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Patterns of organic contaminants in marine mammals with reference to sperm whale strandings

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Abstract

Discriminant analysis has been applied to organochlorine contaminant data from a small number (*ca* 3-25) of 12 different marine mammal species to discriminate between the species on the basis of the chlorobiphenyls (CB) patterns in blubber and account for the effects of age, sex, condition and location of the mammals. The raw data are normalised to a single congener, CB 153, to reduce the effect of life history and sex, after which the discriminant factors are plotted to display the differences between species in relation to the intake and the metabolism of chlorobiphenyls. An understanding of these differences gives a better knowledge of the relative sensitivity of these species. The sperm whales were found to have the least ability to metabolise CBs when compared with other cetaceans, although the concentration range observed for ΣCB was relatively low (265-6,313 μg/kg lipid weight).

Keywords: sperm whale, strandings, marine mammals, organochlorines, contaminant, discriminant analysis.

Résumé

La technique d'analyse discriminante a été appliquée à des données de contaminants organoghlorés provenant d'un petit nombre (environ 3-25) d'individus de 12 espèces différentes de mammifères marins pour établir des distinctions entre espèces sur base du tableau que présentent les biphenyls chlorés (CBs) dans le lard et pour rendre compte des effets de l'âge, du sexe, de la forme physique et de l'origine de ces mammifères. Les données brutes sont normalisées par rapport à un congénère unique, CB 153, pour réduire l'effet du sexe et de l'histoire personnelle des individus, après quoi les facteurs discriminants sont mis en graphique pour mettre en évidence les différences entre espèces liées à l'absorption et au métabolisme des CBs. Une compréhension de ces différences permet de mieux cerner la sensibilité relative de ces espèces. Par rapport aux autres cétacés, le cachalot apparait comme le moins apte à métaboliser les CBs, bien que l'éventail des concentrations en ΣCB observées soit relativement faible (265-6.313 μg/kg poids en lipides).

Mots-clés: cachalot, échouage, mammifères marins, organochlorés, contaminant, analyse discriminante.

Introduction

Chlorinated biphenyls (CBs) are ubiquitous, lipophilic environmental contaminants which bioaccumulate through the food chain resulting in high concentrations in top predators which can pass across generations to progeny (ERICKSON, 1986; ADDISON and BRODIE, 1987; TANABE *et al.*, 1987; TANABE *et al.*, 1988; TANABE *et al.*, 1992; CREASER *et al.*, 1992; WELLS, 1993; BORRELL *et al.*, 1995). Elevated CB concentrations have been linked to mass mortalities of marine mammals worldwide and, at high concentrations, CBs in cetaceans and pinnipeds have been reported to affect reproduction and immunity (DELONG, 1973; REIJNDERS,1994; ROSS *et al.*, 1996).

The principal toxicity resulting from experimental exposure to CBs has been associated with congeners possessing a planar-like structure. The mono- and non-ortho chlorinated CBs (CBs 105, 118, 156 and CBs 77, 126 and 169 respectively) have a more planar structure and produce toxic effects similar to those of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (SAFE *et al.*, 1989; DE VOOGT, 1990). The concern over the toxicity of these CBs is primarily because they occur in marine mammal tissue at much higher concentrations than TCDD.

CB patterns in marine mammals can differ markedly from those in their prey (TANABE *et al.*, 1988). MUIR *et al.* (1988) showed that large differences in CB patterns were apparent through the food chain of arctic animals owing to the different ability of each animal in the chain (cod ringed seal polar bear) to metabolise individual CB congeners. BOON *et al.* (1994) have devised a pharmokinetic model to investigate the relative metabolic degradation of CBs in marine mammals. Similar comparisons had been made earlier by WELLS and ECHARRI (1992), who found that the relative extent of the metabolism of a number of CB congeners varied with species (grey seal >> harbour porpoise $\simeq$ bottlenose dolphin >> sperm whale), indicating that in different species there are considerable differences in the activities of xenobiotic metabolising enzymes.

Differentiation between metabolic patterns of CBs in animals at a similar trophic level, however, requires careful analysis of many factors. Differences in diet of marine mammals, body condition, age, sex and possibly habitat (geographic location) resulting from different sources of the original contaminant mixture must be taken into consideration (ELSKUS *et al.*, 1994; STORR-HANSEN *et al.*, 1995). Data reduction is essential to identify patterns in the data more readily and to provide a descriptive overview.

Principal Components Analysis (PCA) is normally

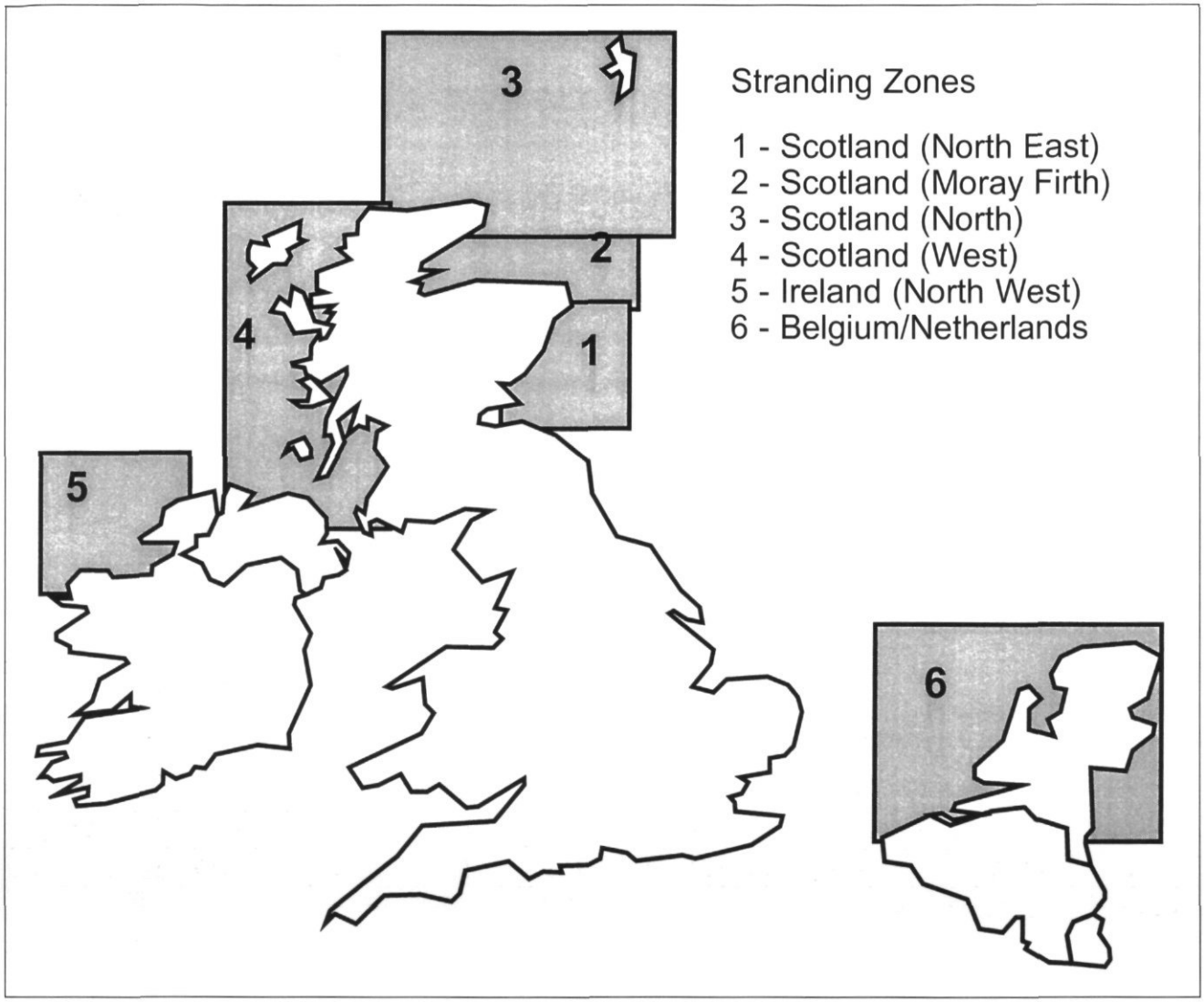

Fig. 1 – Distribution of selected marine mammal strandings around the Scottish and northern European coast (1990-1995).

