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Bioaccumulation of persistent organic pollutants in female common dolphins (*Delphinus delphis*) and harbour porpoises (*Phocoena phocoena*) from western European seas: Geographical trends, causal factors and effects on reproduction and mortality

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High PCB levels were recorded in porpoises and common dolphins from European coasts.

Abstract

Concentrations of polychlorinated biphenyls (PCBs) in blubber of female common dolphins and harbour porpoises from the Atlantic coast of Europe were frequently above the threshold at which effects on reproduction could be expected, in 40% and 47% of cases respectively. This rose to 74% for porpoises from the southern North Sea. PCB concentrations were also high in southern North Sea fish. The average pregnancy rate recorded in porpoises (42%) in the study area was lower than in the western Atlantic but that in common dolphins (25%) was similar to that of

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the western Atlantic population. Porpoises that died from disease or parasitic infection had higher concentrations of persistent organic pollutants (POPs) than animals dying from other causes. Few of the common dolphins sampled had died from disease or parasitic infection. POP profiles in common dolphin blubber were related to individual feeding history while those in porpoises were more strongly related to condition.

Keywords: Phocoena phocoena; *Delphinus delphis*; Persistent organic pollutants; Reproduction; Diet

1. Introduction

Long-lived apex predators are particularly at risk from effects of persistent organic pollutants (POPs), e.g. polychlorinated biphenyls (PCBs) and dichlorodiphenylethanes (e.g. DDT), due to bioaccumulation (increasing concentration with age in individuals) and biomagnification (higher levels higher up the food chain, especially when moving from gill-breathing animals like fish and cephalopods to air-breathing animals like marine mammals). POPs are lipophilic compounds that tend to accumulate in the lipid-rich blubber (although lipid-normalised concentrations of POPs in different body compartments tend to be very similar). In marine mammals, POPs enter the body almost exclusively through the diet.

Amounts of POPs in marine mammal tissues will vary in relation to input (reflecting levels of environmental contamination, trophic position and the type of prey eaten), elimination in faeces, transformation to non-toxic forms and, in the case of lipophilic organic compounds, transfer from mother to offspring during pregnancy and lactation. Aguilar et al. (1999) reviewed the main biological factors responsible for variation in pollutant concentrations in cetaceans, highlighting the importance of diet, body size (which affects excretion rate, activity of detoxifying enzymes and metabolic rate), body composition (especially, in the case of lipophilic POPs, the mass of blubber), nutritive condition, disease, age, sex, and duration of lactation.

The harmful consequences of bioaccumulation of POPs in marine mammals include depression of the immune system (e.g. De Swart, 1995; Ross, 1995), increased risk of infection (Hall et al., 2006) and reproductive failure (Helle et al., 1976; Reijnders, 1986), potentially adversely affecting population status (Reijnders, 1984). Reijnders (1986) showed that reproductive failure in harbour seals (*Phoca vitulina*) was linked to feeding on contaminated fish: seals fed on fish from the Wadden Sea showed a decreased reproductive rate at an average total-PCB level of 25–27 $\mu g\ g^{-1}$ lipid, whereas a control group showed normal reproductive rates at mean PCB levels of 5–11 $\mu g\ g^{-1}$ lipid. However, Addison (1989) argued that reproductive failure in several wild marine mammal populations could not be conclusively attributed to effects of contaminants. Jepson et al. (2005) found that total PCB levels in porpoises from UK waters were significantly higher in animals that had died from infectious diseases than in those dying as a result of physical trauma. They suggested that these results supported a causal (immunotoxic) relationship between PCB exposure and infectious disease mortality. De Guise et al. (1995) report evidence of immunosuppression related to organochlorine bioaccumulation in belugas (*Delphinapterus leucus*) in the Gulf of St. Lawrence.

Not all POPs that are present in food find their way equally into blubber. For example, within the PCBs, some are subject to enzyme-mediated metabolism, related to their structural characteristics, and bioaccumulate to a much lesser degree than the persistent congeners. Certain chlorinated biphenyls can be metabolised by cytochrome P-450. Although the ability to metabolise PCBs was previously thought to be less well developed in cetaceans than in pinnipeds (Boon et al., 1997), implying that cetaceans may be more sensitive to effects of exposure to POPs, more recent evidence (reviewed by Hall et al., 2006) suggests that this may not be the case. Immunoreactive proteins recognised by heterologous CYP2B antibodies are present in several cetaceans including harbour porpoises (White et al., 1994; Goksøyr, 1995; Hummert et al., 1995) and CYP1B-like amino acid sequences are present in striped dolphin cDNA (Godard et al 2000).

Even though use of some harmful organic compounds has decreased or even ceased as the associated dangers have become recognised, new classes of chemicals are of concern, notably the brominated flame retardants (De Boer et al., 1998). Initially, studies focused on the brominated diphenyl ether formulations (PBDEs). Their acute toxicity is low, but critical sub-lethal effects include neurodevelopmental toxicity and altered thyroid hormone homeostasis, but further studies are needed (Darnerud, 2003). The production and use of the penta- and octa-mix PBDE formulations was banned in the EU in 2004.

Recently, attention has focused on hexabromocyclododecane (HBCD), which is the principal brominated flame retardant in polystyrene foams used in the building industry (Law et al., 2005, 2006a). With a worldwide production of 16,700 tons in 2001, of which the majority (9500 tons) was used in the European market, it is recognised as a priority pollutant by the European Union. Retrospective analyses of eggs of the guillemot (*Uria aalge*) from the Baltic Sea demonstrated that HBCD residues were already detectable in the early 1970s, although levels started to increase sharply after 1980 (Sellström et al., 2003). A recent study has shown rising concentrations from 2001 to 2003 in blubber of harbour porpoises from the UK (Law et al., 2006b).

Toxic elements such as cadmium (Cd) and mercury (Hg) are also known to bioaccumulate in the tissues of marine mammals. Again, this will reflect diet: for example, species that feed primarily on cephalopods may be expected to accumulate higher levels of cadmium than those feeding on fish (Bustamante et al., 1998; Lahaye et al., 2005). Another element of interest is zinc (Zn), which plays an important role in mammalian immune systems. High concentrations of Zn in the liver have previously been associated with poor health in harbour porpoises (Das et al., 2004) and in humans (e.g. Amdur et al., 1991) and may thus provide an index of health status.

The links between feeding, reproduction, condition and contaminant burdens in marine mammals are undoubtedly complex. Important insights have been provided from studies on populations in which individual reproductive history is known (e.g. bottlenose dolphins in Sarasota Bay, Wells et al., 2005) but there have been no experimental studies on captive cetaceans comparable to the work on seals undertaken by Reijnders (1986). For a large-scale survey, the use of stranded animals has several advantages over taking biopsies from living animals in the wild. Sampling from dead animals is less expensive, raises no ethical issues, and provides access to all tissues, not simply blubber, as well as a wealth of ancillary information on size, age, reproductive status, condition and pathology. Restricting sampling to relatively fresh carcasses can assure high sample quality, while analysis of the ancillary data can assist in interpretation of contaminant data, including helping to control for possible biases associated with such opportunistic sampling.

The aim of the present study was to survey geographical variation in concentrations of persistent organic contaminants in body tissues of small cetaceans in European Atlantic waters, specifically two of the most commonly occurring species, common dolphin *Delphinus delphis* and harbour porpoise *Phocoena phocoena*, and to identify biological factors (e.g. diet) responsible for observed patterns of variation in concentrations. The patterns of bioaccumulation in female marine mammals are more complex than those in males due to the transfer of POPs to the offspring during pregnancy and lactation. However, the consequences for reproductive output are more readily observed in females and are, arguably, much more important at the population level. We therefore focused on females.

In modelling regional variation in concentrations of the main categories of POPs in the two cetacean species, we controlled for effects of individual length, age, reproductive status, and condition. We used blubber thickness as an indicator of condition. Since blubber thickness varies seasonally in cetaceans (Elsner, 1999; Lockyer et al., 2003; Learmonth, 2006), we included season as an additional explanatory variable, so that any marginal effect of blubber thickness should be related to condition.

We tested whether POP concentrations were related to diet (as proxied by fatty acid profiles in the inner blubber layer) and analysed variation in POP levels in samples from a range of putative prey species. We tested for a link between POP and trace element (Hg, Cd) concentrations in tissues.

Finally we examined evidence of possible consequences of POP bioaccumulation for reproductive output and health. We tested whether the incidence of pregnancy was related to POP concentrations. Regional variation in POP concentrations in the two cetacean species is also summarised and interpreted in relation to data on average pregnancy rate. We tested whether Zn levels in the liver can provide an indicator of health status (as suggested by Das et al., 2004) and whether POP concentrations were related to cause of death and/or liver Zn concentrations.

