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Long-term dietary segregation of short-beaked common dolphins (*Delphinus delphis*) in the Bay of Biscay determined using cadmium as an ecological tracer

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ABSTRACT: Dietary studies in marine mammals are traditionally performed by stomach contents analyses, which may be insufficient to determine long-term dietary preferences of these upper-level predators. The primary objective of this study was to test the efficiency of trace metal measurements as complementary tools in dietary studies. Variations in cadmium (Cd) exposure through the diet and its effective renal levels in the short-beaked common dolphin Delphinus delphis were investigated to study the long-term feeding ecology of this predator in the neritic and oceanic waters of the Bay of Biscay. Based upon previous stomach contents analyses, the main prev occurring in the diet of common dolphins were analysed for their Cd contents. Results showed that cephalopods, and especially oceanic Cranchids and Histioteuthids, constituted a major source of Cd for common dolphins. Estimated Cd intake would therefore be 12 times higher in oceanic common dolphins (1400  $\pm$  65  $\mu$ g day and below the dolphins (1400  $\pm$  65  $\mu$ g day day) compared to neritic ones (120  $\pm$  30  $\mu$ g day ). Accumulation of renal Cd concentrations with age was 5 times higher in by-caught oceanic dolphins than neritic ones (p < 0.0001). At the neritic area scale, renal Cd accumulation rate was 2 times more important in by-caught individuals compared to strandings (p = 0.002). Thus, the use of Cd concentrations in by-caught individuals constituted an efficient complementary tool to assess the existence of dietary segregation between neritic and oceanic common dolphins from the Bay of Biscay. On the contrary, the use of Cd data in stranded animals to infer about their feeding ecology would have to be considered cautiously.

KEYWORDS: Trace elements; Marine mammals; Diet; Cephalopods; Exposure; North Eastern Atlantic

#### INTRODUCTION

The short-beaked common dolphin Delphinus delphis is generally considered to be the most common small cetacean inhabiting the temperate waters of the north eastern Atlantic. Indeed, its distribution covers a large area, which extends from about 35°N to 60°N, with most sightings off south western England, southern Ireland, Brittany, and in the Bay of Biscay (Forcada et al. 1990, Evans 1994, Hammond et al. 1995). No overall population data are available, but the MICA (Mesure de l'Impact des Captures Accessoires, 1993) and SCANS (Small Cetacean Abundance in the North and adjacent Seas, 1994) surveys provided an estimate for the summer period of about 120 000 individuals in the Celtic Sea and south west of this area, from 6 to 20°W and 43 to 51°N (Goujon 1996). The short-beaked common dolphin is a pelagic species that mainly occurs over the continental shelf, notably around the 100-200m depth contour, or over areas with prominent underwater topographic features (Evans 1982). The presence of this species in offshore waters has also been reported, e.g. in northern Scotland (Weir et al. 2001) and in the northern Pacific Ocean (Ferrero & Walker 1995). In the Bay of Biscay, short-beaked common dolphins show a wide distribution since they are observed both over the continental shelf, the shelf-edge and in deep oceanic waters (Forcada et al. 1990, Brereton et al. 1999). In addition, Forcada et al. (1990) suggested a bimodality in distribution by depth for the North part of the Bay of Biscay and proposed the occurrence of two different common dolphin populations in the north-east Atlantic, one neritic and one oceanic.

Although distributions of cetacean species can often be related to physical oceanographic features such as water depth, it is likely that prey abundance and

movements are the most important factors in determining the occurrence and movements of cetaceans (Gaskin 1982, Evans 1987, Selzer & Payne 1988). Furthermore, varied diet exhibited by short-beaked common dolphins in different geographic regions (Klinowska 1991, Young & Cockcroft 1994, Santos et al. 1996) has been taken to imply that they are opportunistic, i.e. that their diet is likely to reflect the fish and cephalopod occurrence in the area where they feed. Hence, dietary studies could also be used to monitor the habitat preferences of these long-lived upper-level predators. Consequently, if the spatial segregation of common dolphins previously proposed by Forcada et al. (1990) exists in the Bay of Biscay, oceanic dolphins may exhibit different diet composition than neritic ones, without yet excluding gene flows between populations. Such dietary differences are expected to be emphasised since the neritic and oceanic areas constitute two different ecosystems.

The present study aimed at comparing dietary preferences of common dolphins from the oceanic and the neritic areas. The "oceanic" samples originated from fishery by-catch in the deep waters off the Bay of Biscay (summer 1992-1993) while the "neritic" samples comprised by-caught animals from the continental shelf (winter 2004-2005) plus stranded animals from the French Atlantic coast (2001-2003). Some previous stomach content analyses were carried out on the samples collected from 1992 to 2003 (Pierce et al. 2004). Results showed that the prey species present in the stomachs of stranded and "oceanic" common dolphins were characteristic of each habitat. In addition, "oceanic" common dolphins would consume 10 times more cephalopods than the "neritic" ones. Although stomach content analyses can effectively provide valuable data about the food consumed, results are likely to reflect the diet only on a short time-scale, i.e. a few days. Such a "snapshot" of the diet can be problematic since these

dietary studies relied on stomach contents from stranded and by-caught animals. The difficulty of ensuring that samples are representative of the population is all the more important for strandings, which can include a high number of sick animals. Unhealthy animals may have fed on prey or prey sizes that do not represent the diet of the healthy specimens (Pierce & Boyle 1991). Furthermore, by-catches could be biased towards animals feeding on commercially important fish in areas frequented by fishermen (Santos et al. 2001). Moreover, food preferences evaluated by this method can vary with both season and year (Santos et al. 2004), which can limit valuable long-term insights into dietary preferences. The use of both stranded and by-caught dolphins, combined with the fact that the "oceanic" sample was collected over a limited period (summer months) compared to the "neritic" one (around the year), means that we cannot be sure that the animals sampled offshore did not move to inshore area in other seasons. For those reasons, additional analytical techniques were required to provide a longer time-scale view of a cetacean's diet and to identify potential dietary segregation between neritic and oceanic common dolphins.

Analyses of the fatty constituents of blubber and of stable isotopes of carbon and nitrogen in tissues have recently received considerable attention since these techniques may potentially overcome some of the difficulties that arise using stomach content analyses, providing insight into food preferences (e.g. Rau et al. 1992, Borobia et al. 1995, Abend & Smith1997, Iverson et al. 1997, Hobson et al. 1997, Guitart et al. 1999, Dahl & al. 2000, Das et al. 2000a, Walton et al. 2000, Hooker et al. 2001, Lea et al. 2002, Born et al. 2003). However, the integration of these elements generally occurs over some weeks in the case of fatty acids in the blubber (Thiemann et al. 2003) and 1-2 months for stable isotopes in the muscle (Hobson & Clark 1992), which remains

insufficient to assess the existence of a long-term dietary segregation between habitat for these long-lived predators; common dolphins typically reaching 20-30 years old (Ferrero & Walker 1995). Stable isotope analyses in hard tissues (teeth and bone) can yet provide information about diet on a much longer time-scale (e.g. Tieszen et al. 1983, Hobson 1993) but no hard tissues from the "oceanic" sample was available for dietary analyses.

