Chemosphere
May 2022, Volume 294 Pages 133676 (11p.)
https://doi.org/10.1016/j.chemosphere.2022.133676
https://archimer.ifremer.fr/doc/00748/85998/

Two cetacean species reveal different long-term trends for toxic trace elements in European Atlantic French waters

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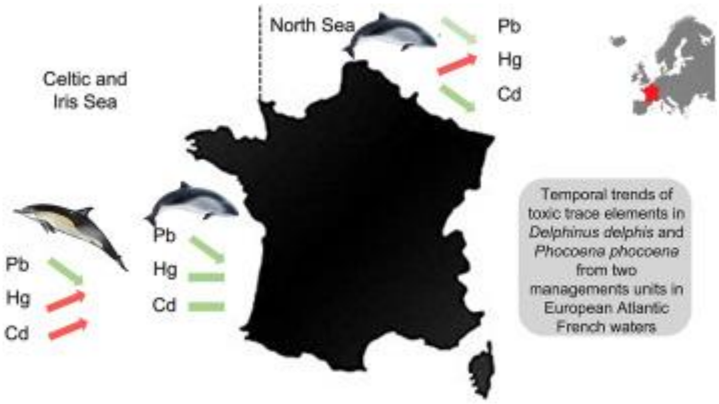
Abstract :

Cetaceans have been naturally exposed to toxic trace elements (TEs) on an evolutionary time scale. Hence, they have developed mechanisms to control and/or mitigate their toxic effects. These long-lived species located at high trophic positions and bioaccumulating toxic elements are assumed to be good biomonitoring organisms. However, anthropogenic emissions have strongly increased environmental levels of toxic TEs in the last decades, questioning the efficiency of the detoxication mechanisms in cetaceans. In this context, temporal trends of mercury (Hg), cadmium (Cd) and lead (Pb) concentrations were studied through the analysis of 264 individuals from two cetacean species the common dolphin (Delphinus delphis) and the harbour porpoise (Phocoena phocoena) and belonging to two different Management Units (MUs) for the latter. These individuals stranded along the French Atlantic coasts from 2000s to 2017. All the trends presented were age- and sex-corrected and stable isotope ratios of carbon (δ13C) and nitrogen (δ15N) were measured as proxies of their feeding ecology. Results showed that Pb concentrations clearly decreased over time in both species and MUs. This decrease agrees with the lead petrol regulation after 2000s, supporting the use of these species as valuable bioindicators of changes for TE levels in the marine environment. A significant long-term increase of total Hg concentrations was only observed in common dolphins. Cadmium concentrations also revealed different trends over the period in both species. The different Hg and Cd trends observed in the two species, probably reflected a contrasted contamination of habitat and prey species than a global increase of the contamination in the environment. These results highlight the necessity and gain of using different species to monitor changes in marine environments, each of them informing on the contamination of its own ecological niche. Lastly, the Se:Hg molar ratios of species suggested a low risk for Hg toxicity over time.

Graphical abstract



Highlights

► Temporal trends of Pb, Hg and Cd in two cetacean species and management units. ► Eco-biological factors were included in models to assess temporal trends. ► Pb showed the same trend for both species with few effects of ecological factors. ► Hg and Cd showed different trends for both species and porpoise management units. ► The Se:Hg molar ratios evolution over time suggested low risk for Hg toxicity.

Keywords : Temporal variation, Detoxification process, Dynamic linear models, Common dolphin, Harbour porpoise, North-east atlantic

Introduction

Over the last decades, industrial and technological development together with the density of human populations around coastal areas have intensified, leading to a sharp increase in pressures on the marine environment. Chemical pollution is recognized as one of the main drivers for ecological changes on marine ecosystems due to anthropogenic disturbances (Halpern et al. 2008). Among chemical pollutants, trace elements (TEs) are naturally present in the environment, however, human activities contribute to increase their concentrations in marine waters. The worldwide refinery activities and mine production have substantially increased these last decades, particularly since the beginning of 2000s (Tiess 2010), to cope with the global increase in mineral demand due to the economic and industrial development of emerging countries (Daughton 2005, Ilyn et al. 2007). Facing the threats to ecosystems and human health, various policies have been implemented to regulate the potential impacts of toxic elements. In this context, the European Union adopted in 2008 the Marine Strategy Framework Directive 2008/56/EC (MSFD), which had the main objective of maintaining or restoring the "Good Environmental Status" (GES) of European marine waters by 2020. To achieve this objective, an initial assessment and the subsequent monitoring of the contamination state of marine ecosystems were necessary. To this end, the MSFD recommended monitoring contamination levels in different marine species. However, the bioindicator species commonly used for monitoring contaminants in the environment, belong to numerous taxonomic groups (e.g micro- and macro-algae, seagrasses, polychetes, crustaceans, bivalve and gastropod molluscs, seabirds, fishes, marine reptiles and mammals, Eilser 2009a, 2009b), each one showing some special merits when compared to the others (Zhou et al. 2008). Cetaceans are part of these bioindicator species since they are long-lived species, located at the top of marine food webs, likely incorporating high concentrations of contaminants. They are therefore considered as integrative and reliable tracers of environmental TE contamination (Capelli et al.

2000). In these species, the trophic route is the main source of exposure rather than direct contact with the environment, even in highly contaminated habitats. Inter- and intra-species variations in contaminant concentrations are therefore related to food preferences, feeding areas and biological factors such as age, sex or sexual maturity (Muir et al. 1988, André et al. 1990a,b, Aguilar et al. 1999, Caurant et al. 1994).

Cetaceans have been exposed to toxic TEs at an evolutionary time scale. As such, they have developed mechanisms to limit their toxic effects. For instance, their capacity to demethylate methyl-mercury (MeHg) and to sequester Hg with selenium (Se) in a non-toxic form named tiemanite, is a well-known process (Martoja and Berry 1980, Wagemann et al. 1984). Liver is the tissue where inert HgSe crystals mainly accumulate. However, Se could be involved in the detoxification process of Hg in other tissues than just liver as was confirmed by Nakazawa et al. (2011). But, this binding process may also cause indirect physiological problems older than act as a defensive mechanism against the direct effects of Hg exposure. Spiller (2018) also suggested that the "protective effect" may in fact produce a deficient state in Se, with the sequestration of Se by MeHg directly affecting both the synthesis and activity of important Se-dependent enzymes for other biological functions (Ralston et al. 2012). Therefore, the strong increase of TE environmental levels, as a consequence of anthropogenic emissions, may question the efficiency of detoxication mechanisms in cetaceans.

The objective of this work is to assess the temporal evolution of toxic TE concentrations (i.e. Hg, Cd and Pb) in two common cetacean species from European Atlantic waters, the common dolphin (*Delphinus delphis*) and the harbour porpoise (*Phocoena phocoena*). The harbour porpoises analysed in this study belong to two Management Units (MUs) among the five MUs that have been delineated within the North-east Atlantic for this species (ICES WGMME 2013, 2014; IAMMWG 2015), namely the Celtic and Irish Seas and the North Sea. These borders were based on genetic analysis and measurements of time-integrated ecological tracers and morphological differences. The two cetacean species have different prey sources and feed on different habitats. In fact, common dolphins can be found in both coastal and offshore waters up to more than 1000m depth, while porpoises (for any MU) frequently visit shallow bays, estuaries and tidal channels with less than 200m in depth and the majority of sightings occur within 10km of land. The diet of the harbour porpoise is mostly composed of small schooling fish living close to the seafloor (98 percent by mass) (e.g. blue whiting *Micromesistius poutassou*, sardine *Sardina pilchardus*, scads *Trachurus* spp., and whiting *Merlangius merlangus;* Spitz et al. 2006, Santos and Pierce 2003). The diet of the continental shelf common dolphins is dominated by small schooling fish (e.g. scads *Trachurus* spp., sardine, anchovy *Engraulis encrasicolus* and mackerel *Scomber scombrus*) (Meynier et al. 2008, Santos et al. 2013).

