Characterization of legacy persistent organic pollutants (POPs) in northern bottlenose whales of the Western North-Atlantic

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Abstract

Desforges, JP., Hooker, S.K., Feyrer, L., Ferguson, S. H. 2021. Characterization of legacy persistent organic pollutants (POPs) in northern bottlenose whales of the Western North-Atlantic. Can. Tech. Rep. Fish. Aquat. Sci. 3436: viii + 32 p.

Northern bottlenose whales (*Hyperoodon ampullatus*) are a medium sized toothed whale of the North Atlantic. Two Canadian populations exist, one along the Scotian Shelf ("The Gully"; *Endangered*) and the other around Baffin Bay. The objective of this study was to characterize the concentrations and patterns of persistent organic pollutants (POPs) in northern bottlenose whales as related to sex, location, and sampling year. Samples from a stranded individual allowed opportunistic analysis of blubber stratification of POPs. Concentrations of POPs in northern bottlenose whales were similar to other toothed whales in the North Atlantic. Contrary to most marine mammals, levels of dichlorodiphenyltrichloroethane (DDT) were higher than polychlorinated biphenyls (PCBs), suggesting a local source of DDT in the region. Females were found to have lower blubber concentrations of most POPs compared to males due to reproductive offloading. Levels of POPs were also typically higher in Scotian Shelf compared to Arctic animals. Full-depth blubber analysis revealed higher concentrations and proportion of less recalcitrant POPs in the inner blubber relative to middle and outer sections. Future monitoring of these populations would benefit from greater sample sizes, more consistent temporal sampling, and complimentary analysis of biogeochemical tracers to decipher aspects of feeding ecology and habitat use.

Résumé

Desforges, JP., Hooker, S.K., Feyrer, L., Ferguson, S. H. 2021. Characterization of legacy persistent organic pollutants (POPs) in northern bottlenose whales of the Western North-Atlantic. Can. Tech. Rep. Fish. Aquat. Sci. 3436: viii + 32 p.

La baleine à bec commune (*Hyperoodon ampullatus*) est une espèce de baleine à dents de taille moyenne présente dans l'océan Atlantique Nord. Deux populations au Canada ont été désignées; une le long du plateau néo-écossais (« The Gully »; *en voie de disparition*) et l'autre autour de la baie de Baffin. L'objectif de cette étude était de caractériser les concentrations et les tendances de polluant organique persistent (POP) chez la baleine à bec commune au niveau de sexe, à l'emplacement et à l'année d'échantillonnage. Des échantillons provenant d'un individu échoué ont permis de réaliser une analyse fortuite de la stratification des POPs dans la graisse. Dans l'ensemble, les concentrations de POP hérités chez les baleines à bec communes étaient similaires à celles des autres baleines à dents de l'Atlantique Nord. Contrairement à la plupart des mammifères marins, les niveaux de dichlorodiphényltrichloroéthane (DDT) étaient plus élevés que les niveaux de biphényles polychlorés (PCB), ce qui suggère une source locale de DDT dans la région. On a observé que les femelles présentaient des concentrations plus élevées de POPs comparativement aux mâles à cause du transfert reproductif. Les niveaux de POP étaient aussi plus élevés chez les individus du plateau néo-écossais que ceux dans l'Arctique. Des sous-échantillons prélevés sur toute la profondeur de la graisse ont révélé des concentrations plus élevées et une proportion plus importante de POP moins récalcitrants dans la section intérieure de la graisse par rapport à la section médiane et extérieure. La surveillance future de ces populations bénéficierait d'échantillons de plus grande taille, d'un échantillonnage temporel plus cohérent, et des analyses complémentaire de traceurs biogéochimiques afin de mieux comprendre les aspects de l'écologie alimentaire et de l'utilisation de l'habitat.

Introduction

Persistent organic pollutants (POPs) are a class of organic chemical that share physicochemical properties, show long term persistence in the environment and biota, are widespread in distribution , accumulate in tissue of organisms, and are toxic to humans and wildlife. It is the dangerous combination of these properties that led to national regulation of many POPs starting in the 1970s and continuing today and signing of a global treaty to eliminate the manufacture and use of select POPs in 2001 named the Stockholm Convention on POPs (http://www.pops.int/). Major classes of POPs include polychlorinated biphenyls (PCBs), an industrial chemical used primarily as heat exchange fluids and additives in commercial products, and organochlorine pesticides such as dichlorodiphenyltrichloroethane (DDT), Chlordane, Dieldrin, Toxaphene, hexachlorobenzene (HCB), and hexachlorocyclohexane (HCH), among others.

Due to their widespread production and use around the world, POPs are ubiquitous in the natural environment and can be detected in biotic and abiotic samples from pole to pole. Because of their propensity to accumulate in fatty tissue, POPs are readily taken up by biota and accumulate to greater and greater concentrations throughout the food chain through a process known as biomagnification (Mackay and Fraser 2000; Macdonald et al. 2002). Marine mammals are particularly susceptible to accumulate elevated levels of POPs for several reasons (Muir and Norstrom 1994; Ross et al. 2000). First, they have a relatively long lifespan such that persistent chemicals like POPs can accumulate year after year. Second, they are often top predators in long marine food webs, resulting in high biomagnification potential. Third, they have large lipid reserves stored as blubber tissue that preferentially accumulate and store lipophilic POPs. Lastly, they lack the metabolic capacity to efficiently breakdown most POPs. As such, marine mammals inhabiting locally contaminated areas are among the world's most POP contaminated organisms (Martineau et al. 1987; Ross et al. 2000; Jepson et al. 2016). Accumulation of high levels of POPs is of concern because of their demonstrated broad toxicity, including effects on reproduction, immunity, carcinogenicity, and ultimately survival and population growth (Helle et al. 1976; De Guise et al. 1995; Ross et al. 1996a; Letcher et al. 2010).

Beyond indicating potential health risks, POPs have been used as intrinsic biogeochemical tracers, often in combination with other tracers such as fatty acids and stable isotopes, to study wildlife feeding ecology, habitat use, movement patterns, and population delineation (Herman et al. 2005; Krahn et al. 2007; Ramos and González-Solís 2012; Ryan et al. 2013). As with other tracers, the utility of using POPs to understand the ecology of marine mammals stems from the understanding that these persistent compounds are transferred from prey relatively unchanged, thus representing a spatiotemporal signal for that particular species in their environment. For instance, POP patterns have been shown to differ markedly among pacific killer whale (*Orcinus orca*) ecotypes, allowing for classification of individuals into correct groups using POP pattern analysis alone (Herman et al. 2005). Combining information from POPs and other dietary tracers in tissues of varying turnover rates can provide a wealth of information about animal ecology.

Marine mammals are considered ideal sentinels for ecosystem health and global change. Sentinel or indicator species can be defined broadly as an animal that can identify potential health hazards to other animals and humans, and thus indicate the health of the environment or ecosystem (e.g. Basu et al. 2007). Importantly, sentinels can provide warning signals about potential impacts on individuals, populations, and ecosystems that can help identify, manage, and mitigate ecological and anthropogenic stressors (Bossart 2011; Fossi et al. 2017). Marine mammals are representative sentinel species because of their long lifespan, high trophic level, and propensity to respond to or accumulate environmental stressors (Fossi et al. 2017). Their long lifespan allows for the study of chronic or slow acting stressors such as diseases, contaminant accumulation, and climate change. Their high trophic level results in biomagnification of contaminants and environmental toxins which may otherwise be transitory or below detection limits in lower trophic organisms or abiotic media. Marine mammals have been shown to respond to various stressors both physiologically and behaviorally, including spatial dynamics linked to human activities (e.g. underwater noise disturbance) or biomarkers of exposure and effects of POPs (Ramos and González-Solís 2012). Ultimately, marine mammals are good sentinels for environmental health because they integrate data on multiple stressors for both exposure (e.g. type, amount, availability) and cumulative effects (e.g. sublethal and clinical health responses) (Basu et al. 2007).

Northern bottlenose whales (*Hyperoodon ampullatus*) are medium sized toothed whales and members of the least known family of marine mammals, beaked whales. They are found only in the North Atlantic, and in Canada are primarily distributed from Nova Scotia to Baffin Bay (Harris et al. 2013) (Figure 1). Two major populations have been delineated in the Western North-Atlantic. The Scotian Shelf population, listed as Endangered under the Species at Risk Act, is reliably found in deep waters of a submarine canyon off the coast of Eastern Canada called The Gully. Scotian Shelf individuals appear genetically distinct from individuals in Arctic waters around the Davis Strait (Feyrer et al. 2019). The deepwater habitat use of this species is related to the distribution of their primary prey, the deepwater squid from the genus *Gonatus* (Hooker et al. 2001).

The objective of this study was to characterize the concentrations and patterns of POPs in northern bottlenose whales in the Western North-Atlantic, with particular attention to differences due to sex, location, and sampling year. Blubber biopsy samples were analyzed for a suite of legacy POPs in Scotian Shelf and Davis Strait male and female adult northern bottlenose whales, sampled from 1997 to 2019. A stranded animal in the Scotian Shelf population provided an additional opportunity to examine possible stratification of POPs throughout the entire blubber layer.

Materials and Methods

Sample collection

Biopsy samples were collected from northern bottlenose whales in The Gully submarine canyon (n=24) and in the Davis Strait (n=7) (Figure 1). Gully sampling took place in August 1996, July-

August 1997, August 2002, August-September 2003, and July-August 2018. Davis Strait sampling took place in August 2003, September 2018, and July 2019. For the purpose of this study, individuals were grouped into two populations based on sample location, namely the Scotian Shelf (samples from The Gully and one stranded individual from Newfoundland) and Arctic (samples from Davis Strait). One female individual (whale ID 288) stranded live and in poor body condition in Fortune Bay (Newfoundland) and later died on August 12th 2019. The cause of death was unknown, with plastic debris and squid beaks found in her digestive tract, suggesting she was still feeding even if emaciated. A full depth blubber core was taken from the abdomen and divided into three sections (inner, middle, and outer blubber).

Sampling details are provided in (Hooker et al. 2008) and briefly described here. Biopsy darts (2.5 cm x 0.6 cm) were fired from a 150 lb crossbow or air rifle at ranged of 5-15 m. Darts targeted the flank near the dorsal fin and only in healthy adult animals (i.e. good body condition). Biopsy material was subdivided within an hour of collection, and a small blubber sample was stored at -20^{o}C in hexane-washed glassware until contaminant analysis.

Contaminant analysis

The samples from 1996 to 2003 were analyzed at Trent university following standard procedures as reported in Hooker et al. (2008). Briefly, blubber was ground with sodium sulfate and extracted on a glass column with 50:50 dichloromethane:hexane. Lipids were removed by gel permeation chromatography and sample subfractionation for POP analysis was done by silica-gel column chromatography. Final extracts were analyzed by gas chromatography with an electron capture detector (GC-ECD). Procedural blanks and a National Institute for Standards and Technology cod liver oil reference material (SRM 1588) were analyzed for quality control/quality assurance purposes.

