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POPs in free-ranging pilot whales, sperm whales and fin whales from the Mediterranean Sea: Influence of biological and ecological factors

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ABSTRACT

The pilot whale *Globicephala melas*, the sperm whale *Physeter macrocephalus,* and the fin whale *Balaenoptera physalus* are large cetaceans permanently inhabiting the Mediterranean Sea. These species are subjected to numerous anthropogenic threats such as exposure to high levels of contaminants. Therefore, selected persistent organic pollutants POPs (29 PCBs, 15 organochlorine compounds, 9 PBDEs and 17 PCDD/Fs) were analysed in blubber biopsies of 49 long-finned pilot whales, 61 sperm whales and 70 fin whales sampled in the North Western Mediterranean Sea (NWMS) from 2006 to 2013. Contamination profile and species feeding ecology were then combined through the use of stable isotopes. $\delta^{13}C$, $\delta^{15}N$ values and POPs levels were assessed through IR-MS and GC–MS respectively. To assess the toxic potency of the dioxin-like compounds, the TEQ approach was applied. $\delta^{15}N$ values were 12.2 ± 1.3‰ for sperm whales, 10.5 ± 0.7‰ for pilot whales and 7.7 ± 0.8‰ in fin whales, positioning sperm whales at higher trophic levels. $\delta^{13}C$ of the two odontocetes was similar and amounted to -17.3 ± 0.4‰ for sperm whales and -17.8 ± 0.3‰ for pilot whales; whilst fin whales were more depleted (-18.7 ± 0.4‰). This indicates a partial overlap in toothed-whales feeding habitats, while confirms the differences in feeding behaviour of the mysticete. Pilot whales presented higher concentrations than sperm whales for ΣPCBs ($38{,}666 \pm 25{,}731$ ng g^{-1} lw and $22{,}849 \pm 15{,}566$ ng g^{-1} lw respectively), ΣPBDEs (712 ± 412 ng g^{-1} lw and 347 ± 173 ng g^{-1} lw respectively) and ΣDDTs ($46{,}081 \pm 37{,}506$ ng g^{-1} lw and $37{,}647 \pm 38{,}518$ ng g^{-1} lw respectively). Fin whales presented the lowest values, in accordance with its trophic position (ΣPCBs: 5721 ± 5180 ng g^{-1} lw, ΣPBDEs: 177 ± 208 ng g^{-1} lw and ΣDDTs: 6643 ± 5549 ng g^{-1} lw). Each species was characterized by large inter-individual variations that are more related to sex than trophic level, with males presenting higher contaminant burden than females. The discriminant analysis (DA) confirmed how DDTs and highly chlorinated PCBs were influential in differentiating the three species. Pollutant concentrations of our species were significantly higher than both their Southern Hemisphere and North Atlantic counterparts, possibly due to the particular Mediterranean geomorphology, which influences pollutants distribution and recycle. Dioxin-like PCBs accounted for over 80% of the total TEQ. This study demonstrated (1) an important exposure to pollutants of Mediterranean cetaceans, often surpassing the estimated threshold toxicity value of 17,000 ng g^{-1} lw for blubber in marine mammals; and (2) how the final pollutant burden in these animals is strongly influenced not only by the trophic position but also by numerous other factors such as sex, age, body size and geographical distribution.

1. Introduction

The Mediterranean Sea is threatened by numerous factors, mainly related with the high urbanization of the coasts (estimated to reach 529 million inhabitants by 2025 – UNEP/MAP, 2012) and

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all the activities deriving from these settlements, such as wastewaters release, shipping, fishing, and chemical pollution (Halpern et al., 2008). Despite the existence of regulations and bans since the late 1970s, persistent organic pollutants (POPs), including polychlorinated biphenyls PCBs, polybrominated diphenyl ethers PBDEs, polychloro dibenzo-dioxins and furans PCDD/Fs and organochlorine compounds (OCPs) remain widespread in the Mediterranean marine environment through air and water transport from Asia and Eastern Europe, where large amount of these compounds are still produced and discharged (Ryan et al., 2013b; Marsili et al., 2014). Moreover, due to their lipophilic nature POPs can biomagnify in marine food webs, reaching very high levels among top predators (Aguilar et al., 2002). Elevated concentrations of these compounds are believed to affect the endocrine and immune systems of mammalian species, potentially affecting the overall survival of the animals (Ross et al., 2000; Reijnders, 2003; Tanabe, 2002; Das et al., 2006; Beineke et al., 2007).

The North-western Mediterranean Sea (NWMS) is especially influenced by the release of man-made substances (Halpern et al., 2008; Moulins et al., 2008) due to the large naval traffic and offshore oil activities in the area (Moulins et al., 2008). Moreover, large rivers such as Rhone and Ebro, determine the discharge of several substances produced in agricultural and industrial areas (UNEP/MAP, 2012). Large top predators including fin whales (*Balaenoptera physalus*), sperm whales (*Physeter macrocephalus*), and long-finned pilot whales (*Globicephala melas*) are commonly sighted in the area all year round (Laran and Ganniers, 2008; Moulins et al., 2008; Praca and Gannier, 2008). Fin whales are mainly distributed in deep, offshore waters of Western and Central Mediterranean, but can be rarely sighted in other regions (Reeves and Notarbartolo di Sciara, 2006; Laran and Gannier, 2008; Bentaleb et al., 2011). Long-finned pilot whales are found mostly in oceanic areas, while sperm whales seem to have a wider habitat, being present over the entire continental slope and on some occasion offshore (Azzellino et al., 2008; Praca and Gannier, 2008).

Beside the threat of POPs potential hazard to marine mammal populations, the levels and profile of these contaminants can serve as an intrinsic marker from which population structure can be defined (Praca et al., 2011; Ryan et al., 2013b). Indeed, these compounds reflect the ecosystem conditions under which marine mammals live and feed and therefore can infer their geographical distribution (Aguilar et al., 2002; Borrell et al., 2006; Praca et al., 2011). In addition, as the chemical analysis requires only a small amount of sample, it can be performed using biopsies; allowing the study of living and healthy animals (Praca et al., 2011).

Other chemical tracers such as stable isotope ratios of carbon ($^{13}C/^{12}C$ or $\delta^{13}C$ in delta notation) and nitrogen ($^{15}N/^{14}N$, $\delta^{15}N$ in delta notation) demonstrated their usefulness in exploring feeding ecology of cetaceans (Frodello et al., 2000; Capelli et al., 2008; Newsome et al., 2010; Praca et al., 2011; Ryan et al., 2013b). As terrestrial and marine carbon sources differ in their ^{13}C composition, with terrestrial and coastal areas more enriched than less-productive offshore regions, $\delta^{13}C$ permits to discriminate between offshore/nearshore and benthic/pelagic foraging behaviour (Newsome et al., 2010; Praca et al., 2011). Moreover, the depletion in marine organic matter with increasing latitude can be used for investigating seasonal changes in foraging areas (Bentaleb et al., 2011). On the other hand, $\delta^{15}N$ isotope ratio shows an increase with each trophic level and can be used to model the position of the consumers in the marine food web (Newsome et al., 2010). The combination of pollution studies with SI analysis, thus, allows time-integrated measure of sources, pathways, and degree of biomagnification of the different organic contaminants (Das et al., 2004; Newsome et al., 2010; Van De Vijver et al., 2003).

Although very important for species conservation, data on stock structure of fin whales, long-finned pilot whales and sperm whales populations of NWMS are still highly fragmented (Praca and Gannier, 2008; Budzinski et al., 2011; Bentaleb et al., 2011). In this regard, intrinsic markers such as $\delta^{13}C$, $\delta^{15}N$ and POPs may provide deeper insights into species connectivity and permit the long-term analysis of their eco-toxicological status (Ryan et al., 2013b).

The aim of this study was to conduct an eco-toxicological analysis of long-finned pilot whale *G. melas* (Traill, 1809), sperm whale *P. macrocephalus* (Linnaeus, 1758) and fin whale *B. physalus* (Linnaeus, 1758) from the Mediterranean Sea, in order to understand how the particular ecology of each species influences the contamination pattern. Firstly, we assessed the occurrence of several POP classes including PCBs, dioxin-like compounds PCDD/Fs and DL-PCBs, PBDEs and OCPs in blubber biopsies collected between 2006 and 2013; secondly, we combined the POPs analysis with $\delta^{13}C$ and $\delta^{15}N$ values in skin, to gain further understanding on their trophic levels and feeding behaviour. To our knowledge, this was the first time that such impressive number of samples (180 different blubber biopsies collected over a period of 8 years) from a relatively confined area (off of the South coast of France) was available. This permitted a complete and in-depth comparative study among our three species in the Mediterranean Sea and with other regions of the world, and gather new important information on the ecology of these elusive, but yet severely endangered, species.

