Presence of organochlorine pollutants in fat and scats of pinnipeds from the Antarctic Peninsula and South Shetland Islands, and their relationship to trophic position

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GRAPHICAL ABSTRACT
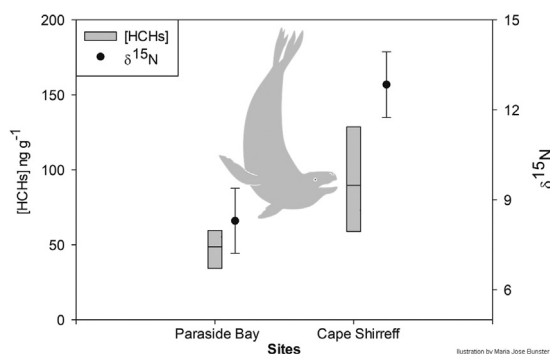
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ABSTRACT

Antarctica is still considered one of the few pristine areas in the globe. Despite this, several studies have shown phased out organic pollutants are present in several environmental abiotic and biological compartments. This study, based on blubber and fecal samples collected from five species of Antarctic pinnipeds, assessed the relationship between organochlorine pesticide (OCs) levels and trophic characterization using stable isotope analysis (δ^{13}C and δ^{15}N). The prevailing pollutants found in blubber were hexachlorocyclohexane isomers (HCHs), hexachlorobenzene (HCB), Heptachlor and Aldrin (0.84–564.11 ng g^{-1} l.w.). We also report a high presence of HCHs, Endrin, Dichlorodiphenyltrichloroethane (DDTs) and Methoxychlor (4.50–363.86 ng g^{-1} d.w.) in feces suggesting a detoxification mechanism. All the species tend towards high trophic positions (3.4–4.9), but with considerable variation in trophic niche and organochlorine pesticide concentrations per sampling site. This finding suggests that differences in pesticide levels in individuals are associated to foraging ecology.

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1. Introduction

Organochlorine pesticides (OCs) are harmful for wildlife due to their persistence, bioaccumulation capacity, toxicity, and worldwide distribution (Barra et al., 2005). For instance, OCs have been included in the Stockholm Convention on Persistent Organic Pollutants, a global treaty to protect human health and the environment from chemicals that remain intact in the environment for long periods and become widely distributed geographically as a result of their volatility, and susceptibility to Long Range Atmospheric Transport. Due to these characteristics these compounds can reach polar areas far from their sources (SCAR, 2010; Stockholm Convention, 2008).

Organochlorine pesticides have been reported in several Antarctic environmental matrices (Borghini et al., 2005; Negri et al., 2006; Geisz et al., 2008; Klánová et al., 2008; Galbán-Malagón et al., 2013; Cabrerizo et al., 2013), including their bioconcentration, bioaccumulation, and biomagnification in terrestrial and marine food webs (Borghesi et al., 2008; Bargagli, 2005; Galbán-Malagón et al., 2013 and 2018). In consequence, there is enough evidence to show an increase in chemical concentration in organisms of increasing trophic levels (Kelly et al., 2007; Goerke et al., 2004). It has been reported that marine mammals such as pinnipeds, commonly known as seals, accumulate high levels of OCs (Goerke et al., 2004; Hoekstra et al., 2003), but little is known on the differences in pollutant accumulation, biomagnification, and the possible role of different diets in these processes in polar environments.

In Antarctica there are six species of seals with very different food sources depending on the species ecology; their diets may even change depending on the prey availability in the feeding area (Lowther, 2018). The only member of the Otariidae family, the Antarctic fur seal (AFS; *Arctocephalus gazella*) has a diet that varies seasonally and spatially. It feeds on adult krill, fish, and occasionally penguins (Jefferson et al., 2007). A female specimen ranges in size between 1 and 1.7 m, while a male can reach 2.5 m in length, which could lead to dietary differences (Boyd, 2009). The other five pinniped species are of the Phocidae family, with a very wide range of ecological and trophic behavior. The Crabeater seal (CS; *Lobodon carcinophagus*) has a diet mainly based on Antarctic krill, *Euphausia* spp. with a very wide home range; on the other hand, the Weddell seal (WS; *Leptonychotes weddellii*) is less mobile, with isolated populations around Antarctic coasts, and preys on local fish and occasionally squids and other invertebrates. At the same time, the Leopard seal (LS; *Hydrurga leptonyxs*) has shown the greatest prey diversity, including krill, fish, squid, and penguins, a variety of seabirds, and juvenile specimens of other pinniped species, depending on the foraging area. The largest pinniped in the Antarctica, the Southern elephant seal (SES; *Mirounga leonina*), has a high metabolic capacity for diving, allowing it to feed on fish and deep-sea squid (Hindell and Perrin, 2008); and the Ross seal (*Ommatophoca rossii*) has been poorly described due to the difficulty in finding it. Due to these wide ranges in ecology and trophic behavior, pinnipeds are likely to be good biomonitors of chemical marine pollution in Polar Regions (Croxall 1992; Hodgson et al. 1998; Becker, 2000), as they are high in the trophic chain and present extended life spans. Most of these mammals have high lipid reserves which act as a reservoir for hydrophobic organic pollutants (Mössner and Ballschmiter, 1997). Despite this fact, there is still scarce knowledge regarding OCs accumulation processes in Antarctic biota (Bengtson-Nash et al., 2010).