The samples from 2018/19 were analyzed for POPs using USEPA Method 1699 and 1668 (US EPA 1999, US EPA 2007, US EPA 2010). Details on the application of these methods to marine mammal blubber can be found in the supporting information in Houde et al. (2019). In brief, biopsy samples were homogenized and spiked with ^{13}C- or deuterated OCPs (15 analytes) and 23 ^{13}C-labelled PCBs prior to Soxhlet extraction with dichloromethane (DCM). Spiking standards also included ^{13}C12-PCB-133 as a recovery standard for gel permeation chromatography (GPC) performance. Extracts were reduced by rotary evaporation to 2 ml and then subjected to lipid removal by GPC using Biobeads SX3. Extracts were further cleaned up and fractionated on activated silica. PCBs were determined by gas chromatography-high resolution mass spectrometry (GC-HRMS), and OCPs by GC-high resolution MS, in electron-ionization mode. Separation was achieved on a HP-5ms column (30m length x 0.25mm id x 0.25 µm film thickness). Two exact m/z's for each PCB homolog and OCP analyte were monitored throughout a pre-determined retention time window. OCPs for which a labeled analog was not available were determined using labeled compounds as internal standards an internal standard technique. Using the isotope dilution method all analytes were recovery corrected. Analyses were conducted by ALS Environmental (Burlington ON).

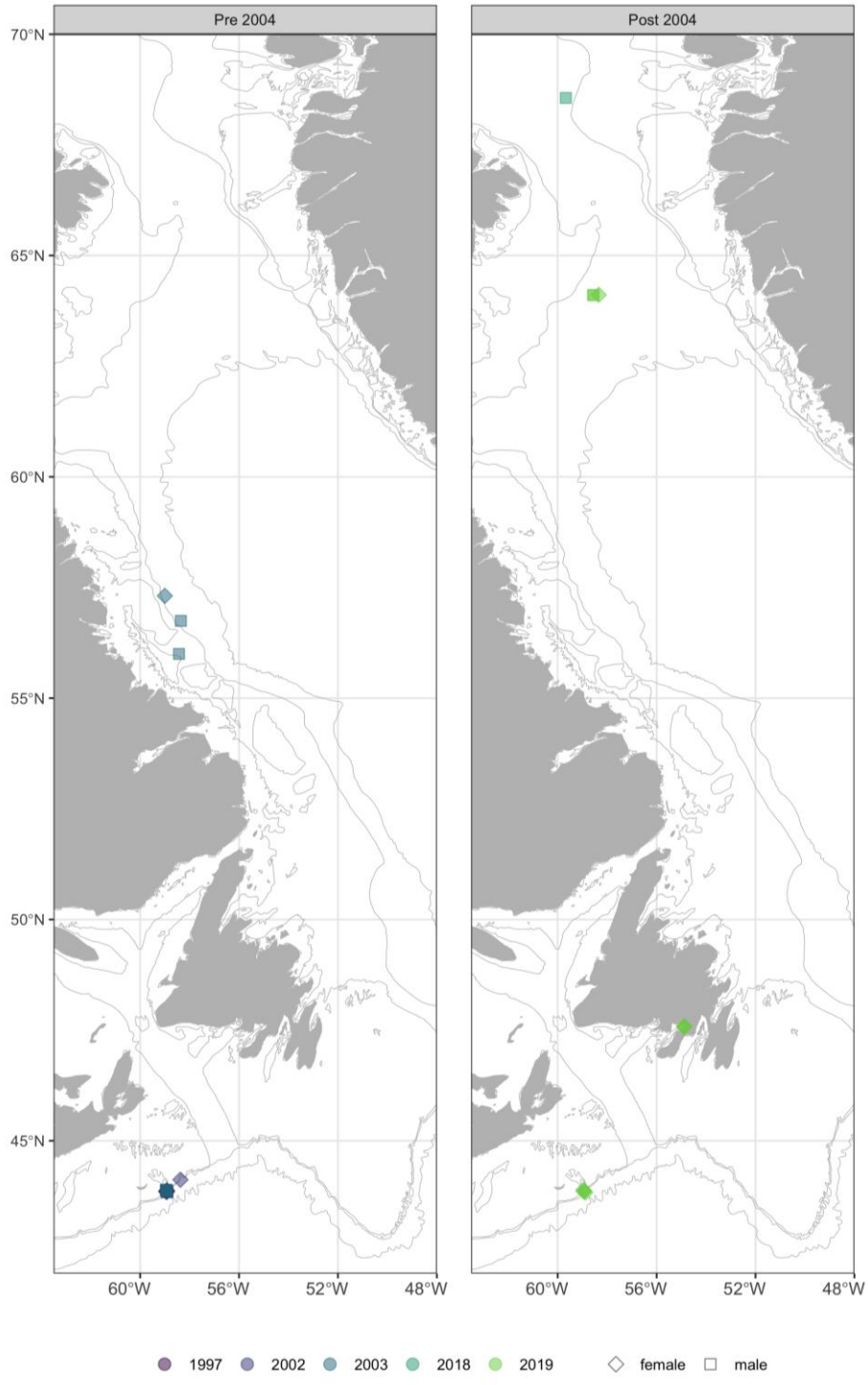

Figure 1. Map of samples collected from northern bottlenose whales for this study.

Samples were analyzed for PCBs (up to 162 congeners), DDTs (o,p'-DDE, p,p'-DDE, o,p'-DDD, p,p'-DDD, o,p'-DDT, and p,p'-DDT), Chlordanes (CHL; trans and cis Chlordane), hexachlorocyclohexane (HCH; α-, β and γ isomers), endosulfan (α- and β-endosulfan, endosulfan sulfate), heptachlor epoxide B (HEB), heptachlor, aldrin, dieldrin, endrin, methoxychlor, mirex, hexachlorobenzene (HCB), toxaphene (Parlar26, Parlar50, Parlar62), pentachloroanisole, and octachlorostyrene (OCS). A different number of PCB congeners were analyzed in 1996-2003 (n=18) and 2018/19 (n=162), thus the final set of congeners included only the 18 from the earlier analyses to maintain consistency between datasets (i.e. sumPCB = Σ_{18}PCB). These included PCB-52, 49, 44, 101, 99, 87, 110, 149, 118, 153, 105, 138, 156, 180, 170, 195, 194, and 209. Detection limit substitutions were made for compounds that were detected in over 70% samples, else they were given values of zero.

All POP concentrations were lipid weight corrected and raw data are available online at the following open access data repository: DOI: 10.5281/zenodo.4432402.

Data analysis

All tables and figures show the mean and standard deviation of lipid weight corrected POP concentrations. Differences in the concentration of POPs between sample groups (e.g. sex, year, location) was assessed using analysis of variance (ANOVA), followed by post hoc Tukey Honest Significant Difference tests. ANOVA analyses were carried-out excluding samples from year 2018/19 as both sexes were not present in each location. Prior to the ANOVA, a Shapiro-Wilks Test was used to verify the assumption of normality and a Levene's F Test for Equality of Variances to verify homogeneity of variance among groups. Log-transformations were performed when necessary to meet assumptions. Principal component analysis (PCA) was used to compliment concentration-based analyses through exploration of POP patterns among individual whales. Two PCAs were carried-out, the first using all quantified POPs and the second using only PCBs. For the PCB PCA, individual congener concentrations were first converted to proportions of Σ_{18}PCB. Data were centered and scaled prior to PCA. All data analysis and graphics production used the software program R (R core Team 2020).

Results

Blubber stratification

The concentration of ten abundant POPs in sectioned full depth blubber from WhaleID 288 is shown in Figure 2. Concentrations were highest in the inner blubber layer and lowest in the middle layer for most POP classes (Figure 2), though differences were relatively minor compared to the overall variability across individuals (see Table 1 for range across the sampled population).

To get more detailed insight into the stratification of POPs throughout the blubber, the compositional profile of specific POP classes can be compared between layers. The congener profile for PCBs differed very little between blubber layers (Figure 3). Of the small noticeable differences, there was a relatively higher proportion of heavy and more persistent congeners in the outer blubber layer compared to inner and middle layers. Similarly, the compositional pattern of DDT compounds differed little between blubber layers (Figure 4).

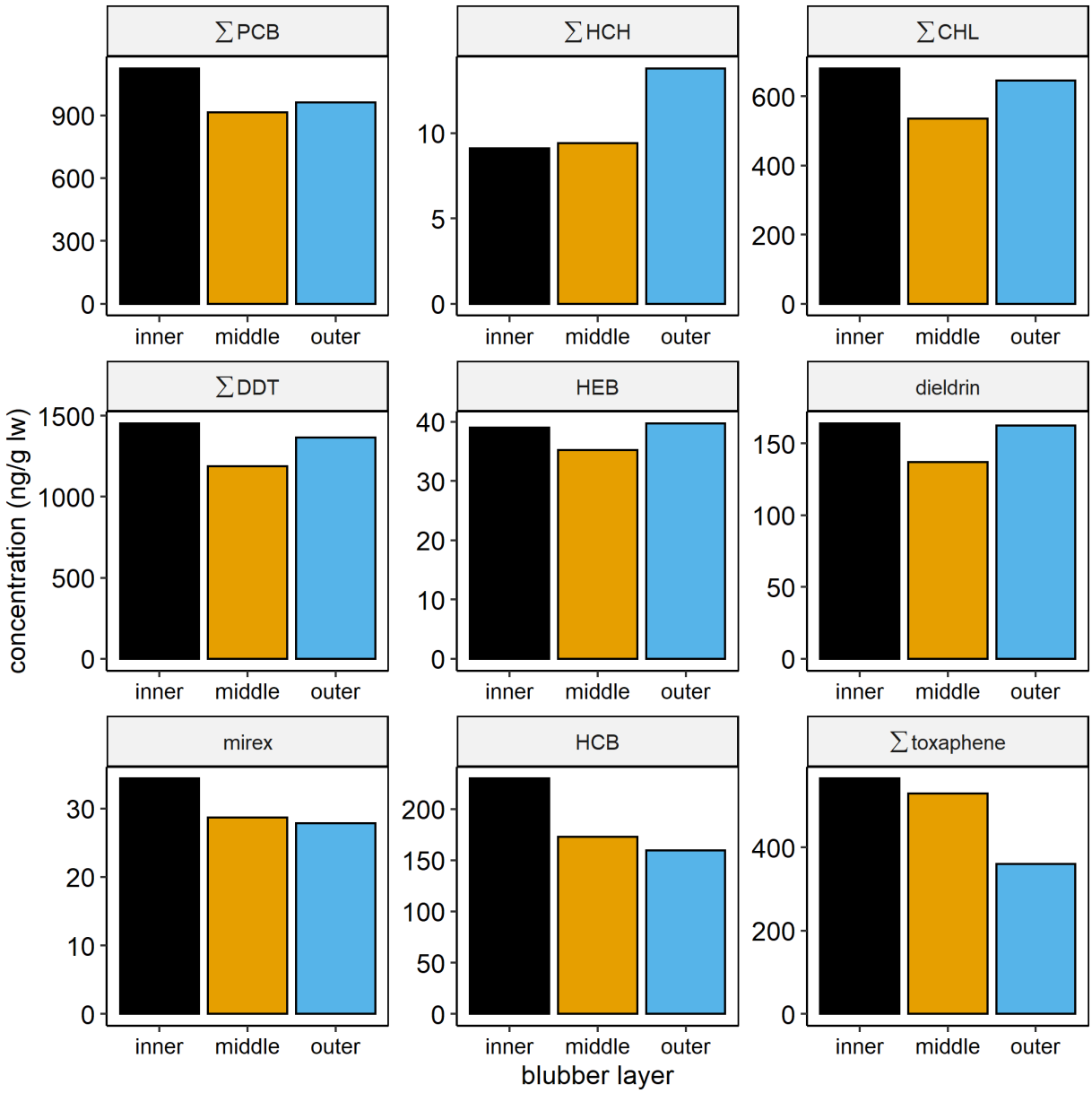

Figure 2. Blubber stratification of abundant persistent organic pollutant (POP) concentrations in northern bottlenose whale ID 288.

