Polychlorinated biphenyls are associated with reduced testes weights in harbour porpoises (*Phocoena phocoena*)

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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, here we 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, we suggest the inclusion of such effects in population level impact assessments involving PCB exposures. Given the re-emergent PCB threat our findings are globally significant, with potentially serious implications for long-lived mammals. We 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.

Keywords: *Phocoena phocoena*; Polychlorinated biphenyls; testes weights; male reproduction; marine mammals; fertility

1. Introduction

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.

Here, we have used the largest cetacean toxicology strandings dataset globally available 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). Our 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.

2. Materials and Methods

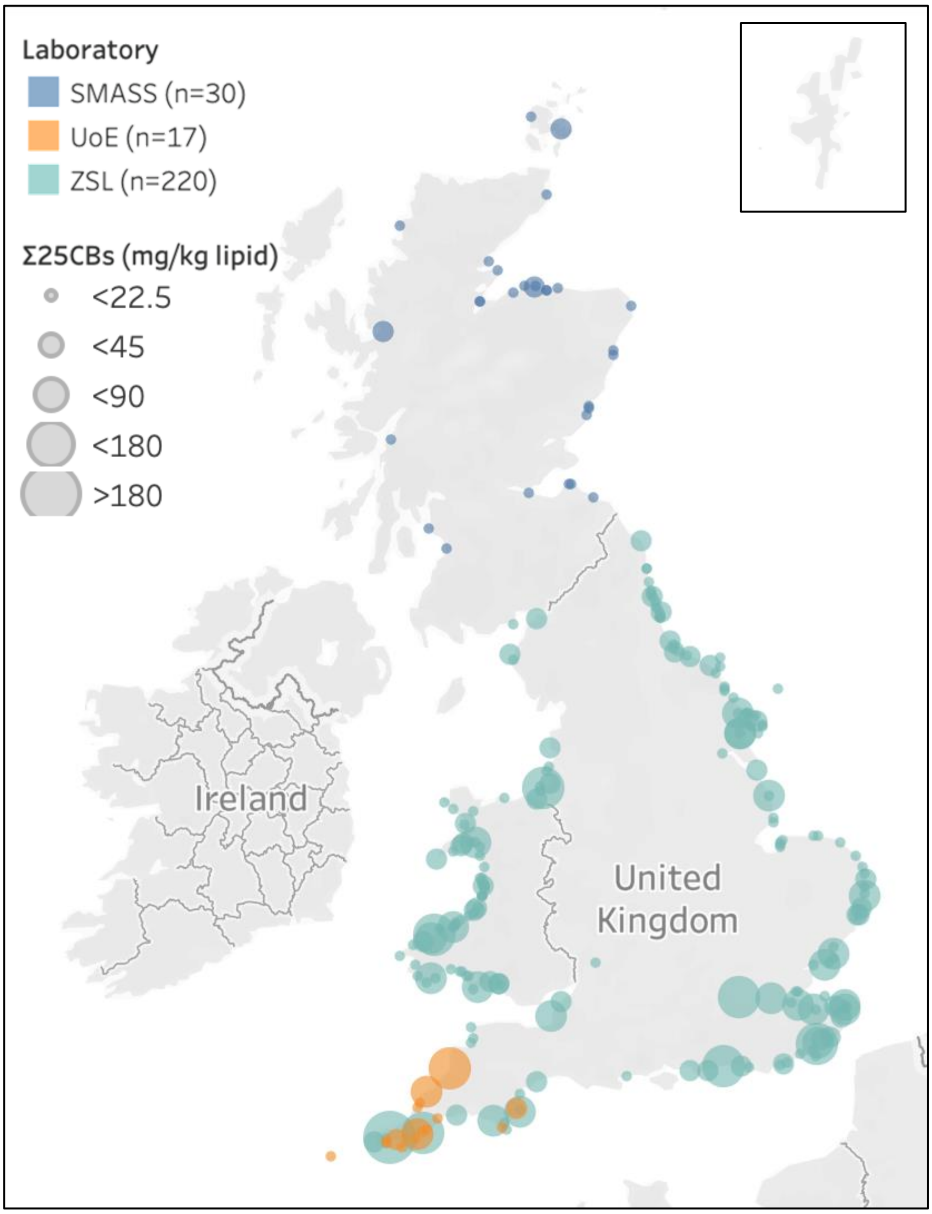

Figure 1:Geographic locations of the adult male individuals that stranded and were analysed to obtain blubber concentrations for the sum of 25 selected congeners of polychlorinated biphenyls (Σ25CBs). The colours of the dots represent the laboratories where the animals were necropsied: Scottish Marine Animal Stranding Scheme (SMASS), University of Exeter (UoE), Zoological Society of London (ZSL). The size of the dots represent 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.

2.1 Sampling

We determined 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, 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 (n=30); the University of Exeter (n=17); the Zoological Society of London (n=220) (Figure 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.

2.2 PCB Analysis

We used a standardised methodology 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), we set the concentration at half the limit (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. 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).

The CEFAS laboratory (Lowestoft) participates biannually in the QUASIMEME proficiency testing scheme for quality assurance and quality control. All the analyses were conducted under full analytical quality control procedures, including the analysis of a blank sample and certified reference material with each batch of 10 samples to assess performance of the methods. In every case the blanks were always below the limit of quantification. When target analytes were beyond the range of instrumentation calibration, the extracts were diluted and re-analysed. The reference material BCR349 (cod liver oil; European Bureau of Community reference) was used and the reference material results were plotted as Shewhard quality control charts for each compound. The charts were previously created from repeated analysis of the reference material using the North West Analytical Quality Analyst software™ (Northwest Analytical Inc., USA). All certified reference materials for each of the samples analysed were within the control and warning limits for each compound, defined as 2σ and 3σ – 2x and 3x the standard deviation from the mean.