2. Materials and methods

2.1. Sampling

Skin and blubber biopsies were sampled from 61 sperm whales (14 females and 47 males), 49 long-finned pilot whales (23 females and 26 males), and 70 fin whales (35 females and 35 males) in the North Western Mediterranean sea during 8 WWF campaigns between 2006 and 2013 (Fig. 1 – permit 10/370/EXP delivered by the French Ministry of Ecology and Sustainable Development). The biopsies of skin and the first 3-4 cm of blubber were operated when the animals were accessible at the surface, using a 150 lb crossbow (Panzer, US). The arrows for the biopsies were produced by Finn LARSEN (CETADART®); while the tips by Jean-Charles Aragnostou (AFT-Micromécanique). Three types of points were used depending on the species: 10 mm in diameter and 50 mm in length for fin whales, 5 mm in diameter and 40 mm in length for sperm whales, and 5 mm in diameter and 25 mm in length for pilot whales. To avoid infection of cetaceans and contamination of the samples, the sampling heads were sterilized in boiling water and sprayed with 90% alcohol before the use. Together with each sample, additional information on the time and date of sampling, the geographical coordinates and the structure of the group in which the individual was found was noted. Samples of skin and blubber were frozen at -20 °C on-board.

2.2. Sex determination

The determination of sex for all the species was conducted by the Genome and Selection laboratory at the French Agricultural Research Centre for International Development (Montpellier – GS/CIRAD). DNA was extracted from the skin of each biopsy using commercial extraction kits (QIAGEN DNeasy® blood and tissue kit). Sex was determined genetically following the protocol of Bérubé and Palsbøll (1996) using primers designed for mysticetes. A fragment of the zinc finger protein coding gene, specific to the X (ZFX) and Y (ZFY) chromosome, was amplified using 2 sets of primers, which permitted to anneal to both loci separately, yielding amplification products of different lengths. Gel

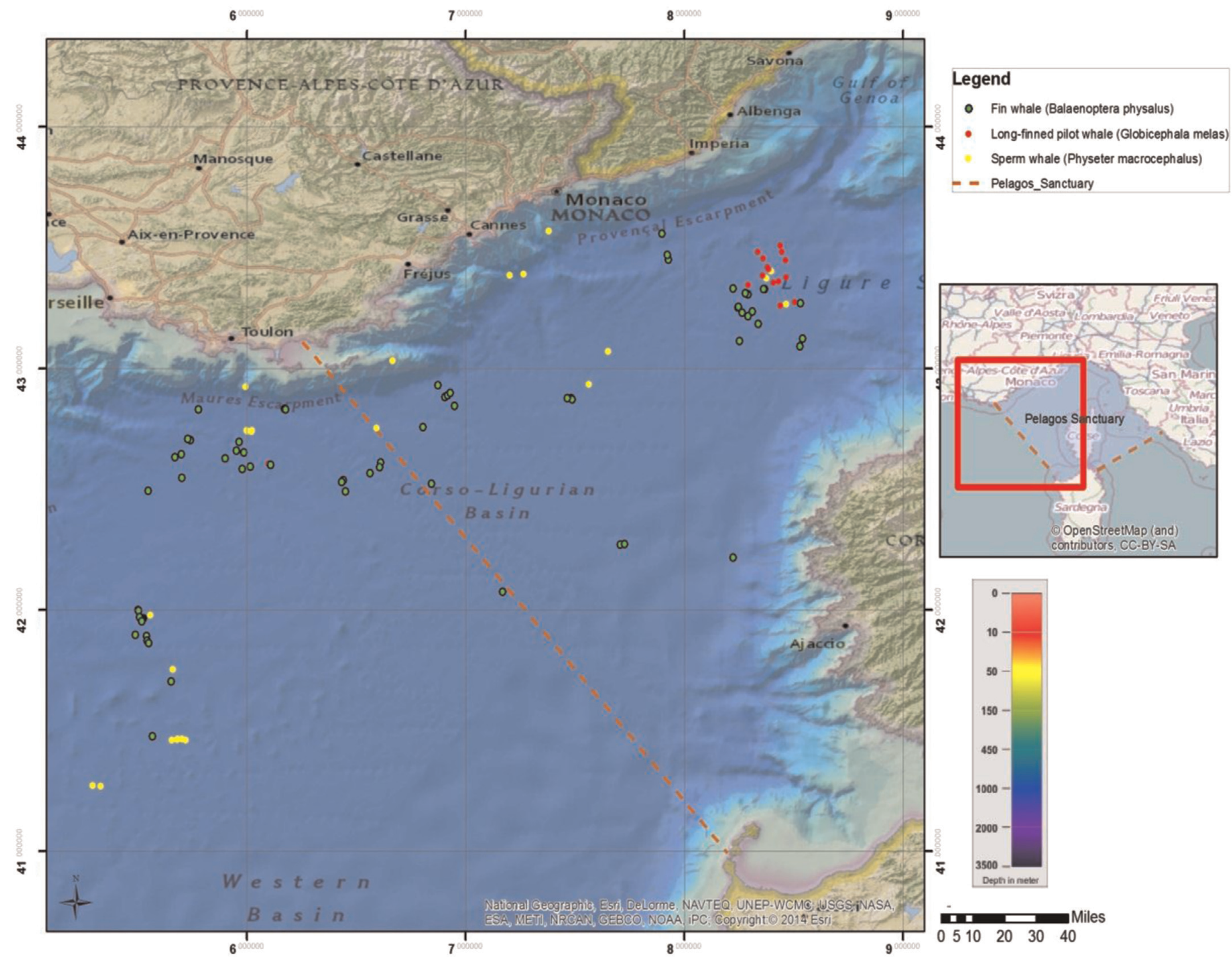

Fig. 1. Sampling sites of skin and blubber biopsies in 2013 campaign. Green dot: fin whale; red dot: long-finned pilot whale; yellow dot: sperm whale. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

electrophoresis of the PCR products through 2% agarose was used to separate the ZFX and ZFY amplification products. Once terminated with the PCR, results were read with UV light.

2.3. Persistent organic pollutants

2.3.1. 2006–2011 samples

Blubber biopsies of 70 fin whales collected between 2006 and 2009, 31 sperm whales and 31 long-finned pilot whales collected between 2008 and 2011 were analysed at the University of Bordeaux (EPOC – UMR 5805). The details of the entire procedures and quality assurance are given in the Supporting Information and elsewhere (Budzinski et al., 2011; Tapie et al., 2011). Dried samples were extracted and purified through accelerated solvent extraction (ASE) by the ASE 200 Dionex system. The concentration of the extract was conducted using a Rapid-Vap vacuum evaporation system (Labconco, USA) before a second purification step on acidic silica gel column. POPs measurement was performed on an HP 5890 series II gas chromatograph (Hewlett-Packard, USA) coupled to a ^{63}Ni electron-capture detector (ECD). The analysis included the PCBs congeners 8, 18, 50+28, 52, 44, 66, 101, 87, 118, 153, 105, 138, 187, 128, 180, 170, 195, 206 and 209; PBDEs 47, 99 and 153; pp'-DDT, pp'-DDE; and pp'-DDD; dieldrin and γ-HCH. Recovery percentages ranged between 70% and 122% for PCBs, between 55% and 126% for DDTs and between 83% and 115% for PBDEs.

2.3.2. 2012–2013 samples

Samples of 30 sperm whales and 17 pilot whales collected between 2012 and 2013 were instead analysed at the University of Liège (LMS/LEAE – CART, ULg). The analysis was conducted for 6 non-dioxins like (NDL) PCBs no. 28, 52, 101, 138, 153 and 180; the 12 WHO dioxins-like (DL) PCBs and the 17 WHO PCDD/Fs (Table SI.1); 9 PBDEs no. 28, 47, 66, 99, 100, 153, 154, 183 and 209; and selected pesticides pp'-DDT, pp'-DDE; and pp'-DDD; α-HCH, β-HCH, γ-HCH, aldrin, dieldrin, HCB, β-Endosulfan. The analysis of DL compounds was not possible on all fin whales, sperm whales and long-finned pilot whales from 2008–2012; therefore these samples are not included in the comparative analysis and discussion on these particular pollutants (Table SI.3).

