Stratification of lipids, fatty acids and organochlorine contaminants in blubber of white whales and killer whales

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ABSTRACT

The biopsy – via dart, trocar or surgery – is becoming the preferred protocol for sampling skin and blubber of many cetacean species, because a small sample from a healthy animal may provide better information than a larger sample collected via necropsy from an ill or emaciated animal. Furthermore, the biopsy is often the only means of obtaining samples (e.g. for threatened or endangered species). Because biopsy darts collect only a small sample of tissue – and blubber can be heterogeneous in structure and composition – it is essential to compare the results obtained from biopsies to those found by analysing full-thickness blubber samples obtained via necropsy. This manuscript compares blubber stratification in two odontocete species, white whales (*Delphinapterus leucas*) and killer whales (*Orcinus orca*). Five parameters (i.e. lipid percent and classes, contaminant concentrations and profiles, fatty acid profiles) were measured by blubber depth. Results of these comparisons strongly suggest that biopsy results must be interpreted with caution and in conjunction with results from species-specific blubber depth profiling. For example, lipid classes measured in biopsy samples of white whales and killer whales were similar to those for equivalent-depth samples obtained by necropsy. In addition, lipid-adjusted contaminant concentrations measured in dart or trocar samples adequately represented those obtained by necropsy of both species. Conversely, the lipid content in biopsy samples was lower than that found in same-depth necropsied samples due to loss of lipid during sampling. Also, because of the high level of fatty acid stratification observed, fatty acid profiles from the outer blubber layer collected via biopsy from both species are less likely than the metabolically active inner layer to be useful in determining the prey species consumed by these odontocetes. This study demonstrates, for white and killer whales, that properly interpreted results from blubber biopsies can provide valuable information about the body condition, health and life history of individual animals.

KEYWORDS: POLLUTANTS; ORGANOCHLORINES; STRANDINGS; BIOPSY SAMPLING; KILLER WHALE; WHITE WHALE; PACIFIC OCEAN

INTRODUCTION

Lipid (fat) comprises a large proportion of the body mass of many cetaceans, with most of the lipid consolidated as a blubber layer. Analysis of blubber can provide a great deal of information about the body condition and health of marine mammals. For example, blubber thickness and lipid content can be indicative of the nutritive condition of cetaceans (Aguilar and Borrell, 1990) and profiles of fatty acids in blubber can be used to infer the diet of marine mammals (Adams *et al*., 1997; Iverson *et al*., 1997; 2002; Walton *et al*., 2000). In addition, measuring concentrations of lipophilic organochlorine contaminants (OCs) in the blubber of top predators (e.g. odontocetes) provides information on potential adverse health effects resulting from contaminant exposure, because high OC concentrations have been associated with immunosuppression, reproductive impairment, alteration in bone development and growth and increased susceptibility to disease (Reijnders, 1986; Olsson *et al*., 1994; De Guise *et al*., 1996; 1997; De Swart *et al*., 1996; Kamrin and Ringer, 1996; Ross *et al*., 1996; Zakharov *et al*., 1997; Beckmen *et al*., 1999; 2003). However, when measuring OC concentrations, changes in lipid class profiles (i.e. proportions of triglycerides, free fatty acids, phospholipids, wax esters and cholesterol) should be considered because these factors may influence the concentrations of contaminants in a blubber layer (Koopman *et al*., 1996).

Collecting biopsy samples (i.e. blubber and epidermis) from wild cetaceans – through surgical or punch biopsies on captured-released individuals and through remote biopsy darting – is becoming routine as part of an effort to develop non-destructive techniques for studying genetics, contaminant exposure and feeding ecology (stable isotope ratios and fatty acid profiles) of populations (Barrett-Lennard *et al*., 1996; Fossi, F.C. *et al*., 1997; Fossi, M.C. *et al*., 1997; 1999; 2000). In addition, biopsy samples from presumably healthy animals are preferred over samples collected from necropsies of stranded cetaceans, because strandings often involve animals in compromised health that are not representative of the overall population (Brown, 1985; Aguilar *et al*., 1999). However, only a few studies have tested whether blubber biopsies provide a sample that is representative of the entire blubber layer in cetaceans (Aguilar and Borrell, 1991; Gauthier *et al*., 1997) and these studies have looked primarily at the distribution of OCs within the blubber of mysticetes.

Differences in OC concentrations by blubber depth appear to be species-specific, i.e. some species show pronounced stratification and others have blubber that is more homogenous. Aguilar and Borrell (1991) found higher lipid-normalised PCB and DDT concentrations in the outer blubber layer compared to the inner layer in fin whales (*Balaenoptera physalus*) and in male sei whales (*B. borealis*) (by factors of 1.1 to 1.5), but female sei whales showed little variation in OC concentrations between these layers. The authors concluded that blubber samples collected from cetaceans for pollutant analyses should include all layers in order to be representative of an individual animal's pollutant load. In another study, Gauthier *et al*. (1997) found no statistically significant differences in lipid-normalised OCs among the outer,

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middle or inner blubber layers in minke (*B. acurostratra*) and blue whales (*B. musculus*).

Recently, as interest in using blubber fatty acids to provide information about diet has increased, researchers have questioned whether biopsy samples can provide adequate samples for fatty acid signature analysis. Koopman (1996; 2001; 2002) found that significant vertical stratification of fatty acids was evident between the inner and outer blubber layers in odontocetes, suggesting that the inner blubber layer is more active metabolically than the outer layer in terms of lipid deposition and mobilisation. Similarly, Olsen and Grahl-Nielsen (2003) observed significant vertical stratification of fatty acids in minke whale blubber and concluded that studies to trace dietary influence of fatty acids should be made using the inner blubber layer. Furthermore, Hooker *et al.* (2001) reported that fatty-acid stratification was present throughout the depth of the blubber in northern bottlenose whales (*Hyperoodon ampullatus*), but was much less pronounced than that found in the blubber of smaller cetaceans (Koopman, 2001; Koopman *et al.*, 2002).

Because the dart biopsy is becoming a standard protocol for obtaining tissue samples from many cetacean species, it is essential to compare the results obtained from analysing biopsy samples to those for blubber collected by necropsy. This paper compares blubber stratification in two odontocete species — white whales (*Delphinapterus leucas*) and killer whales (*Orcinus orca*) — by measuring five parameters (i.e. percent lipid, lipid classes, OC concentrations, OC patterns and fatty acid profiles) by blubber depth in samples obtained both by biopsy and necropsy. In addition, two biopsy-sampling techniques — darts and trocars — have been compared.

METHODS

Cetaceans sampled

Blubber samples from five white whales from the genetically distinct Cook Inlet and Bristol Bay populations (O'Corry-Crowe *et al.*, 1997), as well as from three stranded killer whales, were studied (Table 1). Two of the killer whales were marine mammal-eating transient ecotypes and one was a piscivorous resident ecotype (Black *et al.*, 1997; Ford *et al.*, 2000). Although non-stranded animals are preferred for blubber stratification studies, neither of the killer whales stranded in 2002 (L60 and CA189) was believed to have died from a chronic illness resulting in emaciation, so the results should not be biased from this cause. The cause of death of the Alaskan transient killer whale that stranded in 2003 (ATx; a currently unidentified member of the AT1 pod; see Table 1) is not known, but lipid content of the blubber from this animal was in the same range as previously found for wild Alaskan transients (Ylitalo *et al.*, 2001), so emaciation was unlikely.

Subsampling of blubber by depth and position on animal

Thickness of blubber from the necropsied white whales CI-73, CI-76 and BB-75 was sufficient to allow subdividing the samples by depth. However, because of differences in blubber thickness, blubber from CI-73 and CI-76 was divided into fourths and blubber from BB-75 (a juvenile) was divided into thirds.

Initial attempts to obtain full-thickness blubber samples from white whales using trocars were unsuccessful due to the fluidity of the blubber. Lipid seeped from the trocar tip, so these samples have not been included in this paper. However, samples CI-01-05 and CI-01-06 (6mm core) were frozen rapidly in the trocar and were removed while still frozen. The resulting samples were of sufficient length and mass to allow division into halves — near-epidermis and near-muscle subsamples.

Blubber was collected from the dorsal region of killer whales CA189 and ATx and also from the mid-lateral region of CA189. Subsequently, blubber was subdivided by depth into three layers from 0-2cm (from the epidermis), 2-4cm and >4cm. Blubber from L60 was collected from the dorsal and lateral body regions and from the anterior, central and posterior (to the dorsal fin) regions of the saddle patch. Blubber from each region was subdivided into three layers by depth from 0-2cm (from the epidermis), 2-4cm and >4cm (the lateral sample was not thick enough to include a third layer; see Table 4). At the same time, biopsy samples were taken using a dart (5 × 20mm) that was thrust fully into the anterior, central and posterior regions of the saddle patch. The samples were estimated to be ~2cm in depth. To simplify references, the near-epidermis blubber layer was termed the 'outer' layer, the layer nearest to the muscle the 'inner' layer and the other layer(s) were 'middle' layer(s).

