# USING POPs TO DISTINGUISH HARBOUR SEAL (*Phoca vitulina)* COLONIES OF ATLANTIC CANADA

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## Introduction

Most persistent organic pollutants (POPs) present in the environment accumulate at high concentrations in lipid-rich tissues such as the blubber of marine mammals. Contaminant burdens in marine mammals are determined by various factors including the level of pollution in the environment where the animals live, the contamination level of their diet, but also their sex, age, condition, trophic position and their capacity to metabolise and excrete certain POPs[1]. On this basis, POP levels and patterns can likely be used to distinguish colonies of a certain species of marine mammals.

Harbour seals (*Phoca vitulina)* from Atlantic Canada are considered as sedentary throughout their range and normally associated with discrete haul-out sites. Distances between these haul-out sites can be quite large and information on movements between sites is limited or non-existent. Several small isolated colonies of harbour seals have been proposed in eastern Canada based on pelage patterns and variation in the number of post-canine teeth[2]. Obviously, these tools have limited capability to discriminate among seal populations.

The objective of this study was to investigate the possibility of using POP contamination to differentiate among harbour seals collected from three sites in eastern Canada. Levels and patterns of two families of POPs, polychlorinated biphenyls (PCBs) and polybrominated diphenylethers (PBDEs), were determined in the blubber of each seal and then compared among sites. In addition, biological parameters, including among others the tropic position of each animal, were also determined.

## Methods and Materials

*Samples*. Blubber samples were obtained from adult male harbour seals hunted between 1999 and 2002 in the vicinity of Bic in the St. Lawrence Estuary (SLE; n=8), Charlottetown in the southern Gulf of St. Lawrence (SG; n=10) and Placentia Bay and the surrounding south coast of Newfoundland (NF; n=10) (Table 1). Locations of these seal sampling sites are separated from each other by at least 600 km. A large piece of skin-blubber-muscle sample was collected from the dorso-lateral region of each individual. Samples were wrapped in solvent rinsed aluminium foil and placed in a sealed plastic bag, and then stored at –20°C until analysis.

*Analysis of POPs*. PCBs and PBDEs were determined in blubber samples according to the methods reported in Hobbs et al.[3] and Lebeuf et al.[4], respectively. In brief, a sample of blubber

extending the depth of the blubber layer, from the skin to the muscle, was first taken from a larger piece of skin-blubber-muscle sample. Blubber samples were then chemically dried with sodium sulphate before being transferred to a glass column. A single $^{13}C_{12}$ PCB was added to the column before the extraction procedure. Lipids and lipophilic compounds were extracted from the sample with dichloromethane-hexane (50:50). The extraction solution received a mixture of four $^{13}C_{12}$ PBDEs and five $^{13}C_{12}$ PCBs and was prepared for purification. Lipids were removed from the remaining extract by gel permeation chromatography. The extract was further cleaned by elution through a two-layer column packed with neutral silica and alumina. The final extract was reduced in volume and spiked with an instrument performance solution containing two $^{13}C_{12}$ PCBs.

Nine PBDEs and thirty-one individual PCBs were measured in each sample. Quantification of PCBs was performed using a Varian 3400CX series gas chromatograph equipped with a Varian Saturn IV ion trap, a Varian 1078 split/splitless programmable injector (5 µl injection volume) operated in splitless mode, and a Varian 8200CX autosampler. Quantification of PBDEs was performed using a ThermoQuest Trace GC gas chromatograph equipped with a Finnigan PolarisQ ion trap, a ThermoQuest PTV split/splitless programmable injector (5 µl injection volume) operated in splitless mode, and a ThermoQuest AS2000 autosampler. For both PCBs and PBDEs, the chromatographic separation was achieved using a 30m DB-5MS column (0.25 mm ID, 0.25 µm film thickness) with helium as the carrier gas. The ion source was operated in electron impact ionisation mode and the ion trap in MS/MS mode. Concentrations of PCB and PBDE congeners were calculated using relative response factors (RRFs) determined from a multiple-point calibration curve.

*Analysis of nitrogen stable isotopes*. Animals analysed for POPs were also analysed for stable nitrogen isotopes, $^{14}N$ and $^{15}N$, except that hair samples were used instead of blubber. Stable nitrogen isotopes were determined according to the method reported in Lesage et al.[5]. In brief, dried samples were combusted in an elemental analyser and sample gases introduced in an isochrom continuous-flow stable isotope mass spectrometer coupled to Carlo Erba elemental analyser. All samples were standardised against $N_2$ in air and stable nitrogen isotope ratios ($\delta^{15}N$) were expressed, by convention, in delta notation (‰).

*Statistical analysis*. Differences in biological parameters and contaminant levels among seals collected from the different sampling sites were assessed by analysis of variance (ANOVA), on logarithmic transformed data for $\delta^{15}N$, sum of PCBs (ΣPCBs) and sum of PBDEs (ΣPBDEs), followed by a Bonferroni's post-hoc comparisons test. Differences in patterns of PCBs and PBDEs were assessed for by principal component analysis (PCA). Principal component scores were also subjected to an ANOVA followed by a Bonferroni's post-hoc comparisons test. Statistical significance was set at $\alpha$=0.05. Outliers, characterised by studentised residual higher than three, were removed from the ANOVA analyses. Linear discriminant function analyses were used to determine the proportion of seals that were correctly classified to the appropriate sampling site based on the level and pattern variables (parameters) for which significant differences were observed among seal sampling groups. Statistical analyses were conducted using SYSTAT for Windows (version 10, SPSS Inc., 2000).

