Science of the Total Environment 650 (2019) 1521–1528

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

First assessment of persistent organic pollutant contamination in blubber of Chilean blue whales from Isla de Chiloé, southern Chile

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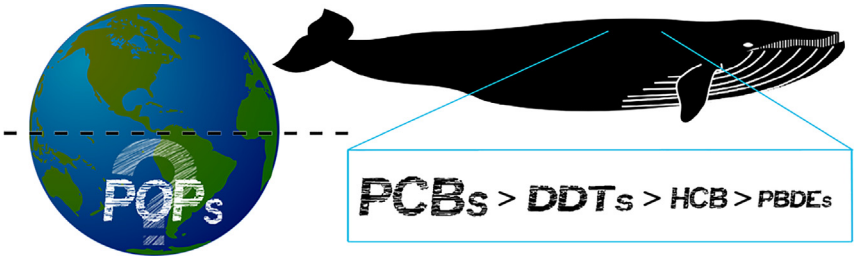
HIGHLIGHTS

- First data on POPs from Southern Hemisphere blue whales
- PCBs, most abundant among study POPs
- High contributions of lower substituted PCB and PBDE congeners
- Marked contribution of BDE-28, never reported in any cetacean species

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 6 July 2018
Received in revised form 4 September 2018
Accepted 5 September 2018
Available online 6 September 2018

Editor: D. Barcelo

Keywords:
Baleen whales
POPs
Southern Hemisphere
PCBs
PBDEs
DDTs

ABSTRACT

Persistent organic pollutants (POPs) were assessed for the first time in blue whales from the South Pacific Ocean. Concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), hexachlorobenzene (HCB) and dichlorodiphenyltrichloroethane and its main metabolites (DDTs), were determined in 40 blubber samples from 36 free-ranging individuals and one stranded, dead animal along the coast of southern Chile between 2011 and 2013. PCBs were the most abundant pollutants (2.97–975 ng/g l.w.), followed by DDTs (3.50–537 ng/g l.w.), HCB (nd–77.5 ng/g l.w.) and PBDEs (nd–33.4 ng/g l.w). There was evidence of differences between sexes, with lower loads in females potentially due to pollutants passing to calves. POP concentrations were higher in specimens sampled in 2013; yet, between-year differences were only statistically significant for HCB and PBDEs. Lower chlorinated (penta > tetra > tri) and brominated (tetra > tri) congeners were the most prevalent among PCBs and PBDEs, respectively, mostly in agreement with findings previously reported in blue and other baleen whales. The present study provides evidence of lower levels of contamination by POPs in eastern South Pacific blue whales in comparison to those reported for the Northern Hemisphere.

1. Introduction

It has been long recognized that persistent organic pollutants (POPs) are threats to public health and ecosystems (Jones and de Voogt, 1999). This lead to the enactment of the Stockholm Convention in 2001 under the United Nations Environment Programme, which seeks to ban and/or

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https://doi.org/10.1016/j.scitotenv.2018.09.070
0048-9697/

minimize POP production and use internationally (UNEP, 2001). Aside from their toxicity, these pollutants are bioaccumulative, resistant to degradation (metabolic or otherwise) and capable of long-range transportation. In consequence, a large number of studies has proven a generalized distribution of these chemicals in marine wildlife from all oceans and depths (Alonso et al., 2014; Burreau et al., 2006; Corsolini et al., 2002; Crain et al., 2009; Fisk et al., 2001; Lukyanova et al., 2014; Tanabe, 2002; Tanabe et al., 1994; Tolosa et al., 1997). The impact of POPs in marine mammal populations is, however, difficult to assess due to the logistics of collecting samples from highly mobile, ocean-dwelling animals and confounding factors from other cumulative anthropogenic impacts (Crain et al., 2009; Reijnders et al., 2009). Yet, studies to date have suggested a link between high levels of POPs such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) or organochlorine pesticides (*e.g.* hexachlorobenzene (HCB) or dichlorodiphenyltrichloroethane (DDT)), and adverse health effects on marine mammals. Specifically, a potential deleterious influence on their immune and endocrine systems, reproduction and offspring survivorship rates, and ultimately on population growth has been documented (Bossart, 2011; Desforges et al., 2016; Hall et al., 2006; Hunt et al., 2013; Yordy et al., 2010). For instance, PCBs are thought to have caused higher susceptibility and played an important role in the development of the epizootic infection by a morbillivirus involved in a massive striped dolphin (*Stenella coeruleoalba*) die-off in the Mediterranean Sea (Aguilar and Borrell, 1994; Aguilar and Raga, 1993; Kannan et al., 1993).

Among cetaceans, that is, mysticetes (baleen whales) and odontocetes (dolphins, porpoises and toothed whales), the highest POP concentrations have been described in odontocetes as consequence of being apex predators in marine food webs. A paradigmatic example is that of the Orca (*Orcinus orca*), for which levels reported in different studies make the species the most contaminated with PCBs on Earth (Jepson and Law, 2016). Concern about these heightened PCB concentrations is ongoing as a possible cause for current declines in marine apex predators globally (Jepson and Law, 2016; Stuart-Smith and Jepson, 2017). Concurrently, monitoring of POPs in cetaceans continues to increase, with most studies reporting on samples from stranded specimens rather than free-ranging animals due to the undeniable complexity and associated cost with sampling free-ranging cetaceans (García-Alvarez et al., 2014). In many cases the blubber, which represents the largest fat compartment in cetacean species, is analyzed. This tissue accounts for 70–95% of the whole body burden of lipophilic pollutants such as most POPs (Reijnders et al., 2009; Yordy et al., 2010). Moreover, bubbler biopsies can be obtained in a non-lethal and minimally invasive way from free-ranging animals by means of biopsy darts or poles (Bilgmann et al., 2007; Krützen et al., 2002), which becomes a strategy of the utmost importance for hazard assessment in endangered species of marine mammals (Fossi et al., 2014; Hunt et al., 2013; Marsili et al., 2000).

To date, most of the available data about POPs in cetaceans originate from environmental surveys or monitoring activities focused on both the Northern Hemisphere (mostly from North Atlantic and European waters), and different species of toothed whales. On the other hand, there is a paucity of studies on POPs in the Southern Hemisphere and in baleen whales in general. It is commonly assumed that mysticetes face a lower chemical risk in comparison to odontocetes derived from their lower trophic level; and thereby, from a reduced biomagnification of POPs, among other pollutants (Bengtson Nash et al., 2013). Nonetheless, baleen whales often experience prolonged fasting periods owing to reproduction, breeding and migration, when they rely on their lipid storage as capital breeders. It is during these periods when an important mobilization of their lipid resources takes place along with their previously sequestered lipophilic pollutants. In consequence, these phases of heightened fat mobilization represent times of increased chemical risk for these species, including their offspring (Bengtson Nash et al., 2013; Desforges et al., 2016; Polischuk et al., 2002).

