Agenda Item 6.1

Project Funding through ASCOBANS
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Document 6-04

Project Report: Effects of contaminants on reproduction in small cetaceans, Phase II

Action Requested

- Take note of the report

Submitted by

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Contract Report

EFFECTS OF CONTAMINANTS ON REPRODUCTION IN SMALL CETACEANS, Phase II

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ZSL LIVING CONSERVATION

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

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SUMMARY

Within the current report we present a preliminary assessment of reproductive tract pathologies in common dolphins and harbour porpoises inhabiting the North-east Atlantic. To date genital ulcers, (possible) genital papilloma lesions, vaginal plaques, vaginal abscesses and wall lesions and ovarian abnormalities were observed in harbour porpoises. Vaginal calculi and numerous ovarian abnormalities including tumours, an ovarian cyst, and an ovotestis were observed in the common dolphin. This is the first reported case of an ovotestis in a cetacean species.

Issues arose over the diagnosis of ovarian abnormalities based purely on haematoxylin and eosin stained sections. Therefore, additional work is required, including undertaking immunohistochemical staining for specific cell types, and this work is currently been carried out. As a result only preliminary results are presented here - and a number of additional possible ‘abnormality’ cases are not described. Consequently, a full assessment of, contaminant induced reproductive (morphological and functional) dysfunction and, the indirect effects of contaminants through lowering immunity, could not be undertaken. However what is apparent, when comparing the results to date to other published studies focusing on cetaceans, and solely assessing the female reproductive system, the number of observed cases of reproductive tract pathologies (possible genetic and congenital abnormalities, tumours, and endocrine disorders, infections, and structural changes) presented here (and those cases not presented as still under investigation) is considerable. Until the ovarian abnormalities are correctly identified it is unknown if they are resulting from abnormalities of genetic or chromosomal origin, inappropriate hormone exposure, chemical exposure, or disease – though corresponding contaminant data are not available for a number of females exhibiting disorders of the genital tract.

There were no observed cases of premature newborns within the harbour porpoise or common dolphin sample. There were some instances of ovarian hyper- and hypoplasia which are currently been investigated.

Results support those of Phase I - high contaminant burdens, above a threshold level for adverse effects on reproduction, were not inhibiting ovulation, conception or implantation in female harbour porpoises or common dolphins. All mature female harbour porpoises (n = 57) showed evidence of current or previous gravidity. However, there were a number of cases where although the female porpoise had been previously pregnant they had not successfully offloaded their contaminant burdens, suggesting: (1) the female had aborted her calf, or (2) the calf did not survive after birth. This is based on the fact that 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. Furthermore, one female harbour porpoises with high total PCB concentrations recently aborted a foetus. All

suggesting that high contaminant levels impacted on foetal survival. There were a number of additional abortion cases presented in the report with no available corresponding contaminant data. Further a number of other ‘possible’ abortion cases are currently been assessed within the harbour porpoise sample.

Within the common dolphin control group sample of ‘healthy’ individuals (with corresponding contaminant data), 8.9% of mature females showed evidence of recent abortion during their second trimester. These females had high a contaminant burden, above the threshold level of 17 $\mu g\ g^{-1}$ lipid for adverse health effects in marine mammals. One of the three primiparous females identified during Phase I with a high total PCB concentration was re-classified as recently aborted – based on histological assessment of the corpus luteum. There were two cases of resting mature (non-pregnant and non-lactating) females with high total PCB burdens, ranging from 60.4 to 103.5 $\mu g\ g^{-1}$ lipid. These two females ranged in age from 15 to 30 years, in corpora scar number from 5 to 11 and both were previously pregnant. However they did not offload a substantial proportion of their contaminant burdens, indicting foetal or newborn mortality. In mink, although ovulation, conception and implantation occurred, similar to the current study, PCBs increased foetal mortality. This was mediated through affecting maternal vasculature in the placenta and producing degenerative changes in the trophoblast and foetal vessels, which lead to foetal growth retardation or death. A similar situation may be occurring in individuals within the current study.

The association between high contaminant burdens and the incidence of abortion within the harbour porpoise and common dolphin contaminant sample in the current study cannot be discounted, nor the number of observed cases where previously gravid females did not offload their contaminant burdens, due to either foetal or newborn mortality.

Whether or not contaminants and/or other stressors (e.g. nutritional and immune) are instrumental in instigating abortion and/or impacting newborn survival rates in common dolphins and harbour porpoises, inhabiting the North-east Atlantic, requires further investigation. This is currently been undertaken as part of the Marie Curie International Outgoing Fellowship project ‘CETACEAN-STESSRORS’. In addition to further investigations into contaminant (and other stressor) induced reproductive (morphological and functional) dysfunction.

INTRODUCTION

The endocrine and reproductive effects of persistent organic pollutants 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).

The effects of organochlorine compounds (OCs), such as polychlorinated biphenyls (PCBs) and DDT, on the North-east Atlantic continuous system harbour porpoise population that ranges from France to Norway, including the North Sea (Fontaine et al. 2007, ICES WGMME 2009), is a particular concern. Primarily due to the low pregnancy rates (Scotland: 34%, (Learmonth et al. in review), England and Wales: 50.9%, (Murphy 2008); sampling between 1990-2006) recently reported for this population within UK waters. Although the vast majority of individuals sampled within these studies were stranded animals that died as a result of numerous pathological reasons, such as generalized bacterial infection, pneumonia and starvation, in addition to trauma, it is not known if they are really representative samples of the extant population. The large numbers of diseased porpoises that strand on a yearly basis through-out the North-east Atlantic (Siebert et al. , Siebert et al. 2001, Jauniaux et al. 2002, Jepson et al. 2005, Jepson 2005) would suggest either a high incidence of diseased porpoises in this region or just an increased propensity for ill health porpoises, and possibly older animals, to washed up dead or live-strand on the shore. Using data from the UK and Denmark and a sample of ‘healthy’ harbour porpoises (animals that died from incidental capture or trauma, 1986-2005), Winship (2008) estimated a higher pregnancy rate of 59% for the North Sea – using data from all females the estimated rate was 41% for this region. Nevertheless, this is still considerably lower than the 98% pregnancy rate estimated for porpoises off Iceland (Ólafsdóttir et al. 2003), and the 95% pregnancy rate estimated for porpoises in the Gulf of Maine and Bay of Fundy in the North-west Atlantic (Read and Hohn 1995; data obtained between 1990 and 1993).

Siebert et al. (2006) found that bycaught and hunted harbour porpoises from Greenlandic, Icelandic and Norwegian waters were healthier than bycaught porpoises from the North and Baltic Seas. Overall lesions associated with parasitic infection were milder and severe secondary bacterial infections were infrequent. A follow up study assessed the regional differences in bacterial flora in harbour porpoises within the North-east Atlantic by sampling bycaught, hunted and stranded animals over an 18-year period. Significantly less bacterial growth and fewer associated pathological lesions were observed in porpoises from Icelandic and Greenlandic waters, compared to animals inhabiting the German North Sea, Baltic Sea and Norwegian waters (Siebert et al. 2009). The observed differences were attributed to possibly higher stress due to anthropogenic activities, such as chemical pollutants, in porpoises inhabiting the German North Sea, Baltic Sea and

Norwegian waters. This possibly resulted in immunosuppression and increased risk of infection from pathological bacteria in these individuals (Siebert et al. 2009). Porpoises in Icelandic and Greenlandic waters had significantly lower PCB, PBDE (Iceland assessed only), toxaphene and organotins (Greenland assessed only) compared to other areas (Siebert et al. 2009 and ref. therein).

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 into 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). Although the use and production of PCBs was phased out decades ago, diffuse inputs continue and environmental levels are declining slowly (Law et al. 2010, and ref. therein). In UK harbour porpoises, blubber PCB levels are also only declining slowly, which suggests that their toxic impacts will continue for some time (Law et al. 2010). An initial decline in Σ25CBs concentrations was observed in the mid-1990s, but this then plateaued off. Within the sample of 440 porpoises in the Law et al. (2010) study, concentrations of Σ25CBs were higher in animals sampled in England and Wales. However, interestingly, levels have been increasing in Scotland since 1995 (Law et al. 2010).

Stranded common dolphins do provide a representative sample of the NE Atlantic population for estimating biological parameters (Murphy et al. 2009). Primarily as a large proportion of individuals were incidentally captured in fishing nets (and subsequently washed ashore), and older individuals (>20 yr) were not over-represented in the stranding data (Murphy 2004, Jepson 2005, Murphy et al. 2009). In addition there is a lower incidence of disease in stranded common dolphins compared to stranded porpoises (Jepson 2005, Deaville and Jepson 2011). Nevertheless, a low pregnancy rate of 26% was also reported for the NE Atlantic common dolphin population (Murphy et al. 2009). Significantly higher pregnancy rates of 47% and 40.2% were reported in *D. delphis* inhabiting the Eastern Tropical Pacific and the long-beaked common dolphins *D. capensis* off South Africa, respectively (Mendolia 1989, Murphy et al. 2009). Even though a low pregnancy rate of 26% may well in fact be the natural rate for this species in a temperate region, environmental and anthropogenic activities may also be contributing factors (Murphy et al. 2009).

There have been a relatively low number of reported incidences of neoplasms in marine mammals, resulting from either not being assessed due to autolytic changes or other reasons, animals dying without pathological investigations or individuals dying before attaining an older age, namely when most cancers occur (Newman and Smith 2006). The highest estimated annual rate of cancer in cetaceans was reported in the beluga whale

(*Delphinapterus leucas*) inhabiting and St Lawrence Estuary in Canada (Martineau et al. 2002). Cancer was the second leading cause of death, with tumours identified in 27% of sampled adult whales (Martineau et al. 2002, McAloose and Newton 2009). Putative causes include environmental exposure to carcinogenic substances, and/or reduced immune function due to limited genetic diversity (Martineau et al. 2002, Newman and Smith 2006). In addition to three ovarian tumours observed in the St Lawrence beluga whale population (see Table 1), adenocarcinomas were observed in mammary gland tissue of three individuals and the uterus of another (Martineau et al. 2002). An atypical bilateral true hermaphrodite (De Guise et al. 1994a), and a male pseudohermaphrodite (Reijnders 2003) have been reported within the population, and a follicular cyst (measuring 6 x 6 x 5 cm) was found on the left ovary of the true hermaphrodite (De Guise et al. 1995). Further, incidences of mastitis have also been reported within this population that may have affected milk production (De Guise et al. 1995).

Table 1. Individual cases of ovarian neoplasms in cetaceans.

	Ovarian neoplasms		
	Cetaceans		
Dysgerminoma (*germ cell tumour*)	Dusky dolphin	Peru	Van Bressem et al. 2000
Dysgerminoma[A]	Beluga Whale	St. Lawrence Est.	De Guise et al. 1994b, Martineau et al. 2002
Granulosa cell tumour (*sex-chord-stromal tumour*)	Beluga whale	St. Lawrence Est.	Martineau et al. 1988
Granulosa cell tumours	Beluga whale	St. Lawrence Est.	De Guise et al. 1994b
Granulosa cell tumour	Pilot whale	Japan	Benirschke and Marsh 1984
Granulosa cell tumour	Fin whale	Antarctica	Rewell and Willis 1950, Geraci et al. 1987
Granulosa cell tumour	Fin whale	Antarctica, S. Georgia	Rewell and Willis 1950, Geraci et al. 1987
Granulosa cell tumour	Blue whale	Antarctica, S. Georgia	Rewell and Willis 1950, Geraci et al. 1987
Ovarian carcinoma (granulosa cell tumour?[B])	Fin whale*	Antarctica	Stolk 1950, Geraci et al. 1987
Mucinous cystadenoma (*epithelial tumour*)	Blue whale	Antarctic	Rewell and Willis 1950, Geraci et al. 1987

[A] Originally classified as a granulosa cell tumour, and reclassified as a dysgerminoma by Martineau et al. 2002
[B] Reclassified as a (possible) granulosa cell tumour in Geraci et al. 1987

Viruses and carcinogenic contaminants are the putative causes of the high incidence of metastatic carcinomas of genital origin in California sea lions (*Zalophus californianus*).

A herpesvirus-associated metastatic genital carcinoma was reported in 18% (66 of 370 individuals) of stranded sexually mature sea lions between 1979 and 1994 (Gulland et al. 1996, Lipscomb et al. 2000). Genital epithelial neoplasia were also observed in 6.3% of California sea lions that died during a series of unusual mortality events caused by harmful algal blooms (Gulland 2000). Otarine herpesvirus-1 is the proposed cause of genital carcinoma in California sea lions (King et al. 2002). Interestingly though, an 85% higher level of PCBs (30% in relation to DDT) was reported in blubber tissue of individuals with genital carcinoma relative to those without genital carcinoma (Ylitalo et al. 2005). This demonstrates an association between OCs and carcinoma, through possibly affecting the prevalence of carcinomas by acting as immunosuppressive agents or by genotoxic mutation and tumour promotion (Ylitalo et al. 2005).

A number of other reproductive abnormalities have been linked to the exposure to organochlorines including 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), and premature pupping in sea lions (DeLong et al. 1973); 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).

Overview of project - Phase I

Samples were analysed from a control group of 43 'healthy' female common dolphins, and also 91 female harbour porpoises that stranded along the English and Welsh coastlines. 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) were inhibiting ovulation, conception or implantation. Results suggested that high contaminant burdens, above a threshold level for adverse effects on reproduction, were not inhibiting ovulation, conception or implantation in female common dolphins or harbour porpoises, though the impact on the foetal survival rate (in both species) required further investigation.

During Phase I, supplementary analysis was carried out with colleagues on data produced by the EC BIOCET (Bioaccumulation of persistent organic pollutants in small cetaceans in European waters: transport pathways and impact on reproduction) project. Results were published, in conjunction with those from the UK common dolphin control group study, in Murphy, S., G. J. Pierce, R. J. Law, P. Bersuder, P. D. Jepson, J. A. Learmonth, M. Addink, W. Dabin, M. B. Santos, R. Deaville, B. N. Zegers, A. Mets, E. Rogan, V. Ridoux, R. J. Reid, C. Smeenk, T. Jauniaux, A. López, J. M. A. Farré, A. F. González, A. Guerra, M. García-Hartmann, C. Lockyer, and J. P. Boon. 2010. Assessing the effect of persistent organic pollutants on reproductive activity in common dolphins and harbour porpoises. NAFO/ICES/NAMMCO symposium "The Role of Marine Mammals in the Ecosystem in the 21st Century". Journal of Northwest Atlantic Fishery Science 42:153-173 (http://journal.nafo.int/42/murphy/11-murphy.pdf).

Phase I also reported contrasting results between the EC BIOCET and the UK common dolphin control group (Phase I) studies. Within the BIOCET sample, a significant increase in corpora number and PCB burden was observed for sexually mature *D. delphis.* The majority of individuals with contaminant burdens above the threshold level for adverse health effects were resting mature females (83%), 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.

For BIOCET *P. phocoena*, 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 (Murphy et al. 2010).

Overview of project – Phase II

Phase II has two main objectives:

(1) *Assessment of reproductive abnormalities within English and Welsh D. delphis and P. phocoena and, where data are available, investigate their association with pollutants*

This study will assess abnormalities associated with the reproductive tract. Gonadal inactivity or lesions can be caused by many factors including genetic defects, infectious disease, degenerative changes, neoplasia or aging (senescence), or secondary to other primary problems such as nutritional or environmental stress, systemic infection and central nervous system disease (Reeves et al. 2001). 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 (Reeves et al. 2001). Within the proposed study, genital pathology will be linked to other data such as age, nutritional status, disease, as well as contaminant levels. Further, evidence of abortions, stillbirths, and premature births will be assessed.

(2) *Assessment of the direct and indirect effects, through lowering immunity, of contaminants on reproduction in female P. phocoena*

This will be accomplished by increasing the harbour porpoise sample size to 140 individuals. Statistical analysis will incorporate data (where available) on health status and nutritional condition, along 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).

METHODS

This study was undertaken in collaboration with colleagues of the UK Department of Environment, Food, and Rural Affairs (Defra) funded UK Cetacean Strandings Investigation Programme (CSIP) and Robin Law at the UK Centre for Environment, Fisheries, and Aquaculture Science (Cefas).

