BIOACCUMULATION OF POLLUTANTS IN GALAPAGOS SEA LIONS AND MARINE MAMMALS FROM BRITISH COLUMBIA, CANADA

by

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ABSTRACT

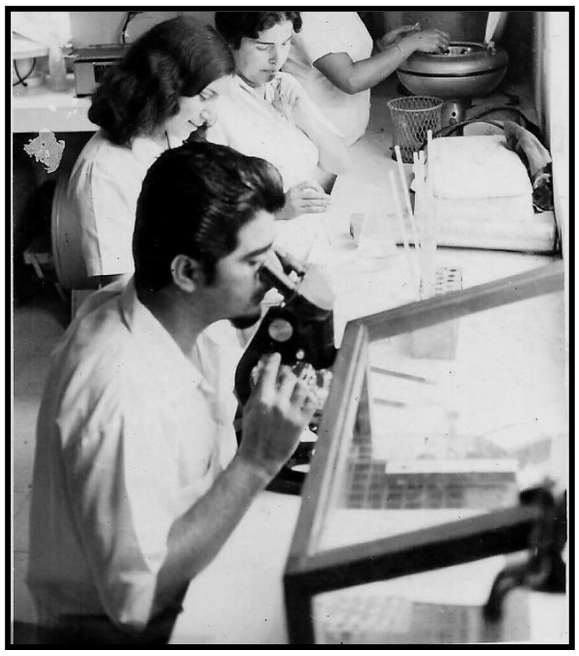
Bioaccumulation is a key criterion to assess and manage commercial chemicals and pollutants recognized internationally in the United Nations Stockholm Convention for Persistent Organic Pollutants, the Registration, Evaluation, Authorization and Restriction of Chemicals Program in the European Union, the Toxic Substances Control Act in the USA and nationally the Canadian Environmental Protection Act. Bioaccumulation is the process by which chemical concentrations achieve high levels in wildlife and humans, which can cause health effects and elevated health risks. To assess the degree of bioaccumulation and health effects of persistent organic pollutants in marine mammals, field studies of the bioaccumulation and health effects of these pollutants were conducted in a remote marine environment (Galapagos Islands, Ecuador) and in local marine ecosystems of British Columbia, Canada. The main findings of this work indicate that a number of persistent organic pollutants, including PCBs, DDTs and several other organochlorine pesticides biomagnify in Galapagos sea lions but are generally below concentrations associated with known effects. An increase in DDT concentrations was observed in Galapagos sea lions from 2005 to 2008, which may be related to the renewed use of DDT in malaria affected regions endorsed by the World Health Organization in 2006. PCB and PBDE concentrations were higher in Steller sea lions than in Galapagos sea lions. PCBs in Steller sea lions exceeded immunotoxic and endocrine disruption thresholds. To provide science-based tools for the management of pollutants, a bioaccumulation model for marine mammals was developed and tested. The model was applied to derive sediment target values for sediment remediation and for the derivation of ocean disposal permits in British Columbia. The application of the model shows that current sediment quality guidelines in Canada are not protective of the health of killer whales and Steller sea lions. Based on the model results, I recommend values that can be used as a basis for the derivation of sediment quality criteria for the protection of marine mammals in British Columbia. The findings support environmental management plans to mitigate chemical stressors of marine mammalian ecosystems in the Galapagos Islands and British Columbia.

***Keywords*: Galapagos Islands, British Columbia; Galapagos sea lion, Steller sea lion, killer whale, food web; ecosystem, bioaccumulation, biomagnification, model; immunotoxicity, endocrine disruption, health effects; sediment quality guidelines, management; persistent organic pollutants, POPs, DDT, PCBs, PBDEs, PCCDs, PCDFs, organochlorine pesticides.**

DEDICATION

First of all, I dedicate this work from the bottom of my heart to my father, the late Juan José Alava Parraga, who was the source of inspiration to conduct this work and encouraged me to be a man of science, a man of service in benefit of humanity, but above all to be a good person to sacrifice everything for others. Second, I extend this dedication to the effort and sacrifice of my family, specially my wife, Nastenka, my children (Nastenkita, Juan Jose and Joshua), my mother, Ana Mila, my siblings, Juan Manuel, Ana Johanna, and Ana Melina, who were also supporting and encouraging me to reach my dreams. Finally, I dedicate this to the unique creatures of the Galapagos where they struggle for survival and where evolution is still underway. All of them made this dream come true.

In memory of Dad



Juan José Alava Parraga (1942–2008)

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GLOSSARY

Bioaccumulation The process by which the chemical concentration in an aquatic organism achieves a level that exceeds that in the water, as a result of chemical uptake through all possible routes of chemical exposure, including dietary absorption, transport across the respiratory surface (e.g., gills), dermal absorption and inhalation. Bioaccumulation takes place under field conditions.

Biomagnification The process in which the chemical concentration in an organism achieves a level that exceeds that in the organim's diet (prey), due to dietary absorption.

Biotransformation The process by which chemical substances undergo chemical or biochemical reactions in organisms. The rate of transformation usually is expressed in terms of a rate constant or half life.

BMF Biomagnification Factor is described as the ratio of the chemical concentration in the organism to the concentration in its diet:

$$BMF = C_B/C_D$$

Where the chemical concentration in the organism (C_B) and the diet (C_D) are usually expressed in units of mass of chemical per kg of the organism (in wet weight or in a lipid basis) and mass chemical per kg of food (in wet weight or in a lipid basis).

BSAF The Biota Sediment Accumulation Factor describes bioaccumulation in sediment dwelling organisms, fish and marine mammals relative to chemical concentrations in sediments. It is the ratio of chemical concentration in an organism to that in the sediments:

$$BSAF = C_B/C_s$$

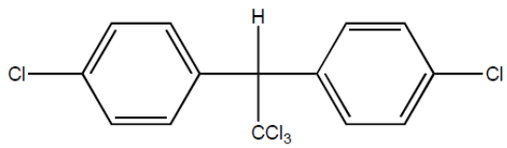
Where C_B is the chemical concentration in the organism (g chemical/kg organism) and C_s is the chemical concentration in the sediments (g chemical/kg dry weight). The BSAF expressed in a lipid (g lipid/ g organism) and organic carbon (g organic carbon/g dry weight sediment) normalized basis is more universal in its application because it accounts for differences in lipid content between organism and the organic carbon content of sediments.

CEPA The Canadian Environmental Protection Act

CUP Current Use Pestcide

DDT

Dichloro-diphenyl-trichloroethane (1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane) is an organohclorine pesticide, which is a white amorphous powder in appearance. Generally, when referring to DDT, it is referring to *p,p'*-DDT. Technical-grade DDT is a mixture of three forms, including the active ingredient *p,p'*-DDT (65-85%), nearly inactive *o,p*'-DDT (15-21%), *p,p'*-DDD (4%) and *o,o*'-DDT (trace amounts). All of these are white, crystalline, tasteless, and almost odourless solids. Technical grade DDT may also contain DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene) and DDD (1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethane) as contaminants. Both DDE and DDD are breakdown products (metabolites) of DDT. DDT was widely used during World War II to protect soldiers and civilians from malaria, typhus, and other diseases spread by insects. After the war, DDT continued to be used to control disease, and it was sprayed on a variety of agricultural crops, especially cotton. DDT continues to be applied against mosquitoes in several countries to control malaria. Due to its stability and persistence, it can remain as much as 50% in the soil 10-15 years after application.



DDT chemical structure

DSL

Domestic Substances List

Endocrine Disruptor Chemical (EDC)

Endocrine disruptors are synthetic chemicals having the potential for disrupting the delicate balance of the endocrine system by mimicking, blocking, deactivating and interfering with the synthesis, release, transport, elimination and binding of natural hormones.

Equilibrium

Chemical equilibrium is achieved when chemical is distributed among environmental media (including organisms) according to the chemical's physico-chemical partitioning behaviour. Thermodynamically, equilibrium is defined as a condition where the chemical's potentials (also chemical activities and chemical fugacities) are equal in the environmental media. At equilibrium, chemical concentrations in static environmental media remain constant over time.

Food Web

Food web" is defined as the network of organisms and species-specific feeding relationships that control the flow of energy and contaminants in the ecosystems studied. In some cases, the term "food chain" is used to represent the overall transfer of contaminants from primary producers to top predators of a given food web (e.g., marine mammalian food chain: phytoplankton to invertebrate to fish to mammal).

K_{OA}

The Octanol-Air partition coefficient is the ratio of concentrations of a chemical in octanol and air, representing how a chemical would thermodynamically distribute between the lipids of biological organism and air. It further represents the lipophilicity and the hydrophobicity of the

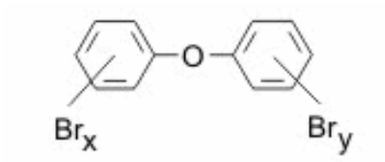
chemical substance. It usually is referred in its 10-based logarithmic form as log K_{OA}, and is unitless.

K_{OW}

The Octanol-Water partition coefficient is the ratio of concentrations of a chemical in octanol and water, representing how a chemical would thermodynamically distribute between the lipids of biological organism and water. It further represents the lipophilicity and the hydrophobicity of the chemical substance. It usually is referred in its 10-based logarithmic form as log K_{OW}, and is unitless.

PBDEs

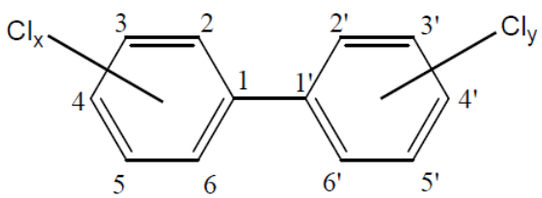
Polybromodiphenyl ethers comprise a class of halogenated organic compounds consisting of 209 possible congeners with 1–10 bromine atoms attached to the biphenyl molecule. PBDEs are used as additive flame retardants to inhibit or suppress combustion in organic materials. PBDEs are found in three commercial mixtures, typically referred to as Pentabromodiphenyl Ether (PeBDE), Octabromodiphenyl Ether (OBDE) and Decabromodiphenyl Ether (DBDE). PeBDE is predominantly a mixture of pentaBDE, tetraBDE and hexaBDE congeners, but may also contain trace levels of heptaBDE and tribromodiphenyl ether (triBDE) congeners. OBDE is a mixture composed mainly of heptaBDE, octaBDE and hexaBDE, but may also contain small amounts of nonaBDE and decaBDE. Current formulations of DBDE are almost completely composed of decaBDE and a very small amount of nonaBDE.



PBDEs chemical structure
(where x + y = 1 to 10 bromine atoms)

PCBs

Polychlorinated biphenyls are a class of halogenated organic compounds in which 2-10 chlorine atoms are attached to the biphenyl molecule. Monochloronited biphenyls (i.e., one chlorine atom attached to the biphenyl molecule) are often included when describing PCBs. These compounds are used in industry as heat exchange fluids, in electric transformers and capacitors, and as additives in paint, carbonless copy paper, and plastics. Of the 209 different types of PCBs, 13 exhibit a dioxin-like toxicity. Their persistence in the environment corresponds to the degree of chlorination, and half-lives can vary from 10 days to one-and-a-half years.

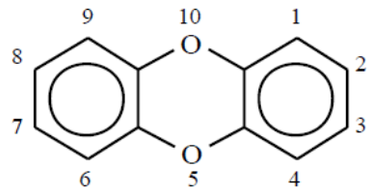


PCBs chemical structure

(where x + y = 1 to 10 chlorine atoms)

PCDDs

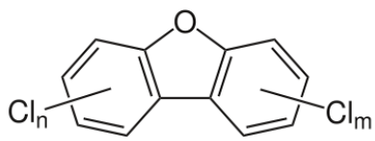
Polychlorinated dibenzo-*p*-dioxins are a group of halogenated organic compounds or related chlorinated hydrocarbons (i.e., 75 different congeners) which are structurally similar. Dioxins are unintentionally produced as by-products by industries, municipals and domestic incineration and combustion processes. They exist as colorless solids or crystals in the pure state. The compound 2,3,7,8-TCDD is one of the most toxic PCDDs to mammals and has received the most attention. Thus, 2,3,7,8-TCDD serves as a prototype for PCDDs. PCDDs with toxic properties similar to 2,3,7,8-TCDD are called “dioxin-like” compounds. The basic structure is a dibenzo-*p*-dioxin (DD) molecule, comprised of two benzene rings joined at their *para* carbons by two oxygen atoms.



PCDDs chemical structure
(The numbers indicate the positions for chlorine substitutions, excluding position 5 and 10)

PCDFs

Polychlorinated dibenzofurans are a class of halogenated organic compounds in which 1-8 chlorine atoms are attached to the benzene ring positions (carbon atoms) of a dibenzofuran structure (parent chemical). PCDFs are colorless solids and are not deliberately produced by industries. Due to the molecular asymmetry, PCDFs have 135 congeners compared to 75 for PCDDs.



PCDFs chemical structure
(where n+m =1 to 10 chlorine atoms)

POPs

Persistent Organic Pollutants are organic (carbon-based) chemical substances possessing a particular combination of physical and chemical properties such that, once released into the environment, they: a) remain intact for exceptionally long periods of time (many years); b) become widely distributed throughout the environment as a result of natural processes involving soil, water and, most notably, air; c) accumulate in the fatty tissue of living organisms including humans, and are found at higher concentrations at higher levels in the food chain; and, d) are toxic to both

	humans and wildlife.
REACH	REACH is the Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals created by the European Union.
Rate Constant	Rate constant describe the fraction of the total chemical mass or concentration in a particular medium or organism that is transported from and/or transformed per unit of time. It has units of 1/day or 1/hour or 1/year.
Steady State	A mass balance process in which the total flux of chemical into (input) an organisms equals the total flux out (output) with no net change in mass or concentration of the chemical over time. Steady-state differs from equilibrium in that it is achieved as a result of a balance of transport and transformation processes acting upon the chemical, while equilibrium is the end result of a physical-chemical partitioning process.
TEC	Threshold Effect Concentration
TL	Trophic Level of an organism
TMF	Trophic Magnification Factor is a bioaccumulation criterion and an approach to measure biomagnification of pollutants in food chains and food webs using log transformed, lipid normalized concentrations of contaminants measured in biota versus trophic levels of organisms at each step of the food web.
TSCA	Toxic Substances Control Act of 1976 (USA)
UNEP	United Nations Environmental Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
WHO	World Health Organization

Definition of POPs, PCBs, PCDDs, PCDFs, DDT, and PBDEs was retrieved from The Stockholm Convention on Persistent Organic Pollutants website: (http://chm.pops.int/Convention/The%20POPs/tabid/673/language/en-US/Default.aspx), and from the Agency for Toxic Substances and Disease Registry (ATSDR) website: (http://www.atsdr.cdc.gov/toxprofiles/index.asp).

CHAPTER 1

INTRODUCTION

1.1 Background

Global contamination by persistent organic pollutants (POPs) is an issue of great concern because these contaminants are ubiquitous in the environment, detected at relatively high concentrations, and driven by the long range atmospheric transport from temperate, subtropical and tropical areas to remote, oceanic regions and to both the northern and southern hemispheres (Wania and Mackay 1993; Iwata *et al.* 1993; Iwata *et al.* 1994; Tanabe *et al,* 1994; Wania and Mackay 1996). The effects of POPs on human health and wildlife are of major concern because these compounds bioaccumulate and cause toxic effects (e.g., endocrine disrupting nature) in organisms (Colborn *et al*. 1993).

POPs are “a set of organic compounds that: a) possess toxic characteristics; b) are persistent; c) are liable to bioaccumulate; d) are prone to long-range atmospheric transport and deposition; and e) can result in adverse environmental and human health effects at locations near and far from their sources” (UNEP 2002). Although some POPs, including polychlorinated biphenyls (PCBs) and dichloro-diphenyl-trichloroethanes (DDT) were banned in developed and industrialized countries long ago during the 1970s, some of the pesticides are still used in developing countries to control malaria-vectors and crop pests (i.e., DDT). For instance, the World Health Organization recently recommended the use of DDT once again to combat the malaria vector (*Anopheles*) due to re-emerging malaria in developing nations (WHO, 2006). The reactivation of DDT use was also endorsed by the 34th G8 summit in July 2008.

The levels of various POPs such as DDTs, dieldrin and PCBs were detected and documented for first time in birds in North America (Barnett 1950; Mitchell *et al*. 1953; Barker 1958; Bernard 1963), and birds and seals in Britain, the Netherlands and Sweden (Moore and Ratcliffe 1962; Koeman and van Genderen 1966; Jensen *et al.* 1969). In the time since these discoveries of PCBs in wildlife fat tissues, detectable levels have been found in most samples analyzed, from marine organisms living in the deep ocean to polar bears in the Arctic (Jensen 1966; Jensen 1972). Similarly, DDTs were found to bioaccumulate across food chains, as evidenced by high levels in predators at the top of ecological food pyramids (Jensen *et al.* 1969). These endocrine-disrupting chemicals have been released into the global environment following application as agricultural pesticides (e.g., organochlorines including DDT), stable industrial lubricants and oils (e.g., PCBs), and polybrominated diphenyl ether flame retardants (PBDEs), representing a particular threat to marine mammals, other wildlife and humans at the top of the food chain as these substances are persistent in the environment, bioaccumulate and biomagnify in food chains, and toxic at low to moderate concentrations (Tanabe *et al.* 1994; Colborn *et al.* 1993; Colborn and Smolen 1996; Colborn and Smolen 2003; Kelly *et al.* 2007).

Some POPs such as dioxins/furans and polychlorinated biphenyls (PCBs) were found (e.g., OCDF and PCB 180) to be widely distributed in the global ocean, including tropical zones, where they fall out from long distances through advective transport including wet and dry deposition (Baker and Hite 1999; Jurado *et al.* 2005). This is supported by the fact that high concentrations of hexachlorocyclohexanes (HCHs) and DDTs (DDT and its metabolites) have been found in several environmental matrices (i.e., sediment, river water and air) sampled near to tropical developing countries from southern Asia and Oceania and in oceanic surface water samples (Iwata *et al.* 1993; Wania and Mackay 1993; Iwata *et al.* 1994). Similarly, relatively high levels of chlordane

compounds and PCBs, which were found in high concentrations in the northern hemisphere, were also irregularly detected in the tropics, suggesting that these POPs are spreading southward to the tropical countries (Iwata *et al.* 1993; Wania and Mackay 1993; Iwata *et al.* 199; Tanabe *et al.* 1994). Substantial levels of DDTs are still detected in African lakes (Kidd *et al.* 2001; Manirakiza *et al.* 2002) and in the Amazon Basin (Azeredo *et al*. 2008; Torres *et al*. 2009). Goldberg (1975) was the first author in postulating the "grasshopper effect" as one of the major mechanisms of atmospheric transport of POPs to remote areas, using DDT as an example. At present, this global distillation process has been confirmed by a recent modelling work as the multi-hopping effect, involving northward transport from mid-latitudes (Guglielmo *et al.* 2009).

However, the highest concentrations still tend to be reported from locations in temperate countries where usage was very intense or POPs were manufactured, stored or disposed (e.g., Palos Verdes in Southern California Bight) (Blasius and Goodmanlowe 2008).

1.2 POPs in marine mammals

Research on the exposure and toxic effects of persistent organic pollutants (POPs) in marine mammals is an ongoing and growing field within environmental toxicology, immunotoxicology and human–ecological risk assessment arenas (Kannan *et al.* 2000; Ross 2000; Ross 2002; Ross and Birnbaum 2003; O'Shea *et al.* 2003). Through the recent history on POPs in marine mammals and their environment (Figure 1.1), several studies have widely demonstrated the presence (exposure levels), behaviour, accumulation, and health endpoints effects (e.g., endocrine disruption, emerging infectious diseases) of environmental organic contaminants in different species of marine mammals elsewhere (Tanabe *et al.* 1994; Martineau *et al.* 1994; Aguilar and Borrell 1994; Ross *et al.* 1995; Ross *et al.* 1996; Ross *et al.* 2000; O'Shea

and Tanabe 2003). Likewise, other species such as killer whales (*Orcinus orca*), polar bears (*Ursus maritimus*), belugas (*Delphinapterus leucas*), ringed seals (*Pusa hispida*) and Californian sea lions (*Zalophus californianus*) have been used as natural indicators or sentinels to monitor seasonal, temporal and spatial trends of POPs in some urbanized and remote areas from northern latitudes and arctic regions (Muir *et al.* 1996a; Muir *et al*. 1996b; Norstrom *et al.* 1998; Muir *et al.* 2000; Ross *et al.* 2000; Lieberg–Clark *et al.* 1995; Le Boeuf *et al.* 2002; Le Boeuf *et al.* 2003; Lie *et al.* 2003; Hobbs *et al.* 2003; Dietz *et al.* 2004; Kannan *et al.* 2005; Verreault *et al.* 2005; Smithwick *et al.* 2006). Most of these studies have demonstrated that cetaceans around the world exhibit the highest POP concentrations among wildlife species; for example, average concentrations of PCBs in transient male killer whales, *Orcinus orca*, have been found to be about 250 ± 55 mg/kg lipid weight (Ross *et al.* 2000).

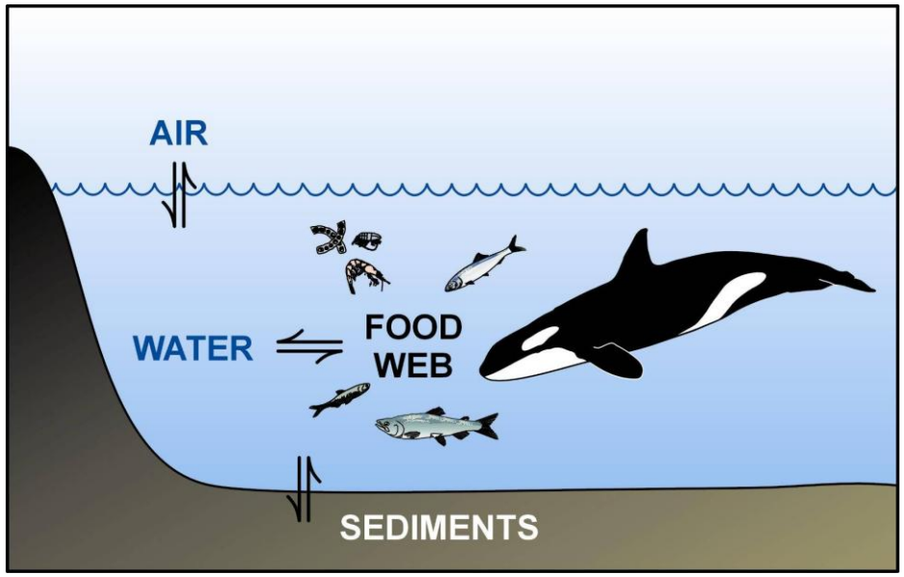


Figure 1.1 Marine mammals bioaccumulate Persistent Organic Pollutants, which partition among compartments in the marine environment and biomagnified in the food web (adapted from Lachmuth *et al.* 2010).

Particularly, pinniped species (e.g., seals and sea lions) are also among the most contaminated marine mammals worldwide because of its diet preferences (mostly fish-eaters), foraging strategies, high trophic levels in the food chain, global distribution (both industrialized-urban areas and remote regions) and the POPs absorbing nature of their thick blubber–tissue burden (Ross and Troisi 2001). For example, extremely high levels of DDTs, with concentrations averaging 1452 mg/kg lipid weight and ranging 417–5,077 mg/kg, were reported in Californian sea lions at beginning of the 70s by Le Boeuf and Bonnell (1971). Phocid seals have been identified as crucial biological matrixes for ecotoxicological studies. Indeed, O'Shea and Tanabe (2003) pointed out that more than 75% of samples collected from pinnipeds belong to grey (*Halichoerus grypus*), harp (*Pagophilus groenlandicus*), harbour (*Phoca vitulina*) and ringed seals (*P. hispida*). Recently, various species of interest have been proposed as models and key sentinels of toxicological health effects and coastal pollution involving phocids such as the harbour seal (*P. vitulina*) and grey seal (*H. grypus*), and otariids such as Steller (*Eumetopias jubatus*) and California sea lions (*Z. californianus*) (O'Shea *et al.* 2003). This is based on a well known weight of evidence obtained from different studies in either field work (live capture, strandings) or captive and semi-field experiments.

1.3 International Policy and Regulation of POPs

At the International level, The Stockholm Convention is a global treaty to protect human health and the environment from POPs through their reduction and eventual elimination. It has also been called the Stockholm Convention, POPs Convention, or POPs Treaty. The Convention was officially adopted in Stockholm, Sweden, on 23 May 2001 (UNEP 2002; UNEP 2005). The Convention entered into force on 17 May 2004, becoming international law. By April 2005, over

90 countries had joined as Parties and many more are expected to become members over the next several years (UNEP 2005). The Convention addresses the challenge posed by past use and intentionally produced POPs by targeting the 12 most toxic chemicals ever created. Nine of these POPs are pesticides: aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene (HCB), mirex, and toxaphene (Table 1.1). The others POPs are industrial chemicals, including the classic polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and hexachlorobenzene (HCB), which is a pesticide as mentioned above, but it can also be a byproduct of pesticide manufacture (UNEP 2002; UNEP 2005). Article 3 of the Stockholm Convention addresses the banning and elimination of these chemicals, including their production, use and trade, except for DDT which has restricted use, but not prohibition until substitute products can replaced it to control mosquito–malaria vectors (Annexes A and B of the Convention). Within the Convention, Article 8 and Annexes D, E, and F address the inclusion of additional POPs to the Treaty. Nine new compounds have recently been added to the list (Table 1.1), including emerging compounds such as PBDE flame retardants (i.e., treta, penta, hexa and heptabromodiphenyl formulations), and perfluorooctane sulfonate compounds or PFOS (i.e., perfluorooctane sulfonic acid and perfluorooctane sulfonyl fluoride).

Table 1.1 List of POPs under the Stockholm Convention

Initial 12 POPs[a]

Pesticides	aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene
Industrial chemicals	hexachlorobenzene, polychlorinated biphenyls (PCBs);
By-products	hexachlorobenzene; polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/PCDF), and PCBs

New POPs[b]

Pesticides	chlordecone, alpha hexachlorocyclohexane, beta hexachlorocyclohexane, lindane, pentachlorobenzene
Industrial chemicals	hexabromobiphenyl, tetrabromodiphenyl ether, pentabromodiphenyl ether, hexabromodiphenyl ether and heptabromodiphenyl ether, pentachlorobenzene, perfluorooctane sulfonic acid, its salts and perfluorooctane sulfonyl fluoride.
By-products	alpha hexachlorocyclohexane, beta hexachlorocyclohexane and pentachlorobenzene.

[a]**Initial 12 POPs:** Initially**,** twelve POPs have been recognized as causing adverse effects on humans and the ecosystem and these can be placed in 3 categories.

[b]**Nine new POPs**: At its fourth meeting held from 4 to 8 May 2009, the Conference of the Parties (COP), by decisions SC-4/10 to SC-4/18, adopted amendments to Annexes A (elimination), B (restriction) and C (unintentional production) of the Stockholm Convention to list nine additional chemicals as persistent organic pollutants.

Source: Stockholm Convention on Persistent Organic Pollutants (POPs).
http://chm.pops.int/Convention/The%20POPs/tabid/673/language/en-US/Default.aspx

The screening criteria used by the Stockholm Convention involve chemical identity, persistence, bioaccumulation, potential for long-range environmental transport and adverse effects. Persistence refers to the length of time a substance resides in the environment. A substance's persistence is commonly measured by its half-life, that is, the time required for the quantity of a substance to diminish or degrade to half of its original amount in a particular environmental medium. The persistence of a substance in each of the relevant media (e.g., soil, water, or air) must be evaluated and compared against the categorization half-life criteria. Substances that have the potential to be transported to remote areas of the globe are considered persistent, and the relevant evidence for long-range transport (LRT) is taken into consideration in determining the persistence of substances.

Bioaccumulation is a general term describing a process by which substances are accumulated in organisms directly from exposure to water and through consumption of food containing the substances (Gobas *et al*. 2009). The regulations express preference for bioaccumulation factors (BAFs) over bioconcentration factors (BCFs) or log octanol water partition coefficient (log K_{OW}). Adverse effects refer to the toxicity of a substance and include: a) evidence of adverse effects to human health or to the environment that justifies consideration of the chemical within the scope of this Convention; or b) toxicity or ecotoxicity data that indicate the potential for damage to human health or to the environment.

Bioaccumulation is also one of the key criteria used by the Regulation for Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH program) in the European Union, the Toxic Substances Control Act (TSCA) in United States and the Canadian Environmental Protection Act (CEPA) in Canada to asses and manage the production of chemicals and pollutants that have the potential to bioaccumulate in organisms and food webs.

In Canada, CEPA is the the major federal environmental protection legislation for the regulation and control of POPs. Two key aspects depicting the spirit of CEPA (Section 2; Part 1) are the prevention of pollution and the protection of environmental and human health. This indicates that CEPA is considered by both the Ministry of Environment and the Ministry of Health, reflecting essential duties and potential conflicts among these two Ministers when considering the environment, non-human organisms, public health and human health within the regulation of POPs. Herein, it is important to mention that “pollution prevention” means the use of processes, practices, materials, products, substances or energy that avoid or minimize the creation of pollutants and waste, and reduce the overall risk to the environment or human health (CEPA 1999). Therefore, pollution prevention should be the common goal of these Ministers.

Approximately 50% of the compounds presently listed in the Domestic Substance List (DSL) of Canada (23 000 chemicals) are organic substances, including POPs, which are released in the environment (terrestrial ecosystems, rainwater, lakes, rivers, oceans and atmosphere) and bioaccumulated in freshwater, marine, terrestrial and arctic food chains of Canada (Arnot and Gobas 2006; Kelly *et al.* 2007). A small fraction of chemicals included in the DSL are being assessed for persistence, bioaccumulation and toxicity criteria under the CEPA. Based on the results of a screening assessment, the Ministers can propose taking no further action with respect to the substance, adding the substance to the Priority Substances List (PSL) for further assessment, or recommending that the substance be added to Schedule 1 of CEPA 1999 and, where applicable, the implementation of virtual elimination.

However, recent bioaccumulation and biomagnification studies in terrestrial and marine mammalian food webs of the Canadian Arctic have demonstrated that the screening criterion for bioaccumulation used at both the international (i.e., Stockholm Convention) and national (e.g.,

CEPA) levels only protect gill−ventilating, cold−blooded organism, but not air−breathing, warm−blooded animals, including marine mammals (Kelly *et al.* 2007). In addition, there is currently a need for the implementation of new Sediment Quality Guidelines (SQGs) to protect high trophic levels organisms (e.g., top avian and mammalian predators) and critical habitat of threatened species under the Species at Risk Act (SARA) mandate in Canada as the existing SQGs are protective for low trophic levels organisms or benthic invertebrates (Lachmuth *et al.* 2010). Because of these caveats, it is important to continue conducting eco-toxicological research and assessments in key species of animals at the top of the food webs and susceptible to the bioaccumulation of pollutants to provide science in support of risk management and decision makings.

In Ecuador, including the Galapagos Islands, the Ministry of Environment through the National Normative for the Management of Hazardous Chemical Products (Book VI of Environmental Quality) has recently commenced an assessment to monitor and control the use of POPs by implementing the National Plan for the Management of POPs in Ecuador (http://www.ambiente.gov.ec/), including the recent National Inventory of POPs (Ministerio del Ambiente 2004; Ministerio del Ambiente 2006). In addition, the Ecuadorian Regulation for the Prevention and Control of Pollution by Hazardous Wastes is the legal body in charge to protect the atmosphere, air and soils from chemical contamination. Although POPs are not produced in Ecuador, they were shipped into the country in the past and are still used at the industrial level (i.e., PCBs) and for public health campaigns to control the malaria vector (i.e., DDT). For instance, the national inventory of POPs revealed that presence of PCBs as cooling fluids or PCB contaminated oils in electric transformers and capacitors used by Ecuadorian Electric Companies to provide energy, as well as the presence of stocks of DDT at the National Malaria Eradication

Service to be used for emergency responses against re-emerging malaria mosquitoes (Ministerio del Ambiente 2004; Ministerio del Ambiente 2006). Also found was that while the imports of aldrin, chlordane, dieldrin, hexachlorobenzene, and endrin were prohibited, DDT was excluded and can be imported to the country with authorization of the Ministry of Public Health. Similarly, heptachlor, mirex and toxaphene are not enlisted in the list of chemical that are forbidden to enter the country as import products and suggesting no restrictions for their use. For example, mirex was used as an insecticide in baits to eliminate ants as supported by the import of 25.5 kg between 1997 and 1998 in Ecuador. Therefore, it is very possible to find POPs in marine, freshwater and terrestrial ecosystems of Ecuador, including the remote Galapagos Islands.

1.4 Rationale, Theory and Research Questions

While substantial work has been carried out on the fate, behaviour of POPs in the northern hemisphere and their atmospheric transport into the polar regions, as well as regulator efforts of these substances in those regions, very little has been conducted to investigate equatorial deposition, bioaccumulation and control of POPs (e.g., DDT) in tropical remote areas such as the Galapagos Islands.

From a global perspective, the protection of coastal food webs from contamination by both chemical and biological pollutants is critical to the long term conservation of the biodiversity and native inhabitants residing in unique places of the Earth such as the Galapagos Islands and the marine regions of British Columbia. Coastal waters that are contaminated with persistent chemicals and pathogens can lead to human illness and adverse health, reduced fisheries quality and quantity, and impacts of the health of marine wildlife. This had obvious social and economic consequences. Conversely, coastal waters that are protected from chemical pollutants provide for

an abundance of clean fisheries products and wildlife, and essential foundation for the well-being of the local biodiversity, human residents and the ecotourism sector.

At the top of the marine-coastal food chain, marine mammals can provide an 'integrated' overview of ecosystem health. As aquatic animals, they are also vulnerable to infection by pathogens of terrestrial origin. By documenting the presence of chemical pollutants in this species, we are able to deliver science-based advice to conservationists, managers, regulators and stakeholders, on the implementation of best management practices. Equivalent to the role of killer whales as global sentinels of pollution in the Northeastern Pacific, the Galapagos sea lion, for example, can be used as a sentinel of environmental contamination and a key indicator of not only the coastal marine health, but the public health in Galapagos Islands. Therefore, this work aims to characterize the chemical pollutants that accumulate and occur in coastal–marine food chains of the Galapagos sea lion, and its use as a sentinel–model species of environmental pollution by POPs.

The major questions in regard to the spirit of this research to elucidate if there is a POPs problem in the Galapagos marine ecosystems are depicted as follow: What are the concentration levels and patterns of POPs in Galapagos sea lions? What are the levels of POPs in major diet items of the Galapagos sea lion? Does bioaccumulation and biomagnification of POPs occur in the Galapagos sea lion marine food chain and to what extent? Is the exposure to environmental pollution by POPs both local and external associated with anthropogenic activities affecting the health of sea lions in Galapagos Islands, Ecuador? Are there any geographical differences in the levels and patterns of POPs between Galapagos sea lions and pinnipeds or marine mammals (i.e., Steller sea lion and killer whales) from northern latitudes (i.e., British Columbia)? Can food

web bioaccumulation assessments of POPs (e.g., PCBs) using marine mammals provide science in support of risk management and decision making?

Under this premise and within the context of the environmental resource management paradigm, this dissertation relies on eco-toxicological studies and risk assessment of organic pollutants with the aim to use and apply knowledge to develop managerial approaches for environmental stewardship and conservation of threatened marine mammals.

1.5 Objectives

The general goal of this thesis is primarily to assess the exposure levels, pattern and biomagnification of priority 'chemical pollutants' in the sentinel species Galapagos sea lion. Objectives were to measure the concentrations and assess the health effects of legacy and emerging contaminants of concern, including industrial chemicals, pesticides and flame retardants: polychlorinated biphenyls (PCBs), dioxins (PCDDs/PCDFs), dichlorodiphenyltrichloroethanes (DDTs), organochlorine pesticides (OC pesticides) and polybrominated diphenyl ethers (PBDEs). To tailor the global implications from the regional/local scale, this was followed by an assessment of PCB and PBDEs in Steller sea lions and the development of a PCB bioaccumulation modelling in resident killer whales and Steller sea lions inhabiting more contaminated areas from British Columbia.

Research objectives were stated as follow:

1) Assess the presence and health effects of chemical pollutants in the sentinel species Galapagos sea lion, by measuring the concentrations of legacy and emerging contaminants

of concern, including industrial chemicals, pesticides and flame retardants: PCBs, dioxins (PCDDs/PCDFs), DDTs, OC pesticides, and PBDEs.

2) Determine the levels of POPs (i.e., PCBs, DDTs, and OC pesticides) in the local and major Galapagos sea lions' food–diet items such as thread herrings (*Opisthonema* sp.), and mullets (*Mugil* sp.).

3) Assess and predict the trophic transfer biomagnification of POP for the Galapagos sea lion food chain based on $\delta^{15}N$ stable isotope measurements (trophic levels).

4) Conduct geographical comparisons of concentrations and signature patterns of POPs between a pinniped species from a tropical-equatorial area, the Galapagos sea lions (Galapagos Islands, Ecuador), and pinnipeds from northern latitudes, including Steller sea lions.

5) Contribute to the improvement of regional sediment quality guidelines for British Columbia based on a PCB food web bioaccumulation model for killer whale and Steller sea lions based on biota sediment accumulation factors (BSAF), empirical PCB sediment concentrations and PCB threshold effect concentrations (TEC).

1.6 Thesis Scope and Organization of Chapters

In an effort to characterize and understand the bioaccumulation and health effects of POPs in tropical regions around mid latitudes, an assessment of legacy and emerging POPs was conducted in the Galapagos Islands, using the Galapagos sea lion as a biotic compartment and environmental sentinel of global pollution by POPs, as described above. Furthermore, an assessment of current levels of POPs and food web bioaccumulation modelling of PCBs in marine mammals, including Steller sea lions and killer whales, of the northern hemisphere was carried out to be used as a reference and compared with the POPs assessment in the Galapagos. To accomplish this work, this thesis dissertation is encompassed and arranged by five major chapters, which are a series of separated papers or journal articles presented as independent manuscripts. Each chapter is integrated by its own introduction and discussion sections, list of references, figures and tables. Therefore, this work included completion of a review paper (**Chapter 2**) showing an overall environmental impact assessment of pollution as a conservation threat in the Galapagos Islands based on the limited, existing body of literature and personal research and findings by the author. This is followed by the baseline information, analyses, results and discussion on POPs in Galapagos sea lions resulting from field studies and the first empirical data ever reported for the species, including PCBs and PBDEs (**Chapter 3**; published as a peer reviewed paper on *Environmental Toxicology and Chemistry*), and DDT (**Chapter 4**; published as a peer reviewed paper in *Marine Pollution Bulletin*). These findings (**Chapter 3 and 4**) were further examined by investigating the biomagnification of POPs (i.e., trophic magnification factors or TMFs) and measurements of stable isotopes (i.e., $\delta^{13}C$ and $\delta^{15}N$) in a specific food chain of the Galapagos sea lion, involving thread herrings and mullets (**Chapter 5**). To complement and

compare the POPs study in Galapagos sea lions relative to current levels found in other species of marine mammals in the northern hemisphere, Steller sea lions and killer whales were also assessed for POPs, including the first study of PCBs and PBDEs in overwintering Steller sea lions in British Columbia (**Chapter 6**) and the development of PCB bioaccumulation models in the food webs of the resident killer whale and Steller sea lion of British Columbia (**Chapter 7**). The modelling in the latter chapter was also done with the aim of providing guidance for health risk assessment and management of POPs (i.e., derivation of target sediment quality guidelines) at the local level in British Columbia, and its feasible application and adaption for priority POPs (i.e., DDT) in tropical systems such as the Galapagos Islands. Finally, the overall conclusions of this thesis are depicted in a final chapter (**Chapter 8**), reflecting the summary of major findings and perspectives of this original research for future work.

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CHAPTER 2

TOWARD AN ENVIRONMENTAL ASSESSMENT OF POLLUTION AS A CONSERVATION THREAT FOR THE GALAPAGOS ISLANDS

Abstract: The Galapagos Archipelago is one of the last natural living museums to be preserved since its designation in 1979 as a UNESCO World Heritage Site. While tourism and fisheries activities stand by the islands' economy, several anthropogenic activities threaten the Galapagos ecosystem. A critical survey on the literature was conducted to identify and characterize the coastal-marine pollution impacts caused by organic wastes and plastics, hydrocarbons and oil spills, emerging pathogens and invasive species (i.e., biological pollution), currently use pesticides (CUPs) and environmental transport of persistent organic pollutants (POPs) from distant sources that may affect endemic species such as sea lions, marine iguanas and sea birds in the Galapagos. Under this premise, municipal waste incineration of organic waste and plastics in open dump areas were identified as a potential source of unintentional produced POPs such as dioxins (PCDDs) and furans (PCDFs), although levels were expected to be low. Plastic is the second most abundant solid waste at sea and shore lines representing 25% of the total marine debris. More than 50% of CUPs applied in the agriculture zone of the inhabited islands were found as belonging to the category of endocrine disrupting chemicals (EDCs). Oil spills and traces of hydrocarbons threaten the survival of marine iguanas in the long term. Among the biological pollutants, canine distemper virus (CDV) carried by domestic dogs threaten the endemic Galapagos sea lions and fur seals, while avian pox−like viruses hosted by domestic birds has

already been detected in Darwin's finches. Concerted local and global management strategies and international policy instruments are strongly needed into the decision-making processes to protect the Galapagos Archipelago from chemical and biological pollution.

***Keywords**:* **Galapagos Islands; marine-coastal pollution, POPs, pesticides, municipal waste; diseases, virus.**

2.1 Introduction

Since Charles Darwin wrote "*The Origin of the Species*" in 1859, the Galapagos Islands have become a living laboratory for the study of natural history. The roots of their unique nature can be attributed to their remote, oceanic geography. The Galapagos comprises an Archipelago with 13 major volcanic islands, situated approximately 1000 km from the Ecuadorian coast, between 01°40´N-01°25´S and 89°15´W- 92°00´W (Figure 2.1). At present, 2,909 marine species have been identified, of which 18.2% are endemic to the Galapagos (Bustamante *et al*. 2002).

Several oceans currents influence the regional climate and drive the population dynamics of native and endemic species. The most important oceanic surface currents are the Panama (El Niño) current, coming from the Northeast and bringing warm, nutrient-poor waters and, and the Peru (Humboldt) current, arriving from the Southern Ocean, and transporting cold, nutrient rich waters. Both current systems merge to form the South Equatorial Current (SEC), which drives surface marine waters to the west of the islands and which has been proposed as the major mean of transportation bringing species from mainland Ecuador to the Galapagos (Banks 2002; Bustamante *et al.* 2002).

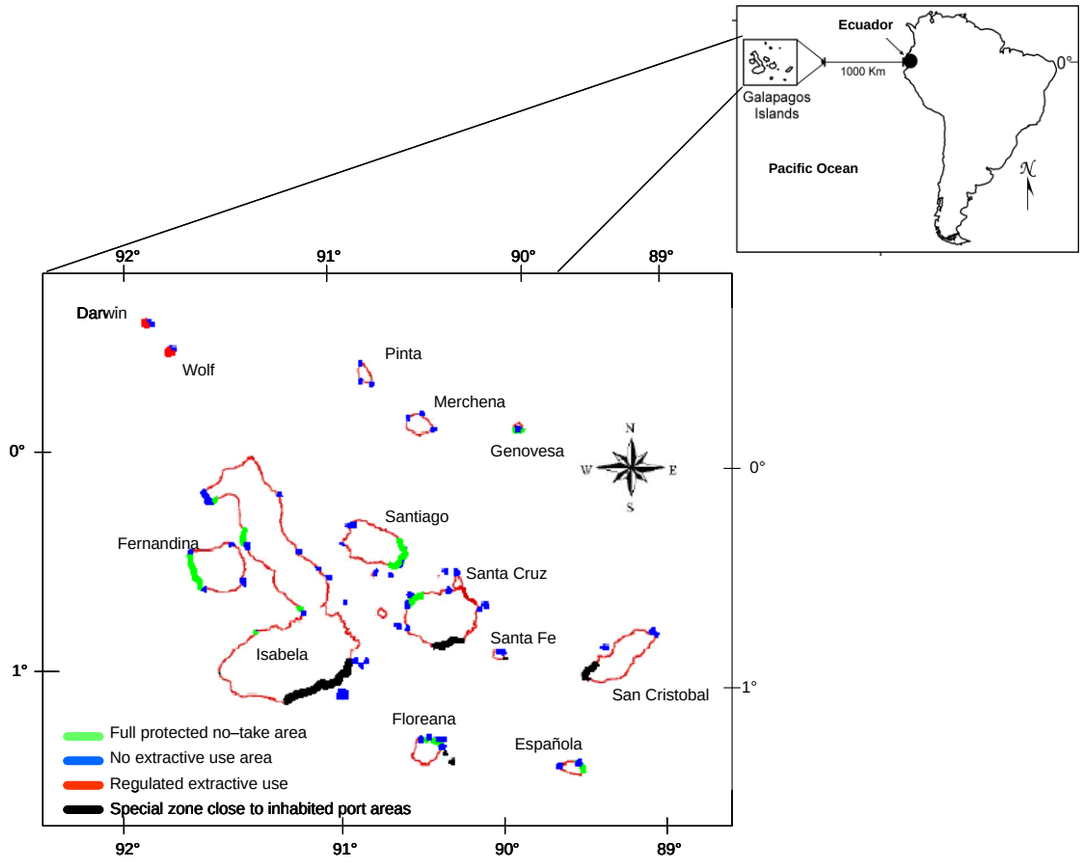

Figure 2.1 Location of the Galapagos Islands relative to continental Ecuador, South America. The coastal zoning scheme for the Galapagos Marine Reserve (GMR) is also shown. The zones are fully-protected 'no-take' area, in green; non-extractive use areas, in blue; regulated extractive uses, in red; and, special zones nearby the inhabited port areas, in black. Adapted from Charles Darwin Foundation and World Wildlife Fund (2002).

In addition, the Equatorial Undercurrent or Cromwell current, rich in nutrients (i.e., dissolved iron), flows from west to east enhancing upwelling conditions around the western platform of the Galapagos. Only two kinds of seasons occur in this region, a warmer, wet-rainy season from December to May or June, and a cold, dry ("*garúa*") season from June to November or December (Snell and Rea 1999; Banks 2002). Periodically, El Niño event can disrupt the Galapagos regional climate, where in the last 20 years it has showed up with more intensity and reflecting an intense peak frequency (Snell and Rea 1999).

The Galapagos National Park and the Galapagos Marine Reserve have been designated a United Nations Educational, Scientific and Cultural Organization (UNESCO 1979) −World Natural Heritage Site and Biosphere of the Earth, containing a critical biodiversity and reflecting the evidence of evolutionary theory such as natural selection, adaptation, speciation and radiation processes, as well as endemism. These tropical remote islands still conserving 95% of its biodiversity has also been recently enlisted as a Heritage in risk in 2007 due to the rising number of invasive species, emergent human population growth and increasing tourism (Watkins and Cruz 2007).

Shortly after its declaration as a National Park (≈7900 km^2 of the terrestrial Galapagos Islands) in 1959, Rachel Carson in her well known publication "*Silent Spring*" was probably the first person to draw global attention to the potential effects of man–made chemicals on wildlife populations (e.g., raptors) and human health (Carson 1962). Both intentional (operational) and unintentional (accidental) releases occur around the islands from ships, with the former occurring in the long-term causing chronic degradation and latter resulting in acute impacts to the marine environment (Lessmann 2004). Oil spills offer perhaps the most visible example of pollutant impacts on sea life. Less visible and more insidious global toxicants of concern involve persistent organic pollutants (POPs), which have not been assessed in the Galapagos.

Coastal development, fisheries overexploitation and chemical and biological pollution have been identified as the major threats to the world's oceans and marine protected areas (Boersma and Parrish 1999). In these islands, most of the resident population obtains its economic incomes either directly or indirectly from the ecotourism, which is the major economic activity, based on the observation of native fauna and flora of the Islands, while others are benefited from fisheries exploitation of reef fishes, lobster, sea cucumber and even illegal shark finning (Merlen 1995;

MacFarland and Cifuentes 1996; Bensted–Smith *et al.* 2002). During the last 15 years, the Galapagos Islands Archipelago has undergone drastic economic, social, cultural and ecological changes. The principal cause of these changes has been economic growth driven by tourism whose gross income has increased by an average 14% each year (Watkins and Cruz 2007). Tourism and population growth stimulate the arrival of more flights and more cargo ships, diminishing the degree of isolation of these remote islands and therefore increasing the arrival of invasive species (Watkins and Cruz 2007) and augmenting the risk of pollution.

The coastal environment and food webs in the Galapagos may be at risk due to anthropogenic impacts. Contaminations by both chemical and biological pollutants are critical to the long term conservation of Galapagos biodiversity and native inhabitants. Coastal waters that are contaminated with persistent chemicals and pathogens can lead to human illness, reduced fisheries quality and quantity, and impacts on the health of marine wildlife. This can have serious obvious social and economic consequences. Conversely, coastal waters that are protected from environmental pollutants provide food humans and wildlife, and provide a foundation for biodiversity, the human population and the ecotourism sector. In 2000, Galapagos tourism alone earned US $ 210 million for the Ecuadorian economy (Fundación Natura and World Wildlife Fund 2002). For the Ecuadorian government and the people of the Galapagos, therefore, a rigorous evaluation of past, current and potential environmental impacts is a crucial part of the social and economic integrity of the archipelago.

In this article, a review was conducted to explore evidences of conservation threats by identifying and assessing environmental and marine pollution pressures as current risks for endemic wildlife of the Galapagos Islands. An identification of local and external pollution sources and their potential impacts in the health of wildlife populations with the goal to develop and

recommend precautionary mitigation strategies with implications for the environmental management plan of the Galapagos are described.

2.2 Declining wildlife in Galapagos: El Niño and other environmental stressors

Several populations of endemic wildlife and marine species (e.g., marine mammals, sea birds and marine iguanas) are being affected by both natural and anthropogenic factors in the Galapagos. The Galapagos wildlife is affected for different environmental stressors, including both natural and anthropogenic, as those depicted in Figure 2.2. These include the Galapagos sea lions (*Zalophus wollebaeki*) and Galapagos fur seals (*Arctocephalus galapagoensis*), which have declined from 40,000 and 30,000-40,000 to 16,000 (50-60%) and 6,000-8,000 (80-85%) animals, respectively, since the late 1970s. without showing signs of recovery in most of the islands. This implies a decline of 60% for Galapagos sea lions and 80-85% for Galapagos fur seals from the late 1970s to 2000 (Alava and Salazar 2006). As a result, these species are listed under the IUCN endangered (EN) category (Aurioles and Trillmich 2008a; Aurioles and Trillmich 2008b). Among the potential causes of these declines are the El Niño event, nutritional stress, fisheries interactions, illegal sealing, and diseases.

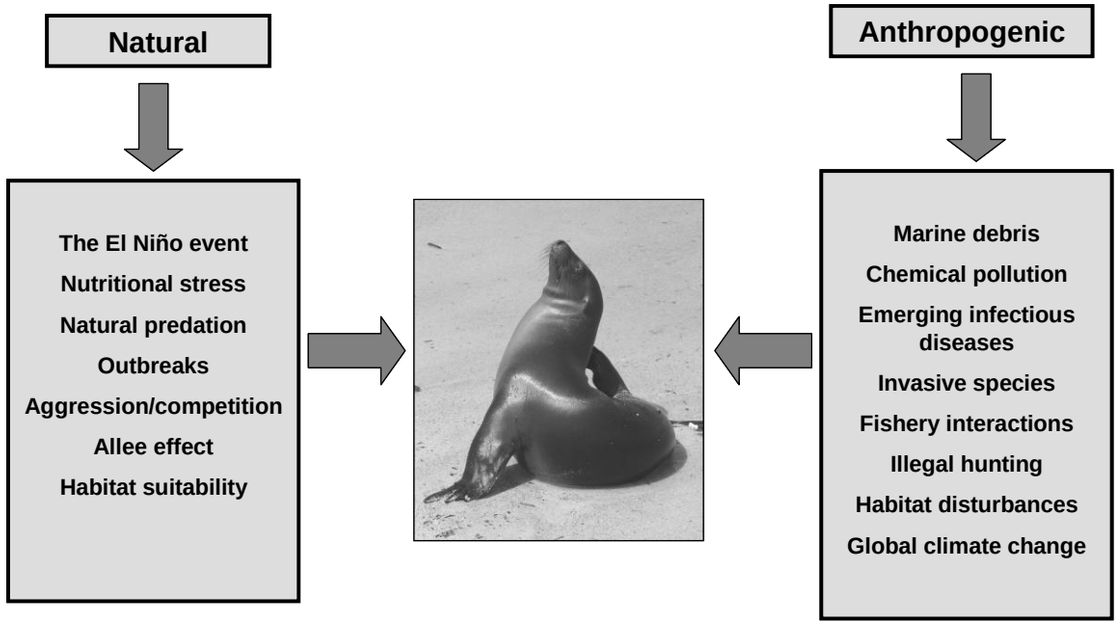

Figure 2.2 Environmental stressors, both natural and anthropogenic factors, influence the population dynamics of marine wildlife in the Galapagos Islands. In this illustration, the Galapagos sea lion is shown as an example (Picture: J. J. Alava).

The El Niño phenomenon has also affected sea birds population, including the flightless cormorants (*Phalacrocorax harrisi*) and Galapagos penguin (*Spheniscus mendiculus*). For instance, the 2004 penguin population (≈1,500 birds) was estimated to be less than 50% of that prior to the strong 1982–1983 El Niño event (Vargas *et al.* 2005; Vargas *et al.* 2006; Vargas *et al.* 2007). Fishery interactions and plastic threaten the critically endangered Waved albatross (*Phoebastria irrorata*) and Galapagos petrels (*Pterodroma phaeopygia*) in oceanic waters outside of the limit of GMR. Additional anthropogenic and catastrophic factors such as introduced predators (particularly rats, cats and dogs), competition from fisheries, introduced diseases (i.e., outbreaks) and oil spills could further contribute to population declines or accelerate the probability of extinction of Galapagos seabirds (Vargas *et al.* 2005; Vargas *et al.* 2006).

Whereas the effect of oceanographic−climate phenomena such as the El Niño events are well known as a cause of declining in sea lions, fur seals and sea birds, the role of marine pollution has not been fully investigated although it is among them. An exception to this was the obvious case of high mortality of the unique vulnerable population of Galapagos marine iguanas (*Amblyrhynchus cristatus*) due to the chronic toxic effects of the 2001−Jessica oil spill's residues, which has been well documented elsewhere (Wikelski *et al.* 2001; Romero and Wikelsky 2002; Wikelski *et al.* 2002).

2.3 Pollution sources and impacts

2.3.1 Anthropogenic impacts identification

A characterization of anthropogenic impacts resulting in major conservation threats and environmental effects for the marine and terrestrial components of the Galapagos Islands are presented as an environmental impact assessment matrix in Appendix A (Table A-1) The information was compiled and integrated based on the existing literature and lines of evidences from peer reviewed/scientific articles, technical reports and web sites available elsewhere and discussed as follows.

2.3.2 Production and incineration of solid waste

The human population has recently increased in the Galapagos, having approximately 19,000 people (tourists not included) by 2006 and showing an annual population growth rate of 6.4% during the period 1990-1998 (Fundación Natura *et al.* 2000; Kerr *et al.* 2004; Epler 2007). Between 1974 and 1998, the population in Galapagos showed more than a three fold increase,

from 4,078 to 15,311 inhabitants (Epler 2007). Likewise, tourism has drastically increased with a rise in the number of visitor to Galapagos from 40,000 in 1990 to 145,000 tourists in 2006 (Watkins and Cruz 2007; Epler 2007). As population increases in these islands, the waste generation has been increasing in magnitude, resulting in increasing burning of waste. Total human population and waste production for three of the islands harbouring urbanized centres are showed in Table 2.1. From 1995 to 1997, the generation of waste in Isabela, San Cristóbal and Santa Cruz ranged was approximately 0.6─1.3 kg/day/person, which exceeded the national waste production average of 0.4 kg/day/person for continental Ecuador (Table 2.2; Fundación Natura and WWF 1999). It also appears that the proportion of organic matter estimated from the total waste production is higher in San Cristóbal when compared to Santa Cruz and Isabela islands.

Table 2.1 Population and waste production in three islands of the Galapagos (data obtained and adapted from Fundación Natura and WWF 1999; Kerr *et al*. 2004).

Island (year of survey)	Population*	kg/day/person	tonnes/year	% organic matter
Isabela (1998)	1619	0.6	284	≈ 70
San Cristóbal (1997)	5633	1.3	2034	> 70
Santa Cruz (1995)	11,388	0.8	2375	≈ 60

*2001-human population census for the Galapagos Islands (obtained from INEC 2007).

The disposal of municipal waste in open dumps in rural areas close to coastal zones of urbanized islands of the Galapagos is an environmental issue of concern (Kerr *et al.* 2004). The leachate and incineration of local, municipal organic solid waste, polyvinyl chlorine (PVC) plastics and bleached paper without appropriate treatment represents an unquantified source of toxic

POPs such as PCDDs and PCDFs, which enter aquatic systems (Czuczwa *et al.* 1984; Czuczwa and Hites 1984). These are by-products and unintentional POPs generated from anthropogenic sources by incomplete combustion or thermal processes involving organic matter and chlorine. In continental Ecuador, the estimated total emission of dioxins and furans is about 98 g TEQ/year, from which uncontrolled combustion processes contribute approximately 51% (Ministerio del Ambiente 2006). As current practices do not prevent the by-production of PCDDs and PCDFs, an as yet uncharacterized risk exists to aquatic biota.

Most of the solid waste is organic and is disposed of in open areas assigned for this purpose. These areas are a short distance from the main ports, 4 km from Puerto Ayora and 3 km from Puerto Baquerizo (Kerr *et al.* 2004). During the last three years, efforts has been carried out to improve the waste management of municipal organic waste to avoid the generation of dioxins by banning the burning of this kind of waste in open areas close to harbours and coastal zone.

2.3.3 Marine debris

Marine pollution by debris in Galapagos waters is emerging as a significant concern for biota. A beach-shoreline cleanup program around the Galapagos in 1999 retrieved 22,140 kg of debris, with plastics and metals being the predominant objects at 25 and 28% of the total (Figure 2.3; Fundación Natura and WWF 2000). At sea, the accidental or deliberate disposal of solid waste (e. g., plastic, fishery gear) from both tourism and fishing vessels represent a threat for marine vertebrates such as large pelagic fish, sea turtles, cetaceans, sea lions, fur seals and sea birds. For example, Galapagos sea lions have been found to interact with floating objects and debris on the sea surface, including hooks, plastic, nylon and rope (Figure 2.4; Alava and Salazar 2006). Fish hooks were the predominant object (22%) affecting sea lions, followed by plastics,

which represented almost 20% of the total. This particularly causes concern because although the level of municipal waste collection is high in the islands, no appropriate waste management program exists onboard vessels to ensure a low impact on the marine environment.

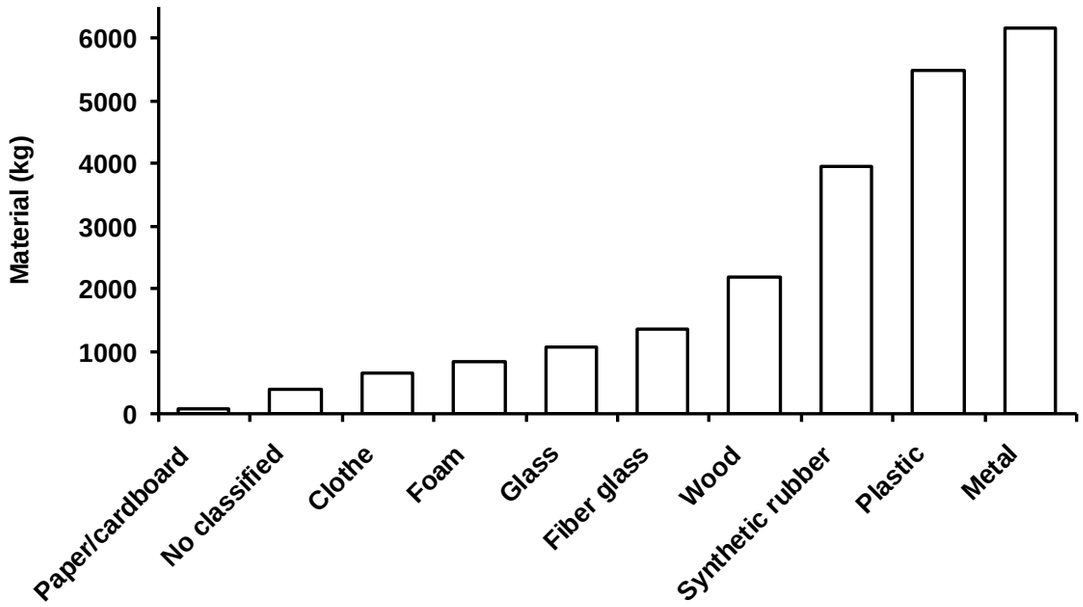

Figure 2.3 Amount of marine and coastal debris collected in Galapagos during shoreline cleanups in 1999 (Data adapted from Fundación Natura and WWF 2000). See legends for definitions of items: plastics (bags, plastic wraps, containers, bottles and plastic mesh); metals: (cans, and aerosol-can containers); synthetic rubber (gum, waxes, gloves, shoes, tires and toys); wood (boxes and tables); glass (bottles, containers, and light/fluorescent bulbs); foam (buoys, floaters, packing material, and disposable dishes); and, paper/card board (boxes, cups, containers, and newspaper).

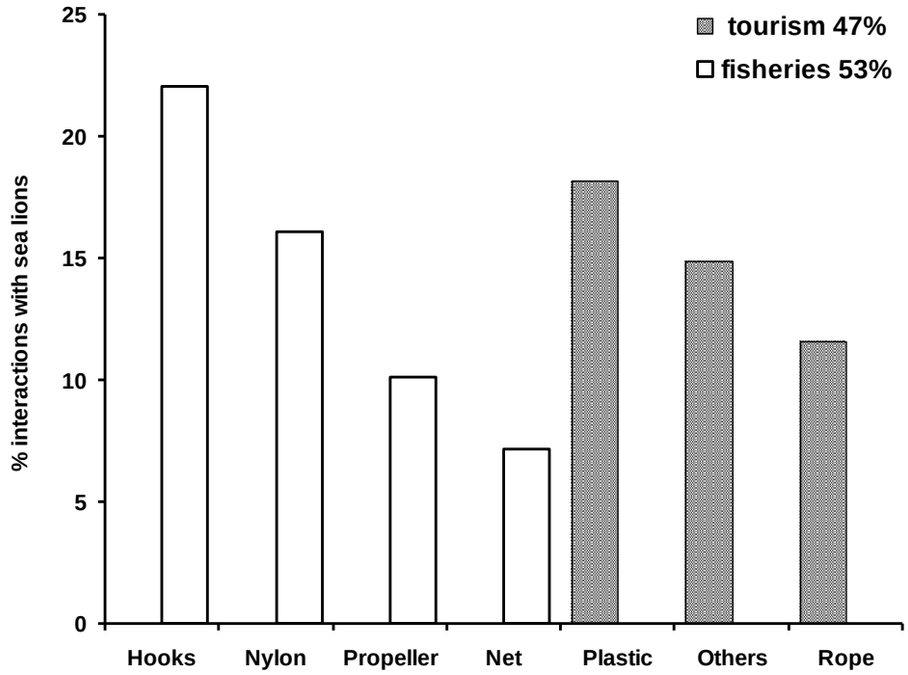

Figure 2.4 Type of objects and contribution by type of marine economic activities (tourism and fisheries) interacting with Galapagos sea lions in marine and terrestrial environments of the Galapagos. (Data adapted from Alava and Salazar 2006; Merlen and Salazar 2007).

2.3.4 Marine pollution by oil spills and hydrocarbons

Oil spills are one of the major threats to marine ecosystems, both in offshore and coastal zones. The transportation of crude oil or refined products, including distribution activities, results in the spill of an average estimated between 150,000 and 160,000 tonnes of petroleum worldwide annually (National Research Council 2003; ITOPF 2005). Biodiversity, fisheries and ecotourism can be threatened when oil spills of severe magnitude occur. The use of fuels such as diesel, high octane gasoline and liquefied petroleum gas transported from continental Ecuador has increased risks in the Galapagos. In 2000, a total of about 22 million L of fuel (20% gasoline and 80% diesel) were delivered to the Galapagos (Fundación Natura 2003). Tourism and electric power generation are the major energy usage sectors for diesel consumption, whereas fishing (i.e. outboards

motors) and motor vehicle transportation consume most of the gasoline in the islands (Table 2.2; Fundación Natura 2003).

Table 2.2 Consumption of Diesel (17.6 x 10^6 L) and Gasoline (4.4 x 10^6 L) by sector in the Galapagos in 2001 (Data adapted from Fundación Natura 2003).

Economic Sector	Diesel in L	(%)	Gasoline in L	(%)
Tourism (inboard, outboard and bus engines, tourist hotels)	10.6 x 10^6	(60)	1.012 x 10^6	(23)
Fishing (outboard engines, truck motors)	0.704 x 10^6	(4)	1.364 x 10^6	(31)
Overland transportation (motorcycle/car/truck/bus engines)	0.352 x 10^6	(2)	1.804 x 10^6	(41)
Electricity (electric power facilities, diesel generators)	4.60 x 10^6	(26)	No usage	(0)
Institutions (car engines and diesel generators)	1.41 x 10^6	(8)	0.220 x 10^6	(5)

During the last two decades, several oil spills have taken place in the Galapagos (Table 2.3). A major oil spill that threatened a significant part of the Galapagos Marine Reserve was the *MV Jessica* spill on 16 January 2001 at the entrance of Naufragio Bay (89 37'15"W, 053'40"S), San Cristóbal Island. The oil tanker released almost 100% of its total cargo consisting of 302,824 L of IFO 120–bunker fuel (Fuel Oil 120) and 605,648 L of Diesel oil # 2 (DO#2) (Lougheed *et al.* 2002; Edgar *et al.* 2003). In early July 2002, a second oil spill took place in the Galapagos, when a small tanker (*BAE/Taurus*) sank and spilled diesel fuel in waters off the coast of Puerto Villamil, Isabela Island. Fortunately, no sign of fuel was found on the beaches or on marine animals

(including sea lions), due to mitigation efforts conducted by the GNPS and CDRS. Other low magnitude oil spill events have also occurred (Lessmann 2004).

Table 2.3 Inventory of oil and diesel spills in the Galapagos from 2001 to 2006

Boat/Tanker	Date	Site	Quantity (L)
Motor Yacht Iguana	June 1988	Santa Cruz Island	189,265
MV/Jessica	16 January 2001	Naufragio Bay, San Cristóbal	908,472
BAE/Taurus	4-7 July 2002	Puerto Villamil, Isabela Island	7571
MV/Galapagos-Explorer	13-14 September 2005	Academia Bay, Puerto Ayora, Santa Cruz Island	Not reported*

*151,412 L of fuel were estimated to be contained in the boat, but actual volume spilled was not reported.

In addition, the Galapagos sea lion (*Z. wollebaeki*) was an impacted species of concern within the Charles Darwin Research Station (CDRS) and in the GNPS monitoring and management plans for marine fauna since some colonies were relatively close to the Jessica oil spill (Salazar 2003a). About 79 oil-affected individuals, showing different degree of oil presence on their bodies, were rescued, cleaned and released, and one fatality was recorded. On the other hand, no significant declines in the numbers of individuals were observed in the rookeries monitored after the spill (Salazar 2003a).

Measurements of hydrocarbons in sedimentary shores of the Galapagos right after the *Jessica* oil spill showed low levels or no detectable concentrations (Figure 2.5), ranging from 0.4 to 48.9 µg/g dry weight, with evidences of residual hydrocarbon contamination from sources other than the oil spill and suggesting absence of heavy oiling contamination (Kinstong *et al.* 2003). In

general, concentrations of dissolved and dispersed oil hydrocarbons measured in water samples from five bays of the Galapagos Islands (Figure 2.6) about one year before the aftermath were below threshold levels, 3-10 µg/L (Rodriguez and Valencia 2000).

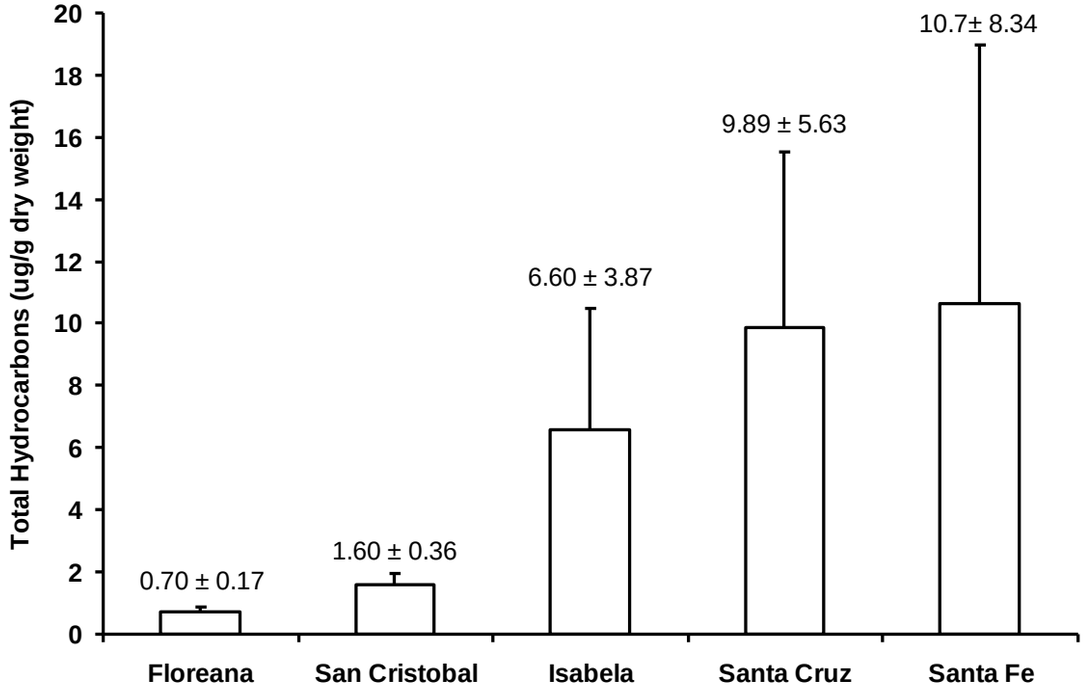

Figure 2.5 Mean of total hydrocarbon concentrations measured in sediment samples collected from oil impacted sandy shores of five islands of Galapagos Islands after the 2001—Jessica oil spill. Error bars are standard errors. (Data adapted from Kingston *et al*. 2003).

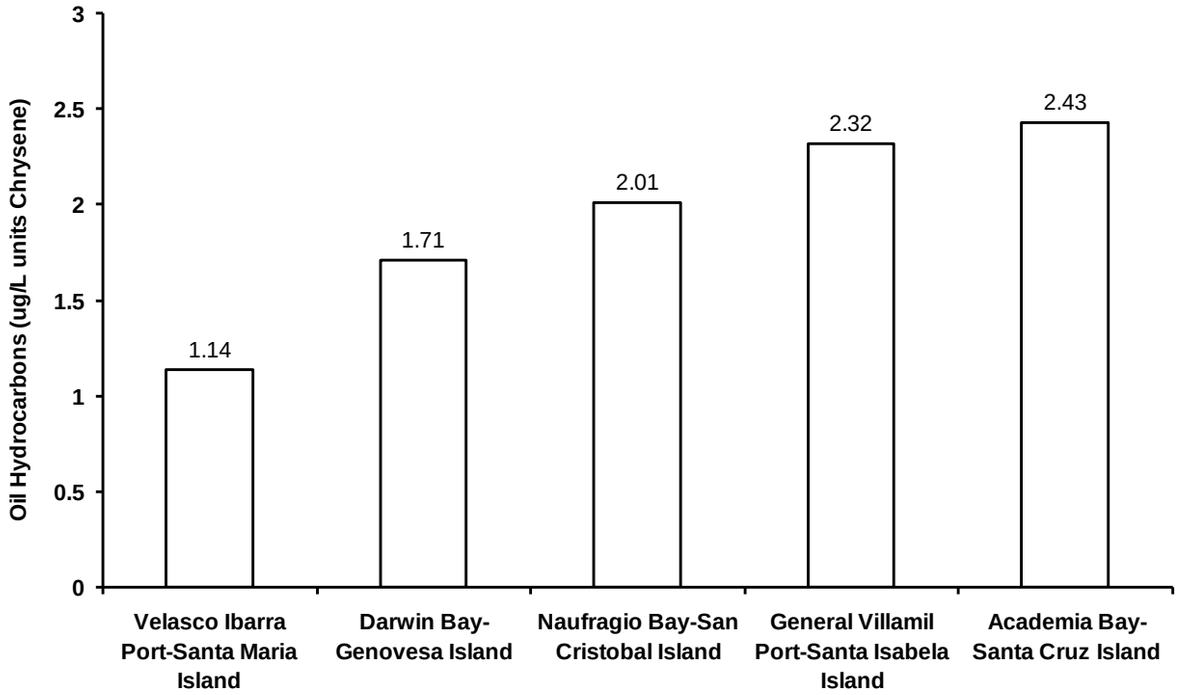

Figure 2.6 Levels of oil hydrocarbons detected in marine water from fives sites of the Galapagos Islands. (Data adapted from Rodriguez and Valencia 2000).

Recent studies of the endemic Galapagos marine iguanas (*A. cristatus*) found elevated plasma corticosterone levels, impaired development (i.e. reduction of growth) and high mortality in individuals exposed to low levels or residual hydrocarbon traces during and/or after the *Jessica* oil spill (Wikelski *et al.* 2001; Romero and Wikelsky 2002; Wikelski *et al.* 2002). This suggests that even low levels or traces of oil hydrocarbons are of critical negative effects for marine, endemic species of the Galapagos. Fortunately, the populations of endangered sea birds such as Galapagos penguins and flightless cormorants were not affected for the direct impact of this spill; however, the chemical exposure of these birds to chronic residue levels of oil hydrocarbons in the long term is unknown.

2.3.5 Impact of Persistent Organic Pollutants (POPs)

The Galapagos Islands and surrounding ocean waters might be susceptible to the global pollution by POPs. It is likely that organic contaminants transported from Asia, South America and Western industrialized countries are atmospherically delivered to these remote tropical islands. This implies the need of research and field work studies to elucidate the fate and transport of POPs in the Southeastern Tropical Pacific region, where the Galapagos are located.

In semi-urbanized centres (i.e., Santa Cruz and San Cristóbal), the presence of electric facilities/equipments and the grid electric wires' system containing transformers, capacitors, and cooling-insulator fluid to provide energy to human settlements are likely to represent potential sources of PCBs. PCB−contaminated dielectric fluid-oil found in transformers and tanks of the grid electric system and facilities of human centres of the Galapagos are likely to be the minor, local sources of these contaminants, which need a management plan to treat and remove them from the islands (Ministerio del Ambiente 2006). To our understanding, Aroclor mixtures have not been yet identified. In Ecuador, PCBs have never been produced for any chemical industry. Ecotoxicological studies on PCBs have never been conducted at continental Ecuador, except for some recent measurements of these industrial compounds in dielectric oil-fluid used in transformers and capacitors/tanks of some electric station facilities of the Guayaquil's Electric Corporation (CATEG) (CEMA 2005). The PCB levels found are below 10 mg/L (CEMA 2005). More recently, the preliminary national inventory of PCBs in Ecuador reported a total volume of about 5,473,000 L of PCB contaminated oil-fluid used in abandoned, unused and used electric transformers by the Electric Corporations (Ministerio del Ambiente 2006).

In the past, the biomonitoring and ecotoxicological risk assessment of POPs was never conducted in the Galapagos; therefore, data on concentrations, patterns, distribution, and fate is scarcely available for these contaminants. Despite of the potential conservation impact and risk in

the Galapagos Islands, environmental pollution by POPs has not fully been characterized in wildlife from this archipelago. Recently, a study assessing the levels of PCBs and PBDE flames retardants in the Galapagos reported that Galapagos sea lions are not exempt from the global contamination by POPs (Figure 2.7). The mean concentration of PCBs measured in Galapagos sea lion pups was 104 µg/kg lipid, ranging from to 49 to 384 µg/kg lipid (Alava *et al.* 2009). The global distribution of POPs, their persistence in the environment/biota, their risk to both human and biota, and, in some cases, continued production (deliberate or inadvertent) emphasize the need for an integrated approach to manage issues of POPs production, waste, remediation and exposure (Tanabe *et al.* 1994; Ross and Birnbaum 2003). This implies the need of baseline research on POPs in the Galapagos. For example, while threats associated with oil spills are visible and unlikely to cause a long-term decline of the Galapagos sea lion population due to their metabolic capacity to biotransform polycyclic aromatic hydrocarbon (PAHs) or non-halogenated hydrocarbons, the possible negative impacts (e.g., long-term chronic toxicity an sublethal effects) of POPs and other contaminants on health endpoints of this species are becoming more evident (Alava and Salazar 2006; Alava *et al.* 2009; Figure 2.7).

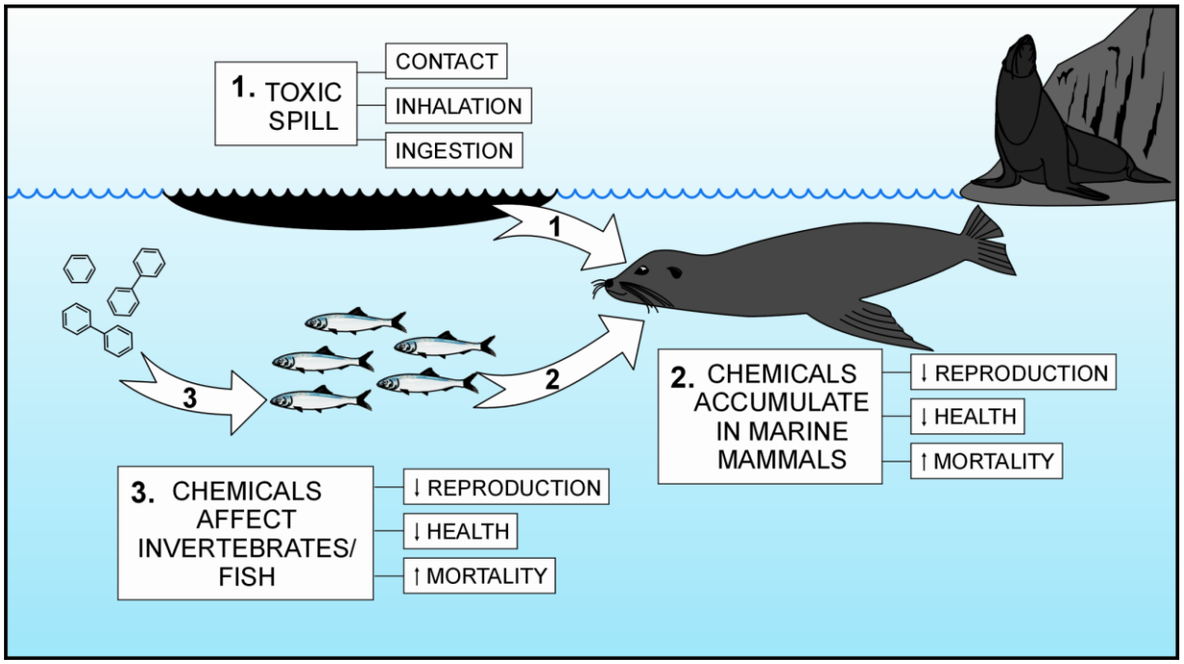

Figure 2.7 Galapagos sea lions can be exposed to chemicals assaults, including oil spills, which can possess acute and chronic toxic effects, and Persistent Organic Pollutants (1), which can be accumulated mainly through dietary ingestion and by inhalation, causing potential health effects (2) due to contamination of diet items (fish preys) in the food chain (3).The prey can be also affected by contaminants (3).

Given that it is well documented that marine mammals are key biological compartments to assess the concentrations, fate, distribution and toxic effects of POPs (Ross and Birnbaum 2003; O'Shea *et al*. 2003), a potential coastal sentinel to biomonitoring and investigate marine pollution and bioaccumulation by POPs is the Galapagos sea lions, which is an endemic, resident species as well as a top predator of the Galapagos marine food web (Alava and Salazar 2006). A considerable weight of evidence indicates that environmental pollution by POPs is affecting and jeopardizing the health and survival of pinnipeds (e.g., harbour seals, California sea lions) and cetaceans (e.g., killer whales and belugas). This is supported by several lines of evidence in toxicological research (Ross 2000). For example, the exposure to POPs has been linked to effects

on the immune (impairments in T-lymphocytes proliferation/count, and phagocytosis) and endocrine systems (i.e., disruption of Vitamin A and thyroid hormones) in harbour seals (Ross *et al*. 1995; Ross *et al*. 1996; Simms and Ross 2000; Tabuchi *et al*. 2006; Mos *et al*. 2006), in grey seals (Hall *et al*. 2003; Jensen *et al*. 2003) and in California sea lions (Debier *et al*. 2005). Recently, the deleterious effects of high levels of POPs (PCBs and DDTs) have been significantly linked to high prevalence of neoplasms and carcinoma, associated with mortality, in California sea lions (Ylitalo *et al*. 2005). Therefore, the Galapagos sea lion represents a novel marine mammal to be used as a potential biological compartment and eco-marker of coastal pollution by assessing the concentration and effect of POPs (i.e., measurements of POPs in blubber or blood samples and biomarker endpoints of the immune/endocrine systems).

2.3.6 Agriculture and pesticide use

In the Galapagos, agriculture occurs on all four human inhabited islands (Santa Cruz, Santa Cristóbal, Floreana and Isabela). These activities occur mainly in the highlands, where the highly biodiverse humid zone has largely been cleared (Table 2.4; Snell *et al*. 2002). Currently, approximately 3.96% (23,400 ha) of land area have been dedicated for agricultural use in the Galapagos and the proportion of humid zones is diminishing (Kerr *et al*. 2004). Furthermore, local use of pesticides can lead to runoff and the contamination of coastal food webs. For example, farmers from the agriculture sector use insecticides, herbicides, fungicides and fertilizers to control pests, while organic agriculture is partially practiced in the Galapagos (Dr. Alan Tye, pers. comm., former Head Scientist of the Department of Plant and Invertebrate Science, Charles Darwin Research Station, Puerto Ayora, Santa Cruz, Galapagos Islands).

Table 2.4 Total areas for agricultural and habitat (humid and transition*) zones in km^2 and the proportion of clearance affected by agriculture occupancy in humid and transition zones in four islands of the Galapagos (adapted from Snell *et al*. 2002).

Island	Agriculture	Humid zone	(% affected)	Transition zone	(% affected)
Santa Cruz	122	118	(74)	127	(26)
San Cristóbal	82	83	(93)	40	(9)
Floreana	5	31	(15)	39	(2)
Isabela	52	641	(8)	1323	(0)
Sierra Negra**	52	370	(14)	460	(0)

*****Transition zone: woodland communities dominated by *Pisonia floribunda*, *Psidium galapageium* (Guayabillo woodland), *P. galapageium/Scalesia* tree *spp.* (Scalesia-Guayabillo forest).
**This is a specific site represented by a volcano on Isabela Island where the human settlements are located.

As seen in Appendix A (Table A-2), some current use pesticides (CUPs) are used for agriculture in rural areas (highlands) in islands with human centres. According to this list, no legacy organochlorine pesticides (OC pesticides such as DDTs, dieldrin, mirex, Heptachlor and chlordanes) are currently used in the Galapagos. However, DDT was used in significant amounts by military personnel from the US Navy (former American Air Force and Naval Base in Baltra, Santa Cruz Island, used during the second World War) to eliminate introduced rats as invasive species in human housing from urbanized areas and into the Islands between 1940s and 1950s in the last century (M. P. Harris, Centre for Ecology and Hydrology, Banchory Research Station, Banchory, UK, pers. comm.; M. Cruz, GGEPL-Galapagos National Park, pers. comm.). More recently, the insecticide Deltamethrin is being used to control the dengue-mosquito vector (*Aedes*

aegypti) in the Galapagos (Dr. Hugo Jurado, pers. comm., National Center for Tropical Medicine, University of Guayaquil and Technical Director of the National Malaria Eradication Service Centre -SNEM, Guayaquil, Ecuador).

Many of these pesticides have been identified as causing reproductive and endocrine disrupting effects (see superscript EDC in bold pesticides listed in Table A-2) in both wildlife and human populations (Colborn *et al*. 1993; Colborn 1998; WWF Canada 1999; Lyons 1999). Furthermore, Chlorothalonil and its metabolites are highly toxic to fish, aquatic invertebrates, and marine organisms. Levels lower than 1mg/L can cause negative effects in rainbow trout, bluegill and channel catfish (see review by Verrin *et al*. 2004). Similarly, Malathion is extremely toxic for aquatic invertebrates, to some species of fish (<1 mg/L) and to some aquatic life stages of amphibians, whereas Carbaryl is moderately toxic to fish (1.3 −10 mg/L) (Verrin *et al*. 2004).

The application of pesticides in the agricultural zones of these human-inhabited islands may also introduce dioxins (polychlorinated dibenzo-*p*-dioxins or PCDDs) and furans (polychlorinated dibenzofurans or PCDFs) to the marine environment, as these have been found as contaminants in a number of pesticide products. While no risk assessments have been carried out to elucidate on the levels and potential health effects of CUPs in the Galapagos, there are reasons for urgent concern.

2.3.7 Biological pollution and invasive pathogens

Biological invasions are considered a leading cause of extinctions in terrestrial and marine ecosystem of marine protected areas (Boersma and Parrish 1999; Bax *et al*. 2003) as emerging marine diseases in marine organisms have been linked to anthropogenic factors (Harvell *et al*. 1999). For the purpose of this review, biological pollution is defined as the accidental or deliberate

introduction of viruses, bacteria and parasites, as well as terrestrial, exotic species of vertebrates, invertebrates and plants. Information on terrestrial exotic species (i.e., animals and plants) is not discussed in this review since it has been well reported elsewhere (Snell *et al*. 2002). The introduction of exotic marine species and pathogens (viruses, bacteria and parasites) represents major threats for biodiversity and ecosystem functions, with potentially serious implications for fisheries resources, tourism, human health in marine protected areas and biosphere reserves (Carlton 1989; Carlton and Geller 1993; Carlton 1996; Bax *et al*. 2003). The Hawaiian Islands represents an extraordinary example of the negative effects of the biological invasion on endemic and native species (Vitousek *et al*. 1987). This is supported by the fact that Hawaii contains a large proportion of the imperilled USA endemic birds (43%) and plants (40%) threatened by alien species (Gurevitch and Padilla 2004). Similarly, alien pathogens represent 34% of the birds affected by aliens of all kinds (Coles *et al*. 1999), and 91 of approximately 400 marine species present in Pearl Harbour are aliens (Gurevitch and Padilla 2004). The Galapagos Islands are facing a similar fate unless control and conservation strategies take place to mitigate biological invasion. The number of registered introduced species in the archipelago has increased 10 times from 112 species in 1900 to 1321 in 2007 (Watkins and Cruz 2007). This does not include introduced pathogens. Among the invasive pathogens, viruses, bacteria and parasites are the ones possessing serious risk to the endemic fauna.

Some introduced viral diseases from domestic animals such as avian virus or avipoxvirus by domestic birds, fowlpox virus infecting chicken and canine distemper virus-CDV epidemic in domestic dogs have threatened endemic species of birds (e.g., Darwin's finches) and marine mammals (e.g., Galapagos sea lions) in the Galapagos (Wikelski *et al*. 2004; Salazar *et al*. 2001; Cruz *et al*. 2002). Thiel *et al*. (2005) has recently found presence of canarypox-like viruses in pox-

like lesions of endemic passerine birds (Yellow Warblers, *Dendroica petechia*; finches, *Geospiza* spp.; and Galápagos mockingbirds, *Nesomimus parvulus*) from the inhabited islands of Santa Cruz and Isabela. A seroprevalence of 66% (29/44) to adenovirus group 1 has been found in waved albatrosses (*Phoebastria irrorata*) inhabiting Espanola Island (Padilla *et al*. 2003).

In the Galapagos, a CDV outbreak killed about 400 domestic dogs on Santa Cruz and Isabela islands accounting for 69.2 and 31%, of the CDV cases, respectively (Cruz *et al*. 2002). In Santa Cristóbal Island only one case of CDV was found. A serological survey determined the seropositive response of antibodies against CDV (50% or 7/14), Parvovirus (14% or 1/7) and Adenovirus (Canine Hepatitis virus, 100% or 1/1) in the canine population of Santa Cruz during 2001-2002 (Cruz *et al*. 2002).

Newcastle disease, Marek's disease virus (herpes) and mycoplasmosis detected in domestic chickens farmed on the islands (Vargas and Snell 1997), has the potential to cause declines of the flightless cormorant (*Phalacrocorax harrisi*), lava gull (*Larus fuliginosus*), and Galapagos penguin (*Spheniscus mendiculus*), species with small population sizes. West Nile Virus (WNV) is expected to reach Ecuador anytime and there is a high probability risk of its introduction into Galapagos unless strict control and preventive strategies are implemented prior to the arrival of the disease (GGEPL 2004). If WNV is introduced in to Galapagos it is likely to cause catastrophic mortality of endemic birds, reptiles and mammals, leading to irreparable ecological and economic damage to the islands (GGEPL 2004). Disease introduction is most likely to occur through the human transport of infectious mosquitoes, particularly via inadvertent transport in airplanes. The incidental transport of mosquitoes by boat or of infected vertebrate hosts is also significant risks for WNV invasion.