Thus, the present work explores the utility of trace element measurements to provide additional information on diet. Some previous studies underlined the potential efficiency of trace elements to provide long-term insights into both feeding and habitat preferences of such upper-level predators (e.g. Noda et al. 1995, Sanpera et al. 1996, Monaci et al. 1998, Das et al. 2000a, Dietz et al. 2000, Kunito et al. 2002, Born et al. 2003, Das et al. 2004a). Indeed, the natural origin of trace elements (Nriagu 1996) has involved adaptations of marine organisms facing the presence of toxic metals in their environment. Thus, marine mammals have developed capacities to accumulate high levels of some of these metals during their life (Wagemann & Muir 1984, Aguilar et al. 1999) with the ability to detoxify them through different mechanisms (see reviews by Cuvin-Aralar & Furness 1991 and Das et al. 2000b). Thus, the concentrations of cadmium (Cd) can be particularly elevated in the kidneys of marine mammals (Dietz et al. 1998), in which the biological half-life of Cd is more than 10 years for mammals (Elinder 1982, Stoeppler 1991). Given that the main pathway of exposure to this metal for marine mammals is diet (Aguilar et al. 1999) and that the major source of Cd is feeding on cephalopods (Bustamante et al. 1998, 2002a), Cd concentrations in the kidneys of these top predators may constitute a signature of the consumption of cephalopods over a long time-scale. Indeed, the use of Cd as tracer of diet has already been shown when applied on grey seals from the Faroe Islands (Bustamante et al. 2004). The author highlighted the fact that Cd levels in seals were not in accordance with the stomach contents analyses (limited to the summer months), emphasising also that the use of stomach contents data alone is not sufficient when considering feeding over a longer time-scale.

Therefore, the present study employs a relatively new approach to investigate common dolphin feeding preferences by examining both short-term exposure to cadmium through the diet and effective levels in common dolphin tissues. The approach is based on the hypothesis that if common dolphins have a long-term affinity to specific feeding areas, which are characterised by different biotic or/and chemical environments, significant differences in long-term metal signatures might allow us to distinguish common dolphins from each area. The objectives of the paper were: (1) to provide baseline data on levels of cadmium concentrations in the prey previously found in stomach contents; (2) to estimate a short-term value of the exposure to Cd through the diet for each area, based upon the proportion of prey found in stomachs; (3) to compare renal Cd levels in common dolphins from each area. Since common dolphins from nertic and oceanic areas differ in their consumption of cephalopods on a short-time scale, the hypothesis that Cd concentrations in their kidneys could discriminate dietary preferences on a long-time scale can be tested for these two Atlantic marine food webs.

#### **MATERIAL AND METHODS**

## Study area

The Bay of Biscay is a sector of the northeastern Atlantic between 1 to 10°W and 43 to 48°N. This area is characterised by a variable distance of the shelf-edge from the coastline (Fig. 1). Indeed, continental shelf width varies from 60 to 100 nautical miles (NM) in the northern part of the bay (up to 45°N) and is reduced to 25-30 NM in the southern part, reaching 3 NM at the latitude of the Capbreton trough. In this study, the oceanic and neritic areas have been distinguished as potential habitats for common dolphins for the north part of the Bay of Biscay, i.e. up to 45°N.

## **Dolphin sampling**

Between 2001 and 2003, 37 stranded common dolphins (17 males and 20 females) were sampled by the *Réseau National Echouage* (RNE), co-ordinated by the *Centre de Recherche sur les Mammifères Marins* (CRMM) from La Rochelle (France), along the North French Atlantic coast, i.e. from 45 to 48°N (Fig. 1). The state of decomposition was noted as: 1) very fresh, less than 48 hours after death (blood still separates serum); 2) fresh (blood does not separate serum, cloudy eyes) or 3) slightly decomposed (skin peeling, moderate smell of decomposition). Given that the continental shelf covers a large area at these latitudes and that the sampled animals were relatively fresh, these dolphins were assumed to have been feeding in the neritic area. After the necropsies, all the collected tissues (i.e. kidney for Cd analyses and teeth for age determination) were

stored at –20°C until processed in the laboratory. In addition, this "neritic" sample was completed by 10 by-caught animals (2 males and 8 females) which were collected over the continental shelf-edge in winter 2004-2005. The "oceanic" sample comprised 10 individuals (5 males and 5 females) by-caught during albacore drifnet fisheries in oceanic waters from May to September 1993, between 46° and 50°N and 9° and 20°W (Fig. 1). This collection was conducted by the *Institut Français de Recherche pour l'Exploitation de la Mer* (IFREMER) from Brest (France) as part of a study on the impact of this fishery on small cetaceans (GERDAU program, see Goujon et al. 1993). Tissue samples were taken at the fishing site and frozen immediately in liquid nitrogen and then kept in a freezer (-20°C) until metal analyses in the Oceanology Laboratory (Liege, Belgium; Das et al. 2000a).

Other parameters determined during the sampling included total length (cm) and gender. Age was determined at the CRMM of La Rochelle. The procedure consisted of counting Growth Layer Groups (GLGs) from tooth sections, assuming that one GLG equals one year (as described by Perrin & Myrick 1980).

# **Prey sampling**

Results from previous stomach content analyses allowed the collection of the main prey of common dolphins, aiming to cover the range of sizes found in stomach contents. This prey sampling was composed of 7 species of cephalopod and 11 species of fish (see Table 1). These prey were collected during IFREMER groundfish surveys in the Bay of Biscay, from the continental shelf to the shelf-edge in autumn 2001 to 2003 (EVHOE cruises survey). During these campaigns, oceanic species were also collected by pelagic

trawling over the shelf-edge in the early night. Prey samples were immediately frozen at -20°C on board and then stored at the laboratory until use. After species determination, each individual was weighed and measured and, owing to relatively small sample sizes, prey of similar size from a same trawling for several species were pooled. Metal analyses were carried out on whole prey in order to reflect the exposure to predators.

#### Heavy metal analyses

All equipment used in the sample processing was cleaned, and subsequently decontaminated for 24 h in a solution comprised of 35 ml HNO<sub>3</sub> (65%) and 50 ml HCl (36%) to 1 L of Milli-Ro quality water. Fresh samples were freeze-dried and ground to powder. Each sample was then treated in duplicate.

Prior to Cd analyses, 2 aliquots of approximately 200 mg of each homogenised dry sample were digested with 3.5 ml of 65% HNO<sub>3</sub> 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) with deuterium background correction, and graphite furnace (Hitachi Z-5000) atomic-absorption spectrophotometer with Zeeman background correction. Metal concentrations in tissues are reported in  $\mu g g^{-1}$  wet weight (wet wt).

Quality control was assessed using dogfish liver DOLT-2 and DOLT-3 (NRCC). These standards were treated and analysed under the same conditions as the samples. Our results were in agreement with the certified values (respectively  $20.6 \pm 0.4 \ vs \ 20.8 \pm 0.5$ 

for DOLT-2 and  $19.2 \pm 0.4 \ vs \ 19.4 \pm 0.6 \ \mu g \ g^{-1}$  dry wt for DOLT-3). Detection limits were 0.002 and 0.4  $\mu g \ g^{-1}$  dry wt, for furnace and flame AAS, respectively.

