observed in gerbil fur. Hobson et al. (1996) also observed a relatively constant enrichment in $\delta^{15}N$, within 1‰, for all tissues except blood which is consistent with these findings. Tissues having the largest amount of lipid also had the greatest fractionation in $\delta^{13}C$ from muscle. This is to be expected because lipid synthesis discriminates against the incorporation of the heavier isotope, ^{13}C (DeNiro and Epstein 1978).

The food web analyses previously described for wild seals indicated that multiple food sources were likely and may have consisted of different isotope ratios. We have also shown that prey from different regions (i.e. inside vs outside PWS) appeared to have different isotope ratios as well. Because of the often unknown isotopic variations and metabolic activity in wild seal tissues, fractionation values relative to muscle tissue may be better to use to predict dietary isotopic values. Further studies on fractionation determination for all pinnipeds are recommended using both captive and wild animals for comparison.

Table 2. $\delta^{13}C$ and $\delta^{15}N$ fractionation of harbor seal tissues relative to muscle using least square means. Harbor seals were collected in Ketchikan, Sitka, and Prince William Sound, Alaska, 1995 - 1997.

Tissue	n	^{13}C Mean,	SE	^{15}N Mean,	SE
blood, whole	5	0.40	0.22	-0.29	0.29
blubber	53	-5.99	0.26	--	--
brain	31	-1.04	0.30	0.52	0.10
collagen	24	-0.39	0.20	0.31	0.07
fur	39	0.69	0.16	-1.0	0.18
heart	27	-0.06	0.12	0.40	0.08
kidney	33	-0.38	0.17	0.38	0.11
liver	37	-0.24	0.21	0.25	0.10
lung	2	-0.68	0.06	0.65	0.20
skin	40	-1.83	0.24	0.86	0.12
vibrissae	14	1.90	0.72	0.10	0.67

Temporal Variation in ^{13}C of Pinnipeds

The differences observed in the $\delta^{13}C$ of harbor seals between 1975 and 1995 (Schell and Hirons 1997) were further defined in seals and sea lions dating back to 1951, and the results were submitted as a manuscript (Appendix 1). Harbor seal, Steller sea lion, and northern fur seal skeletons archived at the University of Alaska Museum and the Kodiak Historical Society had collagen extracted from bone samples free of humus and tissues. No significant change was found in the $\delta^{15}N$ values for the 47 years for which samples were available. This does not mean that prey variability did not occur but that likely the predominant prey in the diets of these phocids and otariids were consistently from the same trophic level. A significant decline in the $\delta^{13}C$ of approximately 2l in Steller sea lions took place during this time while no significant change in harbor seals or fur seals could be detected.

This decrease in $\delta^{13}C$ over time, with no accompanying change in $\delta^{15}N$, suggests an environmental change affecting the base of the food web rather than a trophic level change due to prey switching. The carbon isotope ratios in pinnipeds result from the carbon composition in phytoplankton in the food webs and a decline in productivity can lead to a decrease in $\delta^{13}C$ (Laws et al. 1995; Bidigare et al. 1997). A decrease in the winter mixed depth layer (Freeland et al. 1997) and increasing stability in the water column would reduce the available nutrients to the phytoplankton, and hence, productivity. Foraging at a lower trophic level or switching from a benthic diet to a more pelagic diet can also decrease the $\delta^{13}C$ but no evidence exists for either scenario (Hobson and Welch 1992; France and Peters 1997). The timing of this shift corresponds with the time of other observed changes in the physical and biological environment from the North Pacific Ocean (Ebbesmeyer et al. 1991, Trenberth and Hurrell 1994). The decline in the $\delta^{13}C$ of the pinniped bone collagen occurred during the same period that Schell (in prep.) observed declining $\delta^{13}C$ values in bowhead whales in the Bering Sea. These combined data may

be indicators that the carrying capacity of the North Pacific Ocean has declined since the 1960s. The reasons for this change in the $\delta^{13}C$ are not yet known but likely result from changes in the physical environment.

Growth Rates in Pinniped Vibrissae

Captive adult harbor seals and Steller sea lions were given $\delta^{13}C$- and $\delta^{15}N$-labeled glycine to act as a marker in all vibrissae on the animals. The peaks created by the large quantity of stable isotopes acted as temporal markers and growth rates were calculated between time periods. Harbor seals showed seasonal growth in the vibrissae, growing rapidly in early spring prior to the onset of breeding and then having little or no growth for the remainder of the year. There is now some evidence that harbor seals may rapidly replace some or all of their vibrissae annually during the molting period. This is in contrast to Steller sea lion vibrissae which appear to grow continually throughout the year and are maintained for several years. These data have been compiled into a manuscript as Appendix 2.

CONCLUSIONS

Geographic Gradients in Isotope Ratios: Our zooplankton sampling and that of Kline (in press 1999) show that isotope ratios for both carbon and nitrogen decreased markedly in the Gulf of Alaska with increasing distance from shore. Calanoid copepods showed a decrease of approximately 2-3 l in $\delta^{15}N$ between Prince William Sound and pelagic waters of the Gulf of Alaska. Figures 1 and 2 show the isotopic contours constructed from the available data for copepods. Data for euphausiids showed much more variability, which may reflect omnivory by these organisms. Values for $\delta^{13}C$ ranged from approximately -22l to near -20l with little evidence of offshore-onshore trends. The decrease in isotope ratios with distance offshore is evident in the vibrissae of Steller sea lions and a small number of individual harbor seals, indicating movements into offshore feeding areas during part of the annual cycle. These isotope data are consistent with radio-tracking data that showed relatively limited movements by most harbor seals but occasional extensive movements into the Gulf of Alaska by some individuals.

Harbor Seal Trophic Energetics: Harbor seal tissues have been analyzed to identify the isotopic fractionation that occurs among differing tissues. These data will be useful in establishing the average isotopic makeup of particular harbor seals where available tissues are limited. Large fluctuations in some harbor seal vibrissae were compared with food webs in and outside Prince William Sound. The fluctuations indicate that the seals are relying upon more than one food web, shifting between pelagic vs. benthic or Prince William Sound vs. Gulf of Alaska. With the data available, we are uncertain as to the definitive causes for these fluctuations.

Captive Seal Growth Studies: Data from the captive harbor seal at Mystic MarineLife Aquarium indicate the $\delta^{15}N$-labeled glycine is an effective marker in the vibrissae for the growth rate studies. Because of the much larger carbon and $\delta^{13}C$ content in organisms, $\delta^{13}C$-labeled glycine is much less effective as a marker for a given weight of label. Only if carbon reallocation information is essential would the much higher experimental cost be warranted. Growth rate data have been

calculated and contrasted with growth rates from a wild harbor seal, captive Steller sea lions, and wild Steller sea lions. Growth rates in the captive harbor seal may surpass those of wild phocids and otariids and captive otariids, but the limited experimental markings over the annual cycle make this conclusion tentative. The growth rate experiments are ongoing and will be continued at the Alaska Sealife Center. If the data on the two labeled seals are representative, harbor seals have faster whisker growth, which is tied to seasons and may even reflect annual replacement. In contrast, the data from the Steller sea lions showed a slower growth rate that continued over multiple years in the whisker.

Temporal Indications of Ecosystem Change: Comparisons of archived and modern seal and sea lion tissues indicate that a decrease in $\delta^{13}C$ has taken place over the past 47 years, although the scatter in the data prevents more precise timing of the shift. This shift is concurrent with other observed changes in the physical and biological environment of the North Pacific Ocean, defined as the 1976 regime shift. The regime shift appears to be associated with shifts in the Aleutian Low Pressure system eastward into the Gulf of Alaska, and although the direct linkages between the biota and the shifts in climatic patterns have not been established, the correlations have spurred further research into this area.

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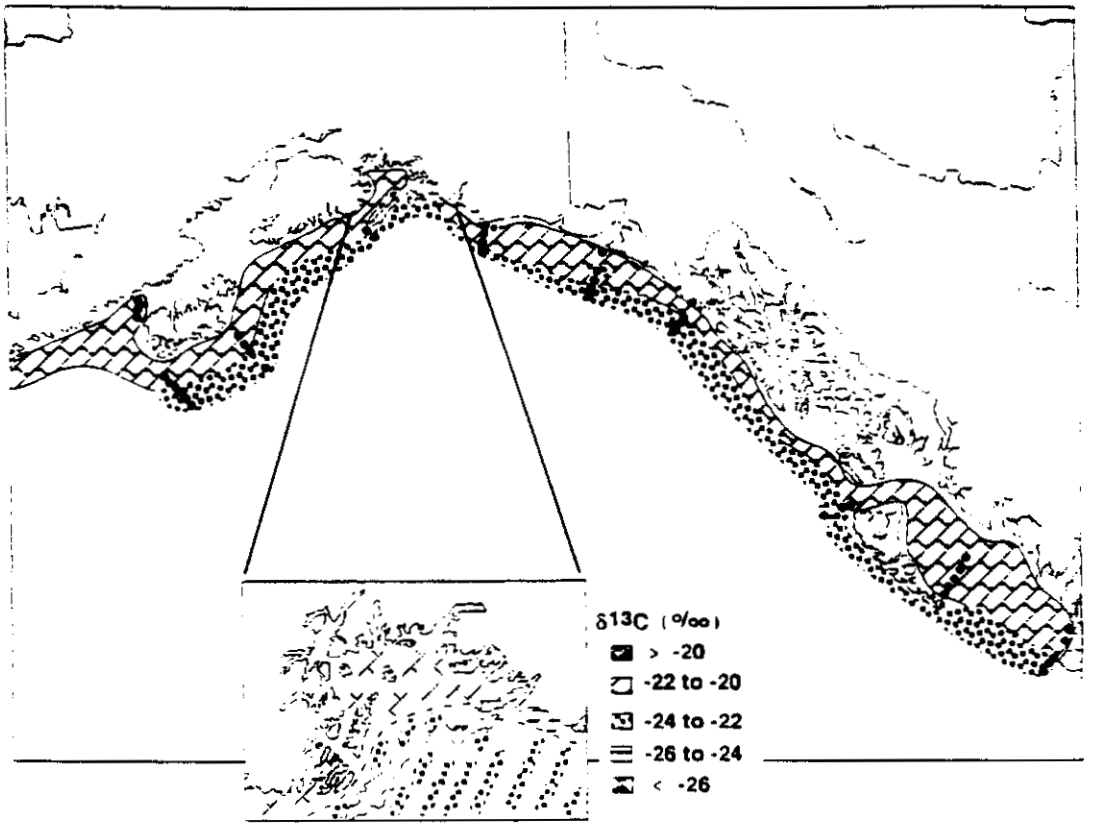

Figure 1. $\delta^{13}C$ isotope contours for calanoid copepods in the Gulf of Alaska and Prince WIlliam Sound.

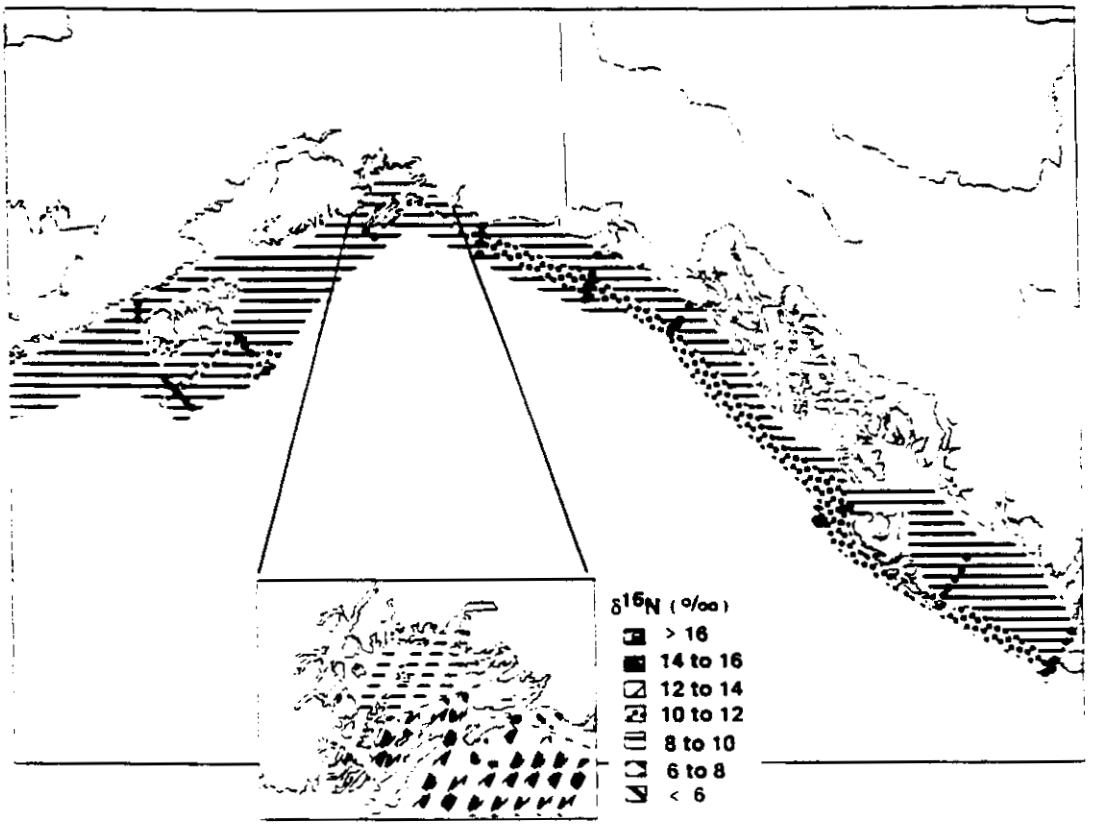

Figure 2. $\delta^{15}N$ isotope contours for calanoid copepods in the Gulf of Alaska and Prince William Sound.

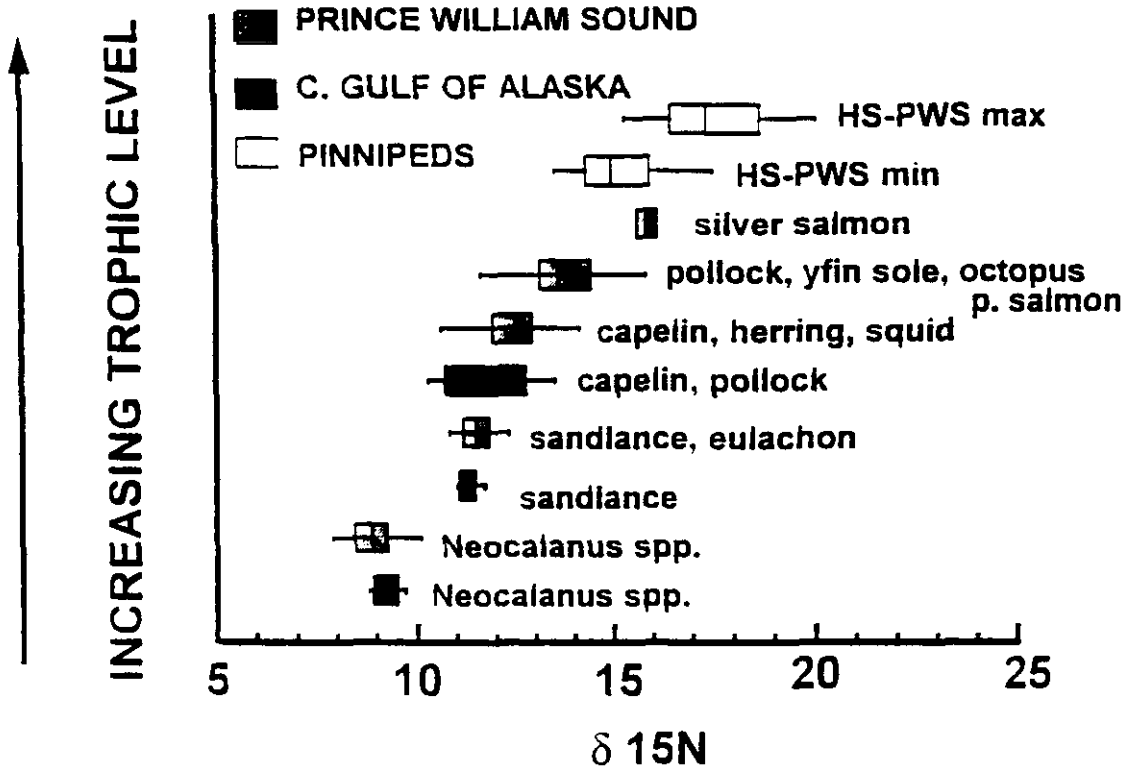

Figure 3. Maximum (max) and minimum (min) mean (± SE) $\delta^{15}N$ from Prince William Sound harbor seals (PWS HS) and mean (± SE) $\delta^{15}N$ from Prince William Sound and Gulf of Alaska fishes and invertebrates. Vibrissae values have been normalized to muscle. Sample sizes are ≥ 5.

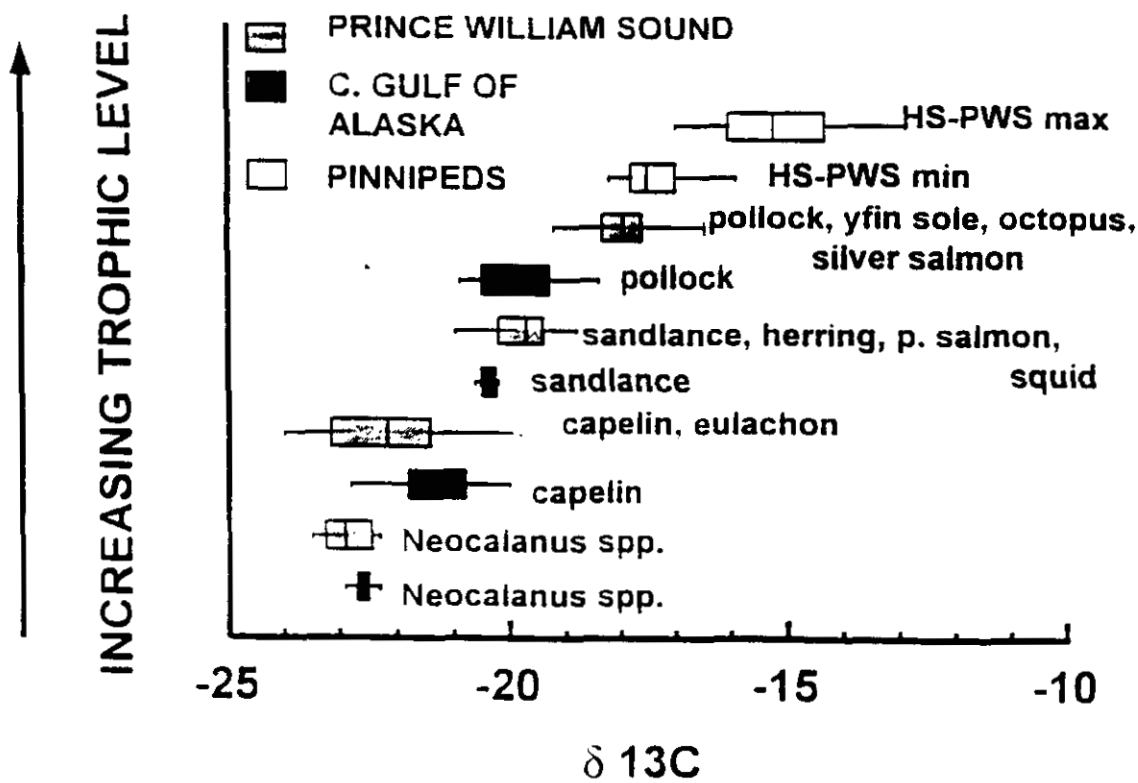

Figure 4. Maximum (max) and minimum (min) mean (± SE) $\delta^{13}C$ from Prince William Sound harbor seals (PWS HS) and mean (± SE) $\delta^{13}C$ from Prince William Sound and Gulf of Alaska fishes and invertebrates. Vibrissae values have been normalized to muscle. Sample sizes are ≥ 5.

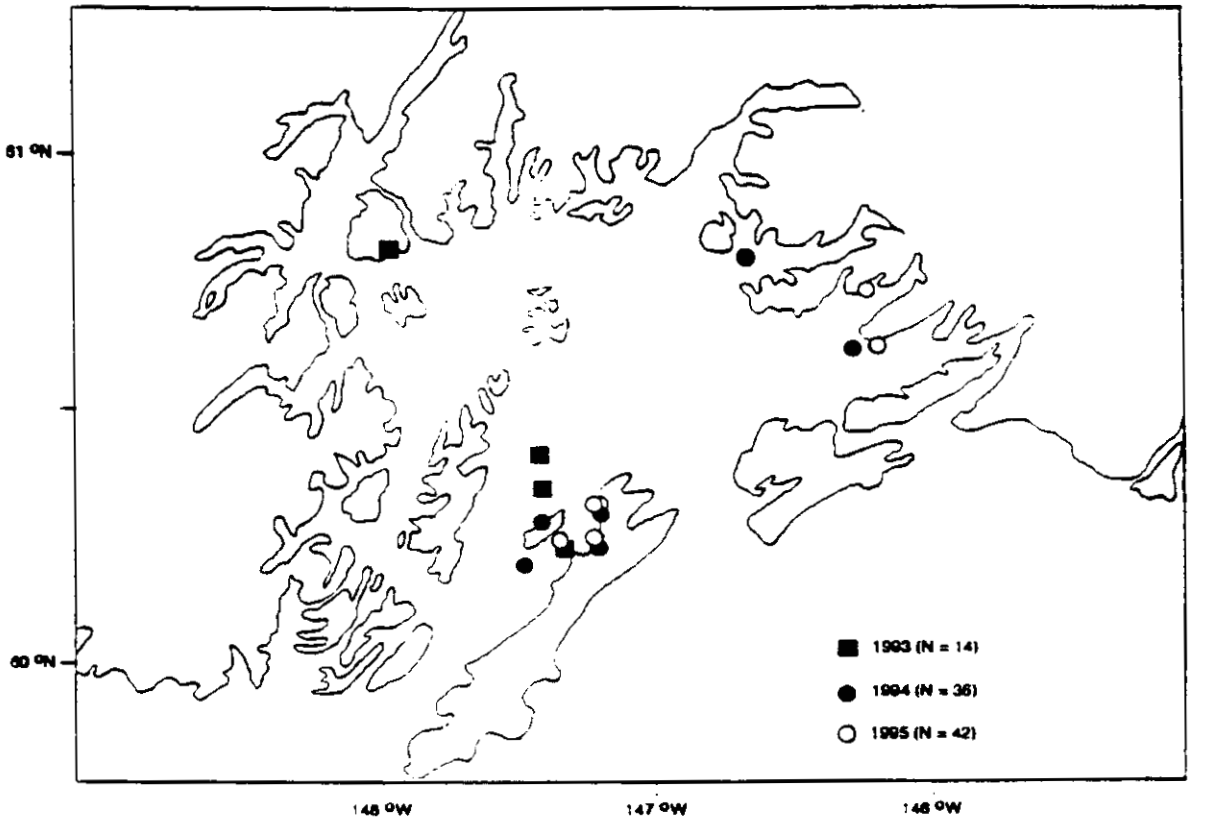

Figure 5. Sample locations for harbor seals in Prince William Sound, 1993-1995.

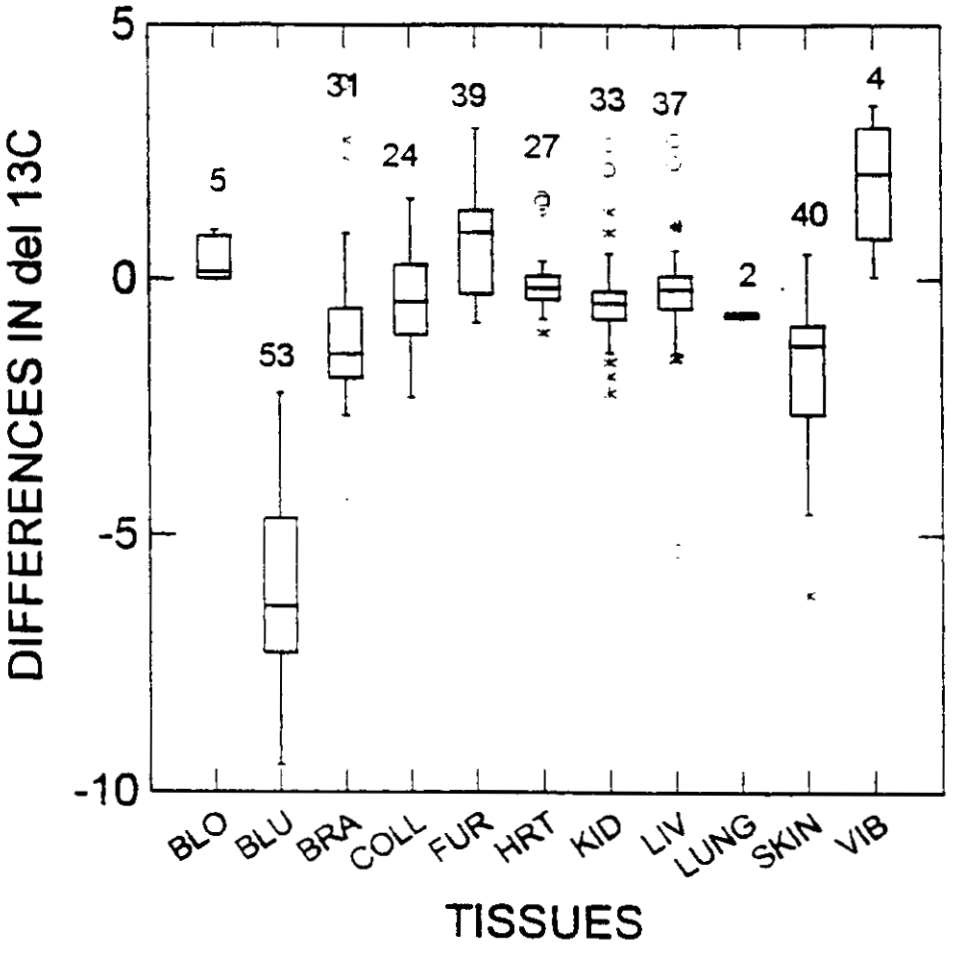

Figure 6. $\delta^{13}C$ fractionation of harbor seal tissues. blo = whole blood, blu = blubber, bra = brain, coll = collagen, hrt = heart, kid = kidney, liv = liver, vib = vibrissae. The sample size of each tissue is given above the box plot. * indicates values outside the first and third quartile of all values. o indicates values lower that 12.5% and greater than 87.5% of all values.

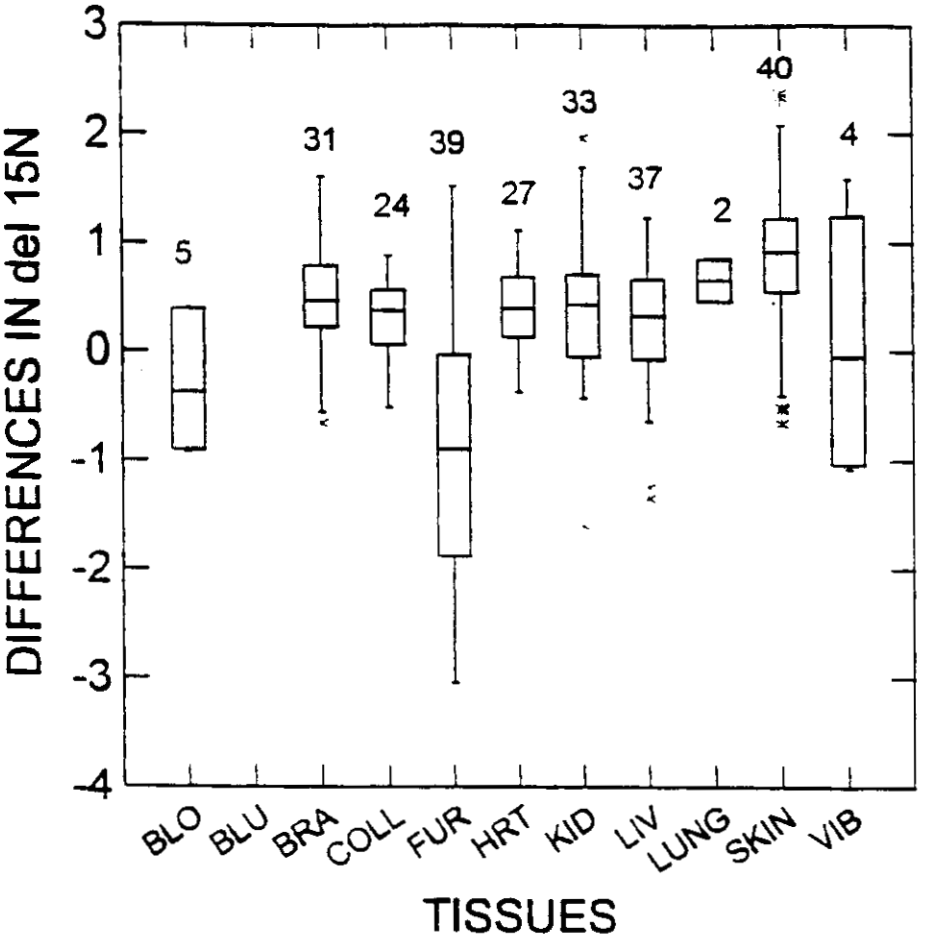

Figure 7. $\delta^{15}N$ fractionation of harbor seal tissues. blo = whole blood, blu = blubber, bra = brain, coll = collagen, hrt = heart, kid = kidney, liv = liver, vib = vibrissae. The sample size of each tissue is given above the box plot. * indicates values outside the first and third quartile of all values. o indicates values lower that 12.5% and greater than 87.5% of all values.