A serological survey of sea lions from different colonies of the Galapagos Islands in 2001 revealed that no CDV antibodies were present in this species (Salazar 2001). This indicates that they have not had any recent infection by Morbilliviruses and that they are vulnerable to infection by this genus of viruses. Mortalities among pinnipeds caused by Morbilliviruses CDV, phocine distemper virus (PDV) have been documented in harbour (*P. vitulina*), grey (*H. grypus*), Baikal (*Phoca siberica*) and Caspian *(P. caspica*) seals in industrialized regions (Osterhaus *et al*. 1988; Dietz *et al*. 1989; Osterhaus *et al*. 1989; Osterhaus *et al*. 1990; Visser *et al*. 1991; Kennedy *et al*. 2000). For instance, about 10,000 Caspian seals died due to CDV in 2000, and more than 23,000 and 30,000 harbour seals died in 1988 and 2002, respectively (Härkönen *et al*. 2006).

In 1999, a microbiological survey of total and fecal coliforms bacteria conducted in several sites of the Galapagos reported concentrations ranging from 2 to 240 CFU/100 mL and from 5 to 15 CFU/100 mL, respectively (Table 2.5; Rodriguez and Valencia 2000). At that time, these levels were below the Ecuadorian Water Quality Guidelines for the Prevention and Control of Environmental Contamination passed out in 1989. However, the impact of spill-over pathogens and antibiotic resistant bacteria in endemic organisms inhabiting this remote area warrants further microbiological and pathological research.

Recently, several kinds of bacteria have already been detected in endemic sea bird and pinnipeds of the Galapagos. For example, while antibodies to avian adenovirus type 1 and *C. psittaci* were found in 31% (21/68) and 11% (7/65) of flightless cormorants, respectively, seventy-five of 84 (89%) Galapagos penguins had antibodies to *Chlamydophila psittaci,* but chlamydial DNA was not detected via polymerase chain reaction in samples from 30 birds (Travis *et al*. 2006a; Travis et al. 2006b). Waved albatrosses showed a seroprevalence of 9% (4/44) to avian encephalomyelitis; however, cloacal swabs were negative for *C. psittaci*-DNA. (Padilla *et al*.

2003). *Salmonella* sp. was reported in domestic pigeons (introduced rock doves, *Columba livia*) on San Cristóbal and may cause severe disease in species such as Galapagos doves (*Zenaida galapagoensis*) and other native birds (Harmon *et al*. 1987; Wikelski *et al*. 2004; Padilla *et al*. 2004).

Table 2.5 Values of fecal and total coliforms (CFU/100mL) at coastal marine sites, Galapagos (Data from Rodriguez and Valencia 2000), relative to the current recreational marine water quality standards (US Environmental Protection Agency 1986).

Sites	Fecal coliforms	Total coliforms	US EPA (1986) fecal coliform standard (200 CFU/100mL)	US EPA (1986) total coliform standard (1000 CFU/100mL)
Academia Bay (Las Ninfas), Santa Cruz Island	15	240	Not exceeded	Not exceeded
Naufragio Bay, San Cristóbal Island	8.8	16	Not exceeded	Not exceeded
Santa Maria, Isabela and Genovesa islands	5.0	2.0	Not exceeded	Not exceeded

A serological survey determined that 5 out of 6 domestic dogs were seropositive (83%) to *Leptospira* on Santa Cruz in 2001-2002 (Cruz *et al*. 2002). This implied that Galapagos pinnipeds may be at risk of infection by this bacterial pathogen. Shortly after, a health surveys showed that Galápagos sea lions were susceptible to nine strains of the bacterium *Leptospira*, whereas Galápagos fur seals were susceptible to two strains, but there was no immunological response to brucellosis (Salazar 2002; Salazar 2003b). Recently, a conjunctivitis associated with bacillo-cocci bacteria, with a prevalence in Galapagos sea lion pups of 60–100% appears to be related to the presence of a new species of ocular parasite (*Philophtalmus zalophi*) (Dailey *et al*. 2005).

Among parasites, *Haemoproteus* sp., the only hemoparasite identified, was found in 89% of the Galapagos doves sampled but not in the rock doves (Padilla *et al*. 2004). In marine mammals, ectoparasites such as lice (*Antarctophthirius microchir*) and nasal mites (*Orthohalarachne diminuata*) were identified in various individuals of pinnipeds (Salazar 2002; Salazar 2003b). Domestic and feral animals introduced from the continent poses a major threat as potential sources for horizontal transmission of ecto and endoparasites to local, endemic species. Currently, the major parasitic disease that could cause widespread mortality of native, endemic birds is the avian malaria, if it is introduced into Galapagos ecosystems. This parasite has caused severe mortality and decimation of a significant proportion of Hawaiian's endemic birds since it was introduced at beginning of 20th century (Wikelski *et al*. 2004). At present, despite its vector, the mosquito *Culex quinquefasciatus* (Diptera: Culicidae), is already established on the Galapagos Islands (Peck *et al*. 1998; Whiteman *et al*. 2005), there has been no report or detection of *Plasmodium relictum* (Wikelski *et al*. 2004; Thiel *et al*. 2005). A protozoan, *Trichomonas gallinae*, was reported in domestic pigeons (introduced rock doves, *Columba livia*) on San Cristóbal and may cause severe disease in species such as Galapagos doves (*Zenaida galapagoensis*) and other native birds (Harmon *et al*. 1987; Wikelski *et al*. 2004; Padilla *et al*. 2004). Because the Galapagos endemic species were not exposed to alien parasites transmitted by invasive species prior human occupation of the islands, they are more susceptible to the pathogenesis generated by parasitic diseases with potential risk at the population health level.

Because of the presence of livestock, antibiotics are used for cattle ranching and domestic farms in rural zones (Francisco Torres, pers. comm., Centro de Estudios de Medio Ambiente, Escuela Superior Politécnica del Litoral, Guayaquil, Ecuador). Antibiotic resistance results from the broad use of antibiotics, both in humans and in animals (Pruden *et al*. 2006). Residual

antibiotics from animals' feces as well as from of septic tank overflow and sewage effluent may enter coastal marine areas. This may had antibiotic resistance in both human-introduced pathogens and natural strains of bacteria (i.e., antibiotic-resistant pathogens). Recently, antibiotic resistance genes (ARGs) from tetracycline and sulfonamide ARGs have been categorized as emerging contaminants, showing higher concentrations in urban/agricultural impacted river sediments (Pruden *et al*. 2006).

The threat and development of emerging infection diseases and microbial invasions can be further exacerbated in endemic fauna exposed to immunotoxic and endocrine disruptor chemicals (e.g., POPs) causing impairments in the immunological (e.g., decreased proliferation of white cells) and endocrine (e. g., disrupted regulation of thyroid hormone) systems and making them more susceptible to pathogens. This can be worsened and more lethal in nutritional stressed animals.

2.4 Management Implications and Research Needs

Environmental pollution in the Galapagos has, in the past, typically been described as an aesthetic and minor issue of concern rather than a significant conservation problem (Snell *et al*. 2002; Bustamante *et al*. 2002). However, increases in human migration and tourism, introduction of exotic and invasive species, solid waste generation, lack of sewage systems and water pollution are some the central degrading activities in the Galapagos Islands in the last three decades (Merlen 1995; MacFarland and Cifuentes 1996; Watkins and Cruz 2007). The threats for the Galapagos conservation and mitigation strategies in terms of environmental pollution are summarized as follow.

1.4.1 Conservation Threats

The Galapagos is a heritage at risk not only because of the massive tourism, human migration and invasive species but due to potential chemical assaults and the spreading out of pathogens, as supported for the lines of evidences found in this review. A series of major developments in recent years, including oil spills, expansion of agriculture and tourism sector, and the emerging of new pathogens and other biological pollutants, should serve as a wake up call for decision makers in the Galapagos.

Of important concern is the release of solid wastes (e.g., plastics) and leaking of hydrocarbons from tourism sector and the fishing industry, which are likely to be the major local sources of contamination in the Galapagos marine environment (MacFarland and Cifuentes 1996; Lessmann 2004). Overflow from rudimentary septic tanks and runoff of sewage waters around the islands threaten the water quality near urbanized centres. Both large and small fuel spills take place on a regular basis in the Islands during the transport and delivery of fuel to tourist boats (Okey *et al*. 2004). The existence of localized sources (waste incineration in open dumps in the recent past) and atmospheric inputs (continental or global inputs) might be contributing to the migration and deposition of POPs to the Galapagos environment, as evidenced for the levels of PCBs found recently in Galapagos sea lion pups.

1.4.2 Management Actions and Mitigation Measures

Several laws, regulations, policies and plans have been enacted recently by the Ecuadorian government in benefit of the conservation and management of both the Galapagos

Marine Reserve and the Galapagos National Park (e.g., Special Law for the Conservation and Sustainable Development of the Galapagos Province, 1998). However, the control and management of environmental pollution in the Galapagos warrants additional efforts. At continental Ecuador, efforts have already been undertake through the Ecuadorian Guidelines for the Control and Management of Environmental Pollution.

Meanwhile, the lessons learned from the oil spills and from the remedial actions taken in response to them was a topic of particular concern for the Ecuadorian government and regional commissions involved with marine protected areas and environmental pollution. Because of this, the Galapagos was recently designated as a Particularly Sensitive Sea Area (PSSA) by the International Maritime Organization (IMO) in 2005 under Resolution MEPC-135(53) to prevent marine pollution by spills and hazardous contamination coming from ships. At this level, the application of the precautionary principle would help to avoid and mitigate pollution in the Galapagos Archipelago.

During the last three years, the local waste management of municipal organic waste is being improved and treated appropriately to avoid the generation of dioxins by banning the burning of this kind of waste in open areas close to harbours and coastal zone. Also, the implementation of an environmental impact assessment of current use pesticides (CUP) and past use pesticides in the urbanized centres should be a priority aspect in the regional management plan and environmental monitoring of the Galapagos Marine Reserve and National Park to assess the levels and potential health effects to wildlife, aquatic/marine organisms and humans dwelling there. Local effluents need to be controlled to avoid biological pollution and spread of infectious diseases to local wildlife and native human. Alternative approaches to dispose and treat sewage water effluents and oil leaking are required at the domestic and economic sectors (fisheries and

tourism). Local hotels and restaurants should incorporate best management practices (BMPs) through environmental management systems (EMS), which will promote green certification as an add value. The periodical maintenance and monitoring (i.e., Environmental Audits to fix irregularities) of outboard motors, boat engines and oil tankers can contribute in the reduction of marine pollution by hydrocarbons.

The management concerning with POPs (i.e., dioxin/furans generated form organic waste incineration, pesticides) and biological pollution so far analyzed in this review need to be focused both at the local/regional and international levels regarding environmental and marine policy. Ecuador is a recent signatory country of the Stockholm Convention since May 2001 and ratified it on 7 June 2004. Since then, the National Plan for the Implementation of the POPs Management in Ecuador was undertaken in this country by commencing with a national inventory of COPs, including PCBs, dioxins/furans and OC pesticides (Ministerio del Ambiente 2006). Therefore, the use of international policy instruments such as the Stockholm Convention on Persistent Organic Pollutants and The Convention on Long-Range Transboundary Air Pollution (CLRTAP)–POPs protocol must be emphasized to protect this pristine, remote area of the world.

We propose the use of endemic marine species such as pinnipeds (Galapagos sea lions and fur seals) and sea birds (e.g., Waved albatrosses, Galapagos penguins and flightless cormorants) to assess and bio-monitor the current exposure levels, patterns, fate and effects of contaminants in the Galapagos. These charismatic, top predator species can be used potentially as regional sentinels of marine pollution and coastal health in these remote islands. For example, the eco-toxicological research on POPs (e.g., dioxins/furans, PCBs, DDTs and other organochlorines pesticides) can be focused in the measurement and assessment of these compounds in blubber biopsies and blood samples of sea lions and sea birds to elucidate both

local and regional contamination. To look for evidences on health effects, biomarkers such as Vitamin A and thyroid hormones can be evaluated to examine potential endocrine disruption by POPs in the Galapagos sea lion. This needs to be accompanied by ecotoxicological modeling to predict and better assess these contaminants (i.e., toxicity, persistence, and bioaccumulation/biomagnification) in marine food webs. This should be coupled with the use of model bias and uncertainty analyses, as a tool to account for variability and uncertainty. In fact, the use and application of models has tremendously contributed to the progress of science in environmental toxicology and chemistry, and contributed to the management of toxic chemicals by helping to understand their origin, behaviour, distribution, fate, exposure and toxic impacts on the environment (Gobas and Muir 2004).

This is the last remote, evolutionary natural lab to protect and conserve for future generations. While it is not too late to undertake international and local environmental stewardship and management strategies to mitigate and control pollution, the presence of anthropogenic stressors and coastal-marine pollution is a sign that the Galapagos Marine Reserve is not immune to the contamination either local or global.

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CHAPTER 3

POLYCHLORINATED BIPHENYLS AND POLYBROMINATED DIPHENYL ETHERS IN GALAPAGOS SEA LIONS (*ZALOPHUS WOLLEBAEKI*)

Abstract: Concentrations of polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) were measured in muscle-blubber biopsy samples from 21 Galapagos sea lion (*Zalophus wollebaeki*) pups that were live captured in the Galapagos Islands (Ecuador) using gas chromatography/high-resolution mass spectrometry. Only traces of PBDEs were detected in one male pup, whereas PCDDs and PCDFs were not detected in any sample. The total concentration of PCBs (∑PCB) in the pups averaged 104 µg/kg lipid (range, 49–384 µg/kg). No statistically significant differences in ∑PCB were observed among the four study sites in the Galapagos Islands. Concentrations of PCB congeners in Galapagos sea lion pups were dominated by low-molecular-weight congeners. These results suggest that global transport is the main source for PCBs in Galapagos sea lions. The ∑PCB levels were below immunotoxic and endocrine-disruption thresholds in pinnipeds, suggesting a limited risk of adverse health effects. The present study indicates that Galapagos sea lions can serve as a useful sentinel of pollutants with a long-range transport capacity and that Galapagos Islands are not exempt from the threats of global pollutants despite its remote locale.

Keywords: **PBDEs, PCBs; Galapagos sea lions; Galapagos Islands; Atmospheric transport.**

3.1 Introduction

Polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), polychlorinated dibenzo-*p*-dioxins (PCDDs), and polychlorinated dibenzofurans (PCDFs) represent persistent, bioaccumulative, and toxic compounds of global concern. Whereas the legacy PCB, PCDD, and PCDF production or by-production has been curtailed, in part because of the global Stockholm Convention on persistent organic pollutants (UNEP 2001), PBDEs represent chemicals of emerging environmental concern (de Boer *et al*. 1998; de Wit *et al*. 2002; Alaee *et al.* 2003; Alock *et al*. 2003).

In the industrialized world, PCBs were banned during the late 1970s as a result of concerns about their persistence, bioaccumulation, and toxicity to wildlife (UNEP 2001). Polychlorinated biphenyls are found at relatively high concentrations in the northern hemisphere, but their presence in the tropics can be attributed, in part, to long-range transport (Shen *et al*. 2006; Wania and Mackay 1993; Iwata *et al*. 1993; Jurado *et al*. 2005). Polychlorinated biphenyl concentrations in pinniped species have declined since the 1970s, as source control and regulations served to reduce inputs into the environment (Blasius and Goodmanlowe 2008). Before national controls, both PCDDs and PCDFs were formed as by-products of pulp and paper mill processes (Hagen *et al*. 1997), but they also can be formed as by-products of combustion (Czuczwa and HIte s 1984). Assessing PCB, PCDD, and PCDF exposure is an important part of marine wildlife conservation, because these compounds have been associated with effects on the immune and endocrine systems of marine mammals (Ross *et al*. 1995; Debier *et al*. 2005; Tabuchi *et al*. 2006; Mos *et al*. 2006), which can compromise survival and reproduction.

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants in foams, textiles, coatings, furniture, construction materials, electronic devices (e.g., television sets, appliances, and computers), plastics, and paints since 1970 (de Boer *et al*. 1988; de Wit 2002; Alaee *et al*. 2003; Nylund *et al*. 1992; van Esch 1994). The production and use of PBDEs have been restricted in Europe and Canada, but the deca-PBDE formulation is still used extensively elsewhere (Alcock *et al*. 2003). The worldwide production of brominated flame retardants, including PBDEs, during the 1990s and the year 2000 was approximately 150,000 to 350,000 tons/year (de Boer *et al*. 1998; de Wit 2002; Alaee *et al.* 2003). Similar to PCBs, a total of 209 PBDE congeners are possible, although commercial mixtures and environmental samples typically contain a small number of dominant PBDE congeners (Alaee *et al*. 2003; Rayne and Ikonomou 2002; Hale *et al*. 2003; La Guardia *et al*. 2006). Polybrominated diphenyl ethers also have been detected in marine mammals, including polar bears (*Ursus maritimus*), seals, and cetaceans (She *et al*. 2002; Wolkers *et al*. 2004; Hall and Thomas 2007; Kelly *et al*. 2008). For example, PBDE concentrations in ringed seals (*Pusa hispida*) increased exponentially in the Canadian Arctic from 1981 to 2000 (Ikonomou *et al.* 2002). In Europe, however, declines in PBDE concentrations have resulted from the regulation of penta- and octa-formulations in 1998 (Nylund *et al.* 1992; Sellström *et al*. 1993; Meironyte *et al*. 1999). Polybrominated diphenyl ethers are relatively persistent environmental contaminants that bioaccumulate in organisms and can undergo long-range transport to remote regions (Ter Schure *et al*. 2004; Shen *et al*. 2006). In addition, PBDEs can cause toxic effects, including neurotoxicity, disruption of steroid and thyroid hormone regulation, teratogenicity, and carcinogenicity (Meerts *et al*. 2001; Darnerud *et al*. 2001; Hallgren and Darnerud 2001).

Evidence for the propensity of PCBs, PBDEs, PCDDs, and PCDFs to undergo long-range transport typically has been gauged by their occurrence in polar regions. The protection of peoples inhabiting Arctic regions from the adverse health effects of persistent organic pollutants is an integral component of the Stockholm Convention, which became international law on May 17, 2004 (UNEP 2001). Long-range transport to tropical regions, however, is not receiving comparable attention. In the case of PBDEs and PCBs, no studies, to our knowledge, have been conducted in pinnipeds from equatorial or tropical areas.

Despite its protected status, the Galapagos sea lion (*Zalophus wollebaeki*) population decreased by 60% between the 1970s and the year 2000 (Alava and Salazar 2006). Several hypotheses have been proposed to explain this decline. These include E1 Niño events, nutritional stress, fisheries interactions, illegal hunting, as well as diseases (e.g., *Leptospira* and *Morbillivirus* sp.) introduced by feral mammals, such as dogs (Alava and Salazar 2006; Merlen and Salazar 2007). As a result, the Galapagos sea lion is listed as “threatened” under the IUCN (World Conservation Union) endangered category (Utreras *et al*. 2001; Aurioles and Trillmich 2008). To our knowledge, the potential impact of endocrine-disrupting persistent organic pollutants has not been investigated and could represent another factor contributing to the decline in Galapagos sea lions.

The present study measured PCB, PBDE, PCDD, and PCDF concentrations in Galapagos sea lions to characterize the presence of these priority contaminants in these pinnipeds and to evaluate any possible risks associated with exposure. The Galapagos Island Archipelago (Ecuador) is a United Nations Educational, Scientific, and Cultural Organization (UNESCO) World Heritage Site that recently was listed as being at risk (Watkins and Cruz 2007). Understanding the

fate and potential health effects of these contaminants on Galapagos sea lions is an important part of protecting biodiversity in this region and enhancing environmental stewardship.

3.2 Materials and Methods

3.2.1 Sampling

Muscle-blubber biopsy samples (100mg; 6 mm biopsy punch) of 21 Galapagos sea lion pups (*Z. wollebaeki*) were obtained from four rookeries of the Galapagos Islands Archipelago (Figure 3.1) at 1000 km (600 miles) off the Ecuadorian continental coast, between 01°40´N-01°25´S and 89°15´W- 92°00´W, during a field research expedition between March 13-21, 2005. Pups were sampled from the Caamaño (*n* = 11) and Plaza Sur (*n* = 4) colonies on Santa Cruz Island, and six from Punta Espinoza (*n* = 3) on Fernandina Island and Cabo Chaimbers (*n* =3) on Pinta Island, respectively. Santa Cruz is a semi-urbanized island, while Fernandina and Pinta islands are noninhabited, pristine environments in the Galapagos. Nursing pups were chosen for the following reasons: they are readily accessible and easy to capture in most of the rookeries of the Galapagos Islands year round; they are approximately of the same age, thus minimizing the influence of life history on contaminants concentration; they are nursed by adult reproductive females with a similar diet item (i.e., milk); and, they are in a high trophic position feeding on mother's tissue (i.e., milk). Galapagos sea lions reproduce year round. The main period of birth is between August and November, and the young are weaned after approximately 12 to 24 months (Trillmich 1986; Trillmich and Wolf 2008). The capture and immobilization of pups followed the field anesthesia methodology for studies of Galapagos sea lions and fur seals developed by Parás *et al*. (2002). Pups were selected based on observed nursing behaviour and estimated age based on size and weight ranged from 2 to 12 months old (i.e., <less than two years old). The animals'

weight, length, and girth were measured. Further details on the pups capture, determination of body condition index (i.e., Fulton condition factor), and immobilization are described in the Appendix B.

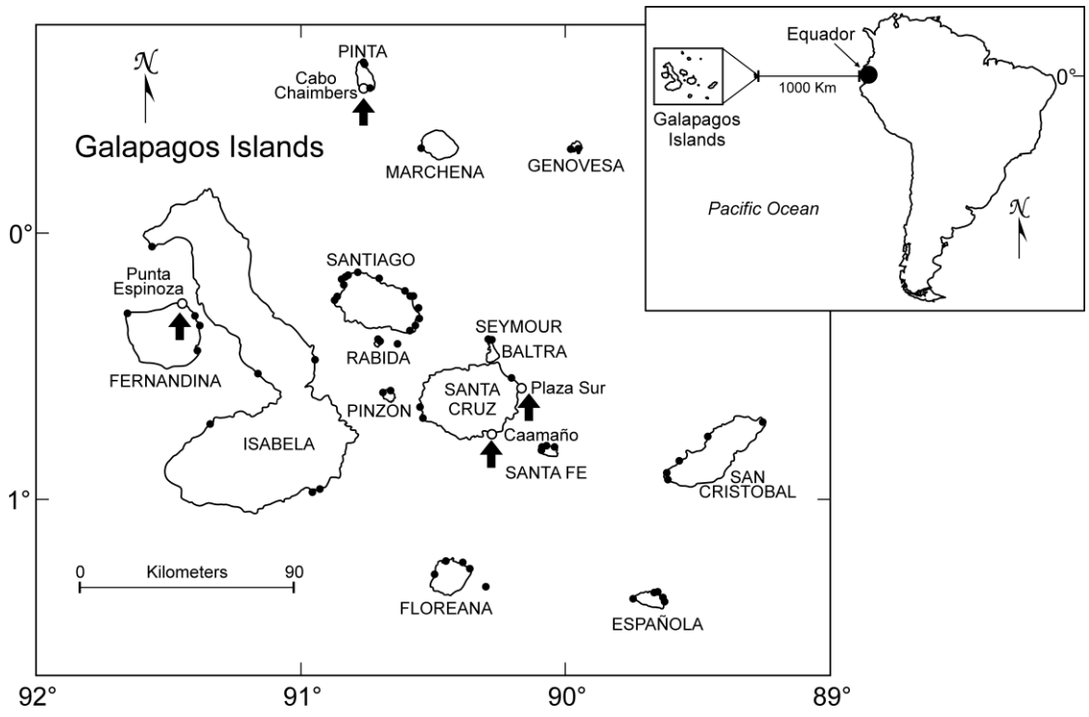

Figure 3.1 Map of the Galapagos Islands in relation to Ecuador and South America showing the sampling sites (white dots) indicated by black arrows and distribution of the Galapagos sea lion rookeries (small black dots).

Biopsy specimens were collected from the supraspinatus muscle, located right above of the flipper, which had been previously cleaned with alcohol and betadine. Biopsy specimens were wrapped in hexane-rinsed aluminum foil and placed into cryovials, which were stored in a cooler with ice during field work and then transferred in a freezer (-20°C) on board of the expedition boat

until transport to the laboratory. In the laboratory, the samples were at -80°C until chemical analysis.

3.2.2 Chemical Analysis

The chemical analyses for all target contaminant classes (PCDDs, PCDFs, PBDEs, and PCBs) were performed by gas chromatography/high-resolution mass spectrometry (GC/HRMS) in the Regional Dioxin Laboratory (RDL) at the Institute of Ocean Sciences (IOS), Fisheries and Ocean Canada (DFO), based on analytical methodologies described elsewhere (Ikonmomou *et al*. 2001). More details regarding the chemical analysis can be found in the Appendix B. Briefly, the entire muscle-blubber biopsy sample (0.004–0.145 g) underwent extraction for the target contaminant classes. One biopsy sample contained some cartilaginous tissue in addition to blubber and exhibited a relatively low lipid content. The intact biopsy samples were spiked with a mixture of surrogate internal standards that contained all seventeen 2,3,7,8-chlorine-containing, $^{13}C_{12}$-labeled PCDDs and PCDFs (except octachlor-odibenzofuran) as well as fifteen $^{13}C_{12}$-labeled PCBs and a suite of nine $^{13}C_{12}$-labeled PBDEs. All surrogate internal standards were purchased from Cambridge Isotope Laboratories. The spiked samples were homogenized with 20 g of Na_2SO_4 in a mortar, transferred quantitatively into an extraction column, and extracted with dichloromethane (DCM)/hexane (1:1, v/v). For some of the samples, the extract formed two layers/phases, a waxy-precipitate layer and the solvent layer. The solvent layer was transferred to a clean flask, and the waxy precipitate was treated with several aliquots of hexane and DCM. Each of these precipitates was then transferred to the flask that contained the solvent layer of the extract. Despite the treatment with additional volumes of hexane and DCM, vortexing, and pulverization, the waxy precipitate did not dissolve in the solvents used. As a result, it was not

included in the corresponding sample extract that was used for lipid and contaminant determinations.

The DCM/hexane sample extracts were evaporated to dryness, and the residue was weighed to determine the total lipid content in the sample. Subsequently, the residue was resuspended in DCM/hexane (1:1) and divided quantitatively into two aliquots. The larger aliquot (75% of the extract) was subjected to sample cleanup for PBDE, PCB, PCDD, and PCDF determinations, whereas the remaining aliquot was stored for future contaminant determinations. Sample extracts were cleaned up by silica gel chromatography (with layers of basic, neutral, acidic, and neutral silica) and activated alumina chromatography and carbon fiber. Two fractions were collected on carbon fiber—namely, the PCB/PBDE fraction (in DCM/hexane) and the PCDD/PCDF fraction in toluene. Each fraction was concentrated to less than 10 µl and spiked with the corresponding ^{13}C-labeled method performance standards before instrumental analysis. Details regarding the quality-assurance/quality-control (QA/QC) protocols followed, the amounts and composition of the surrogate internal and performance standards used, and the sorbents, solvents, and conditions used in all the cleanup steps are reported in detail elsewhere (Ikonomou *et al*. 2001) and in Appendix B. The PCDD/PCDF fraction was analyzed by GC/HRMS for the corresponding analytes. The PCB/PBDE fraction was first analyzed by GC/HRMS for the target (mono- to hepta-) PBDE congeners. Subsequently, the PCB/PBDE fraction was combined with the PCDD/PCDF fraction and analyzed for full congener PCBs by GC/HRMS. For all analyses, the HRMS was operated at 10,000 resolution under positive conditions, and data were acquired in the single-ion resolving mode. The instrumental analyses conditions used for each of the three contaminant classes are provided in Appendix B. Tissue lipid contents were determined

gravimetrically using 0.004 to 0.145 g (wet wt) of sample. Lipid contents were expressed as a percentage of the original wet tissue weight.

3.2.3 Quality Assurance/Quality Control

Rigorous QA/QC protocols were applied for analysis of the Galapagos sea lion blubber samples. Biopsy samples were analyzed in batches of 12 samples, with each containing one or two procedural blanks that were used to determine the method detection limit (MDL), an in-house performance evaluation sample containing known concentrations of the analytes of interest or a certified reference material (CRM), and 9 or 10 biopsy samples. Analyte concentrations were calculated by the surrogate internal standard method using mean relative response factors determined from calibration standard runs before and after each batch of samples. Recoveries of individual internal standards were between 60 to 110% for all analyses. Concentrations of analytes were corrected for the recoveries of internal standards. Method blanks, consisting of Na_2SO_4, were extracted according to the same procedure as used for environmental samples and were analyzed with every batch of 12 samples to check for background contamination throughout the entire analytical procedure. Multipoint (for details, see Appendix B) calibration curves were used to determine instrument detection limits, linearity in detector response, and dynamic range for each target analyte. Method accuracy and precision for all analytes was determined from the analyses of CRMs, participation in intercalibration studies, and analysis of in-house reference samples (spiked or natural matrices) analyzed repeatedly over long periods of time. The CRMs used for PCDD/PCDF and PCB method validation were EDF-2524, EDF-2525, and EDF-2526 (purchased from Cambridge Isotope Laboratories). The method accuracy and precision (i.e., %

deviation from the mean or the certified value as applicable) established from analyses of these standards for all congeners of all four analyte classes was better than 20%.

Concentration analysis involved examining concentration data on a pg/sample basis (wet weight), because the amounts of sample weight for extraction available in the present study were 50- to 100-fold lower (5–50 mg) than those normally used. For PBDEs, the concentrations measured in these samples were close to the levels measured in the procedural blanks. Concentration data therefore were plotted as the mass of PBDEs measured in the sample as a function of sample weight (i.e., on a per-sample basis) to elucidate the contribution of background contamination to the total measured concentration. Measured concentrations of PCDDs, PCDFs, and PCBs were evaluated using the same approach.

Concentrations of all detected PCDDs, PCDFs, PBDEs, and PCBs were blank corrected using the MDL (i.e., MDL on a pg/sample basis), defined here as the mean response of the levels measured in three procedural blanks used plus threefold the standard deviation (SD) of the blanks ($MDL = Mean_{blanks} + 3SD_{blanks}$). The concentration of each congener was determined according to two methods, i.e., based on concentrations above the MDL only and based on all concentrations using half the MDL for those concentrations below the MDL. The total concentration of PCBs (∑PCB) was determined as the sum of the concentrations of all 72 congeners using half the MDL for concentrations below the MDL. The total concentration of PBDEs (∑PBDE) was calculated as the sum of the concentrations of four congeners (BDEs 47, 49, 66, and 183) above the MDL. Concentrations of contaminants were lipid normalized by dividing wet-weight concentration by the lipid content to account for the differences in lipid content among the muscle-blubber biopsies. The normality of the concentration data were explored and reported as geometric mean concentrations with asymmetric standard deviations (SDs).

3.2.4 Statistics

Log-transformed morphometric data (i.e., length, weight, and body condition) of both sexes were compared using a Welch's modified two-tailed *t* test assuming unequal variances (Zar 1999) to determine if any difference in life-history parameters existed between the sexes. To examine possible relationships between ∑PCB in sea lion tissues and age, length, girth, lipid content, and body condition index (i.e., Fulton condition factor [FCF]), correlation analyses were conducted among all variables and contaminant levels (results are presented in a correlation matrix). The occurrence of significant differences between ∑PCB concentrations in male and female pups was investigated using the Welch's approximate *t* test.

The Welch analysis of variance followed by a Tukey-Kramer multiple-comparisons test (Zar 1999) was used to explore the occurrence of statistically significant differences in ∑PCB concentrations among sites, because the variances among sites were unequal (i.e., heteroscedasticity; Bartlett test, $p = 0.0187$). All statistical analyses were conducted using JMP 7.0 (SAS Institute) at a level for significance of $p < 0.05$.

3.2.5 Health Risk Assessment

A preliminary hazard/effect assessment was based on the estimation of total toxic equivalent concentrations (TEQs, ng/kg lipid) using the most recent data on total equivalent factors for dioxin-like PCBs, including planar (non-*ortho*-) PCBs (sum of PCBs 77, 81, 126, and 169) and mono-*ortho*-PCBs (sum of PCBs 105, 114, 118, 123, 156, 157, and 167) reported by Van den Berg *et al*. (1998). Both PCDDs and PCDFs were not included in the TEQ calculations, because these compounds were not detected. The resulting TEQs were then compared to the TEQ threshold levels, including the no-observable-adverse effect level (NOAEL) and the lowest-observable-adverse effect level (LOAEL) for dioxin-like PCBs, derived from immunotoxic action and

endocrine-disruption endpoints assessed in semicaptive harbour seals (Ross *et al.* 1995; Kannan *et al.* 200). Total toxic equivalent concentrations for PBDEs were not assessed at this time, because total equivalent factors have yet to be determined for this group of organic contaminants.

3.3 Results and Discussion

3.3.1 Study animals

The lipid content of our 21 Galapagos sea lion pup biopsy samples was of 72% ± 19% (mean ± standard error) (Table 3.1). The lipid content did not correlate with any of the life-history parameters (regression analysis for all body measurements, $p > 0.05$), including age, length, weight, girth, and corporal condition (Table 3.2). No significant differences were found in lipid content between female and male pups (Welch's approximate *t* test, $p = 0.550$) (Table 3.1). The ages of male and female pups (Welch's approximate *t* test, $p = 0.2350$) were similar, because biopsies were only performed on suckling pups (age, 2–12 months). In contrast, body weight and length of female pups were significantly greater than those of male pups (Welch's approximate *t* test, $p < 0.0001$ and $p < 0.0001$, respectively). Length and weight were highly correlated in these pups ($r = 0.98$, $p < 0.00001$) (Table 3.2). Similarly, girth showed a significant relationship with length and weight (regression analysis, $p < 0.0001$). The body condition index (i.e., FCF) of male pups was higher than that of female pups (Welch's approximate *t* test, $p = 0.004$), reflecting the generally higher body density of male otariid pups (Luque and Aurioles-Gamboa 2001).

Table 3.1 Morphometric data reported as the arithmetic mean ± SE (range) and mean log of sum of polychlorinated biphenyls (∑PCBs) and sum of polybrominated diphenyl ether (∑PBDEs) concentrations (µg/kg lipid weight) ± standard error of the mean. The range of ∑PCBs concentrations is presented in brackets.

	Male	**Female**	**Welch's approximate *t* Test *p* value**
Sample size (*n*)	8	13	
Body weight (kg)	20.6 ± 0.95 (18−25.6)	66.9 ± 7.01 (14.4−98.4)	<0.0001*
Standard length (cm)	102 ± 1.85 (96−109)	155 ± 7.67 (87−177)	<0.0001*
FCF (corporal condition)[a]	1.94 ± 0.03 (1.82−2.07)	1.71 ± 0.06 (1.31−2.19)	0.004*
Lipid (%)	70.2 ± 9.34 (13.5−100)	73.3 ± 3.92 (44.4−92.8)	NS
Log of ∑PCBs	1.98 ± 0.10 (49.0−384)	1.90 ± 0.06 (53.2−353)	NS
Sample size-PBDEs (*n*)	(1)[b]		
BDE-47	33.3		
BDE-49	0.87		
BDE-66	0.33		
BDE-183	0.63		

*Asterisk indicates significant difference; NS = no significant;
[a]FCF = Fulton's Condition Factor (weight x 10^5/standard length^3)
[b]Only a male pup from the South Plaza rookery (Santa Cruz Islands) exhibited detectable concentrations of PBDEs.

Table 3.2 Correlation matrix presenting the correlation coefficients of the Log of sum of polychlorinated biphenyls ∑PCB concentrations (µg/kg lipid) and all the morphological parameters of Galapagos sea lion pups analyzed in the present study.

Variable	∑PCBs (n =21)	% Lipid	Age	Girth	Weight	Standard Length	FCF[c]
∑PCB	1						
% Lipid	-0.76***	1					
Age[b]	0.24	- 0.32	1				
Girth	Male (0.76)* Female (0.47)	- 0.03	0.67*	1			
Weight	Male (0.69) Female (0.50)	0.07	0.61*	0.98***	1		
Standard Length	Male (0.50) Female (-0.62)*	0.09	0.58*	0.96***	0.98***	1	
FCF	Male (0.14) Female (0.68)*	- 0.28	0.19	-0.51*	- 0.53*	-0.69**	1

[b]Lipid content and age were negatively correlated when the pup showing the lowest lipid value (13%) was included (r = -0.59; p = 0.006)
[c]FCF = Fulton's Condition Factor (weight x 10^5/standard length^3)
Asterisks indicate significant correlations: *($p \leq 0.05$); ** ($p < 0.0005$); and *** ($p < 0.0001$).

3.3.2 Concentrations of PCBs, PBDEs, PCDDs and PCDFs

Of a total of 207 PCB congeners included in the analysis, 72 congeners in Galapagos sea lion muscle-blubber biopsies were consistently detected at concentrations above the MDL. Lipid-normalized concentrations and MDLs for individual congeners detected are reported in Appendix B (Table B-1). The sum of the mean PCB congener concentrations based only on detectable concentrations was 104 µg/kg lipid, and the geometric mean concentration of PCBs in the blubber samples using half the MDL for nondetectable PCB congener concentrations (n = 21) was 85 µg/kg lipid (lower geometric SD, 48 µg/kg lipid; upper geometric SD, 150 µg/kg lipid).

Among pups of the four different rookeries, ∑PCB concentrations were not significantly different (Welch analysis of variance, p = 0.4964; Tukey-Kramer test, $p > 0.05$), indicating a

common environmental source for PCBs. This indicates that the majority of the Galapagos sea lions sampled were subject to the same degree of PCB exposure.

Most of the Galapagos sea lion samples did not contain PBDE concentrations that exceeded the MDL. Only one animal (PSP-03) out of 21 Galapagos sea lion pups exhibited detectable concentrations for four congeners, including BDEs 47, 49, 66, and 183 (Table 3.1 and Appendix B, Figure B-1). To evaluate further whether background contamination interfered with the reporting of concentrations, correlations between the concentrations of PBDEs and PCBs were explored (Appendix B, Figure B-2). A strong correlation was observed between concentrations (pg/sample) of PBDEs and PCBs ($r = 0.625$; $p = 0.0024$) (Appendix B, Figure B-2). Such a correlation can occur naturally in animals exposed through similar routes (e.g., diet in female animals). As seen in the Figure B-2 of the Appendix B, the correlation between PBDE and PCB concentrations in procedural blanks has a much steeper slope than the correlation for biopsy samples, indicating a specific source of PBDE contamination in at least one of the blanks. The PBDE and PCB concentration correlations in the biopsy samples did not exhibit this steeper slope. This indicates that the samples with higher PBDE concentrations (e.g., PIP-01, −02, and −08) may not have been affected by this specific source of contamination. Hence, the PBDE concentrations in these samples may actually reflect detectable concentrations even though the concentrations are considered to be nondetectable based on the QA/QC rules regarding the MDL. On the other hand, sample PSP-03 contained an apparent high level of PBDE contamination that does not fit the general relationship between PCB and PBDE concentrations in biopsy samples. This concentration appears to be above the MDL following QA/QC rules but should be treated with caution, because the sample may have been inadvertently contaminated with PBDEs. Because only three procedural blanks were used, and because the procedural blanks suggest the

possibility of significant PBDE contamination of these small samples, the PBDE concentration data should only be viewed in a qualitative way—that is, that the ∑PBDE concentration is low, with concentrations both within the range and below those measured in our procedural blanks.

Of 93 individual PCDD and PCDF congeners measured, none met the criteria for detectability (i.e., all were less than the MDL) in any of the samples examined. The highest MDL was 146 pg/g wet weight, for octachlorodibenzo-*p*-dioxin, whereas the lowest MDL was 51.4 pg/g wet weight, for 1,2,3,4,7,8-hexachlorodibenzofuran. The congener 1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin exhibited a MDL of 67.0 pg/g wet weight, falling within these two values. Because no detectable concentrations were observed for any of the 93 target PCDD/PCDF congeners in any of the samples, it can be concluded that the exposure of Galapagos sea lions to PCDDs/PCDFs is very low.

3.3.3 Composition of PCBs

The ∑PCB concentration was characterized by a dominant contribution of lower-chlorinated PCB congeners (i.e., di-, tri-, tetra-, and pentachloro-PCBs), which made up 56% of ∑PCB (Figure 3.2). In most pinniped species from the northern hemisphere, hexa- and heptachloro-PCBs make up the majority of ∑PCB concentration (Figure 3.3), revealing a different ∑PCB composition compared to our Galapagos sea lion pups and to southern elephant seals (*Mirounga leonina*) from Antarctica (Miranda-Filho *et al.* 2007). In the Galapagos sea lion pups, PCBs 5/8 (2.12%), 16/32 (1.24%), 85 (21.3%), 95 (1.55%), 99 (6.93%), 101 (5.49%), and 118 (2.87%) make up approximately 42% of ∑PCB concentrations, whereas PCBs 153 (7.00%), 138/163/164 (3.1%), and 180 (19.4%) contribute 30% of ∑PCB concentrations (Figure 3.2). The finding of a light PCB

signature suggests comparatively greater inputs from lower-molecular-weight and more volatile PCBs congeners that are more easily transported globally by atmospheric processes.

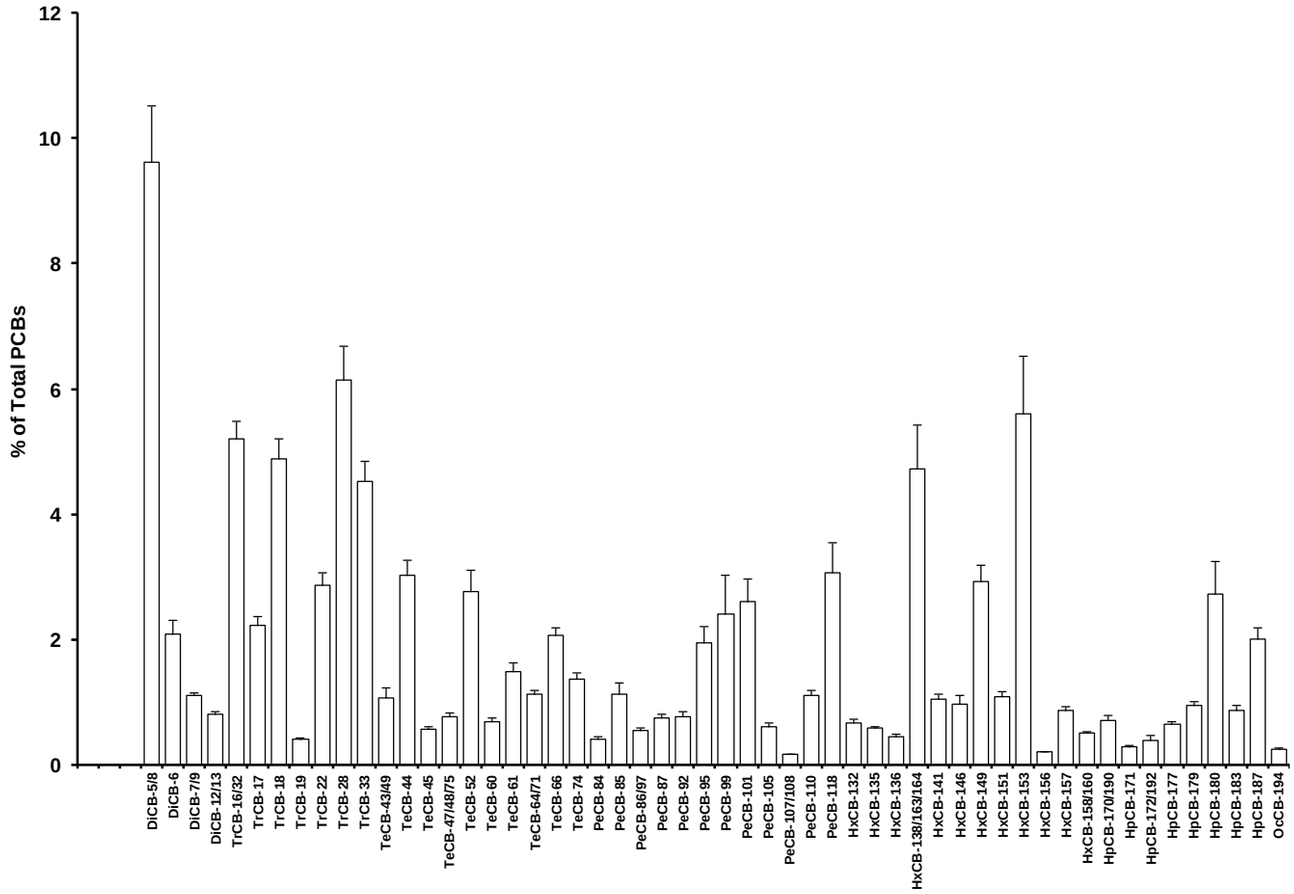

Figure 3.2 Polychlorinated biphenyl (PCB) congener composition in pups of the Galapagos sea lion (*Zalophus wollebaeki*). Error bars indicate the standard error.

Similarly, the ∑PCB concentrations in southern elephant seals from Antarctica also contains a relatively high proportion of low-molecular-weight PCBs (Miranda-Filho *et al.* 2007), with PCBs 18, 28, 31, 44, 49, and 74 contributing 22% of ∑PCB concentrations. In contrast, ∑PCB concentrations in northern elephant seal pups from California (USA) (Debier *et al*. 2005) and in harbour seal pups inhabiting industrialized regions from the Northeastern Pacific (Ross *et al*. 2004) contain a high proportion of heavier PCB congeners, resembling the composition of Aroclor 1260 (Schulz *et al*. 1989) (Figure 3.3).

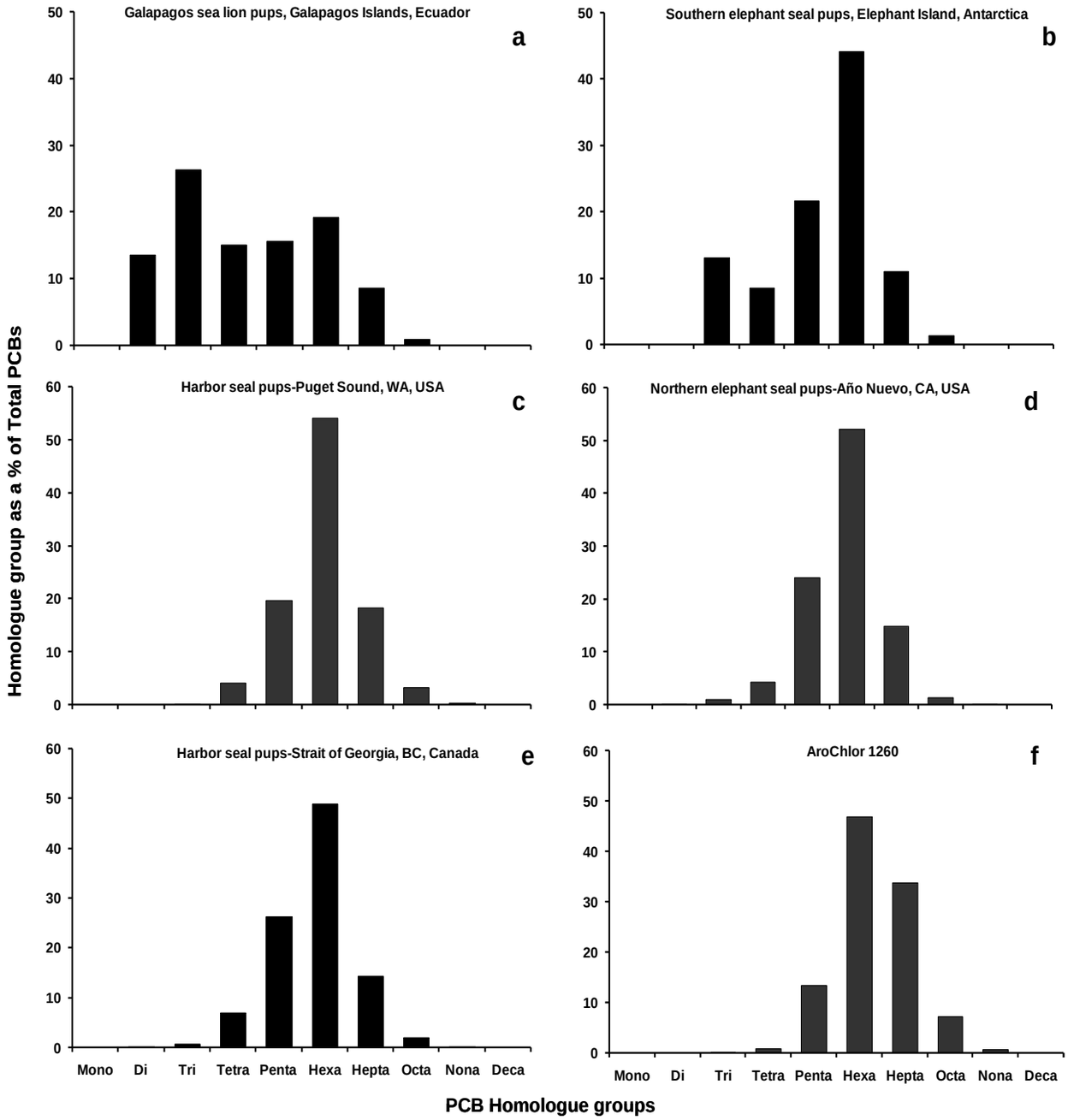

Figure 3.3 Polychlorinated biphenyl (PCB) homologue composition in pups of various pinnipeds species from different locations in relation to that of Aroclor 1260: (**a**) The PCB pattern in Galapagos sea lions (*Zalophus wollebaeki*), (**b**) PCB congeners composition for pups of southern elephant seals (*Mirounga leonina*) from Antarctic (Miranda-Filho *et al*. 2007), (**c**) harbour seal (*Phoca vitulina*) pups from Washington State (USA) (Ross *et al*. 2004), (**d**) northern elephant seal pups (*Mirounga angustirostris*) from California (USA) (Debier *et al*. 2005), (**e**) harbour seal pups from British Columbia (Canada) (Ross *et al*. 2004), and (**f**) Aroclor 1260 (Schulz *et al*. 1989).

Global fractionation of PCB congeners may be playing an important role in the PCB profile differences found among these pinniped species (Wania and Mackay 1993; Iwata *et al*. 1993; Wania and Su 2004; Jurado *et al*. 2005). Low-molecular-weight PCB congeners tend to partition into the atmosphere to a greater extent than high-molecular-weight PCB congeners. These lower-molecular-weight PCBs therefore may be able to travel from their sources to remote locations faster than higher-molecular-weight congeners. The high partitioning tendency in air and the high transport rate in the atmosphere may cause the occurrence of PCB concentrations dominated by low-molecular-weight congeners in remote locations, such as the Galapagos Islands and Antarctica.

3.3.4 Life history and physiological factors as determinants of contaminant concentrations

The influence of age and body condition on contaminant concentrations were minimized by collecting biopsy samples from similarly aged animals (age, one year or younger) at a time when pups were still nursing. Differences were observed in morphometric parameters between male and female pups, but no statistically significant differences in ∑PCB concentrations (Welch's approximate *t* test, $p = 0.4927$) were found between sexes (Table 3.1). The lack of a difference in concentration between male and female pups may be caused by the similarity in prenatal and postnatal PCB exposure (i.e., milk) of male and female pups and by the lack of differences in the life histories of these young animals. Elimination of persistent organic pollutants via transplacental

and milk transfer to their young ultimately will cause differences in PCB concentrations between males and females in sexually mature adult animals (Addison and Smith 1974).

The ∑PCB concentrations in male pups did not show a statistically significant correlation with standard length, weight, or FCF (regression analysis of ∑PCB concentrations versus any of these morphometric parameters, $p > 0.05$) but was positively correlated with girth ($r^2 = 0.571$, $p = 0.030$). A negative relationship was found between the ∑PCB concentrations in biopsy samples of female pups and the standard length ($r^2 = 0.381$, $p = 0.025$), but a positive correlation was observed between ∑PCB concentrations in biopsy samples and FCF in female pups ($r^2 = 0.462$, $p = 0.011$) (Table 3.2). Other studies of marine mammals have found negative relationships or associations between contaminant concentration and length or age (Addison and Smith 1974; Tuerk *et al*. 2005). For instance, PCB concentrations in adult male Atlantic white-sided dolphins (*Lagenorhynchus acutus*) decreased with body length, possibly reflecting a growth dilution phenomenon (Tuerk *et al*. 2005). Under the assumption of growth dilution, a young marine mammal receives a large initial contaminant load through lactation. After the pup is weaned, it experiences a period of growth coupled with a switch in food source from milk to less contaminated prey items. This produces a decline in PCB concentration over time after weaning. Because the sampling design of the present study was aimed at minimizing the effect of life-history factors on contaminant concentration, differences in weight, length, and body condition factors among the sampled animals are small. As a result, we do not associate specific significance to the apparent decrease of contaminant levels with length in female Galapagos sea lion pups.

The metabolic capacity of marine mammals can influence PCB patterns in these animals. Even though coplanar PCBs largely are retained by marine mammals, pinniped species (i.e.,

phocids) are able to metabolize most of the PCB congeners with *meta*- and *para*-vicinal-H atoms and two *ortho*-chlorines because of the enzymatic activity and induction of the cytochrome P450 enzymes CYP1A and CYP2B (Tanabe *et al*. 1998; Boon *et al*. 1997). However, whereas planar (non-*ortho*-)PCBs were not detected in the samples, PCB congeners with *meta*- and *para*- as well as *ortho*- and *meta*-vicinal hydrogens were detected (Appendix B, Table B-1). These observations suggest a relatively poor metabolic capacity or lack of cytochrome P450 enzymatic induction, possibly resulting from the low level of PCB contamination in Galapagos sea lion pups.

Differences in foraging grounds and feeding behaviour among female sea lions can influence PCB concentrations and the composition of ∑PCB. Lactating female Galapagos sea lions spent a significant proportion of time in other islands (i.e., multiple haul-out sites) other than their breeding colonies (i.e., rookery) during foraging trips (Villegas-Amtmann *et al*. 2008). This interisland movement may contribute to the similarity in PCB concentrations in Galapagos sea lions.

3.3.5 Comparisons with other marine mammal species

The ∑PCB concentrations in Galapagos sea lions are among the lowest PCB concentrations reported in pinniped species (Debier *et al*. 2005; Ross *et al*. 2004) (Figure 3.4 and Appendix B, Table B-2). Only southern elephant seal pups from Antarctica (Miranda-Filho *et al.* 2007) had lower ∑PCB concentrations than Galapagos sea lion pups. Even if the recalcitrant PCB 153 is used as a measure of PCB contamination (to eliminate differences in concentrations resulting from differences in the number of congeners monitored and detected), the results are similar (Figure 3.4).

Figure 3.5 and Table B-3 (Appendix B) show that the ∑PBDE concentrations in Galapagos sea lion pups (25.0 µg/kg wet wt or 35.2 µg/kg lipid) also are among the lowest reported

concentrations in pinnipeds (Kalantzi *et al*. 2005; Stapleton *et al*. 2006; Hall and Thomas 2007; Noel *et al*. 2008). Northern fur seals (*Callorhinus ursinus*) from the Pacific coast of Japan (Kajiwara *et al.* 2004) and ringed seals from the Canadian Arctic (Ikonomou *et al*. 2002) also exhibited low ∑PBDE concentrations. The ∑PBDE concentrations detected in the Galapagos sea lion pups was lower than those measured in cetacean species, including harbour porpoises (*Phocoena phocoena*) from England and Wales (Law *et al*. 2002), killer whales (*Orcinus orca*) from the Northeastern Pacific (Rayne *et al*. 2004), beluga whales (*Delphinapterus leucas*) from the Arctic (Wolkers *et al*. 2004) and the St. Lawrence Estuary (Lebeuf *et al*. 2004), and Atlantic white-sided dolphins (Tuerk *et al*. 2005).

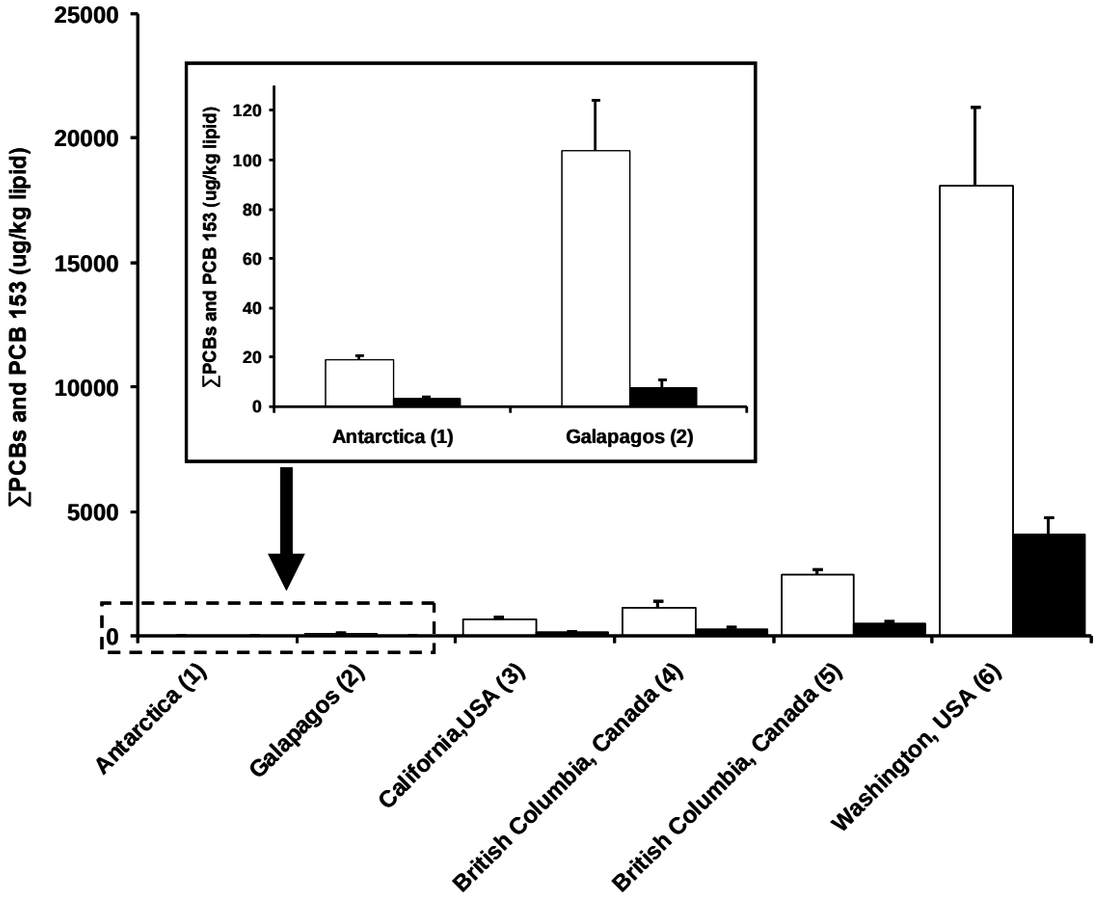

Figure 3.4 Global comparisons of mean total polychlorinated biphenyls (∑PCBs; □) and PCB 153 (■; used here as a reference congener due to its recalcitrance nature) concentrations (µg/kg lipid) in pups from pinnipeds species from different marine-coastal regions. Error bars are standard errors. All values are expressed on a lipid weight basis (µg/kg lipid). (1) Pups of southern elephant seals from Elephant Island Antarctica (25 PCB congeners detected) (Miranda-Filho *et al*. 2007); (2) Galapagos sea lion pups (72 PCB congeners detected) [this study]; (3) northern elephant seal pups from Año Nuevo, California (141 PCB congeners detected) (Debier *et al*. 2005); (4) Harbour seal pups from Queen Charlotte Strait, British Columbia (BC), Canada (Ross *et al*. 2004); (5) Harbour seal pups from the Strait of Georgia, BC, Canada (Ross *et al*. 2004); and (6) Harbour seal pups from Puget Sound, Washington State (WA), United States of America (USA) (Ross *et al*. 2004). For Harbour seals, 109 PCB peaks were detected.

It is difficult to compare PBDE and/or PCB concentrations directly across marine mammal species when gender, age, reproductive status, size and body condition, as well as differences in trophic position, feeding behaviour/ecology, and bioenergetics vary. The comparisons made in the

present study, however, place the degree of contamination of Galapagos sea lions in a global context.

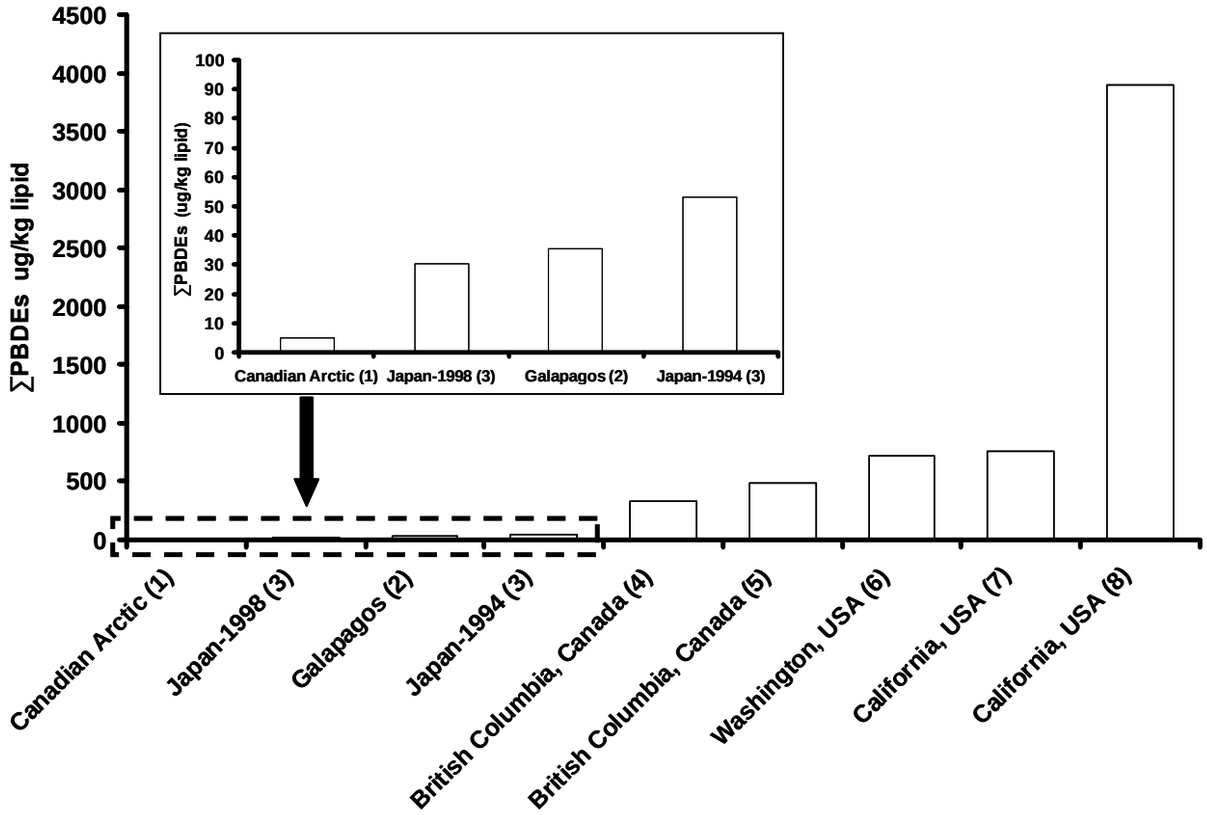

Figure 3.5 Global comparisons of total polybrominated diphenyl ethers concentrations (∑PBDE) measured in pinniped species from different marine regions (see also Appendix B, Table B-3). All values are expressed on a lipid-weight basis (µg/kg lipid). (1) Arithmetic mean concentration (4.62 µg/kg lipid) in ringed seals (*P. hispida*) from Holman Island, Northwestern Territories, Canada (Ikonomou *et al*. 2002); (2) Total concentration (35.2 µg/kg lipid) in Galapagos sea lions (*Z. wollebaeki*) from the Galapagos Islands, Ecuador [this study]; (3) Arithmetic mean concentrations (53 and 30 µg/kg lipid for 1994 and for 1998, respectively) in Northern fur seals (*C. ursinus*) from Sanriku, Pacific Coast of Japan (Kajiwara *et al*. 2004); (4) Steller sea lions (*Eumetopias jubatus*) from the Strait of Georgia (Norris Rocks, Vancouver Island), British Columbia, Canada (geometric mean = 336 µg/kg lipid) [Alava *et al*. unpublished data; see Chapter VI]; (5) Harbour seals (*P. vitulina*) from the Strait of Georgia (Hornby Island and Vancouver), British Columbia, Canada (geometric mean = 493 µg/kg lipid) (Noel *et al*. 2008); (6) Harbour seals from the Juan de Fuca Strait (Smith Island) and Puget Sound (Gertrude Island), Washington State, USA (geometric mean = 726 µg/kg lipid) (Noel *et al*. 2008); (7) Harbour seals from San Francisco Bay, California, USA (geometric mean = 765 µg/kg lipid) (She *et al*. 2002); (8) California sea lions (*Z. californianus*) from different locations of Coastal California, USA (geometric mean = 3900 µg/kg lipid) (Stapleton *et al*. 2006).

3.3.6 Health risks from exposure to contaminants

The ∑PCB concentrations in Galapagos sea lion pups was less than the LOAEL threshold effect concentration of 1,300 µg/kg lipid for risk of immunotoxicity and endocrine disruption in harbour seals (Mos *et al*. 2010). Because non-*ortho*-PCB congeners were not detected, they could not be included in the TEQ. Only mono-*ortho*-PCBs (i.e., PCBs 105, 118, 156, and 157) were detected, making up a total of 0.97 ng TEQ/kg lipid. The TEQ level in Galapagos sea lion pups was well below the LOAEL (286 ng TEQ/kg lipid) and NOAEL (90 ng TEQ/kg lipid) thresholds calculated from the lipid-normalized concentrations measured in harbour seals (Kannan *et al.* 2000). This suggests that these pups are not at risk of immunotoxicity and endocrine disruption as a result of PCBs. A lack of information regarding PBDE toxicity makes it difficult to assess the health risks associated with these flame retardants, but the very low concentrations observed in our Galapagos sea lions suggest limited risk. However, the endocrine-disrupting nature of these compounds has been demonstrated by in vitro studies and in vivo laboratory animal studies (Meerts *et al*. 2001; Darnerud *et al*. 2001; Hallgren and Darnerud 2002). Despite the fact the Galapagos sea lion pups are less contaminated than other pinniped species from the northeastern Pacific Ocean, they may still be at risk for low-level, chronic exposure to PCBs, mainly during the weaning or postweaning fasting, a sensitive period when contaminants in the blubber (e.g., PCBs) can be released into the circulation (Debier *et al*. 2005).

3.3.7 PCB and PBDE transport and fate in the Galapagos

The lack of statistically significant differences in ∑PCB concentrations in Galapagos sea lions among the four sampling locations indicates a common source for these pollutants (Figure 3.6). The remoteness of the Galapagos Islands and the long distances between sources and target organisms may be one of the key factors causing the low ∑PCB and ∑PBDE concentrations

observed. Local sources, which can be expected to produce differences in concentrations between human-inhabited islands (e.g., Santa Cruz, which is a center of ecotourism) and uninhabited islands (e.g., Pinta), do not appear to be significant contributors to current concentrations of PCBs detected in Galapagos sea lion pups. The long-range transport capability of PCBs and PBDEs to remote areas of the world has been well documented (Wania and Mackay 1993; Iwata *et al*. 1993; Ter Shure *et al*. 2004; Jurado *et al*. 2005; Shen *et al*. 2006).

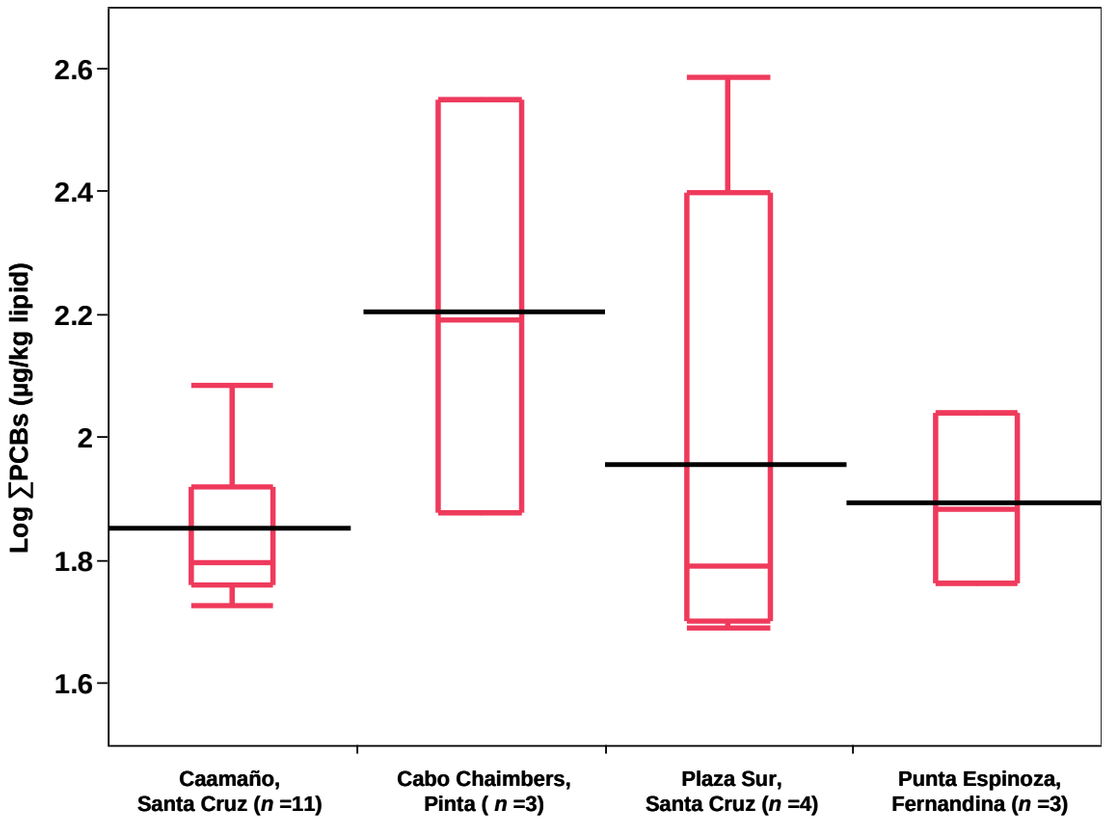

Figure 3.6 Intersite comparisons showing box plots of log total polychlorinated biphenyl concentrations (∑PCB) in sea lion (*Zalophus wollebaeki*) pups sampled from different rookeries of the Galapagos Islands (Ecuador). The internal lines across the boxes identify the median sample values, the ends of the boxes are the 25 and 75% quartiles, and the whisker bars are the minimum and maximum values. The external line crossing the middle on each box plot is the mean sample of log ∑PCBs of each rookery.

Long-range environmental transport comprising both atmospheric and oceanic processes likely explains the route of entry of PCBs and PBDEs into the Galapagos sea lion food web. However, it cannot be ruled out that local sources from urbanized, human-inhabited islands (e.g., Santa Cruz and San Cristobal) may have contributed to the measured concentrations. Past burning of waste products (i.e., computer devices and furniture) containing flame-retardant formulation mixtures in open dumps without treatment can be potential sources of PBDEs. However, local waste management practices of municipal organic waste have improved over the last few years, and burning in open dumps close to harbours and coastal zones has been banned.

Based on the present results, it can be concluded that concentrations of PCBs and PBDEs in Galapagos sea lion pups are still fairly low and below toxicologically relevant concentrations. The present results also suggest that currently, local sources of PCBs and PBDEs likely are small compared to remote sources and that PCB and PBDE concentrations largely may be reflecting global rather than local contamination. The rapid increase in human population and development of the Galapagos Islands (Watkins and Cruz 2007) presents an emerging risk for Galapagos sea lions, because land-based activities may increasingly release pollutants into coastal waters. However, global practices regarding the production, use, and disposal of these chemicals appear to be more important present determinants of PCB and PBDE concentrations in Galapagos sea lions compared with local practices. Both PCDDs and PCDFs were not detected in these samples, suggesting that a combination of low environmental concentration and/or metabolism prevent significant bioaccumulation in Galapagos sea lions.

Results of the present study suggest that whereas PCB, PBDE, and PCDD/PCDF concentrations are relatively low, the remote Galapagos Islands are not immune to the consequences of global environmental contamination. This means that in addition to remote polar

regions, remote equatorial areas, such as the Galapagos Islands, deserve attention and consideration when contemplating the widespread use of commercial chemicals. Sea lions in the Galapagos Islands can serve as a useful sentinel of global pollution.

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CHAPTER 4

A RECURRING LEGACY: DDT IN ENDANGERED GALAPAGOS SEA LIONS (*ZALOPHUS WOLLEBAEKI*)

Abstract: We characterize for the first time the presence of DDT and its metabolites in tropical Galapagos sea lions (*Zalophus wollebaeki*). ∑DDT concentrations in Galapagos sea lion pups sampled in 2005 and 2008 ranged from 16 to 3070 µg/kg lipid. Concentrations of ∑DDT in pups in 2008 averaged 525 µg/kg lipid and were 1.9 times higher than that (281µg/kg lipid) detected in pups in 2005, suggesting a possible temporal increase. These concentrations are lower than those reported in many pinnipeds elsewhere, comparable to those in Hawaiian monk seals, and higher than those in southern elephant seals. The health risk characterization showed that only 1% of the male pups exceeded the *p*,*p*'-DDE toxic effect concentration associated with anti-androgenic effects reported in rats. The findings provide preliminary guidance on the relationship between DDT use and ecological impacts, serving as a reference point against which possible future impact of tropical DDT use can be assessed.

***Keywords*: Galapagos sea lion; Galapagos Islands, Ecuador; DDT, *p*,*p*'-DDE; health risk.**

4.1 Introduction

Global contamination by dichlorodiphenyltrichloroethane (DDT) and other persistent organic pollutants (POPs) remains a serious health concern for protecting biodiversity on the planet. As a result, the Stockholm Convention on POPs was established as an international treaty on 17 May 2004 to eliminate the world's most persistent, bioaccumulative and toxic substances, including DDT (UNEP 2001).

Because of the long range transport characteristics of these substances (Wania and Mackay 1993; Iwata *et al*. 1993), the impact of DDT and other POPs on wildlife and human populations inhabiting remote Arctic regions has remained an active area of research (Muir *et al*. 2000; Kelly *et al*. 2007; Guglielmo *et al*. 2009). However, limited information is available on the status and impacts of DDT on remote tropical regions. This is unfortunate, since DDT is still used in tropical regions for malaria control (Roberts *et al*. 2000; Schenker *et al*. 2008; Van den Berg 2008). Recently, The World Health Organization recommended a renewed indoor use of DDT in human habitations of developing countries (WHO 2006). In addition, an increase in the use of DDT to combat malaria was endorsed by the 34th G8 summit in July 2008. Since its first use in the 1940s, DDT has caused serious impacts to many wildlife populations. For instance, DDT was associated with catastrophic impacts on birds and fish-eating wildlife populations (Hickey and Anderson 1968; Blus 2003). Therefore, the renewed use of DDT renews concerns about the impacts of DDT on human and ecosystem health, especially in tropical regions where DDT may be increasingly used (Blus 2003; Van den Berg 2008).

Several studies have reported high concentrations of DDTs in abiotic media (i.e., soil, sediment, river, water and air), and subsequent volatilization, with pronounced meridional transport (multi-grass hopping) northward, from tropical developing regions in southern Asia and Oceania, including oceanic surface water samples, and western boundaries of Africa and the Americas (Iwata *et al*. 1993; Iwata *et al*. 1994; Guglielmo *et al*. 2009). High concentrations of DDTs resulting from biomagnification of DDT have also been detected in fish of African tropical lakes (Kidd *et al*. 2001; Manirakiza *et al.* 2002), and Amazonian river dolphins (*Inia geoffrensis*) from the Brazilian Amazon (Torres *et al*. 2009). Accumulation of DDT in ospreys (*Pandion haliaetus*) suggests that breeding grounds in North America are still a substantial source for higher DDT exposure (Elliott *et al*. 2007). A recent study in migratory White faced Ibis (*Plegadis chihi*) found higher exposure of DDT on wintering grounds further down in tropical areas (Yates *et al*. 2010). Likewise, DDT levels in White faced Ibis from Mexico and Adélie penguins (*Pygoscelis adeliae*) from the western Antarctic Peninsula have not decreased between 1985 and 2006 (Geisz *et al*. 2008; Yates *et al*. 2010).

Since the early 1970s, reproductive impairment and a high rate of abortions and stillbirths in California sea lions (*Zalophus californianus*) were associated with DDT (Le Bœuf and Bonell 1971; Delong *et al*. 1973). More recently, high levels of DDTs were linked to a high prevalence of neoplasms and carcinoma, and associated mortality, in California sea lions (Ylitalo *et al*. 2005). In addition, POPs have been linked to effects on the immune system (e.g. impairment of T-lymphocyte function, phagocytosis, and respiratory burst) and the endocrine system (e.g., disruption of Vitamin A and thyroid hormones) of several pinnipeds, including harbor seals (*Phoca vitulina*) and California sea lions, as well as small cetaceans (Ross *et al*. 1995; Lahvis *et al*. 1995;

Debier *et al*. 2005; Tabuchi *et al*. 2006). Reduced immune function increases susceptibility to infectious diseases and poses population level risks (Ross 2002).

Since the visit by Charles Darwin aboard the *HMS Beagle* in 1835, the Galapagos Islands have become a living, natural laboratory for evolutionary biologists. Since the Galapagos was designated as a UNESCO–World Heritage site in 1979, it has faced a gauntlet of anthropogenic stresses, and its UNESCO designation was revised in 2007 to an “at risk” category. A burgeoning human population, increased ecotourism, and invasive species, underlie the revised designation (Watkins and Cruz 2007).

Of the two endemic pinnipeds inhabiting the Galapagos Archipelago, the Galapagos sea lion (*Zalophus wollebaeki*) population has decreased by 50-60% since the late 1970s (Alava and Salazar 2006), and is listed as “endangered” by the International Union for the Conservation of Nature (IUCN) (Aurioles and Trillmich 2008). Notable stressors have included the El Niño events of 1982─1983 and 1997─1998, fisheries interactions, illegal hunting, oil spills, enzootic diseases, as well as infectious diseases transmitted by rats and dogs (e. g., *Leptospira* and Morbilliviruses, including Canine Distemper Virus) (Alava and Salazar 2006; Aurioles and Trillmich 2008).

The possible role of DDT and related contaminants in the Galapagos sea lion decline is unclear. There is no historical report on the use DDT in the Galapagos Islands. However, relatively low concentrations of polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polybrominated diphenyl ethers (PBDEs) have been reported in Galapagos sea lion pups (*Zalophus wollebaeki*) (Alava *et al*. 2009). In adjacent areas (≈3350 km to the north), high concentrations of DDTs are still detected in California sea lions, harbor seals and elephant seals (*Mirounga angustirostris*) from California,

USA (Blasius and Goodmanlowe 2008). The extent that the Galapagos are affected by local and atmospherically-transported DDT from such ‘hotspots’ is unknown.

The objective of this study was to investigate the concentrations, patterns, temporal trends and possible health risks of DDT in Galapagos sea lions, with a goal of providing input to the changing international regulations.

4.2 Materials and Methods

4.2.1 Collection of Samples

Muscle-blubber biopsy samples were collected from 41 free−ranging, live captured Galapagos sea lion pups (*Z. wollebaeki*) of 2 to 12 months of age from eight rookeries of the Galapagos Islands Archipelago during two expeditions carried out on March 13−21 in 2005 and March 26-29 in 2008. Pups were sampled at Santa Cruz (Caamaño, *n* = 11; and Plaza Sur, *n* = 4), Fernandina (Punta Espinoza, *n* = 3) and Pinta (Puerto Posada, *n* =3) islands in 2005; and, from Isabela (Loberia Chica, *n* = 5), Floreana, (Loberia, *n* =6) and Santa Cristobal (Puerto Baquerizo, *n* = 4; Isla Lobos, *n* = 5) islands in 2008 (Figure 4.1).

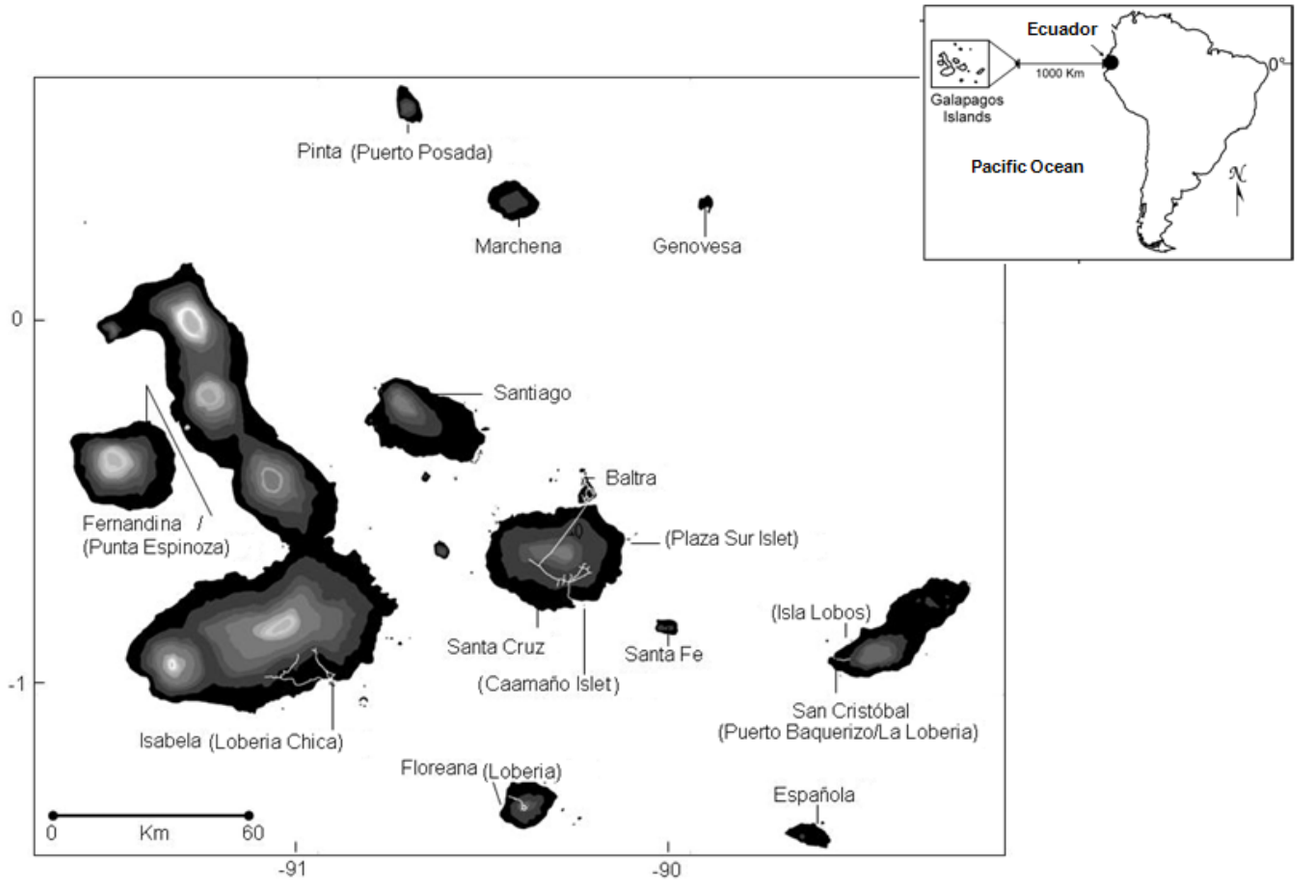

Figure 4.1 Map of Galapagos Archipelago at 1000 km off the Ecuadorian continental coast (01°40´N-01°25´S and 89°15´W- 92°00´W), showing the islands' names and sites harbouring the rookeries (in brackets) of Galapagos sea lions pups (*Zalophus wollebaeki*) sampled during the expeditions carried out in 2005 and 2008.

Reproduction in Galapagos sea lions follows a yearly reproductive cycle, principally during the cold season, with peak pupping taking place between August and November (Villegas-Amtmann *et al*. 2009). The young are weaned after approximately 12–24 months (e.g., Trillmich 1986; Trillmich and Wolf 2008). Nursing pups were chosen because a) the animals are readily accessible and relatively easy to capture in most of the rookeries of the Galapagos Islands year round; b) the animals are approximately of similar age, minimizing the influence of life history parameters on contaminant concentrations; c) as they are nursed by adult reproductive females

they have a high trophic position as they are feeding on mother's milk, analogous to a predator-prey relationship.

Pups sampled in 2005 were captured with hoop nets and immobilized following the field isoflurane gas (0.5 to 2.5%) anesthesia methodology developed by Parás *et al*. (2002) (see Appendix B), while those sampled in 2008 were captured with hoop nets and manually restrained without involving anesthesia. In all circumstances, capture stress and holding time were minimized (< 10-15 min). Biopsies (100 mg; 6mm−Miltex biopsy punch) were collected from the supraspinatus muscle, located just above of the pectoral flipper, or were collected from an area 10-20 cm lateral to the spinal column and anterior to the pelvis (Villegas-Amtmann and Costa 2010). The biopsy site was pre-cleaned with alcohol and betadine. Biopsies were wrapped in hexane-rinsed aluminium foil an placed in a cooler with wet ice and transferred into cryovals placed in a cryoship (-20°C) during the field sampling, and, afterwards stored at -80°C in the laboratory until chemical analysis. Standard length, weight, girth, and sex for each pup were recorded. The body condition of the pups was measured using the Fulton's condition factor (FCF = weight x 10^5/standard length^3) to compare body weight of sea lion pups of different standard length within a given reproductive season and eliminate the effect of size on weight (Luque and Aurioles 2001; Castro-Gonzalez *et al*. 2001). Age was estimated by visual observation of both the size and weight of the animal. Details of morphometric and field data of the pups are in Appendix C (see Table C-1).

4.2.2 Contaminant Analysis

Muscle-blubber biopsy samples (0.004-0.212 g) were analyzed for DDTs by gas chromatography and high resolution mass spectrometry (GC/HRMS) in the Regional Dioxin Laboratory (RDL) at

the Institute of Ocean Sciences (IOS), Fisheries and Ocean Canada (DFO), as discussed elsewhere (Ikonomou *et al*. 2001). The DDT analytes quantified included *o, p'*-DDE, *p, p'*-DDE, *o, p'*-DDD, *p, p'*-DDD, *o, p'*-DDT, and *p, p'*-DDT. The intact biopsy samples were spiked with a mixture of surrogate internal standards which contained $^{13}C_{12}$ *p*,*p*-DDE, $^{13}C_{12}$ o,*p*-DDD, $^{13}C_{12}$ *p*,*p*-DDD, $^{13}C_{12}$ *o*,*p*-DDT, and $^{13}C_{12}$ *p*,*p*-DDT. All surrogate internal standards were purchased from Cambridge Isotope Laboratories (Andover, MA). The spiked samples were homogenized with Na_2SO_4 in a mortar, transferred quantitatively into an extraction column, and extracted with DCM/hexane (1:1 v/v). The solvent layer was transferred to a clean flask and the waxy precipitate was treated with several aliquots of hexane and DCM, and transferred to the flask that contained the solvent layer of the extract. Despite the treatment with additional volumes of hexane and DCM, vortexing and pulverization, the waxy precipitate did not dissolved in the solvents used and as a result it was not included in the corresponding sample extract that was used for lipid and contaminants determinations.

The DCM:Hexane sample extracts were evaporated to dryness and the residue was weighted in order to determine the lipid content of the samples. Subsequently the residue was re-suspended in 1:1 DCM/Hexane and divided quantitatively into two aliquots. The lager aliquot (75% of the extract) was subjected to sample-cleanup for PCBs, PCDDs, PCDFs, and PBDEs determinations and the results have been reported elsewhere (Alava *et al*. 2009). The remaining (25% of the extract) was used for DDT determinations. The lower volume fraction of the sample extract was loaded onto a Florisil column (8 grams of 1.2% water deactivated Florisil slurry packed with hexane into a fritted column) and eluted with 60 mL 1:1 DCM:hexane. Cleaned extracts were concentrated to less than 10 µL and spiked with the ^{13}C-labeled method performance standard ($^{13}C_{12}$- PeCB-111) prior to instrumental analysis. Details on the conditions used for sample clean-

up and the quality assurance quality control protocols followed are reported in detail elsewhere (Ikonomou *et al*. 2001).

The corresponding extracts were analyzed for target organochlorine pesticides by GC/HRMS. The high resolution mass spectrometer was a Micromass Ultima (Micromass, UK) instrument equipped with an HP-6890 gas chromatograph and a CTC autosampler. For the OCPs analyses a DB-5 column was used (45m x 0.25mm, 0.1μm film, J&W Scientific, Folsom CA), initial temperature 80^{o}C for 3 min, increased at 15^{o}C/min to 160 ^{o}C, then at 5 ^{o}C/min to 270 ^{o}C and held for 1 min, and lastly at 15 ^{o}C/min to 300 ^{o}C. The injector temperature was held at 200 ^{o}C. Splitless injection of 1 µL sample and 1 µL air were performed and the purge was activated 2 min after injection. For all analyses the HRMS was operated at 10000 resolutions under positive EI conditions and data were acquired in the Single Ion Resolving Mode (SIR). The source temperature was maintained at 280 ^{o}C and the GC/HRMS interface at 260 ^{o}C.

4.2.3 Quality Assurance/Quality Control Measures

Samples were processed in batches of 12 samples each containing one or two procedural blanks, an in-house performance evaluation sample containing known concentrations of the analytes of interest, and a certified reference material (CRM), i.e., NIST Standard Reference Material (SRM) 1945 (whale blubber homogenate), and nine or ten real samples. Method blanks, consisting of Na_2SO_4, were processed according to the same procedure as the samples and analyzed with every batch of twelve samples to check for potential background contamination. Analytes were identified only when the GC/HRMS data satisfied the following criteria: (i) two isotopes of the analyte were detected by their exact masses with the HRMS operating at 10,000 resolution during the entire chromatographic run; (ii) the retention time of the analyte peak was within 3 seconds of

the predicted time obtained from analysis of authentic compounds in the calibration standards (where available); (iii) the maxima for both characteristic isotopic peaks of an analyte coincided within 2 seconds; (iv) the observed isotope ratio of the two ions monitored per analyte were within 15% of the theoretical isotopic ratio; and (v) the signal-to-noise ratio resulting from the peak response of the two corresponding ions was ≥3 for proper quantification of the analyte. Analyte concentrations were calculated by the internal standard isotope-dilution method using mean relative response factors (RRFs) determined from calibration standard runs made before and after each batch of samples was analyzed. Concentrations of analytes were corrected for the recoveries of the surrogate internal standards. The validity of data correction was confirmed from the tight accuracy and precision data obtained from the analyses of CRM and in-house reference samples. The recoveries of all pesticide surrogate internal standards were between 65 and 110% and the accuracy of determining the target DDT analytes in spiked samples was between 15 and 20%.

