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Impact of Killer Whale Predation on Harbor Seals in Prince William Sound: A Preliminary Assessment of Diet Using Stable Isotope and Fatty Acid Signature Analysis on Blubber Biopsies

Restoration Project 96012A-2
Final Report

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Study History: The effect of predation on the recovery rates of injured resources has been defined as a priority research issue by the EVOS Trustee Council. In Prince William Sound, predation by killer whales occurs on at least three injured resources: harbor seals (*Phoca vitulina*), salmon (*Ochorhynchus* sp), and herring (*Clupea pallasi*). To predict the relative impact that killer whale predation may have on these injured resources, the level of predation and the relative proportion of each species consumed by killer whales must be quantified. Current information on the feeding habits of killer whales are based either on observations of feeding events or examination of stomach contents of stranded whales. Although both methods are valid, each approach has serious limitations. The current paradigm is that transient whales feed primarily on marine mammals and that resident whales feed on fish. The objective of the present study was to assess whether stable isotope signature analysis and fatty acid signature analyses could be applicable in the resolution of this problem. This preliminary attempt was designed to ascertain whether standard biopsy samples, which are commonly derived for genetic analysis, would be appropriate for use in the assessment of feeding habits using these techniques.

Abstract: Data on levels of $\delta^{15}N$ and $\delta^{13}C$ were obtained from blubber samples collected from resident and transient killer whales found in Prince William Sound AK. One difficulty in interpreting these data relates to a lack of information for turnover rates of these isotopes in cetaceans. Significant differences in $\delta^{15}N$ values and in the calculated trophic level are consistent with the suggestion that transient whales may feed on marine mammals, but are not inconsistent with them feeding on piscivorous fish such as coho salmon. These data do not allow us to calculate what proportion of their diet may be represented by these prey items. The data do suggest that there is likely intra-pod, as well as inter-pod, variability in feeding strategy. One significant result of the present study was the confirmation that the outer layer of blubber is inappropriate for fatty acid signature analysis and that a deeper sample is necessary. Fatty acid analysis of the outer layer of blubber indicated the presence of long-term structural components rather than those which would indicate recent feeding history. At present the appropriate depth cannot be estimated, however, on-going research will attempt to define the necessary dart design.

Key words: feeding ecology, stable isotope analysis, biopsy samples, fatty acid signature analysis, killer whales, *Orcinus orca*, Prince William Sound AK

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Table of Contents:

List of Appendices:

Introduction: Killer whales are classified as top predators in the marine ecosystem with diets that vary regionally and seasonally (Heyning and Dahlheim 1988). Two life-history patterns, involving two forms of killer whales termed resident and transient, have been suggested for whales occupying the waters of Puget Sound, Washington and British Columbia (Matkin and Saulitis 1994). One criterion used to differentiate the two forms is diet. Resident whales are thought to feed primarily on fish, whereas transients are thought to feed primarily on marine mammals. Both forms of killer whale have been described in Prince William Sound AK (PWS) (Heise *et al.* 1992), but there is only anecdotal evidence suggesting consistent feeding differences. In PWS, predation by killer whales occurs on at least three injured resources: harbor seals (*Phoca vitulina*), salmon (*Ochorhynchus* sp), and herring (*Clupea pallasi*). To predict the relative impact that killer whale predation may have on these injured resources, the level of predation and the relative proportion of each species consumed by killer whales must be quantified.

Although studied for decades, relatively little is known about feeding habits of killer whales. Most information has been obtained from stomach content analysis of shot or stranded animals (Nishiwanka and Handa 1958, Rice 1968, Ivashin 1981, Jefferson *et al.* 1991) whose stomachs are usually empty or would only indicate food consumed within the past 6-8 hours. Transient killer whales along the Pacific Northwest coast, in particular Puget Sound and southwest British Columbia, have been described as feeding extensively on marine mammals with a trend to exploitation of harbor seal haulouts during the pupping season (Heimlich-Boran 1988). In contrast, it has been suggested that resident whales feed primarily on salmon and other fish (Bigg *et al.* 1987, Ford *et al.* 1994, Nichol and Shakleton 1996). This latter suggestion is at least partially based on the identification of fish scales found floating at the surface after foraging behavior was observed. It has also been suggested, based on some observational data, that resident whales in Prince William Sound consume primarily fish, whereas transient whales preferentially consume other marine mammals such as harbor seals (Craig Matkin pers. observ.). This has led to the suggestion that transient killer whales may be impacting the post-EVOS recovery of harbor seal populations within Prince William Sound.

The determination of feeding preferences through either direct observation of feeding or the analysis of stomach contents has serious limitations. In recent years there have been a number of studies undertaken using stable isotopes of carbon and nitrogen to determine trophic relationships of a variety of birds and mammals (*e.g.*, Hobson 1987, Schell *et al.* 1989, Hobson and Montevecchi 1991, Sukumar and Ramesh 1992, Hobson *et al.* 1994, Hobson *et al.* 1996, Abend and Smith 1995, Abend and Smith 1997). Since the stable isotope ratio of a consumer reflects its diet (*e.g.*, DeNiro and Epstein 1978, 1981), whale tissues analyzed for these ratios have the potential to indicate dietary history. This approach would overcome the temporal biases inherent in stomach content analysis or strict observational evidence and has been used for other cetacean species (*e.g.*, Schell *et al.* 1989 Ostrom *et al.* 1993, Abend and Smith 1995, Abend and Smith 1997).

Isotope ratios are determined by the general type of food (original method of carbon fixation, number of trophic levels) that has been incorporated into the animal over the past several weeks or months and it gives a good overall idea of the average diet. With multiple types of food

generally available, isotope ratios can indicate, but cannot prove, that a certain type of food was used; they can however, sometimes prove when a food has not been eaten and assimilated (Gearing 1991). In general, the $\delta^{13}C$ and $\delta^{15}N$ values of animals reflect the isotopic values of their diets, even though animals appear to incorporate dietary ^{15}N over ^{14}N. Isotopic analysis of an animal can therefore be used to reconstruct the diet when food sources have different $\delta^{15}N$ values. Furthermore, the ^{15}N enrichment between trophic levels ranges from 1.3 to 5.3‰, averaging 3.4 ± 1.1‰ irrespective of biochemical differences or differences in habitat (DeNiro and Epstein 1978; 1981). This characteristic elevation in nitrogen isotope ratio is thought to be due to isotopic fractionation associated with catabolic metabolism (Ehleringer *et al.* 1986). This suggests that the $\delta^{15}N$ of the animal's body would be a useful parameter in assessing its trophic level in an ecosystem. On the other hand, the $\delta^{13}C$ values of animal tissue are very close to those in their diet, and only a small increase in ^{13}C content (about 1‰ enrichment) occurs with increasing trophic level (DeNiro and Epstein 1978; 1981). The primary theoretical basis of using $\delta^{13}C$ as a tracer is that the characteristic ratios of different sources are preserved as the carbon is cycled through organisms and detritus. Consequently, the differences in $\delta^{13}C$ values have been used for the food habit analysis of animals in an ecosystem.

