CONTAMINANTS IN BALTIC SEA MALE AND FEMALE GREY SEALS (*HALICHOERUS GRYPUS*) OF DIFFERENT AGES

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Introduction

The grey seal (*Halichoerus gryphus*) is the most abundant marine mammal in the Baltic Sea with approximately 25000 individuals today[1]. They are piscivorous mammals and as top predators receive contaminants via their food. Due to biomagnification concentrations of contaminants might reach harmful levels[2] for example the contaminants PCB and DDT that caused a severe disease complex among grey seals, mostly seen in the 1980s and 1990s[3, 4].

The ultimate aim of our research is to model biomagnification processes in the Baltic Sea food web, but in doing so it is important to know the variation in contaminant concentrations among individual animals. In this study, we determine concentrations of organochlorines (OCs) and brominated flame retardants (BFRs) in the blubber of grey seals and investigate the contaminant levels and patterns in seals of different ages and sexes by using multivariate data analyses.

Materials and Methods

Thirty individual grey seals (*Halichoerus gryphus*) from the Baltic Sea collected during autumn in 1999-2003, were sampled from the Environmental Specimen Bank at the Swedish Museum of Natural History. Twenty-five of the seals were either by-caught in fishing gear or shot during the annual hunt and five individuals (3 males and 2 females) were found dead. The females and males, respectively, were divided into three age groups; up to 2 years, between 5-9 years and 12 years or older, giving 6 groups. Sample treatment, analytical methods for quantification of individual OCs and BFRs isomers/congeners as well as laboratory QA/QC procedures have previously been described in detail, for OCs [2, 5-7] and for BFRs [5, 8, 9]. In short, the OCs was determined by high resolution gas chromatography (GC) and the BFRs by GC connected to a mass spectrometer operating in the electron capture/negative ion mode. By using a combination of different capillary columns all compounds could be separated except for CB138 where an interfering peak caused by CB163 meant that CB138 could be overestimated by 20-30%. To facilitate reading, CB138+CB163 are henceforth written as CB138. All samples were homogenized, the lipids were extracted and the lipid content (F%) determined gravimetrically at ITM. Blubber samples were analysed on an individual basis. Muscle samples were analysed as 6 pooled homogenates where the 2 sexes and the three age groups had been pooled separately (muscle results are not given in this abstract). The analysed contaminants were: DDTs (*p,p*'DDT, *p,p*'DDE, *p,p*'DDD), polychlorinated biphenyls (PCBs: CB28, CB52, CB101, CB105, CB118; CB138, CB153, CB156 and CB180), hexachlorocyclohexanes (αHCH, βHCH, and, γHCH), hexachlorobenzene (HCB), *trans*-Nonachlor (t-noCl), polybrominated diphenylethers (PBDEs: BDE47, BDE99, BDE100, BDE153, and BDE154) and hexabromocyclododecane (HBCD). The multivariate data analyses were done by using the software Simca-P+ 11.0 (www.umetrics.com) and the statistics by using GraphPad Prism 5.01 (www.graphpad.com).

Results and Discussion

The animals' biological data are given in Table 1. The two sexes consist of comparable individuals in the three age groups. In general the older males were slightly leaner (lower body weight, BW) than their female counterparts although the males were longer (higher body length, BL). Further, the males do not live as long as the females, and the older males had thinner blubber thickness (BlubTh) with lower percentage fat in it (F%). Some of the concentrations of the total 23 different contaminants analysed are shown in Table 2.

Table 1. Grey seals (*Halichoerus gryphus*) sampled 1999-2003 in the Baltic Sea. Biological variables; body weight (BW), body length (BL), age in years (Age), blubber thickness (BlubTh) and percent fat (lipids) in blubber (F%). Values are given as mean ± standard deviation.

		Young seals (0-2 years)		**Adult seals** (4-9 years)		**Old seals** (12-34 years)	
		Females	*Males*	*Females*	*Males*	*Females*	*Males*
		n= 5	*n= 5*	*n= 5*	*n= 5*	*n= 4*	*n= 5*
BW	kg	54.2 ± 36.8	53.2 ± 30.4	151 ± 38.1	137 ± 19.1	117 ± 44.3	100 and 86.4 (n=2)
BL	cm	121 ± 20	141 ± 29	169 ± 12	176 ± 18	171.5 ± 5.2	192 ± 10.4
Age	year	1.3 ± 0.8	1.6 ± 0.8	6.1 ± 1.2	5.7 ± 1.9	20.1 ± 9.6	15.4 ± 2.3
BlubTh	cm	3.1 ± 1.4	3.0 ± 0.9	4.8 ± 2.1	4.4 ± 1.1	4.2 ± 0.8	2.1 ± 0.8
F%	%	85.3 ± 2.4	85.3 ± 2.8	75.12 ± 15.7	86.1 ± 3.5	87.4 ± 3.6	69.7 ± 27.9