The effect of OCs in Antarctic pinnipeds is uncertain. However, we could refer to effects in pinnipeds from other coastal zones. Evidence relating dichlorodiphenyltrichloroethane (DDT) concentrations (100–800 pg g^{-1}) with shorter gestation times in Californian sea lion (*Zalopus californianus*) implies that we could expect other effects on the reproductive systems of these species (Addison, 1989). Due to OCs long-distance transportation ability, toxic potential, and bioaccumulation in marine species and the importance of these organisms in the Antarctic ecosystem, an increase of OCs in Antarctica (Bengtson-Nash et al., 2010; Cabrerizo et al., 2013; Kang et al., 2012; Zhang et al.,

2015) contribute to the vulnerability of Antarctic pinnipeds health (Kovacs et al., 2011; Forcada and Hoffman, 2014).

The objectives of the present work were to report the occurrence of a wide variety of OCs (Hexachlorocyclohexane isomers -HCHs-, Hexachlorobenzene -HCB-, Heptachlor, Heptachlor, Aldrin, Endrin, Endosulfan, Dichlorodiphenyltrichloroethane isomers -DDT-, and Methoxychlor) in biological samples of Southern elephant seals, Antarctic fur seals, Leopard seals, Weddell seals, and Crabeater seals from two locations in the Antarctic Peninsula; and to study the relationship between their trophic positions (TP) and foraging habitats through the use of stable nitrogen and carbon isotopes.

2. Methods

2.1. Study area

Samples were collected during the 2013–2014 austral Summer in two geographic study areas in the Antarctic Peninsula: Cape Shirreff Field Station (CSFS; Antarctic Specially Protected Area No. 149) located on Livingston Island in the South Shetland Islands archipelago (62°27′0″ S 60°47′0″; Fig. 1); and Presidente Gabriel González Videla Station (GGVS; 64°49′S 62°51′O; Fig. 1) located on the coast of the Strait of Gerlache, connected with the Antarctic Peninsula by a small isthmus, called Danco's Coast.

2.2. Sampling

Biopsies and fecal matter samples were taken from adult individuals and sexed by direct observation when possible. Blubber biopsies of subcutaneous samples from Southern elephant seals, Leopard seals, and Crabeater seals were collected using specialized darts thrown from ≥25 m distance with a crossbow (Fossi et al., 2010); in the case of Antarctic fur seals and Weddell seals, samples were collected with a single stick with a surgical punch, because of their smaller size. All methods are considered nonlethal and are approved by the National Oceanic and Atmospheric Administration (NOAA), the Endangered Animal Protection Law (Sec. 407. MMPA. NOAA, 2007), and the Chilean Antarctic Institute (INACH). Biopsies were stored in cryovials in liquid nitrogen and kept at −80 °C until analysis. Approximately 30 g of feces were collected fresh from the animal's resting platforms and stored in aluminum bags, previously rinsed 3× with acetone, and preserved at −20 °C until analysis. In CSFS, blubber samples were collected from Southern elephant seals, Crabeater seals, Weddell seals, Antarctic fur seals, and Leopard seals, along with fecal samples from Southern elephant seals, Antarctic fur seals, and Leopard seals. In GGVS, biopsies were taken from Crabeater seals, Weddell seals, and Leopard seals. Details on the number of individuals sampled and their sex is given in Table S1 in the Supporting information.

2.3. Organochlorine pesticide chemical extraction and analysis

From both blubber and feces samples, 19 OCs were analyzed: Hexachlorocyclohexane (HCHs; isomers α, β, γ, δ-HCH), Hexachlorobenzene (HCB), Drins (Aldrin, Dieldrin, Endrin, Endrin Aldehyde), Chlordanes (Heptachlor and Heptachlor Epoxide), Endosulfan (α, β, Endosulfan Sulfate or Endos), Methoxychlor, and Dichlorodiphenyltrichloroethane (DDT; isomers 4,4′- DDE, 4,4′- DDD, o,p′- DDT, and p,p′- DDT).