TE concentration in cetaceans can reflect a broader marine habitat than the coastal habitats usually represented by sessile species such as bivalve mollusks, commonly used in biomonitoring programs. We can thus expect at least two different scenarios: a same trend of TE concentrations over time for both species, likely reflecting a global marine contamination, or, in opposition, different temporal trends for the two species, MUs and/or TEs considered, likely reflecting an heterogenous marine contamination of the studied cetaceans depending on habitats, intrinsic factors (age, sex, maturity, etc.) or prey consumed. Therefore, these potential intrinsic factors were all measured and considered to investigate the temporal trends. In addition, the stable isotope approach is suited to better interpret contaminant exposure and levels in historic and contemporary samples. This is all the more important in changing ecosystems where cetacean species may have modified their feeding area or movements. Therefore, $\delta^{13}C$ and $\delta^{15}N$ values were also measured as proxies of their feeding ecology and integrated as variables in the temporal trend models for TEs in both species.

Lastly, in order to evaluate the question alluded above on the efficiency of the MeHg detoxification process, the molar ratio Se:Hg was calculated for both species and its temporal evolution explored.

Materials and Methods

Sample collection

Pieces of muscle, liver and kidneys of a total of 184 individuals of common dolphin (*Delphinus delphis*) and 80 harbour porpoises (*Phocoena phocoena*) were collected from stranded or by-caught animals on the French Atlantic coasts (Fig. 1) for the different analyses.

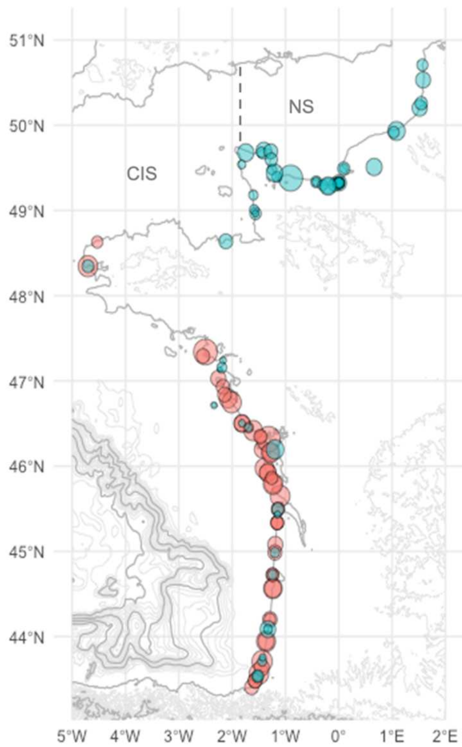


Figure 1. Map showing the location where the animals were stranded. Red and blue dots represent common dolphins (*Delphinus delphis)* and harbour porpoises (*Phocoena phocoena)*, respectively. The dash line indicates the harbour porpoise management unit boundaries, namely the Celtic and Irish Seas (CIS) and the North Sea (NS).

First, animals were identified to species, measured and sexed by external observation of the genitals cavities. Then, only fresh or slightly decomposed animals (i.e. decomposition state ≤ 3 based on the classification proposed by Kuiken and Garcia Hartmann 1993 and more recently by Ijsseldijk et al. 2019) were dissected by the trained staff from the French stranding network ("Reseau National Echouage", RNE). Common dolphins' and harbour porpoises' samples were collected from 2001 to 2017 and from 2004 to 2015, respectively. However, the conducted analysis differ depending on the chemical element and management unit for porpoises related with tissues availability (Fig. 2).

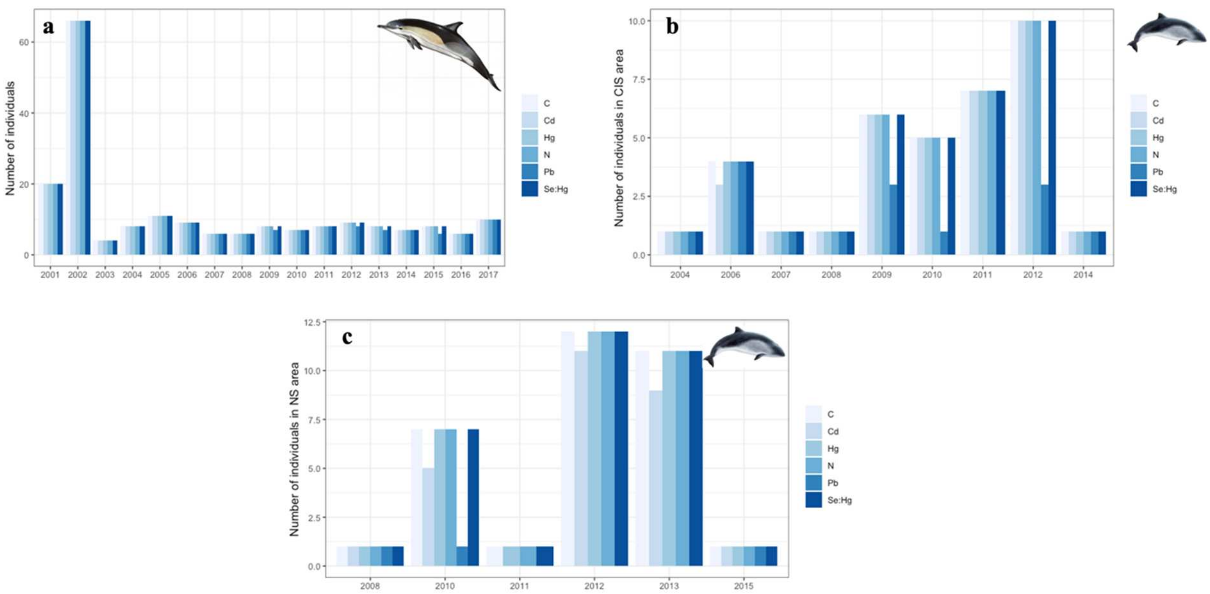


Figure 2. Temporal sampling distribution for a) common dolphins (*Delphinus delphis*), b) harbour porpoises (*Phocoena phocoena*) of the Celtic and Irish Sea (CIS) management unit and c) harbour porpoises of the North Sea (NS) management unit. C (carbon stable isotope analyses), N (nitrogen stable isotope analyses), Se:Hg (Se:Hg molar ratio).

After dissection, all samples of muscle, liver and kidney were stored in polyethylene bags and stored frozen at −20 °C until required for carbon (C) and nitrogen (N) stable isotope and total TE analyses. Teeth of the same individuals were also collected for age determination.

Trace element analyses

Total TE concentrations were analysed in the main storage tissues for marine mammals, namely the liver for Hg and Se, and kidneys for Cd (e.g. Honda et al. 1983, Wagemann and Muir 1984, Frodello et al. 2000). Lead has a strong affinity for calcified tissues, thus they are more appropriated than soft tissues for studying bioaccumulation, however, among soft tissues, liver and kidney exhibit the higher concentrations (Thompson 1990, Ma 1996). Due to the small number of bones available for analyses, liver has been selected for Pb concentrations in this study.