2.3 Pathological and Statistical Analyses

As part of the pathological investigations, certain attributes were determined for each animal in the study. We determined sexual maturity using gonadal appearance and, where undertaken, looking for histological evidence of spermatogenesis in male testes (Murphy, 2008). We 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) we 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 (Lockyer, 1995).

Testes were removed from the 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 we 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 we assumed death occurred during the same season that the animal stranded. We 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 2). 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, we fitted a power regression model and extracted the residuals to obtain a metric that could be used as a proxy for body condition (see Appendix A).

We 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). We 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. We validated this approach by modelling testes weights against selected covariates for immature individuals and this analysis is shown in Appendix A.

We carried out all of the analyses using the statistical software R (version 3.4.3) (R Core Team, 2016). Prior to model fitting we carried out extensive data exploration to test for collinearity between variables and remove individuals with missing body weight, length or testes weights (Appendix B Table 6). We 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 biological rationale that they could impact testes weights. These were nutritional condition, breeding season and log of PCB blubber concentration, with a three-way interaction. We included laboratory as a random effect (Figure 1) in the model to account for any sources of variation between laboratories, including whether testes were weighed with or without the epididymis. We assumed that the inclusion or exclusion of the epididymis would only impact the intercepts and would have no effects on the coefficient estimates. We validated this approach by ensuring that the relationship between length and mean testes weight was consistent across the laboratories (Appendix B Figure 3). We did not include the longitude and latitude of the stranding location in the model as we did not observe any spatial variation in testes weights (see Appendix B Tables 3 & 4). 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 harbour porpoises (Williams et al., 2020b). The log of body length was included as an offset term to scale testes weights. We used body length as opposed to body weight because body weight included testes weights and was correlated with nutritional condition (Pearson's correlation, $r=0.92$, $p=<0.01$).

We 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). Our final prediction was obtained by averaging the set of plausible models ($\Delta AIC < 4$) from the candidate models. We validated the models by checking the distribution of the residuals and plotting them against selected variables and assessing the variance (see Appendix B Figure 1).