Organochlorine pesticide extractions were performed at the Environmental Chemistry Laboratory of the EULA research center (University of Concepcion, Chile); samples of 0.1–0.15 g of fat (dried with 25 g of sodium sulfate anhydrous granulate for trace analysis EMSURE® Merck) and 0.2 g of fecal matter (previously freeze-dried) were extracted by Soxhlet in 150 mL of n-hexane (for gas chromatography MS SupraSolv® Merck) at 60–70 °C for 24 h. Once the extraction was finished and the solvent evaporated, the lipid percentage was calculated by gravimetry. The sample was reconstituted with 10 mL of n-hexane.
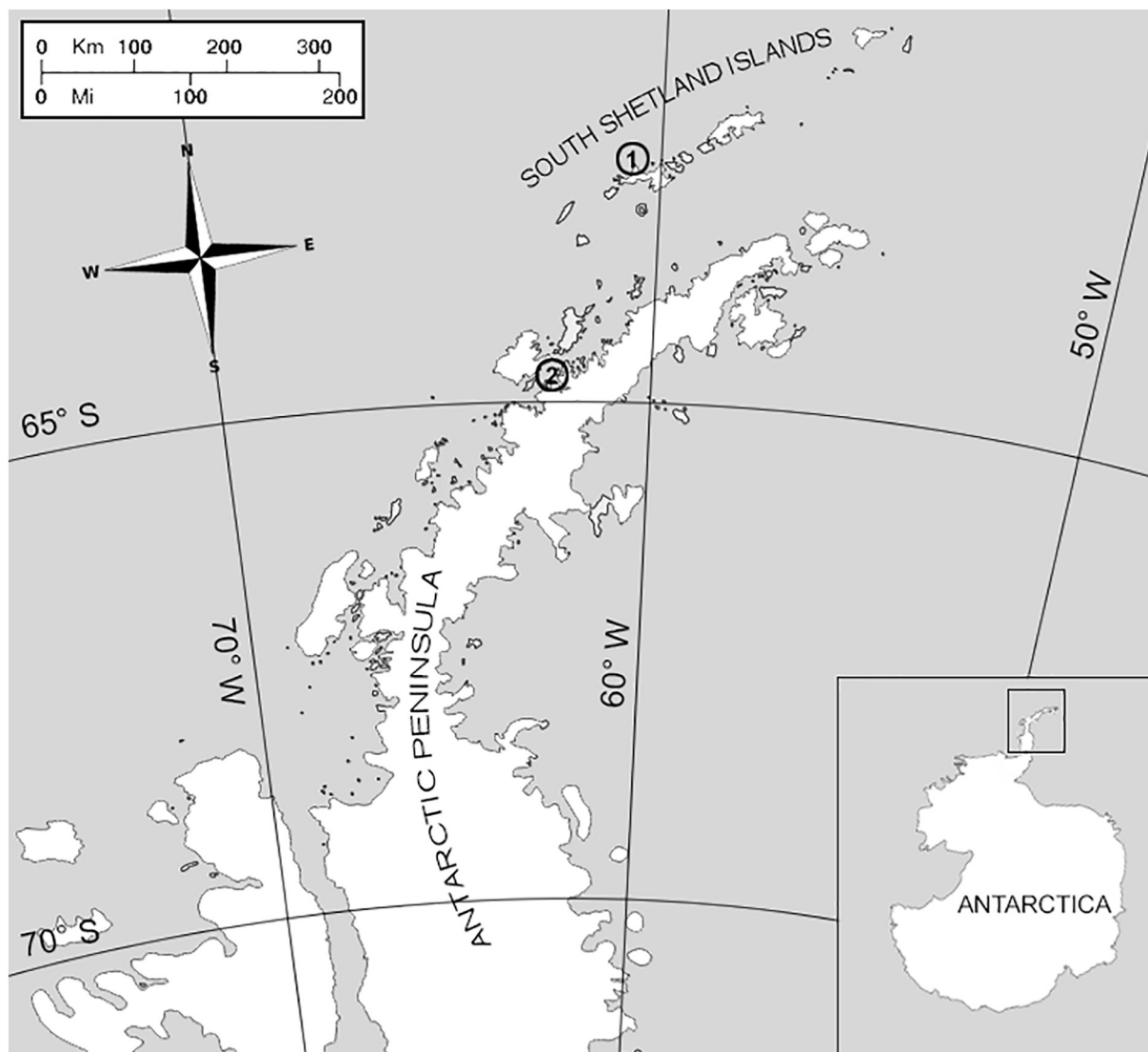
Fig. 1. Sampling sites (1) CSFS and (2) GGVS.

The solution was concentrated using a rotary evaporator at 40 °C to get a final volume of 10 mL for feces extraction and 1.5 mL for blubber extraction. The clean-up procedure for blubber extraction consisted in a mix of n-hexane/sulfuric acid 70% reagent (20 mL; 1:1) to degrade the lipids, so the organic phase could be diluted, concentrated, and passed through columns with separate layers of differently treated silica gel (sodium sulfate, neutral silica gel, and basic silica gel). For the feces extractions, the clean-up method was used with a different column composition (Merck sodium sulfate, basic silica, Merck neutral silica, and acid silica). The resulting solution was concentrated to 1.5 mL and transferred into amber vials to be concentrated using a N_2 gentle stream. The final solution was filled with 15 μL of Pentachloronitrobenzene internal standard (PNCB 0.0075 μg L^{-1}) and 1485 μL of n-hexane. The quantification and identification of pesticides was performed using a gas chromatograph (Agilent 7890A Concepción, Chile) coupled to an electron capture detector (^{63}Ni). The carrier gas used was nitrogen in splitless mode with 2 mL min^{-1} flow (1. 29 psi) with temperature conditions for the injector and the detector (ECD) of 240 °C and 300 °C, respectively.