used to reduce the variability in a data set to a few components and has been used to identify differences between three harbour seal populations (STORR-HANSEN and SPLIID, 1993). A complementary technique to PCA is discriminant analysis (DA), which provides a series of discriminant factors that maximise the separation between similar populations. Once a DA model has been established it is possible to identify the origin of individual unknown samples. Both of these techniques are used in the present work to elucidate differences in CB patterns of sperm whales and other marine mammals and study the within sperm whale variance.

Materials and Methods

Marine mammals are regularly stranded along the Scottish coast and, since 1990, these animals have been sampled as part of the UK national survey. Sperm whales (*Physeter macrocephalus*) were stranded along the Orkney coastline in December 1994, the Belgian coast in November 1994, and the Netherlands coast in January 1995. Other single sperm whale strandings in Scotland occurred in November 1993 on Skye and Loch Ailort (west coast) and Nairn (east coast) in March 1995. Chlorobiphenyl (CB) residues have been measured in blubber to determine the concentration of toxic contaminants, as part of the information base on stranded animals.

Samples

Dorsal blubber samples of marine mammals stranded on the Scottish coast were obtained in cooperation with the Veterinary Centre at the Scottish Agricultural College, Inverness. Frozen blubber samples from sperm whales that stranded in the Netherlands in 1994/95 were obtained from Dr. J. P. BOON, Netherlands Institute for Sea Research (NIOZ), Texel; samples from the sperm whales that stranded in Belgium were obtained from P. ROOSE (Rijkstation voor Zeevisserij, Oostende). With the exception of the Belgian strandings complete blubber cores,

Table 1 – Sperm whale strandings 1993-1995

Lab code (PCA)	Lab code (DA)	Date	Location	Age (years)	Stranding zone	Sex	Length (cm)	%Extractable lipid
O1	PM1	11 12 94	Orkney	22	3	M	1,230	45
O2	PM2	11 12 94	Orkney	25	3	M	1,240	45
O3	PM3	11 12 94	Orkney	22	3	M	1,280	36
O4	–	11 12 94	Orkney	27	3	M	1,320	31
O5	–	11 12 94	Orkney	24	3	M	1,260	39
O6	PM4	11 12 94	Orkney	25	3	M	1,340	40
O7	PM5	11 12 94	Orkney	21	3	M	1,240	31
O8	PM6	11 12 94	Orkney	21	3	M	1,200	34
O9	–	11 12 94	Orkney	23	3	M	1,280	*46
O10	PM7	11 12 94	Orkney	23	3	M	1,330	49
O11	PM8	11 12 94	Orkney	22	3	M	1,250	46
S1	PM12	21 11 93	Loch Airlort	>40	4	M	1,500	47
S2	PM13	24 11 93	Skye	>40	4	M	1,565	45
S3	PM18	23 03 95	Nairn	>21	2	M	1,370	*12.5
N1	PM15	14 01 95	The Hague	15-25	6	M	1,450	53
N2	PM16	13 01 95	The Hague	15-25	6	M	1,450	31
N3	PM17	14 01 95	The Hague	15-25	6	M	1,450	25
B4	PM19	18 11 94	Koksijde (B)	20-30	6	M	1,800	36
B3	PM20	18 11 94	Koksijde (B)	20-30	6	M	1,440	8
B2	PM21	18 11 94	Koksijde (B)	20-30	6	M	1,480	24
B1	PM22	18 11 94	Koksijde (B)	20-30	6	M	1,540	38

* The mean of two duplicate lipid determinations

from the skin to the muscle layer were taken. In the case of the Belgian samples < 2 g was obtained. Blubber samples were wrapped in hexane-washed aluminium foil and stored at -20° C prior to analysis (WELLS *et al.*, 1994).

Chemical Analysis

Chemical analysis used standard preparative and clean-up procedures for animal tissues. Briefly, blubber was extracted by Soxhlet using methyl tertiary butyl ether (MTBE). The lipid content of the extract was determined gravimetrically and an aliquot containing 200 mg lipid was cleaned up using alumina and silica columns. Non-ortho chlorinated CBs were separated using pyrenyl-silica high performance liquid chromatography at 0° C. Final determination was by gas chromatography with electron capture detection (Varian 3500) (WELLS *et al.*, 1994; WELLS *et al.*, 1995).

Results

The animals for this study were selected from a larger data set (WELLS *et al.*, 1994; MCKENZIE *et al.*, in press). This selection was based upon the availability of a complete set of data for all selected CBs for each blubber sample. Table 1 gives the geographic location of each stranding and the date of tissue sampling for the sperm whales, together with the sex, age and length, and the percentage of extractable lipids determined in the blubber. Stranding zones have been identified and are illustrated in Figure 1. A summary of the CB data (normalised to CB 153) is for the 12 species presented in Table 2. For the sperm whales, the concentration range on a lipid weight basis for monitoring seven CBs, total CB, total DDT (o,p'-DDE, + o,p'-DDD + o,p'-DDT + p,p'-DDE + p,p'-DDD + p,p'-DDT), hexachlorobenzene (HCB) and dieldrin, as well as non-ortho chlorinated CBs, are presented in detail in Table 3.

Blubber samples from the Belgian sperm whales (B1-B4) have recently been analysed by other researchers. Dr. J. BOON, NIOZ, Texel gave significantly different results for three of the samples B1, B2 and B3 (J. BOON, pers. comm.). As well as differences in CB concentrations, large differences in extractable lipid contents were measured. No major analytical differences have been observed between the two laboratories previously (BOON *et al.*, in press). The differences observed in these samples

