

Subject: estimated PCBs and PBDEs in the SRKWs
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Hello,

I recently graduated with a Masters degree from the School of Aquatic and Fishery Sciences, UW, and my thesis was on PCB and PBDE accumulation in the southern residents. I have attached my thesis for the 5 year review. In general, I used a modeling approach to estimate contaminant levels in each individual, and I projected those levels forward. I also examined how life history traits influence concentration levels in these whales and provide a discussion regarding adverse health effects of these contaminants on the whales. Please feel free to contact me with any questions.

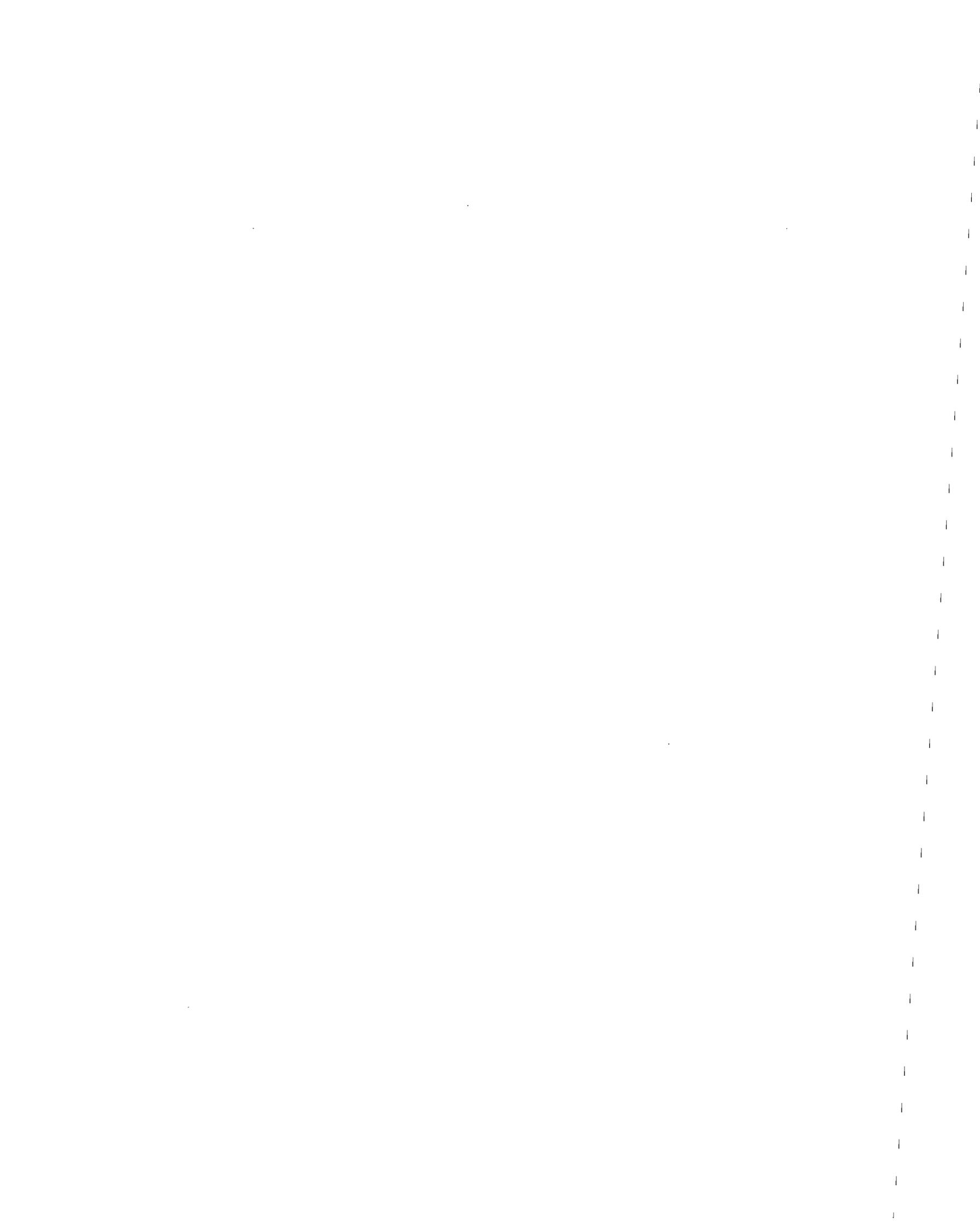
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Estimated polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE)
accumulation in Southern Resident killer whales

Teresa Mishael Mongillo

A thesis
submitted in partial fulfillment of the
requirements for the degree of

Master of Science

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University of Washington
Graduate School

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Abstract

Estimated polychlorinated biphenyl (PCB) and polybrominated diphenyl ether (PBDE) accumulation in Southern Resident killer whales

Teresa Mishael Mongillo

Chair of the Supervisory Committee:
Professor Glenn R. VanBlaricom
School of Aquatic and Fishery Sciences

Southern Resident killer whales (*Orcinus orca*) that reside in the coastal waters of British Columbia and Washington State in the summer months consist of three pods (J, K, and L) and were recently listed as “endangered” as defined by the U.S. Endangered Species Act. One potential threat to this population is high contaminant concentrations. Persistent organic pollutants (POPs) are man-made lipophilic contaminants that bioaccumulate in upper trophic level species. Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are POPs of particular concern because they can potentially cause immunosuppression, reproductive damage, and neurotoxicity. An individual-based modeling approach was used to estimate the accumulation of PCBs and PBDEs in specific individuals in Southern Resident killer whales (SRKWs). The current accumulated levels were also projected into the future under various assumptions and scenarios. Similarities in the predicted PCB and PBDE concentrations in individuals were assessed to identify any natural groupings in the model output. In addition, the respective influences of the life history traits that lead to the pattern of the predicted contaminant levels across demographic categories were also determined.

The PCB accumulation model scenario that best fit the data included a “mixed” diet for all three pods of Southern Resident killer whales. In general, J pod individuals had higher PCB concentrations than K or L pod individuals. The predicted PBDE accumulation doubling time for all three pods ranged from 3 to 4 years. Model projections indicate that PCBs are slowly declining but the killer whales will continue to be exposed for some generations to come. In contrast, PBDEs are projected to increase

rapidly, and individuals may experience levels equal to current PCB levels and surpass the health effects threshold in a short period of time. The uncertainty in the model parameters appeared to have little effect on model results excluding the missed calf factor and rate of accumulation of PBDEs. In male SRKWs, no primary factor significantly influenced the PCB concentrations predicted across the individuals in the current SRKW population. In contrast, the birth year, age, and birth order significantly influenced PBDE concentrations. Age was found to have a generally positive association with PCBs and a significant negative association with PBDEs in male killer whales. In addition, birth order had a generally negative association with PCBs and a significant positive association with PBDE concentrations. Among male killer whales, individuals with the highest PCB concentrations included primarily older individuals with low birth order and individuals with high PBDE concentrations included primarily younger individuals born recently with high birth order. The contaminant levels in female killer whales were primarily influenced by individual reproductive status and were not strongly associated with age or birth order. Reproductive females were found to have distinct contaminant concentrations compared to all other life history groups.

Detrimental biological effects from exposure to PCBs and PBDEs have the potential to hinder the recovery of the SRKWs. Ultimately, determination of exposure levels and potential resulting risks posed by these persistent organic pollutants in the SRKWs are essential for the effective protection of this endangered species. It is not possible to biopsy all individuals, yet estimates for all individuals are necessary if scientists are to tease out the effects of contaminant loading on individual reproductive output and survival. The results are important to the conservation and management of this population and provide a template for future inquiries on the effects of lipophilic contaminants.

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DEDICATION

I would like to dedicate this work to my beautiful daughter, Lucia Grace, and my supportive husband, Peter.

Chapter 1: Introduction

1.1 Overview

Killer whales (*Orcinus orca*) that reside along the west coast of the United States and Canada are considered ecologically, economically, and culturally important in the region. The Southern Resident killer whales (SRKWs), a community of killer whales found in the inland waters of British Columbia and Washington State in the summer months (Bigg, 1982; Osborne, 1999; Hauser et al., 2007) were listed as “endangered” as defined by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2001, and by the U.S. Endangered Species Act (ESA; 16 U.S.C.A. §§ 1531 *et seq.*, as amended) in 2005. Several hypotheses have been proposed for the population decline including reduced prey availability (Ford et al., 2005, 2009; Ward et al., 2009); disturbance from noise pollution and concentrated vessel traffic (Bain et al., 2006); and high contaminant body burdens (Ross et al., 2000; Krahn et al., 2007, 2009).

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), are a family of chemicals that are persistent, lipophilic, and will biomagnify up the food chain. In general, species at the top of the food chain will have higher contaminant concentrations than species at lower trophic levels in the food chain. PCBs and PBDEs are POPs of particular concern because they may cause reproductive damage, endocrine disruption and immunotoxicity (Gilmartin et al., 1976; Reijnders, 1986; de Swart et al., 1994; Hallgren et al., 2001; Fossi and Marsili, 2003; Legler and Brouwer, 2003). Unfortunately, current contaminant concentrations are known for only a few individuals in the SRKWs (Ross et al., 2000; Rayne et al., 2004; Herman et al., 2005; Krahn et al., 2007, 2009) and the effects of such loads are unknown. Therefore, it is critical to estimate past and present contaminant concentrations in order to assess current and future potential population-level effects. The goal of this thesis is to address several questions of concern regarding the accumulation, chronic exposure, and effects of PCBs and PBDEs in the SRKW population.

1.2 Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls (PCBs) are xenobiotic compounds with 209 potential congeners. The number and position of chlorine atoms in PCB molecules play a large role in bioavailability, volatility, and toxicity. In general, PCBs are persistent in nature, resist metabolic degradation, bioaccumulate in upper trophic level species, and may cause adverse health effects in many species (Jones and de Voogt, 1999).

1.2.1 Use and Production

Historically, PCBs were used as industrial lubricants and coolants for transformers and capacitors, as flame retardants, and in paints and sealants. They are manufactured by chlorination of biphenyl in the presence of a catalyst. Between 1930 and 1993, the total global production was at least 1.324 million t, of which approximately 97% of the historical global use was in the Northern Hemisphere (Breivik et al., 2002a). PCBs were produced and discharged in the U.S. beginning in the 1920s and 1930s and reached a peak in production by the 1960s and 1970s (Lefkovitz et al., 1997; Breivik et al., 2002a) prior to being banned in 1977. Although PCBs have been banned in many countries due to their adverse health effects, they still persist in the environment.

1.2.2 Environmental Fate/Trends

PCBs enter the environment through several processes including direct release, atmospheric transport, ocean transport, and runoff (Iwata et al., 1993; Grant and Ross, 2002). Several physicochemical properties of PCBs influence their fate in the environment. These properties include persistence, volatility, water solubility, and bioaccumulation potential (Grant and Ross, 2002). The more highly chlorinated PCBs are more environmentally persistent, less volatile, and have a higher bioaccumulation potential than the lesser chlorinated PCBs (Grant and Ross, 2002). In addition to the physicochemical properties of PCBs, temperature can strongly influence the total PCB emissions and emission patterns (Breivik et al., 2002b).

Sediments in Washington and British Columbia have been routinely monitored for contaminant exposure because of their potential to accumulate high levels of contaminants. PCB levels in sediment in the inland waters of Washington state have been estimated since the early 1900s (Lefkovitz et al., 1997). In general, Lefkovitz et al. (1997) found a steady increase in PCB concentration in Puget Sound sediment beginning in 1930. Peak levels in the early 1960s were close to 4 times the current levels of PCB sediment contamination (Lefkovitz et al., 1997). Contaminants in the sediment can become remobilized and transfer through the food web to high trophic level species. Puget Sound harbor seals (*Phoca vitulina*) have also been sampled since the mid 1980s (Calambokidis et al., 1999). Puget Sound harbor seal PCB concentration trends have indicated a dramatic decrease of levels from 1970 and relatively constant levels from 1985 to the late 1990s (Calambokidis et al., 1999).

The storage of PCBs in marine mammals is highly dependent on the lipid content of the body tissue. For example, levels in fin whales (*Balaenoptera physalus*) are highest in the blubber, followed by bone, muscle, kidneys and then the liver (Aguilar and Borrell, 1994a). PCB concentrations have been shown to vary within the blubber layer as well. After fasting, the inner blubber layer of a northern elephant seal (*Mirounga angustirostris*) is more contaminated than the outer blubber layer (Debier et al., 2006). PCB concentrations in the serum also increase significantly at the end of the fast (Debier et al., 2006) indicating a mobilization of PCBs from blubber to blood during times of nutritional stress. PCBs can also become mobilized during pregnancy (O'Shea, 1999) allowing females to offload their contaminant loads to offspring via transplacental transfer and lactation. For instance, Fukushima and Kawai (1980) found transfer rates in striped dolphins (*Stenella coeruleoalba*) from gestation and lactation to be 3.8% and 88%, respectively.

1.2.3 Health Effects/Toxicity

High PCB loads have been shown to be associated with several adverse health responses in marine mammals. Some of these responses include premature births in California sea lions (*Zalophus californianus*; Gilmartin et al., 1976) and reproductive failure in bottlenose dolphins (*Tursiops truncatus*; Schwacke et al., 2002). In addition, reproductive success was found to be significantly lower in harbor seals fed fish from the more polluted waters of the western part of the Wadden Sea compared to seals fed fish from the less polluted waters from the north-east Atlantic (Reijnders, 1986). Suppressed immune systems were found in striped dolphins (Aguilar and Borrell, 1994b) and harbor seals (de Swart et al., 1994; Ross et al., 1995). Similarly, Jepson et al. (1999) found an association between high PCB congener levels in the blubber of harbor porpoises (*Phocoena phocoena*) and mortality due to infectious disease providing additional evidence that PCBs may cause immunosuppression. Thyroid hormones, such as thyroxine (T4) and triiodothyronine (T3), are produced in the thyroid gland and target tissues and play an essential role in metabolism and developmental processes by binding with two nuclear receptors, TR- α and TR- β . Tabuchi et al. (2006) found high levels of PCBs correlated with TR- α in harbor seals suggesting that these lipophilic contaminants can also affect the thyroid hormone system and may alter gene expression.

1.3 Polybrominated diphenyl ethers (PBDEs)

Polybrominated diphenyl ethers (PBDEs) are structurally similar to PCBs and have been identified as a growing concern. They have a ubiquitous distribution with increasing levels found in the air, sediment, wildlife, and humans. In fact, these xenobiotic compounds were present in sperm whales (*Physeter macrocephalus*) indicating that they are present even in the deep ocean (de Boer et al., 1998).

1.3.1 Use and Production

PBDEs are additive flame retardants used in many products since the 1960s (Siddiqi et al., 2003) including electronics, textiles and plastics. They are manufactured by bromination of diphenyl ether in the presence of a catalyst. PBDEs have been

manufactured around the globe including countries such as the United States, the Netherlands, Japan, Great Britain, France, and Israel (Siddiqi et al., 2003). PBDEs have been released into the environment in North America since at least the 1970s (Hale et al., 2003). In 2001, the global demand was over 70,000 metric t. Almost half the production of the deca- and octa- forms, and over 95% of the penta-products was used in North America (Hale et al., 2003; Hites, 2004). The production of penta-BDE and octa-BDE was phased out in the U.S. at the end of 2004. Deca-BDE now accounts for all PBDE production in North America (congener BDE-209 is the primary congener).

1.3.2 Environmental Fate/Trends

Little is known about the environmental fate of PBDEs, however, the PBDE carbon-bromine bond is weaker than the carbon-chlorine bond in PCBs, and therefore is more susceptible to environmental degradation. Additionally, PBDEs are not covalently bound to the products; therefore, they can enter the environment via leaching (de Wit, 2002; Siddiqi et al., 2003) as well as from point sources such as manufacturing sites. In the absence of sunlight, the larger molecular weighted congeners, such as deca-BDEs, likely persist in sediments (Hooper and McDonald, 2000). BDE-209 is not as volatile as the lighter weighted congeners and hence it is less likely to be transported long distances from point sources (Hale et al., 2003). Although congener BDE-209 is considered less toxic because it is a larger molecule and absorption into the body is less likely, the main concern for this congener is its ability to degrade in the environment into more toxic forms (such as the penta- and octa- forms).

Uptake of PBDEs is most likely from food consumption, inhalation, and dermal absorption in some species (Siddiqi et al., 2003). PBDEs can also transfer from mother to offspring via gestation and lactation. Ikonomou and Addison (2008) describe total PBDE concentrations in British Columbia harbor seals and mother-pup pairs of grey seals (*Halichoerus grypus*) from Sable Island. They found a decline in transfer efficiency among the different congeners with increasing molecular weight. Tetra-BDEs were

highest in concentration in both mothers and pups followed by penta- and hexa-, respectively (Ikonomou and Addison, 2008).

Many marine species have recently experienced an almost exponential increase in accumulated concentrations of PBDEs (Ikonomou et al., 2002, 2006; Rayne et al., 2003). Mountain whitefish (*Prosopium williamsoni*) from the Columbia River experienced an exponential increase in PBDEs from 1992 to 2000 with an unprecedented doubling time of 1.6 years (Rayne et al., 2003). Ikonomou et al. (2002) found an exponential increase in penta-, hexa-, and tetra-BDEs in Canadian Arctic ringed seals (*Phoca hispida*) from 1981 to 2000 with doubling times of 4.3, 4.7, and 8.6 years, respectively. Ikonomou et al. (2006) presented an assessment of PBDE trends in British Columbia waters from 1992 to 2002. They found a steady increase in PBDEs in Dungeness crab (*Cancer magister*) between 1994 and 2002. Stranded beluga whales (*Delphinapterus leucas*) in the St. Lawrence Estuary were sampled for PBDEs between 1988 and 1999 (Lebeuf et al., 2004). Lebeuf et al. (2004) found no influence of age, sex, or blubber thickness on PBDE levels in the whales, which they suggest could be due to the rapid accumulation of total PBDEs with a doubling time of only 3 years for males and 2.2 years for females.

Norén and Meironyté (2000) found that PBDE levels in Swedish women's breast milk increased exponentially with a 5 year doubling time between 1972 and 1997. Because companies phased out PBDE use, there was a decline in PBDEs in Swedish breast milk after 1997 by almost 30%. In contrast, PBDEs in humans in the U.S. and Canada are 10 to 100 times that found in Europe (Schechter et al., 2003). For example, women from the San Francisco Bay area in the late 1990s had higher penta- and hexa-BDE levels than women from Europe and Canada (She et al., 2002). Indeed, PBDE levels in women from California appear to be the highest to date (She et al., 2002). Human serum pools collected in the U.S. from 1985 to 2002 showed a steady increase through time of six PBDE congeners (tetraBDEs to hexaBDEs) (Sjödín et al., 2004). Despite the fact that BDE-209 is a larger molecule making it less bioavailable compared to the other congeners, Schechter et al. (2003) found detectable levels of this deca-form in human breast milk.

1.3.3 Health Effects/Toxicity

Although the impacts of high PBDE loads in marine mammals are unknown, they may be similar to what has been observed in marine mammals with high PCB loads. Due to their similar molecular structure and persistence as PCBs, some of the PBDE congeners have a potential to cause cancer, neurodevelopmental toxicity, thyroid hormone imbalance, and adverse effects on reproductive organs in laboratory species (Hooper and McDonald, 2000; Hallgren et al., 2001; Legler and Brouwer, 2003; Ceccatelli et al., 2006). Penta-BDEs are more toxic, in general, compared to the octa- and deca- forms. Effects of greatest concern from penta- products include developmental neurotoxicity and thyroid hormone disruption (Darnerud, 2003). Developmental effects, such as a departure from “spontaneous behavior” (i.e. behavior important for survival such as hunting and predator avoidance), and affected learning and memory function, were found in rats from neonatal exposure of congeners BDE-47 and BDE-99 (Eriksson et al., 2001). In addition, Kuriyama et al. (2005) found that exposure to low doses of BDE-99 caused permanent reproductive effects such as low sperm counts in male rats. Decreased thyroid hormone levels (T4) were also found in rats from exposure to penta-products. These data as well as other studies suggest that PBDEs are endocrine disruptors (Zhou et al., 2002; Legler and Brouwer, 2003). Octa-BDEs have also shown to be endocrine disruptors by affecting the thyroid hormone system (Zhou et al., 2001) and fetotoxicity was the endpoint found in rats from an octa-BDE product (Darnerud, 2003). Even with deca-BDEs, absorption has been shown to induce developmental neurotoxicity in adult mice (Viberg et al., 2003) and cause thyroid changes in adult animals (Darnerud, 2003).

1.4 Study Population: Southern Resident Killer Whales (SRKWs)

Killer whales can accumulate high concentrations of POPs because they are long-lived apex predators. In the Pacific Northwest, three sympatric killer whale ecotypes termed the “resident”, “transient”, and “offshore” killer whales (Krahn et al., 2002) inhabit the region. While little is known about the offshore killer whales, the resident and transient types differ in many aspects of their behavior and ecology. These differences include the

frequency of vocalizations and specific call types (residents tend to be more vocal than transients; Barrett-Lennard et al., 1996; Deecke et al., 2005); matrilineal dispersal patterns (resident killer whales remain in their natal group while some transient killer whales leave their natal group; Baird and Whitehead, 2000); and pod size (resident pods are larger than transient pods; Ford et al., 2000). There are also differences in diet (residents tend to be fish-eaters while transients tend to be mammal-eaters; Ford et al., 1998; Herman et al., 2005), and there is genetic isolation between the two ecotypes (Hoelzel et al., 1998; Barrett-Lennard, 2000). Some researchers even propose that speciation is occurring between the resident and transient killer whales (Baird et al., 1992). On a smaller scale, several isolated populations exist within the killer whale ecotypes. The study reported in this thesis estimates the long-term bioaccumulation of PCBs and PBDEs in one of the resident killer whale populations.

The Southern Resident killer whales (SRKWs) are a resident population that consists of three pods (J, K, and L) whose summer geographic range is primarily from the Georgia Strait to Puget Sound (Bigg, 1982; Hauser et al., 2007). The winter ranges are less known but appear to be different among the three pods. Since the late 1970s, J pod has been seen in the Puget Sound area at least one week a month in the winter months (Osborne, 1999), whereas L and K pods have been spotted more recently along the outer coast, from California to Vancouver Island, during the winter months (Krahn et al., 2004).

The SRKWs have been studied since the early 1970s following several live-captures for the aquarium trade in the 1960s (Bigg and Wolman, 1975). A major research goal was to census the population. During the population surveys, researchers found that individuals could be identified by their distinctive dorsal fin and saddle patch colorations (Bigg et al., 1990). These unique features permitted researchers to track individual whales from birth to death using photo-identification. These studies provided a wealth of information regarding life histories and population dynamics (e.g. Olesiuk et al., 1990; Brault and Caswell, 1993).