2.4. Quality assurance and quality control

Each set was analyzed with two blank samples (n-hexane) and a control (spiked sample) to test the method used to analyze pesticides looking for any interferent, contamination, or loses during the extraction and clean-up. The control was prepared with 37.5 μL of pesticide standard for 19 organochlorine compounds (HCHs, isomers α, β, γ, δ-HCH, HCB, Aldrin, Dieldrin, Endrin, Endrin Aldehyde, Heptachlor, Heptachlor Epoxide, Endosulfan isomers α and β, Endosulfan Sulfate, Methoxychlor, and DDTs, 4,4'- DDE, 4,4'- DDD, o,p'- DDT, and p,p'- DDT; 20 μg L^{-1}) to calculate recoveries after the analysis method process and cleanup. In the OCs analysis, recoveries for extractions and cleanup used on blubber samples were between 84.6 and 106.9%. Meanwhile, for the methods used on fecal samples, the recoveries were between 79.7 and 110.8%. These results are considered acceptable; therefore, the results obtained in this study are representative. Limits of detection (LOD) and limits of quantification (LOQ) were calculated for each compound to be measured (Table S2, Supporting information), using the following algorithm stipulated by the International Union of Pure and Applied Chemistry (IUPAC, 1995):

$$LOD = Y_{bl} + 3 * S_{bl}/m \qquad (1)$$

$$LOQ = Y_{bl} + 10 * S_{bl}/m \qquad (2)$$

where Y_{bl} is the average of the position coefficients obtained for the preparation of 4 curves, S_{bl} represents the average of the standard

deviation obtained for the preparation of 4 curves, and m is average of the slope of the curves used.

2.5. Stable isotopes analysis

Stable isotopes studies to establish trophic position and trophic breadth from blubber samples were analyzed at the Stable Isotopes in Nature Laboratory (SINLAB) from the Canadian Rivers Institute, University of New Brunswick, Fredericton, New Brunswick, Canada. Samples of 200 μg were analyzed to establish trophic position and possible foraging ground by δ^{15}N and δ^{13}C through a continuous flow mass spectrometer (Matt Finnigan Delta Plus, Thermofinnigan, Bremen, Germany) with a Thermoquest elements analyzer (Carlo Erba NC2500, Italy). The isotopic ratio was calculated following Post (2002) algorithms, as the ratio C: N (C: N = %C/%N) that was used to determine δ^{13}C corrected by lipid weight (l.w.), when necessary, according to the recommendations by Post (2002). The simplest model for estimating the trophic position of a secondary consumer is:

$$Trophic\ position = \lambda + \left(\delta^{15}N_{secondary\ consumer} - \delta^{15}N_{base}\right)/\Delta n \tag{3}$$

In this study Antarctic Krill (*Euphausia superba*) was considered as the base consumer ($\lambda = 2$ and $\delta^{15}N_{base} = 3.4$ in Cape Shirreff Field Station; and $\delta^{15}N_{base} = 3.8$ in Gabriel Gonzalez Videla Field Station; Dunton, 2001; Polito et al., 2013). We assumed Δn as 3.4 according to Post (2002). Trophic breadth was determined through analysis of δ^{13}C variance. With a variance higher than 1 the diet was considered a generalist diet and with a variance below 1 it was considered a specialist diet (Bearhop et al., 2004).

2.6. Statistical analysis

Non-parametric tests were used due to the lack of uniformity of the sample size by species and the abnormal data distribution. A Kruskal-Wallis test was used to determine differences between results, considering animal species, site, and sex as factors. When differences were met we applied a post hoc pairwise Wilcox test, where the concentration of pollutants was considered as the dependent variables. Correlations were determined by Spearman test. The statistical analysis was performed with statistics software R and a significant difference was considered when p-value was equal and/or below 0.05.
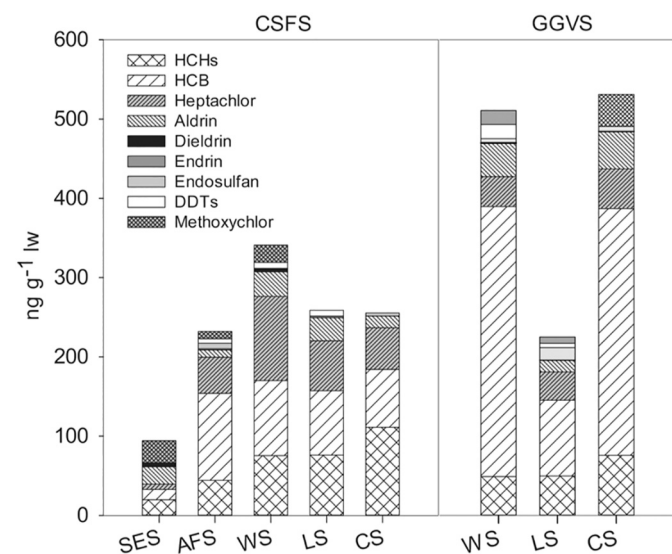
3. Results

