PERSISTENT ORGANIC POLLUTANTS: ASSESSING THE THREAT TO CETACEANS

A thesis submitted for the degree of Doctor of Philosophy

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Abstract

Persistent organic pollutants (POPs) are a group of chemical compounds that are toxic to humans and wildlife. They are persistent in the global environment, can be transported over large distances and have the ability to biomagnify and bioaccumulate. Their extensive use in the 20th century led to widespread environmental contamination, causing harm to many wildlife populations. Despite their ban and restriction under the 2001 Stockholm Convention, one of the most critical international agreements administered by the UN Environment, pervasive and widespread contamination of the environment still occurs. As long-lived apex predators, cetaceans are known to be particularly vulnerable to accumulating high body burdens of POPs. To assess the effectiveness of pollution mitigation strategies and ensure cetaceans are adequately protected it is vital to determine the impacts of exposure to POPs in cetaceans. This thesis uses the world's largest marine mammal strandings toxicology database, collected from stranded cetaceans between 1990 and 2018, to demonstrate that despite their ban under the Stockholm Convention in 2001, POPs are still a significant threat to cetaceans that inhabit the waters of the United Kingdom. I have demonstrated that although levels of POPs are declining, several species are still exposed to concentrations that are deemed to be a toxicological threat. Further, rates of decline vary between taxa and regions, hence some populations are more vulnerable than others. I have also demonstrated that, of the contaminants investigated, polychlorinated biphenyls (PCBs) are present at the highest concentrations, declining at the slowest rate and appear to be the greatest threat to cetacean health. Using harbour porpoises as a model species, this thesis has demonstrated that PCBs are significantly associated with increased disease prevalence and reduced testes weights, which suggests that male fertility may be impacted by exposure to POPs. This could have serious implications on the long-term population viability of harbour porpoises and similar species that are exposed to higher concentrations of PCBs. I also found age and sex related differences in the PCB congener profiles that harbour porpoises are exposed to, which suggests juveniles may be at a higher risk of experiencing neurotoxic effects than adults. Taken together, I believe these findings demonstrate that improved international POP elimination, mitigation and compliance strategies are required alongside conservation and management plans to adequately protect cetaceans from the deleterious effects of POPs.

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Table of Contents

TABLES

FIGURES

Publications

Chapter 3

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Chapter 4

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Chapter 5

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Chapter 1 - Introduction

1.1 Overview

1.1.1 Ecotoxicology

Widespread concern for the impact of pollution on the environment and wildlife began in 1962 following the publication of Rachel Carson's revolutionary book Silent Spring (Carson, 2002). Carson's work highlighted the adverse environmental effects of the widespread use of synthetic pesticides, which are now part of a wider group of toxic chemicals known as persistent organic pollutants (POPs) (Carson, 2002; UNEP, 2017). The term ecotoxicology was first coined six years after the publication of Carson's work (Truhaut, 1977) and is broadly defined as the study of the harmful effects of chemicals upon ecosystems. The field integrates toxicology and ecology to understand the effects of chemicals on natural communities under realistic exposure scenarios. This can range from examining molecular level effects through to studying effects on the biosphere as a whole (Chapman, 2002; Lynch et al., 2001)

1.1.2 Persistent Organic Pollutants Overview

Persistent organic pollutants (POPs) are ubiquitous environmental contaminants that pose an insidious threat to humans and wildlife (Miniero et al., 2015). Their global use in agriculture and industry has led to pervasive and widespread contamination and caused considerable harm to several wildlife populations (Méndez-Fernandez et al., 2018; Reijnders, 1980; Sonne et al., 2020). They are broadly characterised as chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to humans and wildlife (UNEP, 2017). Organohalogen contaminants (OHCs) are among the group of chemicals defined as POPs and have received worldwide attention due to their toxicological effects and resistance to environmental degradation. The chemical properties and environmental impact of OHCs varies widely across the group with the more persistent dichlorodiphenyltrichloroethane (DDT) compounds and polychlorinated biphenyls (PCBs) causing the greatest concern. Despite national and international action to regulate the use of POPs, large amounts still require disposal and continue to enter the marine environment (Stuart-Smith and Jepson, 2017).

POPs are, by definition, persistent chemicals on account of their highly stable structure. Typically, they are hydrocarbons, with a strong aromatic or cyclic structure, that are halogenated by chlorine or bromine atoms (Miniero et al., 2015). This carbon-halogen bond is extremely stable and causes lipophilicity, giving them highly desirable properties, such as fire resistance, heat absorbance and resistance to pests, which led to their widespread use (Fitzgerald and Wikoff, 2014). These structural characteristics however, are also what makes them so damaging to the environment as they are extremely resistant to degradation and accumulate in fatty tissues (Miniero et al., 2015). Despite these shared characteristics, each POP is distinct from every other and their toxicity, persistence and capacity for long-range transport can vary considerably therefore, they tend to be evaluated individually (Fitzgerald and Wikoff, 2014).

The deleterious effects of POPs, on humans and wildlife, have been of considerable concern to governments, the scientific community and non-governmental organisations across the globe. In 2001, this international concern led to the signing of the *Stockholm Convention* (a multilateral treaty to protect human health and the environment from POPs), with the aim of eliminating or restricting the production and use of POPs (UNEP, 2017). Initially the treaty set out to tackle the worst twelve POPs (referred to as the Dirty Dozen) however, structurally similar compounds (including polybrominated diphenyl ethers (PBDEs)) have since been added and there are several emerging environmental contaminants currently under review (Table 1-1) (UNEP, 2019a, 2017).

Table 1-1: Persistent organic pollutants (POPs) currently listed on the UNEP Stockholm Convention (UNEP, 2017)

Pollutant Class	**Applications**	**Year added**
Aldrin	Insecticide	2001
Chlordane	Insecticide	2001
Chlordecone	Pesticide	2009
Dichlorodiphenyltrichloroethane (DDTs)	Insecticide	2001
Dicofol	Pesticide	2019
Dieldrin	Insecticide	2001
Endrin	Insecticide	2001
Heptachlor	Insecticide	2001
Hexabromobiphenyl	Flame retardant	2009
Hexabromocyclododecane (HBCD)	Flame retardant	2009
Hexachlorobenzene (HCB)	Fungicide	2001

Hexachlorobutadiene	Industrial chemical, by-product	2015
Hexachlorocyclohexanes (HCHs)	Pesticide	2009
Lindane	Insecticide	2009
Mirex	Insecticide	2001
Pentachlorobenzene	Flame retardant	2009
Pentachlorophenol and its salts and esters	Herbicide, insecticide, fungicide, algaecide	2015
Perfluorooctanoic acid (PFOA), its salts and PFOA-related compounds	Industrial chemical, surfactant	2019
Perfluorooctane sulfonic acid (PFOS), its salts and perfluorooctane sulfonyl fluoride (PFOSF)	Industrial chemical, surfactant	2009
Polychlorinated biphenyls (PCBs)	Industrial chemical	2001
Polybrominated diphenyl ethers (PBDEs)	Flame retardants	2009
Polychlorinated naphthalenes	Industrial chemical	2015
Short-chain chlorinated paraffins (SCCPs)	Industrial chemical	2017
Technical endosulfan and its related isomers	Insecticide	2011
Toxaphene	Insecticide	2001

Despite the initial success of the *Stockholm Convention,* in reducing concentrations of POPs in the environment and wildlife, parties have failed to eliminate pollutants at a sufficient rate and levels remain high in some species (Jepson et al., 2016; Law et al., 2012a; Stuart-Smith and Jepson, 2017). A 2016 United Nation Environment Programme (UNEP) assessment estimated that 83% (14 million tonnes) of PCB-contaminated equipment and material has yet to be destroyed (Figure 1-1) and if rates of elimination are not dramatically increased, several parties to the Convention will fail to meet their 2025 and 2028 targets (UNEP/DTIE, 2016). The Convention is also failing on its commitment to implement a framework to address compliance and enforcement (*Article 17*) because, after two decades, parties have failed to conclude negotiations on this issue (UNEP, 2019b).

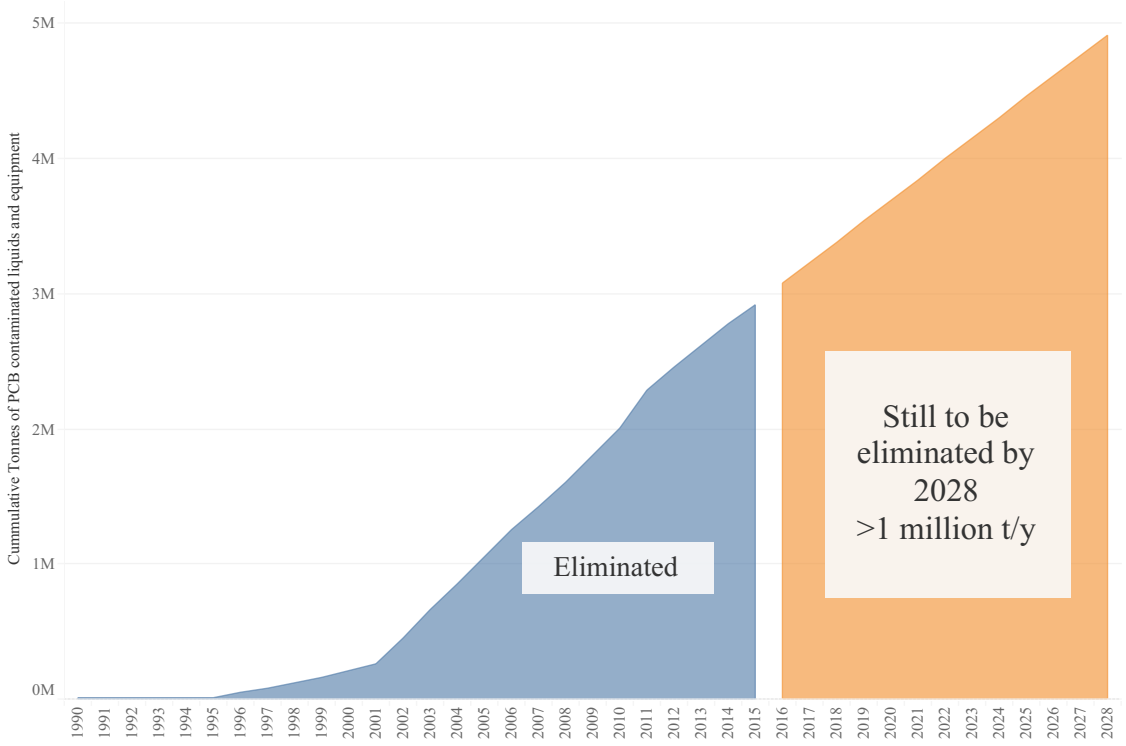


Figure 1-1: Cumulative tonnes of PCB contaminated liquids and equipment that have been eliminated up to 2015 (blue area), estimated amount that must be elimiated to meet 2028 Stockholm Convention target (orange area). Estimates are based on data from (UNEP/DTIE, 2016).

Persistent organic pollutants have a broad range of applications across agriculture, industry and domestically, as a result of their shared structural properties and characteristics (Table 1-1) (Miniero et al., 2015). Their production at an industrial scale began in the 20th century, with the manufacture of polychlorinated biphenyls (PCBs), which were widely used in industry as electrical transformer fluids, sealants, paints, lubricants and plasticisers (Brinkman and De Kok, 1980). This was followed by the manufacture of organochlorine pesticides (OCPs), which were used extensively in agriculture to protect crops and prevent diseases such as malaria (Miyagawa et al., 2016). Following mounting evidence of their toxicity and environmental persistence, the production and use of PCBs and many OCPs was banned in several countries in the late 1970s and 1980s, leading to decreases in environmental concentrations (de Boer and Wells, 1997; Miyagawa et al., 2016). However, despite the initial success of national and international regulations, POPs are still present in some wildlife at concentrations that pose a health risk and continue to be a pervasive threat (Jepson et al., 2016; Law et al., 2012a; Murphy et al., 2015). Further, the list of

chemicals that are recognised as POPs continues to grow as new compounds are synthesized for commercial use and released into the environment (Miniero et al., 2015).

POPs continue to enter the environment from a number of anthropogenic sources, including terrestrial run off, dredging, degrading waste-products and industrial emissions (Stuart-Smith and Jepson, 2017). Following environmental release, POPs are dispersed from their point source via environmental transport mechanisms including ocean currents, rain and vapour phase deposition (Lohmann et al., 2007). The dispersal rates, mechanisms and environmental fates of individual POPs vary according to their chemical structure and this partly explains why there is substantial variation in spatial and temporal trends observed between some pollutant classes (Lohmann et al., 2007). Dispersal of legacy pollutants over time has been well documented and, to date, two mechanisms have been proposed. These state that dispersal is driven either by concentration gradients, from areas with high concentrations to low concentrations, or by latitudinal temperature gradients, from warmer to cooler areas (von Waldow et al., 2010; Wania and Mackay, 1993). These global dispersal mechanisms can cause levels of pollutants to accrue in previously uncontaminated and pristine environments, including the Antarctic and Arctic, where high concentrations of POPs have been recorded in several species (AMAP, 2004; Miniero et al., 2015).

To assess the global fate of POPs and their risk in the environment, long term monitoring of POPs in air, water and biota is required. However, there are very few long term datasets that can be used to monitor global trends as concentrations must be monitored in a remote environment without influence from local sources (Lohmann et al., 2007). One example of a suitable programme is the Arctic Monitoring and Assessment Programme (AMAP), which has derived 20 years of global temporal trends by monitoring background air concentrations. They report that since the introduction of national and international regulations, concentrations of most POPs, listed under the *Stockholm Convention*, are gradually declining (Hung et al., 2016). However, they found concentrations of HCB and some PCB congeners were increasing, and suggest this is likely to be in response to regional warming and continued primary emissions (Hung et al., 2016).

In the United Kingdom (UK), evidence is conflicting as to whether concentrations of POPs are declining. Findings appear to vary according to several factors including, pollutant class, the

environmental matrix being analysed, and the region being studied. For example, concentrations of PCBs measured in air, soil, sewage sludge and breast milk at various locations around the UK have declined (Schuster et al., 2010; Zennegg et al., 2013; Zhang et al., 2014). However, concentrations of PCBs in some coastal and estuarine sediments appear to have increased, suggesting ongoing releases or remobilisation of PCBs into the environment (Nicolaus et al., 2015). Further evidence of contemporary releases of PCBs has been demonstrated by monitoring exposures in cetaceans: despite declining levels of other POPs, PCB blubber concentrations in UK-stranded harbour porpoises (*Phocoena phocoena*) have stabilized (Law et al., 2012a).

1.1.3 Persistent Organic Pollutants and Cetaceans

Cetaceans are considered to be an effective sentinel group for assessing ocean and human health (Bossart, 2011). Many species of cetaceans are long-lived coastal residents that feed at a high trophic level, often sharing their food sources with humans. The long-term monitoring of their exposure to contaminants and associated health effects can, therefore, reflect the health and status of the wider ecosystem and provide an early warning signal of potential threats to marine life and human health. It has also been hypothesised that research outcomes in this field may receive increased attention as a result of humans' affinity to marine mammals (Moore, 2008). Therefore, research in this area may gain greater public interest and have increased impact. An even clearer connection between marine mammals and human health exists in the Arctic, where PCBs have been accumulating in human populations probably in response to the consumption of marine mammals by indigenous people (O'Hara et al., 2005; Sandau et al., 2000). Given that the health of ocean sentinels is inextricably linked to planetary and human health, it is important to monitor the health of marine mammals. Half of the extant species of cetaceans are now listed on the IUCN red list as "Critically Endangered", "Endangered", "Vulnerable", "Near Threatened" or "Data Deficient". Therefore, it is more important than ever to understand the threats these species face and take necessary actions to conserve them.

The Cetacea order comprises whales, dolphins and porpoises and is currently made up of 90 different species (IUCN - SSC Cetacean Specialist Group, 2020). These extant species are divided into two sub-orders: *Mysticeti* and *Odontoceti*. The *Mysteceti* sub order, commonly known as baleen whales, comprises fifteen species of cetaceans and the *Odontoceti* (toothed whales) sub

order is made up of 75 species (Lockyer, 2002). Cetaceans vary widely in size from the vaquita porpoise (*Phocoena sinus*), which can grow to around 1.4m, to the blue whale (*Balaenoptera musculus*), which can grow to 30m in length and is the largest animal ever to have lived (Evans & Raga 2012).

The relationship between the toxic effects of contaminants on organisms and the observed effects at population level is one of the central themes of ecotoxicology. The health effects of exposures on wildlife have been studied in the laboratory, using controlled exposures on organisms, and in the environment, by correlating concentrations with population level effects (Desforges et al., 2018; Folland et al., 2016). These studies have demonstrated that exposure to POPs can cause a broad spectrum of biochemical and toxic responses in humans and wildlife (Miniero et al., 2015). Exposure to POPs has been associated with a range of effects, across a wide range of concentrations, including, neurotoxicity and developmental and carcinogenic effects (Liu et al., 2010; Pessah et al., 2019). Most scientific and public attention has however, focussed on their endocrine disrupting effects, which have been shown to impair the immune and reproductive systems (Godfray et al., 2019). POPs can disrupt the endocrine system by antagonizing and mimicking hormones and altering hormonal synthesis and metabolism (Godfray et al., 2019). For example, DDTs and PCBs have been shown to bind to estrogenic receptors and cause estrogenic and antiestrogenic effects resulting in numerous toxic impacts (Godfray et al., 2019; Kelce et al., 1995; Loomis and Thomos, 1999; Uslu et al., 2013).

Extensive toxicology testing on laboratory animal models has provided convincing evidence of the toxicological effects of POPs (Ahmad et al., 2003; Uslu et al., 2013; Vitalone et al., 2010). Exposure has been shown to impair the immune system (Canesi et al., 2003), disrupt endocrine homeostasis (Brouwer et al., 1989) and impair reproduction and development (Ahmad et al., 2003; Allen et al., 1980). The body of evidence of these effects in marine mammals is less comprehensive, owing to the obvious ethical and economical constraints of carrying out these types of studies. Despite this, interspecies extrapolation of harmful effects is possible as mammals share fundamental features of their reproductive and immune systems (Berta et al., 2005; Levin et al., 2006). Laboratory research has shown that POPs have endocrine disrupting effects whereby they interfere with hormone action and alter endocrine function causing adverse effects on human

and wildlife health. The endocrine system is responsible for controlling a number of developmental processes and most tissue and organ functions (Bergman et al., 2013). Hence, endocrine disruptors can interfere with several processes because they interact with multiple hormone receptors affecting a variety of physiological processes (Wingfield, 2008). Most of these hormone receptors are common across all mammals and therefore common mechanisms of actions occur across mammalian species (Ross, 2002). By combining research on laboratory species with numerous *in vitro, in vivo* and epidemiological studies on marine mammals, causal relationships between POPs and health effects have been identified and there is a convincing body of evidence that shows their harmful effects on marine mammals.

Several immunological investigations of marine mammals have demonstrated relationships between exposure to contaminants and altered function of the immune system. For example, immune function tests (*in vitro* and *in vivo*) in common seals (*Phoca vitulina*) found that those exposed to higher levels of dietary POPs experienced impaired immune function including, impaired natural killer cell activity, reduced T-lymphocyte function and delayed antibody responses (Ross et al., 1996). Similar harmful effects (including altered phagocytosis, reduced natural killer cell proliferation and cytoxic effects) have also been demonstrated in killer whale (*Orcinus orca*) and polar bear (*Ursus maritimus*) cells (Desforges et al., 2017).

The effects of contaminants on immune system function in individuals can also have cascading effects resulting in population level consequences. Despite inherent difficulties in establishing direct links between tissue burdens and disease outbreaks there is a strong weight of evidence to suggest a link between blubber concentrations of contaminants and disease outbreaks (Ross, 2000). For example, epizootic outbreaks of morbillivirus in common seals (*Phoca vitulina*) and striped dolphins (*Stenella coeruleoalba)* in heavily polluted European waters indicate that there is a link between contaminant tissue burdens and viral susceptibility (Aguilar and Borrell, 1994a; Ross, 2000). Further, cumulative pathological investigations have suggested that exposure to high concentrations of organochlorines is a key factor in reducing host resistance (Hall et al., 2006; Jepson et al., 1999; Law et al., 2012a). It is also important to note that the impact of immunosuppression on marine mammals is likely be exacerbated by ocean warming because

higher sea temperatures can increase host susceptibility, rates of disease transmission and pathogen development (Kutz et al., 2005; Moore, 2009; VanWormer et al., 2019).

The endocrine disrupting effects of POPs have also been shown to impact reproductive success in marine mammals. Impaired reproduction has been demonstrated experimentally in captive common seals (*Phoca vitulina*) that were fed mackerel or flatfish from the Atlantic or the more heavily polluted Wadden Sea (Brouwer et al., 1989; Reijnders, 1986). Over two years, seals that were fed polluted fish had reduced fecundity rates, demonstrated implantation failure and had lower levels of estradiol, retinal and thyroid hormones (Brouwer et al., 1989; Reijnders, 1986). In addition, several field studies have demonstrated relationships between high tissue burdens and reproductive failure. In free-living bottlenose dolphins (*Tursiops truncatus*), blubber concentrations of PCBs were positively correlated with increased rates of first-born calf mortality (Wells et al., 2005). More recently, necropsied harbour porpoises (*Phocoena phocoena*) that stranded in the UK were found to have much lower pregnancy rates and reach sexual maturity later than other (less contaminated) populations (Murphy et al., 2015).

The lack of controlled experimental data on the direct effects of chemical contaminants on marine mammals remains a significant issue and has led to difficulty in definitively identifying cause-effect relationships and establishing species-specific toxicity thresholds. Despite this, work has been carried out to collate existing data to establish toxicity reference values that can be used to assess and compare risks. The most widely established threshold for toxic effects of POPs was derived for PCBs by combining data from *in vitro* laboratory studies with data obtained from captive animal studies on seals, otters and mink to derive a threshold tissue residue concentration for marine mammals of 17mg/kg lipid weight (Kannan et al., 2000). The endpoints for the threshold concentrations were defined using effects on concentrations of thyroid hormone and hepatic vitamin A, suppression of natural killer (NK) cell activity and proliferative response of lymphocytes to mitogens (Kannan et al., 2000). A more recent *in vitro* analysis of the relative binding affinities of PCBs to the AHR in beluga whales (*Delphinapterus leucas*) and mice suggested that the beluga AHR had a greater affinity than the mouse for most PCBs tested (Jensen et al., 2010). This comparatively high binding affinity of the beluga implies that beluga, and perhaps all cetaceans, may be more sensitive to the toxic effects of PCBs than other mammals

(Jensen et al., 2010). Taken together, there is substantial evidence to suggest that individual PCB contaminants and commercial PCB mixtures disrupt the endocrine systems of both humans and wildlife. In addition, it is evident that all organisms are exposed to complex mixtures of pollutants that contain several harmful and/or endocrine disrupting compounds rather than to individual chemicals. Moreover, it is now well established that the antagonistic and synergistic actions of multiple pollutants can exert harmful effects, even when each pollutant is present at a level deemed to be safe toxicologically (Carvalho et al., 2014; Silva et al., 2002). Therefore, it is important to consider that the toxicological risk that marine mammals face is likely to be underestimated by risk evaluations that only consider a single pollutant.

As long lived, apex predators with large stores of fatty tissue, marine mammals tend to accumulate higher levels of POPs than other wildlife (Loseto and Ross, 2011). In Swedish waters, many wildlife populations such as otters (*Lutra lutra*), grey seals (*Halichoerus grypus*) and the white-tailed eagle (*Haliaeetus albicilla*), have experienced population recoveries that coincide with a decrease of PCB concentrations in their tissues (Roos et al., 2012). However, levels of POPs in marine mammals have been shown to be much higher than other wildlife (Jepson et al., 2016; Law et al., 2012a). The potential health risks of contaminants are also likely to be compounded by the multitude of other threats that affect marine mammal populations. These include habitat degradation, overfishing, by-catch and acoustic pollution (Avila et al., 2018). At present, 20 marine mammal species are listed as threatened or endangered on the IUCN red list and there is a strong desire to protect and conserve these species (IUCN - SSC Cetacean Specialist Group, 2020). Therefore, it is important to monitor contaminant exposure and consider factors that may influence exposure and increase the vulnerability of marine mammals to contaminants.

Diet, through the ingestion of prey, is considered to be the primary route of exposure to contaminants in marine mammals (Beyer and Meador, 2011). As such, dietary differences have a substantial influence on inter and intraspecific variation in contaminant concentrations and degrees of exposure vary according to dietary preferences and levels of environmental contamination (Andvik et al., 2020; Pulster et al., 2009). The influence of dietary preferences is demonstrated by the much higher concentrations observed in marine mammal eating versus fish eating killer whales (*Orcinus orca*) and the relatively low concentrations found in mysticetes compared to odontocetes

(Andvik et al., 2020; Pinzone et al., 2015). The impact of variation in levels of environmental contamination on body burdens has been demonstrated in populations of bottlenose dolphins (*Tursiops truncatus*) with high site fidelity. Blubber concentrations of PCBs were ten-fold higher in animals sampled near a contaminated site compared with those that were sampled at a less contaminated site and accumulation profiles of each population closely reflected those found in their prey (Pulster et al., 2009; Pulster and Maruya, 2008).

Marine mammals appear to be particularly vulnerable to accumulating high concentrations of POPs as a result of a combination of factors (Loseto and Ross, 2011). The lipophilic nature of POPs and their resistance to biological degradation causes them to partition into lipids and bioaccumulate (concentrations increase with age and body size) and biomagnify (concentrations increase with trophic feeding levels) (Pierce et al., 2008). Therefore, marine mammals' long-life spans and high trophic positions make them more likely to accumulate high concentrations of contaminants. Marine mammals also have several anatomical features that contribute to their comparatively high body burdens. Their lipid-rich blubber acts as a store for POPs and in periods of starvation or fasting these compounds are remobilized, from the blubber, negatively impacting adult and perinatal health (Bossart, 2011). Their vulnerability is further compounded by their reduced capability to metabolise some POPs caused by the reduced activity of their CYP2B enzyme in comparison to other mammals (Boon et al., 1997; Tanabe et al., 1994).

Age, sex and reproductive history are also strong drivers of pollutant concentrations in marine mammals (Hall et al., 2006; Kratofil et al., 2020). Contaminant exposure in marine mammals begins during gestation when foetuses are exposed to POPs through placental transfer (Tanabe et al., 1981). This maternal offloading continues after birth, via lactational transfer (Tanabe et al., 1981). Consequently, neonates have been shown to accumulate very high concentrations due to their small body size and the affinity of POPs to bind to the lipid rich milk (Borrell et al., 1995; Schnitzler et al., 2019). This pollutant offloading is positive for the mother's health as they are able to offload a significant proportion of their body burden (Borrell et al., 1995). However, the high pollutant load, relative to body size, experienced by calves can have severe consequences at population level. Consequently, to increase offspring survival rates the period of maternal care they provide tends to be protracted (Lockyer, 2002). This long period of maternal care (in some

cases up to three years) results in slow sexual maturation, which further limits the rate at which cetaceans can reproduce (Lockyer, 2002). Therefore, high exposures in calves are thought to be the cause of observations of reduced or zero reproduction rates, as a result of an increase in occurrence of stillbirths and calf mortality (Schwacke et al., 2002). Following weaning, concentrations in juveniles tend to decrease as a consequence of a less-contaminated diet and the dilution of contaminants caused by increases in body and blubber mass (Hickie et al., 1999). Once sexual maturity is reached the accumulation profiles of males and females diverge. Males and nulliparous females that are not able to offload their body burden continue to accrue contaminants over their lifespans and their tissue concentrations have been shown to positively correlate with age (Borrell et al., 1995).

As discussed, there are several factors that can influence susceptibility to contaminant exposure, causing some populations to be at a greater risk of experiencing toxic effects than others. Populations that occupy areas with high pollutant levels (e.g. the Baltic Sea) or feed at higher trophic levels (e.g. killer whales) tend to face the biggest threat (Jepson et al., 2016; Sonne et al., 2020). For example, PCB loads in UK bottlenose dolphins are often higher than those observed in other, less contaminated, areas (Jepson et al., 2016; Schwacke et al., 2002; Wells et al., 2005). These high and stable exposures are often associated with small populations and population declines that cannot be explained by other anthropogenic impacts such as bycatch (Jepson et al., 2016). This is demonstrated by the latest abundance estimates for inshore bottlenose dolphins, in the North Sea and Biscay, that show small or contracting populations (Hammond et al., 2002; ICES, 2010). The UK's resident killer whale population provides further evidence of this association as they have extremely high levels of PCBs, have failed to reproduce for over 25 years and only eight individuals remain in the population (Jepson et al., 2016; McHugh et al., 2007). Given the relatively low numbers of these species that strand, monitoring contaminants in more abundant species, such as the harbour porpoise, can provide insight into the risks of exposure in more vulnerable species. Therefore, in chapters 3, 4 and 5, I use harbour porpoises, that have stranded around the UK coast, as a model species to investigate possible health effects of contaminants and thereby gain a better understanding of the risks of exposure in other, more vulnerable, species.

Harbour porpoises inhabit cold temperate to sub-polar waters across the Northern Hemisphere (Ridgway, 1998). They generally live in coastal waters and can be found across Europe, although they are not regularly observed in the Mediterranean Sea (Fontaine, 2016). In the UK, they grow to around 150cm in length and females are generally larger than males (Lockyer, 1996). They become sexually mature after 3-5 years, have a gestation period of 10-11 months and calves are weaned at around six months (Read, 1999). Estimates of lifespan have shown some individuals can live up to 23 years however, estimates of typical lifespan vary from 5 to 12 years, with only 5% estimated to live beyond 12 years (Kesselring et al., 2017; Christina Lockyer, 1995; Lockyer and Kinze, 2003). In general, harbour porpoises feed on small fish, crustaceans and cephalopods but main prey items vary regionally (Santos et al., 2004). There are no complete abundance estimates covering the range of porpoises in Europe. However, in 2016 the number of harbour porpoises in the North Sea and off the west coast of Scotland was estimated to be 345,373 [CV=0.18] and 24 370 [CV=0.23] respectively (Hammond et al., 2017).

Harbour porpoises face a wide number of anthropogenic threats in Europe and are now included in the Oslo/Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) list of threatened and/or declining species for the Greater North Sea and Celtic Seas (OSPAR, 2016). Accidental entanglement in fishing gear (by-catch) is the primary cause of anthropogenic mortality across the North-East Atlantic and porpoises are thought to be particularly vulnerable, as their habitats tend to occupy productive coastal waters (OSPAR, 2016). Harbour porpoises have also been shown to be threatened by depleted prey stocks as a consequence of overfishing and climate change (ASCOBANS, 2020). Prey depletion due to overfishing may explain why porpoises in the North Sea have been shown to feed at a lower tropic level than during the preceding century (Christensen and Richardson, 2008). In addition, observed changes in sand eel abundance (an important prey item), caused by climate change, have been associated with increased numbers of stranded porpoises that have died from starvation (MacLeod et al., 2007). As previously discussed, malnutrition in marine mammals has been linked to the remobilisation and redistribution of PCBs from blubber. Therefore, any threat from overfishing or climate change would be exacerbated by an increased risk of detrimental effects from contaminants (Houde et al., 2005).

A number of studies have documented high levels of persistent organic pollutants in this population of harbour porpoises (Jepson et al., 2016; Law et al., 2012b, 2012a). In addition, associations between blubber concentrations of PCBs and health indices such as infectious disease mortality and pregnancy rates have been identified. For example, it is thought that exposure to higher concentrations of PCBs may explain why the reproductive output of healthy females in this population is almost half of that observed in other, less contaminated, populations (Murphy et al., 2015). Exposure to higher concentrations of PCBs has also been associated with an increased risk of infectious disease mortality such that high exposure levels may be causing significant excess mortality in the population (Hall et al., 2006). Hence, it is vital to investigate the current threat to harbour porpoises that inhabit UK waters to assess whether POP exposures are still impacting the health of this population. In this thesis, I use data collected as part of the UK Cetacean Strandings Investigation Programme (CSIP) to assess the impacts of POPs on cetaceans that live near industrialised coastlines.

The CSIP was established in 1990, by the UK Department for Environment, Food & Rural Affairs (Defra) to begin long-term monitoring of UK-stranded marine mammals, conduct systematic post-mortem investigations (necropsies) and determine major causes of death. The programme has collected over 30 years of pathological and observational data on UK marine mammal strandings, which can provide valuable insights into the threats of cetaceans that occupy or visit British waters. The harbour porpoise is the most abundant cetacean in Northern Europe and is the most commonly stranded cetacean around the UK (Deaville and Jepson, 2010). Consequently, the programme now has the largest collection of harbour porpoise necropsy and toxicology data in the world, allowing rigorous and robust studies to be carried out to assess their threats (Hall et al., 2006; Jepson et al., 2016; Murphy et al., 2015).

Figure 1-2: Satellite image of the United Kingdom and surrounding countries

The UK is representative of several regions around the world where marine mammals live in close proximity to heavily industrialised coasts (Avila et al., 2018; Robbins et al., 2017). The marine environments that surround these areas continue to be polluted by a barrage of legacy and emerging pollutants as a result of poor regulation and waste management and several marine mammal populations are known to be in decline (Magera et al., 2013; Stuart-Smith and Jepson, 2017). Therefore, it is important to understand more about pollutant exposures in marine mammals in these areas to quantify the risk posed to these species and understand whether current elimination and mitigation actions are sufficient to protect them.

1.2 Thesis overview

In this thesis I assess the impact of persistent organic pollutants (POPs) on UK-stranded/necropsied cetaceans using pathological and toxicological data collected between 1990 and 2018 as part of the UK CSIP.

In **Chapter 2** I quantify the temporal and spatial trends of seven classes of POP in eleven species of marine mammal. I examine the influence of spatiotemporal factors and interspecific variation on concentrations and relative abundances. I also determine the relative risk of each pollutant to highlight those of greatest concern and demonstrate that polychlorinated biphenyls (PCBs) present the greatest threat.

In **Chapter 3** and all following chapters, I explore the particular threat of PCBs using harbour porpoises as a sentinel species. Chapter 3 determines the spatiotemporal trends of PCBs in harbour porpoises and demonstrates the impact of current exposures on infectious disease mortality risk. I show that despite a decrease in PCB concentrations, in harbour porpoise blubber, concentrations are still associated with increased rates of infectious disease mortality. I also demonstrate that there is considerable regional variation in the rates of decline of PCB concentrations in the UK.

In **Chapter 4** I investigate whether exposure to PCBs impacts male reproduction by determining the relationship between PCB blubber concentrations and testes weights in harbour porpoises. I advocate that current risk assessments, which do not account for the possible impacts of reduced male fertility, may underestimate the risk posed to cetacean populations.

In **Chapter 5** I investigate whether relative abundances of PCB congeners vary according to age class and sex to understand whether toxicity thresholds that assume congener profiles are similar across all individuals are appropriate. I quantify the relative abundances of PCB congeners in harbour porpoises to determine whether relative abundances differ by age and sex, in response to pollutant offloading from mothers to calves. I also investigate whether relative abundances of individuals vary according to their stranding location. I use these results to discuss the implications of exposure to developing calves and suggest that current approaches that use toxicity thresholds,

which assume congener profiles are similar across all age classes, may result in an over or underestimation of risk.

Chapter 2 - Levels and Trends of Contaminant Exposure in Multiple Marine Mammal Species

2.1 Abstract

Despite international attempts to eliminate or restrict the production of the most persistent and toxic pollutants, environmental releases still occur and their impact on marine mammals remains a global concern. To assess the efficacy of mitigation strategies comprehensively, knowledge of the long-term impacts of pollution across multiple species, pollutant classes and regions is required. In this chapter, using the world's largest marine mammal toxicology database, I examine persistent organic pollutant (POP) burdens in eleven marine mammal species from the North-East Atlantic spanning 30 years. I show that although levels of legacy pollutants are in decline, they are still present at levels known to be a significant threat to marine mammal populations (e.g., at concentrations that are associated with deleterious effects on immunity, reproduction and development), with considerable variation in rates of decline among taxa, pollutant classes and regions. I also found a longitudinal shift in total POP concentrations over the 30-year study period such that, in 1992, concentrations were highest on the west coast of the UK whilst, in 2018, concentrations were highest on the east coast. Levels of polychlorinated biphenyls (PCBs), some of the most toxic, are declining at the slowest rate with 57% of individuals exhibiting a burden known to be a toxicological threat. Pollutant concentrations in common dolphins (*Delphinus delphis*), which also face a substantial threat from bycatch in this region, are declining at a significantly slower rate than other taxa. I conclude that current efforts to reduce environmental concentrations of POPs have failed and that the threat is not yet under control. Therefore, improved international elimination, mitigation and compliance strategies are needed to address this critical issue for the global health of the marine environment.

2.2 Introduction

Since the 1920s, the global agricultural and industrial use of persistent organic pollutants (POPs) has led to pervasive and widespread environmental contamination, causing considerable harm to several wildlife populations (Méndez-Fernandez et al., 2018; Reijnders, 1980; Sonne et al., 2020; Wiemeyer and Porter, 1970). POPs are a group of chemicals that are of grave concern as they are toxic to humans and wildlife, widespread and persistent in the global environment, can be transported over large distances and have the ability to biomagnify and bioaccumulate throughout trophic webs (UNEP, 2017). Despite the initial success of national and international regulatory agreements, such as the Stockholm Convention (a multilateral treaty to protect human health and the environment from POPs), tissue concentrations appear to remain at hazardous levels in several species as a consequence of their persistent nature, continued used in some regions and the failure of governments to eliminate these pollutants at a sufficient rate (Stuart-Smith and Jepson, 2017; Williams et al., 2020b). POPs continue to enter the marine environment from diffuse sources and those still in 'open application', such as in paints and sealants, are thought to contribute most to contemporary environmental releases (Defra, 2013, Jartun, 2011, Stuart-Smith and Jepson, 2017). At present rates of elimination, it is expected that several parties to the Convention will fail to meet their 2025 and 2028 targets (European Commimission - DG Environment, 2014). Therefore, it is important to urgently assess the threat posed to marine mammals, so that governments can implement greater and more coordinated efforts to reduce contamination levels.

As highly mobile top predators with long lifespans, marine mammals tend to accumulate higher concentrations of POPs than other species and are therefore considered effective sentinels of ocean health (Bossart, 2011). Organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs), in particular, have been shown to cause suppression of the immune and reproduction systems in several mammalian species (Desforges et al., 2016; Folland et al., 2016; Reijnders, 1986; Sonne et al., 2020; Williams et al., 2020a), and are thought to be contributing to population declines and lower recruitment observed in several marine mammal populations (Jepson et al., 2016; Roos et al., 2012).

Populations that inhabit heavily contaminated, semi-industrial marine habitats such as the North-East Atlantic, Mediterranean and Gibraltarian Strait, are thought to be most vulnerable (Desforges et al., 2018; Jepson et al., 2016). The North-East Atlantic surrounds a heavily industrialised area where environmental releases of legacy pollutants still take place and large stockpiles of pollutants are yet to be destroyed (Stuart-Smith and Jepson, 2017). This area is characteristic of several other regions around the globe where marine mammals live in close proximity to highly industrialised coasts and therefore, face a number of anthropogenic threats alongside pollution (Avila et al., 2018; Robbins et al., 2017). The marine environment, in these areas, continues to be contaminated, with a barrage of legacy and emerging pollutants, as a consequence of inadequate regulation and poor waste management and several marine mammal populations are known to be in decline (Magera et al., 2013; Stuart-Smith and Jepson, 2017). Understanding more about pollutant burdens in these regions is essential to be able to rigorously and robustly determine whether current elimination and mitigation actions are sufficient and to aid the development of effective conservation and management strategies for marine ecosystems.

In this chapter, I explore the extent of pollutant exposure in eleven North-East Atlantic species of marine mammal, using the largest marine mammal toxicological dataset available globally. I examine the influence of spatiotemporal factors and interspecific variation on accumulation profiles of six pollutant classes (Dichlorodiphenyltrichloroethane (DDTs), Hexachlorocyclohexanes (HCHs), Hexachlorobenzene (HCB), Dieldrin; Polychlorinated biphenyls (PCBs) and Polybrominated diphenyl ethers (PBDEs)) using pathological and blubber pollutant data collected from 1077 individuals. To identify those of most concern I also determine the relative risks to the immune and endocrine systems, from each class of pollutant. I show that POPs continue to pose a health risk to marine mammals in the North-East Atlantic and that current national and international efforts to mitigate this threat are inadequate.

2.3 Materials and Methods

2.3.1 Sampling Regime

Pathological Analyses

Between 1990 and 2018 necropsies were carried out, by the United Kingdom Cetacean Strandings Investigation Programme (CSIP), on over 4000 carcasses according to standard procedures for marine mammals (Law et al., 2006). Carcasses were then prioritised for pollutant analysis according to their state of decomposition using the classification system set out by Deaville et al., 2019, leading to a sample size of 1077 individuals: Atlantic white-sided dolphins (*Lagenorhynchus acutus*); bottlenose dolphins (*Tursiops truncatus*); common seals (*Phoca vitulina*); grey seals (*Halichoerus grypus*); harbour porpoises (*Phocoena phocoena*); killer whales (*Orcinus orca*); Risso's dolphins (*Grampus griseus*); short-beaked common dolphins (*Delphinus delphis*); striped dolphins (*Stenella coeruleoalba*); sperm whales (*Physeter macrocephalus*) and white-beaked dolphins (*Lagenorhynchus albirostris*). Of the carcasses analysed for pollutants, 87% were classified as extremely fresh or slightly decomposed. Carcasses were prioritised in this way to minimise the impact of changes in pollutant concentrations and lipid dispersion that are associated with decomposition. Data exploration showed that the individuals analysed were a representative sample of the strandings that occurred over the study period.

To investigate any differences due to age, individuals were categorised into two age classes (juveniles and adults) according to body length and sexual maturity (Jepson et al., 2016). Sexual maturity was determined using gonadal appearance, which has been shown to be an accurate method for marine mammals (Kesselring et al., 2019). As part of the pathological investigations dorsal, ventral and lateral blubber thickness were measured and the mean thickness for each individual was calculated. A linear regression model was fitted to the natural logarithm of mean blubber thickness for each species and the model residuals were used as a proxy for body condition (Table 2-1). The log of mean blubber thickness was the response variable and species was the predictor variable. The plot of model residuals against cause of death and body weight to length ratio was used to verify that this approach was suitable (Figure 2-1).

Table 2-1: Summary statistics of the linear model fitted to the strandings data. The natural logarithm of mean blubber thickness was the response variable, species was the predictor variable.