PCDD/Fs, DL-PCBs, NDL-PCBs and PBDEs were quantitatively analysed by the isotope dilution technique using ^{13}C labelled analogues (Cambridge Isotopes Laboratories, Tewksbury, MA, USA and Wellington Laboratories, Ontario, Canada; Table SI.2). Briefly, lipids were extracted via accelerated solvent extraction (ASE) with methylene chloride; then evaporated until dryness under nitrogen flux. The lipids amounts were then determined gravimetrically prior to a multicolumn liquid–solid chromatography (acid, neutral, basic silica gel; basic alumina and carbon) clean-up performed

Table 1
Lipid-content, sums of PCBs, PBDEs, pesticides and different ratios for long-finned pilot whales, sperm whales and fin whales from the North-Western Mediterranean Sea. Lipid-content data are represented as percentages (%); POPs concentrations are expressed as ng g^{-1} lw. Data are showed as Mean (Median) ± standard deviation (Min–Max values). Intersex differences are shown as *p*-values from the Mann–Whitney non-parametric t-test ($p < 0.05$). Interspecific differences are shown as p-values from the Kruskal–Wallis ($p < 0.05$) analysis. The *H*-statistic values are shown between brackets. Significant differences are shown in bold.

	Long-finned pilot whale			**Sperm whale**			**Fin whale**			**Kruskal– Wallis**
	M	**F**	***p*-value**	**M**	**F**	***p*-value**	**M**	**F**	***p*-value**	
N	26	23		32	11		35	35		
Lipids%	23(19) ± 16 (4-82)	22(22) ± 10 (5-42)	0.733	10(6) ± 8 (2-32)	18(18) ± 8 (3-32)	**0.017 (U=90)**	31.9(32.6) ± 9.6 (8.8–46.5)	28.2(27.6) ± 11.8 (2.9–55.0)	0.110	**< 0.0001 (X^2=55)**
ΣPCBs[a]	40,683(41,051) ± 19,941 (1555–88053)	36,386(21,280) ± 31,345 (1566–102,967)	0.362	24,237(17,206) ± 17,421 (1581–68,475)	16,877(13,799) ± 7237 (8731–27,244)	0.277 (U=145)	7957(7132) ± 4613 (2119–250.65)	3776(2651) ± 5024 (405–24,781)	**< 0.0001 (U=138)**	**< 0.0001 (X^2=91)**
ΣICES7	29,783(30,304) ± 12,193 (1501–57,084)	24,485(13,748) ± 20,535 (1523–68,013)	0.225	18,879(13,316) ± 12,509 (946–45,876)	11,902(9091) ± 6323 (5624–25,127)	0.053	5824(5353) ± 3282 (1631–17979)	2622(1829) ± 3505 (252–17,362)	**0.0001 (U=126)**	**< 0.0001 (X^2=94)**
ΣPBDEs	750(762) ± 291 (205–1591)	670(406) ± 519 (43.0–1755)	0.362	382(387) ± 176 (0.0–781)	248(218) ± 106 (132–479)	**0.011 (U=82)**	245(207) ± 179 (51.0–841)	119(63.2) ± 223 (6.8–1194)	**< 0.0001 (U=143)**	**< 0.0001 (X^2=73)**
p,p′DDE	42,395(38,348) ± 20,661 (7908–96,134)	38,107(23,363) ± 41,990 (395–165,521)	0.125	41,382(26,694) ± 37,067 (5396–147,253)	15,320(10,541) ± 10,191 (5310–31,472)	**0.013 (U=0.0)**	7518(6837) ± 4551 (1143–19,163)	2672(2316) ± 2693 (138–16,458)	**< 0.0001 (U=134)**	
p,p′DDD	1399(1434) ± 1222 (23.4–3713)	1488(579) ± 1687 (0.0–6087)	0.976	848 (282) ± 1484 (32–7633)	367(307) ± 238 (144–847)	0.746	872(790) ± 537 (70.1–2319)	174(131) ± 116 (29.1–579)	**< 0.0001 (U=65)**	
p,p′DDT	1566(1375) ± 606 (604-2888)	1333(1211) ± 1111 (140–3860)	0.257	3155(1510) ± 3268 (340–9765)	961(642) ± 828 (278–2926)	0.114 (U=98)	300(259) ± 174 (71.7–804)	105(81.9) ± 84.2 (6.5–342)	**< 0.0001 (U=141)**	
ΣDDTs	57,315(51,715) ± 24,266 (25,114–106,926)	45,340(23,758) ± 49,247 (421–185,209)	0.176	44,173(27,836) ± 41,907 (272–169,869)	17,369(12,327) ± 11,172 (6343–34,939)	**0.039 (U=98)**	10,370(8955) ± 6246 (1744–26,695)	3239(2716) ± 2896 (245–18095)	**< 0.0001 (U=99)**	**< 0.0001 (U=74)**
ΣHCHs	23.8(11.6) ± 30.2 (0.8–94.7)	28.9(15.8) ± 34.2 (0.0–71.7)	0.725	85(59) ± 92 (8–330)	208(208) ± 0.0 (208–208)[b]	–	Not analysed	Not analysed	–	
HBCD	167(148) ± 66.4 (100–354)	173(153) ± 122 (22.0–401)	0.910	121(134) ± 57.6 (31.2–214)	58.4(56.5) ± 25.5 (26.8–88.4)	**0.007 (U=26)**	19.9(21.3) ± 12.7 (0.0–43.2)	11.4(9.5) ± 10.7 (0.0–39.9)	**0.007 (U=280)**	
ΣICES7/ΣPCBs	0.8(0.7) ± 0.2 (0.5–1.0)	0.7(0.6) ± 0.1 (0.6–1.0)	**0.007 (U=165)**	0.8(0.8) ± 0.1 (0.6–1.0)	0.7(0.7) ± 0.1 (0.6–1.0)	0.072	0.7(0.7) ± 0.04 (0.7–0.8)	0.7(0.7) ± 0.06 (0.4–0.8)	**0.004 (U=367)**	0.238
ΣDDTs/ΣPCBs	1.6(1.1) ± 2.4 (0.7–13.4)	1.0(0.9) ± 0.5 (0.3-2.3)	0.085	2.1(1.3) ± 2.8 (0.0-13.8)	(0.9) ± 0.2 (0.6–1.3)	**0.014 (U=94)**	1.3(1.2) ± 0.4 (0.6–2.3)	1.6(1.4) ± 0.8 (0.2–4.3)	0.0932	0.375
DDE/DDT[c]	15.3 (14) ± 3.3 (11.3–21.2)	16.5(16.6) ± 5.3 (10.2–34.1)	0.910	9.6(9.1) ± 3.3 (4.6–15.7)	9.6(9.4) ± 3.0 (6.8-16.6)	0.667	6.35(5.0) ± 4.5 (1.2–27.3)	12.4(5.5) ± 11.1 (2.6–39.2)	0.347	**< 0.0001 (X^2=42)**

[a] Sum of NDL congeners n. 8,18,28,52,44,66,101,87,153,138,187,128,180,170,195,206,209 and DL congeners n. 118,105,77,81,126,169,144,123,156,157,167,189.
[b] Only 1 sample.
[c] Calculated as sum of p,p′DDE + o,p′DDE/Sum of p,p′DDT + o,p′DD.

with an automated purification system: Power Prep™ (FMS, Waltham, USA). Finally, the samples were analysed by GC–HRMS on an Autospec Ultima (Micromass/Waters, Manchester, UK) coupled to an Agilent 6890 GC (GMI, Minnesota, USA). The injection was processed in splitless mode and the mass spectrometry via electron ionization using a selected ion-monitoring (SIM) mode.

For the analysis of pesticides 25 μl were extracted from the mono-*ortho* samples and added in another vial. The vials were then spiked with 25 μl of MIREX (100 pg μl^{-1}) that was used as reference standard. The analysis was performed in the Thermo Quest Trace 2000 as Chromatograph, equipped with a ^{63}Ni ECD detector (Thermo Quest, Trace 2000, USA). Quantification was performed by comparison with the internal standards in a certified calibration mixture (Corbetanone curve).