As part of the Phase I project, and funded by the Defra Marine Research Program, blubber samples from a control group of 'healthy' 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 bycatch during detailed post-mortem examinations. Pathological investigations such as gross examination, histological, bacteriological and/or virological analyses identified that these dolphins were not suffering from any infectious or non-infectious diseases that might inhibit reproduction. In addition to these analyses, animals were assessed for evidence of exposure to Brucella infection through bacteriological assessment, including serological evaluation of pericardial fluid. Results of these investigations are presented here. The majority of individuals within the control group were found stranded between the months December and March (88%), along the southwest coast of the UK (95%). Supplementary to these individuals, all available common dolphin reproductive material supplied by the UK CSIP was assessed during Phase II. This included samples from an additional 37 individuals (were both gonads were collected), of which 70% were sampled between November and April. The sampling period ranged from 1991 to 2006.

Reproductive material from 91 female harbour porpoises were examined during Phase I. Blubber tissues were assessed for a range of contaminants as part of earlier studies such as Law et al. (2001) and Jepson et al. (2005). Reproductive material from a further 49 females were analysed within the current study and of these, 19 individuals had corresponding contaminant data. All individuals were either found stranded along the English and Welsh coastlines or obtained as part of the UK fisheries bycatch observer programme. For the whole harbour porpoise sample, no bias in sampling of individuals occurred between quarters, and samples were obtained in all months. The sampling period ranged from 1991 to 2004.

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, nutritional condition (good, moderate, poor) 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 were collected and preserved in 10% neutral formalin, and the uterus was examined for presence of a foetus.

A number of abnormalities were screened for within the reproductive tract, including uterine tumours, uterine occlusions and leiomyomas, genital warts and ulcers and the presence of vaginal calculi. 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 and in some individuals, histological assessment of mammary gland tissue activity. 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 analysed 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.

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 derived the total PCB concentration (Jepson et al. 2005). This is determined by calculating the "ICES7" value (the sum of concentrations of CB28, CB52, CB101, CB118, CB138, CB153, CB180) as three times this value is equivalent to the Aroclor 1254 value (see Jepson et al. 2005). Using thresholds in this way warrants caution owing to possible differences in species sensitivities. However, as stated in Jepson et al. (2005), 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

During the current project, teeth from 25 individuals were processed for ageing. Ages for other common dolphins and harbour porpoises were estimated during previous projects (Murphy 2008, Murphy 2009, 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.

Classification of ovarian scars and maturity status

Before examination, the preserved ovaries were rinsed in water for 24 hours and then replaced in their containers with 70% ethanol. For each ovary, maximum length, width and height (mm) were recorded to the nearest 0.1 mm using vernier calipers, and the weight was recorded to the nearest 0.1 gram. Corpora scars present on each ovary were classified into a corpus luteum (CL), regressing corpus luteum, and corpus albicans (CA). CLs were measured on three plains that were all perpendicular to each other. The colour, texture and position of corpora albicantia were noted and maximum diameters were recorded. The activities of both ovaries were recorded to assess symmetry. Ovaries were then hand sectioned into 0.5-2 mm slices and examined under a binocular microscope for the presence of additional corpora scars. The presence of yellow-orange bodies was also noted during gross assessment. Yellow bodies, which are lipid-laden cells of degenerating follicle atresia, are deep yellow-orange or tan colour and usually stellate or amoeboid in shape (Lockyer 1987, Brook et al. 2002). Yellow bodies do not protrude from the ovary and do not have a stigma (Lockyer 1987).

The corpus luteum (CL) is a temporary endocrine gland produced from cellular components of the follicle, after release of the oocyte during ovulation. It 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. Histologically, corpora lutea are composed of granulosa lutein cells (luteinised granulosa cells) and theca-lutein cells (modified theca interna cells), interspersed with connective tissue and blood vessels. CL regression has been defined as luteal structures that undergo autolysis and are phagocytosed by macrophages. A gradual invasion of connective tissue occurs with masses of collagen fibres (the main supportive protein of connective tissue) (Peters and Kenneth 1980; see Figure 1). Involution of the tissue is accompanied by progressive fibrosis and shrinkage (Fraser et al. 1999). The CL produces the hormone progesterone, which in responsible for maintaining pregnancy, and CL regression occurs after parturition or unsuccessful fertilization of the oocyte.

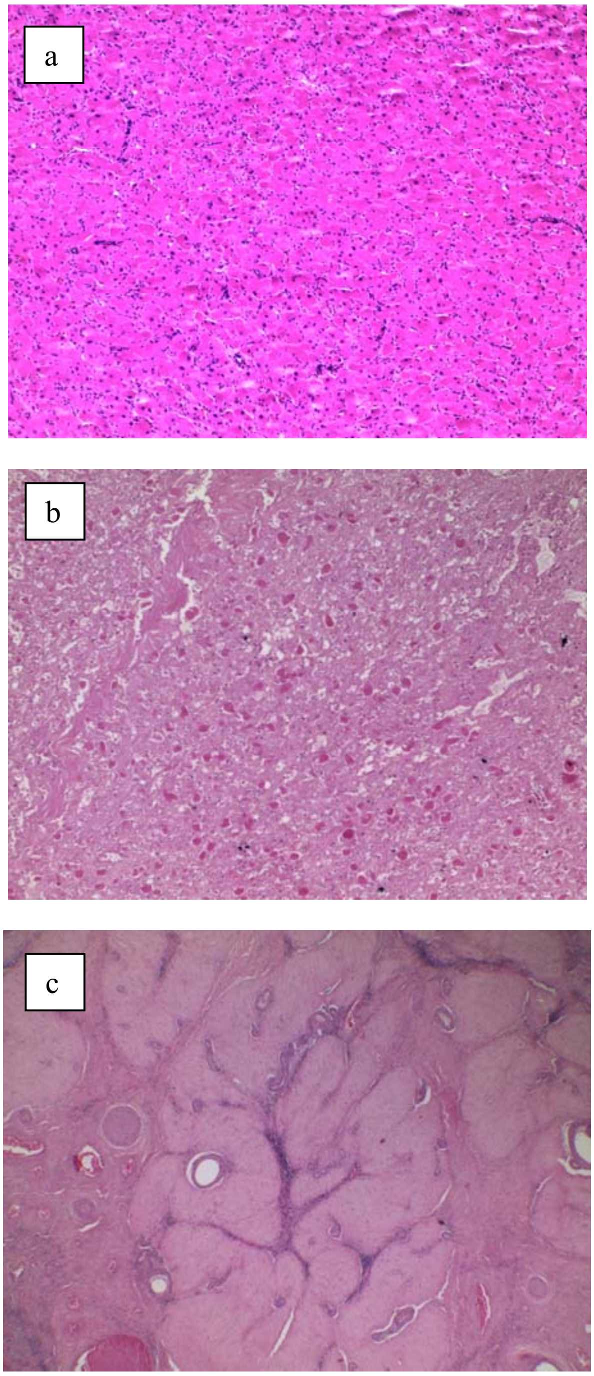


Figure 1. Active and regressing Corpora lutea in common dolphins. (A) An active corpus luteum of pregnancy; (B) regressing corpus luteum of a recent pregnancy, note the increase in connective tissue and autolysis of lutein cells; (C) a corpus albicans composed primarily of collagen fibers, fibroblast-like cells and remnants of blood vessels.

A regressing corpus luteum eventually gives rise to a tissue scar called corpus albicans (CA). In cetaceans, corpora albicantia consist of acellular connective tissue largely composed of unpigmented collagen, or acellular fibrioid material that contains patches of PAS reaction-positive material, or collagenous fibers and amorphous material containing granules (see Takahashi et al. 2006 and ref. therein). On gross assessment corpora albicantia appear as spherical knobs or as raised, wrinkled scars. Internal structure can vary between white tissue either loosely constructed or solid, or yellow tissue sometimes interspersed with white connective tissue, and some remnants of vascularisation (Akin et al. 1993). In common dolphins, many corpora albicantia have a cauliflower appearance on the surface of the ovary, and are easily recognisable on dissection as pale fibrotic areas, composed of white connective tissue that becomes fragmented with age (Murphy 2004).

It was proposed 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, and therefore provide an index of the number of past ovulations (Perrin and Donovan 1984). However, in recent years questions have arisen towards the legitimacy of this assertion (Brook et al. 2002, Takahashi et al. 2006, Dabin et al. 2008), though further investigations did not achieve a definitive resolution on the matter (Murphy et al. 2010).

Collet and Harrison (1981) reported that histologically, corpora albicantia in common dolphins could be divided into two types: (a) considerable acellular hyaline (transparent-translucent tissue containing little fibrous tissue) material arranged in lobules, and (b) mainly coils of obliterated blood vessels and sparse hyaline material. It was suggested that CAs which consisted of little more than coils of blood vessels were resulting from either a CL of unfertilised ovulation or of a luteinised follicle, while those with considerable acellular hyaline material resulted from a CL of pregnancy (Fisher and Harrison 1970, Collet and Harrison 1981, Takahashi et al. 2006). However, Marsh and Kasuya (1984) could not differentiate, histologically, CAs of ovulation and pregnancy in pilot whales (*Globicephala macrorhyuchus*), and only found evidence of CAs composed of coiled blood vessels.

Following these earlier studies, Takahashi et al. (2006) assessed the composition of corpora albicantia in common dolphins and noted that CAs were relatively acellular and consisted of collagen fibers and elastin-like material. In contrast to the Collet and Harrison study, Takahashi identified that the shrinking of CAs may be due to the progressive loss of collagen through hyalinisation. The proportion of collagen tissue decreased with decreasing CA size, while the portion of elastin increased. Elastin material was found (through immunohistochemical staining) near the central part of the CA and at the walls of the blood vessels and the perivascular (tissues surrounding blood vessels) connective tissue. As elastin has a long half-life 40–70, suggests persistence of

CAs within common dolphin ovarian tissue – maximum age reported in the North-east Atlantic population is 30-years. For the purpose of the current study, both CA scar types identified by Collet and Harrison were recorded as corpora albicantia – though on histological examination corpora scars had some vascular component.

Corpora scars were assessed in individuals' included within Phase II (n = 86), and re-assessed in individuals that were part of the Phase I study (n = 134). 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 six reproductive states: immature, pregnant, pregnant & lactating, lactating, resting mature (not pregnant or lactating), and recently aborted (not-lactating). For harbour porpoises, two additional categories were created, including abortion & stillbirth, and lactating, abortion & stillbirth. During necropsies, the development of the blood vessels in the broad ligaments and the presence of striations within the smooth musculature of the uterine wall were noted, as these can indicate if mature (non-pregnant) females had been previously gravid. In addition, the degree of involution of uteri was noted. In humans, uteri become fully involuted 4-6 weeks after parturition.

Assessing reproductive abnormalities and evidence of reproductive failure

As noted earlier, in other marine mammal species, uterine stenosis, occlusions and leiomyomas, cancer and hermaphroditism have linked to exposure to organochlorines. Using data and samples provided by the UK CSIP, an assessment of reproductive tract abnormalities was undertaken on female (stranded and bycaught) common dolphins (n = 80) and harbour porpoises (n = 140). Abnormalities associated with the reproductive tract were assessed; including tumours, uterine stenosis, occlusions and leiomyomas and vaginal calculi. Ovaries were examined for evidence of ovarian cysts and tumours through gross and histopathological assessment. The sample was also assessed for evidence of hermaphroditism, and other disorders of genital development. Ovarian lesions and abnormalities were measured and shape, colour, texture and position were recorded. Genital pathologies were linked to other data such as nutritional status, disease, and contaminant levels. Further, evidence of abortions, stillbirths and premature births were assessed.

Histopathology

Sections of various types of ovarian scars, including active and regressing corpora lutea, corpora albicantia, follicles, nodules, and yellow bodies were processed for histological examination. In addition to any scar tissue that could not be correctly identified on gross assessment, either due to size, colour or texture. Histopathological assessment was undertaken on all corpora lutea present on the ovaries of animals that died from dystocia (abnormal or difficult birth due abnormally large or misplaced foetus) or were in the process of aborting a foetus prior to death, in order to identify if CLs were already undergoing involution.

Histopathological assessment was also undertaken on any ovarian lesion and abnormality identified during gross assessment. This included ovaries that exhibited evidence of either hyper-or hypoplasia based on relative size. In addition to any ovarian tissue that appeared slightly necrotic or congested.

115 and 228 common dolphin and harbour porpoise, respectively, ovarian tissue samples were processed for histopathology. Samples of ovarian tissue were 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. All histological slides were assessed on two separate occasions, and assistance was provided by a veterinary pathologist in the assessment of ovarian abnormalities.

RESULTS AND DISCUSSION

As part of the current study a full gross and histological assessment and categorisation of ovarian scars, yellow bodies, atretic follicles etc. in both the common dolphin and harbour porpoise was undertaken. This work, focusing on describing ovarian characteristics in both species, will be presented in a subsequent paper that will be submitted to a peer reviewed journal. Within the current report, we present descriptions of reproductive tract pathologies in both species. Issues arose over the diagnosis of ovarian abnormalities based purely on haematoxylin and eosin stained sections. Therefore, additional work is required, including undertaking immunohistochemical staining for specific cell types, and this work is currently been carried out. As a result only preliminary results are presented here - and a number of additional possible ‘abnormality’ cases are not described. Consequently, a full assessment of, contaminant induced reproductive (morphological and functional) dysfunction and, the indirect effects of contaminants through lowering immunity, could not be undertaken.

COMMON DOLPHIN

Dystocia and abortion

A unimodal summer calving/mating period occurs in common dolphin in the North-east Atlantic, extending over approximately 5 months from May to September, inclusive, with possibly a more active period in July and August (Murphy et al. 2009). A lactation period of approximately 10.35 months has been determined for the population (Murphy 2004), and females attain sexual maturity at an average age of 8.22 years (Murphy et al. 2009).

There were no cases of reported dystocia within the sample of 57 mature female *D delphis*, though four animals representing 7% of the mature sample had recently aborted. These included a primiparous female (SW1992/20) from the control group that was identified with a high contaminant burden (total PCB concentration = 44.67 $\mu g\ g^{-1}$ lipid) during Phase I. This non-lactating female was bycaught during her second trimester and it was believed that the foetus was lost prior to examination due to lesions associated with the bycatch event. However, histopathological assessment of the corpus luteum of pregnancy revealed that it already started to undergo involution, i.e. animal had miscarried prior to the incidental capture event. This female was in good nutritional condition, and fat was present in the subcutaneous tissue on the dorsal flank.

SW1996/16, another female from the control group sample, bore a corpus luteum that exhibited early signs of regression, and measured 30 x 23.3 x17.9 mm. This female died in January and thus would have been in her second trimester, and the ovaries contained no prominent follicles. Mammary glands were not well developed and showed no gross evidence of lactation.

SW1997/15, a bycaught dolphin, was in moderate nutritional condition, with some subcutaneous fat. This animal died in the month of January, was not lactating, and no foetus was found - though the uterus was large and flaccid which is an indication of a recent pregnancy. A large regressing corpus luteum was identified, through histological examination, on the left ovary. A number of anomalies were observed within the ovaries of this individual which are currently under investigation.

SW2004/257 was a 23-year old female that died in the month of August. Cause of death was attributed to a live-stranding event, and the animal exhibited signs of starvation. The uterus was not quite fully involuted, contained mucoid material, and the cervix was fully closed. This animal was not lactating or ovulating, and a large regressing CL was present on the right ovary. Although this animal died during the calving/mating season, the state of the uterus, large regressing CL, lack of evidence of recent ovulation, and as the female was not lactating, all suggests an early miscarriage.

Twinning

Within the sample of 57 mature females, only one individual was pregnant with twins. This 12-year old female was in excellent nutritional condition (with subcutaneous fat) and health status and died as a result of incidental capture in fishing gear in December 1994. Both foetuses were male, measuring 27 cm and 31 cm, and were located in the left uterine horn. Two corpora lutea were present on the left ovary. This is only the second reported case of twinning in the common dolphin. A female measuring 201 cm in body length was found stranded in June 1998 along the Galician coastline, and described in González et al. (1999). A female foetus measuring 72 cm in body length, which had a curved caudal area, was located in the left uterine horn close to the genital aperture. A male foetus measuring 46 cm in body length was found in the uterus and its external morphology was slightly deformed – nose shorter than the female foetus (González et al. 1999).

