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Evaluation of PCDD/Fs, dioxin-like PCBs and PBDEs in sperm whales from the Mediterranean Sea

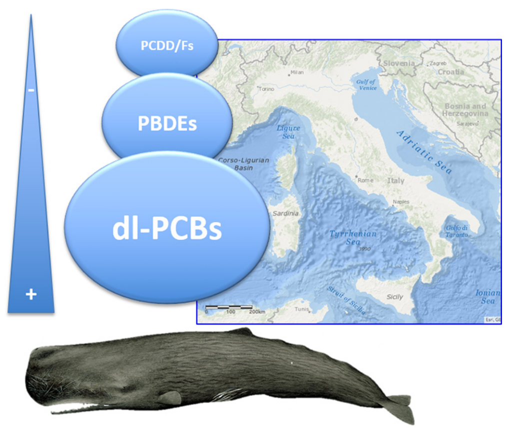
A. Bartalini [a], J. Muñoz-Arnanz [b,*], L. Marsili [a], S. Mazzariol [c], M.C. Fossi [a], B. Jiménez [b]

[a] Department of Environmental, Earth and Physical Sciences, University of Siena, Via Mattioli 4, Siena, Italy
[b] Department of Instrumental Analysis and Environmental Chemistry, Institute of Organic Chemistry, CSIC. Juan de la Cierva, 3, 28006 Madrid, Spain
[c] Department of Public Health, Comparative Pathology and Veterinary Hygiene, University of Padova, Viale dell'Università 16, 35020 Legnaro, PD, Italy

HIGHLIGHTS

- dl-PCBs ranking at the top of the reported values for this species worldwide
- Mediterranean Sea still today a global PCB "hotspot" for marine mammals
- Non-ortho-dl-PCB pattern (126 > 169 > 77)
- TEQ contribution: Σnon-*ortho*-dl-PCBs > Σ*ortho*-dl-PCBs > PCDDs > PCDFs

GRAPHICAL ABSTRACT



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ABSTRACT

Numerous studies to date have reported concentrations of Persistent Organic Pollutants (POPs) in different marine mammal species worldwide. Yet data on sperm whales are scarce from rich and unique biodiverse areas such as the Mediterranean Sea. This work aimed to assess levels of dioxin-like polychlorinated biphenyls (dl-PCBs), polybrominated diphenyl ethers (PBDEs), and polychlorodibenzo-*p*-dioxins and furans (PCDD/Fs) in blubber of sperm whales stranded along the Italian coast between 2008 and 2016. POP mean concentrations (dl-PCBs: 6410 ng/g l.w.; PBDEs: 612 ng/g l.w.; PCDD/Fs: 57.8 pg/g l.w.) were mostly in line with what has been previously reported on the same species in the Mediterranean environment and tended to be higher than those reported from other geographical regions. The relative abundance followed the order dl-PCBs > PBDEs ≫ PCDD/Fs. Interestingly, the non-*ortho* dl-PCB pattern (126 > 169 > 77) was similar to that described in other studies worldwide and different from what is described in its main prey. This could be linked to particular metabolic activities in sperm whales against these highly toxic contaminants. Total TEQs ranged from 275 to 987 pg/g l.w. and showed the pattern Σnon-*ortho*-dl-PCBs > Σ*ortho*-dl-PCBs > PCDDs > PCDFs, with PCBs' contribution about 96%. These findings highlight the high abundance of PCBs still found in the Mediterranean environment despite having been banned for decades. All sperm whales analyzed in this study surpassed the threshold of 210 pg WHO-TEQ/g l.w. proposed as starting point of

* Corresponding author.
E-mail address: juan.ma@iqog.csic.es (J. Muñoz-Arnanz).

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immunosuppression in harbour seals; a level of contamination that may have contributed to an impairment of their immune system.

1. Introduction

The Mediterranean Sea is a complex and rich ecosystem that accounts for a heightened degree of marine biodiversity (Coll et al., 2012). Concurrently, it is an area under intense anthropogenic pressures (Castro-Jiménez et al., 2013), and as such, it is considered a sink for many environmental contaminants, partially due to its geo-morphological characteristics (e.g. semi-enclosed marine basin), and the high urbanization rate of its coasts along with related activities derived from it (wastewater releases, shipping, fishing and chemical pollution) (Marsili et al., 2018). Persistent organic pollutants (POPs) rank among the most relevant environmental contaminants. Aside from their toxicity, their unique physical-chemical properties are responsible for their global distribution and persistence in the environment. Due to their lipophilic nature they can biomagnify in marine food webs reaching high levels among top predators (Aguilar et al., 2002). Even though the Stockholm Convention banned or restricted the production and use of different POPs since 2004 (UNEP, 2001), they continue to be present in the environment and biota. POP concentrations measured in the Mediterranean Sea reach this area by different pathways; among the most important of these are long-range transportation (LRT) from Asia (Lelieveld et al., 2002) and western, central and eastern Europe (Berrojalbiz et al., 2014; Iacovidou et al., 2009), and from river discharges and sedimentation (Albaigés, 2005; Gómez-Gutiérrez et al., 2006). In consequence, important POP levels are today reported for different animal species (Guerranti et al., 2014; Maisano et al., 2016; Muñoz-Arnanz et al., 2011; Pinzone et al., 2015; Roscales et al., 2016; Tekin and Pazi, 2017), which justifies unceasing research on these pollutants. Concern raises not only for emerging contaminants such as polybrominated diphenyl ethers (PBDEs), for which regulations on their use are more recent, but also for legacy chemicals such as polychlorinated biphenyls (PCBs). Concentrations of the latter in different Mediterranean dolphin species have been recently reported surpassing previously known PCB toxicity thresholds for marine mammals (Stuart-Smith and Jepson, 2017). In consequence, it appears the declining concentrations recorded after the wide ban on PCBs in the European environment in the mid-1980s, followed by the enforcement of the Stockholm Convention in 2004, had somehow halted, leaving in general still high concentration values in most European biota (Jepson et al., 2016; Jepson and Law, 2016; Law and Jepson, 2017; Stuart-Smith and Jepson, 2017).

