Author(s)/Höf:

Anaïs Remili, Rune Dietz, Christian Sonne, Filipa I. P. Samarra, Robert J. Letcher, Audun H. Rikardsen, Steven H. Ferguson, Cortney A. Watt, Cory J. D. Matthews, Jeremy J. Kiszka, Aqqalu Rosing-Asvid, Melissa A. McKinney*

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Varying diet composition causes striking differences in legacy and emerging contaminant concentrations in killer whales across the North Atlantic

Anaïs Remili [1*], Rune Dietz [2], Christian Sonne [2], Filipa I.P. Samarra [3], Robert J. Letcher [4], Audun H. Rikardsen [5], Steven H. Ferguson [6], Cortney A. Watt [6], Cory J.D. Matthews [6], Jeremy J. Kiszka [7], Aqqalu Rosing-Asvid [8], Melissa A. McKinney [1*]

[1] Department of Natural Resource Sciences, McGill University, Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada

[2] Department of Ecoscience, Arctic Research Centre, Aarhus University, DK-4000 Roskilde, Denmark

[3] University of Iceland, 900 Vestmannaeyjar, Iceland

[4] Ecotoxicology and Wildlife Health Division, Wildlife and Landscape Science Directorate, Environment and Climate Change Canada, Carleton University, Ottawa, ON K1A 0H3, Canada

[5] Faculty of Biosciences, Fisheries and Economics, UiT The Arctic University of Norway, 9037 Tromsø, Norway

[6] Arctic Aquatic Research Division, Fisheries and Oceans Canada, Winnipeg, MB R3T 2N6, Canada.

[7] Institute of Environment, Department of Biological Sciences, Florida International University, North Miami, FL 33181, USA

[8] Greenland Institute of Natural Resources, GR-3900 Nuuk, Greenland

*corresponding authors

E-mail contact: anais.remili@mail.mcgill.ca or melissa.mckinney@mcgill.ca

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Abstract

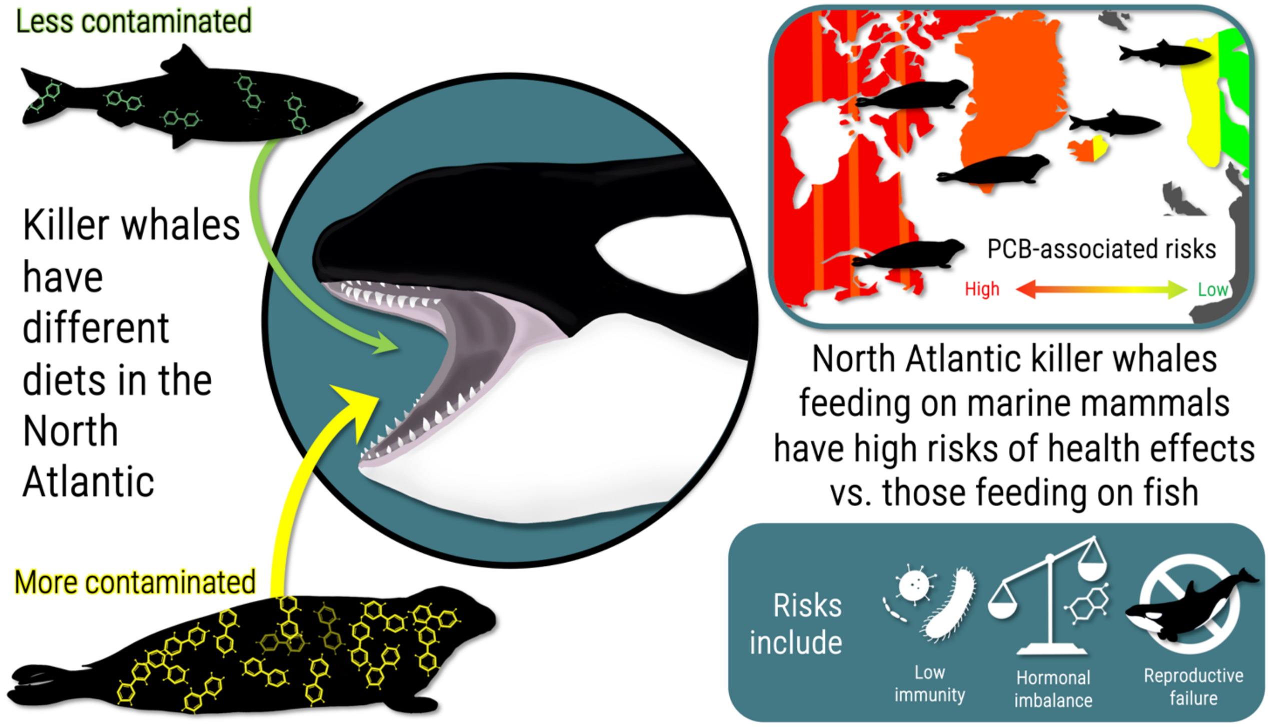
Lipophilic persistent organic pollutants (POPs) tend to biomagnify in food chains, resulting in higher concentrations in species such as killer whales (*Orcinus orca*) feeding on marine mammals compared to those consuming fish. Advancements in dietary studies include the use of quantitative fatty acid signature analysis (QFASA) and the differentiation of feeding habits within and between populations of North Atlantic (NA) killer whales. This comprehensive study assessed the concentrations of legacy and emerging POPs in 162 killer whales from across the NA. We report significantly higher mean levels of polychlorinated biphenyls (PCBs), organochlorine pesticides, and flame retardants in Western NA killer whales compared to eastern NA conspecifics. Mean ∑PCBs ranged from ~100 mg/kg lipid weight (lw) in the Western NA (Canadian Arctic, Eastern Canada) to ~50 mg/kg lw in the mid-NA (Greenland, Iceland), to ~10 mg/kg lw in the Eastern NA (Norway, Faroe Islands). The observed variations in contaminant levels were strongly correlated with diet composition across locations (inferred from QFASA), emphasizing that diet, and not environmental variation in contaminant concentrations among locations, is crucial in assessing contaminant-associated health risks in killer whales. These findings highlight the urgency for implementing enhanced measures to safely dispose of POP-contaminated waste, prevent further environmental contamination, and mitigate the release of newer and potentially harmful contaminants.

Keywords: Orcinus orca, biomagnification, PCBs, health risks, top predator, diet specialization, blubber, marine mammals

Synopsis (30 words)

Throughout the North Atlantic, killer whales feeding on dolphins and seals have higher contaminant concentrations than fish-feeding individuals, thereby increasing contaminant-associated health risks for these apex predators.

