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Lead contamination of small cetaceans in European waters – The use of stable isotopes for identifying the sources of lead exposure

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Abstract: Lead concentrations and isotopic composition have been measured in bone and teeth of small cetaceans belonging to three species (*Delphinus delphis*, *Phocoena phocoena* and *Stenella coeruleoalba*), to evaluate the toxicological risk and to determine sources of lead in the European waters. Lead concentrations, far lower than threshold value inducing toxic effects in human, were higher in teeth than in bones, but highly correlated between the two tissues ($r = 0.92$, $p < 0.001$). Large variations of $^{206}\text{Pb}/^{207}\text{Pb}$ values in bone tissue showed that cetaceans must be submitted to various atmospheric influences. No geographical differences appeared which is consistent with studies on their distribution indicating seasonal movements between Brittany waters and the Bay of Biscay. The negative correlation between $^{206}\text{Pb}/^{207}\text{Pb}$ ratios and age of the individuals reflected the decrease in the production of alkyl lead in Europe, i.e., the increasing use of unleaded gasoline.

Keywords: Biomonitoring; Temporal trends; Northeast Atlantic; Common dolphins; Striped dolphins; Harbour porpoises; Heavy metals; Hard tissues; Lead

1. Introduction

Among heavy metals, lead (Pb) shows a particularly elevated anthropogenic enrichment factor (Lantzy and Mackenzie, 1979). This metal has been mined and smelted by humans for centuries, and the use of Pb-based products has greatly increased since the industrial revolution. Moreover, the volatile character of Pb, associated with many emission sources, has resulted in Pb being not only a local air pollutant but also a pollutant on a global scale (Charalampides and Manoliadis, 2002). More than 90% of environmental Pb is as a result of past anthropogenic activities and nowadays, there is a global background concentration of this metal in the environment due to these past activities (Cossa et al., 1993). Consequently, Pb is ubiquitous in air, water and soil, in both urban and rural environments (Nriagu, 1978) and its dynamic nature in the environment, including transport, distribution and elimination, has long been a matter of concern. In the marine ecosystems, the neritic domain is more likely to be subject to discharges of numerous toxic components such as organic pollutants or heavy metals including Pb. Although to date, no biological requirement has been demonstrated for this metal suggesting that it is not an essential element, Pb is bioaccumulated in biota and some species, especially those living close to anthropogenic sources, exhibit relatively high concentrations (Cossa et al., 1993; Law et al., 1991, 1992). Due to their long-life span and their position at the top of marine food webs, marine mammals are considered to be good indicators for long-term monitoring of metal accumulation in the marine environment (Honda et al., 1983). However, evidence of bioamplification of Pb in the marine food web has never been shown (Cossa et al., 1993; Dietz et al., 1996). As an example, a bioaccumulation factor of 0.07 has been shown between fish and cetaceans in the Arctic food web (Muir et al., 1992). Most of the studies dealing with Pb in marine mammals have been carried out on soft tissues, in which Pb concentrations are often very low (less than $1 \mu\text{g g}^{-1}$ wet weight) (Honda et al., 1983; Wagemann et al., 1983; Storelli et al., 1999; Cardellicchio, 1995; Cardellicchio et al., 2002). Among the soft tissues, liver and kidney exhibit the higher Pb concentrations (Thompson, 1990; Ma, 1996), followed by skin (Fujise et al., 1988; Yang et al., 2002), and lastly brain and muscle (Thompson, 1990; Ma, 1996). Nevertheless, because of the strong affinity of Pb for calcified tissues, hard tissues such as bones or teeth are more appropriated than the soft ones for studying Pb bioaccumulation. Overall, investigations on Pb bioaccumulation in bones and/or teeth of cetaceans are rare, but all show that Pb concentrations in these hard tissues are usually higher than in the soft ones (Fujise et al., 1988; Honda et al., 1982). Moreover, Pb half-life varies from 5 to 20 years in the hard tissues according to the authors, whereas it is only a few weeks or months in the soft ones (e.g., Ma, 1996). For these reasons, Pb analysis in tissues such as bones or teeth should provide information on long-term trends of bioaccumulation throughout the life of a cetacean.

Furthermore, calcified tissues appear to be excellent archives, which allow us to use stable isotopes of Pb for the determination of the different sources of exposure (Aberg et al., 1998; Outridge et al., 1997; Stewart et al., 2003). Lead is a mixture of four stable isotopes: ^{204}Pb , ^{206}Pb , ^{207}Pb and ^{208}Pb . Among them, only ^{204}Pb is not the product of the radioactive decay of other elements and its abundance in the earth crust does not vary with time, whereas the three other isotopes result from the decay of ^{238}U , ^{235}U , and ^{232}Th , respectively. Therefore, in contrast to many elements whose isotopic composition is fixed during the formation of the universe, the isotopic composition of Pb varies until it is extracted from the geochemical cycle by formation of the ore. Thus, each Pb ore displays its own isotopic signature given by the relative proportions of ^{204}Pb , ^{206}Pb , ^{207}Pb and ^{208}Pb depending on the original composition and the age of the ore bodies (Doe, 1970; Sangster et al., 2000). Isotope analyses were first carried out to

explain petrogenetical and petrochemical processes which are involved in ore prospecting. However, since the isotopic composition of the ore is not affected to a measurable extent by physical or chemical fractionation processes, these isotopes can be used as natural tracers, thereby providing an ideal tool in environmental studies for determining potential sources of Pb exposure and pathways of pollution (Charalampides and Manoliadis, 2002; Véron et al., 1998; Bollhöfer and Rosman, 2001). Nowadays stable isotopes are increasingly used in many fields of environmental research and they have even been applied to wild animals such as birds (Scheuhammer and Templeton, 1998) and marine mammals (Outridge et al., 1997; Stewart et al., 2003).

The main objectives of this study were: (i) to determine the more appropriate hard tissue (bone or teeth) for long-term monitoring of Pb in small cetaceans; (ii) to investigate potential differences in Pb concentrations within small cetaceans from European waters; (iii) to determine the factors which could explain these differences; and (iv) to discriminate potential sources of Pb exposure in small cetaceans by using stable isotope ratios. Lead was analysed in individuals belonging to three species of small cetaceans, the common dolphin (*Delphinus delphis*), the harbour porpoise (*Phocoena phocoena*) and the striped dolphin (*Stenella coeruleoalba*), collected along the Dutch, Irish, French and Spanish coasts.