TLC/FID lipid percent and lipid class determinations

Blubber samples were analysed for total lipids by thin layer chromatography coupled with flame ionisation detection (TLC/FID) using an Iatroscan Mark 5 (Shantha, 1992). The lipid sample extracts (i.e. a portion of the extract prepared for fatty acid analyses; see below) were spotted on Chromarods (Type SIII) and developed in a solvent system containing 60:10:0.02 hexane:diethyl ether:formic acid (v/v/v). The various classes of lipids (i.e. wax esters, triglycerides, free fatty acids, cholesterol and phospholipids) were separated based on polarity, with the nonpolar compounds (e.g. wax esters) eluting first, followed by the more polar lipids (e.g. phospholipids). The Iatroscan was operated with a hydrogen flow rate of 160mL min^{-1} and airflow of 2000m min^{-1}. A four-point linear external calibration was used for quantitation. Duplicate TLC/FID analyses were performed for each sample extract and the mean value reported. Total lipid concentrations were calculated by adding the concentrations of the five lipid classes for each sample and reported as total percent lipid relative to the original sample.

Fatty acid concentrations and profiles

The analytical method used to measure fatty acid concentrations in these tissues, which was recently developed in the Northwest Fisheries Science Center laboratory, integrates several methodologies reported in the literature. Briefly, the method involves: (1) extracting approximately 1g of tissue (mixed with sodium and magnesium sulphates to remove water) by Accelerated Solvent Extraction (ASE) using 50ml methylene chloride at 100°C and 2000psi (Sloan *et al.*, 2004); (2) partitioning the extract into three fractions [approximately 46% for OC analysis, 46% for gravimetric lipid and 8% for fatty acid and lipid class (Iatroscan) analysis]; (3) derivatising the fatty acid fraction to fatty acid methyl esters (FAMEs) using 3% sulphuric acid in methanol; (4) extracting the FAMEs into iso-octane; (5) drying the extract over a bed of sodium sulphate; and finally (6) separating and analysing the FAME extracts on a DB-23 capillary column using quadruple gas chromatography/mass spectrometry (GC/MS) operated in the selected ion monitoring (SIM) mode. In most cases, the molecular ion was chosen for quantitation and a confirmation ion was also monitored. Eighty-three different fatty acids were determined (as methyl esters; Table 2). A

Table 1
Life history and other parameters of the white whales and killer whales sampled.

	Sample type	Date sampled/ stranded	Location	Age class	Sex	Length (cm)	Ecotype	Comments
White whales								
CI-73	Subsistence/ necropsy	7/2001	Cook Inlet	Adult	F	345		Lactating
CI-76	Subsistence/ necropsy	7/2002	Cook Inlet	Adult	M	457		
BB-75	Subsistence/ necropsy	5/2002	Bristol Bay	Juvenile	M	287		
CI-01-05	Trocar/live	8/2001	Cook Inlet	Adult	F	362		Captured for satellite tagging and released.
CI-01-06	Trocar/live	8/2001	Cook Inlet	Adult	F	401		Captured for satellite tagging and released.
Killer whales								
CA189	Stranded/ necropsy	1/2002	Inland waters of Washington state	Adult	F	671	Transient	According to necropsy results, CA189 was 'fresh-dead' with a 'good' body condition and no emaciation. CA189 may have given birth to a stillborn calf or aborted a late-term foetus. Most often observed in California waters (Black et al., 1997).
L60	Stranded/ necropsy (biopsy*)	4/2002	Outer coast of Washington state	Adult	F	606	Resident	Southern Resident from a pod that inhabits the inland waters of Puget Sound in the summer months (Black *et al.*, 1997; Ford *et al.*, 2000). She had given birth to at least two calves, the first died (1990-1998), but the second calf is still alive (1995-) (Ford *et al.*, 2000).
ATx	Stranded/ necropsy	4/2003	Prince William Sound, AK	Adult	M	700	Transient	Likely a member of the AT1 pod that frequents the waters of Prince William Sound and the Kenai Fjords. Genetic analyses are currently underway to assist in identifying this individual.

* Biopsy samples were simulated by thrusting a biopsy dart into the saddle patch area of the killer whale (see Methods).

standard nomenclature system was used for naming these fatty acids, where 'n' followed by a number refers to the position of the first double bond relative to the alkyl end of the molecule.

OC contaminant concentrations and profiles

Blubber samples (1 to 3g) of both cetacean species were extracted, following addition of internal standards, using the ASE procedure (Sloan *et al*., 2004). The methylene chloride extract was then filtered through a column of silica gel and alumina and concentrated for further cleanup by high-performance liquid chromatography (HPLC) using a size-exclusion column that separated lipids and other biogenic material from the OC fraction (Krahn *et al*., 1988; Sloan *et al*., 2004). Finally, the fraction containing OCs was analysed by GC/MS to measure analyte concentrations. ΣPCB is the sum of the following 46 congeners (only 40 peaks because some congeners elute together) −17, 18, 28, 31, 33, 44, 49, 52, 66, 70, 74, 82, 87, 95, 99, 101/90, 105, 110, 118, 128, 138/163/164, 149, 151, 153/132, 156, 158, 170, 171, 177, 180, 183, 187/159/182, 191, 194, 195, 199, 205, 206, 208, 209; and ΣDDTs is the sum of *o,p'*-DDD, *p,p'*-DDD, *o,p'*-DDE, *p,p'*-DDE, *o,p'*-DDT and *p,p'*-DDT.

Statistical analyses

Prior to all statistical calculations, both OC and fatty acid concentration data were tested for normality by computing the skew and kurtosis of the individual analyte distributions; all were found to be substantially non-normal. In an attempt to improve the homogeneity of the data, the concentration data were both arcsine and square root transformed but neither of these transforms resulted in a consistent improvement in data normality nor resulted in an appreciable difference in any of the final PCA results. Therefore, the untransformed data were retained in the final analyses. All statistical comparisons among univariate variables were computed using a two sample, one-tailed, t-test of significance assuming unequal variances.

Principal Component Analysis (PCA) of OCs in blubber was conducted using JMP Statistical Discovery Software® (Macintosh version 5.01) to determine the similarity of contaminant and fatty acid patterns. Analyte concentrations were normalised by dividing concentrations of each OC analyte by total OCs (sum of the PCBs and pesticides measured). PCA was then computed on both the correlation and covariance matrices of these normalised data. When PCA is used, the number of samples should exceed the number of variables, preferably by a factor of two (McGarigal *et al*., 2000). Because of the need to further reduce the number of variables (64 variables and 15 samples for white whales; 58 variables and 23 samples for killer whales), only those OC analytes exhibiting the largest positive and largest negative eigen vector projections along the first three principal component axes were used for the final PCA analysis. For white whales, these analytes were PCB congeners 28, 52, 70, 118, 151, dieldrin, *p,p'*-DDD, *o,p'*-DDT; and for killer whales, analytes were congeners 52, 74, 99, 149, 156, 183, beta-HCH, alpha-chlordane, t-nonachlor, mirex, *o,p'*-DDE, *p,p'*-DDE. Also, all analytes that had values below the limits of quantitation were excluded from the dataset because values below this limit distort the pattern and strongly affect the PCA analysis.

Fatty acid concentrations were normalised by dividing concentrations of each analyte by the sum of all FAME analytes measured. All analytes that had values below detection limits were excluded and PCA was computed on both the correlation and covariance matrices. For white whales, the eight fatty acids that had the largest contribution to the first three principal components were used in the final PCA analysis – C16:1n7, iso-C18:0, C18:1n9, C18:1n13,

Table 2

Fatty acids analysed (as methyl esters) in the blubber of marine mammals. The fatty acid number (as shown in figure 3), abbreviation, systematic name and trivial (common) name are provided.