Table 2 – Summary of CB data normalised to CB 153

Species		CB 28	CB 52	CB 44	CB 70	CB 101	CB 149	CB 118	CB 105	CB 138	CB 158	CB 128	CB 156	CB 180	CB 170	CB 194	CB 77	CB 126	CB 169
PP	Median	0.019	0.209	0.013	0.003	0.175	0.397	0.176	0.056	0.788	0.035	0.082	0.024	0.194	0.082	0.034	0.0002	0.0001	0.0002
	Max	0.054	0.324	0.055	0.030	0.386	1.153	0.407	0.108	1.091	0.053	0.120	0.045	0.327	0.135	0.067	0.0083	0.0018	0.0024
	Min	0.002	0.092	0.003	0.001	0.034	0.278	0.054	0.012	0.665	0.020	0.035	0.005	0.112	0.046	0.011	0.0000	0.0000	0.0000
HG	Median	0.007	0.061	0.008	0.006	0.165	0.096	0.031	0.034	0.715	0.033	0.051	0.020	0.267	0.124	0.052	0.0001	0.0001	0.0001
	Max	0.018	0.097	0.009	0.016	0.266	0.135	0.046	0.036	0.819	0.038	0.062	0.028	0.486	0.226	0.111	0.0003	0.0002	0.0004
	Min	0.003	0.019	0.003	0.002	0.055	0.067	0.011	0.002	0.631	0.024	0.030	0.012	0.146	0.069	0.014	0.0000	0.0000	0.0000
SC	Median	0.042	0.154	0.035	0.017	0.299	0.459	0.309	0.125	0.912	0.041	0.154	0.044	0.474	0.197	0.108	0.0002	0.0000	0.0004
	Max	0.076	0.183	0.051	0.035	0.399	0.536	0.374	0.152	0.979	0.047	0.197	0.053	0.571	0.239	0.181	0.0020	0.0009	0.0037
	Min	0.022	0.134	0.013	0.003	0.173	0.416	0.227	0.077	0.769	0.035	0.056	0.031	0.400	0.165	0.073	0.0000	0.0000	0.0002
LAC	Median	0.013	0.154	0.010	0.002	0.202	0.445	0.177	0.092	0.912	0.032	0.106	0.025	0.287	0.127	0.047	0.0009	0.0001	0.0001
	Max	0.036	0.340	0.064	0.009	0.511	0.532	0.424	0.133	1.004	0.051	0.171	0.040	0.393	0.167	0.055	0.0002	0.0002	0.0004
	Min	0.005	0.078	0.003	0.001	0.087	0.407	0.064	0.005	0.747	0.027	0.083	0.017	0.264	0.119	0.026	0.0000	0.0000	0.0002
LAL	Median	0.007	0.167	0.014	0.006	0.251	0.346	0.222	0.082	0.885	0.043	0.118	0.033	0.288	0.118	0.049	0.0003	0.0001	0.0003
	Max	0.044	0.206	0.059	0.011	0.392	0.456	0.442	0.157	1.014	0.114	0.153	0.057	0.480	0.195	0.194	0.0018	0.0009	0.0010
	Min	0.002	0.087	0.006	0.001	0.125	0.297	0.131	0.058	0.863	0.031	0.104	0.014	0.274	0.063	0.037	0.0000	0.0000	0.0000
GG	Median	0.063	0.080	0.018	0.042	0.213	.288	0.292	0.081	0.773	0.048	0.100	0.051	0.482	0.191	0.313	0.0040	0.0017	0.0013
	Max	0.073	0.102	0.038	0.047	0.240	0.295	0.307	0.112	0.863	0.063	0.124	0.056	0.688	0.200	0.334	0.0056	0.0023	0.0015
	Min	0.035	0.075	0.015	0.038	0.187	0.220	0.250	0.075	0.764	0.034	0.086	0.050	0.379	0.178	0.073	0.0005	0.0002	0.0006
PM	Median	0.021	0.271	0.066	0.063	0.476	0.472	0.474	0.135	0.883	0.047	0.099	0.057	0.414	0.171	0.087	0.0006	0.0010	0.0006
	Max	0.083	0.621	0.157	0.240	0.705	0.520	0.541	0.166	0.977	0.057	0.128	0.073	0.452	0.195	0.109	0.0107	0.0023	0.0029
	Min	0.007	0.090	0.015	0.019	0.102	0.309	0.092	0.031	0.756	0.021	0.032	0.011	0.360	0.115	0.022	0.0001	0.0003	0.0001
TT	Median	0.010	0.135	0.023	0.003	0.242	0.378	0.287	0.090	0.887	0.057	0.129	0.039	0.359	0.134	0.055	0.0002	0.0002	0.0002
	Max	0.044	0.206	0.059	0.011	0.392	0.456	0.442	0.157	1.014	0.114	0.153	0.057	0.480	0.195	0.194	0.0017	0.0009	0.0005
	Min	0.002	0.087	0.006	0.001	0.125	0.297	0.131	0.058	0.863	0.031	0.104	0.014	0.274	0.063	0.037	0.0000	0.0001	0.0001
MB	Median	0.022	0.136	0.037	0.006	0.294	0.379	0.308	0.081	0.818	0.039	0.093	0.062	0.632	0.233	0.225	0.0002	0.0005	0.0002
	Max	0.025	0.148	0.043	0.009	0.320	0.433	0.344	0.104	0.853	0.042	0.100	0.068	0.668	0.241	0.260	0.0004	0.0006	0.0003
	Min	0.020	0.099	0.018	0.002	0.169	0.333	0.272	0.072	0.760	0.036	0.064	0.049	0.442	0.179	0.090	0.0001	0.0003	0.0002
GM	Median	0.012	0.187	0.019	0.007	0.322	0.443	0.358	0.106	0.908	0.045	0.097	0.042	0.365	0.152	0.069	0.0003	0.0001	0.0005
	Max	0.017	0.207	0.020	0.008	0.347	0.449	0.368	0.113	0.931	0.046	0.110	0.043	0.418	0.162	0.085	0.0004	0.0002	0.0006
	Min	0.005	0.156	0.016	0.006	0.307	0.423	0.344	0.104	0.879	0.041	0.093	0.038	0.335	0.139	0.060	0.0002	0.0001	0.0004
PV	Median	0.018	0.147	0.016	0.022	0.150	0.115	0.060	0.025	0.721	0.019	0.068	0.019	0.295	0.130	0.050	0.0001	0.0001	0.0000
	Max	0.040	0.245	0.047	0.051	0.217	0.227	0.114	0.041	0.784	0.027	0.081	0.027	0.355	0.156	0.092	0.0033	0.0013	0.0002
	Min	0.006	0.061	0.009	0.010	0.121	0.105	0.052	0.019	0.784	0.016	0.079	0.001	0.209	0.087	0.050	0.0001	0.0000	0.0000
OO	Median	0.012	0.262	0.017	0.006	0.271	0.473	0.236	0.058	0.968	0.045	0.090	0.028	0.443	0.149	0.082	0.0002	0.0000	0.0001
	Max	0.017	0.189	0.011	0.006	0.222	0.450	0.183	0.041	0.909	0.038	0.070	0.036	0.513	0.146	0.085	0.0003	0.0000	0.0002
	Min	0.008	0.189	0.011	0.006	0.222	0.450	0.183	0.041	0.909	0.038	0.070	0.020	0.374	0.146	0.078	0.0001	0.0000	0.0001

* Key to Table 2:

Species	Code	Species	Code	Species	Code	Species	Code
Grey seal	HG_x	Bottlenosed dolphin	TT_x	White beaked dolphin	LAL_x	Long-finned pilot whale	GM_x
Common seal	PV_x	Striped dolphin	SC_x	White sided dolphin	LAC_x	Sowerby's beaked whale	MB_x
Harbour porpoise	PP_x	Risso's dolphin	GG_x	Sperm whale	PM_x	Killer whale	OO_x

Table 3 – Sperm whale strandings (μg/kg lipid weight)

