1 Predicting the effects of polychlorinated biphenyls on cetacean populations through impacts
2 on immunity and calf survival.

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Abstract

The potential impact of exposure to polychlorinated biphenyls (PCBs) on the health and survival of cetaceans continues to be an issue for the conservation and management, yet few quantitative approaches for estimating population level effects have been developed. An individual based model (IBM) for assessing effects on both calf survival and immunity was developed and tested. Three case study species (bottlenose dolphin, humpback whale and killer whale) in four populations were taken as examples and the impact of varying levels of PCB uptake on achievable population growth was assessed. The unique aspect of the model is its ability to evaluate likely effects of immunosuppression in addition to calf survival, enabling consequences of PCB exposure on immune function on all age-classes to be explored. By incorporating quantitative tissue concentration-response functions from laboratory animal model species into an IBM framework, population trajectories were generated. Model outputs included estimated concentrations of PCBs in the blubber of females by age, which were then compared to published empirical data. Achievable population growth rates were more affected by the inclusion of effects of PCBs on immunity than on calf survival, but the magnitude depended on the virulence of any subsequent encounter with a pathogen and the proportion of the population exposed. Since the starting population parameters were from historic studies, which may already be impacted by PCBs, the results should be interpreted on a relative rather than an absolute basis. The framework will assist in providing quantitative risk assessments for populations of concern.

Keywords: Individual based model, risk assessment, marine mammal, contaminants

Capsule

Current exposure levels of particular cetaceans to PCBs may significantly affect their population growth rates, through effects on immunity as well as calf survival.

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Introduction

Polychlorinated biphenyls (PCBs) are ubiquitous persistent organic pollutants that biomagnify through the food chain, resulting in high concentrations in the blubber of marine mammals, particularly piscivorous cetaceans (Jepson et al., 2016; Yordy et al., 2010b). These compounds are known to cause a range of adverse health effects that are likely to have consequences for cetacean abundance (Hall et al., 2006a; Kannan et al., 2000; Schwacke et al., 2012) through impacts on reproduction and survival. Often organic pollutants are only one of many anthropogenic stressors facing endangered wildlife populations (Côté et al., 2016). For example, three anthropogenic threats – namely prey limitation, noise and disturbance from vessels and chemical contaminants – have been identified as factors in the at-risk status of resident, fish-eating killer whales (*Orcinus orca*) in the northeastern Pacific Ocean (Canada, 2011; Krahn et al., 2004). The effects of prey limitation on survival and reproduction have been quantitatively assessed (Ford et al., 2010) but pollutants have only been treated in a qualitative way in conservation and management plans, thereby making it difficult to rank threats.

An individual-based model (IBM) framework was developed (Hall et al., 2006b) to simulate the impact of PCBs on the achievable growth rate (λ) of cetacean populations over a number of decades. Density dependence is not included in the model so the comparisons made are on a relative achievable population growth rate basis rather than an absolute basis. IBM approaches have been used to assess the population consequences of other harmful agents, including pathogens and parasites, as well as pollutants, for terrestrial and fish species (Ajelli and Merler, 2009; Gaba et al., 2010; Murphy et al., 2008). An initial framework was previously developed which modelled the effects of maternal PCBs on calf survival probability (Hall et al., 2006b), an exposure pathway that remains of concern. In certain cetacean populations, where have females with high concentrations of PCBs in their blubber, there continues to be an association between low-recruitment and declining abundance (Jepson et al., 2016), consistent with uptake affecting reproduction. However, adverse effects of PCBs on the immune system are also well-established and are of particular concern for marine mammals (De Guise et al., 1995; Ross et al., 1996). A number of disease epidemics, primarily involving morbillivirus, have led to large-scale mortalities in marine mammal populations over the past several decades (Van Bressem et al., 2014). The magnitude of these events has raised questions as to whether PCBs or other pollutants could be increasing the impact of natural infections by suppressing immune function and decreasing host resistance thus decreasing the probability of survival (Ross et al., 1996).

In the current study, the tissue concentration-response function for calf survival from the initial IBM framework was expanded to also include tissue concentration-response functions for the effects on immunity. This approach was chosen as empirical exposure data for these species is generally only available as levels of PCBs in blubber samples (Balmer et al., 2011). The approach taken here does not explicitly model the toxicokinetics of PCBs in cetaceans which has been carried out in a number of previous studies (Hickie et al., 2000; Hickie et al., 2013; Hickie et al., 1999; Weijs et al. 2013). Often the diet composition and consumption rate of prey for the cetaceans of interest is unknown and whilst including a bioenergetics and toxicokinetic model into the IBM might be desirable, empirical data for model comparison in cetaceans over time is generally only available as blubber concentrations (Law, 2014). Thus the starting point here is taken as the PCBs assimilated into the blubber as an indication of exposure, using the tissue concentration-response

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90   functions available for model species (Fuchsman et al., 2008), rather than the ingested
91   dose-response functions, to estimate the impact of PCBs on cetacean calf survival and
92   immunity.

93   The model was applied to three cetacean species and four populations as examples;
94   bottlenose dolphins (*Tursiops truncatus*), two populations of killer whales and humpback
95   whales (*Megaptera novaeangliae*). The additional complexity and originality in this approach
96   was to include PCB effects on immune status. However, for such effects to be evaluated at
97   the population level, the model must allow for animals to be subsequently exposed to an
98   infection with an associated survival probability. The population consequences of varying
99   the proportion of the population that encounter a novel infectious pathogen each year was
100 explored. This was achieved by integrating the relationship between an *in vitro* immune
101 function assay, T lymphocyte proliferation in response to concanavalin A (Con A)
102 stimulation, and exposure to PCBs in bottlenose dolphins from field studies (Schwacke et al.,
103 2012) with the results of the U.S. National Toxicology Program studies (Luster et al., 1993)
104 that quantified the link between this immune assay and host resistance in mice. This
105 improved the reality of the model whilst also capturing the level of uncertainty around the
106 resulting population trajectories.

107 PCB concentrations in the blubber of bottlenose dolphins are among some of the highest
108 concentrations reported in wildlife globally (Balmer et al., 2011; Hansen et al., 2004; Pulster
109 et al., 2009; Fair et al., 2010; Schwacke et al., 2012), and studies in this species have
110 documented adverse health effects in association with high PCB uptake. For example,
111 samples of blubber from free-ranging dolphins along the southern coast of Georgia, on the
112 east coast of the US, had concentrations up to 2900 mg/kg lipid (Balmer et al., 2011; Pulster
113 and Maruya, 2008). Health evaluations among free-swimming captured and released
114 dolphins in this region found that thyroid hormone levels (hypothyroidism) were significantly
115 negatively correlated with increased blubber PCB concentrations (Schwacke et al., 2012)
116 and that T-lymphocyte proliferation and indices of innate immunity were also significantly
117 negatively correlated (Schwacke et al., 2012). Based on their study findings, the authors
118 concluded that bottlenose dolphins are vulnerable to PCB-related toxic effects mediated
119 through the endocrine system. This is in contrast to other populations, such as those
120 inhabiting Sarasota Bay and the Indian River Lagoon, Florida that have much lower PCB
121 levels in their blubber (mean total PCBs in males ~70 - 80 mg/kg lipid as compared to 170
122 and 450 mg/kg lipid from two sites along the southern Georgia coast, (Fair et al., 2010;
123 Kucklick et al., 2011; Schwacke et al., 2014; Wells et al., 2005)).

124 Killer whales can also be significantly exposed to PCBs and concentrations of approximately
125 400 mg/kg lipid have been reported in blubber samples from animals in Japanese waters
126 (Ono et al., 1987) and the west coast of the North America (Hayteas and Duffield, 2000)
127 since the late 1980s. During this same time frame, high mean PCB concentrations (> 250
128 mg/kg lipid) were also reported in the blubber of transient male killer whales from British
129 Columbia (Ross et al., 2000) and the west coast of the U.S. (Krahn et al., 2007b) and
130 transient females from British Columbia had mean levels exceeding 50 mg/kg lipid (Ross et
131 al., 2000). These concentrations are above estimated thresholds for endocrine disruption,
132 effects on reproduction and immunity in cetaceans (~17-20 mg/kg lipid) (Hickie et al., 2013;
133 Kannan et al., 2000). Transient killer whales feed on marine mammals (Baird and Dill,
134 1995), unlike the fish-eating resident killer whales, and the higher trophic level of the
135 transient population would help to explain these very high levels. In contrast, large mysticete

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136 cetaceans such as humpback whales, have lower blubber PCB concentrations (2-4 mg/kg
137 lipid) (Elfes et al., 2010; Metcalfe et al., 2004), as they feed at a lower trophic level, on
138 copepods (Simon et al., 2012), schooling fish and crustaceans (Witteveen et al., 2011).
139 Long term studies on the population dynamics of humpback whales in the Gulf of Maine
140 indicate that their abundance has been increasing since the 1980s (Robbins, 2007) and
141 combined with data on their blubber PCB concentrations, this population provided an
142 example of a species and population with lower exposure. Thus, these species were chosen
143 as examples for the model because not only do they have contrasting PCB concentrations in
144 their blubber and therefore different levels of exposure, but four populations also have
145 published population vital rates that could be used to parameterize the model.

146

147 **Methods**

148 *Model Structure*

149 The overall structure of the model is shown in Fig. 1. The model has been constructed using
150 the statistical and modelling package R (R Development Core Team, 2014) and it simulates
151 the fate of individual females using published fecundity and survival data for each cetacean
152 species to construct an initial, appropriately sized, population of animals with a stable age
153 structure. The population parameters used in a Leslie matrix model to construct these initial
154 populations for each species are given in Table 1. Since the model predicts what effects
155 PCBs may have on achievable population growth into the future, starting population
156 parameters were chosen using historical rather than current data. This allowed for the
157 model outputs and projections to be compared, as far as possible, with the dynamics of the
158 various populations in the intervening years. However, it should be noted that these
159 populations and vital rates may already have been influenced by exposure to PCBs which
160 were ubiquitous and maximal in the environment during the 1960s and 70s. So whilst the
161 parameters are not from populations in pristine environments, the aim here is to provide a
162 framework to investigate the impact of exposures across a continuum, starting at some point
163 in time, using reasonable values from the literature, in which the result of varying the annual
164 accumulation of PCBs into the blubber on potential population growth can be explored.

