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New insights from age determination on toxic element accumulation in striped and bottlenose dolphins from Atlantic and Mediterranean waters

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Abstract:

This study aimed at comparing toxic element (Hg, Cd) bioaccumulation in relation to age for bottlenose (*Tursiops truncatus*) and striped dolphins (*Stenella coeruleoalba*) from Mediterranean and Atlantic waters. Metal concentrations were also measured in selected prey to infer metal exposure through the diet. As expected, Mediterranean prey exhibited the highest Hg levels, probably as a consequence of the Hg enrichment of the Mediterranean Sea. Comparing the predators from each area and taking age into account, Mediterranean bottlenose dolphins displayed higher Hg levels than Atlantic dolphins ($p = 0.032$), whereas Mediterranean striped dolphins did not ($p = 0.691$). The consumption of Myctophid fish, which showed the highest Hg levels (105 ± 80 ng g^{-1} w.wt.) among Atlantic prey, may explain the high Hg levels in the liver of the Atlantic striped dolphins and suggested a preferential offshore feeding behaviour in this area. Concerning Cd, no clear differences were found between geographical areas.

Keywords: *Stenella coeruleoalba*; *Tursiops truncatus*; Mercury; Cadmium; Diet; Exposure

1. Introduction

Due to their terminal position in the food web and their long life span, carnivorous marine mammals accumulate particularly high levels of mercury (Hg) and cadmium (Cd) (Wagemann and Muir, 1984; Aguilar et al., 1999). The natural occurrence of these trace elements in seawater (Nriagu, 1996) has involved adaptations of marine organisms over geological time scales to the presence of toxic metals in their environment. Thus, marine mammals have developed efficient detoxification capacities to support elevated exposure to these metals (see reviews by Cuvin-Aralar and Furness, 1991 and Das et al., 2000a). The demethylation of organic Hg in the liver leads to the production of non-toxic granules of tiemannite (Koeman et al., 1973; Martoja and Berry, 1980). The accumulation of tiemannite granules makes the liver the ultimate organ of retention of Hg (Wagemann et al., 1998; 2000). Since these granules are not excreted (Nigro and Leonzio, 1996), inorganic Hg would be stored in this organ for nearly the whole life. This detoxification process results in particularly elevated Hg concentrations in the liver, which is not the case when considering levels in the kidneys, where no storage of Hg under tiemannite would occur (Cuvin-Aralar and Furness, 1991). Like Hg in the liver, Cd levels can also be particularly elevated in the kidneys of marine mammals (Wagemann and Muir, 1984; Dietz et al., 1998). Its potential toxic effects are mitigated by the binding to metallothioneins both in the liver and the kidneys (Klaassen and Liu, 1997; Teigen et al., 1999). In other mammals such as humans, the biological half-life of Cd has been estimated to be a few months in liver and between 10 to 30 years in kidneys (Friberg et al., 1974), and Cd could also be stored over very long time in the renal tissue of marine mammals.

In addition, Cd and Hg concentrations display an important variability in marine mammal tissues, which may be the result of biological and ecological influences (Aguilar et al., 1999). At the individual scale, the most important parameter to be considered is the age, since these metals potentially accumulate throughout the life. Other factors such as growth features, gender, reproductive status and/or health aspects may also be important. At the population scale, feeding preference is probably the key factor controlling Cd and Hg levels because upper-level predators are mainly exposed to metals through their food (Aguilar et al., 1999). Feeding on cephalopods is probably a major source of Cd for small cetaceans both because of the high Cd levels in these prey and because Cd is present mainly in bioavailable forms in their tissues (Bustamante et al., 1998; 2002). In both fish and cephalopods, Hg occurs mainly in organic forms which are highly bioavailable for the predators (Bloom, 1992; Bustamante et al., submitted). As a result, species or populations that consume an important proportion of cephalopods can be expected to exhibit higher Cd levels in their tissues than piscivorous upper-level predators. The effects of feeding preferences on metal exposure may also be combined with other characteristics inherent to the species, the habitat and the geographical location, as concentrations in prey would reflect concentrations in their environment (e.g. Law et al., 1991; 1992).

The present study aims at determining the main biological and ecological factors influencing Cd and Hg concentrations in two small cetacean species inhabiting the French waters, the striped dolphins (*Stenella coeruleoalba*) and the bottlenose dolphins (*Tursiops truncatus*) (e.g. Forcada et al., 1990; Goujon et al., 1993; Gannier, 1995; Liret et al., 1998). The two geographical areas involved are the Mediterranean Sea and the Bay of Biscay. These areas are characterised by different oceanological features which are likely to affect metal exposure to small cetaceans. Indeed, the Mediterranean Sea is well-known for its natural Hg enrichment (e.g. Bacci, 1989; Cossa et al., 1997). Furthermore, a previous study has reported that striped dolphins from the Mediterranean Sea exhibited much higher Hg levels than those from the Atlantic (André et al., 1991). However, this report did not take age into account, only body length, which may introduce a bias. Indeed, the use of length as a covariate to detect differences in pollutant levels between areas does not permit comparisons between individuals having reached their asymptotic length or/and between groups with different growth curves (Monaci et al., 1998). Striped dolphins from the Mediterranean Sea are smaller than those from the Atlantic (Di-Méglio et al., 1996), which may affect Hg levels in these two populations.

As a consequence, understanding accumulation of toxic elements with age is mandatory to establish the effective metal impregnation state of small cetaceans from the Mediterranean and Atlantic waters and to allow further comparison between both areas. Renal Cd and hepatic Hg are measured in order to provide a long-term overview of the impregnation. In addition, the influence of diet on metal exposure to striped and bottlenose dolphins is investigated using metal concentrations in reported prey from each area. Thus, the influence of diet on metal levels in these small cetaceans could be clarified both at the intra- and inter-specific scale.

2. Material and methods

2.1. Studied areas

The Bay of Biscay is situated in the north-eastern Atlantic and extends from 1 to 10°W and 43 to 48°N (Fig. 1). This area is characterised by a variable distance of the shelf-edge from the coastline, the continental shelf width varying from 60 to 100 nautical miles (NM) in the northern part of the Bay (up to 45°N) to 25-30 NM in the southern part, and reaching 3 NM at the latitude of the Capbreton trough. Within this area, Arcachon Bay (see Fig. 1) constituted a semi-enclosed shallow habitat for bottlenose dolphins until 2001, when the last individual of this small resident population was found stranded (Ferrey et al., 1993; J.J. Boubert, pers. comm.).

In contrast, the continental shelf width is very limited along the French Mediterranean coasts, with the largest width encountered in the Gulf of the Lion (15 to 45 NM, see Fig. 1), as compared to 2.5-10 NM at the eastern of 5°30 E. These latter areas are characterised by an abrupt shelf-edge, with deep canyons (up to - 2000m) close to the coastline.