The annual survey data indicate that the SRKWs experienced a 20% population decline from 1996 to 2001 (Krahn et al., 2004). In response to this decline and their low numbers, they were listed as “endangered” as defined by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2001 and by the U.S. Endangered Species Act of 1973 (ESA; 16 U.S.C.A. §§ 1531 *et seq.*, as amended) by the end of 2005. Several studies have examined possible hypotheses for the population decline including reduced prey availability (Ford et al., 2005, 2009; Ward et al., 2009); disturbance from noise pollution and concentrated vessel traffic (Bain et al., 2006); and high POP concentrations (Hayteas et al., 2000; Ross et al., 2000; Krahn et al., 2007, 2009). Recently, blubber biopsy samples were obtained from a few individual SRKWs (e.g. Ross et al., 2000; Rayne et al., 2004; Herman et al., 2005; Krahn et al., 2007, 2009). Analyses of all samples indicate that the SRKWs are currently one of the most contaminated cetacean populations in the world (Ross et al., 2000; Krahn et al., 2007).

Although the exact processes by which SRKWs accumulate PCBs and PBDEs are unknown, many of the general pathways are known or suspected. Contaminant concentrations in individual killer whales are dependent on diet, age, sex, and birth order (Ross et al., 2000; Ylitalo et al., 2001). Following weaning, individuals accumulate PCBs and PBDEs from their food. Because these contaminants bioaccumulate up the food chain, killer whales that consume prey in higher trophic levels, such as marine mammals, generally have higher contaminant loads than killer whales that consume prey at lower trophic levels, such as fish. Chinook salmon (*Oncorhynchus tshawytscha*), the SRKWs primary summer prey (Ford et al., 1998; Ford and Ellis, 2006; Hanson et al, in press) also carry relatively higher levels of lipophilic contaminants than other potential prey (e.g. other salmonids) in the SRKWs distribution range (O’Neill et al., 2006).

Physiological exposure to persistent organic pollutants increases throughout an individual consumer’s life because POPs resist metabolic degradation. However, age, sex, and reproductive status can explain a lot of the variability in observed contaminant levels. PCBs and PBDEs are primarily stored in the blubber but are mobilized during nutritional stress or during pregnancy (O’Shea, 1999). Therefore, females offload their contaminant

loads to their offspring via gestation and lactation while males retain virtually all of their contaminants. Consequently, reproductive females have lower contaminant levels than adult male killer whales. First born individuals acquire the majority of their mother's contaminant load, whereas subsequent offspring have lesser burdens (Ylitalo et al., 2001). Therefore, birth order also influences the contaminant concentrations in individuals.

1.5 Rationale and Objectives

Subsequent to a species being listed as “endangered” as defined by ESA, the agency with designated responsibility, in this case the National Marine Fisheries Service, is required to designate critical habitat and develop and implement a recovery plan. The objectives listed in the recovery plan take an adaptive management approach that includes continuous research and monitoring to address potential threats to the population. The SRKW Recovery Plan specifies monitoring and reducing the input of contaminants in the SRKWs habitat (National Marine Fisheries Service, 2006).

Determining the long-term effects of contaminants on a killer whale population is difficult because contaminant accumulation and effects occur over many years, and acquisition is age-, sex-, and birth-order specific. In these situations, quantitative models can be used to forecast the long-term burdens and effects of contaminant exposure on survival and reproduction of the individuals. This approach has been used successfully to estimate contaminant accumulation for beluga whales in the St. Lawrence Estuary (Hickie et al., 2000), arctic ringed seals (Hickie et al., 2005), North Atlantic right whales (*Eubalaena glacialis*; Klanjscek et al., 2007), the average resident killer whale (Hickie et al., 2007), and effects on reproduction in bottlenose dolphins (Schwacke et al., 2002; Hall et al., 2007).

This thesis describes a quantitative modeling approach to estimate PCB and PBDE concentrations in SRKWs and forecast long-term contaminant burdens. The current estimated levels were projected into the future and run under various assumptions and

scenarios to predict the potential long-term contaminant exposure on the population. Hickie et al., (2007) used a deterministic individual-based modeling approach to estimate PCB levels in the average resident killer whale. However, they did not account for individual variation and had a low sample size ($n = 4$) to validate their model results. This thesis uses an individual-based modeling approach to estimate both PCBs and PBDEs in specific individuals in the SRKWs that accounts for parameter uncertainty and validates the model with current data ($n = 21$ individuals; Krahn et al., 2007; 2009). This thesis will contribute to the recovery of the SRKWs because the results illustrate the dynamics of contaminant accumulation within an individual throughout its entire life, and allow assessment of interactions of life history traits and consequent patterns of contaminant levels seen across demographic categories. Projecting contaminant levels in individuals and in the population under various scenarios provide the basis for predicting the long-term effects of contaminants on the survival and fecundity of the population. The results of this thesis provide useful information for the recovery of this endangered species. Below is a description of the remainder of the thesis, divided into two additional chapters. Because the chapters will be submitted for publication as separate papers, there is some unavoidable repetition of text, particularly in the introductory sections.

1.5.1 Chapter 2 Objectives

Chapter 2 describes the PCB and PBDE individual-based models and PCB and PBDE projection models. The individual-based models describe the uptake of PCBs and PBDEs for each individual in the SRKW population for each year of the individual's life. The projection models forecast future levels and predict potential contaminant exposure. The specific objectives of my research were to

1. develop a life-cycle model for the accumulation of polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) in Southern Resident killer whales (SRKWs) that incorporates current research on energetics, diet, and contaminant levels in prey-fish.

2. use this model to forecast the long-term burdens of contaminant exposure in the SRKWs and estimate the historical contaminant levels.

1.5.2 Chapter 3 Objectives

Chapter 3 describes the respective influences of diet, gender, age and birth order on the predicted PCB and PBDE concentration levels from the individual-based model using a multivariate approach. The specific objectives of this chapter were to

1. assess similarities among individuals in the SRKW population and explore and identify any natural groupings in the model output (i.e. identify groups of individuals with similar life history traits and estimated contaminant levels).
2. determine the respective influences of life history traits that lead to the pattern of contaminant levels predicted in male and female killer whales.

To accomplish these objectives, multivariate techniques such as clustering and principal components analysis (PCA) were used. These techniques also provided information on the structure of the model and created succinct summaries of the model output.

Chapter 2: Past, Present, and Future Accumulation of PCBs and PBDEs in Southern Resident Killer Whales

2.1 Introduction

Three sympatric killer whale (*Orcinus orca*) ecotypes that inhabit the Pacific Northwest are termed the “resident”, “transient”, and “offshore” killer whales (Krahn et al., 2002). Southern Resident killer whales (SRKWs) are a fish-eating resident population that consists of three pods (J, K, and L) whose summer geographic range is primarily from the Georgia Strait to Puget Sound (Bigg, 1982; Osborne, 1999; Hauser et al., 2007). The winter ranges are less known but appear to differ among the three pods (Osborne, 1999; Krahn et al., 2004, 2007). Annual surveys have been conducted on the SRKWs since the early 1970s (Bigg, 1982). The annual survey data (courtesy of the Center for Whale Research, Friday Harbor, WA) indicate that the SRKWs experienced a 20% population decline from 1996 to 2001 (Krahn et al., 2004). In response to this decline and their low numbers (less than 100 individuals), they were listed as “endangered” as defined by the U.S. Endangered Species Act (ESA; 16 U.S.C.A. §§ 1531 *et seq.*, as amended) in 2005. Several studies have examined possible hypotheses for the population decline including reduced prey availability (Ford et al., 2005, 2009; Ward et al., 2009); disturbance from noise pollution and concentrated vessel traffic (Bain et al., 2006); and high persistent organic pollutant (POP) concentrations (Ross et al., 2000; Krahn et al., 2007, 2009). SRKWs accumulate relatively high concentrations of POPs because they are long-lived, their prey are distributed near point sources, and mothers transfer a significant proportion of their burden to their offspring. Recently, blubber biopsy samples were obtained from a few individuals of the SRKW population (e.g. Ross et al., 2000; Rayne et al., 2004; Herman et al., 2005; Krahn et al., 2007, 2009). Analyses of all samples indicate that the SRKWs are currently one of the most contaminated cetacean populations in the world (Ross et al., 2000; Krahn et al., 2007, 2009).

Polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs) are POPs of particular concern because they resist metabolic degradation, are persistent in

nature, bioaccumulate in upper trophic level species, and may cause deleterious biological effects in many species (Jones and de Voogt, 1999). Adverse health responses in marine mammals have been associated with high levels of PCBs; some of these responses include suppressed immune systems, endocrine disruption, weakened reproduction and premature births (Gilmartin et al., 1976; Reijnders, 1986; Aguilar and Borrell, 1994b; de Swart et al., 1994; Ross et al., 1995; Schwacke et al., 2002; Fossi and Marsili, 2003). Although the impacts of high PBDE loads in marine mammals are unknown, they may have similar impacts to high PCB loads because of their similar molecular structure and mode of action. Studies have found that relatively high levels of PBDEs cause endocrine disruption, particularly to thyroid hormone systems as well as adverse effects on reproductive organs in laboratory species (Hallgren et al., 2001; Legler and Brouwer, 2003; Ceccatelli et al., 2006). PCBs and PBDEs are primarily stored in the blubber of marine mammals but can become mobilized during times of nutritional stress (O'Shea, 1999). Thus the effects of contaminants may interact with and be compounded by additional stresses associated with nutritional limitation.

There are several processes by which persistent organic contaminants enter the environment, including direct release, atmospheric and ocean transport, and runoff (Iwata et al., 1993; Grant and Ross, 2002). PCBs were produced and discharged in the U.S. beginning in the early 1920s and reached a peak in production by the 1960s and 1970s (Lefkovitz et al., 1997; Breivik et al., 2002a). They had been primarily used as industrial lubricants and coolants for transformers and capacitors prior to being banned in the U.S. in 1977 due to their adverse health effects. In contrast, the production of PBDEs began in the 1970s and these chemicals are currently used as additives in flame retardants (Yogui and Sericano, 2009). PBDEs have a ubiquitous distribution and many marine species have shown an exponential increase in their bodily PBDE loads (Ikonomou et al., 2002, 2006; Rayne et al., 2003).

Previous studies have found that contaminant concentrations in killer whales are dependent on several factors (Ross et al., 2000; Ylitalo et al., 2001). For example, diet can affect the POP concentration in an individual. The SRKWs primary summer prey is

Chinook salmon (*Oncorhynchus tshawytscha*; Ford et al., 1998; Ford and Ellis, 2006; Hanson et al., in press). Chinook salmon carry relatively high levels of lipophilic contaminants (O'Neill et al., 2006) and these high contaminant levels in the prey have led to the high contaminant levels found in the SRKWs. Due to prey consumption, contaminant concentrations in an individual tend to increase with age. However, females have the ability to offload their contaminant loads to their offspring via gestation and lactation while males retain virtually all of their contaminants (Subramanian et al., 1987; Aguilar and Borrell, 1994a; Tuerk et al., 2005). Consequently, reproductive females have lower contaminant levels than adult male killer whales. As a result, a pattern is seen in which concentrations increase throughout the individual's life for males but decrease with age for females until they become post-reproductive at which point concentrations begin increasing (e.g. Subramanian et al., 1987; Aguilar and Borrell, 1994a). Offloading by females to their offspring also means that birth order can influence the contaminant concentrations in individuals because first born individuals acquire the majority of their mother's contaminant load, whereas subsequent offspring receive lesser burdens (Ylitalo et al., 2001).

The objectives of this study were to use a quantitative modeling approach to estimate PCB and PBDE concentrations in individual SRKWs and forecast the long-term burdens of contaminant exposure. A quantitative modeling approach has been used successfully to estimate contaminant accumulation for beluga whales (*Delphinapterus leucas*) in the St. Lawrence Estuary (Hickie et al., 2000), arctic ringed seals (*Phoca hispida*; Hickie et al., 2005), North Atlantic right whales (*Eubalaena glacialis*; Klanjscek et al., 2007), and the average resident killer whale (Hickie et al., 2007). This study extends the information from Hickie et al. (2007) by providing predicted PCB and PBDE concentrations in specific SRKW individuals (n=182).

2.2 Methods

2.2.1 Individual-Based Model

The individual-based model describes the uptake of PCBs and PBDEs for each individual in the SRKW population for each year of the individual's life. Model components are organized and described as follows: model description, parameterization, validation and model runs, and sensitivity analysis.

2.2.1.A Model Description

The model simulates the total accumulation and transfer of PCBs and PBDEs in individual SRKWs. There were 182 known individuals that had been identified in the annual surveys (data courtesy of the Center for Whale Research). Growth, energetics, and contaminant accumulation were tracked in these 182 individuals for each year of the individual's life. The model's time-step is one year and the estimated contaminant accumulation occurs at the end of each year. Subscript and parameter descriptions are listed in Tables 2.1 and 2.2 respectively. Predicted killer whale body burdens are reported in *ng* and contaminant concentration values are reported in *ng/g* lipid blubber weight (*hw*).

Figure 2.1 shows the input pathways for PCBs and PBDEs for an individual female *i*, in year *y*, in pod *p*, and age-class *x*. The total contaminant intake of an individual includes contaminant burdens from the prey ($PI_{c,y,p,x}$), contaminant burdens from year *y*-1 ($totC_{c,i,y-1}$), and contaminant burdens from gestation and nursing ($totC_{c,k,y,x=0}$; $totC_{c,k,y,x=1}$). Prey intake is a function of contaminant *c*, year *y*, pod *p*, and age-class *x*. The transplacental transfer and lactation offloading percentages vary among individual females *i*. The PCB model includes an excretion or elimination rate constant *E* (yr^{-1}) parameter as an output pathway via urine and feces but this is not included in Figure 2.1.

In general, individuals acquire contaminant loads throughout their life. A newborn acquires a percentage of its mother's total body burden via transplacental transfer during pregnancy. In the subsequent year, the calf nurses and accumulates an increased

percentage of the mother's total body burden. From age 2 years, individuals accumulate PCB and PBDE loads from prey consumption. In the PCB model, individuals excrete a small amount of the total PCBs via urine and feces. Elimination rates have not been quantified in marine mammals, however Hickie et al. (2007) estimated a PCB elimination half life of 28.7 to 43.1 years or a rate of approximately 0.016 to 0.024 yr^{-1} . Currently there are no excretion rates for the PBDE model. However, because the accumulation rate of PBDEs is assumed to be exponential, it is assumed that the amount excreted by an individual would be considerably less than the intake and essentially E would have a trivial impact on an individual's final concentration. The details and equations for each pathway are provided below.

Total PCB and PBDE Accumulation ($totC_{c,i,y}$)

The total contaminant c (PCBs or PBDEs) accumulation for an individual i (of age 2+) in year y ($totC_{c,i,y}$; reported in ng) equals the total contaminant accumulation in year $y-1$ ($totC_{c,i,y-1}$) plus the contaminant accumulation via prey intake ($PI_{c,y,\rho,x}$; equation 2.7) minus PCB excretion ($E(totC_{c=PCB,i,y})$):

$$totC_{c=PCB,i,y} = totC_{c=PCB,i,y-1} + PI_{c=PCB,y,\rho,x} - E(totC_{c=PCB,i,y}) \quad [\text{equation 2.1}]$$

$$totC_{c=PBDE,i,y} = totC_{c=PBDE,i,y-1} + PI_{c=PBDE,y,\rho,x} \quad [\text{equation 2.2}]$$

where E is a uniform random variable with lower and upper bounds of 0.016 and 0.024, respectively (Hickie et al., 2007). Approximately 80% of the total contamination ($totC_{c,i,y}$) is assumed to be in the blubber (Ross et al., 2000; Hickie et al., 2007) therefore the total contamination is reduced by 20% at the end of each year. The 20% reduction of the total contamination is assumed to be in the body core (e.g. brain, liver, and kidneys) and is no longer tracked by the model.

If the individual is female and gave birth in year y , she offloaded a percentage TT_i of her contaminant loads via transplacental transfer and her total PCB and PBDE body burdens were reduced to:

$$totC_{c,i,y} = (totC_{c,i,y})(1 - TT_i) \quad [\text{equation 2.3}]$$

For any given year y , the PCB and PBDE burdens for the age 0 offspring k ($totC_{c,k,y,x=0}$) are the amounts offloaded from the mother (or individual) due to transplacental transfer after converting the mother's concentration level (ng/g lipid weight in the blubber in an adult female, $L_{i,y}$) to the calf's concentration level (ng/g lipid weight in the blubber of a calf, $L_{k,y}$).

$$totC_{c,k,y,x=0} = [(totC_{c,i,y})(TT_i)] \quad [\text{equation 2.4}]$$

If the individual was female and gave birth in year $y-1$, she offloaded a percentage (LO_i) of her contaminant load via lactation and her PCB and PBDE burdens were reduced to:

$$totC_{c,i,y} = (totC_{c,i,y})(1 - LO_i) \quad [\text{equation 2.5}]$$

The contaminant load for age 1 offspring k ($totC_{c,k,y,x=1}$) increases from age 0 to age 1 from lactation because the mother's load is being transferred to her nursing offspring:

$$totC_{c,k,y,x=1} = totC_{c,k,y-1,x=0} + (totC_{c,i,y})(LO_i) \quad [\text{equation 2.6}]$$

The contaminant accumulation via prey intake ($PI_{c,y,p,x}$) is the product of the contaminant concentration in the prey for year y for pod p in the Puget Sound/Georgia Basin, $P.S.$, and off the outer coast, $O.C.$ ($PC_{c,y,p,S/O.C.}$; reported in $\mu g/kg$ wet weight),

and the annual biomass of fish ($BIO_{x,g}$; reported in kg) consumed by an individual i in year y .

$$PI_{c,y,p,x} = PC_{c,y,P.S.I.O.C.} * BIO_{x,g} \quad \text{[equation 2.7]}$$

To convert an individual's body burden to a concentration, the total body burden ($totC_{c,i,y}$) was divided by the lipid blubber weight ($L_{i,y}$) for the individual and ($L_{k,y}$) for the calf.

Parameters were varied within a biological range in different simulations to explore the sensitivity of the results to the precise parameter values. The parameters that were varied in different simulations (see Table 2.2) included the percentage offloaded via gestation and lactation (TT_i, LO_i), and the PCB elimination rate E . Within a simulation, some parameters had yearly variation and other parameters had variation across individuals (see Table 2.2). The parameters that varied by year included the prey's caloric content (CC_y) and the prey's contaminant concentration ($PC_{c=PCB,y,P.S.I.O.C.}, PC_{c=PBBE,y,p}$). Lastly, the parameters that varied across individuals included the total body mass ($M_{x,g}$), blubber mass ($B_{i,y}$), lipid blubber weight ($L_{i,y}$), energetic requirements ($DPER_{x,g}$), annual biomass consumed ($BIO_{x,g}$), and prey intake ($PI_{c,y,p,x}$). Section 2.2.1.B describes each parameter used in equations 2.1 to 2.7.

2.2.1.B Parameterization

Killer Whale Mass and Energetic Requirements ($M_{x,g}, DPER_{x,g}$)

In this section, the calculations behind the mass of an individual in age-class x and gender g ($M_{x,g}$) and the daily prey energetic requirements for a male or female in age-class x

($DPER_{x,g}$) are shown. Values for $M_{x,g}$ and $DPER_{x,g}$ resulting from these calculations are presented in Tables 1 and 2 of Appendix A.

The energetic requirement of an individual ($DPER_{x,g}$) is dependent on its mass as well as its activity or behavior states (Kleiber, 1975; Costa, 2002). To obtain an estimate of minimum and maximum DPERs for free-ranging killer whales, Noren (in revision) used published field metabolic rates (FMRs)—or the metabolic rates of active free-ranging individuals—of marine mammals and estimated that daily energy expenditure in killer whales is between 5 (minimum) and 6 (maximum) times the predicted basal metabolic rate (BMR).

BMR is the amount of energy required by a non-reproductive adult at rest. Kleiber (1975) determined that BMR for terrestrial mammals can be predicted by one equation:

$$BMR = 70M_{x,g}^{.75} \quad \text{[equation 2.8]}$$

where $M_{x,g}$ is the total body weight in kg of an individual in age-class x and of gender g .

Following Noren (in revision), the estimates of minimum and maximum daily energy expenditure (*i.e.*, 5 to 6 times BMR) were converted to minimum and maximum daily prey energetic requirements ($DPER_{x,g}$, reported in *kcal/day*) by assuming a digestive efficiency of 84.7% for killer whales (Williams et al., 2004).

$$\min DPER_{x,g} = 413.2 * M_{x,g}^{.75} \quad \text{[equation 2.9]}$$

$$\max DPER_{x,g} = 495.9 * M_{x,g}^{.75} \quad \text{[equation 2.10]}$$

The DPER equations (equations 2.9 and 2.10) require an estimate of the mass of an individual of age-class x and gender g . Clark et al. (2000) used the Gompertz model to

compute mass in captive female killer whales in age-class x using measured lengths and weights of known-aged individuals:

$$M_x = W[\exp(-b * \exp(-k * age))] \quad \text{[equation 2.11]}$$

where age is in *days* and mass for age-class x (M_x) is in *kg*. W is the asymptotic weight, b is the integration constant, and k is the growth rate constant. Noren (in revision) used the parameters developed by Clark et al. (2000) to predict total body mass for male and female killer whales aged 1-12 years old ($W = 2,763$ *kg*, $b = 2.3$, $k = 0.0007$). Females aged 13 through 20 years were assumed to grow at a constant rate of 107 *kg year*⁻¹ and females ≥ 20 years were assumed to maintain a body mass of 3338 *kg* (following Noren, in revision). A pregnant female's weight was calculated as equal to the weight of a sexually mature female in age-class x , plus the weight of a newborn calf ($M_0 = 155$ *kg*; Clark et al., 2000). The result of this assumption is that the predicted DPER of pregnant females is 3 – 5% greater than those of non-pregnant females of the same age for the entire gestation period. This is because the model assumed that the mass of a pregnant female would be equivalent to that at full-term for the entire year. A more accurate approach to predict the energetic requirements of pregnant females might be to track the monthly growth of individuals and their calves *in utero*. However, the sensitivity analysis revealed the model output is robust to small changes in the DPER (see sections 2.2.2 and 2.3.2). Thus, the simpler approach of an annual time step was used. For males aged 13 through 20 years, Noren (in revision) assumed a constant rate of growth of 244 *kg year*⁻¹, and that males ≥ 20 years maintained a total mass of 4434 *kg*. The individual age data from the annual surveys (data courtesy of the Center for Whale Research) with the mass-at-age estimates from Noren (in revision) were used to estimate the mass for each individual in the population at each year during its life. The estimated mass for each individual was then used to estimate the energetic requirements for each individual at age x .