#	Abbreviation	Systematic name	Trivial name	#	Abbreviation	Systematic name	Trivial Name
1	C10:0	n-Decanoic acid§	Capric	43	C18:1n5	13-Octadecenoic acid	
2	C11:0	n-Undecanoic acid§	Hendecanoic	44	C18:1n7	11-Octadecenoic acid§	Vaccenic
3	C11:1	10-Undecenoic acid§	Hendecenoic	45	C18:1n9	9-Octadecenoic acid§	Oleic
4	C12:0	n-Dodecanoic acid§	Lauric	46	C18:2n4	11,14-Octadecadienoic acid	
5	C12:1	11-Dodecenoic acid§		47	C18:2n6	9,12-Octadecadienoic acid§	Linoleic
6	Me4812C13:0	4,8,12-Trimethyltridecanoic acid		48	C18:2n7	8,11-Octadecadienoic acid	
7	C14:0	n-Tetradecanoic acid§	Myristic	49	C18:3n1	11,14,17-Octadecatrienoic acid	
8	isoC14:0	12-Methyltridecanoic acid§		50	C18:3n3	9,12,15-Octadecatrienoic acid§	alpha-Linolenic
9	Me11C14:0	11-Methyltetradecanoic acid		51	C18:3n4	8,11,14-Octadecatrienoic acid	
10	C14:1n5	9-Tetradecenoic acid§	Myristoleic	52	C18:3n6	6,9,12-Octadecatrienoic acid§	gamma-Linolenic
11	C14:1n7	7-Tetradecenoic acid		53	C18:4n1	8,11,14,17-Octadecatetraenoic acid	
12	C14:1n9	5-Tetradecenoic acid		54	C18:4n3	6,9,12,15-Octadecatetraenoic acid§	
13	C15:0	n-Pentadecanoic acid§		55	C19:0	n-Nonadecanoic acid§	
14	isoC15:0	13-Methyltetradecanoic acid§		56	C20:0	n-Eicosanoic§	Arachidic
15	anteisoC15:0	12-Methyltetradecanoic acid		57	C20:1n11	9-Eicosenoic acid	Gadoleic
16	tMeC15:0	2,6,10,14-Tetramethylpentadecanoic acid		58	C20:1n15	5-Eicosenoic acid§	
17	C15:1n5	10-Pentadecenoic acid§		59	C20:1n5	15-Eicosenoic acid	
18	C16:0	n-Hexadecanoic acid§	Palmitic	60	C20:1n7	13-Eicosenoic acid	
19	isoC16:0	14-Methylpentadecanoic acid§		61	C20:1n9	11-Eicosenoic acid§	Gondoic
20	anteisoC16:0	13-Methylpentadecanoic acid		62	C20:2n11	6,9-Eicosadienoic acid	
21	Me7C16:1	7-Methylhexadecenoic acid		63	C20:2n6	11,14-Eicosadienoic acid§	
22	Me78C16:1na	7,8-Dimethylhexadecenoic acid		64	C20:2n9	8,11-Eicosadienoic acid	
23	C16:1n11	5-Hexadecenoic acid		65	C20:3n3	11,14,17-Eicosatrienoic acid§	
24	C16:1n5	11-Hexadecenoic acid		66	C20:3n6	8,11,14-Eicosatrienoic acid§	homo-gamma-Linolenic
25	C16:1n7	9-Hexadecenoic acid§	Palmitoleic	67	C20:4n3	8,11,14,17-Eicosatetraenoic acid	
26	C16:1n9	7-Hexadecenoic acid		68	C20:4n6	5,8,11,14-Eicosatetraenoic acid§	Arachidonic
27	C16:2n4	9,12-Hexadecadienoic acid		69	C20:5n3	5,8,11,14,17-Eicosapentaenoic acid§	EPA
28	C16:2n6	7,10-Hexadecadienoic acid		70	C21:5n3	6,9,12,15,18-Heneicosapentaenoic acid	
29	C16:3n4	6,9,12-Hexadecatrienoic acid		71	C22:0	n-Docosanoic acid§	
30	C16:3n6	4,7,10-Hexadecatrienoic acid		72	C22:1n11	11-Docosenoic acid	
31	C16:4n1	6,9,12,15-Hexadecatetraenoic acid		73	C22:1n5	17-Docosenoic acid	
32	C16:4n3	4,7,10,13-Hexadecatetraenoic acid		74	C22:1n7	15-Docosenoic acid	
33	C17:0	n-Heptadecanoic acid§	Margaric	75	C22:1n9	13-Docosenoic acid§	Erucic
34	anteisoC17:0	14-Methylhexadecanoic acid		76	C22:2n6	13,16-Docosadienoic acid§	
35	isoC17:0	15-Methylhexadecanoic acid§		77	C22:3n3	13,16,19-Docosatrienoic acid§	
36	C17:1n7	10-Heptadecenoic acid§		78	C22:4n3	10,13,16,19-Docosatetraenoic acid	
37	C17:1ne	Heptadecenoic acid		79	C22:4n6	7,10,13,16-Docosatetraenoic acid§	
38	C18:0	n-Octadecanoic acid§	Stearic	80	C22:5n3	7,10,13,16,19-Docosapentaenoic acid§	DPA
39	isoC18:0	16-Methylheptadecanoic acid§		81	C22:6n3	4,7,10,13,16,19-Docosahexaenoic acid§	DHA
40	anteisoC18:0	15-Methylheptadecanoic acid		82	C24:0	n-Tetracosanoic acid§	
41	C18:1n11	7-Octadecenoic acid		83	C24:1n9	15-Tetracosenoic acid§	Nervonic
42	C18:1n13	5-Octadecenoic acid					

§ These fatty acids were identified using known standards. The other fatty acids (unmarked) were tentatively identified based on GC retention time and mass spectral data.

C18:2n7, C18:3n3, C19:0, C20:1n7. For killer whales, the final PCA analysis was performed using the nine fatty acids exhibiting the largest statistically significant differences in concentrations when comparing all transient blubber samples (at all depths and locations) with all resident blubber samples. Those were 2,6,10,14-Me-C15:0, iso-C15:0, C16:3n4, iso-C18:0, C18:3n3, C18:1n13, C20:1n11, C22:1n11, C24:1n9.

Quality assurance

Quality assurance procedures for determining OC and fatty acid concentrations and percent lipid included use of Standard Reference Materials (SRMs), certified calibration standards, method blanks, solvent blanks and replicate samples. SRMs used (from the National Institute of Standards and Technology) were SRM 1945 for OC contaminants and percent lipids and SRM 1946 for fatty acids. Acceptance criteria for analyses of NIST SRM 1945 and SRM 1946 were those that NIST uses for its Intercomparison Exercises. In addition, our laboratory has successfully participated in NIST and other quality assurance intercomparison exercises each year.

To determine whether differences by depth in the lipid content and OC concentrations were larger than the variability inherent in the analyses, relative standard deviations for replicate analyses (n=8) of these analytes were determined for the SRM analysed with each set of samples. RSDs were relatively low (RSDs = 3.7% for lipid content, 8.3% for ΣPCBs and 8.4% ΣDDTs) and generally less than the differences reported between different depths or techniques. Replicates of samples analysed in the same

set showed lower OC variability (RSDs = 0.4-2.8% for ΣPCBs and 0.2-2.3% ΣDDTs) than reported for the SRMs that were analysed over a period of months with each set.

RESULTS

Percent lipid in blubber

Percent lipid did not vary greatly by blubber depth in the samples from the necropsied white whales (RSDs = 1.6-14%; Table 3), although the middle layer(s) tended to have higher percent lipid compared to the outer (near-epidermis) or inner (near-muscle) layers. For each individual, the outer layer had percent lipid that was very close to the mean of the three or four layers analysed (Table 3). In contrast, blubber samples collected by trocar showed a large variation in percent lipid between the halves (Table 3). Furthermore, both the inner and outer layers sampled by trocar had much lower percent lipid values than those in the corresponding layers of blubber samples collected via necropsy.

There was greater variability in total lipid by blubber depth in the necropsied resident killer whale L60 (Table 4) than in the necropsied white whales. For the body positions (i.e. anterior, central, posterior, dorsal) having blubber thick enough to be split into three layers, RSDs for mean percent lipid ranged from 12-53%. The middle layer generally had the highest percent lipid and the inner layer had the same or lower percent lipid than was found in the outer layer. For each body position sampled, the outer layer had percent lipid that was similar to the mean of the two or three layers analysed (Table 4). Samples of L60 collected by dart biopsy showed substantially lower percent lipid (8.3-10%) compared to the samples from the same body positions and depth (0-2cm) collected by necropsy (28-40%).

Percent lipid varied less by depth in the transient killer whales (RSDs = 14-22%; Table 4) than in the resident whale, but more than in the white whales. For CA189, the middle layer from each of the two positions sampled had the highest percent lipid. However, the inner layer had lower percent lipid than the outer layer in the dorsal sample and the reverse was true for the mid-lateral sample. For ATx, the highest percent lipid was found in the middle layer (dorsal position), whereas lipid content was lower and approximately equal in the inner and outer layers. As found for the resident killer whale, the outer layers from the transient whales generally had percent lipid that was similar to the mean of the three layers analysed.

Lipid classes

Triglycerides were the predominant lipid class found in blubber samples from all the white whales, irrespective of depth in the blubber layer (Fig. 1). Necropsy samples CI-73 and CI-76 and trocar sample CI-01-05 also showed a small proportion (~5%) of free fatty acids.

The lipid classes in killer whale blubber varied substantially by depth at all body positions (Fig. 2). Both the resident and transient animals had a high proportion (>50%) of wax esters in the outer layer (0-2cm) and that proportion decreased rapidly with depth. As the wax ester proportion decreased, the triglyceride proportion increased to >80% in the layer nearest the muscle. The lipid in blubber sampled by dart biopsy from the resident killer whale contained about 40% wax esters and most resembled the equivalent layer (outer) in the sample taken by necropsy from the same animal. Two of the samples showed a small proportion (<5%) of free fatty acids (L60 0-2cm necropsy and L60 biopsy).