2. Materials and methods

2.1. Tissue preparation and sampling procedure

Tissue samples were obtained from 61 adult cetaceans and 9 common dolphin fetuses stranded along the European coasts between 2001 and 2002. All the French common dolphins belonged to a single group, stranded on the North coast of Brittany and have been identified as the “France West Channel” school (see map Fig. 1); the individuals of other species were from single strandings and belonged to the “Bay of Biscay” sampling. In each specimen, teeth and bones (i.e., the entire foetus bones and the fifth rib for adults) were removed for Pb determinations. After collection, the samples were stored at -20 °C in individual plastic bags until analyses were performed. Teeth were also used for age determination by counting the growth layer groups (GLGs) based on the methodology described by Lockyer (1995). The sample size and the characteristics of the individuals for each country are given in Table 1.

2.2. Analytical procedures

Prior to analysis, bones and teeth were thawed just sufficiently to be cut and cleaned totally from flesh with a stainless steel knife. Subsequently, the surface of the bones and tooth samples were rubbed with high-purity acetone and then gently washed with ultrapure Milli-Q water. Teeth samples were dried over night at room temperature. All the dry samples were stored in polyethylene bags until Pb analysis.

Whenever possible, two pieces of less than 0.5 g of each dry sample were digested in a microwave digester (MARS 5) in open conditions with 5 ml of 65% HNO₃ (Normatom Suprapur®). The digested contents were made up to 50 ml with Milli-Q water and stored in plastic flasks until analysis by inductively coupled plasma-mass spectrometry (ICP-MS) with a VARIAN ULTRAMASS 700. Matrix effects and instrumental drift of the ICP-MS were corrected using the element Rhodium as the internal standard.

Certified samples from the National Institute of Standards and Technology (*Bone Meal*) were treated using the same procedure as the other samples. Results of quality controls showed a good agreement with certified data and recoveries of the metal were $95 \pm 6\%$. Lead concentrations are reported as $\mu\text{g g}^{-1}$ dry weight (dwt) and the detection limit is $0.008 \mu\text{g g}^{-1}$ dwt.

The Pb isotopic composition was also measured by ICP-MS. The measurements were corrected for isotopic fractionation using NIST SRM981 (common Pb reference material for ^{204}Pb , ^{206}Pb , ^{207}Pb and ^{208}Pb relative abundance). The accuracy of the method (expressed as the Relative Standard Error in %) was 4.6% when standard Pb concentration was $0.07 \mu\text{g l}^{-1}$ and 1% when standard Pb concentration was $1.69 \mu\text{g l}^{-1}$.

2.3. Statistical analysis

XLStat 7.5 (Microsoft_) was used to perform statistical analyses. Multiway ANCOVA analyses have been used to determine the influence of the different parameters on Pb bioaccumulation. Fisher multicomparison tests were then performed in order to identify the significant different groups of individuals. Correlation analyses were performed using Spearman product-moment correlation. The significance levels for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1. Levels and relationships between tooth and bone Pb concentrations

Lead concentrations in the hard tissues of small cetaceans from European waters are shown in Table 2. In both bone and teeth tissues, mean concentrations were lower than $1 \mu\text{g Pb g}^{-1}$ dwt, except for the French common dolphins, which exhibited mean concentration of $1.471 \mu\text{g Pb g}^{-1}$ dwt in teeth. However, the maximum Pb concentration in teeth reached $1.028 \mu\text{g g}^{-1}$ dwt in one striped dolphin from Spain, $1.520 \mu\text{g g}^{-1}$ dwt in one porpoise from France, $1.371 \mu\text{g g}^{-1}$ dwt in one common dolphin from Spain and $2.251 \mu\text{g g}^{-1}$ dwt in one harbour porpoise from the Netherlands.

A significant positive relationship between tooth and bone Pb occurred in all the species examined ($r^2 = 0.898$; $p < 0.0001$, $n = 47$; Fig. 2), and Pb concentrations in teeth were about two times higher than in bones (Table 2).

3.2. Factors influencing contamination levels

No sex-related difference in Pb concentrations was observed in teeth ($p = 0.793$) or bone ($p = 0.265$), hence this parameter was discharged as a variable in the further statistical treatment. An ANCOVA analysis has been carried out which showed that Pb concentrations in teeth was positively related to age ($p < 0.0001$, $r^2 = 0.514$), with area of stranding and species explaining a further 13.8% of the variation ($p < 0.001$, multiple $r^2 = 0.652$). However, the rate of accumulation did not differ between areas and/or species, and no significant different groups were found. Hence the bioaccumulation of Pb in teeth with age is shown Fig. 3a without differentiating species or areas of stranding. In the same way, Pb concentrations in bone was positively related to age ($p < 0.0001$, $r^2 = 0.591$), with area of stranding and species explaining a further 16.3% of the variation ($p < 0.0001$, multiple $r^2 = 0.754$). Neither species, nor areas of

stranding exhibited significant differences and the rate of accumulation were the same for all the individuals (Fig. 3b).

3.3. Pb concentration in foetuses

Mean concentration of Pb found in the bones of nine common dolphin foetuses belonging to the school stranded on the West Channel coasts of France, was $0.081 \pm 0.040 \mu\text{g g}^{-1}$ dwt. Compared to adults (1.024 ± 0.267) from the same area, this value was about 10 times lower. Because foetus length only ranged between 37 and 60 cm, Pb accumulation with growth could not be studied during all the foetal period and no significant correlation was found between Pb concentrations in bone and foetus length. It should be noted that the smallest foetus of 37 cm length exhibited the highest Pb concentration, i.e., $0.16 \mu\text{g g}^{-1}$ dwt. Moreover, Pb concentrations in foetuses were not correlated to Pb concentrations in their mother.

