Assessing the Population Consequences of Pollutant Exposure in Cetaceans (Pollution 2000+) – from Ingestion to Outcome.

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ABSTRACT

This risk assessment framework for investigating the population consequences for cetaceans of exposure to pollutants has been produced as part of the IWC Pollution 2000+ initiative. In this phase we have continued to refine the model and assess where further efforts to reduce model uncertainty would be best directed. We have also embedded a physiologically based toxicokinetic model into the framework so that data on contaminants in cetacean prey can be used to determine exposure. Whilst this adds a layer of complexity to the framework it increases the flexibility and applicability of the model to populations where contaminants in fish prey may be more readily available than blubber or live biopsy samples. Here we found that:

1. Empirical data (total PCB concentrations measured in blubber biopsy samples) recently collected from humpback whales in the Gulf of Maine suggest that the health risks from PCB exposure for this population are low. Concentrations in immature animals were higher than in adults and if over 10% of this immature age class were exposed to a pathogen that would reduce their survival probability resulting in up to a 1% decrease in the population growth rate. However, at lower pathogen exposure levels no population level effects would be seen.

2. The effect of uncertainty in the underlying population model was assessed to determine the relative importance of the various vital rates in generating a stable age-structured population as the basis for the individual based model (or IBM). Both the model sensitivities and elasticities suggested that adult survival had the largest proportional effect on the population growth rate (λ) estimation. This therefore indicates that where resources are limited and decisions about how and where to best deploy them have to be made, effort should be directed towards reducing the uncertainty in this vital rate when compared to the others (such as female fecundity and age at first reproduction) required for the underlying model.

3. In terms of the uncertainty in the concentration-response functions, the degree of variability (i.e. the width of the 95% confidence limits on the estimated decrease in population growth that exposure to contaminants may cause) increased as the annual contaminant accumulation (in this case PCB or polychlorinated biphenyl) and thus level of contaminant exposure via the prey, increased. This may be due at least in part to the lack of empirical dose-response data from the laboratory animal model studies at the higher exposure levels.

4. The level of uncertainty was higher around the fecundity effects than it was around the immune effects. However, not all of the sources of uncertainty have been captured here. For the relationship between the Con A immune function assay and the probability of survival we used data from the National Toxicology Program studies (Luster et al. 1993). These extensive and expensive studies are unlikely to be repeated in the short term so the best estimates for the functional relationship have been included here and attention focussed on the other sources of variability that are more likely to be addressed in further studies.

5. These model uncertainties could be reduced in future with increased toxicological studies. It was noted that the degree of uncertainty generated in the final estimates of potential population growth rate was not prohibitively large. However, this conclusion is, of course, accepting that there are no major issues with the underlying model assumptions.

5. The embedded toxicokinetic model (Jongeneelen and ten Berge 2011) resulted in total PCB blubber concentrations in adult females of the order expected from the empirical data, suggesting this approach has value for risk assessment in cetaceans. Improvements to the method will certainly be gained from determining species-specific kinetic constants for the hepatic metabolism of PCBs, particularly CB153 as the most recalcitrant congener, by cetaceans.

Finally this combined risk assessment model framework (both the individual based model and the embedded toxicokinetic model) will be available as a web based program for use by the community in future.

INTRODUCTION

This paper is the final in a series of three that describe and report on the final two phases of the IWC Pollution 2000+ initiative. In the first (Hall et al. 2011) we developed and implemented an individual based model (IBM) framework to explore the consequences of exposure to pollutants (specifically polychlorinated biphenyls or PCBs) on potential population growth rate in cetaceans. Two species for which vital rate and contaminant data are available were included in the model demonstrations, the bottlenose dolphin (*Tursiops truncatus*) and the humpback whale (*Megaptera novaeangliae*). The concentration-response function, describing the relationship between total blubber PCBs and effects on physiology, embedded in the model was derived from the results of a wide range of relevant studies using laboratory model species (namely mink, which are sensitive to the effects of PCBs). The function considered the impacts of maternal PCBs on calf survival. In the second phase, a more detailed investigation of the effects of blubber PCBs on immune function were considered (Hall et al. 2012). Relationships which allowed for the translation of the results of *in vitro* immune function assays, available for free-ranging bottlenose dolphins (Schwacke et al. 2012a), into meaningful host resistance and survival probabilities were generated. These were then incorporated as additional concentration-response functions. Model runs varying the proportion of the population exposed to a pathogen and the degree of pathogen virulence were carried out, to determine the impact immunity effects would have on population growth rate in addition to impacts on calf survival. These analyses suggested that annual accumulation rates of PCBs into the blubber of between 2 and 4 mg/kg lipid were likely to cause declines in the demonstration populations of both bottlenose dolphins and humpback whales. If pathogens were then introduced into a naive population, with between 10-20% of the population being exposed, then the impacts would be seen at lower annual accumulation rates of between 1 and 2 mg/kg lipid. For the most virulent pathogens this could be as low as <0.5 mg/kg lipid as increased pathogenicity outweighed PCB immunosuppression.

Thus these model frameworks provide tools by which researchers and mangers can investigate the effects of contaminants on cetaceans beyond the individual level. To complete the work here we report on the findings of the final, phase III of the study. The objectives as agreed with the Pollution 2000+ Steering Committee were:-

1. To carry out a sensitivity analysis to determine which parameters in the model are having the largest influence on the outcome (i.e. the potential population growth rate). The effect of varying the vital rates in the underlying Leslie Matrix population model; the concentration response functions (both impacts of maternal PCBs on calf survival and the effect of PCBs on immune function); the relationship between the immune function assays and the host resistance tests will be explored. We will investigate what effect a proportional change in each will have

on the final population growth rate estimates. This will allow users of the final models to determine where more data are required or where parameter estimates need narrowing.

2. Investigate embedding a bioaccumulation model into the individual based model framework to allow concentrations of blubber PCBs in cetaceans to be estimated from information about levels in their prey. In this way effects of contaminants on populations of cetaceans could be investigated based on contaminant concentrations in prey without the need for invasive biopsy sampling.

3. Continue to investigate implementation of a concentration-response component for PBDEs

4. Produce a user-friendly web based portal for the model so that the community can use the code and investigate the effect of different scenarios and simulations. The raw R code will also be made freely available for anyone to use and modify should they wish to do so.

Here we report on the findings of objectives 1-3. The final user-friendly web based portal is currently under construction and will be finalised following discussions with the Steering Group at an intersessional meeting.

In addition to the objectives above, we also report on a comparison between the outputs from the model for humpback whales and recent data on PCB concentrations in blubber biopsy samples from animals in the Gulf of Maine. This has enabled us to estimate what the annual accumulation of PCBs by the whales in this region is currently expected to be and whether this is likely to have any impacts at the population level.

POLYCHLORINATED BIPHENYLS (PCBS) IN BLUBBER OF HUMPBACK WHALES FROM THE GULF OF MAINE - POPULATION RISK ASSESSMENT.

PCB Concentrations in Blubber Biopsies from Female Humpback Whales

Blubber biopsy samples from female humpback whales in the Gulf of Maine collected between 2004 and 2012 were analysed for a range of persistent organic pollutants, including PCBs. Samples were collected from 38 individuals, ranging from calves to adults. Standard GC/MS methods were used to quantify the PCB concentrations, as described in Mongillo et al. (2012). The sum of 40 PCB congeners (listed in Appendix 1) by age class was then used to compare with the estimated blubber PCB concentrations from model outputs. The estimated blubber concentration in adult females is obtained from the model runs and an equivalent annual accumulation concentration obtained, where model levels are comparable to those measured. The ability to be able to compare model simulations with empirical data is clearly invaluable in grounding the model in reality.

The geometric mean of total PCBs (sum 40 congeners, on a lipid weight basis) are given in Table 1 by age class. There was no difference in the mean concentrations among the adult females by year ($p>0.05$, the only age class for which there was sufficient data) so all years were grouped together.

Age class	**Geometric mean ± Geometric SD (mg/kg lipid wt)**	**n**
Calves	2.59 ± 2.65	2
Juveniles	5.60 ± 1.61	8
Subadults	3.10 ± 1.41	7
Adults	1.62 ± 1.63	20

Table 1. Geometric mean total PCBs (mg/kg lipid weight) in blubber biopsy samples from female humpback whales in the Gulf of Maine.

As in other cetacean species, concentrations were highest among the subadults and juveniles which were significantly higher than the adult females (Fig 1, generalised linear model, $p<0.005$). There was also no

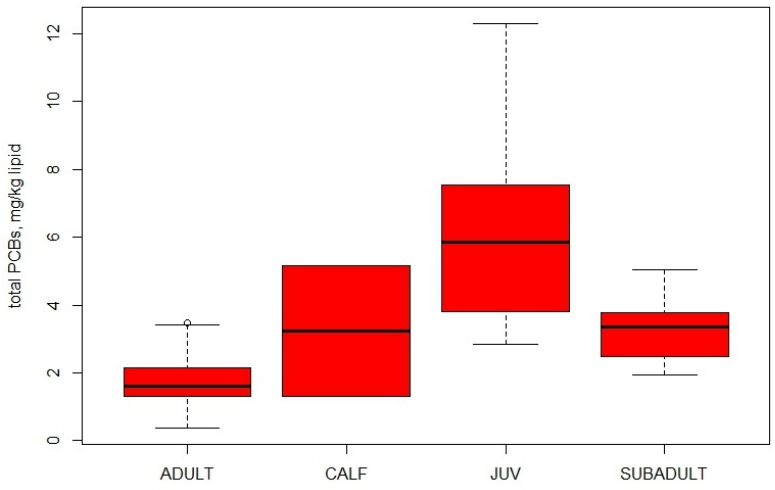


Fig. 1. Boxplot showing concentration of total PCBs in the blubber of humpback whales by age class (black horizontal lines show median concentration, red box interquartile range, whiskers minimum and maximum).

difference between the lactating and pregnant females although sample sizes for animals where this was known was small (4 lactating and 6 pregnant).