Cetaceans at high trophic levels, long life spans and large fat deposits tend to accumulate high levels of POPs. Tissue concentrations of environmental contaminants depend on the contamination levels of the source and on many biological factors (diet, body size, body composition, nutritive condition, incidence of disease, age and sex) (Aguilar et al., 1999), and once ingested, the ability of each species to metabolize and excrete contaminants through biotransformation systems (Hakk, 2003; Tanabe et al., 1988). These systems are divided in two phases: phase I, characterized by the introduction or modification of xenobiotics' functional groups, and phase II, involving conjugation or synthetic reactions that increase compounds' solubility. Both phases aim to transform hydrophobic lipid-soluble organic xenobiotics into water-soluble excretable metabolites. In this sense, cetaceans are known to have low phase I biotransformation activity, which translates into low capability to metabolize contaminants (Boon et al., 1997; Hoydal et al., 2018; Tanabe et al., 1988).

Deleterious effects on marine mammals' health from elevated POP concentrations have been strongly suggested in numerous investigations to date, e.g. potential adverse influences on the endocrine and immune systems, and on the reproduction and offspring survivorship rates (Bossart, 2011; Desforges et al., 2016; Hall et al., 2006; Yordy et al., 2010). Thus, high POP levels have been proposed as potential factors involved in mass mortality and stranding events (Evans et al., 2004). However, even when tissue pollutant concentrations and negative health effects have been suggested in pinnipeds and cetaceans (Béland et al., 1993; Reijnders, 1994; Ross et al., 1996; Skaare et al., 2000), establishing a direct association between levels and toxic effects is quite complex owing to the large number of confounding factors involved (e.g. specimen's sex, age, diet, health status, metabolic and excretion capabilities, etc.). This points out the need of a multidisciplinary approach to reach an understanding on the cause of stranding and death (Mazzariol et al., 2016), for which ecotoxicological investigations are essential to understand the real role played by contaminants on these events.

The sperm whale (*Physeter macrocephalus*) is a marine mammal with a cosmopolitan distribution (Whitehead, 2018). This large toothed whale, feeds mainly on meso- and bathypelagic cephalopods (Wong and Whitehead, 2014). Males tend to eat larger prey than females and more likely demersal fishes, such as sharks and rays (Wong and Whitehead, 2014). Although the biggest threat to sperm whales - commercial whaling - has stopped, there are other anthropogenic activities, such as contamination, entanglement in fishing gears and noise pollution that continue to threaten this species. Sperm whales exist as a genetically distinct subpopulation in the Mediterranean Sea, where it is widely distributed and potentially geographically isolated (Dulau et al., 2004; Engelhaupt et al., 2009). This subpopulation accounts for fewer than 2500 mature individuals; a number that is still declining and grouped in one undivided subpopulation, and for all these reasons it is listed by the International Union for Conservation of Nature (IUCN) as endangered (IUCN, 2006). Safeguard and protective measures have been adopted to protect Mediterranean sperm whales, mainly from entanglement in fishing gears and ship collisions. However, other anthropogenic activities such as oil and gas prospecting (seismic airguns), military operations, illegal dynamite fishing and very specially, contamination, are today sources of concern.

Numerous studies have reported to date POP levels in different marine mammal species all over the world (Aguilar et al., 2002; Alonso et al., 2014; Tanabe, 2002). Yet, data on sperm whales are scarce, including the Mediterranean Sea where very few studies have investigated POP contamination on this species. To the best of our knowledge, there exist fifteen studies worldwide from 1993 to 2018 about POPs in sperm whales and only four of them focused on the Mediterranean Sea (Marsili et al., 2014; Pinzone et al., 2015; Praca et al., 2011; Zaccaroni et al., 2018). This work aims to assess levels of dioxin-like polychlorinated biphenyls (dl-PCBs), PBDEs, polychlorodibenzo-*p*-dioxins and furans (PCDD/Fs) and their congener profiles in blubber of sperm whales stranded along the Italian coast. These data will help 1) to expand our knowledge about the current contamination status of the sperm whale Mediterranean sub-population in order to better understand the threat posed by these POPs, 2) to investigate the toxic load represented by dioxin-like PCBs, PCDDs and PCDFs, for which toxic equivalency factors (TEFs) are available. This approach based on the different contribution of each pollutant to total toxic equivalent (TEQ), makes it possible to carry out a risk assessment for this species.

