DIRECT ORDINATION ANALYSIS OF ORGANOCHLORINES IN POLAR BEAR: DETERMINING THE RELEVANCE OF EXPLANATORY VARIABLES

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Introduction

Since first discovered merely four decades ago, the presence of persistent organochlorine (OC) pollutants in the Arctic ecosystem has been monitored with concern. This highly lipophilic group of contaminants is known to accumulate in the marine environment and tend to biomagnify up the marine food chain.[1] High levels of OCs, particularly polychlorinated biphenyls (PCB), have been found in top predators such as the polar bear (*Ursus maritimus*).[2] Particularly high concentrations of PCBs have been detected in polar bears at Svalbard and in the Russian Arctic as compared to the North American Arctic.[2-6] The organochlorine pesticides (OCPs) have shown greater geographical variation as compared to PCBs: the highest levels of oxychlordane, trans-nonachlor and *p,p'*-DDE were found in polar bears at Franz Josef Land and in the Kara Sea, whereas the highest concentrations of α-HCH and β-HCH were detected in the Chukchi Sea.[6] Spatial and temporal variations, dynamic ecological interactions, and the introduction of new persistent organic pollutants (POPs) to the Arctic[7], urge the need for a comprehensive and effective methodology to monitor and evaluate levels and potential effects of environmental contaminants in the region. Ordination techniques may offer an effective tool to elucidate intrinsic patterns in a data matrix (e.g. the relationship between classes of contaminants, environmental variables affecting composition in species/contaminants etc.).[8] An ordination diagram obtained from a direct constraint ordination technique, Redundancy Analysis (RDA), provides a visualization of the main pattern of both OC variation explained by environmental variables, and correlation coefficients between species and environmental variables, in the same diagram.[9] The advantage of using RDA instead of a series of separate univariate and multivariate techniques, e.g. regression analysis, ANOVA etc. is that it provides a sensible and convenient summary of the relationship between species and environmental variables.[8,9] The objective of the present paper is to demonstrate that a linear response ordination technique such as RDA is a useful and an effective tool to evaluate the role and effect of multiple environmental and biological variables on the composition of contaminants in polar bears. The database of a circumpolar study of OC levels in polar bears from the Arctic was tested by RDA, reviewed in light of univariate and other multivariate techniques, and evaluated towards previous findings.

Materials and methods

Blood samples from 176 adult (≥6 years old) female polar bears were collected during two main periods, March-May and August-September, between 1987 and 1998 at seven different circumpolar locations (Table 1) and grouped accordingly.[3] Similar capture protocols were used at all locations.[10] All capture and handling methods used were approved by the Norwegian Experimental Animal Committee.[3,6,10] OC analyses were performed at the Laboratory of Environmental Toxicology at the Norwegian School of Veterinary Science. OCs in polar bears from Franz Josef Land, Kara Sea and East-Siberian Sea were quantified using the same methods as the other locations, but with some analytical modifications.[3] Equal quality-assurance/quality-control procedures were used for all analysis in order to ensure comparable results. Biological parameters such as body size and weight, reproductive status, health condition etc. were not determined at all locations and are subsequently excluded from analysis in the present report. Further details on capture, sampling, and OC analysis are described in depth elsewhere.[2,3,6,10] The analysis included the 15 OCs that were found at all seven locations; PCB-99, -118, -138, -153, -156, -157, -170, -180 and -194; and hexachlorobenzene (HCB), α- and β-hexachlorocyclohexanes (HCHs), oxychlordane, trans-nonachlor and *p,p'*-DDE [1,1-dichloro-2,2-bis(4-chlorophenyl) ethylene].

Table 1 Geographic regions where adult female bears were captured, year and month of capture, capture location (range), sample size at each region, age (mean; range in brackets), percent extractable fat (fat%) in blood samples (mean; range in brackets), geometric mean with 95% confidence interval (in brackets below) for sum$_9$ (∑) polychlorinated biphenyls (PCBs) and sum$_6$ organochlorine pesticides (OCPs) concentrations (ng/g lipid weight).

