Agenda Item 6.1

Project Funding through ASCOBANS
Progress of Supported Projects

Document 6-05

Project Report: Effects of Contaminants on Reproduction in Small Cetaceans

Action Requested

- Take note of the report
- Comment

Submitted by

Secretariat

NOTE:
IN THE INTERESTS OF ECONOMY, DELEGATES ARE KINDLY REMINDED TO BRING THEIR OWN COPIES OF DOCUMENTS TO THE MEETING

Secretariat's Note

This report was previously attached as Annex 1 to AC17/Doc.6-01 rev.1. It has now been published separately in order to enable easy access and printing.

Contract Report

EFFECTS OF CONTAMINANTS ON REPRODUCTION IN SMALL CETACEANS

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Final Report of Phase One to ASCOBANS
Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas

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INTRODUCTION

Organochlorine compounds (OCs), such as polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichloroethane (DDT), accumulate in the blubber of marine mammals, and a large number of these lipophilic substances are known to be hormone or endocrine disrupting chemicals (EDCs). Endocrine functions can be altered by interference with the synthesis, secretion, transport, binding, action, or elimination of the endogenous natural hormones. The production of PCBs and DDTs has been limited or completely banned since 1970s in most developed countries. However, organochlorine compounds including PCBs are still being released into the environment by (1) use, disposal or accidental release from previously produced material, (2) volatilization of previously released material, and (3) creation of PCBs and dioxins during combustion processes (Breivik et al. 2002; Katami et al. 2002; Toft et al. 2004). Furthermore, some developing countries are still using DDT as vector control (Toft et al. 2004).

Reproductive effects linked with exposure to PCBs and associated DDT-like compounds include decreased fecundity, implantation failure and sterility (caused by uterine stenosis, occlusions and leiomyomas) in seals (Helle 1976; Helle et al. 1976; Reijnders 1986; Olsson et al. 1994; Reijnders 1999; Bredhult et al. 2008); premature pupping in sea lions (DeLong et al. 1973); and also severe reproductive dysfunction through the development of cancer and possibly hermaphroditism in beluga whales (*Delphinapterus leucas*) (Martineau et al. 1987; De Guise et al. 1994; Reijnders 1999). However, the findings of these studies, although strongly suggestive, have not been conclusive as the etiology of the observed disorder has usually been uncertain (Reijnders 2003). OCs have also been reported to increase susceptibility to infection (Jepson et al. 2005; Hall et al. 2006a), which may have consequences not only on adult survival but also on uterine and placental health and, subsequently, foetal health and survival (Hohn et al. 2007).

Uptake of OCs in marine mammals occurs predominately through prey consumption. Contaminants are reported to both biomagnify and bioaccumulate, as their concentration increases from one trophic level to the next, within the food chain. The high tissue concentrations of persistent organic pollutants (POPs) reported in some species such as killer whales (*Orcinus orca*) (Hickie et al. 2007) are a consequence of these animals' high trophic level and lipid-rich blubber that acts as a reservoir for lipophilic chemicals, leading to retention and accumulation of contaminants over time. Ylitalo et al. (2001) suggested that higher contaminant concentrations found in transient killer whales compared to residents could be attributed to dietary differences between the two ecotypes, i.e. transient killer whales feed on marine mammals with elevated POP levels, while resident animals are primarily piscivorous.

Reproductive failure in female harbour seals (*Phoca vitulina*) has been connected to feeding on contaminated fish. Average pup production per female harbour seal in the Dutch Wadden Sea population declined by approximately 30%, and toxicology studies revealed that, of all the organochlorines analysed, PCB levels were significantly higher (by 5 to 7 times) in the Dutch Wadden Sea population compared to other contiguous populations (Reijnders 1980). Experimental studies revealed that 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 μg g^{-1} lipid (Reijnders 1986). Hormone profiles of non-pregnant animals fed fish from the Wadden Sea indicated that the effects occurred at the stage of implantation, whereas the follicular, luteal and post-implantation phases were not affected. On the whole, oestradiol-17β levels in seals fed with fish of a higher contaminant burden were lower than those of the control group. Lower levels of oestradiol could have impaired endometrial receptivity and prevented successful implantation of the blastocyst (Reijnders 2003).

A morbillivirus epizootic caused a mass die-off of more than 1000 striped dolphins in the Mediterranean Sea between 1990 and 1992. It was viewed that PCBs and other organochlorine pollutants with potential for immunosuppressive effects may have triggered the mass die off event, or enhanced its spread and lethality (Aguilar and Borrell 1994). In additional to a high number of abortions during the epizootic, unusual luteinized cysts, with the potential to impede ovulation, were reported on the ovaries; these cysts were associated with high levels of PCB exposure (Munson et al. 1998). Luteinized cysts occur when ovulation is impeded, and it has been suggested they were caused by the effects of, PCBs or morbillivirus on hypothalamic/pituitary function or, PCBs on ovarian responsiveness (Munson et al. 1998). It has been suggested that the occurrence of the cysts and the reproductive impairment induced by PCBs may be depressing reproductive rates in the population and inhibiting recovery, along with decreased food availability caused by overexploitation by fisheries (Reeves and Notarbartolo di Sciara 2006).

Not only does an individual's contaminant burden reflect its dietary preferences, it is influenced by its body size, body condition, nutritive condition, disease, metabolism, excretion, age and sex (Aguilar et al. 1999; Pierce et al. 2008). Furthermore, it is an indication of the conditions it experienced in early life: contaminant levels in its mother, the duration of nursing, birth order and the length of the calving interval preceding its birth (Hickie et al. 2007). Females, through mobilization of lipid-associated toxins from the blubber during periods of high energy requirements, transfer toxic compounds to their offspring during gestation (via the placenta) and lactation (via their lipid rich-milk), resulting in a high exposure of newborns to those chemicals (O'Hara and O'Shea 2005). In free ranging bottlenose dolphins (*Tursiops truncatus*), concentrations of OCs declined with reproductive activity: blubber OC concentrations of nulliparous females were significantly greater than those of primiparous and multiparous females (Wells et al. 2005). The majority (c. 80% of OCs) of a female's contaminant burden is believed to be transferred to first born calves during the first seven weeks of lactation (Cockcroft et al. 1989). In captive *T. truncatus*, ∑PCB was more than 2.5 times higher and ∑DDT was three times higher in females whose calves died compared with females whose calves survived beyond six months (Reddy et al. 2001).

Even though female mammals are capable of transferring their contaminant load to their offspring during gestation and lactation, males are unable to do so and accumulate high contaminant levels; the effects of which are not fully understood in male cetaceans. In humans, it has been suggested that EDCs can cause lower sperm counts, quality and motility; reproductive abnormalities (morphological and functional gonadal dysfunction) which may cause infertility; and congenital malformations (altered embryonic and fetal intrauterine development) (Mostafa et al. 2007). Dallinga et al. (2002) reported an inverse correlation between the concentration of PCB metabolites in blood and seminal plasma and sperm motility, as well as sperm concentration (Mostafa et al. 2007). Whereas Guo et al. (2000) concluded that heavy exposure to PCBs resulted in negative effects on sperm morphology and motility, but not on sperm concentration. Other studies on humans indicate that high concentrations of persistent OCs may adversely induce menstrual cycle abnormalities and cause spontaneous abortions, prolong waiting times before pregnancy, reduce birth weights, skew sex ratio's, and alter the age of sexual development (Toft et al. 2004).

In marine mammals, a negative correlation between testosterone levels and tissue concentrations of DDE in Dall's porpoise *Phocoenoides dalli* has been reported (Subramanian et al. 1987). A possible explanation for the observed lower hormone levels in Dall's porpoise, and as mentioned earlier decreased oestradiol-17β levels in female harbour seals, could be an increased break down of steroids as a consequence of PCBs, or PCB metabolite induced enzyme activity. Another explanation may be that PCB or DDE, or metabolites thereof, bind to hormone carrier proteins and/or hormone receptors. Although both mechanisms mentioned above could operate in tandem (Reijnders 2003).

Common dolphins and Harbour porpoises

Piscivorous (and carnivorous) marine mammals inhabiting the mid-latitudes of Europe and North America are reported to have the highest DDT and PCB burdens (Aguilar et al. 2002). These findings are consistent with those previously reported on the geographical distribution of OCs in the atmosphere and surface waters, and are related to the extensive production and use of OCs in industrialized countries (see Aguilar et al. 2002, and ref. therein). Marine mammals provide information on the chemicals which present the greatest risk to consumers at the top of the food chain, something that cannot be adequately described or predicted in laboratory models (Ross 2000). Therefore, the current study will focus on two cetacean species that feed on commercially important fish species in the Northeast Atlantic, the common dolphin (*Delphinus delphis*) and harbour porpoise (*Phocoena phocoena*).

Common dolphins and harbour porpoises are the two most abundant top predators in the Northeast Atlantic. Although both species have been found to consume similar prey species, for example *Trisopterus* spp., sandeels (Ammodytidae), herring (*Clupea harengus*), hake (*Merluccius merluccius*) and whiting (*Merlangius merlangus*) (Learmonth et al. 2004), there are a number of population level differences between the species, including seasonal variations in diet and a number of life history traits. In UK waters, female *P. phocoena* attain sexual maturity at 4.51 years and the calving interval is c. 2 years (Learmonth 2006; Murphy 2008) compared to 8.23 yrs and 3.79 years, respectively, in *D. delphis* (Murphy et al. 2009). A recent study undertaken by Murphy et al. (2009) reported a low annual pregnancy rate (26%) for the Northeast Atlantic common dolphin population. Results suggested the level of anthropogenic mortality during the period of the study (1990-2006) did not cause a substantial population level decline, or that the low annual pregnancy rate, reported throughout the study period, could be a result of high contaminant burdens causing reduced fertility in females (Murphy et al. 2009).

Harbour porpoises are found predominately on the continental shelf in the Northeast Atlantic, including the North Sea. A single continuous population, with significant isolation by distance, has been reported ranging from waters off France, northward to Norway, (Fontaine et al. 2007). Separate sub-populations have been proposed within this region (Walton MJ 1997; Andersen et al. 2001; Andersen 2003). One common dolphin population with low genetic differentiation has been reported to inhabit both the continental shelf (it is rarely reported in the North Sea) and adjacent oceanic waters, ranging from Portugal to Scotland (see Murphy et al. 2008, and ref. therein). The *D. delphis* population exhibits seasonal movements, possibly due to the migratory pattern of its preferred prey species (ICES WGMME 2005).

The EC BIOCET (Bioaccumulation of persistent organic pollutants in small cetaceans in European waters: transport pathways and impact on reproduction) analysed samples from 70 stranded female common dolphin and 60 stranded female harbour porpoises that stranded along coastlines in the Northeast Atlantic (Ireland, Scotland, the Netherlands, France, and Galician, Spain) between 2001 and 2003. 18 PCB congeners and brominated flame retardants such as brominated diphenyl ether formulations (PBDEs) and hexabromocyclododecane (HBCD) - which is the principal brominated flame retardant in polystyrene foams used in the building industry - were analysed. Pierce et al., (2008) reported that a number of individuals in the BIOCET sample had contaminant levels above a threshold PCB level that has been reported to have adverse health effects. The threshold in question is 17 $\mu g\ g^{-1}$ PCB lipid weight, which was derived by Kannan et al. (2000) and is based on experimental studies of both immunological and reproductive effects in seals, otters, and mink. In the BIOCET sample, this threshold was frequently exceeded in both porpoises (47% of individuals) and common dolphins (40%), especially porpoises from the southern North Sea (74%) and common dolphins inhabiting waters off the French coast (50%). Pierce et al., (2008) stated though that there may be an issue with the study, as it was not known to what extent the sampled animals were representative of the population – a higher proportion of the sampled porpoises had died due to disease or parasitic infection as compared to common dolphins.

Further analysis of the effects of POPs on reproduction activity (analysing gonadal material) within the BIOCET dataset was undertaken by Murphy et al. (re-submitted; see Annex I). Results identified that common dolphins with the highest contaminant burdens were resting mature females (not pregnant or lactating). Further, these individuals also had the highest number of scars of ovulation on their ovaries, which suggested that (a) due to high contaminant burdens female common dolphins may be unable to reproduce and thus, continue ovulating; or (b) females are not reproducing for some other reason, either physical or social, and started accumulating higher levels of contaminants in their blubber. The high associated POP burdens may thus be either (or both) the cause of infertility or the consequence of infertility. In contrast in harbour porpoises, although sample sizes were small, once the effect of age was taken into account, the data so far suggests that higher POP concentrations tended to be associated with lower numbers of corpora scars, possibly indicating that high contaminant levels were inhibiting ovulation (Murphy et al. re-submitted).

Overview of project – Phase one

To investigate these results further, in the current study data were analysed from a control group of 'healthy' common dolphins, and also using a larger sample size of harbour porpoises with detailed pathological records (gross examination, histological, bacteriological and/or virological analyses). Variations in contaminant burdens between mature females in different reproductive states (resting mature, pregnant and lactating) and, between nulliparous, primiparous and multiparous females were assessed. Investigations were undertaken to determine whether increased contaminant levels (PCBs and DDT) are inhibiting ovulation, conception or implantation in common dolphins and harbour porpoises. Preliminary analysis was undertaken on ovarian lesions and other abnormalities of the genital tract, in order to investigate their association with contaminant levels.

The research undertaken in the current study has important implications for the conservation of both these species in the Northeast Atlantic. If the results identify that contaminants have an adverse effect on individual reproductive capabilities, the species would be more vulnerable to exploitation than is normally assumed, especially from other anthropogenic activities such as incidental capture, and would not necessarily recover from exploitation in a predictable way. Furthermore, assessing the effects of contaminants on wildlife are not only important in their own right, but are also significant to human health concerns, because of the information that may be conveyed regarding possible parallel changes in humans (Philips and Harrison 1999).

EC BIOCET data

During the current project, supplementary analysis was carried out with colleagues on data produced by the EC BIOCET project. This lead to the production and submission of a research paper to the NAFO/ICES/NAMMCO symposium proceedings entitled "The Role of Marine Mammals in the Ecosystem in the 21st Century", which will be published in a special issue of the Journal of Northwest Atlantic Fishery Science. Results from laboratory analysis undertaken during the current project is presented in the main text of this report, and results from the additional analysis of the EC BIOCET data is presented in Annex A - Murphy S, Pierce GJ, Law RJ, Bersuder P, Jepson PD, Learmonth JA, Addink M, Dabin W, Santos MB, Deaville R, Zegers BN, Mets A, Rogan E, Ridoux V, Reid RJ, Smeenk C, Jauniaux T, López A, Farré JMA, González AF, Guerra A, García-Hartmann M, Lockyer C, Boon JP (re-submitted). Assessing the effect of persistent organic pollutants on reproductive activity in common dolphins and harbour porpoises.

Adjustment to original aims for phase one of the project:

Although the project start date was 1st January 2009, the contract between ASCOBANS and the University of St Andrews was not signed until April 2009 and as a result, funding for salaries and laboratory costs were not made available until the end of April 2009. Due to the delay in funding, samples could not be processed for histopathology analysis until the final few weeks of the project, and adequate time was not available for microscopic examination of all histological slides of reproductive abnormalities. Therefore, results from this aspect of the project will be reported at a later stage. Histological slides of corpora scar (*albicantia* and *lutea*) tissues were assessed. In light of the results from the additional analysis of the EC BIOCET data (see introduction and ANNEX A), preliminary investigations were undertaken on ovaries from female harbour porpoises that stranded along the English and Welsh coastlines - gross examination of ovarian material from 91 individuals. Analysis (gross and histological) of the complete English and Welsh female harbour porpoise gonadal sample (with contaminant data) will be completed during phase two of the project.

METHODS

This study was undertaken in collaboration with Paul Jepson, Rob Deaville and colleagues at the Institute of Zoology, London (IOZ) and Robin Law at the UK Centre for Environment, Fisheries, and Aquaculture Science (Cefas).

Reproductive samples (and detailed post-mortem examination reports) from 96 female common dolphins collected by the UK Department of Environment, Food, and Rural Affairs (Defra) funded Cetacean Strandings Investigation Programme (CSIP) were provided for analysis. As part of the current project, and funded by the Defra Marine Research Program, blubber samples from a control group of common dolphins collected between 1992 and 2004 were processed for contaminant analysis by Cefas. Blubber samples were analysed for 25 polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), hexachlorocyclohexane (HCH; alpha, beta and gamma), and organochlorine pesticides (OCPs) such as DDT, DDE and TDE. The control group of 43 stranded females were individuals diagnosed as incidentally bycaught during detailed post-mortem examinations (Figure 1a). Pathological investigations such as gross examination, histological, bacteriological and/or virological analyses, identified whether dolphins were suffering from any infectious or non-infectious diseases that might inhibit reproduction (Jepson 2005). The majority of the control group were found stranded between the months December and March (88%), along the southwest coast of the UK (95%).

Samples from 564 English and Welsh harbour porpoises, including 261 females and 303 males have been processed for all or a combination of the following contaminants: 25 PCBs, 13 heavy metals, three butyltin compounds, 5 OCPs and 15 polybrominated diphenyl ethers as part of earlier studies such as Jepson et al., (2005) and Law et al., (2001). To date, ovaries from 91 female harbour porpoises, with corresponding contaminant data, were made available to the Sea Mammal Research Unit for analysis (Figure 1b). These individuals were either found stranded along the English and Welsh coastlines or obtained as part of a bycatch observer programme. Full pathological investigations, such as gross examination, histological, bacteriological and/or virological analyses were undertaken. No bias in sampling of individuals occurred between quarters, and samples were obtained in all months.

Data collection protocols followed European Cetacean Society guidelines for gross post-mortem examination and tissue sampling (Kuiken and Garcia 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). Teeth (N≥5) 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 and any abnormalities. Milk glands were examined for evidence of lactation by cutting through the mammary glands, and noting if milk or colostrum was present in the sinuses. Between 90 and 95% of the total burden of many POPs, particularly PCBs and DDTs, are found in the blubber because of its high lipid content (Aguilar 1985). Blubber samples for POP analysis were taken from the left side in front of the dorsal fin, and preserved using the standardised methodology.