3. Results

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 1).

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

Equation 1: The final form of the model 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 B Table 7.

From the averaged model, we 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 3, Figure 4,Table 1). We found that animals in poor nutritive condition were predicted to have the lowest testes weights (Figure 4). 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 Appendix B Table 3.

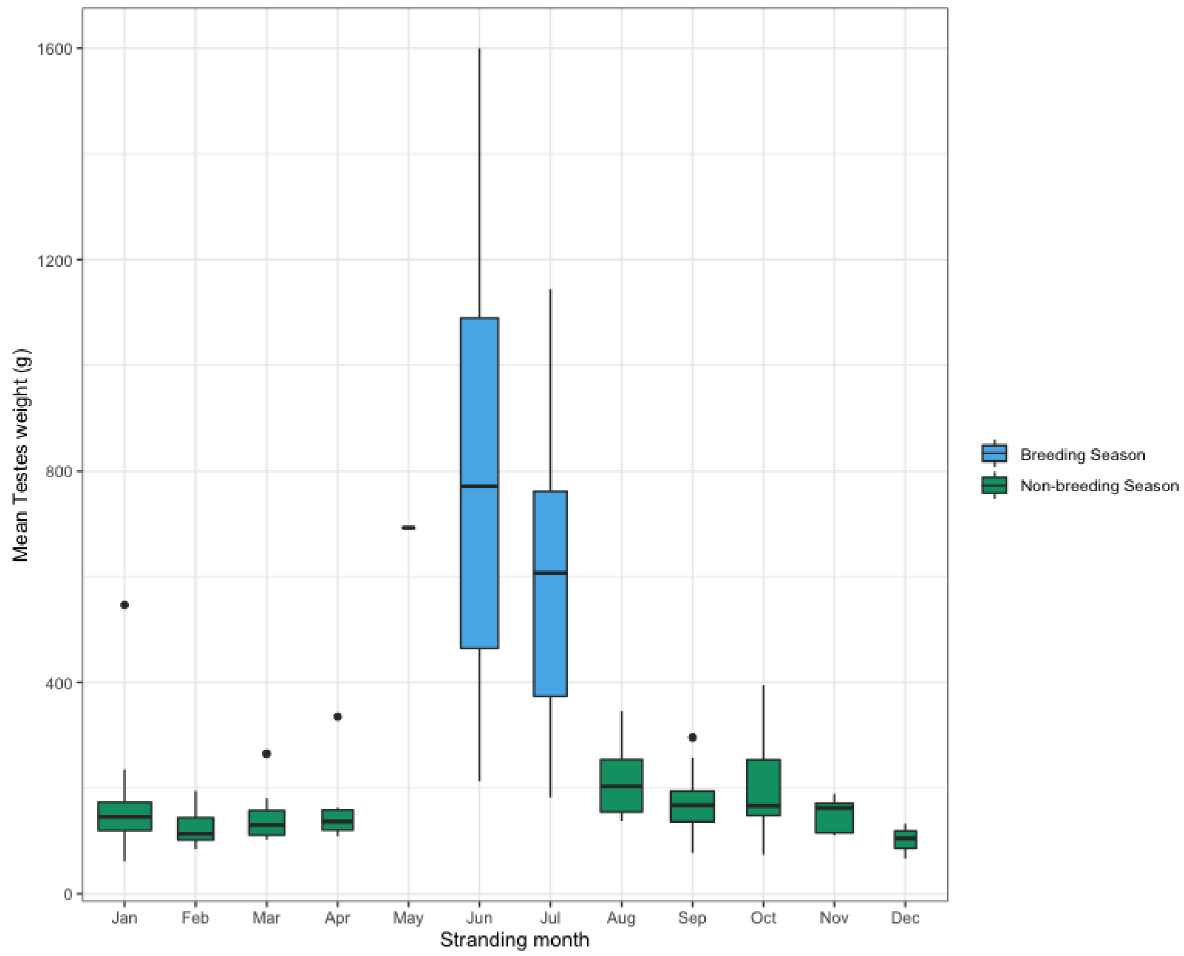

Figure 2: Mean testes weights (g) of harbour porpoises (*Phocoena phocoena)* stranded in the UK between 1991 and 2017 by month of stranding for mature individuals (n=99). The width of the boxes is proportional to the sample size. In months that do not contain more than one data point a dash is displayed. 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.