To assess the toxic potency of the dioxin-like CBs in the whale blubber, samples concentrations of PCDD/Fs and dioxin-like PCBs were multiplied by the appropriate toxic equivalency factor (TEF – Table SI.1), recommended by World Health Organization for human and wildlife health (Van den Berg et al., 2006). In this way the toxicity of each dioxin was weighted relative to the most toxic one, the 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD).

Recovery rates for NDL-PCBs ranged between 20% and 86%, for PBDEs between 10% and 50% and for DL compounds between 30% and 100%. Results were not corrected for recoveries as quantitation is based on isotope dilution technique. In order to express our results on lipid weight basis (lw), samples presenting a lipid percentage lower than 2% were excluded from the statistical analysis (18 out of 61 samples of sperm whales).

2.4. Stable isotopes analysis

30 samples from sperm whales, 16 from long-finned pilot whales and 12 fin whales were analysed from 2012–2013 biopsies. δ^{15}N and δ^{13}C values for other 164 fin whales (years 2010–2012) were extracted from a previous study (Das et al., unpublished data). Skin samples were freeze-dried for 24 h and homogenized. Lipids were extracted in a 2:1 chloroform/methanol solution (Folch et al., 1957). This process was conducted in order to minimize the possible error given by the presence of ^{13}C-depleted lipids (Post et al., 2007; Lesage et al., 2010).

Isotopic ratios were expressed in delta (δ) notation (hereafter, noted as δ^{15}N and δ^{13}C, for nitrogen and carbon stable isotopic composition, respectively) in parts per thousand (‰) using Vienna Pee Dee Belemnite (vPDB) and atmospheric nitrogen as international standard (IAEA, Vienna, Austria). IAEA-C_6 and IAEA-N_2 (NH_4SO_4 IAEA-N_2 $\delta^{15}N = 20.3 \pm 0.2$‰; Sucrose IAEA-C6: $\delta^{13}C = -10.8 \pm 0.5$‰) were used as certified internal standards. Standard deviations on multi-batch replicate measurements of glycine were 0.3‰ and 0.2‰ for δ^{15}N and δ^{13}C, respectively. The small amount of skin from pilot whales precluded any lipid extraction. Therefore, we applied the lipid normalization equation (McConnaughey and McRoy, 1979) adapted by Post et al. (2007) for aquatic animals:

$$\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99 \times (C: N)$$

Where the numerical values represent respectively the line ($b = -3.32$) and the intercept ($a = 0.99$) of the regression line equation, and C:N the explanatory variable. Following Post et al. (2007) recommendations, the normalization was conducted only on samples presenting a C:N ratio between 3 and 4. In our case, all the pilot whale samples could be normalized. Samples were analysed using an EA-IRMS (Optima, Isoprime, U.K.) coupled in continuous flow to an elemental analyser (Vario microtube, Elementar, Germany).

2.5. Statistical treatment

The normality of the data was checked by a D'Agostino & Pearson omnibus normality test. The non-parametric Kruskal–Wallis analysis of variance was used for interspecific comparisons of all the chemical tracers. If a significant difference was obtained (p-value < 0.05), the post hoc Dunn's multiple comparison was performed for individual comparisons of species. The Discriminant Analysis (DA) was carried out for structuring the three species resting on their congener's profile. Significant differences in the profiles were then assessed with 2-way ANOVA test with the Bonferroni post hoc multiple comparison of means. The analysis of variance between the two sexes of each species was conducted using the unpaired two-tailed Mann–Whitney t-test ($p < 0.05$). The unpaired t-test with Welch's correction was then applied to test for difference of variances in POPs concentrations among sexes. Correlation between stable isotopes and ΣPCBs, ΣPBDEs and ΣDDTs was performed with the Spearman correlation ρ ($p < 0.05$). All the statistical analyses were conducted with the software Graph Pad, Prism 5. DA was conducted using the software package JMP statistical discovery software.

3. Results

Analyses of 29 PCBs congeners (17 NDL-PCBs, 12 DL-PCBs), 10 PBDEs, 6 DDTs, HBCD, α-HCH, β-HCH, γ-HCH, HCB, aldrin, dieldrin and β-endosulfan were conducted on 61 sperm whales and 49 long-finned pilot whales (Table 1). For fin whales ($n = 70$) the analysis included 19 PCBs (17 NDL-PCBs and 2 DL-PCBs), 3 PBDEs, 6 DDTs, HBCD and γ-HCH. The ΣICES7 includes priority PCBs congeners 28, 52, 101, 118, 138, 153 and 180, listed by the International Council for the Exploration of the Sea for International Comparisons. The ΣPCBs is the total sum of the 31 PCB congeners that were analysed between 2006 and 2013; the ΣDDTs is the sum of p,p′DDT, o,p′DDT, p,p′DDE, o,p′DDE, p,p′DDD and o,p′DDD; and the ΣPBDEs is the sum of the 9 PBDEs congeners analysed. We also calculated the following ratios: ΣICES7/ΣPCBs, ΣDDE/ΣDDT and ΣDDTs/ΣPCBs. The ΣDDE/ΣDDT was measured as (p,p′DDE+o,p′DDE)/(p,p′DDT+o,p′DDT).

3.1. Range of lipids and POP concentrations

For long-finned pilot whales and sperm whales lipids percentage differed significantly between species and year of sampling (Kruskal–Wallis test: $p = 0.032$, $X^2 = 10.55$; $p < 0.0001$, $X^2 = 30.75$ respectively). Lipid content of fin whales instead remained similar with time (Kruskal–Wallis $p = 0.586$, $X^2 = 1.936$). Long-finned pilot whales sampled in 2009 presented lower lipid percentage than pilot whales from 2010 (8% and 35% respectively). Similarly, lipid percentages of sperm whales sampled in 2012 were lower compared to 2010 and 2013 ones (2%, 18% and 11% respectively). For the other years the difference was less important.

All the analysed compounds were detected at quantifiable levels (> LOQ) in our samples excepted for Aldrin and β-endosulfan in long-finned pilot whales and sperm whales (< LOQ). ΣPCBs, ΣICES7, ΣDDTs and ΣPBDEs concentrations were significantly different among the three species with long-finned pilot whales showing the highest values (Table 1). Male fin whales displayed significantly higher POP concentrations than females (Table 1). Male sperm whales displayed significantly higher concentrations of ΣPBDEs, ΣDDTs and HBCD than females, while no difference was observed for ΣPCBs (Table 1). ΣHCHs was analysed in one female sperm whale, precluding any comparison between sexes. POP concentrations remained similar between males and females of long-finned pilot whale, with however a higher variability for

Table 2
Blubber PCDD (ng g^{-1} lw), PCDF (ng g^{-1} lw) and dioxin-like PCBs (ng g^{-1} lw), total TEQ (pg WHO-TEQ g^{-1} lw), and percentage to T-TEQ of sperm whales and long-finned pilot whales from the Mediterranean Sea. Data are showed as Mean (Median) ± standard deviation (Min – Max values) and n = numbers of samples. Interspecies comparison is shown as p-values and the statistical U of the Mann–Whitney test.