Threshold level for effects on reproduction

Concentrations of 25 individual CBs congeners concentrations, determined on a wet-weight basis, were measured using methodology routinely used in the Cefas. The individual International Union of Pure and Applied Chemistry (IUPAC) CB congeners analyzed were numbers 18, 28, 31, 44, 47, 49, 52, 66, 101, 105, 110, 118, 128, 138, 141, 149, 151, 153, 156, 158, 170, 180, 183, 187, and 194. The sum of the concentrations of the 25 CB congeners determined (∑25CB) was then converted to a lipid basis ($\mu g\ g^{-1}$ lipid) using the proportion of hexane-extractable lipid in individual blubber samples.

As mentioned previously, a Σ-PCB level of 17 $\mu g\ g^{-1}$ lipid has been reported as a threshold level for health effects in marine mammals (Kannan et al. 2000; 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). Using thresholds in this way warrants caution owing to possible differences in species sensitivities and, as in Jepson et al. (2005), it is proposed that this threshold blubber concentration for adverse health effects should provide a benchmark for interpreting whether associations between reproductive activity and PCB exposure are biologically significant.

Determination of age and reproductive status

During the current project, teeth from 12 common dolphins within the control group were processed for ageing. Ages for other common dolphins and harbour porpoises were estimated during previous projects (Murphy 2008: Murphy et al. 2009). Age was determined by analysing growth layer groups (GLGs) in the dentine of teeth, following Lockyer (1995). 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.

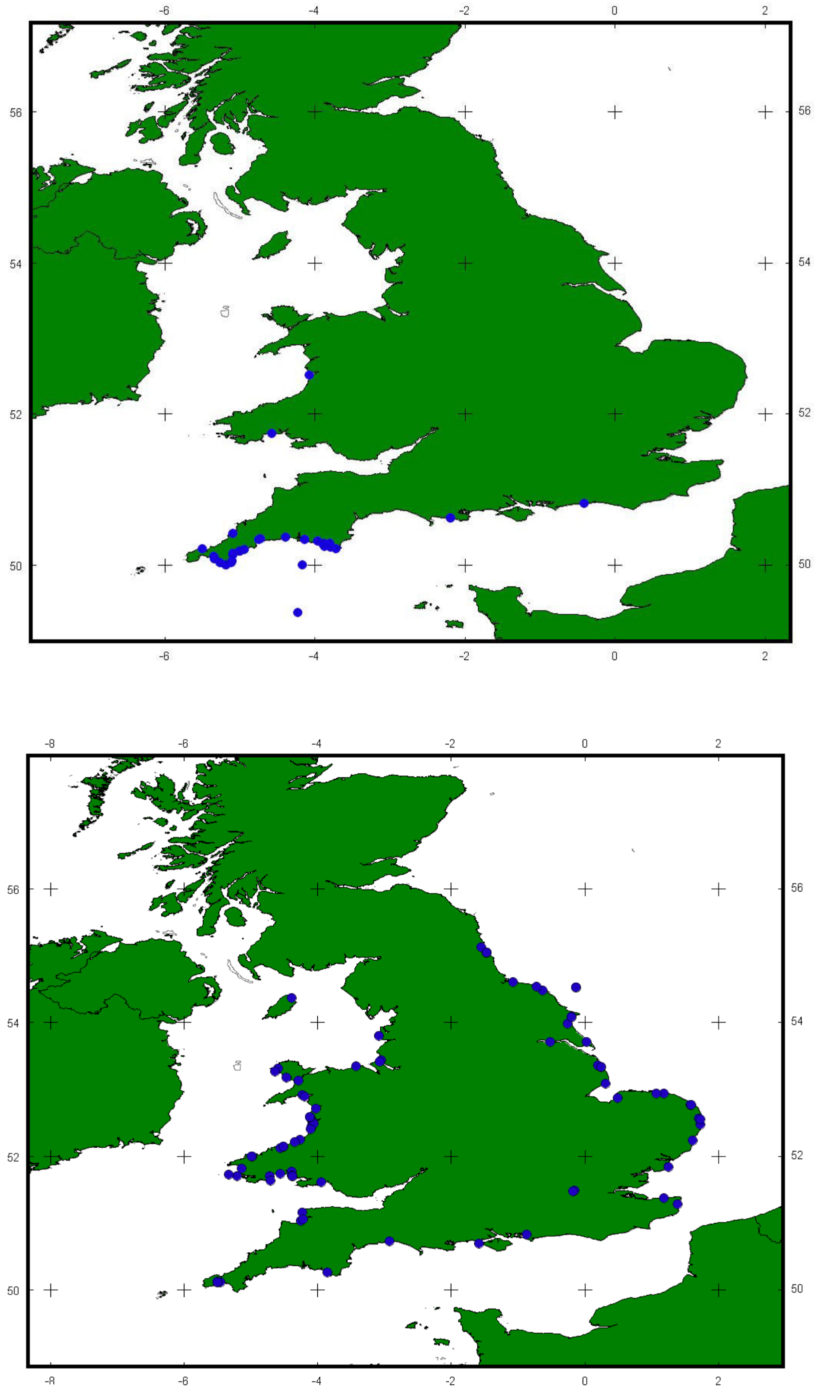


Figure 1. Distribution of sample locations of female (a) common dolphins (n = 43, 1992-2006) and (b) harbour porpoises (n = 91, 1991-2004).

Before examination, the preserved ovaries were rinsed in water for 24 hours and then replaced in their containers with 70% ethanol. For each ovary, the maximum length, height, width (mm) and weight (g) were recorded. Corpora scars present on the ovary were classified into *corpora lutea*, regressing *corpora lutea*, and *corpora albicantia*. The *corpus luteum* (CL) is an endocrine gland and is easily recognisable on the ovary as a pronounced distension, usually yellow in colour as a result of the yellow pigments of the carotenoid luteins. A regressing CL has been defined as luteal structures that have started to regress, and appears faintly yellowish in gross observation. A regressing *corpus luteum* CL eventually gives rise to a tissue scar called a *corpus albicans*. A *corpus albicans* (CA) can appear as a spherical knob or as raised, wrinkled scar and it is easily recognisable on the cut surface as pale fibrotic areas. CAs are composed of white connective tissue that becomes fragmented with age. Ovaries were hand sectioned into 0.5-2mm slices and examined internally under a binocular microscope for the presence of additional corpora scars.

It has been reported that *corpora albicantia* persist throughout the life of some marine mammals, as a consequence of the large amount of connective tissue present and poor vascularisation (Stewart and Stewart 2003), and therefore provide an index of the number of past ovulations (Perrin and Donovan 1984). However, contrasting results have highlighted inconsistencies with this theory (Brook et al. 2002; Takahashi et al. 2006). Recently, Dabin et al. (2008) and Murphy et al. (re-submitted) investigated the significance (in terms of our understanding of individual reproductive history) of *corpora albicantia* (scars of ovulation and pregnancy) in the ovaries of small cetaceans in the Northeast Atlantic. Although the results in the Murphy et al. (re-submitted) study were inconclusive, for the purpose of the current analysis, it is proposed that *corpora albicantia* do provide a lifetime record of past ovulations.

Females were considered sexually mature if the ovaries contained at least one *corpus luteum* or *albicans*. Pregnancy was established by the presence of an embryo/foetus due to the difficulty, during gross and histological examinations, in distinguishing a CL of pregnancy from a CL of ovulation. Females were classified into five reproductive states: immature, pregnant, pregnant & lactating, lactating, and resting mature (not pregnant or lactating).

Assessing reproductive abnormalities and evidence of reproductive failure

A review of causes of reproductive failure in animals was undertaken by Reeves et al. (2001). It was reported that gonadal inactivity or lesions (i.e. abnormalities) can be caused by many factors including genetic defects, infectious disease, degenerative changes, neoplasia or aging (senescence). In addition, gonadal problems can be secondary to other primary problems such as nutritional or environmental stress, systemic infection, central nervous system disease or toxins. Abnormal genital tract structure can be the result of developmental defects (genetic, disease- or toxin-induced) or acquired abnormalities due to hormone deficiencies or excesses, toxic exposure or infection. Foetal development or survival can be impaired by genetic defects, nutritional deficiencies or excesses, toxic exposure or infection. Post-partum neonatal death can be caused by inherited or congenital defects, poor nutrition, environmental stress or infectious disease.

Using data provided by the UK CSIP, an initial analysis of reproductive abnormalities in the UK (stranded and bycaught) common dolphin sample was carried out, in order to undertake a case-control study. This study will assess abnormalities associated with the reproductive tract, where data are available. Genital pathology will be linked to other data such as nutritional status, disease, and contaminant levels. Further, evidence of shortened reproductive spans, abortions, stillbirths, premature births and evidence of low birth size/weight in newborns will be assessed within the UK common dolphin sample.

Ovaries are assessed for evidence of atrophy and early senescence, ovarian cysts including luteinized cysts, and tumours. Other abnormalities of the reproductive tract include tumours, uterine stenosis, occlusions and leiomyomas and vaginal calculi. The sample will also be assessed for evidence of hermaphroditism. Completion of the assessment for reproductive abnormalities in UK female common dolphins, and a full assessment of the English and Welsh female harbour porpoise sample, will be undertaken during phase two of the project.

Phase two of the project will also encompass the effects of contaminants on male reproduction. Using the English and Welsh male harbour porpoise sample, the impact of high contaminant levels will be investigated through histopathology analysis of testicular tissue in order to assess sperm production and the presence of disorders in the male reproductive tract; contaminant data available for 25 PCB congeners, 13 heavy metals, three butyltin compounds, 5 OCPs and 15 polybrominated diphenyl ethers.

Histological Processing

Within the whole common dolphin reproductive sample, sections of all types of ovarian scars and reproductive abnormalities were taken for histopathology. The tissue was dehydrated using 30%, 50%, 70%, 80%, 95% graded ethanol solutions, absolute ethanol and butanol. Tissues were embedded in paraffin wax, sectioned at 7µm, stained with haematoxylin and mounted on a glass slide using DPX. Histological analysis was carried out on tissue samples from abnormalities, active and regressing *corpora lutea*, *corpora albicantia*, follicles, nodules, yellow bodies and any scar tissue that could not be assessed on gross examination. 136 tissue samples from common dolphin ovaries were processed for histology during the current study. However, as mentioned earlier, adequate time was not available for microscopic examination of all histological slides.

RESULTS AND DISCUSSION

Common dolphin control group study – English and Welsh data

In order to eliminate any affects of infectious and non-infectious disease on reproductive output, contaminant data and gonadal material were analysed from a "control group" of healthy common dolphins. The control group sample was divided into three health status categories; category 1 - healthy individuals; category 2 - health of individuals only mildly compromised (but may still be capable of successfully reproducing); and category 3 - individuals suffering from severe (and potentially fatal) infectious or non-infectious disease. 93% of the sample was assessed as category 1 (see Table 1).

The sample was composed of 20 immature and 23 mature females. Sexually immature females ranged from 0 to 11 years (n = 19) in age, 107 to 210 cm (n = 20) in length, and 0.78 to 5.46 g (n = 16) in combined gonadal weight. ∑25CB and total DDT values ranged from 9.22 to 48.05 (n = 20) and 1.26 to 13.7 (n = 20) µg g^{-1} lipid weight, respectively. Sexually mature individuals ranged from 7.5 to 30 years (n = 19) in age, 186 to 221 cm (n = 22) in length and 1.88 to 17.48 g (n = 22) in combined gonadal weight. ∑25CB and total DDT values ranged from 1.65 to 53 (n = 23) and 0.17 to 13.7 µg g^{-1} lipid (n = 23), respectively.

In the control group, although the sample size was small (n=19), a significant increase in corpora scar number with age was observed ($p = 0.002$, $r^2 = 0.44$) in sexually mature individuals. Corpora scar number ranged from 1 to 16 (n = 23) in sexually mature females.

Table 1. Health status categories in the control group *D. delphis* sample.

	Sample size	Cat. 1	%	Cat. 2	%	Cat. 3
Immature	20	19	0.95	1	0.05	0
Lactating	7	7	1	0	0	0
Pregnant	3	3	1	0	0	0
Pregnant & Lactating	2	2	1	0	0	0
Resting Mature	11	9	0.82	2	0.18	0

Total blubber PCB levels (as Aroclor 1254) were also calculated [(Σ-ICES7 PCB congeners)*3], enabling direct comparison with a proposed threshold for adverse health effects in marine mammals of 17 µg g^{-1} lipid, thus providing a benchmark for interpreting whether associations between reproductive activity and PCB exposure are biologically significant. All sexually immature (nulliparous) females (range 17.2-93.6 $\mu g\ g^{-1}$ lipid) and the three "primiparous" pregnant females (range 32.3-77.82 $\mu g\ g^{-1}$ lipid) had total blubber PCB levels above the threshold level (Figure 2a, b). Although not significant, a decline in total blubber PCB levels with increasing corpora scar number was observed (see Figure 2b), and a similar plot was obtained when ∑25CBs was plotted against corpora scar number (not shown). Further, a non-significant decline in DDT burden against corpora scar number was also observed (Figure 2c).

As all sexually immature (nulliparous) females and the three "primiparous" pregnant females had total blubber PCB levels above the threshold level suggests that that high PCB burdens are not inhibiting ovulation, conception or implantation in *D. delphis*. As mentioned previously, Reijnders (1986) reported a decrease in reproductive success in harbour seals which was possibly due to implantation disruption. However, as pinnipeds experience delayed implantation/embryonic diapause, they may be more vulnerable than cetaceans at this stage of the reproductive cycle. Studies on mink (*Mustela vison*) have also reported that PCBs can impair reproduction; although ovulation, conception and implantation occur, fetues died during gestation or shortly after birth (Jensen et al. 1977; Reijnders 1986; Backlin and Bergman 1992; Backlin and Bergman 1995; Schwacke et al. 2002). This was attributed to either hormonal disturbance, direct dominant-lethal action or to an embryo lethal effect caused by toxicants (Reijnders 1986).

Relative low-level exposures to some chemicals at critical life stages or "critical windows of exposure" (e.g. early foetal development and puberty) can result in dramatic effects on individuals, and/or subtle but important population-wide impacts, by affecting population growth, maintenance and/or health (O'Hara and O'Shea 2005). Effects of contaminants can occur in foetuses at doses levels that are orders of magnitude below those that effect adult reproductive function. Exposure to an endocrine disrupter during a sensitive stage in development or differentiation may also result in non-reversible and usually latent sexual dysfunction or physical abnormalities (Kavlock et al. 1996; Hohn et al. 2007). It appears that in cetaceans, the first born offspring tends to be the most susceptible to exposure of contaminates, as first time mothers have a higher contaminant load, accumulated over many years. Wells *et al*., (2005) reported that high rates of first born calf mortality were correlated with higher concentrations of PCBs in the blubber and plasma of primiparous female *T. truncatus*, inhabitating Sarasota Bay, Florida. Subsequent calves exhibited higher survival rates; only 50% of first-born calves survived through their first year, whereas more than 70% of calves born to multiparous mothers survived (Wells et al. 2005). It cannot be ruled out though that the high mortality rate of first born calves may be due to other reasons, such as predation and human interactions, along with first time mothers being less capable at successfully rearing offspring due to being physiologically (significantly smaller) and behaviourally inexperienced (Wells et al. 2005).

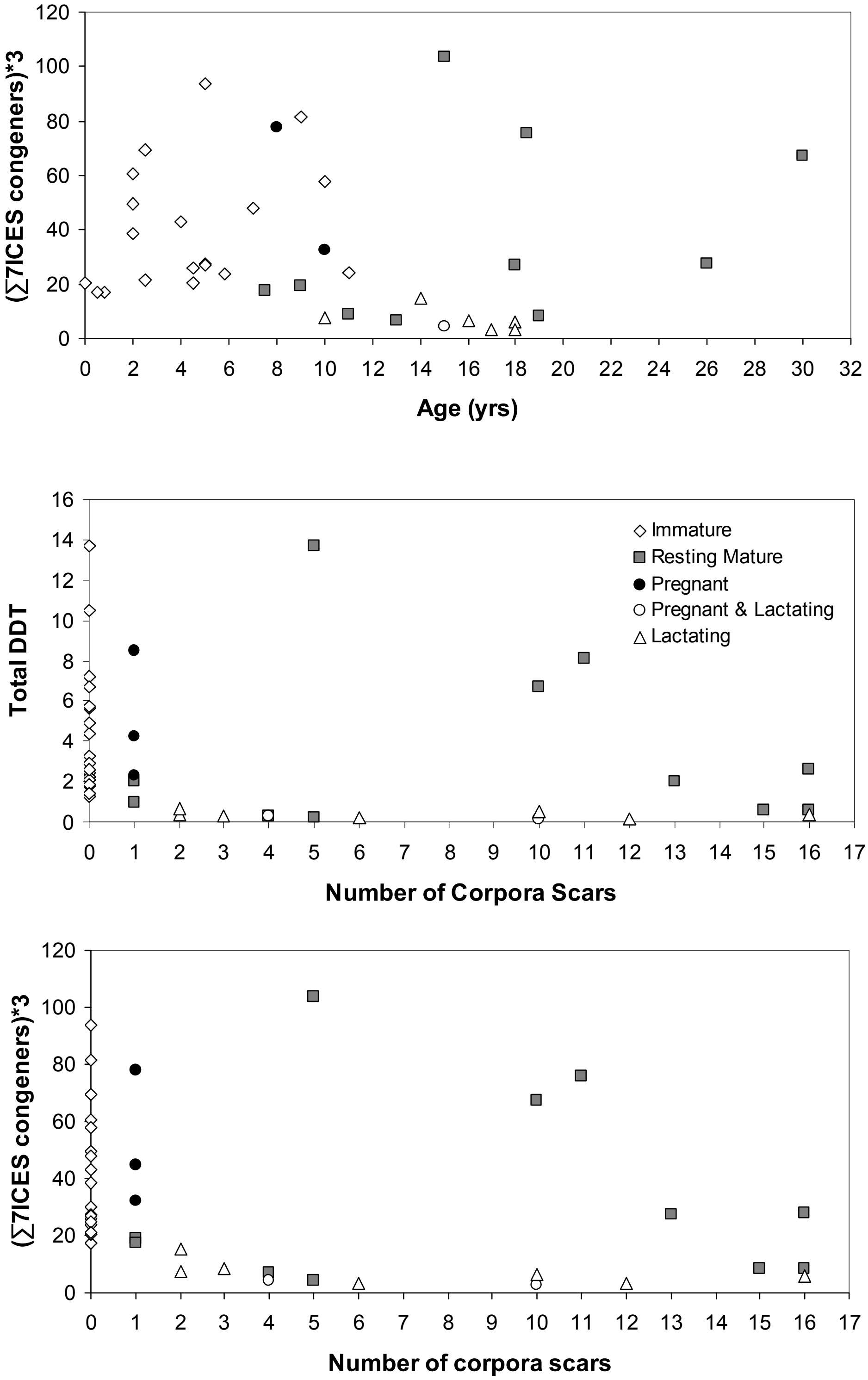


Figure 2. PCB burden [(∑7ICES congeners)*3] ($\mu g\ g^{-1}$ lipid) as a function of (a) age (n = 38) and (b) number of corpora scars (n = 43); (c) total DDT burden ($\mu g\ g^{-1}$ lipid) as a function of number of corpora scars (n = 43) in the *D. delphis* control group sample.