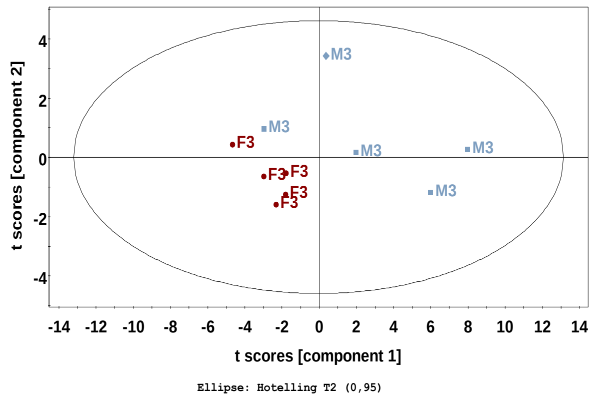
Table 2. Some of the analysed contaminants (as ng/g lw) in blubber of female and male grey seals (*Halichoerus gryphus*) sampled 1999-2003 in the Baltic Sea. ∑DDT as the sum of *pp'*-DDE, *pp'*-DDD and *pp'*-DDT; ∑PCB as the sum of ICES seven marker PCBs: IUPAC nos. CB28, CB52, CB101, CB118, CB138, CB153 and CB180; ∑PBDE as sum of BDE congeners IUPAC nos. BDE47, BDE99, BDE100, BDE153, and BDE154; and HBCD. Values are given as mean ± standard deviation.

	Young seals (0-2 years) [1] Mean±StDev ng/g lw		**Adult seals** (4-9 years) [2] Mean±StDev ng/g lw		**Old seals** (12+) [3] Mean±StDev ng/g lw	
	Females	*Males*	*Females*	*Males*	*Females*	*Males*
	n= 5	*n= 5*	*n= 5*	*n= 5*	*n= 5*	*n= 4*
∑DDT	5740 ± 1700	7150 ± 3660	13000 ± 15 200	9510 ± 4080	8500 ± 4050	64800 ± 75100
∑PCB	7720 ± 2600	9540 ± 4100	27900 ± 36500	26300 ± 20600	28760 ± 23880	421700 ± 720100
∑PBDE	102 ± 38	106 ± 57,8	139 ± 130	122 ± 53.0	127 ± 50.5	454 ± 423
HBCD	64 ± 25	72 ± 8,7	92 ± 77	72.3 ± 18.8	86.3 ± 52.4	214 ± 197

[1] Mean age = 1.4 years, [2] Mean age = 5.9 years, [3] Mean age = 17.5 years

Sex differences. When analyzing the data with multivariate data analysis techniques interesting patterns emerge. For instance, when performing partial least-squares discriminant analyses (PLS-DA) with female *vs.* male contaminant concentrations, there were no significant models for the four groups with young and adult seals (< 10 years, *n*=20). This means that the contaminant concentrations do not differ between the younger females and males. However, for the oldest animals (ages between 12-34 years for females and 12-18 years for the males) there were differences between the sexes creating a significant model (R^2X= 0.83, R^2Y=0.73, Q^2=0.51, two components) (Figure 1).

