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Stable isotope ratios of carbon, nitrogen and sulphur and mercury concentrations as descriptors of trophic ecology and contamination sources of Mediterranean whales

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HIGHLIGHTS

- Hg and trophic ecology interplay was explored by quantitative niche modelling.
- Neither trophic position nor variety of consumed food items influenced Hg exposure.
- A generalist prey selection leads to a larger and more variable Hg contamination.
- Combining stable isotopes with T-Hg efficiently describes accumulation processes.

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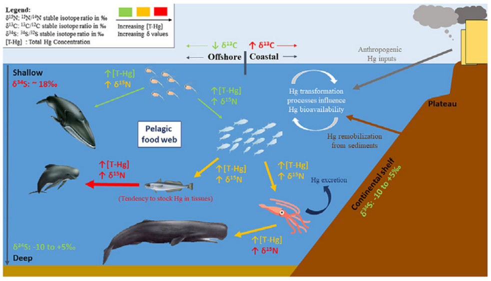
GRAPHICAL ABSTRACT



ABSTRACT

The Mediterranean Sea remains a complex system for mercury (Hg) cycling and accumulation in marine vertebrates. The extremely high levels these animals present demand for an urgent understanding of such processes and the development of new analytical techniques that go beyond the simple contamination monitoring. It was often proposed that prey selection or habitat use may affect Hg contamination in animals; however, it was never possible to measure which factor influences more rates and pathways of contamination. In this paper, we directly integrate toxicological information (Hg levels) and ecological tracers (stable isotopes of C, N and S) into a common data analysis framework (isotopic niches), with the aim of quantifying the influence of species' trophic behaviour on Hg contamination. The analysis was conducted on skin biopsies of fin whales *Balaenoptera physalus*, long-finned pilot whales *Globicephala melas* and sperm whales *Physeter microcephalus.* Their different trophic modes and residency in the area make them model species for the analysis of Hg accumulation along NWMS food webs. We measured Total Hg (T-Hg) concentrations through absorbance spectrometry with the DMA80 Milestone. Carbon, nitrogen and sulphur isotope compositions were measured via mass spectrometry in an IRMS coupled to an Elemental Analyser (EA) Isoprime.

Comparison of ecological and contamination niches allowed to explain Hg accumulation in Mediterranean marine predators. Factors such as food web complexity, trophic position, hunting distribution or habitat use (e.g., foraging depth) did not influence Hg exposure. It is rather the selection of prey type,

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which determines the range of potential Hg sources and as a consequence the rates of accumulation in whales' tissues. A generalist piscivorous species such as the pilot whales will bioaccumulate more Hg than specialised sperm whales feeding mostly on cephalopods.

1. Introduction

Mercury (Hg) is an ubiquitous metal that has both natural and anthropogenic origin (Outridge et al., 2018; Kim et al., 2016) whose cycle in marine ecosystems is very complex and far from being understood (Bradley et al., 2017; Mason et al., 2012; Fitzgerald et al., 2007). Within the organisms, Hg distributes between most tissues where it accumulates in its most toxic form, methylmercury (MeHg). MeHg bioaccumulates along the food chain (Lavoie et al., 2013; Clarkson, 2007). Biological factors such as age, diet, reproductive status and nutritional conditions can influence Hg contamination patterns (Ramos and Gonzalez-Solis, 2012). Up to now there is not real understanding of Hg cycling in water and its pathways of entry in food webs (Gallo, 2012). For example, in the Mediterranean Sea no direct relationship between the total mercury in water and the methylmercury content in fish seems to occur (Mason and Newman, 2002; Cossa et al., 1997; Harmelin-Vivien et al., 2009). Hg levels measured in Mediterranean coastal or deep waters are not necessarily higher than in other regions (Harmelin-Vivien et al., 2009). However, several studies have demonstrated how Mediterranean marine vertebrates, such as fish or cetaceans, present Hg levels among the highest in the world (Savery et al., 2013; Storelli et al., 2002; Cossa et al., 2012). In the last 50 years, this Mediterranean "anomaly" was alleged to be of geochemical origin, with large cinnabar and volcanic-related deposits being considered as the main sources of remobilised Hg in the water column (Cossa et al., 2005). In 2005, Cossa & colleagues reviewed the role of processes such as sediments mineralisation, riverine outflow, external watery inputs and air-seawater exchanges, on Hg accumulation in the marine ecosystem (Cossa et al., 2005). They recognised them as not instrumental in attaining the particularly elevated levels found in Mediterranean food webs (Cossa et al., 2005). On the contrary, biotic and ecological processes were proposed as the main causes for the Mediterranean Hg "anomaly". Due to their particularly long life expectancy, large marine mammals such as cetaceans are more susceptible to longer and higher accumulation of contaminants like Hg (Bossart, 2006). Understanding the potential mechanisms behind such large exposure to contaminants in Mediterranean top predators is today more urgent than ever, especially if we take into consideration the precarious status of these populations (Notarbartolo-di-Sciara, 2013; Notarbartolo-di-Sciara, 2002; Roussel, 2002).

The North Western basin of the Mediterranean Sea (NWMS) is an important feeding ground for many marine mammals (Praca and Gannier, 2008; Praca et al., 2009), but at the same time constitutes the main corridor for several anthropogenic activities (e.g., commerce, naval transport and urbanisation) (Coll et al., 2012). The most common resident species are the fin whale *Balaenoptera physalus* (Linnaeus 1758), the long-finned pilot whale *Globicephala melas* (Traill, 1809) and the sperm whale *Physeter macrocephalus* (Linnaeus 1758) (Notarbartolo-di-Sciara et al., 2015; Notarbartolo-di-Sciara et al., 2003; Canadas, 2012). The "IUCN Red List of Species" reports in 2012 and 2015 have stated the Mediterranean subpopulations of these species as "endangered" (Notarbartolo-di-Sciara et al., 2015; Canadas, 2012; Panigada and di Sciara, 2012). From 2006 to 2014, a monitoring research project funded by the French WWF led to a massive collection of skin biopsies from fin whales, long-finned pilot whales and sperm whales resident in the NWMS. The analysis of organic pollutants (POPs) concentrations in their blubber, showed how NWMS resident cetaceans present among the highest contamination levels in all marine animals of the world (Pinzone et al., 2015). Even though habitat use and diet of NWMS fin, pilot and sperm whales is relatively known (Praca et al., 2009; Druon et al., 2012; Bentaleb et al., 2011; Santos et al., 1999; Squadrone et al., 2015), the link between their trophic ecology and the rates of Hg bioaccumulation in their tissues is still not understood.