165 The model simulates the accumulation of PCBs through transplacental transfer, suckling and
166 prey ingestion, and the loss of PCBs from the mature females' blubber during gestation and
167 lactation. Maternal blubber PCB concentrations then affect the calf survival probability in a
168 dose-dependent matter. Additional exposure-response relationships are included to
169 simulate the impact of PCB uptake on immune function. The model is stochastic so that
170 each of the birth and survival outcomes are determined by whether a random number
171 (drawn from a uniform distribution) is less than or equal to the probability associated with
172 that event.

173 Each animal is assigned a state variable of 1 (alive), or 0 (dead), an age and blubber PCB
174 concentration (mg/kg lipid). The model is a post-breeding census and age class 1 is
175 equivalent to newborn calves. Each model simulation spans a period of 100 years and a
176 starting abundance is based on the specific populations being simulated. For any given set
177 of fecundity or survivorship values, the stable age structure is calculated by multiplying a
178 random seed age structure by the appropriate Leslie matrix 100 times. Fecundity here also
179 accounts for differences in calving intervals between the different species. The stable age

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180   structure is used as the underlying population structure of the initial population of n females
181   that is then projected forward in the simulations. At first, each animal is assigned zero PCB
182   level and after the first year, animals are then allocated an appropriate blubber PCB
183   concentration depending on their age class and reproductive status (i.e. calves, juveniles
184   and adults, Wells et al., 2005; Ross et al., 2000; Metcalf et al., 2004) following a simulation
185   run-in. A plausible range of annual accumulation of PCBs into the blubber is chosen, which
186   includes uptake from contaminated prey. This is combined with the concentrations obtained
187   through maternal legacy (*in utero* and lactational transfer). The annual accumulations ranged
188   from 1 to 5 mg/kg lipid and the different achievable population growth rates from each set of
189   simulations were compared. Whilst these accumulation rates are not equivalent to PCB
190   ingestion rates (Hickie et al., 2013), the resulting concentrations in the blubber of the
191   females from the model outputs can be compared to the empirical data. The annual
192   accumulation concentrations are arbitrarily chosen, however additional information on the
193   slope of the linear relationship between blubber PCB concentrations and age in males gives
194   some indication of the annual accumulation for a given population (since unlike females,
195   males do not depurate PCBs through gestation and lactation processes and show a general
196   increase in blubber concentrations with age (Wells et al., 2005; Ross et al., 2000)). These
197   age-specific male data provide annual accumulation rates that implicitly include metabolism
198   and excretion, as the blubber concentrations include these processes since they are only
199   what ends up stored in the blubber. Whilst this is a simplification of the variation in
200   concentrations that could occur in an individual during a year, for the purposes of this
201   blubber-based model they are indicative of the general pattern of blubber PCB
202   concentrations that are seen in the empirical data. The aim of this model framework is to
203   allow researchers and conservation managers to investigate the impact of variation in the
204   annual accumulation rate, indicative of PCB exposure, for the different cetacean species.
205   Thus, for comparative purposes each accumulation rate (from 1 to 5 mg/kg lipid) was
206   investigated for each case study and the model outputs (population growth and age-specific
207   female blubber concentrations) were compared with empirical data (historical or current).

208   The model is a female-only individual based population model. When females reach sexual
209   maturity they become pregnant with a certain probability then during gestation and lactation
210   offload a proportion of their blubber PCB to the calf (Tanabe et al., 1982). The probability of
211   survival of the offspring is modified by a tissue concentration-response function relating
212   maternal PCB to offspring survival estimates. The variation in achievable population growth
213   rate with varying annual PCB accumulation rates can then be investigated, incorporating
214   uncertainty from the tissue concentration-response relationships. For each 100-year
215   simulation, this is achieved by the model choosing random tissue concentration-response
216   model coefficients from a set of 500 coefficients generated by data resampling. Juvenile and
217   adult survival are then also modified using the blubber PCB immune suppression tissue
218   concentration-response function following exposure of a specified proportion of the
219   population to a pathogen.

220   After approximately the 40th simulation year, the effect of the PCB concentrations on
221   achievable population growth stabilises. From the population trajectories after the first 40
222   years, the mean achievable growth rate is calculated, and the 2.5 and 97.5 percentiles are
223   estimated from the ranked individual simulation growth rates.

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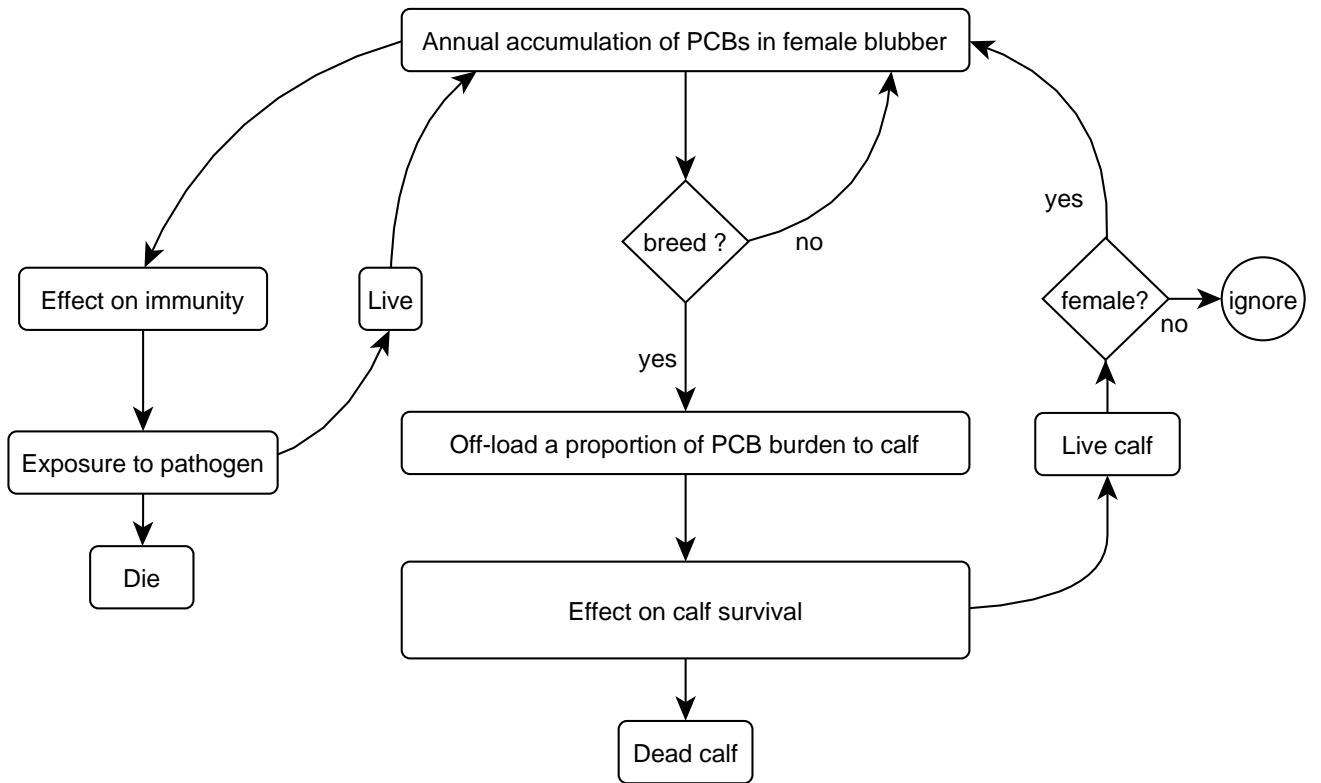
Fig. 1. Schematic diagram of individual based model to estimate impact of PCB exposure on cetacean population growth.

Tissue concentration-response relationship for maternal PCB concentration and calf survival
probability

230 It has been well demonstrated in a number of laboratory animal models that PCB exposure
231 can, in addition to other effects, reduce offspring early survival probability (Barsotti et al.,
232 1976; Kihlstrom et al., 1992). The studies carried out on mink provided data for the tissue
233 concentration-response relationship used in the first probabilistic risk assessment study into
234 the effects of PCBs on bottlenose dolphin populations published by Schwacke et al. (2002).
235 More recently Folland et al. (2016) also used mink as an appropriate model for cetaceans
236 due to the logistical constraints posed by using homologous species and the fact that
237 genomically mink are more closely related to marine mammals than rodents and they
238 occupy upper aquatic trophic levels. Further considerations in using the surrogate mink data
239 are also given in the Discussion. Fuchsman et al. (2008) reported a comprehensive
240 quantitative analysis of published results of PCB effects on mink reproduction. A subset of
241 six studies where concentrations of total PCBs in the maternal tissues and details of off
242 spring survival were listed (Bursian et al., 2006; Heaton et al., 1995; Jensen et al., 1977;
243 Kihlstrom et al., 1992; Platonow and Karstad, 1973; Restum et al., 1998). These raw data
244 produced the tissue concentration-response relationship shown in Fig. 2. A generalized
245 linear quasibinomial model with a logit link function, weighted by the number of animals in
246 each study, was fitted to the data. The uncertainty around the relationship was again
247 estimated using resampling with replacement (n=500, also shown in Fig 2). The resulting
248 EC50 from the best fit relationship was 46.5, SE 8.8 mg/kg.
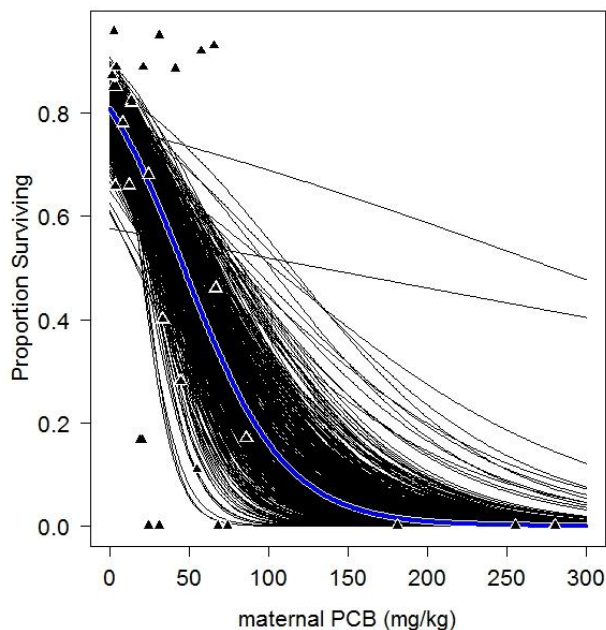

249 Fig. 2.   Logistic regression model predicting probability of kit survival in relation to maternal
250 blubber PCB concentration using a subset of the mink studies. The triangles represent the
251 data points from the six individual published studies (Barsotti et al., 1976; Fuchsman et al.,
252 2008; Heaton et al., 1995; Jensen et al., 1977; Platonow and Karstad, 1973; Restum et al.,
253 1998), black lines show 500 resampled regression models and the blue line shows the best
254 fit.