A



B

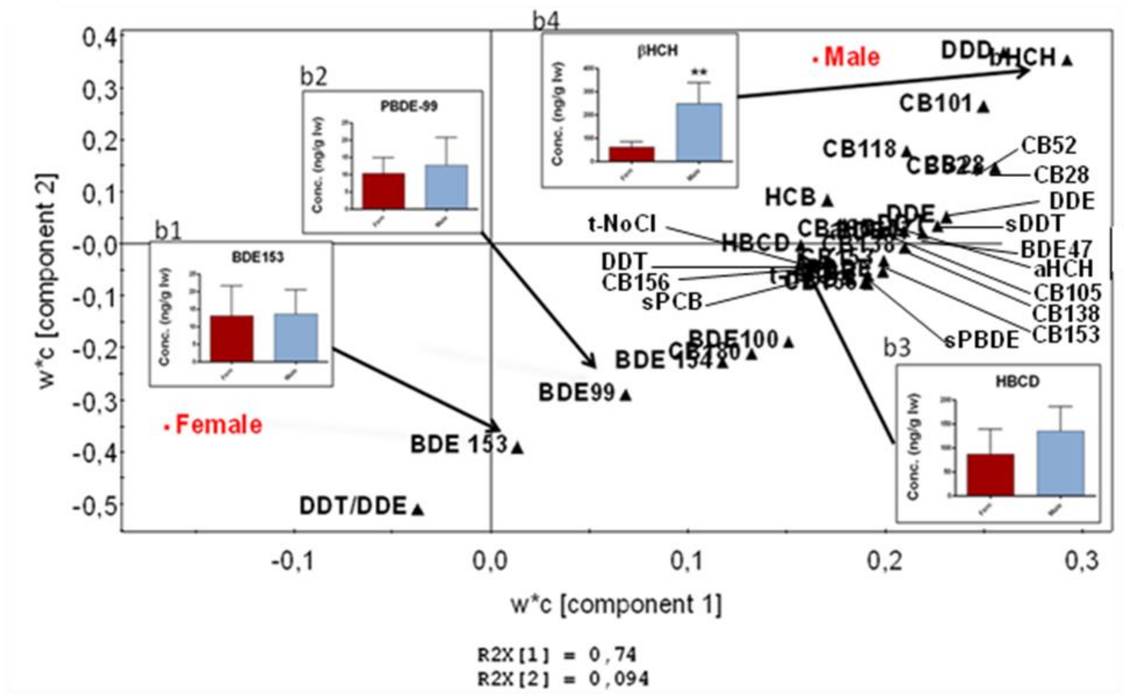


Figure 1. PLS discriminant analysis (PLS-DA) with contaminant concentrations in grey seal (*Halichoerus grypus*) females *versus* males give a model with two components (R^2X= 0.83, R^2Y=0.73, Q^2=0.51). A, score plot with the old females (F3) and the old males (M3) and B, loading plot.

To further illustrate the result from the PLS-DA, four variables (BDE153, BDE99, HBCD and βHCH) have graphs inserted on top of the loading plot (Figure 1B). In these graphs, bars show the mean concentrations +SD in the older females and in males, respectively. For the brominated flame retardants there were no differences between the sexes (see b1 for BDE153, b2 for BDE-99, and b3 for HBCD, with p-values (t-test) of ~ 0.77- 0.2. For βHCH (see b4; p= 0.002, t-test), that according to the PLS-DA was the variable having the largest influence on the model (and for several of the other organochlorines) there were significant differences between the sexes with males having higher concentrations. When performing the same analysis but with the contaminant concentrations on a wet weight basis instead of on a lipid weight basis the patterns for the two sexes are remarkably similar. There are not sufficient differences to separate between the sexes for the two younger age classes but there are for the older animals, giving a significant model that showed that the older males had higher concentrations of the same contaminants as in the “lipid-weight” model (shown in Figure 1).

Age differences. When performing a PLS with age of the animals as Y and their contaminant concentrations as X one gets a good model for the males ($R^2X= 0.85$, $R^2Y=0.78$, $Q^2=0.68$, two significant components) that showed that 8 (CB138, CB153, CB156, CB180, DDE, BDE153, BDE154 and γHCH) out of the 23 contaminants increase significantly in concentration with age though that 15 of 23 contaminants do not change with age. The same pattern emerges for the males when performing the modelling using the concentrations on a wet weight basis. However, for the females the results are not the same, as there are no significant first components, either with the contaminants on a lipid or on a wet weight basis. This reflects gender differences in age-dependent bioaccumulation of contaminants due to the lactational transfer of lipophilic contaminants from females to their pups[10].

Comparisons with earlier studies. When comparing the levels of the contaminants between different studies there are always problems with the comparability of the results. Nevertheless, the levels of PBDEs in these samples seem to be lower when compared to samples of Baltic Sea grey seals from the years 1980-1990[11] in all the age groups and the same seems to be the case for DDT and PCB[12]. However, for the PBDE analyses, then Bromcal 70-5 DE was used as standard as compared to the congener specific standards used today so it might be possible that the decline is due to the different methodology used. To further complicate the matter, one factor resulting in the lower contaminant loads in these seals compared with those in seals during the 1980s could be the observed change in the grey seal diet[13, 14] due to the decline of large fish in the Baltic Sea. However, there has been a general decline in the organohalogens' levels during the last 20 years in other animals from the Baltic Sea[2], so this might be noticeable also in the grey seal.

Acknowledgements
Thanks to: the Department of Contaminant Research at the Swedish Museum of Natural History, Stockholm, Sweden; the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (FORMAS); the Foundation for Strategic Environmental Research (MISTRA); and the Swedish EPA.

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