Estimated annual accumulation of PCBs in Gulf of Maine humpback whales

The IBM is described in detail in Hall et al. (2012). In order to compare the model output with empirical humpback whale PCB data the following model conditions were applied:

1. The underlying Leslie matrix population model used the published vital rates from Barlow and Clapham (1997) to construct a stable age-structured population. This gave us a population with a growth rate (λ) of 1.065. NOTE: these vital rates may not be applicable to the current population as they were estimated in the 1990s. They are used to provide a baseline to determine by how much the *relative* population growth rate might change. The absolute values of λ should not be taken as the present status.

2. The effect of maternal PCB on calf survival was included (with model uncertainty) using the concentration-response function from studies in mink.

3. The effect of PCB exposure on immunity was also included with 3% of the population being exposed to a relatively virulent pathogen. It is very difficult to estimate the *rate* at which wild animals might encounter a pathogen in a population. However, an exposure level of 3% was taken based on the observation by Murdoch et al. (Murdoch et al. 2008) that the annual incidence rate (that is the rate at which new cases were occurring) of lobomycosis (lacaziosis) was 2.66%. This might indicate the rate of pathogen exposure in a population outside an unusual mortality event.

An annual PCB accumulation of 0.2 mg/kg would result in blubber PCB concentrations among the adult females (>9 years old) of approximately 1.57 mg/kg lipid. This is comparable to the level found in the adult female blubber biopsy samples (Table 1, 1.63 mg/kg lipid). The estimated concentration in the blubber of the females of all age classes is shown in Fig. 2. The red line is the mean concentration by age. Whilst the average levels in the females are in line with the empirical data, the concentrations in the calves and juveniles compared to the empirical data (1-4 years) are low (geometric mean calves and juveniles $\leq$ 4 years old empirical data = 4.8 mg/kg, model = 1.43 mg/kg). This may mean that the proportion of PCBs transferred intra-uterine and during lactation may be underestimated in this species or that juveniles are feeding on different, more contaminated prey than adults.

Overall the adult female humpback whales had very low concentrations of PCBs in their blubber, well below the threshold for any effects to be observed at a population level. At the individual level, the model predicted that some individuals would have blubber levels > 8mg/kg lipid weight, whereas the highest concentration in an adult

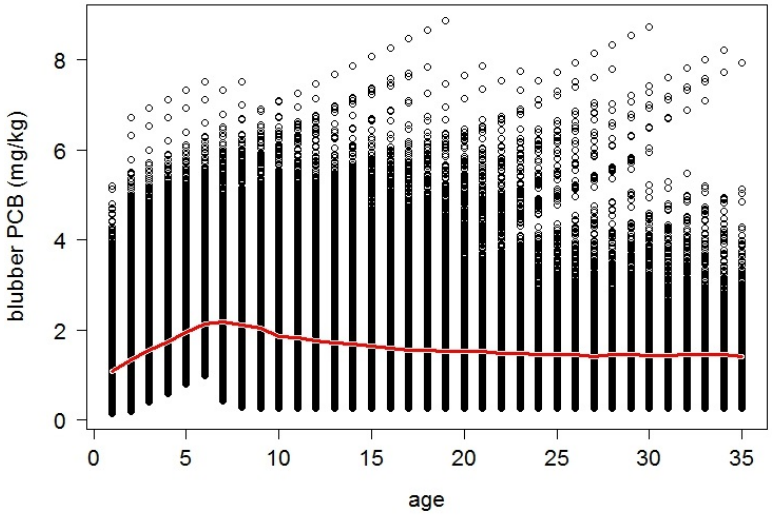


Fig. 2. Estimated concentration of PCBs in the blubber of Gulf of Maine female humpback whales with an annual accumulation of 0.2 mg/kg lipid weight (red line shows mean for each age group, adult females = 1.57 mg/kg lipid weight).

female from the empirical dataset was 3.5 mg/kg lipid weight. However, the empirical dataset was small. For reproductive effects, the EC50 estimate from the mink data was 20 mg/kg lipid weight, 10 times the concentration seen in the humpbacks. No discernible effects at the population level incorporating both reproductive and immune effects would be detected until the level in reproducing humpback females reached an annual accumulation rate of >2mg/kg lipid weight, four times the level (0.5 mg/kg lipid) predicted by the model as the current annual PCB accumulation rate.

For immune effects the concentration of 5.6 mg/kg seen in the juveniles could translate to an approximately 5% decrease in individual survival probability, *after* the exposure of the animals to a class 2 pathogen. We then modified the model to see what impact this effect on juvenile survival would have on the potential population growth rate. For this simulation we increased the proportion of animals exposed to the pathogen to 10% of the juveniles (up to 4 years old). This reduced the estimated population growth from 1.065 to 1.056 (95% CI 1.054, 1.059), representing a 1% decrease in λ. Thus, the PCB levels in the juveniles could represent a risk for the population but a relatively large proportion of the population would have to be exposed to a somewhat virulent pathogen (as might be expected during a viral epidemic perhaps) for it to have any impact.

The model framework is therefore flexible enough to allow the effect of the different dose-response relationships (i.e. affecting reproduction and immunity) and thus the relative importance of each at the population level, to be investigated.

MODEL SENSITIVITY ANALYSIS

In any model framework it is important to understand which parameters are having the greatest influence on the model outputs, in other words how much would the model results and conclusions change if the basic data was slightly inaccurate? Much of the data for these population models will of course be inherently uncertain so it is critical to understand how much this really matters to the outcome. In addition, collecting basic data for the estimation of vital rates is costly, time consuming and logistically difficult. Therefore it is important that any resources available for this work are directed towards reducing uncertainty in the parameters that have the greatest effect.

Variation in the Vital Rates

The individual based model (IBM) framework we have developed requires priming with a stable age structure. This seeds the IBM with an appropriate number of individuals within each age class. The numbers are calculated

based on a Leslie matrix model (Leslie 1948), a standard way of projecting population growth. Empirical data on vital rates such as survival, fecundity and age at first reproduction are therefore also required in order for the model to provide some realistic scenarios for testing. For example, many of the vital rates used in the underlying population matrix were based on data published some time ago (Wells and Scott 1990; Barlow and Clapham

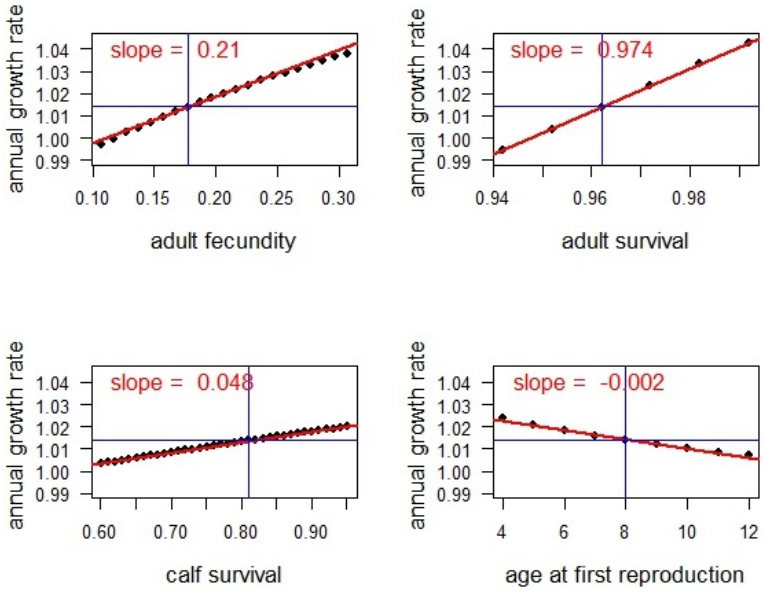


Fig. 3. Sensitivity plots showing impact of varying each vital rate on population growth rate estimates for the bottlenose dolphin model. The blue lines shows the values used in the model and the resulting growth rates estimate of **1.014**.

1997). If more recent data were to be collected for these population studies, this analysis indicate which has the largest impact on the population growth rate (λ).

The plots shown in Fig. 3 indicate the results of an analysis carried out to investigate this for the bottlenose dolphin model. Four vital rate and model parameters were included; adult fecundity, adult survival, calf survival and age at first reproduction. These were varied over a range spanning the values used in the model (from (Wells and Scott 1990) and were plotted against the resulting estimates for the population growth rate, λ, keeping everything else except that parameter equal. The red line shows the slope for the relationships between the vital rates and population growth rates. This indicates how sensitive each is and therefore how much impact it would have if it's value were uncertain; the steeper the slope, the greater the impact. This analysis indicates that adult survival probably has the largest influence.