#### **Data treatment**

Statistical analyses were performed using XL-STAT. All the concentrations which were below the detection limit were replaced with "dummy values" that were half of the detection limit (Gibbons & Coleman 2001).

# Calculation of exposure to metal through the diet

Cd levels in whole prey were used to calculate the exposure to this metal through the diet. This calculation took into account the basal metabolic rate of common dolphins (BMR; eq.1), the field metabolic rate (FMR; eq.2) and the daily consumption rate of prey (DCR; eq.3).

- (1) BMR =  $70.W^{0.75}$
- (2) FMR = BMR . AE . AM
- (3)  $DCR_i = (FMR \cdot F_i) / EV_i$
- (4) Exposure i to  $j = \Sigma_i$  to j DCR $_i$  . [Cd] $_i$

The basal metabolic rate (BMR, in kJ day ) was estimated according to the standard mammalian metabolic model of Kleiber (1975), re-investigated for marine mammals by Hunter et al. (2000). An average body mass (W, in kg) of 90 kg has been used for

common dolphins (Collet 1981). BMR was converted to field metabolic rate (FMR, in kJ day ) by multiplying BMR by factors accounting for assimilation efficiency (AE) and active metabolism (AM). Assimilation efficiency was assumed to be 80% following Hinga (1979), resulting in a multiplication factor of 1.25. Active metabolism in cetaceans has been established at approximately 2 to 5 times BMR (Hinga 1979, Santos 1998). A value of 2.5 times BMR was chosen at near the bottom of that range (Kenney et al. 1997). The daily consumption rate (DCR, in g day ) was obtained by multiplying the reconstituted biomass of each prey (f<sub>i</sub>; see Pierce et al. 2004) in the diet by the FMR, divided by the energetic value of each prey (EV<sub>i</sub>, in kJ g ; see Table 1) according to the values of Evans (1987) and Desmercières (unpublished data, see Table 1). Finally the exposure to Cd (eq.4, in µg day ) through the diet was obtained by multiplying the DCR by the level of Cd in whole prey ([Cd], in µg g wet wt) for all prey occurring in the diet of common dolphins. Estimates of uncertainty are based upon Cd concentrations standard deviation for each prey.

## Variations of metal concentrations in common dolphins

The variations of Cd concentrations in dolphins were investigated using analysis of covariance (ANCOVA, Quinn & Keough 2002). As the age is an important factor influencing the accumulation of metals (Aguilar et al. 1999), this parameter was used as the co-variate in the ANCOVA. The factors tested were successively the parameters *state of decomposition, gender* and *origin of samples* and their first-order interaction with *age*, depending of the suitability of each sampling. When significant, the ANCOVA was followed by Fisher multi-comparison tests.

#### **RESULTS**

## Exposure to Cd through the diet

Cd levels of the prey species examined in this study are given in Table 1, along with the mean length of the individuals sampled, their energetic value and their proportion and size in the diet of common dolphins from neritic and oceanic areas.

Cephalopods exhibited much higher levels of Cd than fishes, with respective mean concentrations of  $0.291 \pm 0.302$  and  $0.022 \pm 0.016 \, \mu g \, g^{-1}$  wet wt. The high variability exhibited among cephalopods (104%) is partly due to the specimen of *Teuthowenia megalops*, which exhibited the highest concentration of  $1.32 \, \mu g \, g^{-1}$  wet wt (Table 1). Moreover, a comparison between families of cephalopod revealed decreasing levels of Cd with Cranchids > Histioteuthids > Ommastrephids > Loliginids (Fig. 2). In the case of fish, the maximal levels were obtained in Clupeids, Scombrids and Carangids, in which concentrations were at least twice those of the other fishes (Table 1).

Taking into account the contribution by weight of each prey to the diet, a 12 times more elevated exposure to Cd was obtained for oceanic common dolphins compared to neritic ones (Fig. 3). This particularly elevated estimation for offshore ( $1400 \pm 65 \mu g \, day^{-1}$ ) is linked to the important contribution of cephalopods in the calculation (98%, see Fig. 3), and especially that of *Teuthowenia megalops* (86%). On the contrary, the relative contribution of fish in the calculation for inshore reached 88% (Fig. 3), which accounts for the low exposure obtained. Furthermore, it should be underlined that the calculation

of exposure through the prey analysed is representative of the diet for neritic dolphins but the exposure may be under-estimated for oceanic dolphins since the total proportions in mass of the prey in diet analysed were 94 % for inshore and 53.6% for offshore (Table 1). The lower percentage of the prey analysed for the oceanic area is linked to the difficulty of collecting all the oceanic species for which both occurrence and diel vertical migration pattern are poorly known.

Given that the specimen of *Teuthowenia megalops* was the prey which showed the highest contribution in the calculation for oceanic dolphins, the same approach was conducted without this species. The under-estimation of the resulted exposure was also emphasised since a further 17% of prey were not used (Table 1). However, this second calculation indicated a 2 times higher Cd exposure for oceanic dolphins compared to neritic ones (Fig. 4).

## **Renal Cd levels in predators**

Table 2 shows the results of ANCOVA on Cd concentrations found in the kidney of common dolphins from both the neritic and oceanic areas of the Bay of Biscay. Firstly, ANCOVA confirmed the significant influence of age on Cd concentrations (p < 0.0001). The state of decomposition of stranded dolphins did not significantly influence Cd concentrations (p = 0.938). In addition, there were no significant differences in Cd bioaccumulation between male and female for by-caught "oceanic" dolphins and stranded animals (p > 0.05), which enabled us to pool all individuals from each area for the further comparison.

Thus, the origin of samples and the age were the most important factors explaining renal Cd variability in dolphins (p < 0.0001) with 52.5% of the observed variation being explained by age and 43.2% by the interaction of this factor and the origin of samples. Indeed, Cd concentrations in "oceanic" by-caught dolphins were about 4 times higher than "neritic" ones (p < 0.0001), with respective mean levels of  $16.3 \pm 14.0$  and  $4.35 \pm 2.89 \ \mu g \ g^{-1}$  wet wt. The high standard deviations are the consequence of the increase of concentrations with age, with a 5 times higher rate of Cd bioaccumulation in "oceanic" by-caught dolphins than in "neritic" ones (Fig. 5).

Moreover, among "neritic" samples, stranded animals displayed 2 times less Cd levels (and  $1.66 \pm 1.64~\mu g~g^{-1}$  wet wt) than by-caught ones (p = 0.002; Table 2) although the range of concentrations was similar for the two samples (i.e. 0.15 - 9.34 and 0.34 – 9.08  $\mu g~g^{-1}$  wet wt. Respectively for stranding and by-catch).

#### **DISCUSSION**

Elevated concentrations of Cd in marine mammals are usually ascribed to a diet in which cephalopods and/or crustaceans predominate (Hanamaka et al. 1982, Honda & Tatsukawa 1983, Noda et al. 1995, Sanpera et al. 1996, Das et al. 2000a, Szefer et al. 2002, Kunito et al. 2002, Bustamante et al., 2004). Yet, attempts to compare the intake through the diet with effective levels in predators remain scarce (Caurant & Amiart-Triquet 1995, Bustamante et al. 1998). Hence, this study aimed at calculating a global Cd exposure for common dolphins from the Bay of Biscay examining a combination of prey species which occur in a high mass proportion in the diet. Although the selection of these prey species was based upon stomach analysis from stranded and by-caught

individuals, the reliability of the prey chosen as representative of the short-term diet can be supported by the relatively high number of stomach contents analysed (n > 60 for each area), which may avoid too high an influence of intra-individual variation. Note that the individuals analysed for metals constituted a sub-sampling of the one used for stomach content analyses.