Table 3

Concentrations of ∑PCBs and ∑DDTs determined by GC/MS in white whale blubber sampled at various depths.

		ng/g, wet weight		ng/g, lipid	
Blubber depth*	%Lipid**	∑PCBs	∑DDTs	∑PCBs	∑DDTs
BB-75; necropsy; juvenile male					
Outer third	66	1,100	1,000	1,700	1,500
Middle third	85	1,500	1,300	1,800	1,500
Inner third	69	2,400	2,000	3,500	2,900
BB-75 necropsy (mean)	73	1,700	1,400	2,300	2,000
SD	10	670	510	1,000	810
RSD (%)	14	39	36	43	41
CI-73; necropsy; adult female					
Outer quarter	71	860	920	1,200	1,300
2nd quarter	73	820	790	1,100	1,100
3rd quarter	73	650	610	890	840
Inner quarter	71	590	560	830	790
CI-73 necropsy (mean)	72	730	720	1,000	1,000
SD	1.2	130	170	170	240
RSD (%)	1.6	18	24	17	24
CI-76; necropsy; adult male					
Outer quarter	68	2,200	3,000	3,200	4,400
2nd quarter	85	2,600	3,200	3,100	3,800
3rd quarter	73	1,300	1,400	1,800	1,900
Inner quarter	74	1,200	1,400	1,600	1,900
CI-76 necropsy (mean)	75	1,800	2,300	2,400	3,000
SD	7.2	690	990	840	1,300
RSD (%)	9.6	38	43	35	43
CI-01-05; trocar biopsy; adult female					
Outer half	10	66	70	660	700
Inner half	37	120	120	320	320
CI-01-05 trocar biopsy (mean)	24	93	95	490	510
SD	19	38	35	240	270
RSD (%)	79	41	37	49	53
CI-01-06; trocar biopsy; adult female					
Outer half	5.8	110	150	1,900	2,600
Inner half	13	230	280	1,800	2,200
CI-01-06 trocar biopsy (mean)	9.4	170	220	1,800	2,400
SD	5.1	85	92	70	280
RSD (%)	54	50	42	3.9	12

*A full-thickness portion of blubber from the necropsy samples was subdivided into quarters or thirds; the 'outer' portion was the layer nearest the epidermis and the 'inner' was nearest the muscle; CI-73's blubber layer was 5 cm thick, BB-75's was 2.2 cm and CI-76's was 6 cm. The trocar biopsy samples were divided in half; the outer half was the layer nearest the epidermis and the inner was nearest the muscle; CI-01-05's sample measured 6.8 cm and CI-01-06's was 3.3 cm (see Methods). **TLC/FID lipid analyses (see Methods).

Fatty acid profiles

Fig. 3 shows the fatty acids in the blubber of the white whale CI-73 (divided into four layers), those from the 'central' position on the saddle patch of the resident killer whale L60 (divided into three layers) and those from the transient killer whale ATx (divided into three layers). The identities of the 83 fatty acids are given in Table 2. The lines at 25%, 50% and 75% (white whale sample) and at 33.33% and 66.66% (killer whale samples) indicate the proportion of each fatty acid that would be expected if these acids were homogeneously distributed among the layers. Some fatty acids appeared to be fairly evenly distributed (e.g. 29, 45, 46 and 66 in the white whale; 6, 21 and 35 in the resident killer whale; and 1, 24, 34, 35 and 37 in the transient killer whale). Other fatty acids appeared to be more heterogeneously distributed among layers (e.g. 1 and 4 in the white whale; 80 and 81 in both killer whales). Furthermore, the lower molecular weight fatty acids (those having lower identification numbers) were found in higher proportions in

Table 4

Concentrations of ∑PCBs and ∑DDTs determined by GC/MS in blubber sampled at various depths and positions in one resident and two transient killer whales.

			ng/g, wet weight		ng/g, lipid	
Blubber position	Blubber depth*	%Lipid**	∑PCBs	∑DDTs	∑PCBs	∑DDTs
Female resident (L60) - necropsy						
Anterior	0 - 2 cm	40	5,800	6,400	15,000	16,000
	2 - 4 cm	40	8,800	8,600	22,000	22,000
	> 4 cm	12	3,700	4,800	31,000	40,000
	Anterior mean	31	6,100	6,600	23,000	26,000
	SD	16	2,600	1,900	8,000	12,000
	RSD (%)	53	43	29	35	46
Central	0 - 2 cm	28	5,300	6,100	19,000	22,000
	2 - 4 cm	35	7,300	8,200	21,000	23,000
	> 4 cm	11	6,200	6,900	56,000	63,000
	Central mean	25	6,300	7,100	32,000	36,000
	SD	12	1,000	1,100	21,000	23,000
	RSD (%)	50	16	15	66	64
Posterior	0 - 2 cm	37	5,900	6,400	16,000	17,000
	2 - 4 cm	46	8,600	8,400	19,000	18,000
	> 4 cm	38	8,800	9,300	23,000	24,000
	Posterior mean	40	7,800	8,000	19,000	20,000
	SD	5	1,600	1,500	3,500	3,800
	RSD (%)	12	21	19	18	19
Dorsal	0 - 2 cm	42	6,800	7,700	16,000	18,000
	2 - 4 cm	51	9,400	10,000	18,000	20,000
	> 4 cm	17	3,700	4,100	22,000	24,000
	Dorsal mean	37	6,600	7,300	19,000	21,000
	SD	18	2,900	3,000	3,000	3,000
	RSD (%)	48	44	41	16	14
Lateral	0 - 2 cm	31	6,000	7,500	19,000	24,000
	2 - 4 cm	38	6,700	7,500	18,000	20,000
	Lateral mean	35	6,400	7,500	19,000	22,000
	SD	5	500	0	700	3,200
	RSD (%)	14	8	-	3.7	15
	L60 - necropsy (mean n=14)	33	6,600	7,300	22,000	25,000
	SD	12	1,800	1,600	11,000	12,000
	RSD (%)	37	27	23	47	49
Female resident (L60) - dart biopsy* **						
Anterior	0 - ~2 cm	10	2,600	2,700	26,000	27,000
Central	0 - ~2 cm	9.0	2,600	2,700	29,000	30,000
Posterior	0 - ~2 cm	8.3	2,300	3,000	28,000	36,000
	L60 - dart biopsy (mean n=3)	9	2,500	2,800	28,000	31,000
	SD	0.9	170	170	1,500	4,600
	RSD (%)	9.9	6.8	6.1	5.4	15
Female transient (CA189) - necropsy						
Dorsal	0 - 2 cm	48	480,000	1,800,000	1,000,000	3,800,000
	2 - 4 cm	64	690,000	2,600,000	1,100,000	4,100,000
	> 4 cm	42	540,000	2,600,000	1,300,000	6,200,000
	Dorsal mean	51	570,000	2,300,000	1,100,000	4,700,000
	SD	11	110,000	460,000	150,000	1,300,000
	RSD (%)	22	19	20	14	28
Mid-lateral	0 - 2 cm	42	670,000	2,600,000	1,600,000	6,200,000
	2 - 4 cm	64	770,000	3,000,000	1,200,000	4,700,000
	> 4 cm	58	760,000	3,100,000	1,300,000	5,300,000
	Mid-lateral mean	55	730,000	2,900,000	1,400,000	5,400,000
	SD	11	55,000	260,000	210,000	750,000
	RSD (%)	21	8	9	15	14
	CA189 (mean n=6)	50	650,000	2,600,000	1,300,000	5,100,000
	SD	13	120,000	460,000	210,000	1,000,000
	RSD (%)	26	18	18	16	20
Male transient (ATx) - necropsy						
Dorsal	0 - 2 cm	26	110,000	170,000	420,000	540,000
	2 - 4 cm	34	140,000	200,000	410,000	590,000
	> 4 cm	28	140,000	190,000	640,000	790,000
	ATx (mean n=3)	29	130,000	190,000	490,000	640,000
	SD	4	17,000	15,000	130,000	130,000
	RSD (%)	14	13	8	27	20

*Measured from bottom of epidermis. **TLC/FID lipid analyses (see Methods). ***Dart was thrust into the skin/blubber by hand. ∑DDTs (wet weight) were measured as described by Ylitalo *et al.* (2001).

