Assessing the Population Consequences of Pollutant Exposure to Cetaceans using an Individual Based Modelling Framework.

Hall, Ailsa J[1]; Schwacke, Lori H.[2]; McConnell, Bernie J.[1]; Rowles, Teri K.[3]

Sea Mammal Research Unit, Scottish Oceans Institute, University of St Andrews, St Andrews, KY16 8LB, UK

National Marine Fisheries Service, Marine mammal health and stranding response program, 1315 East-West highway, Silver Spring, Maryland, 20910, USA

NOAA's Ocean Service, Center for Human Health Risks, 331 Fort Johnson Road, Charleston, South Carolina, 29412, USA

ABSTRACT

The population level consequences of pollutant exposure in cetaceans is very difficult to determine directly and therefore requires a comprehensive risk assessment approach in order to estimate at what level of exposure individual impacts translate into detrimental effects on populations. Here we describe and demonstrate, using two case study populations and species, an individual based model framework that can be used to simulate a variety of pollutant impacts and their effect on potential population growth. Bottlenose dolphins (*Tursiops truncatus*) and humpback whales (*Megaptera novaeangliae*) were used to construct simulated populations using previously published life history data and vital rate parameters. The model is female-based and, under various pollutant exposure circumstances, the *potential* population growth rates (*lambdas*) can be compared. Density dependent effects are not included, so the emphasis is on relative rather than actual population growth rates. The model is demonstrated using the relationship between maternal polychlorinated biphenyl (PCB) uptake and its effect on early calf survival. The model is stochastic and includes uncertainty in the concentration-response relationship. Here we include two examples using data from studies in mink (*Mustela vison*) which have been the preferred laboratory model species for determining the effects of PCBs on reproductive success. We found that using the results from over 50 experiments reported in 15 papers (synthesised by Fuchsman et al. 2008) produced wide uncertainty bounds and that by restricting the studies to a fewer number of more directly comparable studies which reported tissue concentrations measured in the females, the degree of uncertainty was reduced. However, this also increased the EC50 (median effective concentration) from 20 to 46.5 mg/kg lipid weight, thereby increasing threshold at which effects were observed. Nevertheless, the simulations indicated that at annual PCB exposure levels of above ~ 2-4 mg/kg, depending on the species and population scenario, are likely to affect the potential population growth rate. Further work needs to be carried out to determine which concentration-response function to use in the model or whether to allow this to vary depending on how conservative the risk management measures must be for a given species and situation.

KEYWORDS: POLLUTANTS; CONTAMINANTS; POLYCHLORINATED BIPHENYLS; RISK ASSESSMENT; POPULATION DYNAMICS; CONCENTRATION-RESPONSE

INTRODUCTION

Following the IWC Pollution 2000+ Intersessional Workshop held at the Marine Mammal Center, Sausalito, CA, USA from 22nd-24th February, 2010, the goals and objectives for Pollution 2000+ Phase II were agreed as follows:

1. **Develop integrated modelling approaches and a risk assessment framework for evaluating the cause and effect relationships between pollutant exposure and cetacean populations**
2. Develop a prioritization hazard identification framework to evaluate the broad number of environmental pollutants
3. **Identify data needs and available datasets or case studies that would be appropriate for the models that are exposure driven, source driven or effects driven.**

Objectives 1 and 3 are the current priorities of this section of the Phase II project.

There are a number of different modelling approaches that could be taken to simulate and estimate the impact of pollutant exposure on cetacean populations. We chose to use an individual-based model (IBM) framework (Hall et al. 2006), an approach that has also been used in a variety of different fields to determine the consequences of many other harmful agents on population dynamics including pathogens and parasites as well as pollutants (Murphy et al. 2008; Ajelli and Merler 2009; Gaba et al. 2010). In the first iteration of the model we investigated the impact of polychlorinated biphenyls (PCBs), on calf survival probability and how this may therefore ultimately affect potential population growth rate. We do not include density dependence in the model (due to lack of data) so the comparisons made are on a relative rather than an absolute basis.

It has been well demonstrated in a number of laboratory animal models that PCB exposure can, in addition to other effects, reduce offspring early survival probability (Barsotti, Marlar and Allen 1976; Kihlstrom et al. 1992). These studies, and primarily those carried out on mink, provided data for the concentration-response relationship used in the first probabilistic risk assessment study into the effects of PCBs on bottlenose dolphin populations (*Tursiops truncatus*) published by Schwacke et al. (2002). Initially we also used the bottlenose dolphin as a study species, specifically the Sarasota Bay, Florida population as an example of our IBM approach (Hall et al. 2006), emphasising that this was for illustrative purposes to test the model framework and its sensitivity. The initial species choice was dictated to some extent by the availability of vital rate and population dynamics data as well as data on PCB exposure levels. By using only one population to illustrate the applicability of the approach we were able to maintain, as far as possible, internal data consistency. To this end we also chose to use concentration-response data from captive born bottlenose dolphins (Reddy et al. 2001) with additional data from the Sarasota Bay dolphins (Wells et al. 2005) where maternal blubber PCB concentration and offspring survival to six months of age was known. However, this was a very limited dataset which therefore introduced a large amount of uncertainty into the model. The initial sensitivity analysis on the performance of the model and resulting potential population growth rates indicated that this uncertainty exerts a large influence on the results.

At the Intersessional Pollution 2000+ workshop it was recognised that the IBM approach for assessing pollutant impacts at the population level was likely to have wider and more general applicability to other cetacean species. The ultimate goal is therefore to develop the model framework into a more robust and flexible format, using open source software with a user-friendly interface that would allow researchers and managers to investigate, using their own simulated scenarios, potential impact of pollutants on cetaceans. This will allow them to compare such impacts to the many other competing threats facing these populations.

Here, we expand, develop and refine the IBM approach further and introduce a second demonstration species and population, the humpback whale (*Megaptera novaeangliae*) as a further case study. At the same time we also identify data needs and available datasets that would be appropriate for the risk assessment model (particularly in relation to refining and reducing the uncertainty in the concentration-response relationship).

The specific aims of the Phase II modelling project are therefore:

1. Improve the existing concentration-response (CR) function for PCB-related reproductive effects in cetaceans. This involves re-initiating efforts to derive a CR function based on surrogate species for reproductive effects in relation to PCB exposure. The CR component will be improved by conducting a literature search and integrating additional data into the model from recent studies.

2. Derive additional CR functions to address other toxicological endpoints in relation to PCB exposure. This requires a multi-stage modelling approach, e.g., a series of functions that provide a connection from PCB exposure $\rightarrow$ functional immune endpoints $\rightarrow$ increased pathogen susceptibility $\rightarrow$ increased likelihood of mortality.

3. Integrate improved concentration-response components into a population risk model (i.e. individual-based model) for two case study species: bottlenose dolphin and humpback whale. These two species have been chosen as demonstration cast studies since they represent a small and large cetacean species for which sufficient relevant data already exist on both exposure and vital rates for specifically defined populations. The model will be developed so that it can be distributed throughout the community for use and development using other species and endpoints where sufficient life history, contaminant exposure and vital rate data exist. The overall objective is to determine the magnitude of the risk to a population (as measured at least in the first instance as changes in potential population growth rate or *lambda*) from contaminant exposure at various levels, which would ultimately allow the 'pollution risk' to be compared with other risks that may result in changes in their population dynamics (e.g. the impact of bycatch or prey availability).

4. Implement a CR component for at least one additional contaminant of concern (COC). The COC would be determined by the steering committee based on given knowledge for likelihood of exposure and toxicity. This will involve a literature search to parameterise the additional CR component and investigate changes in model outcome assuming both additive and synergistic effects.

This paper outlines the modelling approach and the progress made on fulfilling the above aims, particularly 1 to 3 above.