Predictably, 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 1, Figure 2). Nutritional condition also heavily influenced testes weights such that individuals with better body condition had higher mean testes weights (Figure 3). The effect of nutritional condition on testes weights was greater during the breeding season than during the non-breeding season.

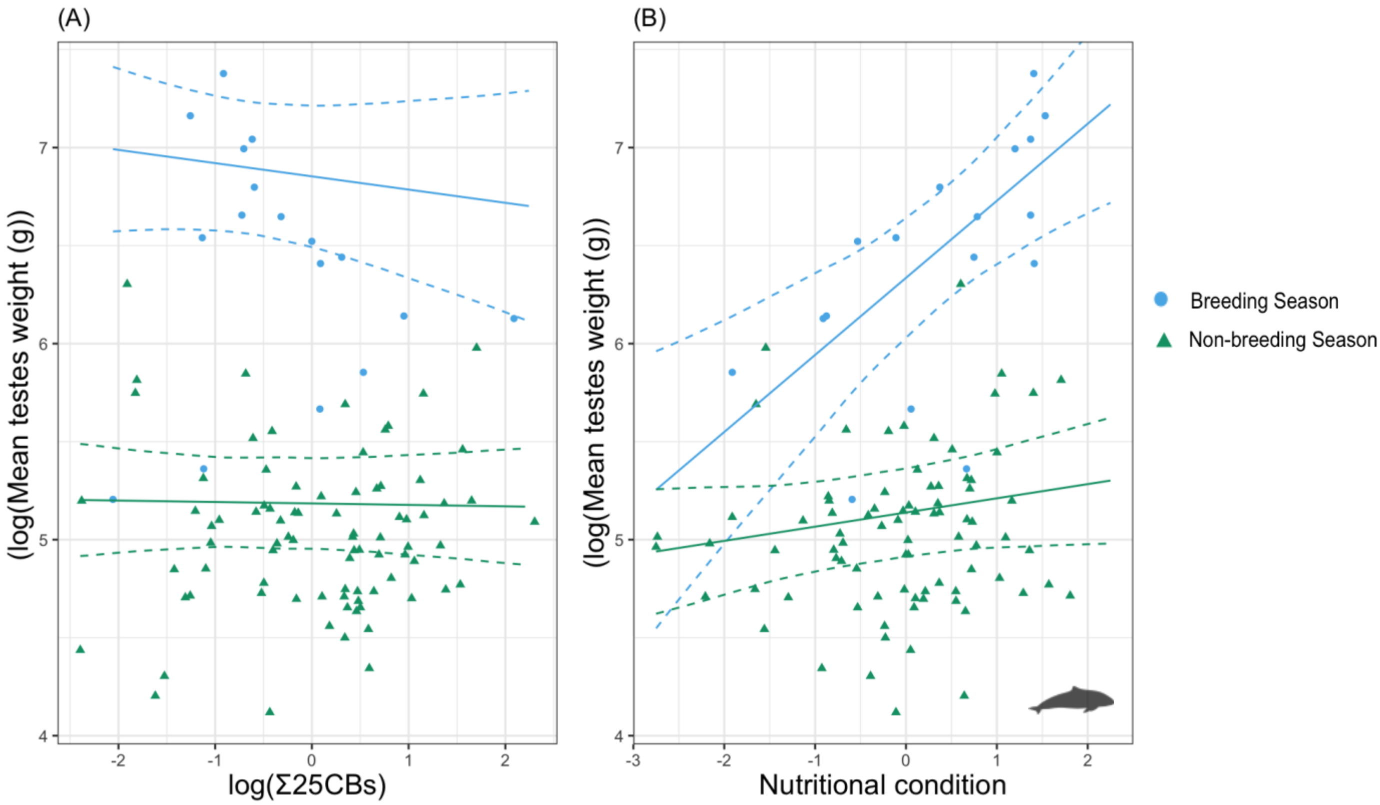

Figure 3: Individual mean testes weights (g) of 99 adult harbour porpoises (Phocoena phocoena) stranded in the UK between 1991 and 2017 plotted against (A) the log of blubber concentrations of the sum of 25 chlorobiphenyl congeners (∑25CBs) (mg kg^{-1} lipid) with nutritional condition at the third quadrant value (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 1: 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	1.389	0.146	0.148	9.377	0.000*

Season:Non-breeding	-1.222	0.110	0.111	11.013	0.000*
Nutritional Condition	0.364	0.109	0.110	3.299	0.001*
Log(Σ25CBs)	0.093	0.063	0.064	1.450	0.147
Non-breeding:Nutritional Condition	-0.306	0.103	0.104	2.949	0.003*
Nutritional Condition:log(Σ25CBs)	-0.147	0.041	0.042	3.494	0.000*