Variable	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	2.94	0.06	46.5	0
Common dolphin	-0.17	0.08	-2.28	0.02
Harbour porpoise	-0.18	0.06	-2.83	0
Risso's dolphin	0.13	0.11	1.21	0.22
Striped dolphin	-0.45	0.13	-3.57	0
White-beaked dolphin	-0.22	0.11	-1.93	0.05
White-sided dolphin	-0.25	0.12	-2.04	0.04

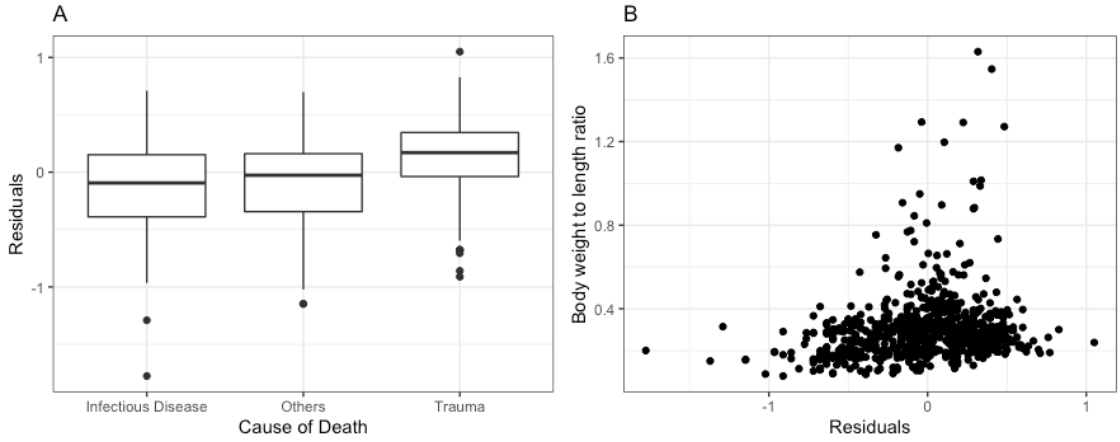


Figure 2-1: Residuals (that were used as a proxy for nutritional condition) from the linear regression model fitted to the natural logarithm mean blubber thickness and species against: (A) Cause of death (B) Body weight to length ratio

Toxicological Analyses

Blubber concentrations of six pollutant classes were determined across all eleven species of marine mammals examined. The six pollutant classes have widespread applications either as crop treatments: 1) Isomers of Dichlorodiphenyltrichloroethane (DDTs), 2) Hexachlorocyclohexanes (HCHs), 3) Hexachlorobenzene (HCB), 4) Dieldrin; or as industrial chemicals: 5) (PCB congeners) and 6) flame retardants (Polybrominated diphenyl ethers (PBDEs). All groups are known to be toxic to marine life (Table 2-2).

For each individual, a full thickness blubber sample was taken (from the dorsolateral region close to the insertion of the dorsal fin in cetaceans and from the ventral thorax in pinnipeds), wrapped in catering grade foil and preserved at −20 °C using established protocols (Law et al., 2006). Contaminant concentrations were determined (on a mg kg^{-1} wet weight basis) at the Cefas laboratory (Lowestoft) using methods that follow the recommendations of the International Council for the Exploration of the Sea (ICES) and validated under the QUASIMEME laboratory proficiency scheme (de Boer and Law, 2003; de Boer and Wells, 1997; ICES, 1998; Webster et al., 2013). The POP content of samples was measured using gas chromatography electron capture detection (Agilent 6890) now listed as the EU reference method for lipophilic toxins. In cases where concentrations were below the limit of quantification, the concentrations were set at half the detection limit (Law et al., 2012a). The pollutants and their respective congeners/isomers investigated here cover a wide range of physical and chemical properties and include congeners that can be compared with other studies and measurement standards (e.g., seven PCBs prioritised for international monitoring by ICES) and have a wide range of applications (Table 2-2). The measured contaminants also vary in terms of historical use, persistence and toxicities (Table 2-2).

For Quality Assurance and Quality Control the CEFAS laboratory (Lowestoft) participates biannually in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) proficiency testing scheme. In addition, the laboratory participated successfully in the 2008 marine mammal interlaboratory exercise organised by the National Institute of Standards and Technology in the USA. All analyses were carried out under full analytical quality control procedures, which included the analysis of a blank sample and the analysis of a certified reference material with every batch of 10 samples to assess the performance of the methods. Blanks for individual POPs and congeners were always below the limit of quantitation. Where the levels of target analytes were beyond the range of the instrument calibration, the extract was diluted and re-analysed. The reference material BCR349 (cod liver oil; European Bureau of Community reference) was used to ensure precision and accuracy and for each compound the reference material results were plotted as Shewhart quality control charts. The charts were created previously from repeated analysis of the reference material using the North West Analytical Quality Analyst software™ (Northwest Analytical Inc., USA). The warning and control limits for the charts were defined as 2σ and 3σ – 2x and 3x the standard deviation from the

mean for each compound. For each of the samples analysed the certified reference materials were within the limits set by the control charts. Therefore, all results were deemed to be valid. For example, the expanded uncertainty MU (calculated as 2*standard deviation of the control charts for the BCR349 reference material from the last 10 years) for the ICES7 PCBs ranges from 11.9% for CB153 to 17.9% for CB28, which is well within the requirement to be < 50% (SANCO/12495/2011, 2011) .

Table 2-2: The congeners, isomers and historical applications of each class of persistent organic pollutant (POPs) analysed in the harbour porpoise blubber. The nested tables show the maximum concentrations of each pollutant class and the percentage of each species with blubber concentrations of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) above derived toxicity thresholds (PCBs= 9 mg/kg lipid[5], PBDES 1.5 mg/kg lipid[32]). The percentages shown were calculated for the most recent five years of data (2014-2018). ND = no data available. NT= No toxicity threshold available.

Pollutant Class	**Congeners/isomers analysed**	**Applications**	**Year ratified in the Stockholm Convention (Annex)**	**Toxicity and concentrations by species**				
				Species (N=)	**% over toxicity threshold between 2014-2018**	**Concentration (mg/kg lipid)**		
						Mean	**Max**	**Min**
Polychlorinated biphenyls (PCBs)	CB18, CB28, CB31, CB44, CB 47, CB49, CB52, CB66, CB101, CB105, CB110, CB118, CB128, CB138, CB141, CB149, CB151, CB153, CB156, CB158, CB170, CB180, CB183, CB187, CB194	Used in construction joint sealants, paints, transformers and capacitors, lubricants, plasticizers3	2004 (Annex A Elimination & Annex C Unintentional Production)	Atlantic white-sided dolphin (22)	0	11.9	54.87	1.59
				Bottlenose dolphin (63)	80	75.85	697.99	0.82
				Common seal (16)	31	18.61	158.45	1.2
				Grey seal (21)	17	8.17	34.22	0.64
				Harbour porpoise (731)	42	16.31	159.68	0.46
				Killer whale (15)	100	263.81	956.37	11.76
				Risso's dolphin (26)	31	8.46	31.3	0.36
				Short-beaked common dolphin (124)	70	31.27	225.1	0.46
				Sperm whale (6)	17	6.93	11.98	4.41

				Striped dolphin (22)	13	37.19	183.61	1.87
				White-beaked dolphin (24)	85	26.9	124.35	5.19
Dichlorodiphenyl-trichloroethane (DDTs)	*p,p′*-DDE, *p,p′*-DDT, *p,p′*-TDE	Insecticide[19]	2004 (Annex B Restriction)	Atlantic white-sided dolphin (12)	NT	11.04	31.03	0.89
				Bottlenose dolphin (33)	NT	25.47	219.02	0.5
				Common seal (16)	NT	1.61	4.65	0.32
				Grey seal (7)	NT	1.38	4.67	0.16
				Harbour porpoise (658)	NT	3.45	42.78	0
				Killer whale (10)	NT	297.38	1203.41	27.44
				Risso's dolphin (18)	NT	2.24	15.61	0.07
				Short-beaked common dolphin (80)	NT	5.45	33.49	0.09
				Sperm whale (6)	NT	7.81	17.74	3.53
				Striped dolphin (12)	NT	17.69	99.28	1.5
				White-beaked dolphin (15)	NT	12.26	51.13	2.51
Polybrominated diphenyl ethers (PBDEs)	BDE17, BDE28, BDE47, BDE66, BDE85, BDE99, BDE100, BDE138, BDE153, BDE154, BDE183	Flame retardants used in electrical equipment, construction materials, textiles,	2009 (Annex A Elimination)	Atlantic white-sided dolphin (11)	0	0.38	0.61	0.09
				Bottlenose dolphin (28)	21	3.01	15.37	0.08
				Common seal (9)	0	0.18	0.8	0.03

		furniture foam, plastics[20]		Grey seal (7)	0	0.08	0.14	0.03
				Harbour porpoise (420)	0	0.9	15.65	0.02
				Killer whale (8)	100	8.41	25.53	0.69
				Risso's dolphin (15)	0	0.58	2.53	0.03
				Short-beaked common dolphin (10)	0	0.59	1.46	0.03
				Sperm whale (0)	ND	ND	ND	ND
				Striped dolphin (11)	0	0.51	1.94	0.07
				White-beaked dolphin (14)	44	3.06	12.63	0.45
Hexachlorocyclohexane-hexanes (HCHs)	α-HCH, γ-HCH	Pesticide[19]	2009 (Annex A Elimination)	Atlantic white-sided dolphin (12)	NT	0.11	0.9	0.02
				Bottlenose dolphin (33)	NT	0.05	0.12	0
				Common seal (9)	NT	0.03	0.05	0.01
				Grey seal (7)	NT	0.03	0.04	0.03
				Harbour porpoise (658)	NT	0.1	2.02	0
				Killer whale (10)	NT	0.17	0.58	0
				Risso's dolphin (18)	NT	0.04	0.1	0
				Short-beaked common dolphin (80)	NT	0.13	0.9	0

				Sperm whale (6)	NT	0.02	0.02	0.01
				Striped dolphin (12)	NT	0.07	0.37	0.02
				White-beaked dolphin (15)	NT	0.07	0.27	0.03
Hexachloro-benzene (HCB)	HCB	Fungicide, combustion by-product[19]	2001 (Annex A Elimination & Annex C Unintentional Production)	Atlantic white-sided dolphin (22)	NT	0.37	1.19	0.2
				Bottlenose dolphin (63)	NT	0.39	1.44	0.01
				Common seal (16)	NT	0.01	0.03	0.01
				Grey seal (21)	NT	0.01	0.05	0.01
				Harbour porpoise (736)	NT	0.24	1.88	0
				Killer whale (15)	NT	2.24	8.63	0.33
				Risso's dolphin (26)	NT	0.25	1.08	0.02
				Short-beaked common dolphin (125)	NT	0.17	0.81	0
				Sperm whale (6)	NT	0.4	0.63	0.09
				Striped dolphin (23)	NT	0.3	0.98	0.15
				White-beaked dolphin (24)	NT	0.47	0.85	0.21
Dieldrin	Dieldrin	Insecticide[21]	2004 (Annex A Elimination)	Atlantic white-sided dolphin (12)	NT	0.89	7.5	0.03
				Bottlenose dolphin (33)	NT	0.66	3.94	0.02

	Common seal (9)	NT	0.03	0.08	0.01
	Grey seal (7)	NT	0.04	0.12	0.01
	Harbour porpoise (658)	NT	0.78	13.41	0
	Killer whale (10)	NT	20.62	88.03	0.11
	Risso's dolphin (18)	NT	0.22	1.09	0.01
	Short-beaked common dolphin (80)	NT	0.48	6.71	0.01
	Sperm whale (6)	NT	0.06	0.11	0.03
	Striped dolphin (12)	NT	0.55	2.82	0.04
	White-beaked dolphin (15)	NT	0.89	5.14	0.05

Statistical Analyses

All analyses were carried out using the statistical computer programme R (version 4.0) (R Core Team, 2016).

2.3.2 Contaminant Concentrations

Initial analyses of blubber pollutant concentrations covered all eleven species. Killer whales (*Orcinus orca*), sperm whales (*Physeter macrocephalus*), grey seals (*Halichoerus grypus*) and common seals (*Phoca vitulina*), were included in the preliminary statistical analysis (n=1077) but were excluded from the models (n=745) because of their low sample size and high variance (Table 2-5). The flame retardants (PBDEs) were also excluded from statistical modelling because analysis for these pollutants was only carried out on some of the species included in the models. Prior to model fitting, extensive data exploration was carried out to test for collinearity between variables and to remove individuals with missing biological data or those for which there were incomplete results for any of the five of the pollutant classes, included in the models. This resulted in a total sample size of n= 745 (Table 2-3).

Table 2-3: Sample sizes for each species that were included in the statistical models

Species	**n ()**
Atlantic white-sided dolphin	11
Bottlenose dolphin	28
Harbour porpoise	604
Risso's dolphin	14
Short-beaked common dolphin	63
Striped dolphin	11
White-beaked dolphin	14

To investigate the factors that influence contaminant concentrations, generalised linear models (GLMs) with normal distributions were fitted to selected variables that could explain the variability in the data (Chambers and Hastie, 1992; Venables et al., 2002). Models were fitted to the summed

and individual concentrations of pollutants (PCBs, DDTs, HCHs, dieldrin and HCB). For each model the natural logarithm of contaminant concentration was the response variable. The predictor variables included in the full models were selected according to the biological rationale that they could influence contaminant concentrations. These were year of stranding, age class, sex, latitude, longitude, species and mean blubber thickness, including two-way interaction terms between age class and sex and a four-way interaction term between latitude, longitude, species and year of stranding (Miniero et al., 2015).

For each model, all possible variable combinations were tested to obtain several candidate models. Final predictions were obtained by averaging the set of plausible models (Δ Akaike's Information Criterion (AIC) < 4) from the candidate models (Akaike, 1973; Barton, 2015; Richards, 2005). The models were validated by assessing the normality of the residuals and by plotting them against selected variables and assessing the variance. The top 20 candidate models for the summed pollutant concentration model can be found in the Appendix Table A- 1.

2.3.3 Relative abundance of contaminants

Differences in the relative abundance of contaminants in all eleven species were assessed using Kruskal-Wallis tests and post-hoc Dunn's tests (n=1077). If significant differences were found, a post hoc Dunn's test was performed to identify which species were significantly different. Differences in PCB contamination profiles were investigated by examining patterns in variation for individual congeners and congener groupings. Congeners were grouped according to their degree of chlorination and their dioxin-like properties. Similarly, variations in PBDE profiles were assessed for individual congeners and for congeners grouped according to their degree of bromination.

Analysis of the drivers of variation in relative abundance covered seven species and five pollutant classes, as per the analyses for contaminant concentrations. Drivers of variation were assessed by scaling the concentrations of each contaminant class by the total concentration of pollutants and carrying out principal component analysis (PCA) to aggregate the variation. PCA was carried out to enable the possible drivers of variation to be modelled against the principal component that described the largest amount of variation. A generalised linear model (GLM) was fitted to the first

component of the PCA, using the same model averaging technique and predictors described for contaminant concentrations. The same methodology was used to assess accumulation patterns for PCBs grouped by their degree of chlorination.

2.3.4 Calculation of critical risk quotients

Where thresholds for toxicity in marine mammals existed (PCBs & PBDEs), toxicity was assessed by assessing pollutant concentrations in all individuals of all eleven species in relation to these thresholds (Hall et al., 2003; Kannan et al., 2000). To compare the relative risks of exposure from each pollutant class, comparative risk quotients (CRQs) were derived for each contaminant and taxon (n=1077), using the method outlined by Mos et al. (2010). Sperm whales were excluded from this comparative analysis as PBDEs were not analysed in this species (n=6). Individuals were also excluded from this analysis if complete contaminant data was unavailable, this resulted in a sample size of n=858. CRQs are not absolute measures of risk but are relative values that can be used to assess the risk of a contaminant relative to others and allow for contaminants to be prioritised according to risk. Mice and rats were chosen as the reference organisms for toxicity because toxicity reference values (TRVs) do not exist for marine mammals but the primary mechanisms of toxicity of persistent organic pollutants have been shown to be similar among mammals (Ross, 2000). TRVs were taken from the US Agency for Toxic Substances and Disease Registry (ATSDR) Toxicological Profiles data set (Agency for Toxic Substances and Disease Registry, 2007). CRQs were derived in relation to endocrine disruption and immunosuppression and calculated by dividing tissue concentrations by TRVs for no observed adverse effects levels (NOAELs) for oral intakes in mice. Where data was not available for mice, the NOAELs in rats were used (Mos et al., 2010). The TRVs used to calculate CRQs are shown in Table 2-4.

*Table 2-4: Toxicity reference values (TRVs) used to derive Comparative Risk Quotients (CRQs) for each contaminant. *TRVs were taken from (Agency for Toxic Substances and Disease Registry, 2007)*

Contaminant	**Toxicity reference values* (mg kg−1 day−1))**	
	Immunotoxicity	Endocrine disruption
PCBs	0.50	4.00
Dieldrin	0.65	0.50
DDTs	10.50	5.40
HCB	22.00	9.50
HCHs	20.00	0.50
PBDEs	0.13	10.00

2.4 Results

2.4.1 Contaminant Concentrations

Analysis of pollutant concentrations over time shows that burdens of pollutants in marine mammals in the North-East Atlantic have declined over the last three decades, across all species. However, there is considerable variation in pollutant concentrations and rates of decline among taxa and geographical regions (Figure 2-2 A,B). Of all the pollutants analysed, PCBs are declining at the slowest rate and present in the highest concentrations across all species. In the most recent five years of the analysis (2014-2018) PCB burdens in 48% (88/184) of individuals, exceed the accepted threshold for the onset of immunological effects in marine mammals (Table 2-2, Table 2-5). The mean and maximum concentrations of each pollutant class and species are shown in Table 2-5. Killer whales have the highest mean concentration of pollutants (657 mg/kg lipid), two orders of magnitude greater than grey seals, which have the lowest mean concentration (8 mg/kg lipid) (Figure 2-3). Mean concentrations of PBDEs exceed the only published threshold for toxic effects (1.5 mg/kg lipid total PBDEs (Hall et al., 2003)) in killer whales, bottlenose dolphins and white-beaked dolphins (Table 2-2).

Table 2-5: Sample size, mean and maximum concentrations of each persistent organic pollutant in each species. The bold number represents the sample size included in the statistical models.

	Pollutant class and lipid content							
	N(sample size) *bold number indicates sample size included in models							
	LIPID	PCBs	DDTs	DDT/DDE	Dieldrin	PBDEs	HCHs	HCB
Atlantic white-sided dolphin	23	22 **(11)**	12 **(11)**	12 **(11)**	12 **(11)**	11	12 **(11)**	22 **(11)**
Bottlenose dolphin	63	63 **(28)**	33 **(28)**	33 **(28)**	33 **(28)**	28	33 **(28)**	63 **(28)**
Common seal	16	16	9	9	9	9	9	16
Grey seal	21	21	7	7	7	7	7	21
Harbour porpoise	784	731 **(604)**	658 **(604)**	657 **(604)**	658 **(604)**	420	658 **(604)**	736 **(604)**
Killer whale	15	15	10	10	10	8	10	15
Risso's dolphin	26	26 **(14)**	18 **(14)**	18 **(14)**	18 **(14)**	15	18 **(14)**	26 **(14)**
Short-beaked common dolphin	125	124 **(63)**	80 **(63)**	80 **(63)**	80 **(63)**	10	80 **(63)**	125 **(63)**
Sperm whale	6	6	6	6	6	0	6	6
Striped dolphin	24	22 **(11)**	12 **(11)**	12 **(11)**	12 **(11)**	11	12 **(11)**	23 **(11)**
White-beaked dolphin	25	24 **(14)**	15 **(14)**	15 **(14)**	15 **(14)**	14	15 **(14)**	24 **(14)**
	Mean blubber concentration (mg/kg lipid)							
	LIPID	PCBs	DDTs	DDT/DDE	Dieldrin	PBDEs	HCHs	HCB
Atlantic white-sided dolphin	76.67	11.9	11.04	0.84	0.89	0.38	0.11	0.37
Bottlenose dolphin	56.25	75.85	25.47	0.81	0.66	3.01	0.05	0.39

Common seal	75.98	18.61	1.61	0.91	0.03	0.18	0.03	0.01
Grey seal	77.75	8.17	1.38	0.88	0.04	0.08	0.03	0.01
Harbour porpoise	83.56	16.31	3.45	0.62	0.78	0.9	0.1	0.24
Killer whale	51.05	263.81	297.38	0.91	20.62	8.41	0.17	2.24
Risso's dolphin	63.34	8.46	2.24	0.79	0.22	0.58	0.04	0.25
Short-beaked common dolphin	77.13	31.27	5.45	0.68	0.48	0.59	0.13	0.17
Sperm whale	51.42	6.93	7.81	0.82	0.06	NA	0.02	0.4
Striped dolphin	71.37	37.19	17.69	0.84	0.55	0.51	0.07	0.3
White-beaked dolphin	76.56	26.9	12.26	0.86	0.89	3.06	0.07	0.47

Maximum blubber concentration (mg/kg lipid)

	LIPID	PCBs	DDTs	DDT/DDE	Dieldrin	PBDEs	HCHs	HCB
Atlantic white-sided dolphin	91.33	54.87	31.03	0.93	7.5	0.61	0.9	1.19
Bottlenose dolphin	89.6	697.99	219.02	0.97	3.94	15.37	0.12	1.44
Common seal	91.05	158.45	4.65	0.93	0.08	0.8	0.05	0.03
Grey seal	97.4	34.22	4.67	0.95	0.12	0.14	0.04	0.05
Harbour porpoise	100	159.68	42.78	0.97	13.41	15.65	2.02	1.88
Killer whale	73.72	956.37	1203.41	0.98	88.03	25.53	0.58	8.63
Risso's dolphin	88	31.3	15.61	0.9	1.09	2.53	0.1	1.08
Short-beaked common dolphin	92	225.1	33.49	0.92	6.71	1.46	0.9	0.81

Sperm whale	59.6	11.98	17.74	0.86	0.11	NA	0.02	0.63
Striped dolphin	92.41	183.61	99.28	0.96	2.82	1.94	0.37	0.98
White-beaked dolphin	93.24	124.35	51.13	0.96	5.14	12.63	0.27	0.85

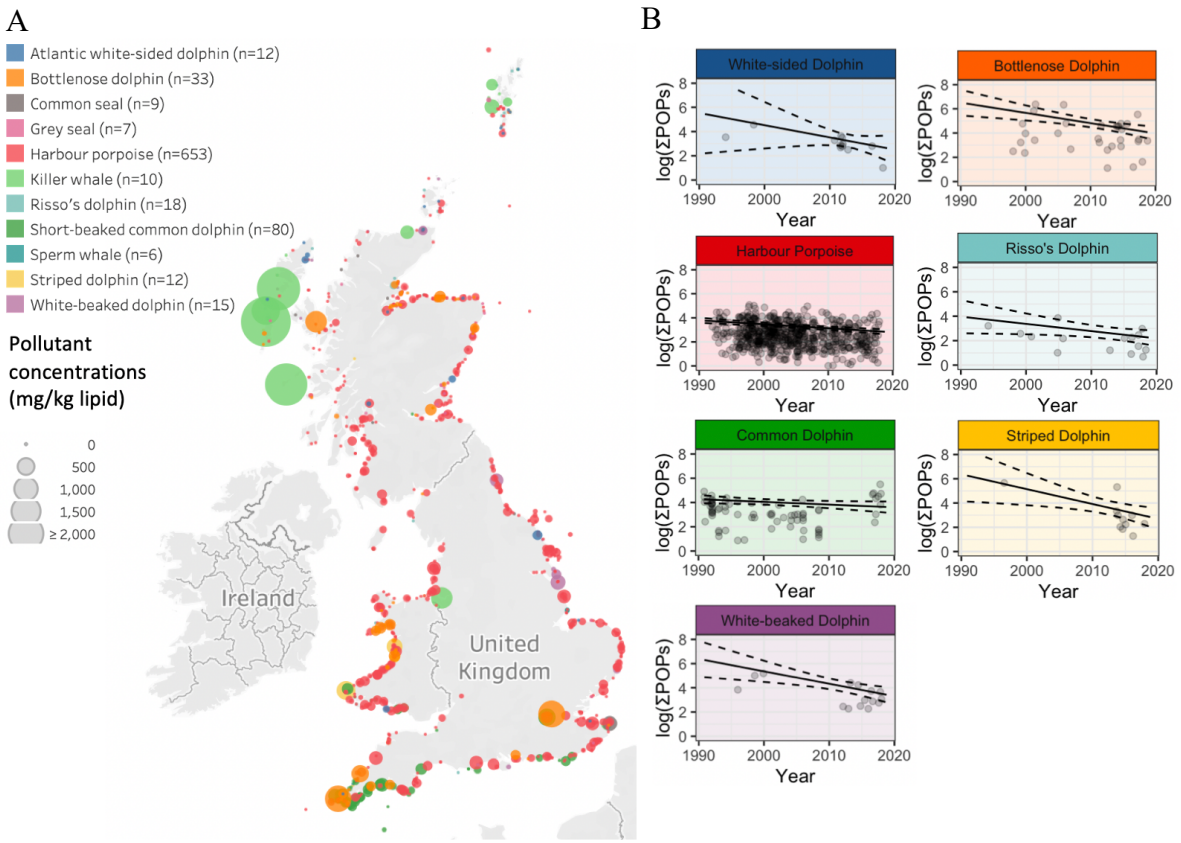


Figure 2-2: Spatiotemporal trends of measured and modelled pollutant concentrations. (A) Geographic locations of the stranded individuals that were analysed to obtain pollutant blubber concentrations (HCB, Dieldrin, ΣDDTs, ΣCBs, ΣHCHs). The colours of the dots represent the different species and the dots are sized by the summed blubber concentrations of pollutants. (B) Modelled temporal trend in summed pollutant concentrations (ΣPOPs) for each species. The solid lines represent the model predictions for each year and the dashed lines represent 95% confidence intervals (twice the standard error). The dots show the measured pollutant concentrations.

Table 2-6: Summary statistics of the averaged linear model fitted to the strandings data with the natural log of total pollutant concentrations as the response variable. The continuous variables were zero centred and scaled. Coefficient estimates were calculated relative to a female adult bottlenose dolphin.

Model term	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)
(Intercept)	3.76	0.24	0.24	15.87	0.00
Juvenile	0.56	0.09	0.09	6.51	0.00
Neonate	0.08	0.23	0.23	0.33	0.74
Blubber Thickness	-0.32	0.03	0.03	10.65	0.00
Latitude	-0.46	0.15	0.15	3.02	0.00
Longitude	0.02	0.03	0.03	0.53	0.60
Number of days (NOD) since 1/1/90	-0.70	0.21	0.21	3.39	0.00
Male	1.43	0.09	0.09	16.00	0.00
Common dolphin	-1.87	0.41	0.41	4.59	0.00
Harbour porpoise	-1.83	0.23	0.23	7.78	0.00
Risso's dolphin	-1.97	0.39	0.39	5.10	0.00
Striped dolphin	-0.69	0.52	0.52	1.34	0.18
White-beaked dolphin	-0.35	0.39	0.39	0.90	0.37
White-sided dolphin	-1.04	0.64	0.64	1.63	0.10
Juvenile:Male	-1.47	0.12	0.12	12.23	0.00
Neonate:Male	-1.39	0.29	0.29	4.81	0.00
Latitude:Longitude	-0.04	0.04	0.04	1.06	0.29
Longitude:NOD	-0.08	0.03	0.03	2.69	0.01
NOD:Common dolphin	0.52	0.23	0.23	2.23	0.03
NOD:Harbour porpoise	0.46	0.21	0.21	2.17	0.03
NOD:Risso's dolphin	0.31	0.30	0.30	1.03	0.30
NOD:Striped dolphin	-0.22	0.36	0.36	0.60	0.55
NOD:White-beaked dolphin	0.10	0.31	0.31	0.34	0.74
NOD:White-sided dolphin	-0.04	0.50	0.50	0.08	0.93
Latitude:Common dolphin	-0.05	0.23	0.23	0.20	0.84
Latitude:Harbour porpoise	0.09	0.15	0.15	0.63	0.53
Latitude:Risso's dolphin	0.20	0.28	0.28	0.71	0.48
Latitude:Striped dolphin	0.29	0.38	0.38	0.76	0.44
Latitude:White-beaked dolphin	0.12	0.24	0.24	0.49	0.62
Latitude:White-sided dolphin	0.35	0.60	0.60	0.58	0.56
Latitude:NOD	0.02	0.03	0.03	0.55	0.58
Latitude:Longitude:NOD	0.01	0.02	0.02	0.31	0.76

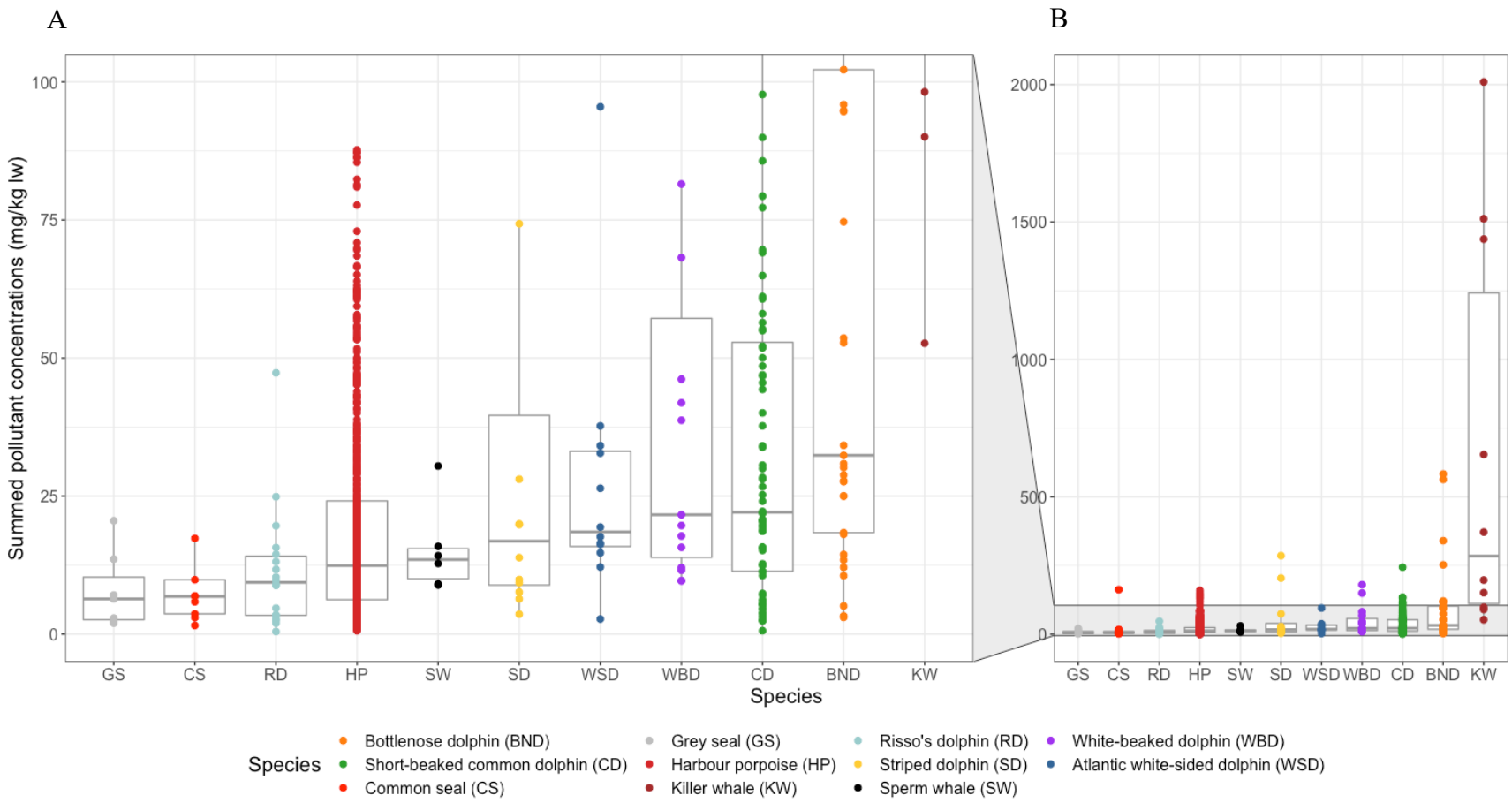


Figure 2-3: Total pollutant concentrations (mg/kg lipid) (HCB, dieldrin, DDTs, CBs, HCHs) in the blubber samples for each species. (A) Shortened scale for contaminant concentrations (0-100 mg/kg lipid) omitting some data points for ease of reading (B) Full scale for contaminant concentrations (0-2000 mg/kg lipid). The horizontal lines represent the median value. The lower and upper hinges correspond to the first and third quartiles. The upper whisker extends from the upper hinge to the largest value unless the largest value is greater than 1.5 times the interquartile range (IQR) in which case the upper whisker is limited at 1.5 x IQR. The lower whisker extends from the lower hinge to the smallest value unless the smallest value is greater than 1.5 times the interquartile range (IQR) in which case the lower whisker is limited at 1.5 x IQR. Data beyond the end of the whiskers are outliers and are plotted individually.

Bottlenose dolphins have the highest modelled concentrations across all pollutant classes (Figure 2-2B, Table 2-5), with the exception of HCB. However, it is important to note that killer whales have a far higher mean concentration of POPs than all other species (Figure 2-3) but their low sample size and high variance meant they were excluded from the modelling analyses to preserve statistical robustness. All species showed the same pattern of exposure for age class and sex: adult males > juveniles > neonates > adult females. Modelled rates of decline are significantly slower in harbour porpoises (*Phocoena phocoena*) and common dolphins (*Delphinus delphis*) (GLM, $p<0.05$) than other species, across all contaminants, with the exception of HCH (Tables 2-7, 2-8, 2-9, 2-10, 2-11, Figures 2-5, 2-6, 2-7, 2-8, 2-9, 2-10).

Summed pollutant concentrations are highest at lower latitudes and the rate of decline is negatively associated with longitude such that levels are declining at a faster rate on the North Sea coast of the UK compared to the Atlantic seaboard. White-beaked and bottlenose dolphins within the English Channel, the Irish Sea and Southern North Sea OSPAR (Oslo and Paris Conventions) assessment areas face a substantial threat as modelled mean concentrations exceed the highest known threshold for toxic effects in marine mammals (Kannan et al., 2000) (Figure 2-4B).

Of the pollutant classes modelled, PCBs are declining at the slowest rate and have the greatest latitudinal concentration gradient whereby, concentrations are higher further south. (Figure 2-6, Figure 2-10, Table 2-8). In contrast, DDTs are declining at a rate almost twice that of PCBs and concentrations are not significantly associated with latitude. Instead, they exhibit a longitudinal gradient whereby, concentrations are higher on the west coast (Figure 2-4C, Table 2-7). With the exception of PCBs, the spatial distributions of all pollutants have shifted longitudinally over time across all species. In 1990, concentrations are highest in eastern regions whilst, in the more recent years of the study, concentrations are highest at western longitudes (Figure 2-4C).

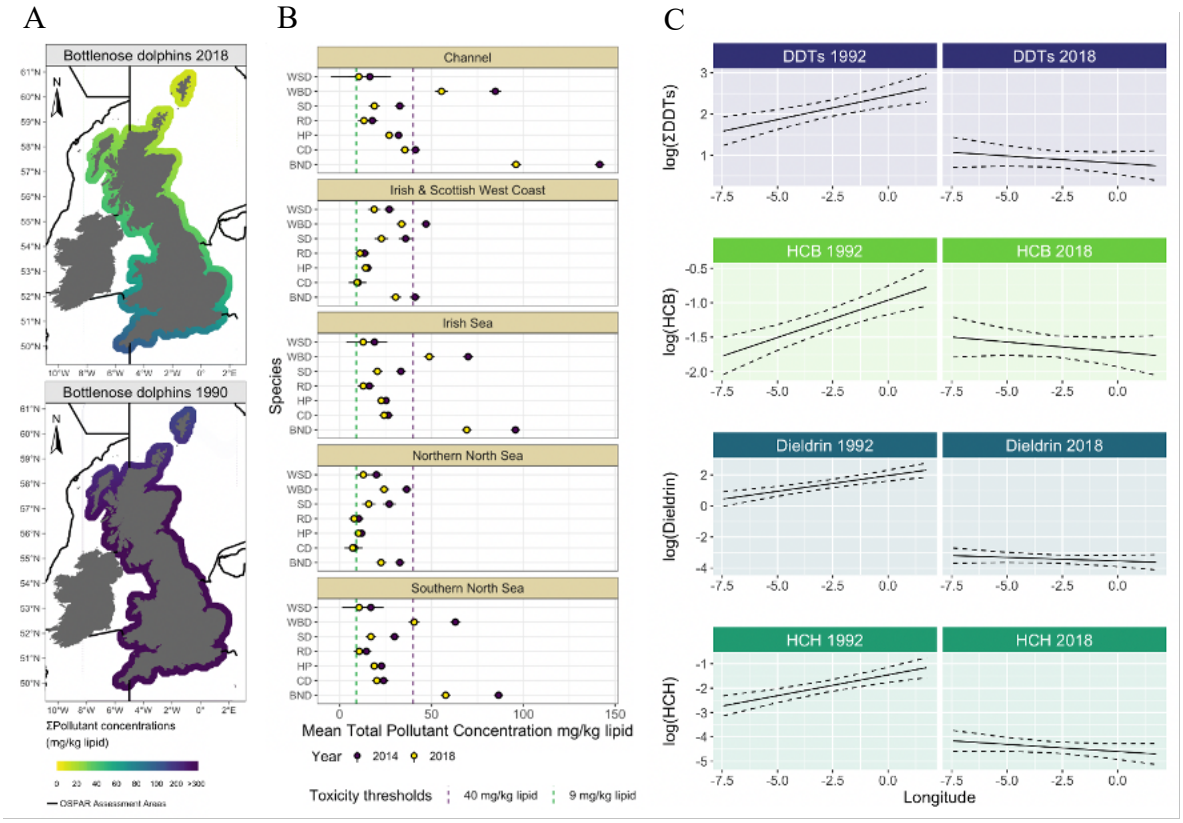


Figure 2-4: Modelled pollutant concentrations (A) Modelled spatial distribution of summed pollutant concentrations along the UK coast in adult male bottlenose dolphins (BND) in 2018. (B) Modelled mean PCB concentrations for each species in each OSPAR assessment area in 2014 and 2018. The horizontal bars represent twice the standard error. (C) Modelled latitudinal trends in concentrations for 1992 and 2018 for each pollutant class. Only significant pollutant classes have been included (GLM, $p<0.05$). The dotted lines represent twice the standard error.

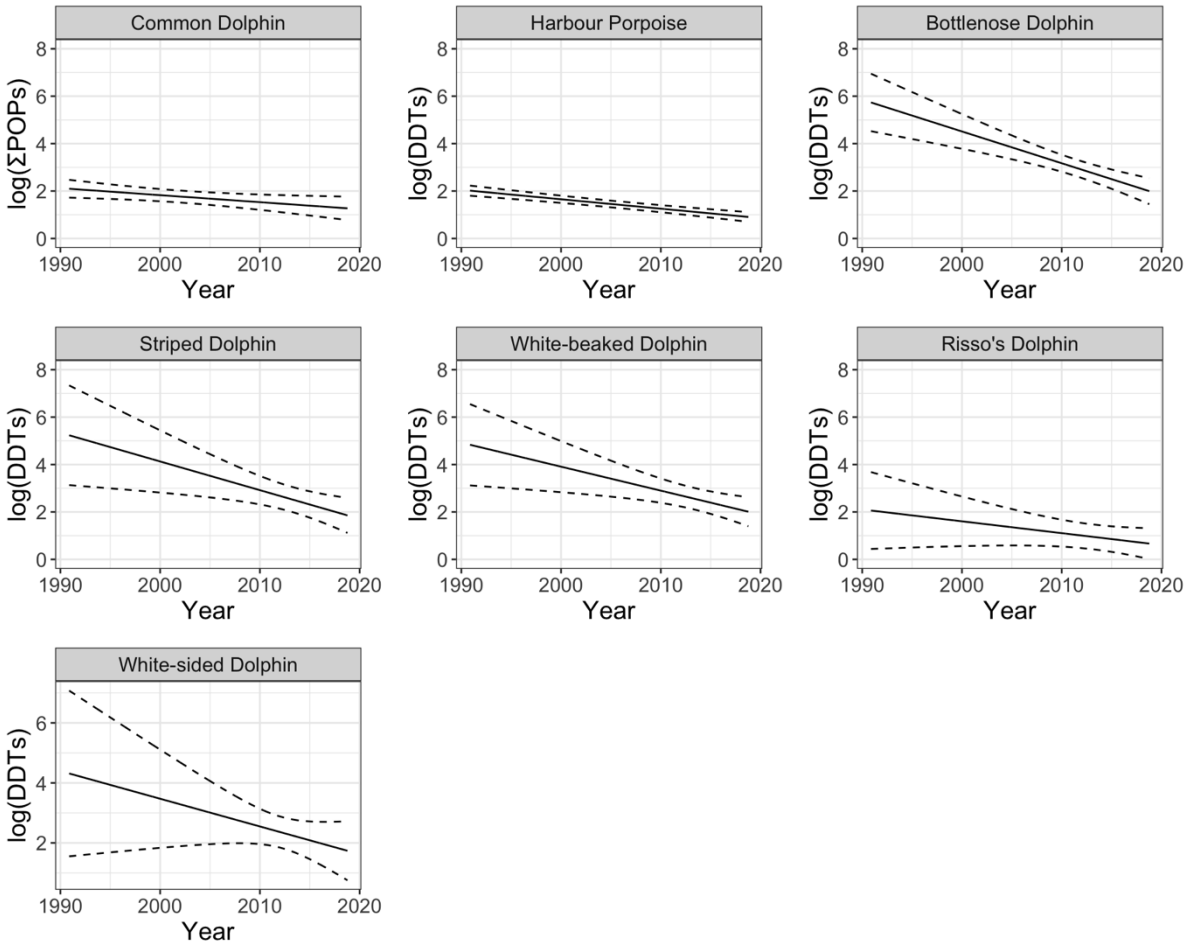


Figure 2-5: Model predictions for the temporal trend of the log of ΣDDT concentrations (mg/kg lipid) for each species. The solid lines represent the model predictions for each year and the dashed lines represent 95% confidence intervals (twice the standard error).

Table 2-7: Summary statistics of the averaged linear model fitted to the strandings data. The natural log of ΣDDT concentrations (mg/kg lipid) was the response variable. The continuous variables were zero centred and scaled. Coefficient estimates were calculated based on a female adult bottlenose dolphin.