4.2.4 Data and Statistical Analyses

Concentrations of pesticides measured were blank-corrected using the method detection limit (i.e., MDL on a pg/sample basis), defined here as the mean response of the levels measured in three procedural blanks used plus three times the standard deviation (SD) of the blanks (MDL = $Mean_{blanks} + 3 \times SD_{blanks}$) (Alava *et al*. 2009). Concentrations below the MDL were substituted using half of the MDL. Concentrations were lipid normalized to account for differences in the lipid content of the samples (µg/kg lipid) and were log-transformed before conducting statistical analyses. ∑DDT concentrations were calculated as the sum of *o, p'*-DDE, *p, p'*-DDE, *o, p'*-DDD, *p, p'*-DDD, *o, p'*-DDT, and *p, p'*-DDT. To examine whether morphometric factors or sex affected

contaminant concentrations, life history parameters (i.e., length, weight, corporal condition or FCF) and lipid content of both sexes were compared through the Welch ANOVA assuming unequal variances (Zar 1999). Linear regression (Pearson correlation) was used to determine whether life history parameters are correlated with contaminant concentrations.

To determine differences in contaminant concentrations between females and males, a Welch two-tailed t-test for unequal variances was used. Differences in contaminant concentrations and percent lipid among sea lion rookeries were evaluated using analysis of variance (ANOVA), where variances among sites were equal (i.e., homoscedastic as tested by the Levene's test and Bartlett test, $p > 0.05$), or Welch ANOVA, where variances were unequal (i.e., heteroscedastic; Levene's test or Bartlett test, $p < 0.05$). This was followed by a Tukey-Kramer honestly significant difference (HSD) multiple comparison test, which is a post-hoc method recommended to test differences between pairs of means among groups that contain unequal sample sizes (Zar 1999). Concentrations were expressed in term of the geometric mean with an upper and lower standard deviation (± SD) unless otherwise specified (i.e., arithmetic mean ± SD). Statistical analyses were conducted using JMP 7.0 (SAS Institute Inc.; Cary, NC, USA, 2007) at a level of significance of $p < 0.05$ ($\alpha = 0.05$).

4.2.5 Health risk assessment

In absence of toxicological and health studies of DDT on Galapagos sea lions, we attempted to interpret observed DDT concentrations in terms of potential DDT related health effects by comparing *p,p'*-DDE concentration distributions to *p,p'*-DDE circulatory levels related to immunotoxicity in bottlenose dolphins (*Tursiops truncatus*) (Lahvis *et al*. 1995) and anti-androgenic effect in mammalian (i.e., rat) cell cultures (Kelce *et al*. 1995). To make comparable

these reference values, we normalized them to lipid and protein content of blood reported for bottlenose dolphins (e.g., Bossart *et al*. 2001; Woshner *et al*. 2006; Houde *et al*. 2006; Yordi *et al*. 2010) and rats (Poulin and Krishnan 1996; DeBruyn and Gobas 2007) to express the concentrations in equal units and in similar media, using the following equation:

$$TEC_{\text{BLOOD-LIPID NORMALIZED}} = TEC_{\text{BLOOD-WET WEIGHT}} / (f_{\text{L,BLOOD}}) + (f_{\text{P,BLOOD}})\,0.05$$

where $TEC_{\text{BLOOD-LIPID NORMALIZED}}$, and $TEC_{\text{BLOOD-WET WEIGHT}}$ are the circulatory toxic effect concentrations of *p*, *p*'-DDE in a lipid and wet weight basis, respectively; $f_{L,BLOOD}$ is the fraction of lipid in blood, and $f_{P,BLOOD}$ is the fraction of protein in the blood. The coefficient 0.05 is the sorptive capacity of proteins in relation to that of lipids (DeBruyn and Gobas 2007). Lipid, protein fractions and lipid normalized effect concentrations for bottlenose dolphin and rats are available in Table C-2 (Appendix C). In an effort to conduct the health risk characterization, the relative frequency of the population sampled (i.e., pups), here expressed as the normal probability density distribution function of the log *p*,*p*'-DDE concentrations measured in a lipid weight basis in pups, were plotted (Gaussian distribution) against the lipid normalized log values of *p*, *p*'-DDE toxic effect concentrations above documented to assess what proportion of the pups (i.e., frequency) exceed target threshold *p*, *p*'-DDE concentrations

4.3 Results and Discussion

4.3.1 Morphometrics and lipid content.

The mean ± SD of the standard length, body weight, corporal condition and lipid content of the 41 pups is showed in Table 4.1. When compared to males sampled in 2005 and pups (males and females) sampled in 2008, female pups sampled in 2005 were significantly longer (Barlett test, p = 0.0007; Welch ANOVA, $p < 0.0001$; Tukey-Kramer test, $p < 0.05$) and heavier (Barlett test, p = 0.0009; Welch ANOVA, $p < 0.0001$; Tukey-Kramer test, $p < 0.05$). Because of differences in body size (i.e., length and weight), the corporal condition of 2005-females was significantly different from the body condition of 2005-males (Welch t-test =3.343, p = 0.0036, df =18); 2008-males (t-test =2.580, p = 0.0179, df =20); and, 2008-females (t-test =2.942, p = 0.0081, df =20). This likely reflects the more rapid growth and the higher body density of male otariid pups, because they allocate a larger fraction of milk energy to muscular and skeletal growth than females (Luque and Aurioles 2001).

Pups appeared nutritionally healthy (i.e., lipid measurements >50%). No significant differences were observed in lipid content among any group of pups (Welch ANOVA, p = 0.7358; Tukey-Kramer test, $p > 0.05$). The mean ± SD lipid content of the pup samples ranged from 70.2% ± 9.34% to 77.8% ± 2.45% (Table 4.1).

Table 4.1 Sample size, lipid content, length, weight, corporal condition and Pearson correlation coefficients (*r*) with *p* values resulting from the linear regression analyses of the log transformed lipid concentrations of ∑DDTs versus morphometric parameters by sex categories in Galapagos sea lion pups, *Zalophus wollebaeki*.

Sex	year	Sample size (*n*)	Lipid (%)	Weight (kg)	Standard length (cm)	Body Condition (FCF)[a]	Standard length vs ∑DDTs	Weight vs ∑DDTs	FCF vs ∑DDTs
Males	2005	8	70.2 ± 9.34	20.6 ± 0.95	102 ± 1.85	1.94 ± 0.03	*r*=-0.373 *p*=0.3622	*r*=-0.409 *p*=0.3144	*r*=0.124 *p*=0.7696
Females	2005	13	73.3 ± 3.92	66.9 ± 7.01*****	155 ± 7.67*****	1.71 ± 0.06*****	*r*=-0.894 *p*<0.0001*****	*r*=-0.777 *p* =0.0018*	*r*=0.686 *p*=0.0096*
Males	2008	10	77.8 ± 2.45	22.3 ± 2.34	105 ± 3.03	1.94 ± 0.06	*r*=0.2041 *p*= 0.5716	*r*=0.1910 *p* =0.6225	*r*=-0.280 *p* = 0.4667
Females	2008	10	75.9 ± 3.50	21.1 ± 2.21	102 ± 3.28	2.01 ± 0.08	*r*=0.1698 *p*= 0.6390	*r*=-0.1769 *p*= 0.6488	*r*=-0.413 *p* = 0.2693

[a]FCF is the Fulton's Condition Factor (FCF = weight x 10^5/standard length^3).
*Asterisk indicates a statistically significant comparison or correlation.

4.3.2 Biological factors as determinants of ∑DDT concentrations in pups.

To reduce the possible influence of age and body condition on DDT concentrations, only biopsy samples from nursing animals of similar age (i.e., < 2 year) were collected. DDT concentrations in Galapagos sea lion pup females captured in 2005 were significantly lower than the DDT concentration found in the 2005 males (*t*-test =2.320, $p = 0.0316$, df =19) and in pups, both males (*t*-test = 2.873, $p = 0.0091$, *df* =21) and females (*t*-test = 4.126, $p = 0.0005$, *df* =21), sampled in 2008 (Figure 4.2, Table 4.2). Since the study animals were immature, differences in concentrations between male and female pups due to reproductive losses (i.e., milk secretion and parturition) (e.g., Addison and Smith 1974; Addison and Brodie 1987) can be ruled out as a cause.

Regression analyses showed that there were no significant correlations between measured life history parameters and ∑DDT concentrations in male pups captured in 2005 and pups sampled in 2008 (regression analysis for all pup groups, Table 4.1; $p > 0.05$). In contrast, concentrations of ∑DDTs in female pups sampled in 2005 were negatively correlated with increasing length and weight ($p < 0.005$; Table 4.1; Figure C-1 in Appendix C). The low concentrations of DDT in the 2005 females can be explained due to the growth dilution effect since negative, significant correlation were observed between DDT concentration and body size in this particular group of pups (Table 4.1). Under the assumption of growth dilution, an apparent dilution on contaminant concentrations occurs in the body mass as a result of isometrical or linear growth and possible shift to diet items containing lower levels of contaminants (Alava *et al*. 2009; Gobas and Arnot 2010).

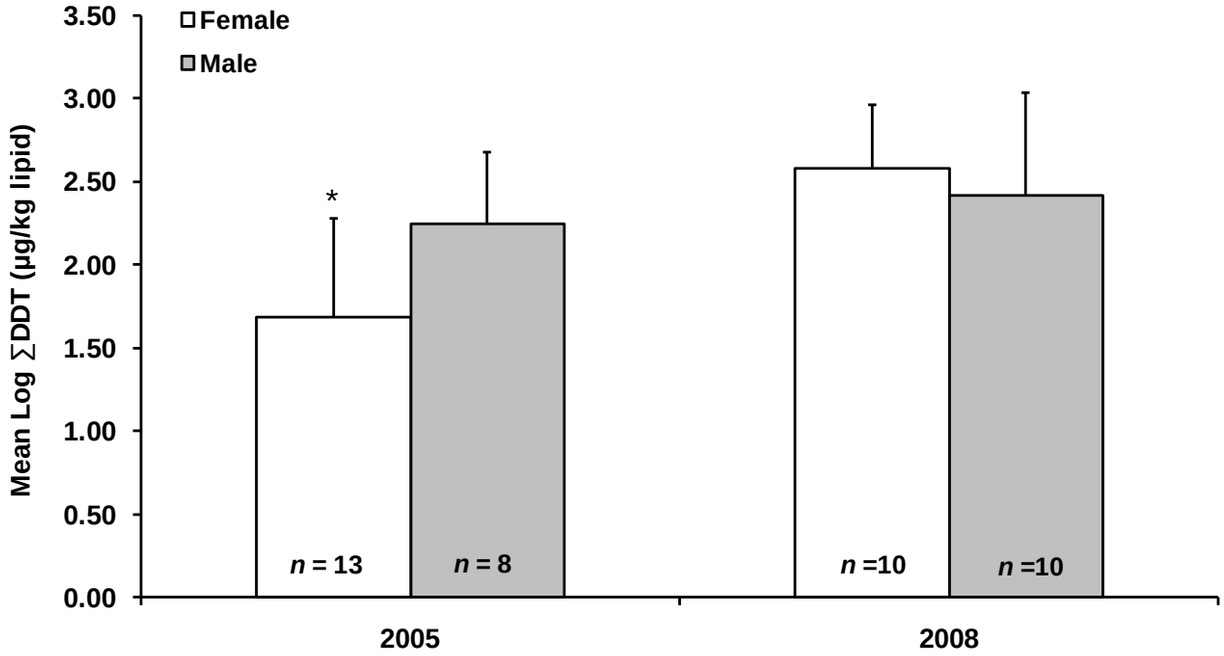

Figure 4.2 Temporal comparisons of mean ∑DDT concentration by sex categories. The asterisk indicates that the concentration was significantly different from the other concentrations. Error bars are standard errors.

Table 4.2 Overall and arithmetic mean ± standard error (SE) concentrations of ∑DDTs (µg/kg lipid) and metabolites (µg/kg lipid) in muscle-blubber samples of Galapagos sea lion pups collected in 2005 and 2008.

Sample	sex	*o,p*-DDE	*p,p*-DDE[a]	*o,p*-DDD	*p,p*-DDD	*o,p*-DDT	*p,p*-DDT	∑DDTs[b]
2005								
PIP-02	M	0.06	1140	2.10	33.5	1.60	25.60	1200
PIP-08	F	3.10	2900	8.30	106	9.15	39.15	3070
PIP-10	M	2.70	134	1.70	11.0	1.15	10.30	161
PEP-01	F	2.13	115	0.450	6.50	0.100	2.50	130
PEP-03	M	2.70	390	0.850	14.2	1.10	3.05	412
PEP-07	M	2.70	67.5	0.500	7.20	0.700	7.00	85.5
PSP-01	M	1.80	107	1.300	11.2	0.800	7.35	130
PSP-02	M	1.10	90.0	0.300	6.35	0.200	8.70	107
PSP-03	M	2.50	181	0.900	10.1	1.10	5.20	200
PSJ-06	M	13.2	20.1	2.300	2.20	5.60	7.60	50.0
CAAF-01	F	3.20	23.0	0.600	1.05	1.40	1.80	30.8
CAAF-02	F	2.72	28.6	0.500	3.20	1.20	1.60	38.0
CAAF-03	F	2.20	32.0	0.100	2.70	0.900	1.30	40.0
CAAF-04	F	2.00	11.2	0.300	0.700	0.800	1.10	16.0
CAAF-05	F	2.72	65.3	0.500	0.600	1.20	1.60	72.0
CAAF-06	F	2.21	32.0	0.400	1.90	0.950	1.50	38.5
CAAF-07	F	2.22	26.5	0.400	2.50	0.950	1.30	33.8
CAAF-08	F	2.14	14.3	0.400	0.450	0.900	1.20	19.4
CAAF-09	F	2.20	21.0	0.400	1.10	0.900	1.80	27.0
CAAF-10	F	4.00	7.00	0.700	0.800	1.70	2.30	16.3
CAAF-11	F	2.20	0.150	0.400	0.450	0.900	32.6	37.0
females		2.50 ± 0.16	252 ± 221*	1.02 ± 0.60	9.80 ±8.00*	1.60 ± 0.60	6.90 ± 3.60*	274 ±233*
males		3.40 ± 1.40	266 ± 130	1.20 ± 0.30	12.1 ± 3.30	1.50 ± 0.60	9.30 ± 2.45	293 ± 135
2008								
IZS-01	F	1.11	1058	1.18	44.1	1.06	16.8	1122
IZS-02	M	0.00	193	0.34	9.31	0.24	8.80	212
IZP-04	F	0.20	65.4	0.16	2.97	0.76	1.69	71.2
IZP-05	M	0.13	13.6	0.17	0.96	0.37	0.97	16.3
IZP-06	F	0.00	143	0.11	1.88	0.40	2.29	148
FPZ-01	F	0.00	293	0.16	9.9	0.27	17.4	320
FPZ-02	F	0.16	231	0.22	4.05	0.16	5.04	241
FSZ-03	M	0.00	1647	0.00	9.44	0.00	9.44	1666
FPZ-04	M	0.28	81.9	0.35	7.25	0.33	5.91	96.0
FPZ-05	M	0.69	147	1.98	20.5	1.56	9.66	181
FPZ-06	M	1.06	132	1.97	16.7	1.79	9.10	163
SCPZ-01	F	0.62	1183	0.00	26.2	0.11	21.6	1231
SCPZ-02	F	2.08	637	3.03	38.0	1.92	16.6	699
SCSP-03	F	2.02	273	2.19	25.1	3.46	13.6	320

Sample		*o,p*-DDE	*p,p*-DDE[a]	*o,p*-DDD	*p,p*-DDD	*o,p*-DDT	*p,p*-DDT	∑DDTs[b]
SCPZ-04	M	0.52	947	0.74	16.3	0.56	12.4	977
ILPZ-01	M	1.74	1172	2.32	53.6	2.13	11.6	1243
ILPZ-02	F	0.63	542	1.61	17.7	0.85	7.03	570
ILSP-03	M	1.53	89.6	1.89	11.9	0.99	6.45	112
ILPZ-04	F	0.00	377	1.97	27.8	2.31	29.4	438
ILPZ-05	M	1.78	625	2.56	22.2	1.18	11.5	664
females		0.68 ± 0.25	480 ±120	1.06 ± 0.34	20.0 ± 4.70	1.10 ± 0.35	13.0 ± 2.85	516 ± 125
males		0.77 ± 0.22	505 ± 180	1.20 ±0.315	17.0 ± 4.60	0.92 ± 0.20	8.60 ±1.10	533 ± 183

[a]The mean log ± standard deviation of ∑*p,p*'-DDE concentrations for males and females were 2.14 ± 0.52 µg/kg lipid and 1.39 ± 0.93 µg/kg lipid in 2005, and 2.36 ± 0.65 µg/kg lipid and 2.55 ± 0.39 µg/kg lipid in 2008, respectively
[b]The mean log ± standard deviation of ∑DDT concentrations for males and females were 2.25 ± 0.43 µg/kg lipid and 1.69 ± 0.60 µg/kg lipid in 2005, and 2.42 ± 0.62 µg/kg lipid and 2.58 ± 0.38 µg/kg lipid in 2008, respectively.

4.3.3 DDT contamination and patterns

Mean concentrations of ∑DDT and ∑*p, p'*-DDE ranged from 274 ± 233 to 533 ± 183 µg/kg lipid, and from 252 ± 221 to 505 ± 180 µg/kg lipid, respectively (Table 4.2). The range of concentrations for ∑DDT and ∑*p, p'*-DDE in sea lion pups were 16.0−3070 µg/kg lipid, and 0.15−2900 µg/kg lipid, respectively. ∑DDT concentrations detected in pups sampled in 2005 were lower than the concentrations of ∑DDT measured in 2008, suggesting a possible temporal increase in DDT concentrations, as illustrated in Figure 4.2. Male pups showed significantly higher concentrations of major DDT metabolites, *p,p*'-DDD (t-test, 2.92 $p = 0.0087$), *p,p*'-DDT (t-test, 2.45; $p = 0.0239$) and, *p,p*'-DDE (Welch t-test = 2,37, $p = 0.0286$), compared to females in 2005. The metabolite *p, p'*-DDE contributed the highest proportion (>90%) of ∑DDT compounds (Figure 4.3). The second most dominant metabolite was *p, p'*-DDD, followed by *p, p'*-DDT.

The composition pattern of each DDT metabolite did not differ between males and females in 2005 (Welch two-tailed t-test for all comparisons, $p > 0.05$), or between males and females in 2008 (Welch two-tailed t-test for all comparisons, $p > 0.05$). However, significant differences were

observed when comparing the temporal (2005 and 2008) composition of DDT metabolites among all groups of pups (Figure 4.3). While the contribution of *p*,*p*'-DDE to the total of DDT compounds in the 2005 females was significantly lower to that observed in females sampled in 2008 (Barlett test, $p < 0.0001$; Welch ANOVA, $p = 0.0271$; Tukey-Kramer test, $p < 0.05$), the contributions of *o*,*p*-DDE and *o*,*p*-DDT were significantly higher in females sampled in 2005 compared to male and female pups sampled in 2008 (Barlett test, $p < 0.0001$; Welch ANOVA, $p = 0.0019$; Tukey-Kramer test, $p < 0.05$ for *o*,*p*-DDE; and, Barlett test, $p < 0.0001$; Welch ANOVA, $P = 0.0085$; Tukey-Kramer test, $p < 0.05$ for *o*,*p*-DDT).

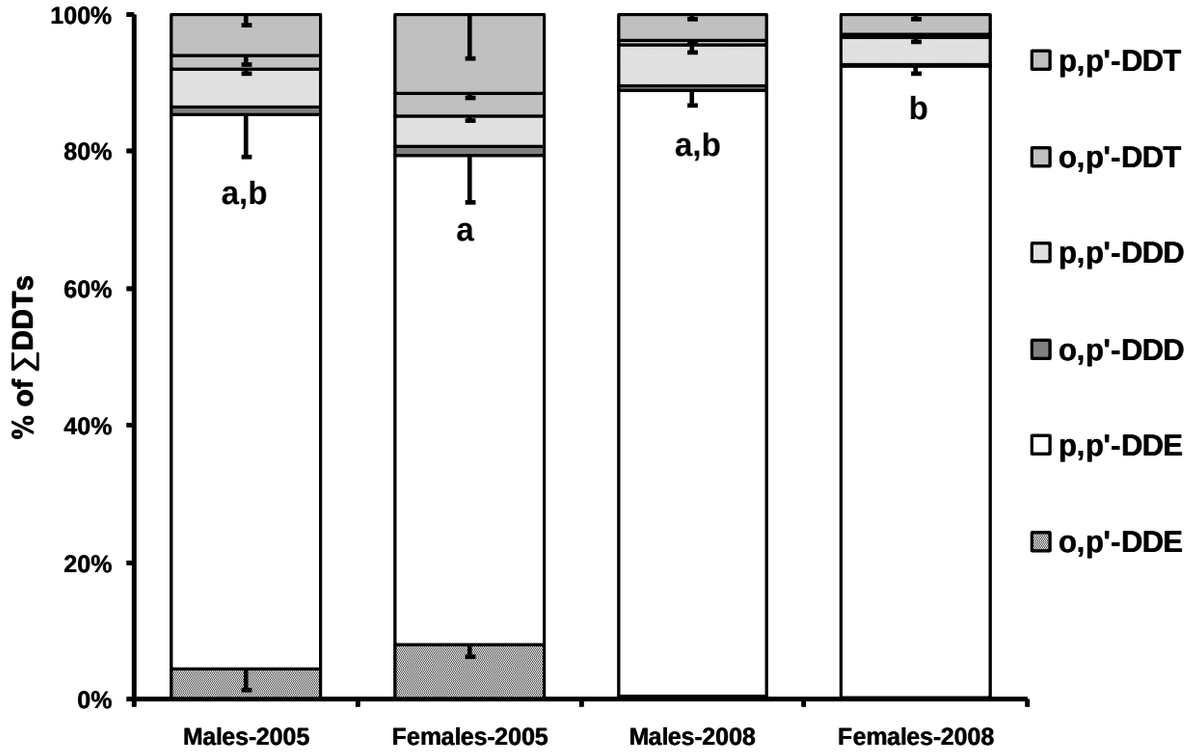

Figure 4.3 Composition pattern of DDT metabolites (i. e., *o, p*-DDE, *p, p*-DDE, *o, p*-DDD, *p, p*-DDD, *o, p*-DDT, and *p, p*-DDT) in males and females of Galapagos sea lion pups (*Zalophus wollebaeki*). Error bars are standard errors.

No significant differences in the composition pattern of *p*,*p*-DDD (ANOVA, $p = 0.2528$; Tukey-Kramer test, $p > 0.05$) and *p*,*p*-DDT (Barlett test, $p < 0.0001$, Welch ANOVA, $p = 0.2224$; Tukey-Kramer test, $p > 0.05$) were observed among pups. This indicates that male and female pups were exposed to DDT mixtures of similar composition in either 2005 or 2008, although temporal differences in composition pattern (e.g., *p*,*p*'-DDE) were detected possibly due to the historical or former use of DDT in the past or recent times.

4.3.4 Site differences of DDT concentrations

Inter-site comparisons showed that concentrations of ∑DDT detected in pups from Caamaño (Santa Cruz) exhibited the lowest levels and were significantly lower than ∑DDT concentrations measured in pups from Puerto Posada (Pinta), Punta Espinoza (Fernandina) and Plaza Sur (Santa Cruz) (Levene's test, $p = 0.0310$; Welch ANOVA, $p = 0.0238$; Tukey-Kramer test, $p < 0.05$), sampled in 2005; and, also significantly lower than those measured in pups from rookeries of San Cristobal Island (Isla Lobos y Puerto Baquerizo) and La Loberia (Floreana), when all sites, sampled in both 2005 and 2008, were compared (ANOVA, $p < 0.0001$; Tukey-Kramer test, $p < 0.05$) (Figure 4.4) . Concentrations of ∑DDTs in pups from Plaza Sur were also significantly lower than DDT concentrations in pups of Pinta Island in 2005 (Tukey-Kramer test, $p < 0.05$). ∑DDT concentrations in the four sites sampled in 2008 were not significantly different from each other (ANOVA, $p = 0.1357$; Tukey-Kramer test, $p > 0.05$). Pups from Pinta Island (pups PIP-02 and PIP-08; Table 4.2), one of the most remote and uninhabited islands (Figure 4.1), exhibited the highest concentrations of ∑DDTs compared to the rest of the samples. Although it cannot be ruled out that newborns and youngest pups of marine mammals can have low contaminant concentrations, concentrations of contaminants increase as newborns and pups nurse and absorb contaminant from lipid rich milk during lactational transfer. This contaminant load is especially high for first born calves (Ylitalo *et al*. 2001; Hickie *et al*. 2007), which might be the case in the two pups from Pinta Island.

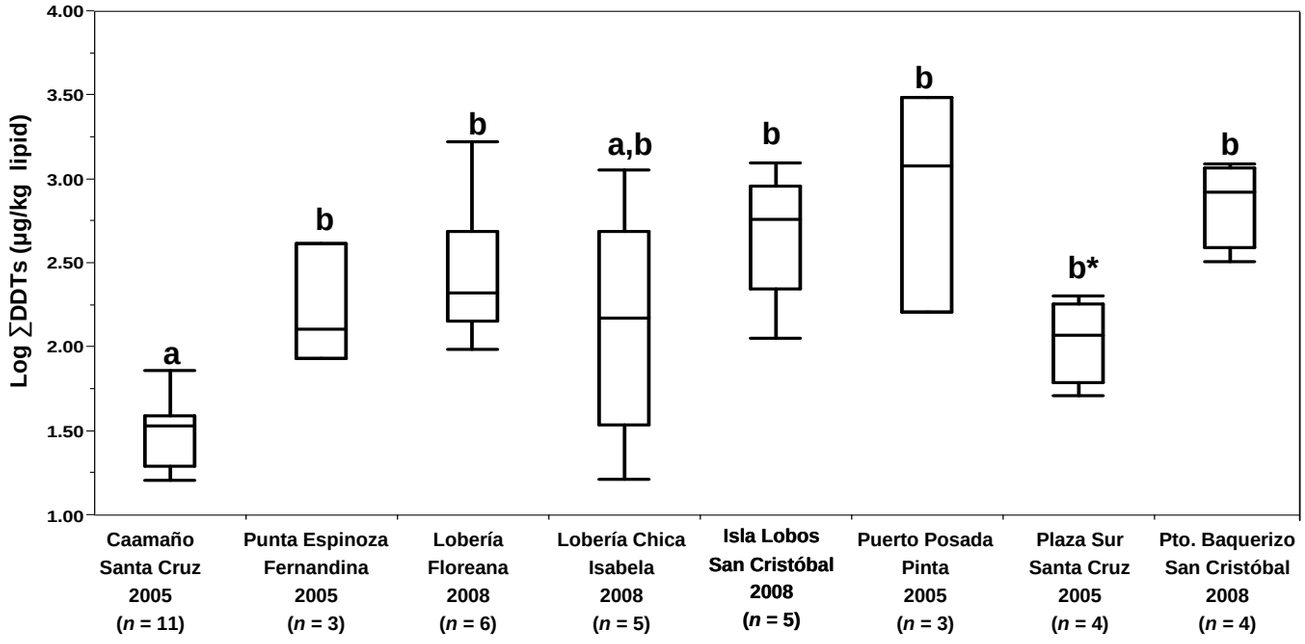

Figure 4.4 Inter-site comparisons showing box plots of log DDT concentrations among rookeries of Galapagos sea lion pups**.** The internal line across the middle of the box identifies the median sample values; the ends of the box are the 25% and 75% quartiles; and the whisker bars are the minimum and maximum values. Concentrations in rookeries not connected by the same letter are significant different. An asterisk right after the letter indicates that the concentration was also significantly different from the preceding box plot. When congeners were undetectable, half of the method detection limit was assigned in samples.

Gender and size of pups (e.g., females from Caamaño sampled in 2005), and sample size as well as inter-island sea lion movements (i.e., home range) and foraging trips (feeding areas) of Galapagos sea lion adult females might partly explain the spatial differences in DDT contamination of Galapagos sea lions. A recent study confirmed that adult females undertake trips to the sea to forage and spend a significant proportion of time on islands (i.e., multiple haul-out sites) other than their breeding colonies (Villegas-Amtmann *et al.* 2008; Villegas-Amtmann and Costa 2010). Proximity to populated urban areas in some islands (e.g., Santa Cruz, San Cristobal and Floreana) seems not to influence or elevate the concentration of DDT as the pups sampled

from rookeries close to human centres exhibited either lower or similar levels compared to those existing on more remote islands (e.g., Pinta and Fernandina; Figures. 4.1 and 4.4).

4.3.5 Global Comparison.

∑DDT concentrations in Galapagos sea lion pups are lower than those detected in pinnipeds from the Northern Hemisphere (Kajiwara *et al*. 2001; Kannan *et al*. 2004; Debier *et al*. 2005; Del Toro *et al*. 2006; Blasius and Goodmanlowe 2008; Mos *et al*. 2010), but greater than those detected recently in adult subdominant males, adult females, juveniles and pups of southern elephant seals (*Mirounga leonina*) from Elephant Island, Antarctica (Miranda-Filho *et al*. 2007) (Table 4.3; Figure 4.5). Interestingly, Galapagos sea lion pups exhibited ∑DDT concentrations similar to those detected in juveniles of Hawaiian monk seals (*Monachus schauinslandi*) from several subpopulations in the Northwestern Hawaiian Islands (Ylitalo *et al*. 2008). The maximum concentrations (i.e., 1000−3000 µg/kg lipid) observed in our study pups are similar to DDT concentrations observed in adult individuals of California sea lions (*Z. californianus*) from Baja California, Mexico, (Del Toro *et al*. 2006), but lower than those found in California sea lions from the coast of California, USA (Figure 4.5).

The DDT concentrations measured in some of the animals (for example, pups from Pinta Island; mean = 1490 µg/kg lipid, ranging 177−3097 µg/kg lipid) are comparable or higher to the DDT levels detected in adult male spinner dolphins (*Stenella longirostris*; 2553 µg/kg lipid) from the Eastern Tropical Pacific (Prudente *et al*. 1997), captured northwest of the Galapagos Archipelago, and in Amazonian River dolphins (*Inia geoffrensis*; 1624 µg/kg lipid) from the Brazilian Amazon, where DDT has been sprayed (Torres *et al*. 2009). These observations might

indicate a resident “background” DDT contamination of the Eastern Tropical Pacific Ocean and the Americas region.

The apparent increase of DDT levels from 2005 to 2008 in remote Galapagos sea lions is not an isolated event since concentrations of DDT in Adélie penguins (*Pygoscelis adeliae*) from remote areas of the western Antarctic Peninsula have not decreased between 2004 and 2006 (Geisz *et al*. 2008). Likewise, concentrations of DDT in human breast milk from Japan have not decreased since 1998 (Kunisue *et al*. 2006).

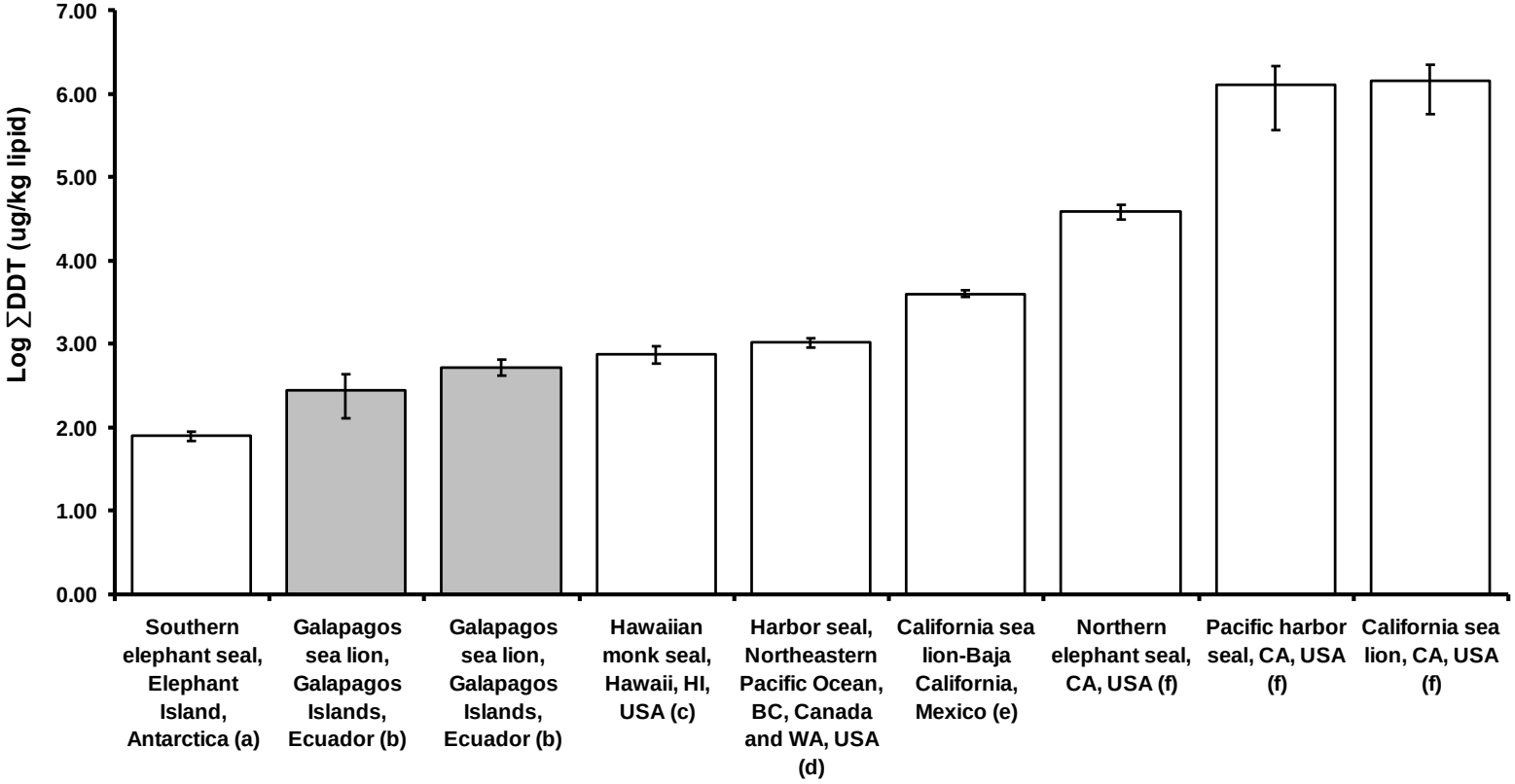

Figure 4.5 Global comparisons of Log ∑DDT mean concentrations (µg/kg lipid) among pinniped species from the Pacific and Antarctica: (a) Miranda-Filho *et al*. (2007); (b) Present study (2005 and 2008 samplings, respectively); (c) Ylitalo *et al*. (2008); (d) Mos *et al*. (2010); (e) Del Toro *et al*. (2006); (f) Blasius and Goodmanlowe (2008). Except for California sea lions from Baja California (Mexico), used here as reference, all the individuals are pups. Error bars are standard errors (SE).

Table 4.3 Global comparisons of mean concentrations (mg/kg lipid) of ∑DDT in muscle-blubber of pinniped species

species	stage/sex	∑DDT	Reference
location and year of collection			
Zalophus californianus	adult female and male	1450	Le Boeuf and Bonell (1971)
Coastal California, USA, 1970			
Z. californianus	full term parturient female	120	Delong *et al*. (1973)
San Miguel Island, California, USA, 1970	premature term parturient female	980	
Z. californianus	adult male	830	Kajiwara *et al*. (2001)
Coastal California, USA, 1991−1997	adult female	110	
	subadult male	870	
Mirounga angustirostris	yearling male	9	Kajiwara *et al*. (2001)
Coastal California, USA, 1991−1997	yearling female	62	
Z. californianus	adult male	140	Kannan *et al*. (2004)
North, Central and South California Coast, USA, 2000	adult female	283	
	subadult male	63	
Z. californianus[a]	juvenile (e. g. yearlings)	28	Debier *et al*. (2005)
Año Nuevo, Central California, USA, 2002			
Z. californianus	stranded adult male	380	Ylitalo *et al*. (2005)
Central California Coast, USA, 1993−2003	stranded adult female	250	Ylitalo *et al*. (2005)
Z. californianus	stranded adult and subadult male	4	Del Toro *et al*. (2006)
Baja California, Mexico, 2000−2001			
Z. californianus[b]	pup	2500	Blasius and Goodmanlowe (2008)
Southern California Bight, USA, 1994–1996			
Phoca vitulina	pup	1940	Blasius and Goodmanlowe (2008)
Southern California Bight, USA, 1994–1996			
Mirounga angustirostris	pup	77	Blasius and Goodmanlowe (2008)
Southern California Bight, USA, 1994–1996			

species	stage/sex	∑DDT	Reference
Phoca vitulina	pup	1.0	Mos *et al*. (2010)
Northeastern Pacific Ocean: British Columbia, Canada, and Washington State, USA, 1996–1997			
Mirounga leonina	adult male	0.20	Miranda-Filho *et al*. (2007)
Shetland Islands, Elephant Island, Antarctica,			
1997−2000	adult female	0.20	
	juvenile	0.10	
	pup	0.10	
Monachus schauinslandi[c]	juvenile	0.56−0.90	Ylitalo *et al*. (2008)
Hawaiian Islands: French Frigate Shoals, Laysan Island and Midway Atoll, 1997−2002			
Zalophus wollebaeki (this study)	pup	0.28	Present study
Galapagos Islands, Ecuador, 2005			
Zalophus wollebaeki (this study)	pup	0.53	Present study
Galapagos Islands, Ecuador, 2008			

[a] Concentrations detected in the serum of juvenile California sea lions
[b]Mean concentrations for pups of the three pinniped species from the Southern California Bight (CA, USA) were calculated as the sum of the mean concentrations reported for pup males and females and divided by the total number of pups; see Table 2 in Blasius and Goodmanlowe (2008); [c]Range of means concentrations of *p,p'*-DDE for Hawaiian monk seals; see Table 1 in Ylitalo *et al*. (2008)

4.3.6 DDT health effects assessment.

Marine mammals are at a particular risk of endocrine disruption and reduced immune function due to their high trophic position in the food-chain and long lifespan (Ross *et al*. 2000; Ross 2002; Mos *et al*. 2010). Experimental studies using *in vitro* tests and laboratory animals have demonstrated estrogenic and anti-androgenic effects of DDT metabolites (Kelce *et al*. 1995; Andersen *et al*. 1999; Freyberger and Ahr 2004). For example, transcriptional activity of androgen receptors in mammalian cell cultures is inhibited at *p,p'*-DDE concentrations of 64 µg/kg wet weight (Kelce *et al*. 1995). Also, *p,p'*-DDE concentrations ranging between 13 to 536 µg/kg wet weight have been associated with decreased proliferative responses of lymphocytes in free ranging bottlenose dolphins (Lahvis *et al*. 1995) and splenocytes in beluga whales (De Guise *et al*. 1998). The risk characterization showed that while > 99% of the concentrations were below the *p,p'*-DDE anti-androgenic effect reference value in pup sampled in 2005, the *p,p'*-DDE concentrations in 2% of females and 3% of males were above the minimum *p,p'*-DDE immunotoxic effect concentration in bottlenose dolphins (Figure 4.6a). In 2008, 8% of males and 9% of females exceeded the minimum *p,p'*-DDE immunotoxic effect threshold, while close to 100% of females are below the *p,p'*-DDE anti-androgenic reference value; however, 1% of the males surpass the *p,p'*-DDE anti-androgenic effect (Figure 4.6b). This indicates that DDT concentrations in Galapagos sea lion pups are near levels expected to be associated with impacts on the immune systems, and in minor degree on the endocrine systems in males. Other pollutants with a similar mode of toxicity such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ether (PBDEs) flame retardants, which were also detected in these animals (Alava *et al*. 2009), can further elevate the

immune and endocrine response. A compromised immune and endocrine system affects the ability of animals to combat disease and to successfully reproduce.

Since our study animals comprised only pups aged 2−12 months, our risk categorization here may be considered as a conservative estimate at the population level. Adult male Galapagos sea lions can be expected to have DDT concentrations that are higher than those in pups as DDTs accumulate throughout the animal's life (Addison and Smith 1974; Addison and Brodie 1987; Ross *et al*. 2000).

The 50% decline in the Galapagos sea lion population between the 1970s and 2001 continues to raise questions about underlying causes. While malnutrition and starvation associated with the El Nino events of 1982−1983 and 1997−1998 can cause large-scale populations declines, DDT metabolites can contribute to population level declines through immunotoxicity and developmental impacts of nutritionally stressed animals (Alava and Salazar 2006). A return to heavy reliance on DDT may represent a significant long-term health risk for Galapagos sea lions.

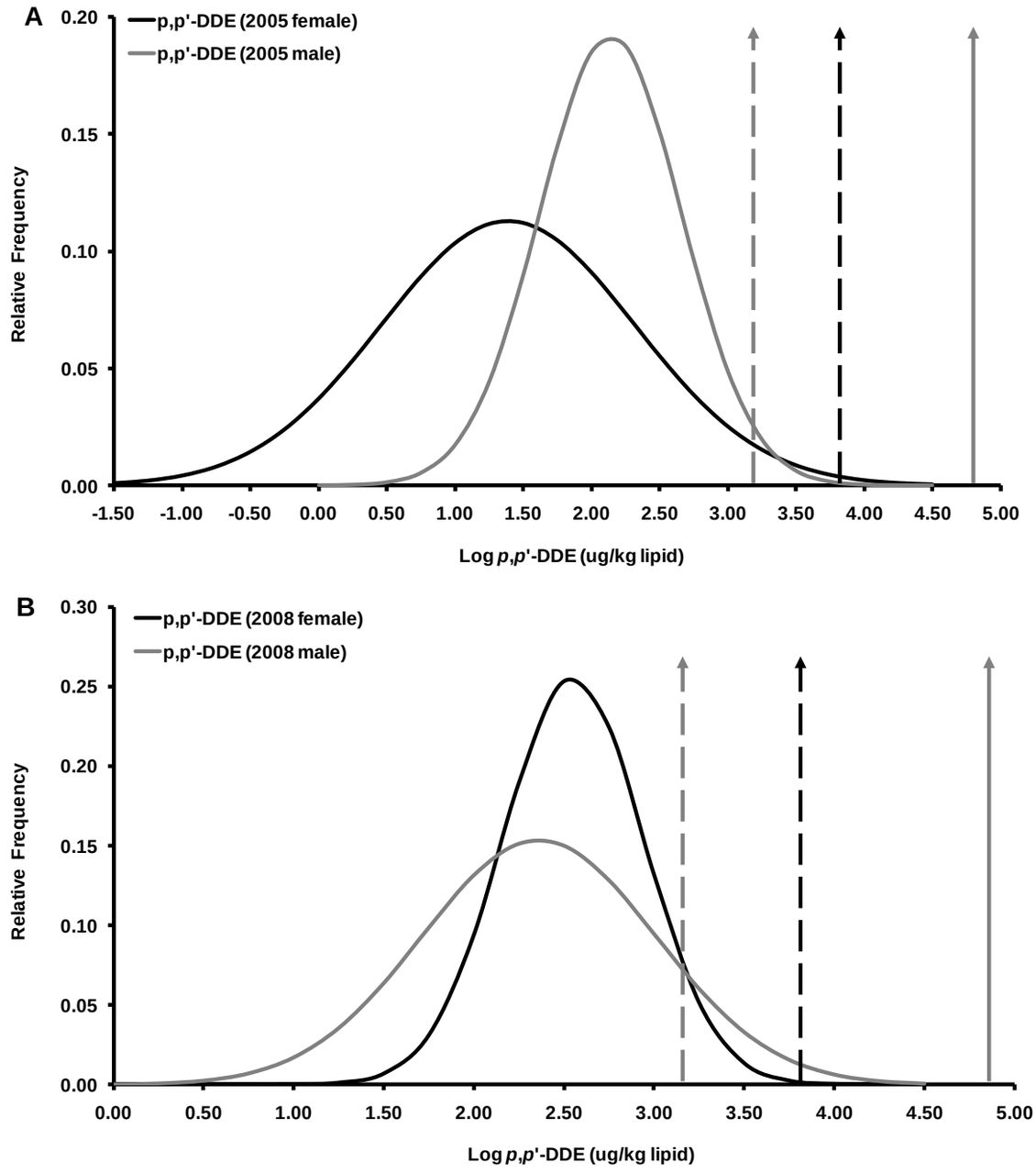

Figure 4.6 Normal probability density distributions of *p*,*p*'-DDE concentrations (i.e., cumulative frequency) of log-transformed *p*,*p*-DDE concentrations (µg/kg lipid) in biopsy samples of Galapagos sea lion pups sampled in 2005 (A) and 2008 (B) shown in relation to the *p*,*p*-DDE anti-androgenic effect concentration 64 µg/kg wet weight (Kelce *et al*. 1995) in mammalian species, equivalent to 6890 µg/kg lipid and represented by the black dashed arrow; and, the range of *p*,*p*-DDE concentrations (13–536 µg/kg wet weight) associated with a decreased lymphocyte proliferation response in bottlenose dolphins (Lahvis *et al*. 1995), equivalent to 1430 µg/kg lipid (minimum concentration represented by grey dashed arrow) and 58,900 µg/kg lipid (maximum concentration represented by the solid grey arrow). (A) The cumulative distribution of *p,p'*-DDE concentrations is shown by the grey solid curve in males and by the black solid curve in females in 2005; and, (B) The cumulative distributions of *p,p'*-DDE concentrations is shown by the grey solid curve in males and by the black solid curve in females in 2008.

4.3.7 Regional versus global transport of DDT.

DDT in the Galapagos sea lion pups likely originate from continental sources since there are no historical records indicating the use of DDT in the Galapagos. DDT was never imported to the islands (Dr. H. Jurado, Servicio Nacional de Erradicacion de la Malaria (SNEM)-National Malaria Eradication Service Centre of Ecuador, pers. comm.). This is supported by the fact that malaria and its mosquito vector (*Anopheles* sp.) have never been found in the Galapagos, although historical, anecdotic communications suggest that DDT was used in huge amounts by military personnel from the US Navy (former American Base in Baltra, Santa Cruz Island, used during the Second World War) to eliminate introduced rats as invasive species in human housing from urbanized areas and into the Islands between 1940s and 1950s in the last century (M. P. Harris, Centre for Ecology and Hydrology, Banchory Research Station, Banchory, UK, pers. comm.; M. Cruz, GGEPL-Galapagos National Park, pers. comm.). In continental Ecuador, DDT was applied inside homes (intra-domestic applications) and in agriculture between 1957 and 1999 to control malaria and crop pests (Ministerio del Ambiente 2004). The national inventory of organochlorine pesticide use in continental Ecuador reported that approximately 134,000 kg/year DDT was used in 1993. DDT use then dropped to approximately 1400 kg/year in 1998 (Appendix C; Figure C-2). Ecuador stopped importing DDT in 1994. At present, a stock of 1636 kg of DDT is available for emergency malaria control (Ministerio del Ambiente 2004; Ministerio del Ambiente 2006).

The high ratio *p, p*'-DDE/∑DDT (0.91−0.94) suggests a scenario of past DDT contamination and insignificant contributions from recent or fresh DDT sources. However, it must be emphasized that biota and in particular marine mammals are able to metabolize DDT to *p, p*'-DDE (Jensen and Jansson 1976; Letcher *et al*. 1995), which may also explain the high proportion of *p, p*'-DDE detected in Galapagos sea lion pups. The concentration ratio is similar to that found (0.93) in

southern elephant seals of Antarctica (Miranda-Filho *et al*. 2007). In comparison, *p, p*'-DDE/∑DDT concentration ratios measured in sediment and aquatic organisms of the Taura River in Continental Ecuador are 0.66 in sediments and 0.14 in fish (Montaño and Resabala 2005), and indicate a more recent DDT contamination and a potential regional source of DDT contamination.

Although linking the use of DDT in Ecuador and other Central and South American countries to the concentrations detected in the Galapagos sea lion pups is difficult, it is not unrealistic to assume that DDT use in continental Ecuador contributes to current concentrations of DDT in Galapagos sea lions. Recent estimates of annual DDT emissions from 1940 to 2005 (Schenker *et al*. 2008) indicate that the major use of DDT on the latitudinal band between 6ºN and 6ºS, encompassing part of the tropics and the equator (i.e., latitude 0º), took place from 1945 and 1965, as shown by the steep increase of DDT emissions (Appendix C; Figure C-3). Annual DDT emissions have since decreased slowly from 1965 to 2005 in this latitudinal zone, with a reduction of approximately 94% (Figure C-3).

In the mid 1970s, Goldberg (1975) described a global fractionation process, commonly known as “the Grasshopper Effect”, to illustrate the atmospheric transfer of DDT from continents to oceans (i.e., global distillation), which has been recently confirmed (Guglielmo *et al*. 2009). While substantial work has been carried out on the fate and behaviour of POPs and their atmospheric transport into the polar regions, very little has been conducted to investigate equatorial deposition of DDT from high-use regions. Despite the fact that the Galapagos are located 1000 km from continental Ecuador or more than 3000 km from legacy DDT hot spots in California, it cannot be ruled out that this mechanism might be playing a role in DDT transport to and contamination in the Galapagos.

The regional atmospheric-oceanic system, including the confluence of the NE and SE trade winds (i.e., the Inter-Tropical Converge Zone-ITCZ), winds from the west and oceanographic currents (i.e., Panama and Humboldt currents, and the Equatorial undercurrent or Cromwell current coming from the west) may contribute to the distribution of these contaminants in this particular region of the Southeastern Pacific Ocean. DDT in Galapagos might also originate from tropical countries in Asia by means of trans-Pacific air pollution (Wilkening *et al*. 2000). This is supported by the fact that tropical Asia is a significant global emission source of contaminants, including the long-range atmospheric transport of POPs (Iwata *et al*. 1993).

Recent modelling work reports that residence times and proportions of the total global masses of DDT are 10-15 days and 2% in the atmosphere, and 1.2 years and 26% in the global ocean with 30% of the DDT mass bounded to the organic matter phase in the equatorial Pacific Ocean, where high primary productivity is found due to existence of wind driven upwelling delivering nutrient enriched waters (Guglielmo *et al*. 2009), as those found in Galapagos waters (Alava, 2009). These observations portray that the physical-chemical properties of DDT, oceanographic conditions and atmospheric inputs are the driven forces explaining the presence of DDT in the islands.

4.3.8 Management Implications.

The management of DDT involves international policy instruments such as the Stockholm Convention on Persistent Organic Pollutants and the Convention on Long-Range Transboundary Air Pollution (CLRTAP). Ecuador has been a signatory country of the Stockholm Convention since May 2001. Since the ratification of the Stockholm Convention on POPs by Ecuador, the National Plan for the Implementation of the POPs Management in Ecuador was undertaken, commencing

with a national inventory of POPs, including PCBs, dioxins/furans, DDT and OC pesticides (Ministerio del Ambiente 2004; Ministerio del Ambiente 2006). Continuation of this initiative will help to control DDT contamination in the Galapagos.

While DDT is indeed among the 12 POPs (i.e., dirty dozen) listed under the Stockholm Convention, an exception has been granted for DDT use for malaria control. After nearly 30 years of restraint on the use of the DDT, the WHO has recently recommended indoor use of DDT once again to mitigate malaria in Africa (WHO 2006). This recommendation was encouraged by the 34th G8 summit in July 2008, where an increase in DDT use was proposed as one of the sanitation and health strategies. While DDT can save human lives, it can also adversely affect wildlife, local food production and opportunities for ecotourism. DDT use requires that trade offs are made between the conservation of valued, sensitive wildlife (i.e., Galapagos sea lions) and public health objectives to control malaria. The toxicological paradigm that the “dose makes the poison” provides a theoretical foundation for an approach that minimizing ecological damage while optimizing human health benefits. However, the application of this approach requires rigorous control of DDT use and emissions while continuously monitoring the concentrations and ecological effects of DDT in wildlife. Programs for monitoring DDT emissions and ecological effects in tropical areas do not exist at this time, but will be instrumental to achieving human health and environmental objectives.

DDT may be come a significant factor shaping the evolutionary processes that are so keenly studied in the Galapagos Islands. While we recognize that our study is imitated in scope, due to the highly protective measures in place on the Galapagos Islands and the difficult sampling and analysis protocols, it provides a unique and timely warning signal to the dangers of an increased reliance of DDT for malaria control in tropical countries. The results from this study may

help to provide preliminary guidance on the relationship between DDT use and ecological impacts and serve as a reference point against which possible future impact of tropical DDT use can be measured.

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CHAPTER 5

BIOMAGNIFICATION OF POPS AND ASSESSMENT OF STABLE $\delta^{15}N$ ISOTOPES IN THE GALAPAGOS SEA LION FOOD CHAIN.

Abstract: The WHO recently re-committed to the use of the organochlorine pesticide DDT to address the rising malaria cases in tropical countries. A significant increase in the use of DDT in malaria regions is likely to cause increases in DDT concentrations in wildlife species in both nearfield and in remote locations. In an effort to assess the degree of biomagnification of Persistent Organic Pollutants (POPs), including organochlorine pesticides and PCBs, and health risks in the Galapagos Islands, we collected blubber biopsies from the endemic and endangered Galapagos sea lions, *Zalophus wollebaeki*, sampled in 2008 and homogenized samples of their prey (thread herring, *Ophistonema* sp. and mullets, *Mugil* sp.). Stables isotope analysis ($\delta^{15}N$ and $\delta^{13}C$) in sea lion hair and fish homogenates were used to estimate trophic levels (TLs) and feeding ecology. Field derived Biomagnification Factor ratios (BMFs) and predator-prey Biomagnification Factor (BMF_{TL}) were used to evaluate biomagnification of POPs. The signatures of $\delta^{15}N$ in thread herring, mullets and sea lions were 9.38 (TL =3.1), 12.7 (TL = 4.1), and 13.0 (TL =4.2). The $\delta^{15}N/\delta^{13}C$ profile for the Galapagos sea lions showed reliance on pelagic sources of carbon and offshore foraging habits. Lipid normalized concentrations for all contaminant groups in Galapagos sea lions were significantly higher than those detected in prey items ($p < 0.05$). BMFs and BMF_{TL} for ∑DDT ranged from 132 to 172 kg/kg lipid and from 122 to 1631 kg/kg lipid, respectively; while BMFs and BMF_{TL} for ∑PCBs were lower, ranging between 7.85 and 28.0 kg/kg lipid. The BMFs

for organochlorine pesticides measured in this study were higher than those reported in harp seals from the Barents Sea, while BMFs for PCB congeners in Galapagos sea lions were lower than BMFs of PCBs reported for harp seals. Our results suggest that PCB, DDTs and other several organochlorine pesticides, including mirex, dieldrin, β-HCH and chlordanes, biomagnify in the Galapagos sea lion food chain. This is the first assessment of biomagnification of pollutants in an isolated, tropical region of the world around 0º latitude and suggests that endangered species in remote tropical areas are not immune to the risks associated with long range environmental transport of POPs.

Keywords: **Biomagnification, Biomagnification factor; Galapagos sea lion; stable isotopes, $\delta^{15}N$, $\delta^{13}C$, trophic level; DDT, PCBs, organochlorine pesticides**

5.1 Introduction

Bioaccumulation of persistent organic pollutants (POPs) represents a risk to the environment, including endangered wildlife and humans (Elliott *et al.* 1989; Ross *et al*. 2000, Kelly *et al.* 2007; Elliott *et al*. 2007). Biomagnification is the process by which thermodynamic activities of chemical subtances (often measured by the lipid normalized concentration or fugacity) in consumer and higher trophic level organisms exceed those concentrations in the diet or organism's prey (Gobas *et al.* 1993; Gobas *et al*. 1999; Gobas *et al*. 2009). This process can occur at each step in a food chain, potentially producing very high and toxic concentrations in upper-trophic-level species (Gobas *et al*. 2009).

In addition to persistence and toxicity, bioaccumulation and biomagnification are part of the screening criteria to conduct risk assessment of chemical compounds under the treaty of the Stockholm Convention for POPs and regulatory and management efforts in several nations such

Canadian Environmental Protection Act Canada (CEPA; Government of Canada 1999), the Toxic Substances Control Act (TSCA; USEPA 1976) in the United States and the Registration, Evaluation, Authorisation and Restriction of Chemicals program (REACH) in the European countries (Council of the European Union 2006). Due to the long-range atmospheric transport and global fractioning of POPs northward from low or mid latitudes (Wania and Mackay 1993; Guglielmo *et al*. 2009), the Arctic and northern hemisphere have remained as active regions of research to study biomagnification of POPs in trophic chains and food webs (Muir *et al*. 2003; Kelly and Gobas 2003; Borga *et al*. 2004; Kelly *et al.* 2007). However, very little is known on the bioaccumulative behaviour and fate of these substances in tropical zones of the planet.

There are several measures that have been used express the degree of biomagnification. The simplest measure is the Biomagnification Factor (BMF), which is described as the ratio of the chemical concentrations in the organism (C_B) and the diet of the organism (C_D), i.e., BMF = C_B/C_D, where the chemical are usually expressed in units of mass of chemical per kg of the organism (in wet weight or in a lipid basis) and mass chemical per kg of food (in wet weight or in a lipid basis) (Gobas and Morrison 2000). Biomagnification of organic contaminants and foraging preferences in aquatic and marine food webs can also be investigated using stable nitrogen isotope as biomarkers of trophic level (Kidd *et al.* 2001; Fisk *et al*. 2001; Borga *et al*. 2004; Christensen *et al.* 2005; Cullon *et al*. 2009). Stable isotope analysis (SIA) has emerged as a tool in foraging ecology/habitat use, physiology and ecotoxicology, and is strongly applied to study marine mammal ecology (Newsome *et al.* 2010). Stable nitrogen isotope analysis is a known well established technique for assessing predator–prey interactions and organism trophic levels (TL) in food webs (Peterson and Fry 1987; Hobson and Welch 1992; Hanson *et al.* 1997; Hobson *et al*. 2002). Specifically, $\delta^{15}N$, the concentration ratio of $^{15}N/^{14}N$, expressed relative to a standard (i.e.,

atmospheric N_2), has been shown to increase with increasing trophic level due to the preferential excretion of the lighter nitrogen isotope (DeNiro and Epstein 1981). Likewise, carbon isotope signatures ($\delta^{13}C$) provide information on habitat use and general sources of diet of organisms, i.e., marine/freshwater, coastal/oceanic, pelagic/benthic (Burton and Koch 1999).

Studies of the biomagnification and food web transport of POPs in tropical systems such as tropical remote islands around the equatorial Pacific Ocean are lacking. Due to the remoteness and isolation of the Galapagos Islands relative to other better studied geographical areas, the Galapagos Island food web offers an unique opportunity to undertake research related to the transport, bioaccumulative nature and biomagnification of globally distributed contaminants in tropical environments.

The Galapagos sea lion is an endemic marine mammal species residing year round in the islands and exhibiting a high degree of dietary plasticity, consuming several groups of fish prey (99% of the diet). The Galapagos sea lion diet includes Cupleidae (thread herrings and sardines), Engraulidae (anchovies), Carangidae (bigeye scad), Serranidae (groupers, whitespotted sand bass or camotillo), Myctophidae (lantern fish), Mugilidae (mullets) and Chlorophtalmidae fishes, and a low proportion of squid, as reported in the existing literature (Dellinger and Trillmich 1999; Salazar 2005; Páez-Rosas 2008; Aurioles-Gamboa *et al*. 2009). Although the information about diet and trophic level is limited for sea lions at several rookeries in the Galapagos Islands, it is known that the dietary preferences of Galapagos sea lions are also a function of the local variation in prey availability and regional climate-oceanic variability such as the El Niño events, when sea lions can switch their diet composition to more abundant fish items (Salazar and Bustamante, 2003; Alava and Salazar, 2006; Páez-Rosas, 2008). Because of its high trophic position, relative

abundance in the islands and nonmigratory behaviour, Galapagos sea lions can serve as local sentinels of food web contamination (Alava *et al*. 2009).

With the aim to contribute to the understanding of the behaviour of POPs in marine food webs of tropical regions, a biomagnification assessment of POPs was conducted in the Galapagos Islands by measuring the levels of legacy PCBs and organochlorine pesticides (e.g., DDT) and stable isotopes ($\delta^{15}N$) in Galapagos sea lions and major fish preys. In this study, we test the hypothesis postulating that a set of POPs, including PCBs, DDT and several other organochlorine pesticides do biomagnify in the Galapagos sea lions. To quantify the degree of biomagnification in this species, several biomagnification factor methods were used. Insights on the use of different approaches to calculate biomagnification and the effect of the magnitude in the trophic level difference are also investigated.

5.2 Materials and Methods

5.2.1 Tissue collection from Galapagos sea lion pups

Blubber biopsy (6 mm biopsy punch) and hair samples of 20 Galapagos sea lion pups (*Zalophus wollebaeki*) were obtained from four rookeries in the Galapagos Islands Archipelago between March 24-29, 2008. Pups were sampled at Isabela (Loberia Chica, *n* = 5), Floreana, (Loberia, *n* =6) and Santa Cristobal (Puerto Baquerizo, *n* = 4; Isla Lobos, *n* = 5) islands. Pups were captured with hoop nets and manually restrained. In all circumstances, capture stress and holding time were minimized (< 10-15 min). Hair samples were obtained using a sterile scissor to trim or a scalpel to shave the region to be used prior to the biopsy collection and deposited into labelled zipper bags. Biopsies (100 mg; 6mm−Miltex biopsy punch) were collected from an area

10-20 cm lateral to the spinal column and anterior to the pelvis. The biopsy site was pre-cleaned with alcohol and betadine. Biopsies were wrapped in hexane-rinsed aluminum foil and placed in a cooler with wet ice and transferred into cryovals placed in a cryoship (-20°C) during the field sampling, and, afterwards stored at -80°C in the laboratory until chemical analysis.

5.2.2 Fish collection and Homogenization

Two species of fish (mullets, *Mugil curema*; *n* = 11; and, Galapagos thread herrings, *Ophistonema berlangai*; *n* = 4), which are major prey items of Galapagos sea lions, were collected from Galapagos waters by fishers during March–April 2008. Mullets are coastal fish, inhabiting nearshore habitats, and demersal-benthic feeders (detritivorous), grazing on detritus and bottom sediments and digesting the nutritive matter (iliophagous foraging), while Galapagos thread herrings are endemic, pelagic and schooling fishes that filter-feed (planktivorous) mainly on tiny planktonic organisms (e.g., phytoplankton) in open waters (Grove and Lavenberg 1997).

After field collection, fish specimens were frozen until further transportation to the lab, where the fish were stored at -80ºC. Each fish was measured, weighed and sexed (for morphometrics see supporting information). Muscle biopsies were extracted from the dorsal, lateral muscle of each fish, using a 6mm–biopsy punch (Accuderm, USA), and saved in vials for stable isotope analysis.

Each individual fish was homogenized using a clean, hexane-acetone rinsed, meat grinder (Omcam Inc., Italy). The ground fish was then further homogenized in a homogenizer (Omni, USA and/or Polytron, Kinematica, GmbH, Switzerland) at dial position 5-6 for ≈1 min until material was well mixed and homogenous in appearance. Homogenized samples and subsamples were transferred to clean glass jars and stored at -80 ºC until further chemical analysis.

5.2.3 Chemical Analysis

Contaminant analyses were conducted in the Regional Dioxin Laboratory (RDL) at the Institute of Ocean Sciences (IOS), Fisheries and Ocean Canada (DFO), based on analytical methodologies described elsewhere (Ikonmomou *et al*. 2001). The muscle-blubber biopsy samples of Galapagos sea lion pups (0.053 to 0.212 g wet weight) and subsamples of fish homogenate (9.23 to 10.5 g) were spiked with a mixture of surrogate internal standards which contained all fifteen $^{13}C_{12}$-labeled PCBs, and a mixture of labelled organochlorine pesticides (OCPs): D_3 1,2,4-Trichlorobenzene, $^{13}C_6$ 1,2,3,4 Tetrachlorobenzene, $^{13}C_6$ Hexachlorobenzene, $^{13}C_6$ β-HCH, $^{13}C_6$ γ-HCH, $^{13}C_{10}$ trans Nonachlor, $^{13}C_{12}$ TeCB-47, $^{13}C_{12}$ *p*,*p*'-DDE, $^{13}C_{12}$ Dieldrin, $^{13}C_{12}$ *o*,*p*-DDD, $^{13}C_{12}$ *p*,*p*'-DDD, $^{13}C_{12}$ *o*,*p*-DDT, $^{13}C_{12}$ *p*,*p*'-DDT, $^{13}C_{10}$ Mirex. All surrogate internal standards were purchased from Cambridge Isotope Laboratories (Andover, MA). The spiked samples were homogenized with Na_2SO_4 in a mortar, transferred quantitatively into an extraction column, and extracted with DCM/hexane (1:1 v/v). For some of the samples the extract formed two layers/phases, a "waxy-precipitate" layer and the solvent layer. The solvent layer was transferred to a clean flask and the waxy precipitate was treated with several aliquots of hexane and DCM. Each of these were transferred to the flask that contained the solvent layer of the extract. Despite the treatment with additional volumes of hexane and DCM, vortexing and pulverization, the waxy precipitate (for sea lions) did not dissolved in the solvents used and as a result it was not included in the corresponding sample extract that was used for lipid and contaminants determinations.

The DCM:Hexane sample extracts were evaporated to dryness and the residue was weighted in order to determine the total lipid in the samples. Subsequently the residue was re-suspended in 1:1 DCM/Hexane and divided quantitatively into two aliquots. The larger aliquot

(75% of the extract) was subjected to sample-cleanup for PCBs determinations. The remaining (25% of the extract) was used for OCP determinations.

5.2.4 PCB analyses

Sample extracts were analyzed for PCB congeners by gas chromatography/high-resolution mass spectrometry (GC/HRMS). To obtain quantitative data for a maximum number of PCBs congeners the extracts were analyzed twice under GC/HRMS conditions using two different GC columns. The columns and the conditions used were: a) DB-5 column (50m x 0.25mm, 0.1μm film, J&W Scientific, Folsom CA), initial temperature 80^{o}C for 2 min, increased at 8^{o}C/min to 150 ^{o}C, then at 4 ^{o}C/min to 300 ^{o}C and held for 2 min; and b) CP-19 column (WCOT fused silica coating CP-SIL 19CB, 60m x 0.25mm, 0.15μm film, Varian, USA), initial temperature 100 ^{o}C for 2 min, increased at 20^{o}C/min to 200 ^{o}C, then at 1.5 ^{o}C/min to 268 ^{o}C, and 12.5 ^{o}C/min to 280 ^{o}C held for 2 min. For all analyses the HRMS was operated at 10,000 resolution under positive EI conditions and data were acquired in the Single Ion Resolving Mode (SIR). The source temperature was maintained at 300 ^{o}C the injector at 285 ^{o}C and the GC/HRMS interface at 260 ^{o}C. Splitless injection of 1 µL sample and 1 µL air were performed and the purge was activated 2 min after injection. Five point calibration curves were used and the PCB calibration solutions used for GC/HRMS quantitation covered a range from 0.77 pg/µL to 460 pg/µL

5.2.5 OC pesticides analyses

The lower volume fraction of the sample extract was loaded onto a Florisil column (8 grams of 1.2% water deactivated Florisil slurry packed with hexane into a fritted column) and eluted with 60 mL 1:1 DCM:hexane. Cleaned extracts were concentrated to less than 10 µL and spiked with the

^{13}C-labeled method performance standard ($^{13}C_{12}$- PeCB-111) prior to instrumental analysis. The corresponding extracts were analyzed for target OCPs by GC/HRMS. The high resolution mass spectrometer was a Micromass Ultima (Micromass, UK) instrument equipped with an HP-6890 gas chromatograph and a CTC autosampler. For the OCPs analyses a DB-5 column was used (45m x 0.25mm, 0.1μm film, J&W Scientific, Folsom CA), initial temperature 80^{o}C for 3 min, increased at 15^{o}C/min to 160 ^{o}C, then at 5 ^{o}C/min to 270 ^{o}C and held for 1 min, and lastly at 15 ^{o}C/min to 300 ^{o}C. The injector temperature was held at 200 ^{o}C. Splitless injection of 1 µL sample and 1 µL air were performed and the purge was activated 2 min after injection. For all analyses the HRMS was operated at 10000 resolution under positive EI conditions and data were acquired in the Single Ion Resolving Mode (SIR). The source temperature was maintained at 280 ^{o}C and the GC/HRMS interface at 260 ^{o}C. The mass spectrometry conditions used for all the analyses, the composition of the linearity calibration solutions, the criteria used for congener identification and quantification and the quality assurance – quality control procedures used for the quantification of OCPs were those described in detail elsewhere (Ikonomou *et al.* 2001).

5.2.6 Quality Assurance/Quality Control Measures

Samples were processed in batches of 12 samples each containing one or two procedural blanks, an in-house performance evaluation sample containing known concentrations of the analytes of interest, and nine or ten real samples. Method blanks, consisting of Na_2SO_4, were processed according to the same procedure as the samples and analyzed with every batch of twelve samples to check for potential background contamination.

Analytes were identified only when the GC/HRMS data satisfied the following criteria: (i) two isotopes of the analyte were detected by their exact masses with the HRMS operating at

10,000 resolution during the entire chromatographic run; (ii) the retention time of the analyte peak was within 3 seconds of the predicted time obtained from analysis of authentic compounds in the calibration standards (where available); (iii) the maxima for both characteristic isotopic peaks of an analyte coincided within 2 seconds; (iv) the observed isotope ratio of the two ions monitored per analyte were within 15% of the theoretical isotopic ratio; and (v) the signal-to-noise ratio resulting from the peak response of the two corresponding ions was ≥3 for proper quantification of the analyte. Analyte concentrations were calculated by the internal standard isotope-dilution method using mean relative response factors (RRFs) determined from calibration standard runs made before and after each batch of samples was analyzed. Concentrations of analytes were corrected for the recoveries of the surrogate internal standards. The recoveries of all surrogate internal standards were between 60 and 110% and the accuracy of determining PCBs in spiked samples was between 15 and 20%. The levels of individual PCBs congeners measured in the procedural blanks were between 2 and 60 pg/sample wet weight. For the dichloro- and trichloro-PCBs the range was a bit higher, between 60 and 200 pg/sample wet weight. For all target analytes the concentrations reported were within the linear range of the multipoint calibration range established. The recoveries of all OCP surrogate internal standards were between 65 and 110% and the accuracy of determining the target OCPs in spiked samples was between 15 and 20%. For all target analytes the concentrations reported were within the linear range of the multipoint calibration range established.

5.2.7 Sample preparation for Stable Isotopes Analysis (SIA)

Each set of hair samples collected from Galapagos sea lion pups were cleaned for lipids and particles removal by washing the hair three times with a chloroform:methanol 2:1 v/v solution

using a clean Pasteur glass pipette. Samples were transferred into labelled scintillation vials and desiccated overnight, and, then, lyophilized using a freeze drier (Free Zone ® Plus 4.5 Liter Cascade; Labconco, Kansas City, MO) for 24 hr (Vacuum pressure set point: 0.01 mBar).

Fish biopsies were freeze dried overnight (Vacuum pressure set point: 0.01 mBar). Biopsy samples were weighed and freeze dried again to determine if there were differences in weights after the second freeze drying. Once the sample weight was constant (i.e., no presence of moisture), one set of freeze dried samples were stored in the desiccator until further analysis for $\delta^{15}N$. The set of freeze dried replicates underwent an extraction protocol to remove lipids to be used for $\delta^{13}C$ analysis. First, freeze dried samples were pulverized using a mortar and transferred into a glass tube for lipid extraction by adding 5ml of chloroform:methanol 2:1 v/v; and, then vortex mixed for 30 seconds. Solids were dispersed with sonification in bath sonicator for 10 min. Samples were allowed to settle for 30 min at room temperature, followed by an additional 30 second vortex and sonification. Samples were centrifuged for 5 minutes at 1000 rpm (model GS6R, Beckman, USA) to enhance pellet. The solvent was carefully removed with glass Pasteur pipette (pipette was changed for each sample), without transferring any particulate matter, and the solvent was disposed in the waste bottle. A second extraction was repeated. The supernatant was carefully removed with pipette and the residue was left at -20^{o}C overnight. Samples were dried under Nitrogen and transferred to a clean, amber vial for analysis of stable isotopes of carbon and nitrogen.

5.2.8 Stable Isotopes Analysis (SIA).

Carbon and nitrogen isotopic analyses on fish biopsies and Galapagos sea lion hair were accomplished by continuous flow, isotopic ratio mass spectrometry (CF-IRMS) using a GV-Instruments® IsoPrime attached to a peripheral, temperature-controlled, EuroVector® elemental

analyzer (EA) (University of Winnipeg Isotope Laboratory, UWIL). One-mg samples were loaded into tin capsules and placed in the EA auto-sampler along with internally calibrated carbon/nitrogen standards. Nitrogen and carbon isotope results are expressed using standard delta (δ) notation in units of per mil (‰).The delta values of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) represent deviations from a standard. $\delta^{15}N$ isotope ratios (‰) were determined using the following equation (DeNiro and Epstein 1981; Newsome *et al.* 2010):

$$\delta^{15}N = [(^{15}N/^{14}N_{SAMPLE}/^{15}N/^{14}N_{STANDARD}) - 1] \times 1000$$

where $^{15}N/^{14}N_{SAMPLE}$ is the isotope ratio of the tissue sample analyzed; and, $^{15}N/^{14}N_{STANDARD}$ represents the ratio of the international standard of atmospheric N_2 (air), IAEA-N-1 (IAEA, Vienna), for $\delta^{15}N$. The equivalent equation for $\delta^{13}C$ isotope ratios (‰) is:

$$\delta^{13}C = [(^{13}C/^{12}C_{SAMPLE}/^{13}C/^{12}C_{STANDARD}) - 1] \times 1000$$

The standard used for carbon isotopic analyses was the Vienna PeeDee Belemnite (VPDB). Analytical precision, determined from the analysis of duplicate samples, was ±0.13‰ for $\delta^{13}C$ and ±0.6‰ for $\delta^{15}N$. The analytical precision based on standards, which are more isotopically homogeneous than samples, was ± 0.19‰ for $\delta^{13}C$ and ±0.24 for $\delta^{15}N$.

5.2.9 Trophic Level Estimations.

The trophic positions ($TP_{CONSUMER}$) of the prey species (i.e. fish) and the predator (Galapagos sea lion) were determined relative to the baseline $\delta^{15}N$ (assumed to occupy a trophic

level 2), using the algorithm proposed by Vander Zanden and Rasmussen (1999); Vander Zanden *et al*. (1997):

$$TP_{CONSUMER} = \frac{\left(\delta^{15}N_{CONSUMER} - \delta^{15}N_{BASELINE}\right)}{3.4} + 2$$

Where $\delta^{15}N_{CONSUMER}$ is the average $\delta^{15}N$ signature value of the predator; $\delta^{15}N_{BASELINE}$ is the $\delta^{15}N$ signature at the base of the food web; and 3.4‰ is the isotopic, trophic level enrichment factor ($\Delta^{15}N$), recommended to be used for constructing food webs when a priori knowledge of $\Delta^{15}N$ is unknown (Jardine *et al*. 2006). The $\delta^{15}N_{BASELINE}$ was set up as the $\delta^{15}N$ signature of the particulate organic matter (POM) of bottom sediments in the eastern equatorial Pacific Ocean (250 km south of the islands) with a value of 5.5‰ (Farrell *et al*. 1995; Aurioles-Gamboa *et al*. 2009). The rationale for using this signature is supported by the fact that the assimilation of nitrogen (i.e., NO_3^-) up taken from near surface marine waters by phytoplankton is reflected by $\delta^{15}N$ values of POM, which is also a major component of the carbon flux and sediments (Farrell *et al*. 1995).

Although pups instead of adult individuals of sea lions were sampled in this study, the $\delta^{15}N$ signature in the pup is expected to reflect the isotopic nitrogen signature of the mother, as pups feed only on mothers' tissue (i.e., milk proteins) analogous to a predator-prey relationship, resulting in a $\delta^{15}N$ isototipc enrichment of 2.1‰ and 0.9‰ $\delta^{13}C$ enrichment in relation to adult females (Fogel *et al.* 1989; Porras-Peters *et al.* 2008). Because of lactation, pups can be at a higher trophic level than their mothers. Therefore, this allows inferring indirectly the $\delta^{15}N$ signature and foraging habits (i.e., diet) in adult animals (females) (Páez-Rosas and Aurioles-Gamboa 2010).

5.2.10 Bioaccumulation parameters

Three approaches were used to quantify biomagnification in the Galapagos sea lions relative to prey items (i.e., thread herring and mullet) and to explore the effect of the magnitude of trophic level differences on the BMF measures.

5.2.10.1 *Field derived Biomagnification Factor (BMF)*

To quantify biomagnification in the Galapagos sea lion the mean lipid normalized concentration of each contaminant measured in the pups were divided by the mean lipid adjusted concentration in the prey. Pups were considered as the predator as they feed on mother tissues (e.g., milk), equivalent to a predator-prey relationship.

$$\boldsymbol{BMF} = \boldsymbol{C}_{PREDATOR}/\boldsymbol{C}_{PREY}$$

Where the chemical concentrations in the predator ($C_{PREDATOR}$) and the prey (C_{PREY}) are expressed in units of mass of chemical (µg) per kg of the predator and mass chemical (µg) per kg of prey in a lipid normalized basis ($BMF_{LIPID\ WEIGHT}$), respectively. The criterion used to indicate the capability of the chemical to biomagnify is BMF > 1. A BMF statistically greater than 1 indicates that the chemical is a probable bioaccumulative substance (Gobas *et al*. 2009)

5.2.10.2 *Predator-Prey Biomagnification Factor (BMF_{TL})*

The biomagnification factor can be adjusted to represent exactly one trophic level in difference using the trophic level estimated from $\delta^{15}N$. Therefore, the field based predator-prey

biomagnification factor normalized to trophic position or $BMF_{TROPHIC\ LEVEL}$ (BMF_{TL}) was also calculated using the following equation (Borga *et al*. 2004):

$$BMF_{TL} = \frac{(C_{PREDATOR} / C_{PREY})}{TL_{PREDATOR} - TL_{PREY}}$$

Where $C_{PREDATOR}$ and C_{PREY} are appropriately normalized (e.g. lipid normalized) chemical concentrations in the predator and prey, and $TL_{PREDATOR}$ and TL_{PREY} are the trophic levels of the predator and prey. The BMF_{TL} values were used to measure biomagnification in the tropical food chain between two adjacent trophic levels (i.e., the difference in TL between predator and prey is small), assuming steady state in contaminant concentrations between predator and prey. Since BMF_{TL} can be related to the trophic magnification factor (TMF), which describes the increase of contaminants from one trophic level to the other (derived from the slope, b, of the relationship between an organism's log lipid normalized chemical concentration), it can also be expressed as BMF_{TL}* (Conder *et al*. 2011):

$$BMF_{TL}^{*} = 10^{\left[\frac{\log_{10}([C_{PREDATOR}]/[C_{PREY}])}{TL_{PREDATOR} - TL_{PREY}}\right]}$$

Where $C_{PREDATOR}$ and C_{PREY} are appropriately normalized (e.g., lipid normalized) chemical concentrations in the predator and prey, and $TL_{PREDATOR}$ and TL_{PREY} are the trophic levels of the

predator and prey. In essence, the BMF_{TL} is the biomagnification factor normalized to a single trophic level increase in the food-web (Conder *et al*. 2011).

5.2.11 Data Treatment and Supporting Statistical Analysis

Concentrations of all detected POPs were blank corrected using the method detection limit (MDL), defined as the mean response of the levels measured in three procedural blanks used plus three times the standard deviation (SD) of the blanks ($MDL = Mean_{BLANKS} + 3*SD_{BLANKS}$). Following this methodology, the concentration of each PCB congener and OC pesticide was determined based on concentrations above the MDL only. Only PCBs detected in 100% of samples and above the MDL were used for data analysis and calculations of BMFs. Contaminant concentration data were log-transformed to fit assumption of normality criteria before statistical analysis. ∑PCB concentrations were calculated as the sum of PCB-52, PCB 74, PCB 95, PCB-99, PCB-101, PCB-105, PCB-118, PCB 128, PCB -138/163/164**,** PCB-146, PCB 153, PCB 156, PCB 174, PCB 180, PCB 183, PCB 187, PCB 201 and PCB 202. ∑DDTs were defined as the sum of *o, p'*-DDE, *p, p'*-DDE, *o, p'*-DDD, *p, p'*-DDD, *o, p'*-DDT and *p, p'*-DDT, and ∑chlordanes as the sum of *trans*-chlordane, *cis*-chlordane, *trans*-nonachlor and *cis*-nonachlor.

To further support the analysis of biomagnification of POPs in the tropical food chain of the Galapagos, statistical comparisons between the concentrations of selected PCBs (e.g., PCBs 153, 180), ∑DDTs, *p*,*p*'-DDE and other organochlorine pesticides measured in the Galapagos sea lion and those detected in diet items (i.e., mullet and thread herring) were conducted. These comparisons were conducted using analyses of variance (ANOVA) if variances were homoscedastic (i.e., equal variances) or Welch's analyses of variance if variances or standard deviations were heteroscedastic (i.e., unequal variances as tested by Levene's test or Bartlett test,

$p < 0.05$), and a Tukey-Kramer honestly significant difference (HSD) test, which is a post-hoc method recommended to test differences between pairs of means among groups that contain unequal sample sizes (Zar 1999). Inter-site comparisons among rookeries samples followed the same statistical methods. Statistical comparison tests were conducted at a level of significance of $p < 0.05$ ($\alpha = 0.05$).

Principal Components Analyses (PCAs) were conducted on the fractions of PCBs and organochlorine pesticides relative to total concentrations by contaminant group (i.e., contaminants expressed as a fraction of total) for each sample to visualize spatial differences in patterns in sea lion pups from different sites within the Galapagos Archipelago and elucidate potential sources (i.e., local versus global-atmospheric). First, samples with undetectable values were replaced by a random number between the lowest and the highest concentration that were detectable (> MDL) before PCA (i.e., *trans*-chlordane and PCB 110 showed zero values in blanks in three and two samples out of 20, respectively; therefore; there was not possible to calculate MDLs), or otherwise removed from the PCAs. Secondly, samples were normalized to the concentration total before PCA to remove artifacts related to concentrations differences between samples. Finally, the centered log ratio transformation (division by the geometric mean of the concentration-normalized sample followed by log transformation) was then applied to this compositional data set to produce a data set that was unaffected by negative bias or closure (Ross *et al*. 2004). Regressions, statistical comparisons and PCAs were run using JMP 7.0 (SAS Institute Inc.; Cary, NC, USA, 2007).

5.3 Results and Discussion

5.3.1 Stable Isotope profiles and trophic levels

Stable isotope ratios of $\delta^{15}N$ and $\delta^{13}C$ are reported in Table 5.1. The values of $\delta^{15}N$ and $\delta^{13}C$ found here are consistent to those reported in Galapagos sea lion pups (i.e., 13.1‰ ± 0.5‰ for $\delta^{15}N$, and -14.5‰ ± 0.5‰ for $\delta^{13}C$) in a recent study (Aurioles-Gamboa *et al*. 2009). No significant relationship was observed between isotopic values and length of the pups ($\delta^{15}N$: r = 0.005, p =0.7594; $\delta^{13}C$: r = 0.18, p = 0.0626) or weight ($\delta^{15}N$: r = 0.0001, p =0.9645; $\delta^{13}C$: r = 0.18, p = 0.0752). Although female pups appeared to exhibit higher values of $\delta^{15}N$ compared to male pups (t-test = 2.3767, p = 0.0288), $\delta^{13}C$ values between males and females were similar (t-test = -0.3326, p = 0.7433; Table 1). In addition, no significant inter-site differences in $\delta^{15}N$ (ANOVA, p = 0.4235) and $\delta^{13}C$ (ANOVA, p = 0.8378) values were found among rookeries (Table 1; Figure D-1 in Appendix D). This indicates that site or foraging location had minimal influence on the isotope ratios. The lack of differences was further minimized by sampling similar ontogenetic stages (i.e., pups of similar age, development and size), and a metabolically inactive tissue (i.e., fur hair), which is corroborated by the fact that hair is an inert tissue containing physiological and dietary information (isotopic signals) (Darimont and Reimchen 2002).

The $\delta^{15}N/\delta^{13}C$ profile indicates that Galapagos sea lions possess offshore foraging habits relying on pelagic sources of Carbon as shown in Figure 5.1. The isotopic profiles for fish species indicates that while thread herring are offshore feeders dependent on pelagic Carbon, mullets are nearshore foragers relying on benthic sources of Carbon (Figure 5.1).

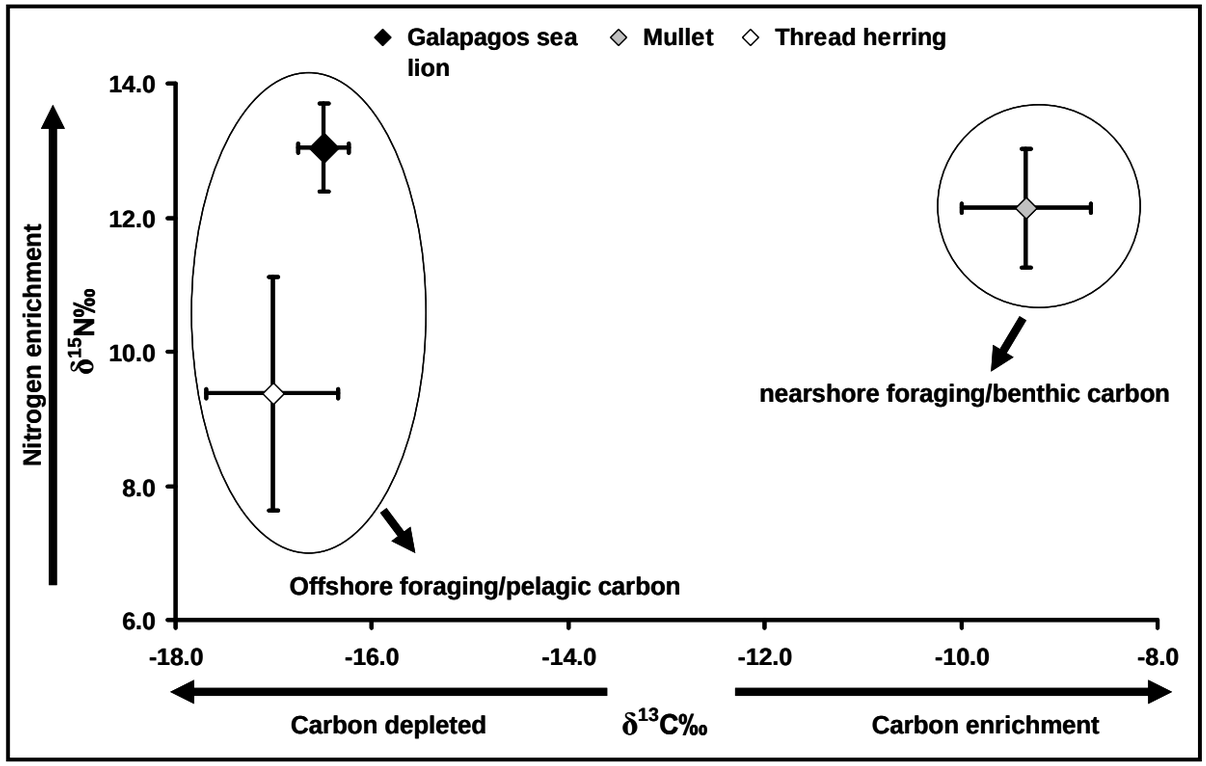

Figure 5.1 Biplot showing comparisons of mean $\delta^{15}N$ and $\delta^{13}C$ values measured in samples collected (Galapagos sea lions' fur and fish homogenate) in the Galapagos Islands in 2008. Error bars are 95% confidence intervals.

The trophic position for Galapagos sea lions and fish prey based on their $\delta^{15}N$ signatures are provided in Table 5.1. The $\delta^{15}N$ signature and trophic level measured here for the Galapagos sea lion ($\delta^{15}N$ = 13.0; TL = 4.2) are similar to those recently reported (i. e., $\delta^{15}N$ =12.6−13.4; TL = 4.1−4.4) by Aurioles-Gamboa *et al*. (2009), and Páez-Rosas and Aurioles-Gamboa (2010).

Table 5.1 Stable isotope values (mean ± standard deviation) for $\delta^{15}N$ and $\delta^{13}C$ (‰), trophic level (TL) estimates, and sample size for Galapagos sea lion pups (fur samples), fish species and by sampling location (sea lion pups) in the Galapagos Islands.

	n	$\delta^{13}C$	$\delta^{15}N$	TL
Galapagos sea lion				
Female pup	10	-16.4 ± 0.52	13.7 ± 1.64	4.4
Male pup	10	-16.5 ± 0.66	12.3 ± 0.96	4.0
all pups	20	-16.5 ± 0.58	13.0 ± 1.50	4.2
Location				
Isabela (Lobería Chica)	5	-16.6 ± 0.68	13.3 ± 1.44	4.3
Floreana (Lobería)	6	-16.3 ± 0.74	12.2 ± 1.26	4.0
San Cristóbal (Pto. Baquerizo)	4	-16.4 ± 0.24	13.4 ± 1.65	4.3
San Cristóbal (Isla Lobos)	5	-16.6 ± 0.58	13.6 ± 1.69	4.4
Fish				
Mullet (*Mugil* sp.)	6	-9.34 ± 0.82	12.7 ± 1.10	4.1
Thread herring (*Ophistonema berlangai*)	4	-17.0 ± 0.68	9.4 ± 1.77	3.1

5.3.2 POP concentrations in animals and inter-site comparisons

5.3.2.1 *Galapagos sea lions*

Observed concentrations of selected POPs in Galapagos sea lion and two of its main prey items are summarized in Table 5.2. Galapagos sea lions represented the largest number of organisms sampled in this study (n = 41) and exhibited the highest concentrations of PCBs and OC pesticides. The multi-comparison post hoc analysis, including sea lions and prey fish, showed that no significant differences in OC pesticides and PCB congener concentrations were observed between male and female pups. Fish preys commonly exhibited significantly lower concentrations than Galapagos sea lion pups (ANOVA and multi-comparisons Tukey-Kramer (HSD) post-hoc test, $p < 0.05$) (Table 5.2, Figure 5.2).