Compounds	**Species**				**Mann–Whitney**
	Sperm whales		**Long-finned pilot whale**		
	ng g^{-1} lw	**pg WHO-TEQ g^{-1} lw**[a]	**ng g^{-1} lw**	**pg WHO-TEQ g^{-1} lw**	
N	15		15		
ΣPCDDs	0.36 (0.35) ± 0.31 (0.12–0.13)	40.9 (42.0) ± 24.0 (14.0–100)	0.06 (0.04) ± 0.06 (0.002–0.2)	4.5 (4.7) ± 1.0 (0.8–5.5)	**< 0.0001 (*U* = 11.0)**
ΣPCDFs	0.16 (0.11) ± 0.17 (0.06–0.74)	15.7 (14.1) ± 7.4 (8.3–38.7)	0.08 (0.04) ± 0.08 (0.01–0.3)	7.8 (5.8) ± 8.8 (1.3–36.5)	**0.016 (*U* = 54.0)**
Σnon-ortho PCBs	6.3 (5.7) ± 2.17 (3.7–12.0)	343 (311) ± 148 (181–784)	2.4 (2.5) ± 1.0 (0.4–3.7)	75.3 (92.1) ± 50 (0.0–181)	**< 0.0001 (*U* = 0.0)**
Σortho PCBs	2114(1796) ± 1066 (961–4735)	63.4 (54.0) ± 32.0 (29.0–142)	1813 (1814) ± 1250 (87–3627)	50.7 (52.3) ± 37 (2.6–109)	0.308 (*U* = 100.0)
Total TEQ (pg WHO-TEQ g^{-1} lw)	812 (726) ± 349 (447–1833)		223 (244) ± 130 (12.2–472)		**< 0.0001 (*U* = 19.0)**
ΣPCDD/Fs to T-TEQ (%)	13 (13) ± 3 (8–21)		10 (8) ± 6 (4–20)		0.064 (*U* = 67.4)
Σnon-ortho PCBs to T-TEQ (%)	13 (13) ± 2 (9–19)		31 (34) ± 15 (7–71)		**< 0.0001 (*U* = 30.5)**
Σortho PCBs to T-TEQ (%)	73 (73) ± 4 (65–80)		59 (60) ± 12 (25–74)		**< 0.0001 (*U* = 18.5)**

[a] TEQ (toxic equivalents) are calculated from TEFs values (toxic equivalent factors) as follow: TEQ = Σ_{n1}[$PCDD_i$ x TEF_i] + Σ_{n2}[$PCDF_i$ x TEF_i] + Σn[PCB_i x TEF_i]. TEFs values by Van den Berg et al. (2006).

females (Unpaired t test with Welch's correction – ΣPCBs $p = 0.031$, $t = 0.564$; ΣDDTs $p = 0.006$, $t = 0.304$; ΣPBDEs $p = 0.006$, $t = 0.661$).

The ΣICES7/ΣPCBs and ΣDDTs/ΣPCBs ratio did not vary among the species (Table 1). On the contrary, the DDE/DDT ratio highly differed, with long-finned pilot whales presenting the highest values. ΣICES7/ΣPCBs ratio highly differed between males and females for both fin whales and long-finned pilot whales. By contrast, these ratios remained similar between male and female sperm whales. Long-finned pilot whales and fin whales did not display any difference in ΣDDTs/ΣPCBs ratio between the two sexes; male sperm whales, instead, presented significantly higher values than females. Finally, the DDE/DDT ratio demonstrated to not be influenced by sex-related differences for all the three species.

3.2. *Concentration of dioxin-like compounds*

Sperm whales displayed the highest concentrations for Σnon-*ortho* PCBs, ΣPCDDs and ΣPCDFs (Table 2), whereas Σ*ortho* PCBs concentrations (ng.g^{-1} lw) remained similar between species. When data were expressed on a TEQ basis (pg WHO-TEQ g^{-1} lw) the same differences were found and accentuated. PCDDs and PCDFs accounted for 10 to 13% of the total TEQ (Table 2) without important differences between the species. The Σ*ortho* PCBs accounted for 73% and 59% of the total TEQ in sperm whales and long-finned pilot whales respectively; and finally, the Σnon-*ortho* PCBs accounted for 13% and 31% of the total TEQ for sperm whales and long-finned pilot whales respectively.

3.3. *Congeners profiling*

The three species showed a similar profile with respect to the different contribution of DL-PCBs, NDL-PCBs, DDTs, and PBDEs (2-way ANOVA, $p = 0.944$ F = 0.058, Fig. SI.1). DDTs resulted to be the most abundant pollutant (in average 54%), followed by NDL-PCBs (40%) and DL-PCBs (5%) (Fig. SI.1). PBDEs and the sum of HCHs, instead, represented less than 2%. On the other hand, among the DL compounds almost 100% of the profile was dominated by the Σortho-PCBs in both the two species when expressing the data on lipid weight. When expressed in pg WHO-TEQ.g^{-1} lw Σortho-PCBs remained the dominant pollutants, followed by non-ortho PCBs and ΣPCDD/Fs.

The most abundant NDL-congener was PCB153 (6 Cl), known to be the most difficult to metabolize and therefore usually the most bio-accumulated, followed by PCB138 (6 Cl), PCB180 (8 Cl), and PCB 187 (8 Cl), in all the three species (Fig. SI.2A). The two most abundant DL-PCBs were, instead, PCB118 (5 Cl) and PCB105 (5 Cl) representing respectively the 59% and 31% in long-finned pilot whales and 61% and 22% in sperm whales (Fig. SI.2B). In all the species the PCBs profile was not influenced by sex-related differences. The most abundant PBDE congeners were the BDE47 and 99 in all the three species (Fig. SI.2C), which represented respectively 65–75% and 13–18% in all the three species. No difference between the sexes was observed in the two odontocetes (Mann–Whitney, Gm $p = 0.341$, $U = 239.5$ for BDE47 and $p = 0.162$, $U = 218.0$ for BDE99; Pm $p = 0.061$, $U = 149.0$ for BDE47 $p = 0.34$, U = 191.0 for BDE99), while male and female fin whales manifested highly significant variation in the profile for both the congeners (Mann–Whitney, $p < 0.0001$, $U = 126.0$; $p < 0.0001$, $U = 203.5$, respectively). Finally DDTs congeners (Fig. SI.2D) did not show a significant difference between the three species (2-way ANOVA, $p = 0.999$ $F = 0.0001$); and no variation (2-way ANOVA, $p = 1.000$ $F = 0.009$) was observed when taking in consideration males and females separately.

Among all DDTs congeners, 4,4′DDE was the most abundant representing the 85.3% in long-finned pilot whales, 90.1% in sperm whales and 75.0% in fin whales (Fig. SI.2D). To better characterise our species a discriminant analysis (DA) was used. The first principal component (PC1) explained the 71% of the total variability in the dataset, while the second principal component (PC2) expressed the 29% (Fig. 2). The eigenvalues loadings showed that along the PC1 the highly chlorinated PCB congeners 138, 153, and 187, 180 and 206 were the most influential in differentiating our three species, whilst for PC2 most of the variability was explained by 2,4′DDT and BDE99. Sperm whales, pilot whale and fin whales resulted very well differentiated in three groups (Fig. 2). The horizontal axis neatly separates fin whales (red points) from the odontocetes (pilot whales: green points; sperm whales: blue points); whereas the second axis strongly differentiates sperm whales from the other two species.

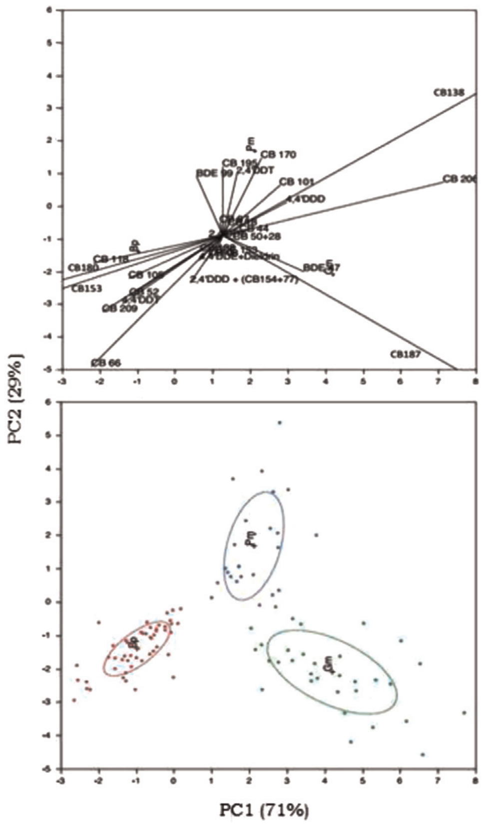

Fig. 2. Discriminant analysis bi-plot of the different analysed congeners and the different species resulted from the DA analysis. Long finned-pilot whales are represented by the purple diamonds; sperm whales by the orange up-triangles; the fin whales by the blue circles. Data are expressed as ng g^{-1} lw.