MODELLING APPROACH

Details of the initial model framework are given in Hall et al. (2006). The model has been constructed using the statistical and modelling package R (R Development Core Team, 2011) and it simulates the fate of individual females using published fecundity and survival data for each species of interest to construct an initial, appropriately sized, population of animals with a stable age structure. The model then simulates the accumulation of pollutants, in this case polychlorinated biphenyls (PCBs) through transplacental transfer, suckling and prey ingestion and loss of PCBs from the mature females' blubber. Maternal blubber PCB concentrations then affect the first-year calf survival probability in a dose-dependent matter. An outline flow diagram of the model is shown in Fig. 1. The model is stochastic so that each of the birth and survival outcomes are determined by whether a random number (generated from a uniform distribution) was less than or equal to the probability associated with that event.

Model Structure

Each animal is assigned a state variable of 1, alive or 0, dead, an age and blubber PCB concentration (mg/kg lipid weight). The model is a post-breeding census and age class 1 is equivalent to newborn calves. Each model simulation spans a period of 100 years and a starting abundance is chosen based on the specific populations being simulated. For any given set of fecundity or survivorship values the stable age structure is calculated by multiplying an arbitrary seed age structure by the appropriate Leslie matrix 100 times. The stable age structure is then used as the underlying population structure of the initial population of *n* females. At first each animal is assigned zero PCB level and after the first year, animals are then allocated an appropriate blubber PCB concentration depending on their age class and reproductive status. After approximately the 40th simulation year, the relationship between PCB concentrations and age stabilises. From the population trajectories after the first 40 years the mean potential growth rate of the population is calculated and the 2.5 and 97.5 percentile growth rates estimated from the ranked individual growth rates. The variation in potential population growth rate with varying annual PCB accumulation rates can then be investigated, incorporating uncertainty into the concentration-response relationship. This is achieved by each 100-year simulation the model choosing random concentration-response model coefficients from a set of 500 coefficients generated by data resampling.

Model parameters and case study populations

The vital rates (fecundity and survival) and other explicit model parameters such as initial population size and maximum age class used in the model simulations for the two case study species are given Table 1. Where possible, species and population specific parameters were used. Those listed for the Sarasota Bay, Florida bottlenose dolphin population are the same as the parameters used in the initial simulations reported in Hall et al. (2006).

For the humpback whale, population parameters were collated from the various publications listed with the main source of survival and fecundity rates being obtained from Barlow and Clapham (1997). The recent publication by Zerbini, Clapham and Wade (2010) lists all the plausible life history parameters for various humpback whale populations. The population in the Gulf of Maine has been extensively studied and therefore provides reliable life history parameters for this species. In addition, data on blubber PCB concentrations needed for model comparisons and, if possible, estimates of annual accumulation rates (e.g. in the bottlenose dolphin example the annual accumulation was estimated from the rate of increase in blubber PCBs with age in the males since they do not offload their burden each year). A study published in the 1975 reported levels of chlorinated hydrocarbons in a number of cetacean species in the north Atlantic including humpback whales (Taruski, Olney and Winn 1975) and in 1997 a more detailed study was reported levels in four female humpback whales from the Gulf of St Lawrence (Gauthier, Metcalfe and Sears 1997). More recently Elfes et al. (2010) published data on levels in males collected from the North Atlantic (Gulf of Maine) population although no information on age was available. Given the consistency of the information from the Gulf of Maine population and because there have been various estimates of life history parameters over time which will allow us to simulate a historic as well as a recent population, we chose to use them as the second case study.

In both case studies data from various sources is used to estimate the proportion of PCBs transferred form the female to the calf *in utero* (0.6) and during lactation (0.77) (Tanabe et al. 1982; Cockcroft et al. 1989; Salata et al. 1995). Where the calf (of either sex) dies within its first year we assume death occurs at 6 months and the depuration for that year is halved to 0.38. Subsequently the birth of male calves is ignored by the model.

Table 1. Model parameters used to simulate effect of maternal PCB concentrations on first calf survival and population growth rate for bottlenose dolphin and humpback whale.

Parameter	Bottlenose Dolphin	Reference	Humpback Whale	Reference
No. females	300		500	
Maximum age	40 years	(Wells and Scott 1990)	35 years	Barlow and Clapham, 1997
1st year calf survival	0.811	(Wells and Scott 1990)	0.875	Barlow and Clapham, 1997
Adult survival	0.962	(Wells and Scott 1990)	0.960	Barlow and Clapham, 1997
Fecundity	0.177	(Wells and Scott 1990)	0.111 – 0.241, depending on age	Barlow and Clapham, 1997
Calf sex ratio	1:1	(Wells and Scott 1990)	1:1	Barlow and Clapham, 1997
Length of lactation	2 years	Oftedal 1997	1 year	(Oftedal 1997)

Concentration-response function

Since the degree of uncertainty in the concentration-response function has a large influence on the resulting potential population growth rate simulations, it is critical that the most robust data are used. As with human risk assessments it is often impossible to obtain data on the particular species of interest so data from surrogate (laboratory animal models) are commonly used. This is now widely accepted as the best available strategy, despite the obvious drawbacks and caveats.

In the first IBM (Hall et al. 2006) we chose to use bottlenose dolphin data published by Reddy et al. (2001) and Wells et al. (2005) to estimate a concentration-response relationship using a generalized linear model with a logit link function (p=0.0395). Uncertainty around this relationship was then estimated by resampling with replacement from the original data and recalculating the regression. This was repeated 500 times and the resulting relationship with uncertainty is shown in Fig. 2. (intercept = 1.77, SE 0.95 p= 0.0623 , PCB = -0.193, SE 0.094, p=0.04). This relationship resulted in an EC50 (median effective concentration) of 9.2 mg/kg, SE 2.95 which is significantly lower than the EC50 of 33 mg/kg estimated by Schwacke et al (2002) from two mink exposure studies included in their risk assessment and, with the additional uncertainty, this was a priority area for model refinement.

RESULTS

Improving the concentration-response function

As recommended by the Intersessional Pollution 2000+ Workshop, a wider literature search was carried out to see what other, perhaps more recent studies have been published that report informative concentration-response function data. A recent paper by Fuchsman et al. (2008) reported a comprehensive quantitative analysis of published results from over 50 tests of PCB effects on mink reproduction. Their meta-analysis of the combined data from these studies reported a variety of dose (being the amount of contaminants <u>fed</u> to the animals) and concentration-response (being the amount of contaminants measured <u>in</u> the animals during the experiment) parameters, including a potentially useful measure for our purposes of whole-body total PCBs (mg/kg). The relevant data from their paper are reproduced in Table 2. More than one experiment with different administered dose rates was often reported in many of the papers thus

the results of the individual experiments are listed. The authors then used nonlinear regression models fitted to the various PCB exposure metrics against production of surviving kits (n=27). Their relationship for whole-body total PCBs is shown in Fig. 3. The data were fitted using the model form:-

y = p0 + ((1-p0) / (1+exp(-(a+b*ln(whole-body PCBs)))))   (Fuchsman et al. 2008)
where
a = -1.0763, b =2.830,
EC50 = 1.5, 95% confidence limits 0.97 – 1.8, residual standard error = 24.9,
R^2=0.71

and P0 = upper asymptote (100 %) and y = surviving offspring per mated female as % of control (values > 100% were assigned as 100%).