2. Methods

2.1. Sampling programme

During 2001–2003, in collaboration with national strandings schemes, stranded harbour porpoises and common dolphins were sampled from Scottish

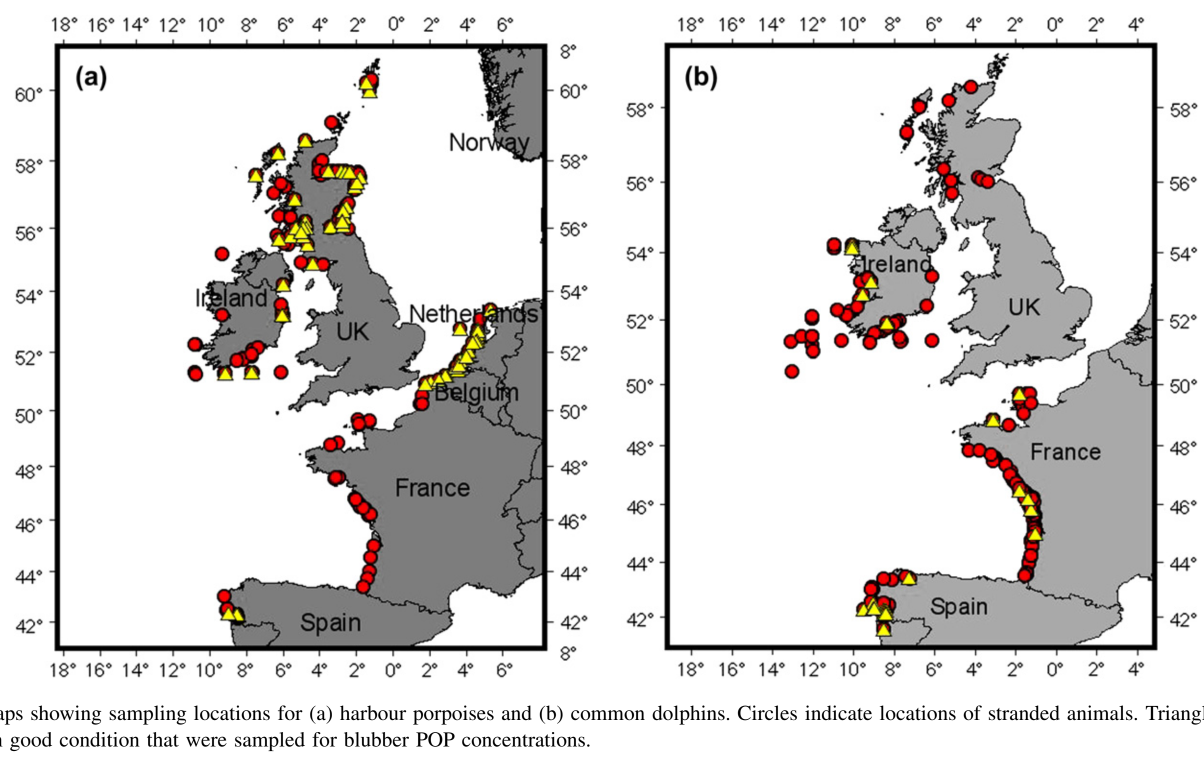

Fig. 1. Maps showing sampling locations for (a) harbour porpoises and (b) common dolphins. Circles indicate locations of stranded animals. Triangles indicate females in good condition that were sampled for blubber POP concentrations.

(UK), Irish, Dutch, Belgian, French and Galician (NW Spain) Atlantic coasts (see Fig. 1). In Ireland, the sample included a substantial proportion of fishery by-catches. Priority was given to females recovered in good condition, from which all necessary samples could be obtained, but data and samples were collected from other animals when possible. Samples obtained from France included those originating from a mass live stranding that occurred in February 2002 at Pleubian, Brittany. The nursery group comprised adult (7+ years old) females accompanied by their unweaned calves. Of 53 individuals found dead, 52 were fully necropsied.

Data collection protocols followed European Cetacean Society guidelines for gross post-mortem examination and tissue sampling (Kuiken and Hartmann, 1991). Basic data collected from each animal included stranding location, date, species, sex, total length and blubber thickness (measured immediately in front of the dorsal fin in dorsal, midline and ventral positions). Animals sampled ranged in decomposition state from extremely fresh (point 2a on the ECS scale) to moderately decomposed (point 3). Pathological and histopathological analyses were routinely carried out in Scotland, Netherlands, Belgium and Galicia. Pathological and histopathological analyses were also carried out for some samples from France and Ireland. Infectious disease mortality is generally regarded as a consequence rather than a cause of high contaminant burdens (see Jepson et al., 2005).

Blubber samples for POP analysis were taken from the left side in front of the dorsal fin. Samples were complete vertical cross-sections, to prevent any possible effects of stratification of the blubber. An additional (adjacent) blubber sample was collected for fatty acid analysis. Samples of liver and kidney, for trace element analysis, were removed and stored in polythene bags. All samples for pollutant analysis were frozen at −20 °C until required for analysis. During transport, samples were packed in insulation boxes with dry ice to ensure that they remained frozen.

At least 5 teeth were collected from each sampled individual, selecting the least worn/damaged and least curved teeth, to ensure sufficient material for replicate preparations. Teeth were preserved frozen or in 70% alcohol. The ovaries and associated reproductive tract were collected and preserved in 10% neutral formalin. The uterus was examined for presence of a foetus. Milk glands were examined for evidence of lactation.

Samples were also collected of some of the main prey species of common dolphins and harbour porpoises in each region, to allow measurement of POP in prey tissues (Table 1). This sampling made use of fish and squid collected during trawling surveys, as well as market sampling, or material collected for other projects. Selection of species was based on identification of the main prey species from the literature (Santos and Pierce, 2003; Santos et al., 2004a,b, 2005; De Pierrepont et al., 2005; Pusineri et al., 2007) and unpublished data held by the authors, although minor prey species were also included where material was available. Variation in contaminant concentrations in prey tissues was analysed in relation to taxonomic group, geographical location and body size.

Table 1
Prey samples analysed for persistent organic pollutants (POP) concentrations: (a) fish and (b) cephalopods

Taxon	Sources
(a) Fish	
Ammodytidae	1, 5
Clupea harengus	1
Gadiculus argenteus	4
Lampanyctos festivus	4
Limanda limanda	1
Melanogrammus aeglefinus	1
Merlangius merlangus	1, 2, 3
Merluccius merluccius	5
Micromesistius poutassou	5
Notoscopelus kroyeri	4
Pleuronectes platessa	1
Pollachius virens	1
Pomatoschistus spp	3
Sardina pilchardus	4, 5
Scomber scombrus	1
Sprattus sprattus	1
Trachurus trachurus	4
Triglidae	1
Trisopterus esmarkii	1
Trisopterus luscus	4, 5
(b) Cephalopods	
Illex coindetii	5
Loligo vulgaris	4, 5
Octopus vulgaris	5
Todaropsis eblanae	5

Region codes: 1 = UK (Scotland), 2 = Ireland, 3 = Netherlands/Belgium, 4 = France, 5 = Spain (Galicia). Note: for Spanish fish samples, separate POP analyses were carried out on liver and muscle. Otherwise, whole animals were used.

2.2. POP measurements

Because POP analysis was budget-limited, effort was focused on the best sample sets (i.e. 20+ individuals per region per species), concentrating on those females for which most data were available on other variables. Thus, for porpoises, analysis focused on samples from Ireland, Scotland, and the southern North Sea (Netherlands, Belgium and northern France). For common dolphins, analysis focused on samples from Ireland, France and Galicia. POPs measurements were made on 70 female common dolphins and 67 female porpoises (out of 531 common dolphins and 243 porpoises collected, the latter figures including individuals both sexes and all decomposition states). During the sampling programme, additional funding became available to measure levels of HBCD in some of the samples (see Zegers et al., 2005, for further details).

Analysis of POP concentrations in cetacean and prey samples was carried out at the Royal Netherlands Institute for Sea Research (NIOZ), with some Scottish cetacean samples analysed at the Centre for Environment, Fisheries and Aquaculture Science (Cefas). For prey samples, analysis was normally carried out on homogenates of whole animals. For small species, samples from several individuals sometimes had to be combined. The samples were thawed and homogenised, extracted with a mixture of pentane/dichloromethane/water and lipid content determined gravimetrically. Samples were cleaned by sulphuric acid treatment and elution over silica columns to separate the contaminants of interest from the lipids used.