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Tissue concentration-response relationship between blubber PCB concentration and T-lymphocyte proliferation (Con A response) in bottlenose dolphins

258 A two stage process was implemented whereby the functional response between the
259 proportional decrease in T-lymphocyte response to Con A stimulations and decrease in
260 survival (Luster et al., 1993) was combined with the function relating T-lymphocyte
261 proliferation response to Con A to blubber PCB concentrations from wild bottlenose dolphins
262 from several sites along the east coast of the US using the data from Schwacke et al. (2012)
263 The steps involved in this process are shown in Fig. 3.
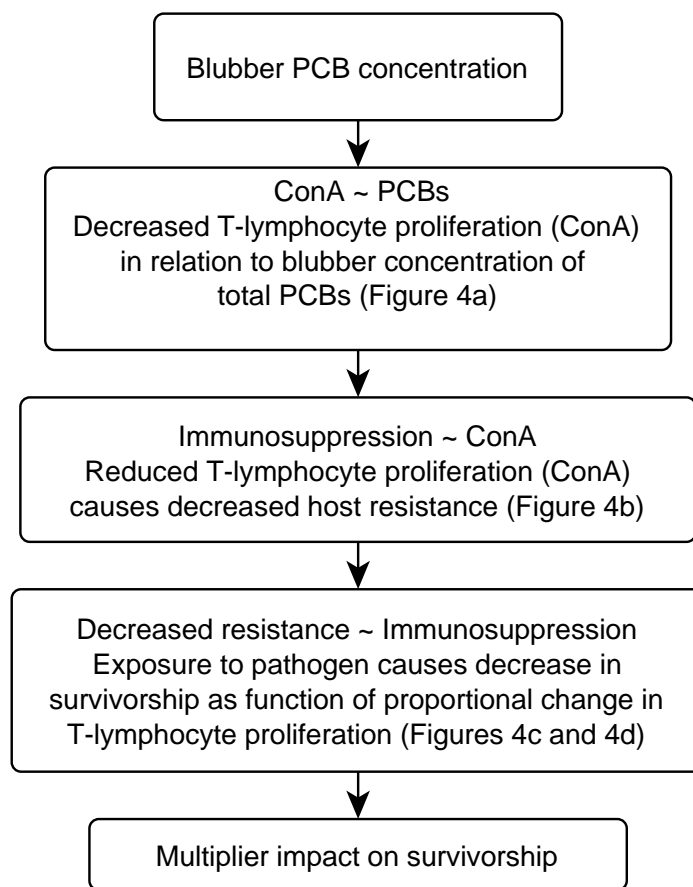
264

265 Fig. 3. Steps involved in estimating the expected change in survival probability in relation to
266 exposure to PCBs through immune suppression.
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268 In order to utilise the Luster et al. (1993) predictive relationships, data from Schwacke et al.
269 (2012) were converted to a *proportional* change in response to Con A in relation to an
270 estimated maximal response. Thus the "control" was taken as the T-lymphocyte response to
271 Con A at the intercept (Fig. 4a). This relationship was then converted to an estimate of
272 whole animal immunosuppression (Luster et al., 1993) (Fig. 4b). This was given in terms of
273 the dose of an immunosuppressant compound (cyclophosphamide) administered to the
274 animals. Both cyclophosphamide and PCBs act on T cells and while at high doses

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cyclophosphamide can completely eradicate haematopoietic cells, both compounds act on the same arms of the immune system (Harper et al.,1993; Ahlmann and Hempel 2016). The final step was to estimate a parameter that could be used in the model taking the previous relationship and converting it to a decrease in host resistance following exposure to a pathogen, either of low (Fig. 4c) or higher virulence (Fig. 4d) (Luster et al., 1993). These three steps resulted in a multiplier, which was used to modify the probability of survival – so a factor of 1 did not change the background survival probability even after exposure to a pathogen but a factor of 0.5 resulted in a halving of the survival probability. Figures 4e and 4f show the overall error associated with predicting the decrease in host resistance from PCBs in blubber (500 predictions were carried out for each PCB level) for low and high virulence pathogens.

The effect of exposure of either 5% or 20% of the population to a higher virulence, class II pathogen was assessed. It was assumed that a novel pathogen was introduced into the population, affecting the specified proportion of individuals each year. Such novel pathogens may have a dramatic effect on a naïve population, causing an epidemic in a single year and then fading from the population. An exploration of this effect on a slowly increasing cetacean population was also included.
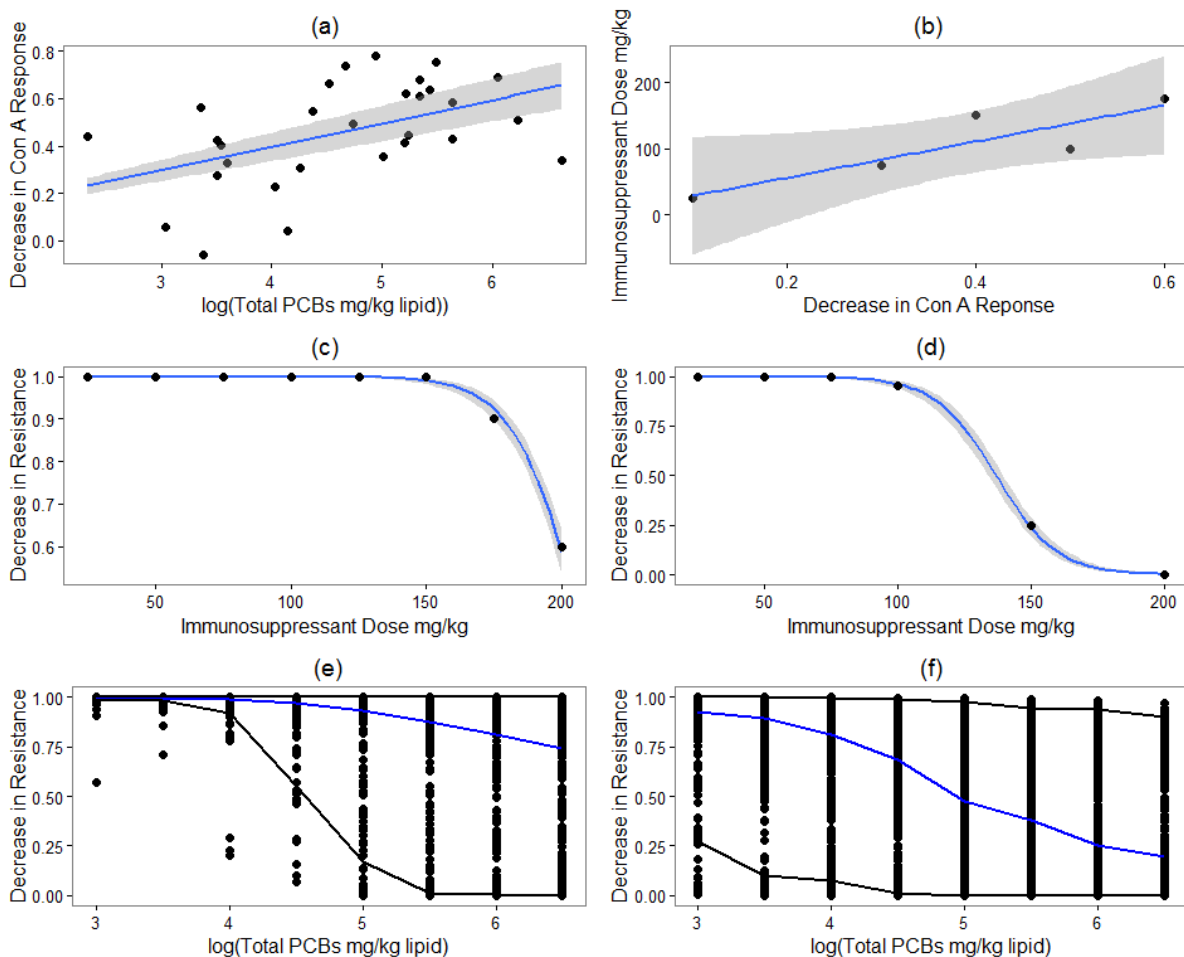
Fig. 4. (a) Relationship between change in T-Lymphocyte response to Con A and log blubber PCBs in bottlenose dolphins (Schwacke et al., 2012); (b) proportional decrease in Con A response in relation to immunosuppressant dose in mice (Luster et al., 1993); (c) decrease in host resistance (probability of survival) in relation to immunosuppressant dose

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297 for a pathogen with low virulence (Luster et al., 1993); (d) decrease in host resistance
298 (probability of survival) in relation to immunosuppressant dose for a pathogen with high
299 virulence (Luster et al., 1993); (e and f) the error associated with predicting decrease in host
300 resistance from blubber PCBs, low and high virulence pathogens respectively, black lines
301 connect the 95% intervals for each PCB level prediction. The blue line indicates the mean.

302 *Model parameters and case study populations*

303 The vital rates (fecundity and survival) and other explicit model parameters such as age at
304 first reproduction and maximum age class used in the Leslie matrices for the baseline
305 populations for the four case study species are given Table 1.

306 Table 1. Model parameters, including those used in a Leslie matrix model for a baseline
307 population with a stable age structure to then simulate effect of maternal PCB concentrations
308 on achievable population growth rate.

Parameter	Bottlenose Dolphin	Humpback Whale	Northern Resident Killer Whale	Southern Resident Killer Whale
Maximum age (years)	40	35	50	50
First year calf survival	0.811	0.875	0.97	0.97
Adult survival	0.962	0.960	0.999	0.990
Fecundity at sexual maturity	0.177	0.111 – 0.241, depending on age	0.200	0.180
Length of lactation (years)	2	1	2	2
Age at sexual maturity(years)	8	8	14	14
Population growth (baseline λ)	1.014	1.065	1.019	1.013
Starring population size	100	1000	200	100
Source Reference	Wells and Scott, 1990 (Wells and Scott, 1990)	Barlow and Clapham, 1997 Zerbini, Clapham and Wade 2010 (Barlow and Clapham, 1997; Zerbini et al., 2010)	Olesiuk et al 1990 (Olesiuk et al., 1990)	Olesiuk et al 1990 (Olesiuk et al., 1990)

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Bottlenose dolphins

The population of bottlenose dolphins in Sarasota Bay, Florida has been well studied and both historical vital rate and contaminant data exist for this population (Wells et al., 2005).