3.4. Stable isotopes analysis

$\delta^{13}C$ ranged between -17.3 ± 0.4‰ in sperm whales and -17.8 ± 0.3‰ in long-finned pilot whales and differed significantly from fin whales (-18.7 ± 0.4‰ – Fig. 3). The highest $\delta^{15}N$ values were measured in sperm whales (12.2 ± 1.3‰), followed by long-finned pilot whales (10.5 ± 0.7‰) and fin whales (7.7 ± 0.8‰), showing a significant difference between the three species (Kruskal–Wallis $p<0.0001$, $X^2=97.54$). No significant differences were found between males and females of long finned-pilot whales (Mann–Whitney $\delta^{15}N$, $p=0.713$, $U=26.0$; $\delta^{13}C$ $p=0.955$, $U=26.0$) and fin whales (Mann–Whitney $\delta^{15}N$ $p=0.331$, $U=3413$; $\delta^{13}C$ $p=0.318$, $U=2985$). Due to the presence of only a female sperm whale, gender-related differences could not be investigated. For fin whales it was possible to evaluate $\delta^{15}N$ and $\delta^{13}C$ differences between pregnant and non-pregnant females, but no significant variation was found for both isotopes (Mann–Whitney $\delta^{15}N$ $p=0.523$, $U=663.5$; $\delta^{13}C$ $p=0.833$, $U=640.5$).

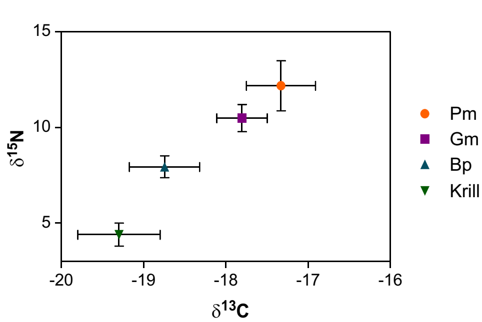

Fig. 3. Mean ($\pm$SD) $\delta^{13}C$ and $\delta^{15}N$ values (‰) of sperm whale (Pm), long-finned pilot whale (Gm), fin whale (Bp) and krill Meganyctyphanes norvegica. For the $\delta^{15}N$ values the raw data (non-lipids extracted and non-normalized) were used for all species. For odontocetes, data from samples normalized in ^{13}C were used; for fin whales ^{13}C values from lipid extracted samples were used. Data on krill were integrated from Bentaleb et al. (2011).

3.5. Correlation between stable isotopes and pollutants

In sperm whales $\delta^{15}N$, ΣPCBs and ΣICES7 correlated significantly ($\rho=0.657$, $p=0.004$, $R^2=0.492$; $\rho=0.588$, $p=0.013$, $R^2=0.458$ respectively). A positive but not significant correlation was instead observed for ΣPBDEs and ΣDDTs ($\rho=0.435$, $p=0.092$, $R^2=0.045$; $\rho=0.061$, $p=0.830$, $R^2=0.105$ respectively). No significant relationship was found between $\delta^{15}N$ and POPs for long-finned pilot whales (Fig. SI.3). Likewise, the correlation analysis did not show any relationship between $\delta^{13}C$ and POPs concentrations for both species (Fig. SI.4).

4. Discussion

4.1. Diet and trophic position

Among the odontocetes, sperm whales displayed the highest $\delta^{15}N$ values than long-finned pilot whales. As expected instead, $\delta^{13}C$ and $\delta^{15}N$ values in skin of fin whales were lower, reflecting the different dietary behaviour. Indeed, fin whales mainly forage during summer on a small euphausiacean, *Meganyctyphanes norvegica* (Bentaleb et al., 2011; Druon et al., 2012), while long-finned pilot whales and sperm whales are teuthophageous, feeding exclusively or preferentially on bathypelagic cephalopods of the Histioteuthidae and Ommastrephidae family (Astruc and Beaubrun, 2005; Praca and Gannier, 2008). Stomach content analysis from stranding events in the French and Italian coasts, showed that sperm whales are true specialists in terms of food selection, as 90% of their diet consists on species of the Histioteuthidae family (Praca and Gannier, 2008; Praca et al., 2011). Long-finned pilot whales are more generalist feeders as 40–50% of their diet consists of *Todarodes sagittatus*, 10–20 % of *H. bonnelii and H. reversa* and 10% is made up by several other prey species (Praca and Gannier, 2008; Praca et al., 2011). Thus, the higher $\delta^{15}N$ values for sperm whales may be related with higher proportion of Histioteuthidae among their prey. Indeed, Histioteuthidae present higher $\delta^{15}N$ values than other squids, octopods or cuttle-fish (Cherel et al., 2009; Praca et al., 2011). $\delta^{13}C$ values change among food sources (terrestrial *vs.* marine) and habitat distribution (nearshore *vs.* offshore), therefore two species that feed on the same prey in the same area, should present equal ^{13}C signatures (Layman et al., 2012). This is the case for the two odontocetes, which had similar $\delta^{13}C$ values, suggesting a partial overlap of their feeding habitats. Different studies had already shown that sperm whales are highly opportunistic in their habitat use, being not only present along the entire continental slope but also extending in more oceanic waters where long-finned pilot whales usually occur (Drout et al., 2007; Praca and Gannier 2008; Praca et al., 2011). The low $\delta^{13}C$ values displayed by fin whales could be, instead, a consequence of their lower trophic level, since $\delta^{13}C$ values demonstrated to slightly increase from prey to predator (<2‰; McCutchan et al., 2003; Bentaleb et al., 2011); but also of specific migratory paths presented by this species in the NWMS. Indeed, previous $\delta^{13}C$ results on baleen plates and satellite tracking showed how fin whales and krill inhabiting the Atlantic Ocean are depleted in ^{13}C and how some Mediterranean fin whales tend to migrate during winter towards the Gibraltar Strait and feed on ^{13}C-depleted krill. This explains, then, the lower $\delta^{13}C$ values found in our study (Cotte et al., 2009; Bentaleb et al., 2011).

One of the main factors influencing contaminant concentrations in marine mammals is the trophic level at which they feed (Caban and Rasmussen, 1994; Fisk et al., 2001). Hydrophobic POPs tend to biomagnify along the food webs resulting in greater concentrations with increasing trophic level (Fisk et al., 2001; Newsome et al., 2010). Subsequently, higher concentrations of POPs have been often related with higher $\delta^{15}N$ values (Cabana and Rasmussen, 1994; Fisk et al., 2001; Newsome et al., 2010). This seems not to be the case for our odontocetes species, for which in contrast with trophic data, the most contaminated species for all classes of POPs is the long-finned pilot whale. Fin whales, not surprisingly, presented the lowest concentrations. Several biotic and non-biotic factors such as gender, age, specific blubber metabolism, body size and species distribution, can influence marine mammals final burden of contaminants, and explain interspecies differences (Aguilar 1987; Aguilar et al., 1999; Voorspoels et al., 2003; Weijs et al., 2013).

4.2. Sources of variation

4.2.1. Sex and the age

While both sperm whales and fin whales showed sex-related differences in pollutants burden, concentrations between the two sexes of pilot whales were similar, although higher variability was observed in females. The transfer of organic pollutants from females to offspring and the consecutive lower concentrations in females are well documented for marine mammals (Abarnou et al. 1986, Aguilar and Borrell, 1988; Borrell et al., 1995; Voorspoels et al. 2003; Vos et al. 2003; Weijs et al., (2013). Studies on long-finned pilot whales reported transfer rates of DDTs or PCBs from mother to calf of 70% via lactation and circa 8% during gestation (Borrell et al., 1995). In the same way, fin whales, presented transfer rates ranging between 72% and 98% during lactation (Aguilar and Borrell, 1988). Therefore, while POPs concentration in males either remain stable or increase with age, in sexually mature females contaminant burdens generally decline as long as they are reproductively active (Aguilar and Borrell, 1988; Borrell et al., 1995; Schwacke et al., 2002). The high variability observed in POPs concentrations in female pilot whales from the Mediterranean Sea could be explained by the fact that these transfer mechanisms are negatively affected by age and number of reproductive cycles (Borrell et al., 1995; Weijs et al., 2013). The highest transfer rates of pollutants from mother to calf were reported from primiparous females, indicating that the major load of contaminants is imparted to their firstborn calf (Aguilar and Borrell, 1988; Aguilar and Borrell, 1994a, 1994b; Schwacke et al., 2002; Weijs et al., 2013). During the successive reproductive cycles, the overall pollutants burden transferred from the mother to the calf seems to substantially decrease (Aguilar and Borrell, 1994a, 1994b; Evans et al., 2004; Weijs et al., 2013). For example, it was demonstrated that young females of long-finned pilot whales can transfer concentrations of pollutants between 3 and 6 times higher than old females (Borrell et al., 1995).