Extensive literature exists on the correlation of Hg concentrations with ecological tracers such as stable isotopes to understand how foraging depth (Peterson et al., 2015; Chouvelon et al., 2012), age-related diet shift (Chouvelon et al., 2014), food web structure (Braune et al., 2014), distribution (Das et al., 2004a) or hunting behaviour (Aubail et al., 2010) may influence Hg dynamics within marine mammals. Stable isotopes integrate dietary information over periods ranging from days to years, depending on target tissues, without the biases linked to prey digestibility that occur in stomach content analysis (Ramos and Gonzalez-Solis, 2012; Jardine et al., 2006). Primary producers at the base of food webs often imprint the biological molecules that they manufacture with distinct carbon, nitrogen, and sulphur isotopic composition (Newsome et al., 2007a). Nitrogen isotopes ($^{15}N/^{14}N$) undergo strong fractionation as a consequence of organism's metabolic processes such as digestion and assimilation of ingested prey (O'Connell, 2017; Hansson et al., 1997). This results in a stepwise ^{15}N enrichment along the food web. The $^{15}N/^{14}N$ ratio can be used to assess trophic position of a species (Newsome et al., 2010). Carbon isotopic ratios ($^{13}C/^{12}C$) are less modified during transfer up the food web. This allows to identify direct or indirect producers supporting animal populations, and to study species potential distribution and habitat use (Torniainen et al., 2017). Recent studies showed how sulphur isotopic composition can be efficiently used to trace food source inputs and help refining the information given by a dual-isotopes approach (Fry and Chumchal, 2011; Pizzochero et al., 2018).

Newly developed techniques allow using stable isotope ratios to build geometric spaces (or "isospaces") that can be used as proxies of species' or populations' ecological niches commonly referred to "isotopic niches" (Newsome et al., 2007a). Each dimension is linked with environmental and/or resource requirements at both intra- and inter-individual level (Bearhop et al., 2004; Layman and Allgeier, 2012). Previous studies showed how the isotopic niche of rorqual whales clearly differed between regions (Das et al., 2017; Ryan et al., 2013; Gavrilchuk et al., 2014). A further step could involve the inclusion of toxicological information into the created isospace, so that pollutants would become an additional dimension in explaining the structure and trophic contamination pattern of a specific population (Cransveld et al., 2017). This method would allow a useful assessment of species' ecology and potential patterns of contamination, which is of huge importance in the field of marine mammals' conservation.

The general objective of this work was to understand how the trophic ecology of a species influence Hg exposure and bioaccumulation in marine vertebrates such as common cetacean species (fin whales, long-finned pilot whales and sperm whales) in the North Western Mediterranean Sea (NWMS). Specifically, we aimed to answer the following questions: (1) is Hg contamination correlated with cetacean trophic level, or, in other terms, is biomagnification a key driver of mercury contamination in the NWMS? And (2) can the joint use of stable isotope ratios of C, N, S and Hg levels help us to highlight other ecological traits that could explain differences in Hg concentrations between the three studied species? We used isotopic and contamination niches, to unravel influences of feeding behaviour, feeding strategy (generalist *vs.* specialist), and choice of prey type or habitat use (geographical and depth-related habitat segregation) on Hg contamination.

2. Materials and methods

2.1. Samples collection and preparation

A detailed explanation of sampling techniques and sample preparation methods is given in Pinzone et al. (2015) (Pinzone et al., 2015) and Das et al. (2017) (Das et al., 2017), from which some of the individuals included in this study were also integrated. Briefly, skin-biopsies of 29 sperm whales *Physeter macrocephalus*, 36 long-finned pilot whales *Globicephala melas*, and 67 fin whales *Balaenoptera physalus* were collected in the North Western Mediterranean Sea during 5 WWF campaigns between 2010 and 2014. Biopsies were collected using a 150 lb crossbow (Panzer, US) (Table 1 – S1). At sea, samples were stored in an isolate foam box and kept frozen using technique coolers. Storing temperature was always kept (in the field and laboratory) at −20 °C. Samples were freeze-dried (~24 h), and then homogenized with mortar and pestle into powder. Sex was determined genetically using primers designed for mysticetes (Bérubé and Palsbøll, 1996). Each skin sample was separated in two fractions: between 1 and 2 mg were used for stable isotope analysis, and the remainder was used for mercury analysis.

2.2. Stable isotope analysis

Isotopic ratios of N, C and S were measured as described in Pinzone et al. (2015) (Pinzone et al., 2015) and are hereafter expressed in delta (δ) notation in parts per thousand (‰) (Coplen, 2011). The isotopic ratios were estimated relative to international references Vienna Pee Dee Belemnite (VPBD) for carbon, Atmospheric Air for nitrogen and Vienna Canyon Diablo Troilite (VCDT) for sulphur. We used International Atomic Energy Agency (IAEA, Vienna, Austria) certified reference materials sucrose (IAEA-C_6, $\delta^{13}C = -\ 10.8 \pm 0.5$‰; mean ± SD), ammonium sulfate (IAEA-N_2, $\delta^{15}N = 20.3 \pm 0.2$‰; mean ± SD) and silver sulfide (IAEA-S_1, mean $\delta^{34}S = -\ 0.3$‰) as primary standards, and sulphanilic acid ($\delta^{13}C = -25.6 \pm 0.4$‰; $\delta^{15}N = -0.1 \pm 0.5$‰; $\delta^{34}S = 5.9 \pm 0.5$‰; mean ± SD in each case) as secondary analytical standard. Samples were analysed using an IRMS (IsoPrime 100, Isoprime, U.K.) coupled in continuous flow to an elemental analyser (EA, vario MICRO cube, Elementar, Germany). Since skin of cetaceans is very close to animals external layers of blubber, the high percentage of fat content (typically strongly depleted in ^{13}C) may influence its $\delta^{13}C$ values (Takimoto et al., 2007). In order to eliminate this bias, we corrected the $\delta^{13}C$ values using the normalisation equation (McConnaughey and McRoy, 1979) adapted by Post et al. (2007) (Post et al., 2007) for aquatic animals ($\delta^{13}C_{normalised} = \delta^{13}C_{untreated} - 3.32 + 0.99 \text{ x } (C:N)$). To validate the use of such equation we also extracted lipids in samples for which we had enough material. No difference was found between $\delta^{13}C$ normalised values and lipids-extracted ones (Mann-Whitney, $U = 2653$, $p = 0.371$), validating the application of the normalisation equation in our analysis. More details on lipids extraction can be found in Supporting Information (paragraph S.1).