# Dietary sources of Cd for common dolphins in the Bay of Biscay

As expected, cephalopods exhibited 13 times higher Cd levels than fish (Table 1), which is in good agreement with previous studies (Hanamaka et al. 1982, Sapunar et al. 1989, Bustamante 1998). Cd is mainly bioaccumulated in the digestive gland of cephalopods reaching up to 98% of the total body Cd in some species (e.g. Miramand & Bentley 1992, Bustamante et al. 2002b). The high bioavailability of Cd in the digestive gland indicates a high potential for the trophic transfer of this metal to top predators such as marine mammals (Bustamante et al. 2002a). Thus, given that crustaceans occurred at less than 1% in mass in stomach (Pierce et al. 2004), cephalopods may constitute a major source of Cd for common dolphins in the Bay of Biscay.

Among cephalopods, the highest Cd level was found for a single specimen of the Cranchid squid *Teuthowenia megalops* with a concentration of 1.32 µg g<sup>-1</sup> wet wt. Even if the number of the prey analysed was low and did not allow any robust statistical analyses, Cd levels in squids would be different between families with a decrease of Cd concentrations in the following pattern: Cranchids > Histioteuthids > Ommastrephids > Loliginids (Fig. 2). Minimal data exists for Cranchids and Histioteuthids, but the Ommastrephids have also higher burdens of Cd than the Loliginids for the Bay of

Biscay, the Celtic Sea and around Faroe Islands (Bustamante et al. 1998). Loliginids are considered as a neritic family while Histioteuthids and Cranchids are rather found in the oceanic area (Clarke 1985, Nesis 1987). Given that Ommastrephids occur in both inshore and offshore waters, with migrations over long distances between feeding and breeding grounds during their life cycle (Nesis 1987), they are likely to integrate the exposure to Cd from both areas and to exhibit also an intermediate burden compared to the strictly oceanic and neritic cephalopods.

Both Teuthowenia megalops and Histioteuthis sp. are found mostly in the productive waters of northern temperate Atlantic Ocean (Voss 1985, Voss et al. 1998). Likewise, it is now well-established that the geographical distribution of Cd in offshore waters is similar to that of the labile micronutrient phosphorus (Boyle et al. 1976, Frew & Hunter 1992). Like phosphate, Cd is enriched in the surface waters of upwelling regions relative to areas of lower productivity and is depleted in the surface ocean relative to the deeper waters because of its uptake by organisms at the surface and regeneration from sinking biological debris deeper in the water column (Boyle et al. 1976). As the main Cd source for cephalopods is dietary (Koyama et al. 2000, Bustamante et al. 2002b) and the oceanic squids of this study were collected over the shelf-edge, the elevated concentrations of Cd in Histioteuthids and Cranchids may be due to the consumption of prey highly contaminated in Cd in the mesopelagic waters. However, the question arises about the validity of using prey caught over the shelf-edge while dolphins have been sampled over deeper waters. As the vertical water column distribution of Cd over the shelf-edge did not differ than that from the open ocean (Cotté-Krief et al. 2002), cephalopods from the oceanic part of the Bay of Biscay may also feed in productive waters which may expose them to high levels of Cd. Given that Cd has a long biological half-life in cephalopods (Bustamante et al., 2002b), it is likely that most of the Cd assimilated through the diet is stored during the short-life of squid, leading to the very high levels observed. This hypothesis is supported by other studies on oceanic cephalopods which also reported very high Cd concentrations in their tissues (e.g. Martin & Flegal 1975).

#### Dietary segregation between oceanic and neritic common dolphins

The exposure estimated for common dolphins in the oceanic area reached  $1400 \pm 65 \,\mu g$  day with 98% of this intake owing to the consumption of cephalopods (Fig. 3), and especially *T. megalops* (compare Fig. 3 and Fig. 4). This estimation of exposure to Cd in the oceanic area may be biased since only one specimen of *T. megalops* could be collected. However, this estimation could be expected to remain under-estimated since only 53.4% of the prey occurring in the diet of "oceanic" dolphins were analysed (Table 1) and that the uncollected prey comprised cephalopods (Gonatidae and Onychoteuthidae, i.e. 22% of the overall diet). Despite uncertainties of the estimation, the exposure to Cd calculated for the oceanic area was 12 times higher than the one obtained for the neritic area (Fig. 3).

In contrast to the oceanic dolphins, the exposure estimated for neritic mammals took into account a larger proportion of their diet, with 94% (by mass) of the prey present in stomachs being analysed (Table 1). Although the contribution of fish for neritic dolphins was elevated (88%), cadmium levels were nevertheless increased (Fig. 4) by the important consumption of the fatty fishes *Sardina pilchardius* (35.6%), *Trachurus trachurus* (18.3%) and *Engraulis encrasicolus* (11.7%), which exhibited the highest

levels of Cd among fishes (Table 1).

Such an elevated global exposure for offshore dolphins compared to inshore ones is the consequence of a far higher importance of cephalopods in the diet of dolphins from the oceanic area compared to the neritic one, i.e. 45.7% vs 4.8% (Pierce et al. 2004). Yet, the difference in Cd exposure through the consumption of cephalopods may be emphasised by the higher impregnation of Cranchids, Histioteuthids and Ommastrephids consumed by oceanic dolphins compared with that of Loliginids, being the main source of cephalopods for neritic dolphins (Table 1). The consideration of variations of the exposure between gender, age classes and/or seasons would be necessary to better understand the transfer of Cd through the diet at the population scale.

When looking at effective renal Cd concentrations in common dolphins, age and origin of samples were the two factors explaining the Cd variations in dolphins (p < 0.0001; Table 2). At the neritic scale, stranded animals displayed 2 times less Cd levels than by-caught ones (p = 0.002). The Cd levels found in the kidney of the stranded common dolphins collected between 2001 and 2003 were yet similar to those previously obtained by Holsbeek et al. (1998) for common dolphins stranded between 1977 and 1990 in the same area (Fig. 6). Therefore, the observed differences of renal Cd levels between stranded and by-caught "neritic" animals may also rather be the result of the sample source than inter-annual variations. Indeed, stranded animals may not be really representative of a healthy population, which is likely to affect metal concentrations for this biological material.

However, the Cd levels in by-caught common dolphins displayed a similar trend to that obtained by the calculation of the exposure, the mean Cd levels being 5 times higher in

"oceanic" dolphins compared to "neritic" ones. Such differences in Cd levels between areas could be attributed to temporal variations of Cd in the environment since "oceanic" dolphins were collected in 1992-93 whereas "neritic" ones were sampled in 2004-2005. The combination of our data with those of Holsbeek et al. (1998) would suggest that Cd levels in the kidney of common dolphins from the Bay of Biscay would have not changed during these last 20 years (Fig. 6). As well, Cd levels in the neritic prey from the present study are similar to those previously reported (e.g. Amiart-Triquet et al. 1983; Bustamante et al. 1998). Thus, the observed difference of renal Cd levels between "neritic" and "oceanic" common dolphins may also be the consequence of a long-term integration of Cd in two distinct food webs.