Particularly, among mysticetes, very little is known concerning POP contamination in blue whales (*Balaenoptera musculus*) despite being the largest and one of the most emblematic extant inhabitants of the ocean. The blue whale is a cosmopolitan species whose worldwide current numbers are diminished, primarily as consequence of the massive hunt carried out during the whaling era. Hence, today it is classified as *endangered* in the Red List of Threatened Species of the International Union for Conservation of Nature and Natural Resources (IUCN, 2008). Despite their low trophic level as strict feeders on krill, blue whales may face an important health threat derived from accumulation of POPs based upon 1) their long lifespan of up to ninety years (Sears and Perrin, 2009), and 2) the sheer magnitude of bioconcentration processes as a result of massive amounts of ingested prey. To date only four studies have reported POP concentrations in blue whales, all of them from the Northern Hemisphere: Gulf of St. Lawrence, Canada (Gauthier et al., 1997; Metcalfe et al., 2004), Gulf of California, Mexico (Fossi et al., 2014) and Santa Barbara, USA (Trumble et al., 2013), finding relatively high concentrations for some pollutants such as DDTs and PCBs.

Three subspecies of blue whales are currently recognized in the Southern Hemisphere: the pygmy blue whale (*Balaenoptera musculus brevicauda*) in the sub-Antarctic zone; the Antarctic blue whale (*B. m. intermedia*), which summers in the Antarctic Zone (Rice, 1998), and an unnamed subspecies, the Chilean blue whale off Chile that is intermediate in size between the other two. This unnamed subspecies has been accepted by the Taxonomy Committee of the Society for Marine Mammalogy (SMM, 2018), considering morphometric (Branch et al., 2007a), acoustic (McDonald et al., 2006) and genetic (LeDuc et al., 2007; Torres-Florez et al., 2014) evidences.

Isla Grande de Chiloé, in southern Chile, is known as an important austral summer and autumn feeding ground for this population with among the highest sighting rates in the Southern Hemisphere (Branch et al., 2007b; Galletti Vernazzani et al., 2012). Capture-recapture open population models estimated that ~570–760 whales are feeding seasonally in this region with high inter-annual return rates. This suggests the number of whales using this feeding ground is relatively small and show a high degree of site-fidelity to Isla de Chiloé (Galletti Vernazzani et al., 2017).

The main objective of this work was to gauge for the first time -to the best of our knowledge- the degree of contamination by PCBs, HCB, DDTs and PBDEs in Chilean blue whales. This sought to inform about the health status of this recovering subspecies, pave the way for future studies of blue whales in the Southern Hemisphere, and showcase the potential for baleen whales to act as sentinel organisms of ocean contamination.

2. Material and methods

2.1. Sampling

Forty-one integument blubber samples of blue whales were obtained in March 2011 (n = 13), 2012 (n = 3) and 2013 (n = 25). Forty samples were biopsies collected from free-ranging specimens sampled in Chilean waters (41.8300–42.3364S, 73.7679–74.4747W, Fig. 1). One sample was obtained from a dead animal stranded on the coast of southern Chile (41.5584S, 73.7679W). Marine surveys were conducted within 12 km from the coastline, on board the 7 m *Alfaguara* research vessel. The primary/main survey area was off northwestern Isla de Chiloé (Fig. 1) and data collected during marine surveys included photo-identification, biopsy samples, group composition, behavior, weather and sea conditions, associated fauna and sea surface temperatures (SST). The position of a whale or group of whales was determined using GPS.

Biopsy samples were collected at a distance of approximately 20 m using biopsy darts (5 mm diameter and 4 cm long) attached to aluminum and carbon fiber arrows (Easton Superlite A/C/C), fired with a crossbow. Biopsies were obtained from the flank of the whales anterior

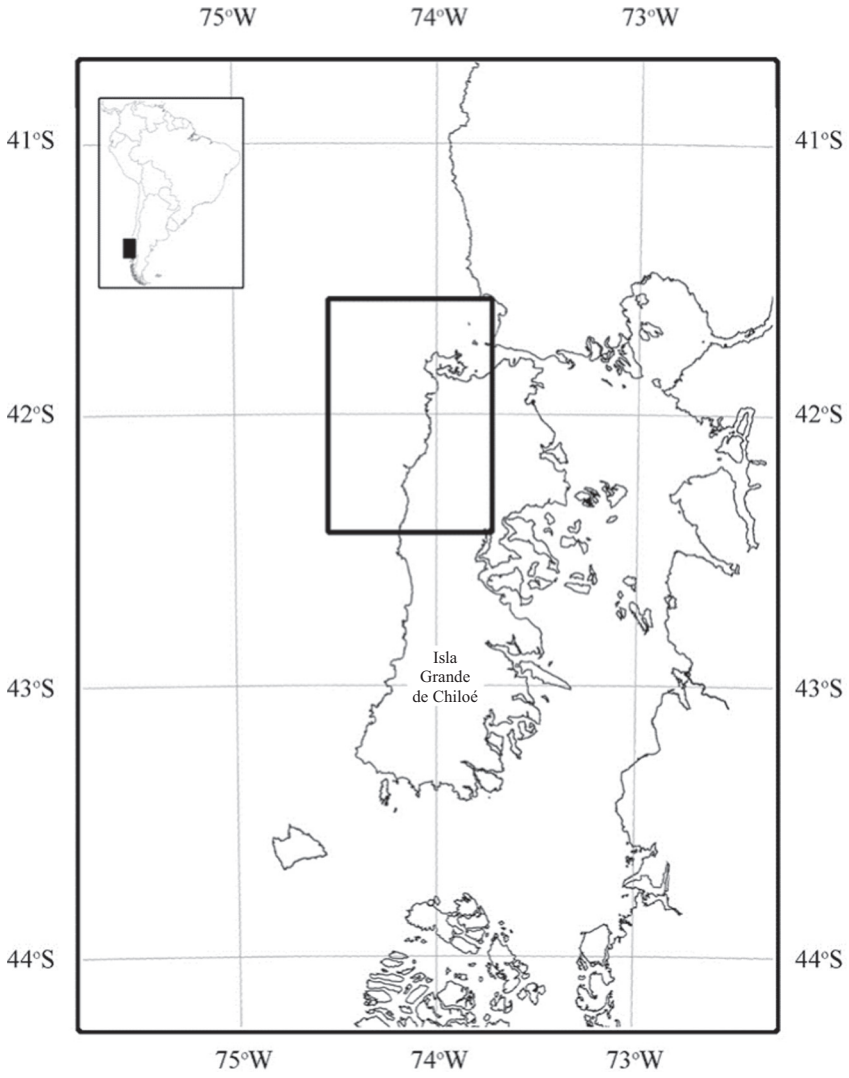

Fig. 1. Map of the sampling area.

to the dorsal fin. After collecting the floating dart, each sample was carefully extracted from the tip with clean forceps. Skin was removed from the sample and stored separately in 20% dimethyl sulfoxide saturated with NaCl or 95% ethanol for sex determination, whereas blubber was wrapped in acetone rinsed aluminum foil and placed in sterile Eppendorf tubes, then stored in a cooler with ice packs and subsequently at −20 °C in the laboratory. Ulterior photo ID and genetic analysis proved that four specimens were double-sampled: three of them twice in 2013 and the fourth in 2011 and again in 2013.