Since the turnover rate of an isotope in any single tissue is dependent on the tissue's metabolic rate (Tieszen *et al.* 1983, Hobson and Clarke 1992, Hobson *et al.* 1996), measuring the isotopic signature of several tissues from the same individual can provide short-, intermediate-, and long-term dietary information (*e.g.*, Hobson 1993, Hobson *et al.* 1996, Abend and Smith 1997). Analysis of metabolically inactive tissues, such as hair, baleen, teeth, or whiskers, will reflect the diet as it existed during the period of tissue growth (*e.g.*, Schell *et al.* 1989), while tissues which are very metabolically active, such as blood, will reflect the signature of the most recent meal. One difficulty in examining isotopic signatures of cetaceans is the lack of knowledge about the turnover rates of any tissues. There is some turnover data available for seals (*e.g.*, Hobson *et al.* 1996) and some basic information on cetaceans (*e.g.*, Ostrom *et al.* 1993, Abend and Smith 1995), but it is difficult to extrapolate tissue turnover data for other species to cetaceans because of suggestions that cetacean skin has higher turnover rates than other mammals (Bruce-Allen and Geraci 1985). There is also the question about the structural differences between different portions of the blubber layer. Three distinct layers of blubber have been described for other odontocetes and these studies suggest that the middle layer, which has the highest concentration of fat cells, is likely the most "active" layer (Cowan and Worthy 1991). This layer would presumably also best reflect the recent diet of the animal. In addition to this lack of compositional homogeneity, different layers may also have different turnover rates (Koopman *et al.* 1996) further complicating their interpretation.

Recently there has been increasing interest in a new technique which uses the analysis of fatty acid signatures to identify the particular prey being consumed by a predator. There have been a number of studies suggesting a strong relationship between the fatty acid composition of storage tissues in an animal and the fatty acid composition of its prey (*e.g.*, Iverson 1993, Iverson *et al.* 1995, Iverson *et al.* 1997). Ultimately, this approach requires knowledge of the fatty acid signatures of all potential prey species, but it has the potential to answer the question of whether a

particular whale had been feeding on a specific species. This approach has shown very promising results in initial applications to harbor seals in Prince William Sound AK (Iverson *et al.* 1997) and, while it has yet to be validated in controlled studies with cetaceans, should have a great deal of potential for resolving the question of feeding habits in killer whales.

Objectives: The goal of the present study was to undertake a preliminary investigation into the question of feeding preferences of killer whales by utilizing skin and blubber samples collected using biopsy darts. The relatively shallow depth of the dart means that the samples will originate in the outer layer of blubber nearest the skin. The goal of the present study was to examine the feasibility of using the techniques of stable isotope signature and fatty acid signature analysis with these types of tissue samples to attempt to answer the question of whether transient and resident killer whales differ in their dietary preferences.

Materials And Methods:

1) Sample Collection and Field Processing: Samples were obtained using biopsy darts fired from an air pistol powered by a CO_2 cartridge. The dart size was 6 mm x 25 mm (diameter x depth) and the dart was made of plastic to reduce the weight and improve flotation. All darts were flame sterilized prior to use.

Samples were collected by North Gulf Oceanic Society. During sampling, vessel approach was gradual at a speed matching that of the focal whale. Whales were only sampled when there was no chance that a non-focal animal would be struck. The dorsal surface immediately below the dorsal fin was targeted and darts were retrieved once the whales had moved away from the area. Identity of any individual whale was ascertained prior to sampling, but photographs were also taken to confirm identities later. Blubber samples (approximately 1 g) were immediately separated from skin and stored frozen in glass vials for subsequent analysis. Skin was used in genetic analysis and unfortunately became unavailable for further analysis.

2) Stable Isotope Signature Analysis: Carbon and nitrogen naturally occur in two stable forms. Lighter forms, ^{14}N and ^{12}C, are more abundant than the heavier forms, ^{15}N and ^{13}C. The common vernacular is to refer to the heavier isotope concentration as a ratio in δ notation (‰) as determined from:

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

where X is either ^{15}N or ^{13}C and R is the corresponding ratio of either $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The ^{13}C ratios are expressed relative to PeeDee limestone and the ^{15}N ratios are expressed relative to atmospheric nitrogen gas.

Samples were freeze-dried, then thoroughly ground. Approximately 2-4 mg of powdered sample was loaded into quartz combustion tubes, along with 2.5 g of precombusted CuO and 5 g of copper wire to fully combust the sample by pyrolytic decomposition. Tubes were sealed under vacuum and combusted in a preset temperature regime for approximately 15 hours. Using a vacuum line, CO_2 and N_2 were isolated for analysis on the mass spectrometer using cryogenic

distillation. A Nuclide 3/60-RMS and Finnigan Delta S isotope ratio mass spectrometer were used for nitrogen and carbon analyses respectively. Measurement error was 0.2‰ for nitrogen and 0.1‰ for carbon. To compare our blubber data with muscle data available from other sources - our values were "corrected" by subtracting 2.5‰ for $\delta^{15}N$ (a factor derived by comparing blubber and muscle data in Abend and Smith 1995) and by adding 2.5‰ for $\delta^{13}C$ (DeNiro and Epstein 1977, Tieszen *et al.* 1983).

3) Fatty Acid Signature Analysis: Blubber samples were extracted in 2:1 chloroform/methanol (v:v) with 0.01% BHT (w:v) by the Folch method (Folch *et al.* 1957) as modified by Iverson (1988). Fatty acid methyl esters were prepared directly from aliquots of the chloroform extract by the addition of borontrifluoride in methanol, sealing under nitrogen, and heating at 100°C for one hour. Following transesterification, methyl esters were extracted and purified in hexane.