Such differences in Cd levels between different areas have already been reported and were attributed to variations in feeding behaviour among areas (Hanamaka et al. 1982, Szefer et al. 2002, Born et al. 2003), chemical water properties (André et al 1990, Noda et al. 1995, Monaci et al. 1998), or metabolism (Sanpera et al. 1996). Concerning common dolphins from the Bay of Biscay, the variations of renal Cd observed may indeed be the consequence of different dietary preferences between areas, but the high concentrations exhibited by "oceanic" dolphins may also reflect a signature of the particular geochemical conditions that would occur offshore. As well, the low carbon stable isotope "delta" reported for muscle of "oceanic" common dolphins were consistent with an oceanic feeding behaviour on a mid-time scale (i.e. some months; Das et al. 2000a). Therefore, we can assess the existence of a long-term dietary segregation between common dolphins from inshore and offshore waters of the Bay of Biscay. The higher rate of Cd bioaccumulation in the kidney of "oceanic" dolphins compared to "neritic" ones (p < 0.0001; Fig. 5) could be linked to the clear difference in bioavailability of Cd in the prey (Bustamante et al. 2002a) and to their different

feeding behaviour. However, the eventuality of a difference in metabolism of dolphins between areas could also be envisaged. In this context, the analysis of both morphometric data and growth curves will enable the investigation of biological characteristics for these groups of dolphins. More than that, the combination of dietary studies, biological characteristics and genetic will allow us to determine the degree of differences between these two groups of common dolphins and to assess the existence of two populations in the Bay of Biscay, as previously suggested by Forcada et al. (1990). The identification of common dolphin stocks in the north-eastern Atlantic would also be relevant in term of conservation and management of these populations.

#### CONCLUSIONS

This investigation showed that the consumption of cephalopods was a major Cd exposure route to common dolphins, providing insights in the feeding behaviour of both prey and predators. At the prey scale, the elevated levels of Cd obtained in oceanic cephalopods would be the consequence of their feeding behaviour and the chemical particularity of the area where they feed. The collection of more samples of oceanic squids is however mandatory to confirm the different burdens seen in coastal and oceanic cephalopods, and to determine the Cd bioavaibility in oceanic cephalopods in order to evaluate its potential for the transfer to top predators.

The concordance of the estimation of the Cd exposure through the diet and effective Cd levels in predators constitute a preliminary stage for the modelling of the bioaccumulation of this metal in marine mammals. In the future, the estimation of the exposure to Cd through the diet would have to take into account the variability of each

parameter involved in the calculation of the FMR, as well as variations linked to age classes and gender.

In addition, this approach appeared to be an efficient tool to get a better knowledge of the ecology of common dolphins in the Bay of Biscay since the use of Cd as a metallic tracer confirmed the existence of a dietary segregation between oceanic and neritic dolphins on a long-term scale. Genetic techniques will have to be used to improve knowledge in the state of the two identified groups of common dolphins in the Bay of Biscay. Finally, the differences of Cd levels between stranded and by-caught animals would have to be confirmed with a larger sampling, but the obtained results make metals in stranded animals to be considered cautiously.

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## **CAPTION TO FIGURES**

Fig. 1. *Delphinus delphis*. Locations of sampling of oceanic (n = 10) and neritic (n = 37) dolphins from the Bay of Biscay, the 200m isobath being indicated with a black line.

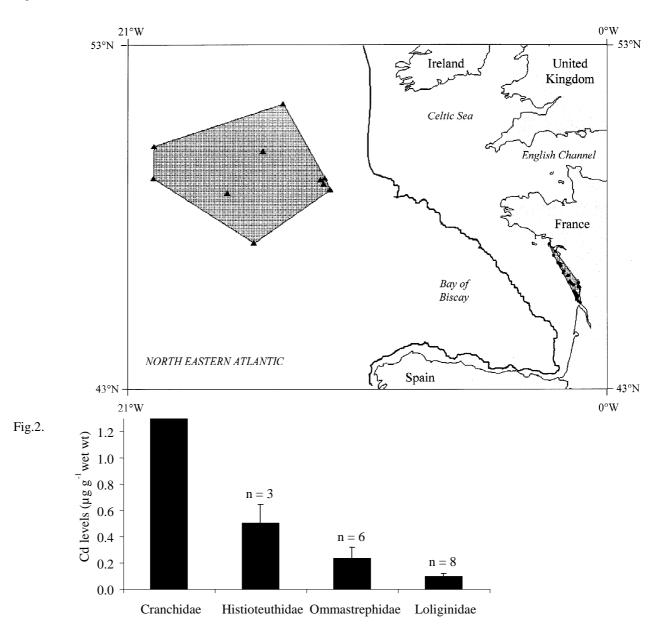
Fig. 2. Mean  $\pm$  SD Cd concentrations ( $\mu g g^{-1}$  wet wt) in whole body of 4 cephalopods families from the bay of Biscay.

Fig. 3. *Delphinus delphis*. Contribution of fish (in white) and cephalopod (all families, in black) in the Cd exposure (µg day ) of neritic and oceanic dolphins, with respective percent of explanation through the diet of 94% and 53.6%.

Fig. 4. *Delphinus delphis*. Contribution of fish (in white) and cephalopod (without *Teuthowenia megalops*, in black) in the Cd exposure (µg day of oceanic and neritic dolphins with respective percent of explanation through the diet of 94% and 36.6%.

Fig. 5. *Delphinus delphis*. Renal Cd ( $\mu g g^{-1}$  wet wt) as a function of age in neritic (in white) and oceanic (in black) dolphins.

Fig.1.



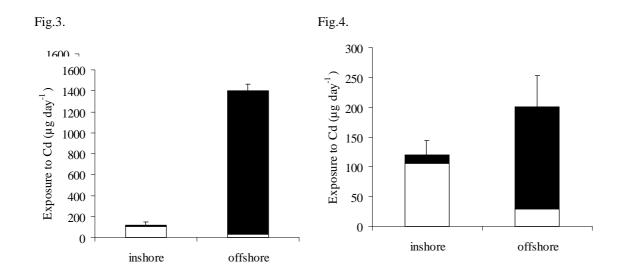


Fig.5.

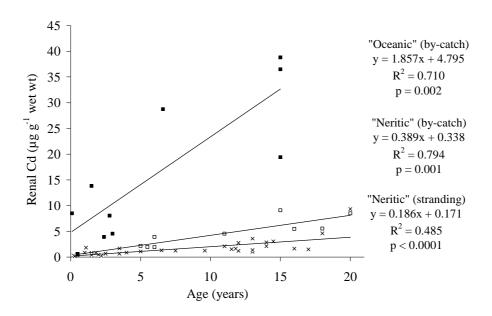


Fig.6.