Concentrations of ∑DDTs in Galapagos sea lions ranged from 16.0 to 1700 µg/kg lipid and ∑DDTs were the predominant OC pesticide in Galapagos sea lion pups. ∑Chlordanes were the second most abundant group of contaminants present. *Trans*-nonachlor represented 68% of ∑chlordanes, followed by *cis*-chlordane, *cis*-nonachlor and *trans*-chlordane (Tables 5.2), a pattern comparable to that reported in pups of southern elephant seals (*Mirounga leonina*) (Miranda-Filho *et al*. 2007) and Wedell seals (Kawano *et al*.1998). This indicates that *trans*-nonachlor is a predominant chlordane compound in pinnipeds.

Table 5.2 POP concentrations (µg/kg lipid) in Galapagos sea lion, thread herring and mullet sampled in 2008. Lipid contents are arithmetic mean ± standard deviations (SD). Concentrations are mean ± standard error (SE), and range between brackets. Different letters (i.e. A, B, and C) indicate significant differences among sea lion pups and fish species (ANOVA and multi-comparisons Tukey-Kramer (HSD) post-hoc test, $p < 0.05$).

	Galapagos sea lion (predator)		**Fish (prey)**		***p*-value**
	Female pups	**Male pups**	**Thread herring**	**Mullet**	
	(*n* = 10)	(*n* = 10)	(*n* = 4)	(*n* = 6)	
Lipid (%)	75.9 ± 3.50	77.8 ± 2.45	1.22 ± 0.86	2.86 ± 2.00	
***p*,*p*'-DDE**	480 ± 120 **A**	505 ± 180 **A**	3.30 ± 1.00 **B**	2.22 ± 0.700 **B**	<0.0001*
	(65.4–1183)	(13.6–1650)	(0.669–5.00)	(0.620–5.20)	
***p*,*p*'-DDT**	13.0 ± 2.85 **A**	8.60 ± 1.08 **A**	0.070 ± 0.046 **B**	0.130 ± 0.051 **B**	<0.0001*
	(1.70–29.0)	(0.974–12.0)	(ND–0.195)	ND–0.300	
***p*,*p*'-DDD**	20.0 ± 4.73 **A**	17.0 ± 4.60 **A**	0.440 ± 0.140 **B**	0.550 ± 0.170 **B**	<0.0001*
	(1.88–44.0)	(0.965–54.0)	(0.036–0.70)	(0.155–1.30)	
∑DDT	516 ± 125 **A**	533 ± 183 **A**	4.00 ± 1.26 **B**	3.00 ± 0.910 **B**	<0.0001*
	(71.2–1230)	(16.3–1666)	(0.705–6.05)	(0.820–6.80)	
mirex	8.60 ± 1.76 **A**	6.40 ± 2.20 **A**	0.330 ± 0.030 **B**	0.040 ± 0.008 **C**	<0.0001**
	(2.50–21.0)	(0.850–24.0)	(0.250–0.400)	(0.028–0.080)	
dieldrin	31.0 ± 7.26 **A**	22.0 ± 4.80 **A**	0.600 ± 0.204 **B**	0.880 ± 0.128 **B**	<0.0001**
	(9.00–83.0)	(9.00–63.0)	(0.005–0.90)	(0.400–1.30)	
***β*-HCH**	34.2 ± 4.00 **A**	26.0 ±7.05 **A**	0.440 ± 0.090 **B**	0.495 ± 0.095 **B**	<0.0001**
	(18.3–52.0)	(7.75–78.0)	(0.229–0.620)	(0.041–0.650)	
***trans*-chlordane**	0.410 ± 0.100 **A**	0.65 ± 0.10 **A**	0.070 ± 0.027 **B**	0.040 ± 0.015 **B**	0.0277**
	(ND–0.840)	(0.273–1.03)	(ND–0.130)	(ND–0.110)	
***cis*-chlordane**	17.2 ± 2.67 **A**	15.0 ± 2.75 **A**	0.455 ± 0.140 **B**	0.250 ± 0.053 **B**	<0.0001*
	(6.800–34.0)	(3.60–31.0)	(0.049–0.670)	(0.120–0.482)	
***trans*-nonachlor**	73.0 ± 12.0 **A**	65.0 ± 22.0 **A**	0.860 ± 0.191 **B**	0.40 ± 0.072 **B**	<0.0001**
	(37.0–146)	(11.0–214)	(0.430–1.30)	(0.160–0.570)	
***cis*-nonachlor**	16.0 ± 3.20 **A**	10.0 ± 2.10 **A**	0.300 ± 0.109 **B**	0.195 ± 0.050 **C**	<0.0001*
	(3.7–31.8)	(3.56–25.8)	(ND–0.510)	(0.075–0.380)	
∑Chlordanes	107 ±15.0 **A**	90.5 ± 25.2 **A**	1.70 ± 0.445 **B**	0.870 ± 0.175 **B**	<0.0001*
	(48.1–180)	(18.8–255)	(0.481–2.50)	(0.372–1.50)	
PCB 52	3.20 ± 0.530 **A**	2.10 ± 0.610 **A**	0.210 ± 0.030 **B**	2.20 ± 1.85 **B**	<0.0001**
	(1.13–5.60)	(0.332–7.05)	(0.136–0.270)	(0.055–11.0)	
PCB 74	2.60 ± 0.410 **A**	2.00 ± 0.510 **A**	0.100 ± 0.009 **B**	0.280 ± 0.220 **B**	<0.0001**
	(1.40–5.10)	(0.340–4.40)	(0.050–0.085)	(0.012–1.40)	
PCB-95	2.80 ± 0.303 **A**	2.20 ± 0.320 **A**	0.300 ± 0.090 **B**	2.02 ± 1.70 **B**	0.0325**
	(1.63–4.83)	(0.873–3.75)	(0.018–0.413)	(0.026–10.4)	
PCB-99	11.0 ± 2.07 **A**	8.30 ± 2.70 **A**	0.570 ± 0.073 **B**	2.62 ± 2.14 **B**	<0.0001**
	(4.99–27.0)	(1.30–23.0)	(0.390–0.740)	(0.090–13.0)	

	Galapagos sea lion (predator)		Fish (prey)		*p*-value
	Female pups	**Male pups**	**Thread herring**	**Mullet**	
	(*n* = 10)	(*n* = 10)	(*n* = 4)	(*n* = 6)	
PCB-101	8.70 ± 1.38 **A**	4.30 ±1.38 **A**	0.630 ± 0.186 **B**	3.35 ± 2.70 **B**	0.0015**
	(4.36–18.3)	(1.79– 16.4)	(0.115–0.980)	(0.090–17.0)	
PCB-105	2.05 ± 0.630 **A**	1.30 ± 0.445 **A**	0.205 ± 0.070 **B**	0.760 ± 0.600 **B**	0.0129**
	(0.715–7.40)	(0.140–4.10)	(0.062–0.374)	(0.020–3.70)	
PCB-118	14.0 ± 3.50 **A**	9.70 ± 3.40 **A**	1.00 ± 0.170 **B**	3.80 ± 3.00 **B**	<0.0001**
	(5.70–43.0)	(1.26–32.0)	(0.710–1.46)	(0.118–19.0)	
PCB 128	2.50 ± 0.750 **A**	1.60 ± 0.570 **A**	0.180 ±0.060 **B**	0.560 ± 0.450 **B**	0.0026**
	(0.740–8.76)	(0.201–5.25)	(0.071–0.350)	(0.015–2.80)	
PCB 138/163/164	24.0 ± 6.70 **A**	15.50 ± 5.60 **A**	1.30 ± 0.360 **B**	3.30 ± 2.60 **B**	0.0005*
	(7.80–80.0)	(2.080–50.0)	(0.690–2.20)	(0.150–16.0)	
PCB 146	6.00 ± 1.40 **A**	2.80 ± 1.10 **A**,**B**	0.40 ± 0.078**B**,**C**	0.600 ± 0.460 **C**	0.0001**
	(2.10–16.0)	(0.620–11.5)	(0.210–0.570)	(0.030–3.00)	
PCB 153	35.0 ± 8.90 **A**	25.0 ± 9.80 **A**	1.60 ± 0.580 **B**	3.80 ± 3.00 **B**	<0.0001*
	(11.3–99.3)	(2.60–95.4)	(0.601–3.10)	(0.180–19.0)	
PCB-156	0.610 ± 0.137 **A**	0.40 ± 0.110 **A**	0.17 ± 0.035**A**,**B**	0.400 ± 0.320 **B**	0.0473**
	(0.170–1.60)	(0.090–1.07)	(0.075–0.240)	(0.012–1.96)	
PCB-174	0.680 ± 0.110 **A**	0.420 ± 0.096 **A**	0.090 ± 0.050 **B**	0.370 ± 0.300 **B**	0.0192**
	(0.140–1.30)	(0.100–0.860)	(0.025–0.230)	(0.014–1.80)	
PCB 180	16.0 ± 4.24 **A**	12.0 ± 4.40 **A**	1.66 ± 0.420 **B**	1.90 ± 1.50 **B**	<0.0001*
	(3.90–44.0)	(1.00–44.0)	(0.600–2.60)	(0.130–9.10)	
PCB-183	2.20 ± 0.669 **A**	1.40 ± 0.536 **A**	0.215 ± 0.072 **B**	0.440 ± 0.350 **B**	0.0008*
	(0.516–7.45)	(0.170–5.26)	0.008–0.330	0.030–2.20	
PCB 187	3.40 ± 0.812 **A**	1.45 ± 0.43 **A,B**	0.620 ± 0.130 **B**	0.930 ± 0.680 **B**	0.0007*
	(0.965–9.50)	(0.470–4.55)	(0.230–0.840)	(0.080–4.32)	
PCB 201	1.20 ± 0.515 **A**	0.60 ± 0.20 **A,B**	0.140 ± 0.04**A**,**B**	0.370 ± 0.280 **B**	0.0284*
	(0.140–5.60)	(0.050–2.00)	(0.060–0.240)	(0.030–1.80)	
PCB 202	0.355 ± 0.180 **A**	0.160 ± 0.050 **A**	0.070 ±0.020 **A**	0.120 ± 0.090 **A**	0.1597*
	(0.022–1.90)	(0.008–0.470)	0.033–0.126	0.010–0.600	
∑PCBs	136 ± 32 **A**	91.0 ± 30.0 **A**	9.35 ± 1.90 **B**	28.0 ± 22.0 **B**	<0.0001**
	(50.2–384)	(16.0–282)	(5.40–14.0)	(1.20–138)	

*Homocedastic: Welch's analysis of variances not used; **Heteroscedastic: Welch's analysis of variances used; ND = non-detectable concentration

Within the hexachlorocyclohexanes (HCHs), *β*-HCH was the only isomer detectable in all pups (>MDL). *β*-HCH was the dominant HCH isomer in blubber samples of California sea lions from Baja California (Del Toro *et al*. 2006) and in toothed cetaceans from tropical and temperate waters of the Indian and North Pacific oceans (Prudente *et al*. 1997) due to the greater biomagnification of the most bioaccumulative *β*-HCH versus *γ*-HCH (Kelly *et al*. 2007; Cullon *et al*. 2009). Interestingly, the mean *β*-HCH concentration in Galapagos sea lions was higher than the mean ∑HCH concentrations measured in spinner dolphins (*Stenella longirostris*) (21.3 µg/kg lipid) captured in a marine area of the Eastern Tropical Pacific (Prudente *et al.* 1997) in offshore waters further north of the Galapagos.

Both dieldrin and mirex were detected in all pups with concentrations ranging from 0.85 to 24 µg/kg lipid for mirex and from 9.00 to 83.0 µg/kg lipid for dieldrin. Concentrations of ∑PCBs (i.e., sum of 20 PCB congeners) ranged between 16.0 and 380 (µg/kg lipid) in pups and from 1.0 to 140 (µg/kg lipid) in fish preys (Table 5.2).

5.3.2.2 *Fish prey*

OC pesticides, including ∑DDTs, chlordanes, *β*-HCH, dieldrin and mirex, and individual PCB congeners detected in Galapagos sea lion pups were also detected (> MDL) in all sampled thread herrings and mullets. Significantly lower concentrations of OC pesticides and PCBs were found in thread herrings and mullets than in Galapagos sea lion pups (ANOVA and multi-comparisons Tukey-Kramer (HSD) post-hoc test, $p < 0.05$; Table 5.2). PCB 202 was the only congener exhibiting similar concentrations in sea lions and fish (ANOVA, $p > 0.05$), suggesting a lack of bioaccumulation in the food chain. Although thread herrings and mullets showed differences in $\delta^{15}N$ values or trophic levels and foraging strategies, concentrations of POPs in

these two fish species were similar (Figure 5.2) with the exception of mirex and *cis*-nonachlor, which were higher in planktivorous thread herrings than in mullets. Endosulfan sulphate was detected in all mullet samples ranging from 0.07 to 0.22 µg/kg lipid, with an arithmetic mean of 0.16 µg/kg lipid. Only two thread herring samples exhibited detectable concentration of this pesticide (0.002–0.05 µg/kg lipid). Endosulfan sulphate was not detected in any of the biopsy samples of pups.

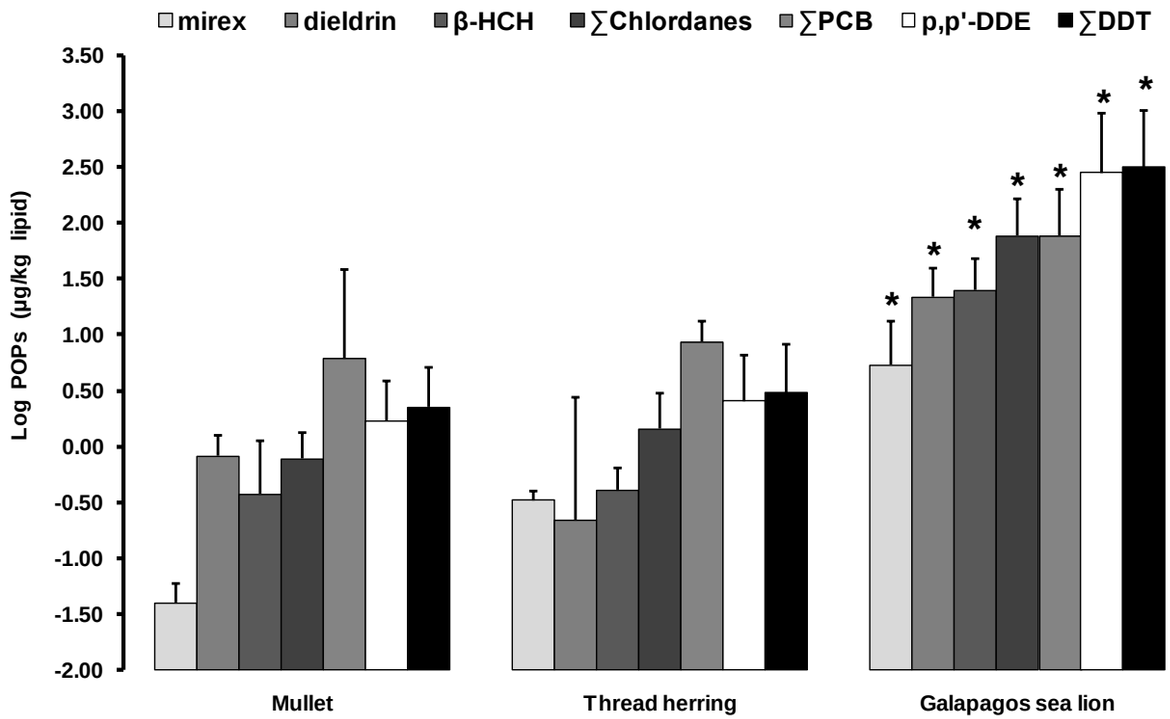

Figure 5.2 Inter-species comparisons of ∑PCBs and organochlorine pesticides (mirex, dieldrin, *β*-HCH, ∑Chlordanes, *p*,*p*-DDE, ∑DDT) concentrations. Asterisks indicate that concentration in the Galapagos sea lion were significantly higher ($p < 0.05$) than those found in mullets and thread herrings. Error bars are standard deviations.

Figure 5.3 illustrates that PCB composition in preys showed a different composition of PCB congeners compared to that of sea lions pups. Higher chlorinated PCBs, i.e., Hepta, Octa and Nona-chlorinated biphenyls (PCBs 180–201) are more abundant in thread herrings and mullets

than in Galapagos sea lion pups. This indicates the possible role of biotransformation, reduced uptake of PCBs, or a natural placental barrier for heavier PCBs in sea lions. Lower chlorinated PCB congeners, ranging from PCB 43/44 to PCB 118 (Tetra to Penta- chlorinated biphenyls), make up an important contribution (≈ 37% ± 7.25%) to the total PCB concentrations suggesting a lighter PCB signature ("equatorial fingerprint") in the Galapagos sea lion, mullet and thread herring compared to that observed in many arctic biota.

5.3.2.3 *Intersite comparisons*

The relative concentrations of contaminants observed in all sites exhibited a general common pattern, ∑DDT > ∑Chlordane > ∑PCBs > *β*-HCH> dieldrin > mirex, which was dominated by ∑DDTs, followed by chlordanes and PCBs, and secondly by *β*-HCH, dieldrin and mirex (Figure D-2 in Appendix D). Concentrations of ∑PCBs and OC pesticides detected in Galapagos sea lion pups showed no significant differences among rookeries (ANOVA for all comparisons, $p > 0.05$), as shown in Figure D-2. This might suggest a common, global source of contamination delivering POPs to the animals, and that localized sources play a little role in contributions of POPs.

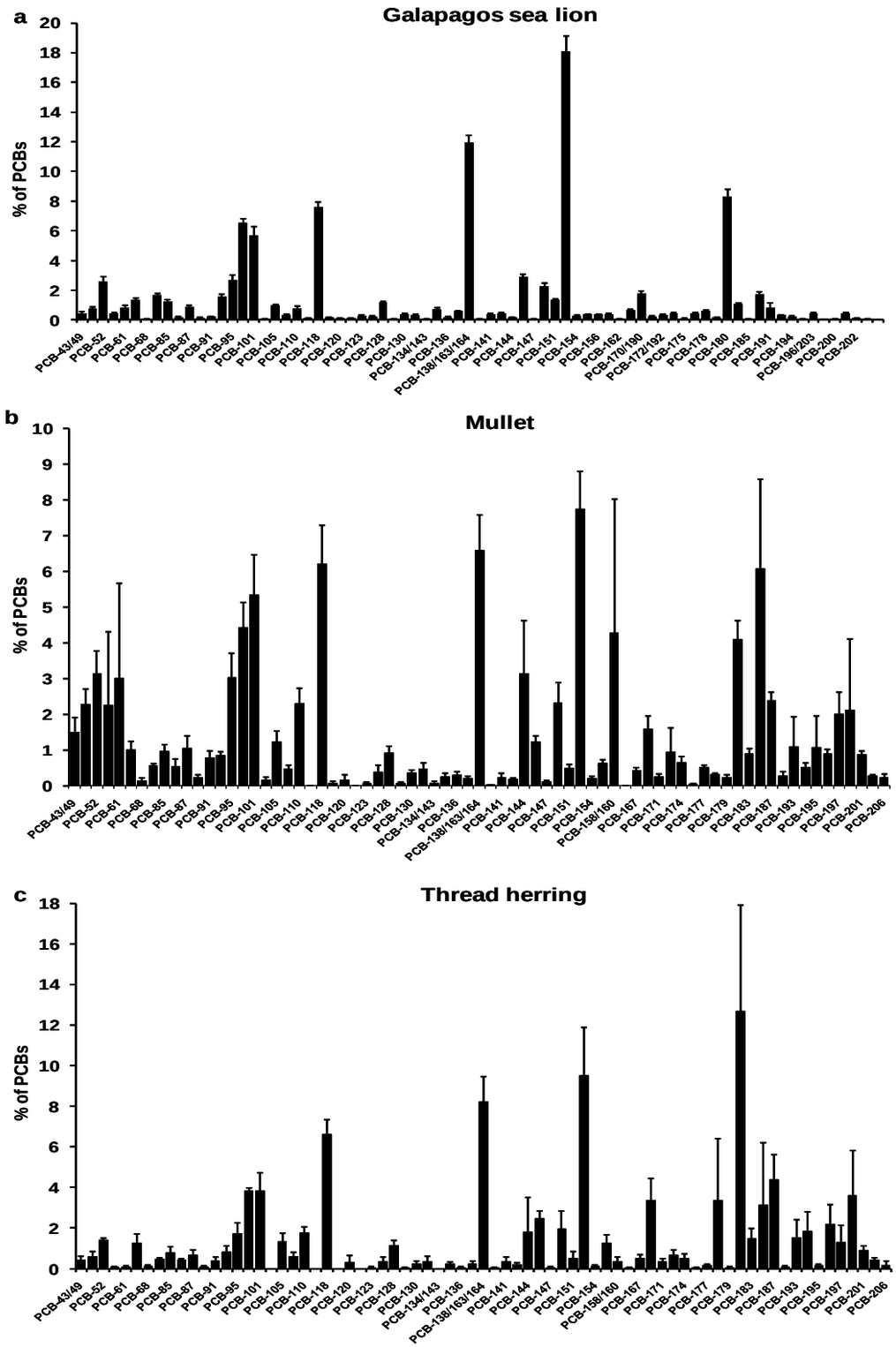

Figure 5.3 Composition of PCB congeners in Galapagos sea lion pups (a), mullet (b) and thread herring (c). Error bars are standard errors.

5.3.3 BMF measures

The results yielded from the application of the three approaches to calculate biomagnification factors in the Galapagos sea lions are provided in Table 5.3 (Figures D-5 and D-6 in Appendix D). When the BMF is calculated for the Galapagos sea lion/thread herring case, the BMF values are consistent among the methodologies used. In contrast, the three methods differed markedly from 9 to 9.5 $x10^{18}$ orders of magnitude higher for OC pesticides and from 4.8 to 1.9 $x10^{7}$ orders of magnitude higher for PCBs when the predator-prey BMF_{TL} approaches versus the conventional $C_{PREDATOR}/C_{PREY}$ ratio in the Galapagos sea lion/mullet relationship are compared. These fluctuations appear to be driven by the effect of the magnitude resulting from the differences in trophic levels. While the trophic level difference (= 1.1) between the Galapagos sea lion and the thread herring is large, the trophic level difference (= 0.11) between the Galapagos sea lion and the mullet is statistically insignificant (p <0.05) and cannot be used in the calculation of the predator-prey BMF_{TL} .Thus, the predator-prey biomagnification factor methodologies (BMF_{TL}) are sensitive to small differences in trophic levels (i.e., Galapagos sea lion-mullet). Based on this observation, the best way of expressing the BMF is the calculation of the BMF calculated as the $C_{PREDATOR}/C_{PREY}$ ratio**,** which was similar between the Galapagos sea lion/herring and Galapagos sea lion/mullet cases.

Table 5.3 Biomagnification factors (BMF), Predator-prey Biomagnification factors (BMF_{TL}) and Log Predator-prey Biomagnification factors (BMF_{TL}*) in units of kg/kg lipid for organochlorine pesticides and PCB congeners in the Galapagos sea lion.

Compound	Log K_{OW} 25-26 ºC	Log K_{OA} 37 ºC	BMF (sea lion/ thread herring)	BMF (sea lion/ mullet)	BMF_{TL} (sea lion/ thread herring)	BMF_{TL} (sea lion/ mullet)	BMF_{TL} * (sea lion/ thread herring)	BMF_{TL} * (sea lion/ mullet)
OC pesticides								
p,p'-DDE	6.93	6.96	150	220	140	2000	100	2.10E+21
p,p'-DDT	6.39	6.91	150	84.0	140	760	106	3.00E+17
p,p'-DDD	6.30	6.50	41.0	33.0	38	300	31.0	6.60E+13
∑DDT	6.41	6.91	132	180	122	1630	92.0	3.10E+20
***β*-HCH**	3.81	10.5	68.5	60.7	60	550	50.0	1.60E+16
***trans*-chlordane**	6.27	10.1	7.90	14.0	7.0	130	6.80	2.67E+10
***cis*-chlordane**	6.20	10.1	35.0	65.0	33	590	27.0	2.86E+16
***trans*-nonachlor**	6.35	10.0	80.0	177	74	1610	57.5	2.70E+20
***cis*-nonachlor**	6.08	8.38	44.0	68.0	40	615	33.0	4.30E+16
∑Chlordanes			58.0	113	54.0	1030	43.0	4.70E+18
Mirex	7.50	7.96	22.0	176	21	1600	18.0	2.50E+20
dieldrin	5.48	8.73	45.0	30.0	41	270	34.0	2.70E+13
PCBs								
PCB-52	5.9	7.64	12.5	1.21	12.0	11.0	10.0	5.80E+00
PCB 74	7.7	8.41	32.0	7.87	30.0	72.0	25.0	1.40E+08
PCB 95	7.3	8.98	8.78	1.25	8.10	11.0	7.50	7.50E+00
PCB-99	6.6	9.36	16.7	3.64	15.5	33.1	13.5	1.30E+05
PCB-101	6.3	9.11	10.3	1.90	9.53	18.0	8.66	4.20E+02
PCB-105	6.8	9.56	8.10	2.20	7.50	20.0	6.95	1.28E+03
PCB-118	6.7	8.24	12.0	3.17	11.0	29.0	10.0	3.60E+04
PCB 128	7.0	9.16	11.4	3.60	10.5	33.0	9.50	1.10E+05
PCB -138/163/164	7.2	10.0	15.0	5.90	14.0	54.0	12.0	1.10E+07
PCB-146	7.3	9.22	11.8	7.33	11.0	67.0	9.80	7.30E+07
PCB 153	6.9	9.79	19.0	7.90	18.0	72.0	15.0	1.50E+08
PCB 156	7.4	9.74	2.95	1.28	2.70	12.0	2.72	9.15E+00
PCB 174	7.0	9.62	6.05	1.50	5.60	14.0	5.30	3.90E+01
PCB 180	7.2	9.83	8.30	7.40	7.70	67.0	7.10	7.60E+07
PCB 183	7.0	9.88	8.50	4.10	7.90	38.0	7.30	4.00E+05
PCB 187	7.25	9.71	3.95	2.60	3.70	24.0	3.60	6.90E+03
PCB 201	7.1	10.3	6.26	2.35	5.80	21.0	5.50	2.40E+03
PCB 202	7.1		3.80	2.10	3.55	19.0	3.50	9.40E+02
∑PCBs			12.0	4.10	11.0	37.0	10.0	3.65E+05

5.3.4 Biomagnification Factors

The interpretation of the data resulting from the use of biomagnification factors are focused on BMF and BMF_{TL} as the BMF_{TL}* was used in this study as an optional approach for evaluation of BMF methods (Section 5.3.3).

BMF. Calculated biomagnification factors of OC pesticides and PCB congeners are shown in Table 5.3. The BMF of OC pesticides ranged from 7.9 (*trans*-chlordane) to 150 (*p*,*p*'-DDT) kg/kg lipid in Galapagos sea lion/thread herring, and from 14 (*trans*-chlordane) to 220 (*p*,*p*'-DDE) kg/kg lipid in Galapagos sea lion/mullet. BMF values for PCBs were lower, ranging from 2.9 (PCB 156) to 32 (PCB 74) kg/kg lipid in Galapagos sea lion/thread herring and from 1.2 (PCB 52) to 7.9 (PCB 153) kg/kg lipid in Galapagos sea lion/mullet. No relationship was found between the BMF of OC pesticides and log K_{OW} (Figure 5.4a,b). Table 5.3 shows that the BMF_{TL} of OC pesticides ranged from 7.3 (*trans*-chlordane) to 140 (*p*,*p*'-DDT) kg/kg lipid in Galapagos sea lion/thread herring and from 130 (*trans*-chlordane) to as high as 2000 (*p*,*p*'-DDE) kg/kg lipid in Galapagos sea lion/mullet, while BMF_{TL} for PCB congeners ranged from 2.7 (PCB 156) to 30 (PCB 74) kg/kg lipid in Galapagos sea lion/thread herring, and from 11 (PCB 52) to 72 (PCB 153) kg/kg lipid in Galapagos sea lion/mullet (Table 5.3).

BMF_{TL}. In a similar fashion to BMF, no correlation was found between the BMF_{TL} of OC pesticides and K_{OW} (Figure 5.5a,b). BMF_{TL} values decrease for some pesticides (e. g., *p*,*p*'-DDD; trans-chlordane) when a K_{OW} of $10^{5.5}$ or $10^{6.0}$ is exceeded. As a function of the octanol-air partition coefficient (K_{OA}), both the BMF and the BMF_{TL} for OC pesticides increased markedly as the K_{OA} increased from $10^{6.5}$ to $10^{6.9}$, and then dropped for the rest of pesticides as K_{OA} exceeds $10^{7.0}$ (Figures 5.4c,d and 5.5c,d).

No correlation was found between the BMF or BMF_{TL} of PCBs and log K_{OW} for the Galapagos sea lion/thread herring feeding relationships (Figures 5.6a,b and 5.7a,b). BMF and BMF_{TL} of PCBs showed different trends when looking a different prey items in terms of K_{OA}. While no correlation was found between the BMF or BMF_{TL} of PCBs and log K_{OA} in the Galapagos sea lion/ mullet relationship (Figures 5.6d and 5.7d), BMF and BMF_{TL} for PCBs increased as the K_{OA} increased from $10^{7.6}$ to $10^{8.4}$, and afterwards the biomagnification factor appeared to decrease gradually with increasing log K_{OA} in the Galapagos sea lion/thread herring relationship (Figures 5.6c and 5.7c).

When comparing the plots of BMF and BMF_{TL} of PCBs versus log K_{OW} or versus log K_{OA} similar patterns were observed for both Galapagos sea lion/thread herring or Galapagos sea lion/mullet feeding relationships (Figures 5.6 and 5.7). This is explained by the strong correlation usually observed between log K_{OA} and log K_{OW} of PCBs (Gobas *et al*. 2003).

These observations demonstrate that these halogenated substances biomagnify and achieve concentrations in Galapagos sea lions that exceed those in their prey, although physiological processes and biotransformation may limit the biomagnification of some contaminants.

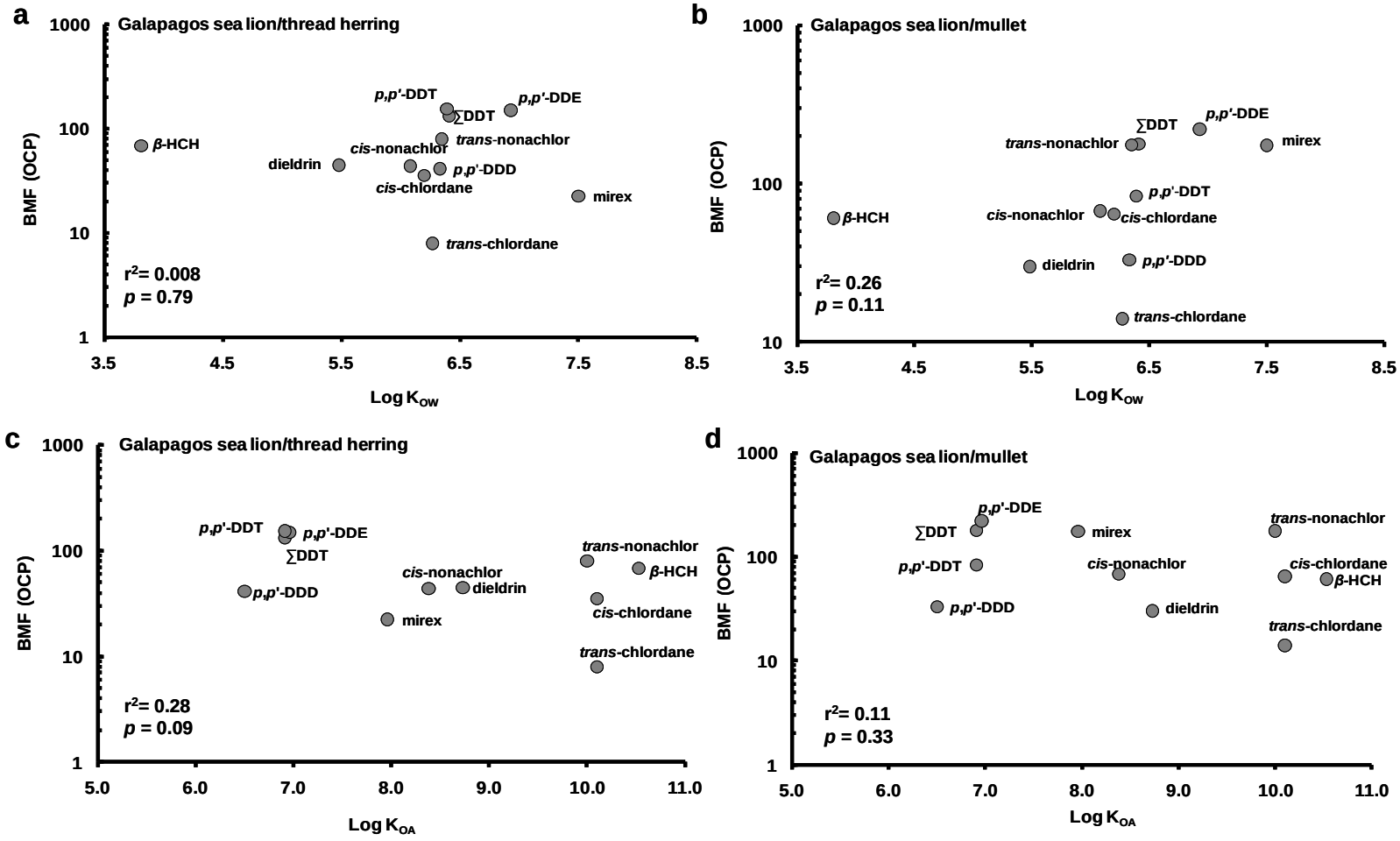

Figure 5.4 Biomagnification factor (BMF) in the Galapagos sea lion as expressed by the concentration ratios sea lion/thread herring (a,c) and sea lion /mullet (b, d) relative to mullet and thread herring for OC pesticides as a function Log K_{OW} (a, b) and Log K_{OA} (c, d).

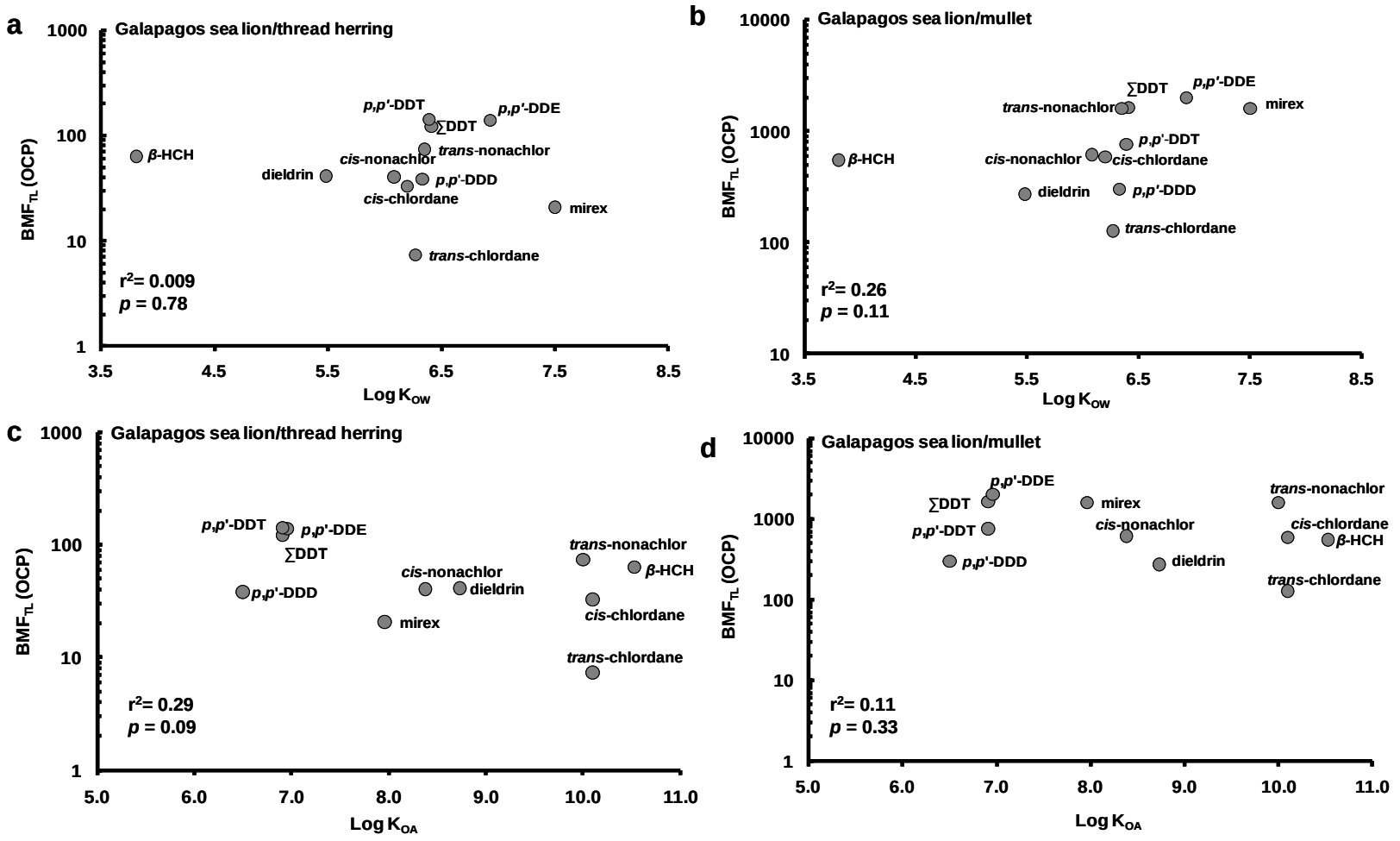

Figure 5.5 Predator-prey biomagnification factors (BMF_{TL}) in the Galapagos sea lion as expressed by the concentration ratios sea lion/thread herring (a,c) and sea lion/mullet (b, d) relative to mullet and thread herring for OC pesticides as a function of Log K_{OW} (a, b) and Log K_{OA} (c, d).

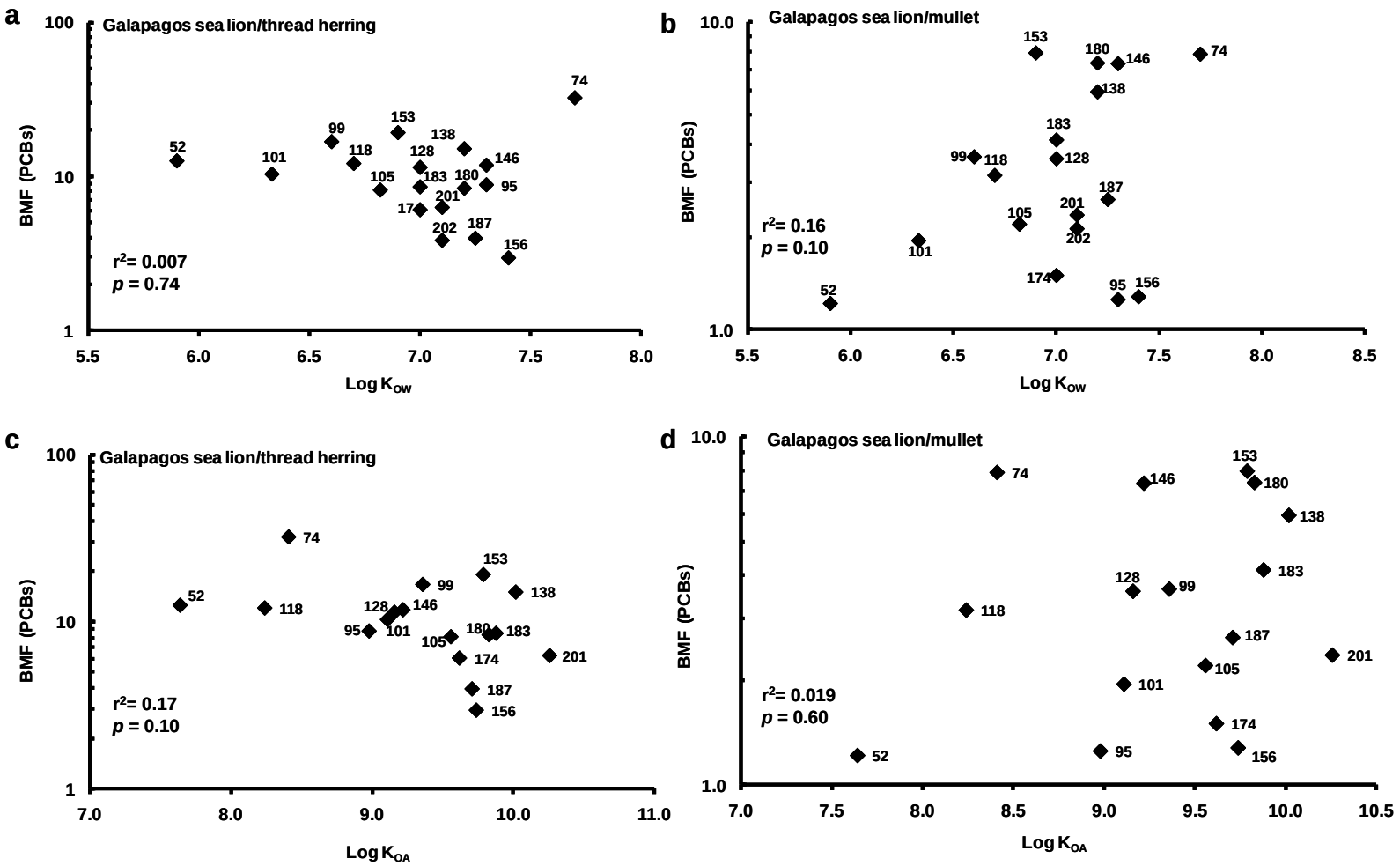

Figure 5.6 Biomagnification factor (BMF) in the Galapagos sea lion as expressed by the concentration ratios sea lion/thread herring (a,c) and sea lion /mullet (b, d) relative to mullet and thread herring for PCBs as a function of Log K_{OW} (a, b) and Log K_{OA} (c, d). Numbers are PCB congeners based on the IUPAC system

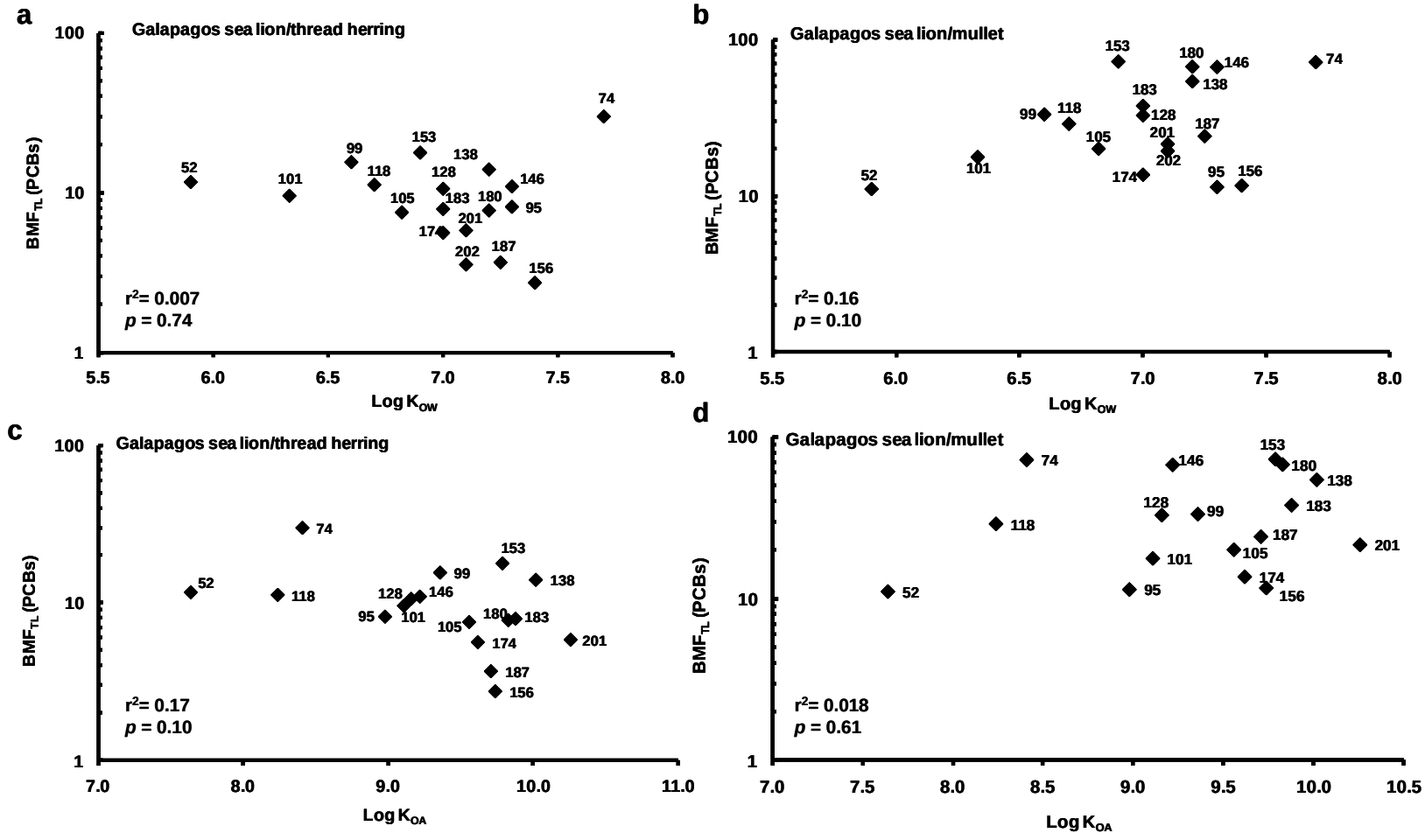

Figure 5.7 Predator-prey biomagnification factors (BMF_{TL}) in the Galapagos sea lion as expressed by the concentration ratios sea lion/thread herring (a,c) and sea lion/mullet (b, d) relative to mullet and thread herring for PCBs as a function of Log K_{OW} and Log K_{OA}. Numbers are PCB congeners based on the IUPAC system.

The BMF and BMF_{TL} for organochlorine pesticides expressed by the concentration ratios sea lion/thread herring and sea lion/mullet of the Galapagos sea lion are higher than those reported for harp seals (*Pagophilus groenlandicus*) from the contaminated Barents Sea (Borga *et al*. 2004), with the exception of the BMF of *p*,*p*'-DDE which is similar for the Galapagos sea lions (BMF = 220) and for the harp seal (BMF = 250) (Table 5.4). However, the BMF and BMF_{TL} for PCBs of the Galapagos sea lion are lower than those reported for harp seals. This indicates the biomagnification predominance of organochlorine pesticides in tropical-equatorial regions versus the predominant biomagnification of PCBs in Arctic regions. To further explore these comparisons, the ratio of the BMF for *p*,*p*'-DDE (the DDT dominant metabolite) to the BMF for PCB 153 (used here as the most recalcitrant PCB congener) was calculated for both species of pinnipeds and then compared. As shown in Table 5.4, the ratio *p*,*p*'-DDE BMF/PCB 153 BMF was much higher in the Galapagos compared to that of the Barents Sea, which is driven by the predominance of *p*,*p*'-DDE biomagnification in the Galapagos. Vapor pressures of organic contaminants are expected to be higher in tropical systems due to warmer/higher temperature in comparisons to cold/lower temperature in the Arctic; and, therefore, higher thermodynamic gradients are likely to occur during the trophic transfer of contaminant mass from prey to predator, resulting in a high biomagnification factor.

Table 5.4 Comparison of BMF and BMF_{TL} for remote marine food chains between the Galapagos Islands and Arctic regions for selected organochlorine pesticides and PCBs. BMF and BMF_{TL} for Galapagos sea lions are expressed as the range of concentration ratios of both sea lion/thread herring and sea lion/mullet feeding relationships.

	Galapagos Islands (Ecuador) **Galapagos sea lion**		**Barents Sea** **Harp seal**[a]	
	BMF	BMF_{TL}	BMF	BMF_{TL}
***p*,*p*'-DDE**	150−222	139−2014	21.3−250	319
***p*,*p*'-DDT**	83.6−154	142−760	5.4	NR
∑DDT	132−179	122−1631	11.0	NR
***β*-HCH**	60.7−68.5	63.0−552	3.0−4.3	4.1
***cis*-chlordane**	35.3−64.6	32.7−587	0.2	NR
***trans*-chlordane**	7.93−14.0	7.34−128	0.2	NR
***trans*-nonachlor**	79.6−177	73.7−1609	8.2−111	141.7
∑Chlordanes	58.4−113	54.1−1029	4.5	NR
PCB 52	1.21−12.5	11.0−11.6	4.1	NR
PCB 99	3.64−16.7	15.5−33.1	19.7−115.0	147.0
PCB 101	1.94−10.3	9.53−17.7	5.7	NR
PCB 105	2.20−8.11	7.51−20.0	6.2−14.0	18.1
PCB 118	3.17−12.1	11.2−28.8	9.7−33.0	41.6
PCB 138	5.93−15.0	13.9−53.9	22.1−256.0	327.7
PCB 153	7.94−19.1	17.7−72.2	22.1−325	416
PCB 180	7.36−8.33	7.72−66.9	17.8	NR
∑PCBs	4.09−12.1	11.2−37.2	NR	NR
Ratio BMF *p*,*p*'-DDE to BMF PCB 153	7.85−28.0	7.85−27.9	0.77−0.96	0.77

NR= non reported
[a](Borga *et al*. 2004)

5.3.5 Biomagnification Behaviour of POPs

Trophic magnification is driven by the diet's contaminant concentrations in top predators, which further shape the composition of contaminants through toxicokinetics processes (i.e., uptake, metabolism, respiration and excretion), influencing the persistence and food-web biomagnification of POPs. In this study, the biomagnification capacity of organochlorine contaminants in the tropical food chain of the Galapagos sea lion is established (i.e. $C_{PREDATOR} > C_{PREY}$, BMF > 1). At the organism level, gastrointestinal magnification of contaminants is the driven force and mechanism explaining gastrointestinal uptake, accumulation and biomagnification of organic chemicals in food chains (Gobas *et al*. 1993; Kelly and Gobas 2003).

Various factors affect contaminant exposure and accumulation in predators, including complex ecologies and physiologies, feeding preferences, life history parameters (sex, age, body size and corporal condition), reproduction, geographic locations and stochastic-climatic events. Due to these factors, it is complex to elucidate whether a wild predator is at a steady state with its diet; therefore, calculated BMFs may not always reflect actual biomagnification (Christensen *et al*. 2009). As shown in this study, BMFs revealed the biomagnification capacity of POPs in the food chain of the Galapagos sea lions, which is an apex predator possessing flexible feeding preferences (dietary plasticity).

Efficient uptake and dietary assimilation and slow depuration/excretion rates of these compounds (PCBs with K_{OW} ranging 10^5–10^7, and OC pesticides K_{OW} ranging $10^{3.8}$–$10^{7.0}$) explain the high degree of biomagnification in the Galapagos marine food chain. Dietary absorption efficiencies of Penta and Hexachlorobiphenyls are typically between 50-80% in fish and 90-100% in mammals (Kelly *et al*. 2004) and chemical half-lives ($T_{1/2}$) or recalcitrant PCBs such as PCB 153

in organisms exceed 1000 days (Mackay *et al*. 1992). The comparative analysis of BMF estimates of PCBs and OC pesticides (Figures 5.9 and 5.10) indicates that OC pesticides and PCBs are accumulated by fish and sea lions and also biomagnify in the food chain. Based on contaminants' BMFs, the DDT metabolites, *p*,*p*'-DDT and *p*,*p*'-DDE, followed by *trans*-nonachlor (Figures 5.4 and 5.5), are the most bioaccumulative pesticides, while PCB 74 and 153 are the most bioaccumulative PCB congeners in the Galapagos sea lion (Figures 5.6 and 5.7). The less bioaccumulative compounds are *trans*-chlordane and PCB 156.

Of particular attention is the biomagnification behaviour of *β*-HCH with a $K_{OW} < 10^4$ ($K_{OW} = 10^{3.8}$; Figures 5.4a, b and 5.5a, b), but with a K_{OA} of $10^{8.9}$—$10^{10.5}$ (Figures 5.4c, d and 5.5c, d), contrasting with the regulatory criteria and current management policies for POPs that consider only chemicals with K_{OW} values $>10^5$ as bioaccumulative substances. The biomagnification factors (BMF = 60.7−68.5) and predator-prey biomagnification factors (BMF_{TL} = 63—552) of *β*-HCH in Galapagos sea lions exceed equivalent biomagnification factors of PCB 153 (BMF =7.94 —19.1; BMF_{TL} =18.0—72.2) and PCB 74 (BMF = 7.9—32.2; BMF_{TL} =30.0−72.0), as shown in Table 5.3. This portrays that *β*-HCH, a relatively hydrophilic and nonmetabolizable chemical, biomagnifies in the tropical marine mammalian food chain of an air breathing organism (the Galapagos sea lions), which is explained by the relatively high K_{OA} of *β*-HCH ($K_{OA} > 10^{7.0}$) and its negligible respiratory elimination. Biomagnification of *β*-HCH was evident in the lichen-caribou-wolf terrestrial food chain, in the maritime and interior grizzly bears' food chains, and in a marine mammalian food web (including water-respiring and air-breathing organisms) from temperate regions of Canada and the Canadian Arctic (Kelly *et al*. 2003; Christensen *et al*. 2005; Kelly *et al*. 2007).

5.3.6 Potential sources and pathways of contaminants

Lack of significant differences and consistent uniformity of PCBs and OC pesticides, particularly for PCBs (see Chapter 3), among sites might indicate common source of contamination. Possible sources and origin of PCBs and DDTs in the islands were discussed in more details in Chapters 3 (see also Alava *et al*. 2009) and 4.

Furthermore, Principal Components Analysis represented a more comprehensive approach for exploring spatial differences and behaviour of POPs. The two first principal components (i.e., PC 1 and PC2) accounted for 55.2% of the total variation in Galapagos sea lion pups. PCA score plot results for the 2008 data further revealed that contaminants follow a similar trend, aggregated near to the centre of the axes, among sites, showing lack of discrimination and differentiation in contaminant patterns (Figure 5.8a). The first principal component (i.e., loading plots, PC1: 40.1% of the total variance) segregated in a significant degree the heavier PCB congeners (upper and lower left quadrants) from the lighter PCBs (upper and lower right quadrants; as seen in Figure 5.8b). A high positive PC1 score was correlated with higher percentages of low chlorinated PCBs (e.g., PCBs 43/49, 47/48/49, 52, 60, 61, 66, 74, 85, 86/97,87, 92, 95, 101, 110, 123, 132, 135, 136, 141, 144, 149) and *p*,*p*'-DDD, *p*,*p*'-DDT, dieldrin, *cis*-nonachlor, *trans*-chlordane, *cis*-chlordane and *β*-HCH, while a high negative score in PC 1 (upper and lower left quadrant) was correlated with a lower proportion of heavily and several, more persistent chlorinated PCBs (e. g. PCBs 118, 138/163/164, 137, 153, 158/160, 171, 177, 180, 183, 170/190, 172/192, 193, 194, 195, 196/203, 201, 202), as well as the semi-volatile and more bioaccumulative *p*,*p*'-DDE. These patterns show that PC1 appeared to be related to vapour pressure (Henry's Law constant or H) due to a high contribution of more volatile halogenated contaminants (pesticides) and less chlorinated (lighter) PCB congeners. A significant correlation is also observed between the log of

the Henry's law constant (Log H) for the PCBs and PC1 (the variable loadings of the first principal component; $p < 0.05$, $r = 0.27$; Figure 5.9), suggesting that log H represents an important factor influencing the transport pathways and partitioning of PCB mixtures in remote environments; and, therefore, affecting the ultimate composition pattern observed in Galapagos sea lions. The Henry's law constant for each PCB is a fundamental parameter that represents the air-water equilibrium partitioning between surface waters and the atmosphere (Fang *et al.* 2006). This indicates that local sources of exposure for high chlorinated PCBs are minimal in the Galapagos and that most of the contamination by POPs is coming from common atmospheric or continental sources.

Dieldrin is a metabolite of aldrin, which was used for agriculture and public health purposes at beginning of the 1950s until its production was cancelled in 1989 in North America, but as with other pesticides, it continues to enter the environment via erosion of soils contaminated in the past and atmospheric deposition (ATSDR 2002). Mirex is a very unreactive and hydrophobic insecticide that was used in North America to control fire ants and as a fire retardant, persisting in the environment due to chronic small inputs from the atmosphere (Sergeant *et al.*1993). The presence of this compound in these blubber samples might be related to the past use of mirex in continental Ecuador (Solórzano *et al*. 1989) due to the possible use as insecticide (bait) to control invasive ants in the Galapagos and continental Ecuador.

β-HCH is a major constituent of technical HCHs, which is likely one of the sources of this residue. Another potential source of *β*-HCH can be lindane (i.e., γ-HCH) since this pesticide is currently being used in several countries in the southern hemisphere as evidenced by its detection in blubber samples of southern elephant seals and minke whales (*Balaenoptera acutorostrata*) from the Antarctic Ocean (Miranda-Filho *et al*. 2007; Aono *et al.* 1997). At the continental coast of

Ecuador, lindane has recently been detected in sediments and aquatic organisms from the Taura River in the Gulf of Guayaquil (Montaño and Resabala 2005). The atmospheric influx of HCHs source formulations used in the Asian and South American tropics (i.e., lindane) and North America (i.e. technical HCH) might explain the incidence of *β*-HCH in these samples.

No clear records of use of legacy OC pesticides exist for the Galapagos, although anecdotic accounts pointed out the use of CUP for agriculture (Dr. Alan Tye, former Head Scientist, Department of Plant and Invertebrate Science, Charles Darwin Foundation, Galapagos Islands), and the use of DDT to eliminate introduced rats in the Galapagos by the US Navy during the 1940s and 1950s (Dr. M. P. Harris, Centre for Ecology and Hydrology, Banchory, UK).

The long range atmospheric transport coupled with global fractionation have usually been described as the major mechanism delivering POPs from lower or mid latitudes to the polar regions (Wania and Mackay 1993; Iwata *et al*. 1993; Iwata *et al*. 1994), but it is likely that a similar mechanism or redistribution from mid latitudes may be also expanding or delivering volatile or semi-volatile pesticides such as HCHs and DDTs to isolated islands around the equator (i. e. the Galapagos Archipelago). These observations suggest that the contamination by organochlorine pesticides might be coming from both local and continental sources due to pesticides used either currently or in the recent past in countries in the southern hemisphere (Blus 2003; Miranda-Filho *et al*. 2007). Trans-Pacific air pollution of contaminants from tropical Asia to the eastern Pacific (Iwata *et al*. 1993; Wilkening *et al*. 2000) cannot be ruled out as a global and common pathway of POPs of atmospheric origin.

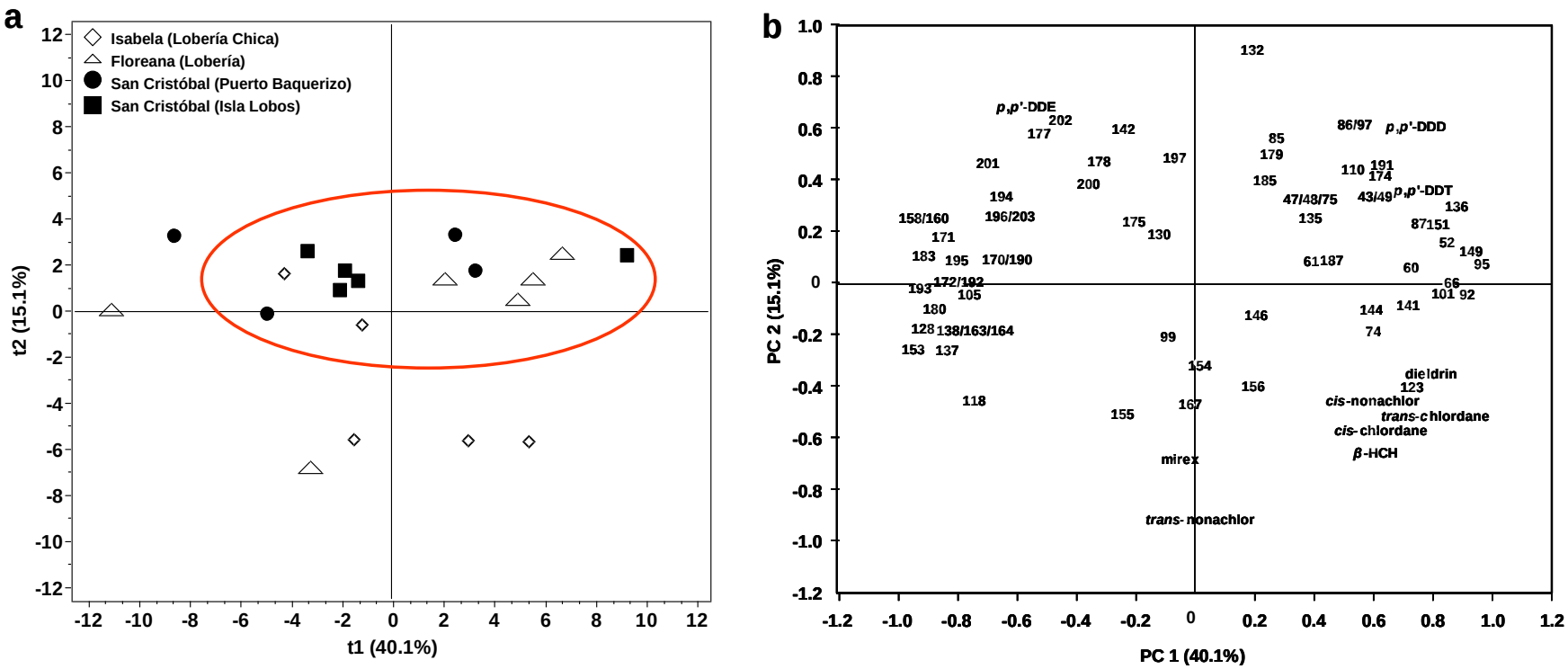

Figure 5.8 Principal components analysis where the variance accounted for by each principal component is shown in parentheses after the axis label: (a) score plots for patterns of POPs for the first two principal components shows that most of the pups from different rookeries have a similar contaminant pattern, as demonstrated here by the sample scores plot (t1 and t2) of 20 individuals; b) loadings plots (PC1 and PC2) showing values of individual PCB congeners and pesticides in Galapagos sea lion pups, where numbers are PCB congeners based on the IUPAC system

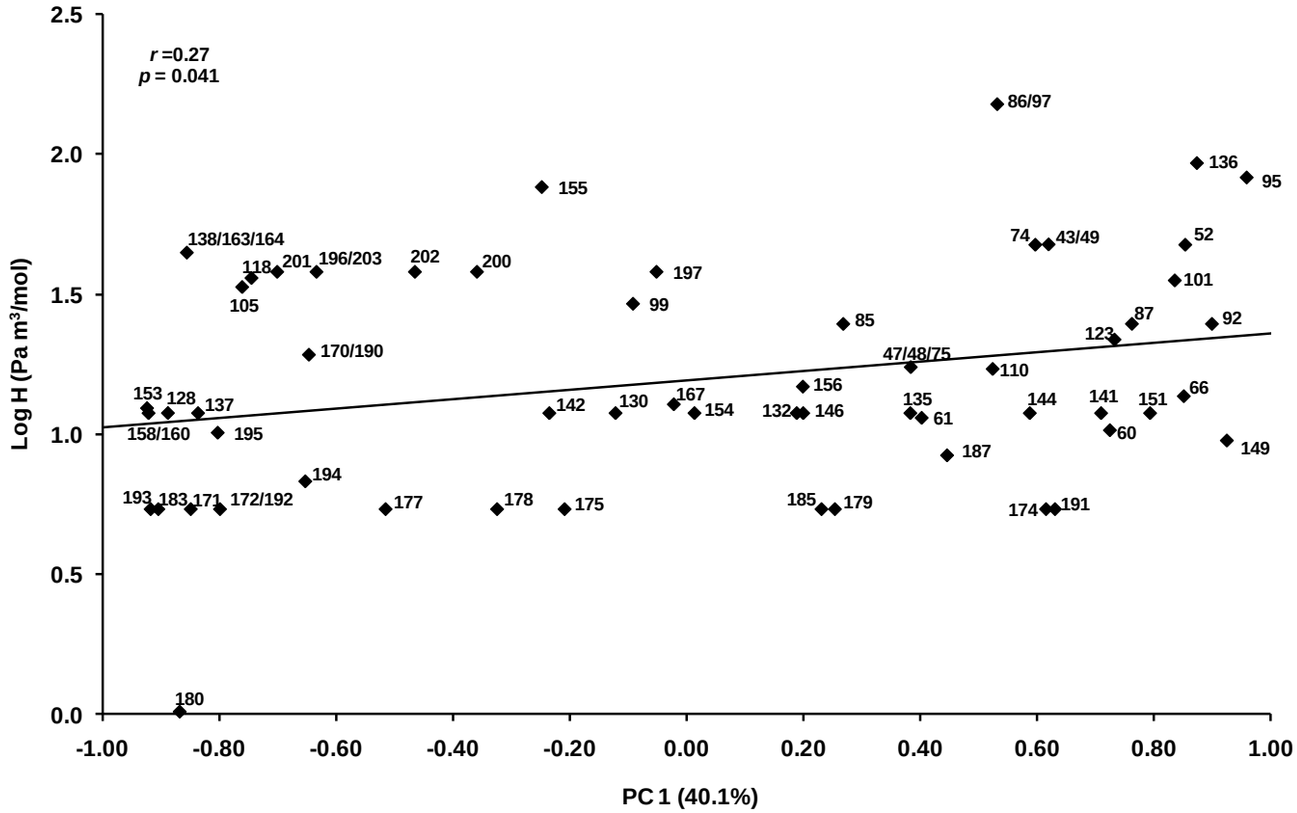

Figure 5.9 Relationship between the Henry's law constant (Log H) for polychlorinated biphenyl (PCB) congeners and the first principal component (PC1). PC1 is significantly correlated with Log H for PCB congeners, suggesting that Galapagos sea lions from the remote Galapagos Islands are more exposed to light PCB mixtures, consistent with atmospheric signals. Numbers are PCB congeners based on the IUPAC system.

5.4 Conclusion

Based on this study, it is concluded that POPs biomagnify in a significant degree in a tropical marine food chain of the Galapagos Islands. This has important implications for management and control of organochlorine pesticides in tropical regions. While the concentrations of DDT and associated health risks in wildlife are generally believed to be declining, this may no longer be the case in tropical countries where DDT is increasingly used and can biomagnify in food chains. A renewed use of DDT to combat malaria is likely to increase DDT concentrations in

the Southern Hemisphere and put in particular bird and marine mammal populations at greater risk due to the biomagnification of these substances in their food webs.

The use of different biomagnification factor measures showed that BMF_{TL} and BMF_{TL}* are more appropriate to assess biomagnification if differences in trophic levels of predator/prey relationships are large (i.e., >1). The use of trophic magnification factors (TMFs) is currently an emerging approach to better assess the biomagnification of POPs in marine food webs (Borga *et al.* 2011). An important number of studies in the northern hemisphere have relied on the use of the TMF for this purpose (Fisk *et al*. 2001; Hop *et al*. 2002; Hoekstra *et al*. 2003; Houde *et al*. 2008; Kelly *et al.* 2008). Thus, the use of TMF coupled with stable isotope analysis (SIA) to track the amplification and transport of POPs in food webs is a recommended methodology in eco-toxicology to study the biomagnification of POPs. The lack of prey samples and minimal trophic levels required (≥ 3) preclude our undertaking a trophic magnification factor study at the moment. Therefore, additional research and field sampling efforts may include other organisms integrating the trophic guilds of the Galapagos sea lion food web by measuring legacy and new POPs, stable isotopes and subsequent estimations of trophic levels. This will allow assessing in a higher degree the trophic biomagnification of these substances in the remote Galapagos Islands

This study provides sound scientific information on food chain contamination in the Galapagos that can be used for conservation plans of endangered and endemic species, and portrays the implications for environmental management and control of bioaccumulative, persistent and toxic contaminants (e. g. DDT) and the use of more environmental friendly and alternative substances to control pests and vectors in developing countries.

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CHAPTER 6

POLYBROMINATED DIPHENYL ETHERS AND POLYCHLORINATED BIPHENYLS IN STELLER SEA LIONS (*EUMETOPIAS JUBATUS*) FROM BRITISH COLUMBIA, CANADA

Abstract: We measured polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in blubber biopsy samples collected from 22 live-captured migratory Steller sea lions (*Eumetopias jubatus*) that were feeding in the Strait of Georgia, British Columbia, Canada. ∑PBDE ranged from 50 µg/kg (lipid weight) in adult females to 3780 µg/kg in subadult individuals, while ∑PCBs ranged from 272 µg/kg in adult females to 14,280 µg/kg in subadult individuals. While some PBDE and PCB congeners were transferred through placenta and milk to fetus and pup, PCBs with log K_{OW} > 7.0, as well as BDE 49, were constrained. The ratio of individual PCB congeners by metabolic group (Groups I, II, III, IV and V) to PCB-153 regressed against length of males suggested poor biotransformation of these compounds (i.e., slopes were not significantly different from zero, $p > 0.05$). The dominance of the single congener, BDE-47 (64% of total PBDEs) reduced our ability to explore congener-specific dynamics of PBDEs in these pinnipeds. With 80% of our Steller sea lions exceeding a recent toxicity reference value for PCBs, the fasting-associated mobilization of these contaminants during their annual migration raises questions about a heightened vulnerability to adverse effects.

***Keywords*: Steller sea lions; PBDEs, PCBs; metabolism, accumulation; British Columbia.**

6.1 Introduction

Persistent organic pollutants (POPs) represent a threat to marine mammals due to their recalcitrance, bioaccumulative nature and toxicity. Polychlorinated biphenyls (PCBs) are legacy industrial POPs that were banned during the late 1970s in North America and are subject to the terms of the Stockholm Convention. More recently, polybrominated diphenyl ethers (PBDEs) have emerged as a significant concern, having been extensively used as flame retardants in foams, textiles, coatings, furniture, construction materials, electronic devices, plastics and paints since the 1970s (de Boer *et al*. 1998; de Wit 2002; Alaee *et al*. 2003). There are three commercial PBDE products, including the penta-BDE, octa-BDE and deca-BDE formulations (La Guardia *et al*. 2006). In Europe and North America, production of two PBDE products (penta- and octa-BDE formulations) ceased in 1998 and 2004, respectively. The third (deca) formulation was recently banned in Europe and Canada, and is subject to some state-based bans in the U.S. (La Guardia *et al*. 2006; Ross *et al*. 2009; de Boer *et al*. 2009; Birnbaum *et al*. 2009). The treta, penta, hexa and heptabromodiphenyl mixtures are currently classified as POPs under the terms of the Stockholm Convention, and the octa BDE formulation may be added eventually to the list of banned POPs (de Boer *et al*. 2009).

Despite having been banned, PCBs are still found at high concentrations in some marine biota of northern hemisphere (Kelly *et al*. 2007; Hall and Thomas 2007). The NE Pacific Ocean is no exception, with very high PCB concentrations having been observed in killer whales, *Orcinus orca* (Ross *et al*. 2000; Ylitalo *et al*. 2001) and to a lesser extent harbour seals, *Phoca vitulina* (Ross *et a*l. 2004). PBDEs have also been detected in marine mammals from the NE Pacific Ocean, although at lower concentrations than the PCBs (Rayne *et al*. 2004; Krahn *et al*. 2007).

High levels of POPs have been implicated in adverse effects on immune and endocrine systems of marine mammals, with the PCBs, in particular, being of concern (Ross *et al*. 1995; Ross *et al*. 1996; DeGuise *et al*. 1998; Simms and Ross 2000; Tabuchi *et al*. 2004; Mos *et al*. 2006; Mos *et al*. 2010). While many of the measured endpoints are considered sub-lethal, the fitness of individuals is also being affected. High levels PCBs have been associated with a high prevalence of neoplasms and carcinoma, causing mortality in California sea lions, *Zalophus californianus* (Ylitalo *et al*. 2005). While less is known about the toxicity of PBDEs, this flame retardant has been implicated in carcinogenicity and the disruption of steroid and thyroid hormones (Meerts *et al*. 2000; Meerts *et al*. 2001; Hallgren and Darnerud 2002).

The Steller sea lion (*Eumetopias jubatus*) is a piscivorous pinniped that inhabits the Pacific coastal waters of Canada, the USA and Asia. There are two populations, with the Eastern and Western stocks being genetically distinct and geographically separated at approximately 145° W longitude (Bickham *et al*. 1996). While the eastern stock is considered stable, the western stock has declined during the last 30 years across its entire range. In addition to the hypotheses involving nutritional stress and shifts in ocean–climate, which might explain this decline (Rosen and Trites 2000; Tites *et al*. 2007), contaminants have also been suggested as a possible contributing factor (Barron *et al*. 2003). While low to moderate concentrations of PCBs have been observed in Steller sea lions from both the declining western stock (Varanasi *et al*. 1992; Lee *et al*. 1996; Krahn *et al*. 1997; Krahn *et al*. 2001), as well as some animals from the eastern stock (Krahn *et al*. 1997; Krahn *et al*. 2001), localized PCB hotspots may reflect historical contamination by military installations. To date, there have been no reports of PCBs or PBDEs in Steller sea lions from British Columbia and adjacent southern coastal US states.

The total Steller sea lion population in British Columbia during the breeding season is estimated to be approximately 20,000 individuals, including pups, breeding and non-breeding animals, with an overall growth rate of 3.5% per year (Olesiuk 2008). Of these, approximately 3,000 Steller sea lions migrate into the waters off southern Vancouver Island and into the Strait of Georgia (Olesiuk 2004; A. Trites, pers. comm.). Although the British Columbia population has been increasing, Steller sea lions are listed as of "Special Concern" under the terms of the Species at Risk Act (SARA) because of human disturbance, oil spills and environmental contaminants (COSEWIC 2003; Olesiuk 2008).

As part of a larger effort to characterize the feeding ecology of Steller sea lions frequenting the Strait of Georgia, British Columbia, 22 animals were live-captured and telemetry devices attached prior to release (Jeffries *et al*. 2004; North Pacific Universities Marine Mammal Research Consortium 2006). This provided a valuable opportunity to evaluate contaminants and to characterize this potential conservation threat.

6.2 Materials and Methods

6.2.1 Capture and Sampling

Free-ranging Steller sea lions were live-captured at Norris Rock in the Strait of Georgia, British Columbia, Canada, in February 2005 and January 2006, using a floating mobile trap described elsewhere (Jeffries *et al*. 2004). After capture, sea lions were moved into a transfer cage and weighed, and then moved into a squeeze cage, where they were physically immobilized. Valium was administered (0.02 to 0.11 mg/kg, mean dosage of 0.06 mg/ kg) intramuscular around the shoulder area to those individuals upon which telemetry devices were being installed. Valium was

given 10-20 minutes prior to general anesthetic using isoflorane, administered via a cone over the head. Intubation of the stomach was performed if a stomach sensor was to be inserted. Monitoring was done with a Heska G2Digital pulse-oximeter and temperature probe.

Blubber biopsy samples were collected from 22 individuals, including a freshly aborted fetus, pups (*n* =3), subadults (*n*=10), adult females (*n*=6) and adult males (*n*=2). Blubber samples were obtained with a 6 mm-biopsy punch from a cleansed (betadine and isopropyl alcohol) site 20 cm lateral to the spinal column and anterior to the pelvis as described elsewhere (Tabuchi *et al*. 2004; Mos *et al*. 2006). These samples were temporarily stored on wet ice in the field, frozen within 4 hours and stored at –80º C at the Institute of Ocean Sciences (Fisheries and Ocean Canada) until further analysis.

We defined a nursing pup as an individual with an estimated age of 0—1.5 years (the deceased pup was a known-age 1.5 year individual, WDFW0206-01, which had milk in its stomach at the time of sampling). Nursing dependency can last up to three years in Steller sea lions (Pitcher and Calkins 1981). Sampling data, including dates, age and sex categories, morphometrics and lipid content are reported in Table 6.1.

Table 6.1 Life history and collection data of Steller sea lions captured at Norris Rock, Strait of Georgia, British Columbia, Canada.

Code ID	Capture Date	Weight (kg)	Length (cm)	Girth (cm)	Age class	Age (years)	Sex	Blubber sample size (g)	% Lipid
WDFW 0206-02	02/08/06	2	NR	NR	fetus[a]	<0.0	M	0.158	8.51
WDFW 0206-01	02/07/06	91	NR	NR	pup[b]	1.5	F	0.441	83.4
EJ 05-07	01/28/05	115	171	127	pup	1.5	F	0.08	24.3
EJ 05-03	01/26/05	101	162	117	pup	1.5	M	0.049	27.0
EJ 05-04	01/28/05	221	214	153	subadult	3.5	M	0.074	27.0
EJ 05-05	01/28/05	233	222	155	subadult	4.5−5.5	M	0.089	9.44
EJ 05-09	01/28/05	164	207	148	subadult	2.5	M	0.068	1.94
EJ 05-13	02/09/05	214	219	137	subadult	3.5	M	0.092	30.5
EJ 05-14	02/09/05	270	242	164	subadult	5.5	M	0.112	13.5
EJ 05-15	02/09/05	166	207	132	subadult	2.5-3.5	M	0.084	18.5
EJ 05-17	02/10/05	195	208	139	subadult	3.5−4.5	M	0.108	15.6
EJ 06-02	01/11/06	216	209	143	subadult	3-4	M	0.088	15.8
EJ 06-08	01/25/06	298	242	157	subadult	6.5	M	0.1	62.7
EJ 06-10	01/25/06	140	188	116	subadult	2.5-3.5	F	0.074	55.4
EJ 05-01	01/26/05	278	227	162	adult	NR	F	0.117	46.5
EJ 05-18	02/10/05	223	217	142	adult	3.5−4.5	F	0.106	18.4
EJ 06-03	01/11/06	200	203	138	adult	3−5	F	0.078	11.4
EJ 06-07	01/24/06	187	211	142	adult	3.5−5.5	F	0.109	44.8
EJ 06-09	01/25/06	155	197	124	adult	3.5−4.5	F	0.103	51.6
EJ 06-11	02/08/06	332	233	167	adult	8-15	F	0.083	43.3
EJ 06-01	01/11/06	479	278	184	adult	7-10	M	0.098	6.81
EJ 06-05	01/23/06	385	247	169	adult	6.5-8.5	M	0.075	15.0

[a]aborted;
[b] fresh dead animal found inside cage;
NR = No reported

6.2.2 Contaminant Analyses

Blubber samples were analysed by AXYS Analytical Services Ltd (Sidney, BC, Canada), using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS) as described elsewhere (Christensen *et al*. 2005). Briefly, blubber samples (ranging 75−440 mg wet weight) were analyzed using an Ultima HRMS equipped with a Hewlett-Packard 5890 GC and a DB-5 Durabond capillary column (60m x 0.25 mm, 0.10 µmfilm). Percent of lipid in samples was determined at using the gravimetric lipid determination by weight of extract method with dichloromethane (DCM).

Briefly, samples were spiked with ^{13}C-labeled surrogate standards (*n* = 12 PBDEs; *n* = 29 PCBs) and then ground with anhydrous sodium sulfate. Samples were transferred to a Soxhlet thimble, surrogate standard was added, and samples were refluxed for 16 h with DCM. The extract was eluted through a gel permeation column with 1:1 DCM:hexane. The extract was applied to a partially deactivated Florisil column and eluted with hexane followed by 15:85 DCM:hexane. Elutes were then combined and eluted with 1:1 DCM:hexane and each fraction was concentrated. Included with each batch of samples was a procedural blank. The lab blank had concentrations above detectable levels (<25 pg/g) for 20 PBDE congeners, while for most of the PCB congeners the lab blank had concentrations above <5 pg/g. Limits of detections (LODs) for PBDE congeners generally ranged from <10 to < 60 pg/g wet weight, with exception to BDE-209 which had LODs ranging from 66.2 to 2480 pg/ g. For PCBs, the LODs were in general <10 pg/g, and, in most cases, <5 pg/g. For PBDEs, a total of 34 individual PBDE congener peaks ranging from dibromodiphenyl ethers through decabromodiphenyl ether and six co-eluting bands (each composed of two congeners) were identified and quantified in the blubber samples, constituting a data set of 40 congeners overall: BDE-7, -8/11, -10, -12/13, -15, -17/25, -28/33, -30, -32, -35, -37,

-47, -49, -51, -66, -71, -75, -77, -85, -99, -100, -105, -116, -119/120, -126, -128, -138/166, -140, -153, -154, -155, -181, -183, -190, -203, -206, -207, -208, -209.

6.2.3 Data treatment and Statistical Analysis

Concentrations of PBDEs and PCBs were calculated and reported as the sum of the concentrations congeners (i.e., ∑PBDE and ∑PCB) that were detectable in at least 15 out of 22 individual sea lions (≥ 68% of samples). When congeners were not detected, detection limit substitutions were made using half the limit of detection. Where less than 15 animals had detectable concentrations of an analyte (<68% of samples), 0 ng/kg was substituted for non-detect concentrations. Contaminants were not reported if there were low non-detectable ranges (NDRs) in combination with non-detectable levels (< LOD) in all sea lion samples. PBDE concentrations were calculated as the sum of the concentrations of 11 congeners, including co-eluting congeners, that were detectable >=68% of samples (∑PBDEs = BDE -7/25, -28/33, -47, -49, -66, -99, -100, -153, -154, -155 and -183*).*

For all PBDE and PCB congeners, reported concentrations were adjusted based on their respective recoveries, as well as concentrations found in the laboratory blank (i.e., the blank concentration was subtracted from the actual concentration measured in the sample). The method of detection limit (MDL) was calculated as the standard deviations of the two procedural blanks analyzed with the samples times three (i.e., 3 x SD_{blanks}), and then compared to the blank-subtracted concentrations of samples. Samples with concentrations above the MDL were reported. Corrected concentrations of contaminants were lipid normalized by dividing wet weight concentrations by the lipid content (µg/kg lipid) to adjust for differences in the lipid content of the samples.

Morphometric data were log-transformed to meet the assumption of normality prior to statistical analyses. Analysis of variance (ANOVA) followed by a Tukey-Kramer multiple comparison post-hoc test were used to make inter-group comparisons of weight, length and lipid content among subadults, adult females and adult males. The fetus was not included in the ANOVA as only one individual was available.

Inter-group comparisons of PBDEs and PCBs were conducted using ANOVA, with Tukey-Kramer multiple-comparison post-hoc test. When the variances of each group were significantly different or unequal (Bartlett test $p < 0.05$), a Welch analysis of variance (ANOVA) was carried out, followed by a Tukey-Kramer multiple-comparison test. Age categories with $n = 1$ were not included in this analysis (i.e., fetus). Confounding effects of life history parameters were assessed by plotting the log transformed data of PBDE and PCBs concentrations of subadult and adult males versus length, used here as a proxy for age. Adult females were not included due to the influence of the onset of sexual maturity and reproduction, including gestation, parturition and nursing. Thus, analysis of linear regression was used for each contaminants class to determine if length has a significant influence in contaminants concentration. Statistical analysis was carried out with JMP 7.0 (SAS Institute Inc.; Cary, NC, USA, 2007) at a level of significance of $\alpha = 0.05$. Data were presented as the mean plus or minus the Standard Deviation of the mean (SDM).

6.2.4 Congener-specific metabolism

The role of biotransformation of individual PBDE and PCB congeners was explored as an indication of CYP1A and CYP2B metabolic induction in male sea lions. First, the relative presence of each PBDE congeners, expressed as a percent of PCB 153 (i.e., PBDEx/PCB 153 ratios), was

calculated and regressed against length (i.e., length was used as proxy for age) to assess accumulation and metabolism of PBDEs.

For PCBs, the five metabolic groups (i.e., structure-activity groups) of PCBs according to position of vicinal H atoms and number of chlorine substitutions in the *ortho* position described by Boon *et al*. (1997) were used to explore PCB metabolism. Group I is comprised of congeners without any vicinal hydrogen atoms on carbons of either phenyl ring (PCBs 111, 133, 146, 153/168, 162, 165, 167, 172, 175, 178, 180/193, 187, 189, 191, 194, 196, 198/199, and 201–209). Group II is comprised of congeners with vicinal H atoms exclusively on *ortho*- and *meta*-carbons in combination with two or more *ortho*-chlorine atoms (PCBs 44/47/65, 83/99, 85/116/117, 128/ 166, 129/138/160/163, 130, 137, 158, 170, 177, 190, and 195). Group III congeners have *ortho*- and *meta*-hydrogen pairs with less than two *ortho*-chlorines (PCBs 20/28, 37, 60, 61/70/74/76, 63, 66, 68, 77, 105, 114, 118, 123, and 156/157). Group IV congeners have *meta*- and *para*-hydrogen pairs with two or fewer *ortho*-chlorines (PCBs 21/33, 26/29, 31, 49/69, 52, 59/62/75, 64, 86/87/97/108/119/125, 90/101/113, 92, 109, and 110/115). Group V congeners have *meta*- and *para*-hydrogen pairs with more than two *ortho*-chlorines (PCBs 93/95/98/100/102,135/151/154, 136, 144, 147/149, 174, 176, 179, and 197/200). The ratio of individual congeners within each group (i.e., Group I, Group II, Group III, Group IV and Group V) relative to PCB 153 were calculated (PCBx/PCB153), and then regressed against length, respectively. PCB-153 was used as a reference congener to assess the biotransformation of PCBs in sea lions because this congener is one of the most recalcitrant and bioaccumulative congeners in marine mammals (Tanabe *et al*. 1988; Boon *et al*. 1997; Wolkers *et al*. 2004). PCB congeners were further classified as persistent, biotransformed by the CYP1A and 2B enzyme subfamily (groups II and III), biotransformed by the CYP2B enzyme subfamily (groups I and IV); or likely to be

biotransformed by the CYP1A or/and 2B enzyme subfamily (Group V) according to the classification scheme developed by Boon *et al*. (1997).

The following criteria were used to examine metabolism: a) Poor biotransformation is observed for any of the PBDEx/PCB 153 or PCBx/PCB153 ratios versus length when the slope = 0 (i.e., slope is not significantly different from zero); b) Retention of contaminant was observed, if a significant positive relationship between the ratios versus length was observed when the slope > 0 (i.e., the chemical accumulates due to the lack of metabolism); and, c) Metabolism or biotransformation was observed when slope < 0 or if a significant negative relationship existed (i.e., the chemical decreases due to elimination), and this was an indicative of CYP1A and CYP2B metabolic induction.

6.2.5 Health Risk Assessment

The toxic equivalency quotient (TEQ) was calculated for PCB congeners in these samples based on toxic equivalency factors (TEFs) established for dioxin-like PCBs, including the planar (non-*ortho*) PCBs (ΣPCBs 77, 81, 126, and 169) and mono-*ortho* PCBs (ΣPCBs 105, 114, 118, 123, 156, 157, 167 and 189)(Van den berg *et al*. 2006). For the ∑TEQ calculations, substitutions using half of detection limits were made when congener-specific PCBs (i. e. planar, non-*ortho* PCBs) were not detected. The TEQs were then compared to the TEQ threshold levels, including the no observable adverse effect level (NOAEL) and the lowest observable adverse effect level (LOAEL) for dioxin-like PCBs, derived from immunotoxic action and endocrine disruption endpoints assessed in semi-captive harbour seals (Ross *et al*. 1995; Kannan *et al*. 2000). In addition, the health risk characterization using the normal probability density function of log PCB concentrations (i.e., relative frequency) measured in the animals and compared to the revised

PCB threshold effect concentration of 1300 µg/kg lipid for risk of immunotoxicity and endocrine disruption in harbour seals was assessed (Mos *et al*. 2010).

6.3 Results and Disscussion

6.3.1 Biological influences on contaminant level

Weight and length were significantly correlated in all Steller sea lions ($r = 0.94$, $p < 0.0001$), as well as in subadult ($r = 0.89$; $p = 0.00004$) and adult ($r = 0.98$; $p = 0.00001$) age categories. Although male subadults and female adults did not differ in weight and length, the two male adults were heavier (ANOVA, $p = 0.0052$; Tukey-Kramer multicomparisons test, $p < 0.05$) and longer (ANOVA, $p = 0.0058$; Tukey-Kramer multicomparisons test, $p < 0.05$) when compared to male subadults and female adults (Table 6.1).

Despite some morphometric differences, concentrations of ∑PBDEs did not differ among age categories (ANOVA, $F = 0.4883$; $df = 3$; $p = 0.6950$; Table 6.2). No correlation was found between ∑PBDE concentrations and length in male subadults and adults (∑PBDE: $r = 0.31$; $p = 0.3476$). In contrast, ∑PBDE concentrations decreased as the length of female adults increases (∑PBDE: $r = 0.83$; $p = 0.0386$).