The same approach was taken for the humpback whale model. Fig. 4 shows the sensitivity analyses for this underlying population in which the population growth rate was much higher (1.065). Again this indicates that effort should be directed towards estimating adult survival. However, this does not account for the fact that the

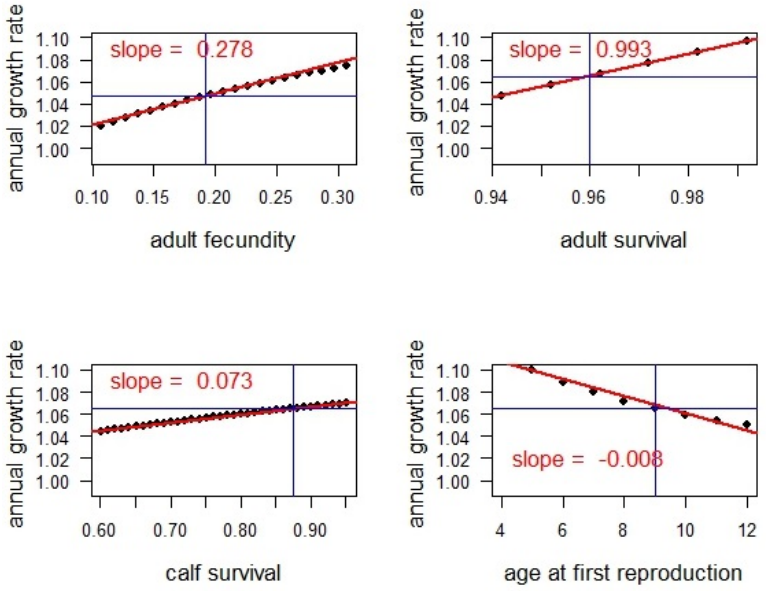


Fig. 4. Sensitivity plots showing impact of varying each vital rate on population growth rate estimates for the humpback whale model. The blue lines shows the values used in the model and the resulting growth rates estimate of **1.065**.

magnitude and scale of these vital rates are very different which means the results could be misleading. To deal with this problem a measure known as elasticity can be calculated.

Leslie Matrix Elasticities

Elasticities are a measure of the relative contribution of each element of the Leslie matrix (i.e. survival and fecundity estimates) to the growth rate (λ), tested by a unit change in each, all other elements being constant. They sum up to one for a given matrix. The package *primer* (Stevens 2009) in the program R (Team 2011) was used to determine the elasticities. Fig 5 shows the elasticities for (a) the bottlenose dolphin model and (b) the humpback whale model. Each square on the figure represents a cell in the Leslie matrix model. Along the top are the fecundity estimates and across the diagonals are the survival estimates into the next age class. The elasticity values for each element are colour coded according to the legend. In both cases the early survival estimates (including the calf survival up to approximately 10 years old for the dolphins and 5 for the humpbacks) are the most important parameters affecting the growth rate estimates. Uncertainty in the fecundity estimates would have much less impact on the population growth rate.

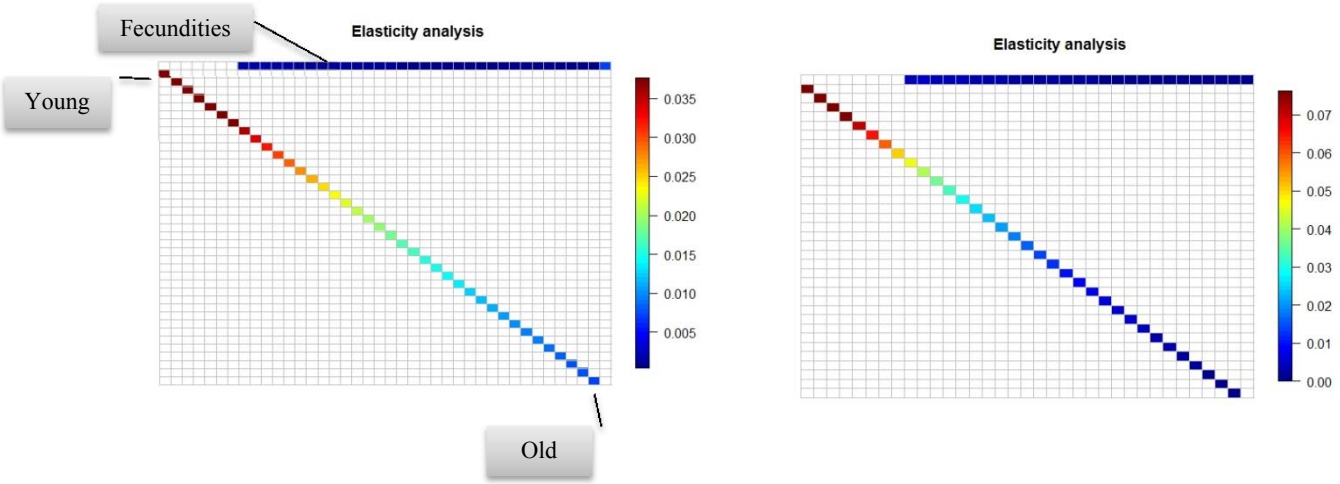


Fig. 5. Elasticities plots showing the relative contribution the survival and fecundity estimates have on the population growth rates. The grid represents the Leslie matrix in which the fecundity rates are entered on the top line and the survival rates on the diagonal, with each cell representing a single age-class with the early age classes at the top left and the older age classes at the bottom right. Red cells indicate the rates contribute relatively more to the population growth rate than the blue squares.

Thus both approaches are in general agreement. And whilst the elasticities evaluate the effect of varying a single age-specific survival or fecundity estimate at a time, for most cetacean populations it would be very difficult to estimate survival by year class. Mostly these data are only available by age-classes (calves, juveniles and adults). Thus the first approach, in which the group-based survival estimates vary, is likely to be more pragmatic.

Uncertainty in the Concentration-Response Functions

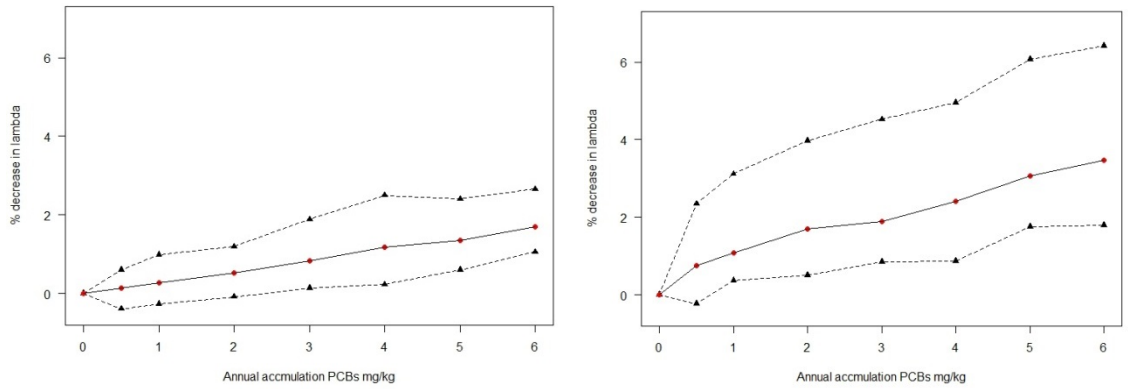
In addition to variation in the vital rate estimates further uncertainty is included in the error associated with the different PCB concentration-response functions, both in the effects on fecundity and the effects on immunity. Whilst it is not possible to investigate the impact of all the uncertainty in these relationship (i.e. all the measurement and observation error) some of the effects were investigated as follows.

Effect of maternal PCBs on calf survival

In order to investigate the impact of uncertainty around the concentration-response function for the effect of maternal PCBs on calf survival (i.e. on fecundity), we kept the effect on immunity constant. The uncertainty in the fecundity concentration-response function (i.e. in the data from the mink model species studies) was then incorporated using bootstrap with resampling (500 times) then choosing at random one of the resulting

concentration-response curves for each of the 100 individual based model runs. This was then carried out for a range of PCB annual accumulation rates, taking the mean and 2.5 and 97.5 percentiles for the resulting population growth rates to indicate the impact of any uncertainty. The concentration-response function for the effects on immunity was kept constant for all model runs, with 5% of the population being exposed to a relatively pathogenic organism. This exposure level was taken based on the observation by Murdoch et al (Murdoch et al. 2008) that the annual incidence rate of lobomycosis (lacaziosis) was 2.66%. This might indicate the rate of pathogen exposure in a population outside an unusual mortality event. To be on the conservative side this was therefore increased to 5% with the exposure to an organism as pathogenic as *Streptococcus pneumoniae.*

The results for the bottlenose dolphins (a) and humpback whales (b) are shown in Fig. 6. As the annual accumulation rate increases so does the uncertainty and associated proportional decrease in lambda. For the dolphin model, particularly at low accumulation rates, the amount of uncertainty in the resulting population growth rate estimate is ± < 1%, increasing to ± 1% at the highest levels. For the humpback whales the uncertainty is much greater (± 1 - 4%), particularly in the upper confidence bound at the higher level of exposure.



(a) bottlenose dolphins (b) humpback whales

Fig. 6 Effect of uncertainty in the concentration-response function for effects of maternal PCB on calf survival in (a) bottlenose dolphins and (b) humpback whale on the population growth rate estimates (dotted lines = upper and lower confidence limits, solid line = mean)

Effect of PCBs on immune function and survival following pathogen exposure

To investigate the population level effects of PCBs on immune function, firstly the relationship between an *in vitro* immune function test (known as the Con A stimulation index (Schwacke et al. 2012b)) and the concentration of PCBs in the blubber was evaluated. This index is the percent increase in number in stimulated compared to

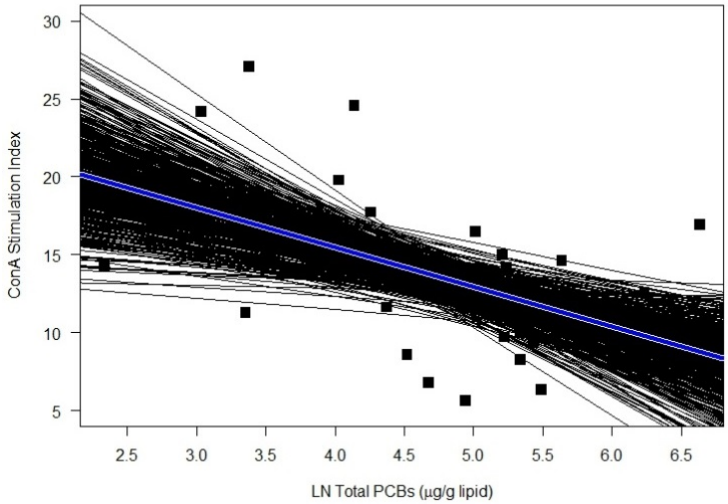


Fig. 7. Uncertainty in the relationship between loge(total blubber PCBs) and the in vitro immune function assay known as the Con A response. Black lines show 500 bootstrapped resampled regression models. The blue line shows the best fitting linear m

unstimulated cells. Thus normal cells have a high index whereas dysfunctional cells will have a low index. The effect of uncertainty around this relationship was investigated for the bottlenose dolphin model alone, as this is the only species for which data was available from free-living individuals. Again the uncertainty was incorporated by bootstrap with resampling (500 times) the relationship shown in Fig. 7 (NOTE: here the PCB concentrations are on a ***log*** scale). For the IBM, this relationship had to then be translated into a proportional decrease in survival probability following exposure to a pathogen which was possible using data published by the US National Toxicology Program (NTP) from a wide range of immunotoxicological experiments in mice (Luster et al. 1993).

