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# A multi-elemental approach to identification of subpopulations of North Atlantic minke whales *Balaenoptera acutorostrata*

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A combination of heavy metals, organochlorines (OC) and fatty acids (FA) that reflect long-term deposition (1+ year) in tissues was used in a Canonical Discriminant Analysis (CDA) exploring population substructure in 104 minke whales *Balaenoptera acutorostrata* that were sampled in West Greenland, the Central and Northeast Atlantic Sea and in the North Sea in 1998. Using a CDA that included mercury and cadmium in muscle, liver and kidney, and eight OCs and four unsaturated FAs in blubber we were able to separate the whales into four subpopulations: 1) a West Greenland group, 2) a Central Atlantic group represented by whales from Jan Mayen, 3) a Northeast Atlantic group (Svalbard, Barents Sea and northwestern Norway), and 4) a North Sea group. During an assignment test based on the data transformation developed by the CDA, about 84% of the individuals were correctly assigned to the area where they had been caught. The highest degree of misassignment was between Jan Mayen and the Northeast Atlantic groups. The differences among the four groups likely reflected regional differences (i.e. sea water chemistry, prey type and prey availability) among the marine ecosystems within the range studied. The study indicated that a multi-elemental approach based on long-term deposited compounds with different ecological and physiological pathways can be used for identification of subpopulations of marine mammals.

*Key words:* *Balaenoptera acutorostrata*, minke whale, multi-elemental analysis, North Atlantic, subpopulation

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Proper identification of subpopulations or 'biological stocks' is a prerequisite for management that will ensure long-term sustainability of exploitation of wild animal species (e.g. Anon. 2002c). A biological population encompasses all the individuals in an area that are part of the same reproductive process. They form a self-contained unit, with emigration and immigration rates far lower than the initial rate of population growth (ibid.). A subpopulation may be defined as a geographically or otherwise distinct group within the population between which there is little exchange (Molloy et al. 2002). Minke whales *Balaenoptera acutorostrata* range widely in the North Atlantic Ocean from the eastern coasts of the North American continent to Novaya Zemlya in the east (Fig. 1). The International Whaling Commission (IWC) divided North Atlantic minke whales into four major management areas ('stocks') based mainly on segregation by sex and length, catch distribution, marking data and the distribution of the whales at their summer feeding grounds, and considerations of ecological conditions. These four 'stocks' were: Canadian east coast, West Greenland, Central Atlantic (East Greenland-Iceland-Jan Mayen) and Northeast Atlantic (Svalbard-Norway-British Isles; Donovan 1991a).

These areas have been further divided into 10 'management subareas' or 'small areas' (Anon. 1994, 2004; see Fig. 1).

Several studies involving analyses of genetics, morphometrics, and distributional and catch data have aimed at determining the population substructure of North Atlantic minke whales (reviewed in Anon. 1998, Waerebeek et al. 1999, Andersen et al. 2003, Anon. 2004). The studies indicated some substructuring, but generally failed to find a clear distinction between minke whales from various regions of the North Atlantic. Most recent studies supported the hypothesis that minke whales from West Greenland and the North Sea differ from those in other subareas (Andersen et al. 2003, Møller et al. 2003, Born et al. 2002, 2003, Hobbs et al. 2003, Anon. 2004).

Four distinct groups of minke whales were identified genetically by Andersen et al. (2003) based on material sampled from West Greenland during 1996-1999 and across the Northeast Atlantic to the North Sea (1998). Using the suite of samples from 1998, the population substructure of minke whales was also studied using regional variation in muscle  $^{137}\text{Cs}$  concentrations (Born et al. 2002), organochlorine (OC) burdens (Hobbs et al. 2003), fatty acid (FA) compo-

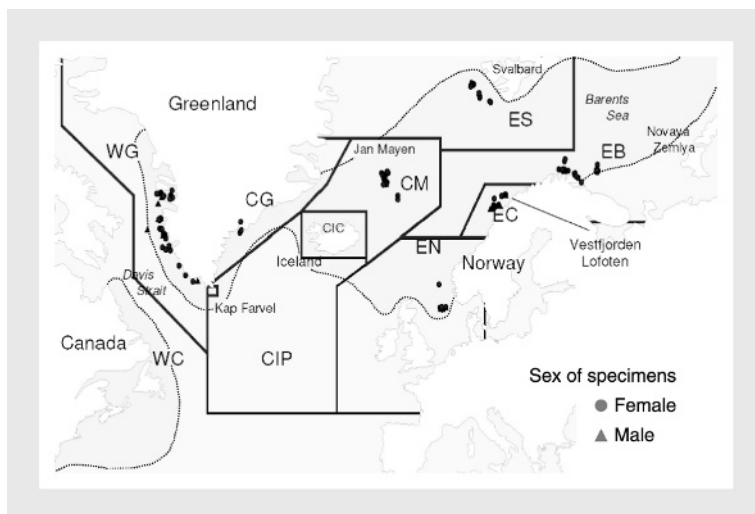


Figure 1. Locations at which sampling of tissues from 159 minke whales (125 females and 34 males) were collected in 1998. A subset of 104 of these whales was included in this paper (see Table 1). Boundaries of the seven International Whaling Commission (IWC) North Atlantic management areas (Anon. 1993) are shown, and the approximate minke whale summer range (Stewart & Leatherwood 1985, Donovan 1991a,b, Anon. 1997) is delineated by the dotted line. IWC acronyms of different management areas are: WC (West Canada), WG (West Greenland), CG (Central Greenland), CIC (Central Island, Coastal), CIP (Central Iceland, Pelagic), CM (Central Jan Mayen); ES (East Svalbard), EB (East Barents Sea), EC (East Coastal), EN (East North Sea).

sition (Møller et al. 2003), and various elements including mercury (Hg) and cadmium (Cd) in soft tissues and baleen (Born et al. 2003).

