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Humpback whales (*Megaptera novaeangliae*) breeding off Mozambique and Ecuador show geographic variation of persistent organic pollutants and isotopic niches☆

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ABSTRACT

Humpback whales (*Megaptera novaeangliae*) from the Southern Hemisphere carry information on persistent organic pollutants (POPs) from their feeding zones in Antarctica to their breeding grounds, making this species a sentinel of contaminants accumulation in the Southern Ocean. This study aimed to evaluate driving factors, namely feeding areas, trophic level, and sex, affecting POP concentrations in the blubber of humpback whales breeding off Mozambique and off Ecuador. Biopsies of free-ranging humpback whales including blubber and skin were collected in 2014 and 2015 from Ecuador ($n = 59$) and in 2017 from Mozambique ($n = 89$). In both populations, HCB was the major contaminant followed by DDTs > CHLs > PCBs > HCHs > PBDEs. POP concentrations were significantly higher in males compared to females. HCB, DDTs, HCHs and PBDEs were significantly different between whales from the Mozambique population and the Ecuador population. Sex and feeding habits were important driving factors accounting for POP concentrations in Ecuador whales. The whales from our study had some of the lowest POP concentrations measured for humpback whales in the world. These whales fed predominantly on krill as reflected from the low $\delta^{13}C$ and $\delta^{15}N$ values measured in the skin. However, the isotopic niches of whales from Mozambique and Ecuador did not overlap indicating that the two populations are feeding in different areas of the Southern Ocean.

1. Introduction

Persistent organic pollutants (POPs) accumulate in polar regions like Antarctica (Wania and Mackay, 1993). They can be found everywhere on our planet in measurable concentrations and can be transported far from their emission sites via long-range environmental transport, mainly through the atmosphere, to redeposit close to the poles (Corsolini et al., 2006). POPs are not easily broken down and can accumulate in the tissue of living organisms. Legacy POPs, such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and brominated flame retardants (BFRs), can biomagnify as they move up the food web (Kelly et al., 2007). Polar POP contamination can occur through re-deposition of atmospheric POPs or local emission of these contaminants, by scientific stations for example (Risebrough et al., 1990). This long-range transport and redeposition of POPs results in different contaminant patterns not only throughout Antarctica but also throughout Antarctic food webs (Nash, 2011). Marine mammals accumulate high levels of contaminants through the food web. PCBs, OCPs, and polybrominated diphenyl ethers (PBDEs) affect the endocrine, immune and reproductive systems of marine mammals (Desforges

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et al., 2016; Ross et al., 2000). Thus, it is important to monitor contaminant accumulation and patterns in marine mammal sentinel species that forage in different regions around Antarctica.

A good marine mammal sentinel for Antarctica is the humpback whale (*Megaptera novaeangliae*) (Bengtson Nash et al., 2018). Humpback whales feed in Antarctic waters during the austral summer, preferentially on Antarctic krill (*Euphausia superba*) (Ryan et al., 2014). Their extensive feeding on krill ensures their successful migration and reproduction (Silva et al., 2013). Humpback whales in the Southern Hemisphere undertake northward migrations from Antarctica to warmer waters where they breed throughout the austral winter. These migrations are known to reach 10 000 km and are among the longest of any mammal (Stevick et al., 2011). Humpback whales feed opportunistically and at a reduced rate during their migrations to and from the breeding grounds as well as on the breeding grounds (Cerchio et al., 2013; Fossette et al., 2014; Silva et al., 2013).

The International Whaling Commission (IWC) defined seven stocks (A to G) of Southern Hemisphere humpback whales, based on where they breed (Fig. 1). A high breeding site fidelity for southern humpback whales was highlighted by genetic studies (Baker et al., 1994, 1993; Constantine et al., 2012; Jackson et al., 2014). The south-eastern Pacific Ocean corresponds to stock G with a breeding ground extending from north of Peru to Costa Rica and Panama in Central America (Castro et al., 2013; Pacheco et al., 2009; Rasmussen et al., 2007; Scheidat et al., 2000; Acevedo et al., 2007). The southwestern Indian Ocean is considered to be home to stock C with four sub stocks: C1, along with Mozambique and the eastern coast of South Africa; C2, the islands off Mozambique; C3, Madagascar; and C4, La Reunion (Best et al., 1998; Rosenbaum et al., 2009; Dulau-Drouot et al., 2012, 2011; Ersts et al., 2011). In the Southern Ocean, humpback whale feeding areas are separated into six longitudinal zones around Antarctica, named Areas I to VI (Rosenbaum et al., 2017). Breeding stock G (southeast Pacific) feeds in Area I (110°W-50°W), off the West Antarctic Peninsula, the South Shetland Islands, Sandwich Islands, and in the Magellan Strait (Acevedo et al., 2007; Branch, 2011; Castro et al., 2015). Area III (10°E–60°E) is the feeding area for humpback whales from breeding stock C (Branch, 2011). Out of the seven breeding stocks of southern humpback whales, only three were previously analysed for POP concentrations.

POP concentrations in the Southern Hemisphere were previously quantified in the blubber of humpback whales breeding off eastern Australia (stock E1), the western Antarctic Peninsula (stock A), and La Reunion island (stock C4) (Bengtson Nash et al., 2013; Das et al., 2017; Dorneles et al., 2015). Concentrations of POPs and other harmful chemicals were low and the result of humpback whales foraging at a low trophic level. Bulk stable isotopes like $\delta^{13}C$ and $\delta^{15}N$ have been used for decades to provide information on the diet, trophic level, and resource partitioning of marine mammals (Newsome et al., 2012, 2010). Briefly, $\delta^{15}N$ can be used to assess the trophic position of a consumer, while $\delta^{13}C$ gives information on the geographic location of the primary producers (Post, 2002). Stable isotopes measured in combination with POPs in migratory species can further characterize their trophic ecology, feeding habits, and population structure (Witteveen et al., 2009a,b).

Here, we present the first results of POP concentrations and bulk stable isotopes in humpback whales breeding off Mozambique and Ecuador. The objective of our study was to describe and compare these populations' concentrations of various legacy POPs, taking into account their sex, trophic level, and feeding locations. We hypothesized that the isotope values and contaminant concentrations would differ among the populations due to the geographic differences separating the two stocks.

2. Methodology

2.1. Sampling

Sampling was conducted on the whales' breeding grounds after they arrived from Antarctica. Incorporation of stable isotopes from the diet has been estimated for cetacean species and skin stable isotopes seem to reflect the diet of cetaceans two to six months before sampling (Busquets-Vass et al., 2017; Giménez et al., 2016). Therefore, we focused our sampling efforts on the wintering season to guarantee an accurate feeding habit representation from the stable isotope data. Whales from Ecuador (n = 59) were sampled after their arrival on their wintering grounds from 27 August to