In the NTP experiments the relationships between host resistance and immune function responses were reported as a *proportional* decrease in survival probability. Thus, the data from Schwacke et al. (2012) was also converted to a proportional change in response to Con A in relation to an estimated maximal response (at an index of 25.6), taken as the intercept in the linear relationship shown in Fig. 7 (the blue line). However, as can be seen from the 500 bootstrapped linear models (black lines) there is considerable uncertainty in this intercept. To capture this uncertainty, an intercept generated from the 500 bootstrapped models was chosen at random as the estimated maximal response. Then the relationship between a new proportional change in Con A response and total PCBs was recalculated and converted to an estimated decrease in probability of survival using the functions published by Luster et al. (1993). This step was also repeated 500 times as shown in Fig. 8 to generate a family of slopes to be used in the IBM to modify survival in relation to total blubber PCBs. The relationship was forced through the origin since there should be no effect on survival in clean animals.

The fecundity effects were kept constant in all model runs with 5% of the population being exposed to a relatively pathogenic organism. The effect of annual accumulations of between 0.5 and 6 mg/kg lipid per year were investigated. Fig 9a shows the results from the model runs with uncertainty in the effects of immunity as described above included but with no variation in the effects on fecundity and Fig 9b shows the effect on the estimated population growth rate when uncertainty in both immune and fecundity effects are included for the bottlenose dolphin model.

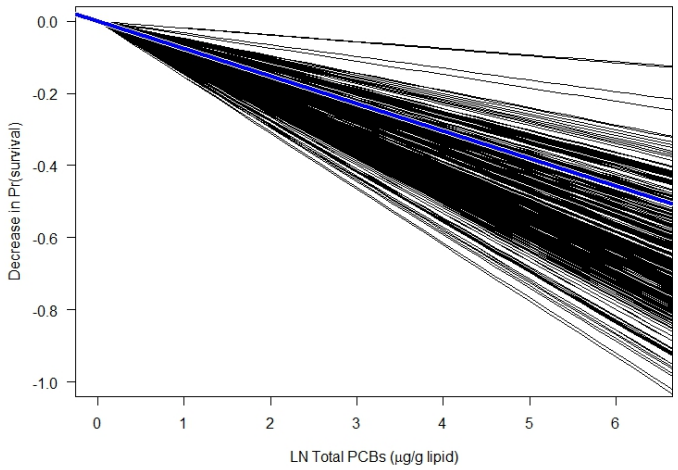


Fig. 8. Relationship between decrease in survival probability and $\log_e$(total blubber PCBs) incorporating variability in the relationship between Con A immune function and $\log_e$(total blubber PCBs, Fig. 7). Black lines show 500 resampled linear models, blue line shows best fitting model.

In the majority of the model runs there was a small effect on the estimated population growth (lambda) as reflected by the lower limits and the mean decrease in lambda being relatively small (generally <1%). When both sources of error and uncertainty were included the mean percentage decrease in lambda was very similar but the confidence limits were much wider, particularly at the highest annual accumulation rates. It seems that the uncertainty in the effects on fecundity may be having a greater effect on the reliability of the model than the errors in the effects on immune function, host resistance and survival probability especially when a lower level of population pathogen exposure (5%) is incorporated.

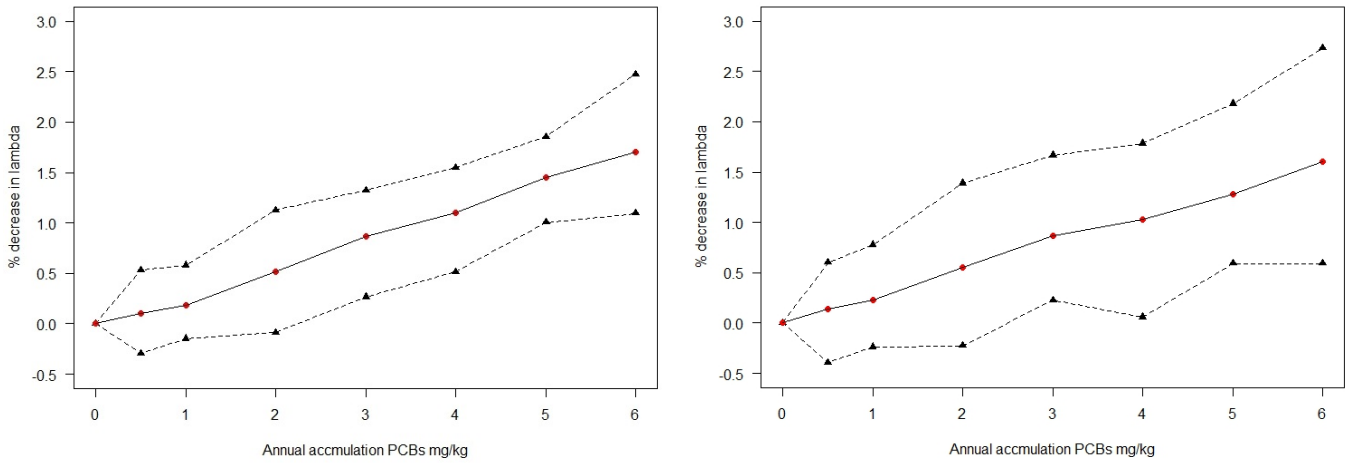


Fig. 9. Effect of uncertainty in the relationship between immune function and PCBs on estimated population growth rate in bottlenose dolphins (dotted lines = upper and lower confidence limits, solid line = mean) (a) without fecundity uncertainty (b) with fecundity uncertainty

TOXICOKINETIC MODEL

In this section we explored using a toxicokinetic model (also known as physiology based toxicokinetic or PBTK) into the individual based model framework which would allow concentrations of total blubber PCBs in cetaceans to be estimated from information about levels in their prey. In this way effects of contaminants on populations could be investigated without the need for invasive biopsy sampling, using data on prey and daily energy requirements instead. These models require detailed information on various physiological parameters, many of which have already been used to model the absorption, metabolism and excretion of PCBs in odontocete cetaceans (Hickie, MacKay and De Koning 1999; Hickie et al. 2000; Hickie et al. 2005). We therefore restricted this analysis to the bottlenose dolphin model which was carried out in three stages. Firstly data on the diet of bottlenose dolphins was reviewed and summarised and secondly, data on the concentration of PCBs in the fish was needed. Finally a PBTK model to link PCB prey levels to tissue concentrations was required.

Bottlenose dolphin diet

Bottlenose dolphins have been described has having a diverse diet and are thus opportunistic, generalist predators that take prey based on its availability in the environment (Barros and Odell 1990; Jefferson, Leatherwood and Webber 1993; Berens McCabe et al. 2010). For example, 43 taxonomically diverse prey species were identified in 76 stomachs of stranded dolphins from the southeast US (Barros and Odell 1990). They have been shown to feed on a wide variety of fish as well as some cephalopods, and occasionally small sharks and rays (Connor et al. 2000). Based on an extensive literature review of marine mammal diet, it was concluded that bottlenose dolphin diet generally consisted of 60% mesopelagic fishes, 20% small squids, 15% small pelagic fishes and 5% large squids, and that their trophic position was only marginally lower than killer whales (*Orcinus orca*) (Pauly et al. 1998).

Bottlenose dolphin diet and foraging strategies also vary between regions and between sympatric populations, so here we focus on the diet of dolphin populations along the East coast of the US. Diets of coastal bottlenose dolphins along the East coast have been shown to differ between estuarine and oceanic habitats (Gannon and Waples 2004). In an extensive study of the stomach contents of 146 stranded bottlenose dolphins from North Carolina, sciaenid fishes were the most common prey type accounting for 73% of all food items eaten. By mass, Atlantic croaker (*Micropogonias undulates*) dominated the diet of dolphins that stranded inside estuaries whereas weakfish (*Cynosicon regalis*) dominated the diet of those stranded on ocean beaches (Gannon and Waples 2004). In addition, inshore squid (*Loligo* sp.) were commonly found in the stomachs of the oceanic dolphins, but not the estuarine animals. This further supports the hypothesis that some members of the coastal populations principally forage in estuaries while others remain in more open waters. There was also some evidence that the diets of males and females differed slightly with males eating more croaker than females, and adults eating more than juveniles, but there was no significant pattern in prey size associated with dolphin demography (Gannon and Waples 2004).