There is annual variability in the energetic requirements of an individual killer whale as well as variability among individuals in similar age- and sex-classes. For example, a juvenile female may need more energy due to growth than an adult female during senescence. A pregnant or lactating female will also have higher energy demands than another female in the same age-class that is not pregnant or lactating. However, it is assumed that pregnant and lactating females have a DPER that is within the range of 5 to 6 times the predicted BMR based on the Kleiber relationship (Noren, in revision). Therefore, the model randomly selected values from a uniform distribution between the minimum and maximum DPER values for each individual i in each year y regardless of reproductive status:

$$DPER_{x,g} = U(\min DPER_{x,g}, \max DPER_{x,g}) \quad [\text{equation 2.12}]$$

Prey Caloric Content and Consumption ($CC_y, BIO_{x,g}$)

While Chinook salmon (*Oncorhynchus tshawytscha*) are not the only fish consumed by the SRKWs (Ford et al., 1998), and the different pods are likely to consume different proportions of different salmon stocks, Chinook salmon are thought to be the primary summer diet of SRKWs based on available data (Ford et al., 1998; Ford and Ellis, 2006, Hanson et al., in press). For simplicity, it was assumed that the SRKWs consume solely Chinook salmon for the prey caloric content estimates. J pod is seen in the Puget Sound/Georgia Basin region throughout the year with winter observations less often than summer observations (Osborne, 1999), therefore one model scenario (“uniform” diet scenario) assumed 100% of their prey consists of Puget Sound/Georgia Basin Chinook salmon. The model defines Puget Sound/Georgia Basin region Chinook salmon as salmon from the Fraser, Duwamish, Nooksack, Nisqually, and Deschutes Rivers. K and L pods, however, have been seen along the outer coast from California to Vancouver Island during the non-summer months, or approximately 2/3 of the year, and are observed approximately 1/3 of the year in the inland waters (Krahn et al., 2004). Therefore, another model scenario (“mixed” diet scenario) assumed K and L pod diets included outer coast Chinook salmon for 2/3 of their diet and included Puget Sound/Georgia Basin

Chinook salmon for 1/3 of their diet. The model defines outer coast Chinook salmon as fish from the Sacramento, Columbia (fall and spring runs), and Skeena rivers.

Using the DPERs for killer whales (equation 2.12) and the caloric content (*kcal/kg*) of Chinook salmon in year *y* (CC_y), the average annual biomass of Chinook salmon consumed by each individual in age-class *x* and gender-class *g* was estimated as:

$$BIO_{x,g} = (DPER_{x,g} / CC_y)365 \quad \text{[equation 2.13]}$$

The caloric content (CC_y) used in the simulations was based on the caloric content of Chinook salmon sampled in Puget Sound/Georgia Basin and on the outer coast (O'Neill et al., 2006). It is reasonable to assume that the caloric content of fish varies seasonally as well as annually and therefore the average caloric content of Chinook salmon consumed by a killer whale, CC_y , will vary among years. Indeed, the killer whales are consuming different salmon stocks and the caloric content of those stocks varies between years depending on factors such as the productivity of salmon prey at sea. Due to this variability, the model randomly drew from a uniform random variable for CC_y for each year, with an upper bound of 1804 *kcal/kg* and a lower bound of 1643 *kcal/kg*:

$$CC_y = U(1643,1804) \quad \text{[equation 2.14]}$$

The range from 1643-1804 *kcal/kg* reflects the 95% CI for the average Chinook salmon sampled in years 2000 and 2004 from the Puget Sound/Georgia Basin region and along the outer coast from western Vancouver Island to California (O'Neill et al., 2006).

Blubber Mass and Total Lipid Blubber Mass ($B_{i,y}$, $L_{i,y}$)

The standard unit of contaminant concentration is reported in *ng/g* total lipid blubber weight, the determination of which requires an estimate of total blubber mass ($B_{i,y}$) and

total lipid mass ($L_{i,y}$) in the blubber of an individual. A blubber mass range (equations 2.15 and 2.16) was estimated for each age group, where age x is ≤ 20 , using an adapted relationship established from the blubber and age relationship found in bottlenose dolphins sampled in the winter (Noren and Wells, 2009). Individuals aged ≥ 20 are assumed to be physically mature and maintained total blubber mass within the range of a 20 year old. The adapted relationship encompassed the estimated blubber mass of 30% total body mass found in adult killer whales (Christensen, 1982):

$$B\%_{i,y,lower} = 39 * x_i^{-10} \quad \text{[equation 2.15]}$$

$$B\%_{i,y,upper} = 44 * x_i^{-10} \quad \text{[equation 2.16]}$$

The model then drew from a uniform distribution between the lower and upper bounds to estimate the blubber mass as a percentage of total body mass in the individual:

$$B\%_{i,y} = U(B\%_{i,y,lower}, B\%_{i,y,upper}) / 100 \quad \text{[equation 2.17]}$$

The blubber mass of individual i during year y ($B_{i,y}$) is the product of the mass of the individual of age-class x and gender g ($M_{x,g}$) and the estimated percentage blubber ($B\%_{i,y}$):

$$B_{i,y} = M_{x,g} * B\%_{i,y} \quad \text{[equation 2.18]}$$

The percentage lipid in the blubber was measured for a limited number of killer whale samples (Krahn et al., 2004, 2007, 2009; Herman et al., 2005; Koopman, 2007). Krahn et al. (2004) collected samples from an L pod individual via both biopsy (post-mortem) and necropsy. They found that the percentage lipid in the biopsy sample (post-mortem) ranged from 8-10% whereas the percent lipid from the necropsy ranged from 28-40% at a similar depth (0-2 cm). Biopsy samples from free-ranging resident killer whales in the

eastern North Pacific had an average of 10% lipid in the blubber (Herman et al., 2005). Lower lipid levels found in biopsy samples may result from leaching of lipid from the sample during ejection of the biopsy dart from the animal, or when the dart enters the water (Krahn et al., 2004). Therefore, the model generated the percent lipid for every individual i in each year y ($L\%_{i,y}$) using the range of lipid percentages established from the necropsy (28-40%; Krahn et al. 2004) where leaching was not a concern:

$$L\%_{i,y} = U(.28,.40) \quad \text{[equation 2.19]}$$

The percentage lipid ($L\%_{i,y}$) was multiplied by the estimated blubber mass ($B_{i,y}$) to calculate the reported total lipid mass in the blubber ($L_{i,y}$) for each individual in the population in each year:

$$L_{i,y} = L\%_{i,y} * B_{i,y} \quad \text{[equation 2.20]}$$

Total concentration values were predicted by dividing the total body burden ($totC_{c,i,y}$) by the estimated total lipid mass in the blubber ($L_{i,y}$).

Transplacental Transfer and Lactation Offloading (TT_i and LO_i)

Contaminant concentrations differ among individual killer whales not only because of the influence of age and sex (Ross et al., 2000), but also because of recruitment or birth order (Ylitalo et al., 2001). The total contaminant load accumulated in a calf prior to weaning depends solely on the mother's contaminant load. Females offload their contaminant burdens via transplacental transfer and lactation. In general, a female's first-born calf will acquire a much higher load from the mother than any subsequent calf (Ylitalo et al., 2001).

Currently, the amount of contaminants typically offloaded from mother to calf in killer whales is unknown and may vary among individuals. However, PCB offload data are available for other delphinids. Female bottlenose dolphins off South Africa offload almost 80% of their total PCB body burden to their first-born offspring via transfer during gestation and then lactation (Cockcroft et al., 1989). Cockcroft et al. (1989) calculated that approximately 4% of a female's total body burden can be transferred daily during lactation, indicating the mother's full load would be transferred after approximately 7 weeks of lactation. Salata et al. (1995) estimated a transplacental transfer of 3.7% of the total body burden in female bottlenose dolphins (*Tursiops truncatus*) off the Gulf of Mexico. Fukushima and Kawai (1980) found a maximum transfer of the total body burden to be approximately 88% from lactation in striped dolphins (*Stenella coeruleoalba*), while the percent of total load transferred during gestation were estimated to be approximately 3.8%. Tanabe et al. (1981) also reported a transplacental transfer percentage of 3.7% and lactation offload percentage up to 90% of the total body burden for striped dolphins.

The amount of contaminants offloaded due to lactation varies within a year (Cockcroft et al., 1989). For simplicity, offloading was assumed to occur in one time step at the end of the first year. The calf was assumed not to consume fish until the beginning of the second year (Heyning, 1988) although weaning can be variable among individuals within a species. It is possible that the calf will begin to consume fish prior to turning age one. However, the amount of contaminants acquired from fish compared to the amount acquired from the mother in the first year is probably negligible.

Based on information in the published literature, it is reasonable to assume that the proportion of a mother's total contaminant body burden offloaded to a fetus during gestation is between 3% and 5% of the mother's total load, while the amount offloaded to a dependent calf during lactation is between 70% and 90% of the mother's load. Offloaded amounts probably vary by individual female, however it is unknown if these amounts change over time as females age and their contaminant loads change. The model also assumes that the PBDE offload parameters have the same range as the PCB

offload parameters with the caveat that little is currently known about the transfer rate of PBDEs in killer whales or in other delphinids. However, comparable PCB and PBDE transfer rates are a reasonable assumption because of the similar chemical structure and mode of action of these two classes of contaminants.

For each female in each simulation, the model randomly drew the transplacental transfer percentage for individual i (TT_i) from a uniform distribution with a lower bound of 3% and an upper bound of 5%. The transfer percentage for a given individual was constant throughout the female's life, but varied among individuals and simulations:

$$TT_i = U(3\%,5\%) \quad \text{[equation 2.21]}$$

The model also randomly drew the lactation offload percentage for female i (LO_i) from a uniform distribution with lower and upper bounds of 70% to 90%, respectively, for each female in each simulation.

$$LO_i = U(70\%,90\%) \quad \text{[equation 2.22]}$$

A missed calf (MC) factor was added to the model to compensate for any calves that were missed during the annual surveys. Calves can be missed if they are born during the winter and die prior to the annual summer surveys. In general, females mature sexually and give birth to their first calf between the ages of 12 and 17 years, and reproductive senescence usually occurs by age 40 (Olesiuk et al., 2005). The majority of these females have a calving interval of 3 to 7 years (Olesiuk et al., 2005). If a reproductive female was not observed with a calf within 5 years of her previous calf, the model assumes a calf was missed and generates a "missed calf". The MC factor was set to offload via "gestation" and "lactation" in a reproductive female aged 12 to 40 years if that female was not seen to have given birth to a calf in 5 years (see Appendix B for the pseudo code). It was assumed females stop producing calves by the age of 40. The MC factor is important not only for missed calves that may have been born and died before the annual summer

surveys, but also prior to the initial surveys in the early 1970s. It is probable that a current reproductive female that was sexually mature prior to the initial surveys and has not been observed with offspring gave birth several times and lost the calves prior to the initial surveys.

PCB Concentration Levels in Killer Whale Prey ($PC_{c=PCB,y,P.S/O.C.}$)

PCB accumulation via prey intake ($PI_{c=PCB,y,p,x}$; equation 2.7) is the product of the PCB concentration in the prey consumed in year y (for Puget Sound/Georgia Basin Chinook salmon, $PC_{c=PCB,y,P.S.}$; for outer coast Chinook salmon, $PC_{c=PCB,y,O.C.}$) and the annual biomass of prey consumed ($BIO_{x,g}$, equation 2.13). Thus, to calculate the contaminant intake for an individual older than 1 year in a given year y , estimates of contaminant concentrations in prey during year y were needed. In the calculations described below, prey is assumed to be Chinook salmon exclusively.

A recent study examining contaminant patterns in Chinook salmon from the Puget Sound/Georgia Basin and the outer coast of Washington indicate that contaminant concentrations vary by geographic location (O'Neill et al., 2006). The average concentration of PCBs was found to be 47 $\mu\text{g}/\text{kg}$ wet weight ($w\text{w}$) in Puget Sound/Georgia Basin Chinook salmon sampled in the 1990s and the early 2000s, and 23 $\mu\text{g}/\text{kg}$ $w\text{w}$ in outer coast Chinook salmon (O'Neill et al., 2006).

Although there is no historical time series of data for contaminant concentrations in Chinook salmon in the Puget Sound/Georgia Basin region or along the outer coast, PCB levels in Puget Sound sediment have been estimated since the early 1900s (Lefkovitz et al., 1997) and in Puget Sound harbor seals (*Phoca vitulina*) since the mid 1980s (Calambokidis et al., 1999). Lefkovitz et al. (1997) found a steady increase in PCB concentration in the sediment beginning in 1930 with levels peaking in the early 1960s. Levels in the 1960s were close to 4 times the current levels of PCB sediment contamination. Puget Sound harbor seal PCB concentrations decreased dramatically

from 1970 to the middle 1980s, and have been relatively constant from the mid 1980s to the late 1990s (Calambokidis et al., 1999).

The sediment and harbor seal PCB historical time series were used as proxies for the PCB contaminant loads in Puget Sound/Georgia Basin Chinook salmon. Current proxy levels were set to current average Chinook salmon PCB concentration levels by multiplying the proxy levels by scaling factors. To estimate the PCB trend in Puget Sound/Georgia Basin Chinook salmon, a time series for the proxies for the period 1931 to 2007 was developed, using piecewise linear function divided into 3 time periods. The first time period (1931:1959 or years 1:29) showed an increasing trend, the second time period (1960:1988 or years 30:58) showed a decreasing trend, and the third time period (1989:2007 or years 59:77) had no trend. Negative concentrations, such as those predicted for 1931 and in 1932, were set to equal zero. Proxy values were multiplied by the scaling factors (5.4 and 7.6 for equations 2.23 and 2.24, respectively) to estimate the 1931 to 2007 average Chinook salmon PCB contaminant concentrations. The outer coast Chinook salmon were assumed to have a different contaminant history. Outer coast Chinook salmon were assumed to have PCB levels that increased linearly from 1930 (no PCBs) to current levels because there is no evidence that the outer coast had three distinct time periods as the Puget Sound/Georgia Basin data did. Moreover, during model validation, PCB concentrations along the outer coast fit the data better when assuming a linear trend. Scaling factors for outer coast Chinook salmon trends were 2.8 and 3.6 for equations 2.26 and 2.27, respectively.

To allow yearly variability in the contaminant concentrations of Puget Sound/Georgia Basin Chinook salmon ($PC_{c=PCB,J,P,S}$; reported in $\mu g / kg$ wet weight), the annual Puget Sound/Georgia Basin Chinook salmon concentration level was selected from a uniform distribution with lower and upper bounds that equaled the lower and upper 95% confidence limits ($CI_{ps,low}, CI_{ps,high}$), respectively, for the average PCB levels found in Puget Sound/Georgia Basin Chinook salmon (O'Neill et al., 2006).

$$CI_{ps,low} = \begin{cases} 5.6y - 11.7 \\ -4.1y + 279 \\ 38.7 \end{cases} \quad \text{[equation 2.23]}$$

$$CI_{ps,high} = \begin{cases} 7.88y - 16.49 \\ -5.78y + 393 \\ 54 \end{cases} \quad \text{[equation 2.24]}$$

$$PC_{c=PCB,y,P.S.} = U(CI_{ps,low}, CI_{ps,high}) \quad \text{[equation 2.25]}$$

The PCB concentration in outer coast Chinook salmon was selected from a uniform distribution with lower and upper bounds equal to the lower and upper 95% confidence limits, respectively, from outer coast Chinook salmon ($PC_{c=PCB,y,O.C.}$ reported in $\mu\text{g} / \text{kg}$ wet weight; O'Neill et al., 2006).

$$CI_{outer,low} = .35y - .35 \quad \text{[equation 2.26]}$$

$$CI_{outer,high} = .456y - .456 \quad \text{[equation 2.27]}$$

$$PC_{c=PCB,y,O.C.} = U(CI_{outer,low}, CI_{outer,high}) \quad \text{[equation 2.28]}$$

PBDE Concentration Levels in Killer Whale Prey ($PC_{c=PBDE,y,P}$)

There are no sediment data available for PBDEs. However, PBDE levels were tracked in two fish species from the Columbia River system from 1992 to 2000 (Rayne et al., 2003) and in Dungeness crab (*Cancer magister*) in British Columbia waters from 1994 to 2000 (Ikonomou et al., 2006). Results from these studies indicate that PBDE concentration levels have increased exponentially. These local increases in PBDE concentrations match general trends in levels of PBDEs on a global scale. For example, PBDE congener doubling times ranged from 4.3 years and 8.6 years in arctic ringed seal (*Phoca hispida*)

blubber in the Canadian Arctic (Ikonomou et al., 2002). Hites (2004) found that total PBDEs (Σ PBDEs) in human blood, milk, and tissue had a doubling time of approximately 5 years, while marine mammals throughout the world had concentration doubling times of approximately 5 to 7 years. In addition, PBDE accumulation in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Canada, had a doubling time of almost 3 years (Lebeuf et al., 2004).

Following the PBDE trends seen locally and worldwide, the model assumes that PBDE intake via prey consumption for an individual is the product of the concentration of PBDEs in the prey consumed by pod p ($PC_{c=PBDE,y,p}$; reported in $\mu\text{g}/\text{kg}$ wet weight) and the annual biomass of prey consumed ($BIO_{x,g}$). The PBDE concentration accumulation from the prey ($PC_{c=PBDE,y,p}$) was assumed to be exponential with a doubling time (T) ranging from 3.2 to 4.0 years (with variations among the pods and from year to year). These doubling times were chosen because they are consistent with what is seen in other local marine species and were also found to be a reasonable assumption during model validation. To allow for annual variability, the model drew the doubling time parameter (T_y) from a uniform distribution for each year. The first year ($y = 0$) was 1970 and the initial PBDE concentration was $PC_{c=PBDE,y=0,p} = .01$.

$$PC_{c=PBDE,y,p} = PC_{c=PBDE,y=0,p} \exp(ry) \quad [\text{equation 2.29}]$$

$$r = \ln(2)/T_y \quad [\text{equation 2.30}]$$

2.2.1.C Validation and Model Runs

The individual-based model was validated by comparing the model results to the available biopsy data ($n = 21$; Krahn et al., 2007, 2009) consisting of PCB and PBDE concentrations in SRKWs. Only a few biopsy samples have been collected from individuals in the SRKW population. However, the samples cover both sexes and a range

of ages and birth histories. The measured PCB and PBDE concentrations from the biopsy samples are shown in Appendix A, Table 3.

The contaminant concentrations in an individual for each year were calculated by averaging 100 simulations. For each simulation in the PCB and PBDE models, the female offload parameters (TT, LO_i) were randomly drawn and remained constant throughout the individual's life, changing only among simulations because it is unknown if these amounts change over time as females age and their contaminant loads change. In contrast, the caloric content of the prey (CC_p) changed in each year, but was constant in a given year across individuals. The PCB values of Puget Sound/Georgia Basin prey were also drawn for each year from the 95% confidence intervals in the piecemeal linear functions (equation 2.25) and the PCB values for the outer coast prey were generated from the linear equations 2.26 and 2.27. PBDE values were generated each year using equation 2.29. Ultimately, this model assumes each female will offload a different percentage of her total body burdens to her offspring, and that these percentages are similar for each individual's pregnancy. Furthermore, the model assumes that Chinook salmon have inter-annual variation in their size and contaminant loads, but no variation in caloric content of fish or contaminant levels per kg fish consumed among killer whale individuals within a given year. For further clarification, pseudo codes for the PCB and PBDE estimations are provided in Appendix B.

Two diet scenarios were run to simulate PCB accumulation. Both scenarios assumed a simplistic diet for all three pods where the SRKWs consumed solely Chinook salmon. Although Chinook salmon have been identified as the preferred summer prey (Ford et al., 1998; Ford and Ellis, 2006; Hanson et al., in press), Chum salmon (*Oncorhynchus keta*) were found to be an important prey item in the fall for Northern residents (Ford and Ellis, 2006) and Southern residents (NWFSC unpubl.data). The result of this simplistic diet assumption is that the PCB accumulation is higher than that of a more complex diet scenario consisting of both Chinook and Chum salmon. This is because POP concentrations and lipid levels in Chum salmon were found to be lower than those measured in Chinook salmon (O'Neill et al., 2006). However, the sensitivity analysis

revealed the model output is robust to small changes in the prey contamination (see section 2.2.2). Thus, the simpler diet approach was used. In the “uniform” scenario, J pod consumed Chinook salmon from the Puget Sound/Georgia Basin region whereas K and L pods consumed Chinook salmon from the outer coast. The “mixed” scenario assumed J pod consumed Chinook salmon from the Puget Sound/Georgia Basin region 2/3 of the time and consumed outer coast Chinook salmon 1/3 of the time to correspond with their seasonal distribution (Osborne, 1999; Hauser, 2006). Conversely, K and L pods consumed Chinook salmon from the Puget Sound/Georgia Basin region 1/3 of the time and consumed Chinook salmon from the outer coast 2/3 of the time also reflecting their seasonal distribution (Osborne, 1999; Krahn et al., 2004; Hauser, 2006).

An exponential equation was used to estimate the accumulation of PBDEs for each individual in each year. The doubling time of PBDE accumulation was varied among the pods to best fit the available data. However, the maximum difference in doubling time is 6 months and does not necessarily indicate a difference in PBDE accumulation among the pods. J and K pod’s PBDE accumulation doubling time range was set at 3.2 to 3.5 years, whereas L pod’s accumulation doubling time was varied between 3.7 and 4.0 years. The results of the sensitivity analysis for variable PBDE doubling times are presented in section 2.2.2.

2.2.2 Sensitivity Analyses

Due to the uncertainty concerning the average parameter values (e.g. offload amounts, caloric content of prey, historic and current contaminant levels of prey) a sensitivity analysis was conducted to determine how the results of the model were influenced by variability in these values. The confidence interval ranges of the parameter values were sequentially extended by $\pm 10\%$. In addition, the initial year of PBDE accumulation was altered by ± 10 years. The output of each trial was compared to the original output values.

The parameters from the PCB and PBDE models that were modified in the sensitivity analysis included transplacental transfer percentage (TT_i), lactation transfer percentage (LO_i), elimination rate (E), caloric content of the prey (CC_y), historical PCB concentrations in Chinook salmon ($PC_{c=PCB,y,P.SIO.C.}$), energetic requirements ($DPER_{x,g}$), mass of the individual in each age-class ($M_{x,g}$), percentage of contaminant sequestered in the blubber (approximately 80%), percentage blubber ($B\%_{i,y}$), percentage lipid in the blubber ($L\%_{i,y}$), and lastly the factor that adjusts for any missed calves (MC). In the PBDE model, the doubling time (T_y), the start year when PBDEs were present (y), and the initial PBDE concentration where $y = 0$ ($PC_{c=PBDE,y=0,p}$) were also altered.

2.2.3 Projection Model

The second objective of this study was to use the individual-based accumulation model to project future contaminant levels (post 2007) in the SRKWs. The contaminant scenarios that best predicted the PCB and PBDE data (section 2.2.1) were used in the projection model simulations. First- and second-born whales with unknown mothers and therefore unknown transplacental and lactation offload amounts were excluded from the projection models and subsequent analyses because it was found that the amount of contaminants obtained through nursing was a significant portion of the total contaminant load in the individual. PCB and PBDE projection model pseudo codes are provided in Appendix B.

