Stock identity of beluga (*Delphinapterus leucas*) in Eastern Canada and West Greenland based on organochlorine contaminants in their blubber

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** This paper was completed after the untimely death of the senior author while conducting field research. The surviving authors have tried to remain true to Stuart's directions with it but, of course, can not anticipate how he might have brought it to conclusion himself. In this and many other aspects of our lives, Stuart is missed.*

ABSTRACT

Belugas (*Delphinapterus leucas*) caught by hunters from various hamlets in the Arctic differed in the concentrations of organochlorine contaminants in their blubber. By applying Canonical Discriminant Analysis (CDA) it was possible to separate all seven sampling locations from each other. Over 90% of the samples could be classified back to their landing location based on the data transformations developed by CDA. This analysis suggested that "stock" or management unit for belugas is best described by the culturally transmitted behaviour of their migration route. The analysis also provides evidence that most belugas caught by hunters from Grise Fiord are not the same as belugas caught while migrating along West Greenland; that some belugas caught in Sanikiluaq are not the same as beluga caught in the Nastapoka River estuary; and that the belugas caught in Kimmirut are not the same as belugas caught in Cumberland Sound. There is a need to redefine the stock descriptions of some belugas in Canada and Greenland.

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INTRODUCTION

It has been recognised since the late 1960's that most piscivorous marine mammals have high concentrations of lipophilic organochlorine (OC) pollutants in their blubber, and that monitoring these populations could be used to identify geographical and temporal trends in concentrations (Holden 1970, Muir *et al.* 1992, AMAP 1997). Organochlorine contamination has been used to differentiate between schools of eastern Atlantic long-finned pilot whales (*Globicephala melas*) on the basis of differences in the concentrations of total (∑) PCBs and ∑DDTs and ratios of two groups of contaminants (∑DDE/∑DDT and ∑DDT/

∑PCB) (Aguilar 1987, Aguilar *et al*. 1993). Similarly, Stern *et al*. (1994) found significant differences in the concentrations of ∑DDTs and ∑PCBs and ratios of summed OC classes among beluga (*Delphinapterus leucas*) blubber samples collected in West Greenland, Jones Sound, Hudson Bay, Cumberland Sound, the Beaufort Sea and the St. Lawrence River estuary. While the mean concentration of OCs such as ∑DDTs and ∑PCBs were different, the distributions of any contaminant concentration in beluga blubber from the Canadian Arctic and Greenland overlapped. Thus it was difficult to assign a beluga to a stock based on only one contaminant. Additionally, the large number of OCs identified in modern analyses makes it possible to find univariate differences between sampling locations or sampling times due to statistical chance, to differences in the sex ratio, or to the age of animals taken in the hunt.

Muir *et al*. (1996) showed that belugas from the St. Lawrence River estuary could be distinguished from belugas found dead on the east coast of Newfoundland, and from a limited number of Arctic beluga, based on principal components analysis of OCs in blubber. Here we apply a multivariate technique to OC concentrations in blubber samples to distinguish stocks of Arctic belugas.

We hypothesise that both absolute and relative proportions of each contaminant will vary among stocks of belugas due to the combined effects of transfer through lactation and the difference in feeding ecology associated with differences in migration routes. The differences in OC concentrations represent local and regional contamination levels, the composition, length and structure of associated food webs, the age, sex, and reproductive status of the beluga, and the season and year of sampling.

MATERIALS AND METHODS

Samples

Samples of blubber were obtained from belugas caught by hunters from seven locations in the Canadian Arctic and West Greenland and from biopsies of live-captured belugas near Churchill, Manitoba (Table 1, Fig. 1). Greenland samples were stored in cleaned aluminium foil and polyethylene plastic bags; Canadian samples were stored in polyethylene bags. Samples were stored at –30 to –40 degrees C until analysed. Tissues analysed were taken from the interior of the sample by paring away the outer several millimeters. Sex was determined by the samplers. Age was estimated by counting growth layer groups in a section of a tooth from the lower jaw (see Heide-Jørgensen *et al*. 1994). The summary of univariate analyses of the OC pesticide and PCB concentrations for most of these samples have been published in Muir *et al*. (1990), Hansen *et al*. (1990), and Stern *et al*. (1994), but have not been used to determine stock boundaries or affiliations. Results for samples from Kimmirut (formerly Lake Harbour), Cumberland Sound, Grise Fiord and Churchill have not been previously reported.

Organochlorine analysis

Determinations of OC pesticides and PCBs in blubber tissues followed Muir *et al*. (1990). Briefly, samples of blubber (2 g) were extracted with hexane. The extract was centrifuged and a portion (1/10) removed for lipid determination. A second portion equivalent to 100 mg lipid was chromatographed on a Florisil column to separate PCBs, chlorobenzenes, 4,4'-DDE and Mirex (all in the hexane fraction) from most toxaphene components, chlordane-related compounds and 4,4'-DDT (hexane:dichloromethane; 85:15). A third fraction (hexane:dichloromethane; 1:1) contained heptachlor epoxide and dieldrin. Samples were injected (splitless mode) on a 60 m x 0.25 mm i.d. DB-5 column (film thickness 0.25 mm) with H_2 carrier gas (Muir *et al*. 1990). PCB's and OC pesticides were identified and quantified with authentic analytical standards as described previously (Muir *et al*. 1990). All samples were analysed at the same laboratory (Freshwater Institute) between 1988 and 1996.

More than 100 OCs can be separated and quantified from extracts of marine mammal blubber by high-resolution capillary GC (Duinker *et al*. 1989, Muir *et al*. 1988). We selected a subset of 64 compounds representing 24 OC pesticides and metabolites and 40 PCB congeners. The major criterion for selection was that these compounds were consistently detected in all samples, avoiding the problem of missing data.

Table 1. Sampling locations and times, sex and mean age of sampled beluga from five putative stocks and three other locations. Mean ages with the same superscript letter were not significantly different from each other ($P = 0.05$).

Putative Stock	Stock	Sampling Location	Mean Age (by Stock)	Males	Females	Unknown Sex
Baffin Bay						
Greenland	1a	Sassat -Disko Bay ice entrapment (1992)	5.7[a]	4	4	
	1a	Saqqaq -Disko Bay (1992)		1	4	
	1a	Qeqertarsuaq -Disko Island (19??)		6	0	
	1a	Kitsissuarsuit –*Kronprinsens Ejland* (1992)		2	2	
	1b	Nuussuaq – *Kraulshavn* (1985)		12	8	
	1b	Nuussuaq – *Kraulshavn* (1990)		33	54	
Canada	2	Grise Fiord (1984)	5.3[a]	8	7	
	2	Grise Fiord (1985)		4	1	
	2	Grise Fiord (1987)		6	2	
West Hudson Bay	3	Arviat (1986)	11.6[b]	4	4	
	3	Churchill (1993)		5	4	1
East Hudson Bay	4	Nastapoka River (1984)	11.9[b]	4	5	
	4	Nastapoka River (1985)		4	3	
	5	Sanikiluaq (1994)	15.0[b,c]	5	6	
S.E. Baffin Island	6	Cumberland Sound (1993/94)	18.1[c]	5	6	
	7	Kimmirut *Lake Harbour* (1994)	29.3	6	5	