Humpback whales

For the humpback whale, the main source of survival and fecundity rates were obtained from Barlow and Clapham (1997). The population in the Gulf of Maine has been extensively studied (Clapham et al., 1995; Payne et al., 1986) and therefore provides reliable life history parameters for this species.

Northern and Southern resident killer whales

Using published historic population parameters for the northern (NRKW) and southern resident populations of killer whales (SRKW), which inhabit the coasts of British Columbia, Canada and Washington State, USA (Ford et al., 2000), the outcome for the same species which have slightly different population dynamics and contaminant burdens can be compared.

The population of SRKW has not increased at the same rate as the NRKW population and the trend from 1975-1987 indicated that the population was increasing at approximately 1.3% per annum during that period (Olesiuk et al., 1990). However, it should be noted that the parameters from this era are likely to already include PCB-induced effects and that this should be taken into consideration when interpreting changes in potential population growth over time.

In all four case studies, data from various sources was used to estimate the proportion of PCBs transferred from the female to the calf *in utero* (0.6) and an additional proportion during lactation (0.77) (Cockcroft et al., 1989; Salata et al., 1995; Tanabe et al., 1982). Where the calf died within its first year, we assumed death occurred at 6 months and the depuration for that year was halved to 0.38. Subsequently the fate of male calves was ignored by the model.

Validation using empirical data

One output from the model was the estimated PCB concentration in each individual female. By comparing these with distributions of concentration found in the mature females within a given example population, it was possible to estimate the equivalent annual accumulation of PCBs and resultant achievable population growth, assuming the source concentration is not changing substantially over time which could be an oversimplification.

Results

Population model simulations

For each population, 100 model simulations were run for each PCB annual accumulation value. An example of the model output population trajectories from the simulations is given in the Supplementary material (Fig. S1). Fig. 5a-5d shows the change in achievable population growth rate for different annual accumulations of blubber PCBs for the four

examples. Firstly, in each case achievable population growth rates taking only the effects of PCBs on calf survival into account were generated and compared to the population growth without accounting for the impact of PCB uptake.

Bottlenose dolphins

For the bottlenose dolphin example, an increase from 0 to 5 mg/kg lipid PCB annual accumulation was predicted to cause a decrease in annual achievable population growth rate from 1.4 to 0.43%. The population trajectory declines from a growing to a static population (Fig. 5a), representing an approximately 69% (95% CI 53% - 85%) decrease in the annual population growth, a significant reduction between the baseline unexposed population and the population with an annual PCB accumulation of 5 mg/kg lipid.

Secondly, achievable population growth rates were estimated taking effects on immunity into account and with two example pathogen exposure levels (i.e., 5 or 20%). As expected, this caused the population to decline at lower PCB annual accumulation levels. When 5% of the population were exposed to a novel pathogen, it did not start to decline until the annual accumulation was between 4 and 5 mg/kg lipid. However, when 20% of the population was exposed, the population started to decline at annual accumulation levels of between 1 and 2 mg/kg lipid (Fig. 5a). By 5 mg/kg lipid annual accumulation, the achievable annual population growth had declined by 230% (95% CI 211% - 248%) compared to the baseline annual population growth (Fig. 5a).

Humpback whales

The achievable population growth rate for the baseline population in this example was high at ~6.5% per annum, resulting in exponential trajectories. The impact of PCB annual accumulations of again between 1 and 5 mg/kg lipid on population growth for all three scenarios was less pronounced (Fig. 5b). Although the population growth rates declined as expected, these were proportionally lower than for the bottlenose dolphin example, being between approximately 10% (95% CI 6% - 15%) up to a maximum of 76% (95% CI 69% – 83%) decline in achievable population growth

Northern resident killer whale

This baseline population was growing at ~2% per annum without the effects of PCBs and in the first set of simulations with impacts on calf survival only, the mean estimated potential population growth declined by between 2% (95% CI 15% - +19%) and 37% (95% CI 20% - 55%) at the 5 mg/kg lipid weight annual accumulation concentration. However, the mean estimated λ at this level was greater than 1.0 (Fig. 5c) indicating the population would still be increasing by ~0.9% per annum.
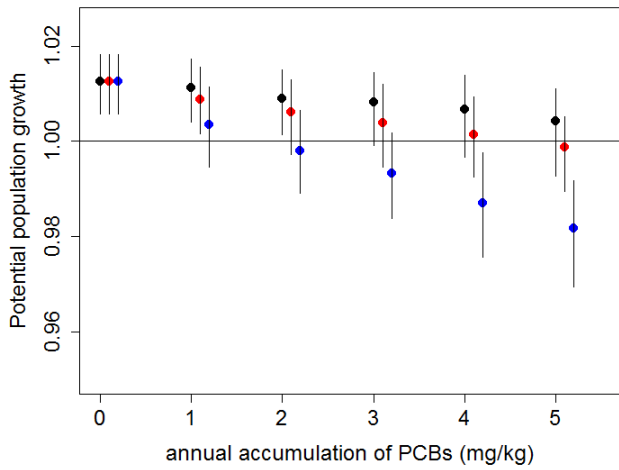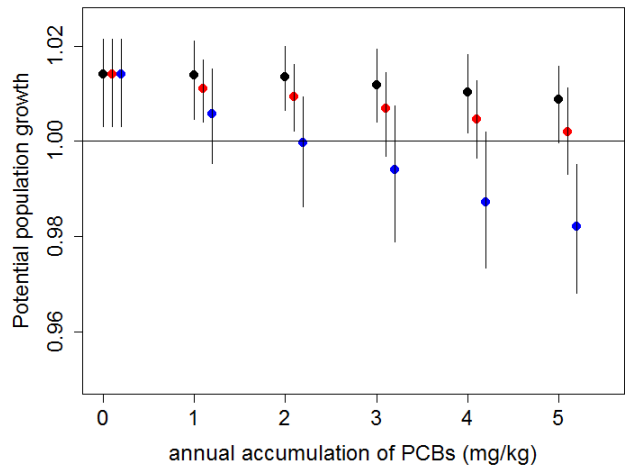
Fig. 5a Bottlenose dolphin

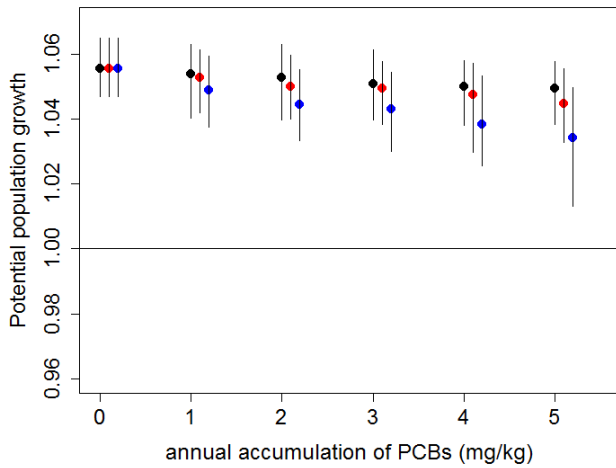Fig. 5c Northern Resident Killer Whale
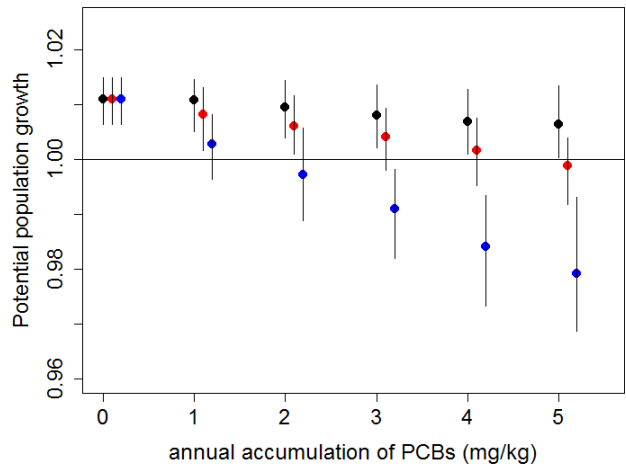
Fig. 5b Humpback whale

Fig. 5d Southern Resident Killer Whale

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394   Fig 5. Change in the achievable population growth for different annual accumulations of
395   blubber PCBs in (a) bottlenose dolphins, (b) humpback whales, (c) Northern resident killer
396   whales and (d) Southern resident killer whales with different proportions of the population
397   exposed to a class II pathogen. The vertical line indicates the 95% range obtained from 100
398   simulations. Calf survival effects only = black circles, 5% exposed to a pathogen = red
399   circles, 20% exposed to a pathogen = blue circles. Horizontal line = stable population, λ=1.0.

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402 In the second set of simulations, PCB effects on immunity were also included in the model
403 (Fig. 5c). At 5% of animals exposed to a pathogen the achievable population growth rate
404 decreased up to 86% (95% CI 68% - 104%) per annum at the highest accumulation rate
405 resulting in a mean achievable population growth of only 0.2% per annum but with
406 confidence limits spanning 1.0. A similar result was observed when the proportion of the
407 population exposed to a high virulence pathogen increased to 20%. The population declined
408 further, up to 226% (95% CI 203% – 250%) at the highest annual uptake level. Under this
409 most extreme scenario, the mean achievable population growth rate fell below 1.0, indicating
410 that the population is expected to decline at a rate of ~2% per annum.

411 *Southern resident killer whale*

412 The results of the simulations for the SRKW population are shown in Fig. 5d and indicate
413 that when only calf survival effects are included in the model the population would still
414 increase slightly even at the highest uptake of 5 mg/kg lipid annual accumulation, with an
415 achievable λ just above 1.0. However, when immunity effects are taken into consideration
416 with 5% of the population exposed to a novel pathogen, at the highest uptake level, the
417 population is likely decline with a mean λ of 0.999 (although the confidence limits span 1.0,
418 indicating that in some simulation runs the populations did not decline Figure 5d). In terms
419 of a percentage change in λ from the baseline however, this represents a decrease of up to
420 110% (95% CI 97% - 124%) at the 5 mg/kg level.

421 When 20% of the population was exposed to a novel pathogen, the mean λ fell below 1.0 at
422 the 2 mg/kg annual accumulation level, representing a ~75% decrease compared to the
423 baseline. By the 5 mg/kg level, the mean λ was 0.979 (95% confidence limits 0.969, 0.993),
424 representing an annual population decline of ~2% and a decrease in λ of 289% (95% CI
425 265% - 312%) compared to baseline.