As with PBDEs, no variations in ∑PCB concentrations were observed among age or sex classes (ANOVA, $F = 0.6095$; $df = 3$; $p = 0.6179$; Tukey-Kramer multicomparisons test, $p > 0.05$; Table 6.3). There was no correlation between ∑PCB and length in the combined male subadult and adult category (∑PCB: $r = 0.36$; $p = 0.2796$). However, ∑PCB concentrations decreased with increasing length of adult females (∑PCB: $r = 0.85$; $p = 0.0314$). Because life history parameters such as age, sex and size affect PCB concentrations and patterns (Addison and Smith 1974;

Aguilar et al., 1999; Ross et al. 2000), the small sample size and lack of detailed age information may have precluded a full exploration of these factors in Steller sea lions.

Table 6.2 Lipid content and concentration means (range) for the top six PBDEs and the top six PCB congeners (µg/kg lipid) detected in blubber samples of Steller sea lions. Data are arranged by age/sex categories.

	Fetus male (*n* =1)	**Pups (*n* = 3)**	**Subadults (*n*= 10)**	**Adult females (*n* = 6)**	**Adult males (*n* = 2)**
Lipid %	8.51	45.0 (24.0—83.0)	25.0 (1.90—63.0)	36 (11.0 —52.0)	10.9 (6.80—15.0)
BDE-28/33	2.50	12.0 (2.00−20.8)	10.1 (0.93−45.6)	3.80 (1.12−6.25)	4.32 (1.40−7.25)
BDE-47	88.5	558 (57.8−1158)	740 (70.3−2777)	323 (30.5−832)	364 (1.36−593)
BDE-99	20.8	92.7 (9.37−199)	69.2 (11.5−159)	47.8 (5.12−106)	86.9 (64.9−109)
BDE-100	22.3	130 (15.0−275)	171 (13.0−600)	90.1 (6.69−230)	116 (54.2−177)
BDE-153	3.90	13.4 (1.61−29.7)	14.3 (2.69−31.3)	10.6 (1.25−21.4)	15.0 (14.4−15.7)
BDE-154	5.61	19.5 (2.17−42.1)	27.4 (2.78−92.7)	17.3 (2.03−42.5)	19.0 (13.3−24.7)
∑PBDE[a]	151	843 (91−1759)	1049 (110-3776)	503 (49.7−1258)	620 (336−904)

[a] Total PBDE concentrations included the 11 congeners detected in Seller sea lion blubber samples

Table 6.3 Lipid content and concentration means (range) for top six PCB congeners (µg/kg lipid) detected in blubber samples of Steller sea lions. Data are arranged by age/sex categories.

	Fetus male (*n* =1)	**Pups (*n* = 3)**	**Subadults (*n*= 10)**	**Adult females (*n* = 6)**	**Adult males (*n* = 2)**
Lipid %	8.51	45.0 (24.0–83.0)	25.0 (1.90–63.0)	36 (11.0 –52.0)	10.9 (6.80–15.0)
PCB-153/168	82.3	614 (70.1−1328)	967 (90.6−2960)	446 (39.9−1081)	583 (260−906)
PCB-129/138/160/ 163	62.5	436 (53.2−871)	627 (59.8−1946)	277 (30.0−647)	399 (180−619)
PCB-118	33.2	217 (26.2−432)	325 (36.7−1009)	125 (17.3−308)	172 (75.3−269)
PCB-180/193	30.9	197 (21.4−456)	276 (20.1−771)	140 (11.2−345)	188 (81.9−295)
PCB-83/99	27.8	188 (20.5−378)	288 (28.7−920)	123 (13.8−294)	141(62.4−219)
PCB-147/149	28.4	110 (16.0−176)	109 (11.0−456)	43.2 (12.8−82.9)	90.0 (30.7−149)
∑PCB[b]	572	3158 (393−6296)	4294 (398−14277)	1895 (272−4386)	2713(1114−4311)

[b]Total PCB concentrations included the 93 congeners detected in Seller sea lion blubber samples

6.3.2 PBDE and PCB concentrations and patterns

A total of 11 out of 40 PBDE congeners sought were detected in at least 68% of Steller sea lion samples. ∑PBDEs measured as the sum of these 11 congeners ranged from 49.7 to 3776 µg/kg lipid (Table 6.2), with a geometric mean of 464 µg/kg lipid (lower value geometric SD= 149 µg/kg lipid; upper value geometric SD = 1445 µg/kg lipid). BDE 47 (2, 2', 4, 4'-tetrabromodiphenyl ether) was the dominant congener in all samples, representing 64.4% ± 1.7% of the ∑PBDE concentrations. Other predominant congeners were BDE 100 (2,2',4,4',6-pentabromodiphenyl ether) and BDE 99 (2,2',4,4',5-pentabromodiphenyl ether), which made up 16.5% ± 0.5 % and 10.5% ± 1.2% of ΣPBDE concentrations, respectively (Figure 6.1a; Figure E-1 in Appendix E).

The patterns of PBDE congeners among age classes and sex categories were similar, except for subadult animals and adult males which showed variations in the composition of BDE-47 and BDE-99 (Figure 6.1a). While the contribution of most congeners (e.g., BDE-28/33, -66, -100, -153, -154, 183) remained constant among different age classes (ANOVA with Tukey-Kramer test for BDE 17/25, 100 and 183, $p > 0.05$; and Welch ANOVA with Tukey-Kramer test for BDE 28/33, 47, 49, 66, 99, 153, 154, and 155, $p > 0.05$), the proportion of BDE-47 was higher in subadults compared to adult males (Tukey-Kramer test, $p < 0.05$; t-test, $p = 0.0100$) (Figure 6.1a; Figure E-1). In addition, the BDE-99 fraction was lower in subadults than in adult males (Tukey-Kramer test, $p < 0.05$; t-test, $p = 0.0109$). Lipid normalized BDE-47 decreased by a factor of 2.0 from subadult aged animals to adult males, whereas BDE 99 was 1.3 fold greater in adult males compared to subadults (Figure 6.1a; Table 6.2).

The PBDE composition in the Steller sea lion is similar to the PBDE patterns reported for other marine mammals from the northern hemisphere (Wolkers *et al*. 2004; Tuerk *et al*. 2005;

Rayne *et al*. 2004). The importance of BDE-47 at upper trophic levels of the marine food web reflects a combination of the propensity of this congener to biomagnify and/or its generation through debromination pathway of other PBDE congeners (Sellström *et al*. 1993; Boon *et al*. 2002; Wolkers *et al*. 2004; Stapleton *et al*. 2004a; Kelly *et al*. 2008). BDE-209 was not detected in our sea lions, likely as a result of its preferential binding to the particle phase in the water column and sediments (Johannessen *et al*. 2008; Ross *et al*. 2009) and the subsequent lack of biomagnification of this high log K_{ow} congener in aquatic food webs (Wolkers et al., 2004; Kelly et al., 2008). Some studies have demonstrated that BDE-209 is quickly debrominated to lower brominated congeners (BDE- 154, -155) in fish (Stapleton *et al*. 2004b; Stapleton *et al*. 2006b) and grey seals (*Halichoerus grypus*) (Thomas *et al*. 2005*),* perhaps explaining in part our observations.

A total of 93 PCB congeners were detected out of the 159 analytes sought in at least 68% of Steller sea lion samples. The concentration of ∑PCBs in Steller sea lions ranged from 272 to 14, 277 µg/kg lipid, with a geometric mean of 1,893 µg/kg lipid (lower value geometric SD= 618 µg/kg; upper value geometric SD = 5,797 µg/kg lipid; Table 6.3). The coeluting PCB congeners 153/168 accounted for 21% of ∑PCBs, followed by PCBs 129/138/160/163 (14.1%), PCB 118 (7.0%), PCB 180/193 (6.5%) and PCBs 83/99 (6.1%) (Figure E-2 in Appendix E). While patterns of PCB homologue groups in subadult and adult individuals (females and males) were similar, and were dominated by the more heavily chlorinated PCB homologue groups, the PCB composition in the fetus and pups was relatively lighter (Figure 6.1b). The PCB pattern in the Steller sea lions is similar to that observed in marine mammals in the Northeastern Pacific Ocean (Ross *et al*. 2000; Ross *et al*. 2004), and reflects the distribution and fate of this contaminant class in marine food webs.

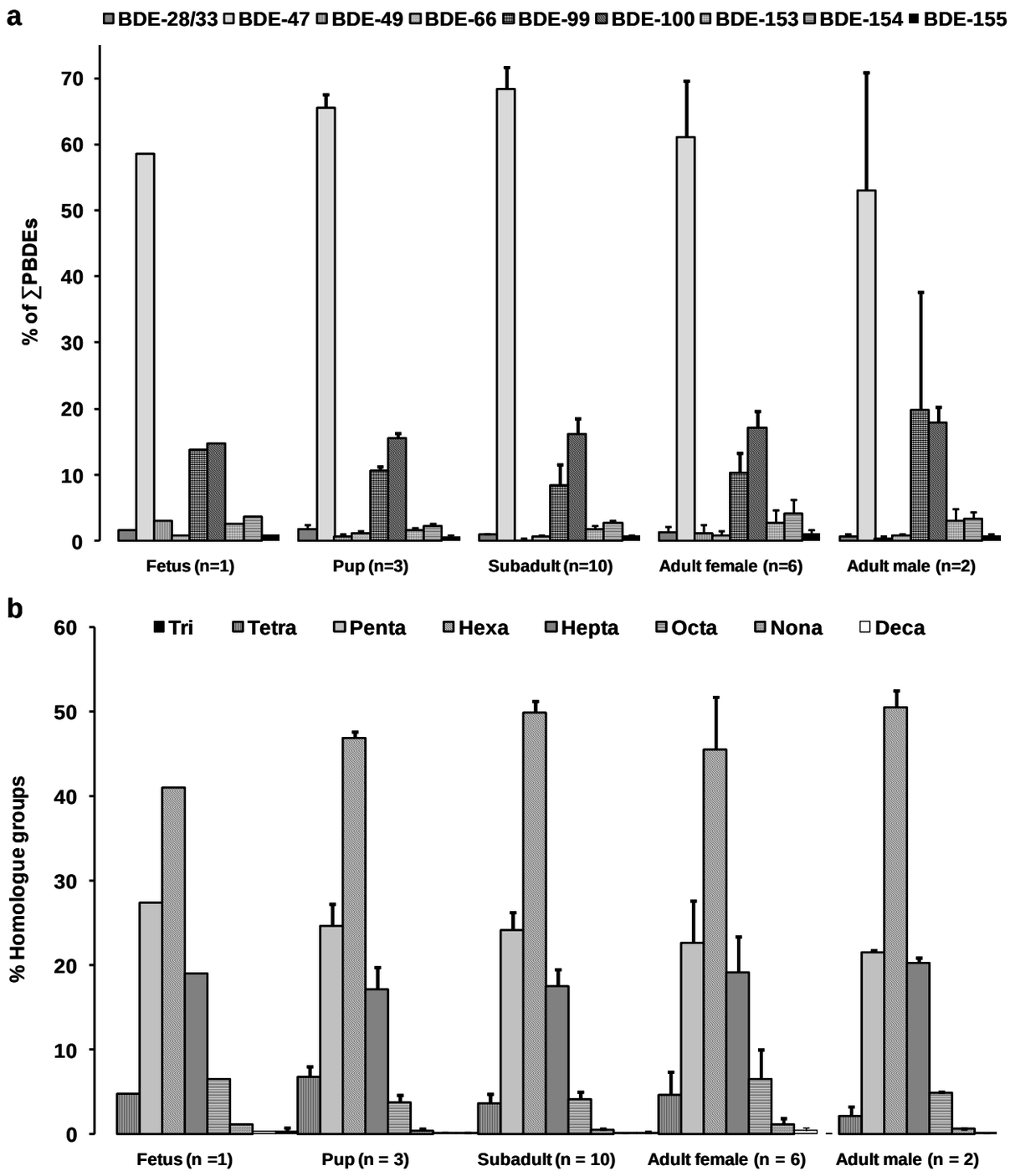

Figure 6.1 Patterns of PBDEs and PCBs by age class of Steller sea lion sampled in British Columbia, Canada (fetus, pups, subadults, females, and males: a) PBDE congener composition; b) PCB homologue group patterns. Results are expressed as mean ± Standard Deviation.

Concentrations of ∑PBDEs and ∑PCBs were significantly correlated (r = 0.99; p < 0.0001; Figure E-3 in Appendix E), indicating that despite some differences in physical-chemical properties, PBDEs and PCBs both bioaccumulate in Steller sea lions. However, ∑PCB concentrations were four times higher than ∑PBDE concentrations in Steller sea lions, underscoring the continued and widespread contamination of the marine environment by these legacy chemicals.

6.3.3 Lipid content and PBDE and PCB concentrations

Lipid content in Steller sea lion varied considerably among blubber biopsy samples (from 1.94 to 83.4%), with a mean of 28.7 % ± 4.52% (Table 6.1). The lipid fraction of the blubber samples was less than 50% in about 80% of the Steller sea lions (Tables 6.1), suggesting that animals underwent a fasting period during their migration into the Strait of Georgia. In California sea lions, a lipid content < 50% indicates that an animal is nutritionally stressed or fasting (Stapleton *et al*. 2006). Interestingly, the nursing pup in our study had the highest lipid values measured (83%).

This indication of fasting was supported by reduced or normal blood urea nitrogen (BUN) values (range 10-35 mg/dl or 4–12.5 mmol/l), which suggests protein utilization in subadult and adult individuals (D. Lambourn, unpublished data). These values were consistent with those reported for fasting-adapted pinnipeds (Bossart *et al*. 2001). This indicates that energy was primarily being obtained from the catabolism of lipid body stores (i.e., hypodermal lipid or blubber) rather than from body protein catabolism.

Fasting periods are a normal physiological process in the natural life history of pinnipeds, which occurs during migrations, reproductive cycles and/or molting periods (Costa 1995). Food consumption by free ranging Steller sea lions likely fluctuates seasonally in response to changes in energy requirements (Winship *et al*. 2002), coupled with activities such as breeding (Pitcher and Calkins 1981), periods of growth (lean tissues and energy reserves) and molting (Lager *et al*. 1994; Costa 1995). Sea lions spend proportionally more time feeding at sea during winter and spring compared to summer (Merrick and Loughlin 1997; Trites and Porter 2002). Foraging time for lactating females is also longer in winter than in summer, suggesting a greater effort is required to obtain sufficient food in winter due to dispersed fish distribution and/or increased energy needs (Merrick and Loughlin 1997; Trites and Porter 2002).

No differences in lipid content were found among age groups (Kruskal Wallis Test; χ^2 = 8.8904; df= 6; p = 0.1798; Table 6.2). Lipid content did not differ between subadult individuals (the only female subadult was grouped with the subadult males since its lipid content did not differ from those of males; Mann Whitney Test; χ^2 = 1.4848; df= 1; p = 0.2230), or between female and male adults (Welch ANOVA, p = 0.1983; Tukey-Kramer multicomparisons test, $p > 0.05$).

Fasting presumably mobilizes PBDEs and PCBs from depleted blubber lipid into the bloodstream, resulting in a consequent increase in lipid-based concentrations in circulation (De Swart *et al*. 1995) and blubber (Hall *et al*. 2008). While we did not measure circulating concentrations of PBDEs or PCBs in blood, concentrations (wet weight) of both PBDEs (r = 0.60; p = 0.003) and PCBs (r = 0.62; p =0.002) decreased in Steller sea lions (all animals combined) with diminishing lipid content in blubber (Figure E-4, Appendix E). When exploring this by age/sex categories no relationships were found between PCBs or PBDEs (wet weight) and lipid content in any age class ($p > 0.05$), except for a positive relationship in subadults (PBDEs, r = 0.68, p =

0.031; PCBs, r = 0.70, p = 0.023). Since fasting has been implicated in increased POP concentrations in the blubber of otariid species (Hall *et al*. 2008), our observations may reflect either a period of feeding on more contaminated prey coupled with high dietary uptake efficiency of lipophilic contaminants as the sea lions entered the industrialized Strait of Georgia.

6.3.4 Maternal transfer of PBDEs and PCBs to fetus and pups

The ∑PBDE: ∑PCB concentration ratios for fetus relative to adult females were 0.30, suggesting limited transfer efficiencies for these compounds across the placenta. However, there was considerable variation among congeners. Congener-specific BDE ratios between the Steller sea lion fetus and adult females were low and did not relate to log K_{OW} (p > 0.05; Figure 6.2a). The one exception was BDE 49 (log K_{OW} = 7.3), which exhibited a ratio of 2.07, indicating that this congener is preferentially transferred through the placenta. Likewise, no relationship was observed between PCB ratios versus log K_{OW} (p > 0.05). However, a few PCB congeners did appear to be readily transferred from female to fetus (Figure 6.2a). These included PCB congeners 88, 90/101, 110 and 134, with ratios that ranged from 1.13 for PCB 134 (log K_{OW} = 7.3) to 1.41 for PCB 88 (log K_{OW} = 6.5). While these observations may indicate that factors other than lipid solubility influence the transfer of these contaminants via placenta, a matched mother: fetus pair might clarify this question, as the aborted fetus sampled here was not identified, and that of an average female signal was used as a proxy.

Compared to the constrained transplacental transfer of PBDEs and PCBs, transfer from mother to pup via milk appeared to readily occur. Both ∑PBDEs and ∑PCB concentrations in nursing pups were higher than those in adult females (ratio pups/females ≈1.7). Although the ratios of PBDE congeners in pups to adult females were high, they did not relate to log K_{OW},

despite a negative trend with increasing log K_{OW} (r = -0.60, p = 0.118; Figure 6.2b). All ratios were higher than 1.0, with BDE 28 and BDE 66 exhibiting ratios above 3.0, which suggest that all major BDE congeners appear to be transferred through lactation. However, PCB congener concentration ratios between pups and adult females were high and were related to log K_{OW} (r =-0.60, p < 0.0001; Figure 6.2b). Interestingly, most ratios for PCB congeners were above 1 (>100%), and only PCB 206, 207, 208, and 209 had ratios below 1. In general, lower ratios for PCBs are observed above a log K_{OW} of 7.5. This suggests that the log K_{OW} is a constraining factor for lactational transfer of PCBs, but not the PBDEs, in Steller sea lions.

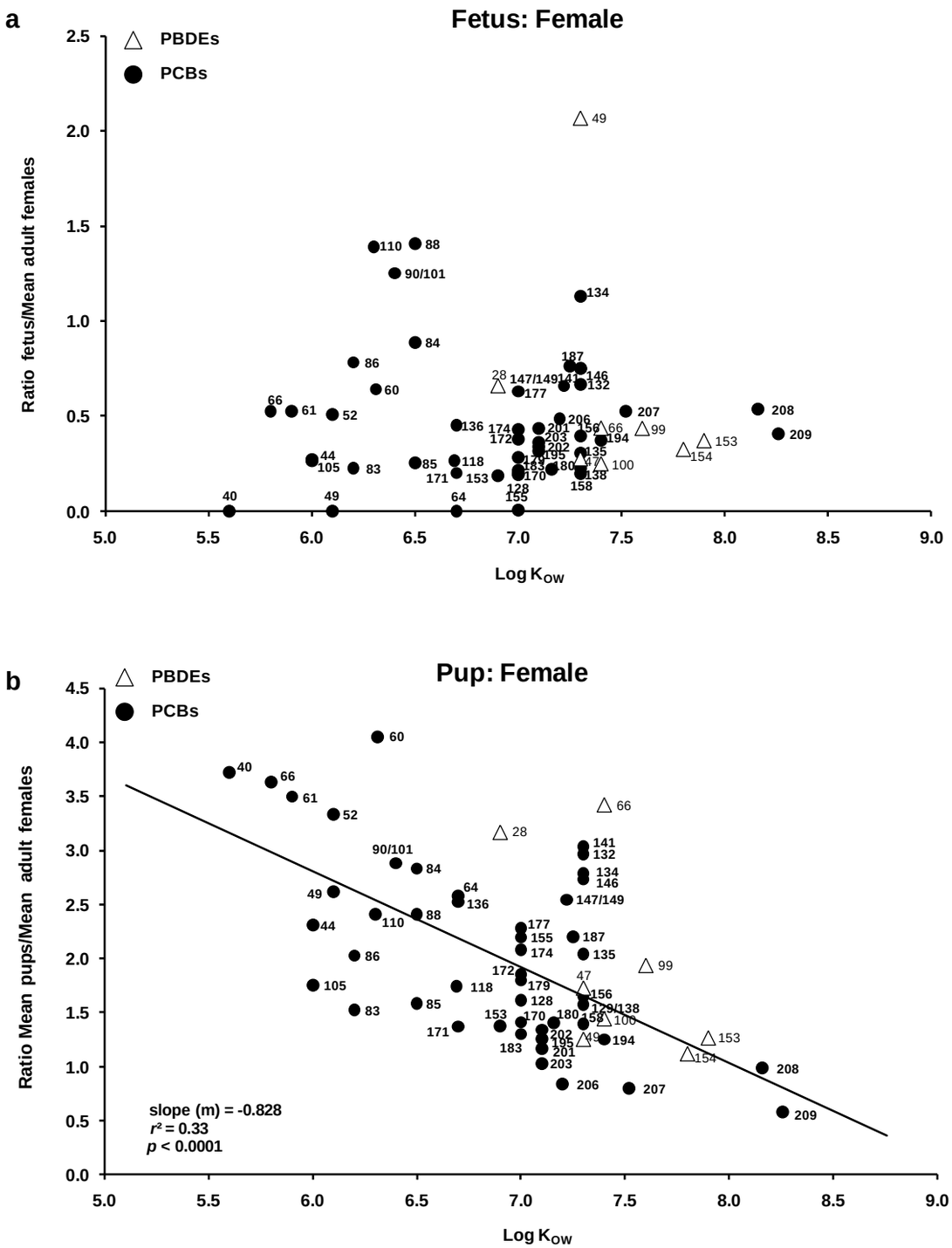

Figure 6.2 Assessment of maternal transfer for PBDEs and PCBs. a) Ratios of major PBDE congeners (BDE 28, -47, -49, -66, -99, -100, -153, -154) and PCB congeners measured in the fetus (n= 1) to mean concentrations detected in adult female Steller sea lions (n= 6) versus the Log K_{OW} of PBDE and PCB congeners; and, b) Ratios of the mean of PBDE and PCB congeners measured in pups (n= 3) relative the mean concentrations detected in adult females (n= 6) versus the Log K_{OW} PBDE and PCB congeners.

In cetaceans, maternal transfer of organochlorines (i.e., PCBs and DDT) to offspring during lactation was found to deliver as much as 60 to 95% of the mother's burden (Borrell *et al*. 1995; Hickie *et al*. 2007). However, some feeding on prey by the older pups in our study may confound the lactational transfer assessment of PCBs and PBDEs. However, transplacental and lactational transfer of persistent organic contaminants is thought to be constrained by the physico-chemical properties of the congener in question (Addison and Smith 1974; Addison and Brodie 1987).

In an effort to further characterize maternal transfer of PCBs in these animals, the PCB pattern differences resulting from the comparison of ratios for individual congener profiles corrected to PCB 153 between adult females and fetus or pups clearly revealed that the females at reproductive age delivers a significant proportion of low chlorinated PCBs (Figure E-5a, Appendix E), followed by heavier PCBs, while pups receive a mixture of less chlorinated PCBs (lower K_{OW}) through nursing (Figure E-5b), which might be confounded by occasional feeding. This suggest that the partitioning of PCB congeners from the mother to fetus is not limited by the same physical-chemical properties as observed during the nursing period where the more heavily chlorinated demarcated approximately at PCB 170 are limited, as observed in grey seals, previously (Addison and Brodie 1987). Major caveats here include our small sample size and the fact that we did not sample mother-pup pairs.

Pattern differences between fetus, pups and adult females are also evident for PCBs, but not PBDEs, in a cursory examination of homologue groups. For example, PCB profiles were dominated by lighter homologue groups (e.g., pentachlorobiphenyls) in fetus relative to adult females, while pups had similar PCB and PBDE patterns compared to adult females (Tables 6.2-6.3; Figure 6.1a,b).

6.3.5 Contaminant Metabolism and Accumulation

Metabolism can also play an important role in shaping the POP composition in the tissues of marine mammals. Pinnipeds are able to metabolize most PCB congeners with *meta* and *para* vicinal-H atoms and two *ortho*-chlorines because of their induction of CYP1A and CYP2B cytochrome P450 enzymes (Tanabe *et al*. 1988; Boon *et al*. 1997; Routti *et al*. 2008). Although less studied, similar enzymatic induction and metabolic pathways have been proposed and/or observed for PBDEs (de Wit 2002; Hallgren and Darnerud 2002).

The regression between the ratios of individual PBDE congeners to PCB-153 versus length showed significant positive relationships (i.e., slope > 0) for four PBDE congeners, including BDE 49, 99, 153 and 183, suggesting bioaccumulation potential of these congeners relative to PCB 153 (Figure 6.3). The slopes for PBDE congeners 17/25, 28/33, 47, 66, 100, 154 and 155 were not significantly different from zero (Table 6.4), suggesting lack of lack of metabolism of these compounds. However, the important contribution of PBDE 47 relative to ΣPBDEs renders it difficult to evaluate the metabolic vulnerability of different PBDE congeners.

Table 6.4 Regression statistics for the relationships between the ratios of individual PBDE congeners relative to PCB 153 versus length in male Steller sea lions.

	slope	r^2	*p* value
PBDEs/PCB 153			
BDE 17/25	1.5×10^{-05}	0.193	0.1768
BDE 28/33	-6.6×10^{-05}	0.211	0.1550
BDE 47	-2.9×10^{-03}	0.129	0.2780
BDE 49	7.2×10^{-05}	0.503	**0.0146***
BDE 66	4.8×10^{-05}	0.274	0.0988
BDE 99	3.8×10^{-03}	0.622	**0.0039***
BDE 100	1.8×10^{-04}	0.008	0.7899
BDE 153	4.2×10^{-04}	0.584	**0.0062***
BDE 154	2.2×10^{-04}	0.249	0.1185
BDE 155	1.6×10^{-05}	0.050	0.5080
BDE 183	1.5×10^{-05}	0.406	**0.0351***

*Slope was significantly different from zero

The regression slopes for individual PCB congeners within each metabolic groups (I, II, III, IV and V) were not significantly different from zero ($p > 0.05$) (Table E-1 in Appendix E), except for PCB 137 of Group IV which had a positive slope significantly greater than zero ($r^2 = 0.57$, $p = 0.007$). This suggests that the Steller sea lion possesses poor biotransformation capabilities and indicates lack of induction of cytochrome P450 enzymes (i.e., CYP1A and CYP2B).

Slow uptake and excretion rates of PBDEs may be also contributing to the bioaccumulation of PBDEs in the Steller sea lion through its food web, implying that some congeners (e.g., BDE-47) might require longer time periods to reach the steady state, while others PBDE congeners exhibit a relatively rapid rate of depuration likely by debromination and/or cytochrome P450 enzyme mediated oxidative metabolism (McKinney *et al*. 2006; Kelly *et al*. 2008). Measurements

of PBDE and PCB metabolites (e.g., hydroxylated PCBs and PBDEs: OH–PCBs and OH–PBDEs) in sea lions would substantiate the inferences illustrated here.

6.3.6 PBDEs and PCBs related health risks

Total toxic equivalents (∑TEQ) for non-*ortho* and mono-*ortho* (planar) ∑PCB concentrations in the Steller sea lions (10.2 ± 2.23 ng TEQ/kg lipid) are below the NOAEL-TEQ thresholds of 90 ng TEQ/kg and 209 ng TEQ/kg for immunotoxic effects reported in harbour seals (Kannan et al. 2000; Ross et al., 1995). The ∑TEQ in Steller sea lions is higher than the ∑TEQs reported for Northern elephant seals (*Mirounga angustirostris*) from California (Debier *et al*. 2005), harbour seals from Queen Charlotte Strait and those inhabiting the Strait of Georgia, British Columbia (Ross *et al*. 2004), but lower than that reported in harbour seals from Puget Sound, Washington (Ross *et al*. 2004). Concentrations of PCBs in 80% of our study animals exceeded the latest PCB-immunotoxicity and endocrine disruption toxicity reference value (1300 µg/kg lipid) for harbour seals (Mos *et al*. 2010; Figure 6.4; see also Figure E-6).

PBDEs are also of concern due to potential endocrine disruption mechanisms, including thyroid hormone and vitamin A disruption, estrogenic effects and immunotoxicity (Meerts *et al*. 2001; Hallgren and Darnerud 2002; Hall and Thomas 2007), although there currently exists limited information about risks or effects concentrations.

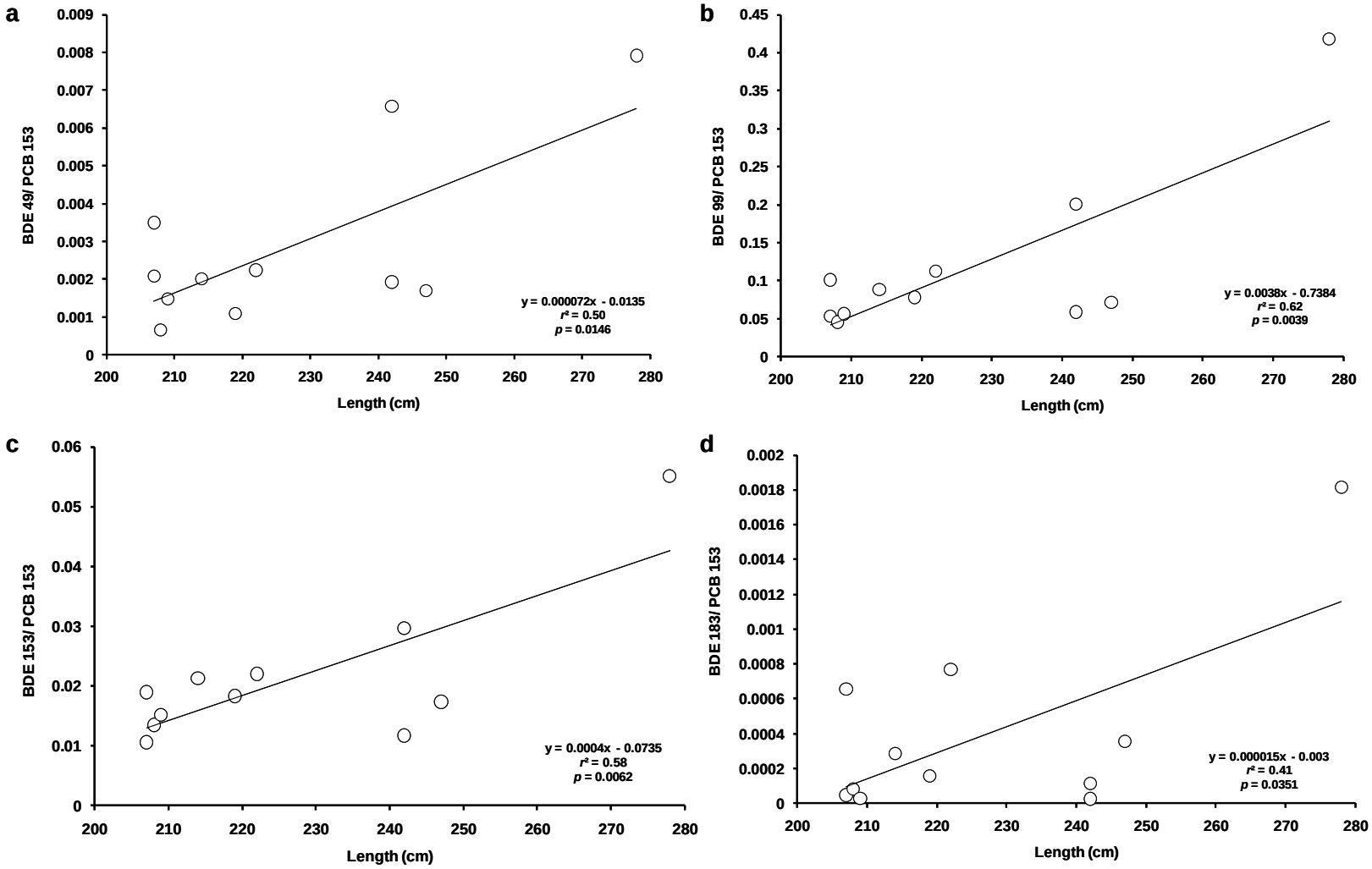

Figure 6.3 Relationship between the ratios of selected PBDE congeners [(a) BDE 49, (b) BDE 99, (c) BDE 153, and (d) BDE 183] relative to PCB 153 versus length in male Steller sea lions.

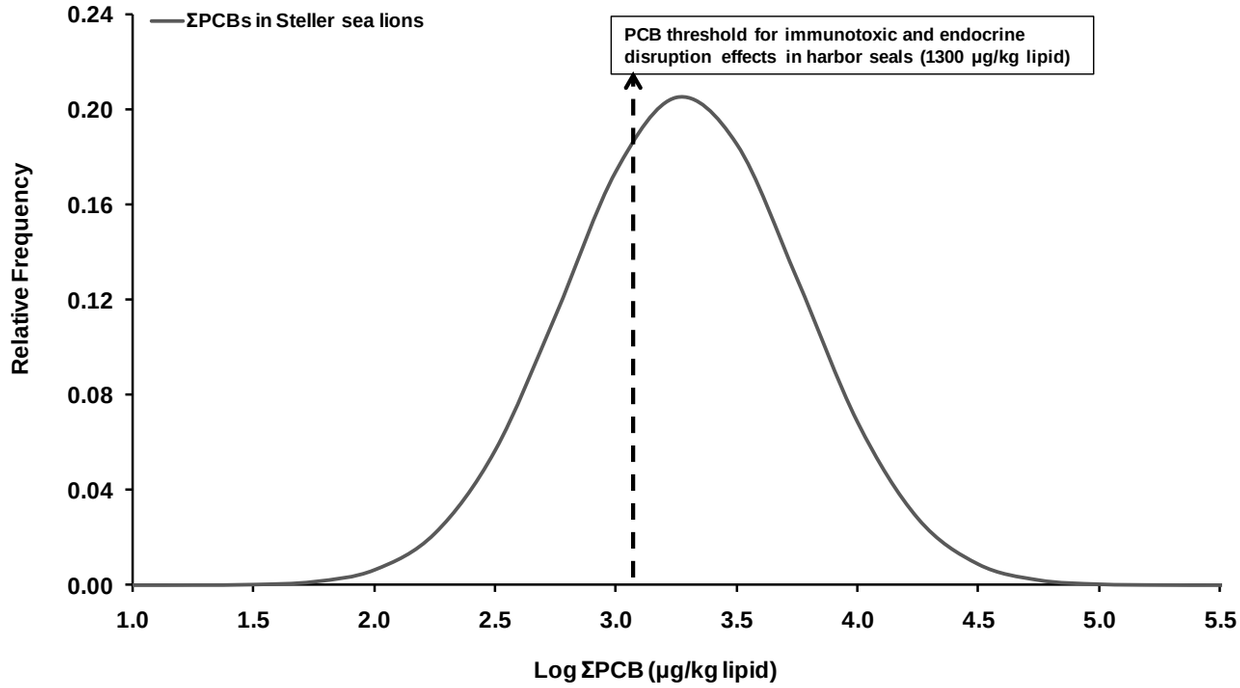

Figure 6.4 Normal probability density curve showing the frequency distribution of PCB concentrations measured in Steller sea lion. The dashed line represents the revised harbour seal toxicity threshold (Mos *et al*. 2010).

6.3.7 Comparisons with other marine mammals and regional trends

Concentrations of PBDEs in Steller sea lions were lower or comparable to those in harbour seals from San Francisco (She *et al*. 2002), California sea lions from coastal California (Stapleton *et al*. 2006) and harbour seals from Puget Sound (Noel et al., 2008) (Table E-1 in Appendix E), but similar to those found in resident and transient killer whales from the Northeastern Pacific Ocean (Rayne *et al*. 2004; Krahn *et al*. 2007). While PCB concentrations in Steller sea lions were lower than those reported for individuals from the western stock (Krahn 1997; Krahn *et al*. 2001; Table E-2) and from resident harbour seals in the Strait of Georgia (Ross *et al*. 2004), they were

higher than PBDE concentrations recently detected in Galapagos sea lions (Alava *et al*. 2009). Differences in contaminant concentrations among pinniped species in North Pacific Ocean are likely due to differences in feeding preferences, the use of foraging habitat, and the relative contamination of prey.

Although PCB bans several decades ago have improved habitat quality for marine mammals in the Pacific, concerns linger about health risks associated with some heavily contaminated populations. While PBDEs increasingly face regulation today for many of the same reasons PCBs were phased out in the 1970s, increasing environmental concentrations, coupled with potentially unstable sediment-bound reservoirs of PBDEs (notably decaBDE), represent an emerging threat (Ross *et al*. 2009). Our results suggest that migrating Steller sea lions are exposed to contaminants that are amplifying in North Pacific food webs, and that these are readily transferred to offspring.

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CHAPTER 7

MODELLING THE BIOACCUMULATION OF POLYCHLORINATED BIPHENYLS IN THE KILLER WHALE (*ORCINUS ORCA*) AND STELLER SEA LION (*EUMETOPIAS JUBATUS*) FOOD WEBS FROM BRITISH COLUMBIA, CANADA

Abstract: Threatened northern resident killer whales (NRKWs), and endangered southern resident killer whales (SRKWs) from the northwest Pacific coast of North America feed primarily on Pacific salmon (96% of their diet), of which Chinook salmon (*Oncorhynchus tshawytscha*) accounts for 70%. Steller sea lion are also marine mammals of special concern that overwinter in the Strait of Georgia, where the feed on abundant herring. Because of their high trophic level, long lifespan and high lipid content in blubber, killer whales and Steller sea lions are particularly vulnerable to heavy contamination by persistent organic pollutants, including PCBs. We modeled the bioaccumulation of PCBs in the resident killer whale and Steller sea lion food webs in coastal British Columbia, Canada, including such geographic areas as the open Pacific Ocean, Queen Charlotte Strait, Critical Habitat for NRKWs and SRKWs, Strait of Georgia and Puget Sound (Washington, USA). The aims of this study were to conduct an eco-toxicological risk assessment by modeling the role of salmonid fish as biological vectors of pollutants to top predators (orcas), and to improve our understanding of the bioaccumulation and health effects of PCBs. The model makes use of: PCB sediment data measured for the study areas; physical-chemical properties of specific PCB congeners; biological parameters of killer whales, Steller sea lions and their prey;

diet composition, trophic interactions, and PCB uptake/elimination rates. These were integrated through the use of steady state mass balance equations. The Biota Sediment Accumulation Factor (BSAF) is the major outcome of the model to predict concentrations in biota and compared against PCB toxicity thresholds. Field observed habitat distribution (%) of resident killer whales was used to adjust predicted concentrations. Predicted ΣPCB concentrations in females and males of the northern resident population were 8.3 and 54 mg/kg lipid, respectively, and lower than those predicted in female and male of the southern resident population (50 and 95 mg/kg lipid, respectively). ∑PCB concentrations predicted in SRKWs and male NRKWs as well as in Steller sea lions exceed PCB-immunotoxic and endocrine disruption thresholds reported in harbour seals (1.3 and 17 mg/kg lipid) and the effect concentration (10 mg/kg lipid) reported to reduce the population growth rate in bottlenose dolphins. The performance of the model was evaluated against independent empirical PCB concentrations, resulting in comparable values and showing a mean model performance bias for ∑PCBs ($MB \pm SD_{MB}$) of 1.23 ± 0.36 for male northern resident orcas and of 1.12 ± 0.49 for male Steller sea lions, which corroborated the use of the model.

Key words: **Killer whale, Steller sea lion; PCBs; Bioaccumulation, food web, modelling; BSAF, sediment quality guideline, toxicity effect concentration.**

7.1 Introduction

Killer whales (*Orcinus orcas*) from British Columbia have been identified as the marine mammals exhibiting the highest levels of polychlorinated biphenyls (PCBs) in the world, surpassing the endangered St Lawrence beluga whales (*Delphinapterus leucas*) by a factor of 2-5 times (Ross *et al.* 2000). Bioethical, logistical and legal constraints prevent mechanistic toxicological studies from being carried out on killer whales, and limit the ability to determine the

precise health impacts of their very high PCB burdens. The list of difficulties for an assessment of population-level consequences of high PCB exposures is long. Killer whales are: a) exposed to a complex mixture of contaminants; b) long-lived, meaning that they are exposed to a cumulative history of chemical use; c) have large habitat needs as do their primary prey (Chinook salmon); d) difficult to study, such that collecting blood (or many other tissue samples) for toxicological evaluation is not possible; and, e) protected under the terms of SARA in BC waters.

Three ecotypes of killer whales inhabit the marine environment of southern British Columbia (BC), Canada, and northern Washington State (WA), USA, including resident, transient, and offshore ecotypes (Ford *et al.* 1998). Resident killer whales are further distinguished into two groups, i.e., northern residents (NRKW) that are often found in the waters off northeast Vancouver Island, BC, and southern residents (SRKW) that are often found in the waters off southeast Vancouver Island (Ford *et al.* 1998).

In 2001, SRKWs were listed as Endangered under the Canadian *Species at Risk Act* (SARA; Government of Canada 2010a), and in 2005 under the United States *Endangered Species Act* (NOAA 2010). The NRKW population is listed as Threatened in Canada (Government of Canada 2010b). Critical Habitat has been identified for both populations (Figures 2 and 3) and an evaluation of the threats to both the individuals and their Critical Habitat is currently under way. The sizes of the two small and reproductively isolated populations have fluctuated since photo-identification studies first shed light on their demographics in the early 1970s (Bigg *et al.* 1990; Bigg 1982; Ford *et al.* 1994; Ford *et al.* 2000a). However, the northern residents have fared better than their southern counterparts, with a 2.44% increase in population numbers per year between 1974 and 2003 compared to just 0.71% increase in population numbers per year between 1973 and 2003 for the southern residents (Fisheries and Oceans Canada 2008). This is explained by

southern residents having a lower female age at sexual maturity (as indicated by estimated female age at first successful calf), apparently reduced reproductive females among their peers, and higher mortality rates, compared to northern residents (Olesiuk *et al.* 1990). The critical importance of Chinook salmon has been highlighted as a major driver of birth and mortality rates among resident killer whales (Ford *et al.* 2010), although PCBs could exacerbate food shortages through a variety of mechanisms.

The marine water of British Columbia also harbour a population of Steller sea lions (*Eumetopias jubatus*), which are part of the Eastern stock in North America. Although the British Columbia population has been increasing, Steller sea lions are listed as of “Special Concern” under the terms of the Species at Risk Act (SARA) and COSEWIC. Conservation concerns include human disturbance, oil spills and exposure to environmental contaminants (COSEWIC, 2003; Olesiuk 2008). While the eastern stock is considered stable or increasing, the western stock has declined during the last 30 years across its entire range. Several hypotheses have been formulated to explain this (Trites and Larking 1996; Eberhardt *et al.* 2005). In addition to the hypotheses involving nutritional stress and shifts in ocean–climate (Rosen and Trites 2000; Trites and Donnelly 2003; Tites *et al*. 2007), contaminants have been suggested as an environmental stressor contributing to the decline of the western stock (Barron *et al*. 2003). While low to moderate concentrations of PCBs have been generally observed in Steller sea lions from the declining western stock, as well as some animals from the eastern stock (Krahn *et al.* 1997; Krahn *et al*. 2001; Chapter 6 in this thesis), the assessment and health risks of PCB bioaccumulation and contamination in the Steller sea lion food web is unknown.

PCBs have been implicated in the disruption of endocrine and immune systems in pinnipeds (De Swart *et al.* 1994). Such observations explain at least partly the increased

incidence of reproductive impairment (Helle *et al.* 1976; De Guise *et al.* 1995) and disease outbreaks (Ross *et al.* 1996a) in free-ranging populations of seals and whales. There are a number of established effects of PCBs in mammals, including reproductive impairment (Addison 1989), immunotoxicity (Brouwer *et al.* 1989; De Swart *et al*.1996; Ross *et al.* 1995; Ross *et al.* 1996b; Mos e*t al.* 2006), skeletal abnormalities (Bergman *et al*.1992; Ross *et al*. 2000), and endocrine disruption (Brouwer *et al.* 1989; De Swart *et al.* 1996; Ross *et al.,* 1996b; Ross *et al.* 2000; Tabuchi *et al.* 2006). PCBs have been linked to cancer in both humans (Bertazzi e*t al.* 2001) and California sea lions (Ylitalo *et al.* 2005), and are listed as probable human carcinogens by the US EPA and International Agency for Research on Cancer (ATSDR 2000).

In addition, studies of free-ranging harbour seals and bottlenose dolphins have generated more insights into the effects of PCBs on marine mammal health (see Table 1). While PCBs represent one chemical class found in complex environmental mixtures, they have been viewed by some researchers as the pre-eminent contaminant threat at the top of aquatic food webs in the northern hemisphere over the past three decades (Elliott *et al.* 1989; Elliott and Norstrom 1998; Ross *et al*. 2000; Ross and Birnbaum 2003; Best *et al*. 2010). In British Columbia, a comprehensive risk-based assessment of different POPs in harbour seals clearly identified the PCBs as the top concern (Mos *et al.* 2010). While a similar exercise has not yet been conducted in killer whales, this ranking is not expected to differ markedly.

PCB concentrations measured in adult northern and southern resident killer whales range from 9,300-146,000 µg/kg lipid weight (Ross *et al.* 2000), which readily exceed thresholds for the onset of adverse health effects determined for other marine mammals that range from 10,000-77,000 µg/kg PCB in blubber or liver *(*Hall *et al.* 2006; Kannan *et al.* 2000; Reijnders 1986; Ross e*t al.* 1996a*)*. Given the special vulnerability of killer whales to contamination by PCBs and related

contaminants and their associated health effects, it is important that current *Canadian Environmental Protection Act* (CEPA) regulations for disposal at sea be critically evaluated in this regard, with an emphasis on contamination within the species' Critical Habitat. Studies, such as those by Hickie *et al.* (2007) and Natale (2007), have evaluated the protectiveness of sediment guidelines and regulations (e.g., CEPA Action Levels) for upper trophic level organisms and the results indicate that the guidelines and regulations are often not protective for biomagnifying contaminants. However, most sediment quality guidelines and regulations were not designed to protect wildlife subject to high degree of food web bioaccumulation, and do not consider upper trophic levels. To protect 95% of the population of male harbour seals in Burrard Inlet, Natale (2007) found that total PCB concentrations in sediments would need to be below 1.13 µg/kg dry weight. This value is 20 times lower than the current CCME Interim Sediment Quality Guideline for total PCBs of 21.5 µg/kg dry weight (CCME 1999)

This study develops and applies a food web bioaccumulation model approach based on the previous PCBs model for San Francisco Bay developed by Gobas and Arnot (2010) with the aims of conducting an eco-toxicological risk assessment by modelling the role of salmonid fish (i. e. Chinook salmon) and herrings as biological vectors of pollutants to top predators (killer whales and Steller sea lions), and of improving our understanding of the bioaccumulation and health effects of PCBs to determine if PCB-contaminated sediments in British Columbia (e. g. Strait of Georgia) poses a threat to marine mammals via harm to individuals (see Section 32 of the *Species at Risk Act*) as PCBs biomagnify up the food web.

In addition, since the Canadian Sediment Quality Guidelines (SQGs) for contaminants were designed to be protective only for benthic organisms, without taking into account bioaccumulation, and were not designed to protect top predators, including marine mammals and sea birds, from

contaminants, such guidelines do not currently exist. However, the SQGs are the only broadly available sediment quality criteria for the management and assessment of sediment contamination in Canada, and are routinely used in site-specific risk assessment and remediation efforts to protect aquatic biota. Therefore, an assessment of their value in protecting upper trophic level wildlife such as killer whales and Steller sea lions was also conducted.

Table 7.1 POP-related health effects have been characterized in a series of captive and free-ranging studies of marine mammals. These studies have largely implicated the PCBs as the dominant cause of reported effects.

Species	Health Endpoint Affected	PCB Estimated Effects Concentration (lipid weight)	Reference
Harbour seal	Reproduction	25 mg/kg	Reijnders (1986)
	Vitamin A and thyroid hormones		
Harbour seal	Immune function	17 mg/kg	Ross *et al.* (1996b) ; Ross *et al.* (1995);
	Natural killer cell activity		
	T-cell function		De Swart *et al.* (1994) ; De Swart *et al.* (1996)
	Antibody responses		
	Vitamin A and thyroid hormones		
Bottlenose dolphin	Population growth rate	10 mg/kg	Hall *et al.* (2006)
Harbour seal	EC_5*	1.3 mg/kg	Mos *et al.* (2010); Tabuchi *et al.* (2006); Mos *et al.* (2007).
	Immune function		
	Vitamin A and thyroid hormones		
	Thyroid hormone receptors		

*EC_5 is the upper confidence limit of the 5% exposure concentration equivalent to a tissue residue dose (TRD) of 1.3 mg/kg lipid weight in harbour seal blubber, as measured in seals in biomarker studies and considered as a high protection-level risk tool for the assessment of sublethal effects in free-ranging marine mammals (Mos *et al.* 2010).

7.2 METHODS

7.2.1 Model Theory and Development

The development of the PCB bioaccumulation models of the coastal and oceanic food webs for killer whale critical habitats and that of the Strait of Georgia for Steller sea lions were based on the application of a food web bioaccumulation model for PCBs developed for San Francisco Bay, CA, USA (Gobas and Arnot 2010). The aim of this model is to characterize the relationship between the concentrations of PCBs in sediments and key biological species (i.e., herring, Chinook salmon, Resident killer whales and Steller sea lion) in residents killer whale critical habitats located in southern British Columbia (BC), Canada, and northern Washington State (WA), USA .The relationship between the PCB concentrations in biota (C_B in ng PCB/kg wet weight organism) and the sediment (C_S in ng PCB/kg dry weight sediment), developed for each species i, is represented by the Biota-Sediment Accumulation Factor (BSAF in kg dry weight/kg wet weight):

$$BSAF_i = C_{B,i}/C_S \quad (1)$$

The BSAF is the main output of the model and provides a method to calculate, in a "forwards" manner, the chemical concentration in selected biological species from the chemical concentration in the sediments as $C_B = BSAF \cdot C_S$. The BSAF can also be used in a "backwards" calculation, to derive a chemical concentration in the sediment that is expected to cause a particular concentration C_B as $C_S = C_B / BSAF$. The BSAF basically depends on the food web structure, species diet composition, biomass, lipid content and congener specific composition.

7.2.3 PCB Inputs and study areas

There are 209 theoretically possible PCB congeners, of which 136 having been detected in killer whales in BC (Ross *et al.* 2000). Properties of individual congeners vary, causing them to have different distributions, different levels of toxicity and half-lives in the environment ranging from a few years to a hundred years. Even though PCBs are no longer used in Canada, they are persistent and are transported atmospherically from areas that continue to use them and cycling has produced stable concentrations in the environment (Johannessen *et al.* 2008a).

PCBs enter killer whale habitat in a variety of ways: atmospheric deposition, urban runoff, sewage outfalls, ground water, watersheds such as the Fraser River, and smaller tributaries. Sediment PCB concentrations range from very low or non-detectable (outer coast) to extremely high levels as in Puget Sound's Everett Harbour (4658 µg/kg dry weight) (Long *et al.* 2005). Therefore, it is important to capture the distribution of PCB congeners in the environment in the model. Empirical studies have found a wide range of congeners in resident killer whale habitat and biota; however, we have restricted those included in the model to the ones with the most data in the areas of interest (see Appendix F-1). These tables summarize the PCB congener octanol-water (Log K_{OW}) and octanol-air (Log K_{OA}) partition coefficients used in the model areas. The tables also contain the freshwater-based K_{OW} at the mean ambient water temperature of the areas of interest. These were used to calculate the saltwater-based K_{OW} values based on the approach of Xie *et al.* (1997), which were used to determine the PCB distribution between fish and water in the areas of interest. Freshwater-based K_{OW} values at 37.5°C were used to describe partitioning between lipids and aqueous media (e.g., urine) in killer whales. Also included in the table are K_{OA} values corrected to 37.5°C, which were used in the calculation of PCB transfer between killer whales and air, via their lungs.

The model was designed to focus on seven specific areas that make up the habitat of northern and southern resident killer whales in BC and WA (Figure 7.1). These areas were: Outer coast, Queen Charlotte Strait, NRKW Critical Habitat, Strait of Georgia, SRKW Critical Habitat in Canada, SRKW Critical Habitat in the USA (summer core and Juan de Fuca Strait); and, SRKW Critical Habitat in the USA (only Puget Sound*).* For the Steller sea lion, only the Strait of Georgia was designated as the study area for modelling purposes.

PCB sediment concentration monitoring programs have included a significant distribution of PCB sediment concentration hot spots throughout the Strait of Georgia and transboundary areas of Puget Sound (Grant *et al.* 2010). A fairly large number of independent sediment PCB concentration measurements have been collected from the region and can provide a reasonable representation of the spatial distribution of the PCB concentrations in the Critical Habitats. The PCB data of sites where empirical sediment concentration were obtained and then used in the food web model are provided in Lachmuth *et al.* (2010). Total PCB (ΣPCB) concentration is calculated as the sum of the concentrations of the congeners included in the model.

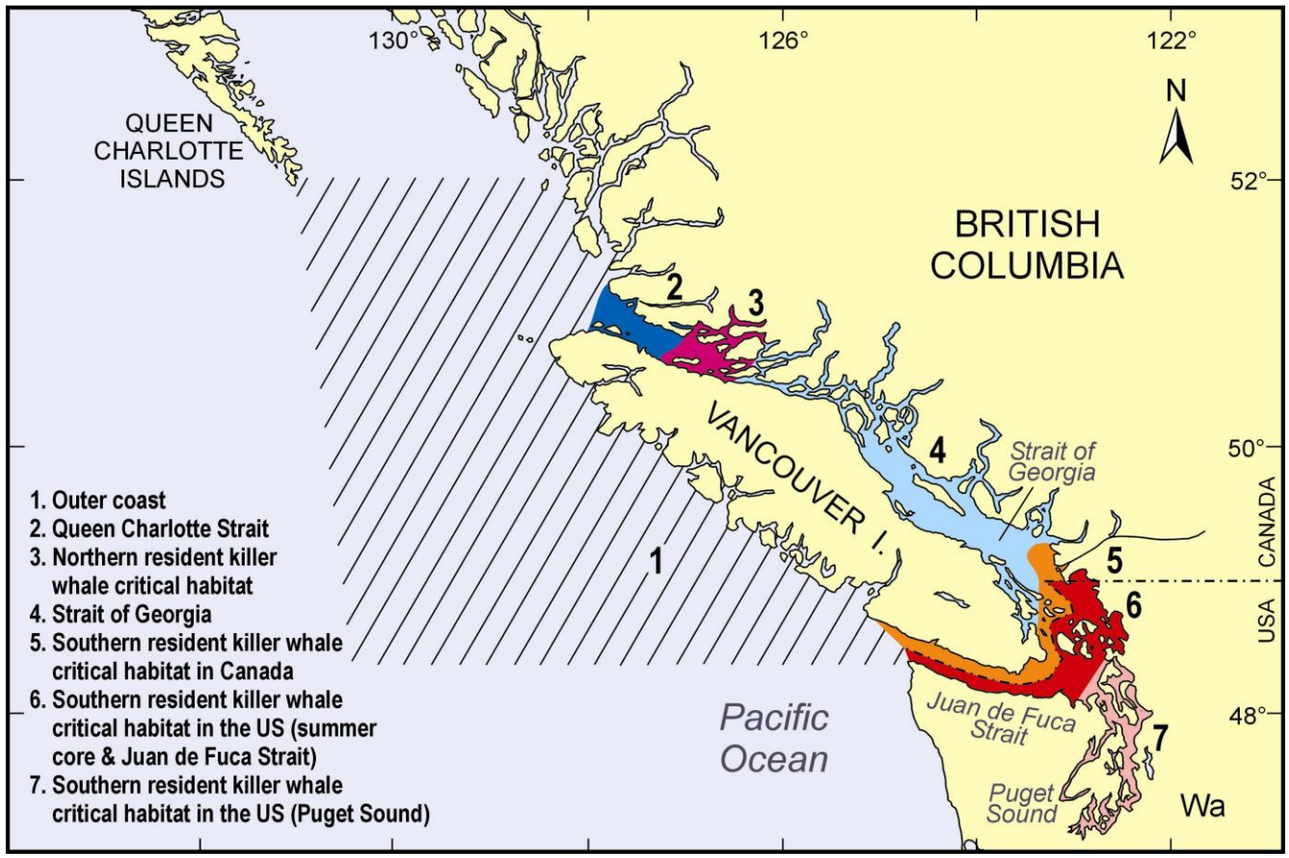


Figure 7.1 The seven areas included in the food web bioaccumulation model. Designated Critical Habitat for northern (Area 3) and southern (Area 5) resident killer whales in British Columbia and in the US (Areas 6 and 7) are also depicted in the figure. The Strait of Georgia area was used for the bioaccumulation model in Steller sea lions (taken from Lachmuth *et al.* 2010).

Since killer whales and Steller sea lion are warm-blooded, air-breathing organisms, in which the chemical inhalation and exhalation are important routes for uptake and elimination of PCBs, PCB air concentrations were also incorporated in the food web models. Concentrations of total PCBs in air were obtained from the near urban Saturna Island station to represent air concentration (9.3×10^{-6} ng/L) in Critical habitats within the Strait of Georgia, and the remote Ucluelet station for air concentration (8.9×10^{-6} ng/L) in offshore habitat at the west coast of

Vancouver Island (Noël *et al.* 2009). These PCB concentrations in air are very low and may not represent a direct source to the marine mammals' burden through inhalation. Although the model builds on the assumption that an increase in sediment PCBs would lead to a consequent increase in delivery of PCBs to the killer whale and Steller sea lion food webs, increases in PCB concentrations in water was also tested to asses the impact in bioaccumulation of PCBs in the marine food web.

7.2.4 Environmental Conditions of Areas Included in the Model

The environmental condition input variables used in the seven model areas are reported in Appendix F-2. In water, PCBs can be freely dissolved or absorbed to particulate organic matter (POM) and dissolved organic carbon (DOC). These values were obtained from the literature or were estimated based on the relationship that most organic carbon (~80%) in water is in the form of DOC (Lachmuth *et al.* 2010).

7.2.5 Steady-State Assumption

Steady state models assume that contaminant concentrations have enough time to exchange between the water column, the sediments, and biota in the food web and reach a dynamic "equilibrium" (contaminant concentrations no longer change over time). An important implication of the selection of the steady-state approach is that PCB concentrations in biota are directly proportional to the PCB concentrations in the habitat sediments. However, seasonal changes and the effect of age on PCB concentrations can still be captured with a steady state approach by using the appropriate parameters. A steady state rather than time dependent approach was adopted for the resident killer whale food web bioaccumulation model because the

time response of sediment PCB concentrations to changes in loadings and external conditions is slow compared to that in biota. The environmental half-life for PCBs has been estimated to range from a few years to 100 years (Jonsson *et al.* 2003; Sinkkonen and Paasivirta 2000), while the half life of PCB 126 in rainbow trout (a salmonid) ranges from 82-180 days (Brown *et al.* 2002). This assumption is valid for small aquatic organisms (e.g., plankton) as equilibrium between uptake and elimination is quickly reached; however, this process can be much longer for larger organisms (e.g., seals and killer whales), as their body burden often lags behind changing environmental conditions (Hickie *et al.* 2007). Thus steady-state models often overestimate concentrations in larger organisms because those concentrations are not likely to be reached in the short time-span that the model considers (Natale 2007). To maintain simplicity in the model we applied a steady state approach, and included different age classes for certain organisms in the food web to account for age specific differences in PCB concentration. The temporal response of PCB concentrations in the sediments is the “rate controlling” step in the model. The model is designed to predict the steady state concentrations in biota due to exposure to PCBs in air, water, and sediments.

7.2.6 Structure of Killer Whale and Steller Sea Lion Food Webs

The structure of the resident killer whale food web is complex and varies spatially and temporally. Not all species and interactions present in the food web are known or were included in the model. The model is a simplification of the real world and focuses on a few key species. The model is based on the assumption that organisms at the same trophic level tend to have similar PCB concentrations, thus can be grouped as one trophic guild as long as the organisms included have similar feeding behaviours. One food web refereed as the coastal food web was used in the

Critical Habitat areas defined in Figure 7.1, in the Queen Charlotte Strait and the Strait of Georgia. The other food web was developed for the outer coast area (Figure 7.1), which has a pelagic food web that differed slightly from the coastal food web as seen in Figure 7.2. Feeding behaviour is affected by prey abundance, prey size, and predator size, and the model is designed to account for these factors. The following criteria were applied during the development of the food web structure for modeling PCB bioaccumulation in resident killer whales habitat and Steller sea lions:

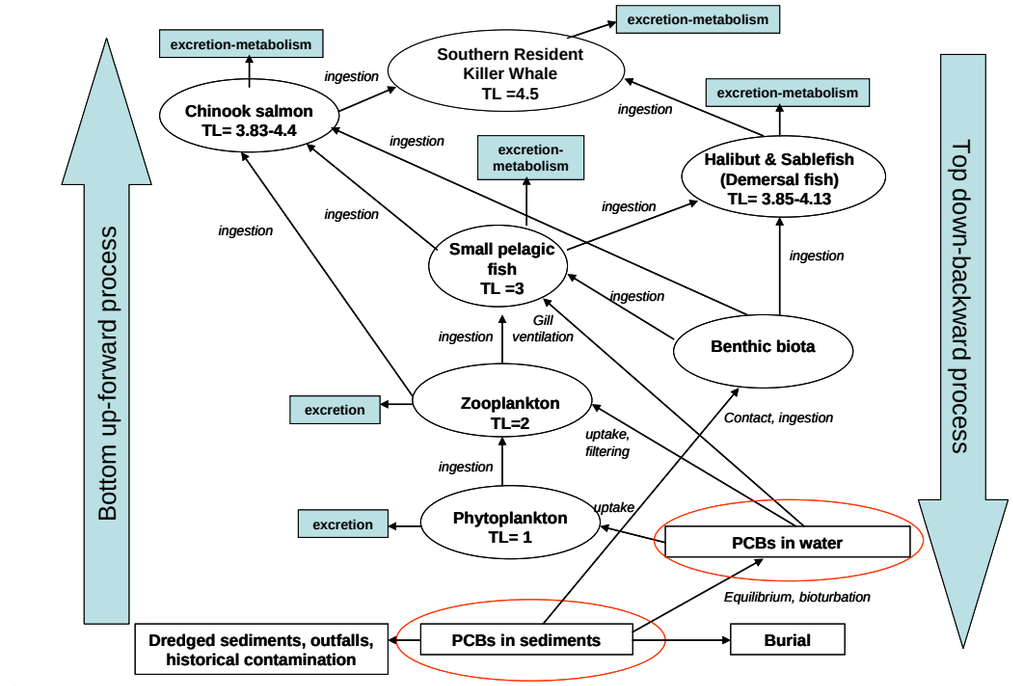
1. Species of primary interest were included. This included northern and southern resident killer whales (*Orcinus orca*), Steller sea lions (*Eumetopias jubatus*), Pacific salmon (*Oncorhynchus* spp.), including Chinook (*O. tshawytscha*), coho (*O. kisutch*) and chum (*O. keta*), Pacific halibut (*Hippoglossus stenolepis*), sablefish (*Anoplopoma fimbria*), and Pacific herring (*Clupea pallasi*).
2. Species considered local to the areas were included in the model. These species forage primarily in the areas considered. For instance, resident killer whales have been documented to spend up to 12 months per year in the coastal waters of BC and WA, feeding on fish, principally salmonids (Ford *et al.* 1998). In addition, realistic habitat distribution for both killer whales and Chinook salmon were also incorporated in the model as killer whales have seasonal movements in the study region and Chinook salmon is a migratory species.
3. Species from different trophic guilds relevant to the transfer and bioaccumulation of PCBs in the food web were included. Relevant trophic guilds include phytoplankton and algae, zooplankton (i.e., copepods), filter feeding invertebrates (i.e., mussels and oysters), benthic detritivores (i.e., amphipods, crabs, shrimp, and polychaetes), juvenile and adult forage and predatory fish, Steller sea lions and resident killer whales.

4. Important trophic guilds were represented by one or two species to simplify the model and render calculations transparent.

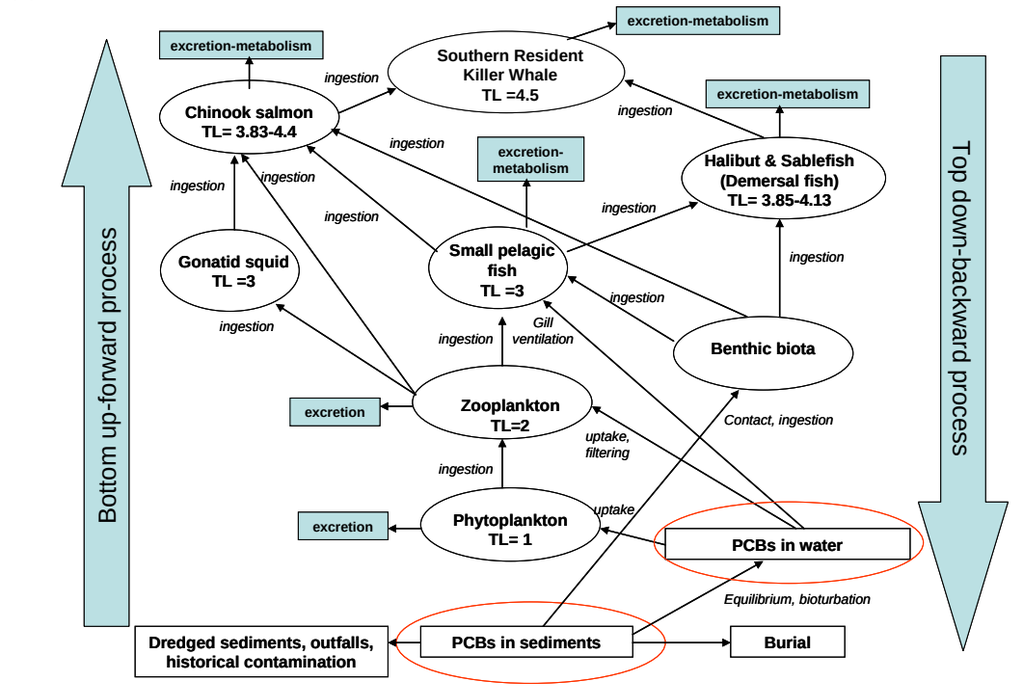
5. Species with available empirical PCB concentration data were included to allow evaluation of the accuracy of the model predictions. Empirical PCB concentration data were available for Chinook salmon, northern resident killer whales and wintering Steller sea lion from the Strait of Georgia.

The number of species in the model was further minimized to keep the model simple and make model calculations more transparent. Simplifications of the food web (i.e., exact feeding preferences of fish) are consistent with evaluations of food webs that are sediment-driven (von Stackelberg *et al.* 2002b). Only the most abundant prey items for each fish species to represent their feeding behaviour and dietary preferences were included. This approach produced a food web bioaccumulation model that included one category for phytoplankton, one category for zooplankton, eight invertebrate species (including detritivores and filter feeders), 12 fish species, and male, female, juvenile and newborn resident killer whales. Most of the data on ecology, feeding habits/diet composition and trophic position for fish and other aquatic biota were retrieved from www.fishbase.org (Froese and Pauly 2010) and www.sealifebase.org (Palomares and Pauly 2010), respectively. In addition, various peer-reviewed papers were consulted when information on life history parameters, prey items, and diet composition were unavailable in the web link sources. Weight and lipid content of Chinook salmon for killer whale Critical Habitats (i.e., Johnstone Strait, Strait of Georgia, and Puget Sound), for example, were obtained from Cullon *et al.* (2009). The biological and physiological parameters used in the food web bioaccumulation model are listed in Appendix F-3.

The species that were included in the model, diet composition and their feeding relationships are listed in Appendix F-4 (Feeding Preferences Matrix - dietary composition and trophic levels for coastal and oceanic food webs). Coastal and oceanic food webs are illustrated in Figure 7.2, respectively. Figure 7.2a is a schematic diagram of organisms included in the coastal food web and the representative trophic interactions considered, while Figure 7.2b is a conceptual diagram of the oceanic food web. The main difference between the two food webs is that Chinook salmon primarily feed on squid in the outer coast, rather than herring.



(a)



(b)

Figure 7.2 Conceptual diagram illustrating organisms included in the model and their trophic interactions and trophic level for coastal (a) and oceanic (b) food webs. The figure also highlights the pathways PCBs move from sediments and the water column to biota. Steller sea lions occupy a trophic position similar to that of resident killer whales, but with a different diet composition.

7.2.7 Resident Killer Whales

Southern resident killer whales are composed of three pods: J, K and L. These pods range from Monterey Bay, California to Langara Island, BC, which is approximately 2000 km along the Pacific coast (Ford 2006). From early summer to late fall they are common off the coast of southeastern Vancouver Island and Puget Sound (Ford 2006), and in July and August 90% of their time is spent in their Critical Habitat in Canada and the US (Ford *et al.* 2010; Figure 7.1). In winter and spring SRKWs travel extensively in outer coastal waters (Ford *et al.* 2000b; Nichol and Shackleton 1996; Wiles 2004). However, J pod is often sighted in inshore waters all months of the year. K and L pods usually return to the Georgia Basin in May or June and leave in October or November. From May to November all three pods make excursions to outer coastal areas for several days at a time (Ford 2006). From this information and based on the data reported by Lachmuth *et al.* (2010), it was estimated that the annual distribution of SRKWs in the areas included in the food web bioaccumulation model are as follows:

- Time spent in outer coast is ~37% of the year.
- Time spent in Canadian Critical Habitat is ~18% of the year.
- Time spent in US Critical Habitat (summer core and Juan de Fuca Strait) is ~36% of the year.
- Time spent in US Critical Habitat (Puget Sound) is ~6% of the year.
- Time spent in the Strait of Georgia is ~3% of the year.

Northern resident killer whales range and forage in coastal waters from Glacier Bay, Alaska, to Gray's Harbour in Washington (Ford 2006). During summer and fall they are often found in nearshore waters off the coast of northeastern Vancouver Island (Ford 2006). Like SRKWs, during

winter and spring NRKWs travel extensively in outer coastal waters (Ford *et al.* 2000b; Nichol and Shackleton 1996; Wiles 2004). The Johnstone Strait Critical Habitat area is used by NRKWs all months of the year, but they are most often seen there from July-October, and are seen infrequently there from March-May (Ford 2006). On average 14.5% of the average 222 animals in the population are present in Critical Habitat from July to August (Ford *et al.* 2010). Based on the information reported elsewhere (Lachmuth *et al.,* 2010), the annual distribution of NRKWs in the areas included in the food web bioaccumulation model were estimated as follows:

- Time spent in Critical Habitat is ~8% of the year.
- Time spent in Queen Charlotte Strait is ~17% of the year.
- Time spent in outer coast is ~75% of the year.

For modeling purposes it was assumed that annual pod distributions were the same for all pods. The distributions in the model areas described above for NRKWs and SRKWs were used as "realistic" model scenarios, whereas "hypothetical" model scenarios consider killer whales spend 100% of their time in one of the model areas. This approach provides a range of scenarios for management purposes.

To characterize the resident killer whale food web, published information on killer whale diet was used to determine which fish species to include. Salmonid species comprise 96% of the diet of resident killer whales, of which 71.5% is Chinook salmon (Ford and Ellis 2006). The only non-salmonid species in killer whale diet identified by Ford and Ellis (2006) were Pacific herring (*Clupea pallasi*), sablefish (*Anoplopoma fimbria*), yelloweye rockfish (*Sebastes ruberrimus*), quillback rockfish (*Sebastes maliger*), and Pacific halibut (*Hippoglossus stenolepis*). Ford and Ellis (2006) suspected that the herring and rockfish are not targeted as prey items by killer whales but

that halibut and sablefish are consumed by killer whales. Rockfish were observed to be only partially eaten by killer whales and then discarded. Herring are likely consumed by salmon which are then consumed by the killer whales (Ford and Ellis 2006). The main prey items of resident killer whales are therefore Chinook salmon, while halibut and sablefish constitute only a small fraction of killer whales diet. In “realistic” model scenarios we set the resident killer whale diet as: 96% Chinook salmon, 2% halibut, and 2% sablefish.

More recent data collection and analyses by Ford *et al*. (2010) confirm earlier findings (Ford and Ellis 2006). This study found that resident killer whales consumed 71% Chinook salmon, 24% chum salmon, and other salmonids comprised less than 3% each to the overall diet (Ford *et al.* 2010). However, significant variation in the percentages occurs seasonally, for example chum salmon are more important than Chinook in October and November (Ford *et al.* 2010). Under this premise and using the data provided by Lachmuth *et al.* (2010), the resident killer whale diet was refined with the aim to include more species that they are likely consuming in winter months when little prey sampling studies are conducted, as provided in Table 4. We considered the revised resident killer whale diet to be: 70% Chinook salmon, 15% other salmonids (10% chum, 5% coho), and 15% groundfish (3% halibut, 3% sablefish, 3% lingcod, 3% dover sole, 3% gonatid squid).

The majority of Chinook salmon consumed by SRKWs originate from the south Thompson River, but killer whales also consume south Fraser River Chinook (Ford *et al.* 2010). Resident killer whales consume approximately 75% ocean-type Chinook salmon as stream-type Chinook migrate directly from natal rivers to the open ocean off the continental shelf and do not spend a significant amount of time in coastal waters (Ford *et al.* 2010). During winter when Chinook salmon abundance is low, ground fish such as sablefish can become prey items for resident killer whales and SRKW spend more time feeding on salmon in Puget Sound (Ford *et al.* 2010). During

July and August they are likely eating close to 100% Chinook. During this time, SRKWs spend approximately 90% of their time in Critical Habitat, while NRKWs only spend 14.5% of their time in Critical Habitat during July and August (Ford *et al.* 2010). Both northern and southern resident killer whales leave Critical Habitat and head out of coastal areas, and have been found foraging at Swiftsure Bank, just outside the mouth of Juan de Fuca Strait, the extent of Critical Habitat (Ford *et al.* 2010). However, resident killer whales likely do not stray beyond the continental shelf to open ocean areas as salmon distribution is extremely patchy in those waters (John Ford, Fisheries & Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Rd., Nanaimo, BC V9T 6N7, pers. comm., 2010; Lachmuth *et al.* 2010).

There is high variability in PCB concentrations in killer whales related to age, sex, reproductive status and birth order (Ross *et al.* 2000a; Ylitalo *et al.* 2001). Newborns have low contaminant concentrations. However, concentrations of contaminants increase as newborns nurse and absorb contaminant from lipid rich milk, and the contaminant load is especially high for first born calves (Ylitalo *et al.* 2001; Hickie *et al.* 2007). One year old killer whales tend to be the most contaminated members of the population, and as killer whales grow and switch to a less contaminated fish diet, their PCB concentration is diluted (Ylitalo *et al.* 2001). At approximately 15 years of age, PCB concentrations in male killer whales tend to increase, whereas females transfer a substantial fraction of their contaminant burden to their offspring (Ylitalo *et al.* 2001; Hickie *et al.* 2007). The mean lifetime of female killer whales is approximately 50 years and males 29 years (Olesiuk *et al.* 1990).

7.2.8 Steller Sea Lions

In coastal waters of British Columbia, the Steller sea lion is the only member of the family Otariidae that resides year-round and breeds in Canadian waters (Olesiuk 2008). Steller sea lions breed at traditional rookeries on the Scott Islands off the north tip of Vancouver Island, at Cape St. James off the southern tip of the Queen Charlotte Islands, on the Sea Otter Group off the central coast, and on North Danger Rocks off the northern mainland coast. There is also a major rookery situated north of the BC border on Forrester Island in Alaska (Olesiuk 2008).

During summer, non-breeding animals are found at year-round haulout sites. There are 23 such sites distributed off B.C., primarily along the outer exposed coast. In August, animals disperse from rookeries to feed, and begin to occupy numerous winter haulout sites, many of which are located in inside protected waters. Nursing of pups and young animals can last up to 2 or 3 years. Although the species is considered non-migratory, there are well-defined local seasonal movements in some areas. In the southern part of their range, Steller sea lions migrate north along the Oregon and Washington coast (Olesiuk 2008; Figure 7.3). This coincides with a dramatic increase in the number of sea lions wintering off the coast of southern Vancouver Island. Non-breeding animals can disperse distances of up to 1,700 km from where they were born.

In 2006, the total Steller sea lion population counted during the breeding season in British Columbia was 19,800 individuals, including pups, breeding and non-breeding animals (Olesiuk 2008). The number of pups has increased from about 1000 animals in the early 1970s to more than 3400 individuals in 2002 and about 4800 animals in 2006 (Olesiuk 2004; Olesiuk 2008). Based on estimated pup production and life table statistics there were 20,000-28,000 Steller sea lions inhabiting coastal waters of British Columbia in 2006, with an overall growth rate of 3.5% per year (Olesiuk 2008). Current aerial surveys in 2010 indicated that the population has increased

by 25% (P. Olesiuk, pers. comm.). The number of Steller sea lions wintering off southern Vancouver Island increased steadily from less than 1000 animals in the 1970s to more than 3000 individuals in 2004 (Olesiuk 2004), which is about 15% of the total population inhabiting the marine water of British Columbia. These animals are highly mobile and disperse widely during the non-breeding season, and numbers in the Strait of Georgia fluctuate, thus “several thousand” typically winter in the Strait of Georgia (Figure 7.3)

The diet of the Steller sea lions from British Columbia has been scarcely studied. Pacific herring, hake and salmon are major prey species consumed by Steller sea lions, including those from Southeast Alaska, British Columbia and Oregon (Bredsen *et al.* 2006; Trites *et al.* 2007; Trites and Calkins 2008). Recent data on scat analysis showed that Steller sea lions from British Columbia are predominately piscivorous, foraging on Pacific herring, salmon, rockfish and sandlance (Olesiuk 2004; A. Trites, pers. comm.). In fact the seasonal movement of the Steller sea lion into the Strait of Georgia is linked to the seasonal abundance of herring (Olesiuk 2004).

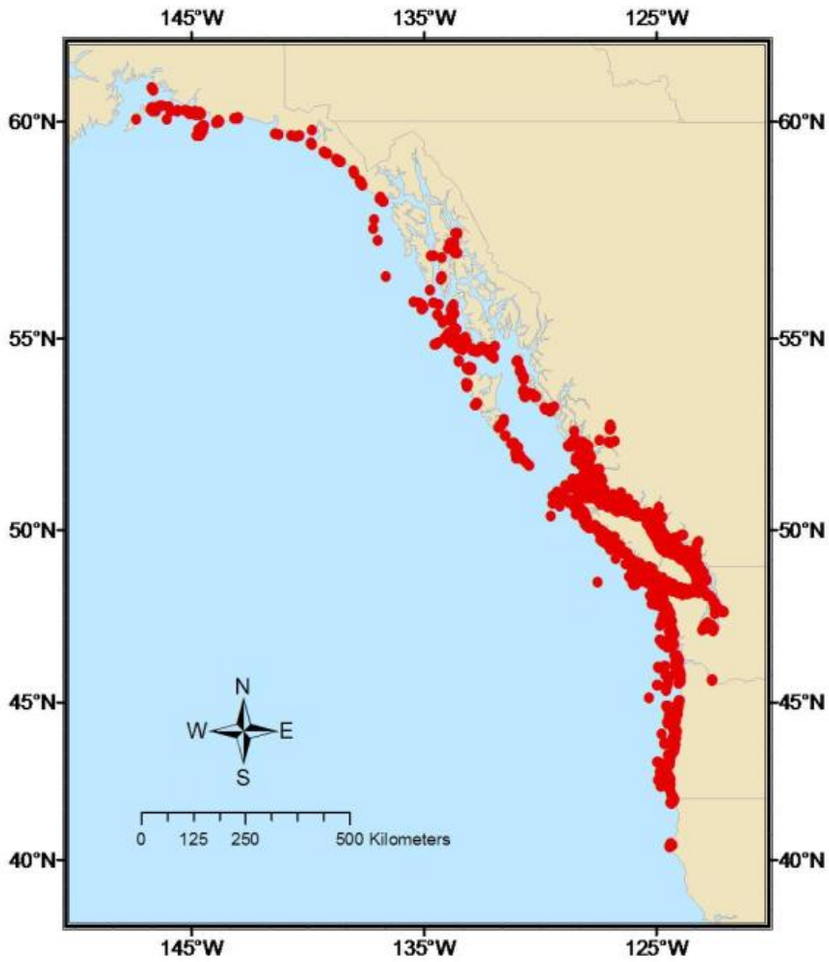


Figure 7.3 Map of satellite locations (ARGOS) showing the movements and distribution of Steller sea lions (red dots) tagged at Norris Rock, Strait of Georgia (BC, Canada). Due to the widely disperse home range, these animals can be considered representative of the Eastern population of Steller sea lions (Courtesy of P. Olesiuk, Pacific Biological Station, DFO).

To model the bioaccumulation of PCBs in the Steller sea lion food web, a “hypothetical” scenario was set up, assuming that Steller sea lion spend 100% of their time in the Strait of Georgia area. The rationale for this assumption in the modelling wok was adopted because these animals are extensively distributed with long movements outside and inside the Strait of Georgia (Figure 7.3). In addition, it was assumed that Steller sea lions feed exclusively on non-migratory

herring populations (*Clupea pallasi*) found in the Strait of Georgia (Therriault *et al.* 2009) and Pacific salmon (*Oncorhynchus* spp.), as studies on the diet of Steller sea lion from the Strait of Georgia still need more work. Therefore, the composition diet for the purpose of this modelling exercise was 80% Pacific herring, 6.7% Chinook salmon; 6.7% chum salmon; and 6.7% coho salmon.

7.2.9 Chinook Salmon

Chinook salmon (*Oncorhynchus tshawytscha*) are anadromous, spending most of their life at sea and returning to natal streams to spawn (Healey 1991). They can accumulate PCBs from the water via gill uptake, and from dietary uptake (Qiao *et al.* 2000). While some PCB exposure may occur during their time in freshwater, estuarine and coastal environments, approximately 97-99% of PCBs is derived from global sources during their time outside of their natal streams, in marine waters (Cullon *et al.* 2009). During the migration back to natal streams, Chinook salmon can loose more than 80% of their lipid reserves (Brett 1995), which magnifies their PCB burden as PCBs are lipid-soluble (DeBruyn *et al.* 2004). SRKWs feed on Chinook salmon in waters that are relatively more contaminated, near-urban, and closer to natal streams than NRKWs, thus are likely eating fish that are more contaminated and have fewer lipids (Cullon *et al.* 2009). Adult Chinook salmon primarily feed on forage fish, such as herring, sardine, anchovy, smelt, and groundfish, but also eat krill, squid, and crab (Brodeur 1990). Two food webs for Chinook salmon were created. One food-web represents the diet of coastal-marine habitats while in continental shelf waters (coastal phase). The other food-web represents their time during their oceanic life stage when they are off the continental shelf (pelagic phase). In the Strait of Georgia, juvenile Chinook mainly eat herring, but they also consume crab megalops, amphipods, euphausiids, and

insects (Healey 1980). The diet of juvenile Chinook further north in the Strait of Georgia is much less reliant on fish. While in their pelagic phase, Chinook salmon primarily eat gonatid squid (which are micronektonic), they also forage on mid-water fish and euphausiids (Pearcy *et al.* 1988).

There are two behavioural forms of Chinook salmon life history in BC, the “stream-type” and “ocean-type”, with the ocean-type being most common (Healey 1991). The stream-type Chinook rear in freshwater for a year or more and then migrate to the ocean where they travel extensively off the continental shelf for a year or longer before returning to their natal stream several months before they spawn (Healey 1991). The ocean-type Chinook usually migrate to the ocean as juveniles within three months of emergence and usually do not disperse more than 1,000 km from their natal river, and return to their natal river a few days or weeks before spawning (Healey 1983; Healey 1991). Approximately 75% of the Chinook salmon that resident killer whales eat are ocean-type, and 25% are stream-type (Ford *et al.* 2010).

Approximately 58% of Chinook salmon eaten by resident killer whales in all areas of the BC coast are composed of stocks from the Fraser River system (Ford *et al.* 2010). This predominance of Fraser River Chinook is especially pronounced in NRKW Critical Habitat (64%) and SRKW Critical Habitat (75%) (Ford *et al.* 2010). Of these Fraser River stocks, resident killer whales primarily eat South Thompson River and Lower Fraser River Chinook (Ford *et al.* 2010). South Thompson River Chinook migrate north after leaving freshwater, and spend the least amount of time of any Chinook stock in southern BC (Lachmuth *et al.* 2010). Fraser River Chinook stocks are the most prominent Chinook stock on the coast and once they enter saltwater they do not follow northward migration route, but are found at all life-stages in southern BC, from the Queen Charlotte Islands to Oregon, Puget Sound, and they also spend time offshore in the open ocean

(Lachmuth *et al.* 2010). To simplify the modeling process, we assumed that resident killer whales only eat South Thompson and Fraser River stocks of Chinook salmon.

Fishing mortality distribution tables (from 1985 to 2007) for Chinook salmon in different fishery regions were used as a proxy for estimating the annual fraction or percent of time that Chinook spend in the model areas. These estimates were provided by Gayle Brown (Fisheries & Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Rd., Nanaimo, BC V9T 6N7), as reported by Lachmuth *et al*. (2010). As seen in Table 2, the average annual distribution (% time) for South Thompson and Fraser River Chinook salmon in the areas included in the model were labelled as “realistic” scenarios. Hypothetical scenarios occurred when we considered the salmon to occupy a model area for 100% of its life to obtain best and worst case results.

Table 7.2 Average annual distribution (% time) of South Thompson and Fraser River Chinook in the areas included in the model (Gayle Brown, Fisheries & Oceans Canada, Pacific Biological Station, 3190 Hammond Bay Rd., Nanaimo, BC V9T 6N7, pers. comm., 2010; Lachmuth *et al*. 2010).

Area	South Thompson Chinook	Fraser River Chinook
Outer coast	80%	55%
Queen Charlotte Strait	8%	2%
NRKW Critical Habitat (CH)	3%	14%
Strait of Georgia	3%	8%
SRKW CH in Canada	3%	8%
SRKW CH in US (summer core and Juan de Fuca Strait)	2%	4%
SRKW CH in US (Puget Sound)	0.2%	9%

7.2.10 Chum Salmon

Chum salmon (*Oncorhynchus keta*) are benthopelagic and anadromous, as they inhabit coastal streams before moving to the ocean (Riede 2004). Migrating fry form schools in estuaries and remain close to shore for a few months before dispersing into the ocean (Scott and Crossman 1973). The diet of juveniles and adults is composed mainly of copepods, tunicates, euphausiids, pteropods, squid, and small fishes (Scott and Crossman 1973). The diet is 17-40% pteropods, 17-60% euphausiids, 52% fish, 10% salps, and 10% mixed items (Birman 1960). The order of abundance of food items is (1) amphipod / euphausiid / pteropod / copepod, (2) fish; and, (3) squid larvae (Kanno and Hamai 1971).

7.2.11 Coho Salmon

Coho salmon (*Oncorhynchus kisutch*) are demersal and anadromous (Riede 2004). They are found in oceans and lakes, and adults return to their natal rivers to spawn (Morrow 1980). Immature fish emerge in the spring and usually remain in fresh water for 1-2 years (sometimes up

to 4 years) (Morrow 1980), and after that time they migrate at night to freshwater lakes or to the sea (Scott and Crossman 1973).

Overall, Chinook salmon and coho salmon have a more coastal marine distribution along the continental shelf than do sockeye salmon, pink salmon, and chum salmon (Quinn 2005). When smolts reach the sea they remain close to the coast and feed on planktonic crustaceans, and as they grow they move farther out to sea and feed upon larger organisms (Morrow 1980) such as jellyfish, squid, and fishes (Coad and Reist 2004). Herring and sandlance comprise ~32% of their diet, amphipods ~34%, and crab megalops ~26% (Sandercock 1991). Adult coho and Chinook have very similar diets, except invertebrates comprise approximately one-fifth of the coho diet, and less than 3% for Chinook (Sandercock 1991).

7.2.12 Pacific Halibut

The maximum reported age of a Pacific halibut (*Hippoglossus stenolepis*) is 42 years (Armstrong 1996). It is one of the largest flatfish in the world, and the maximum reported size is 3 m and over 200 kg (Mecklenburg *et al.,* 2002). This species lives near the bottom of the ocean, and adults spend the winter in deep waters (250-600 m) along the edge of the continental shelf, where spawning occurs in late January to mid-March (Armstrong 1996; Loher and Blood 2009a; Loher and Seitz 2008). British Columbian Halibut aggregate to spawn off Langara Island and Cape St. James (Skud 1977; St. Pierre 1984). In the summer they move to shallow coastal waters (<200 m deep) (Loher and Seitz 2008) to feed on fishes, crabs, clams, squid, and invertebrates (Hart 1973). Halibut can also move alongshore seasonally, and some of British Columbia's summer biomass may join spawning groups in southern Alaskan waters, while halibut from Washington

and Oregon may move north to Canadian waters (Loher and Blood 2009b; Loher and Blood 2009a).

7.2.13 Sablefish

Sablefish (*Anoplopoma fimbria*) are found on mud bottoms in waters deeper than 200 m (Allen and Smith 1988), with adults usually at the continental shelf-slope margin (Harvey 2009). They tend to be localized but some juveniles migrate more than 2,000 miles over 6-7 years (Armstrong 1996). They are a long lived species with a maximum reported age of 114 years (Beamish and MacFarlane 2000), and can reach up to 57 kg in weight (Eschmeyer *et al.* 1983), and one meter in length (Schirripa and Colbert 2005). Their diet is composed of crustaceans, worms, and small fishes (Clemens and Wilby 1961).

7.2.14 Lingcod

Lingcod (*Ophiodon elongates*) are demersal, ranging from the intertidal to depths of 475 m (Allen and Smith 1988), with adults typically found near rocks, and young found on sand or mud bottom of bays and inshore areas (Eschmeyer *et al.,* 1983). They are oceanodromous (Riede 2004), and both migratory and non-migratory populations exist (Hart 1973). The average weight of lingcod is 30 kg (Stock and Meyer 2005), and the maximum reported age is 20 years (Miller and Geiber 1973). Young feed on copepods and other small crustaceans (Hart 1973); while adults mainly eat other fishes but they also take crustaceans, octopi, and squid (Clemens and Wilby 1961).