Analysis of fatty acid methyl esters were performed according to Iverson *et al.* (1997) using temperature-programmed capillary gas liquid chromatography on a Perkin Elmer Autosystem II Capillary FID Chromatograph fitted with a 30 m x 0.25 mm i.d. column (J&W DB-23) and linked to a computerized integration system (Turbochrom 4 software). Identification of fatty acids and isomers were determined from known standard mixtures (Nu Check Perp., Elysian MN) and silver nitrate chromatography (Iverson 1988, Iverson *et al.* 1997). Fatty acids are designated by shorthand IUPAC nomenclature of carbon chain length:number of double bonds and location (n-x) of the double bond nearest the terminal methyl group.

Results:

1) Animals Sampled: Biopsy samples were obtained from representatives of four pods of transient whales (AT, AU, AS, and AC pods) and four pods of resident whales (AK, AE, AD, and AB pods) during 1994 and 1995 (Table 1). To eliminate possible annual variability, only samples from an equal number of resident and transient whales sampled in 1995 were used in the current analysis.

2) Stable Isotope Signature Analysis: Uncorrected values for the resident whale population were -19.8 ± 1.1‰ for $\delta^{13}C$ and 18.9 ± 0.8‰ for $\delta^{15}N$, compared to the transient whale values of -18.7 ± 1.5‰ and 19.9 ± 1.0‰ respectively (Table 2). These values were subsequently "corrected" to facilitate comparison with other published data for muscle values resulting in a shift of +2.5‰ for carbon and -2.5‰ for nitrogen (Table 2). There was no significant difference in $\delta^{13}C$ values between these two groups of whales, but $\delta^{15}N$ values were significantly greater in the transient whales (t-test, $p < 0.05$).

When the corrected $\delta^{13}C$ and $\delta^{15}N$ data for the transient whales were examined - three individual animals appeared to be different. AT1, AT14, and AU3 had significantly ($p < 0.05$) more enriched $\delta^{13}C$ and $\delta^{15}N$ values than other transient whales or resident whales (Figure 1). Mean $\delta^{13}C$ for these three animals (referred to as "transient whales group 2") was -14.8 ± 0.06‰ and mean $\delta^{15}N$ was 18.0 ± 0.7‰. Removing their data from the balance of the transient whale population (referred to as "transient whales group 1") resulted in a change in the mean value for

$\delta^{13}C$ to -17.5 ± 0.6‰ and for $\delta^{15}N$ to 17.0 ± 1.0‰. The removal of transient whales group 2 data also eliminated any significant differences between the resident animals and those in transient whales group 1.

Trophic level was calculated by using the equation: $TL = 1 + [(D_m - 5.4)/3.8]$, where TL is trophic level and D_m is the $\delta^{15}N$ value for muscle (Hobson and Welch 1992). When these values were calculated for each of the two presumed whale ecotypes, the trophic level for resident whales was 3.89 ± 0.20 (n = 7) and for the combined transient whales was 4.18 ± 0.25 (n=7) (Table 3). These values are significantly different ($p < 0.05$). When assessed separately, the mean trophic level for transient whales group 2 (AT1, AT14, and AU3) was 4.33 ± 0.15 and that for transient whales group 1 was 4.08 ± 0.26. This new value for transient whales group 1 is not significantly different from that of the resident whales ($p > 0.05$), while the value for the transient whales group 2 is significantly different from both other groups ($p < 0.05$).

3) Fatty Acid Signature Analysis: Very few of the long chain fatty acids which are critical for the identification of dietary history (Iverson 1993) were found and therefore no interpretation of feeding history was possible (Table 4, Figure 2, Appendix 1). There were high levels of two fatty acids (14:1 n-5 and 16:1 n-7) which are indicative of sequestered, biosynthesized components. All of the fatty acids which are indicative of diet were greatly reduced or absent (Table 4, Figure 2, Appendix 1).

Discussion: Transient killer whales have been reported to feed on almost every marine mammal species available to them, with predation having been reported on 15 cetacean and 7 pinniped species which are distributed in Alaskan waters (Jefferson *et al.* 1991). In addition transients have been described as feeding on sea otters (*Enhydra lutris*), river otters (*Lutra canadensis*), a deer carcass (*Odocoileus hemionus sitkensis*), a live moose (*Alces alces*), and several species of marine birds (Matkin and Saulitis 1994). Resident whales in PWS have never been observed to consume marine mammals (Matkin and Saulitis 1994), however, a resident pod in Puget Sound has been seen to attack a Dall's porpoise (*Phocoenoides dalli*), a harbor porpoise (*Phocoena phocoena*) and a harbor seal (Felleman *et al.*, 1991). This latter observation suggests that proposed dietary differences between resident and transient whales may not be clearly defined. Stomach content analysis of some dead whales do appear to support the suggestion, with stomachs containing either all marine mammal parts or all fish (Rice 1968), but since killer whales rarely strand very few stomachs have been analyzed. Stomach content data also represent a one time and fairly recent feeding event and could misrepresent the relative contribution of any prey item found in the stomach.

One advantage of studying a large predator feeding on relatively large prey, such as most marine mammals, is that they are easy to observe. This is true when killer whales are feeding on large schooling fish such as herring or salmon, or when they feed on marine mammals. Difficulties in interpreting these types of observational data relate to extrapolating observations which are temporally limited. There is also an inability to estimate actual mass of prey consumed. Obviously, alternative techniques are needed to determine the seasonal and regional dietary

preferences of killer whales.

Isotope data derived for the present study suggest that some transient killer whales had been feeding at a higher trophic level than resident whales. This could imply that they may have been feeding on marine mammals, but based only on isotopic data we cannot exclude the possibility that they may have been feeding on piscivorous fish. The $\delta^{13}C$ data suggest that the source of the carbon may have been from a system associated with the nearshore environment, since $\delta^{13}C$ values are typically higher in coastal or benthic foodwebs than pelagic ones (Rau *et al.* 1983, Hobson 1993, Hobson *et al.* 1997). Hobson *et al.* (1997) found that harbor seals and Steller sea lions from the Washington coast were more enriched in $\delta^{13}C$ than pelagic animals from the Gulf of Alaska. Not surprisingly, these data indicate that PWS whales may have been feeding on things living in Prince William Sound.