Region	Svalbard	Barents Sea	Franz Josef Land	Kara Sea	East-Siberian Sea	Chukchi Sea	Churchill
Sampling year(s)	1991-1998	1997-1998	1995	1991, 1994	1992-1993	1987-1992	1998
Sampling month(s)	March-May	April-May	April	April-May	April	March-April	August-Sept.
Capture	77-79ºN	74-78ºN	76-82ºN	74-80ºN	70-72ºN	62-71ºN	57-59ºN
location	16-26ºE	37-44ºE	46-69ºE	82-118ºE	163-173ºE	179ºE -206ºW	93-94ºW
N	71	24	21	17	8	11	15
Age	11 (6-26)	12 (6-23)	12 (6-22)	13 (7-28)	11 (6-23)	10 (6-18)	15 (6-27)
Fat%	1,05 (0,48-1,80)	1,11 (0,49-1,57)	0,53 (0,35-0,77)	0,84 (0,76-1,13)	0,75 (0,55-0,97)	0,55 (0,39-0,76)	1,0 (0,63-1,44)
∑PCB$_9$[a]	6 760	8 770	14 700	11 900	4 250	2 950	2 110
	(6 630-6 880)	(8 590-8 970)	(14 400-14 900)	(11 600-12 200)	(4 140-4 360)	(2 910-3 000)	(2 090-2 130)
∑OCP$_6$[b]	1 870	2 010	3 850	3 580	3 610	3 140	1 630
	(1 840-1 910)	(1 980-2 040)	(3 810-3 900)	(3 520-3 650)	(3 530-3 680)	(3 100-3 180)	(1 620-1 650)

a) ∑PCB$_9$ represents the sum of the nine PCB congeners (PCB-99, -118, -138, -153, -156, -157, -170, -180 and -194); b) ∑OCP$_6$ represent the sum of six organochlorine pesticides (HCB, α-HCH, β-HCH, oxychlordane, trans-nonachlor and *p,p'*-DDE).