	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr(>|z|)**
(Intercept)	2.27	0.24	0.24	9.56	0.00
Juvenile	0.72	0.09	0.09	7.97	0.00
Neonate	0.19	0.24	0.24	0.77	0.44
Blubber Thickness	-0.36	0.03	0.03	11.49	0.00
Latitude	-0.13	0.14	0.14	0.90	0.37
Longitude	0.10	0.03	0.03	2.93	0.00
Number of days (NOD) since 1/1/90	-1.00	0.20	0.20	5.00	0.00
Male	1.52	0.09	0.09	16.39	0.00
Common dolphin	-2.24	0.47	0.47	4.73	0.00
Harbour porpoise	-2.28	0.23	0.23	9.71	0.00
Risso's dolphin	-2.62	0.40	0.40	6.50	0.00
Striped dolphin	-0.04	0.51	0.51	0.08	0.94
White-beaked dolphin	-0.35	0.41	0.41	0.84	0.40
White-sided dolphin	-0.85	0.60	0.60	1.41	0.16
Juvenile:Male	-1.54	0.13	0.13	12.27	0.00
Neonate:Male	-1.42	0.30	0.30	4.70	0.00
Latitude:Longitude	-0.11	0.04	0.04	3.02	0.00
Longitude:NOD	-0.10	0.03	0.03	3.09	0.00
NOD:Common dolphin	0.71	0.22	0.22	3.19	0.00
NOD:Harbour porpoise	0.70	0.20	0.20	3.43	0.00
NOD:Risso's dolphin	0.59	0.31	0.32	1.87	0.06
NOD:Striped dolphin	0.06	0.38	0.38	0.15	0.88
NOD:White-beaked dolphin	0.32	0.33	0.33	0.99	0.32
NOD:White-sided dolphin	0.32	0.50	0.50	0.65	0.52
Latitude:Common dolphin	-0.12	0.27	0.27	0.45	0.66
Latitude:Harbour porpoise	0.08	0.15	0.15	0.53	0.59
Latitude:Risso's dolphin	0.19	0.31	0.31	0.62	0.54
Latitude:Striped dolphin	0.13	0.24	0.24	0.53	0.60
Latitude:White-beaked dolphin	0.01	0.18	0.18	0.08	0.94
Latitude:White-sided dolphin	0.16	0.47	0.47	0.35	0.73
Latitude:NOD	0.01	0.02	0.02	0.34	0.73
Latitude:Longitude:NOD	0.01	0.02	0.02	0.40	0.69

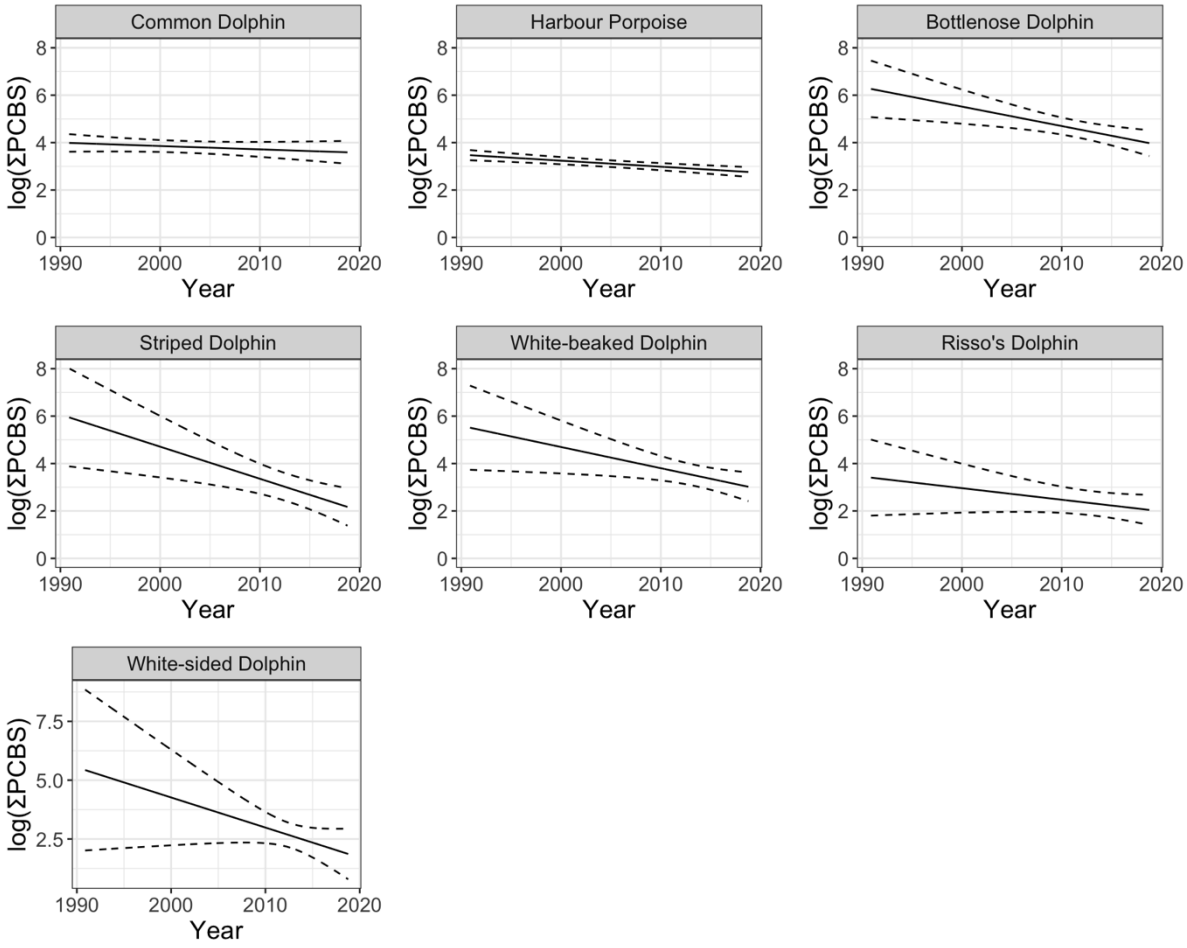


Figure 2-6: Model predictions for the temporal trend of the log of ΣPCB concentrations (mg/kg lipid) for each species. The solid lines represent the model predictions for each year and the dashed lines represent 95% confidence intervals (twice the standard error).

Table 2-8: Summary statistics of the averaged linear model fitted to the strandings data. The natural log of ΣPCB concentrations (mg/kg lipid) was the response variable. The continuous variables were zero centred and scaled. Coefficient estimates were calculated based on a female adult bottlenose dolphin.

	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr(>|z|)**
(Intercept)	3.43	0.23	0.23	14.61	0.00
Juvenile	0.51	0.09	0.09	5.76	0.00
Neonate	0.04	0.24	0.24	0.15	0.88
Blubber Thickness	-0.31	0.03	0.03	10.01	0.00
Latitude	-0.65	0.18	0.18	3.62	0.00
Longitude	0.00	0.03	0.03	0.15	0.88
Number of days (NOD) since 1/1/90	-0.60	0.20	0.20	3.07	0.00
Male	1.39	0.09	0.09	15.01	0.00
Common dolphin	-1.88	0.44	0.44	4.23	0.00
Harbour porpoise	-1.73	0.23	0.23	7.46	0.00
Risso's dolphin	-1.88	0.40	0.40	4.72	0.00
Striped dolphin	-0.76	0.54	0.54	1.42	0.15
White-beaked dolphin	-0.33	0.41	0.41	0.80	0.42
White-sided dolphin	-1.39	0.75	0.75	1.85	0.06
Juvenile:Male	-1.45	0.12	0.12	11.66	0.00
Neonate:Male	-1.36	0.30	0.30	4.59	0.00
Latitude:Common dolphin	-0.01	0.31	0.31	0.03	0.97
Latitude:Harbour porpoise	0.20	0.18	0.18	1.10	0.27
Latitude:Risso's dolphin	0.40	0.31	0.31	1.27	0.20
Latitude:Striped dolphin	0.62	0.42	0.42	1.46	0.14
Latitude:White-beaked dolphin	0.30	0.32	0.32	0.94	0.35
Latitude:White-sided dolphin	0.76	0.75	0.75	1.02	0.31
Longitude:NOD	-0.06	0.04	0.04	1.39	0.16
NOD:Common dolphin	0.47	0.22	0.22	2.17	0.03
NOD:Harbour porpoise	0.41	0.20	0.20	2.07	0.04
NOD:Risso's dolphin	0.21	0.31	0.31	0.67	0.50
NOD:Striped dolphin	-0.38	0.38	0.38	1.02	0.31
NOD:White-beaked dolphin	-0.02	0.33	0.33	0.05	0.96
NOD:White-sided dolphin	-0.34	0.58	0.58	0.58	0.56
Latitude:Longitude	-0.02	0.04	0.04	0.66	0.51
Latitude:NOD	0.02	0.03	0.03	0.62	0.54
Latitude:Longitude:NOD	0.00	0.02	0.02	0.27	0.79

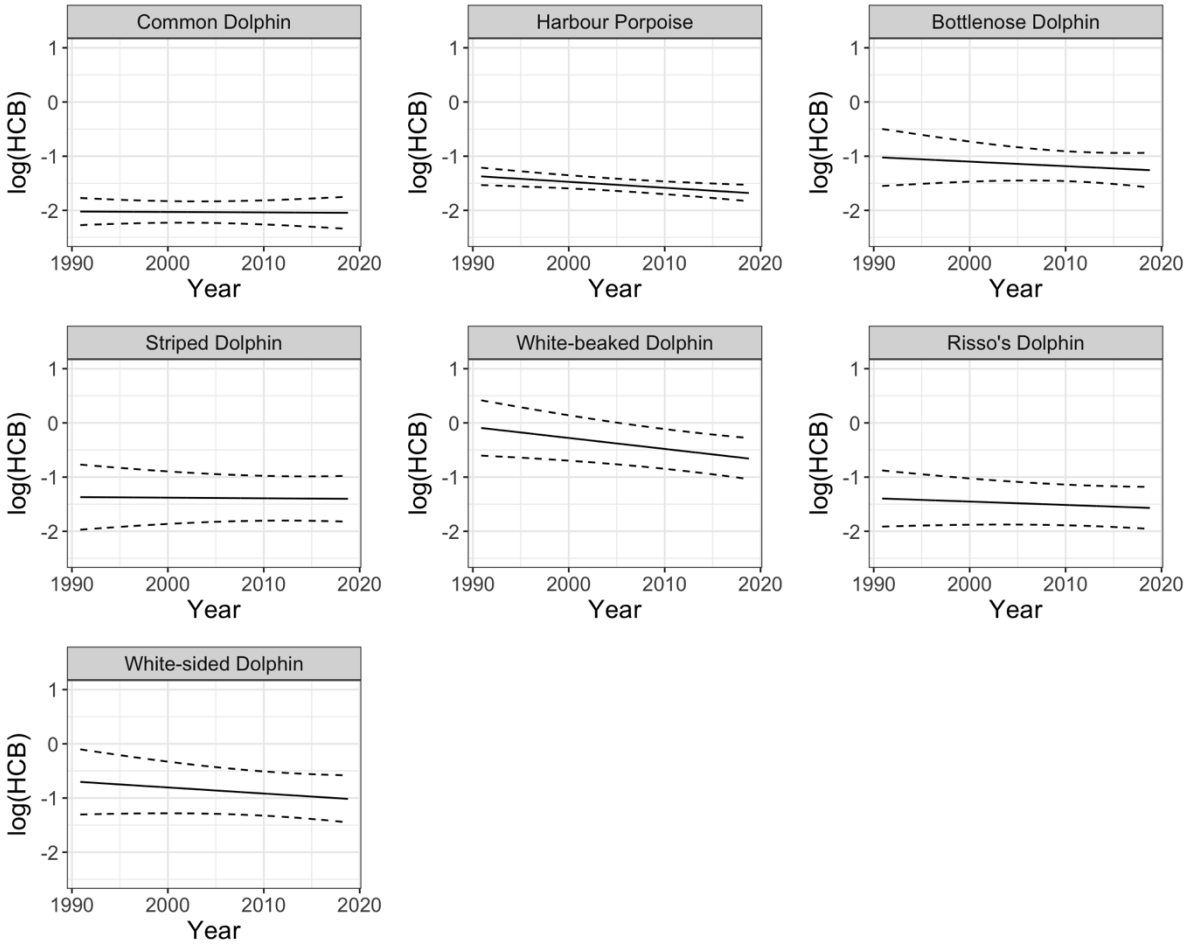


Figure 2-7: Model predictions for the temporal trend of the log of HCB concentrations (mg/kg lipid) for each species. The solid lines represent the model predictions for each year and the dashed lines represent 95% confidence intervals (twice the standard error).

Table 2-9: Summary statistics of the averaged linear model fitted to the strandings data. The natural log of HCB concentrations (mg/kg lipid) was the response variable. The continuous variables were zero centred and scaled. Coefficient estimates were calculated based on a female adult bottlenose dolphin.

	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr(>|z|)**
(Intercept)	-2.25	0.15	0.15	14.89	0.00
Juvenile	1.22	0.07	0.07	16.89	0.00
Neonate	0.80	0.19	0.19	4.14	0.00
Blubber Thickness	-0.31	0.02	0.02	12.53	0.00
Latitude	0.05	0.03	0.03	1.83	0.07
Longitude	0.08	0.03	0.03	3.14	0.00
Number of days (NOD) since 1/1/90	-0.11	0.08	0.08	1.46	0.15
Male	1.15	0.07	0.07	15.69	0.00
Common dolphin	-0.76	0.17	0.17	4.36	0.00
Harbour porpoise	-0.36	0.15	0.15	2.49	0.01
Risso's dolphin	-0.49	0.24	0.24	2.06	0.04
Striped dolphin	-0.05	0.26	0.26	0.20	0.84
White-beaked dolphin	0.63	0.23	0.23	2.76	0.01
White-sided dolphin	0.18	0.26	0.26	0.69	0.49
Juvenile:Male	-1.21	0.10	0.10	12.00	0.00
Neonate:Male	-1.08	0.24	0.24	4.45	0.00
Latitude:Longitude	-0.03	0.03	0.03	0.97	0.33
Longitude:NOD	-0.09	0.03	0.03	3.67	0.00
Latitude:NOD	0.00	0.01	0.01	0.02	0.98
Latitude:Longitude:NOD	0.00	0.02	0.02	0.31	0.76
NOD:Common dolphin	0.02	0.10	0.10	0.21	0.84
NOD:Harbour porpoise	0.01	0.07	0.07	0.19	0.85
NOD:Risso's dolphin	0.02	0.10	0.10	0.19	0.85
NOD:Striped dolphin	0.02	0.11	0.11	0.18	0.86
NOD:White-beaked dolphin	0.01	0.07	0.07	0.13	0.90
NOD:White-sided dolphin	0.02	0.11	0.11	0.16	0.87

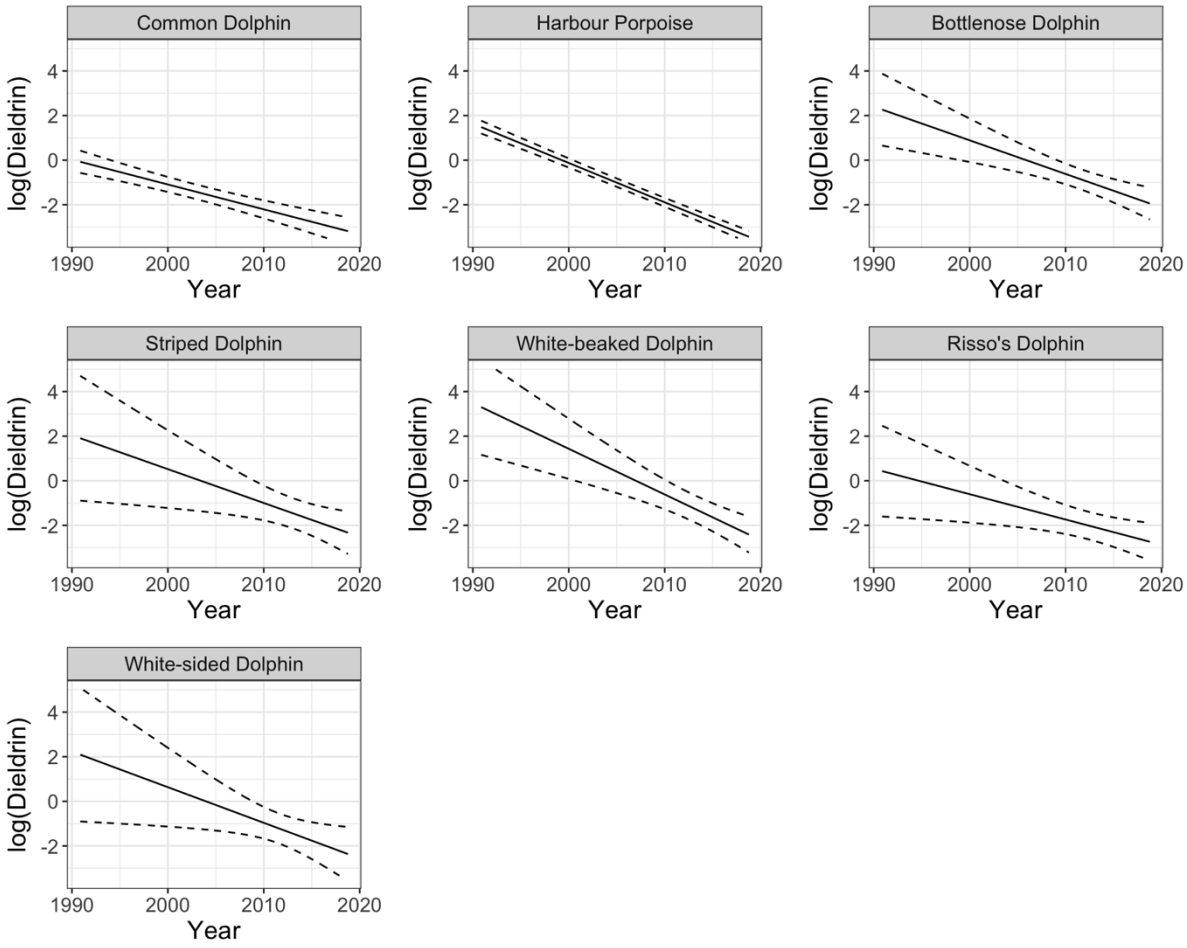


Figure 2-8: Model predictions for the temporal trend of the log of dieldrin concentrations (mg/kg lipid) for each species. The solid lines represent the model predictions for each year and the dashed lines represent 95% confidence intervals (twice the standard error).

Table 2-10: Summary statistics of the averaged linear model fitted to the strandings data. The natural log of total dieldrin concentrations(mg/kg lipid) was the response variable. The continuous variables were zero centred and scaled. Coefficient estimates were calculated based on a female adult bottlenose dolphin.

	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr(>|z|)**
(Intercept)	-1.64	0.31	0.32	5.22	0.00
Juvenile	0.89	0.12	0.12	7.39	0.00
Neonate	0.76	0.32	0.32	2.36	0.02
Blubber Thickness	-0.38	0.04	0.04	9.07	0.00
Latitude	-0.20	0.05	0.05	4.42	0.00
Longitude	0.17	0.04	0.04	3.91	0.00
Number of days (NOD) since 1/1/90	-1.07	0.27	0.27	4.02	0.00
Male	1.72	0.12	0.12	13.94	0.00
Common dolphin	-1.82	0.35	0.35	5.17	0.00
Harbour porpoise	-1.10	0.31	0.31	3.52	0.00
Risso's dolphin	-1.05	0.53	0.53	1.99	0.05
Striped dolphin	-0.29	0.66	0.66	0.43	0.67
White-beaked dolphin	0.33	0.55	0.55	0.61	0.54
White-sided dolphin	0.07	0.63	0.63	0.10	0.92
Juvenile:Male	-1.83	0.17	0.17	10.97	0.00
Neonate:Male	-1.90	0.40	0.40	4.73	0.00
Latitude:Longitude	-0.06	0.06	0.06	1.08	0.28
Latitude:NOD	0.20	0.04	0.04	4.54	0.00
Longitude:NOD	-0.15	0.04	0.04	3.44	0.00
NOD:Common dolphin	0.43	0.30	0.30	1.45	0.15
NOD:Harbour porpoise	-0.17	0.27	0.27	0.64	0.52
NOD:Risso's dolphin	-0.07	0.41	0.41	0.16	0.87
NOD:Striped dolphin	0.08	0.51	0.51	0.16	0.87
NOD:White-beaked dolphin	-0.46	0.42	0.42	1.08	0.28
NOD:White-sided dolphin	-0.40	0.57	0.57	0.70	0.48
Latitude:Longitude:NOD	-0.01	0.03	0.03	0.36	0.72

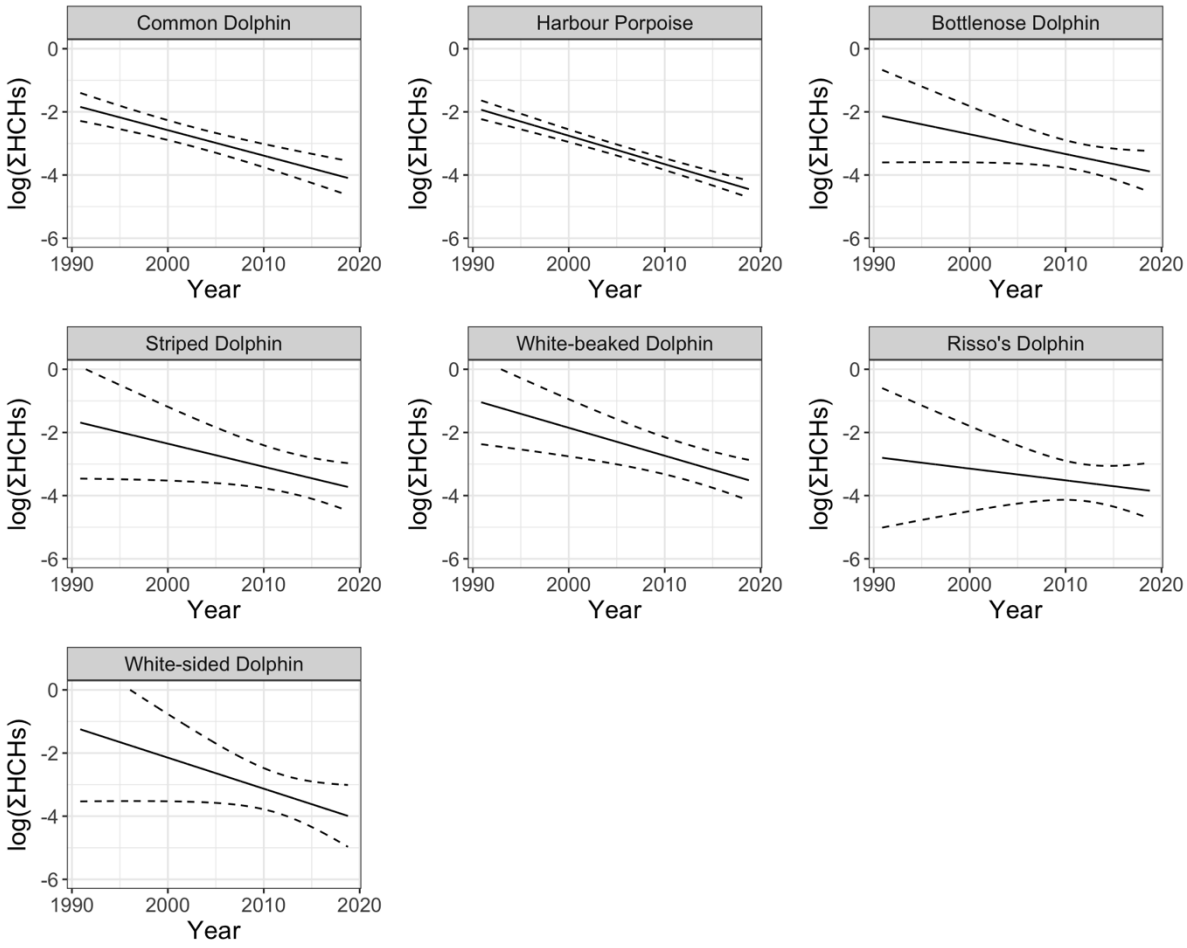


Figure 2-9: Model predictions for the temporal trend of the log of ΣHCH concentrations (mg/kg lipid) for each species. The solid lines represent the model predictions for each year and the dashed lines represent 95% confidence intervals (twice the standard error).

Table 2-11: Summary statistics of the averaged linear model fitted to the strandings data. The natural log of ΣHCH concentrations (mg/kg lipid) was the response variable. The continuous variables were zero centred and scaled. Coefficient estimates were calculated based on a female adult bottlenose dolphin.

	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr(>|z|)**
(Intercept)	-3.46	0.29	0.29	12.01	0.00
Juvenile	0.63	0.11	0.11	5.59	0.00
Neonate	0.49	0.30	0.30	1.60	0.11
Blubber Thickness	-0.10	0.04	0.04	2.64	0.01
Latitude	0.01	0.04	0.04	0.25	0.80
Longitude	0.10	0.04	0.04	2.58	0.01
Number of days (NOD) since 1/1/90	-0.48	0.25	0.25	1.89	0.06
Male	0.46	0.12	0.12	3.99	0.00
Common dolphin	0.09	0.33	0.33	0.26	0.80
Harbour porpoise	-0.21	0.29	0.29	0.72	0.47
Risso's dolphin	-0.33	0.42	0.42	0.78	0.44
Striped dolphin	0.37	0.48	0.48	0.78	0.43
White-beaked dolphin	0.61	0.43	0.44	1.40	0.16
White-sided dolphin	0.33	0.60	0.60	0.54	0.59
Juvenile:Male	-0.63	0.16	0.16	4.06	0.00
Neonate:Male	0.18	0.38	0.38	0.48	0.63
Latitude:NOD	0.08	0.05	0.05	1.43	0.15
Longitude:NOD	-0.12	0.04	0.04	2.88	0.00
NOD:Common dolphin	-0.09	0.20	0.20	0.43	0.66
NOD:Harbour porpoise	-0.17	0.27	0.27	0.62	0.53
NOD:Risso's dolphin	0.05	0.24	0.24	0.22	0.83
NOD:Striped dolphin	-0.06	0.30	0.30	0.20	0.84
NOD:White-beaked dolphin	-0.15	0.31	0.31	0.48	0.63
NOD:White-sided dolphin	-0.36	0.58	0.58	0.62	0.53
Latitude:Longitude	-0.01	0.03	0.03	0.21	0.83
Latitude:Longitude:NOD	0.00	0.02	0.02	0.20	0.84

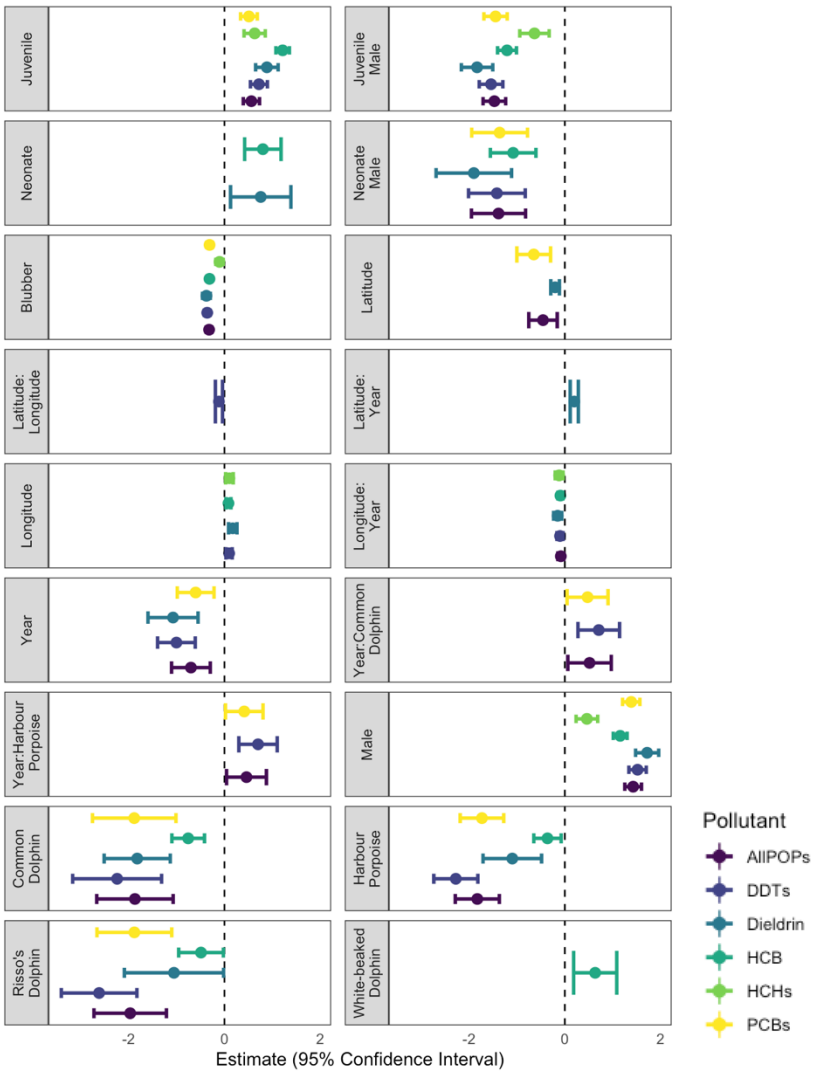
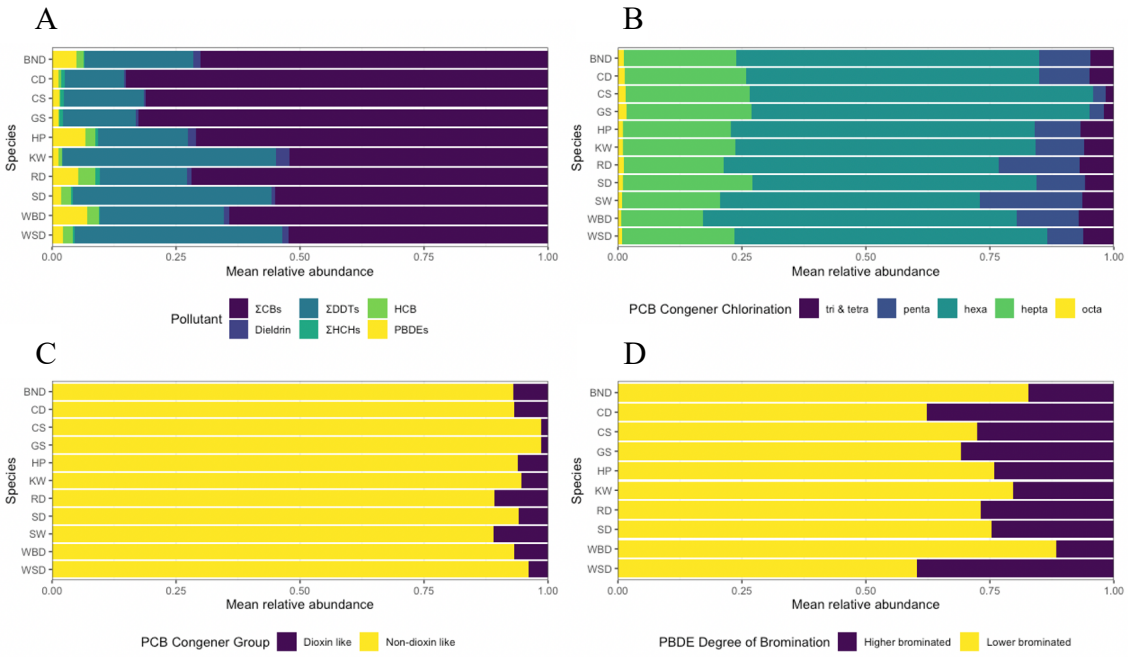


Figure 2-10: Forest plot of the averaged model coefficients for each pollutant class. The dots represent the mean coefficient estimates from the model, the upper and lower limits represent the 95% confidence intervals. Only significant terms have been included (GLM, $p<0.05$)

2.4.2 Relative Abundance of Pollutants

Relative abundances of pollutants and congeners are highly variable between species, however, the three most abundant pollutants follow the same trend in all but one of the taxa I examined: PCBs > DDTs (pp'DDE> pp'DDT) > PBDEs (Table 2-12, Figure 2-11A). The remaining pollutants (dieldrin, HCHs and HCB) each contributed less than 3% to the overall burden. The contribution of PCBs to overall burden is highest in grey seals (84%), and lowest in sperm whales (48%). In all

of the taxa I investigated, CB153 is the most abundant PCB compound. The median contribution of lower (*tri-, tetra- and penta-*) chlorinated PCBs is significantly lower in pinnipeds compared with odontocetes (Kruskal-Wallis, $\chi 2$ (1, N=1128)=159, p<2.2e-16, Dunn test, z=12.6, p=0) (Figure 2-11B). BDE47 was the most abundant PBDE congener and the profiles, across all taxa, were dominated by lower brominated PBDEs (Figure 2-11D). Analysis revealed that relative abundances of pollutants are predominantly influenced by taxa, age class and latitude and that, in contrast to pollutant concentrations, sex and body condition are not significant predictors (Table 2-11).



Species Code	BND	CD	HP	RD	SD	WBD	WSD
Species	Bottlenose dolphin	Common dolphin	Harbour porpoise	Risso's dolphin	Striped dolphin	White-beaked dolphin	White-sided dolphin

Figure 2-11: Mean relative abundance plots for each species (A) Pollutant class (B) Polychlorinated biphenyls congeners grouped by chlorination group (C) Polychlorinated biphenyls congeners grouped by dioxin or non-dioxin group (D) Polybrominated diphenyl ethers congeners grouped by degree of bromination

Table 2-12: Mean relative abundances of the pollutant classes for each of the species

Species	Pollutant	mean
Bottlenose dolphin	PCBs	74.13
Bottlenose dolphin	Dieldrin	1.44
Bottlenose dolphin	DDTs	22.90
Bottlenose dolphin	HCHs	0.20
Bottlenose dolphin	HCB	1.33
Common dolphin	PCBs	84.69
Common dolphin	Dieldrin	1.18
Common dolphin	DDTs	12.96
Common dolphin	HCHs	0.60
Common dolphin	HCB	0.56
Common seal	PCBs	83.37
Common seal	Dieldrin	0.31
Common seal	DDTs	15.84
Common seal	HCHs	0.27
Common seal	HCB	0.21
Grey seal	PCBs	84.93
Grey seal	Dieldrin	0.56
Grey seal	DDTs	13.37
Grey seal	HCHs	0.90
Grey seal	HCB	0.24
Harbour porpoise	PCBs	75.11
Harbour porpoise	Dieldrin	3.37
Harbour porpoise	DDTs	18.85
Harbour porpoise	HCHs	0.72
Harbour porpoise	HCB	1.95
Killer whale	PCBs	51.11
Killer whale	Dieldrin	2.86
Killer whale	DDTs	45.18
Killer whale	HCHs	0.08
Killer whale	HCB	0.77

Risso's dolphin	PCBs	76.73
Risso's dolphin	Dieldrin	1.77
Risso's dolphin	DDTs	17.43
Risso's dolphin	HCHs	0.86
Risso's dolphin	HCB	3.20
Striped dolphin	PCBs	58.14
Striped dolphin	Dieldrin	0.91
Striped dolphin	DDTs	38.73
Striped dolphin	HCHs	0.36
Striped dolphin	HCB	1.86
Sperm whale	PCBs	47.83
Sperm whale	Dieldrin	0.43
Sperm whale	DDTs	48.90
Sperm whale	HCHs	0.15
Sperm whale	HCB	2.70
White-beaked dolphin	PCBs	69.24
White-beaked dolphin	Dieldrin	1.18
White-beaked dolphin	DDTs	27.12
White-beaked dolphin	HCHs	0.21
White-beaked dolphin	HCB	2.25
White-sided dolphin	PCBs	53.90
White-sided dolphin	Dieldrin	1.79
White-sided dolphin	DDTs	41.90
White-sided dolphin	HCHs	0.29
White-sided dolphin	HCB	2.12

Table 2-13: Summary statistics of the averaged linear model fitted to the first principal component (PC1) of the principal component analysis (PCA) carried out on all pollutant classes. PC1 was the response variable, the continuous variables were zero centred and scaled. Coefficient estimates were calculated based on an adult female bottlenose dolphin.

	Estimate	**Std. Error**	**Adjusted SE**	**z value**	**Pr(>|z|)**
(Intercept)	-1.06	0.30	0.30	3.54	0.00
Juvenile	0.85	0.09	0.09	9.45	0.00
Neonate	1.18	0.21	0.21	5.59	0.00
Latitude	1.05	0.06	0.06	18.58	0.00
Longitude	0.22	0.05	0.05	4.83	0.00
Number of days (NOD) since 1/1/90	-0.47	0.23	0.23	2.06	0.04
Male	0.26	0.34	0.35	0.76	0.45
Common dolphin	0.51	0.30	0.30	1.69	0.09
Harbour porpoise	0.39	0.52	0.52	0.75	0.46
Risso's dolphin	2.70	0.87	0.87	3.10	0.00
Striped dolphin	1.14	0.52	0.52	2.17	0.03
White-beaked dolphin	1.52	0.62	0.62	2.46	0.01
Latitude:Longitude	-0.27	0.06	0.06	4.76	0.00
Latitude:NOD	-0.14	0.05	0.05	2.75	0.01
Longitude:NOD	-0.14	0.05	0.05	3.04	0.00
NOD:Common dolphin	0.00	0.25	0.25	0.01	1.00
NOD:Harbour porpoise	-0.11	0.24	0.24	0.47	0.63
NOD:Risso's dolphin	0.18	0.37	0.37	0.49	0.63
NOD:Striped dolphin	0.71	0.69	0.69	1.03	0.30
NOD:White-beaked dolphin	0.05	0.35	0.35	0.15	0.88
NOD:White-sided dolphin	0.27	0.52	0.52	0.53	0.60
Latitude:Longitude:NOD	-0.22	0.05	0.05	4.41	0.00
Blubber	-0.02	0.07	0.07	0.27	0.79
Male	-0.01	0.05	0.05	0.22	0.83

2.4.3 Toxicity

PCBs pose the greatest risk to marine mammal health across both of the immunotoxic and endocrine disrupting endpoints I investigated (Figure 2-12). I found considerable variation in relative toxicities between the endpoints; pollutants that were typically used in agriculture (DDTs, dieldrin, and HCHs) showed a greater relative impact on the endocrine system than the immune system whereas those typically used in industry and manufacturing (PCBs and PBDEs) have a greater relative immunotoxic impact. The contribution of PCBs towards immunotoxicity ranged from 69% in white-sided dolphins to 93% in grey seals, whilst their contribution towards endocrine disruption ranged from 49% in killer whales to 80% in grey seals. Although PCBs represent the greatest risk across both end points, I found that rankings, in terms of risk, for the other pollutants varied depending on the end point being investigated. Of the other pollutants, DDTs represent the next highest risk to endocrine disruption with values ranging from 9% in grey seals to 32% in white-sided dolphins (Figure 2-12A). However, DDTs pose a much smaller risk with respect to immunotoxicity, with the proportion of risk ranging from 1% in common dolphins to 5% in sperm whales (Figure 2-12B).

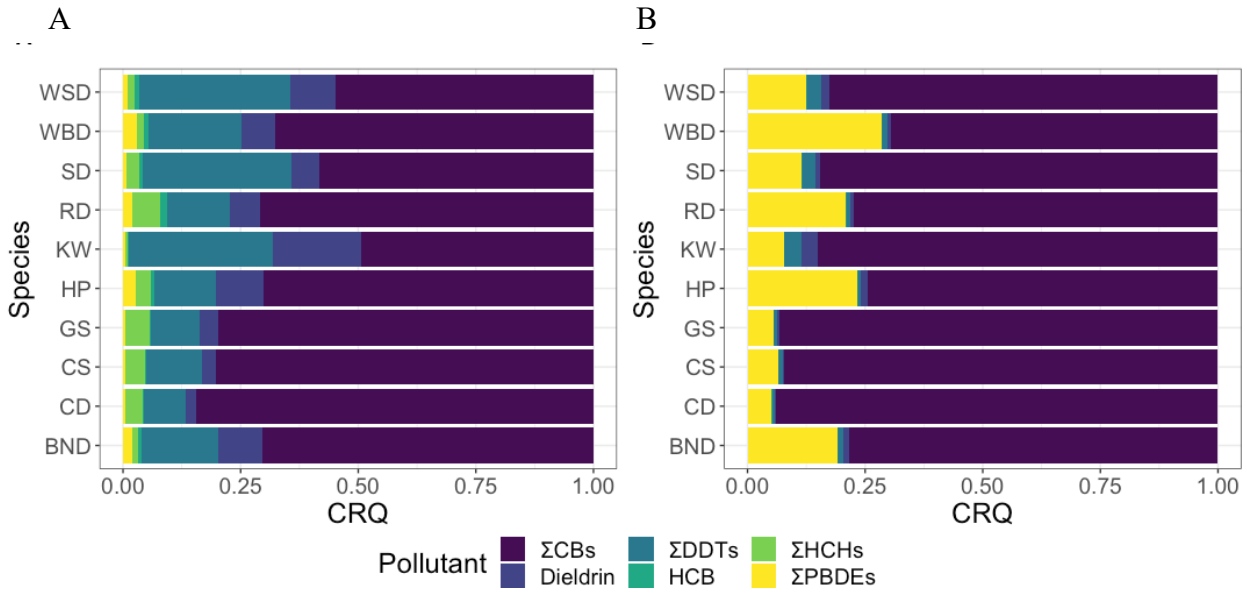


Figure 2-12: Relative contributions of each pollutant class to the critical risk quotient (CRQ) for each species for (A) endocrine disruption and (B) immunotoxicity. CRQs were derived using toxicity reference values (TRVs) derived from murine models.

2.5 Discussion

Here I show that, despite their world-wide ban and restriction 20 years ago, many marine mammals remain exposed to a widespread, persistent and toxic chemical threat from persistent organic pollutants (POPs) in the North-East Atlantic. Although pollutant burdens are declining, POPs are still present at concentrations that pose a significant threat to marine mammal health, and there is considerable variation in concentrations and rates of decline across species, pollutant classes, and regions. This heterogeneity in contamination demonstrates that assessing individual species and aggregating data over large spatial extents can mask significant impacts at population and ecosystem level. By monitoring sentinel species to assess the impacts of past and current elimination and mitigation actions I have demonstrated that efforts in the North-East Atlantic region have failed to protect marine mammals. It is vital that governments do not lose sight of this issue, amid the context of other global pressures such as, climate change, impact from fisheries and plastic pollution, as these threats can interact with POPs and increase environmental burdens (Ma et al., 2011; Wasser et al., 2017). Therefore, ahead of the 2021 Conference of the Parties (COP) to the *Stockholm Convention* on POPs, I call on governments to provide adequate resources to further reduce environmental contamination and ensure signatories meet their international obligations.