3.4. Isotopic composition

Geographical trends of stable Pb isotopic ratios in the bone of the different species are plotted in Fig. 4. Cetaceans exhibited a large variability of $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{207}\text{Pb}/^{208}\text{Pb}$ ratios, which ranged from 1.104 to 1.271 and 2.264 to 2.503, respectively. For comparison, different potential sources of natural and anthropogenic Pb as well as different geographical atmospheric aerosols have been plotted. An ANCOVA analysis showed that $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in bone was positively related to $^{208}\text{Pb}/^{207}\text{Pb}$ ratios ($p < 0.001$, $r^2 = 0.405$), with area of stranding and species explaining a further 15.4% of the variation ($p = 0.006$, multiple $r^2 = 0.559$). However, no significant differences were found between species, or between areas. On the other hand, a significant negative relationship between $^{206}\text{Pb}/^{207}\text{Pb}$ ratios and Pb concentrations in bone is shown Fig. 5 ($r = -0.805$, $p = 0.0001$). Individuals with the lowest Pb concentrations exhibited the highest $^{206}\text{Pb}/^{207}\text{Pb}$ ratios. As Pb concentrations were related to age, the temporal trends of $^{206}\text{Pb}/^{207}\text{Pb}$ are shown Fig. 6 by representing the relationship between these ratios and the age of the individuals. An ANCOVA analysis showed that $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in bone were negatively related to age ($p < 0.001$, $r^2 = 0.39$), with area of stranding and species explaining a further 37.4% of the variation ($p < 0.0001$, multiple $r^2 = 0.712$). Interaction between age and species was significant ($F = 9.67$, $p < 0.001$), individuals of common dolphin (mainly belonging to the group from the West Channel) exhibiting higher mean age compared to the other species, and thus higher $^{206}\text{Pb}/^{207}\text{Pb}$ ratios.

4. Discussion

4.1. Pb concentrations

Lead concentrations found in this study ranged from 0.07 to $1.79 \mu\text{g g}^{-1}$ dwt in bones and from 0.05 to $3.33 \mu\text{g g}^{-1}$ dwt in teeth (Table 2). Although information on this topic is scarce, our values appear to be consistent with data reported in the current literature for marine mammal bones: $<1 \mu\text{g g}^{-1}$ dwt in common dolphins from the Australian waters (Kemper et al., 1994), from 0.27 ± 0.06 to $0.74 \pm 0.26 \mu\text{g g}^{-1}$ dwt in striped dolphins from Japan (Honda et al., 1986). As expected, Pb concentrations in bones and teeth were higher than Pb concentrations currently found in soft tissues, which are very often lower than the limit of detection (Falconer et al., 1983; Beck et al., 1997) or lower than $0.3 \mu\text{g g}^{-1}$ wet weight in liver and kidney of cetaceans (Honda et al., 1982; Wagemann et al., 1983; Fujise et al., 1988; Wagemann et al., 1996). Only studies concerning species from the Mediterranean Sea reported Pb concentrations in liver or kidney higher than concentrations found in this study (Storelli et al., 1999; Cardellicchio et al.,

2002). It has to be noted that values ranging from 0.38 to 7 $\mu\text{g g}^{-1}$ wet weight in the liver of four bottlenose dolphins, one common dolphin and one striped dolphin from the Irish Sea have been reported (Law et al., 1991, 1992). These values indicate that Pb concentrations in bones or teeth of these individuals would have been higher than those found for cetaceans stranded in Ireland in this current study.

From a toxicological point of view, the Pb concentrations found in small cetaceans from the European waters are probably not a matter of concern. Indeed, they were 10–100 times lower than Pb concentrations reported for some wild avian species for which Pb is known as a mortality factor (Van Eeden and Schoonbee, 1996), and were generally below the baseline values reported in human bones (<19 years old), i.e., from 1.5 to 3 $\mu\text{g g}^{-1}$ dwt (Samuels et al., 1989). However, the knowledge of the dose-effect relationships is necessary to assess the no-adverse-effect levels. In humans, biological effects of Pb including early reversible changes such as interference with different enzyme systems are related to blood Pb (WHO, 1995, 2001) which reflects a short-term exposure. Once it has been absorbed, Pb is not distributed homogeneously throughout the body. The non-excreted fraction of absorbed Pb is distributed among three compartments: blood, soft tissues and the mineralising tissues (bone and teeth). About 95% of the Pb body burden in adults is located in the bones, where the biological half-time has been reported to be about 30 years (WHO, 2001). Considering the case of an environmental long-term exposure, Pb concentrations in bone or teeth provide retrospective information about integrated absorption in the past. Different studies have been carried out on shed deciduous teeth in children aged 6–12 years from different countries (WHO, 1995). Pb concentrations in the whole teeth or in the dentine varied from values <2 $\mu\text{g g}^{-1}$ up to more than 20 $\mu\text{g g}^{-1}$. In most studies a negative association between Pb measures and IQ measures is found in uncontrolled data but when the social and other confounding factors are controlled, the effect has, in most cases, been reduced (WHO, 1995). Pb concentrations in dolphin teeth are much lower than in children and confirm that Pb is probably not a matter of concern for small cetacean in European waters.

Compared to adults, Pb concentrations in foetus bones were very low (see results). However, they confirm that a placental transfer of Pb occurs as early as the early embryonic stage, which has already been reported for striped dolphins (Honda et al., 1986). After birth, Pb accumulation in hard tissues was age-related (Fig. 3). However, contrary to the Honda et al. study on striped dolphin (1986), accumulation rate does not seem to be faster during the suckling period which indicates that absorption efficiency and accumulation of Pb via milk are not higher than those via food. Despite the lower number of males analysed, the similarity of accumulation rate between the two sexes (see results) would confirm this by indicating that milk is not an efficient way of Pb excretion for females. Even when only intraspecific variation is considered, Pb concentrations were highly variable among the same age class of adult dolphins of this study, and no clear general pattern could be shown regarding to the geographical origin of stranding or the species. Among factors affecting individual variation, diet is probably one of the most important, and most persistent contaminants are incorporated into the body of mammals via food (Aguilar et al., 1999). However, in the case of Pb, several studies carried out on Pb accumulation in fish or squids from different geographical areas show very little variation, and Pb concentrations in prey muscle are often <1 $\mu\text{g g}^{-1}$ dwt (Cossa et al., 1993; Miramand and Bentley, 1992; Falandysz, 1989). Along the coasts of France, the biomonitoring of contaminants carried out by Ifremer through the National Network of Observation does not show clear difference of Pb contamination between the West Channel and the Atlantic coasts.

The highest Pb levels are encountered in two estuaries: the Seine estuary in the English Channel coast and the Loire estuary in the Atlantic coast (RNO, 1995, 2000). Dependent on these estuary contributions, Pb concentrations in marine organisms tend to be higher in species inhabiting coastal and estuarine environments while Pb values are low in species living far from any industrialised outputs (Law et al., 1991, 1992; Parsons, 1998). The three species studied here occupy different habitats: the harbour porpoise is more coastal, and the striped dolphin has a more oceanic distribution than the common dolphin. However, as with geographical origin, habitat type (i.e., neritic or oceanic) did not appear to influence Pb bioaccumulation and harbour porpoises did not exhibit higher Pb concentrations as would have been expected. Thus, the very wide variation of Pb concentrations in adult individuals, despite the homogeneity of Pb levels in prey and the absence of biomagnification of this metal in the marine environment (Cossa et al., 1993), suggests that factors other than diet and age would influence Pb concentrations in dolphins.