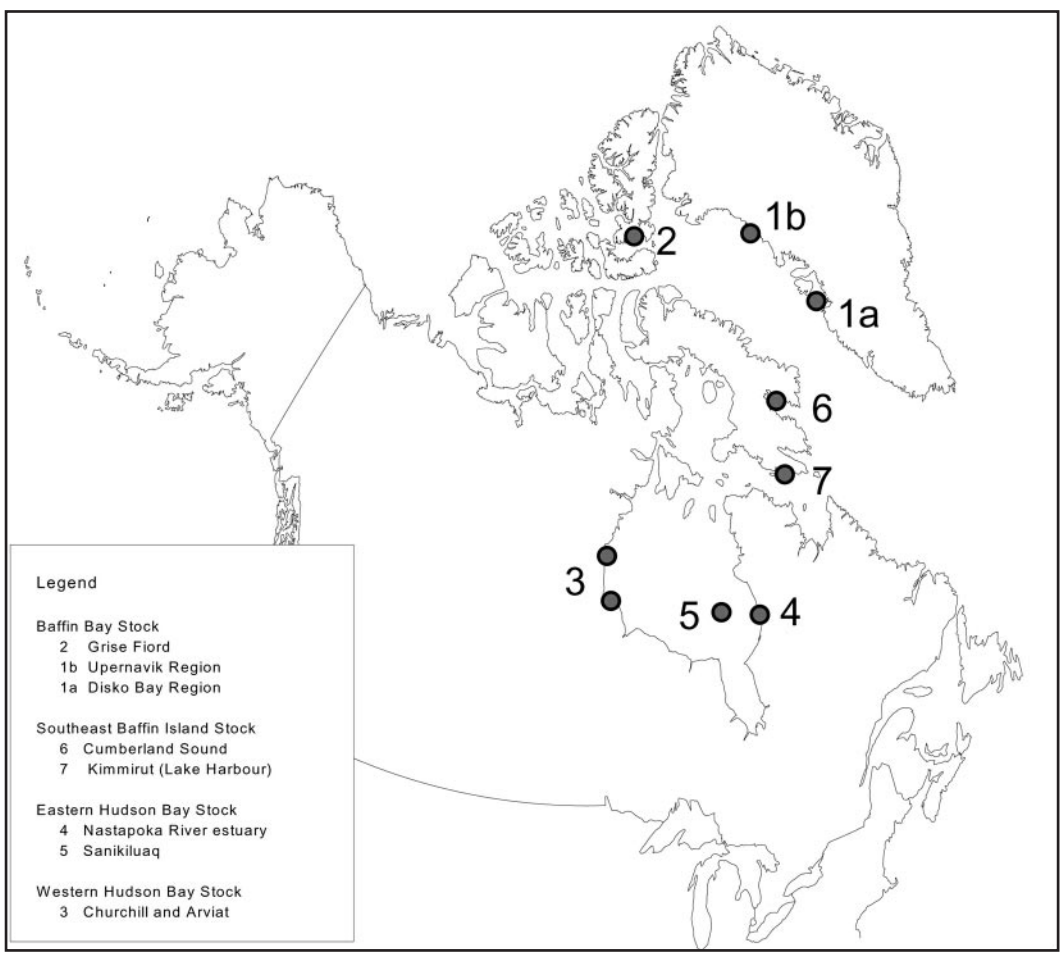

Fig. 1. *Sampling locations used in this study.*

The second criterion was that co-eluting components were avoided as much as possible.

Statistical analyses

Differences in the concentrations of OC contaminants in blubber samples among sampling locations and mean age estimates of the beluga included in the samples were tested by ANOVA (PROC GLM with Duncan's Multiple Range, SAS Institute Inc. 1989). No transformations or covariates were used in these tests. Age estimates and sex information were missing for some samples.

Multivariate analyses

Organochlorine patterns among sampling locations (*i.e.* eigenvectors) were determined using Canonical Discriminant Analysis (PROCs CANDISC, SAS Institute Inc. 1989). Canonical Discriminant Analysis (CDA) was used as a data summarising step to produce fewer but composite observations of OC composition that captured important differences between sampling locations. Each of the canonical axes and variates represents a simplification of the available information about the contaminants present in the belugas' blubber by transforming the observations, then weighting each variable to produce the highest multiple correlation with the groups (SAS Institute Inc. 1989). These new composite observations were then used to determine the ability to assign observations to a sampling location (using PROC DIST, SAS Institute Inc. 1989).

A series of preliminary analyses was carried out as a data investigation step. The first investigative step determined if a sampling location represented one natural grouping or a mixed sample. The status of each of the locations was determined by running the CDA and then using the component loadings to select OC compounds that were correlated with variation captured by that canonical axis. Then the concentrations of these compounds were plotted and compared within sampling locations and examined for possible mixed stock samples.

The final comparisons were made for the sampling locations in two ways. The first was to perform a CDA using all the samples and then to discriminate based on these canonical variates. The second was to remove one sample from the CDA and then classify it based on the canonical weights determined from the remaining samples. This was to determine the power of the discrimination for small samples.

In addition to examining all sample locations over the broad geographic range, CDA was used to examine the variation within smaller geographical areas. These were *Hudson Bay - Southeast Baffin Island* (Eastern Hudson Bay, Western Hudson Bay, Sanikiluaq, Kimmirut, and Cumberland Sound sampling locations), *Baffin Bay - Southeast Baffin Island* (West Greenland, Grise Fiord, Kimmirut and Cumberland Sound), *Grise Fiord vs. West Greenland*, and *Upernavik vs Disko Bay* in West Greenland. Divisions were chosen by combining neighbouring sampling locations using hunter-based (Remnant and Thomas 1992, Thomsen 1993) and science-based summaries (Richard *et al.* 1990, Heide-Jørgensen 1994).

RESULTS

Univariate analyses

Each of the 64 OC compounds (or co-eluting PCB congeners) (Table 2) had at least one significant difference (greatest difference was significant at $P < 0.005$) between sampling locations. Nearly all had significant main effects associated with sex or age of the beluga from which the sample was taken. There were 650 location to location significant differences out of the total of 2,730 comparisons, considerably more than the 137 significant differences (19.5 per community) expected by chance ($P = 0.05$). Generally, the concentrations declined from Kimmirut (193 significant comparisons), to Sanikiluaq (165), to Cumberland Sound (106) and West Greenland (103); 5 to 10 times more than expected by chance. Western Hudson Bay and Nastapoka River Estuary samples had only 7 and 17 significantly greater concentrations of OC compounds in the location by location comparisons, less than expected by chance (Table 2). No difference was found between the number of significant differences for the pesticide residues in relation to the PCBs ($\chi 2 = 0.3$, $P > 0.5$, df =1). Sixteen of the compounds had either zero means for one or more of the locations or were combinations of compounds.

Table 2. Number of significant differences ($P < 0.05$) found between sampling locations for the 64 organochlorine compounds measured in beluga blubber. SQ - Sanikiluaq, Na - Nastapoka River estuary, WHB - W. Hudson Bay, Kim - Kimmirut, CSd - Cumberland Sound, GF - Grise Fiord, WG - West Greenland.

