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1 **Humpback whales (*Megaptera novaeangliae*) breeding off Mozambique**
2 **and Ecuador show geographic variation of persistent organic pollutants**
3 **and isotopic niches**
4

5 Anaïs Remili ^{1,4*}, Pierre Gallego ^{1,6*}, Marianna Pinzone ¹, Cristina Castro ⁵, Thierry Jauniaux
6 ², Mutien-Marie Garigliany ², Govindan Malarvannan ³, Adrian Covaci ³, Krishna Das ^{1#}
7

8 1. Freshwater and Oceanic sciences Unit of reSearch (FOCUS – Oceanology) University of
9 Liege, 4000 Liege, Belgium

10 2. Department of Pathology, Veterinary College, University of Liege, Sart Tilman B43, 4000
11 Liege, Belgium

12 3. Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium

13 4. Department of Natural Resource Sciences, McGill University, Sainte-Anne-de-Bellevue,
14 QC H9X 3V9, Canada

15 5. Pacific Whale Foundation Ecuador, Malecón Julio Izurieta y Abdón Calderón. Palo Santo
16 Travel, Puerto López - Manabí - Ecuador

17 6. Odyssea asbl., 37 rue du Nord, L-4260 Esch-sur-Alzette, Luxembourg
18

19 # **Corresponding author(s): Krishna.das@uliege.be**

20 *Anaïs Remili and Pierre Gallego contributed equally to this work.
21
22

23 **Highlights:**
24

- 25 - POP concentration varied with sex, geographic zones, and trophic levels
- 26 - HCB and DDTs were the major POPs in humpback whale blubber
- 27 - Stable isotopes revealed whales feed on krill but in different feeding areas
- 28 - Whales in our study had some of the lowest POPs ever measured for humpback whales
- 29
- 30
- 31

32 **Abstract**

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

50

51 **Keywords**

52 Persistent organic pollutants, stable isotopes, humpback whales

53

54 **Capsule:** POP concentrations in humpback whale blubber differed between populations off
55 Ecuador and Mozambique in relation to feeding areas.

56

57 **Introduction**

58 Persistent organic pollutants (POPs) accumulate in polar regions like Antarctica (Wania and
59 Mackay, 1993). They can be found everywhere on our planet in measurable concentrations and
60 can be transported far from their emission sites via long-range environmental transport, mainly
61 through the atmosphere, to redeposit close to the poles (Corsolini et al., 2006). POPs are not
62 easily broken down and can accumulate in the tissue of living organisms. Legacy POPs, such
63 as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and brominated flame
64 retardants (BFRs), can biomagnify as they move up the food web (Kelly et al., 2007). Polar
65 POP contamination can occur through re-deposition of atmospheric POPs or local emission of
66 these contaminants, by scientific stations for example (Risebrough et al., 1990). This long-
67 range transport and redeposition of POPs results in different contaminant patterns not only
68 throughout Antarctica but also throughout Antarctic food webs (Nash, 2011). Marine mammals
69 accumulate high levels of contaminants through the food web. PCBs, OCPs, and
70 polybrominated diphenyl ethers (PBDEs) affect the endocrine, immune and reproductive
71 systems of marine mammals (Desforges et al., 2016). Thus, it is important to monitor
72 contaminant accumulation and patterns in marine mammal sentinel species that forage in
73 different regions around Antarctica.

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

82 migrations to and from the breeding grounds as well as on the breeding grounds (Cerchio et
83 al., 2013; Fossette et al., 2014; Silva et al., 2013).

84 The International Whaling Commission (IWC) defined seven stocks (A to G) of Southern
85 Hemisphere humpback whales, based on where they breed (Figure 1). A high breeding site
86 fidelity for southern humpback whales was highlighted by genetic studies (Baker et al., 1994,
87 1993; Constantine et al., 2012; Jackson et al., 2014). The south-eastern Pacific Ocean
88 corresponds to stock G with a breeding ground extending from north of Peru to Costa Rica and
89 Panama in Central America (Castro et al., 2013; Pacheco et al., 2009; Rasmussen et al., 2007;
90 Scheidat et al., 2000). The southwestern Indian Ocean is considered to be home to stock C with
91 four sub stocks: C1, along with Mozambique and the eastern coast of South Africa; C2, the
92 islands off Mozambique; C3, Madagascar; and C4, La Reunion (Best et al., 1998; Rosenbaum
93 et al., 2009; Dulau-Drouot et al., 2012, 2011; Ersts et al., 2011). In the Southern Ocean,
94 humpback whale feeding areas are separated into six longitudinal zones around Antarctica,
95 named Areas I to VI (Rosenbaum et al., 2017). Breeding stock G (southeast Pacific) feeds in
96 Area I (110°W-50°W), off the West Antarctic Peninsula, the South Shetland Islands, Sandwich
97 Islands, and in the Magellan Strait (Acevedo et al., 2007; Branch, 2011; Castro et al., 2015).
98 Area III (10°E-60°E) is the feeding area for humpback whales from breeding stock C (Branch,
99 2011). Out of the seven breeding stocks of southern humpback whales, only three were
100 previously analysed for POP concentrations.

101 POP concentrations in the Southern Hemisphere were quantified in the blubber of humpback
102 whales breeding off eastern Australia (stock E1), the western Antarctic Peninsula (stock A),
103 and La Reunion island (stock C4) (Bengtson Nash et al., 2013; Das et al., 2017; Dorneles et
104 al., 2015). Concentrations of POPs and other harmful chemicals were low and were the result
105 of humpback whales foraging at a low trophic level. Bulk stable isotopes like $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$
106 have been used for decades to provide information on the diet, trophic level, and resource

107 partitioning of marine mammals (Newsome et al., 2012, 2010). Briefly, $\delta^{15}\text{N}$ can be used to
108 assess the trophic position of a consumer, while $\delta^{13}\text{C}$ gives information on the geographic
109 location of the primary producers (Post, 2002). Stable isotopes measured in combination with
110 POPs in migratory species can further characterize their trophic ecology, feeding habits, and
111 population structure (Witteveen et al., 2009).

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

118

119 **Methodology**

120 Sampling

121 Sampling was conducted on the whales' breeding grounds after they arrived from Antarctica.
122 Incorporation of stable isotopes from the diet has been estimated for cetacean species and skin
123 stable isotopes seem to reflect the diet of cetaceans two to six months before sampling
124 (Busquets-Vass et al., 2017; Giménez et al., 2016). Therefore, we focused our sampling efforts
125 on the wintering season to guarantee an accurate feeding habit representation from the stable
126 isotope data. Whales from Ecuador ($n = 59$) were sampled after their arrival on their wintering
127 grounds from 27 August to 14 September 2014 (18 days) and from 2 to 17 September 2015
128 (12 days) in collaboration with the Pacific Whale Foundation Ecuador. Sampling was
129 performed around La Plata Island off Machalilla National Park. Whales from Tofo,
130 Mozambique ($n = 89$) were biopsied after their arrival on their wintering grounds from 17 July
131 to 15 September 2017 (60 days) in collaboration with Odyssey (Figure 2). Sampling took place

132 on small boats (5-12 m) and was performed using a crossbow (Barnett Panzer V®, 150 lb draw-
133 strength) with bolts (Mikkel Villum TM) and 40 mm steel tips. Only adult whales were
134 sampled, and we focused our effort mainly on males although females were occasionally
135 sampled. Biopsies were collected under permits from the respective governments. Skin and
136 blubber biopsies were kept at -20°C until they were transferred to Liège, Belgium using CITES
137 permits (N° IM085/2014/A and N° MZ786/2017 for Ecuador and Mozambique, respectively)
138 issued by the Luxembourg Government.

