



POPs in long-finned pilot whales mass stranded in Iceland as a proxy for their physiological condition

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ABSTRACT

Long-finned pilot whales (*Globicephala melas*) are the most frequently stranded cetaceans in the world; however, the predominant drivers of these events are poorly understood. In this study the levels of persistent organic pollutants from pilot whales stranded in North-east Iceland were quantified and compared to historical data and physical parameters to investigate whether contaminant load may have influenced the physiological state of stranded individuals, how these loads fluctuate with sex and age group, and if this is consistent with the literature. Historical comparison was also carried out to discern how pollutant contamination has changed throughout the past few decades. DDE, transnonachlor and PCB-153 were the top three pollutants respectively. The accumulation of POPs was greater on average in immature individuals than adults, whilst among adults, males had higher concentration than females. Moreover, despite an indication of decreasing POP loads throughout the years, knowledge of harmful thresholds remains exceedingly limited.

1. Introduction

The long-finned pilot whale (*Globicephala melas*) is a large delphinid that inhabits subtropical to subarctic waters. Males are larger than females, reaching a maximum of 7.62 m and 5.7 m respectively, and weigh up to 2300 kg (Ridgway et al., 1998; Perrin et al., 2009; Minton et al., 2018). This species lives in matrilineal family groups ranging from 50 to 100 whales but can accrue to over 1000 individuals in rare events (Amos et al., 1993; Bloch et al., 1993a, 1993b). Sex ratios typically favour females but pods completely composed of males have also been recorded (Desportes et al., 1992). Their continuous, broad distribution across the North Atlantic is influenced by seasonality where they migrate to deeper waters in winter and shallower, shelf and slope waters during the summer months (Payne and Heinemann, 1993; Nelson and Lien, 1996; Fullard et al., 2000). They are deep diving, feeding mainly on various species of cephalopods (Gannon et al., 1997; Santos et al., 2014), but fish, such as mackerel and herring, can also be a substantial part of their diet (Gannon et al., 1997).

Despite being classified as of 'least concern' under the IUCN Red List

of Threatened species, *G. melas* still faces considerable threats to its population integrity and habitat (Minton et al., 2018). The foremost threat to pilot whales is mass strandings, the causes of which remain obscure at best, but can include navigation errors which lead pods to abruptly shallow areas, geomagnetic anomalies that disrupt orientation, and distress caused by human interference. These mass mortality events are further exacerbated by adverse anthropogenic activities including fishing gear entanglement, trawler net bycatch, and pollutant bioaccumulation such as persistent organic pollutants (POPs) and heavy metals (Muir et al., 1988; Minton et al., 2018).

Persistent organic pollutants (POPs) are a significant threat to marine mammals because cetaceans tend to occupy high trophic levels in marine ecosystems and consume large amounts of marine fauna resulting in the bioaccumulation and magnification of pollutants within their bodies. This, coupled with their thick layer of blubber and long lifespans, makes them exceedingly prone to lipophilic POP accumulation, which may be exacerbated in odontocetes since they tend to consume larger prey that accumulates palpable amounts of pollutants themselves (Hoekstra et al., 2003; Krahn et al., 2007; Noël et al., 2009; Ylitalo et al.,

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2009; Dam, 2011; Bachman et al., 2014).

POPs are a diverse assortment of contaminants distinguished by extensive environmental half-lives and considerable potential for bioaccumulation. Their immense dispersal ability within atmospheric and oceanic currents, as well as through trophic levels is directly proportional to their volatility. Despite heavy restrictions and bans imposed on their use in developed countries, some POPs are still used by developing countries where use is unregulated and education about their effects is limited. Here they are predominantly used to mitigate the persistence of vector borne diseases (Wania and Mackay, 1996; Jones and De Voogt, 1999; Kelly et al., 2007; Harrad, 2010; Alharbi et al., 2018; Krasnobaev et al., 2020). Several international conventions have been established to control their use and impacts including the Vienna Convention (1989), the Basel Convention (1992), the Convention on Long-Range Transboundary Air Pollutants (LRTAP) (2003), the Stockholm Convention (2004), the Rotterdam Convention (2004), the International Convention on the Control of Harmful Anti-fouling Systems on Ships (2008) as well as the Globally Harmonized System (GHS) (UNEP, 1989; UNEP, 1992; U. S. Department of State, 2003; UNEP a, 2004; UNEP b, 2004; IMO, 2008; UNECE, 2019).

The danger of these compounds lies within their lipophilicity and persistence, since they have extensive environmental residence times, with many lasting decades. This enables them to obstinately accumulate within the lipids of organic matter and organisms, especially those in higher trophic levels (Jones and De Voogt, 1999; Kelly et al., 2007). Because of this biomagnification, POPs can potentially cause adverse health effects including cancers, allergies, hypersensitivity, developmental changes, neural damage, and endocrine and immunological disruption (Alharbi et al., 2018; Guo et al., 2019). They are especially insidious due to their vertical transmission from mother to embryo and through lactation (Guo et al., 2019). However, due to the vast assortment of compounds, it is difficult to discern which POP causes which ailment (Jones and De Voogt, 1999). A cocktail effect might also arise, where the mixture of several compounds results in alternate or unforeseen consequences (Panseri et al., 2019).

Pilot whale mass stranding events have regularly been recorded in the North Atlantic (Abend and Smith, 1999). Records from Iceland date back to at least 1809, when around 1000 individuals stranded near Akranes, SW Iceland. The largest episode ever recorded involving around 2000 individuals occurred on the Snæfellsnes peninsula, West Iceland in 1813 and another large one in the same region in 1943 with around 700 individuals (Kristjánsson, 1983). Other significant events in Iceland saw 148 individuals strand at Þorlákshöfn in 1986, and 280 whales at Rif on Snæfellsnes, (Sigurjónsson et al., 1993; Rawlings, 2019a). Moreover, pilot whale strandings in Iceland tend to fluctuate with low and high frequency years, which has been proposed to be related to squid migrations into shallower waters (Sæmundsson, 1937). Little is known of the movements and site fidelity of pilot whales in Iceland due to lack of studies, although their occurrence appears to be increasing in recent years, at least in some regions (Selbmann et al., 2022). 2019 was a year with high stranding frequency, as three substantial incidents with a total mortality of around 136 individuals were recorded (Enoksen, 2019; Elliott, 2019; Hafstað, 2019; Rawlings, 2019a; Rawlings, 2019b). One of these events consisted of 62 pilot whales stranded at Ytra Lón on the Langanes peninsula, northeast Iceland on the 6th of September, where none survived. Due to the uncompromised integrity of the carcasses from the latter incident, the collection of blubber and muscle samples from several individuals was carried out under the supervision of the Marine and Freshwater Research Institute (Elliott, 2019).

The aim of the study was to investigate the physiological condition of long-finned pilot whales stranded in Icelandic waters with a focus on POPs and seek evidence for possible physiological drivers of the strandings. The contaminant levels were quantified and compared with other studies to gain an understanding of the relative physiological status of the stranded individuals used in this study. To investigate the

current situation of POP accumulation in long-finned pilot whales in the North Atlantic, the levels obtained from this study are compared with historical records of POP levels from the region.