The projection model used the same conceptual framework as the individual-based model. The projection model was started in 2007 with the estimated individual PCB and PBDE contaminant loads for killer whales with known mothers. Essentially, for each individual in the population for each year they were alive, the model generated the developmental growth and energetic requirement for the specific sex-and age-class (using the average parameter values including average DPER, blubber mass, and lipid mass); estimated the prey's contaminant concentration running three accumulation scenarios for each contaminant; and for females offloaded the contaminant levels during gestation and

lactation using average offload values (4% offload via transplacental transfer, and 80% offload via lactation). Average values were used in the projection analysis because the sensitivity analyses revealed the model was robust to changes in the parameter values and because the range of parameter values resulted in relatively small changes in the predicted values. For example, an individual that had an average PCB concentration of 30,000 *ng/g* lipid weight (*lw*) would have a confidence interval of approximately ± 300 *ng/g lw*.

Survival was set to equal 1.0 for all age- and sex-classes in order to track an individual's potential accumulation and compare contaminant levels among individuals in the same life history stage. Female SRKWs typically give birth for the first time between the ages of 12 and 17, stop reproducing by age 40, and have an average calving interval between 3 and 7 years (Olesiuk et al., 2005). For each female, projection simulations used an average of 14 years until maturation and a calving interval of 5 years. The caloric content of the fish was determined by calculating the average between the upper and lower bound 95% CI found in the Puget Sound/Georgia Basin and outer coast Chinook salmon (between 1643 and 1804 *kcal/kg*; O'Neill et al., 2006). The PCB elimination rate was set to the average of the lower and upper bound ranges ($E=0.020$; Hickie et al., 2007). Results include PCB and PBDE concentrations in specific individuals that were alive in 2007 (excluding the individuals with unknown mothers), as well as the average simulated individual, or the novel calves produced by the projection model, for each projected year through 2050.

Since 1977, PCBs have no longer been produced in the U.S. and PCB concentrations in the environment have since declined. Consequently, contaminant levels in the SRKW prey are projected to decline in the future. PCB congeners have a range of estimated environmental half-lives from a few years to over 100 years (Sinkkonen and Paasivirta, 2000; Jönsson et al., 2003). Several factors can influence the degradation rates ranging from microbial communities in the sediments to environmental temperature patterns. Given that there are several factors that can influence these degradation rates (Sinkkonen and Paasivirta, 2000) three scenarios were run for PCB accumulation. The first scenario

($s = 1$) assumed a constant PCB concentration in the prey (equation 2.31). The second scenario ($s = 2$) assumed PCB levels in the prey are reduced in half by 2050 (equation 2.32). The third scenario ($s = 3$) assumed that prey PCB concentrations will be undetectable by 2050 (equation 2.33).

$$PC_{c=PCB,y,s=1} = 35 \quad \text{[equation 2.31]}$$

$$PC_{c=PCB,y,s=2} = -.41y + 35 \quad \text{[equation 2.32]}$$

$$PC_{c=PCB,y,s=3} = -.82y + 35 \quad \text{[equation 2.33]}$$

In the individual-based model (section 2.2.1), the PBDE accumulation doubling times ranged from 3.2 to 3.5 for J and K pods, and 3.7 to 4.0 years for L pod. The projection model assumed a continued exponential increase in PBDEs for all scenarios (equation 2.36; where the first projected year is 2008, $y_0 = 39$ and $PC_{c=PBDE,y=0,s} = 0.01$), however, the accumulation doubling time was varied. In the projection model, three scenarios were run for PBDE accumulation: the first scenario assumed a doubling time of 3.2 years for all pods, the second scenario assumed a doubling time of 3.6 years, and the third scenario set the doubling time at 4 years (equations 2.35, 2.36, and 2.37 respectively).

$$PC_{c=PBDE,y,s} = PC_{c=PBDE,y=0} \exp(T_s * y) \quad \text{[equation 2.34]}$$

$$T_{s=1} = \ln(2) / 3.2 \quad \text{[equation 2.35]}$$

$$T_{s=2} = \ln(2) / 3.6 \quad \text{[equation 2.36]}$$

$$T_{s=3} = \ln(2) / 4 \quad \text{[equation 2.37]}$$

2.3 Results

2.3.1 Individual-Based Model Validation

The results from the PCB and PBDE models were analyzed separately. Observed biopsy data are provided in Table 3 of Appendix A, courtesy of Krahn et al. (2007, 2009).

Prediction of PCB concentration levels in J pod was best under the “mixed” diet scenario ($R^2 = 0.79$; Figure 2.2). The “uniform” diet scenario was expected to result in higher predicted PCB levels compared to the measured PCB levels because PCB concentrations are relatively higher in Puget Sound/Georgia Basin Chinook salmon than in other salmon (O’Neill et al., 1998). Moreover, J pod’s distribution extends outside the Puget Sound/Georgia Basin region during a small part of the year. Therefore, whales in J pod likely consume prey with lower PCB levels than Puget Sound/Georgia Basin Chinook salmon for part of the year. “J39” had PCB levels that were over-predicted in both scenarios, possibly indicating that his mother may have had a calf that died just prior to giving birth to “J39”, resulting in a lower measured PCB concentration (Figure 2.2). Minute changes in the mother’s burden could result in the discrepancy seen between predicted and observed PCB levels in “J39”.

The “mixed” diet scenario better predicted K and L pod PCB concentrations with the exception of three individuals, “L87”, “L78”, and “L73” ($R^2 = 0.63$ for the “uniform” model; Figure 2.2). These individuals may be consuming prey at a lower trophic level with lower PCB concentrations for part of the year, or they may have received less of a burden from their mothers during gestation and lactation. In general, the predicted levels more closely resembled the measured values when the model assumed K and L pod individuals consumed their prey from the outer coast 2/3 of the time and consumed prey from the Puget Sound/Georgia Basin 1/3 of the time.

The predicted PBDE concentrations for J, K, and L pod individuals were consistent with an exponential increase in PBDE accumulation with doubling times between 3 and 4 years ($R^2 = 0.83$; Figure 2.3). PBDE concentrations in J and K pods were best predicted

using a shorter accumulation doubling time range of 3.2 to 3.5 years, whereas the PBDE doubling time range for L pod was set at 3.7 to 4.0 years. However, “K21” was overestimated when the accumulation doubling times were similar to J pod’s doubling time. “K21”, an adult male, was subsequently better predicted with an accumulation doubling time similar to L pod individuals. It may be that K pod individuals have similar accumulation rates as L pod individuals because they have a more similar geospatial distribution and the two K pod juveniles, “K34” and “K36”, received maternal burdens higher than predicted. Unfortunately, a small sample size of K pod individuals precludes a more accurate prediction of PBDE accumulation doubling times.

The effects of age and year are apparent in male killer whales as demonstrated by the average PCB and PBDE historical profiles from the simulations of four males with different birth years (Figure 2.4.a, b). From the average simulated profiles, there is no clear evidence of a decline of PCBs through time from the 1970s to the present although the male’s PCB concentrations have shown a general increase with age following a growth dilution (Figure 2.4.a). Growth dilution occurs when the total body mass significantly increases in a short period of time, thereby causing the contaminant concentration to be diluted or reduced. Indeed, the adult male aged 23 years old in the 1990s was estimated to have a slightly higher level of PCBs than the predicted PCB levels in the current juvenile or sub-adult males aged 12 and 18 years old, respectively (Figure 2.4.a). In contrast, PBDE concentrations among male killer whale calves have significantly increased over time (Figure 2.4.b). PBDE concentrations continue to increase with age within males after the period of growth dilution. However, it appears that there is almost a negative relationship with age when comparing a current calf, juvenile or sub-adult to an adult from the 1990s (Figure 2.4.b).

The effects of birth order are apparent as demonstrated by the average PCB and PBDE historical profiles of a matriline from J pod (Figures 2.5.a, b). In the simulations, individuals have decreasing PCB concentrations with increasing birth orders prior to the 3rd offspring while subsequent calves have relatively similar PCB levels (Figure 2.5.a). In contrast, these same individuals have increasing PBDE concentrations with increasing

birth order, or more specifically, have increasing PBDE concentrations through time, although there was no difference in current PBDE concentrations between the 2nd and 3rd born individuals (Figure 2.5.b).

2.3.2 Sensitivity Analyses

The predicted PCB concentrations within the biopsied individuals changed by less than 10% when the gestation, lactation, elimination, caloric content, prey contaminant concentration, DPER, percent acquired in the blubber, percentage blubber mass and percentage lipid in the blubber parameter value ranges were extended by $\pm 10\%$ (Table 2.3). PCB concentrations changed by a maximum difference of 31% when the missed calf (*MC*) factor was removed from the PCB model (Table 2.3). The *MC* factor also changed PBDE concentrations in individuals but the differences were less than 10% (Table 2.4). Indeed, the predicted PBDE concentrations within the biopsied individuals had changed by less than 10% when most of the parameter values were changed by $\pm 10\%$ (Table 2.4). The contaminant predictions from the PBDE model were sensitive to the start year (causing the PBDE concentrations to change by more than 100% in all individuals across models with different start years; Table 2.4) and the doubling time (causing the PBDE concentrations to differ between 38% and 71% across different models; Table 2.4). To offset the sensitivity to the doubling time and start year, the parameter values used in the final model were adjusted simultaneously to best fit the observed values. In general, the sensitivity results indicate that the PCB model is most sensitive to the mother's total burden prior to offloading as reflected by the removal of the *MC* factor and the PBDE model is most sensitive to the rate of accumulation. In the projection model below, individuals with unknown mothers or unknown birth order were therefore excluded and a range of PBDE doubling time values was simulated.

2.3.3 Projection Model

The projection results include predicted contaminant concentrations for specific individuals and average individuals for the three PCB and PBDE diet scenarios. Specific

individuals are defined as identified individuals that were alive in 2007 with known life histories (i.e. sex, age, and mother). Average individuals (i.e. the simulated novel individuals) were also examined in the analysis. Contaminant concentrations in these average individuals were calculated as the average PCB and PBDE concentrations for each age-class in each year in the simulated individuals from 2008 to 2050.

Contaminant concentrations from the six diet scenarios were compared in two specific individuals: a 4 year-old calf and an 18 year old sub-adult male (Figure 2.6.a, b.). In projections, both killer whales doubled their current concentrations by year 2060 when PCB concentrations in the prey were constant (Figure 2.6.a). PCB concentrations no longer increased by years 2040 and 2050 for the sub-adult male and calf, respectively, when the model assumed PCB concentrations in the prey would be reduced in half by the end of the simulation (Figure 2.6.a). Lastly, the sub-adult male showed a reduction in concentration beginning around 2030 and had a concentration similar to current levels by 2060 when the model assumed the prey's PCB concentration would be essentially zero by 2050 (Figure 2.6.a). The calf had a reduced PCB concentration by year 2040, and by year 2060 had the lowest projected levels for this scenario (Figure 2.6.a). In all three PBDE diet scenarios, the calf and sub-adult male had similar PBDE concentration levels until approximately year 2020 (Figure 2.6.b). By year 2020, the individuals had PBDE concentrations of approximately 250,000 ng/g *lw* when the doubling time was set to 3.2 years, whereas they had approximately 100,000 ng/g *lw* and 50,000 ng/g *lw* when the doubling times were 3.6 and 4.0 years, respectively (Figure 2.6.b).

PCB concentrations in the average calf were most often higher than PCB levels found in older animals (Figures 2.7, 2.8). When the PCB concentration in the prey was reduced, the average male experienced an increase in PCBs with age following the growth dilution, but a decrease in PCBs through time among similar age-classes (Figure 2.7). The average female experienced a reduction in PCB concentration following the growth dilution and experienced an increase in PCB concentration during reproductive years (Figure 2.8). Post-reproductive females had variable PCB concentration levels through time and did not show a decrease in concentration until after year 2040 (Figure 2.8).

The average male and female had similar PBDE concentration trends with age, time, and diet scenario (Figures 2.9. and 2.10). PBDE concentration levels increased through time and with decreasing doubling times (Figures 2.9 and 2.10). There did not appear to be a significant difference in PBDE concentrations between a sub-adult and an adult male before the year 2020 (Figure 2.9). In contrast, the average reproductive female had lower PBDE concentration levels than the average post-reproductive female by year 2015 (Figure 2.10).

2.4 Discussion

2.4.1 Model Scenarios and Sensitivities

Modeling is a non-invasive tool that can be used to estimate contaminant levels in an endangered species and the corresponding sensitivity analyses can guide future research. In this study, various scenarios were run encompassing a wide range of parameter values and the results were compared to the available data to provide an assessment of confidence in the model's validity. In general, the PCB model scenario that best fit the data included a "mixed" diet for all three pods of Southern Resident killer whales. The "mixed" scenario assumed J pod consumed Chinook salmon from the Puget Sound/Georgia Basin region 2/3 of the time and consumed outer coast Chinook salmon 1/3 of the time. Conversely, K and L pods consumed Chinook salmon from the Puget Sound/Georgia Basin region 1/3 of the time and consumed Chinook salmon from the outer coast 2/3 of the time. In general, J pod individuals had higher contaminant concentrations than K or L pod individuals because the Puget Sound/Georgia Basin Chinook salmon have relatively higher contaminant levels than the outer coast Chinook salmon (O'Neill et al., 2006). Evaluation of the "uniform" diet scenario, likely unrealistic because it does not correspond to the killer whale's known geospatial distribution (Osborne, 1999; Krahn et al., 2004; Hauser, 2006), illustrates that the whales probably have a more variable and complex dietary pattern. Indeed, Hickie et al. (2007) estimated PCB concentrations in average killer whales and found that the effect of an

individual's historical exposure is complex, and using a steady-state exposure approach can underestimate concentrations in the individuals. The "uniform" diet scenario overestimates concentration levels in J pod individuals because J pod is probably consuming prey with lower PCB levels, such as Chum salmon and salmon from the outer coast, for part of the year. In contrast, the "uniform" diet scenario underestimates the majority of K and L pod individuals because K and L pods are probably consuming prey with higher PCB loads than the Chinook salmon from the outer coast for part of the year. J pod appears to consume prey with shorter predicted PBDE doubling times of 3.2 to 3.5 years, whereas L pod appears to be accumulating PBDEs at a slightly slower rate of 3.7 to 4.0 years. Increasing the sample size so that all life history stages are represented for each pod would provide for a more thorough examination of the distinction in diet among the pods, and the consequences thereof for patterns of accumulation and loss of PCBs and PBDEs.

Uncertainty in the model parameters appeared to have little effect on model results excluding the missed calf factor and rate of accumulation of PBDEs (i.e. the predicted doubling time and initial year of contamination). Removing the missed calf factor from the model causes the predicted PCB and PBDE levels in some individuals to be higher than the measured levels. One suggested explanation for the overestimation is that a reproductive female that has not been observed with a calf within the predicted 5 year calving interval (Olesiuk et al., 2005), has probably given birth, but the calf subsequently died prior to being observed in the summer annual surveys. Accurate data for calf mortalities and calving intervals are important because it has been suggested in bottlenose dolphins that high levels of PCBs from the mother can affect the calf's mortality (Schwacke et al., 2002; Hall et al., 2006). Along with the missed calf factor, the sensitivity analyses determined that the PBDE doubling time greatly altered model results. Most notable was a strong sensitivity to a change in doubling time in the range of 3.0 years and 3.2 years. While data for mountain whitefish from the Columbia River indicated PBDE doubling times of under 2 years (Rayne et al., 2003), this study predicted the Southern Residents have accumulation doubling times of approximately 3 to 4 years assuming individuals started accumulating PBDEs in the 1970s. Projection results

suggest that PBDEs will surpass current PCB levels in the near future (approximately 5 to 20 years), emphasizing the need for an accurate estimate of current and future PBDE doubling times in the killer whales.

2.4.2 Toxic Effects

In the absence of PCB and PBDE thresholds for health effects in killer whales, it is difficult to determine the toxicological risk of past, present and future concentration levels. Indeed, a cause-and-effect association has not been established in killer whales because of numerous confounding factors. In addition, the adverse effects from contaminant exposure are species-specific and dose-dependent. However, the tissue residue guidelines established for a marine mammal species that has reproductive physiological traits similar to killer whales can potentially be used to estimate risk in killer whales (Kannan et al., 2000). For example, Kannan et al. (2000) derived a health-effects threshold concentration for PCBs in marine mammal blubber of 17,000 *ng/g lw*. The Southern Residents have surpassed this threshold in PCB concentration and are close to surpassing it in PBDE concentration. In general, projection scenarios indicate that the PCB concentrations in male and female killer whale calves and adult male killer whales will continue to exceed this health-effects threshold except in the zero diet scenarios in the year 2060. Sub-adult male killer whales exceed the health-effects threshold when contaminants in the prey remain constant, but are below this threshold by year 2040 in the half and zero diet scenarios. In contrast, reproductive female killer whales are below the health-effects threshold following their first or second offspring, but may have levels equal to or above prior to giving birth. By year 2060, post-reproductive females have PCB concentrations below 17,000 *ng/g lw* and are no longer considered at risk for adverse health effects. In contrast, Hickie et al. (2007) estimated that 95% of the Southern Resident population will drop below this threshold only after 2089. Although this study predicts individuals will have concentrations below the health-effects threshold by 2060, both studies highlight the persistence and exposure legacy of PCBs in individual killer whales.

In general, Southern Resident killer whale calves had the highest predicted PCB and PBDE concentrations compared to any other age-class in most scenarios. Calves tended to have higher contaminant concentrations not only because they received relatively high contaminant loads from their mother, but also because of their smaller size. In addition, calves are potentially at a higher risk of health effects from contaminant exposure than adults because high contaminant levels accumulate during a period of rapid biological development (Krahn et al., 2009). Eriksson et al. (2006) demonstrated that when neonatal mice were exposed to a PCB and a PBDE congener during a critical period of brain development, the exposure enhanced neurobehavioral defects. However, exposure during non-critical periods produced no effect (Eriksson et al., 2002). Deviations from normal or spontaneous behavior due to the exposure were also shown to increase with age. It is unknown if contaminant exposure in developing killer whales causes neurological effects. The behavior of an individual is an appropriate endpoint to study when evaluating potential health effects because the nervous system can be influenced by a contaminant, thereby causing a change in behavior (Eriksson et al., 2006). Noteworthy is the fact that when PCB and PBDE congeners interact, the reaction can be more than additive (Eriksson et al., 2006). Southern Resident killer whale calves have relatively high contaminant concentration levels of both PCBs and PBDEs, thereby increasing their susceptibility to detrimental biological health effects resulting from biochemical interactions of multiple contaminant categories.

In most current and projected reproductive females, PCB concentrations exceeded the health-effects threshold prior to giving birth to their first calf. PCB concentrations in these reproductive females can potentially affect calf survival. In fact, Hall et al. (2006) estimated a 50% probability of calf mortality in bottlenose dolphins when the maternal blubber burden was approximately 10,000 ng/g. Furthermore, when the maternal blubber burden doubled, the probability of a calf surviving the first 6 months dropped to almost 10% (Hall et al., 2006). Although Hall et al. (2006) emphasized the uncertainty with their model, their study demonstrates that the maternal burden has the potential to alter population growth and stresses the importance of calculating the probability of missing a calf. In a separate study, a concentration-response curve was estimated for PCBs in

bottlenose dolphins and the median effective concentration (EC50) for calf mortality occurred when the total body concentration was $33 \mu\text{g}/\text{g lw}$ (Schwacke et al., 2002). Most female Southern Resident killer whales currently giving birth to their first- and second-born offspring have these concentration levels in their blubber and could potentially be at or approaching a concentration threshold for effects on health or mortality.

The reconstructed historical PCB concentrations had reached peak levels in the habitats of the Southern Resident killer whale population prior to the 1990s. However, the reconstructed historical PCB concentrations in most age-classes in the 1990s were well above the threshold level found for PCB-related health effects, whereas the reconstructed historical PBDE concentrations were just reaching minimum detection levels. In the 1990s, the Southern Resident killer whales experienced a large population decline (Krahn et al., 2004). Chinook salmon also experienced a regional decline (Ford et al., 2005) indicating the potential for nutritional stress in the whales. In addition, fecundity rates were found to be highly correlated with Chinook salmon abundance (Ward et al., 2009) which may have restricted population growth. During times of nutritional stress, the lipids stored in the blubber will become mobilized (O'Shea, 1999) liberating lipophilic contaminants into circulation in the body core (Debiec et al., 2003, 2006). In bottlenose dolphins, immunosuppression was correlated with increased PCB concentrations in the blood (Lahvis et al., 1995). Furthermore, chronic contaminant exposure was demonstrated to cause immunotoxicity in captive harbor seals following the completion of the study, although seals remained healthy during the course of the study and a short fast did not appear to aggravate the suppression (de Swart et al., 1994; Ross et al., 1995). If an individual killer whale was subject to nutritional stress in the 1990s, it may have experienced decreased fecundity, and increased mortality because of a lack of prey resources coupled with contaminant-induced adverse health effects.

2.4.3 Factors Influencing PCB and PBDE Accumulation

While several challenges prevent an accurate assessment of the total annual PCB and PBDE exposure to the Southern Resident killer whales, Chinook salmon have been used as a proxy for POP exposure (Hickie et al., 2007; Cullon et al., 2009). Chinook salmon have a complex life history which makes assessing the factors that affect contaminant accumulation in killer whales difficult. For instance, O'Neill et al., (1998) found Chinook salmon had higher levels of PCBs compared to Coho salmon (*Oncorhynchus kisutch*) which they suggest may be a result of differences in age, diet, distribution, and tissue lipid content. These factors can also influence contaminant concentrations in individual killer whales (Ross et al., 2000; Ylitalo et al., 2001; Hickie et al., 2007) in addition to the seasonality, habitat use, differences in biotransformation, and individual killer whale body size and growth (Borgå et al., 2004).

Males and females in the Southern Resident killer whale population tend to have very different age-related contaminant patterns. Life history traits such as sex, age, birth order, and reproductive status have been shown to influence persistent organic pollutant concentrations in individuals (Ross et al., 2000; Ylitalo et al., 2001). Specifically, PCB and PBDE concentrations in male killer whales increase with age following a growth dilution, whereas PCB and PBDE concentrations in female killer whales remain relatively low following the growth dilution and increase in the post-reproductive years. The reduction in contaminant concentrations in reproductive females reflect what is known about a female's ability to transfer lipophilic contaminants to her offspring (Duinker and Hillebrand, 1979; Ridgeway and Reddy, 1995; Wells et al., 2005). As expected, PCB concentrations decreased with increasing birth order. In contrast, these same individuals had increasing PBDE concentrations with increasing birth order. The influence of the contaminant exposure history, or accumulation rate, of PBDE concentrations in individuals is probably the driving factor influencing this unexpected relationship. In the average male killer whale, there was no clear evidence of a decline of PCBs through time from the 1970s to the present. In contrast, PBDE concentrations among male killer whales have significantly increased over time.

