

INVESTIGATING TROPHIC RELATIONSHIPS OF PINNIPEDS IN ALASKA AND WASHINGTON USING STABLE ISOTOPE RATIOS OF NITROGEN AND CARBON

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ABSTRACT

We measured stable-nitrogen ($\delta^{15}\text{N}$) and stable-carbon ($\delta^{13}\text{C}$) isotope ratios in muscle and hair from 7 northern fur seals (*Callorhinus ursinus*) from the Pribilof Islands, Alaska, and 27 Steller sea lions (*Eumetopias jubatus*), and 14 harbor seals (*Phoca vitulina*) from the Gulf of Alaska and coast of Washington State, in order to contrast dietary information derived from isotopic *vs.* available conventional dietary studies. Stable-nitrogen-isotope analysis of muscle revealed that harbor seals were enriched over sea lions (mean $\delta^{15}\text{N} = 18.6\text{‰}$ *vs.* 17.5‰) which were in turn enriched over northern fur seals (mean $\delta^{15}\text{N} = 16.6\text{‰}$). Trophic segregation among these species likely results primarily from differential reliance on herring (*Clupea harengus*), Atka mackerel (*Pleuragrammus monopterygius*), and large *vs.* small walleye pollock (*Theragra chalcogramma*). According to their $\delta^{15}\text{N}$ values, adult male Steller sea lions showed a higher trophic position than adult females (mean $\delta^{15}\text{N}$: 18.0‰ *vs.* 17.2‰), whereas adult female northern fur seals were trophically higher than juvenile male fur seals (mean $\delta^{15}\text{N}$: 16.5‰ *vs.* 15.0‰). Each of these observed differences likely resulted from differential reliance on squid or differences in the size range of pollock consumed. Three northern fur seal pups showed higher $\delta^{15}\text{N}$ enrichment over adults (mean 17.7‰ *vs.* 15.8‰) due to their reliance on their mother's milk. Stable-carbon isotope measurements of hair revealed a cline toward more negative values with latitude. Segregation in hair $\delta^{13}\text{C}$ between Steller sea lions and harbor seals off the coast of Washington (mean $\delta^{13}\text{C}$: -13.6‰ *vs.* -15.0‰) reflected the greater association of harbor seals with freshwater input from the Columbia River. Our study dem-

onstrates the utility of the stable isotope approach to augment conventional dietary analyses of pinnipeds and other marine mammals.

Key words: northern fur seal, Steller sea lion, harbor seal, *Callorhinus ursinus*, *Eumetopias jubatus*, *Phoca vitulina*, stable-isotope analysis, carbon-13, nitrogen-15.

Populations of Steller sea lions (*Eumetopias jubatus*), northern fur seals (*Callorhinus ursinus*), and harbor seals (*Phoca vitulina*) have declined significantly in the Bering Sea and Gulf of Alaska during the past two decades (Pitcher 1990; Loughlin *et al.* 1992; NMFS 1992, 1993, 1995). It is suspected that decreased availability or reduced abundance of major prey species, such as juvenile walleye pollock (*Theragra chalcogramma*) and capelin (*Mallotus villosus*), may have contributed to these declines, or lack of recovery in the case of northern fur seals (Merrick *et al.* 1987, Loughlin and Merrick 1989, Alverson 1991, Springer 1992, Trites 1992, Alaska Sea Grant 1993, Merrick 1995). However, a detailed understanding of the feeding ecology of these animals is lacking, a situation common to marine mammals in general (Beddington *et al.* 1985). This lack of information is based primarily on limitations associated with sampling in remote marine environments, as well as with conventional approaches to dietary analyses (Hobson and Welch 1992). However, the use of naturally occurring stable isotopes of carbon and nitrogen in marine food-webs may provide new insights to feeding ecology of pinnipeds and other marine mammals (Schell *et al.* 1989; Hobson and Welch 1992; Abend and Smith, in press; Hobson *et al.* 1996).

Diets of pinnipeds typically have been described from prey remains recovered from stomach or colon contents, stomach lavage, fecal material, or vomitus (Imler and Sarber 1947; Spalding 1964; Fiscus and Baines 1966; Pitcher 1980a, 1981; Brown and Mate 1983; Kajimura 1985; Antonelis *et al.* 1986; Perez and Bigg 1986; Merrick and Calkins, in press). The number and size distribution of particular prey species is obtained usually through quantification and identification of otoliths and other bones, scales, and beaks. However, such approaches involve several disadvantages and potential biases. Historically, large samples have been amassed by shooting pinnipeds and examining the gastrointestinal tracts (*e.g.*, Pitcher 1980a, 1981). Most research now avoids techniques that require killing animals, particularly when depleted, threatened, or endangered species are involved. Stranded marine mammals and other opportunistic samples (*e.g.*, incidental catch in fisheries) are not likely to provide large sample sizes or may not be representative of healthy animals. Large samples of fecal material can be collected with minimal or no disturbance to animals, but identification of food habits for particular age and sex groups may not be possible. In addition, otoliths, small bones, and cephalopod beaks in feces may not provide an accurate assessment of species composition in diet (Pitcher 1980b, Olesiuk *et al.* 1990, Harvey and Antonelis 1994). It is also impossible to assess the importance to the diet of soft-bodied prey that may be missed entirely in feces. An additional problem with "conventional"

dietary techniques is that unless such techniques are applied over long time periods, each analysis presents a "snapshot" of food consumed only during the most recent feeding trip(s), and dietary evaluations are based on ingested and not necessarily assimilated foods (Hobson *et al.* 1994).