TOC



(Illustrations by A. Remili)

1. Introduction

Recent studies have highlighted the critical threat posed by consistently high concentrations of persistent organic pollutants (POPs) in at least some populations of killer whales [1, 2] (*Orcinus orca*), due to their high trophic positions [3] and limited biotransformation and elimination capacities [4, 5]. As the ocean's ultimate apex predators, killer whales from certain populations exhibit among the highest POP concentrations in the animal kingdom [3, 6, 7]. High levels of legacy contaminants like polychlorinated biphenyl (PCBs), organochlorines (OCs), polybrominated diphenyl ethers (PBDEs) and emerging flame retardants (FRs) have been associated with health issues that include increased risks of altered immune, endocrine, and reproductive functions in marine mammals, as well as carcinogenicity [3, 8, 9]. Toxicity effects from PCB exposure are estimated to occur past the 9 mg/kg lipid weight (lw) threshold in marine mammals and reach a high risk of reproductive failure past 41 mg/kg lw [3, 10-12]. While the thresholds for risks of health effects have not been established for other POP classes in marine mammals, previous *in vitro* research using killer whale and polar bear (*Ursus maritimus*) immune cells suggest that the immunotoxic effects of POP mixtures are greater than for a single compound [13]. Thus, there is a need to assess both the levels, toxicity, and risks caused by POPs for marine mammal populations around the globe.

Trophic ecology has been shown to play a large role in the accumulation of POPs in various killer whale populations [14-16]. Indeed, the lipophilic legacy POPs and legacy (e.g., PBDEs) and emerging FRs have tendencies to biomagnify, i.e., to increase in concentration with each trophic position. Thus, individuals feeding on high-trophic marine mammals such as pinnipeds and cetaceans may accumulate levels of contaminants putting them at higher risk compared to their conspecifics primarily feeding on fish [7]. Individual variation in diet can also occur within a population. Recent studies measuring POPs (including legacy and emerging classes) in killer whales from Norway and Iceland reported high PCB levels for mixed-diet individuals, i.e., those feeding on marine mammals and fish, as opposed to those only known to feed on herring, a mid-trophic level fish (*Clupea harengus*) [17, 18]. In both populations, levels of PCBs in mixed-diet individuals were typically above the maximum threshold for risks of health effects. Conversely, fish-eating individuals had PCB concentrations associated with low risk for health effects.

Although tissue concentrations of legacy POPs and their relationship with diet habits are well documented for Northeast Pacific killer whales [6, 15, 19], considerably less is known for North Atlantic (NA) conspecifics and on emerging POPs [20]. Although killer whales sampled in Greenland exhibited elevated POP concentrations (averaging ~40-70 mg/kg lw), those sampled in Iceland and Norway demonstrated comparatively lower levels (averaging ~10-50 mg/kg lw) [17, 18, 21]. However, POP concentrations remain unknown in killer whales from the Western NA, including in the

Eastern Canadian Arctic and Eastern Canada, and the influence of diet composition on POPs in killer whales across the NA has not been quantified. Other marine mammals in these regions showed moderate levels of POPs (~2 mg/kg lw to ~10 mg/kg lw for ∑PCBs) in these regions for other marine mammals [22, 23]. As a result of biomagnification, marine mammal-eating killer whales could be exposed to high levels of contaminants in the Eastern Canadian Arctic and Eastern Canada, although this remains an understudied area requiring further investigation.

Although POP concentrations are influenced by feeding habits, quantitative estimates of killer whale diet composition, especially in remote areas of the NA Ocean, were not available until recently. The recent use of quantitative fatty acid signature analysis (QFASA) on ~200 NA killer whales spanning from Eastern Canada to Norway revealed important differences in their diet between and within populations [24]. The diet estimates obtained in Remili et al. 2023 revealed that killer whales sampled in the Eastern NA feed on a high proportion of herring, while mid-NA killer whales feed on a mixture of Arctic seals and mackerel (*Scomber scombrus*) and Western NA killer whales prey largely on marine mammals such as baleen whales and porpoises (*Phocoena phocoena*) in Eastern Canada, and belugas (*Delphinapterus leucas*), narwhals (*Monodon monoceros*), and ringed seals (*Pusa hispida*) in the Canadian Arctic [24]. Nonetheless, these estimates calculated for each individual showed some marked differences among individual killer whales within populations. For example, all Arctic Canadian killer whales fed mainly on cetaceans or ringed seals, while Greenlandic killer whales showed more generalist feeding patterns that may suggest opportunistic foraging. Killer whales sampled in Norway and Iceland generally showed a strong preference for herring, but some individuals also consumed porpoises or seals around Iceland, and seals or lumpfish (*Cyclopterus lumpus*) in Norway. These inter and intra-population differences may result in different POP exposure and associated risks in killer whales across the NA.

Here, we first compare legacy and new POP concentrations (PCBs, OCs, PBDEs and non-BDE FRs) in 162 individuals across the NA, including, for the first time, Western NA killer whales. To our knowledge, this represents the largest NA killer whale contaminant dataset to date. We then assess the relationship between POP concentrations, sex, age class and diet composition, using diet estimates previously inferred from QFASA for the same individuals [24]. Finally, we assess the risks associated with PCBs for all individuals sampled across the NA Ocean, depending on their sex and diet types.

2. Materials and Methods

Sampling:

Killer whale blubber samples were collected from 162 individuals across the NA (details can be found in Table S1, S6). From the Western NA, thirty killer whales were sampled in the Eastern Canadian Arctic (Pond Inlet and Pangnirtung from 2013 to 2019), and five in Eastern Canada (off the French territory of Saint-Pierre & Miquelon and Newfoundland from 2019 to 2022). In the mid-NA, nineteen killer whales were sampled in Greenland (Tasiilaq, Scoresby Sund and Nuuk from 2012 to 2021), and from the Eastern NA, two killer whales were sampled in the Faroe Islands (2008), forty-eight in Iceland (Vestmannaeyjar and Grundarfjörður from 2014 to 2016) and fifty-eight from Norway (Skjervøy from 2017 to 2019). Sampling was performed via dart biopsies in the Canadian Arctic, Eastern Canada, Iceland and Norway. Briefly, skin and blubber biopsies were collected from free-ranging killer whales using an ARTS pneumatic darting system (LKARTS-Norway, Norway) or a crossbow and stainless-steel biopsy tips (CetaDart, Denmark) ranging from (*depth* x *diameter*) 25 x 7 mm to 40 x 5 mm, depending on the location. In Greenland, blubber samples were collected from individuals after subsistence-harvest, and in the Faroe Islands, samples were collected from two stranded individuals. All samples were stored at -80 °C until analysis. Upon arrival in their respective extraction laboratories, samples were cut in half longitudinally: one half was used for fatty acid analysis [24], while the other half was kept for contaminant analyses. Contaminant analyses could only be performed when the sample weight was sufficient for both analyses. Sexing and age class was assessed in the field thanks to photo-identification or detailed field observation (in Iceland and Norway), directly on the animal when harvested or stranded (in Greenland and the Faroe Islands) or genetically (for Western NA individuals, as part of the federal Canadian Department of Fisheries and Oceans's ongoing monitoring program).