2.2. Biological material

2.2.1. Small cetaceans

Between 1999 and 2004, 55 stranded small cetaceans were sampled along the whole French Mediterranean (n = 15) and Atlantic coasts (n = 40) by the *Réseau National Echouage* (RNE) co-ordinated by the *Centre de Recherche sur les Mammifères Marins* (CRMM) of La Rochelle (France). Striped dolphins were the most common species (n = 40) whereas only 15 bottlenose dolphins were collected. Among the 10 Atlantic bottlenose dolphins, 3 animals were identified as the last bottlenose dolphins from the small resident group of Arcachon Bay (J.J. Boubert, pers. comm.), the history of which is well-known (Ferrey et al., 1993). During the necropsies, teeth (n = 55) were collected for age determination, and liver (n = 55) and kidney (n = 48) for trace element analyses (Table 1). These samples were stored at –20°C until being processed in the laboratory.

Age was determined at the CRMM, following the recommendations of Perrin and Myrick (1980). Briefly, this procedure consists of counting Growth Layer Groups (GLGs) from teeth sections, assuming that one GLG equals one year. All carcasses were in good post-mortem condition, except a single striped dolphin from the Mediterranean Sea. Owing to the small number of dolphins collected along the Mediterranean coast (see Table 1), the single putrefied animal has been included in the study. This individual can be identified in figures since it is the oldest striped dolphins (i.e. 27 years-old).

2.2.2. Prey

Selected prey from each area were also sampled for toxic element analyses. The selection of species and their size was based upon previous dietary studies on bottlenose and striped dolphins originating from the study areas (Würz et al., 1992; Blanco et al., 2001; Spitz, 2004; Astruc et al., in press; Pusineri et al., in press; Spitz et al., submitted). Prey sampling occurred during IFREMER groundfish surveys, in winter and spring 1997 and in autumn 2001-2003 (RESSGASC and EVHOE campaigns, respectively). For the Mediterranean Sea, sampling was conducted in May and October 2004 (MERMED campaigns). Whereas RESSGASC and MERMED surveys occurred only on the continental shelf, EVHOE surveys extended to the shelf-edge, allowing the collection of mesopelagic species. All prey samples were immediately frozen at -20°C on board and then stored until being processed in the laboratory.

After species determination, each prey was weighed and measured. Metal analyses were carried out using the whole prey in order to reflect the exposure to predators. As cephalopods represent a major pathway of Cd exposure (Bustamante et al., 1998; 2002) and since Cd levels are particularly low in fish (Lahaye et al., in press), Cd levels were assayed in cephalopods but not in fish. In contrast, the presence of Hg in its bioavailable form in both fish and cephalopod (Bloom, 1992; Bustamante et al., submitted) necessitates measurements of Hg concentrations in both prey items.

2.3. Metal analyses

All equipment used in the sample processing was cleaned, and subsequently decontaminated for 24 h in a solution composed of 35 ml HNO_3 (65%) and 50 ml HCl (36%) for 1 L of Milli-Ro quality water. Fresh samples were freeze-dried and ground to powder. The mean ratio between dry weight (d.wt.) and wet weight (w.wt.) was 0.28 for liver and 0.23 for kidney. Each sample was then treated in duplicate.

For total Hg measurements, aliquots ranging from 0.2 to 2 mg of dried-material were analysed in an Advanced Mercury Analyser spectrophotometer (Altec AMA 254). Hg determination in the AMA 254 involved evaporation of Hg by progressive heating to 800°C under oxygen atmosphere for 3 min, and subsequent amalgamation on a gold net. The net was then heated to liberate the collected Hg, which was measured by UV atomic-absorption spectrophotometry.

For Cd analyses, 2 aliquots of approximately 200 mg of each homogenised dry sample were digested with 3.5 ml of 65% HNO_3 at 60°C for 3 days. The digested contents were then diluted to 10 ml in milli-Q quality water. Then Cd contents were assayed using a flame (Varian 250 Plus) and graphite furnace (Hitachi Z-5000) atomic absorption spectrophotometer with deuterium and Zeeman background correction, respectively.

Quality controls were made using standard reference materials (National Research Council of Canada). These standards were treated and analysed under the same conditions as the samples and results were in good agreement with the certified values (Table 2). In addition, intercalibration exercices were carried out on material from the International Atomic Energy Agency. Detection limits were determined with blank analyses and the obtained values were 0.005 $\mu g.g^{-1}$ d.wt. for Hg, 0.4 and 0.002 $\mu g.g^{-1}$ d.wt. for Cd, respectively using flame and furnace AAS.

2.4. Data treatment

Metal concentrations were expressed as $\mu g.g^{-1}$ wet weight (w.wt.). All concentrations below the detection limit were replaced with "dummy values" that were half of the detection limit in order to allow further statistical comparisons (Gibbons and Coleman, 2001). Figures showing metal concentrations against age were presented on a logarithmic scale in order to better describe the relationship of metals with age in the youngest dolphins since they constituted a large part of the sampling (see Table 1). Statistical analyses were performed using XL-STAT Pro 7.0. Metal concentrations were natural log-transformed in order to reduce skewness when striped dolphins were taken into account. When the relationship of metal concentrations with age was linear, analysis of covariance (ANCOVA, Quinn & Keough 2002) was used to determine the influence of gender and area, with age as the covariate. When no influence of age on metal concentrations was established, analyse of variance (ANOVA) was used to compare groups (i.e. per gender or area).

3. Results

3.1. Metal levels in bottlenose dolphins

Concentrations of both metals displayed high variability: overall coefficients of variations (CV) were 96% for Hg in liver and 103% for Cd in kidneys. Hepatic Hg concentrations ranged from 1.5 to 331 μg^{-1} w.wt. (Fig. 2a) and renal Cd concentrations ranged from 0.06 to 3.05 $\mu g.g^{-1}$ w.wt. (Fig. 2b).

Correlation with age was significant for both hepatic Hg ($p = 0.001$) and renal Cd ($p < 0.0001$, Table 3). As the three bottlenose dolphins from Arcachon Bay constituted a particular social unit, these individuals were not taken into account for the global geographical comparison (Mediterranean Sea *vs* Atlantic Ocean). Figure 2 shows that Mediterranean bottlenose dolphins exhibited much higher Hg levels in liver than Atlantic ones ($p = 0.032$, Table 3) whereas Cd levels were rather similar in these two groups ($p = 0.207$, Table 3).

The three 3 individuals from Arcachon Bay exhibited very low Cd levels compared to the other Atlantic animals (Fig. 2b). Among the Arcachon Bay animals, two individuals exhibited hepatic Hg concentrations as high as those of Mediterranean bottlenose dolphins (Fig. 2a).

3.2. Metal levels in striped dolphins

The global CV of hepatic Hg was particularly elevated for striped dolphins (i.e. 221%). The highest concentration was found in a 27-year-old Mediterranean striped dolphin (1033 $\mu g.g^{-1}$ w.wt; see Fig. 3a), which was also the single putrefied specimen of the overall sampling. Concentration variability was much lower for renal Cd, with a CV of 94%. Surprisingly, the maximal value of renal Cd was obtained in a relatively young Atlantic striped dolphin (age estimated to be 1.3-year-old) reaching 40.2 µg Cd.g^{-1} w.wt. (see Fig. 3b).