2. Materials and methods

2.1. Sample collection

All pilot whale blubber samples were collected from stranded individuals that died on the shore at Ytra Lón on the Langanes peninsula (Fig. 1), Northeast Iceland, on the 6th of September 2019, in cooperation with the Marine and Freshwater Research Institute (MFRI). Out of the 60 whales that stranded (Fig. 2), the blubber and muscle of 42 individuals were taken, but 20 were ultimately used for this study. The samples were cut from the dorsal side of each individual shortly after they expired, around the dorsal fin, cutting from the skin down to the muscle to collect the entire blubber layer. The samples were placed in plastic zip-lock bags, labelled, and stored at -20 °C. Individuals were divided into adult or juvenile according to their body length, where juveniles had lengths below 378.5 cm, whilst adults were above. Juveniles also included young calves which could have still been nursing.

2.2. Chemical analysis of persistent organic pollutants in blubber

The following chemicals and mixtures were used during the lipid extraction procedure to separate and purify the lipid containing the POPs from the rest of the blubber tissue for analysis, as well as for cleaning equipment: hexane, acetone, diethyl ether, NaCl, 85 % ortho-phosphoric acid, 95–97 % sulfuric acid, Isooctane (2,2,4-trimethylpentane), Tetrachloronaphthalene (TCN). The mixtures were: 0.9 % NaCl / 0.1 M phosphoric acid mixture, and Hexane / ether (9:1) mix. Recovery standards (E-HCH, PCB-112, o,p-DDD, PCB-198 all at 100 pg/μL), quantitative analysis standards containing 31 POPs, (hexachlorobenzene (HCB), α-, β- and γ-hexachlorocyclohexane (HCH), α- and γ-chlordane, trans-nonachlor, oxychlordane, three toxaphene congeners (Parlar nos. 26, 50 and 62), p,p'-dichlorodiphenyl dichloroethene (p,p'-DDE), p,p'-dichlorodiphenyldichloroethane (p,p'-DDD), p,p'-dichlorodiphenyl trichloroethane (p,p'-DDT) and o,p'-DDT and eleven PCB-congeners (#28, 31, 52, 101, 105, 118, 138, 153, 156, 170, 180), and five PBDEs (#47, 99, 100, 153,154)) and the internal standard (30 ng/mL (pg/μL) of TCN diluted in isooctane), were stored in a refrigerator. Each component was obtained from Accustandard, USA or LGC-standards, UK.

The homogenized blubbers were extracted as described earlier (Olafsdottir et al., 1995). In short, approx. 3 g of tissue were extracted with hexane/acetone/diethyl ether, solvents evaporated at 40 °C under N₂, the residue resuspended in isooctane containing the internal standard (TCN). The fat content was determined gravimetrically and the samples were cleaned by treatment with concentrated sulfuric acid. The POPs were determined using GC-MSD from Thermo (Thermo Trace 1310 GC with ISQ LT mass detector). Column: DB5MS from JW, 25 m 0,20 mm i.d., 0,35 μm film. Following the quantification of the peaks using the Chromeleon™ Chromatography Data System (CDS) software, from Thermo, and a six level standard curve (0.5–250 pg/μl), these values, were imported into Excel and R-studio for statistical analysis.

For quality control and quality assurance, a sample of certified whale blubber (SRM1945) from the National Institute of Standardization and Testing (NIST), USA was analysed with the samples and all analytes were within 80–120 % of the reported values in the SRM sample. Further, by participating successfully 2×/year in laboratory performance studies by Quasimeme-BT-2 (biota). Recovery standards PCB-112, PCB-198, E-HCH and o,p'-DDD were added to the samples prior to the extraction. Analytes in the blanks were subtracted from the same found in the samples.

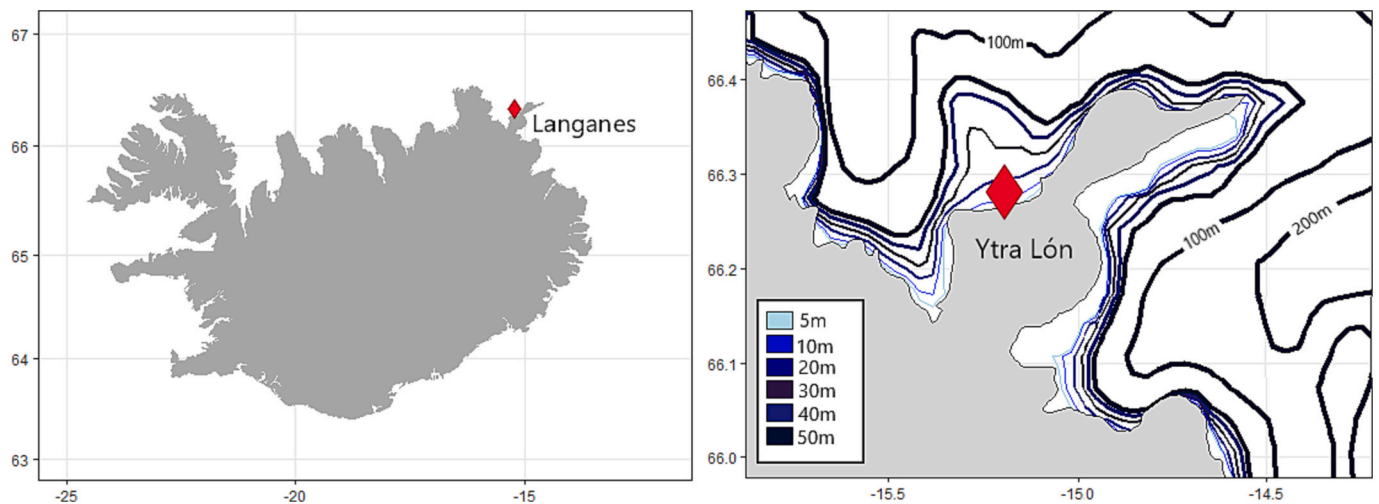


Fig. 1. The location of the pilot whale (*Globicephala melas*) stranding on the 6th of September 2019, at Ytra Lón on the Langanes peninsula, NE Iceland.



Fig. 2. Some of the dead long-finned pilot whales (*Globicephala melas*) stranded at Ytra Lón, on the Langanes Peninsula, NE Iceland, on the 6th of September 2019. Photo by Charla Jean Basran, 2019.

2.3. Statistical methods

The investigation of contaminant data was carried out with the purpose of exploring the possible relationships between the levels of pollutants and the physiological parameters of sex and age group (juvenile or adult). One-way permutation tests and linear regression analyses were employed using R Studio (V1.2.5033) to explore any significant relationships between the categorical variables of sex and age group, and all other variables. Shapiro-wilks test were also carried out to assess the normality of the data, and log functions were subsequently used to normalise data that was skewed. Review of the literature concerned with pollutant loads in long-finned pilot whales and other delphinids in the North Atlantic was the basis of comparison of POP levels with time.

3. Results

3.1. Persistent organic pollutant levels

The present study screened for 31 POPs, with the eight most abundant listed in Table 1, including the sums of multiple congener families. DDE was the highest in concentration by far with an average of 5850 ng/g lw, followed by transnonachlor at 1280 ng/g lw. PCA biplots were created to show the variance in males vs females, and adults vs juveniles in relation to the analysed pollutants (Fig. 3). Here, the sexes did not show a significant difference in variation, which is reflected by the lack of significant relationships in subsequent tests between the sex of the whales and contaminant concentrations. However, adults and juveniles exhibited a clear difference in variance, with juveniles having a much larger spread than the adults (Table 2).

DDE had the highest concentration of any POP analysed, with levels far beyond the scale of the other pollutants. Thus, separate bar plots were created to clearly illustrate the varying levels of different

Table 1

Pilot whale (*Globicephala melas*) physical parameters and POP levels in ng/g lw obtained from GC-MS analysis. . J = Juvenile, A = Adult, M = Male, F = Female.