2.3. Mercury analysis

Dried skin samples (~0.01 g) were accommodated in quartz boats (Volume: 1500 μL) prior the analysis (0.001 mg balance precision). The Total Hg (T-Hg) content was determined using atomic absorption spectrometry at 254 nm, in a Direct Mercury Analyser (DMA 80 Milestone, Minnesota, USA) according to the US EPA standard method 7473 and expressed in ng g^{-1} of dry weight (dw). Quality assessment was operated using replicates, standards (T-Hg 100 ng g^{-1}), blanks (HCl 1%) and Reference Certified Materials (DORM-2 Dogfish, muscle = 4640 ± 140 ng g^{-1} dw) at the beginning and the end of each series. The percentage recovery for DORM-2 ranged between 101% and 105% showing optimal run of the analyses. Hg Standard precision equalled to ±0.3 ng g^{-1} ww.

2.4. Calculation of trophic position (TP)

Following Quezada-Romegialli et al. (2018) (Quezada-Romegialli et al., 2018) we measured the trophic position of sperm whales, long-finned pilot whales and fin whales from NWMS and the Atlantic sub-populations using the package tRophicPosition (version 0.7.6) in R (version 3.5.1) (Quezada-Romegialli et al., 2018). We did so to check for possible geographical differences on food web structure and its consequence on Hg accumulation patterns. To run the model, we integrated $\delta^{15}N$ and $\delta^{13}C$ values of whales and their sources from this study and previous research. The $\delta^{15}N$ and $\delta^{13}C$ values for NWMS whales were analysed in the present study. $\delta^{15}N$ and $\delta^{13}C$ values of Atlantic sperm whales derive from Mendes et al. (2007) (Mendes et al., 2007) and Borrell et al. (2013) (Borrell et al., 2013). The $\delta^{15}N$ and $\delta^{13}C$ values of Atlantic pilot whales were extracted from Abend and Smith (1997) (Abend and Smith, 1997); while data for Atlantic fin whales were extracted from Das et al. (2017) (Das et al., 2017). We used a single C and N source as baseline: the krill species *Meganyctiphanes norvegica* (M. Sars, 1857). Baseline values for the NWMS were extracted from Ferraton (2007) (Ferraton, 2007), while for the Atlantic Ocean we used the values presented in Bentaleb et al. (2011) (Bentaleb et al., 2011). We set our model with an *a priori* lambda = 2 since our baseline is constituted by a zooplankton species. When possible, we

Table 1
Sampling table by species, the study in which the analysis was conducted, sampling period in years and number of individuals divided by sexes.

Species	Study of analysis	Sampling period	Females	Males	Unknown
Fin whale	Pinzone et al., 2015	2010–2013	5	4	0
	Das et al., 2017	2010–2011	6	2	0
	This study	2014	23	26	1
Long-finned pilot whale	Pinzone et al., 2015	2013	6	9	0
	This study	2014	3	18	0
Sperm whale	Pinzone et al., 2015	2012–2013	1	27	1

selected the literature values based on tissue and sampling period. Our isotopic analysis was conducted on skin biopsies collected in summer. Thus, isotopic values for Atlantic pilot and fin whales derived from summer-collected skin. However, we did not find any study presenting stable isotope ratios in skin of Atlantic sperm whales. We therefore used data from stable isotope analyses in dentine layers, keeping in mind the potential difference linked with tissues' turnover during the interpretation of the results. Also for the baseline we used isotope ratios measured in krill sampled during the summer period only. Fractionation factors of 0.4 ± 1.2‰ and 2.3 ± 1.61‰ were applied for δ^{13}C and δ^{15}N, respectively (after McCutchan et al., 2003) (McCutchan et al., 2003). Direct pairwise comparisons were performed, and were considered meaningful when probability of occurrence (e.g., number of model solutions where a given situation was found) exceeded 95%. The number of run iterations was set to 10^6.

2.5. *Niche width analysis using SIBER*

Following Jackson et al. (2011) (Jackson et al., 2011) we used the Stable Isotopes Bayesian Ellipses (SIBER) package (version 2.1.3) in R (version 3.5.1 (R Core Team. R, 2018)) to explore variation in δ^{13}C, δ^{15}N, δ^{34}S and T-Hg values of our species. We examined the isotopic niche distribution of each species in either a δ^{13}C *vs.* δ^{15}N or a δ^{15}N *vs.* δ^{34}S biplot, and used it as proxy of the ecological niche. Then, we used δ^{13}C *vs.* T-Hg and δ^{15}N *vs.* T-Hg biplots as proxy of contamination variability (Ryan et al., 2013; Jackson et al., 2011; Layman et al., 2007). In this paper, we will refer to these last models as "contamination niches". It was not possible to model T-Hg *vs.* δ^{34}S ellipses due to the small numbers of samples for which both data were available (e.g., less than 5 individuals for sperm whales). For the quantification of contamination niches, we corrected the δ values (∂k_{st}, "standardised δ value" in equation a) and T-Hg ($[c]_{st}$, "standardised T-Hg concentration" in equation b) as suggested by Cucherousset and Villéger (2015), to avoid bias given by the large difference between T-Hg and isotope range values:

$$\partial k_{st} = \frac{(\partial k - \min(\partial k))}{(\max(\partial k) - \min(\partial k))} \qquad \text{a}$$

$$[c]_{st} = \frac{([c] - \min([c]))}{(\max([c]) - \min([c]))} \qquad \text{b}$$

In this way, we created a standardised multidimensional space where each axis is scaled to have the same range (0–1) for each stable isotope ratio (∂k) or contaminant concentration ($[c]$). For consistency, we applied the scaling also in the ecological niches.