Metal accumulation with age in striped dolphins was only apparent for hepatic Hg (Fig. 3a; $p < 0.0001$, Table 3). In addition, no influence of the geographical area on the bioaccumulation rates was observed for this metal ($p = 0.691$, Table 3). In contrast, renal Cd concentrations would increase until 2 years-old, and then would remain independent of the age (Fig. 3b). However, when looking at calves of 2 years-old and less, Cd concentrations in the kidneys were more influenced by the geographical area ($p = 0.018$) than the age ($p = 0.635$) and the interaction between these two parameters was not significant ($p = 0.741$, Table 3). In addition, although the Mediterranean sampling was limited compared to the Atlantic sampling, the two adult Mediterranean striped dolphins exhibited quite similar renal Cd concentrations to the Atlantic ones (Fig. 3b).

The high number of striped dolphins sampled along the Atlantic coast ($n = 30$; see Table 1) allowed us to consider the influence of gender on metal accumulation. No influence of gender on metal concentrations was established for either hepatic Hg ($p = 0.637$) or renal Cd ($p = 0.515$, Table 4).

3.3. Inter-specific comparisons

Owing to the limited Mediterranean sampling, inter-specific comparisons were carried out only for the Atlantic. Since bottlenose dolphins from Arcachon Bay had lived isolated for a long time and therefore displayed different metal concentrations compared to the other individuals from the Atlantic coast, they were not included in the inter-specific comparison.

Bioaccumulation rates of Hg in liver with age were similar in Atlantic bottlenose and striped dolphins ($p = 0.774$) whereas Cd bioaccumulation in the kidneys with age was significantly higher in striped dolphins than in bottlenose dolphins ($p = 0.026$, Table 5).

3.4. Metal levels in prey

Mediterranean prey displayed higher Hg levels than Atlantic ones, the mean length of the prey analysed being equivalent for both areas. In addition, cephalopod and fish exhibited similar Hg concentrations, the highest level being reached in a cephalopod specimen, *Illex coindetii*, from the Mediterranean Sea (490 $ng.g^{-1}$ w.wt). Among fish, the Myctophid *Notoscopelus kroeyeri* from the Atlantic displayed the highest Hg level (105 ± 80 $ng.g^{-1}$ w.wt.). However, no Myctophids from the Mediterranean area could be sampled.

Cd concentrations in cephalopods displayed high intra- and inter-specific variability, and especially for the Mediterranean Sea where samples had the lowest variability in their mean length (Table 6). For example, the squid *Loligo vulgaris* was characterised by the lowest Cd levels in both Atlantic and Mediterranean Sea (132 ± 54 and 99 ± 111 $ng.g^{-1}$ w.wt., respectively) but also by the highest CV (41 and 112%, respectively). In the Atlantic, Cd levels in cephalopods could be described following this pattern: *Teuthowenia megalops* (Cranchids) > *Histioteuthis reversa* (Histioteuthids) > *I. coindetii* (Ommastrephids) > *L. vulgaris* (Loliginids).

4. Discussion

Metal concentrations displayed high variability in storage tissues of striped and bottlenose dolphins from the Mediterranean and Atlantic areas. The extreme CV values obtained in our study (i.e. 94 to 221%) have previously been reported in other marine mammal species for these toxic elements (e.g. Caurant et al., 1994; Bustamante et al., 2004). This underlines the fact that metal levels in upper-level predators are likely to be influenced by numerous factors, such as age, gender, body condition, geographical location and/or dietary preferences (Aguilar et al., 1999).

Concerning the influence of gender, no effect was detected in striped dolphins stranded along the Atlantic coast for either hepatic Hg or renal Cd concentrations (Table 4). This result is consistent with those obtained for striped dolphins from the Pacific Ocean (Honda et al., 1983). Some differences of Cd concentrations between genders have been reported for other marine mammals, e.g. long-finned pilot whales *Globicephala melas* and grey seals *Halichoerus grypus*, and were attributed to their sexual dimorphism (Caurant et al., 1994; Bustamante et al., 2004). Although male striped dolphins from the Atlantic Ocean are slightly bigger than females (Di-Méglio et al., 1996), this sexual dimorphism might not be sufficient to induce differences in Cd levels between genders. This also suggests that the transfer of Cd to offspring during pregnancy and lactation is not an important route of excretion for female striped dolphins.

4.1. Mercury

Age was the major factor affecting hepatic Hg concentrations in bottlenose and striped dolphins from the Atlantic and Mediterranean waters (Table 3). Such an increase of Hg concentrations with age has previously been demonstrated in several cetacean species (e.g. Honda et al., 1983; Paludan-Müller et al., 1993; Caurant et al., 1994; Monaci et al., 1998). Comparisons between the two studied species do not reveal any difference in Hg accumulation in the Atlantic (Table 5). Such similar Hg accumulation trends between bottlenose (i.e. mainly fish-eaters in this area: Spitz, 2004) and striped dolphins (i.e. mixed item-eaters: Pusineri et al., in press; Spitz et al., submitted) are consistent with the high bioavailability of Hg in both fish and cephalopods (Bloom, 1992; Bustamante et al., submitted). However, geographical comparisons highlighted two different patterns: 1) bottlenose dolphins from the Mediterranean Sea displayed higher Hg concentrations than Atlantic ones whereas 2) Mediterranean and Atlantic striped dolphins exhibited similar Hg concentrations at a given age (Fig. 2a, Fig. 3a, Table 3).

Firstly, the Hg accumulation in Mediterranean bottlenose dolphins compared to Atlantic animals resulted in mean Hg levels about 5 times higher in Mediterranean bottlenose dolphins than Atlantic animals (i.e. 204 ± 121 *vs* 38 ± 41 $\mu g.g^{-1}$ w.wt., respectively). Mediterranean prey displayed higher Hg levels than Atlantic prey (Table 6). Thus, higher Hg levels in Mediterranean prey and Mediterranean bottlenose dolphins were in agreement with the Hg enrichment in Mediterranean food webs, due to higher methylation rates of Hg in the anoxic layer waters of this area (Bacci, 1989; Cossa et al., 1997).

Surprisingly, and conversely to bottlenose dolphins, no difference in Hg bioaccumulation was established between Mediterranean and Atlantic striped dolphins. The highest Hg concentration (1033 $\mu g.g^{-1}$ w.wt.) was encountered in a putrefied 27 year-old Mediterranean dolphin. Such an extreme elevated level has been previously reported for several striped dolphins from the Mediterranean Sea (see Table 7), but without taking into account their age or body condition. In their previous report on Hg levels in striped dolphins from the Atlantic and the Mediterranean Sea, André et al. (1991) used mostly mature animals, which had reached their asymptotic length. As the life-span of striped dolphins might reach 40 years (Miyasaki, 1981; Honda et al., 1983), these individuals could also have been from about 10 to 40 years old, which makes it difficult to establish geographical comparisons for these individuals. Therefore, the previous report of higher Hg levels in Mediterranean striped dolphins compared to Atlantic ones (André et al., 1991) can not be assessed. This underlines the fact that relationships of metal concentrations with body length is not appropriate for geographical comparisons, especially when growth curves are clearly different (Di-Méglio et al., 1996). However, if the environmental chemistry of water mass was considered alone, the Mediterranean striped dolphins would display higher Hg concentrations than the Atlantic ones. Hence, feeding and habitat preferences need to be taken into account.