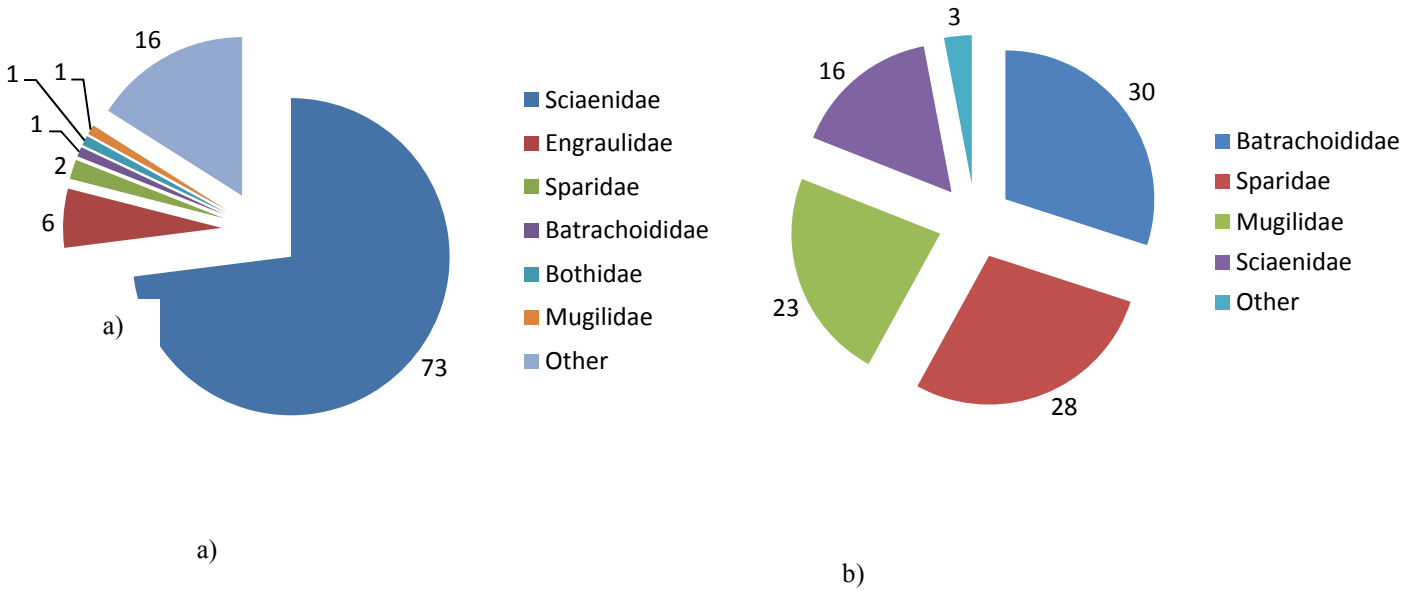
There also appears to be a difference between near shore and offshore dolphin populations (Mead and Potter 1995). Again from stomach contents analysis, it was seen that offshore individuals fed on pelagic squid and fish, mainly of the *Myctophidae* family, while the diet of the near shore individuals consisted primarily of Sciaenid fishes (Mead and Potter 1995). Squid were rarely found in the stomachs of the near shore animals, and when they were, these were *Loligo* species.

Fish Family	**Species**	**Source**
Sciaenidae	Weakfish (*Cynoscion regalis*) Spot (*Leiostomus xantburzls*) Atlantic croaker (*Micropogonias undulates)* Silver perch *(Bairdiella chrysoura)* White perch (*Bairdiella chrysura*) Spotted seatrout (*Cynoscion nebulosus)*	(Barros and Wells 1998; Gannon and Waples 2004; Berens McCabe et al. 2010)
Sparidae	Pigfish (*Ortbopristis chrysoptera*) Pinfish (*Lagodon rhomboids*)	(Barros and Wells 1998; Gannon and Waples 2004; Berens McCabe et al. 2010)
Bothidae	Flounder species (*Parafichthys sp.*)	(Gannon and Waples 2004)
Mugilidae	Striped mullet *(Mugil cephalus*)	(Barros and Wells 1998)
Engraulidae	Striped anchovy (*Anchoa hepsetus*)	(Gannon and Waples 2004)
Batrachoididae	Gulf Toadfish (*Opsanus beta)*	(Berens McCabe et al. 2010)

Table 2. Important prey species of bottlenose dolphins along the East Coast of the United States

A study on the diet of bottlenose dolphins specifically in Sarasota Bay, Florida, showed considerably less prey diversity than those further up the coast as it was dominated by a few species (Barros and Wells 1998). Pinfish made up almost 70% of all prey eaten. Striped mullet, pigfish and spot were other important prey species in terms of their high frequency of occurrence. This population appeared to be exclusively piscivorous (Barros and Wells 1998). In another study of stomach contents analysis of Sarasota Bay bottlenose dolphins, Batrachoididae fishes were the most abundant (34.8%), followed by Sciaenidae (11.9%) and Sparidae (10.8%) and it was seen, based on fish abundance in trawl surveys, that there was significant positive selection for sciaenids especially (Berens McCabe et al., 2010). Whether stomach contents samples from stranded animals, are representative of the diet of free-ranging animals has been a source of debate. However, molecular prey detection techniques using faecal and gastric samples collected from live animals during a health study showed results consistent with previous stomach contents analysis (Dunshea et al. 2013). Prey species composition and relative amounts were comparable between the two datasets of the Sarasota Bay bottlenose dolphins (Dunshea et al. 2013), so the existing data on stomach contents from stranded dolphins is used here to estimate the diet of free-ranging animals.

Overall, across the studies conducted to date, a number of important prey species appear consistently in the diets of the bottlenose dolphins along the eastern coast of the US, and these are summarised in Table 2. Focus here is on the prey species reported for the estuarine and near-shore dolphins, and as such, PCB concentrations were investigated in fish from these six families of fish; Sciaenidae, Sparidae, Bothidae, Mugilidae, Engraulidae and Batrachoididae. Their average percentage contributions to the diet along the south east coast of the United States and in the Gulf of Mexico are summarised in Fig 10. These are approximations based on the combination of the different datasets.



a) b)

Fig. 10. a) Percentage contribution of different prey fish families to bottlenose dolphin diet along the South East coast of the US (Gannon and Waples 2004). b) Approximate percentage contribution of different prey fish families to bottlenose dolphin di diet in the Gulf of Mexico (Barros and Wells 1998; Berens McCabe et al. 2010).

PCB Concentrations in Prey Fish of Bottlenose Dolphins

There is a considerable amount of data on PCBs in marine fish in the US published and reviewed from the 1970s and the 1980s (Eisler 1986; Franklin 1987; Mearns et al. 1988; Schwartz 1988), but up to date data on persistent organic pollutants in North American waters are harder to find. More recent research efforts have focussed on contaminants in marine fish in other parts of the world including China (Liu et al. 2011; Xia et al. 2012; Shi et al. 2013), the Mediterranean (Geyer, Freitag and Korte 1984.; Bayarri et al. 2001; Bocio et al. 2007) , Africa (Kelechi 2012), Alaska (Hardell et al. 2010), the Black Sea (Georgieva, Stancheva and Makedonski 2012), Brazil (Ferreira 2013), the Arctic (Hoekstra et al. 2003) and also the Antarctic (Caio et al. 2013). In addition, since the 1980s, work on POPs in North America has shifted to sampling and monitoring fresh water habitats, notably lakes and rivers (Eisler and Belisle 1996; Blocksom et al. 2010). The majority of marine species monitored in the North Atlantic or North Pacific are also either farmed species or wild species that are caught for human consumption (Domingo and Bocio 2007), but, these species often do not form a large part of bottlenose dolphin diet. For example, a number of studies have focused wild and farmed salmon (Missildine et al. 2005; O'Neill and West 2009; Hardell et al. 2010).

However, in one recent study, 736 sampling sites across the US in the Northeast, Southeast, Gulf of Mexico and West coast estuaries were surveyed by the US Environmental Monitoring Program's National Coastal Assessment to examine the contaminants in a variety of fish species (Harvey, Harwell and Summers 2008). The included a

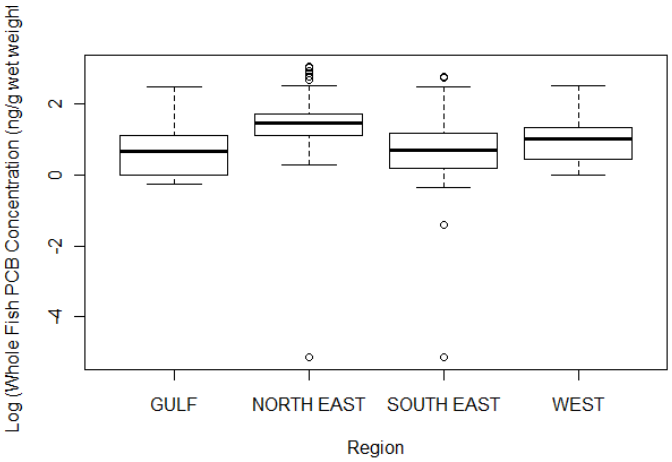


Fig. 11 . Log_e (Total PCB) concentrations in prey fish of bottlenose dolphins from different regions of North America. The North East had a significantly higher average concentration of PCBs that the other three areas.

number of species that form a major part of bottlenose dolphin diet. In this study, 31% of samples evaluated for total PCBs were found to exceed recreational fisher guidelines, and overall, PCBs, mercury, PAHs, and DDT contaminants were the most prevalent, both nationally and regionally (Harvey, Harwell and Summers 2008). The raw data of PCB concentrations used in this extensive study were provided by Harvey for the purposes of this project. Here, fish contaminant data, including data provided by Harvey, was collated from five studies of PCB, PBDE and heavy metal contamination from specific areas along the West coast of the US (Brown et al. 2006), as