2.2. Sex determination

DNA was extracted from the skin tissue following a modified salting-out protocol (Sunnucks and Hales, 1996). The sex of each specimen was determined by PCR amplification of a fragment of the genes ZFX/ZFY and SRY using primers designed by Aasen and Medrano (1990) and Fain and LeMay (1995), respectively, and following the method of Gilson et al. (1998). The sex of four animals could not be determined due to insufficient quality or quantity of the DNA.

2.3. Analysis of POPs

2.3.1. Sample treatment

Blubber samples (~200 mg on average) were lyophilized and then extracted and purified following the procedure detailed in Muñoz-Arnanz et al. (2016) with some modifications. Briefly, lyophilized blubber samples were homogenized with 5 g of anhydrous Na_2SO_4, spiked with a suite of $^{13}C_{12}$-PCBs (2500 pg), $^{13}C_{12}$-BDE-138 (3750 pg), $^{13}C_{12}$-HCB (5000 pg) and D_8-DDTs (5000 pg of *o*,*p*′-, *p*,*p*′-DDT, and *p*,*p*′-DDE). Soxhlet extraction was carried out for 24 h using pre-cleaned cellulose thimbles and a mixture of n-hexane:dichloromethane (9:1 v/v). Lipid content of each sample was determined gravimetrically. Purification of extracts was achieved by low pressure chromatography on neutral and acidic silica gel multilayer. Final extracts were evaporated using a TurboVap® system until ~1 mL, transferred to vials and reduced to incipient dryness under a gentle nitrogen steam. Samples were reconstituted in a solution of 25 μL of $^{13}C_{12}$-PCB-111,170,178, $^{13}C_{12}$-BDE-139 and $^{13}C_{10}$-*p*,*p*′-DDT in nonane as internal standards for instrumental analysis. Information about all materials and standards used is provided in Table S1.

2.3.2. Instrumental determination

Twenty *ortho* and mono-*ortho* PCB congeners (# 28, 52, 95, 101, 105, 114, 118, 123, 132, 138, 149, 153, 156, 157, 167, 170, 180, 183, 189, 194), six DDTs (*p*,*p*′- and *o*,*p*′-DDT, -DDE and -DDD), and HCB were analyzed by gas chromatography low resolution mass spectrometry (GC-LRMS) using a 7890N gas chromatograph coupled with a 5975C quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) operated in selected ion monitoring mode (SIM) and electronic impact (EI) as ionization mode. Quantification of the target analytes was based on the isotopic

dilution technique. Fifteen brominated BDE congeners, from tri- to deca-substituted (# 17, 28, 47, 66, 85, 99, 100, 153, 154, 183, 184, 191, 196, 197, 209), were analyzed by GC-LRMS using a 6890N gas chromatograph coupled with a 5975 quadrupole mass spectrometer (Agilent, Palo Alto, CA, USA) operated in SIM with negative chemical ionization (NCI). Quantification of PBDEs was based on the internal standard ($^{13}C_{12}$-BDE-139) technique. Full details on each chromatographic and MS method can be found in Muñoz-Arnanz et al. (2016) and the Supplementary material.

2.4. QA/QC

Metal and glassware material was thoroughly cleaned (3×) with three solvents of decreasing polarity: acetone, dichloromethane and n-hexane. A procedural blank was analyzed within each batch of five samples covering each analytical step. Care was taken to minimize exposure to UV light throughout the entire analytical procedure. Quantification was carried out according to the following criteria: (a) ratio between the two monitored ions within ±15% of the theoretical value, and (b) limits of quantification (LOQs) corresponding to S/N of 10. Quantifications based on the isotopic dilution technique were inherently recovery corrected and when quantifiable levels of a given analyte were found in a procedural blank, these were subtracted from the corresponding batch of samples. Calibration curves (ten points from 1 to 1000 pg/μL for PCBs, DDTs and HCB and seven points from 1 to 250 pg/μL for PBDEs) were daily checked. Satisfactory analyses (n = 3) of the certified standard reference material SRM 1945 ("Organics in Whale Blubber", NIST) were achieved. Further information pertaining QA/QC including surrogate recoveries, reference material values and limits of detection of the target compounds is provided in Tables S2 and S3.

2.5. Data analysis

All concentrations are given in ng/g on lipid weight (l.w.) basis. Samples with concentrations below quantification limits were assigned a value of zero and regarded as not detected (ND). Statistical analyses were carried out with SigmaPlot for Windows version 12.0 (Systat Software Inc., CA, USA). Since data were not normally distributed (Shapiro–Wilk test, $p < 0.001$), they were $\log_{10}$-transformed in order to meet normality. Differences in pollutant loads between years and sexes were studied by means of *t*-tests. Pearson's correlations were used to explore relationships among the four groups of pollutants. The minimum significance level was set at $\alpha = 0.05$. Both samples from the four specimens sampled twice were analyzed. In the case of the three specimens sampled two times in March 2013, average concentration values were obtained and used in statistical analyses. In the case of the specimen sampled in 2011 and 2013, concentration values from each year were kept and regarded as different specimens.

3. Results and discussion

3.1. Pollutant concentrations

All target POPs were detected in the analyzed blubber samples. The average, median and range of concentration values are summarized in Table 1. It is noteworthy highlighting the broad range of values found, especially for PCBs and DDTs, which is likely to be attributed to differences in age, sex and reproductive status of the sampled specimens. The relative abundance of the study contaminants followed the order PCBs > DDTs > HCB > PBDEs. This pattern is similar to what has been described in liver of penguins (*Spheniscus magellanicus*) from south-central Chile (Baldassin et al., 2016); yet, the lack of studies on POPs in blue whales, or most species of cetaceans from the South Pacific Ocean for that matter, makes it challenging to analyze the measured contamination in a comparative way with closely related species. The very few data available for blue whales on the same POPs are shown in Table 2. Any comparison between data obtained in this study with those reported in the literature must be exerted with caution given the important differences in the number of sampled specimens, sampling techniques (biopsies *vs.* stranded animals), years and geographical areas. However, blue whales from Chilean waters showed markedly lower levels –of at least one order of magnitude- for all study pollutants compared to other studies. The greatest difference was in the case of DDTs, for which concentrations found in Chilean specimens were up to two orders of magnitude lower than those reported in blue whales from Canada and Mexico (Table 2). In consequence, the present study provides further evidence for a lower degree of contamination by POPs in the eastern South Pacific compared to the Canadian North Atlantic in the 90s and to the Gulf of California in recent years. This scenario is also in agreement with the reduced degree of contamination found by numerous studies in marine mammals from South Pacific waters relative to those from Northern Hemisphere regions (Aguilar et al., 2002; Alonso et al., 2014; Bengtson Nash et al., 2013; Connell et al., 1999; O'Shea and Brownell, 1994).