7.2.15 Dover Sole

Dover Sole (*Microstomus pacificus*) are demersal, with a depth range from 10 - 1370 m (Russian Academy of Sciences 2000). They are found on mud bottoms and move into deep water in winter (Eschmeyer *et al.* 1983). The average male weight is 245 g, and female weight is 508 g (Choromanski *et al.* 2005), and the maximum reported age is 45 years (Beddington *et al.* 1985). The diet of adults is 10.5-42.7% polychaetes, 41.4-84% ophiuroids, 3.5-14.5% mollusks, and 1.5-2.1% crustaceans (Gabriel and Pearcy 1981).

7.2.16 Pacific Herring

Pacific herring (*Clupea pallasii pallasii*) populations in Puget Sound and the east side of the Strait of Georgia are non-migratory (Therriault *et al.* 2009). However, most herring populations in the Strait of Georgia are migratory and spend late spring, summer, and fall in feeding grounds (shelf waters <200 m deep) on the west coast of Vancouver Island (Tanasichuk 1997;Therriault *et al.* 2009). There is also a herring stock on the west coast of Vancouver Island, which comingles with the Strait of Georgia stock on the summer feeding grounds (Megrey *et al.* 2007). During the fall, herring form dense concentrations and then congregate in spawning areas in February and March (Therriault *et al.* 2009). Spawning occurs from February to May (mainly in March and April) and is concentrated on the east side of Vancouver Island between Saltspring and Denman islands. Juvenile herring (at least one year of age) do not migrate until after their second summer in the Strait of Georgia (Therriault *et al.* 2009). Thus migratory herring populations spend approximately half the year in the Strait of Georgia. Adult herring feed primarily on zooplankton, larval invertebrates, and small fish (Robinson 2000; Iverson *et al.* 2002).

Resident herrings in Puget Sound exhibited PCB contamination levels 3-9 times higher compared to herrings in the Strait of Georgia, which is likely due to their year-round proximity to near-urban areas (West *et al.* 2008). Herring populations from northern British Columbia are composed of three spawning stocks: Queen Charlotte Islands, Prince Rupert, and Central Coast (Megrey *et al.* 2007). These three populations spawn in locations different than the southern populations.

7.2.17 Gonatid Squid

Squid (*Gonatius sp.*) were included in the revised resident killer whale diet, and in the outer coast food web as the oceanic life stage of Chinook salmon feed predominantly (~70%) on gonatid squid, and to a lesser extent on fish and zooplankton (Ito 1964; Brodeur 1990).

7.2.18 Pollock

Pollock (*Theragra chalcogramma*) are small (max length 91 cm) (Eschmeyer *et al.* 1983) benthopelagic (depth range 0-1280 m), non-migratory fish (Fedorov *et al.* 2003) that can live up to 15 years old (Cohen *et al.* 1990). Pollock undergo diurnal vertical migrations (Cohen *et al.* 1990), and although their diet is predominantly composed of krill, they also eat fish and crustaceans (Hart 1973).

7.2.19 Shiner Surfperch

Shiner surfperch (*Cymatogaster aggregata*) are small (max length 20 cm) (Morrow 1980) demersal, non-migratory fish (Eschmeyer *et al.* 1983) that can live up to 9 years of age (Shanks

and Eckert 2005). Juveniles mainly eat copepods, and adults mainly eat various small crustaceans, mollusks, and algae (Morrow 1980).

7.2.20 Northern Anchovy

Most populations of Northern Anchovy (*Engraulis mordax*) remain off the west coast of Vancouver Island, and are unlikely to be significant forage fish in the Strait of Georgia (Therriault *et al.* 2009). Adult anchovy mainly feed on zooplankton such as euphausiids, copepods, and decapod larvae (Kucas 1986).

7.2.21 Benthic Invertebrates

Benthic organisms have a wide variety of feeding strategies (e.g., deposit feeding, suspension feeding, filter feeding, scavenging), processing PCBs bound to organic matter in the sediments and water column (Burd *et al.* 2008a).

7.3 FOOD WEB MODELS

7.3.1 Description of Food Web Bioaccumulation Model for Phytoplankton, Zooplankton, Aquatic Invertebrates, and Fish

A conceptual representation of the main routes of PCB uptake and depuration in aquatic organisms that obtain oxygen from the water is shown in Figure 7.4. The food web bioaccumulation model is based on the assumption that PCB exchange between an aquatic organism and the ambient environment can be sufficiently described by:

$$dM_B/dt=[W_B\cdot(k_1\cdot [m_O\cdot \Phi \cdot C_{WT,O} + m_P\cdot C_{WD,S}] + k_D\cdot\Sigma(P_i\cdot C_{D,i}))] - (k_2 + k_E + k_M)\cdot M_B \quad (1)$$

Where the mass (g) of the PCB congener in the organism is M_B, the net flux of PCB congener uptake and elimination by the organism at any point in time t (d) is dM_B/dt, the weight of the organism (kg) at time t is W_B, the elimination rate constant (L/kg/d) for uptake from the respiratory organ (i.e., gills or skin) is k_1, the fraction of respiratory ventilation of overlying water is m_O, the fraction of respiratory ventilation of sediment associated pore water is m_P, the fraction of the total chemical concentration in overlying water that is freely dissolved and can be absorbed via membrane diffusion is $\dot{\Phi}$ (unitless), the total concentration (g/L) of the PCB congener in the water column above the sediments is $C_{WT,O}$, the freely dissolved PCB congener concentration (g/L) in the sediment associated pore/interstitial water is $C_{WD,S}$, the clearance rate constant (kg/kg·d) for chemical uptake via ingestion of food and water is k_D, the diet fraction consisting of prey item i is P_i, the PCB congener concentration (g/kg) in prey item i is $C_{D,i}$, the PCB elimination rate constant (1/d) via the respiratory area (i.e., gills and skin) is k_2, the PCB elimination rate constant (1/d) via excretion into egested feces is k_E, and the PCB metabolic transformation rate constant (1/d) is k_M.

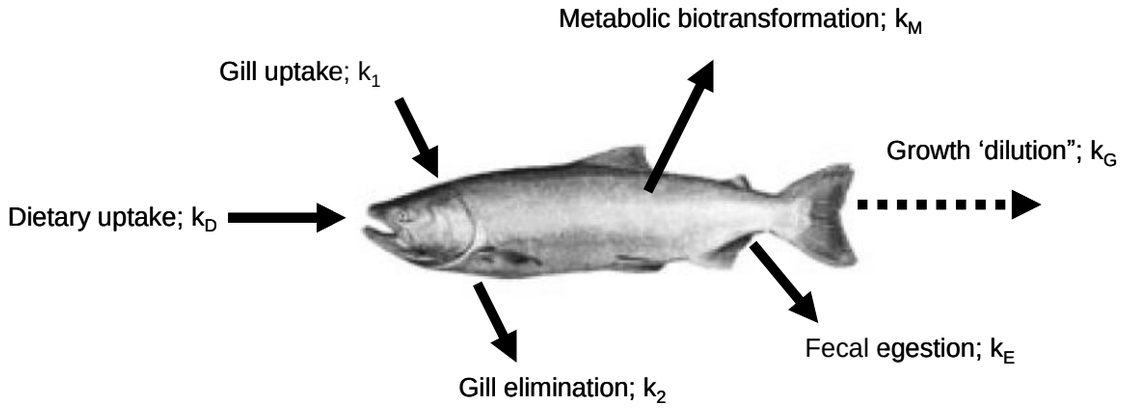


Figure 7.4 Conceptual diagram of major routes and associated rate constants of chemical (i.e., PCBs) uptake and elimination processes in fish (in this case Chinook salmon is used as an example).

For phytoplankton and algae the value of k_D is zero, and k_E is considered insignificant. There are several important assumptions in the model:

1. As long as differences in tissue composition and phase partitioning are accounted for, the PCB congeners are assumed to be homogeneously distributed in the organism (Arnot and Gobas 2004; Gobas and Arnot 2010).

2. Organisms can be characterized as a single compartment that experience exchange with the surrounding environment (Arnot and Gobas 2004; Gobas and Arnot 2010).

3. The model assumes that steady-state has been achieved between the organisms and its environment (i.e., water and sediment). This assumption is most suitable for situations such as this where variations in PCB concentrations in sediments and water are rather slow over time.

4. PCB congeners can be eliminated via egg deposition or sperm ejection but lipid-normalized concentrations of PCB congeners within the organism remain the same. This process is captured in the form of growth dilution associated with egg formation in the adult female, which is counteracted by uptake of PCBs from water and the diet. A balance of uptake and elimination processes determines the ultimate PCB concentration in the female.

The steady state assumption ($dM_B/dt = 0$) simplifies equation 1 to:

$$C_B = (k_1 \cdot (m_O \cdot \dot{\Phi} \cdot C_{WT,O} + m_P \cdot C_{WD,S}) + k_D \cdot \dot{\sum} P_i \cdot C_{D,i}) / (k_2 + k_E + k_G + k_M) \quad (2)$$

Where the organism's PCB congener concentration ($g \cdot kg^{-1}$, wet weight) (i.e., M_B/W_B) is C_B. It is reasonable to assume steady-state for organisms that have been exposed to the PCB congener for a long period of time and during their entire life. However, an implications of this assumption is

that the organism's growth has to be described as a growth rate constant (k_G), which is $dW_B/(W_B \cdot dt)$. Inherent in the growth rate constant is that for the duration of time that the model applies, the organism's growth is a constant fraction of its body weight. The methods for derivation of the model state variables can be found in Appendix F-5 (I).

7.3.2 Description of Food Web Bioaccumulation Model: Killer Whales

Figure 7.5 is a conceptual overview of the primary PCB uptake and elimination routes in killer whales. PCB uptake occurs via inhalation and dietary uptake (expected to be the main source for killer whales). Elimination of PCBs in killer whales occurs via exhaled air, fecal matter, urine, and metabolism. Female killer whales can also transfer PCBs into calves and via lactation (Hickie *et al.* 2007; Ross *et al.* 2000). Killer whale females give birth and nurse their calves for a period of approximately 12-24 months (Ford 2002). PCB concentrations can also be affected by growth periods. Uptake and elimination processes occur at different times of year and include continuous and non-continuous processes.

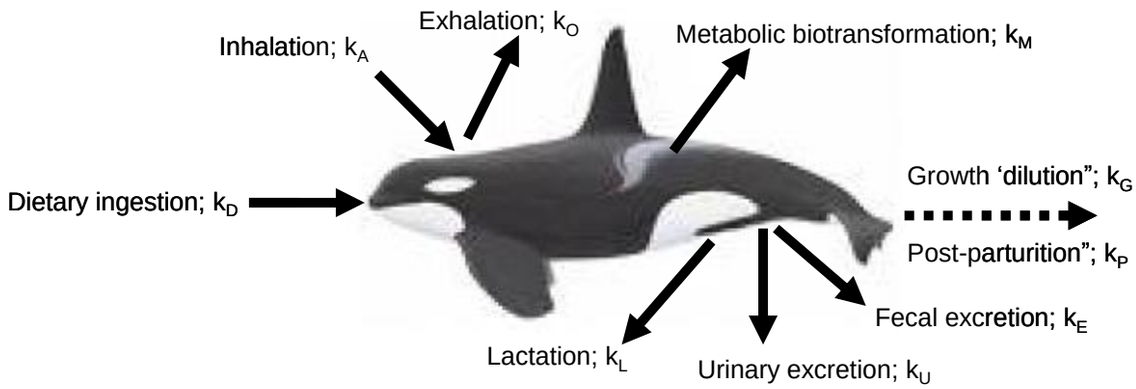


Figure 7.5 Conceptual diagram of the major chemical (i.e., PCBs) uptake and elimination processes in the killer whale and associated rate constants.

Certain PCB congeners can be metabolized in killer whales, although limited metabolic capacity/elimination in these long-lived animals contributes to sustained and prolonged PCB body burdens (Hickie *et al.* 2007; Ross *et al.* 2000). To keep the model simple and capture uptake and elimination processes by killer whales, key PCB characteristics were considered. PCBs are lipophilic and accumulate to high concentrations in organism lipids. Killer whales have significant quantities of fat in their blubber (i.e., the lipid content of healthy killer whales is about 64% (Ross *et al.* 2000), and most PCBs are found in lipid tissues. PCBs tend to establish chemical equilibrium, which means that PCBs distribute equally between various parts of the organism's lipids (lipid-normalized concentration is approximately equal). This chemical equilibrium is of especially relevance to the transfer of PCBs from female whales into their calves as lipid normalized concentrations in female whales are assumed not to change upon parturition. This means that the transfer of PCB mass from the mother to the calf upon giving birth is associated with a proportional drop in lipid mass of the mother, resulting in no change in lipid-normalized concentration. This transfer also occurs during lactation. If one assumes that PCBs are equally distributed among fats in the nursing female, then during lactation there is no change in PCB concentration since proportional declines in PCB mass and lipid mass occur. However, in the model, offspring production and lactation do have a long-term concentration effect in killer whales because of growth dilution. These processes require that killer whales grow body mass in addition to any net (year-to-year) changes in mass. Growth dilution occurs gradually over the killer whale's life cycle and can be described by a continuous process of elimination. Uptake and elimination are represented by the following mass balance equation:

$$dC_{KW,I}/dt = k_A C_{AG} + k_D \cdot \Sigma(P_i \cdot C_{D,i}) - (k_O + k_E + k_U + k_G + k_P + k_L + k_M) \cdot C_{KW,I} \qquad (3)$$

Where the lipid-normalized PCB congener concentration in the killer whale is $C_{KW,l}$, and the net change in lipid-normalized concentration over time t (d) is $dC_{KW,l}/dt$. The gaseous aerial concentration (g/L) is C_{AG}. The inhalation rate constant (L/kg lipid/d) is k_A. The clearance rate constant (kg/kg lipid/d) for PCB uptake via ingestion of food and water is k_D. The fraction of the diet consisting of prey item i is P_i and the concentration of the PCB congener (g/kg) in prey item i is $C_{D,i}$. The rate constant (1/d) for PCB exhalation via the lungs is k_O. The rate constant (1/d) for PCB congener elimination via excretion into feces is k_E. The rate constant for urinary PCB excretion is k_U. The rate constant for growth dilution is k_G, and it accounts for net growth increases year-to-year. The rate constant for PCB transfer into the calves is k_P, and it represents the lipid mass increase (equal to the calf's post-parturition lipid mass) during the gestation period. The rate constant for PCB transfer to the calf via lactation is k_L, and it represents the lipid mass increase of the female whale over the year that is transferred to the calf during lactation. k_G, k_P, and k_L (1/d) are fixed annual proportional increases in body lipid weight (i.e., $dW_{KW,l}/(W_{KW,l} \cdot dt)$) where the weight of the lipids in the killer whale is $W_{KW,1}$. The rate constant for metabolic PCB congener transformation is k_M. At steady-state, we can simplify equation 3 to:

$$C_{KW,l} = (k_A C_{AG} + k_D \cdot \Sigma(P_i \cdot C_{D,i})) / (k_O + k_E + k_U + k_G + k_P + k_L + k_M) \quad (4)$$

The lipid-normalized concentration can be used to calculate a whole organism wet weight based concentration in the killer whale C_{KW}:

$$C_{KW} = L_{KW} \cdot C_{KW,l} \quad (5)$$

During the year, considerable changes occur in the whole organism's lipid content. The wet weight based concentration is expected to experience changes of the same magnitude. In the model this is captured by varying L_{KW}. Since killer whales have a high lipid content, non-lipid organic matter does not play a significant role in PCB storage. Further description of the model state variables for killer whales is included in Appendix F-5 (II).

7.3.3 Description of Food Web Bioaccumulation Model: Steller Sea Lions

Basically, the PCB food web bioaccumulation model and the rationale for the Steller sea lion describing mass balance processes, including dietary ingestion, inhalation, metabolism, growth rate and excretion, are similar to those of killer whales, as seen in Figure 7.6. However, the state variables describing the animal characteristics (e.g., weight, lipid content, new borns per mothers, lactation rates) differ between killer whales and Steller sea lions.

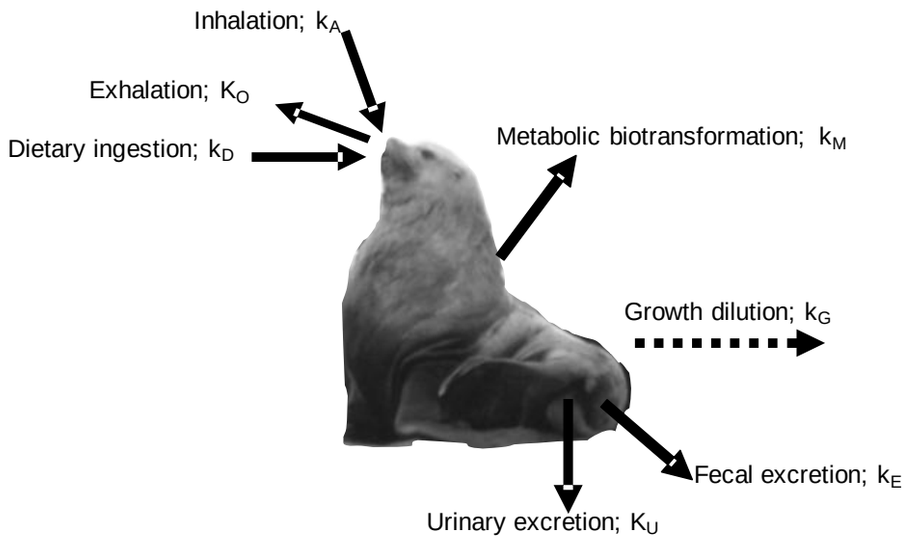


Figure 7.6 Conceptual diagram of the major chemical (i.e., PCBs) uptake and elimination processes in a male Steller sea lion and associated rate constants.

Uptake and elimination are represented by the following mass balance equation:

$$dC_{SSL,l}/dt = k_A C_{AG} + k_D \cdot \Sigma(P_i \cdot C_{D,i}) - (k_O + k_E + k_U + k_G + k_P + k_L + k_M) \cdot C_{SSL,l} \quad (6)$$

Where the lipid-normalized PCB congener concentration in the Steller sea lions is $C_{SSL,l}$, and the net change in lipid-normalized concentration over time t (d) is $Dc_{SSL,l}/dt$. The definition and description of rate constants is the same as that discussed earlier for the killer whale model. At steady-state, we can simplify the mass balance equation (6) to:

$$C_{SSL,l} = (k_A C_{AG} + k_D \cdot \Sigma(P_i \cdot C_{D,i})) / (k_O + k_E + k_U + k_G + k_P + k_L + k_M) \quad (7)$$

The lipid-normalized concentration can be used to calculate a whole organism wet weight based concentration in the Steller sea lion C_{SSL}:

$$C_{SSL} = L_{SSL} \cdot C_{SSL,l} \quad (8)$$

Further description of the model state variables for Steller sea lions is included in Appendix F-5 (II).

Details in the selection of biological parameters and species specific model state variables for each organism that require parameterization in the food web models are provided in Appendix F-5 (Table F-5.5a,b).

7.4 MODEL IMPLEMENTATION

The model was built in Microsoft Excel 2003-2007® and updated with Microsoft Excel XLS ®. The model includes submodels, data compilations, calculations (e.g., BSAFs), and results that were used in the evaluations of model performance analysis.

7.4.1 Forward Calculation: Total PCB Concentration Estimations in Fish and Wildlife

The model was initially used to predict PCB concentrations in Chinook salmon, Steller sea lions and resident killer whales based on empirical PCB sediment concentration data or sediment quality guideline values, assuming each species spends 100% time in each of the seven areas evaluated. The resulting BSAF values were employed to generate weighted BSAF values using realistic habitat distribution (%) or actual time spent in each of the seven areas of interest to predict PCB concentrations in salmon and killer whales. For the case of the Steller sea lion, only the hypothetical scenario (100% time spent in the Strait of Georgia) was used. BSAF concentrations were also predicted for two major Chinook salmon stocks (South Thompson and Lower Fraser). This approach also enabled us to attribute PCBs in killer whales to each of the seven areas of interest. Killer whales had diet of 96% Chinook salmon, 2% halibut, and 2% sablefish.

The forward calculation determines PCB concentrations in fish and wildlife (C_B) based on measured or predicted PCB concentrations in the sediment (C_S) (in this case sediment concentrations are the model input), as illustrated in Figure 7.7. Sediment PCB concentrations are in logarithmic format (log C_S) so that the lognormal distributions of sediment concentrations are able to be depicted as normal distributions of log C_S. Sediment and water PCB congener concentrations included in the model to represent the areas are listed in Appendix F-6. The BSAF

(model output) is also depicted in logarithmic format (log BSAF) based on the same reasoning. The calculation is:

$$\log C_B = \log C_S + \log BSAF \quad (9)$$

And C_B then follows as:

$$C_B = 10^{\log(BSAF \cdot Cs)} \quad (10)$$

Mathematically this is equivalent to:

$$C_B = BSAF \cdot C_S \quad (11)$$

Log C_B contains the propagation of variability and error from model input parameters (i.e., log C_S) and error in the model calculations (i.e., log BSAF). Uncertainty in organism concentrations is described by the geometric mean concentration's standard deviation (SD_{CB}), which is calculated from the log BSAF standard deviations (SD_{BSAF}) and the sediment concentration standard deviations (SD_{CS}):

$$SD_{CB} = \sqrt{(SD_{CS}^2 + SD_{BSAF}^2)} \quad (12)$$

C_B is calculated for each PCB congener and total-PCBs in the forward calculations, and its uncertainty is based on uncertainty in sediment total-PCB concentrations and BSAFs. The

variability and uncertainty in BSAF are described in more detail later. Uncertainty is derived using a model performance analysis involving a comparison of observed and predicted total-PCB BSAFs. Because one of the goals of the modeling process is to conduct a risk assessment of current Sediment Quality Guidelines for PCBs in their Critical Habitats, three sediment quality guidelines were used in the forward application, i.e. the CCME Interim Sediment Quality Guideline of 21.5 µg/kg dry weight (CCME 1999); the CEPA Action Level Low of 100 µg/kg dry weight (CEPA 2001); and the BCMWLAP Sediment Quality Criteria for sensitive species (SQC_{SCS}) of 120 µg/kg dry weight (BCMWLAP 2004a).

The log-normal cumulative distribution of predicted PCB concentrations in killer whales was compared to thresholds for toxicity determined in other marine mammal species with the goal to determine the frequency with which PCB concentrations exceed target threshold PCB concentrations. As illustrated in Table 7.3, several toxicity thresholds in marine mammals were considered. They included the harbour seal toxicity threshold for PCBs of 17,000 µg/kg lipid (Ross *et al.* 1996a), the toxicity threshold for bottlenose dolphins of 10,000 µg/kg lipid weight (Hall *et al.* 2006), and the revised harbour seal toxicity reference value (TRV) of 1,300 µg/kg lipid weight tissue residue in blubber (Mos *et al.* 2010). Related health endpoints affected by these toxic effect concentrations are provided in Table 7.1. Furthermore, two toxicity thresholds in Chinook salmon were evaluated. They included the tissue residue guideline for fish-eating wildlife of 50 µg/kg derived for PCBs from the CCME guideline for dioxin-like toxicity (Hickie *et al.* 2007), and the newly-derived value of 8 µg/kg wet weight PCBs in killer whale prey for 95% of the killer whale population falling below the 17,000 µg/kg toxicity threshold (Hickie *et al.* 2007). PCB concentrations in killer whale prey below these two toxicity thresholds would reduce PCB concentrations in killer whales to levels deemed to be protective of health effects.

Table 7.3 Toxic effect concentrations of total PCBs in marine mammals. All studies involved free-ranging or captive fed marine mammals, wherein PCBs represented the dominant concern and the contaminants which best correlated with observed effects.

Toxic Effect Concentrations (TEC)	TEC (µg/kg lipid)	Log TEC (µg/kg lipid)
Harbour seal PCB toxicity (Ross *et al.* 1996b)	17000	4.23
Bottlenose dolphin PCB toxicity (Hall *et al.* 2006)	10000	4.00
Revised harbour seal PCB toxicity (Mos *et al.* 2010)	1300	3.11

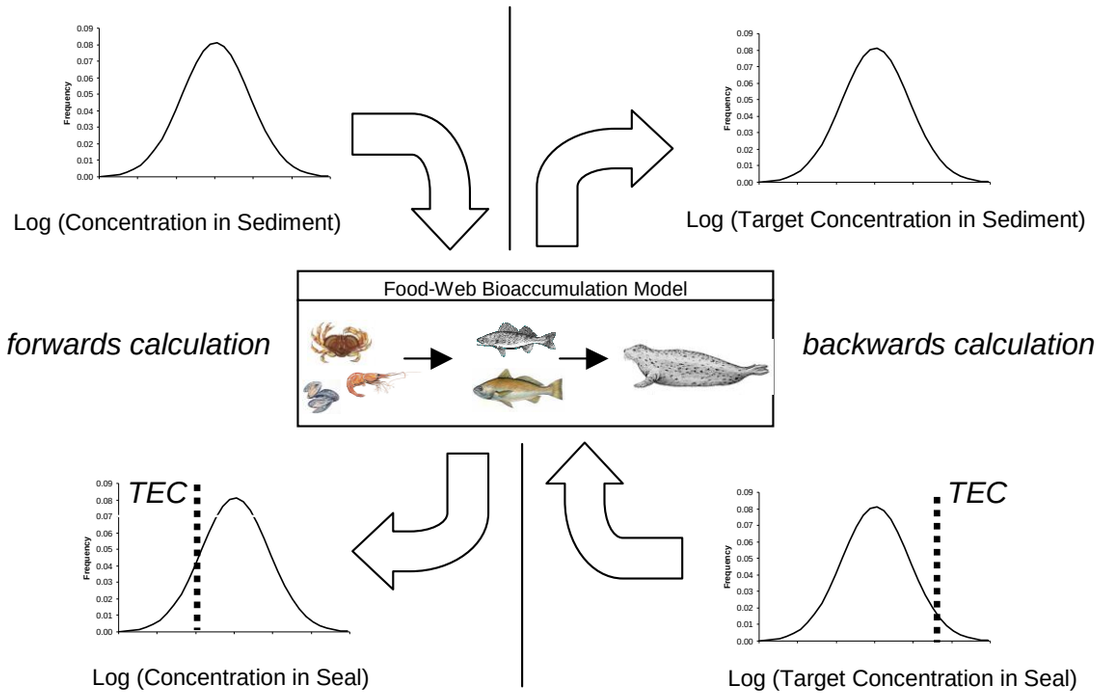


Figure 7.7 Illustration of the forward and backward applications of the BSAF in the food web bioaccumulation model for PCBs (adapted from Gobas and Arnot 2010). TEC is the toxic effect concentration.

7.4.2 Backward Calculation: Estimating Total PCB Concentrations in Sediments from PCB Concentration in Fish and Wildlife

In an effort to derive new Sediment Quality Guidelines protective of killer whales and Steller sea lions, we conducted a backward application of the BSAF model. We used realistic habitat distributions for resident killer whales (and a diet of 96% Chinook salmon, 2% halibut, and 2% sablefish) and Chinook salmon, as well as hypothetical scenarios where the animals spend 100% of their time in the area. For the Steller sea lion only a hypothetical scenario (100% of their time in the Strait of Georgia) was used.

The backward calculation uses PCB concentrations in fish or wildlife (C_B) to calculate the PCB concentration in sediments (C_S). This provides target sediment PCB concentrations that meet ecological criteria expressed as a PCB concentration C_B. The calculation is:

$$\log C_S = \log C_B - \log BSAF \quad (13)$$

Which is mathematically equivalent to:

$$C_S = C_B / BSAF \quad (14)$$

Where C_B is the external input variable and the model calculates the BSAF. Backwards calculations were conducted for ∑PCBs. Model error uncertainty is captured in backwards calculations in the uncertainty in the BSAF, which the model calculates as described above. When entering the biota PCB concentrations it is also possible to include accepted variability in the target biota concentration by combining the uncertainty in the BSAF and C_B to obtain a distribution

of sediment PCB concentrations expected to produce the entered distribution of PCB concentrations in fish or wildlife.

The backward calculation can be used to derive sediment target levels (e.g., new Sediment Quality Guidelines) using toxicity tissue thresholds reported for marine mammals (e.g., harbour seals) to protect predator species at the top of the food web such as killer whales and Steller sea lions (Figure 7.7). Therefore, sediment ΣPCB concentrations expected to meet ΣPCB concentrations in fish and wildlife associated with various ecological risks were calculated as:

$$\log C_S = \log (TEC) - \log BSAF - 1.96 \cdot (SD_{MB}) \qquad (15)$$

Where, TEC is the toxic effect concentration in biota and 1.96 is the confidence value to have 95% probability for the observations in a normal distribution to fall below the target sediment concentration, and SD_{MB} is the standard deviation of the model bias (MB) for biota obtained from the model testing/performance analysis. BSAFs were calculated in the forwards calculations based on the current composition of sediment PCB congeners in the areas included. The calculated sediment ΣPCB concentration (C_S) assumes that the composition of PCB concentrations in the areas is the same as entered in forward calculations to represent current conditions. Thus, ΣPCB concentrations can be used to calculate congener specific concentrations assuming that the PCB congener profile is similar to that in current sediments. The resulting predicted sediment concentrations from these thresholds may be considered as ecologically relevant targets for management, which can guide remediation, pollution control, and suitability of disposal sites with respect to resident killer whales' Critical Habitat and Steller sea lion habitat.

7.5 MODEL SENSITIVITY

The model by Gobas and Arnot (2010) has had extensive use and testing to determine by which parameters the model is most affected. The sensitivity analysis assesses the impact of variability or error in the model's state variables (e.g., organism weight, lipid content, temperature) on the model outcome, i.e., the BSAF of total PCBs in bay fish and wildlife (Gobas and Arnot 2010). A general overview of relative sensitivity of the various parameters is shown in Table 7.4. To avoid repetitiveness in the sensitivity analysis of parameters previously tested by Gobas and Arnot (2010), the parameters of the killer whale food web bioaccumulation model evaluated in the sensitivity analysis for this study included the variation in the killer whale's diet composition and changes in water and sediment concentrations (Section 7.83). The approach used for the sensitivity analysis of water and sediment concentrations is detailed in Appendix 5. In addition, the PCB food web model for killer whales and Steller sea lions was evaluated by using a model performance analysis, and an uncertainty analysis.

Table 7.4 Food web bioaccumulation model sensitivity to various parameters (adapted from Gobas and Arnot 2010).

Parameter	Model Sensitivity
Dietary preference[1]	High
Body weight[1]	High
Lipid content[1]	High
Gill ventilation rate[1]	Low
Gill uptake efficiency[1]	Low
Feeding rate[1]	Low for chemicals with log $K_{OW} \leq 6.5$
	High for PCBs with log $K_{OW} > 6.5$
PCB dietary uptake efficiency[1]	Low
Growth rate[1]	Low but increases in importance for larger organisms (fish & marine mammals) and higher K_{OW} PCB congeners
Metabolism[1]	Low – unless metabolic transformation rates are high compared to other elimination routes
K_{OW}[1]	High
Food digestibility[1]	High
Diet lipid content[1]	High
Diet composition (killer whale)[2]	Low for the coastal food webs
	High for the oceanic food web
Concentration in water[2]	High
Concentration in sediments[2]	Low
Organic carbon content in sediments[2]	High

[1](Gobas and Arnot 2010)
[2](this study)

7.6 MODEL TESTING AND PERFORMANCE ANALYSIS

Model performance analysis compares each PCB congener's (i) model predicted sediment-receptor concentration relationship ($BSAF_{P,i}$), to the observed sediment-receptor concentration relationship ($BSAF_{O,i}$). Measured sediment and estimated water PCB congener concentrations were input parameters for the calculation of PCB concentrations in biota, then $BSAF_{P,i}$ was calculated dividing the calculated biota concentration by the sediment concentration. Measured

PCB concentrations in biota were divided by measured concentrations in sediments to obtain the $BSAF_{O,i}$. This measure of model performance is described quantitatively by the model bias (MB), which is species-specific:

$$MB_j = 10^{\left(\sum_{i=1}^{n} \frac{\left[\log(BSAF_{P,i} / BSAF_{O,i}) \right]}{n} \right)} \quad (16)$$

Assuming a log-normal distribution of the ratio $BSAF_{P,i}$ / $BSAF_{O,i}$, the MB_j is the geometric mean of the ratio of predicted and observed BSAFs for all PCB congeners (i) in a particular species (j). MB indicates the model's systematic over- (MB>1) or under-prediction (MB<1), where an MB = 2 means that the model over-predicted the species empirical PCB congener concentrations by a factor of 2 on average. Over- and under-estimations of observed PCB congener BSAFs tend to cancel out while calculating MB, which causes MB to track the central tendency of the model's ability to predict PCB congener concentrations. The standard deviation of MB represents the variability of the over- and under-estimation of measured values.

To quantitatively express model performance for ΣPCBs, the model bias was used MB*, which is derived for each species as:

$$MB_j^* = 10^{\left(\sum_{i=1}^{m} \frac{\left[\log(BSAF_{P,\Sigma PCB} / BSAF_{O,\Sigma PCB}) \right]}{m} \right)} \quad (17)$$

Assuming a log-normal distribution of the ratio $BSAF_{P,\ \Sigma PCB}$ / $BSAF_{O,\ \Sigma PCB}$, MB_j* is the geometric mean of the ratio of predicted and observed BSAFs for ΣPCB in species j. MB* indicates the model's systematic over- (MB*>1) or under-prediction (MB*<1) of the BSAF for ΣPCB. The variability of over- and under-estimation of measured values is represented by the standard deviation of MB*, and is an indication of the variability and uncertainty of model predictions. The error of MB* can be described as a factor (rather than a term) of the geometric mean because of the log-normal distribution of the ratio of predicted and observed BSAFs.

7.7 UNCERTAINTY ANALYSIS

Uncertainty in the model input parameters (i.e., log C_S) and in the model calculations (i.e., log BSAF) are propagated in the estimate of log C_B in terms of the standard deviation SD_{CB} of log C_B (i.e., the geometric mean concentration). Spatial distribution of sediment PCB congener concentrations were represented by the standard deviation (SD_{CS}) of the mean log C_S (i.e., of the geometric mean of the concentration in the sediments). The standard deviation (SD_{BSAF}) of log BSAF (i.e., geometric mean of the BSAF) was used to represent variability in log BSAF. The standard deviation (SD_{CB}) of log CB (i.e., geometric mean of the ∑PCB concentration in the biota) was used to express the effect of variability and error in sediment PCB congener concentrations and uncertainty in BSAF estimates of biota PCB congener concentrations. SD_{CB} is calculated from the standard deviations (SD_{BSAF}) of log BSAF estimates and the standard deviation (SD_{CS}) of log C_S as:

$$SD_{CB} = \sqrt{(SD_{CS}^2 + SD_{BSAF}^2)} \qquad (18)$$

Using empirical data to describe model calculation uncertainty improves model credibility since model calculations are directly compared to empirical data, but any problems with the empirical data are reflected in the model's uncertainty estimate. One important limitation of the empirical data is that there was limited spatial coverage. For example, biota concentrations were only obtained for some of the areas included in the model, thus the biota PCB concentrations may not accurately represent the concentrations in all areas. Also, empirical PCB concentrations likely do not accurately represent temporal variations in PCB concentrations because of their limited temporal coverage. Thus it is beneficial to assess model uncertainty with a second method that attempts to incorporate geographical and temporal variations in PCB concentrations.

Uncertainty analysis through Monte Carlo simulation was considered but found to be problematic because of the interdependence of state variables and lack of data to define uncertainty distributions for several state variables. The interdependence of several state variables including feeding rates, growth rates, fecal egestion rates and feeding preferences caused inconsistencies in the energy and mass balance of the model. The associated error was deemed to be too large for the Monte Carlo simulations to provide meaningful estimates of model uncertainty. One of the advantages of using empirical observation to assess uncertainty is that it includes many sources of uncertainty while Monte Carlo simulation is limited to model parameterization uncertainty. Because uncertainty in observed ΣPCB concentrations reflects to some degree spatial variation in PCB concentrations in sediments, which is also specifically considered by SD_{CS}, the estimated uncertainty in C_B may be somewhat overestimated by this method.

7.8 RESULTS AND DISCUSSION

7.8.1 Model Testing and Performance

The ability of the model to estimate PCB congener concentrations in biota was tested by comparing predicted concentrations in biota (i.e., Johnstone Strait Chinook salmon, male northern resident killer whale and male Steller sea lion from the Strait of Georgia) to available empirical concentrations. Model predicted and empirical PCB congeners included are shown in Figures 7.8-7.10. The PCB congeners' mean model bias (MB) for Johnstone Strait Chinook salmon was 1.30 ± 0.31 (Figure 7.8), 1.23 ± 0.36 for male northern resident killer whales (Figure 7.9), and 1.12 ± 0.49 for male Steller sea lion (Figure 7.10). In all cases, the model bias values for biota were close to one (MB ≈1), and the over prediction was small or negligible. These comparisons are an indication that the predicted concentrations of PCB congeners are in good agreement with the observed PCB congener concentrations and within the range of observed PCB concentrations in Chinook salmon, resident killer whales and Steller sea lions. Figures 7.8−7.10 also illustrate that congener patterns of PCBs in Chinook, killer whales and Steller sea lions are reasonably well reproduced by the model when compared against the empirical profiles found for the three species. The standard deviation of log MB (SD_{MB}) is 0.31 for Chinook salmon, 0.36 for male NRKW, and 0.49 for male Steller sea lion, indicating a standard deviation of the mean MB equal to a factor of $10^{0.31}$,$10^{0.36}$, and $10^{0.49}$ for Chinook salmon, male NRKW and male Steller sea lion, respectively.

Likewise, the mean model bias (MB*) for total PCBs (∑PCBs) was 1.24 ± 0.18 for Chinook salmon, 0.94 ± 0.33 for male resident killer whale and 1.62 ± 0.41 for male Steller sea lions (Figure 7.11). This indicates that the performance of the model conducted both in a PCB congener basis and as ∑PCBs generates a small error bias and corroborates the ability of the models to predict chemical concentrations in biota.

Predicted BSAF values were also very similar to empirical data observed in Chinook salmon and male killer whales from the Northern resident killer whale Critical Habitat and in Steller sea lions from the Strait of Georgia (Figure 7.12). The model, therefore, produces little systematic over- or under-estimation of PCB congener concentrations.

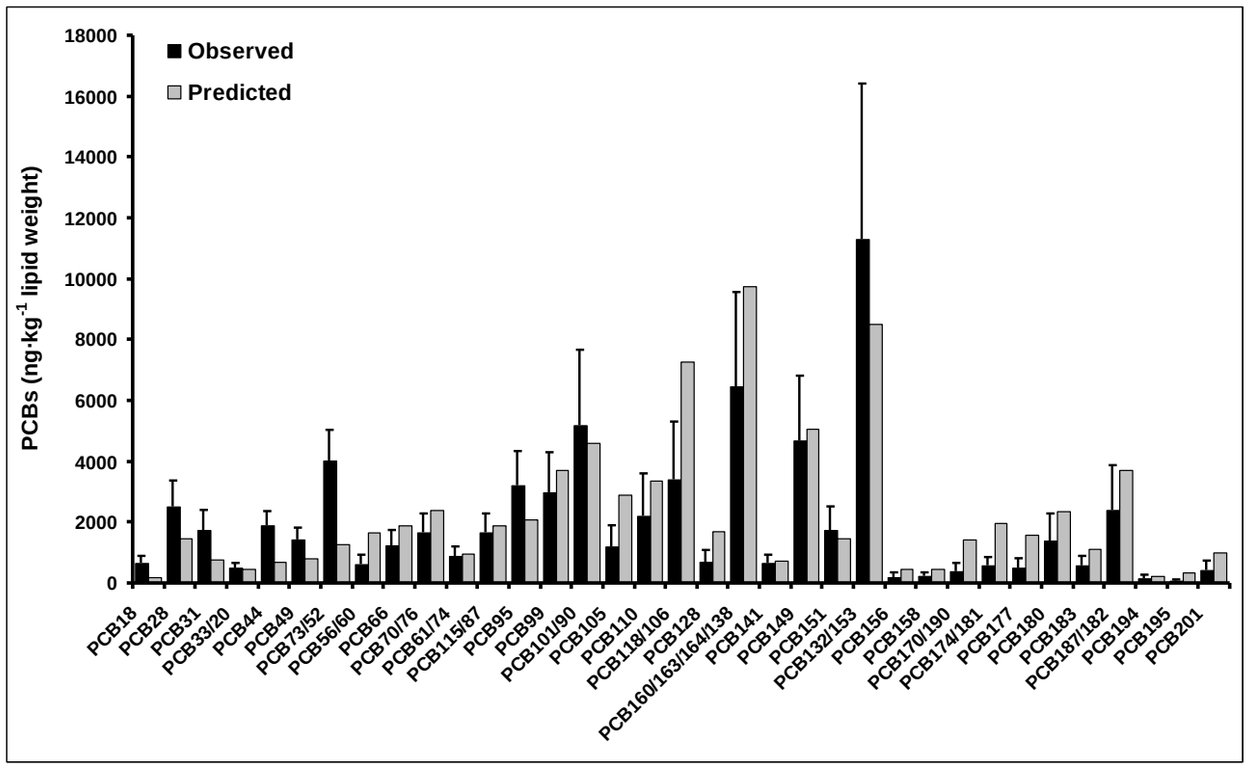


Figure 7.8 Model predicted and observed concentrations for specific PCB congeners (ng/kg lipid) of approximately 35 PCB congeners in Chinook salmon in the northern resident killer whale Critical Habitat. Error bars is the standard deviation of observed concentrations.

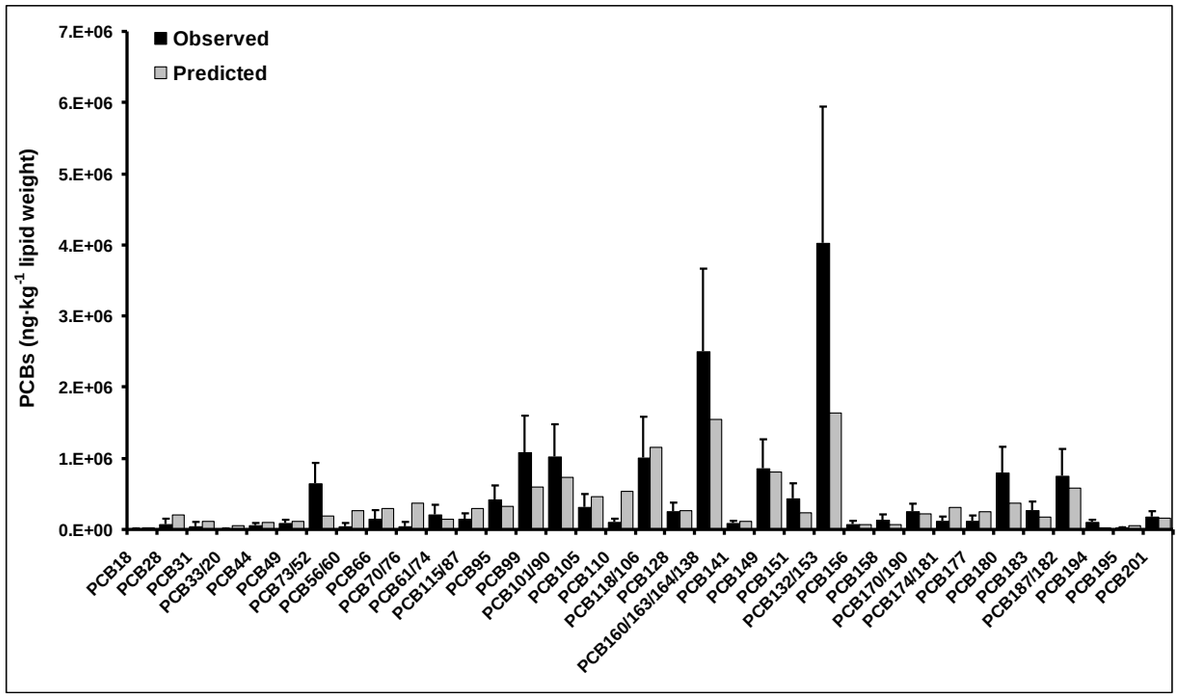


Figure 7.9 Model predicted and observed concentrations for specific PCB congeners (ng/kg lipid) of approximately 35 PCB congeners in male NRKW in the northern resident killer whale Critical Habitat. Error bars are the standard deviation of observed concentrations.

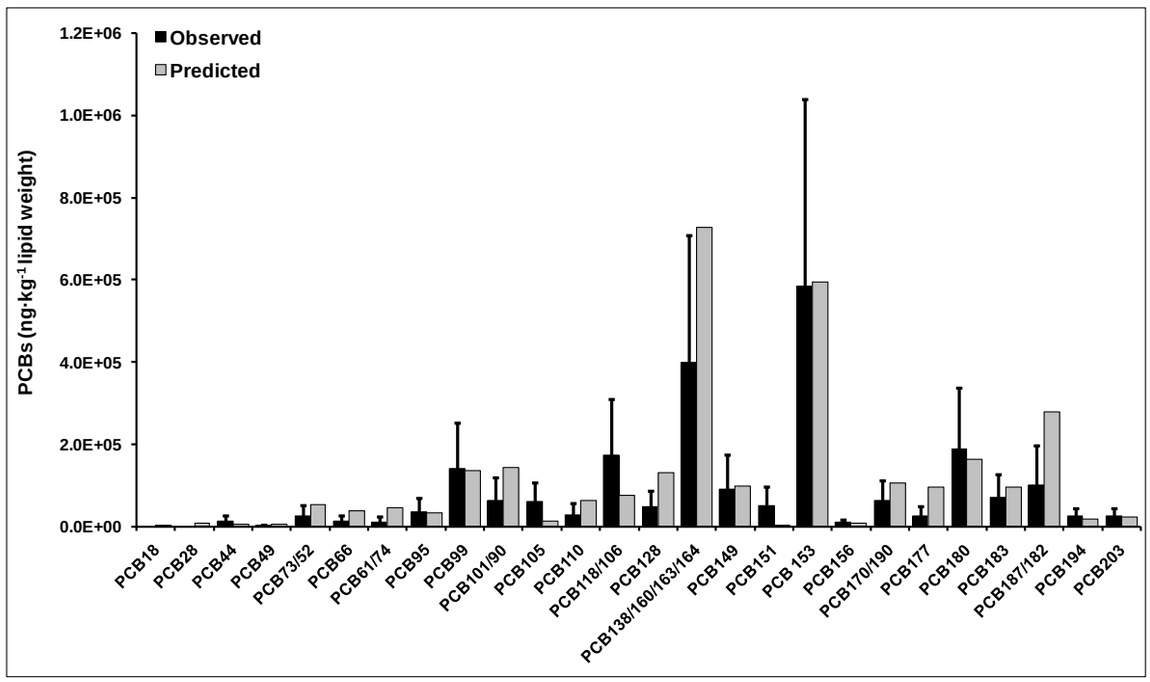


Figure 7.10 Model predicted and observed concentrations for specific PCB congeners (ng/kg lipid) of approximately 35 PCB congeners in male Steller sea lion from the Strait of Georgia. Error bars are the standard deviation of observed concentrations

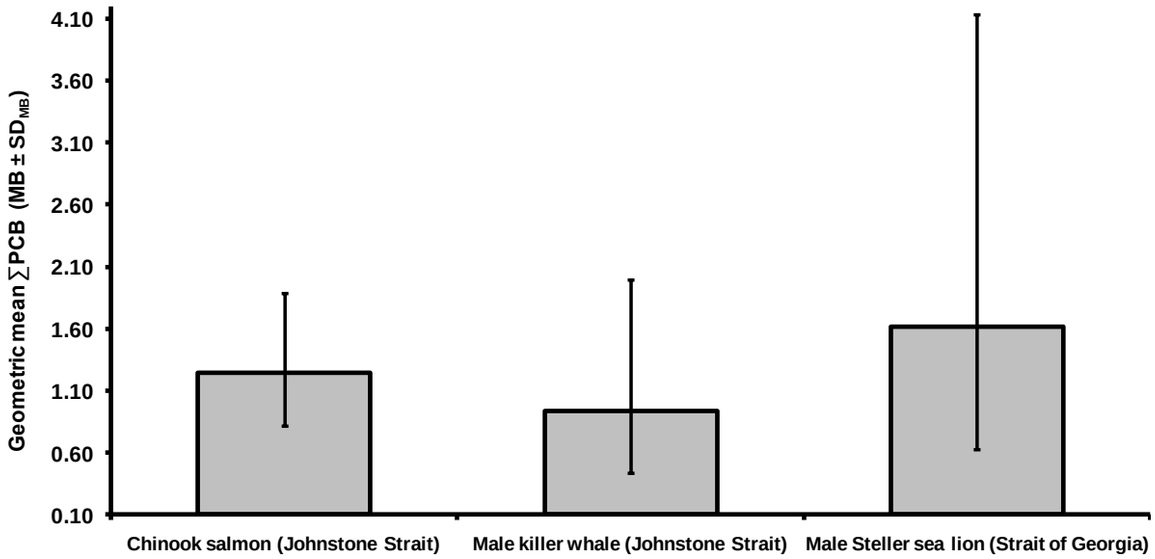


Figure 7.11 Outcomes of the model bias (MB*) analysis for total PCBs (∑PCBs) in Chinook salmon, male resident killer whale and male Steller sea lions. Error bars are asymmetric standard deviations of the geometric mean (upper and lower standard deviations).

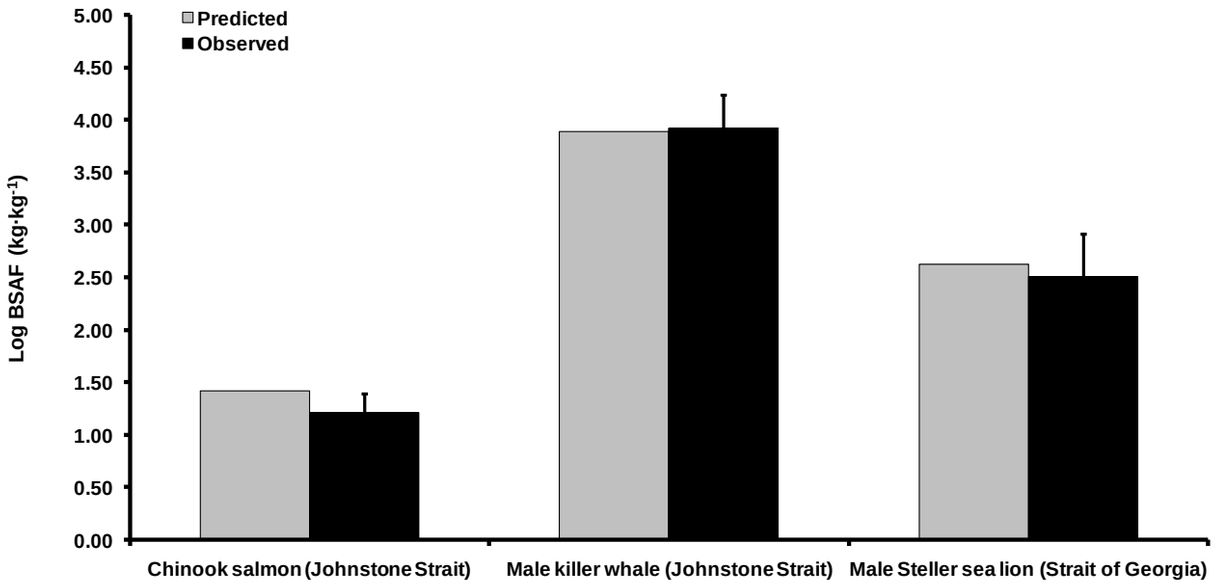


Figure 7.12 Predicted BSAF values in Chinook salmon, male resident killer whale and Steller sea lion were similar to empirical data observed for these species in the Northern resident killer whale Critical Habitat (Johnstone Strait) and the Strait of Georgia. Error bars are standard deviations of observed values.

7.8.2 Uncertainty Analysis

The uncertainty analysis involves the standard deviation of the mean model bias (BSAF) and standard deviations of the empirical sediment concentration data to determine the uncertainty of the model outcome. The empirical PCB concentration in sediment exhibit a wide range of values representing considerable spatial variation), as shown in Table 7.5. The mean PCB sediment concentrations ranged from $10^{-3.62 \pm 0.74}$ mg/kg dry weight in NRKW Critical Habitat to $10^{4.55 \pm 1.17}$ mg/kg dry weight for the SRKW Critical Habitat in the USA (Puget Sound). Table 7.5 shows that the uncertainty in the model outcomes (i.e., C_B) in terms of the standard deviation SD $_{\log C_B}$ (i.e., the geometric mean concentration of ∑PCBs in biota) ranged from 0.32 in SRKW critical habitat in Canada to 1.24 in SRKW critical habitat in USA (Puget Sound) for Chinook salmon (i.e., $SD_{\log C_{fish}}$), and from 0.42 in SRKW critical habitat in Canada to 1.30 in SRKW critical habitat in USA (Puget Sound) for male resident killer whales (i.e., SD $_{\log C_{kw\text{-}male}}$). The standard deviation SD $_{\log C_B}$ for male Steller sea lions in the Strait of Georgia was 0.65 (Table 7.6). In addition, the uncertainty in C_S is higher than the uncertainty in BSAF, implying that the spatial variation in sediment concentrations is a larger contributor to uncertainty in model outcomes than model error.

This portrays that over- and under-estimations of PCB congeners for specific species can be considerable even if the predicted mean concentration values are close to the observed values. The standard deviations can be viewed as the magnitude of the uncertainty analysis in the model outcomes (BSAF estimates and ∑PCBs).

Table 7.5 Uncertainty values showing the standard deviations of the Log ∑PCB concentrations for Chinook salmon and male resident killer whales in the model areas.

	Mean log C_S (mg/kg dry weight)	**$SD_{log\ Cs}$**	**$SD_{log\ BSAFfish}$**	**$SD_{log\ Cfish}$ (mg/kg wet weight)**	**$SD_{log\ BSAFkw\text{-}male}$**	**$SD_{log\ Ckw\text{-}male}$ (mg/kg wet weight)**
NRKW Critical Habitat (CH)	-3.62	0.74	0.18	0.77	0.33	0.81
Queen Charlotte Strait	-3.63	1.08	0.18	1.09	0.33	1.13
Outer coast	-3.63	1.08	0.18	1.09	0.33	1.13
Strait of Georgia	-3.58	0.51	0.18	0.54	0.33	0.60
SRKW CH in Canada	-3.68	0.27	0.18	0.32	0.33	0.42
SRKW CH in USA (Puget Sound)	-1.85	1.23	0.18	1.24	0.33	1.30
SRKW CH in USA summer core & Juan de Fuca Strait)	-2.53	0.40	0.18	0.47	0.33	0.54

Table 7.6 Uncertainty values showing the standard deviation of the Log ∑PCB concentration for male Steller sea lions in the Strait of Georgia.

	Mean log C_S (mg/kg dry weight)	$SD_{log\ Cs}$	$SD_{log\ BSAFssL\text{-}male}$	$SD_{log\ CssL\text{-}male}$ (mg/kg wet weight)
Strait of Georgia	-3.58	0.51	0.41	0.65

7.8.3 SENSITIVITY ANALYSIS

7.8.3.1 Evaluating the Effects of the Resident Killer Whale Diet Composition on Model Outcomes

In the risk assessment forward calculations (Section 7.8.5), it is assumed that resident killer whales have an initial diet that includes 96% Chinook salmon, 2% halibut, and 2% sablefish. However, these percentages can fluctuate during the year as killer whales can target other fish species. In the model, it is also assumed that the Chinook salmon consumed by resident killer whales are from the South Thompson and Lower Fraser River stocks. Again, this is a simplification of the actual situation and further modeling efforts can be conducted to include more of the salmon stocks killer whales eat. Studies that target these Chinook stocks and test for PCB concentrations would be very beneficial for a food web exercise such as this, as it would provide the PCB concentrations of the stocks that resident killer whales primarily consume, which may provide more accurate predictions of PCB concentrations in killer whales.

Further model scenarios were conducted with the aim to incorporate more fish species in the NRKW diet (i.e. the addition of chum and coho salmon, lingcod, squid, and dove sole) while the killer whales are in Critical Habitat. The sensitivity analysis shows only minor changes in PCB congener concentrations in the coastal food web of resident killer whales as a result of changing

the diet of killer whales. No significant differences were observed in the outcomes (i.e. biota concentrations) by changing the killer whale diet (i.e., 70% Chinook salmon; 10% chum salmon; 5% coho salmon; 3% halibut; 3% sablefish; 3% lingcod; 3% dover sole; and 3% gonatid squid) in the coastal NRKW Critical Habitat. Under this premise, it was stated that the predicted PCB concentrations in biota in the coastal food web models for all habitat areas are not significantly affected by changing the diet composition of the coastal food web in terms of adding salmonid species and several other fish species. The lack of differences further indicates that prey items are of approximately the same trophic levels in the two coastal food webs (Appendix F-4a, c). On the contrary, when changing the killer whale diet composition (i.e., 70% Chinook salmon; 10% chum salmon; 5% coho salmon; 3% halibut; 3% sablefish; 3% lingcod; 3% dover sole; and 3% gonatid squid) in the oceanic food web (i.e., outer coast model), the concentration of PCB congeners predicted in killer whales using the initial diet is significantly higher than the PCB congener concentrations in killer whales using a different or updated diet composition.

7.8.3.2 NRKW Critical Habitat

Males*.* No significant differences (t-test, t = 0.5682; *p* = 0.5716) were found between the PCB congener concentrations predicted in the coastal food web model for the NRKW Critical Habitat using the resident killer whale diet composition of 96% Chinook salmon, 2% halibut, and 2% sablefish, and the concentration predicted using the diet composition of 70% Chinook salmon, 10% chum salmon, 5% coho salmon, 3% halibut, 3% sablefish, 3% lingcod, 3% dover sole and 3% gonatid squid. When comparing the PCB congener concentrations of the two diets, the relative frequencies of the outcomes were similar as shown in Figure 7.13 (see also Appendix F-7a).

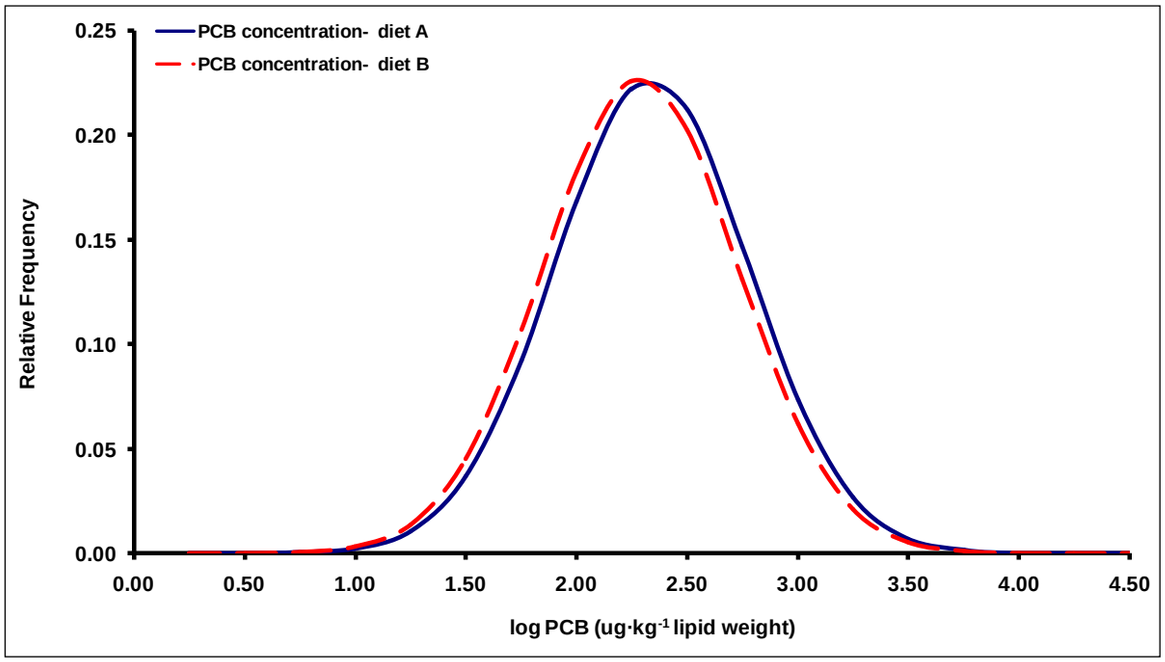


Figure 7.13 Normal probability density curves showing the comparisons of PCB concentrations predicted in male killer whale with the coastal PCB food web bioaccumulation model using a diet composition of 96% Chinook salmon; 2% halibut; and 2% sablefish (diet A, solid line) versus a diet consisting of 70% Chinook salmon; 10% chum salmon; 5% coho salmon; 3% halibut; 3% sablefish; 3% lingcod; 3% dover sole; and 3% gonatid squid (diet B, dashed line) in northern resident killer whale critical habitat.

Females. No significant differences (t-test, t = 0.5831; *p* = 0.5616) were found between the PCB congener concentrations predicted in the coastal food web model for the NRKW Critical Habitat using the resident killer whale diet composition consisting of 96% Chinook salmon, 2% halibut, and 2% sablefish, and the concentration predicted using the diet composition of 70% Chinook salmon, 10% chum salmon, 5% coho salmon, 3% halibut, 3% sablefish, 3% lingcod, 3% dover sole, and 3% gonatid squid. When comparing the PCB congener concentrations of the two diets, the relative frequencies of the outcomes were similar as shown in Figure 7.14 (also shown in Appendix F-7b).

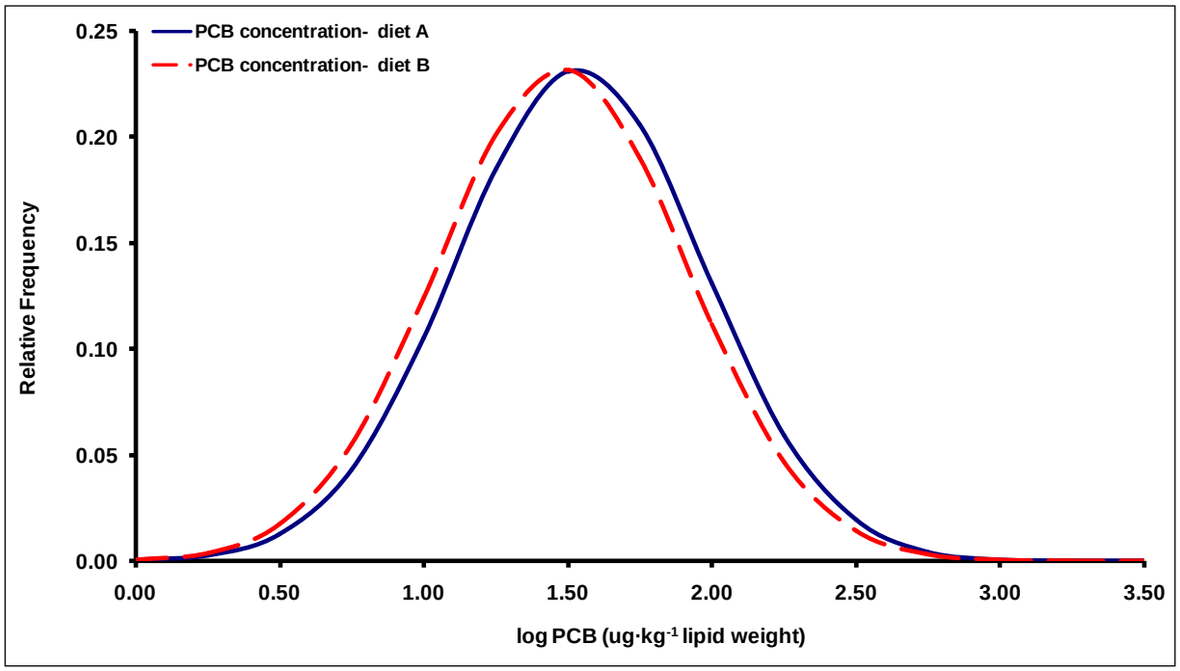


Figure 7.14 Normal probability density curves showing the comparisons of PCB concentrations predicted in female killer whale with the coastal PCB food web bioaccumulation model using a diet composition of 96% Chinook salmon, 2% halibut and 2% sablefish (diet A, solid line) versus a diet consisting of 70% Chinook salmon, 10% chum salmon, 5% coho salmon, 3% halibut, 3% sablefish, 3% lingcod, 3% dover sole, and 3% gonatid squid (diet B, dashed line) in northern resident killer whale critical habitat.

7.8.3.3 Outer Coast Habitat

Males. A significant difference (t-test, t = 3.0781; *p* = 0.003) was found between the PCB congener concentrations predicted in the oceanic food web model for the Outer coast habitat using the resident killer whale diet composition of 96% Chinook salmon 2% halibut and 2% sablefish, and the concentration predicted using the diet composition of 70% Chinook salmon, 10% chum salmon, 5% coho salmon, 3% halibut, 3% sablefish, 3% lingcod, 3% dover sole, and 3% gonatid squid. When comparing the distributions of the PCB congener concentrations between the two diets, the concentration of the diets are significantly different (Figure 7.15; also shown in Appendix F-7c).

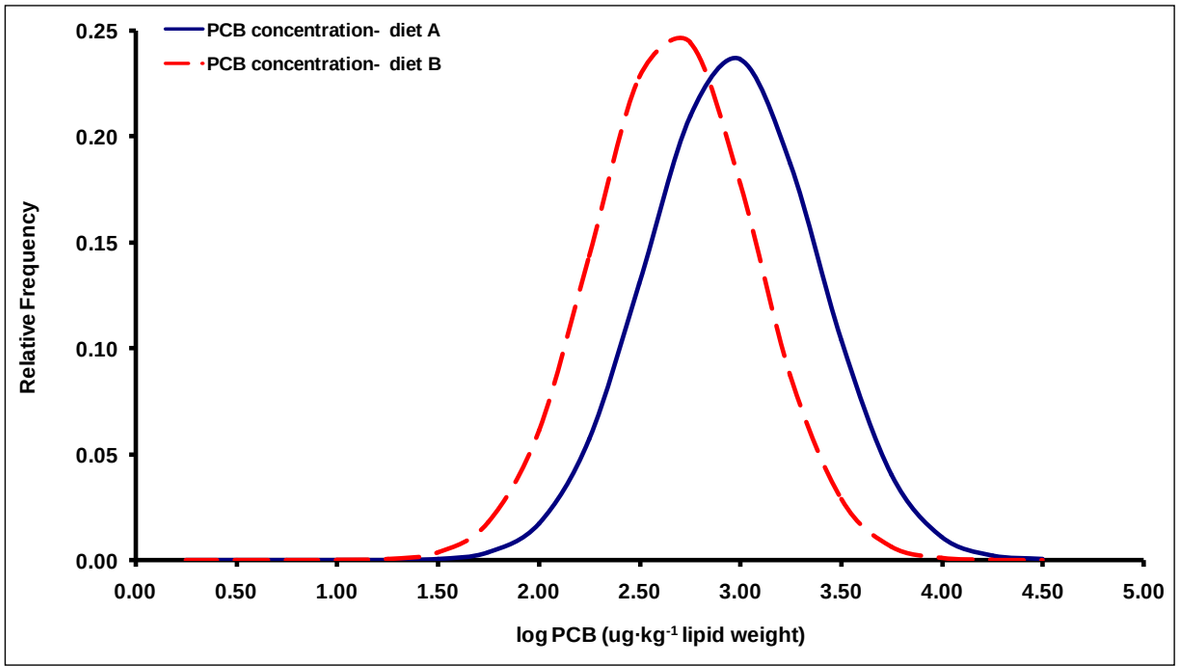


Figure 7.15 Normal probability density curves showing the comparisons of PCB concentrations predicted in male killer whale with the oceanic PCB food web bioaccumulation model using a diet composition of 96% Chinook salmon, 2% halibut and 2% sablefish (diet A, solid line) versus a diet consisting of 70% Chinook salmon, 10% chum salmon, 5% coho salmon, 3% halibut, 3% sablefish, 3% lingcod, 3% dover sole, and 3% gonatid squid (diet B, dashed line) in the outer coast habitat.

Females. A significant difference (t-test, t = 3.1311; p = 0.0025) was found between the PCB congener concentrations predicted in the oceanic food web model for the Outer coast habitat using the resident killer whale diet composition of 96% Chinook salmon; 2% halibut; and, 2% sablefish, and the concentration predicted using the diet composition of 70% Chinook salmon; 10% chum salmon; 5% coho salmon; 3% halibut; 3% sablefish; 3% lingcod; 3% dover sole; and 3% gonatid squid. When comparing the distributions of the PCB congener concentrations between the two diets, the concentration of the diets are significantly different (Figure 7.16; Appendix F-7d).

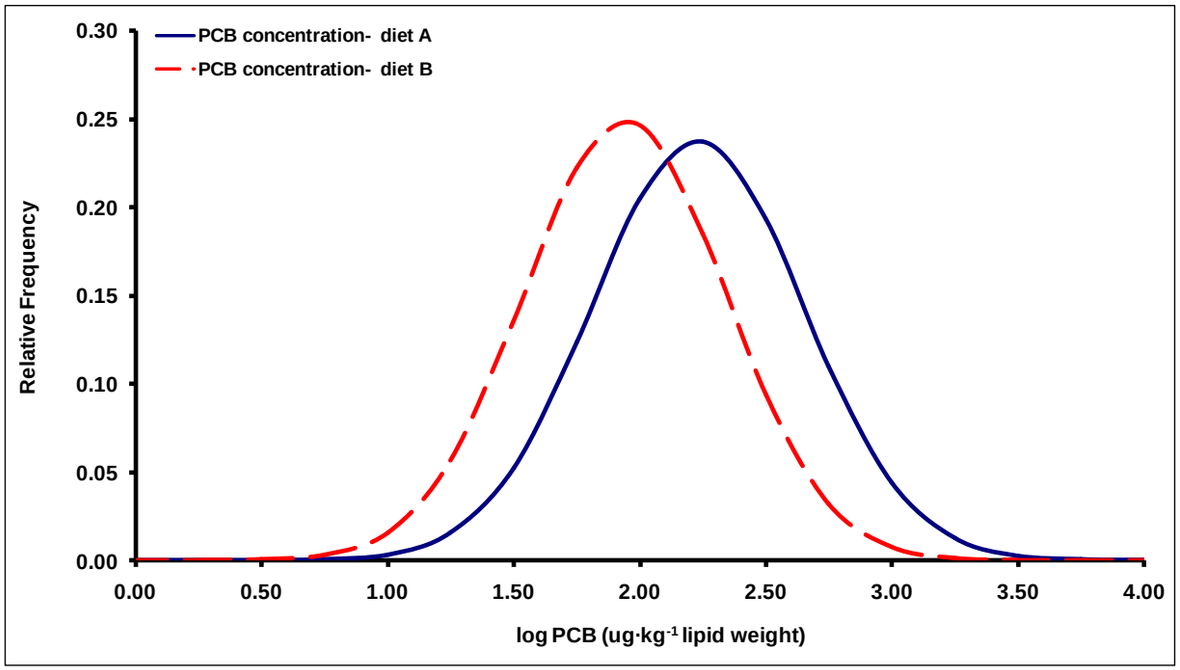


Figure 7.16 Normal probability density curves showing the comparisons of PCB concentrations predicted in female killer whale with the oceanic PCB food web bioaccumulation model using a diet composition of 96% Chinook salmon, 2% halibut and 2% sablefish (diet A, solid line) versus a diet consisting of 70% Chinook salmon, 10% chum salmon, 5% coho salmon, 3% halibut, 3% sablefish, 3% lingcod, 3% dover sole, and 3% gonatid squid (diet B, dashed line) in the outer coast habitat.

While no differences was observed in predicted PCB concentration in resident killer whales for the coastal food web (NRKW Critical Habitat) by changing the diet composition, the differences found in PCB concentrations in the oceanic food web involving the same change in diet composition is attributed to the reliance of the killer whales' major prey item (i.e., Chinook salmon) on Gonatid squid, which makes up most of the Chinook diet composition (70% in initial diet) during its oceanic-offshore life stage. In addition, the killer whale diet consisting of 70% Chinook salmon, 10% chum salmon, 5% coho salmon, 3% halibut, 3% sablefish, 3% lingcod, 3% dover sole and 3% gonatid squid produced changes in the diet composition for killer whales and Chinook salmon in the outer coast by redistributing diet proportions (squid comprised 3% in the killer whale diet, and 10% in the Chinook diet items composition). Conversely, in the killer whale diet consisting of

96% Chinook salmon, 2% halibut and 2% sablefish, squid accounts for 70% of the Chinook salmon diet composition. This implies that squid might be a major biovector delivering significant concentrations of PCBs to oceanic Chinook salmon.

7.8.3.4 Evaluating the Effects of Water PCB Concentrations on Model Outcomes

In an effort to assess whether water or sediment are the main sources delivering PCBs to the aquatic food web, a sensitivity analysis on the water and sediment concentrations was conducted. The sensitivity analysis involved changes in the concentrations of PCBs in water and sediments to determine changes in the PCB concentration in Chinook salmon and killer whales in the NRKW critical habitat (coastal food web) and the outer coast (oceanic food web) models. Unfortunately, there is little information in PCB concentrations in water of the study area. However, there is a study indicating that the water column concentrations of PCBs ranges from 28 pg/L below the halocline to 35 pg/L above the halocline with a mean of 32 pg/L in the Strait of Georgia (Dangerfield *et al.* 2007). The data on the PCB concentration in water and sediment in the Strait of Georgia were used to calculate an empirical sediment: water PCB concentration ratio. The study by Dangerfield *et al.* (2007) indicates that the PCB concentration in the water does not vary with depth (i.e., there is no significant PCB concentration gradient in the water column). This implies that all organisms including phytoplankton are exposed to approximately the same PCB concentration and that the thermocline and halocline do not appear to have a major impact on the PCB concentration in the water column. However, the empirical PCB concentration in the water column (32 pg/L = 0.032 ng/L) reported by Dangerfield *et al.* (2007) was 9 or 10 times greater than the PCB concentration in the water (0.003 ng/L) calculated in the model. Based on this

observation, the PCB concentration in the water was increased 10 times in the model sensitivity analysis.

The results of the sensitivity analysis are shown in Table 7.7. The sensitivity analysis showed that a 10-fold increase in the PCB concentration in water caused the predicted PCB concentration in biota to increase by 9.5 times. This indicates that PCBs in the water column are the main source of PCBs in killer whales and suggests that the main pathway of killer whale exposure to PCBs is via the water column. However, the only route of uptake of PCBs is from the killer whale diet as killer whales are not gill ventilating organisms. PCBs in the water column are absorbed by phytoplankton, zooplankton and fish directly from the water and indirectly from the water as a result of dietary exposure, with the exception of phytoplankton. PCB concentrations in sediment dredgeate in excess of those current present can be expected to increase PCB concentrations in the water column and the food web. Through this process, the concentration of PCB in lipid-rich, low trophic level zooplankton can be a million times higher than that in water (Macdonald *et al.* 2005).

For the specific case of the outer coast model, the results of the sensitivity analysis indicate that the bioaccumulation of PCB in the ocean food web is likely to be driven by PCB water concentration. A similar conclusion was found for killer whales in the NRKW critical habitat model using a coastal food web. Recent studies suggest that the net flux of PCBs in the Strait of Georgia is from the atmosphere to seawater (Noël *et al.* 2009) and from seawater into the sediments (Johannessen *et al.* 2008). The outcome of the models (i.e., ΣPCB concentrations in killer whales) was sensitive to a 10% reduction or 10% increase of PCB water concentration (i.e. S = 0.95 for the coastal model and S= 0.97 for the oceanic model), but less sensitive when there was either a 10% reduction or a 10% increase in PCB sediment concentration (i.e., S = 0.05 for the coastal

model and S= 0.03 for the oceanic model). Variations in total organic carbon content in sediments also play a role in the bioavailability and bioaccumulation of PCBs in the food web and concentrations of PCBs in killer whales. The PCB concentration in killer whales increased as the total organic carbon in sediments decreased.

This supports the notion that air and water may be delivering a major portion of PCBs to the aquatic food web, notably in more remote areas. Within the aquatic ecosystem, the concentrations in water and sediments are related. However, these relationships are complex and dependent on the relationship between PCB concentrations in the water column and sediments, and hence is affected by sediment diagenesis and organic carbon cycling in the system, sorption and desorption rates and the source materials (Gobas and Maclean 2003).

Table 7.7 Effect of PCB water and sediment concentrations in predicted PCB concentration in biota

Habitat/food web	PCB water concentration used in the model (ng/L)	10 times increase in PCB water concentration (ng/L)	Initial PCB concentration predicted in Chinook salmon (ng/kg wet weight)	PCB concentration (ng/kg wet weight) predicted in Chinook after 10 times increase in water concentration	Initial PCB concentration predicted in male killer whale (ng/kg wet weight)	PCB concentration predicted in male killer whale (ng/kg wet weight) after increase in water concentration
NRKW critical habitat (coastal food web)	0.003	0.034	11700	111620	3.44×10^{6}	3.28×10^{7}
Outer coast (oceanic food web)	0.009	0.090	47160	459545	1.36×10^{7}	1.33×10^{8}

Ratio PCB concentration after 10 times increase in PCB water concentration/Previous PCB concentration in biota

	Chinook salmon	Resident killer whale
NRKW critical habitat	9.5	9.5
Outer coast	9.7	9.7

Sensitivity Analysis (S*) of abotic state variable (i.e. 10% reduction or 10% increase), including PCB concentrations in water and sediment, and organic carbon content in sediment, on ΣPCB concentrations in male resident killer whales of the NRKW critical habitat and outer coast area

	NRKW critical habitat (coastal food web)	Outer coast (oceanic food web)
Concentration in water	0.95	0.97
Concentration in sediment	0.05	0.03
Organic carbon content – sediment ($OC_{SEDIMENT}$)	-0.026	-0.014

$^{*}S = \left(\frac{(\Delta O/O)}{(\Delta I/I)} \right)$ (See Appendix 5 for a description of the sensitivity analysis approach used here).

7.8.4 Model Applications to Chinook Salmon, Resident Killer Whales and Steller Sea Lions

7.8.4.1 Hypothetical Scenarios

Predicted PCB concentrations in Chinook salmon, resident killer whales and Steller sea lions and calculated BSAF values under the hypothetical scenario (assuming 100% presence in each model area) for each of the areas of interest using empirical sediment PCB values are provided in Table 7.8 for Chinook salmon, Tables 7.9 and 7.10 for killer whales, and Table 7.11 for Steller sea lion. The lowest concentrations of PCBs in biota and the lowest BSAF value were predicted in the northern resident killer whale Critical Habitat (NRKW-Critical Habitat), while the highest PCB concentration in biota and the highest BSAF value were predicted in the southern resident Critical Habitat in the USA (Puget Sound). The BSAFs above calculated were used as baseline data for subsequent modeling calculations which rely on realistic geographical distributions of predator/prey (killer whale and Chinook salmon), and contribute to our exploration of PCB bioaccumulation in the killer whale food web, assessment of health impacts and sound scientific information for decision making and environmental management (i.e., derivation of more protective SQGs). Therefore, life history-based BSAF values based on predicted time spent by Chinook salmon and killer whales in each of the seven areas of interest were derived using the hypothetical BSAFs.

Table 7.8 Empirical PCB concentrations in sediments (C_S, mg/kg dry weight) and predicted PCB concentrations in Chinook salmon (C_{fish}, mg/kg wet weight), with calculated $BSAF_{fish}$ (kg dry weight /kg wet weight) in assessed model areas (assuming 100% presence in model areas).

Model Areas	C_S	$BSAF_{fish}$	C_{fish}
NRKW -Critical Habitat	4.40×10^{-04}	26.5	0.010
Queen Charlotte Strait	6.95×10^{-04}	38.0	0.030
Outer coast	6.95×10^{-04}	68.0	0.050
Strait of Georgia	1.05×10^{-03}	63.0	0.100
SRKW-Critical Habitat in Canada	5.20×10^{-04}	63.5	0.03
SRKW-Critical Habitat in USA (Puget Sound)	7.40×10^{-02}	51.0	3.80
SRKW-Critical Habitat in USA (summer core & Juan de Fuca Strait)	6.10×10^{-03}	92	0.558

Table 7.9 Empirical PCB concentrations in sediments (C_S, mg/kg dry weight) and predicted PCB concentrations in a male killer whale ($C_{KW\text{-}male}$, mg/kg wet weight), with calculated $BSAF_{KW\text{-}male}$ (kg dry weight /kg wet weight) in assessed model areas (assuming 100% presence in model areas; and a realistic diet: 96% Chinook salmon, 2% halibut; and 2% sablefish).

Model Areas	C_S	$BSAF_{KW\text{-}male}$	$C_{KW\text{-}male}$
NRKW -Critical Habitat	4.40×10^{-04}	7790	3.40
Queen Charlotte Strait	6.95×10^{-04}	11160	7.80
Outer coast	6.95×10^{-04}	19640	14.0
Strait of Georgia	1.05×10^{-03}	18800	20.0
SRKW-Critical Habitat in Canada	5.20×10^{-04}	18845	10.0
SRKW-Critical Habitat in USA (Puget Sound)	7.40×10^{-02}	15370	1140
SRKW-Critical Habitat in USA (summer core & Juan de Fuca Strait)	6.10×10^{-03}	27670	168

Table 7.10 Empirical PCB concentrations in sediments (C_S, mg/kg dry weight) and predicted PCB concentrations in a female killer whale ($C_{KW\text{-}female}$, mg/kg wet weight), with calculated $BSAF_{KW\text{-}female}$ (kg dry weight /kg wet weight) in assessed model areas (assuming 100% presence in model areas; and a realistic diet: 96% Chinook salmon, 2% halibut; and 2% sablefish).

Model Areas	C_S	$BSAF_{KW\text{-}female}$	$C_{KW\text{-}female}$
NRKW -Critical Habitat	4.40×10^{-04}	1100	0.480
Queen Charlotte Strait	6.95×10^{-04}	1820	1.26
Outer coast	6.95×10^{-04}	3200	2.20
Strait of Georgia	1.05×10^{-03}	3045	3.20
SRKW-Critical Habitat in Canada	5.20×10^{-04}	3050	1.60
SRKW-Critical Habitat in USA (Puget Sound)	7.40×10^{-02}	2490	185
SRKW-Critical Habitat in USA (summer core & Juan de Fuca Strait)	6.10×10^{-03}	4475	27.0

Table 7.11 Empirical PCB concentrations in sediments (C_S, mg/kg dry weight) of the Strait of Georgia and predicted PCB concentrations in male ($C_{SSL\text{-}male}$, mg/kg wet weight) and female ($C_{SSL\text{-}female}$, mg/kg wet weight) Steller sea lion, with calculated BSAF (kg dry weight /kg wet weight), assuming 100% presence in the Strait of Georgia and a diet consisting of 80% Pacific herring, 6.7% Chinook salmon; 6.7% chum salmon; and 6.7% coho salmon.

Model Area	C_S	$BSAF_{SSL\text{-}male}$	$C_{SSL\text{-}male}$	$BSAF_{SSL\text{-}female}$	$C_{SSL\text{-}female}$
Strait of Georgia	1.05×10^{-03}	423	0.444	134	0.140

7.8.4.2 Realistic Scenarios

The more realistic outcomes are provided for Chinook salmon (Lower Fraser stocks in Table 7.12 and South Thompson stocks in Table 7.13, and for killer whales (northern resident males and females in Table 7.14; and, southern resident males and females in Table 7.15). Similar to the scenarios assuming 100% presence in each model area, the lowest PCB concentrations in biota and the lowest BSAF value were predicted in the northern resident killer whale Critical Habitat (NRKW-Critical Habitat), while the highest PCB concentrations were predicted in the southern resident Critical Habitat in the USA (Puget Sound). This reflects a combination of ambient sediment PCB concentrations in the different areas, combined with relative time spent by Chinook salmon and resident killer whales in the different areas studied.

Predicted PCB concentrations for major diet items of resident killer whales, including Chinook salmon, halibut, and sablefish, as well as for herrings (assumed to be major diet item for Steller sea lion of the Strait of Georgia) are provided in Appendix F-8.

Table 7.12 Realistic and total BSAF values for Lower Fraser River Chinook salmon based on the observed distribution in the model areas.

Lower Fraser River Chinook areas	% Time spent per area	BSAF (100% presence)	BSAF per area
Queen Charlotte Strait	1.71	38.0	0.650
Outer coast	55.0	68.0	37.0
NRKW Critical Habitat	14.47	26.5	3.80
SRKW Critical Habitat in Canada	7.68	63.5	4.90
Strait of Georgia	7.68	63.0	4.90
SRKW Critical Habitat in USA (summer core & Juan de Fuca Strait)	4.07	92.0	3.75
SRKW Critical Habitat in USA (Puget Sound)	9.41	51.0	5.0
Total	100		60.0

BSAF per area = (% Time spent)*(BSAF)

Table 7.13 Realistic and total BSAF values for South Thompson Chinook salmon based on the observed distribution in the model areas.

South Thompson Chinook areas	% Time spent per area	BSAF (100% presence)	BSAF per area
Queen Charlotte Strait	7.99	38.0	3.00
Outer coast	79.9	68.0	54.0
NRKW Critical Habitat	3.47	26.5	0.90
SRKW Critical Habitat in Canada	3.45	63.5	2.20
Strait of Georgia	3.45	63.0	2.20
SRKW Critical Habitat in USA (summer core & Juan de Fuca Strait)	1.63	92.0	1.50
SRKW Critical Habitat in USA (Puget Sound)	0.17	51.0	0.10
Total	100		64.0

BSAF per area = (% Time spent)*(BSAF)

Table 7.14 Realistic and total BSAF values for northern resident killer whales (males and females) based on field observed distributions in the model areas.

	Outer Coast	Queen Charlotte Strait	NRKW Critical Habitat	Total
% Time spent per area	**75.0**	**17.0**	**8.0**	**100**
	Northern Resident Killer Whale (male)			
BSAF (100% presence)	19600	11160	7790	
BSAF per area	14700	1900	620	17250
% of PCBs attributable to area	**85.4**	**11.0**	**3.6**	**100**
	Northern Resident Killer Whale (female)			
BSAF (100% presence)	3190	1820	1080	
BSAF per area	2390	310	86	2790
% of PCBs attributable to area	**85.7**	**11.1**	**3.1**	**100**

BSAF per area = (% Time spent)*(BSAF)

Table 7.15 Realistic and total BSAF values for southern resident killer whales (males and females) based on field observed distributions in the model areas.

	Outer coast	SRKW Critical Habitat in Canada	Strait of Georgia	SRKW Critical Habitat in USA (Puget Sound)	SRKW Critical Habitat in USA (summer core & Juan de Fuca Strait)	Total
% Time spent per area	**37.0**	**18.0**	**3.0**	**6.0**	**36.0**	**100**
Southern Resident Killer Whale (male)						
BSAF (100% presence)	19640	18845	18800	15370	27670	
BSAF per area	7266	3392	564	922	9960	22105
% of PCBs attributable to area	**32.9**	**15.3**	**2.6**	**4.2**	**45.1**	**100**
Southern Resident Killer Whale (female)						
BSAF (100% presence)	3190	3050	3045	2490	4475	
BSAF per area	1180	550	90	150	1610	3580
% of PCBs attributable to area	**33.0**	**15.4**	**2.5**	**4.2**	**45.0**	**100**

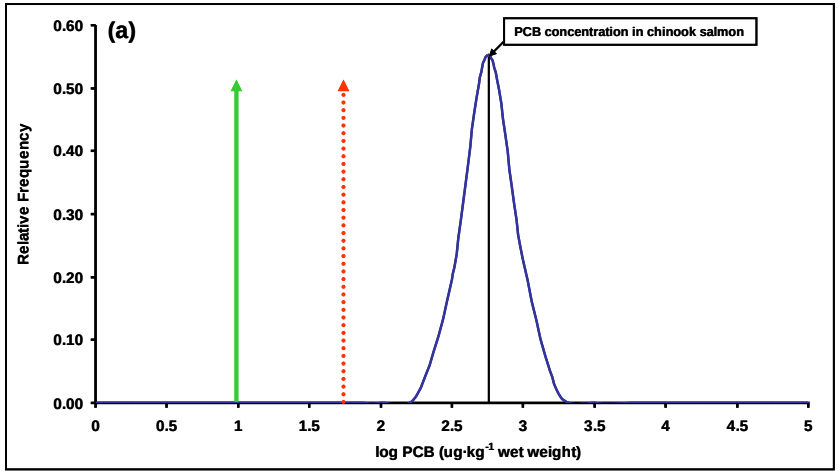
BSAF per area = (% Time spent)*(BSAF)

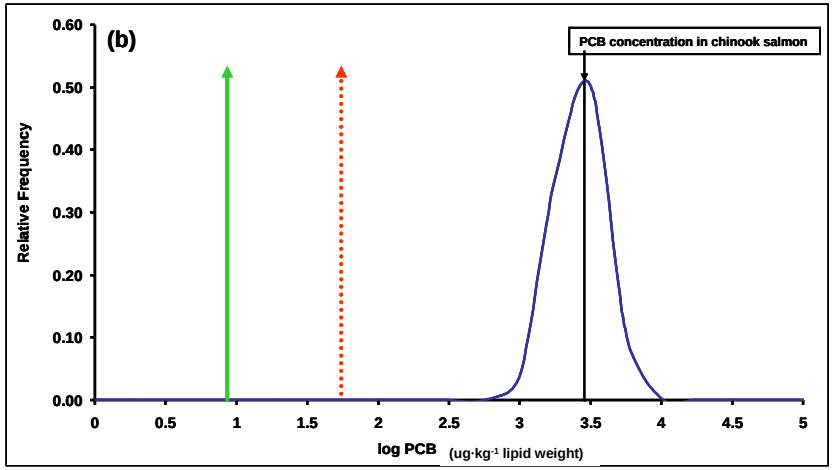
7.8.5 Forward Calculations

7.8.5.1 Chinook Salmon

The predicted PCB concentration in Chinook salmon assuming 100% presence in the areas included in the model, exceeded the tissue residue guideline for fish-eating wildlife (50 µg/kg wet weight) and the Chinook salmon concentration for 95% of the killer whale population to fall below the toxicity threshold of 8 µg/kg wet weight for the CCME ISQG, CEPA Action Level Low and BC-MWLAP SQC_{SCS} tested. As an example, this is illustrated in Figure 7.17 for the northern resident killer whale critical habitat (i.e., Johnstone Strait in Canada) and in Figure 7.18 for the southern resident Killer whale habitat in US (Puget Sound).