In vertebrates, calcified tissues constitute the main target tissues for Pb accumulation and around 90% of the total Pb body burden is contained in the skeleton (WHO, 1995), from which this element is released very slowly (Ma, 1996; Mason, 2000). This specific storage in calcified tissues and low elimination rates of Pb are typically explained by the close chemical behaviour between Pb^{2+} and Ca^{2+} ions, which display a similar affinity to those tissues. However, throughout life, there is a continuous process of bone turnover or remodelling (Berglund et al., 2000). Thus, the skeleton is a potential source of endogenous Pb, which may be slowly remobilised from the bones into the blood and then eliminated. This phenomenon is particularly strong for females during pregnancy and lactation in response to hormonal changes (Mason, 2000; Berglund et al., 2000; Gulson et al., 2003). Moreover, remobilisation of Pb may occur for post-menopausal human females showing bone resorption (Ma, 1996; Berglund et al., 2000). Hence some biological processes such as pregnancy, lactation and growth rates of bones must also influence Pb concentrations in the bone. Unlike bones, teeth do not remobilise accumulated elements and continue to incrementally deposit calcium layers throughout the life of the individuals (Perrin and Myrick, 1980). The absence of such a Pb turnover in the dental tissue would explain the higher Pb concentrations in teeth than in bones of cetaceans. Therefore, teeth appear to be easier to analyse and to constitute a better archive tissue of the long-term Pb accumulation. Further studies investigating Pb concentrations in marine mammals should therefore consider teeth as the preferential tissue to monitor Pb accumulation.

4.2. Isotopic composition

Pb isotope ratio signatures have been successfully used to differentiate the sources of environmental Pb (Charalampides and Manoliadis, 2002), and for tracing sources of pollution and the movement of air-masses on a regional or a global scale (Véron et al., 1998, 1999; Bollhöfer and Rosman, 2001). Few studies have employed this technique for wildlife. Smith et al. (1990) reported that the Pb isotope ratios in the teeth of modern Alaskan sea otters (*Enhydra lutris*) reflected anthropogenic contamination compared to samples from pre-industrial times. In the same way, Outridge et al. (1997) compared modern teeth of beluga (*Delphinapterus leucas*) and walrus (*Odobenus rosmarus rosmarus*) with museum specimens in order to evaluate the historical trends of anthropogenic influence. More closely related to this study, Scheuhammer and Templeton's objectives (1998) were to distinguish sources of Pb exposure in wild birds and the use of stable Pb isotopes allowed them to conclude that Pb shot ingestion was the cause of most of the elevated Pb exposure in waterfowl and their predators in Canada.

In the present study, the wide range of $^{206}\text{Pb}/^{207}\text{Pb}$ ratios exhibited by the small cetaceans from European waters indicates that the Pb incorporated into European marine food chains, originates from a great number of sources (Fig. 5). On a global scale, the atmosphere would be the main source of pollution for the Ocean since the estuaries contribute only in a very weak part to it (Elbaz-Poulichet et al., 1984). Therefore, studies considering the Pb isotopic composition of the atmosphere have been examined to identify the possible indirect sources of Pb for the small cetaceans from the European waters. To this end, various aspects can be considered: (i) the temporal variations of the composition of the atmosphere, (ii) the geographical variability related to the fact that various areas can be under the influence of air masses exhibiting different isotopic compositions.

On the scale of Europe, the combination of the $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ ratios allowed Hopper et al. (1991) to clearly differentiate four regions: Northwest (Norway, Sweden), East (Russia, Finland), Eastern Europe and Western Europe (see Fig. 4). However, although the dolphins of this study are closed to the Western Europe area, they exhibited much more variable ratios than those which characterise it; individuals must be submitted to various atmospheric influences, including all Europe and Eastern United States as shown by isotopic composition of atmospheric aerosols from these areas (Hopper et al., 1991; Véron et al., 1999; Bollhöfer and Rosman, 2001; Flament et al., 2002). The observed differences in the Pb isotope ratios arise from the use of Pb from different ore bodies for gasoline additives and industrial operations and the signatures reflecting the mixing among different sources, will vary between two extremes in Europe. The natural imprint is represented by contribution from Holocene loess from Sahara which exhibits $^{206}\text{Pb}/^{207}\text{Pb}$ ratio of 1.198 (Grousset et al., 1994) and pre-industrial North Atlantic sediments which exhibit $^{206}\text{Pb}/^{207}\text{Pb}$ ratios comprised between 1.197 and 1.220 (Sun, 1980) (see Fig. 4). The other fraction is anthropogenic and is derived mostly from gasoline consumption (Elbaz-Poulichet et al., 1984; Hopper et al., 1991; Monna et al., 1997). In 1995, the Associated Octel Company (AOC), U.K. controlled about two-thirds of the worldwide production of alkyl leads. Although the origin of the Pb added to the gasoline may vary slightly from one European country to another, AOC uses a mixture of 206 -depleted ores derived from Precambrian ores, mostly originating from the Broken Hill ores in Australia ($^{206}\text{Pb}/^{207}\text{Pb} = 1.03 - 1.04$, see Fig. 4) (Hopper et al., 1991; Grousset et al., 1994; Monna et al., 1997). Flament et al. (2002) reported that the mean British signature of air masses ($^{206}\text{Pb}/^{207}\text{Pb} = 1.122 \pm 0.038$) was less radiogenic than the mean continental signature ($^{206}\text{Pb}/^{207}\text{Pb} = 1.155 \pm 0.022$). This has to be related to the higher automotive content in Great Britain aerosols (23–62%) compared to Continental Europe (10–36%). Moreover, European countries have not banned Pb from petrol at the same time, and France and Great Britain were the last in 2000 (Flament et al., 2002). Despite these differences, no geographical discrimination has been found in cetaceans stranded in the European waters and the individuals analysed in this work exhibited highly variable ratios and were distributed along a mixing curve that links the two extremes described above (Fig. 4). These results are consistent with studies on the distribution of common dolphins. The available data indicate that there is a marked seasonal movement along the continental shelf. During the winter, common dolphins are at their most abundant in the shallow Brittany waters, in the adjacent English Channel and nearby Celtic Shelf (Brereton et al., 1999; Tregenza et al., 1997) whereas during the spring/early summer the deep waters of the middle of the Bay of Biscay support large number of common dolphins (Tregenza et al., 1997). Therefore, despite the difference between the European countries and especially Great Britain and continental Europe, these migrations would lead to the exposure to a large variability of isotopic composition. The

analysis in the bone does not allow distinguishing between dolphins which would have different rates of migration between the two areas and different times of residence in each of them. Moreover, age is another parameter which would interfere with the geographical differences.