In the Bay of Biscay, striped dolphins are mainly found in oceanic waters, where they primarily feed upon on mesopelagic fish and cephalopods (Pusineri et al., in press). Some intrusions over the continental shelf could also occur (Forcada et al., 1990; Kiszka et al., in press), as underlined by the simultaneous occurrence of neritic and oceanic species in stomachs of stranded animals (Spitz et al., submitted). In contrast, in north-western Mediterranean waters, striped dolphins would occur equally over the continental shelf, the shelf-edge and in deeper waters (Gannier, 1995). The diet of stranded Mediterranean striped dolphins is mainly based on cephalopods and demersal fish from the upper shelf, at comparable proportions in terms of reconstructed biomass (Astruc et al., in press). Regarding Hg levels in the Atlantic prey, Myctophid fish were the prey with the highest concentrations (Table 6). Indeed, these mesopelagic fish are well-known to exhibit particularly high Hg levels (Monteiro et al., 1996; Thompson et al., 1998). No Mediterranean Myctophids could be sampled in this study but these mesopelagic fish could be expected to also present elevated mercury levels, probably higher than those from the Atlantic as a result of the Hg enrichment in this area (Bacci 1989). Our results concerning Hg concentrations in the liver of the striped dolphins strongly suggest that they do not feed on mesopelagic prey on a long time-scale in the Mediterranean waters in contrast to those from the Atlantic. Thus, different feeding behaviour could explain similar Hg tissue concentrations because mesopelagic prey species in the Atlantic display similar Hg levels to demersal prey in the Mediterranean, leading to similar Hg exposure in both areas.

4.2. *Cadmium*

Contrary to Hg, different patterns of Cd bioaccumulation occurred between the two studied species. Indeed, despite the limited number of bottlenose dolphins sampled (see Table 1), comparisons between the two species in the Atlantic revealed far lower Cd levels at a given age in bottlenose dolphins than in striped dolphins (Table 5). Some piscivorous marine mammals such as harbour porpoise (*Phocoena phocoena)*, belugas (*Delphinapterus leucas*), grey seals (*H. grypus*) and bottlenose dolphins from other areas also accumulate low levels of Cd (e.g. Falconer et al., 1983; Wagemann et al., 1996; Szefer et al., 2002; Bustamante et al., 2004; Decataldo et al., 2004). The low Cd levels encountered in bottlenose dolphins would be induced by a diet dominated by fish (Spitz, 2004), which contain low Cd levels (Amiart-

Triquet et al., 1983; Bustamante, 1998; Lahaye et al., in press). In contrast, regarding elevated Cd levels in striped dolphins, high Cd concentrations have also been reported in the tissues of teuthophageous species (e.g. Caurant et al., 1994; Holsbeek et al., 1999; Bustamante et al., 2003). As feeding on cephalopods constitutes a major source of Cd for small cetaceans (Bustamante et al., 1998; 2002), the elevated Cd concentrations in striped dolphins well-reflected their important squid consumption in the Atlantic area (Pusineri et al., in press; Spitz et al., submitted).

No difference in Cd accumulation was found in bottlenose dolphins from the Atlantic and Mediterranean areas (Table 3). However, the 3 individuals from Arcachon Bay displayed particularly low Cd concentrations compared to the individuals stranded along the Atlantic coast (Fig. 1b). Consequently, the low Cd levels encountered in the resident bottlenose dolphins from Arcachon Bay may be the result of a specialised diet resulting from particular prey availability in this restricted area. Most of the fish encountered are coastal species rather than demersal ones and the main source of cephalopods in this bay is common cuttlefish (*Sepia officinalis*). Indeed, Arcachon Bay is a mating area for this species, which die after the spawning, adults therefore being present in this area only during a very limited period of the year (Poulard and Léauté, 2002). Consequently, the Cd levels obtained in the bottlenose dolphins from Arcachon Bay were clearly due to the even more limited occurrence of cephalopods in their feeding regime compared to the other individuals from the Atlantic area, for which stomachs generally contain about 10% of cephalopods in term of reconstructed biomass (Spitz, 2004).

In addition, Cd accumulated significantly with age in bottlenose dolphins which was not the case for striped dolphins (Table 3). Like bottlenose dolphins, harbour porpoises and belugas would exhibit increasing concentration of renal Cd during their life (Falconer et al., 1983; Wagemann et al., 1996; Szefer et al. 2002). Concerning striped dolphins, the apparent increase of Cd in the kidneys until 2 years old, and then followed by a plateau (Fig. 3b), can not be statistically confirmed with our sampling. Indeed, when looking at calves of 2 years-old and less, renal Cd concentrations were more influenced by the geographical area than the age (Table 3). Because it was opportunistic, our sampling is however limited to a few individuals for the Mediterranean area (see Table 1). Our results fall within the range of Cd concentrations reported in previous studies in the Mediterranean Sea but such a comparison with previous studies also showed that immature Mediterranean striped dolphins from our study were smaller and also younger than those from other studies (see Table 7). The particularly low Cd levels encountered in Mediterranean calves compared to Atlantic calves may also be the consequence of the sampling characteristics of this study. Indeed there is no significant difference when interaction between age and area is considered (Table 3). In addition, the proportion of cephalopods in the diet did not seem to differ between Atlantic and Mediterranean striped dolphins, as revealed by stomach content analyses (Astruc et al., in press; Pusineri et al., in press; Spitz et al., submitted). Given that Mediterranean cephalopods displayed similar or even higher Cd levels compared to Atlantic cephalopods (Table 6), the similar Cd concentrations measured in the kidneys of two Mediterranean striped dolphins compared to Atlantic ones may reflect a similar Cd impregnation state for these two populations.

A larger sample size is also required to better understand Cd accumulation with age in Atlantic and Mediterranean striped dolphins. Indeed, a logarithmic pattern of Cd accumulation was previously described for Pacific striped dolphins (Honda et al., 1983), as well as in long-finned pilot whales *G. melas* (Caurant et al., 1994), and arctic narwhals

Monodon monoceros (Dietz et al., 2004), which consume a large proportion of squids. A rapid increase of Cd concentrations in calves could be explained by transfer of the metal through milk during the suckling period (Honda et al., 1983) as well as a higher absorption efficiency generally exhibited by young mammals (Underwood, 1977). Hence, Cd concentrations increased rapidly in calves to levels closed to the threshold likely to induce toxic effects (Dietz et al., 1998) (i.e. up to 40 $\mu g.g^{-1}$ w.wt., see Fig. 3b). The fact that these concentrations would be maintained constant for the rest of the striped dolphin's life (Fig. 3b) suggests an efficient adaptation of accumulation processes in response to high exposure to Cd through the consumption of cephalopods.