Whales	Age/sex/length (cm)/Blubber thickness (cm)	HCB	PCB-153	∑10 PCBs	Tox-50	∑3 tox	DDE	∑DDTs	Oxy-Chlordane	Trans-Nonachlor	PBDE-47	∑5 PBDEs
28	J/M/170/2.19	394	494	1670	654	1100	1940	2700	77.6	666	63.5	98.3
10	J/F/232/2.00	1200	2250	7960	2430	4320	16,200	19,000	369	3050	386	551
12	J/F/256/3.98	683	4480	15,100	2350	4990	28,300	32,900	760	5720	586	820
8	J/F/280/2.12	432	1984	6830	1240	2470	12,000	14,500	280	2350	292	411
5	J/F/300/3.62	179	804	2420	689	1220	3630	4560	76.1	820	87.2	159
14	J/F/330/2.90	339	945	3240	875	1510	3490	4820	129	955	152	224
13	J/F/352/3.54	215	1040	3650	704	1350	5620	7200	161	1290	169	247
38	J/F/355/3.10	319	1010	3580	571	1180	4090	5600	126	1050	145	228
20	A/F/385/4.12	41.4	354	1100	270	456	948	1330	29	330	35.8	53.2
11	A/F/386/5.11	170	1550	5150	810	1560	7870	9780	176	1770	196	313
25	A/M/386/4.10	179	1240	4360	558	1100	7130	8810	151	1430	131	199
15	A/M/389/3.31	168	1900	6380	825	1730	11,600	14,000	266	2260	186	288
41	A/M/402/5.44	149	929	2790	492	923	3500	4450	73.4	888	107	172
6	A/F/417/4.09	23.8	391	1180	261	463	448	886	17.5	256	34.6	51.5
40	A/F/417/4.65	132	296	957	315	550	760	1130	26	305	29.6	61.9
9	A/F/420/4.45	164	446	1480	522	937	1370	2030	52.1	531	70.6	141
26	A/M/427/3.99	33	168	545	163	278	447	634	15.3	153	15.3	26.4
17	A/F/434/4.81	139	299	1040	421	774	1010	1500	39.1	354	37.3	46.9
22	A/F/440/5.81	118	243	825	323	563	754	1100	24.9	286	36.2	44.2
16	A/M/518/6.52	156	1090	3730	501	1030	5860	7170	114	1230	111	190
	mean	262	1100	3700	749	1430	5850	7200	148	1280	144	216
	min	23.8	168	545	163	278	447	634	15.3	153	15.3	26.4
	max	1200	4480	15,100	2430	4990	28,300	32,900	760	5720	586	820
	stdev	270	1010	3440	616	1220	6880	7950	174	1310	140	196

contaminants (Fig. 4). Whale 12, a calf which could have still been nursing, had the highest concentrations for several pollutants, particularly DDE (28,300 ng/g lw), transnonachlor (5720 ng/g lw), and PCB-153 (4480 ng/g lw), the three most abundant pollutants among all individuals. Moreover, most individuals had a similar pattern of contaminant concentrations, with juveniles typically having the highest values for all pollutants. Nevertheless, toxaphenes, particularly tox-50, had the most inconsistent relationship with the other pollutants. To investigate linear relationships between all the variables available, Kendall's correlation tests were run on every contaminant, individual length, and blubber fat percentage combination. All POPs were positively correlated with each other, showing a significant relationship. Fat percentage had no significant relationship with any variable, and length had a significant relationship and a negative correlation with all the pollutants (Table 3).

3.2. Variability of contaminant concentrations between the sexes and age groups

Six males and 14 females were used for this study, however, when comparing sexes, only the adults were used with five males and seven females. Since only one juvenile male was available, the investigation of sex differences among juveniles was not viable, so these were treated as one group. Contaminant concentration was generally higher in juveniles than in adults, the pollutants, which proved significantly higher in juveniles are listed in Table 4 and shown in Fig. 5. The smallest juvenile, a male, differs from the other juveniles in having low levels of contaminants (Table 1). Additionally, blubber thickness was significantly lower in juveniles than adults (p -value < 0.01) whilst PCB-153 (p -value = 0.055) and PBDE-154 (p -value = 0.054) did not have a significant relationship with age group. These values pointed to a relationship between the age groups of the whales and the concentration of pollutants within them, but not with sex, which was investigated further below.

Oxychlordane, transnonachlor, DDE, toxaphene-50, and PBDE-47 all had significantly higher values in juveniles compared to adults as well, with each linear model corroborating these relationships. Linear regression models with each contaminant as a response variable also revealed some possible relationships between sex, age group, and specific pollutants. The means of the compared groups (female vs. male and

adult vs. juvenile) had considerable disparity between them, however, the linear models indicated that there could be a difference between the compared groups with almost significant relationships based on the p -value. Furthermore, outliers are indeed present in each group, thus, affecting the test results. The figures and complimentary regression models for each of these can be found in the supplementary Appendix A.

Fig. 6 shows the average concentrations of pooled mean contaminant levels, highlighting how juveniles had the highest average concentration of the three groups. However, juvenile, and male mean concentrations did not appear to be significantly different when all pollutants were pooled together even though some juveniles had exceedingly higher values. Due to the much higher values among certain pollutants, their results may have overshadowed the differences between groups with regards to the lower value pollutants in Fig. 6.

3.3. Historical POP level comparisons

PCB and DDT levels were measured within the muscle and blubber of 211 Faroese *G. melas* specimens hunted in 1987 (Borrell, 1993, Table A1). Their lipid-based analysis yielded a total mean DDE levels of 14,110 ng/g lw, which is considerably higher than the mean DDE levels found in the samples from this study, at 5850 ng/g lw. Borrell (1993) total mean PCB was 37,540 ng/g lw, which was obtained from averaging the ten most abundant PCB congeners. The mean ∑PCB of the same congeners in the whales examined for the present study was about one order lower at 3700 ng/g lw (± 440). Here, the same patterns between males and females persisted, where the latter contained lower concentrations than males.

Dam and Bloch (2000) quantified several pollutants within the blubber of Faroese long-finned pilot whales and ten of the 14 PCB congeners analysed were the same as those in the present study. For juveniles, most PCB concentrations from the year 1997 were almost double that of the present study except for PCB-28, which was lower than the concentration found in juveniles of the present study (Table A2). The difference in concentration between individual females was much greater than found by Dam and Bloch (2000). Also, PCB-28 showed higher load in juveniles in the present study compared to Dam and Bloch (2000). For males, the difference was even higher than for females, except for PCB-28, which was only slightly higher. Here, the