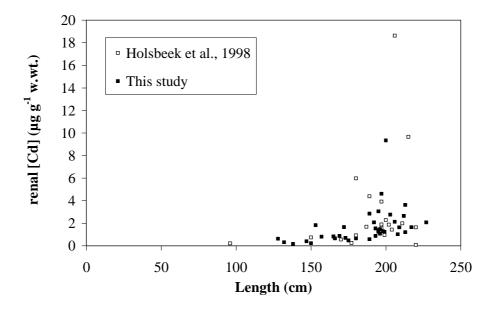


Table 1. Characteristics and Cd levels of the prey occurring in the diet of common dolphins from the bay of Biscay.

	PREY IN DIET			PREY ANALYSED FOR Cd				
	Mass frequency		T (1. ()	I	Cd levels (µg g <sup>-1</sup> w. wt)			EV
	Inshore		Mean ± SD	Length (cm) Mean ± SD	Cα ieveis (με Mean ± SD	Range	n	<b>EV</b> (kJ g <sup>-1</sup> )
	manore	OHSHOIC	Wican ± 5D	INICALI T SD	Wican ± 5D	Runge		(NJ E )
FISH								
Carangidae								
Trachurus trachurus	18.3		$11.7 \pm 3.5$	$14.1 \pm 0.03$	$0.039 \pm 0.007$	0.034 - 0.046	3*	5.8
Clupeidae								
Sardina pilchardius	35.6		$19.2 \pm 3.2$	$19.8 \pm 0.2$	$0.048 \pm 0.017$	0.029 - 0.060	3	10.5
Sprattus sprattus	4.7		$11.4 \pm 2.1$	$7.5 \pm 0.04$	$0.028 \pm 0.001$	0.027 - 0.029	3*	8.3
Engraulidae								
Engraulis encrasicolus	11.7		$12.1\pm2.6$	$11.7 \pm 0.2$	$0.019 \pm 0.004$	0.015 - 0.022	3*	6.7
Gadidae								
Merlangius merlangus	3.1		$19.7 \pm 4.1$	$14.1 \pm 0.3$	$0.005\pm0.000$		3*	4.1
Micromesistius poutassou	6.9		$12.2 \pm 4.2$	$14.1 \pm 0.2$	$0.012\pm0.003$	0.009 - 0.014	3*	4.5
Trisopterus minutus	3.7		$8.0\pm3.6$	$8.5 \pm 0.1$	$0.016\pm0.003$	0.013 - 0.019	3*	5.7
Gobididae	1.0							
Pomatochistus minutus			$4.5\pm0.5$	$6.4 \pm 0.11$	< 0.002	< 0.002	3*	4.8
Myctophidae								
Notoscopelus kroeyeri		29.5	$4.6\pm2.0$	$7.6 \pm 0.9$	$0.017 \pm 0.010$	0.009 - 0.028	3	6.5
Paralepididae								
Arctozenus risso		0.6	$13.0\pm2.7$	$13.1\pm1.8$	$0.020 \pm 0.008$	0.011 - 0.026	3	5
Scombridae								
Scomber scombrus	6.2		$29.0 \pm 7.7$	$27.5 \pm 1.1$	$0.041 \pm 0.006$	0.034 - 0.046	3	9.2
CEPHALOPOD								
Cranchiidae								
Teuthowenia megalops		17.0	$11.6 \pm 2.7$	18.1	1.32		1	5
Histioteuthidae								
Histioteuthis sp		6.3	$2.3 \pm 8.0$	$2.63 \pm 0.55$	$0.500 \pm 0.146$	0.385 - 0.665	3	5
Loliginidae	2.8		$18.5 \pm 11.5$					
Loligo forbesi				$19.1 \pm 3.2$	$0.077 \pm 0.015$	0.060 - 0.090	3	5
L. vulgari				$16.8 \pm 6.3$	$0.113 \pm 0.010$	0.102 - 0.121	3	5
Ommastrephidae		0.2	14.3					
Illex coindetii				$13.7 \pm 4.8$	$0.220 \pm 0.026$	0.200 - 0.250	3	4.9
Todarodes sagitattus				13.7	0.28	0.230 - 0.320	2	5
Todaropsis eblanae				$14.9 \pm 1.3$	$0.216 \pm 0.147$	0.121 - 0.385	3	5.5

Length refers to standard length for fish and to mantle length for cephalopods; n: number of samples analysed;  $n^*$ : when samples were pooled; EV, energetic value.

Table 2. *Delphinus delphis*. Significance of the factors influencing Cd concentrations in the kidneys of neritic and oceanic dolphins (analyses by ANCOVA).

Factor	Sampling Areas							
Co-variable								
Interaction	Inshor	e		Offshore				
State of decomposition	n – 1	(0)						
State of decomposition  Age	p = 1 $p < 0.0001$			-				
State of decomposition x Age	_			-				
Gender	p = 0.669	(0.8)		p = 0.915	(0.1)			
Age	p < 0.0001	(96.7)		p = 0.006	<b>(97)</b>			
Gender x Age	p = 0.443	(2.5)		p = 0.495	(2.9)			
Area		р	= 0.002 (3.7)					
Age		p	< 0.0001 (57.7)					
Area x Age		р	< 0.0001 (38.6)					

Significant values are in bold characters and the percent of the variability explained by each factor variable is indicated in brackets.

## LITERATURE CITED

Abend AG, Smith TD (1997) Difference in stable isotope ratios of carbon and nitrogen between long-finned pilot whales (*Globicephala melas*) and their primary prey in the western north Atlantic. ICES J Mar Sci 54:500-503

Aguilar A, Borrel A, Pastor T (1999) Biological factors affecting variability of persistent pollutant levels in cetaceans. J Cetacean Res Manag Special Issue 1:83-116

André JM, Amiard JC, Amiard-Triquet C, Boudou A, Ribeyre F (1990) Cadmium contamination of tissues and organs of delphinids species (*Stenella attenuata*) – Influence of biological and ecological factors. Ecotoxicol Environ Saf 20:290-306

Born EW, Outridge P, Riget FF, Hobson KA, Dietz R, Øien N, Haug T (2003) Population substructure of North Atlantic minke whales (*Balaenoptera acutorostrata*) inferred from regional variation of element and stable isotopic signatures in tissues. J Mar Syst 43:1-17

Borobia M, Gearing PJ, Simard Y, Gearing JN, Beland P (1995) Blubber fatty acids of finback and humpback whales from the Gulf of St. Lawrence. Mar Biol 122:341-353

Boyle EA, Sclater F, Edmond JM (1976) On the marine geochemistry of cadmium. Nature 263:42-44

Brereton TM, Williams AD, Williams R (1999) Distribution, and relative abundance of the common dolphin (*Delphinus delphis*) in the Bay of Biscay. Europ Res Cet 13:295-299

Bustamante P (1998) Bioaccumulation des éléments traces (métaux et terres rares) chez les mollusques céphalopodes et bivalves pectinidés. Implication de leur biodisponibilité pour le transfert vers les prédateurs. PhD thesis, University of La Rochelle, France

Bustamante P, Caurant F, Fowler SW, Miramand P (1998) Cephalopods as a vector for the transfer of cadmium to top marine predators in the north-east Atlantic Ocean. Sci Tot Environ 220:71-80