POPs analyses:

POPs were extracted and quantified in four different laboratories (see Table S2), with each laboratory having slight variations in the suite of target compounds. We thus only reported concentrations for those compounds analyzed in all four different laboratories. This included thirty PCB congeners, and seventeen OCs, and for a subset of individuals, brominated and non-brominated flame retardants (twenty-four BDE and twenty-one non-BDE FRs) in the killer whale biopsies (see supplementary text for the detailed list of targeted compounds and methods). Concentrations of POPs are reported in mg/kg (ppm) of lipid weight (lw) and sums for each contaminant class were calculated including only the compounds analyzed in all four labs. Given the small sample weights for the biopsies, we were unable to perfom interlab comparisons. However, Pedersen et al. re-extracted in our laboratory (McGill) PCB and OC compounds in the subsistence-harvested Greenlandic killer whales' blubber previously analyzed by Pedro et al., using the QuEChERS method and reported no significant differences between the two extraction methods, and the two laboratory analyses [21, 25]. Additionally, while an interlaboratory difference might result in a small bias in the contaminant concentrations, the killer whale blubber showed orders of magnitude variation, which is well beyond what might be expected from interlaboratory differences [25]. Therefore, any potential minor bias should not lead to a significant influence on the results or interpretation. Details on each procedure, and instrument analyses can be found in the SI.

QA/QC:

The standard reference materials (NIST 1945 "pilot whale blubber" or 1946 "Great Lakes fish homogenate") were run with each batch of ten samples and checked for precision and accuracy. Accuracies for each laboratory can be found in Table S2. Method limits of detection (MLODs) and quantification (MLOQs) were defined as the minimum amount of analyte which produced a peak with a signal-to-noise ratio of 3 and 10, respectively. A procedural blank was run with each batch as well. Only a small contamination of heptachlor epoxide was reported for the Greenlandic samples (see SI for details) [21]. For these, the blank concentrations were subtracted from the sample concentrations. Recoveries for spiked internal standards (^{13}C-labelled compounds) are reported in Table S2.

Fatty acid analyses and QFASA:

All fatty acid data was obtained from Remili et al. (2023) and can be found on the Polar Data Catalogue: https://doi.org/10.21963/13299. Fatty acid analyses were performed on the same individuals as previously described [26]. QFASA diet estimates representing the estimated percentage of each prey species from the prey library in the diet of each predator were obtained using the *QFASAR* package in R [27]. To calculate the diet estimates, killer whale calibration coefficients were used and developed by Remili et al. (2022) as well as 900+ prey in the prey library as described earlier [24, 28]. Model diagnostics were validated using the *leave_one_prey_out* and the *prey_beyond_pred* functions of the *QFASAR* package.

Statistical analyses:

All statistical analyses were performed in R (version 4.2.3). The five main contaminant classes, i.e., ∑PCBs, dichlorodiphenyltrichloroethane (∑DDTs), chlordane (∑CHLs), and chlorobenzenes (∑ClBz) were quantified in all samples, while ∑HCHs were detected in > 90% of the samples. Contaminant concentrations were log-transformed ($\log x + 1$) to improve normality which was evaluated and confirmed with qqplots on residuals and/or Shapiro-Wilk tests. Any non-detects (N.D.) were assigned a random value between 0 and the MLOD of the compound before inferential statistical analysis. Compounds detected, but below the MLOQ, were assigned a random value between MLOD and MLOQ.

Before applying statistical tests and GLM models on our dataset, we had to remove certain individuals from the datasets prior to analyses. In Norway, eleven individuals had to be excluded from modelling because their sex could not be identified (they were identified in the field as "females or juveniles"). We also had to remove the two Faroese females, since they were under the minimum number for statistical analyses. Two extra individuals in Norway, one in Eastern Canada, and one in the Eastern Canadian Arctic had to be removed because their sex was unknown. Finally, six individuals were removed from Eastern Canadian Arctic, and one from Iceland due to no diet estimates being available for these individuals, bringing the total number of individuals included in the statistical analyses to 138. Because the diet varied significantly across the NA [24], we first tested the impact of *location* on POP class concentrations through ANOVAs and *post-hoc* Tukey tests. This analysis could only be done in Norway and Iceland for ∑PBDEs and ∑non-BDE FRs because they were the only locations where we had a sufficient number of sampled individuals.

We employed generalized linear models (GLM), using the *MuMin* and *Jtools* packages, to examine the influence of multiple factors contributing to the variability in PCB and OC classes among killer whales in the NA Ocean. The following variables were considered to determine the strongest influence on variations in the log-transformed concentrations of ∑PCBs, ∑DDTs, ∑CHLs, ∑ClBz, and ∑HCHs: *location*, *sex/age class* (adult males, adult females, and juveniles), and *diet-type*. Due to the variations in sampling years across different locations, we couldn't incorporate the "*year sampled*" as a variable in our analyses. Nonetheless, we carried out separate Pearson's correlation tests for each location, where the sample size was adequate, to explore the temporal patterns of the primary classes of contaminants. These tests revealed that, apart from a rise in the Canadian Arctic between 2013 and 2019, which we refrained from interpreting further due to a limited sample size, there were no notable differences among the years. To investigate how diet influenced POP concentrations within and among killer whale groups, we first separated the individuals into feeding types. This categorization was necessary because QFASA estimates for each prey species are interdependent and would violate model assumptions. Consequently, we separated our individuals into a “fish-dominant”, “mixed-diet” (i.e.: mix of marine mammals and fish), “pinniped-dominant”, “baleen whale-dominant” and “toothed whale-dominant” feeding types, based on their QFASA estimates. Mixed-diet individuals (i.e.: fish and marine mammals) were classified as such if their percentage of fish was < 65% and their marine mammal percentage was > 35%. In the Canadian Arctic, individuals with toothed whale percentages > 50 % were identified as toothed whale-dominant, while individuals with > 50 % of pinnipeds in their diets were identified as “pinniped-dominant”. The details on the diet composition estimates of each individual included in this analysis can be found in the SI of Remili et al. (2023), as well as in Table S9 of this study and the diet-type assigned to each individual can be found in Table S12 of this present study [24]. To prevent overparameterization of the models, we did not test any interactions between variables. We utilized the Akaike information criterion corrected for small sample sizes (AICc) scores to select the most appropriate models. When multiple models had a difference in AICc (ΔAICc) of less than 4, we averaged all the models with a ΔAICc of 4 or lower, to obtain an average effect for each variable (Tables S10-11). For ∑PBDEs and ∑non-BDE FRs, we again used ANOVAs and *post hoc* Tukey tests to investigate the impact of sex/age and diet-type on killer whales sampled in Norway and Iceland.

We then visualized how PCB and OC profiles of compounds that were detected in > 70 % of the individuals varied by dietary habits, by computing a principal component analysis (PCA) on the scaled percentage contaminant concentrations, as described previously [18]. For FRs, since the detection percentages were lower, we included the compounds detected in > 50 % of the individuals. The 27 legacy compounds included in this analysis were: Hexachlorobenzene, Oxychlordane, *cis*-Chlordane, *trans*-Nonachlor, *cis*-Nonachlor, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, Heptachlor Epoxide, Dieldrin, CB-52, -74, -95, -99, -101, -105, -118, -138, -149, -151, -153, -156, -158, -170, -180, -183 and -187. The eight FR compounds included BDE-47, -85/-155, -99, -100, -153 and -154, BB-153, and α- Hexabromocyclododecane (HBCDD) (Tables S3-4). PCAs were also computed on the log-transformed PCB, OC and FR concentrations (not the profiles) to test for visual intra-population variations (Fig. S2).