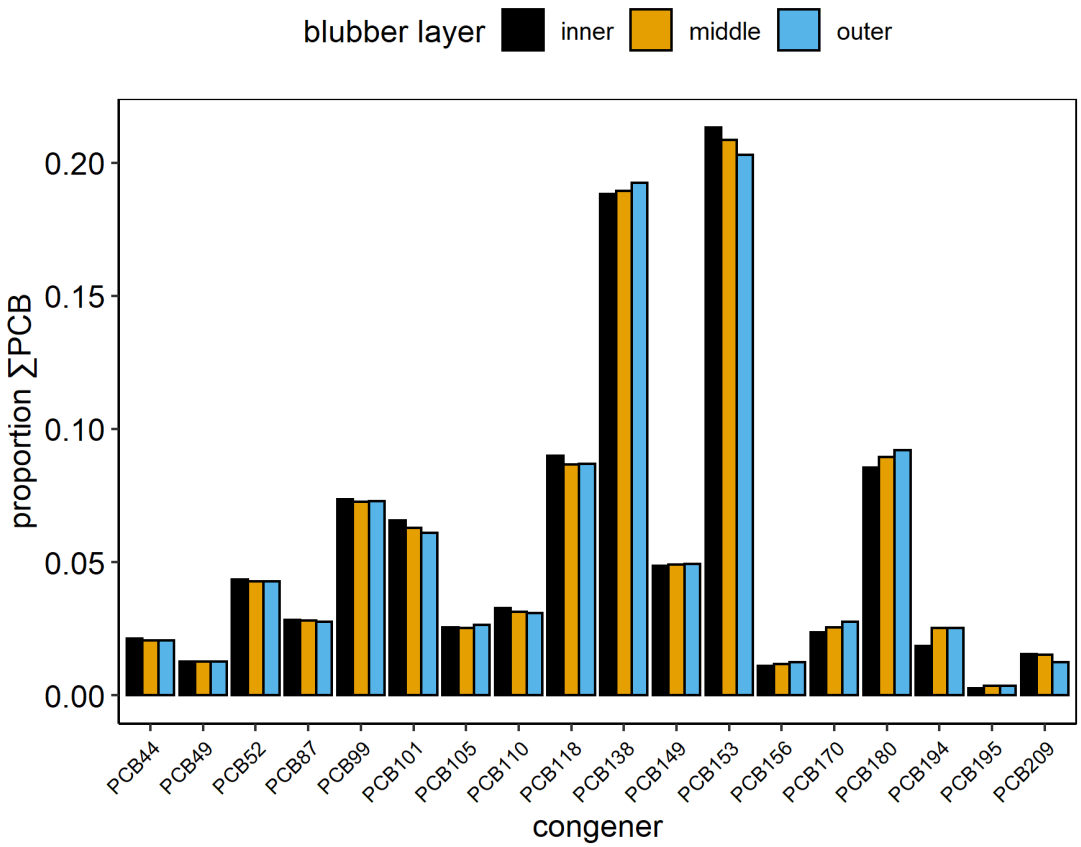

Figure 3. Blubber stratification in the composition of polychlorinated biphenyls (PCBs) in northern bottlenose whale ID 288. Composition is depicted as the concentration of each PCB congener relative to the sum of all congener concentrations (Σ_{18}PCB).

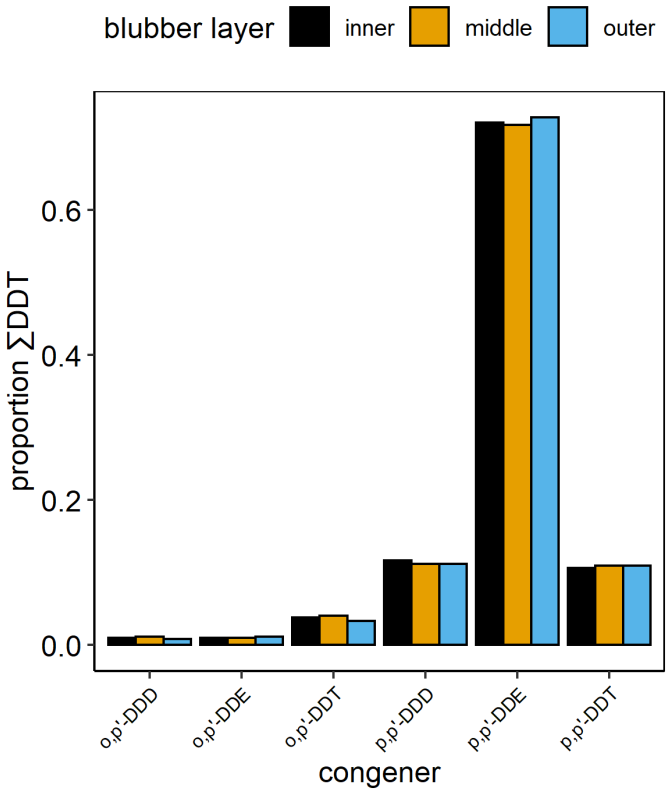

Figure 4. Blubber stratification in the composition of Dichlorodiphenyltrichloroethane (DDT) compounds in northern bottlenose whale ID 288. Composition is depicted as the concentration of each DDT compound relative to the sum of all compound concentrations (ΣDDT).

Individual temporal variability

Two individuals were sampled at least twice over time, allowing for analysis of individual variability in contaminant concentrations and composition. In addition to multiple yearly sampling, whale180 was sampled twice in the same year, separated only by a few days. Unexpectedly, the concentration of POPs varied widely between samples collected in the same year (Figure 5). For example, ΣDDT doubled between sampling events, while Σ_{18}PCB differed very little. Similar to intra-annual variation, the inter-annual variability of POP concentrations was marked for both individuals, but was not consistent across POPs (Figure 5). Σ_{18}PCB decreased slightly for whale180 between 1997 and 2002/3, while it increased in whale181. ΣChlordane increased substantially in whale180, but not whale181, while the opposite was observed for heptachlor epoxide B, mirex, and HCB.

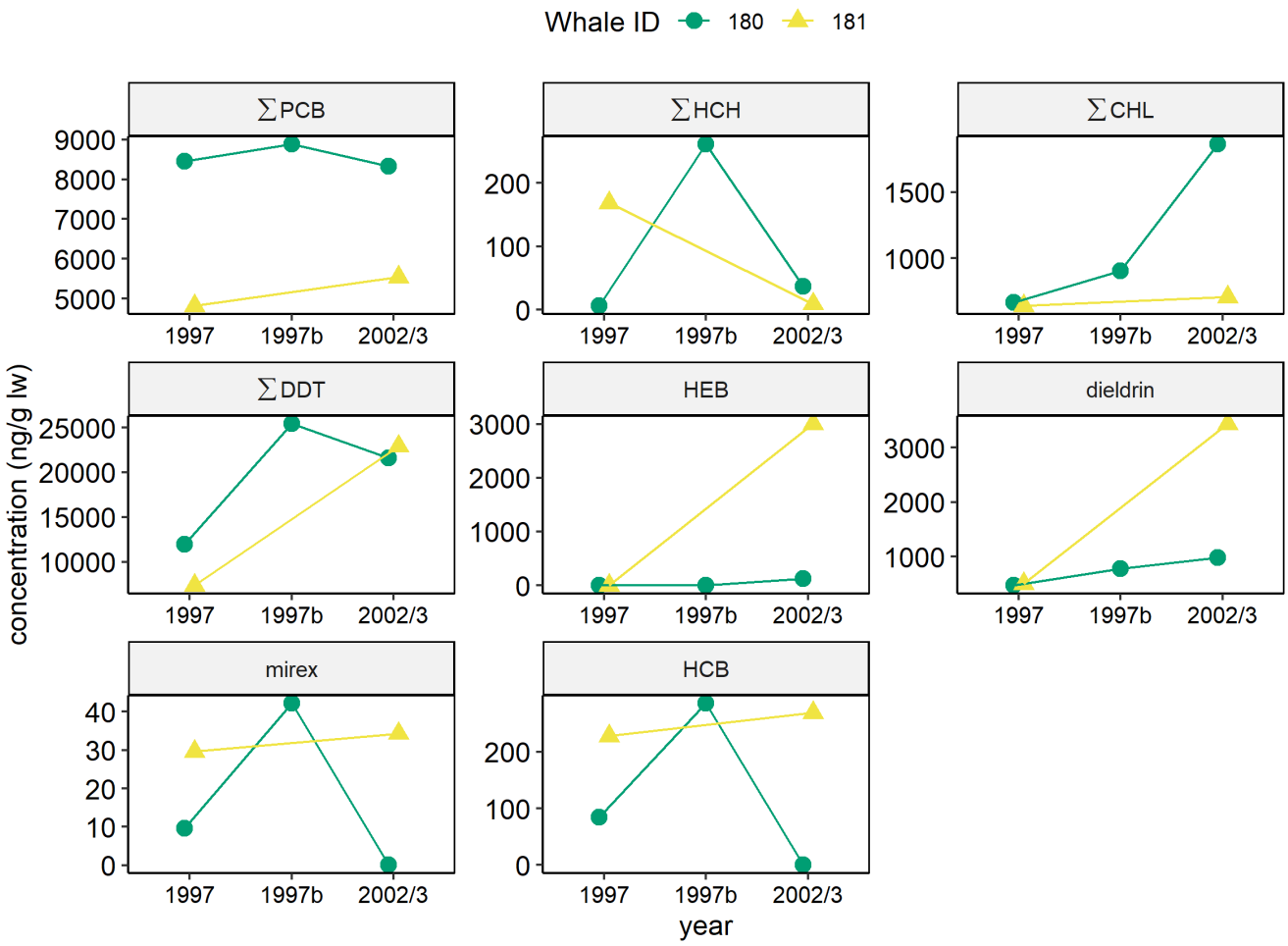

Figure 5. Temporal variability in persistent organic pollutant (POP) concentrations in re-sampled northern bottlenose individuals. WhaleID 180 was sampled twice in 1997 and the second instance is denoted by '1997b'.