Thus considering the temporal trend, the Pb isotopic composition of the dolphin bone represents the integration of the exposure over variable periods according to the age of the individuals. Some have been exposed for 24 years (for the oldest dolphin) whereas others are exposed since less than one year. Thus the individuals exhibiting the highest concentrations which are the oldest individuals (see Fig. 3) also showed the lowest $^{206}\text{Pb}/^{207}\text{Pb}$ ratios (Fig. 4). This would mean that during the last 20 years, the isotopic composition of Pb has changed and that the $^{206}\text{Pb}/^{207}\text{Pb}$ ratios would have increased in the trophic webs of the European waters. Thus whereas most of the individuals born before 1990 exhibit Pb signatures which are roughly halfway between the natural and the anthropogenic end members, individuals born after 1990 (less than 10 years old) display $^{206}\text{Pb}/^{207}\text{Pb}$ ratios that are very close to the upper crust ratio represented by the pre-industrial sediments ((Sun, 1980), Fig. 6). This is in accordance with the results found by Grousset et al. (1994), who showed that since the early 1980s, there has been a general increase in the $^{206}\text{Pb}/^{207}\text{Pb}$ ratios as recorded by European aerosols. This increase parallels the decrease in anthropogenic lead in the south western European atmosphere and the decrease in the production of alkyl-Pb in Europe, i.e., the increasing use of unleaded gasoline (Grousset et al., 1994).

5. Conclusion

Age was the most important factor influencing total Pb concentrations in hard tissues of small cetaceans in European waters, but neither species nor geographical areas were discriminated by the levels of this metal. The Pb isotopic signatures in whole bone reflect the mixing between different sources and do not allow discriminating different groups of common dolphins which is consistent with the large scale movement of this species in European waters. Finally the analysis of Pb isotopes in cetacean bone tissue have shown that the temporal variability encountered in top predators of marine food webs exhibited the same tendency as the European aerosols with an increase of the $^{206}\text{Pb}/^{207}\text{Pb}$ since the early 1980s. The temporal trend that occurred in the eastern Atlantic Ocean would thus be the same as the European atmosphere. However, this could only be confirmed either in a few years by analysing bone from individuals born at the end of the 1990s or by using Laser Ablation-Inductively Coupled Plasma-Mass Spectrometry. Applied to the teeth, which provide an unique chemical archive of an individual, it may be possible to reconstruct the temporal trends as well as the frequency of the individual's movements between different areas by comparing Pb isotopes in tooth cementum growth layer groups which are continually added through life (Perrin and Myrick, 1980).

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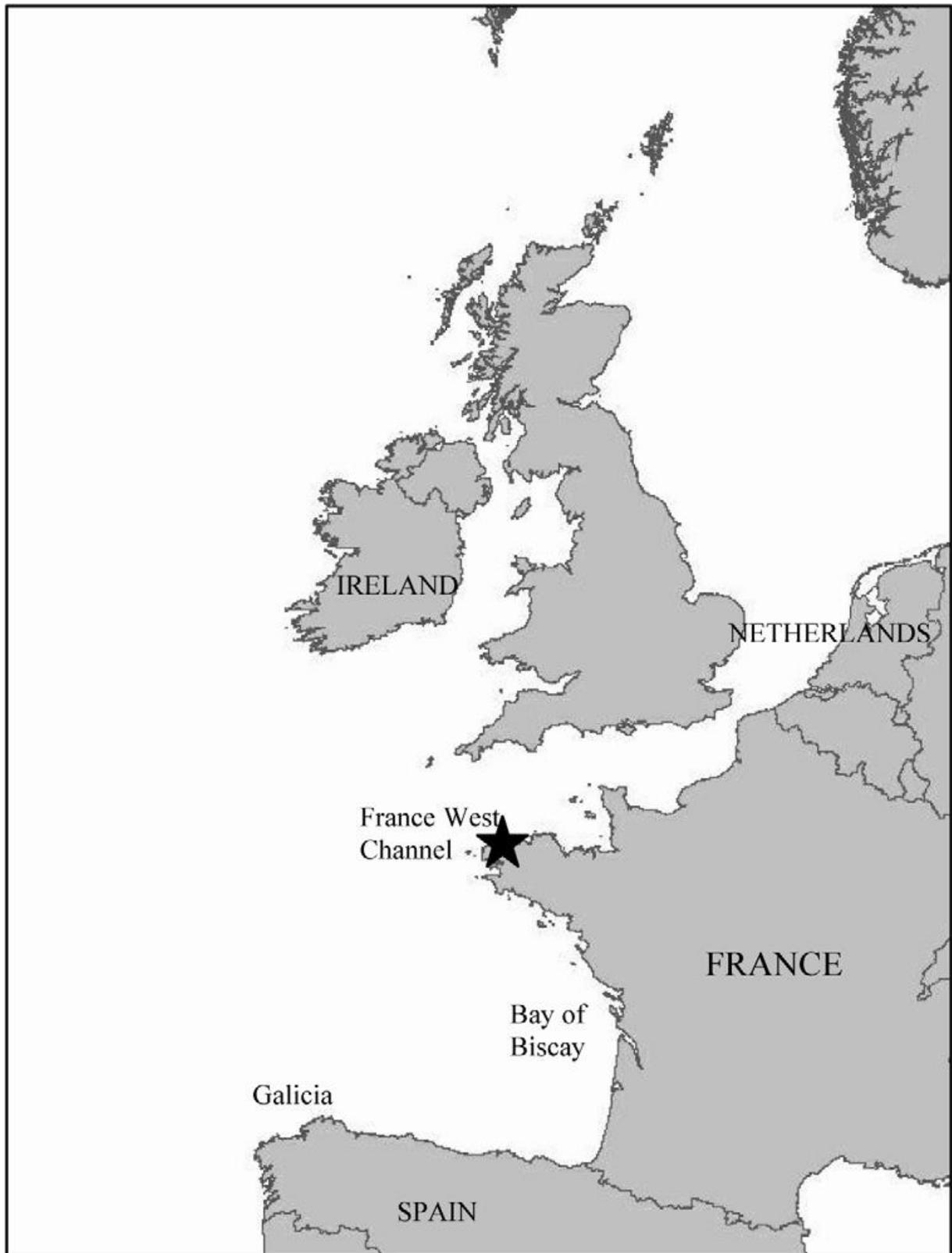


Fig. 1. Map of the European waters showing the station of the mass stranding group of common dolphins.