Interestingly, lower concentrations were described for PCBs and PBDEs in the earplug cerumen from a single specimen sampled in California waters, while higher values were found for DDTs (Table 2). The authors described how the total POP burden in blubber equaled about 90% of the total accumulative burden in the earplug of that particular specimen (Trumble et al., 2013). Nonetheless, the value of this comparison is limited and subjected to the fact of being only one animal and having no knowledge about the differential transfer and accumulation of each specific pollutant in blubber *vs.* cerumen. Additionally, data are provided in ng/g cerumen basis instead of ng/g lipid weight basis. When comparing with data from other baleen whale species from other Southern Hemisphere geographical areas caution is, again, mandatory. However, blubber data from both southern right whales (SRWs, *Eubalaena australis*) sampled in Argentinian waters (Torres et al., 2015) and humpback whales (HWs, *Megaptera novaeangliae*) from Australian waters (Bengtson Nash et al., 2013) seem to be loosely comparable in magnitude for the target compounds (Table 2). Based on the similar length of their food chains, as bioindicator organisms, the variability for each species in the relative abundance of one or another type of pollutants (PCBs > DDTs in SRWs and HCB > DDTs > PCBs in HWs) is likely a direct reflection of the contamination patterns in their different feeding grounds.

Table 1
Average, median, range, detection frequencies (% > LOQ) of total PCBs, DDTs, HCB and PBDEs detailed by males (n = 18), females (n = 16), unknown sex (n = 4) and total specimens (n = 38).

	Sex	Average (ng/g l. w.)	Median (ng/g l. w.)	Range (ng/g l. w.)	>LOQ (%)
PCBs (20 congeners)	Male	136	118	8.66–470	100
	Female	167	78.5	2.97–975	100
	Unknown	136	81	28.7–352	100
	Total	149	95.3	2.97–975	100
HCB	Male	20.4	15.2	7.84–77.5	100
	Female	10.8	11.0	ND–17.0	88
	Unknown	27.3	18.8	8.79–63.1	100
	Total	17.4	12.7	ND–77.5	95
DDTs (6 isomers)	Male	49.6	34.6	11.0–196	100
	Female	16.7	11.9	3.50–54.3	100
	Unknown	170	58.3	28.0–537	100
	Total	48.4	26.7	3.50–537	100
PBDEs (15 congeners)	Male	9.48	6.05	1.32–33.4	100
	Female	4.00	3.63	ND–14.2	81
	Unknown	12.9	11.7	1.06–26.1	75
	Total	7.69	4.95	ND–33.4	90

Table 2
Average reported concentrations in the literature for PCBs, DDTs, HCB and PBDEs in blubber samples and earplug cerumen from blue, humpback and southern right whales.

Samples	Species	PCBs (ng/g l.w.)	HCB (ng/g l.w.)	DDTs (ng/g l.w.)	PBDEs (ng/g l.w.)	Sampling area & year	Type of blubber sample	Reference
Males (n = 18)	*Balaenoptera*	136	20.4	49.6	9.48	Southern Chile	Biopsy	This study
Females (n = 16)	*musculus*	167	10.8	16.7	4.00	2011–2013	(1 stranding)	
Unknown (n = 4)		136	27.3	170	12.9			
Unknown sex (n = 3)	*Balaenoptera musculus*	2220	125	3130	–	Gulf of St. Lawrence (Canada) 1992	Stranding	Gauthier et al. (1997)
Males (n = 38)	*Balaenoptera*	2020	226	3420	–	Gulf of St. Lawrence (Canada)	Biopsy	Metcalfe et al. (2004)
Females (n = 27)	*musculus*	1220	90.0	1350	–	1992–1997		
Males (n = 3)	*Balaenoptera*	4910	–	3930	32.9	Gulf of California (Mexico)	Biopsy	Fossi et al. (2014)
Females (n = 3)	*musculus*	2550	–	902	19.7	2010		
Male (n = 1)[a]	*Balaenoptera musculus*	5.9–30	–	120–830	0.19–5.9	Santa Barbara (USA) 2007	Stranding (earplug cerumen)	Trumble et al. (2013)
Males (n = unknown)	*Megaptera novaeangliae*	18.0	91	51.0	–	Moreton Bay Marine Park Australia 2008–2011	Biopsy	Bengtson Nash et al. (2013)
Males (n = 15)[b]	*Eubalaena australis*					Península Valdés	Stranding	Torres et al. (2015)
Females (n = 18)		7.5	–	5.75	–	Argentina		
Unknown (n = 2)						2003–2011		

[a] Concentration given as a range of ng/g d.w. (cerumen basis) along ~25 cm earplug cerumen.
[b] Average concentration for the whole collection of samples in ng/g w.w.

Among the scarce known data on PBDEs in blubber from Pacific baleen whales to date are those reported in stranded juvenile HWs in Hawaiian islands, for which a median level of 7.05 ng/g l.w. and range 2.08–39.2 ng/g l.w. were found (Bachman et al., 2014). Interestingly, these values are remarkably similar to those from southern Chile blue whales, and contrast with the rest of target pollutants, found in these same HWs at greater levels. Notably, HCB median concentrations (115 ng/g l.w.) and range (167–336 ng/g l.w.) were one order of magnitude higher in HWs, which mostly reflects the heightened degree of contamination in their North Pacific feeding grounds. Nevertheless, it is plausible that differences in species, specimen's age and sampling techniques could play an important role confounding direct comparisons among all contaminant families, as it might be the case of PBDEs.

Statistically significant sex differences were found (*t*-test, $p < 0.05$) among contaminant concentrations for all groups of pollutants save for PCBs (Fig. 2A), with males exhibiting the highest burdens. Lower loads in females were expected owing to the pollutant transfer taking place from mothers to calves during pregnancy and lactation periods (Aguilar et al., 1999; Tanabe et al., 1994). It is worth noting, however, how PCB concentrations -the most prevalent group among the study pollutants- were not significantly higher in males, and for which there is no clear explanation. When contrasting 2011 against 2013 –the only two years with a large enough sample size for a valid comparison- a common pattern of greater concentration in 2013 was found for all groups of pollutants (Fig. 2B). Nonetheless, these differences were only statistically significant in the case of HCB and PBDEs. Relationships among the four groups of pollutants were explored by means of Pearson's correlations (Table 3), finding positive and statistically significant ($p < 0.05$) correlations among HCB, DDTs and PBDEs. On the contrary, PCBs were only weakly and positively correlated with DDTs ($p = 0.020$) and marginally with PBDEs ($p = 0.052$). Contamination of the marine media by POPs reflects inputs from both local sources and long-range transportation mechanisms. Thus, the different behavior found for PCBs in relation to sex and sampling years in comparison to the rest of target pollutants, together with its lower degree of correlation to all of them, might highlight the existence of specific sources and/or differential transport mechanisms for PCBs involved in the Chilean blue whales' body burdens.