	N		Monitoring "7" CBs	Total CB	Total DDT	HCB	Dieldrin	CB77	CB 126	CB 169
Orkney (O) strandings	11	Median	1,051	1,399	2,729	101	199	0.23	0.51	0.31
		Min	265	308	1,184	33	34	0.16	0.24	0.09
		Max	6,313	9,332	15,501	413	140	0.70	1.03	0.77
Scottish (S) strandings	3	Median	5,307	7,917	11,483	472	385	1.05	1.26	0.66
		Min	3,864	5,427	11,376	357	379	0.27	0.54	0.28
		Max	5,566	8,388	11,590	559	389	2.84	2.35	0.79
Belgian (B) strandings	4	Median	4,599	6,411	6,906	341	311	8.91	2.07	2.07
		Min	3,037	4,400	5,301	248	171	2.37	0.42	0.37
		Max	16,375	21,217	12,660	416	1,538	14.37	4.45	2.83
Dutch (N) strandings	3	Median	4,788	6,547	N/A	519	N/A	0.16	4.39	0.68
		Min	2,776	3,911	N/A	291	N/A	0.11	0.97	0.48
		Max	4,925	7,011	N/A	534	N/A	0.24	1.85	0.72

Monitoring seven CBs - sum (CBs 28, 52, 101, 118, 138 (+163), 153, 180)
Total CB - sum (CBs 28, 31, 44, 52, 70, 101, 105, 118, 128, 138 (+163), 149, 153, 156, 158, 170, 180, 189, 194, 209)
Total DDT - sum (o,p'-DDE, o,p'-DDD, o,p'-DDT, p,p'-DDE, p,p'-DDD, p,p'-DDT)

are most likely to have been caused by sampling error as very small samples, comprising incomplete cores, were provided for each laboratory and differences may have been exacerbated by the condition of the animals at the time of sampling. For this reason the Belgian strandings were removed from the subsequent within-species analysis.

Discussion

Following the phocine distemper virus epizootic in the North Sea in 1988 and 1989, the UK began a national survey to monitor stranded marine mammals to establish the causes of death and to identify any contributory factors. A small number of each species have been sampled. Although the number of observations for many of the species was relatively small, this study offers one of the more comprehensive sets of data for a wide variety of organochlorine contaminants in marine mammals in Europe. The within and between species variability of contaminant concentrations results from the history and the varying metabolic capacity of the individual animals sampled. The main factors which account for this variability are: age, sex, condition of the animal, reproductive status, habitat, food source, and physiological differences between species (TANABE *et al.*, 1987; WELLS *et al.*, 1994). In view of the restricted numbers of observations for each species it was necessary to devise an approach that kept account of these variables whilst clarifying any underlying CB patterns that might indicate a toxic effect. This was achieved by normalising the CB data using CB 153 as a reference compound. CB 153 is unmetabolise-able and present in most marine mammals at a relatively high concentration (TANABE *et al.*, 1988; BOON *et al.*, 1992; WELLS and ECHARRI, 1992).

Normalising all congeners to CB 153 accounts for most of the within-species variance associated with the life history and so allows a more critical evaluation of the between species differences. In general the normalisation procedure used decreases the within-species variance, but occasionally this may not be the case. Increased variance may arise from differences in CB patterns between males and females due to reproductive transfer mechanisms, the presence of a concentration-dependent metabolism of the xenobiotic, and differences in the condition of the animals.

Within-Species Variation

Concentration dependence of metabolism

A relationship between the induction of the P450 1A isoenzymes in marine mammal species and the total CB concentration was identified in previous studies. It was inferred that higher concentrations of total CBs resulted in greater enzyme induction (TANABE and TATSUKAWA, 1991; CORSOLINI *et al.*, 1995). Here a larger data set from harbour porpoises, sampled from 1988-1992, was examined to investigate the dependence of the relative ratios of CBs on the concentration of CB 153 (WELLS *et al.*, 1994; MCKENZIE *et al.*, in press). We found that the ratio of the concentration of two hexochlorobiphenyls (CBs 138 and 153), resistant to metabolism in the harbour porpoise, remained constant with increasing concentration of CB 153. However, the ratios are *not* constant for those CBs that can be metabolised by harbour porpoise. Congeners with vicinal hydrogen atoms in the ortho- and meta-positions of at least one phenyl ring in combination with a chlorine atom in the ortho position are expected to be metabolised by P450 1A1 as a result of induced isoenzyme activity. Congeners with this molecular structure include CBs 28, 70, 118, 105 and 156. The non-ortho substituted congeners, CBs 77, 126 and 169, are pure 3-MC-type inducers and by contrast induce the P450 1A2