Our study was made with the purpose to identify minke whale subpopulations that on a long-term scale have been geographically separated at the North Atlantic summering grounds where Greenland and Norway catch this species for human consumption (e.g. Grønvik 1998, Witting 2000, Anon. 2002a). We took a relatively novel approach to investigations of population substructure by combining information on regional variation in certain FAs, OCs and heavy metals (Hg and Cd). These substances are presumably acquired from food on the summer feeding grounds and their concentrations reflect long-term accumulation. Hence, our study explores the feasibility of using several different diet-related compounds in combination for identification of subpopulations of North Atlantic minke whales. The rationale is that if: a) minke whales feed little if at all during winter (*cf.* Horwood 1990), b) groups of minke whales have a long-term affinity to specific summer feeding grounds, c) these feeding areas differ substantially in minke whale prey availability and prey choice (Neve 2000, Sigurjonsson et al. 2000, Olsen & Holst 2001, Haug et al. 2002), and d) if the combinations and concentrations of FAs, OCs and heavy metals that are transmitted to the whales via the food differ, it may be expected that this is reflected in different signatures in the whale tissues indicating the existence of ecologically separated groups, or different subpopulations.

In our study, we analysed the same suite of samples from 1998 as used in Hobbs et al. (2003), Møller et al. (2003) and Born et al. (2003) for regional variation in the patterns of a combination of different dietary-related compounds in North Atlantic minke whales. These compounds included eight specific OCs (e.g. PCB congeners, mirex and dieldrin, four unsaturated FAs in blubber, as well as Hg and Cd in muscle, kidney and liver. All are thought to represent long-term accumulation (1+ years) in tissues (Aguilar & Borrell 1988, Norstrom et al. 1992, Dietz et al. 1998, Hickie et al. 2000, Koopman et al. 2002).

A main purpose of our study was to investigate whether the regional variation in this combination of long-term signatures reflect the existence of profound differences in the major marine ecosystems within the geographical range studied; and therefore whether this multi-elemental approach can be used for identification of subpopulations of whales. The four regions considered were: a) West Greenland, b) the Central Atlantic represented by whales from the

Jan Mayen area, c) the Northeast Atlantic (Svalbard, Barents Sea and Vestfjorden/Lofoten of coastal Norway), and 4) the North Sea.

## Ecology of minke whales in the North Atlantic region

To supply the reader with a background for the study, the ecology of minke whales in the North Atlantic is briefly summarised.

Apparently, North Atlantic minke whales feed little, if at all, when wintering between about 11° and 45° N latitude. Pairing likely takes place from December to May, and calving predominantly from October to March, during a period when minke whales are mostly absent from North Atlantic waters. During spring, the minke whales migrate north to their boreal, subarctic and arctic summer feeding grounds; some, likely few, individuals may stay farther south during summer. Female minke whales tend to summer farther north than males. Although the whales may occur in areas with deep water in the North Atlantic during summer (e.g. Anon. 1997), they concentrate on traditional feeding grounds such as eastern Canada (Gulf of St. Lawrence, Nova Scotia, Newfoundland-Labrador), off West and Southeast Greenland, around Iceland and Jan Mayen, off Svalbard and in the Barents Sea, off western Norway and in the North Sea (Mackintosh 1965, Jonsgård 1962, 1966, Øien 1988, Larsen & Øien 1988, Horwood 1990, Mitchell 1991, Folkow & Blix 1991, Anon. 1997, Waerebeek et al. 1999).

Within the North Atlantic, no single organism forms the dominant food supply for minke whales (e.g. Skaug et al. 1997). The greater variety of food in the northern hemisphere as compared to the food in the southern can be partly attributed to the more complex topography and water conditions in the north (Mackintosh 1965). Although the shallow continental shelf-areas in which minke whales feed are areas of great productivity, they differ substantially with respect to oceanography (Mann & Lazier 1991, Anon. 2003, Macdonald et al. 2003): 1) the West Greenland area is influenced by a mixture of waters from the cold East Greenland Current and the warmer and more saline Irminger Current; 2) the East Greenland - Jan Mayen area is dominated by the East Greenland Current that brings cold, low-saline polar water south along the eastern coast of Greenland resulting in heavy pack ice almost all year round; 3) the western coast of Svalbard is an area where the waters of Polar

origin mix with a branch of the warm North Atlantic Current; 4) the Barents Sea is a relatively shallow area that is dominated by the North Atlantic Current. These latter two areas are ice-covered for part of the year; 5) the northwestern coast of Norway is greatly influenced by the North Atlantic Current and the Norwegian Coastal Current which results in relatively high water temperatures; and 6) the North Sea is confined between the British Isles, southern Norway and Denmark, and is influenced by water from the North Atlantic Current as well as land runoff from the surrounding countries. Ice never occurs along western Norway and in the North Sea.

These regions also differ with respect to fish and crustacean fauna which again is reflected in differences among areas in minke whale prey preferences (Folkow et al. 2000, Neve 2000, Anon. 2001, Olsen & Holst 2001, Haug et al. 2002).