During the last decade, stable-isotope analysis has emerged as a powerful tool that can augment more conventional dietary studies because isotope ratios in animal tissues are related to those of their food (DeNiro and Epstein 1978, 1981; Peterson and Fry 1987; Rundel *et al.* 1989; Tieszen and Boutton 1989). Nitrogen-15 typically shows a stepwise increase with trophic level, thus allowing comparison among various consumers within an ecosystem (Fry 1988, Hobson and Welch 1992, Rau *et al.* 1992, Hobson *et al.* 1994). This enrichment is likely due to the preferential excretion of ^{15}N -depleted nitrogen usually in the form of urea, ammonia, or uric acid (reviewed by Michener and Schell 1994; see also Minagawa and Wada 1984, Hobson and Clark 1992). While differences in ^{15}N retention are apparent according to species, diet, and nutritional stress (Hobson *et al.* 1993, Koch *et al.* 1994), an average $\delta^{15}\text{N}$ trophic enrichment value of 3.2‰ has been found in field studies (Michener and Schell 1994). Measurement of $\delta^{15}\text{N}$ values in the tissues of consumers can thus allow the estimation of $\delta^{15}\text{N}$ values of their average diet, and the isotopic measurement of tissues from both consumer and potential prey allows the determination of trophic relationships in often complex foodwebs (Hobson *et al.* 1994).

Rather than being a reliable indicator of trophic level, carbon isotope ratios can reflect the relative input of dietary resources from fresh water *vs.* marine, inshore *vs.* offshore, or pelagic *vs.* benthic food webs (McConnaughey and McRoy 1979; Rau *et al.* 1983; Rounick and Winterbourn 1986; Dunton *et al.* 1989; Hobson 1990, 1993; Mizutani *et al.* 1990; Hobson *et al.* 1994; France 1995). Thus, while stable-isotope analyses are rarely used to identify the importance of individual prey species, they are used to indicate trophic level of feeding or the relative importance of different food webs or sources of nutrients to the diet. Because stable-isotope ratios in a consumer's tissues are derived from assimilated foods, the tissues reflect dietary input integrated over time, not just during the last feeding trip. In fact, the metabolic rates and the subsequent turnover of elements in different tissues varies (Tieszen *et al.* 1983), allowing the evaluation of diet integrated over different periods of time (*e.g.*, days, weeks, or months; Tieszen *et al.* 1983; Hobson and Clark 1992; Hobson *et al.*, in press).

The work presented here compares results from stable-isotope analyses with existing information about diets of northern fur seals near the Pribilof Islands, Alaska, and Steller sea lions and harbor seals in the Gulf of Alaska and along the Washington coast. Our primary objective was to demonstrate the utility of the stable-isotope approach for investigating trophic relationships and sources of feeding in pinnipeds and other marine mammals and to contrast information based on isotopic and conventional dietary data.

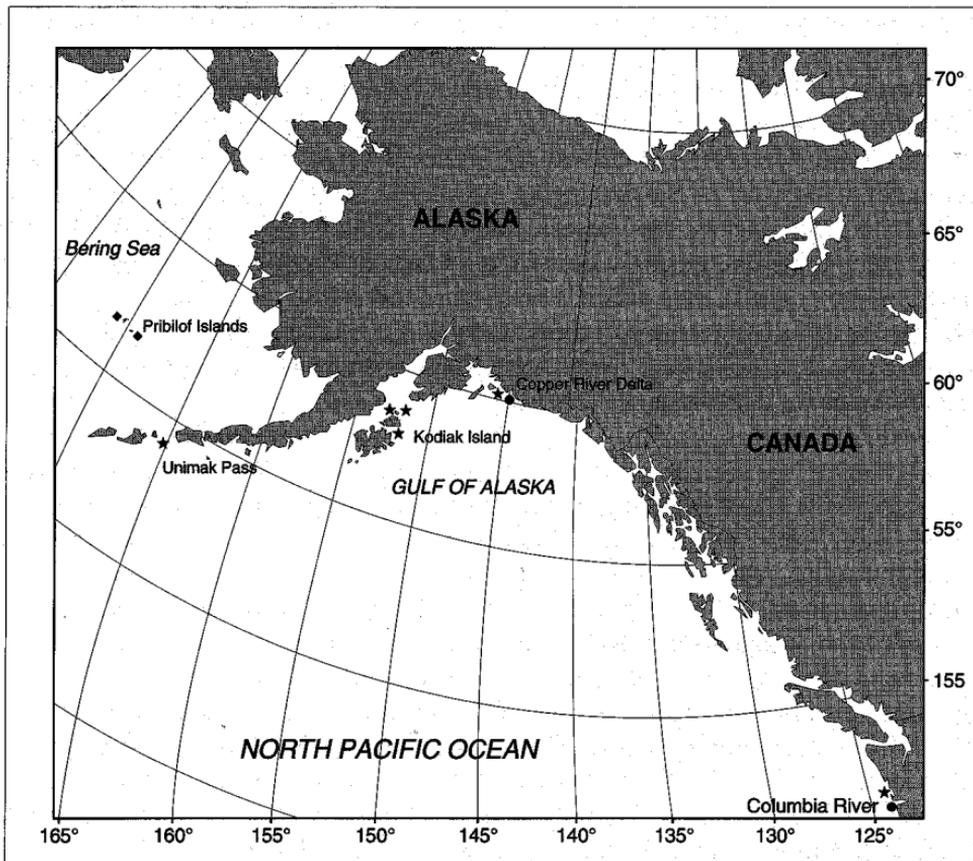


Figure 1. Locations where samples were collected from northern fur seals (◆), Steller sea lions (★), and harbor seals (●).