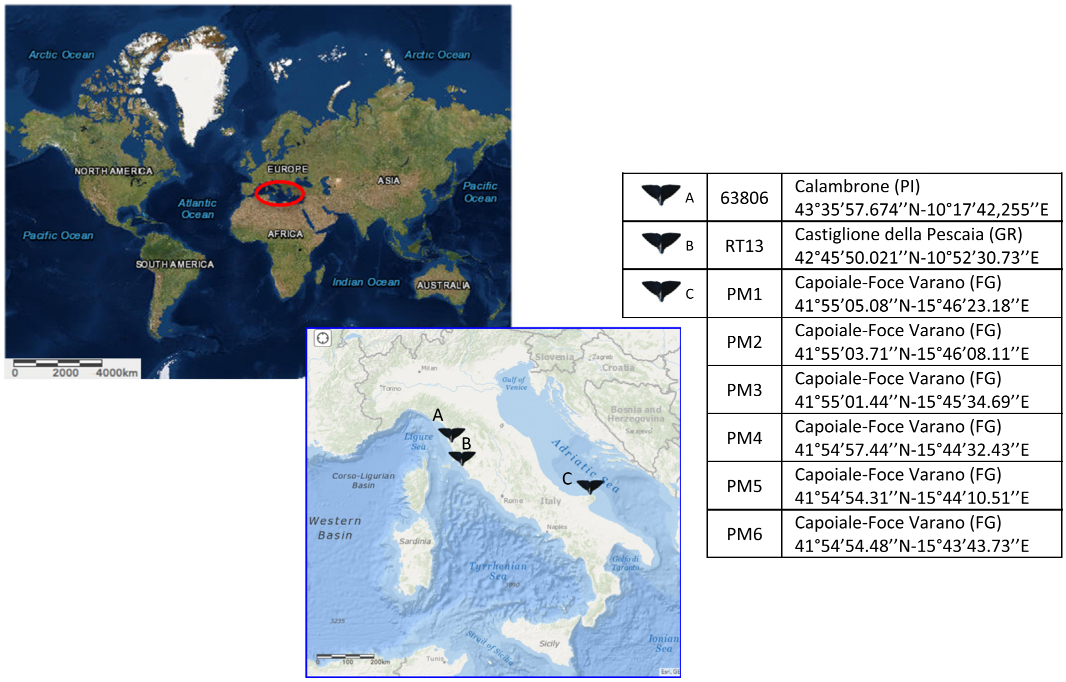

Fig. 1. Map of the three stranding sites (2008, 2009 and 2016) on the coast of Italy.

2. Material and methods

2.1. Sampling

Blubber was collected from nine stranded sperm whales, all males. Seven stranded in 2009 during a mass stranding along the Apulian coast (southern Adriatic Sea) and two stranded in 2008 and 2016 along the coast of Tuscany (Fig. 1). Detailed information about the nine specimens is listed in Table 1. The seven sperm whales stranded in 2009 were part of the same pod and members of the Mediterranean subpopulation (Mazzariol et al., 2011). A multidisciplinary study (histopathology, virology, bacteriology, parasitology, toxicology, genetic and screening of veins looking for gas emboli) was conducted on these individuals and a multi-factorial cause was proposed for this mass stranding (Mazzariol et al., 2011). Determination of organochlorine compounds (PCBs, HCB and DDTs), polycyclic aromatic hydrocarbons and biomarker responses (CYP1A1 and CYP2B) can be found in (Marsili et al., 2014).

All samples were taken from the dorsal area, front the cranial insertion of the dorsal fin, wrapped in aluminum foil, stored in ice on site and then frozen at −20 °C until residue analysis. For the seven sperm whales involved in the mass stranding, age was determined by counting dentin growth layer groups (GLG), and weight (corrected weight) was calculated considering also postmortem body fluid and tissue leakages (Mazzariol et al., 2011). Each of the two sperm whales involved in individual strandings were assigned to a class age according to each specimen's length; weight was estimated using the animal total length (Lockyer, 1976).

2.2. Analytical procedure

Fresh samples were weighed, homogenized with anhydrous sodium sulfate (Na_2SO_4) and spiked with a suite of ^{13}C-labeled standards of dl-PCBs, PBDEs and PCDD/Fs, prior to Soxhlet extraction (24 h) with a *n*-hexane: DCM (9:1) mixture. Extracts were rota-evaporated and cleaned-up by using the automated sample preparation system DEXTech+ (LCTech GmbH, Dorfen, Germany). Final extracts were evaporated using a TurboVap® system until ~1 mL, transferred to vials, and dried under a gentle nitrogen stream. Fractions were reconstituted in a few microliters of ^{13}C-labeled injection standards of dl-PCBs, PBDEs and PCDD/Fs prior to instrumental analysis. Samples' lipid content was determined gravimetrically. Comprehensive details on the analytical procedure are provided in the Supplementary Information.

2.3. Instrumental determination

Samples were analyzed for 12 dl-PCBs (#77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189), twenty-seven PBDEs (# 3, 7, 15, 17, 28, 47, 49, 66, 71, 77, 85, 99, 100, 119, 126, 138, 153, 154, 156, 183, 184,

Table 1
Detailed information about sperm whales stranded along the coast of Italy in 2008, 2009 and 2016.