APPENDIX 1

TEMPORAL VARIATION IN THE $\delta^{13}C$ OF NORTH PACIFIC PINNIPEDS: INDICATION OF ENVIRONMENTAL CHANGE?

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ABSTRACT

Sea lion and seal populations in Alaskan waters have declined for over two decades and the cause(s) for the declines remain unknown. The stable carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios in bone collagen from wild Steller sea lions *(Eumetopias jubatus)*, northern fur seals *(Callorhinus ursinus)* and harbor seals *(Phoca vitulina)* from the Bering Sea and Gulf of Alaska were measured for the period 1951-1997 to test the hypothesis that a change in trophic level may have occurred during this interval and contributed to the population declines. A significant change in $\delta^{15}N$ in pinniped tissues over time would imply a marked change in trophic level. No significant change in bone collagen $\delta^{15}N$ was found for any of the three species during the past forty-seven years in either the Bering Sea or the Gulf of Alaska, but the ^{15}N in the Steller sea lion collagen was significantly higher than both northern fur seals and harbor seals. A significant decline in $\delta^{13}C$ (almost 2 ‰ over the 47 years) was evident for Steller sea lions and harbor seals were only significant at the 90% level. This decrease in $\delta^{13}C$ over time, with no accompanying change in $\delta^{15}N$, suggests an environmental change affecting the base of the foodweb rather than a trophic level change due to prey switching. Evidence exists of a decline in the seasonal primary production in the region which would exhibit itself as a decline in $\delta^{13}C$. Declining production could be an indication of a reduced carrying capacity in the North Pacific Ocean and sufficient quantities of optimal prey species may have fallen below threshold sustaining densities for these pinnipeds, particularly for yearlings and subadults.

KEY WORDS: Stable isotope analysis, Steller sea lions, northern fur seals, harbor seals, bone collagen

INTRODUCTION

Declining pinniped populations in the northeastern Pacific Ocean have drawn into question the role of the changing physical environment and its impact on biological organisms. We hypothesized that seals and sea lions made dietary changes as a result of changing prey abundances and the $\delta^{13}C$ and $\delta^{15}N$ values after the mid 1970s, when the latest major climatic shift occurred, would reflect the changes in trophic level.

Pinniped declines

Steller sea lions (*Eumetopias jubatus*), northern fur seals (*Callorhinus ursinus*) and harbor seals (*Phoca vitulina*) are generally found in coastal waters and along the continental shelf throughout the North Pacific Ocean, including the Bering Sea and the Gulf of Alaska (NRC 1996). These populations have drastically declined for more than two decades, particularly in the western Gulf of Alaska and Bering Sea (Pitcher 1990; Loughlin 1993; ADFG 1996; Strick et al. 1997). Food limitation has been hypothesized as the likely cause behind the declines in the pinniped populations, resulting from decreases in prey populations and emigration of certain species (Merrick et al. 1987; Alverson 1991; Trites 1992; Alaska Sea Grant 1993; Merrick 1995; Anderson et al. 1997; Merrick et al. 1997).

Isotope ratios in food webs

The isotopic ratios of animal tissues, particularly in marine organisms, are slightly more enriched in ^{13}C (0.5 to 1‰) and ^{15}N (3 to 5‰) than those found in the diet. Isotopic variations observed in organisms throughout the marine environment are believed to result from differences in organic carbon at the base of food webs and metabolic pathways in the organisms (DeNiro and Epstein 1978, 1981; McConnaughey and McRoy 1979; Rau et al. 1983; Fry and Sherr 1984; Minigawa and Wada 1984; Sholto-Douglas et al. 1991; Hobson and Welch 1992; France and Peters 1997). Herbivorous zooplankton, consisting primarily of calanoid copepods and euphausiids in the North Pacific Ocean, are first-order consumers of primary productivity. Any changes affecting the stable isotope ratios within the phytoplankton, such as carbon source and growth rate, would be carried through the food web and be reflected in foraging pinnipeds. Recent studies in phytoplankton have shown a close correlation between cellular growth rates and carbon isotope ratios ($\delta^{13}C$). Laws et al. (1995) have shown a close linear

relationship between diatom growth rates and isotopic fractionation in the laboratory and Bidigare et al. (1997) extended the findings to both laboratory cultures of haptophytes and samples from various world ocean environments. Increased growth rate and production in both the diatoms and haptophytes were correlated with increased $\delta^{13}C$ values.

Bone collagen is a tissue that has a relatively slow turnover rate, as much as ten years in large adult mammals. Depending on the age of the animal, the stable isotope ratios in the collagen may be integrated over much of its life (Stenhouse and Baxter 1977; Hobson and Clark 1992; Ambrose and Norr 1993). This tissue acts as a long-term integrator of isotope ratios and moderator of sporadic isotopic fluctuations, a factor which is useful when comparing isotope ratios of many individuals over long periods of time (Schoeninger and DeNiro 1984; Lee-Thorp et al. 1989). Episodic or short-term changes in dietary isotope ratios are dampened in the collagen record, leaving only changes in the long-term trends as an indicator of the organism's trophic status in its environment.

Physical and biological changes

Little emphasis has been placed on the effects of changing environmental conditions on pinnipeds in the North Pacific Ocean. An abrupt climatic change occurred in the Pacific Ocean in the mid-1970s and the new "regime" continued through the 1980s. Changes in atmospheric circulation have reportedly altered wind patterns and intensity, mixed layer depth, sea surface temperatures, ice extent and depth of ocean current patterns (Royer 1989; Trenberth and Hurrell 1994; Freeland et al. 1997).

The biological responses to these physical changes have manifested themselves in fluctuating phytoplankton abundance, zooplankton production and shifting migration patterns and biomass of commercial and non-commercial organisms (Venrick et al. 1987; Ebbesmeyer et al. 1991; Brodeur and Ware 1992; Hollowed and Wooster 1992; Francis and Hare 1994; Polovina et al. 1994; Hollowed and Wooster 1995; Polovina et al. 1995; Quinn and Niebauer 1995; Anderson et al. 1997). Sugimoto and Tadokoro (1997) reported chlorophyll concentrations and zooplankton biomass in the western, central and eastern subarctic Pacific and the Bering Sea from 1954 -1994. They found evidence of declining chlorophyll concentrations and zooplankton biomass during the mid-1970s and late 1980s in the eastern

Pacific and Bering Sea while the central Pacific experienced peaks in both chlorophyll and zooplankton in the late 1960s and continual declined after that point.

Many groundfish stocks have dramatically increased while forage fishes have declined in the Gulf of Alaska since the mid-1970s. The species composition for the region has shifted from an environment dominated by clupeid fishes and panaeid shrimp to one currently dominated by gadids and pleuronectids (Anderson et al. 1997). In 1951 and 1964, samples from Steller sea lion stomachs from the Bering Sea showed that walleye pollock was the fourth most prevalent prey species (Fiscus and Baines 1966) but by 1976, pollock was the dominant prey item (Lowry et al. 1989). Stomach content analyses of Pribilof Island fur seals in the early 1980s showed a predominance of juvenile walleye pollock and squid. Pacific herring and capelin, previously considered important prey, were absent (Sinclair et al. 1994). Kenyon (1965) noted harbor seals from Amchitka Island in the Aleutian Archipelago had remains of octopus and Atka mackerel in their stomachs while harbor seals sampled in 1979 from the Alaska Peninsula had primarily walleye pollock and octopus in their stomachs (Pitcher 1980).

If any of these physical parameters causes a decline in primary production, then the carrying capacity for the entire food web declines as less prey become available for each successive trophic level. For top consumers such as seals and sea lions, prey availability may fall below threshold densities necessary to sustain recruitment into the population.

METHODS

Pinniped samples

Seal and sea lion bone samples were collected from current native-harvested animals and museum skeletal collections for a total of 31 Steller sea lions, 13 northern fur seals and 63 harbor seals from regions throughout the Gulf of Alaska and the Bering Sea. Pinniped bones of all three species were provided by the University of Alaska Museum and the Kodiak Historical Society. Specimens were collected during the years 1951-1997 from coastal areas of southeast Alaska westward through the Gulf of Alaska and into the central Bering Sea (Figure 1). The Gulf of Alaska was separated into three regions for statistical analyses. The western Gulf of Alaska was defined as the area between 152°W and 175°W. The

central Gulf of Alaska was the area between 144°W and 152°W and the southeastern Gulf of Alaska was the area between 130°W and 144°W.

Sex and age of the animal were not recorded for most of the specimens which prevented some categorical analyses of the data. One skeleton was suspected as being from a pup and data from that animal would not have been sufficient to test for trophic differences either over time or in comparison with other age animals. The remaining skeletons were either labeled as adults and subadults or labeled as age unknown. More than half of the sampled sea lions (61%) and northern fur seals (62%) came from the Bering Sea while the remainders were split between the western and central portions of the Gulf of Alaska. Harbor seal samples were evenly distributed from the Bering Sea and through the western, central and southeastern Gulf of Alaska. Samples for both the sea lions and the harbor seals were almost evenly distributed throughout the forty-seven year study period, but there were years when, at a minimum, no samples were available, and at a maximum, six samples were available. An average of two specimens were available per year.

Collagen extraction

Bone samples were well preserved and free of humus and tissues. Collagen was extracted following the procedure described in detail in Matheus (1997). Approximately 1 gm of bone was either cut as a solid piece or shaved from the mandible or the shaft of a long bone; only cancellous bone was used. The bone samples had lipids removed with a methanol/chloroform procedure described in Bligh and Dyer (1959) prior to demineralization. The bone was allowed to demineralize in 1N HCl for approximately seven days at 5°C; fresh acid was added to the samples every day. The remaining material was rinsed and then boiled in deionized water for approximately eight hours to dissolve the collagen and precipitate peptides. The solution was passed through a 0.45μ filter and the filtrate was dried in an aluminum dish at 60°C for a minimum of 48 hours.

Mass spectrometry

Subsamples of each tissue (1-1.5 mg) were combusted and analyzed for stable isotope ratios with a Europa 20/20 continuous flow isotope ratio mass spectrometer. All samples were analyzed in duplicate. Stable isotope ratios were expressed in the following standard notation:

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

where X is ^{13}C or ^{15}N and R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ respectively. $R_{standard}$ for ^{13}C is Pee Dee Belemnite; for ^{15}N it is atmospheric N_2 (air). If the difference between replicates was greater than 0.5‰, samples were re-analyzed. Analytical error for samples was approximately ± 0.1‰ for both carbon and nitrogen.

RESULTS

The three pinniped species segregated isotopically for only $\delta^{15}N$ ($P = 0.002$) when $\delta^{13}C$ and $\delta^{15}N$ were considered together (MANOVA: Wilks Lambda $F_{4, 130} = 3.30$, $P = 0.013$). Bonferroni correction tests for $\delta^{15}N$ showed Steller sea lions differed from harbor seals and northern fur seals ($P = 0.015$ and $P = 0.014$, respectively). The mean nitrogen isotope ratios in the Steller sea lions was greater than those in both harbor seals and northern fur seals. No differences in either isotope were detected among the defined regions for any of the three species ($P = 0.750$). When all years were considered for $\delta^{13}C$ and $\delta^{15}N$, only $\delta^{13}C$ showed a significant difference (MANOVA: Wilks Lambda $F_{70,130} = 1.558$, $P = 0.015$). Steller sea lions had a significant difference in $\delta^{13}C$ (Kruskal-Wallis $P = 0.004$) while harbor seals and northern fur seals did not ($P = 0.298$ and $P = 0.072$, respectively). Regression analysis of $\delta^{15}N$ revealed no change in the slope of ratios over the forty-seven year period either in combined or individual species (Figure 2). Regression analysis of $\delta^{13}C$ showed a significant decline in the Steller sea lions ($P < 0.001$) (Figure 3) and a decline, although not significant, in both the harbor seals and northern fur seals ($P = 0.108$ and $P = 0.375$, respectively) (Figure 4 and 5). The sea lion $\delta^{13}C$ declined an average of 1.9‰ from 1951 through 1997.

Nitrogen isotope values for harbor seals ranged from 14.0 to 20.5‰ with a mean of 17.2 ± 1.6‰. The $\delta^{15}N$ for northern fur seals ranged from 15.2 to 20.1‰ with a mean of 17.2 ± 1.5‰ and the $\delta^{15}N$ for Steller sea lions ranged from 16.2 to 21.9‰ with a mean of 18.5 ± 1.4‰. Harbor seal collagen $\delta^{13}C$ values ranged from -12.0 to -16.4‰. The $\delta^{13}C$ of collagen from northern fur seals ranged from -13.0 to -16.7‰ and the Steller sea lion collagen $\delta^{13}C$ ranged from -12.5 to -15.8‰ (Table 1). The annual variance in the $\delta^{13}C$ of all three species ranged from less than 1‰ to as much as 5‰.

DISCUSSION

The diets of Steller sea lions, northern fur seals and harbor seals consist of similar prey species but the composition differs based on preferred prey items and locally abundant species. Harbor seal diets appear to consist of mostly pelagic and semidemersal fishes and benthic invertebrates. Fur seal and sea lion diets appear to rely on pelagic and demersal fishes and some pelagic invertebrates (Pitcher 1980, Kajimura 1985, Sinclair et al. 1997).

The $\delta^{15}N$ values obtained from the pinniped collagen suggest that Steller sea lions may feed at a slightly higher trophic level than the harbor seals and northern fur seals. Hobson et al. (1997) found similar results for Steller sea lions and northern fur seals. They concluded that the sea lions were consuming more large-size pollock which were enriched in $\delta^{15}N$ relative to the juvenile pollock and squid that the fur seals were predominantly relying on. Perez and Bigg (1986) noted that northern fur seals in the Aleutians and Gulf of Alaska between 1958 and 1974 fed largely on sandlance, capelin and herring. The diets of both the forage fish and juvenile pollock consist primarily of zooplankton and this similarity could result in comparable $\delta^{15}N$ values. Pitcher (1980) noted that walleye pollock was the predominant prey in both the Steller sea lion and harbor seal diets in the Gulf of Alaska during the mid-1970s but that each species foraged on different size pollock. Steller sea lions were eating pollock significantly larger than those eaten by harbor seals. Larger pollock are largely piscivorous while the smaller pollock are planktivorous; this should have resulted in higher $\delta^{15}N$ in the sea lions.

These pinnipeds have heterogeneous diets which often include prey from different trophic levels and regions. Schell et al. (1998) noted the existence of isotopic gradients in the $\delta^{13}C$ and $\delta^{15}N$ of zooplankton from the Bering Sea and recently identified isotopic gradients in the continental shelf waters of the Gulf of Alaska (Schell unpublished). Regions of high primary productivity, including the shelf break in the Bering Sea and coastal upwelling zones in the Gulf of Alaska, are more enriched in ^{13}C and ^{15}N than areas low in productivity. These enriched values in the zooplankton would be passed through the foodwebs to the upper trophic organisms. Pelagic organisms that feed in and travel through several isotopically distinct regions would exhibit a composite of those isotope ratios.

Variations in the year-to-year movement patterns of both pinnipeds and prey, as well as differences in prey availability, likely cause some fluctuations in the isotope ratios among individual pinnipeds. Pinniped species, and the prey they assimilate, are often separated geographically. The isotope ratios in harbor seals, which tend to have a strong site affinity, would represent the prey items in the seals' coastal and deep water feeding locations (Pitcher 1980). The nitrogen isotopes in the Steller sea lions would reflect the prey often found in offshore waters off California and Oregon and in the Gulf of Alaska and while the isotope ratios in northern fur seals could reflect prey foraged in the Bering Sea, the Gulf of Alaska and as far south as the offshore waters of California (Goebel et al. 1991; Loughlin 1993; Merrick 1995).

Unlike the nitrogen isotope data which showed no significant change during the 47 year period, carbon isotopes values showed a decrease of ~ 2 ‰ for all three species, although only the decline in Steller sea lions was statistically significant at the $P = 0.05$ level. Changes in prey composition during the 1970s, as previously described, may have altered the isotope ratios in the pinnipeds but a more enriched isotope signature would be expected. Pollock, which is currently predominant in the diets of these animals, generally have more enriched $\delta^{13}C$ and $\delta^{15}N$ values than the once prevalent clupeid fishes (Anderson et al. 1997; Merrick et al. 1997). If these pinnipeds began to forage in different trophic levels than they once occupied, any change in the nitrogen isotope ratios would likely have a corresponding change in the carbon isotopes (DeNiro and Epstein 1978, 1981; Rau et al. 1983) A decrease in $\delta^{13}C$ in consumers can result if marine organisms begin foraging at a lower trophic level or switch from an enriched benthic diet to a more depleted pelagic diet (Hobson and Welch 1992; France and Peters 1997) althought no evidence exists for this scenario to have occurred in any one or all of these pinnipeds.

Steller sea lions and female and juvenile northern fur seals from the subarctic Pacific are found foraging in the offshore waters along the California and Oregon coasts for a portion of the year (Kajimura 1980; NRC 1996). Roemmich and McGowan (1995) noted that zooplankton abundance in the California Current declined more than 70% beginning around 1977 and has remained low. McGowan et al. (1998) have also reported large declines in oceanic seabirds and commercial pelagic catch and smaller kelp forests along the California coast. Reduced primary and secondary production in the region would likely

lead to a reduction in food available for the upper trophic level organisms, including pinnipeds. This regional decline in production may partially explain why Steller sea lions exhibit declining $\delta^{13}C$ values. The suggested decline in the $\delta^{13}C$ may not be as apparent in the samples of northern fur seals because only one animal was available after 1976 and this cannot be considered an adequate sample for drawing conclusions regarding declining carbon isotopes. However, when that seal from 1995 was removed from the pool of samples and a regression analysis was performed, a similar decreasing slope existed for the fur seals from 1951 through 1976.

Although we cannot determine the migratory ranges and life histories of individual seals and sea lions, we assume that the $\delta^{13}C$ values in the bone collagen represent the prey derived from primary production in the northeastern Pacific. The implied decline in the carrying capacity, resulting from a decrease in primary production, would result in diminished resources for populations of top trophic level organisms, such as seals and sea lions. Steller sea lions were the only species to show significant long-term declines in their carbon isotope ratios and this may be due to the spatial variation between the sea lions and the other two species.

The decline in the $\delta^{13}C$ of Steller sea lion bone collagen occurred during the same period that Schell (in prep.) observed the declining $\delta^{13}C$ values in bowhead whales in the Bering Sea. Schell has used the findings of Laws et al. (1995) and Bidigare et al. (1997), in conjunction with the average carbon isotope ratios in bowhead whale baleen laid down in the Bering and Chukchi seas, to estimate the relative interannual changes in primary production in the Bering Shelf ecosystem. If the correlation between the measured haptophyte growth rates and changes in $\delta^{13}C$ is similar to phytoplankton growth in the Bering Sea, the isotope ratios in baleen imply a decline of up to 40% in ecosystem productivity between 1965 and 1995.

The carbon isotope ratios in top trophic level marine organisms can result from the carbon composition in phytoplankton at the base of the food web. The isotopic composition of the phytoplankton is affected by the isotopic composition of inorganic carbon and the fractionation during growth of the plant cells. A rapid use of CO_2 during photosynthesis can lead to a temporal increase in the $\delta^{13}C$ of the plant cells if the rate of CO_2 replenishment is slower than usage (Goericke et al. 1994). The Bering Sea

and upper reaches of the North Pacific Ocean are regions of high nutrients and low chlorophyll (HNLC). Excessive quantities of nitrogen and phosphorus are available in the North Pacific throughout the year but, until recently, summer phytoplankton in the North Pacific have not fully utilized all the available nitrate for production. The lack of a micronutrient may be limiting the phytoplankton productivity (Martin and Fitzwater 1988). Boyd et al. (1996) identified iron, possibly from atmospheric input, as limiting the nitrate utilization by phytoplankton. However, recent evidence by Freeland et al. (1997) indicates that summer nitrate concentrations may now be limiting. Rapidly growing phytoplankton would draw down the available nitrate and a deficit would take place which would limit the amount of primary production.

Changes in the wind intensity and the mixed depth layers in the northeast Pacific appear to have affected the amount of production in the region. Brodeur and Ware (1992) found increases in zooplankton biomass in the region of the Pacific subarctic gyre between 1956 and 1989 which they attributed to increased wind intensity. They surmised that the intensification of the winds either provided a limiting micronutrient to surface waters or a deepening of the mixed layer that would slow primary production and allow zooplankton time to graze more completely. Venrick et al. (1987) noted cooler sea surface temperatures during 1980-1985 between 30° and 50°N in the North Pacific Ocean which probably resulted from wind mixing from greater than average winter storminess. Yet Polovina et al. (1995) observed a shallowing of the winter mixed layer depth in the subarctic North Pacific from 1977-1988 and attributed the change to an intensification of the Aleutian Low Pressure System. Data presented by Freeland et al. (1997) for Station Papa (50°N 145°W) also indicated a shallowing of the mixed layer depth. However, they suggested that changes in the position and strength of the Alaska gyre circulation are related to the changes in the Aleutian Low.

There seems little doubt that the mixed layers in the north Pacific Ocean are changing. The shallowing of the winter mixed layer allows phytoplankton to receive more light energy and, as Freeland et al. (1997) suggested, an earlier spring bloom would result due to larger concentrations of overwintering phytoplankton and greater light intensity. This increased production during the summer months would reduce nitrate levels over time. If winter mixing has decreased during the past several decades and

nutrients are not replenished at the same rate, a decline in the available nitrate in the upper water column could result due to entrainment of phytoplankton.

A large amount of evidence exists that the environment of the North Pacific Ocean has changed during the past several decades and changes in the physical environment may be associated with changes in the primary production in the region. The Committee on the Bering Sea Ecosystem assessed the likelihood of various potential causes on the declines of these three pinniped species and found that climate effects and environmental changes were likely factors affecting the fish community and food availability for these animals (NRC 1996). Reduction in food, which subsequently leads to population declines if food depletion is great enough, seems to be supported for northern fur seals and Steller sea lions (Loughlin et al. 1987; Trites 1992; Merrick and Loughlin 1993; Merrick 1995).

Marine mammal populations can be expected to change with time in response to environmental perturbations. The large scale declines seen in the Bering Sea and Gulf of Alaska pinnipeds are unusual because they appear to have happened in a short time whereas some populations of the same species have remained stable or increased in other areas of the North Pacific. Short term environmental changes, such as El Niño events, would have only a limited impact on these pinniped populations by reducing food availability (Trillmich and Ono 1991). Short term changes that could alter the carbon isotope ratios in the marine food webs would likely be tempered in the bone collagen records due to the relatively slow turnover rate of isotopes in this tissue. The magnitude of changes observed in the North Pacific seem to warrant further investigation on their impact to marine mammal populations and the use of isotope ratios should enhance our understanding of these changes.

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Table 1. Mean stable isotope ratios of bone collagen from harbor seals, northern fur seals and Steller sea lions. SEGOA = southeastern Gulf of Alaska, CGOA = central Gulf of Alaska, WGOA = western Gulf of Alaska, BS = Bering Sea.

Species	n	Year	Location	Mean $\delta^{13}C$	Mean $\delta^{15}N$
Harbor seal	2	1951	CGOA	-13.9 ± 0.5	20.2 ± 0.0
	1	1952	CGOA	-13.6	17.7
	1	1952	WGOA	-14.6	14.7
	1	1953	BS	-14.4	18.9
	1	1954	WGOA	-14.6	18.4
	2	1955	CGOA	-14.8 ± 0.2	17.9 ± 1.4
	2	1956	CGOA	-14.8 ± 0.8	17.3 ± 0.2
	1	1962	CGOA	-14.9	17.2
	2	1964	WGOA	-13.9 ± 0.9	16.1 ± 0.2
	5	1965	SEGOA	-13.2 ± 0.7	16.4 ± 0.7
	1	1965	WGOA	-13.1	16.1
	1	1966	CGOA	-13.4	16.9
	1	1966	WGOA	-14.8	17.7
	1	1966	BS	-14.2	19.4
	2	1968	BS	-14.3 ± 2.1	14.8 ± 0.8
	1	1969	BS	-15.4	14.4
	1	1970	WGOA	-12.0	16.9
	1	1970	BS	-13.6	19.5
	1	1971	WGOA	-13.2	17.4
	1	1971	BS	-14.4	16.9
	1	1972	CGOA	-13.6	20.1
	2	1972	BS	-13.7 ± 0.2	16.5 ± 0.2
	1	1973	CGOA	-14.3	16.4
	1	1973	BS	-15.6	20.5
	1	1974	BS	-14.4	16.5
	3	1975	CGOA	-14.7 ± 1.2	16.4 ± 1.2
	1	1976	WGOA	-13.7	17.0
	2	1977	WGOA	-14.7 ± 0.3	16.4 ± 0.5
	2	1978	CGOA	-13.5 ● 0.8	16.9 ± 0.3
	1	1978	WGOA	-14.4	18.4
	2	1979	BS	-14.1 ± 0.5	18.4 ± 0.6
	2	1980	WGOA	-15.6 ± 0.5	16.3 ± 0.2
	1	1981	CGOA	-12.2	15.6
	1	1981	BS	-13.5	20.0
	2	1985	WGOA	-14.8 ± 0.6	18.9 ± 1.5
	1	1989	CGOA	-14.0	18.7
	1	1993	CGOA	-15.8	17.2
	2	1995	SEGOA	-15.0 ± 0.6	19.0 ± 1.4
	1	1995	BS	-13.4	15.6
	1	1996	SEGOA	-14.4	15.8
	5	1996	CGOA	-15.0 ± 0.9	16.2 ± 0.6
Northern fur seals	2	1952	BS	-13.2 ● 0.2	16.1 ± 0.8
	1	1954	WGOA	-13.7	16.9
	1	1955	BS	-15.2	16.3
	1	1957	CGOA	-13.1	20.1
	1	1957	WGOA	-13.7	19.9
	1	1960	BS	-15.2	16.5

Table 1. - cont.