The geometric representation of a niche in a SIBER model is the standard ellipse area (SEA). It incorporates 40% of studied individuals, representing a bivariate equivalent of standard deviation. As such, it contains only the "typical" individuals of a population without being influenced by outliers (Newsome et al., 2007b). For this reason, it can be used as a proxy of the trophic and habitat resources most commonly used by the population (Layman and Allgeier, 2012). To limit calculation biases when comparing small and/or unbalanced samples, standard ellipses areas were corrected for small sample size (SEA_C) when the analysed groups contained less than 30 individuals (Syväranta et al., 2013). This choice does not cause bias in standard ellipse areas estimation for larger sample sizes, as SEA and SEA_C tend to converge when sample size increases (Jackson et al., 2011). Niche areas of each species were also estimated using Bayesian modelling (SEA_B). SEA_B involves the use of an iterative model based on Bayesian inference to estimate the covariance matrix from the data. SEA_B is more effective at taking into account both natural and analytical variability in the data and provides a distribution of solutions rather than a single value, providing error estimates as well as pairwise comparisons. Here, the number of iterations was set to 10^7. Model solutions were presented using credibility intervals of probability density function distributions (Fig. 2). Direct pairwise comparisons were performed and considered meaningful when probability of occurrence (e.g., number of model solutions where a given situation was found) exceeded 95%. Finally, the geometric overlap among ellipses and differences between SEA_C was also measured and compared between species.

2.6. *Statistical treatment*

The normality of data was assessed using a Shapiro test and the homoscedasticity using a Bartley test. Because the majority of data deviated from a normal distribution and did not present homogeneity of variances, the non-parametric Kruskal-Wallis and Mann-Whitney U tests were used for species and sex comparisons of δ^{13}C, δ^{15}N, δ^{34}S and T-Hg values. Correlations between mercury concentrations and the different isotopic values or between T-Hg in the different tissues were performed with the Spearman's rank correlation ρ. For all tests, rejection of the null hypothesis was set at $p < 0.05$. Statistical analyses were conducted with Statistica (version 12) and R version 3.5.1 (R Development Core Team. R, 2011).

3. Results

3.1. *Trophic position and T-Hg levels*

Atlantic fin whales presented higher TP (Mode ± SD [C.I. 2.5%–97.5%]: 3.6 ± 0.1 [3.3–3.9]) than Mediterranean ones (Mode ± SD [C.I.: 2.5%–97.5%]: 2.9 ± 0.4 [2.3–4.4]) in 95% of model runs. Conversely, the Atlantic and Mediterranean sub-populations of sperm and pilot whale species showed similar TP, with Atlantic pilot and sperm whales presenting higher TP (respectively 4.0 ± 0.1 [3.7–4.3] and 4.9 ± 0.7 [3.1–6.0]) than Mediterranean ones (respectively 3.9 ± 0.7 [3.0–5.4] and 4.2 ± 0.5 [3.4–5.3]) in only 23% and 33% of model runs respectively. Among our Mediterranean whales, sperm whales presented the highest values of TP while fin whales presented the lowest. As shown in Table 2, long-finned pilot whales were the most contaminated species, followed by sperm and fin whales (Kruskal-Wallis: $p < 0.001$, $W = 92.6$).

3.2. *Isotopic niches*

When considering δ^{13}C *vs.* δ^{15}N isotopic niches, we did not observe any overlap between the fin whales and the two others species (Fig. 1A). Sperm whales and long-finned pilot whales were characterised by higher δ^{15}N values. Between the two odontocete species, only a very small ellipse overlap was found (overlapping area: 0.001‰^{2}), representing 3% of sperm whale total area and 5% of long-finned pilot whale area (Fig. 1A). Sperm whales presented the largest δ^{13}C *vs.* δ^{15}N SEA (SEA: 0.034‰^{2}), followed by fin whales (SEA: 0.031‰^{2}) and long-finned pilot whales (SEA: 0.021‰^{2}) in 98.1% of model runs (for all) (Fig. 2A). Fin whales presented an intermediate δ^{13}C *vs.* δ^{15}N niche area in 97.7% of model runs (for all) (Fig. 1A). Finally, long-finned pilot whales showed the smallest δ^{13}C *vs.* δ^{15}N isotopic niche in 98% of model runs (for all).

When considering δ^{34}S and δ^{15}N values, the isotopic niche of fin whales (SEA_C: 0.15) was larger than the one of long-finned pilot whales (SEA_C: 0.05‰^{2}) in 99.7% of model runs, and the one of sperm whales (SEA_C: 0.07‰^{2}) in 96.5% of model run (Fig. 2B). We observed no overlap at all between fin whale and sperm whale ellipses; and a negligible one between fin whale and long-finned

Table 2
Stable isotope ratios (in ‰), C:N ratios and T-Hg concentrations in skin (ng g^{-1} dw) in three cetaceans species from the Mediterranean Sea. Data are shown as Mean (Median) ± Standard Deviation, (Min − Max) and n.

	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)	$\delta^{34}S$ (‰)	C:N	T-Hg_{skin} (ng g^{-1} dw)
Fin whale	−19.2 (−19.2) ± 0.5 (-20.2 − −17.8) 67	7.8 (7.7) ± 1.0 (4.8–11.0) 67	16.4 (16.7) ± 1.9 (12.8–19.2) 9	5.5 (5.4) ± 1.3 (4.0–12.4) 67	814 (828) ± 229 (262–1363) 66
Long-finned pilot whale	−17.7 (−17.9) ± 0.6 (-18.6 − −16.1) 36	10.5 (10.4) ± 0.5 (9.6–12.3) 36	16.3 (17.0) ± 2.4 (8.6–18.4) 15	3.6 (3.6) ± 0.3 (3.1–4.6) 36	33028 (32175) ± 7480 (17235–49496) 29
Sperm whale	−17.4 (−17.4) ± 0.5 (-18.2 − −15.9) 39	11.9 (11.6) ± 1.0 (10.6–15.6) 42	16.0 (16.7) ± 2.5 (9.2–19.2) 26	3.4 (3.3) ± 0.2 (2.8–4.0) 41	10763 (9782) ± 3774 (4235–18080) 19