Reproductive tract abnormalities and anomalies

The following is a summary of reproductive abnormalities and anomalies within the common dolphin sample of 80 individuals. As a large proportion of the sample was composed of the control group of ‘healthy’ animals (54%), the incidences of abnormalities reported here may be somewhat lower than what is occurring within the wider population. For a number of abnormalities only a tentative diagnosis is presented. Primarily as immunohistochemical staining is required for verification of cells. This work is currently being undertaken and results will be published in a subsequent paper on reproductive abnormalities. Of the eleven individuals (13.8% of sample) listed below with reproductive tract pathologies, corresponding contaminant data were only available for two females.

No incidences of uterine stenosis, occlusions or leiomyomas were observed within the common dolphin sample.

Vaginal calculi

Of the eighty female common dolphins assessed within the current study, 6 presented with vaginal calculi within their reproductive tracts (see Table 2), an occurrence of 7.5% within the whole common dolphin sample and 9% within the mature sample. These included one sexually immature, four resting mature, and one resting mature and lactating individual. In mature individual's exhibiting calculi, corpora scar numbers ranged from 13 to 34 scars. As common dolphins are reported to possibly ovulate up to 5 times within one oestrus period (Murphy 2004, Murphy et al. 2010), suggests that reproductive dysfunction (large number of ovulations without fertilisation) may occur in some individuals, conceivably due to the relative size of the calculi. SW2004/131, a bycaught resting mature female, had the highest corpora scar number within the whole mature sample. However these data are only tentative, as corpora scars ranged in size, colour and texture. Histological examination would have to be undertaken on all scars on the ovaries of this female for verification purposes, which was not undertake within the current study.

Some vaginal calculi were believed to represent incomplete abortion with retention of part or all of a foetus that subsequently crystallized and coalesced (Benirschke et al. 1984, Woodhouse and Reinne 1991). Others are possibly produced by one foetal bone that acted as a nidus for calcium phosphate deposition (Benirschke et al. 1984, Woodhouse and Reinne 1991). However within the current study, a sexually immature bycaught female common dolphin (SW1993/43) bore a calcified body within her vagina. This 7-year old individual was in good nutritional condition, though it had a high contaminant burden (total PCB = 47.73 $\mu g\ g^{-1}$ lipid), and its ovaries were heavily congested (see Figure 2). This is not the first study to report a vaginal calculus in a sexually immature cetacean. Van Bressem et al. (2000) observed a struvite calculus in an immature Peruvian dusky dolphin (*Lagenorhynchus obscurus*), and suggested an infectious etiology. McFee and Osborne (2004) also reported that a urinary tract infection might have been the underlying cause of a struvite (magnesium-ammoniumphosphate hexahydrate) calculus observed in the vagina of a four year-old bottlenose dolphin.

Table 2. Abnormalities and anomalies within the reproductive tract of 80 UK female *D. delphis*. *COD* = Cause of death, BY = Bycatch, NE = Not Established, = Live-stranding, NA = Not Available.

	Code	COD	Body length (cm)	Age (yr)	Maturity status	Corpora scar no.	Previously Gravid	Total PCB (μg g^{-1} lipid)
Reproductive tract								
Vaginal Calculi	SW1993/43	BY	195	7	Immature	0	No	47.73
Vaginal Calculi	SW1995/4	NE	207	>7	Resting mature	13	NA	NA
Vaginal Calculi	SW1996/98	NE	201	>18	Resting mature & lactating	23	Yes	NA
Vaginal Calculi	SW2004/131	BY	205	>10	Resting mature	c.34	Yes	NA
Vaginal Calculi	SW2004/336	LS	196	25.5	Resting mature	19	Yes	NA
Vaginal Calculi	SW2005/65	LS	196	>10	Resting mature	21	Yes	NA
Ovary								
Ovotestis	SW2006/289A	NE	191	6	Immature	0	No	NA
Tubule-like elements	SW1994/24	NE	174	2	Immature	0	No	38.6
Possible fibroma	SW1994/147	BY	192	c.14	Resting mature	12	Yes	NA
Possible tumour	SW1998/148	LS	209	24	Resting mature	26	Yes	NA
Ovarian Cyst	SW2005/2	LS	195	19	Resting mature	27	Yes	NA

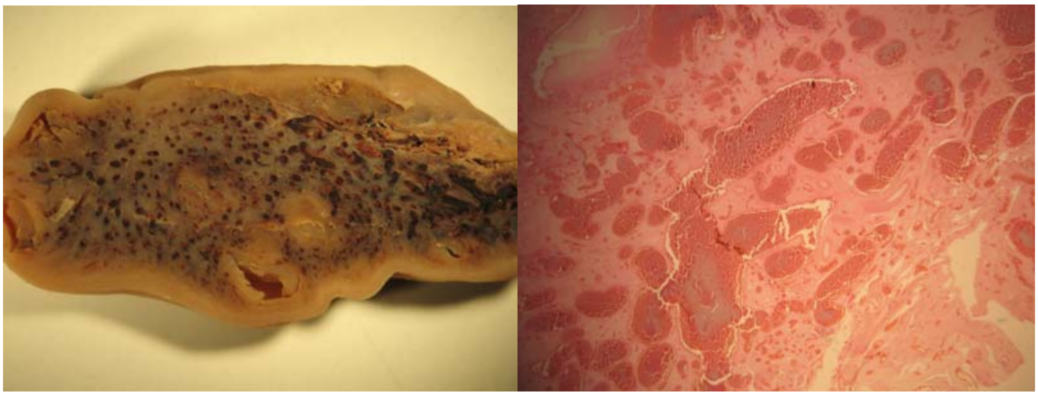


Figure 2. Congested ovary of an immature bycaught female (SW1993/43). This individual possessed a vaginal calculus and a high total PCB burden.

A large calculus was reported within the vagina of SW1995/4, weighing approximately 100 g, and may have prevented breeding. In SW1996/98, the anterior vagina contained 5 highly polished faceted calculi that could be assembled into an egg-shaped mass - with one section missing. The largest calculus was 32 x 29 x 26 mm and the smallest 17 x 19 x 15 mm. Analysis revealed they were mainly composed of phosphate salts. This individual was in moderate nutritional condition, though both ovaries appeared slightly congested on gross appearance. Histological assessment of the ovaries revealed numerous fluid filled cystic spaces without granulosa cells or an oocyte (see Figure 3). Cysts were either filled with eosinophilic like material or blood. Cause of death was not established for this individual, and the animal was not frozen prior to the necropsy. This female was more than 18 years of age and possessed 23 corpora scars on her ovaries.

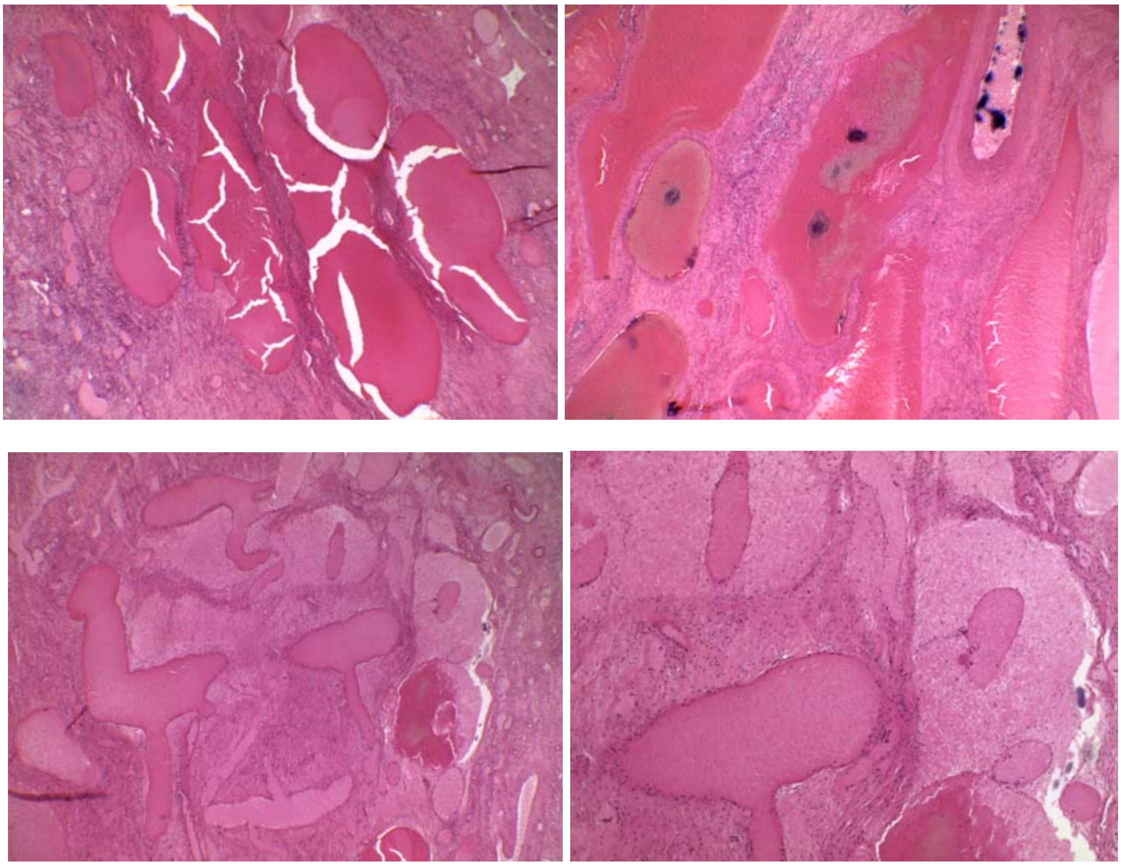


Figure 3. Fluid-filled cyts in an ovary of a female common dolphin with a vaginal calculus (SW1996/98). On gross assessment these areas appeared congested. Cause of death was not established for this individual.

SW2004/131 had a single large vaginal calculus present within the lumen. It was 102 mm in length, 56 mm at its widest part, and weighed 321g. Its cranial extremity protruded into the distal cervix, and the entire structure filled the lumen of the distended vagina. The calculus was easily detached from the vaginal mucosa which appeared relatively smooth walled. The ovaries were heavily congested and fibrous adhesions were present on the ovarian surface. This bycaught female was in good nutritional condition and health status, though exhibited adrenal cysts (bilateral).

SW2004/336, a live-stranded animal in good nutritional condition, had a small oval shaped vaginal calculus (approx. 20 mm in length) within the cranial end of the vagina, adjacent to a large mucus plug situated next to the entrance to the uterus. A large egg-shaped off-white vaginal calculus was present within the markedly distended vagina of SW2005/65. The anterior part of the calculus was moulded into the shape of the mucosal folds within the anterior vagina. The complete calculus weighed 491g. This female live-stranded in an emaciated condition, and 21 corpora scars were present on her ovaries.

Baker (1992) noted a vaginal calculus in a UK common dolphin collected prior to 1992. The calculus, which was also composed of phosphate salts, was flat and discoid and measured 40 x 4 mm. A lower incidence (3.2%) of vaginal calculi was reported in 435 female common dolphins that stranded along the French coast. Struvite calculi ranged from 3.6 to 1460 g in weight, and 12 of the 14 calculi were big enough to prevent successful copulation by occlusion of reproductive tract (Dabin et al. 2007). Dabin et al. (2007) also suggested that supersaturated urine and associated urinary tract infection with urease-producing microbes were the most likely cause. A struvite calculus was also recently reported in a one-year old harbour porpoise (Norman et al. 2011).

Ovotestis – True hermaphrodite

True hermaphroditism is defined as the simultaneous presence in a single individual of both testicular and ovarian tissue, which may exist either in separate gonads or in the same gonad (ovotestis; (Krob et al. 1994). True hermaphroditism can be bilateral (testicular and ovarian tissue identified on both sides, usually as ovotestes), unilateral (ovotestis on one side and an ovary or testis on the other side), or lateral (testis and contralateral ovary) (Tangner et al. 1982, De Guise et al. 1994a). An immature 6 year old unilateral hermaphrodite common dolphin (SW2006/289A) was found stranded on the southwest coast of the UK. The external phenotype was that of a female, but internally there was one ovotestis containing both ovarian follicles and testicular tubular elements, and a contralateral ovary. Ovarian portions of the ovotestis appeared normal and demonstrated follicular development, whereas the testicular tissue exhibited hypoplasia and degeneration. On gross assessment, the portion of the ovotestis was not initially noted as an abnormality. It appeared tan-to-yellow in colour and measured only 6.83 × 5.49 mm. As the individual was at an age approaching sexual maturity, a sample of this tissue was taken for histology to assess whether the scar tissue represented a corpus albicans - see Annex A for a full description of this abnormality within the ovarian tissue.

This is the first case of an ovotestis in a cetacean species and it is not known if this disorder of genital development is due to abnormalities of genetic or chromosomal origin or inappropriate hormone exposure. Cytogenetic analysis has not been undertaken, nor have levels of lipid soluble contaminants been assessed. As mentioned earlier contaminants have endocrine disrupting activities that can lead to reproductive disorders during fetal and neonatal development (Delbes et al. 2006, Murphy et al. 2011).

An atypical bilateral hermaphrodite beluga whale was reported in the St Lawrence Estuary, Canada (De Guise et al. 1994a). That animal appeared externally to be male with the presence of a penis in the genital slit, located halfway between the anus and the umbilicus. On dissection, two testicles with associated epididymis and vas deferens, two separate ovaries, and the complete ducts of each sex were found; although the cervix, vagina and vulva were absent. An ovary was located dorsally to the caudal pole of each testicle (De Guise et al. 1994a). The whale was sexually mature, with evidence of both spermatogenic and follicular development. This condition was attributed to hormonal disturbance in early pregnancy, whereby normal differentiation of male and female organs was disrupted (De Guise et al. 1994a, Reijnders 2003). Out of the 93 remaining beluga whales (48 female and 45 male) examined in the St Lawrence population, one individual appeared to be a male pseudohermaphrodite (Reijnders 2003). Prior to this investigation, pseudohermaphroditism had also been reported in two bowhead whales (*Babaena mysticetus*; (Tarpley et al. 1995), a fin whale *(Balaenoptera physalus*; (Bannister 1962) and a striped dolphin (*Stenella coeruleoalba* formerly *Prodelphinus coeruleoalba*; (Nishiwaki 1953). In all of these cases, although the external phenotype was that of a female, the internal reproductive organs, or elements thereof, were male. In the case of the bowhead whales, which were both apparently normal male karyotypes (40 + XY), findings suggested a failure of androgen (testosterone or dihydrotestosterone) expression consistent with the syndrome of complete testicular feminisation (Tarpley et al. 1995).

Tubule-like elements

SW1994/24 was a sexually immature 2-year old common dolphin with a high total PCB burden (total PCB level = 38.6 $\mu g\ g^{-1}$ lipid). On gross appearance, one ovary was slightly larger than the other (see Figure 4a), and a cross section was obtained for histopathological assessment. Although lots of small developing and primordial follicles (Figure 4b, d) were observed on one side of the section/ovary, on the other side tubule-like structures were observed in the ovarian stroma (see Figure 4b, c, e, f). In this unencapsulated tissue, tubules ranged in size and were in various stages of disintegration.

Small tubules were observed in aggregations in the outer cortex and larger tubules closer to the medulla. Tubules that are not disintegrated were well differentiated, had well-formed basement membranes and showed some resemblance to juvenile male seminiferous tubules with, in some cases, cells lining the basement membrane (Figure 4f). In the ovotestis case, testicular tissue appeared to be slightly compartmentalized, with connective tissue separating the ovarian and testicular components (see Annex A). In addition, there was evidence of spermatogenesis with the presence of spermatocytes within the seminiferous tubules. In contrast, the tubule-like elements in SW1994/24 were interspersed within the ovarian stroma, throughout the majority of one side of the section/ovary.