3.2. Pollutant profiles

In terms of abundance profiles, the average PCB content (Fig. 3) was mostly dominated (~15%) by lower-medium chlorinated congeners, notably penta-chlorinated PCB-95 and -101. Important contributions (5–10%) were also found for congeners 153 > 52 > 28 > 149 > 138–132. This is somewhat similar to that described by

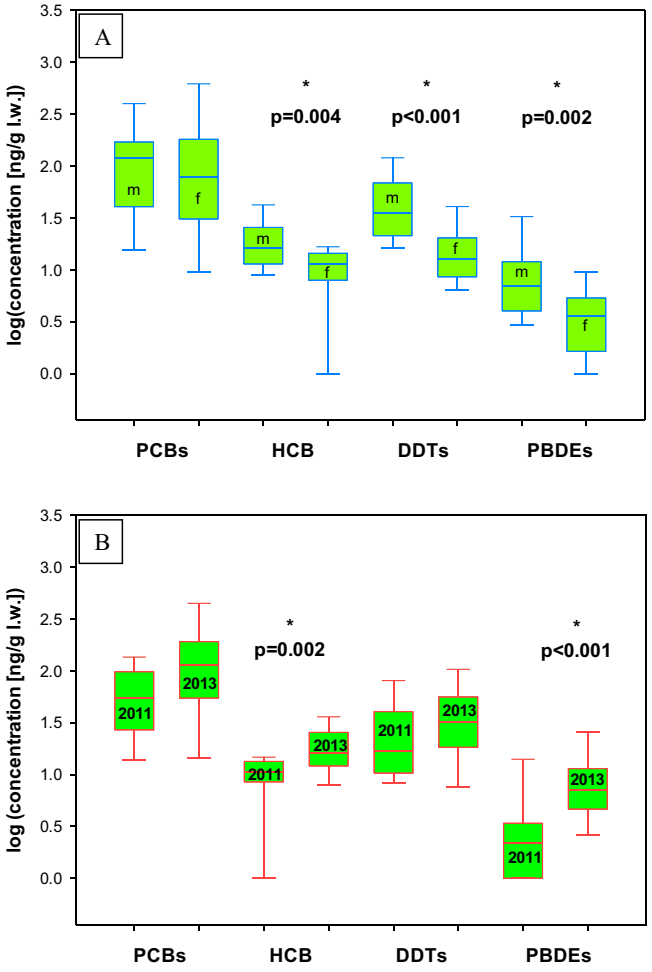

Fig. 2. Box-and-whisker plots of PCB, HCB, DDT and PBDE concentrations by A) sex [males (m) and females (f)], and B) year (2011 and 2013). Boxes are depicted as first and third quartiles with the drawn median and whiskers corresponding to 5 and 95% percentiles. Statistically significant differences between groups (*t*-test) are indicated with an asterisk and p-value.

Table 3
Pearson correlation results among target contaminants. **Correlation coefficients** and p values are shown.

	Log (HCB)	Log (DDTs)	Log (PBDEs)
Log (PCBs)	**0.282** p = 0.086	**0.376** p = 0.020	**0.318** p = 0.052
Log (HCB)		**0.649** p < 0.001	**0.692** p < 0.001
Log (DDTs)			**0.489** p = 0.002

Gauthier et al. (1997) in Canadian blue whales, at least in the predominance of PCB-101 (with PCB-95 not analyzed) over hexa- (153,138) and hepta-chlorinated (180) congeners. Furthermore, penta-chlorinated PCBs (followed by tri- and tetra-) were found dominant as well by Torres et al. (2015) in Argentinian SRWs and by Bengtson Nash et al. (2013) in Australian HWs. This is interesting since PCBs such as 153, 118 and 180 are examples of the most recalcitrant PCB congeners and, therefore, generally found as the most abundant owing to their high resistance to biodegradation (Borja et al., 2005). However, it is consistent with surface oceans being enriched in lower chlorinated PCB congeners (Jurado et al., 2004), and also consistent with the shortness of the baleen whales' food chains. This last fact could account for the absence of clear biomagnification of higher chlorinated congeners. While the PCB content of krill in the study area was not assessed, the profile with the highest contribution from penta- and tetra-chlorinated congeners is also in good agreement with that reported in mussels (*Perumytilus purpuratus*) from southern Chile (Mendoza et al., 2006). These are regarded as indicators of pollutants' bioavailability in the water media due to their filter feeding behavior and their low level of metabolic activities (Tanabe et al., 2008).

For DDTs the relative contribution to the average total content followed the pattern: *p,p′*-DDE (~72%) ≫ *p,p′*-DDD (~15%) > *p,p′*-DDT (~7%) > *o,p′*-DDT (5.5%) > *o,p′*-DDD (0.5%) > *o,p′*-DDE (not detected). The relatively high abundance of *p,p′*-DDD is in line with what has been previously observed in blue whales (Gauthier et al., 1997; Metcalfe et al., 2004) and other baleen species (Bachman et al., 2014; Bengtson Nash et al., 2013). In consonance, the average value and range for the ratio $R_{p,p'}$ (=[*p,p′*-DDE + *p,p′*-DDD]/[*p,p′*-DDT]) was 28.2 and 3.26–375 ng/g l.w., respectively. Values >1 for this ratio are commonly regarded as indication of legacy inputs of this pesticide (Muñoz-Arnanz and Jiménez, 2011). In terms of DDT levels in cetaceans from the study area, it exists only one study reporting concentrations on blubber from Bryde's whales (*Balaenoptera edeni*) and fin whales (*Balaenoptera physalus*) in Chilean waters (Pantoja et al., 1984), back in 1983. Both species are phylogenetically close to the blue whale, and the average DDT levels (∑DDTs) established in that study were 589 ng/g (fresh weight, n = 2) and 54.4 ng/g (fresh weight, n = 2), respectively. The heighten decrease of DDT levels in the area along with the prevalence of *p,p′*-DDE among DDTs suggests a likely reduction in the use and input of this pesticide in the environment accordingly to its worldwide ban that Chile implemented in 1984 (Henriquez et al., 2006).