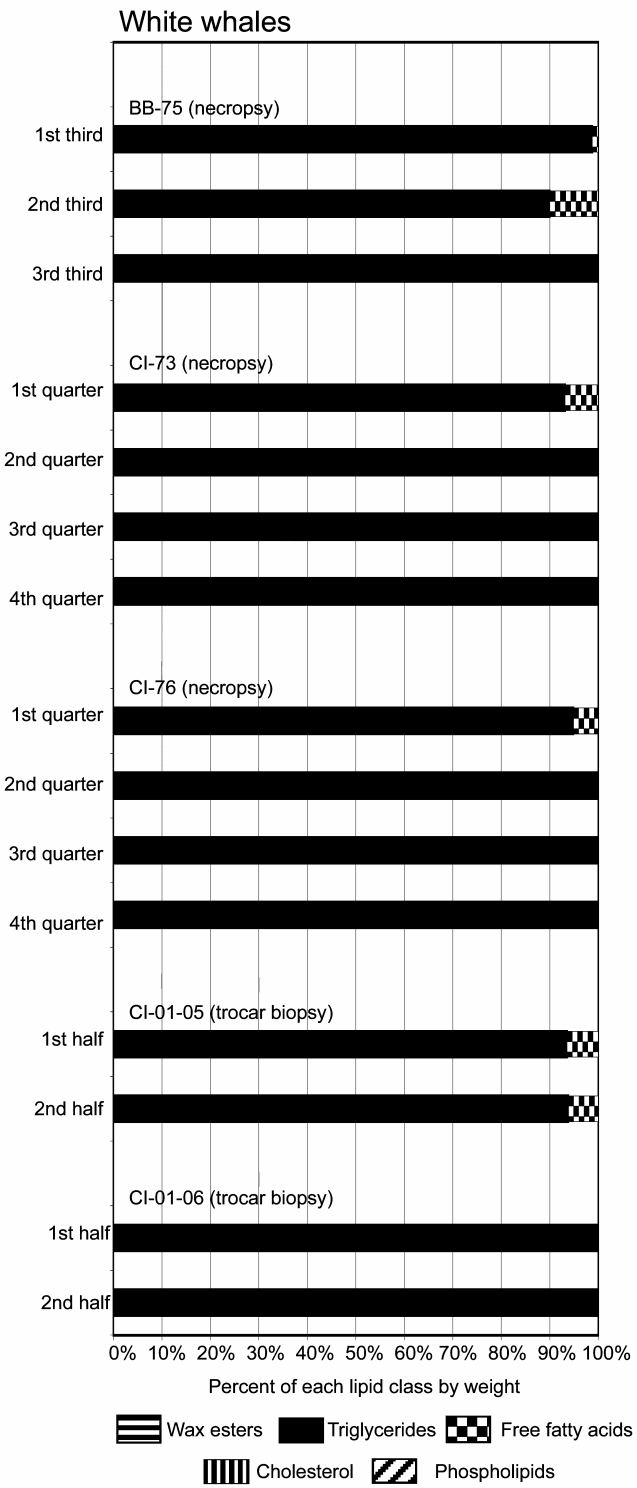

Fig. 1. Lipid classes (i.e. wax esters, triglycerides, free fatty acids, cholesterol and phospholipids) determined in each layer of white whale blubber by depth (i.e. each half, third or quarter). The layer labeled '1st' is the one nearest the epidermis.

the outer (epidermis) layer than in the inner layer — particularly in the white whales and also to a lesser extent in the killer whales.

PCA was used to further assess the homogeneity of the fatty acids profiles in the white whale blubber samples. In this plot (Fig. 4A), the eigen vectors exhibiting the highest factor loadings on the first three principal component axes were: C16:1n7 and C18:2n7 (PC1); C18:3n3 and C18:1n9 (PC2); and iso-C18:0 (PC3). The fatty acid profiles for each white whale generally did not group closely by depth in the blubber layers (evenly dashed ovals — Fig. 4A), again indicating a high degree of depth stratification for these

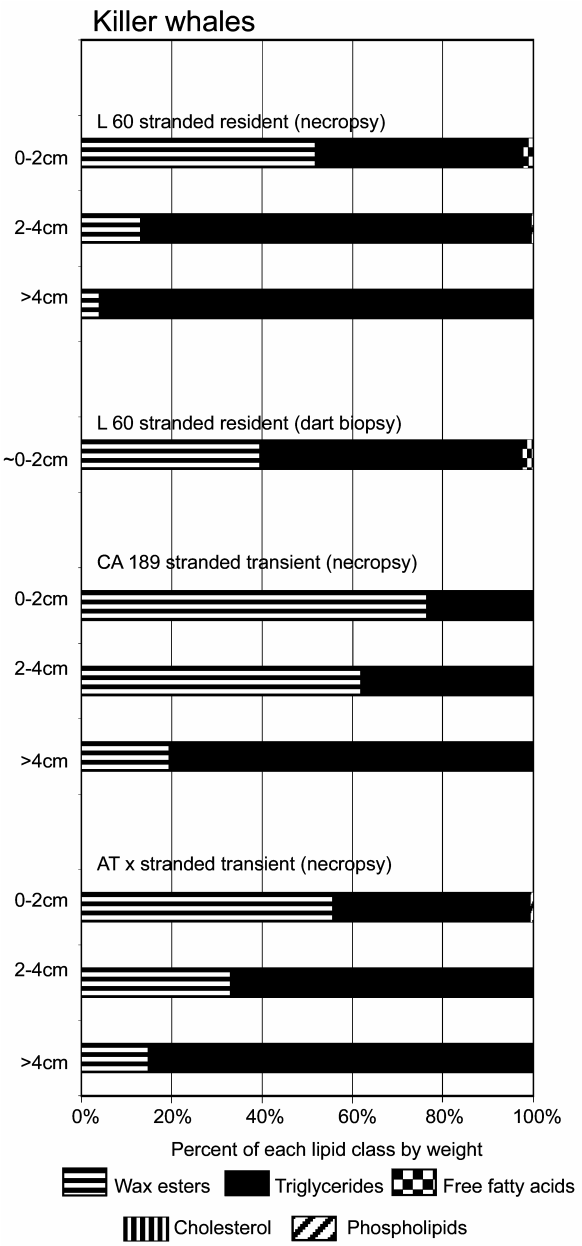

Fig. 2. Lipid classes (i.e. wax esters, triglycerides, free fatty acids, cholesterol and phospholipids) determined in each layer of killer whale blubber by depth from the epidermis (i.e. 0-2cm, 2-4cm, >4cm). The data for each depth represent the mean from all body positions sampled.

animals. However, the four animals from Cook Inlet could be distinguished from the single animal from Bristol Bay by their fatty acid profiles, regardless of which blubber depth was used for comparison (unevenly dashed ovals – Fig. 4A). This distinction was predominantly due to a larger relative abundance of branched-chain fatty acids in BB-75 (in particular, iso-C18:0). A pair-wise univariate analysis of the relative concentration of iso-C18:0 in each of the five animals in Fig. 4A indicated that this compound was significantly higher (by approximately a factor of 2) in the animal from Bristol Bay than in any of the four Cook Inlet animals, regardless of the sampling depth or lateral location of the blubber sample ($p<0.02$). Because replicate measurements of iso-C18:0 in the standard reference material ($n=5$), indicated that the analytical uncertainty in this particular fatty acid was low (RSD = 10.0%), the observed 2-fold difference cannot be attributed to measurement error.

When PCA was used to depict patterns for the killer whale fatty acids, all body positions sampled on each killer whale were clustered when grouped by depth (solid ovals – Fig. 4B). For example, the fatty acid profiles of the five body positions sampled for the resident killer whale (L60) were highly similar and clustered when grouped by depth (solid ovals). Similarly, the fatty acid profiles for the dorsal and mid-lateral positions of the transient CA189 were grouped by depth. However, in all the whales, it is the outer layer that is the most different from all the other layers sampled (Fig. 4B). In this plot, the eigen vectors exhibiting the highest factor loadings along the first three principal component axes were: C20:1n11 and C22:1n11 (PC1); C22:1n11 and iso-C15:0 (PC2); C18:3n3 and C18:1n13 (PC3). Regardless of the blubber depth or body position at which the blubber sample was acquired, the two killer whale ecotypes could be readily distinguished from one another by means of their fatty acid profiles (unevenly dashed ovals in Fig. 4B). Separation of the two ecotypes appeared to be greatest along PC1 indicating that the concentrations of C20:1n11 and C22:1n11 were substantially different between the transient and resident killer whale(s). A univariate analysis of the concentrations for these two fatty acids in each of the three animals indicated both fatty acids were significantly greater in the resident animal than either of the two transients ($p<0.01$). Again, the analytical uncertainty in each of these fatty acids measured from replicate analyses of the SRM material (RSDs = 1.3% and 5.5%, respectively) was negligible and contributed little to the observed separation of the two ecotypes.

Contaminant concentrations

Concentrations of selected contaminants (ΣPCBs and ΣDDTs) for all depths and positions sampled are given in Table 3 (white whales) and in Table 4 (resident killer whale L60 and transient killer whales CA189 and ATx). In white whales, the highest wet weight contaminant concentrations were found in CI-76 (an adult male), next was BB-75 (a juvenile male) and then CI-73 (a lactating female). The lowest concentrations were found in the two adult female white whales sampled by trocar. Although reproductive status of these females is unknown, they were not accompanied by calves. Concentrations of ΣPCBs and ΣDDTs (wet weight) in the outer layer of white whales differed from concentrations in the inner layer, but generally by no more than a factor of two, with higher concentrations sometimes in the outer layer and other times in the inner layer (Table 3). 'Normalisation' of wet weight concentrations to lipid (i.e. concentrations reported in ng/g lipid in Table 3) did not equalise the distribution of the contaminants among layers. Instead, the outer layer had consistently higher concentrations (ng/g lipid) of ΣPCBs and ΣDDTs than those found in the inner layer for Cook Inlet white whales and the reverse was true for the Bristol Bay whale.