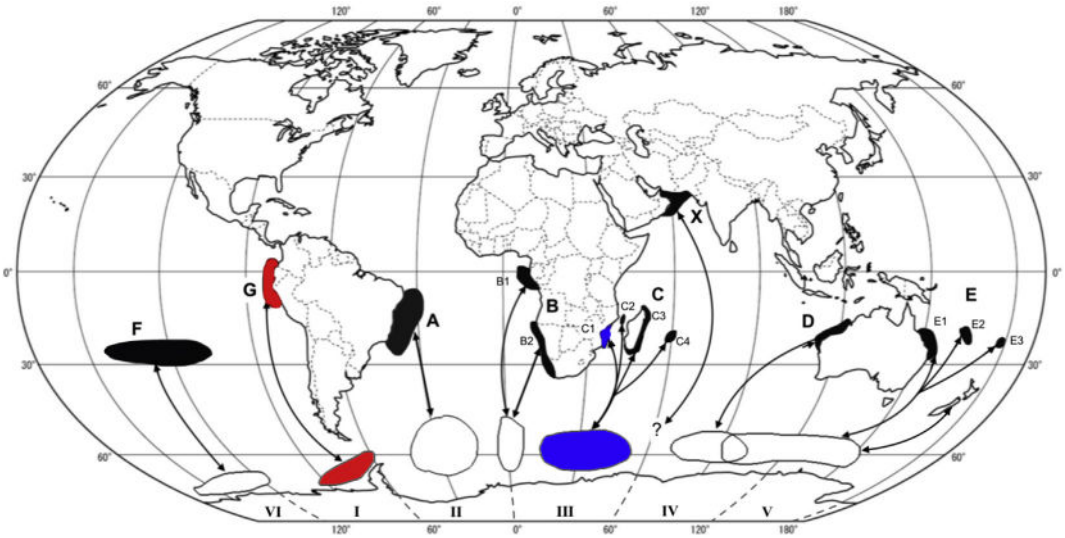

Fig. 1. Breeding and feeding areas for the different stocks of humpback whales in the Southern Hemisphere (after International Whaling Commission, 1998). Letters indicate the breeding areas and roman numerals indicate the feeding areas. The breeding and foraging areas for the present study are shown in red (Ecuador) and blue (Mozambique). Additional subpopulations/populations are shown in black (breeding regions) and gray (foraging regions). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

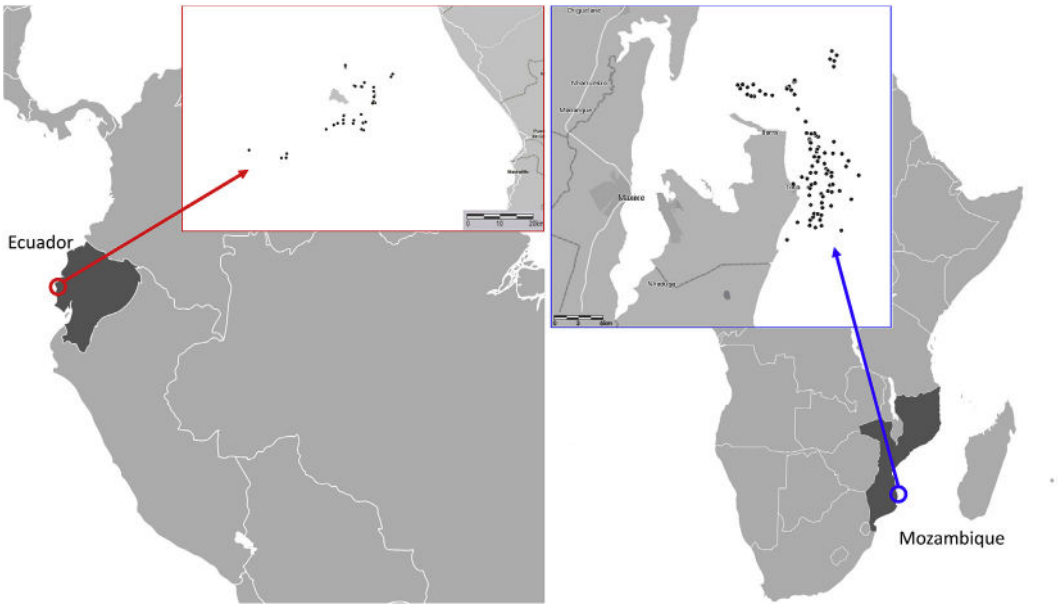

Fig. 2. Sampling map of whale skin biopsies collected in Ecuador (n = 59, 2014–2015) and Mozambique (n = 89, 2017).

September 14, 2014 (18 days) and from 2 to September 17, 2015 (12 days), in collaboration with the Pacific Whale Foundation Ecuador. Sampling was performed around La Plata Island off Machalilla National Park. Whales from Tofo, Mozambique (n = 89) were biopsied after their arrival on their wintering grounds from 17 July to September 15, 2017 (60 days), in collaboration with Odyssea (Fig. 2). Sampling took place on small boats (5–12 m) and was performed using a crossbow (Barnett Panzer V®, 150 lb draw-strength) with bolts (Mikkel Villum TM) and 40 mm steel tips. Only adult whales were sampled, and we focused our effort mainly on males although females were occasionally sampled. Biopsies were collected under permits from the respective governments. Skin and blubber biopsies were kept at −20 °C until they were transferred to Liège, Belgium using CITES permits (N° IM085/2014/A and N° MZ786/2017 for Ecuador and Mozambique, respectively) issued by the Luxembourg Government.

2.2. Sample processing

Upon reception in Liège, the biopsies were cut into three parts using sterilized scalpels. The first part of the biopsy corresponded to the skin, which was placed in a glass tube for stable isotope analysis. The second part of the biopsy corresponded to the blubber and was placed in an Eppendorf tube. The last part was the smallest and corresponded to the blubber/skin interface and this was stored in 70% ethanol for genetic determination of the sex. All three parts were then stored at −20 °C until their respective analyses.

2.3. Genetic determination of sex

Sex was determined genetically following the method described previously (Macé and Crouau-Roy, 2008). Briefly, we extracted DNA from the samples using a XYZ kit (according to manufacturer's instructions). The reaction mix contained 0.5 μM of each primer (Primers SC1: 5′-CAAGCATGCATTTCAATTCCC and SC2: 5′-CTGCATGGGGAACATCGGAG), 2 μl of DNA, and 10 μl of HotStarTaq Master Mix (Qiagen) bringing the total volume to 20 μl. PCR was achieved through the following steps: 1) initial activation at 95 °C for 5 min; 2) denaturing through 45 cycles (95 °C for 1 min); 3) annealing at 55 °C for 45 s; 4) elongation at 72 °C for 1 min; 5) final elongation at 72 °C for 5 min. PCR products were run on 1% agarose gel stained with Midori Green Advance (Nippon Genetics).

2.4. Bulk stable isotope analysis

The skin was cut and freeze-dried for easier grinding. Samples were then ground using a mortar and pestle until fully homogenized. In cetaceans, there is an association of skin with lipids present in the blubber; these lipids are more enriched in ^{12}C compared to proteins, which decreases the $\delta^{13}C$ values in the skin (DeNiro and Epstein, 1978; Ryan et al., 2012). Additionally, the variation of lipid percentage between samples is an important factor of variation in $\delta^{13}C$ values and, therefore, lipid extraction is recommended (Ryan et al., 2012). Solvent lipid extraction increases $\delta^{15}N$ values, thus requiring two distinct measures of isotope ratios (one with lipid extraction, one without lipid extraction) (Lesage et al., 2010; Ryan et al., 2012; Sweeting et al., 2006). The stable isotope analysis followed the methods of Pinzone et al. (2019) and is described in the supplementary information.

2.5. Contaminant analysis

The persistent organic pollutants analysed in our study are available in Table 1. The analysis was conducted following Das et al. (2017), and is fully described in the supplementary information. Briefly, we extracted contaminants and lipids from blubber (~200 mg) using hexane: dichloromethane (1:1, v/v). We used an aliquot (~1/10) of the extract to measure the lipid percentage in the blubber. Following the removal of the lipids from the rest of the extract, we measured the contaminant concentrations through a GC-MS system in electron ionization (EI) mode for low chlorinated PCBs and DDTs, and through a GC-MS system in electron capture negative ionization (ECNI) mode for PBDEs, high chlorinated PCBs, and the remaining OCPs.