				Sampling location with a significantly greater concentration					
Location with a significantly smaller concentration		SQ	Na	WHB	Kim	CSd	GF	WG	Total
Sanikiluaq	SQ		3	0	23	0	7	13	46
Nastapoka River estuary	Na	41		1	48	33	16	36	175
W. Hudson Bay	WHB	38	3		32	23	17	17	130
Kimmirut	Kim	15	3	3		9	6	6	42
Cumberland Sound	CSd	16	3	0	24		6	9	58
Grise Fiord	GF	29	2	2	37	23		22	115
West Greenland	WG	26	3	1	29	18	7		84
Totals		165	17	7	193	106	59	103	650

Table 3. Canonical structure and Standardized (mean = 0, s =1) Canonical Coefficients for the 49 organochlorine compounds used in the analysis. Other compounds used in the univariate analysis and not selected for CDA are also shown. The magnitude of the coefficient indicates its relative contribution to the discrimination of the sampling locations.

	Total Canonical Structure			Total Standardised Coefficients		
Compound/homolog group[1]	CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
1245-tetrachlorobenzene (T1245Bz)						
1234-tetrachlorobenzene (1234TBz)	-0.340	0.238	-0.297	-0.334	0.210	0.270
Pentachlorobenzene (P5CBz)	-0.118	0.153	-0.396	-0.298	-0.206	-0.205
Hexachlorobenzene (HCB)	0.012	0.147	-0.321	-1.790	-0.620	0.620
a-Hexachlorocyclohexane (HCH)	0.374	-0.022	-0.178	0.445	-0.298	-1.214
b-HCH	-0.140	0.475	-0.261	-0.223	0.322	-0.102
g-HCH	0.487	0.095	-0.152	1.342	0.213	0.387
"C" –heptachlor isomer[2]	0.657	-0.005	0.276	0.597	0.082	1.068
MC3 - heptachloro chlordane isomer						
MC5 - octachloro chlordane isomer	-0.087	0.216	-0.033	0.330	0.936	-0.545
MC6 - nonachlor isomer						
Oxychlordane	0.296	0.192	-0.077	-0.804	2.278	0.481
Trans-chlordane	-0.065	0.208	-0.091	-0.174	-0.140	0.098
Cis-chlordane	0.169	0.223	-0.109	0.177	0.691	0.054
Trans-nonachlor	0.345	0.182	-0.245	2.448	-0.089	-1.333
Cis-nonachlor	0.120	-0.370	0.365	-0.052	-1.347	1.145
Heptachlor epoxide	0.199	0.378	-0.230	0.222	0.380	0.780
Dieldrin	0.011	0.339	-0.291	0.209	-0.312	-0.483
P,p'-DDE	0.212	0.190	-0.027	0.030	0.132	0.086
O,p'-DDT	0.418	0.347	0.053	0.712	-0.527	-0.964
P,p'-DDD	0.000	0.113	-0.170	-0.766	-1.221	-0.963
P,p'-DDT	0.388	0.143	0.187	-1.044	0.091	1.455
Mirex	0.717	0.034	0.148	0.984	-0.075	0.620
Toxaphene	0.161	0.377	-0.143	-0.899	0.542	0.634
CB16/32						
CB52	-0.251	0.204	-0.068	0.473	-0.664	0.712
CB49	0.203	0.078	-0.129	-0.045	0.488	0.059
CB47						
CB44	-0.136	0.245	-0.229	-0.227	-0.243	-0.329
CB42	0.430	-0.203	-0.476	0.382	-0.624	-0.557
CB41/71						
CB40						
CB74	0.038	-0.163	-0.231	-0.326	-0.280	-0.335

Table 3. Continued

	Total Canonical Structure			Total Standardised Coefficients		
Compound/homolog group[1]	CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
CB70/76						
CB95						
CB91	0.571	0.023	-0.341	0.558	-0.127	-0.252
CB101						
CB99	-0.065	0.310	-0.106	0.465	0.343	0.173
CB83	-0.194	0.191	-0.388	0.335	0.009	-0.513
CB97	0.060	0.267	-0.296	0.041	0.109	0.152
CB87	0.404	-0.344	-0.082	-0.138	-0.152	0.250
CB85	0.049	0.179	0.009	-0.137	-0.206	0.031
CB136						
CB110	0.169	0.259	-0.249	0.517	0.401	-0.073
CB144	0.507	-0.406	-0.068	0.034	-1.493	-1.610
CB149	0.511	0.165	-0.150	0.669	-0.820	-0.061
CB118	-0.151	0.431	-0.221	0.200	0.043	-1.331
CB114	-0.121	0.540	-0.189	-0.117	0.209	-0.374
CB146	0.135	0.272	-0.059	-0.532	-0.935	-0.648
CB153	0.028	0.212	-0.011	-1.459	-0.383	2.695
CB105						
CB141	0.075	0.438	-0.054	-0.219	0.339	0.399
CB137						
CB138	-0.035	0.219	-0.016	0.553	-0.169	1.729
CB158	0.174	0.249	-0.035	-0.779	0.950	-0.521
CB187	0.156	0.280	-0.060	-0.752	0.944	-0.334
CB183	0.464	0.044	0.098	0.079	0.505	-0.054
CB174	0.300	0.342	0.080	0.281	0.804	0.233
CB177						
CB200	0.415	0.239	0.088	0.265	-0.311	0.350
CB172						
CB170	0.187	0.209	0.091	0.193	-0.833	-2.121
CB199	0.612	0.341	0.180	0.636	1.346	-0.395
CB194	0.628	0.073	0.366	0.284	-0.572	0.682

[1]Of the 64 compounds determined in all samples (first column) 49 were selected for Canonical discriminant analysis and are assigned canonical coefficients
[2]Heptachloro ($C_{10}Cl_7$) compound first reported in marine biota by Norstrom *et al*. (1988).

These were excluded from the multivariate analysis leaving 49 compounds for these analyses (Table 3).

Beluga from Kimmirut were significantly older than beluga from all other sampling locations (Table 1). The mean age of beluga sampled in Cumberland Sound was not different from that for the samples from Sanikiluaq, but was greater than for samples from all other locations except for Kimmirut. Mean ages of beluga from Western Hudson Bay, Nastapoka River and Sanikiluaq were not significantly different from each other. The mean ages of beluga from West Greenland and Grise Fiord were similar to each other, but significantly less than those from all other sampling locations.

Multivariate analyses

The initial Canonical Analysis differentiated samples from Cumberland Sound collected and analysed in the 1980s from those taken and analysed in the 1990s, and was strongly correlated with much lower cis-nonachlor concentrations in the 1980's samples. This difference was due to small refinements in analytical equipment and methods. In the 1980s the cis-nonachlor partially coeluted with other OCs but was fully separated in the 1990 analyses. To account for this difference, the data set was reduced to samples analysed in recent years on the same equipment with the same methods. Samples from each of the sampling locations appeared to be a homogeneous group with no obvious mixtures of two or more groupings.