Finally, to estimate the risks associated with ∑PCBs, we calculated the risk quotient (RQ; RQ = Body Residue /Critical Body Residue) for each individual in this trans-Atlantic study, based on a conservative 10 mg/kg lw critical body residue, as previously described [3, 29]. This threshold, established by Dietz et al. (2019) considers immunotoxic effects as well as endocrine disrupting effects, which also corresponds to the upper limit reported as the immune threshold modelled for cetaceans [9]. Hence, if future studies reveal lower critical daily doses, it is likely that the RQs observed in this study would be higher.

3. Results and Discussion

Concentrations of persistent organic contaminants in NA killer whales:

This study is the most comprehensive assessment of legacy and emerging contaminant concentrations in killer whales across the NA. Mean concentrations of PCBs ranged from a high of ~ 100 mg/kg lw in the Western NA (mean: 92.0 ± SE: 9.8 mg/kg lw in the Canadian Arctic; 106.1 ± 31.1 mg/kg lw in Eastern Canada) to about 50 mg/kg in the mid NA (mean: 66.1 ± SE: 10.6 mg/kg lw in Greenland; 42.1 ± 11.1 mg/kg lw in Iceland) to lower levels in the Eastern NA (mean: 2.9 ± SE: 1.5 mg/kg lw in the Faroe Islands; 12.2 ± 10.8 mg/kg lw in Norway). Killer whales sampled in the Eastern Canadian Arctic had mean ∑PCB concentrations 7-fold higher than killer whales sampled in Norway, while the difference for ∑DDTs and ∑CHLs were 16-fold and 32-fold between the same two populations (Fig. 1, Table S5-7). A notable observation was the prevalence of higher DDT concentrations surpassing PCB concentrations in the Eastern Canadian Arctic (mean: 108.1 ± 28.7 mg/kg lw for DDTs), whereas DDT levels were lower than PCBs in other regions and significantly lower in Iceland and Norway (Fig. 1). Although this difference between DDTs and PCBs in the Canadian Arctic was non-significant, this pattern resembles the typically higher DDT concentrations than PCBs found for killer whales of the North Pacific killer whales and Southern

Ocean [7, 16, 30, 31].

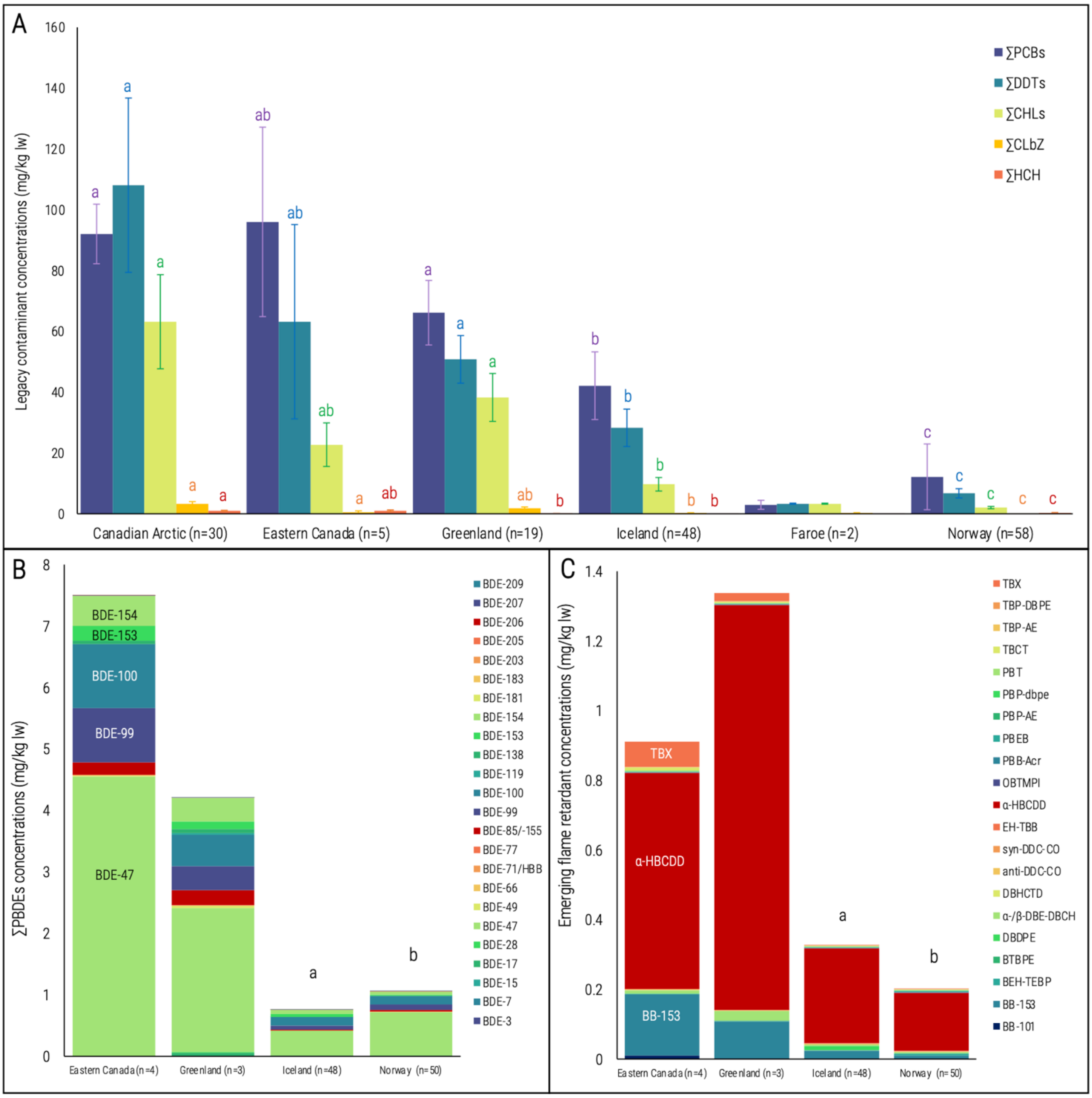


Figure 1: Total concentrations of mean (±SE) A) legacy polychlorinated biphenyls (PCB) and organochlorine contaminant classes (mg/kg lw), B) Polybrominated diphenyl ethers (PBDEs) (in mg/kg lw) and C) Emerging flame retardants (in mg/kg lw) in North Atlantic killer whales sampled from 2008 to 2022. Legacy contaminants were measured in 162 individuals while the ∑PBDEs and FRs analyses were conducted only on a subset of individuals (n = 105). (Note: the legend is in the same order as the bars). The letters indicate the results of the Tukey post-hoc tests on the various contaminant classes tested against *location* (*p*-value threshold set at 0.05).