Variable historical contaminant levels in SRKW prey combined with the effects of gender, birth order, and reproductive history (for females), lead to a wide range of contaminant levels within individuals. This variation in contaminant concentration can limit our ability to generalize about the concentration levels in individuals within the same life history stage. Nevertheless, an individual-based model based on the biological processes that cause individuals to both acquire and lose contaminants over time allows one to estimate contaminant concentrations in specific individuals using their known history (e.g. birth order, reproductive history, pod membership, birth year, and sex). This allows one to project contaminant levels across all individuals in the population and to tease out the effects of various life-history traits on concentration levels.

2.4.4 Conclusions and Recommendations for Future Research

Southern Resident killer whales frequent the marine waters off British Columbia, Washington, Oregon and California where relatively high levels of PCBs and PBDEs are found. Detrimental biological effects from exposure to PCBs and PBDEs have been shown to alter reproduction, immune function, neurodevelopment, and disrupt the endocrine system in many species. These biological effects have the potential to hinder the recovery of the Southern Resident killer whales. An individual-based model is a way to represent the biological processes by which individuals acquire and lose contaminants to estimate the contaminant levels across the entire population – using known life-history information about the individuals. Model projections indicate that “legacy” PCBs are slowly declining but the killer whales will continue to be exposed for some generations to come. Alternatively, PBDEs are projected to increase rapidly, and individuals may experience levels equal to current PCB levels in a short period of time. The sensitivity analyses determined that the results of the model were most sensitive to the missed calf factor and the PBDE doubling times. Hence, establishing accurate data on intervals between births, calf survival rate, and environmental PBDE doubling times should be a research priority for the Southern Resident killer whale population. A more accurate estimation of calf mortality could clarify the proximity of these individuals to contamination thresholds for physiological damage. Ultimately, determination of

exposure levels and potential resulting risks posed by these persistent organic pollutants in the Southern Resident killer whales are essential for the effective protection of this endangered species.

Table 2.1.

Descriptions and values of parameter subscripts in the individual-based model.

Subscript	Description	Value
<i>c</i>	Contaminant	PCB, PBDE
<i>i</i>	Individual	1:182
<i>y</i>	Year	1910:2050
<i>p</i>	Pod	J, K, L
<i>x</i>	Age-class	1:100
<i>g</i>	Sex-class	Male, Female
<i>k</i>	Calf of individual	Ages: 0, 1

Table 2.2. Descriptions, equation number, and reference page of parameter variables used in the individual-based model for PCBs and PBDEs.

Variable	Description	Equation (Reference Pg.)
$totC_{c=PCB,i,y}$	Total PCB	2.1 (17)
$totC_{c=PBDE,i,y}$	Total PBDE	2.2 (17)
$PI_{c,v,p,x}$	Prey intake	2.7 (19)
$DPER_{x,g}$	Daily prey energetic requirement	2.12 (22)
$BIO_{x,g}$	Biomass of fish consumed	2.13 (23)
CC_y	Caloric content of prey	2.14 (23)
$B_{i,y}$	Blubber mass	2.18 (24)
$L_{i,y}$	Lipid mass in blubber	2.20 (25)
TT_i	Offload via gestation	2.21 (27)
LO_i	Offload via lactation	2.22 (27)
$PC_{c=PCB,y,P,S}$	PCB concentration in Puget Sound/Georgia Basin prey	2.25 (30)
$PC_{c=PCB,y,O,C}$	PCB concentration in outer coast prey	2.28 (30)
$PC_{c=PBDE,y,P}$	PBDE concentration in prey for J, K, and L pods	2.29 (31)
T_y	Doubling time	(31)

Table 2.3. Percent difference between the original predicted PCB concentrations in the individual killer whales (*Ind.*) and the altered PCB concentrations from expanding the parameter ranges $\pm 10\%$. The parameter ranges were expanded sequentially and included: percent offloaded due to gestation (TT_i), percent offloaded due to lactation (LO_i), elimination (E), prey's caloric content (CC_y), prey's concentration ($PC_{c=PCB,y,P.S/O.C.}$, represented as "PC" in the table), energetic requirement ($DPER_{x,g}$), total body mass ($M_{x,g}$), percent of contaminant in the blubber (%), percent blubber mass ($B\%_{i,y}$), percent lipid mass ($L\%_{i,y}$), and the missed calf (MC) factor.

<i>Ind.</i>	TT_i	LO_i	E	CC_y	PC	$DPER_{x,g}$	$M_{x,g}$	%	$B\%_{i,y}$	$L\%_{i,y}$	MC
J39	1%	1%	0%	-1%	-3%	0%	1%	3%	3%	4%	0%
J38	0%	4%	-2%	-2%	0%	0%	-2%	0%	2%	2%	0%
J27	0%	2%	2%	2%	-2%	-2%	0%	0%	0%	2%	-2%
J1	1%	2%	0%	1%	-1%	1%	-1%	0%	1%	2%	-1%
K36	0%	0%	0%	0%	-2%	-2%	-2%	-2%	0%	-2%	-31%
K34	0%	0%	0%	0%	-3%	0%	0%	0%	-3%	-3%	0%
K21	0%	0%	0%	-2%	-2%	-2%	0%	0%	2%	2%	-14%
L78	-3%	0%	0%	-3%	-6%	-3%	-3%	-3%	-3%	-3%	-8%
L85	0%	-2%	0%	0%	-2%	-2%	-2%	-2%	2%	2%	-11%
L87	2%	0%	0%	-2%	0%	0%	-2%	0%	0%	0%	-2%
L71	3%	0%	0%	0%	-3%	0%	-3%	0%	0%	3%	-2%
L74	2%	2%	2%	2%	0%	2%	0%	5%	2%	5%	2%
L73	0%	0%	0%	0%	-2%	0%	-2%	-2%	2%	-2%	-2%
L57	0%	2%	0%	2%	0%	0%	-2%	0%	2%	0%	-8%

Table 2.4. Percent difference between the original predicted PBDE concentrations in the individual killer whales (*Ind.*) and the altered PBDE concentrations from expanding the parameter ranges $\pm 10\%$. The parameters were expanded sequentially and included: percent offloaded due to gestation (TT_i), percent offloaded due to lactation (LO_i), prey's caloric content (CC_y), doubling time (T_y), energetic requirement ($DPER_{x,g}$), total body mass ($M_{x,g}$), percent of contaminant in the blubber ($\%$), percent blubber mass ($B\%_{i,y}$), percent lipid mass ($L\%_{i,y}$), the missed calf (*MC*) factor, the initial PBDE value in 1970 ($PC_{c=PBDE,y=0,p}$, represented as "*I*" in the table), and the initial start year (y_0).

<i>Ind.</i>	TT_i	LO_i	CC_y	T_y	$DPER_{x,g}$	$M_{x,g}$	$\%$	$B\%_{i,y}$	$L\%_{i,y}$	<i>MC</i>	<i>I</i>	y_0
J39	0%	0%	0%	-67%	-7%	-7%	0%	0%	0%	0%	0%	>100%
J38	0%	0%	0%	-62%	0%	0%	0%	8%	0%	0%	0%	>100%
J27	0%	1%	-1%	-62%	-3%	0%	1%	0%	-1%	0%	-1%	>100%
J1	2%	2%	6%	-46%	1%	4%	2%	4%	1%	4%	3%	>100%
K36	0%	0%	0%	-71%	0%	0%	0%	0%	0%	-7%	0%	>100%
K34	0%	0%	0%	-64%	0%	0%	0%	0%	0%	0%	0%	>100%
K21	0%	1%	-2%	-61%	0%	0%	1%	1%	2%	0%	1%	>100%
L78	0%	0%	-5%	-43%	0%	0%	0%	-5%	0%	0%	0%	>100%
L85	3%	0%	-3%	-45%	0%	-3%	0%	0%	0%	0%	3%	>100%
L87	0%	0%	0%	-45%	0%	0%	0%	0%	0%	0%	5%	>100%
L71	0%	0%	0%	-38%	0%	0%	0%	5%	0%	0%	5%	>100%
L74	0%	5%	0%	-38%	0%	5%	0%	0%	0%	0%	5%	>100%
L73	0%	0%	0%	-51%	3%	3%	0%	0%	3%	0%	3%	>100%
L57	3%	3%	0%	-42%	3%	0%	3%	3%	3%	3%	6%	>100%

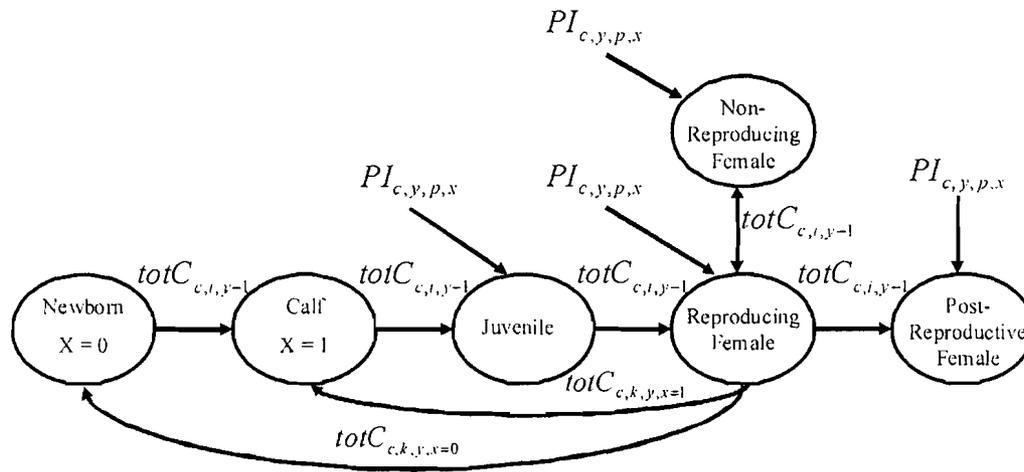


Figure 2.1. Conceptual framework of the individual-based model. The life-cycle model for a female killer whale has six stages: newborn age = 0; calf age = 1; juvenile age = 2 to sexual maturity; reproducing, non-reproducing, and post-reproducing female. The arrows indicate the direction of contaminant transfer through time. $totC_{c,k,y,x=0}$ is the contaminant offloaded via transplacental transfer; $totC_{c,k,y,x=1}$ is the contaminant load offloaded via lactation, $totC_{c,i,y-1}$ is the contaminant load where $c = \text{PCB, PBDE}$ for individual i in year $y-1$; $PI_{c,y,p,x}$ is the contaminant load via prey intake for contaminant c in year y for pod p and age-class x . A male killer whale's life-cycle model is similar, but excludes the offloaded via transplacental transfer and lactation. The output of PCBs via elimination is not shown but is included for each individual at the rate E times the individual's current contaminant load in the blubber.

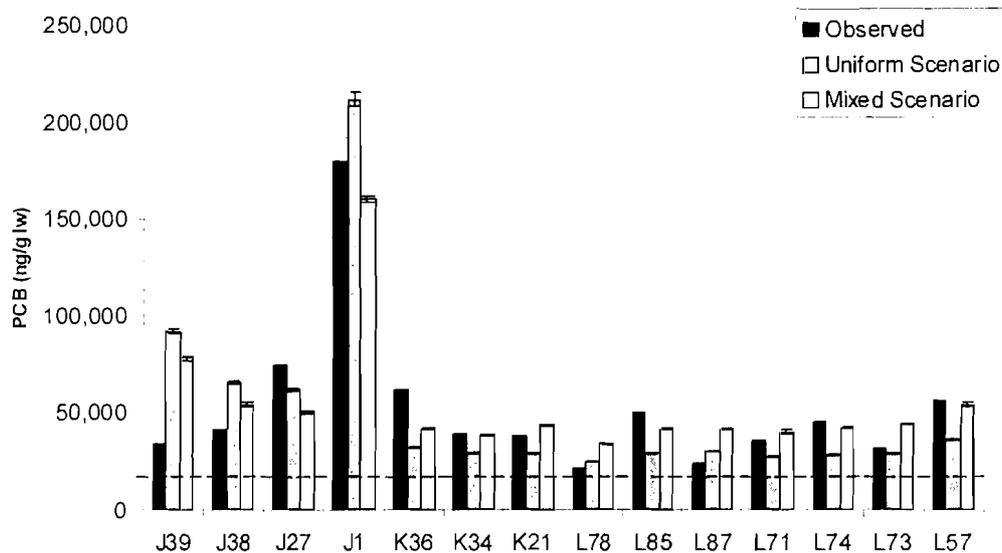


Figure 2.2. Measured concentrations of $\sum PCBs$ in blubber biopsy samples in Southern Resident killer whales from Krahn et al. (2007; 2009) compared to predicted $\sum PCB$ concentrations from the individual-based model. The two diet scenarios are shown: the “uniform” scenario assumed the diet for J pod individuals was 100% from the Puget Sound/Georgia Basin region and the diet for L and K pods were 100% from the outer coast ($R^2 = 0.63$). The “mixed” scenario assumed J pod had a diet that consisted of 2/3 Puget Sound/Georgia Basin Chinook salmon and 1/3 Chinook salmon from the outer coast, whereas K and L pod individuals had a diet that consisted of 1/3 Puget Sound/Georgia Basin Chinook salmon and 2/3 Chinook salmon from the outer coast ($R^2 = 0.79$). Concentration levels are given in ng/g lipid weight in the blubber. The horizontal dashed line indicates the threshold for PCB health-related effects in marine mammals (Kannan et al., 2000). Standard error bars are shown.

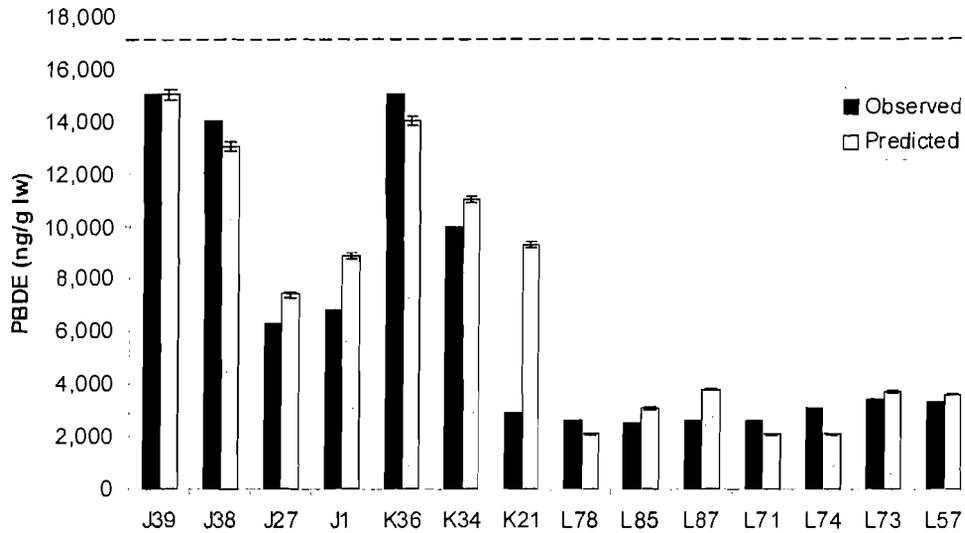


Figure 2.3. Measured concentrations of $\sum PBDEs$ in blubber biopsy samples in Southern Resident killer whales from Krahn et al. (2007; 2009) compared to predicted $\sum PBDE$ concentrations from the individual-based model ($R^2 = 0.83$). J and K pod individuals had a PBDE doubling time range of 3.2 to 3.5 years, and L pod individuals had a doubling time range of 3.7 to 4.0 years. Concentration levels are given in *ng/g* lipid weight in the blubber. The horizontal dashed line indicates the threshold for PCB health-related effects in marine mammals (Kannan et al., 2000). Standard error bars are shown.

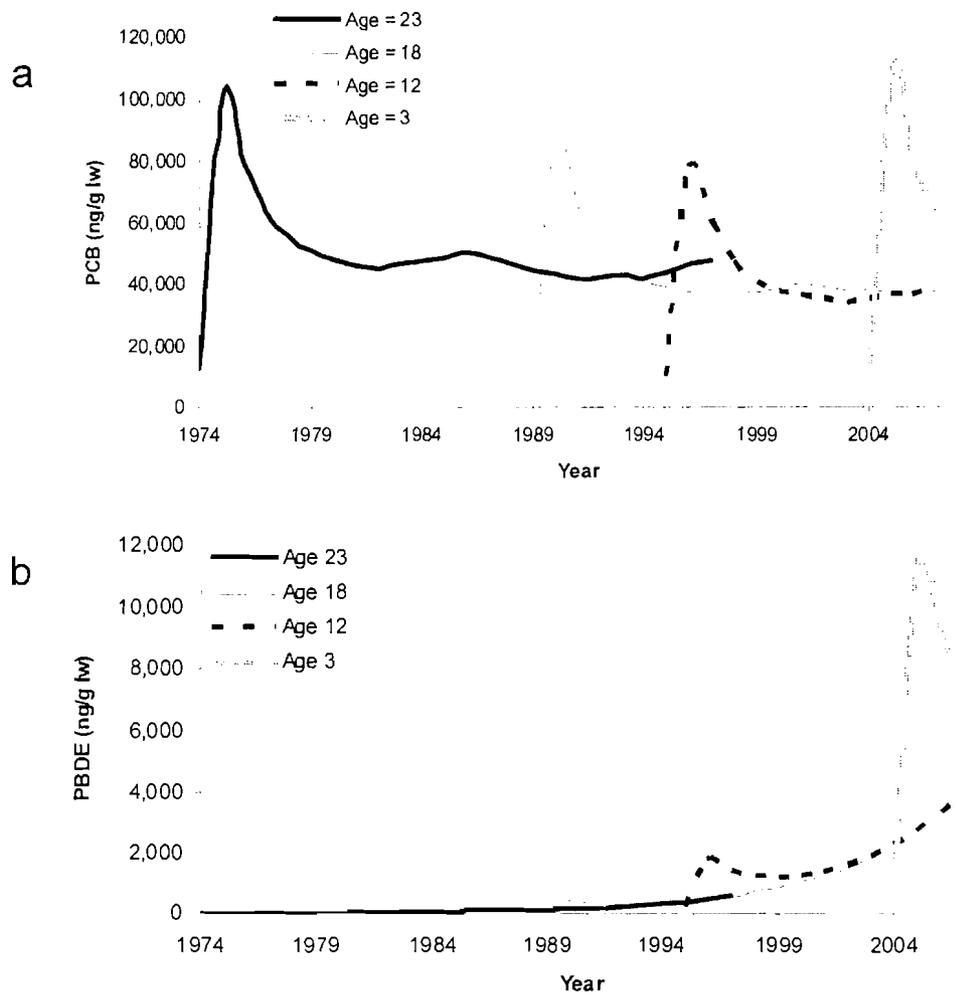


Figure 2.4. Four male PCB profiles (a) and PBDE profiles (b) throughout the reconstructed history from 1974 to present. The vertical line for each individual represents the accumulation of PCBs from the mother and the drop in concentration in young juveniles represents the growth dilution. The four profiles are from non-first born individuals (2nd born or later). The ages indicate the current age of the individual, or the age of the individual when they died.

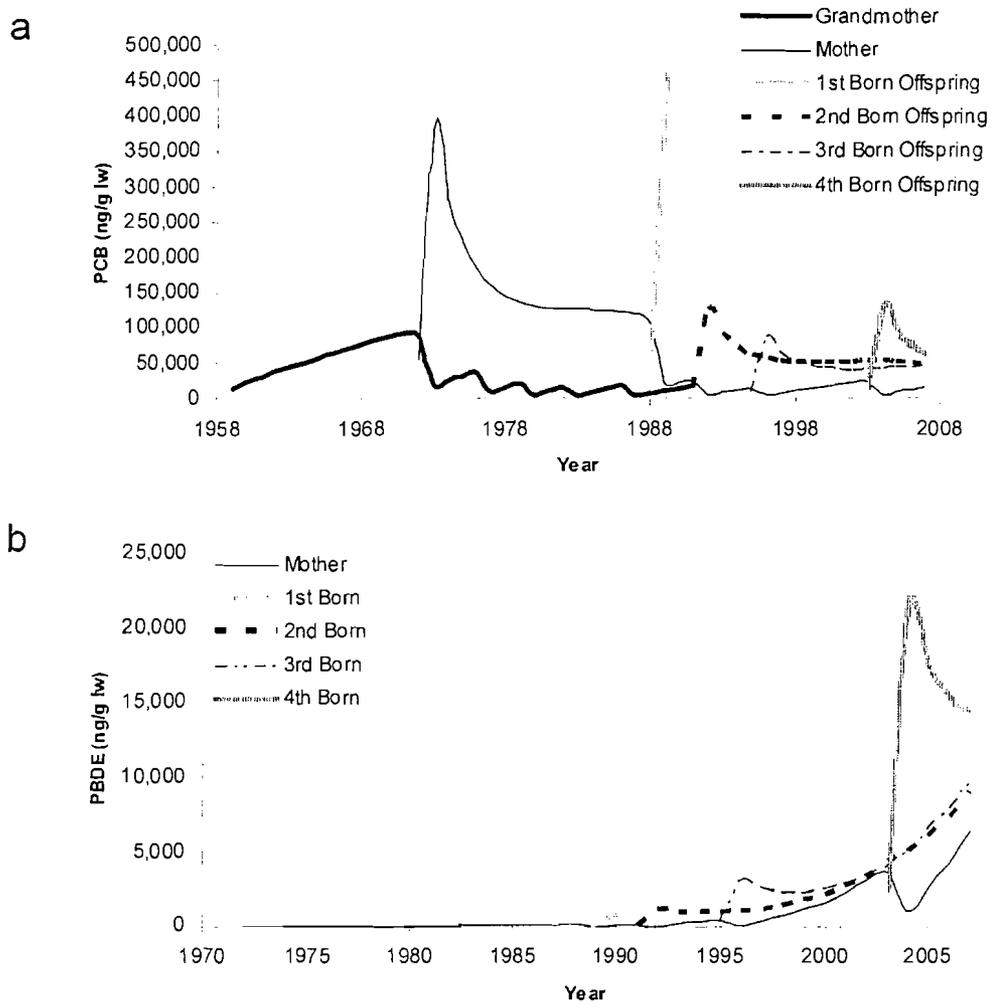


Figure 2.5. Historical PCB profiles (a) and PBDE profiles (b) for 6 members of a matriline. For the grandmother and mother, the decrease in concentration represents a birth. The grandmother died in the early 1990s and the first-born offspring died after lactation by the end of the first year. The drops in concentrations in the offspring represent a growth dilution.