Realistic scenarios based on the total BSAF values per habitat distribution showed that PCB concentrations predicted in Lower Fraser River and South Thompson Chinook salmon also exceeded tissue residue guidelines for the CCME ISQG, CEPA Action Level Low and BC-MWLAP SQC_{SCS} tested. For example, both Chinook salmon stocks exhibited PCB concentrations above tissue residue guidelines when the CEPA Action Level Low was tested (Figures 7.19 and 7.20).





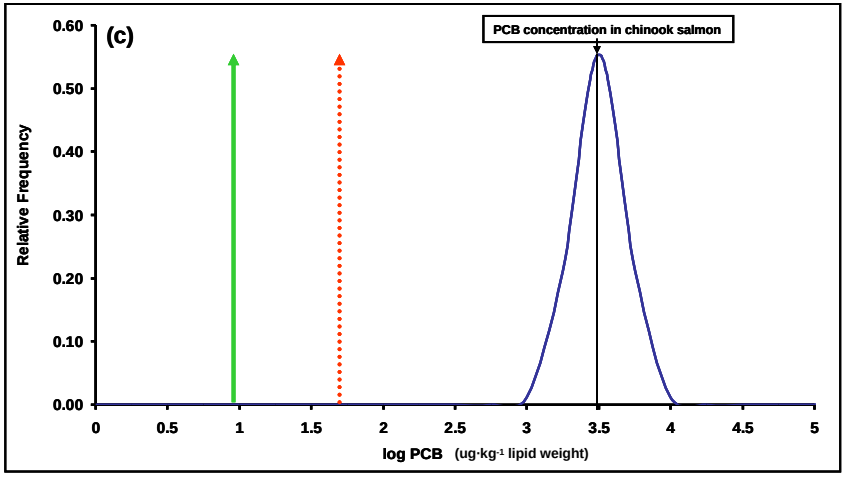
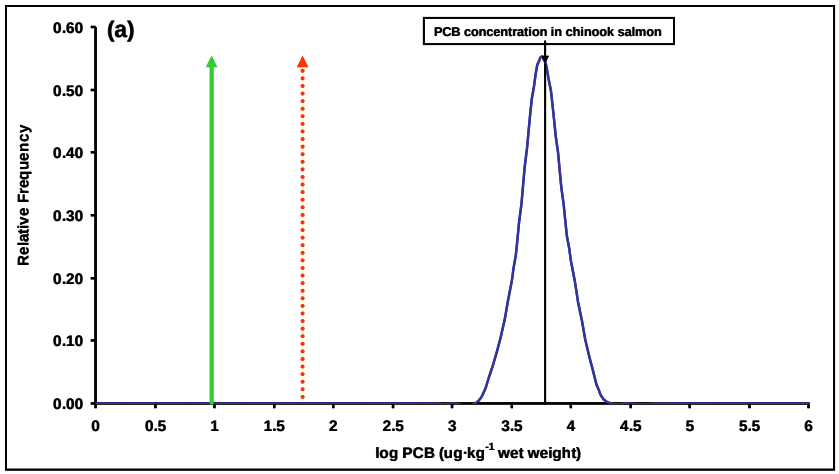
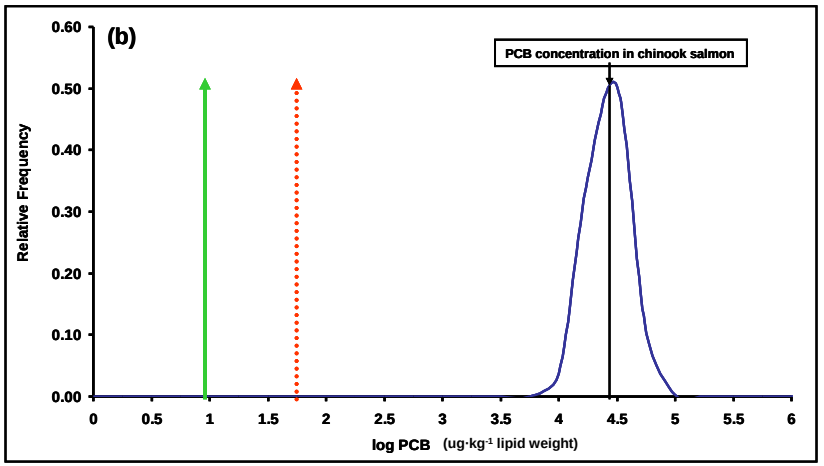


Figure 7.17 Normal probability density functions of predicted log PCB concentration in Chinook salmon in NRKW Critical Habitat (assuming 100% presence in modeled areas) based on (a)The CCME ISQG for total PCBs; (b) The CEPA Action Level Low for total PCBs; and (c) The BC-MWLAP SQC_{SCS}. The solid arrow represents the Chinook salmon concentration (8 µg/kg wet weight) proposed for killer whale prey tissue residue guideline and protective for 95% of the killer whale population (Hickie *et al*. 2007); and the dotted arrow is the established tissue residue guideline for fish-eating wildlife (50 µg/kg we tweight; CCME).





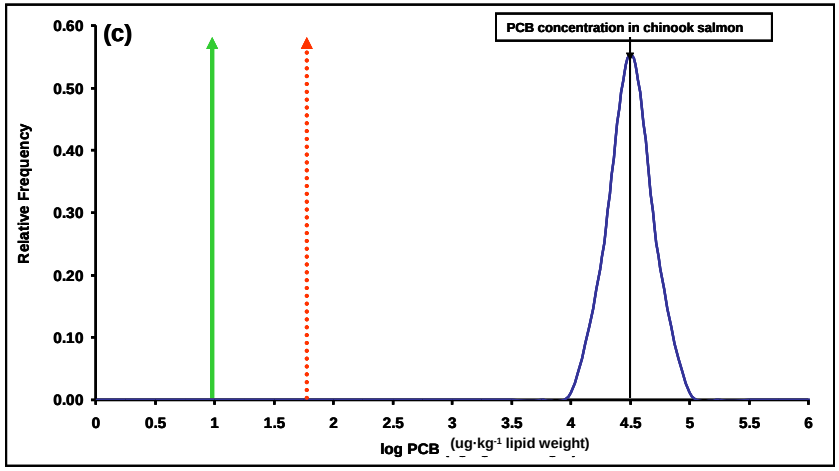


Figure 7.18 Normal probability density distributions of predicted log PCB concentration in Chinook salmon in SRKW Critical Habitat in USA, Puget Sound (assuming 100% presence in modeled areas) based on (a)The CCME ISQG for total PCBs; (b) The CEPA Action Level Low for total PCBs; and (c) The BC-MWLAP SQC_{SCS}. The solid arrow represents the Chinook salmon concentration (8 µg/kg wet weight) proposed for killer whale prey tissue residue guideline and protective for 95% of the killer whale population (Hickie *et al*. 2007); and the dotted arrow is the established tissue residue guideline for fish-eating wildlife (50 µg/kg wet weight; CCME).

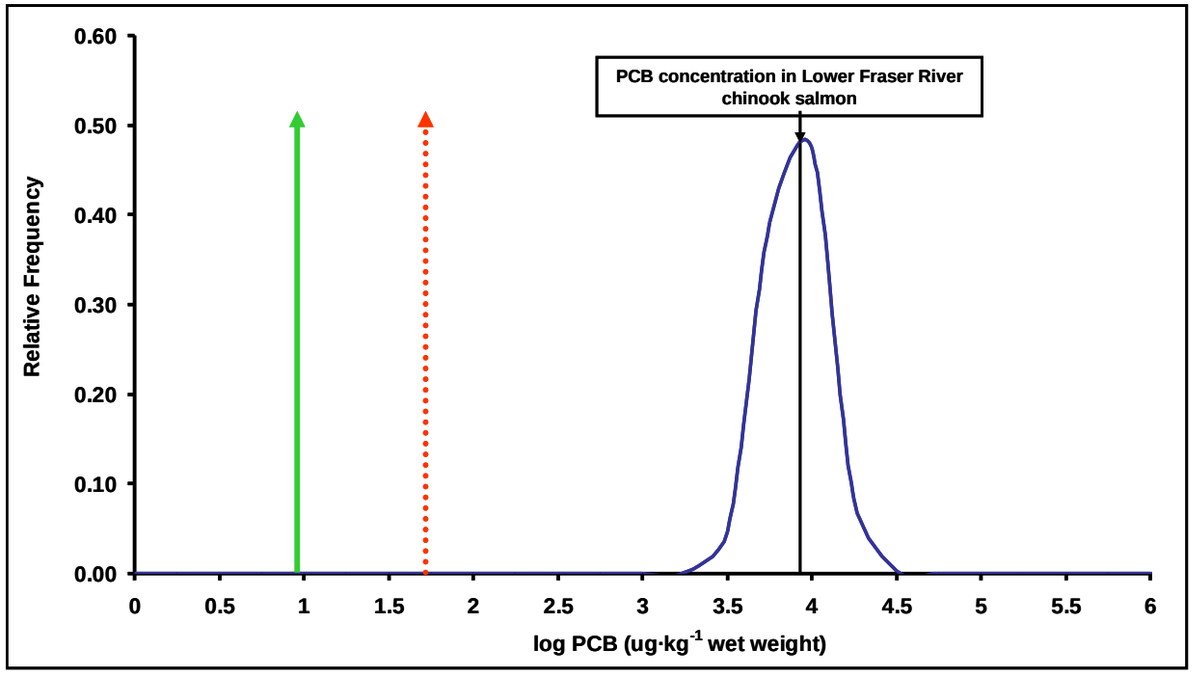


Figure 7.19 Normal probability density distribution of predicted log PCB concentrations in Lower Fraser River Chinook salmon based on the CEPA Action Level Low. The solid arrow represents the Chinook salmon concentration (8 µg/kg wet weight) proposed for killer whale prey tissue residue guideline and protective for 95% of the killer whale population (Hickie *et al*. 2007); and the dotted arrow is the established tissue residue guideline for fish-eating wildlife (50 µg/kg wet weight; CCME).

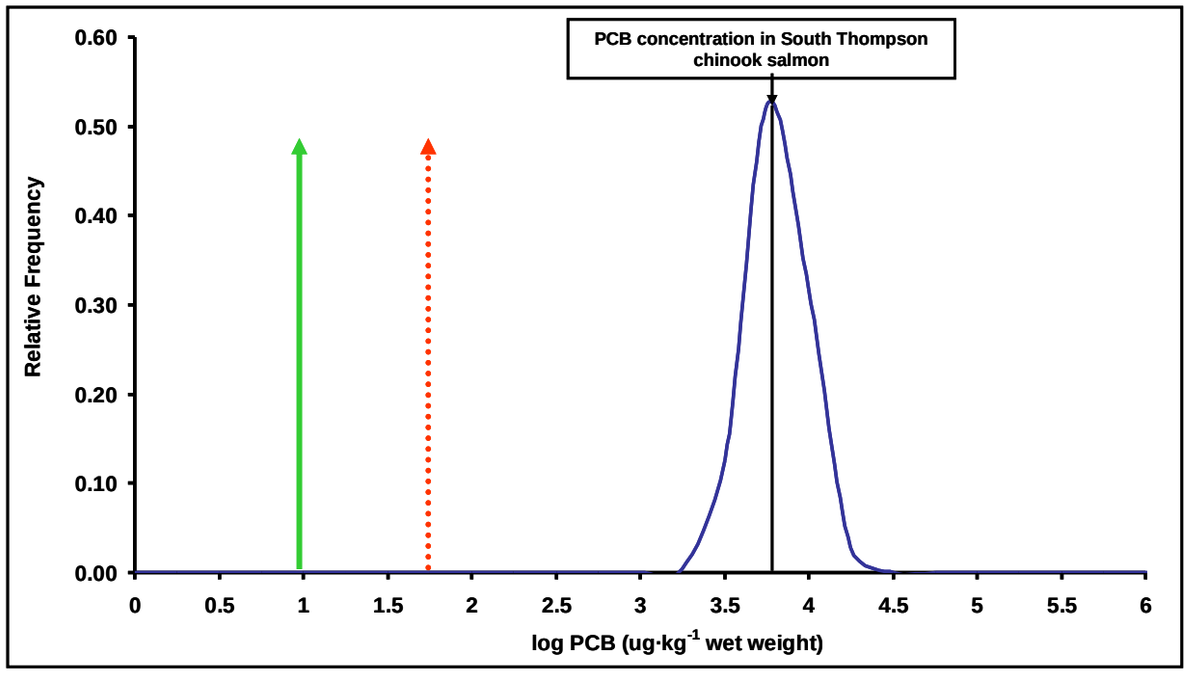


Figure 7.20 Normal probability density distribution of predicted log PCB concentrations in South Thompson Chinook salmon based on the CEPA Action Level Low. The solid arrow represents the Chinook salmon concentration (8 µg/kg wet weight) proposed for killer whale prey tissue residue guideline and protective for 95% of the killer whale population (Hickie *et al*. 2007); and the dotted arrow is the established tissue residue guideline for fish-eating wildlife (50 µg/kg wet weight; CCME).

7.8.5.2 Killer Whales

In a similar fashion, resident killer whales exceeded toxicity health effect thresholds under the assumption that they spend 100% of their time in the model areas for the CCME ISQG and CEPA Action Level Low tested. As an example, both male and female resident killer whale exceeded these thresholds when the CCME ISQG and CEPA Action Level Low are tested (Figure 7.21).

Since several habitat areas can be used by the two populations of resident killer whales, the load of PCB levels in killer whales can be attributed as a contribution from different areas. Thus, realistic scenarios for northern and southern resident killer whales based on habitat distribution data (i.e., the proportion of time invested in each area; Tables 7.13 and 7.14) were generated by testing the CCME ISQG and CEPA Action Level Low. As seen in Figures 7.22 and 7.23, the two resident populations of killer whales are well above the toxic effect concentrations reported for marine mammals.

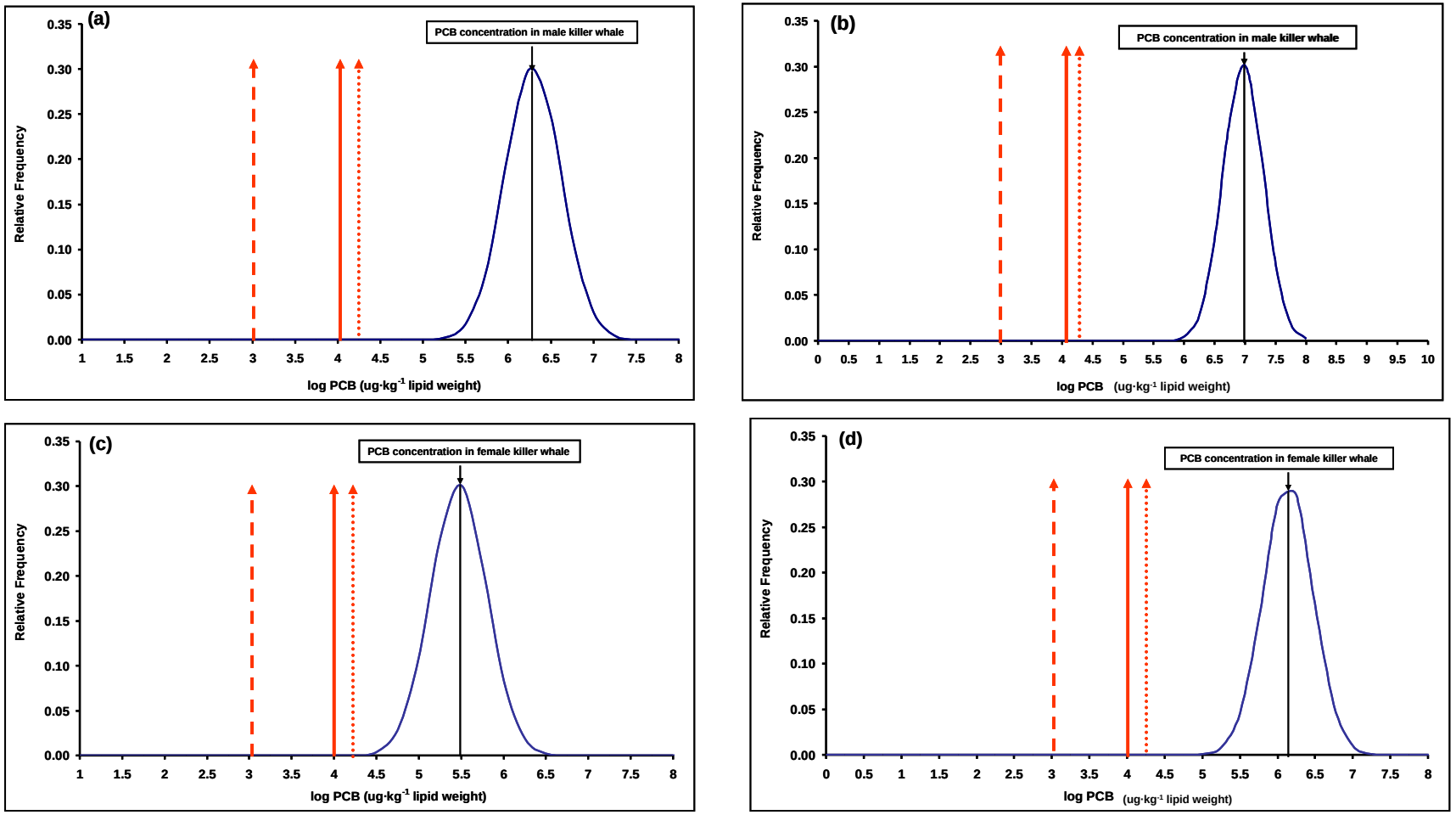


Figure 7.21 Normal probability density distributions of predicted PCB concentrations in resident killer whales spending 100% time in the areas included in the model, with a 96% Chinook salmon diet, based on (a) the CCME ISQG in male resident killer whale; (b) The CEPA Action Level Low in male resident killer whale; (c) Testing the CCME ISQG in female resident killer whale; and, (d) The CEPA Action Level Low in female resident killer whale. The dashed arrow represents the revised harbour seal PCB toxicity threshold (1,300 µg/kg lipid; Mos *et al.* 2010); the solid arrow represents the bottlenose dolphin PCB toxicity threshold (10,000 µg/kg lipid; Hall *et al.* 2006); and, the dotted arrow represents the previous harbour seal PCB toxicity threshold (17,000 µg/kg lipid; Ross *et al*. 1996).

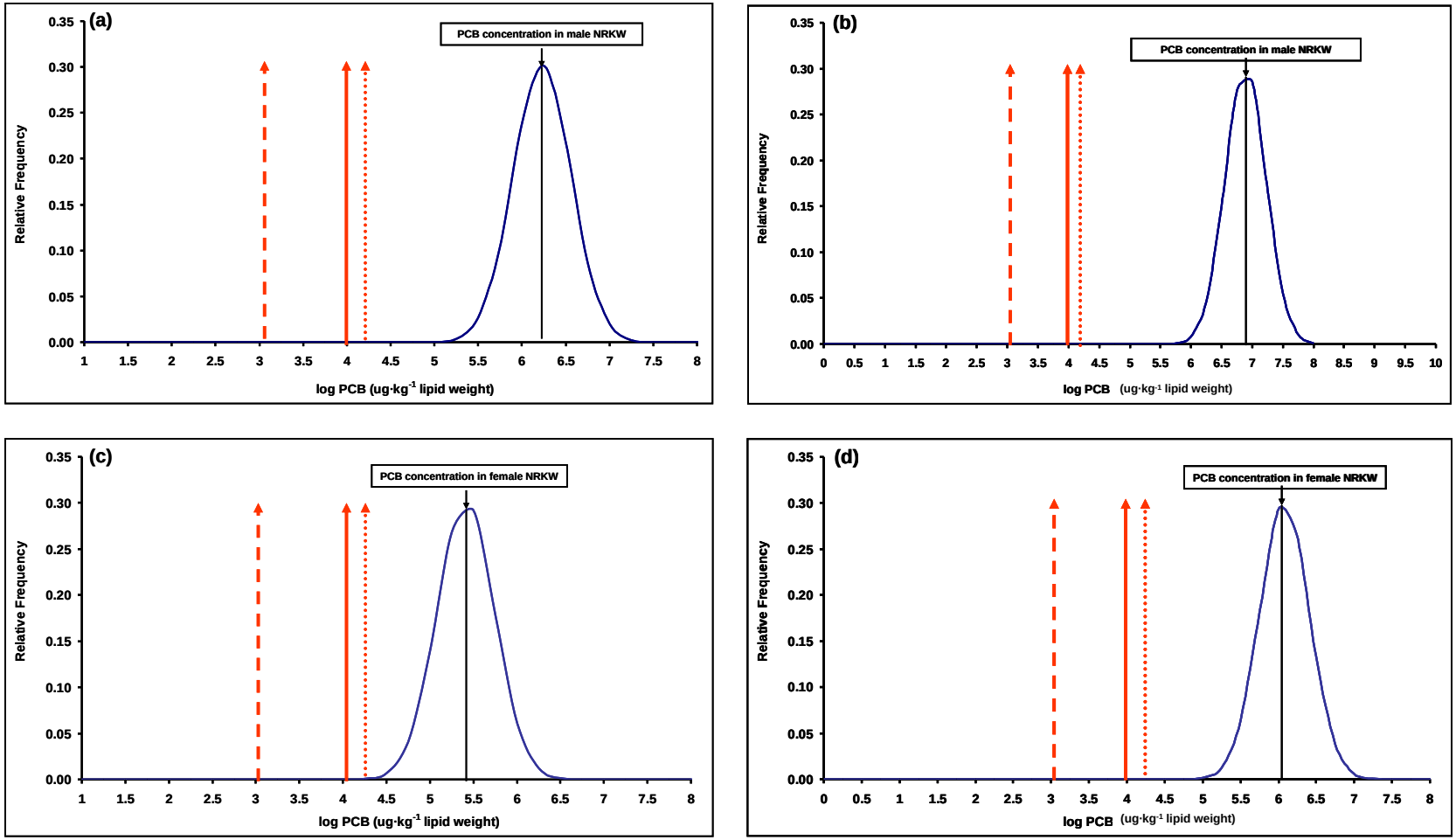


Figure 7.22 Normal probability density distributions of predicted PCB concentrations for realistic scenarios (real habitat distribution % included) in NRKWs (males and females) in model areas, with a 96% Chinook salmon diet, based on (a) The CCME ISQG in male NRKW; (b) The CEPA Action Level Low in male NRKW; (c) The CCME ISQG in female NRKW; and, (d) The CEPA Action Level Low in female NRKW. The dashed arrow represents the revised harbour seal PCB toxicity threshold (1,300 µg/kg lipid; Mos *et al.* 2010); the solid arrow represents the bottlenose dolphin PCB toxicity threshold (10,000 µg/kg lipid; Hall *et al.* 2006); and the dotted arrow represents the previous harbour seal PCB toxicity threshold (17,000 µg/kg lipid; Ross *et al.* 1996).

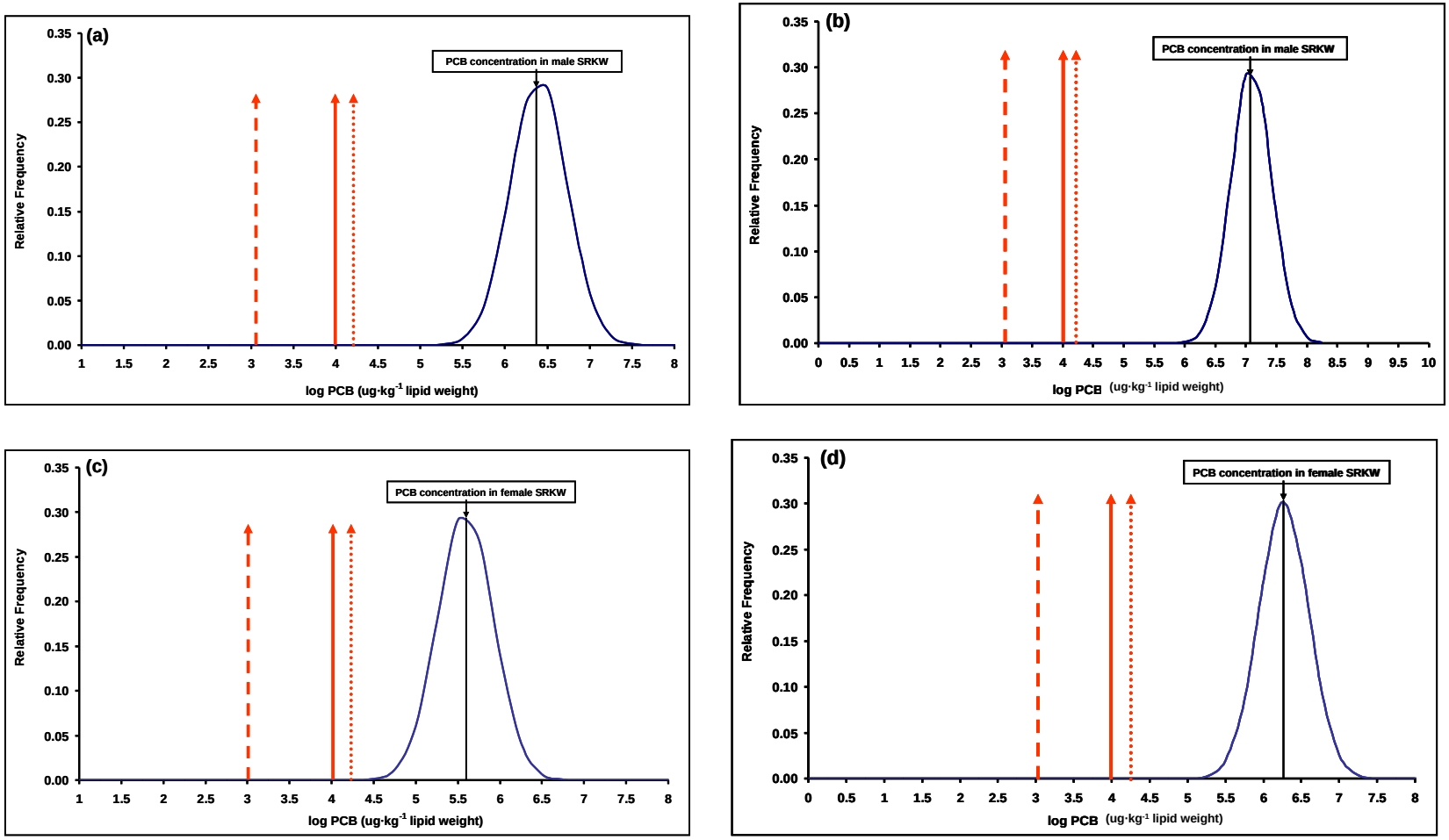
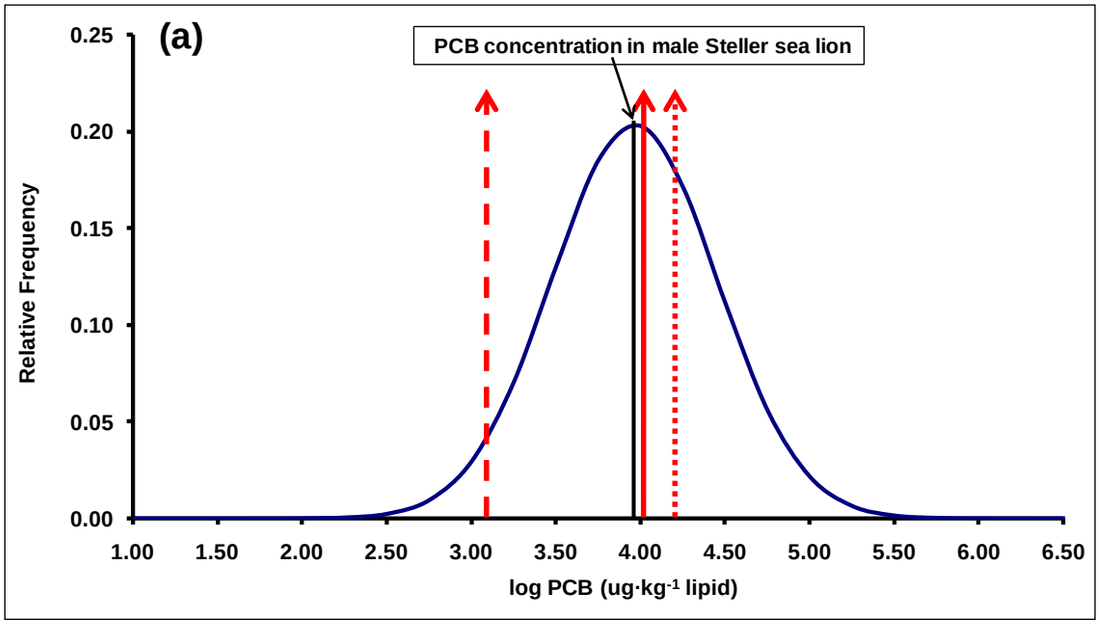


Figure 7.23 Normal probability density distributions of predicted PCB concentrations for realistic scenarios (real habitat distribution % included) in SRKWs (males and females) in model areas, with a 96% Chinook salmon diet, based on (a) The CCME ISQG in male SRKW; (b) The CEPA Action Level Low in male SRKW; (c) The CCME ISQG in female SRKW; and, (d) The CEPA Action Level Low in female SRKW. The dashed arrow represents the revised harbour seal PCB toxicity threshold (1,300 µg/kg lipid; Mos *et al.* 2010); the solid arrow represents the bottlenose dolphin PCB toxicity threshold (10,000 µg/kg lipid; Hall *et al.* 2006); and, the dotted arrow represents the previous harbour seal PCB toxicity threshold (17,000 µg/kg lipid; Ross *et al.* 1996).

7.8.5.3 Steller Sea Lions

Similar to killer whales, a cumulative distribution of predicted PCB concentrations in male Steller sea lions from the Strait of Georgia shows that 95% and 100% of the expected concentrations exceed the revised harbour seal toxicity threshold value as a result of exposure to PCB concentrations in sediments equal to the CCME and CEPA quality guidelines (Figure 7.24a, b). Predicted PCB concentrations in female Steller sea lions showed that 87% and 95% of females exceeded the revised harbour seal toxicity threshold when the CCME ISQG and CEPA Action Level Low were tested, respectively (Figure 7.25a, b). PCB concentrations predicted in male and female Steller sea lions are available Appendix F-9.



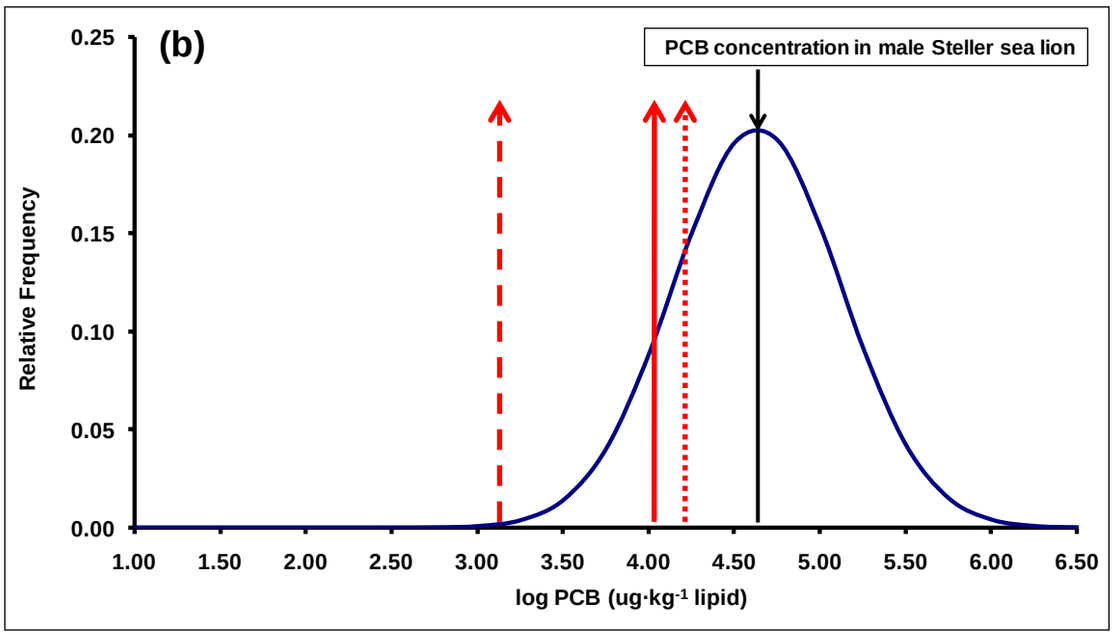
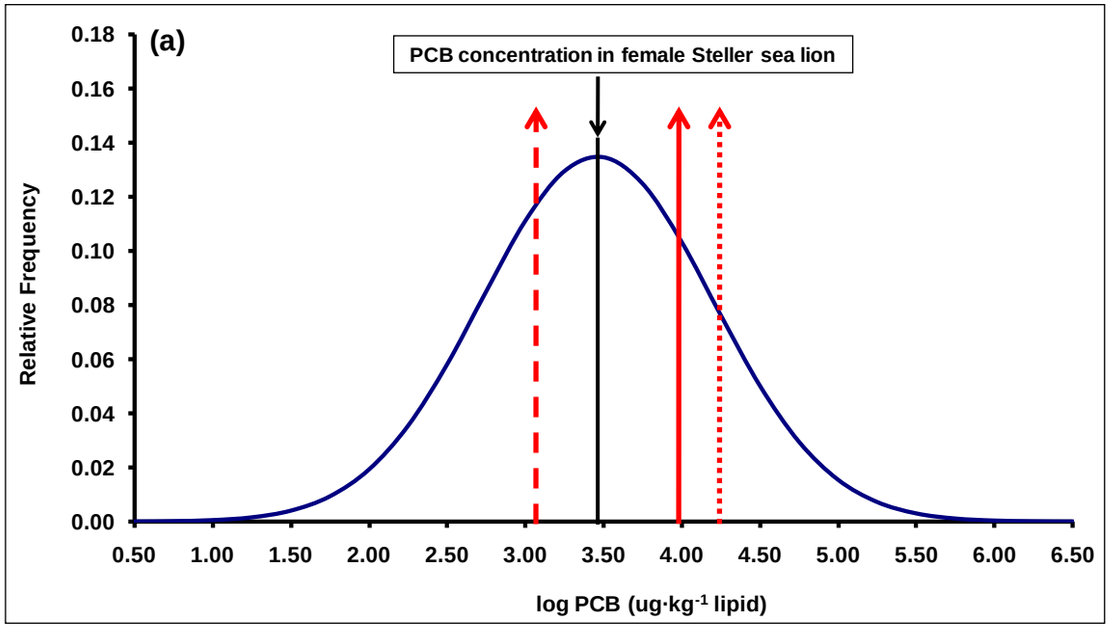


Figure 7.24 Normal probability density distributions of predicted PCB concentrations in male Steller sea lions that spent all their time in the Strait of Georgia and have a diet that includes 80% Pacific herring, 6.7% Chinook, 6.7% chum and 6.7% coho salmon, based on (a) The CCME ISQG; and (b) The CEPA Action Level Low. The dashed arrow represents the revised harbour seal PCB toxicity threshold (1,300 µg/kg lipid; Mos *et al.* 2010); the solid arrow represents the bottlenose dolphin PCB toxicity threshold (10,000 µg/kg lipid; Hall *et al.* 2006); and, the dotted arrow represents the previous harbour seal PCB toxicity threshold of 17,000 µg/kg lipid (Ross *et al.* 1996).



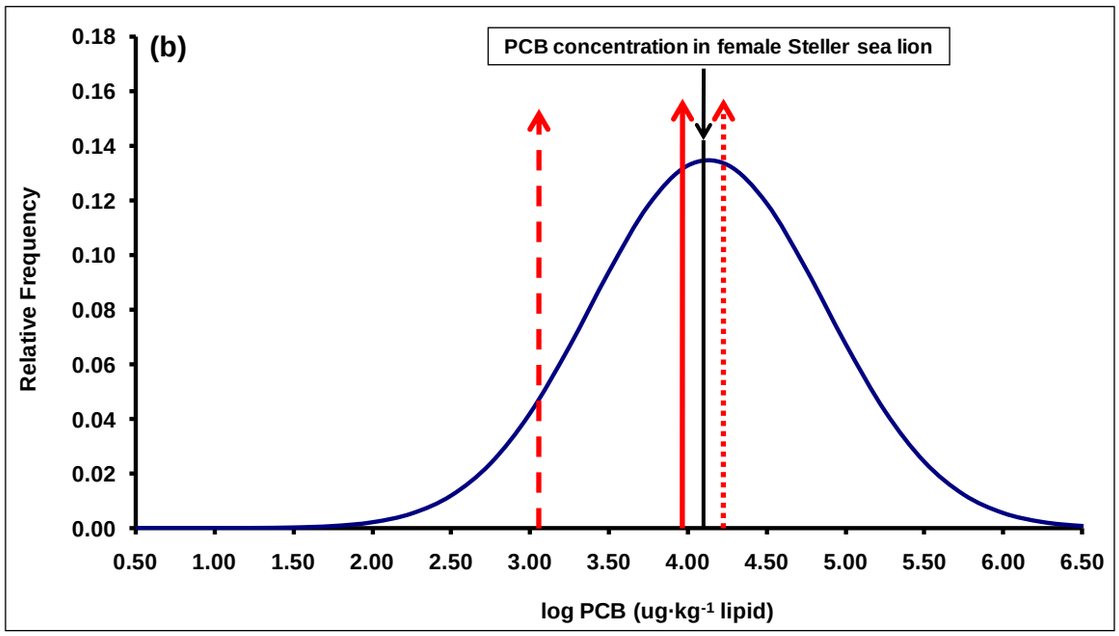


Figure 7.25 Normal probability density distributions of predicted PCB concentrations in female Steller sea lions that spent all their time in the Strait of Georgia and have a diet that includes 80% Pacific herring, 6.7% Chinook, 6.7% chum and 6.7% coho salmon, based on (a) The CCME ISQG; and (b) The CEPA Action Level Low. The dashed arrow represents the revised harbour seal PCB toxicity threshold (1,300 µg/kg lipid; Mos *et al.* 2010); the solid arrow represents the bottlenose dolphin PCB toxicity threshold (10,000 µg/kg lipid; Hall *et al.* 2006); and, the dotted arrow represents the previous harbour seal PCB toxicity threshold (17,000µg/kg lipid; Ross *et al.* 1996).

These scenarios portray that current Interim Sediment Quality Guideline thresholds for PCBs do not protect Chinook salmon, Steller sea lions or resident killer whales and likely other predator organisms at the top of the marine food webs.

7.8.6 Backward Calculations: Deriving Target Sediment Levels

In an effort to generate Sediment Quality Guidelines (SQGs) that are protective of killer whales, the backward application of the BSAF model was carried out. Under this premise, proposed target sediment concentrations to protect 95% of resident killer whales involving realistic scenarios of habitat distribution are shown in Table 7.16. Table 7.16 shows that the overall mean target sediment concentrations ranged from 0.012 to 0.20 µg/kg dry weight. Similar sediment target values were observed under the assumption that resident killer whales spend 100% of their time in model areas (i.e., resident killer whale with 100% presence in model areas), as shown in Table 7.17. This is because the food web structure and killer whale diet composition is basically the same in the realistic scenarios and scenarios assuming 100% time spent in modeled areas.

Derived target sediment levels from hypothetical scenarios of male and females Steller sea lions ranged from 0.05 to 1.70 µg/kg dry weight. The overall mean target sediment concentrations involving for both killer whales and Steller sea lions that spent 100% of their time in model areas ranged from 0.05 to 0.680 µg/kg dry weight (Table 7.17).

Table 7.16 Derivation of target Sediment Quality Guidelines (SQGs) to protect 95% of the population of northern and southern resident killer whales using realistic geographical distributions of killer whales in model areas and a diet of 96% Chinook salmon, 2% halibut, and 2% sable fish. For toxicity threshold values see Table 7.3.

	Toxic Effect Thresholds used		
	Harbour seal PCB toxicity	**Bottlenose dolphin PCB toxicity**	**Revised Harbour seal PCB toxicity**
Resident killer whale population	**Target SQG (µg/kg dry weight)**	**Target SQG (µg/kg dry weight)**	**Target SQG (µg/kg dry weight)**
NRKW male	0.050	0.030	0.004
NRKW female	0.320	0.200	0.024
SRKW male	0.040	0.020	0.003
SRKW female	0.250	0.150	0.020
Average	0.200	0.100	0.012
SD	0.140	0.080	0.010

These calculations are dependent on the food web and marine mammal species assessed. To support this notion, differences in SQG values were observed between the resident killer whale and Steller sea lion coastal food webs assuming that these marine mammals spent 100% in modeled areas, as seen in Table 7.17. To illustrate this, the ratio of the target SQG in Steller sea lion relative to that in killer whales, based on the revised PCB toxic effect threshold for harbour seals, show that the mean target SQG value for Steller sea lions is 6.0 times greater, ranging from 3.0 for females to 12.5 for males, than the mean target SQGs for resident killer whales. These differences in SQGs are the result of assumptions related to food-web structure, reported trophic levels (i.e., Steller sea lion, TL = 4.5; and resident killer whales, TL = 5.0), species feeding preferences, exposure scenarios and degree of contamination in marine mammal species (i.e., killer whales are more contaminated by PCBs than Steller sea lions).

Table 7.17 Derivation of target Sediment Quality Guidelines (SQGs) to protect 95% of resident killer whales and Steller sea lions, assuming 100% presence in model areas. For toxicity threshold values see Table 7.3.

	Toxic Effect Thresholds used		
	Harbour seal PCB toxicity	**Bottlenose dolphin PCB toxicity**	**Revised Harbour seal PCB toxicity**
Resident killer whale and Steller sea lion populations	**Target SQG (µg/kg dry weight)**	**Target SQG (µg/kg dry weight)**	**Target SQG (µg/kg dry weight)**
Male resident killer whale male (100% time in model areas) [a]	0.050	0.030	0.004
Female resident killer whale (100% time in model areas) [a]	0.350	0.210	0.030
Average	0.200	0.120	0.015
Male Steller sea lion (100% time in the Strait of Georgia)	0.670	0.390	0.050
Female Steller sea lion (100% time in the Strait of Georgia)	1.70	0.980	0.130
Average	1.164	0.685	0.090
Overall average (killer whales and Steller sea lions)	**0.680**	**0.400**	**0.050**

[a]Model areas included: Outer coast, Queen Charlotte Strait, NRKW Critical Habitat, Strait of Georgia, SRKW Critical Habitat in Canada, SRKW Critical Habitat in the USA (summer core and Juan de Fuca Strait); and, SRKW Critical Habitat in the USA (only Puget Sound).

7.9 Conclusions

In all seven areas investigated, including the Critical Habitats of both northern and southern resident killer whales, predicted PCB concentrations in Chinook salmon from measured PCB concentrations in sediments exceeded the tissue residue guideline for PCBs in fish-eating wildlife (equivalent to the Canadian Council of Ministers of the Environment or CCME ISQG of 50 µg/kg wet weight). In addition, modelled sediment PCB concentrations equivalent to the CCME Interim Sediment Quality Guideline (ISQG; 21.5 µg/kg dry weight) and CEPA Action Level Low for disposal at sea (100 µg/kg dry weight) resulted in PCB concentrations in Chinook salmon that exceeded tissue residue guidelines for fish-eating wildlife (8 µg/kg wet weight) derived by Hickie *et al.* (2007). Scenarios based on BSAF values in each of the seven areas predicted that Lower Fraser River and South Thompson Chinook salmon also exceeded the CEPA Action Level Low, tissue residue, and the ISQG Guideline evaluated.

The model predicted that more than 95% of resident killer whales can be expected to exceed health effect thresholds established for marine mammals (i. e. 1300, 10000, and 17000 µg/kg lipid) if killer whale are exposed throughout their lives to PCB concentrations in sediments that are equivalent to the CCME ISQG or the CEPA Action Level Low. Similar to killer whales, 100% of predicted PCB concentrations in Steller sea lions are above the harbour seal toxicity threshold of 1300 µg/kg lipid if concentrations in sediments are equivalent to the CEPA Action Level Low. Realistic scenarios reflecting estimated foraging times spent of killer whales and their prey in all seven areas revealed that PCB concentrations in northern and southern resident killer whale populations exceed toxicity health effect thresholds established for marine mammals. These findings are consistent with measured observations of PCBs in killer whales (Ross *et al*. 2002). The fraction of PCBs in the killer whale body burden attributed to PCBs in Critical Habitat

was approximately 3% for northern residents and 15% for southern residents. In most areas, measured PCB concentrations in sediment are below the CCME ISQG. However, it was found that the ISQG to be inadequate to protect resident killer whales and the CEPA guideline also fails to protect Steller sea lions. These scenarios highlight the notion that the current Interim Sediment Quality Guideline (CCME) and the Action Level Low (CEPA) values for PCBs do not protect resident killer whales or their habitat and prey, and underscores the need for refined sediment-based approaches to protect higher trophic level wildlife. This has important implications and relevance for other wildlife species such as aquatic and terrestrial birds.

The model estimated the PCB concentration in sediments expected to protect 95% of resident killer whales, within a concentration range of 0.012 to 0.200 µg/kg dry weight. To protect 95% of Steller sea lions, the derived target sediments ranged from 0.09 to 1.60 µg/kg dry weight. The overall outcomes for derived sediment target levels that would protect both 95% of resident killer whales and 95% of Steller sea lion ranged from 0.050 to 0.680 µg/kg dry weight. Results revealed the vulnerability of killer whales and Steller sea lions to accumulation of persistent contaminants, since only 4/61 (6.6%) of the sediment sites for which we have PCB measurements (Lachmuth *et al.* 2010) fall below the least protective of these sediment values (0.200 µg/kg dry weight). This suggests that there has been widespread contamination of resident killer whale habitat by the legacy PCBs, as the sediments are contaminating their prey, which in turn contaminates the whales. While we could not evaluate PBDE risks using present models, the doubling of this class of POPs every 3.5 years in coastal British Columbia (Lachmuth *et al.* 2010) represents an emerging concern.

7.10 Recommendations for Sediment Quality Guidelines and Ocean Disposal

The model can be used and applied to explore the impact of dredging material and disposal at sea of contaminated marine sediments with the aim to generate revised or new Sediment Quality Guidelines and Action Levels for the explicit protection of high trophic level organisms. This can help to establish Sediment Quality Guidelines and regulated limits for other marine pollution prevention programs to address other contaminants that bioaccumulate, such as PBDEs.

From the environmental monitoring standpoint, biomonitoring of biotic indicators (birds and mammals) and increased sediment sampling for the entire suite of PCB congeners, especially in background or reference areas (especially outside of the Strait of Georgia), would be of value to provide more empirical PCB sediment data. It is also recommended to measure PCB concentrations from material disposed at sea to enable food web-based bioaccumulation models to assess the environmental impact of marine-coastal disposal.

Increased sampling of PCBs in surface waters and air would improve model predictions and accuracy. In addition, sampling of PCBs in several other organisms (especially biota included in the modelled food web, e.g., South Thompson Chinook salmon) will help to verify model output and determine model bias/error. Finally, it is important to design field studies to better capture Chinook salmon and Steller sea lions (Strait of Georgia) annual distributions and feeding ecology to improve model predictions.

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CHAPTER 8

CONCLUSIONS

8.1 Sound science information and environmental management

The management and control of environmental pollution at the global or regional levels must rely on regulations, legal approaches and international agreements based on science-based policy and principles focused on sustainable development and precautionary actions. The contribution of sustainable development can have a positive impact through pollution prevention and the protection of the global environment and biodiversity. This principle is traditionally defined by the Brundtland Report ("Our Common Future") as "*development that meets the needs of the present without compromising the ability of future generations to meet their own needs*" (Brundtland 1987). Defining sustainable development in terms of POPs management requires that we must avoid and mitigate the potential toxic and deleterious effects of organic contaminants in both the environmental and human health of the present and future generations.

Moreover, when controlling and managing contaminants, it is of particular interest and strongly recommended the use and application of the precautionary principle, which according to the CEPA (Bill C-32; Section 2, Part 1) states that "*where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measures to prevent environmental degradation, and promotes and reinforces enforceable pollution prevention approaches"* . In other words, as pointed out by Vanderzwaag

(1994), this principle illustrates that even in the absence of conclusive proof of harm, to human health or ecosystem integrity, emissions of contaminants to the environment be eliminated or prevented when there is reason to suspect harm.

The findings and outcomes of this work provide science-based data that can be used to support environmental management plan and policy, as well as risk assessment in the Galapagos Islands (Ecuador) and British Columbia (Canada) coupled with precautionary actions in order to mitigate and minimize the negative impacts of POPs in wildlife and the health of the marine environment. The overall conclusions and recommendations of this work are described as follows:

- Persistent organic pollutants (POPs) are still present in marine organisms at a global scale, which is further confirmed in this study by the first baseline (i. e. empirical measurements) and trophic biomagnification (as predicted with stable isotopes) assessment of POPs, particularly DDT, in Galapagos sea lions and fish preys. The lessons learned from studies conducted in the northern hemisphere in the past demonstrated clearly the persistence, toxic and bioaccumulative nature of POPs, which provide sufficient weight of evidence to undertake precautionary actions in developing countries. However, we are just beginning to understand the fate and transport of POPs in tropical systems, which have been poorly studied. This portrays the need to change our view in how we use and manage substances that can be harmful for endemic and endangered animals in tropical regions where studies and biomonitoring of POPs have received little attention. Precautionary approaches, science-based policy and the use of more environmental friendly substances need to be incorporated to protect and conserve biodiversity, including the human environment.
- Concentrations of DDT and associated health risks in wildlife are generally believed to be declining but this may no longer be the case in tropical countries where DDT is increasingly

used, as demonstrated here by the temporal levels of DDT found in Galapagos sea lions at the regional level (Galapagos Islands-Ecuador). The toxicological principle "*the dose makes the poison*" provides the scientific rationale for benefiting from the anti-malaria properties of DDT while minimizing or avoiding damage to local and global wildlife and advocates the implementation of source control**.** However, its precautionary application requires a commitment to monitoring remote environments, including the much-valued Galapagos Island Archipelago, and impact assessment and source control programs that have received scant attention in developing nations to date.

- Several other organochlorine pesticides (i. e. dieldrin, mirex, *β*-HCH and chlordanes) listed by the Stockholm Convention for POPs as deleterious substances and banned in Canada and USA were found to be biomagnified in the Galapagos sea lion food chain, suggesting that they have been used recently and probably still used in the region. Management and control actions by local authorities need to be enforced and empowered to mitigate the imports and use of OC pesticides forbidden elsewhere.

- As it is the case elsewhere, levels of POPs are in general lower in tropical, equatorial regions and mid latitudes compared to regions at higher latitudes in the northern hemisphere. This is evident when comparing the concentrations of PCBs detected in Galapagos sea lions versus those in Steller sea lions from British Columbia. Likewise, DDT levels in Galapagos sea lions were relatively lower compared to concentrations detected in pinnipeds from northern latitudes, but similar and higher than those detected in Hawaiian monk seals and southern elephant seals from Antarctica.

- PBDE flame retardants were scarcely detected in Galapagos sea lion; however, in contrast to Galapagos sea lions, PBDEs were readily detected in considerable levels in Steller sea lions,

emphasizing that marine mammals from more industrialized regions are contaminated by PBDEs, as expected. The low levels of these emerging POPs in Galapagos sea lions must be considered as an early signal and warning of the ubiquitous nature and long-range environmental transport of PBDEs in the global ocean and the need of appropriate waste management of electronic waste and products containing PBDEs in tropical areas.

- Polychlorinated biphenyls continue bioaccumulating in marine food webs of marine mammals of the Northeastern Pacific, including Steller sea lions and threatened resident killer whales, despite these compounds were banned more than 30 years ago. The PCB bioaccumulation modelling work and the use of the BSAF conducted in this thesis indicates that levels found in resident killer whales and Steller sea lions of British Columbia are of priority health concerns for apex predators, requiring the implementation of environmental actions through the application of recommended (targeted) sediment quality guidelines to protect organisms at the top of the food webs (Chapter VII) as the guidelines currently available are only protective for benthic invertebrates or lower trophic level organisms. This has important implications for ocean disposal and dredging operations in the Strait of Georgia and other marine environments of British Columbia.

- Similar to PCBs, the development and application of a PBDE bioaccumulation model for marine mammals (e. g. killer whales, harbour seals and Steller sea lions) of the Northeastern Pacific might provide science and expertise in support of risk assessment and management of PBDE congeners that bioaccumulate in food webs with accumulation routes and toxicity similar to PCBs.

- The development and successful application of the PCB food web bioaccumulation modelling in marine mammals from industrial regions in the Northeastern Pacific is promising in terms of

its adaptability and applicability to conceive and build up a food web bioaccumulation model for DDT in tropical regions such as the Galapagos Islands (i. e. Galapagos sea lions as the sensitive apex predator) and the Gulf of Guayaquil (using Coastal bottlenose dolphins as the top predator compartment) in Ecuador. This will supports decision makers of regulatory bodies to set up environmental quality guidelines and thresholds with the aim to protect regional biodiversity of tropical marine ecosystems using umbrella species.

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APPENDICES

Appendix A

Table A-1 Environmental assessment matrix depicting human impacts in the Galapagos Islands.

Anthropogenic Impacts		Environmental Components			
		Abiotic			Biotic
		Air	Water	Soil/sediments	Wildlife: marine and terrestrial
Socio-economic forces	Increasing human population growth/tourism	Increasing use of fossil fuel (gas, diesel); and, increasing production of CO_2 emissions from land transportation and airplanes. Acoustic noise from land and aerial transportation.	Increasing in the amount of marine debris and volume of oil and sewage effluents	Rising production of solid waste; accumulation in open dumps/landfills; land claiming for agriculture and pesticide use	Reduction of habitat suitability and habitat/ecological niche disturbances
Solid waste	Human trash and marine debris	Air emission from solid waste incineration in open dumps and backyards	Floating or submerged artifacts/objects and nets in surface and water column	solid waste contamination of beaches and visual impact	Ingestion and gut obstruction; hazardous interaction; entanglements and mutilations
Chemical Pollution	Polycyclic Aromatic Hydrocarbons (PAHs)	PAH gas emission from outboard motors (boat) and engine cars/trucks/motorcycles	Accidental and intentional oil spills from boats and re-fuel stations at harbours and marinas	Hydrocarbon spills from stored, old tanks/bins on land	Hydrocarbon exposure: narcotic, acute and chronic health effects
	Persistent Organic Pollutants (POPs)	Long-range atmospheric transport and global/regional distillation from continental sources	Oceanic transport from continental sources and binding to organic suspended solids/particles	Atmospheric deposition and binding to organic particulate matter	POPs exposure: long-term and chronic/sublethal adverse health effects (i.e., immunotoxicity and endocrine disruption)

Anthropogenic Impacts		Environmental Components			
		Abiotic			Biotic
		Air	Water	Soil/sediments	Wildlife: marine and terrestrial
Biological Pollution	Invasive species: vertebrate and invertebrate alien species				Predation of endemic species; competition for habitat and resources; and, horizontal transmission of pathogens
	Alien pathogens: viruses, bacteria and parasites	Air contamination and mechanic/abiotic vector for transmitting pathogens (i. e., viruses, bacteria and fungi) to wildlife	Water contamination by spillovers; run-off effluents; and, mechanic/abiotic vector for transmitting pathogens (i. e., virus, bacteria, parasites and fungi) to wildlife	Contamination and reservoir/compartment to spread out pathogens (i. e., bacteria, parasites and fungi) to wildlife	Emerging infectious diseases (EID) by viruses, bacteria, parasites and fungi; wildlife outbreaks. Antibiotic resistance.

Table A-2 Current use pesticides (CUPs) applied to agricultural lands in the Galapagos (Source: Massachusetts Institute of Technology. 2008. Mission 2008: Galapagos Islands. http://web.mit.edu/12.000/www/m2008/teams/lastortugas/v_agriculture.html)

Pesticide type	Chemical class	Chemical product (trade name)	EDC*	LOAEL or LEL**
Insecticide	Mixture of avermectins[1]	Avermectin B1 (Abamectin)		0.40mg/kg/day
	Neonicotinoid	Acetamiprid		N/A
	Pyrethroid	Cyhalothrin-lambda (Karate)	EDC	1.5 mg/kg/day
		Deltamethrin	EDC	N/A
	Carbamate	Carbaryl (Sevin)	EDC	15.6mg/kg/day
	Thiourea	Diafenthiuron		N/A
	Organophosphate	Malathion	EDC	0.34mg/kg/day
Herbicide	Chlorinated phenoxy compound	2,4-D Amine (Salvo)[2,]	EDC	0.75mg/kg/day
	Organophosphate	Glyphosate (Rodeo)	EDC	30 mg/kg/day
	Quaternary ammonium	Paraquat (Gramoxone)	EDC	0.93mg/kg/day
	Pyridine [3]	Picloram (Grazon and Tordon)	EDC	35 mg/kg/day
Fungicide	ß-methoxyacrylates [4]	Azoxistrobin (Heritage)		N/A
	Chloronitrile	Chlorothalonil (Bravo, Ole)		3 mg/kg/day
	Dithiocarbamate[5]	Maneb	EDC	15 mg/kg/day
	Dithiocarbamate	Mancozeb	EDC	N/A
	Substituted dimethyl aniline	Metalaxyl		25 mg/kg/day
	Copper compound	Copper hydroxide		N/A
	Copper compound	Copper Sulphate		N/A
	Non-metal chemical element	Pentahydrated Sulphur (micro-ionized)		N/A

[1]Containing more than 80% avermectin B1a and less than 20% avermectin B1b. Avermectins are insecticidal or anthelmintic compounds derived from the soil bacterium *Streptomyces avermitilis.*
[2] 2,4-Dichlorophenoxyacetic acid
[3] Chlorinated derivative of picolinic acid used in combination or formulations with 2,4-D or 2,4,5-T (Agent Orange) against perennials on non-croplands for brush control.
[4] Derived from the naturally-occurring strobilurins.
[5] Ethylene-(bis)-dthiocarbamates (EBDC) group of fungicides
***EDC**: Endocrine Disrupting Chemical according to Colborn *et al*. (1993); Colborn (1998) and WWF − Canada (1999).
****LOAEL (Lowest-Observed-Adverse-Effect Level):** The lowest exposure level at which there are biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control group; **LEL (Lowest-Observed-Effect Level):** In a study, the lowest dose or exposure level at which a statistically or biologically significant effect is observed in the exposed population compared with an appropriate unexposed control group (US EPA 2008. Integrated Risk Information System (IRIS) Database. www.epa.gov/ncea/iris. Accessed 16 January 2008).

Appendix B:

Immobilization of pups

Galapagos sea lion pups were immobilized following the field anaesthesia methodology developed by Parás *et al*. (2000; 2002). Each pup was caught by its rear fins, and subsequently anesthetized. A maximum of 5 individuals were caught before starting the process of anesthesia. The first animal caught was the first one anesthetized. An Isote 3 (Ohmeda UK) vaporizer was used to administer 5% Isoflurane in oxygen (1-2L/min), adapted to a small animal anesthetic apparatus that consists of an oxygen column with a flow regulation capacity of 0.2 to 5 L per minute and a one-liter ventilation bag. This anesthetic procedure is currently the approach allowed for the Galapagos National Park to collect tissue samples (e.g. blubber and blood) from live captures. Once the animal was relaxed, measurements and samples were taken. Standard length, weight, girth, and determination of sex for each pup were taken. The corporal condition of the pups was measured using the Fulton's condition factor, FCF = weight x 10^5/standard length^3, which is an appropriate index to compare the weight of sea lions pups of different standard lengths and eliminates the size effect on weight (Castro-Gonzalez *et al*. 2001).

Chemical Analysis

PCDD/PCDFs analyses

The PCDD/PCDF fractions of the extracts were analyzed for full congener PCDD/Fs by gas chromatography/high-resolution mass spectrometry (GC/HRMS). The high resolution mass spectrometer was a Micromass Ultima (Micromass, UK) instrument equipped with an HP-6890 gas chromatograph and a CTC autosampler. The GC/HRMS conditions, the criteria used for congener identification and quantification and the quality assurance – quality control procedures

used for the quantification of PCDD/Fs are described in detail elsewhere (Ikonomou *et al*. 2001). For all analyses the HRMS was operated at 10,000 resolution under positive EI conditions and data were acquired in the Single Ion Resolving Mode (SIR). Five point linearity calibration curves were established and the PCDD/PCDF calibration solutions used for GC/HRMS quantitation covered the range 0.25 pg/µL to 1000 pg/µL. The recoveries of all surrogate internal standards were between 75 and 110% and the accuracy of determining PCDD/Fs in the performance evaluation standard was between 10 and 20%.

The samples were diverse in lipid content and in size and the concentrations of most target contaminants monitored were close to the levels measured in the blanks and as such could be impacted by back ground contamination. Data treatment was first conducted by examining the concentration data of the target contaminants on a pg/sample basis (wet weight). For PCDDs and PCDFs, the concentrations measured in these samples were lower or in some cases close to the concentrations measured in the procedural blanks. The MDL was defined as the mean response of the levels measured in three procedural blanks used plus three times the standard deviation of the blanks ($MDL = Mean_{blanks} + 3*SD_{blanks}$). Accordingly, the MDLs for individual PCDDs congeners on a pg/sample basis were 1.03 and 3.03 pg/sample. For PCDFs the same MDLs range applied.

PBDEs analyses

The PCB-PBDE fraction of the sample extracts was analyzed for a suite of 40 target mono to hepta PBDE congeners by GC/HRMS. The GC/HRMS conditions and the criteria used for congener identification and quantification and the quality assurance are described in (Ikonomou *et al*. 2001). The GC conditions used for the mono- to hepta- BDEs analysis were: 15m DB5-HT x

0.25mm ID x 0.1um film thickness column, and the temperature program 100^{o}C (hold 1min) at 2^{o}C/min to 140^{o}C, at 4^{o}C/min to 220^{o}C, at 8^{o}C/min to 330^{o}C (hold 0.5min). The temperatures for the GC injector, the GC/HRMS interface and the MS ion source were 300^{o}C, 260^{o}C, and 300^{o}C respectively. Splitless injection of 1 μL sample and 1 μL air were performed and the purge was activated 2 min after injection. For all analyses, the HRMS was operated at 10,000 resolution under positive EI conditions and data were acquired in the Single Ion Resolving Mode (SIR). Three point linearity calibration curves were established and the PBDE calibration solutions used for GC/HRMS quantitation covered the range 5 pg/µL to 444 pg/µL. The recoveries of all surrogate internal standards were between 75 and 110% and the accuracy of determining PBDEs in the performance evaluation standard was between 10 and 20%. The levels of individual PBDE congeners measured in the procedural blanks were between 2 and 225 pg per sample.

PCBs analyses

The PCB-PBDE fractions of the sample extracts were analyzed for PCB congeners by GC/HRMS. Quality control procedures used for the quantification of PCBs are described in (Ikonomou *et al*. 2001). To obtain quantitative data for a maximum number of PCBs congeners the extracts were analyzed twice under GC/HRMS conditions using two different GC columns. The columns and the conditions used were: a) DB-5 column (50m x 0.25mm, 0.1μm film, J&W Scientific, Folsom CA), initial temperature 80^{o}C for 2 min, increased at 8^{o}C/min to 150 ^{o}C, then at 4 ^{o}C/min to 300 ^{o}C and held for 2 min; and b) CP-19 column (WCOT fused silica coating CP-SIL 19CB, 60m x 0.25mm, 0.15μm film, Varian, USA), initial temperature 100 ^{o}C for 2 min, increased at 20^{o}C/min to 200 ^{o}C, then at 1.5 ^{o}C/min to 268 ^{o}C, and 12.5 ^{o}C/min to 280 ^{o}C held for 2 min. For all analyses the HRMS was operated at 10,000 resolution under positive EI conditions and data were acquired in the Single Ion Resolving Mode (SIR). The source temperature was maintained

at 300 ^{o}C the injector at 285 ^{o}C and the GC/HRMS interface at 260 ^{o}C. Splitless injection of 1 μL sample and 1 μL air were performed and the purge was activated 2 min after injection. Five point linearity calibration curves were established and the PCBs calibration solutions used for GC/HRMS quantitation covered the range 0.77 pg/µL to 460 pg/µL. The recoveries of all surrogate internal standards were between 60 and 110% and the accuracy of determining PCBs in spiked samples was between 15 and 20%. The levels of individual PCBs congeners measured in the procedural blanks were between 2 and 60 pg/sample wet weight. For the dichloro- and trichloro-PCBs the range was a bit higher, between 60 and 200 pg/sample wet weight. For all target analytes the concentrations reported were within the linear range of the multipoint calibration range established.

Table B-1 Mean concentrations (µg/kg lipid ± Standard Error) of 72 PCB congeners and ∑PCB in blubber samples of Galapagos sea lion pups and the method detection limit (MDL µg/sample). The mean of lipid content is 72% (*n* = 21).

PCBs				MDL
PCB-5/8	8.82	±	1.62	0.320
PCB-6	1.91	±	0.34	0.060
PCB-7/9	1.01	±	0.18	0.045
PCB-12/13	0.74	±	0.13	0.023
PCB-16/32	4.78	±	0.85	0.191
PCB-17	2.07	±	0.38	0.084
PCB-18	4.52	±	0.84	0.208
PCB-19	0.39	±	0.07	0.012
PCB-22	2.66	±	0.50	0.090
PCB-28	5.71	±	1.17	0.204
PCB-33	4.20	±	0.79	0.153
PCB-43/49	1.10	±	0.27	0.066
PCB-44	2.72	±	0.55	0.099
PCB-45	0.54	±	0.10	0.019
PCB-47/48/75	0.80	±	0.16	0.030
PCB-52	3.11	±	0.75	0.132
PCB-60	0.71	±	0.13	0.023
PCB-61	1.35	±	0.28	0.058
PCB-64/71	1.04	±	0.18	0.050
PCB-66	2.02	±	0.37	0.071
PCB-74	1.55	±	0.36	0.047
PCB-84	0.38	±	0.08	0.021
PCB-85	1.28	±	0.38	0.000
PCB-86/97	0.51	±	0.10	0.026
PCB-87	0.87	±	0.22	0.031
PCB-92	0.88	±	0.22	0.027
PCB-95	2.11	±	0.46	0.074
PCB-99	3.53	±	1.51	0.053
PCB-101	3.25	±	1.07	0.105
PCB-105	0.73	±	0.19	0.026
PCB-107/108	0.18	±	0.03	0.008
PCB-110	1.13	±	0.24	0.057
PCB-118	4.01	±	1.36	0.077
PCB-132	0.65	±	0.13	0.037
PCB-135	0.58	±	0.10	0.029

PCBs				MDL
PCB-136	0.42	±	0.08	0.024
PCB-138/163/164	6.35	±	2.30	0.163
PCB-141	0.97	±	0.18	0.051
PCB-146	1.16	±	0.41	0.024
PCB-149	2.80	±	0.56	0.163
PCB-151	1.12	±	0.23	0.062
PCB-153	7.64	±	2.95	0.181
PCB-156	0.21	±	0.04	0.009
PCB-157	0.79	±	0.15	0.039
PCB-158/160	0.51	±	0.09	0.019
PCB-170/190	0.90	±	0.25	0.025
PCB-171	0.32	±	0.07	0.014
PCB-172/192	0.40	±	0.11	0.000
PCB-177	0.67	±	0.14	0.034
PCB-179	0.87	±	0.16	0.049
PCB-180	3.52	±	1.33	0.076
PCB-183	0.81	±	0.16	0.041
PCB-187	1.90	±	0.39	0.106
PCB-194	0.25	±	0.05	0.015
PCB-196/203	0.28	±	0.06	0.018
PCB-201	0.37	±	0.09	0.020

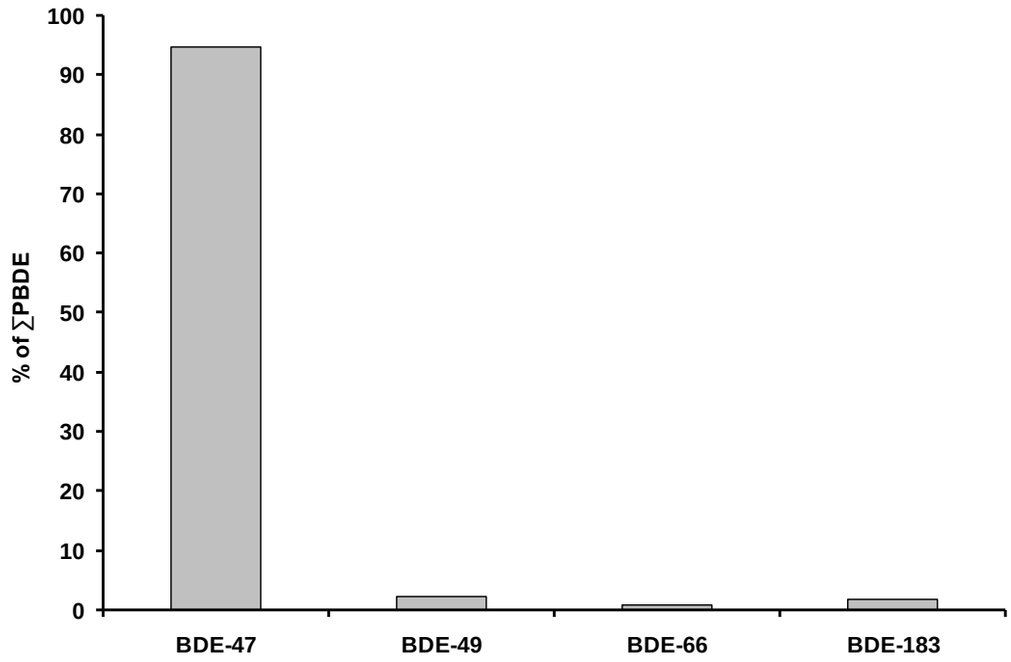

Figure B-1 PBDE congener composition detected in one blubber sample of a Galapagos sea lion pups, *Zalophus wollebaeki*.

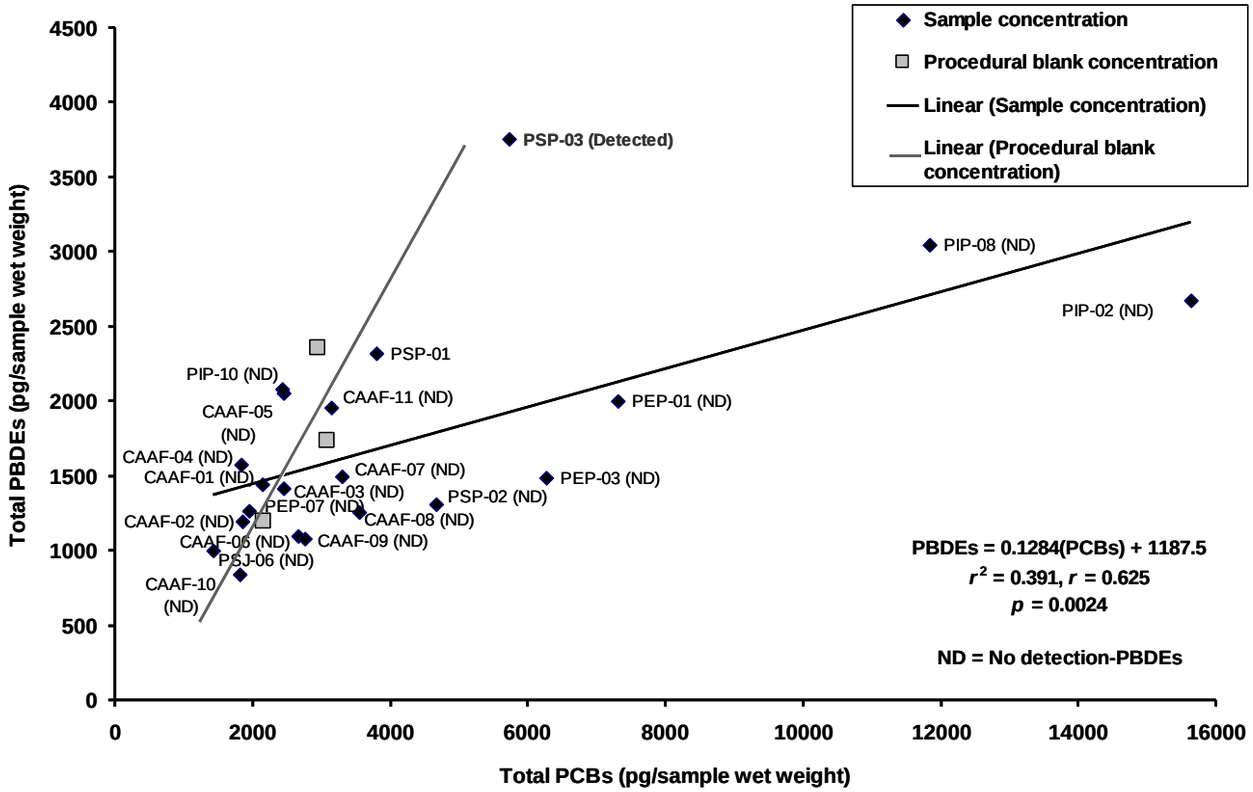

Figure B-2 Regression line showing the relationship between the Log ∑PBDEs and Log ∑PCBs in Galapagos sea lion pups (n = 21) in a pg/sample basis to explore the behaviour of the lab blanks. Sample PSP-03 was the only one showing detectable concentrations of PBDEs. The regression line of procedural blank concentrations used during the lab analysis of both groups of contaminants is also shown as a dashed.

Table B-2 Mean Concentrations of PCBs (mg/kg lipid) in Blubber of Pinnipeds from the Northeastern−Central Pacific Ocean and southern elephant seals from Antarctica (1971–2005)

species location and year of collection	stage/sex	∑PCB	Reference
Zalophus californianus	full term parturient female	20	Delong *et al*. (1973)
San Miguel Island, California, USA, 1970	premature term parturient female	134	
Z. californianus	adult male	356	Kajiwara *et al*. (2001)
Coastal California, USA, 1991−1997	adult female	299	
	subadult male	910	
Mirounga angustirostris	yearling male	5.55	Kajiwara *et al*. (2001)
Coastal California, USA, 1991−1997	yearling female	33	
Z. californianus	adult male	45	Kannan *et al*. (2004)
North, Central and South California Coast, USA, 2000	adult female	83	
	subadult male	20	
Phoca vitulina			
Queen Charlotte Strait, British Columbia, Canada, 1997	pup	1	Ross *et al*. (2004)
Strait of Georgia, British Columbia, Canada, 1996−1997	pup	2	Ross *et al*. (2004)
Puget Sound, Washington State, USA, 1996	pup	18	Ross *et al*. (2004)
Mirounga angustirostris	pup	14	Debier *et al.* (2005)
Año Nuevo, Central California, USA, 2002			
Z. californianus	stranded adult male	77	Ylitalo *et al*. (2005)
Central California Coast, USA, 1993−2003	stranded adult female	83	
Z. californianus	stranded adult and subadult male	3.4	Del Toro *et al*. (2006)
Baja California, Mexico, 2000−2001			
Mirounga leonina	adult male	0.10	Miranda-Filho *et al*. (2007)
Shetland Islands, Elephant Island, Antarctica, 1997−2000	adult female	0.10	
	juvenile	0.04	
	pup	0.02	
Eumetopias jubatus	fetus/pup/subadult/adult	3.17	Alava *et al*. [Chapter VI]
Strait of Georgia, Vancouver Island, British Columbia 2005 – 2006	pup	3.16	
Zalophus wollebaeki	pup	0.10	Alava *et al*. (2009)
Galapagos Islands, Ecuador, 2005			

Table B-3 Comparisons of measured ∑PBDE concentrations, mean or geometric mean (SD) [range of means], in µg/kg wet weight between the Galapagos sea lion and other pinniped species of the world.

Collection year	Collection region	Species	Age category	Sex	*n*	∑PBDEs	Reference
1972-1998	Sanriku, Pacific coast of Northern Japan.	*Callorhinus ursinus*	adult	females	35	[0.23-40][b]	Kajiwara *et al*. (2004)
1981-2000	Holman Island, Northwest Territories, Canada	*Pusa hispida*	pup/juvenile/subadult /adult	mixed	50	[0.57-4.62][c]	Ikonomou *et al.* (2002)
1989-1998	San Francisco Bay, CA, USA	*Phoca vitulina*	adult[a]	mixed	34	512[d]	She *et al*. (2002)
1991-2005	Northwestern Atlantic coast	*P. vitulina concolor*	pups	NR	13	3645 [e]	Shaw *et al.* (2007)
1998-2000	Farne Islands, east coast of United Kingdom	*H. grypus*	pup and juvenile	NR	110	240[f]	Kalantzi *et al*. (2005)
1993-2003	Coastal California, USA	*Z. californianus*	subadult and adult[a]	males	25	1470	Stapleton *et al*. (2006)
2003	United Kingdom	*P. vitulina*	subadult and adult	mixed	60	[80-520][g]	Hall and Thomas *et al*. (2007)
2003	British Columbia, Canada	*P. vitulina*	pup	mixed	16	383	Noel *et al*. (2008)
2003	Washington State, USA	*P. vitulina*	pup	mixed	16	472	Noel *et al*. (2008)
2005-2006	Strait of Georgia, British Columbia, Canada	*Eumetopias jubatus*	pup/subadult/adult	mixed	14	98.3	Alava *et al*. [Chapter VI]
			pup	mixed	3	181	
2005	Galapagos Islands, Ecuador	*Z. wollebaeki*	pup	mixed	1	25.0[h]	Alava *et al*. (2009)

[a] stranded animals; [b] range indicates the lower and higher means of ∑PBDE concentrations observed in blubber/fat samples of female Northern fur seals (*C. ursinus*) collected between 1972 and 1998 (wet mass concentrations depicted here were calculated by multiplying these means in lipid weight by their respective mean lipid fraction; see Table 1 in Kajiwara *et al*. (2005).

[c] included as a range of lipid normalized arithmetic means since the original article reported concentrations only on a lipid normalized basis and lipid content was not reported.

[d] The geometric mean in wet mass was calculated from the actual lipid normalized concentrations of ∑PBDEs and lipid fractions in Harbour seal blubber samples available in Table 2 in She *et al*. (2002).

[e] lipid normalized arithmetic mean

[f] geometric mean in wet mass (the original geometric mean in lipid weight was multiplied by the mean lipid fraction, 83%, as reported by Kalantzi *et al*. (2005).

[g] range of lipid normalized geometric means calculated from the lower and higher geometric means in lipid weight observed in blubber biopsy samples of five Harbour seal populations (wet mass concentrations were calculated by multiplying these geometric means in lipid weight by their respective mean lipid fraction; see Table 2 in Hall and Thomas (2007).

[h] Total PBDE concentration in one out of 21 Galapagos sea lion pups.

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Appendix C:

Table C-1 Sampling sites, morphometric data and lipid content on live-captured, free ranging Galapagos sea lion pups (*Zalophus wollebaeki*) sampled in March 2005 and March 2008 in the Galapagos Islands.

Sample	Sampling site	Sampling Date	Standard length (cm)	Weight (kg)	Sex	Age (months)	FCF	Fraction lipid
PIP-02	Pinta, Puerto Posada	March 17, 2005	96	18	M	2	2.03	0.877
PIP-08	Pinta, Puerto Posada	March 17, 2005	87	14.4	F	3	2.19	0.575
PIP-10	Pinta, Puerto Posada	March 17, 2005	105	21.8	M	4	1.88	0.663
PEP-01	Punta Espinoza, Fernandina	March 18, 2005	103	18	F	3	1.65	0.833
PEP-03	Punta Espinoza, Fernandina	March 18, 2005	108.5	23.2	M	4	1.82	0.665
PEP-07	Punta Espinoza, Fernandina	March 18, 2005	103	20.2	M	4	1.85	0.661
PSP-01	Plaza Sur, Santa Cruz	March 21, 2005	100	18.8	M	3	1.88	1.00
PSP-02	Plaza Sur, Santa Cruz	March 21, 2005	97.5	18.8	M	3	2.03	0.907
PSP-03	Plaza Sur, Santa Cruz	March 21, 2005	96.5	18.6	M	3	2.07	0.710
PSJ-06	Plaza Sur, Santa Cruz	March 21, 2005	109	25.6	M	12	1.98	0.135
CAAF-01	Caamaño, Santa Cruz	March 13, 2005	161	78	F	>8	1.87	0.559
CAAF-02	Caamaño, Santa Cruz	March 13, 2005	161	78	F	5	1.87	0.653
CAAF-03	Caamaño, Santa Cruz	March 13, 2005	171	95.6	F	>8	1.91	0.812
CAAF-04	Caamaño, Santa Cruz	March 12, 2005	177	72.4	F	NR	1.31	0.928
CAAF-05	Caamaño, Santa Cruz	March 14, 2005	150	55	F	4	1.63	0.653
CAAF-06	Caamaño, Santa Cruz	March 14, 2005	157	61.8	F	≈5	1.60	0.802
CAAF-07	Caamaño, Santa Cruz	March 14, 2005	177	98.4	F	>10	1.77	0.799
CAAF-08	Caamaño, Santa Cruz	March 14, 2005	163	72.6	F	6	1.68	0.831
CAAF-09	Caamaño, Santa Cruz	March 14, 2005	166	71.6	F	4-6	1.57	0.819
CAAF-10	Caamaño, Santa Cruz	March 15, 2005	168	78.2	F	8-10	1.65	0.444
CAAF-11	Caamaño, Santa Cruz	March 15, 2005	168	75.4	F	>6	1.59	0.822
IZS-01	Isabela, Loberia Chica	March 26, 2008	116	ND	F	9	N/A	0.657
IZS-02	Isabela, Loberia Chica	March 26, 2008	114	ND	M	9	N/A	0.728
IZP-04	Isabela, Loberia Chica	March 26, 2008	89	16	F	3	2.27	0.858
IZP-05	Isabela, Loberia Chica	March 26, 2008	99	21	M	6	2.16	0.792

Sample	Sampling site	Sampling Date	Standard length (cm)	Weight (kg)	Sex	Age (months)	FCF	Fraction lipid
IZP-06	Isabela, Loberia Chica	March 26, 2008	97	19	F	6	2.08	0.790
FPZ-01	Floreana, Loberia	March 27, 2008	107	21	F	5	1.71	0.681
FPZ-02	Floreana, Loberia	March 27, 2008	115	30	F	7	1.97	0.693
FSZ-03	Floreana, Loberia	March 27, 2008	124	38	M	10	1.99	0.639
FPZ-04	Floreana, Loberia	March 27, 2008	95	15.5	M	5	1.81	0.895
FPZ-05	Floreana, Loberia	March 27, 2008	109	25	M	5	1.93	0.733
FPZ-06	Floreana, Loberia	March 27, 2008	107.5	22	M	5	1.77	0.750
SCPZ-01	San Cristóbal, Pto. Baquerizo	March 28, 2008	88	14	F	3	2.05	0.730
SCPZ-02	San Cristóbal, Pto. Baquerizo	March 28, 2008	99	15	F	4	1.55	0.677
SCPZ-03	San Cristóbal, Pto. Baquerizo	March 28, 2008	114	33.5	F	8	2.26	0.794
SCPZ-04	San Cristóbal, Pto. Baquerizo	March 28, 2008	101	17	M	4	1.65	0.762
ILPZ-01	San Cristóbal, Isla Lobos	March 29, 2008	97	17.5	M	3	1.92	0.830
ILPZ-02	San Cristóbal, Isla Lobos	March 29, 2008	103	21	F	5	1.92	0.698
ILSP-03	San Cristóbal, Isla Lobos	March 29, 2008	110	27	M	6	2.03	0.762
ILPZ-04	San Cristóbal, Isla Lobos	March 29, 2008	96	20	F	4	2.26	1.016
ILPZ-05	San Cristóbal, Isla Lobos	March 29, 2008	94	18	M	4	2.17	0.888

NR = no reported; N/A = no available as weight was not reported for these pups; FCF = Fulton's Condition Factor (weight x 10^5/standard length^3)
.

Table C–2 Toxic effect concentrations (*p*,*p*,'-DDE) with lipid and protein contents reported for the bottlenose dolphin and rat cell culture.

Species	TEC[a] (µg/kg wet weight)	$f_{L,BLOOD}$ Lipid fraction	$f_{P,BLOOD}$ Protein fraction	Equivalent lipid normalized TEC (µg/kg lipid)
Bottlenose dolphin	13[b]	0.005 [c]	0.082[d]	1430
Bottlenose dolphin	536[b]	0.005 [c]	0.082[d]	58,900
Cell culture (rat)	64[e]	0.001[f]	0.1587[f]	6890

[a]TEC = Toxic effect concentration
[b]range of *p*,*p*,'-DDE effect concentrations (13-536 µg/kg wet weight) associated with immunotoxicity in bottlenose dolphins, causing decrease in lymphocyte proliferative responses (Lahvis *et al*. 1995).
[c]The percent lipid for bottlenose dolphin was retrieved from Houde et al. (2006) and Yordi *et al*. (2010).
[d]The protein content was estimated by dividing the plasma protein value reported elsewhere (Bossart et al., 2001; Woshner et al., 2006) to the blood density (a density of 105.3 g/100mL reported for *Macaca fascicularis* was used, Ageyama *et al*. 2001)
[e]Level of *p*,*p*,'-DDE causing potent anti-androgenic effect, inhibiting the transcriptional activity of androgen receptors in mammalian cell cultures (Kelce *et al*. 1995).
[f]Lipid and protein fractions for rats were retrieved from Poulin and Krishnan (1996) and DeBruyn and Gobas (2007).

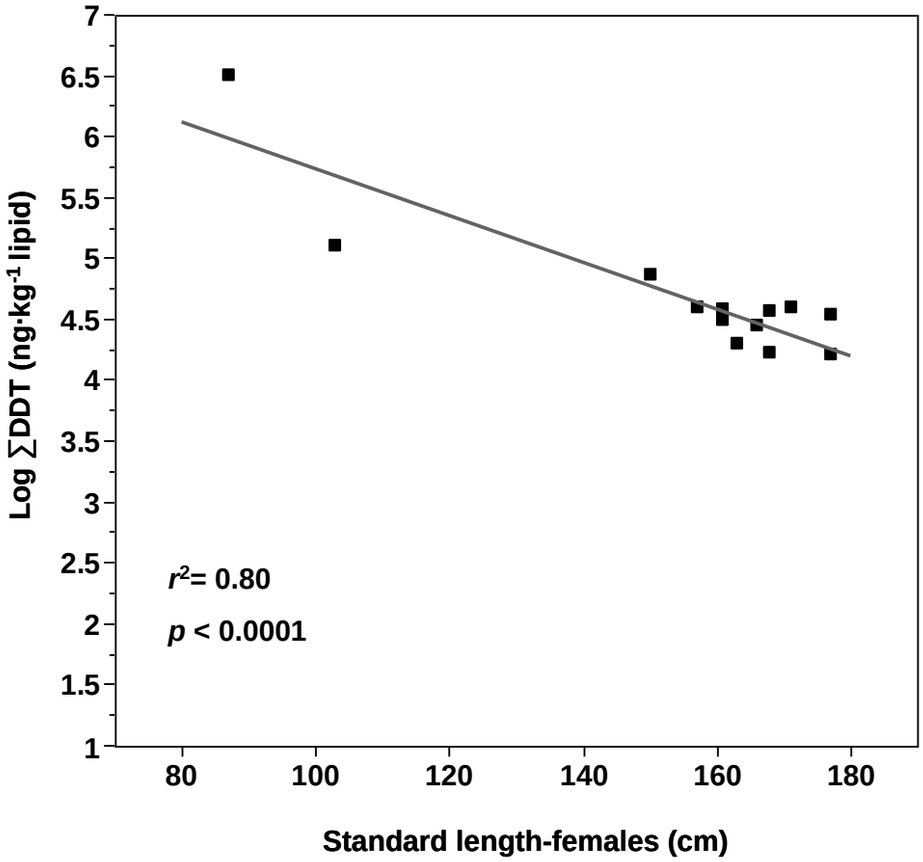

Figure C-1 Relationship between standard length and the logarithm of concentration of ∑DDT, sum of *o, p*-DDE, *p, p*-DDE, *o, p*-DDD, *p, p*-DDD, *o, p*-DDT, and *p, p*-DDT, in female Galapagos sea lion (*Zalophus wollebaeki*) [i. e., log (∑DDTs) = 7.65 - 0.019*Standard length (cm)]**.**

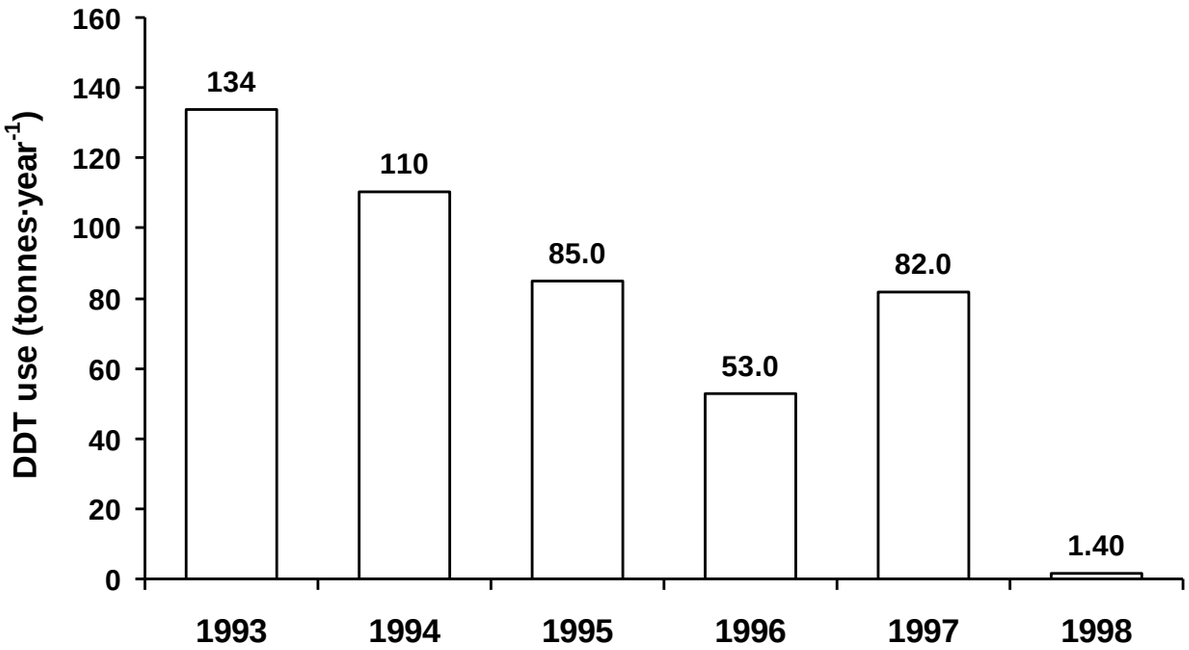

Figure C-2 Annual use of DDT in mainland Ecuador (tonnes/year) to combat the malaria vector (*Anopheles*) from 1993 to 1998.