Although much lower than for the legacy POPs, for ∑PBDEs and non-BDE FRs, killer whales in the Western NA again showed higher concentrations than those in the Eastern NA (Fig. 1, Tables S5-7). The predominant PBDE congeners were BDE-47 followed by BDE-99 and 100 at all locations and ∑PBDEs were significantly higher in Norway than in Iceland ($F = 8.1$; $p = 0.005$). For emerging BFRs, α-HBCDD dominated across locations with levels in Iceland being statistically higher than in Norway ($F = 10.1$; $p = 0.002$), reaching the highest concentration in Greenlandic killer whales at 1.2 ± 0.4 mg/kg lw. This compound had a similar concentration in mixed-diet individuals from Iceland, reported in our previous study, which found a mean concentration of 1.0 mg/kg lw in the mixed-diet individuals [18]. In Eastern Canada, α-HBCDD had a mean concentration of 0.6 ± 0.3 mg/kg lw. These α-HBCDD concentrations in Iceland, Eastern Canada and Greenland are among the highest reported for any marine mammal (including killer whales) to date, far exceeding HBCDD concentrations reported in southern resident killer whale blubber (0.1 mg/kg lw) or transient killer whale liver (0.2 mg/kg lw) [6, 32]. Despite being added to the Stockholm Convention in 2013, as it was increasing in the environment [33]. Subsequent to the ban, due to its stable structure and widespread distribution, HBCDD concentrations have not significantly decreased in the environment [34]. α-HBCDD is the primary HBCDD congener in biota and exhibits biomagnification [35].

Prior to discussing the effects of sex and diet composition on contaminant concentrations in NA killer whales, it is worth considering how historic usage of legacy contaminants may influence contaminant variation in killer whales across the NA. In this study, killer whales located in the Western part of the NA, specifically the Eastern Canadian Arctic and Eastern Canada, exhibited the highest concentrations of legacy POPs and ∑PBDEs. This distribution pattern contrasts with the findings in other Arctic biotic and abiotic compartments, where POP levels are typically higher in Greenland and Norway rather than in the Canadian Arctic [36-38]. The prevailing pattern of higher concentrations in the Eastern NA can be attributed to historical practices of contaminant usage in North America, subsequently leading to their transportation through the atmosphere and oceans towards the East [39]. Because we observed an opposite spatial pattern in the contaminant concentrations in NA killer whales, it is imperative to consider the influence of sex, diet and age.

Effect of sex on POP concentrations:

Male killer whales were significantly more contaminated than females, with the GLM effect for "adult males" being significant and positive in PCBs, DDTs, CHLs and ClBz, but not HCHs (Fig. 2, Table S10-S11). For FRs, we found that *sex* in Iceland and Norway had a significant effect on PBDEs concentrations (F: 14.06, $p < 0.01$). However, this test resulted in non-significant differences in non-BDE FR concentrations. Lower concentrations in females are most likely attributed to the maternal offloading of lipophilic contaminants from mammalian mothers to their offspring. During gestation and lactation, adult female cetaceans transfer approximately 10% and 60% of their body burdens to their offspring, respectively [14, 21, 40-42]. Since a significant portion of these burdens is unloaded during the first pregnancy and nursing period, contaminant levels may also differ depending on the number of births, the interval between births and, consequently, the age of the individuals. Our study did not include samples collected from young juveniles (~1-1.5 years old) but these young juveniles may have among the highest POPs concentrations due to nursing. These concentrations may decrease rapidly after weaning due to growth dilution and the shift to a diet of prey, which may have lower concentrations than milk. Juveniles included in our analyses did not significantly differ from adult females (Fig. 2, Table S10-11), but future studies should include the actual age of the individuals to account for this likely source of variation. To do so, precise age estimates could be obtained from photo-identification for long-term monitored populations, or in future studies through the development of DNA-methylation methods to age killer whales [43].

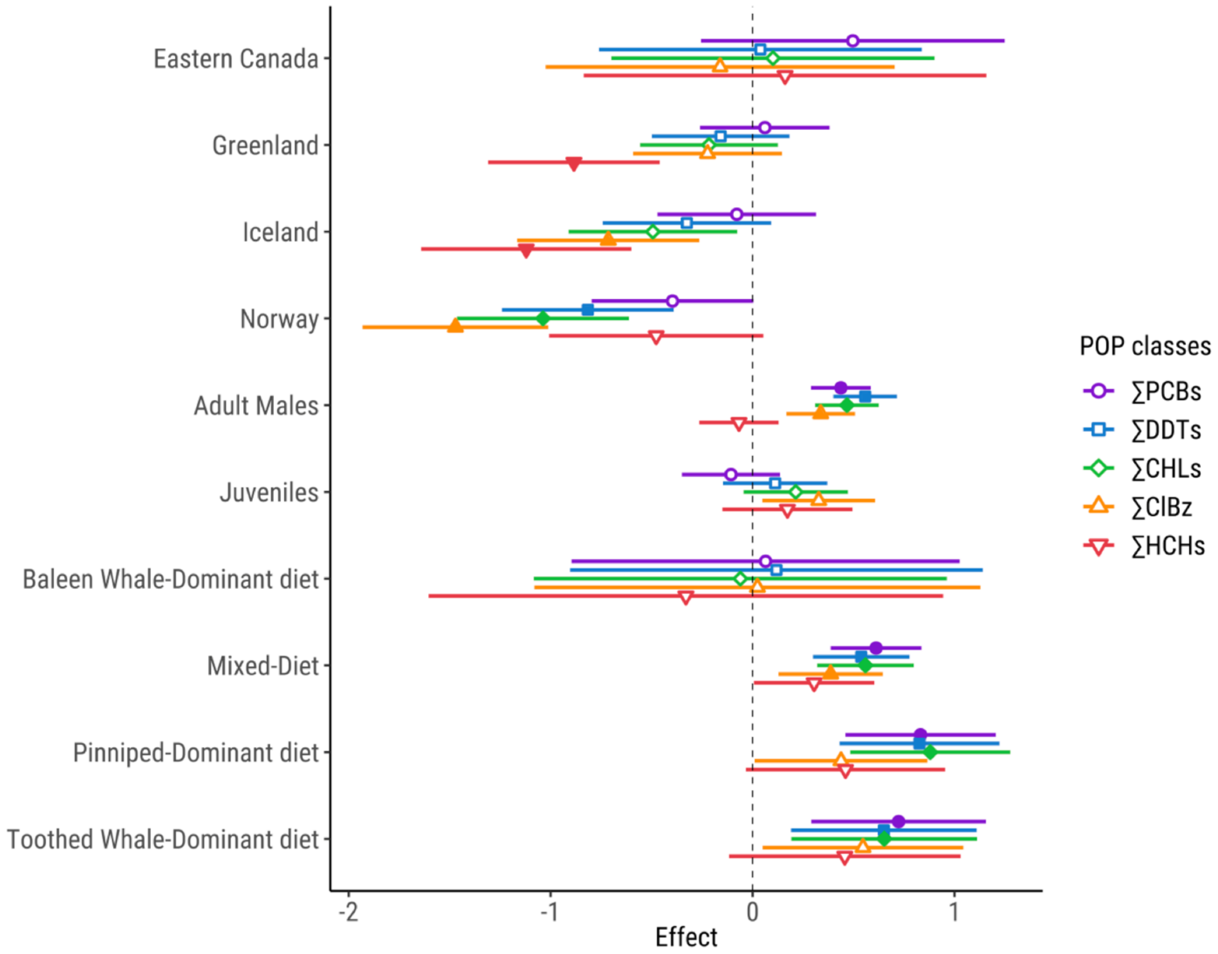


Figure 2: Summary results from the generalized linear modelling approach testing the effects [and 95% confidence intervals] of the following independent variables: *location*, *sex / age*, and *diet-type* (inferred from QFASA) on the log-transformed concentrations of ∑PCBs, ∑DDTs, ∑CHLs, ∑HCHs, and ∑ClBzs in the blubber biopsies of North Atlantic killer whales. The intercept represents adult females (for *sex / age*), Eastern Canadian Arctic (for *location*) and fish-dominant diets (for *diet-type*). Filled shapes represent significant variation in the contaminant classes. Model selection table and precise effects, confidence intervals and significance are available in the SI (Table S10-S11).