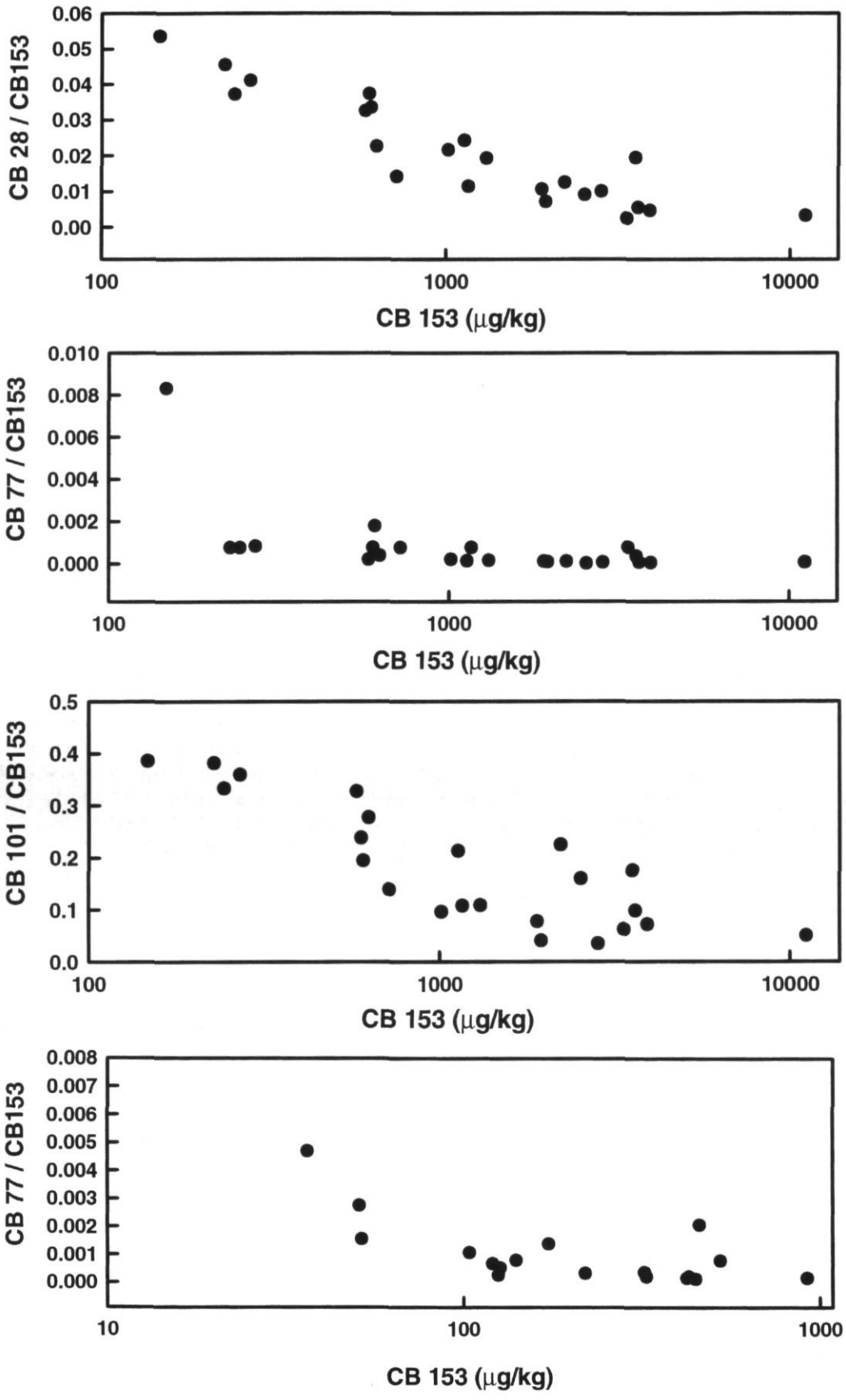

Fig. 2 – a) Relationship of CB 28/CB 153 with absolute CB concentration (harbour porpoise). b) Relationship of CB 77/CB 153 with absolute CB concentration (harbour porpoise). c) Relationship of CB 101/CB 153 with absolute CB concentration (harbour porpoise). d) Relationship of CB 77/CB 153 with absolute CB concentration (sperm whales).

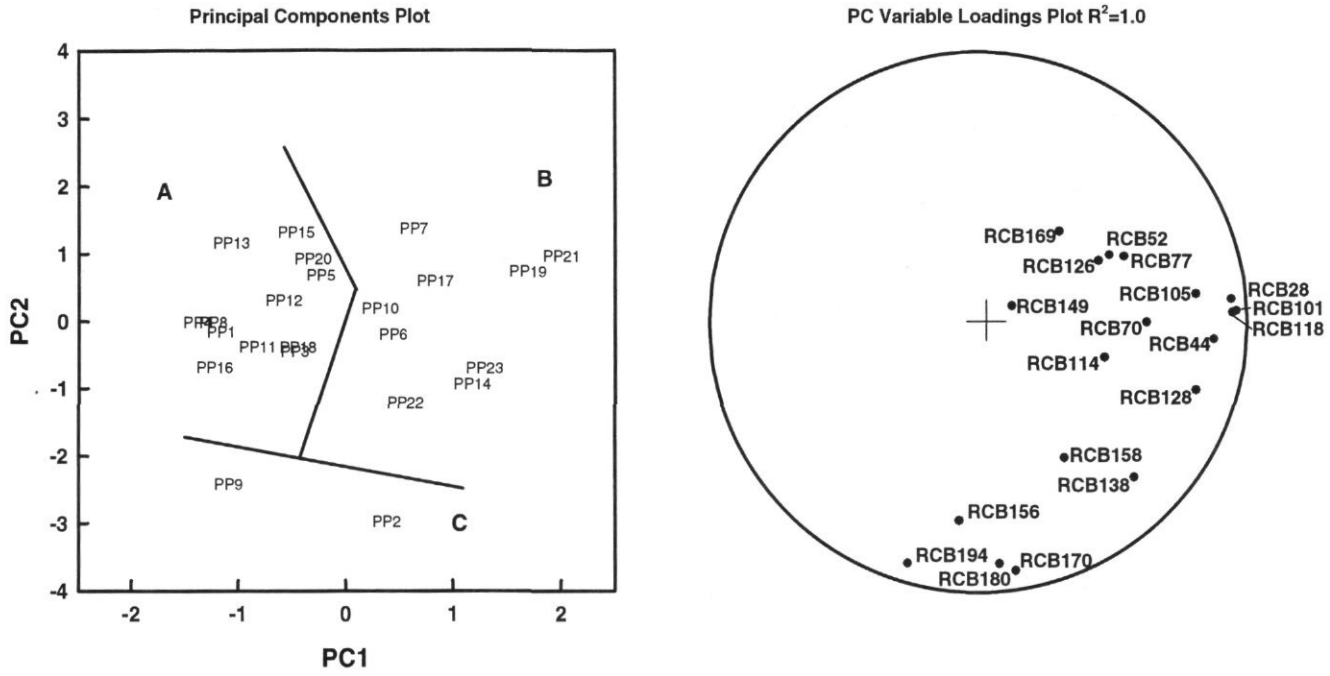

Fig. 3 – Biplot of the first and second principal components of the normalised CB harbour porpoise data set.

isoenzymes. The inverse relationship found between the relative concentrations of CB 28 and the absolute concentration of CB 153 is depicted in Figure 2a. This relationship may reflect a concentration-dependent induction of an enzyme system. There is little evidence for this relationship for the non-ortho CBs, although the animal with the highest CB 77 ratio has the lowest contaminant burden. If this animal is removed there is no relationship (Fig. 2b)

Other P450 subfamilies are also inducible. Two CBs, CB 44 and CB 101 which have vicinal hydrogen atoms in the meta- and para- positions only, are only metabolised by P450 2B isoenzymes. Both CBs follow the same decrease in the relative concentration as the absolute amount of the CB 153 increases (Fig. 2c). There is no clear evidence that harbour porpoises as yet possess this limited P450 2B enzyme system but other workers have observed lower ratios of these congeners (Boon *et al.*, 1994; Bruhn *et al.*, 1995). Of all the cetaceans only Beluga whales have been proved capable of metabolising CBs with m-, p-vicinal H atoms (White *et al.*, 1994). Even so, for the sperm whales, no apparent concentration dependence of metabolism was observed for P450 1A1 inducers. A possible linear relationship of high variance, however, was found for the sperm whales in relation to the non-ortho CBs (Fig. 2d) but this was highly influenced by the higher non-ortho CB ratios observed in three of the Orkney strandings which had lower CB burdens.

The hypothesis that the pattern of metabolisable congeners is dependent on the absolute body burden of CBs was tested using PCA on the normalised harbour porpoise data (Fig. 3). The biplot shows a cluster of vectors for CBs 28, 44, 70, 77, 105 and 118, where the variance is explained by the first principal component. A second cluster of vectors occurs for CBs 156, 170, 180 and 194. With the exception of CB 156, all the CBs represented by vectors in the top half at the biplot are the persistent congeners. There are three main groups of animals in the plane of the first two principal components:

- animals in Group A have a significantly (f test) higher absolute CB concentrations than Group B;
- the relative concentrations of the CBs which have the structural requirements for metabolism are lower in group A than in group B;
- the two harbour porpoises in group C (PP2 and PP9) are the only samples originating from the west coast of Scotland (south). The most likely cause for a difference in the ratio of persistent CBs is a different diet (Storr-Hansen *et al.*, 1993).

These findings are consistent with the hypothesis that there may be a concentration dependency in the metabolism of CBs.

PCA was also used to investigate the CB 153 normalised sperm whale data (Fig. 4). All the animals with negative scores in the first principal component were found to have relatively low absolute burdens of total CB (< 1,000 μg/kg, wet weight) compared to the other sperm whales. These sperm whales had higher relative amounts of the non-ortho chlorinated CB's (CB 77, 126, 169) as observed previously. Animals O10 and O8 had higher relative concentrations of CBs 28, 44 and 105, the prior CBs being metabolisable by the P450 1A1 isoenzyme system.