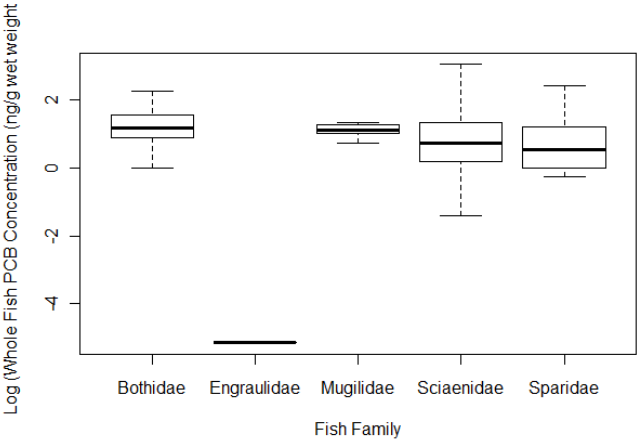


Fig. 12. Log_e (Total PCB) concentrations in prey fish of bottlenose dolphins in North America grouped by family. The Sciaenids had a significantly higher average PCB concentration than the other four families and showed considerably more variation.

well as the East coast, the Gulf of Mexico and the North Atlantic as a whole (Johnson-Restrepo et al. 2005; Pulster, Smalling and Maruya 2005; Munshi et al. 2009; Storelli et al. 2011). Specific species of interest that are known to form part of bottlenose dolphin diet (Table 2) were focussed on, and geometric mean total PCB concentrations in ng/g of wet weight were calculated for each species along the West coast, the South East, the North East and the in Gulf of Mexico.

There were differences in PCB concentration by region. The North East had the highest concentrations of PCBs in its fish species ($p < 0.0005$) while there were no significant differences between the other three areas (Fig 11). The different species were then grouped into their respective families, Bothidae, Engraulidae, Mugilidae, Sciaenidae and Sparidae. No information was found on the PCB concentrations in species from the Batrachoididae, so this was excluded from the analysis. Overall the Scaenids had the highest PCB concentrations than the other four groups (mean differences, $p=0.011$, Fig 12), which was largely a result of higher PCB concentrations in the White Perch and both the Atlantic and White Croakers, although their geometric mean concentrations were very similar. Thus, there are both regional and family specific differences in PCB concentrations in the prey species of the dolphins which are summarised in Table 3. These differences will, in turn, affect the contaminant burdens of the dolphins and may help to highlight regions of greatest concern in terms of the negative health effects on individuals and thus the potential population consequences. As the diet of the

dolphins was shown to vary by region, but data on PCB concentrations for all fish families were not available for each region, where missing, average values across all regions are used for modelling purposes (Table 4).

Region	Family	Geometric Mean Whole Fish Total PCBs (ng/g wet weight)
Gulf	Mugilidae	2.99 ± 1.23
	Sciaenidae	1.73 ± 1.86
	Sparidae	1.86 ± 2.27
North East	Bothidae	3.86 ± 1.45
	Sciaenidae	8.98 ± 2.32
	Engraulidae	0.0058
South East	Bothidae	3.20 ± 1.97
	Sciaenidae	2.13 ± 1.96
	Sparidae	3.52 ± 1.33
	Engraulidae	0.0058
West	Bothidae	2.52 ± 1.74
	Sciaenidae	2.99 ± 2.82

Table 3 Geometric mean (±SD) PCB concentrations in dolphin prey species by family and by region.

Fish Family	Geometric Mean Whole Fish Total PCBs (ng/g wet weight)
Bothidae	3.21
Engraulidae	0.0057
Mugilidae	2.99
Sciaenidae	2.41
Sparidae	1.99

Table 4 Geometric mean PCB concentrations in dolphin prey species across all regions

Bottlenose Dolphin Food Intake

Annual food consumption estimates based on observations of captive study animals were derived from a longitudinal study of historical data from two aquaria in the UK and the Netherlands between 1979 and 1991. The animals were fed on a mixture of herring, mackerel, whiting, sprat and squid (Kastelein et al. 2002). These estimates are presented in Table 5 and should be viewed as rough weight estimates of what wild conspecifics might eat depending on their diet and energy expenditure. Unfortunately in this study, the caloric value of the diet was variable, and as such, not recorded, however, a rough estimate of energy intake of 8800 kJ/kg was calculated using average values for the mixture the fed fish and squid species (Kastelein et al. 2002). Overall, the average annual food consumption was estimated at 2000kg of fish for adult males, non-pregnant and non-lactating females (Kastelein et al. 2002). Fish consumption showed little increase during gestation, but there was a 58–97% increase in consumption during lactation (Kastelein et al. 2002). A significant increase in daily food intake was also observed in lactating compared to pregnant females in captive Indian Ocean bottlenose dolphins (Cheal and Gales 1991), and should therefore be taken into consideration when modelling fish consumption by wild dolphins.

While there have been multiple studies of food intake in captive bottlenose dolphins, mostly for animal husbandry purposes, basic bioenergetic models predicting the total prey consumption and mean feeding rates of wild dolphins are much harder to estimate. In one study however, the kilocalorie intake, assuming a general rule of 54 kcal/kg required for maintenance, was estimated for free-ranging spinner dolphins of various sizes (Benoit-Bird 2004). With an average adult female bottlenose dolphin for the purposes of this study weighing approximately 150 kg (Hart, Wells and Schwacke 2013), using the 54 kcal/kg estimate, a dolphin would require 8,100 kcal/day in

maintenance costs (33,890 kJ/day), or 3.85 kg/day of fish (using an average of 8800 kJ/kg (Kastelein et al. 2002)). Actual consumption rates are likely to be higher when energetic expenditure is considered (Cheal and Gales 1991; Cheal and Gales 1992; Kastelein et al. 2002) and higher for a larger animal.

Thus, daily food intake is likely to vary based on the diet, foraging effort and reproductive status of the dolphins with average estimates for adults ranging from ~ 4 kg/day up to 13 kg/day (Table 5). In addition, water temperature has been shown to significantly affect the food intake of captive bottlenose dolphins whereby intake increases with decreasing water temperature (Cheal and Gales 1992). Annual variations in water temperature may therefore result in seasonal changes in food intake in wild bottlenose dolphins, but this potential increase/decrease is difficult to quantify.

Age / Sex Class	**Annual Food Intake (kg)**	**Annual Energy Intake (kJ)**	**Daily Food Intake (kg)**	**Daily Energy Intake (kJ)**	**Paper**
Adult Males / Females	2000	176×10^5	5.48	482.2×10^2	(Kastelein et al. 2002)
Immature Males / Females	1700	150×10^5	4.66	231.9×10^2	
Pregnant Females	2000	176×10^5	5.48	482.2×10^2	
Lactating Females	3500	308×10^5	9.59	843.8×10^2	
Pregnant Females			8.7		(Cheal and Gales 1991)
Lactating Females			13.2		
Adult Males / Females			10.2	903.7×10^2	(Benoit-Bird 2004)

Table 5. Ranges of Estimated Annual and Daily Food and Energy Intake by Bottlenose Dolphins

Physiologically Based Toxicokinetic Model

Toxicokinetic (or PBTK) modelling is used to predict the absorption, distribution, metabolism, storage and excretion of synthetic or natural chemical substances around the body and has proved to be an invaluable technique for interpreting and assessing the effects of environmental contaminants in terrestrial mammals (Menzel 1989). More recently, these modelling approaches have been applied to a number of marine mammal species as our understanding of their physiology expands and concerns over the harmful effects of high levels of hydrophobic contaminants continue to be an issue (Hickie, MacKay and De Koning 1999; Klanjscek et al. 2007; Weijs et al. 2009a; Weijs et al. 2009b; Weijs et al. 2010; Weijs et al. 2011). The model equations follow the principles of mass transport, fluid dynamics, and biochemistry in order to simulate the fate of a substance within the body through partitioning and transformation in various organs and in the blood. Conventional PBTK models generally consider the uptake and distribution of chemicals over a relatively short time period, but some models have been developed and applied to marine mammals that consider the accumulation of contaminants over the life-span of individuals (Hickie, MacKay and De Koning 1999; Weijs et al. 2010).

The estimated intake of chemicals is modelled using information on contaminant concentrations in the prey. The quantity of prey consumed is then estimated using bioenergetic balance equations that incorporate the energy demands of the metabolism, somatic growth and an activity factor. PBTK models then function by separating the body into different compartments (e.g. lungs, liver, gut, muscle, blubber etc.) and estimating the contaminant flow in the blood through these compartments. Using physiological information, including mass and volume for example, the amounts of chemical conserved or metabolised in each of these compartments is then modelled along with sources of chemical loss such as excretion and lactation. Different life stages are also treated separately to categorise the animals according to their status as normal growth, pregnancy, lactating or neonates feeding

exclusively on milk. Thus, through a combination of equations, the models calculate the status of the animal and its contaminant uptake, release, and accumulation over a specific time frame.

In the current IBM model framework we are mainly concerned with estimating the concentration of PCBs that accumulate in the blubber through oral exposure from the prey. Whilst other organs may also store PCBs, the currency used in the model is blubber concentration of total PCBs. To estimate this we used a PBTK model available as open source known as IndusChemFate (Jongeneelen and ten Berge 2011). This flexible model is specifically targeted at understanding the fate of chemical exposure in a variety of animals and humans. The model has the ability to estimate blood, fat and urine concentrations for chemicals and their metabolites, even where substances are relatively data-poor. It is a generic, cross-chemical predictive model and more details are published in Jongeneelen and Berge (2011) and; Jongeneelen and ten Berge (2012). The model holds quantitative structure-property relationships (QSPR) algorithms to minimize the number of input parameters. These are limited to physico-chemical properties (e.g. molecular weight, density, vapour pressure, water solubility) and metabolic kinetic parameters (e.g. maximum velocity of metabolism ($=V_{max}$) and the Michaelis-Menten constant ($=K_m$) which is defined as the concentration of substrate that produces one half of the maximum metabolic velocity. For many chemicals these are available using internet resources (e.g. the NIH PubChem database http://pubchem.ncbi.nlm.nih.gov/ and ChemSpider http://www.chemspider.com/). Distribution over the body is largely determined by tissue:blood partition coefficients obtained from laboratory animal models (DeJongh, Verhaar and Hermens 1997). This means that the model does not require compound-specific tissue:blood partitioning information. Oral intake of compounds is considered as a dose applied directly to the stomach and then transferred to the intestinal tissue at a first order rate.