426

427 *Comparisons with empirical data*

428 In order to determine the annual accumulation concentration relevant to each case study
429 population, an estimate of the total PCB concentrations in the blubber of the adult females
430 from the various case study populations was used. These were compared to the age-
431 specific concentrations estimated by the model runs. In addition, the relationship between
432 the annual accumulation rates (1 – 5 mg/kg) and the mean concentration in the blubber of
433 the adult females (above the age at sexual maturity), estimated from 25 model runs including
434 only effects of PCBs on calf survival is shown in Figs. 6a-6d. This allows the accumulation
435 rates to be interpreted in relation to blubber PCB concentrations. A positive linear
436 relationship was seen for all four case studies, within similar ranges.

437 <u>Bottlenose dolphins</u>

438 For populations that have underlying vital rates similar to those published for the Sarasota
439 Bay population and used in these simulations, the resulting estimated annual accumulation
440 would be approximately 0.5 mg/kg lipid for the lower exposed populations such as those
441 monitored in Florida and the Gulf of Mexico (Fig. 6a, Schwacke et al., 2014) whereas it
442 would be almost 6 mg/kg lipid for more highly exposed populations, such as those in
443 Georgia (Schwacke et al., 2012). In these situations, a decline in the abundance of animals

15

444 would be predicted, given no compensatory population inputs or changes in vital rates over
445 time.

446 <u>Humpback whales</u>

447 A study published in 1975 reported levels of chlorinated hydrocarbons in a number of
448 cetacean species in the north Atlantic including humpback whales (Taruski et al., 1975) and
449 in 1997 a more detailed study reported levels in four female humpback whales from the Gulf
450 of St Lawrence (Gauthier et al., 1997) which ranged between ~2 and 4 mg/kg lipid. Although
451 these data were collected some years ago from animals outside the Gulf of Maine Region,
452 this equated to an annual accumulation of only between 0.2 and 0.4 mg/kg lipid (Fig. 6b).
453 This suggests exposure levels are considerably lower than for the other species and
454 populations included here. More recently (Elfes et al., 2010) published data only reported on
455 levels in males collected from the North Atlantic (Gulf of Maine) population.

456 <u>Northern Resident Killer Whales</u>

457 The model runs resulted in an estimated concentration of PCBs in NRKW adult females
458 (aged 14 to the maximum age class 50 years). For the 1 mg/kg and 3 mg/kg lipid annual
459 accumulations this resulted in a mean concentration for the females of 10.43 mg/kg lipid and
460 30.53 mg/kg lipid, respectively. Empirical data (Ross et al., 2000; Ylitalo et al., 2001)
461 reported total PCBs in adult females in the order of ~10 mg/kg lipid which would suggest an
462 annual accumulation of ~1 mg/kg although this comparison assumes sampled animals come
463 from a population with a similar age structure as the modelled population (Fig 6c).

464 <u>Southern Resident Killer Whales</u>

465 The model outputs suggest that accumulations are unlikely to be very much higher than ~5
466 mg/kg in SRKWs, because at this rate the mean level of total PCBs in the adult females was
467 ~50 mg/kg lipid weight (Fig 6d and Supplementary Fig. S2). This is in line with the small
468 amount of published data for adult female SRKWs of ~ 45-55 mg/kg lipid weight (Krahn et
469 al., 2007a; Ross et al., 2000).

16

(a)

(b)

(c)

(d)
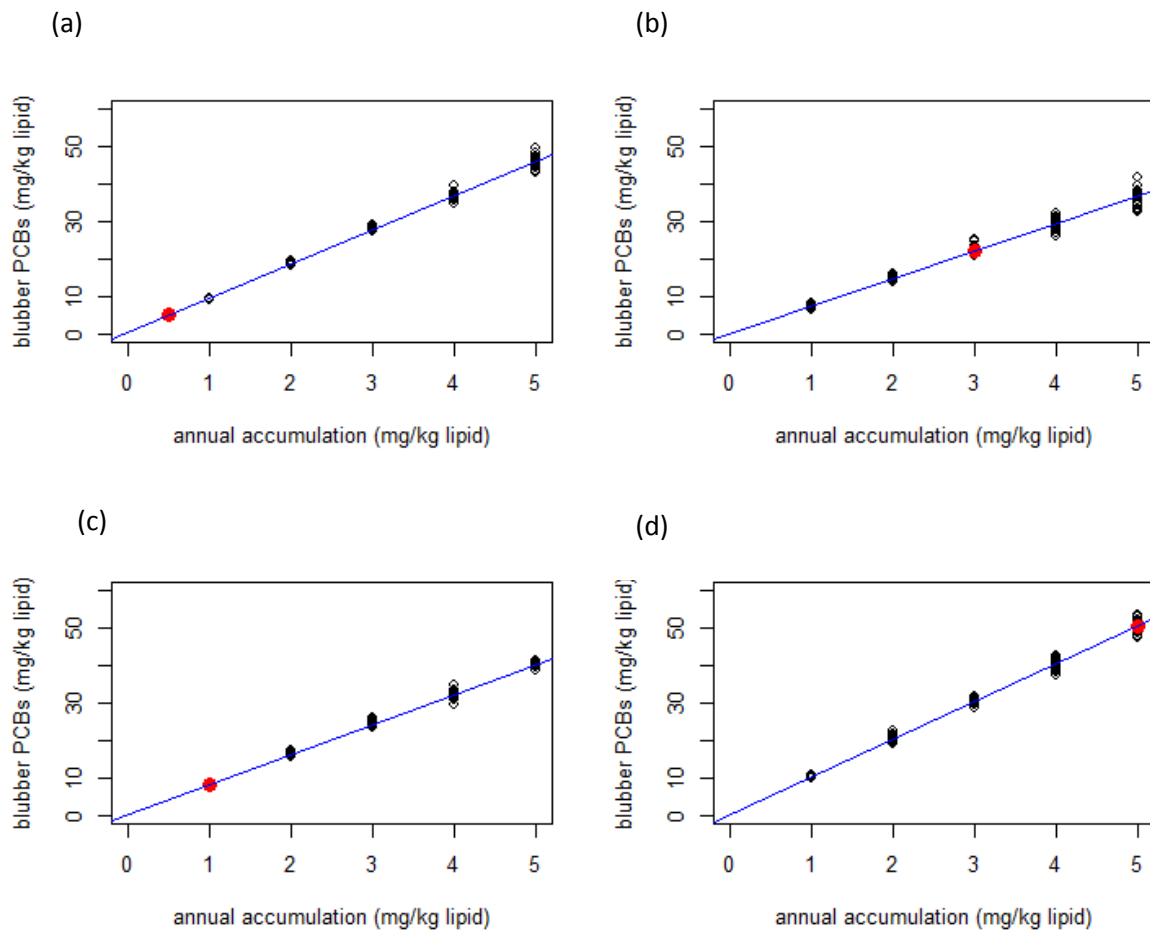
Fig. 6. Relationship between annual accumulation of PCBs and mean concentration in adult females for the four case studies (a) Bottlenose dolphin (BND) (b) Humpback whale (HW) (c) Northern Resident Killer whale (NRKW) (d) Southern Resident Killer whale (SRKW). Black circles indicate the results from the 25 model simulations. Red dots indicate the concentrations of PCBs and estimated annual accumulations reported for each of the case studies.

Effect of an epidemic

The effect of pathogen exposure during an epidemic in a given year was also be investigated using this model framework. An example of the impact of increasing the proportion of individuals exposed to a pathogen in a population of bottlenose dolphins is shown in Fig. 7. Here, the annual accumulation was set at 3 mg/kg lipid and the in a given year (here year 60 of the 100 year timeline) 80% of the population was exposed to a pathogen at some time during the year. The population trajectories showed a stable or slightly increasing population then a steep decrease in abundance in year 60 of the simulations when the outbreak is clearly seen as a step in the population trajectories in the year when the epidemic occurred. Interestingly, due to the stochastic nature of the model, not all the simulated population trajectories showed a step decline in the epidemic year. Clearly, the impact will be dictated by the virulence of the pathogen and the proportion of the population exposed.

490
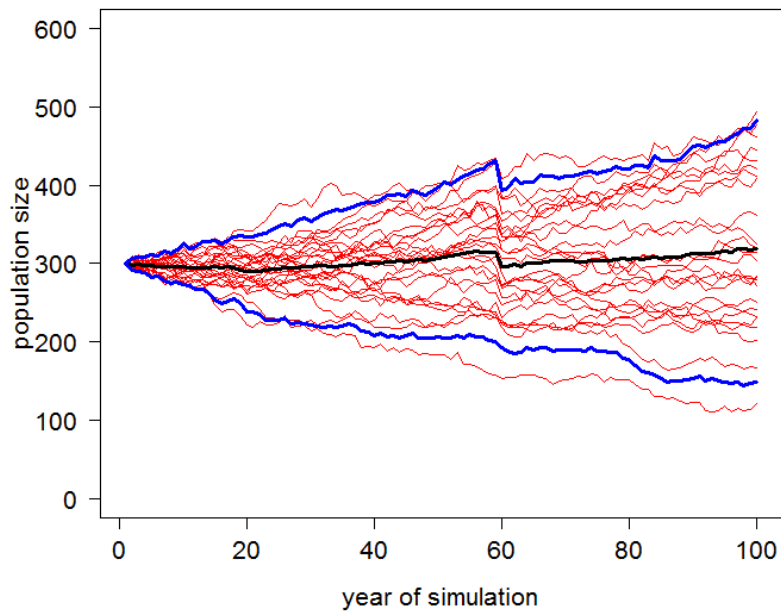
Fig. 7. Population trajectories from the individual based model with simulations showing the effect of an epidemic with 80% of the population exposed to a higher virulence pathogen in year 60. 100 simulations were run but the results for 25 are shown for clarity. Black line shows median population growth, blue lines 2.5th and 97.5th percentiles from the ordered population growth trajectories.

496

498 **Discussion**

499

500 The IBM described here was used to explore the achievable population level impacts of PCB
501 uptake by cetaceans, mediated through calf survival and immunosuppression. The model
502 provides an important insight into the likely effects of PCBs on achievable population growth
503 in a range of species, using four case studies as examples. However, it should be
504 recognised that the starting population parameters for these cases are generally from
505 historic studies which means in some cases the parameters may already be affected by
506 exposure to PCBs. This may have resulted in a more pessimistic outcome than is currently
507 the case, thus we would caution against interpreting the findings in absolute terms rather
508 that they represent relative changes in potential population growth at different levels of PCB
509 exposure mediated through different effect endpoints.