The three primiparous *D. delphis* females in the current study were within their second trimester, and it is not known whether all three females would have successfully given birth and/or the survival rate of their first born calves. In female Californian sea lions, associations have been documented between high OC levels in post parturient individuals and miscarriages, and premature pupping during the last two trimesters of pregnancy. The majority of premature pups are born alive during the third trimester, but all die within several hours of birth (Marine Mammal Commission 1999). However, the association between OCs and prematurity is confounded by the presence of disease capable of inducing abortions: serological evidence of leptospirosis and calicivirus has been found. Further, the frequency of prematurity was higher during El Nino years, indicating that the nutritional status of the females also influences the probability of prematurity (Marine Mammal Commission 1999).

It should be noted that the Kannan et al. (2000) threshold of 17 μg g^{-1} lipid is less protective than that proposed of 10 μg g^{-1} lipid PCBs, which was associated with increased calf mortality in wild bottlenose dolphins (Hall et al. 2006b; Hickie et al. 2007). Further, the analysis in the current study did not include the most immunotoxic IUPAC congeners, such as CB77 and CB126.

The most parsimonious interpretation of the negative (non-significant) relationship between POP (DDT and PCB congeners) concentrations with increasing corpora scar number in the *D. delphis* control group, is that a high number of corpora scars indicates infertility or a high level of miscarriages/abortions (repeated ovulations as the animal does not get pregnant, or loses the foetus during gestation or soon after), and some females may go through a large number of infertile ovulations prior to a successful pregnancy, birth, and survival of their first offspring during early lactation - as mentioned previously, females offload c.80% of their OC burden during the first seven weeks of lactation (Cockcroft et al. 1989). A high foetal morality rate in the first trimester (40-67%) has been reported in other small delphinids (*Stenella longirostris and Stenella attenuata*), which was attributed to adverse interactions with purse seine fisheries in the eastern tropical Pacific; induction of miscarriage due to physiological stress of chase and capture or indirectly through depletion of energy stores (Perrin *et al.,* 2003). Data in the current study may also suggest the existence of non-breeding (ovulating) females in the population, though it appears that almost all females eventually become pregnant – due to a decline in the contaminant levels with increasing corpora scar number. Although the number of corpora scars increased with age within the control group *D. delphis* sample, the problems in deciphering whether or not *corpora albicantia* provide a lifetime record of past ovulations has caused difficulties in correctly interpreting these data.

Harbour porpoise data – English and Welsh data

Preliminary analysis of reproductive data from 91 English and Welsh harbour porpoises is presented in the current report. The harbour porpoise contaminant sample was composed of individuals that died from incidental capture (39%, n = 32), physical trauma (9.6%, n = 8), infectious and non-infectious disease (31%, n = 26), live stranding (4.8%, n = 4), starvation (6%, n = 5), dystocia and still birth (4.8%, n = 4), and other reasons (4.8%, n = 4). Females ranged from 0 to 21 years (n= 79) in age, 70 to 191 cm (n = 90) in length, and 0.29 to 12.15 g (n=79) in combined gonadal weight. ∑25CB and total DDT values ranged from 0.48 to 159.68 (n =83) and 0.17 to 11.7 (n = 65) μg g^{-1} lipid weight, respectively. The sample was composed of 62 immature and 29 mature females. Sexually immature individuals ranged in age, length and combined gonadal weight from 0 to 6 yrs (n = 58), 70 to 157 cm (n = 61) and 0.29 to 3.94 g (n = 53). Sexually mature individuals ranged from 4 to 21 years (n = 21) in age, 138 to 191 cm (n = 29) in length, 1.93 to 12.15 g (n = 26) in combined gonadal weight, and 1 to 22 (n = 29) in corpora scar number (*corpora albicantia* and *lutea*). ∑25CB and total DDT values ranged from 0.48 to 159.68 (n = 55) and 0.45 to 11.7 (n = 43) μg g^{-1} lipid in sexually immature individuals, and from 1.29 to 42.17 (n =28) and 0.17 to 5.53 (n = 22) μg g^{-1} lipid, respectively, in sexually mature females.

The mature sample was composed of seven resting mature, four pregnant, seven pregnant and lactating and eleven lactating individuals (Table 2). Corpora scars were reported on the right ovary in only one mature individual, of unknown age; 14 *corpora albicantia* were observed on the left ovary and one *corpus albicans* was reported on the right ovary. No significant correlation was observed between corpora scar number and age within the mature female *P. phocoena* sample (spearman's rho coefficient = 0.353, p = 0.116, n = 21). Overall, no apparent variation in accumulation of corpora scars was observed between reproductive status groups within the English and Welsh harbour porpoise sample (see Figure 3).

The youngest sexually mature females, both aged at four years, had two and three corpora scars present on their ovaries. Individuals aged at 5 years reported between 5 and 15 corpora scars, suggesting numerous ovulations during an oestrus period. The female with the highest number of corpora scars (n = 22) was a 14-year old live stranded lactating individual, that was suffering from severe gastropathy &/or enteropathy.

Table 2. Reproductive status of all sexually mature females that stranded along the English and Welsh coastlines (1991 to 2004). Categories: pregnant (foetus and a *corpus luteum* of pregnancy present); simultaneously pregnant and lactating, sexually mature and lactating, and resting mature individuals that were not pregnant or lactating.

Reproductive status	**Age (yr)**	**Length (cm)**	**Combined gonadal weight (g)**	**Corpora scar number**	**∑25CB μg g^{-1} lipid**
Resting Mature	4 - 9 (n = 5)	145 – 164 (n = 7)	2.65 – 6. 72 (n = 5)	1 - 10 (n = 7)	1.47 – 42.17 (n = 7)
Pregnant	4 – 21 (n = 3)	143 – 176 (n = 4)	7.4 – 12.15 (n = 4)	2 -15 (n = 4)	4.17 – 15.16 (n = 3)
Pregnant & Lactating	5 - 15 (n = 5)	146 – 190 (n = 7)	6.52 – 10.82 (n = 6)	5 - 15 (n = 7)	1.44 – 7.41 (n = 7)
Lactating	5 – 14 (n = 8)	138 – 191 (n = 11	1.93 – 9.07 (n = 11)	1- 22 (n = 11)	1.29 – 21.4 (n = 11)

Large variations in contaminant burdens in calves less than one year of age were observed (Figure 4a) which reflects the differences in: accumulated contaminant levels in their mothers, the duration of nursing, birth order, and the length of the calving interval preceding their birth. Ten immature females had had contaminant levels >50 μg g^{-1}lipid and of these, 60% died from infectious or non-infectious diseases. Neonate calves with extremely high contaminant burdens may suggest first born offspring. The highest contaminant burden (310.88 µg g^{-1}lipid) was reported in a neonate calf measuring 90 cm in length. This individual was reported to have been in a very poor nutritional condition, and died of starvation soon after its birth (teeth were unerupted and papillae were prominent on the tip of the tongue).

A significant negative relationship was observed between total blubber PCB levels [(∑7ICES congeners)*3] and age ($p = 0.042$, $r^2 = 0.058$, $n = 71$; Figure 4a) and length ($p = 0.004$, $r^2 = 0.097$, $n = 81$; Figure 4b) in the *P. phocoena* sample. Furthermore, a significant negative relationship was observed between total blubber PCB levels and corpora scar number ($p = 0.014$, $r^2 = 0.021$, $n = 27$) and total DDT and corpora scar number ($p = 0.046$, $r^2 = 0.018$, $n = 21$) in sexually mature individuals (Figure 5). All resting mature female harbour porpoises had ≤10 corpora scars. All individuals with ≥ 10 corpora scars (n = 4) were lactating and pregnant females, with contaminant levels <9 µg g^{-1}lipid. In the sexually immature sample, only 4% of females had total PCB levels <9 µg g^{-1}lipid.

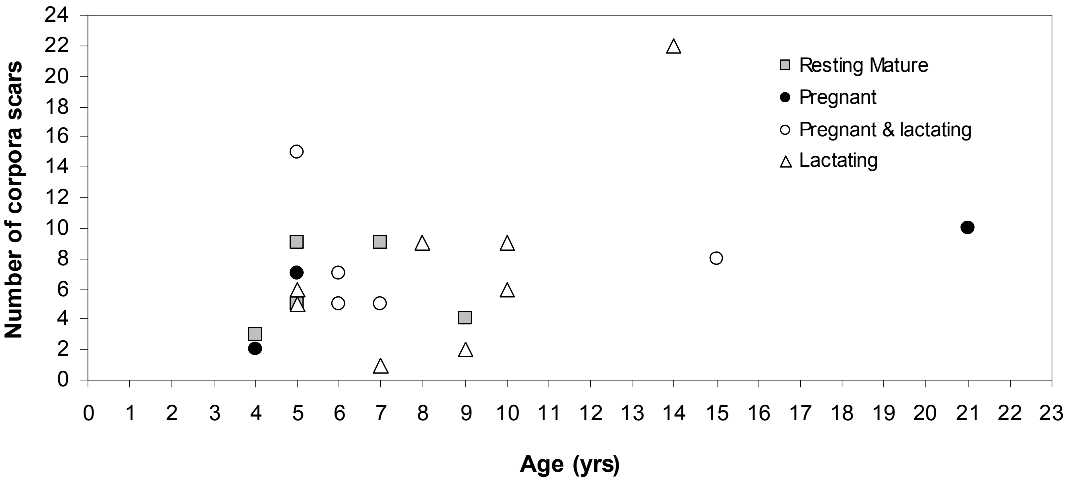


Figure 3. Number of corpora scars against age in the English and Welsh sexually mature female harbour porpoise sample (1991-2004, n = 21).

As mentioned earlier all sexually immature common dolphins within the control group sample had total blubber PCB levels above the threshold, of 17 µg g^{-1}lipid, for adverse effects on reproduction. However, only 64% of the immature harbour porpoise sample was observed above this level (Figure 4). This may be attributed to the fact that common dolphins attain sexually maturity at a much older age than harbour porpoises (8.23 yrs vs. 4.51 years), therefore lengthening the period for accumulation of contaminants during the immature phase through dietary input and, subsequently, leading to a higher maternal contaminant burden.

Preliminary assessment of the harbour porpoise data suggests that increased contaminant burdens, above the threshold level, are not inhibiting ovulation, conception or implantation. For example, a resting mature female of unknown age measuring 161cm in length had a contaminant burden of 87 µg g^{-1}lipid. This female had been pregnant on one prior occasion (by assessing the state of the uterus), although it is not know if the foetus had successfully come to term. The resting mature female live stranded and was euthanised following unsuccessful reflotation attempts, and a post-mortem examination revealed the animal was suffering from pneumonia (parasitic and bacterial). Based on the average total blubber PCB level of 33.9 μg g^{-1}lipid (converted data) in sexually immature female harbour porpoises, the contaminant level reported in this resting mature individual suggests that its foetus had either aborted, or died very soon after birth.

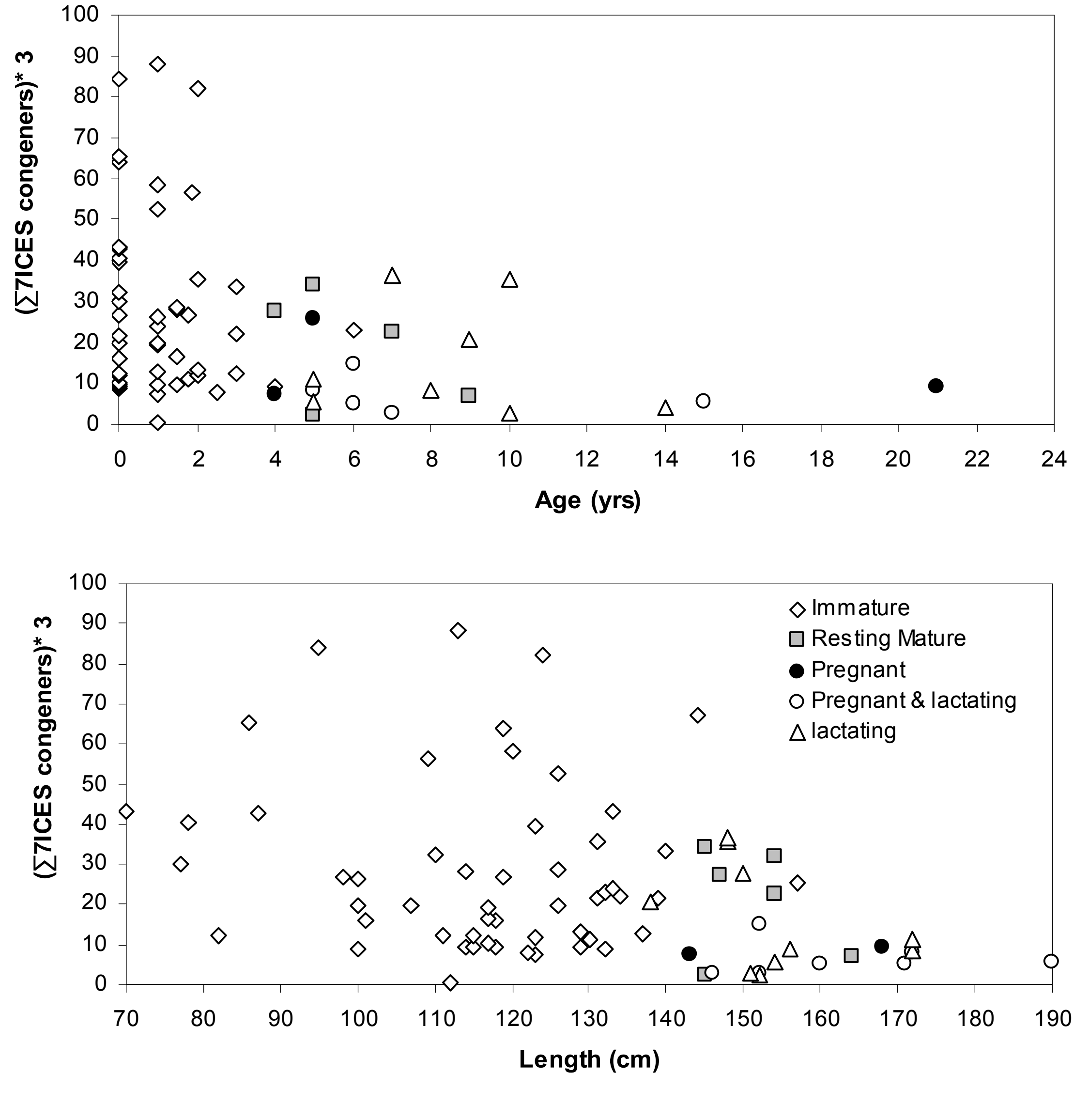
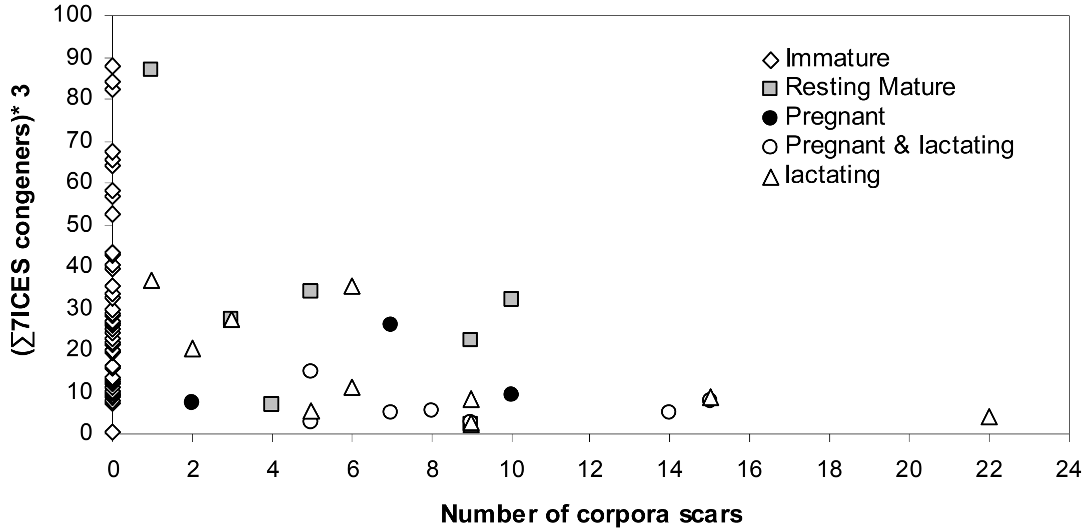


Figure 4. PCB burden [(∑7ICES congeners)*3] (μg g^{-1} lipid) as a function of (a) age (n = 72) and (b) length (n = 82) in the *P. phocoena* sample (excluding the neonate female measuring 90 in length, with a contaminant load of 310.88 μg g^{-1}lipid)

The preliminary results from the English and Welsh harbour porpoise study are similar to those obtained from the common dolphin control group study, where it appears that high contaminant burdens are not disrupting the reproductive cycle prior to or during implantation. However, they are in contrast to the results from *P. phocoena* BIOCET study where once the effect of age was taken into account, the data suggested that higher POP concentrations tended to be associated with lower numbers of corpora scars, thus indicating that high contaminant levels were possibly inhibiting ovulation (Murphy et al. re-submitted). The differing results from the two harbour porpoise samples may reflect the sampling biases in the BIOCET study towards individuals that died from a variety of infectious diseases. Whereas the English and Welsh *P. phocoena* sample was composed of a large proportion of individuals (48.6%) that died from an acute physical trauma (e.g. bycatch).