4.2.2. Species behaviour and physiology

The same contamination pattern with long-finned pilot whales as the most polluted species was reported in Borrell et al. (1995). The authors suggested that the pollutants burden is positively correlated with trophic levels and negatively correlated with body size (Borrell et al., 1995). Body size influences metabolic rates and consequently the pollutants load (Aguilar et al., 1999; Borgå et al., 2004). The high mass-specific metabolism of small sized cetaceans force these animals to consume large amount of food on a daily basis, thus they are exposed to higher intake rates of lipophilic contaminants (Aguilar et al., 1999). Moreover, the larger size of an organism may result in dilution of the accumulated pollutants in the lipids store (Borgå et al., 2004). Therefore, the highest pollutants burden showed by long-finned pilot whales could be linked, in the Atlantic as well as in the NWMS, to their smaller body size (length $\approx$ 5.4 m in adult females and 6.2 m in adult males) in comparison to sperm whales (length $\approx$ 17 m in adult males) (Borrell et al., 1995).

In the DA scatterplot (Fig. 2) our species are distributed along the first or second principal component resting on their characteristics in POPs profile. The length of each congener line reflects the importance of that congener along the axis and the angle between the line and the axis shows the direction of their correlation ($<90°$ = positive correlation; $90°$ = no correlation; $>90°$ = negative correlation). The loadings of the eigenvalues analysis reported how the highly chlorinated PCBs were the most influencing along PC1, while 2,4'DDT and BDE99 were the most influential along PC2. The net separation between the fin whale and the two odontocetes along PC1, then, very well reflects the difference in concentrations of the most recalcitrant PCB congeners (7, 6 and 5 chlorinated): with the two odontocetes associated to the highest levels and the fin whales characterised by lower levels. This is coherent with the reported concentrations and their trophic distribution (see Table 1 and Section 4.1). On the other hand, along PC2 sperm whales strongly differentiate from the other two species. This could be a consequence of the seasonal migrations of sperm whales, especially young males, in the eastern basin of the Mediterranean Sea, where higher concentrations of DDTs are still discharged in the marine environment (Mendes et al., 2007; Ryan et al., 2013b; Marsili et al., 2014).

The importance of species distribution influence in species contamination patterns can be additionally confirmed by differences in pollutants ratios (Aguilar and Borrell, 1988; Ross et al., 2000). In our case, The only ratio to express variation among the species was the ΣDDE/ΣDDT, which displayed the highest values in long-finned pilot whales. DDE is a derivate product of the DDT metabolism, which is produced through mechanisms of dehydrochlorination taking place in both living organisms and in abiotic environmental systems, and consequently becomes more abundant over time and food webs (Aguilar and Borrell, 1988). Therefore, an increasing proportion of DDE in relation to the total DDT can reflect a decreasing exposure to new sources of DDT pollution (Aguilar and Borrell, 1988). Thus, the high trophic level of pilot whales and their preferential oceanic distribution, which limits exposure to new DDTs input, can explain the higher ratios of ΣDDE/ΣDDT showed by pilot whales in this study.

4.2.3. Geographical distribution

The overall mean concentrations in Mediterranean long-finned pilot whales, sperm whales and fin whales demonstrated to be relatively higher compared to those from the Southern hemisphere (Table 3) and the North Atlantic Ocean (*e.g.* Faroe Islands and Gulf of St. Lawrence) (Aguilar and Borrell, 1988; Borrell et al., 1995; Gauthier et al., 1997b; Rotander et al., 2012). The only exception was given by PBDEs concentrations, which resulted higher in the North-Atlantic than in Mediterranean and Tasmania (Praca et al., 2011; Law et al., 2014; Weijs et al., 2013; Marsili et al. 2014). This is possibly related to the greater historical usage of these compounds in North America (Law et al., 2014). Also ΣPCDD/Fs and ΣDL-PCBs values of our two toothed-whales were exceeding concentrations found in Tasmanian sperm whales and long-finned pilot whales, in harbour seals from British Columbia and harbour porpoises from the Skarregat-Kattegat (Table 4; Bergreena et al., 1999; Ross et al., 2004; Gaus et al., 2005). Noteworthy, both sperm whales and pilot whales presented the same concentration ranges of ΣPCDD/Fs, and higher levels of ΣDL-PCBs, than Franciscana dolphins from the Guanarama Bay in Brazil which, due to the

Table 3
PCBs, PBDEs and DDTs (ng g^{-1} lw) in blubber samples of the selected species from previous literature. Data are expressed as Mean ± SD (when possible), number of individuals of each species/geographic zone of cetaceans sampling, sex and year of collection. F: females; M: males; IM: immature males; AM: adult males; AFNL: adult females not lactating; AFL: adult females lactating.

References (Year)	Geographic area	Species	N	Sex	ΣPCBs	ΣPBDEs	ΣDDTs
Aguilar (1983)[a,b]	North Atlantic	Sperm whale	7	M	9930	–	7730
				F	15,550		5100
Borrell et al. (1995)[b]	North Atlantic	Long-finned pilot whale	52	M	48,810 ± 23,130	20230 ± 15180	31,390 ± 9230
		Sperm whale	10	M	10,510 ± 2070	4160 ± 1040	7800 ± 1510
		Fin whale	48	M	1260 ± 610	490 ± 290	850 ± 460
Gauthier et al. (1997b)	North-eastern Atlantic	Fin whale	15	nd	2677 ± 2850	–	3812 ± 3908
Hobbs et al. (2001)[b]	North-eastern Atlantic	Fin whale	6	F	2490	–	6880
			6	M	3790		4470
Pettersson et al. (2004)[b]	Italy	Fin whale	1	F	–	483	–
		Long finned pilot whale	1	M		886	
Evans et al. (2004)[bc]	Southern Australia	Sperm whale	32	F	800 ± 400	–	1700 ± 1900
			5	M	1300 ± 1200		3100 ± 3600
Rotander et al. (2012)[b]	Atlantic	Long finned pilot whale	3	nd	–	1041(840-1307)	–
		Fin whale	3			22 (20–31)	
Praca et al. (2011)[b]	NWMS	Long finned pilot whale	4	F/M	66,020 ± 57,910	–	35,380 ± 33,630
		Sperm whale	12	F/M	107,810 ± 108,720		115,980 ± 112,350
Marsili et al. (2014)[b]	Italy	Sperm whale	7	F/M	190,635 ± 340,091	–	205,176 ± 337,634
Weijs et al. (2013)[b]	Tasmania	Long finned pilot whales	10	IM	404 ± 53	10 ± 3	983 ± 141
			5	AM	380 ± 88	13 ± 3	997 ± 223
			9	AFNL	244 ± 150	8 ± 4	528 ± 408
			8	AFL	167 ± 114	6 ± 4	347 ± 311
Our study (2015)	NWMS	Long-finned pilot whales	49	F/M	38,666 ± 25,731	712 ± 412	46,081 ± 37,506
		Sperm whale	42	F/M	22,849 ± 15,566	347 ± 173	37,647 ± 38,518
		Fin whales	75	F/M	5721 ± 5180	177 ± 208	6643 ± 5549

[a] Expressed in μg g^{-1} lw.
[b] Stranded or caught animals.

particularly heavy urbanization of that area, are exposed to massive levels of toxic substances and are, nowadays, considered as one of the most polluted cetacean in the world (Dorneles et al., 2013).

Despite the differences in pollutants concentrations, congeners profile of both DL and NDL compounds of long-finned pilot whales, sperm whales and fin whales did not vary either among the three species in the NWMS or in comparison with other geographical regions (Dam and Bloch, 2000; Evans et al., 2004). The ΣDDTs resulted as the most abundant pollutants in all three species. This was in accordance with concentrations reported in sperm whales and long finned pilot whales studied in the Southern hemisphere, but highly differed from animals from the eastern North Atlantic and the Mediterranean (Borrell et al., 1995; Pettersson et al., 2004; Mazzariol et al., 2011; Praca et al., 2011; Marsili et al., 2014). As for PBDEs, the higher concentrations of DDTs with respect to PCBs of our species compared to the North Atlantic may be a reflection of the different pollutant usage and global sources distribution (Evans et al., 2004). In fact, in the Mediterranean Sea new input of PCBs and pesticides still occur from the southern countries, where international regulations for these compounds are not yet applied (UNEP/MAP 2012). Moreover, the extremely elevated levels generally presented by our species in all classes of POPs in comparison with other parts of the world, may be related to the geomorphological structure of the Mediterranean Sea. Indeed, this semi-enclosed Sea receives new input of water only from the shallow and narrow Gibraltar Strait, which leads to slow water recycle and, consequently, limited dispersal of discharged hazard-substances (UNEP/MAP, 2012).