Organochlorines were determined by gas chromatography with electron capture detection (GC-ECD). The external standard mixture for the PCBs contained 39 congeners. Since concentrations of many compounds were often below the limit of detection, we finally selected 18 PCB congeners for further analysis (CB28, CB49, CB52, CB99, CB101, CB118, CB128, CB138; CB141, CB149, CB151, CB153, CB170, CB177, CB180, CB183, CB187 and CB194). Data available from Cefas (for Scottish porpoises) excluded values for CB99 and CB177, which were therefore dropped from the majority of the analyses of data for porpoises. Other OCs analysed were p,p'-DDE, which is the most persistent metabolite and the major representative of the insecticide DDT-group, the fungicide hexachlorobenzene (HCB), and pentachlorobenzene (PeCBz), a fire retardant and precursor for the fungicide pentachloronitrobenzene.

Based on results of studies on mink, otters and seals, a Σ-PCB level of 17 μg g^{-1} lipid in blubber has been estimated as the threshold level for effects on reproduction in aquatic mammals (Kannan et al., 2000) and this value was previously applied in a study of bottlenose dolphins by Schwacke et al. (2002). For comparison with this figure, which was based on the commercial PCB mixture Aroclor 1254, we also derived the "ICES7" value (the sum of concentrations of CB28, CB52, CB101, CB118, CB138, CB153, CB180), since three times this value is equivalent to the Aroclor 1254 value (Jepson et al., 2005).

Brominated flame retardants were determined by gas chromatography with electron-capture negative ion mass spectrometry (GC−ECNIMS). The compounds were detected on the basis of selective ion recording at the masses of the two bromine isotopes with masses 79 and 81, which occur in the environment in approximately a 1:1 ratio. Our external standard mixture for the polybrominated diphenyl ethers (PBDEs) contained 11 PBDE congeners and HBCD. Since many compounds were often below their limit of detection, we finally selected five PBDE congeners (BDE47, BDE99, BDE100, BDE153 and BDE154) for further analysis. For the determination of total (α-, β-, and γ-) hexabromocyclododecanes (HBCDs) at NIOZ, elution of the silica column was performed with 30 ml of an 85% pentane/15% diethyl ether mixture, an alteration required particularly for measurement of the β-isomer (Boon et al., 2002; Zegers et al., 2003). Cefas conducted analyses for HBCD on an individual diastereoisomer basis using LC–MS (Law et al., 2006b). Funding for HBCD analysis did not become available until after sample processing was underway and sample sizes are therefore smaller. Consequently HBCD data are not included in all analyses.

At regular intervals, certified reference materials were analysed for PCBs and DDE and laboratory reference materials were analysed for PBDEs (since certified reference materials were not at the time available for this class of compounds). The values obtained fell within the accepted normal ranges. Both NIOZ and Cefas participated in tests of analytical protocols in which both laboratories performed up to the current standard. Results from duplicate samples from the same animals analysed by both laboratories were similar.

2.3. Determination of trace element levels

Kidney and liver samples were freeze-dried and then ground to powder. Total Hg in liver was directly determined using a mercury analyser AMA 254. Prior to renal Cd and hepatic Zn analyses, two 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. Cd and Zn were analysed by Atomic Absorption Spectrometry (AAS) using flame (Varian spectrophotometer Vectra 250 Plus with deuterium background correction). Graphite furnace (Hitachi Z5000 with Zeeman correction) was also used when low Cd levels were detected in samples.

Organic Hg in the liver is normally detoxified through demethylation by selenium (Se), and conversion to tiemannite (Martoja and Berry, 1980). Thus the Hg:Se ratio may provide an indicator of the extent to which Hg has been successfully detoxified. Since the atomic masses of Hg and Se are 200.59 g mol^{-1} and 78.96 g mol^{-1} respectively, when concentrations of both elements are expressed as μg g^{-1} wet weight, ratios greater than approximately 2.5 suggest the presence of toxic Hg. Determination of Se in liver was carried out by graphite furnace AAS (Hitachi Z5000 with Zeeman correction).

Quality controls were ensured by analysis of reference materials (TORT-2, DOLT-2 and -3) from the Canadian National Research Council (CNRC). Concentrations of Hg, Cd, Se and Zn were expressed in μg g^{-1} wet weight.

2.4. Determination of age and reproductive status

Age was determined by analysing growth layer groups (GLGs) in the dentine of teeth, following the methods of Hohn and Lockyer (1995) and Lockyer (1995). Teeth were decalcified and sectioned using a freezing microtome. The most central and complete sections (including the whole pulp cavity) were selected from each tooth, stained, mounted on glass slides, and allowed to dry. GLGs were counted under a binocular microscope and on enhanced computer images of the sections. All readings were initially made blind (with no access to other data on the animals) and replicate counts were made by at least two readers. As ages were recorded by a number of different researchers, cross-calibration exercises were carried out under the direction of ER and CL.

Methods for examining and assessing female reproductive status are described in Murphy (2004) and Learmonth (2006). The ovaries were rinsed in water for 24 h and transferred to 70% ethanol. For each ovary the maximum length, height, width (mm) and weight (g) were recorded. Both ovaries were examined externally to record the presence of a *corpus luteum* (CL) of pregnancy and *corpora albicantia* (CA) of ovulation. Ovaries were hand sectioned into 0.5–2 mm slices and examined internally under binocular microscope for the presence of additional *corpora albicantia* and follicles. Females were normally considered sexually mature if the ovaries contained at least one *corpus luteum* or *albicans*. An overall pregnancy rate was derived for each species in each region based on animals sampled during the present study. We also compare these results to the best estimates of pregnancy rate available from wider sampling.

Pregnancy was established by the presence of an embryo/foetus. It is difficult to be certain whether a *corpus luteum* is associated with a pregnancy, e.g. one will be present even if the pregnancy was lost to an early miscarriage. In common dolphins there was good agreement in the final data set (animals for which POPs data were available) between presence of foetuses (11 instances) and of a *corpus luteum* (12 instances). In the final harbour porpoise data set there were only 6 females carrying foetuses and 11 with a *corpus luteum* and the latter variable was selected for use in analysis as it resulted in a less unbalanced data set.

2.5. Determination of fatty acid profiles

Fatty acid data were considered to be a more reliable indicator of average individual diet than stomach contents, since many stomachs were empty and food remains in the stomach normally represent a single meal whereas fatty acids in blubber represent dietary input integrated over a time-scale of weeks to months. The inner layer from each blubber sample, which is more metabolically active than the outer layer and contains higher levels of fatty acids derived primarily from the diet (Koopman et al., 1996), was analysed for fatty acids. Lipids were extracted from blubber samples (approximately 1 g) and homogenised whole fish samples (approximately 10 g) using the method of Bligh and Dyer (1959) as modified by Hanson and Olley (1963). Fatty acid methyl esters (FAMEs) were prepared by acid catalysis and analysed by gas chromatography with flame ionisation detection (GC–FID). Further details on the methods for determining fatty acid profiles are described in Learmonth (2006).

Individual fatty acids were identified using mass spectrometry and commercial standards. The normalised area percentage (NA%) was calculated for 31 fatty acids: 12:0, 14:0, 14:1n-5, 15:0, 16:0, 16:1n-7, 16:2n-6, 16:3n-6, 16:4n-3, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-6, 18:3n-3, 18:4n-3, 20:0, 20:1n-11, 20:1n-9, 20:2n-6, 20:4n-6, 20:3n-3, 20:4n-3, 20:5n-3, 22:0, 22:1n-11, 22:1n-9, 21:5n-3, 22:5n-3, 22:6n-3 and 24:1n-9.

Since it was impractical to continue analysis with 31 explanatory variables related to diet, fatty acid variation was summarised using PCA. For common dolphin data, the first two PCA axes explained 28.6% and 18.7% of variation, respectively, in fatty acid profiles. Axis 1 scores related most strongly to relative amounts of fatty acids 14:1n-5, 16:1n-7, 12:0, 22:6n-3 and 18:0 (in descending order of importance, all with absolute coefficient values greater than 0.25). Axis 2 scores related most strongly to relative amounts of fatty acids 16:4n-3, 20:5n-3, 16:3n-6, 18:3n-6, 16:2n-6 and 21:5n-3. For harbour porpoise data, the first two PCA axes explained 40.2% and 9.3% of variation, respectively, in fatty acid profiles. Axis 1 scores related most strongly to relative amounts of fatty acids 22:6n-3, 21:5n-3, 14:0 and 20:5n-3. Axis 2 scores related most strongly to relative amounts of fatty acids 22:1n-11, 20:1n-11, 18:2n-6, 18:3n-3, 16:0, 18:4n-3 and 18:1n-9.