Small differences were present between sampling locations either between years, such as for Western Hudson Bay or between locations within West Greenland. In addition, there were a few outliers which were not clearly associated with any grouping.

Canonical Analysis and Discriminant Analysis were performed on all sampling locations, pooling ages and sexes. The first three Canonical axes accounted for 89% of the variation explained by the six possible axes (43, 27, and 18% respectively), and all six possible canonical axes were significant. The first three were retained (eigenvalues 12.1 to 5.1, $P < 0.0001$) for discrimination between locations. The sampling locations were all significantly different from each other. All sampling locations but West Greenland had small sample sizes (11 to 29 samples) so the analysis was centred by the large sample from West Greenland. The samples from Greenland are nearest to the combined sample centroid location (*i.e.*, 0, 0, 0) and differences will be relative to this sampling location. The first canonical axis created a cline between Kimmirut, Sanikiluaq and Cumberland Sound which were separate from Western Hudson Bay, Nastapoka River estuary, Grise Fiord and West Greenland sampling locations. The second axis produced a cline between West Greenland, Cumberland Sound, and Sanikiluaq which were separate from Western Hudson Bay, Kimmirut, and Nastapoka River estuary, which were separate from Grise Fiord. The third axis produced a cline between Sanikiluaq, Cumberland Sound, Grise Fiord and Western Hudson Bay, Nastapoka River estuary, and West Greenland which were separated from Kimmirut (Fig. 2).

The first Canonical Axis was strongly and positively correlated with mirex ($r = 0.71$), C ($r = 0.66$), CB194 ($r = 0.63$) and CB199 ($r = 0.61$). These had moderate standardised canonical weightings (0.98, 0.60, 0.28 and 0.64 respectively). The first axis was also moderately and positively correlated with trans-nonachlor ($r = 0.35$), g-HCH ($r = 0.48$) and p,p'-DDT ($r = 0.39$) which had high standardised canonical weights (2.45, 1.34, and -1.04, respectively). C concentrations in beluga from Kimmirut (54 ng/g), Cumberland Sound (37 ng/g), and Sanikiluaq (27 ng/g) were significantly different from each other and all greater than those samples from Western Hudson Bay (15 ng/g),

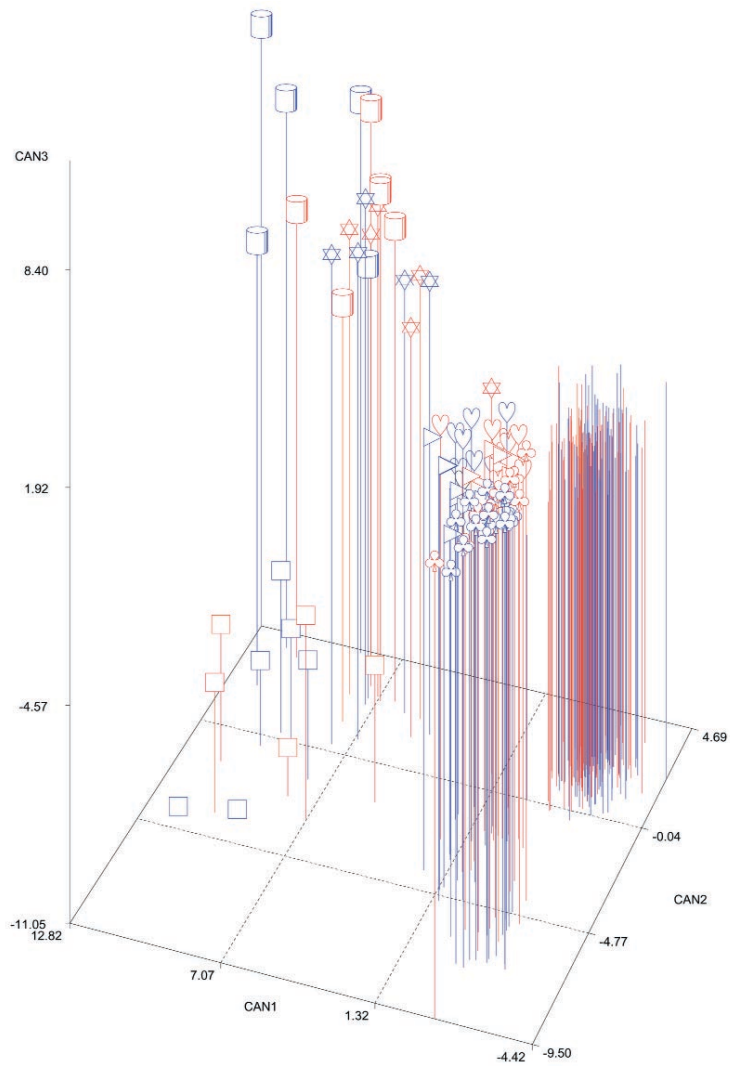

Fig. 2. *Distribution of Canonical values from the first three axes of the analysis for beluga blubber samples from West Greenland (point), Grise Fiord (club), Cumberland Sound (star), Sanikiluaq (cylinder), Kimmirut (square), Nastapoka River estuary (flag), and Western Hudson Bay (heart). Males: blue; females: red*

Grise Fiord (13 ng/g), Nastapoka River estuary (7 ng/g) and West Greenland (4 ng/g) which were not different from each other. Similarly, for trans-nonachlor, Sanikiluaq (2,117 ng/g) was significantly greater than Kimmirut (1,502 ng/g) and Cumberland Sound (1,230 ng/g) which in turn were greater than West Greenland (976 ng/g), Grise Fiord (764 ng/g) Western Hudson Bay (623 ng/g) and Nastapoka River estuary (434 ng/g).

Canonical Axis 2 was most strongly correlated with CB114 (r = 0.54) with a low standardised canonical weight (0.21) and was moderately correlated with cis-nonachor (r = -0.37) which had a high canonical weight (-1.35). Canonical Axis 3 was most strongly correlated with CB42, P5CBz, CB194 and cis-nonachlor (r = 0.47, -0.40, 0.37 and 0.37, respectively). Of these, only cis-nonachlor had a high standardised canonical weight (-0.31) (Table 3).

The canonical values, the composite values for each sample, had highly significant sampling location treatment effects ($P < 0.001$), but none had a significant sex related treatment effect (Can 1: P = 0.52, Can 2: P = 0.28, Can 3: P = 0.45), or age related treatment effects (*i.e.*, 7 years or less, and greater than 7 years of age - Can 1: P = 0.055, Can 2: P = 0.81, Can 3: P = 0.41), although Can 1 was nearly significant. Again the large sample from Greenland was dominant. In some other sampling locations there appeared to be sex related differences. Samples from male belugas from Kimmirut were more negative than those from females on Axis 1; samples from male belugas from Western Hudson Bay, Grise Fiord and Nastapoka River estuary were more negative than most females from their respective sampling locations on Axis 2. However, samples from one location were more similar to each other than they were to other sampling locations in spite of any age and sex effects.

Ninety-four percent (221 of 236) of the samples were correctly classified to their sampling location. Of the fifteen samples that were misclassified, the most common misclassifications were between Cumberland Sound and Sanikiluaq, and between Western Hudson Bay and Nastapoka River estuary (Table 4).