Bustamante P, Cosson RP, Gallien I, Caurant F, Miramand P (2002a) Cadmium detoxification processes in the digestive gland of cephalopods in relation to accumulated cadmium concentrations. Mar Environ Res 53:227-241

Bustamante P, Teyssié JL, Fowler SW, Cotret O, Danis B, Miramand P, Warnau M (2002b) Biokinetics of zinc and cadmium accumulation and depuration at different stages in the life cycle of the cuttlefish *Sepia officinalis*. Mar Ecol Prog Ser 231:167-177

Bustamante P, Morales CF, Mikkelsen B, Dam M, Caurant F (2004) Trace element bioaccumulation in grey seals *Halichoerus grypus* from the Faroe Islands. Mar Ecol Prog Ser 267:291-301

Caurant F, Amiard-Triquet C (1995) Cadmium contamination in pilot whales Globicephala melas: source and potential hazard to the species. Mar Pollut Bull 30(3):207-210

Clarke MR (1985) Cephalopods in the diet of cetaceans and seals. Rapp Comm Int Mer Médit 29(8):211-219

Collet A (1981) Biologie du dauphin commun *Delphinus delphis* L. en Atlantique Nord-Est. PhD Thesis, University of Poitiers, France

Cotté-Krief MH, Thomas AJ, Martin JM (2002) Trace metal (Cd, Cu, Ni and Pb) cycling in the upper water column near the shelf edge of the European continental margin (Celtic Sea). Mar Chem 79:1-26

Cuvin-Aralar MLA, Furness RW (1991) Mercury and selenium interaction : a review Ecotoxicol Environ Saf 21:348-364

Dahl TM, Lydersen C, Kovacs KM, Falk-Petersen S, Sargent J, Gjertz I, Gulliksen B (2000) Fatty acid composition of the blubber in the white whales (*Delphinapterus leucas*). Polar Biol 23:675-678

Das K, Lepoint G, Loizeau V, Debacker V, Dauby P, Bouquegneau JM (2000a) Tuna and dolphin associations in the North-East Atlantic: evidence of different ecological niches from stable isotope and heavy metal measurements. Mar Pollut Bull 40(2):102-109

Das K, Debacker V, Bouquegneau JM (2000b) Metallothioneins in marine mammals. Cell Mol Biol 46(2): 283-294

Das K, Holsbeek L, Browning J, Siebert U, Birkun Jr A, Bouquegneau JM (2004a) Trace metal and stable isotope measurements ( $\delta^{13}$ C and  $\delta^{15}$ N) in the harbour porpoise *Phocoena phocoena relicta* from the Black Sea. Environ Pollut 131:197-204

Das K., Siebert U., Fontaine M., Jauniaux T., Holsbeek L., Bouquegneau J.M. (2004b) Ecological and pathological factors related to trace metal concentrations in harbour porpoises *Phocoena phocoena* from the North Sea and adjacent areas. Mar Ecol Prog Ser 281:283-295

Dietz R, Pacyna J, Thomas DJ, Asmund G, Gordeev V, Johansen P, Kimstach V, Lockhart L, Pfirman S, Riget FF, Shaw G, Wagemann R, White M (1998) Heavy metals. AMAP 1998. In: Dietz R, Pacyna J, Thomas DJ (eds) AMAP Assessment Report: Arctic Pollution Issues. Arctic Monitoring and Assessment Program (AMAP), Oslo, Norway, p 373-524

Dietz R, Riget F, Born EW (2000) Geographical differences of zinc, cadmium, mercury and selenium in polar bears (*Ursus maritimus*) from Greenland. Sci Tot Environ 245:25-47

Elinder CG (1982) Cadmium and health: a survey. Int J Environ Stud 19:187-193

Evans PGH (1987) The Natural History of Whales and Dolphins, 1<sup>st</sup> edn. Christopher Helm Ltd, London

Evans WE (1982) Distribution and differentiation of stocks of *Delphinus delphis* Linnaeus in the northeastern Pacific. FAO Fish Ser 5:45-66

Evans WE (1994) Common Dolphin, White-bellied Porpoise - *Delphinus delphis* Linnaeus, 1758. In: Ridgway SH, Harrison R (eds) Handbook of marine mammals vol 5. Academic Press, London, p 191-225

Ferrero RC, Walker WA (1995) Growth and reproduction of the common dolphin, Delphinus delphis Linnaeus, in the offshore waters of the Pacific Ocean. Fish Bull 93:483-494

Forcada J, Aguilar A, Evans PGH, Perrin W (1990) Distribution of common and striped dolphins in the temperate waters of the Eastern North Atlantic. Europ Res Cet 4:64-65

Frew RD, Hunter KA (1992) Influence of Southern Ocean waters on the cadmium-phosphate properties of the global ocean. Nature 360:142-144

Gaskin DE (1982) The ecology of whales and dolphins, 1<sup>st</sup> edn. Heinemann Educational Books Ltd, London

Gibbons RD, Coleman DE (2001) Statistical methods for detection and quantification of environmental contamination. John Wiley & Sons, New York

Goujon M, Antoine L, Collet A (1993) Incidental catches of cetaceans by the French albacore tuna fishery: preliminary results. Int Counc Explor Sea CM 1993/N:13

Goujon M (1996) Captures accidentelles du filet maillant dérivant et dynamique des populations de dauphins au large du Golfe de Gascogne. PhD thesis, ENSAR Rennes, France

Guitart R, Silvestre AM, Guerrero X, Mateo R (1999) Comparative study on the fatty acid composition of two marine vertebrates: striped dolphins and loggerhead turtles. Comp Bioch Physiol Part B 124:439-443

Hammond PS, Benke H, Berggren P, Borchers DL, Buckland ST, Collet A, Heide-Jorgensen MP, Heimlich-Boran S, Hiby AR, Leopold MF, Oien N (1995) Distribution and abundance of the harbour porpoise and other small cetaceans in the North Sea and adjacent waters. Final report to the European Commission under contract LIFE 92-2/UK/O27.

Hanamaka T, Itoo T, Mishima S (1982) Age-related change and distribution of cadmium and zinc concentrations in the steller sea lion (*Eumetopias jubata*) from the coast of Hokkaido, Japan. Mar Pollut Bull 13(2):57-61

Hinga KR (1979) The requirements of whales in the southern hemisphere. Deep-Sea Res 26:569-577

Hobson KA, Clark RG (1992). Assessing avian diets using stable isotopes: I. Turnover of carbon-13. Condor 94:181-188

Hobson KA, Sease JL, Merrick RL, Piatt JF (1997) Investigating trophic relationships of pinnipeds in Alaska and Washington using stable isotope rations of nitrogen and carbon. Mar Mamm Sci 13(1):114-132

Honda K, Tatsukawa R (1983) Distribution of cadmium and zinc in tissues and organs, and their age-related changes in striped dolphins, *Stenella coeruleoalba*. Arch Environ Contam Toxicol 12:543-550

Hooker SK, Iverson SJ, Ostrom P, Smith SC (2001) Diet of northern bottlenose whales inferred from fatty-acid and stable isotope analyses of biopsy samples. Can J Zool 79:1442-1454

Hunter AMJ, Trites AW, Pauly D (2000) Estimates of basal metabolic and feeding rates for marine mammals from measurements of maximum body length. Proc Comp Nutr Soc 2000:103-106

Iverson SJ, Frost KJ, Lowry LF (1997) Fatty acid signatures reveal fine scale structure of foraging distribution of harbor seals and their prey in Prince William Sound, Alaska.