5. Conclusions

The present study underlines the importance of considering age when making geographical comparisons of toxic element accumulation in upper-level predators. Hence, taking into account age as a covariate has permitted us to control for biometric differences in the two populations of striped dolphins considered in this study. Thus, the similar Hg levels obtained in Atlantic and Mediterranean striped dolphins highlighted the influence of feeding behaviour on Hg concentrations and suggested that striped dolphins feed in mesopelagic waters of the Atlantic whereas striped dolphins from the Mediterranean Sea feed on other species over shallower waters. Further investigations on feeding behaviour of striped dolphin and quantitative estimation of exposure to metals through the diet are needed, and especially for other areas of the Mediterranean Sea. Moreover, Cd concentrations in the kidneys would well-reflect the occurrence of cephalopods in the diet on a long time-scale, as underlined by the particular Cd impregnation state of animals that have inhabited Arcachon Bay throughout their life. Concerning striped dolphins, the similar Cd impregnation state in Mediterranean and Atlantic populations obtained when taking age into account will have to be re-investigated in the future with a larger sample size. Overall, our results suggest that metal measurements could constitute efficient additional tracers to better understand long-term feeding preferences of these pelagic upper-level predators.

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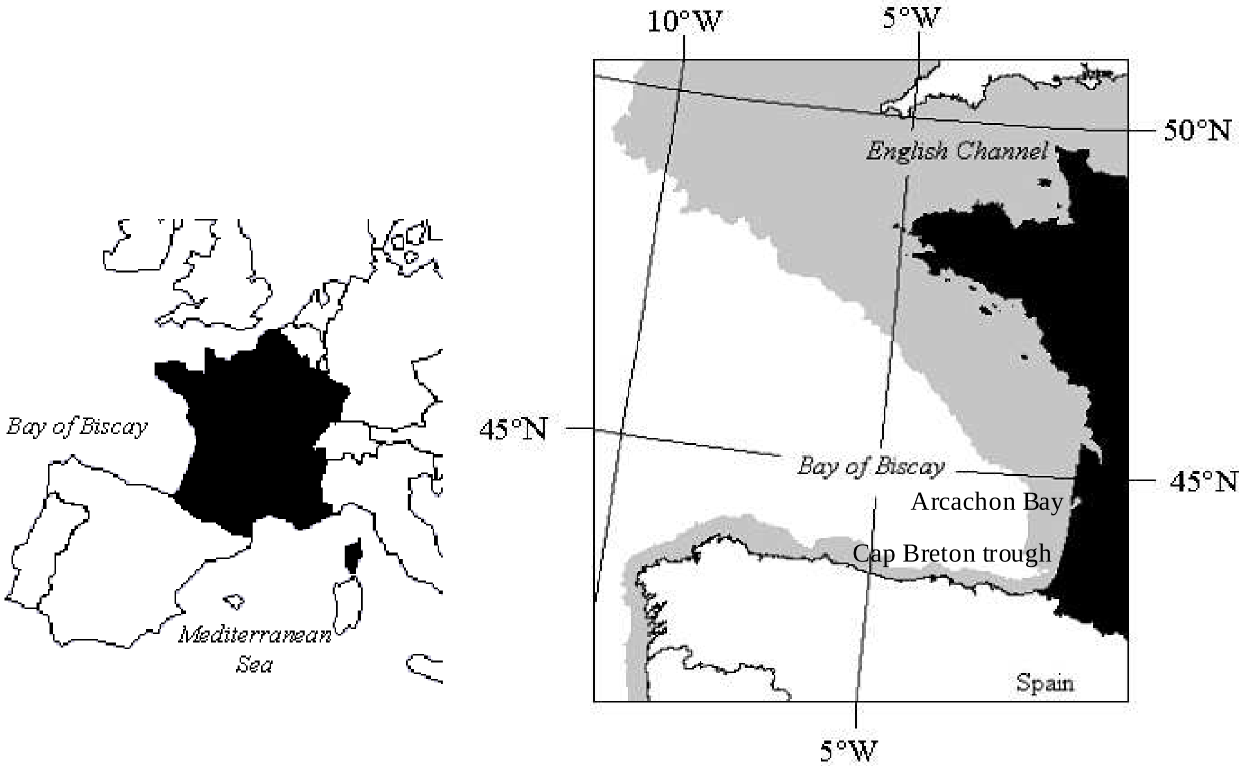
Figure captions

Figure 1. Studied areas in the north-eastern Atlantic waters and the north-western Mediteranean Sea. The continental shelf is represented in grey.

Figure 2. Relationships between age and (a) hepatic Hg and (b) renal Cd concentrations ($\mu g.g^{-1}$ w.wt.) in bottlenose dolphins stranded along the French Atlantic (white squares) and Mediterranean (black squares) coasts. Crosses represent the 3 individuals from Arcachon Bay.

Figure 3. Relationships between age and (a) hepatic Hg and (b) renal Cd concentrations ($\mu g.g^{-1}$ w.wt.) in striped dolphins stranded along the French Atlantic (white squares) and Mediterranean (black squares) coasts.

Figure 1


Bay of Biscay
Mediterranean
Sea
10°W
5°W
50°N
English Channel
45°N
Bay of Biscay
45°N
Arcachon Bay
Cap Breton trough
Spain
5°W