STUDY AREA AND METHODS

The primary study areas were the Cape St. Elias-Copper River Delta region of the eastern Gulf of Alaska and the northern Kodiak Archipelago in the central Gulf of Alaska. Additional comparisons and inferences were made using samples collected at the Pribilof Islands in the Bering Sea, the Krenitzin Islands-Unimak Pass region in the eastern Aleutian Islands, and two locations along the coastline of Washington (Fig. 1).

We measured stable-nitrogen and stable-carbon isotope ratios in samples of skeletal muscle collected opportunistically from pinnipeds found dead on beaches. Some samples were recovered from tissues collected and archived during other studies. Sampled pinnipeds included 13 Steller sea lions and nine harbor seals from the eastern Gulf of Alaska during May 1990 through September 1993, five northern fur seals from the Pribilof Islands during July and August 1992, and five harbor seals from the Washington coast near the Columbia River during October 1992. We also sampled muscle from two juvenile male fur seals taken for subsistence by Alaska natives on St. Paul Island, Alaska. Muscle was collected in sufficiently large pieces (5–10 g) that small,

clean subsamples could be removed under laboratory conditions at the time of isotopic analysis.

Samples of hair were available from several of the pinnipeds described above: one of the sea lions, five of the fur seals, and four of the harbor seals from Washington. We collected additional Steller sea lion hair samples from three animals in the Gulf of Alaska during July 1992, from five animals in the Gulf of Alaska and one in the eastern Aleutian Islands during February-March 1993, and from five animals near Grays Harbor, Washington, during March 1993. Most of the Alaska animals were alive and restrained temporarily during the course of other research; others were found dead on the beach. Hair was removed with a razor or clipped with scissors from an area approximately 5 cm × 5 cm on the middorsal region.

Most of the fishes, squids, and other invertebrates were collected in research trawls during spring and summer of 1992 and 1994. The largest fishes were collected with hook and line during July 1994. Additional samples of small-sized fishes were collected from food items recovered from seabird nest sites during August 1992 (Hobson *et al.* 1994). In the laboratory, muscle tissue was freeze-dried and then powdered in an analytical mill. Copepods and euphausiids were analyzed whole in batch samples of many individuals. Invertebrate samples were treated with HCl to remove carbonates prior to isotopic analysis. Lipids were removed from all samples using a modified methanol/chloroform extraction technique (Bligh and Dyer 1959), and the samples were then oven-dried at 60°C for at least 24 h. Sample combustion was based on the Dumas method (see Knowles and Blackburn 1993). Powdered samples were sealed in evacuated Vycor tubing with 1-g CuO wire, 1-g Cu wire, and Ag foil and combusted at 850°C for 2 h. Combusted samples were allowed to cool slowly for 18 h in the furnace. CO₂ and N₂ gas were separated cryogenically and analyzed using a VG OPTIMA isotope ratio mass spectrometer (National Hydrology Institute, Saskatoon, Saskatchewan, Canada).

Differences in isotopic composition were expressed in δ notation as the proportional deviation in parts per thousand (‰) of the isotope ratio in a sample from that of the appropriate standard:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1,000$$

where X is ¹⁵N or ¹³C, and R_{sample} and R_{standard} are the ratios of ¹⁵N/¹⁴N or ¹³C/¹²C for the sample and the standard, respectively. The standards for stable-isotope ratio analysis are atmospheric air (AIR) for nitrogen and Pee Dee belemnite (PDB) for carbon. The conventional use of these internationally accepted standards with relatively low and high levels of ¹⁵N and ¹³C, respectively, results in positive nitrogen- and negative carbon-isotope values for most biological materials. Based on replicate measurements of laboratory standards, measurement error was ± 0.3‰ and ± 0.1‰ for ¹⁵N and ¹³C, respectively.

RESULTS

Stable isotope ratios in pinniped muscle—The three pinniped species in Alaska segregated isotopically when $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ were considered together (MAN-

Table 1. Mean (\pm SE) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values for skeletal muscle of northern fur seals, Steller sea lions, and harbor seals from the Pribilof Islands (Bering Sea), the Copper River Delta, Gulf of Alaska, and the Columbia River estuary, Washington.

Species	Location	n	$\delta^{15}\text{N}$ (‰)	$\delta^{13}\text{C}$ (‰)
Northern fur seal				
females	Pribilof Islands, AK	2	16.5 \pm 0.2	-18.1 \pm 0.7
males	Pribilof Islands, AK	2	15.0 \pm 0.4	-19.9 \pm 0.6
pups	Pribilof Islands, AK	3	17.7 \pm 0.3	-18.8 \pm 0.1
all		7	16.6 \pm 0.5	-18.9 \pm 0.4
Steller sea lion				
females	Copper R. Delta, AK	8	17.2 \pm 0.2	-18.1 \pm 0.3
males	Copper R. Delta, AK	5	18.0 \pm 0.2	-18.2 \pm 0.5
all		13	17.5 \pm 0.2	-18.2 \pm 0.2
Harbor seal				
females	Copper R. Delta, AK	2	19.0 \pm 0.9	-17.6 \pm 0.1
males	Copper R. Delta, AK	6	18.6 \pm 0.3	-17.4 \pm 0.2
pup	Copper R. Delta, AK	1	18.0	-18.9
all		9	18.6 \pm 0.3	-17.6 \pm 0.2
females	Columbia R., WA	3	16.1 \pm 0.2	-17.5 \pm 0.2
males	Columbia R., WA	2	16.8 \pm 0.3	-16.6 \pm 0.3
all		5	16.4 \pm 0.2	-17.1 \pm 0.3