A new approach to determining which specific prey species are being consumed is that of Fatty Acid Signature Analysis (see Iverson *et al.* 1997). Unfortunately, blubber fatty acid data reported in the present study are consistent with those obtained by Koopman *et al.* (1996), whereby the signature of the outer blubber layer is consistent with a tissue undergoing very slow turnover (Figure 3) suggesting the tissue was likely more structural than a dynamic energy storage area. This is consistent with the histological observations of Cowan and Worthy (1991) which suggest that the outer layer of the blubber is primarily structural. These blubber samples did not contain any of those classes of fatty acids which are necessary to determine prey species (Iverson 1993, Iverson *et al.* 1997) and the results were therefore inconclusive. The apparently inadequate depth of the blubber samples and the unavailability of skin for analysis, collectively meant that the interpretative power of the fatty acid signature component of this study was limited.

Conclusions: Despite the inadequacies of the samples, stable isotope signature analysis was partially successful. The stable isotope data do suggest that some transient whales were feeding at higher trophic levels than other transients or residents, however, no whales were consistently feeding at the same trophic level as polar bears feeding on seals. This suggests that none of the whales were feeding solely on other marine mammals. These data do suggest that there is likely intra-pod, as well as inter-pod, variability in feeding strategy. They do not allow us to reject the hypothesis that at least some transient killer whales may feed on marine mammals, but also cannot confirm that they do. The failure of the fatty acid signature technique to elucidate specific prey species was an unfortunate by-product of the sampling protocol and clearly, in the future, biopsy samples need to be deeper. A small pilot project using archived killer whale blubber would be very informative as to what depths would be required to fully take advantage of the power of both the stable isotope signature and fatty acid signature techniques in the future.

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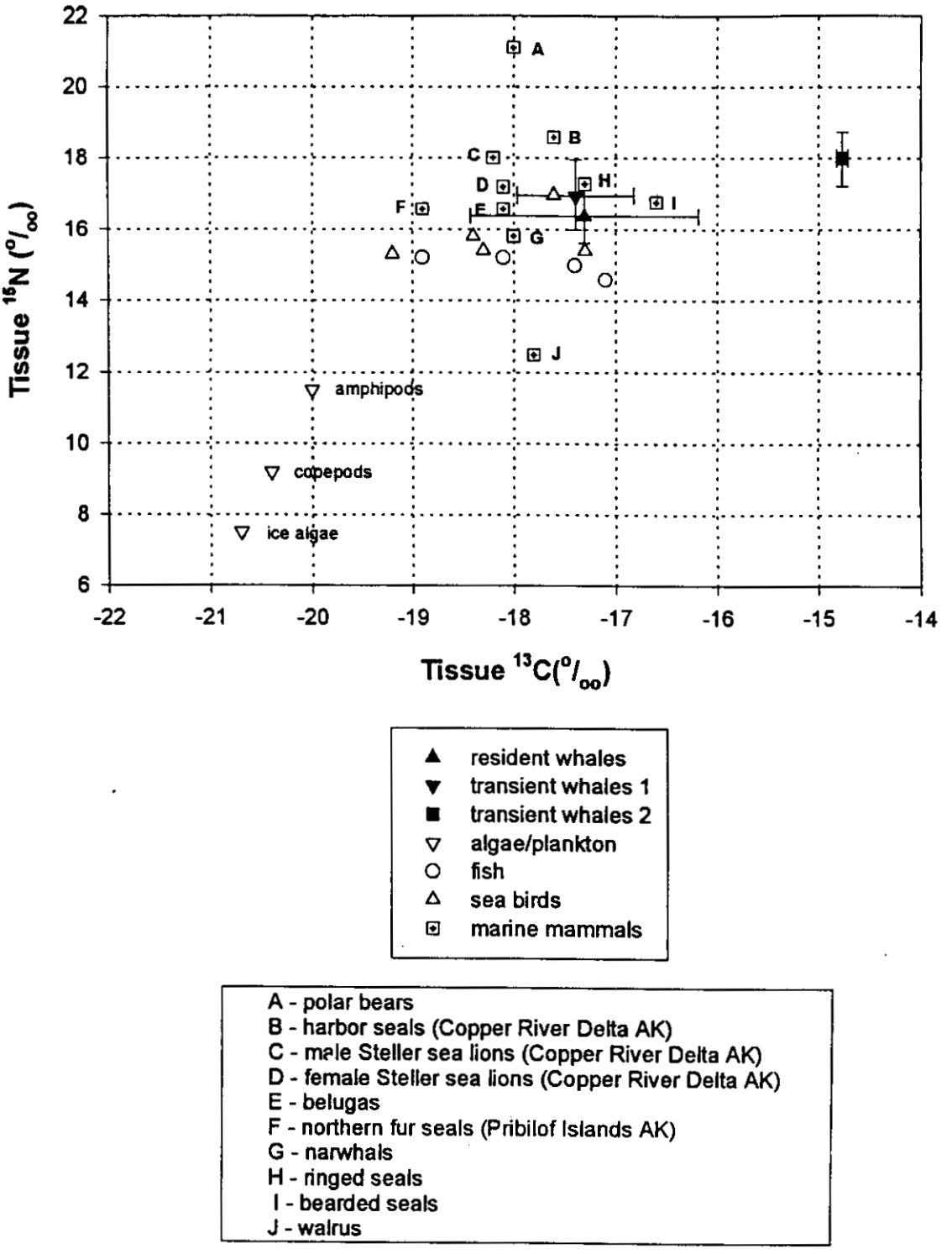

Figure 1: Relationship between $\delta^{15}N$ and $\delta^{13}C$ values for groups of marine food-web organisms. Mean values for "corrected" data (standard deviations are indicated by error bars) for resident and both groups of transient killer whales (see text for explanation) are shown, as well as mean values for other arctic data taken from the literature for comparison (Hobson and Welch 1992, Hobson *et al.* 1997).