Effect of the diet on contaminant concentrations:

Across the NA, the *diet-type* predictor had a stronger effect than *sex* or *location* for PCBs, DDTs, CHLs and ClBz, but not HCHs (Fig. 2, Tables S10-S11). Specifically, and compared to fish-dominant diets, diets including pinnipeds and toothed whales (mixed-diet included) resulted in significantly higher concentrations for these contaminant classes, with pinniped-dominant diets having a stronger effect (mean effect across PCBs, DDTs, CHLs and ClBz: 0.75) compared to toothed whale-dominant diets (mean effect: 0.64) or mixed-diets including fish and pinnipeds or toothed whales (mean effect: 0.53). These results were supported by figs. S2-4 that clearly showed a gradual increase of all contaminant concentrations across diet types, from fish diets to marine mammal diets. Baleen whale diets did not differ significantly from the fish diets in terms of contaminant concentrations, probably due to both prey types having a similar trophic position [44], but interpretation is challenging due to low sample size and high variability in the concentrations across the individuals feeding on baleen whales (for example, the only female in this location had the highest contaminant concentrations, higher than the four other males). The stronger effect for *diet-type*, in relation to *sex* or even *location* is of particular interest, and shows that, at least for the most abundant POP classes, dietary habits impact contaminant accumulations more than sex differences or geographical variations in contaminant distribution for NA killer whales. For FRs, we found that *diet-type* in Iceland and Norway influenced PBDEs concentrations (F: 4.3, $p = 0.04$). However, this effect was lower than for sex. While statistical testing was not conducted to determine the impact of diet types on α-HBCDD, the most detected emerging FR, due to a low sample size in Greenland (n = 3) and Eastern Canada (n = 4), important variations in α-HBCDD concentrations were observed between killer whales that primarily feed on marine mammals and those that primarily feed on fish. Individuals feeding on fish had mean α-HBCDD concentrations of 0.2 ± 0.1 mg/kw lw, while individuals feeding on marine mammals had mean concentrations of 0.7 ± 0.1 mg/kg lw. PCAs focused on the log-transformed concentrations of PCB, OC and FR compounds showed a striking trend of compound concentrations across the NA, with fish feeding individuals having lower contaminant concentrations, followed by the mixed-diet individuals, and toothed whale and pinniped-feeding individuals having the highest concentrations (Fig. S2-4).

Future work could further assess the influence of particular diet/tissue types on bioavailability of POPs in killer whales, as it has been shown to be important for other predators [45].

Within the different diet-types, we observed that PCB and OC profiles (expressed as % contribution of congeners to the ∑PCBs or ∑OCs) differed between diets including pinnipeds, fish or toothed whales (Fig. 3). Specifically, for PCBs, pinniped-dominant diets were associated with higher concentrations of highly chlorinated compounds (e.g., CB-138, -170, -180, -183 and -187). Conversely, toothed whale-dominant diets were associated with lower chlorinated compounds (e.g., CB-74, -95, -99, -101 and -149) (Fig. 3A). Interestingly, contaminant profiles were reported to show similar patterns between serum samples of harbor seals (a pinniped) and harbor porpoises (a toothed whale) [46] Additionally, ringed seal have demonstrated a capacity to biotransform CB-101, -149, and -151, which could explain why killer whales feeding on pinnipeds had lower proportions of these compounds than killer whales feeding on toothed whales. Other research showed higher CB-118 proportions in herring compared to marine mammals [47], which may explain its higher percentage in the fish-feeding killer whales (Fig. 3-A). Pinniped-dominant diets showed higher proportions of oxychlordane and heptachlor epoxide among the OC compounds, while toothed whale-dominant diets exhibited higher percentages of DDT. Previous reports indicated that pinnipeds in the Arctic generally have relatively higher proportions of CHL (within their overall OC levels) compared to toothed whales [48]. This finding may explain why killer whales feeding on pinnipeds displayed higher CHL percentages than those feeding on toothed whales (Fig. 3B). The higher percentages of DDT in diets primarily consisting of odontocetes (toothed whales) is perhaps not surprising, considering the comparatively lower contaminant-eliminating capacities of cetaceans when compared to pinnipeds (DDE being a metabolite of DDT) [5, 49]. For fish-based diets, previous reports indicated that herring have higher percentages of DDE (relative to total DDTs) compared to harbor porpoises [50]. This observation suggests a potential higher metabolism capacity for DDT in herring than in cetaceans, which could explain the high proportions of DDE found in fish-feeding killer whales (Fig. 3-B). Killer whales who had baleen whale-dominant diets (specifically in Eastern Canada) overlapped with toothed whale-dominant diets and fish-dominant diets for PCB and OC profiles (Fig. 3A-B), suggesting little distinction in POP patterns between toothed and baleen whale feeding killer whales.