Blubber samples (necropsy) from the resident killer whale L60 had mean concentrations of ΣPCBs and ΣDDTs (Table 4) that were about 3-fold higher (wet weight) or 9-fold higher (lipid weight) than those found in the most heavily contaminated white whale (CI-76). In general, the highest concentrations of OCs (wet weight) in L60 occurred in the middle blubber layer. Lipid normalisation of ΣPCBs

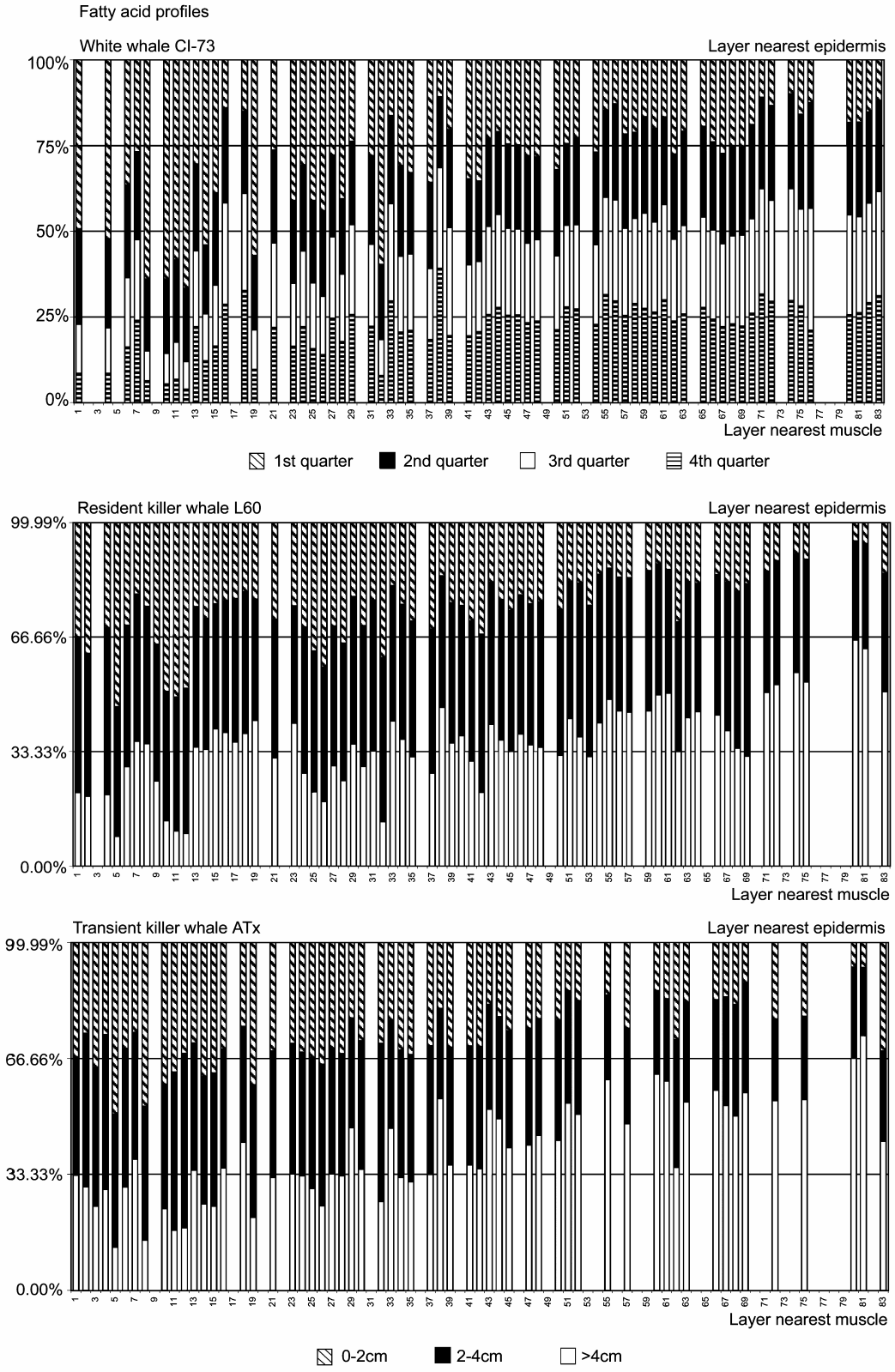

Fig. 3. Proportions of 83 fatty acids (see Table 2 for identification) found in the blubber layers of a white whale from Cook Inlet (CI-73), the resident killer whale L60 and the transient killer whale ATx. Blubber from CI-73 was divided into quarters and that from L60 and ATx was divided into three layers (0-2cm, 2-4cm and >4cm). Data are not shown when fatty acids concentrations were below the limit of quantitation for one or more layers.

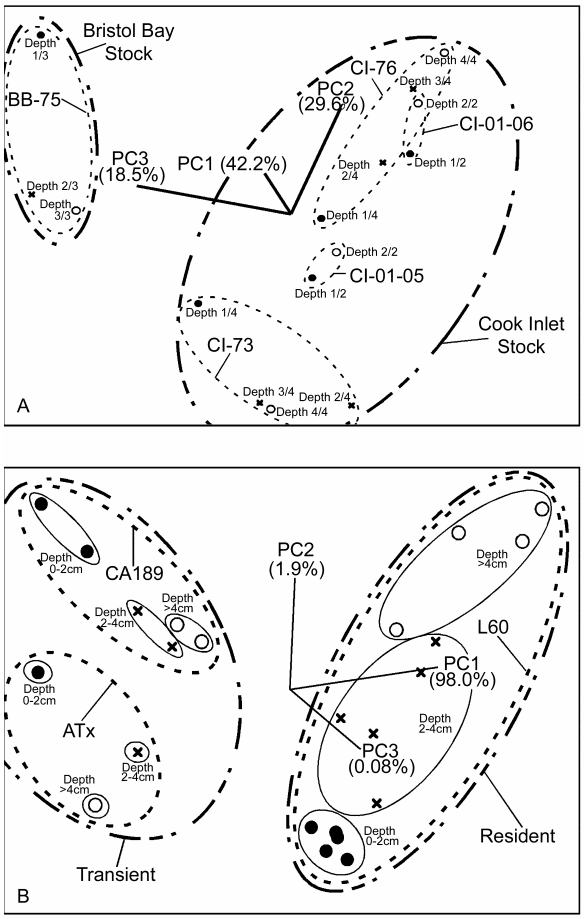

Fig. 4. Plot of the first three principal components derived from the fatty acid composition of (A) white whale blubber and (B) killer whale blubber. The quartered white whale blubber samples are grouped by animal (evenly dashed ovals) and by stock (unevenly dashed ovals). Killer whale blubber samples from each body position (individual symbols) were divided into thirds and grouped by depth from epidermis (solid ovals), by animal (dashed ovals) and by ecotype (unevenly dashed ovals). The percent of the total variation among samples explained by each principal component is given on the label for each axis. Depth 1 is the layer closest to the epidermis and depth 4 is closest to the muscle.

and ΣDDTs contributed to increased (or equal) contaminant homogeneity (decreased RSDs) for the posterior, dorsal and lateral samples, whereas the anterior and central positions showed decreased homogeneity (increased RSDs). Except for the lateral position, the lipid-adjusted OC concentrations were highest in the inner layer. The samples taken by dart biopsy had wet weight concentrations of ΣPCBs and ΣDDTs that were about half the concentrations in the necropsied samples from the same depth and body position. When the concentrations of ΣPCBs and ΣDDTs were lipid-adjusted, the biopsy samples had OC concentrations that were 1.5-2 times those in the necropsied samples.

The transient killer whales, CA189 and ATx, had mean concentrations of ΣPCBs and ΣDDTs (wet weight) in blubber that were much higher than those of the necropsied resident killer whale (by a factor of about 100 for ΣPCBs and about 400 for DDTs for CA189 and by about 20 for both ΣPCBs and ΣDDTs for ATx; Table 4). For samples from the two body positions of CA189, variability by depth in mean wet weight concentrations of ΣPCBs and ΣDDTs was low (RSDs = 8-20%). When the concentrations were expressed as lipid weight, the variability among layers increased (RSDs = 14-28%). The layer with highest lipid-normalised concentrations of ΣPCBs and ΣDDTs differed between the two sampling positions — the inner layer had higher lipid normalised concentrations than the outer layer for the dorsal sampling position, but the reverse was true for the mid-lateral position. For ATx, variability in wet weight concentrations of ΣPCBs and ΣDDTs among the layers was also low (RSDs = 8-13%), but increased when the concentrations were expressed using lipid weight (RSDs = 20-27%). As found for the dorsal samples in CA189 and L60, ATx's inner layer sample had higher lipid normalised concentrations of ΣPCBs and ΣDDTs than were found in the outer layer.