The average PBDE congener profile was noticeably dominated by lower brominated congeners, namely, BDE-47 (~42%) followed by BDE-28 (~26%) and important contributions (>10%) from BDE-99 and -85 (Fig. 4). In general, these results are congruent with the blue whale's low trophic level, given that these congeners have been found prevalent in zooplankton from an Arctic food web (de Wit et al., 2006) and in Antarctic krill (Chiuchiolo et al., 2004; Corsolini et al., 2006). The predominance of lower brominated congeners such as BDE-47 and -99 is in agreement too with what has been reported in Pacific HWs (Bachman et al., 2014). Conversely, such a marked contribution of BDE-28 has never been reported –to the best of our knowledge- in any cetacean species. It could be the result of biodegradation from higher brominated congeners or a singular lack of this congener's metabolization and/or elimination in blue whales, which warrants further research on this issue.

4. Conclusions

By means of a non-lethal and minimally invasive approach such as blubber biopsies, this study has assessed for the first time -to the authors' knowledge- the POP burden of blue whales in the Chilean coast and elsewhere in the Southern Hemisphere. Given the few existing works reporting chemical contamination on blue whales, this study has contributed to mitigate the lack of scientific data on environmental pollution by POPs in both this species and the South Pacific Ocean as geographical area. Important differences in sampling techniques, number and age of specimens, and sampling years were acknowledged among previous investigations and this study. Nonetheless, comparisons of PCB, DDT, HCB and PBDE levels against published data on blubber from Northern Hemisphere blue whales clearly suggested a lower degree of contamination by these pollutants in the southern Chilean environment. Comparison to other South Hemisphere phylogenetically close species -baleen- showed variability in terms of the relative abundance of each pollutant, which in turn, reflected on the type and degree of contamination in geographically different feeding grounds. Thus, it seems that despite their legacy nature, PCBs are still lingering in the coast of southern Chile, being prevalent among the rest of investigated POPs. The bioaccumulation patterns of different pollutant congeners were in agreement with expectations based on the short food chain in blue whales: there were high contributions of lower chlorinated PCB congeners and lower brominated PBDE congeners. This altogether suggests that blue whales and other baleen whales could be valid and useful sentinel organisms of ocean contamination.

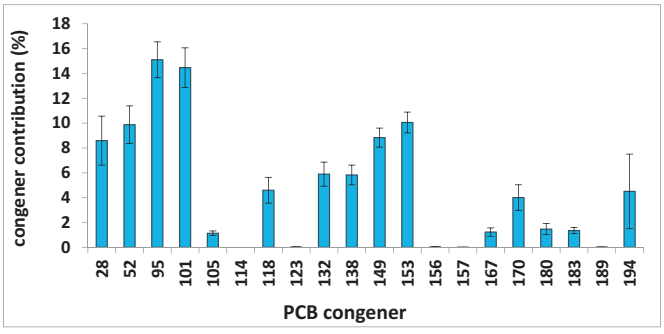

Fig. 3. Relative contribution of each PCB congener to the total PCB content. Error bars represent the standard error.

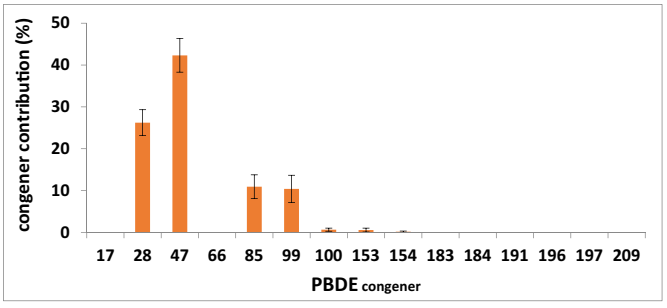

Fig. 4. Relative contribution of each PBDE congener to the total PBDE content. Error bars represent the standard error.

Acknowledgements

J. M-A acknowledges his contract under project 15CAES004. Ms. Alicia Jiménez Almarza is greatly acknowledged for her valuable help in sample analysis. Alba Vicente is thanked for the graphical abstract. We thank the Directorate General of the Maritime Territory and Merchant Marine (DIRECTEMAR) from the Chilean Navy and the Ministry of Foreign Affairs of Chile for their Official Support to the Alfaguara (Chilean blue whale) Project. We would also like to thank the valuable support of Global Greengrants Fund, Rufford Foundation, Mohamed Bin Zayed Species Conservation Fund and Pacific Whale Foundation to the Alfaguara Project. We thank Moira Brown for providing the crossbow to collect biopsies and Instituto de Conservación de Ballenas (Argentina) for financial support to MS. Special thanks to José Aviles Puen, skipper of the Alfaguara research vessel for all the marine surveys conducted to get blue whale data and samples.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.09.070.