Northern fur seals	1	1961	CGOA	-16.7	16.7
	1	1961	BS	-15.2	16.8
	1	1965	BS	-15.2	15.5
	1	1976	WGOA	-13.8	18.4
	1	1976	BS	-13.8	16.8
	1	1995	BS	-15.3	17.2
Steller sea lions	2	1953	BS	-14.1 ± 0.6	20.3 ± 0.9
	3	1956	CGOA	-13.0 ● 0.3	18.0 ± 0.6
	1	1957	CGOA	-14.5	18.5
	1	1958	BS	-12.5	17.0
	2	1960	WGOA	-12.8 ± 0.1	18.5 ± 0.4
	1	1960	BS	-14.2	16.4
	1	1961	BS	-13.2	21.9
	1	1965	BS	-14.6	18.8
	1	1966	CGOA	-13.1	20.4
	1	1969	CGOA	-14.3	18.7
	2	1971	BS	-14.9 ± 0.3	18.9 ± 1.6
	1	1974	BS	-12.9	20.0
	1	1977	BS	-14.9	18.5
	1	1978	WGOA	-15.2	17.5
	1	1979	BS	-15.0	17.0
	1	1988	CGOA	-14.6	18.5
	1	1989	WGOA	-15.6	17.0
	2	1993	BS	-15.7 ± 0.1	18.1 ± 1.9
	2	1994	BS	-14.9 ± 0.1	18.0 ● 0.1
	3	1995	BS	-14.2 ● 0.2	18.7 ● 0.8
	1	1996	BS	-15.4	17.0
	1	1997	WGOA	-15.8	17.8

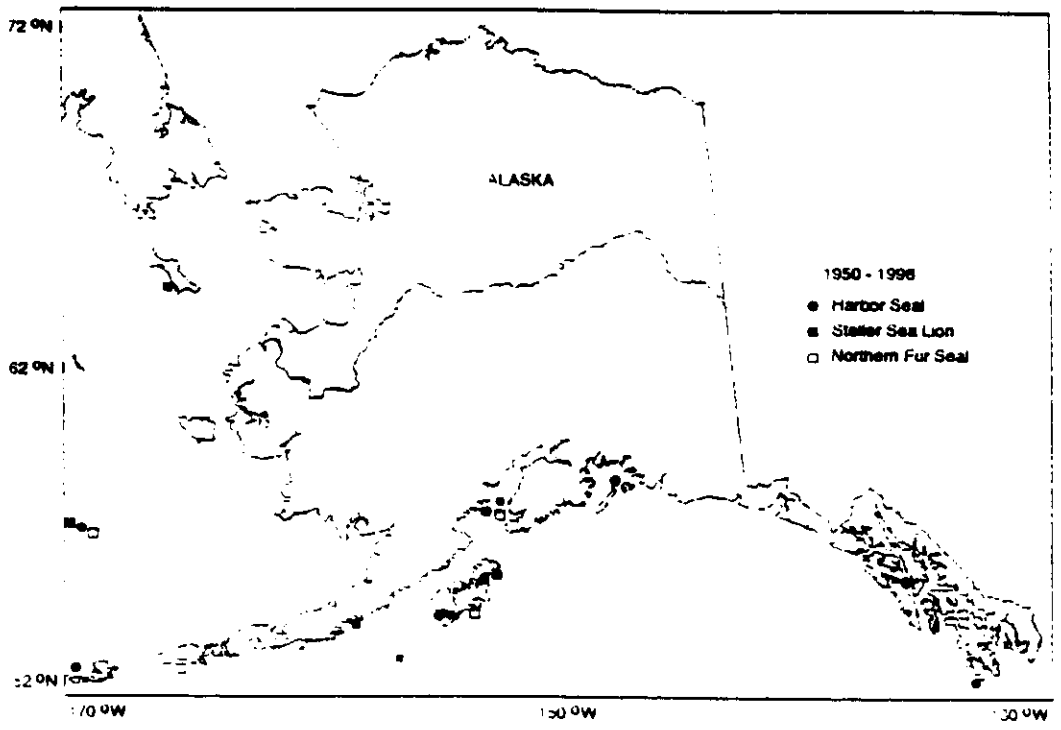

Figure 1. Collection locations of bone samples from Steller sea lions, northern fur seals and harbor seals, 1951-1997.

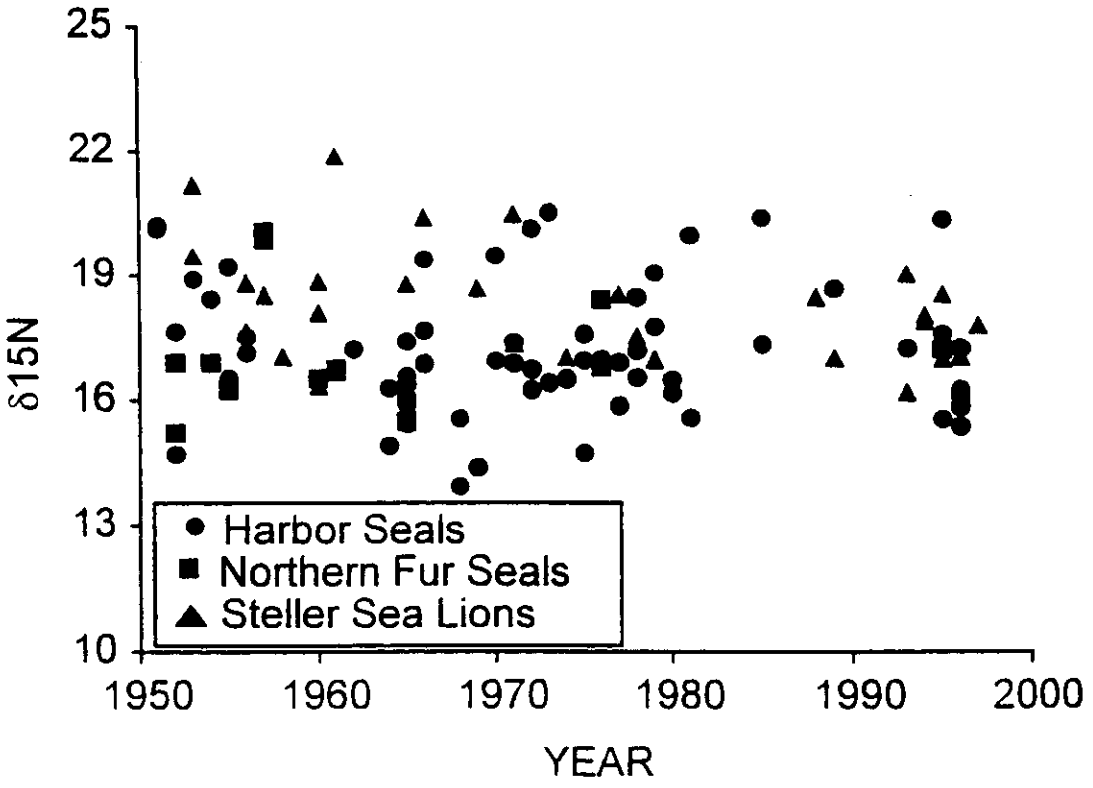

Figure 2. $\delta^{15}N$ values of bone collagen for Steller sea lions, northern fur seals and harbor seals from the Bering Sea and Gulf of Alaska, 1951-1997.

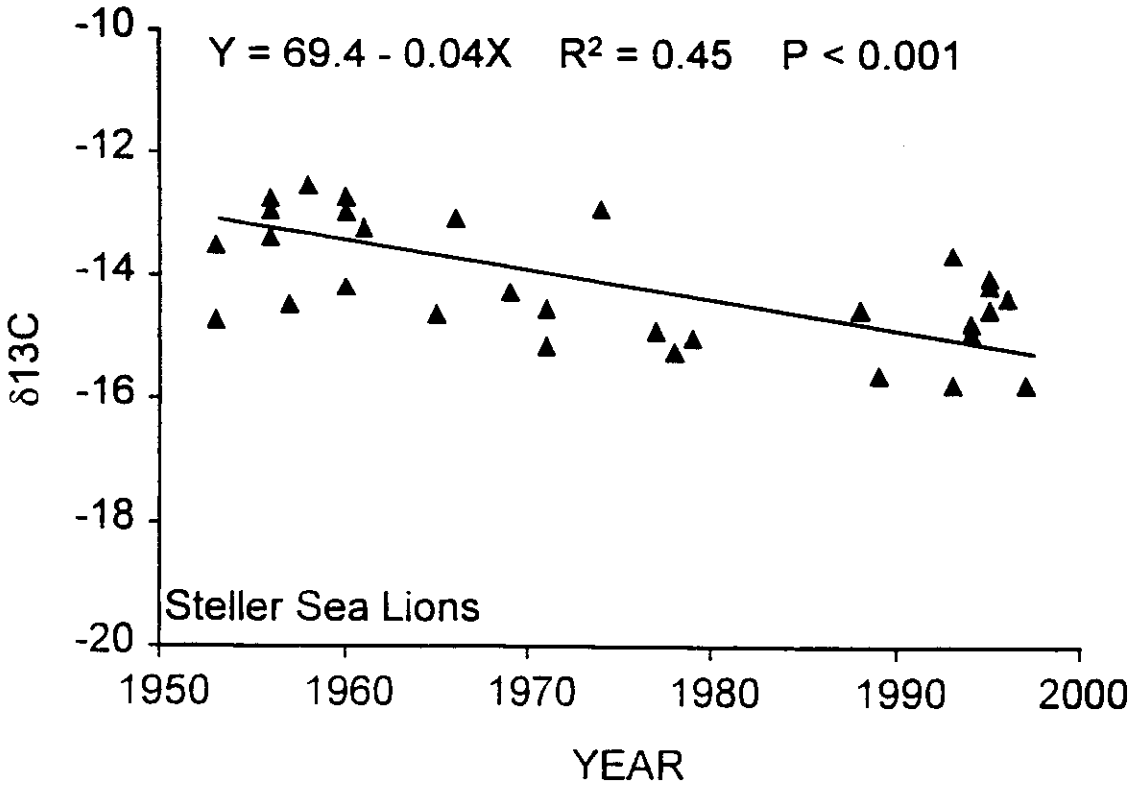

Figure 3. $\delta^{13}C$ values of bone collagen for Steller sea lions for the Bering Sea and Gulf of Alaska, 1951-1997.

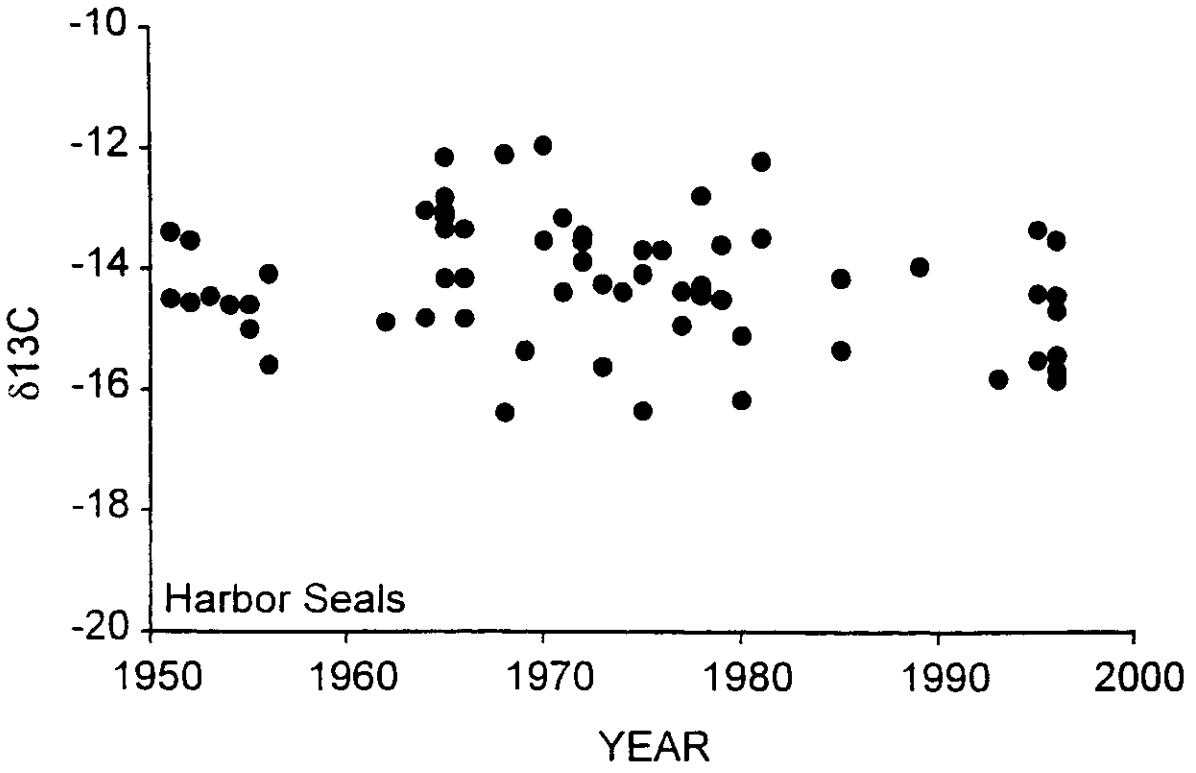

Figure 4. $\delta^{13}C$ values of bone collagen for harbor seals from the Bering Sea and Gulf of Alaska, 1951-1997.

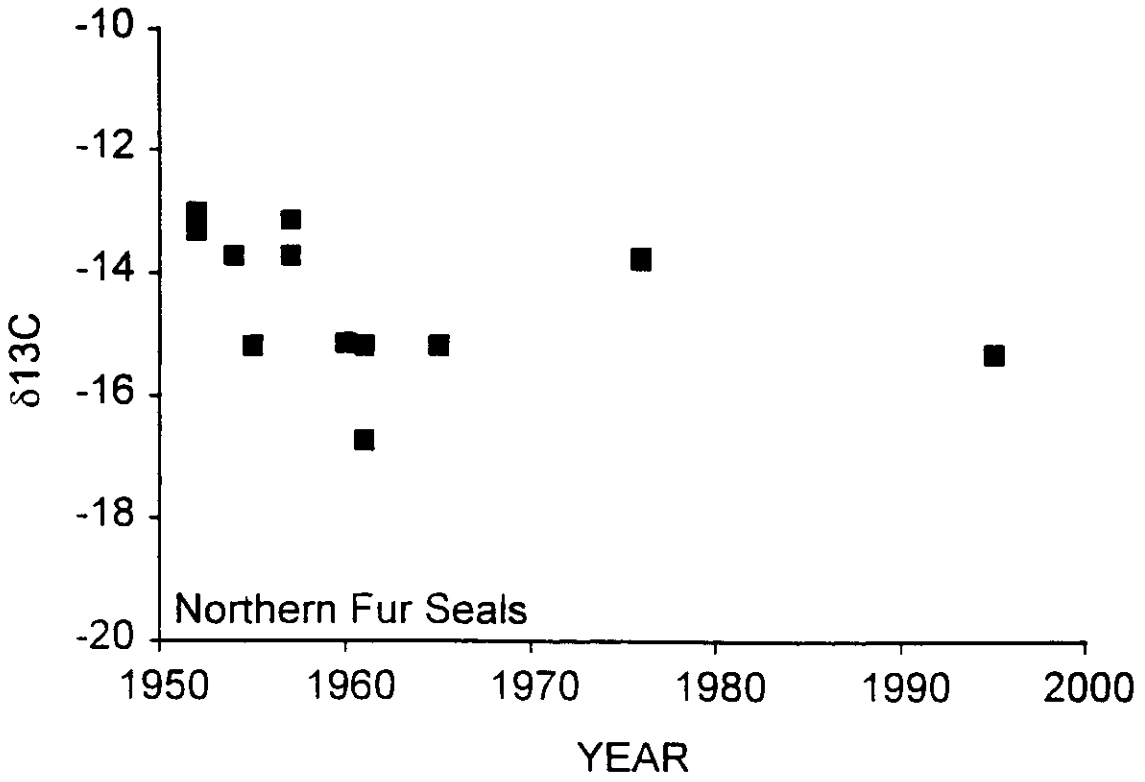

Figure 5. $\delta^{13}C$ values of bone collagen for northern fur seals from the Bering Sea and Gulf of Alaska, 1951-1997.

APPENDIX 2

VIBRISSAE GROWTH RATES OF HARBOR SEALS (*PHOCA VITULINA*) AND STELLER SEA LIONS (*EUMETOPIAS JUBATUS*)

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KEY WORDS: Vibrissae, stable isotope ratios, harbor seals, Steller sea lions, glycine

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Vibrissae growth rates of harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*)

ABSTRACT

Vibrissae, which act as a temporal record of feeding in harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*), had growth rates estimated using ^{13}C- and ^{15}N-labeled glycine and subsequent stable isotope analysis. The labeled glycine was incorporated into the keratin and served as a temporal marker for growth rate calculation. One captive seal received two doses 147 days apart while a second seal received only one dose; vibrissae were analyzed after 86 and 154 days. The positions of the peaks indicate growth begins in late fall or winter, continues into spring but ceases by June. Two captive sea lions each received two labeled doses during a 308 day period. After 427 days vibrissae showed two peaks corresponding to the markers and growth rates of 0.05 - 0.07 mm/day. Similar growth rates in captive juvenile and wild adult sea lions, 0.05 - 0.18 mm/day, supported the assumption that the major isotopic oscillations were annual. The multi-year records imply that Steller sea lions retain their vibrissae while harbor seal vibrissae, in contrast, have periods of rapid growth and minimal or no growth at other times and appear to be shed, at least in part, annually.

INTRODUCTION

Severe declines in harbor seal (*Phoca vitulina*) and Steller sea lion (*Eumetopias jubatus*) populations have been recorded in the Bering Sea and Gulf of Alaska for more than two decades (Pitcher 1990, Strick et al. 1997). No cause and effect relationships have yet been established despite the concurrent increased commercial fishing pressure and decline in pinniped populations that compete for many of the same resources. Food limitation has still been hypothesized as the likely cause behind the declines in the 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 et al. 1997). Vibrissae (whiskers) from seals and sea lions contain a timeline of stable isotope ratios derived from prey items. By comparing the isotope ratios found along the lengths of vibrissae with the isotope ratios 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 the study we attempted to determine how growth rates patterns changed in the vibrissae of seals and sea lions throughout the year.

Vibrissae (whiskers) are hairlike organs but differ considerably from pelage (hair). Vibrissae follicles are like pelage follicles in structure but differ by being larger overall, highly innervated, having large blood sinuses and are controlled by voluntary muscles. The whiskers on pinnipeds occur in the musculature on the muzzle and above the eyes and most of these muscles control the positioning of the vibrissae (Ling 1977). Dehnhardt and Kaminski (1995) described how the vibrissae of harbor seals could discriminate diameter differences among disks by touching them with their mystacial vibrissae. The vibrissae from harbor seals and Steller sea lions have some anatomical differences from each other. The vibrissal shaft in the otariids, including Steller sea lions, are outwardly smooth while harbor seals and other phocids have a waved surface. No known information exists regarding the significance of this characteristic in different species but differences in the vibrissae structure may be associated with slightly different functions.

Ling (1966) determined that elephant seal vibrissae were not shed during their annual pelage molt but were shed periodically only after the seals were older than two years of age. However, the marsupial *Tricosurus vulpecula*, as noted by Lyne et al. (1974), had prolonged but variable vibrissae

growth cycles compared to its pelage. Because vibrissae appear to function as sensory organs, periodic replacement or renewal due to physical damage could be selectively more advantageous than seasonal replacement. Based on this information, we hypothesized that these two species of pinnipeds would maintain their vibrissae from year-to-year and grow continually. Understanding the relationship between growth and isotope ratios in the vibrissae will facilitate interpretation of the temporal record of food consumption in these animals.

MATERIALS AND METHODS

Administration of labeled glycine

Glycine enriched with ^{13}C and ^{15}N (98%) (Cambridge Isotope Laboratories, Andover, Mass,) was employed to mark vibrissae. Two adult harbor seals and two adult Steller sea lions at the Mystic Marinelife Aquarium in Connecticut were intravenously administered both singly- (^{15}N) and doubly-labeled (^{13}C and ^{15}N) glycine. Glycine was chosen as the carrier for the isotope label due to the high mole percentage found in keratin. The amount of labeled-amino acid sufficient enough to create an easily measurable isotope marker was based on the approximate weight of each animal. The concentration of glycine in the injected solution was 100 mg/ml and the dose chosen was approximately 5 mg glycine/kg of body weight. The amino acid was administered in sterile normal saline solution. All procedures were approved by the Institutional Animal Care and Use Committees of both the Mystic Marinelife Aquarium and the University of Alaska Fairbanks and were carried out in accordance with guidelines established by the Canadian Council on Animal Care.

The labeled glycine was administered to two seals (Norton and Peter) and two sea lions (Lucia and Stella). Norton received two doses of ^{13}C and ^{15}N-labeled glycine 143 days apart, while Peter was given just one dose of ^{13}C and ^{15}N-labeled glycine. Lucia received one dose of doubly-labeled and one dose of ^{15}N-labeled amino acid. Stella was given two doses of ^{15}N-labeled glycine. Table 1 details the sequence of label additions and whisker clipping. The glycine was metabolically incorporated into the keratin during growth of the whiskers and the large addition of ^{15}N or ^{13}C provided a temporal marker. Whole blood samples of 1-2 ml were collected prior to dosing and at twenty-four hour intervals for several days after the dosing in order to monitor the loss of the label. Whiskers were allowed to grow for several

months before a second dose of glycine was administered. The second peak was desired to establish two known dated markers in order to calculate growth rate. After several more months, a whisker was cut as close to the skin as possible from each animal and analyzed for stable isotope ratios at close intervals along its length to locate the markers (Table 1).

Whisker growth in subadult sea lions

A second type of growth rate experiment was conducted simultaneously at the Vancouver Aquarium in British Columbia, Canada on subadult Steller sea lions. Vibrissae were clipped from the muzzle of each of the six animals periodically during a three year period. The vibrissae were analyzed for the normal variability in natural abundance stable isotope ratios and all the whiskers from an animal are plotted together. Overlap in growth from one vibrissae to the next was measured from an inflection point obvious on at least two separate segments. The date of each clipping was known and the growth rate calculated.

All the mystacial vibrissae from a subsistance-harvested sea lion were pulled and analyzed for carbon and nitrogen isotope ratios. The patterns of isotope ratios were compared among the vibrissae, particularly the shorter, anterior vibrissae versus the longer, posterior whiskers to determine if growth rate varied among sea lion vibrissae.

Whisker growth in wild harbor seals

An adult harbor seal in southeastern Alaska was recaptured seven months after a vibrissae had been removed for analysis. A second vibrissae was removed and analyzed and the patterns in the isotope ratios were compared in an effort to determine the growth rate during that time period.

All the mystacial vibrissae from a subsistance-harvested harbor seal were pulled and analyzed for carbon and nitrogen isotope ratios. The patterns of isotope ratios were compared among the vibrissae, particularly the anterior versus posterior whiskers to determine if growth rate varied among seal vibrissae.

Laboratory procedures

Vibrissae were scrubbed with steel wool to remove any debris. The first vibrissae from the harbor seal Norton was segmented at 1.5 mm while the remaining vibrissae were segmented at 2.5 mm intervals. Blood samples were dried for several days at 60°C and then ground for homogeneity. Each sample was

combusted at high temperature using a Europa Roboprep CHN analyzer and the nitrogen and carbon dioxide gases were separated and purified by gas chromatography. All samples were then analyzed for stable carbon and nitrogen isotope ratios with a Europa 20/20 continuous flow isotope ratio mass spectrometer. Results are reported in the standard $\delta^{13}C$ and $\delta^{15}N$ notation. Stable isotope ratios were expressed in the following standard notation:

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

where X is ^{13}C or ^{15}N and R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ respectively. $R_{standard}$ for ^{13}C is Pee Dee Belemnite; for ^{15}N it is atmospheric N_2 (air). Analytical error for samples was approximately ± 0.1‰ for both carbon and nitrogen

RESULTS

Isotopic analyses of blood samples in both captive seals and sea lions showed a rapid increase in both $\delta^{13}C$ and $\delta^{15}N$ following administration. The nitrogen isotope ratio changes were the most pronounced and reflected the relative quantities of the element in the body composition. Decreases in the blood $\delta^{13}C$ over time indicated loss of the isotope through respiration or excretion and incorporation into body proteins. Vibrissae growth rates are summarized in Table 2.

Harbor seals

The vibrissae of the first harbor seal tested, Norton, showed only one peak after 143 days (29 August 1996) following injection of the labeled glycine in January 1996. An identical peak was in the same location after an additional 68 days (November 1996) (Fig. 1). The second harbor seal (Peter) had the label administered in June 1996 and had a whisker cut in November after 155 days. No marker was evident in Peter's whisker (Fig. 2) but the large quantities of stable isotopes from the label were visible in the blood samples confirming that the seal did receive the labeled amino acid (Fig. 3). No marker was evident in either seal from the time between the last administered label (June 1996) and the last cutting of the whiskers, 765 days later (July 1998) (Figs. 1 and 2). Because Peter did not receive a labeled injection

in January and Norton demonstrated only a single isotopic peak, the labeled glycine peak evident in Norton's whiskers was thought to have resulted solely from the January injection.

The blood serum washout in Figure 3 showed evidence of the carbon isotope ratios returning to pre-injection levels thirty days later while the nitrogen isotope ratios maintained a slight enrichment. The starting point of the increasing isotope ratios in Norton's vibrissae until the point when the isotope ratios returned to constant levels was presumed to be a thirty day period and the growth rate during this period from mid-January to mid-February was 0.70 mm/day. If the growth rate remained constant for the entire vibrissae, Norton's whiskers represented growth from December 1995 to mid-April 1996 and then appeared to stop. Growth had not resumed as of early November when the second whisker was analyzed. A third whisker removed from Norton 20 months later showed no evidence of any carbon and nitrogen enrichment.

An adult harbor seal from southeastern Alaska that was originally tagged 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 that seven months, the whiskers had a calculated growth rate of 0.07 mm/day assuming continually growth (Fig. 4). In 1997 a second harbor seal was recaptured and a whisker removed for isotope analysis two years after a whisker was initially sampled. No overlap in isotope ratios occurred between the two vibrissae. A third harbor seal, a yearling, was also recaptured one year after it had been initially sampled as a pup and the stable isotope ratios showed no similarity or overlap between the two vibrissae. The subsistence-harvested seal showed no distinct change in isotope ratios between anterior and posterior vibrissae and between vibrissae along the left and right sides of the muzzle (Fig. 5).

Steller sea lions

The two captive sea lions each received two doses of labeled glycine as shown in Table 1. Whiskers were allowed to grow over a 610 and 735 day period, respectively. The first sea lion, Lucia, received one dose of doubly-labeled and one dose of ^{15}N-labeled amino acid. The stable isotope ratios in the vibrissae showed both δ^{15}N and δ^{13}C in the first enriched peak and the single label of δ^{15}N in the second peak (Fig. 6). Stella's vibrissae exhibited two enriched peaks in the δ^{15}N that represented the two

doses of ^{15}N-labeled glycine administered (Fig. 7). Growth rates between the two markers ranged from 0.05 - 0.07 mm/day for both sea lions. The vibrissae were retained by the sea lions for more than 735 days and these data imply the sea lions retain their whiskers for several years.

Six juvenile Steller sea lions held in captivity at the Vancouver Aquarium in British Columbia, Canada, had their vibrissae clipped periodically during a three year period. One animal had two whiskers cut successively which had an adequate overlap of growth to estimate its rate of growth. The daily growth rate, averaged over fourteen months, was 0.14 mm/day (Fig. 9). A second sea lion had a much shorter overlap of growth in two successive whiskers. The daily growth rate for the second animal, averaged over two winter months, was 0.17 mm/day.

The growth rates of these captive sea lions were compared with the limited growth information for Steller sea lions in the wild. Subadult and adult sea lions (n = 27) sampled from wild stocks had consistent isotopic oscillations along their vibrissae with growth rates ranging from 0.05 - 0.18 mm/day and averaging 0.10 - 0.14 mm/day, assuming that the major oscillations evident were annual (Fig. 8). These regular isotopic oscillations (we observed up to seven on a vibrissae) seem to indicate the animals continue to grow their whiskers for several years before the whiskers are broken or lost. Oscillation length varied from animal to animal and year to year. All the sea lions sampled in the Gulf of Alaska were adult females while 72% of the sea lions from the Pribilof Islands in the Bering Sea were less than 5 years of age and almost exclusively male. Growth rates averaged over twelve months were 0.11 - 0.12 mm/day for all sea lions combined. The subsistence-harvested sea lion 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 experiments indicate that the vibrissal growth characteristics between harbor seals and sea lions are remarkably different. The harbor seal growth rates indicated an irregular growth rate throughout the year and this may also apply to different vibrissae on the muzzle. The harbor seal had only one peak after being given one dose in January and the second

application in June was not evident in either of the two seals. Rosen and Renouf (1995) observed an 84% increase in the resting metabolic rates (RMR) of captive adult harbor seals from November to April and a higher than average RMR than the August estimates for the animals. These variations in metabolic rates may have some connection to the rapid growth of the vibrissae from Norton which appeared to have grown from the end of November until mid-April. Because the peak remained in approximately the same location on whiskers sampled from Norton in August and November, growth was assumed to have decreased to some minimal level or ceased altogether some time prior to June. At some time during the next twenty months, however, most or all of the whiskers were lost and the marker was no longer evident.

Recaptured wild seals

The vibrissal growth rate in the recaptured adult wild seal was one-tenth the spring growth rate of the captive seal. The first sampling of the wild seal took place at the end of September 1996 at which time the seal showed signs of having completed molting. The two other recaptured seals showed no evidence of similarity in their natural patterns of isotope ratios from the previous one and two years respectively. The two vibrissae analyzed from the seal following recapture two years later showed close similarities in the patterns indicating growth during the same feeding times. These data lend support to the idea that harbor seals may be lose all or most of their vibrissae annually. Bowen (pers. comm.) 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 on grey seals throughout the year. Growth rate in the harbor seal vibrissae is assumed to be consistent among all the vibrissae.