2.6. Quality assurance/quality control

The limit of detection (LOD) was established for each compound

Table 1
Persistent organic pollutant target analytes included thirty PCBs, fourteen OCs, seven PBDEs, and two MeO-PBDEs.

Polychlorinated biphenyls (PCBs)	IUPAC numbers: CB 18, 28, 49, 52, 87, 95, 99, 101, 105, 110, 118, 128, 138, 146, 149, 153, 156, 170, 171, 172, 174, 177, 180, 183, 187, 194, 195, 199, 205, and 209
Dichlorodiphenyltrichloroethane (DDT) and metabolites	*p,p′*-DDD, *p,p′*-DDE, *p,p′*-DDT, *o,p′*-DDD, and *o,p′*-DDT
Chlordanes (CHL) and metabolites	*cis*-chlordane (CC), *trans*-chlordane (TC), *trans*-nonachlor (TN), *cis*-nonachlor (CN), oxychlordane (OxC)
Hexachlorocyclohexane (HCHs)	α-HCH, β-HCH, and γ-HCH
Hexachlorobenzene (HCB)	HCB
Polybrominated diphenyl ethers (PBDEs)	IUPAC numbers: BDE 28, 47, 99, 100, 153, 154, and 183
Methoxylated PBDEs (MeO-PBDEs)	2′-MeO-BDE68 and 6-MeO-BDE47

and corresponded to three times the standard deviation (SD) of the mean of the blank measurements. Procedural blanks (n = 12) were analysed with every batch of samples to check for lab contamination. Blanks were consistent (RSD < 20%) and the mean value calculated for each compound was subtracted from the sample values. Mean ± SD recoveries for the internal standards PCB 143, ε-HCH, ^{13}C-HCB, and BDE 77 were 86 ± 6%, 98 ± 8%, 85 ± 10%, and 93 ± 10%, respectively. Analytical procedures were validated through the analysis of certified material SRM (Standard Reference Material) 1945: organic contaminants in whale blubber. Deviations from certified values were less than 10%. Contaminant values are presented in ng/g lipid weight (lw).

2.7. Data analysis

One outlier in stable isotope data was removed ("EQ7", Suppl. Info). We used the Stable Isotope Bayesian Ellipses (SIBER) package (v2.1.3), run in R (v3.5.0) to compare the isotopic niches of humpback whales from Ecuador and humpback whales from Mozambique. The stable isotope data analysis is fully described elsewhere (Pinzone et al., 2019). Standard ellipses included 40% of the data to represent the core of the population (Jackson et al., 2011). Bayesian modelling (SEA_B) was run to calculate the area of each population's niche and calculate the potential overlap of the niches. The number of iterations for the Bayesian model was set to 10^5. To compare isotopic values between each population, we used a Student's *t*-test. To understand the intra-population variability in stable isotopes, we used a partitioning cluster analysis (k-means) on δ^{15}N and δ^{13}C to determine different groups. This analysis was followed by a general linear modelling (GLM) analysis on the cluster groups (response variable) and included the following predictors: *sex*, *lipid percentage*, *contaminant concentrations* and *time*. Before the contaminant analysis, pollutant data were lipid normalized and values under the limit of detection (LOD) were assigned a value corresponding to half the compound's LOD. Statistics were run in R (v3.5.0). The sex ratio was similar between the two populations (75% males in Mozambique; 80% males in Ecuador) so the concentrations were not sex corrected. The lipid percentages were compared between the two populations using a Student's *t*-test.

To account for baseline geographic variation in δ^{15}N, we took into account mean krill δ^{15}N data averaged from two studies in feeding area III and four studies from feeding area I. As δ^{15}N values for krill from each feeding zone did not vary considerably (3‰ for feeding area I, 2.5‰ in feeding area III) we used the data to calculate the trophic position of each humpback whale using the trophic position equation $TP = ((\delta^{15}N\ whale - \delta^{15}N\ krill)/2.8) + 1$, where 2.8 is the mean trophic enrichment factor for the incorporation of krill bulk nitrogen isotopes into fin whale skin (Borrell et al., 2012). This trophic position was only used in the contaminant analysis to evaluate the impact of the trophic position and not δ^{15}N values because δ^{15}N values vary across ocean basins. We used a GLM approach with a Gamma (link = log) distribution to determine which factors were responsible for the contaminant variations. We included three predictors: *sex*, *population*, and *trophic position*; a *sex:population* and a *trophic position:population* interaction. δ^{13}C was not included as a predictor since it was confounded with the Population factor. Every possible model combination was run in the MuMIn package. To compare the different models, the Akaike's information criterion corrected for small sample size (AICc) was calculated and models within $\Delta AIC \leq 2$ were averaged to estimate predictors and their significance. When the *sex:population* effect was significant, we conducted a Tukey contrasts test from the multcomp package to conduct some pairwise comparisons between the sexes and populations. When the interaction between the *trophic position* and *population* was significant, we tested for the correlation between the contaminant class and the trophic position for each population with Pearson's correlation test. The Variance Explained (1-(Residual Deviance/Null Deviance)) was also calculated to determine how the different models explained the variance in pollutant classes (McFadden's Pseudo R2). Finally, to visualize the variation in POPs profiles between the two populations of humpback whales, we used a principal component analysis (PCA) on log-transformed and centre-scaled POP classes.

3. Results

The genetic sex determination resulted in 32 females, 10 in Ecuador and 22 in Mozambique; and 109 males, 44 in Ecuador and 65 in Mozambique. Sex could not be determined for 8 whales.

3.1. Dietary tracers – stable isotopes

We only considered lipid-extracted δ^{13}C and non-lipid-extracted δ^{15}N for statistical analyses. δ^{13}C after lipid extraction measured in Ecuador whales had a mean value of −24.5‰ (range: −25.49‰ to −23.05‰). We found that Ecuador whales had lower δ^{13}C values than Mozambique whales, which had a mean δ^{13}C of −26.03‰ (range: −27.67 to −23.57‰) (t = 13.963, p < 0.001). δ^{15}N values were lower in whales from Mozambique (t = 2.5126, p = 0.013) and had a mean value of 8.02‰ (range: 6.25–12.29‰) while Ecuadorian whales had a mean δ^{15}N value of 8.68‰ (range: 6.62–11.97‰) (Table 2). Male and female δ^{13}C and δ^{15}N values in Mozambique whales did not differ (t = 0.5569,

Table 2
δ^{13}C and δ^{15}N values (‰) and C:N ratio in skin from humpback whales *Megaptera novaeangliae* from Mozambique and Ecuador. Results are expressed in mean ± standard deviation. (* indicates P-value <0.05; ** indicates P-value <0.01 for Student's *t*-test comparison between populations).