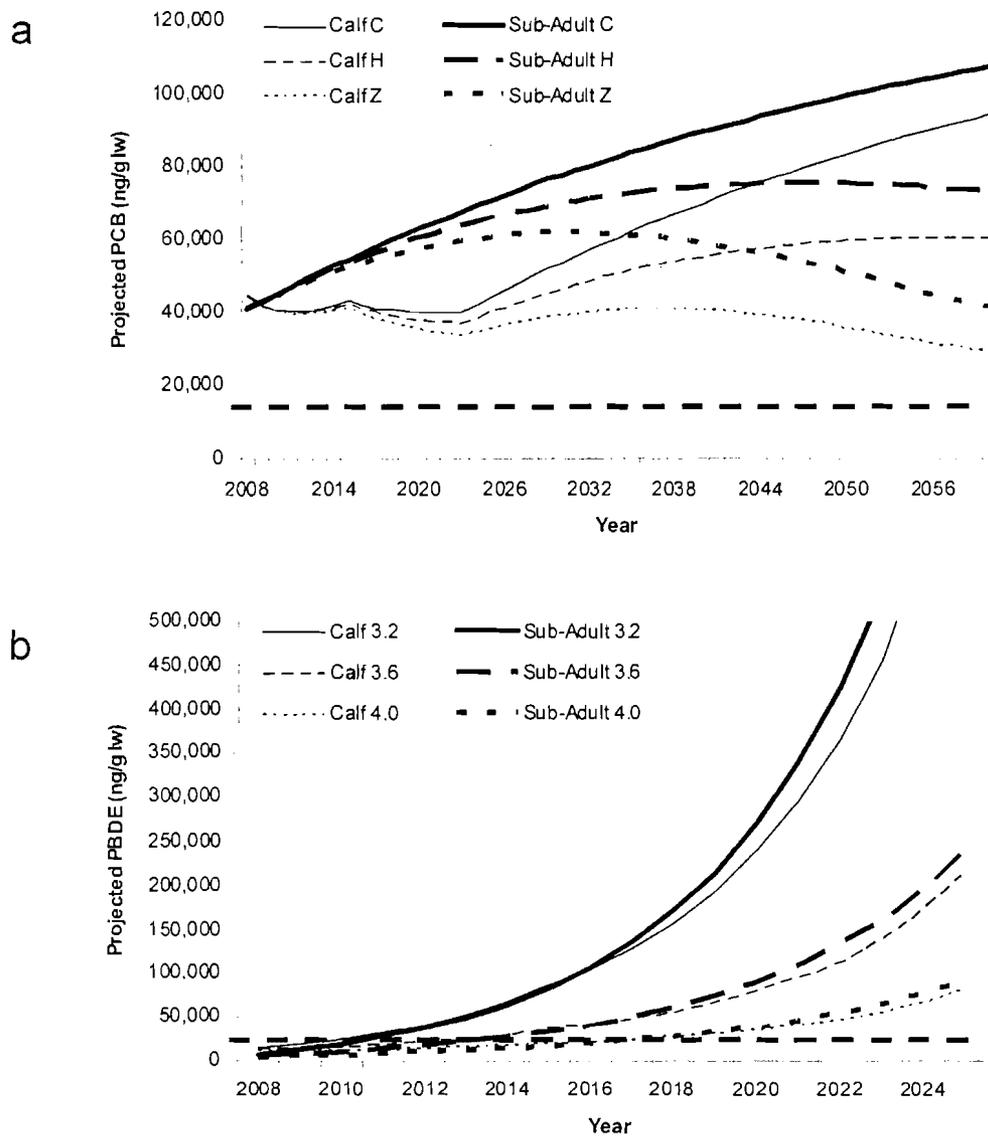


Figure 2.6. Projected PCB profiles (a) and PBDE profiles (b) for a male calf (age 4) and sub-adult male (age 18). The three PCB diet scenarios include a constant diet, C, a diet where the PCB concentration in the prey is reduced in half by year 2050, H, and a diet where the PCB concentration in the prey is essentially zero by year 2050, Z. The three PBDE diet scenarios assume an exponential increase with doubling times equal to 3.2, 3.6, and 4.0 years. The horizontal dashed line indicates the threshold for PCB health related effects in marine mammals (Kannan et al., 2000).

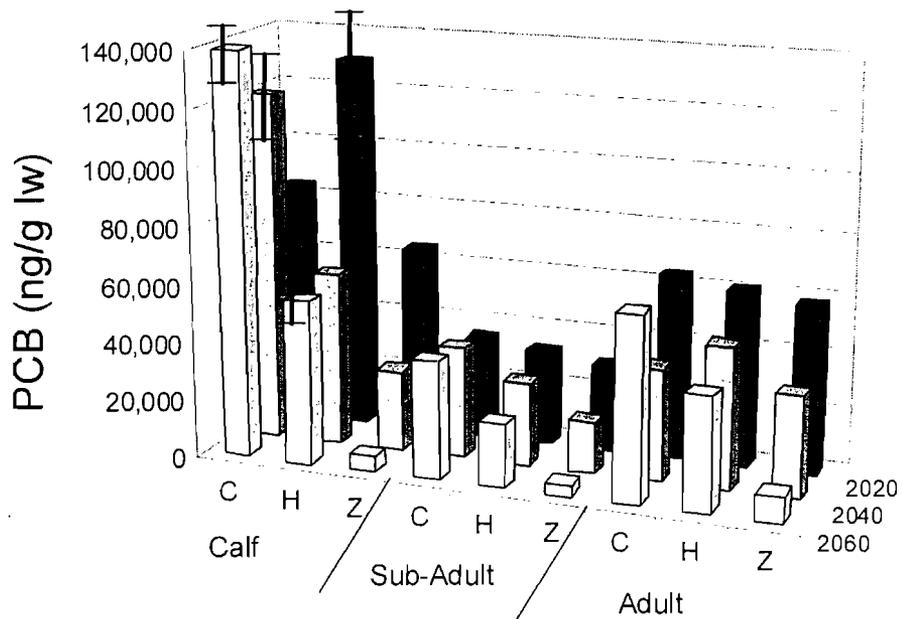


Figure 2.7. Average simulated male projected PCB concentrations in all three diet scenarios in years 2020, 2040, and 2060. Age-classes and diet scenarios are indicated on the x axis and include ages 2-4 (calf), 17 (sub-adult), and 33-38 (adult) years old; the three diet scenarios include a constant diet, C, a diet where the PCB concentration in the prey is reduced in half by year 2050, H, and a diet where the PCB concentration in the prey is essentially zero by year 2050, Z. Standard error bars are shown for standard error values greater than 6,000 $ng/g\ lw$.

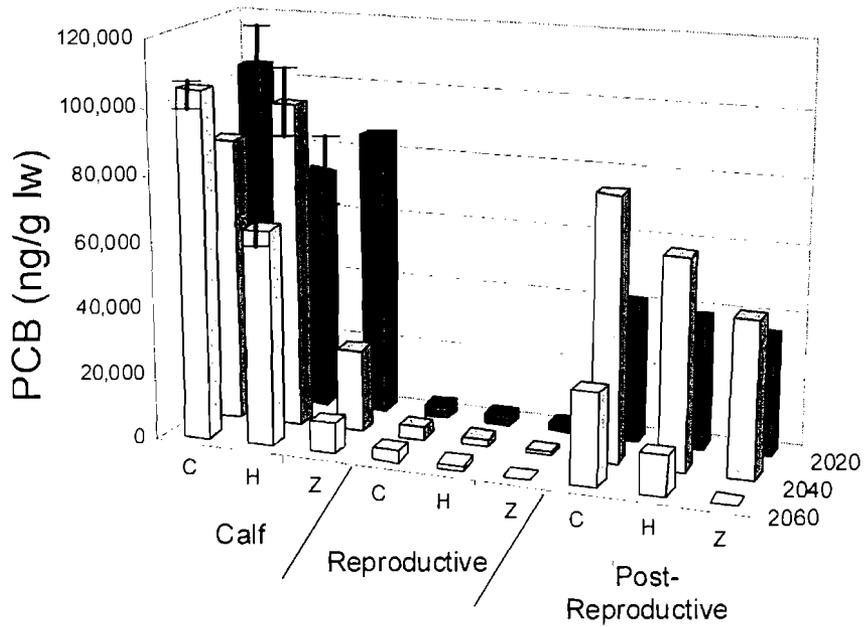


Figure 2.8. Average simulated female projected PCB concentrations in all three diet scenarios in years 2020, 2040, and 2060. Age-classes and diet scenarios are indicated on the x axis and include ages 1 (calf), 25 (reproductive), and 49 (post-reproductive) years old; the three diet scenarios include a constant diet, C, a diet where the PCB concentration in the prey is reduced in half by year 2050, H, and a diet where the PCB concentration in the prey is essentially zero by year 2050, Z. Standard error bars are shown for standard error values greater than 3,000 $ng/g\ lw$.

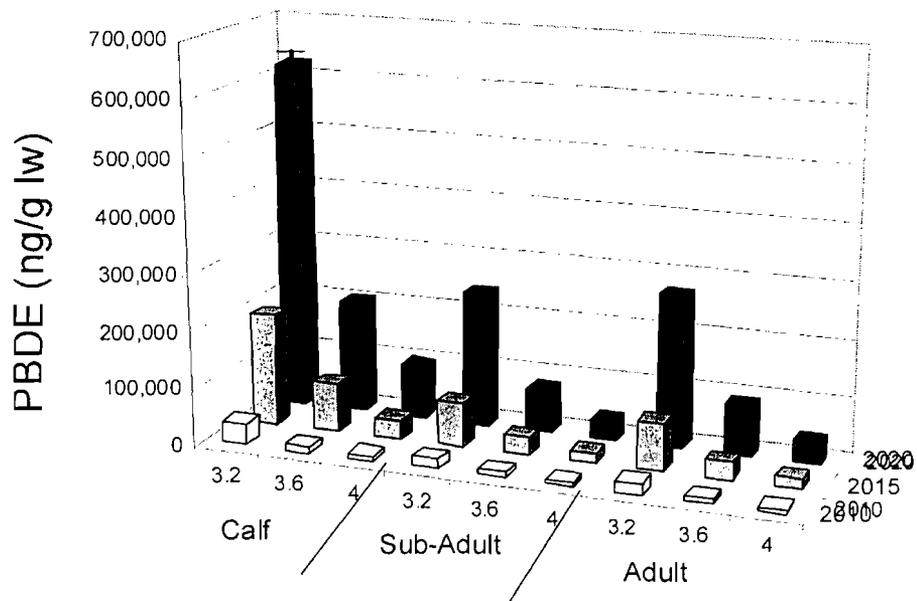


Figure 2.9. Average simulated male projected PBDE concentrations in all three diet scenarios in years 2010, 2015, and 2020. Age-classes and diet scenarios are indicated on the x axis and include ages 2-4 (calf), 17 (sub-adult), and 33-38 (adult) years old; the three diet scenarios assume an exponential increase with doubling times equal to 3.2, 3.6, and 4.0 years. Standard error bars are shown for standard error values greater than 13,000 ng/g lw.

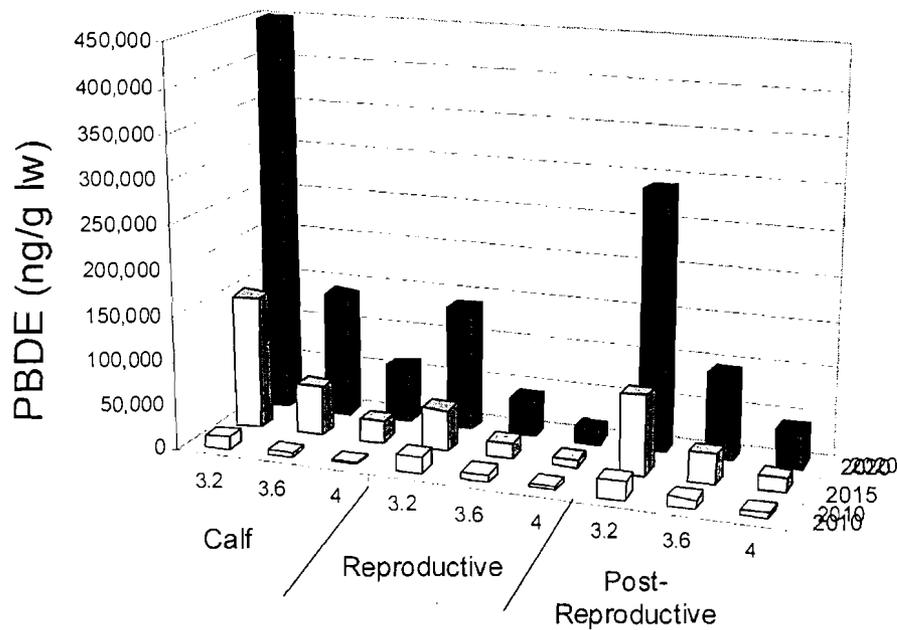


Figure 2.10. Average female projected PBDE concentrations in all three diet scenarios in years 2010, 2015, and 2020. Age-classes and diet scenarios are indicated on the x axis and include ages 3-5 (calf), 17 (reproductive), and 49-51 (post-reproductive) years old; the three diet scenarios assume an exponential increase with doubling times equal to 3.2, 3.6, and 4.0 years. Standard error bars are not shown because all standard error values are less than 7,000 ng/g lw.

Chapter 3: Influence of Life History Traits on the Predicted PCB and PBDE levels in the Southern Resident Killer Whale

3.1 Introduction

Killer whales (*Orcinus orca*) are a cosmopolitan species that occupy a variety of ecological niches. The Southern Resident killer whales (SRKWs) are a distinct community of killer whales with a distribution off the west coast of Canada and the United States (Bigg, 1982; Osborne, 1999; Hauser et al., 2007). They consist of three pods (J, K, and L pods), with little to no observed matrilineal dispersal (Bigg et al., 1990). The population has been surveyed annually since the 1970s. From these annual surveys each individual in the population has been identified by dorsal fin markings and saddle patch colorations (Bigg et al., 1990), permitting researchers to track individual whales from birth to death using photo-identification techniques. The annual surveys have provided a unique opportunity to identify individual life history traits (Olesiuk et al., 1990; Center for Whale Research, 2009).

The SRKWs were listed as “endangered” as defined by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) in 2001 and by the U.S. Endangered Species Act of 1973 (ESA; 16 U.S.C.A. §§ 1531 *et seq.*, as amended) in 2005 due to an average annual population decline of 4.3% from 1996 to 2001 (Krahn et al., 2004). One potential reason for this decline is high concentrations of persistent organic pollutant concentrations (e.g. the polychlorinated biphenyls, PCBs, and polybrominated diphenyl ethers, PBDEs) found in individual killer whales (Ross et al., 2000; Krahn et al., 2007, 2009). PCBs and PBDEs are xenobiotic compounds that persist in nature and bioaccumulate in upper trophic level species. These persistent organic pollutants are of particular concern because they may cause reproductive damage (Reijnders, 1986; Kuriyama et al., 2005), endocrine disruption (Brouwer et al., 1999; Zhou et al., 2001) and immunotoxicity (Aguilar and Borrell, 1994b; de Swart et al., 1994, 1996; Ross et al., 1995).

Chapter 2 of this thesis described an individual-based model that was used to estimate historic and future accumulations of PCBs and PBDEs in the SRKWs, using known or estimated life history traits of each individual. In addition, the model used information on individual growth, energetics, and diet to estimate contaminant accumulation for each individual in the population for each year of the individual's life. The model output consisted of a suite of variables (i.e. sex, age, birth year, death year, estimated birth order, estimated number of offspring, and annual contaminant uptake values) for each individual in the population.

Contaminant levels in the blubber of marine mammals are known to vary with diet, age, sex, birth order, and reproductive status (Aguilar and Borrell, 1994a; Borrell et al., 1995; Addison and Ross, 2000; Ross et al., 2000; Ylitalo et al., 2001; Siddiqi et al., 2003; Krahn et al, 2009). Potential health risks associated with PCB and PBDE exposure are difficult to predict in the SRKWs because there is a wide range of predicted PCB and PBDE concentrations in individuals and there is a lack of dose-response information. However, the first steps in predicting risk are to assess similarities in individuals. Subsequent to assessing similarities, examining the respective influences of the life history traits on the total contaminant burden (i.e. both PCB and PBDE concentrations) may prove to be useful for predicting future contaminant patterns across demographic categories.

In this chapter my specific objectives are to

1. assess and clarify similarities among known living and deceased individuals in the SRKW population and explore and identify any natural groupings in the model output (i.e. identify groups of individuals with similar life history traits and estimated contaminant levels);
2. determine the respective influences of life history traits that lead to the pattern of contaminant levels predicted in male and female killer whales.

3.2 Methods

3.2.1 Data Set and Assumptions

To accomplish the objectives, two multivariate techniques, clustering and principal components analysis (PCA), were used. The input matrices used in the analyses were derived from the individual-based model. The objects (or rows) of the original matrix were the 147 living and dead individuals with known mothers, and the descriptors (or columns) were the estimated log PCB and PBDE concentrations in living individuals in 2007, or concentrations at time of death for deceased individuals. To address the first objective, the original matrix was further divided by pod (“J”, “K”, and “L” matrices) to identify any pod-specific diet effects due to their different winter distributions (Krahn et al., 2004; Zamon et al., 2007). To address the second objective, the original matrix was divided by sex (“living males in 2007” and “living females in 2007”). The male and female matrices included additional descriptors such as age, birth year, estimated birth order, and estimated number of offspring. All analyses were performed in R: A Language and Environment for Statistical Computing (R Development Core Team, 2009).

The elements in the matrices have a wide range of variation. For example, the predicted contaminant concentrations can vary $\pm 100,000$ ng/g, whereas birth order can vary ± 5 . Therefore, standardized Z scores were calculated for each continuous matrix element:

$$Z = (X_{i,j} - \mu_i) / S_i, \quad \text{[equation 1]}$$

where $X_{i,j}$ is the original data element in the j th row and i th column, μ_i is the mean of all the elements in the i th column, and S_i is the standard deviation of all elements in the i th column.

Several assumptions must be met in order for most multivariate analyses to be applicable for inferential testing. For example, the data must be multivariate normal, the data should be derived from an independent random sampling procedure, outliers may need to be

excluded, and variables need to have linear relationships among each other (McGarigal et al., 2000). These assumptions can be relaxed when performing descriptive analyses such as clustering techniques and PCA (McGarigal et al., 2000). The best way to test for multivariate normality is to test for normality in each variable, with the caveat that the entire model output may not be multivariate normal even when all individual variables are normal.

Prior to performing the analyses for this chapter, the Shapiro-Wilk normality test was used to determine consistency of the model output with the underlying multivariate normality assumption. The descriptors were log transformed to facilitate consistency with this assumption prior to the analyses. However, only the contaminant concentrations met the normality assumption subsequent to the log transformation. In addition, a few individuals could be considered outliers. Some were substantially older than others, and some gave birth to more than the average number of offspring. Although outliers exert a pronounced influence on principal component axes (McGarigal et al., 2000), the few individual outliers were retained in the analyses because it is important to have a full range of ages and fertility in order to determine the influence of such life history traits on contaminant levels. Ultimately, excluding the outliers would create a loss of meaningful information in this case.

3.2.2 Clustering Analyses

The first objective of this study was to clarify the relationships among the individual killer whales and identify natural groupings in the estimated contaminant levels from the individual-based model. The assumption was that individuals in similar life history groups would have similar predicted contaminant concentrations and that these similar individuals may have similar health risks associated with contaminant exposure. For example, we would expect first-born adult males to have higher predicted contaminant levels than first-born adult females. Consequently, the first-born adult males would be in a separate health risk category than first-born females. One way to test for similarities in contamination among specific life history groups is to perform clustering analyses. As

mentioned previously, the input matrices (“J”, “K”, and “L” matrices) contained the predicted PCB and PBDE concentrations from the 147 individuals with known mothers. If the individual was alive, the predicted concentrations were from 2007, if the individual was dead, the predicted concentration levels were from the year the individual died.

The clustering analyses used an exclusive sequential hierarchical agglomerative polythetic technique. In general, this technique places each individual in a single group and applies a sequence of fusion strategies until all groups merge (Everitt, 1980). The technique also considers all information for each individual as opposed to considering only one variable (McGarigal et al., 2000).

Resemblance matrices for the clustering analyses were computed using Gower’s coefficient. The similarity coefficient (S) calculates the similarity among individuals 1 and 2 by summing the similarities for all the descriptors for individuals 1 and 2 and averaging them over all the descriptors.

$$S(x_1, x_2) = \frac{1}{p} \sum_{j=1}^p s_{12j} \quad \text{[equation 2]}$$

$$s_{12j} = 1 - [|y_{1j} - y_{2j}| / R_j] \quad \text{[equation 3]}$$

Where: x is the individual;
 p is the total number of descriptors;
 s is the partial similarity between the two individuals for each descriptor j ;
 y is the score of individual 1 or 2 on variable j ; and
 R_j is the largest descriptor distance.

These similarities were transformed to dissimilarities, or distances.

After computing the resemblance matrices, a space-conserving fusion strategy which preserved the multidimensional structure, average linkage (Unweighted, Pair-Group Method using Arithmetic means; UPGMA), was chosen to group similar objects together. Average linkage assigns the distance values between clusters to equal the average distance between all individuals in the two groups (Everitt, 1980). More specifically, for UPGMA, the dissimilarity of two cluster groups (1 and 2) is equal to the average of all dissimilarities between individuals of cluster 1 and cluster 2 (Kaufman and Rousseeuw, 1990). This space-conserving method is used most often in ecology and maximizes the correlation between the input and output dissimilarity matrices.

3.2.3 Principal Components Analyses

The second objective of this study was to explain the variation in the 2007 estimated contaminant concentration levels by determining the respective influences of life history traits on the predicted total contaminant body burdens in males and females. It has been well documented that life history traits play a role in the accumulation of POPs (Aguilar and Borrell, 1994a; Borrell et al., 1995; Addison and Ross, 2000; Ross et al., 2000; Ylitalo et al., 2001; Siddiqi et al., 2003; Krahn et al., 2009), but the relative influence of these life history traits on the total contamination (i.e. PCBs and PBDEs) is unknown. To accomplish this objective, an unconstrained ordination technique that reduces the dimensionality of the model output (Dunteman, 1999; PCA), was used to integrate the life history traits and estimated contaminant levels of the SRKW population into a smaller suite of derived variables. PCA is a widely used ordination technique that reduces the number of variables into a smaller number of dimensions or components (McGarigal et al., 2000). Similar to clustering analysis, PCA can aid in revealing similarities among individual killer whales. In addition, PCA can examine which life history traits are the primary drivers of contaminant concentration and accumulation. Individuals that died prior to 2007 were excluded in the PCA.