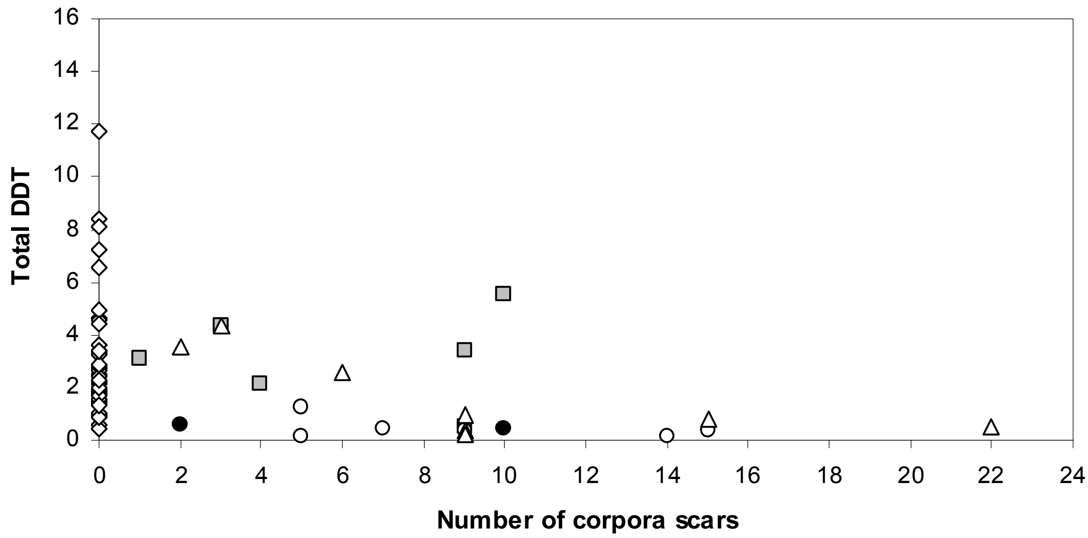


Figure 5. PCB burden [(∑7ICES congeners)*3] ($\mu g\ g^{-1}$ lipid) as a function of (a) number of corpora scars (n = 82 (excluding the neonate female with a contaminant load of 310.88 $\mu g\ g^{-1}$ lipid) and (b) total DDT burden ($\mu g\ g^{-1}$ lipid) as a function of number of corpora scars (n = 64) in the *P. phocoena* sample

It has been reported that chronic exposure to environmental contaminants, accumulated through the food chain, possibly affects the immune system function in marine mammals (e.g. de Swart et al. 1996; Jepson et al. 22005). A causal (immunotoxic) relationship has been reported between PCB exposure and infectious disease mortality in UK harbour porpoises (Jepson et al. 2005). Among stranded adult female harbour porpoises, PCB levels were significantly higher in individuals classified into the infectious disease group than in animals classified in the physical trauma group. Further, females dying of infectious disease had significantly poorer nutritional status (relative body wt and mean blubber thickness) compared to the physical trauma group (Jepson et al. 2005). In the current study, two lactating female harbour porpoises, aged 10 and 7 years, which died from a severe acute interstitial pneumonia and generalised bacterial infection (*Streptococcus canis*), had total blubber PCB levels of 35.5 and 36.6 $\mu g\ g^{-1}$lipid, respectively. Although it appears that both these females had successfully pregnancies, the impact of the high maternal contaminant levels on the offspring's survival rate may have been detrimental - if PCBs had comprised the immune system function in these individuals - as in one of the cases the female had just recently given birth.

A single continuous harbour porpoise population, with significant isolation by distance, has been reported ranging from waters off France, northward to Norway, (Fontaine et al. 2007). Within English and Welsh waters two separate management stocks have been proposed; (1) Celtic Sea (plus South-west Ireland, Irish Sea & Western Channel) and (2) southwestern North Sea & eastern Channel stocks (Evans and Teilmann 2009). The current report presents preliminary results from the English and Welsh harbour porpoise sample. Future work will analyse the remaining reproductive material from this region with available contaminant data and investigate, using statistical analysis, both population and stock level effects of contaminants on reproductive output; taking into account the health status, age, length, condition, reproductive status, reproductive abnormalities, as well as other contaminant data (13 heavy metals, three butyltin compounds and 15 polybrominated diphenyl ethers).

In conclusion, results to date suggest that high contaminant burdens, above the threshold level for adverse health effects from PCBs, were not directly inhibiting ovulation, conception or implantation in female *D. delphis* or *P. phocoena*, though the impact on the foetal and newborn survival rates requires further investigation. To date, research has focused on the effects of PCBs and DDT, and future work should include other contemporary contaminants. Due to the problems in deciphering whether or not *corpora albicantia* provide a lifetime record of past ovulations, further investigations into this subject area are required.

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Annex A

Assessing the effect of persistent organic pollutants on reproductive activity in common dolphins and harbour porpoises

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Abstract

As top predators, marine mammals can provide information on the accumulation of anthropogenic toxins which present the greatest risk to consumers. We assessed the impacts of persistent organic pollutants (POPs) on two cetacean species that feed on commercially important fish species in the eastern North Atlantic; the common dolphin *Delphinus delphis* and the harbour porpoise *Phocoena phocoena*. In order to evaluate the possible long-term effects of POPs on the continued viability of these populations, we investigated their effects on reproductive activity in females, using ovarian scars as an index of reproductive activity. In harbour porpoises, high POP burdens tended to be associated with lower ovarian scar number, possibly indicating that high contaminant levels were inhibiting ovulation, or some females may go through a number of infertile ovulations prior to a successful pregnancy, birth, and survival of their first offspring during early lactation. In contrast, initial results identified that the common dolphins with contaminant burdens above a threshold level for adverse health effects in marine mammals (17 $\mu g\ g^{-1}$ total PCBs lipid) were resting mature females, with high numbers of ovarian scars. This suggests that (a) due to high contaminant burdens, females may be unable to reproduce, thus continue ovulating, or (b) females are not reproducing for some other reason, either physical or social, and started accumulating higher levels of contaminants. Additional analyses were carried out on a control group of "healthy" *D. delphis*, i.e. stranded animals diagnosed as bycatch and were assessed for evidence of any infectious or non infectious disease that would inhibit reproduction. Results suggested that high contaminant burdens, above the threshold level, were not inhibiting ovulation, conception or implantation in female *D. delphis*, though the impact on the foetal survival rate (in both species) requires further examination. Investigations into accumulation and persistence of ovarian scars and use as an index of reproductive activity were also undertaken within this study.

Keywords: Phocoena phocoena, *Delphinus delphis*, ovarian scars, *corpora albicantia*, *corpora lutea*, persistent organic pollutants, reproduction, health

1. Introduction

Organochlorine compounds (OCs), such as polychlorinated biphenyls (PCBs) and DDT, accumulate in the blubber of marine mammals, and a large number of these lipophilic substances are known to be hormone or endocrine disrupters. The endocrine and reproductive effects of these chemicals are believed to be due to their ability to: (a) mimic the effect of endogenous hormones; (b) antagonize the effect of endogenous hormones; (c) disrupt the synthesis and metabolism of endogenous hormones; and (d) disrupt the synthesis of hormone receptors (Amaral Mendes, 2002). OCs have been reported to increase susceptibility to infection (Jepson *et al.*, 2005; Hall *et al.*, 2006a), which may have consequences not only on adult survival but also on uterine and placental health and, subsequently, foetal health and survival (Hohn *et al.*, 2007). Reproductive effects linked with exposure to endocrine disruptors such as PCBs and associated DDT-like compounds include decreased fecundity, implantation failure and sterility (caused by stenosis, occlusions and leiomyomas) in seals (Helle, 1976; Helle *et al.*, 1976; Reijnders, 1986; Olsson *et al.* 1994; Reijnders, 1999; Bredhult et al. 2008); premature pupping in sea lions (DeLong *et al.*, 1973); and also severe reproductive dysfunction through the development of cancer and possibly hermaphroditism in beluga whales (*Delphinapterus leucas*) (Martineau *et al.*, 1987; De Guise *et al.*, 1994; Reijnders, 1999). The findings of these studies however, although strongly suggestive, have not been conclusive as the etiology of the observed disorder has usually been uncertain (Reijnders 2003).

PCBs and DDT are persistent organic pollutants (POPs), substances that persist in the environment, and uptake of POPs in marine mammals occurs predominately through prey consumption. POPs are reported to both biomagnify and bioaccumulate, as their concentration increases from one trophic level to the next, within the food chain. Not only does an individual's POP burden reflect its dietary preferences, it is influenced by its body size, body condition, nutritive condition, disease, metabolism, excretion, age and sex (Aguilar *et al.*, 1999). Furthermore, it is an indication of the conditions it experienced in early life: contaminant levels in its mother, the duration of nursing, birth order and the length of the calving interval preceding its birth (Ylitalo *et al.,* 2001; Hickie *et al.,* 1999; Hickie *et al.,* 2000; Ross *et al.,* 2000; Hickie *et al.,* 2007). Females, through mobilization of lipid-associated toxins from the blubber during periods of high energy requirements, transfer toxic compounds to their offspring during gestation (via the placenta) and lactation (via their lipid rich-milk), resulting in a high exposure of newborns to those chemicals (O'Hara and O'Shea, 2005). In contrast, male cetaceans become increasingly contaminated as they grown older. In free ranging bottlenose dolphins (*Tursiops truncatus*), concentrations of OCs declined with female reproductive activity: blubber OC concentrations of nulliparous females were significantly greater than those of primiparous and multiparous females (Wells *et al.*, 2005). In this species, approx. 80% of OCs are transferred to first born calves during the first seven weeks of lactation (Cockcroft *et al.*, 1989). In captive *T. truncatus*, $\sum$PCB was more than 2.5 times higher and $\sum$DDT was three times higher in females whose calves died compared with females whose calves survived beyond six months (Reddy *et al.*, 2001).

Reproductive failure in female harbour seals (*Phoca vitulina*) has been connected to feeding on contaminated fish. Average pup production per female harbour seal in the Dutch Wadden Sea population declined by approximately 30%, and toxicology studies revealed that, of all the OCs analysed, PCB levels were significantly higher (by 5 to 7 times) in the Dutch Wadden Sea population compared to other contiguous populations (Reijnders, 1980). Experimental studies revealed that 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 (Reijnders, 1986). Hormone profiles of non-pregnant animals fed fish from the Wadden Sea indicated that the effects occurred at the stage of implantation, whereas the follicular, luteal and post-implantation phases were not affected. On the whole, oestradiol-17β levels in seals fed with fish of a higher contaminant burden were lower than those of the control group. Lower levels of oestradiol could have impaired endometrial receptivity and prevented successful implantation of the blastocyst (Reijnders, 2003).

Piscivorous (and carnivorous) marine mammals inhabiting the mid-latitudes of Europe and North America are reported to have the highest PCB and DDT burdens (Aguilar *et al.*, 2002). These findings are consistent with those previously reported on the geographical distribution of OCs in the atmosphere and surface waters, and are related to the extensive production and use of OCs in industrialized countries (see Aguilar *et al.,* 2002, and ref. therein). Common dolphins (*Delphinus delphis*) and harbour porpoises (*Phocoena phocoena*) are the two most abundant top predators in the eastern North Atlantic (ENA). Harbour porpoises are found predominately on the continental shelf, including the North Sea. A single continuous population, with significant isolation by distance (i.e. the greater the distance the smaller the genetic correlation), has been reported ranging from waters off France, northward to Norway, (Fontaine *et al.*, 2007). Separate sub-populations have been proposed within this region (Walton, 1997; Andersen *et al.*, 2001; Andersen, 2003; Evans *et al.,* 2008). One common dolphin population with low genetic differentiation has been reported to inhabit both the continental shelf (it is rarely reported in the North Sea) and adjacent oceanic waters, ranging from Portugal to Scotland (see Murphy *et al.*, 2009, and ref. therein). The *D. delphis* population exhibits seasonal movements, possibly due to the migratory pattern of its preferred prey species (ICES WGMME, 2005).

Due to a decline in abundance and high bycatch rates, the harbour porpoise has been included on OSPAR's (The Convention for the Protection of the Marine Environment of the North-east Atlantic) "list of threatened and/or declining species and habitats" in regions II (the greater North Sea) and III (the Celtic Sea, and waters off the west coast of Ireland and the UK, www.ospar.org). Both *D. delphis* and *P. Phocoena* are listed under Annex IV of the EU Habitats Directive, and are afforded protection as European Protected Species (EPS). For any EPS, it an offence to deliberately or recklessly kill, capture, injure or disturb any such animal. It also requires the establishment of a system to monitor incidental capture and killing of individuals and to take measures to ensure that these activities do not have significant negative impacts on the species concerned. The harbour porpoise is also listed as an Annex II species in the EU Habitats Directive, which requires the establishment of Special Areas of Conservation (SAC). Signatory countries to ASCOBANS (Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas) are required to assess the status and seasonal movements of the populations and stocks for the harbour porpoise and common dolphin; locate areas of special importance to their survival; identify present and potential threats to the different species; and establish efficient systems for reporting and retrieving bycaught and stranded specimens, in order to carry out full autopsies for collecting tissues for further studies, and reveal possible causes of death and to document food composition.

Although both *P. phocoena* and *D. delphis* have been found to consume similar prey species, for example *Trisopterus* spp., sandeels (Ammodytidae), herring (*Clupea harengus*), hake (*Merluccius merluccius*) and whiting (*Merlangius merlangus*) (Santos et al., 2004a, b, c), there are a number of population level differences between the species, including seasonal variations in diet and a number of life history traits. In UK waters, female *P. phocoena* attain sexual maturity at c.4.5 years and the calving interval is c.2 years (Learmonth *et al.*, 2004; Murphy, 2008) compared to 8.2 yrs and 3.8 years, respectively, in *D. delphis* (Murphy *et al.*, 2009). A low pregnancy rate of 26% has been reported for the ENA *D. delphis* population, and although it has been suggested that the pregnancy rate may well in fact be the natural rate for this species in a temperate region, it cannot be ruled out that environmental and other anthropogenic activities, such as chemical and physical pollutants, may be contributing factors to the low reproductive output (Murphy *et al.*, 2009). Further, *P. phocoena* in UK waters exhibit among the lowest pregnancy rates reported for this species; ranging from 34% in Scottish waters (Learmonth *et al.,* in prep.), 36% in western UK waters (English Channel, Irish and Celtic Seas) to 60% in the English North Sea (Murphy, 2008). This is in stark contrast to pregnancy rates of 95% for the Gulf of Maine and Bay of Fundy in the western North Atlantic (Read & Hohn 1995; data obtained between 1990 and 1993), 98% for Icelandic waters (Ólafsdóttir et al. 2003), and 73% for Danish waters (Sørensen & Kinze 1994). However, a lower pregnancy rate of 59% has been observed in Dutch waters (Addink *et al.,* unpublished)[1] and Learmonth *et al.* (in prep) point out that the high proportion of animals in the Scottish sample that had died from pathological causes may have resulted in the pregnancy rate being underestimated.

The EC-funded BIOCET (BIOaccumulation of persistent organic pollutants in small CETaceans in European waters: transport pathways and impact on reproduction) project analysed samples from female *D. delphis* and *P. phocoena* that stranded along coastlines in the ENA. Results on geographic variation in POP burdens, and relationships between POP burdens and age, fatty acid profiles, health status and reproduction, were presented in Pierce *et al.* (2008). The most important variable explaining POP profiles in common dolphin blubber was individual feeding history, while those in porpoises were more strongly related to individual condition. A substantial proportion of individuals in the BIOCET sample had contaminant levels above a threshold PCB level that has been reported to have adverse health effects. The threshold in question is 17 $\mu g\ g^{-1}$ PCB lipid weight, which was derived by Kannan *et al.,* (2000) and is based on experimental studies of both immunological and reproductive effects in seals, otters, and mink. This threshold was frequently exceeded in both *P. phocoena* (47% of individuals) and *D. delphis* (40%), especially *P. phocoena* from the southern North Sea (74%) and *D. delphis* inhabiting waters off the French coast (50%). Within the *D. delphis* sample, the incidence of pregnancy was negatively related to contaminant burdens. However, this relationship did not conclusively demonstrate that high POP concentrations inhibit pregnancy in this species since, for example, infertility may allow high levels of POPs to bioaccumulate

[1] Based on 27 mature females, 1988-1995 (M. Addink, T.B. Sørensen, M. Garcı´a Hartmann, H. Kremer, unpublished data).

(Pierce *et al.*, 2008). The analysis of effects of POPs on reproduction in Pierce *et al.* (2008) was restricted to consideration of pregnancy rates. However, additional information on reproductive status is available from examination of ovarian scars.

In order to further investigate the effects of POPs on reproduction in both these species, contaminant levels have to be assessed against an index of reproductive activity. It has been reported that *corpora albicantia* (ovarian scars of ovulation and pregnancy) persist throughout the life of some marine mammals, as a consequence of the large amount of connective tissue present and poor vascularisation (Stewart and Stewart, 2003) and therefore provide an index of the number of past ovulations (Perrin and Donovan, 1984), i.e. complete reproductive activity. However, contrasting results have highlighted inconsistencies with this theory. In some small delphinids, it has been proposed that CAs of infertile ovulations are more likely to be resorbed than those of pregnancy, which has been suggested in young mature female bottlenose dolphins (Harrison *et al.*, 1972; Perrin and Reilly, 1984, and ref. therein). Whereas, the ovaries of senescent female *Stenella* spp. are withered with fewer CAs present compared to ovaries of younger females, suggesting that some CAs may eventually be resorbed (Perrin and Reilly, 1984, and ref. therein). More recent studies by Brook *et al.,* (2000) and Dabin *et al.,* (2008) have further questioned the assumptions of CA persistence in the ovaries of small delphinids.