I have revealed that, despite the decline in mean total pollutant burdens in marine mammals over the last 30 years, concentrations in a substantial proportion of individuals are still above toxicity thresholds, particularly in species feeding at higher trophic levels that are stranded along southerly coastlines. Although pollutant concentrations are dependent on a number of factors (e.g., bioavailability and feeding ecology) I expect geographical differences in historical use, contemporary discharges and atmospheric transport dynamics are likely to be the primary drivers of the spatial variation I observed. Levels of industrialisation are substantially greater at lower latitudes in the North-East Atlantic and, in the United Kingdom, PCBs were manufactured at a single site on the Bristol channel (Robin, 2010). As such, legacy and contemporary releases are more likely to have occurred at lower latitudes, as reflected by the spatial distribution in PCB concentrations and congener relative abundances. Spatial patterns of the other pollutants, which were primarily used as crop treatments, also appear to reflect their historical use. Concentrations

are higher and have declined at a slower rate in the North Sea, which is associated with greater arable farming effort (Defra, 2020).

The analysis also revealed temporal shifts in the spatial distribution of pollutants. Dispersal of legacy pollutants over time has been well documented and is thought to be driven either by concentration gradients or by latitudinal temperature gradients, from warmer to cooler areas (von Waldow et al., 2010; Wania and Mackay, 1993). The spatial distribution of pollutant concentrations can also vary according to animal movement and carcass drift, causing animals to accrue pollutants in a different location to where they strand. While it was not possible to determine an animal's movements over its lifespan, I was able to minimise the impact of carcass drift by prioritising samples that were fresh or only slightly decomposed (933/1077 = 87%). These findings suggest that the dominant dispersal mechanism varies across the pollutant classes. Dispersal of PCBs over time is more strongly influenced by latitudinal temperature gradients while, the dispersal of the other pollutants is driven by concentration gradients. This is demonstrated by the shift in distribution of the agricultural pollutants, with higher concentrations found on the North Sea compared to the Atlantic coast earlier in the study and vice versa in more recent years. This evidence of atmospheric transport in the North-East Atlantic is also concerning against the backdrop of recent increases in some pollutant concentrations in pristine environments such as the Arctic (Hung et al., 2016). This problem is likely to be exacerbated as increased temperatures increase the volatility of pollutants causing them to remobilise into the atmosphere and increase exposures (Teran et al., 2012).

Inter and intraspecific differences such as lifespan, metabolic capabilities, foraging strategies and trophic level can cause some species to be at greater risk of high pollutant burdens than others (Aguilar et al., 1999). Burdens tend to be greater in longer lived species that feed at a high trophic level as evidenced by the high concentrations I found in killer whales and bottlenose dolphins. Concentrations were higher in juveniles than adult females, which is reflective of lactational transfer of pollutant burdens from mothers to calves and has been associated with reduced calf survival (Schwacke et al., 2002). Differences in pollutant burdens between taxa can also occur if the home ranges of some taxa are more contaminated than others (Pulster et al., 2009). I found concentrations were highest on the southwest coast of the UK, an area inhabited by a large

population of common dolphins (Hammond et al., 2017). This may explain why concentrations in this species are falling at a significantly slower rate than other species.

By analysing a broad spectrum of pollutants, I was able to determine relative abundances of pollutants to infer spatial differences in contamination sources as well as intraspecific differences in metabolism and lifespan. Burdens in long-lived species can represent multi-decadal exposure and hence relative abundances in these species often lag behind those found in the environment and shorter-lived biota (Hickie et al., 2007). The higher ΣDDTs to ΣPCBs ratio I found in longer-lived taxa provides evidence of this and reflect the faster rate of decline in ΣDDTs concentrations, which may be due to the greater persistence of PCBs or continued environmental contamination. The distribution of PCB congener proportions that I found may indicate differences in initial timings of releases. Proportions of heavier congeners are decreasing overtime and greater at lower latitudes and longitudes. This variance aligns with the location of a PCB manufacturing plant and suggests inputs into the environment are decreasing over time (Robin, 2010). I found BDE47 was the most abundant PBDE congener, which is reflective of the likely contamination source as BDE47 is associated with the legacy production of the penta-BDE commercial mixture. It is estimated large reservoirs of this compound are still in circulation (Alcock et al., 2003). Ratios of pollutants can also vary according to differing metabolic capabilities between taxa. For example, pinnipeds are able to metabolize some pollutants more easily than odontocetes (Weijs et al., 2009) and have vastly shorter lactation periods (Berta et al., 2005), as demonstrated by the differences in PCB congener abundances I observed. Pollutant concentrations and abundance profiles can also be affected by loss of blubber mass, as a consequence of chronic negative energy balance (Borrell and Aguilar, 1990; Williams et al., 2020b). I was able to minimise this by controlling for variation in body condition.

I have shown that marine mammals are exposed to a barrage of legacy pollutants and it is now well documented that antagonistic and synergistic actions of pollutants can create toxic mixtures, even when each pollutant is present at a level deemed to be safe (Kortenkamp and Faust, 2018). Therefore, toxic thresholds can be considered to be conservative, as the toxicological risk that marine mammals face is likely to be exacerbated by the mixture effects of the pollutants to which they are exposed. I identified exposure to PCBs as the greatest risk to health, in terms of their

contribution to toxicity and slow decline in comparison to other pollutants. Other pollutants are, however, present at toxic concentrations and account for a substantial proportion of risk to the immune and endocrine systems. In lieu of marine mammal toxicity reference values I have used values derived for murine models. I note there are likely to be considerable levels of uncertainty however, this approach is routinely used in ecotoxicology to compare effects of pollutants and is widely accepted (Awkerman et al., 2008). It is clear that exposure to multiple pollutants is likely to increase the risk of harmful effects and should be accounted for when determining acceptable environmental concentrations and managing contamination. Scientific evidence for heightened toxicity from such mixtures is mounting, but regulation is lagging behind (Kortenkamp and Faust, 2018).

Aside from exposures to pollutants, marine mammals face an increasing number of threats, and population level impacts are likely to be exacerbated in areas where high levels of contamination coincide with other pressures (e.g., acoustic disturbance, prey depletion, climate change). For example, I have shown that pollutant concentrations in common dolphins are a persistent threat and are declining at a significantly slower rate than other species. When combined with the substantial threat they face from by-catch and their conservation status of 'unknown' in the UK and Atlantic and Unfavourable-Bad in the Mediterranean (Joint Nature Conservation Committee (JNCC), 2018), these findings raise concerns about the long-term health of this population. The impact of pollutant exposure may also be greater in areas where high levels of contamination overlap with areas of biological importance. I have shown that Cardigan Bay, one of a network of protected sites set out by the European Union's Habitat Directive known as Special Areas for Conservation (SACs), is located in an area associated with higher pollutant exposures. Further, this SAC is in close proximity to a major UK PCB manufacturing site and so may be more vulnerable to contemporary releases (Robin, 2010). I have demonstrated that a harmonised and integrated approach is needed to monitor and assess marine mammal health in relation to pollution and other combined pressures, particularly in areas of high ecological value such as Marine Protected Areas and SACs.

These findings are particularly important in the context of the *Stockholm Convention*, as many European and non-European countries are failing to take the necessary remedial actions to meet

their 2025 and 2028 commitments (Stuart-Smith and Jepson, 2017). As a priority, the Convention must conclude negotiations on a compliance mechanism, proposed almost two decades ago, to provide stronger incentives for targets to be met. In addition, I urge for renewed action in Europe to allocate sufficient resources to increase elimination and mitigation rates. For example, destruction rates of POPs (stockpiled or in open application) should be increased and regulation should be improved to ensure contaminated products are not present in recycling streams and that those present in waste streams are treated appropriately. I have shown that overall burdens of POPs are declining and highlighted the importance of examining pollutant burden heterogeneity within populations alongside overall spatiotemporal trends to assess whether mitigation actions are sufficient to protect vulnerable wildlife and ecosystems. Given the pervasive and persistent threat of chemical contamination in marine mammals, these findings are globally significant and highlight the need to continue to hold nations accountable to their commitments to protect the marine environment.

Chapter 3 - Spatiotemporal Trends of Polychlorinated Biphenyls and Associations with Infectious Disease Mortality in Harbour Porpoises (*Phocoena phocoena*)

3.1 Abstract

Polychlorinated biphenyls (PCBs) are toxic, persistent and lipophilic chemical compounds that accumulate to high levels in harbour porpoises (*Phocoena phocoena*) and other cetaceans. It is important to monitor PCBs in wildlife, particularly in highly exposed populations, to understand if concentrations are declining and how levels relate to toxicological thresholds and indices of health like infectious disease mortality. In this chapter I show, using generalised additive models and tissue samples of 814 UK-stranded harbour porpoises collected between 1990 and 2017, that mean blubber PCB concentrations have fallen below the proposed thresholds for toxic effects. However, I found they are still associated with increased rates of infectious disease mortality such that an increase in PCB blubber concentrations of 1 mg kg^{-1} lipid corresponds with a 5% increase in risk of infectious disease mortality. Moreover, rates of decline and levels varied geographically, and the overall rate of decline is slow in comparison to other pollutants. I believe this is evidence of long-term preservation of PCBs in this population and continued environmental contamination from diffuse sources. These findings have serious implications for the management of PCB contamination in the UK and reinforce the need to prevent PCBs entering the marine environment to ensure that levels continue to decline.

3.2 Introduction

This chapter builds on the conclusions of **Chapter 2** in which, of the five persistent organic pollutants (POPs) I investigated, the most persistent polychlorinated biphenyls (PCBs) were shown to be of most concern to marine mammals in UK waters and have been shown to induce reproductive and immunotoxicity in the marine environment across Europe (Desforges et al., 2016; Law and Jepson, 2017; Murphy et al., 2015; Stuart-Smith and Jepson, 2017). This chapter explores the spatiotemporal trends of PCB accumulation and associations between PCB exposure and disease using harbour porpoises as a sentinel species.

Despite the European ban on PCBs in the mid-1980s, large amounts still require disposal (Defra, 2013; Stuart-Smith and Jepson, 2017). Legacy PCBs continue to enter the marine environment via several mechanisms such as terrestrial run off, dredging and atmospheric transport and deposition (Jartun, 2011; Minh et al., 2006). In Swedish waters, many wildlife populations such as otters (*Lutra lutra*), grey seals (*Halichoerus grypus*) and the white-tailed eagle (*Haliaeetus albicilla*), have experienced population recoveries that coincide with a decrease of PCB concentrations in their tissues (Roos et al., 2012). However, trends in the concentrations of PCBs in cetaceans in the United Kingdom (UK) have not been analysed since 2012, when it was reported that concentrations in harbour porpoises (*Phocoena phocoena*) had stabilised in 1998, at levels still deemed to be a toxicological threat (Jepson et al., 2016).

Determining the toxicological threat from PCBs is a challenging task. Whilst there are well-established dose-response relationships for many terrestrial species, which can be studied in laboratories, the direct impact that PCBs have on marine apex predators remains uncertain (Kannan et al., 2000). Direct evidence of immune system impairment in marine mammals in captivity has been demonstrated in a limited number of cases. Immune function tests both *in vitro* and *in vivo* in captive harbour seals (*Phoca vitulina*) showed seals exposed to higher levels of dietary organochlorines (including PCBs) experienced a reduction in host defence against viral infections (Ross et al., 1996). Indirect evidence of the link between high PCB tissue burdens and immune system impairment has also been demonstrated by multiple epizootic outbreaks of morbillivirus in harbour seals and striped dolphins (*Stenella coeruleoalba)* in European waters

(Aguilar and Borrell, 1994a; Dietz et al., 1989; Duignan et al., 2014). Cumulative pathological investigations suggest that exposure to high concentrations of organochlorines (including PCBs) is a key factor in reducing host resistance (Hall et al., 2006; Jepson et al., 1999; Law et al., 2012a). Thus, observed adverse effects of PCB exposure in cetaceans are consistent with effects reported from laboratory studies on other mammals (Schwacke et al., 2012).

While it is ethically and economically not viable to carry out controlled captive exposure experiments on marine mammals, the risks associated with pollutant exposure have been estimated in human health and wildlife epidemiology using logistic regression modelling. In 2006 it was estimated that there was an increased risk of infectious disease mortality in harbour porpoises of 2% associated with each mg kg^{-1} lipid increase in PCB blubber concentrations (Hall et al., 2006). However, no studies have assessed whether this risk assessment is still appropriate for the UK population, given that this analysis was carried out fifteen years ago.

It is important, therefore, to reassess the current trends and levels of PCBs in UK cetaceans and understand how these relate to infectious disease mortality. In this chapter, I used the Cetacean Strandings Investigation Programme (CSIP) dataset to investigate the relationship between PCB concentrations and infectious disease mortality to quantify the change in risk at current exposure levels. I determined the temporal trends and current levels of PCBs in the blubber of UK-stranded/necropsied harbour porpoises using data collected between 1990-2017, which incorporated unpublished data from 2012-2017 with historical published data. In addition, the previously published analysis may have been confounded by the high proportion of underweight animals, present at the beginning of the study, as nutritional stress may have affected the concentrations of PCB in their blubber (Hall et al., 2008). Hence, it is important to determine the trend, and control for confounding factors, to understand the effectiveness of remediation strategies and the level of threat now posed to the population.

3.3 Materials and Methods

3.3.1 Sampling

Between the years 1990 and 2017 blubber PCB concentrations were determined for 814 UK-harbour porpoises from necropsies carried out according to standard cetacean post-mortem procedures (Law et al., 2006). The animals that were necropsied had stranded around the UK coast and so were opportunistically sampled. As part of these investigations, individuals' length, weight, girth, sex, age class and the latitude and longitude of the stranding location were recorded. Toxicological analysis was only conducted on blubber samples from animals that had undergone minimal to moderate levels of decomposition, according to the condition scoring guide outlined in the post-mortem protocol (Law et al., 2006). This was to minimise the impact of changes in pollutant tissue dispersion and levels associated with decomposition (Law, 1994). The animals that were analysed for PCBs were otherwise assumed to be a random sample of the strandings that occurred over the study period. However, it should be noted that by prioritising fresher carcasses the sampling may be biased towards animals that died closer to shore, which may be skewed towards certain causes of death. I tested whether this was significant by fitting a linear model to the toxicological strandings dataset (n=814) and the overall strandings dataset (n=6734) and used cause of death as the response variable and dataset as the predictor variable. I found there was no statistical difference between the proportions of each cause of death in the datasets (F-value = 15.914, p-value > 0.05).

3.3.2 PCB Analysis

A standardised methodology was used, over the entire sampling period, to extract and preserve the blubber samples for contaminant analysis (Law, 1994). The Cefas laboratory (Lowestoft) determined the concentrations of ∑25 CB congeners (on a mg kg^{-1} wet weight basis) using a method that was validated by continuing participation in the QUASIMEME laboratory proficiency scheme and followed the recommendations of the International Council for the Exploration of the Sea (ICES) (de Boer and Law, 2003; de Boer and Wells, 1997; ICES, 1998; Webster et al., 2013). In cases where the congener/isomer concentrations were below the limit of quantification (<0.0003 or <0.0004 mg kg^{-1} wet weight), concentrations were set at half the limit, as per Law et al. (2012).

The numbers of the International Union of Pure and Applied Chemistry CBs congeners analysed were: 18, 28, 31, 44, 47, 49, 52, 66, 101, 105, 110, 118, 128, 138, 141, 149, 151, 153, 156, 158, 170, 180, 183, 187, 194. These congeners were analysed because they were relatively abundant in commercial PCB mixtures and have a broad range of chlorination. They also incorporate the seven PCBs prioritised for international monitoring by ICES. The sum of the individual congener concentrations was calculated and normalized to a lipid basis (mg kg^{-1} lipid) by solvent extracting lipids from the blubber and calculating the hexane extractable lipid content (Webster et al., 2013).

3.3.3 Pathological and Statistical Analysis

As part of the pathological investigations certain biological attributes were recorded including information on weight, length, age and sex. For smaller cetaceans like the harbour porpoise, a basic index of weight to length ratio is thought to be the most appropriate metric of body condition and is widely acknowledged as a good predictor of fitness in marine mammals (Beauplet and Guinet, 2007; Christiansen et al., 2014; Kershaw et al., 2017). The weight and length data variable for the individuals in this study followed a power relationship and so a power regression model was fitted to obtain a metric that could be used as a proxy for body condition (Figure 3-2). The residuals from the best-fit regression line were extracted and used for further modelling whereby, values above the model fit represented cases in good nutrition and individuals below the line represented cases in poorer nutritional condition. Body length and sexual maturity were used to categorise the individuals into age classes. Neonates were defined as individuals with a body length less than 90cm, juveniles were defined as individuals with a body length greater than 90cm that were sexually immature and adults were defined as individuals with a body length greater than 90cm that were sexually mature (Jepson, 2003). For the purposes of this study the neonates (n=57) and juveniles (n=395) were grouped together and classed as subadults (n=452). This was to reduce the number of categories in the age class variable so that the statistical power of the model was greater. Cause of death was divided into three categories: “trauma”, “infectious disease” and “other” (including not established, starvation, neoplasia and live strandings). Date of stranding was used to categorise strandings into seasons (Dec-Feb “Winter”, Mar-May “Spring”, Jun-Aug “Summer”, Sept-Nov “Autumn”). The latitude and longitude of the stranding location of each animal was collected and used to investigate geographical variation. I used the coordinates to categorise the individuals into three geographic areas, Scotland, West England & Wales and East England

(Figure 3-1), that were previously defined in a study of contaminants in stranded cetaceans in the UK (Law et al., 2012a).

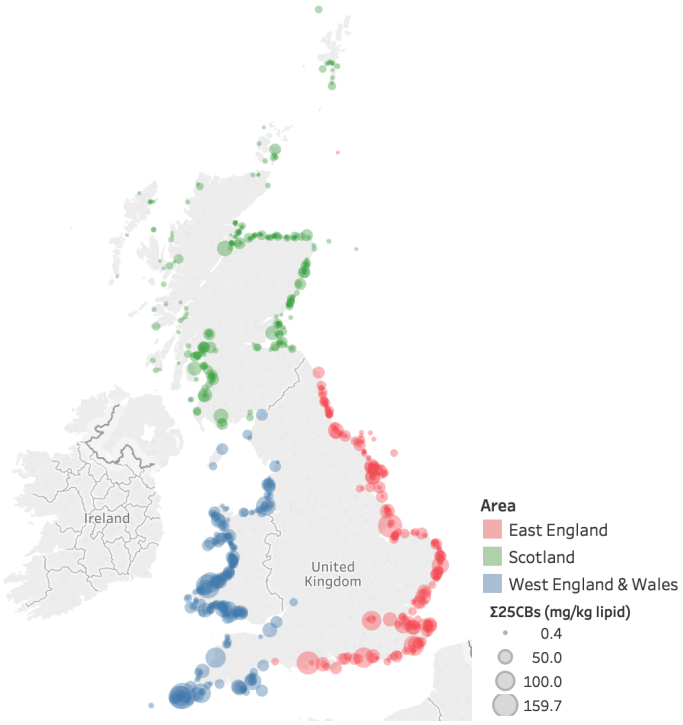


Figure 3-1: Geographic locations and area classifications of the individuals that stranded and were analysed to obtain blubber concentrations for the sum of 25 selected congeners of polychlorinated biphenyls (Σ25CBs). The colours of the dots represent the area classification and the dots are sized by the blubber concentrations of Σ25CBs.

Temporal and spatial trends

I carried out all of the analyses using the statistical software R (version 3.4.3) (R Core Team, 2016). Prior to model fitting, I carried out extensive data exploration to identify collinearity between the variables, detect outliers and remove individuals with missing values. This resulted in a subset of 777 individuals being included in the analysis. Previous analyses have shown that ∑25 CB concentrations are heavily influenced by factors such as nutritional condition, age class and sex that may confound temporal trends (Aguilar et al., 1999; Tanabe et al., 1981). To account for this, I modelled ∑25 CBs with covariates, which were selected because there was existing evidence that they could affect ∑25 CBs concentrations, and used the model residuals for the temporal analysis. Following extensive data exploration, I established that there was a linear relationship between ∑25 CBs and other covariates. Therefore, I fitted several multiple linear regression

models to the variables, which could explain the variability in the data using ∑25 CBs as the response variable. The variables included in the full model were nutritional condition, sex, age class, cause of death, latitude, longitude and an interaction term between age class and sex. I tested all possible variable combinations to obtain several candidate models which were ranked according to their AIC (Akaike's Information Criterion) values. I selected the model with the fewest predictors whereby the difference in AIC relative to the minimum AIC was <4 (Akaike, 1973). The complete model selection table is shown in the Appendix Table A- 2. I performed model validation by assessing the diagnostic plots and plotting the model residuals against selected variables to assess the variance.

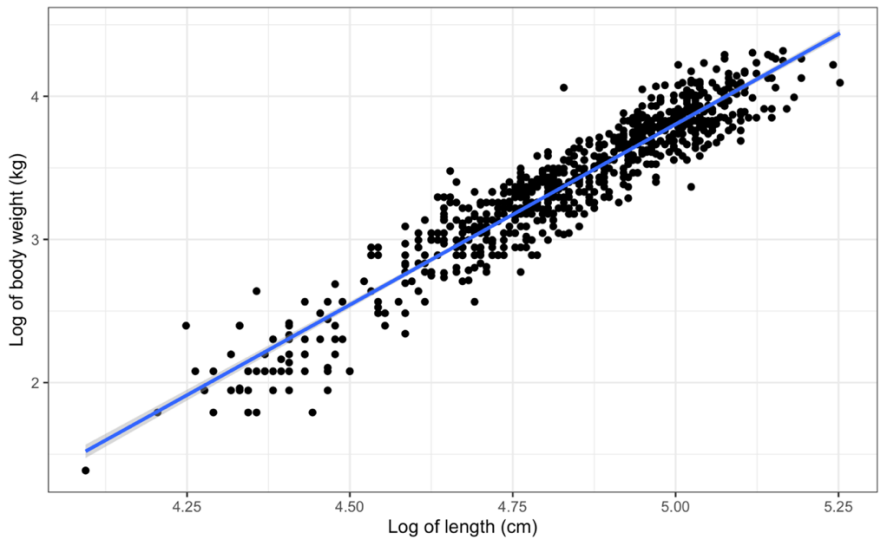


Figure 3-2: Natural log of body weight (kg) plotted against the natural log of body length (cm). The blue line represents the linear model fitted to the data. The residuals of the model were used as a proxy for nutritional condition. The shaded area represents the 95% confidence interval.

To model temporal trends in ∑25 CB concentrations, generalised additive models (GAMs), with an identity link function were fitted, to the smoothed number of days since the 1st of January 1990 and the residuals of the model fitted to ∑25 CBs and confounding covariates, using the *gam* function available within the R library *mcgv* and *nlme* (R Core Team, 2016; Wood, 2006). Thin plate regression splines were applied to smooth the number of days to prevent over fitting of the model. To investigate geographical variation, I fitted GAMs to a subset of the data for each geographical area. The GAMs were fitted to the residuals of the same linear regression model,

between ∑25CBs and selected covariates, which was used for the whole of the UK. I chose to subset the data rather than add area as a variable to the model because of limited data availability in Scotland between 1990 and 1993. As a consequence, the trend for Scotland was modelled from 1994 onwards, data from previous years were excluded (n=8).

For all GAMs, the basis dimension to determine the degree of smoothing was determined using the integrated smoothness estimation within *mgcv* (R Core Team, 2016; Wood, 2006). This was validated using generalised cross validation and visual assessment of the smoothing splines to assess whether the value of the smoothing dimension was appropriate (Wood, 2006). To ensure the models were not over fitted the smoothing penalty term was set at 1.4 as per Kim and Gu (2004). Diagnostic plots were used to assess the models' assumptions of normality, heterogeneity and independence and the variances of residuals were examined for further model validation.

3.3.5 Infectious disease mortality

To calculate the PCB exposure odds ratio of infectious disease mortality, a subset of 641 of the 814 harbour porpoises were chosen for analysis (based on cause of death). Animals whose cause of death could not be established and those that died from neoplasia, starvation or live stranded were excluded (n=126). Individuals were also excluded if their body weight, girth or length data were missing (n=47) to ensure the effect of nutritional status could be investigated. I used a case-controlled approach to compare animals that died of infectious disease (cases) with animals that died of trauma (controls) to investigate whether there was a relationship between high concentrations of PCBs and infectious disease mortality. There were 267 individuals in the infectious disease category and 374 individuals in the trauma category. The complete classification of cases and controls and detailed causes of death are shown in Table 3-1.

Table 3-1: Detailed causes of death for cases and controls

	Cause of Death	n
Cases	(Meningo)encephalitis	7
	Gastritis and/or Enteritis	31
	Generalised Bacterial Infection	63
	Generalised Mycotic Infection	4
	Generalised Fungal Infection	1
	Morbillivirus	1
	Others	12
	Pneumonia, Bacterial	4
	Pneumonia, Bacterial and Mycotic	2
	Pneumonia, Mycotic	5
	Pneumonia, Parasitic	90
	Pneumonia, Parasitic and Bacterial	36
	Pneumonia, Parasitic and Mycotic	9
	Pneumonia, Unknown Aetiology	2
Controls	Bycatch	126
	Bycatch (known)	67
	Dystocia & Stillborn	10
	Entanglement	1
	Physical Trauma	36
	Physical Trauma, Boat/Ship Strike	5
	Physical Trauma, Bottlenose dolphin Attack	125
	Physical Trauma, Grey seal Attack	4

PCB blubber concentrations are influenced by an individual's nutritional condition and blubber mass to the extent that large variation in condition and blubber mass can make concentrations incomparable (Kajiwara et al., 2008). To minimise the impact of this variation on the results I standardised the PCB concentrations, of nutritionally stressed individuals, according to an individual's blubber mass and condition as per the method defined by Hall et al. (2006a). Hall et al. used data available for 156 harbour porpoises to fit a linear regression model to estimate total blubber mass from an individual's mass, length and girth (Equation 3-1). I used this equation to estimate the total blubber mass of each individual in the analysis. This resulted in eight individuals being excluded because there was not sufficient data to estimate total blubber mass.

Estimated total blubber mass (kg) = 0.35 + [0.17 × mass (kg)] – [0.05 × length (cm)] + [0.14 × girth (cm)] – [1.0 × sex].

Equation 3-1: Equation used to estimate total blubber mass, which was then used to adjust PCB concentrations to account for the impact of blubber loss.

I then used the linear regression model, previously described (Figure 3-2) to estimate a body weight for each individual. Therefore, for each given length of an individual their body weight was calculated from the fitted model to standardise body weights against the population mean. Individuals were then classified as nutritionally stressed if their actual body weights were less than the predicted body weights. It was then assumed that this difference in predicted versus actual body weights was as a result of blubber depletion. The assumption then followed that for animals that were nutritionally stressed the concentration of PCBs in their blubber was higher than it would have been had they not suffered from blubber depletion. To adjust for this a standard blubber mass was calculated by adding the previously estimated blubber mass to the difference between the predicted and actual body weights for nutritionally stressed individuals. Adjusted PCB concentrations were calculated by multiplying the PCB concentrations by the ratio of estimated blubber mass to standardized blubber mass. Concentrations were only adjusted for animals deemed to be nutritionally stressed whereby their predicted body mass was greater than their actual body mass.

I investigated the relationship between PCB blubber concentrations and infectious disease mortality by fitting generalised linear models with binomial distributions and logit link functions. Cause of death was used as the response variable; PCB concentrations and other selected covariates were used as potential predictors. The potential predictors were selected according to the biological rationale that they could impact cause of death. The variables included in the full model were nutritional condition, sex, age class, latitude, longitude, season, year of stranding with interaction terms between age class and sex and between season and year. I used the same approach, described in the temporal and spatial trends methods section, to extract a set of plausible models from the candidate models. The final prediction model was obtained by averaging the set

of plausible models where ΔAIC<4. The model selection table is shown in the Appendix Table A-3. To validate the model, I plotted the residuals of the model against other variables and assessed the variance. I assessed the model for over dispersion using the ratio of deviance and residual deviance (1.051) and the value was within the proposed acceptable limits outlined in the literature (<1.5) (Mangiafico, 2015). Further model validation was carried out by conducting the Hosmer Lemeshow Goodness of Fit test, which indicated a good fit (Hosmer et al., 2013).

3. 4 Results

3.4.1 Long term trends in blubber PCB concentrations in UK-stranded harbour porpoises

The results clearly illustrate that in 2007, modelled mean PCB concentrations (∑25 CBs) in the blubber of harbour porpoises fell below the most widely used threshold for toxic effects (9 mg kg^{-1} lipid) derived by Jepson et al. (2016), when the UK was treated as a single geographical region (Figure 3-3). However, 39% (n=15/38) of the individuals sampled in 2016 and 2017 still exceeded this threshold. Moreover, when I modelled the sub-regions in the UK separately, I found geographic variation in PCB concentrations and rates of decline. I show that at the beginning of the study period (1990-1998) blubber PCB concentrations appeared to be in decline (Figure 2-3). This decline appeared to stop around 1998 after which concentrations were stable until 2006. In the most recent years of the study PCB blubber concentrations have begun to decline again and, in 2007, fell below the established threshold for toxic effects in marine mammals (Kannan et al., 2000).

When I modelled the geographic sub-regions separately, I found inter-regional variation as well as variation between the regions and the whole of the UK (Figure 2-3). I found that levels in animals that stranded on the east coast of England and west coast of England and Wales showed a steady decline over the entire period, however, the rate of decline was greatest in animals that stranded on the east coast of England. Levels in animals that stranded on the east coast of England appear to have fallen below the most widely used threshold for toxic effects (9 mg kg^{-1} lipid) in 2007, approximately two years later than the UK as a whole. Levels in animals that stranded on the west coast of England and Wales appear to have fallen below the threshold in 2017 however, the standard errors span the threshold. I found that, unlike the other two areas, modelled mean

concentrations in Scotland did not experience a continuous decline over the study period. I found that PCB blubber concentrations increased at the beginning of the study period and peaked around 2004 after which they declined steadily. I found that modelled mean concentrations were higher on the west coast of Scotland than the east coast (Figure 3-4).

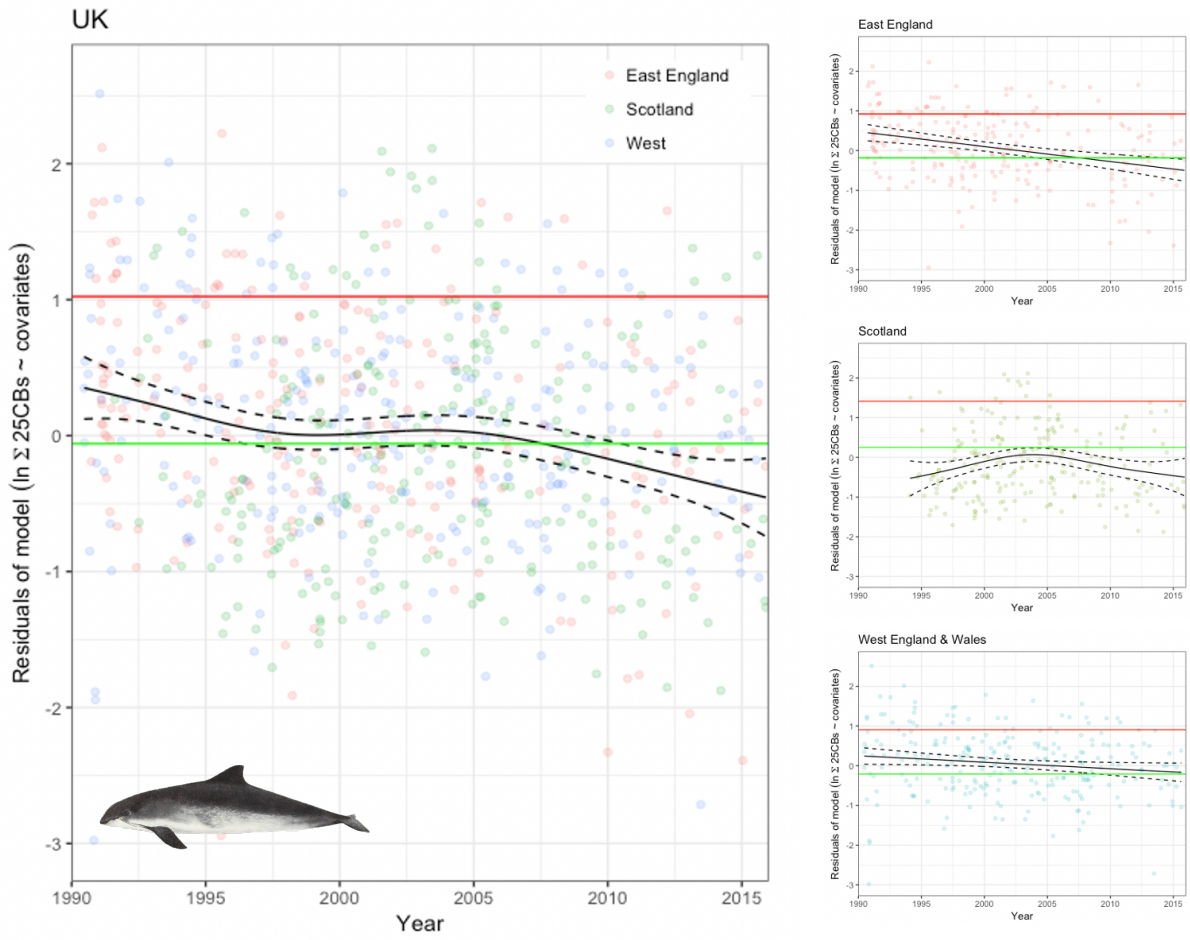


Figure 3-3: The smoothing splines from the generalised additive models fitted to the residuals of the linear regression model (Equation 3-2) against number of days since the 1st of January 1990 for the UK and three sub-regions. The solid line represents the smoothed trend and the dashed lines represent twice the standard error. The green lines represent the most widely used proposed threshold for toxicological effects of polychlorinated biphenyls in cetaceans (9 mg kg^{-1} lipid) (Kannan et al., 2000). The red lines represent the highest proposed threshold for toxicological effects (41 mg kg^{-1} lipid) (Helle et al., 1976). For clarity points less than -3 were removed (UK n=3, East England n=2, Scotland n=0, West England & Wales n=1).

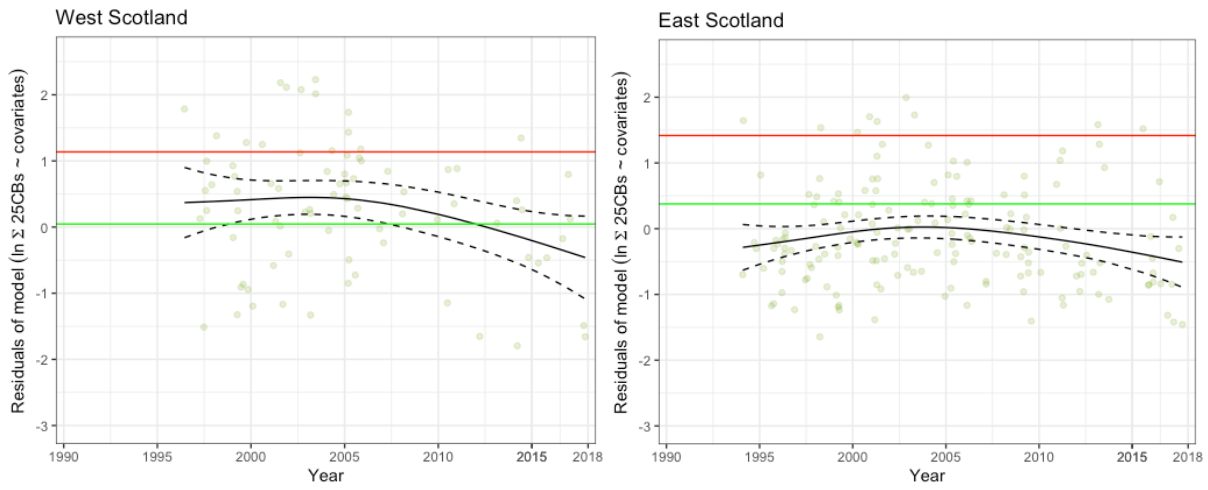


Figure 3-4: The smoothing splines from the generalised additive models fitted to the residuals of the linear regression model (Equation 3-2) against number of days since the 1st of January 1990 for the (A) West coast of Scotland (B) East coast of Scotland. The solid line represents the smoothed trend and the dashed lines represent twice the standard error. The green lines represent the most widely used proposed threshold for toxicological effects of polychlorinated biphenyls in cetaceans (9 mg kg-1 lipid) (Kannan et al., 2000). The red lines represent the highest proposed threshold for toxicological effects (41 mg kg-1 lipid) (Helle et al., 1976).

The final form of the model used to extract residuals that were fitted to the GAMs was linear and included nutritional condition, latitude and an interaction term between sex and age class as explanatory variables (Equation 3-2). Summary statistics are shown in Table 3-2.

$$\log \sum 25CBs \sim \beta_0 + \beta_1 Nutritional\ condition + \beta_2 Sex + \beta_3 Age\ Class + \beta_4 Latitude + \beta_5 Sex * Age\ Class$$

Equation 3-2: The final form of the model fitted to the log of PCB blubber concentrations and selected covariates that were used to extract residuals that were fitted to the GAMs.

3.4.2 Association between PCB exposure and infectious disease mortality

The results clearly show that PCB exposure is associated with an increased risk of infectious disease mortality in harbour porpoises (Table 3-3). I found that the exposure odds ratio for ∑25 CBs blubber concentrations and infectious disease mortality was 1.05 (97.5% CI: 1.03-1.07). Hence, for a 1mg kg-1 lipid increase in ∑25 CBs blubber concentrations there is an increased risk of death from infectious disease of 5%. I found that nutritional condition was the biggest predictor

of death from infectious disease (Table 3-3). Subadults were predicted to have a lower risk of death from infectious disease than adults and males were predicted to have a lower risk than females. Animals that stranded in winter were also predicted to have a significantly higher risk of infectious disease mortality.

*Table 3-2: Summary statistics of the linear model fitted to the natural log of PCB blubber concentrations and selected covariates. Nutritional condition and latitude were zero centred and scaled. (The coefficient estimates were calculated in relation to a female adult.) * indicates statistical significance*

Coefficient	Estimate	Std. Error	Z value	P value
(Intercept)	10.24	0.66	15.47	0.00*
Nutritional condition	-1.32	0.18	-7.25	0.00*
Sex (male)	1.26	0.10	13.20	0.00*
Age class (subadult)	0.40	0.09	4.37	0.00*
Latitude	-0.16	0.01	-12.90	0.00*
Sex * age class (male, subadult)	-1.29	0.12	-10.35	0.00*

*Table 3-3: Summary statistics of the logistic regression model fitted to the data where cases were defined as animals that died of infectious disease and controls were defined as animals that died from trauma. All continuous variables were centred and scaled. (The coefficient estimates were calculated in relation to a female adult that stranded in Autumn.) * indicates statistical significance*

	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)
(Intercept)	0.32	0.28	0.28	1.14	0.25
Subadult	-1.41	0.32	0.32	4.44	0.00*
Nutritional condition	-1.15	0.12	0.12	9.70	0.00*
Latitude	0.21	0.12	0.12	1.82	0.07
Longitude	0.17	0.12	0.12	1.39	0.17
Spring	0.38	0.28	0.28	1.33	0.18
Summer	-0.26	0.29	0.29	0.87	0.38
Winter	1.23	0.29	0.29	4.26	0.00*
Male	-1.10	0.36	0.36	3.09	0.00*
Adjusted ∑25 CBs concentrations	0.67	0.13	0.14	4.97	0.00*
Subadult Male	0.72	0.50	0.50	1.45	0.15
Year	0.04	0.08	0.08	0.47	0.64

To investigate the variation in mortality risk over different ranges of blubber PCB concentrations the increase in risk was calculated across various concentration differences (Figure 3-5). I found

that at the population mean adjusted concentration of 11.5 mg kg^{-1} lipid there is an increased risk of death from infectious disease of 59% (97.5% CI:36%-82%). At the population mean concentration from the final year of the study (8.09 mg kg^{-1} lipid) there is an increase in risk of 41% (97.5% CI:25%-58%).

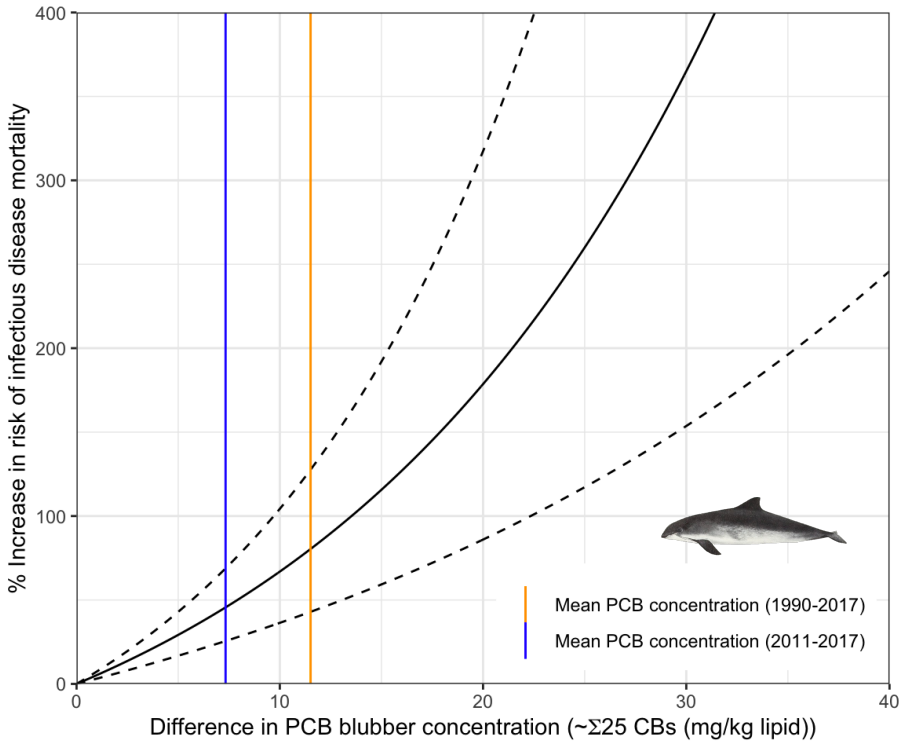


Figure 3-5: The increased risk of death by infectious disease against the adjusted sum of 25 chlorobiphenyl congeners (∑25 CBs (mg kg^{-1} lipid)) blubber concentrations as predicted by the logistic regression model. The population mean over the entire study period (orange line) and over the last five years of the study (blue line) have been included for reference. The dashed lines represent the 97.5% confidence intervals.