Liver and kidney were first freeze-dried and ground to a fine powder. For Cd, Pb and Se analyses, a Varian Vista-Pro for Inductively Coupled Plasma Atomic Emission Spectrophotometry (ICP-AES) and a Thermofisher Scientific XSeries 2 for Inductively Coupled Plasma Mass Spectrometry (ICP-MS) were used. For ICP measurements, aliquots of dried samples (0.1 to 0.3 g) were digested in a 3:1 mixture of 65% HNO_3 (Merck, suprapur quality) and 37% HCl (Merck, suprapur quality). Acid digestion of the samples was carried out overnight at room temperature and then in a Milestone microwave oven. After digestion, each sample was made up to a final volume of 50 mL with milli-Q water. Procedural blanks and certified reference materials (CRMs) – dogfish liver (DOLT-5, National Research Council Canada / NRCC) and lobster hepatopancreas (TORT-3, NRCC) – were treated and analysed in the same way as the samples. Recoveries of the elements ranged from 86 to 118% and the limits of quantification (LOQ) for Cd and Pb were 0.025 µg/g based on 0.2 g. All the equipment used in the sample processing was cleaned, and subsequently decontaminated for 24h in a solution composed of 35 mL HNO_3 (65%) and 50 mL HCl (36%) mixed into 1 L of Milli-Ro quality water.

Total Hg was determined in the liver using an Advanced Mercury Analyser (ALTEC AMA 254, with a detection limit of 0.05 ng), which does not require an acid-digestion of the samples. Aliquots of 0.5 ± 0.2 mg dried samples were directly analysed. One blank and one standard sample of CRM DOLT 5 (Dogfish liver; NRCC) were analysed every 10 samples (mean recovery rate of the CRM = 109%).

All TE concentrations in tissues are reported in µg/g dry weight (dry wt).

Carbon and nitrogen stable isotope analyses in the muscle

Muscle samples were cut, freeze-dried and ground into a fine powder before isotopic analyses. Lipids being highly depleted in ^{13}C relative to other tissue components (DeNiro and Epstein 1978), they were extracted from all muscle samples using cyclohexane to avoid any bias in $^{13}C/^{12}C$ ratios. To this end, 50 mg of muscle powder were agitated with 2 mL of cyclohexane for 1 h. Next, the samples were centrifuged for 10 min at 2500 g, and the supernatant containing the lipids was discarded. This procedure was repeated 1-3 times depending on the sample lipid content. Then, the samples were dried in an oven at 45°C for 48h, and 0.35 ± 0.05 mg subsamples of lipid-free dry powder were weighed in tin capsules for stable isotope analyses. Analyses were performed with an isotope ratio mass spectrometer (Delta V Advantage with a Conflo IV interface, Thermo Scientific) coupled to an elemental analyzer (Flash EA 2000, Thermo Scientific). Results were expressed with the classical δ notation relative to the deviation from international standards (Vienna Pee Dee Belemnite for $\delta^{13}C$ values, and atmospheric nitrogen for $\delta^{15}N$ values), in parts per thousand (‰).

Based on replicate measurements USGS-61 and USGS-62 used as laboratory internal standards, experimental analytical precision was <0.10‰ and <0.15‰ for $\delta^{13}C$ and $\delta^{15}N$ values, respectively.

Determination of age

At least five teeth were collected from each sampled individual, selecting the least worn/damaged and least curved teeth to ensure sufficient material for replicate preparations. Teeth were preserved frozen or in 70% alcohol.

Tooth preparation was adapted from the protocol described by Lockyer (1995). Teeth were immersed in a decalcifying agent (DC3   Labonord, Z.I. de Templemars, f-59175 Templemars, France) before sectioning and staining in toluidin blue (Martoja and Martoja 1967) and then washed overnight in running tap water. Sections were fixed in a synthetic medium (Isomount Labonord ®) and observed with a stereomicroscope with transmitted polarized light (Olympus® BH2, Olympus Optical Co., Ltd.). Growth layer groups (GLG) were counted, assuming that one GLG equals one year (Gurevich et al. 1980, Perrin and Myrick 1980, Klevezal 1996). Three independent readings were done for each tooth section (Hohn and Fernandez 1999). All readings were recorded to the nearest whole year and age was expressed as mean for each individual.

Data treatment

All statistical analyses and plotting of results were performed using R studio version 1.4.1106 (R Core Team, 2019). Firstly, the mean concentrations of the three toxic TEs analysed and the mean of $\delta^{13}C$ and $\delta^{15}N$ values were statistically compared among species and/or MUs (for porpoises) using the non-parametric Mann Whitney Wilcoxon test.

Dynamic linear models (DLMs) were used to investigate the temporal trends of toxic TEs and identify the variables that better explained their specific trends. Isotopic ratios (i.e. ecological variables) were also modeled with DLMs in order to identify potential environmental changes (or at least changes in the cetacean feeding behavior). However, as these ratios showed a strong correlation, they were separately added to the models.

Only the individuals for which we had data for all accompanying (explanatory) variables and toxic TE concentrations in the dedicated tissue were used for fitting DLMs. The sample size, the studied time range and the number of individuals thus varied in the models, depending on the TE studied. The models were all constructed with year, age (or length for Pb models using harbour porpoise's dataset since not enough age data were available) and sex as biological explanatory variables. For Pb models using porpoise's dataset, sex was not included since not enough females were available. Moreover, for harbour porpoises, models were split by MUs depending on the location where animals were stranded, the North Sea (NS) located east of the French Cotentin, and the Celtic and Irish Seas (CIS) located west of the French Cotentin (Fig. 1). This geographical division was based on the recommended Management Units for harbour porpoise delineated by the ICES Working Group on Marine Mammal Ecology (WGMME) (ICES 2014).

A general script for dataset analysis using DLMs was written in Stan (Carpenter et al. 2017). Then, it was incorporated into an R script using the rstan library (Stan Development Team 2020). DLMs are linear models that reveal trends over time (such as 'shifts') in a dataset (Petris et al. 2009). The model is based on the decomposition of a data y_{it} (on a log scale for TE concentration values) about an individual i at time t into a component related to the individual's own characteristics (e.g. a set of p covariates x_p) and another one specific to time α_t (and a residual ε_{it}):

$$y_{it} = \mu + \sum_{k=1}^{p} \beta_k \times x_{ik} + \alpha_t + \varepsilon_{it} \tag{1}$$

The equation (1) is a decomposition of the response variable into time-invariant characteristics of an individual i (for example sex or age at death) modelled with linear regression, and a time-varying component α_t modelled with a random walk of order 1:

$$\begin{cases} \alpha_1 = 0 \\ \alpha_{t+1} \sim \mathcal{N}(\alpha_t, \sigma_\alpha) \end{cases} \tag{2}$$

where σ_α is a scale parameter controlling the magnitude of temporal change from one year to the next.

The R script was then adapted for the different TEs (i.e. Pb, Hg and Cd) and systematically run taking the above presented co-variables in the following order: age, sex, $\delta^{13}C$ and $\delta^{15}N$.

The WAIC (widely applicable information criterion, a measure of the goodness of fit of a statistical model; Gelman et al. 2014) was taken and compared between models: the smaller WAIC revealed the better model in terms of parsimony and fit to the dataset. The results of the inter-model comparisons were used to determine the influence of co-variables on the TE temporal trends. The models with the smallest WAIC were selected, and the linear trend between the first and last year was estimated from the posterior distribution of the parameters. Data and code to reproduce the analyses are available at https://gitlab.univ-lr.fr/pelaverse/pelaMSFD.

Finally, the Se:Hg molar ratios were also calculated in liver from each individual and modelised over time.