Marine mammals provide information on the chemicals which present the greatest risk to consumers at the top of the food chain, something that cannot be adequately described or predicted in laboratory models (Ross, 2000). Therefore, the current study will focus on two cetacean species that feed on commercially important fish species in the ENA, the common dolphin and harbour porpoise, incorporating data produced by the EC-funded 5th Framework BIOCET project and data from a control group study which was funded in-part by the UK Department of the Environment, Food and Rural Affairs (Defra) Marine Research Program and also by ASCOBANS. In order to assess the possible long-term effects of POPs on the continued viability of these populations, we investigated the impact of POPs on reproductive activity in females. Initially, investigations were undertaken to assess the significance, in terms of our understanding of individual reproductive history, of *corpora albicantia* in the ovaries of *D. delphis* and *P. phocoena*. The relationship between accumulation of corpora scars and contaminant burdens was examined, taking into account the health status of the individual. Further, variations in contaminant burdens were assessed between mature females in different reproductive states (resting mature, pregnant and lactating) and, between nulliparous, primiparous and multiparous females. Finally, we investigated whether increased contaminant burdens inhibit ovulation or pregnancy.

The research undertaken in the current study has important implications for the conservation of both these species in the ENA. If the results identify that contaminants have an adverse effect on individual reproductive capabilities, the species would be more vulnerable to exploitation than is normally assumed, especially from other anthropogenic activities such as incidental capture, and would not necessarily recover from exploitation in a predictable way.

2. Methods

2.1 Sampling programme

In all cases, data collection protocols followed European Cetacean Society guidelines for gross post-mortem examination and tissue sampling (Kuiken and Garcia Hartmann, 1991). Phase one analysed samples collected by the EC-funded BIOCET project, which included partners from a number of European national marine mammal strandings schemes. Stranded *P. phocoena* and *D. delphis* were sampled between 2001 and 2003 (see Pierce *et al.,* 2008 for further information and distribution maps), and ranged in decomposition states from fresh (CC2) to moderately decomposed (CC3) (see Kuiken and Garcia Hartmann, 1991). Females recovered in fresh condition, from which all necessary samples could be obtained, were prioritised for contaminant analysis; resulting in sample sizes of 70 *D. delphis* obtained from Ireland, France and Spain and 67 *P. phocoena* from Ireland, Scotland, southern North Sea (the Netherlands, Belgium, France) and Galicia[2]. Due to funding constraints, health status and cause of death were not determined for all individuals with estimated body burdens of contaminants and therefore these variables were not included in the statistical analysis. Data and samples for assessing reproductive status, such as gonads and teeth, were collected from all stranded females, when possible, throughout the sampling period of the project; resulting in sample sizes of 177 *D. delphis* and 99 *P. phocoena*. The *D. delphis* samples obtained from France included those originating from a mass live stranding event that occurred in February 2002 at Pleubian, Brittany. The group comprised adult (7+ years old) females accompanied by their unweaned calves. Of the 53 individuals found dead, 52 were fully necropsied (Dabin *et al.*, 2008; Viricel *et al.*, 2008),

[2] Galician sample was composed of only three immature females

and reproductive data from 49 females were available for the current study. In a previous paper, Dabin *et al.* (2008) assessed the use of ovarian scars to reconstruct individual reproductive histories. Their analysis included data from the Pleubian mass live stranding event.

In order to account for the effects of health status and cause of death in the analysis, a follow-up control group study was undertaken during phase two. 43 female *D. delphis* taken incidentally as bycatch in fishing gear, and which subsequently stranded along the UK coastline, were designated as the control group. Cause of death was determined during detailed post-mortem examinations carried out by the UK Defra-funded Cetacean Strandings Investigation Programme (CSIP; Jepson *et al.,* 2005). Individuals stranded between 1992 and 2004, and the majority were found along the southwest coast of the UK (95%), between December and March (88%). Pathological investigations, including gross examination and histological, bacteriological and/or virological analyses, identified whether dolphins were suffering from any infectious or non-infectious diseases that might inhibit reproduction (see Jepson, 2005). The control group sample was divided into three health status categories: category 1 - healthy individuals; category 2 - health of individuals mildly compromised (but may still be capable of successfully reproducing); and category 3 - individuals suffering from severe (and potentially fatal) infectious or non-infectious disease. 93% of the sample was assessed as category 1 (see Table 1). Furthermore, where nutritional information was available (n = 40), 82% were classified in good and 15% in moderate condition, and only one individual was in poor nutritional condition (3%).

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). Between 90 and 95% of the total body burden of many POPs, particularly PCBs and DDTs, is found in the blubber because of its high lipid content (Aguilar, 1985). 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 lamination of the blubber, and were stored frozen at -20° C. During transport, samples were packed in insulation boxes with dry ice to ensure that they remained frozen.

Teeth (N≥5) 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 uterus was examined for presence of a foetus, and the ovaries were collected and preserved in 10% neutral buffered formalin. Milk glands were examined for evidence of lactation by cutting through the mammary glands, and noting if milk or colostrum was present in the sinuses.

2.2. POP measurements

For the BIOCET data, analysis of POP concentrations in cetaceans was carried out at the Royal Netherlands Institute for Sea Research (NIOZ), with some Scottish harbour porpoise samples analysed at the UK Centre for Environment, Fisheries and Aquaculture Science (Cefas). For information on the methodologies used in determining POP concentrations, and comparisons between laboratories, see Pierce *et al.,* (2008). Eighteen PCB congeners were selected for analysis within the BIOCET project (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 using porpoise data. Other chemicals analysed were *p,p'*-DDE, which is the most persistent metabolite and the major representative of the insecticide DDT-group, and brominated flame retardants such as brominated diphenyl ethers (PBDE congeners: BDE47, BDE99, BDE100, BDE153 and BDE154) and hexabromocyclododecane (HBCD isomers: α, β, and γ) - the principal brominated flame retardant in polystyrene foams used in the building industry. Due to funding constraints, data on HBCD concentrations were only available for 44 *P. phocoena* and 60 *D. delphis*, whereas for other POPs the sample size increased to 67 and 70, respectively. For the *D. delphis* control group study (n = 43), the sixteen selected PCB congeners and *p,p'*-DDE were analysed by Cefas.

As mentioned previously, a Σ-PCB level of 17 $\mu g\ g^{-1}$ lipid has been reported as a threshold level for adverse health effects in marine mammals (Kannan *et al.,* 2000; 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). Using thresholds in this way warrants caution owing to possible differences in species sensitivities; however, as in Jepson *et al*. (2005), it is proposed that this threshold blubber concentration for adverse health effects should provide a benchmark for interpreting whether associations between reproductive activity and PCB exposure are biologically significant.

2.3. Determination of age and reproductive status

All teeth and gonadal material obtained during the BIOCET project were analysed, irrespective of whether contaminant burdens were not investigated. Where data were available, teeth and gonadal samples from the control group were also analysed. Age was determined by analysing growth layer groups (GLGs) in the dentine of teeth, following Lockyer (1995). 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, usually from separate labs. In cases where there was disagreement, teeth were re-examined by readers and an age and/or an age range was agreed. As ages were recorded by a number of different researchers, cross-calibration exercises were carried out - for further information see Rogan *et al.* (2004).

Before examination, the preserved ovaries were rinsed in water for 24 hours and then replaced in their containers with 70% ethanol. For each ovary, the maximum length, height, width (mm) and weight (g) were recorded. Corpora scars present on the ovary were classified into *corpora lutea*, regressing *corpora lutea*, and *corpora albicantia*. The *corpus luteum* (CL) is an endocrine gland and is easily recognisable on the ovary as a pronounced distension, usually yellow in colour as a result of the yellow pigments of the carotenoid luteins. A regressing CL has been defined as a luteal structure that has started to regress, and appears faintly yellowish in gross observation. A regressing *corpus luteum* CL eventually gives rise to a tissue scar called a *corpus albicans*. A *corpus albicans* (CA) can appear as a spherical knob or as raised, wrinkled scar and it is easily recognisable on the cut surface as a pale fibrotic area. CAs are composed of white connective tissue that becomes fragmented with age. Ovaries were hand-sectioned into 0.5-2mm slices and examined internally under a binocular microscope for the presence of additional corpora scars. Females were considered sexually mature if the ovaries contained at least one *corpus luteum* or *albicans*. Pregnancy was established by the presence of an embryo/foetus due to the difficulty, during gross and histological examinations, in distinguishing a CL of pregnancy from a CL of ovulation. Females were classified into five reproductive states: immature, pregnant, pregnant & lactating, lactating, and resting mature (not pregnant or lactating). For the BIOCET dataset an additional category “pathological” was included, based on gross (and occasionally histological) examination of abnormalities of the reproductive system; i.e. ovarian cysts, uterine bodies, mastitis, and early mammary gland development and lactation in immature individuals.

2.4. Data analysis

For the BIOCET dataset, generalised linear models (GLM) and generalised additive models (GAM) were used to model individual variation in numbers of CAs in relation to a series of explanatory variables, namely reproductive status (pregnancy), average CA size, geographic location (region), age, POP concentrations and condition (proxied by dorsal blubber thickness) for both *D. delphis* and *P. phocoena*. Seasonality was not taken into account within the analysis due to sampling biases in the strandings data, as the majority of individuals stranded during the first quarter (Jan-Mar). In principle, CA numbers are expected to follow a Poisson distribution but models were checked for over-dispersion of the response variable, and a quasi-Poisson distribution was used when slight over-dispersion as detected. Average CA size (mean length of CAs on the left ovary) was modelled as a function of age and the number of CAs present, using GAM, in this case assuming a Gaussian distribution. For analysis of geographical variation, BIOCET samples were grouped into five regions: Scotland, Ireland, southern North Sea (Netherlands, Belgium and the French coast north of Calais), France (Biscay coast of France) and Galicia. Concentrations of PBDEs and HBCDs in harbour porpoise were log-transformed due to their highly skewed distributions.

The advantage of GAM over other regression-type models is that it is not necessary to assume linear relationships between response and explanatory variables. Non-linear relationships are captured as "smoothers" (Hastie and Tibshirani, 1990; Zuur *et al.*, 2007). However, if all relationships prove to be approximately linear, GLM can then be used instead. When fitting smoothers, the maximum complexity of the resulting curve can be constrained by setting an upper limit to the number of "knots". We used a maximum value of 4 to avoid over-fitting, i.e. to avoid the fitting of unrealistically complex relationships. Explanatory variables to be retained in the final model were selected using a combination of forwards and backwards selection. Several alternative methods are available to evaluate goodness of fit and thus select the best model. We selected the model with the lowest value for the Akaike Information Criterion (AIC), in which all remaining explanatory variables have significant effects (as determined by F, t or Chi-squared tests, depending on the distribution assumed for the response variable and whether the explanatory variable was assumed to have a linear effect or not), and there are no obvious patterns in the residuals. Where an explanatory variable was marginally significant (p~0.05), an F test was used to compare models with and without the variable in question and if the difference was not significant, the simpler model was accepted (see Zuur *et al.*, 2007). All GAMs were fitted using BRODGAR 2.6.5. (www.brodgar.com), an interface for the R statistical programming language. Additional comparisons of CA numbers were carried out using the Mann-Whitney test.

3. Results

3.1.1 P. phocoena – BIOCET sample

Reproductive status was determined for 99 female harbour porpoises from European waters, of which 62% were immature. Three Dutch female *P. phocoena* were classed as pathological, based on milk gland pathology. Two of these individuals would have been described as mature based on age, body length and development of mammary gland tissue, but no corpora scars were present on the ovaries. Of the 38 mature females, 11 were pregnant, two were pregnant and lactating, six were lactating, 15 were classed as resting mature and four as pathological. Resting mature female *P. phocoena* had the highest average number of corpora scars (average = 7.8 scars, range 1-16, n = 15), followed by lactating (average = 7.5, range 3-11, n = 6) and pregnant females (average = 4.7, range 1-17, n = 10). However, no significant variation in number of corpora scars (*Corpora albicantia* and *lutea*) was observed between these three reproductive groups (Mann-Whitney test, p>0.05).

3.1.2 *D. delphis– BIOCET sample*

Reproductive status was determined for 177 female common dolphins, of which 103 (58%) were sexually mature (See Table 2). Of the 103 mature females, 22 were pregnant, nine were pregnant and lactating, 13 were lactating, 54 were resting mature and five were classified as pathological. Resting mature female *D. delphis* had the highest average number of corpora scars (average = 17.8, range = 2–34, n = 40), followed by pathological (average 16.2, range 3-26, n =5) and lactating (average = 15.5, range 2-26, n=12) females. Pregnant (average = 9.4, range =1-23, n = 20) and pregnant & lactating (average = 9.7, range = 2-19, n = 9) females had the lowest average number of corpora scars. Resting mature female *D. delphis* had a significantly higher number of corpora scars than pregnant (Mann-Whitney test, $p = 0.0003$, $n = 60$) and pregnant & lactating females (Mann-Whitney test, $p = 0.0043$, $n = 49$); 88% of the resting mature sample had ≥ 10 corpora scars. Further, lactating females had a significant higher number of corpora scars than pregnant females (Mann-Whitney test, $p = 0.0493$, $n = 22$).

3.2. Size and number of corpora albicantia – BIOCET sample

Corpora albicantia (CAs) were recorded on both ovaries in *D. delphis*, although very few scars were observed on the right ovary in *P. phocoena* (see Figure 1). In *P. phocoena*, average CA size was independent of the number of *corpora albicantia* present, though the sample size was small ($n = 19$), and there was also no significant relationship with age. In *D. delphis*, the model for average CA size explained 41.4% of deviance ($n = 71$) and included strong effects of age (an asymptotic curve, with a positive effect of age up to around age 15, estimated degrees of freedom = 2.01, $F=5.236$, $p = 0.0077$), and total number of *corpora albicantia* (negative, edf = 1.49, $F=29.167$, $p <0.0001$). Overall, average CA size decreased with increasing number of *corpora albicantia* - due to an increase in the number of smaller CAs present on the ovaries. There was no significant correlation between the number of corpora scars and age for sexually mature *P. phocoena* ($r^2 = 0.004$, $p = 0.735$, $n = 32$; Figure 2a) or *D. delphis* ($r^2 = 0.036$, $p = 0.094$, $n = 79$; Figure 2b).

A GAM fitted to data on both immature and mature porpoises, testing for effects of age, condition, region and pregnancy indicated that the number of CAs in porpoise ovaries was significantly related to age, condition and region. This model, which assumed a quasi-Poisson distribution for the response variable, explained 70.8% of deviance ($n = 81$). The age effect (edf = 2.83, $F = 13.89$, $p <0.0001$, $n = 88$; Figure 3a) unsurprisingly reveals an increase in CA numbers up to an age of around 5 (soon after attainment of sexual maturity), while the effect of blubber thickness (condition) was weakly negative (edf = 2.71, $F = 3.91$, $p = 0.0147$; Figure 3b). Irish porpoises had a higher number of CAs than Scottish porpoises ($t = 2.050$, $p = 0.0440$). Although the overall effect of region was not significant ($F = 3.915$, $p = 0.0641$), its inclusion significantly improved the final model ($F = 2.887$, $p = 0.0386$). It should be noted that the data include one very old French animal (24 years of age) while the next oldest individual was 15 years old.

A (quasi-Poisson) GAM for the number of *corpora albicantia* in *D. delphis* ovaries in relation to age, pregnancy and region explained 72.8% of deviance. The number of CAs reaches an asymptote around age 12 (edf = 2.94, $F = 16.92$, $p < 0.0001$, $n = 123$; Figure 3c) and fewer CAs were present in the ovaries of pregnant females ($F= 23.11$, $p <0.0001$). There was also regional variation ($F = 4.09$, $p = 0.0085$), with animals from Galicia having fewer CAs than animals from Scotland ($t = -2.81$, $p = 0.0059$). Blubber thickness data were missing for Galicia. If blubber thickness is included in the model (reducing sample size to 100 animals), effects of age and pregnancy remain, the regional difference disappears (presumably due to the absence of Galician data) and blubber thickness (condition) is seen to have an almost linear positive effect (edf = 1.45, $F = 6.13$, $p = 0.0074$; Figure 3d).

3.3. Effects of POP on reproductive activity – BIOCET sample

The GAM for the number of *corpora albicantia* on porpoise ovaries was improved by adding an effect of $\sum$16PCB congeners concentrations (87.5 % of deviance explained, $n = 59$). As in the previous model, the number CAs rose to reach an asymptote around age 5 (edf = 3.00, $F = 9.05$, $p < 0.0001$), decreased as blubber thickness increased (edf = 2.15, $F = 10.14$, $p = 0.0001$) and showed significant regional variation ($F = 7.04$, $p = 0.0020$), with Irish animals having more CAs in their ovaries than Scottish animals ($t = 3.31$, $p = 0.0018$). The number of CAs was lower at higher PCB concentrations (edf = 2.02, $F = 9.50$, $p = 0.0003$, Figure 4a). Substituting PBDE concentrations for PCBs, no significant effect of PBDEs was found. However, a significant effect was seen for HBCDs, with fewer CAs present in animals with higher HBCD concentrations (edf = 1.36, $F = 4.80$, $P = 0.0266$; Figure 4b). It should be noted that this latter model contained effects of age and blubber thickness as before but no effect of region, and explained 80.7% of deviance ($n = 36$). A significant effect was also seen for DDE concentration (edf = 1.72, $F = 7.35$, $p = 0.0026$; Fig. 4c). In this model, effects of age and blubber thickness were again retained, and the previously seen regional difference was more pronounced ($F = 5.41$, $p = 0.0075$). The model explained 85.5% of deviance ($n= 59$). Note that models in which the concentration of one category of POPs was included as a predictor were not improved by adding the concentration of a second category of POPs as an additional predictor.