3.5 Discussion

Here I show that modelled mean PCB concentrations in the blubber of harbour porpoises in the UK have fallen below the previously established threshold for toxic effects (9 mg kg^{-1} lipid) (Kannan et al., 2000). Notwithstanding this, my findings show that current concentrations are still associated with increased rates of infectious disease mortality. Moreover, the inclusion of fifteen years of additional data in the epidemiological analysis, has more than doubled the previous

estimate of the increase in risk associated with a 1mg kg^{-1} lipid increase in PCB concentrations, from 2% to 5% (Hall et al., 2006). Moreover, when I compare the trend for PCBs with other persistent organic pollutants (POPs) in marine mammals (e.g. hexabromadecadane, brominated diphenyl ethers, hexachlorobenzene, hexachlorocyclohexanes, dieldrin, dichlorodiphenyltrichloroethane) it is clear that concentrations of other POPs have declined much more rapidly, despite legislation to control the production and disposal of PCBs being implemented at a similar time to other POPs (Law, 2008; Law et al., 2012a). The slower rate of decline in comparison to other POPs is likely to be a combination of higher initial levels of contamination, greater persistence of PCBs and the continued release of PCBs into the marine environment via diffuse inputs (Stuart-Smith and Jepson, 2017).

These results are in agreement with other studies that have investigated the temporal trends of PCB concentrations in fish, soil and the atmosphere in the UK and globally, which observed downward trends (Lu et al., 2017; Schuster et al., 2010; Zhang et al., 2014). However, the most recent Marine Strategy Framework Directive (MSFD) assessment of mussels and four species of fish in the UK found evidence of nuanced geographical and taxonomical trends with declines occurring in some but not all populations (Maes et al., 2018; OSPAR, 2015a). Notwithstanding the finding that levels in the UK harbour porpoise are declining I observed a slower rate of decline in harbour porpoises when compared with overall trends in fish in the UK, which is likely to be the result of a combination of their high trophic feeding position and relatively long-life span causing a lag in any decline (OSPAR, 2015a).

Despite finding that blubber PCB concentrations for the UK have fallen below the most widely used toxicity threshold I have shown that there are still individuals that are above this threshold. I also found distinct geographical differences in the trends and overall levels. I found that levels in animals that stranded on the west coast of England and Wales are experiencing a slower decline than the rest of the UK and may still be above the toxicity threshold. This variation may be explained by spatial ecology for example, individuals in different geographical areas may have different feeding ecologies, which could affect PCB accumulation rates. However, I believe the most likely explanation is that PCBs are continuing to enter the environment at a higher rate in West England and Wales as this is where PCBs were traditionally produced, therefore there may

be higher amounts of legacy PCBs in the region (Harrad et al., 1994). Indeed, the most recent OSPAR assessment of sediment concentrations found that there was no significant downward trend on the Irish east coast and Scottish west coast and that mean concentrations were higher in the Irish Sea than the Northern North Sea and the Irish and Scottish west coast (OSPAR, 2015b). I observed that there was a period where levels increased in Scotland that corresponded with levels decreasing in the other areas. PCB concentrations are dependent on a number of factors including the eutrophication of systems, the variability of sinks such as degradation and the variability of volatilisation rates and run-off from land (Dachs et al., 2000; Harrad et al., 1994; Komprda et al., 2013). However, I believe it is likely that the differences in concentrations between animals that stranded in Scotland and those that stranded on the west coast of England and Wales were partially driven by the dispersal of PCBs over time from areas where they were produced to previously uncontaminated areas (Gioia et al., 2013; Harrad et al., 1994). This phenomenon has been well documented in the Northern Hemisphere whereby, PCBs are transported from midlatitudes, where they were manufactured, to the Artic (Hung et al., 2001). A hypothesis for the mechanisms of PCB dispersal known as the "differential removal hypothesis" states that the dispersal of PCBs is primarily driven by a gradient of contamination levels whilst, a previous hypothesis states that dispersal is driven by latitudinal temperature gradients (von Waldow et al., 2010; Wania and Mackay, 1993). Both of these explanations would fit with the differences observed between the trends in animals that stranded in Scotland and those that stranded on the west coast England and Wales. Therefore, the higher amounts of PCBs entering the environment in West England and Wales may be transported to Scotland, via environmental transport or animal movements, causing the increase in PCB concentrations in animals that stranded in Scotland between 1994-2005. Hence, it is vital to carry out remediation work in the UK to prevent PCBs entering the environment and ensure levels remain below the toxicity threshold.

Thresholds for the toxic effects of PCBs in harbour porpoises are typically derived from toxicological data on other species for a variety of end points and should therefore be interpreted as an approximation in the absence of more accurate toxicology data. The significant increase in risk of infectious disease mortality associated with PCB blubber concentrations supports previous findings that PCBs *in vivo* and *in vitro* can cause immunosuppression in marine mammals (Desforges et al., 2016; Ross et al., 1996; Sormo et al., 2009). When I compared the results with a

previous study I found a higher exposure odds ratio of 1.05 compared with the previous study's odds ratio of 1.02 (Hall et al., 2006). Despite levels now being below established toxicological thresholds, I found mean PCB concentrations, in the most recent year of the study, were associated with a 41% increase in risk of infectious disease mortality. This suggests that PCB contamination may still be causing an increase in the number of deaths from infectious disease (Kannan et al., 2000) and that the currently used threshold may need to be revised.

In addition to the increase in risk associated with PCB concentrations I also found that age class, season, nutritional condition and sex had a significant effect on the risk of infectious disease mortality. The higher mortality risk found in adults may be due to adults being exposed to a greater number of pathogens, as a result of differences in prey choice, and subadults being more vulnerable than adults to other causes of death including starvation and bycatch (Börjesson et al., 2003; Moore and Read, 2008). It is also possible that the model was unable to differentiate between the effects of age class and PCB concentrations. Hence, the effect of age class was confounded by PCB concentrations and so increased PCB levels in adults were the cause of the higher mortality risk. The higher risk of infectious disease mortality found in females could be caused by a possible weakening of the immune system, during reproduction and lactation, causing them to be more susceptible to infectious disease. Seasonal differences in pathogen types and abundance may explain why infectious disease mortality was greatest in winter (Valderrama Vasquez et al., 2008). Moreover, animals' immune systems may be more likely to be compromised in winter because of colder water and reduced prey availability (Santos et al., 2004).

I found that nutritional condition has a large effect on the odds ratio of infectious disease mortality. The relationship between nutritional condition and death by infectious disease are, however, intrinsically linked as nutritional stress can inhibit the immune system whilst infectious disease can cause nutritional stress. This stress can trigger blubber loss as the animal uses energy stores and this in turn can cause PCBs held in the blubber and other fat-rich body tissues to mobilise into the bloodstream where they are more toxic and can increase the likelihood of the animal contracting and dying from an infectious disease (Hall et al., 2008; Kajiwara et al., 2008). Therefore, in an attempt to control for changes in PCB concentrations in nutritionally stressed animals the PCB concentrations were standardised according to nutritional condition. Whilst there

are still levels of uncertainty in this approach it is still reasonable to conclude that the increased risk of infectious disease mortality is because of higher PCB exposure in the cases than in the controls as opposed to nutritional stress causing increased PCB concentrations.

These findings make an important contribution to understanding more about the temporal trends of PCBs and the possible drivers of infectious disease mortality in cetaceans. However, the scope of the study did not include whether risk varies according to different pathogens or parasites and the analysis does not include non-fatal infections. I also cannot completely rule out that selection bias in the controls may have impacted the findings. While I attempted to select the cases and controls independently of PCB exposure there is a possibility that animals that died of physical trauma had a higher or lower mean PCB exposure than the general population, which could result in an under or over estimation of the odds ratio. However, I suspect that this is likely to have a minimal effect as there is no evidence to suggest that animals that die from physical trauma have altered PCB concentrations. It is also important to note that animal movement and carcass drift may have affected the results. Very little is known about the home range size of harbour porpoises in the UK. Therefore, large home ranges or the movement of carcasses in ocean currents may cause individuals to accrue contaminants in a different location to where they strand. However, a tracking study of harbour porpoises in the Bay of Fundy and Gulf of Maine, Canada, found that none of the tracked individuals left the Gulf of Maine during the 66 day tracking period (Read and Westgate, 1997). Hence, if UK porpoise movements are similar to those in the Gulf of Maine then the geographical boundaries that I have used should be large enough to minimise the impact of animal movement on the results. Moreover, the effect of carcass drift should be minimised by selecting recently deceased carcasses because this increases the likelihood that an animal died close to where they stranded, as only decomposed, gas-filled carcasses can float and drift long distances (Santos et al., 2018).

The association between PCB exposure and infectious disease mortality in cetaceans is well documented in the literature (Desforges et al., 2016). Moreover, there are several epidemiological studies that add to the weight of evidence (Jepson et al., 1999; Ross et al., 1996). This study confirms this association and further indicates an increased risk of mortality from exposure at lower levels than have been previously suggested (Hall et al., 2006; Kannan et al., 2000). However,

it is important to consider that logistic regression modelling attributes a measure of risk for each unit increase in concentration. Yet, toxicity thresholds are typically based on a fixed level at which negative effects occur. If this is the case with PCBs and immunosuppression, then there may be no increased risk below a certain concentration. However, a number of cetacean studies both *in vivo* and *in vitro* have demonstrated that PCBs cause immunosuppression in a dose-dependent manner (Desforges et al., 2016; Ross et al., 1996; Sormo et al., 2009). Further effort is required to understand whether risk increases from zero or whether there are safe limits whereby no negative effects occur. Nonetheless, I have found a significant association between PCB blubber concentrations and infectious disease mortality, which is particularly important in the context of other cetacean species. Specifically, in very coastal species such as bottlenose dolphins, which have been shown to have high PCB concentrations, in the UK and in some enclosed Mediterranean areas (Jepson et al., 2016). Similarly, killer whales have been shown to accumulate the highest concentrations of PCBs in cetaceans, and populations in the UK and Strait of Gibraltar face an immediate threat of extinction from exposure at population level (Desforges et al., 2018).

This is the first epidemiological study to show that PCBs are still a threat to harbour porpoises in the UK despite mean concentrations having fallen below established levels of toxicological concern (9 mg kg^{-1} lipid) (Kannan et al., 2000). I have shown for the first time that although levels of PCBs in UK harbour porpoises are declining, concentrations still appear to be associated with an increased risk of infectious disease mortality. Moreover, the rate of decline of PCBs appears to be slow when compared with studies on other pollutants. In addition, I found considerable variation in concentrations and rates of decline between the sub-regions, which suggests PCBs are continuing to enter the environment. These findings have serious management implications as they suggest that more remediation action is required to reduce or prevent further discharges to ensure that levels continue to decline and remain below the thresholds for toxic effects. I also suggest that the risk of contamination from secondary sources should be mitigated via strict international compliance with the Stockholm Convention (UNEP, 2017). This study makes an important contribution towards understanding the trends of pollutant exposure in cetaceans and assessing associated risks; however, further research is required to quantify more robust toxic thresholds for chronic exposure to PCBs.

Chapter 4 - The Relationship Between Polychlorinated Biphenyls and Testes Weights in Harbour Porpoises (*Phocoena phocoena)*

4.1 Abstract

Polychlorinated biphenyls (PCBs) are highly toxic and persistent aquatic pollutants that are known to bioaccumulate in a variety of marine mammals. They have been associated with reduced recruitment rates and population declines in multiple species. Evidence to date documents effects of PCB exposures on female reproduction, but few studies have investigated whether PCB exposure impacts male fertility. Using blubber tissue samples of 99 adult and 168 juvenile UK-stranded harbour porpoises (*Phocoena phocoena*) collected between 1991 and 2017, I show that PCBs exposures are associated with reduced testes weights in adults with good body condition. In animals with poor body condition, however, the impact of PCBs on testes weights was reduced, conceivably due to testes weights being limited by nutritional stress. This is the first study to investigate the relationship between PCB contaminant burden and testes weights in cetaceans and represents a substantial advance in our understanding of the relationship between PCB exposures and male reproductive biology in cetaceans. As testes weight is a strong indicator of male fertility in seasonally breeding mammals, I suggest the inclusion of such effects in population level impact assessments involving PCB exposures. Given the re-emergent PCB threat these findings are globally significant, with potentially serious implications for long-lived mammals. I show that more effective PCB controls could have a substantial impact on the reproductive health of coastal cetacean species and that management actions may need to be escalated to ensure adequate protection of the most vulnerable cetacean populations.

4.2 Introduction

In this chapter, I build on the findings of **Chapters 2 and 3**, where I demonstrated that PCBs pose the greatest threat to marine mammal health and are associated with increased risks of infectious disease mortality to further explore the impacts of PCBs on cetaceans by investigating the impacts of PCBs on reproductive physiology.

Polychlorinated biphenyls (PCBs) are a group of toxic chemicals compounds that were banned in the EU in the mid-1980s and have been linked to numerous health effects in humans and wildlife (Folland et al., 2016; Liu et al., 2010). PCBs continue to enter the marine environment from diffuse sources and those still in 'open application', such as in paints and sealants, are thought to contribute most to contemporary environmental releases (Defra, 2013; Jartun, 2011; Stuart-Smith and Jepson, 2017). Several wildlife populations in Europe, both terrestrial and marine, have experienced decreases in PCB tissue concentrations (e.g Williams et al., 2020b), which in some instances have coincided with population recoveries (Roos et al., 2012). However, PCB concentrations in European cetaceans still pose a toxicological threat and are associated with suppression of the immune and reproductive systems (Jepson et al., 2016; Murphy et al., 2015; Williams et al., 2020b).

Numerous studies have found associations between PCB exposure and reduced reproductive output through reduced fertility in females, increased embryonic loss and increased calf mortality (Murphy et al., 2015; Schwacke et al., 2002). The possible impacts of PCB exposure on male fertility have yet to be investigated and remain largely unknown. Studies on other mammals have, however, shown that PCB exposure inhibits the male reproductive system. For example, human epidemiological studies have found negative associations between PCB exposure, sperm motility and circulating testosterone levels in men (Goncharov et al., 2009; Meeker and Hauser, 2010). In other mammals, PCB exposure has been shown to cause: smaller seminal vesicles, epididymides and testes; decreased sperm levels and spermatid counts; and reduced plasma testosterone levels (Ahmad et al., 2003; Kuriyama and Chahoud, 2004).

Determining the effect of PCB exposure on measures of male fertility is a challenging task in cetaceans. Measuring sperm quality parameters and circulating hormones would require live capture, which is ethically and logistically unfeasible. However, testes weights, of harbour porpoises (*Phocoena phocoena*) and other marine mammals, have been shown to correlate with sperm production, which is a widely used measure of male fertility (Neimanis et al., 2000; Stewardson et al., 1998). Therefore, testes weights, measured in stranded animals examined post-mortem, may provide a valid proxy for reproductive fitness and provide useful insights into the relationship between PCB exposure and male fertility.

Testes weights in harbour porpoises vary greatly between breeding and non-breeding seasons as a consequence of changes in spermatogenic activity (Neimanis et al., 2000; Orbach et al., 2019). Harbour porpoises are referred to as sperm competitors whereby their only known form of competition is the process by which the spermatozoa of two or more males compete to fertilise a given set of ova (Fontaine and Barrette, 1997). Selective forces for sperm competition in mammals are thought to have caused increased relative testes sizes, to sustain the greater rates of spermatogenesis required, to maximise ejaculate volume and number of inseminations (Dixson and Anderson, 2004). Greater testes weights have also been associated with increased sperm motility in primates as a consequence of gamete level changes (Anderson and Dixson, 2002). Therefore, in mammals that are sperm competitors, a reduction in relative testes weights may reduce an individual's chances of successful reproduction, which could have wider impacts on the fitness of the entire population (Fontaine and Barrette, 1997). If PCB burdens can impact both male and female fertility this could have serious consequences on the long-term population viability of marine apex predator populations that are highly exposed to PCBs.

In this chapter, I have used the Cetacean Strandings Investigation Programme (CSIP) dataset to investigate, for the first time, the relationship between PCB blubber concentrations and testes weights in harbour porpoises. It has been shown previously, in this population, that the reproductive output of healthy females is almost half that of other, less contaminated, populations and it has been hypothesised that reproductive dysfunction in these individuals may be related to PCB exposure (Murphy et al., 2015; Ólafsdóttir et al., 2003). This work is an essential first step towards improving our understanding of the possible effects of PCBs on male reproduction. This

will help determine whether current risk assessments, which do not account for the possible compounding impacts of reduced male fertility, are appropriate or whether they potentially underestimate the risk posed to populations.

4.3 Materials and Methods

4.3.1 Sampling

The blubber PCB concentrations and testes weights of 99 adult and 168 juvenile male harbour porpoises that stranded in the UK between 1991 and 2017, were determined from necropsies carried out according to standard post-mortem procedures for cetaceans (Law et al., 2006). The post-mortems were carried out at the following three institutes: the Scottish Marine Animal Stranding Scheme (SMASS) (n=30); the University of Exeter (UoE) (n=17) and the Zoological Society of London (ZSL) (n=220) (Figure 4-1). The individuals selected for PCB analysis were prioritised according to their state of decomposition using the scoring system set out by (Law et al., 2006). Ninety-two percent of the carcasses were classified as extremely fresh (*"as if just died, no bloating"*) or slightly decomposed ("*slight bloating, blood imbition visible*"). Fresher carcasses were prioritised to minimise the impact of changes in pollutant tissue concentrations and dispersion that are associated with decomposition (Law et al., 2006). The individuals analysed were otherwise a representative sample of the strandings that occurred over the period.

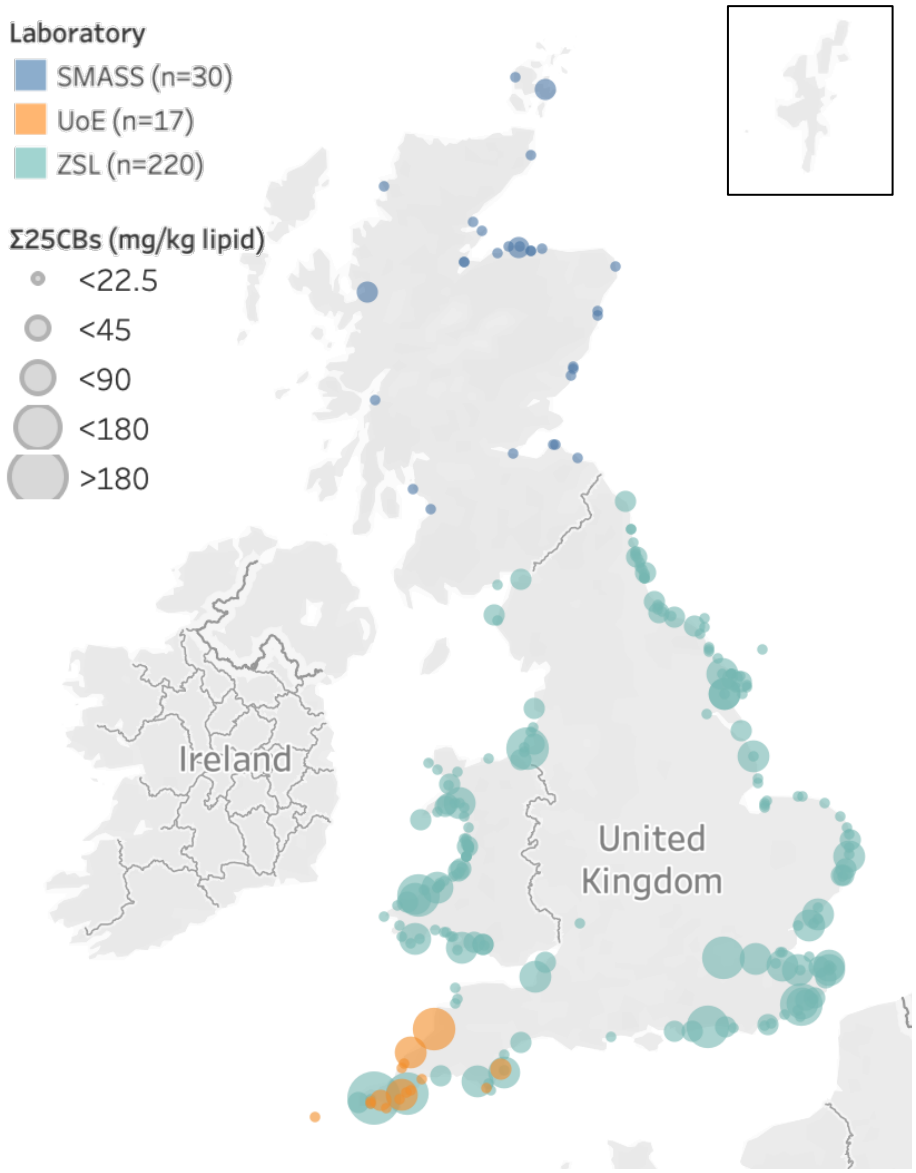


Figure 4-1: Geographic locations of the adult male individuals that stranded and were analysed to obtain blubber concentrations for the sum of 25 selected congeners of polychlorinated biphenyls (Σ25CBs). The colours of the dots represent the laboratories where the animals were necropsied: Scottish Marine Animal Stranding Scheme (SMASS), University of Exeter (UoE) and the Zoological Society of London (ZSL). The size of the dots represents the concentration of Σ25CBs (mg/kg lipid) measured in the blubber. The scaling sizes were chosen to reflect the findings of Hall et al., (2006) whereby Σ25CBs concentrations of 45 mg/kg lipid equate to a doubling of risk of infectious disease mortality.

4.3.2 PCB Analysis

A standardised methodology was used to extract and preserve the blubber samples for PCB analysis (Law, 1994). Briefly, blubber samples were taken from the left side of the body, at the caudal insertion of the dorsal fin and preserved at −20 °C (Law, 1994). The Cefas laboratory (Lowestoft) determined the concentrations of the sum of 25 individual chlorobiphenyl (CB) congeners (∑25 CBs) (on a mg kg^{-1} wet weight basis) using a method that was validated following participation in the QUASIMEME (Quality Assurance of Information for Marine Environmental Monitoring in Europe) laboratory proficiency scheme and followed the recommendations of the International Council for the Exploration of the Sea (ICES) (de Boer and Law, 2003; de Boer and Wells, 1997; ICES, 1998; Webster et al., 2013). In cases where the congener concentrations were below the limit of quantification ((<0.0003 or <0.0004 mg kg^{-1} wet weight), I set the concentration at half the limit (Law et al., 2012a). The numbers of the International Union of Pure and Applied Chemistry CBs congeners analysed were: 18, 28, 31, 44, 47, 49, 52, 66, 101, 105, 110, 118, 128, 138, 141, 149, 151, 153, 156, 158, 170, 180, 183, 187, 194. This selection was chosen to ensure incorporation of the seven PCBs prioritised for international monitoring by ICES (∑ICES7) and included those that are relatively abundant in commercial PCB mixtures with a broad range of chlorination. The sum of the 25 individual CB congener concentrations was calculated and normalized to a lipid basis (mg kg^{-1} lipid) by extracting hexane from the blubber and calculating the hexane extractable lipid content (Webster et al., 2013).

4.3.3 Pathological and Statistical Analyses

As part of the pathological investigations, certain attributes were determined for each animal in the study. Sexual maturity was determined using gonadal appearance and, where undertaken, looking for histological evidence of spermatogenesis in male testes (Murphy, 2008). I validated this classification by looking at the differences in testes weights between mature and immature individuals. In cases where immature individuals had testes weights that were greater than the minimum testes weight for mature individuals (n=4) I used age data to further validate the classification. Exact age was determined by quantification of growth layer groups from analyses of decalcified tooth sections using the methods outlined by (Rogan et al., 2004) and (C Lockyer, 1995).

Testes were removed from each animal and weighed as per standard post mortem protocols (Law et al., 2006). For each individual, the arithmetic mean of the right and left testes weights was calculated. In some cases (n=33/267) only one testis was weighed, either as result of protocol variations and time constraints (n=32) or due to the absence of the testis as a result of scavenger damage (n=1). In these cases, the weights of the single testes were used, as I found there was no statistical difference between left and right testes weights (two sample t-test , p=0.77). Date of stranding was used to categorise strandings into breeding and non-breeding seasons and I assumed death occurred during the same season that the animal stranded. I defined the breeding season as the 1st May to the 31st of July (Kesselring et al., 2019) and compared mean testes weights across all months of the year (Figure 4-3). For smaller cetaceans like the harbour porpoise, a basic index of weight to length ratio is thought to be the most appropriate metric of body condition and is widely acknowledged as a good predictor of fitness in marine mammals (Beauplet and Guinet, 2007; Christiansen et al., 2014; Kershaw et al., 2017). Body weight and length followed a power relationship therefore, I fitted a power regression model and extracted the residuals to obtain a metric that could be used as a proxy for body condition, as described in **Chapter 3**.

I excluded immature individuals from further statistical analysis because sperm production, which is associated with testes weights and fertility, only occurs in mature individuals (Kesselring et al., 2019). I did not expect to observe any effect of PCBs on testes weights in immature individuals because they are not sexually active so there is no known mechanism by which PCBs could affect testes weight. I validated this approach by modelling testes weights against selected covariates for immature individuals and this analysis is shown at the end of the results section.

I carried out all of the analyses using the statistical software R (version 3.4.3) (R Core Team, 2016). Prior to model fitting I carried out extensive data exploration to test for collinearity between variables and remove individuals with missing body weight, length or testes weights (Table 4-1). I investigated the relationship between the mean testes weight (g) and PCB blubber concentrations (mg kg^{-1} lipid wt.) by fitting linear mixed models (LMMs) to selected variables that could explain the variability in the data (Chambers and Hastie, 1992; Venables et al., 2002). Mean testes weights and PCB blubber concentration were natural logarithm transformed prior to statistical analysis so that the assumptions of homoscedasticity and normality were met. Mean testes weight was the

response variable. The potential predictor variables included in the full model were selected according to the biological rationale that they could impact testes weights. These were nutritional condition, breeding season and the natural logarithm of PCB blubber concentration, with a three-way interaction. I included laboratory as a random effect (Figure 4-1) in the model to account for any sources of variation between laboratories, including whether testes were weighed with or without the epididymis. I assumed that the inclusion or exclusion of the epididymis would only impact the intercepts and would have no effects on the coefficient estimates. I validated this approach by ensuring that the relationship between length and mean testes weight was consistent across the laboratories (Figure 4-2). I did not include the longitude and latitude of the stranding location in the model as I did not observe any spatial variation in testes weights (see Table 4-2, Table 4-3). Furthermore the inclusion of latitude and longitude in the model was likely to confound any effect from PCB exposure as PCB blubber concentrations have been shown to vary spatially in UK-stranded harbour porpoises (Williams et al., 2020b). The natural logarithm of body length was included as an offset term to scale testes weights. I used body length as opposed to body weight because body weight included testes weights and was correlated with nutritional condition (Pearson's correlation, $r=0.92$, $p<0.01$).

Table 4-1: Results of the Pearson's product-moment correlation tests to test for collinearity between model variables

Test	**Correlation Coefficient**	**t**	**df**	**p-value**
MATURE: Nutritional condition ~ log(PCB concentrations)	-0.26	-2.68	97	0.01
IMMATURE: Nutritional condition ~ log(PCB concentrations)	-0.02	-0.26	165	0.80
MATURE: Nutritional condition ~ Length	0.00	0.01	97	0.10
IMMATURE: Nutritional condition ~ Length	0.08	-0.98	165	0.32

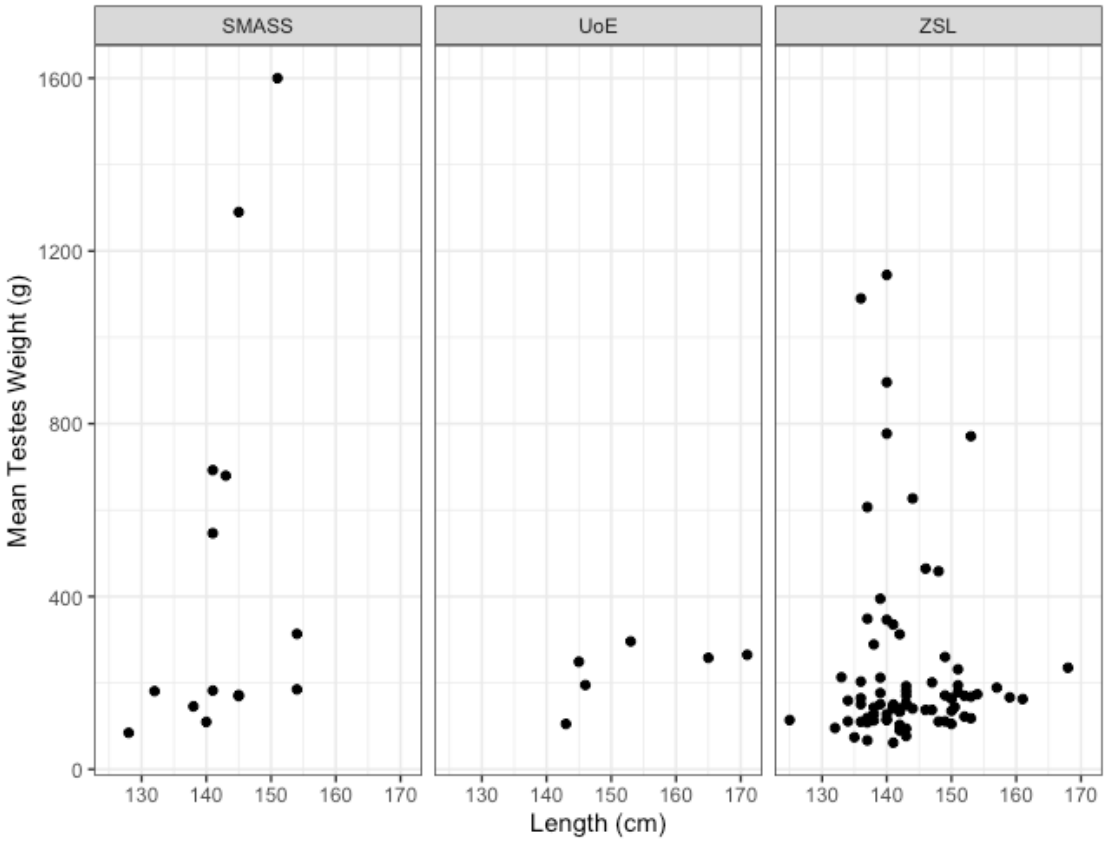


Figure 4-2: Body length (cm) plotted against mean testes weight (kg) for each of the three UK laboratories (SMASS: Scottish Marine Animal Stranding Scheme, UoE: University of Exeter, ZSL: Zoological Society of London)

Table 4-2: Results from analysis of variance testing of average testes weight against longitude for all individuals that had available testes weights and stranding location date (n=1091).

Variable	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Longitude	1	1.5	1.46	0.58	0.45
Residuals	257	654.6	2.55		

Table 4-3: Results from analysis of variance testing of average testes weight against longitude for all individuals that had available testes weights and stranding location date (n=1091).

Variable	**Df**	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Latitude	1	1	0.71	0.23	0.63
Residuals	1085	3303	3.04		

I tested all possible variable combinations to obtain several candidate models, which were ranked according to their AIC (Akaike's Information Criterion) values (Akaike, 1973; Barton, 2015). The final prediction was obtained by averaging the set of plausible models ($\Delta AIC < 4$) from the candidate models. I validated the models by checking the distribution of the residuals and plotting them against selected variables and assessing the variance.

To model the testes weights of immature harbour porpoises I used the same model averaging approach and covariates as described for mature harbour porpoises. However, in the model for immature individuals the natural logarithm of body length was included as a predictor variable rather than an offset term. This was because, unlike in mature individuals, mean testes weight variance increased with length. In addition, length varied significantly between the breeding and non-breeding seasons. This is likely to be as a consequence of the calving season coinciding with the breeding season (Christina Lockyer, 1995). Therefore, including length as an offset term could have had a confounding influence on the effect of breeding season on immature individuals.

Table 4-4: Results of the Kruskal-Wallis rank sum tests for the natural logarithm of length (cm) against breeding season for immature and mature individuals

Test	**X^2**	**df**	**p-value**
MATURE: Log(Length) ~ Breeding Season	0.95	1	0.33
IMMATURE: Log(Length) ~ Breeding Season	17.12	1	0.00

4.4 Results

4.4.1 Mature individuals

The final form of the model, obtained by averaging the set of plausible candidate models, included breeding season, nutritional condition, PCB blubber concentrations and two-way interaction terms between breeding season, PCB concentrations and nutritional condition (Equation 4-1).

$$\log \sum \text{Mean testes weight} \sim \beta_0 + \beta_1 Breeding\ Season + \beta_2 Nutritional\ condition + \beta_3 \Sigma 25\text{CBs} + \beta_4 Breeding\ Season * Nutritional\ Condition + \beta_5\ Nutritional\ Condition * \log(\Sigma 25\text{CBs}) + \text{offset}(\log(\text{Length})) + |\ Laboratory$$

Equation 4-1: The final form of the model for testes weight against seleected covariates obtained by averaging the set of plausible candidate models. The coefficients are weighted according to the frequency of their presence in the plausible candidate models as per the model selection table available in the Appendix Table A- 4.

From the averaged model, I found that the relationship between PCB blubber concentrations and testes weights is dependent on nutritional condition, whereby PCBs have a greater influence on testes weights in animals that are in good body condition (Figure 4-4, Figure 4-5, Table 4-6). I found that animals in poor nutritive condition were predicted to have the lowest testes weights (Figure 4-5). Animals in good nutritional condition with relatively high PCB concentrations also had suppressed testes weights, while animals with good nutritional condition and low PCB blubber concentrations had the highest testes weights. The mean concentrations of each congener and the PCB Toxic Equivalencies (TEQs) for mature and immature individuals are shown in Table 4-5.

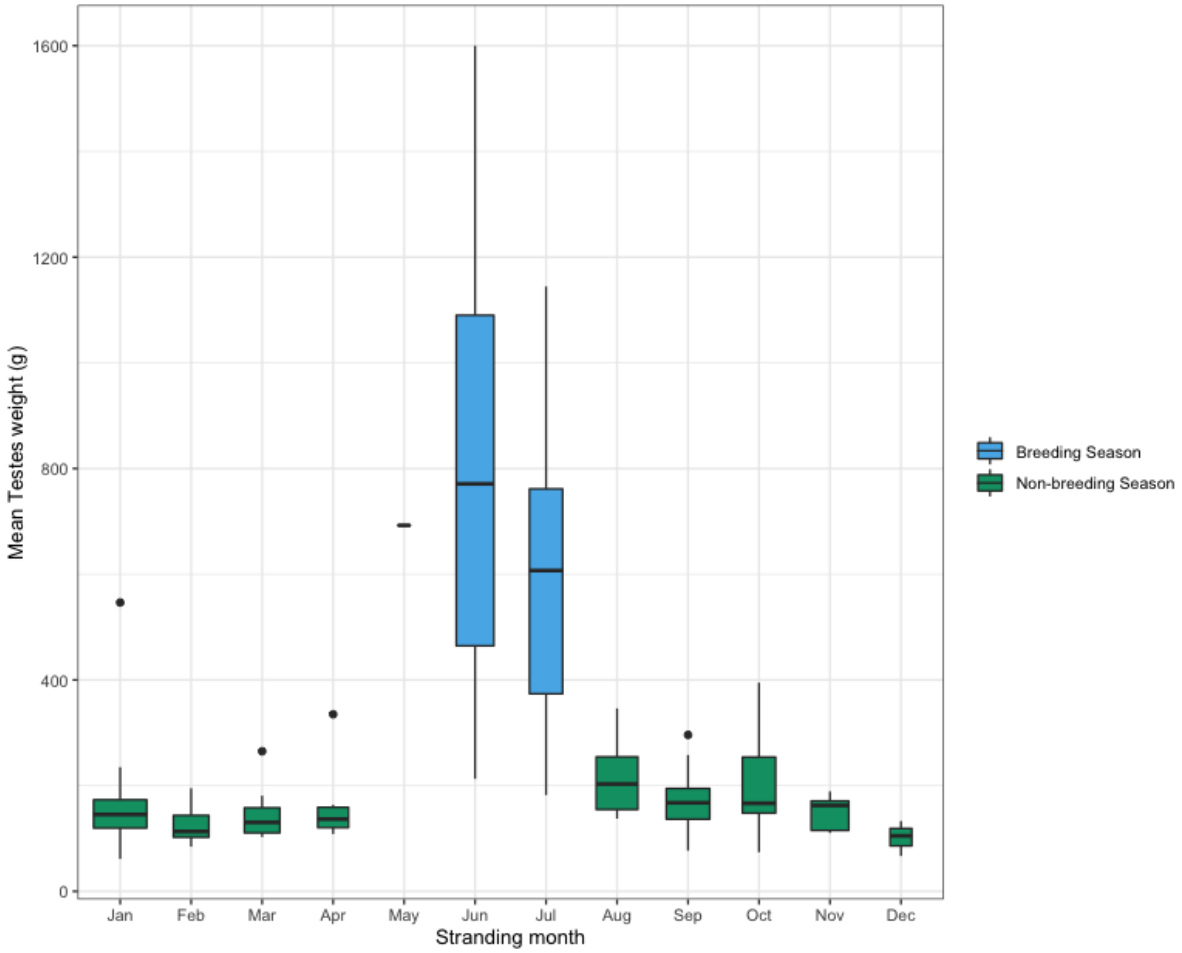


Figure 4-3: Mean testes weights (g) of harbour porpoises (Phocoena phocoena) stranded in the UK between 1991 and 2017 by month of stranding for mature individuals (n=99). The width of the boxes is proportional to the sample size. In months that do not contain more than one data point a dash is displayed. The boxes are coloured by the breeding season classification, green for non-breeding season, blue for breeding season. The horizontal lines represent the median value. The lower and upper hinges correspond to the first and third quartiles. The upper whisker extends from the upper hinge to the largest value unless the largest value is greater than 1.5 times the interquartile range (IQR) in which case the upper whisker is limited at 1.5 × IQR. The lower whisker extends from the lower hinge to the smallest value unless the smallest value is greater than 1.5 times the interquartile range (IQR) in which case the lower whisker is limited at 1.5 × IQR. Data beyond the end of the whiskers are plotted individually as points.

Table 4-5: Mean concentrations of each polychlorinated biphenyl congener for immature and mature individuals. Summed concentrations of the seven International Council for the Exploration of the Sea PCBs (ΣICES7) and the 25 PCB congeners (Σ25CBs) are detailed. Asterisks denote congeners for which toxic equivalency factors (TEFs) exist and were therefore included in the toxic equivalency (TEQ) calculations shown at the bottom of the table.

IUPAC Congener number	**Immature (n=167)**	**Mature (n=99)**
	Mean Concentration (mg/kg lipid)	**Mean Concentration (mg/kg lipid)**
CB.18	0.04	0.05
CB.28*	0.02	0.01
CB.31	0.01	0.01
CB.44	0.03	0.02
CB.47	0.10	0.10
CB.49	0.07	0.04
CB.52*	0.42	0.79
CB.66	0.30	0.45
CB.101*	0.43	0.31
CB.105	0.16	0.21
CB.110	0.09	0.08
CB.118	0.64	0.45
CB.128	0.31	0.37
CB.138*	2.56	5.14
CB.141	0.05	0.03
CB.149	1.45	2.62
CB.151	0.41	0.67
CB.153*	3.43	7.51
CB.156	0.15	0.17
CB.158	0.13	0.22
CB.170	0.40	0.95
CB.180*	0.93	2.25
CB.183	0.33	0.71
CB.187	1.05	2.54
CB.194	0.10	0.25
ΣICES7	8.41	16.45
Σ25CBs	13.57	25.92
	Toxic equivalencies (TEQ)	
	$2.83\text{^}10^{-5}$	$2.48\text{^}10^{-5}$

Predictably, the season of stranding had the largest effect on adult testes weights whereby individuals that stranded during the breeding season had significantly higher testes weights than animals that stranded in the non-breeding season (Table 4-6, Figure 4-3). Nutritional condition also heavily influenced testes weights such that individuals with better body condition had higher mean testes weights (Figure 4-4). The effect of nutritional condition on testes weights was greater during the breeding season than during the non-breeding season.

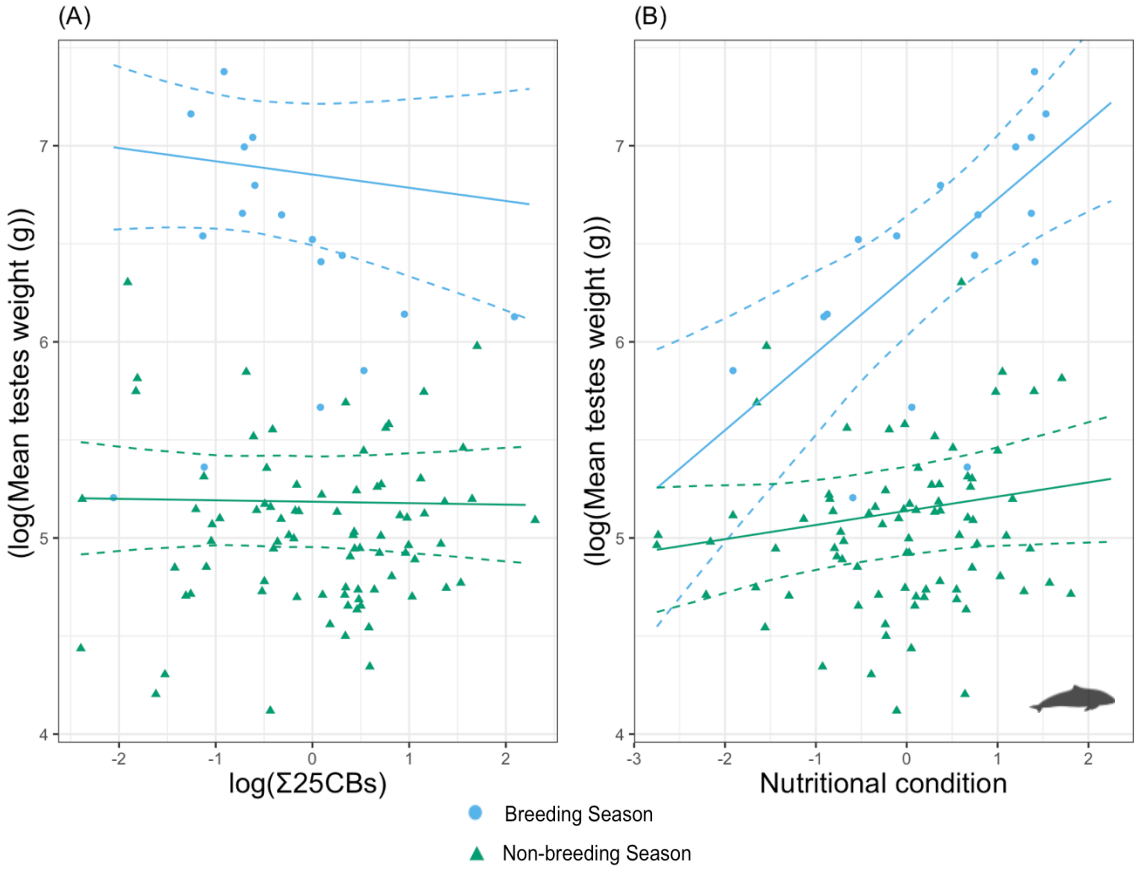


Figure 4-4: The log of individual mean testes weights (g) of 99 adult harbour porpoises (Phocoena phocoena) stranded in the UK between 1991 and 2017 plotted against the log of blubber concentrations of the sum of 25 chlorobiphenyl congeners (∑25CBs) (mg kg^{-1} lipid) with: (A) Nutritional condition set at the mean value of the third quartile, (B) Nutritional condition at the mean concentration of ∑25CBs. The solid lines represent the model predictions for each season and the dashed lines represent 95% confidence intervals (twice the standard error).