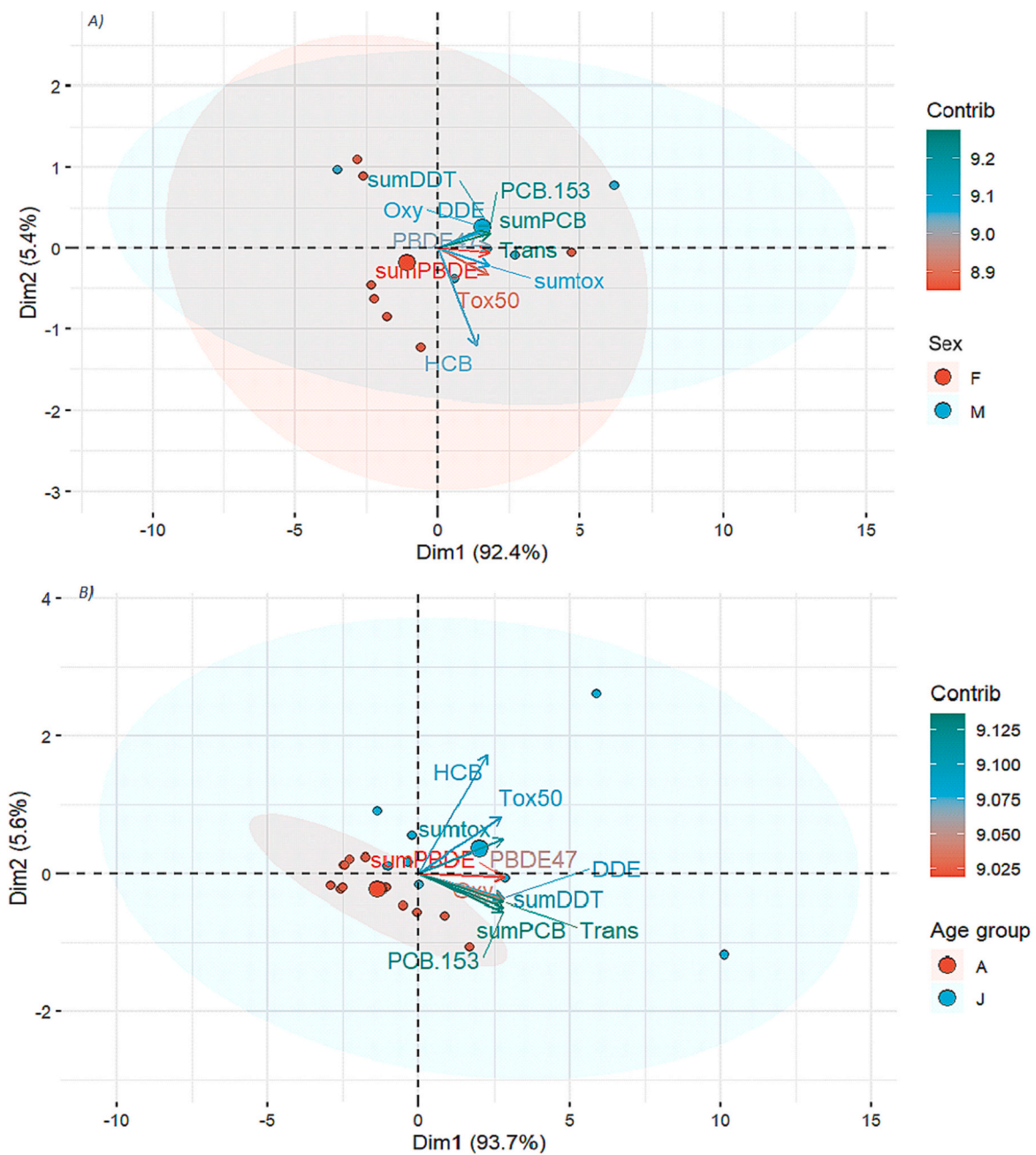


Fig. 3. PCA biplots of individual pilot whales (*Globicephala melas*) and variables contaminants grouped by sex (A) and age group (B), using colour and confidence ellipses across the first two principal components, with contribution of the individuals by point size and contribution of the variables of colour. Age group A represents adults and the group J represents juveniles.

Table 2

Mean, minimum and maximum values in ng/g lw for each contaminant by age group as measured in the blubber of pilot whales (*Globicephala melas*).

Pollutants	Juvenile mean (n = 8)	Juvenile min	Juvenile max	Adult mean (n = 12)	Adult min	Adult max
HCb	469	179	1202	123	23.8	178
PCB-153	1630	494	4470	742	167	1900
∑10 PCB	5550	1670	15,060	2460	545	6380
Tox-50	1190	571	2430	454	162	824
∑3 tox	2270	1100	4990	864	278	1730
DDE	9420	1940	28,300	3480	448	11,600
∑DDT	11,400	2700	32,900	4400	634	14,000
oxychlorodane	247	77.6	759	82.0	15.1	265
transnonachlor	1990	666	5720	816	151	2260
PBDE-47	235	63.5	586	82.5	15.3	196
∑5 PBDE	342	98.3	819	132	26.4	313

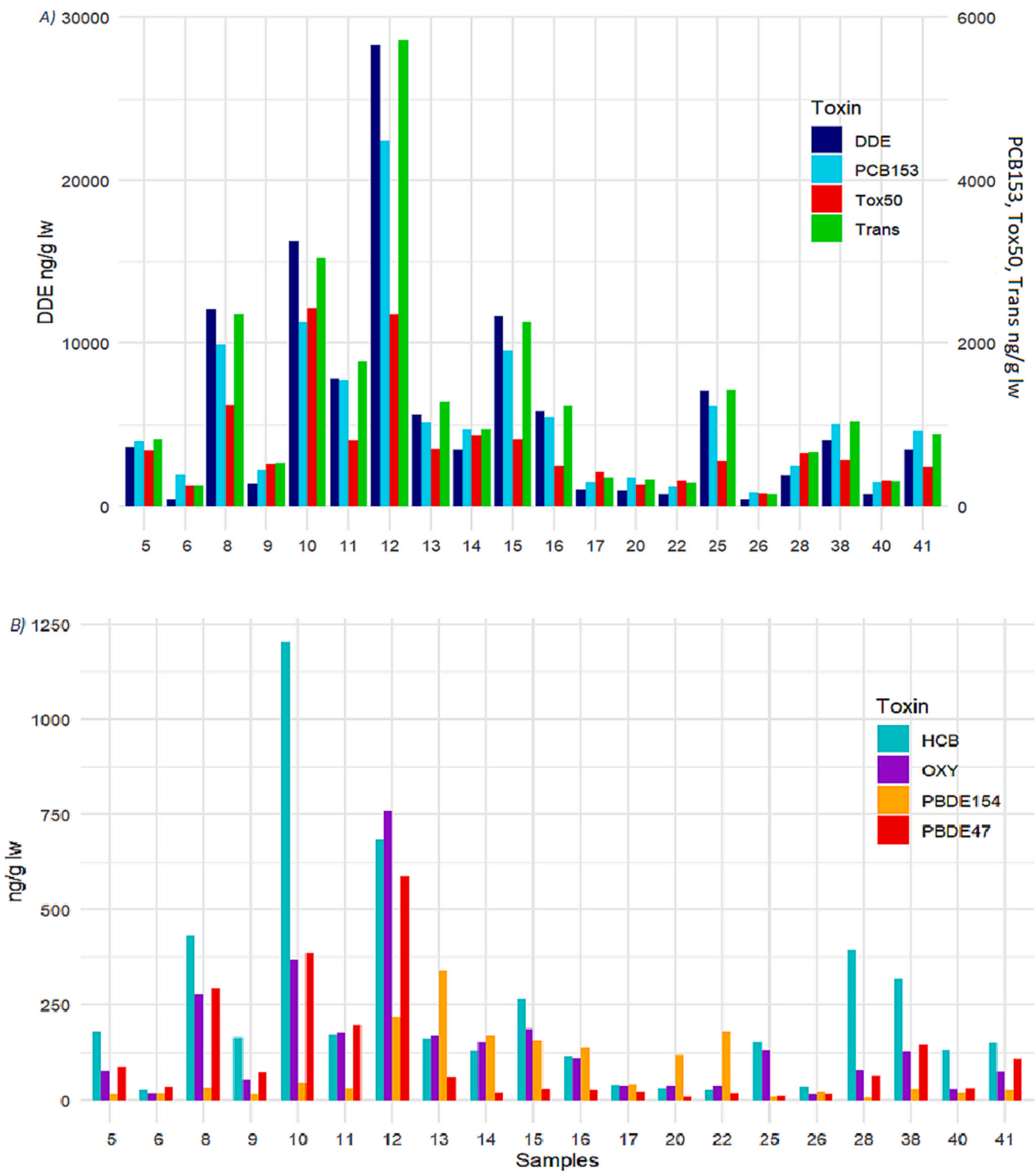


Fig. 4. Barplots of main POPs analysed in the pilot whale (*Globicephala melas*) samples divided between high concentrations (A) and lower concentrations (B) to enhance clarity.

pattern of concentration between sexes and age groups was only consistent when it came to the juveniles and females, as the males sampled in 1997 had very comparable levels to the juveniles sampled that same year. This could also be seen in most of the other pollutants quantified in Dam and Bloch (2000), except for tox-50, tox-62, and HCB (Tables S4 & S5).