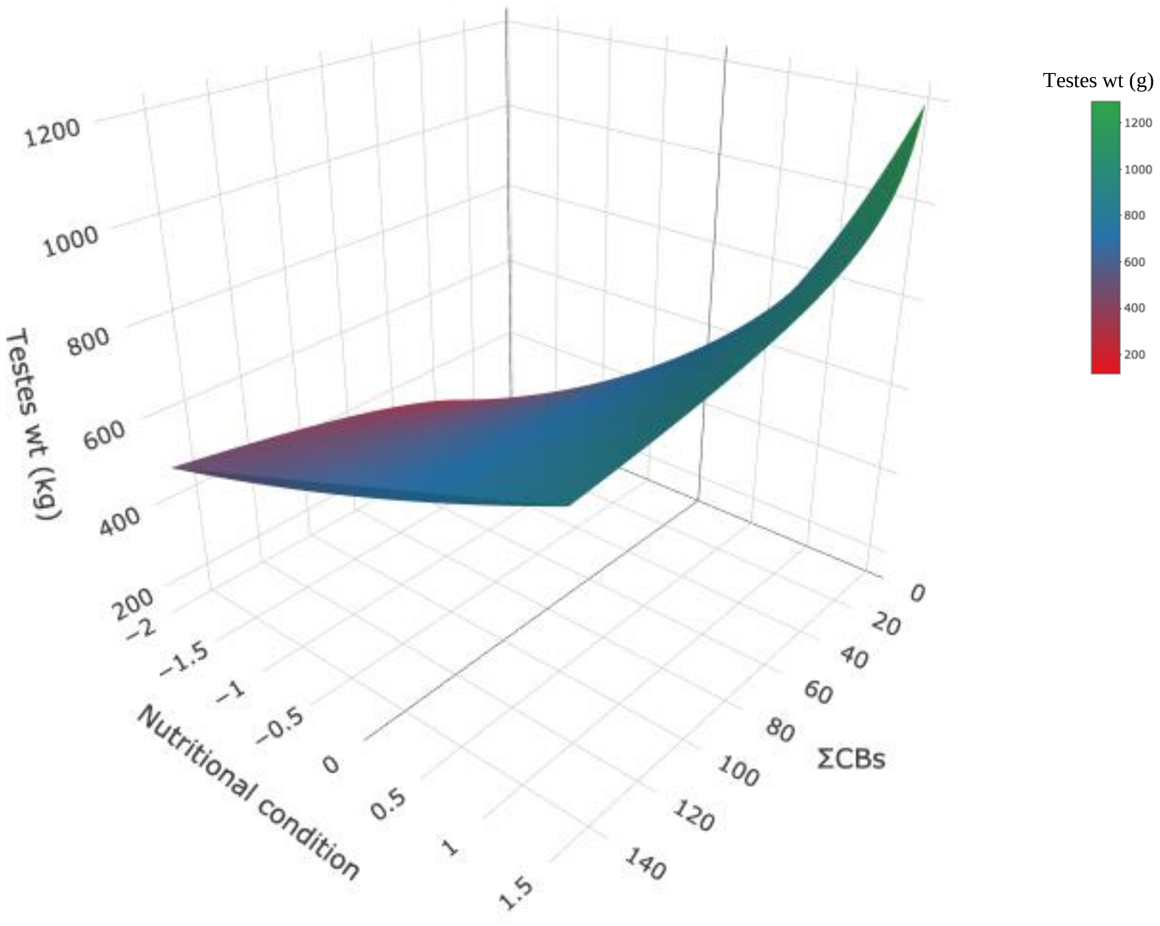

Figure 4: Surface plot of predicted testes weights (kg) against nutritional condition and PCB blubber concentrations, (∑CBs) (mg kg^{-1} lipid), for mature individuals during the breeding season. The surface plot is

colour graded according to predicted testes weights (kg). Red indicates the lowest weights; green indicates the highest weights.

4. Discussion

Here we 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 our 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). Our 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 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 we have shown that poor body condition is the predominant driver of reduced testes weights, previous work, using the same dataset, has shown 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.

We 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 our 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) our sample of mature individuals had very few individuals above the age of 15 (n = 2), our sample should represent a fertile portion of the population (Appendix B Table 2). To ensure our findings were not affected by individual variation in timing of the breeding season, our classification 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 we 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 we have included infectious disease and trauma cases in our analysis. This has allowed us 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 B Table 1.

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 (*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 our findings to answer these questions perhaps 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.

ACKNOWLEDGEMENTS

The authors would like to thank Department for Environment, Food and Rural Affairs and the UK’s devolved administrations of Scotland and Wales for funding the Cetacean Strandings Investigation Programme (CSIP) as part of the UK government’s commitment to a number of international conservation agreements. The