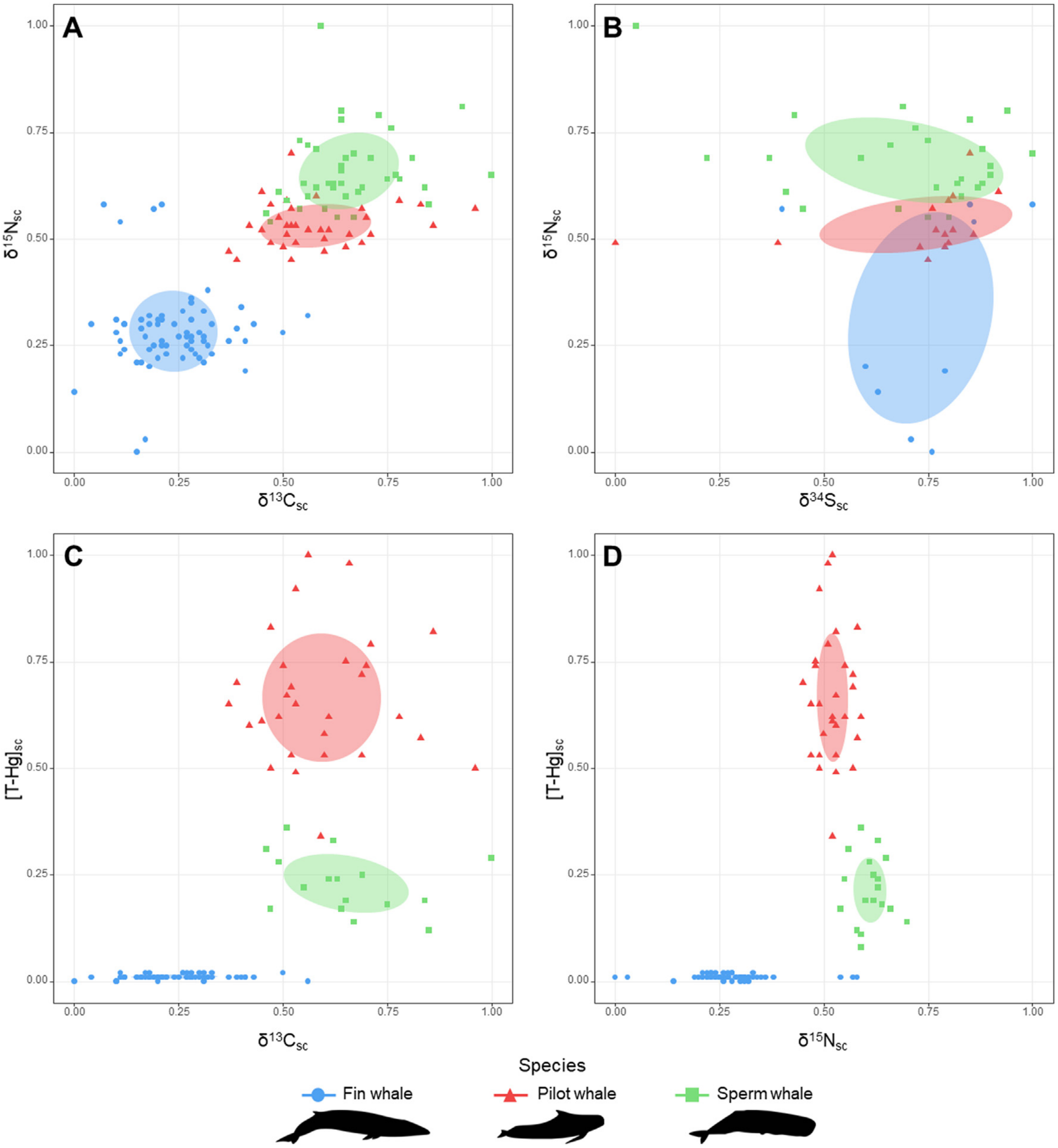

Fig. 1. $\delta^{13}C$ *vs.* $\delta^{15}N$ (**A**), $\delta^{34}S$ *vs.* $\delta^{15}N$ (**B**), T-Hg *vs.* $\delta^{13}C$ (**C**) and T-Hg *vs.* $\delta^{15}N$ (**D**) biplots in skin of fin whales (blue), long-finned pilot whales (red) and sperm whales (green). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

pilot whale niches (overlapping area: 0.022‰^{2}) representing 0.04% of the fin whale ellipse area and 0.09% of the pilot whale ellipse area (Fig. 1B). The overlap between the two odontocetes in the $\delta^{34}S$ *vs.* $\delta^{15}N$ isospace was minimal (overlapping area = 0.003‰^{2}; sperm

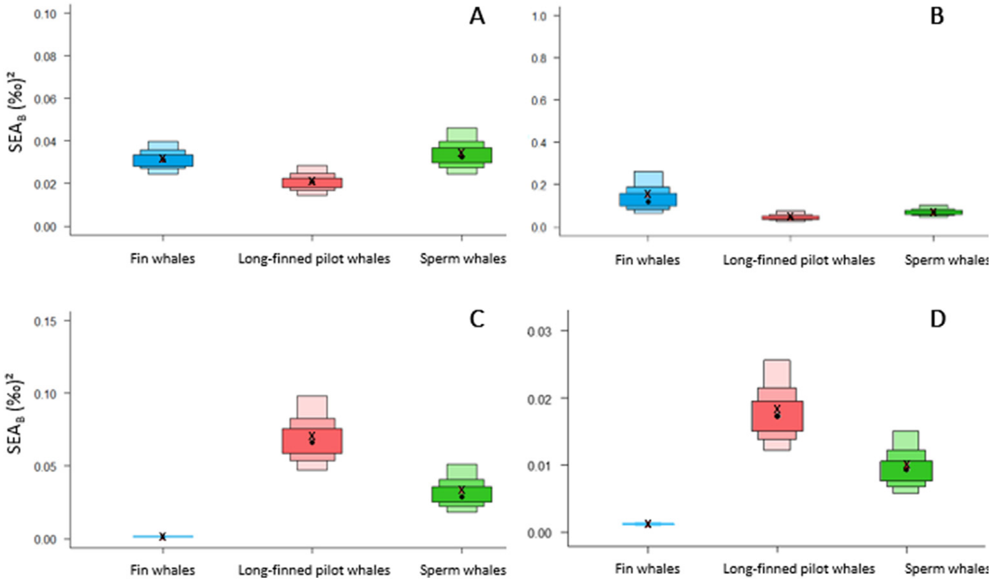

Fig. 2. Estimates of standard ellipse area (SEA) for fin whales, long-finned pilot whales and sperm whales. Boxplots are posterior probability distributions of model estimations of standard ellipse areas (SEA$_B$). The dark, intermediate and light boxes are the 50%, 75% and 95% credibility intervals, respectively, and the black dot is the mode of each distribution. The cross is the standard ellipse area calculated using correction for small sample size (SEA$_C$).

whale: 6% of ellipse area; pilot whale: 4% of ellipse area; Fig. 1B).