References

Aasen, E., Medrano, J.F., 1990. Amplification of the ZFY and ZFX genes for sex identification in humans, cattle, sheep and goats. Nat. Biotechnol. 8, 1279.
Aguilar, A., Borrell, A., 1994. Abnormally high polychlorinated biphenyl levels in striped dolphins (*Stenella coeruleoalba*) affected by the 1990–1992 Mediterranean epizootic. Sci. Total Environ. 154, 237–247.
Aguilar, A., Raga, J.A., 1993. The striped dolphin epizootic in the Mediterranean Sea. Ambio 524–528.
Aguilar, A., Borrell, A., Pastor, T., 1999. Biological factors affecting variability of persistent pollutant levels in cetaceans. J. Cetacean Res. Manag. 1, 83–116.
Aguilar, A., Borrell, A., Reijnders, P.J.H., 2002. Geographical and temporal variation in levels of organochlorine contaminants in marine mammals. Mar. Environ. Res. 53, 425–452.
Alonso, M.B., Azevedo, A., Torres, J.P.M., Dorneles, P.R., Eljarrat, E., Barceló, D., et al., 2014. Anthropogenic (PBDE) and naturally-produced (MeO-PBDE) brominated compounds in cetaceans — a review. Sci. Total Environ. 481, 619–634.
Bachman, M.J., Keller, J.M., West, K.L., Jensen, B.A., 2014. Persistent organic pollutant concentrations in blubber of 16 species of cetaceans stranded in the Pacific Islands from 1997 through 2011. Sci. Total Environ. 488–489, 115–123.
Baldassin, P., Taniguchi, S., Gallo, H., Maranho, A., Kolesnikovas, C., Amorim, D.B., et al., 2016. Persistent organic pollutants in juvenile Magellanic penguins (*Spheniscus magellanicus*) in South America. Chemosphere 149, 391–399.
Bengtson Nash, S.M., Waugh, C.A., Schlabach, M., 2013. Metabolic concentration of lipid soluble organochlorine burdens in the blubber of southern hemisphere humpback whales through migration and fasting. Environ. Sci. Technol. 47, 9404–9413.
Bilgmann, K., Griffiths, O.J., Allen, S.J., Möller, L.M., 2007. A biopsy pole system for bow-riding dolphins: sampling success, behavioral responses, and test for sampling bias. Mar. Mamm. Sci. 23, 218–225.
Borja, J., Taleon, D.M., Auresenia, J., Gallardo, S., 2005. Polychlorinated biphenyls and their biodegradation. Process Biochem. 40, 1999–2013.
Bossart, G.D., 2011. Marine mammals as sentinel species for oceans and human health. Vet. Pathol. Online 48, 676–690.
Branch, T.A., Abubaker, E.M.N., Mkango, S., Butterworth, D.S., 2007a. Separating southern blue whale subspecies based on length frequencies of sexually mature females. Mar. Mamm. Sci. 23, 803–833.
Branch, T.A., Stafford, K.M., Palacios, D.M., Allison, C., Bannister, J.L., Burton, C.L.K., Cabrera, E., Carlson, C.A., Galletti Vernazzani, B., Gill, P.C., Hucke-Gaete, R., Jenner, K.C.S., Jenner, M.N.M., Matsuoka, K., Mikhalev, Y.A., Miyashita, T., Morrice, M.G., Nishiwaki, S., Sturrock, V.J., Tormosov, D., Anderson, R.C., Baker, A.N., Best, P.B., Borsa, P., Brownell Jr, R.L., Childerhouse, S., Findlay, K.P., Gerrodette, T., Ilangakoon, A.D., Joergensen, M., Kahn, B., Ljungblad, D.K., Maughan, B., Mccauley, R.D., Mckay, S., Norris, T.F., Rankin, S., Samaran, F., Thiele, D., Van Waerebeek, K., Warneke, R.M., 2007b. Past and present distribution, densities and movements of blue whales *Balaenoptera musculus* in the Southern Hemisphere and northern Indian Ocean. Mammal Rev. 37 (2), 116–175.
Burreau, S., Zebühr, Y., Broman, D., Ishaq, R., 2006. Biomagnification of PBDEs and PCBs in food webs from the Baltic Sea and the northern Atlantic Ocean. Sci. Total Environ. 366, 659–672.
Chiuchiolo, A.L., Dickhut, R.M., Cochran, M.A., Ducklow, H.W., 2004. Persistent organic pollutants at the base of the Antarctic marine food web. Environ. Sci. Technol. 38, 3551–3557.
Connell, D.W., Miller, G.J., Mortimer, M.R., Shaw, G.R., Anderson, S.M., 1999. Persistent lipophilic contaminants and other chemical residues in the Southern Hemisphere. Crit. Rev. Environ. Sci. Technol. 29, 47–82.
Corsolini, S., Romeo, T., Ademollo, N., Greco, S., Focardi, S., 2002. POPs in key species of marine Antarctic ecosystem. Microchem. J. 73, 187–193.
Corsolini, S., Covaci, A., Ademollo, N., Focardi, S., Schepens, P., 2006. Occurrence of organochlorine pesticides (OCPs) and their enantiomeric signatures, and concentrations of polybrominated diphenyl ethers (PBDEs) in the Adélie penguin food web, Antarctica. Environ. Pollut. 140, 371–382.
Crain, C.M., Halpern, B.S., Beck, M.W., Kappel, C.V., 2009. Understanding and managing human threats to the coastal marine environment. Ann. N. Y. Acad. Sci. 1162, 39–62.
de Wit, C.A., Alaee, M., Muir, D.C.G., 2006. Levels and trends of brominated flame retardants in the Arctic. Chemosphere 64, 209–233.
Desforges, J.-P.W., Sonne, C., Levin, M., Siebert, U., De Guise, S., Dietz, R., 2016. Immunotoxic effects of environmental pollutants in marine mammals. Environ. Int. 86, 126–139.
Fain, S., LeMay, J., 1995. Gender identification of humans and mammalian wildlife species from PCR amplified sex linked genes. Proceedings of the American Academy of Forensic Science 1, p. 34.
Fisk, A.T., Hobson, K.A., Norstrom, R.J., 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the Northwater Polynya marine food web. Environ. Sci. Technol. 35, 732–738.
Fossi, M.C., Panti, C., Marsili, L., Maltese, S., Coppola, D., Jimenez, B., et al., 2014. Could feeding habit and migratory behaviour be the causes of different toxicological hazard to cetaceans of Gulf of California (Mexico)? Environ. Sci. Pollut. Res. 21, 13353–13366.
Galletti Vernazzani, B., Carlson, C.A., Cabrera, E., Brownell Jr., R.L., 2012. Chilean blue whales off Isla Grande de Chiloe, 2004–2010: distribution, site-fidelity and behaviour. J. Cetacean Res. Manag. 12, 353–360.
Galletti Vernazzani, B., Jackson, J.A., Cabrera, E., Carlson, C.A., Brownell Jr., R.L., 2017. Estimates of abundance and trend of Chilean blue whales off Isla de Chiloé, Chile. PLoS ONE 12 (1), e0168646. https://doi.org/10.1371/journal.pone.0168646.
García-Alvarez, N., Martín, V., Fernández, A., Almunia, J., Xuriach, A., Arbelo, M., et al., 2014. Levels and profiles of POPs (organochlorine pesticides, PCBs, and PAHs) in free-ranging common bottlenose dolphins of the Canary Islands, Spain. Sci. Total Environ. 493, 22–31.
Gauthier, J.M., Metcalfe, C.D., Sears, R., 1997. Validation of the blubber biopsy technique for monitoring of organochlorine contaminants in balaenopterid whales. Mar. Environ. Res. 43, 157–179.
Gilson, A., Syvanen, M., Levine, K., Banks, J., 1998. Deer gender determination by polymerase chain reaction: validation study and application to tissues, bloodstains, and hair forensic samples from California. Calif. Fish Game 84, 159–169.
Hall, A.J., McConnell, B.J., Rowles, T.K., Aguilar, A., Borrell, A., Schwacke, L., et al., 2006. Individual-based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. Environ. Health Perspect. 114, 60.
Henriquez, M., Becerra, J., Barra, R., Rojas, J., 2006. Hydrocarbons and organochlorine pesticides in soils of the urban ecosystem of Chillan and Chillan Viejo, Chile. J. Chil. Chem. Soc. 51, 938–944.
Hunt, K.E., Moore, M.J., Rolland, R.M., Kellar, N.M., Hall, A.J., Kershaw, J., et al., 2013. Overcoming the challenges of studying conservation physiology in large whales: a review of available methods. Conserv. Physiol. 1.
IUCN, 2008. The IUCN Red List of Threaten Species. *Balaenoptera musculus*. http://www.iucnredlist.org/details/2477/0 (last accessed, May 2018).
Jepson, P.D., Law, R.J., 2016. Persistent pollutants, persistent threats. Science 352, 1388–1389.
Jones, K.C., de Voogt, P., 1999. Persistent organic pollutants (POPs): state of the science. Environ. Pollut. 100, 209–221.
Jurado, E., Lohmann, R., Meijer, S., Jones, K.C., Dachs, J., 2004. Latitudinal and seasonal capacity of the surface oceans as a reservoir of polychlorinated biphenyls. Environ. Pollut. 128, 149–162.
Kannan, K., Tanabe, S., Borrell, A., Aguilar, A., Focardi, S., Tatsukawa, R., 1993. Isomer-specific analysis and toxic evaluation of polychlorinated biphenyls in striped dolphins affected by an epizootic in the western Mediterranean Sea. Arch. Environ. Contam. Toxicol. 25, 227–233.
Krützen, M., Barré, L.M., Möller, L.M., Heithaus, M.R., Simms, C., Sherwin, W.B., 2002. A biopsy system for small cetaceans: darting success and wound healing in *Tursiops* spp. Mar. Mamm. Sci. 18, 863–878.
LeDuc, R.G., Dizon, A.E., Goto, M., Pastene, L.A., Kato, H., Nishiwaki, S., et al., 2007. Patterns of genetic variation in Southern Hemisphere blue whales and the use of assignment tests to detect mixing on the feeding grounds. J. Cetacean Res. Manag. 9, 73–80.
Lukyanova, O., Tsygankov, V.Y., Boyarova, M., Khristoforova, N., 2014. Pesticide biotransport by Pacific salmon in the northwestern Pacific Ocean. Dokl. Biol. Sci. 456, 188–190 (Springer).
Marsili, L., Fossi, M.C., Neri, G., Casini, S., Gardi, C., Palmeri, S., et al., 2000. Skin biopsies for cell cultures from Mediterranean free-ranging cetaceans. Mar. Environ. Res. 50, 523–526.
McDonald, M.A., Mesnick, S.L., Hildebrand, J.A., 2006. Biogeographic characterisation of blue whale song worldwide: using song to identify populations. J. Cetacean Res. Manag. 8 (1), 55–66.
Mendoza, G., Gutierrez, L., Pozo-Gallardo, K., Fuentes-Rios, D., Montory, M., Urrutia, R., et al., 2006. Polychlorinated biphenyls (PCBs) in mussels along the Chilean coast. Environ. Sci. Pollut. Res. 13, 67–74.
Metcalfe, C., Koenig, B., Metcalfe, T., Paterson, G., Sears, R., 2004. Intra- and inter-species differences in persistent organic contaminants in the blubber of blue whales and humpback whales from the Gulf of St. Lawrence, Canada. Mar. Environ. Res. 57, 245–260.
Muñoz-Arnanz, J., Jiménez, B., 2011. New DDT inputs after 30 years of prohibition in Spain. A case study in agricultural soils from south-western Spain. Environ. Pollut. 159, 3640–3646.