*Table 4-6: Summary statistics of the averaged linear mixed model fitted to the strandings data for mature harbour porpoises (Phocoena phocoena). Natural log transformed mean testes weight (g) was the response variable. The continuous variables were zero centred and scaled. Coefficient estimates were calculated based on an animal that stranded during the breeding season. *indicates statistical significance (p<0.01)*

Variable	Estimate	Std. Error	Adjusted SE	z value	Pr(>|z|)
Intercept	1.39	0.15	0.15	9.38	0.00*
Season:Non-breeding	-1.22	0.11	0.11	11.01	0.00*
Nutritional Condition	0.36	0.11	0.11	3.30	0.00*
Log(Σ25CBs)	0.09	0.06	0.06	1.45	0.15
Non-breeding:Nutritional Condition	-0.30	0.10	0.10	2.95	0.00*
Nutritional Condition:log(Σ25CBs)	-0.14	0.04	0.04	3.49	0.00*

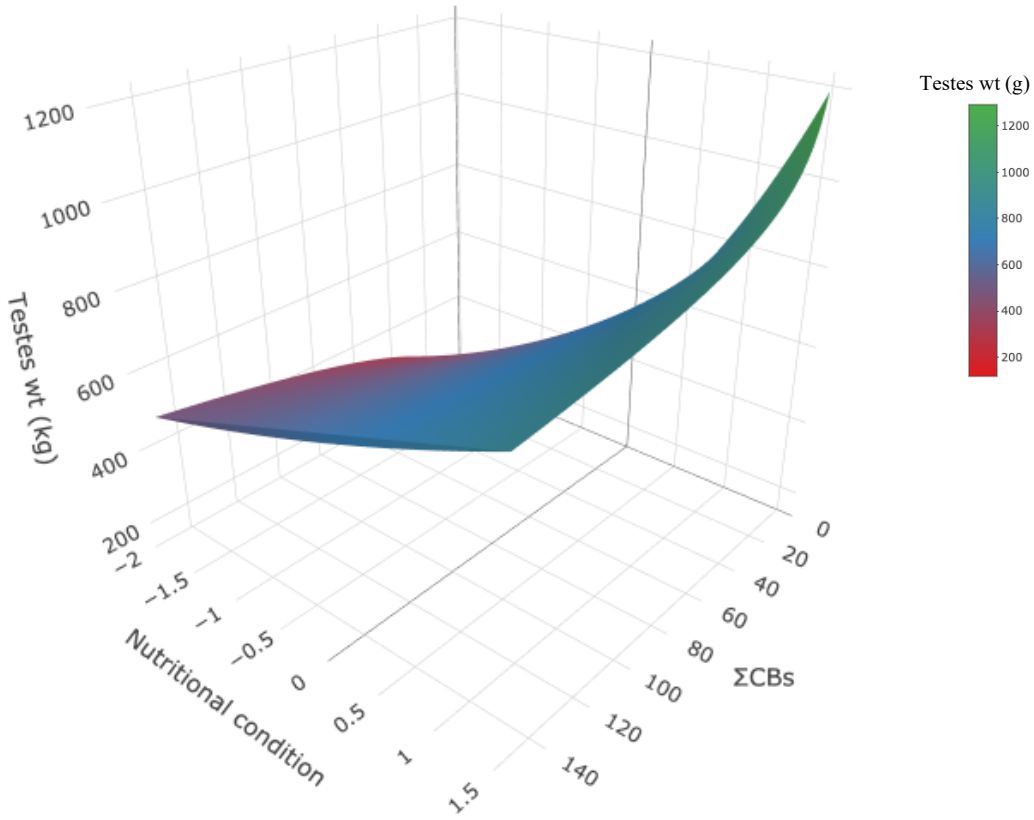


Figure 4-5: Surface plot of predicted testes weights (kg) against nutritional condition and PCB blubber concentrations, (∑CBs) (mg kg^{-1} lipid), for mature individuals during the breeding season. The surface plot is colour graded according to predicted testes weights (kg). Red indicates the lowest weights; green indicates the highest weights.

4.4.2 Immature individuals

The final form of the averaged model for immature individuals included breeding season and length (Table 4-7). Unlike for mature individuals, nutritional condition, Σ25CBs were not included. Length was the only significant predictor in the model.

*Table 4-7: Summary statistics of the generalised linear model fitted to the strandings data for immature harbour porpoises (Phocoena phocoena). Mean testes weight (g) was the independent variable. Coefficient estimates were calculated based on an animal that stranded during the breeding season. *indicates statistical significance (p<0.01)*

Variable	Estimate	Std. Error	Adjusted SE	z value	PR(>|Z|)
Intercept	-16.19	1.06	1.06	15.25	0.00*
Log(length)	3.95	0.23	0.23	16.93	0.00*
Season:non-breeding	-0.18	0.08	0.09	2.11	0.04

4.5 Discussion

Here, I have shown that PCB concentrations found in the blubber of mature harbour porpoises in good nutritional condition, are negatively associated with testes weights. The available scientific literature clearly documents that mammalian testes weights are likely to be a good indicator of reproductive potential (Fontaine and Barrette, 1997) in a great number of species as they correlate with sperm production rates (Moller, 1989), which are associated with fertility and reproductive health. Moreover, reduced testes weights, either associated with or as a consequence of PCB exposures, have been widely reported along with other indicators of reproductive toxicity (reduced sperm counts and motility, semen volume and serum testosterone concentrations) in humans, rats and other vertebrates (Kuriyama and Chahoud, 2004; Meeker and Hauser, 2010). If lower testes weights are indicative of reduced fertility in cetaceans, then these findings are extremely concerning as they suggest that the reproductive abilities of animals in good nutritional health, exposed to high levels of PCBs, are reduced. These 'healthy' individuals are, arguably, the individuals that are most likely to reproduce in the population therefore, exposure to PCBs may cause individuals that would have successfully reproduced to be outcompeted. If a sufficient number of males were impacted in this region, this may have a direct impact of fecundity and

reduce population fitness, as a consequence of lower genetic diversity through reduced competition.

Despite the global ban on PCB use and manufacture over three decades ago, blubber concentrations in cetaceans are still associated with low recruitment and increased infectious disease mortality, which have been linked to population declines (Desforges et al., 2018; Jepson et al., 2016; Williams et al., 2020b). These results suggest the impacts of PCB exposure on male fertility may offer a partial explanation as to why pregnancy rates, in this population of harbour porpoises, are less than half of those observed in other less contaminated populations (Murphy et al., 2015; Ólafsdóttir et al., 2003). Similarly, impacts on male fertility may be an additional driver for reduced birth rates that are associated with PCB exposure in bottlenose dolphins (Schwacke et al., 2002). This is important in the context of other higher trophic level species, such as killer whales, that accumulate the highest concentrations of PCBs and therefore, face the greatest toxicological threat (Jepson et al., 2016). The impacts of PCB exposure in killer whales are compounded by their low birth rates, as a consequence of their protracted periods of maternal care, which make it difficult for populations to respond rapidly to increases in mortality rates (Evans and Stirling, 2002). Consequently, several populations that live close to industrialised areas face an immediate threat from exposure (Desforges et al., 2018).

Nutritional condition and breeding season were significant predictors of testes weights. Testes weights were significantly higher during the breeding season, which is reflective of the increase in spermatogenic activity known to occur during this period (Neimanis et al., 2000). Individuals with poorer nutritional condition were predicted to have lower testes weights than individuals in good nutritional condition. Investing in reproduction is only possible when energy demands are met and chronic nutritional stress, in marine mammals, has been linked to population declines and poor pregnancy success rates (Trites and Donnelly, 2003; Wasser et al., 2017). Prolonged fasting has similarly been shown to reduce sperm count and decrease testes weights in rodents (Eliza et al., 1997; Samuel et al., 2015). This is likely to be because of a lack of availability of nutritional elements that are vital for spermatogenesis (Cheah and Yang, 2011). While I have found that poor body condition is the predominant driver of reduced testes weights, I have shown, in chapter 3, that PCB concentrations are higher in nutritionally compromised individuals (Williams et al.,

2020b). Hence, the effect of PCBs is unlikely to be directly observed in animals with reduced body condition but may still contribute to reduced reproductive output within the population.

I have shown that in animals with good nutritional condition, adult testes weights are negatively associated with PCB concentrations. However, there are some biases associated with strandings data that are important to consider. Strandings data may be overrepresented by older animals with naturally lower fertility and reduced testes weights as a consequence of reduced spermatogenic activity. This could confound the results because PCB levels in cetaceans accumulate with age therefore, older animals tend to have higher PCB concentrations. However, although senescence has not been well documented in harbour porpoises, pregnancies have been documented in animals older than 15 (Learmonth et al., 2014). Thus, given that (where age data was available n = 45) the sample of mature individuals had very few individuals above the age of 15 (n = 2), the sample should represent a fertile portion of the population (Appendix Table A- 5). To ensure the findings were not affected by individual variation in timing of the breeding season, the classification of breeding season was based on the consensus of a number of sources (Fontaine and Barrette, 1997; Kesselring et al., 2019; Learmonth et al., 2014; Neimanis et al., 2000), which was consistent with the seasonal variation in testes weights I observed in the data. Strandings data can also be overrepresented by individuals in poor nutritional condition or ill health. This can influence results as animals suffering from disease have higher PCB concentrations as a consequence of blubber loss (Hall et al., 2006; Kajiwara et al., 2008). An important strength of this study is that I have included infectious disease and trauma cases in the analysis. This has allowed me to compare animals in poor and good nutritive condition and reveal the complex relationship between nutrition, PCB exposure and testes weights. The sample size for each cause of death category is comparable and shown in the Appendix Table A- 6.

The timing of exposure to contaminants can have a profound impact on the overall effects throughout an individual's lifetime. There is a weight of evidence suggesting that *in utero* exposure to endocrine disrupting chemicals, in humans for example, can cause permanent reproductive suppression by disrupting development of the male reproductive organs (Bergman et al., 2013). Therefore, the impact of PCB exposure on testes weights in male cetaceans could be partially driven by the level of exposure of their mothers to PCBs during pregnancy and lactation (Borrell

et al., 1995; Williams et al., 2020). Exposure in adults can be considered to cause transient effects, yet foetal or neonatal exposure can result in permanent effects because contaminants impact development of the endocrine and physiological systems (Bergman et al., 2013). These effects can also be transgenerational as chromosomal damage will often be inherited (Skinner et al., 2011). This means exposure to PCBs may cause long term damage to the reproductive health of a population that will persist regardless of current exposure levels.

Despite being banned over 35 years ago in the UK (*Control of Pollution (Supply and Use of Injurious Substances) Regulations 1986),* PCBs continue to enter the marine environment and remain at levels still associated with reduced recruitment rates in several cetacean populations. It is imperative that more is done to reduce the input of legacy PCBs into the environment. Strict international compliance with the *Stockholm Convention on Persistent Organic Pollutants* (UNEP, 2017) and EU legislation (*Regulation (EU) 2019/1021 of the European Parliament and of the Council*, 2019) would help to minimise the risk of contamination from secondary sources and ensure stockpiled PCBs and PCBs in 'open application' are destroyed. Thereby, preventing further discharge into the environment. At present, many parties are falling short of their commitments to the Convention and many European nations are unlikely to achieve their 2025 and 2028 targets. Harbour porpoises are a coastal species and therefore UK-managed effective PCB controls could have a substantial impact on their population health and should be prioritised accordingly. Further research is urgently required to identify the potential mechanisms by which PCBs may reduce testes weights and explore other possible PCB mediated impacts on male reproductive health. Future research can build on the findings I have presented here to answer these questions through the use of histopathological examination or other markers of reproductive fitness (Holt et al., 2004; Kesselring et al., 2019). This would help to establish whether current risk assessments, which do not account for impacts on male fertility, are underestimating the risk of PCBs, and provide vital information to improve the management of cetacean populations both in the UK, and around the globe.

Chapter 5 - The Influence of Age, Class, Sex and Stranding Location on Polychlorinated Biphenyl Congener Profiles in Harbour Porpoises (*Phocoena phocoena)*

5.1 Abstract

Polychlorinated biphenyls (PCBs) are a group of 209 persistent and bio-accumulative toxic pollutants present as complex mixtures in human and animal tissues. Harbour porpoises accumulate some of the highest levels of PCBs because they are long-lived mammals that feed at a high trophic level. Studies typically use the sum of a suite of individual chlorobiphenyl congeners (CBs) to investigate PCBs in wildlife. However, toxic effects and thresholds of CB congeners differ, therefore population health risks of exposure may be under or over-estimated dependent on the congener profiles present. In this chapter, I found congener profiles varied with age, sex and stranding location, particularly between adult females and juveniles. I found that adult females had the highest proportions of *octa*-chlorinated congeners whilst juveniles had the highest proportions of *tri*- and *tetra*-chlorinated congeners. This is likely to be a consequence of pollutant offloading between mothers and calves during lactation. Analysis of the individual congener toxicities found that juveniles were exposed to a more neurotoxic CB mixture at a time when they were most vulnerable to its effects. These findings are an important contribution towards our understanding of variation in congener profiles and the potential effects and threats of PCB exposure in cetaceans.

5.2 Introduction

Polychlorinated biphenyls (PCBs) are a large group of chemically related persistent and bio-accumulative pollutants known to cause neurotoxic and endocrine disrupting health effects in humans and wildlife (Faroon et al., 2003; Folland et al., 2016). PCBs typically occur in the food chain as complex mixtures and their mechanisms of toxicity and the potencies of individual PCB congeners vary according to differences in chemical structure (Hansen, 1998). Having demonstrated in the previous chapters that PCBs post the greatest risk to marine mammal health and that they are associated with impacts of reproductive physiology and increased risk of infectious disease mortality; in this chapter, I investigate whether the relative abundances of PCB congeners vary within the UK harbour porpoise population.

All 209 chlorinated biphenyl (CB) congeners have the same basic structure: two connected phenolic rings with between one and ten chlorine molecules at different positions around the rings Figure 5-1. For coplanar or non-ortho congeners, also known as dioxin-like (DL) PCBs, the chlorine atoms are bonded in the *meta-* or *para-* position and the two phenyl rings are on the same plane (Devito, 2012). DL PCBs (similar in structure to dioxin) bind to the aryl hydrocarbon receptor (AHR) and induce the cytochrome P450 monooxygenase enzyme (CYP) 1A subfamily. DL PCBs were classically thought to be the most toxic and anti-estrogenic congeners due to their persistent and high-level stimulation of the AHR (Kodavanti and Loganathan, 2014). However, numerous toxic effects have been reported for non-dioxin-like (NDL) PCBs that act independently of the AHR (Hansen, 1998; Pessah et al., 2019). NDL PCBs have chlorine atoms bonded at the *ortho* positions and are also known as noncoplanar PCBs as the phenyl rings are on different planes (Figure 5-1) (Devito, 2012). A review of several neurotoxicity studies concluded that NDL lower chlorinated (*di-* and *tri-*) congeners were the most neurotoxic (Hansen, 1998). NDL *tetra-*chlorinated congeners (e.g. CB47) and less chlorinated mixtures have also been shown to cause greater disruption to thyroid homeostasis than highly chlorinated congeners (e.g. CB99, CB153) (Hansen, 1998). Whilst, NDL lower chlorinated PCBs have been shown to cause greater oxidative stress in cerebellar neurons than DL PCBs (Pessah et al., 2019). Hence, studies that estimate PCB mixture toxicities based on dioxin-like congeners and toxic equivalent factors (TEFs) may be inadequate.

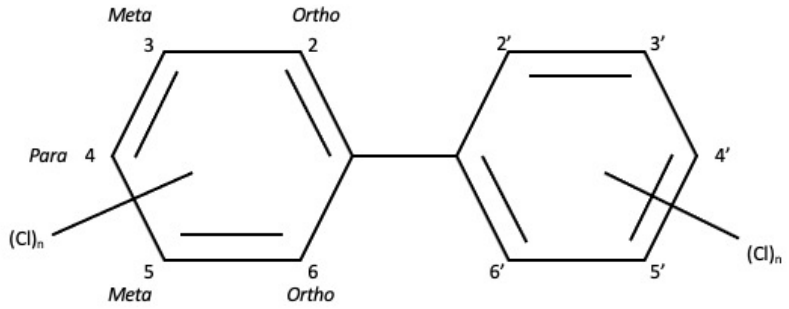


Figure 5-1: The chemical structure of a polychlorinated biphenyl (PCB) showing the ten possible chlorine (Cl) locations (2-5 and 2'-5') on the two phenyl rings. The positions of the chlorines form different congeners. Each congener varies in structure according to the number of hydrogen atoms that are replaced by chlorine atoms on the phenyl rings and the position of the chlorine atoms on the phenyl rings. If the chlorines are bonded on the meta- or para- positions then congeners are defined as coplanar or dioxin-like PCBs. If the chlorines are bonded on the ortho-positions then the congeners are defined as noncoplanar or non-dioxin-like PCBs.

To standardise and simplify monitoring of PCBs in human and animal tissues, the sum of concentrations of certain CB congeners are traditionally used to determine PCB body burdens. Risk assessments for these PCB exposures in marine mammals are carried out using toxicity thresholds for summed concentrations of PCBs derived by combining results from laboratory studies and field studies on otters, mink and seals (*Lutra lutra, Mustela vison, Phoca vitulina*) (Kannan et al., 2000). Unfortunately, as these thresholds are based on summed congener concentrations, the CB congeners and their ratios in the mixtures vary between published studies and are often not published in full. As a consequence, the total toxicity of PCB burdens in marine mammals may be more or less toxic than predicted using current approaches, dependent on the complete profile of PCB congeners present (Peñín et al., 2018). This may be particularly important during pregnancy and lactation where energetic demands cause the mobilization of contaminants into circulation and some of the maternal toxicant burden to be transferred to the calf via lactation, which has been associated with reduced calf survival in cetaceans (Schwacke et al., 2002). Variation in congener profiles between mothers and their offspring, as well as differences in the proportion of highly chlorinated congeners transferred during gestation compared with lactation, could profoundly affect PCB toxicity (Hamers et al., 2011; Hansen, 1998; Iwata et al., 2004; Pěnčíková et al., 2018). Therefore, to understand whether calves are at a different risk to adults

and whether current toxicity thresholds are adequate, it is important to determine not just the total burden, but also the congener profile of the PCB mixture to which they are exposed.

To date no studies have addressed this question in cetaceans at the population level. To investigate this question, I used the cetacean strandings investigation programme (CSIP) toxicology dataset, to obtain the blubber PCB congener concentrations of harbour porpoises stranded between 1992 and 2015. I used the PCB blubber concentrations to determine whether the congener profiles of individuals differ by age class and sex, in response to possible differences in pollutant offloading from mothers to calves. I also considered whether the congener profiles of individuals vary according to their stranding location, possibly resulting from different chemical compositions of polluting sources, timings of release and atmospheric transport.

5.3 Materials and Methods

5.3.1 Sampling

Between 1992 and 2015 blubber congener concentrations were determined for 696 UK stranded harbour porpoises. Detailed and standardised necropsies were carried out according to standard procedures for cetaceans (Law et al., 2006). Blubber samples were taken from the left side of the body, at the caudal insertion of the dorsal fin. To minimise the impact of changes in pollutant levels and tissue dispersion with body decomposition, toxicological analysis was prioritised for blubber samples from freshly dead cases as defined in the necropsy protocol (Law, 1994; Law et al., 2006). Animals chosen for toxicological analysis were otherwise assumed to be a random sample of all the strandings that occurred over the study period.

5.3.2 PCB Analysis

Blubber samples for contaminant analysis were taken and preserved at -20°C using established protocols (Law, 1994). The concentrations of 25 PCB congeners, were determined (on a mg kg^{-1} wet weight basis) by the Cefas laboratory (Lowestoft) using a method that followed the recommendations of the International Council for the Exploration of the Sea (ICES) and had been validated following participation in the QUASIMEME laboratory proficiency scheme (de Boer and Law, 2003; de Boer and Wells, 1997; ICES, 1998; Webster et al., 2013). In cases where the

congener/isomer concentrations were below the limit of quantification (<0.0003 or <0.0004 mg kg^{-1} wet weight), concentrations were set to zero. The numbers of the International Union of Pure and Applied Chemistry CB congeners analysed were: 18, 28, 31, 44, 47, 49, 52, 66, 101, 105, 110, 118, 128, 138, 141, 149, 151, 153, 156, 158, 170, 180, 183, 187, 194. These congeners were chosen for analysis based on their relatively high concentrations in commercial PCB mixtures and their wide range of chlorination. The individual congener concentrations were calculated and normalized to a lipid basis (mg kg^{-1} lipid) by solvent extracting lipids from the blubber and calculating the hexane extractable lipid content.

5.3.3 Statistical Analysis

All analyses were carried out using the statistical computer programme R (version 3.4.3) (R Core Team, 2016). I used a subset of the harbour porpoise stranding data (n=696), which only included trauma cases with complete age class data (n=347). This was to control against any confounding influence from the remobilisation of PCBs that may occur in sick and underweight animals (n=349). I expressed the congener concentrations as fractions of the sum of the 25 congeners that were measured (∑25 CBs). I investigated any differences due to age class and sex by categorising all animals by age class and the adults by sex. Individuals were categorized into age classes according to their body length and sexual maturity. Sexual maturity was determined using gonadal appearance and by looking for histological evidence of spermatogenesis in male testes. To ensure that the method of classification was reliable over the extended time period of the study I established that there was no temporal trend in body length. Individuals with body lengths greater than 90cm that were sexually mature were classified as adults, juveniles were classified as individuals with body lengths greater than 90cm that were sexually immature and individuals with body lengths less than 90cm (Jepson, 2003). Exact age was determined for a subset of individuals (n=236) by quantification of growth layer groups from analyses of decalcified tooth sections using the methods outlined by (Rogan et al., 2004) and (C Lockyer, 1995). The pooled standard deviation associated with this technique has been shown to be <0.5 years therefore, associated errors should not impact age class determination (Bjoerge et al., 1995). I did not categorise the juveniles by sex as I wanted to investigate the possible effect of pollutant offloading from mothers to calves. Levels of PCBs in females accumulate until they reach sexual maturity and successfully reproduce and offload some of their PCBs to their calves, after which their body burdens decline following each

successful pregnancy. Females that do not successfully reproduce and males continue to accumulate PCBs over their lifetime via prey ingestion (Tanabe et al., 1981). Therefore, differences in mean PCB burdens between the sexes only occurs in sexually mature animals. I conducted analysis that verified that there were no significant differences between male and female juveniles (Appendix Table A-13). Thus, it was only relevant to categorise adult animals by sex. I was unable to categorise females according to whether or not they had successfully reproduced however, if congener variation is associated with maternal transfer of pollutants, the large sample size should ensure this is still apparent even if some non-reproducing females are present in the data.

My approach led to three groups being investigated (juveniles, adult males and adult females) whereby I compared adult females with adult males and adult females with juveniles to test whether there was evidence of selective transfer of congeners via lactation. To investigate geographical variation, I categorized the stranding locations into three geographic areas that were previously defined in a study assessing temporal trends of contaminants in stranded cetaceans (Law et al., 2012a). The three areas were Scotland, the West (defined as the west coast of England and Wales including the south-west coast up until Dorset and the Isle of Man) and East England (defined as the east coast of England and the south-east coast up to and including Dorset). I chose to categorise the locations in this way because the division of the east and west coasts in England and Wales allowed for any longitudinal differences to be observed. While the division of Scotland from England and Wales allowed for any latitudinal differences to be observed.

To determine whether the degree of chlorination and the metabolic pathway of congeners affected the PCB profiles I carried out analyses using two separate classifications (Table 5-1). First, I grouped the congeners according to their degree of chlorination. This method of classification allowed every assayed congener to be assigned a group and has been proposed in the literature as the most suitable classification method (Moysich et al., 1999; Warner et al., 2012). Furthermore, increasing degree of chlorination corresponds with increasing lipophilicity and can therefore highlight certain accumulation patterns that may not be apparent when analysing congeners individually (Safe and Hutzinger, 1984). Second, I classified the congeners according to their metabolic pathway/structural activity group (SAG). This classification scheme was proposed in a

study that suggested cetaceans have reduced capability of metabolising non-dioxin-like PCBs, in comparison to other mammals, because the activity of their CYP2B enzyme is lower (Boon et al., 1997).

To investigate congener profile variation, I used principal component analysis (PCA) to examine the data before grouping any congeners. I then grouped the congeners by degree of chlorination and carried out PCA to further assess variation between age class and sex. I fitted linear models and carried out Tukey's Honestly Significant Difference (HSD) tests to identify which differences were statistically significant. I investigated the association between the metabolic pathway of the congeners and congener profiles by conducting PCA on the congeners grouped by their structural activity group (SAG). To explore the differences further, the five SAGs were grouped into two further groups according to their reported persistence in cetaceans (highly persistent and less persistent) (Boon et al., 1997). SAGs 1, 2 & 5, which are not readily metabolised in cetaceans, were grouped together and SAGs 3 and 4, which are metabolised by CYP-450, were grouped together. SAGs 1, 2 & 5 are dominated by the more highly chlorinated *hexa*- and *hepta*-chlorinated congeners whilst SAGs 3 & 4 are dominated by the less chlorinated *tri*- and *tetra*-chlorinated congeners. Where age data was available (n=236) I fitted linear regression models to the data where the proportion of ∑25 CBs in each group was the dependent variable and age was the predictor variable. The proportion of ∑25 CBs was log transformed to reduce variance and meet the assumption of normality. I selected the best models by comparing models with different variables and forms and chose the models with the fewest predictors whereby the difference in AIC (Akaike's Information Criterion) relative to the minimum AIC was <2 (Akaike, 1973).

Table 5-1: Congener classification schemes. Dioxin like congeners are marked with an asterisk (*)

Structural activity group (SAG)	**IUPAC Congener Number**	**Definition**	**Persistence grouping**
1	153, 180 ,183. 187, 194	No vicinal hydrogen atoms, more than 2 *ortho*-chlorines	Highly persistent
2	128, 138, 170	Only vicinal hydrogen atoms in *ortho* and *meta* positions, 2 or more *ortho*-chlorines	Highly persistent
3	66, 105*, 118*	Only vicinal hydrogen atoms in *ortho* and *meta* positions, less than 2 *ortho*-chlorines	Less persistent
4	18, 44, 49, 52, 101	Only vicinal hydrogen atoms in *meta* and *para* positions, 2 or less *ortho*-chlorines	Less persistent
5	149, 151	Only vicinal hydrogen atoms in *meta* and *para* positions, more than 2 *ortho*-chlorines	Highly persistent
Degree of chlorination	**IUPAC Congener Numbers**	**Definition**	
Tri-/Tetra-	18, 28, 31, 44, 47, 49, 52, 66	Congeners contain 3 or 4 chlorine atoms	
Penta-	101, 105*, 110, 118*	Congeners contain 5 chlorine atoms	
Hexa-	128, 138, 141. 149, 151, 153, 156*,158	Congeners contain 6 chlorinate atoms	
Hepta-	170, 180, 183, 187	Congeners contain 7 chlorine atoms	
Octa-	194	Congeners contain 8 chlorine atoms	

To ensure there was not an overrepresentation of animals from certain locations, time-periods, age classes or sexes, which could affect the results, I calculated the proportions in each category to assess the likelihood of bias. I found that there were no large overrepresentations (Appendix Table A- 14, Table A- 15). The relatively large sample size should also help to mitigate this risk of bias. I also carried out separate PCAs, for age class and sex, using subsets of the data. I subsetted the

data spatially and temporally to ensure the conclusions were consistent and not confounded by overrepresentation (Appendix Figure A- 1, Figure A- 2, Figure A- 3).

For all of the analyses detailed above, I used the mean proportion of the ∑25CBs made up by each congener or group of congeners. For all of the PCAs, the congeners were zero centred and scaled to have unit variance. For each analysis I plotted the first two components of the PCA and calculated the 70% confidence ellipses for the variables that I was investigating. Ellipse overlap between groups was estimated as per (Jackson et al., 2011).

5.4 Results

5.4.1 Variation of Individual Congeners Profiles Between Age Class and Sex

I found that 25 components were required to describe the variation in congener profiles. The first two components accounted for 25% and 19% of the variance respectively and revealed some clustering by age class and sex (Figure 5-2A). I found relatively little overlap between the groups (overlap: adult-females/juveniles = 0.19, adult-females/adult-males = 0.14, adult-males/juveniles=0.28). Therefore, it is reasonable to conclude that age class and sex drove some of the variation in congener profiles between individuals. The congener specific loadings across the first two components (PC1 &PC2) are shown in Figure 5-3. CBs 170, 180, 183, 187 and 194 had high positive scores across both components. The other congeners had positive scores in PC2 and negative scores in PC1 with the exceptions of CB 138 and 153, which had positive scores for PC1, and CBs 52, 66, 138,149,151 and 153, which had negative scores for PC2.

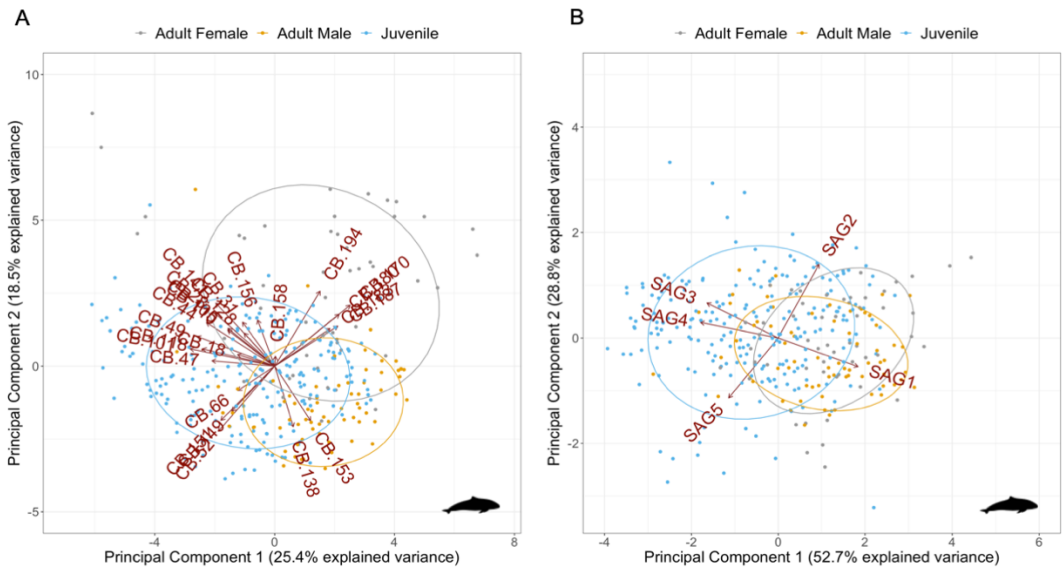


Figure 5-2:(A) Principal Component 1 plotted against Principal Component 2 for the PCA carried out on the individual congeners, coloured by age class and sex. The sizing of the confidence ellipses was set at 70%. (B) Principal Component 1 plotted against Principal Component 2 for the PCA carried out on the congeners grouped by their by their structural activity group. The sizing of the confidence ellipses was set at 70%.

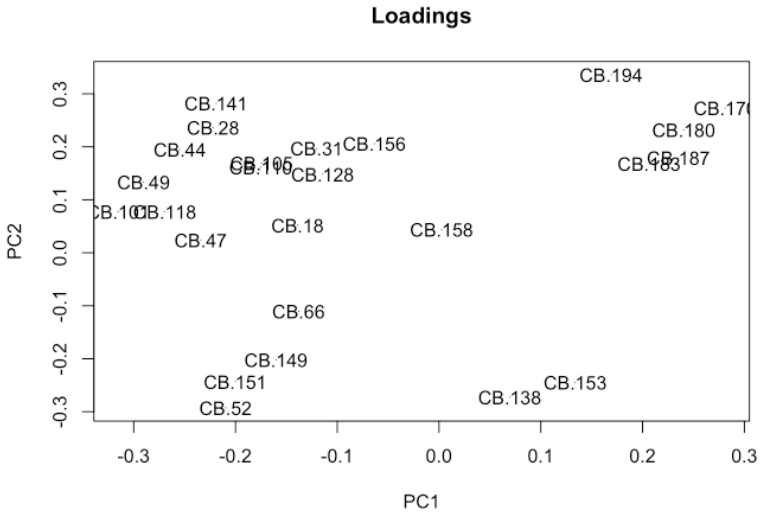


Figure 5-3:The loadings of each congener for principal component 1 (PC1) and principal component 2 (PC2) from the principal component analysis carried out for each congener.

The loadings arrows show that adult females had a greater proportion of the highly chlorinated congeners while juveniles had a greater proportion of the less chlorinated congeners (Figure 5-3A).

I tested the differences for significance between adult males and adult females and also between adult females and juveniles. I found adult males had significantly higher proportions of CBs 52, 138, 149, 151 and 153 when compared with adult females ($p<0.05$) (Appendix Table A- 7). I found adult females had significantly higher proportions of CBs 156, 170, 180, 183, 187 and 194 when compared with juveniles and juveniles had significantly higher proportions of CBs 52, 101, 118, 138, 149, 151, 153 when compared with adult females ($p<0.05$).

5.4.2 Variation of Congener Chlorination Profiles Between Age Class and Sex

I found that five components were required to describe the variation in congener profiles (when grouped by their degree of chlorination). The first two components accounted for 53% and 29% of the variance respectively and also revealed clustering by age class and sex (Figure 5-4). I found relatively little overlap between the groups (overlap: adult-females/juveniles = 0.20, adult-females/adult-males = 0.16, adult-males/juveniles=0.29). Analysis of the mean proportions of congeners (classified by degree of chlorination) showed that adult males had significantly higher proportions of hexa-chlorinated congeners than adult females ($p<0.05$) (Figure 5-5, Appendix Table A- 8). When I compared adult females with juveniles, I found juveniles had significantly higher proportions of *tri-*, *tetra-* and *penta*-chlorinated congeners whilst adult females had significantly higher proportions of *hepta-* and *octa*-chlorinated congeners ($p<0.05$).

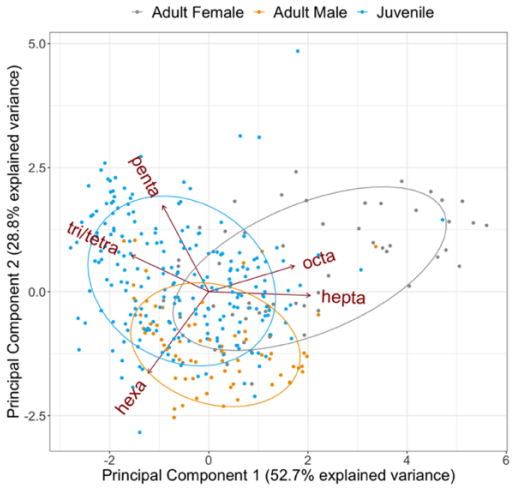


Figure 5-4: Principal Component 1 plotted against Principal Component 2 for the PCA carried out on the congener grouped by their degree of chlorination, coloured by age class and sex. The sizing of the confidence ellipses was set at 70%.

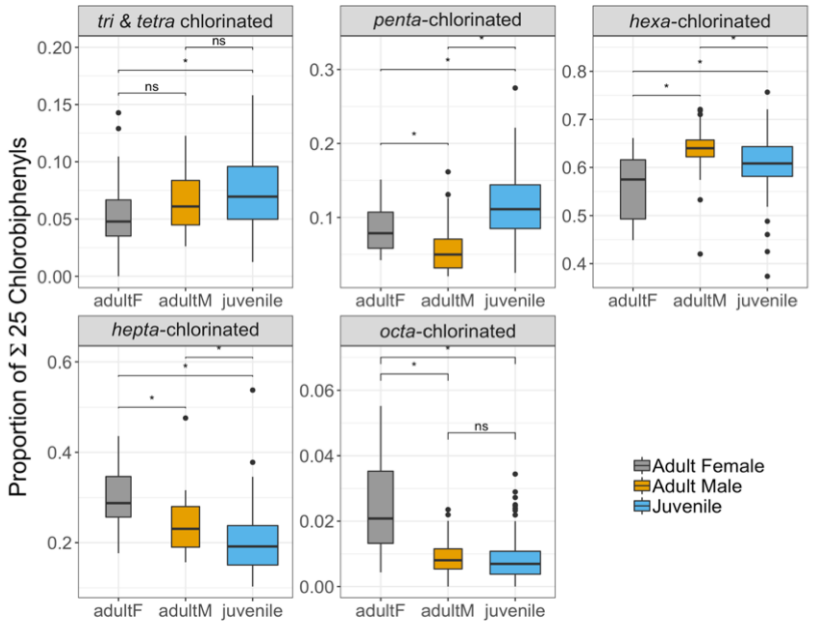


Figure 5-5: Congener proportions (grouped by degree of chlorination) for each age class and sex. The width of the boxes is proportional to the sample size. The horizontal lines represent the median value. The lower and upper hinges correspond to the first and third quartiles. The upper whisker extends from the upper hinge to the largest value unless the largest value is greater than 1.5 times the interquartile range (IQR) in which case the upper whisker is limited at 1.5 x IQR. The lower whisker extends from the lower hinge to the smallest value unless the smallest value is greater than 1.5 times the interquartile range (IQR) in which case the lower whisker is limited at 1.5 x IQR. Data beyond the end of the whiskers are outliers and are plotted individually.

5.4.3 Variation of Congener Structural Activity Groups (SAGs) Between Age Class and Sex

When the congeners were grouped by their structural activity group (SAG) I found that five components were required to describe the variation in congener profiles. The first two components accounted for 57% and 21% of the variance respectively. The overlap between the ellipses was greater compared with the ellipses in the PCA plot for the individual congeners, particularly between adult males and females, suggesting less variation (overlap: adult-females/juveniles = 0.27, adult-females/adult-males = 0.64, adult-males/juveniles=0.29) (Figure 5-2B). The loadings for the first two components (PC1 &PC2) are shown in Figure 5-6. SAGs 1 & 2 had high positive scores in PC1 whilst SAGs 3, 4 & 5 had negative scores on PC1. SAGS 2,3, & 4 had positive scores on PC2 whilst SAGs 1 & 5 had negative scores on PC2. I found adult males had significantly higher proportions of SAG5 than adult females ($p<0.05$) (Appendix Table A-9). I found juveniles

had significantly lower proportions of SAG1 and significantly higher proportions of SAGs 3, 4 and 5 when compared with adult females ($p<0.05$).

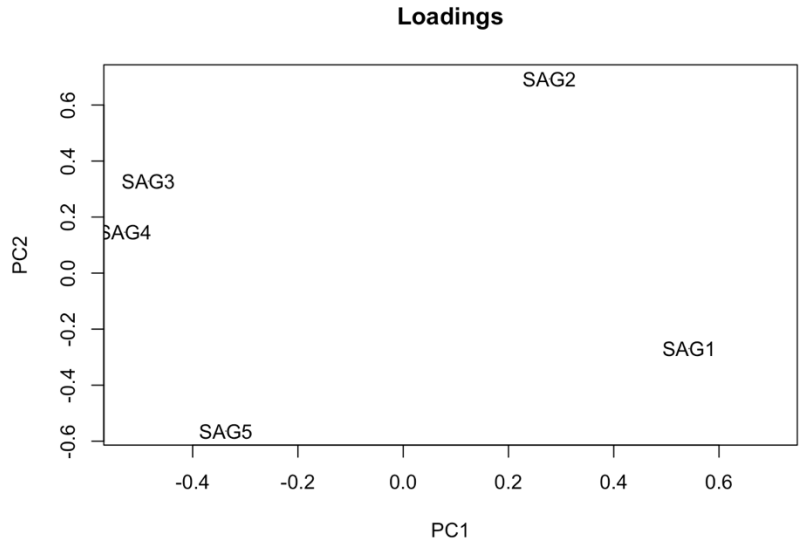


Figure 5-6: The loadings of each congener for principal component 1 (PC1) and principal component 2 (PC2) from the principal component analysis carried out for the congeners grouped by their structural activity group.

After fitting linear models to the SAGs grouped by their reported persistence in cetaceans, (highly persistent [SAGs 1,2&5] and less persistent [SAGs 3&4]), I found there was a significant relationship with age ($p<0.05$). For highly persistent congeners the best fitting model included sex, location, year of stranding and age (Table 5-2). However, for the less persistent congeners sex was not included in the final model (Table 5-3). I found that proportions of the less persistent congeners declined with age and the proportions of the highly persistent congeners increased with age (Figure 5-7). Similarly, the proportion of highly persistent congeners increased over time (year of stranding) and the proportion of less persistent congeners decreased (Table 5-2, Table 5-3). Geographically, the proportion of highly persistent congeners was significantly higher in animals that stranded on the west coast of England and Wales than those that stranded in Scotland and the east coast of England and vice versa for the less persistent congeners ($p<0.05$) (Table 5-2, Table 5-3, Appendix Table A-10).