		δ^{13}C	δ^{15}N	C:N
Mozambique	Non extracted	−27.2 ± 0.8	8.0 ± 1.4	3.9
	Extracted	−26.0 ± 0.8**	7.4 ± 0.4	3.5
Ecuador	Non extracted	−24.7 ± 2.8	8.7 ± 1.7*	3.8
	Extracted	−24.5 ± 0.5	8.2 ± 0.3	3.2

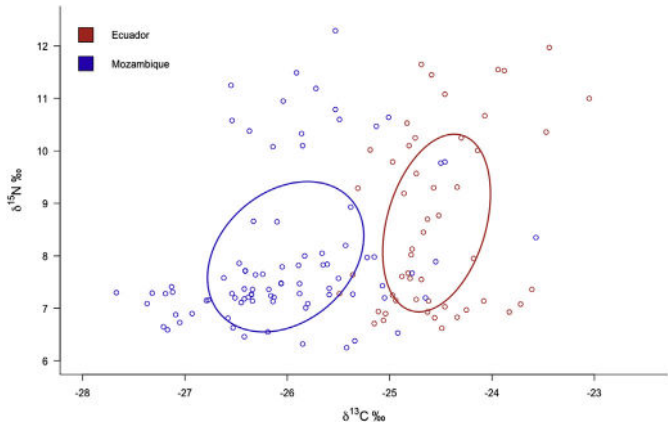

Fig. 3. $\delta^{13}C$ and $\delta^{15}N$ biplot in skin of humpback whales from Ecuador and Mozambique. Each little circle represents an individual (from Mozambique in blue; from Ecuador in red). Solid lines represent the standard ellipses (40% of the data, representing the core of the population) associated to each population. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

$p = 0.5819$ for $\delta^{13}C$ and $t = 0.055601$, $p = 0.956$ for $\delta^{15}N$). However, $\delta^{13}C$ and $\delta^{15}N$ values were both significantly lower in females from Ecuador compared to males from Ecuador ($t = -4.9814$, $p < 0.001$ for $\delta^{13}C$ and $t = -3.5438$, $p < 0.001$ for $\delta^{15}N$).

The SIBER analysis for nitrogen and carbon revealed that the two $\delta^{15}N$ *vs.* $\delta^{13}C$ core (40%) ellipses from the Mozambique population and the Ecuador population did not overlap. This could also be observed on the isotopic biplot in Fig. 3. The Mozambique Bayesian standard ellipse area (SEAb) (mode = 3.23; CI 95%: 2.62 to 4.03) was larger than the Ecuador one (mode = 2.68; CI 95%: 2.07 to 3.50) in 86% of the model runs (Supplementary Information). The whales from Ecuador showed a continuous distribution both on the $\delta^{15}N$ axis and $\delta^{13}C$ axis. Even though there were significant differences in Males *vs.* Females, the partitioning cluster analysis (k-means) on stable isotope data did not separate the Ecuador population into clusters. However, we observed two subgroups in the Mozambique ellipse and the partitioning cluster analysis on $\delta^{15}N$ and $\delta^{13}C$ revealed that the Mozambique whales were separated into two clusters (Supplementary Information). Cluster 1 included whales that had higher $\delta^{15}N$ values (above 8.9‰; mean = 10.4‰); cluster 2 included whales that had lower $\delta^{15}N$ values (below 8.9‰, mean = 7.3‰). We ran a GLM model selection on predictors expected to impact the cluster separation of the values. Our predictors included *sex*, *time* (number of days since start of sampling), *lipid percentage* and all *contaminant classes*. No model had a lower AICc than the null model (AICc = 85.75). Five models separated from the null model by a ΔAICc lower than 2 were averaged but no predictor effect was significant, meaning no factor in our dataset could explain why Mozambique whales were separated into two isotopic clusters.

3.2. Persistent organic pollutants

The lipid percentage in the blubber was not statistically different between Mozambique and Ecuador populations: 42% (11–70%) *vs.* 40% (20–58%) respectively ($t = -1.3436$, $p = 0.18$). Lipid percentages within each population were not different between sexes ($t = 1.0211$, $p = 0.31$ for Mozambique; $t = 1.0464$, $p = 0.31$ for Ecuador) (Table 3). The trophic positions were calculated to account for geographic baseline $\delta^{15}N$ variation between whales from Mozambique and Ecuador by using krill $\delta^{15}N$ values from the two feeding areas. Trophic levels were successful in getting rid of the

Table 3
Lipid percentage and concentrations of PCBs, HCB, HCHs, CHLs, PBDEs, DDTs, and MeO-PBDEs in blubber samples from humpback whales from Mozambique and Ecuador. Results are expressed as mean (median) ± standard deviation; range. POP concentrations are expressed as mean in ng/g lw. Total number includes males + females + unidentified sex.

	% Lipids	∑PCBs	HCB	∑HCHs	∑CHLs	∑PBDEs	∑DDTs	∑MeO-PBDEs
Mozambique males (n = 63)	**41.2** (42.0) ± 12.2 11–68	**2.5** (1.8) ± 3.2 0.4–22.7	**68.6** (68.0) ± 20.5 8.7–127.0	**0.3** (0.3) ± <0.1 0.3–0.5	**4.3** (3.6) ± 2.8 1.0–16.9	**0.4** (0.4) ± 0.1 0.3–0.8	**8.4** (8.0) ± 3.7 0.4–26	**0.3** (0.3) ± 0.2 0.10–0.6
Mozambique females (n = 22)	**43.9** (41.0) ± 10.1 25–70	**1.8** (1.7) ± 0.6 1.2–3.6	**59.6** (59.8) ± 14.9 32.8–95.5	**0.3** (0.3) ± <0.1 0.3–0.3	**3.7** (2.9) ± 2.6 1.2–10.9	**0.4** (0.4) ± <0.1 0.4–0.4	**7.3** (6.9) ± 2.8 2.1–13.7	**0.3** (0.3) ± 0.1 0.10–0.6
Mozambique total (n = 87)	**41.8** (41.0) ± 11.6 11–70	**2.3** (1.7) ± 2.8 1.2–22.7	**66.5** (65.7) ± 19.3** 8.7–126.7	**0.3** (0.3) ± <0.1** 0.3–0.5	**4.1** (3.2) ± 2.7 1.0–16.9	**0.4** (0.4) ± 0.1** 0.4–0.8	**8.0** (7.4) ± 3.5** 0.4–26.1	**0.3** (0.3) ± 0.1 0.1–0.6
Ecuador males (n = 44)	**38.5** (39.0) ± 9.5 20–58	**2.0** (1.8) ± 1.1 0.6–7.4	**42.1** (37.8) ± 15.6 9.1–77.3	**0.7** (0.5) ± 0.8 0.3–4.9	**5.2** (5.0) ± 2.5 1.5–10.8	**0.4** (0.3) ± 0.1 0.3–0.9	**26.7** (24.6) ± 21.9 6.1–153.0	**0.4** (0.3) ± 0.3 0–2.0
Ecuador females (n=10)	**41.1** (40.5) ± 6.3 32.0–53.0	**1.0** (1.0) ± 0.4 0.6–1.6	**20.1** (15.3) ± 15.5 6.1–59	**0.6** (0.5) ± 0.2 0.3–1.0	**2.3** (1.7) ± 2 0.70–6.8	**0.3** (0.3) ± <0.1 0.3–0.4	**18.9** (17.7) ± 6.1 11.4–28.6	**0.2** (0.2) ± 0.1 0–0.3
Ecuador total (n = 59)	**49.5** (40) ± 8.9 20–58	**1.8** (1.7) ± 1.0 0.6–7.4	**36.5** (36.2) ± 17.8** 6.1–77.3	**0.7** (0.5) ± 0.7** 0.3–4.9	**4.9** (4.0) ± 2.7 0.7–10.8	**0.4** (0.3) ± 0.10** 0.3–0.9	**24.0** (19.8) ± 19.7** 4.8–153.9	**0.3** (0.3) ± 0.3 0–2.0

** indicates P-value < 0.01 and shows a difference between the Mozambique and Ecuador populations from the GLM analysis when the predictor *population* was significant.

geographic differences in $\delta^{15}N$ as trophic positions did not differ between the two populations (mean = 2.98 for Ecuador, mean = 2.94 for Mozambique, $p = 0.71$).