Removing a single animal from small samples may dramatically alter the mean of the remaining samples when samples sizes are small and, as expected, a few more (22) samples were misclassified (89% properly classified) but no major differences were found. Most of misclassifications were again between Cumberland Sound and Sanikiluaq, or between Western Hudson Bay and Nastapoka River estuary sampling locations (Table 4).

The organization of the first three canonical axes suggested that there could be value in examining differences within smaller geographic areas, possibly allowing for greater power in assigning samples to a location. This was done by segregating sampling locations into smaller, geographically related groups: *Hudson Bay-Southeast Baffin Island* (which included Western Hudson Bay, Nastapoka River estuary, Sanikiluaq, Kimmirut and Cumberland Sound

Table 4. Samples discriminated into sampling locations based on the first three Canonical components. Initially all samples were used (All data), and then each sample was run as an unknown relative to the other samples as a test of power.

		Into location													
		All data							One against the rest						
From location		WG	GF	CSd	Kim	SQ	NaR	WHB	WG	GF	CSd	Kim	SQ	NaR	WHB	Total
Greenland	WG	140							138						2	140
Grise Fiord	GF		28				1			27				2		29
Cumberland Sound	CSd			8		2		1			5		5		1	11
Kimmirut	Kim				11							10			1	11
Sanikiluaq	SQ			4		7					6	1	4			11
Nastapoka River	NaR						11	5		1				10	5	16
Western Hudson Bay	WHB		1				1	16		1			2	15	18	
Total		140	29	12	11	9	13	22	138	29	11	11	9	14	24	236

sites); *Baffin Bay - Southeast Baffin Island* (Kimmirut, Cumberland Sound, Grise Fiord, and West Greenland); *Grise Fiord - West Greenland*; and *Upernavik - Disko Bay*.

Hudson Bay-Southeast Baffin Island

The organochlorine compounds examined during a single analysis were separated into OC pesticides or PCBs because of the small number of samples associated with this region (N = 67). Little improvement was found in examining samples from each location in the region compared to pooling all locations for the entire sample region, except that most (10 of 11) of the Cumberland Sound samples and all of the Sanikiluaq samples were assigned to Sanikiluaq. Three to 6 of the samples from Western Hudson Bay and Nastapoka River estuary were misclassified to the other. Eigenvalues ranged from 10.7 to 7.3 for the pesticides and 22.8 to 3.7 for PCBs. The first 3 axes explained greater than 90% of the variation in the 4 possible data summarising rotations.

There is considerable management interest about the relationship between beluga sampled in Sanikiluaq and the Nastapoka River estuary due to the *threatened* status of Eastern Hudson Bay beluga assigned by COSEWIC (Reeves and Mitchell 1989). When only these two sampling locations were compared using pesticide and pesticide metabolic products, and the weightings determined from the remaining samples, none of the samples was misclassified. Sample sizes associated with this comparison were small, limiting the available degrees of freedom, so not all of the information in the OC concentrations could be used. Mirex, b-HCH, g-HCH, cis-chlordane and heptachlor epoxide were strongly and negatively correlated with the canonical axis ($r > -0.76$), and only T1234Bz was moderately and positively correlated with the canonical axis. The eigenvalue for the single axis was 343 ($P < 0.0001$), and the class means were -21 for Sanikiluaq and 15 for Nastapoka River estuary samples. Samples from the Nastapoka River estuary and Sanikiluaq had the same relationship between heptachlor epoxide and b-HCH, but the sample from Nastapoka River estuary had lower concentrations than those from Sanikiluaq (Fig. 3). The only sample from Sanikiluaq that overlapped those from the Nastapoka River was an older female (Fig. 3). In both sampling locations females had lower concentrations than males and concentrations declined with age.

Baffin Bay - Southeast Baffin Island

When sample locations were restricted to Baffin Bay and Southeast Baffin Island, all samples but two were properly classified back to their sampling location based on the first two Canonical axes. One sample from Cumberland Sound was classified as West Greenland and one sample from Kimmirut was classified to Cumberland Sound. When each sample was classified independently using the first two axes an additional three samples were misclassified. None of the samples was misclassified when three Canonical Axes were used.

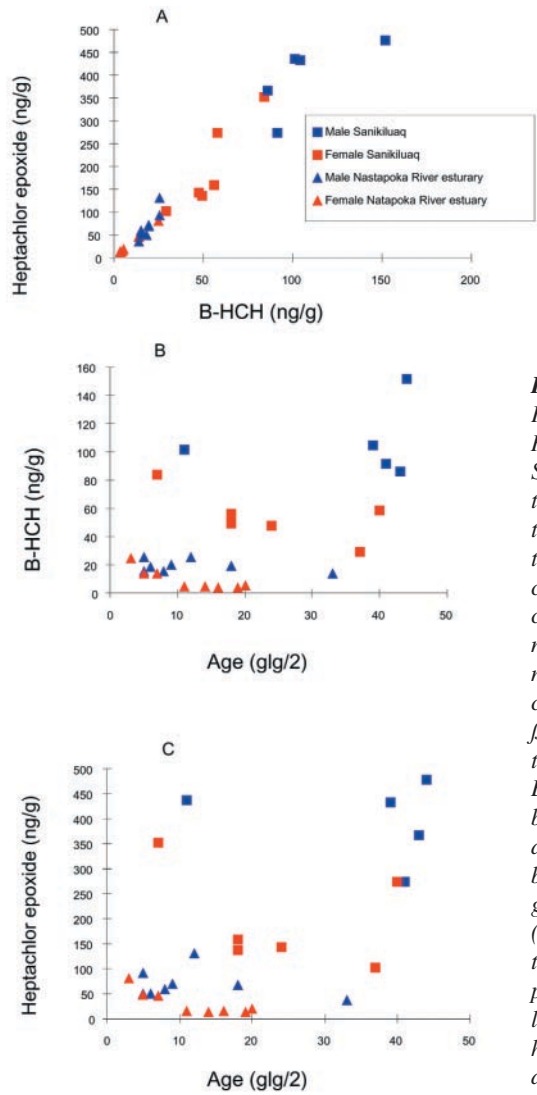

Fig. 3. *For Nastapoka River estuary and Sanikiluaq belugas, the relationships between ß-HCH, heptachlor epoxide (a chlordane-related compound), age and males (blue) and females (red). A: the correlation between ß -HCH and heptachlor epoxide . B: the relationship between ß -HCH and age determined by counting the growth layer groups (glg) and assuming that two are formed per year. C: the relationship between heptachlor epoxide and age.*

The first Canonical Axis separated West Greenland and Grise Fiord samples from Cumberland Sound and Kimmirut; the second Canonical Axis separated West Greenland and Grise Fiord samples; and the third separated Cumberland Sound from Kimmirut. The three axis explained 48, 32 and 20% of the explained variation and had eigenvalues of 14.5, 9.4, and 6.0 which were significant. The first Canonical Axis was highly correlated with C (a heptachlor isomer reported by Norstrom *et al*. 1988), mirex, and the CBs 144 and 149 which all had relatively high canonical weights. The second axis was correlated with cis-nonachlor and β-HCH and the third was correlated with C, and CBs 42 and 91.