However, in order to embed this relationship into our IBM we needed to convert, where necessary, the mink whole-body PCB concentrations back to tissue concentrations (liver or adipose since the lipid weight PCB concentrations for these two tissues is approximately equal, see Schwacke et al., 2002). Fuchsman et al. (2008) estimated whole body PCBs either from the tissue concentrations or ingested doses using the method of Leonards et al. (1995) to account for differences in the bioaccumulative potential of different PCB mixtures as well as differences in exposure duration. We then used the linear relationship between the whole body PCB and the tissue concentrations using the raw data given in the paper to estimate a tissue concentration for all the studies reported (n=32). The resulting relationship is shown in Fig. 4 (p < 0.0001, R^2 = 0.96) and the tissue concentration estimates for each study are also given in Table 2. This allowed us to construct a concentration-response relationship using the same non-linear model form of Fuchsman et al., (2008) as above for maternal total PCB tissue concentrations against offspring survival probability as shown in Fig. 5a and 5b with the following parameter estimates:-

a = -4.782, SE = 2.055, p=0.027;
 b=1.5875, SE=0.6431, p=0.0195.

This resulted in an EC50 estimate of 20.59 mg/kg PCB tissue concentration (95% confidence limits 7.4 – 54.6 mg/kg). The uncertainty around the relationship was again estimated using resampling with replacement (n=500, Fig 5b). This uncertainty was then incorporated into the IBM as before, by choosing, at random, a regression from the set of 500 regressions calculated by resampling.

Mink Studies Subset

There was considerable uncertainty in the resulting concentration-response relationship reflecting the variation in the different study designs, both in terms of PCB mixtures to which the mink were exposed (commercial mixtures, individual congeners or contaminated prey) and the duration of each study. In addition, the calculation of estimated tissue concentrations from whole body PCBs introduced an additional degree of variation. Thus, in order to reduce some of the variability, six of the papers which had the most relevant study designs were used as a subset. These were the study where concentrations of total PCBs in the tissues of the mothers (again either liver or adipose) and raw details of the survival of the individual offspring were listed (Platonow and Karstad 1973; Jensen et al. 1977; Kihlstrom et al. 1992; Heaton et al. 1995; Restum et al. 1998; Bursian et al. 2006b). The raw data extracted from these papers produced the concentration-response relationship shown in Fig. 6. As in (Schwacke et al. 2002) who also used the Heaton et al. 1995 data, adjustments were made where necessary to convert wet weight liver concentrations to lipid weight using the average lipid weight for mink liver of 4.7% and estimates of PCB liver concentrations in the control and low

dose groups using the equation of (Leonards et al. 1995) to predict tissue concentration from dietary intake.

A generalized linear quasibinomial model with a logit link function, weighted by the number of animals in each study, was then fitted to the data giving model coefficients of:-
intercept = 1.442, SE 0.368   p=0.0005,
PCBs = -0.031, SE 0.008, p=0.0012.

The uncertainty around the relationship was again estimated using resampling with replacement (n=500, also shown in Fig. 6). The resulting EC50 from the best fit relationship was 46.5 mg/kg, SE 8.8. This is again significantly higher than the EC50 estimated from the bottlenose dolphin data and somewhat higher than the Fuchsman et al. (2008) complete dataset (20.59 mg/kg) and two datsasets used by (Schwacke et al. 2002) namely (Heaton et al. 1995; Restum et al. 1998) (33 mg/kg).

Case Study 1 – Bottlenose Dolphins

In order to compare the results using the surrogate concentration-response relationships with the findings of our initial study (Hall et al., 2006), we carried out simulations using the same Sarasota Bay, Florida population bottlenose dolphin life history and population parameters as listed in Table 1 which resulted in a stable age structure with a population growth rate (*lambda*) of 1.014.

Firstly, the population was modelled with an annual accumulation rate of 2.96 mg/kg PCBs as estimated from the relationship between male blubber PCBs concentrations measured in this population and age (PCB concentration = 2.96 x age + 19.4, p<0.0001; R^2=0.56, (Wells et al. 2005)) and using the Fushman et al. (2008) concentration-response relationship. This resulted in a mean potential population growth rate after the first 40 years of 0.997 (95% confidence interval (CI) 0.984, 1.006). The effect of uncertainty in the concentration-response relationship can clearly be seen in Fig. 7a (100 simulations) where although a decline in the population is most likely to occur at this level of exposure (after a slight increase in abundance in the early years), some simulations resulted in the population continuing to increase. By comparison Fig. 7b shows the results for simulations using only the best-fit concentration-response relationship without uncertainty (100 simulations). In this scenario the population growth rate again increases over the first few years but then in almost all simulations the population gradually declines over time (mean growth rate after the first 40 years 0.995, 95% CI 0.989, 1.001).

Secondly, we investigated the how variation in the PCB annual accumulation rate affected the potential population growth rates (again 100 simulations over 100 years, Fig. 8). At annual accumulation rates above approximately 2 mg/kg the likelihood that the potential population growth rate will be negatively affected increases up to a maximum of approximately a 4.5% decrease compared to an uncontaminated population. As exposure increases the impact on *lambda* reaches an asymptote as maternal transfer to the calves reaches a universally fatal threshold.

A second set of simulations were then carried out using the selected subset mink concentration-response studies and the relationship shown in Fig 6. In this scenario, the populations did not start to decline until annual accumulation rates were >4mg/kg (Fig. 9). In addition the degree of uncertainty using this concentration-response relationship is reflected in the smaller difference between the 95% confidence limits calculated with and without the additional variation. The two sets of results were not widely different, particularly at the lower end of annual accumulation rates and when compared to the previous scenarios using the Fuchsman et al. (2008) data (Fig. 8).

Case study 2 – Humpback whales

For the humpback whale population, the life history and population parameters shown in Table 2 were incorporated into the model and again two sets of population growth rate simulations were run. An range of age-specific fecundity estimates based on those published by Barlow and Clapham (1997, Table 2) were used in the model. The Leslie matrix stable age structure which initially fed into the model gave an identical convergent population growth rate (*lambda*) to that reported by Barlow and Clapham (1997) of 1.065. This gave us a check of model performance. The first set of simulations were again run using the concentration-response relationship from the Fuchsman et al. (2008) mink dataset and the second set using the selected subset of mink studies, as for the bottlenose dolphins. Again, both included 100 simulations with and without concentration-response model uncertainty.

Although there are data available on the concentration of PCBs in male humpback whales (Elfes et al. 2010) age-specific data from which an annual accumulation could be estimated was not available. We therefore only investigated the change in potential population growth rate over a range of annual accumulation rates. In the simulations using the Fuchsman et al. (2008) concentration-response relationship the population continued to increase at accumulation rates of <2 mg/kg but above this level the population began to decline as shown in Fig. 10. The results of the simulations for humpback whales using the subset of mink studies are shown in Fig. 11. Here the potential population growth rate began to decline at annual accumulation rates of >3 mg/kg with a maximum depression at the highest accumulation rates (10mg/kg) of approximately 3% compared to the uncontaminated population.

DISCUSSION

The approach demonstrated here investigates the population consequences of PCB exposure in cetaceans using an individual based model framework linking maternal PCB uptake to early calf survival. We have refined our initial model to incorporate concentration-response relationships based on studies in mink as a surrogate model species and we have focussed on two case studies species, bottlenose dolphins and humpback whales. The model simulation outputs indicate the level of annual PCB exposure and accumulation likely to affect the potential population growth rates.