OVA: Wilks Lambda $F_{4,50} = 4.92$, $P = 0.002$, Table 1, Fig. 2). Ratios of both isotopes in harbor seals were consistently greater than those in Steller sea lions, which in turn were greater than those in northern fur seals. Males and females were significantly different for subadult and adult Steller sea lions from Alaska (MANOVA: Wilks Lambda $F_{2,10} = 3.91$, $P = 0.056$), but not for northern fur seals ($P = 0.330$), harbor seals from Alaska ($P = 0.291$), or harbor seals from Washington ($P = 0.271$). However, for fur seals and harbor seals, small sample sizes preclude any strong inferences with respect to possible dietary differences between the sexes.

The lowest and highest observed values for $\delta^{15}\text{N}$ in individual pinnipeds were 14.6‰ and 20.1‰ for a juvenile male northern fur seal from the Pribilof Islands and a subadult harbor seal from the eastern Gulf of Alaska, respectively. For $\delta^{13}\text{C}$, the lowest and highest individual observations were -20.5‰ from a juvenile male northern fur seal and -16.8‰ from a harbor seal from the eastern Gulf of Alaska. For subadult and adult male and female Steller sea lions, muscle $\delta^{15}\text{N}$ differed (Mann-Whitney $P = 0.027$) whereas $\delta^{13}\text{C}$ did not ($P = 1.0$). Northern fur seals showed separation in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ among juvenile males, pups, and adult females (Kruskal-Wallis 1-way ANOVA, $P = 0.069$ and $P = 0.107$, respectively). For harbor seals, we were unable to test for differences between ages or sexes due to small sample sizes. However, our results suggest that some segregation may be found with further sampling.

Carbon isotope ratios in pinniped hair—In the 24 individual samples, $\delta^{13}\text{C}$ of hair ranged from -18.7‰ in a juvenile northern fur seal from the Pribilof

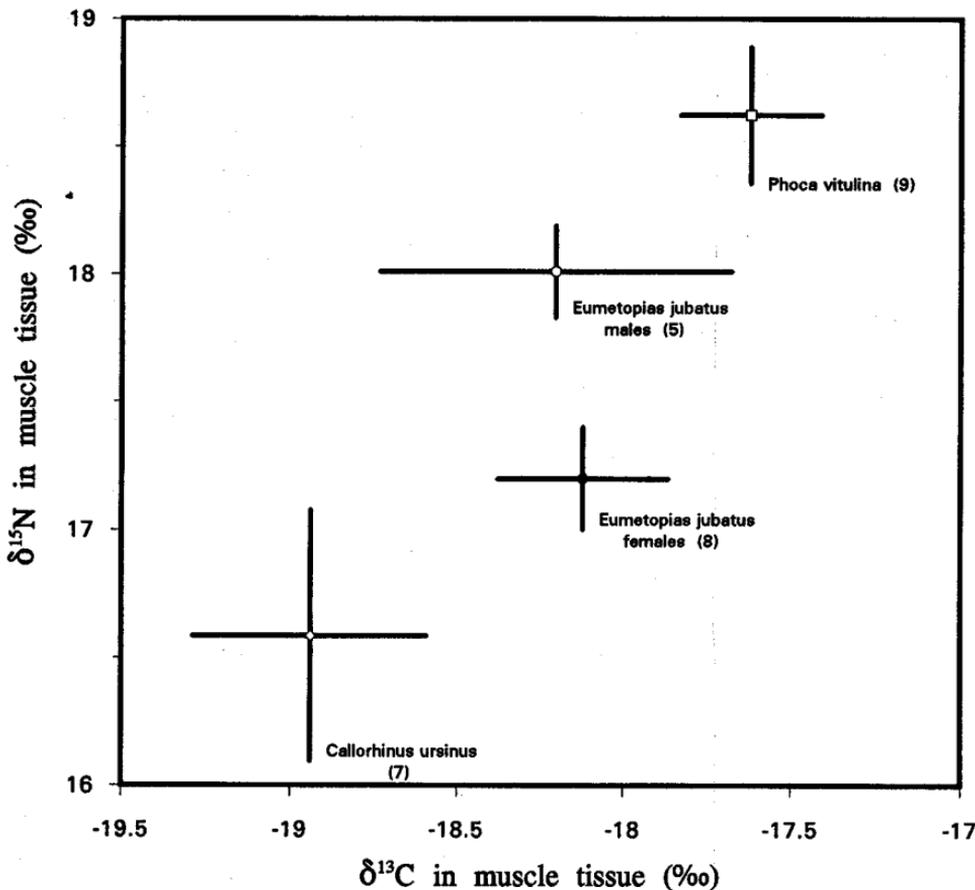


Figure 2. Mean (\pm SE) stable-carbon and stable-nitrogen isotope ratios in skeletal muscle from northern fur seals (*Callorhinus ursinus*) from the Pribilof Islands, Alaska, and from Steller sea lion (*Eumetopias jubatus*) females and males and harbor seals (*Phoca vitulina*) from the eastern Gulf of Alaska. Sample sizes given in parentheses.