POP concentrations were quantified in order of highest to lowest as HCB > DDTs > CHLs > PCBs > HCHs > PBDEs > MeO-PBDEs for all whales except 11 whales in Ecuador that had higher ∑DDT concentrations than HCB (Table 3). In Mozambique, the two major classes of contaminants were HCB and ∑DDTs. HCB had a mean concentration of 66.5 (8.7–126.7) ng/g lw, DDTs had a mean concentration of 8.0 (0.4–26.1) ng/g lw. The predominant compounds in each class of chemicals present in Mozambique whales were HCB, *p,p*'-DDE, *trans*-nonachlor (TN), PCB-153, γ-HCH, BDE-47, and 6-MeO-BDE47. HCB accounted for 81% and ∑DDTs for 10% of POPs. In Ecuador, the two major pollutant classes were also HCB and ∑DDTs. HCB had a mean concentration of 36.5 (6.1–77.3) ng/g lw and ∑DDTs had a mean concentration of 24.0 (4.8–153.9) ng/g lw. The predominant compounds in each class of chemicals present in Ecuador whales were HCB, *p,p*'-DDE, TN, PCB-138, α-HCH, BDE-47, and 6-MeO-BDE47. HCB and DDTs represented 54% and 35% of all POPs, respectively.

The GLM analysis revealed that different predictors were responsible for the variation of contaminants (Table 4). The best models for ∑PCBs explained up to 18% of the deviance but none of the averaged effects were significant, meaning ∑PCB concentrations were not statistically different between sexes, populations or across trophic positions. The best model for ∑DDTs explained 64% of the deviance. Mozambique whales had lower ∑DDT concentrations than Ecuador whales ($\beta = -0.37$, $p < 0.01$). *Trophic position* was a significant factor in variation of ∑DDTs ($\beta = 0.0.59$, $p < 0.01$). Since the interaction between the *trophic position* and *population* was significant, we tested for the correlation between DDTs and the trophic level in each population. This correlation test revealed ∑DDTs were only correlated with the *trophic position* in Ecuador whales ($R = 0.29$, $p = 0.03$) not in Mozambique whales ($R = 0.1$, $p = 0.26$). The best model for ∑CHLs explained 12% of the deviance. Males had higher ∑CHL concentrations ($\beta = 0.75$, $p < 0.01$) and although the two populations were statistically not different, the interaction *sex:population* was significant. The Tukey's contrasts test revealed that males had higher ∑CHLs concentrations than females in Ecuador ($\beta = 0.87$, $p < 0.01$) but not in Mozambique ($\beta = 0.12$, $p = 0.85$). The best model for HCB explained 43% of the deviance, included all predictors and all the predictors were significant. Tukey's contrasts tests revealed that males had higher HCB concentrations in Ecuador ($\beta = 0.75$, $p < 0.01$) but not in Mozambique ($\beta = 0.13$, $p = 0.47$). *Trophic position* was a significant factor of HCB variation, yet only in whales from Ecuador ($R = 0.44$, $p < 0.01$), not in Mozambique ($R = -0.13$, $p = 0.26$). *Population* was the only predictor in the best model for ∑HCHs and explained 46% of the deviance; Mozambique whales had lower concentrations than Ecuador whales ($\beta = -0.63$, $p < 0.01$). Finally, the model that best explained ∑PBDE concentration variations revealed that concentrations were higher in Mozambique ($\beta = 0.87$, $p < 0.01$). We conducted a Pearson correlation test on ∑PBDEs and the trophic position in each population since the interaction was significant. These two tests revealed whales ∑PBDE concentrations were associated with *trophic position* in Ecuador ($R = 0.33$, $p = 0.01$) but not in Mozambique ($R = -0.06$, $p = 0.58$).

We performed a principal component analysis to test for the

Table 4
Results of the generalized linear modelling approach that assessed the independent variables (Sex, Trophic Position, and Population) that explained the variability in each of the six response variables (contaminant classes). Only models that have an ΔAICc below or equal to 2 are presented since they were averaged to determine the predictors coefficients. The deviance explained is calculated as: 1–(Residual Deviance/Null Deviance) and is similar to R^2. ** indicates a p-value < 0.001.

Models	AICc	ΔAICc	Deviance Explained	Intercept	Population	Sex	Sex:Population	Trophic Position	Population:Trophic Position
PCBs ~ sex + population + trophic position + population:trophic position	390.03	0.00	0.17	−0.69	1.88	0.4	−0.23	0.37	−0.59
PCBs ~ sex + population + trophic position + sex:population + population:trophic position	391.35	1.32	0.18						
DDTs ~ population + trophic position + trophic position:population	818.16	0.00	0.64	1.58	−0.37 **	0.08	–	0.59 **	−0.54**
DDTs ~ sex + population + trophic position + trophic position:population	819.40	1.24	0.64						
CHLs ~ sex + population + sex:population	584.71	0.00	0.12	0.7	0.36	0.85**	−0.7**	0.09	0.25
CHLs ~ sex + population + trophic position + sex:population	585.24	0.53	0.13						
CHLs ~ sex + population + trophic position + sex:population + population:trophic position	586.20	1.49	0.14						
HCB ~ sex + population + trophic position + sex:population + population:trophic position	1157.10	0.00	0.43	2.04	2.09**	0.56**	−0.44**	0.4**	−0.42**
HCHs ~ population	−169.97	0.00	0.46	−0.54	−0.63**	0.03	–	−0.02	–
HCHs ~ sex + population	−168.10	1.87	0.47						
HCHs ~ trophic position + population	−168.00	1.97	0.47						
PBDEs ~ population + trophic position + trophic position:population	−317.40	0.00	0.12	−1.61	0.67**	0.07	−0.07	0.21**	−0.25**
PBDEs ~ sex + population + trophic position + trophic position:population	−317.30	0.10	0.13						
PBDEs ~ sex + population + trophic position + sex:population + trophic position:population	−309.27	2.15	0.14						