Mar Ecol Prog Ser 151:255-271

Kenney RD, Scott GP, Thompson TJ, Winn HE (1997) Estimates of prey consumption and trophic impacts of cetaceans in the USA northeast continental shelf ecosystem. J Northw Atl Fish Sci 22:155-171

Kleiber M (1975) The fire of life, an introduction to animal energetic, 2<sup>nd</sup> edn. RE Kreiger publishing Co, New York

Klinovska MM (1991) Dolphins, porpoises and whales of the world. The IUCN Red Data Book. IUCN, Cambridge, United Kingdom, Gland

Koyama J, Nanamori N, Segawa S (2000) Bioaccumulation of waterborne and dietary cadmium by oval squid, *Sepioteuthis lessoniana*, and its distribution among organs. Mar Pollut Bull 40(11):961-967

Kunito T, Watanabe I, Yasunaga G, Fujise Y, Tanabe S (2002) Using trace elements in skin to discriminate the populations of minke whales in southern hemisphere. Mar Environ Res 53:175-197

Lea M, Cherel Y, Guinet C, Nickols PD (2002) Antarctic fur seals foraging in the polar frontal one: inter-annual shifts in diet as shown from fecal and fatty acid analyses. Mar Ecol Prog Ser 245:281-297

Martin JH and Flegal AR (1975) High copper concentrations in squid livers in association with elevated levels of silver, cadmium and zinc. Mar Biol 30:51-55

Miramand P, Bentley D (1992) Concentration and distribution of heavy metals in tissues of two cephalopods, *Eledone cirrhosa* and *Sepia officinalis*, from the french coast of the English Channel. Mar Biol 114:407-414

Monaci F, Borrel A, Leonzio C, Marsili L, Calzada N (1998) Trace elements in striped dolphins (*Stenella coeruleoalba*) from the western Mediterranean. Environ Pollut 99:61-68

Nesis KN (1987) Cephalopods of the world: squid, cuttlefishes, octopuses, and allies. Neptune City TFH Publications, New Jersey

Noda K, Ichihashi H, Loughlin TR, Baba N, Kiyota M, Tatsukawa R (1995) Distribution of heavy metals in muscle, liver and kidney of northern fur seal (*Callorhinus ursinus*) caught off Sanriku, Japan and from the Pribilof islands, Alaska. Environ Pollut 90(1):51-59

Nriagu JO (1996) A history of global metal pollution. Science 272:223-224

Perrin WF, Myrick Jr AC (1980) Age determination of toothed whales and sirenians. Rep Int Whal Comm, special issue 3

Pierce GJ, Boyle PR (1991) A review of methods for diet analysis in piscivorous marine mammals. Oceanogr Mar Biol 29:409-486

Pierce GJ, Santos MB, Learmonth JA, Smeenk C, Addink M, García Hartmann M, Boon JP, Zegers B, Mets A, Ridoux V, Caurant F, Bustamante P, Lahaye V, Guerra A, González A, López A, Alonso JM, Rogan E, Murphy S, Van Canneyt O, Dabin W, Spitz J, Dorémus G, Meynier L, 2004. Bioaccumulation of persistent organic pollutants in small cetaceans in European waters: transport pathways and impact on reproduction. Final Report to the European Commission's Directorate General for Research on Project EVK3-2000-00027

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists, 1<sup>st</sup> edn. Cambridge University Press, Cambridge

Rau GH, Ainley DG, Bengtson JL, Torres JJ, Hopkins TL (1992) <sup>15</sup>N/<sup>14</sup>N and <sup>13</sup>C/<sup>12</sup>C in Weddel sea birds, seals and fish: implication for diet and trophic structure. Mar Ecol Prog Ser 84:1-8

Sanpera C, Gonzales M, Jover L (1996) Heavy metals in two populations of North Atlantic fin whales (*Balaenoptera physalus*). Environ Pollut 91(3):299-307

Santos MB, Pierce GJ, Lopez A, Barreiro A, Guerra A (1996) Diets of small cetaceans stranded NW Spain. Int Counc Explor Sea CM1995/N:11

Santos MB (1998) Feeding ecology of harbour porpoises, common and bottlenose dolphins, and sperm whales. PhD thesis, University of Aberdeen, Scotland

Santos MB, Clarke MR, Pierce GJ (2001) Assessing the importance of cephalopods in the diets of marine mammals and other top predators: problems and solutions. Fish Res 52:121-139

Santos MB, Pierce GJ, Learmonth JA, Reid RJ, Ross HM, Patterson IAP, Reid DG, Beare D (2004) Variability in the diet of harbor porpoises (*Phocoena phocoena*) in scottish waters 1992-2003. Mar Mamm Sci 20(1):1-27

Sapunar J, Jusic M, Bazulic D (1989) Total mercury and cadmium in some cephalopods and fish from the Adriatic Sea. Mar Pollut Bull 20(11):576-577

Selzer LA, Payne PM (1988) The distribution of white-sided (*Lagenorhynchus acutus*) and common dolphins (*Delphinus delphis*) vs. environmental features of the continental shelf of the northeastern United States. Mar Mamm Sci 4(2):141-153

Stoeppler M (1991) Cadmium. In: Merian E, Clarson TW (eds) Metal and their compounds in the environment. VCH, New York, p 803-851

Szefer P, Zdrojewska I, Jensen J, Lockyer C, Skora K, Kuklik I, Malinga M (2002) Intercomparison studies on distribution and coassociations of heavy metals in liver, kidney and muscle, of harbor porpoise, *Phocoena phocoena*, from southern Baltic and coastal waters of Denmark and Greenland. Arch Environ Contam Toxicol 42:508-522

Thiemann GW, Budge SM, Iverson SJ. (2004) Determining blubber fatty acid composition: a comparison of *in situ* direct and traditional methods. Mar Mamm Sci 20:284-295.

Voss NA (1985) Systematics, biology and biogeography of the cranchiid cephalopod genus *Teuthowenia* (Oegopsida). Bull Mar Sci 36:1-85

Voss NA, Nesis KN, Rodhouse PG (1998) The cephalopod family Histioteuthidae (Oegopsida): Systematics, biology, and biogeography. Smithson Contr Zool 586(2):293-372

Wagemann R, Muir DCG (1984) Concentrations of heavy metals and organochlorines in marine mammals of northern waters: overview and evaluation. Can Tech Rep Fish Aquat Sci 1279, 97p.

Walton MJ, Henderson RJ, Pomeroy PP (2000) Use of blubber fatty acid profiles to distinguish dietary differences between grey seals *Halichoerus grypus* from two UK breeding colonies. Mar Ecol Prog Ser 193:201-208

Weir CR, Pollock C, Cronin C, Taylor S (2001) Cetaceans of the Atlantic Frontier, north and west Scotland. Contin Shelf Res 21:1047-1071

Young DD, Cockcroft VG (1994) Diet of common dolphins (*Delphinus delphis*) off the south-east coast of southern Africa: opportunism or specialization? J Zool 234:41-53