Although this model could consider the fate of up to four metabolites, for simplicity we only consider the fate of the parent compound and its estimated uptake into the blubber. The residence time of the compound of interest is determined by the rate of circulation, storage in tissues and rate of excretion. Only metabolism in the liver is considered. The model contains 9 relevant body compartments (lung, heart, brain, adipose, muscles, bone, stomach/intestines, liver and kidney). All physiological parameters are estimated from published sources for bottlenose dolphins in particular or were scaled from other small cetaceans such as harbour porpoise or beluga whale (Ridgway and Johnston 1966; Leatherwood and Reeves 1989; McLellan et al. 2002; Turner et al. 2004; Weijs et al. 2010; Weijs et al. 2011). The key physiological scaling parameters are given in Table 6. Cardiac output was estimated at 10 x total body mass.

Body Mass	150 kg	(Hart, Wells and Schwacke 2013)
Blood volume	0.071*body mass	(Ridgway and Johnston 1966)
Blubber volume	0.22*body mass	(Struntz et al. 2004)
Brain volume	0.009*body mass	(Turner et al. 2004)
Heart volume	0.005*body mass	(Turner et al. 2004)
Kidney volume	0.006*body mass	(Turner et al. 2004)
Liver volume	0.026*body mass	(Turner et al. 2004)
Lungs volume	0.037*body mass	(Turner et al. 2004)
Muscle volume	0.26*body weight	(Dearolf et al. 2000)
Intestine and stomach volume	0.05*body weight	(Leatherwood and Reeves 1989)

Table 6 Compartment volumes and body mass used in the PBPK model to estimate oral contaminant exposure in bottlenose dolphins.

Compound specific chemical property data are therefore also required for the model. Whilst there was data on the density and molecular weight of a range of PCB congeners, surprising little data was available for the kinetic constants for PCB metabolism (V_{max} and K_m) for any species, including humans. However, a study by Schnellmann (1984) estimated these constants for one of the PCB congeners found at relatively high levels in bottlenose dolphins, CB138 (2,2',3,4,4',5 hexachlorobiphenyl). This congener made up approximately 12% of

the total PCBs in the blubber of bottlenose dolphins (Hall et al. 2004). In the absence of data for other recalcitrant congeners, the kinetic constants estimated for human liver metabolism for CB138 were therefore used as a surrogate for the dolphins in this study as shown in Table 7.

	2,2',3,4,4',5 Hexachlorobiphenyl (CB138)
CAS	035065-28-2
Density (mg/cm3 or grams/litre)	1566
Molecular weight	360.388
Vapour Pressure (Pa)	7.74E-05
Log(Kow) at skin pH 5.5	7.44
Log(Kow) at blood pH 7.4	7.44
Water solubility (mg/litre)	0.00236
Enterohepatic removal (relative to liver venous blood)	0
Vmax Liver (parent[total] µmol/kg tissue/hr)	12.24
Km Liver (parent[total] µmol/litre)	8.8

Table 7 Physico-chemical properties for 2,2',3,4,4',5 hexachlorobiphenyl (CB138) used in the toxicokinetic model.

Accumulation of total PCBs from prey into blubber

Total PCB concentrations in blubber based on simulated diet

As has been shown above, there is considerable variability in (i) the estimated energy requirements for wild dolphins, (ii) the concentrations of PCBs in their fish prey and (iii) the diet composition of bottlenose dolphins. Here, we test two scenarios: (a) what the estimated concentrations of PCBs in the blubber of adult females would be if they were consuming a combination of contaminated fish with the concentrations shown in Table 3 and (b) what concentration of PCBs in the diet would result in blubber contaminant concentrations reported for the Sarasota Bay, Florida adult females (~ 8 mg/kg lipid weight, (Wells et al. 2005))

Using the above data on diet and PCBs in fish from all areas as a guide, the estimated concentrations of PCBs in the blubber were determined using the PBPK model and the data shown in Table 8.

Simulated diet	33% Sparidae 33% Mugilidae 33% Sciaenidae
PCB concentrations in fish	1.99 ng/g Sparidae 2.99 ng/g Mugilidae 2.41 ng/g Sciaenidae
Daily requirements	10 kg fish

Table 8. Simulated diet composition and estimated oral intake of PCBs by bottlenose dolphins.

The model was run simulating the annual intake in a single daily meal, although it would be possible to input the intake as a number of meals in a day. The PBTK model output showing the concentration of total PCBs in the blubber (mg/kg lipid weight, assuming a lipid proportion of ~ 60%, (Koopman 2007)) is shown in Fig. 13.

After 40 days of exposure the animals reach a steady state in which the blubber concentrations stabilise (with small daily fluctuations due to ingestion and minor metabolism of the PCBs). Thus on an annual intake basis of approximately 2.4 mg/kg of total PCBs in the daily diet (i.e. the concentration if the diet were comprised of the combination of fish shown in Table 8), the total PCB blubber concentrations would be ~ 9.5 mg/kg lipid. However, if the daily requirements were as low as 5 kg fish, this would reduce the total concentration in the blubber to ~ 3.5 mg/kg lipid, almost 1/3rd of the concentration seen at the higher intake rate.

Comparing the results of a simulation to the empirical data published for the Sarasota Bay, Florida population (Wells et al. 2005), the concentration of total PCBs in the prey that would result in a steady state concentration in the blubber of 8mg/kg (as was seen in the adult females in this population) would be ~ 2 ng/g wet weight fish.

However, what seems to not be reflected in this version of the PBTK model is the annual increase in total blubber PCBs that is observed in juvenile and non-lactating females and in males, i.e. life history stages that do not annually depurate their contaminants. This is probably largely due to the lack of species-specific kinetic constants (V_{max} and K_m) which dictate the rate of metabolism in the liver and the storage of PCBs in the blubber. The effect of this can be seen in Fig 14. If the V_{max} is arbitrarily halved then the asymptote continues to increase, as does the concentration of PCBs in the blubber.

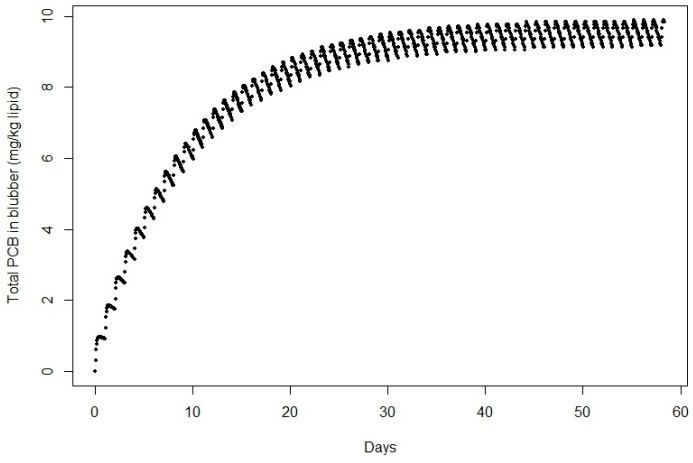


Fig. 10. Estimated total blubber PCB concentrations in adult female bottlenose dolphins following consumption of contaminated fish as shown in Table 8 at a consumption rate of 10 kg fish per day

Since the value of K_m (the rate at which the maximum metabolism is reached) also varies by compound and

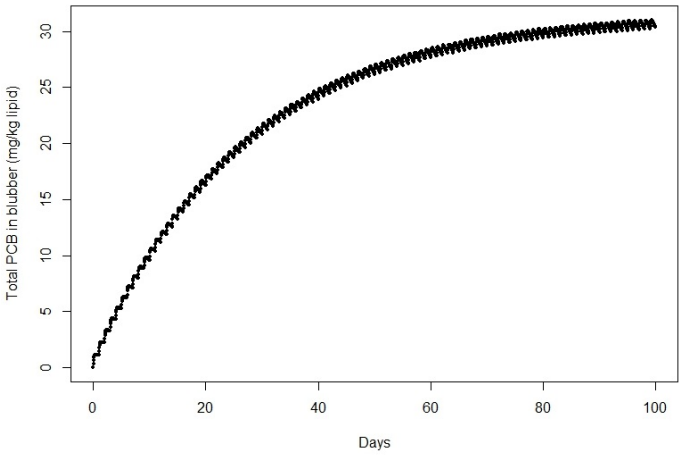


Fig. 11. Decreasing the estimated Vmax, i.e. the maximum the rate at which the liver can metabolise PCBs, increases the concentration in the blubber and the rate at which the contaminants accumulate over time.

species, (Schnellmann 1984) without further empirical data it is difficult to fully explore the effect of these kinetic parameters.