Due to the state of autolysis in the tissue/section, it was difficult to decipher the cell-types within the tubules in the haematoxylin stained sections. Additional sections will be obtained from this possible tumour for immunohistochemical (marker) staining for specific cells, such as granulosa, Sertoli and leydig cells. Granulosa-theca cell tumours have been noted to develop a tubular pattern similar to that of the Sertoli cell tumour (Kennedy and Miller 1993). Considering the age of the female, this disorder could be due to genetic or chromosomal origin or inappropriate hormone exposure.

Possible fibroma tumour

A possible fibroma (benign) tumour was observed on the ovary of SW1994/147. The tumour measured 20 x 25 mm and consisted of reddish/black tissue resembling splenic tissue in gross appearance. The individual was incidentally captured in fishing gear and in moderate nutritional condition. The ovoid mass was attached to the hilus of that right ovary by a band of mesothelial connective tissue. Fibrous stroma comprising the core of the mass was moderately vascular and variably composed of spindle-shaped or more ovoid and irregular fibroblastic cells frequently orientated at random. These cells were densely packed towards the periphery of the mass and more loosely arranged centrally. Unfortunately tissue autolysis and poor staining made final diagnosis problematic. The individual in question was a bycaught 14-year old resting mature female in moderate nutritional condition.

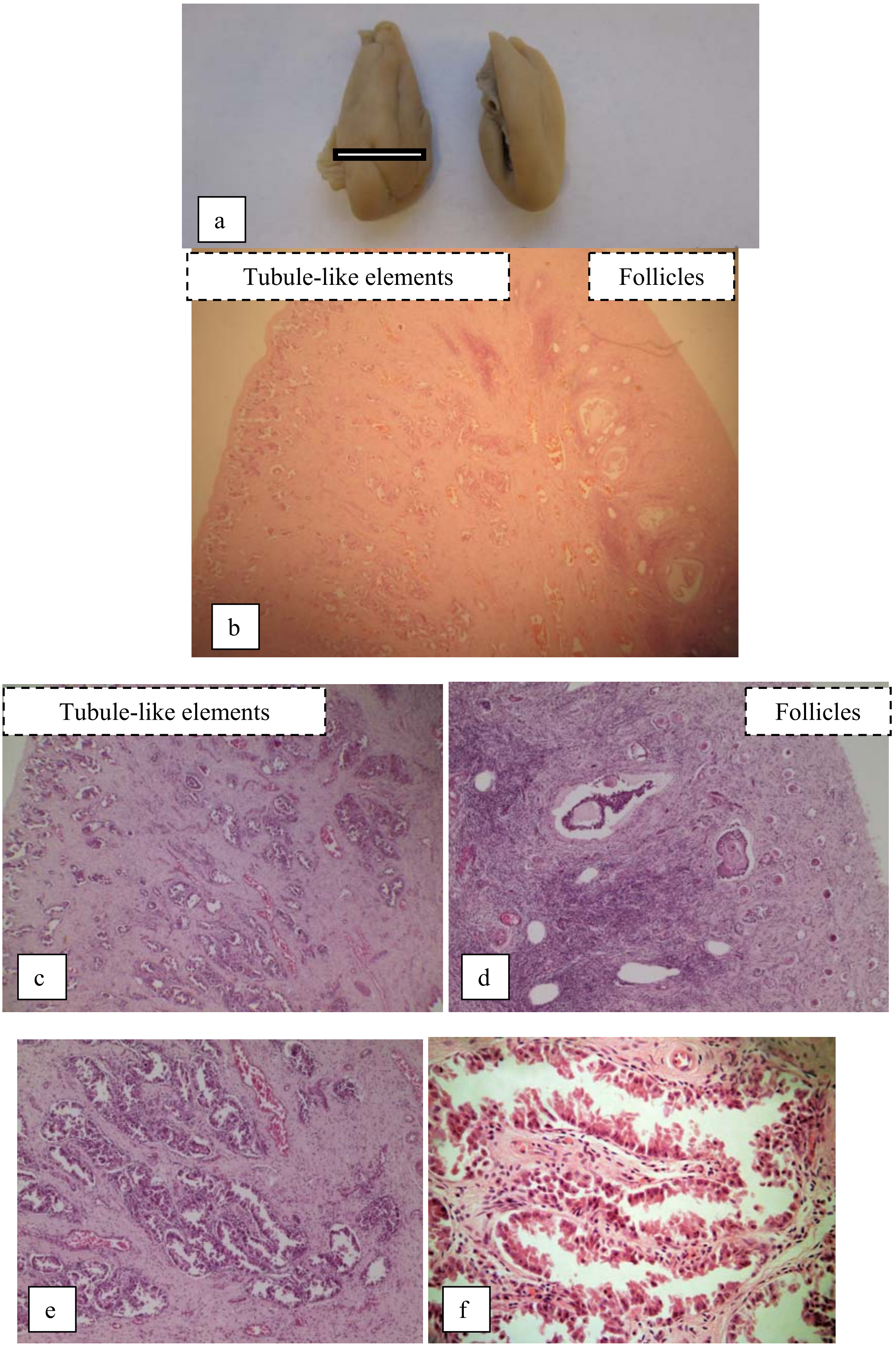

a
Tubule-like elements
Follicles
b
Tubule-like elements
Follicles
c
d
e
f

Figure 4. (a) Ovaries of SW1994/24, and location of cross section taken; (b) Cross section of ovary stained with haematoxylin, note the tubule like elements on the left-hand side and follicles on the right; (c) Tubule-like elements; (d) ovarian follicles; (e, f) Tubule-like elements within the ovarian stroma, note cells lining the membranes.

Possible tumour

SW1998/148, a 24-year old resting mature female, live-stranded in the month of August and possessed 26 ovarian scars. The largest follicle measured 2.93 mm and was present on the right ovary. The left ovary contained a possible tumour cyst-like structure. The thick walled multilocular cyst was approximately 11 mm in diameter, and, on gross assessment appeared to have two-three compartments. Histologically these appeared to have both solid and cystic components (see Figure 5). All compartments were separated by thick walled septa. The main outer chamber contained cells a diffuse pattern, within some infiltration of fibrous tissue. Cells exhibited pleomorphism, varying in size and shape ranging from round to polyhedral to elongated. Cells were well defined with some undergoing mitosis. On the whole, nuclei appeared oval and some were grooved. Chamber two contained a number of cystic spaces (some fluid-filled) and collagenous fibrous tissue. There are not available corresponding pollutant data for this individual.

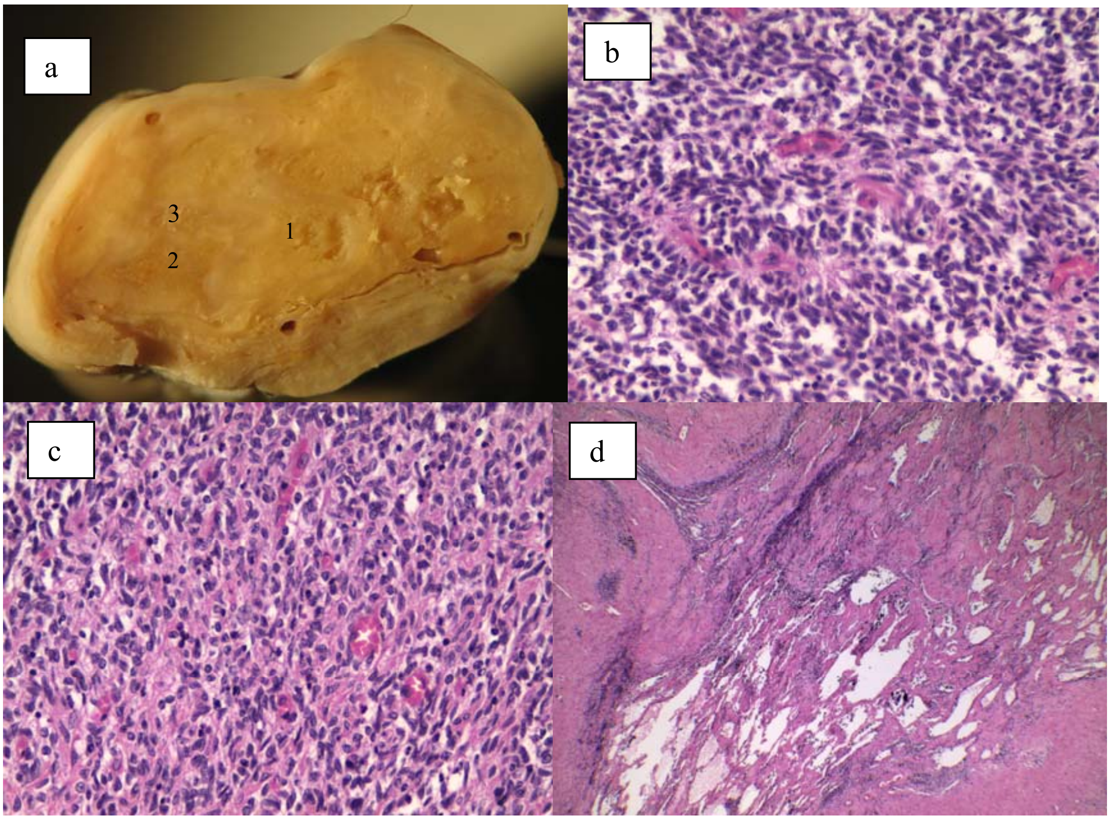


Figure 5 (a) Cyst in left ovary of SW1998/148, containing possibly three chambers (b, c) possible tumour cells in a diffuse pattern in chamber 1, (d) cystic spaces in chamber 2.

Ovarian cyst

Ovarian cysts, such as follicular and luteinized cysts (though mainly the former), have been reported in the ovaries of the dusky dolphin, striped dolphin (*Stenella coeruleoalba*), Pacific white-sided dolphin (*Lagenorhynchus obliquidens*), short-finned pilot whale (*Globicephala macrorhynchus*), and southern minke whale, among others (Marsh 1984, Lockyer 1987, Munson et al. 1998, Van Bressem et al. 2000, Robeck et al. 2009). Cystic follicles have been known to produce estrogens and progesterone, depending on the degree of luteinisation (Robeck et al. 2001).

A large thin walled fluid-filled cyst was noted on the lateral aspect of the left ovary of SW2005/2 (Figure 6a). The right ovary exhibited signs of congestion (Figure 6b), and both ovaries had a total corpora scar count of 24. The maximum length of the cyst was c.47 mm, while the maximum length of the ovary was only 43 mm. Prevalence of ovarian cysts in the mature sample was 1.75%. This 19-year old non-lactating dolphin was in good nutritional condition, though live-stranded in the month of January – i.e. outside the mating period. Nothing abnormal was detected in the uterus or vagina upon gross assessment.

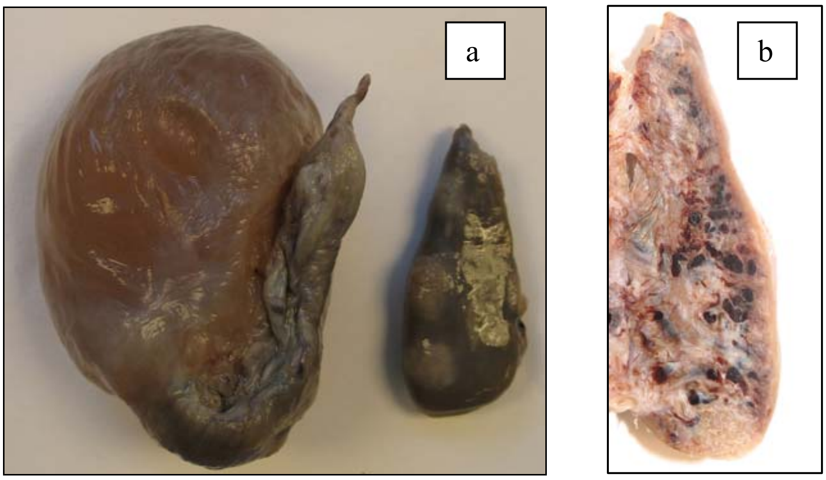


Figure 6. (a) Lateral fluid-filled ovarian cyst on the left ovary of SW2005/2, measuring 47 mm; (b) cross section of congested right ovary.

Cysts were also reported on an ovary of a Peruvian sexually mature lactating long-beaked common dolphin (Van Bressem et al. 2006). A large (suggested) follicle cyst measured 19 x 17 x 15 mm and also projected from the left ovary. The prevalence of ovarian cysts in the *D. capensis* sample was 4.2%, similar to that observed in *Lagenorhynchus obscurus* from the same region (3.06%, n = 98; Van Bressem et al. 2000). In cattle, deviation of the preovulatory surge of luteinizing hormone, either the absence or mistiming of the surge, is thought to cause follicular cysts (McEntee 1990, Kennedy and Miller 1993, Van Bressem et al. 2006).

Luteinised cysts, with the potential to impede ovulation, were reported on the ovaries of four striped dolphins that died during a morbillivirus epizootic in the Mediterranean Sea (Munson et al. 1998). Associated with high levels of PCB exposure, they occur when ovulation is impeded. It was suggested they were caused by the effects of, PCBs or morbillivirus on hypothalamic/pituitary function or, PCBs on ovarian responsiveness (Munson et al. 1998). A luteinized cyst was also reported in a dusky dolphin (Van Bressem et al. 2000).

Congested ovaries

A number of ovaries appeared congested with areas of vascular haemorrhage (e.g. 1993/43) and/or fluid-filled cysts (e.g. 1996/98); the significance of which is not yet known. None of individuals were frozen prior to necropsy. A significant number these individuals however died as a result of incidental capture and congestion, oedema, and haemorrhaging were noted in other tissues, such as the lungs, findings consistent with death due to immersion. Ovarian congestion and/or a necrotic appearance were noted in the ovaries of three individuals that presented with vaginal calculi. In some of the other individuals, Brucella was detected (weak positive) in the pericardial fluid, an ovarian cyst was present, or the female recently aborted.

Brucella

Brucella is a virus that has been reported to induce abortion in marine mammals (Miller et al. 1999); most recently suggested to have occurred in a harbour porpoise that stranded on the coast of Belgium (Jauniaux et al. 2010). Out of the fifteen individuals tested for Brucella within the current study, four (27% of the sample) tested positively either in blood or pericardial fluid (ELISA test) and/or in various tissues. Brucella was detected in the blood and uterine tissue of one common dolphin within the control group. This 13-year old resting mature female (SW2006/58c) had four ovarian corpora scars and a low total PCB burden (6.77 $\mu g\ g^{-1}$ lipid). The ovaries were slightly congested, and certain anomalies within the ovaries that were identified histologically are still under investigation. As noted earlier, another female with noted ovarian congestion (SW1994/25) gave weak positive result (in pericardial fluid) for Brucella with the ELISA test. This was a lactating female with a low contaminant burden (7.56 $\mu g\ g^{-1}$ lipid). Neither of the two mature females showed evidence of recent abortion. SW2005/295, tested positively for the Brucella antibody in blood and pericardial fluid. Brucella was also recovered from the lung and kidney in this immature dolphin. Finally, the ELISA examination of pericardial fluid for antibody to Brucella proved positive in SW2006/30a, though it was not recovered from any of the tissues removed from the animal at post mortem. Post mortem examination of this immature animal revealed extensive chronic pleural adhesions.

EFFECTS OF CONTAMINANTS ON REPRODUCTION IN COMMON DOLPHINS – CONTROL GROUP SAMPLE

Within the control group of 'healthy' females, all ovarian corpora scars were re-assessed and where uncertainties existed, verified through histological examination. As a result, corpora scar count data were updated for four individuals. In addition, a primiparous pregnant female and a resting mature female were reclassified as recently aborted. The sample of 43 'healthy' females (not suffering from any infectious and non-infectious disease that might inhibit reproduction) was composed of 20 immature, 10 resting mature, two primiparous pregnant, two pregnant & lactating, seven lactating, and two non-lactating females that recently aborted.