2.6. Data analysis: description of patterns in the data set

For analysis of geographical variation, samples were grouped into five regions: Scotland, Ireland, Southern North Sea (Netherlands, Belgium and the French coast north of Calais), France (south of Calais, including the entire Biscay coast of France) and Galicia. Average POP concentrations, age and pregnancy rates were summarised by region for both species. Data on HBCD concentrations were available for 60 female common dolphins from Ireland, France and Galicia. For other POPs, sample size increased to 70 (see Table 2). Data on HBCD concentrations were available for 44 female harbour porpoises (mainly from Scotland, Ireland and the southern North Sea) while data on other POPs were available for 67 animals (see Table 3).

To minimise underestimation of pregnancy rate, only mature animals obtained during October to May were included in these estimates, since foetuses present during June to September may be missed during necropsy due to their small size. Since sample sizes are small, where available, literature values for pregnancy rate are also given.

To summarise relationships between POP concentrations in blubber and the set of potential explanatory factors we used redundancy analysis (RDA), as implemented in Brodgar 2.5.1 (www.brodgar.com). Common dolphin and harbour porpoise data were analysed separately. Sample sizes for HBCD concentrations were lower than for other POPs and further analysis of the HBCD data appears in Zegers et al. (2005). Therefore, the RDA excluded the HBCD data. The explanatory factors selected were: geographical location (region), season (quarter of year), size (body length), condition (blubber thickness), age, reproductive characters indicative of maturity (combined ovary weights,

Table 2
Results of redundancy analysis (RDA) on concentrations of POPs (excluding HBCDs) in blubber of female small cetaceans

Explanatory variable	*F*	*p*
(a) Common dolphins		
France	10.01	0.000
Fatty acid profile (PCA axis 1)	8.41	0.001
Number of *corpora albicantia*	5.21	0.007
Quarter 2	5.18	0.007
Fatty acid profile (PCA axis 2)	4.65	0.009
Length	3.67	0.022
Pregnancy	3.40	0.029
Ireland	2.23	0.090
Age	1.97	0.119
Hg:Se ratio in liver	1.95	0.122
Zinc concentration in liver	1.54	0.188
Cadmium concentration in kidney	1.08	0.305
Quarter 1	0.89	0.387
Mercury concentration in liver	0.33	0.825
Combined ovary weights	0.30	0.850
Quarter 3	0.12	0.971
(b) Harbour porpoises		
Zinc concentration in liver	4.79	0.009
Mercury concentration in liver	5.00	0.011
Quarter 3	2.92	0.034
Cadmium concentration in kidney	2.73	0.065
Netherlands/Belgium	2.38	0.065
Length	2.36	0.070
Ireland	2.38	0.072
Galicia	2.30	0.080
Mercury:selenium ratio in liver	2.21	0.091
Dorsal blubber thickness	1.32	0.233
Number of *corpora albicantia*	1.32	0.238
France	0.80	0.409
Fatty acid profile (PCA axis 2)	0.79	0.469
Quarter 1	0.78	0.476
Quarter 2	0.53	0.686
Fatty acid profile (PCA axis 1)	0.35	0.860
Combined ovary weights	0.19	0.970
Age	0.14	0.989
Presence of a *corpus luteum*	0.05	1.000

Values of F and associated probability (p) are tabulated. For nominal variables (season or quarter, region, pregnancy), one value is always excluded and used as a basis for comparison.

number of *corpora albicantia*), pregnancy (presence of foetus in common dolphins or a *corpus luteum* in porpoises, see above), diet (axis 1 and 2 scores from PCA on fatty acid concentrations in blubber) and trace element concentrations (Cd in kidney, Hg in liver, Zn in liver and the Hg:Se ratio in liver).

RDA requires that the number of explanatory variables is smaller than the number of samples. We had 67–70 samples and 16–18 explanatory variables, which is an acceptable ratio. RDA assumes that the underlying relationships between variables are generally linear, which was supported by initial data exploration. Significance testing in RDA is based on a permutation test and no assumption of normality is required and collinearity between explanatory variables is not an issue. The analysis makes no assumption that links found represent causal relationships; indeed some of these variables may vary as a consequence of variation in POP burdens rather than being causal factors. Between-species variation in POP concentrations in prey was also analysed using RDA.

2.7. Modelling individual variation in POP burdens and reproductive status

To answer specific questions about relationships between variables we used generalised additive models (GAMs). GAM is basically a smoothing

Table 3
Results of RDA on POPs in prey species

Explanatory variable	*F*	*p*
Netherlands	11.15	0.000
France	4.19	0.004
Fatty acid profile (PCA axis 1)	1.95	0.099
Spain	1.11	0.342
Clupeioid	1.12	0.310
Gadoid	1.136	0.325
Mackerel/scad	0.87	0.383
Cephalopod	0.86	0.466
Sandeel	0.84	0.434
Fatty acid profile (PCA axis 2)	0.43	0.771
Scotland	0.21	0.907
Myctophid	0.18	0.961

The analysis was based on species averages (so size variation within species is not taken into account), $n = 30$. Values of F and associated probability (p) are tabulated.

equivalent of generalised linear modelling (GLM) (see McCullagh and Nelder, 1989; Hastie and Tibshirani, 1990). Although data exploration suggested that the assumption of linearity was generally sound, this approach ensured that no non-linear effects were missed. If smoothing curves for effects of any explanatory variables were found to be approximately linear, a linear (parametric) term was used in place of the smoother and if all smoothing curves were approximately linear we used a GLM. The effects represented by smoothers and parametric terms are marginal effects, i.e. effects of the explanatory variables once effects of all other variables in the model have been taken into account.

In most of the models, the response variable was the (summed) concentration for a class of POPs. The data distributions for log-transformed POP concentrations in cetacean tissues were approximately normal, so a Gaussian distribution with identity link was applied. For models in which pregnancy was the response variable, a binomial distribution and logit link was used. In each case, forwards and backwards selection was applied to find the optimum models. Degrees of freedom for the smoothers were determined using a cross-validation procedure. Generally, the best model is that with the lowest value for the Akaike Information Criterion (AIC), in which all remaining explanatory variables have significant effects, and there are no obvious patterns in the residuals.

The specific questions asked were:

(1) Is there geographic variation in blubber POP (summed PCBs, summed PBDEs and HBCD) concentrations once we control for effects of sample composition, i.e. taking into account length, age, reproductive status and season? We used summed ovary weights as a proxy for reproductive status: ovary weights increase as animals mature and are highest in pregnant females. We also tested whether adding a condition indicator (dorsal blubber thickness) to the model improved the fit, on the basis that POP concentrations may increase when blubber reserves are mobilised. Missing values resulted in reduced available sample sizes for some of these analyses since GAM requires complete data for all variables (in RDA, missing values are replaced by averages). No blubber thickness data were available for Galician animals so sample size was reduced for all analyses using this variable.
(2) In common dolphins (but not porpoises), RDA analysis indicated a relationship between POP burdens and fatty acid profiles. We therefore fitted GAMs to quantify this relationship. Again we tested the effect of including blubber thickness as an additional explanatory variable to control for the possibility that, when blubber is mobilised, different fatty acids may be utilised at different rates. We also tested whether adding dietary data would improve the models developed to answer question 1.
(3) In harbour porpoises, RDA analysis suggested that POP concentrations were related to trace element concentrations. We therefore fitted GAMs to quantify these relationships.
(4) Are POP concentrations related to health status? Since full pathology data were not available for all animals, we compared POP concentrations

(using ANOVA) between broad cause of death categories, in particular distinguishing deaths due to disease or parasites (‘‘pathological’’ causes) from other known ‘‘non-pathological’’ causes (mainly trauma, including porpoises killed by bottlenose dolphins). Cause of death was unknown in many cases so we also tested whether liver Zn concentration could be used as a proxy for health status by comparing Zn concentrations for different cause of death categories (using Kruskal–Wallis tests). Lastly we used GAMs to quantify the relationships between POP concentrations and Zn concentrations.

(5) Do POP concentrations affect the incidence of pregnancy?

3. Results

3.1. Patterns of variation in concentrations of POPs

Explanatory variables related to diet, location, reproductive status and season all affect the overall pattern of variation in POP concentrations in common dolphins (Table 2a). French (but not Irish) animals differed from Galician animals and the significant effects of both fatty acid variables suggest that diet plays an important role in determining the POP profile. Overall, the set of explanatory variables used explained 53% of the overall variation in POP levels, with RDA axes 1 and 2 accounting for 35% and 8.3% of variation respectively. Such relatively ‘‘low’’ values are common in ecological field studies (Zuur et al., 2007). While caution is needed in interpretation, since the first two RDA axes explain only 43% of variation in POP concentrations, it can be seen from Fig. 2 that the variable ‘‘pregnancy’’ is related (negatively) to the concentrations of many of the CB congeners (as indicated by the approximately 180° angle on the plot between the vectors for the CB congeners and the position of the symbol for pregnancy). Similarly, the effect of combined ovary weight (and/or cadmium levels in the kidney, since they were highly correlated with each other) appears to relate most strongly (and negatively) to concentrations of CB congeners CB28 and CB49. It can also be seen that dietary variation (as proxied by the first PCA axis for the fatty acid profile) relates most strongly to CB28, CB49, HCB and PeCBz.