510 Nonetheless some general patterns have emerged. When populations are growing at a
511 modest rate of 1 - 2% per annum (as in the bottlenose dolphin and killer whale examples),
512 incorporating only calf survival effects into the model was not sufficient to cause a population
513 decline until relatively high levels of annual accumulation of PCBs, and correspondingly high
514 levels of PCBs in the blubber of females, had been reached (annual accumulation
515 concentrations > 5 mg/kg lipid). However, the very high levels of blubber PCB
516 concentrations that would result in accumulation concentrations above 5 mg/kg lipid are
517 seen in some populations of bottlenose dolphins (Balmer et al., 2011; Pulster et al., 2009),
518 and for at least one of these populations, significant adverse health conditions have been
519 documented (Schwacke et al., 2012). In light of these findings and the result of our IBM
520 simulations, this population would be expected to decline over time.

521 In addition, impacts of PCBs on adult survival (i.e., with immunocompromised individuals
522 showing increased vulnerability to novel pathogens) strengthen these effects. Recent
523 analysis in 2014 reported the NRKW population to be composed of 290 whales with a mean
524 annual growth rate of 2.2% since 1974 and 2.9% since 2002 (range -0.4 – 8.6%) (Towers et
525 al., 2015). The maximum intrinsic growth rate for this species is estimated to be 2.6%
526 (Olesiuk et al., 2005). By contrast, and in line with our predictions, the SRKW population has
527 hovered below 90 individuals since the late 1990s (Center for Whale Research, unpublished
528 data). This indicates that current accumulation rates are ~5 mg/kg lipid, resulting in females
529 with blubber PCB concentrations of ~ 50 mg/kg lipid (Ross et al., 2000, Krahn et al. 2009)
530 and inferring that the continued high exposure of this population to PCBs is one of the
531 factors constraining its recovery, particularly in conjunction with other highlighted issues
532 such as dietary limitation (Ford et al., 2010). Conversely, the population of humpback
533 whales, increasing near its maximum plausible growth rate, is unlikely to suffer a decline
534 even at the highest PCB concentrations measured in Gulf of Maine or Gulf of St Lawrence
535 humpback whales. The minimal risk for this population is primarily driven by the lower
536 trophic level of their prey.

537 The model is stochastic and whilst it captures some of the uncertainty in the model
538 parameters not all the potential sources of error have been included. For example, the vital
539 rates used to generate the baseline population are fixed, as are the depuration and
540 lactational transfer approximations estimated from various sources (Cockcroft et al., 1989;
541 Tanabe et al., 1982) and inclusion of the uncertainty associated with these parameters

542 would increase the variability of population growth estimates. The model also does not
543 include all potential health effects of PCB uptake, such as effects on fecundity (Barsotti et
544 al., 1976), which would potentially increase estimated risks of decline. This is a female
545 based model and the fate of males is excluded. However, males may be similarly impacted
546 by effects of immunosuppression. In addition, the tissue concentration-response relationship
547 for PCBs and calf survival and associated uncertainty was estimated from published
548 laboratory studies of a surrogate species (mink). Additional uncertainty for the application of
549 this tissue concentration-response function stems from potential interspecies differences in
550 metabolism of the various PCB congener groups, which may be a particular issue when
551 dosing is conducted using non-weathered technical mixtures of PCBs (e.g., commercially
552 sold Aroclor mixtures) or specific PCB congeners. While the uncertainty resulting from
553 interspecies extrapolation could not be included in the model due to the lack of empirical
554 data, uncertainty was reduced by focusing on laboratory studies where dosing was
555 conducted via contaminated prey (i.e. environmentally relevant mixtures), the results from
556 which contributed the majority of the data to the tissue concentration-response function.

557 Incorporating effects of PCBs on immunity in this model required including a three-stage
558 process. This was necessary in order to relate the concentration of PCBs in the blubber of
559 cetaceans to the ability of an individual animal to respond to infection (host resistance). The
560 only data currently available are from an extensive study carried out by the US National
561 Toxicology Program (NTP) in the 1990s using laboratory animal models (Luster et al., 1993)
562 and from a study of free-living dolphins from various populations for which the relationship
563 between blubber PCBs and a single immune function assay, the *in vitro* response to Con A
564 stimulation, was available (Schwacke et al., 2012). The NTP studies relating immune
565 function assays to proportional changes in host resistance and survival, suggested that,
566 given the different magnitude of responses between different immune function assays and
567 between innate and acquired immunity, more than one assay should be included in a battery
568 of tests. As such, we would recommend the future inclusion of a second assay. For
569 example, investigating natural killer cell activity in relation to blubber PCB concentrations in
570 cetaceans would provide a further insight into the impact on an arm of the innate immune
571 system important in defence against viral infection (De Guise et al., 1997).

572 Setting a realistic level at which to set the proportion of the population exposed to a
573 pathogen is also problematic and the 5% level chosen here is arbitrary. Most studies on
574 disease occurrence in marine mammals are based on serological studies which, whilst
575 indicating the prevalence of exposure to a pathogen in a population, do not measure the
576 occurrence or incidence of disease (i.e. the number of new cases of infection occurring in a
577 particular time period). Prevalence studies can only suggest how many animals have
578 historically been in contact with a particular pathogen but not when contact occurred.
579 However, a study of bottlenose dolphins in Florida reported that the annual incidence rate of
580 lobomycosis (lacaziosis) was 2.66% (Murdoch et al. 2008). This might indicate the rate of
581 pathogen exposure in a population outside an unusual mortality event. To be on the
582 conservative side this was therefore increased to 5%. However, in a free-ranging population
583 of cetaceans even exposing 5% of the population each year to a relatively virulent pathogen
584 may be an overestimation. And other aspects for a given species should be considered,
585 such as social organisation and pod structure which could affect pathogen exposure
586 dynamics. The laboratory animal model data are based on controlled exposure of caged
587 mice in which pathogen uptake is highly likely due to the dosing regimen. However, this may
588 ensure a degree of precaution in the model outputs and the conclusions drawn from them. If

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a novel pathogen were to be introduced into this population or particularly during an epidemic (as recently occurred during the 2013-2105 cetacean morbillivirus event that occurred along the US east coast (Morris et al., 2015)), the risk of observing a reduction in population growth may be considerably higher, depending on the persistence and transmission of the pathogen in the population, as increased mortality may be experienced by all age classes of animals, in addition to increased calf mortality.   Including the potential impact that a single year epidemic may have on a population could be investigated empirically, particularly in populations for which vital rates before and after an infectious disease outbreak are available.

This model only investigates the effect of a single class of persistent organic pollutants, the PCBs and it should be noted that cetaceans are likely to be simultaneously exposed to many other compounds, including heavy metals, polycyclic aromatic hydrocarbons and pesticides (Yordy et al., 2010a). Effects caused by these pollutant mixtures are not being considered here, because data are only available from PCBs to quantify relationships between lipid concentration and effects on vital rates. However, the fact that we have included data which relates Con A response to blubber PCB concentrations combined with the observation that many persistent organic pollutant concentrations in cetacean blubber co-vary (Krahn et al., 2009) would suggest that we are indirectly including the potential impact of other contaminants. In the meantime, the use of toxic equivalency factors to simulate potential effects (van den Berg et al., 2013) may provide some guidance but this is likely to be problematic for emerging and poorly studied contaminants but there may be cases for which it is better to test plausible scenarios in the absence of data than to ignore entire classes of contaminants altogether. Whilst in the scenarios presented here are based on fixed annual exposures over time, the model can be modified to include a reduction in PCB exposure level over time, as has been seen in some populations and species (Lebeuf et al., 2014).

In conclusion, this approach allows broad and general achievable population dynamic predictions to be made for specific populations when estimates of PCB concentrations, particularly in mature, breeding females, are known. These impacts can then be compared to other population pressures (such as interactions with boats, shipping and fisheries) so that the overall effect of pollutant exposures can be placed into a relative management context (Williams et al., 2016). Interest in understanding the cumulative impacts of man's activities on cetacean populations is growing (Côté et al., 2016). The approach presented in this study will provide an important contribution to these initiatives, by placing the effects of contaminants in the same demographic currency as other anthropogenic stressors.

Acknowledgments

References

Ahlmann, M., Hempel, G., 2016. The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Cancer Chemother. Pharmacol. 78, 661-671.

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

Baird, R.W., Dill, L.M., 1995. Occurrence and Behavior of Transient Killer Whales - Seasonal and Pod-Specific Variability, Foraging Behavior, and Prey Handling. Can. J. Zool. 73, 1300-1311.

Balmer, B.C., Schwacke, L.H., Wells, R.S., George, R.C., Hoguet, J., Kucklick, J.R., Lane, S.M., Martinez, A., McLellan, W.A., Rosel, P.E., Rowles, T.K., Sparks, K., Speakman, T., Zolman, E.S., Pabst, D.A., 2011. Relationship between persistent organic pollutants (POPs) and ranging patterns in common bottlenose dolphins (*Tursiops truncatus*) from coastal Georgia, USA. Sci. Tot. Environ. 409, 2094-2101.

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

Barsotti, D.A., Marlar, R.J., Allen, J.R., 1976. Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). Food Cosm. Toxicol. 14, 99-103.

Bursian, S.J., Sharma, C., Aulerich, R.J., Yamini, B., Mitchell, R.R., Beckett, K.J., Orazio, C.E., Moore, D., Svirsky, S., Tillitt, D.E., 2006. 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.

Canada, F.a.O., 2011. Recovery Strategy for the Northan and Southern Resident Killer Whales (*Orcinus orca*) in Canada, Species at Risk Strategy Series. Fisheries and Oceans Canada, Ottawa, p. 80.
Clapham, P.J., Berube, M., Mattila, D.K., 1995. Sex-Ratio of the Gulf of Maine Humpback Whale Population. Mar. Mam. Sci. 11, 227-231.

Cockcroft, V.G., De Kock, A.C., Lord, D.A., Ross, G.J.B., 1989. Organochlorines in bottlenose dolphins (*Tursiops truncatus*) from the East coast of South Africa. South African Journal of Marine Science 8, 207-217.