Eigenvalues were calculated for each matrix. The eigenvalues measure the amount of variation in the matrix represented on the components or axes. The first principal

component has the largest eigenvalue and therefore defines the gradient with the most variance. The percentage of variation explained by each component was calculated by dividing the eigenvalue for each component by the sum of all eigenvalues and multiplied by 100. The calculated eigenvalues were compared with a null distribution of eigenvalues derived from the broken-stick model to determine if they were higher than expected by chance. The calculated eigenvalues that were greater than the expected null eigenvalues were considered to explain a significant amount of the variation. The principal weights, which indicate the respective contribution of the original variables to each principal component, were also calculated. A general rule of thumb (Hair et al., 2006) was used to determine if the component weights were significant: loadings greater than 0.30 or less than -0.30 are important, loadings greater than 0.40 or less than -0.40 are significant, loadings greater than 0.50 or less than -0.50 are very significant.

A more quantitative approach to test for significant loadings was not used in this analysis because the descriptors did not meet the underlying normality assumption. When the results are used in statistical inference, the concern over meeting the assumptions mentioned previously is reasonable. However, (Gauch, 1982) warns that even when the analysis is for descriptive purposes, there should be caution when relaxing the assumptions because the underlying model in the analysis may be valid to one data set but not another.

3.3 Results

3.3.1 Clustering Analyses

The clustering analyses reveal that patterns of contaminant concentrations in living individuals and dead individuals are distinct from one another in all three pods (Figures 3.1 to 3.3). Furthermore, total contamination in dead individuals in K and L pods that died in the 1970s and 1980s are distinct from those of dead individuals that died in the 1990s and early 2000s (Figures 3.2 and 3.3). Due to a smaller sample size, dead J pod animals did not cluster by time as they do in K and L pods. There do not appear to be

any other discrepancies in the cluster formations among the pods, and therefore slight differences in diet among the pods did not greatly influence clustering.

In living individuals, predicted contaminant concentrations resulted in clustering of reproductive females (Figures 3.1 to 3.3). However, there were a few reproductive L pod females that clustered with other life history groups. In general, the living male and female calves and juveniles formed clusters with the sub-adult (Figure 3.1) and adult males (Figures 3.2, 3.3) although there were exceptions or outliers in each grouping.

3.3.2 Principal Components Analyses

Males

The first three principal components (PC1, PC2, and PC3) describe a total of 90% of the variation in contaminant levels and life history traits (PC1 describes 50%, PC2 describes 24%, and PC3 describes 16% of the variation). PC1 and PC3 are above the null broken stick values indicating the variance explained by these components is significant. The factors that define PC1 include birth year, age, and PBDEs with a gradient from younger individuals with high PBDE concentrations to older individuals with low PBDE concentrations (Figure 3.4). The factors that define PC3 include birth order and PBDEs with a gradient from high PBDEs and high birth order to low PBDEs and low birth order (Figure 3.4). PBDEs were positively associated with birth year and birth order, and negatively associated with age (Table 3.1). PCBs were the primary factor defining PC2 and had a generally positive association with age and a generally negative association with birth order and birth year (Table 3.1). Males with the highest PCB concentrations included older first-born individuals whereas males with the highest PBDE concentrations included young individuals who were born more recently and were not first-born.

Females

The first three principal components in the female PCA describe a total of 94% of the variation (PC1 describes 49%, PC2 describes 28%, and PC3 describes 17%). The first three components are above the null broken stick values indicating they describe a significant amount of the variation. The factors that define PC1 include age, birth year, and the number of offspring with a gradient from older individuals with more offspring to younger individuals with few to no offspring (Figure 3.5). The factors that define PC2 include the PCB and PBDE concentrations with a gradient from high PCB and PBDE concentrations to low PCB and PBDE concentrations (Figure 3.5). Birth order was the only descriptor that significantly defined PC3 with a principal weight of 0.99 (Table 3.2). Therefore, birth order had no significant relationship with any other descriptor (i.e. birth year, age, number of offspring, and PCB and PBDE concentrations). The life history descriptors lacked a significant association with the PCB or PBDE concentrations in female killer whales (Table 3.2). PCBs had a generally negative association with age and the number of offspring, whereas PBDEs lacked an association with age and number of offspring (Table 3.2). Both PCBs and PBDEs lacked an association with birth order in female killer whales (Table 3.2). Similar to the male analysis, the female killer whales were quite similar in ordinate space with only a few outliers (Figure 3.5).

3.4 Discussion

Life history traits such as age, sex, birth order, and reproductive status can influence the contaminant concentrations in individuals (Ross et al., 2000; Ylitalo et al., 2001). The goal of this analysis was to clarify similarities in individuals based on their total contaminant burden. In addition, the respective influences of these life history traits on the combined PCB and PBDE concentrations in the SRKWs was determined to describe the pattern observed in the predicted contamination in male and female killer whales.

3.4.1 Similarities Among Individuals

Males and females in the Southern Resident killer whale population tend to have very different age-related contaminant patterns. PCBs and PBDEs can become mobilized

during pregnancy (O'Shea, 1999) allowing females to offload their body burdens via transplacental transfer and lactation whereas males lack the ability to offload their contaminants to their offspring. Ross et al., (2000) found PCB levels in female killer whales to be reduced around the time of the first calf, remain low until approximately age 50, and then begin to increase again with age, suggesting reproductive senescence.

In the hierarchical clustering analyses, reproductive females were distinct from all other individuals. Surprisingly, there was not enough distinction in calf total concentrations to form an isolated cluster. In addition, there did not appear to be a distinction among female and male juveniles. In fact, calves and juveniles of both sexes had similar concentrations as adult males and these life history groups clustered together. Subsequent to receiving the maternal burden from birth, individuals experience a growth dilution in contamination whereby their total contaminant concentration is reduced when the total body mass significantly increases in a short period of time. These results are similar to those found in calves and adult male killer whales that were stranded in sea ice off Japan (Kajiwara et al., 2006). Indeed, the stranded killer whale calves had similar concentrations as adult males. These concentrations were approximately 1.5-fold higher than PCB concentrations and 2-3-fold higher than PBDE concentrations in adult females (Haraguchi et al., 2009). The relatively high contaminant concentrations in calves indicate the ability for females to transfer a significant amount of their total body burden to their offspring (Kajiwara et al., 2006).

There is currently not enough information to assign relative health-associated risk to the two cluster groupings, reproductive females versus all other individuals. However, future risk assessments should estimate risk for individuals in these two groupings separately. For instance, adverse health effects in reproductive females from high exposure may include reproductive failure or increased stillbirths and calf mortalities (Schwacke et al., 2002) whereas health risks associated with individuals in all other life history categories may include immunosuppression (de Swart et al., 1994, 1996) or other health-related effects.

The hierarchical clustering analyses also demonstrated that dead killer whales were distinct from living killer whales in terms of contaminant concentrations. Moreover, individual killer whales that were born and died prior to the 1990s were different from all other individuals. It is believed that PBDE concentrations play a large role in these findings. PBDEs have been shown to increase exponentially in several marine species (Ikonomou et al., 2002; Rayne et al., 2003; Lebeuf et al., 2004). Individuals alive after the 1990s were estimated to accumulate substantially more PBDEs than individuals alive prior to the 1990s. Furthermore, each additional year's accumulation increases at an exponential rate causing current individuals to be exposed to relatively large PBDE levels. The clustering of individuals that died prior to the 1990s is, in part, a reflection of the predicted exponentially increasing PBDE trend.

3.4.2 Relative Influential Factors on Total Contamination

There is no significant primary factor that influences PCB concentrations in male killer whales. Although not a significant factor, age was found to influence PCB levels in male killer whales. Congruent with other studies (e.g. Subramanian et al., 1987; Ross et al., 2000), this study found that there was generally a positive relationship between PCBs and age in male killer whales. Birth order had a generally negative association with PCB concentrations in male killer whales. The negative association is consistent with evidence from biopsy samples taken from first-born killer whales from Kenai Fjords/Prince William Sound, Alaska (Ylitalo et al., 2001). However, the organochlorine concentrations in first-born male Alaskan resident killer whales were found to be significantly higher than in non-first-borns (Ylitalo et al., 2001). First-born individuals are generally exposed to higher contaminant levels than subsequent offspring because the mother's contaminant burden declines with each successive offspring via gestation and lactation. In addition, it may be that female killer whales offload a declining percentage of their contaminant burden as they age, similar to the pattern found in bottlenose dolphins (Ridgeway and Reddy, 1995), although the model assumed a continuous offload percentage throughout the female's life. The total contamination predicted in male SRKWs is uniformly influenced by birth year, age, and birth order.

The significant primary factors that influence PBDE levels in male SRKW are birth year, age, and birth order. There was a significant negative association found between PBDEs and age in male killer whales. Indeed, the SRKW biopsy sample results indicate that calves have significantly higher levels of PBDEs than adult males (Krahn et al., 2009). The relationship between PBDEs and age is confounded by the fact that individuals have only been exposed since the 1970s (biasing levels in adult males) and due to the non-linear input. Individuals born prior to the 1970s have similar concentration levels as individuals born in the 1970s. A somewhat similar PCB trend has been reported in male fin whales (*Balaenoptera physalus*; Aguilar and Borrell, 1994a). PCB levels in adult fin whales were found to approach asymptotic levels as the individual aged, suggesting that adults were not exposed to PCBs as juveniles (Aguilar and Borrell, 1994a). Similar to PCB exposure in male fin whales, it is probable that the extreme increase in PBDE concentrations in the killer whales, with PBDE accumulation doubling times between 3 and 4 years, may have masked the age-related increase in contaminants in male killer whales. Age can be a proxy for contaminant exposure trends and therefore the initial year of PBDE accumulation in killer whales can be predicted. As PBDE exposure continues, it is likely that an age-related association will emerge and adult males will have higher PBDE concentrations than juveniles or sub-adults. A positive association with age can also indicate PBDEs are no longer increasing exponentially. In contrast to PCBs in male killer whales, birth order had a positive association with PBDE concentrations. This is likely due to the exponential increase in environmental contamination over time. For example, a fourth-born individual born in the 2000s can have a higher PBDE body burden than a first-born individual born in the 1990s.

Aside from the reproductive status, there is no significant factor that influences PCB concentrations in female killer whales. PCBs had a generally negative association with age and number of offspring in female killer whales, reflecting what is known about a female's ability to transfer lipophilic contaminants to her offspring, thereby reducing her body burden with age (Duinker and Hillebrand, 1979; Ridgeway and Reddy, 1995; Wells et al., 2005) until post-reproduction. Contrary to age-related contaminant levels found in

many marine mammals, Borrell et al. (1995) found no relationship between PCB concentrations and age in juvenile female long-finned pilot whales and found no evidence of an increase in PCBs in older females. Indeed, PCBs decreased with age in adult female pilot whales (Borrell et al., 1995) perhaps illustrating the possibility that females in that population may remain reproductive throughout their lifetime. In this study, a female's reproductive status was most influential in PCB contamination, and age and number of offspring had similar influences on concentration levels. PBDE levels were generally influenced by the birth year, but lacked a significant association with all other life history traits. The lack of association with any life history traits and PBDEs in current individuals is likely due to the exponential increase in contamination.

3.4.3 Caveats

There are several caveats that should be recognized when performing multivariate analyses. In general, the main disadvantage in agglomerative hierarchical clustering is that once an entity has been classified in the beginning of the analysis, there is no method for reallocation (Kaufman and Rousseeuw, 1990). As a result, an entity that had been poorly classified early on in the analysis could drastically alter the outcome in a negative way. There should be caution when considering the validity and biological significance of the formed clusters because the different clustering techniques can give rise to different solutions. It is important to validate the results of cluster analysis by comparing results among various clustering techniques when there is interest in future analyses such as in hypothesis testing (McGarigal et al., 2000). However, in this case, the interest was to explore the model output, or more specifically to explore the estimated individual contaminant levels by grouping the individuals for clarifying purposes. Therefore, validation included comparing hierarchical clustering results with results from PCA. Principal components analysis can be sensitive to outliers, but this limitation can be avoided by standardizing the model output and removing any outlier with little or no biological meaning.

3.5 Conclusions

The model output mirrors results suggested by previous contaminant studies. But few of the previous studies, particularly on SRKWs, had a large enough sample size to statistically analyze how life history traits influence PCB and PBDE levels. In male SRKWs, no primary factor significantly influenced the PCB concentrations predicted across the individuals in the current SRKW population. In contrast, the birth year, age, and birth order significantly influenced PBDE concentrations. Age was found to have a generally positive association with PCBs and a significant negative association with PBDEs in male killer whales. In addition, birth order had a generally negative association with PCBs and a significant positive association with PBDE concentrations. The latter, perhaps counterintuitive result, is due to the exponential increase of PBDEs in the SRKWs. For example, a third-born individual born in 2007 will have a higher PBDE concentration than a first-born individual born in 2000. Among male killer whales, individuals with the highest PCB concentrations included primarily older individuals with low birth orders and individuals with high PBDE concentrations included primarily younger individuals born recently with high birth orders. The contaminant levels in female killer whales were primarily influenced by individual reproductive status and were not strongly associated with age or birth order. Reproductive females were found to have distinct contaminant concentrations compared to all other life history groups. The results of this study are important and valuable in the context of conservation risk assessment for the endangered Southern Resident killer whale population because they help us predict the contaminant levels across all individuals within the current SRKW population. It is not possible to biopsy all individuals, yet estimates for all individuals are necessary if scientists are to tease out the effects of contaminant loading on individual reproductive output and survival in this endangered species.

Table 3.1. Principal loadings, or weights, for each principal component in the PCA for living males. The life history descriptors included the individual's birth year, age, and estimated birth order. The estimated PCB and PBDE concentrations were predicted levels in 2007.

Descriptors	PC1	PC2	PC3
Birth Year	-0.56	0.39	-0.05
Age	0.57	-0.33	-0.06
Birth Order	0.32	0.31	0.85
PCB	-0.25	-0.76	0.20
PBDE	-0.45	0.26	0.48

Table 3.2. Principal loadings, or weights, for each principal component in the PCA for living females. Similar to the analysis for living males, the life history descriptors included birth year, age, and estimated birth order. The estimated number of offspring produced by each female was also included as a descriptor in the PCA. The estimated PCB and PBDE values were predicted levels in 2007.

Descriptors	PC1	PC2	PC3
Birth Year	0.51	-0.33	0.01
Age	-0.55	0.15	-0.10
Birth Order	-0.06	-0.04	0.99
Offspring #	-0.54	0.15	-0.02
PCB	0.31	0.62	-0.02
PBDE	0.20	0.68	0.09



Figure 3.1. Dendrogram from the hierarchical cluster analysis performed on J pod killer whales. Clustering was determined using a space-conserving fusion strategy (average linkage). Juveniles are between the ages of 2 and 11 years, reproductive females are between the ages of 12 and 40 years, sub-adult males are between the ages of 12 and 20 years, and adult males are 21 years and older.

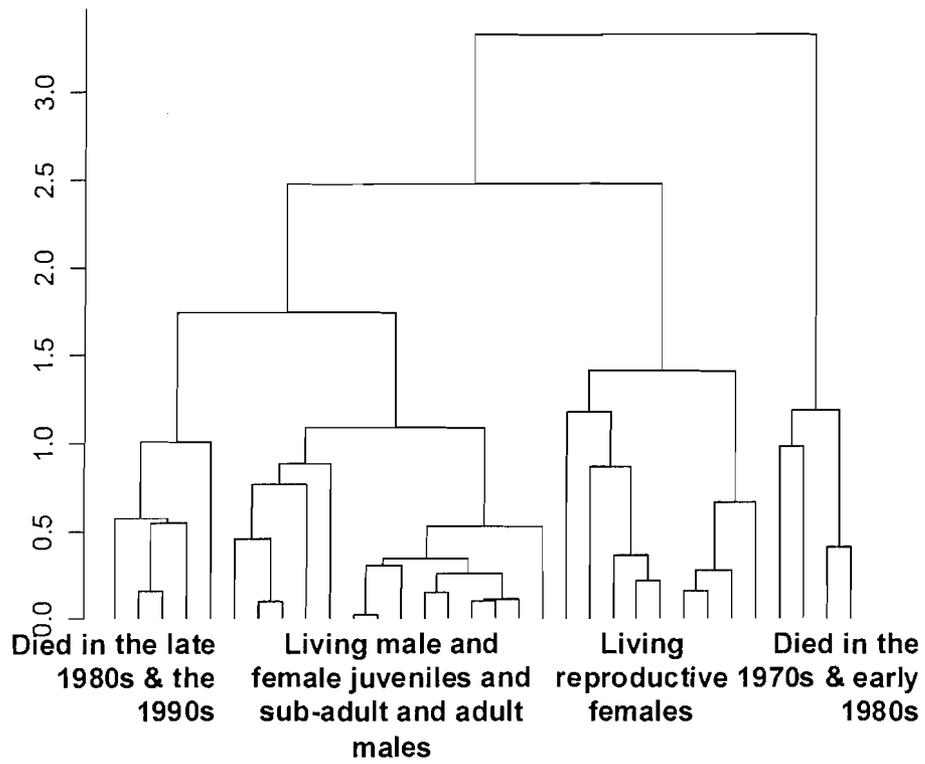


Figure 3.2. Dendrogram from the hierarchical cluster analysis performed on K pod killer whales. Clustering was determined using a space-conserving fusion strategy (average linkage). Juveniles are between the ages of 2 and 11 years, reproductive females are between the ages of 12 and 40 years, sub-adult males are between the ages of 12 and 20 years, and adult males are 21 years and older.

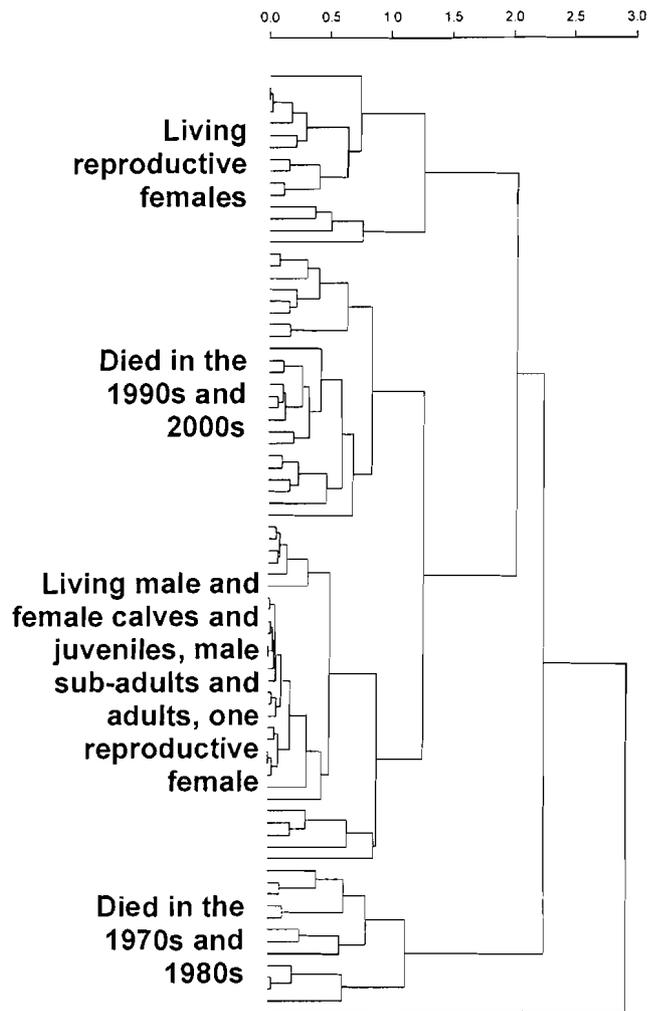


Figure 3.3. Dendrogram from the hierarchical cluster analysis performed on L pod killer whales. Clustering was determined using a space-conserving fusion strategy (average linkage). Calves are between the ages of 0 and 1, juveniles are between the ages of 2 and 11 years, reproductive females are between the ages of 12 and 40 years, sub-adult males are between the ages of 12 and 20 years, and adult males are 21 years and older.

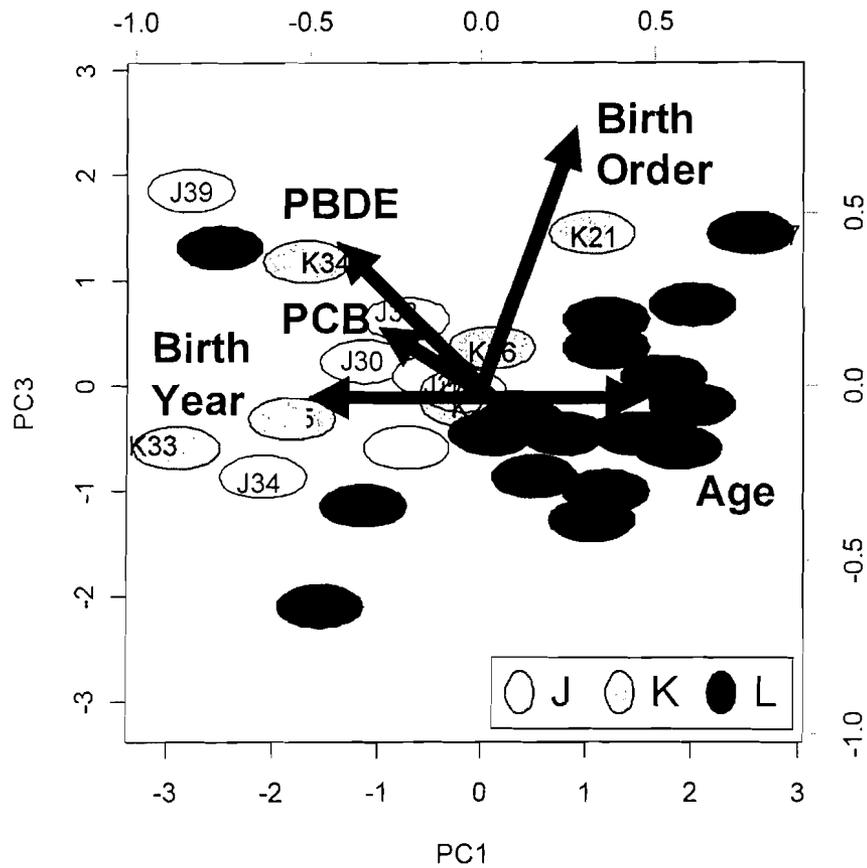


Figure 3.4. Ordination plot showing the first and third components from the living male principal components analysis. The descriptors included PBDEs, PCBs, age, birth order, and birth year. Pods are distinguished in the ordination plot by color.