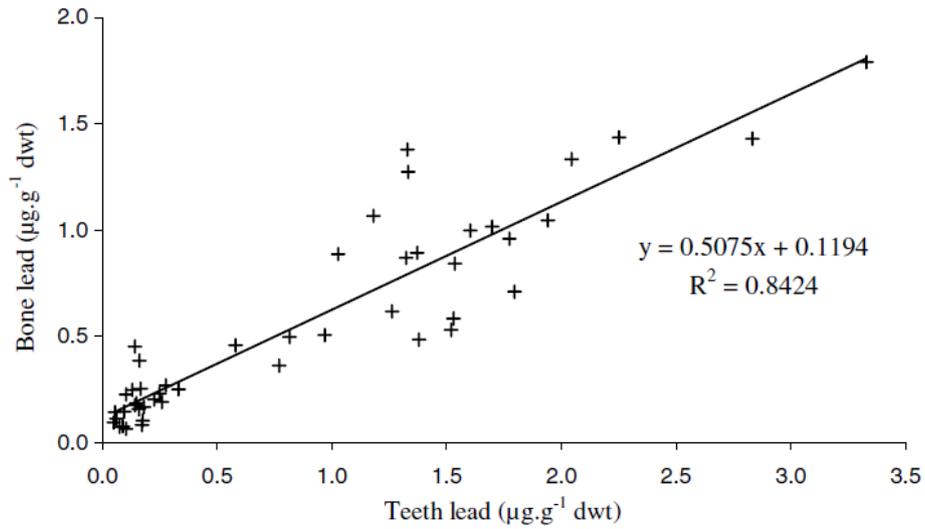


Fig. 2. Small cetaceans from the European waters: relationship between Pb concentrations in bones ($\mu\text{g g}^{-1}$ dry weight) and Pb concentrations in teeth ($\mu\text{g g}^{-1}$ dry weight). All species (*Delphinus delphis*, *Stenella coeruleoalba* and *Phocoena phocoena*) and all origins (France, Spain, Ireland and Holland) have been combined.

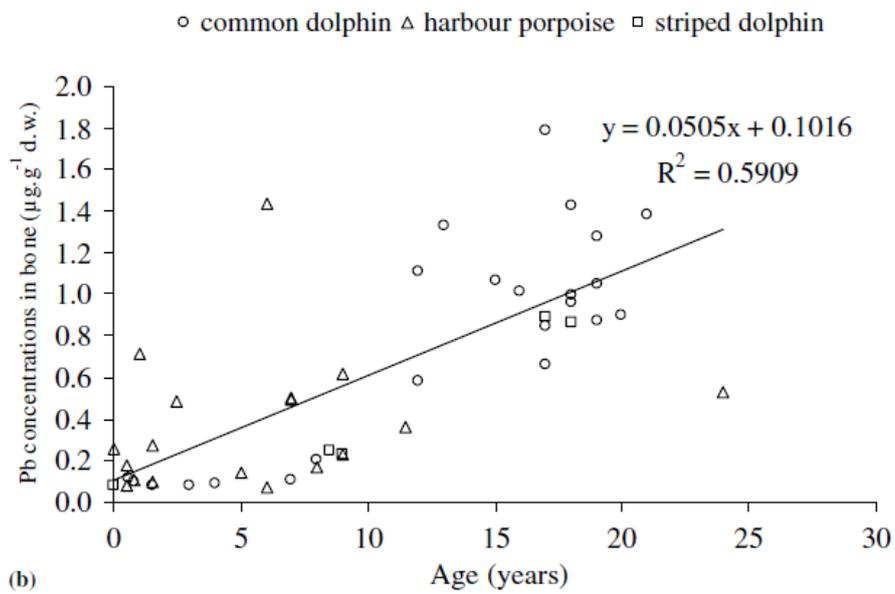
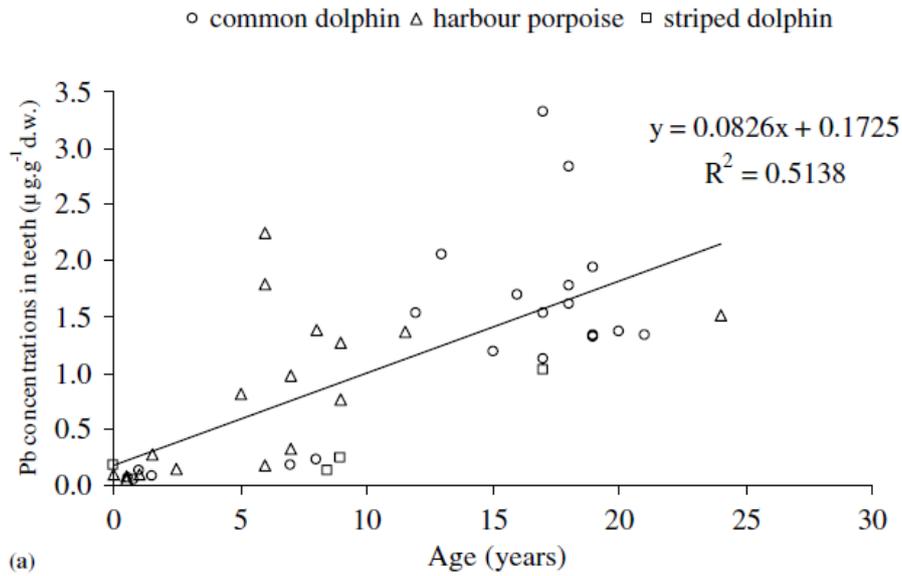


Fig. 3. Relationship between Pb concentrations in teeth ($\mu\text{g g}^{-1}$ dry weight) and age (in years) (a), Pb concentrations in bone ($\mu\text{g g}^{-1}$ dry weight) and age (in years) (b) in common dolphin, *Delphinus delphis*, striped dolphin, *Stenella coeruleoalba* and harbour porpoise, *Phocoena phocoena* from European waters.