## Material and methods

### Collection of samples

Tissue samples were available from a total of 159 minke whales that were taken during Greenland and Norwegian licensed whaling operations during 6 May - 31 October 1998 in seven IWC management areas (see Fig. 1). The character of the whaling operations determined the sampling areas visited and the aggregate locations within areas exploited by Norwegian whalers (i.e. CM, ES, EB, EC, EN in Fig. 1). However, overall the seasonal and spatial distribution of samples in the present study is representative of the Greenland (*cf.* Witting 2000) and Norwegian catches in 1998 (N. Øien, unpubl. data). The samples were analysed for  $^{137}\text{Cs}$  (Born et al. 2002), OC (Hobbs et al. 2003), FAs (Møller et al. 2003), several elements including Hg, Cd and Se (Born et al. 2003), and variation in mitochondrial and nuclear DNA (Andersen et al. 2003). For details on collection of samples and treatment in the laboratory see these sources. Because in some cases not all tissues were sampled from each whale, or not all substances were analysed in the individual whale, a total of 104 individual whales (21 males and 83 females), for which we had complete data, were included in the present analyses (Table 1).

For statistical analyses, the data from the various subareas were combined into groups that represented the four major marine ecological regimes within the range covered: West Greenland (WG), a Central Atlantic group represented by samples from Jan Mayen (CM); two samples from East Greenland were

Table 1. Number by area and sex of North Atlantic minke whales sampled in 1998 and included in the analyses of regional differences in various dietary-related compounds. For explanation of the acronyms see Fig. 1. The Northeast Atlantic sample consisted of whales from Svalbard (ES: one male and 13 females), the Barents Sea (EB: one male and 23 females) and Vestfjorden/Lofoten (EC: three males and three females).

Area	Acronym	Number of specimens		Period of collection
		Females	Males	
West Greenland	WG	19	6	6 May -31 October
Jan Mayen	CM	16	3	7 June -1 July
Northeast Atlantic	NE	39	5	15 May -14 August
North Sea	EN	9	7	15 May -8 June
Total		83	21	

omitted), a Northeast Atlantic (NE) group that consisted of samples from Svalbard (ES), the Barents Sea (EB) and Vestfjorden/Lofoten (EC) on the northwestern coast of Norway, and a North Sea group (EN), see Table 1.

### Chemical analyses and selection of compounds for statistical analyses

Cadmium and mercury concentrations in muscle, liver and kidney were included in the present analysis because these elements represent a long-term dietary response with a biological half-life of 2-30 years (Dietz et al. 1998).

Among 102 PCB congeners and several other OCs in the blubber of the same minke whales (Hobbs et al. 2003), the following eight were selected for the analysis: PCB153, PCB138 and PCB180, *p,p'*-DDE, HCB, trans-nonachlor, mirex and dieldrin. These OCs are known to have long half-lives in mammals (Matthews & Dedrick 1984, Dearth & Hites 1991).

A total of 43 FAs have been identified in minke whale blubber (Møller et al. 2003). Among these, the following four unsaturated FAs found in the outer blubber layer (immediately under the skin) were included in our study: C14:1n-5, C16:1n-7, C18:1n-9 and C20:1n-11. The reasons for selecting these FAs were that: a) FAs in the outer blubber layer are thought to represent a longer-term dietary accumulation than the inner layer, which is more labile (Møller et al. 2003 and references therein, Olsen & Grahl-Nielsen 2003), b) unsaturated FAs generally reflect long-term dietary response better than saturated FAs do (Gurr et al. 2002), c) there was no possibility for transformation of one of these FAs into another by e.g. 2-carbon chain elongation (*ibid.*), and d) they were present in a relatively high proportion in all individuals.

Metal concentrations were expressed on dry matter basis ( $\mu\text{g} \cdot \text{g}^{-1}$ ), and OC concentrations on lipid



weight basis ( $\text{ng} \cdot \text{g}^{-1}$ ). FAs were expressed as mass percentage of total FAs.

### Statistical methods

Statistical analyses aimed at determining whether the patterns of the compounds in combination, expressed as relative levels, rather than their individual levels differed from one summering area to another. The statistical package SAS (SAS Institute 1999-2001) was used for all analyses.

A principal components analysis (PCA) of the three groups of variables (i.e. heavy metals, OCs and FAs) gave preliminary insights into their relationships.

Then we explored the correlation between the selected compounds by clustering them based on the correlation matrix (SAS procedure PROC VARCLUS). The number of clusters was determined so that each cluster only had a single eigenvalue greater than one.

Canonical Discriminant Analysis, CDA (procedure PROC CANDIST), was then conducted for all selected metals, OCs and FAs to determine patterns, similarities and differences among the four groups of whales. CDA summarises the data into few canonical variables that capture important differences among sampling locations. The first canonical variable is a linear combination of the compounds that has the highest overall power to discriminate between the groups. The second canonical variable is another linear combination of the compounds, in the sample uncorrelated with the first canonical variable that has the highest possible multiple discrimination between the groups (SAS Institute 1999-2001).

The correlations were explored between the compounds and the canonical variables together with the standardised (mean = 0, SD = 1) canonical coefficients. The correlation coefficients measure the univariate relationship between the compounds and the canonical variable, whereas the standardised coefficients show the contribution of the compounds in the presence of each other compound, and therefore provide a multivariate approach to interpretation of the contribution of the variables acting in combination (Rencher 1995).