Vibrissae from Steller sea lions at the Vancouver Aquarium had been collected when the animals ranged in age from two to four years old. Periodic changes in the animals' diets are evident in the shifts in the stable isotopes along their vibrissae. The growth rate in the juvenile sea lions was twice the rate exhibited by the adult sea lions and metabolic studies on various mammalian species have shown higher metabolic rates in juvenile animals versus adults of the same species (Schmidt-Nielsen 1979). The increased growth rates in the juvenile sea lions may not be unusual.

Vibrissae growth rates in wild adult sea lions had a range equal to the captive animals. The isotopic oscillations in the vibrissae from wild adult sea lions throughout the Bering Sea and Gulf of

Alaska resulted from feeding on prey in different geographic regions with different isotopic signatures. Sea lions seasonally travel hundreds of kilometers from haul-out sites to feeding locations, returning to the same rookery each year to breed (Merrick 1995, Merrick et al. 1997). These behaviors resulted in the oscillating patterns along the vibrissae and, likewise, each isotopic oscillation represented one years' forage information. The similar vibrissae growth rates in both wild and captive sea lions, combined with the repetitive isotopic patterns in wild sea lions, provide evidence that sea lions retain their vibrissae for several years and likely replace them only when broken or worn.

ACKNOWLEDGMENTS

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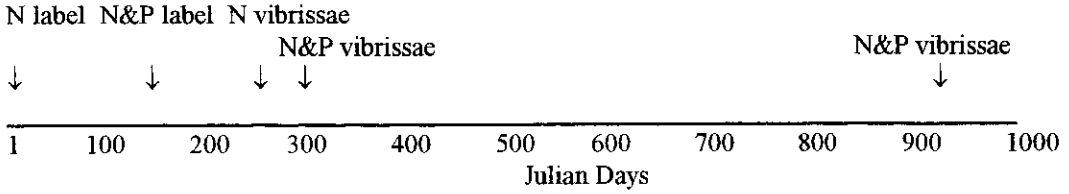
Table 1. Sequence of vibrissae growth rate experiment in harbor seals, Norton and Peter, and in Steller sea lions, Lucia and Stella, 1 January 1996 through 9 July 1998.

	Norton (N)	**Peter (P)**	**Lucia (L)**	**Stella (S)**
Delivery	Injection	Injection	Injection	Injection
Glycine label	2 - $^{13}C\&^{15}N$	1 - $^{13}C\&^{15}N$	1 - $^{13}C\&^{15}N$ 1 - ^{15}N	2 - ^{15}N
Tissues	Blood, Vibrissae	Blood, Vibrissae	Blood, Vibrissae	Blood, Vibrissae
Delivery date	9 Jan. 1996 4 June 1996	4 June 1996	18 June 1996 22 Apr. 1997	20 Aug. 1996 22 Apr. 1997
Sample date (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

Harbor seals (Norton and Peter)



Steller sea lions (Lucia and Stella)

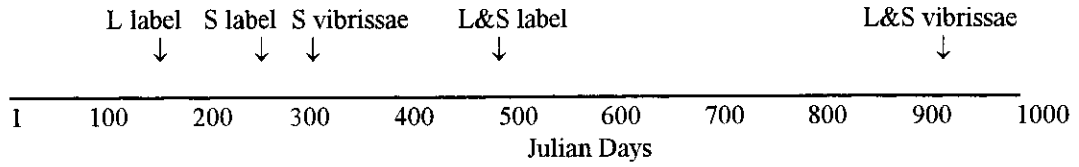


Table 2. Vibrissae growth rates in harbor seals and Steller sea lions.

Species	Location	Age	Aver. growth (mm/day)
Harbor seals	captive (Mystic, CT)	adult	0.37 - 0.60
	wild (Alaska)	adult	0.07
Steller sea lions	captive (Mystic, CT)	adult	0.05 - 0.09
	captive (Vancouver, BC)	juvenile	0.14 - 0.17
	wild (Alaska)	adult	0.10 - 0.14

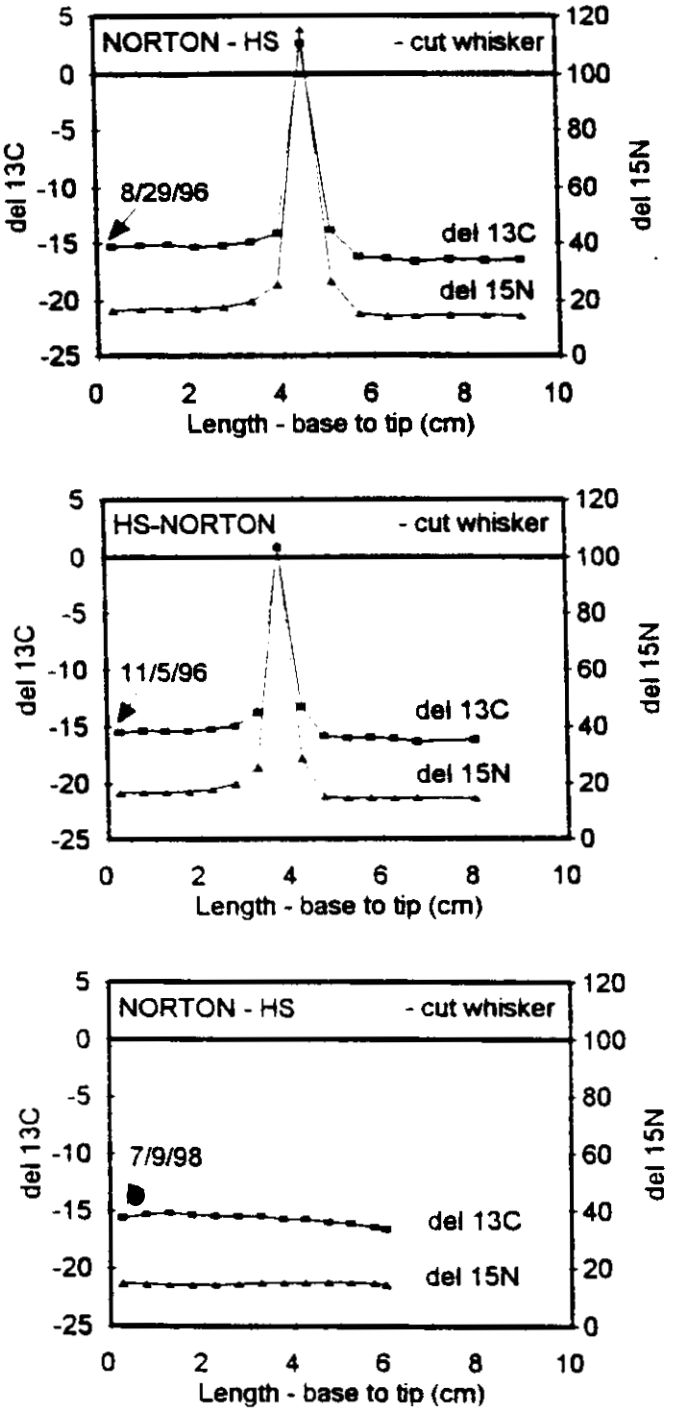


Fig. 1. Stable isotope plots of vibrissae from an adult harbor seal, Norton, in captivity. The doubly-labeled ($\delta^{13}C$ and $\delta^{15}N$) glycine peak is visible in the vibrissae cut in August 1996 (top). The vibrissae cut in November 1996 reveals the same peak in approximately the same location (middle). No peak is evident from a vibrissae cut in July 1998 (bottom).

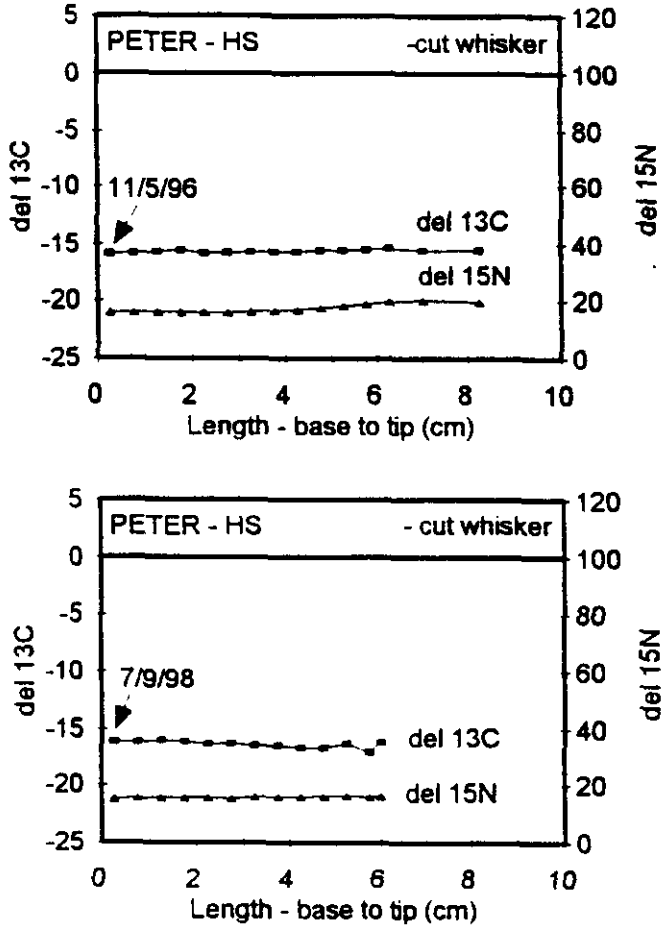


Fig. 2. Stable isotope plots of vibrissae from an adult harbor seal, Peter, in captivity. No glycine peak was visible in the vibrissae cut in November 1996 (top) or in the vibrissae cut in July 1998 (bottom).

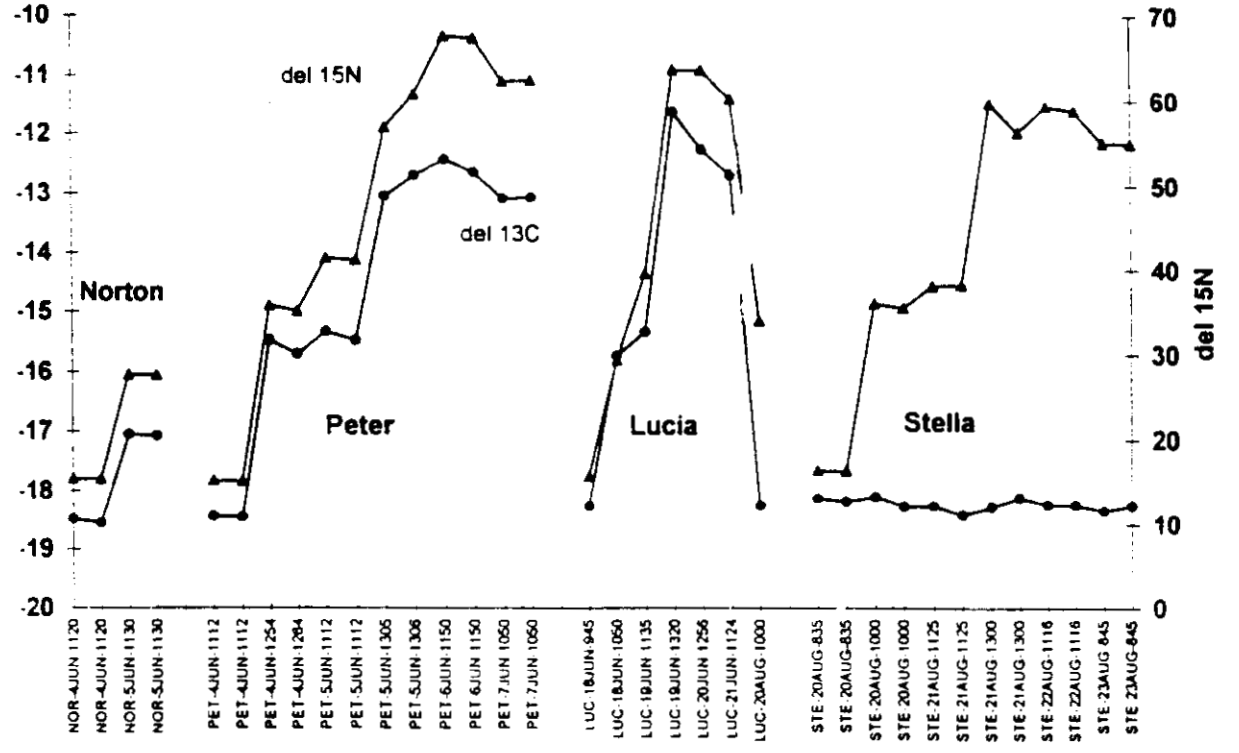


Fig. 3. Stable isotope ratios in blood serum from two captive adult harbor seals, Norton and Peter, and two captive adult Steller sea lions, Lucia and Stella. Serum samples show evidence of the doubly-labeled ($\delta^{13}C$ and $\delta^{15}N$) glycine in the animals' blood streams.

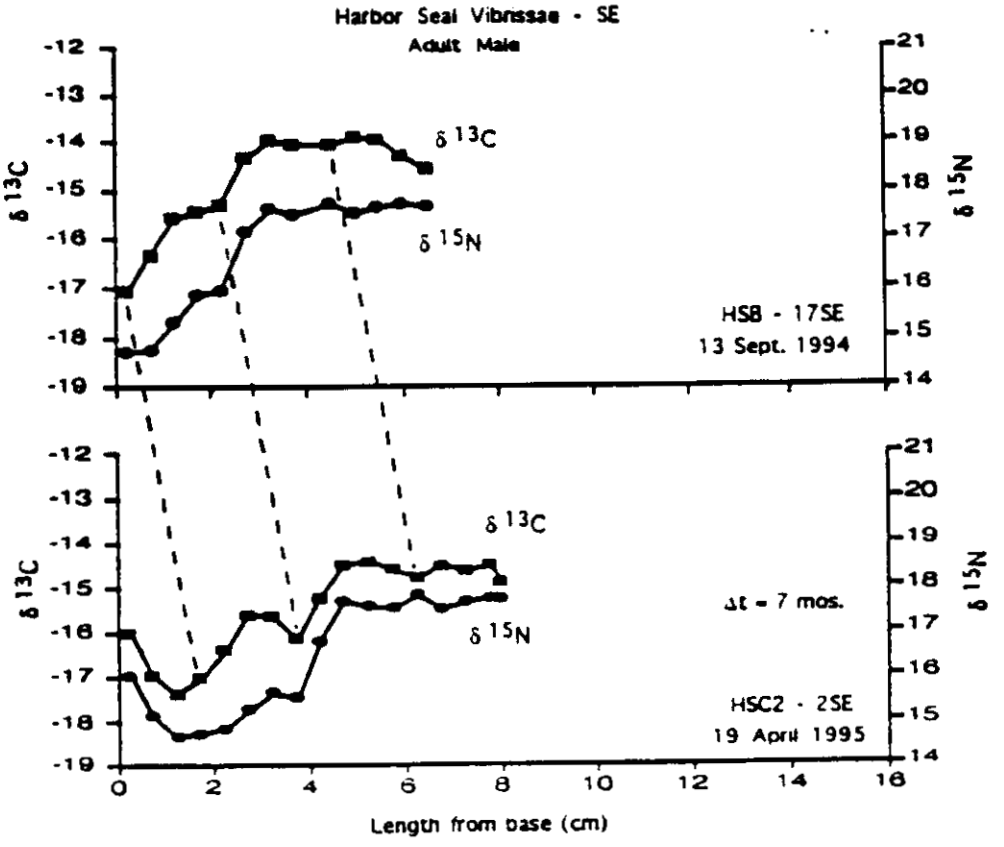


Fig. 4. Stable isotope plots of vibrissae from a recaptured adult harbor seal in southeastern Alaska. A vibrissae sampled in September 1994 (upper plot) is contrasted with a vibrissae taken from the same seals seven months later (lower plot).

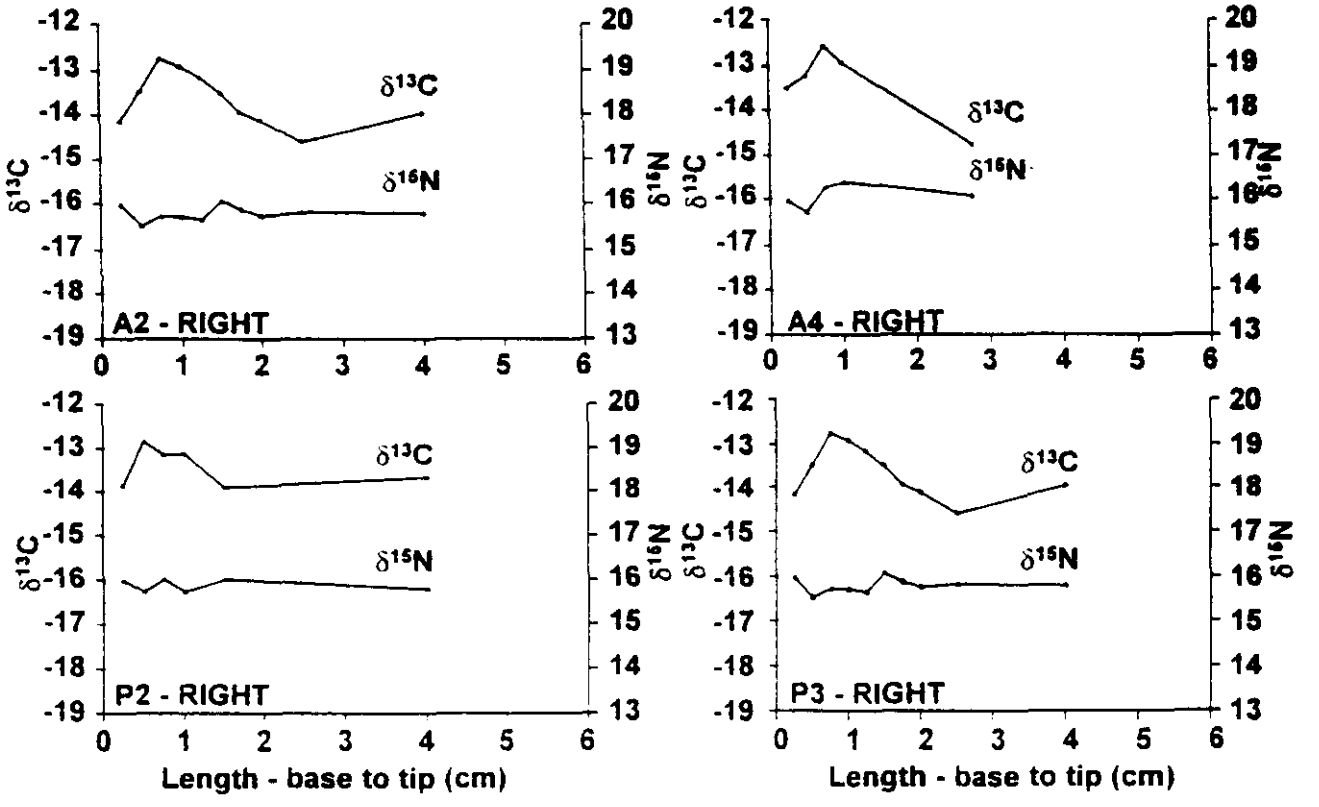


Fig. 5. Stable isotope plots of vibrissae from a killed adult harbor seal in Prince William Sound, Alaska. Anterior (top plots) and posterior (lower plots) vibrissae and left (left plots) and right-sided (right plots) vibrissae were contrasted.

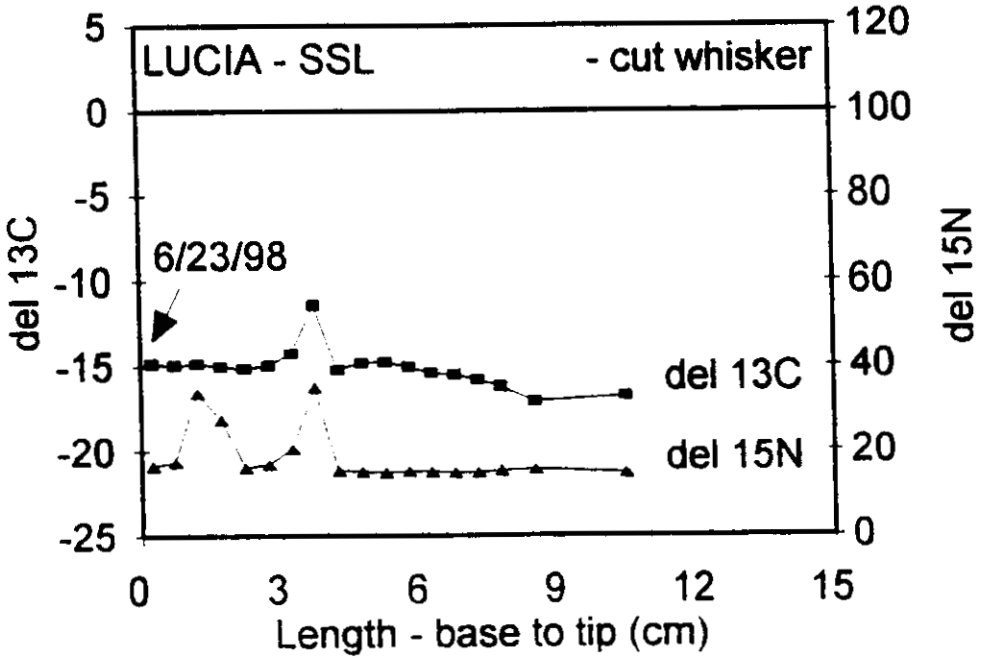


Fig. 6. Stable isotope plot of a vibrissae from an adult Steller sea lion, Lucia, in captivity. The peaks furthest to the right in the $\delta^{13}C$ and $\delta^{15}N$ represent the doubly-labeled glycine administered in June 1996 while the left peak in the $\delta^{15}N$ represents the $\delta^{15}N$-labeled glycine administered in April 1997. The vibrissae was cut in June 1998.

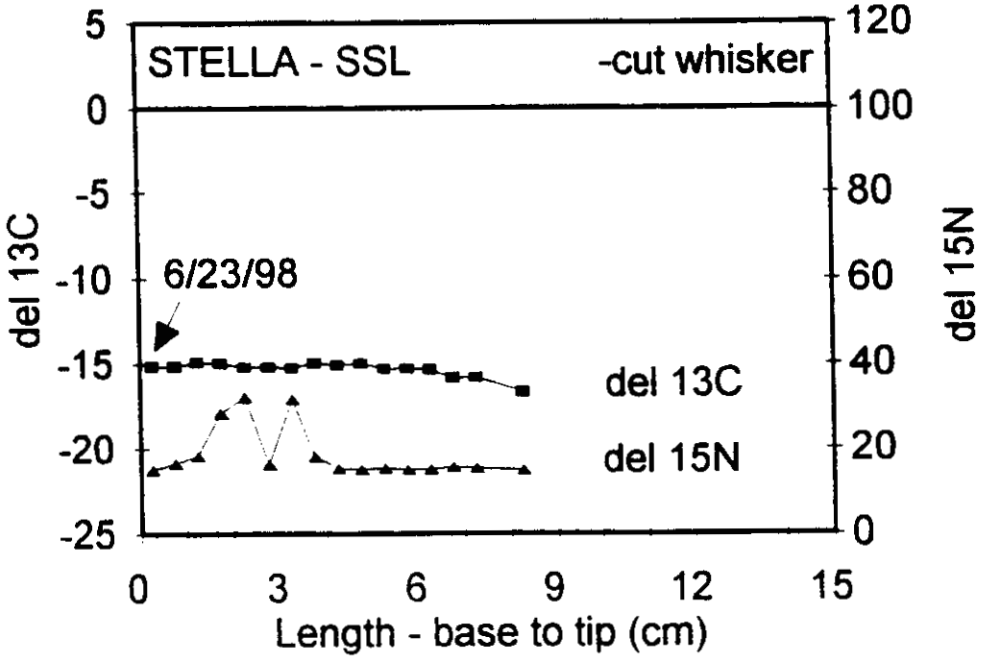


Fig. 7. Stable isotope plot of a vibrissae from an adult Steller sea lion, Stella, in captivity. The peak furthest to the right in the $\delta^{15}N$ represents the $\delta^{15}N$-labeled glycine administered in August 1996 while the left peak represents the $\delta^{15}N$-labeled glycine administered in April 1997. The vibrissae was cut in June 1998.

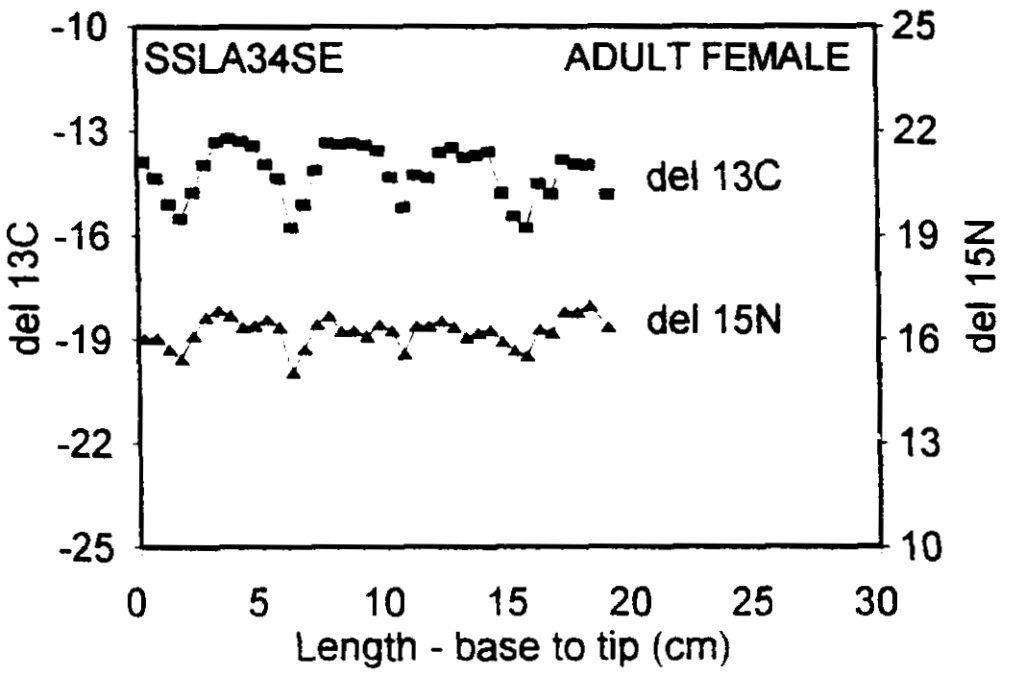


Fig. 8. Stable isotope plot of a vibrissae from an adult Steller sea lion in southeastern Alaska collected June 1993. Each oscillation represents one years' growth in the vibrissae.

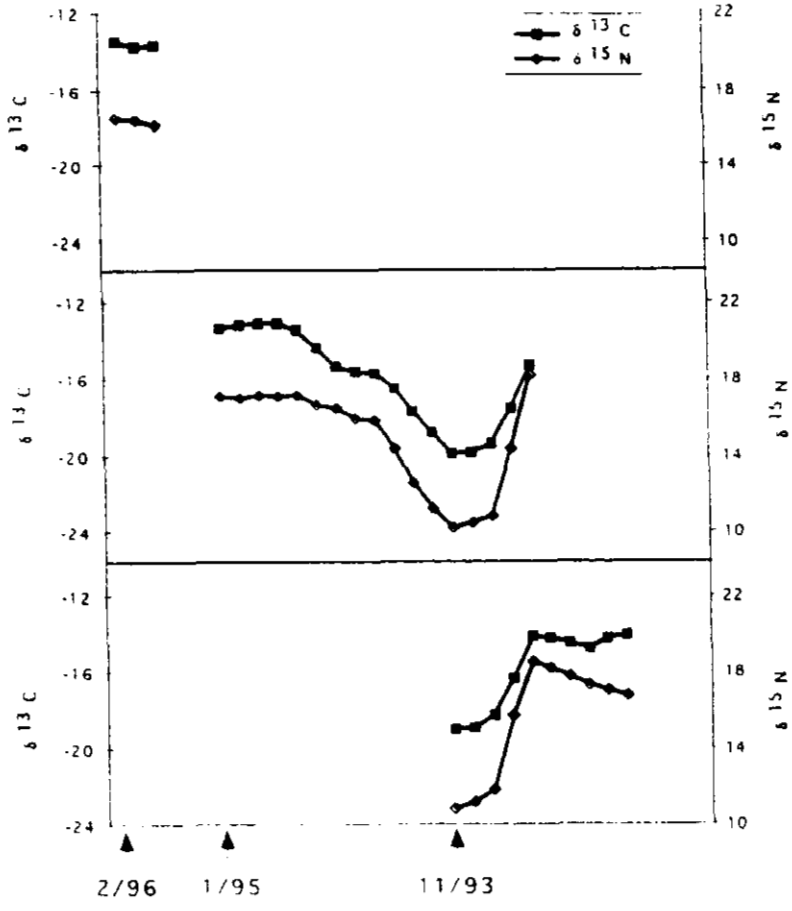


Fig. 9. Stable isotope plots of vibrissae from a juvenile Steller sea lion in captivity. The first vibrissae (furthest to the right) was cut in November 1993. The second vibrissae was cut in January 1995 and shows overlap in growth with the first vibrissae. The third vibrissae (furthest to the left) was cut in February 1996 and was too short to reveal any overlap in growth.