Islands to -13.0‰ in an adult female Steller sea lion from Washington State. Stable-carbon isotope ratios in hair differed (Kruskal-Wallis one-way ANOVA, $P = 0.0002$) among the four groups of pinnipeds: northern fur seals, Steller sea lions from Alaska, Steller sea lions from Washington state, and harbor seals from Washington state (Table 2, Fig. 3). Within each group there were no significant differences between sexes or age groups ($P > 0.27$ for all comparisons). Location was not a source of significant difference for $\delta^{13}\text{C}$ in Steller sea lions from the western, central, and eastern Gulf of Alaska (Kruskal-Wallis one-way ANOVA, $P = 0.487$).

Other foodweb components—The measurement of $\delta^{15}\text{N}$ values in tissues of potential prey in addition to those in marine mammals allows greater insight into possible trophic relationships within the marine foodwebs we investigated. For example, by adding 3‰ to prey $\delta^{15}\text{N}$ values, an estimate of the corresponding $\delta^{15}\text{N}$ value for marine mammals feeding exclusively on that prey type can be approximated. Stable-nitrogen isotope ratios in muscle from

Table 2. Mean (\pm SE) $\delta^{13}\text{C}$ values measured in hair of northern fur seals, Steller sea lions, and harbor seals from the Pribilof Islands, Gulf of Alaska, eastern Aleutian Islands, and Washington.

Species	Location	<i>n</i>	$\delta^{13}\text{C}$ (‰)
Northern fur seal	Pribilof Islands, AK	5	-17.8 ± 0.3
Steller sea lion	Gulf of Alaska and eastern Aleutian Islands	10	-16.3 ± 0.2
Steller sea lion	Grays Harbor, WA	5	-13.6 ± 0.2
Harbor seal	Columbia River, WA	4	-15.0 ± 0.2

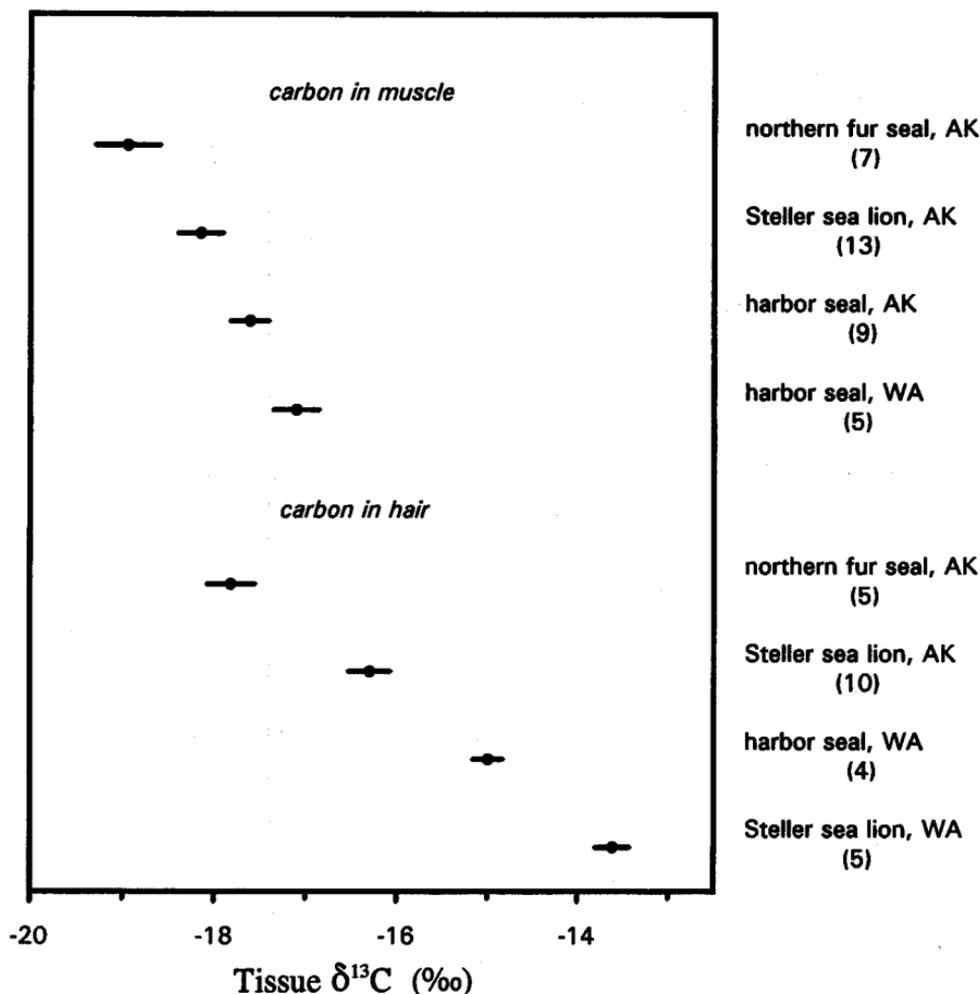


Figure 3. Mean (\pm SE) stable-carbon isotope ratios in skeletal muscle and in hair from northern fur seals, Steller sea lions, and harbor seals from the Pribilof Islands, the Gulf of Alaska, and the coast of Washington State. Sample sizes given in parentheses.

Table 3. Mean (\pm SE) $\delta^{15}\text{N}$ values measured in muscle of fishes and invertebrates from the Gulf of Alaska and the North Pacific off of Washington and Oregon.