Muñoz-Arnanz, J., Roscales, J.L., Ros, M., Vicente, A., Jiménez, B., 2016. Towards the implementation of the Stockholm Convention in Spain: five-year monitoring (2008–2013) of POPs in air based on passive sampling. Environ. Pollut. 217, 107–113.

O'Shea, T.J., Brownell Jr., R.L., 1994. Organochlorine and metal contaminants in baleen whales: a review and evaluation of conservation implications. Sci. Total Environ. 154, 179–200.

Pantoja, S., Pastene, L., Becerra, J., Silva, M., Gallardo, V.A., 1984. DDTs in balaenopterids (Cetacea) from the Chilean coast. Mar. Pollut. Bull. 15, 451.

Polischuk, S., Norstrom, R., Ramsay, M., 2002. Body burdens and tissue concentrations of organochlorines in polar bears (*Ursus maritimus*) vary during seasonal fasts. Environ. Pollut. 118, 29–39.

Reijnders, P.J., Aguilar, A., Borrell, A., 2009. Pollution and marine mammals. Encyclopedia of Marine Mammals, second edition Elsevier, pp. 890–898.

Rice, D.W., 1998. Marine mammals of the world: systematics and distribution. Mar. Mamm. Sci. (Spec. Publ.) 4, 67–78.

Sears, R., Perrin, W.F., 2009. Blue whale: *Balaenoptera musculus*. Encyclopedia of Marine Mammals, second edition Academic Press, London, pp. 120–124.

SMM. Society for Marine Mammalogy. Committee on Taxonomy, 2018. List of marine mammal species and subspecies. www.marinemammalscience.org (last accessed, June 18).

Stuart-Smith, S.J., Jepson, P.D., 2017. Persistent threats need persistent counteraction: responding to PCB pollution in marine mammals. Mar. Policy 84, 69–75.

Sunnucks, P., Hales, D.F., 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I–II in aphids of the genus *Sitobion* (Hemiptera: Aphididae). Mol. Biol. Evol. 13, 510–524.

Tanabe, S., 2002. Contamination and toxic effects of persistent endocrine disrupters in marine mammals and birds. Mar. Pollut. Bull. 45, 69–77.

Tanabe, S., Iwata, H., Tatsukawa, R., 1994. Global contamination by persistent organochlorines and their ecotoxicological impact on marine mammals. Sci. Total Environ. 154, 163–177.

Tanabe, S., Ramu, K., Isobe, T., Takahashi, S., 2008. Brominated flame retardants in the environment of Asia-Pacific: an overview of spatial and temporal trends. J. Environ. Monit. 10, 188–197.

Tolosa, I., Readman, J.W., Fowler, S.W., Villeneuve, J.P., Dachs, J., Bayona, J.M., et al., 1997. PCBs in the western Mediterranean. Temporal trends and mass balance assessment. Deep-Sea Res. II Top. Stud. Oceanogr. 44, 907–928.

Torres, P., Miglioranza, K.S.B., Uhart, M.M., Gonzalez, M., Commendatore, M., 2015. Organochlorine pesticides and PCBs in southern right whales (*Eubalaena australis*) breeding at Península Valdés, Argentina. Sci. Total Environ. 518-519, 605–615.

Torres-Florez, J.P., Hucke-Gaete, R., LeDuc, R., Lang, A., Taylor, B., Pimper, L.E., et al., 2014. Blue whale population structure along the eastern South Pacific Ocean: evidence of more than one population. Mol. Ecol. 23, 5998–6010.

Trumble, S.J., Robinson, E.M., Berman-Kowalewski, M., Potter, C.W., Usenko, S., 2013. Blue whale earplug reveals lifetime contaminant exposure and hormone profiles. Proc. Natl. Acad. Sci. 110, 16922–16926.

UNEP, 2001. Stockholm Convention on Persitent Organic Pollutants. United Nations Environment Programme Chemicals (UNEP).

Yordy, J.E., Pabst, D.A., McLellan, W.A., Wells, R.S., Rowles, T.K., Kucklick, J.R., 2010. Tissue-specific distribution and whole-body burden estimates of persistent organic pollutants in the bottlenose dolphin (*Tursiops truncatus*). Environ. Toxicol. Chem. 29, 1263–1273.