Results

General levels of toxic trace elements and $\delta^{13}C$ and $\delta^{15}N$ values

Liver Pb concentrations were significantly higher in harbour porpoises than in dolphins (p-value < 0.05), and similar between MUs (p-value = 0.7705). Mean liver Hg concentrations were similar in both species (p-value = 0.5145) but different between MUs for harbour porpoises (p-value < 0.05) (Table 1). On the contrary, Cd concentrations in kidneys were significantly higher in common dolphins (p-value < 0.05) (Table 1) and similar between areas for porpoises (p-value = 0.7462). Concerning C and N stable isotope ratios, both species exhibited significantly different values (p-value < 0.005), but the values were similar between areas for harbour porpoises (Table 1). Regarding the mean Se:Hg molar ratio in liver, it was of 3.8 (± 3.7) in common dolphins and 2.4 (± 1.3) and 2.8 (± 1.3) in CIS and NS porpoises, respectively. These ratios were not significantly different between species, nor between MUs (p-value = 0.8614 and 0.5077, respectively).

Table 1. Toxic trace element concentrations in µg/g of dry weight, Se:Hg molar ratio and $\delta^{13}C$ and $\delta^{15}N$ values in ‰ (means ± standard deviations) measured in common dolphins (*Delphinus delphis*) and harbour porpoises (*Phocoena phocoena*) from European Atlantic waters. "n" represents the total number of individuals analysed per species and management units (MUs). The different letters in parentheses indicate when significant differences (p-value < 0.005) were observed between species or MUs for porpoises.

Chemical elements & Stable isotope ratios	*Delphinus delphis*		*Phocoena phocoena*			
			Celtic & Irish Sea		North Sea	
	n	mean ± SD	n	mean ± SD	n	mean ± SD
Pb in liver	87	0.035 ± 0.03 (a)	31	0.061 ± 0.036 (b)	26	0.060 ± 0.042 (b)
Hg in liver	201	28.6 ± 43.2 (a)	36	42.9 ± 59.7 (b)	33	21.0 ± 24.6 (a)
Cd in kidney	201	5.18 ± 7.66 (a)	35	2.64 ± 2.39 (b)	27	1.75 ± 1.49 (b)
Se in liver	201	26.7 ± 36.2 (a)	36	21.0 ± 25.3 (a)	33	30.8 ± 50.8 (a)
Se:Hg molar ratio	201	3.8 ± 3.7 (a)	36	2.4 ± 1.3 (a)	33	2.8 ± 1.3 (a)
$\delta^{13}C$	201	-17.6 ± 0.6 (a)	36	-17.1 ± 0.5 (b)	33	-17.2 ± 0.5 (b)
$\delta^{15}N$	201	12.2 ± 0.8 (a)	36	13.2 ± 0.9 (b)	33	15.7 ± 1.2 (b)

Temporal trends of toxic trace elements

The temporal evolution of the three toxic TEs were studied using DLMs, with biological and ecological data (i.e. stable isotope ratios) used as co-variables that may explain these trends while they can correct their effect. Thus, the models with the best statistical values for each species and MUs (herein called the "best models") are summarized in Table 2.

Table 2. Results for the "best models" selected to explain temporal trends of (log transformed) TE concentrations and Se:Hg molar ratios in harbour porpoises (*Phocoena phocoena*, from the two MUs studied) and common dolphins (*Delphinus delphis*). WAIC values and the best model performed are indicated together with the sample size "n".

Species	**n**	**Celtic and Irish Sea (CIS)**	**North Sea (NS)**
Harbour porpoise (*Phocoena phocoena*)	31/26	Log [Pb] ~ year + sex + length WAIC = 47.58	Log [Pb] ~ year + sex + length WAIC = 44.04
	37/33	Log [Hg] ~ year + sex + age WAIC = 89.42	Log [Hg] ~ year + sex + age + δ^{13}C WAIC = 85.85
	36/29	Log [Cd] ~ year + sex + age + δ^{15}N WAIC = 74.37	Log [Cd] ~ year + sex + age + δ^{15}N WAIC = 68.26
	36/33	Log [Se:Hg] ~ year + sex + age WAIC = 27.35	Log [Se:Hg] ~ year + sex + age + δ^{15}N WAIC = 6.05
Common dolphin (*Delphinus delphis*)	95	Log [Pb] ~ year + sex + age + δ^{15}N WAIC = 145.77	
	184	Log [Hg] ~ year + sex + age + δ^{13}C WAIC = 459.78	
	184	Log [Cd] ~ year + sex + age + δ^{15}N WAIC = 421.31	
	180	Log [Se:Hg] ~ year + sex + age + δ^{15}N WAIC = 246.30	

For porpoises from both MUs, the best Pb concentration models (smallest WAIC) were those including year, sex and length as co-variables. The $\delta^{13}C$ and $\delta^{15}N$ values were not considered in these models (Table 2). Both MUs showed a high probability to have a negative trend, however, these trends were not significant (Table 3 and Figure 3). For common dolphins, we observed a clear decreasing trend (Figure 4) confirmed by a probability to have a negative trend of 0.999 (Table 3). Moreover, this negative trend was statistically significant, with a p-value of 0.008 (Table 3). The best Pb model for dolphins included year, sex, age and $\delta^{15}N$ values as co-variables influencing its temporal trend (Table 2).

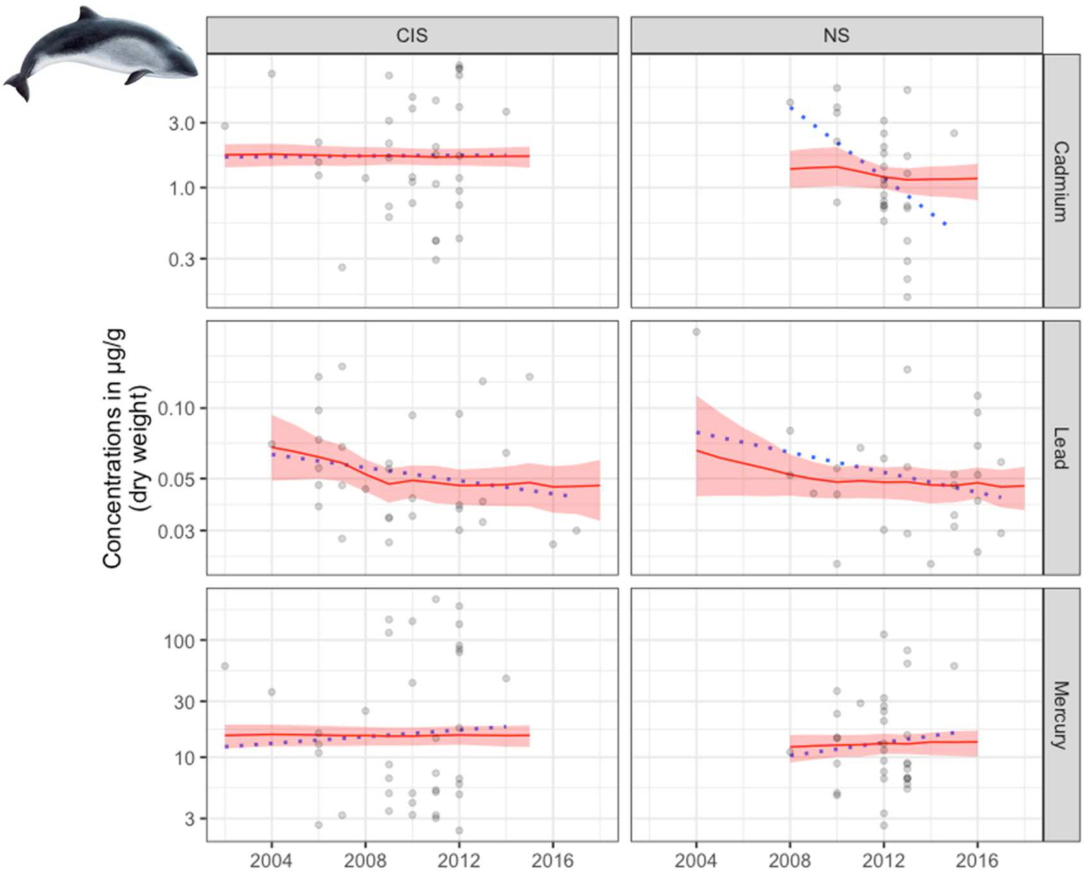


Figure 3. Dynamic linear best models for log-transformed TE concentrations (µg/g dry weight) measured in harbour porpoises (*Harbour porpoise*) from the two management units, namely Celtic and Irish Sea (CIS) and North Sea (NS). Grey dots represent the raw data, the red shaded areas the trajectory estimated by the model and the blue dotted line the trend.