Côté, I.M., Darling, E.S., Brown, C.J., 2016. Interactions among ecosystem stressors and their importance in conservation. Proceedings. Biological Sciences / the Royal Society 283.
De Guise, S., Martineau, D., Beland, P., Fournier, M., 1995. Possible mechanisms of action of environmental contaminants on St. Lawrence beluga whales (*Delphinapterus leucas*). Environ. Health Perspect. 103 Suppl 4, 73-77.

De Guise, S., Ross, P.S., Osterhaus, A.D.M.E., Martineau, D., Béland, P., Fournier, M., 1997. Immune functions in beluga whales (*Delphinapterus leucas*): evaluation of natural killer cell activity. Vet. Immunol. Immunopathol. 58, 345-354.

Elfes, C.T., VanBlaricom, G.R., Boyd, D., Calambokidis, J., Clapham, P.J., Pearce, R.W., Robbins, J., Salinas, J.C., Straley, J.M., Wade, P.R., Krahn, M.M., 2010. Geographic Variation of Persistent Organic Pollutant Levels in Humpback Whale (*Megaptera*

23

novaeangliae) Feeding Areas of the North Pacific and North Atlantic. Environ. Toxicol. Chem. 29, 824-834.

Fair, P.A., Adams, J., Mitchum, G., Hulsey, T.C., Reif, J.S., Houde, M., Muir, D., Wirth, E., Wetzel, D., Zolman, E., McFee, W., Bossart, G.D. 2010. Contaminant blubber burdens in Atlantic bottlenose dolphins (*Tursiops truncatus*) from two southeastern US estuarine areas: Concentrations and patterns of PCBs, pesticides, PBDEs, PFCs and PAHs. Sci. Tot. Environ. 408, 1577-1597.

Folland, W.R., Newsted, J.L., Fitzgerald, S.D., Fuchsman, P.C., Bradley, P.W., Kern, J., Kannan, K., Remington, R.E., Zwiernik, M.J., 2016. Growth and reproductive effects from dietary exposure to Aroclor 1268 in mink (*Neovison vison*), a surrogate model for marine mammals. Environ. Toxicol. Chem. 35, 604-618.

Ford, J.K., Ellis, G.M., Olesiuk, P.F., Balcomb, K.C., 2010. Linking killer whale survival and prey abundance: food limitation in the oceans' apex predator? Biol. Lett. 6, 139-142.

Ford, K.J., Ellis, G.M., Balcomb, K.C., 2000. Killer whales: the natural history and genealogy of Orcinus orca in British Columbia and Washington. UBC Press, British Columbia.

Fuchsman, P.C., Barber, T.R., Bock, M.J., 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, J.M., Metcalfe, C.D., Sears, R., 1997. Chlorinated organic contaminants in blubber biopsies from northwestern Atlantic balaenopterid whales summering in the Gulf of St Lawrence. Mar. Environ. Res. 44, 201-223.

Hall, A.J., Hugunin, K., Deaville, R., Law, R.J., Allchin, C.R., Jepson, P.D., 2006a. The risk of infection from polychlorinated biphenyl exposure in harbor porpoise (*Phocoena phocoena*) - A case-control approach. Environ. Health Perspect. 114, 704-711.

Hall, A.J., McConnell, B.J., Rowles, T.K., Aguilar, A., Borrell, A., Schwacke, L., Reijnders, P.J., Wells, R.S., 2006b. Individual-based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. Environ. Health Perspect. 114 Suppl 1, 60-64.

Hansen, L.J., Schwacke, L.H., Mitchum, G.B., Hohn, A.A., Wells, R.S., Zolman, E.S., Fair, P.A., 2004. Geographic variation in polychlorinated biphenyl and organochlorine pesticide concentraitons in the blubber of bottlenose dolphins from the US Atlantic coast. Sci. Tot. Environ. 319, 147-172.

Harper, N., Howie, L., Connor, K., Dickerson, R., Safe, S., 1993. Immunosuppressive effects of highly chlorinated biphenyls and diphenyl ethers on T-cell dependent and independent antigens in mic. Toxicology, 85, 123-125.

Hayteas, D.L., Duffield, D.A., 2000. High levels of PCB and p,p '-DDE found in the blubber of killer whales (*Orcinus orca*). Mar. Poll. Bull. 40, 558-561.

Heaton, S.N., Bursian, S.J., Giesy, J.P., Tillitt, D.E., Render, J.A., Jones, P.D., Verbrugge, D.A., Kubiak, T.J., Aulerich, R.J., 1995. Dietary exposure of mink to carp from Saginaw Bay,

Michigan. 1. Effects on reproduction and survival, and the potential risks to wild mink populations. Arch. Environ. Contam. Toxicol. 28, 334-343.

Hickie, B., Kingsley, M.C.S., Hodson, P.V., Muir, D.C.G., Beland, P., Mackay, D., 2000. A modelling-based perspective on the past, present and future polychlorinated biphenyl contamination of the St. Lawrence beluga whale (*Delphinapterus leucas*) population. Can. J. Fish. Aq. Sci. 57, 101-112.

Hickie, B.E., Cadieux, M.A., Riehl, K.N., Bossart, G.D., Alava, J.J., Fair, P.A., 2013. Modeling PCB-Bioaccumulation in the Bottlenose Dolphin (*Tursiops truncatus*): Estimating a Dietary Threshold Concentration. Environ. Sci. Technol. 47, 12314-12324.

Hickie, B.E., Mackay, D., de Koning, J., 1999. Lifetime pharmacokinetic model for hydrophobic contaminants in marine mammals. Environ. Toxicol. Chem. 18, 2622-2633.

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

Jepson, P.D., Deaville, R., Barber, J.L., Aguilar, A., Borrell, A., Murphy, S., Barry, J., Brownlow, A., Barnett, J., Berrow, S., Cunningham, A.A., Davison, N.J., ten Doeschate, M., Esteban, R., Ferreira, M., Foote, A.D., Genov, T., Gimenez, J., Loveridge, J., Llavona, A., Martin, V., Maxwell, D.L., Papachlimitzou, A., Penrose, R., Perkins, M.W., Smith, B., de Stephanis, R., Tregenza, N., Verborgh, P., Fernandez, A., Law, R.J., 2016. PCB pollution continues to impact populations of orcas and other dolphins in European waters. Sci. Reports 6, 18573.

Kannan, K., Blankenship, A.L., Jones, P.D., Giesy, J.P., 2000. Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. Hum. Ecol. Risk Assess. 6, 181-201.

Kihlstrom, J.K., 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.

Krahn, M., Herman, D., Ylitalo, G., Sloan, C., Burrows, D., Hobbs, R., Mahoney, B., Yanagida, G., Calambokidis, J.C., Moore, S., 2004. Stratification of lipids, fatty acids and organochlorine contaminants in blubber of white whales and killer whales. J. Cetacean Res. Manage. 6, 175-189.

Krahn, M.M., Hanson, M.B., Baird, R.W., Burrows, D.G., Emmons, C.K., Ford, J.K.B., Jones, L.L., Noren, D.P., Ross, P.S., Schorr, G.S., Collier, T.K., Boyer, R.H., 2007a. Persistent organic pollutants and stable isotopes in biopsy samples (2004/2006) from Southern Resident killer whales. Mar. Pollut. Bull. 54, 1903-1911.

Krahn, M.M., Herman, D.P., Matkin, C.O., Durban, J.W., Barrett-Lennard, L., Burrows, D.G., Dahlheim, M.E., Black, N., LeDuc, R.G., Wade, P.R., 2007b. Use of chemical tracers in assessing the diet and foraging regions of eastern North Pacific killer whales. Mar. Environ. Res. 63, 91-114.

Krahn, M.M., Hanson, M.B., Schorr, G.S., Emmons, C.K., Burrows, D.G., Bolton, J.L., Baird, R.W., Ylitalo, G.M., 2009. Effects of age, sex and reproductive status on persistent organic pollutant concentrations in "Southern Resident" killer whales. Mar. Pollut. Bull. 58, 1522-1529.

Kucklick, J., Schwacke, L., Wells, R., Hohn, A., Guichard, A., Yordy, J., Hansen, L., Zolman, E., Wilson, R., Litz, J., Nowacek, D., Rowles, T., Pugh, R., Balmer, B., Sinclair, C., Rosel, P.,

792 2011. Bottlenose dolphins as indicators of persistent organic pollutants in the western North
793 Atlantic Ocean and northern Gulf of Mexico. Environ. Sci. Technol. 45, 4270-4277.
794
795 Law, R.J., 2014. An overview of time trends in organic contaminant concentrations in marine
796 mammals: Going up or down? Mar. Pollut. Bull. 82, 7-10.
797
798 Lebeuf, M., Measures, L., Noel, M., Raach, M., Trottier, S., 2014. A twenty-one year
799 temporal trend of persistent organic pollutants in St. Lawrence Estuary beluga, Canada. Sci.
800 Tot. Environ. 485, 377-386.
801
802 Luster, M.I., Portier, C., Pait, D.G., Rosenthal, G.J., Germolec, D.R., Corsini, E., Blaylock,
803 B.L., Pollock, P., Kouchi, Y., Craig, W., et al., 1993. Risk assessment in immunotoxicology.
804 II. Relationships between immune and host resistance tests. Fund. Appl. Toxicol. 21, 71-82.
805
806 Metcalfe, C., Koenig, B., Metcalfe, T., Paterson, G., Sears, R., 2004. Intra- and inter-species
807 differences in persistent organic contaminants in the blubber of blue whales and humpback
808 whales from the Gulf of St. Lawrence, Canada. Mar. Environ. Res. 57, 245-260.
809
810 Morris, S.E., Zelner, J.L., Fauquier, D.A., Rowles, T.K., Rosel, P.E., Gulland, F., Grenfell,
811 B.T., 2015. Partially observed epidemics in wildlife hosts: modelling an outbreak of dolphin
812 morbillivirus in the northwestern Atlantic, June 2013–2014. Journal of the Royal Society
813 Interface 12, 20150676.
814
815 Murdoch, ME, Reif JS, Mazzoil M, McCulloch SD, Fair PA, Bossart GD. 2008. Lobomycosis
816 in bottlenose dolphins (*Tursiops truncatus*) from the Indian River Lagoon, Florida: estimation
817 of prevalence, temporal trends, and spatial distribution. EcoHealth 5:289-297.
818
819 Murphy, C.A., Rose, K.A., Alvarez Mdel, C., Fuiman, L.A., 2008. Modeling larval fish
820 behavior: scaling the sublethal effects of methylmercury to population-relevant endpoints.
821 Aquat. Toxicol. 86, 470-484.
822
823 Olesiuk, P.F., Bigg, M.A., Ellis, G.M., 1990. Life history and population dynamics of resident
824 killer whales (*Orcinus orca*) in the coast waters of British Columbia and Washington State.,
825 Report of the International Whaling Commission (special issue), pp. 209-243.
826
827 Olesiuk, P.F., Ellis, G.M., Ford, J.K.B., 2005. Life history and population dynamics of
828 noerthern resident killer whales (*Orcinus orca*) in British Columbia. Canadian Science
829 Advisory Secretariate, Fisheries and Oceans, Canada, Ottawa, Canada.
830
831 Ono, M., Kannan, N., Wakimoto, T., Tatsukawa, R., 1987. Dibenzofurans a Greater Global
832 Pollutant Than Dioxins - Evidence from Analyses of Open Ocean Killer Whale. Mar. Pollut.
833 Bull. 18, 640-643.
834
835 Payne, P.M., Nicolas, J.R., Obrien, L., Powers, K.D., 1986. The Distribution of the
836 Humpback Whale, Megaptera-Novaeangliae, on Georges Bank and in the Gulf of Maine in
837 Relation to Densities of the Sand Eel, *Ammodytes americanus*. Fish. Bull. 84, 271-277.
838
839 Platonow, N.S., Karstad, L.H., 1973. Dietary effects of polychlorinated biphenyls on mink.
840 Can. J. Comp. Med. 37, 391-400.
841
842 Pulster, E.L., Maruya, K.A., 2008. Geographic specificity of Aroclor 1268 in bottlenose
843 dolphins (*Tursiops truncatus*) frequenting the Turtle/Brunswick River Estuary, Georgia
844 (USA). Sci. Tot. Environ. 393, 367-375.
845