In porpoises, which were sampled mainly from Scotland, Ireland and the southern North Sea, RDA results indicated significant relationships with Hg and Zn concentrations in the liver and a weak seasonal effect (Table 2b). Overall, the set of explanatory variables entered into the RDA explained 42% of variation in POP concentrations between samples, with RDA axes 1 and 2 accounting for 23.8% and 9% of variation respectively. The biplot (Fig. 3) shows that Zn concentration in liver is strongly positively correlated with the variable ‘‘southern North Sea’’, as well as with scores on the first PCA axis derived from fatty acid profiles, and negatively correlated with dorsal blubber thickness, Cd concentration in kidney and the variables ‘‘Ireland’’ and ‘‘Galicia’’. Thus, although the effects of the individual location variables were not statistically significant, it appears that there are important geographical trends in the data. Zinc concentrations in liver are also highly correlated with concentrations of CB49, CB101, CB118, BDE99 and DDE in the blubber.

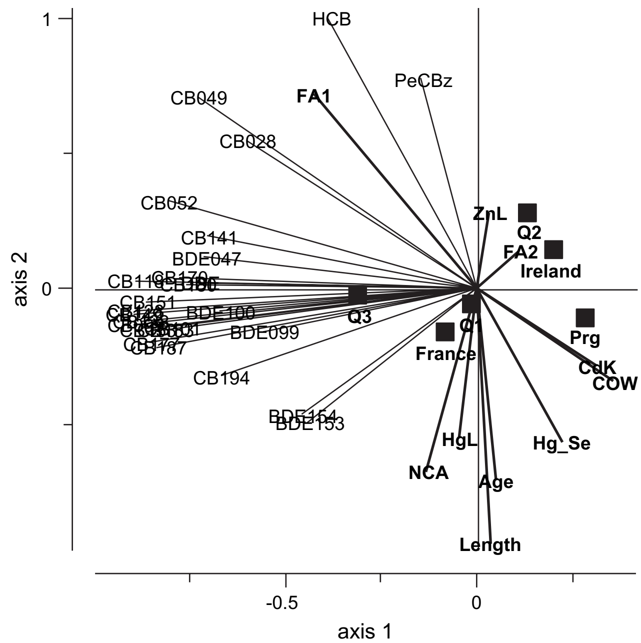

Fig. 2. Results of redundancy analysis (RDA) on persistent organic pollutant (POP) concentrations (excluding hexabromocyclododecane, HBCD) in blubber of female common dolphins: bi-plot of explanatory and response variables. CdK, cadmium concentration in kidney; COW, combined ovary weight; FA1, FA2, scores on 1st and 2nd PCA axes in an ordination of fatty acid data; HgL, mercury concentration in liver; Hg_Se, ratio of mercury to selenium concentrations in liver; NCA, total number of *corpora albicantia*; Prg, pregnancy; Q2, Q3, Q4, 2nd, 3rd and 4th quarters of the year (as compared to quarter 1); ZnL, zinc concentration in liver. Results for France and Ireland are expressed in relation to those from Galicia.

Variation in POP profiles of prey species was significantly related to geographic location but not to taxonomic groupings (see Table 3). Overall, 57% of variation in POP concentrations was explained by the set of explanatory variables. Particularly high levels of CB49 and CB101 were recorded in southern North Sea prey samples (23 and 168 $\mu g\ g^{-1}$ respectively in whiting, and 39 and 156 $\mu g\ g^{-1}$ in gobies, as compared to averages of 3 and 28 $\mu g\ g^{-1}$ respectively across all prey taxa from other areas).

3.2. Regional variation in POP concentrations

Average summed PCB concentrations in common dolphin blubber were highest in the French sample and lowest in Ireland. The threshold summed PCB concentration at which effects on cetacean reproduction would be expected (which, given the high correlation between summed concentrations of the ICES7 PCBs and all 18 PCBs recorded here, is equivalent to a [Σ18PCB] of 9.4 $\mu g\ g^{-1}$ lipid) was frequently exceeded in both French and Galician common dolphins. Concentrations of PBDEs were rather similar across all countries while HBCD concentrations were higher in Ireland than elsewhere (Table 4a). GAM results for PCBs indicated that both French and Galician dolphins had significantly higher PCB concentrations in their blubber than did Irish animals, there was no region effect on PBDE concentrations and

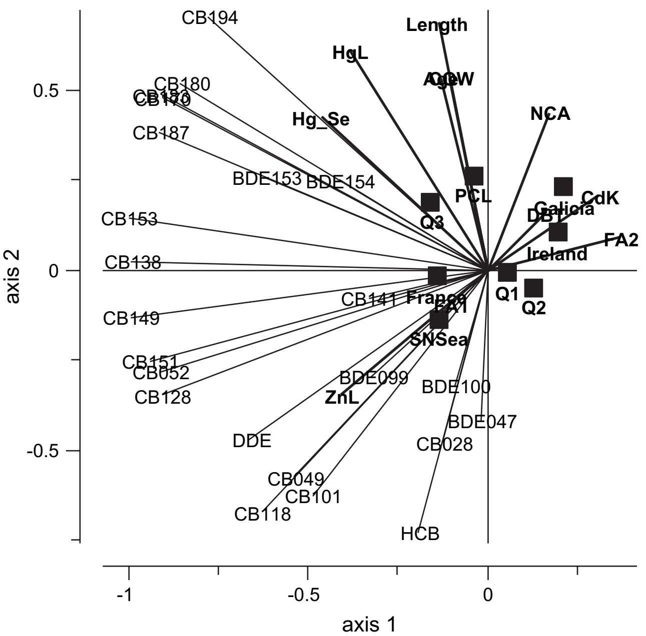

Fig. 3. Results of RDA on POP concentrations (excluding HBCD) in blubber of female harbour porpoises: bi-plot of explanatory and response variables. Labels are as in Fig. 2 except: DBT, dorsal blubber thickness. Results for Southern North Sea (SN Sea), France and Galicia are expressed in relation to those for Scotland.

HBCD concentration were significantly lower in Galicia than Ireland (Tables 4a and 5a). Effects of season, age and length were not significant in any of the models. Adding blubber thickness (thereby including a condition effect but excluding Galician data) did not improve the models. However, all three models included a significant and generally negative effect of ‘‘maturation’’ (lower POP concentrations at higher ovary weights, see Fig. 4a,b). The smoother in Fig. 4a shows a markedly negative effect of ovary weight on blubber PCB concentration for combined ovary weights over 15 g. Most pregnant dolphins had combined ovary weights over 15 g while the highest combined ovary weight for a non-pregnant animal was around 14 g.

Although the French sample of common dolphins had the highest pregnancy rate, the sample size for the other areas was small, and a pregnancy rate for Irish animals calculated using a larger data set (see Murphy, 2004) was similar to that obtained for the French animals. In all regions except Galicia, the proportion of animals that had died due to disease, among those for which cause of death was diagnosed, was very low. In Galicia, although there was a high proportion of undiagnosed deaths (over 60%), where cause of death was diagnosed almost 50% of the animals had died due to disease or parasite infection (see Table 4a).

In female harbour porpoises, average summed PCB concentrations were highest in samples from the southern North Sea. PCB concentrations exceeded the threshold for effects on reproduction in almost three-quarters of the southern North Sea sample and over one-third of the Scottish sample. PBDE concentrations were higher in porpoises from Scotland than in those from Ireland and Galicia. HBCD concentrations were highest in the samples from Ireland and Scotland, particularly animals from the coast of the Irish Sea (Table 4b). GAM results confirmed that there were significant between-region differences in concentrations of all three categories of POP in porpoise blubber. PCB levels were significantly higher in southern North Sea samples than in Scottish samples. PBDE levels were lower in both Irish and Galician samples than in Scotland, while HBCD concentrations were lower in Galicia than in Scotland (Tables 4b and 5b). Note however that the Galician sample was very small (3 animals). Effects of maturation, length, age and season were all non-significant. When dorsal blubber thickness was added to these models, in all cases its effect was non-significant.