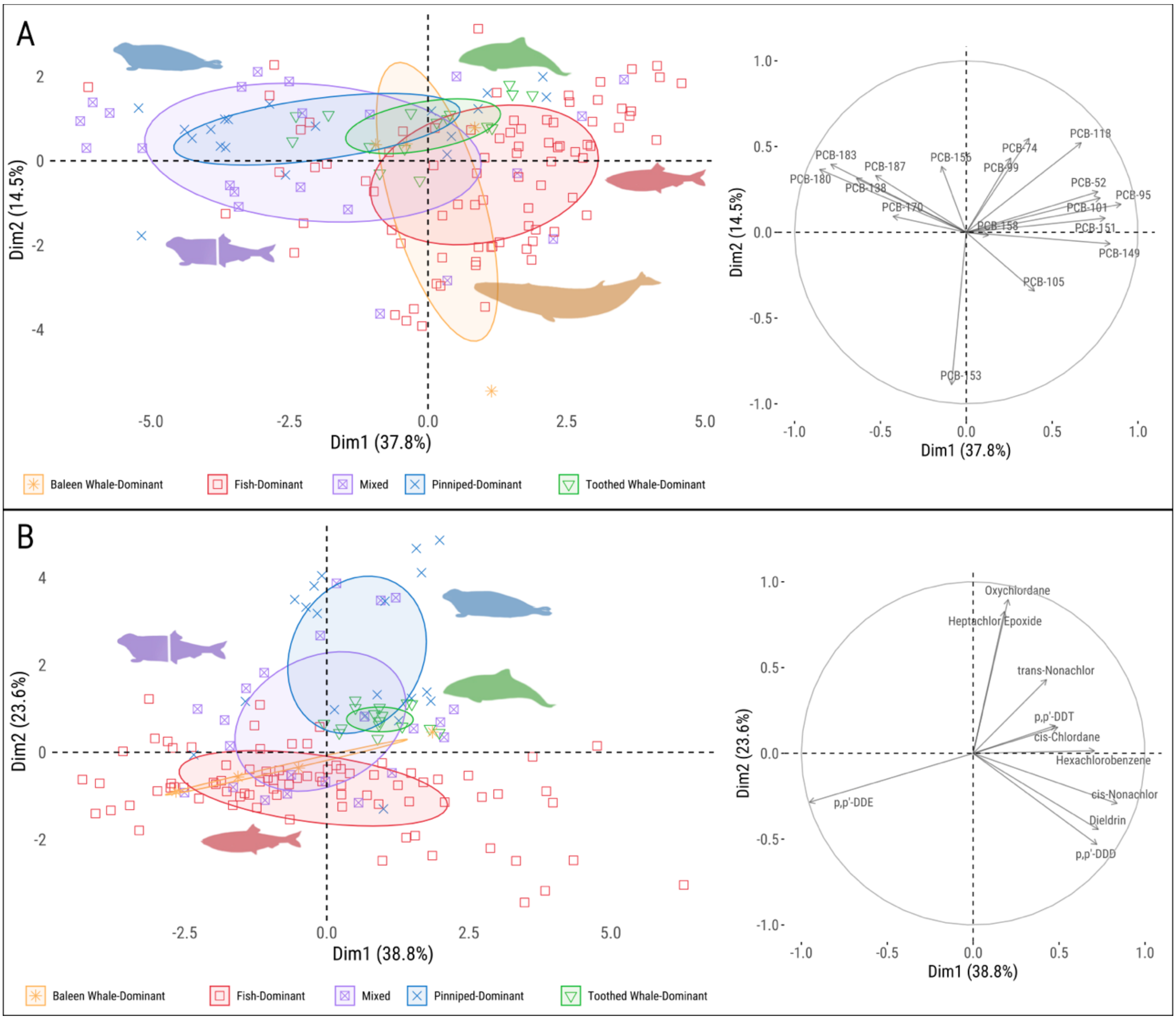


Figure 3: Principal component analysis on the proportional contribution of individual compounds for A) polychlorinated biphenyls (PCBs) and B) organochlorine pesticides (OCs) in the blubber of North Atlantic killer whales (Panels A and B share a legend). Only the PCB and OC compounds detected in > 70 % of the individuals were included. The FR equivalent of this PCA can be found in the SI (Fig. S1) Each point represents an individual killer whale. The animal shapes represent the diet-types of killer whales, inferred from QFASA (fish for fish-dominant diets, mixed fish/seal for mixed diets, porpoise for toothed whale-dominant diets, etc.)

Intra-population diet variation estimated using QFASA (Remili et al. 2023) allowed for interpretation of the differences in accumulation of PCBs, OCs and FRs within several populations. In Iceland, killer whales with mixed diets showed significant variation in contaminant concentrations. Their concentrations of POPs sometimes overlapped with fish-eating killer whales in the same location and with killer whales in Greenland and Western NA (Fig. S2-4). In terms of PCBs and OCs specifically (Fig. S2-3), the overlap between the two ellipses representing the two diet types in Iceland was negligible or non-existent (for example, both diet-types overlapped more in Norway), indicating more pronounced diet-related differences in contaminant accumulation within this location [17]. These dietary variations among killer whales in Iceland, and the rest of the NA deserve further research, especially for the individuals known to consume marine mammals [18, 24, 51].

Risk Assessment for PCBs:

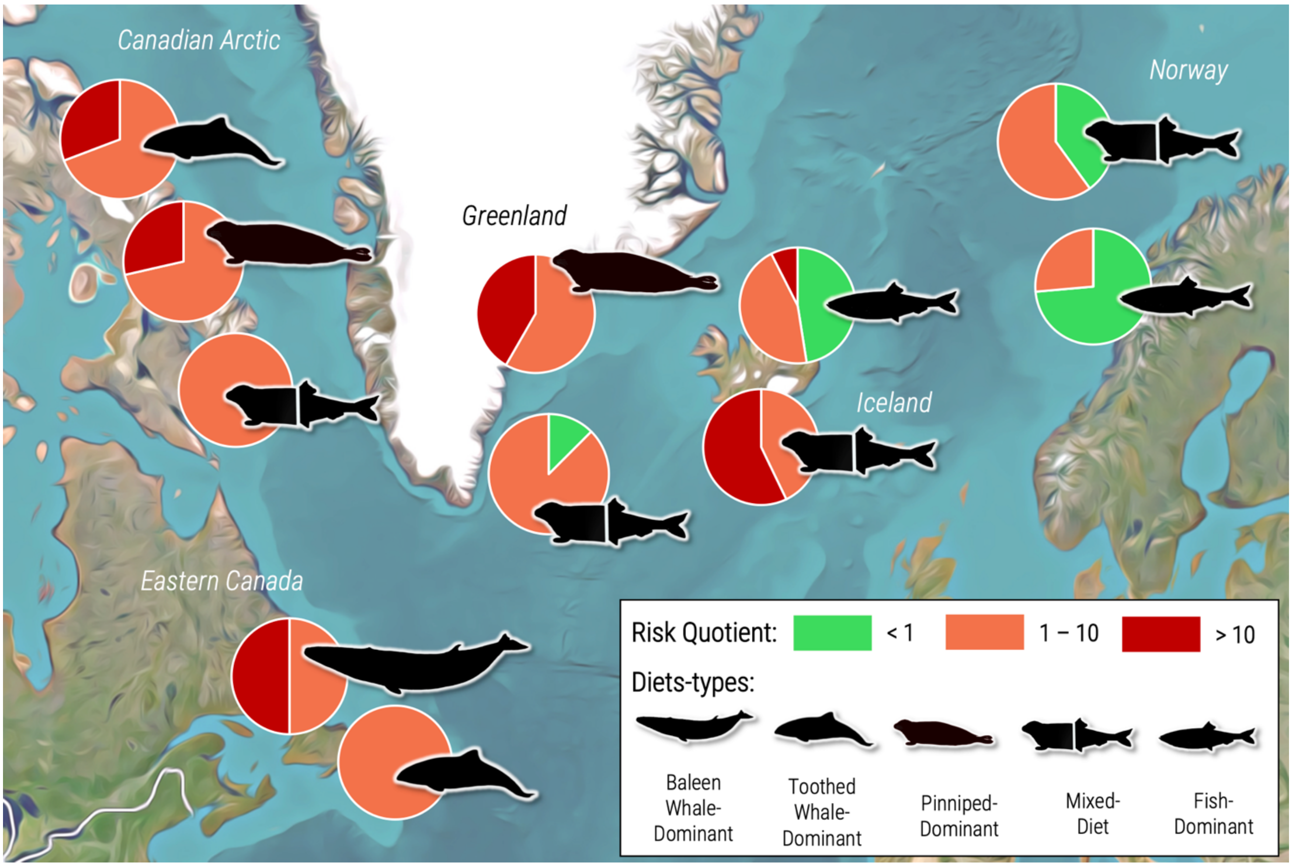


Figure 4: Risk Quotient estimated for ∑PCBs based on a conservative 10 mg/kg lw threshold for

immunotoxic and hormonal imbalance effects (from Dietz et al. 2019) in North Atlantic killer whales separated by location and by diet-type of each killer whale, as inferred from quantitative fatty acid signature analysis on the same individuals. See Table S12 for more information and see Fig. S5 for the same map with RQ differences based on sex/age.