As for DDTs, it was evident that Faroese whales analysed by Dam and Bloch (2000) had significantly higher concentrations than those stranded in Iceland in 2019 (Table A3), apart from the DDE

concentration in juveniles which was similar for both studies, or 9420 ng/g lw (± 8490) in 2019 and 10,171 ng/g lw (± 3430) in 1997. The DDTs' concentrations differed, however, less between studies than what was found for the PCBs. In both studies, females had lower mean concentrations than both males and juveniles, with the latter two having relatively similar levels of DDT congeners.

Comparison of HCBs, oxychlorodane, and transnonachlor from blubber sampled in 1997 and 2019 showed that the Faroese pilot whales from 1997 had considerably higher concentrations of these pollutants

Table 3
Correlation matrix of all POPs, length and fat percentage of each individual pilot whale (*Globicephala melas*, n = 20).

	HCB	PCB-153	Tox-50	DDE	Oxy	Trans	PBDE-47	PBDE-154	Fat %	length
HCB	–	R = 0.55 p ≤ 0.01	R = 0.81 p ≤ 0.01	R = 0.6 p ≤ 0.01	R = 0.69 p ≤ 0.01	R = 0.62 p ≤ 0.01	R = 0.67 p ≤ 0.01	R = 0.49 p ≤ 0.01	R = 0.21 p = 0.21	R = –0.66 p ≤ 0.01
PCB-153		–	R = 0.69 p ≤ 0.01	R = 0.92 p ≤ 0.01	R = 0.86 p ≤ 0.01	R = 0.94 p ≤ 0.01	R = 0.84 p ≤ 0.01	R = 0.67 p ≤ 0.01	R = 0.05 p = 0.77	R = –0.4 p = 0.014
Tox-50			–	R = 0.74 p ≤ 0.01	R = 0.81 p ≤ 0.01	R = 0.76 p ≤ 0.01	R = 0.81 p ≤ 0.01	R = 0.56 p ≤ 0.01	R = 0.17 p = 0.32	R = –0.55 p ≤ 0.01
DDE				–	R = 0.91 p ≤ 0.01	R = 0.96 p ≤ 0.01	R = 0.84 p ≤ 0.01	R = 0.62 p ≤ 0.01	R = 0.09 p = 0.59	R = –0.39 p = 0.016
Oxy					–	R = 0.93 p ≤ 0.01	R = 0.89 p ≤ 0.01	R = 0.63 p ≤ 0.01	R = 0.08 p = 0.63	R = –0.47 p ≤ 0.01
Trans						–	R = 0.88 p ≤ 0.01	R = 0.65 p = 0.68	R = 0.07 p = 0.016	R = –0.39 p = 0.016
PBDE-47							–	R = 0.67 p ≤ 0.01	R = 0.13 p = 0.46	R = –0.43 p ≤ 0.01
PBDE-154								–	R = 0.18 P = 0.27	R = –0.43 p ≤ 0.01
Fat %									–	R = –0.18 P = 0.27
Length										–

Table 4
Significant relationships exhibited by linear models between pollutants (ng/g lw) and age groups of pilot whales (*Globicephala melas*).

	HCB	Oxylchlordane	Tox-50	Transnonachlor	PBDE-47	DDE
Age group	0.002	0.033	0.005	0.047	0.013	0.0369
p-value						
intercept	0.059	0.086	0.006	0.031	0.03	0.048
R ² /R ²	0.424/	0.229/	0.360/	0.201/	0.298/	0.200 /
adjusted	0.392	0.186	0.324	0.157	0.259	0.160

compared to the whales sampled in 2019 apart from b-HCB. The b-HCB concentrations were very similar between the different years, with the females sampled in 2019 having slightly higher levels of b-HCB than the ones sampled in 1997 or 34 ng/g lw (±10) and 27 ng/g lw (±16) respectively (Table A4). The concentrations of these pollutants were also higher for juveniles than for adult males and females, with the latter having the lowest levels of pollutants (Table A4).

Comparisons of toxaphenes concentrations measured in Dam and Bloch (2000) and this study showed that juveniles had the highest mean concentrations in both studies, followed by males and then females. One exception was Tox-62, where females had slightly higher concentrations (94 ng/g lw ±48) than males (85 ng/g lw ±43) in the present study (Table A5). Here, females and males had similar concentrations of toxaphenes, but juveniles had significantly higher levels for all three parlars. Whereas in Dam and Bloch (2000), the juveniles and males had similar concentrations, and the females had much lower levels. Nevertheless, it was clear that overall Toxaphene concentrations were lower in the present study, especially for Tox-50.

A comparison of PBDE concentrations in pilot whales between multiple studies conducted in the Faroe Islands indicated a drastic increase in all five of the most prominent PBDE congeners until 1997, after which a decrease started (Table A6). Notably, the results from the present study were still very high compared to those from 1986 (Fig. 7). These values showed a considerable spike in PBDEs in the early 1990s, which started to subside after a peak in 1997. A visible difference in concentration was seen between the 2006 and the 2010 study, after which the PBDE levels seemed to stabilise, with subsequent years having very similar concentrations to the present study. We did not have the opportunity to analyse the PBDE209, the major PBDE still produced, but as it breaks down into a more persistent, bioaccumulative, toxic, and mobile PBDE congeners in the environment we should be including at least some of that source (Ross et al., 2009).

Pinzone et al. (2015) found that North-western Mediterranean pilot whale blubber had very high levels of most POPs. One comparable contaminant between our studies was the DDT complex (Table A7),

which was found to be in considerably higher concentrations within the Mediterranean long-finned pilot whales. Mono-ortho PCBs (PCB-105, PCB-118, and PCB-156) were also investigated in both studies, with the Mediterranean pilot whales having a mean concentration of 2114 ng/g (±1066), markedly higher than the 204 ng/g (±164) found within the pilot whales sampled in this study (Pinzone et al., 2015).

Garcia-Cegarra et al. (2021) found the concentrations of various POPs within the blubber of long-finned pilot whales stranded in 2016, in Chilean Patagonia, revealed that pollutant concentrations in the Southern Pacific Ocean were relatively low. The levels of POPs here were far lower than those considered toxic for cetaceans, and those found in other areas, particularly, the HCBs, PCBs, and DDTs, which were markedly lower when compared to the present study (Table A8).