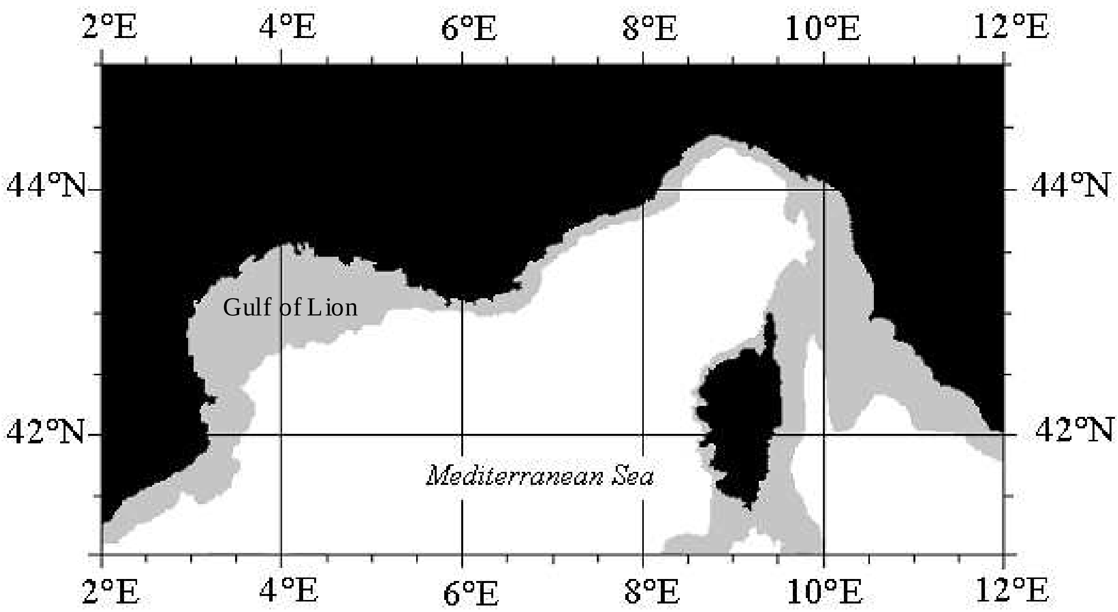
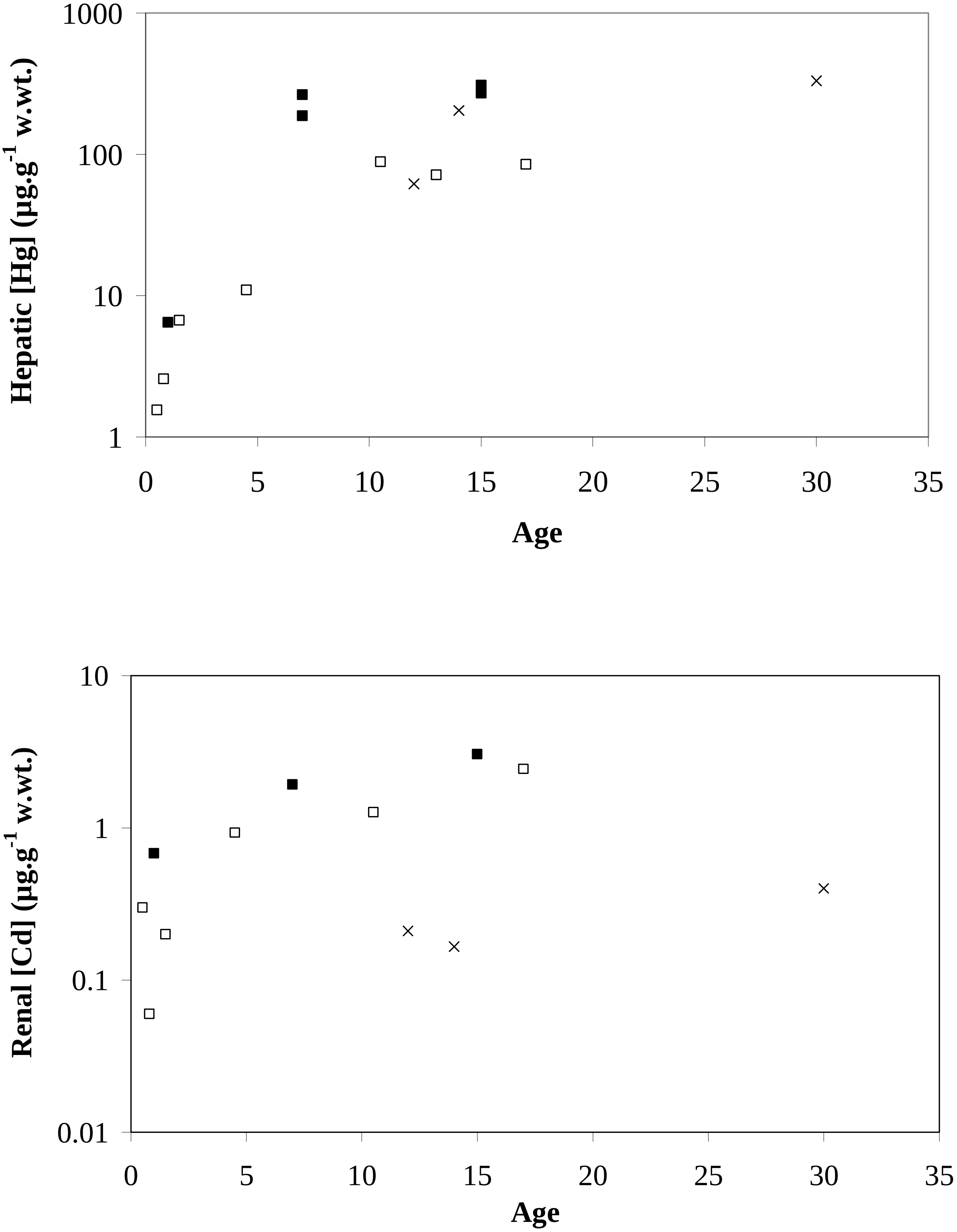
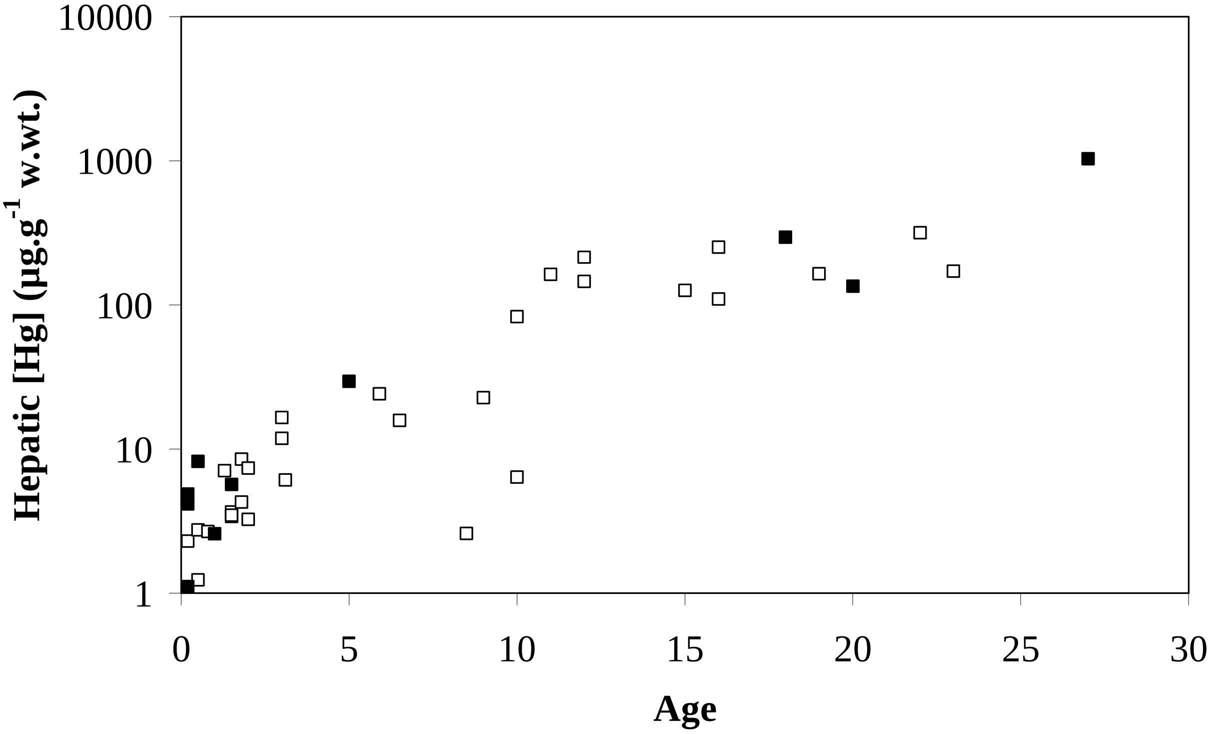