Species	<i>n</i>	$\delta^{15}\text{N}$ (‰)
Gulf of Alaska samples		
large-sized fishes and squid		
squid	1	16.7
walleye pollock	2	15.7 \pm 0.7
Atka mackerel	6	11.7 \pm 0.2
small-sized fishes and squid		
capelin	13	12.4 \pm 0.1
Atka mackerel	3	12.2 \pm 0.2
prowfish	12	12.2 \pm 0.2
rockfish	2	12.0 \pm 0.02
sandlace	8	11.9 \pm 0.1
mackerel	5	11.9 \pm 0.7
polychaete	1	11.7
Pacific cod	19	11.4 \pm 0.2
euphausiid	9	11.2 \pm 0.5
walleye pollock	24	10.9 \pm 0.2
squid	4	9.6 \pm 0.5
copepod	1	8.5
Washington-Oregon Specimens		
large-sized fishes		
flathead sole	1	14.6
Pacific herring	2	14.5 \pm 0.3
squid	2	13.3 \pm 0.4
English sole	1	12.6
rex sole	3	12.5 \pm 1.0
arrowtooth flounder	1	11.9
walleye pollock	1	11.3

fishes sampled in the Gulf of Alaska ranged from $15.7 \pm 0.7\text{‰}$ to $10.9 \pm 0.2\text{‰}$ for large- and small-sized walleye pollock, respectively (Table 3). The extreme $\delta^{15}\text{N}$ values observed in the Gulf of Alaska were from invertebrates: 16.7‰ for a gonatid squid and 8.5‰ for a composite sample of copepods (Fig. 4). $\delta^{15}\text{N}$ from fishes sampled off the Washington-Oregon coast ranged from 14.6‰ for a flathead sole to 11.3‰ for a walleye pollock (Table 3).

DISCUSSION

Trophic relationships based on nitrogen-isotope ratios—The inferred trophic relationships of the various taxa sampled, based on the nitrogen-isotope content of muscle tissue, generally match what we know of these species from other studies. That is, these results show that the three species of pinnipeds are trophically higher than the various species of fish and invertebrates, many of which are known to be prey species. Some new insights into the trophic relationships of pinnipeds in this area also have been obtained using the stable-

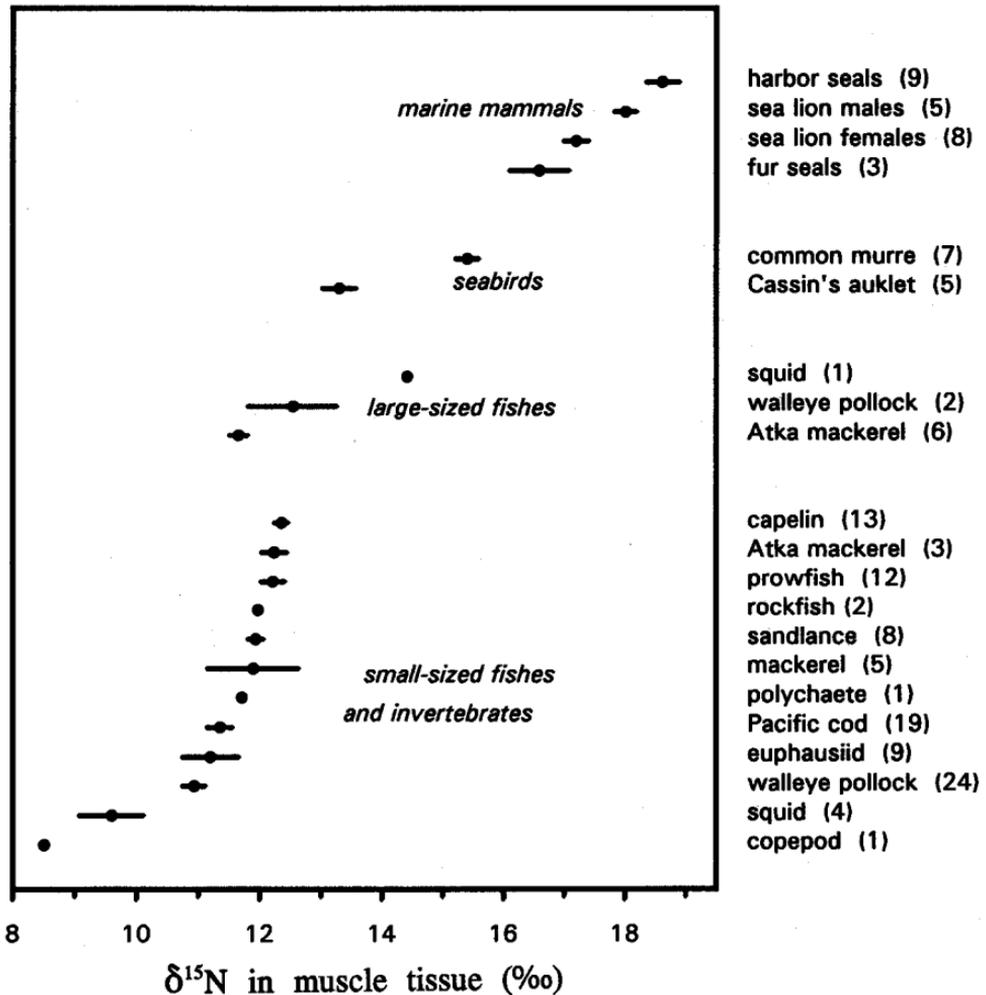


Figure 4. Mean (\pm SE) stable-nitrogen isotope ratios in skeletal muscle from pinnipeds, fishes, and invertebrates in Alaska. Sample sizes given in parentheses.

isotope approach. For example, our results imply that harbor seals sampled in Alaska fed at a higher trophic level than Steller sea lions, which, in turn, fed trophically higher than northern fur seals. Our results also suggest that male Steller sea lions fed trophically higher than females.