Pregnancy rate data for porpoises arising directly from the present study were limited. Only one of seven mature females from the southern North Sea was pregnant (Table 4b). Based on larger sample sets (Learmonth, 2006; Addink et al., unpublished data), the pregnancy rate for the southern North Sea during 1988–1995 (0.59) is higher than that (0.42) recorded for Scotland during 1992–2004. A relatively high proportion of diagnosed deaths was due to disease or parasite infection in all areas except Ireland (0.05), with the highest proportion in the southern North Sea (0.67) (Table 4).

Concentrations of POPs in the two cetacean species can be compared only in Ireland, where sufficient common dolphin and harbour porpoise strandings occurred to provide an adequate sample size. The average PCB and HBCD concentrations in harbour porpoises were higher than those common dolphins.

3.3. POP concentrations and diet in common dolphins

GAMs for POP concentrations in common dolphin blubber in relation to fatty acid profiles (PCA scores for axes 1 and 2, i.e. FA1, FA2) explained between 10% (for PBDEs) and 22% (for PCBs) of variation (see Table 6). None of these models was significantly improved by including dorsal blubber thickness.

Adding dietary (fatty acid profiles, FA1) data to the GAMs for between-region differences in POPs in common dolphin blubber resulted in improved model fits, with a positive effect of age also remaining in the new final models (Table 6). Regional differences and effects of combined ovary weight were similar to those found previously. Adding blubber thickness as an additional explanatory variable did not improve these models.

3.4. POPs, Hg and Cd concentrations in harbour porpoises

Prior to fitting GAMs for effects of metals on POP concentrations in porpoises, the Cd and Hg values were square root transformed to reduce the influence of the relatively few very high values. The final model for summed PCB concentrations explained 46% of deviance ($n = 39$) and included a negative linear effect of Cd concentration ($p = 0.0008$) and positive effect of the Hg:Se ratio ($\mathrm{Df} = 3.8$, $p = 0.0269$; although

Table 4
Regional summaries of blubber POP concentrations female small cetaceans

Parameter	Scotland	Ireland	Southern North Sea	France	Galicia	All regions
(a) Common dolphins						
Σ18[PCBs], ng/g lipid	–	3649 (3394, 11)	–	13692 (12721, 36)	10955 (11563, 23)	11215 (11779, 70)
GAM coefficient for PCBs	–	Reference	–	0.651	0.644	–
3 × Σ[ICES7 PCBs]	–	6919 (6404, 11)	–	24644 (22938, 36)	19875 (20797, 23)	20292 (21194, 70)
Proportion above critical	–	0.09 (11)	–	0.50 (36)	0.39 (23)	0.40 (70)
Σ5[PBDEs], ng/g lipid	–	758 (505, 11)	–	612 (413, 36)	422 (182, 23)	573 (384, 70)
GAM coefficient for PBDEs		Reference	–	nsd	nsd	–
[HBCD], ng/g lipid	–	1086 (1137, 7)	–	433 (211, 31)	185 (101, 23)	415 (478, 61)
GAM coefficient for HBCD		Reference	–	nsd	−0.485	–
Age, years	8.40 (8.38, 5)	9.03 (8.45, 27)	–	11.31 (6.24, 95)	6.43 (5.27, 51)	9.48 (6.72, 178)
Disease/parasite deaths	0.08 (13)	0 (42)	–	0 (252)	0.49 (51)	0.07 (358)
Pregnancy rate	–	0.14 (7)	–	0.30 (66)	0.06 (16)	0.25 (91)
Pregnancy rate (literature)		0.282[a]	–			
(b) Harbour porpoises						
Σ16[PCBs], ng/g lipid	10525 (13152, 31)	5347 (4750, 12)	15021 (8574, 19)	13809 (10582, 2)	5306 (4199, 3)	10737 (10811, 67)
GAM coefficient for PCBs	Reference	nsd	0.287	nsd	nsd	–
3 × Σ[ICES7 PCBs]	20320 (25243, 31)	10492 (9451, 12)	30598 (17994, 19)	27600 (20872, 2)	10266 (7972, 3)	21242 (21277, 67)
Proportion above critical	0.39 (31)	0.25 (12)	0.74 (19)	0.50 (2)	0.33 (3)	0.46 (67)
Σ5[PBDEs], ng/g lipid	1369 (1352, 31)	656 (492, 12)	1056 (803, 19)	1398 (939, 2)	284 (44, 3)	1105 (1079, 67)
GAM coefficient for PBDEs	Reference	−0.289	nsd	nsd	−0.549	–
[HBCD], ng/g lipid	2236 (2562, 20)	2961 (2716, 7)	1080 (354, 12)	1533 (1101, 2)	121 (37, 3)	1860 (2154, 44)
GAM coefficient for HBCD	Reference	nsd	nsd	–	−1.127	
Age, years	2.92 (2.79, 56)	3.36 (3.32, 21)	4.97 (3.92, 15)	6.61 (8.10, 7)	0.92 (0.20, 6)	3.44 (3.69, 105)
Disease/parasite deaths	0.54 (105)	0.05 (22)	0.67 (21)	0.32 (19)	0.25 (4)	0.46 (171)
Pregnancy rate	0.5 (10)	0.4 (5)	0.14 (7)	–	–	0.42 (26)
Pregnancy rate (literature)	0.42[b]		0.59[c]			

Values tabulated are arithmetic means, with standard deviations (where available) and sample sizes in parentheses. Also given are "region" coefficients for the GAM models (see Table 5), indicating differences in POP concentrations relative to samples from the reference region, taking account of the effects of reproductive status. Positive coefficient values indicate significantly higher levels, negative values indicate significantly lower levels and "nsd" indicates no significant difference. "Southern North Sea" data for porpoises include data from the Netherlands, Belgium and northern France, the latter data therefore being excluded from the "France" category. The table also shows average age, pregnancy rate and the proportion of (known cause) deaths recorded that were due to disease or parasites.

[a] Based on 37 sexually mature females, 1991–2004, see Murphy (2004).

[b] Based on 33 mature females, 1992–2004 (Learmonth, 2006).

[c] Based on 27 mature females, 1988–1995 (M. Addink, T.B. Sørensen, M. García Hartmann, H. Kremer, unpublished data).

non-linear, the smoother was monotonic). No satisfactory model could be fitted to the PBDE or HBCD data. The model for PCBs in porpoises in relation to metal concentrations was not improved by adding age and/or ovary weights as additional explanatory variables.

3.5. POP concentrations, cause of death and liver Zn concentrations

In common dolphins, summed PCB and summed PBDE concentrations did not differ significantly between pathological and non-pathological cause of death categories. HBCD concentrations were significantly higher in dolphins that had died of non-pathological causes (ANOVA, $p = 0.0049$). In contrast, in porpoises, PCB ($p = 0.0007$), PBDE ($p = 0.016$) and HBCD ($p = 0.0083$) concentrations were all significantly higher in animals that had died of pathological causes.

Based on the entire set of samples collected during the project, liver Zn concentrations and cause of death data were available for 172 common dolphins and 73 porpoises. In common dolphins, only 12 animals had died from pathological causes and there was no significant difference in liver Zn concentrations between these animals and those in the 160 animals that died from other known causes (Kruskal–Wallis test, $H = 1.14$, $p = 0.285$). In contrast, 34 porpoises had died from pathological causes and on average these had significantly higher liver Zn concentrations than animals that died from other known causes (Kruskal–Wallis test, $H = 13.12$, $p < 0.001$).

In common dolphins, POP concentrations (PCBs, PBDEs or HBCD) were unrelated to Zn concentration in liver (no satisfactory GAM or GLM could be fitted). In harbour porpoises, PCB concentrations were weakly positively related to liver Zn concentration ($p = 0.0428$) but there was no significant relationship between PBDE or HBCD concentrations and Zn concentration.

Table 5
GAM results for regional patterns in POP concentrations in blubber of female small cetaceans

Response	n	%dev	AIC	Region	COW
(a) Common dolphin (reference region: Ireland)					
Σ18[PCB]	58	42.9	64.4	France: +, $p < 0.0001$ Galicia: +, $p = 0.0002$	Df = 4.2, $p = 0.0169$
Σ5[PBDE]	58	11.4	32.2		−, $p = 0.0096$
HBCDs	50	50.5	−3.0	Galicia: −, $p < 0.0001$	Df = 1.6, $p = 0.0372$
(b) Harbour porpoise (reference region: Scotland)					
Σ16[PCB]	66	17.8	74.0	SN Sea: +, $p = 0.0164$	
Σ5[PBDE]	66	19.6	40.4	Ireland: −, $p = 0.0081$ Galicia: −, $p = 0.0049$	
HBCDs	43	44.5	37.2	Galicia: −, $p < 0.0001$	

The original set of explanatory variables was: region, maturation/reproductive status (proxied by combined ovary weight, COW), age, length and season. Model summaries contain the following information: sample size (n), %deviation explained (%dev), Akaike Information Criterion (AIC) value, and effects of region and COW, with associated probabilities (p). Degrees of freedom (Df) are indicated for smoothers and the direction of the effect is indicated for categorical and linear terms. For region effects, the direction of the effect is expressed relative to a reference region. Only the significant regional differences are reported.