139 Sample processing

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

146 Genetic determination of sex

147 Sex was determined genetically following the method described previously (Macé and Crouau-
148 Roy, 2008). Briefly, we extracted DNA from the samples using a XYZ kit (according to
149 manufacturer's instructions). The reaction mix contained $0.5\ \mu\text{M}$ of each primer (Primers SC1:
150 5'-CAAGCATGCATTTCAATTCCC and SC2: 5'-CTGCATGGGGAACATCGGAG), $2\ \mu\text{l}$ of
151 DNA, and $10\ \mu\text{l}$ of HotStarTaq Master Mix (Qiagen) bringing the total volume to $20\ \mu\text{l}$. PCR
152 was achieved through the following steps: 1) initial activation at 95°C for 5 min; 2) denaturing
153 through 45 cycles (95°C for 1 min); 3) annealing at 55°C for 45 sec; 4) elongation at 72°C for
154 1 min; 5) final elongation at 72°C for 5 min. PCR products were run on 1% agarose gel stained
155 with Midori Green Advance (Nippon Genetics).

156 Bulk stable isotope analysis

157 The skin was cut and freeze-dried for easier grinding. The skin was ground using a mortar and
158 pestle until fully homogenized. In cetaceans, there is an association of skin with lipids present
159 in the blubber; these lipids are more enriched in ^{12}C compared to proteins, which decreases the
160 $\delta^{13}\text{C}$ values in the skin (DeNiro and Epstein, 1978; Ryan et al., 2012). Additionally, the
161 variation of lipid percentage between samples is an important factor of variation in $\delta^{13}\text{C}$ values
162 and, therefore, lipid extraction is recommended (Ryan et al., 2012). Solvent lipid extraction
163 increases $\delta^{15}\text{N}$ values, thus requiring two distinct measures of isotope ratios (one with lipid
164 extraction, one without lipid extraction) (Lesage et al., 2010; Ryan et al., 2012; Sweeting et al.,
165 2006). The stable isotope analysis followed the methods of Pinzone et al. (2019) and is
166 described in the supplementary information.

167 Contaminant analysis

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

177 Quality assurance/quality control

178 The limit of detection (LOD) was established for each compound and corresponded to three
179 times the standard deviation (SD) of the mean of the blank measurements. Procedural blanks
180 ($n = 12$) were analysed with every batch of samples to check for lab contamination. Blanks
181 were consistent ($\text{RSD} < 20\%$) and the mean value calculated for each compound was subtracted

182 from the sample values. Mean \pm SD recoveries for the internal standards PCB 143, ϵ -HCH,
183 ^{13}C -HCB, and BDE 77 were $86 \pm 6\%$, $98 \pm 8\%$, $85 \pm 10\%$, and $93 \pm 10\%$, respectively.
184 Analytical procedures were validated through the analysis of certified material SRM 1945
185 (organic contaminants in whale blubber) for which deviations from certified values were less
186 than 10%. Contaminant values are presented in ng/g lipid weight (lw).

187 Data analysis

188 One outlier in stable isotope data was removed (“EQ7”, Suppl. Info). We used the Stable
189 Isotope Bayesian Ellipses (SIBER) package (v2.1.3), run in R (v3.5.0) to compare the isotopic
190 niches of humpback whales from Ecuador and humpback whales from Mozambique. The
191 stable isotope data analysis is fully described elsewhere (Pinzone et al., 2019). Standard ellipses
192 included 40% of the data to represent the core of the population. Bayesian modelling (SEA_B)
193 was run to calculate the area of each population’s niche and calculate the potential overlap of
194 the niches. The number of iterations for the Bayesian model was set to 10^5 . To compare isotopic
195 values between each population, we used a Student’s *t*-test. To understand the intra-population
196 variability in stable isotopes, we used a partitioning cluster analysis (k-means) on $\delta^{15}\text{N}$ and
197 $\delta^{13}\text{C}$ to determine different groups. This analysis was followed by a general linear modelling
198 (GLM) analysis on the cluster groups (response variable) and included the following
199 predictors: *sex*, *lipid percentage*, *contaminant concentrations* and *time*. Before the contaminant
200 analysis, pollutant data were lipid normalized and values under the limit of detection (LOD)
201 were assigned a value corresponding to half the compound’s LOD. Statistics were run in R
202 (v3.5.0). The sex ratio was similar between the two populations (75% males in Mozambique;
203 80% males in Ecuador) so the concentrations were not sex corrected. The lipid percentages
204 were compared between the two populations using a Student’s *t*-test.

205 To account for baseline geographic variation in $\delta^{15}\text{N}$, we took into account mean krill $\delta^{15}\text{N}$
206 data averaged from two studies in feeding area III and four studies from feeding area I

207 (Figure 5). As $\delta^{15}\text{N}$ values for krill from each feeding zone did not vary considerably (3‰ for
208 feeding area I, 2.5‰ in feeding area III) we used the data to calculate the trophic position of
209 each humpback whale using the trophic position equation $\text{TP} = ((\delta^{15}\text{N whale} - \delta^{15}\text{N krill})/2.8)$
210 $+ 1$, where 2.8 is the mean trophic enrichment factor for the incorporation of krill bulk nitrogen
211 isotopes into fin whale skin (Borrell et al., 2012). This trophic position was only used in the
212 contaminant analysis to evaluate the impact of the trophic position and not $\delta^{15}\text{N}$ values because
213 $\delta^{15}\text{N}$ values across ocean basins. We used a GLM approach with a Gamma (link = log)
214 distribution to determine which factors were responsible for the contaminant variations. We
215 included three predictors: *sex*, *population*, and *trophic position*; a *sex:population* and a *trophic*
216 *position:population* interaction. $\delta^{13}\text{C}$ was not included as a predictor since it was confounded
217 with the Population factor. Every possible model combination was run in the MuMIn package.
218 To compare the different models, the Akaike's information criterion corrected for small sample
219 size (AICc) was calculated and models within $\Delta\text{AIC} \leq 2$ were averaged to estimate predictors
220 and their significance. When the *sex:population* effect was significant, we conducted a Tukey
221 contrasts test from the multcomp package to conduct some pairwise comparisons between the
222 sexes and populations. When the interaction between the *trophic position* and *population* was
223 significant, we tested for the correlation between the contaminant class and the trophic position
224 for each population with Pearson's correlation test. The Variance Explained (1-(Residual
225 Deviance/Null Deviance)) was also calculated to determine how the different models explained
226 the variance in pollutant classes (McFadden's Pseudo R²). Finally, to visualize the variation in
227 POPs profiles between the two populations of humpback whales, we used a principal
228 component analysis (PCA) on log-transformed and centre-scaled POP classes.