The composition of PCBs was more similar in blubber samples collected in the same year than between years (Figure 6). The temporal variation in PCB congeners was consistent between individuals and characterized by increased proportions of the dominant congeners PCB99, PCB138, and PCB153, and decreased proportions of PCB44, PCB49, PCB101, PCB105, PCB110, PCB170, PCB194, PCB195, and PCB209 over time. The composition of DDT compounds varied intra-annually and inter-annually (Figure 7). All the dominant DDT compounds fluctuated widely between the first and second sampling within the same year for whale180, characterized by decreased o,p'-DDT, p,p'-DDD, and p,p'-DDT, and increased p,p'-DDE. Sampling of the same individual five years later showed the proportion of p,p'-DDD and p,p'-DDT decreased further and p,p'-DDE increased. The pattern was reversed for the second re-sampled individual in which p,p'-DDE decreased over time and p,p'-DDT increased (Figure 7).

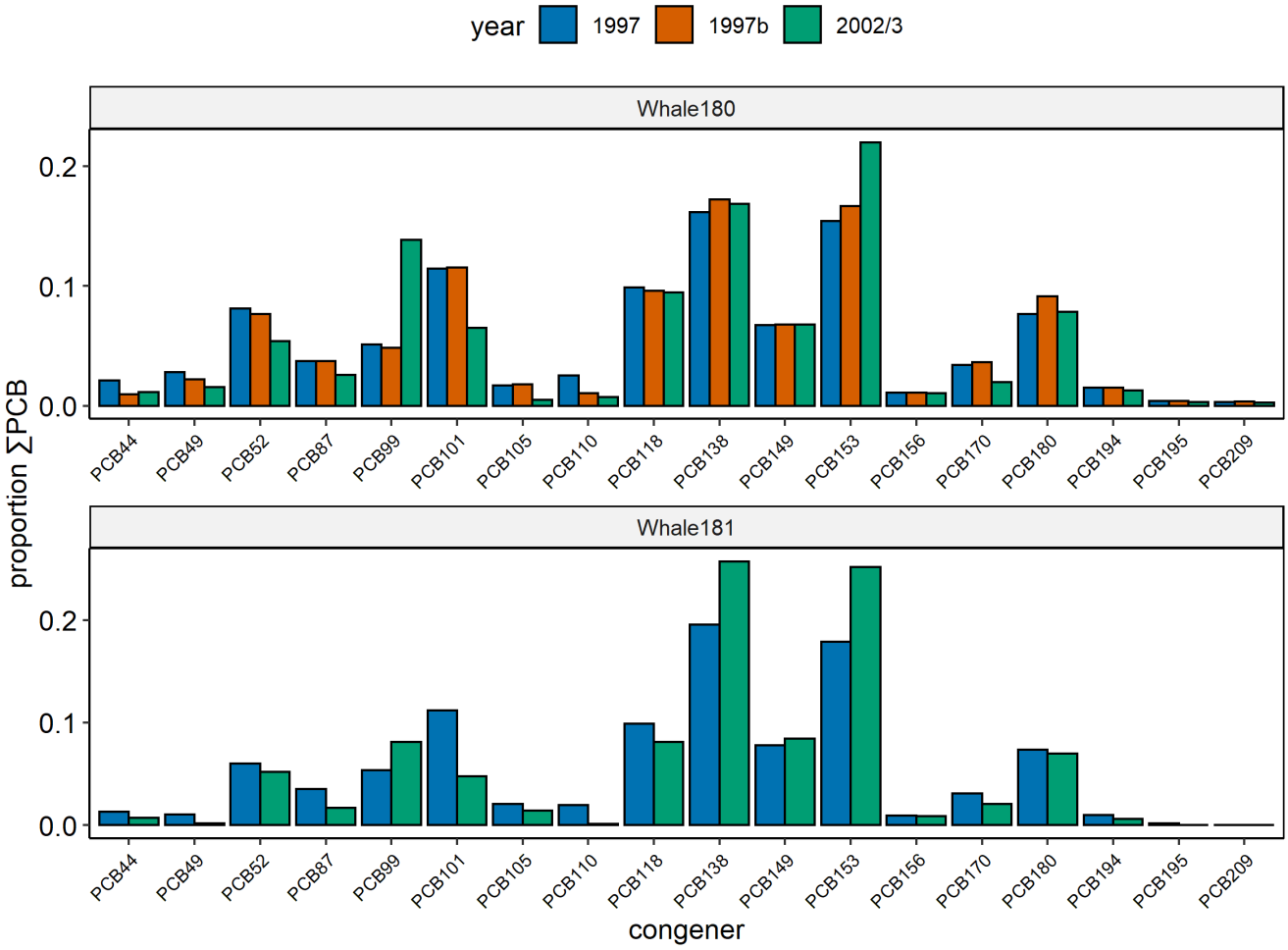

Figure 6. Temporal variability in the composition of polychlorinated biphenyls (PCBs) in re-sampled northern bottlenose individuals. Composition is depicted as the concentration of each PCB congener relative to the sum of all congeners (Σ_{18}PCB). WhaleID 180 was sampled twice in 1997 and the second instance is denoted by '1997b'.

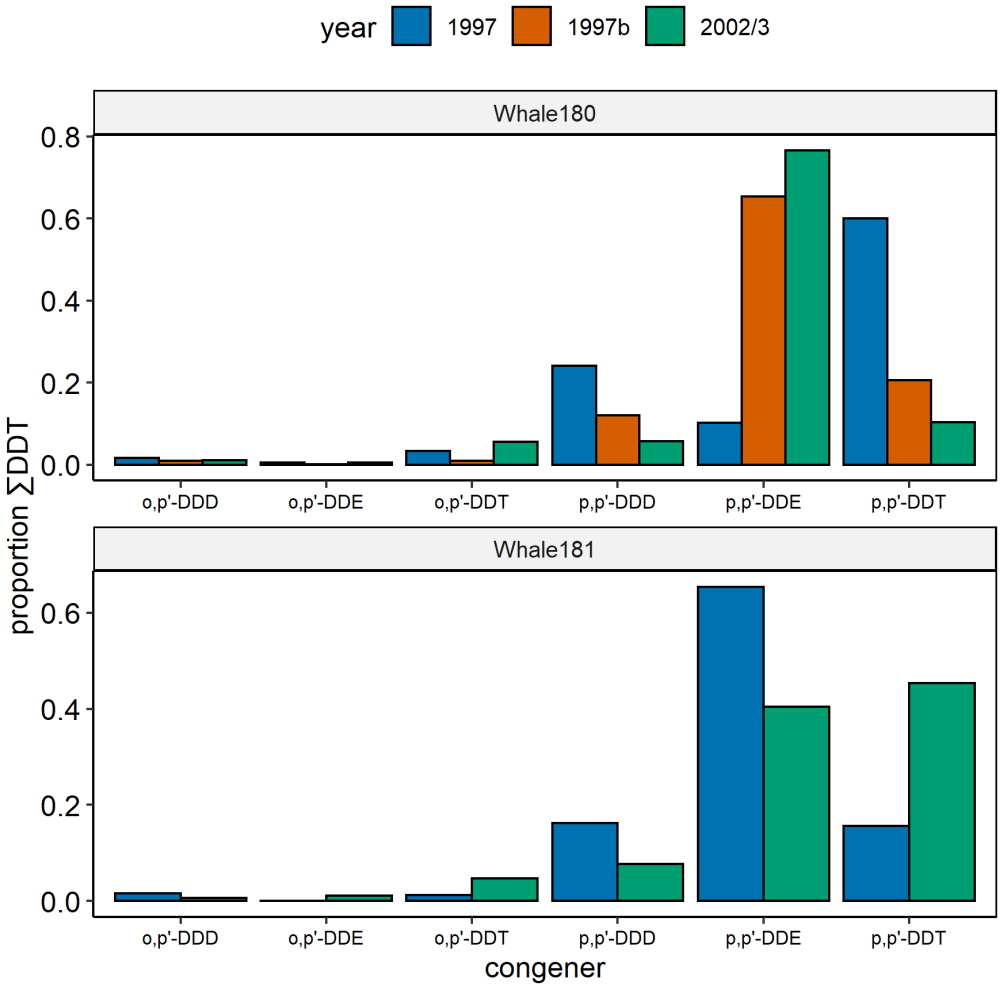

Figure 7. Temporal variability in the composition of dichlorodiphenyltrichloroethane (DDT) in re-sampled northern bottlenose individuals. Composition is depicted as the concentration of each DDT compound relative to the concentration of the sum of all compounds (ΣDDT). WhaleID 180 was sampled twice in 1997 and the second instance is denoted by '1997b'.

Spatiotemporal variation in the population

A broad range of POPs were quantified in the blubber of northern bottlenose whales (Figure 8, Table 1). The major POP classes included Σ_{18}PCB, ΣHCH, ΣChlordane, ΣDDT, Σendosulfan, heptachlor epoxide B, dieldrin, endrin, methoxychlor, mirex, HCB, octachlorostyrene, and ΣToxaphene. The rank order of the top five POPs based on total concentrations was ΣDDT > Σ_{18}PCB > ΣChlordane > dieldrin > HCB (Table 1). There was marked variability in POP concentrations, as evidenced by the large standard deviations for each POP relative to mean concentrations, even after reducing for confounding factors such as sex, sampling year, and location (Table 1, Figure 8).

The concentrations of major POPs separated by location, sex, and sample year are shown in Figure 8. It is evident that males had higher concentrations than females for almost all POPs, though statistically significant differences were only found for Σ_{18}PCB ($F_{1,19} = 13.88$, $p = 0.003$), ΣDDT ($F_{1,19} = 22.89$, $p = 0.0001$), and ΣChlordane ($F_{1,19} = 4.37$, $p = 0.05$); all other compounds were not significantly different between sex after accounting for location: ΣHCH ($F_{1,19} = 0.021$, $p = 0.89$), dieldrin ($F_{1,19} = 1.58$, $p = 0.22$), mirex ($F_{1,19} = 1.16$, $p = 0.29$), HCB ($F_{1,19} = 1.14$, $p = 0.30$), and heptachlor epoxide B ($F_{1,19} = 1.06$, $p = 0.32$). The lack of significant differences for these POPs can likely be attributed to small sample sizes (Table 1).