3.6. Pregnancy and POP burdens

Data on pregnancy was available for 102 mature female common dolphins over the study period, of which 29 were pregnant. Only four of these 102 animals had died from ‘‘pathological’’ causes, none of which were pregnant, while 80 had died from ‘‘non-pathological causes’’, of which 25 were pregnant. Thus we cannot test for an association between pregnancy and cause of death category. In harbour porpoises, data on pregnancy were available for 37 mature females, 14 of which were pregnant. Of nine mature females that had died from ‘‘non-pathological’’ causes, five were pregnant while of 19 that had died from ‘‘pathological’’ causes, 5 were pregnant. Although this suggests that there is an association between pregnancy and cause of death category, the association is not significant ($\chi^2 = 2.274$, Df = 1, $p = 0.132$).

In those common dolphins for which POPs data were available, a binomial GLM indicated that the incidence of pregnancy was positively related to age ($p = 0.013$, N = 64, deviance explained = 13.7%). This model was improved by adding a linear effect of summed PCB concentrations (deviance explained = 29.7%). In this model, age has a positive effect ($p = 0.0076$), and summed PCB concentration has a weak negative effect ($p = 0.0289$), on the incidence of pregnancy. Using summed PBDE concentrations as an explanatory variable, instead of summed PCBs, age dropped out of the final model. The fitted effect of PBDEs on the incidence of pregnancy was linear and weakly negative ($p = 0.0330$). This model explained only 11.5% of deviance but was not improved by adding PCB concentrations as an additional explanatory variable. No satisfactory model of the incidence of pregnancy could be obtained using HBCD concentration as an explanatory variable.

Only six of the sampled porpoises for which POPs data were available were definitely pregnant and, as might be expected given the imbalance between the numbers of pregnant and non-pregnant models, no satisfactory binomial GAMs using age and POP concentrations could be fitted. The analysis was repeated using incidence of a *corpus luteum* as the response variable but again no satisfactory model based on age and POP concentrations could be fitted.

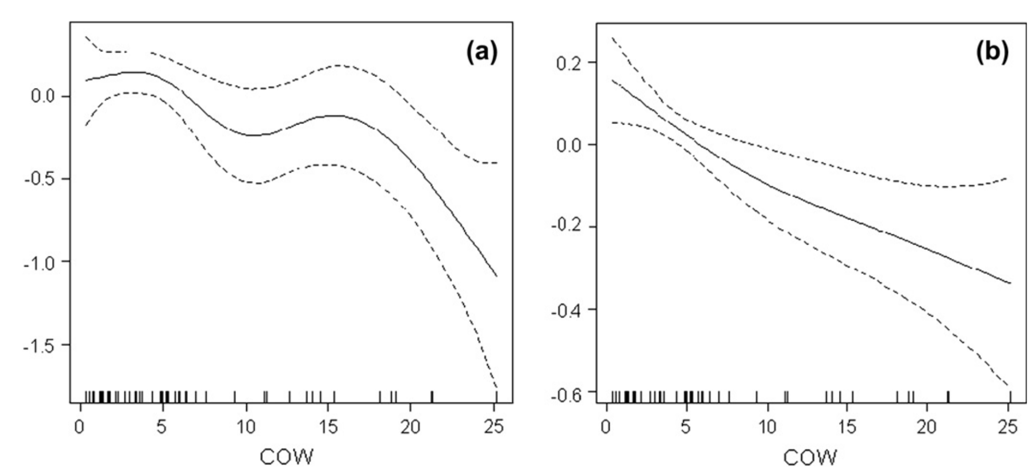

Fig. 4. Illustration of generalised additive model (GAM) results for analysis of POP concentrations in common dolphin and porpoise blubber in relation to country, season, age, length, maturity and condition: (a) smoother for partial effect of combined ovary weight (COW) on summed polychlorinated biphenyl (PCB) concentrations in common dolphin blubber, (b) smoother for partial effect of combined ovary weight on HBCD concentration in common dolphin blubber.

Table 6
GAM results for dietary patterns in POP concentrations in blubber of female common dolphins

Compounds	n	%dev	AIC	Region	FA1	FA2	Age	COW
(a) Models using fatty acid data only								
Σ18[PCB]	67	21.7	86.3		Df = 3.8, $p = 0.0137$			
5[PBDE]	67	9.9	40.0		+, $p = 0.0096$			
HBCDs	58	14.8	38.6			+, $p = 0.0029$		
(b) Models also including effects of region, length, age and maturation (reference region: Ireland)								
Response	N	%dev	AIC		FA1			
Σ18[PCB]	54	56.7	47.4	France: +, $p < 0.0001$ Galicia: +, $p = 0.0023$	Df = 3.8, $p = 0.0050$		+, $p = 0.0348$	Df = 1.8, $p = 0.0318$
Σ5[PBDE]	54	38.5	19.0		Df = 1.8, $p = 0.0130$		+, $p = 0.0206$	−, $p = 0.0132$
HBCDs	47	78.8	−22.2	Galicia: −, $p < 0.0001$	Df = 2.2, $p = 0.0094$		Df = 7.6, $p = 0.0121$	−, $p = 0.0104$

The explanatory variables were the 1st and 2nd axis scores from a PCA on fatty acid data (FA1, FA2). In addition, the table presents the final models from Table 5 revised to include dietary information. Model summaries contain the following information: sample size (n), %deviation explained (%dev), Akaike Information Criterion (AIC) value, and effects of explanatory variables, with associated probabilities (p). Degrees of freedom (Df) are indicated for smoothers and the direction of the effect is indicated for categorical and linear terms. For region effects, the direction of the effect is expressed relative to a reference region. Only the significant regional differences are reported.

4. Discussion

The focus of this study was on sub-lethal effects of POP bioaccumulation: most of the sampled animals almost certainly died of other causes, although secondary effects cannot be ruled out. In particular, we were interested in possible effects on reproduction.

A Σ-PCB level of 17 μg g^{-1} lipid in liver has been reported as a threshold level for effects on reproduction in aquatic mammals (Kannan et al., 2000). Since this value was based on comparison with the main peaks in the commercial PCB mixture Aroclor 1254, this level cannot be directly compared with our Σ_{18}-PCB (or Σ_{16}-PCB) levels. Following Jepson et al. (2005), we derived the summed concentration of the ICES7 CBs in each sampled animal. Multiplying this figure by three gives a figure that is equivalent to the Aroclor 1254 value reported by Kannan et al. (2000). On this basis, the threshold was frequently exceeded in both porpoises (47% of individuals) and common dolphins (40%) in the present study, especially porpoises from the southern North Sea (74%) and common dolphins inhabiting waters off the French coast (50%). The threshold was least frequently exceeded in the cetaceans from off Ireland (9% of common dolphins, 25% of porpoises). The highest average PCB levels were recorded in porpoises from the southern North Sea. Some caution is however needed in applying this threshold to cetaceans, since the published experimental data all derive from mammals of the order Carnivora (mink, otters and seals). Another issue is the extent to which the sampled animals were representative of the population. Thus, there was a higher proportion of animals that had died due to disease or parasitic infection among the sampled porpoises than among the common dolphins sampled and it is difficult to know whether this reflects the condition of animals in the extant populations.

The present study generated insufficient data to compare pregnancy rates between regions within the study area. However, in common dolphins, the high PCB concentrations recorded in the French sample were associated with a pregnancy rate (0.30) that was slightly higher than the value for Ireland (0.28) reported by Murphy (2004). These figures and the overall pregnancy rate for common dolphins in this study (0.25) are consistent with recently published results for this species in the western North Atlantic, in which annual pregnancy rate was estimated to be between 25% and 33% (Westgate and Read, 2007).

In common dolphins, the incidence of pregnancy was negatively related to the concentrations of PCBs and PBDEs in blubber. These relationships do not conclusively demonstrate that high POP concentrations inhibit pregnancy since, for example, infertility may allow high levels of POPs to bioaccumulate. Female cetaceans are normally able to offload some of their POP burden to their offspring during pregnancy and lactation. In harbour porpoises, the sample included few pregnant animals and a larger sample size would be needed to detect a link between POP concentrations and pregnancy.