All sexually immature (nulliparous) females (range 17.2-93.6 μg g^{-1} lipid), the two "primiparous" pregnant females (range 32.3-77.82 μg g^{-1} lipid), and two females that recently aborted (44.7-73.6 μg g^{-1} lipid) had total blubber PCB levels above the threshold level of 17 μg g^{-1} lipid for adverse health effects (Figure 7). Apart from these individuals all other females with total PCB burdens above the threshold level were resting mature females. Two resting mature individuals, 7.5 and 9 years old, with total PCB levels just above the threshold had not been previously gravid and had only one corpora scar of ovulation. A decline in total blubber PCB levels with increasing corpora scar number was observed, which was approaching significance ($p = 0.08$) (see Figure 7a), and a similar plot was obtained when ∑25CBs was plotted against corpora scar number (not shown).

Two females within the mature control group sample (sample $n = 23$) recently aborted (8.9% of the mature sample). Both had high a contaminant burden, above the threshold level of 17 μg g^{-1} lipid for adverse health effects in marine mammals (Kannan et al. 2000, Schwacke et al. 2002). SW1992/20 was a primiparous female, with a total PCB concentration of 44.67 μg g^{-1} lipid. As noted earlier, this non-lactating female was bycaught during her second trimester. This individual was in good nutritional condition, though suffered from generalised chronic pleuritis. Morbillivirus was not detected in the lung tissue. SW1996/16 died in the month of January and showed evidence of a recent abortion. Although a regressing corpus luteum and ten corpora albicantia were observed on the left ovary, the 18.5 year old individual had a total PCB concentration of 75.6 μg g^{-1} lipid. The association between high contaminant burdens and the incidence of abortion in these two individuals cannot be discounted.

Within the resting mature group, there were two cases where females had been previously gravid, though had not successful offloaded their contaminant burdens, i.e. either aborted or their offspring did not survive during the initial few weeks of lactation. This is based on the fact that the majority (c. 80% of OCs) of a females contaminant burden is believed to be transferred to first born calves during the first seven weeks of lactation (Cockcroft et al. 1989). SW1994/57 was a resting mature female, 15-years in age with 5 corpora scars and a high total PCB burden of 103.5 μg g^{-1} lipid – the highest in whole *D. delphis* sample. The second individual, SW2005/289, was a 30-year old resting mature female with a total PCB burden of 67.4 μg g^{-1} lipid and 10 corpora scars. Brucella was not detected in various tissues from either of these two individuals. Two other resting individuals with high corpora scar numbers (14, 15) were also previously pregnant, and had total PCB burdens above the threshold level (27.2 - 27.7 μg g^{-1} lipid).

Elevated PCB levels may have consequences on uterine and placental health and, subsequently, foetal health and survival (Hohn et al. 2007, Murphy et al. 2010). An association has been reported between PCB-153 (at serum concentrations of > 200 ng/g lipid) and increased risk of foetal loss (miscarriages or stillbirths) in humans (Toft et al. 2010); however some other studies have not reported this relationship (e.g. Meeker et al. 2011). Unlike the previous studies on mink, PCB-153 and ΣPCBs have been associated with a significantly higher likelihood of failed implantation in humans, and these relationships demonstrated dose-dependent trends (e.g. Meeker et al. 2011). It was suggested that the impact of PCBs on uterine receptivity may contribute to the increased risk of implantation failure (Meeker et al. 2011). A similar situation to seals where 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, estradiol-17β levels in seals fed with fish of a higher contaminant burden were lower than those of the control group. Lower levels of estradiol could have impaired endometrial receptivity and prevented successful implantation of the blastocyst (Reijnders 2003). In the current study it appears that high PCB burdens are not inhibiting ovulation, conception or implantation in common dolphins as all sexually immature (nulliparous) females and the three primiparous females had total blubber PCB levels above the threshold level. As noted during Phase I, since pinnipeds experience delayed implantation/embryonic diapause, they may be more vulnerable than cetaceans at this stage of the reproductive cycle.

In the control group sample of 'healthy' common dolphins high total PCBs concentrations may have increased the risk of foetal mortality. In mink (*Mustela vison*), although ovulation, conception and implantation occurred, similar to the current study, PCBs increased foetal mortality. This was mediated through affecting maternal vasculature in the placenta and producing degenerative changes in the trophoblast and foetal vessels, which lead to foetal growth retardation or death (Backlin et al. 1997, 1998). A similar situation may be occurring in individuals within the current study.

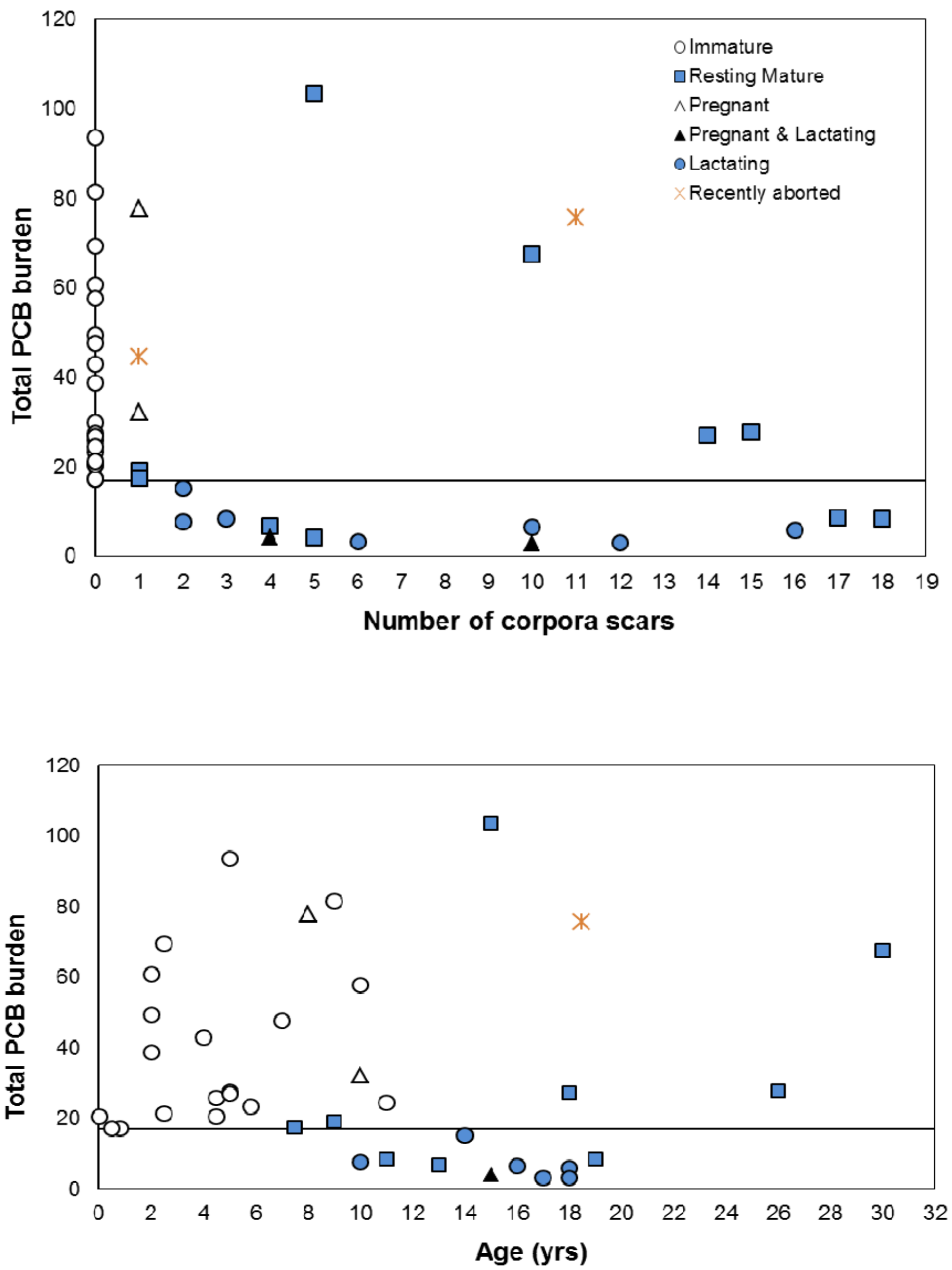


Figure 7. Total PCB burden [(∑7ICES congeners)*3] (µg g^{-1} lipid) as a function of (a) corpora scar number (n = 43) and (b) age (n = 38) in the *D. delphis* control group sample. Threshold for adverse health effects on reproduction of 17 µg g^{-1} lipid is marked on the graph.

Reproductive pathologies were observed in two females within the control group sample. SW1993/43 was a 7-year old female with a vaginal calculus and a high total PCB burden (47.73 µg g^{-1} lipid). As this female was sexually immature, the etiology of the calculus is more than likely an infectious one. SW1994/24 was also a sexually immature individual, two-years in age, with a total PCB burden of 38.6 μg g^{-1} lipid. Both primordial and developing follicles were present in the ovaries in the latter individual in addition to tubule-like elements. The etiology of this condition is unknown, and could be caused by a congenital disorder.

HARBOUR PORPOISE

Average age at attainment of sexual maturity in female harbour porpoise inhabiting English and Welsh waters is 4.24 years, and individuals predominately attain sexual maturity between three and five years of age (Murphy 2008). In UK waters, a unimodal summer mating and caving period has been reported for this species, with the main period between May to July (Learmonth et al. in review). Mean length at birth is 78.8 cm (Learmonth et al. in review).

The sample of 140 harbour porpoise was composed of animals that died from variety of causes including generalised bacterial infection and pneumonia (see Table 3). 51% of the sample included animals that died from incidental capture or physical trauma – in the majority of cases the latter was attributed to adverse interactions with bottlenose dolphins. The porpoise sample was composed of 81 immature, 11 resting mature, nine pregnant, six pregnant & lactating, 21 lactating, three aborting & stillbirth, six lactating, aborting & stillbirth, and two females that recently aborted. In the six 'lactating, aborting & stillbirth' cases, four females were lactating for their near-full-term foetuses.

Of the 59 mature females, 29 (49%) died as a result of bycatch or trauma. All mature females showed evidence of previous or current gravidity.

Females ranged from 0 to 21 years (n= 116) in age, 70 to 191 cm (n = 139) in length, and 0.29 to 23.17 g (n = 131) in combined gonadal weight. Sexually immature females ranged from 0 to 5 years (n= 77) in age, 70 to 158 cm (n = 80) in length, and 0.29 to 3.94 g (n = 75) in combined gonadal weight. Sexually mature females ranged from 4 to 21 years (n= 39) in age, 138 to 191 cm (n = 59) in length, 1.93 to 23.17 g (n = 56) in combined gonadal weight, and 1 to 21 in corpora scar number.

Harbour porpoises can have multiple ovulations within their unimodal mating period (see Murphy et al. 2010). Within the mature sample, two lactating females had the highest corpora scar number (see Figure 8). SW2000/128, a 12-year old lactating female that had recently given birth had 20 ovarian scars. The individual died from lesions sustained by dystocia (see below). SW1996/160 was a 14-year old lactating harbour porpoise with 21 ovarian scars. This individual live stranded in the month of October in poor body condition and cause of death was attributed to gastropathy and/or enteropathy and also suffered from mastitis.

Table 3. Cause of death categories for female harbour porpoises assessed within the current study (n = 140).

Cause of Death	Number
Bycatch	57
Pneumonia	18
Physical trauma	14
Generalised bacterial infection	14
Starvation	10
Live stranded	5
Other	5
Dystocia	3
Gastropathy&/or enteropathy	3
Neoplasia	2
Not Established	9

Within the sample of 140 females, there were no observed cases of prematurity – assessed during gross examination. Mean length at birth for this species in UK waters is 78.8 cm (Learmonth et al. in review). The smallest porpoise in the sample (SW1997/97) measured 70 cm, and died from starvation in the month of June. The foetal folds were still present and the umbilicus was not completely healed. SW1994/115 was 77 cm in length, died in the month of July, and cause of death was not established. The stump of the umbilical cord was also present; it was transparent and free of blood. There were three cases of dystocia (abnormal or difficult birth; see below), and foetuses measured between 74 and 84 cm in length.

Within the mature sample there a number of cases of abortion (see below). Additional cases are currently being assessed, and the samples size may be higher than what is presented below. In addition there were noted cases of ovarian hyer- and hypoplasia (some outlined below), and we are currently evaluating these cases further.

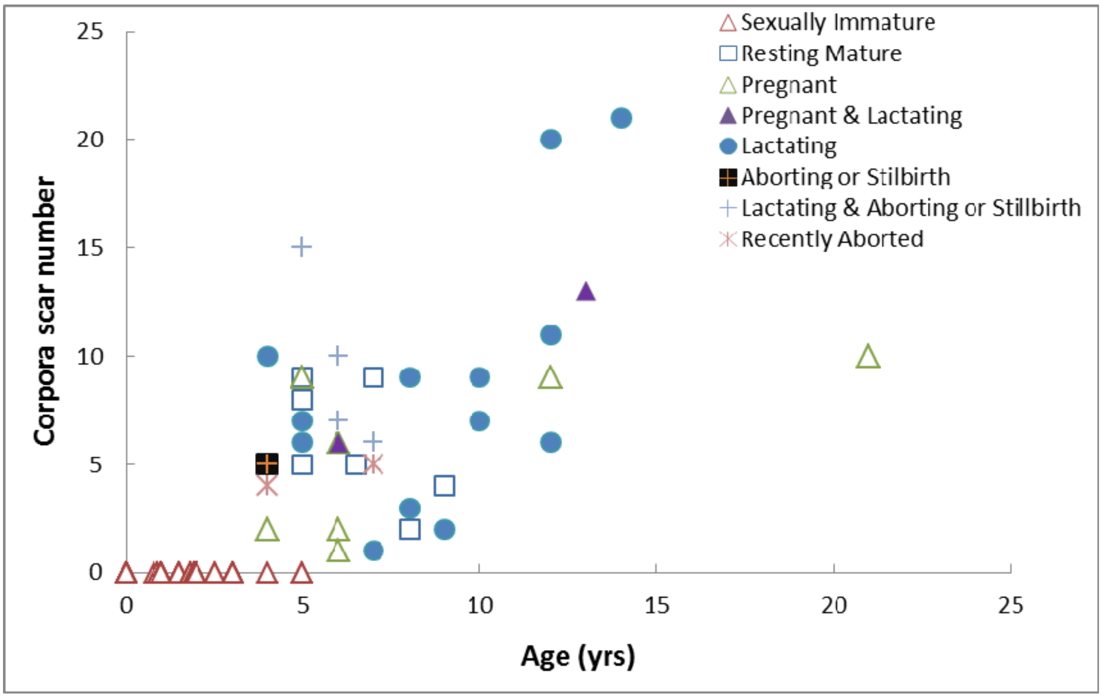


Figure 8. Number of corpora scars against age in the English and Welsh sexually mature female harbour porpoise sample (1991-2004, n = 116).

Dystocia and abortion

For three mature females, cause of death was attributed to dystocia (associated with death of full-term foetus; 5.1% of mature sample). There were two cases where parturition, through lesions caused by dystocia, caused significant disease believed to contribute to the death of the animal, four cases where the mature female died while aborting a foetus, two instances of recent abortion, and two incidences where the mature pregnant female died from physical trauma and histological examination of the corpus luteum showed evidence of involution, i.e. foetus had died prior to the mother/incidental capture event. These cases in all composed 23% of the mature sample; and in the majority of cases females were in their second or third trimester. Of these 13 cases, four died from either bycatch or physical trauma. However, these results are only preliminary, as within the mature sample there are a number of additional abortion cases that are still under investigation.

In the three dystocia and stillbirth cases, foetuses ranged between 7 and 15.6 kg in weight. SW1997/93, a 6-year old porpoise, suffered a uterine rupture, and developed acute septic metritis and circulatory (septic) shock. In the case of the 5-year old SW1997/94, its foetus weighed 15.6 kg (average weight of newborns in Scottish waters was 7 kg; Learmonth et al. in review), was in a breech presentation and showed very marked autolytic change, far more than the maternal carcass. The uterine wall contained many small-to-medium sized haemorrhages. A similar situation to SW2003/274, where the body of its foetus was at a more advanced stage of decomposition, indicating that the calf may have died in utero and the mother had been trying to abort. The foetus was still in the folded position within the vagina, though the bulk of the curved tailstock - which was grossly swollen - was protruding from the entrance to the vagina. The foetus had also incurred injuries to the front of the head. In all three cases, the corpora lutea were undergoing early regression, confirming that the foetuses had died prior to their mother.