Samples from Grise Fiord and West Greenland were investigated further. These two locations were compared to identify organochlorine compounds responsible for the differences. In addition, there is a contention that samples from the Upernavik District of West Greenland in early fall are from a different stock from those caught in Disko Bay and south of West Greenland during late fall to spring (JCNB 1997) so samples from these areas were analysed separately.

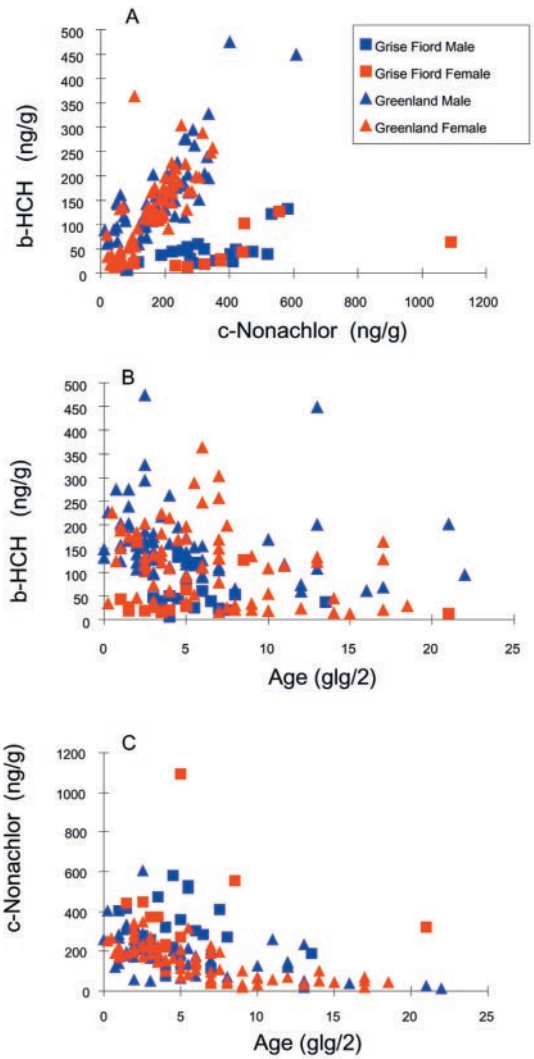

Fig. 4. *The relationships between ß -HCH, cis-nonachlor (chlordane-related), age and males (blue) and females (red) sampled at Grise Fiord and West Greenland. A: the correlation between ß -HCH and cis-nonachlor. B: the relationship between ß -HCH and age. C: the relationship between cis-nonachlor and age.*

Samples from Grise Fiord and West Greenland separated clearly (Fig. 4). The single canonical axis was associated with a eigenvalue of 13.6 ($P < 0.001$) and was positively correlated ($r > 0.4$) with b-HCH and CB 114 and negatively correlated ($r < -0.5$) with cis-nonachlor and CB 144. West Greenland samples had more b-HCH per unit of cis-nonachlor than samples from Grise Fiord (Fig. 4).

Samples landed within West Greenland were divided into those killed in the Upernavik Region (*i.e*., Nuussuaq) and those killed near or in Disko Bay (Qeqertarsuaq, Saqqaq, and Kitsissuarsuit). The eigenvalue for the single axis was 1.3 ($P < 0.0002$), and much smaller than most first axes in the other analyses. This axis was highly correlated with T1234Bz ($r = 0.62$) which had a moderate weight (0.88), and moderately ($r = 0.40$ to 0.30) correlated with g-HCH, trans-nonachlor and p,p'-DDD which had high weights (1.33, 1.49, and -1.57, respectively). Based on this canonical axis 96% of the samples taken near Disko Bay were assigned to this sampling location and 93% of the samples taken at Nuussuaq were assigned to Nuussuaq. The samples from Upernavik Region had higher levels of T1234Bz than those from Disko Bay area. Many Upernavik samples had up to twice the p,p'-DDD levels of those from Disko Bay area (Fig. 5).

DISCUSSION

The patterns or relative proportions of OC contaminants in belugas landed at each of the seven locations were so different from each other that there is virtually no chance that they came from fewer than seven different subsets of belugas. Based on the original stock descriptions there would have been only four sub-sets or stocks representing these seven sampling locations: Baffin Bay (Grise Fiord and West Greenland), Southeast Baffin Island (Kimmirut, Cumber-

land Sound), Eastern Hudson Bay (Sanikiluaq and Nastapoka River estuary) and Western Hudson Bay (Arviat and Churchill) (Sergeant and Brodie 1975, Stewart 1994). Within the geographic range of the samples used in this study, there are two different types of habitat usage by the four putative stocks. Belugas in the Baffin Bay stock were thought to use one summering area but then separate into two wintering groups (Finley and Renauld 1980, Richard *et al*. 1998, Heide-Jørgensen 1994, JCNB 1997). The belugas in Hudson Bay and the Southeast Baffin Island area are thought to use separate summering locations but one wintering area (Reeves and Mitchell 1989, Richard *et al*. 1990). Even prior to this analysis, it was expected that there were more stocks than the four. It is possible, even likely, that samples from communities such as Sanikiluaq, Kimmirut and Grise Fiord are a mixture of more than one migration group.

Not all the results fit with either of these stock summaries. For example, belugas from Kimmirut were different from both Southeast Baffin and Western Hudson Bay belugas. Whales taken at Sanikiluaq differed from those hunted from the coastal communities of Eastern Hudson Bay and from Western Hudson Bay whales, indicating that they were not Western Hudson Bay whales migrating to their summer range. Not only were belugas hunted in Grise Fiord different than those hunted in West Greenland, but there was separation among Greenland belugas. Some whales hunted in Upernavik were not available to hunters near Disko Bay. These differences require alteration in the stock descriptions in the Arctic, even though they do not present a complete picture.

"Stock" is a commonly used term in fisheries management, however there are great differences of opinion about the actual definition of a stock and how it should be determined (see Brooke 1981, Gauldie 1991, Dizon *et al*. 1992). One important attribute of a stock is that there is an interaction between hunters or fishers with potentially exploited individuals (Gauldie 1991) and the boundaries are defined by which animals are exposed to a fishery. In slightly different terms, "stock" is the unit, in space and time, that is affected at the group level (density, reproduction rates *etc*.) by the removal of an individual by a fishery. While it is likely that those individuals removed by a fishery will have some biological association (*i.e*., deme, spawning stream, metapopulation, population, subspecies, or species), biological descriptions are not sufficient to define a stock, and are not necessary as long as each biological unit responds the same way to the removal (see Ricker 1958).