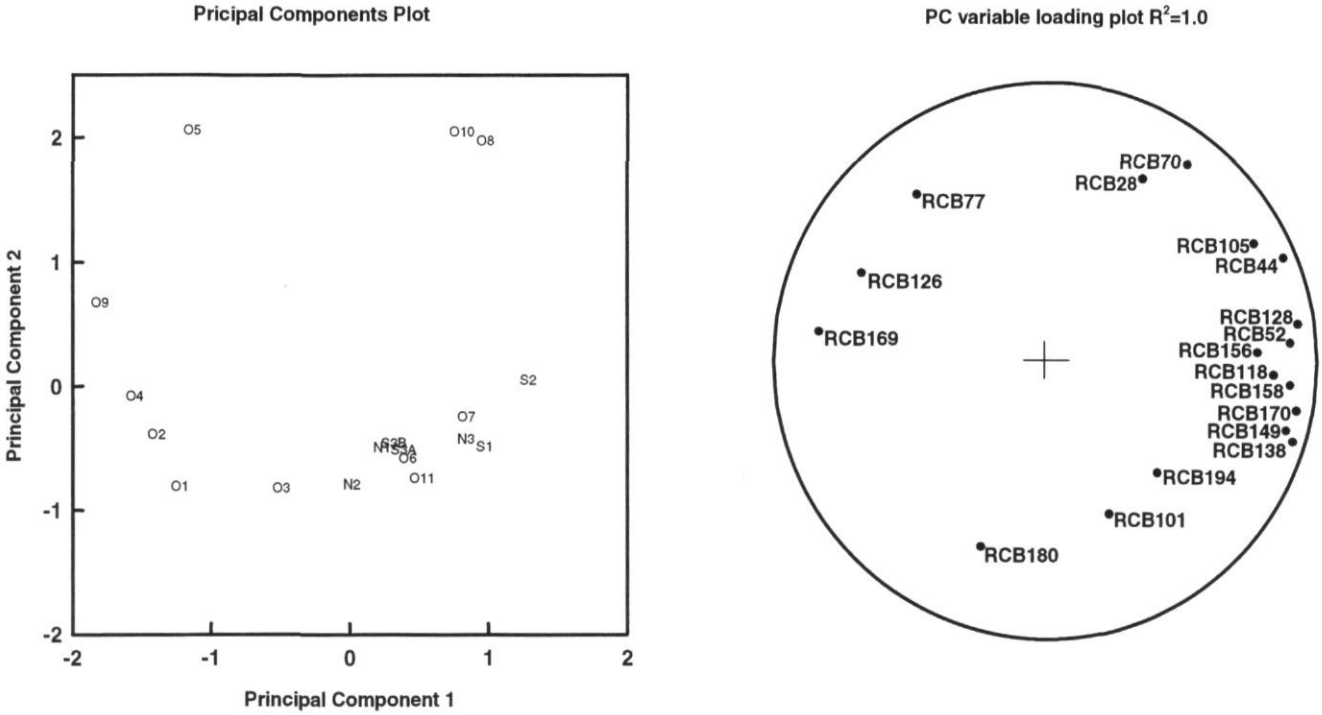

Fig. 4 – Biplot of the first and second principal components of the normalised CB sperm whale data set.

Most of the within-species variance in CB patterns can, therefore, be accounted for by concentration-dependent metabolism, with possible species-specific variability. The separation of the sperm whales using PCA does not appear to be linked to the location of strandings, as the Orkney sperm whales (Ox) are separated into two groups, one with low total CB burdens and the other with higher CB burdens (Fig. 4).

Animal condition

The physical condition of the animals prior to stranding can have a strong influence both on the absolute concentration and relative values of metaboliseable CBs. If an animal were unable to feed prior to death, fat stores would be mobilised and the blubber thickness would decrease (WELLS *et al.*, 1994). Reduced blubber thickness correlates with increased absolute concentrations of persistent CB congeners whilst the CB 153 ratios of metabolisable CBs are lower in starved animals than in healthy animals (BOON *et al.* 1994).

An example of these effects can be seen in the harbour porpoise PP15 from the east coast of Scotland (Fig. 3) (Group A). The lipid content in the blubber was only 36% compared to the more normal range of 65-85%. Although the concentrations of metabolisable CBs are low, starvation did not cause the animal to have a CB pattern different from the rest of the group *as a whole*. However, within the group the pattern changed from that of a less polluted animal into one with a higher burden, despite there being little difference in the total CB burden expressed on a lipid weight basis. Starvation effectively ''ages'' the CB pattern in the animal, mimicking the mode of accumulation of CBs over time in such predators. It is not possible to suggest whether an ''ageing'' effect occurs in sperm whales as seen with the harbour porpoise, as the age range of the sperm whale samples is very limited and preliminary investigation has failed to show a significant relationship between CB concentrations and length.

Sample S3 from Nairn and O9 from Orkney were analysed in duplicate and were consistently found to have different levels of extractable lipid (14 and 11% (S3) and 40% and 54% (O9)). Lipid determination in the blubber would appear either to have a much higher variance than the CB determination or more likely there is a degree of heterogeneity in the samples, which is observed even when complete blubber cores are analysed from the same sampling site on the animal.

Between Species Variation

Visual inspection of the normalised data show that there are observable differences for some CBs between marine mammal species. For example, there is a clear distinction between grey seals and sperm whales on the basis of a CB 149 (Fig. 5). Separation between two species so different could be predicted, but difficulty arises when trying to identify small differences between conspecifics.

Discriminant analysis (DA) assists in visualising differences between species in their relative congener patterns. The DA factors for the data set are spread over M-1 functions, where M is the number of species, in a way that maximises separation between the proposed

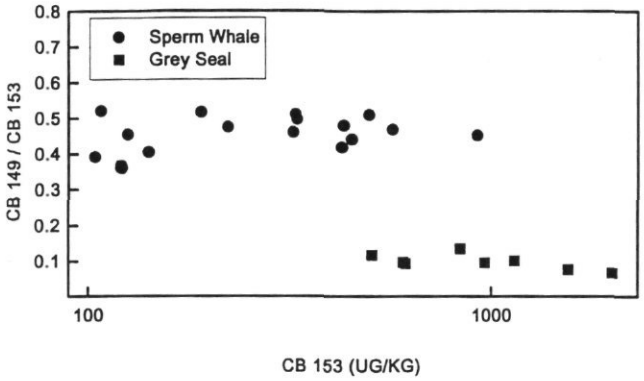

Fig. 5 – Separation of grey seals and sperm whales using CB 149.

grouping variables (species). The biplots are initially viewed in 12-dimensional multivariate space, which maximises the likelihood of discernable patterns. Some species may not appear to be separated from each other in the two dimensional biplots (e.g. Fig. 6), but are actually well separated in 12-dimensional multivariate space.

Figure 6 shows the biplot for all the species in the data set for the first two discriminant factors (Df). Only two groups of animals are clearly separated from all other animals in this DA plane. These are the grey and common seals (PG and PV) and the sperm whales (PM). All animals, with three exceptions, fall into species groups, indicating that the species are distinguishable to different extents between each other on the basis of CB patterns. To visualise the separation of the remaining species the DA was re-calculated after removing the seals and sperm whales, which are clearly separated. The resulting biplot (DA2) gave a clear separation of Risso's dolphins (GGx) from the other species. The Risso's dolphins were removed and DA was rerun (DA3). The biplot for DA3 is given in Figure 7. Correct group classification of each species of each of the discriminant plots is given along with the total number of animals in that species (Table 4). The exceptions are one sperm whale from Belgium (PM22) and two bottlenosed dolphins from the east coast of Scotland (TT1 and TT8).