229

230 **Results**

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

234 Dietary tracers – Stable isotopes

235 We only considered lipid-extracted $\delta^{13}\text{C}$ and non-lipid-extracted $\delta^{15}\text{N}$ for statistical analyses.
236 $\delta^{13}\text{C}$ after lipid extraction measured in Ecuador whales had a mean value of -24.5‰ (range: $-$
237 25.49‰ to -23.05‰). We found that Ecuador whales had lower $\delta^{13}\text{C}$ values than Mozambique
238 whales, which had a mean $\delta^{13}\text{C}$ of -26.03‰ (range: -27.67 to -23.57‰) ($t = 13.963$, $p <$
239 0.001). $\delta^{15}\text{N}$ values were lower in whales from Mozambique ($t = 2.5126$, $p = 0.013$) and had a
240 mean value of 8.02‰ (range: 6.25 to 12.29‰) while Ecuadorian whales had a mean $\delta^{15}\text{N}$ value
241 of 8.68‰ (range: 6.62 to 11.97‰) (Table 2). Male and female $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in
242 Mozambique whales did not differ ($t = 0.5569$, $p = 0.5819$ for $\delta^{13}\text{C}$ and $t = 0.055601$, $p = 0.956$
243 for $\delta^{15}\text{N}$). However, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were both significantly lower in females from
244 Ecuador compared to males from Ecuador ($t = -4.9814$, $p < 0.001$ for $\delta^{13}\text{C}$ and $t = -3.5438$, p
245 < 0.001 for $\delta^{15}\text{N}$).

246 The SIBER analysis for nitrogen and carbon revealed that the two $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ core (40%)
247 ellipses from the Mozambique population and the Ecuador population did not overlap. This
248 could also be observed on the isotopic biplot in Figure 3. The Mozambique Bayesian standard
249 ellipse area (SEAb) (mode = 3.23 ; CI 95%: 2.62 to 4.03) was larger than the Ecuador one
250 (mode = 2.68 ; CI 95%: 2.07 to 3.50) in 86% of the model runs (Supplementary Information).
251 The whales from Ecuador showed a continuous distribution both on the $\delta^{15}\text{N}$ axis and $\delta^{13}\text{C}$
252 axis. Even though there were significant differences in Males vs. Females, the partitioning
253 cluster analysis (k-means) on stable isotope data did not separate the Ecuador population into
254 clusters. However, we observed two subgroups in the Mozambique ellipse and the partitioning
255 cluster analysis on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ revealed that the Mozambique whales were separated into

256 two clusters (Supplementary Information). Cluster 1 included whales that had higher $\delta^{15}\text{N}$
257 values (above 8.9‰; mean = 10.4‰); cluster 2 included whales that had lower $\delta^{15}\text{N}$ values
258 (below 8.9‰, mean = 7.3‰). We ran a GLM model selection on predictors expected to impact
259 the cluster separation of the values. Our predictors included *sex*, *time* (number of days since
260 start of sampling), *lipid percentage* and all *contaminant classes*. No model had a lower AICc
261 than the null model (AICc = 85.75). Five models separated from the null model by a ΔAICc
262 lower than 2 were averaged but no predictor effect was significant, meaning no factor in our
263 dataset could explain why Mozambique whales were separated into two isotopic clusters.

264

265 Persistent organic pollutants

266 The lipid percentage in the blubber was not statistically different between Mozambique and
267 Ecuador populations: 42% (11 to 70%) vs. 40% (20 to 58%) respectively ($t = -1.3436$, $p =$
268 0.18). Lipid percentages within each population were not different between sexes ($t = 1.0211$,
269 $p = 0.31$ for Mozambique; $t = 1.0464$, $p = 0.31$ for Ecuador) (Table 3). The trophic positions
270 were calculated to account for geographic baseline $\delta^{15}\text{N}$ variation between whales from
271 Mozambique and Ecuador by using krill $\delta^{15}\text{N}$ values from the two feeding areas. Trophic levels
272 were successful in getting rid of the geographic differences in $\delta^{15}\text{N}$ as trophic positions did not
273 differ between the two populations (mean = 2.98 for Ecuador, mean = 2.94 for Mozambique,
274 $p = 0.71$).

275 POP concentrations were quantified in order of highest to lowest as HCB > DDTs > CHLs >
276 PCBs > HCHs > PBDEs > MeO-PBDEs for all whales except 11 whales in Ecuador that had
277 higher $\sum\text{DDT}$ concentrations than HCB (Table 3). In Mozambique, the two major classes of
278 contaminants were HCB and $\sum\text{DDTs}$. HCB had a mean concentration of 66.5 (8.7 to
279 126.7) ng/g lw, DDTs had a mean concentration of 8 (0.4 to 26.1) ng/g lw. The predominant
280 compounds in each class of chemicals present in Mozambique whales were HCB, *p,p'*-DDE,

281 *trans*-nonachlor (TN), PCB-153, γ -HCH, BDE-47, and 6-MeO-BDE47. HCB accounted for
282 81% and \sum DDTs for 10% of POPs. In Ecuador, the two major pollutant classes were also HCB
283 and \sum DDTs. HCB had a mean concentration of 36.5 (6.1 to 77.3) ng/g lw and \sum DDTs had a
284 mean concentration of 24 (4.8 to 153.9) ng/g lw. The predominant compounds in each class of
285 chemicals present in Ecuador whales were HCB, *p,p'*-DDE, TN, PCB-138, α -HCH, BDE-47,
286 and 6-MeO-BDE47. HCB and DDTs represented 54% and 35% of all POPs, respectively.
287 The GLM analysis revealed that different predictors were responsible for the variation of
288 contaminants (Table 4). The best models for \sum PCBs explained up to 18% of the deviance but
289 none of the averaged effects were significant, meaning \sum PCB concentrations were not
290 statistically different between sexes, populations or across trophic positions. The best model
291 for \sum DDTs explained 64% of the deviance. Mozambique whales had lower \sum DDT
292 concentrations than Ecuador whales ($\beta = -0.37$, $p < 0.01$). *Trophic position* was a significant
293 factor in variation of \sum DDTs ($\beta = 0.059$, $p < 0.01$). Since the interaction between the *trophic*
294 *position* and *population* was significant, we tested for the correlation between DDTs and the
295 trophic level in each population. This correlation test revealed \sum DDTs were only correlated
296 with the *trophic position* in Ecuador whales ($R = 0.29$, $p = 0.03$) not in Mozambique whales
297 ($R = 0.1$, $p = 0.26$). The best model for \sum CHLs explained 12% of the deviance. Males had
298 higher \sum CHL concentrations ($\beta = 0.75$, $p < 0.01$) and although the two populations were
299 statistically not different, the interaction *sex:population* was significant. The Tukey's contrasts
300 test revealed that males had higher \sum CHLs concentrations than females in Ecuador ($\beta = 0.87$,
301 $p < 0.01$) but not in Mozambique ($\beta = 0.12$, $p = 0.85$). The best model for HCB explained 43%
302 of the deviance, included all predictors and all the predictors were significant. Tukey's
303 contrasts tests revealed that males had higher HCB concentrations in Ecuador ($\beta = 0.75$, $p <$
304 0.01) but not in Mozambique ($\beta = 0.13$, $p = 0.47$). *Trophic position* was a significant factor of
305 HCB variation, yet only in whales from Ecuador ($R = 0.44$, $p < 0.01$), not in Mozambique (R