Table 3. Results of the trend assessment between the first and last year for Hg, Cd and Pb concentrations and for Se:Hg molar ratios. The trend corresponds to the trend direction coefficient, the standard error gives information on the interval where the trend direction coefficient is most likely to be found, and the p-value gives information on the statistical significance of this coefficient (the significance threshold being set at 0.05). Finally, the trend probabilities provide information on the probability that the trend is positive or negative and these probabilities are between 0 and 1. The highest probability are in bold.

	Chemical element	**Trend**	**se**	**p-value**	**Probability of positive trend**	**Probability of negative trend**
Delphinus delphis						
	Pb	-0.002	0.001	0.008	0.001	**0.999**
	Hg	1.806	0.293	0	**1**	0
	Cd	0.366	0.046	0	**1**	0
	Se:Hg	0.164	0.023	0	**1**	0
Phocoena phocoena						
	Pb	-0.002	0.002	0.312	0.112	**0.888**
Celtic & Irish Sea	Hg	-0.006	0.281	0.983	0.504	0.496
	Cd	-0.003	0.032	0.915	0.476	0.523
	Se:Hg	0.037	0.048	0.445	**0.804**	0.196
	Pb	-0.002	0.004	0.656	0.283	**0.717**
Nort Sea	Hg	0.229	0.796	0.774	**0.619**	0.382
	Cd	-0.024	0.13	0.855	0.334	**0.665**
	Se:Hg	-0.006	0.058	0.915	0.462	**0.538**

For porpoises, the best models selected for Hg concentrations included year, sex and age as co-variables. Moreover, for NS porpoises, $\delta^{13}C$ values also affected the Hg trend (Table 2). No visual trend was observed for both MUs (Fig. 3), that is confirmed by the non-statistically significant p-value observed for both trends (Table 3). However, NS porpoises showed a higher probability to have a positive trend than CIS porpoises (0.619 positive trend *vs* 0.382 negative trend, Table 3). In opposition, dolphins showed a clear and statistically significant positive trend for Hg concentrations, with a probability to be positive of 1 (Fig. 4 and Table 3). The best model for this species included year, sex, age and $\delta^{13}C$ values as co-variables having an effect (Table 2).

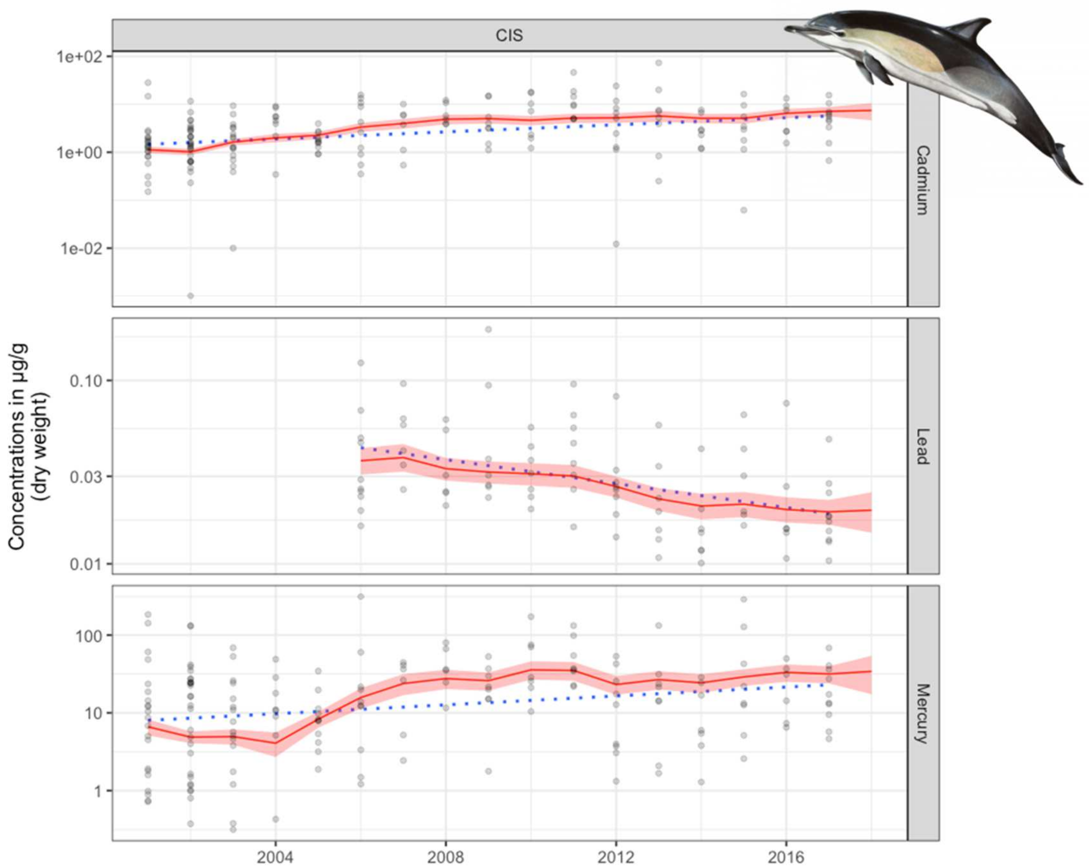


Figure 4. Dynamic linear best models for log-transformed TE concentrations (µg/g dry weight for common dolphins (*Delphinus delphis*). Grey dots represent the raw data, the red shaded areas the trajectory estimated by the model and the blue dotted line the trend.

As for Hg, porpoises did not reveal a clear trend for Cd concentrations (Fig. 3). The trend was not statistically significant for both MUs, but NS porpoises showed a higher probability to have a decreasing trend (0.665 *vs* 0.334) than CIS porpoises (0.504 *vs* 0.496, Table 3). Moreover, the best models for both MUs included the same co-variables year, sex, age and δ^{15}N values (Table 2). In contrast to porpoises, dolphins revealed a positive and significant trend for Cd (p-value < 0.05), with a probability of 1 to be positive (Table 3). The best model selected for this species also included δ^{15}N values, in addition to year, sex and age (Table 2).

Temporal trends of Se:Hg ratios

Both species showed mean hepatic Se:Hg molar ratio higher than 1 (Table 1), exhibiting an excess of Se compared to Hg. However, while no porpoise presented values lower than 1, nine common dolphins showed ratios below 1. The best Se:Hg models revealed the importance of biological variables for both species (Table 2) and, moreover, the effect of δ^{15}N values for NS porpoises and common dolphins. In addition, the Se:Hg trend was significant and positive for common dolphins while no significant for porpoises (Fig. 5 and Table 3). However, the CIS porpoises showed a probability to have a positive trend of 0.804.