The models for numbers of *corpora albicantia* in common dolphin ovaries (with or without blubber thickness included) were not improved by including PCB burden ($\sum$18PCB congeners) as a predictor. Similarly, the models were not improved by including PBDE, HBCD or DDE concentrations as predictors.

3.4.1 Threshold level – BIOCET sample

No significant relationship between age and PCB burden [(∑7ICES congeners)*3] was observed for either *P. phocoena* (r^2 = 0.008, p = 0.490, n = 60) or *D. delphis* (r^2 = 0.000, p = 0.965, n = 66). The highest PCB burden was reported in *P. phocoena*, with 120 µg g^{-1} lipid reported in individual, compared to a maximum of 84.54 µg g^{-1} lipid in *D. delphis* (see Figure 5). High concentrations were reported in immature *P. phocoena*, ranging from 1.93 to 60.9 µg g^{-1} lipid, of which 42% had contaminant loads above the threshold level of 17 µg g^{-1} lipid for adverse health effects. All pregnant *P. phocoena* sampled had contaminant loads below 20 µg g^{-1} lipid (Figure 5a). A decline in contaminant load with increasing corpora number (*Corpora albicantia and lutea*) was observed, which was significant for resting mature females (p = 0.010, n = 9), though not for the whole sexually mature female *P. phocoena* sample (p = 0.105, n = 21; excluding two "pathological" females). In contrast, a significant increase in corpora number and PCB burden was observed for sexually mature *D. delphis* (r^2 = 0.1263, p = 0.029, n = 38; Figure 5b). When the threshold level was applied to these data, resting mature females (not pregnant or lactating) composed 83% of the mature sample above this level. In general, *D. delphis* with high contaminant burdens above the threshold level for adverse health effects were resting mature females with high numbers of corpora scars.

3.4.2 Threshold level – Control group sample

In the control group of "healthy" bycaught *D. delphis* not suffering for any infectious or non-infectious disease that would inhibit reproduction, a significant increase in corpora number with age was observed in sexually mature individuals - although the sample size was small (p = 0.002, r^2 = 0.44, n=19; Figure 6). Corpora number ranged from 1 to 16 (n = 23) in mature females, which is in contrast to the high scar numbers observed within the BIOCET data (Table 2). 51% of mature female BIOCET *D. delphis* had ≥17 corpora scars and 84% had ≥ 11 corpora scars (range 1-34). Within the control group, all sexually immature (nulliparous) females (range 17.2-93.6 µg g^{-1} lipid) and the three "primiparous" pregnant females (range 32.3-77.82 µg g^{-1} lipid) had total blubber PCB levels above the threshold level (Figure 7a, b). The two aged "primiparous" pregnant females were 8 and 10 years old. Although not significant - though similar to the results obtained using the BIOCET harbour porpoise data - a decline in blubber PCB levels with increasing corpora number was observed in mature females (see Figure 7b), and a similar plot was obtained when ∑18PCBs was plotted against corpora number (not shown).

Further, a non-significant decline in DDT burden against corpora number was also observed (Figure 7c).

4. Discussion

4.1 Accumulation and Persistence of corpora scars

Many of the common dolphins and harbour porpoises in the present study had high numbers of *corpora albicantia* in their ovaries and we can be reasonably certain that these could not all indicate past pregnancies, which suggests that CAs from infertile ovulations are common in free-ranging small cetacean populations. Takahashi *et al.* (2006) reported that collagenous fibrous tissue in regressing *corpora lutea* from *D. delphis* off Japan was replaced by elastin tissue, a material which has a reported half life of 40-70 years. They were unable to differentiate CAs from pregnancy with those from ovulation, and it was assumed that smaller CAs - containing <15% elastin - were derived from infertile ovulations. With a reproductive period of c.19 years reported for the ENA *D. delphis* population (Murphy et al. 2009), results from Takahashi *et al*. (2006) study suggest that CAs, of pregnancy at least, do persist throughout the lifetime of a female common dolphin.

Pregnant and pregnant & lactating female BIOCET *D. delphis* had significantly lower number of corpora scars than resting mature females. In contrast, a lack of significant variation was observed in corpora number between reproductive groups in BIOCET *P. phocoena*. In addition, the number of corpora scars in porpoise ovaries tended to be lower than in common dolphins, at a given age after attaining sexual maturity (see Figure 2). Within the BIOCET sample, the estimated annual pregnancy rate (APR) in *P. phocoena* (42%) was higher than in *D. delphis* (25%) (Pierce *et al.,* 2008), and the calving interval (using the BIOCET data) for *P. phocoena* (1/APR = 2.4 years) is much shorter than in *D. delphis* (4 years). Note however that the APR for *P. phocoena* is almost certainly underestimated from strandings, being incompatible with mortality rate data (see Learmonth et al, in prep.), whereas the APR for *D. delphis* is similar to that proposed for the ENA population (26%; Murphy et al. 2009). These data suggest that harbour porpoises are pregnant more of the time than common dolphins, and would therefore possibly ovulate less; common dolphins are more likely to undergo repeated unsuccessful ovulations during their extended calving interval. This difference in number of corpora scars may also be related to social organisation, i.e. porpoises do not form large social groups (outside the breeding period) so the occurrence of fertile non-breeders undergoing multiple ovulations is less likely, or as a result of a number of other life history and population traits (and/or variations in species capabilities to metabolise PCBs by cytochrome P-450).

In *D. delphis* off the French Atlantic coast, Dabin *et al*. (2008) suggested that all ovarian scars do not persist, and their number at any one time would be a function of rates of ovulation and healing, the latter being defined here as the resorption or disintegration of CA tissue. CA counts differed between individuals of distinct reproductive status. Pregnant (CA counts = 8.8 ± 5.9, range 1–22, n = 32) and pregnant/lactating females (CA counts = 11.3 ± 4.9, range 5–18, n = 6) exhibited lower numbers of CAs than those observed in resting mature females (CA counts = 15.4 ± 8.6, range 1–34, n = 60), which is similar to the BIOCET sample in current study. Dabin *et al*. (2008) proposed that most CAs would heal quickly, with a half-life of <1 year (the time after which half of the CA has disappeared) - though larger CAs (possibly from pregnancy) may persist longer than smaller CAs (possibly from unsuccessful ovulations). This was based on the following observations: CA number did not increase significantly with age; pregnant *D. delphis* had c. 40% fewer scars than non-pregnant animals; and since all pregnant individuals were sampled between January and March, prior to the estimated calving period for the population. In contrast, studies undertaken on other populations of *D. delphis*, by Danil and Chivers (2007) in the eastern tropical Pacific, Westgate and Read (2007) in the western North Atlantic and Takahashi *et al.,* (2006) in waters off Japan have all reported a significant increase in corpora number with age.

Both *D. delphis* and *P. phocoena* in the ENA appear to have a more extended mating/conception period than other populations inhabitating temperate waters. Mating and calving periods in *D. delphis* and *P. phocoena* have been reported from May to September (Murphy, 2004) and May to August (Learmonth, 2006), respectively, although peaks in reproductive activity were noted in both populations (Murphy, 2004; Murphy *et al.*, 2005; Learmonth, 2006). Repeated ovulations during a nine month period (7 ovulations) have been observed in a captive *D. delphis* (Kirby and Ridgeway, 1984), though it is not known if this individual was obtained from a population that exhibited reproductive seasonality. Off the Irish coast, mature and pubertal females were only reported ovulating during the mating period, May to September (6 out of 45 individuals examined; Murphy, 2004). With a reproductive cycle lasting c.27 days (range 17-36 days; Atkinson and Yoshioka, 2007; Robeck *et al.,* 2005) in *Tursiops truncatus*, *P. phocoena* and *D. delphis* in the ENA could undergo four and five infertile ovulations, respectively, within their mating periods. However shorter or longer estrus cycles may occur - estrus cycles of 31 days (n = 22) have been reported in Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) (Robeck *et al.,* 2009). Gross and histological examination of ovarian material for *D. delphis* off the Irish coast suggested that two individuals had ovulated five times within one estrus period (Murphy, 2004). In both cases these dolphins died during September, and for one individual the final ovulation had resulted in pregnancy.

In the Dabin *et al.* (2008) study, lactating *D. delphis* had the highest numbers of CAs with an average number of 20 scars (range 11–26, n = 8), slightly higher than that obtained in lactating BIOCET *D. delphis* in the current study (average = 15.5, range 2-26, n=12). Although these sample sizes are small, results do not suggest a CA half-life of less than one year; taking into account the estimated lactation period for this population is 10.4 months (Murphy, 2004), a gestational period of c.12 months (Murphy *et al.,* 2009) and reproductive seasonality i.e. not ovulating outside the mating season. In contrast, a significantly lower number of corpora scars (Mann-Whitney test, p = 0.046, n = 19) were reported in the ovaries of lactating *D. delphis* in the control group sample (average = 7.3, range = 2-16, n = 7) compared to lactating BIOCET *D. delphis*. Furthermore, a significantly lower number of corpora scars (Mann-Whitney test, p = 0.0007, n = 51) were observed in the ovaries of resting mature *D. delphis* in the control group (average = 8.8, range = 1-16, n = 11), compared to the resting mature BIOCET *D. delphis* (average = 17.8, range = 2–34, n = 40). Interestingly, there was no significant difference in corpora scar number between resting mature *D. delphis* in the control group, and pregnant *D. delphis* in the BIOCET sample (average = 9.4, range =1-23, n = 20). One BIOCET French resting mature individual, which was part of the mass live stranding event at Pleubian, had 31 ovarian scars and was aged at only 8 yrs. It cannot be ruled out that some *corpora albicantia* present on the ovaries of this individual could be as a result of accessory *corpora lutea* and/or ovulatory disorders, though further (histological) analysis is required to fully account for the high scar number in this young mature female.

In *D. delphis*, it has been suggested sexual maturity can be marked by the onset of a variable number of successive estrus cycles not resulting in pregnancy (Collet and Harrison, 1981). Gaskin *et al.* (1984) reported multiple CAs in four and five-year old *P. phocoena* from the Bay of Fundy, ranging from 1-12 and 2-15 scars, respectively; individuals attain sexual maturity between two and four years of age in this population (Read, 1990). Further, as can be seen in Figure 2, large-scale individual variation also occurred in corpora count data at a given age for both BIOCET *P. Phocoena* and *D. delphis*. For example, at four years of age corpora number ranged from 1 to 7 scars in mature *P. phocoena*. These data suggest multiple infertile ovulations at the onset of sexual maturity in some individuals. However, within the control group sample only one corpora scar was present in the ovaries of the three "primiparous" females, aged 8 and 10 years, thus indicating that *D. delphis* can become pregnant after only one estrus cycle.

The variation in number of ovarian scars at a given age and/or ovulation rates between individuals and the lack of correlation between age and corpora number for the *D. delphis* and *P. phocoena* BIOCET samples, does suggest the possibility of resorption of corpora scars. In addition to resorption though, the large individual variation in corpora scar count data may also be as a consequence of (1) variation in age at attainment of sexual maturity between individuals; (2) variations in estrus cycle length; (3) ovulatory disorders; (4) health status of the female, some females might not be capable in conceiving or carrying foetuses to full term due to poor nutritional condition, disease, infection, or other pathological reasons; (5) length of time before a young mature female attains the status of breeding "cow" within the social structure, as during this time a variable number of successive estrus cycles not resulting in pregnancy could occur (Collet and Harrison, 1981); and (6) the possibility of breeding and non-breeding mature females within *D. delphis* social groups, as non-pregnant females may not reproduce but help in the nursing of calves. Therefore, females that were unable to become pregnant either as a result of age, dominance hierarchies (a higher probability for *D. delphis*) or poor health could continue to ovulate (infertile ovulations) and accumulate higher numbers of corpora scars.

It has been noted in short-finned pilot whales *Globicephala macrorhynchus*) that, although CAs are believed to be retained indefinitely, estimating the rate of accumulation of scars is difficult because of variation in the age at attainment of sexual maturity and variation in the ovulation rate within an individual's reproductive lifespan (Marsh and Kasuya, 1984). Interestingly, within the control group of "healthy" individuals, a significant positive correlation between age and corpora number was observed in mature female *D. delphis*, though some data scattering was observed (see Figure 6). Further, a significantly lower number of CAs were observed in the ovaries of the *D. delphis* control group sample compared to the BIOCET *D. delphis* sample (Mann-Whitney test, $p = 0.0001$, $n = 108$; see Table 2). As 52% of the mature BIOCET *D. delphis* sample with corpora count data was obtained from a single mass live stranding event in Pleubian in 2002, this may have caused potential biases within that sample. For the majority of mature individuals in the control group an increase in corpora scar number (≥4 CAs) was not obvious until after 15 years of age. Therefore once other factors that may effect reproduction such as various infectious and non-infectious diseases and poor nutritional status were taken account, individuals appeared to ovulate less. Whether or not this reflects a lower number of unsuccessful ovulations in these "healthy" females, i.e. pregnant more often, necessitates further investigation. Only one animal was reported in poor nutritional condition in the control group, which is the oldest aged female within the ENA population. The individual in question was a 30-year old resting mature female with a high contaminant burden of 67.4 μg g^{-1} lipid [(Σ-ICES7 PCB congeners)*3], and corpora scar number (10 CAs). Following a live stranding event the female was euthanised and the postmortem examination revealed evidence of recent net entanglement; the poor nutritional status was attributed to a period of starvation as a result of the traumatic injuries sustained.

Inconsistencies were observed between both species in the current study. Apart from *P. phocoena* displaying lower numbers of corpora scars at a given age after attaining sexual maturity, they also exhibited lower right ovarian activity. There may also be inter-species differences in persistence of ovarian scars, as the presence of elastin has not been assessed in harbour porpoise CAs. In Hawaiian monk seals (*Monachus schauinslandi*), as collagenous fibrous tissues are not replaced by elastic fibrous tissues, CAs (including pregnancy) do not persist (Iwasa and Atkinson, 1996). Further, data obtained from a long-term study using ultrasound on a captive bottlenose dolphin indicated that *corpus albicans* derived from a *corpus luteum* of pregnancy persist in the ovaries, while those arising from infertile ovulations completely disintegrate and, are ultimately resorbed (Brook *et al.*, 2002). The individual examined ovulated 18 times over a 12-year period in captivity, and produced three calves. At death, aged 18 years, only three *corpora albicantia* were recorded, measuring 3-4 mm in diameter. In contrast, gross and histological examinations revealed c.15 CAs on the ovaries of another bottlenose dolphin, that was known to have at least five ovulations and one calf in captivity (Kirby and Ridgeway, 1984).

Data within the current study do not add further proof to theory of resorption of corpora scars, nor suggest an average half-life (the time after which half of the CAs has disappeared) of <1 year for a CA. The higher number of corpora scars present on the ovaries of resting mature females within the BIOCET sample could be attributed to a higher number of infertile ovulations for a number of reasons, as outlined earlier. In addition: (1) corpora scar number increased significantly with age in the *D. delphis* control group sample of "healthy" individuals, (2) a lack of significant variation in corpora count data between resting mature *D. delphis* in the control group and pregnant *D. delphis* in the

BIOCET sample, and (3) no significant variation in corpora count data between pregnant and resting mature *P. phocoena.* Even though taking in account these results, we have not fully disproved the theory of resorption and further investigations are required to assess the disintegration/resorption of CAs at a histological level in both species. Based on these results we believe that it is acceptable to use number of corpora scars as an index of reproductive activity, though we do take into consideration the biases that may result from this approach.

4.2 Effects of POPs on reproductive activity

For the BIOCET data in the current study, *D. delphis* with the highest PCB burdens (and above the threshold level) were resting mature females (not pregnant or lactating). Further, these individuals also had the highest number of scars of ovulation on their ovaries, which suggested that due to high contaminant burdens female common dolphins may be unable to reproduce and thus, continue ovulating; or females are not reproducing for some other reason, either physical or social, and started accumulating higher levels of contaminants in their blubber. The high associated PCB burden may thus be either (or both) the cause of infertility or the consequence of infertility. In contrast in harbour porpoises, once the effect of age and nutritional condition were taken into account, the data so far suggests that higher POP concentrations (PCB, HBCD and DDE) tended to be associated with lower numbers of corpora scars, possibly indicating that high contaminant levels were inhibiting ovulation.

Redundancy analysis undertaken on the BIOCET data by Pierce *et al.,* (2008) indicated that the number of *corpora albicantia* ($p = 0.007$) and incidence of pregnancy ($p = 0.029$) were related to concentrations of POPs (PCBs, PBDEs and DDE; excluding HBCDs) in the blubber tissue of *D. delphis*. In contrast, reproductive variables were not related to concentrations of POPs in the blubber of harbour porpoises although there were relationships between the latter and concentrations of zinc and other heavy metals. In humans, infection has been associated with zinc redistribution, and high concentrations observed in the liver were due to acute-phase protein synthesis (Scott, 1985; Amdur *et al.*, 1991; Pierce *et al.,* 2008). Further, as high concentrations of Zn have previously been associated with poor health in *P. phocoena* (Das *et al.*, 2004), it was used to provide an index of the poor health status in this species in the Pierce *et al.* (2008) study; cause of death was determined for 68% of the *P. phocoena* BIOCET POP sample and of this, 57% died from pathological causes. A causal (immunotoxic) relationship has been reported between PCB exposure and infectious disease mortality in UK harbour porpoises (Jepson *et al*., 2005). Among adult stranded female *P. phocoena*, PCB levels were significantly higher in individuals classified in the infectious disease group than in animals classified in the physical trauma group (died from incidental capture and bottlenose dolphin attacks), and females dying of infectious disease had significantly poorer nutritional status (relative body wt and mean blubber thickness) compared to the physical trauma group (Jepson *et al*., 2005). This casual immunotoxic relationship may therefore have masked any direct affects of POPs, through lowering immunity, on reproductive activity/output in *P. phocoena* in the current study.