306 = -0.13, $p = 0.26$). *Population* was the only predictor in the best model for Σ HCHs and
307 explained 46% of the deviance; Mozambique whales had lower concentrations than Ecuador
308 whales ($\beta = -0.63$, $p < 0.01$). Finally, the model that best explained Σ PBDE concentration
309 variations revealed that concentrations were higher in Mozambique ($\beta = 0.87$, $p < 0.01$). We
310 conducted a Pearson correlation test on Σ PBDEs and the trophic position in each population
311 since the interaction was significant. These two tests revealed whales Σ PBDE concentrations
312 were related to *trophic position* in Ecuador ($R = 0.33$, $p = 0.01$) but not in Mozambique ($R = -$
313 0.06 , $p = 0.58$).

314 We performed a principal component analysis to test for the differences in POP profiles
315 between the two populations. PC1 and PC2 explained 57.1% of the variance between samples
316 (Figure 4). PC1 axis was represented by Σ PCBs, HCB, and Σ CHLs (28%, 25%, and 25%
317 contribution to PC1 respectively), while PC2 axis was represented by Σ DDTs and Σ HCHs
318 (43% and 34% contribution to PC2 respectively). Σ DDTs and Σ HCHs were a factor of
319 separation between the two populations on the PC2 axis. HCB concentrations were an
320 important factor of variance, highlighting the fact that Mozambique whales had higher HCB
321 concentrations than Ecuador whales.

322

323 **Discussion**

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

328

329 *Feeding habits of humpback whales on both sides of the Equator*

330 Humpback whales have higher $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values in the Northern Hemisphere than in the
331 Southern Hemisphere (Witteveen et al., 2009). Differences in $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values from the
332 two hemispheres could be attributed to geographic variation in particulate organic matter and
333 planktonic $\delta^{15}\text{N}$ values (McMahon et al., 2013). Prey species of northern humpback whales
334 were found to have higher $\delta^{15}\text{N}$ values: krill had a mean $\delta^{15}\text{N}$ of 8‰ in Alaska (compared to
335 ~3‰ in the Southern Ocean). Higher $\delta^{15}\text{N}$ values in northern humpback whales also illustrates
336 the fact that Northern Hemisphere whales feed on fish like herring, pollock, haddock, mackerel,
337 capelin, salmon, and various other fish (Todd et al., 1997). Eutrophication caused by higher
338 human activity in the Northern Hemisphere could also increase $\delta^{15}\text{N}$ values in local fish and
339 invertebrates (Griffin, 2001; McClelland et al., 1997).

340

341 *Interpopulation variation in feeding habits in Ecuador versus Mozambique*

342 Cetacean skin stable isotopes represent the diet of the last 2-6 months before the biopsies are
343 collected (Busquets-Vass et al., 2017; Giménez et al., 2016). Thus, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ value
344 analyses from Ecuador and Mozambique whales represent their feeding habits from feeding
345 areas I and III, respectively. The low $\delta^{15}\text{N}$ values in our study imply that our whales feed at a
346 low trophic level, agreeing with other southern humpback whale studies (Bengtson Nash et al.,
347 2018; Das et al., 2017; Dorneles et al., 2015). Values of $\delta^{13}\text{C}$ for our whales are in agreement
348 with $\delta^{13}\text{C}$ measured in particulate organic matter and Antarctic krill (Francois et al., 1993;
349 Frazer, 1996; Hodum and Hobson, 2000; Polito et al., 2013; Schmidt et al., 2003; Stowasser et
350 al., 2012; Zhang et al., 2017). $\delta^{13}\text{C}$ values for krill in feeding area I ranged from -24.5‰ to -
351 29.3‰ (Dunton, 2001; Frazer, 1996) while values ranged from -25‰ to -31.2‰ in krill from
352 feeding area III (Hodum and Hobson, 2000; Schmidt et al., 2003) (Figure 5). Our SIBER
353 analysis showed no overlap in $\delta^{15}\text{N}$ vs. $\delta^{13}\text{C}$ ellipses from Mozambique and Ecuador suggesting
354 a different geographic origin of the primary production, supported by a significant difference

355 in $\delta^{13}\text{C}$ values between our two populations (Figure 3). Differences in $\delta^{15}\text{N}$ values in
356 populations from the Southern Hemisphere could be attributed to geographic variations in
357 planktonic $\delta^{15}\text{N}$ (Lorrain et al., 2009) since their trophic position, taking into account the
358 baseline $\delta^{15}\text{N}$ variation, resulted in no differences between the populations. Although the
359 equation we used to calculate the trophic position has now been replaced by complex Bayesian
360 modelling approaches, this simple equation was only used to remove the effect of baseline
361 variation (Quezada-Romegialli et al., 2018).

362

363 *Intrapopulation variation in stable isotopes*

364 Within the Ecuador population, females had significantly lower $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values than
365 males. A possible difference between male and female feeding habits deserves further
366 investigation to better understand the intra-population structure of southern humpback whales.
367 $\delta^{15}\text{N}$ varied across the population, ranging from 6 to 12‰. These values expressed in the
368 trophic level were correlated with DDTs and HCB concentrations and could illustrate a large
369 dietary spectrum for these whales. Climate change and the reduction of sea ice impacts
370 phytoplankton biomass and, through a bottom-up feedback, reduces krill communities on
371 which humpback whales feed (Flores et al., 2012). By feeding mainly on a single species,
372 humpback whales are in a precarious trophic position and will most likely need to shift their
373 dietary preferences to survive the effects of climate change (Bengtson Nash et al., 2018).
374 Additionally, recent studies have found that migrating humpback whales could feed on their
375 breeding grounds, as demonstrated by observation or higher isotopic values (Eisenmann et al.,
376 2016; Findlay et al., 2017). For future research on feeding habits of southern humpback whales,
377 we recommend a study focusing on compound-specific stable isotopes since they account for
378 baseline isotopic variation and are more accurate at estimating consumers' feeding habits

379 (Chikaraishi et al., 2009). We also recommend measuring stable isotopes both on the breeding
380 and feeding grounds to assess potential opportunistic feeding at a higher trophic level.