We have incorporated uncertainty into the concentration-response relationship to determine what effect variability in this function will have on our conclusions. At this stage we concentrated on collating data from a single surrogate species to investigate how much variation exists within one laboratory model. Using the set of toxicological data for mink collated by Fuchsman et al. (2008) we found that, despite the large number of studies and individual exposure experiments, there is still considerable uncertainty around the relationship. This is probably due to the disparity between the individual experiments. They exposed mink to PCBs in various different forms and mixtures (from commercially produced compounds to naturally contaminated feed), used different exposure durations and different exposure metrics to determine the exposure-response relationship. When we focussed on six studies that reported concentrations of total PCBs in the tissues of the mink in relation to kit survival, which match the exposure metrics available for cetaceans, the degree of uncertainty was reduced. However, the EC50 from these two datasets varied surprisingly widely, ranging from 20-46 mg/kg lipid weight which clearly had influenced the level at which negative potential population growth was seen. Nevertheless, it seems that annual PCB accumulation rates above between 2 and 4 mg/kg, for both the humpback whale and bottlenose dolphin populations are potentially detrimental to the population growth, depressing it by between 1- 3% when compared to uncontaminated populations.

Evidently more work is needed to determine the most appropriate concentration-response relationship to use in this model. The literature review also investigated the results reported from studies in other species including rats and mice (Arnold et al. 1997; Arnold et al. 1998a; Arnold et al. 1998b; Feeley and Jordan 1998; Voltura and French 2007; Cocchi et al. 2009). However, the results were not given in a form suitable for inclusion in a combined species function (for example only average litter size was reported or no treatment effects were seen). Other studies using monkeys (Barsotti, Marlar and Allen 1976) and pigs (Brunstrom, Kihlstrom and Lundkvist 1982) did report the tissue concentration of PCBs in relation to offspring survival and these will be included in future refinements. Since there are also discrepancies in using data from model species that give birth to litters rather than single offspring further discussions with the steering group on this issue will be required.

For the bottlenose dolphin simulations based on the data from the Sarasota Bay, Florida population, an annual accumulation of 2.96 mg/kg (based on the information from the males) is likely to be too high, since this resulted in a mean PCB level in adult females of 23.8 mg/kg. Data from blubber biopsies collected from females >9 years of age was 8 mg/kg (SD 6.8, (Wells et al. 2005) which equates to an annual accumulation of 0.9 mg/kg. For the humpback whales in the Gulf of St Lawrence data reported in the literature suggest that adult females may have concentrations of approximately 2-4 mg/kg lipid weight PCBs (Gauthier, Metcalfe and Sears 1997) although these data were collected some years ago and are from animals outside the Gulf of Maine region. However, with these caveats in mind, this equates to an annual accumulation rate of between 0.2 and 0.4 mg/kg.

Further refinements of the model framework are also being undertaken. Additional sources of variation, particularly in the estimates of *in utero* transfer and lactational transfer of PCBs from mother to calf will be incorporated and other toxicological endpoints will be investigated, as outlined in the objectives above. In particular effects on fecundity and immunity will be included. Data on fecundity effects are also available from the mink toxicological data and concentration-response data on immune function effects may be gleaned from studies in various species including bottlenose dolphins and seals.

In conclusion the most appropriate concentration-response functions to use in these models remains an issue and a priority for discussion as uncertainty still high, despite the large number of mink studies that have been carried out since the 1970s. The model framework is flexible and we are currently working on a user friendly interface so that different concentration-response models can be more easily embedded in the model, together with the ability to easily vary the life history and population parameters.

Table 2 Summary of studies testing effects of PCBs on mink reproduction from (Fuchsman, Barber and Bock 2008)

Authors	Form of PCBs Administered	Exposure Duration (days)	Total PCB Concentration in Food (mg/kg)	Daily intake Total PCBs (µg/kg-day)	Whole-body Total PCBs (mg/kg)	Estimated Tissue Concentration Total PCBs (mg/kg)	Surviving kits/mated female (% of control)
(Aulerich and Ringer 1977)	Aroclor 1242:1248:1254	156	10:10:10	3913	26	414.5	0
	Aroclor 1254	280	5	724	7.5	119.6	0
	Aroclor 1254	280	10	1491	15	239.1	0
	Aroclor 1254	130	15	1957	20	318.8	0
	Aroclor 1221	297	2	261	0.27	4.3	235
	Aroclor 1242	297	2	261	0.30	4.7	203
	Aroclor 1254	297	2	261	2.7	43.0	0
(Jensen et al. 1977)	Clophen A50/A60	66	11	3300	48	765.2	0
(Bleavins, Aulerich and Ringer 1980)	Aroclor 1242	247	5	938	1.1	17.5	0
	Aroclor 1242	247	10	1875	2.2	35.1	0
	Aroclor 1242	192	20	3750	4.3	68.6	0
	Aroclor 1242	138	40	7500	8.6	137.1	0
(Den Boer 1984)	Clophen A60	400	0.25	25	0.6	9.6	99
	Colphen A60	35	60.8	6076	93	1482.6	0
	Clophen A60	51	20.3	2025	37	589.9	0
	Clophen A30	51	60.8	6076	6.3	100.4	0
	Clophen A30	51	20.3	2025	2.1	33.5	0
(Aulerich et al. 1985)	Aroclor 1254	102	2.5	307	3.0	47.8	0
(Wren et al. 1987)	Aroclor 1254	185	1	180	1.9	30.3	111
(Kihlstrom et al. 1992)	Clophen A50	88	11.8	2094	20	318.8	0
	Aroclor 1254	94	9.65	1308	13	207.3	0
(Brunstrom et al. 2001)	Clophen A50	550	0.77	81	0.84	13.4	57
	Clophen A50	550	2.31	267	2.8	44.6	0
(Kakela et al. 2002)	Aroclor 1242	147	2.88	826	0.95	15.1	73

(Bursian et al. 2006a)	Carp (low)	120	0.83	86	0.86	13.7	97
	Carp (medium)	120	1.1	115	0.97	15.5	113
	Carp (high)	120	1.7	177	1.5	23.9	114
(Aulerich and Ringer 1977)	Aroclor 1016	297	2	261	0.19	3.0	89
(Bleavins, Aulerich and Ringer 1980)	Aroclor 1016	247	20	3750	2.8	44.6	41
(Aulerich et al. 1985)	PCB 169	102	0.1	14	0.42	6.7	0
	PCB 169	72	0.5	78	2.1	33.5	0
(Kihlstrom et al. 1992)	Synthetic non-ortho PCBs	88	0.009	1.4	6.2	98.8	92

REFERENCES

Ajelli, M, Merler S. 2009. An individual-based model of hepatitis A transmission. J Theor Biol 259:478-488.

Arnold, DL, Bryce F, Miller D, Stapley R, Malcolm S, Hayward S. 1998a. The toxicological effects following the ingestion of Chinook salmon from the Great Lakes by Sprague-Dawley rats during a two-generation feeding-reproduction study. Regul Toxicol Pharm 27:S18-S27.

Arnold, DL, Nera EA, Stapley R, Bryce F, Fernie S, Tolnai G, Miller D, Hayward S, Campbell JS, Greer I. 1997. Toxicological consequences of aroclor 1254 ingestion by female rhesus (*Macaca mulatta*) monkeys and their nursing infants. Part 3: Post-reproduction and pathological findings. Food and Chemical Toxicology 35:1191-+.

Arnold, DL, Stapley R, Bryce F, Mahon D. 1998b. A multigeneration study to ascertain the toxicological effects of Great Lakes salmon fed to rats: Study overview and design. Regul Toxicol Pharm 27:S1-S7.

Aulerich, RJ, Bursian SJ, Breslin WJ, Olson BA, Ringer RK. 1985. Toxicological Manifestations of 2,4,5-Hexachlorobiphenyl, 2',4',5'-Hexachlorobiphenyl, 2,3,6,2',3',6'-Hexachlorobiphenyl, and 3,4,5,3',4',5'-Hexachlorobiphenyl and Aroclor 1254 in Mink. Journal of Toxicology and Environmental Health 15:63-79.

Aulerich, RJ, Ringer RK. 1977. Current Status of Pcb Toxicity to Mink, and Effect on Their Reproduction. Arch. Environ. Contam. Toxicol. 6:279-292.