4.2.4. Analytical methods

The difference in pollutants profile between our species and the other studies conducted in the Mediterranean Sea may anyway be linked to different analytical methods. Previous authors, in fact, analysed POPs concentration on stranded animals, instead of using biopsies. Several papers demonstrated how POPs concentrations in cetaceans blubber are strongly influenced by its thickness, stratification and lipid profile (Aguilar, 1987; Gauthier et al., 1997a; Evans et al., 2003; Koopman et al., 2007). The sampling from stranded animals permits the collection of all the three layers of blubber, while with biopsies only the few cm of the outermost layers are usually gathered. This could strongly influence the resulting concentrations of pollutants and explain differences between our study and previous literature (Aguilar, 1987; Gauthier

Table 4
Mean dioxin-like PCBs (ng g^{-1} lw) and PCDD/Fs concentrations (pg g^{-1} lw) in blubber samples from previous literature. Data are expressed as Mean ± SD (when possible), number of individuals of each species/geographic zone of cetaceans sampling, sex and year of collection. F: females; M: males.

Reference (Year)	Geographic area	Species	N	Sex	ΣPCDD/Fs	ΣDL-PCBs
Berggrena et al. (1999)	North Sea Kattegat-Skarregat	Harbour Porpoises	47	M	18	1085
Ross et al. (2004)	British Columbia	Harbour Seals	22	F/M	216	–
Gaus et al. (2005)	Tasmania (Southern Australia)	Sperm whales	7	F/M	40	29
		Long-finned pilot whales	1		19	66
Dorneles et al. (2013)	Guaranama Bay (Brazil)	Franciscana Dolphin	19	F/M	398	288
Our Study (2015)	North-Western	Sperm whales	42	F/M	523 ± 414	2120 ± 1490
	Mediterranean Sea	Long-finned pilot whales	49		139 ± 119	1816 ± 1281

et al., 1997a; Evans et al., 2003; Ryan et al., 2013a). Finally, the type of extraction used during the experimental procedure also demonstrated to be influential in final lipid percentages (Evans et al., 2003; Tapie et al., 2008). Hexane-based methods (as in the present study) proved to be less efficient in extracting hydrophobic and complex lipids like wax esters (WE) as chloroform-based methods (Evans et al., 2003). Thus, in the case of sperm whales, which present the external blubber layer composed mainly of WE, our extraction method could have partly influenced the resulting concentrations (Lockyer, 1991; Evans et al., 2003).

4.3. Toxicity

Assessment of toxicity of persistent organic pollutants in marine mammals has always been very complex, because obvious logistical and ethical considerations preclude any definite cause-effect relationships (Schwacke et al., 2002; Ross, 2002). However, post-mortem investigation of marine mammals combined to pollutant analysis (Jepson et al., 1999; Siebert et al., 1999; Das et al., 2004, Weijs et al., 2013), *in vitro* experiments (Ross, 2002; Vos et al., 2003), probability risk assessment (Schwacke et al., 2002) and exposure of other mammals have suggested that these compounds may affect immune and endocrine systems of the species with juveniles placed at higher risk because of the maternal transfer and immature immune system (Weijs et al., 2013).

For example, in the Mediterranean Sea, the impairment of immunologic response was associated with high mortality rates of local cetacean populations during the Cetaceans Morbillivirus epizootic (CeMV) between 2006 and 2008 (Aguilar and Borrell, 1994a, 1994b; Van Bressem et al., 2014). This epidemic, believed to have spread through the Gibraltar Strait, was found in stranded striped dolphins (*Stenella coeruleoalba*) and bottlenose dolphins (*Tursiop truncatus*), as during the first epizootic event in 1990-1991. For the first time DMV was reported in 25 long-finned pilot whales and four fin whales stranded along the Tuscan coast of Italy (Aguilar and Borrell, 1994a, 1994b; Fernàndez et al., 2008; Keck et al., 2010; Di Guardo et al., 2013; Mazzariol et al., 2012). Histo- and toxicological analysis showed how all the individuals which succumbed to the infection were highly contaminated, suggesting that accumulation of high levels of hazard substances increase the sensibility of marine mammals to viral infections (Aguilar and Borrell, 1994a, 1994b; Gabrielsen, 2007; Mazzariol et al., 2012). No threshold values for POPs toxicity potential have been yet defined for fin, pilot and sperm whales. However in this study it appears that 23 out of 42 sperm whales and 35 out of 49 pilot whales displayed ΣPCB concentrations higher than the 17 mg/kg lw threshold set by Jepson et al. (2005) and Kannan et al. (2000), above which deleterious effects on the individuals health may occur.

The use of TEFs to achieve TEQ constitutes an accurate procedure for the assessment of the toxic risk associated with a complex mixture of pollutants that are capable of triggering Aryl hydrocarbon (Ah) receptor mediated effects, such as DLCs (Dorneles et al., 2013). This method permits to operate risk assessment of cetaceans based on the different contribution of each pollutant to the total TEQ (Van der Berg et al., 2006). In our case, dioxin-like PCBs accounted for over 80% of the total TEQ for all cetaceans, showing that most of the toxic effect of POPs pollution in NWMS cetaceans is exercised by the presence of PCBs. It raises concern to see how these compounds, banned more than 40 years ago, are still today, the main source of possible health impairment. Conversely from NDL-compounds pattern, the higher concentrations of PCDD/Fs and non-*ortho* DL-PCBs were displayed by sperm whales, indicating that this species is more subjected to exposure to new input of contaminants. This is in accordance with the previously described difference in geographical distribution of the two toothed whales (see Section 4.2.2; Azzellino et al., 2008; Praca and Gannier, 2008).

It is important to notice how all the sperm whales and long-finned pilot whales in this study were in the range of TEQs capable of eliciting physiological effects in mammals (160–1400 pg WHO-TEQ g^{-1} lw) and, in the case of some sperm whales, even surpassing it. Moreover, both the species were in average surpassing the threshold of 210 pg WHO-TEQ g^{-1} lw in blubber, proposed as starting point of immunosuppression in harbour seals by Ross et al. (1995). Every species responds to contaminant toxic effects in different ways, resting on their health status, nutrition state, body size and age; therefore caution must be made in applying such threshold level for toxicity assessment of large cetaceans (Kannan et al., 2000; Schwacke et al., 2002). Nevertheless, the concomitance of such important concentrations of DL and NDL compounds in Mediterranean cetaceans and the spread of CeMV to species for which it was never reported before (e.g fin whales) raise the urgency of better understanding the toxicological status of these animals and the ecological and biological factors that influence it.

5. Conclusions

The large decrease in population numbers of long-finned pilot whales, sperm whales and fin whales observed in the NWMS in last 20 years, led to the necessity of better understanding the health status, ecology and distribution of these resident large cetaceans. The objectives of this study were to give a complete overview of different POP concentrations and their possible relationship with marine mammals ecology. However, the elusive behaviour of these species makes very difficult any kind of interpretation. This study showed that long-finned pilot whales, sperm whales and fin whales from the NWMS present alarming concentrations of both DL and NDL compounds, often surpassing the threshold of POPs toxic effects of 17,000 ng g^{-1} lw. These concentrations are much higher not only compared to the Southern hemisphere, but also in relation with countries of the North Atlantic Ocean. The data presented by this study raise also apprehension on the possibility of human health problem, due to consumption of fish in the Mediterranean Sea. The levels of DL compounds found in our species were much higher than the 2 pg WHO-TEQ g^{-1} lw limit for human lifetime intake that can be ingested without appreciable health risk (Charnley and Doull, 2005). This apprehension is augmented when it is considered that these marine mammals can prey on many fish species that are also consumed by humans. Therefore, toxicological and risk assessment studies on marine mammals are of fundamental importance not only for the conservation of local populations, but also to predict the human exposure to these hazard substances in the environment.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2015.06.021.

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