In two adult female porpoises, there were indications that peritonitis, due to a ruptured cervix (SW2000/128), and peritonitis and metritis (SW2000/98) were sequel to dystocia. The former individual also suffered from necrotising vaginitis. In both cases, the animals live stranded (see Table 4).

There were four cases where a mature female live stranded while aborting (expulsion of foetus before the normal end of pregnancy) a foetus. SW1999/10 live stranded in the month of November, and was in a poor nutritional condition. The animal was in the process of aborting a dead male foetus measuring 38 cm in length. A uterine salmonella species infection was possibly the cause of the abortion and the presence of purulent fluid within the uterus. The internal viscera of the foetus were slightly more decomposed than the tissues of the maternal carcass, and histological assessment of the corpus luteum identified it was undergoing early involution. Maternal cause of death was attributed to generalised bacterial infection (Salmonella sp.). SW1999/40, a four year old harbour porpoise was heavily parasitised and in the process of aborting (dilated cervix, ruptured placental membranes) a dead foetus measuring 46 cm when it stranded alive in the month of March. There were severe lesions (of probably parasitic origin) in the lungs, which may have severely compromised normal pulmonary respiratory function. The foetus again appeared to be in a more advanced state of decomposition than the maternal carcass. SW2000/5, a pregnant lactating female porpoise in good nutritional condition live stranded in the month of January. This female was under the process of aborting a foetus measuring 36cm in length. Upon examination, foetal fluid was flowing from the genital slit, the cervix was open, and the mucus plug was missing.

External examination of SW1996/177 revealed what appeared to be placental membranes extending from the vulva. A small foetus was found to be present within one horn of the uterus. This foetus was moderately decomposed and no placental tissue was found in association with it. The uterine mucosa appeared to be thickened and possibly necrotic. Histological examination of the corpus luteum revealed that the endocrine gland was undergoing involution and lutein cells had undergone complete autolysis; indicating that the foetus died some time before its mother.

Within the mature sample of 59 females, there were instances of recent abortion. Upon examination of the reproductive system, SW1997/174 and SW1994/21 showed evidence of being recently gravid, in addition to the presence of a regressing (histologically assessed) corpus luteum – in both cases lutein cells had undergone complete autolysis. Females were not actively lactating, and both died from injuries sustained from physical trauma in the months of November and February – i.e. outside the calving season.

Finally, there were two cases (SW1994/175, SW1996/94) where pregnant females died as a result of incidental capture in fishing gear in the months of December and May, and the corpora lutea showed clear evidence of regression. Lutein cells were undergoing autolysis at more of an advanced stage than females who recently gave birth to full-term offspring. SW1996/94 died in May and was pregnant with a full term foetus, weighing 6 kg. Mammary gland tissue was well developed and there was about 50ml of viscid yellow secretion in each gland, in addition to the presence of cholesterol clefts.

Reproductive tract abnormalities and anomalies

No vagina calculi were reported in any of the140 harbour porpoises assessed within this study. However, calculi have been observed in other UK female harbour porpoises (CSIP, unpublished data). There were no incidences of uterine stenosis, occlusions or leiomyomas within the harbour porpoise sample. None of the females listed here with reproductive tract abnormalities were individuals identified to-date to either have aborted or died from dystocia.

Genital ulcers

In the sample of 140 female harbour porpoises, five presented with genital ulcers, including three sexually immature and two mature females (See Table 4).

SW1999/39 was a neonate, measuring 104 cm in length and cause of death was attributed to starvation. This porpoise live stranded in the month of March. On gross examination, acute inflammation of the genital slit epidermis and some erythematous ulceration of the vaginal mucosa was noted. Histological examination revealed acute dermatitis of the genital slit region and localised sub-acute to chronic vaginal ulceration associated with mild-moderate lymphocytic/plasmacytic cellular infiltration. Minimal volume serous fluid was also noted in the uterine lumen upon gross assessment, though no bacteria were detected. A significant ovarian abnormality was detected, which will be discussed later. Unfortunately, no contaminant data were available for this individual.

Table 4. Abnormalities and anomalies within the reproductive tract of 140 UK female *P. phocoena*. NA = not available.

	Code	Body Length (cm)	Age (yr)	Maturity status	Scar no.	Previously Gravid	Total PCB burden ($\mu g\ g^{-1}$ lipid)
Reproductive tract							
Genital Ulcer	SW1994/185	145	5	SM	5	Yes	34.09
Genital Ulcer	SW1995/61	148	7	SM	1	Yes	36.6
Genital Ulcer	SW1995/85	86	0	IM	0	No	65.39
Genital Ulcer	SW1999/39*	104	0	IM	0	No	NA
Genital Ulcer	SW2000/33	109	1.9	IM	0	No	56.54
Genital Papilloma	SW2000/73	152	6	SM	6	Pregnant	14.93
Genital Papilloma	SW2001/21	171	>17	SM	13	Pregnant	5.08
Genital Papilloma	SW2001/218	144	6	SM	1	Pregnant	46.65
Genital Papilloma	SW2002/95	156	6.5	SM	5	Yes	16.36
Vaginal plaques	SW1997/36	156	7	SM	9	Yes	22.38
Vaginal plaques	SW2000/50	148	10	SM	7	Yes	35.52
Vaginal wall lesion	SW1998/129	154		SM	11	Yes	31.9
Vaginal abscess	SW1998/211	149	12	SM	11	Yes	NA
Endometritis	SW1997/178	156		SM	14	Yes	8.78
Peritonitis and metritis	SW2000/98	165		SM	10	Yes	NA
Peritonitis and vaginitis	SW2000/128	162	12	SM	20	Yes	NA
Ovary							
Tubule-like elements	SW1999/39*	104	0	IM	0	No	NA
Possible tumour	SW2003/296	154	12	SM	6	Yes	4.86

* same individual

For the remaining porpoises exhibiting genital ulcers, three of the individuals had total PCB concentrations above the threshold level for adverse health effects – contaminant data were not available for one female (see Table 4). SW1995/85, also a neonate, measured only 86 cm in length and live stranded in good nutritional condition in the month of July. The umbilicus had fully healed and the vibrissae were absent, suggesting this animal was not just born. On gross assessment a small cutaneous ulcer of the vulva was found adjacent to the clitoris. Histology assessment revealed an ulcer of epithelium with mixed (mainly PMN) inflammatory cell infiltration and hyperplasia of adjacent epithelium. A small quantity of normal-looking sero-mucoid fluid was present within the uterus, and contained three colonies of Pasteurella sp. This individual possessed proportionally large ovaries for its relative size. SW2000/33 was a 1.9-year old harbour porpoise that live stranded in poor nutritional condition. Cause of death was attributed to pneumonia (parasitic) and the animal died in the month of February. This individual presented with two virtually confluent irregular/star-shaped cutaneous ulcers (covering an area 1.7cm x 0.4cm) on the inner aspect of the left genital slit lip. Three small fresh erythematous ulcers were seen more deeply on the vulval mucosa. There were also some slightly reddened areas of the vaginal mucosa.

SW1994/185, a sexually mature non-lactating 5- year old female, died due to pneumonia (parasitic and bacterial), and possibly live stranded, in the month of December. A number of ulcers were noted on the epidermis near the genital slit. Histological assessment revealed ulcerative necro-purulent dermatitis associated with acanthosis of adjacent epidermis. SW1995/61, a 7-year old mature female, live stranded in the month of June and had just given birth to her first offspring. Cause of death was attributed to generalised bacterial infection (*Streptococcus canis*). On gross assessment, haemorrhaging was noted from the genital slit, appearing to originate from multiple small, circular or linear bleeding ulcers (up to 5mm long) situated at the mucocutaneous junction around the opening of the vulva and caudal to the clitoris. The genital lesion produced a mixed growth overgrown with *Proteus mirabilis*.

Genital papilloma

Papillomaviruses can be host- and tissue-specific, causing epithelial and fibroepithelial benign tumours of the skin and mucous membranes (warts, condylomas, papillomas and fibropapillomas) in a variety of vertebrate species including humans (Van Bressem et al. 1999). In marine mammals, genital warts possibly caused by papillomaviruses, have been reported in the long-beaked common dolphin, dusky dolphin, bottlenose dolphin, and Burmeister's porpoise *Phocoena spinipinnis*, with lesions being more prevalent in males (Van Bressem et al. 1996, Van Bressem et al. 2006, Van Bressem et al. 2007). In these species it was suggested that the virus was venereally transmitted. Interestingly, within the current sample, three of the females exhibiting possible papillomavirus lesions were pregnant. Papillomavirus associated lesions typically regress spontaneously, though tumours induced by specific papillomaviruses genotypes may persist and progress to invasive carcinomas (Van Bressem et al. 1999).

Within the sample of 140 individuals there were four possible cases of genital papilloma, a prevalence of 2.9%. As noted above, three individuals were pregnant and the fourth was a resting mature female. The prevalence of possible papilloma lesions within the mature sample was 6.8%. SW2000/73, a 6-year old pregnant female in good nutritional condition, was incidentally captured in fishing gear in the month of April with a foetus in its third trimester. Multiple raised papillomatous lesions were noted in the vaginal mucosa and clitoris. Histologically the lesions were characterised by localised epithelial hyperplasia and patchy mild subepithelial (mainly) mononuclear cell infiltration. SW2001/21 a pregnant female was more than 17 years in age and died of pneumonia (parasitic). A proliferative cauliflower-like lesion (2 cm x 3 cm) with a reddened (abraded) surface was located on the ventral clitoris. A smaller area of ulceration with a red base was found on the clitoral surface adjacent to this lesion. Histological assessment revealed a squamous papilloma-like lesion in the clitoral epithelium, with scant mononuclear cells within the sub-epithelium. The third pregnant female, SW2001/218, was primiparous with a high total PCB concentration (see Table 4). This female died from incidental capture in the month of October, and was in a good nutritional state. Two, off-white raised (1-1.5 cm wide) cauliflower-like papillomatous lesions were seen on the surface of the clitoris. Histology was not carried out on these lesions. Finally, SW2002/95 a 6.5-year old resting mature porpoise, died in relatively poor nutritional condition. Cause of death was attributed to pneumonia (parasitic). A single circular and slightly raised whitish plaque was located on the vulval epithelium adjacent to the clitoris resembling a papilloma-like lesion. This lesion was not processed for histological assessment.

Vaginal plaques

Vaginal plaques were observed in two individuals; both sexually mature with total PCB burdens above the threshold level. In the bycaught SW1997/36, several small whitish raised mucosal plaques were present within the anterior vagina. Plaque consisted of hyperplastic epithelium with superficial necrosis. Numerous single eosinophilic intra-cytoplasmic inclusion bodies were present and there was mild, mainly plasmacytic cellular infiltration within the underlying lamina propria. SW2000/50, a lactating female, died from pneumonia and a small white mucosal plaque was present on the mucosal surface of the inner vagina just distal to the pseudocervix. There were widespread localised areas of mucosal reddening and possible haemorrhage throughout the vagina (including the pseudocervix). The plaque consisting of thickened (hyperplastic) stratified epithelium, with moderate mixed (mainly mononuclear) cellular infiltration.

Others

An epi-mural nodule was noted in the vagina of SW1998/129. This single small, yellowish nodule was located on the serosal surface of the dorsal wall of the vagina. This animal live stranded in a poor nutritional state, and cause of death was attributed to generalised *Mycobacterium fortuitum* infection. SW1998/211, a lactating female, presented with five abscesses of variable size (approx. 1cm - 2.5cm dia.) protruding from the outer aspect of the dorsal wall of the vagina either on, or slightly to the right side, of midline. The abscesses each had fibrous walls that encapsulated beige creamy pus. *Corynebacterium* sp., *Clostridium perfringens*, *Clostridium sordellii*, *Pseudomonas fluorescence* were isolated from the pus, and *Corynebacterium* sp from the abscess itself. Cause of death was not established for this individual. SW1997/178, a lactating female presented with mild multifocal eosinophilic endometritis, the etiology of which was also not established. Cause of death was attributed to starvation.

Ovarian abnormalities and anomalies

A number of ovarian abnormalities were identified within the harbour porpoise ovarian tissue. Only two cases are presented here, and a number of others are currently being investigated.

Tubule-like elements

SW1999/39 was a neonate, measuring 104 cm in length and cause of death was attributed to starvation. As noted earlier, the porpoise suffered from acute dermatitis of the genital slit region and localised sub-acute to chronic vaginal ulceration associated with mild-moderate lymphocytic/plasmacytic cellular infiltration. As this animal exhibited ovarian hypoplasia – smallest ovaries within the whole sample, even though 15 porpoises were smaller in size - a tissue sample from the right ovary was taken for histology (see Figure 9).

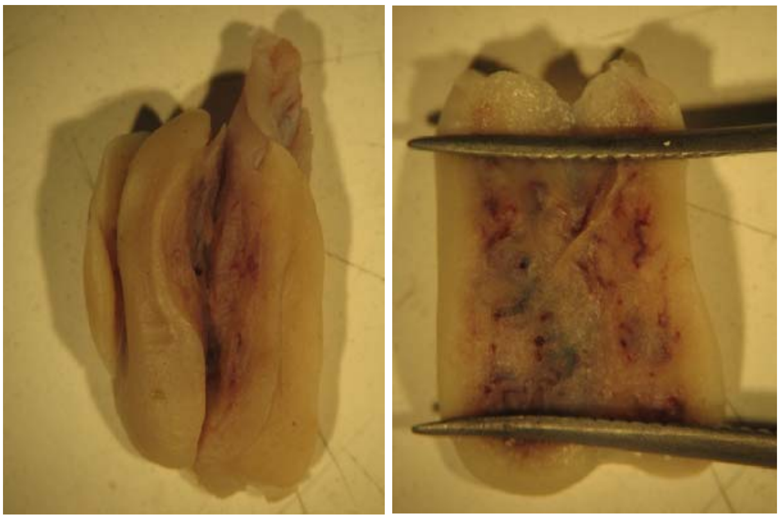


Figure 9. Left and right ovaries of SW1999/39.

On histological assessment, a distinction between the medulla and cortex was observed, and the rete ovarii lined the complete ovary (see Figure 10). No 'typical' ovarian primordial or primary follicles were observed within the cortex, i.e. a single oocyte surrounded by either flattened or cuboidal granulosa cells, respectively. Instead, tubule-like elements were present composed of at least two cell types, with multiple cells. Immunohistochemical staining will be undertaken on to identify the cells within the tubules. In addition a tissue sample will be processed from the left ovary. As with the common dolphin SW1994/24, it is unknown at this point in time if this condition is due to abnormalities of genetic or chromosomal origin, inappropriate hormone exposure, chemical exposure, or disease. Blubber tissue has not been processed for contaminant analysis.

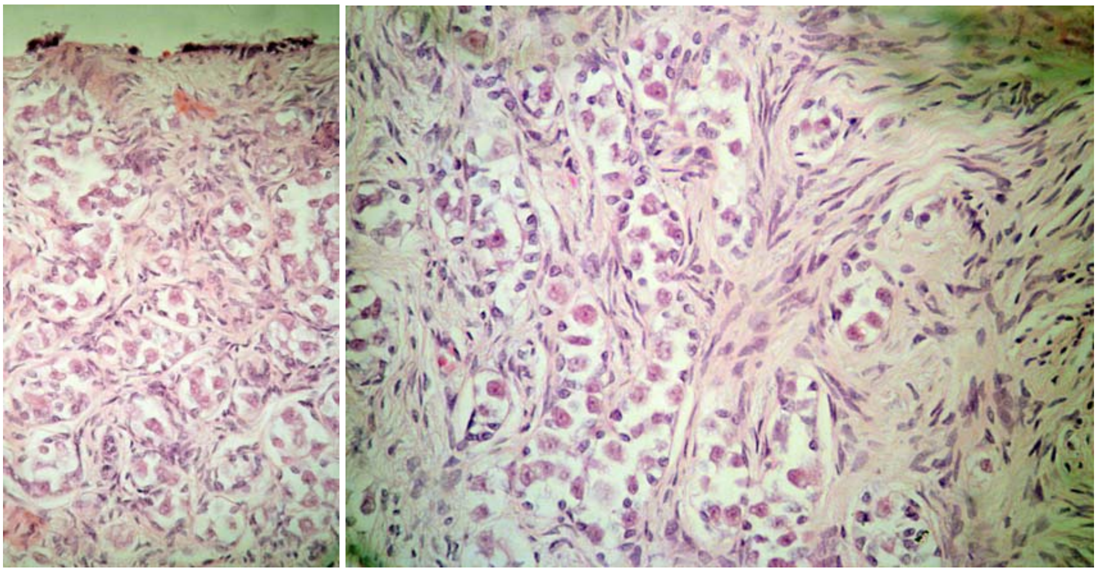


Figure 10. Tubule-like structures in the cortex of the right ovary of SW1999/39.