For belugas, "stock" may be best described by the annual migration path and the hunters who have access to whales during their migration. It is then a cultural trait of the beluga (*e.g*., migration path), not a biological population, that defines which individual belugas are hunted by which hunters. Beluga summering areas and

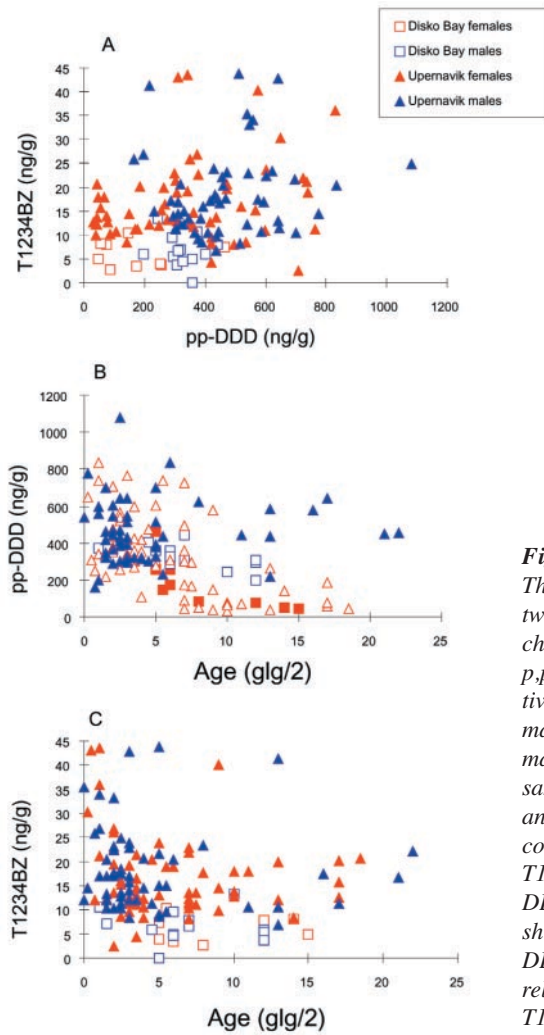

Fig. 5.
The relationships between T1234Bz (a chlorobenzene), and p,p'-DDD (a derivative of DDT), age and male (blue) and female (red) belugas sampled at Nuussuaq and Disko Bay. A: the correlation between T1234Bz and p,p'-DDD. B: the relationship between p,p'-DDD and age. C: the relationship between T12345Bz and age.

migration routes are traditional, with a female beluga returning to them each year, with her offspring learning the migration route to and from summering and wintering habitats (Caron and Smith 1990). The consistency of migration path is also reflected in the mtDNA evolution of this species (Brown Gladden *et al*. 1998) and the population structure of the species as determined using microsatellite markers (Brown Gladden *et al*. 1999). Generally, most belugas at a summering location share similar maternal lineages and there appears to be little immigration from relatively nearby stocks. In addition, there appears to be little interbreeding among whales from different summering locations (de March pers. comm.).

Belugas that summer in the Canadian High Arctic were originally given their own stock definition based on their use of the estuaries in the Canadian High Arctic near Sommerset Island (Sergeant and Brodie 1975, Bodaly *et al*. 1992). Later, belugas from the Canadian High Arctic were grouped with belugas that migrate, in the fall, south along the coast and winter off West Greenland (Doidge and Finley 1993, Remnant and Thomas 1992, Thomsen 1993) with some stragglers overwintering in the North Water polynya (Finley and Renaud 1980). Based on the differences in the types and concentrations of OC contaminants found between those whales killed in Grise Fiord and Greenland (Stern *et al*. 1994, this study) and between Upernavik and the Disko Bay area, a sub-division of the original stock description into at least three new stocks is required.

Satellite-linked radio tagging has suggested that most of the belugas found near Somerset Island during July migrate along the south coast of Devon Island in the fall and then to Jones Sound and remain in the North Water until at least October when most of the tags failed. Only one beluga has migrated to West Greenland out of about 20 beluga with tags still working as the major migration of belugas passed the Upernavik Region (Richard *et al*. 1998). It also appears that few of the belugas that migrate to West Greenland from the Canadian High Arctic are available to hunters at Grise Fiord. None of the 28 Grise Fiord beluga analysed in this study had a Greenlandic distribution of OC contaminants. Combining the contaminant and radio-telemetry data with the most recent survey results (Heide-Jørgensen and Acquarone 2002, Innes *et al*. 2002) suggests that a significant number of these belugas winter somewhere other than off West Greenland. From their contaminant load, it appears that they remain in an Arctic water mass feeding on something at the level of polar cod *(Boreogadus saida*) since they share similar amounts of some Arctic contaminants with belugas killed off West Greenland.

While each of the seven sampling locations was different from each other, they fall generally into three areas in canonical space along the first two dimensions (Fig. 2). The first canonical axis separated belugas caught in Cumberland Sound, Sanikiluaq and Kimmirut from those caught elsewhere, while the second axis separated belugas caught in Grise Fiord, Western Hudson Bay and Nastapoka River estuary from those caught elsewhere. The first axis can be summarised by positive values having relatively higher concentrations of some chlordanes (C and trans-nonachlor), mirex and highly chlorinated PCBs (octachlorobiphenyls–CB194 and 199). Beluga from West Greenland had significantly greater amounts of chlordanes than Western Hudson Bay and Nastapoka River estuary. In a general way, this first axis represents a sum of contamination with the most contaminated stocks to the more positive end of the axis (Fig. 2).

The second axis separated Western Hudson Bay, Nastapoka River estuary and Grise Fiord from other locations. It was consistent with the differences found by Stern *et al*. (1994), who reported significantly greater concentrations of ∑DDTs in blubber samples from male belugas caught off West Greenland than in those from Grise Fiord and Western Hudson Bay. This second axis can be interpreted as a separation between Arctic water and non-Arctic water that summarised several differences. For example, cis-nonachlor concentrations were highest in belugas from Grise Fiord (355 ng/g) and Nastapoka River estuary (322 ng/g) where concentrations were significantly greater than those in belugas from West Greenland (170 ng/g) and Sanikiluaq (123 ng/g). Differences in the nature

of the food webs of different regions will be represented in the OC concentrations of the belugas. It is expected that persistent OCs such as hexa- and heptachlorobiphenyls, nonachlor and DDT-related compounds that biomagnify in marine food chains (Muir *et al*. 1992, de March *et al*. 1998) will be found at greater concentrations in animals that feed on higher trophic levels. Belugas feeding on polar cod should have less of these biomagnifying contaminants than those eating Greenland halibut (*Reinhardtius hippoglossoides*), because small Greenland halibut eat polar cod and Greenland halibut live much longer than polar cod, accumulating more OC contamination in their lipids (Muir *et al*. 1992, AMAP 1997, Bowering and Brodie 1995, Orr and Bowering 1997). There would be relative differences between original contaminants and their metabolites depending on the species being eaten (Muir *et al*. 1995, Letcher *et al*. 1998). Animals remaining in the Arctic water mass would have relatively higher proportions of the more volatile OCs, which are more likely to reach the Arctic via long range atmospheric transport (Muir *et al*. 1992, Wania and Mackay 1993, de March *et al*. 1998). Finally, belugas feeding in the Atlantic water mass are expected to have more OC components from European sources due to the East Greenlandic Current - Labrador Sea transport linkage (de March *et al*. 1998, AMAP 1997) than belugas that feed within the Arctic water mass (see Dunbar 1968) into which contaminants enter primarily from airborne transport, North American river systems, and Pacific water from Bering Strait (AMAP 1997, de March *et al*. 1998).