In order to eliminate any affects of infectious and non-infectious disease on reproductive activity, contaminant data and gonadal material were analysed from a control group of "healthy" *D. delphis.* The threshold for adverse health effects in marine mammal, of 17 $\mu g\ g^{-1}$ lipid was applied to these data [(Σ-ICES7 PCB congeners)*3] in order to provide a benchmark for interpreting whether associations between reproductive activity and PCB exposure are biologically significant. Within the control group, all sexually immature (nulliparous) females (range 17.2-93.6 817 $\mu g\ g^{-1}$ lipid) and the three primiparous pregnant females (range 32.3-77.817 $\mu g\ g^{-1}$ lipid) had contaminant loads above the threshold level, suggesting that high PCB burdens are not inhibiting ovulation, conception or implantation in *D. delphis*. As mentioned previously, Reijnders (1986; 2003) reported a decrease in reproductive success in harbour seals which was possibly due to implantation disruption. However, as pinnipeds experience delayed implantation/embryonic diapause, they may be more vulnerable than cetaceans at this stage of the reproductive cycle. Studies on mink (*Mustela vison*) have also reported that PCBs can impair reproduction; although ovulation, conception and implantation occur, fetues died during gestation or shortly after birth (Jensen *et al.*, 1977; Reijnders, 1986; Backlin and Bergman, 1992; Backlin and Bergman, 1995; Schwacke *et al.,* 2002). This was attributed to either hormonal disturbance, direct dominant-lethal action or to an embryo lethal effect caused by toxicants (Reijnders, 1986). It should be noted that the Kannan *et al.,* (2000) PCB threshold of 17 $\mu g\ g^{-1}$ lipid is less protective than that proposed of 10 $\mu g\ g^{-1}$ lipid, which was associated with increased calf mortality in wild bottlenose dolphins (Hall *et al.*, 2006b; Hickie *et al.,* 2007). Further, the analysis in the current study only assessed the effects of 16 CB congeners on reproductive activity, and did not include the most immunotoxic IUPAC congeners, such as CB77 and CB126.

Relative low-level exposures to some chemicals at critical life stages can result in dramatic effects on individuals, and/or subtle but important population-wide impacts, by affecting population growth, maintenance and/or health (O'Hara and O'Shea, 2005). The three primiparous *D. delphis* females in the current study were within their second trimester, and it is not known whether all three females would have successfully given birth and/or the survival rate of their first born calves. In female Californian sea lions, associations have been documented between high OC levels in post parturient individuals and miscarriages, and premature pupping during the last two trimesters of pregnancy. The majority of premature pups are born alive during the third trimester, but all die within several hours of birth (Marine Mammal Commission 1999). However, the association between OCs and prematurity is confounded by the presence of disease capable of inducing abortions: serological evidence of leptospirosis and calicivirus has been found. Further, the frequency of prematurity was higher during El Nino years, indicating that the nutritional status of the females also influences the probability of prematurity (Marine Mammal Commission 1999).

Interestingly, species-level differences in effects of condition (blubber thickness) on corpora number were observed, with an almost linear positive effect reported in BIOCET *D. delphis* compared to a negative effect in BIOCET *P. phocoena.* Thus *P. phocoena* in good nutritional condition (increased blubber thickness) had lower number of corpora scars whereas for *D. delphis*, individuals in good nutritional condition had higher number of corpora scars. On assessing the BIOCET *D. delphis* data further, 86% (12/14) of mature individuals with contaminant burdens above the threshold level (all but two individuals were resting mature) and corresponding high corpora count data (≥15 scars), were obtained from the mass live stranding event in Pleubian in February 2002. Of the 52 individuals necropsied in this mass stranded group, only one male (calf) was present and all other individuals were female, including four nursing calves. Results from genetic analysis on the Pleubian group reported a lack of evidence for a matriarchal system, with genetic variability within the mass-stranded group similar to variability observed in single strandings i.e. common dolphins were not necessarily genetically related (Viricel *et al.*, 2008). Therefore, the existence of non-reproductive females (based on high contaminant loads and high numbers of ovulations) within this social group is even more remarkable; though it should be noted that the whole mass-stranded group was not sampled for genetic analysis, as 50 other individuals were released alive offshore.

The most parsimonious interpretation of the negative relationship between POP concentrations with increasing corpora number in the *D. delphis* control group and also in resting mature female BIOCET *P. Phocoena*, is that a high number of corpora scars indicates infertility or a high level of miscarriages/abortions (repeated ovulations as the animal does not get pregnant, or loses the foetus during gestation or soon after), and some females may go through a large number of infertile ovulations prior to a successful pregnancy, birth, and survival of their first offspring during early lactation - as mentioned previously, females offload c.80% of their OC burden during the first seven weeks of lactation (Cockcroft *et al.,* 1989). A high foetal mortality rate in the first trimester (40-67%) has been reported in other small delphinids (*Stenella longirostris and Stenella attenuata*), which was attributed to adverse interactions with purse seine fisheries in the eastern tropical Pacific; induction of miscarriage due to physiological stress of chase and capture or indirectly through depletion of energy stores (Perrin *et al.,* 2003). Data in the current study may also suggest the existence of non-breeding (ovulating) females in the population, though it appears that most females eventually become pregnant – due to a decline in the contaminant levels with increasing corpora number in the control group and BIOCET *P. phocoena* sample, though this does not appear to be the case within the Pleubian mass stranding group. Although the number of corpora scars increased with age within the *D. delphis* control group sample, the problems in deciphering whether or not *corpora albicantia* provide a lifetime record of past ovulations has caused difficulties in correctly interpreting these data. Future work will assess the effects of contaminants on foetal survival rates in both species, and also the indirect effects of contaminants, through lower immunity, on reproductive activity.

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Figures

Figure 1. Size distribution of *corpora albicantia* in (a) BIOCET *P. phocoena* (right ovary n = 3, left ovary n = 21) and BIOCET *D. delphis* (right ovary n = 63, left ovary n = 75).

Figure 2. The total number of corpora scars (*corpora albicantia* and *lutea*) in small cetacean ovaries in the BIOCET sample as a function of age (yrs) in (a) *P. phocoena* (n = 86) and (b) *D. delphis* (n = 124).

Figure 3. Smoothers for partial effects of explanatory variables on total number of *corpora albicantia*: (a) age (yrs, n = 88) and (b) dorsal blubber thickness (mm, n=81) in BIOCET *P. phocoena*, and (c) age (yrs, n = 123) and (d) dorsal blubber thickness (mm, n = 100) in BIOCET *D. delphis*. In all cases, the y axis represents the strength and direction of the effect of the explanatory variable. The axis label format indicates the estimated degrees of freedom (edf or "curviness") of the smoother. For example, s(Age, 2.96) indicates a smooth function of age with edf=2.96.

Figure 4. Smoothers for partial effects of explanatory variables on total number of *corpora albicantia* in ovaries of BIOCET *P. phocoena*, for models which include age and blubber thickness (plus regional variation in the first model): partial effects of (a) [PCBs] (ng/g of lipid, n=59), (b) [HBCD] (ng/g of lipid, n=36), and (c) [DDE] (ng/g of lipid, n=59).

Figure 5. The total number of corpora scars (*corpora albicantia* and *lutea*) in sexually mature ovaries as a function of PCB burden [(∑7ICES congeners)*3] $\mu g\ g^{-1}$ lipid in (a) BIOCET *P. phocoena* (n = 24), and (b) BIOCET *D. delphis* (n = 38).

Figure 6. The total number of corpora scars (*corpora albicantia* and *lutea*) as a function of age (yrs, n = 38) in the *D. delphis* control group sample.

Figure 7. PCB burden [(∑7ICES congers)*3] ($\mu g\ g^{-1}$ lipid) as a function of (a) age (yrs, n = 38) and (b) number of corpora scars (n = 43); (c) total DDT burden ($\mu g\ g^{-1}$

lipid) as a function of number of corpora scars (n = 43) in the *D. delphis* control group sample.

Table 1. Health status categories in the control group *D. delphis* sample. Category 1 - healthy individuals; Category 2 - health of individuals only mildly compromised; and Category 3 - individuals suffering from severe infectious or non-infectious disease.

	Sample size	Cat. 1	%	Cat. 2	%	Cat. 3
Immature	20	19	0.95	1	0.05	0
Lactating	7	7	1	0	0	0
Pregnant	3	3	1	0	0	0
Pregnant & Lactating	2	2	1	0	0	0
Resting Mature	11	9	0.82	2	0.18	0
	43	40	100	3	100	0

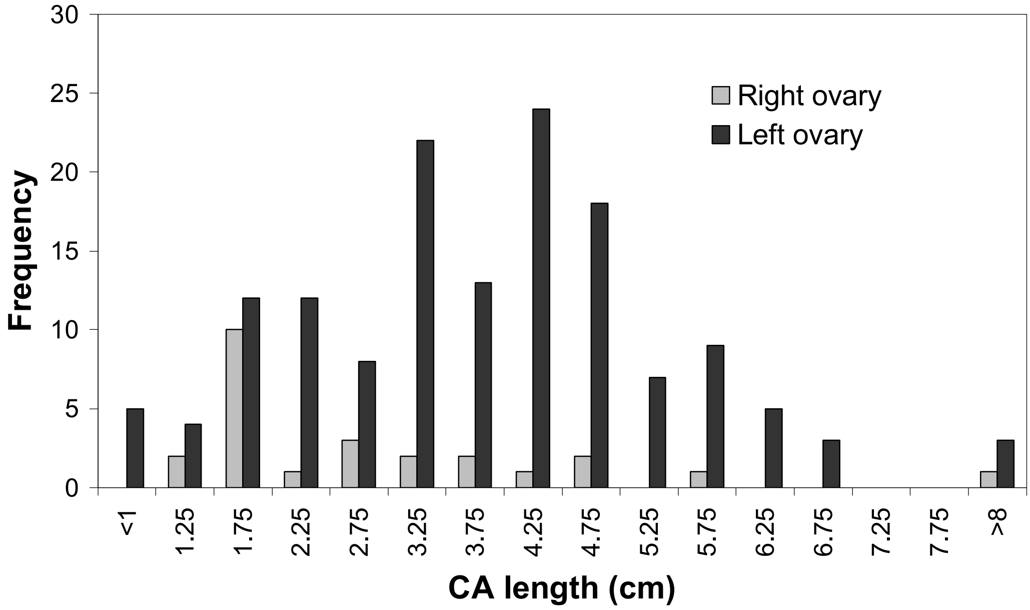
Table 2. Reproductive data from *P. Phocoena* (n = 99) and *D. delphis* (n = 177) in the BIOCET study and the *D. delphis* control group study (n = 43).

	BL (cm)	Age (yrs)	Combined gonadal weight (g)	No of corpora scars[3]	Average no of corpora scars (SE)
Immature BIOCET *P. phocoena*	66-143 (n=59)	0-4.5 (n=59)	0.3-3.2 (n=51)	0	0
Mature BIOCET *P. phocoena*[4]	139-192 (n=36)	3.5-24 (n=32)	1.4-20.7 (n=32)	1-17 (n=34)	6.9 (0.88)
All BIOCET *P. phocoena* data[2]	66-192 (n=95)	0-24 (n=91)	0.3-20.7 (n=83)	1-17 (n=34)	6.9 (0.88)
Immature BIOCET *D. delphis*	91-206 (n=74)	0-10 (n=71)	0.3-7.5 (n=50)	0	0
Mature BIOCET *D. delphis*	170-220 (n=102)	6-26 (n=96)	1.7- 25.3 (n=89)	1-34 (n=86)	14.6 (0.87)
All BIOCET *D. delphis* data	91-220 (n=176)	0-26 (n=167)	0.3-25.3 (n=139)	1-34 (n=86)	14.6 (0.87)
Immature control *D. delphis*	107-210 (n=20)	0-11 (n=19)	0.76-5.46 (n=16)	0	0
Mature control *D. delphis*	186-221 (n=22)	7.5-30 (n = 19)	1.88-17.48 (n=22)	1-16 (n=23)	7.2 (1.16)
All control *D. delphis* data	107-221 (n=42)	0-30 (n=38)	0.76-17.48 (n=38)	1-16 (n=23)	7.2 (1.16)

[3] Including *Corpora lutea* and *albicantia*
[4] Excluding two "pathological" harbour porpoises reported as mature based on milk gland pathology

Figure 1

(a) HP



(b) CD

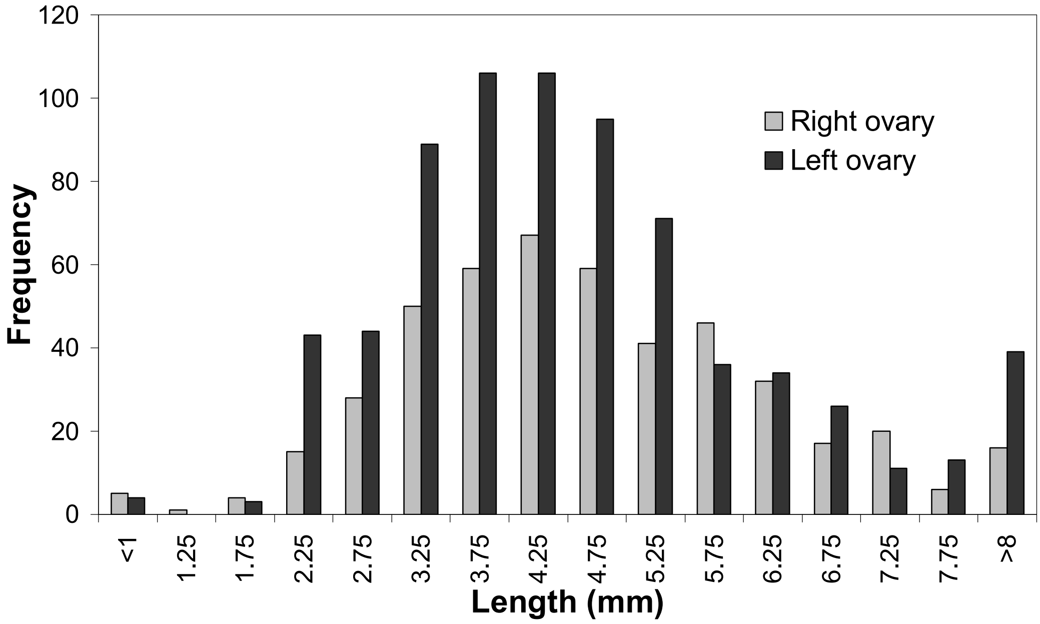
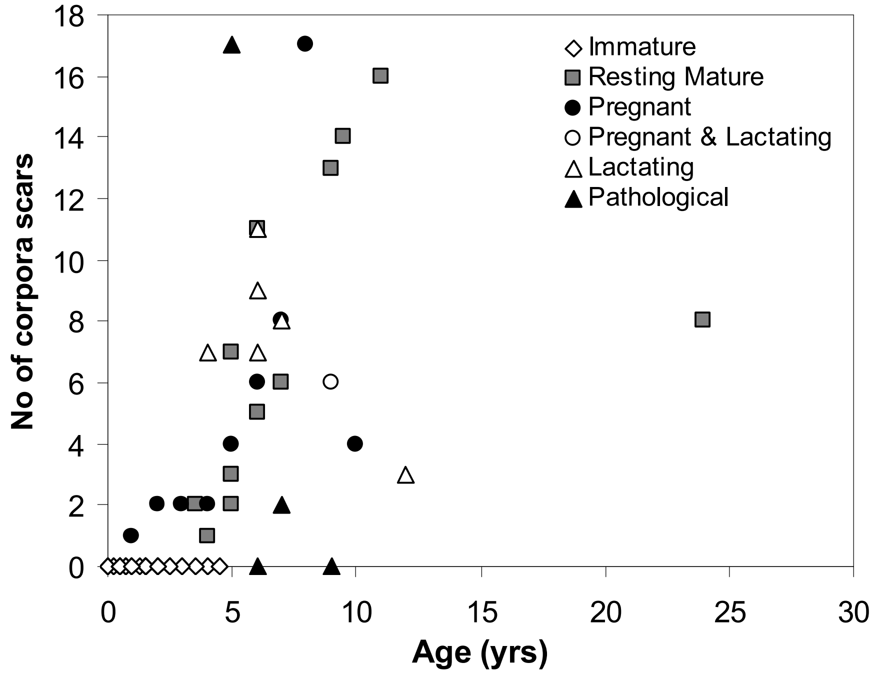


Figure 2

(a) HP



(b) CD

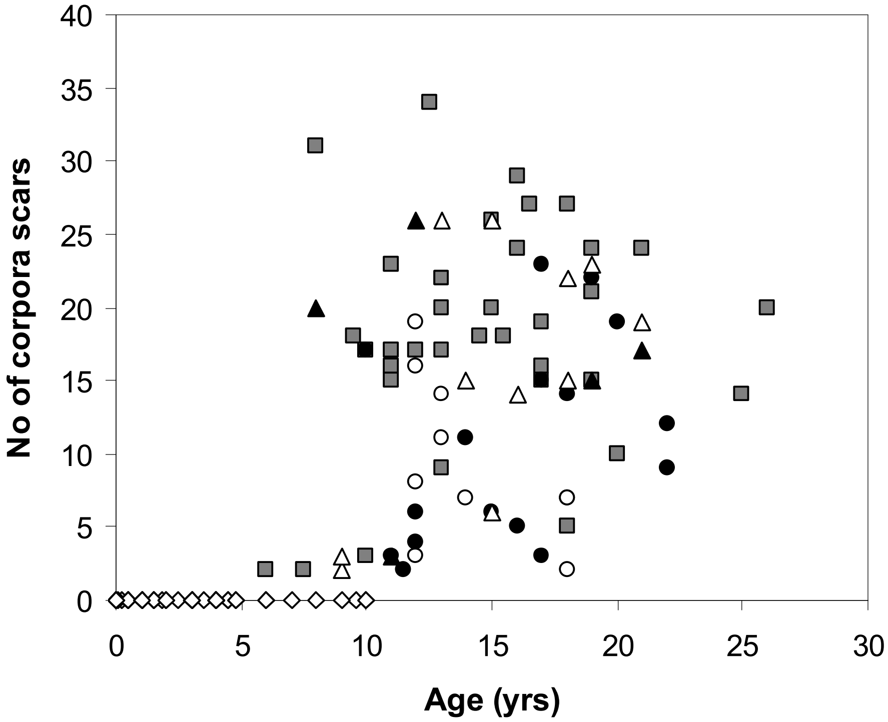
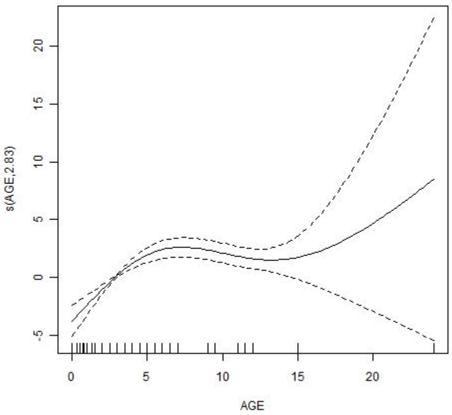
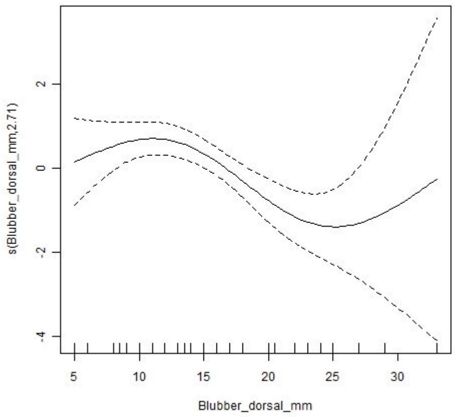