3.1. OCs in fat tissue

The results obtained from the OCs analysis in fat tissue are summarized in Table S3 (Supporting information) and Fig. 2. The data for Cape Shirreff shows high levels of HCB, finding high concentrations of this compound in Antarctic fur seals (109.59 ± 56.24 ng g^{-1} lipid weight (l.w.)), Weddell seals (94.57 ± 8.18 ng g^{-1} l.w.), Leopard seals (81.15 ± 58.37 ng g^{-1} l.w.), and Crabeater seals (72.86 ng g^{-1} l.w.); HCH was also high, with Crabeater seals presenting the highest levels in this area (115.75 ng g^{-1} l.w.), followed by Leopard seals (76.07 ± 48.32 ng g^{-1} l.w.), and Weddell seals (75.41 ± 46.51 ng g^{-1} l.w.); Heptachlor also presented a predominant presence in Weddell seals (106.17 ± 68.79 ng g^{-1} l.w.) and Leopard seals (62.88 ± 69.75 ng g^{-1} l.w.). On the other hand, the data from Gabriel Gonzales Videla Station shows higher levels of HCB (95.45 ± 151.98 ng g^{-1} l.w. in Leopard seals, 311.39 ± 285.70 ng g^{-1} l.w. in Crabeater seals, and 340.66 ± 244.96 ng g^{-1} l.w. in Weddell seals), being the most prevailing pesticides in this area. Meanwhile, the other compounds found in high concentrations in Cape Shirref Station are less abundant in Gabriel Gonzalez Videla Station. The preliminary results concluded there was no significant difference between sexes within species ($p > 0.05$).

As can be seen from the data, Weddell seals are the only species in which we found all of the OCs analyzed. Also, Weddell seals are the species with the highest presence of pesticides in fat tissue, at both research sites. On the contrary, the Southern elephant seals are the species with the lowest levels of pollutants.

Of all the analyzed OCs, the total of HCHs isomers showed statistical differences between a few species regardless of sampling zone (Leopard seals > Southern elephant seals, $p < 0.05$; Weddell seals > Southern
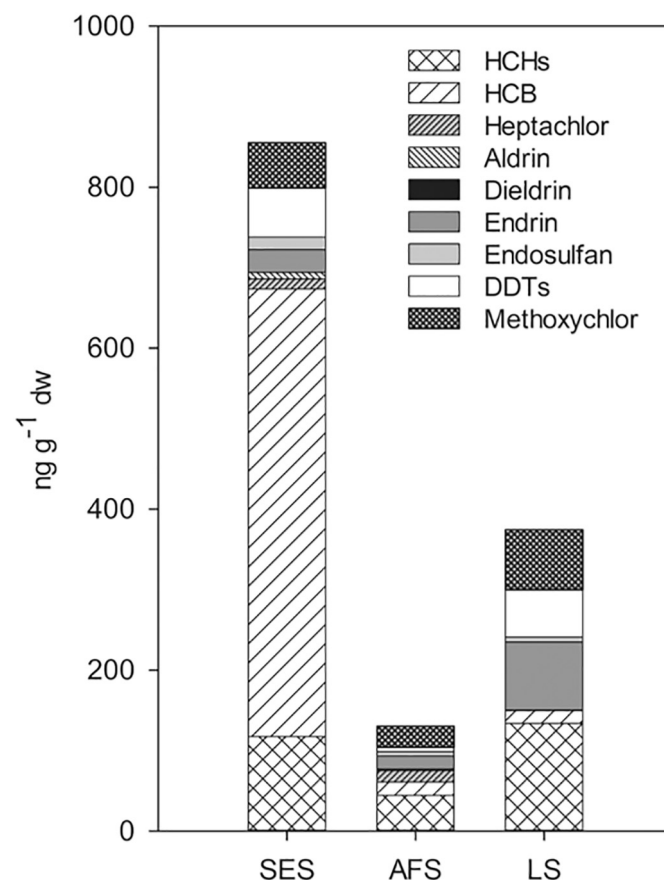

Fig. 2. OCs concentrations (ng g^{-1} lipid weight) in blubber of SES (Southern elephant seal), AFS (Antarctic fur seal), WS (Weddell seal), LS (Leopard seal), and CS (Crabeater seal) in Cape Shirreff Field Station (CSFS) and Gabriel Gonzalez Videla Station (GGVS).

Fig. 3. OCs concentrations (ng g^{-1} dry weight) in feces of SES (Southern elephant seal), AFS (Antarctic fur seal), and LS (Leopard seal) from Cape Shirreff Field Station (CSFS).

elephant seals, $p < 0.005$). When separated by zone, HCH concentration in Cape Shirreff pinnipeds was statistically higher in Weddell seals and Leopard seals than in Southern elephant seals ($p = 0.005$; $p = 0.029$, respectively). For the same species, we were not able to observe statistical differences between sampling zones.

3.2. OCs in feces

The results obtained from the analysis of pesticides in feces are set out in Table S4 (Supporting information) and Fig. 3. From this data we can observe that, differing from the bubbler samples, the Southern elephant seals present considerably high concentrations of HCHs (117.50 ± 61.50 ng g^{-1} dry weight (d.w.)) and of HCB (556.16 ng g^{-1} d.w.). Also, DDTs are more predominant in the feces from Southern elephant seals (61.28 ± 71.45 ng g^{-1} d.w.) and Leopard seals (58.81 ± 55.59 ng g^{-1} d.w.). Antarctic fur seals present considerably lower concentrations of HCB in feces (16.56 ng g^{-1} d.w.) than in blubber (109.59 ± 56.24 ng g^{-1} lipid weight (l.w.)). Comparing Leopard seals to the other two species, we observed that they present the highest concentrations of HCHs (133.72 ± 160.17 ng g^{-1} d.w.), differing from the blubber results, were this species showed the highest levels of several pesticides.