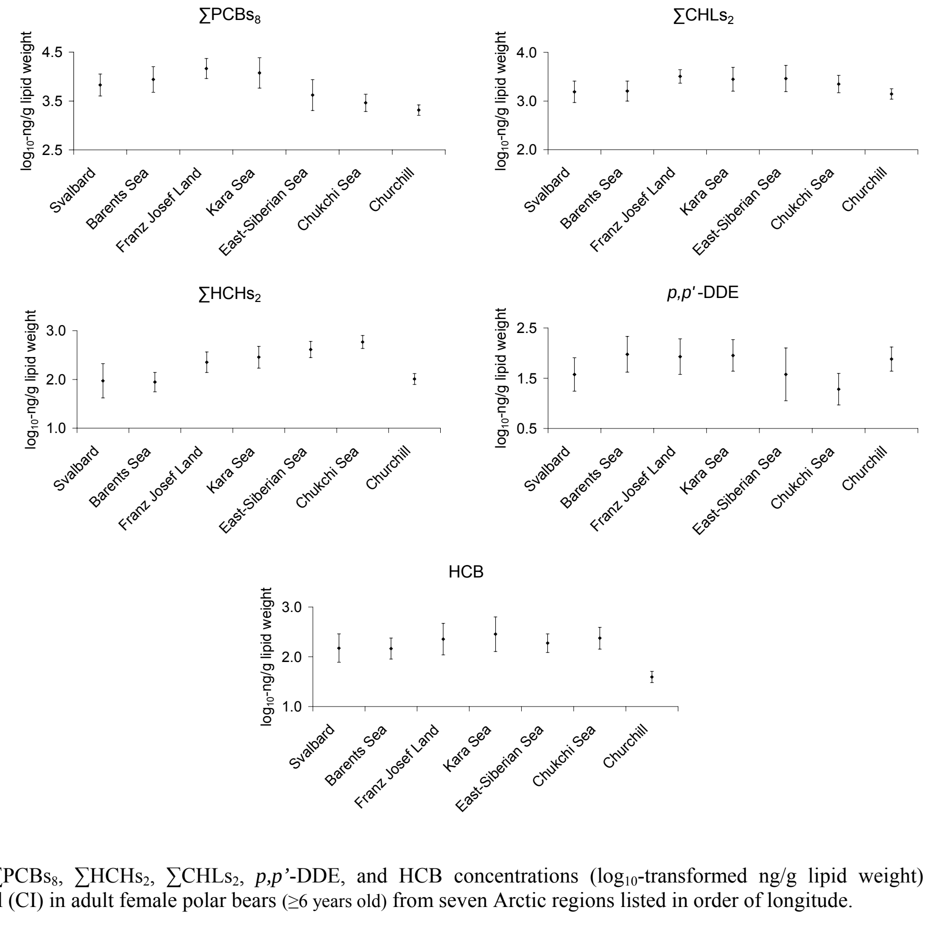

Figure 1 Mean ∑PCBs$_8$, ∑HCHs$_2$, ∑CHLs$_2$, *p,p'*-DDE, and HCB concentrations (log$_{10}$-transformed ng/g lipid weight) plotted with 95% confidence interval (CI) in adult female polar bears (≥6 years old) from seven Arctic regions listed in order of longitude.

Polar bear males, cubs, yearlings and juveniles (<6 years old) and one individual with an extremely high plasma fat percentage were excluded from an originally larger sample size, in order to reduce some of the well documented variance between the sexes, as well as between adults and young bears.[2,12] Univariate and multivariate statistics were performed using JMP 6.0.0 and CANOCO for Windows version 4.54[11], respectively. The significance level for all statistical analysis was set to be 0.05%. The data were lipid normalized prior to statistical analysis. Geometric means and 95% confidence intervals (CI) are back-transformation of arithmetic means and CI of log-transformed data. Pearson correlation coefficient and ANOVA followed by Tukey's post hoc test were used to explore differences between groups (locations, age, year and month of capture). RDA (standard deviation (SD) <<1.5, determined by detrended correspondence analysis (DCA))[8] was used to analyse the pattern of individual OCs, the inter-relation between the OCs, and the OCs relation to explanatory variables (sampling year and month, age, latitude and longitude). The significance of the differences in OC pattern between explanatory variables was analysed with Monte-Carlo permutation tests ($p<0.05$). OC concentrations, presented as a proportion of sum OCs (∑OCs) were used in order to describe a pattern, rather than the absolute levels of OCs. A principal component analysis (PCA) was run to determine whether the variation explained by constrained ordination (RDA) would be in accordance with the variation explained by variables in an unconstrained system (PCA). In order to visualize the relative importance of certain functional PCB-groups at different locations (Figure 3) the PCBs were grouped according to their metabolic affinity.[10,12,13]

Results and Discussion

Relevant environmental and biological data on the seven different locations are presented in Table 1. The levels of the most important contaminants are shown in further detail in Figure 1. The eigenvalues obtained by PCA were 0.99 and 0.01 for the x- and y-axis, respectively. The eigenvalues obtained by RDA were 0.66 and 0.02 for the first and second axis, respectively. This indicates that the first axis is of great importance, in both the PCA and the RDA results. RDA of OCs resulted in four significant explanatory variables: month and year of capture, latitude and longitude. The age variable was not significant and thus excluded from further analysis. The ordination diagram (Figure 2) reveal that certain PCB congeners (PCB-138, -153, -157, -170, -180 and -194) are negatively correlated to longitude, and positively with latitude. OCPs such as α- and β-HCH, and oxychlordane are on the contrary positively