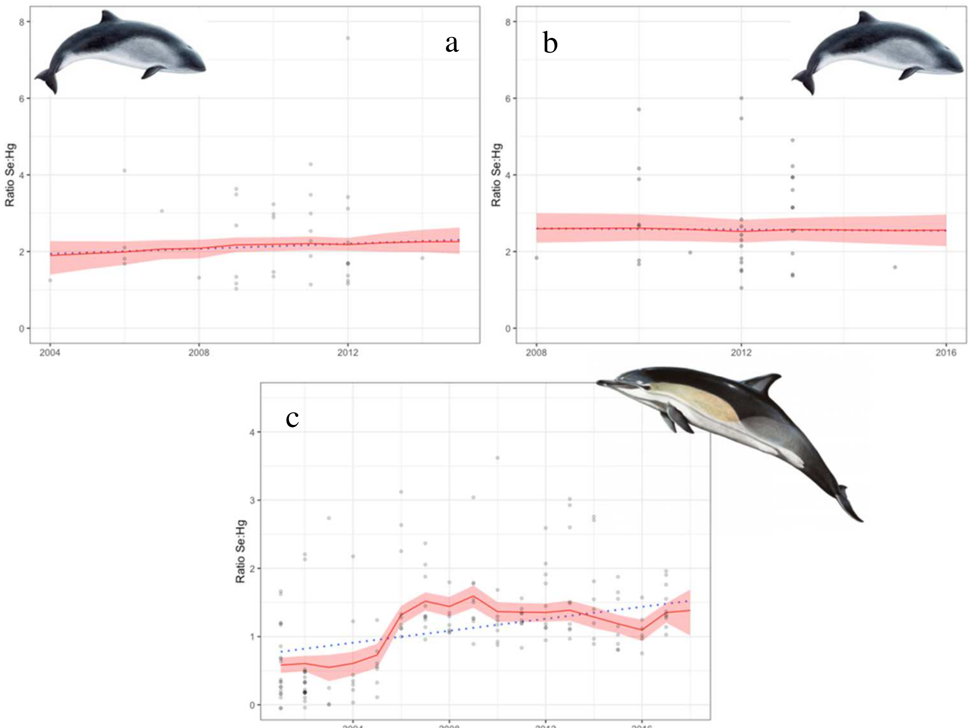


Figure 5. Dynamic linear best models for Se:Hg molar ratio in a) harbour porpoise (*Phocoena phocoena*) from Celtic and Irish Sea (CIS) management unit, b) harbour porpoise from North Sea (NS) management unit and c) in common dolphins (*Delphinus delphis*). Grey dots represent the raw data, the red shaded areas the trajectory estimated by the model and the blue dotted line the trend.

Discussion

Marine mammals are well-known to be considered as good integrators of the environmental contamination on a wide spatial scale. This is explained because marine mammals are long-lived marine apex predators that consume a wide range of prey. They have, moreover, developed mechanisms to control metal concentrations in their bodies or to mitigate their toxic effects. They have the capacity to store anthropogenic chemicals and toxins and they are highly mobile animals (e.g. Honda et al. 1983, Aguirre and Tabor 2004, Bossart 2011). Hence, they are selected as one of the functional groups to assess the marine environmental status of European marine waters in the context of the MSFD, which includes abundance, range and population parameters monitoring, as well as impacts of threats like fishery bycatch, ship strikes, underwater noise, pollution, etc.

Changes in cetacean contaminant concentrations with time may, however, reflect two different events. Changes in the environmental contamination since cetaceans consume the same prey species which, on the contrary, do not present the same levels of contamination due to changes in the environment levels. Or changes in their feeding habits, namely, the prey species ingested and/or the foraging areas where cetaceans feed varied. Therefore, to investigate the temporal trends of toxic TEs and to identify whether these two cetacean species may reflect changes in the environmental contamination *vs* changes in their feeding habits, the evolution of the raw concentrations must be assessed in the light of eco-biological variables (as explained in data treatment section).

Lead

For Pb, the best models showed a decreasing trend with time, in both species and both MUs for porpoises. All models revealed an effect of the biological factors. Moreover, in the case of dolphins, $\delta^{13}C$ values (i.e. proxy of animals' foraging habitat) also had an effect. Anyhow this element showed a common downward trend with a higher effect of the biological factors, such as length, suggesting a decrease of Pb concentrations in cetaceans probably related to a decrease in the environment. This decrease is consistent with the widespread and drastic decrease of Pb in gasoline after the leaded gasoline ban from the European Union market (on 1 January 2000), which has also been observed in several studies carried out on marine and terrestrial dependent species. Caurant et al. (2006) observed a change on the isotopic composition of Pb analysed in teeth and bones of three cetacean species (common dolphin, harbour porpoise and striped dolphin *Stenella coeruleoalba*) from European waters. The stable Pb isotope ratios reflects the geological system from which the metal derives. Grousset et al. (1994) observed an increase of the ratio $^{206}Pb/^{207}Pb$ in the oldest individuals analysed reflecting a decrease in the production of alkyl lead in Europe, i.e., the increasing use of unleaded gasoline. Similarly, Couture et al. (2010) analysed the Pb concentrations and the stable isotope composition of blue mussels (*Mytilus edulis*) from the French Atlantic coast. They also observed difference between the stable Pb isotope ratios measured in the mussels and the Pb emitted in western Europe as a result of leaded gasoline combustion. In fact, the Pb isotope composition of mussels was more like of that of the Pb released to the environment by wastewater treatment plants, municipal waste incinerators and industries, such as metal refineries and smelters. Recently, decreasing Pb concentrations were also observed in bones of a small cetacean species of the southwestern Atlantic, the Franciscana dolphin (*Pantoporia blainvillei*) (Garcia-Garin et al. 2021). Authors also associated this decrease in Pb concentrations with the ban in the use of this element as an additive in gasoline and as component of car batteries. Likewise, such decrease of Pb concentrations have also been shown in the terrestrial environment. Thus, the European moss survey on the temporal trend in atmospheric heavy metal deposition that has been repeated at five-yearly intervals (from 1990 to 2010), showed a decrease of 72% in Pb concentrations between 1995 and 2005 (Harmens et al. 2010). A study carried out in kestrel from rural and urban areas in Spain showed a decrease of Pb concentrations in livers between two periods: 1995-97 and after 2001 when the restrictions on leaded fuel were implemented (García-Fernández et al. 2005).

Consequently, the highest effect of biological co-variables on models and the concordance with literature of the Pb temporal decrease observed in these two cetacean species and in both geographical areas has probably to be related to a reduced environmental contamination linked to Pb restrictions worldwide. Nevertheless, this Pb decrease seems to be slower and later in the high trophic levels to which the cetaceans belong.