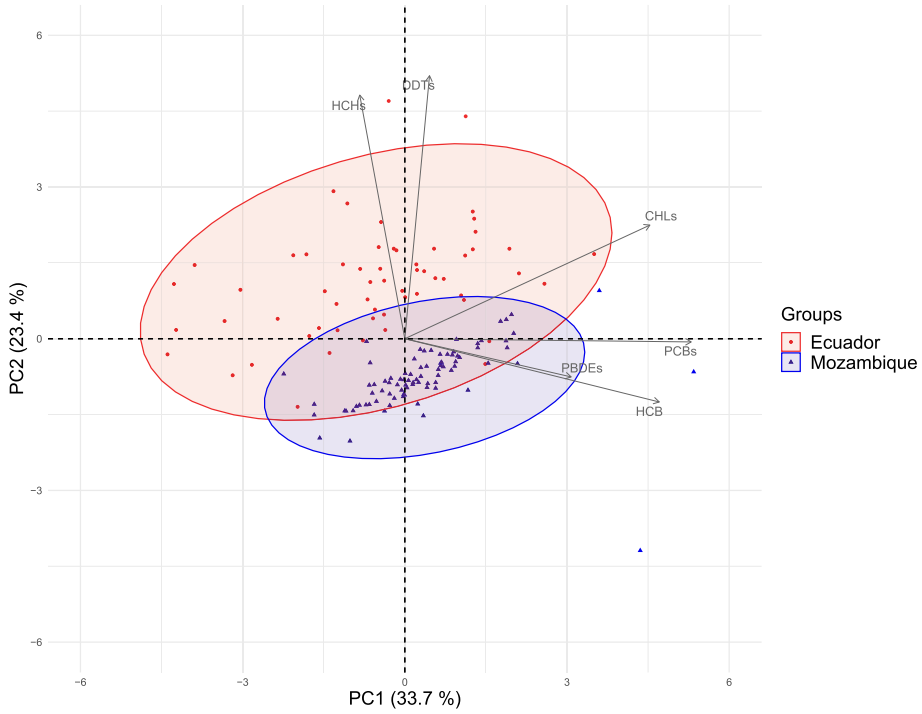

Fig. 4. Principal Component Analysis of POP concentrations with a log-transformed, centre-scaled dataset (PC1 & PC2 account for 57.1% of the POP variation). PC1 axis was represented by PCBs, HCB, and CHLs (28%, 25%, and 25% contribution to PC1 respectively), while PC2 axis was represented by DDTs and HCHs (43% and 34% contribution to PC2 respectively).

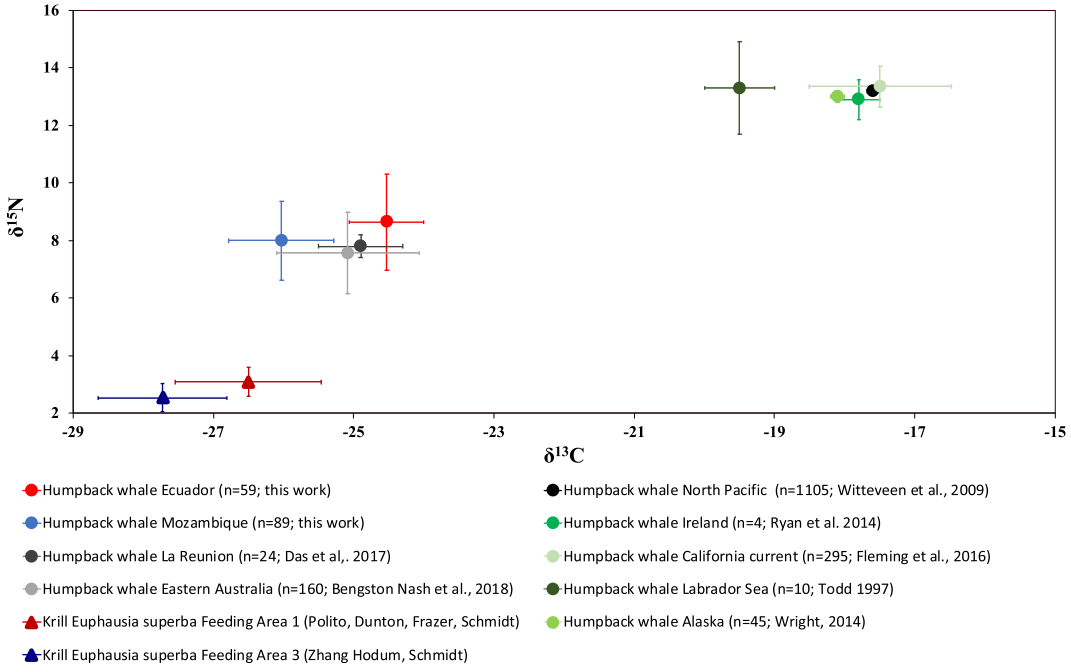

Fig. 5. $\delta^{13}C$ and $\delta^{15}N$ (‰) values in humpback whales, *Megaptera novaeangliae*, off Ecuador and Mozambique (this work) compared to humpback whales off La Reunion Island (Das et al., 2017), eastern Australia (Bengtson Nash et al., 2018,:Supporting Information), the western Antarctic Peninsula (Dorneles et al., 2015), and other populations from the Northern Hemisphere (Witteveen et al., 2009a,b; Ryan et al., 2014; Todd et al., 1997; Fleming and Jackson, 2011; Wright, 2014), and krill populations from feeding area I (stock G) and III (stock C1 & C4) (Dunton, 2001; Frazer, 1996; Polito et al., 2013; Schmidt et al., 2003; Zhang et al., 2017).

differences in POP profiles between the two populations. PC1 and PC2 explained 57.1% of the variance between samples (Fig. 4). PC1 axis was represented by ∑PCBs, HCB, and ∑CHLs (28%, 25%, and 25% contribution to PC1 respectively), while PC2 axis was represented by ∑DDTs and ∑HCHs (43% and 34% contribution to PC2 respectively). ∑DDTs and ∑HCHs were a factor of separation between the two populations on the PC2 axis. HCB concentrations were an important factor of variance, highlighting the fact that

Mozambique whales had higher HCB concentrations than Ecuador whales.

4. Discussion

This study investigated POPs and stable isotopes for the first time in humpback whales breeding off Ecuador and Mozambique. It contributes to the increasing knowledge related to contaminant patterns in Antarctica through the perspective of an Antarctic sentinel species: the humpback whale.

4.1. Feeding habits of humpback whales on both sides of the equator

Humpback whales have higher $\delta^{15}N$ and $\delta^{13}C$ values in the Northern Hemisphere than in the Southern Hemisphere (Witteveen et al., 2009a,b). Differences in $\delta^{15}N$ and $\delta^{13}C$ values from the two hemispheres could be attributed to geographic variation in particulate organic matter and planktonic $\delta^{15}N$ values (McMahon et al., 2013). Prey species of northern humpback whales were found to have higher $\delta^{15}N$ values: krill had a mean $\delta^{15}N$ of 8‰ in Alaska (compared to ~3‰ in the Southern Ocean). Higher $\delta^{15}N$ values in northern humpback whales also illustrates the fact that Northern Hemisphere whales feed on fish like herring, pollock, haddock, mackerel, capelin, salmon, and other fish (Todd et al., 1997). Eutrophication caused by higher human activity in the Northern Hemisphere could also increase $\delta^{15}N$ values in local fish and invertebrates (Griffin, 2001; McClelland et al., 1997).