authors would also like to thank the Centre for Environment, Fisheries and Environmental Sciences for carrying out the chemical analysis and Defra for funding this analysis under a service-level agreement. The authors thank three anonymous reviewers for providing helpful comments on earlier drafts of the manuscript. The first author was funded by the Natural Environment Research Council (NERC) grant NE/L002485/1. D.J.C. is funded by the Bertarelli Foundation through the Bertarelli Programme in Marine Science. P.D.J was partially funded by NERC grant NE/S000100/1 supporting the ChemPop project.

SUPPLEMENTARY MATERIALS

Appendix A: Supplementary Methodology Materials

1. Methods for the derivation of the body condition metric
2. Methodology and results for testes weights modelled against selected covariates for immature harbour porpoises

Appendix B: Supplementary Tables & Figures

Figure B-1: (A) QQ Plot of model residuals; (B) Residuals plotted against Σ25CBs; (C) Residuals plotted against Latitude; (D) Residuals plotted against Longitude Figure 2: (A) QQ Plot of model residuals; (B) Residuals plotted against Σ25CBs; (C) Residuals plotted against Latitude; (D) Residuals plotted against Longitude

Figure B-2: Body length (cm) against mean testes weight (kg) for the 3 UK laboratories

Table B-1: Count of individuals in each cause of death category and sexual maturity status in the strandings sample

Table B-2: Count of individuals in each age class and sexual maturity group

Table B-3: Mean concentrations of each polychlorinated biphenyl congener for immature and mature individuals.

Table B-4: 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).

Table B-5: 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).

Table B-6: Results for Kruskal-Wallis rank sum tests for Length and Breeding Season on immature and mature individuals

Table B-7: Results for Pearson’s product-moment correlation tests

Table B-8: Model selection table for sexually mature individuals

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