Barlow, J, Clapham PJ. 1997. A new birth-interval approach to estimating demographic parameters of humpback whales. Ecology 78:535-546.

Barsotti, DA, Marlar RJ, Allen JR. 1976. Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). Food and Cosmetics Toxicology 14:99-103.

Bleavins, MR, Aulerich RJ, Ringer RK. 1980. Polychlorinated-Biphenyls (Aroclor-1016 and Aroclor-1242) - Effects on Survival and Reproduction in Mink and Ferrets. Arch. Environ. Contam. Toxicol. 9:627-635.

Brunstrom, B, Kihlstrom I, Lundkvist U. 1982. Studies of Fetal Death and Fetal Weight in Guinea-Pigs Fed Polychlorinated-Biphenyls (PCB). Acta Pharmacol Tox 50:100-103.

Brunstrom, B, Lund BO, Bergman A, Asplund L, Athanassiadis I, Athanasiadou M, Jensen S, Orberg J. 2001. Reproductive toxicity in mink (*Mustela vison*) chronically exposed to environmentally relevant polychlorinated biphenyl concentrations. Environ Toxicol Chem 20:2318-2327.

Bursian, SJ, Beckett KJ, Yamini B, Martin PA, Kannan K, Shields KL, Mohr FC. 2006a. Assessment of effects in mink caused by consumption of carp collected from the Saginaw River, Michigan, USA. Arch Environ Contam Toxicol 50:614-623.

Bursian, SJ, Sharma C, Aulerich RJ, Yamini B, Mitchell RR, Beckett KJ, Orazio CE, Moore D, Svirsky S, Tillitt DE. 2006b. Dietary exposure of mink (*Mustela vison*) to fish from the Housatonic River, berkshire County, Massachusetts, USA: effects on organ weights and histology and hepatic concentrations of polychlorinated biphenyls and 2,3,7,8-tetrachlorodibenzo-P-dioxin toxic equivalence. Environ Toxicol Chem 25:1541-1550.

Cocchi, D, Tulipano G, Colciago A, et al. 2009. Chronic treatment with polychlorinated biphenyls (PCB) during pregnancy and lactation in the rat Part 1: Effects on somatic growth, growth hormone-axis activity and bone mass in the offspring. Toxicol. Appl. Pharmacol. 237:127-136.

Cockcroft, VG, De Kock AC, Lord DA, Ross GJB. 1989. Organochlorines in bottlenose dolphins (*Tursiops truncatus*) from the East coast of South Africa. South African Journal of Marine Science 8:207-217.

Den Boer, MH. 1984. Reproduction decline of harbor seals: PCBs in the food and their effect on mink. Annual Report. Leersum, The Netherlands: Rijksinstiuut voor Natuurbeheer. p. 77-86.

Elfes, CT, VanBlaricom GR, Boyd D, et al. 2010. Geographic Variation of Persistent Organic Pollutant Levels in Humpback Whale (*Megaptera novaeangliae*) Feeding Areas of the North Pacific and North Atlantic. Environ. Toxicol. Chem. 29:824-834.

Feeley, MM, Jordan SA. 1998. Dietary and tissue residue analysis and contaminant intake estimations in rats consuming diets composed of Great Lakes salmon: A multigeneration study. Regul Toxicol Pharm 27:S8-S17.

Fuchsman, PC, Barber TR, Bock MJ. 2008. Effectiveness of various exposure metrics in defining dose-response relationships for mink (Mustela vison) exposed to polychlorinated biphenyls. Arch. Environ. Contam. Toxicol. 54:130-144.

Gaba, S, Cabaret J, Sauve C, Cortet J, Silvestre A. 2010. Experimental and modeling approaches to evaluate different aspects of the efficacy of Targeted Selective Treatment of anthelmintics against sheep parasite nematodes. Vet Parasitol 171:254-262.

Gauthier, JM, Metcalfe CD, Sears R. 1997. Chlorinated organic contaminants in blubber biopsies from northwestern Atlantic balaenopterid whales summering in the Gulf of St Lawrence. Marine Environmental Research 44:201-223.

Hall, AJ, McConnell BJ, Rowles TK, Aguilar A, Borrell A, Schwacke L, Reijnders PJH, Wells RS. 2006. Population consequences of polychlorinated biphenyl exposure in bottlenose dolphins - an individual based model approach. Environ. Health Perspect. 114, Suppl. 1:60-64.

Heaton, SN, Bursian SJ, Giesy JP, Tillitt DE, Render JA, Jones PD, Verbrugge DA, Kubiak TJ, Aulerich RJ. 1995. Dietary Exposure of Mink to Carp from Saginaw Bay, Michigan .2. Hematology and Liver Pathology. Arch. Environ. Contam. Toxicol. 29:411-417.

Jensen, S, Kihlstrom JE, Olsson M, Lundberg C, Orberg J. 1977. Effects of PCB and DDT on Mink (*Mustela vison*) during the reproductive season. Ambio 6:239.

Kakela, A, Kakela R, Hyvarinen H, Asikainen J. 2002. Vitamins A1 and A2 in hepatic tissue and subcellular fractions in mink feeding on fish-based diets and exposed to Aroclor 1242. Environ Toxicol Chem 21:397-403.

Kihlstrom, JK, Olsson M, Jensen S, Johansson A, Ahlbom J, Bergman A. 1992. Effects of PCB and different fractions of PCB on the reproduction of the mink. Ambio 21:563-569.

Leonards, PEG, Devries TH, Minnaard W, Stuijfzand S, Devoogt P, Cofino WP, Vanstraalen NM, Vanhattum B. 1995. Assessment of Experimental-Data on Pcb-Induced Reproduction Inhibition in Mink, Based on an Isomer-Specific and Congener-Specific Approach Using 2,3,7,8-Tetrachlorodibenzo-P-Dioxin Toxic Equivalency. Environ. Toxicol. Chem. 14:639-652.

Murphy, CA, Rose KA, Alvarez Mdel C, Fuiman LA. 2008. Modeling larval fish behavior: scaling the sublethal effects of methylmercury to population-relevant endpoints. Aquat Toxicol 86:470-484.

Oftedal, OT. 1997. Lactation in whales and dolphins: evidence of divergence between baleen- and toothed-species. J Mammary Gland Biol Neoplasia 2:205-230.

Platonow, NS, Karstad LH. 1973. Dietary effects of polychlorinated biphenyls on mink. Can J Comp Med 37:391-400.

R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing.

Reddy, ML, Reif JS, Bachand A, Ridgway SH. 2001. Opportunities for using Navy marine mammals to explore associations between organochlorine contaminants and unfavorable effects on reproduction. Sci Total Environ 274:171-182.

Restum, JC, Bursian SJ, Giesy JP, Render JA, Helferich WC, Shipp EB, Verbrugge DA, Aulerich RJ. 1998. Multigenerational study of the effects of consumption of PCB-contaminated carp from Saginaw Bay, Lake Huron, on mink. 1. Effects on mink reproduction, kit growth and survival, and selected biological parameters. J. Toxicol. Env. Health Part A 54:343-375.

Salata, GG, Wade TL, Sericano JL, Davis JW, Brooks JM. 1995. Analysis of Gulf-of-Mexico Bottle-Nosed Dolphins for Organochlorine Pesticides and Pcbs. Environ. Pollut. 88:167-175.

Schwacke, LH, Voit EO, Hansen LJ, Wells RS, Mitchum GB, Hohn AA, Fair PA. 2002. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on bottlenose dolphins (*Tursiops truncatus*) from the southeast United States coast. Environ. Toxicol. Chem. 21:2752-2764.