Mercury

Mercury is listed as one of the "ten leading chemicals of concern" (WHO, 2017) and, in contrast to other metals, it is considered as a "global pollutant" due to the long residence time of some Hg forms that can be transported thousands of miles in the atmosphere around the globe. Mercury is a natural element from the earth's crust, although human activities (e.g. mining, fossil fuel combustion) have led to its widespread pollution. The largest source of anthropogenic mercury emissions are artisanal and small-scale gold mining (37.7%), followed by stationary combustion of coal (21%). Other large sources of emissions are non-ferrous metals production (15%) and cement production (11%) (Steenhuisen and Wilson 2019). Thus, in recent decades, global efforts to reduce anthropogenic Hg emissions and associated risks to ecosystems and human health were set up (such us the Minamata Convention on Mercury, Selin et al. 2018). However, emissions from anthropogenic sources are approximately 2220 metric tons per year, which includes re-emitted Hg (Steenhuisen and Wilson 2019). Therefore, numerous studies around the world have focused on this toxic TE and particularly on its temporal trends in biota. In this study, we observed contrasted trends for dolphins and porpoises from the two MUs studied. Dolphins showed an increasing and significant trend of Hg concentrations with time, NS porpoises also exhibited an increasing but non statistically significant trend while CIS porpoises revealed a stable trend. Additionally, and as expected, age was an important variable for all Hg models. In fact, while the influence of age (or size, as a proxy of age) on Hg concentrations is variable according to the species (and probably related to individual Hg exposure through diet), it remains a major factor driving Hg bioaccumulation. Mercury strongly binds with protein sulfhydryl groups (-SH) once incorporated in organisms, and its elimination or excretion is very slow over time (sometimes equal to zero like in the muscle tissues) due to this affinity (Wang and Wong 2003, Maulvault et al. 2016). Therefore, Hg bioaccumulate with size or age in marine organisms (e.g. Monteiro et al. 1991, Cossa et al. 2009, Chouvelon et al. 2014) and must be considered as confounders in models to correctly interpret concentrations and trends. Another important factor linked to Hg marine biogeochemical cycling and its chemical properties is the habitat of species. The concentrations of dissolved methylmercury (Me-Hg, the most common form of organic mercury in the environment) in ambient waters of cetacean habitats may differ. As an example, mesopelagic species seem to be more exposed to dissolved Me-Hg in the deep-pelagic layers (e.g. Cossa et al. 2009, Heimbürger et al. 2010) reflecting in consequence higher Hg concentrations than epipelagic species (Monteiro et al. 1996, Choy et al. 2009, Chouvelon et al. 2012). In this sense, the best models for dolphins and NS porpoises included $\delta^{13}C$ values as an important factor affecting Hg trends. Both models revealed an increasing trend of Hg concentrations, suggesting a change in prey contamination and likely related to a higher Hg exposure due to changes on the prey ingested or in the feeding areas.

Then, our results agreed with what is observed worldwide for Hg temporal trends, that is different trends depending on species, latitudes and oceans. Thereby, in Artic biota, several studies also revealed contrasted trends for Hg concentrations. Therefore, Aubail et al. (2010) observed a reduction on Hg concentrations of Svalbard polar bears (*Ursus maritimus*) from

1964 to 2000. The authors related this result to a reduction in environmental Hg exposure in absence of trends in $\delta^{13}C$ and $\delta^{15}N$ values that could support the hypothesis of a temporal variation in feeding or foraging habitats. In contrast, Riget et al. (2011) based on 83 different time series from terrestrial, freshwater and marine species indeed revealed an average Hg level change between -8.6% to +10% per year across time series, depending on species and study site. Likewise, in the past century there is a lack of significant and consistent increase of Hg levels in seabird species from the Southern Ocean (Thompson et al. 1993, Becker et al. 2002, Scheifler et al. 2005, Carravieri et al. 2013). This probably suggest that Hg transfer to Southern Ocean predators has relatively remained constant, being in accordance with the characteristic lower anthropogenic emissions and deposition rates of the Southern Hemisphere when compared to the Northern one (e.g. Futsaeter and Wilson 2013). Nevertheless, an increase in Hg concentrations have been detected in subantarctic species by Carravieri et al. (2013). Authors suggested that this increase may be indicative of recent changes in Hg emission and/or deposition patterns, linked to the development of Asian and Southern Hemisphere countries, as shown by increased Hg deposition in peat cores and lake sediments in the Southern Hemisphere (e.g. Hermanns and Biester 2013). In temperate regions, fewer studies focused on Hg temporal trends compared to high latitudes, and results are even less clear. As an example, the moss European survey presented above revealed a significant variability between countries. In general, Hg concentrations did not vary across Europe between 1995 and 2000 (Harmens et al. 2008), either between 2000 and 2005 (Harmens et al. 2010), however a reduction of Hg concentrations was observed between 2005 and 2010 for most of the countries included in this survey (Harmens et al. 2013). Concerning cetacean species, a previous study conducted on harbour porpoise from Japan waters showed constant concentrations since 1985 to 2010 (Yasuda et al. 2012). In the Mediterranean Sea, however, decreasing concentrations were observed in liver samples of striped dolphins (*Stenella coeruleolaba*) stranded between 1990 and 2015, suggesting that measures to reduce Hg emissions in Western European countries have been effective to reduce Mediterranean pollution to Hg (Borrell et al. 2014, Martínez-López et al. 2019a). Later, an overview conducted on more than 101 technical reports and peer-reviewed articles published between 1972 and 2016 considering 284 total Hg measurements in the liver (e.g. main storage tissue) of 43 cetacean species, revealed that no apparent change was detected in reported concentrations between 1975 and 2010 (Kershaw and Hall 2019).

In summary, this literature review suggests that Hg concentrations may vary depending on the food webs, species, habitat and the region studied.

Cadmium

Concerns about the public health effects of Cd concentrations have increased over time, and with the introduction of new technologies, the use of Cd has generally decreased apart from its use in batteries of nickel–cadmium and in Cd telluride solar panels (Cullen and Maldonado 2013). Therefore, it is reasonable to expect that environmental Cd exposure of the general population has decreased. Cadmium temporal trends we found for porpoises from the NS were in accordance with this expected decrease. On the contrary, CIS porpoises showed a stable trend, while common dolphins revealed a significantly increase with a probability of 1 to have a positive trend. As for the other TEs considered, diet is the main source of Cd exposure in cetaceans, which implies that these different trends observed in porpoises and dolphins may be related to both a lower contamination of the environment and/or to changes in their diet preferences. In fact, cephalopods have been shown to be an important vector of Cd to marine top predators in the North-East Atlantic, and especially in high latitude areas (Bustamante et al. 1998). It was also shown that oceanic cephalopods in particular (mainly Cranchids and Histioteuthids) constitute a major source of Cd for common dolphins, representing a Cd intake 12 times higher in oceanic common dolphins compared to neritic ones (Lahaye et al. 2005). The increase in Cd concentrations observed in dolphins may thus reflect a change in Cd exposition through diet. In fact, the best model for this element in dolphins highlighted $\delta^{15}N$ values as the variable that most affected the Cd temporal trend, suggesting that dolphins changed their consumption feeding on Cd-enriched prey species and/or species at different trophic level.

Compared to Hg, fewer studies have focused on the temporal evolution of Cd concentrations over the last decades. However, most of them are consistent with decreasing trends, regardless of the geographical areas and species considered. The European moss survey corroborates this trend, associating it with a slowdown of Cd atmospheric emissions since 1980, due to international regulations and the implementation of efficient capture and recycling initiatives in point sources (Cullen and Maldonado, 2013). Other European studies on terrestrial birds have also shown decreasing concentrations of Cd. Bustnes et al. (2013) found a decreasing trend in tawny owl feathers (*Strix aluco*) from 1986 to 2005 in Central Norway, possibly because of lower atmospheric deposition of Cd. Recently, Manzano et al. (2021) also revealed a decline of Cd concentrations in kestrel (*Falco tinnunculus*) feathers in the southwest of Spain from 1966 to 2016. In artic areas, Outridge et al. (1997) also observed decreasing Cd concentrations from pre-industrial to modern times, through the analyses of Cd in the tooth cementum of walrus (*Odobenus rosmarus*) collected in eastern Arctic Canada (Outridge et al. 1997). They concluded that probably the current Cd levels result exclusively from natural sources. Furthermore, Cd concentrations in Greenland ice and snow cores suggested stable Cd levels from 7760 BP to 1850, after which a sevenfold increased was observed up to 1960–1970, followed by a twofold decrease until 1992 (Candelone et al. 1996).