381

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

392

393 *Geographic variation of persistent organic pollutants across the world*

394 Humpback whales from Ecuador and Mozambique are among the least contaminated
395 populations of humpback whales in the world (Table 5). Thus far, three studies have analysed
396 POPs in humpback whale blubber in the Southern Hemisphere, accounting for three stocks out
397 of the seven stocks defined by the IWC (Bengtson Nash et al., 2013; Dorneles et al., 2015; Das
398 et al., 2017). Contaminant concentrations in southern humpback whales were in the same order
399 of magnitude as our results (Table 5). Whales sampled in the Northern Hemisphere were more
400 contaminated than Southern Hemisphere whales, and POP concentrations were higher by an
401 order of magnitude at least (Metcalf et al., 2004; Elfes et al., 2010; Bachman et al., 2014). The
402 differences between the two hemispheres can be attributed to differences in trophic levels and
403 the historical use of POPs in the Northern Hemisphere. Diet is one of the most important factors

404 of POP variation, as POPs biomagnify through the food web (Corsolini et al., 2006). Humpback
405 whales from the Northern Hemisphere do not only rely on krill, but also on other invertebrates
406 and fish located higher in the food chain, as illustrated in Figure 5. Thus, differences in diet
407 can explain, in part, why humpback whales from the Northern Hemisphere are more
408 contaminated (Gauthier et al., 1997). Additionally, the Northern Hemisphere has historically
409 received more input of POPs than the Southern Hemisphere through industries and pesticide
410 usage. The Northern Hemisphere accounted for almost 97% of the environmental input of
411 PCBs (Breivik et al., 2007). Even though atmospheric and hydrologic transport of POPs is an
412 important redistribution route (Hageman et al., 2015), POPs are more concentrated in food
413 webs close to emission sites and heavily populated areas which also explains the higher
414 contamination in northern humpback whales. Among all populations, \sum PCBs were well below
415 the 17 000 ng/g lw threshold at which animals may demonstrate undesirable biological effects
416 like immune function alterations and reproductive issues (Kannan et al., 2000).

417

418 *Southern Ocean geographic POP variations in humpback whales*

419 Regional differences in POP concentrations contributed to the variation of Southern
420 Hemisphere POP concentrations and profiles in humpback whales. Whales from our study
421 showed the lowest \sum PCB concentrations in the Southern Hemisphere. Whales feeding off the
422 Western Antarctic Peninsula (WAP) displayed higher \sum PCB concentrations than other
423 southern humpback whales (Table 5), most likely due to local contamination in Antarctica
424 (Dorneles et al., 2015). A high number of scientific stations are present in the WAP compared
425 to the rest of Antarctica (Scientific Committee on Antarctic Research - SCAR). These stations,
426 close to the whales that were sampled by Dorneles et al. (2015), have been identified as a source
427 of local PCB contamination due to the discharge of various waste products (Kennicutt et al.,
428 2010). Additionally, whales from Ecuador had lower \sum PCB concentrations than Dorneles et

429 al. (2015)'s whales; although they feed close to the WAP, supposedly further from scientific
430 stations.

431 HCB levels were found to be similar in our study compared to other Southern Hemisphere
432 humpback whales except for whales from Australia (Bengtson Nash et al., 2013). HCB had the
433 highest concentration of all POPs measured in the Southern Hemisphere except for whales
434 from the WAP that had PCB concentrations higher than HCB concentrations (Dorneles et al.,
435 2015). HCB was higher in Mozambique whales than Ecuador whales in our study, although
436 we do not have a clear explanation for higher concentrations in Mozambique. We believe
437 geographic differences in POPs could be a reason for different HCB concentrations in
438 populations of southern humpback whales. HCB is highly volatile and expected to reach
439 worldwide equilibrium faster than other congeners (Bengtson Nash et al., 2008; Kang et al.,
440 2012).

441 Σ DDTs were higher in Ecuador whales than Mozambique whales in our study. Σ DDTs were
442 similar between Ecuador whales and whales sampled feeding close to the WAP (Dorneles et
443 al., 2015). Antarctica still receives inputs of *p,p'*-DDE via redistribution of previously
444 deposited DDT in soil and snow/ice and from ongoing DDT usage in parts of the Southern
445 Hemisphere, e.g. for vector control in disease prevention, which could explain the higher rates
446 in whales feeding close to the WAP, close to South America (Poulsen et al., 2012; Van Den
447 Berg, 2009). *p,p'*-DDE accounted for most of the Σ DDTs found in all humpback whale
448 populations. It is known to be the most persistent DDT metabolite, thus explaining its higher
449 concentration in whales, despite the ban on the intensive use of DDT decades ago (Bengtson
450 Nash et al., 2008).

451 The lowest concentrations of Σ HCHs were found in whales from our study, e.g. from
452 Mozambique and Ecuador. The use of γ -HCH or lindane in vector control is still permitted in
453 South America, likely explaining why Σ HCHs are higher in Ecuador than Mozambique

454 (Dorneles et al., 2015). However, \sum HCH concentrations in the Southern Hemisphere have
455 largely decreased in the last decades, which could explain the lower concentrations in our study
456 (Li et al., 2020). \sum CHLs were found in similar concentrations in our study and populations
457 south of the equator (Bengtson Nash et al., 2013; Das et al., 2017; Dorneles et al., 2015). The
458 lowest \sum PBDE concentrations were found in whales from our study, e.g. from Mozambique
459 and Ecuador where most PBDEs were < LOD. These decreasing \sum PBDE concentrations have
460 also been reported for the rest of the Antarctic atmosphere from 2011 to 2014 (Wang et al.,
461 2017).

462

463 *Factors responsible for the intrapopulation variation of POP concentrations*

464 Sex and trophic level were factors that explained intrapopulation contaminant variations in
465 humpback whales breeding off Ecuador. *Sex* was a factor of variation in Ecuador for HCB, and
466 CHLs. Females had lower concentrations of pollutants than males which can be explained by
467 the maternal transfer of contaminants, widely described in marine mammals (Kajiwara et al.,
468 2008; Pinzone et al., 2015). *Sex* was also a factor of variation in other humpback whale
469 populations in the Southern Hemisphere (Bengtson Nash et al., 2013; Dorneles et al., 2015;
470 Das et al., 2017). Another driver of POP variation in our study was the *trophic position*. Indeed,
471 trophic positions derived from $\delta^{15}\text{N}$ in whales and $\delta^{15}\text{N}$ in krill from their feeding areas were
472 related to the concentrations of \sum DDTs, HCB and \sum PBDEs in Ecuador whales. Males in
473 Ecuador had higher $\delta^{15}\text{N}$ and contaminant values than females which could account for a part
474 of the trophic position's association with contaminants. A higher trophic position and higher
475 $\delta^{15}\text{N}$ values with higher \sum DDT and HCB concentrations could indicate that some whales feed
476 opportunistically at a higher trophic level in Antarctica, although this has not been explicitly
477 demonstrated and deserves further attention.