Metabolism

The cytochrome P450 enzyme system holds a vital position in the biotransformation of CBs metabolic differences between species of marine mammals (NORSTROM *et al.*, 1992; BOON *et al.*, 1994; TANABE *et al.*, 1988). GØKSOYR *et al.* (1992) found that seals have high P450 1A mono-oxygenase activities similar to those found in cetaceans, but that they also have high P450 2B mono-oxygenase activities. In contrast, cetaceans were found to have very low responses, implying lesser ability to metabolise CBs with m,p-vicinal H-atoms (GØKSOYR *et al.*, 1989; BOON *et al.*, 1992). This difference explains the separation of the pinnipeds from the cetaceans (Fig. 6).

The DA factors indicate the extent of the differences between pinnipeds and sperm whales and also between other species. Those congeners which can be metabolised

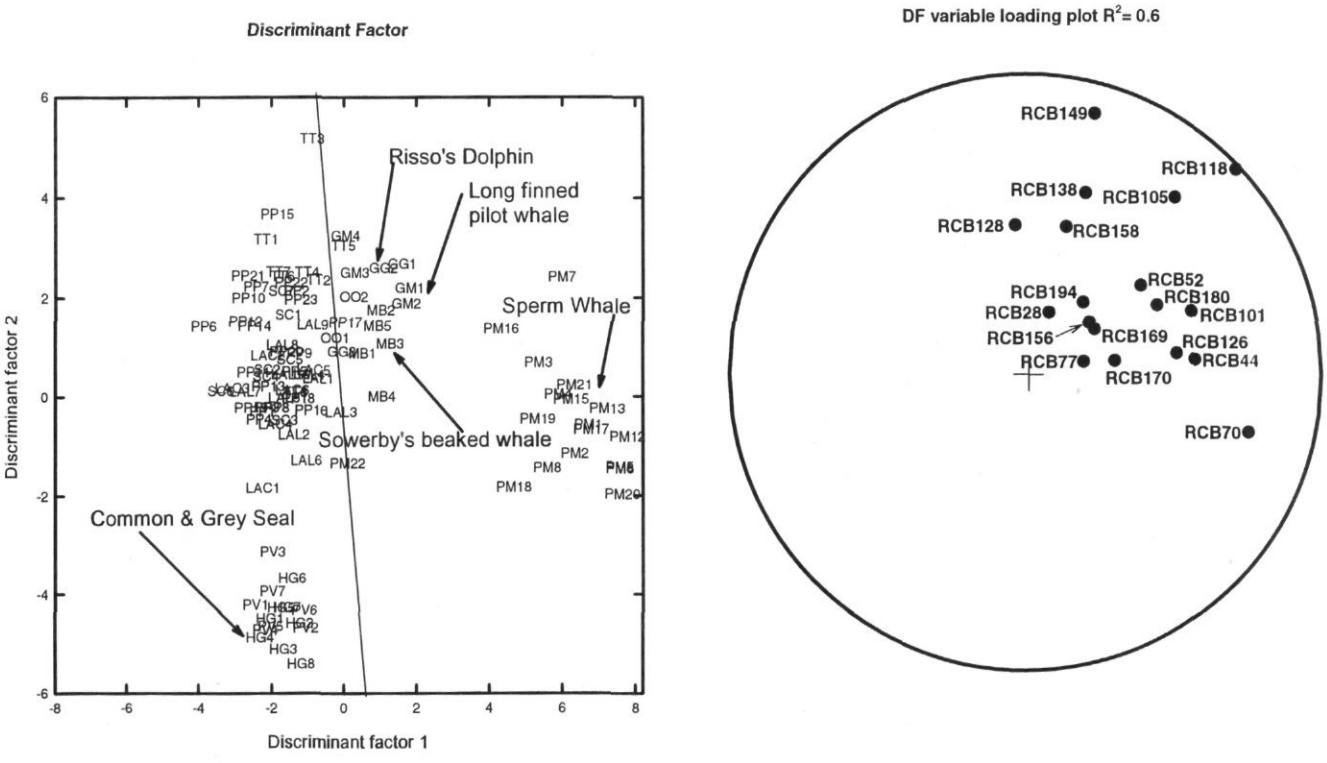

Fig. 6 – DA1 - biplot of the first two discriminant functions (df1 and df2) - all species.

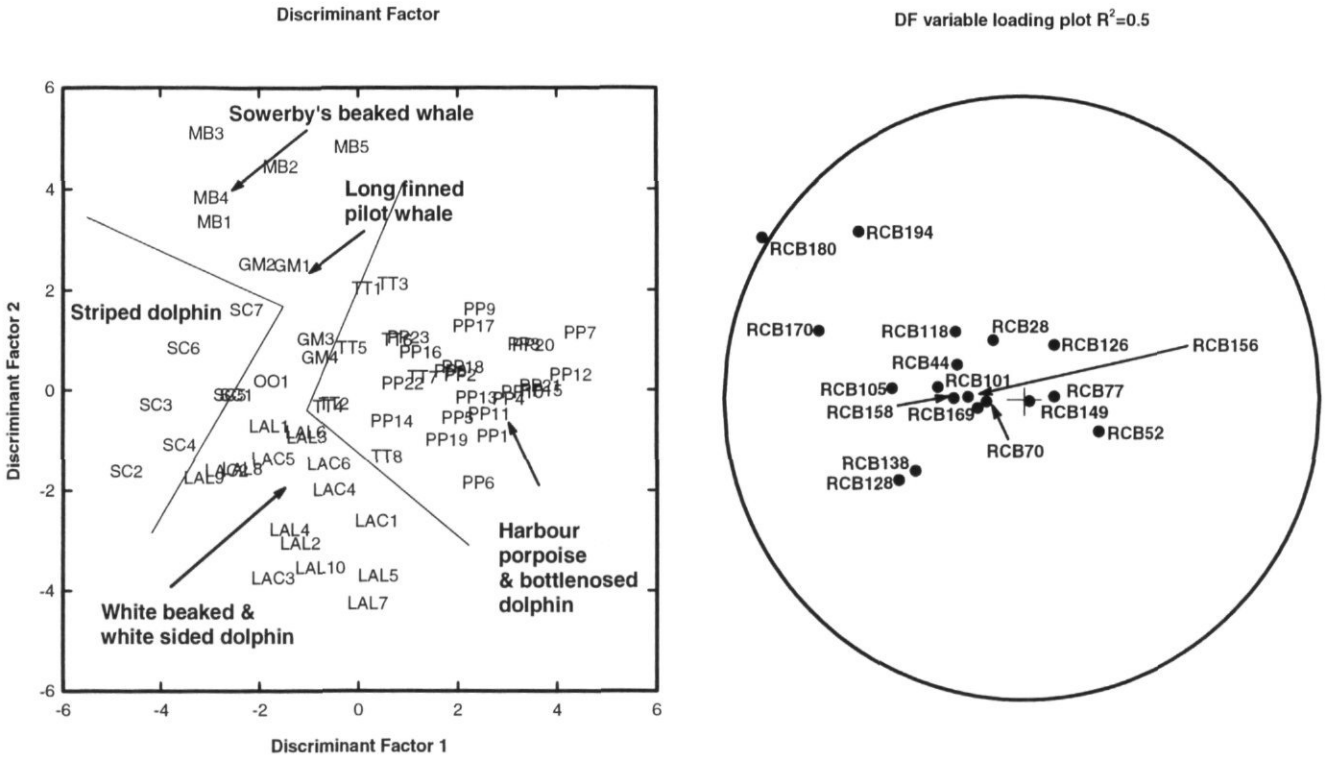

Fig. 7 – DA3 - biplot of the first two discriminant functions (df1 and df2) for selected species