The canonical variables were then used to determine the ability to assign the whales to the four areas (procedure PROC DISCRIM). Based on the generalised squared distance function each whale was placed in the area from which it had the smallest distance. This discrimination was then validated by assigning the single whale based on the discrimination function calculated from all the other whales, and by repeating this procedure for each whale.

Analyses of variance (ANOVA) followed by Tukey *post hoc* tests were used to test for differences in mean canonical variables between areas and sexes.

## Results

### Correlations

Correlation analyses showed a general structure in the data. Mercury (Hg) in muscle, liver and kidney was highly intercorrelated, and (weakly) with cadmium (Cd) in liver and kidney. Cd in muscle, however, was negatively correlated with Hg in all tissues and uncorrelated with Cd in other tissues. Organochlorines were positively correlated with one another, except that dieldrin was negatively correlated with mirex and with mercury in all tissues; they were negatively correlated with Cd in muscle and overall uncorrelated with Cd in other tissues. The fatty acid C16:1n-7 showed a striking negative correlation with all OCs and with mercury; C18:1n-9 showed a similar pattern but the correlations were much weaker. The other two FAs were positively correlated with OCs and metals.

### Principal component analyses

Preliminary PCAs performed separately for the different groups of compounds (metals, OCs and FAs) confirmed the relationships between the variables. For the metals, the first component (explaining 45% of the total variation) was positively correlated with all metals except for Cd in muscle, and the second component (explaining 26%) was positively correlated with Cd in liver and kidney and weakly negatively correlated with Hg in all tissues. The PCA of OCs showed that all compounds were positively correlated with the first component (explaining 60%) although dieldrin had a relatively low correlation. The second component (explaining 17%) was strongly positively correlated with trans-nonachlor and dieldrin and negatively correlated with mirex and hexachlorobenzene (HCB). The PCA of fatty acids showed that C14:1n-5, C16:1n-7 and C18:1n-9 were positively correlated and C20:1n-11 negatively correlated with the first component (explaining 49% of the total variation). The second component (explaining 28%) was mostly positively correlated with C14:1n-5 and C20:1n-11.

### Cluster analysis

The clustering of the compounds based on the correlation matrix resulted in five clusters that explained 70% of the total variance (Table 2). Cluster 1, that explained 36% of the total variance, consisted of all the

Table 2. Results of clustering various compounds in tissues of 104 minke whales that were sampled in the North Atlantic in 1998. The clustering was based on the correlation matrix (see Material and methods). For Hg and Cd, K indicates kidney, M indicates muscle and L indicates liver.

Cluster	Compound	Squared correlation with		(1-own cluster)/ (1-next cluster)	Cumulative proportion of variance explained
		Own cluster	Next cluster		
1	PCB153	0.89	0.17	0.13	0.36
	PCB138	0.70	0.26	0.41	
	PCB180	0.93	0.20	0.09	
	p,p'-DDE	0.71	0.12	0.33	
	trans-nonachlor	0.54	0.28	0.64	
	HCB	0.74	0.04	0.27	
	Mirex	0.70	0.09	0.34	
2	Hg(K)	0.79	0.23	0.27	0.48
	Hg(M)	0.84	0.16	0.19	
	Hg(L)	0.79	0.12	0.24	
	C16:1n-7	0.50	0.16	0.60	
	C20:1n11	0.35	0.04	0.68	
3	Cd(K)	0.75	0.01	0.25	0.57
	Cd(L)	0.71	0.02	0.29	
	Dieldrin	0.20	0.09	0.88	
4	C18:1n-9	0.73	0.03	0.27	0.65
	C14:1n-5	0.73	0.01	0.27	
5	Cd(M)	1.00	0.03	0.00	
Total					0.70

OCs, except dieldrin. Relatively high values of squared correlations with their own cluster together with low values of squared correlations with the next closest cluster indicated that these variables were well separated by this cluster. Low values of the indicator '1-own cluster/1-next cluster' also indicated good separation (see Table 2). PCB180 and PCB153 showed the highest separation from the other clusters while trans-nonachlor showed the lowest separation. Cluster 2, explaining 12% of the total variation, was composed of Hg in kidney, muscle and liver, and two FAs (C16:1n-7 and C20:1n-11). Hg in the three tissues showed the highest separation by this cluster ( $r^2 \geq 0.79$ ), and the two FAs the lowest ( $r^2 \leq 0.50$ ). Cluster 3 explained only 9% of the total variation and was composed of Cd in kidney and liver and the OC, dieldrin. Cadmium in kidney and liver were well separated by this cluster ( $r^2 \geq 0.71$ ), whereas dieldrin was not ( $r^2 = 0.20$ ). Cluster 4 was composed of the two other FAs (C18:1n-9 and C14:1n-5). Cluster 5 consisted only of cadmium in muscle, which was shown to be anomalous in the earlier correlation analyses. In each of the first three clusters, there were variables that fitted poorly into the cluster: trans-nonachlor in cluster 1, the two FAs in cluster 2, and dieldrin in cluster 3.

These results of the cluster analysis were consistent with the PCA of groups of variables. Thus, Cd in mus-

cle had an anomalous loading on the first PC for metals, and fell into a separate cluster, and the different loadings of Cd and Hg in the two components agreed with the separation of Hg and Cd into different clusters. The different loading of dieldrin in the PCA was also consistent with its distinct clustering.