478

479 Other factors that could not be measured in our study could be responsible for variation in POP
480 concentrations and patterns. The better models only explained less than 20% of the variation
481 in Σ PCBs, Σ CHLs, and Σ PBDEs in our two populations. Even when the better models were
482 more efficient at explaining the variation for other POPs like HCB, Σ DDTs, and Σ HCHs, they
483 only accounted for 43 to 66% of the variation. Known factors of POP variation in marine
484 mammals include age, reproductive status, and physiological state. Age is a factor of POP
485 variation since POPs accumulate over time (Desforges et al., 2016). However, age
486 determination using biopsies is still in development for humpback whales. Polanowski et al.
487 (2014) used DNA methylation to determine age in humpback whales, but the technique still
488 needs to be refined to be applied to free-ranging whales in biomonitoring studies. When a well-
489 catalogued population is studied, any information regarding the whales' age should be included
490 in the population comparisons since age can create a bias in pollutant concentrations
491 (Polanowski et al., 2014). The reproductive status as well as the number of offspring per female
492 is also important since females transfer a part of their contaminant load to their offspring (Jeong
493 et al., 2018). Pregnant or lactating females can present different concentrations due to POPs
494 being transferred to the offspring (Krahn et al., 2007). Unfortunately, we had no access to the
495 whales' ages or reproductive status in our study. Additionally, the physiological state of an
496 animal also causes variations in POP concentrations. Bengtson Nash et al. (2013) found that
497 whales on their southward migration (thus, after their breeding period) had less lipids in their
498 blubber and a significantly higher load of POPs.

499

500 Humpback whales have been used as ocean health sentinels in the Southern Hemisphere
501 through the analysis of their POP concentrations and patterns (Bengtson Nash et al., 2018). We
502 demonstrated that POP concentrations and patterns vary between populations of humpback
503 whales feeding in different areas of the Southern Ocean, supported by different $\delta^{13}\text{C}$ values.

504 We also showed that feeding habits differ within populations, supported by the separation of
505 $\delta^{15}\text{N}$ values into clusters in Mozambique and the variation of contaminant concentrations in
506 Ecuador.

507

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519

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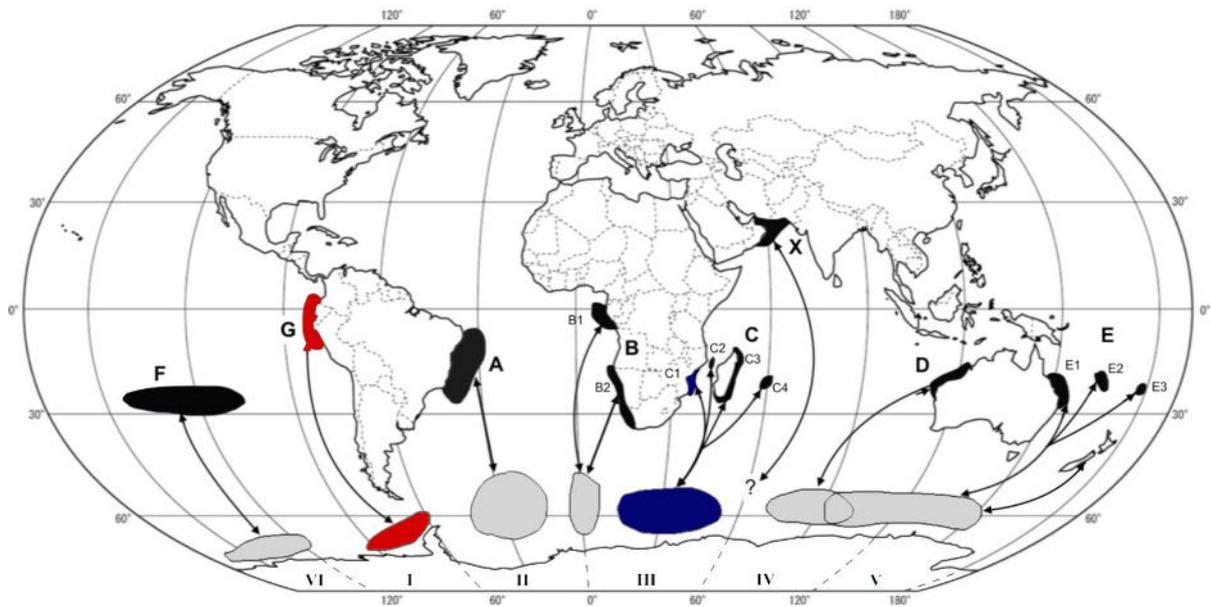
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832 **FIGURES AND TABLES**



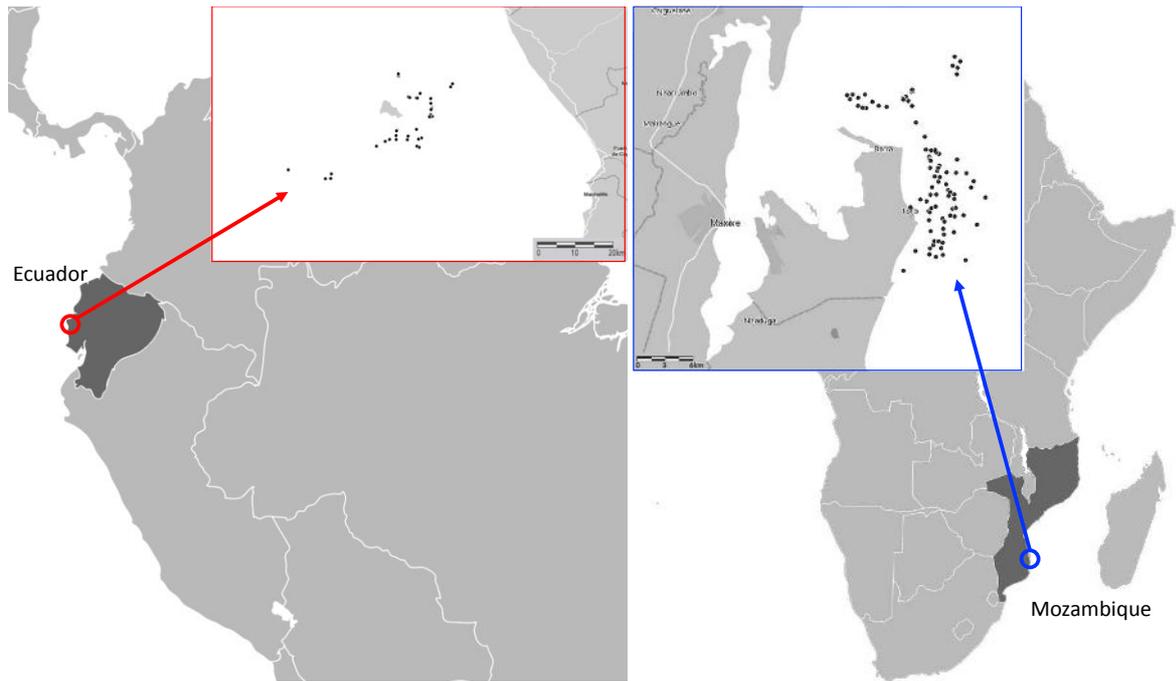
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835 Figure 1: Breeding and feeding areas for the different stocks of humpback whales in the
 836 Southern Hemisphere (after IWC 2006). Letters indicate the breeding areas and roman
 837 numerals indicate the feeding areas. The breeding and foraging areas for the present study are
 838 shown in red (Ecuador) and blue (Mozambique). Additional subpopulations/populations are
 839 shown in black (breeding regions) and gray (foraging regions).