Unfortunately, it was not possible to ascribe the differences observed between the locations to differences in diet and food web. Very limited information is available on OC pesticides and PCB contamination in the marine food webs in various parts of the Arctic (Muir *et al*. 1992, de March *et al*. 1998), the food eaten by belugas while they are in these areas, and the bioaccumulation and the physiological processing of contaminants in these webs. However, it was possible to show that belugas that feed in the Canadian High Arctic have higher relative concentrations of the lower chlorinated PCBs than belugas that may feed on similar webs from lower latitudes. For example, Grise Fiord belugas acquired more lower molecular weight PCBs (*e.g*. trichlorobiphenyls) for each nanogram of higher molecular weight compounds than seen at other locations (Fig. 6). This is consistent with the observed fractionation of PCBs on a global scale (Stern *et al*. 1997, Wania and Mackay 1993). It is tempting to speculate that belugas at Kimmirut, Sanikiluaq, Cumberland Sound feed higher on the food web than belugas from Western Hudson Bay and Nastapoka River estuary, leading to higher levels of contamination. A similar difference has been found in walrus (*Odobenus rosmarus*) (Muir *et al*. 1995).

Several studies have shown differences in the concentrations of OC contaminants related to the sex and the age of the beluga (Muir *et al*. 1990, 1996, Stern *et al*. 1994). Depending on the ingestion rate and the beluga's ability to biotransform and excrete contaminants, the concentration of any specific contaminant can increase, stay the same or decline with age. In addition, female beluga transfer significant amounts of contaminants to their offspring during lactation (Muir *et al*. 1990, 1996, Stern *et al*. 1994) and mature females often have lower contamination levels than males of similar ages. Such differences were expected to have effects on the canonical variate analysis. This was not the case, most likely because the analysis steps within Canonical Discriminant Analysis are more sensitive to changes in the ratios of chemicals than absolute concentrations.

There were no significant increases in the number of stocks that could be discriminated using the more limited regional analyses. This was expected because such limitations reduced the sample sizes available for analyses, reduced the numeric range in concentration of the contaminants, and reduced the number of contaminants that could be compared. Within each of the regional sub-sets, the loadings followed a pattern similar to that of the combined analyses; there was one or more of chlordane, HCH, DDT, and PCB compounds that was highly correlated to the Canoncial axis and separated the sampling locations.

In the regional analyses, the separation of belugas sampled at Nuussuaq from those sampled near Disko Bay a few months later was an inter-

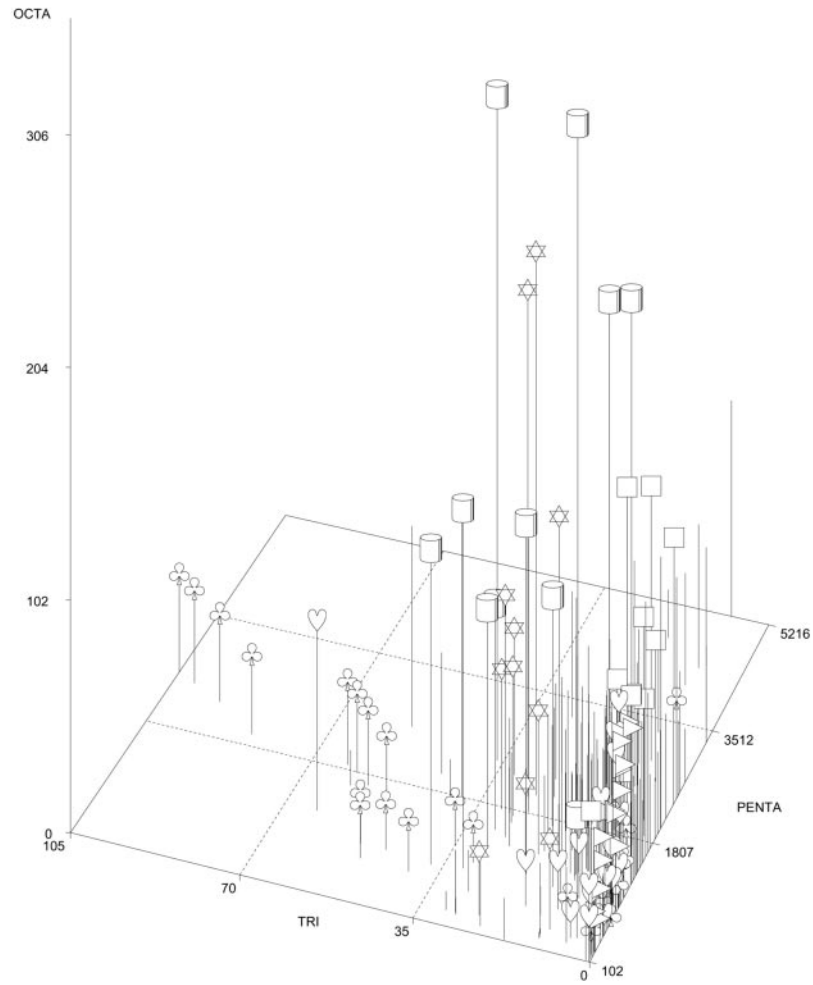

Fig. 6. *Distribution of polychlorinated biphenyls classes with respect to sampling locations. TRI have three chlorine atoms, PENTA have 5, and OCTA have eight. Beluga blubber samples were from West Greenland (point), Grise Fiord (club), Cumberland Sound (star), Sanikiluaq (cylinder), Kimmirut (square), Nastapoka River estuary (flag), and Western Hudson Bay (heart).*

esting result. The main difference was the much lower levels of 1,2,3,4-tetrachlorobenzene (T1234Bz) in the Disko Bay belugas which is likely due to loss of this contaminant from the belugas as they moved from an Arctic food web to the West Greenland food web. T1234Bz is a relatively volatile contaminant and would be expected to have a short half-life in marine mammals. However, the differences in T1234Bz were not the only significant difference. Belugas from Disko Bay also had lower concentrations of p,p'-DDD and a different concentration-to-age relationship (Fig. 5). This is not likely due to a physiological process changing the concentrations of p,p'-DDD over a few months, as could be the case for T1234Bz, but more likely is related to the fact that the belugas that are available to hunters in this area occur closer to the ice edge, and presumably eat different foods than some of those belugas killed in Nuussuaq which are not available to hunters in Disko Bay.

It could also be that the belugas gained significant mass between Nuussuaq and Disko Bay without a concomitant amount of p,p'-DDD. This is unlikely because most of the p,p'-DDD appears to be from the food web off West Greenland; it occurs at much lower concentrations in beluga landed at Grise Fiord. The difference suggests that about 25% of the belugas that are available to the hunters in Nuussuaq are not available to the hunters near Disko Bay. There is also the possiblity that this group has a greater capacity to degrade DDT to DDD that is ultimately genetically related. This might be a more logical explanation given that DDD levels should not be independent but should be correlated to other DDT related compounds supplied via the food web.

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