*Table 5-2: Summary statistics of the linear model fitted to the proportions of highly persistent congeners relative to the overall burden. The proportion of highly persistent congeners was the response variable. Coefficient estimates were calculated based on a female adult that stranded on the east coast of England. *indicates statistical significance (p<0.05)*

Variable	Estimate	Std. Error	t-value	Pr(>|z|)
Intercept	-9.66	1.92	-5.04	0.00*
Age	0.01	0.00	8.85	0.00*
Male	0.02	0.01	2.07	0.04*
Year of stranding	0.00	0.00	4.90	0.00*
Scotland	-0.02	0.01	-1.36	0.18
West coast of England & Wales	0.05	0.01	4.66	0.00*

*Table 5-3: Summary statistics of the linear model fitted to the proportions of less persistent congeners relative to the overall burden. The proportion of less persistent congeners was the response variable. Coefficient estimates were calculated based on a female adult that stranded on the east coast of England. *indicates statistical significance (p<0.05)*

Variable	Estimate	Std. Error	t-value	Pr(>|z|)
Intercept	27.72	8.97	3.09	0.00*
Age	-0.05	0.01	-9.91	0.00*
Year of stranding	-0.01	0.00	-3.28	0.00*
Scotland	0.08	0.05	1.54	0.13
West coast of England & Wales	-0.26	0.05	-5.01	0.00*

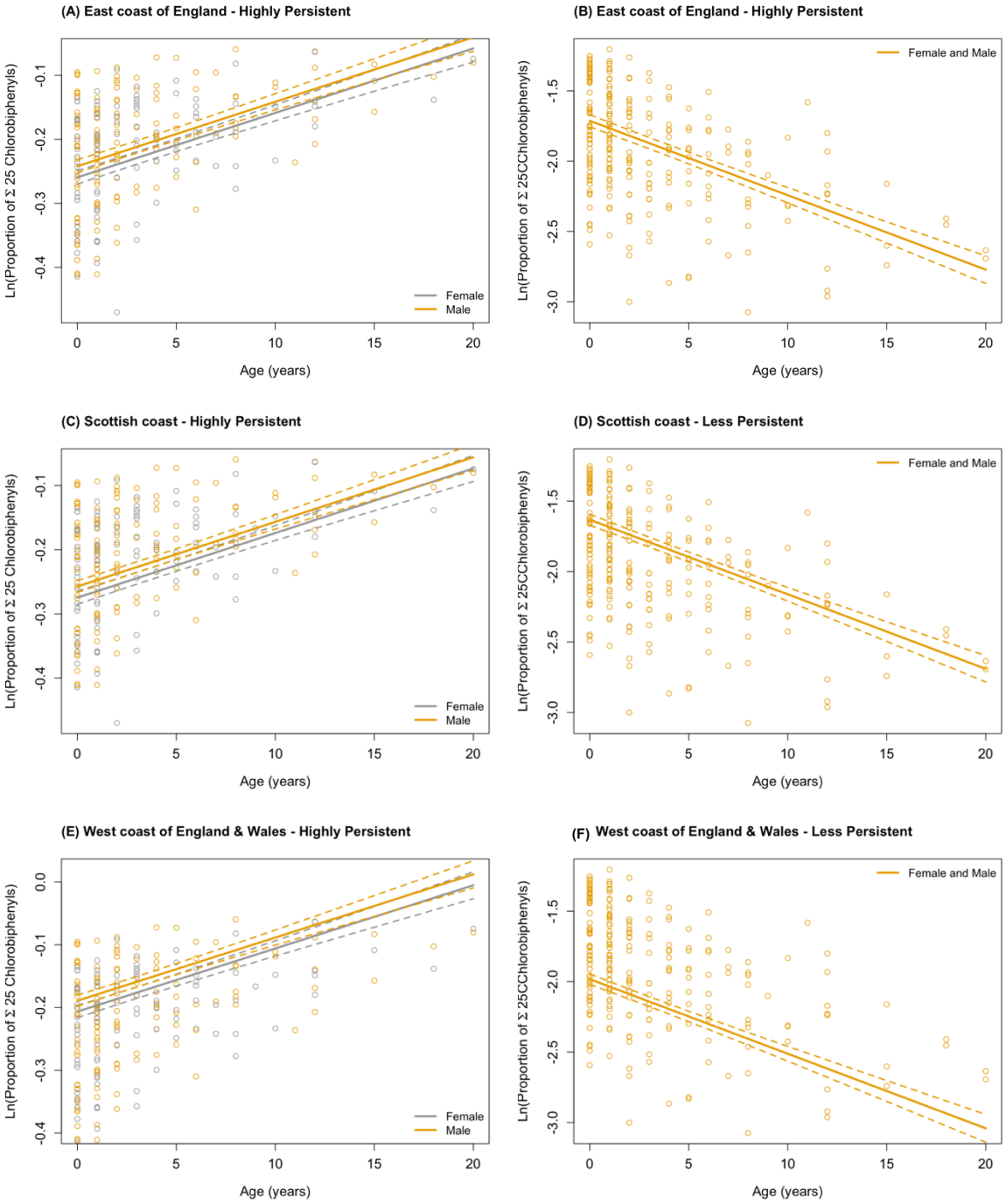


Figure 5-7: Proportion of the sum of 25 congeners (∑25CBs) plotted against age for congeners grouped by the persistence of their structural activity group. The solid line represents the linear regression model fitted to the data ($p<1e^{-12}$). The solid line shows the trend for males and females in the median year of the study period (2001). The dashed lines are 95% confidence intervals. (A) Highly persistent congeners on the east coast of England (B) Less persistent congeners on the east coast of England (C) Highly persistent congeners in Scotland (D) Less persistent congeners in Scotland (E) Highly persistent congeners on the West coast of England & Wales (F) Less persistent congeners on the West coast of England & Wales

5.4.4 Geographical Variation of Congener Chlorination Profiles

As discussed previously, I found that five components were required to describe the variation in congener profiles (when grouped by their degree of chlorination). The first two components of the PCA revealed clustering by geographic area (Figure 5-8). The ellipses show that the profiles of individuals that stranded on the east coast of England had the greatest variance and their profiles overlap with animals that stranded in Scotland and the west coast of England and Wales (overlap: west coast of England and Wales/Scotland = 0.31, west coast of England and Wales/east coast of England = 0.69, east coast of England/Scotland=0.46). The data points and ellipses for Scotland and the west coast of England and Wales overlap the least suggesting greater variation between the profiles of individuals that stranded in these areas (Figure 5-8). The loadings arrows indicate that animals that stranded along the west coast of England and Wales had higher proportions of highly chlorinated congeners whilst animals that stranded in Scotland had higher proportions of less chlorinated congeners.

Analysis of the mean proportions of congeners (classified by degree of chlorination) found that animals that stranded in Scotland had significantly higher proportions of the less chlorinated (*tri-, tetra-, penta-* and *hexa-)* congeners than animals that stranded on the west coast of England and Wales ($p<0.05$) (Appendix Table A- 11). While animals that stranded on the west coast of England and Wales had the highest proportion of highly chlorinated (*hepta-* and *octa-*) congeners ($p<0.05$).

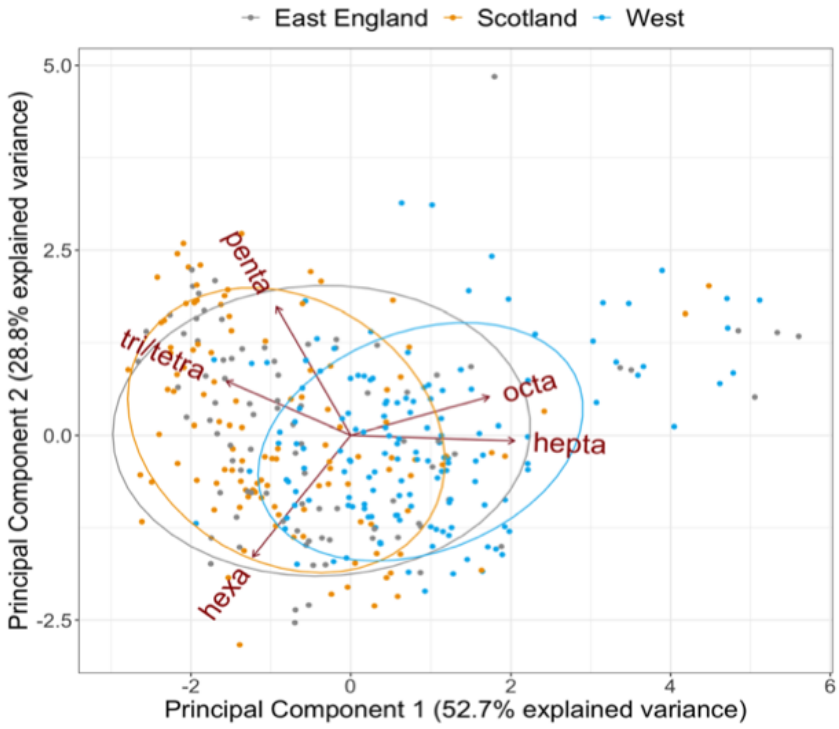


Figure 5-8: Principal Component 1 plotted against Principal Component 2 for the PCA carried out on the congeners grouped by degree of chlorination, coloured by geographical area. The sizing of the confidence ellipses was set at 70%.

Analysis of the mean proportions of congeners (classified by their persistence as defined by their SAG) found that animals that stranded in Scotland had the lowest proportion of highly persistent congeners and the highest proportion of less persistent congeners (Figure 5-9, Appendix Table A-12). While animals that stranded on the west coast of England and Wales had the highest proportion of highly persistent congeners and the lowest proportion of less persistent congeners.

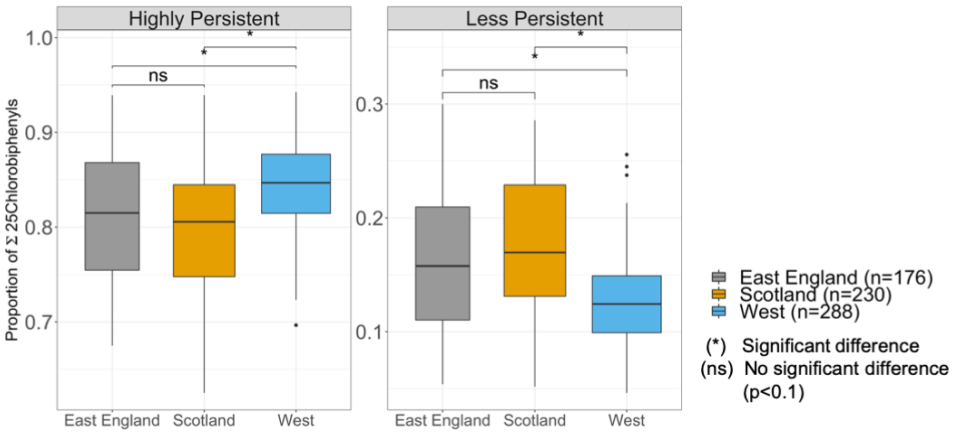


Figure 5-9: Congener proportions of the sum of 25 congeners (∑25CBs) (grouped by their persistence as defined by their SAG) for each area. The horizontal lines represent the median value. The lower and upper hinges correspond to the first and third quartiles. The upper whisker extends from the upper hinge to the largest value unless the largest value is greater than 1.5 times the interquartile range (IQR) in which case the upper whisker is limited at 1.5 x IQR. The lower whisker extends from the lower hinge to the smallest value unless the smallest value is greater than 1.5 times the interquartile range (IQR) in which case the lower whisker is limited at 1.5 x IQR. Data beyond the end of the whiskers are outliers and are plotted individually.

5.5 Discussion

Variation in congener profiles is likely to be driven by a number of factors such as differences in prey profiles, differences in the quantities and congener profiles of initial PCB contamination sources, differences in prey choices as well as differences in the complex physiochemical and physiological processes that occur between individuals. However, maternal offloading of pollutants to calves is the most plausible explanation for the variation I found between adult females and juveniles. The higher proportions of highly chlorinated congeners found in adult females and higher proportions of less chlorinated congeners found in juveniles suggest that the congener profile of the PCB mixture transferred from mother to calf is partially driven by the degree of chlorination because lower degrees of chlorination correspond to lower lipid solubility. However, the dynamics of PCB offloading in cetaceans are poorly understood.

Studies on humans and wildlife have found variation between the congener profiles of mothers, their milk and their offspring (Aguilar and Borrell, 1994b; Debier et al., 2003; Fängström et al., 2005; Ramos et al., 1997). There are several factors driving this variation, including the molecular structure of congeners affecting tissue distribution and accumulation, age-related metabolic

differences, foraging differences, milk fat content, lactation duration and reproductive histories (Addison and Brodie, 1987; Boon et al., 1997; Hall et al., 2006; Reddy et al., 2001; Yordy et al., 2010). Contaminant transfer rates between mothers and calves have been shown to decrease with each successful pregnancy as the first calf receives the highest pollutant load. Therefore, the dynamics of maternal offloading are heavily influenced by progeny number (Wells et al., 2005). Environmental factors such as the regional variation of contaminant concentrations, known to occur in the UK, are also likely to affect the rate and dynamics of maternal offloading (Law et al., 2012a). Investigation of the contaminant profiles of stranded long-finned pilot whales (*Globicephala melas*) showed that the ratio of highly chlorinated congeners transferred between four mother and foetus pairs increased over the gestation period. Following pregnancy, higher proportions of less chlorinated congeners were transferred during lactation than during gestation (Weijs et al., 2013). In grey seals (*Halichoerus grypus*) the proportion of highly chlorinated congeners in mothers and pups has been shown to increase with lactation duration. However, the congener profile of the milk remained constant suggesting highly chlorinated congeners are selectively adsorbed (Debier et al., 2003). The differences between these two marine mammal species further illustrate the complexity of pollutant transfer and accumulation dynamics.

The variation between adult females and males that is indicative of maternal offloading was greater when the congeners were grouped by chlorination or analysed individually than when the congeners were grouped by their structural activity group. Classification by structural activity group is based on cetaceans' capability to metabolise certain congeners more easily than others and so is not directly associated with lipid solubility. Therefore, it is likely that variation is influenced more heavily by age than sex.

For the congeners that were highly persistent and difficult to metabolise sex was a significant predictor, of the proportion of ∑25CBs, in the model. Cetaceans cannot metabolise these congeners easily and so they accumulate high amounts (Boon et al., 1997). Adult females are able to offload some of their burden to their calves. However, adult males continue to accumulate these congeners throughout their life and therefore tend to have higher proportions of these congeners than adult females because they do not detoxify through reproduction. I found that proportions of

the highly persistent congeners increased with age. This was expected because these congeners are difficult to metabolise and excrete and so levels continue to build up over an animal's lifetime.

For the congeners that were less persistent and more easily metabolised, sex was not included in the final model. It is likely that individuals across all age classes and sexes accumulate much lower levels of these congeners. Therefore, relatively small amounts will be transferred from mother to calf causing there to be no significant difference between levels in adult males and females. I found that proportions of the less persistent congeners declined with age. This is consistent with the congener metabolic pathway classification as the congeners are not resistant to biotransformation in cetaceans and so do not accumulate with age (Boon et al., 1997).

Year of stranding was a significant predictor in both models implying that congener profiles vary temporally, and animals are exposed to different PCB mixtures at different points in time. This is consistent with findings that showed that at the point of source congener profiles shift from being similar to the profile of the mixture released to a profile containing heavier congeners as lighter congeners are mobilised or metabolised (Saba and Boehm, 2011). The results show that animals that stranded more recently are likely to have a higher proportion of more persistent PCBs because the less persistent PCBs take less time to be broken down. This suggests that the rate of PCB inputs into the environment is likely to be declining. The most recent Marine Strategy Framework Directive (MSFD) assessment of PCB concentrations in biota and sediment corroborates this finding. The assessment found that concentrations of the less persistent congeners (CB52, CB101) had decreased while all other congeners measured showed no change. Moreover, concentrations of the less persistent congeners (CB52, CB101 and CB118) in biota had decreased in three of the four UK regions investigated while concentrations of the more persistent CB180 had only decreased in one of the regions (Maes et al., 2018; OSPAR, 2015a).

Part of the reason for the temporal shift in congener profiles is that lighter congeners are dispersed more readily (Harner and Bidleman, 1996). I found geographical differences between the congener profiles when they were grouped by degree of chlorination and when they were grouped by metabolic pathway. Dispersal dynamics are governed by several factors including the congener's chemical properties, the presence of other compounds and environmental conditions. Hence, the

toxicity of a PCB mixture at a specific geographic location is a function of both total concentration and the congener profile (Gioia et al., 2013). The geographical variation that I identified could be explained by differences in the congener profiles of the initial PCB sources and the timings of the releases. There may also be regional differences in diets which cause variation because different prey species may have different abilities to metabolise PCB congeners (Santos et al., 2004).

I found that the most significant differences in congener profiles, grouped by degree of chlorination and by structural activity group, were between Scotland and the west coast of England and Wales. PCBs were traditionally produced on the more heavily industrialised west coast (Harrad et al., 1994; Robin, 2010). In the UK it has been shown that PCB mixtures in air are dominated by *tri*- and *tetra*-congeners and that relative atmospheric concentrations of *tetra*-chlorinated PCBs increase with increasing latitude or decreasing temperature (Halsall et al., 1995; Ockenden et al., 1998). Therefore, the higher proportions of less chlorinated congeners in animals that stranded in Scotland could be evidence of atmospheric transport whereby, the less chlorinated congeners are transported more readily than the highly chlorinated congeners. This variation could also be partly explained by the metabolic pathway of the congeners whereby, the less persistent PCBs are bio-transformed over time. Therefore, higher proportions of more persistent congeners remain at the PCB source, as demonstrated by the higher proportions of more persistent congeners found in animals that stranded on the west coast of England and Wales. The significant geographic variation of congener profiles further demonstrates that a single toxicity threshold for the sum of PCB congeners may not be adequate to determine population risk for UK harbour porpoises.

To gain a better understanding of the significance of these findings the toxicities of the profiles need to be quantified. The vast range of mechanisms, toxicities, and synergistic and antagonistic interactions make this a difficult task. Toxicity equivalent factors (TEFs) can be summed to quantify the overall toxicity of a mixture (Van den Berg et al., 2006). However, TEFs only exist for dioxin-like PCBs and so ignore a large number of modes of toxicity. Furthermore, of the congeners analysed in this study only three of them are dioxin-like. However, the literature can provide some insight into how chlorination affects toxicity. A review of congener toxicities collated four studies that determined the congener specific potencies for four different neurochemical end points and found that in every study lower chlorinated congeners were the most

toxic (Hansen, 1998). The same review also looked at thyroid homeostasis and found the *tetra*-chlorinated congener CB47 and lower chlorinated mixtures decreased serum T4 more than highly chlorinated congeners. Lightly chlorinated PCBs have also been shown to impair dopamine signalling, promote tumour growth and cause increased oxidative stress when compared with dioxin-like congeners (Pěnčíková et al., 2018; Pessah et al., 2019). These results are concerning as I found the highest proportions of less chlorinated congeners in juveniles.

Dioxin-like (DL) congeners cause toxicity by binding to the aryl hydrocarbon receptor (AHR). The AHR affects a number of regulatory proteins and so DL congeners can cause numerous toxic effects including immunotoxicity and endocrine disruption (Kodavanti and Loganathan, 2014). The AHR has also been shown to mediate neuro and vascular genesis, which are particularly important processes during development (Lahvis et al., 2000; Larigot et al., 2018). Of the three dioxin-like congeners measured in this study juveniles had significantly higher levels than adult females for CB 118. As neurotoxicity, thyroid and endocrine disruption and enzyme induction are critical end points of toxicity particularly during development, finding higher levels of congeners, which cause these effects in UK juvenile harbour porpoises is a great concern. Furthermore, studies have shown that levels of exposure that cause no effects in adults can cause biological effects in developing animals (Baumann et al., 1983; Vitalone et al., 2010).

I have shown that congener profiles vary by age class and sex in harbour porpoises and that there is a large weight of evidence to suggest different congeners have different toxic thresholds and modes of action. However, when discussing toxicity, it is important to consider this variation in the context of overall concentrations. In the absence of congener specific toxicity thresholds for cetaceans I compared congener concentrations with the Environmental Assessment Criteria (EAC) set out by the UK Marine Strategy Framework Directive used to assess congeners (28, 101, 105, 118, 153 and 180) in fish and shellfish. I found that for CBs 101, 118, 153 and 180 all juveniles exceeded the EAC. And for CBs 28 and 105, 60% and 81% of juveniles exceeded the EAC respectively (Maes et al., 2018; OSPAR, 2015a) (Maes et al., 2018; OSPAR, 2015). Furthermore, several of the juveniles in this study were found to have PCB concentrations that exceed the most widely used toxicity threshold in cetaceans of 9 mg kg^{-1} lipid (Appendix Figure A- 4) (Kannan et

al., 2000). Therefore, it is reasonable to conclude that juveniles in this study were exposed to toxic concentrations of PCBs.

This is the first study to investigate possible drivers of PCB congener profile variation in a UK cetacean and I have shown that profiles vary with age class, sex and geographical location. It should be noted that due to the nature of strandings data there may be some biases present. Congener profiles may be altered by decomposition and therefore profiles of stranded individuals may vary from those of live animals. Similarly, causes of death such as infectious disease or those that cause loss of blubber mass may affect the congener profile (Borrell and Aguilar, 1990). It is also important to note that animal movement is a limitation of the location stranding data. Some individuals may move over their lifespan or die and be carried in ocean currents and strand in a different location causing them to accrue contaminants in a different location to where they strand. However, this effect should be minimised by selecting freshly dead carcasses because this increases the likelihood that an animal died close to where they stranded. It is also important to note that conclusions around the different toxicities of mixtures are limited as this study has only considered variation of a restricted number of CB congeners. Much of the cetacean toxicology work in the UK has focussed on PCBs because levels in cetaceans are much higher than other measured pollutants. Furthermore, levels have not declined at the same rate as other pollutants and analyses have shown that levels may have stabilised (Law, 2008; Law et al., 2012a, 2003). Despite this, it is still important to consider that harbour porpoises are exposed to a wide range of other legacy and emerging contaminants that will also affect the toxicity of pollutant burdens (Andresen et al., 2007; Galatius et al., 2013). Therefore, it is important for future work to take a mixture toxicology approach to identify the emerging substances these animals are exposed to and evaluate the toxicology of the complete pollutant burden to estimate risk.

These results show that congener profiles of harbour porpoises in the UK vary with age class, sex and location. Despite the ban on the production and use of PCBs in Europe in the late 1980s PCBs continue to enter the environment and blubber concentrations in cetaceans remain high (Jepson et al., 2016). Large variations in congener profiles are likely to equate to large variations in the overall toxicities of the congener mixtures and therefore it may no longer be sufficient to just look at overall PCB burden. This analysis has shown that calves are likely to be exposed to a more

neurotoxic PCB mixture than adults. This is particularly concerning as developmental effects of PCBs have been shown to occur at lower levels of exposure (Baumann et al., 1983; Vitalone et al., 2010). Therefore, using toxicity thresholds that assume the same congener profiles across all age classes may result in an over or under estimation of risk. This work is an important contribution towards understanding more about the variation in congener profiles, the drivers of juvenile mortality and the potential effects and threats of pollutant exposure in cetaceans. However, the problem appears to be more complex than first thought. Further work is required to understand how toxic effects vary with different congener mixtures and at different life stages and how dietary factors play a role. This will further our understanding of how to mitigate the risks of legacy pollutants in the marine environment.

Chapter 6 - Discussion

6.1 In summary

Cetacean populations face a number of anthropogenic threats including bycatch, acoustic disturbance, chemical pollution and habitat degradation (Avila et al., 2018). Arguably, exposure to chemical pollution, including persistent organic pollutants (POPs), is one of the least understood threats due to the predominantly chronic nature of both exposures and their resulting effects and the great number of challenges associated with studying biological impacts of pollution in these large and mobile marine animals (Hall, 2002). Cetaceans are particularly vulnerable to suffering toxic effects from POPs as they are long lived, apex predators with a thick layer of blubber where POPs concentrate due to their highly lipophilic nature (Hall, 2002). Despite, international efforts to reduce environmental concentrations of POPs, their impacts on cetaceans remain a global concern (Jepson and Law, 2016; Stuart-Smith and Jepson, 2017). There is an urgent need to gain a better understanding of the impacts of exposure to POPs in cetaceans, particularly in highly exposed populations. This would help to inform the development of conservation and management strategies to protect populations and help scientists, policy makers and conservationists understand whether actions to reduce environmental concentrations are sufficient. In this thesis I set out to address this need by determining current blubber concentrations of POPs, assessing spatiotemporal dynamics and relating these to indices of population fitness, including infectious disease mortality and fertility proxies.

Using pathological and toxicological data, collected as part of a 30-year cetacean strandings program, I have provided new insights on the impacts of POPs on cetaceans. Although I have found that overall POP burdens are in decline, several species are still exposed to concentrations that pose a toxicological risk and there is considerable variation in levels of exposure between species, regions and pollutant classes (Chapter 2). PCBs were present at the highest concentrations, declined at the slowest rate and presented the greatest toxicological risk across all of the species that were analysed. Concentrations of summed POPs were highest on the west coast of England and Wales and also declined at the slowest rate in this area. A similar spatial pattern emerged when PCBs were modelled separately from summed POPs. Harbour porpoises that stranded on the west

coast of England and Wales had the highest concentrations and concentrations declined at the slowest rate in comparison to other areas (Chapter 3). Collectively, these findings indicate that PCBs are entering the environment at a higher rate in this region, suggesting that contemporary releases are likely to be taking place. This area is in close proximity to the only PCB manufacturing site in the UK and so the risk of environmental releases is likely to be heightened (Robin, 2010). This is of particular concern as this area contains a Special Area for Conservation (SAC) that has been deemed by the European Union's Habitat Directive to have high biological importance for bottlenose dolphins and harbour porpoises (Joint Nature Conservation Committee (JNCC), 2018). Therefore, actions to reduce environmental releases of POPs should be prioritised in this area.

Having identified PCBs as being the greatest toxicological threat to cetaceans, I revealed several associations between PCB burdens and impacts on health, using harbour porpoises as a model species. I demonstrated that PCB concentrations are associated with a significant increase in risk of infectious disease mortality such that an increase of 1 mg/kg lipid equated to a 5% increase in risk of death from infectious disease (Chapter 3). This equates to a 41% increase in risk of infectious disease mortality, at the population mean PCB concentration in 2017, the most recent year of the study. Despite the associated increase in risk, it is difficult to estimate the direct effect this will have on population mortality rates when the background rate of infectious disease mortality is unknown. If, in the unexposed population, the background rate of infectious disease mortality is low then a large percentage increase in risk will have a small effect on mortality rates. However, there is a strong weight of evidence to suggest a relationship between exposure to PCBs and infectious disease outbreaks in cetaceans, which is backed up by compelling laboratory evidence of immunosuppression (Desforges et al., 2017, 2016; Ross, 2002). Therefore, a precautionary interpretation of the results would be to conclude that exposure to PCBs is likely to be causing an increase in population mortality rates.

To better assess the risk of exposure to contaminants on populations is it also vital to understand impacts on reproduction. Impacts of PCBs on female reproduction, including reduced fertility, embryonic loss and increased calf mortality are relatively well established (Murphy et al., 2015; Schwacke et al., 2002). However, no studies to date have investigated the possible impacts of exposure to PCBs on male reproduction in cetaceans. In this thesis I have demonstrated that PCB

blubber concentrations are associated with reduced testes weights in males (Chapter 4). Testes weight is a strong indicator of male fertility in seasonally breeding mammals (Fontaine and Barrette, 1997; Moller, 1989). Therefore, reduced testes weights as a consequence of exposure to PCBs could lower male fertility and impact cetacean populations by reducing overall birth rates or by reducing population fitness through reduced competition. These findings could have serious implications for cetaceans as current risk assessments, that only account for impacts on female fertility, may underestimate the risk to the population. Reductions in birth rates have serious implications for cetaceans because their low intrinsic population growth rates hinder rapid recovery in response to anthropogenic stressors (Evans and Stirling, 2002). Therefore, these findings highlight the need for population level impact assessments to consider the compounding impacts of PCBs on male fertility alongside known reproductive impacts on females. Given the well-established effects on calf mortality and female fertility in cetaceans and the possible impacts on male fertility demonstrated here, it is reasonable to conclude that improved management of PCBs could have a substantial impact on the reproductive health of coastal cetacean species.

Population risk assessments may also need to consider the implications of the demographic differences observed in harbour porpoise PCB congener profiles (Chapter 5). Current approaches to quantify risk typically assume the relative abundances of congeners are constant throughout the population (Kannan et al., 2000). However, toxic effects and thresholds for toxicity vary between CB congeners, therefore health risks may be under or over-estimated if the relative abundances of congeners present in the PCB mixture are different (Hansen, 1998; Pessah et al., 2019). I demonstrated that congener profiles vary significantly according to age class and sex, with the most notable differences occurring between adult females and juveniles. Adult females had higher relative abundances of the highly chlorinated congeners whilst juveniles had higher relative abundances of the low chlorinated congeners. These differences are likely to be the consequence of pollutant offloading between mothers and calves during gestation and lactation (Tanabe et al., 1981). The review of the toxicity mechanisms of the congeners revealed that the PCB mixtures that juveniles were exposed to was more neurotoxic than the mixture that adult females and males were exposed to. This finding is particularly concerning as levels of exposure that cause no effects in adults have been shown to cause toxicological effects in developing animals (Baumann et al., 1983; Vitalone et al., 2010). Hence, juveniles in the population are being exposed to a more

neurotoxic mixture at a time when they are likely to be most vulnerable to its effects. These findings suggest that current toxicity thresholds that assume congener profiles are constant across age classes and do not account for lower effect levels in developing animals are likely to underestimate health risks to juveniles.

The blubber concentrations of POPs analysed as part of this thesis are higher than those found in other regions of the world, which is consistent with the findings of other studies that have shown areas in Europe to be global hotspots for PCB burdens in marine mammals (Jepson et al., 2016). Despite Europe being considered a 'world leader' in tackling the risk of POPs, European countries have high levels of PCBs relative to non-European countries on (Stuart-Smith and Jepson, 2017), probably as a result of higher levels of PCB production. Remedial actions in other highly contaminated regions, such as the Hudson River estuary in the United States of America have demonstrated that concentrations in sediment and biota can be significantly reduced through such action (U.S. Environmental Protection Agency, 2019).

This thesis has demonstrated that exposure to POPs is likely to be impacting the health and conservation status of cetaceans in UK waters. These findings are particularly important in the context of ongoing negotiations between the parties to the *Stockholm Convention* and the UK government's commitment to develop a new chemicals strategy imminently (Defra, 2018; UNEP, 2017). The findings of this thesis demonstrate that current mitigation strategies in the UK are not sufficient to protect cetaceans. Destruction rates of POPs need to be increased and waste regulation must be improved to prevent POPs in contaminated products being released into the environment. Furthermore, a compliance mechanism must be established as part of the *Stockholm Convention* to provide incentives for parties to meet their commitments and to enable support and resources to be provided to countries that may require it. From a UK perspective these findings have serious implications for the management of PCBs and demonstrate the need to prevent further environmental contamination to ensure levels decline at sufficient rates to protect vulnerable species. Given the higher concentrations and slower rates of decline of PCBs on the west coast of England and Wales, remediation, elimination and conservation actions should be prioritised in the Special Area of Conservation (SAC) for cetaceans that exists within this region.

6.2 Limitations of this study and future work

This thesis has highlighted the importance of long-term national strandings programs to assess the impacts of POPs on cetaceans. However, there are inherent biases associated with the use of strandings data that are important to consider. Whilst care was taken to minimise the impact of these biases, I cannot rule out that my sample of stranded individuals were not representative of the wider population. For example, there may be an overrepresentation of animals where the cause of their death resulted in loss of blubber mass, such as starvation or infectious disease. This could influence the results as contaminant concentrations may be higher in these individuals as a consequence of blubber loss (Aguilar et al., 1999). However, the impact of this should be minimised because an important strength of this work is the high number of trauma cases that have been included in all analyses. The inclusion of these cases has allowed nutritive condition to be included in the models as a potential confounding factor and has allowed for the complex relationship between contaminant exposures and nutrition to be revealed.

It is also important to consider the possible influence of animal movement and carcass drift when interpreting the results. Large home ranges or the movement of carcasses may cause animals to assimilate contaminants in different locations to where they strand. I have attempted to minimise the impact of carcass drift on the results by only analysing carcasses that were recently deceased to increase the likelihood that animals died close to their stranding location. In addition, the use of harbour porpoises as a model species should minimise the influence of animal movement as tagging studies have shown reasonably high residency in comparison to other cetacean species (Read and Westgate, 1997). However, no tagging studies have been carried out to investigate site fidelity in UK harbour porpoises. In other populations, it has been shown that individuals tend to stay within the continental shelf and inhabit restricted areas where their prey aggregate. However, some individuals have been shown to occasionally travel large distances to inhabit other restricted areas in response to shifts in prey distribution (Nielsen et al., 2018; Sveegaard et al., 2012). The frequency of these movements is unknown; therefore, it is difficult to estimate the influence this may have had on the spatial analyses. However, the scale of the geographical boundaries that have been used in the analyses should minimise any potential impact. In addition, the results are similar to those observed in the UK in other media, therefore, it seems reasonable to assume that the

modelled spatial trends are representative of the population (OSPAR, 2015b). Nonetheless, it would be useful to carry out future work to investigate site fidelity in UK harbour porpoises to confirm this assumption. Tissue decomposition may also influence the results because this can cause contaminant concentrations and profiles measured in stranded animals to differ from those measured when the animals were alive (Aguilar and Borrell, 1994b). However, by only analysing carcasses that are recently deceased I expect the impact of changes as a consequence of tissue decomposition will have been minimal.

A shortcoming of this study was that the analyses focussed on a small number of contaminants (primarily PCBs) and did not consider other environmental contaminants or the impacts of exposure to mixtures. The results of this thesis demonstrated that, of the POPs that were analysed, PCBs make up the majority of contaminant concentrations in the blubber and that levels of PCBs are much higher than other measured pollutants and are declining at a significantly slower rate. Furthermore, the weight of evidence suggests that PCBs present the most significant threat to cetaceans that live around the UK coast (Jepson et al., 2016; Law et al., 2012a). Hepatic concentrations of butyltins and mercury have shown no association with cause of death in UK harbour porpoises and emerging contaminants such as perfluorooctanoic acid and organophosphorus flame retardants have only been detected at low levels (Jepson, 2003; Law et al., 2012a, 2008; Papachlimitzou et al., 2015). Nonetheless, it is still important to consider that cetaceans are exposed to complex mixtures of legacy and emerging environmental contaminants, several of which have been shown to correlate with the contaminants I have investigated here (Jepson, 2003). Therefore, effects are probably caused by the complex mixture to which cetaceans are exposed. Given that PCBs dominate the blubber profile, are endocrine disrupting and have been demonstrated to be the most immunotoxic contaminants of the POPs measured, I expect it is likely that PCB exposure is the greatest contributor to the associations with health and reproduction demonstrated in this thesis. Moreover, I expect if exposure to mixtures had been considered in more detail, PCBs would remain the contaminant that causes most concern and for which elimination and mitigation actions should be prioritised.

A further shortcoming of this study was the limited availability of accurate dose response data and adverse effect thresholds on the exposure effects of POPs on marine mammals. This made it

challenging to accurately assess the impacts of exposures on population health. The existence of species-specific thresholds and dose response concentrations in relation to end points such as mortality, growth and reproduction would have allowed for more accurate assessments of population risk to be carried out. In leu of more accurate toxicity data, for the purpose of the analysis presented in this thesis I used an established threshold for PCB related adverse effects in marine mammals, which was derived by combining data on surrogate laboratory species (European mink (*Mustela lutreola*)) with data from field experiments on otters (*Lutra lutra*) and seals (*Phoca vitulina*) across various end points (Kannan et al., 2000). This threshold is widely used when assessing PCB exposures in marine mammals and therefore allows meaningful comparisons to be made with previous and future studies.

It is also important to recognise that other toxicity thresholds have been derived for marine mammals (Table 6-1). The highest PCB toxicity threshold reported in marine mammals was for profound reproductive impairment in ringed seals (*Phoca hispida*), which was stated to occur at 41 mg/kg lipid for (Helle et al., 1976). More recently, i*n vitro* concentration response curves have been derived for immunotoxic effects in killer whales which suggest lymphocyte proliferation is not impacted until equivalent blubber concentrations exceed 400 mg/kg lipid (Desforges et al., 2017). This effect level is substantially higher than immune effect levels reported in *in vivo* studies (Kannan et al., 2000). It is, therefore, likely that extrapolation from *in vitro* to *in vivo* leads to an overestimate of concentrations or that *in vitro* experiments, which target specific responses, are less sensitive that the immune system as a whole because *in vitro* experiments are unable to recreate cascading effects across different tissues and cell types. A review of immunotoxicity in marine mammals compared *in vivo* and *in vitro* derived concentrations response curves, that were not converted to blubber equivalents, and found that there were no statistical differences between the two therefore, it may not be appropriate to convert concentrations (Desforges et al., 2016). If, as has been suggested, it is inappropriate to convert *in vitro* concentrations to blubber equivalents then Kannan et al's (2000) threshold of 9mg/kg lipid would have resulted in substantially reduced lymphocyte proliferation in killer whales (Desforges et al., 2017). It is clear that thresholds will vary according to several factors including the species being examined, the end point, the congener mixture, the matrix and the mode of study (e.g., *in vitro,* laboratory surrogate species, field). The wide range of thresholds that exist for marine mammals highlights the uncertainties that are

associated with current approaches and demonstrates that further work is required to improve the accuracy of risk assessments in these species.

Table 6-1: Toxicity thresholds for adverse effects of polychlorinated biphenyls (PCBs) derived for marine mammals

Species	Threshold (mg/kg)	Study mode	End-point	Reference
Killer whales	9 *in vitro* concentration 400 blubber conversion	*In vitro*	Lymphocyte proliferation	(Desforges et al., 2017)
Otters, mink, seals	17 (pre conversion to 25CBs)	*In vitro,* laboratory, semi field and field	Various (e.g. lymphocyte proliferation, endocrine disruption, suppression of Natural Killer cells)	(Kannan et al., 2000)
Ringed seals	41	*In vivo*	Reproductive impairment	(Helle et al., 1976)
Ringed seals	1.37	*In vitro*	Gene transcription	(Brown et al., 2014)
Bottlenose dolphins	14.8	Field and surrogate species	10% calf mortality	(Schwacke et al., 2002)
Beluga whales	1.6	*In vitro*	Disruption of vitamin A and E profiles	(Desforges et al., 2013)
Harbour seals	1.3	Field and surrogate species	Immunological and endocrine biomarker endpoints in juveniles	(Mos et al., 2010)

Accurate risk assessments of detrimental effects of POPs on marine mammals are possible but require an interdisciplinary response across ecology, ecotoxicology and analytical chemistry and will require large amounts of resources to obtain accurate estimates for each species. In addition, it may be useful to look at approaches that have been used in other species as these methods could improve the derivation of toxicity data for marine mammals or be used to extrapolate between species. For instance, a recent meta-analysis, developed concentration response curves for the adverse effects of PCBs on growth, mortality and reproduction in fish, using data from controlled

laboratory studies (Berninger and Tillitt, 2019). The dose response curves predicted that, at Kannan et al's (2000) threshold of 9mg/kg lipid, mortality rates would be 38%, growth would be significantly impacted in 15% of individuals and reproduction would be significantly impacted for 39% of individuals. It is of course difficult to compare the concentrations measured in fish with those in marine mammals because of the inherent difficulties in extrapolating between species and because concentrations were measured per kg of total body weight rather than on a lipid basis. However, in the absence of laboratory data in marine mammals, a cautious use of thresholds developed for other species combined with epidemiological studies may improve risk assessments. Given the lack of toxicological information that is available for marine mammals the use of Kannan et al's (2000) threshold is appropriate to assess population risk and allows for comparisons to be made across different studies. However, it is important to consider the wide range of toxicity thresholds that exist and make use of new methodologies and approaches where possible. For instance, future work could compare population level risk using a range of thresholds to generate best case and worst-case scenarios to provide more comprehensive risk assessments.

This work is an important contribution towards understanding more about the impacts of POPs on cetaceans. However, a better understanding of the magnitude of these effects could be gained by assessing the impact on populations in the context of other anthropogenic stressors. This would help to contextualise other pressures and enable conservation actions to be prioritised accordingly. Data deficiency on nearly all demographic parameters have precluded systematic investigations of the relative importance of stressors affecting population viability. More reliable abundance estimates and demography of cetacean species would help to understand whether POPs are impacting population viability. These estimates could allow the impact of combined stressors to be assessed so that recommendations for conservation, such as acceptable limits for exposure to contaminants and total anthropogenic removal rates from bycatch, could be defined in the context of other stressors. The interactive nature of some threats should also be considered when assessing population risk. For example, climate change has been shown to increase the bioavailability of POPs and increase environmental concentrations, which will in turn increase the risk of toxic effects occurring in cetaceans (Teran et al., 2012). Climate change may also impact prey distribution thereby reducing prey availability for some species (Evans and Waggitt, 2020). This

is likely to cause body condition to deteriorate, which has been shown to cause contaminants to be remobilised and increase the likelihood or severity of toxic effects (Aguilar et al., 1999).

The findings of this thesis also highlight the need to better understand the possible impacts of exposure to POPs on male fertility and reproduction. The assessment of the impacts of PCBs on male fertility could be improved by determining the mechanisms by which PCBs may reduce fertility in cetaceans. The possibility of generational effects should also be explored to better understand any population level impacts. The findings of this thesis have also demonstrated that there is a lack of toxicity data for cetaceans particularly in terms of species and age class specific thresholds, so further study in this area is recommended. For example, the impacts of neurotoxic CB congeners on developing cetaceans should be investigated to understand the possible long-term impacts on population health and understand whether separate toxic thresholds, which consider developmental effects, should exist for juveniles.

The findings of this thesis could also be strengthened by analysing a wider range of legacy and emerging contaminants and understanding how these contaminants interact and affect the toxicity of pollutant burdens (Andresen et al., 2007; Galatius et al., 2013). The development of improved tools and methodologies to assess the impacts of exposure to mixtures on cetaceans would provide a greater level of understanding of the impacts of mixtures and help to identify which contaminants pose the biggest threat to health. This would allow for improved monitoring of risk and provide further insights into the impacts of emerging contaminants. In addition, as the data analysed here are spatially limited to the UK, it would be useful to broaden the geographical scope of the study. Including data from carcasses that stranded across areas, which are likely to make up the home ranges of the animals analysed (i.e., across the English Channel, North Sea and Ireland), would yield more robust results, if there is movement between these locations.

6.3 Conclusion

This thesis has provided an original contribution to knowledge regarding the impacts of persistent organic pollutants on cetaceans. This has been demonstrated by the publications that have arisen from three of the chapters, in highly reputable scientific journals. These analyses have shown that

POPs remain at levels that are a toxicological concern and are still associated with increased mortality rates and possible sub-lethal affects that impact reproduction and development. The improved understanding of the spatiotemporal trends of POPs in cetaceans can be used to maximise the impact of actions to mitigate environmental contaminants because actions can be prioritised to target areas where contamination levels are highest. In addition, the findings regarding the variance of PCB congener distributions can be used to increase the efficacy of risk assessments to ensure vulnerable age groups are sufficiently protected. Risk assessment efficacy may be enhanced further by incorporating possible impacts on male fertility when assessing reproductive impacts at the population level. These findings are particularly important in the context of the most vulnerable cetacean populations around the world that have low reproduction rates and face a number of other threats, making recovery difficult.

The findings presented here are globally relevant and highlight the need for improved elimination and mitigation strategies to deal with global POP contamination. They demonstrate that UK-managed effective PCB controls should be prioritised as they could have a substantial impact on the health of coastal cetacean species. This thesis has enhanced the scientific understanding of the impacts of POPs on cetaceans and has highlighted several future research topics in this area. Given the pervasive and persistent threat of emerging and legacy contaminants the findings highlight that further action is required to reduce environmental concentrations of contaminants and protect cetaceans both in the UK and across the globe.