2°E
4°E
6°E
8°E
10°E
12°E
44°N
44°N
Gulf of Lion
42°N
42°N
Mediterranean Sea
2°E
4°E
6°E
8°E
10°E
12°E

Figure 2



1000
100
10
1
Hepatic [Hg] (µg.g-1 w.wt.)
0
5
10
15
20
25
30
35
Age
10
1
0.1
0.01
Renal [Cd] (µg.g-1 w.wt.)
0
5
10
15
20
25
30
35
Age

Figure 3


Hepatic [Hg] (µg.g-1 w.wt.)
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1000
100
10
1
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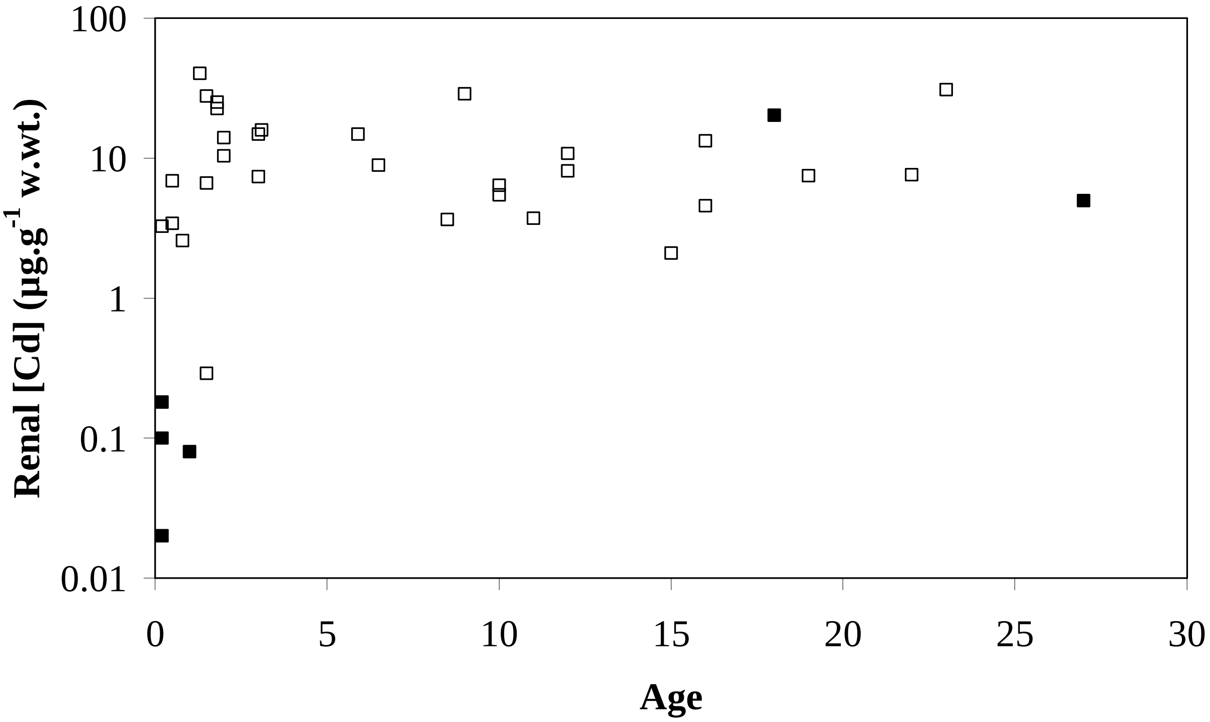
25
30
Age


Renal [Cd] (µg.g-1 w.wt.)
100
10
1
0.1
0.01
0
5
10
15
20
25
30
Age

Tables

Table 1. Characteristics of the striped (n = 40) and bottlenose dolphins (n = 15) sampled between 1999 and 2004 along the Atlantic and Mediterranean French coasts.

Species	Tissue		Age category		Gender		
Sampling area	liver	kidney	< 2 y. old	older	♂	♀	nd
Stenella coeruleoalba							
Bay of Biscay	30	30	12	18	17	13	-
Mediterranean Sea	10	6	6	4	6	3	1
Tursiops truncatus							
Bay of Biscay	10*	9*	3	7	5	5	-
Mediterranean Sea	5	3	1	4	1	4	-

* 3 individuals were originated from the resident group of Arcachon Bay

Table 2. Toxic element levels (mean ± SD; $\mu g.g^{-1}$ d.wt.) in standard reference materials (with 6 replicates).

Metals	Standard reference materials (NRCC)					
	TORT-2		DORM-2		DOLT-3	
	certified value	observed value	certified value	observed value	certified value	observed value
Hg	0.27 ± 0.06	0.28 ± 0.003	4.64 ± 0.26	nd	3.37 ± 0.14	3.27 ± 0.07
Cd	26.7 ± 0.6	26.2 ± 0.2	0.046 ± 0.008	0.046 ± 0.001	19.4 ± 0.6	19.2 ± 0.4

Table 3. Influence of age and area on metal levels in small cetaceans stranded along the French Mediterranean and Atlantic coasts, determined by ANCOVA. Influence of the studied parameters is given with Fisher's F and its associated p-value (in brackets).

Species	Metal	ddl	R^2	Studied parameters		
				Age	*Area*	*Age x Area*
T. truncatus	hepatic Hg	8	0.900	**26.714 (p = 0.001)**	1.286 (p = 0.290)	**6.721 (p = 0.032)**
	renal Cd	5	0.978	**156.768 (p < 0.0001)**	5.198 (p = 0.072)	2.105 (p = 0.207)
S. coeruleoalba	hepatic Hg	36	0.820	**155.5 (p < 0.0001)**	0.116 (p = 0.735)	0.160 (p = 0.691)
	renal Cd*	12	0.767	0.237 (p = 0.635)	**7.453 (p = 0.018)**	0.115 (p = 0.741)

* ANCOVA on renal Cd concentrations refers to young striped dolphins (≤ 2 years old), for which Cd accumulation with age was linear.

Table 4. Influence of age and gender on metal levels in Atlantic striped dolphins. Influence of the studied parameters is given with Fisher's F and its associated p-value (in brackets).

Metal	Statistical test	ddl	R^2	Studied parameters		
				Age	*Gender*	*Age x Gender*
hepatic Hg	ANCOVA	26	0.778	**155.5 ($p < 0.0001$)**	0.442 ($p = 0.512$)	0.229 ($p = 0.367$)
renal Cd	ANOVA	28	0.002	0.051 ($p = 0.822$)	0.435 ($p = 0.515$)	nd

Table 5. Variations of metal concentrations between species at the Atlantic scale, determined using ANCOVA. Influence of the studied parameters is given with Fisher's F and its associated p-value (in brackets).

Metal	ddl	R^2	Studied parameters		
			Age	*Species*	*Age x species*
hepatic Hg	33	0.790	**70.36 (p < 0.0001)**	0.075 (p = 0.786)	0.084 (p = 0.774)
renal Cd	32	0.599	**6.25 (p = 0.018)**	**37.547 (p < 0.0001)**	**5.426 (p = 0.026)**

Table 6. Toxic element levels ($ng.g^{-1}$ w.wt.) in prey from the Atlantic and the Mediterranean Sea.