4.2. Interpopulation variation in feeding habits in Ecuador versus Mozambique

Cetacean skin stable isotopes represent the diet of the last 2–6 months before the biopsies are collected (Busquets-Vass et al., 2017; Giménez et al., 2016). Thus, $\delta^{13}C$ and $\delta^{15}N$ value analyses from Ecuador and Mozambique whales represent their feeding habits from feeding areas I and III, respectively. The low $\delta^{15}N$ values in our study imply that our whales feed at a low trophic level, agreeing with other southern humpback whale studies (Bengtson Nash et al., 2018; Das et al., 2017; Dorneles et al., 2015). Values of $\delta^{13}C$ for our whales are in agreement with $\delta^{13}C$ measured in particulate organic matter and Antarctic krill (Francois et al., 1993; Frazer, 1996; Hodum and Hobson, 2000; Polito et al., 2013; Schmidt et al., 2003; Stowasser et al., 2012; Zhang et al., 2017). $\delta^{13}C$ values for krill in feeding area I ranged from −24.5‰ to −29.3‰ (Dunton, 2001; Frazer, 1996; Polito et al., 2013; Schmidt et al., 2003) while values ranged from −25‰ to −31.2‰ in krill from feeding area III (Hodum and Hobson, 2000; Schmidt et al., 2003; Zhang et al., 2017) (Fig. 5). Our SIBER analysis showed no overlap in $\delta^{15}N$ *vs.* $\delta^{13}C$ ellipses from Mozambique and Ecuador suggesting a different geographic origin of the primary production, supported by a significant difference in $\delta^{13}C$ values between our two populations (Fig. 3). Differences in $\delta^{15}N$ values in populations from the Southern Hemisphere could be attributed to geographic variations in planktonic $\delta^{15}N$ (Lorrain et al., 2009) since their trophic position, taking into account the baseline $\delta^{15}N$ variation, resulted in no differences between the populations. The equation we used to calculate the trophic position has now been replaced by complex Bayesian modelling approaches, thus this simple equation was only used to remove the effect of baseline variation (Quezada-Romegialli et al., 2018).

4.3. Intrapopulation variation in stable isotopes

Within the Ecuador population, females had significantly lower $\delta^{15}N$ and $\delta^{13}C$ values than males. A possible difference between male and female feeding habits deserves further investigation to better understand the intra-population structure of southern humpback whales. $\delta^{15}N$ varied across the population, ranging from 6 to 12‰. These values expressed in trophic level were correlated with DDTs and HCB concentrations and could illustrate a large dietary spectrum for these whales. Climate change and the reduction of sea ice impacts phytoplankton biomass and, through a bottom-up feedback, reduces krill communities on which humpback whales feed (Flores et al., 2012). By feeding mainly on a single species, humpback whales are in a precarious trophic position and will most likely need to shift their dietary preferences to survive the effects of climate change (Bengtson Nash et al., 2018). Additionally, recent studies have found that migrating humpback whales could feed on their breeding grounds, as demonstrated by observation or higher isotopic values (Eisenmann et al., 2016; Findlay et al., 2017). For future research on feeding habits of southern humpback whales, we recommend a study focusing on compound-specific stable isotopes since they account for baseline isotopic variation and are more accurate at estimating consumers' feeding habits (Chikaraishi et al., 2009). We also recommend measuring stable isotopes both on the breeding and feeding grounds to assess potential opportunistic feeding at a higher trophic level.

Mozambique whales showed a large $\delta^{15}N$ range (6–12‰) as well, although this variation could not be explained easily. Two clusters separated by low/high $\delta^{15}N$ values were observed in Mozambique (Fig. 4). No predictors could explain the separation of Mozambique whales into two clusters. Thus, $\delta^{15}N$ variation could be caused by age or physiological differences. Another reason behind the variation in $\delta^{15}N$ values could be geographic since baseline $\delta^{15}N$ can vary at a small geographic scale (Dale et al., 2011). Furthermore, $\delta^{15}N$ values were found to vary latitudinally and longitudinally (range ~0–5‰) across feeding area III in a recent study (Espinasse et al., 2019). The second cluster within this population could correspond to whales feeding in a different area than the other whales. It could represent a foraging habitat difference within the vast feeding area III.

4.4. Geographic variation of persistent organic pollutants across the world

Humpback whales from Ecuador and Mozambique are among the least contaminated populations of humpback whales in the world (Table 5). Thus far, three studies have analysed POPs in humpback whale blubber in the Southern Hemisphere, accounting for three stocks out of the seven stocks defined by the IWC (Bengtson Nash et al., 2013; Dorneles et al., 2015; Das et al., 2017). Contaminant concentrations in southern humpback whales were in the same order of magnitude as our results (Table 5). Whales sampled in the Northern Hemisphere were more contaminated than Southern Hemisphere whales, and POP concentrations were higher by an order of magnitude at least (Metcalfe et al., 2004; Elfes et al., 2010; Bachman et al., 2014). The differences between the two hemispheres can be attributed to differences in trophic levels and the historical use of POPs in the Northern Hemisphere. Diet is one of the most important factors of POP variation, as POPs biomagnify through the food web (Corsolini et al., 2006). Humpback whales from the Northern Hemisphere do not only rely on krill, but also on other invertebrates and fish located higher in the food chain, as illustrated in Fig. 5. Thus, differences in diet can explain, in part, why humpback whales from the Northern Hemisphere are more contaminated (Gauthier et al., 1997). Additionally, the Northern Hemisphere has historically received more input of POPs than the Southern Hemisphere through industries and pesticide usage. The Northern Hemisphere accounted for almost 97% of the

Table 5
Lipid percentage and concentrations of PCBs, HCB, HCHs, CHLs, PBDEs and DDTs in blubber samples from humpback whales from all over the world. Results are expressed as **mean** (median) ± SD; min–max. POP concentrations are expressed as the mean in ng/g lw.

Sampling location	Sample size	Year of sampling	% Lipids	Nutritional State	∑PCBs	HCB	∑HCHs	∑CHLs	∑PBDEs	∑DDTs	Source
Ecuador Pacific Ocean Stock G	**59**	2014-2015	40	Breeding	**1.8** (1.7) ± 1.1 0.6–7.4	**36.5** (36.2) ± 17.7 6.1–77.3	**0.6** (0.5) ± 0.7 0.3–4.9	**4.4** (4.0) ± 2.7 0.7–10.8	**0.4** (0.3) ± 0.1 0.3–0.9	**24.0** (19.8) ± 19.7 4.8–153.9	**This work**
Mozambique Indian Ocean Stock C1	**87**	2017	42	Breeding	**2.3** (1.7) ± 2.8 1.2–22.7	**66.5** (65.7) ± 19.3 8.7–126.7	**0.3** (0.3) ± <0.1 0.3–0.5	**4.1** (3.2) ± 2.7 1.0–16.9	**0.4** (0.4) ± 0.1 0.4–0.8	**8.1** (7.4) ± 3.5 0.4–26.1	**This work**
Reunion Island Indian Ocean Stock C4	**25**	2010-2011	37	Breeding	**3.4** (2.1) ± 3.8 0.7–16.4	**28.8** (23.9) ± 17.7 6.6–66.8	**3.6** (2.4) ± 3.4 0.4–12.2	**8.1** (7.8) ± 6.5 1.4–26.0	**1.4** (0.8) ± 2.4 0.2–12.0	**9.5** (9.0) ± 6.4 2.4–25.7	Das et al., 2017
Western Antarctic Peninsula Southern Ocean Stock G	**15**	2000-2001	40	Feeding	**131.0** (83.3) ± 192.0 4.4–761.0	**35.4** (33.1) ± 20.0 6.8–74.5	**11.5** (9.2) ± 11.0 2.2–43.7	**5.9** (5.7) 73.3 1.9–14.4	**5.8** (1.5) ± 12.6 0.4–50.8	**21.2** (13.2) ± 34.0 4.0–143.0	Dorneles et al., 2015
Eastern Australia Pacific Ocean Stock E	**41**	2008-2011	44.5	Average of northward and southward data	**18**	**160**	**11**	**23**	NA	**51**	Bengston Nash et al. 2013
Hawaii Pacific Ocean	**3**	1998-2009	36	Breeding	**287.0** (104.0) ± 324.0	**141.0** (115.0) ± 45.0	**135.0** (114.0) ± 37.4	**58.3** (55.9) ± 4.1	**16.1** (7.1) ± 20.2	**103.0** (94.7) ± 13.9	Bachman et al. 2014
South East Alaska Northern Pacific	**10**	2003-2004	31	Feeding	**430.0** ± 97.0	NA	**250.0** ± 46.0	**330.0** ± 57.0	**22.0** ± 6.0	**830.0** ± 130.0	Elfes et al., 2010
Gulf of St Lawrence Northern Atlantic	**12**	1993-1999	NA	Feeding	**897.2** ± 596.0	**153** ± 99.8	**108.1** ± 51.7	NA	NA	**1122.2** ± 1255.8	Metcalfe et al., 2004

environmental input of PCBs (Breivik et al., 2007). Even though atmospheric and hydrologic transport of POPs is an important redistribution route (Hageman et al., 2015), POPs are more concentrated in food webs close to emission sites and heavily populated areas which also explains the higher contamination in northern humpback whales. Among all populations, ∑PCBs were well below the 17 000 ng/g lw threshold at which animals may demonstrate undesirable biological effects like immune function alterations and reproductive issues (Kannan et al., 2000).