Possible tumour

SW2003/296 was a 154 cm long harbour porpoise, at least 18 years in age. It was incidentally captured in fishing gear in the month of June, collected as part of the bycatch observer programme. The animal was in good nutritional condition and thick fat deposits were noted beneath the dorsal blubber layer. The animal was lactating and recently pregnant in the left uterine horn. A regressing corpus luteum (11.8x11.3 mm) from pregnancy was present on the left ovary in addition to five corpora albicantia. A mass measuring 12x10.3 mm (see Figure 11) was also present on the left ovary (maximum length of ovary was 34.4 mm). After fixation in formalin, it was tan in colour and presented as a solid mass, though appeared partly cystic after dissection.

Figure 11. Left and right ovaries of SW2003/296. A regressing CL (yellow colour) present on the left side of the let ovary, and a tan coloured tissue mass was present on the right side.

On histological assessment, cells, possibly granulosa type, were arranged in parallel cords separated by fibrous stroma in part of the mass. Cells were not luteinised and appeared to have abundance cytoplasm and very prominent nuclei (see Figure 12). Again immunohistochemistry will be undertaken to assess the cell composition within the possible tumour. Granulosa cell tumours are sex cord–stromal tumours and commonly produce estrogen. They have previously been reported in the beluga, pilot, fin and blue whale (see Table 1).

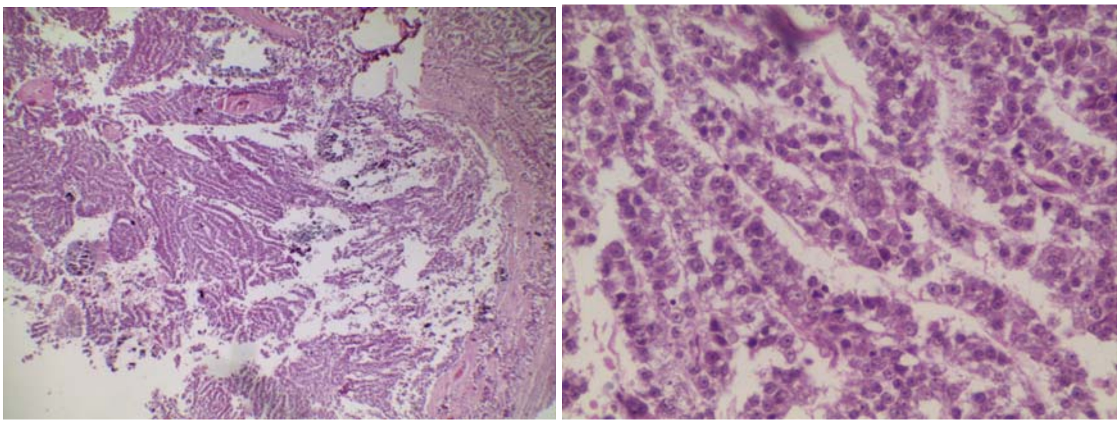


Figure 12. Cells arranged in parallel rows within the possible tumour.

EFFECTS OF CONTAMINANTS ON REPRODUCTION IN HARBOUR PORPOISES

Total PCB burdens ranged from 0.65 to 310.9 µg g^{-1} lipid (n = 66) in sexually immature females, and 2.43 to 87 µg g^{-1} lipid (n = 36) in sexually mature females. The sexually mature female harbour porpoise contaminant sample consisted of seven resting mature, five pregnant, four pregnant & lactating, 14 lactating, one aborting & stillbirth, four lactating, aborting & stillbirth, and one female that recently aborted. A significant negative relationship was observed between total blubber PCB levels [(∑7ICES congeners)*3] and corpora scar number (p = 0.009, n = 100; Figure 13a). A significant negative relationship was also observed between total blubber PCB concentrations and age (p = 0.021, n = 88, Figure 13b). Similar to that observed in the common dolphin control group sample.

Large variations in contaminant burdens in calves less than one year of age were observed (Figure 13b) 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. 59% of the immature sample had a total PCB load above the threshold for adverse health effects (17 µg g^{-1} lipid). Ten immature females had had contaminant levels >50 µg g^{-1}lipid and of these, 60% died from infectious or non-infectious diseases. In mammals, it has been suggested that the foetal and newborn immune systems are more susceptible to immunotoxic effects than the adult immune system (Ropstad et al. 2007). Neonate calves with extremely high contaminant burdens may suggest first born offspring. The highest contaminant burden (310.88 µg g^{-1}lipid) was observed 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). The neonate presented with congenital oesophageal stenosis, and oesophageal (and fundic stomach) ulceration. Samples of ovarian tissues from this individual will be processed for histology in the near future to assess if any abnormality exits.

12 mature females had contaminant levels above the threshold level for adverse health effects, and this group consisted of four resting mature, two pregnant, five lactating, and one female that recently aborted. Females died from physical trauma (2), incidental capture (n = 3), generalised bacterial infection (n =2) and pneumonia (n = 5). Corpora scar number ranged from 1 to 11. Within the resting mature sample, three animals presented with either a genital ulcer, vaginal wall lesion or a vaginal plaque. One of the pregnant females died of a severe bronchopneumonia associated with abscessation and parasitism that may have been facilitated or exacerbated by the stress, and immunosuppression that can be associated with pregnancy and high contaminants

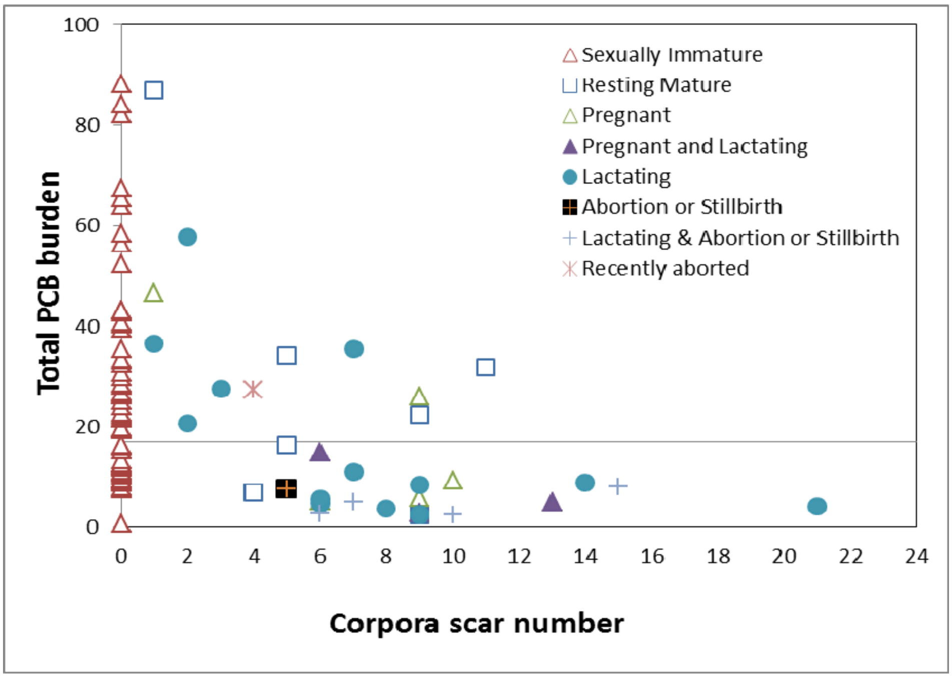
burdens. The other pregnant female died from physical trauma and presented with a possible cervical papillomas lesion. Of the five lactating females, one presented with a vaginal plaque and another with a genital ulcer. Four out of the five lactating females were recently gravid and the fifth individual displayed a mild degree of lactation, and presented with a regressing CA and a vaginal epithelial plaque.

The highest total PCB load in a mature female was reported in a resting mature individual (SW2003/380) that died of pneumonia and had only one corpora scar. This female had been previously gravid and the high contaminant burden of 87 µg g^{-1} lipid suggests that the female either aborted, or her offspring did not survive after birth.

Within the sample of 59 mature females, 13 individuals showed evidence of abortion and/or dystocia. Of these, contaminant data were available for six individuals. All porpoises that died as a result of dystocia had contaminant levels under the threshold level, in addition to two individual that died while aborting a foetus (SW1999/10, SW1999/40). Contaminant data were available for only one other individual that showed evidence of recent abortion. SW1997/174, a 4-year old porpoise had a high total PCB contaminant burden of 27.4 µg g^{-1} lipid and four ovarian corpora scars. Although this animal presented for postmortem with severe traumatic injuries, several disease conditions were identified that would have severely compromised the animal and possibly predisposed it to a (fatal) traumatic incident. These lesions included pulmonary parasitism and associated pneumonic and granulomatous foci, heavy parasitism and associated chronic ulceration of the cardiac stomach, and severe necrotising stomatitis and osteomyelitis of the left mandible. The association between a high total PCB burden and the incidence of abortion in this individual cannot be discounted, nor do the immunosuppressive effects of contaminants (see below).

Preliminary analysis was undertaken on ovarian lesions and other abnormalities of the genital tract, in order to investigate their association with contaminant levels and possibly identify end-points of reproductive toxicity. Due to number of lesions and abnormalities identified, this is work is ongoing and only preliminary results are presented here.

Of the four individuals exhibiting genital ulcers with corresponding contaminant data, all total PCB concentrations were above the threshold for adverse health effects, ranging from 34.1 to 65.4 µg g^{-1} lipid – with the two immature individuals exhibiting the highest levels. All four individuals live-stranded, were in poor nutritional condition and/or suffering from significant disease that contributed to live-stranding incident. SW1995/61 showed evidence of recent pregnancy, though had a total PCB burden of 34.1 µg g^{-1} lipid. This animal was lactating and died in June, all suggesting the animal had died during the early phase of lactation – and had not managed to offload its contaminant burden. Although SW1994/185 had a similar total PCB burden, this individual live-stranded in December, had been previously gravid and presented with five ovarian corpora scars. A total PCB burden of 36.6 μg g^{-1} lipid in this 5-year old porpoise again suggests that this female had not successfully offloaded her contaminant load.



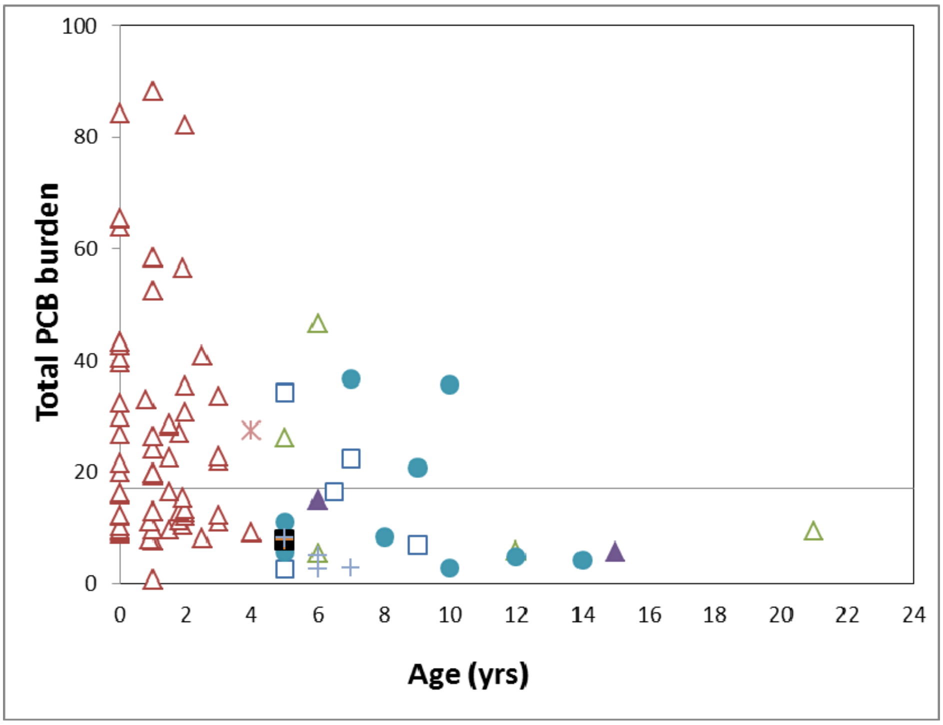


Figure 13. (a) Total PCB burden [(∑7ICES congeners)*3] (μg g^{-1} lipid) as a function of (a) corpora scar number (n = 102), and (b) age (n = 89) in the *P. phocoena* sample (excluding the neonate

female measuring 90 in length, with a contaminant load of 310.9 µg g^{-1}lipid). The threshold level for adverse health effects of 17 µg g^{-1} lipid is marked on the graphs.

Of the four individuals possible exhibiting genital papilloma's, only one individual had a total PCB burden above the threshold level. SW2001/218, a bycaught 6-year old, had a total PCB burden of 46.6 µg g^{-1} lipid and was pregnant with its first calf; a male foetus in its first trimester. High contaminant burdens were also reported in the two females exhibiting vaginal plaques, even though 7 and 9 ovarian corpora scars were present on their ovaries. SW1997/36, a 7-year old non lactating female possessed 9 corpora scars, and had a total PCB burden of 22.38 µg g^{-1} lipid. In addition to the vaginal plaque the individual suffered from chronic diffuse (pyo)granulomatous interstitial mastitis. Although this individual was bycaught, a Group B Salmonella sp. (0,4,12:-:-) was identified in the lungs. SW2000/50 was lactating, recently pregnant, with 7 corpora scars and a total PCB burden of 35.5 µg g^{-1} lipid. Cause of death was attributed to pneumonia (unknown etiology) though the animal may have also live stranded. Finally, SW1998/129 was not a lactating, died of a generalised bacterial infection, had a high PCB burden of 31.8 µg g^{-1} lipid, 11 ovarian corpora scars and presented with a vaginal wall lesion (see Table 4).

Two cases of harbour porpoise ovarian abnormalities were presented in the current report, and contaminant data had only been previously assessed for one of the porpoises. SW2003/296 was recently pregnant and lactating and presented with a low total PCB burden of 4.86 µg g^{-1} and a tumour-type mass. Further investigations are being undertaken to identify the possible tumour cells in this individual, in addition to assessing ovarian anomalies in a number of other individuals.

In summary, there were no incidences of premature birth in any of the newborn harbour porpoises assessed within the current study. There were some instances of ovarian hyper- and hypoplasia which are currently been investigated. Although there were noted cases of female porpoises either aborting a foetus or having recently aborted a foetus, contaminant data were only available for two of those individuals and one had high total PCB burdens. A number of other possible 'abortion' cases are currently being investigated. All mature females within the contaminant sample showed evidence of previous or current gravidity. This again signifies that high contaminant burdens, above the threshold for adverse health effects in marine mammals, did not inhibit ovulation, conception or implantation in harbour porpoises. However, the association between high contaminant burdens and the incidence of abortion within the contaminant sample cannot be discounted. In addition to a number of observed cases where previously gravid females did not offload their contaminant burdens, possibly due to either foetal or newborn mortality.

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SHORT PAPER

True Hermaphroditism: First Evidence of an Ovotestis in a Cetacean Species

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Summary

An immature unilateral hermaphrodite common dolphin (*Delphinus delphis*) was found stranded on the southwest coast of the UK. The external phenotype was that of a female, but internally there was one ovotestis, containing both ovarian follicles and testicular tubular elements, and a contralateral ovary. Ovarian portions of the ovotestis appeared normal and demonstrated follicular development, whereas the testicular tissue exhibited hypoplasia and degeneration. This is the first reported case of an ovotestis in a cetacean species.