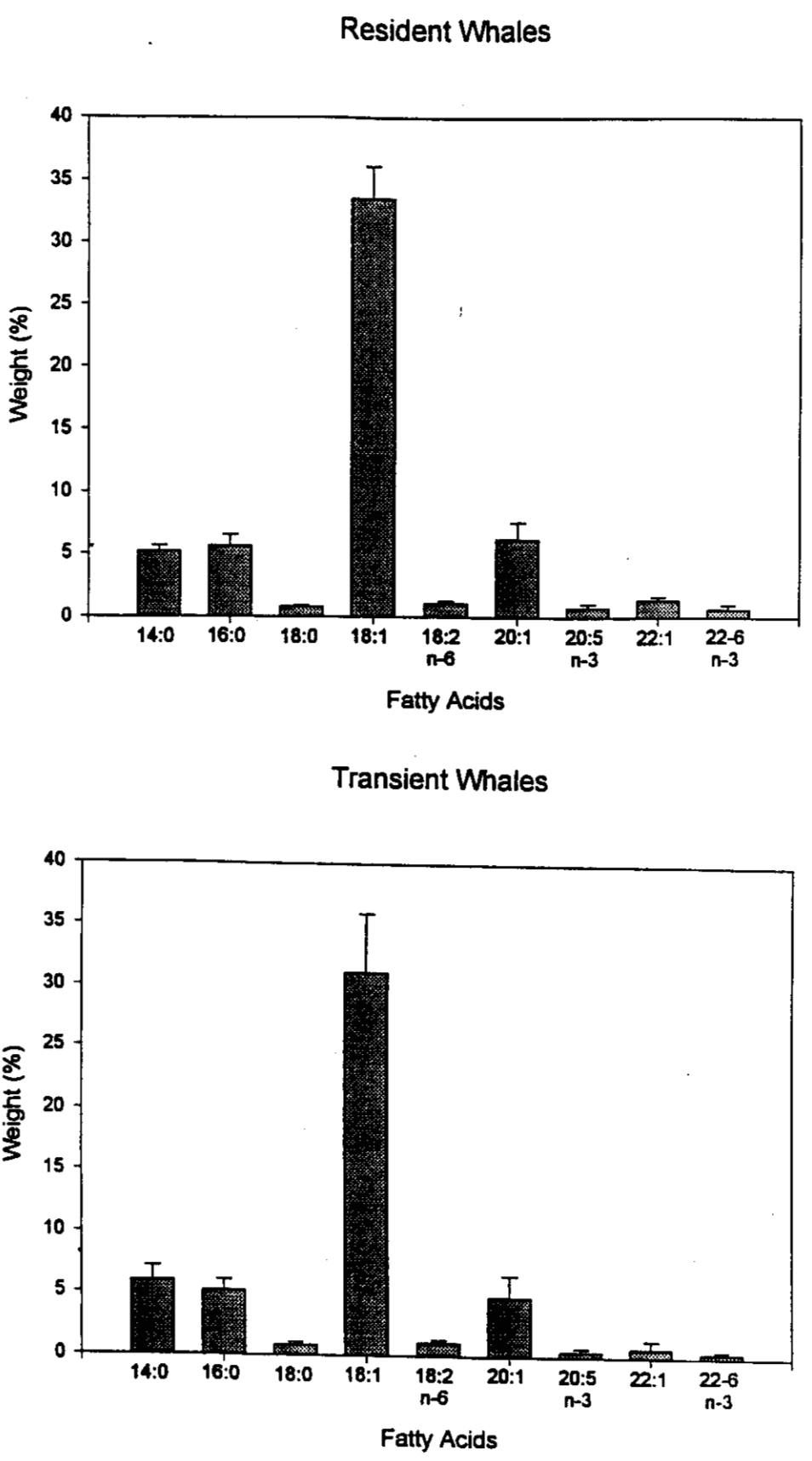

Figure 2: Percent weight of nine selected fatty acids from the outer blubber layer of resident and transient killer whales from Prince William Sound, AK. Standard deviations are indicated by error bars.

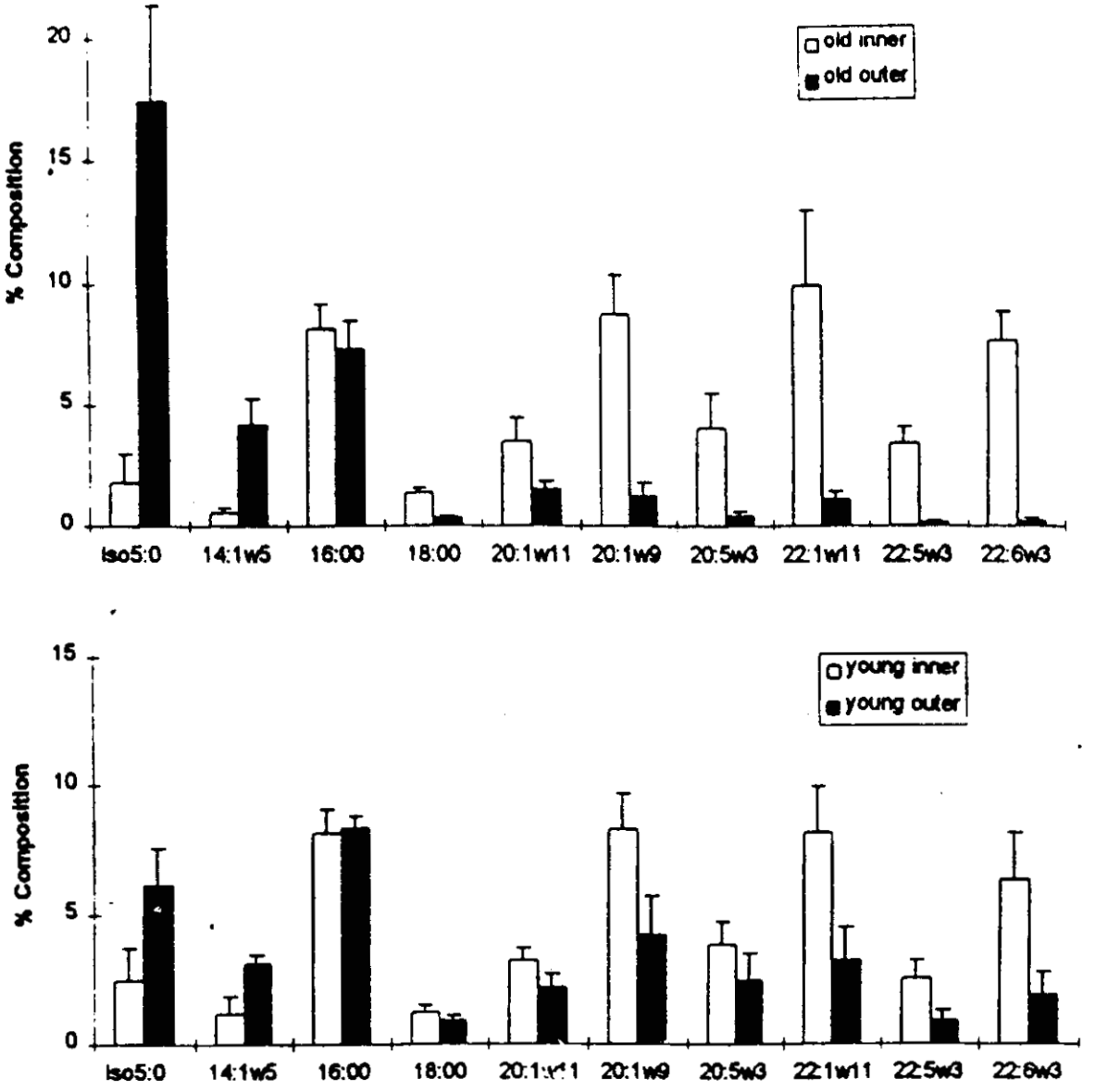

Figure 3: Percent composition of ten selected fatty acids derived from the inner and outer blubber layer of both young and old harbor porpoises (*Phocoena phocoena*) (from Koopman *et al.* 1996). Standard deviations are indicated by error bars.