Contaminant profiles

PCA was used to depict patterns in the OC data for both the white whales and killer whales (Figs 5A and B). For white whales, the OC profiles of samples from different blubber depths of each individual animal were somewhat clustered (evenly dashed ovals in Fig. 5A) and were well separated from each other. The OCs having the highest factor loadings along the first three principal component axes were: PCB 28 and PCB 70 (PC1); PCB 118 and *p,p*'-DDD (PC2); PCB 52 and *o,p*'-DDT (PC3). The two white whale stocks, Cook Inlet and Bristol Bay, were also distinct based on their OC profiles (unevenly dashed ovals), separated primarily along the second principal component axis (PC2). A univariate analysis of the concentration data for the two eigen vectors contributing most to PC2 (i.e. PCB 118 and *p,p*'-DDD) indicated that both were significantly different when comparing the Bristol Bay whale with each of the four whales from the Cook Inlet stock ($p<0.07$). Again, the analytical measurement errors for these particular OCs (RSD = 8.1% for PCB 118 and 9.3% for *p,p*'-DDD) were very small relative to their respective inter-stock concentration differences.

The OC profiles of the five body positions sampled for the resident killer whale L60 were observed to be quite similar when grouped by depth (solid ovals in Fig. 5B), indicating much greater variability in OC patterns by depth than by body position. Depth stratifications of OCs (primarily along PC3) were much lower in the two transient killer whales, with CA189 demonstrating the least stratification. However, OC profiles from all blubber depths of the transient killer whales were well-separated from each other and from those of the resident L60 (evenly dashed ovals in Fig. 5B), principally along PC1. The OCs having the highest factor loadings along the first three principal component axes were: *p,p*'-DDE and PCB 74 (PC1); PCBs 52 and 99 and t-nonaclor (PC2); PCB149 and beta-HCH (PC3). Thus, *p,p*'-DDE and PCB 74 were the OCs primarily responsible for separating these three whales from one another (and perhaps also separating them by ecotype). A univariate analysis of the concentration data for these two OCs indicated both were significantly different in the resident compared to either of the transients ($p<0.01$).

DISCUSSION

Percent lipid in blubber

Percent lipid was fairly uniform by depth in blubber samples for necropsied white whales (Table 3). Furthermore, the lipid percent in the outer layer provided a good measure of the mean for the entire blubber layer for each white whale.

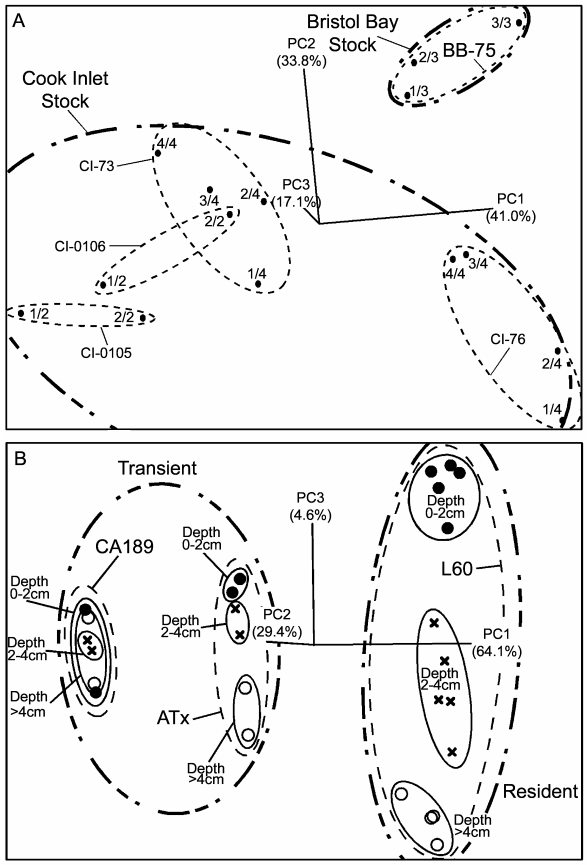

Fig. 5. Plot of the first three principal components derived from the OC composition of (A) white whale blubber and (B) killer whale blubber. The quartered white whale blubber samples are grouped by animal (dashed ovals) and by stock (unevenly dashed ovals). Killer whale blubber samples from each body position (individual symbols) were divided into thirds and grouped by depth from epidermis (solid ovals) and by animal (dashed ovals). The percent of the total variation among samples explained by each principal component is given on the label for each axis. Depth 1 is the layer closest to the epidermis and depth 4 is closest to the muscle.

These results are similar to those found by Gauthier *et al*. (1997) for minke and blue whales and by Aguilar and Borrell (1991) for fin whales, where no significant difference in lipid content was found among blubber layers. In contrast, Aguilar and Borrell (1991) found a significant difference in lipid content between the inner and outer layers of sei whales (the outer layer had higher percent lipid).

The trocar blubber samples collected from live Cook Inlet white whales had comparatively low lipid content, as well as an uneven distribution of lipid between halves, compared to necropsy samples from Cook Inlet whales (Table 3). Blubber thickness and lipid content for the four live-whale samples should, in fact, be similar because they were all sampled during the same season (summer), were of the same approximate age and were feeding within the same geographic location. Although the samples were frozen in the trocar soon after collection, lipid was likely lost when the trocar was removed from the animal due to the consistency of the blubber. Thus, the large differences in measured lipid content between trocar and necropsy blubber samples likely represent a true sampling bias. In contrast, Gauthier *et al*. (1997) reported that their 'deep' (19-26mm) biopsy samples were within the ranges measured in the blubber of necropsied blue and minke whales.

For resident and transient killer whales, the outer blubber layer provided a good estimate of mean percent lipid for the full blubber layer, similar to the results found for white whales. As in the trocar samples from white whales, dart biopsy samples taken from the resident whale had lower percent lipid than found for necropsied samples at equivalent depth (outer layer) and body positions (Table 4). These results are consistent with previous results for dart biopsies of wild cetaceans that showed lower percent lipid than would be expected from blubber sampled via necropsy. For example, Krahn *et al*. (2001) reported that mean lipid levels in gray whale blubber sampled by biopsy (10±1.0%; n=38) were substantially lower than those found for subsistence animals sampled by necropsy (43±2.7%; n= 17).

A number of theories have been advanced to explain the lower percent lipid in dart biopsy samples: (1) lipid seeping from the blubber structural matrix as the biopsy dart is ejected from the animal; (2) lipid washing away when the dart falls into the water before being retrieved; and (3) the dart hitting at an oblique angle so that more epidermis and connective tissue than blubber are collected. Because the laboratory-simulated biopsy dart technique used to acquire the killer whale samples in the current study entered the blubber as vertically as possible and did not fall into water, the low percent lipid in these biopsy samples most likely resulted from lipid that seeped from the blubber matrix as the dart was removed.

Lipid class profiles

For the white whales, triglycerides comprised the greatest proportion of the lipid regardless of depth (Fig. 1). These results agree with previous studies showing that blubber of healthy cetaceans contained primarily neutral lipids, e.g. triglycerides and nonesterified free fatty acids (Kawai *et al*., 1988; Tilbury *et al*., 1997; Krahn *et al*., 2001). Furthermore, the trocar samples showed the same profile of lipid classes as found for the necropsy samples.

Unlike the blubber of white whales, blubber layers of killer whales contained 15-75% wax esters, with greater proportions found in the outer layer (Fig. 2). These results were unexpected because a previous study by Litchfield *et al*. (1975) that examined the lipid composition of fatty tissues of 20 different species of odontocetes (*O. orca* were not sampled) found that the blubber of Delphinidae contained mostly triglycerides, with the exception of false killer whales which contained 96% triglycerides and 4% wax esters. Similarly, other studies (Koopman *et al*., 1996; Koopman, 2001) reported that blubber of odontocetes was composed primarily of triglycerides and contained little or no wax esters. In fact, the only odontocetes that have been reported to contain appreciable proportions of wax esters (>40%) in their blubber were whales from the Ziphiidae (beaked whale) and Physeteridae (sperm whale) families (Litchfield *et al*., 1975; Hooker *et al*., 2001).

The lipid class profile of the resident killer whale from the dart biopsy (outer layer) had only slightly smaller proportions of wax esters than were found in the outer layers from necropsy samples (Fig. 2). Therefore, the biopsy dart sample could provide lipid class information that adequately portrays the profile of the same-depth sample obtained via necropsy. However, unless a deeper biopsy sample were

obtained, a biopsy could not provide information about the manner in which lipid class profiles of killer whales change by blubber depth.