3.3. Contamination niches

A clear distinction of the three cetaceans is observable in the contamination space (Fig. 1C–D) with no overlap occurring among species. To understand "if" and "how much" the habitat could influence Hg contamination, we looked at species contamination niche areas. In the T-Hg *vs.* δ^{15}N analysis, the contamination niche of long-finned pilot whales (SEAc: 0.02‰^{2}) was larger than fin whales (SEA: 0.001‰^{2}) and sperm whale (SEAc: 0.01‰^{2}) niches in 100% and 98% of model runs, respectively (Fig. 2D). In the same way, for the T-Hg *vs.* δ^{13}C space (Fig. 1C) long-finned pilot whales had a larger ellipse (SEAc: 0.07‰^{2}) than the one of fin whales (SEA: 0.002‰^{2}) in 100% of iterations, but SEA of pilot whales and sperm whales (SEAc: 0.03‰^{2}) were similar (Fig. 2C).

4. Discussion

To our knowledge, this study presents for the first time sulphur stable isotope ratios in Mediterranean marine mammals (Table 2 and Figs. 1B and 2B and S2). The absence of overlap between the δ^{34}S *vs.* δ^{15}N niches of our three whales is only due to a difference in species-specific δ^{15}N values, and not by the S isotopic values. This shows no difference in S isotopic composition between fin, sperm and long finned pilot whales. The organic S in animal tissue is derived from the diet, but inorganic S from the environment can also contribute to the total S pool of an animal and its sulphur isotope ratio (McCutchan et al., 2003). Connolly et al., 2004 demonstrated how S stable isotopes discriminate between marine benthic or pelagic producers, depending on their source of S (Connolly et al., 2004). In fact, producers that predominantly utilize seawater sulphates, such as pelagic microalgae and phytoplankton, tend to be enriched in ^{34}S (~18‰). Those using sedimentary sulphides such as marsh plants on the coasts or anaerobic bacteria found in the sea bottom, are instead more depleted (δ^{34}S range: 10 to +5‰) (Connolly et al., 2004). This could help to differentiate a species which relies upon offshore and pelagic food sources from another with more coastal or benthic behaviour (McMahon et al., 2013). All our three species had δ^{34}S comparable to seawater sulphates and pelagic producers. This is consistent with the known offshore distribution of our three species in the Pelagos waters. Overall, the three species studied here seem to reflect more Hg accumulation pathways in offshore and pelagic food webs than in coastal ones. The latter may differ in structure and, therefore, feature other contamination sources and/or Hg transfer processes.

Mercury levels of our species were comparable to previous studies in the Mediterranean Sea (Savery et al., 2013; Frodello et al., 2000), but were much higher than levels found in the Arctic (Wagemann and Kozlowska, 2005), Atlantic (Savery et al., 2013; Stavros et al., 2008) or Southern Pacific species (Yang et al., 2002; Bustamante et al., 2003; Cáceres-Saez et al., 2018). This is consistent with the specific elevated Hg contamination of Mediterranean marine vertebrates reported in literature (Savery et al., 2013; Roditi-Elasar et al., 2003) (Table 3). Cossa et al. (2005) (Cossa et al., 2005) suggested that the elevated temperatures of the region could cause higher rates of Hg methylation processes in the water column and increase its bioavailability to phytoplankton (Cossa et al., 1997; Bacci, 1989; Gehrke and Blum, 2011; Heimbürger et al., 2010). Moreover, the oligotrophic status of the Mediterranean Sea could cause slower growth rates and longer food webs, which could indeed explain the very high levels found in top predators (Harmelin-Vivien et al., 2009; Cossa et al., 2012; Cossa and Pirrone, 2009). As in previous studies (Borrell et al., 2012; Capelli et al., 2008) the oligotrophy of NWMS was reflected by a depletion in both ^{15}N and ^{13}C isotopes in the skin of our three species, compared to their Atlantic (Ruiz-Cooley et al., 2004, 2012; Monteiro et al., 2015) or Pacific (Marcoux et al., 2007) counterparts, as well as the neighbouring subpopulations of the Gibraltar Strait (Borrell et al., 2012; De Stephanis et al., 2008) (Table 4). Hg accumulates in its lipophilic form (MeHg) along the food web, with organisms feeding on higher trophic levels being more polluted (Lavoie et al., 2013; Stavros et al., 2008; Gehrke and Blum, 2011). This results in a positive correlation between δ^{15}N values and T-Hg values that has been extensively reported in literature (Atwell et al., 1998; Das et al., 2004b; Lehnherr, 2014). Oligotrophic waters are known to determine longer food webs (Gallo, 2012). The longer the food web, the larger the amount of Hg incorporated through its multiple

Table 3
Comparison of T-Hg concentrations (ng g^{-1} dw) in skin biopsies between the populations of long-finned pilot whales, sperm whales and fin whales resident in the Mediterranean Sea and other whales from the rest of the world. Data are given as Mean ± SD and, when available, (Min − Max) range.