Table 1: Animals from Prince William Sound, AK which were sampled, for stable isotope and fatty acid signature analysis, during 1994 and 1995. Numbers indicate both individual pod identification numbers as well as individual sample ID numbers. Not all samples were actually used for stable isotope and fatty acid signature analysis. Age/sex data are taken from Dahlheim and Matkin (1994), Matkin *et al.* (1994), and Matkin (1994) and are modified to reflect the year of sampling.

Resident Killer Whales			Transient Killer Whales		
Pod #	ID #	Age/sex	Pod #	ID #	Age/sex
AK 1	95-6	adult:M	AT 1	95-11	adult:M
AK 6	95-7	adult:F	AT 14	95-12	n/a:M
AK 7	95-8	n/a			
AK 12	95-10	n/a	AC 2	95-15	n/a
AE 1	94-13	adult:M	AU 2	95-23	adult:F
AE 2	94-18	n/a	AU 3	95-14	adult:F
AE 5	95-5	n/a	AU 4	95-24	n/a
AE 6	95-4	n/a			
AE 14	94-19	n/a	AS 12	95-16	n/a
AE 15	94-15	n/a	AS ?	95-17	n/a
AE 17	95-19	4 yr old:n/a	AS ?	95-18	n/a
AE 18	95-20	4 yr old:n/a			
AE 19	95-21	n/a			
AD 4	95-25	4 yr old:n/a			
AD 11	95-27	n/a			
AB 14	95-28	>23 yrs old:F			
AB 17	94-4	>20 yrs old:F			
AB 40	94-10	6 yrs old:n/a			
AB 45	95-29	4 yrs old:n/a			

Table 2: Actual and "corrected" stable isotope values (‰)of the outer blubber layer of resident and transient killer whales sampled in Prince William Sound, AK during 1995. "Corrected" values were obtained as described in the Materials and Methods section.

Actual Isotope Values

Resident Killer Whales			Transient Killer Whales		
Whale	$\delta^{13}C$	$\delta^{15}N$	Whale	$\delta^{13}C$	$\delta^{15}N$
AK 1	-18.2	19.0	AT 1	-17.2	21.3
AK 6	-18.6	19.2	AT 14	-17.3	20.4
AE 17	-20.2	19.7	AC 2	-19.8	19.8
AE 18	-21.4	19.7			
			AU 2	-19.2	19.6
AD 4	-19.4	18.6	AU 3	-17.2	19.8
AD 11	-20.3	17.5	AU 4	-20.6	20.4
AB 14	-20.4	18.4	AS 12	-19.9	18.1
	-19.8	18.9		-18.7	19.0
	±1.1	±0.8		±1.5	±1.0

"Corrected" Isotope Values

Resident Killer Whales			Transient Killer Whales		
Whale	$\delta^{13}C$	$\delta^{15}N$	Whale	$\delta^{13}C$	$\delta^{15}N$
AK 1	-15.7	16.5	AT 1	-14.7	18.8
AK 6	-16.1	16.7	AT 14	-14.8	17.9
AE 17	-17.7	17.2	AC 2	-17.3	17.3
AE 18	-18.9	17.2			
			AU 2	-16.7	17.1
AD 4	-16.9	16.1	AU 3	-14.7	17.3
AD 11	-17.8	15.0	AU 4	-18.1	17.9
AB 14	-17.9	15.9	AS 12	-17.4	15.6
	-17.3	16.4		-16.2	17.4
	±1.1	±0.8		±1.5	±1.0

Table 3: Estimated trophic levels for all of the whales sampled in the present study. Values calculated as described in Materials and Methods.

Resident Killer Whales		Transient Killer Whales	
Whale	Trophic level	Whale	Trophic level
AK 1	3.9	AT 1	4.5
AK 6	4.0	AT 14	4.3
AE 17	4.1	AC 2	4.2
AE 18	4.1		
		AU 2	4.1
AD 4	3.8	AU 3	4.2
AD 11	3.5	AU 4	4.3
AB 14	3.8	AS 12	3.7
	3.89 ±0.20		4.18 ±0.25

Table 4: Selected fatty acid values for the outer blubber layer of resident and transient killer whales sampled in Prince William Sound, AK during 1995. See Appendix 1 for full data set.

Pod #	presumed ecotype	14:0	16:0	18:0	18:1	18.2	20:1 (n-6)	20:5	22:1 (n-3)	22:6 (n-3)
AB-14	resident	5.2	6.0	0.8	31.6	1.1	4.9	0.9	1.1	0.8
AD-11	resident	4.6	4.1	0.6	34.7	1.4	6.8	0.8	1.7	0.7
AD-4	resident	4.5	4.6	0.7	33.9	1.1	5.2	0.6	1.1	0.5
AE-17	resident	5.4	6.8	0.9	35.8	1.1	7.0	0.8	1.5	0.6
AE-18	resident	5.1	6.1	1.0	34.7	1.1	8.3	1.4	1.8	1.3
AK-1	resident	5.5	5.7	0.9	35.7	1.2	6.7	0.7	1.4	0.6
AK-6	resident	5.8	5.8	0.8	28.7	0.8	4.4	0.3	1.2	0.2
AC-2	transient	7.2	5.9	0.8	24.0	1.0	3.5	0.2	0.5	0.1
AS-12	transient	5.1	4.8	0.9	35.8	0.8	8.4	0.8	2.1	0.6
AT-1	transient	6.1	5.5	0.8	32.2	1.1	4.2	0.3	0.5	0.4
AT-14	transient	4.5	3.4	0.5	30.6	1.1	3.4	0.3	0.4	0.5
AU-2	transient	7.8	5.8	0.7	25.9	0.8	3.6	0.4	0.5	0.2
AU-3	transient	5.7	4.8	0.7	32.4	1.1	5.2	0.3	0.8	0.3
AU-4	transient	4.9	5.5	1.3	36.9	1.5	4.7	0.9	0.4	0.6

Appendix 1: Fatty acid composition of the outer blubber layer of all killer whales which were analyzed in the present study. Each value is expressed as a percentage of the total fatty acids present.