4.5. Southern Ocean geographic POP variations in humpback whales

Regional differences in POP concentrations contributed to the variation of Southern Hemisphere POP concentrations and profiles in humpback whales. Whales from our study showed the lowest ∑PCB concentrations in the Southern Hemisphere. Whales feeding off the Western Antarctic Peninsula (WAP) displayed higher ∑PCB concentrations than other southern humpback whales (Table 5), most likely due to local contamination in Antarctica (Dorneles et al., 2015). Indeed, a high number of scientific stations are present in the WAP compared to the rest of Antarctica (Scientific Committee on Antarctic Research - SCAR). These stations, close to the whales that were sampled by Dorneles et al. (2015), have been identified as a source of local PCB contamination due to the discharge of various waste products (Kennicutt et al., 2010). Additionally, whales from Ecuador had lower ∑PCB concentrations than Dorneles et al. (2015)'s whales; although they feed close to the WAP, supposedly further from scientific stations.

HCB levels were found to be similar in our study compared to other Southern Hemisphere humpback whales except for whales from Australia (Bengtson Nash et al., 2013). HCB had the highest concentration of all POPs measured in the Southern Hemisphere except for whales from the WAP that had PCB concentrations higher than HCB concentrations (Dorneles et al., 2015). HCB was higher in Mozambique whales than Ecuador whales in our study, although we do not have a clear explanation for this result. We believe geographic differences in POPs could be a reason for different HCB concentrations in populations of southern humpback whales. HCB is highly volatile and expected to reach worldwide equilibrium faster than other congeners (Bengtson Nash et al., 2008; Kang et al., 2012).

∑DDTs were higher in Ecuador whales than Mozambique whales in our study. ∑DDTs were similar between Ecuador whales and whales sampled feeding close to the WAP (Dorneles et al., 2015). Antarctica still receives inputs of *p,p*'-DDE via redistribution of previously deposited DDT in soil and snow/ice and from ongoing DDT usage in parts of the Southern Hemisphere, e.g. for vector control in disease prevention, which could explain the higher rates in whales feeding close to the WAP, close to South America (Poulsen et al., 2012; Van Den Berg, 2009). *p,p*'-DDE accounted for most of the ∑DDTs found in all humpback whale

populations. It is known to be the most persistent DDT metabolite, thus explaining its higher concentration in whales, despite the ban on the intensive use of DDT decades ago (Bengtson Nash et al., 2008).

The lowest concentrations of ∑HCHs were found in whales from our study, e.g. from Mozambique and Ecuador. The use of γ-HCH or lindane in vector control is still permitted in South America, likely explaining why ∑HCHs are higher in Ecuador than Mozambique (Dorneles et al., 2015). However, ∑HCH concentrations in the Southern Hemisphere have largely decreased in the last decades, which could explain the lower concentrations in our study (Li et al., 2020). ∑CHLs were found in similar concentrations in our study and populations south of the equator (Bengtson Nash et al., 2013; Das et al., 2017; Dorneles et al., 2015). The lowest ∑PBDE concentrations were found in whales from our study, e.g. from Mozambique and Ecuador where most PBDEs were < LOD. These decreasing ∑PBDE concentrations have been reported for the rest of the Antarctic atmosphere from 2011 to 2014 (Wang et al., 2017).

4.6. Factors responsible for the intrapopulation variation of POP concentrations

Sex and trophic level were factors that explained intrapopulation contaminant variations in humpback whales breeding off Ecuador. *Sex* was a factor of variation in Ecuador for HCB, and CHLs. Females had lower concentrations of pollutants than males which can be explained by the maternal transfer of contaminants, widely described in marine mammals (Kajiwara et al., 2008; Pinzone et al., 2015). *Sex* was also a factor of variation in other humpback whale populations in the Southern Hemisphere (Bengtson Nash et al., 2013; Dorneles et al., 2015; Das et al., 2017). Another driver of POP variation in Ecuador was the *trophic position*. Indeed, trophic positions derived from $\delta^{15}N$ in whales and $\delta^{15}N$ in krill from their feeding areas were associated with the concentrations of ∑DDTs, HCB and ∑PBDEs in Ecuador whales. Males in Ecuador had higher $\delta^{15}N$ and contaminant values than females which could account for a part of the trophic position's association with contaminants. A higher trophic position and higher $\delta^{15}N$ values with higher ∑DDT and HCB concentrations could indicate that some whales feed opportunistically at a higher trophic level in Antarctica, although this has not been explicitly demonstrated and deserves further attention.

Other factors that could not be measured in our study could be responsible for variation in POP concentrations and patterns. The better models only explained less than 20% of the variation in ∑PCBs, ∑CHLs, and ∑PBDEs in our two populations. Even when the better models were more efficient at explaining the variation for other POPs like HCB, ∑DDTs, and ∑HCHs, they only accounted for 43–66% of the variation. Known factors of POP variation in marine mammals include age, reproductive status, and physiological state (Ross et al., 2000). Age is a factor of POP variation since POPs accumulate over time (Desforges et al., 2016). However, age determination using biopsies is still in development for humpback whales. Polanowski et al. (2014) used DNA methylation to determine age in humpback whales, but the technique still needs to be refined to be applied to free-ranging whales in biomonitoring studies. When a well-catalogued population is studied, any information regarding the whales' age should be included in the population comparisons since age can create a bias in pollutant concentrations (Polanowski et al., 2014). The reproductive status as well as the number of offspring per female is also important since females transfer a part of their contaminant load to their offspring (Jeong et al., 2018). Pregnant or lactating females can present different concentrations due to POPs being transferred to the offspring (Krahn et al., 2007). Unfortunately, we had no access to the whales' ages or reproductive status in our study. Additionally, the physiological state of an animal also causes variations in POP concentrations. Bengtson Nash et al. (2013) found that whales on their southward migration (thus, after their breeding period) had less lipids in their blubber and a significantly higher load of POPs.

Humpback whales have been used as ocean health sentinels in the Southern Hemisphere through the analysis of their POP concentrations and patterns (Bengtson Nash et al., 2018). We demonstrated that POP concentrations and patterns vary between populations of humpback whales feeding in different areas of the Southern Ocean, supported by different $\delta^{13}C$ values. We also showed that feeding habits differ within populations, supported by the separation of $\delta^{15}N$ values into clusters in Mozambique and the variation of contaminant concentrations in Ecuador.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2020.115575.

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