Despite the concentration differences showed between species, we were not able to evidence statistical differences between species or sites, mostly due to the very low statistical power (low sample size and high variance, Tables S3 and S4, Supporting information). The most interesting aspect of this data (Fig. 3) is that feces of the Southern elephant seals showed higher presence of pesticides than the Antarctic fur seals and Leopard seals, contrary to what occurred with these same compounds in the blubber samples.

3.3. Trophic position and trophic range

Given that we were only able to sample one Crabeater seal specimen, said species was not considered for statistical analysis, but overall, TP was higher ($p < 0.05$) near Cape Shirreff Field Station. The results obtained from the isotopic analysis revealed no significant differences (value $p = 0.614$) in trophic position of the species from Cape Shirreff (values between 4.4 and 4.9). However, as we can see, the trophic breadth suggests a difference in dietary behavior between the species, with Southern elephant seals and Leopard seals being generalists, and Weddell seals being specialist species (Fig. S1, in the Supporting information). All species analyzed in Gabriel Gonzales Videla Station presented a specialist diet, and there was a significant difference between the trophic positions of each species ($p = 0.0001$). As a clear example of adaptability, Leopard seals in Cape Shirreff showed a clearly

specialized diet, mostly consisting of Antarctic fur seal pups, despite the greater prey offer compared to Paradise Bay Gabriel Gonzalez Videla Field Station (Field observations, G. Chiang). Still, Weddell seals held the highest trophic position (4.5) compared to Crabeater seals (3.5) and Leopard seals (3.4).

The $\delta^{13}C$ variance analysis provided us with an idea of the niche similarity between species. The results showed that Weddell seals, Antarctic seals, Southern elephant seals, Leopard seals from Cape Shirreff, and Weddell seals from Gabriel Gonzales Videla Station shared a trophic niche (Fig. 4). Meanwhile, Leopard seals from Gabriel Gonzales Videla Station occupy a complete different niche (Fig. 4). There is no data for Crabeater seals near Cape Shirreff Field Station because the sample was prioritized for OCs analysis.

The next section of the survey was concerned with the connection between OCs concentration and trophic position. But no significant correlation was found between OCs concentration scores (in blubber or feces) and the trophic position scores (Table S5 and S6 in the Supporting information).

4. Discussion

4.1. OCs concentrations in blubber

As shown in the results section, the predominant pollutants found in the five species from both sites are, ranked from lowest to highest, Aldrin, Heptachlor, HCHs and HCB. All of these compounds were detected in concerning levels up to 564.11 ng g^{-1} l.w. Despite the low statistical power of the study (Tables S3 and S4), mainly due to scarce sample sizes, we did evidence that Weddell seals bioaccumulate the most OCs, as opposed to our predictions, which pointed at Leopard seals due to their foraging behavior. We will discuss this further on. Another important finding was that when comparing OCs bioaccumulation in Weddell seals, Leopard seals, and Crabeater seals from both sites, the prevailing compounds changed, with significantly higher concentrations of HCB and lower concentrations of HCHs detected in the species from Gabriel Gonzales Videla Station compared to Cape Shirreff Station. Likewise, the difference in pollutant concentration between individuals of the same species but from different sampling areas suggests geographical variations in the concentration of bioavailable OCs for these organisms. These results are consistent with those of Vetter et al. (2003) who describes geographic variations of organochlorine concentrations in the blubber of Weddell seals. On the other hand, ice and snow have been proposed as a secondary source of Persistent Organic Compounds (including OCs) in Antarctica, acting by capturing these pollutants from the atmosphere and then releasing them during ice melting and by diffusion (Kang et al., 2012). The accelerated melting and detachment of glaciers, caused by global warming and the increase in Antarctic temperatures has allowed some of these pollutants, trapped in the ice, to be released and become available for bioaccumulation in the Antarctic food chain (Galbán-Malagón et al., 2013; Geisz et al., 2008). The area around Cape Shirreff Station, located further north, is subjected to periodical changes in the ice and snow cover, "trapping" OCs for a shorter time period. The Gabriel Gonzales Videla Station area, located further south on the Antarctic Peninsula, presents a constant and predominant ice coverage (INACH, 2015), accumulating more pollutants over time, which could now be released given the increase in Antarctic temperature.