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Appendix

Table A- 1: Model selection table for log of summed contaminant concentrations against selected covariables. Only the top 20 of the 1670 models are displayed. (asterisk denotes those the plausible candidate models that were averaged). Lat (latitude); Long (longitude); NOD (Number of Days since 1st January 1990)*

Intercept	Age	Blubber	Lat	Long	NOD	Sex	Species	Age:Sex	Lat:Long	Lat:NOD	Lat:Species	Long:NOD	Long:Species	NOD:Species	Lat:Long:NOD	Lat:Long:Species	Lat:NOD:Species	Long:NOD:Species	Lat:Long:NOD:Species	df	logLik	AIC	delta	weight
3.77*	+	-0.32	-0.36	0.01	-0.72	+	+	+	-0.06	NA	NA	-0.09	NA	+	NA	NA	NA	NA	NA	25	-862.72	1775.44	0.00	0.13
3.75*	+	-0.32	-0.59	0.01	-0.70	+	+	+	-0.06	NA	+	-0.09	NA	+	NA	NA	NA	NA	NA	31	-856.74	1775.49	0.05	0.13
3.78*	+	-0.32	-0.36	0.01	-0.72	+	+	+	-0.06	0.04	NA	-0.08	NA	+	NA	NA	NA	NA	NA	26	-862.03	1776.07	0.63	0.10
3.81*	+	-0.32	-0.36	0.03	-0.74	+	+	+	NA	NA	NA	-0.09	NA	+	NA	NA	NA	NA	NA	24	-864.18	1776.36	0.92	0.08
3.76*	+	-0.32	-0.36	0.01	-0.71	+	+	+	-0.07	0.04	NA	-0.08	NA	+	0.04	NA	NA	NA	NA	27	-861.33	1776.66	1.22	0.07
3.79*	+	-0.32	-0.56	0.03	-0.73	+	+	+	NA	NA	+	-0.09	NA	+	NA	NA	NA	NA	NA	30	-858.37	1776.74	1.30	0.07
3.75*	+	-0.33	-0.61	0.01	-0.70	+	+	+	-0.06	0.03	+	-0.08	NA	+	NA	NA	NA	NA	NA	32	-856.45	1776.89	1.45	0.06
3.81*	+	-0.32	-0.36	0.03	-0.74	+	+	+	NA	0.04	NA	-0.08	NA	+	NA	NA	NA	NA	NA	25	-863.48	1776.96	1.52	0.06
3.74*	+	-0.33	-0.61	0.01	-0.69	+	+	+	-0.07	0.03	+	-0.08	NA	+	0.04	NA	NA	NA	NA	33	-855.66	1777.32	1.88	0.05
3.40*	+	-0.31	-0.37	0.02	-0.27	+	+	+	-0.07	NA	NA	-0.09	NA	NA	NA	NA	NA	NA	NA	19	-870.11	1778.23	2.79	0.03
3.79*	+	-0.33	-0.58	0.03	-0.73	+	+	+	NA	0.02	+	-0.08	NA	+	NA	NA	NA	NA	NA	31	-858.12	1778.24	2.80	0.03
3.40	+	-0.31	-0.37	0.02	-0.27	+	+	+	-0.07	0.03	NA	-0.07	NA	NA	0.05	NA	NA	NA	NA	21	-868.73	1779.47	4.02	0.02
3.40	+	-0.31	-0.37	0.02	-0.27	+	+	+	-0.07	0.02	NA	-0.09	NA	NA	NA	NA	NA	NA	NA	20	-869.77	1779.53	4.09	0.02
3.42	+	-0.31	-0.36	0.03	-0.26	+	+	+	NA	NA	NA	-0.09	NA	NA	NA	NA	NA	NA	NA	18	-871.91	1779.82	4.37	0.01
3.39	+	-0.32	-0.61	0.01	-0.26	+	+	+	-0.07	NA	+	-0.09	NA	NA	NA	NA	NA	NA	NA	25	-865.26	1780.53	5.09	0.01
3.42	+	-0.31	-0.37	0.03	-0.26	+	+	+	NA	0.02	NA	-0.09	NA	NA	NA	NA	NA	NA	NA	19	-871.55	1781.11	5.67	0.01
3.83	+	-0.31	-0.35	NA	-0.72	+	+	+	NA	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	22	-868.72	1781.45	6.01	0.01
3.83	+	-0.31	-0.36	NA	-0.72	+	+	+	NA	0.04	NA	NA	NA	+	NA	NA	NA	NA	NA	23	-867.74	1781.47	6.03	0.01
3.81	+	-0.31	-0.36	0.02	-0.70	+	+	+	-0.06	0.04	NA	NA	NA	+	NA	NA	NA	NA	NA	25	-865.80	1781.61	6.17	0.01
3.80	+	-0.31	-0.35	0.02	-0.70	+	+	+	-0.06	NA	NA	NA	NA	+	NA	NA	NA	NA	NA	24	-866.82	1781.65	6.20	0.01

*Table A- 2: Model selection table for log of summed PCB concentrations against selected covariables. Only the top 20 of the 50 models are displayed. (*asterisk denotes the model that was used)*

(Intercept)	Age Class	Latitude	Longitude	Relative body wt.	Sex	AgeClass: Sex	Latitude: Longtude	df	logLik	AIC	delta	weight
10.24*	+	-0.16	NA	-1.32	+	+	NA	7	-978.15	1970.31	0.00	5.57e-01
10.21	+	-0.16	0.01	-1.31	+	+	NA	8	-977.70	1971.41	1.10	3.21e-01
10.46	+	-0.16	0.10	-1.32	+	+	0	9	-977.68	1973.35	3.05	1.21e-01
10.44	+	-0.16	NA	NA	+	+	NA	6	-1003.75	2019.50	49.19	1.16e-11
10.40	+	-0.16	0.01	NA	+	+	NA	7	-1003.27	2020.54	50.24	6.87e-12
10.64	+	-0.16	0.09	NA	+	+	0	8	-1003.25	2022.50	52.19	2.58e-12
10.38	+	-0.15	NA	-1.28	+	NA	NA	6	-1028.66	2069.33	99.02	1.75e-22
10.34	+	-0.15	0.02	-1.28	+	NA	NA	7	-1027.95	2069.90	99.59	1.31e-22
10.26	+	-0.15	-0.01	-1.28	+	NA	0	8	-1027.95	2071.89	101.59	4.86e-23
9.91	NA	-0.15	NA	-1.24	+	NA	NA	5	-1038.14	2086.29	115.98	3.63e-26
9.86	NA	-0.14	0.02	-1.24	+	NA	NA	6	-1037.52	2087.04	116.73	2.49e-26
10.08	NA	-0.15	0.09	-1.24	+	NA	0	7	-1037.50	2089.01	118.70	9.34e-27
10.58	+	-0.16	NA	NA	+	NA	NA	5	-1049.99	2109.98	139.67	2.60e-31
10.53	+	-0.15	0.02	NA	+	NA	NA	6	-1049.25	2110.51	140.20	2.00e-31
10.45	+	-0.15	-0.01	NA	+	NA	0	7	-1049.25	2112.50	142.20	7.38e-32
1.85	+	NA	0.03	-1.41	+	+	NA	7	-1051.63	2117.26	146.95	6.84e-33
1.75	+	NA	NA	-1.41	+	+	NA	6	-1054.12	2120.25	149.94	1.53e-33
10.13	NA	-0.15	NA	NA	+	NA	NA	4	-1057.93	2123.87	153.56	2.51e-34
10.58	+	-0.15	0.02	-1.19	NA	NA	NA	6	-1056.26	2124.52	154.21	1.81e-34
10.08	NA	-0.15	0.02	NA	+	NA	NA	5	-1057.28	2124.57	154.26	1.77e-34

Table A- 3: Model selection table for cases and controls of infectious disease against selected covariates. Only the top 20 of the 120 models are displayed. (asterisk denotes those the plausible candidate models that were averaged)*

	(Intercept)	Age Class	Relative Body wt.	Latitude	Longitude	Seasn	Sex	Σ25CBs	Year	AgeClass:Sex	Season: Year	df	logLik	AICc	delta	weight
512*	-49.48	+	-6.86	0.10	0.10	+	+	0.05	0.02	+	NA	12	-326.36	677.23	0.00	2.46e-01
384*	-5.10	+	-6.78	0.09	0.10	+	+	0.05	NA	+	NA	11	-327.58	677.58	0.35	2.06e-01
256*	-50.36	+	-6.82	0.09	0.10	+	+	0.05	0.02	NA	NA	11	-328.28	678.98	1.75	1.02e-01
128*	-4.69	+	-6.74	0.08	0.10	+	+	0.04	NA	NA	NA	10	-329.57	679.49	2.27	7.94e-02
376*	-5.01	+	-6.74	0.09	NA	+	+	0.05	NA	+	NA	10	-329.90	680.16	2.93	5.70e-02
504*	-45.24	+	-6.82	0.09	NA	+	+	0.05	0.02	+	NA	11	-328.89	680.19	2.97	5.59e-02
380*	0.00	+	-6.75	NA	0.09	+	+	0.04	NA	+	NA	10	-330.33	681.01	3.78	3.71e-02
508*	-39.53	+	-6.82	NA	0.09	+	+	0.04	0.02	+	NA	11	-329.35	681.11	3.88	3.53e-02
248	-46.13	+	-6.78	0.08	NA	+	+	0.05	0.02	NA	NA	10	-330.66	681.67	4.44	2.67e-02
252	-41.17	+	-6.79	NA	0.09	+	+	0.04	0.02	NA	NA	10	-330.71	681.77	4.54	2.54e-02
120	-4.60	+	-6.69	0.08	NA	+	+	0.05	NA	NA	NA	9	-331.75	681.79	4.56	2.52e-02
124	-0.15	+	-6.71	NA	0.09	+	+	0.04	NA	NA	NA	9	-331.78	681.84	4.61	2.45e-02
372	-0.30	+	-6.71	NA	NA	+	+	0.04	NA	+	NA	9	-332.27	682.83	5.60	1.49e-02
500	-36.36	+	-6.79	NA	NA	+	+	0.05	0.02	+	NA	10	-331.44	683.23	6.00	1.22e-02
1024	-28.99	+	-6.85	0.10	0.10	+	+	0.05	0.01	+	+	15	-326.24	683.25	6.02	1.21e-02
116	-0.44	+	-6.67	NA	NA	+	+	0.04	NA	NA	NA	8	-333.63	683.48	6.25	1.08e-02
244	-37.94	+	-6.75	NA	NA	+	+	0.04	0.02	NA	NA	9	-332.72	683.73	6.50	9.56e-03
768	-32.43	+	-6.82	0.09	0.10	+	+	0.05	0.01	NA	+	14	-328.16	685.00	7.77	5.05e-03
1016	-22.65	+	-6.82	0.09	NA	+	+	0.05	0.01	+	+	14	-328.69	686.05	8.82	2.99e-03
1020	-25.23	+	-6.82	NA	0.09	+	+	0.04	0.01	+	+	14	-329.29	687.25	10.03	1.64e-03

Table A- 4: Model selection table for sexually mature individuals for the log of mean testes weight against selected covariates. Only the top 20 of the 38 models are displayed. (asterisk denotes those the plausible candidate models that were averaged)*

	(Intercept)	**BreedingS**	**Nutritional Condition**	**Log(Σ25CBs)**	**Breeding Season: Nutritional Condition**	**Breeding Season : Σ25CBs**	**Nutritional Condition: Σ25CBs**	**Breeding Season:Nutritional Condition: Σ25CBs**	**offset(log(Length))**	**df**	**logLik**	**AIC**	**delta**	**weight**
176*	1.37	+	0.39	0.08	+	NA	-0.15	NA	+	8	-51.38	118.77	0.00	0.40
140*	1.41	+	0.37	NA	+	NA	NA	NA	+	6	-53.62	119.23	0.47	0.32
192*	1.39	+	0.42	0.17	+	+	-0.14	NA	+	9	-52.25	122.50	3.74	0.06
168*	1.44	+	0.14	0.07	NA	NA	-0.16	NA	+	7	-54.36	122.72	3.95	0.06
132	1.50	+	0.11	NA	NA	NA	NA	NA	+	5	-56.60	123.20	4.43	0.04
130	1.53	+	NA	NA	NA	NA	NA	NA	+	4	-57.71	123.43	4.66	0.04
144	1.43	+	0.39	0.05	+	NA	NA	NA	+	7	-55.22	124.44	5.68	0.02
160	1.47	+	0.47	0.23	+	+	NA	NA	+	8	-55.00	126.00	7.23	0.01
256	1.34	+	0.40	0.13	+	+	-0.22	+	+	10	-53.11	126.21	7.45	0.01
48	6.35	+	0.40	0.11	+	NA	-0.14	NA	NA	8	-55.26	126.52	7.75	0.01
12	6.38	+	0.37	NA	+	NA	NA	NA	NA	6	-57.34	126.68	7.91	0.01
184	1.42	+	0.14	0.01	NA	+	-0.16	NA	+	8	-55.43	126.86	8.09	0.01
136	1.51	+	0.12	0.03	NA	NA	NA	NA	+	6	-58.50	129.01	10.24	0.00
134	1.53	+	NA	0.00	NA	NA	NA	NA	+	5	-59.88	129.75	10.98	0.00
4	6.47	+	0.11	NA	NA	NA	NA	NA	NA	5	-60.03	130.06	11.29	0.00
2	6.50	+	NA	NA	NA	NA	NA	NA	NA	4	-61.05	130.10	11.33	0.00
64	6.37	+	0.43	0.19	+	+	-0.13	NA	NA	9	-56.09	130.19	11.42	0.00
40	6.43	+	0.15	0.09	NA	NA	-0.15	NA	NA	7	-58.10	130.21	11.44	0.00
16	6.41	+	0.40	0.07	+	NA	NA	NA	NA	7	-58.28	130.55	11.78	0.00
32	6.45	+	0.48	0.26	+	+	NA	NA	NA	8	-58.13	132.26	13.49	0.00

Table A- 5: Count of mature individuals in each age class and sexual maturity group

Age (years)	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
N=	0	0	2	1	6	7	6	4	5	2	7	1	1	0	0	2	0	0	2

Table A- 6: Count of individuals in each cause of death category and sexual maturity status in the strandings sample

	Infectious Disease	**Others**	**Trauma**
Immature	34	29	76
Mature	29	10	53

*Table A- 7:Results of analysis of variance and Tukey's HSD tests between the mean proportions of each congener for each age class and sex *indicates significance (p<0.05)*

CB.18 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0	0	0.7	0.5
Residuals	344	0	0		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
adultM-adultF	0	0	0	0.93
juvenile-adultF	0	0	0	0.83
juvenile-adultM	0	0	0	0.50

CB.28 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0	0	10.56	0.00*
Residuals	344	0	0		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
adultM-adultF	-0.00	-0.00	-0.00	0.00*
juvenile-adultF	-0.00	-0.00	0.000	0.10
juvenile-adultM	0.00	0.000	0.00	0.00

CB.31 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0	0	3.04	0.05
Residuals	344	0	0		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
adultM-adultF	0	0	0	0.06
juvenile-adultF	0	0	0	0.07
juvenile-adultM	0	0	0	0.85

CB.44 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0	0	7.83	0.00*
Residuals	344	0	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0	0	0	0.00*
juvenile-adultF	0	0	0	0.85
juvenile-adultM	0	0	0	0.00*

CB.47 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	13.1	0.00*
Residuals	344	0.01	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0	0	0	0.07
juvenile-adultF	0	0	0	0.12
juvenile-adultM	0	0	0	0.00*

CB.49 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	22.78	0.00*
Residuals	344	0.01	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0	-0.01	0.00	0.00*
juvenile-adultF	0	0.00	0.00	0.44
juvenile-adultM	0	0.00	0.01	0.00*

CB.52 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.01	0.01	36.89	0.00*
Residuals	344	0.05	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.02	0.01	0.02	0.00*
juvenile-adultF	0.02	0.01	0.02	0.00*
juvenile-adultM	0.00	-0.01	0.02	0.52

CB.66 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	2.9	0.06
Residuals	344	0.09	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.01	0.00	0.01	0.09
juvenile-adultF	0.01	0.00	0.01	0.06
juvenile-adultM	0.00	-0.01	0.00	0.97

CB.101 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.04	0.02	58.78	0.00*
Residuals	344	0.10	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	-0.01	-0.02	0.00	0.00*
juvenile-adultF	0.01	0.01	0.02	0.00*
juvenile-adultM	0.02	0.02	0.03	0.00*

CB.105 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	10.27	0.00*
Residuals	344	0.04	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.00	-0.01	0.00	0.47
juvenile-adultF	0.00	0.00	0.01	0.04*
juvenile-adultM	0.01	0.00	0.01	0.00*

CB.110 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	8.28	0.00*
Residuals	344	0.01	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0	-0.01	0	0.00*
juvenile-adultF	0	0.00	0	0.66
juvenile-adultM	0	0.00	0	0.00*

CB.118 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.04	0.02	73.13	0.00*
Residuals	344	0.09	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	-0.01	-0.02	0.00	0.00*
juvenile-adultF	0.01	0.01	0.02	0.00*
juvenile-adultM	0.03	0.02	0.03	0.00*

CB.128 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	22.86	0.00*
Residuals	344	0.02	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	-0.01	-0.01	0.00	0.00*
juvenile-adultF	0.00	0.00	0.00	0.16
juvenile-adultM	0.01	0.00	0.01	0.00*

CB.138 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.02	0.01	18.59	0.00*
Residuals	344	0.19	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.03	0.02	0.04	0.00*
juvenile-adultF	0.01	0.01	0.02	0.00*
juvenile-adultM	-0.01	-0.02	0.00	0.00*

CB.141 *Analysis of Variance*

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0	0	30.21	0.00*
Residuals	344	0	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0	-0.01	0	0.00*
juvenile-adultF	0	0.00	0	0.09
juvenile-adultM	0	0.00	0	0.00*

CB.149 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.02	0.01	19.04	0.00*
Residuals	344	0.22	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.02	0.01	0.03	0.00*
juvenile-adultF	0.02	0.01	0.03	0.00*
juvenile-adultM	0.00	-0.01	0.01	0.99

CB.151 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	19.25	0.00*
Residuals	344	0.02	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.00	0	0.01	0.00*
juvenile-adultF	0.01	0	0.01	0.00*
juvenile-adultM	0.00	0	0.00	0.10

CB.153 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.07	0.03	22.6	0.00*
Residuals	344	0.50	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.04	0.03	0.06	0.00*
juvenile-adultF	0.01	0.00	0.03	0.04*
juvenile-adultM	-0.03	-0.04	-0.02	0.00*

CB.156 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	4.38	0.01*
Residuals	344	0.01	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0	-0.01	0	0.04*
juvenile-adultF	0	-0.01	0	0.01*
juvenile-adultM	0	0.00	0	1.00

CB.170 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.01	0.01	65.44	0.00*
Residuals	344	0.03	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	-0.01	-0.02	-0.01	0.00*
juvenile-adultF	-0.02	-0.02	-0.01	0.00*
juvenile-adultM	0.00	-0.01	0.00	0.00*

CB.180 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.08	0.04	30.61	0.00*
Residuals	344	0.45	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	-0.03	-0.04	-0.01	0.00*
juvenile-adultF	-0.04	-0.06	-0.03	0.00*
juvenile-adultM	-0.02	-0.03	0.00	0.01*

CB.183 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.00	0	14.57	0.00*
Residuals	344	0.06	0		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	-0.01	-0.01	0.00	0.00*
juvenile-adultF	-0.01	-0.01	-0.01	0.00*
juvenile-adultM	0.00	-0.01	0.00	0.23

CB.187 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.05	0.02	95.72	0.00*
Residuals	344	0.09	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	-0.02	-0.02	-0.01	0.00*
juvenile-adultF	-0.03	-0.04	-0.03	0.00*
juvenile-adultM	-0.01	-0.02	-0.01	0.00*

CB.194 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.01	0.01	109.97	0.00*
Residuals	344	0.02	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	-0.02	-0.02	-0.01	0.00*
juvenile-adultF	-0.02	-0.02	-0.01	0.00*
juvenile-adultM	0.00	0.00	0.00	0.55

*Table A- 8:Results of analysis of variance and Tukey's HSD tests between the mean proportions of each congener grouped by degree of chlorination for each age class and sex *indicates significance (p<0.05)*

Tri & Tetra-chlorinated Congeners Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0.02	0.01	11.24	0.00*
Residuals	344	0.30	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
adultM-adultF	0.01	0.00	0.03	0.05
juvenile-adultF	0.02	0.01	0.03	0.00*
juvenile-adultM	0.01	0.00	0.02	0.13

Penta-chlorinated Congeners Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0.20	0.10	68.58	0.00*
Residuals	344	0.51	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
adultM-adultF	-0.03	-0.04	-0.01	0.00*
juvenile-adultF	0.03	0.02	0.05	0.00*
juvenile-adultM	0.06	0.05	0.07	0.00*

Hexa-chlorinated Congeners Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0.21	0.11	41.63	0.00*
Residuals	344	0.87	0.00		

Tukey's HSD

Comparison groups	**null value**	**Estimate**	**conf low**	**conf high**
adultM-adultF	0.08	0.06	0.10	0.00*
juvenile-adultF	0.05	0.04	0.07	0.00*
juvenile-adultM	-0.03	-0.04	-0.01	0.00*

Hepta-chlorinated Congeners Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.47	0.23	70.84	0.00*
Residuals	344	1.13	0.00		

Tukey's HSD

Comparison groups	null value	Estimate	conf low	conf high
adultM-adultF	-0.06	-0.09	-0.04	0.00*
juvenile-adultF	-0.10	-0.12	-0.08	0.00*
juvenile-adultM	-0.04	-0.06	-0.03	0.00*

Octa-chlorinated Congeners Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.01	0.01	109.97	0.00*
Residuals	344	0.02	0.00		

Tukey's HSD

Comparison groups	null value	Estimate	conf low	conf high
adultM-adultF	-0.02	-0.02	-0.01	0.00*
juvenile-adultF	-0.02	-0.02	-0.01	0.00*
juvenile-adultM	0.00	0.00	0.00	0.547

Table A- 9:Results of analysis of variance and Tukey's HSD tests between the mean proportions of each structural activity group (SAG) for each age class and sex

SAG1 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0.45	0.22	64.92	0.00*
Residuals	344	1.19	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
adultM-adultF	-0.02	-0.05	0.00	0.07
juvenile-adultF	-0.09	-0.11	-0.07	0.00*
juvenile-adultM	-0.06	-0.08	-0.04	0.00*

SAG2 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0.01	0.00	4.17	0.02
Residuals	344	0.22	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
adultM-adultF	0.01	0.00	0.02	0.13
juvenile-adultF	0.00	-0.01	0.01	0.95
juvenile-adultM	0.01	-0.02	0.00	0.01

SAG3 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Age class and sex	2	0.07	0.03	42.92	0.00*
Residuals	344	0.26	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
adultM-adultF	-0.01	-0.02	0.00	0.27
juvenile-adultF	0.02	0.01	0.03	0.00*
juvenile-adultM	0.03	0.02	0.04	0.00*

SAG4 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.07	0.03	40.66	0.00*
Residuals	344	0.29	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.00	-0.01	0.01	0.96
juvenile-adultF	0.03	0.02	0.04	0.00*
juvenile-adultM	0.03	0.02	0.04	0.00*

SAG5 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Age class and sex	2	0.04	0.02	23.44	0.00*
Residuals	344	0.29	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
adultM-adultF	0.03	0.02	0.04	0.00*
juvenile-adultF	0.03	0.02	0.04	0.00*
juvenile-adultM	0.00	-0.01	0.01	0.82

Table A- 10: Results of analysis of variance and Tukey's HSD tests between the mean proportions of each structural activity group (SAG) grouped by persistence for each age area

Persistent SAGs Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Area	2	0.15	0.07	20.01	0.00*
Residuals	344	1.27	0.00		

Tukey's HSD

Comparison groups	**null value**	**Estimate**	**conf low**	**conf high**	**adjusted p value**
Scotland-East England	0	-0.01	-0.03	0.01	0.34
West-East England	0	0.03	0.02	0.05	0.00*
West-Scotland	0	0.05	0.03	0.06	0.00*

Less Persistent SAGs Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Area	2	0.17	0.08	29.86	0.00*
Residuals	344	0.97	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
Scotland-East England	0.01	-0.00	0.03	0.18
West-East England	-0.04	-0.05	-0.02	0.00*
West-Scotland	-0.05	-0.07	-0.03	0.00*

Table A- 11: Results of analysis of variance and Tukey's HSD tests between the mean proportions of each congener grouped by degree of chlorination for each age area

Tri & Tetra-chlorinated Congeners Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Area	2	0.09	0.04	62.67	0.00*
Residuals	344	0.23	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
Scotland-East England	0.01	0.00	0.02	0.10
West-East England	-0.03	-0.04	-0.02	0.00*
West-Scotland	-0.03	-0.04	-0.03	0.00*

Penta-chlorinated Congeners Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Area	2	0.02	0.01	4.68	0.01*
Residuals	344	0.69	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
Scotland-East England	0.01	-0.01	0.02	0.737
West-East England	-0.01	-0.03	0.00	0.127
West-Scotland	-0.02	-0.03	0.00	0.01*

Hexa-chlorinated Congeners Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Area	2	0.05	0.03	8.95	0.00*
Residuals	344	1.02	0.00		

Tukey's HSD

Comparison groups	Estimate	conf low	conf high	adjusted p value
Scotland-East England	0.01	-0.01	0.03	0.27
West-East England	-0.02	-0.03	0.00	0.06
West-Scotland	-0.03	-0.05	-0.01	0.00*

Hepta-chlorinated Congeners Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Area	2	0.31	0.16	41.37	0.00*
Residuals	344	1.29	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
Scotland-East England	-0.02	-0.04	0.00	0.02
West-East England	0.04	0.03	0.06	0.00*
West-Scotland	0.07	0.05	0.09	0.00*

Octa-chlorinated Congeners Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Area	2	0.00	0.00	14.94	0.00*
Residuals	344	0.03	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
Scotland-East England	0.00	0.00	0.00	0.97
West-East England	0.01	0.00	0.01	0.00*
West-Scotland	0.01	0.00	0.01	0.00*

Table A- 12: Results of analysis of variance and Tukey's HSD tests between the mean proportions of each structural activity group (SAG) grouped by persistence for each age area

Persistent SAGs Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Area	2	0.15	0.07	20.01	0.00*
Residuals	344	1.27	0.00		

Tukey's HSD

Comparison groups	**null value**	**Estimate**	**conf low**	**conf high**	**adjusted p value**
Scotland-East England	0	-0.01	-0.03	0.01	0.34
West-East England	0	0.03	0.02	0.05	0.00*
West-Scotland	0	0.05	0.03	0.06	0.00*

Less Persistent SAGs Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Area	2	0.17	0.08	29.86	0.00*
Residuals	344	0.97	0.00		

Tukey's HSD

Comparison groups	**Estimate**	**conf low**	**conf high**	**adjusted p value**
Scotland-East England	0.01	0.00	0.03	0.18
West-East England	-0.04	-0.05	-0.02	0.00*
West-Scotland	-0.05	-0.07	-0.03	0.00*

Table A- 13: Results of analysis of variance between male and female juveniles for mean proportions of each congener

CB.18 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Males & Female Juveniles	1	0	0	0	1
Residuals	221	0	0		

CB.28 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Males & Female Juveniles	1	0	0	0.33	0.57
Residuals	221	0	0		

CB.31 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Males & Female Juveniles	1	0	0	0.16	0.69
Residuals	221	0	0		

CB.44 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Males & Female Juveniles	1	0	0	0.68	0.41
Residuals	221	0	0		

CB.47 Analysis of Variance

	Df	**Sum Sq**	**Mean Sq**	**F value**	**Pr(>F)**
Males & Female Juveniles	1	0	0	0.34	0.56
Residuals	221	0	0		

CB.49 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0	0	0.48	0.49
Residuals	221	0	0		

CB.52 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	1.57	0.21
Residuals	221	0.04	0		

CB.66 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.82	0.37
Residuals	221	0.06	0		

CB.101 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.08	0.78
Residuals	221	0.09	0		

CB.105 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.84	0.36
Residuals	221	0.03	0		

CB.110 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0	0.99
Residuals	221	0.01	0		

CB.118 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.29	0.59
Residuals	221	0.06	0		

CB.128 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.71	0.4
Residuals	221	0.01	0		

CB.138 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.52	0.47
Residuals	221	0.14	0		

CB.141 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0	0	0	0.96
Residuals	221	0	0		

CB.149 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.46	0.5
Residuals	221	0.17	0		

CB.151 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.01	0.94
Residuals	221	0.01	0		

CB.153 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.01	0.92
Residuals	221	0.28	0		

CB.156 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0	0.99
Residuals	221	0.01	0		

CB.158 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0	0	0.01	0.9
Residuals	221	0	0		

CB.170 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.06	0.81
Residuals	221	0.02	0		

CB.180 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.33	0.57
Residuals	221	0.29	0		

CB.183 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.02	0.89
Residuals	221	0.05	0		

CB.187 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	0.28	0.6
Residuals	221	0.05	0		

CB.194 Analysis of Variance

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Males & Female Juveniles	1	0.00	0	1.25	0.27
Residuals	221	0.01	0		

Table A- 14: Relative frequencies of age class and sex for each area

Area	Age class and sex	n	Relative frequency
East England	adultF	15	0.17
East England	adultM	23	0.26
East England	juvenile	50	0.57
Scotland	adultF	17	0.15
Scotland	adultM	25	0.22
Scotland	juvenile	73	0.63
West	adultF	23	0.16
West	adultM	21	0.15
West	juvenile	100	0.69

Table A- 15: Relative frequencies of age class and sex for each five-year interval

Five-Year Intervals	Age class and sex	n	Relative frequency
1990-1994	adultF	3	0.10
1990-1994	adultM	6	0.21
1990-1994	juvenile	20	0.69
1995-1999	adultF	13	0.14
1995-1999	adultM	20	0.21
1995-1999	juvenile	62	0.65
2000-2004	adultF	18	0.18
2000-2004	adultM	17	0.17
2000-2004	juvenile	66	0.65
2005-2009	adultF	14	0.18
2005-2009	adultM	16	0.20
2005-2009	juvenile	50	0.62
2010-2015	adultF	7	0.17
2010-2015	adultM	10	0.24
2010-2015	juvenile	25	0.60

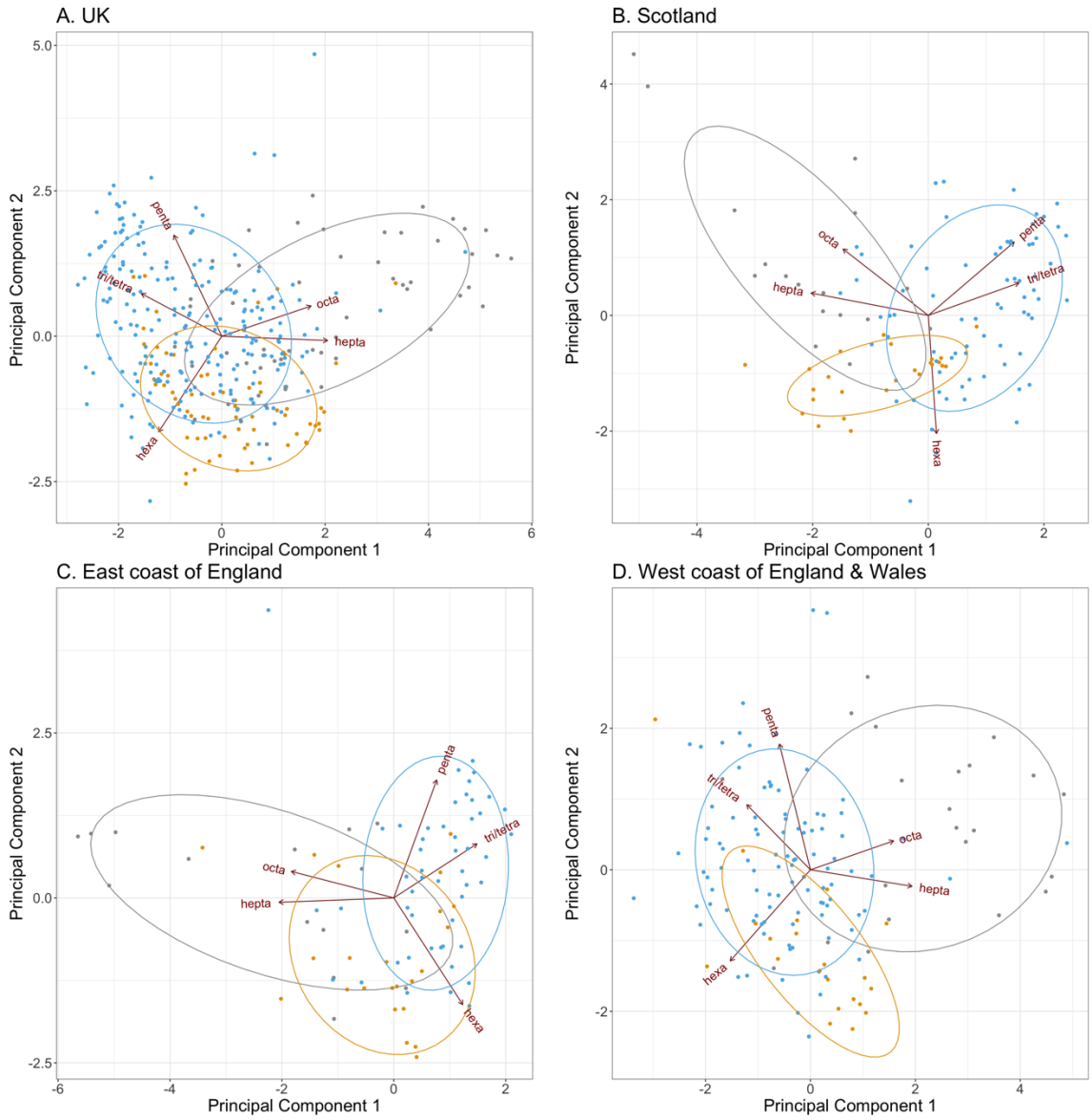

Figure A- 1: Principal Component 1 plotted against Principal Component 2 for the PCA carried out on congeners grouped by their degree of chlorination. The sizing of the confidence ellipses was set at 70%. (A) Data from the whole of the UK (B) Data from Scotland (C) Data from the east coast of England (D) Data from the west coast of England & Wales

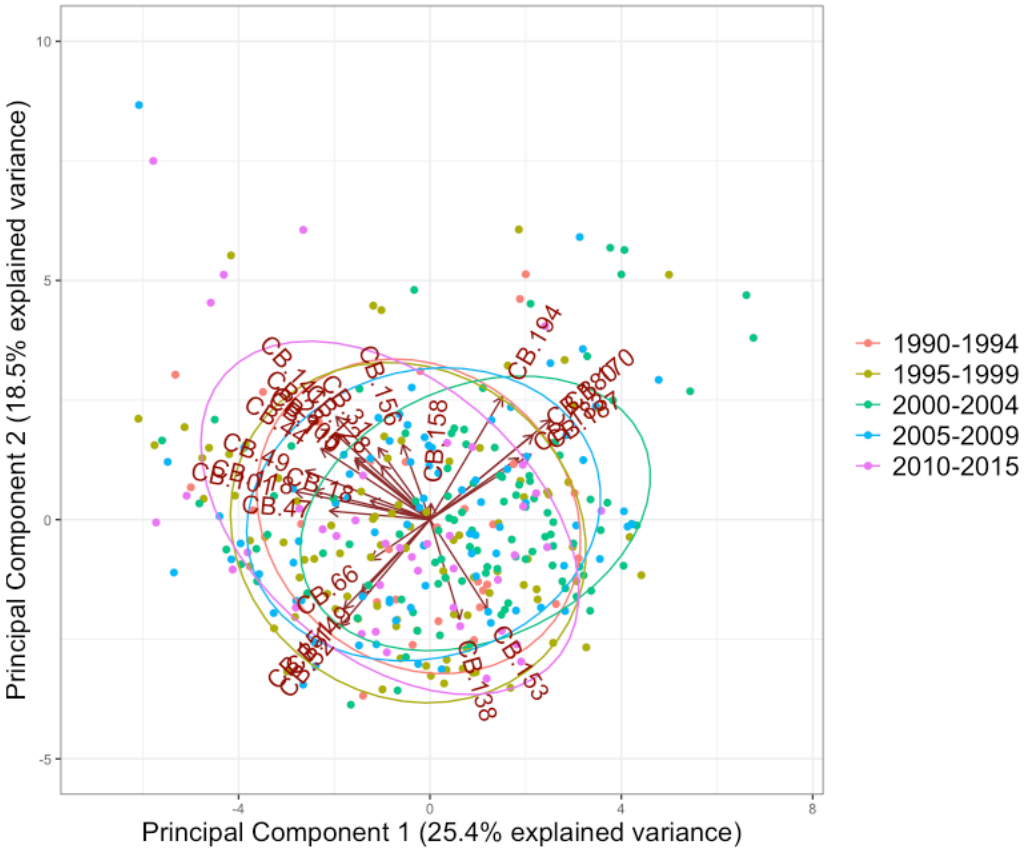

Figure A- 2: Principal Component 1 plotted against Principal Component 2 for the PCA carried out on the individual congeners, coloured by time intervals of five years

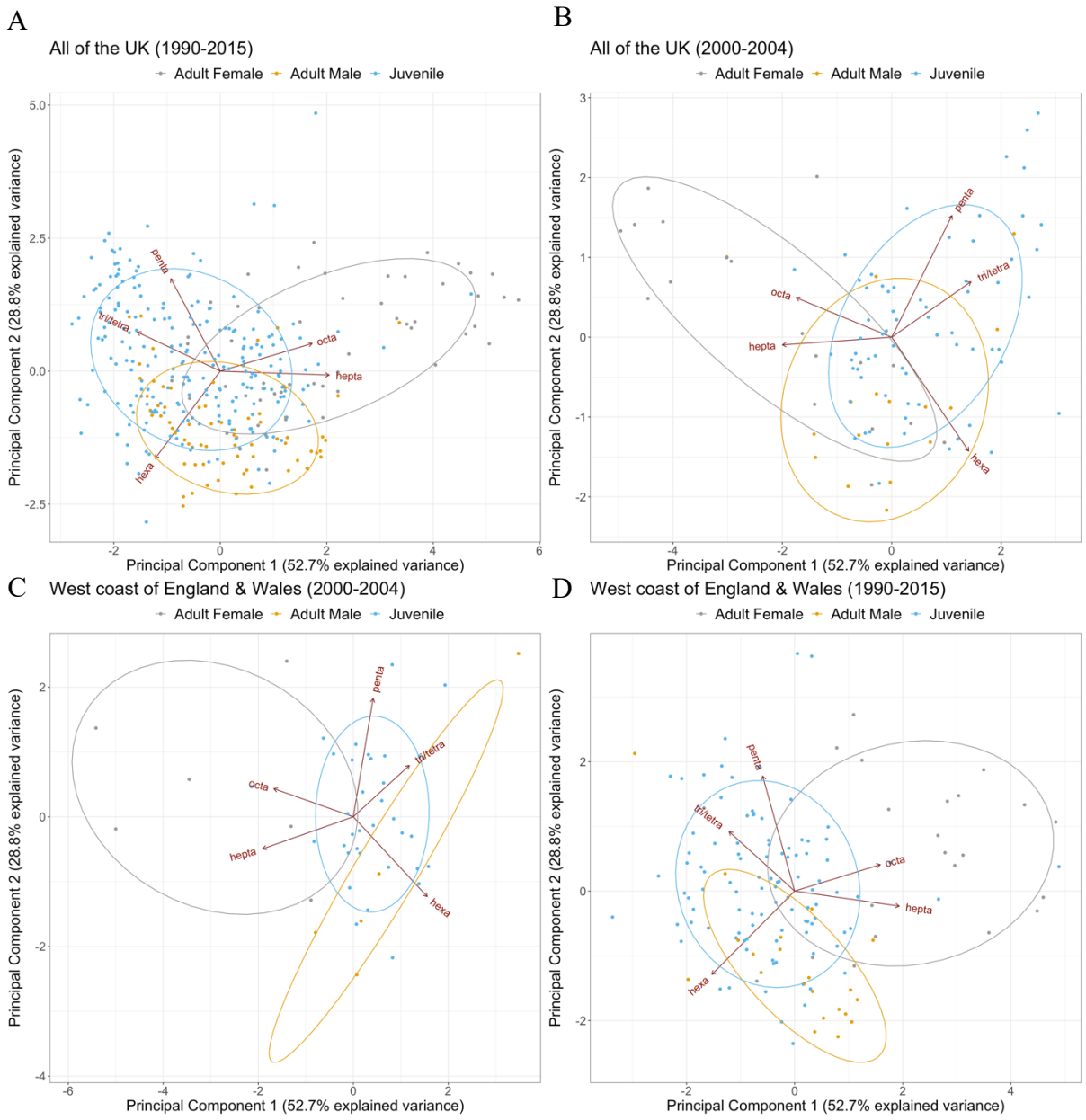

Figure A- 3: Principal Component 1 plotted against Principal Component 2 for the PCA carried out on the individual congeners, coloured by time intervals of five years. (A) Data from the whole of the UK (B) Data from the UK between 2000 and 2004 d (C) Data from the west coast of England & Wales between 2000 and 2004 (D) Data from the west coast of England & Wales

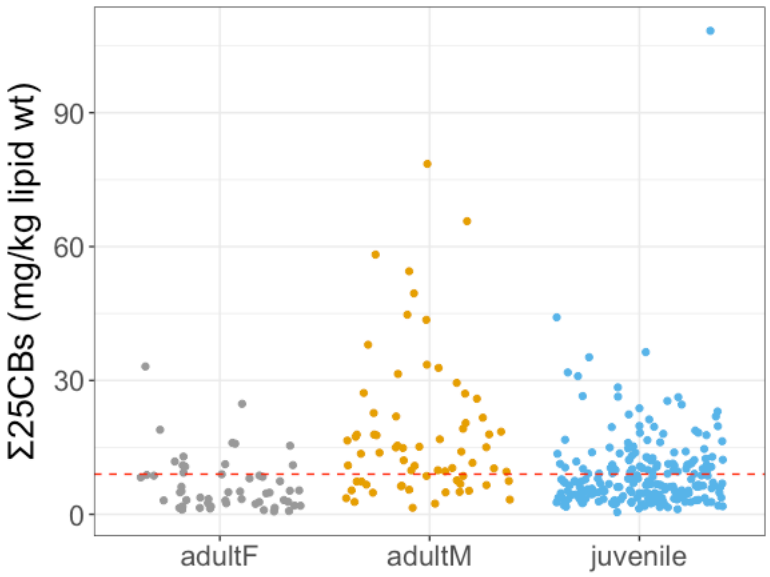

Figure A- 4: Concentrations of the sum of the 25 chlorinated biphenyl congeners measured for each age class and sex. The red horizontal line represents the widely used toxicity threshold for cetaceans of 9mg/ kg lipid weight.