While we have little hard evidence from previous conventional dietary studies to explain the differences in stable-isotope composition observed in pinnipeds from the Gulf of Alaska, some comparisons are possible. Our current knowledge of the food habits of Steller sea lions and harbor seals in the Gulf of Alaska and of northern fur seals in the Bering Sea and the Gulf of Alaska is based on the identification of prey remains (bones, otoliths, beaks, etc.) in stomach contents and in feces, and to a lesser extent on the direct observation of feeding animals. There is substantial overlap in the species composition of diets for these pinnipeds; each has preferred prey, but each also is an opportunist that can take advantage of locally or seasonally abundant prey. Walleye

= 17.5‰). This result is somewhat unexpected, as previous studies of food habits had not suggested any striking differences on a trophic scale. For Steller sea lions and harbor seals collected concurrently during the 1970s (Pitcher 1980a, 1981), pollock was the major food for both species, but the frequency of occurrence was twice as high for Steller sea lions. Cephalopods appeared equally important to both, but sea lions ate more squids whereas harbor seals ate more octopi. Eulachon was an extremely important food for harbor seals near the Copper River Delta during spring and summer, but it was not important for Steller sea lions or for harbor seals elsewhere in the Gulf of Alaska (Pitcher 1980a, 1981).

As noted above, sea lions tend to consume larger pollock than do harbor seals. This difference should contribute to higher $\delta^{15}\text{N}$ in sea lions, as small pollock (FL less than 20–25 cm) are planktivorous whereas older, larger pollock become increasingly piscivorous (Bailey and Dunn 1979, Clausen 1983, Dwyer *et al.* 1987). We did not measure $\delta^{15}\text{N}$ in eulachon, but because this is a predominantly planktivorous species (Hart 1973), the influence of eulachon should not be a relative elevation of $\delta^{15}\text{N}$ in harbor seals.

Muscle $\delta^{15}\text{N}$ in harbor seals and Steller sea lions could be affected by the probable addition of salmon to the diet of both species during the spring and summer in the Copper River Delta region. Salmon can be an important seasonal prey item for Steller sea lions and harbor seals (Imler and Sarber 1947; Pitcher 1980a, 1981; Merrick 1995). Steller sea lions are most abundant in this area during late spring and early summer when sockeye (red) salmon (*Oncorhynchus nerka*) predominate in the spawning runs. Several weeks later, when coho (silver) salmon (*O. kisutch*) predominate, Steller sea lions have largely moved out of the region and harbor seals are the predominant pinniped (K. Wynne, Alaska Sea Grant College Program, University of Alaska Fairbanks, personal communication). Sockeye salmon are largely planktivorous whereas coho salmon are largely piscivorous (Hart 1973, Groot and Margolis 1991). Accordingly, $\delta^{15}\text{N}$ should be elevated in coho salmon relative to sockeye. In fact, previous isotopic studies showed that coho salmon in the Gulf of Alaska were enriched in ^{15}N about 1‰ compared to sockeye salmon (Kline 1991, Welch and Parsons 1993).

The observed difference in $\delta^{15}\text{N}$ between male and female Steller sea lions in the Gulf of Alaska suggests that adult male sea lions are feeding at a higher trophic level than are the females. Unfortunately, we have no evidence from other sources to support this conjecture, as differences between males and females have not been reported (Pitcher 1981; Merrick 1995; Merrick and Calkins, *in press*). While few controlled experiments have been conducted, there is no evidence that differential fractionation of stable isotopes between diet and consumer tissues is related to sex (Hobson and Clark 1992, Hobson *et al.* 1996).

Although differences were not significant for northern fur seals, adult females appeared to feed at a higher trophic level than juvenile males. Suckling pups, in turn, were at a higher trophic plane than the adult females. This latter relationship is the easier to rationalize. The pups were feeding exclusively

on milk produced by their mothers. That milk should have had an isotopic content similar to other tissues in the mothers' bodies. While corroborative experimental evidence is lacking (Hobson *et al.* 1996), as milk proteins are assimilated into the pups' body tissues, fractionation or changes in $\delta^{15}\text{N}$ associated with tissue synthesis are expected to result in pup's tissues having higher $\delta^{15}\text{N}$ values compared with those of their mothers. In this way, suckling pups can be viewed as simply occupying a higher trophic level than their mothers. As noted above, analyses of prey remains in stomachs, colons, and feces indicate that adult females probably consume relatively more squid and more pollock from older age classes than do juvenile males (Antonelis *et al.* 1986; Sinclair *et al.*, in press). We observed $\delta^{15}\text{N}$ to be much higher in a squid and "large-sized" pollock than in "small-sized" (*e.g.*, juvenile) pollock. Accordingly, each of these differences could result in enriched ^{15}N in the adult females.

Sinclair *et al.* (1994) concluded that fur seals do not simply feed on whatever prey is most abundant in the water column, but rather they are size-selective feeders. Prey selection differences also could result from spatial separation, either horizontally or vertically, during feeding. If adult females are feeding to a greater extent in oceanic waters beyond the shelf break, perhaps they would have greater access to oceanic squids than would juvenile males that forage more over the shelf and slope (Kajimura 1985, Sinclair *et al.* 1994).