Author	Area	Specie	N	Skin
Frodello et al., 2000	Western Mediterranean	*Globicephala melas*	1	27000 ± 1000
		Delphinus delphis	1	15000 ± 2000
Yang et al., 2002	Pacific (Japan)	*Phocoenides dalli*	45	2900 ± 1700 (15−8400)
Bustamante et al., 2003	Pacific (New Caledonia)	*Globicephala macrorhynchus*	1	11000 ± 800
		Globicephala macrorhynchus	1	3200 ± 200
[a],Roditi-Elasar et al., 2003	Mediterranean Sea (Israel)	*Stenella coeruleoalba*	5	18900 ± 10000 (5200−28100)
		Tursiops truncatus	13	15500 ± 11100 (1000−37000)
[a],Wagemann and Kozlowska, 2005	Arctic (Canada)	*Delphinapterus leucas*	27	3100 ± 1600 (700−7000)
		Monodon monoceros	20	2200 ± 500 (1200−3200)
Stavros et al., 2008	Atlantic (South Carolina)	*Tursiops truncatus*	74	1700 ± 900 (700−4900)
[a],Savery et al., 2013	Pacific ocean	*Physeter macrocephalus*	32	9700 ± 300 (300−9700)
	Australia		19	3500 ± 300 (1100−6900)
	Atlantic (Canary Islands)		23	1700 ± 100 (700−3800)
	Mediterranean Sea		30	6100 ± 700 (300−16000)
This study	Western Mediterranean	*Balaenoptera physalus*	66	800 ± 200 (300−1400)
		Globicephala melas	29	33000 ± 7500 (17200−49500)
		Physeter macrocephalus	19	10800 ± 3800 (4200−18100)

[a] Concentrations on Hg for skin were presented as wet weight (ww) and were therefore converted in dry weight (dw) for comparison's sake, using the conversion factor of 3.7 as explained in Becker et al. (1995).

Table 4
Stable isotopes composition of sperm whales, long-finned pilot whales and fin whales from different parts of the world[a]. Data are presented in per mill (‰) as Mean ± SD, or (Min − Max) range.

Species	Area	Tissue	N	δ^{13}C	δ^{15}N	References
Fin whales	NWMS	Skin biopsy	67	−19.2 ± 0.5	7.8 ± 1.0	This study
	Celtic Sea	Skin biopsy	21	−18.2 ± 0.5	11.9 ± 1.0	Ryan et al., 2014
	NW coast of Spain	Skin biopsy	9	−18.3 ± 0.4	10.2 ± 0.6	Borrell et al., 2012
	NWMS	Skin biopsy	2	−18.5 − −18.6	8.3 − 9.3	Capelli et al., 2008
	NWMS	Skin biopsy		−18.7 ± 0.4	7.7 ± 0.8	Pinzone et al., 2015
	Gulf of California	Skin biopsy	17	−13.9 ± 0.4	19.8 ± 0.7	Ruiz-Cooley et al., 2004
	Mediterranean Sea	Skin biopsy	136	−18.7 ± 0.4	7.7 ± 0.5	Das et al., 2017
Long-finned pilot whales	NWMS	Skin biopsy	36	−17.7 ± 0.6	10.5 ± 0.5	This study
	North Sea	Skin biopsy	46	−17.7 ± 0.7	12.0 ± 0.7	Monteiro et al., 2015
	Gibraltar Strait	Skin biopsy	56	−16.4 ± 0.4	11.3 ± 0.4	De Stephanis et al., 2008
	NWMS	Skin biopsy		−17.8 ± 0.3	10.5 ± 0.7	Pinzone et al., 2015
Sperm whales	NWMS	Skin biopsy	39 42	−17.4 ± 0.5	11.9 ± 1.0	This study
	Gulf of Mexico	Skin biopsy	53	−17.1 − −15.8	11.2 − 13.6	Ruiz-Cooley et al., 2012
	NWMS	Skin biopsy	1	−18.1	13.1	Capelli et al., 2008
	NWMS	Skin biopsy		−17.3 ± 0.4	12.2 ± 1.3	Pinzone et al., 2015
	Northcentral Gulf of Mexico	Skin biopsy	28	−16.4 ± 0.5	12.2 ± 0.4	Ruiz-Cooley et al., 2012
	Northwestern Gulf of Mexico	Skin biopsy	18	−16.5 ± 0.3	12.2 ± 0.4	Ruiz-Cooley et al., 2012
	South Pacific	Skin biopsy	106	−16.0 ± 0.7	16.8 ± 3.5	Marcoux et al., 2007

[a] Only studies on skin biopsies were included to avoid variability linked with tissue metabolism.

trophic levels. In order to check if the NWMS nutrient depletion may affect the structure of Mediterranean food webs, we measured and compared our species trophic position (TP) to Atlantic subpopulations. With this method, we could use the TP of a species as indicator of the length of the food web it relies upon: a higher trophic position could indicate a longer (or more complex) food web and potentially lead to higher T-Hg levels in top predators (Quezada-Romegialli et al., 2018). The use of baseline δ^{15}N and δ^{13}C values in the tRophicPosition model in R eliminates the influence of geographical distribution on consumers' isotopic composition (Quezada-Romegialli et al., 2018). In this way, a different TP between two sub-populations of the same species purely indicates a reliance on prey from different levels. For example, the higher TP of Atlantic fin whales compared to the Mediterranean subpopulation may be a consequence of this population's foraging on fish, while Mediterranean whales are specialised filter feeders hunting exclusively for zooplankton (Druon et al., 2012; Ryan et al., 2014). Conversely, the similar TP shown by sperm and pilot whales from the Atlantic and Mediterranean subpopulation indicates that they rely on prey from the same TP in both geographic regions. We did not observe any relation between TP values and T-Hg levels. In fact, even if Atlantic fin whales feed on fish, known to be more contaminated in Hg than krill (Druon et al., 2012; Ryan et al., 2014), NWMS fin whales presented higher concentrations (Table 3). The situation for odontocetes was similar: although the TP values did not differ between Atlantic and Mediterranean populations, NWMS pilot and sperm whales presented higher T-Hg levels than whales from other regions of the world (Table 3). These findings suggest that trophic position and food web structure are not the primary cause for higher Hg bioaccumulation in Mediterranean top predators.

Whales' isotopic niches allow the description of their trophic ecology using two parameters: niche area and niche overlap. The former is a proxy of the diversity in resources and/or habitat used by a single predator; the latter captures how the habitat and its resources are shared by several species (Das et al., 2017; Jackson et al., 2011). The lack of isotopic niche overlap between fin whales and the two others species (Fig. 1A) is due to the filter feeding behaviour of fin whales. This leads to a depleted range of both C and N isotopes values, while sperm whales and long-finned pilot whales are characterised by a more ^{15}N-enriched isotopic composition (Boecklen et al., 2011). Between the two odontocetes, only a very small ellipse overlap was found (Fig. 1A). This is interesting considering their close trophic position (only 1.5‰ of difference in $\delta^{15}N$ mean values). This suggests that the two odontocetes may exploit the water column differently (Newsome et al., 2010; Fontaine et al., 2007).