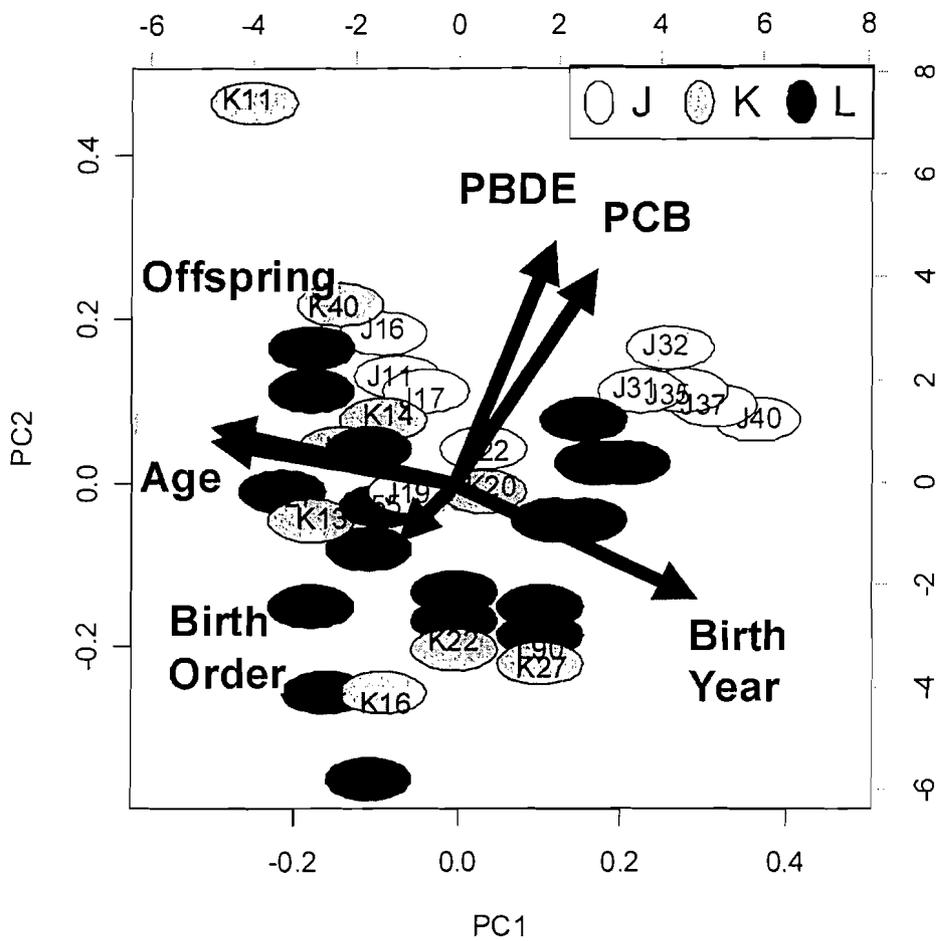


Figure 3.5. Ordination plot showing the first and second components from the living female principal components analysis. The descriptors included PBDEs, PCBs, birth year, birth order, number of offspring, and age. Pods are distinguished in the ordination plot by color.

Appendix A

Data frames for Individual-Based Model

Table 1. Mass-at-age data from Noren (in revision) used in the individual-based model to estimate developmental growth for each individual in the population for each year. Mass is reported in *kg*. Individuals over the age of 20 years are assumed to have a constant mass and are thus not reported here. A pregnant female's mass was estimated by adding a calf's mass at age 0 (155 kg; Clark et al., 2000) to the adult female mass.

Age	Male Mass	Female Mass	Pregnant Female Mass
1	465	465	
2	695	695	
3	949	949	
4	1208	1208	
5	1455	1455	
6	1682	1682	
7	1881	1881	
8	2051	2051	
9	2194	2194	
10	2311	2311	2466
11	2406	2406	2561
12	2482	2482	2637
13	2726	2589	2744
14	2970	2696	2851
15	3214	2803	2958
16	3458	2910	3065
17	3702	3017	3172
18	3946	3124	3279
19	4190	3231	3386
20+	4434	3338	3493

Table 2. Minimum and maximum daily prey energetic requirements ($DPER_{x,g}$) from Noren (in revision) used in the individual-based model to estimate energetic requirements for each sex, age-class, and reproductive status. Individuals over the age of 20 years are assumed to have a constant energy requirement and are thus not reported here. $DPER_{x,k}$ values are reported in *kcal/day*.

Age	Male MinDPER	Male MaxDPER	Female MinDPER	Female MaxDPER	Pregnant Female MinDPER	Pregnant Female MaxDPER
1	40455	48466	40455	48466		
2	54685	65514	54685	65514		
3	69077	82755	69077	82755		
4	82781	99173	82781	99173		
5	95176	114023	95176	114023		
6	106109	127120	106109	127120		
7	115391	138241	115391	138241		
8	123128	147509	123128	147509		
9	129512	155158	129512	155158		
10	134658	161323	134658	161323	141371	169365
11	138788	166271	138788	166271	145436	174236
12	142064	170195	142064	170195	148667	178106
13	152414	182595	146633	175669	153168	183499
14	162536	194721	151155	181086	157627	188840
15	172451	206600	155632	186450	162043	194131
16	182180	218255	160067	191763	166420	199374
17	191738	229706	164461	197027	170758	204572
18	201140	240970	168816	202245	175060	209726
19	210398	252061	173135	207419	179327	214838
20+	219522	262992	177417	212550	183561	219910

Table 3. Total PCBs and PBDEs (*ng/g* lipid) in the blubber of biopsy samples from individual Southern Resident killer whales. Data from Krahn et al. (2007; 2009).

Individual (<i>i</i>)	Pod (<i>p</i>)	Year (<i>y</i>)	Age (<i>x</i>)	Sex (<i>g</i>)	Σ PCBs (<i>c</i>)	Σ PBDEs (<i>c</i>)
J39	J	2006	3	M	34000	15000
J27	J	2006	15	M	74000	6300
J19	J	2006	27	F	45000	7500
J1	J	2006	55	M	180000	6800
J38	J	2007	4	U	41000	14000
J22	J	2007	22	F	4600	880
K36	K	2007	4	U	62000	15000
K34	K	2007	6	M	39000	10000
K21	K	2007	21	M	38000	2900
K13	K	2007	35	F	8900	1200
K7	K	2007	97	F	120000	6700
L78	L	2004	15	M	22000	2600
L71	L	2004	18	M	36000	2600
L74	L	2004	18	M	45000	3100
L85	L	2006	15	M	50000	2500
L57	L	2006	29	M	56000	3300
L87	L	2007	15	M	24000	2600
L73	L	2007	21	M	32000	3400
L67	L	2007	22	F	5600	680
L26	L	2007	51	F	17000	4400
L21	L	2007	57	F	55000	4200

Appendix B

Pseudo code for the PCB and PBDE individual-based and projection models

PCB Individual-Based Model

For simulations 1 to 100

%Draw from a uniform distribution for elimination E

$$E = U(.016, .024)$$

For each female

%Draw from a uniform distribution for offload parameters

$$TT_i = U(3\%, 5\%)$$

$$LO_i = U(70\%, 90\%)$$

End for each female

For each year from 1930 to 2007

%Draw from a uniform distribution for the prey's caloric content

$$CC_y = U(1643, 1804)$$

%Estimate PCB concentration range in P. S. / G.B. prey

$$CI_{ps,low} = \begin{cases} 5.6y - 11.7 \\ -4.1y + 279 \\ 38.7 \end{cases}$$

$$CI_{ps,high} = \begin{cases} 7.88y - 16.49 \\ -5.78y + 393 \\ 54 \end{cases}$$

$$PC_{c=PCB,y,P.S.} = U(CI_{ps,low}, CI_{ps,high})$$

%Estimate PCB concentration range in outer coast prey

$$CI_{outer,low} = .35y - .35$$

$$CI_{outer,high} = .456y - .456$$

$$PC_{c=PCB,y,p=O.C.} = U(CI_{outer,low}, CI_{outer,high})$$

%"uniform" diet Scenario

$$PC_{c=PCB,y,p=J} = PC_{c=PCB,y,P.S.}$$

$$PC_{c=PCB,y,p=K,L} = PC_{c=PCB,y,O.C.}$$

%"mixed" diet Scenario

$$PC_{c=PCB,y,p=J} = \frac{2}{3}(PC_{c=PCB,y,P.S.}) + \frac{1}{3}(PC_{c=PCB,y,O.C.})$$

$$PC_{c=PCB,y,p=K,L} = \frac{2}{3}(PC_{c=PCB,y,O.C.}) + \frac{1}{3}(PC_{c=PCB,y,P.S.})$$

For each individual

If they were born this year or last year or died before this year
Next individual

%Estimate energy requirements

$DPER_{x,g} = U(\min DPER_{x,g}, \max DPER_{x,g})$
%Estimate biomass consumed
 $BIO_{x,g} = (DPER_{x,g} / CC_y) 365$
%Estimate prey intake
 $PI_{c=PCB,y,p,x} = PC_{c=PCB,y,p,S.I.O.C.} * BIO_{x,g}$
%Estimate current PCB burden
 $totC_{c=PCB,i,y} = totC_{c=PCB,i,y-1} + PI_{c=PCB,y,p,x}$
% 80% of total prey intake is located in blubber while the remaining 20% is located in the body core
 $totC_{c=PCB,i,y} = (totC_{c=PCB,i,y}) * .80$
 If they gave birth this year
%Offload via gestation
 $totC_{c=PCB,i,y} = (totC_{c=PCB,i,y})(1 - TT_i)$
 And if they gave birth this year
 If they were born this year by individual *i*
%Receive offload
 $totC_{c=PCB,k,y,x=0} = (totC_{c=PCB,i,y})(TT_i)$
 End if they were born this year
 If they gave birth last year
%Offload via lactation
 $totC_{c=PCB,i,y} = (totC_{c=PCB,i,y})(1 - LO_i)$
 End if they gave birth last year
 If they were born last year by individual *i*
%Receive offload
 $totC_{c=PCB,k,y,x=1} = totC_{c=PCB,k,y-1,x=0} + [(totC_{c=PCB,i,y})(LO_i)]$
 End if they were born last year
%Subtract amount eliminated from total accumulation
 $totC_{c=PCB,i,y} = totC_{c=PCB,i,y} - E(totC_{c=PCB,i,y})$
%Estimate the adjustment term for missed calves to avoid overestimating total PCB in reproductive females
 if female, between the ages 12 and 41, and have not given birth for 3 years, offload via gestation and lactation
 $totC_{c=PCB,i,y} = (totC_{c=PCB,i,y})(1 - TT_i)$
 $totC_{c=PCB,i,y} = (totC_{c=PCB,i,y})(1 - LO_i)$
 End if female adjustment term
 End for each individual
 End for each year
%To Convert PCB burden to PCB concentration
%Estimate blubber mass
 $B\%_{i,y,upper} = 44 * age^{-.10}$
 $B\%_{i,y,lower} = 39 * age^{-.10}$

$$B\%_{i,y} = U(B\%_{i,y,lower}, B\%_{i,y,upper}) / 100$$

$$B_{i,y} = M_{x,g} * B\%_{i,y}$$

%Estimate lipid blubber mass

$$L\%_{i,y} = U(.28, .40)$$

$$L_{i,y} = L\%_{i,y} * B_{i,y}$$

%For each year and each individual, calculate concentration

$$totC_{e=PCB,i,y} = totC_{e=PCB,i,y} / L_{i,y}$$

End for each simulation

PBDE Individual-Based Model

For simulations 1 to 100

%Set up initial conditions for prey's PBDE concentration equation

$y = 0$

$$PC_{c=PBDE,y=0,p} = .01$$

For each female

%Draw from a uniform distribution for offload parameters

$$TT_i = U(3\%, 5\%)$$

$$LO_i = U(70\%, 90\%)$$

End for each female

For each year from 1970 to 2007

%Estimate lower and upper PBDE doubling times for pods

%Doubling time range for J and K pods equals 3.2 to 3.5 years

%Doubling time range for L pod equals 3.7 to 4.0 years

% Estimate PBDE concentration in prey for each pod

%J and K pod lower bound

$$JKlow = PC_{c=PBDE,y=0,p=J,K} \exp(3.2 * y)$$

%J and K pod upper bound

$$JKhigh = PC_{c=PBDE,y=0,p=J,K} \exp(3.5 * y)$$

%Prey PBDE concentration for J and K pods

$$PC_{c=PBDE,y,p=J,K} = U(JKlow, JKhigh)$$

%Lower bound for L pod

$$Llow = PC_{c=PBDE,y=0,p=L} \exp(3.7 * y)$$

%Upper bound for L pod

$$Lhigh = PC_{c=PBDE,y=0,p=L} \exp(4.0 * y)$$

% Prey PBDE concentration for L pod

$$PC_{c=PBDE,y,p=L} = U(Llow, Lhigh)$$

For each individual

If they were born this year or last year or died before this year

Next individual

%Estimate energy requirements

$$DPER_{x,g} = U(\min DPER_{x,g}, \max DPER_{x,g})$$

%Estimate biomass consumed

$$BIO_{x,g} = (DPER_{x,g} / CC_y) 365$$

%Estimate prey intake

$$PI_{c=PBDE,y,p,x} = PC_{c=PBDE,y,p} * BIO_{x,g}$$

%Estimate current PBDE burden

$$totC_{c=PBDE,i,y} = totC_{c=PBDE,i,y-1} + PI_{c=PBDE,y,p,x}$$

% 80% of total prey intake is located in blubber while the remaining 20% is located in the body core

$$totC_{c=PBDE,i,y} = (totC_{c=PBDE,i,y}) * .80$$

If they gave birth this year

%Offload via gestation

$$totC_{c=PBDE,i,y} = (totC_{c=PBDE,i,y})(1 - TT_i)$$

End if they gave birth this year

If they were born this year by individual *i*

%Receive offload

$$totC_{c=PBDE,k,y,x=0} = (totC_{c=PBDE,i,y})(TT_i)$$

End if they were born this year

If they gave birth last year

%Offload via lactation

$$totC_{c=PBDE,i,y} = (totC_{c=PBDE,i,y})(1 - LO_i)$$

End if they gave birth last year

If they were born last year by individual *i*

%Receive offload

$$totC_{c=PBDE,k,y,x=1} = totC_{c=PBDE,k,y-1,x=0} + [(totC_{c=PBDE,i,y})(LO_i)]$$

End if they were born last year

%Estimate the adjustment term for missed calves to avoid

%overestimating total PBDE in reproductive females

If female, between the ages 12 and 41, and haven't given birth for 3 years, offload via gestation and lactation:

$$totC_{c=PBDE,i,y} = (totC_{c=PBDE,i,y})(1 - TT_i)$$

$$totC_{c=PBDE,i,y} = (totC_{c=PBDE,i,y})(1 - LO_i)$$

End if female adjustment term

End for each individual

End for each year

%To Convert PBDE burden to PBDE concentration

%Estimate blubber mass

$$B\%_{i,y,upper} = 44 * age^{-10}$$

$$B\%_{i,y,lower} = 39 * age^{-10}$$

$$B\%_{i,y} = U(B\%_{i,y,lower}, B\%_{i,y,upper}) / 100$$

$$B_{i,y} = M_{x,k} * B\%_{i,y}$$

%Estimate lipid blubber mass

$$L\%_{i,y} = U(.28, .40)$$

$$L_{i,y} = L\%_{i,y} * B_{i,y}$$

%For each year and each individual, calculate concentration

$$totC_{c=PBDE,i,y} = totC_{c=PBDE,i,y} / L_{i,y}$$

End for each simulation

PCB Projection Model

%Set up constant parameter values

%Caloric content of the prey is the average value between the minimum and

%maximum 95% CI

$$CC = 1723.5$$

%Sexual maturity of females is 14 years old

%Calving interval in females is 5 years

%Lipid value is average of range used in the IBM

$$L\% = .34$$

%Offload parameters are the averages from the ranges used in the IBM

$$TT = .04$$

$$LO = .80$$

%Elimination parameter is the average of the range used in the IBM

$$E = .02$$

For each year from 2008 to 2050

%Estimate PCB concentration range in prey, 3 scenarios

%All scenarios (s) assume pods consume similar prey; the PCB values are

%averaged combining Puget Sound/Georgia Basin and outer coast Chinook salmon values; %changes are linear in all scenarios; the first year is 2008

(y=1)

%Scenario 1, average PCBs in prey remain constant

$$PC_{c=PCB,y,s=1} = 35$$

%Scenario 2, average PCBs in prey are half the concentration by 2050

$$PC_{c=PCB,y,s=2} = -.41y + 35$$

%Scenario 3, average PCBs in prey are zero by 2050

$$PC_{c=PCB,y,s=3} = -.82y + 35$$

For each individual

If they were born this year or last year, or died before this year, or if the age > 100 years

Next individual

End If statement

%Estimate energy requirements using the average DPER

$$DPER_{x,g} = (\min DPER_{x,g} + \max DPER_{x,g}) / 2$$

%Estimate biomass consumed

$$BIO_{x,g} = (DPER_{x,g} / CC) 365$$

%Estimate prey intake

$$PI_{c=PCB,y,s} = PC_{c=PCB,y,s} * BIO_{x,g}$$

%Estimate current PCB burden

$$totC_{c=PCB,t,y,s} = totC_{c=PCB,t,y-1,s} + PI_{c=PCB,y,s}$$

% 80% of total prey intake is located in blubber while the remaining 20% is located in the body core

$$totC_{c=PCB,i,y,s} = (totC_{c=PCB,i,y,s}) * .80$$

If the individual is female, has not given birth before, is age > 15 years, then she gives birth for the first time

%Offload via gestation

$$totC_{c=PCB,i,y,s} = (totC_{c=PCB,i,y,s})(1 - TT)$$

End if they gave birth this year for the first time

If the individual is female, gave birth before and the current year is a multiple of the existing interval and her age < 18, then she gives birth

%Offload via gestation

$$totC_{c=PCB,i,y,s} = (totC_{c=PCB,i,y,s})(1 - TT)$$

End if they gave birth not the first time

If they were born this year by individual *i*

%Receive offload

$$totC_{c=PCB,k,y,x=0,s} = (totC_{c=PCB,i,y,s})(TT)$$

End if they were born this year

If individual *i* gave birth last year

%Offload via lactation

$$totC_{c=PCB,i,y,s} = (totC_{c=PCB,i,y,s})(1 - LO)$$

End if they gave birth last year

If they were born last year by individual *i*

%Receive offload

$$totC_{c=PCB,k,y,x=1,s} = totC_{c=PCB,k,y-1,x=0,s} + [(totC_{c=PCB,i,y,s})(LO)]$$

End if they were born last year

End if they were born last year

%Subtract amount eliminated from total accumulation

$$totC_{c=PCB,i,y,s} = totC_{c=PCB,i,y,s} - E(totC_{c=PCB,i,y,s})$$

End for each individual

%To Convert PCB burden to PCB concentration

%Estimate blubber mass

$$B\%_{i,y,upper} = 44 * age^{-10}$$

$$B\%_{i,y,lower} = 39 * age^{-10}$$

$$B\%_{i,y} = (B\%_{i,y,lower}, B\%_{i,y,upper}) / 2$$

$$B_{i,y} = M_{x,g} * B\%_{i,y}$$

%Estimate lipid blubber mass

$$L = L\% * B_{i,y}$$

%For each year and each individual, calculate concentration

$$totC_{c=PCB,i,y,s} = totC_{c=PCB,i,y,s} / L$$

End for each year

PBDE Projection Model

%Set up constant parameter values
%Caloric content of the prey is the average value between the minimum and maximum 95% CI
 $CC = 1723.5$
%Sexual maturity of females is 14 years old
%Calving interval in females is 5 years
%Lipid value is average of range used in the IBM
 $L\% = .34$
%Offload parameters are the averages from the ranges used in the IBM
 $TT = .04$
 $LO = .80$
%Set up initial conditions for prey's PBDE concentration equation
%Start year is 2008, end year is 2050
 $y_0 = 38$
 $PC_{c=PBDE,y=0,s} = .01$

For each year from 2008 to 2050

%Estimate PBDE concentration range in prey, 3 scenarios (s)

%Scenario 1, doubling time (T) = 3.2 years

$$T_{s=1} = \ln(2)/3.2$$

%Scenario 2, doubling time (T) = 3.6 years

$$T_{s=2} = \ln(2)/3.6$$

%Scenario 3, doubling time (T) = 4 years

$$T_{s=3} = \ln(2)/4$$

% Estimate PBDE concentration in prey for each scenario

$$PC_{c=PBDE,y,s} = PC_{c=PBDE,y=0} \exp(T_s * y)$$

For each individual

if they were born this year or last year, died before this year, or if the age > 100 years

Next individual

%Estimate energy requirements

$$DPER_{x,g} = (\min DPER_{x,g} + \max DPER_{x,g})/2$$

%Estimate biomass consumed

$$BIO_{x,g} = (DPER_{x,g} / CC)365$$

%Estimate prey intake

$$PI_{c=PBDE,y,s} = PC_{c=PBDE,y,s} * BIO_{x,g}$$

%Estimate current PBDE burden

$$totC_{c=PBDE,t,y,s} = totC_{c=PBDE,t,y-1,s} + PI_{c=PBDE,y,s}$$

% 80% of total prey intake is located in blubber while the remaining 20% is located in the body core

$$totC_{c=PBDE,i,y,s} = (totC_{c=PBDE,i,y,s}) * .80$$

If the individual is female, has not given birth before, is age 15 years, then she gives birth for the first time

%Offload via gestation

$$totC_{c=PBDE,i,y,s} = (totC_{c=PBDE,i,y,s})(1 - TT)$$

End if they gave birth this year for the first time

If the individual is female, gave birth before and the current year is a multiple of the calving interval, and age < 40, then she gives birth

%Offload via gestation

$$totC_{c=PBDE,i,y,s} = (totC_{c=PBDE,i,y,s})(1 - TT)$$

End if they gave birth not the first time

If they were born this year by individual *i*

%Receive offload

$$totC_{c=PBDE,k,y,x=0,s} = (totC_{c=PBDE,i,y,s})(TT)$$

End if they were born this year

If individual *i* gave birth last year

%Offload via lactation

$$totC_{c=PBDE,i,y,s} = (totC_{c=PBDE,i,y,s})(1 - LO)$$

End if they gave birth last year

If they were born last year by individual *i*

%Receive offload

$$totC_{c=PBDE,k,y,x=1,s} = totC_{c=PBDE,k,y-1,x=0,s} + [(totC_{c=PBDE,i,y,s})(LO)]$$

End if they were born last year

End for each individual

%To Convert PBDE burden to PBDE concentration

%Estimate blubber mass

$$B\%_{i,y,upper} = 44 * age^{-10}$$

$$B\%_{i,y,lower} = 39 * age^{-10}$$

$$B\%_{i,y} = (B\%_{i,y,lower}, B\%_{i,y,upper}) / 2$$

$$B_{i,y} = M_{x,g} * B\%_{i,y}$$

%Estimate lipid blubber mass

$$L = L\% * B_{i,y}$$

%For each year and each individual, calculate concentration

$$totC_{c=PBDE,i,y,s} = totC_{c=PBDE,i,y,s} / L$$

End for each year

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