Fatty acid profiles

Fatty acid profiles of a predator have been statistically linked to fatty acid profiles of potential prey species to provide an estimate of relative proportions of specific prey species consumed (Adams *et al*., 1997; Iverson *et al*., 1997; 2002; Walton *et al*., 2000). Because the inner blubber layer is more metabolically active than the outer layer, it is thought to better indicate prey fatty acid profiles (Hooker *et al*., 2001). Thus, it is important to ascertain whether biopsy sampling of the outer layer can be representative of the fatty acid profiles of the metabolically active inner blubber layer.

In both white whales and killer whales, fatty acids were disproportionately distributed among the blubber layers (Fig. 3), similar to the results reported by other researchers (Hooker *et al*., 2001; Olsen and Grahl-Nielsen, 2003). When PCA was used to determine how the various blubber layers were grouped based on the fatty acids present, profiles from the white whales showed that the inner and outer layers were not highly correlated (Fig. 4A). Similarly, fatty acid profiles of the inner blubber layer were very different from the outer layer for the resident and transient killer whales (Fig. 4B). Thus, a biopsy sample comprising only the outer blubber layer of either species would not accurately represent the metabolically active inner layer and thus would likely fail to correlate well with the fatty acid signatures of their primary prey species.

Olsen and Grahl-Nielsen (2003) have indicated that blubber fatty acid profiles may also be suitable for population (stock) identification in minke whales. Furthermore, a recent study by Moller *et al*. (2003) demonstrated that the blubber fatty acid composition of North Atlantic minke whales supported the existence of a three-region population model, regardless of the depth at which the blubber sample was acquired (deep vs superficial). In the current study, fatty acid profiles were able to distinguish the single Bristol Bay white whale from the Cook Inlet animals at all blubber depths (Fig. 4A). Similarly, the two ecotypes (resident and transient) of killer whales were readily classified from their profiles (Fig. 4B), so ecotype identification based on fatty acid profiles seems possible. Due to the small number of white and killer whales represented in this study, it is impossible to determine with any certainty whether it is differences in diet, genetics or both that are primarily responsible for the separations observed between the white whale stocks, as well as the killer whale ecotypes. However, from these very limited data, it has been observed that the fatty acid profiles of outer blubber layers (thought to contain a higher proportion of endogenous biosynthesised fatty acids, perhaps under genetic control) were nearly as effective as the inner blubber layers (believed to best reflect exogenous diet composition) in resolving white whale stocks, as well as killer whale ecotypes. This preliminary observation suggests that odontocete blubber fatty acid profiles may be subject to both dietary and genetic control. Many additional samples from white whales of different populations and killer whales with identified ecotypes are needed to confirm this hypothesis.

Contaminant concentrations

The adult female white whales had lower concentrations of ΣPCBs and ΣDDTs than were found in the adult male (Table 3), in agreement with previous studies showing maternal transfer of contaminant burdens to offspring during gestation and lactation (Muir *et al*., 1992; Aguilar and Borrell, 1994; Norstrom and Muir, 1994; Ridgway and Reddy, 1995; Krahn *et al*., 1997; 1999; Aguilar *et al*., 1999). Furthermore, OC levels in necropsied Cook Inlet whales (Table 3) were in the same range as those reported previously for animals of the same sex in the Cook Inlet population (Krahn *et al*., 1999).

The ΣPCBs and ΣDDTs found in the female transient killer whale CA189 (Table 4) were higher than any reported previously for either males or females from that ecotype (Ross *et al*., 2000; Ylitalo *et al*., 2001). Transient ATx also had higher levels of ΣPCBs and ΣDDTs than were found in the resident L60, but lower than those found in transient CA189 (Table 4). The high contaminant concentrations found in the transient killer whales compared to those found in the resident animal can be explained by the diets of the two ecotypes, i.e. transient killer whales feed largely on marine mammals and resident animals are primarily piscivores (Baird, 1994). Thus, the transients feed on prey that contain higher contaminant levels as a result of OC bioaccumulation (Muir *et al*., 1988; Kucklick *et al*., 1994; Fisk *et al*., 2001). These results agree with previous studies in Alaska (Ylitalo *et al*., 2001) and in Canadian waters (Ross *et al*., 2000).

ΣPCBs and ΣDDTs in blubber were inconsistently distributed in the white whales — sometimes the outer layer had the highest concentrations, similar to results reported by Aguilar and Borrell (1991), and other times (e.g. the Bristol Bay juvenile male) the inner layer had the highest concentrations (Table 3). Similarly, both ecotypes of killer whales showed an inconsistent OC distribution by depth, with more variation when contaminant concentrations were expressed on a lipid weight basis. These variations in contaminant concentrations within the blubber layer of both species point out the need to have a full-thickness blubber sample, as suggested by Aguilar and Borrell (1991), to accurately represent contaminant concentrations in blubber.

White whales sampled via trocar contained much lower wet weight concentrations of ΣPCBs and ΣDDTs than reported for the necropsied female (Table 3) or previously for subsistence white whales (Krahn *et al*., 1999). However, because these trocar samples also had very low percent lipid, contaminant concentrations expressed as lipid weight increased up to 20-fold, resulting in lipid normalised concentrations of OCs that were in the same ranges as those reported previously (Krahn *et al*., 1999). Thus, OCs in samples collected by trocar should be lipid adjusted to compensate for lipid loss during sampling.

Similar to the white whale trocar samples, samples collected by dart from killer whale L60 contained ΣPCBs and ΣDDTs (wet weight) that were substantially lower than those in necropsied samples from the equivalent depth and position. When these OCs were lipid adjusted, the resulting biopsy concentrations had a high bias relative to their equivalent necropsy samples, thus over-compensating for the lipid loss during sampling. Regardless, lipid-adjusted ΣPCBs and ΣDDTs in biopsies are a sufficiently good measure of these contaminants to allow comparison to OC levels in other marine mammals or to threshold levels for contaminant-related health effects (Nilsson and Huntingdon, 2002).

Additional work on dart and trocar design, as well as on sampling techniques, is warranted to improve their suitability for obtaining more representative OC contaminant data, particularly for wet weight

concentrations. For example, researchers report using darts that are larger in diameter (6.4mm O.D.) (Barrett-Lennard, 2000) or longer (30mm) (Matkin, pers. comm.) than the one used in this study.

Contaminant profiles

PCA analysis of OC contaminants in white whale blubber collected by necropsy have been used to distinguish among stocks, both in Alaska (Krahn *et al*., 1999) and Canada (Muir *et al*., 1996). In this study, OC profiles in white whale blubber (necropsy or trocar) indicated that the intra-animal variation with depth was small relative to the inter-animal differences, such that each individual animal can be readily distinguished from the others (Fig. 5A). Furthermore, OC patterns for the single whale from Bristol Bay differed from those of the Cook Inlet whales (Fig. 5A). However, additional samples from each area will be needed to determine whether these stocks can be distinguished by contaminant patterns alone. Among the Cook Inlet whales, the OC patterns from three females were grouped, whereas the sample from the only male was an outlier. This was not surprising, because white whales have previously demonstrated resolution of OC profiles by sex (Krahn *et al*., 1999).

As found for white whales, the intra-animal variation in OC profiles with depth or body position for the killer whales was small relative to the inter-animal differences (Fig. 5B). Thus, the OC patterns for the resident L60 were different from those of the transients, as would be expected due to their different trophic positions and diets (Baird, 1994). However, because only three animals were sampled, it is difficult to predict whether the resident and transient ecotypes could routinely be distinguished using PCA contaminant profiles.

CONCLUSIONS

This study showed that the degree of stratification varied both by species and parameter measured, so biopsy techniques that sample only an outer blubber layer must be interpreted with caution and in conjunction with results from species-specific blubber depth profiling. However, when carefully interpreted, results from chemical analyses of biopsy blubber samples can provide valuable information for many applications. For example, lipid classes determined in biopsy samples of white and killer whales were similar to those obtained for equivalent-depth samples obtained by necropsy. In addition, lipid-adjusted contaminant concentrations measured in dart or trocar samples were representative of those obtained by necropsy of white and killer whales. Therefore, following lipid normalisation of OCs, biopsy techniques can be used to determine whether these species are highly contaminated and thus at risk for contaminant-related health effects. In contrast, fatty acid profiles from outer blubber layers of these cetacean species were largely different from those in the metabolically active inner layer and are therefore unlikely to be useful in attempting to make correlations with the fatty acid profiles of potential prey.

In spite of the limitations of biopsy sampling, a small biopsy sample from a healthy cetacean may provide better information than a larger sample collected via necropsy from an ill or emaciated animal. In the future, improved design of biopsy darts or trocars could prevent lipid loss during sampling and also increase the size of each sample, allowing increased comparability of biopsy results to those obtained from necropsy. Finally, additional biomarkers and analytical methods should be developed specifically for biopsy samples, so each small sample can provide a large array of data about the animal's life history, body condition and health.

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