The effect of sampling year on the concentration of POPs was assessed only for the Scotian Shelf subpopulations (excluding 2018/19) due to the low sample size and missing sampling year (1997) for the Arctic population. After accounting for the effect of sex, there was a significant effect of sampling year for ΣDDT ($F_{1,16} = 5.36$, $p = 0.034$; 1997 < 2002/3), ΣHCH ($F_{1,16} = 15.59$, $p = 0.001$; 1997 > 2002/3), ΣChlordane ($F_{1,16} = 13.08$, $p = 0.002$; 1997 < 2002/3), heptachlor epoxide B ($F_{1,16} = 441.3$, $p < 0.001$; 1997 < 2002/3), mirex ($F_{1,16} = 8.84$, $p = 0.008$; 1997 > 2002/3), and HCB ($F_{1,16} = 116.13$, $p = 0.013$; 1997 > 2002/3). There was no significant effect for Σ_{18}PCB ($F_{1,16} = 0.005$, $p = 0.95$) or dieldrin ($F_{1,16} = 0.92$, $p = 0.35$).

The effect of population (or sampling location) on the concentration of POPs was assessed considering sex as a confounding factor. Year could not be included as a confounding factor because only 2002/03 data could be used for the analysis: data from 1997 had to be excluded as they were only present for the Scotian Shelf samples and data from 2018/19 had to be excluded as both sexes were not present in both locations. There was a significant difference between populations for Σ_{18}PCB ($F_{1,10} = 8.82$, $p = 0.014$; Scotian Shelf > Arctic), ΣDDT ($F_{1,10} = 31.35$, $p < 0.001$, Scotian Shelf > Arctic), HCH ($F_{1,10} = 7.68$, $p = 0.019$; Scotian Shelf > Arctic), ΣChlordane ($F_{1,10} = 45.24$, $p < 0.001$; Scotian Shelf > Arctic), and dieldrin ($F_{1,10} = 5.98$, $p = 0.035$; Scotian Shelf > Arctic). There was marginal significant difference between populations for heptachlor epoxide B ($F_{1,10} = 4.02$, $p = 0.073$; Scotian Shelf > Arctic) and no differences between populations for mirex ($F_{1,10} = 1.61$, $p = 0.23$) and HCB ($F_{1,10} = 2.08$, $p = 0.18$).

Table 1. Summary of sample details and concentration of abundant persistent organic pollutants (POPs) in northern bottlenose whales. 'Σ' is used to denote sum of many congeners or compounds within a class of POP. Concentrations are reported in ng/g lipid weight.

Location	year	sex	n	ΣPCB	ΣHCH	ΣChlordane	ΣDDT	heptachlor epoxide B	dieldrin	mirex	ΣHCB	ΣToxaphene
Scotian Shelf	1997	female	5	3790 ±1670	57.2 ±23.5	451 ±204	4140 ±1590	0.05 ±0.00	393 ±320	19.7 ±5.59	254 ±224	NA
Scotian Shelf	1997	male	4	6920 ±1740	227 ±159	940 ±314	13700 ±4730	0.05 ±0.00	684 ±205	31.0 ±6.40	249 ±80.8	NA
Arctic	2003	female	1	529	11.2	254	1080	49.9	107	13.7	35.7	NA
Arctic	2003	male	2	3560 ±268	9.78 ±1.74	260 ±8.56	3580 ±1040	117 ±1.05	192 ±22.6	19.7 ±9.94	560 ±16.5	NA
Scotian Shelf	2002/3	female	4	4050 ±1680	39.3 ±3.87	1130 ±239	7600 ±1490	228 ±182	627 ±144	3.55 ±6.93	46.6 ±93.2	NA
Scotian Shelf	2002/3	male	6	6840 ±3870	23.9 ±14.2	1720 ±695	21600 ±10800	1050 ±1200	1160 ±1200	23.7 ±21.8	325 ±413	NA
Arctic	2018/19	male	3	2874 ±2539	31.1 ±24.4	1283 ±1056	5441 ±5418	94.2 ±86.4	338 ±269	51.9 ±28.9	308 ±251	748 ±535
Arctic	2019	intersex	1	2293	31.9	954	4509	81.0	275	36.8	352	600
Scotian Shelf	2019	female	5	4077 ±5016	39.5 ±28.8	1549 ±1538	6104 ±6790	81.8 ±73.5	382 ±346	77.1 ±71.8	321 ±296	893 ±899
All samples			*31*	*4600 ±3200*	*59.6 ±85.4*	*1100 ±867*	*9510 ±8740*	*266 ±632*	*576 ±623*	*32.7 ±37.2*	*273 ±261*	*240 ±522*

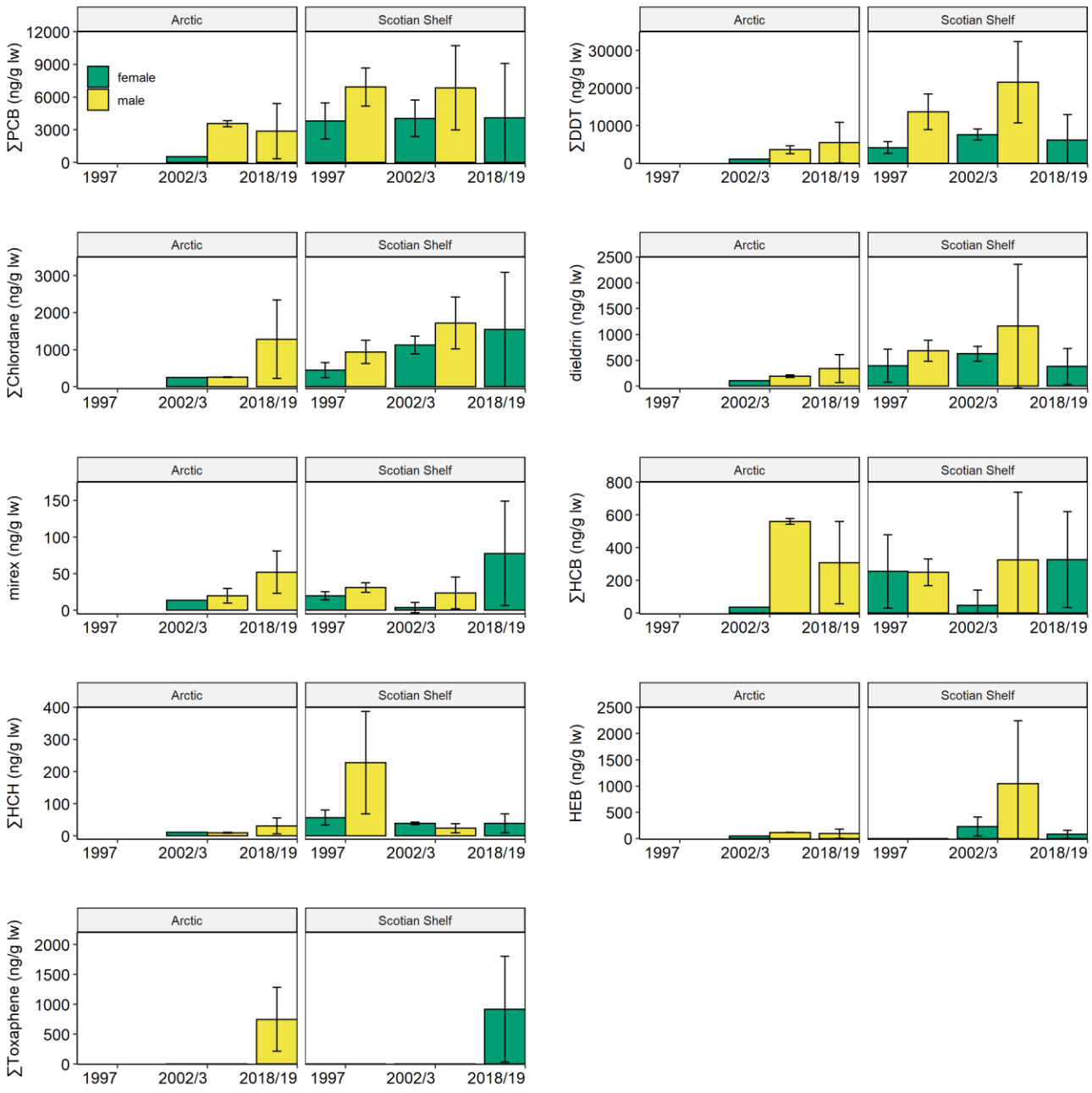

Figure 8. Spatiotemporal variability in the concentration of abundant persistent organic pollutants (POPs) as a function of year, location (population), and sex of northern bottlenose whales. Shown are the mean and standard deviation.

Principal component analysis was carried out to explore the major factors influencing the overall contaminant profile in northern bottlenose whales. While the sample size was low, the PCA using all POPs revealed some noticeable patterns (Figure 9). First, Scotian Shelf samples from 1997 were the only group that did not markedly overlap with the others. The separation of these samples was

driven by PC2, thus by endosulfans (endo), octachlorostyrene (OCS), o,p'-DDD, PCB195 and PCB194. Second, Arctic samples for both sampling years clustered close together, and with 2018/19 Scotian Shelf samples. These clustered furthest away from all POP classes suggesting lower overall concentrations. Overall, the first and second PCs explained ~62% of the variance in the data.

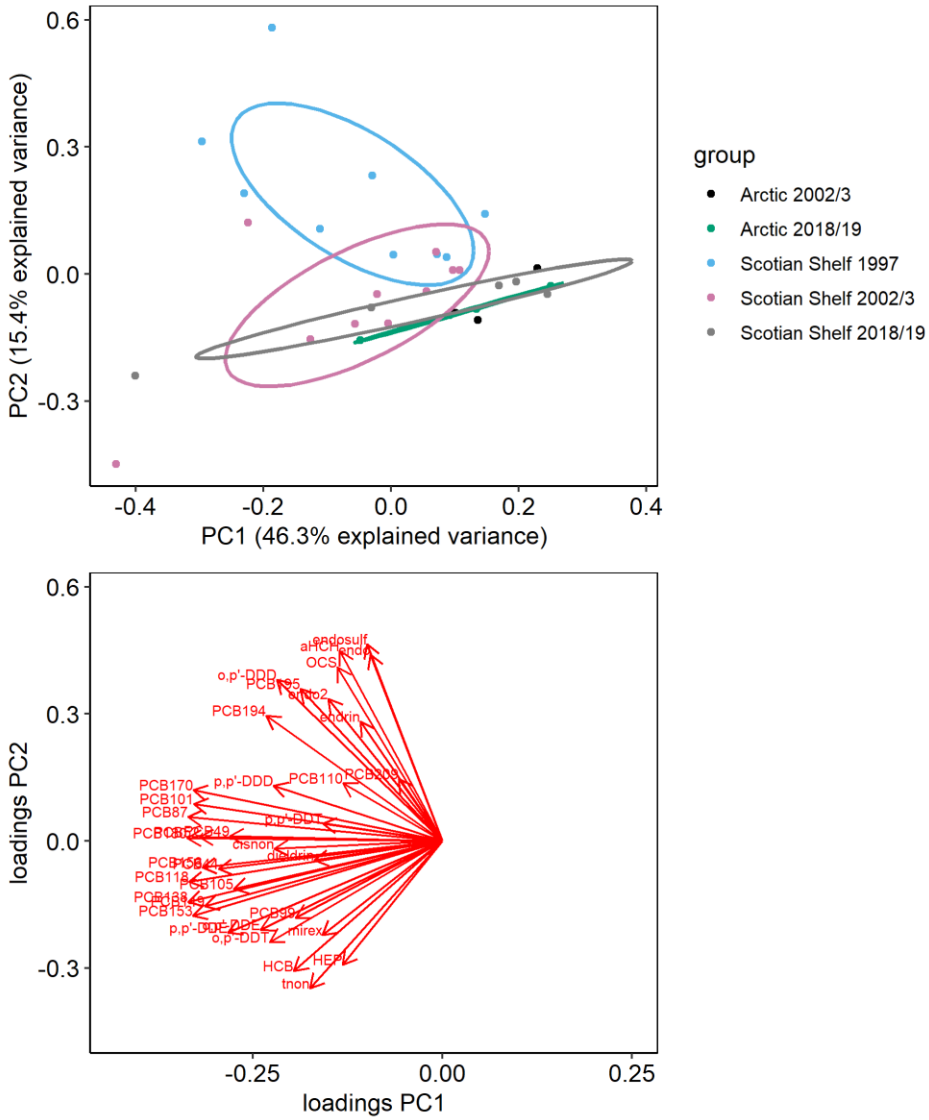

Figure 9. Principal component analysis (PCA) using abundant persistent organic pollutant (POP) concentrations of northern bottlenose whales. Shown are the PCA scores plot (top) and loadings plot (bottom). Ellipses represent a 95% confidence ellipse around each group assuming a multivariate normal distribution of the data. An ellipse could not be draw for Arctic 2002/03 due to insufficient sample size.