Table 1. Biological and contaminant data (average ± 95% confidence interval) of adult male harbour seals from three sampling sites of eastern Canada.

| Sampling site | Bic area, St Lawrence Estuary (SLE) | Charlottetown area, southern Gulf of St Lawrence (SG) | Placentia Bay and the surrounding south coast of Newfoundland (NF) |
|---|---|---|---|
| Number of animals | 8 | 10 | 10 |
| Weight (kg) | 81.6 ± 9.8a | 73.4 ± 7.1a | 81.5 ± 8.8a |
| Length (cm) | 146 ± 5a | 149 ± 4a | 153 ± 5a |
| Lipid content (%) | 93.7 ± 1.5a | 91.8 ± 1.3a | 88.4 ± 1.9b |
| $\delta^{15}N$ (‰) | 16.1 ± 0.2ab | 16.5 ± 0.2a | 15.9 ± 0.4b |
| ΣPCBs (ng/g lipid) | 13900 ± 4350a | 6830 ± 2290b | 5200 ± 2080b |
| ΣPBDEs (ng/g lipid) | 677 ± 127a | 477 ± 129a | 276 ± 45b |

Reading across, non-significant differences are followed by the same letter.

**Results and Discussion**

*Weight, length, lipid content and $\delta^{15}N$.* Biological parameters of seals, such as weight and length, were statistically identical among the three seal groups investigated suggesting that the examined male harbour seals were of comparable age and condition. The average lipid content of blubber samples was significantly lower in NF than in SLE and SG sampling groups. Significant differences were also found in $\delta^{15}N$ among the seal sampling groups. $\delta^{15}N$ increases from prey to predator because of the enrichment of $^{15}N$ relative to $^{14}N$ and therefore represents an index of the trophic position of an organism[5]. It is generally accepted that 2-3 $\delta^{15}N$ units are required to distinguish two trophic levels[6]. The magnitude of the $\delta^{15}N$ differences observed among the three seal groups indicates that all the examined animals occupied essentially the same trophic position.

*Levels.* PCBs were found at higher concentrations in blubber of seals from the SLE than in seals from both SG and NF sampling sites for which no difference was observed (Table 1). Although the use of PCBs in Canada has been regulated since the 1970s, PCBs are still ubiquitous in the environment. The elevated concentrations of PCBs in seals from the SLE population are consistent with the fact that this site is located downstream of Lake Ontario and the St Lawrence River, among the most populated and industrialised watershed in Canada. PBDEs represent a new family of POPs for which no regulation is enforced in North America. PBDEs are mainly found in plastic, foam and paint and as a result, levels are higher near larger industrialised urban centres and would decrease as distance from these areas increases. This could possibly explain the lower PBDE contamination in seals from NF than from other seal sampling locations. Although the average lipid content in blubber of seals from the NF is the lowest, the impact of lipid normalisation on the average levels of POPs is not significant.

*Patterns*. PCA was used to further explore the differences between seals based on PCB and PBDE contaminant patterns, defined as the relative proportion of individual congeners for each contaminant group. For PCBs, two principal components explain 70% of the variance (Figure 1). No differences were observed among seal sampling groups for the first component (PC1) but the second component (PC2) shows clear differences in average scores (PCB patterns) among seals from the three groups. For PBDEs, three principal components explained about 74% of the variance. The first (PC1) principal component indicated differences between seals from SLE and

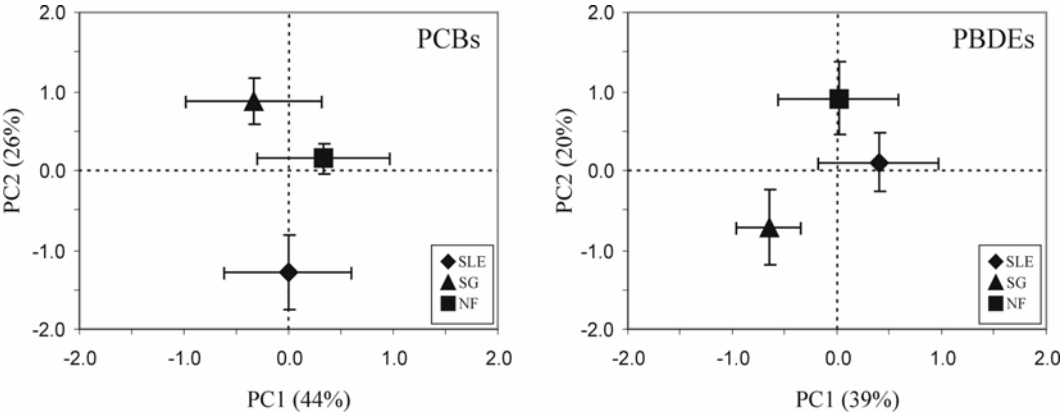


Figure 1. Principal component mean scores (± 95% confidence interval) of PCBs and PBDEs in blubber of harbour seals from St Lawrence Estuary (SLE), southern Gulf of St Lawrence (SG) and Newfoundland (NF).

SG whereas the second (PC2) principal component separated animals between SG and NF sampling groups (Figure 1).

*Classification of seals*. Five variables (parameters), *i.e.* levels of PCBs and PBDEs, and scores from PC1 (PCBs) and PC1 and PC2 (PBDEs), were considered in the classification of each one of the 28 animals examined. A linear discriminant analysis reveals that only three of the five variables, those related to PCA, were necessary to classify the animals into separate colonies. Classification results indicate that about 90% of the seals were correctly assigned to their sampling site. Specifically, one seal from the SLE and one seal from the SG were assigned to the NF colony whereas one seal from NF was assigned to the SG colony. These data indicate that seals collected from the three sampling sites (SLE, SG and NF) of Atlantic Canada were, in large part, adequately classified into distinct colonies on the basis of their patterns of persistent contaminants.

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