TAXON / **Family** / *Species*	Area	n	% H	**Length (mm)*** mean ± sd	range	CV%	**[Hg] ($ng.g^{-1}$ w.wt)** mean ± sd	range	CV%	**[Cd] ($ng.g^{-1}$ w.wt)** mean ± sd	range	CV%
FISH												
Clupeidae												
Sardina pilchardius	Atl.	6	61	154 ± 4	148 - 159	2.6	23 ± 7	19 - 34	26	nd	nd	nd
	Med.	6	61	145 ± 6	137 - 150	3.8	94 ± 22	67 - 127	24	nd	nd	nd
Engraulidae												
Engraulis encrasicolus	Atl.	6	70	118 ± 4	112 - 122	3	28 ± 20	26 - 32	7.2	nd	nd	nd
	Med.	6	73	109 ± 4	104 - 113	4.1	50 ± 9	43 - 61	17	nd	nd	nd
Gadidae												
Micromesistius poutassou	Atl.	3	75	141 ± 2	139 - 144	1.7	16 ± 1	16 - 17	5.2	nd	nd	nd
	Med.	3	75	135 ± 4	131 - 138	2.7	21 ± 2	20 - 23	8.5	nd	nd	nd
Merluccidae												
Merluccius merluccius	Atl.	3	79	257 ± 42	225 - 305	16	33 ± 14	18 - 46	42	nd	nd	nd
	Med.	3	79	222 ± 6	215 - 226	2.7	45 ± 12	34 - 58	26	nd	nd	nd
Myctophidae												
Notoscopelus kroeyeri	Atl.	6	70	93 ± 23	70 - 125	23	105 ± 80	29 - 210	77	nd	nd	nd
CEPHALOPOD												
Cranchidae												
Teuthowenia megalops	Atl.	1	83	181			34			1320		
Histioteuthidae												
Histioteuthis reversa	Atl.	5	85	40 ± 24	20 - 80	59	15 ± 5	10 - 24	34	383 ± 196	148 - 665	21
Loliginidae												
Loligo vulgaris	Atl.	26	77	148 ± 50	73 - 256	34	56 ± 22	24 - 97	40	132 ± 54	58 - 238	41
	Med.	12	74	173 ± 54	120 - 265	31	146 ± 66	68 - 250	45	99 ± 111	20 - 434	112
Ommastrephidae												
Illex coindetii	Atl.	22	79	130 ± 54	73 - 272	42	41 ± 17	14 - 69	41	290 ± 94	150 - 520	32
	Med.	6	67	138 ± 19	118 - 161	14	277 ± 150	110 - 490	54	697 ± 391	380 - 1290	56

With: %H, percent of humidity; nd not determined. * Length refers to standard length for fish and mantle length for cephalopod.

Table 7. Hepatic Hg and renal Cd levels (µg.g^{-1} w.wt.) in striped dolphins obtained in previous works.

Locality / Maturity	Length (cm)	Hepatic Hg (µg.g^{-1} w.wt.) n	mean ± sd	range	Renal Cd (µg.g^{-1} w.wt.) n	mean ± sd	range	Reference
Bay of Biscay								
oceanic waters								
immature	153 ± 27	13	-	-	15	17.3 ± 12.7	0.026 - 43.8	Das et al., 2000b
mature	214 ± 7	5	-	-	8	27.9 ± 11.2	10.7 - 45.8	
stranding								
immature	165 ± 17	17	6.5 ± 6	1.2 - 24.1	17	12.9 ± 10.8	0.29 - 40.2	this study
mature	219 ± 12	13	138 ± 92	6.4 - 317	13	10.6 ± 9.0	2.1 - 30.8	
immature	180 ± 19	3	23.7 ± 20.4	1.2 - 41		-	-	André et al. 1991
mature	211 ± 11	5	68.4 ± 18.3	46 - 87		-	-	
mature	185	2	10.2[a]	9.8 - 10.6[a]		31.3[a]	30.4 - 32.2[a]	Holsbeek et al. 1998
Mediterranean Sea								
NW, France								
immature	105 ± 21	6	4.4 ± 2.5	1.1 - 8.2	4	0.095 ± 0.07	0.02 - 0.18	this study
mature	195 ± 8	4	373 ± 454	29.5 - 1033	2	12.6	4.9 - 20.3	
immature	135 ± 30	5	15.4 ± 20.4	1.2 - 49		-	-	André et al. 1991
mature	198 ±11	20	429 ± 352	4.7 - 1544		-	-	
immature	173	2	32.3[a]	19 - 45.5[a]		-	-	Augier et al. 1993
mature	196 ± 8	9	159 ± 83[a]	48.4 - 290[a]		-	-	
immature	137 ± 36	6	42.9 ± 32.9	3.4 - 81		-	-	Capelli et al. 2000
mature	196 ± 15	9	194 ± 207	37.9 - 692		-	-	
not detailed	-	46	166 ± 314[a,b]	-	39	6.3 ± 7.2[a,b]	-	Monaci et al. 1998
NW, Spain								
not detailed	-	20	292 ± 234[a,b]	-	20	1.9 ± 1.5[a,b]	-	Monaci et al. 1998
NW, Italy								
not detailed	-	19	90.8[a,b]	3.5 - 1232[a]	18	10.3[a,b]	2.5 - 22.7[a]	Leonzio et al. 1992
Southern Italy								
immature	107	1	1.96	-		-	-	Cardellicchio et al. 2000
mature	-	6	189 ± 29	156 - 217	6	7 ± 4.1	1.8 - 12.8	
immature	139 ± 29	3	5.9 ± 5.6	2.3 - 12.3		-	-	Cardellicchio et al. 2002a
mature	198 ± 10	7	241 ± 98	107 - 375		-	-	
not detailed	180 ± 33		-	-	10	6.4 ± 4.3	0.3 - 15.7	Cardellicchio et al. 2002b
immature	134 ± 36	3	9.6 ± 11.5	0.8 - 22.7	3	0.51 ± 0.85	0.01 - 1.5	Decataldo et al. 2004
mature	187 ± 13	6	179 ± 197	13 - 463	6	5.7 ± 4.3	1.5 - 12.6	
not detailed	-	30	277 ± 246	0.58 - 966		-	-	Storelli et al. 1998
Bristish Isles								
mature	-	3	8.9 ± 2.8	5.7 - 11		-	-	Law et al. 1992
Pacific								
not detailed	-	45	205 ± 138	1.7 - 485	30	24.8 ± 16.2	0.06 - 69.6	Honda et al. 1983
immature	-	6	5.81 ± 2.76	-		-	-	Itano et al. 1984
mature	-	15	205 ± 102	-		-	-	

With: [a] d.wt. converted in w.wt. on the basis of the d.wt. : w.wt. ratio obtained in this study; and [b] refers to median. NB: maturity has been assumed to be "immature" when body length was < 175 cm for Mediterranean animals, and < 200 cm for other animals.