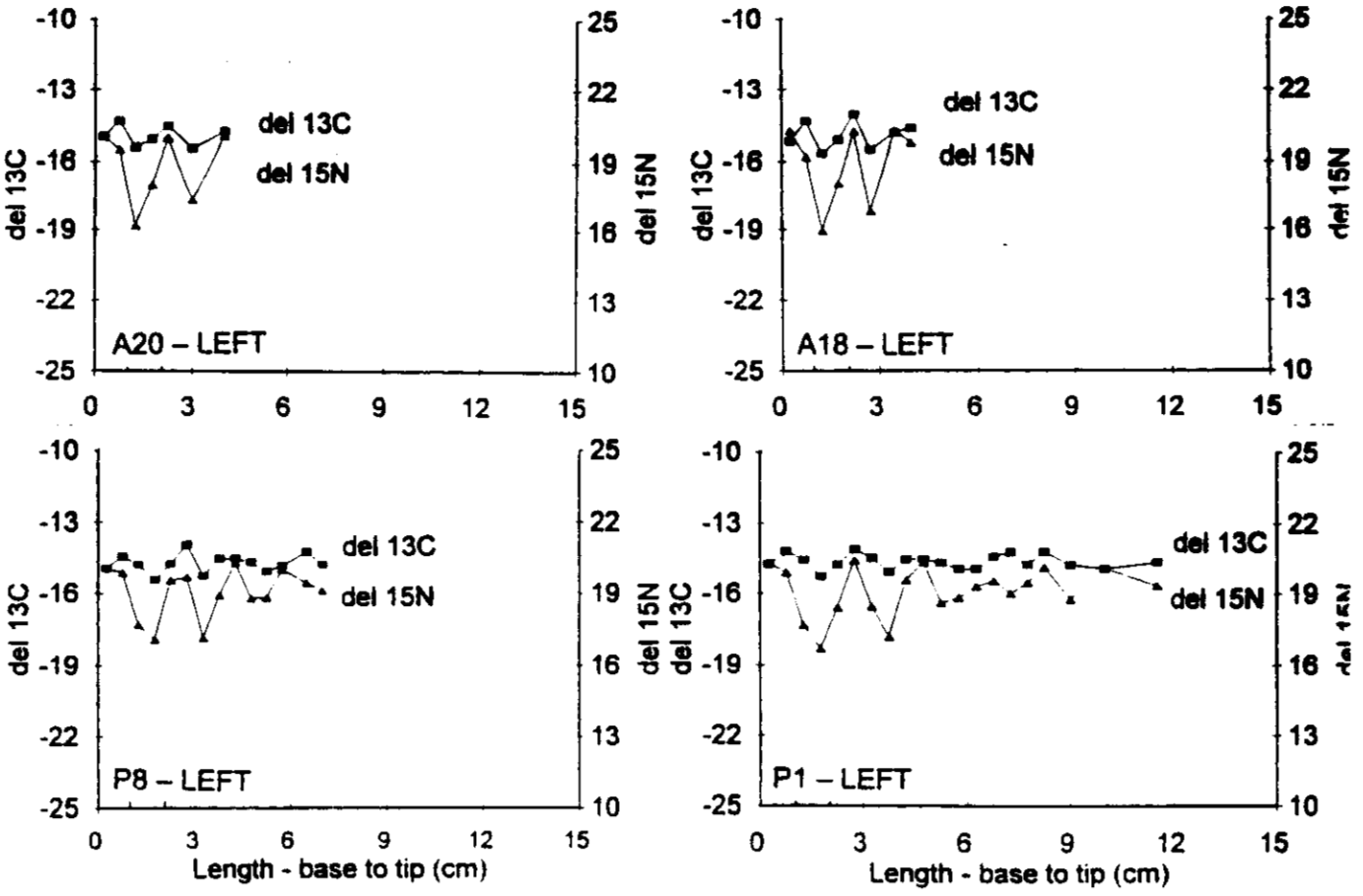
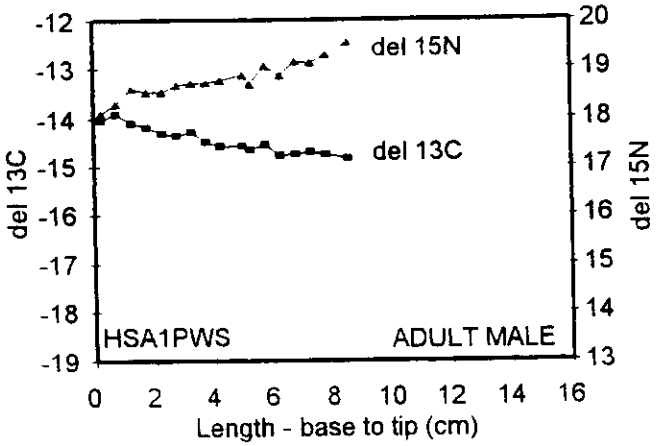


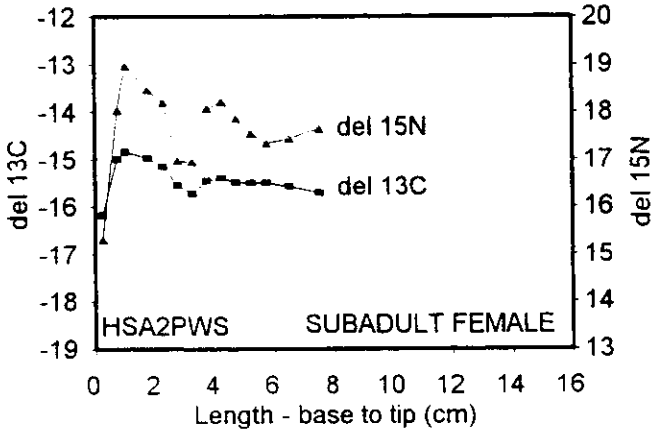
Fig. 10. Stable isotope plots of vibrissae from a killed adult Steller sea lion in the Bering Sea, Alaska. Anterior (top plots) and posterior (lower plots) vibrissae and left (left plots) and right-sided (right plots) vibrissae were contrasted.

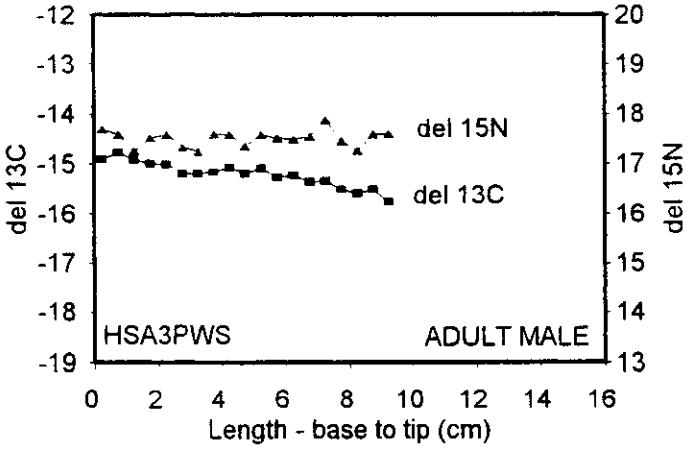
APPENDIX 3

$\delta^{13}C$ and $\delta^{15}N$ in vibrissae of harbor seals from Prince William Sound, Alaska, 1993-1998

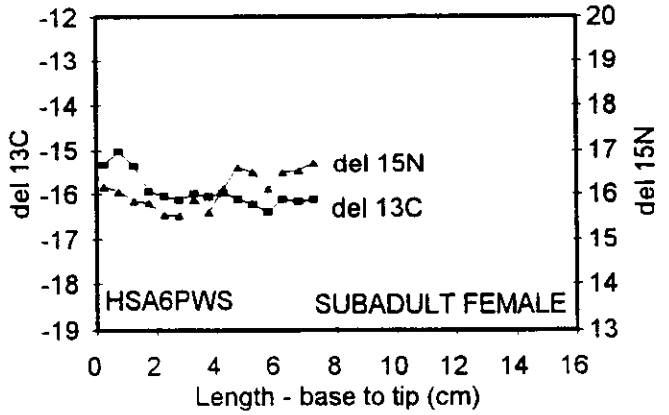
Seal Island, PWS, Alaska - May 1993

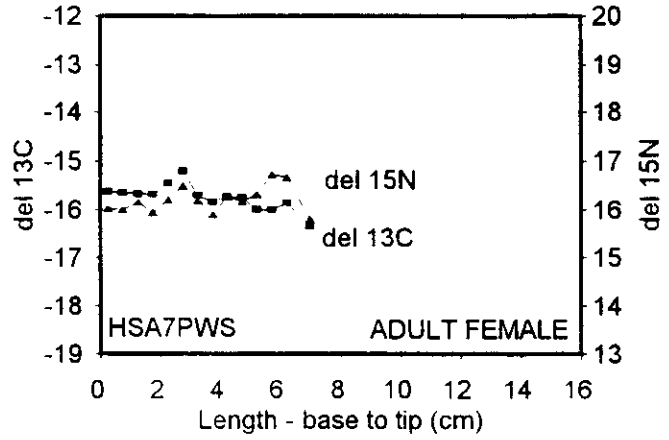


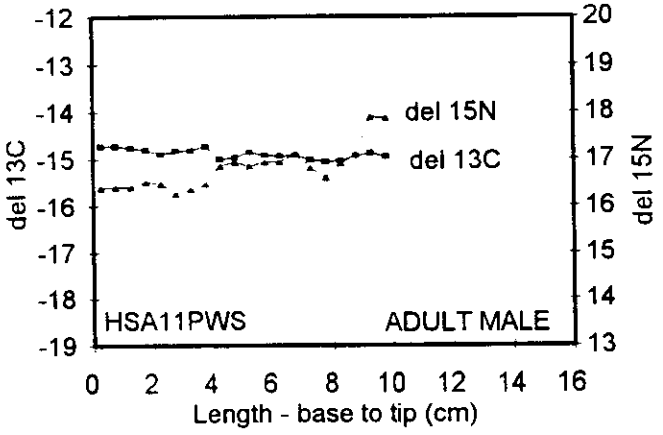




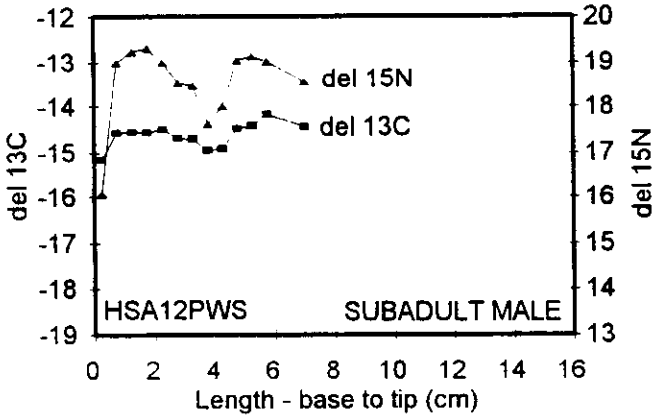
Seal Island, PWS, Alaska - May 1993

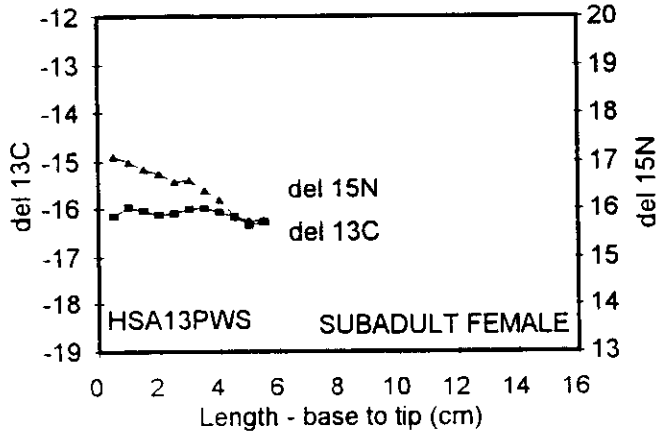




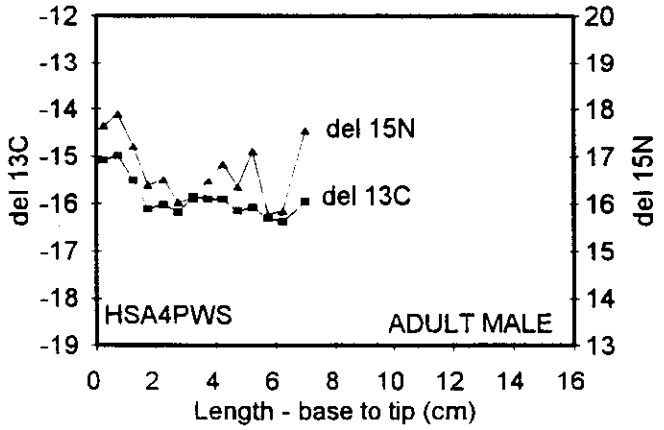


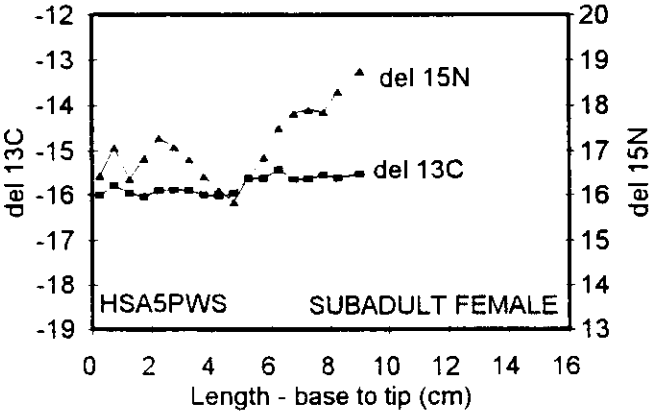
Seal Island, PWS, Alaska - May 1993

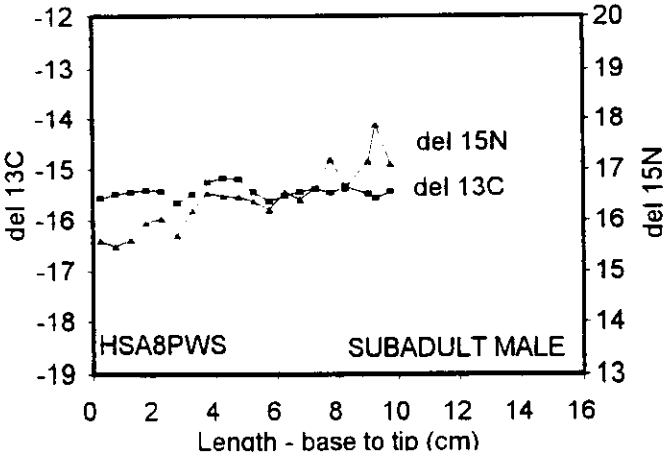




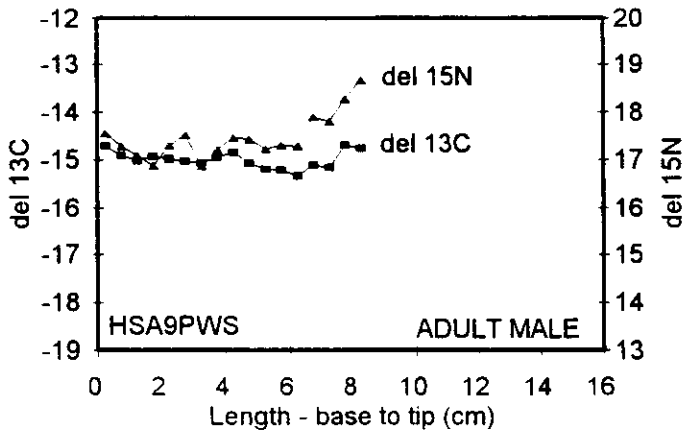
Applegate Rocks, PWS, Alaska - May 1993



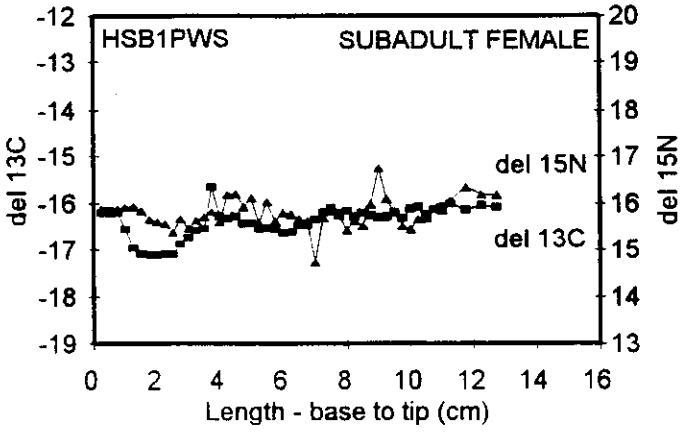


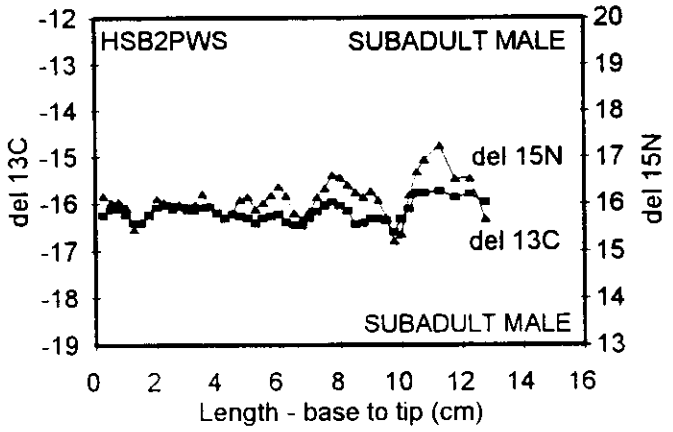


Applegate Rocks, PWS, Alaska - May 1993

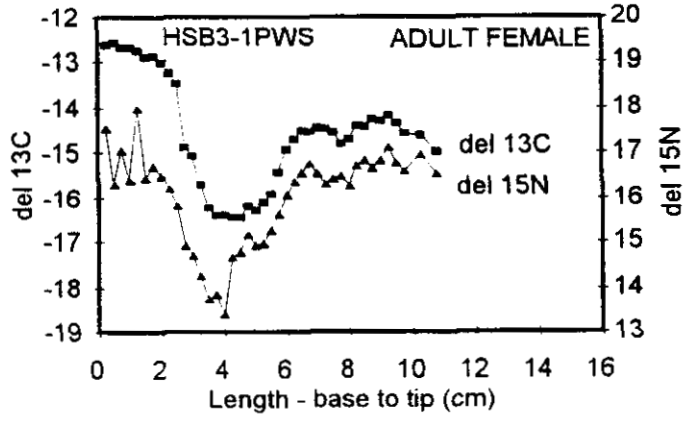


Green Island, PWS, Alaska - April 1994

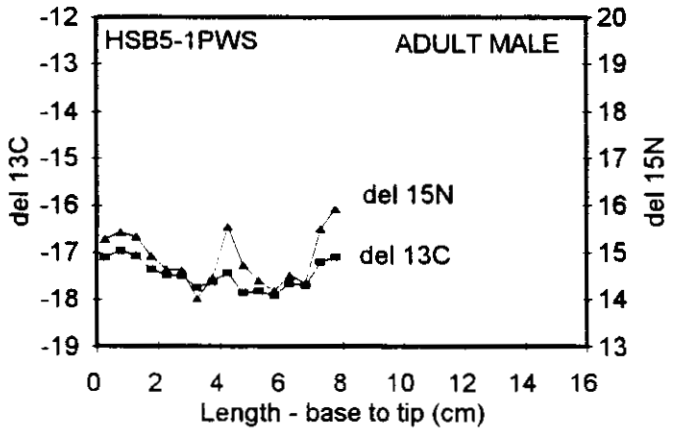


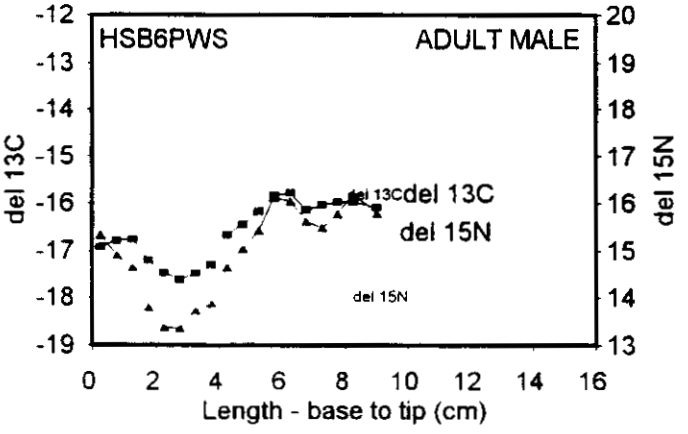


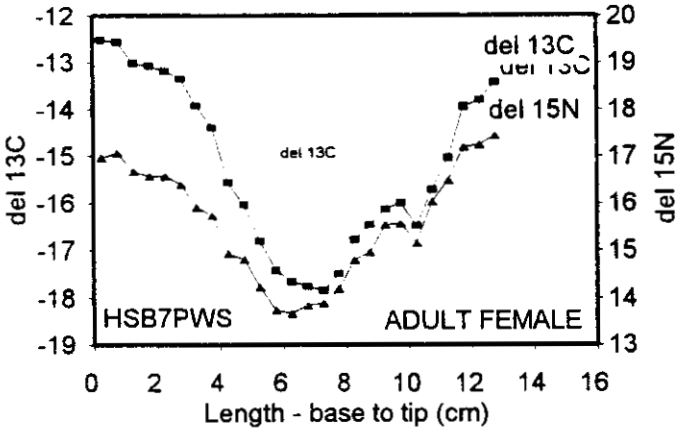
Port Chalmers, PWS, Alaska - April 1994



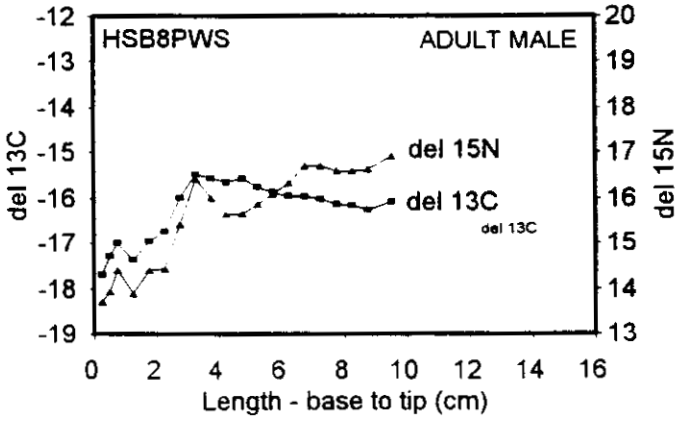
Stockdale Harbor, PWS, Alaska - April 1994

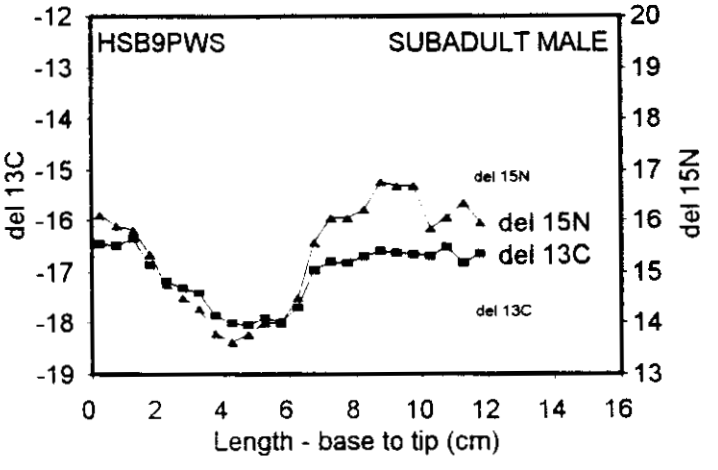


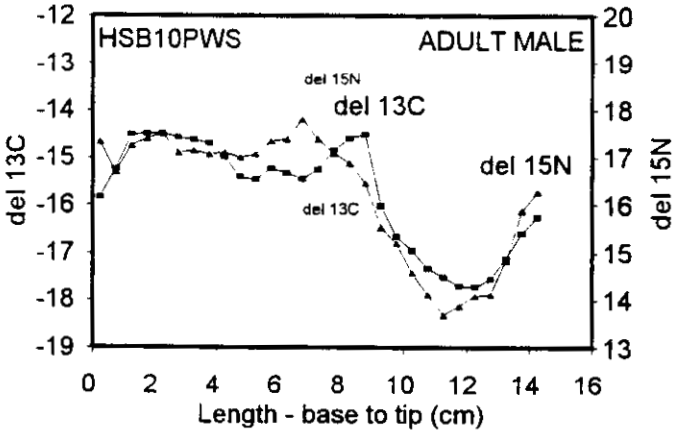




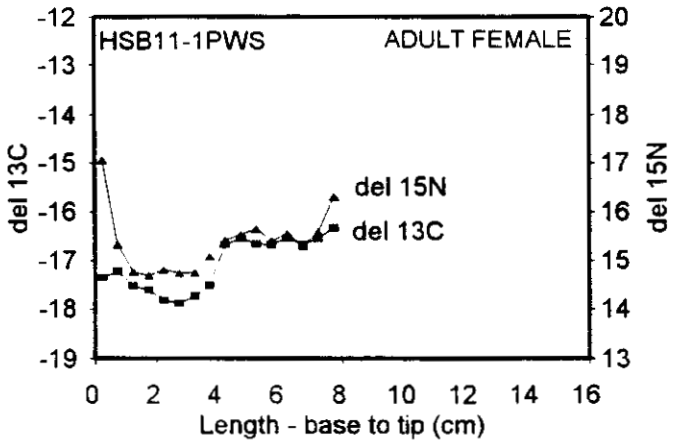
Stockdale Harbor, PWS, Alaska - April 1994

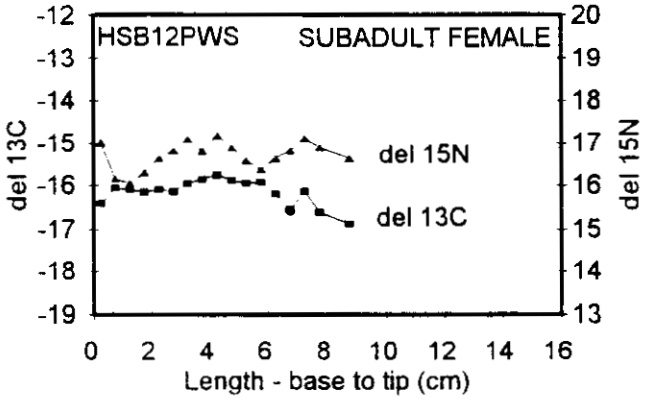


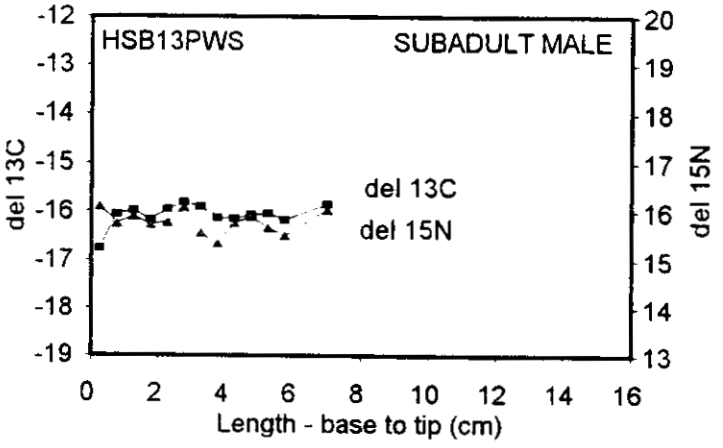




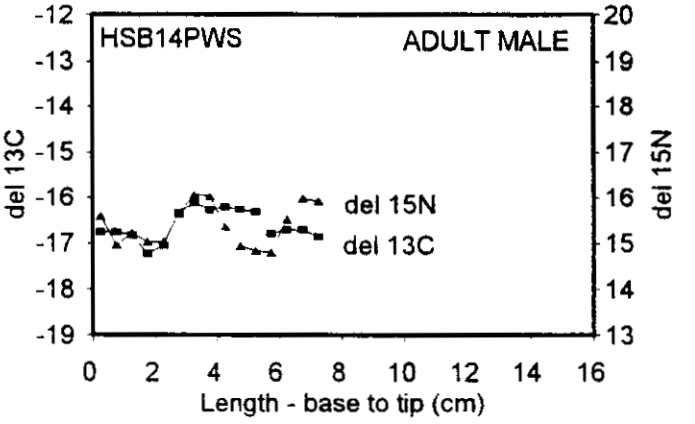
Channel Island, PWS, Alaska - September 1994