Risk quotients for killer whales in the Western NA (Canadian Arctic, Eastern Canada) and mid-NA (Greenland) were consistently higher than those in the Eastern NA, regardless of their sex/age or diet-type, except for the Icelandic mixed-diet type. The risks were similar among killer whales feeding on toothed whales (mean RQ: 11.4 ± SE: 4.4) and killer whales feeding on pinnipeds (mean RQ: 8.6 ± SE: 2.3) in the Eastern Canadian Arctic. Killer whales sampled in Eastern Canada and feeding on baleen whales had a moderate to high risk of health effects (mean RQ: 11.3 ± SE: 3.4). This risk, associated to high PCB concentrations may also be attributed to local sources of PCBs from the contaminated Great Lakes area into the Gulf of Saint Lawrence [52, 53]. Eastern Canadian killer whales thus deserve further attention in future ecotoxicological studies, since preying on pinnipeds or toothed whales may significantly incerase their PCB-associated risks. In Greenland, killer whales feeding on pinnipeds were more at risk (mean RQ: 8.1 ± SE: 1.5) than those having a mixed diet (mean RQ: 4.0 ± SE: 0.7). In the Eastern NA, i.e., Iceland and Norway, killer whales showed lower RQs for ∑PCBs, but these RQs were consistently greater for individuals with a mixed diet compared to those feeding on fish (Fig. 3, Table S12). Previous studies reported that individual killer whales in Iceland and Norway who do prey on marine mammals in addition to fish face significantly greater risks than individuals in the same regions that have fish-dominant diets [17, 18]. Males across these two locations also faced the highest risks of health effects compared to females and/or juveniles (Fig. S5). For example, male Icelandic killer whales who seasonally travel to Scotland to prey on seals or who were photographed preying on porpoises in Iceland (i.e.: individuals IS015, 172, 241, 243 & 256) all had RQs higher than 10 (mean RQ: 22.0 ± SE: 4.4), which represents a high risk of health effects including reproductive failure [10, 11]. In Norway, the two males identified by QFASA as having a mixed diet (i.e.: individuals 17010 and 18025) had an RQ of 4.0 on average, which represents a significantly lower risk than in Iceland. However, it should be noted that an RQ of 4 represents a ∑PCBs concentration of ~ 40 mg/kg lw, similar to the 41 mg/kg lw threshold for risk of reproductive failure [10]. This threshold was established in 1976 based on female seals in the Baltic Sea that showed a high rate of fetal resorption and uterine occlusions. To our knowledge, no threshold has been established for male reproductive failure in marine mammals, which may differ from females. Future research should investigate the reproductive impact of POPs in males. Nevertheless, the findings presented in this study are of concern, particularly when considering that mixtures of contaminants (not just PCBs, but DDTs, CHLs, and newer POPs) may have a greater immunotoxic effect on killer whales compared to individual contaminants alone (e.g., just PCBs) [13]. More research on additional thresholds for other contaminants and mixtures of contaminants is needed to better understand the risks to killer whales' health. These results highlight the necessity for improved risk assessment methods, specific to these ecologically significant top predator species [1, 2].

Our results highlight the need for further efforts in legacy and emerging pollutant management and waste disposal when it comes to reducing the risks faced by the oceans' top predator. The Stockholm Convention will very likely fail to meet its 2025 and 2028 targets for the phase-out of hazardous substances and safe waste disposal [54]. Addressing the more specific issue of pollution in marine mammals will necessitate a pragmatic and systematic approach to mitigate its adverse effects. First, enhancing monitoring programs in the NA and elsewhere is crucial to gather reliable data on pollutant levels in marine mammal populations. Second, interdisciplinary and international collaboration among ecotoxicologists, conservation biologists, policymakers, and other stakeholders is crucial in the near future. Other recommendations regarding emerging chemicals of concern include holding chemical producers responsible for data generation, protecting high-risk populations, avoiding assumptions of "safe" exposure levels, and addressing financial conflicts of interest in assessments of chemical risks [55]. These collaborations and recommendations may facilitate knowledge exchange and resource sharing, enabling the development of targeted strategies for pollution mitigation, and enhanced cetacean conservation.

In this first detailed analysis of POP concentrations in killer whales across the NA, an almost two orders-of magnitude difference in means for PCBs and OCs were found between the individuals of the Eastern NA and Western NA. Contaminant levels were found to be higher in killer whales from the Western NA compared to those from the Eastern NA. This spatial pattern is opposite to the pattern reported in other NA biota, as well as abiotic matrices, suggesting that the pattern is driven by diet and not environmental concentration differences among locations. Across locations, there were also large differences in diets and this diet variation explained the majority of the contaminant differences, indicating how critical this feeding variation among killer whale groups is for their resulting contaminant loads and risks for health effects. Nonetheless, wide intrapopulation differences in contaminant concentrations and associated health risks were also found. The findings of this study support the need for additional measures to be taken to ensure the safe disposal of POP-contaminated waste and to prevent the continued runoff and deposition of these contaminants into the environment and living organisms. It is crucial that these conservation efforts also focus on preventing the release of newer and potentially highly toxic contaminants into the environment.

4. Supporting Information

The tables provide details on sample collection dates and locations (Table S1), laboratory analyses for POPs (Table S2), detection percentages of PCBs and OC compounds (Table S3), and detection percentages of PBDEs and non-BDE flame retardants (Table S4). Concentrations of legacy contaminant classes and emerging flame retardants are presented (Table S5), alongside mean values for flame-retardant and PCB compounds (Tables S6 and S7). Diet estimates from fatty acid analysis are outlined (Table S9), followed by model selection and coefficients related to contaminant classes (Tables S10 and S11). Risk quotients for PCBs are provided, including contextual details about individuals (Table S12). Corresponding figures illustrate principal component analyses for flame retardants (Figure S1), PCBs (Figure S2), and organochlorine pesticides (Figure S3), highlighting overlaps between diet types and locations. Another figure demonstrates concentration differences in flame retardants between fish-feeding and marine mammal-feeding killer whales (Figure S4). Lastly, individual risk quotient estimates for PCBs, divided by location and sex, is presented (Figure S5).

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6. Authors’ contributions

M.A. McKinney, A. Remili, R. Dietz, and C. Sonne designed the study with input from all co-authors. F.I.P. Samarra, A.H. Rikardsen, A. Rosing-Asvid, S.H. Ferguson, C.A. Watt, C.J. D. Matthews; R. Dietz, C. Sonne, and J.J. Kiszka provided the killer whale samples/data. M.A. McKinney, R.J. Letcher, C.J.D. Matthews, C.A. Watt, S.H. Ferguson and A. Remili performed or supervised the contaminant analyses. A. Remili performed the data analysis and wrote the original draft of the manuscript with input from M.A. McKinney. All authors reviewed and edited subsequent versions of the manuscript.

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