Pod #	ID #	12:0	13:0	iso 14	14:0	14:1w9	14:1w7	14:1w5	iso15	anti15	15:0	15:1n-8	15:1n-6
Residents													
AB-14	95-28	1.6	0.0	0.6	5.2	1.6	2.3	6.2	0.6	0.3	0.3	0.2	0.3
AD-11	95-27	0.9	0.3	0.6	4.6	0.8	0.9	6.5	0.6	0.5	0.3	0.2	0.3
AD-4	95-25	1.2	0.0	0.5	4.5	1.3	2.0	6.7	0.5	0.0	0.3	0.3	0.2
AE-17	95-19	0.8	0.0	0.5	5.4	0.2	0.7	4.0	0.7	0.2	0.3	0.1	0.6
AE-18	95-20	0.7	0.0	0.4	5.1	0.5	0.5	4.6	0.6	0.1	0.3	0.1	0.5
AK-1	95-6	1.0	0.0	0.3	5.5	0.7	0.9	5.8	0.5	0.2	0.3	0.2	0.2
AK-6	95-7	1.7	0.2	0.5	5.8	2.5	3.1	6.4	0.5	0.3	0.3	0.2	0.2
Transients													
AC-2	95-15	2.1	0.0	0.7	7.2	2.8	4.2	7.8	0.9	0.3	0.3	0.2	0.3
AS-12	95-16	0.8	0.0	0.3	5.1	0.6	0.6	6.9	0.4	0.3	0.3	0.2	0.1
AT-1	95-11	1.3	0.0	0.4	6.1	1.0	1.8	7.1	0.6	0.2	0.3	0.2	0.2
AT-14	95-12	1.0	0.0	0.5	4.5	1.0	1.7	10.5	0.7	0.2	0.2	0.3	0.3
AU-2	95-23	2.6	0.0	0.5	7.8	2.8	3.4	8.0	0.8	0.3	0.3	0.2	0.2
AU-3	95-14	1.2	0.0	0.3	5.7	1.4	1.4	7.5	0.6	0.2	0.3	0.2	0.2
AU-4	95-24	0.7	0.0	0.4	4.9	0.7	0.6	5.8	1.0	0.1	0.3	0.2	0.4

Appendix 1. Continued

Pod #	ID #	iso16	16:0	16:1n-11	16:1n-9	16:1n-7	7me16:0	16:1n-5	16:2n-6	iso17	16:2n-4	16:3 n-6
Residents												
AB-14	95-28	0.3	6.0	2.6	4.9	22.0	0.3	0.0	0.0	0.1	0.5	0.1
AD-11	95-27	0.2	4.1	1.8	2.6	24.0	0.3	0.0	0.1	0.1	0.4	0.1
AD-4	95-25	0.4	4.6	2.2	4.6	23.4	0.3	0.2	0.2	0.1	0.5	0.1
AE-17	95-19	0.1	6.8	1.5	2.1	23.9	0.3	0.1	0.1	0.1	0.5	0.1
AE-18	95-20	0.1	6.1	1.2	1.6	23.0	0.3	0.1	0.1	0.1	0.5	0.1
AK-1	95-6	0.2	5.7	1.7	2.7	23.3	0.3	0.0	0.1	0.1	0.5	0.1
AK-6	95-7	0.1	5.8	2.6	5.1	23.7	0.3	0.2	0.2	0.1	0.5	0.1
Transients												
AC-2	95-15	0.2	5.9	2.1	5.9	25.6	0.3	0.2	0.2	0.1	0.4	0.1
AS-12	95-16	0.2	4.8	1.5	1.9	22.1	0.3	0.0	0.2	0.1	1.0	0.1
AT-1	95-11	0.6	5.5	1.2	3.4	27.8	0.3	0.1	0.1	0.0	0.8	0.1
AT-14	95-12	0.2	3.4	1.0	3.2	31.5	0.4	0.0	0.1	0.1	0.8	0.1
AU-2	95-23	0.1	5.8	2.2	4.9	24.0	0.3	0.2	0.2	0.1	0.9	0.1
AU-3	95-14	0.2	4.8	1.7	3.2	26.3	0.3	0.1	0.1	0.1	1.0	0.1
AU-4	95-24	0.2	5.5	1.0	1.7	26.0	0.3	0.1	0.3	0.1	0.7	0.1

Appendix 1. Continued

Pod #	ID #	17:0	16:3 n-4	17:1	16:3 n-1	16:4 n-1	18:0	18:1n-13	18:1n-11	18:1n-9	18:1n-7	18:1n-5	18:2d5,7
Residents													
AB-14	95-28	0.0	0.3	0.5	0.0	0.1	0.8	0.2	5.8	22.9	2.5	0.2	0.0
AD-11	95-27	0.0	0.1	0.5	0.0	0.1	0.6	0.3	4.8	26.8	2.6	0.2	0.0
AD-4	95-25	0.2	0.0	0.5	0.0	0.2	0.7	0.2	5.7	25.3	2.5	0.2	0.0
AE-17	95-19	0.1	0.0	0.4	0.1	0.2	0.9	0.3	4.5	27.6	3.2	0.2	0.0
AE-18	95-20	0.1	0.1	0.5	0.0	0.0	1.0	0.3	3.3	27.3	3.5	0.3	0.0
AK-1	95-6	0.1	0.1	0.4	0.0	0.0	0.9	0.2	4.7	27.5	3.1	0.2	0.0
AK-6	95-7	0.0	0.0	0.4	0.7	0.6	0.8	0.2	4.9	21.6	1.9	0.1	0.0
Transients													
AC-2	95-15	0.0	0.1	0.2	0.6	0.2	0.8	0.1	3.8	18.1	1.9	0.1	0.0
AS-12	95-16	0.2	0.1	0.3	0.0	0.0	0.9	0.3	4.0	27.4	3.9	0.2	0.0
AT-1	95-11	0.0	0.0	0.3	0.0	0.0	0.8	0.1	3.3	25.4	3.2	0.2	0.0
AT-14	95-12	0.0	0.1	0.3	0.0	0.0	0.5	0.1	3.0	24.3	3.1	0.1	0.0
AU-2	95-23	0.0	0.1	0.4	0.0	0.5	0.7	0.1	4.0	19.7	2.0	0.1	0.0
AU-3	95-14	0.0	0.0	0.4	0.0	0.1	0.7	0.1	4.4	25.1	2.7	0.1	0.0
AU-4	95-24	0.0	0.0	0.3	0.0	0.0	1.3	0.2	3.0	28.7	4.7	0.3	0.0