Tanabe, S, Tatsukawa R, Maruyama K, Miyazaki N. 1982. Transplacental transfer of PCBs and chlorinated hydrocarbon pesticides from the pregnant striped dolphin *Stenella coeruleoalba* to her fetus. Agr Biol Chem Tokyo 46:1249-1254.

Taruski, AG, Olney CE, Winn HE. 1975. Chlorinated Hydrocarbons in Cetaceans. J Fish Res Board Can 32:2205-2209.

Voltura, MB, French JB. 2007. Effects of dietary PCB exposure on reproduction in the white-footed mouse (*Peromyscus leucopus*). Arch. Environ. Contam. Toxicol. 52:264-269.

Wells, RS, Scott MD. 1990. Estimating bottlenose dolphin population parameters from individual identification and capture-release techniques. In: PS Hammond, SA Mizroch, GP Donovan, editors. Individual Recognition of Cetaceans: Use of Photo-identification and other techniques to estimate population parameters. Cambridge, UK: International Whaling Commission. p. 440.

Wells, RS, Tornero V, Borrell A, Aguilar A, Rowles TK, Rhinehart HL, Hofmann S, Jarman WM, Hohn AA, Sweeney JC. 2005. Integrating potential life-history and reproductive success data to examine relationships with organochlorine compounds for bottlenose dolphins (*Tursiops truncatus*) in Sarasota Bay, Florida. Sci. Total Environ. 349:106-119.

Wren, CD, Hunter DB, Leatherland JF, Stokes PM. 1987. The Effects of Polychlorinated-Biphenyls and Methylmercury, Singly and in Combination, on Mink .1. Uptake and Toxic Responses. Arch. Environ. Contam. Toxicol. 16:441-447.

Zerbini, AN, Clapham PJ, Wade PR. 2010. Assessing plausible rates of population growth in humpback whales from life-history data. Marine Biology 157:1225-1236.

Fig. 1. Flow Diagram of Individual Based Model to assess Population Consequences of Pollutant Exposure
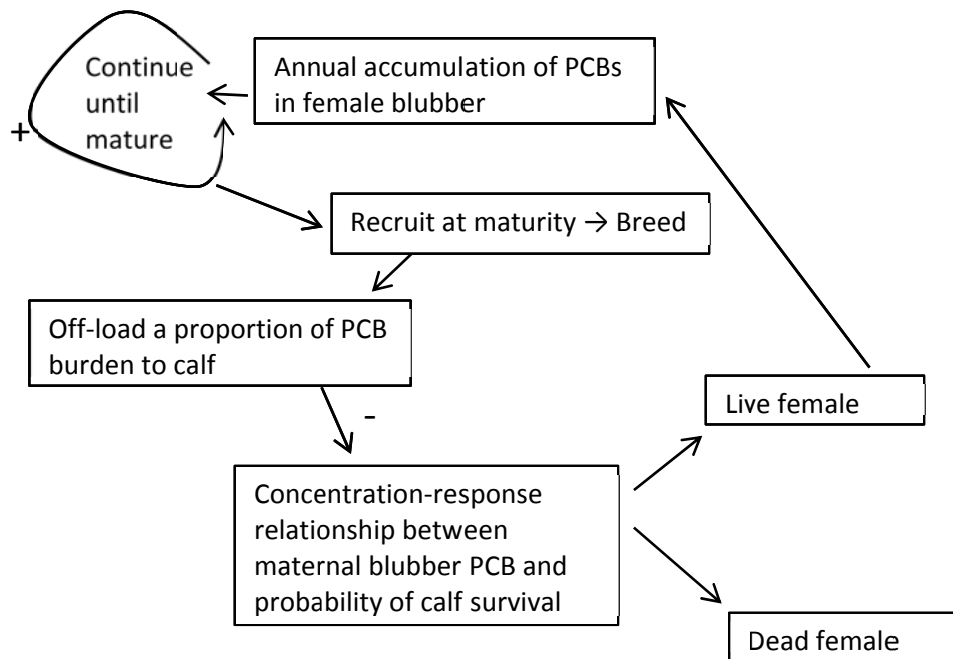

Fig. 2. Logistic regression model predicting probability of 6-month bottlenose dolphin calf survival in relation to maternal PCB concentration. Black lines show 500 resampled regression models.
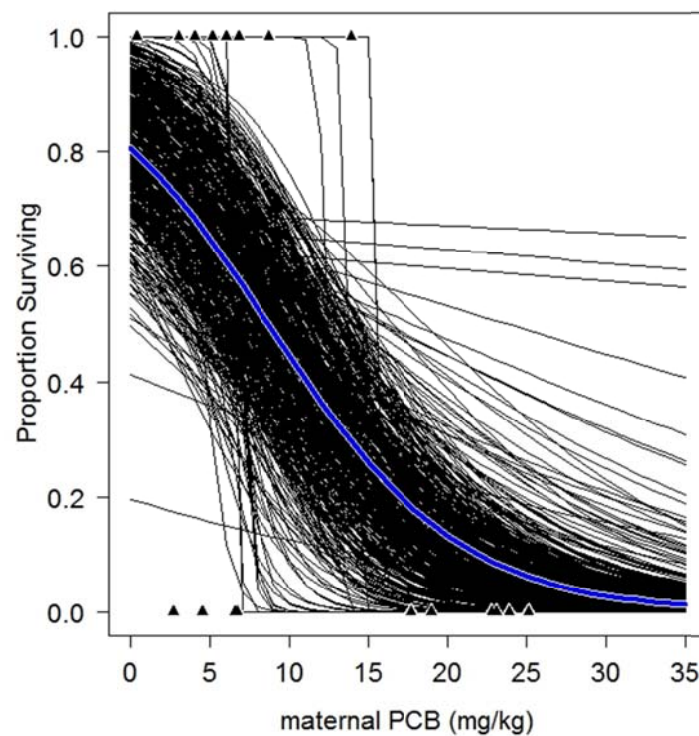
Fig 3. Relationship between PCB exposure as $\log_e$(whole-body PCBs mg/kg) and production of surviving kits per mated female in mink from (Fuchsman, Barber and Bock 2008)
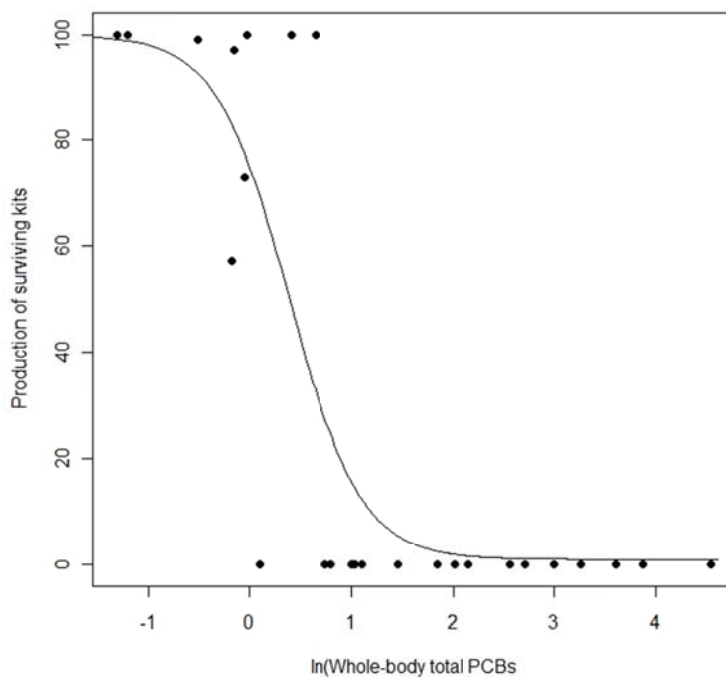