Keywords: common dolphin; *Delphinus delphis*; ovotestis; true hermaphroditism

True hermaphroditism is defined as the simultaneous presence in a single individual of both testicular and ovarian tissue, which may exist either in separate gonads or in the same gonad (ovotestis; Krob *et al.*, 1994). True hermaphroditism can be bilateral (testicular and ovarian tissue identified on both sides, usually as ovotestes), unilateral (ovotestis on one side and an ovary or testis on the other side) or lateral (testis and contralateral ovary) (Tangner *et al.*, 1982; De Guise *et al.*, 1994). The determination of genetic sex (XX or XY) is fixed at the time of fertilization. The mammalian Y chromosome carries a testis determining factor (TDF) gene *SRY*, and at the moment of sex determination stimulates the undifferentiated fetal gonad to develop into a testis (and production of testosterone by the Leydig cells), which subsequently gives rise to the male phenotype. The female gender is the default option, where due to the absence of the TDF (or if the *SRY* function is impaired) the undifferentiated gonad will develop into an ovary and the undifferentiated tubular genital tract will develop into normal female genitalia unless male hormones are present (Kennedy and Miller, 1993). Disorders of genital development, such as sex reversal, are caused by abnormalities of genetic or chromosomal origin or inappropriate hormone exposure. For example, XX mice which, as a result of mutation, carry an autosomal dominant gene *Sxr* that acts like the Y chromosome, develop testes and male tubular genitalia (Kennedy and Miller, 1993). In amphibians it has been reported that oestrogens can induce sex reversal in genetic males, producing either an ovotestis or complete and permanent feminization (Lofts, 1974; Qin *et al.*, 2003).

As part of the UK Cetacean Strandings Investigation Programme a carcase was recovered from the southwest coast of the UK in December 2006 for a routine post-mortem examination. The animal, identified as a common dolphin (*Delphinus delphis*), was of moderate nutritional status with a dorsal blubber thickness of 13 mm. The dolphin measured 191 cm in length and was estimated to be 6 years old on the basis of counting growth layer groups in the dentine as described by Murphy and Rogan (2006). On external appearance the dolphin was sexed as female, due to the presence of a mammary slit with corresponding teat positioned on either side of the genitoanal slit, which housed the anus and urogenital openings.

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Routine necropsy and bacteriological examinations were conducted according to standardized protocols (Jepson, 2005). The post-mortem examination revealed no significant abnormalities of the mammary glands, and the ovaries, uterus and vagina were reported as unremarkable. Both ovaries were retained and fixed in 10% neutral buffered formalin. The cause of death was not established because of scavenger damage and as the carcase was in a moderate state of decomposition. However, one possible net mark was observed on the right side of the thorax, suggesting incidental capture in fishing gear. *Brucella* antibodies were identified in a sample of pericardial fluid by the Rose Bengal plate test, but follow-up testing by enzyme-linked immunosorbent assay proved negative. Bacteriological analysis of a number of tissues did not reveal the presence of *Brucella*.

Ovary A weighed 1.09 g and measured 24.8 × 10.2 × 8.4 mm. Ovary B weighed 1.41 g and measured 26.6 × 13.4 × 7.5 mm. Both ovaries had normal external appearance with no corpus luteum or albicans, consistent with this being an immature female (Fig. 1a). Ovaries were sectioned (slices of 0.5–2 mm) and these sections were examined under a dissecting microscope. There was evidence of follicular growth in both ovaries, with a maximum follicle diameter of 1.75 mm (ovary A). However, the initial dissection of ovary A (a longitudinal transverse cut) revealed a scar-like structure located in the centre of the ovary (within the cortex adjacent to the medulla), which was tan to yellow in colour and measured 6.83 × 5.49 mm (Fig. 1b). Examination of ovary B revealed no other signs of activity (or abnormality) apart from follicular growth.

Tissue samples from both ovaries were processed routinely, embedded in paraffin wax, sectioned (4–6 μm) and stained with haematoxylin and eosin (HE). Microscopical examination of both ovaries revealed the presence of numerous blood vessels within the medulla and primordial and developing follicles (including primary, secondary and secondary-vesicular) within the cortex. Follicles were in various stages of degeneration/autolysis. Female common dolphins in the northeast Atlantic attain sexual maturity at an average length of 188.8 cm and an average age of 8.2 years, and within a sample size of 189 mature females, only one 6-year-old female was classified as sexually mature (Murphy *et al.*, 2009). Based on these data, and an estimated age of 6 years, the dolphin reported here would be classified as sexually immature.

Microscopical analysis of the scar-like tissue in ovary A revealed evidence of testicular tubular elements within the ovarian stroma. This testicular tissue appeared to be slightly compartmentalized, with connective tissue separating the ovarian and testicular components (Fig. 2a). Interstitial tissue and seminiferous tubules with Sertoli cells and spermatogonia were also present. Tubules were slightly atrophic and spindle-shaped Sertoli cells were located both in the basal area and within the lumen of the tubule. Round or polygonal cells (Leydig-like) with granular eosinophilic cytoplasm and small round nuclei were scattered between the seminiferous tubules (Fig. 2b).

Further sections taken from the centre of the testicular tissue revealed evidence of spermatogenesis with the presence of spermatocytes. The proportion of interstitial tissue was relatively high compared with the number of seminiferous tubules, suggesting an immature stage of development. However, the relative diameter of a number of the seminiferous tubules was that of a sexually mature individual. Seminiferous tubules were in various stages of degeneration

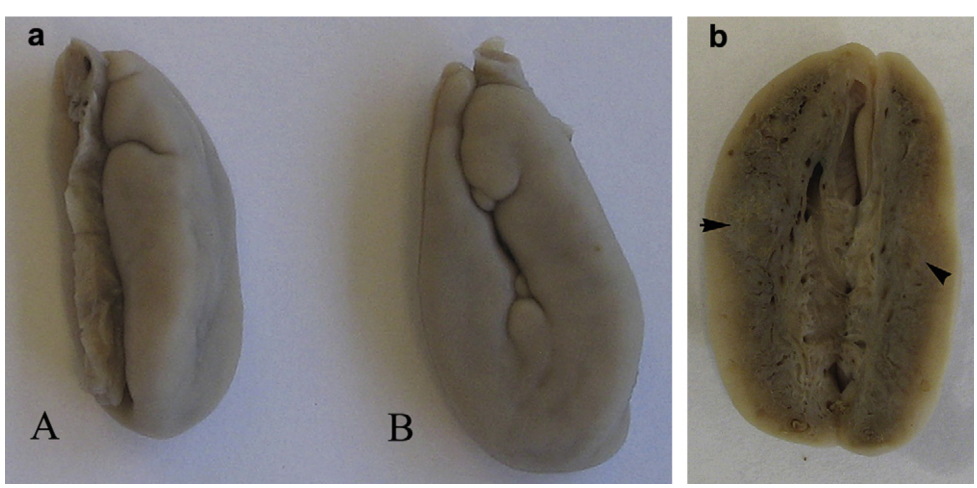

Fig. 1. (a) External appearance of ovaries A and B. (b) Longitudinal transverse cut through ovary A; arrows indicate position of scar-type tissue in the cortex (arrowheads).

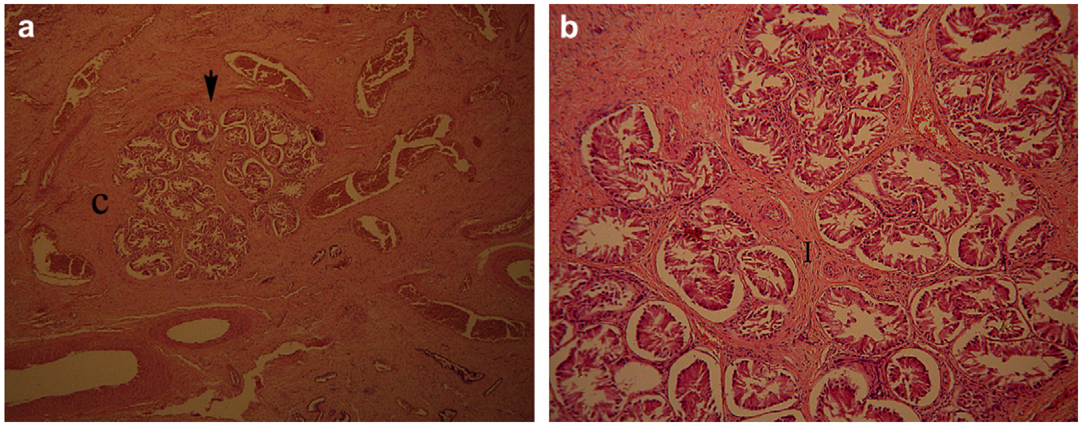

Fig. 2. Outer area of the testicular tissue (arrowhead) in the cortex of ovary A. The testicular tissue is surrounded by connective tissue (C) and includes interstitial tissue (I) and seminiferous tubules with Sertoli cells and spermatogonia. HE. Bar, (a) ×25, (b) ×100.

(Fig. 3a) and exhibited hypoplasia with various stages of sexual development (Fig. 3b). Tubules ranged in size from 100 to 230 μm in diameter. Smaller diameter tubules (including Sertoli cells and spermatogonia) predominated over the larger diameter structures (that included spermatocytes). In this species the tubule diameter of young (approaching maturity) and sexually mature individuals ranges from 80 to 145 μm and 105.3 to 355 μm, respectively (Murphy *et al*., 2005). Large variations in tubule diameters reported in sexually mature common dolphins by Murphy *et al*. (2005) were attributed to seasonal variations in sperm production.

Ladds (1993) defined various degrees of testicular hypoplasia in bulls as: (1) Mild. Occasional tubules comprise only Sertoli cells, but in the majority of active tubules spermatogenesis occurs to at least the spermatocyte stage. Intratubular multinucleate giant cells, resulting from division but not separation of germinal cells, are present; (2) Intermediate. Fifty percent or more of the tubules are hypoplastic, whereas the remaining tubules exhibit varying degrees of spermatogenic activity and occasionally spermatozoa, but in the majority of active tubules differentiation only proceeds to the spermatocyte stage. These active tubules may be of normal diameter. Subsequent degeneration of cells results in obvious vacuolation of the germinal epithelium and, in addition, a variable number of giant cells are present; (3) Severe. Most or all of the seminiferous tubules are of small diameter and are lined by Sertoli cells only, or Sertoli cells and perhaps a basal layer of stem cells or spermatogonia, which do not show mitotic activity. The basement membranes are thickened and hyaline and there is an increase in peritubular connective tissue and the number of interstitial cells of Leydig. Based on these

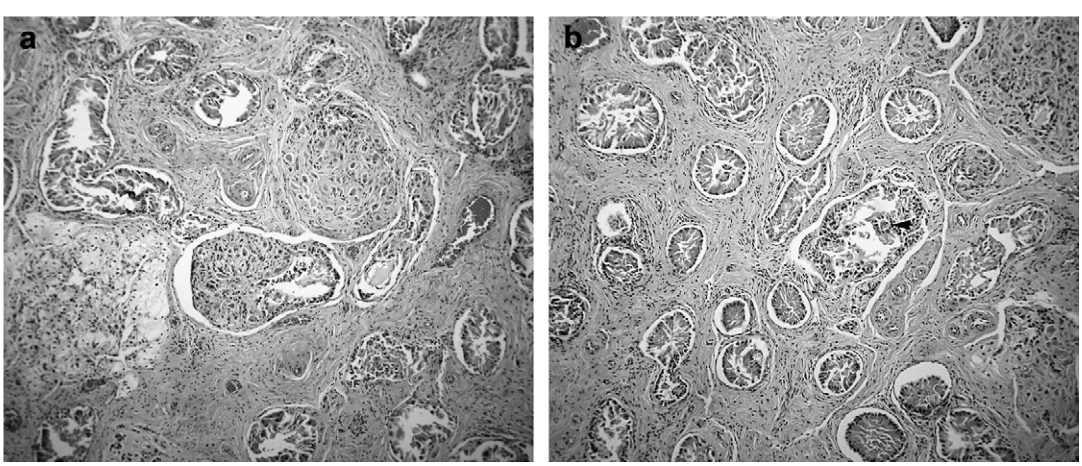

Fig. 3. Centre of the testicular tissue in ovary A. (a) Degeneration of seminiferous tubules. HE. ×100. (b) Hypoplasia with seminiferous tubules of varying diameters and the presence of a giant cell (arrowhead) formed due to fusion of spermatocytes within the large tubule. HE. ×100.

categories, and the presence of giant cells due to fusion of spermatocytes within a number of tubules (Fig. 3), the testicular tissue in the present case exhibited an intermediate form of hypoplasia, with increased (interstitial) peritubular connective tissue and degeneration of cells. There were no testicular tubular elements identified in ovary B, so this phenotypically female dolphin was classified as an immature unilateral true hermaphrodite.

In people, ovaries and ovarian portions of ovotestes appear normal and demonstrate follicular growth and oestradiol production, with around half of ovotestes exhibiting evidence of ovulation (Dayal *et al.*, 2008). Testicular elements show degeneration and hyalinization of the seminiferous tubules, with poor germ cell development. Leydig cell hyperplasia also may occur, although spermatogenesis in the testes is rare. Normally, the presence of oestradiol in developing ovarian follicles usually inhibits the development of spermatogonia in adjacent or contralateral seminiferous tubules (Dayal *et al.*, 2008). In mammals, although cases of sex reversal and ovotestes are rare and develop as a consequence of gene or chromosomal mutations affecting individuals, ovotestes appear to be the main reproductive organ. This is known to be the case in European *Talpid* spp. and Japanese moles (*Mogera wogura*). Females within these species are fertile, as the ovarian tissue is fully functional; however, the testicular tissue is abnormal and sterile, although it can produce high levels of testosterone (Carmona *et al.*, 2008). It has been noted that seasonal changes in testosterone levels occur, with increased levels and aggression outside the breeding season. These seasonal changes are consistent with the hypothesis that high levels of testosterone may have favoured territorial behaviour (Carmona, 2006). A reduction in testosterone level occurs during the breeding season and permits the male to enter a female's territory for reproduction (Whitworth *et al.*, 1999; Carmona *et al.*, 2008).

To the authors' knowledge, this is the first documented case of an ovotestis in a cetacean species. An atypical bilateral hermaphrodite beluga whale was reported from the St Lawrence Estuary, Canada (De Guise *et al.*, 1994). That animal appeared externally to be male, with the presence of a penis in the genital slit, located halfway between the anus and the umbilicus. On dissection, two testicles with associated epididymis and vas deferens, two separate ovaries and the complete ducts of each sex were found, although the cervix, vagina and vulva were absent. An ovary was located dorsally to the caudal pole of each testicle (De Guise *et al.*, 1994). The whale was sexually mature, with evidence of both spermatogenic and follicular development. This condition was attributed to hormonal disturbance in early pregnancy, whereby normal differentiation of male and female organs was disrupted (De Guise *et al.*, 1994; Reijnders, 2003). Of the 93 remaining beluga whales (48 females and 45 males) examined in the St Lawrence population, one animal appeared to be a male pseudohermaphrodite (Reijnders, 2003). Prior to this investigation, pseudohermaphroditism had also been reported in two bowhead whales *(Babaena mysticetus*; Tarpley *et al.*, 1995), a fin whale *(Balaenoptera physalus*; Bannister, 1962) and a striped dolphin (*Stenella coeruleoalba* formerly *Prodelphinus caeruleoalbus*; Nishiwaki, 1953). In all of these cases, although the external phenotype was that of a female, the internal reproductive organs, or elements thereof, were male. In the case of the bowhead whales, which were both apparently normal male karyotypes (40 + XY), findings suggested a failure of androgen (testosterone or dihydrotestosterone) expression consistent with the syndrome of complete testicular feminization (Tarpley *et al.*, 1995).

In the current investigation, cytogenetic analysis and hormone evaluations were not undertaken and levels of lipid soluble contaminants (e.g. polychlorinated biphenyls and DDT) were not assessed. Such contaminants have endocrine disrupting activities that can lead to reproductive disorders during fetal and neonatal development (Delbes *et al.*, 2006).

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