Code	Date of stranding	Site of stranding	Size (m)	Weight (t)	Age (years)	Sex	Remark
PM1	December 2009	Adriatic Coast	11.8	14.8	22–25	M	Found dead
PM2	December 2009	Adriatic Coast	12.2	16.0	20	M	Found dead
PM3	December 2009	Adriatic Coast	11.3	14.8	20	M	Found dead
PM4	December 2009	Adriatic Coast	11.4	13.7	20	M	Found dead
PM5	December 2009	Adriatic Coast	10.5	16.0	15	M	Stranded alive
PM6	December 2009	Adriatic Coast	12.1	17.7	20	M	Stranded alive
PM7	December 2009	Adriatic Coast	11.2	15.7	20	M	Stranded alive
RT13	October 2008	Tyrrhenian Coast	4.5	1.3	Young	M	Found dead
63806	August 2016	Tyrrhenian Coast	12.8	23.6	Adult	M	Found dead

191, 196, 197, 206, 207, 209) and 17 PCDD/Fs (2,3,7,8-susbtituted congeners). Quantification was carried out by the isotopic dilution technique by GC-HRMS on a Trace GC Ultra gas chromatograph (Thermo Fisher Scientific, Milan, Italy) coupled to a high-resolution mass spectrometer (DFS, Thermo Fisher Scientific, Bremen, Germany). A full description of the instrumental parameters can be found in the Supplementary Information.

2.4. QA/QC criteria

Metal and glassware material was cleaned (3×) with three solvents of decreasing polarity: acetone, dichloromethane and *n*-hexane. A procedural blank was analyzed within each batch of six samples. Care was taken to minimize exposure to UV light throughout the entire analytical procedure. Quantification was carried out by the isotopic dilution technique with 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. Final concentrations were blank corrected. Satisfactory analyses ($n = 3$) of the certified standard reference material SRM 1945 ("Organics in Whale Blubber", NIST) were achieved. Further information related to QA/QC including surrogate recoveries, reference material values and limits of detection of the target compounds is provided in the Supplementary information.

2.5. Data handling

All concentrations are given in ng/g (dl-PCBs and PBDEs) or pg/g (PCDD/Fs) on lipid weight (l.w.) basis. Toxic equivalent quantities (TEQ) for dl-PCBs and PCDD/Fs were determined using the World Health Organization (WHO)-2005 toxic equivalency factors (TEF) for mammals (Van den Berg et al., 2006). Data for PCDD/Fs, dl-PCBs and TEQs are reported in upper bound (i.e. substitution of non-detected compounds for detection limit values).

3. Results and discussions

3.1. Detection frequency

The three POP families (dl-PCBs, PBDEs, PCDD/Fs) were detected in all sperm whale samples analyzed. The relative abundance of the study contaminants followed the order dl-PCBs > PBDEs ≫ PCDD/Fs. Five PBDE congeners (3, 7, 15, 119, 126) were consistently not detected in any sample. The most abundant dl-PCBs congeners were PCB118 (5 Cl), PCB156 (6 Cl) and PCB105 (5 Cl) reaching average contributions of 58%, 14% and 12%, respectively, to the total dl-PCB content. The most abundant PBDE congeners were BDE47 (4 Br), BDE100 (5 Br), BDE99 (5 Br), whose average contribution to the total PBDE content was 70%, 10% and 10%, respectively. The most abundant PCDD/F congeners were 2,3,4,7,8-PeCDF (21%) and 1,2,3,6,7,8-HxCDD (17%).

3.2. Concentration values

The wide range of values found for Σdl-PCBs, ΣPBDEs, ΣPCDDs and ΣPCDFs (Table 2) is likely to reflect on unknown differences in age, sampling year and health status of the sampled specimens. In comparison to the most recent available data of POPs in blubber for this Mediterranean species (Table 3), they were in the same order of magnitude as those reported by Pinzone et al. (2015), save for PCDD/Fs, which were found one order of magnitude lower. Instead, PBDE levels (sum of BDE congeners 28, 47, 100, 99, 154, 153, 209 = 600 ± 399 ng/g l.w.) in our study (all males) were up to three times higher than those reported by Zaccaroni et al. (2018) (167 ± 14 ng/g l.w.) for three Mediterranean sperm whales (all females) stranded along the Adriatic coast in 2014. We can presume this dissimilarity between concentration values was highly influenced by sex since each group was made up by males or females, exclusively. Two of the three females had already reached sexual maturity and probably given birth and decreased their contamination load during gestation and lactation (Reijnders et al., 2009). On the contrary, males analyzed in this study were mostly adults and without offloading mechanisms as gestation and lactation serve to females, they could have been accumulating pollutants - especially those with high hydrophobicity and resistance to metabolism - throughout their entire lives.

In comparison to other geographical areas, dl-PCB and PCDD/F levels in the sperm whales analyzed in this study were two orders higher and in the same order of magnitude, respectively, than those reported for sperm whales from Australia (Gaus et al., 2005). This result is congruent with the fact that the Mediterranean Sea, and in particular its western area, is considered for some authors a global PCB "hotspot" for marine mammals (Stuart-Smith and Jepson, 2017).

Regarding PBDE levels, our results were in the same order of magnitude than those reported for sperm whales from Gulf of California (Fossi et al., 2014), but six times lower than those reported from North-Atlantic specimens (Borrell, 1993), which is probably related to the greater historical use of these compounds in North America (Law et al., 2014).