4. Discussion

The present study screened for 31 POPs, including multiple organochlorines and their different forms, of which DDE was the highest in concentration by far at 5850 ng/g lw, followed by transnonachlor at 1280 ng/g lw and PCB-153 at 1100 ng/g lw. This is because these three pollutants have had widespread use in the past as pesticides (DDT and chlordanes) and flame retardants (PCBs), have especially long environmental residence times, are lipophilic and insoluble in water, and are very difficult to degrade (Jensen, 1972; Kutz et al., 1976; Turusov et al., 2002). Thus, despite the establishment of several pieces of legislation banning their use, these compounds are still relatively high in concentration in a variety of forms (Van den Berg, 2009). Particularly wherever anthropogenic activity is more prominent, such as the North Atlantic and the Mediterranean, as these bodies of water are surrounded by highly populated terrestrial environments (Schnitzler et al., 2008; Pinzone et al., 2015; Garcia-Cegarra et al., 2021). This difference is especially unsurprising in the Mediterranean Sea since it is a relatively small and isolated water body with a longer and more intense history of anthropogenic activity than any other marine environment (Gómez-Gutiérrez et al., 2007; Berrojalbiz et al., 2011).

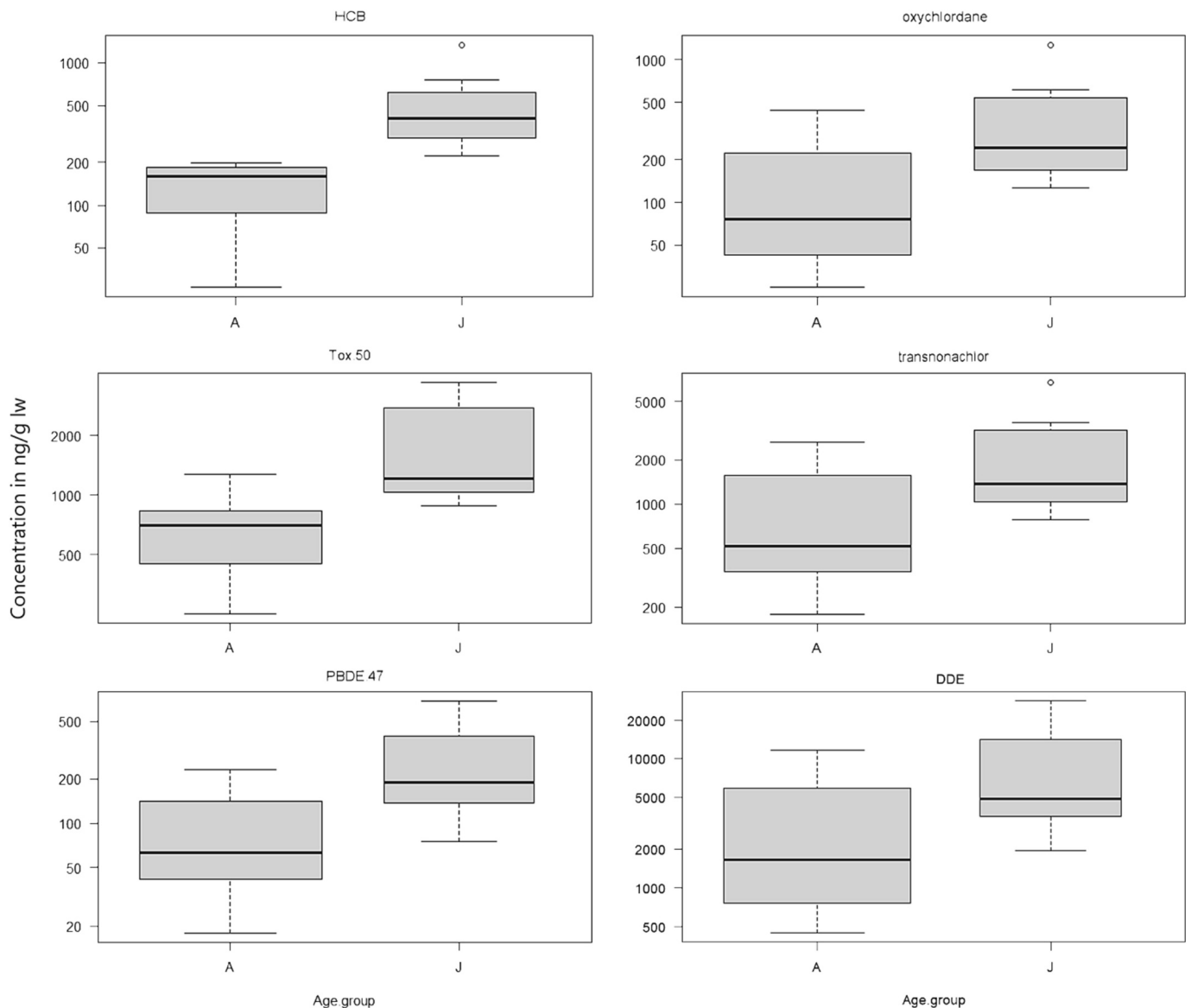


Fig. 5. Main POPs analysed in pilot whale (*Globicephala melas*) samples divided between high concentrations (A) and lower concentrations (B) to enhance clarity. The boxes represent the interquartile range, the whiskers represent the values within 1.5× the lower and upper quartiles, and the horizontal lines represent the median. Outliers are presented as single dots.

Since DDT is still used by 14 countries for disease mitigation, and several more are preparing to reintroduce it into their arsenal of pesticides, it is unsurprising that it is still found in such high concentrations (Van den Berg, 2009). Following its ban by the Stockholm convention in 2004, the highest authority for the control of POPs, DDT use decreased worldwide except for certain countries in Africa, Asia, and South America, where it increased because of its usage for indoor spraying to manage malaria and visceral leishmaniasis vectors (Van den Berg, 2009). This implies that the concentration of DDT and its metabolites in these regions should be higher than areas where it is still banned, such as the North Atlantic. In fact, DDE and ΣDDT concentrations in cetaceans sampled off the coasts of Brazil, Tanzania, South Africa, India, Japan, and the Philippines, were exceedingly high (Kajiwara et al., 2004; Kannan et al., 2005; Mwevura et al., 2010; Yogui et al., 2010; Lailson-Brito et al., 2012; Gui et al., 2016; Trukhin and Boyarova, 2020). However, some studies show that this isn't always the case as certain cetaceans from Argentina and even Brazil had much lower concentrations, sometimes even lower than the present study (Lailson-Brito et al., 2011; Durante et al., 2016). Thus, comparing values from separate

studies is considerably challenging due to the host of different environmental conditions and circumstances experienced within vastly distinct study areas. Especially since the intricate details of global contaminant dispersal are exceedingly difficult to predict because oceanic and atmospheric currents are highly capable of transportation. This, coupled with the unpredictability of animal migration on a regional and local level make comparisons even trickier (Hageman et al., 2015).