### Canonical discriminant analyses

CDAs were performed for four areas (West Greenland, Jan Mayen, the Northeast Atlantic and the North Sea) for each sex separately. However, the discrimination ability was not higher than when the two sexes were pooled. The three canonical variables explained 61, 26 and 13%, respectively, of the variation, and all were significant at the 1% level.

The first canonical axis separated the North Sea (highest mean CAN1) from Jan Mayen and the Northeast Atlantic (in-between values of CAN1), which again were separated from West Greenland (lowest mean CAN1; Fig. 2A). The first canonical variable varied significantly between areas (ANOVA:  $F = 75.9$ ,  $P < 0.0001$ ), but the difference between the Central and the Northeast Atlantic did not make much contribution to this variation (Tukey *post hoc* test, significance level of 5%).

The fatty acids C14:1n-5, C16:1n-7 and C20:1n-11, Cd in liver and Hg in muscle, and HCB and mirex

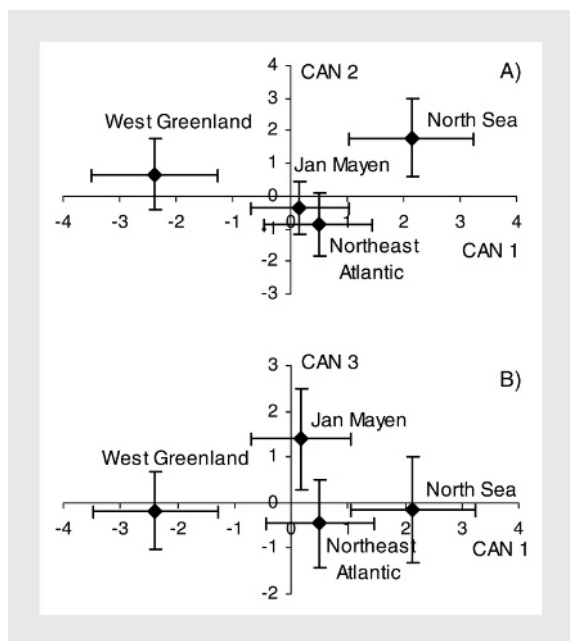


Figure 2. Mean and SD according to sampling region of the two first canonical variables (A; CAN1 and CAN2) and the first and the third canonical variables (B; CAN1 and CAN3) based on the 18 different compounds (given in Table 2) in tissues of 104 minke whales that were sampled in the North Atlantic in 1998. WG = West Greenland, CM = Jan Mayen, NE = the Northeast Atlantic (Svalbard, the Barents Sea and Vestfjorden/Lofoten), EN = the North Sea.

contributed most to the first canonical variable judged from the standardised coefficients (1.10, -0.93, -0.74, -0.83, 0.79, -0.81 and 0.70, respectively; Table 3).

These compounds were all moderately correlated with the first canonical variable ( $r$  between 0.15 and 0.57), except for C20:1n-11 ( $r = 0.06$ ); see Table 3. This means that a whale with a high value of CAN1 (North Sea) had a relatively high concentration of compounds with high positive standardised coefficients (C14:1n-5, Hg in muscle, mirex and PCB153) and a relatively low concentration of compounds with high negative standardised coefficients (C16:1n-7, C20:1n-11, Cd in liver, HCB and PCB180). A whale with a low value of CAN1 (West Greenland) had the opposite concentration pattern.

The compounds C18:1n-9, PCB180, PCB153, dieldrin and Hg in muscle contributed most to the second canonical variable (standardised coefficients of -0.98, -0.87, 0.79, 0.55 and 0.53, respectively; see Table 3). Among these compounds, C18:1n-9 showed the highest correlation coefficient ( $r = -0.80$ ). The second canonical variable separated the minke whales from Jan Mayen plus the Northeast Atlantic (lowest mean CAN2; relative high concentration of C18:1n-9 and PCB180 and relative low concentration of PCB153, dieldrin and Hg in muscle) from those from West Greenland (in between mean CAN2), which again were separated from whales from the North Sea (highest mean CAN2; see Fig. 2A). The second canonical variable varied significantly among areas except, again, between Jan Mayen and the northeastern Atlantic (ANOVA:  $F = 32.5$ ,  $P < 0.0001$ , followed by Tukey *post hoc* test, significance level of 5%).

Table 3. Correlation coefficients and standardised (mean = 0, SD = 1) canonical coefficients between canonical variables (CAN1-CAN3) and various compounds in 104 minke whales that were sampled in the North Atlantic in 1998. For Hg and Cd, K indicates kidney, M indicates muscle and L indicates liver.

Cluster	Compound	Correlation coefficients			Total standardised coefficients		
		CAN1	CAN2	CAN3	CAN1	CAN2	CAN3
1	PCB153	0.364	0.226	-0.116	0.403	0.788	-0.412
	PCB138	0.411	0.257	-0.120	0.219	-0.442	-0.412
	PCB180	0.376	0.193	-0.053	-0.611	-0.870	0.160
	p,p-DDE	0.303	0.234	0.053	-0.154	-0.063	0.692
	trans-nonachlor	0.535	0.369	0.025	0.668	-0.345	-0.109
	HCB	0.152	0.244	-0.082	-0.811	0.427	0.071
	Mirex	0.204	0.212	-0.020	0.703	0.267	-0.352
2	Hg(K)	0.416	0.419	0.568	0.027	0.190	0.901
	Hg(M)	0.569	0.354	0.526	0.793	0.531	0.295
	Hg(L)	0.329	0.300	0.470	-0.331	-0.078	-0.229
	C16:1n-7	-0.446	-0.398	-0.178	-0.929	0.095	0.295
	C20:1n-11	0.055	0.604	0.273	-0.736	0.337	0.608
3	Cd(K)	-0.191	-0.199	0.263	-0.012	-0.123	0.062
	Cd(L)	-0.390	-0.235	0.234	-0.826	-0.190	0.169
	Dieldrin	0.207	0.408	-0.240	-0.256	0.554	-0.469
4	C18:1n-9	0.264	-0.800	0.165	0.136	-0.975	0.631
	C14:1n-5	0.361	-0.270	-0.145	1.098	0.059	-0.674
5	Cd(M)	-0.279	-0.073	-0.169	-0.051	0.005	-0.249