Previous researchers have studied OCs compounds in biopsies from these species and are displayed in Table 1. Karolewski et al. (1987) were the first and the last to report on OCs in Leopard seals in the summer of 1981, where they found low levels of HCHs and HCB (14–25 ng g^{-1} lw) and high DDT concentrations (430 ng g^{-1} lw), contrary to our results that evidenced concentrations of HCHs and HCB around 100 ng g^{-1} lw in Leopard seals. This discrepancy could be attributed to the lack of research in 30 years or to the increasing levels of these contaminants in Antarctica. Weddell seal analyses from samples in 2000 (Yogui, 2002),
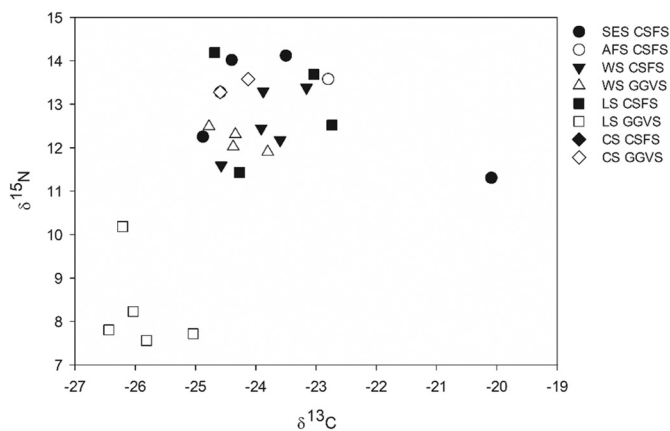

Fig. 4. Isotopic ratio $\delta^{13}C$ and $\delta^{15}N$ (%0) for SES (Southern elephant seal), AFS (Antarctic fur seal), WS (Weddell seal), LS (Leopard seal), and CS (Crabeater seal) from Cape Shirreff Field Station (CSFS); and WS, LS, and CS from Gabriel Gonzalez Videla Station (GGVS).

Table 1

Summary of OCs concentrations (ng g^{-1} lipid weight) determined in blubber from Southern elephant seal (SES), Antarctic fur seal (AFS), Weddell seal (WS), Leopard seal (LS), and Crabeater seal (CS) in Southern Shetland Islands (SSI), Cape Shirreff Field Station (CSFS), and Gabriel Gonzalez Videal Station (GGVS).

	HCHs	HCB	Chlordanes	Drins	Endosulfan	DDTs	Methoxychlor	Year	Site	Reference
LS	14.3	25				432.3		1980	SSI	Karolewski et al., 1987
	76.07 ± 48.32	81.15 ± 58.37	62.88 ± 69.75	31.18 ± 27.54	0.12 ± 0.014	7.29 ± 10.82	N/D	2013	CSFS	Present study
	49.75 ± 28.08	95.45 ± 151.98	35.65 ± 24.86	15.01 ± 15.72	4.65 ± 2.00	5.51 ± 8.98	7.97 ± 1.24	2013	GGVS	Present study
CS	64.2					106		1980	SSI	Karolewski et al., 1987
	0.223	7.23	22.8	18.4	2.09	14.4		2004	SSI	Cipro et al., 2012
	111.15	72.86	52.66	14.76	N/D	N/D	N/D	2013	CSFS	Present study
	75.70 ± 40.34	311.39 ± 285.70	50.10 ± 4.00	61.60 ± 47.16	3.72	N/D	40.13	2013	GGVS	Present study
WS		2	4			460		2000	SSI	Yogui, 2002
	2.59	5.77	9.5	18.5	14	131		2006	SSI	Cipro et al., 2012
	75.41 ± 46.51	94.57 ± 8.18	106.17 ± 68.79	34.72 ± 27.84	0.70 ± 0.66	7.60 ± 6.23	21.92 ± 18.18	2013	CSFS	Present study
	48.96 ± 21.24	340.66 ± 244.96	37.87 ± 29.11	42.13 ± 30.97	3.88 ± 0.05	17.81 ± 12.83	17.62 ± 11.09	2013	GGVS	Present study
SES	1.905	9.89	37.28	10.205	2.85	187.72	2.795	2000	SSI	Miranda-Filho et al., 2007
	1.26	12.6	36.98		1.62	126.53		2000	SSI	Miranda-Filho et al., 2009
	1.41	7.48	37.7	6.88	2.72	98.7		2006	SSI	Cipro et al., 2012
	19.88 ± 7.54	12.88	6.71 ± 1.87	26.08 ± 41.95	0.76	N/D	27.94	2013	CSFS	Present study
AFS	118.5					22.7		1981	SSI	Karolewski et al., 1987
	3.21	4.72	78.2	82.4	21.12	168		2006	SSI	Cipro et al., 2012
	44.27 ± 13.79	109.59 ± 56.24	45.60 ± 66.67	10.77 ± 5.14	7.01 ± 9.57	5.66 ± 4.09	9.11 ± 3.59	2013	CSFS	Present study

2006 (Cipro et al., 2012), and 2013 (current study) from the area of the South Shetland Islands show that HCB and Chlordanes are trending to increase. Meanwhile, DDT levels would be decreasing in this species (Table 1), as might be happening with Crabeater seals. However, this is unlikely for Antarctic fur seals, which show a trend of decreasing HCH levels over time. Findings from Southern elephant seals compared to previous researches show that HCHs and Methoxychlor are twenty times higher in our study, and we detected very low concentrations of Endosulfan and DDTs (Cipro et al., 2012; Miranda-Filho et al., 2009; Miranda-Filho et al., 2007; Table 1).