Fig 4. Relationship between whole body total PCBs and PCB tissue concentrations in mink from (Fuchsman, Barber and Bock 2008)
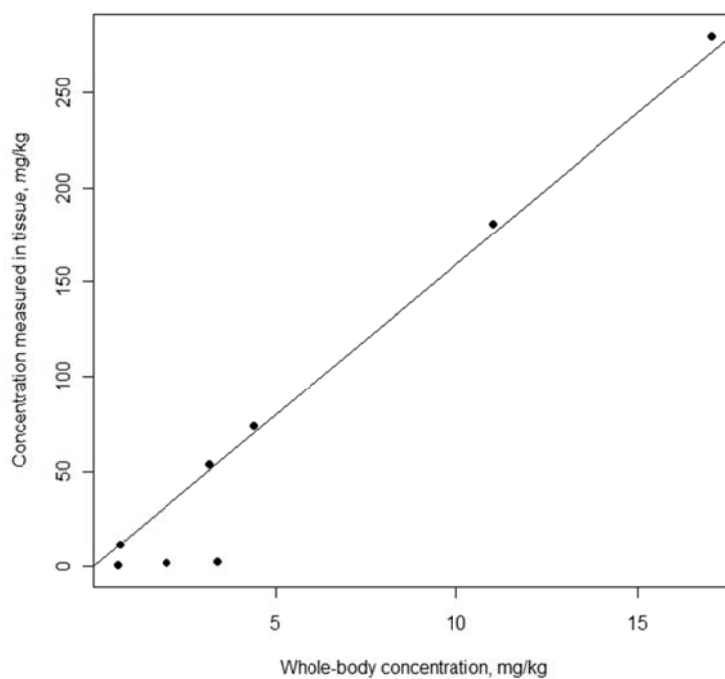
Fig. 5 (a) Relationship between PCB exposure as $\log_e$(estimated tissue concentration) PCBs and production of surviving kits per mated female in mink from (Fuchsman, Barber and Bock 2008) (b) showing 500 resampled non-linear regression models.
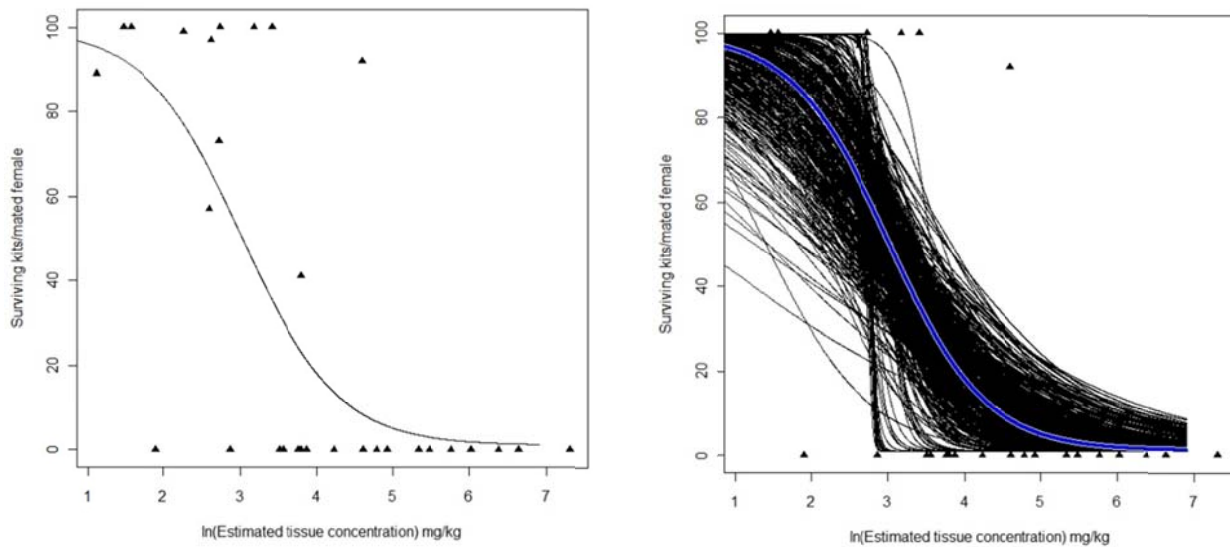
Fig. 6. Logistic regression model predicting probability of kit survival in relation to maternal PCB concentration using a subset of the mink studies. Black lines show 500 resampled regression models.
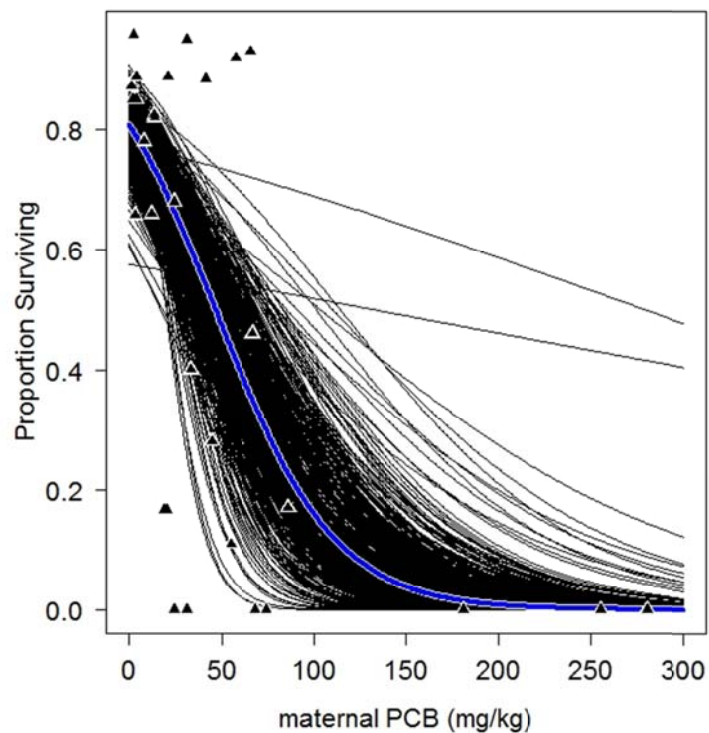
Fig. 7. Potential population growth in Bottlenose Dolphin simulated population with an annual accumulation of 2.96 mg/kg/year and using the Fuchsman et al (2008) mink concentration response relationship (a) with uncertainty (b) without uncertainty
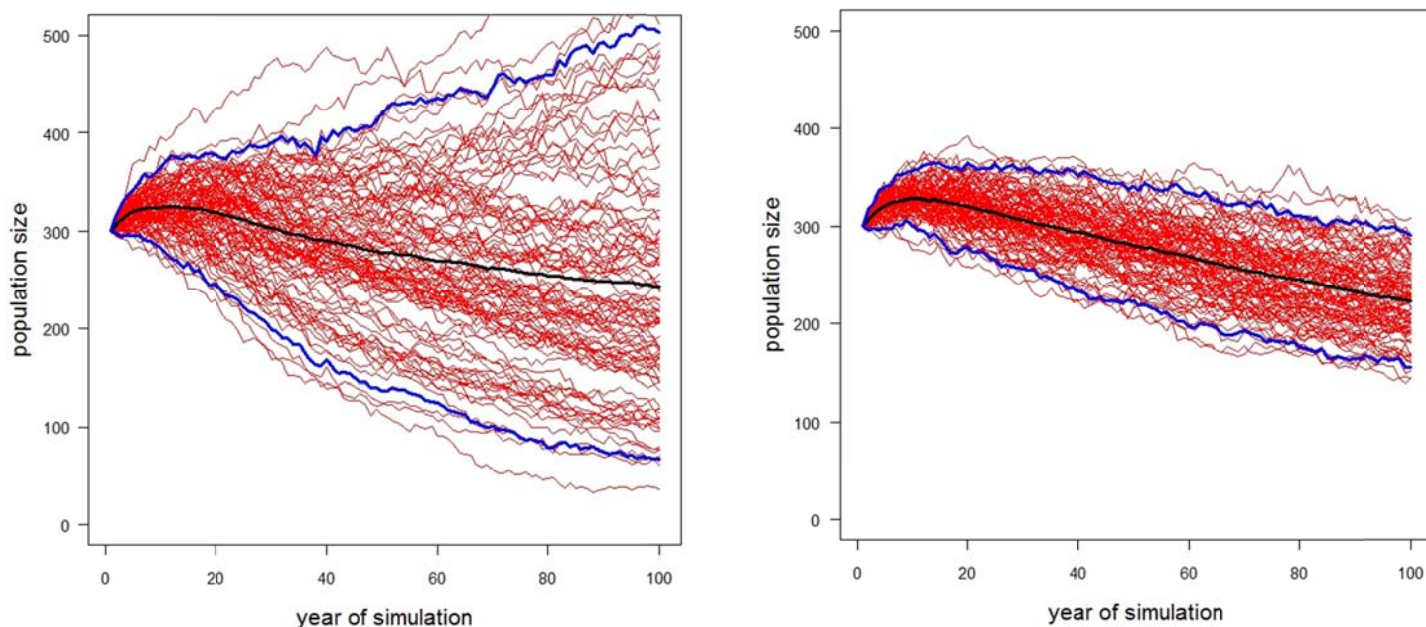