Table 4. Results of the assignment of 104 individual minke whales to the four areas in the North Atlantic based on the three canonical variables and a cross validation of the assignment. Numbers of correctly assigned specimens are italicised. Northeast Atlantic consisted of whales from ES, EB and EC. For location of areas and explanation of acronyms see Figure 1.

Area	Assignment				Cross-validation			
	West Greenland	Jan Mayen	Northeast Atlantic	North Sea	West Greenland	Jan Mayen	Northeast Atlantic	North Sea
	WG	CM	NE	EN	WG	CM	NE	EN
WG	23		2		22	1	2	
CM		16	3		2	10	6	1
NE		6	34	4	2	8	29	5
EN			2	14		3	4	9
Total	23	22	41	18	26	22	41	15

The third canonical variable separated Jan Mayen from all the other areas (see Fig. 2B; ANOVA:  $F = 15.5$ ,  $P < 0.0001$  and Tukey *post hoc* test), including the Northeast Atlantic. Hg in kidney,  $p,p'$ -DDE, C14:1n-5, C18:1n-9 and C20:1n-11 contributed most to the third canonical variable (standardised coefficients of 0.90, 0.69, -0.67, 0.63 and 0.61, respectively). Hg in kidney had the highest correlation coefficient ( $r = 0.57$ ), whereas  $p,p'$ -DDE was only weakly correlated ( $r = 0.05$ ) with the third canonical variable.

In none of the areas did the three canonical variables (CAN 1-3) differ significantly between sexes (two-way ANOVA with the factors 'area' and 'sex' nested 'area'; CAN1:  $F = 1.18$ ,  $P = 0.33$ , CAN2:  $F = 0.65$ ,  $P = 0.63$ , CAN3:  $F = 1.53$ ,  $P = 0.19$ ).

### Assignment test

During the assignment test based on the transformations developed by the CDA, about 84% (87 of 104) of the individual whales were classified to the area where they had been caught (Table 4). The most common misassignment was between the Jan Mayen and the Northeast Atlantic areas. When the assignment of each whale was based on the discrimination function derived from the other whales, the fraction of correctly classified whales was about 67% (70 out of 104). Again the most common mis-classifications were between Jan Mayen and the Northeast Atlantic.

### Discussion

Our study hypothesised that several dietary-related compounds reflect the existence of four markedly different North Atlantic marine environments where minke whales feed during summer. The study tested a) whether variation in patterns of these compounds, that have different origin and ecological and physiological pathways, could identify different groups of

minke whales with long-term affinity to these areas, and therefore b) whether the multi-elemental approach is useful for discrimination of subpopulations or ecologically separated groups of whales.

Basically, the multi-elemental analyses supported the results of the genetic study (Andersen et al. 2003). It was therefore concluded that ecological markers can assist in the identification of subpopulations and can be particularly useful in lack of other evidence of stock separation.

However, premises for this method to be useful are: 1) within the range explored there must exist profound regional variation in the compounds studied (or the combination of compounds); 2) this variation must also be expressed in different minke whale food; and 3) be present in different tissue signatures (i.e. the signal in the whale must be retained over several years).

The spatial occurrence of Cd, Hg and OCs, and their levels and patterns, in the North Atlantic marine ecosystems result from complex processes that differ from compound to compound. In the North Atlantic, both Cd and Hg originate from long-distance transportation of anthropogenic emissions, or from natural sources influencing the 'local' environment (Dietz et al. 1998, Ford et al. 2004). Concentrations of these heavy metals in the marine biota vary on a regional scale. Hg and Cd in liver of the relatively stationary (at least in contrast to minke whales) ringed seal *Phoca hispida* showed significant regional differences among West Greenland, East Greenland, Svalbard and the White Sea. Generally, concentrations were highest in Greenland (Rig  t et al. 2005).

OCs are solely of anthropogenic origin and are mainly brought to the Arctic via long-range transportation in the atmosphere or oceans. However, in areas such as the North Sea, that are closer to urbanised areas, local sources may also be important. Differences in concentrations of PCBs, DDT and chlordane related compounds have been observed between West

Greenland, East Greenland and Svalbard in several arctic species including ringed seals and beluga *Delphinapterus leucas* (Muir et al. 2000, Cleeman et al. 2000, Andersen et al. 2001), polar bears *Ursus maritimus* (Norstrom et al. 1998) and seabirds (de Wit et al. 2004). Higher concentrations of all three OCs were generally more often found in biota from Svalbard and the eastern Barents Sea than in biota from West Greenland. This appears to reflect the influence of European and Russian sources on the Barents Sea and southern Kara Seas (de Wit et al. 2004, Norstrom et al. 1998). Higher levels of PCBs and DDT have also been found in Atlantic cod *Gadus morhua* from the North Sea than in cod from Iceland (Stange & Klungsøyer 1997). Taken together, all this information suggests that there is a gradient of PCB and persistent OCs across the North Atlantic from the North Sea to Greenland, and from the Barents Sea to Greenland, which could influence levels in minke whale tissues. Therefore, it would be reasonable to hypothesise that minke whales feeding in the eastern part of the North Atlantic minke whales summer range could differ significantly in levels and patterns of PCB congeners from those feeding in western Greenland.