846   Pulster, E.L., Smalling, K.L., Zolman, E., Schwacke, L., Maruya, K.A., 2009. Persistent
847   organochlorine pollutants and toxaphene congener profiles in bottlenose dolphins (*Tursiops*
848   *truncatus*) frequenting the Turtle/Brunswick River Estuary (TBRE) in coastal Georgia, USA.
849   Environ. Toxicol. Chem. 28, 1390-1399.

851   R Development Core Team, 2014. R: A language and environment for statistical computing.
852   Vienna, Austria.

854   Restum, J.C., Bursian, S.J., Giesy, J.P., Render, J.A., Helferich, W.G., Shipp, E.B.,
855   Verbrugge, D.A., Aulerich, R.J., 1998. Multigenerational study of the effects of consumption
856   of PCB-contaminated carp from Saginaw Bay, Lake Huron, on mink. 1. Effects on mink
857   reproduction, kit growth and survival, and selected biological parameters. J. Toxicol. Environ.
858   Health A 54, 343-375.

860   Robbins, J., 2007. Structure and dynamics of the Gulf of Maine humpback whale
861   population. PhD thesis, University of St Andrews, St Andrews, UK.

863   Ross, P., De Swart, R., Addison, R., Van Loveren, H., Vos, J., Osterhaus, A., 1996.
864   Contaminant-induced immunotoxicity in harbour seals: wildlife at risk? Toxicol. 112, 157-169.

866   Ross, P.S., Ellis, G.M., Ikonomou, M.G., Barrett-Lennard, L.G., Addison, R.F., 2000. High
867   PCB concentrations in free-ranging Pacific killer whales, *Orcinus orca,*: Effects of age, sex
868   and dietary preference. Mar. Pollut. Bull. 40, 504-515.

870   Salata, G.G., Wade, T.L., Sericano, J.L., Davis, J.W., Brooks, J.M., 1995. Analysis of Gulf-
871   of-Mexico Bottle-Nosed Dolphins for Organochlorine Pesticides and PCBs. Environ. Pollut.
872   88, 167-175.

874   Schwacke, L.H., Smith, C.R., Townsend, F.I., Wells, R.S., Hart, L.B., Balmer, B.C., Collier,
875   T.K., De Guise, S., Fry, M.M., Guillette, L.J., Lamb, S.V., Lane, S.M., Mcfee, W.E., Place,
876   N.J., Turnlin, M.C., Ylitalo, G.M., Zolman, E.S., Rowles, T.K., 2014. Health of Common
877   Bottlenose Dolphins (*Tursiops truncatus*) in Barataria Bay, Louisiana, Following the
878   Deepwater Horizon Oil Spill. Environ. Sci. Technol. 48, 93-103.

880   Schwacke, L.H., Voit, E.O., Hansen, L.J., Wells, R.S., Mitchum, G.B., Hohn, A.A., Fair, P.A.,
881   2002. Probabilistic risk assessment of reproductive effects of polychlorinated biphenyls on
882   bottlenose dolphins (*Tursiops truncatus*) from the southeast United States coast. Environ.
883   Toxicol. Chem. 21, 2752-2764.

885   Schwacke, L.H., Zolman, E.S., Balmer, B.C., De Guise, S., George, R.C., Hoguet, J., Hohn,
886   A.A., Kucklick, J.R., Lamb, S., Levin, M., Litz, J.A., McFee, W.E., Place, N.J., Townsend,
887   F.I., Wells, R.S., Rowles, T.K., 2012. Anaemia, hypothyroidism and immune suppression
888   associated with polychlorinated biphenyl exposure in bottlenose dolphins (*Tursiops*
889   *truncatus*). Proc. Roy. Soc. B 279, 48-57.

891   Simon, M., Johnson, M., Madsen, P.T., 2012. Keeping momentum with a mouthful of water:
892   behavior and kinematics of humpback whale lunge feeding. J. Experi. Biol. 215, 3786-3798.

894   Tanabe, S., Tatsukawa, R., Maruyama, K., Miyazaki, N., 1982. Transplacental transfer of
895   PCBs and chlorinated hydrocarbon pesticides from the pregnant striped dolphin {IStenella
896   coeruleoalba} to her fetus. Agri. Biol. Chem. 46, 1249-1254.

898   Taruski, A.G., Olney, C.E., Winn, H.E., 1975. Chlorinated hydrocarbons in cetaceans. J.
899   Fish. Res. Board Cananda 32, 2205-2209.
900

Towers, J., Ellis, G., Ford, J., 2015. Photo-identification catalogue and status of the northern resident killer whale population in 2014. Canadian Technical Report of Fisheries and Aquatic Sciences 3139, 75vi.

Van Bressem, M.F., Duignan, P.J., Banyard, A., Barbieri, M., Colegrove, K.M., De Guise, S., Di Guardo, G., Dobson, A., Domingo, M., Fauquier, D., Fernandez, A., Goldstein, T., Grenfell, B., Groch, K.R., Gulland, F., Jensen, B.A., Jepson, P.D., Hall, A., Kuiken, T., Mazzariol, S., Morris, S.E., Nielsen, O., Raga, J.A., Rowles, T.K., Saliki, J., Sierra, E., Stephens, N., Stone, B., Tomo, I., Wang, J., Waltzek, T., Wellehan, J.F.X., 2014. Cetacean Morbillivirus: Current Knowledge and Future Directions. Viruses-Basel 6, 5145-5181.

van den Berg, M., Denison, M.S., Birnbaum, L.S., Devito, M.J., Fiedler, H., Falandysz, J., Rose, M., Schrenk, D., Safe, S., Tohyama, C., Tritscher, A., Tysklind, M., Peterson, R.E., 2013. Polybrominated dibenzo-p-dioxins, dibenzofurans, and biphenyls: inclusion in the toxicity equivalency factor concept for dioxin-like compounds. Toxicol. Sci. 133, 197-208.

Weijs, L., Yang, R.S.H., Das, K., Covaci, A., Blust, R. 2013. Application of a Bayesian population physiologically based pharmacokinetic (PBPK) modeling and Markov Chain Monte Carol simulations to pesticide kinetics studies in protected marine mammals: DDT, DDE and DDD in harbor porpoises. Env. Sci. Technol. 47, 4365-4374.

Wells, R.S., Scott, M.D., 1990. Estimating bottlenose dolphin population parameters from individual identification and capture-release techniques, in: Hammond, P.S., Mizroch, S.A., Donovan, G.P. (Eds.), Individual Recognition of Cetaceans: Use of Photo-identification and other techniques to estimate population parameters. International Whaling Commission, Cambridge, UK, p. 440.

Wells, R.S., Tornero, V., Borrell, A., Aguilar, A., Rowles, T.K., Rhinehart, H.L., Hofmann, S., Jarman, W.M., Hohn, A.A., Sweeney, J.C., 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. Tot. Environ. 349, 106-119.

Williams, R., Thomas, L., Ashe, E., Clark, C.W., Hammond, P.S., 2016. Gauging allowable harm limits to cumulative, sub-lethal effects of human activities on wildlife: A case-study approach using two whale populations. Mar. Pol. 70, 58-64.

Witteveen, B.H., Worthy, G.A., Wynne, K.M., Hirons, A.C., Andrews, A.G., Markel, R.W., 2011. Trophic levels of North Pacific humpback whales (*Megaptera novaeangliae*) through analysis of stable isotopes: implications on prey and resource quality. Aquat. Mamm. 37, 101.

Ylitalo, G.M., Matkin, C.O., Buzitis, J., Krahn, M.M., Jones, L.L., Rowles, T., Stein, J.E., 2001. Influence of life-history parameters on organochlorine concentrations in free-ranging killer whales (Orcinus orca) from Prince William Sound, AK. Sci. Tot. Environ. 281, 183-203.

Yordy, J.E., Mollenhauer, M.A., Wilson, R.M., Wells, R.S., Hohn, A., Sweeney, J., Schwacke, L.H., Rowles, T.K., Kucklick, J.R., Peden-Adams, M.M., 2010a. Complex contaminant exposure in cetaceans: a comparative E-Screen analysis of bottlenose dolphin blubber and mixtures of four persistent organic pollutants. Environ. Toxicol. Chem. 29, 2143-2153.

Yordy, J.E., Wells, R.S., Balmer, B.C., Schwacke, L.H., Rowles, T.K., Kucklick, J.R., 2010b. Life history as a source of variation for persistent organic pollutant (POP) patterns in a

community of common bottlenose dolphins (Tursiops truncatus) resident to Sarasota Bay, FL. Sci. Tot. Environ. 408, 2163-2172.

Zerbini, A.N., Clapham, P.J., Wade, P.R., 2010. Assessing plausible rates of population growth in humpback whales from life-history data. Mar. Biol. 157, 1225-1236.