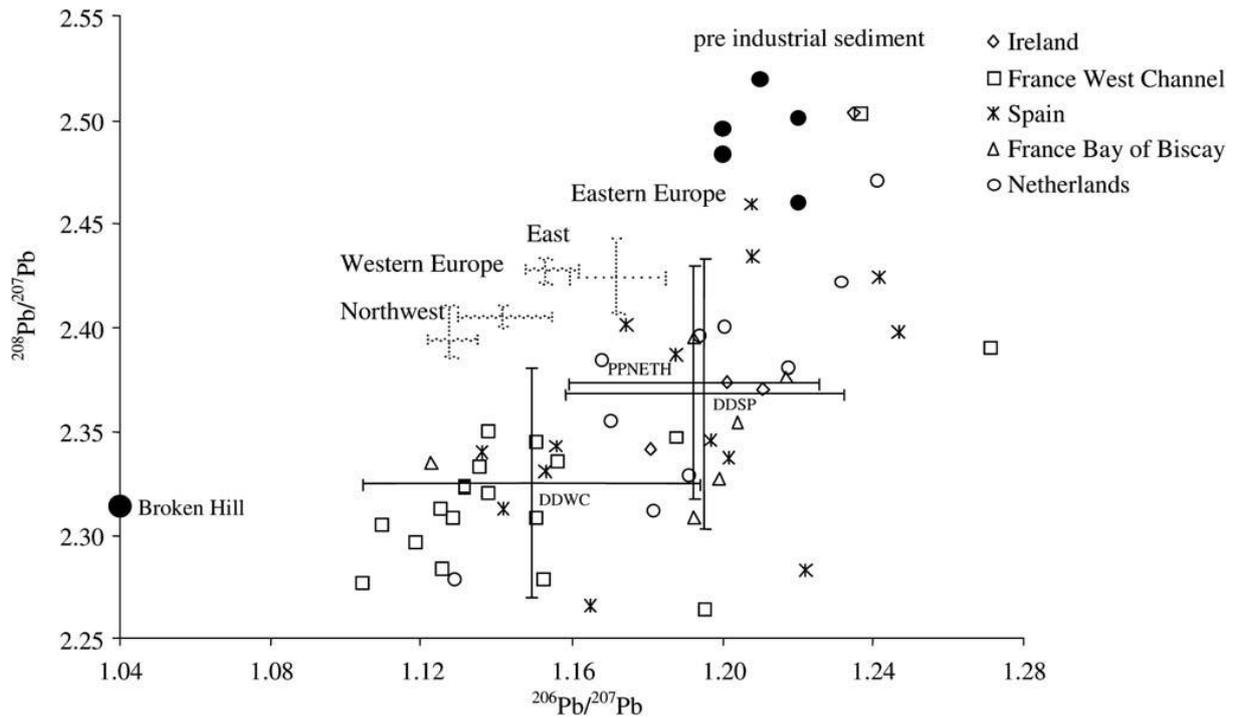


Fig. 4. $^{206}\text{Pb}/^{207}\text{Pb}$ and $^{208}\text{Pb}/^{207}\text{Pb}$ in bone tissue of small cetaceans from the five geographical regions. All species (*Delphinus delphis*, *Stenella coeruleoalba* and *Phocoena phocoena*) combined. The mean value (\pm SD) are represented for the most numerous individuals: *Delphinus delphis* from the West Channel (DDWC), from Spain (DDSP) and *Phocoena phocoena* from the Netherlands (PPNETH). Mean ratios (\pm SD) of the composition of the air originated from the four regions as defined by Hopper et al. (1991) are indicated. Values of pre-industrial sediment (Sun, 1980) and Broken Hill ores – main origin of the Pb added to the gasoline – (Grousset et al., 1994) are also shown.

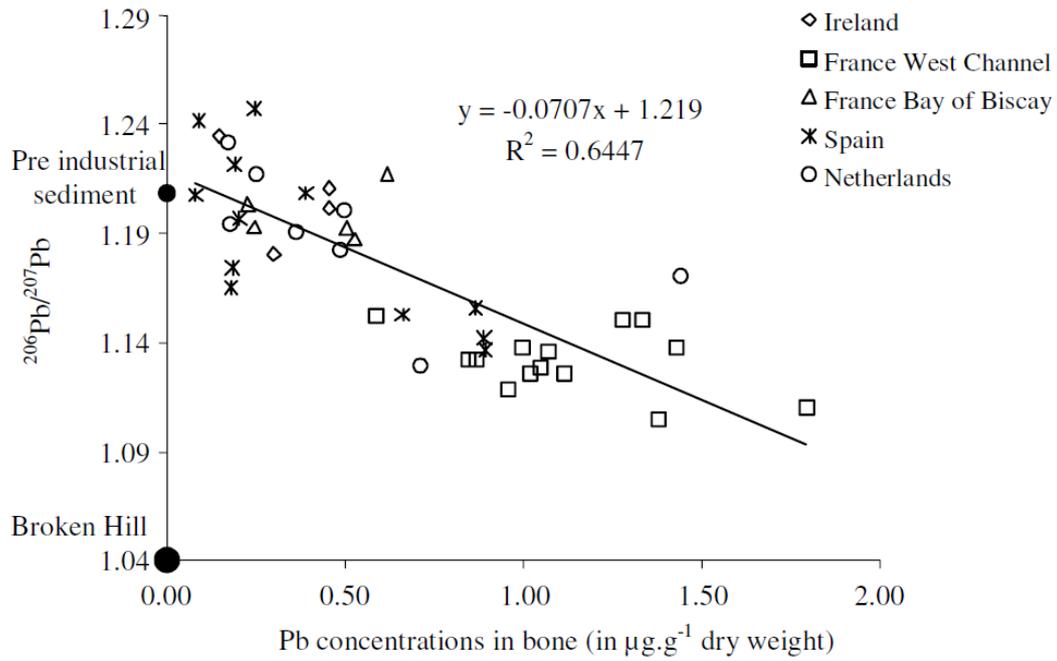


Fig. 5. Relationship between $^{206}\text{Pb}/^{207}\text{Pb}$ ratios and Pb concentrations in bones ($\mu\text{g g}^{-1}$ dry weight) in small cetaceans (common dolphins, striped dolphins and harbour porpoises) from the European Waters. Values of preindustrial sediment (Sun, 1980) and Broken Hill ores – main origin of the lead added to the gasoline – (Grousset et al., 1994) are also shown.