Another reason could be the accelerated degradation of the pollutants in the warmer waters of the tropics, resulting in a higher proportion of DDE to DDT (Aguilar et al., 2002). Thus, it is best to compare DDE or sum DDT amounts instead of the original form and compare DDE/ΣDDT ratios. Since DDT is metabolised into DDE and DDD when it enters the ecosystem, the ratio between the sum of DDTs and DDE can be used to determine the novelty of the compound's entrance into the environment (Lailson-Brito et al., 2011). In the present study, DDE represented 84 % of the ΣDDT, which suggests that the observed DDE levels are a result of old DDT introduction in this region, as it keeps bioaccumulating in this metabolised state. In Dam and Bloch (2000), and Pinzone et al. (2015),

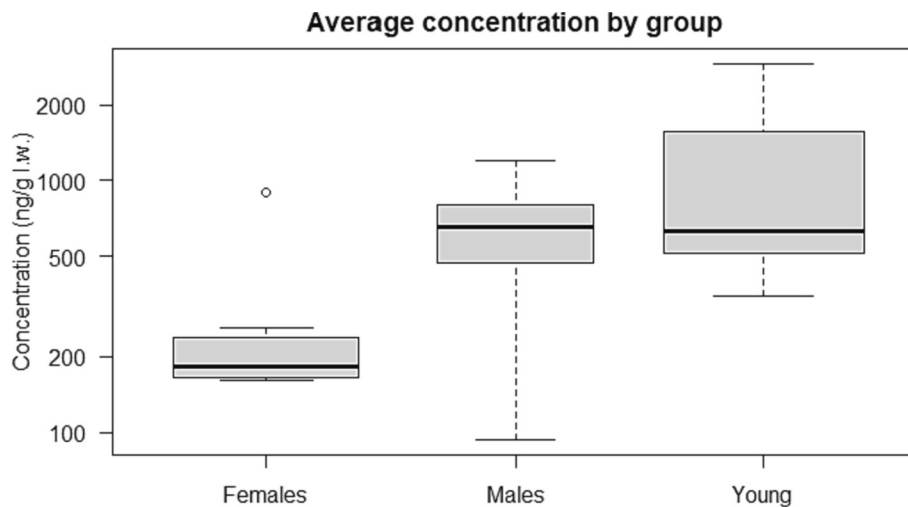


Fig. 6. Mean concentrations of POPs in pilot whale (*Globicephala melas*) blubber divided by age group and sex. Males and females here are considered adults. The boxes represent the interquartile range, the whiskers represent the values within $1.5 \times$ the lower and upper quartiles, and the horizontal lines represent the median. Outliers are presented as single dots.

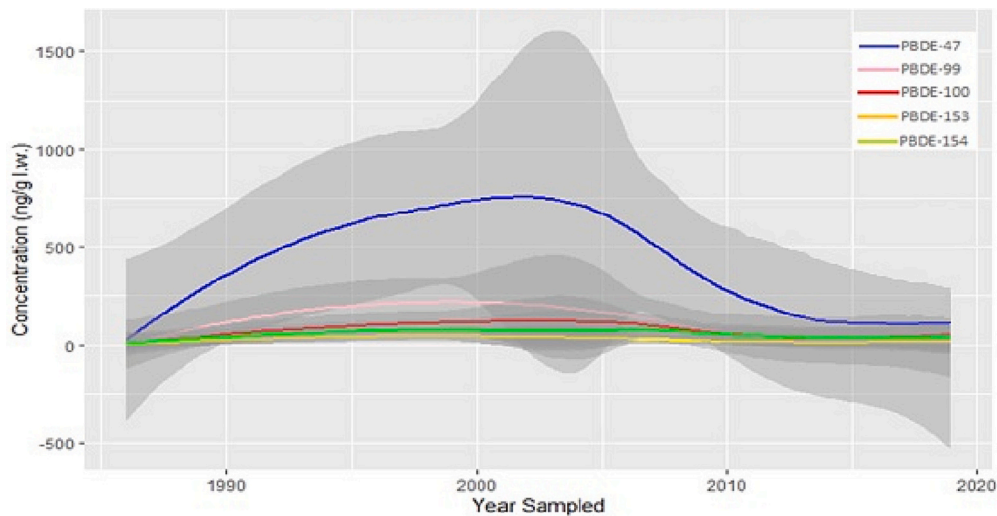


Fig. 7. Five PBDE congener concentrations (Lw within the blubber of pilot whales, *Globicephala melas*, from the Faroe Islands, from 1986 to the present study). Data extrapolated from Fångström (2005), Dam (2011), Rotander et al. (2012), and Bjurlid et al. (2018).

this ratio sits at 65 % and 78 % respectively, indicating that Σ DDT levels are increasingly becoming composed of the metabolised form.

Transnonachlor is a major bioaccumulative component of the organochlorine pesticide chlordane, which was predominantly used for termite control globally. It was first banned in the United States in 1988, after which it started becoming restricted worldwide until it was banned completely by the year 2000. (Bondy et al., 2000; Bondy et al., 2004). Transnonachlor is notoriously resistant to degradation and is highly lipophilic, enabling it to biomagnify within marine mammal adipose tissue for exceedingly long periods of time. As a result, it persists until this day in relatively high concentrations within top ocean predators like *G. melas* despite widespread bans in the late 20th century (Reddy et al., 2001). Due to its resilience to metabolism, it can readily be passed down vertically through lactation and gestation, making its way through generations in significant levels even though there are little to no new sources of chlordane in the ecosystem (Tryphonas et al., 2003; Bondy et al., 2004). When it does metabolize, it turns into oxychlordane, another POP that this study investigated, which was markedly lower in mean concentration (150 ng/g lw) than transnonachlor (1280 ng/g lw), highlighting its recalcitrancy to breakdown (Bondy et al., 2004).

Despite their industrial usage since 1929, PCBs and their bio-accumulation in the ecosystem were unknown until 1966, when the analysis of DDT compounds within marine organisms highlighted large amounts of other unknown substances. This discovery led to the eventual ban of PCB use and manufacturing; however, they persist due to their resistance to degradation. Their robustness is attributed to their chemical stability, giving them a high dielectric constant and low flammability, hence their use in coolants, flame retardants, plasticizers, antifouling paints, and more (Rachdawong and Christensen, 1997; Rudel et al., 2008). Like the above contaminants, PCBs are highly lipophilic and dispersible, and thus, tend to accumulate in considerable concentrations within organisms with high lipid contents such as fatty fish and marine mammals (Tanabe, 1988; Norstrom and Muir, 1994; Mössner and Ballschmiter, 1997; Bayarri et al., 2001).

PCB concentrations presented by this study are far lower than those in long-finned pilot whales sampled in the Faroe Islands in 1997 by Dam and Bloch (2000), approximately by half, which suggests that PCBs are steadily decreasing from marine food webs. Furthermore, the safe Σ PCB threshold proposed in the literature (11,000–17,000 ng/g lw) is substantially higher than the concentrations found within the stranded

whales in the present study, suggesting that it is unlikely that the stranding was caused by toxicity levels (Kannan et al., 2000; Hall et al., 2006; Murphy et al., 2015; Garcia-Cegarra et al., 2021). However, this threshold value range was not developed with data from long-finned pilot whales, but other cetaceans including belugas, bottlenose dolphins, and harbour porpoises, so it should be considered with caution (Kannan et al., 2000; Jepson et al., 2005). Having health risk thresholds for additional POPs would also be a considerable improvement since only those for PCB exist at this point.

These pollutants have been linked to a myriad of diseases and developmental disorders because they interfere with endocrinological systems, since their molecular structure and concentrations are similar to those of the endogenous hormones themselves (Hoydal et al., 2016). Developing fetuses and newborns are particularly susceptible to endocrine disruption, since in-utero nutrient transmission and lactation deposit pollutants from the mother's lipids into theirs (Hoydal et al., 2016), making their impacts even more distressing. The predominant effects of DDTs include steroid homeostasis, endocrine steroidogenesis impairment (Hart et al., 1971; Nelson et al., 1978; Haake et al., 1987; Kelce et al., 1995; You et al., 2001), and HPG (hypothalamic-gonadal) and HPA (hypothalamic-pituitary-adrenal) axes disruption (Galligan et al., 2019). Chlordanes and transnonachlor present serious chronic and terminal illnesses including diabetes (Evangelou et al., 2016), respiratory problems, liver diseases, thyroid, and immunological disorders (Bondy et al., 2000; Tryphonas et al., 2003; Bondy et al., 2004), as well as several forms of cancer (Khanjani et al., 2007; Cook et al., 2011; Lim et al., 2015; Luo et al., 2016). PCB impacts include soft tissue degeneration (Jensen, 1972; Sánchez-Alonso et al., 2003), sex steroid and cell energy metabolism disruption (Ferrante et al., 2014; Abella et al., 2015), genital deformities (Helle et al., 1976), lowered fecundity and birth-weight, impaired neural development of offspring, lowered intelligence quotient (Hussain et al., 2000; Casas et al., 2015), and potentially cancer as well (Cogliano, 1998; Lucena et al., 2001).