Mercury and OCs are known to biomagnify, and therefore the load of these pollutants increases along the food chain (cf. AMAP 1998, Anon. 2002b). Although some studies have indicated that Cd biomagnifies (e.g. Dietz et al. 1996), there is little evidence that Cd biomagnifies when the entire food web is considered, and the study by Campbell et al. (2005) found biodilution of Cd (i.e. a decrease in concentration of an element with increasing trophic level). Minke whales in a feeding area probably act as selective (through their feeding preferences, e.g. piscivory versus carcinophagy) integrators of the occurrence of the compounds in that area.

FAs have been used as a tool to discriminate between populations of various marine mammals (reviewed in Møller et al. 2003) including minke whales (ibid., Olsen & Grahl-Nielsen 2003). FA composition in the blubber reflects not only the feeding preferences of the minke whales but also their ability to synthesise and modify FAs (Møller et al. 2003). Nevertheless, the variations in FA signatures in the outer blubber layer in minke whales from different areas of the North Atlantic are believed to reflect regional differences in types of food available to the whales (ibid.).

The regions studied differ with respect to occurrence of types of minke whale prey. Capelin *Mallotus villosus* and sand eel *Ammodytes* ssp. are important

food for minke whales in West Greenland waters, whereas polar cod *Boreogadus saida* seems to be of greater importance in the East Greenland region (reviewed by Neve 2000). During the last decade or so, Atlanto-boreal species like Atlantic cod *Gadus morhua*, saithe *Pollachius virens*, haddock *Melanogrammus aeglefinus*, herring *Clupea harengus* and mackerel *Scomber scombrus* have either not been present in Greenland waters or have occurred there in such low numbers (e.g. Anon. 2001) that they have been insignificant as minke whale food. Krill *Thysanoessa* sp. and herring are two of the most prominent prey items in the diet of minke whales in the Northeast Atlantic where gadoid fish (cod, saithe, haddock) are also important prey (reviewed by Haug et al. 2002). Within the Northeast Atlantic area there are regional differences in prey preferences. Consumption of herring is almost exclusively confined to the Barents Sea and the northwestern coast of Norway, whereas consumption of krill is more pronounced in the Svalbard area (Folkow et al. 2000, Haug et al. 2002). Herring is a predominant food item in the Norwegian Sea, whereas sand eel dominates the minke whale food in the North Sea. In this latter area, mackerel and other fish (e.g. herring) constitute the remainder of food items (Olsen & Holst 2001). Sand eel and herring are important minke whale food at Scotland (Macleod et al. 2004). It is highly likely that the prey species synthesise and accumulate the various compounds differently and therefore that regional variation in minke whale prey preferences will reflect such differences.

A preliminary exploration of the correlation structure of the selected compounds by cluster analysis separated seven out of eight OCs into one cluster (see Table 2). Highly chlorinated PCB congeners and DDE are known to be often highly correlated in marine mammals (e.g. Weisbrod et al. 2000).

Hg in muscle, liver and kidney also separated into one cluster, which was also the case with Cd (see Table 2). High inter tissue correlations of both mercury and cadmium have often been observed. In animals like minke whales that feed on both fish and crustacean, Hg and Cd concentrations may be negatively correlated (Rigét & Dietz 2000). Hg is known to be present in high concentrations in fish relative to Cd concentrations, whereas the opposite is the case in crustaceans (Dietz et al. 1996).

The variable clustering showed clear separation between OCs as a cluster, mercury in all tissues, and Cd in liver and kidney. However, the correlation analyses also showed that these groups of variables were not independent of one another: there could be iden-

tified a general 'contamination' signature containing all the OCs and mercury. Cadmium, on the other hand, was not part of this signature, and, if anything, was negatively associated with it. The first two canonical variables had generally positive correlations with the OCs and mercury, and negative correlations with cadmium in kidney (CdK) and liver (CdL), and were principally distinguished by having opposite correlations with cluster 4: C18:1n-9 and C14:1n-5. The third canonical variable was distinctive in having only weak correlations with all the OCs, but positive correlations with Hg and with CdK and CdL. Therefore, combining the signals of the compounds that have different ecological and physiological pathways into one analysis is expected to be a stronger tool for separation of groups of minke whales than using the groups of variables in isolation as has been done in Hobbs et al. (2003), Born et al. (2003) and Møller et al. (2003).

Hobbs et al. (2003) used OCs to infer stock structure of North Atlantic minke whales. They found differences among areas in concentrations of certain OCs and suggested that West and Southeast Greenland whales were distinct from whales from Jan Mayen, the Northeast Atlantic and the North Sea. However, principal component analyses (PCA) including a total of 71 PCBs and 20 OC pesticides did not reveal any distinct groupings of animals based on variation in contaminant patterns by region. Møller et al. (2003) studied the regional variation in 43 fatty acid compositions in both deep and superficial blubber. From this analysis, the existence of three regional stocks was inferred: West and East Greenland, the Northeast Atlantic (Jan Mayen, Svalbard, Barents Sea, Vestfjorden/Lofoten) and the North Sea. Using regional variation in concentrations of mercury, selenium and cadmium in various tissues, Born et al. (2003) found significant differences in at least one long-term diagnostic element between several areas. PCAs on 19 elements in baleen suggested that four groups of whales could be distinguished: West Greenland, Jan Mayen, Northeast Atlantic (Svalbard, Barents Sea, Lofoten/Vestfjorden) and the North Sea.