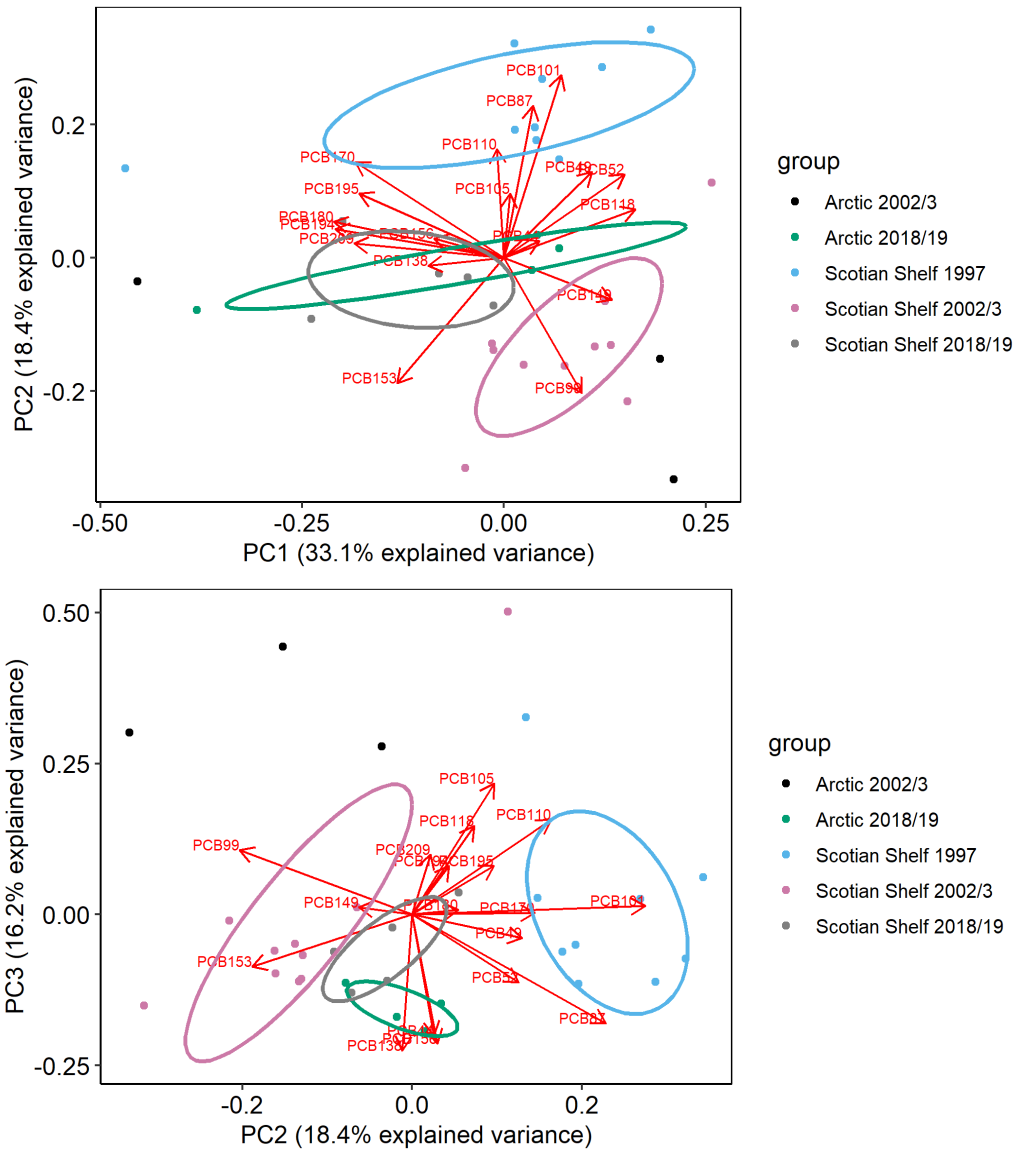

Figure 10. Principal component analysis (PCA) using the composition of polychlorinated biphenyl (PCB) congeners of northern bottlenose whales. Composition is calculated as the concentration of each congener divided by the sum of all congeners. PCA loadings are overlaid on the PC scores using red arrows. Top panel shows PC1 vs PC2 and bottom panel shows PC2 vs PC3. Ellipses represent a 95% confidence ellipse around each group assuming a multivariate normal distribution of the data. An ellipse could not be draw for Arctic 2002/03 due to insufficient sample size.

There was better cluster partitioning from a PCA using the compositional profile of PCBs (Figure 10). Using PC1 and PC2, all 2018/19 samples clustered close together, regardless of location/population, and Scotian Shelf 1997 and 2002/3 clearly separated from all other groups (Figure

10, top panel). The separation for Scotian Shelf 1997 samples was driven by PCB101, PCB87, and PCB110, while Scotian Shelf 2002/3 was driven primarily by PCB149 and PCB99. Further clustering of groups was achieved by plotting the second and third PCs (Figure 10, bottom panel). Here, all samples were clearly separated by year and location. Again, all 2018/19 samples clustered close together, while all other groups separated. Scotian Shelf 1997 samples were driven by PCB87, PCB101, and PCB170, Scotian Shelf 2002/3 by PCB153 and PCB149, and Arctic 2002/3 by PCB99.

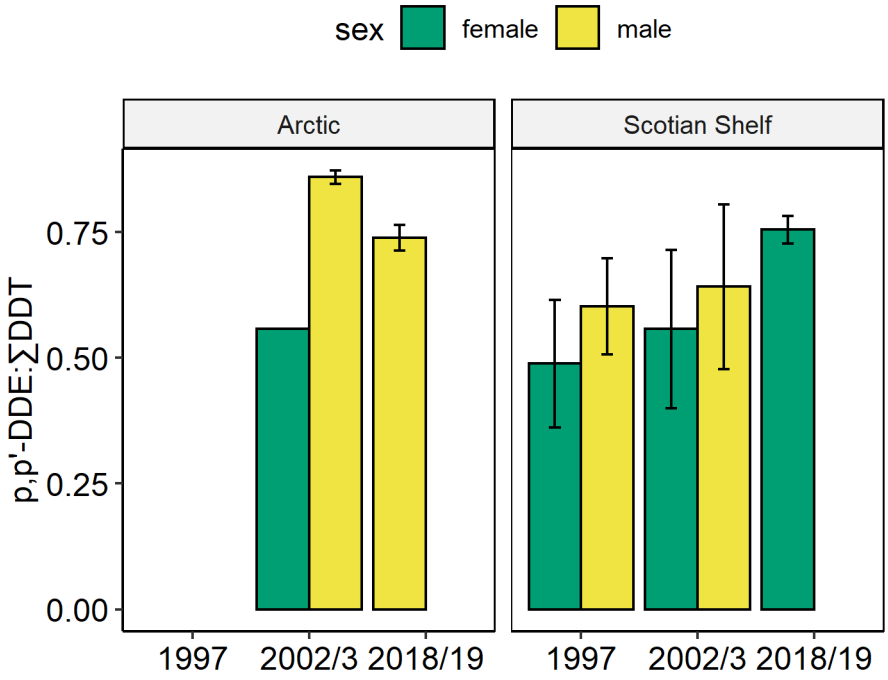

Figure 11. Spatiotemporal variability in the proportion of p,p'-DDE to ΣDDT as a function of year, location, and sex of northern bottlenose whales. Shown are the mean and standard deviation.

The ratio of p,p'-DDE, the dominant breakdown product of DDT, to ΣDDT divided by sex, year, and location (population) is shown in Figure 11. There were no major differences in the DDE/ΣDDT ratios between Scotian Shelf and Arctic individuals, with the exception of the relatively high ratio in 2002/3 Arctic males. Males generally had higher DDE/ΣDDT ratios than females, though there was high variability within location and years for both sexes. Overall, the DDE/ΣDDT ratio increased over time.

Discussion

Bottlenose whale contaminant concentrations

The concentration of POPs in the blubber of northern bottlenose whales of this study were typically greater than concentrations in the blubber of baleen whales of the North-Atlantic, and similar or lower than blubber concentrations in North-Atlantic odontocetes (Table 2). The concentrations of ΣPCB fell in the same range as reported for humpback whales in the Bay of Fundy/Gulf of Maine, harbour porpoises in Newfoundland and around the Faroe Islands, and pilot whales in Cape Cod. All other PCB concentrations in odontocetes were higher than in bottlenose whales, with particularly high levels for harbour porpoises in the Bay of Fundy, long finned pilot whales and white side dolphins from the Faroe Islands, and killer whales from East Greenland (Table 2). As with PCBs, the concentration of ΣDDT in northern bottlenose whales was higher than all baleen whales and similar to some odontocete populations while lower than others (Table 2). ΣDDTs were similar to concentrations reported in harbour porpoises in the St Lawrence and Bay of Fundy, sperm whales in Iceland and the coast of Spain, but lower than the elevated levels reported in East Greenland killer whales, long-finned pilot whales and white sided dolphins from Faroe Islands, and pilot whales from Cape Cod. Unlike PCBs and DDT, ΣChlordane concentrations in northern bottlenose whales were within the range of levels reported for most baleen whales and odontocetes in the North-Atlantic, with the exception of relatively high levels in killer whales of East Greenland and slightly higher levels in harbour porpoises of the St Lawrence and Bay of Fundy (Table 2). ΣHCH was similar across all whales where concentrations were reported in the North Atlantic (Table 2). It is important to note that the congener/compound profile for the different POPs may differ between studies, thus comparisons are mostly qualitative.