DISCUSSION

This paper reports on the final phase of the Pollution 2000+ individual based modelling study, exploring the utility of the model for two species of cetacean (bottlenose dolphin and humpback whale) and comparing the model outputs to empirical data. In a previous paper (Hall et al. 2012) this comparison was carried out for the bottlenose dolphins using empirical data available at the time for the Sarasota Bay, Florida population. This is a very important step in individual or agent based models (also known as pattern-oriented modelling, (Railsback and Grimm 2010)) as it can greatly assist in confirming the structural reality of the model. This helps to ensure the models are both general enough to be widely applicable but also scientifically accurate to allow management decisions and priorities to be determined. Here we again use empirical data to compare with the model outputs and also show that the current levels of PCB exposure in the Gulf of Maine humpback whales are unlikely to be a problem for this species at a population level. The concentrations in the immature animals were higher (up to a maximum of ~12 mg/kg lipid) which may have consequences following exposure of individuals to a pathogen. But in general, the health risks were low.

However, the model outputs did not match the concentrations measured in the juvenile age classes. This may be because there are discrepancies between the estimated intra-uterine and lactational transfer proportions in this species compared to the surrogate data used or because the annual intake in the juveniles is higher than the adults. However, there is no evidence to suggest that juveniles are feeding on different prey to adults in this region (Ramp, C. pers. comm.) so it perhaps more likely to be the former. The level of discrepancy in the transfer rates could be further explored to see what proportions would complement the empirical data.

Another important aspect is to investigate the sources of uncertainty in the modelling process. This was carried out for the underlying population or Leslie Matrix model in which a stable age structure is generated to seed the IBM. For these matrices vital rates, such as calf survival, adult survival, age at first reproduction and female fecundity estimates, are needed. Using both a sensitivity analysis and calculating the elasticities for the survival and fecundity estimates (which are similar to the sensitivities but allow for comparisons between rates which are measured on different scales) we found that adult survival has the largest proportional effect on the subsequent population growth rate estimation. Thus, where resources are limited efforts should be directed towards reducing the uncertainty in this parameter over and above the others required for the model.

As far as uncertainty in concentration-response functions is concerned, we found that the degree of error increased as the annual accumulation level of PCBs, and thus level of contaminant exposure, increased. This may be partly because the degree of uncertainty in the effect of PCBs on fecundity in the model species (i.e. the studies on mink) was higher in the high exposure groups as there were many fewer data points at this end of the range. The effect was particularly marked for the humpback whale population simulation because the population growth rate was much higher in this species. For the bottlenose dolphins, the population growth rate was low in the uncontaminated population (λ=1.014). Thus any effect of PCB exposure on a population with an already low fecundity rate will have the overall effect of further lowering the survival of the small number of calves born. Unlike in the population with a high growth rate, the uncertainty did not increase with increased contamination because the growth rate was already low and at the highest exposure levels, most of the calves born were then not surviving to recruitment.

The levels of uncertainty were higher around the estimated decreases in potential population growth for the fecundity effects than for the effects on immunity. However, as the annual accumulation rate of PCBs into the blubber increased, the effect on survival following exposure to a pathogen had a relatively greater impact on the population. Thus, despite the encounter rate with a pathogen being the same (5% in this scenario) the impact on the population was not linear.

However, it should be noted that not all the uncertainty in the immune effects have been captured here. We included the variability in the relationship between the immune function Con A assay and the concentration of total PCBs in the blubber and translated that uncertainty into variability in the link between host resistance (and thus probability of survival) and total blubber PCBs. However, there is also variability in the relationship between the immune function assay and the estimate of host resistance from the National Toxicology Program studies on

mice reported by Luster (Luster et al. 1993). These studies were extensive and would be very expensive to replicate. It is unlikely that they will be repeated or refined, at least in the short term. The best estimates without additional uncertainty for this relationship have therefore been included here. Efforts to reduce the variation between the Con A immune function assay and the concentration of PCBs in the blubber of bottlenose dolphins and other cetacean species is likely to be a more cost-effective place to begin. As the amount of data available from the type of health assessment studies that generated these data increases, (Schwacke et al. 2012b) so the error around this relationship will decrease.

It is notable that the degree of uncertainty generated in the final estimates of potential population growth rate is perhaps not prohibitively large. However, this conclusion is of course accepting that there are no major issues with the underlying model assumptions.

In the final section of this risk assessment we investigated the use of a toxicokinetic or PBTK model to determine the concentration of PCBs in the blubber from information about contaminants in the prey. In our model scenario we investigated the availability of published data to include in the model for bottlenose dolphins in the US. Whilst there is a reasonably good amount of data on diet and some information on contaminants in prey, there was surprising variation in the estimates of energy requirements for wild bottlenose dolphins. Some effort in improving these values would certainly assist in ensuring the model is as realistic as possible. In addition here we have used average levels of PCB intake for an average adult female dolphin. A more sophisticated approach would be to use the distributions of PCBs in the fish prey to generate distributions of total PCBs in the population of dolphins. This method will be used in the final, web-based version of the model.

But of more concern for the model realism was the lack of information about the rate of PCB metabolism. Using the values available for CB138 as representative of all the PCBs is not realistic because whilst it is one of the most recalcitrant congeners, it is metabolisable to some extent. The ability of the liver to deal with different congeners varies according to their physico-chemical properties. Of most value to this study would be estimate of the V_{max} and K_m values either for a range of PCB congeners or for the most recalcitrant congener found at highest concentrations in the blubber, CB153. Thus the annual accumulation rate has not been fully captured because the V_{max} and K_m values used (estimated for humans) showed that the liver can deal with a rate of PCB intake 10 fold higher that the amount being ingested by the dolphins, i.e. the rate of PCBs being processed by the liver is only 10^{th} of its maximum capacity. This is perhaps overly simplistic and what is really needed are the kinetic constants for dolphin liver (i.e. rates of metabolism within the hepatic microsomes) and for a range of PCB congeners. Both these kinetic parameters can vary be a factor of 10 by both congener type and by species (Schnellmann 1984).

Another approach to this would be to use a PBTK model that does not rely on estimates of these kinetic and Michaelis-Menten parameters and instead estimates of compound –specific tissue:blood partition coefficients are used to determine the kinetics of the compounds through the various body compartments. Indeed this approach has been used for small cetaceans (Weijs et al. 2010; Weijs et al. 2011) but again rely on estimates of hepatic extraction, liver blood flow and the metabolic half-life for the contaminants as estimated for humans or model species (Verner et al. 2008; Verner, McDougall and Johanson 2012). And in the final conclusions the authors also reported that the lack of information on the distribution and uptake processes were the most likely reason for the lack of concordance between their model output and the empirical data for the harbour porpoise model they were using (Weijs et al. 2010). Their estimated elimination half-lives of PCBs in harbour porpoise were much higher compared those estimated using published empirical data. Here, our estimates of the blubber concentrations from ingested contaminated prey were within the expected order of magnitude, suggesting that this approach is worth perusing and refining. It would certainly be feasible to estimate the kinetic parameters for small cetaceans by determining their metabolism by the liver microsomes using the same *in vitro* approaches as has been used for other species (Knaak et al. 1993; Song et al. 2013).

This joint IBM and PBTK modelling approach has provided a risk assessment tool that can be used to determine the population consequences of exposure to contaminants. We have used the example of polychlorinated biphenyls but the model framework has the potential for investigating the impact of a variety of stressors on cetaceans. In order to improve the reliability of the model there are a number of priority areas that have emerged as important:

1. Adult survival estimates have the largest influence on the underlying population model.

2. The incorporation of further data on the relationship between the Con A immune function assay (and indeed other immunity assays) and total blubber PCBs in bottlenose dolphins would improve the model uncertainty.

3. Improved estimates of daily energetic requirements and fish consumption levels for wild cetaceans would assist in producing accurate estimates of blubber PCB concentrations.

3. Species-specific estimates of the kinetic constants for the metabolism of PCBs (particularly CB153) *in vitro* would improve the utility of the PBTK model.

This model framework, built using the open source program R (R Core Development Team 2011) is currently being converted into a web program with a user friendly interface. This will be made widely available to the community for the use and refinement.

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Appendix 1. List of PCB congeners analysed in blubber biopsy samples from humpback whales in the Gulf of Maine.

IUPAC number	Structure	Group
17	2,2’,4	Trichlorobiphenyl
18	2,2’,5	
28	2,4,4’	
31	2,4’,5	
33	2’,3,4	
44	2,2’,3,5’	Tetrachlorobiphenyl
49	2,2’,4,5’	
52	2,2’,5,5’	
66	2,3’,4,4’	
70	2,3’,4’5	
74	2,4,4’,5	
82	2,2’,3,3’,4	Pentachlorobiphenyl
87	2,2’,3,4,5’	
95	2,2’,3,5’,6	
99	2,2’,4,4’,5	
101	2,2’,4,5,5’	
105	2,3’,4,4’	
110	2,3,3’,4’,6	
118	2,3’,4,4’,5	
128	2,2’,3,3’,4,4’	Hexachlorobiphenyl
138	2,2’,3,4,4’,5	
149	2,2',3,4',5’6	
151	2,2',3,5,5',6	
153	2,2',4,4',5,5'	
156	2,3,3',4,4',5	
158	2,3,3',4,4',6	
170	2,2’,3,3’,4,4’,5	Heptachlorobiphenyl
171	2,2’,3,3’,4,4’,6	
177	2,2’,3,3’,4’,5,6	
180	2,2’,3,4,4’,5,5’	
183	2,2’,3,4,4’,5’,6	
187	2,2’,3,4’,5,5’,6	
191	2,3,3’,4,4’,5’6	
194	2,2’,3,3’,4,4’,5,5’	Octochlorobiphenyl
195	2,2’,3,3’,4,4’,5,6	
199	2,2’,3,3’,4’,5,5’,6	
205	2,3,3’,4,4’,5,5’,6	
206	2,2’,3,3’,4,4’,5,5’,6	Nonachlorobiphenyl
208	2,2’,3,3’,4,5,5’,6,6’	
209	2,2’,3,3’,4,4’,5,5’,6,6’	Decachlorobiphenyl