In contrast to the studies by Hobbs et al. (2003), Møller et al. (2003) and Born et al. (2003), our study only included substances that were thought to represent long-term deposition in tissues and hence likely to reflect long-term affinity to a particular summer feeding ground. Furthermore, our study explored the combined difference reflected in compounds of different origin.

All the canonical variables of the CDA reflected complex combined patterns of the three groups of compounds involved, and each canonical variable included substances of importance from different groups. However, while the substances within each group were correlated, the correlations were not perfect, and so the canonical variable had different loadings on the different members of each group. The ecological or physiological interpretation of the specific composition of the canonical variables is very difficult because of the highly different nature and pathways of the compounds involved. We are not able to offer any satisfactory physiological or ecological interpretation of the results of the CDAs.

Different OCs and heavy metals had different loadings, and therefore the differences detected did not reflect a simple picture of regional variation in pollution. The first two canonical variables differed in having opposite correlations with cluster 4 fatty acid variables, so there seemed to be some sort of food level separation involved.

The use of multi-elements is valuable, because each group of variables tends to be correlated, but we see, for example, that both the first and the second canonical variable reflected a possible 'contamination' signature in the same way, but perhaps differed on the fatty-acid signature, while the third canonical variable revealed differences in metal signatures.

The ability of the canonical variables to discriminate among the whales from the four areas where they were caught was relatively good (84% correctly assigned). However, cross validation of the discrimination success rate by analysing the sensitivity of each whale to the discrimination reduced the success rate to about 68%. To some extent, this reflected the sensitivity of the test to small sample size.

A canonical discrimination procedure on OC concentrations has been used to separate 'stocks' of beluga whales in eastern Canada and West Greenland with a success rate of 93% (cross validation success rate of 89%; Innes et al. 2002). However, Innes et al. (2002) included a total of 49 OC congeners in their analyses and did not specifically select those that are likely to represent long-term deposition, and therefore long-term affinity to a certain area. Hence, the classification in Innes et al. (2002) of belugas to an area of catch would inevitably have a higher precision, but the groups or 'stocks' identified by including also short-term, dietary-related OC congeners may more arbitrarily reflect a local and short-term signal and not necessarily stable subpopulations.

In our study, the most common mis-classifications were of whales from the Jan Mayen area to the north-east Atlantic, and *vice versa*, which is consistent with generally poor discrimination of these two groups in the CDA. This may have been caused by several factors: 1) that the Northeast Atlantic represented a mixture of whales from Svalbard, the Barents Sea and Vestfjorden/Lofoten, or 2) that Jan Mayen and Northeast Atlantic whales belong to the same group of whales. The study by Andersen et al. (2003) indicated that whales from Jan Mayen (and East Greenland) were genetically distinct from those in the Northeast Atlantic region (Svalbard, the Barents Sea, Vestfjorden/Lofoten). However, when analysed separately, whales from Jan Mayen, Vestfjorden/Lofoten, Svalbard, the Barents Sea and the North Sea did not differ significantly at the mtDNA level, whereas at the nuclear DNA level (microsatellites), whales from Jan Mayen differed from those sampled at Svalbard (Andersen et al. 2003). The OC levels in whales from Jan Mayen did not differ significantly from those in whales from Svalbard, the Barents Sea and Vestfjorden/Lofoten. Furthermore, FA signatures did not differ among Jan Mayen, Svalbard, Barents Sea and Vestfjorden/Lofoten (Møller et al. 2003). Hence, also when analysed separately, the dietary-related compounds included in our study did not mark a clear distinction between Jan Mayen and Northeast Atlantic minke whales. This lack of a clear distinction, and a small sample size, likely explain the relatively high mis-classification rate found between these two areas in our study.

Based on genetic analyses and analyses of stock boundaries using the Boundary Rank Method, the IWC working group on North Atlantic minke whales concluded in 2004 that: 1) genetic studies have confirmed a distinction between the Central and Northeast Atlantic, and 2) that there is little or no evidence for distinction between whales from the Vestfjorden/Lofoten area and from the waters surrounding it (Anon. 2004). These conclusions are not contradictory to the findings in our study. However, there were indications of a further subdivision of the group of minke whales in the Barents Sea (Anon. 2004).

Several studies have shown differences in the concentrations of OCs related to sex in minke whales (Kleivane & Skaare 1998, Hobbs et al. 2003), and in other baleen whales (Aguilar & Borrell 1998). Sex differences were therefore expected to influence the canonical discriminant analysis. However, this was not the case, probably because the canonical discriminant

analysis is more sensitive to changes in ratios than to change in levels in terms of concentration.

Various elements deposited in baleen (Born et al. 2003) and  $^{137}\text{Cs}$  in muscle (Born et al. 2002) could have been included in the analyses because they represented a relatively long-time dietary response. However, by also including these elements, the number of whales available for the analysis would have been too small. The reason for this is that the basic criterion was that all 14 compounds should have been analysed in each individual whale.

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