The higher levels of POPs in odontocetes compared to baleen whales is consistent with known bioaccumulation and magnification patterns for these highly lipophilic contaminants. As baleen whales feed on lower trophic level prey, they typically accumulate much lower concentrations of POPs than toothed whales that feed on higher trophic level fish or marine mammals. The trophic effect was most evident for PCBs and DDTs, the two dominant contaminant classes and more recalcitrant compounds. Killer whales in Greenland have been found to prey on seals and other marine mammals, explaining the extremely high levels of POPs in that species (Pedro et al. 2017). Beyond the trophic effect, there were evident geographical patterns in the concentration of POPs in North-Atlantic cetaceans. PCBs and DDTs were consistently higher in odontocetes of the eastern Atlantic compare to the western Atlantic, though there were apparent hotspots in the Bay of Fundy and the St Lawrence Estuary. The higher concentration of POPs in these areas are linked to the greater production, use, and environmental release of industrial and agricultural chemicals in highly populated and industrialized regions (Martineau et al. 1987; Westgate et al. 1997; Jepson et al. 2016).

Compared to other beaked whales from various locations around the world, the concentrations of POPs in the blubber of northern bottlenose whales of this study were generally on the lower end (Table 3). The concentrations of ΣPCB were similar to Blainville's beaked whales and Cuvier's beaked whales in Hawai, and Baird's beaked whales in Japan. Particularly high concentrations of ΣPCB were

reported in Japanese Stejneger's beaked whales and Mediterranean Cuvier's beaked whales (Table 3). ΣDDT and ΣChlordane levels in this study were also similar to Hawain beaked whales and lower than the high levels reported again for Mediterranean and Japanese beaked whales.

The ratio of ΣDDT:ΣPCB was ~2 in our study, which is higher than all other North-Atlantic cetaceans, with the exception of pilot whales from Cape Cod (Table 2), and similar to other beaked whales around the world, with the exception of high ratios in Japanese Stejneger's beaked whales (Table 3). It is typical for PCBs to be the dominant compound in marine mammals because it is more resistant to metabolic breakdown then other POPs, thus the ratio of individual POPs to PCBs is expected to decline with time (Borrell 1993). The high ΣDDT: ΣPCB ratio found in this study thus suggests that northern bottlenose whales off the coast of Eastern Canada are exposed to a local source of DDT. DDT was heavily applied by aerial spraying of forests in New Brunswick (1952-1968) and in neighboring areas of Quebec and Maine for control of the spruce budworm (Kurek et al. 2019). DDTs were also produced and used heavily in agriculture in the Northeastern United States (Westgate et al. 1997; Sericano et al. 2014), potentially leaving behind large environmental reservoirs of this compound.

Toxicity thresholds for most POPs are difficult to establish for long-lived marine mammals due lack of controlled exposure studies in these species. PCBs is one exception as it is well studied and several controlled feeding studies using seals, combined with field studies in highly contaminated areas for seals and cetaceans, have documented adverse health effects (Delong et al. 1973; Helle et al. 1976; De Guise et al. 1995; Ross et al. 1996b). Thus, commonly used toxicity thresholds for PCBs in marine mammals are 17 µg/g lipid weight for general immune and reproductive effects, and 41 µg/g lipid weight for reproductive impairment. While a few individuals in this study approached the lower toxicity threshold, there were no instances where blubber PCB levels surpassed it. More recently, a toxicity threshold for PCBs has been set as 1.3 µg/g lipid weight based on a suite of molecular based biomarkers (e.g. gene expression and vitamin levels) in marine mammal studies (Desforges et al. 2013; Brown et al. 2014; Noel et al. 2014). This relatively low toxicity threshold represents an early signal for physiological changes due to PCB exposure, but exposure at this level has not been linked to any adverse effects at more relevant apical endpoints (e.g. growth, reproduction, disease). Most northern bottlenose whales fell above the molecular toxicity threshold, suggesting that PCBs could be modulating physiological responses in these animals at the molecular and cellular level.

Table 2. Review of persistent organic pollutant (POP) concentrations in cetaceans of the North Atlantic. Concentrations are reported in µg/g lipid weight ± standard deviation. Where standard deviation was not available, the range of concentrations is reported.

Species	Feeding type	Location	Year	Sex	Sample size	ΣPCB	ΣDDT	ΣChlordane	ΣHCH	Source
Fin whale	baleen	Spain	1982-1984	m	69	2.03	1.98	.	.	Aguilar and Borrell 1988)
Fin whale	baleen	Spain	1982-1984	f	97	0.83	0.48	.	.	Aguilar and Borrell 1988
Fin whale	baleen	Iceland	1982-1986	m	48	1.26 ± 0.61	1.28 ± 0.46	.	.	Borrell 1993
Fin whale	baleen	Iceland	1982	f	3	0.94 ± 0.12	0.92 ± 0.14	.	.	Borrell 1993
Fin whale	baleen	Canada, St Lawrence	1991	mix	15	2.67 (0.2-10.2)	3.81 (0.6-13.1)	0.62 (0.2-1.9)	0.22 (0.08-0.4)	Gauthier et al. 1997
Humpback whale	baleen	USA, SW Gulf Maine	2005-2006	NA	10	5.40 ± 0.61	2.2 ± 0.26	1.0 ± 0.11	0.036 ± 0.010	Elfes et al. 2010
Humpback whale	baleen	USA, NE Gulf Maine	2005-2006	NA	10	10.00 ± 2.3	4.7 ± 1.3	2.00 ± 0.41	0.035 ± 0.007	Elfes et al. 2010
Minke whale	baleen	West Greenland	1998	m	8	3.49 (0.1-10.2)	0.99 (0.2-1.9)	0.49 (0.2-1.2)	0.099 (0.06-0.2)	Hobbs et al. 2003
Minke whale	baleen	West Greenland	1998	f	34	3.19 (0.4-22.8)	0.90 (0.06-3.3)	0.43 (0.03-1.7)	0.094 (<1-0.5)	Hobbs et al. 2003
Minke whale	baleen	East Greenland	1998	f	4	1.17 (0.7-1.9)	0.33 (0.09-0.7)	0.56 (0.08-1.4)	0.095 (0.04-0.2)	Hobbs et al. 2003
North Atlantic right whale	baleen	Canada, Bay Fundy	1988-1989	m	3	1.5	0.77	.	.	Woodley et al. 1991
North Atlantic right whale	baleen	Canada, Bay Fundy	1988-1989	f	1	2.5	1.44	.	.	Woodley et al. 1991
Sei whale	baleen	Iceland	1982-1985	m	14	0.46 ± 0.26	0.58 ± 0.29	.	.	Borrell 1993
Sei whale	baleen	Iceland	1982-1985	f	26	0.18 ± 0.09	0.16 ± 0.07	.	.	Borrell 1993
Beluga	odontocete	Canada, St Lawrence	2015-2017	m	45	10.51 ±1.19	4.35 ± 0.62	.	.	Simond et al. 2019
Harbour porpoise	odontocete	Canada, NFL	1989-1991	m	18	5.92 ± 2.84	4.59 ± 2.13	4.33 ± 1.99	0.43 ± 0.09	Westgate et al. 1997

Species	Feeding type	Location	Year	Sex	Sample size	ΣPCB	ΣDDT	ΣChlordane	ΣHCH	Source
Harbour porpoise	odontocete	Canada, NFL	1989-1991	f	11	6.20 ± 4.94	3.54 ± 2.56	3.08 ± 2.02	0.42 ± 0.21	Westgate et al. 1997
Harbour porpoise	odontocete	Canada, St Lawrence	1989-1991	m	31	12.02 ± 6.14	7.94 ± 4.45	5.71 ± 2.74	0.58 ± 0.18	Westgate et al. 1997
Harbour porpoise	odontocete	Canada, St Lawrence	1989-1991	f	31	8.08 ± 4.05	5.11 ± 3.11	3.53 ± 2.07	0.40 ± 0.16	Westgate et al. 1997
Harbour porpoise	odontocete	Canada, Bay Fundy	1989-1991	m	55	19.53 ± 12.63	8.71 ± 4.11	6.85 ± 3.66	0.42 ± 0.15	Westgate et al. 1997
Harbour porpoise	odontocete	Canada, Bay Fundy	1989-1991	f	53	12.86 ± 5.44	6.28 ± 2.87	4.34 ± 2.15	0.35 ± 0.18	Westgate et al. 1997
Harbour porpoise	odontocete	Faroe Island	1987	m	3	13.39 ± 2.38	6.55 ± 0.73	.	.	Borrell 1993
Harbour porpoise	odontocete	Faroe Island	1987	f	3	8.83 ± 1.05	4.44 ± 0.38	.	.	Borrell 1993
Killer whale	odontocete	East Greenland	2012-2014	m	1	65.1	55.1	40.2	0.1	Pedro et al. 2017
Killer whale	odontocete	East Greenland	2012-2014	f	6	48.6 (19-65)	30.1 (16-50)	19.3 (1.4-39.1)	0.068 (0.03-0.1)	Pedro et al. 2017
Long-finned pilot whale	odontocete	USA, Cape Cod	1990-1996	mix	21	7.55 ± 7.14	18.34 ± 23.71	2.29 ± 2.24	0.10 ± 0.053	Weisbrod et al. 2000
Long-finned pilot whale	odontocete	Faroe Island	1987	m	52	48.81 ± 23.13	39.83 ± 19.23	.	.	Borrell 1993
Long-finned pilot whale	odontocete	Faroe Island	1987	f	159	26.27 ± 23.12	29.87 ± 16.35	.	.	Borrell 1993
Sperm whale	odontocete	Spain	1979-1980	m	8	9.93	5.1	.	.	Aguilar 1983
Sperm whale	odontocete	Spain	1979-1980	f	6	15.55	7.73	.	.	Aguilar 1983
Sperm whale	odontocete	Iceland	1982	m	10	10.51 ± 2.07	10.23 ±1.51	.	.	Borrell 1993
White sided dolphin	odontocete	Faroe Island	1987	m	8	42.68 ± 18.02	25.79 ± 10.33	.	.	Borrell 1993
White sided dolphin	odontocete	Faroe Island	1987	f	5	25.34 ± 21.22	20.68 ± 14.40	.	.	Borrell 1993

Table 3. Review of persistent organic pollutant (POP) concentrations in beaked whales. Concentrations are reported in µg/g lipid weight ± standard deviation.