It is worth noting how differences in pollutant concentrations and profiles between our study and previous literature could be linked not only to different geographic areas and time periods, but also to distinct analytical methods and type of samples. Specifically, it is known that POP concentration in cetaceans is influenced by blubber thickness (Evans et al., 2003), which could became a significant variable when comparing biopsies taken from free-ranging individuals (few centimetres of the outermost layer) to samples obtained from stranded individuals (usually all three layers of blubber) (Evans et al., 2003; Ryan et al., 2013).

3.3. Congener profiles

The dl-PCB congener profile, showed in Fig. 2, was dominated by mono-*ortho* PCBs, that accounted for about 99.9% of the dl-PCBs.

Table 2
Mean, median, range, detection frequencies (% >LOQ) of total dl-PCBs, PBDEs and PCDD/Fs in blubber from Mediterranean sperm whales (concentrations are expressed in ng/g l.w. save for PCDD/Fs, pg/g l.w.); PCDDs, PCDFs, dioxin-like PCBs, total TEQ and percentage contribution to T-TEQ. Data are expressed in pg WHO-TEQ/g l.w and are showed as mean, median and range.

Compounds	Mean	Median	Range	>LOQ (%)	pg WHO-TEQ/g l.w.			% total TEQ
					Mean	Median	Range	
Σmono-*ortho*-dl-PCBs	6410	3490	2090–20,800	100	192	105	62.9–625	34.1
Σnon-*ortho*-dl-PCBs	4.10	3.74	2.61–7.43	100	281	255	188–527	61.7
Σdl-PCBs	**6420**	**3500**	**2100–20,800**	100	**474**	**374**	**261–968**	**95.8**
ΣPCDDs (pg/g)	29.6	25.5	20.7–47.6	100	13.0	11.9	9.36–21.1	2.9
ΣPCDFs (pg/g)	28.2	27.1	23.9–35.9	100	5.03	4.54	4.26–6.37	1.2
ΣPCDD/Fs (pg/g)	**57.8**	**56.2**	**45.4–83.5**	100	**18.1**	**16.3**	**13.9–27.4**	**4.2**
ΣPBDEs	612	356	312–1390	100	–	–	–	–
Total TEQ					**492**	**394**	**275–987**	–

Bold values indicate the total of dl-PCBs, PCDD/Fs and TEQ.

Table 3
PCBs and PBDEs (ng/g l.w.) in blubber samples of sperm whales from different worldwide studies. Data are expressed as mean ± SD (when possible). Geographic area of sampling, year of collection and sex are reported (when possible).

Reference (year)	Geographic area	Sampling year	N	Sex	ΣPCBs (Σdl-PCBs)	ΣPBDEs
Aguilar (1983)[p]	North Atlantic	–	8	M	9930	–
			6	F	15,550	–
Borrell (1993)[p]	Iceland	1982	10	M	10,510 ± 2070	4160 ± 1040
Holsbeek et al. (1999)[r,a]	Southern North Sea	1994–1995	7	M	3032 ± 547	
Evans et al. (2004)[q,b]	Southern Australia	1998	32	F	800 ± 400	–
			5	M	1300 ± 1200	–
Praca et al. (2011)[r,c]	NWMS	2003–2009	14	1 M/13 U	107,810 ± 108,720	–
Marsili et al. (2014)[q,d]	Italy	2009	7	M	193,608 ± 340,089	–
Pinzone et al. (2015)[r,e]	NWMS	2006–2013	32	M	24,237 ± 17,421	382 ± 176
			11	F	16,877 ± 7237	248 ± 106
				F/M	(2120 ± 1490)	
Gaus et al. (2005)[q,f]	Tasmania (Southern Australia)	–	7	F/M	(28.7 ± 8.73)	–
de Boer et al. (1998)[r,g]	NE Atlantic (North sea)	–	3	M	–	49.6 ± 46.0
Bachman et al. (2014)[q,h]	Pacific Island	2011	1	F	1.470	27.2
Fossi et al. (2014)[r,i]	Gulf of California (Mexico)	2008–2009	14	M	2193 ± 660	30.8 ± 31.7
				F	2294 ± 1180	283 ± 819
Godard-Codding et al. (2010)[r,l]	SC = Sea of Cortez	1999	10		MC: 1514 ± 1693	–
	KR = Kiribati	2000	10		KR: 734 ± 869	–
	GP = Galapagos	2000	10		GP: 1262 ± 1586	–
	PX1 = Pacific Crossing	2000	10		PX1: 777 ± 853	–
	PNG = Papua New Guinea	2001	10		PNG: 1101 ± 1378	–
Law et al. (2003)[q,m]	Orkney Islands (Scotland)	1994	1		–	42.9
	Netherlands	1995	2		–	80.6 ± 90.7
Romero-Romero et al. (2017)[q,n]	Atlantic Ocean (Cantabrian sea)	–	1		1790	149
Zaccaroni et al. (2018)[q,o]	Mediterranean Sea	2014	3	F	–	167 ± 13.9
This study[r]	Mediterranean Sea	2008, 2009, 2016	9	M	6420 ± 6150	612 ± 401