Sperm whales presented the largest $\delta^{13}C$ *vs.* $\delta^{15}N$ SEA, followed by fin whales and long-finned pilot whales (Fig. 2A). This results from the large variability of both $\delta^{13}C$ and $\delta^{15}N$ ranges. This may result from the specialised habitat use and feeding behaviour of sperm whales. In the Mediterranean Sea, young sperm whales males distribute widely between the Western and the Eastern basin (Mendes et al., 2007; Ottensmeyer and Whitehead, 2003). However, stomach content analysis conducted on stranded animals showed that bathypelagic cephalopods such as *H. bonnelli* and *H. reversa* or the Ommastrethidae *Todarodes sagittatus* remain sperm whales' favourite prey at global scale (from the Mediterranean Sea to the Atlantic Ocean (Robert, 2003; Watwood et al., 2006)). Different $\delta^{13}C$ baselines between the Eastern and the Western Mediterranean Sea could determine large spatial $\delta^{13}C$ shifts in preys, and consequently in sperm whales tissues (Mendes et al., 2007). Moreover, the $\delta^{15}N$ values increase with depth as a consequence of changes in biological activities linked with light intensity, oxygen abundance or temperature levels (McMahon et al., 2013). In the Mediterranean Sea, bathypelagic food webs (between 1400 and 1800 m depth) are ^{15}N-enriched with respect to mesopelagic ones (between 200 and 1000 m) (Polunin et al., 2001). Studies based on acoustic recording showed how sperm whales are deep divers that can change their foraging behaviour (depth or hunting technique) based on the topography of the continental slope they feed on (Praca et al., 2009; Teloni et al., 2008; Drouot et al., 2004). Intraspecific specialisations in hunting depth could therefore be responsible for the large interval of $\delta^{15}N$ values seen in sperm whales. Overall, the large isotopic niche of sperm whales seems to be linked more with variability in habitat use (feeding depth and/or spatial segregation of bachelor groups) rather than in consumed food items.

Fin whales presented an intermediate $\delta^{13}C$ *vs.* $\delta^{15}N$ niche area (Fig. 1A). In the Mediterranean, fin whales are regarded as stenophagous, feeding mostly on krill (Bentaleb et al., 2011; Das et al., 2017), which is in accordance with the low trophic level measured here (2.9 ± 0.4). In this context, isotopic variability in this species could be related to feeding movements. In spring, fin whales mostly feed on *Meganyctiphanes norvegica* in the Gulf of Lion in the NWMS. During fall, fin whales were mostly observed preying on *Nyctiphanes couchii* along the Southern coast of Lampedusa Island in the Strait of Sicily (Canese et al., 2006). No data are available on the isotopic composition of the second krill species. However, the distance between the breeding grounds and the differences in both latitude and geochemical characteristics could lead to intraspecific isotopic variability (Newsome et al., 2010). Several studies state that fin whales resident in the Mediterranean Sea feed on other sources than krill, conversely to for example Celtic Sea populations (Notarbartolo-di-Sciara et al., 2003; Druon et al., 2012; Bentaleb et al., 2011; Das et al., 2017; Canese et al., 2006).

Finally, long-finned pilot whales showed the smallest $\delta^{13}C$ *vs.* $\delta^{15}N$ isotopic niche. This may be explained by their generalist diet (Praca and Gannier, 2008; Praca et al., 2011). Pilot whales remain constantly in large groups containing up to 60 individuals, with adult and juveniles, males and females all mixed together (Ottensmeyer and Whitehead, 2003). Such groups seem extremely closed and no mixing occur even with the neighbour subpopulations inhabiting the Alboran Sea (Ottensmeyer and Whitehead, 2003). These strong life-long social structures reduce the rates of individuals' feeding specialisation (De Stephanis et al., 2008). In a tissue with high turnover rates such as skin, incorporation of many different preys may lead to homogenization of animals' isotope composition. Thus, a species with general prey selection behaviour will display lower isotope variability and therefore smaller isotopic niche area (Bearhop et al., 2004).

The integration of T-Hg contamination levels into the previously described isotopic niches (paragraph 3.3) leads to what we define here as "contamination niche". Its use could potentially move us a step forward to understand how ecological factors drive mercury contaminations, and determine the possible causes of its accumulation (Cransveld et al., 2017). Even if not capable of specifically separating the sources, the value of niche overlap may show the extent of sources shared by two species (Cransveld et al., 2017). On the other hand, the contamination niche area could indicate the range of Hg contamination heterogeneity in a species linked with food selection.

In contrast to the isotopic niches, long-finned pilot whales presented the largest contamination area, followed by fin whales and sperm whales (Fig. 1A–D). This shows how the feeding strategy (generalist *vs.* specialist) influence Hg patterns of contamination in our species. Indeed, the generalist behaviour of long-finned pilot whales seems to determine a greater variability of Hg contamination values, while the specialised feeding mode of sperm whales determine a very narrow range of Hg concentrations values. Species' specific metabolism determine the final Hg burden in the entire organism. For example, fish generally accumulate higher levels of T-Hg than cephalopods (Savery et al., 2013; Bustamante et al., 2006; Penicaud et al., 2017). Thus, a generalist predator, which present several fish species in its diet, might be exposed to a more important uptake of Hg. Therefore, the higher proportion of fish among pilot whale preys such as mackerel, herring, cod or turbot, could be the possible cause of the higher accumulation of T-Hg in pilot whales resulted compared to sperm whales.

5. Conclusions and perspectives

The general objective of this work was to understand how the trophic ecology of a species influence Hg exposure and tissue accumulation in fin whales, long-finned pilot whales and sperm whales in the North Western Mediterranean Sea.

We showed that: (1) the trophic position and food web length do not determine the higher Hg levels found in NWMS whales, compared to the Atlantic subpopulations; but (2) it is rather the feeding strategy and prey choice. A generalist piscivorous behaviour expose predators to a larger range of Hg potential sources and consequently to higher accumulation with respect to specialist species feeding on, for example, cephalopods. We also show that (3) the integration of multiple tracers into Bayesian models can be efficiently used to understand the complex ecology of marine vertebrates, in comparison to the simple monitoring of contaminants levels.

The development of new techniques and multidisciplinary approaches such as the one we applied in this research opens a wild new spectrum of possibilities and effective conservation measures through the quantitative tracking of contaminants among food webs.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2019.124448.

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