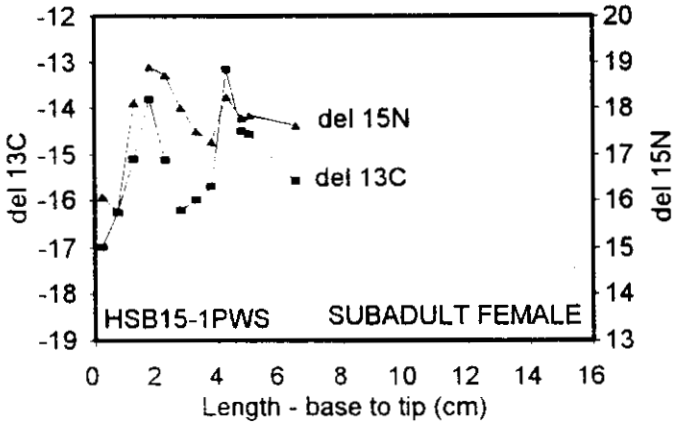


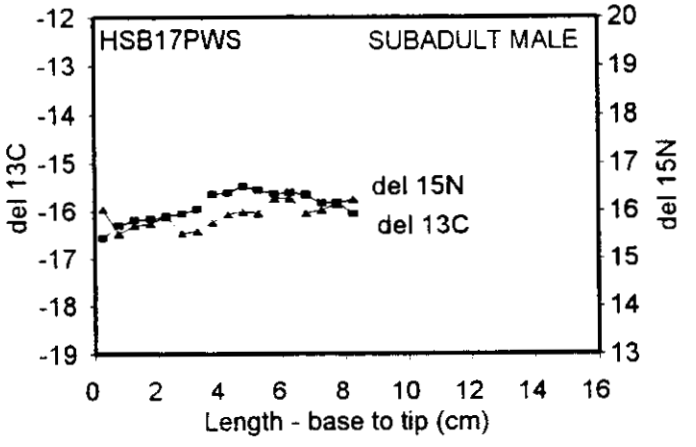




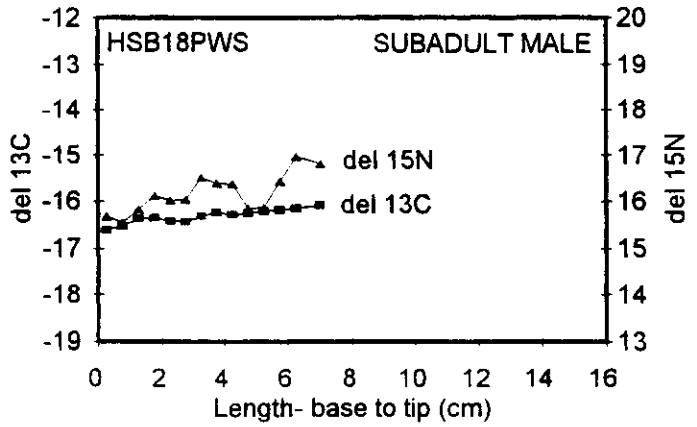
Chaannel Island, PWS, Alaska - September 1994

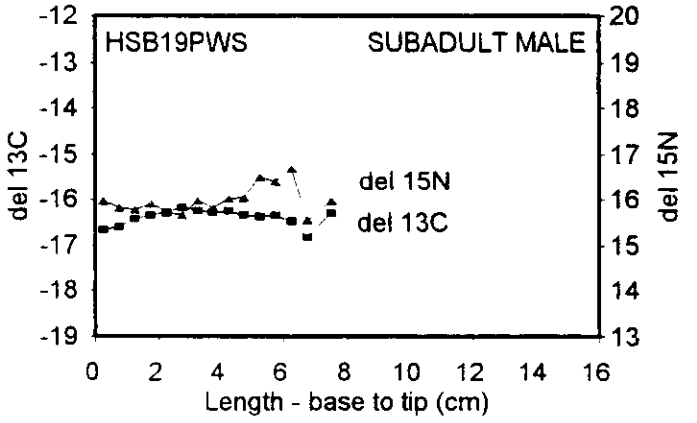


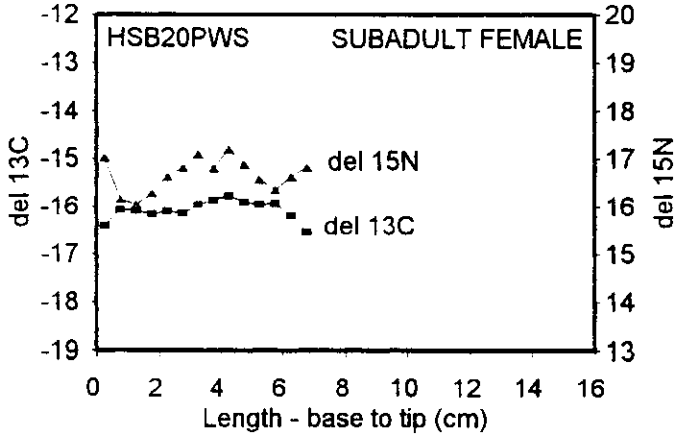




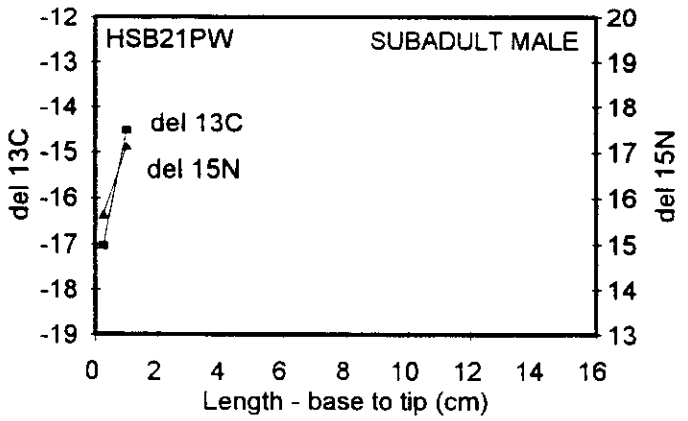
Channel Island, PWS, Alaska - September 1994

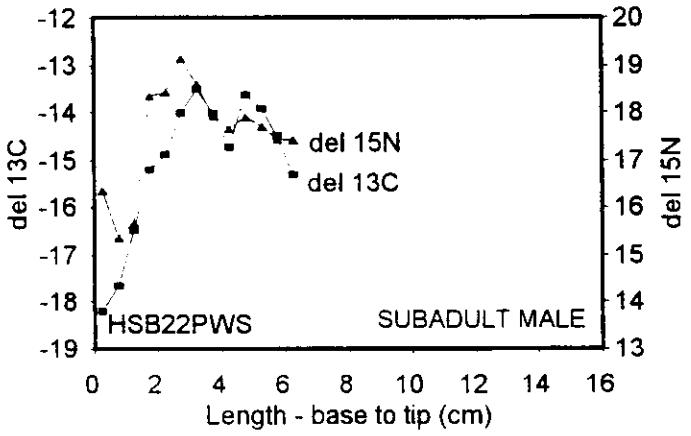


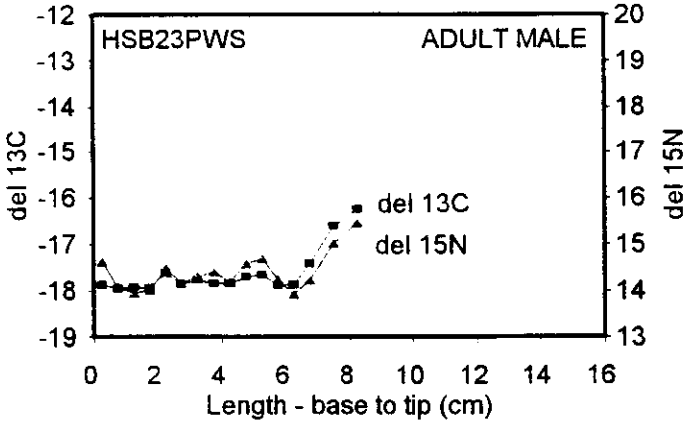




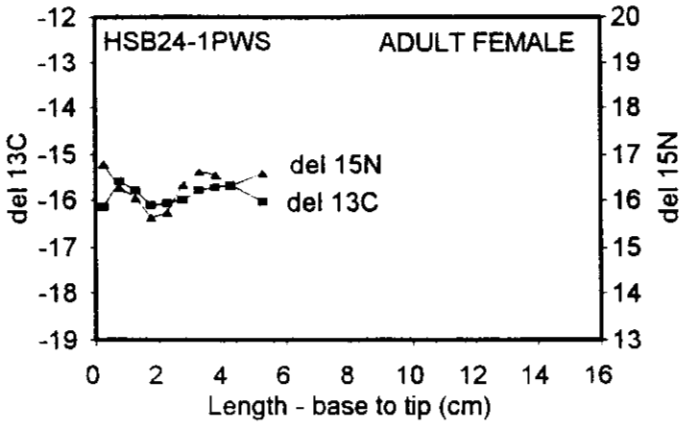
Channel Island, PWS, Alaska - September 1994

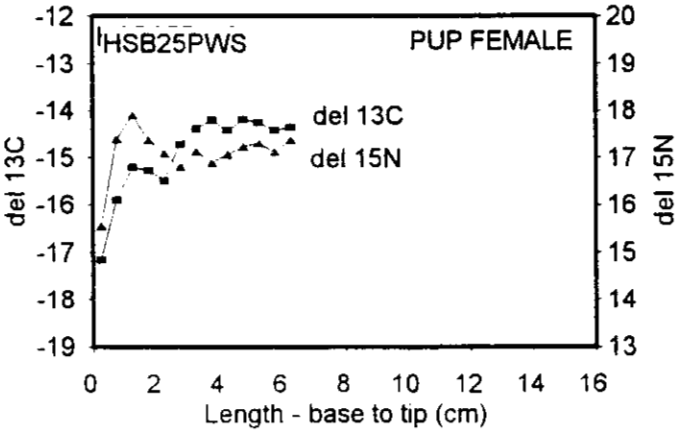


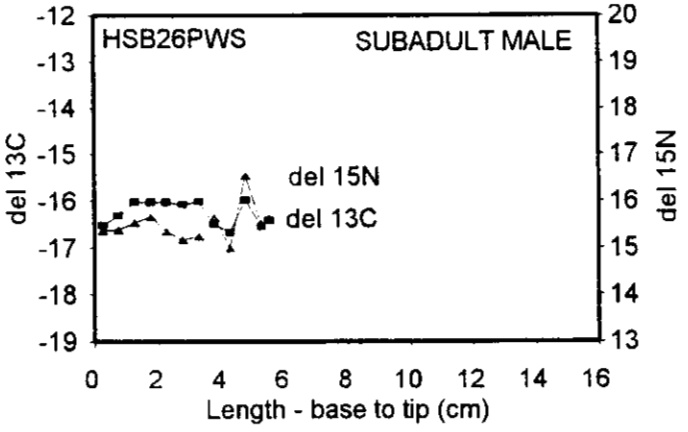




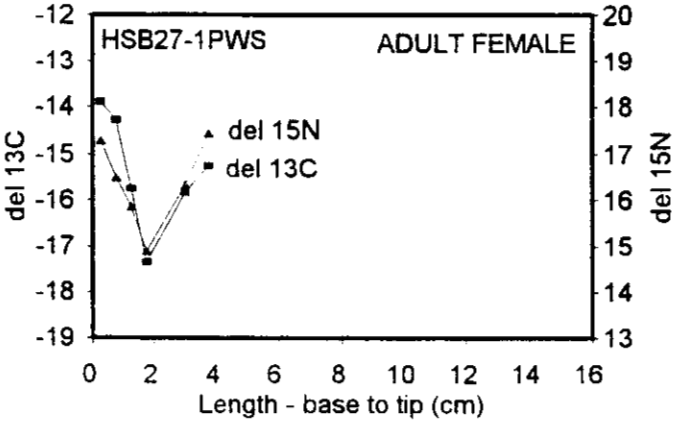
Gravina Island, PWS, Alaska - September 1994

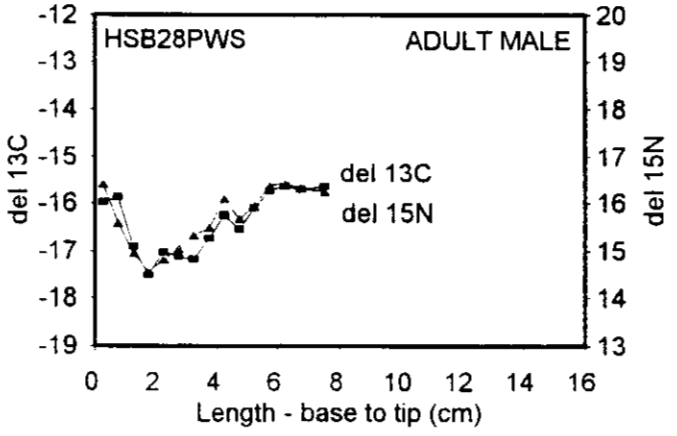


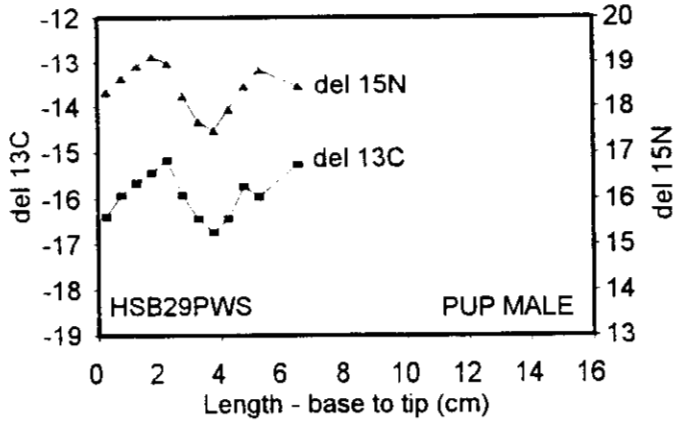




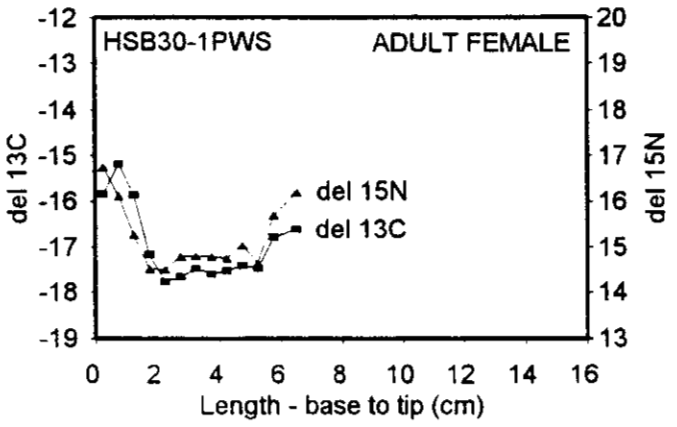
Port Chalmers, PWS, Alaska - September 1994

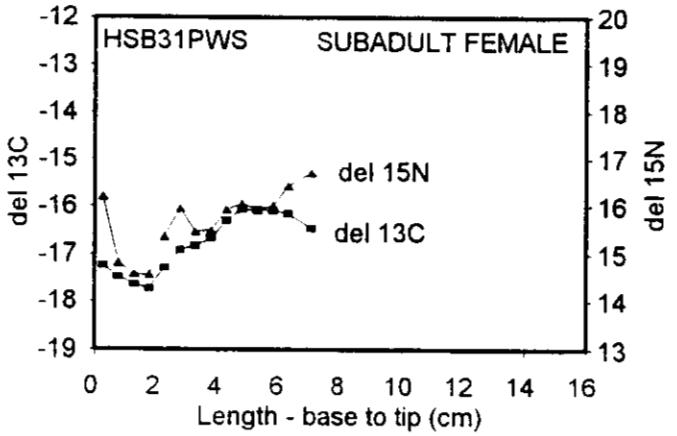


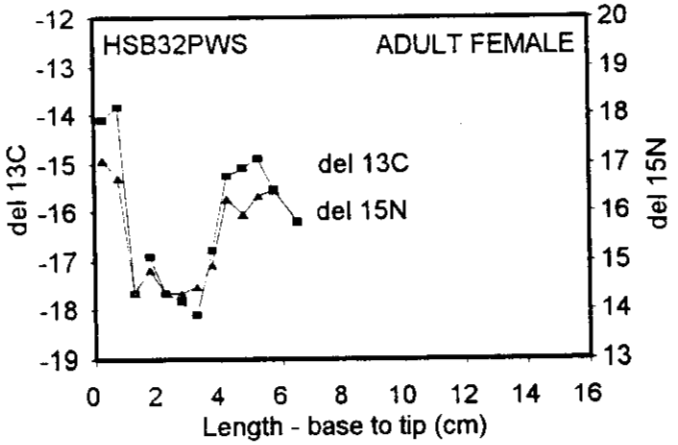




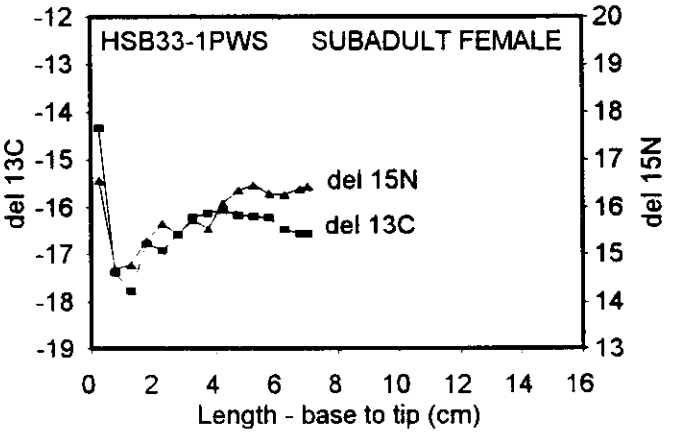
Port Chalmers, PWS, Alaska - September 1994

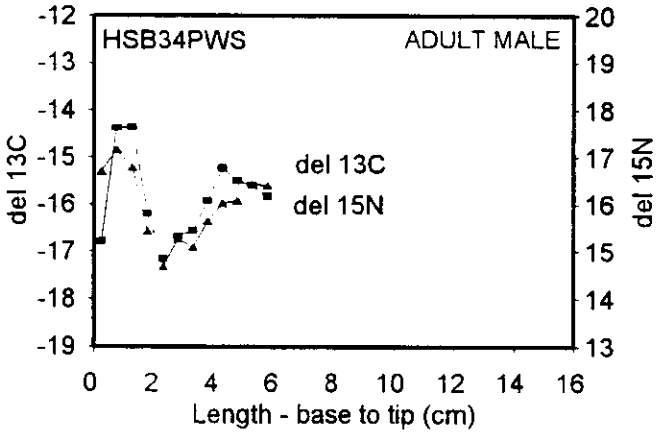


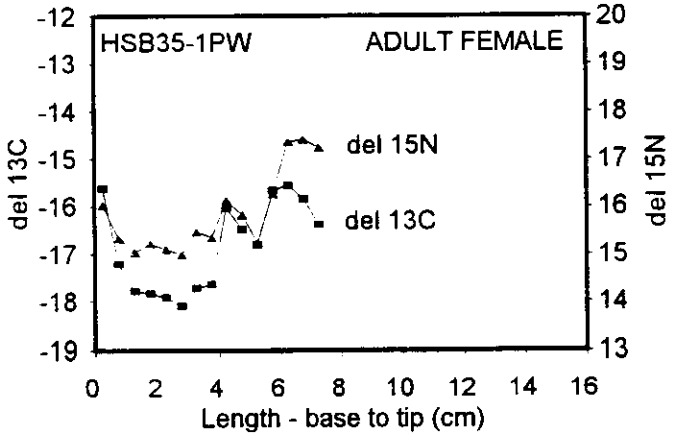




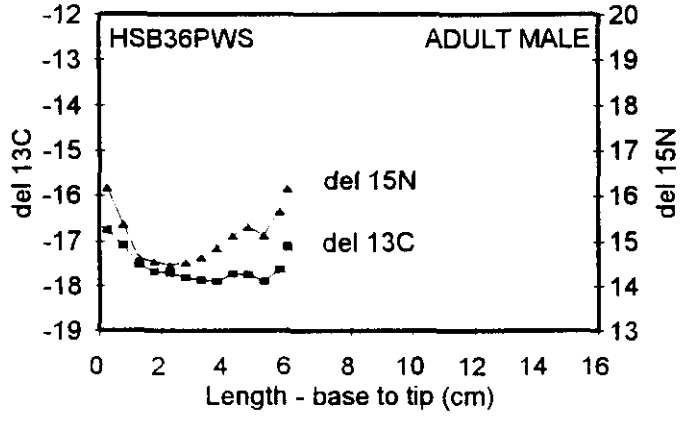
Port Chalmers, PWS, Alaska - September 1994



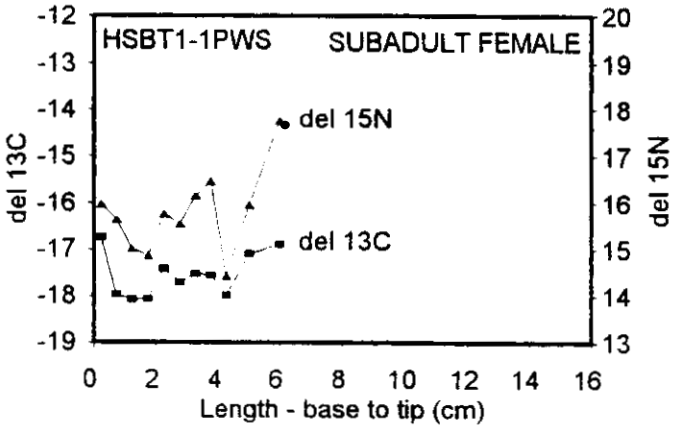


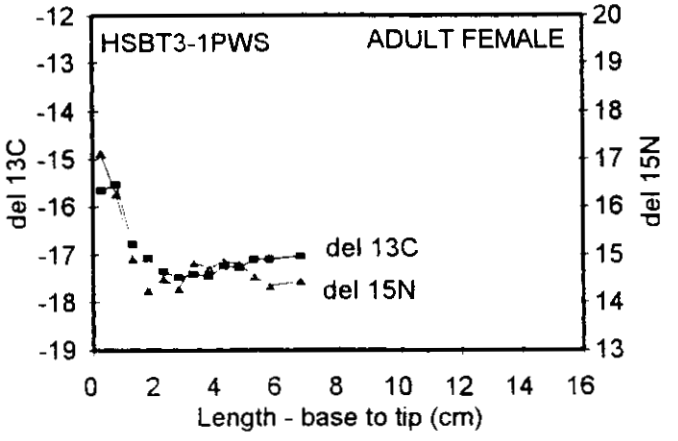


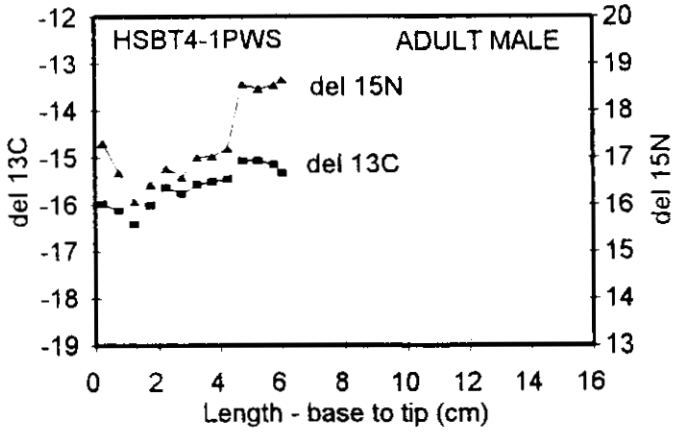
Port Chalmers, PWS, Alaska - September 1994