[a] Sum of PCB congeners n. 28, 52, 101, 118, 153, 138, 156, 180, 170, 194.
[b] Sum of PCB congeners n. 28, 52, 101, 118, 153, 180.
[c] Sum of PCB congeners n. 28, 44, 52, 101, 118, 128, 138, 153, 170, 180, 187, 195, 206.
[d] Sum of PCB congeners n. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206.
[e] Sum of ndl-PCBs congeners n. 8, 18, 28, 52, 44, 66, 101, 87, 153, 138, 187, 128, 180, 170, 195, 206, 209 and dl-PCBs congeners n. 118, 105, 77, 81, 126, 169, 144, 123, 156, 157, 167, 189.
[f] Sum of PCB congeners n. 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189.
[g] Sum of PBDE congeners n. 47, XY‡, 99, 209.
[h] Sum of PCBs congeners n. 8, 18, 28, 29, 31, 44, 45, 49, 50, 52, 56, 63, 66, 70, 74, 79, 82, 87, 92, 95, 99, 101, 104, 105, 106, 107, 110, 112, 114, 118, 119, 121, 127, 128, 130, 132, 137, 138, 146, 149, 151, 153, 154, 156, 157, 158, 159, 163, 165, 166, 167, 170, 172, 174, 175, 176, 177, 178, 180, 183, 185, 187, 188, 189, 191, 193, 194, 195, 196, 197, 199, 200, 201, 202, 203, 205, 206, 207, 208, 209.
[i] Sum of PCB congeners n. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206. Sum of PBDE congeners n. 194, 195, 196, 197, 204, 198, 199, 200, 203, 201, 202, 205, 206, 207, 208, and 209.
[l] Sum of PCB congeners n. 95, 101, 99, 151, 144, 135, 149, 118, 146, 153, 141, 138, 178, 187, 183, 128, 174, 177, 156, 171, 202, 172, 180, 199, 170, 196, 201, 195, 194, 206.
[m] Sum of PBDE congeners n. 28, 47, 66, 71, 75, 77, 85, 99, 100, 119, 138, 153, 154, 190.
[n] Sum of PCB congeners n. (*ortho*) 28, 52, 101, 105, 114, 118, 123, 138, 153, 156, 157, 167, 170, 180, 189, 194; (non-*ortho*) 81, 77, 126, 169. Sum of PBDEs congeners n. 17, 28, 47, 66, 85, 99, 100, 153, 154, 184, 183, 191, 196, 197, 209.
[o] Sum of PBDEs congeners n. 28, 47, 100, 99, 154, 153, 209.
[p] Caught animals.
[q] Stranded animals.
[r] Free-ranging animals.

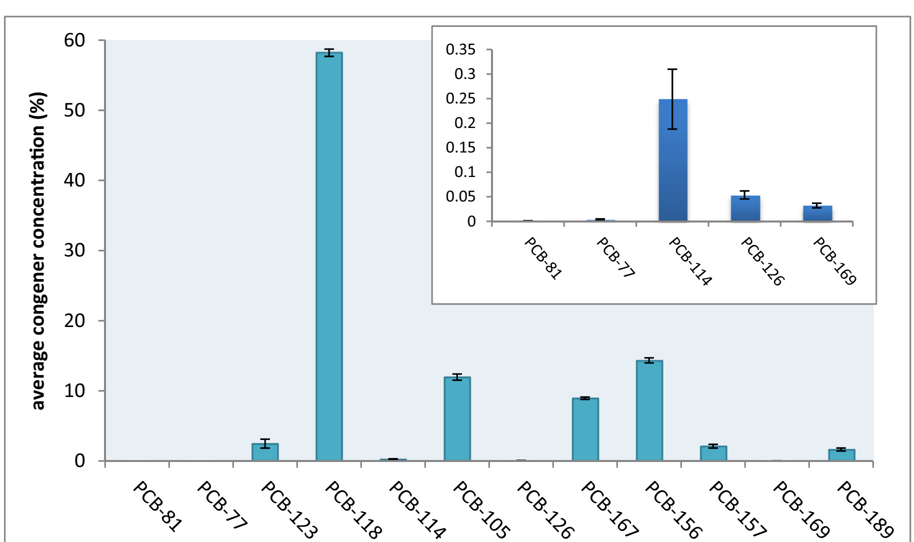

Fig. 2. Average contribution of each dl-PCB congener to the total dl-PCB content. Error bars represent the standard error (SE).

Among them, PCB118 was the most abundant, which is in line with previous studies on sperm whales and other species reporting this congener as frequently the most abundant among dl-PCBs (Bhavsar et al., 2007; Evans et al., 2004; Gaus et al., 2005; Lake et al., 1994; Romero-Romero et al., 2017; Storelli et al., 2011). This was somewhat anticipated since this congener is one of the seven PCBs recommended by The International Council for the Exploration of the Sea (ICES) working groups as indicator congeners (ICES7) for monitoring, based on their relatively clear identification and quantification in gas chromatography and their usual high contribution to the total PCB content in the environmental samples (Boalt et al., 2013).