There is an additional, potentially important factor that we were unable to test. Nutritional stress can result in higher $\delta^{15}\text{N}$ values in consumer tissues, presumably as body proteins are catabolized and redistributed within the body (Hobson and Clark 1992, Hobson *et al.* 1993). One of the adult females that we sampled was carrying a full-term fetus, and the other was several days *post partum* and lactating at the time of death. Certainly the late stages of pregnancy, parturition, and particularly lactation are energy-demanding for adult female fur seals (Perez and Mooney 1986, Costa and Gentry 1986). If these physiological demands were such that these females were undergoing a period of negative energy balance, and if proteins were mobilized from other parts of the body (Swick and Benevenga 1977) for maintenance of skeletal muscle (as well as for maintenance of the fetus), then enrichment of ^{15}N might occur in the muscle of females. Additional samples of muscle tissue from adult females during other times of the year, especially if obtained from captive animals held on an isotopically constant diet (Hobson *et al.* 1996), are needed to test for possible physiological influences on tissue $\delta^{15}\text{N}$ values during pregnancy and lactation.

Geographic and regional clines in carbon isotope ratios—Stable-carbon isotope ratios have proven most useful in identifying where particular organisms feed, and $\delta^{13}\text{C}$ values are typically higher in coastal or benthic food webs than in pelagic food webs (McConnaughey and McRoy 1979, Rau *et al.* 1983, Dunton *et al.* 1989, Hobson 1993). In addition, $\delta^{13}\text{C}$ values are typically lower in riverine systems than in marine systems (Mizutani *et al.* 1990, Hobson 1990, France 1995). Across all samples, including those from Washington, we observed a north-south cline in $\delta^{13}\text{C}$, both in skeletal muscle and in hair (Fig.

3). Muscle tissues least enriched in ^{13}C were those of northern fur seals from the Bering Sea; those most enriched were from harbor seals from the Washington coast near the Columbia River. Steller sea lions and harbor seals from the Gulf of Alaska were intermediate, with harbor seals being slightly more enriched in ^{13}C relative to the sea lions. Similarly, ^{13}C in hair was least enriched in northern fur seals from the Bering Sea, intermediate in Steller sea lions from the Gulf of Alaska, and most enriched in Steller sea lions and harbor seals from the Washington coast. Dunton *et al.* (1989) described a latitudinal and longitudinal gradient in $^{13}\text{C}/^{12}\text{C}$ ratios in northern Alaska, with increasing enrichment of ^{13}C from the eastern Beaufort and East Siberian seas to the western Beaufort and Chukchi seas to the Bering Sea, but little comparative information exists for the Gulf of Alaska. Such natural clines in stable-isotope ratios in marine foodwebs can allow the fingerprinting of animals to specific feeding locations (see Schell *et al.* 1989).

Stable-carbon isotope ratios in hair tended to be more positive than those values found in muscle tissue. This result was expected since isotope fractionation between diet and consumer tissues are known to depend on tissue type, a process reflecting different metabolic pathways during tissue synthesis (Tieszen *et al.* 1983, Hobson and Clark 1992). Moreover, Hobson *et al.* (1996) determined recently that hair of seals raised in captivity on a constant diet showed higher $\delta^{13}\text{C}$ values compared with muscle and other tissues from the same animals. The precise determination of these tissue-dependent isotope fractionation values will allow dietary estimates based on different periods of assimilation (*e.g.*, Hobson 1993). For example, while hair will reflect average diet during the period of growth following molt, muscle tissue represents a dietary average likely based on the last 3–4 wk of the animal's life prior to sampling (Tieszen *et al.* 1983, Hobson *et al.* 1996).

Direct comparisons of $\delta^{13}\text{C}$ in hair between Steller sea lions and harbor seals were possible for two areas: the eastern Gulf of Alaska ($\delta^{13}\text{C}$ in muscle) and the Washington coast ($\delta^{13}\text{C}$ in hair). In the eastern Gulf of Alaska, $\delta^{13}\text{C}$ was lower in Steller sea lions than in harbor seals. Presumably, this reflects a greater reliance on pelagic or offshore food sources by sea lions (Pitcher 1980a; Merrick and Calkins, *in press*). The relative $\delta^{13}\text{C}$ values for these two species were reversed for the Washington animals, however, as $\delta^{13}\text{C}$ was lower in harbor seals. This probably reflected a greater use of Columbia River waters by harbor seals (Smith *et al.* 1996). The harbor seals sampled during this study were found on sand bars adjacent to the mouth of the Columbia River. Harbor seals are present in the Columbia River during most of the year but particularly during autumn and winter (Beach *et al.* 1985). Certainly much of their food is derived from this freshwater system, and low carbon ratios compared to those in animals feeding exclusively in a marine system would be expected.

Future research—Stable-isotope analysis has been applied successfully to delineate trophic relationships in terrestrial and marine systems (Lajtha and Michener 1994) but has only recently been applied to studies involving marine mammals. There are a number of directions for future research. Within the North Pacific, continued sampling of tissues from pinnipeds and their prey is

encouraged in order to better establish trophic segregation between species as well as ages and sexes within species. Such base-line information will enable a better understanding of the influence of large-scale changes to prey populations such as those possibly resulting from commercial fisheries operations. Further studies are also required to determine the influence of nutritional stress on $\delta^{15}\text{N}$ levels in animals due to reproduction, fasting, and reductions in prey availability. The use of captive-rearing studies using controlled diets could facilitate such research and also better define patterns of isotopic fractionation between diet and tissues of marine mammals for a variety of stable isotopes (e.g., C, N, S, H; Hobson *et al.* 1996). Finally, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values within foodwebs are known to change geographically in various ocean systems including the Bering Sea (D. Schell, personal communication). Refinements in our understanding of these isotopic patterns will enhance our ability to apply the stable-isotope approach to studies of marine foodwebs in general and marine mammals in particular.

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