Long-finned pilot whales around the Faroe Islands have been observed to be extensively contaminated with PCBs, DDTs, toxaphene, chlordane, and PBDEs (Borrell, 1993; Hoydal et al., 2015; Hoydal et al., 2016; Hoydal et al., 2017). Pilot whales here differed by age group and gender with higher levels in juveniles than adult females, indicating that the most hydrophobic contaminants transfer to juveniles less readily than smaller, lipophobic ones (Hoydal et al., 2015). Furthermore, in Australian long-finned pilot whales, DDT and its metabolites were the most concentrated, with PCBs and PBDEs being significantly lower. These seemed to decrease with age in males, which might be attributed to dilution by growth or decreasing levels within the environment. The highest concentrations were found in juveniles, with PCB levels being positively correlated with pregnancy duration, indicating that contaminant offloading predominantly takes place in-utero as opposed to during lactation (Weijs et al., 2013). The process of dilution by growth is most likely responsible for the differences in concentration between the youngest juveniles (calves that could have still been nursing) and older juveniles (approaching maturity).

We observed differences between the juveniles in contaminant levels, with the smallest individual (a male) having considerably lower levels than juvenile females, indicating that other factors, such as feeding, may contribute towards various levels of contaminants. de Stephanis et al. (2008) found that, within the same general area (Strait of Gibraltar), some level of specialisation in habitat or prey choice between social units of pilot whales is evident. While information on the feeding ecology of long-finned pilot whales in Iceland is not yet available, studies are underway to investigate the isotopic niche of stranded whales, including the same individuals from our study (Samarra, unpublished data). Given that squid and fish can have substantial differences in contaminant levels (Weisbrod et al., 2001), further knowledge of fish and cephalopod contaminant loads in this region, as well as the movements and social organisation of pilot whales in Iceland, will be necessary to evaluate potential sources of contaminants.

There was no evidence that the stranded long-finned pilot whale from Langanes were emaciated or sick. While body condition could not be assessed on site, visual assessments based on photographs indicated that the whales did not suffer from emaciation. Their blubber thickness ranged from 2.12 to 6.52 cm (smallest to largest), very similar to that of a long-finned pilot whale stranded on the Tasmanian coast in 1998, whose blubber thickness ranged from 3.07 to 7.40 cm (Walters, 2005). A necropsy of a long-finned pilot whale in moderate nutritional state that measured 303 cm found its mean blubber thickness to be 2.6 cm (Wessels et al., 2021), which is very close to our similarly sized whales of 352 cm that had a blubber thickness of 3.1 cm. However, this comparison may be imprecise, as there was no standardised method of measuring the thickness of our samples and the samples from the literature. Nevertheless, the whales sampled for the present study did not exhibit any clear signs of poor health such as emaciation, skin diseases, ectoparasites, congenital malformations, lesions, or other injuries (Joblon et al., 2014; Minton et al., 2018; Raverty et al., 2020), indicating that they were not in poor health prior to stranding.

Throughout the aforementioned studies, as well as those compared to in the results section, some features are evident. Firstly, there is a clear pattern with age group, sex, and contaminant concentrations, where juveniles tend to have the highest concentrations, followed by adult males, then adult females. Secondly, POP levels are related to the degree of human activity in each area, where regions with longer histories of anthropogenic disturbance will have higher amounts of pollutants, as seen in the Mediterranean, which is followed by the North Atlantic and then the Pacific coast of South America (Schnitzler et al., 2008; Pinzone et al., 2015; Garcia-Cegarra et al., 2021). The pattern of concentration variation within sex and age groups is consistent with what has been found in previous studies regarding most cetacean species, where POP loads are high in early life stages but often decrease with age due to tissue growth and subsequent dilution. This is inverted in sub-adult males, resulting in contaminant levels increasing with age until a peak is reached in old age, whilst in females, concentrations typically decrease with increasing age due to vertical transfer to offspring through gestation and lactation (Borrell, 1993; Dam and Bloch, 2000; Fångström, 2005; Hoydal et al., 2015; Bjurlid et al., 2018).

5. Conclusion

This study's aim was to assess the condition of stranded long-finned pilot whales through the investigation of the contaminants within their blubber. This first attempt to quantify and compare POP levels from pilot whales stranded in Iceland found that contaminant concentrations were lower than the potentially harmful thresholds for cetaceans, as proposed by the literature (Kannan et al., 2000; Hall et al., 2006; Murphy et al., 2015; Garcia-Cegarra et al., 2021). However, these thresholds were not based on long-finned pilot whales, and thus, should be considered with caution. This, in combination with relatively good body condition, with respect to blubber thickness, suggested that the stranded individuals analysed in this study were not in poor health.

A clear sex and age pattern also emerged when investigating the contaminant levels among individuals, with juveniles having the highest POP levels, closely followed by males and lastly females. This is supported by the literature, as contaminant offloading during lactation and gestation is a well-known process within marine mammals (Borrell et al., 1995). Therefore, an investigation about how these pollutants might affect development in juveniles would make for an interesting follow-up study. Additionally, a study concerning the quantification of pollutants within live, free-swimming *G. melas* would certainly be valuable, and aid in establishing a baseline for future comparisons. Despite the apparent decrease of POPs within the blubber of *G. melas* in the North Atlantic, the thresholds for harmful levels found in the literature should be re-evaluated and significantly expanded upon as they are very limited.

Several classes of POPs have been linked to the decline of well-studied cetacean populations globally (Krahn et al., 2007; Cullon

et al., 2009; Noël et al., 2009; Ylitalo et al., 2009; Hoguet et al., 2013), therefore, monitoring contaminant loads within cetaceans is a crucial aspect of their conservation. Evidently, the geographic location and distribution range of cetacean populations influences their POP loads. Thus, they could also potentially be used to our advantage as indicators to differentiate between cetacean stocks through the matching of contaminants in their blubber and stable isotope profiles in their skin with those of their prey (Krahn et al., 2007; Krahn et al., 2008; Ryan et al., 2013). No quantitative studies regarding POPs or steroid hormones and their relationships within *G. melas* have been carried out in Iceland so far, leaving ample room for investigation in an area that may have significant implications for the vulnerability and conservation of this species.

CRedit authorship contribution statement

Nicholai Xuereb: Writing – original draft, Methodology, Formal analysis, Investigation, Visualization. **Kristín Ólafsdóttir:** Data curation, Methodology, Formal analysis, Resources, Writing – review & editing, Funding acquisition. **Filipa Samarra:** Resources, Writing – review & editing. **Jörundur Svavarsson:** Supervision, Writing – review & editing. **Edda Elísabet Magnúsdóttir:** Project administration, Supervision, Writing – review & editing, Funding acquisition.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: There are no additional relationships or activities that may have been of conflicting interest in the creation of this research paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marpolbul.2023.115758>.

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