There are no specific previous records of OCs in pinnipeds in the area surrounding Gabriel Gonzales Videla Station; all the studies consider both Cape Shirreff and Gabriel Gonzales Videla Station as belonging to the Antarctic Peninsula (Corsolini, 2009). However, data from Galbán-Malagón et al. (2013) in Bransfield Strait (an area which influences Gabriel Gonzales Videla Station area) would indicate that HCHs and HCB have a greater capacity of dissolving in water, in turn being bioaccumulated by phytoplankton. This also agrees with our earlier observations, which showed high levels of HCB and HCHs in seals living near Gabriel Gonzales Videla Station.

On the other hand, data from previous studies (Kannan et al., 2000; Zaleski et al., 2014) have shown negative effects on the physiology of northern hemisphere pinnipeds with concentrations of organochlorine pollutants (e.g. DDT) starting from 440 ng g^{-1} wet weight, effects such as decreased immune system functioning (Beckmen et al., 2003; Ross et al., 1995). The levels observed in this investigation are far below those observed by Kannan et al., 2000 and Zaleski et al. 2014 and cannot be considered as potentially dangerous (Schiavone et al., 2009). However, further research should be undertaken to investigate the toxicological effect of HCHs, HCB, and Heptachlor which showed a significant increase in Antarctic seal blubber over previous years.

4.2. OCs concentration in feces

In contrast to OCs concentrations in blubber, OCs levels in feces behave differently. We no longer observe a pattern of prevalence of certain pesticides, excepting HCB that was found in concentrations over 100 ng g^{-1} d.w. up to 363.86 ng g^{-1} d.w. in Southern elephant seals, Antarctic fur seals, and Leopard seals. Also, we found HCHs, HCB and DDTs concentrations over 100 ng g^{-1} d.w. in Southern elephant seal feces, and high levels of HCHs, Heptachlor, Endrin, DDTs, and Methoxychlor in Leopards seal fecal matter (specimens with concentrations between 4.50 and 363.86 ng g^{-1} d.w.). These findings suggest that these species could be eliminating some of the pollutants accumulated in their organism through fecal excretion, as suggested by Christensen

et al. (2013), who also found high levels of OCs in grizzly bear (*Ursus arctos horribilis*) fecal matter from a remote coastal watershed in British Columbia, Canada.

4.3. Trophic position and OCs concentration

Prior ecotoxicological studies, and our observations, revealed trophic position ranges between 3.8 and 4.4 for most pinnipeds (Pauly et al., 1998). This placing the seals near the top of the trophic chain, with greater possibilities of presenting biomagnification processes.

In the Cape Shirreff Field Station area species showed no significant differences between their trophic positions, unlike OCs levels per species, which is more variable. On the contrary, in Gabriel Gonzales Videla Station, Weddell seals presented the highest trophic position (4.5) and a high pesticide level, while Crabeater seals and Leopard seals, which presented significantly different pollutant concentrations, occupied lower trophic positions (3.4–3.5). Therefore, the lack of a correlation observed between pollutant concentration and trophic position suggests that there is no relationship between these two factors for these species in the studied area. They can be considered as a functional group (Bates et al., 2017).

The isotope ratio analysis determined that individuals present at Cape Shirreff present a rather uniform carbon source compared to those inhabiting the area near Gabriel Gonzales Videla Station. This could be due to a trophic overlap, where two or more species share food sources (Horn, 1966). It is important to consider that δ^{13}C and δ^{15}N signatures provide information regarding the trophic web which does not identify the prey of each species. Despite these promising results, questions remain. A dietary composition study would be needed to confirm this supposition (García, 1999) and the possible intake routes for the observed pollutants.

5. Conclusions

This study has identified that organochlorine pesticides measured in blubber from South Shetland Islands seals, found before in low concentrations, have increased. It also revealed that these concentrations are equal or higher in other areas of the Antarctic Peninsula. Further studies are needed to assess the long-term effects of high levels of HCB, HCHs, Heptachlor, and Aldrin on the physiology of these species, given the ecological importance of these organisms in the Antarctic ecosystem.

The ability of these organisms to rid themselves of pollutants through excretions is still unknown, but the insights gained from this study may be of assistance to understand how these species metabolize

some of these compounds, such as the Southern elephant seal, which showed higher levels of OCs in feces compared to blubber.

Regarding the relationship between trophic position and pollutant concentration, we can conclude that the trophic position is not a determining factor for the higher bioaccumulation of pollutants among species of higher trophic positions such as pinnipeds, due to the fact that all the studied species belong to the same trophic position or functional group according to the trophic model of the Antarctic Peninsula ecosystem proposed by Cornejo-Donoso and Antezana (2008). Instead, we propose that pollutant levels, such as OCs, may be influenced by the source of the specific diet of each organism rather than by trophic position.

The results of our study are subject to certain limitations. For instance, the difficulty in obtaining samples and reaching a greater sample size, the lack of additional information of every individual (age, size, weight) and a better understanding of each population dietary behavior. This would allow a better understanding of the bioaccumulation-biomagnification-biodilution processes and pharmacokinetics of organochlorine pesticides in seals.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.06.122.

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