The non-*ortho* PCBs (81, 77, 126, 169) made up only a small fraction of total dl-PCBs (0.09%). Among these highly toxic congeners, PCB 126 accounted for (58.0%) more than half of the non-*ortho* PCB content, followed by PCB169 (35.7%) and PCB77 (5.3%). On the contrary, PCB81 contributed with <1%. Interestingly, this profile is similar to those found in sperm whales from Australia (Gaus et al., 2005) and the Atlantic Ocean (Romero-Romero et al., 2017), but differs from the accumulation patterns generally found in fish and their main prey, i.e. cephalopods. In these cases, as well as many other instances of abundance patterns in biota, PCB77 is the predominant congener, followed by PCB126 and PCB169 (Cappelletti et al., 2015; Romero-Romero et al., 2017; Storelli, 2008; Tanabe et al., 1987).

Due to the lack of specific studies about the metabolism of non-*ortho* substituted PCBs in sperm whales, we hypothesize with an enhanced metabolic capability of this species towards PCB77. This hypothesis is not supported by Boon et al. (2000), who showed that PCB77 was not metabolised by sperm whales microsomes in vitro. On the contrary, the higher values of PCB126 and 169 suggested reduced or absent metabolic activities towards these congeners. This points out how more focused investigations are needed on accumulation and contaminant detoxification pathways in sperm whales and cetaceans in general. Moreover, without knowing the exact diet of the analyzed individuals as well as patterns and pollutant concentrations in their prey, caution is mandatory when trying to explain their contamination profiles. Nonetheless, in general the average dl-PCB congener profile (Fig. 2) was alike to that described by Pinzone et al. (2015) for Mediterranean sperm whales sampled between 2008 and 2013.

The PBDE congener profile, showed in Fig. 3, was dominated by lower-medium brominated congeners 47 > 99 > 100, which agrees with the fact of these congeners being generally abundant in aquatic food webs (Fossi et al., 2012; Romero-Romero et al., 2017). In fact, BDE47 is commonly the most abundant congener in most biota samples, including marine mammals (Hites, 2004). The low abundance of BDE-209, which is the main component of the commercial mixture deca-BDE (La Guardia et al., 2006), was somewhat anticipated. Its highest hydrophobicity among BDE congeners explains its heightened sequestration into suspended particulate matter and sediments, which results in low bioavailability (Lee and Kim, 2015). Moreover it shows a higher degree of chemical, microbiological degradation, and a higher degree of metabolization than lighter BDE congeners, which is thought to justify its usual absence - or lower concentrations in comparison to other BDE congeners - in marine mammals (Zhang et al., 2016). Overall, the PBDE congener profile was also similar to those described by Pinzone et al. (2015) and Zaccaroni et al. (2018) for Mediterranean sperm whales sampled between 2008 and 2014.

The PCDF congener profile (penta > hexa > tetra > hepta > octa) (Fig. 4) was relatively similar to those reported for sperm whales from Australia (Gaus et al., 2005) and to those reported in blubber of striped dolphin (*Stenella coeruleoalba*) from the Mediterranean Sea (Fossi et al., 2004). In contrast, the PCDD congener profile (hexa > penta > tetra > hepta > octa) was remarkably different from these two, with a lower concentration of higher chlorinated congeners and higher concentration of lower chlorinated congeners.

3.4. Toxicity assessment

It is well established how the toxicity of PCDD/Fs and dl-PCBs is mediated by the cytosolic receptor AhR, based on their planar spatial configuration (Mandal, 2005). Their potential toxicity is commonly assessed by the toxic equivalent quantity (TEQ) approach, in which each's congener toxicity is relativized to that of the most toxic one, the 2,3,7,8-TCDD (Schecter et al., 2006). In this study, TEQs in sperm whale blubber samples were evaluated using the toxic equivalency factors (TEFs) for mammals provided by the World Health Organization (WHO) in 2005 (Van den Berg et al., 2006). Total calculated TEQs ranged from 275 to 987 pg/g l.w.

Regardless of the wide inter-species sensitivity towards different contaminants, it should be highlighted how all analyzed sperm whales surpassed the threshold of 210 pg WHO-TEQ/g l.w. in blubber, proposed as starting point of immunosuppression in harbour seals (Ross et al., 1995).

All sperm whales in this study exhibited the same TEQ pattern (Σnon-*ortho*-dl-PCBs > Σ*ortho*-dl-PCBs > PCDDs > PCDFs), with dl-PCBs as the most abundant pollutants, in agreement with previous investigations about sperm whales and other cetaceans from the