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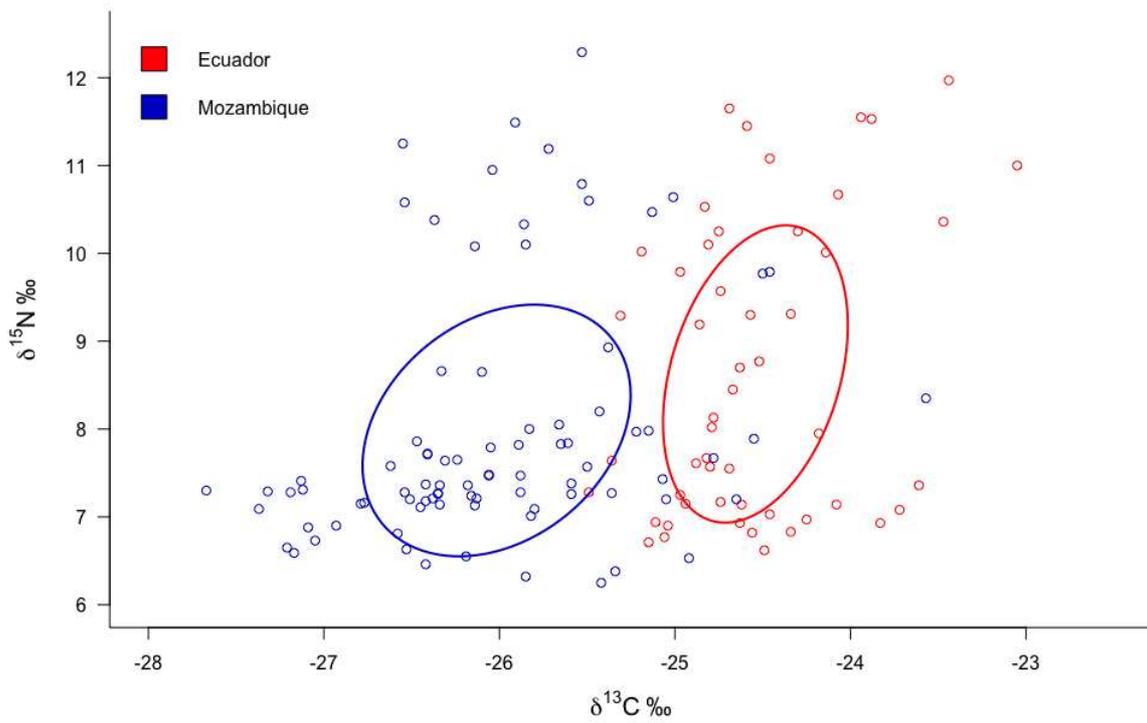
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Figure 2: Sampling map of whale skin biopsies collected in Ecuador (n = 59, 2014-2015) and Mozambique (n = 89, 2017).

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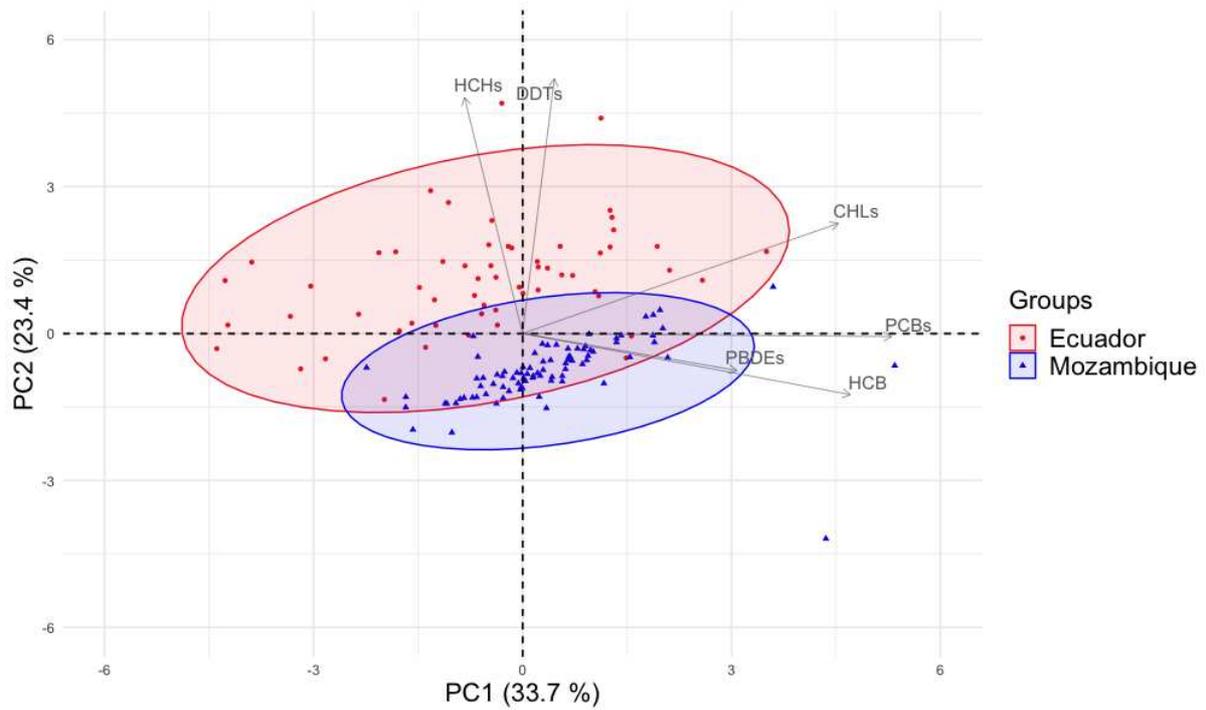
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848 Figure 3: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ biplot in skin of humpback whales from Ecuador and Mozambique.