Although it appears that the overall pregnancy rate in porpoises from the southern North Sea in the present study was unusually low (0.14), the sample size for mature females in this area was very low ($n = 7$). Previous data from porpoises in the Netherlands (1988–1995; M. Addink, T.B. Sørensen, M. García Hartmann, H. Kremer, unpublished data) gave a pregnancy rate of 0.59. Estimated pregnancy rates for Scotland and Ireland from the present study were higher (0.4–0.5) than for the Netherlands but again based on very small sample sizes. Nevertheless, they are consistent with a larger data set for Scotland, based on data from 1991 onwards, for which the pregnancy rate was 0.42 (Learmonth, 2006). The only other published pregnancy rate data available for the southern North Sea is from Danish waters during 1985–1991, where a pregnancy rate of 73% was estimated using the presence of a foetus (Sørensen and Kinze, 1994). Data from the western Atlantic suggest that the latter rate (or higher) may be more typical of porpoises: in the Bay of Fundy (1985–1988, $n = 75$) and Gulf of Maine (1989–1993, $n = 14$) the pregnancy rates were 0.74 and 0.93, respectively (Read, 1990; Read and Hohn, 1995). In Iceland, Ólafsdóttir et al. (2003) estimated the pregnancy rate to be 97% from a sample of by-caught porpoises. It should be noted though that the estimated pregnancy rate for Danish animals could have changed since 1991, and Sorensen and Kinze (1994) analysed samples obtained from all Danish waters, possibly thus including animals from more than one population. Andersen et al. (2001) identified two separate populations (or sub-populations) in Danish waters, based on microsatellite analysis, in the Danish North Sea and in inner Danish waters. Genetic analysis suggests that harbour porpoises from Dutch waters are a mixture of individuals of diverse origin, including a large proportion of migrants from British and Danish waters (Walton, 1997; Anderson et al., 2001). In recent years, there has been a significant increase in the number of harbour porpoises sighted in Dutch waters, which has been attributed to a possible redistribution of harbour porpoises in the North Sea (Camphuysen, 2004; also supported by unpublished results from the SCANS II survey), accompanied by an increase in strandings on the Dutch coast (M.J. Addink, C. Smeenk, E.J.O. Kompanje, unpublished data). Given this evidence of mixing and movements of porpoises, the overall pregnancy rate for porpoises in the North Sea may be more meaningful than figures for smaller areas.

Thus, recent studies mainly suggest that the pregnancy rate in North Sea porpoises is lower than in the western Atlantic or Iceland waters and, coupled with evidence of high PCB levels, this is cause for concern. However, estimates of pregnancy rate are subject to sampling bias (e.g. estimates based on by-caught animals may be higher than those based on strandings) and other biological factors (e.g. nutritional status, population structure) may account for differences in pregnancy rates. Therefore, further investigation of porpoise pregnancy rates in the North Sea and adjacent areas is needed.

Since ingested food represents the only significant post-weaning source of POPs in marine mammal tissues, we would expect to find that POP concentrations vary in relation to diet. However, the initial POP profile of an individual at weaning, accumulated via the placenta and during lactation, presumably reflects the mother's feeding history. POP concentrations in common dolphin blubber were strongly related to the blubber fatty acid profile, which is likely to indicate dependence on diet choice. However, in harbour porpoises there was only weak evidence that diet affects HBCD concentrations and no evidence that it affects PCB and PBDE concentrations.

In the present study we sampled the inner blubber layer to measure fatty acid concentrations since this is the most metabolically active layer and its composition is likely to reflect food intake, as demonstrated for pinnipeds (e.g. Iverson et al., 2004). However, in starving porpoises, thoracic blubber thickness may be reduced by as much as 50%, with lipids being withdrawn mainly from the inner layers (Koopman et al., 2002). It is possible that fatty acids from the inner blubber of porpoises are utilized selectively when blubber reserves are mobilised, so that the dietary signal in the blubber fatty acid profile is confounded.

So called quantitative fatty acid signature analysis (QFASA) has been used to relate fatty acid profiles in predators to diet composition, based on knowledge of the fatty acid profiles of putative prey species (Iverson et al., 2004; Learmonth, 2006). At present it is not possible to use blubber fatty acid profiles to identify the prey species eaten by individual cetaceans. In marine mammals, fatty acids are not deposited in the blubber in proportion to their occurrence in the diet. Although "correction factors" have been derived for some pinnipeds, allowing QFASA to be applied to determine diet (Iverson et al., 2004), no such correction factors are presently available for cetaceans. Our results for porpoises suggest, furthermore, that blubber fatty acid profiles may not be a good indicator of diet when animals are in poor condition. At least it will be necessary to control for variation in condition.

The RDA results suggested that patterns of variation in certain POPs were more strongly related to diet than others. Thus variation in concentrations of CB28, CB49 and HCB was most closely related to dietary variation in common dolphins. The limited survey of prey species carried out in the present study suggested that regional variation in POP profiles outweighed taxonomic variation. However, further more extensive surveys of POP concentrations in putative prey species are needed to quantify variation in POP profiles.

In harbour porpoises, body size and geographical location were the main factors explaining variation in POP concentrations. This general pattern was supported by both RDA (which detects linear effects of explanatory variables on the suite of response variables) and GAM (which allows only one response variable but permits detection of non-linear effects of explanatory variables). Our survey of POP concentrations in prey tissues showed that prey samples from the southern North Sea had high POP concentrations, particularly for CB congeners CB49 and CB101.

This study focused on concentrations of various POPs in the blubber of two species of small cetaceans. Although there have been many previous surveys, the present study was the first to both cover a large proportion of the European Atlantic coast and to evaluate the explanatory variables underlying the observed pattern of variation. In general it is not possible to collect the necessary ancillary data from studies on living animals (except where the history of individual animals in a population is known, c.f. Wells et al., 2005) and the cost of a large-scale, biopsy-based, survey of POP concentrations in European small cetaceans would have been prohibitive. By surveying stranded (and by-caught) animals, all the required data can be collected in a cost-effective and non-invasive manner, while minimising errors due to decomposition prior to sampling by selecting only the freshest carcasses. Sampling biases can to some extent be controlled for in subsequent analysis.

One difficulty when faced with a large set of putative explanatory variables, not all of which are independent, is teasing out effects of each, especially as different variables provide different levels/kinds of explanation. For example, one set of analyses described above suggests that POP concentrations in blubber are strongly related to mercury concentrations in liver and cadmium concentrations in kidney. It is unlikely that this represents cause and effect since, for example, metal concentrations are also related to age and maturity (Lahaye et al., 2005, 2007). Cadmium levels were higher in common dolphins than in porpoises, perhaps related to feeding in offshore waters and/or the presence of oceanic squids (which are known to accumulate large amounts of cadmium) in their diet (Bustamante et al., 1998; Lahaye et al., 2005).

In porpoises, the highest blubber PCB concentrations were recorded from animals sampled on southern North Sea coasts. Results from the present study, as well as published sources and other recent unpublished data (M. Garcia-Hartmann, T. Jauniaux, unpublished data) indicate that a high proportion of porpoises stranded on the coasts of the Netherlands and Belgium suffered from potentially fatal diseases. Pneumonia accounted for a greater percentage (49%) of deaths of stranded harbour porpoise on the Belgian and northern French coasts (1990–2000) (Jauniaux et al., 2002), compared to the Scottish coast (1992–2004), where pneumonia accounted for 11% of known deaths (Learmonth, 2006) and in England and Wales (1991–2002), where 15% of harbour porpoise deaths for which cause was established were attributed to pneumonia (Jepson, 2003). In addition, severe emaciation was the most common condition found in 33 of 55 harbour porpoises examined from Belgian and northern French coasts (Jauniaux et al., 2002).

In general, high concentrations of PCBs are thought to increase susceptibility to disease (e.g. in porpoises, Jepson et al., 2005) and may also be associated with higher parasite burdens (Bull et al., 2006). Only a small proportion of sampled common dolphins had died from pathological causes and no association was found between PCB concentrations and cause of death. Ideally the analysis should be repeated once a larger sample of animals that died from pathological causes is available. In contrast, almost half the porpoises for which cause of death was determined had died from pathological causes and these animals had significantly higher concentrations of all classes of POPs than animals dying from other causes. They also had higher Zn concentrations in their liver, which may be indicative of poor health (Das et al., 2004). Indeed, it is well established that infection is associated with Zn redistribution in humans, and, in particular, that high concentrations in liver rise as a result of acute-phase protein synthesis (Scott, 1985; Hambridge et al., 1986; Amdur et al., 1991). While there was apparently a strong relationship between the overall POP profile and Zn concentration in porpoises, relationships with summed concentrations for individual POP classes were weak. Further study is thus needed to determine which POPs might be linked to effects on health.

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