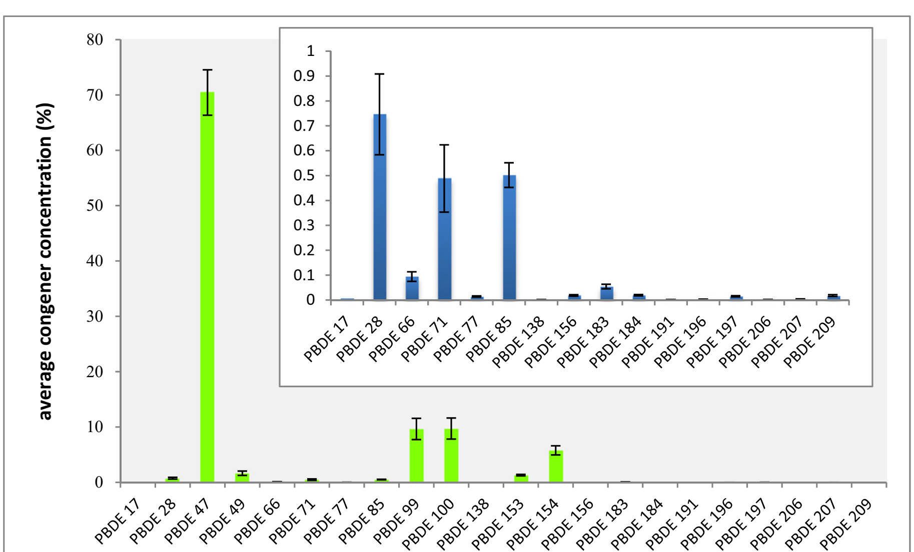

Fig. 3. Average contribution of each BDE congener to the total PBDE content. Error bars represent the standard error (SE).

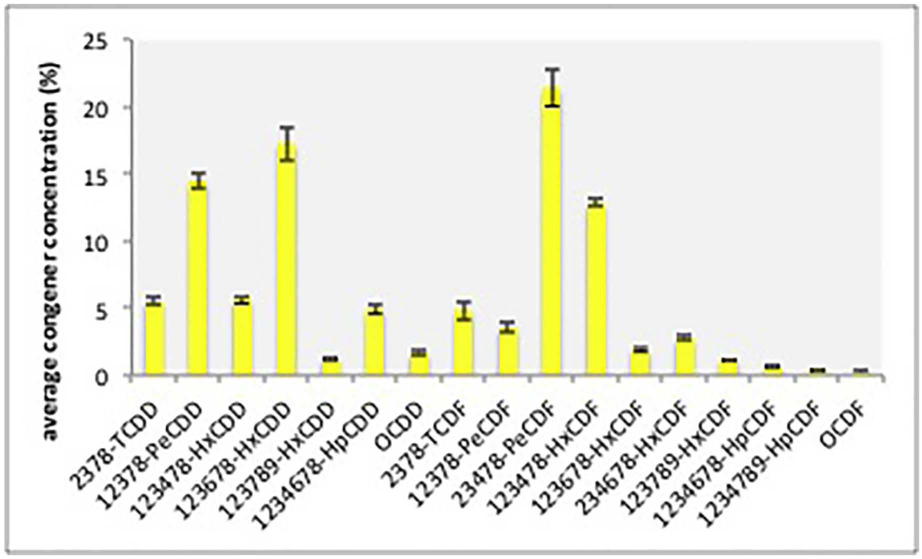

Fig. 4. Average contribution of each PCDD/F congener to the total PCDD/F content. Error bars represent the standard error (SE).

Mediterranean Sea (Fossi et al., 2004; Pinzone et al., 2015). This draws attention to the fact that, even if PCBs were banned around 40 years ago in most European countries, they continue to pose a significant threat to cetaceans in the Mediterranean Sea, where these contaminants have also been related to cetacean populations' declines (Stuart-Smith and Jepson, 2017).

Several adverse health effects have been ascribed to PBDE burdens in mammals; for example, influence on the homeostasis of steroidal and thyroidal hormones (Darnerud, 2003; Zhou et al., 2001), immunotoxicity (Fowles et al., 1994), and reproductive and other neurological disorders (Siddiqi et al., 2003). However, no specific threshold for toxicological effects in sperm whales has been proposed at the moment. Currently, the only available value reported for a marine mammal species is the threshold of 1500 ng/g l.w. established for endocrine disruption in grey seals (Hall et al., 2003). Total PBDE concentrations found in this study ranged from 312 to 1390 ng/g l.w., and therefore were always below the abovementioned reference value, although often in the same order of magnitude. However, the distinct interspecies sensitivity towards these pollutants - currently unknown in the case of sperm whales - cannot be disregarded. Aside from interspecies sensitivity, it is important to underline that, when comparing results with toxicity thresholds, each individual might respond in different ways to contaminant concentrations depending on multiple factors such as sex, age and health status.

4. Conclusions

Concentration levels found in this study were mostly in line with what has been previously reported on the same species in the Mediterranean Sea. Conversely, POPs measured in this study tended to be in higher levels than those reported on sperm whales from other geographical regions. It is important to emphasise that dioxin-like PCBs were, not only found in the highest concentrations among the target POPs, but also ranking at the top of the reported values for the same species worldwide. Our results along with data from recent works, highlight still high levels of PCBs in the Mediterranean Sea biota, which far from showing a decreasing trend during recent years appear to steady linger in this area. This also confirms the role of the Mediterranean Sea as a PCB global "hotspot" for marine mammals, and the need for further mitigation of PCB pollution to protect these sea-dwelling species. As a direct consequence of these concerning high dl-PCB concentrations, all animals in this study surpassed the threshold proposed as starting point of immunosuppression in marine mammals (harbour seals). The lack of a specific threshold for toxicological effects in sperm whales related to the presence of the target POPs does not allow to draw a solid conclusion on the role played by these contaminants in their health status. In turn, this stresses the importance of broadening our knowledge about toxicity, accumulation and detoxification processes, in order to 1) better understand the impact of pollution in the sperm whale's health status and 2) develop more focused conservation strategies, with special attention to the Mediterranean Sea due to its role as a sink for many contaminants of concern.

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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.2018.10.436.

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