849 Each little circle represents an individual (from Mozambique in blue; from Ecuador in red).

850 Solid lines represent the standard ellipses (40% of the data, representing the core of the

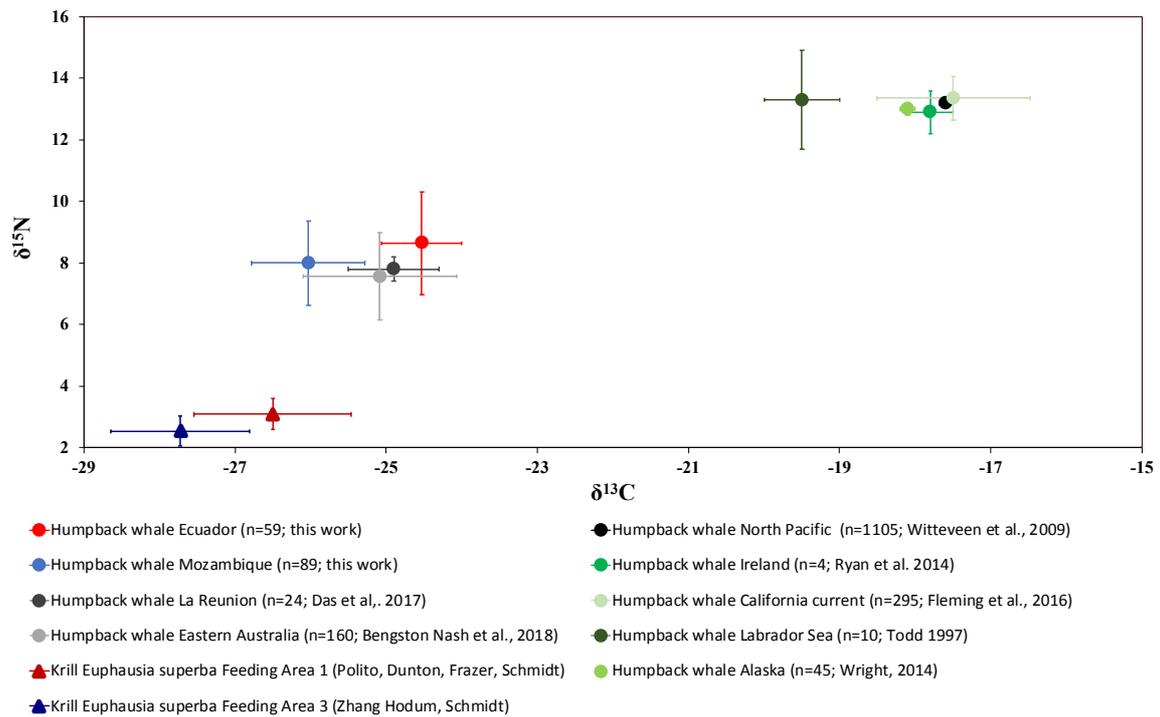
851 population) associated to each population.



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

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860 Figure 5: $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (‰) values in humpback whales, *Megaptera novaeangliae*, off
 861 Ecuador and Mozambique (this work) compared to humpback whales off La Reunion Island
 862 (Das et al., 2017), eastern Australia (Bengtson Nash et al. 2018, Supporting Information), the
 863 western Antarctic Peninsula (Dorneles et al., 2015), and other populations from the Northern
 864 Hemisphere (Witteveen et al., 2009; Ryan et al., 2004; Todd, 1997; Fleming et al. 2016;
 865 Wright, 2014), and krill populations from feeding area I (stock G) and III (stock C1 & C4).
 866

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	<i>p,p'</i> -DDD, <i>p,p'</i> -DDE, <i>p,p'</i> -DDT, <i>o,p'</i> -DDD, and <i>o,p'</i> -DDT
Chlordanes (CHL) and metabolites	<i>cis</i> -chlordane (CC), <i>trans</i> -chlordane (TC), <i>trans</i> -nonachlor (TN), <i>cis</i> -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

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

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

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) \pm standard deviation; range. POP concentrations are expressed as mean in ng/g lw. Total number includes males + females + unidentified sex.

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

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 $\Delta AICc$ below or equal to 2 are presented since they were averaged to determine the predictors coefficients. The deviance explained is calculated as: $1 - (\text{Residual Deviance} / \text{Null Deviance})$ and is similar to R^2 . ** indicates a p-value < 0.001

Models	AICc	$\Delta AICc$	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						
CHLs ~ sex + population + trophic position + sex:population	585.24	0.53	0.13	0.7	0.36	0.85**	-0.7**	0.09	0.25
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						
HCHs ~ sex + population	-168.10	1.87	0.47	-0.54	-0.63**	0.03	—	-0.02	—
HCHs ~ trophic position + population	-168.00	1.97	0.47						
PBDEs ~ population + trophic position + trophic position:population	-317.40	0.00	0.12						
PBDEs ~ sex + population + trophic position + trophic position:population	-317.30	0.10	0.13	-1.61	0.67**	0.07	-0.07	0.21**	-0.25**
PBDEs ~ sex + population + trophic position + sex:population + trophic position:population	-309.27	2.15	0.14						

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

Sampling location	Year of sampling	% Lipids	Nutritional State	Σ PCBs	HCB	Σ HCHs	Σ CHLs	Σ PBDEs	Σ DDTs	Source
Reunion Island Indian Ocean n = 25	2010-2011	37	Breeding	3.4 (2.1) \pm 3.8 0.70 – 16.4	28.8 (23.9) \pm 17.7 6.6 – 66.8	3.6 (2.4) \pm 3.4 0.4 – 12.2	8.1 (7.8) \pm 6.5 1.4 – 26.0	1.4 (0.8) \pm 2.4 0.2 – 12.0	9.5 (9.0) \pm 6.4 2.4 – 25.7	Das et al., 2017
Stock C4										
Mozambique Indian Ocean n = 87	2017	42	Breeding	2.3 (1.7) \pm 2.8 1.2 – 22.7	66.5 (65.7) \pm 19.3 8.7 – 126.7	0.3 (0.3) \pm <0.1 0.3 – 0.5	4.1 (3.2) \pm 2.7 1.0 – 16.9	0.4 (0.4) \pm 0.1 0.4 – 0.8	8.1 (7.4) \pm 3.5 0.4 – 26.1	This work
Stock C1										
Ecuador Pacific Ocean n = 59	2014-2015	40	Breeding	1.8 (1.7) \pm 1.1 0.60 – 7.4	36.5 (36.2) \pm 17.7 6.1 – 77.3	0.6 (0.5) \pm 0.7 0.3 – 4.9	4.4 (4.0) \pm 2.7 0.7 – 10.8	0.4 (0.3) \pm 0.1 0.3 – 0.9	24.0 (19.8) \pm 19.7 4.8 – 153.9	This work
Stock G										
Western Antarctic Peninsula n = 15	2000-2001	40	Feeding	131.0 (83.3) \pm 192.0 4.4–761.0	35.4 (33.1) \pm 20.0 6.8–74.5	11.5 (9.2) \pm 11.0 2.2 – 43.7	5.9 (5.7) 73.3 1.9–14.4	5.8 (1.5) \pm 12.6 0.4–50.8	21.2 (13.2) \pm 34.0 4.0–143.0	Dorneles et al., 2015
Stock G										
Eastern Australia Pacific Ocean	2008-2011	44.5	Average of north-ward and south-	18.0	160.0	11.0	23.0		51.0	Bengston Nash

n = 41			ward migration								et al., 2013
Stock E			data								
Hawaii	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	
Pacific Ocean											
n = 3											
Central North Pacific											
South East Alaska	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	
Northern Pacific											
n = 10											
Central North Pacific											
Gulf of St Lawrence	1993-1999	NA	Feeding	897.2 ± 596.0	153 ± 99.8	108.1 ± 51.7	NA	NA	1122.2 ± 1255.8	Metcalf et al., 2004	
Northern Atlantic											
n = 12											
Northern Atlantic											