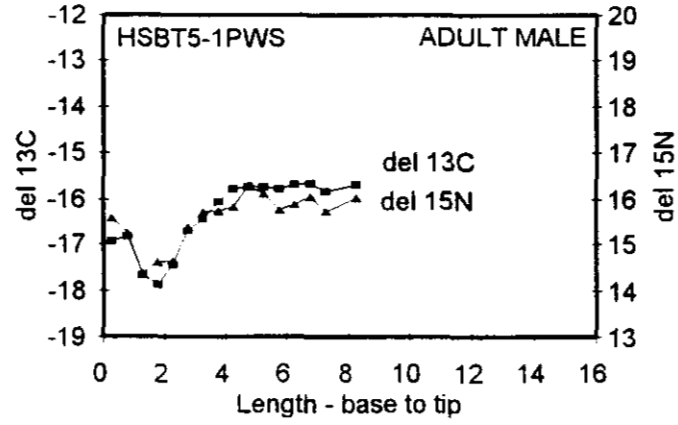
Tatitlik, PWS, Alaska - September 1994

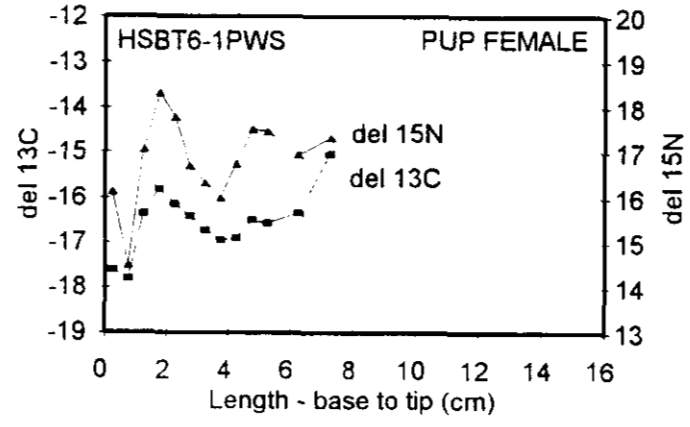


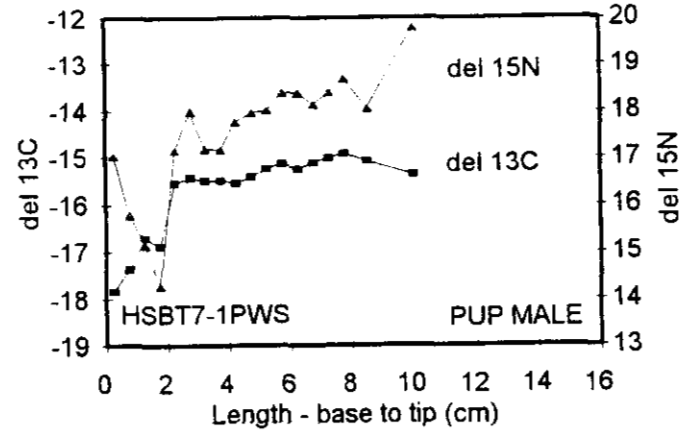




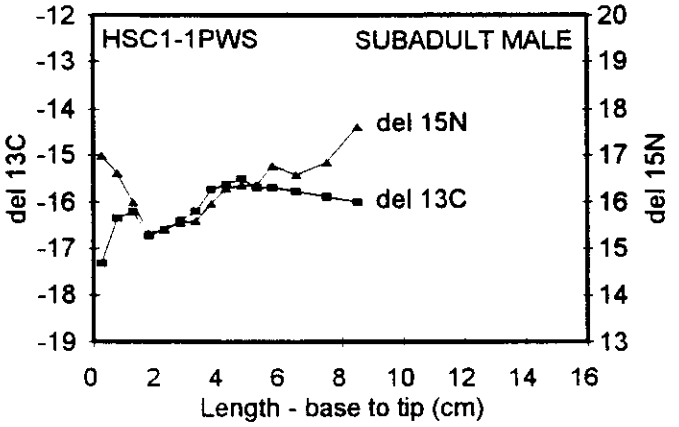
Tatitlik, PWS, Alaska - September 1994

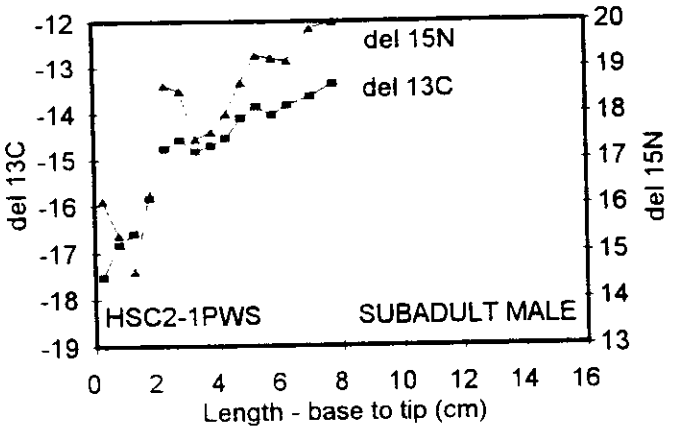


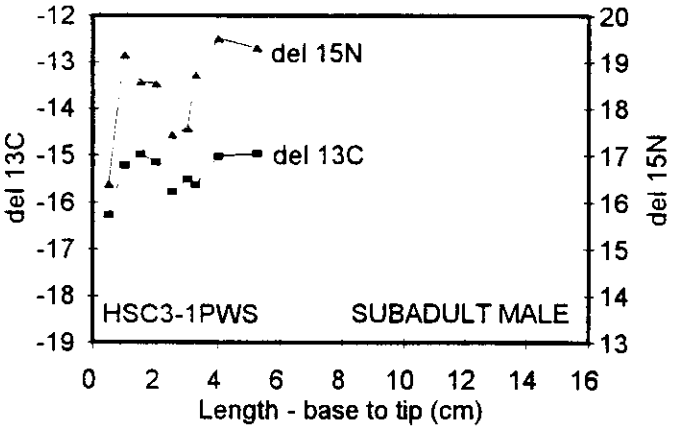




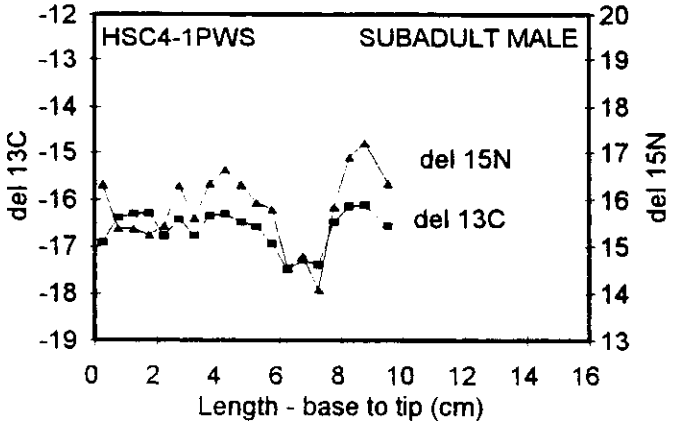
Dutch Group, PWS, Alaska - May 1995

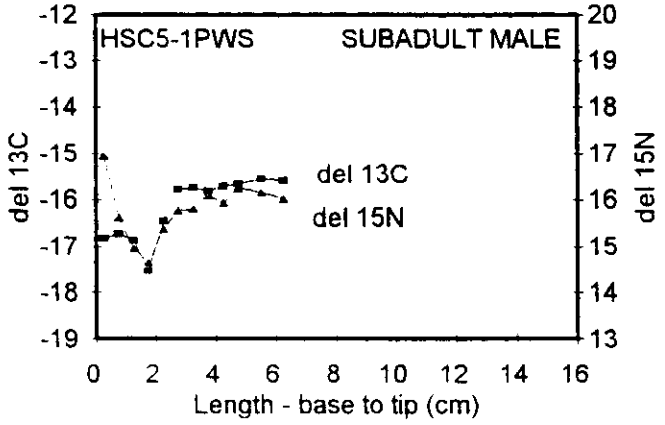




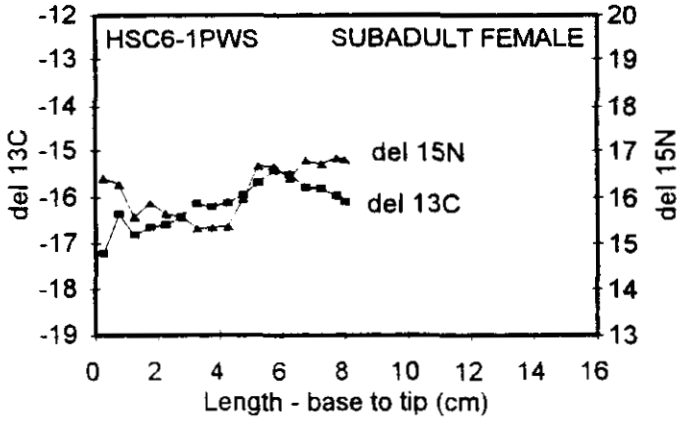


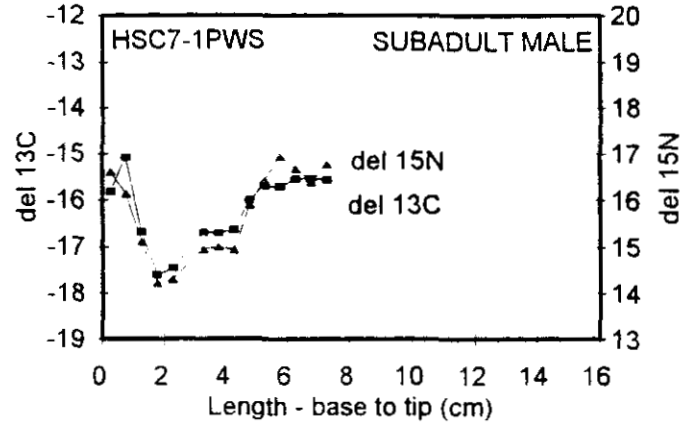
Dutch Group, PWS, Alaska - May 1995



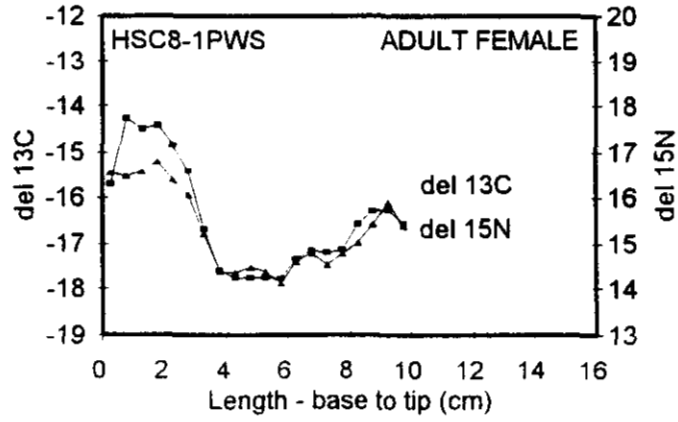


Olsen Bay, PWS, Alaska - May 1995

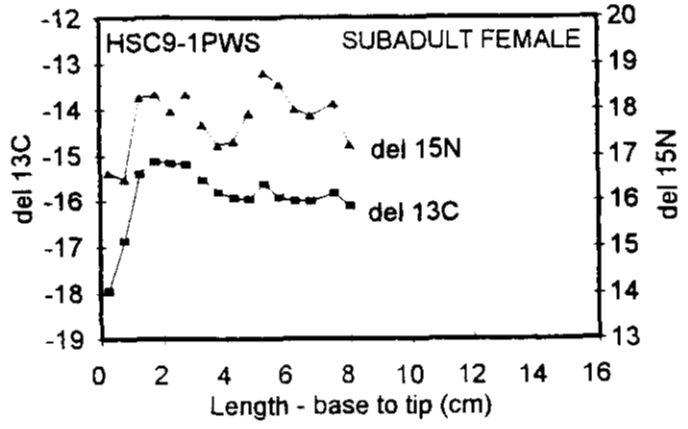


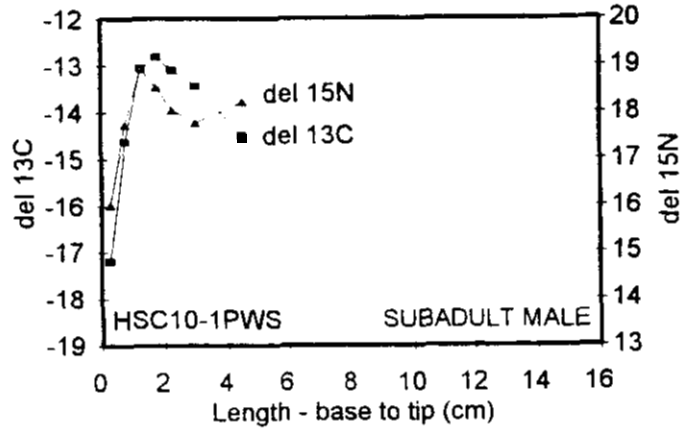


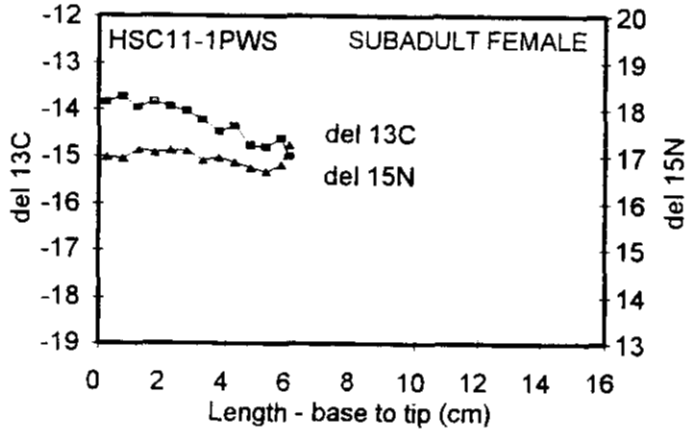
Port Chalmers, PWS, Alaska - May 1995



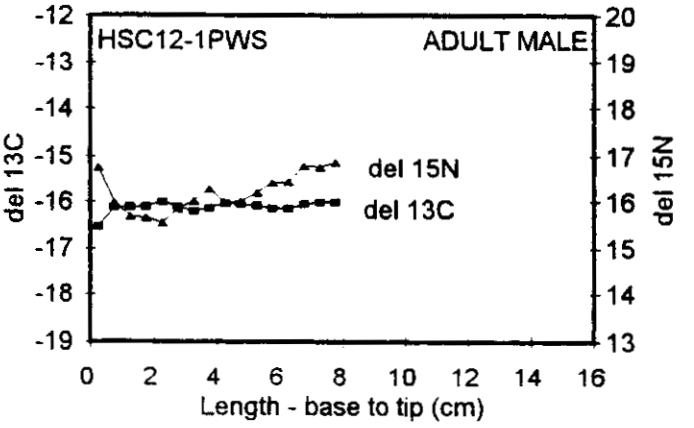
Channel Island, PWS, Alaska - May 1995

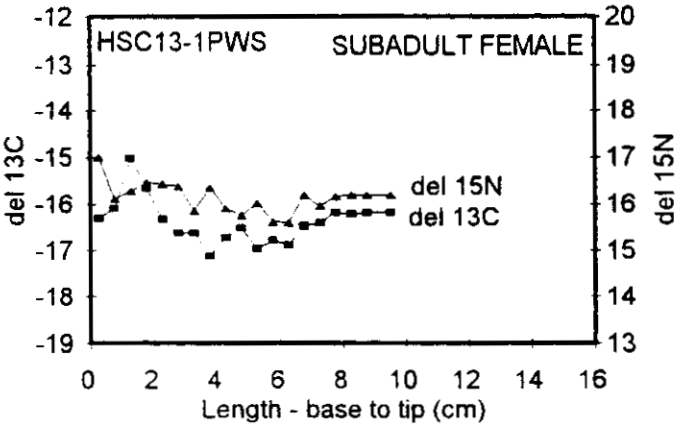


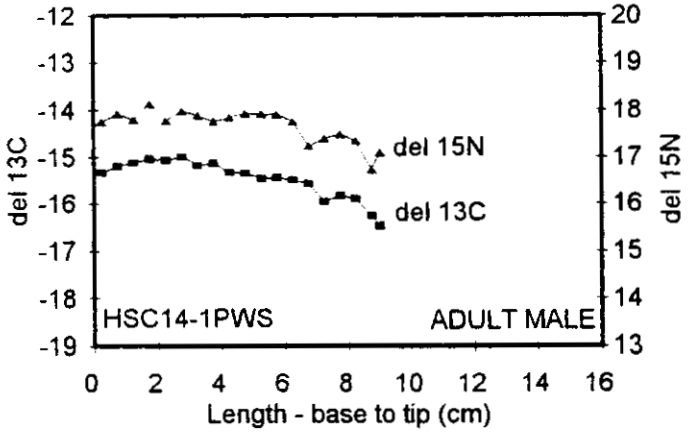




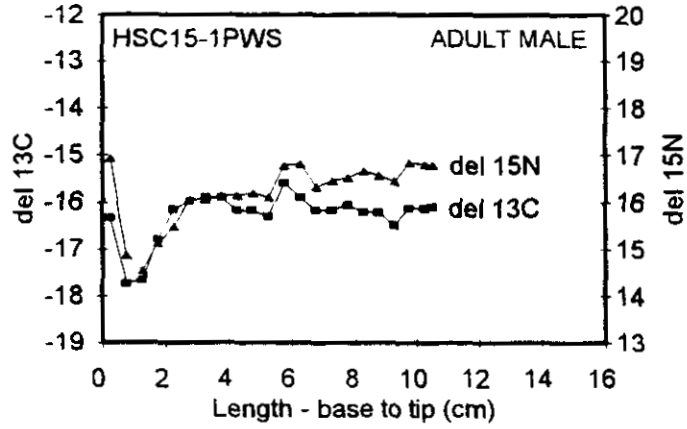
Channel Island, PWS, Alaska - May 1995

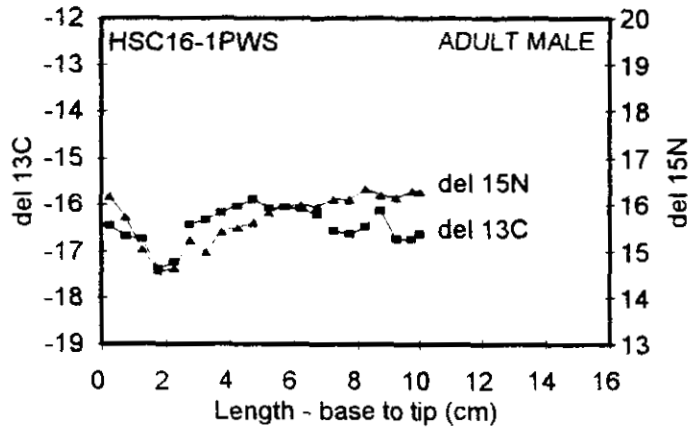


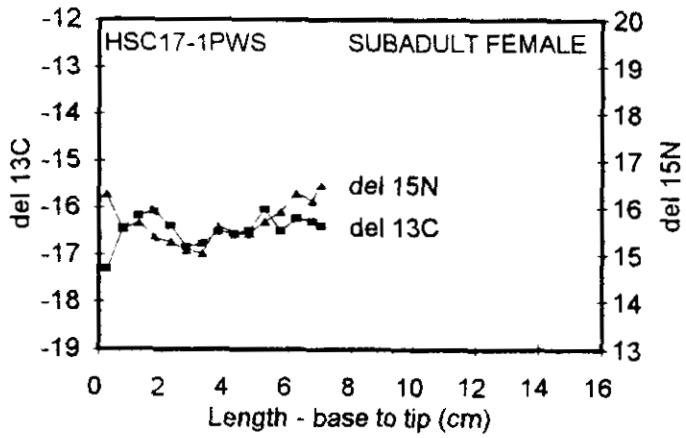




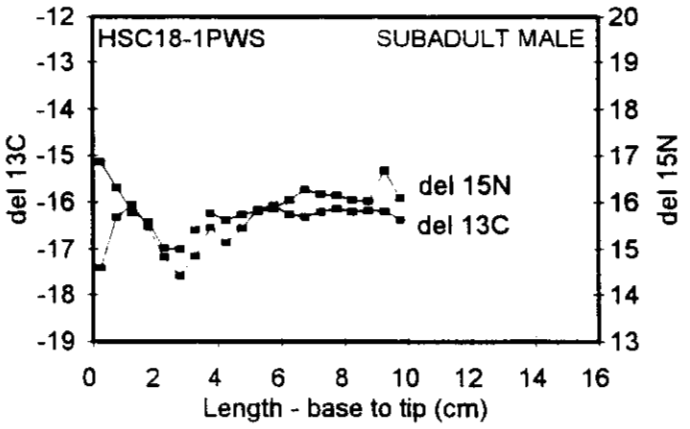
Stockdale Harbor, PWS, Alaska - May 1995

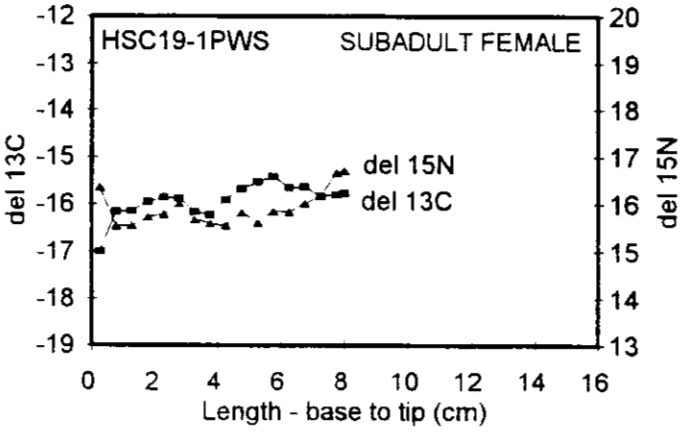




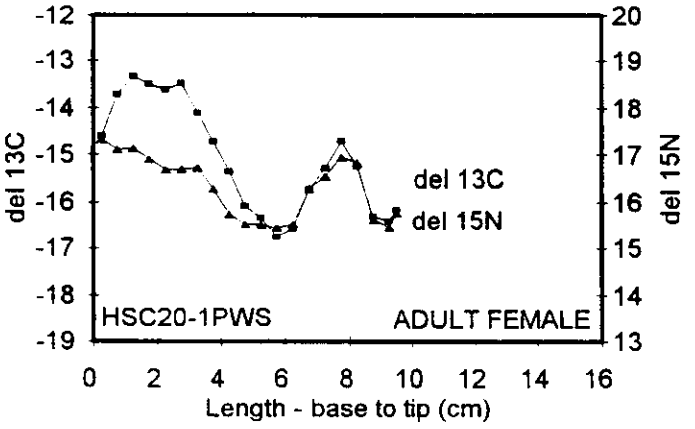


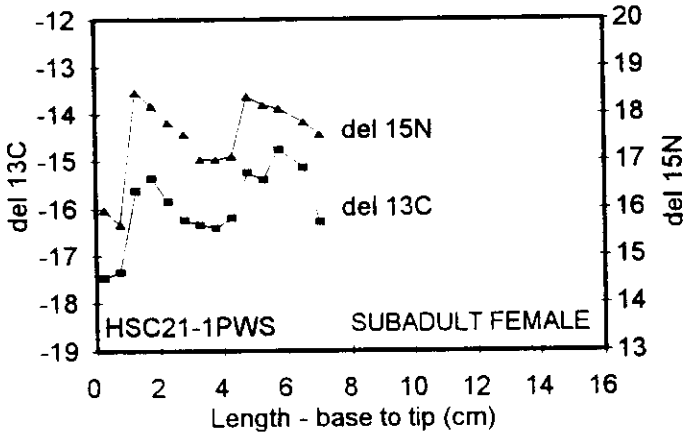
Stockdale Harbor, PWS, Alaska - May 1995

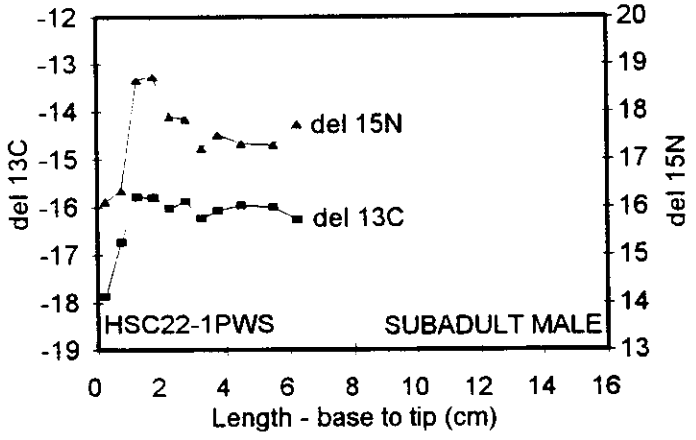




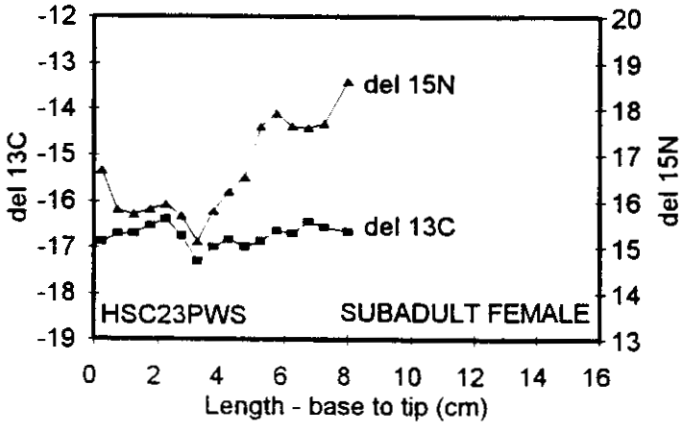
Port Chalmers, PWS, Alaska - May 1995

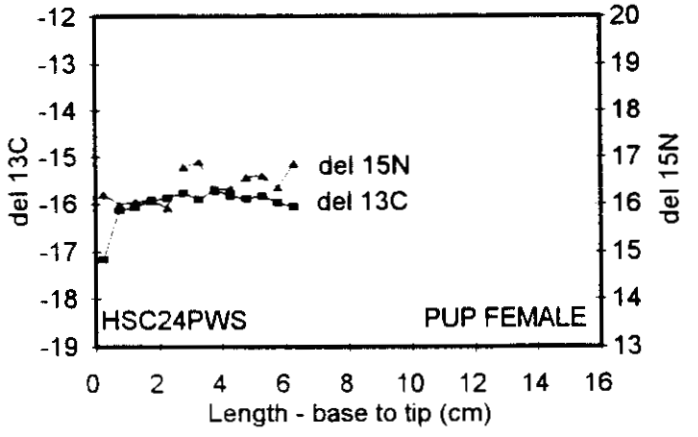




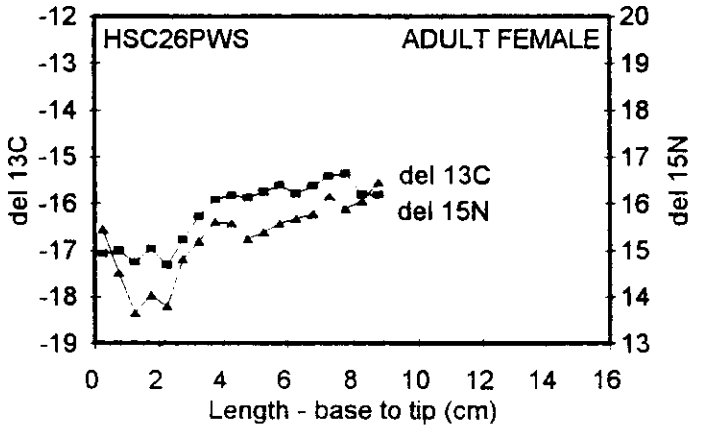


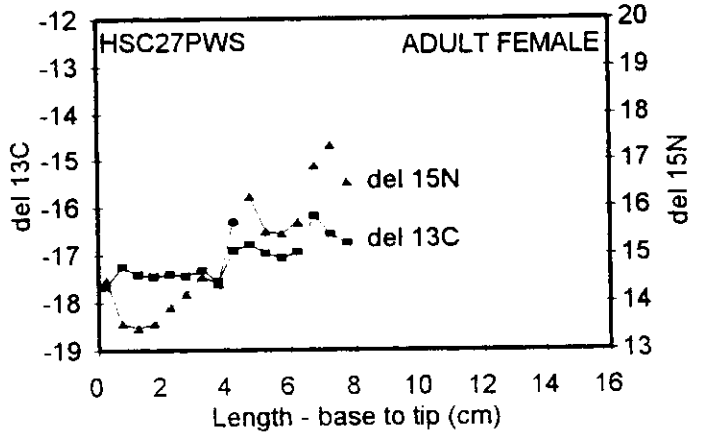
Gravina Island, PWS, Alaska - September 1995



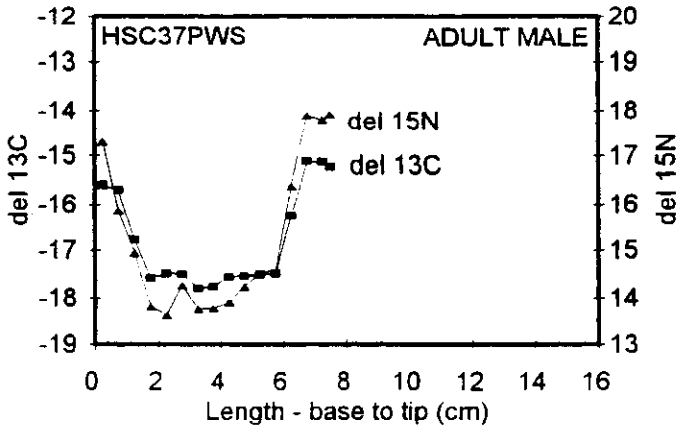


Port Chalmers, PWS, Alaska - September 1995

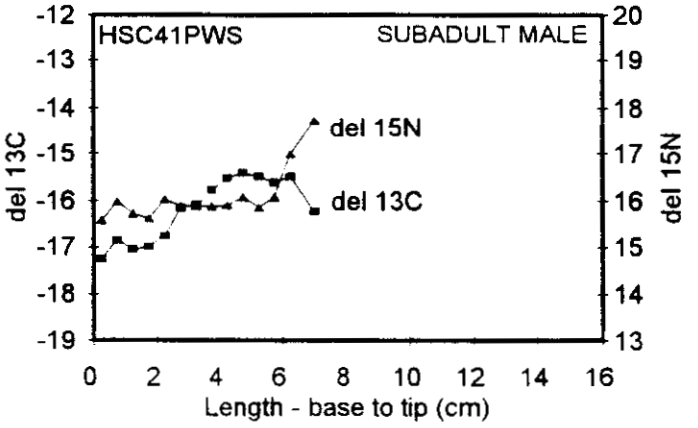


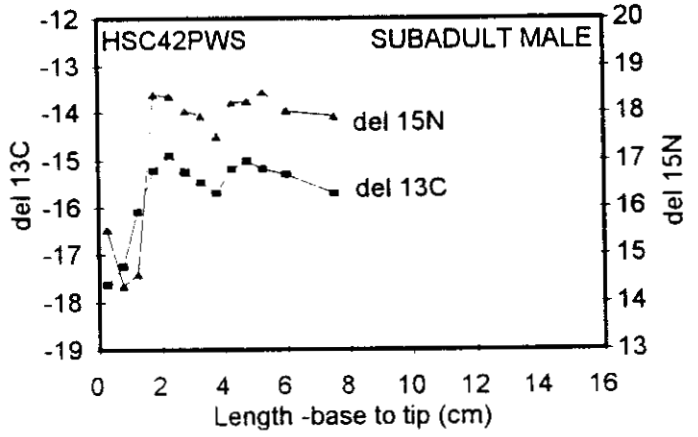


Channel Island, PWS, Alaska - September 1995

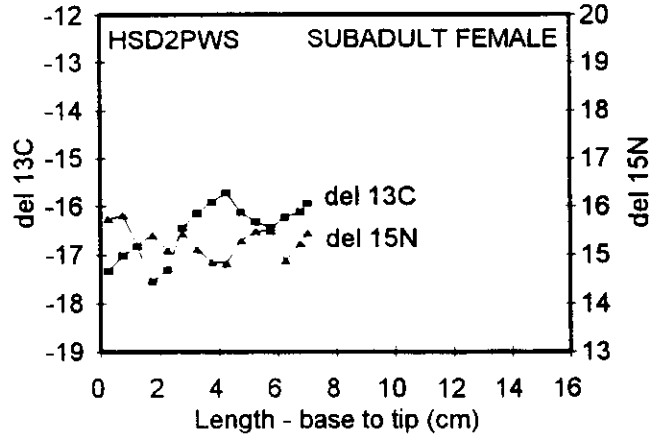


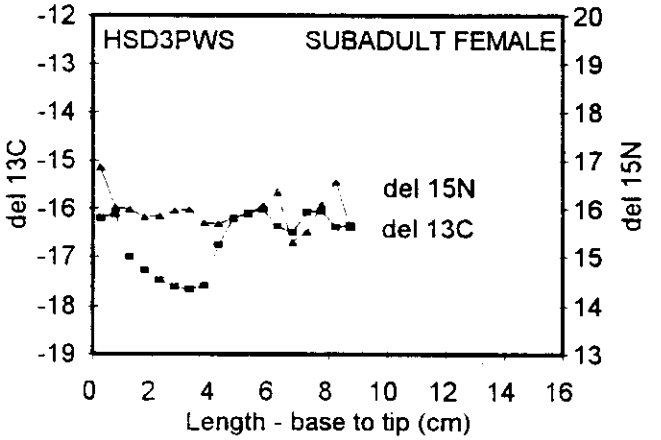
Applegate Rocks, PWS, Alaska - September 1995