The increasing trend observed for Cd concentrations in dolphins is in opposition with the majority trend observed on the literature, which rather confirms an effect of dietary changes and/or feeding areas for common dolphins.

Detoxification processes

The long-term exposure of cetaceans to toxic TEs has enabled them to develop mechanisms to limit their potential toxic effects. The demethylation of MeHg and protection through Se sequestration have been proven as Hg detoxification mechanisms in cetaceans, which may explain the very high concentrations measured in tissues of some species with no apparent acute or long-term toxicity (Wagemann et al. 1998, Caurant et al. 1996). An animal with a liver molar excess of Se (Se:Hg > 1) can be considered at lower risk of Hg toxicity, whereas an animal with a molar excess of Hg (Se:Hg < 1) is at a greater risk of Hg toxicity. Therefore, the Se:Hg molar ratio is of importance when aiming to determine the impact of the contaminant load at an individual or even population level. While both species showed average Se:Hg ratios > 1 (2.4 ± 1.3, 2.8 ± 1.3 and 3.8 ± 3.7 for harbour porpoises and common dolphins, respectively), 9 adult common dolphins stranded between 2001 and 2005 exhibited Se:Hg ratios < 1. In view of the raw data, these ratios are a consequence of the high Hg concentrations found in these individuals rather than a deficiency of Se, which suggests a potential risk of Hg toxicity for these individuals.

The models applied to the Se:Hg molar ratio of both species revealed an increase of these ratios over time for CIS porpoises and dolphins, which in turn showed an increase of Hg concentrations with years. Moreover, $\delta^{15}N$ values affected these models. This suggests a constant exposure to Se through the prey consumed, or at least the homeostasis at the same level, and a probable effect of the trophic level of prey on dolphin Se composition. Lastly, all the best Se:Hg models had age as an important co-variable. In fact, mature animals demethylate MeHg from their diet more efficiently than young animals and in the case of high Hg exposure, a close to 1:1 molar ratio of Se:Hg is maintained in adult animals (e.g. Koeman et al. 1975, Martoja and Berry 1980, Itano et al. 1984, Cuvin-Aralar and Furness 1991, Caurant et al. 1996, Nigro and Leonzio 1996, Yang et al. 2007, Martínez-López et al. 2019b). Thus, the elevated effect of age it is not surprising. Therefore, to reply to our initial question and in light of the stable or increasing temporal trends of Se:Hg ratios observed, the MeHg detoxification process seem to be still efficient for the two cetacean species and MUs considered.

Conclusion

The accumulation of TEs, from natural and anthropogenic sources, in the tissues of marine species together with the potential for indirect effects and synergism with other contaminants, underline the importance of monitoring TEs in the environment to better understand their potential health effects, and how this lead to a risk at the population level. To this aim, investigating temporal trends is an adequate way to evaluate the evolution of the presence of TEs in the environment and/or their transfer to biota over time, and the election of an appropriate bioindicator organism is then essential. In this study, we expected two potential scenarios for toxic TE trends, and we observed both. Lead showed the same trend over time for both species, with few effects of extrinsic factors. This finding was in high accordance with literature and Pb restriction emissions at the end of 1990s, reflecting trends on the global marine Pb contamination and the relevance of the use of these two species as bio-monitoring species. In opposition, different temporal trends for Hg and Cd concentrations were observed for both species and porpoise MUs, with an effect of intrinsic variables but also of extrinsic factors such as habitats and prey consumed. Broadly, these results reflect the complexity to properly assess the contamination exposure of biota without monitoring changes in prey or foraging area preferences, and highlight the necessity and the gain of using different species to monitor changes in marine environments, each of them informing on the contamination of its own ecological niche. Here, common dolphins were thought to reflect a more pelagic and widely spatial exposure, while harbour porpoises rather reflected a finer spatial scale and probably more benthic exposure (CIS and NS).

Lastly, the Se:Hg molar ratios of both species and their evolution over time suggested a low risk for Hg toxicity. Nevertheless, continue monitoring toxic TEs it remains important together with measuring in tandem Se and Hg concentrations in order to develop a more accurate indicator of what these concentrations mean in terms of compromising cetacean health.

Acknowledgments

Authors wish to thank B. Lebreton, G. Guillou and F. Aubert from the Littoral Environnement et Sociétés (LIENSs) Stable Isotope Facility at La Rochelle, France, as well as C. Churlaud et M. Brault-Favrou of the elementary platform from the LIENSs laboratory for their help on chemical analyses. We also wish to warmly thanks all members of the French stranding scheme (RNE) and all members of Pelagis observatory for their continuous effort in collecting data on stranded cetaceans.

The Pelagis observatory is funded by the ministry in charge of the environment, the French Office for Biodiversity (Office Française pour la Biodiversité - OFB), the French National Center for Scientific Research (CNRS) and by Communauté d'Agglomération de la Ville de La Rochelle.

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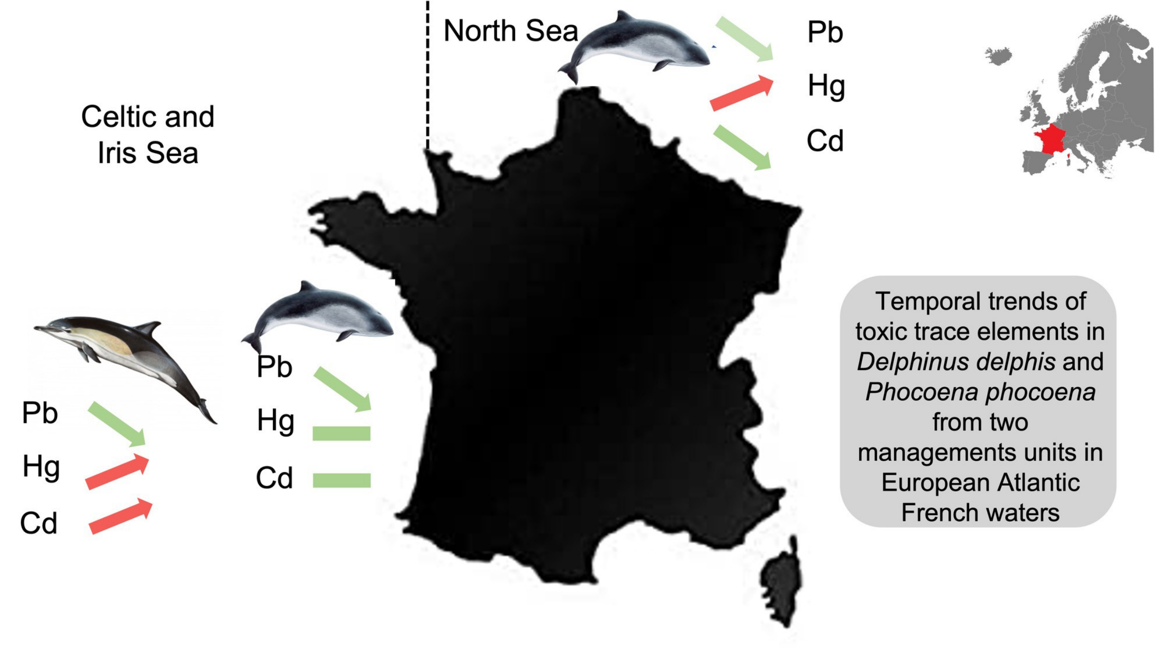
North Sea
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Temporal trends of toxic trace elements in *Delphinus delphis* and *Phocoena phocoena* from two managements units in European Atlantic French waters