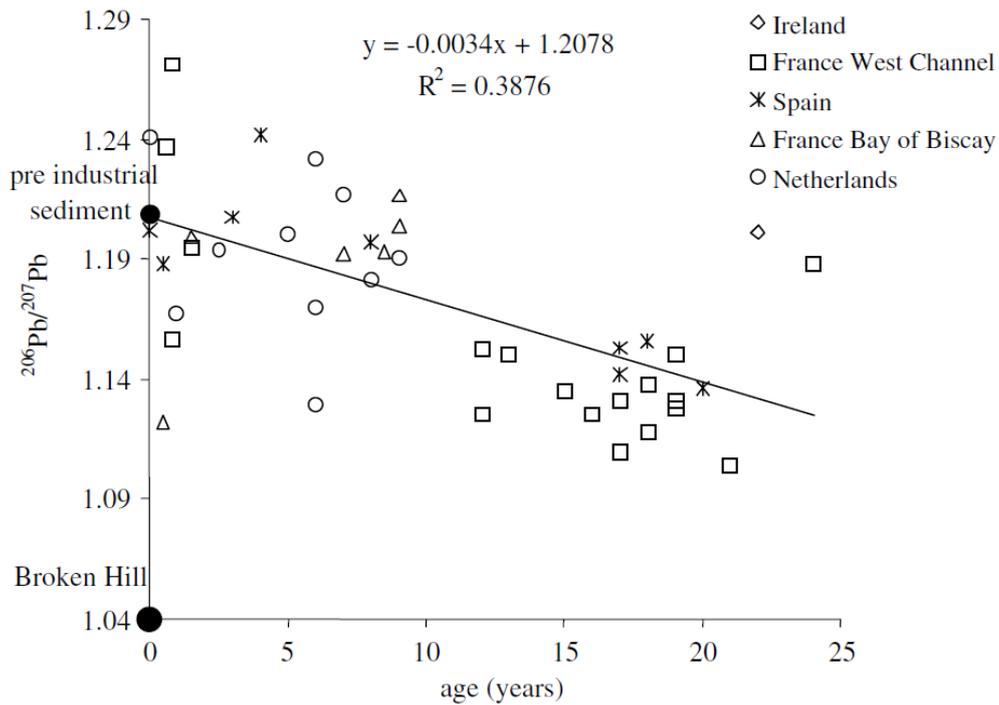


Fig. 6. Relationship between $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in bones and age (in years) in small cetaceans (common dolphins, striped dolphins and harbour porpoises) from the European Waters. Values of pre-industrial sediment (Sun, 1980) and Broken Hill ores – main origin of the lead added to the gasoline – (Grousset et al., 1994) are also shown.

Table 1. Small cetaceans stranded along the European coasts: mean age and length (\pm SD) of individuals sampled for Pb analysis (n = number of individuals).

Origin	Sex	<i>Delphinus delphis</i>			<i>Phocoena phocoena</i>			<i>Stenella coeruleoalba</i>		
		n	Age (years)	Length (cm)	n	Age (years)	Length (cm)	n	Age (years)	Length (cm)
France (n = 35)	♀ (n = 21)	17	12.8 \pm 6.7	193.8 \pm 20.6	3	10.6 \pm 12	164.7 \pm 31.2	1	8.5	165
	♂ (n = 5)	0	–	–	4	3.7 \pm 4.6	135 \pm 12.2	1	9	209
	Fœtus (n = 9)	9	–	50.8 \pm 7.4	0	–	–	0	–	–
Ireland (n = 5)	♀ (n = 2)	1	22	210	1	–	127	1	–	–
	♂ (n = 3)		–	–	2	–	–		–	–
Spain (n = 19)	♀ (n = 11)	8	9 \pm 5.6 (n = 4)	186.1 \pm 17.5	1	0.5	118	2	8.5 \pm 12	163 \pm 72.1
	♂ (n = 8)	7	10.2 \pm 9.6 (n = 4)	175 \pm 31.7	0	–	–	1	18	211
Holland (n = 11)	♀ (n = 10)	0	–	–	10	5.4 \pm 3.5	140.3 \pm 21.6	0	–	–
	♂ (n = 1)	0	–	–	1	8	142	0	–	–

Table 2. Lead concentrations (mean \pm SD) and range in bone and tooth (in $\mu\text{g g}^{-1}$ dry weight) of the small cetaceans stranded along the European coasts.

Tissues	France			Ireland			Spain			Holland			Total		
	Species	<i>N</i>	Mean \pm SD	Range	<i>N</i>	Mean \pm SD	Range	<i>N</i>	Mean \pm SD	Range	<i>N</i>	Mean \pm SD	Range	<i>N</i>	Mean \pm SD
Bone															
<i>Delphinus delphis</i>	17	0.941 \pm 0.486	0.076–1.791	1	0.454	–	12	0.282 \pm 0.250	0.082–0.895	0	–	–	30	0.661 \pm 0.511	
<i>Phocoena phocoena</i>	7	0.315 \pm 0.232	0.078–0.619	3	0.337 \pm 0.107	0.255–0.458	1	0.143	–	0	–	–	21	0.369 \pm 0.309	
<i>Stenella coeruleoalba</i>	2	0.239 \pm 0.013	0.229–0.248	1	0.146	–	3	0.612 \pm 0.458	0.083–0.887	10	0.438 \pm 0.400	0.067–1.437	6	0.410 \pm 0.366	
Total	26	0.719 \pm 0.511	–	5	0.322 \pm 0.134	–	16	0.336 \pm 0.307	–	10	0.438 \pm 0.400	–	57	0.527 \pm 0.449	
Tooth															
<i>Delphinus delphis</i>	16	1.478 \pm 0.899	0.051–3.329	1	0.141	–	10	0.413 \pm 0.450	0.125–1.371	0	–	–	27	1.034 \pm 0.914	
<i>Phocoena phocoena</i>	5	0.823 \pm 0.621	0.088–1.520	2	0.374 \pm 0.292	0.168–0.581	1	0.056	–	0	–	–	19	0.746 \pm 0.677	
<i>Stenella coeruleoalba</i>	2	0.189 \pm 0.084	0.130–0.248	1	0.095	–	2	0.601 \pm 0.605	0.173–1.028	11	0.840 \pm 0.757	0.103–2.251	5	0.335 \pm 0.392	
Total	23	1.223 \pm 0.895	–	4	0.246 \pm 0.225	–	13	0.415 \pm 0.446	–	11	0.840 \pm 0.757	–	51	0.858 \pm 0.813	