Fig. 8. Change in potential population growth rates in Bottlenose dolphins in relation to different annual accumulation rates of PCBs using Fuchsman et al., (2008) mink concentration-response relationship. Red lines indicate 95% CI with uncertainty, blue lines 95% CI without uncertainty.
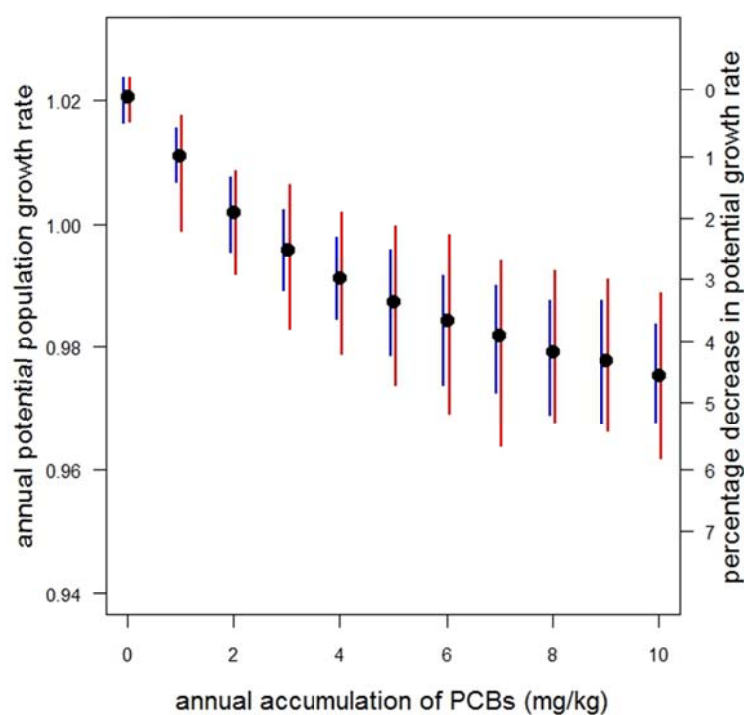
Fig. 9. Change in potential population growth rates in Bottlenose dolphins in relation to different annual accumulation rates of PCBs using subset mink concentration-response relationship. Red lines indicate 95% CI with uncertainty, blue lines 95% CI without uncertainty
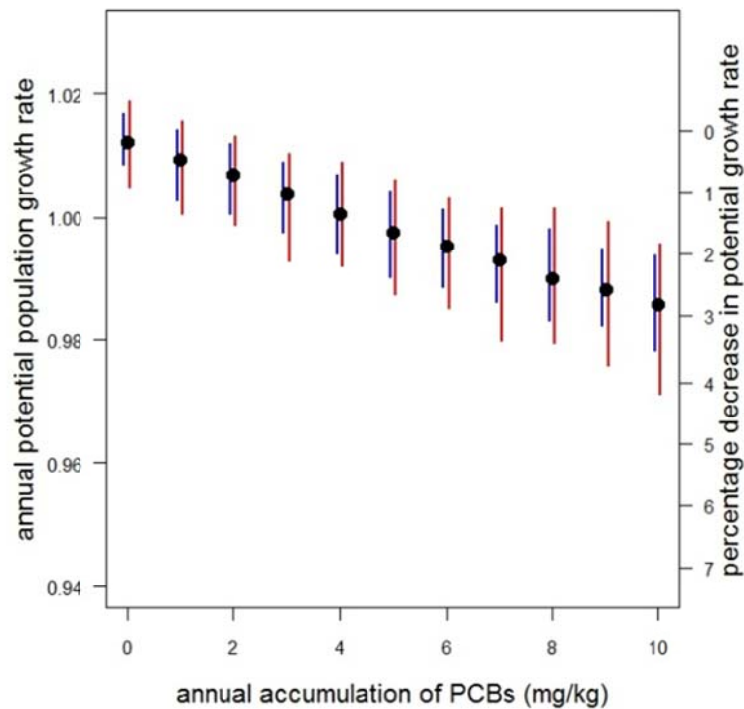

Fig. 10. Change in potential population growth rates in Humpback whales in relation to different annual accumulation rates of PCBs using the Fuchsman et al. (2008) mink concentration-response relationship. Red lines indicate 95% CI with uncertainty, blue lines 95% CI without uncertainty
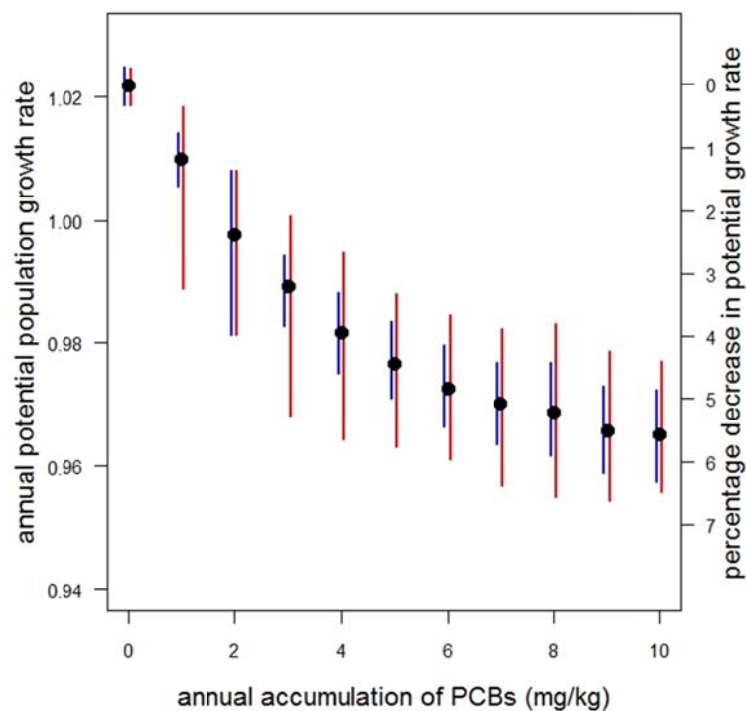
Fig. 11. Change in potential population growth rates in Humpback whales in relation to different annual accumulation rates of PCBs using the subset mink concentration-response relationship. Red lines indicate 95% CI with uncertainty, blue lines 95% CI without uncertainty