Appendix 1. Continued

Pod #	ID #	18:2n-7	18:2 n-6	18:2 n-4	18:3 n-6	18:3 n-4	18:3 n-3	18:3 n-1	18:4 n-3	18:4 n-1	20:0	20:1n-11	20:1n-9
Residents													
AB-14	95-28	0.0	1.1	0.0	0.0	0.0	0.4	0.1	0.7	0.1	0.0	4.3	0.6
AD-11	95-27	0.0	1.3	0.1	0.0	0.0	0.7	0.1	0.3	0.3	0.1	5.7	1.0
AD-4	95-25	0.0	1.1	0.0	0.0	0.0	0.5	0.0	0.9	0.1	0.1	4.4	0.7
AE-17	95-19	0.0	1.0	0.1	0.0	0.0	0.4	0.1	0.7	0.1	0.0	5.8	1.1
AE-18	95-20	0.0	1.0	0.1	0.0	0.0	0.5	0.1	0.2	0.2	0.1	6.5	1.6
AK-1	95-6	0.0	1.1	0.1	0.0	0.0	0.5	0.1	0.1	0.2	0.0	5.5	1.1
AK-6	95-7	0.0	0.8	0.0	0.0	0.0	0.3	0.6	0.1	0.0	0.0	3.9	0.5
Transients													
AC-2	95-15	0.0	0.8	0.0	0.0	0.0	0.2	0.0	0.9	0.0	0.0	3.2	0.3
AS-12	95-16	0.0	1.1	0.1	0.0	0.0	0.5	0.1	0.2	0.1	0.1	6.7	1.5
AT-1	95-11	0.1	1.1	0.0	0.0	0.0	0.4	0.1	0.1	0.0	0.0	3.5	0.6
AT-14	95-12	0.0	1.1	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.0	2.9	0.5
AU-2	95-23	0.0	0.8	0.0	0.0	0.0	0.2	0.8	0.1	0.0	0.0	3.2	0.4
AU-3	95-14	0.0	1.1	0.0	0.0	0.0	0.4	0.1	0.0	0.7	0.0	4.6	0.6
AU-4	95-24	0.1	1.4	0.1	0.0	0.0	0.5	0.1	0.1	0.1	0.1	3.6	1.0

Appendix 1. Continued

Pod #	ID #	20:1n-7	20:1n-5	20:2 n-6	20:3 n-6	20:4n-w6	20:3 n-3	20:4 n-3	20:5 n-3	22:1n-11	22:1n-9	22:1n-7	22:2 n-6
Residents													
AB-14	95-28	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.9	1.0	0.1	0.0	0.0
AD-11	95-27	0.1	0.0	0.1	0.0	0.2	0.0	0.2	0.8	1.5	0.2	0.0	0.0
AD-4	95-25	0.1	0.0	0.0	0.0	0.2	0.0	0.2	0.6	0.9	0.2	0.0	0.0
AE-17	95-19	0.1	0.0	0.1	0.0	0.3	0.0	0.2	0.8	1.3	0.2	0.0	0.0
AE-18	95-20	0.2	0.0	0.1	0.0	0.4	0.0	0.3	1.4	1.6	0.2	0.0	0.0
AK-1	95-6	0.1	0.0	0.0	0.0	0.4	0.0	0.1	0.7	1.2	0.2	0.0	0.0
AK-6	95-7	0.0	0.0	0.0	0.0	0.1	0.0	0.1	0.3	1.0	0.2	0.0	0.0
Transients													
AC-2	95-15	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.5	0.0	0.0	0.0
AS-12	95-16	0.2	0.0	0.1	0.0	0.3	0.0	0.2	0.8	1.9	0.2	0.0	0.0
AT-1	95-11	0.1	0.0	0.0	0.0	0.3	0.0	0.1	0.3	0.4	0.1	0.0	0.0
AT-14	95-12	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.3	0.3	0.1	0.0	0.0
AU-2	95-23	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.4	0.5	0.0	0.0	0.0
AU-3	95-14	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.3	0.8	0.0	0.0	0.0
AU-4	95-24	0.1	0.0	0.0	0.0	0.5	0.0	0.2	0.9	0.4	0.0	0.0	0.0

Appendix 1. Continued

Pod #	ID #	21:5 n-3	22:4 n-6	22:5 n-6	22:4 n-3	22:5 n-3	22:6 n-3	24:1n-11	24:1n-9
Residents									
AB-14	95-28	0.0	0.0	0.0	0.0	0.3	0.8	0.0	0.0
AD-11	95-27	0.0	0.0	0.0	0.0	0.3	0.7	0.1	0.0
AD-4	95-25	0.0	0.0	0.0	0.0	0.2	0.5	0.1	0.0
AE-17	95-19	0.0	0.0	0.0	0.0	0.4	0.6	0.1	0.0
AE-18	95-20	0.0	0.0	0.0	0.0	0.7	1.3	0.0	0.0
AK-1	95-6	0.0	0.0	0.0	0.0	0.4	0.6	0.0	0.0
AK-6	95-7	0.0	0.0	0.0	0.0	0.1	0.2	0.1	0.0
Transients									
AC-2	95-15	0.0	0.0	0.0	0.0	0.1	0.1	0.0	0.0
AS-12	95-16	0.0	0.0	0.0	0.0	0.4	0.6	0.0	0.0
AT-1	95-11	0.0	0.0	0.0	0.0	0.3	0.4	0.0	0.0
AT-14	95-12	0.0	0.0	0.0	0.0	0.3	0.5	0.0	0.0
AU-2	95-23	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0
AU-3	95-14	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0
AU-4	95-24	0.0	0.0	0.0	0.0	0.7	0.6	0.0	0.0