by the CYP1A system and possess a structure predicting their metabolism in cetaceans (CBs 28, 70, 77, 105, 118, 126 and 156) form the basis of the separation in the DA biplots. This discrimination is not likely to be an indication of a difference in metabolic capacity of the P450 1A isoenzyme between species, but a difference between individual animals as a result of the magnitude of contaminant exposure. For example, the sperm whales (PMx) in general have lower contaminant concentrations than most of the other species studied and also appear to be the least able to metabolise those CB congeners via the CYP1A1 system. These observations could be due to a difference in the level of exposure to CBs and therefore a difference in the degree of induction of P450 1A isoenzyme. Consistent with this hypothesis, the Belgian sperm whale PM22 (B1) is completely separate from the sperm whale group (Fig. 6). This animal has the highest CB concentrations of any of the sperm whales and the lowest relative values of the non-ortho chlorinated CB's. Its separation by the DA and subsequent misclassification may be due to unusually high CB burden and greater induction of the P450 1A isozyme system. Alternatively the differences may be due to errors in the sampling of this animal as discussed previously.

Table 4 – Classification of species using discriminant analysis

Species	n	Number correctly classified		
		DA1	DA2	DA3
PP	23	22	23	23
HG	8	8	–	0
SC	7	7	7	6
LAC	6	6	6	6
LAL	10	10	9	10
GG	3	3	3	0
TT	8	6	6	7
MB	5	5	5	5
GM	4	4	4	4
PM	18	17	–	–
PV	7	7	–	–
OO	2	2	2	2

For some species the number of subject animals is relatively low (2-5), but these were the same sex and of similar age in a particular stranding (e.g. four of the five Sowerby's beaked whales were stranded together on the west coast of Scotland). These animals have very similar absolute CB concentrations and therefore very similar patterns, lying close to each other in the biplots. The other Sowerby's beaked whale (MB5) was stranded on the east coast at a different time and had a higher contaminant burden and a slightly different pattern (Fig. 7). A similar observation was made for the bottlenose dolphin from the east coast of Scotland (TT8) which had a much higher CB burden than the other animals in that species, and a different pattern of congeners.

Table 5 – Habitat

Species	Latin names	Habitat	Principal food source
Harbour porpoise	*Phocoena phocoena*	Coastal/migratory	Gadoids, sandeels fish
Grey seal	*Halichoerus grypus*	Coastal	Gadoids and demersal fish, sandeels
Common seal	*Phoca vitulina*	Coastal	Mixed fish, marine mammals
Killer whale	*Orcinus orca*	Coastal/Oceanic	
Striped dolphin	*Stenella coeruleoalba*	Primarily oceanic	Surface fish and cephalopods
Atlantic white sided dolphin	*Lagenorhynchus acutus*	Coastal/continental shelf region	Surface fish and cephalopods
White beaked dolphin	*Lagenorhynchus albirostris*	Coastal	Mixed fish, mainly clupeoids and gadoids, cephalopods
Risso's dolphin	*Grampus griseus*	Deep water	Cephalopods
Bottle-nosed dolphin	*Tursiops truncatus*	Coastal (and offshore)	Salmon, gadoids, water fish and cephalopods
Long-finned pilot whale	*Globicephala melaena*	Oceanic but coastal feeder	Shallow and deep water fish and cephalopods
Sowerby's beaked whale	*Mesoplodon bidens*	Migratory within the North Atlantic	Cephalopods
Sperm whale	*Physeter macrocephalus*	Oceanic	Cephalopods

Body condition

Any significant decline in the condition of an animal will ''age'' the chemical pattern since enzyme induction increases if illness or starvation results in a mobilisation of lipids. This condition-related cause would account for the spread of data observed in the sperm whale patterns where a number of the individuals stranded in poor physical condition.

The increased ability of marine mammals to metabolise CBs when higher contaminant burdens are present will decrease the toxicity of the parent compound. More importantly, the persistent methyl sulphonated metabolites, known to accumulate in lung and uterine fluid and hydroxylated metabolites which are retained in blood plasma are known to exhibit toxicological effects (BROUWER, 1990; LANS *et al.*, 1990; BRANDT and BERGMAN, 1987; MURCK *et al.*, 1994). Metabolism of certain CB congeners may result in the production of metabolites with greater toxicity than the parent CB.

Habitat and Food Source

The habitats of the species examined are summarised in Table 5 (RUDGE *et al.*, 1981). Higher contaminant burdens generally are found in species inhabiting coastal regions due to the close proximity of the animals to possible sources of direct input (BLOMKVIST *et al.*, 1992; STORR-HANSEN and SPLIID, 1993). Any possible differences of relative CB congener patterns as a result of different input sources (i.e. different commercial mixtures) will be effectively blurred by the food web interactions and masked as a result of the mobility of marine mammals.

From the data acquired there appears to be little segregation of the CB patterns within species as a result of the different locations represented by the sampling programme. This is illustrated by the similarities of the CB patterns found in Atlantic white sided dolphins stranded on the west and east coasts of Scotland and those stranded on the northwest coast of Ireland (MCKENZIE *et al.*, in press). The only animals with different patterns were from two harbour porpoises stranded on the west coast (PP2 and PP9, Fig. 3). Marine mammals with similar metabolic capacities, feeding on similar food in localised areas (bottlenosed dolphin, white beaked dolphin) have similar CB congener patterns, but species feeding at different trophic levels (sperm whales) from one another in the same locality are likely to have distinctly different CB patterns.

There are essentially three types of ''feeders'' in this set of mammals. The fish feeders, the mixed feeders and the cephalopod feeders. There is a relationship between the position of an animal in multivariate space to its food source. A particular grouping of the animals in the discriminant analysis plots due to their food source appears in Figure 6. All theoretically cephalopod feeders have positive values of DA factor 1. This group consists of Risso's dolphin, Sowerby's beaked whales and sperm whales that, although biologically similar with regard to possessing a CYP1A and lacking a CYP2B enzyme system, are grouped to the right of other cetaceans although they appear not to lie in the same region of the plot along with the striped dolphin. The principal food of these animals are cephalopods. The difference in diet of these species may be the reason for the observation of different CB patterns. Further research into the contaminant patterns of the prey species

of these species should be carried out before any conclusions are made.

Conclusions

These studies demonstrate the value of using discriminant analysis to distinguish between species differences and PCA to detect those factors which contribute to within species variability on CB patterns in marine mammals. From the analysis of the data the following conclusions can be drawn:

- there is a need for the determination of a wide range of CBs to discern the patterns of contamination; measurement of the seven monitoring congeners is insufficient;
- patterns of congeners in marine mammals are influenced by the total contaminant burden relating to age, sex, location and food source, and as a result the bioaccumulation potential of some CB's are different in different marine mammal species;
- animals with a higher contaminant burden may be at more risk due to increased concentrations of toxic CB metabolites as a result of increased induction of the P450 enzyme system;
- correlation of TEF's (toxic equivalency factors) with biological effects should be based on the sum of the concentrations of a parent CB and it's metabolites rather than the residue alone.

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