Species	Location	Year	Sex-age class	Sample size	ΣPCB	ΣDDT	ΣChlordane	Source
Longman's beaked whale	Hawaii	2010	m	1	7.61	12.00	1.69	Bachman et al. 2014
Blainville's beaked whale	Hawaii	2010	m	1	1.45	2.48	0.31	Bachman et al. 2014
Cuvier's beaked whale	Hawaii	2008-2011	m	2	4.25 ± 5.36	6.18 ± 9.07	0.64 ± 0.73	Bachman et al. 2014
Sowerbys beaked whale	UK	1992-2002	mix	9				Law et al. 2005
Cuvier's beaked whale	UK	2002	m	1				Law et al. 2005
Northern bottlenose whale	UK	2001	f	1				Law et al. 2005
Cuvier's beaked whale	Bermuda	1981	m	3	9.61 ± 2.35	31.19 ± 12.55		Knap & Jickells 1983
Cuvier's beaked whale	Bermuda	1981	f	1	9.03	12.25		Knap & Jickells 1983
Baird's Beaked Whale	Japan	1985	NA	3	2.3 ± 2.8			Kannan et al. 1989
Stejneger's beaked whale	Japan	2000-2001	m	5	19 ± 20	110 ± 140	4.5 ± 4.9	Kajiwara et al. 2006
Stejneger's beaked whale	Japan	1984	m	1	7	44		Miyazaki et al. 1987
Hubbs' beaked whale	Japan	2015	m	1	13			Anezaki et al. 2016
Cuvier's beaked whale	Mediterranean	2014-2015	mix juvenile	4	11.82 ± 4.68			Baini et al. 2020
Cuvier's beaked whale	Mediterranean	2014-2016	m juvenile	8	25.11 ± 19.91			Baini et al. 2020
Cuvier's beaked whale	Mediterranean	2014-2017	m	6	28.17 ± 14.40			Baini et al. 2020
Cuvier's beaked whale	Mediterranean	2014-2018	f	2	5.64			Baini et al. 2020

Factors influencing contaminant profiles

There are many factors that can influence the concentration and composition of POPs in free-ranging marine mammals. One of the most well recognized factors is sex. Most of the POPs in this study were higher in males than females, consistent with the expected pattern. The lower concentrations in females is due to losses during reproduction, including transplacental transfer during gestation and transfer through milk during the lactation period (Aguilar and Borrell 1988; Borrell et al. 1995; Desforges et al. 2012). Since males do not offload a portion of their contaminant burden during reproduction, their tissue concentrations typically increase with age. Two males were sampled several times over the study period, offering potential insight into accumulation over time at the individual level. However, the temporal trends for the different POPs were not consistent between individuals, suggesting that age-related accumulation was not the dominant driver of contaminant patterns. One individual was sampled twice within the same year and displayed variability similar or greater to the inter-annual variation in POP concentrations. Body location (e.g. dorsal, ventral, anterior, posterior, etc.) has been shown to significantly influence wet weight concentrations of POPs in cetaceans, though fewer body location differences are apparent using lipid weight corrected concentrations (Ellisor et al. 2013). All animals in this study were sampled from approximately the same location on the body, though slight differences are expected given the nature of the field collection. It is therefore unlikely that body location is a major factor contributing to the lipid corrected concentration differences observed in re-sampled individuals. It is also assumed that re-sampled individuals did not vary dramatically in body condition (similar blubber lipid content observed), suggesting that fine-scale variation in diet could be the primarily driver of variability in tissue POP levels at the individual level. Diet is the dominant exposure route for highly lipophilic POPs, with trophic level being an important factor in determining the food web magnification of these chemicals (Ross et al. 2000; Macdonald et al. 2002). Thus, feeding on prey of different trophic levels could meaningfully influence POP exposure in bottlenose whales. Alternatively, differential exposure could occur if the whales or their prey move between different regions with different historical contaminant backgrounds. As described above, the Bay of Fundy and the St Lawrence are known contaminant hotspots that could expose prey species to high levels of PCBs and DDTs, which would increase the exposure of whales if they consumed those prey. While northern bottlenose whales are known to have high site fidelity and do not travel far distances to feed (Hooker et al. 2002), their primary prey the squid of genus *Gonatus* may be more widely dispersed.

The other two known factors that could influence POP levels in this study were location (population) and sampling year. The small sample size for each group (e.g. sex, year, and location) precluded strong statistical analysis, though there was a clear pattern of higher concentrations for most POPs in Scotian Shelf whales compared to those sampled in the Arctic. The temporal analysis did not reveal consistent patterns across all POPs, with some showing higher levels in 2002/03 compared to 1997 while others showing the opposite or not statistically significant change. The higher concentrations in animals in the Scotian Shelf is consistent with their closer proximity to urban and industrialized regions of North-America, the primary source of these legacy POPs. A previous study (using a subset of the dataset from this study) suggested that the temporal increase of some POPs may

have been due to offshore oil and gas exploration in the region that may have remobilized sediment-bound POPs, ultimately re-introducing legacy POPs to the food web (Hooker et al. 2008). An alternative explanation could be a change in diet over time, though data is lacking to explore this further. Unfortunately, the compositional profile of POPs (PCA analysis) did not provide additional insight into the temporal shift of POP exposure, revealing only that 1997 samples were relatively enriched in metabolizable POPs, thus all subsequent samples reflect a more 'aged' POP profile.

While only sex, location (population), and year were recorded in this study, a suite of other biological factors could have influenced the concentration of POPs. Important factors include age, body size, body condition, and migration patterns. As described above, POPs tend to accumulate with age (and thus body size) since they typically accumulate faster than they can be broken-down. All animals in this study were presumed adults, but the actual ages were unknown. Body condition, most often described as blubber thickness for marine mammals, is strongly correlated with blubber POP concentrations. As an animal loses blubber mass during periods of starvation, the highly retained POPs become more 'concentrated' in the remaining blubber, resulting in higher concentrations (Macdonald et al. 2002; Tartu et al. 2016). All animals were assumed to be in similar good body condition in this study based on visual observations prior to sampling, nonetheless this remains an unknown confounding factor. Lastly, individual movement patterns and dietary specialization or variation can lead to widely different contaminant exposure, especially when feeding on high trophic level prey or in local contaminant hotspots (Ross et al. 2000; Andvik et al. 2020). This will become increasingly important with shifting prey distributions in the North Atlantic and Arctic oceans due to climate warming (McKinney et al. 2015).

Blubber biopsies and blubber stratification

Biopsy samples typically capture the skin (a few cm) and variable depths of blubber for cetaceans, leading to questions of how representative the outer blubber samples are relative to the whole blubber depth. The results from this study, using a full depth blubber sample from one individual separated into three sections, are consistent with most literature showing that POPs are typically more concentrated in the inner portion of the blubber, while the profile of PCBs in the outer blubber section is relatively enriched in more chlorinated (recalcitrant) congeners (see Pedro et al. 2017 for review). The gradient of POPs is likely the result of variation in blubber lipid content and composition as well as the metabolic capacity between inner and outer portions of the blubber. The outer blubber portion is considered more metabolically inert and important for thermoregulation while the inner blubber is the more metabolically active site for lipid transfer from blood (Koopman et al. 1996; Koopman 2006). It is therefore expected that the inner blubber portion would fluctuate to a greater extent over time in terms of lipid and POP concentrations. Nonetheless, a recent study in killer whales found that lipid correcting blubber layer POP concentrations reduced the layer differences in POP concentrations and concluded that the overall difference between blubber sections was not meaningful for general risk assessments (Pedro et al. 2017). This is consistent with the findings from this study in which blubber

stratification was minor compared to inter- and intra-individual (temporal) variability in POP concentrations within a blubber layer.

While stranded animals offer ample sample opportunities (e.g., full blubber depth, large sample sizes, multiple tissues, etc.), there are important considerations required prior to comparing results with biopsies from live animals. The two most important, and inter-related, factors are cause of death and body condition. As previously discussed, body condition as measured by blubber lipid content and/or thickness can markedly influence concentrations of lipophilic POPs (Macdonald et al. 2002; Tartu et al. 2016). Thus, the blubber of stranded animals in higher states of decomposition is likely disrupted or decomposed such that it can no longer be considered relevant for realistic (i.e., representative of live animal) determination of POP concentrations. This is not the case for all stranded animals, particularly if they are found early after death and the cause of death is not related to nutritional deficiency (i.e., starvation) or diseases that may influence mobilization of blubber reserves. Indeed, cetaceans killed by ship strike or other acute trauma typically have high body condition relative to animals dying of other chronic causes (disease, starvation) (Raverty et al. 2020). The stranded animal in this study was noted to be in poor body condition, however its blubber lipid content was on the higher range of the whole dataset suggesting no major loss of total lipids. Furthermore, the concentrations of POPs in this individual were relatively low, suggesting there was no obvious bioconcentrating effect. It would therefore seem that the poor body condition of this animal did not cause any obvious signs of POP mobilization or change in POP composition expected in severely emaciated marine mammals. In summary, care must be taken to identify whether the cause of death and current state of stranded animals has resulted in substantial loss/mobilization of blubber lipid stores before comparing POP concentrations with biopsies from healthy living animals.

Limitations and recommendations

It is important to note the major caveats of this study to aid in interpretation of the results. Although the collected samples covered a large region and temporal period (1997-2019), sample size limited statistical testing for covariate effects such as sex, location, and sampling year. There were simply too few individuals per group to make meaningful interpretations of spatiotemporal patterns. A greater sample size would facilitate such statistical analysis and provide confidence in the observed patterns herein, which are currently speculative. The current study also lacked data on biological factors such as age, body size, and body condition that are known to influence POP concentrations in marine mammals.

Continued population monitoring for POPs would benefit from a more comprehensive approach that integrates multiple sources of information on animal diet and movement. Biogeochemical tracers, including stable isotopes and fatty acids, can be measured in skin and blubber samples and provide proxies for habitat use and dietary niche or indirectly quantify prey composition (Rubenstein and Hobson 2004; Newsome et al. 2010; Ramos and González-Solís 2012). Indeed, combined POPs and biogeochemical tracers have proven a powerful tool to identify subpopulation structure of marine

mammals (Herman et al. 2005). Tracking studies can further supplement dietary tracer data to confirm spatiotemporal habitat use and activity patterns, with proven implications for POP accumulation (e.g. Blévin et al. 2020). Thus, blubber POPs, biogeochemical tracers, and animal tracking offer complementary approaches to investigate population structure, dietary specialization, and ultimately health.

Blubber samples of northern bottlenose whales can also be used to quantify concentrations of emerging environmental contaminants of concern. Trophic magnification of contaminants results in higher concentrations in top marine predators like the northern bottlenose whale, thus tissue samples provide a unique opportunity to characterize the pollution 'status' of the North Atlantic. Since the legacy POPs quantified in the current study have all been banned for decades, replacement chemicals are now being used and found throughout the environment. One example are perfluorinated compounds (PFCs) like perfluorooctanesulfonate (PFOS) that are now widely found in biota and have been termed 'forever chemicals' because of their persistence (Bossi et al. 2005). Non-target analytical methods have also been used to screen marine mammal blubber samples for a broad range of chemical targets not usually quantified in standard/targeted approaches (Letcher et al. 2017; Escher et al. 2020). In this way, the northern bottlenose whale could serve as a sentinel for the health of the marine environment in Eastern Canada.

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