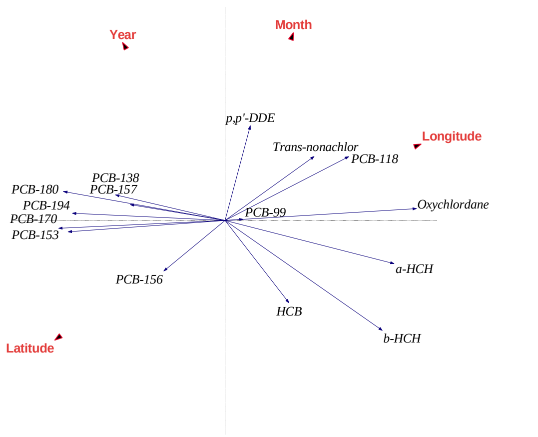

Figure 2 Output of RDA using concentrations of individual OCs (relative to ∑OC) as response variables and latitude, longitude, year of capture as explanatory variables. The eigenvalues for the axes are 0.66 and 0.02, for the x- and y-axis, respectively.

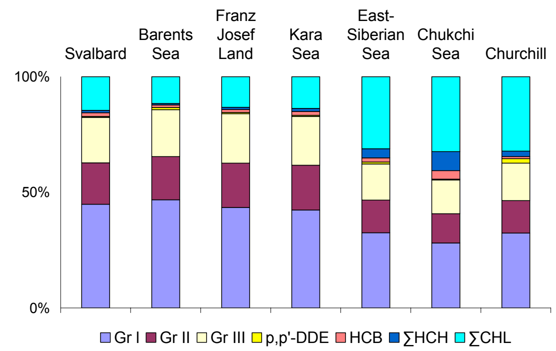

Figure 3 Relative contribution (geometric mean) of six OC groups, group I, II, and III categorized according to their metabolic group.[10,12,13] Group I: PCB-153, -180, and -194; group II: PCB-99, -138, and -170; group III: PCB-118, -156, and -157.

correlated to longitude. Figure 2 further indicates that latitude correlates with PCB 156, and negatively with PCB-118 and trans-nonachlor (supported by regression analysis; r^2=0.39, r^2=0.08 and r^2=0.18, respectively). Longitude and latitude explain a significant part of the analysis (96% of the variance explained by all environmental variables), thus indicating that a difference in patterns of OCs is related to geographical variation. The RDA thereby confirm the geographical pattern found elsewhere.[2,3,6] The remaining significant explanatory variables, month and year of capture, indicate that patterns of OC concentrations additionally is related to seasonal and temporal variations, although to a less extent than longitude and latitude. The year of capture correlates negatively with HCB (r^2=0.19), α-HCH (r^2=0.50) and β-HCH (r^2=0.57). However, the year and month variables are also confounding with geographical variation. Samples gathered in the early nineties mainly consist of samples from East-Siberian Sea and Chukchi Sea, in which HCHs are significant contributors to ∑OCs. Similarly, samples from August/September were exclusively collected at Churchill. To avoid such confounding factors as year and month in present study, the effect of known variables, or variables that are not of interest, can be introduced as co-variables (partial direct ordination).[8] This would allow us to focus more specifically on the effects of the variables of interest. The distinctive behaviour of certain compounds in the ordination diagram such as *p,p'*-DDE, PCB-156 and HCB is yet to be determined. Further investigation of their distinctive structure and metabolic function can perhaps reveal more of how the mechanisms affect the pattern.[13] The relative contribution of six functional groups at each location is provided in Figure 3. The figure confirms the geographical pattern obtained by ordination, but further suggest that *p,p'*-DDE contributes relatively more to ∑OCs at Churchill compared to other locations. This could thus explain why *p,p'*-DDE and latitude points in opposite directions in the ordination diagram (Figure 2). Conclusively, direct ordination analysis provided us with a clear and rigid summary of the polar bear database. The ordination diagram revealed similar trends when OCPs and PCBs were analysed collectively as they did separately, thereby validating a new and more facilitated approach of investigation of OCs in an Artic ecosystem. By this method the explanatory variables and their relative significance for explaining the OC concentration pattern can be easily deduced. This study further suggest that RDA can enable us to efficiently obtain a better picture and understanding of "new" emerging compounds in the Arctic such as brominated flame retardants (BFRs).[7]

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