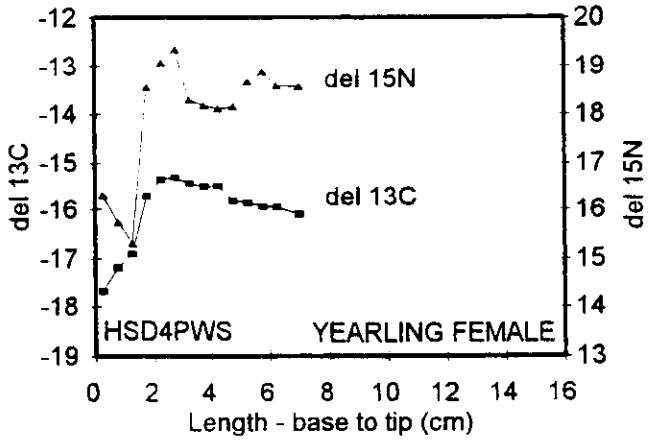


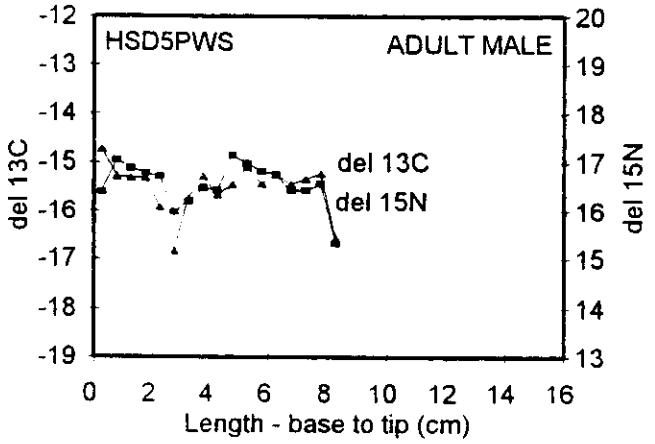
Little Green Island, PWS, Alaska - April 1996



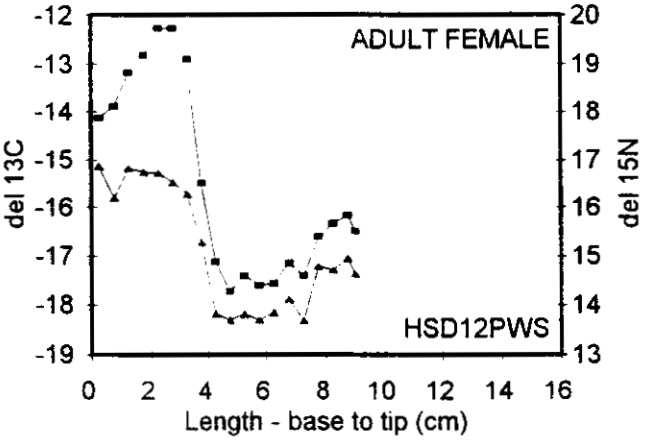


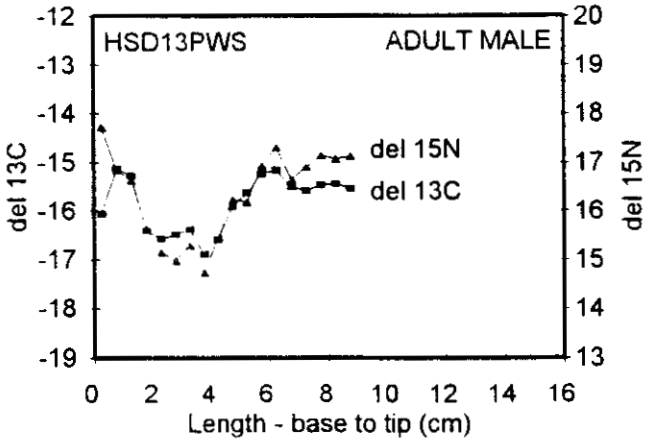
Applegate Rocks, PWS, Alaska - April 1996



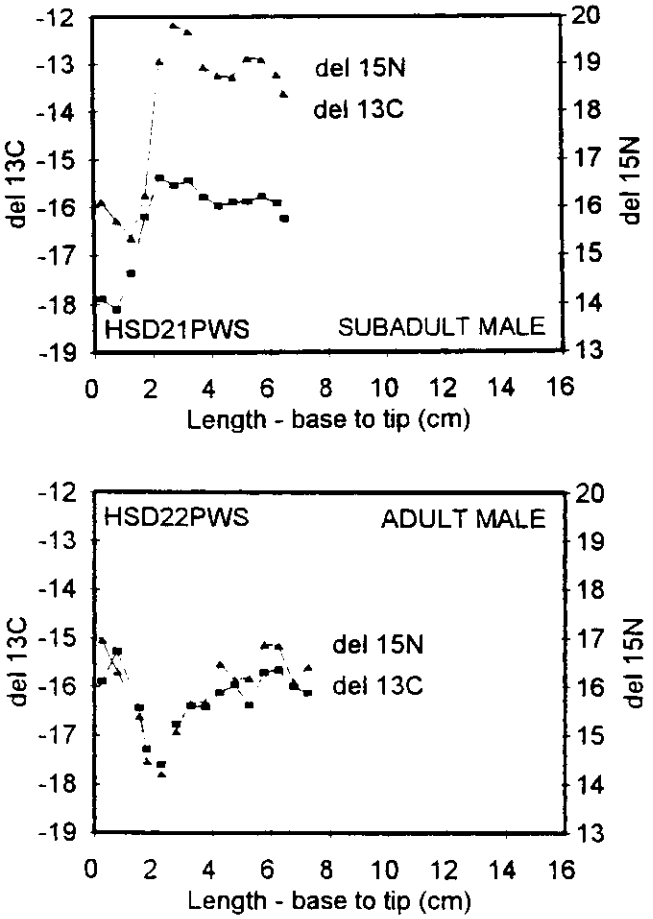


Port Chalmers, PWS, Alaska - April 1996

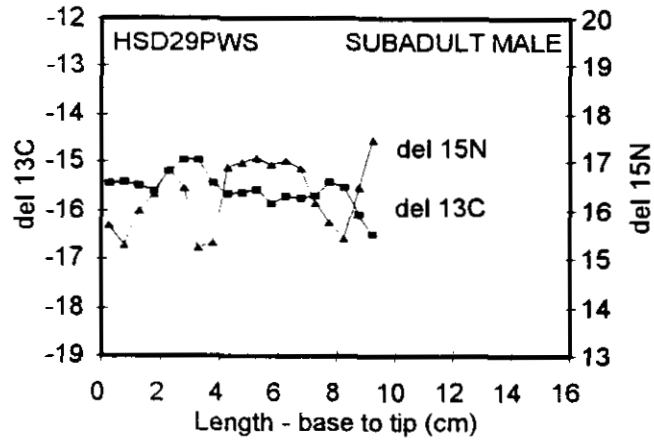


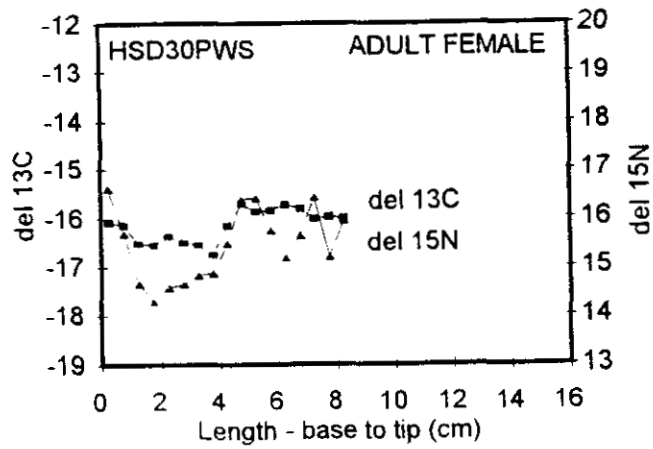


Olsen Bay, PWS, Alaska - May 1996

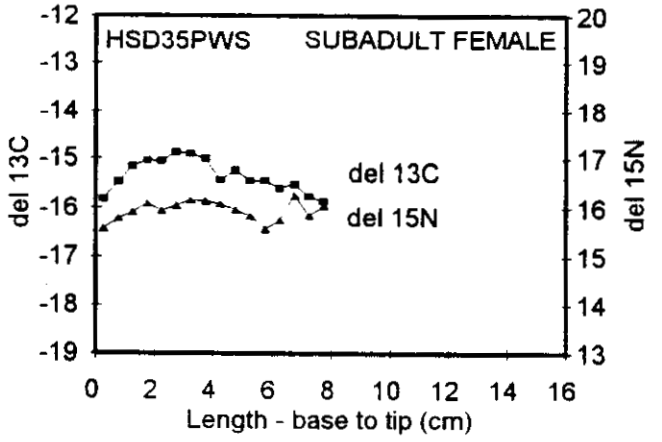


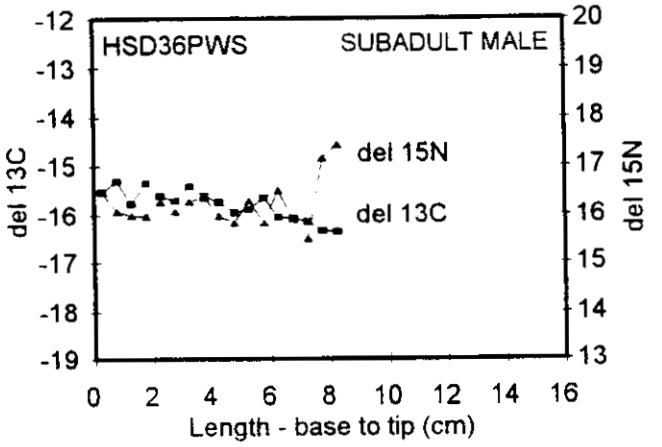
Channel Island, PWS, Alaska - September 1996



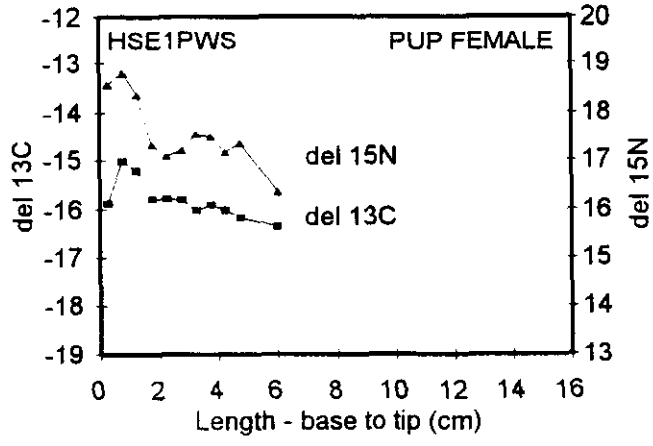


Applegate Rocks, PWS, Alaska - September 1996

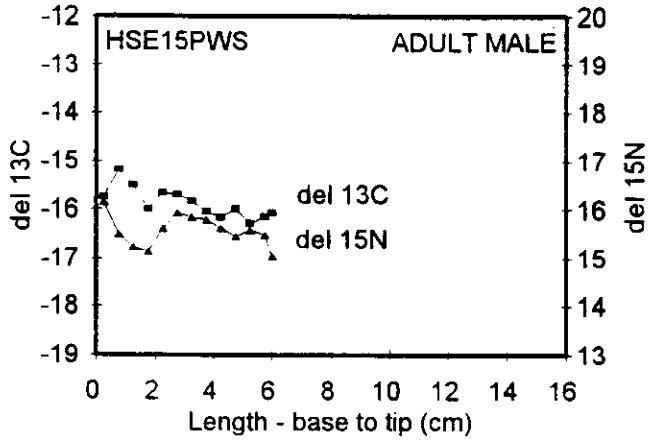


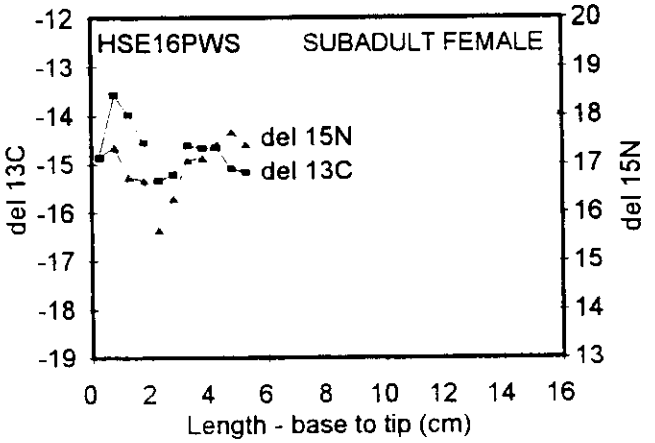


Seal Island, PWS, Alaska - June 1997

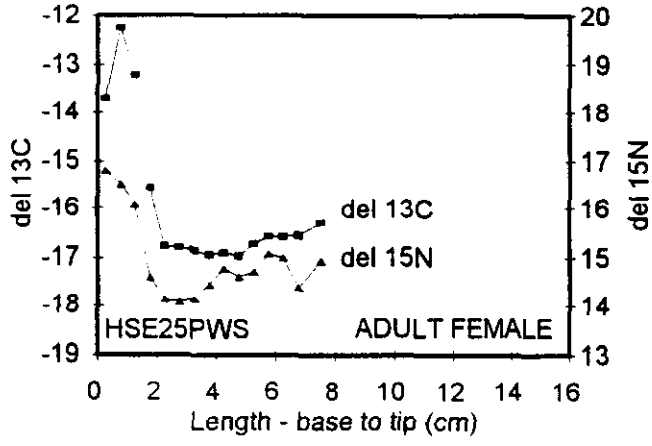


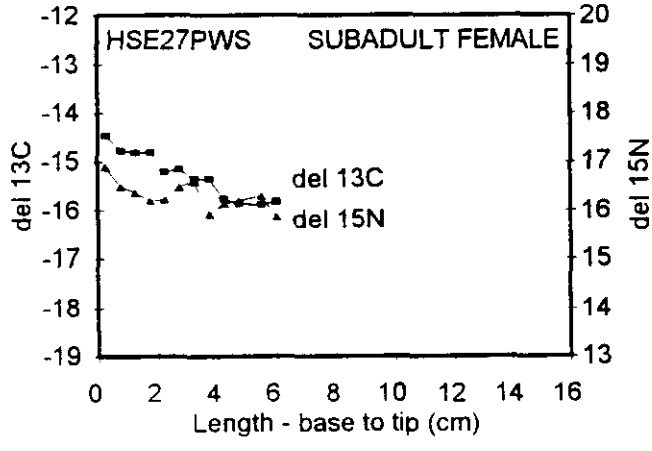
Channel Island, PWS, Alaska - June 1997



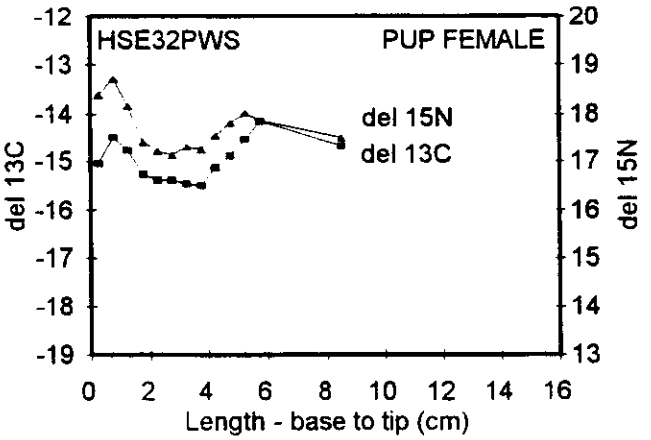


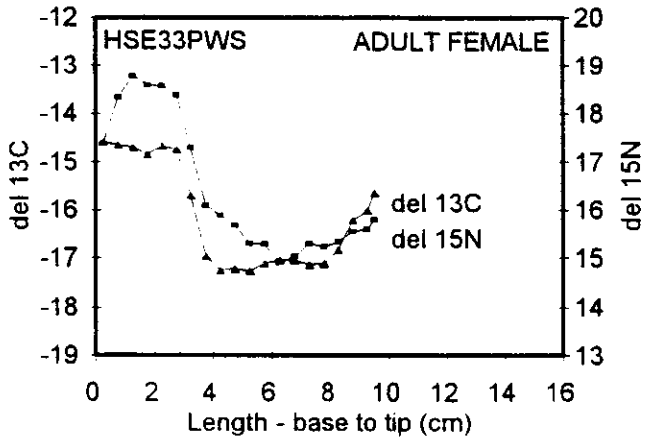
Port Chalmers, PWS, Alaska - June 1997



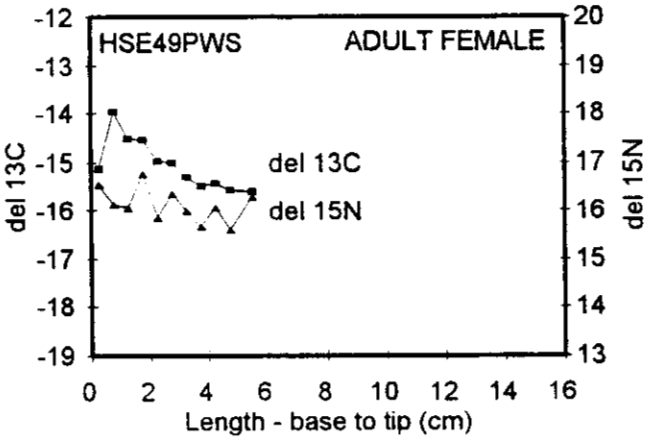


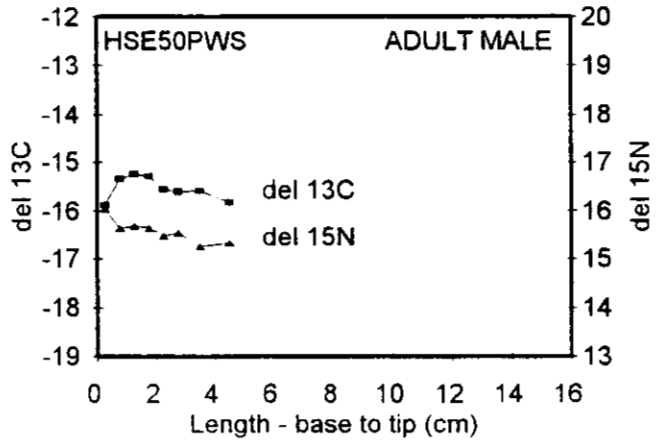
Olsen Bay, PWS, Alaska - June 1997



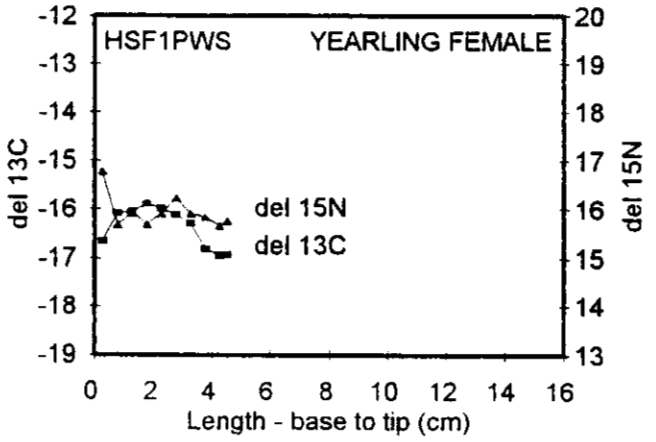


Applegate Rocks, PWS, Alaska - July 1997

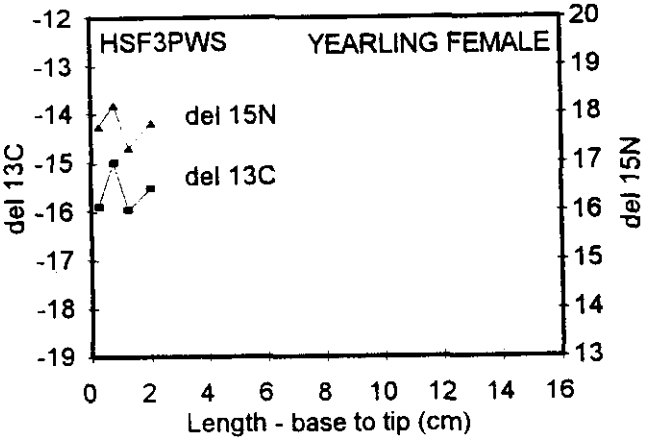


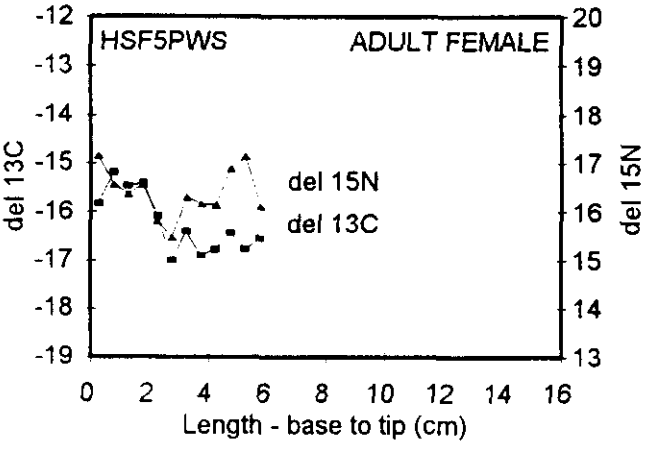


Applegate Rocks, PWS, Alaska - June 1998

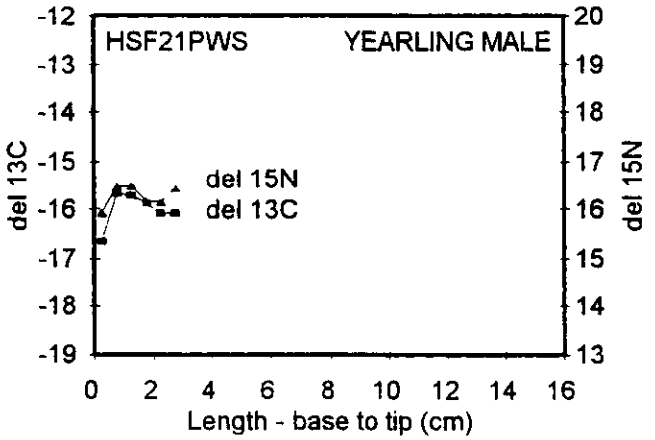


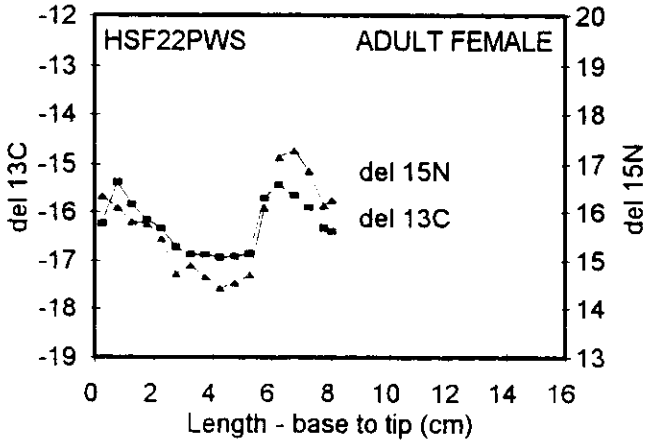
Seal Island, PWS, Alaska - June 1998



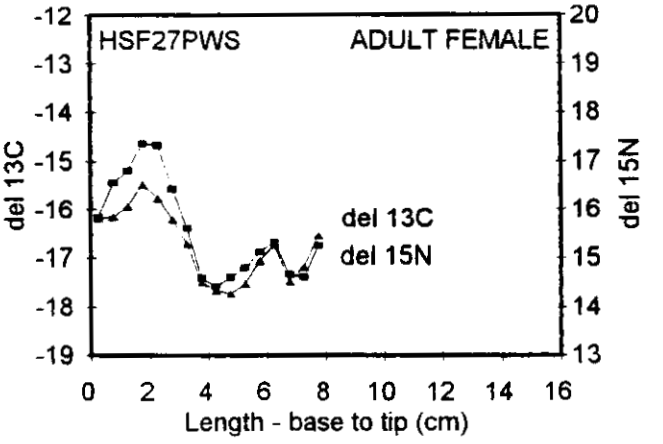


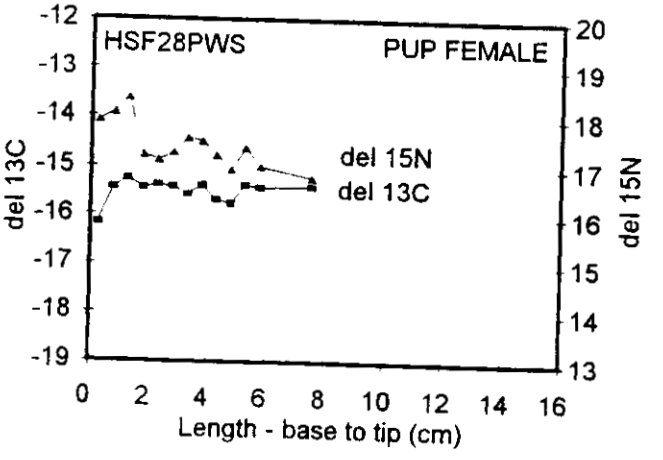
Applegate Rocks, PWS, Alaska - June 1998



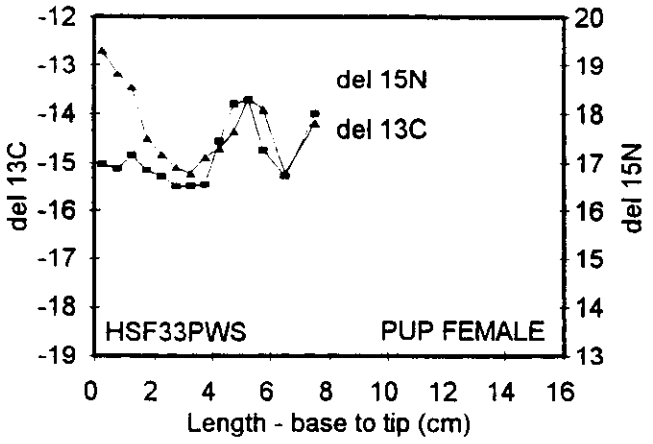


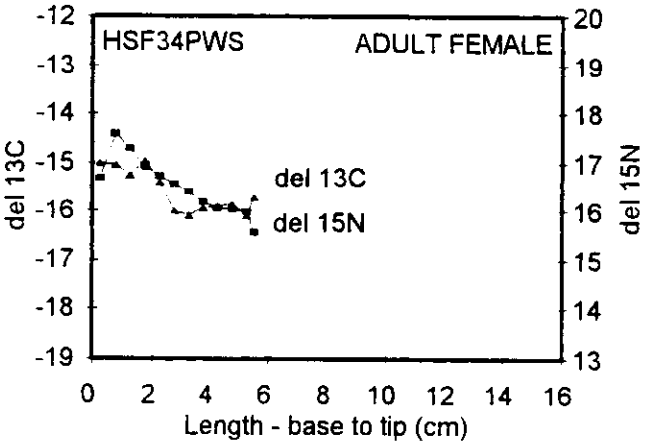
Channel Island, PWS, Alaska - June 1998





Port Chalmers, PWS, Alaska - June 1998





Applegate Rocks, PWS, Alaska - June 1998