Figure 3

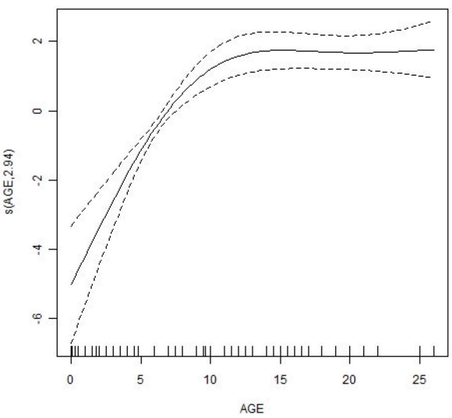
(a) HP, age



(b) HP, blubber thickness



(c) CD, age



(d) CD, blubber thickness

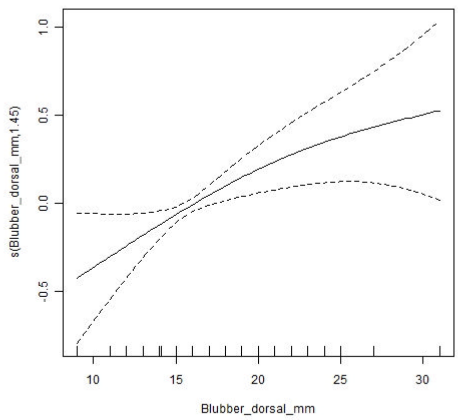
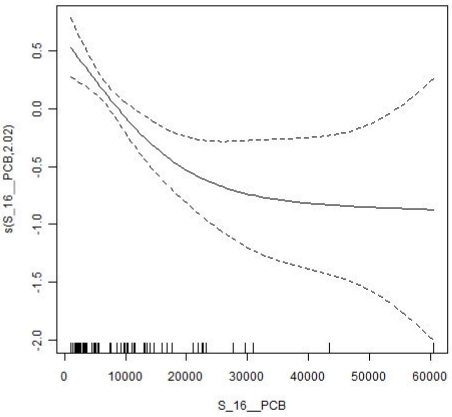
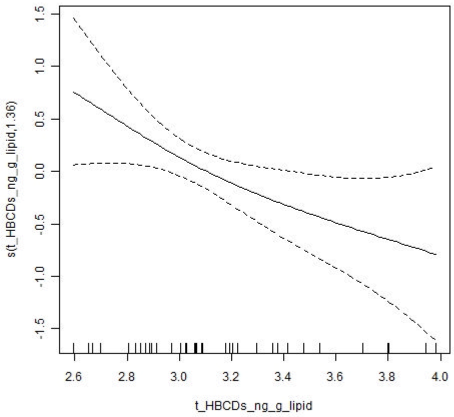


Figure 4

(a) PCBs

(b) HBCDs





(c) DDE

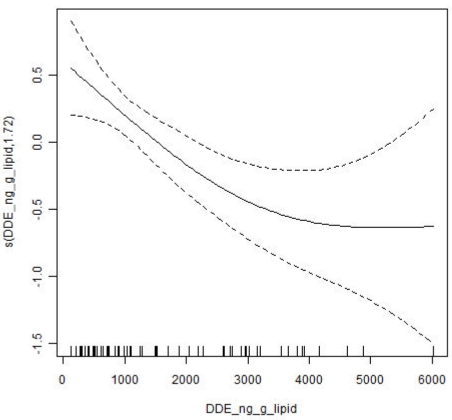
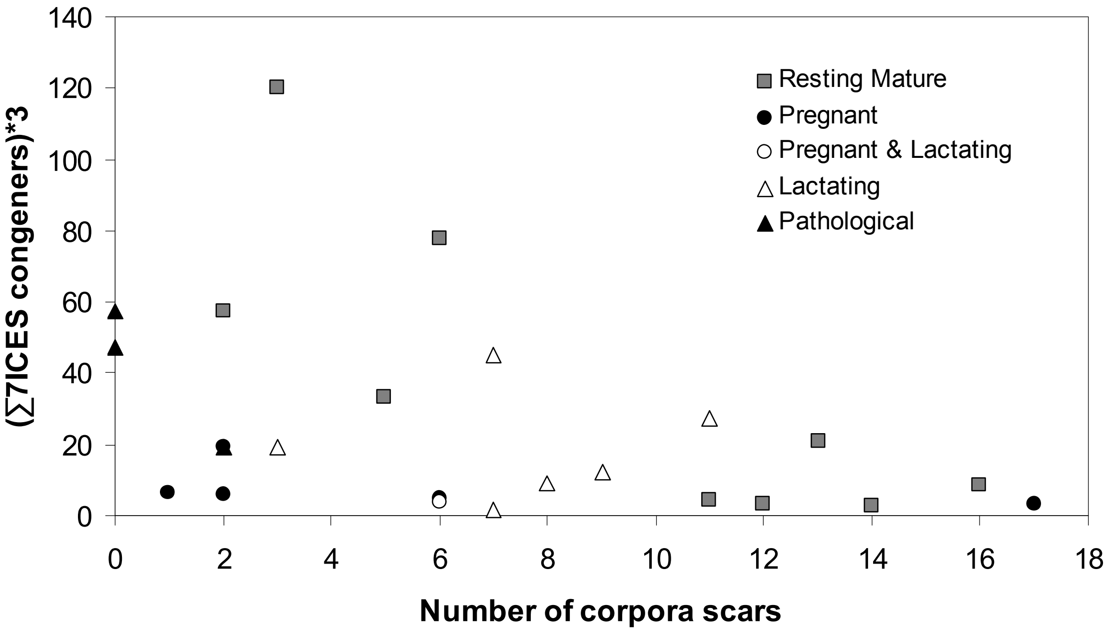


Figure 5

(a) HP BIOCET data



(b) CD BIOCET data

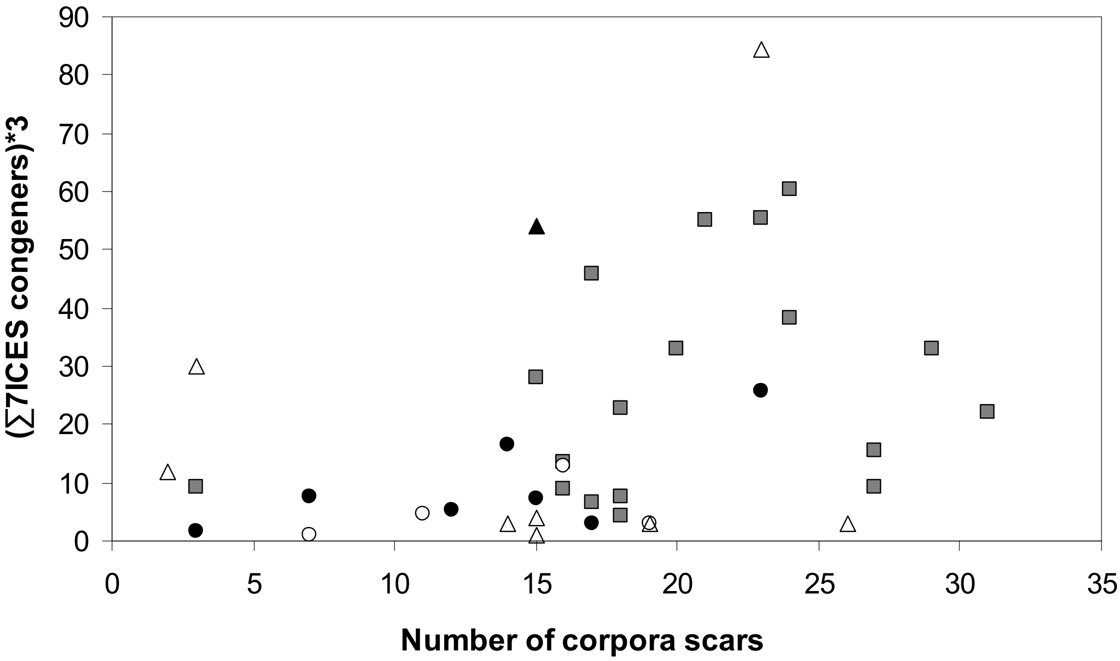
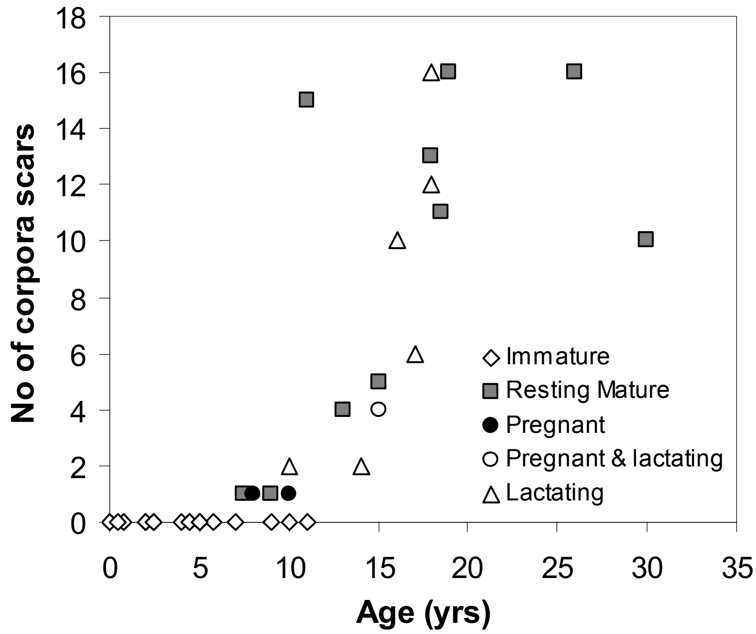
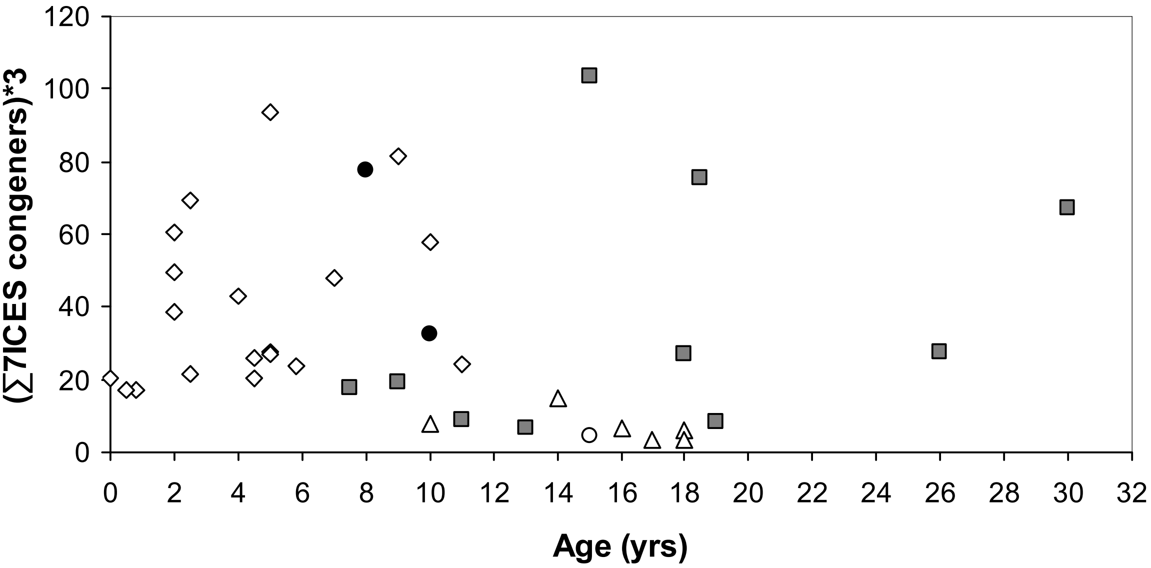
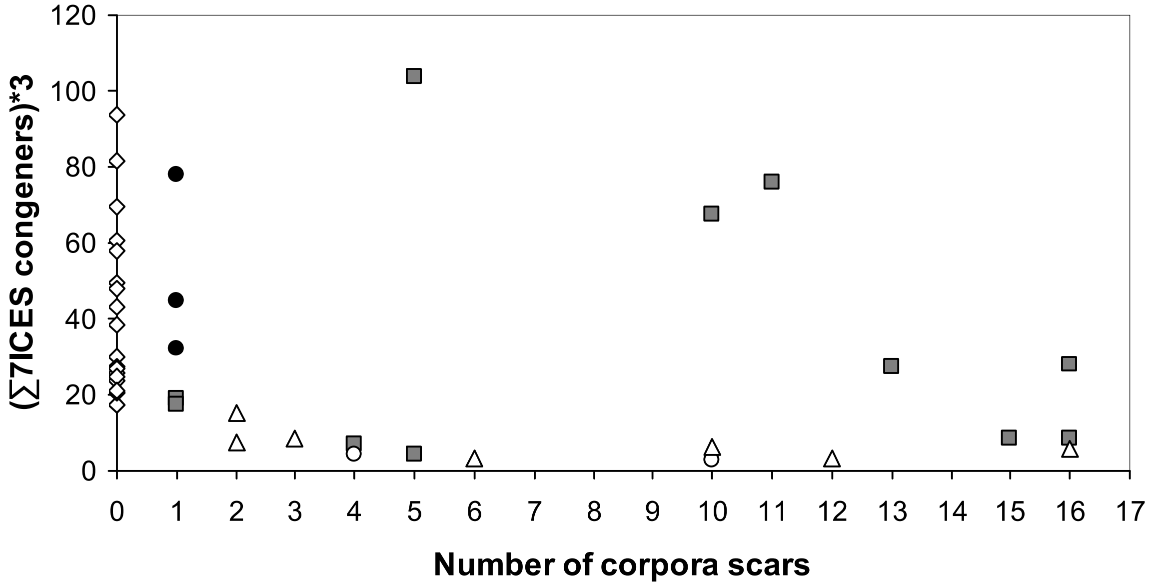


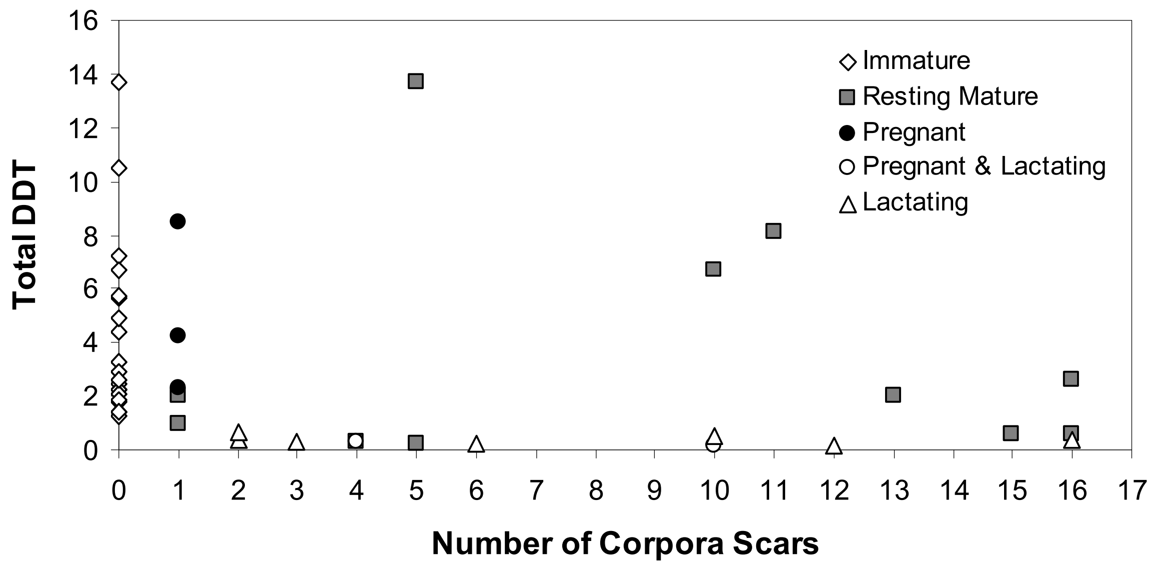
Figure 6

Control Group sample




(∑7ICES congeners)*3
0
20
40
60
80
100
120
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Age (yrs)


(∑7ICES congeners)*3
0
20
40
60
80
100
120
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Number of corpora scars


Total DDT
0
2
4
6
8
10
12
14
16
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
Number of Corpora Scars
Immature
Resting Mature
Pregnant
Pregnant & Lactating
Lactating