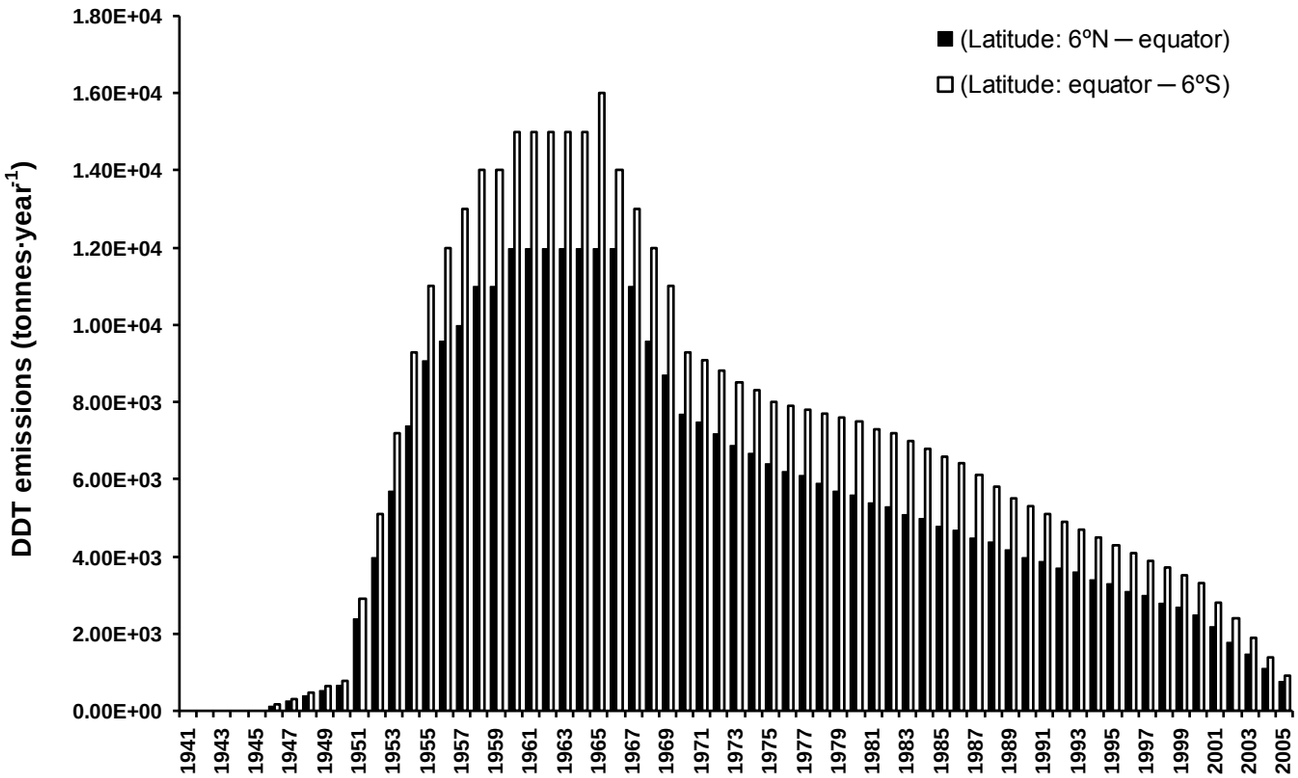

Figure C-3 Yearly DDT emissions in tonnes per year in the equatorial region between 6ºN and 6ºS. Adapted from Schenker *et al*. (2008).

Appendix D:

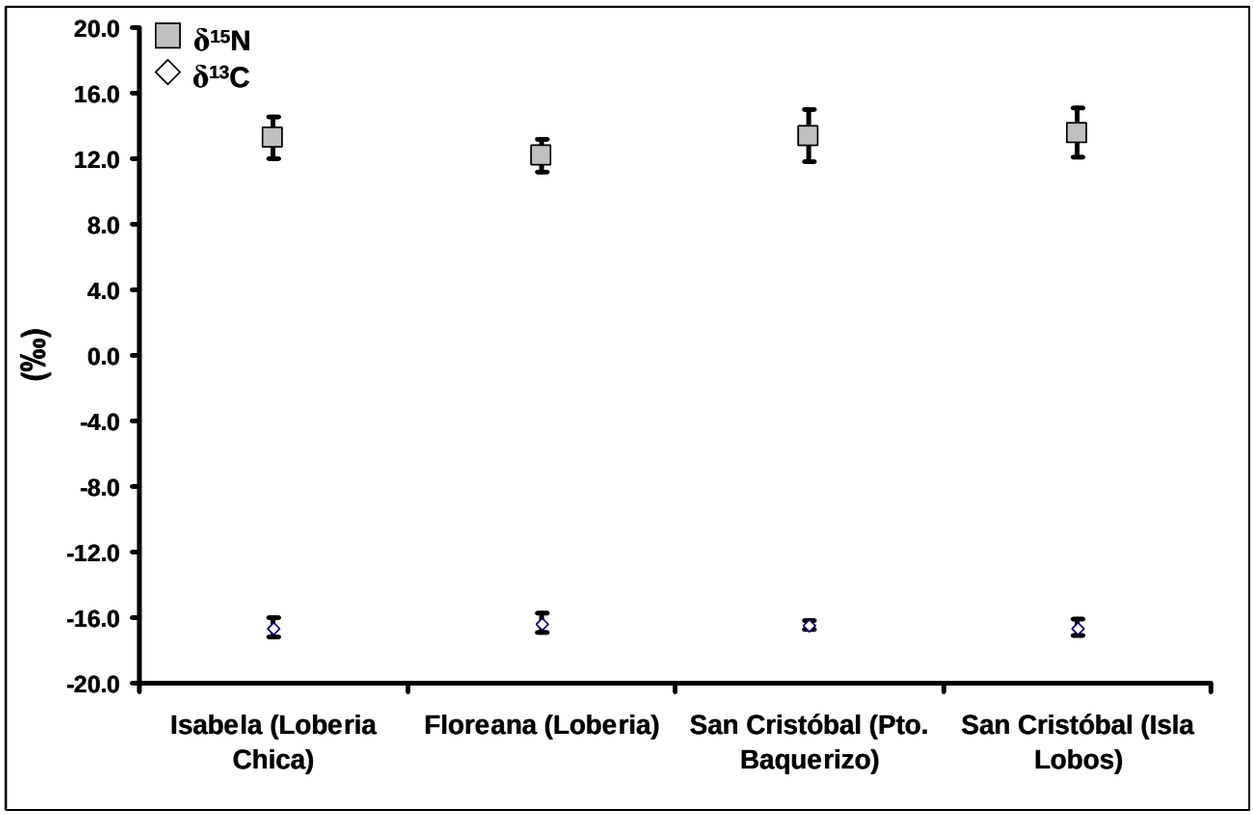

Figure D-1 Stable isotope values by sampling sites. Error bars are 95% confidence intervals. No significant differences were found among sites ($p > 0.05$).

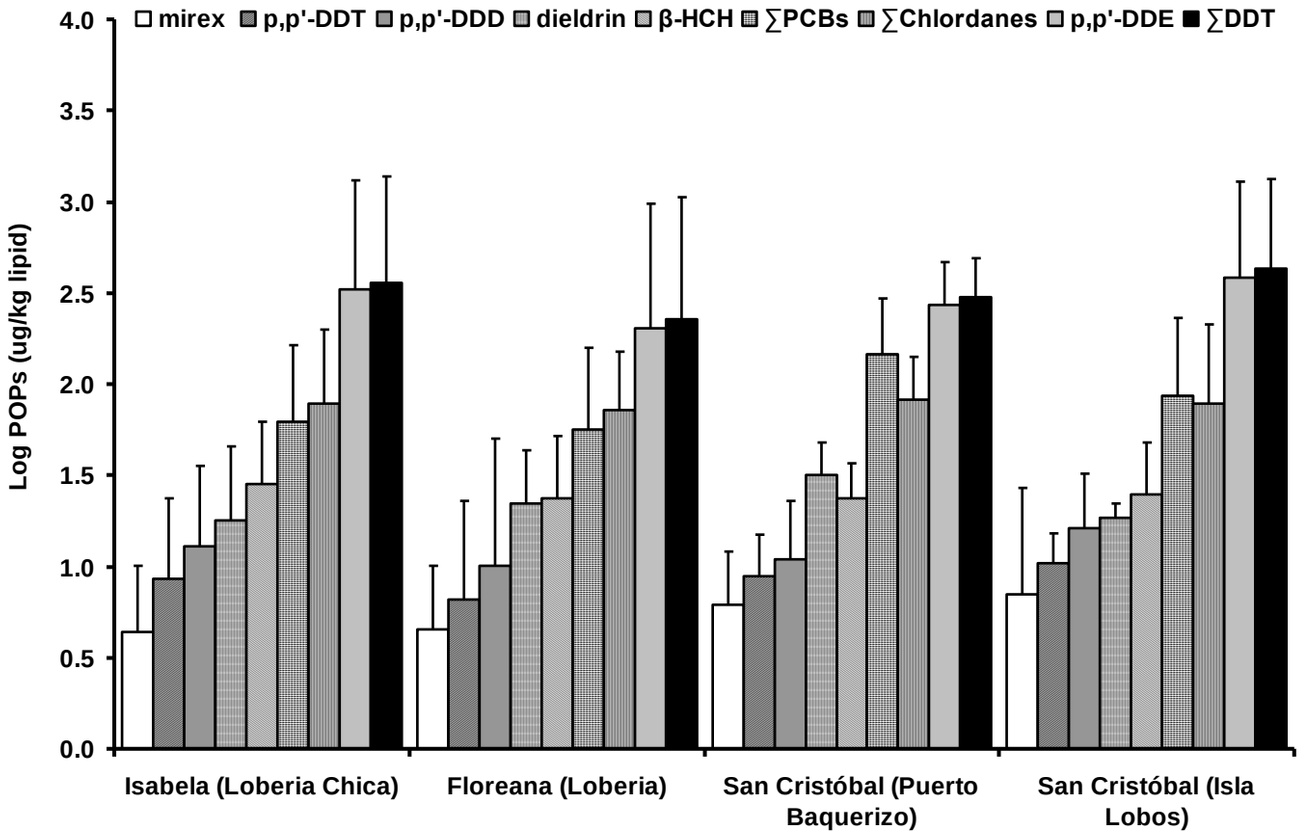

Figure D-2 Inter-site comparisons and relative patterns of POPs of rookeries sampled in 2008. Error bars are standard deviation. No significant differences were found among sites ($p > 0.05$).

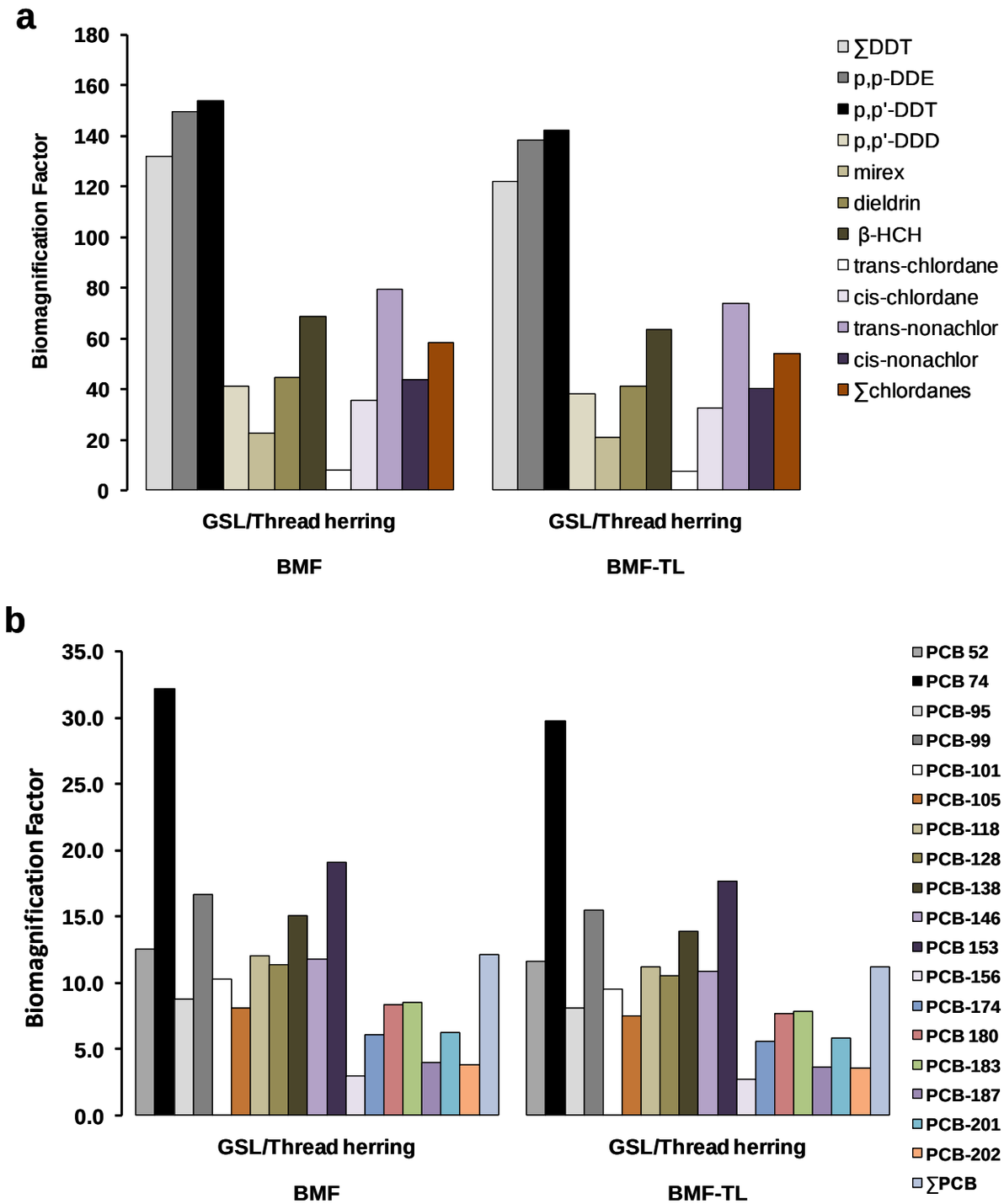

Figure D-3 Comparisons of BMF approaches to calculate the biomagnification of organochlorine pesticides (a) and PCBs (b) in the Galapagos sea lion−thread herring relationship

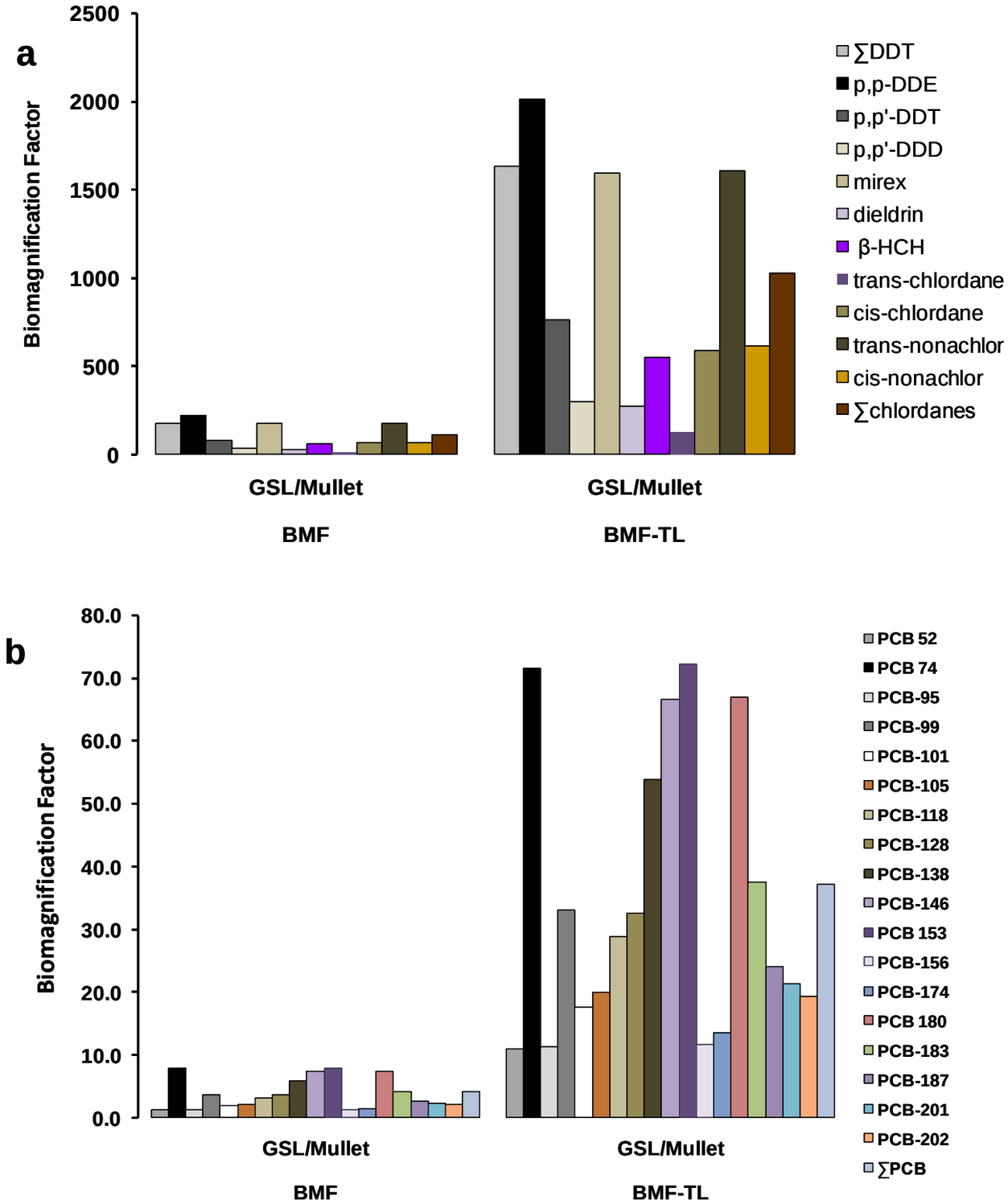

Figure D-4 Comparisons of BMF approaches to calculate the biomagnification of organochlorine pesticides (a) and PCBs (b) in the Galapagos sea lion−mullet relationship.

Appendix E:

Table E-1. Regression statistics for the relationships between the ratios of individual PCB congeners relative to PCB 153 versus length in male Steller sea lions. PCB congeners were classified according to the structure-activity groups (SAGs)* with regards to biotransformation.

	slope	r^2	*p* value		slope	r^2	*p* value
PCBs Group I/ PCB 153				**PCBs Group III/ PCB 153**			
PCB 111	-7.0E-07	0.021	0.6706	PCB 20/28	-2.3E-06	7E-04	0.9375
PCB 133	8.0E-08	1E-06	0.9972	PCB 60	-3.1E-05	0.046	0.5274
PCB 146	2.7E-04	0.102	0.3379	PCB 61/70/74/76	-1.5E-04	0.088	0.3752
PCB 162	-1.0E-06	0.020	0.6791	PCB 66	-1.5E-04	0.096	0.3542
PCB 165	1.0E-06	0.025	0.6385	PCB 105	-5.5E-05	0.008	0.7938
PCB 167	-1.5E-05	0.040	0.5578	PCB 114	-2.4E-05	0.228	0.1375
PCB 172	-5.3E-06	0.002	0.9096	PCB 118	-1.9E-04	0.012	0.7513
PCB 175	-6.5E-06	0.028	0.6219	PCB 123	-1.9E-05	0.241	0.1251
PCB 178	-3.7E-05	0.044	0.5351	PCB 156/157	5.3E-06	0.001	0.9350
PCB 180/193	-2.7E-04	0.022	0.6609				
PCB 187	2.4E-04	0.019	0.6888	**PCBs Group IV/ PCB 153**			
PCB 189	4.1E-07	0.003	0.8726	PCB 49/69	-2.2E-05	0.045	0.5304
PCB 191	4.3E-06	0.033	0.5906	PCB 52	-1.9E-04	0.069	0.4362
PCB 194	-2.8E-05	0.008	0.7992	PCB 59/62/75	-2.3E-05	0.215	0.1506
PCB 196	5.9E-05	0.114	0.3103	PCB 64	-7.8E-06	0.030	0.6114
PCB 198/199	-1.9E-05	0.002	0.8909	PCB 86/87/97/108/119/125	5.9E-05	0.036	0.5768
PCB 201	-2.2E-06	0.001	0.9199	PCB 90/101/113	3.1E-04	0.054	0.4882
PCB 202	-5.2E-05	0.143	0.2524	PCB 92	-1.4E-04	0.090	0.3711
PCB 203	9.3E-05	0.107	0.3271	PCB 109	1.7E-05	0.125	0.2861
PCB 204	5.3E-07	0.065	0.4490	PCB 110/115	1.3E-04	0.060	0.4700
PCB 205	4.9E-06	0.169	0.2094				
PCB 206	4.7E-05	0.123	0.2899	**PCBs Group V/ PCB 153**			
PCB 207	5.9E-06	0.042	0.5433	PCB 93/95/98/100/102	-1.4E-04	0.034	0.5866
PCB 208	-3.9E-07	5E-05	0.9839	PCB 135/151/154	-2.2E-04	0.055	0.4882
PCB 209	2.3E-05	0.113	0.3126	PCB 136	-2.5E-05	0.041	0.5512
				PCB 147/149	3.4E-04	0.064	0.4530
PCBs Group II/ PCB 153				PCB 174	-4.7E-05	0.035	0.5845
PCB 44/47/65	-2.0E-04	0.289	0.0883	PCB 176	-1.7E-05	0.095	0.3551
PCB 83/99	-3.5E-04	0.048	0.5188	PCB 179	-4.4E-05	0.058	0.4772
PCB 85/116/117	-9.2E-05	0.061	0.4619	PCB 197/200	1.9E-06	0.003	0.8754
PCB 128/166	-2.0E-04	0.230	0.1356				
PCB 129/138/160/163	5.4E-04	0.144	0.2489				

PCBs Group II/ PCB 153	slope	r^2	*p* value
PCB 130	8.7E-05	0.352	0.0545
PCB 137	1.2E-04	0.573	**0.0070**
PCB 158	7.2E-05	0.205	0.1616
PCB 170	-5.0E-05	0.009	0.7816
PCB 177	1.1E-04	0.100	0.3444
PCB 190	-3.8E-06	0.002	0.8918
PCB 195	6.9E-06	0.005	0.8375

***Structure-activity groups (SAGs)**: Group I includes congeners without any vicinal hydrogen (H)-atoms and ≥2 *ortho*-substituted chlorine atoms; Group II, congeners have vicinal H-atoms exclusively in the *ortho*- and *meta*-positions in combination with ≥ 2 *ortho*-chlorine substituents; Group III, congeners with vicinal H-atoms in the *ortho*- and *meta*-positions in combination with ≤ 1 *ortho*-substituted chlorine atoms; Group IV, congeners with vicinal H-atoms in the *meta*- and *para*-positions in combination with ≤ 2 *ortho*- substituted chlorine atoms; and Group V, congeners with vicinal H-atoms in the *meta*- and *para*-positions in combination with ≥3 *ortho*- chlorine substituents.

Table E-2 Overview of ∑PBDE concentrations in different pinnipeds expressed as mean ± (SE) or range of means or geometric means in mg/kg wet weight.

Collection year	Collection region	Species	age category	Sex	*n*	PBDEs	Reference
1972-1998	Sanriku, Pacific coast of Northern Japan.	*Callorhinus ursinus*	adult	females	35	0.02 x10^{-2} −0.04[a]	Kajiwara *et al*. (2004)
1989-1998	San Francisco Bay, CA, USA	*Phoca vitulina*	adults (stranded)	mixed	34	0.76 ±0.20[b]	She *et al*. (2002)
1981-2000	Canadian Arctic	*Pusa hispida*	pup, juvenile, subadult and adult	mixed	50	< 0.01 − <0.05[c]	Ikonomou *et al*. (2002)
1998-2000	Farne Islands, east coast of United	*Halichoerus gryphus*	Pup and juveniles	NR	110	0.24[d]	Kalantzi *et al*. (2005)
1993-2003	Coastal California, USA	*Zalophus californianus*	Subadult and adult males	males	25	1.65 ± 0.90[e]	Stapleton *et al*. (2006)
2003	United Kingdom	*Phoca vitulina*	Subadult and adult	mixed	60	0.08-0.52[f]	Hall and Thomas (2007)
2003	British Columbia, Canada	*Phoca vitulina*	pup	mixed	16	0.43 ± 0.05[g]	Noel *et al*. (2008)
2003	Washington State, USA	*P. vitulina*	pup	mixed	16	0.58 ± 0.09[g]	Noel *et al*. (2008
2005	Galapagos Islands, Ecuador	*Zalophus wolleabaeki*	pup	mixed	1	0.025[h]	Alava *et al*. (2010)
2005-2006	Southern Vancouver Island-British Columbia, Canada	*Eumetopias jubatus*	pup, juvenile, subadult and adult	Mixed	14	0.20 ± 0.05[g]	Present study

[a]range indicates the lower and higher means of ∑PBDE concentrations observed in blubber/fat samples of female Northern fur seals (*C. ursinus*) collected between 1972 and 1998. Wet weight concentrations presented here were calculated by multiplying these means in lipid weight by their respective mean lipid fraction Kajiwara *et al*. (2004).
[b] The arithmetic mean concentration in wet mass was calculated from the actual lipid normalized concentrations of ∑PBDEs and lipid fractions in harbour seal blubber samples available in Table 2 in She *et al*. (2002).
[c]range of arithmetic mean ∑PBDE levels (lipid normalized concentrations).
[d]geometric mean in wet weight (the original geometric mean in lipid weight was multiplied by the mean lipid fraction, 83%, as reported by Kalantzi *et al*. (2005)).
[e] The arithmetic mean concentration in wet weight was calculated from the PBDE concentration reported by Stapleton *et al*. (2006); [f]range of lipid normalized geometric means calculated from the lower and higher geometric means in lipid weight observed in blubber biopsy samples of five harbour seal populations (wet weight concentrations were calculated by multiplying these geometric means in lipid weight by their respective mean lipid fraction in Hall and Thomas (2007); [g]arithmetic mean; [h]total concentration in one individual, including only PBDE -47, -49, -66 and -183

Table E-3 Overview of ∑PCB concentrations (mg/kg wet weight) in Steller sea lions in the North Pacific Ocean.

Collection year	Collection region	Age category	Sex	*n*	PCBs	Reference
1976-1981	Gulf of Alaska	Juvenile to adult	Female Males	12 17	12.6 ± 1.90 4.30 ± 4.40	Lee *et al*. (1996)
1985-1990	Prince Williams Sound, Bering Sea	Juvenile to adult	Mixed	8	23.0 ± 37.0	Varanasi *et al*. (1992)
1992-1994	Southeastern Alaska	Juvenile	Mixed	3	6.60 ± 0.50	Krahn *et al*. (1997)
1994-1998	Bering Sea	Juvenile	Mixed	13	2.00 ± 1.20	Krahn and Smolen (unpublished)
1998-2000	Prince Williams Sound, Southeastern Alaska	Juvenile	Mixed	19 10	1.40 ± 0.70 1.60 ± 0.60	Krahn *et al*. (2001)
1997-2004	Western North Pacific (Hokkaido Islands-Japan; Kuril Islands/Olyutorsky Bay-Russia)	Pup-Juvenile-Adult	Mixed	46	1.10 ± 0.80	Hoshino *et al*. (2006)
2005-2006	Southern Vancouver Island, British Columbia, Canada	Pup-Juvenile-Subadult-Adult	Mixed	14	0.70 ± 0.90	Present study

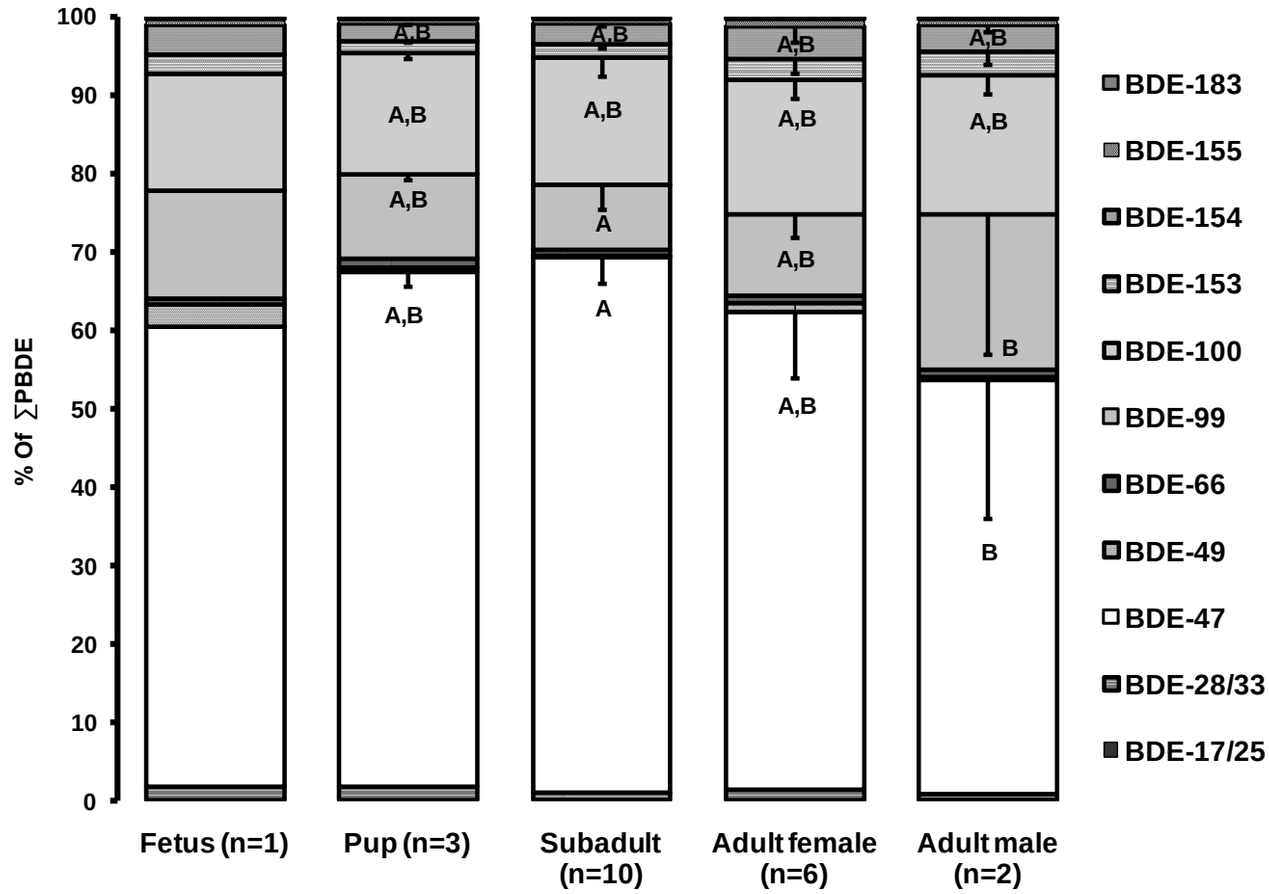

Figure E-1 Pattern of ∑PBDEs showing congeners detected in the blubber of Steller sea lions. Bars no connected by the same letters are significant different. Error bars are standard deviations.

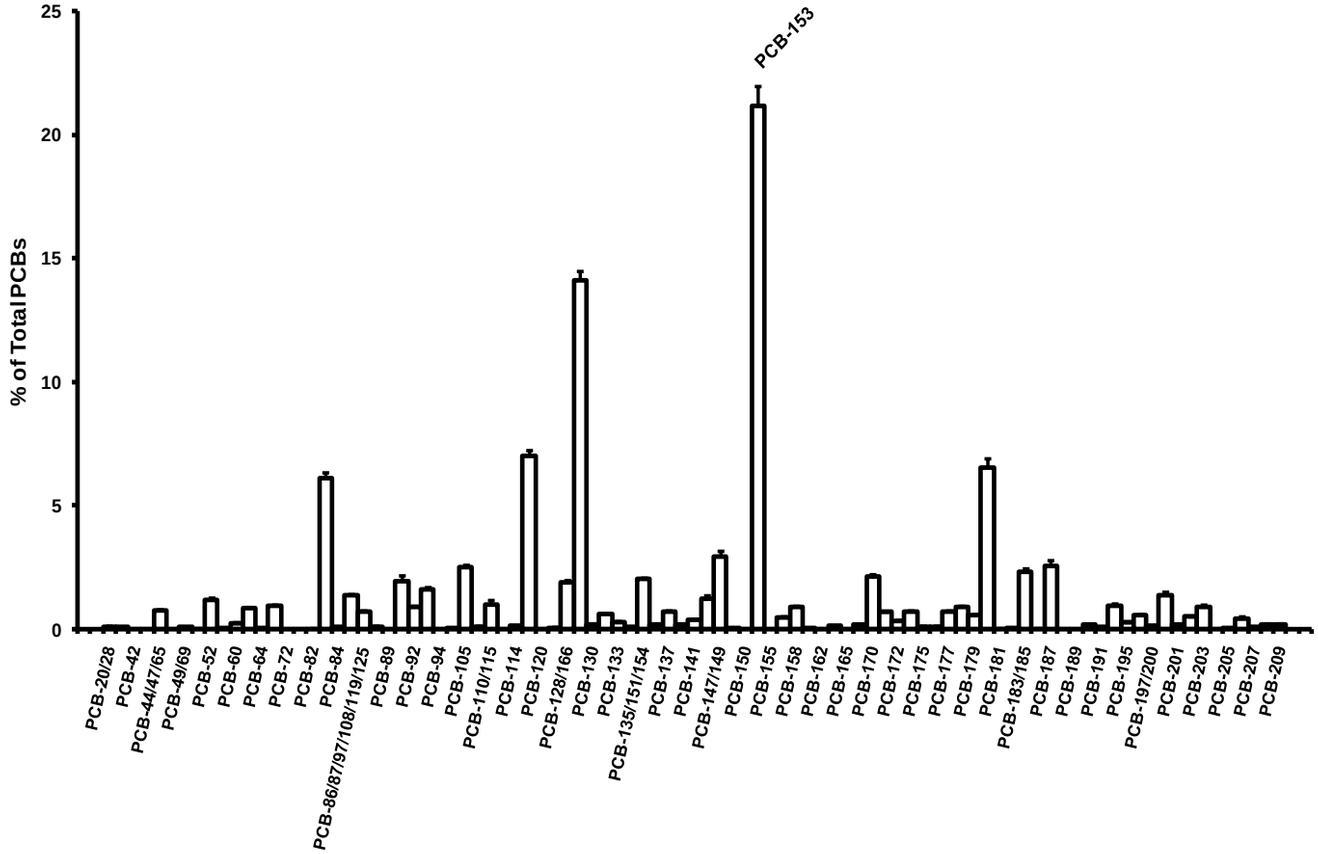

Figure E-2 Profile of the ∑PCB pattern in blubber samples of Steller sea lions from the Strait of Georgia. This pattern is typical of marine mammal inhabiting industrialized regions.

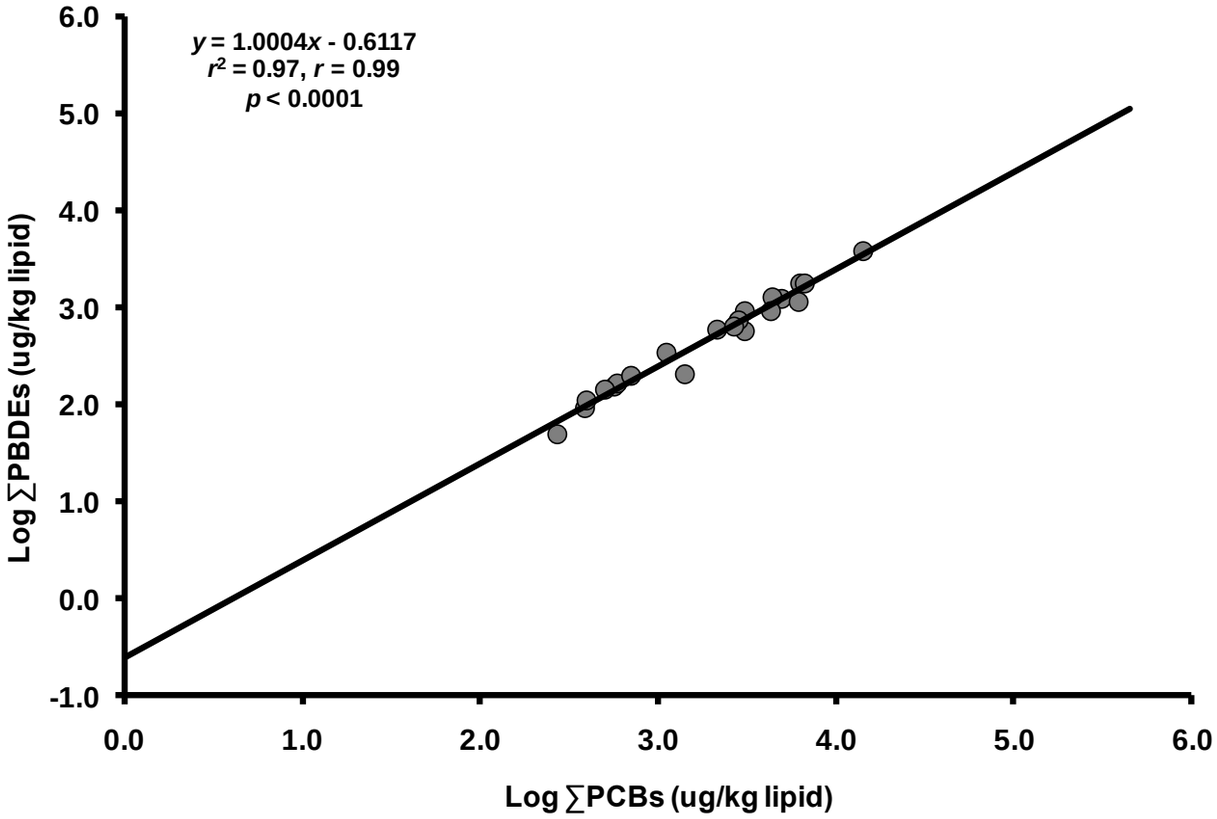

Figure E-3 Linear regression model between log transformed concentrations of ∑PBDEs and ∑PCBs.

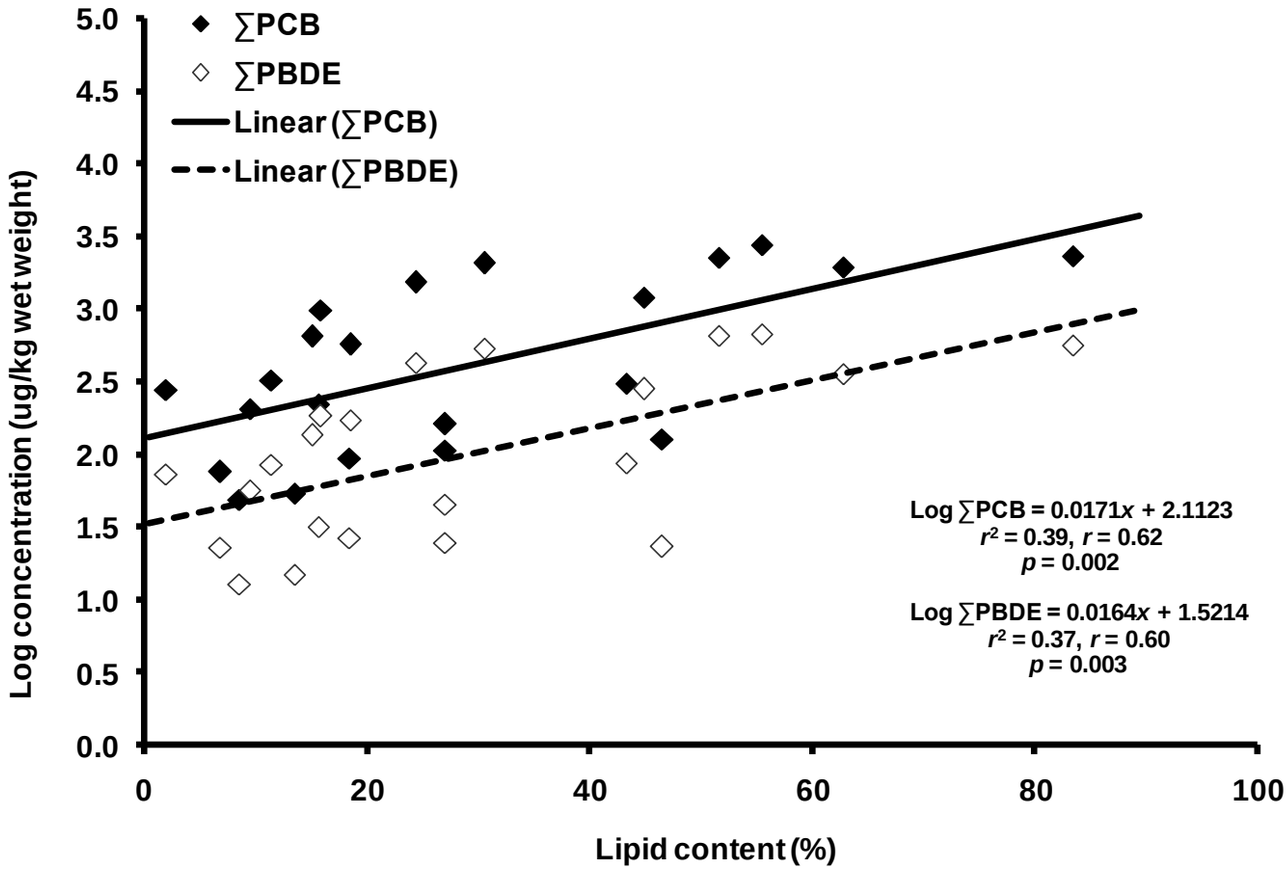

Figure E-4 Logarithms of the ∑PCB and ∑PBDE concentrations (wet weight) in blubber regressed against lipid content in each biopsy sampled in Steller sea lions.

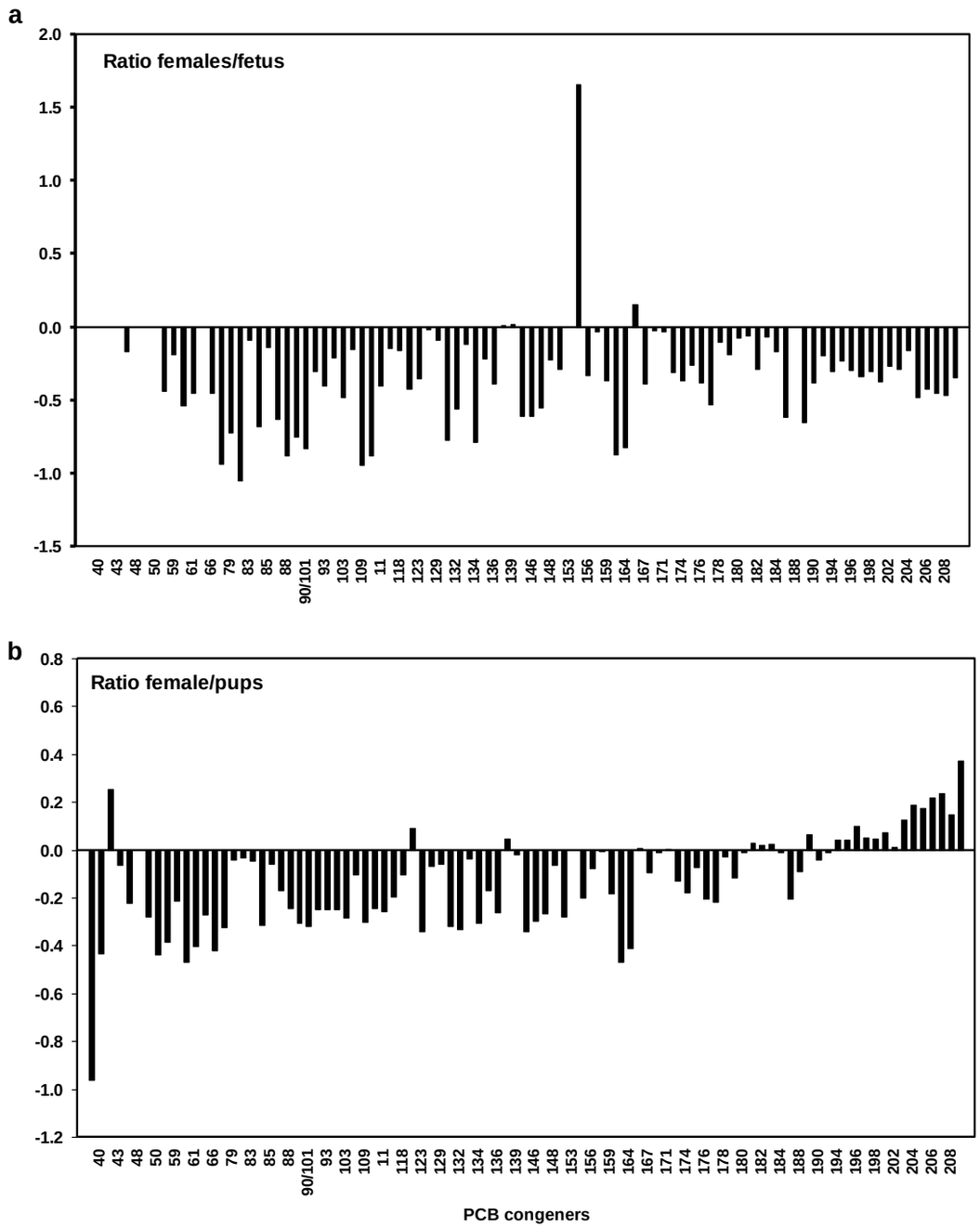

Figure E-5 Ratio of polychlorinated biphenyl (PCB) patterns in adult females to fetus and in adult females to pup in the Steller sea lion: a) The PCB congeners' composition in the fetus showed that basically most PCB congener are readily transferred from adult females (mothers), from which the lower-chlorinated PCBs make up a substantial contribution; and, b) The PCB congeners' pattern in pups is dominated by the lower-chlorinated (lighter) PCBs, while the PCB composition in the adult females is dominated by the higher-chlorinated (heavier) PCBs. We log-normalized the PCB congener data from both adults females/fetus and adult females/pups to PCB-153 to eliminate concentration differences from congener patterns: Log ([adult females (PCB congener)/(PCB-153)]/[fetus (PCB congener)/(PCB-153)]); and, Log ([adult females (PCB congener)/(PCB-153)]/[pups (PCB congener)/(PCB-153)]).

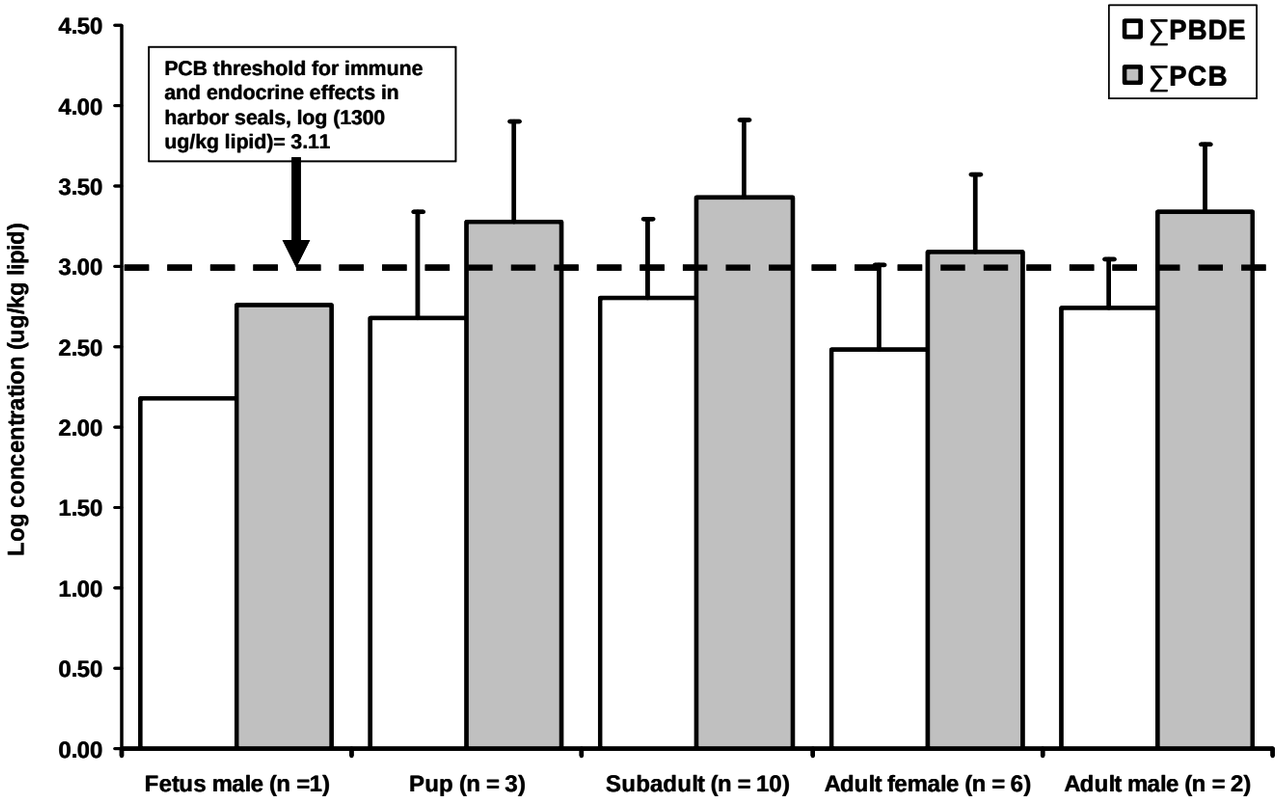

Figure E-6 ∑PCBs and ∑PBDEs concentrations in blubber samples collected from different age and sex categories of Steller sea lions (*Eumetopias jubatus*) from the Strait of Georgia. The concentration detected in the pup-juvenile, subadult males, adult female and males were higher than the 95% LOAEL threshold (dashed line) for endocrine disruption and immunotoxicity of PCBs (1300 µg/kg lipid) reported for harbour seals from the Strait of Georgia by Mos *et al*. (2010).

Appendix F:

Table F-1a PCB congeners and properties' values used in the food web bioaccumulation model for northern resident killer whale Critical Habitat, Queen Charlotte Strait, and outer coast areas.

Chemical Name	Congener CAS #	Molecular Weight (g/mol)	LeBas Molar Volume (cm^3/mol)	log K_{OW} (unitless)	log K_{OW} Temp corrected (37.5 °C) (unitless)	log K_{OA} Temp corrected (37.5 °C) (unitless)
PCB	8	223.1	226.4	5.42	4.96	6.83
PCB	18	257.5	247.4	5.62	5.12	6.82
PCB	28	257.5	247.4	5.99	5.47	7.29
PCB	31	257.5	247.4	6.11	5.60	7.39
PCB	33	257.5	247.4	5.98	5.47	7.40
PCB	44	292.0	268.4	6.16	5.63	7.96
PCB	49	292.0	268.4	6.30	5.76	7.61
PCB	52	292.0	268.4	6.26	5.72	7.64
PCB	56	292.0	268.4	6.39	5.80	8.16
PCB	60	292.0	268.4	6.49	5.91	8.55
PCB	66	292.0	268.4	6.36	5.81	8.58
PCB	70	292.0	268.4	6.46	5.90	8.25
PCB	74	292.0	268.4	6.46	5.91	8.41
PCB	87	326.5	289.4	6.72	6.15	8.51
PCB	95	326.5	289.4	6.43	5.86	8.28
PCB	99	326.5	289.4	6.73	6.16	8.58
PCB	101	326.5	289.4	6.68	6.16	8.25
PCB	105	326.5	289.4	7.20	6.62	8.90
PCB	110	326.5	289.4	6.68	6.11	8.48
PCB	118	326.5	289.4	6.97	6.39	8.74
PCB	128	361.0	310.4	7.18	6.59	9.16
PCB	132	361.0	310.4	6.90	6.36	8.94
PCB	138	361.0	310.4	7.59	7.04	9.05
PCB	141	361.0	310.4	7.13	6.59	9.22
PCB	149	361.0	310.4	6.99	6.44	8.94
PCB	151	361.0	310.4	6.96	6.42	8.99
PCB	153	360.88	310.4	7.28	6.65	8.78
PCB	156	361.0	310.4	7.37	6.85	9.74
PCB	158	361.0	310.4	7.23	6.71	9.43
PCB	170	395.5	331.4	7.56	7.00	9.89
PCB	174	395.5	331.4	7.43	6.83	9.62
PCB	177	395.5	331.4	7.41	6.81	9.73
PCB	180	395.5	331.4	7.57	6.95	9.51
PCB	183	395.5	331.4	7.52	6.92	9.88
PCB	187	395.5	331.4	7.49	6.89	9.71
PCB	194	429.77	352.4	8.18	7.56	10.46
PCB	195	430.0	352.4	7.87	7.25	10.45
PCB	201	430.0	352.4	7.92	7.31	10.26

Table F-1b PCB congeners and properties' values used in the food web bioaccumulation model for Strait of Georgia, southern resident killer whale Critical Habitat in Canada, southern resident killer whale Critical Habitat in USA (Puget Sound), and southern resident killer whale Critical Habitat in USA (summer core and Juan de Fuca Strait) areas.

Chemical Name	Congener CAS #	Molecular Weight (g/mol)	LeBas Molar Volume (cm^3/mol)	log K_{OW} (unitless)	log K_{OW} Temp corrected (37.5 °C) (unitless)	log K_{OA} Temp corrected (37.5 °C) (unitless)
PCB	8	223.1	226.4	5.42	4.96	6.83
PCB	18	257.5	247.4	5.62	5.12	6.82
PCB	28	257.5	247.4	5.99	5.47	7.29
PCB	44	292.0	268.4	6.16	5.63	7.96
PCB	49	292.0	268.4	6.30	5.76	7.61
PCB	52	292.0	268.4	6.26	5.72	7.64
PCB	66	292.0	268.4	6.36	5.81	8.58
PCB	74	292.0	268.4	6.46	5.91	8.41
PCB	95	326.5	289.4	6.43	5.86	8.28
PCB	99	326.5	289.4	6.73	6.16	8.58
PCB	101	326.5	289.4	6.68	6.16	8.25
PCB	105	326.5	289.4	7.20	6.62	8.90
PCB	110	326.5	289.4	6.68	6.11	8.48
PCB	118	326.5	289.4	6.97	6.39	8.74
PCB	128	361.0	310.4	7.18	6.59	9.16
PCB	138	361.0	310.4	7.59	7.04	9.05
PCB	149	361.0	310.4	6.99	6.44	8.94
PCB	151	361.0	310.4	6.96	6.42	8.99
PCB	153	360.9	310.4	7.28	6.65	8.78
PCB	156	361.0	310.4	7.37	6.85	9.74
PCB	170	395.5	331.4	7.56	7.00	9.89
PCB	177	395.5	331.4	7.41	6.81	9.73
PCB	180	395.5	331.4	7.57	6.95	9.51
PCB	183	395.5	331.4	7.52	6.92	9.88
PCB	187	395.5	331.4	7.49	6.89	9.71
PCB	194	429.8	352.4	8.18	7.56	10.5
PCB	203	430.0	352.4	7.95	7.33	10.4

Table F-2a Environmental input parameters for northern resident killer whale Critical Habitat used in the bioaccumulation food web model.

Parameters	Input		Variability	Units	References
Mean Water Temperature	8.67	±	0.5	°C	1
Mean Air Temperature	9	±	0	°C	2
Mean Homeothermic Biota Temperature	37.5	±	1	°C	3
Mean Water Temperature	281.82	±	1.35	K	
Mean Air Temperature	282.15	±	6	K	
Mean Homeothermic Biota Temperature	310.65	±		K	
pH of Water	7.7	±	0.071	Unitless	4
Practical Salinity Units (PSU)	30.38	±	1.34	g/kg	2
Dissolved Oxygen Concentration @ 90% Saturation (DO)	5	±	0	mg O_2/L	5
Dissolved Organic Carbon Content - Water (OCwater)	7.26E-07	±	1.27E-07	kg/L	6
Particulate Organic Carbon Content - Water (POC)	1.56E-07	±	5.09E-08	kg/L	6
Concentration of Suspended Solids (Vss)	8.83E-07	±	1.80E-07	kg/L	6
Percentage of Organic Carbon - Sediment (OCsed)	4.27	±	0.021	%	7, 8
Density of Organic Carbon - Sediment (Docsed)	0.9	±		kg/L	9
Setschenow Proportionality Constant (SPC)	0.0018	±		L/cm^3	10
Molar Concentration of Seawater @ 35 ppt (MCS)	0.5	±		mol/L	10
Absolute Temperature (K)	273.16	±		K	
Ideal Gas Law Constant (Rgaslaw)	8.314	±		$Pa/m^3/mol \cdot K$	

Table References:

1. http://www-sci.pac.dfo-mpo.gc.ca/osap/data/lighthouse/pinet.txt
2. Masson (2006)
3. Gobas and Arnot (2010)
4. Sophie Johannessen (Fisheries & Oceans Canada, Institute of Ocean Sciences, PO Box 6000, Sidney, BC V8L 4B2, pers. comm., 2010)
5. Foreman *et al.,* (2006)
6. Johannessen *et al*., (2008)
7. Burd *et al.,* (2008b)
8. Johannessen *et al.,* (2003)
9. Mackay (1991)
10. Xie *et al.,* (1997)

Table F-2b Environmental input parameters for Queen Charlotte Strait used in the bioaccumulation food web model.

Parameters	Input		Variability	Units	References
Mean Water Temperature	9.4	±	3.11	°C	1
Mean Air Temperature	10.8	±	1.1	°C	2
Mean Homeothermic Biota Temperature	37.5	±	1	°C	3
Mean Water Temperature	282.55	±	1.35	K	
Mean Air Temperature	283.95	±	6	K	
Mean Homeothermic Biota Temperature	310.65	±		K	
pH of Water	7.7	±	0.14	Unitless	4
Practical Salinity Units (PSU)	32.9	±	1.41	g/kg	1
Dissolved Oxygen Concentration @ 90% Saturation (DO)	6.5	±	0.87	mg O_2/L	5
Dissolved Organic Carbon Content - Water (OCwater)	2.6E-07	±	0	kg/L	1
Particulate Organic Carbon Content - Water (POC)	6.5E-08	±	2.12E-08	kg/L	1
Concentration of Suspended Solids (Vss)	2.17E-06	±	0	kg/L	Estimated as POC/3%
Percentage of Organic Carbon - Sediment (OCsed)	3.0	±	0	%	6
Density of Organic Carbon - Sediment (Docsed)	0.9	±		kg/L	7
Setschenow Proportionality Constant (SPC)	0.0018	±		L/cm^3	8
Molar Concentration of Seawater @ 35 ppt (MCS)	0.5	±		mol/L	8
Absolute Temperature (K)	273.16	±		K	
Ideal Gas Law Constant (Rgaslaw)	8.314	±		$Pa/m^3/mol \cdot K$	

Table References:

1. Peña *et al.,* (1999) (estimated from POC reported)
2. Environment Canada (www.climate.weatheroffice.ec.gc.ca)
3. Gobas and Arnot (2010)
4. Sophie Johannessen (Fisheries & Oceans Canada, Institute of Ocean Sciences, PO Box 6000, Sidney, BC V8L 4B2, pers. comm., 2010)
5. Tortell *et al.,* (2005)
6. Conway *et al.,* (2005)
7. Mackay (1991)
8. Xie *et al.,* (1997)

Table F-2c Environmental input parameters for the outer coast area used in the bioaccumulation food web model.

Parameters	Input		Variability	Units	References
Mean Water Temperature	9.4	±	3.11	°C	1
Mean Air Temperature	10.8	±	1.1	°C	2
Mean Homeothermic Biota Temperature	37.5	±	1	°C	3
Mean Water Temperature	282.55	±	1.35	K	
Mean Air Temperature	283.95	±	6	K	
Mean Homeothermic Biota Temperature	310.65	±		K	
pH of Water	7.7	±	0.14	Unitless	4
Practical Salinity Units (PSU)	32.9	±	1.41	g/kg	1
Dissolved Oxygen Concentration @ 90% Saturation (DO)	6.5	±	0.87	mg O_2/L	5
Dissolved Organic Carbon Content - Water (OCwater)	2.6E-07	±	0	kg/L	1
Particulate Organic Carbon Content - Water (POC)	6.5E-08	±	2.12E-08	kg/L	1
Concentration of Suspended Solids (Vss)	2.17E-06	±	0	kg/L	Estimated as POC/3%
Percentage of Organic Carbon - Sediment (OCsed)	3.0	±	0	%	6
Density of Organic Carbon - Sediment (Docsed)	0.9	±		kg/L	7
Setschenow Proportionality Constant (SPC)	0.0018	±		L/cm^3	8
Molar Concentration of Seawater @ 35 ppt (MCS)	0.5	±		mol/L	8
Absolute Temperature (K)	273.16	±		K	
Ideal Gas Law Constant (Rgaslaw)	8.314	±		$Pa/m^3/mol \cdot K$	

Table References:

1. Peña *et al.* (1999) (estimated from POC reported)
2. Environment Canada (www.climate.weatheroffice.ec.gc.ca)
3. Gobas and Arnot (2010)
4. Sophie Johannessen (Fisheries & Oceans Canada, Institute of Ocean Sciences, PO Box 6000, Sidney, BC V8L 4B2, pers. comm., 2010)
5. Tortell *et al.,* (2005)
6. Conway *et al.,* (2005)
7. Mackay (1991)
8. Xie *et al.,* (1997)

Table F-2d Environmental input parameters for the Strait of Georgia used in the bioaccumulation food web model.

Parameters	Input		Variability	Units	References
Mean Water Temperature	9.07	±	2.14	°C	1
Mean Air Temperature	9.25	±	8.13	°C	2
Mean Homeothermic Biota Temperature	37.5	±	1	°C	3
Mean Water Temperature	282.22	±	1.35	K	
Mean Air Temperature	282.4	±	6	K	
Mean Homeothermic Biota Temperature	310.65	±		K	
pH of Water	7.7	±	0.141	Unitless	4
Practical Salinity Units (PSU)	30.4	±	3.03	g/kg	1
Dissolved Oxygen Concentration @ 90% Saturation (DO)	4.11	±	2.03	mg O_2/L	1
Dissolved Organic Carbon Content - Water (OCwater)	6.36E-07	±	1.19E-07	kg/L	5
Particulate Organic Carbon Content - Water (POC)	9.2E-08	±	4.96E-08	kg/L	5
Concentration of Suspended Solids (Vss)	2.62E-06	±	1.21E-06	kg/L	6
Percentage of Organic Carbon - Sediment (OCsed)	1.50	±	0.0071	%	7, 8
Density of Organic Carbon - Sediment (Docsed)	0.9	±		kg/L	9
Setschenow Proportionality Constant (SPC)	0.0018	±		L/cm^3	10
Molar Concentration of Seawater @ 35 ppt (MCS)	0.5	±		mol/L	10
Absolute Temperature (K)	273.16	±		K	
Ideal Gas Law Constant (Rgaslaw)	8.314	±		$Pa/m^3/mol \cdot K$	

Table References:

1. Masson (2006)
2. Environment Canada (2009)
3. Gobas and Arnot (2010)
4. Sophie Johannessen (Fisheries & Oceans Canada, Institute of Ocean Sciences, PO Box 6000, Sidney, BC V8L 4B2, pers. comm., 2010)
5. Johannessen *et al.,* (2008)
6. Komick *et al.,* (2009)
7. Burd *et al.* (2008)
8. Johannessen *et al.,* (2003)
9. Mackay (1991)
10. Xie *et al.,* (1997)

Table F-2e Environmental input parameters for the southern resident killer whale Critical Habitat in Canada used in the bioaccumulation food web model.

Parameters	Input		Variability	Units	References
Mean Water Temperature	9.07	±	2.14	°C	1
Mean Air Temperature	9.25	±	8.13	°C	2
Mean Homeothermic Biota Temperature	37.5	±	1	°C	3
Mean Water Temperature	282.22	±	1.35	K	
Mean Air Temperature	282.4	±	6	K	
Mean Homeothermic Biota Temperature	310.65	±		K	
pH of Water	7.7	±	0.141	Unitless	4
Practical Salinity Units (PSU)	30.4	±	3.03	g/kg	1
Dissolved Oxygen Concentration @ 90% Saturation (DO)	4.11	±	2.03	mg O_2/L	1
Dissolved Organic Carbon Content - Water (OCwater)	6.36E-07	±	1.19E-07	kg/L	5
Particulate Organic Carbon Content - Water (POC)	9.2E-08	±	4.96E-08	kg/L	5
Concentration of Suspended Solids (Vss)	2.62E-06	±	1.21E-06	kg/L	6
Percentage of Organic Carbon - Sediment (OCsed)	1.50	±	0.0071	%	7, 8
Density of Organic Carbon - Sediment (Docsed)	0.9	±		kg/L	9
Setschenow Proportionality Constant (SPC)	0.0018	±		L/cm^3	10
Molar Concentration of Seawater @ 35 ppt (MCS)	0.5	±		mol/L	10
Absolute Temperature (K)	273.16	±		K	
Ideal Gas Law Constant (Rgaslaw)	8.314	±		$Pa/m^3/mol \cdot K$	

Table References:

1. Masson (2006)
2. Environment Canada (2009)
3. Gobas and Arnot (2010)
4. Sophie Johannessen (Fisheries & Oceans Canada, Institute of Ocean Sciences, PO Box 6000, Sidney, BC V8L 4B2, pers. comm., 2010)
5. Johannessen *et al.,* (2008)
6. Komick *et al.,* (2009)
7. Burd *et al.,* (2008)
8. Johannessen *et al.,* (2003)
9. Mackay (1991)
10. Xie *et al.,* (1997)

Table F-2f Environmental input parameters for the southern resident killer whale Critical Habitat in USA (Puget Sound) used in the bioaccumulation food web model.

Parameters	Input		Variability	Units	References
Mean Water Temperature	10.3	±	0.0	°C	1
Mean Air Temperature	9.5	±	0.0	°C	1
Mean Homeothermic Biota Temperature	37.5	±	1	°C	2
Mean Water Temperature	283.45	±	1.35	K	
Mean Air Temperature	282.65	±	6	K	
Mean Homeothermic Biota Temperature	310.65	±		K	
pH of Water	7.7	±	0.0	Unitless	3
Practical Salinity Units (PSU)	30	±	0.0	g/kg	1
Dissolved Oxygen Concentration @ 90% Saturation (DO)	7.5	±	0.0	mg O_2/L	1
Dissolved Organic Carbon Content - Water (OCwater)	1.00E-06	±	0.0	kg/L	1
Particulate Organic Carbon Content - Water (POC)	0.0	±	0.0	kg/L	1
Concentration of Suspended Solids (Vss)	2.4E-06	±	0.0	kg/L	1
Percentage of Organic Carbon - Sediment (OCsed)	1.74	±	0.0	%	1
Density of Organic Carbon - Sediment (Docsed)	0.9	±		kg/L	4
Setschenow Proportionality Constant (SPC)	0.0018	±		L/cm^3	5
Molar Concentration of Seawater @ 35 ppt (MCS)	0.5	±		mol/L	5
Absolute Temperature (K)	273.16	±		K	
Ideal Gas Law Constant (Rgaslaw)	8.314	±		$Pa/m^3/mol \cdot K$	

Table References:

1. Pelletier and Mohamedali (2009)
2. Gobas and Arnot (2010)
3. Sophie Johannessen (Fisheries & Oceans Canada, Institute of Ocean Sciences, PO Box 6000, Sidney, BC V8L 4B2, pers. comm., 2010)
4. Mackay (1991)
5. Xie *et al.,* (1997)

Table F-2g Environmental input parameters for the southern resident killer whale Critical Habitat in USA (summer core and Juan de Fuca Strait) used in the bioaccumulation food web model.

Parameters	Input		Variability	Units	References
Mean Water Temperature	12.5	±	0	°C	1
Mean Air Temperature	9.5	±	0	°C	2
Mean Homeothermic Biota Temperature	37.5	±	1	°C	3
Mean Water Temperature	285.65	±	1.35	K	
Mean Air Temperature	282.65	±	6	K	
Mean Homeothermic Biota Temperature	310.65	±		K	
pH of Water	7.63	±	0	Unitless	1
Practical Salinity Units (PSU)	31.3	±	0	g/kg	1
Dissolved Oxygen Concentration @ 90% Saturation (DO)	4.5	±	0	mg O_2/L	1
Dissolved Organic Carbon Content - Water (OCwater)	1.00E-06	±	0	kg/L	2
Particulate Organic Carbon Content - Water (POC)	0	±	0	kg/L	2
Concentration of Suspended Solids (Vss)	6.00E-06	±	0	kg/L	1
Percentage of Organic Carbon - Sediment (OCsed)	1.14	±	0	%	2
Density of Organic Carbon - Sediment (Docsed)	0.9	±		kg/L	4
Setschenow Proportionality Constant (SPC)	0.0018	±		L/cm^3	5
Molar Concentration of Seawater @ 35 ppt (MCS)	0.5	±		mol/L	5
Absolute Temperature (K)	273.16	±		K	
Ideal Gas Law Constant (Rgaslaw)	8.314	±		$Pa/m^3/mol \cdot K$	

Table References:

1. Wilson and Partridge (2007)
2. Pelletier and Mohamedali (2009)
3. Gobas and Arnot (2010)
4. Mackay (1991)
5. Xie *et al.,* (1997)

Table F-3 General biological and physiological parameter definitions, values, and references used in the food web bioaccumulation model. E_D = dietary chemical transfer efficiency.

General aquatic species input parameter	Mean		SD	Units	Reference
Density of Lipids	0.9	±		kg/L	1
Non-Lipid Organic Matter Content (NLOM)	20%	±	0.01	%	
NLOM proportionality constant (MAF)	0.05	±	5.0E-03	Unitless	2 (modified from)
Fish Growth Rate Factor (PGR)	1.40E-03	±	7.0E-05	Unitless	3 (modified from)
Invertebrate Growth Rate Factor (IGR)	3.50E-04	±	3.5E-05	Unitless	3
Dietary absorption efficiency of lipid in benth-invertebrate (ε_L)	75%	±	0.02	%	4
Dietary absorption efficiency of NLOM in benth-invertebrate (ε_N)	50%	±	0.02	%	4 (modified from)
Dietary absorption efficiency of lipid in fish (ε_L)	92%	±	0.02	%	4
Dietary absorption efficiency of NLOM in fish (ε_N)	60%	±	0.02	%	4, 5
Dietary absorption efficiency of lipid in mammals (ε_L)	98 or 100%	±	0.02	%	5, 7, 8
Dietary absorption efficiency of NLOM in mammals (ε_N)	75 or 98%	±	0.02	%	1 (modified from)
E_D - Constant A - All feeding species except marine mammals	8.5E-08	±	1.4E-08	Unitless	1
E_D - Constant B - All feeding species except marine mammals	2.00	±	0.600	Unitless	1
E_D - Constant A - Mammals	1E-09	±	1.7E-10	Unitless	1
E_D - Constant B - Mammals	1.025	±	1.2E-03	Unitless	1
E_W - Constant A - Water absorption efficiency in fish & invertebrates	1.85	±	0.13	Unitless	1
Water digestion efficiency in marine mammals (E_W)	85%	±		%	1
Lung uptake efficiency in marine mammals(E_L)	0.7	±		Unitless	1
Mean homoeothermic temperature (marine mammals)	37.5	±	1.00	°C	1
Metabolic Transformation Rate Constant (k_M) - All species	0.00	±		1/day	4
Particle Scavenging Efficiency (PSE)	100%			%	Default value

Table References:

1. Gobas and Arnot (2010)
2. Gobas *et al.,* (1999)
3. Thomann *et al.,* (1992)
4. Arnot and Gobas (2004)
5. Kelly *et al.* , (2004)
6. Drouillard and Norstrom (2000)
7. Trumble *et al.,* (2003)
8. Muelbert *et al.,* (2003)

Table F-4a Feeding Preferences Matrix - dietary composition and trophic levels (TL) of 19 predator species / organisms in the Georgia Basin ecosystem for the resident killer whales food web. Prey species and their corresponding trophic levels are identified.

Coastal-Marine Food Web Species (Predators)	TL	Prey[1] (Diet %)																			Sum
		Det	Phy	Zoo	Pol-1	Pol-2	Mus	Oys	Amp	Mys	DCr	Shri	Sper	Herr	Wpol	Anch	WGr	Pmid	Sfish	Hal	Sal	
Zooplankton (Copepoda, *Neocalanus*)	2.0		100																			100
Polychaete-1 *(Neanthes succinea)*	2.1	90	5	5																		100
Polychaete-2 *(Harmothoe imbricata)*	2.1	30	35	35																		100
Blue mussel (*Mytilus edulis*)	2.3	15	60	25																		100
Oyster (*Crassostrea gigas*)	2.3	15	60	25																		100
Amphipods (*Themisto* sp.)	2.4	30	35	35																		100
Mysis sp.	2.5	10	45	45																		100
Dungeness crab (*Cancer magister*)	2.8	43	2	10	5	5	5	5	5	5		5	5	5								100
Crangon sp.*	2.9		30	30						40												100
Shiner surfperch (*Cymatogaster aggregata*)	3.2	5	10	10	10	10			20	15		20										100
Pacific Herring (*Clupea pallasi*)	3.0			98	1				1													100
Walleye pollock (*Theragra chalcogramma*)	3.0			95	2.5				2.5													100
Northern anchovy (*Engraulis mordax*)	3.1		20	20					15	25		20										100
White Spotted Greenling (*Hexagrammos stelleri*)	3.5			10					45	10	10	10	5	5		5						100
Plainfin midshipman (*Porichthys notatus*)	3.5	5			10	5			15	20	15	20	5			5						100
Sablefish (*Anoplopoma fimbria*)	3.8			10	5				5		5	10	6	3	50	6						100
Halibut (*Hippoglossus stenolepis*)	4.0			1	1	1	1	1	1	1	1	14	10	5	47	10		5	1			100
Chinook salmon (*Oncorhynchus tshawytscha*)	4.0			5					1			4	20	25	25	20						100
Killer whale (*Orcinus orca*)	5.0																		2	2	96	100

[1]Legend prey species: Det = Detritus; Phy = Phytoplankton; Zoo = Zooplankton; Pol-1 = Polychaete-1; Pol-2 = Polychaete-2; Mus = Blue Mussels; Oys = Oyster; Amp = Amphipods; Mys = *Mysis*; DCr = Dungeness crab; Shri =Shrimp (*Crangon*); Sper = Shiner Surfperch; Herr = Pacific Herring; Wpol = Walleye Pollock; Anch = Northern Anchovy; WGr= Whitespotted Greenling; Pmid = Plainfin Midshipman; Sfish = Sablefish; Hal = Halibut; Sal = Chinook Salmon. In the models trophic position values for detritus (TL = 1) and phytoplankton (TL = 1) were assigned according to Vander Zanden and Rasmussen (1996).

Table F-4b Feeding Preferences Matrix - dietary composition and trophic levels (TL) of 19 predator species / organisms in the outer coast area for the resident killer whales food web. Prey species and their corresponding trophic levels are identified.

Offshore-Marine Food Web Species (Predators)	TL	Prey[1] (Diet %)																				Sum
		Det	Phy	Zoo	Pol-1	Pol-2	Mus	Oys	Amp	Mys	DCr	Shri	Sper	Herr	Wpol	Anch	Sqd	Pmid	Sfish	Hal	Sal	
Zooplankton (Copepoda, *Neocalanus*)	2.0		100																			100
Polychaete-1 *(Neanthes succinea)*	2.1	90	5	5																		100
Polychaete-2 *(Harmothoe imbricata)*	2.1	30	35	35																		100
Blue mussel (*Mytilus edulis*)	2.3	15	60	25																		100
Oyster (*Crassostrea gigas*)	2.3	15	60	25																		100
Amphipods (*Themisto* sp.)	2.4	30	35	35																		100
Mysis sp.	2.5	10	45	45																		100
Dungeness crab (*Cancer magister*)	2.8	43	2	10	5	5	5	5	5	5		5	5	5								100
Crangon sp.*	2.9		30	30						40												100
Shiner surfperch (*Cymatogaster aggregata*)	3.2	5	10	10	10	10			20	15		20										100
Pacific Herring (*Clupea pallasi*)	3.0			98	1				1													100
Walleye pollock (*Theragra chalcogramma*)	3.0			95	2.5				2.5													100
Northern anchovy (*Engraulis mordax*)	3.1		20	20					15	25		20										100
Gonatid squid (*Gonatius*)	3.5			50					3	5		5	9	9	9	9						100
Plainfin midshipman (*Porichthys notatus*)	3.5	5			10	5			15	20	15	20	5			5						100
Sablefish (*Anoplopoma fimbria*)	3.8			10	5				5		5	10	6	3	50	6						100
Halibut (*Hippoglossus stenolepis*)	4.0			1	1	1	1	1	1	1	1	14	5	5	43	5	4	5	1			100
Chinook salmon (*Oncorhynchus tshawytscha*)	4.0			5										10		14	71					100
Killer whale (*Orcinus orca*)	5.0																		2	2	96	100

[1]Legend prey species: Det = Detritud; Phy = Phytoplankton; Zoo = Zooplankton; Pol-1 = Polychaete-1; Pol-2 = Polychaete-2; Mus = Blue Mussels; Oys = Oyster; Amp = Amphipods; Mys = *Mysis*; DCr = Dungeness crab; Shri =Shrimp (*Crangon*); Sper = Shiner Surfperch; Herr = Pacific Herring; Wpol = Walleye Pollock; Anch = Northern Anchovy; Sqd= Gonatid squid; Pmid = Plainfin Midshipman; Sfish = Sablefish; Hal = Halibut; Sal = Chinook Salmon. In the models trophic position values for detritus (TL = 1) and phytoplankton (TL = 1) were assigned according to Vander Zanden and Rasmussen (1996).

Table F-4c Feeding Preferences Matrix - dietary composition and trophic levels (TL) of 22 predator species / organisms, incorporating the Steller sea lion food web and updated data on new prey items for resident killer whales, including redistribution of diet composition form some fish species. Prey species and their corresponding trophic levels are identified.

Coastal-Marine Food Web Species (Predators)	TL	Prey[1] (Diet %)																						Sum
		Det	Phy	Zoo	Pol-1	Pol-2	Mus	Oys	Amp	Mys	DCr	Shri	Sper	Herr	Wpol	Anch	Dsol	Chum	Sqd	Coho	Lcod	Sfish	Hal	Sal	
Zooplankton (Copepoda, *Neocalanus*)	2.0		100																						100
Polychaete-1 *(Neanthes succinea)*	2.1	90	5	5																					100
Polychaete-2 *(Harmothoe imbricata)*	2.1	30	35	35																					100
Blue mussel (*Mytilus edulis*)	2.3	15	60	25																					100
Oyster (*Crassostrea gigas*)	2.3	15	60	25																					100
Amphipods (*Themisto* sp.)	2.4	30	35	35																					100
Mysis sp.	2.5	10	45	45																					100
Dungeness crab (*Cancer magister*)	2.8	43	2	10	5	5	5	5	5	5		5	5	5											100
Crangon sp.*	2.9		30	30						40															100
Shiner surfperch (*Cymatogaster aggregata*)	3.2	5	10	10	10	10			20	15		20													100
Pacific Herring (*Clupea pallasi*)	3.0			98	1				1																100
Walleye pollock (*Theragra chalcogramma*)	3.0			95	2.5				2.5																100
Northern anchovy (*Engraulis mordax*)	3.1		20	20					15	25		20													100
Dover Sole (*Microstomus pacificus*	3.3				27	27	7.25	7.25	1	10	10	10													100
Chum salmon (*Oncorhynchus keta*)	3.4	12		24	0.5	0.5			9		2			17.5		17.5			17						100
Gonatid squis (*Gonatius*)	3.5			50					3	5		5	9.3	9.3	9.3	9.3									100
Sablefish (*Anoplopoma fimbria*)	3.8			10	5				5		5	10	3	3	45	3	2.5		8						100
Coho salmon (*Oncorhynchus kisutch*)	4.2			26					34		4	4		16		8		8							100
Lingcod (*Ophiodon elongates*)	4.3								10	6.7	6.7	6.7			25		25		20						100
Halibut (*Hippoglossus stenolepis*)	4.0			1	1	1	1	1	1	10	14	14	5	5	38		1		5	1		1			100
Chinook salmon (*Oncorhynchus tshawytscha*)	4.0			5					1			4	10	25	25	10	10		10						100
Steller sea lion (*Eumetopias jubatus*)	4.5													80				6.7		6.7				6.7	
Killer whale (*Orcinus orca*)	5.0																3	10	3	5	3	3	3	70	100

[1]Legend prey species: Det = Detritud; Phy = Phytoplankton; Zoo = Zooplankton; Pol-1 = Polychaete-1; Pol-2 = Polychaete-2; Mus = Blue Mussels; Oys = Oyster; Amp = Amphipods; Mys = *Mysis*; DCr = Dungeness crab; Shri =Shrimp (*Crangon*); Sper = Shiner Surfperch; Herr = Pacific Herring; Wpol = Walleye Pollock; Anch = Northern Anchovy; Dsol = Dove sole; Coho = Coho salmon; Sqd= Gonatid squid; Sfish = Sablefish; Chum = Chum Salmon; Lcod = Lingcod; Hal = Halibut; Sal = Chinook Salmon. In the models trophic position values for detritus (TL = 1) and phytoplankton (TL = 1) were assigned according to Vander Zanden and Rasmussen (1996).

Appendix F-5

Description of Model State Variables (adapted from Gobas and Arnot 2010)

I. Phytoplankton, Zooplankton, Aquatic Invertebrates, Fish

The food web bioaccumulation model is based on the presumption that the exchange of PCB congeners between the organism and its ambient environment can be described by a single equation for a large number of aquatic organisms:

$$C_B = \{k_1 \cdot (m_O \cdot \phi \cdot C_{WT,O} + m_P \cdot C_{WD,S}) + k_D \cdot \sum P_i \cdot C_{D,i}\} / (k_2 + k_E + k_G + k_M)$$

This equation can be simplified by applying a steady-state assumption ($dM_B/dt = 0$), resulting in:

$$C_B = \{k_1 \cdot (m_O \cdot \phi \cdot C_{WT,O} + m_P \cdot C_{WD,S}) + k_D \cdot \sum P_i \cdot C_{D,i}\} / (k_2 + k_E + k_G + k_M)$$

The bioaccumulation factor (BAF) is described by $C_B / C_{WT,O}$ while the wet weight BSAF is C_B/C_S, where the concentration ($g \cdot kg^{-1}$, dry sediment) in the bottom sediment is C_S:

$$BSAF = C_B / C_S$$

The primary output of the food web bioaccumulation model is the BSAF as it allows predictions of PCB concentrations in biota from the PCB concentration in the sediments. Submodels for k_1, k_2, k_E, k_M, k_G and Φ, used to determine the BSAF are described below.

Φ: PCBs are hydrophobic and preferentially bind to organic matter and organic carbon, rendering them unavailable for uptake by biota. Φ describes the ratio of the freely dissolved water concentration C_{WD} (g/L) to the total water concentration C_{WT} (g/L), and is estimated for non-ionizing PCBs as:

$$\Phi = C_{WD} / C_{WT} = 1 / (1 + x_{POC} \cdot D_{POC} \cdot \alpha_{POC} \cdot K_{OW} + x_{DOC} \cdot D_{DOC} \cdot \alpha_{DOC} \cdot K_{OW})$$

Where concentrations of POC and DOC in the water (kg/L) are x_{POC} and x_{DOC} respectively. The disequilibrium factors for POC and DOC partitioning are D_{POC} and D_{DOC} respectively, and represent the degree POC-water and DOC-water distribution coefficients vary from POC-water and DOC-water equilibrium partition coefficients. Values greater than 1.0 for D_{POC} or D_{DOC} indicate distribution coefficients greater than equilibrium partition coefficients, values less than 1.0 indicate conditions where equilibrium has not been reached, and values equal to 1.0 indicate equilibrium partitioning. A variety of organic chemicals (including PCBs) show disequilibria between OC and water in several ecosystems (e.g., Gobas and Maclean 2003) but their values are difficult to predict. We used water and sediment concentration data from the areas of interest to characterize D_{POC} and D_{DOC} in the model. In equation 2.8 above, α_{POC} and α_{DOC} are proportionality constants that characterize the similar phase partitioning of POC and DOC in relation to octanol, and they can differ significantly between types of organic carbon. We assumed that α_{POC} was 0.35 with error bars equivalent to a factor of 2.5 (Seth *et al.* 1999), and α_{DOC} was 0.08 with error bars equivalent to a factor of 2.5 (Burkhard 2000).

k_1 and k_2: The rate the respiratory surface (e.g. gills and skin) absorb chemicals from the water is described by the aqueous uptake clearance rate constant k_1 (L/kg · d). For fish, invertebrates, and zooplankton, it is a function of the ventilation rate G_V (L/d) and diffusion rate of PCBs across the respiratory surface area (Gobas 1993; Walker 1987):

$$k_1 = E_W \cdot G_V / W_B$$

Where the chemical uptake efficiency of the gills is E_W and the wet weight of the organism (kg) is W_B. The chemical uptake efficiency (E_W) is a function of the PCB congener's K_{OW} and was derived from a fish study (Gobas and Mackay 1987):

$$E_W = (A_{EW} + (B_{EW} / K_{OW}))^{-1}$$

Where the constants A_{EW} and B_{EW} are1.85 (± 0.13) and 155 (± 0.50), respectively. Calculations of G_V were based on an allometric relationship between wet weight and oxygen consumption (from a study of 200 fish species ranging in weight between $2.0 \cdot 10^{-5}$ and 60 kg under routine metabolic

test conditions) (Thurston and Gehrke 1990), and on G_V data for zooplankton and aquatic invertebrates:

$$G_V = 1400 \cdot W_B^{0.65} / DO$$

Where the water's dissolved oxygen concentration (mg $O_2 \cdot L^{-1}$) is DO, which was obtained from the literature. A biphasic relationship for k_1 and k_2 based on a water-organic carbon two-phase resistance model was applied for algae, phytoplankton and aquatic macrophytes:

$$k_1 = (A_P + ((B_P / K_{OW}))^{-1}$$

Where the resistance to PCB uptake through the aqueous and organic phases of the algae or phytoplankton are described by the constants A_P and B_P (unit is time), respectively. Numerous data sets were evaluated to obtain A_P and B_P values for phytoplankton. We derived the constant B_P (default value = 5.5 (± 3.7)) by calibration to empirical k_2 values from various phytoplankton, algae and cyanobacteria species over a range of K_{OW} (Koelmans *et al.* ,1993; Koelmans *et al.,* 1995; Koelmans *et al.,* 1999). We derived the constant A_P (default value = 6.0 (± 2.0) $\cdot$ 10^{-5}) from calibration to phytoplankton field BCF data (Oliver and Niimi 1988; Swackhammer and Skoglund 1993). The mean annual k_G value was 0.125/d (Alpine and Cloern 1988; Alpine and Cloern 1992).

The elimination rate constant k_2 (d^{-1}) is similar to k_1 since they both involve water ventilation and membrane permeation:

$$k_2 = k_1 / K_{BW}$$

Where the biota-water partition coefficient is K_{BW} (L/kg, wet weight). PCB partitioning between biota and water is thought to occur in lipids, non-lipid organic matter (e.g., proteins and carbohydrates), and water. Each compartment has its own capacity to sorb PCB congeners, thus for every PCB congener in each organism the organism-water partition coefficient K_{BW} on a wet weight basis (ww) is:

$$K_{BW} = k_1 / k_2 = v_{LB} \cdot K_{OW} + v_{NB} \cdot \beta \cdot K_{OW} + v_{WB}$$

Where the lipid fraction (kg lipid/kg organism ww) is v_{LB}, the non-lipid organic matter (NLOM) fraction (kg NLOM/kg organism ww) is v_{NB}, and the water content (kg water/kg organism ww) of the organism is v_{WB}. The proportionality constant expressing the sorption capacity of NLOM to that of octanol is β, and the value used was 0.035 ±0.004 (Gobas *et al.* 1999). Thus the PCB sorption affinity of NLOM is ~3.5% that of octanol. Compared to lipid, the sorption affinity of NLOM is low but it can be important for controlling partitioning of organic chemicals in organisms with low lipid contents (e.g., phytoplankton).

To calculate the phytoplankton-water partition coefficient (K_{PW}), the value of NLOM in equation 2.14 was replaced by the proportionality constant of 0.35 for non-lipid organic carbon (kg NLOC/kg organism ww) (Skoglund and Swackhamer 1999):

$$K_{PW} = v_{LP} \cdot K_{OW} + v_{NP} \cdot 0.35 \cdot K_{OW} + v_{WP}$$

The BAF is a function of the k_1 and k_2 ratio, thus errors in determining G_V and E_W typically have little effect on the BAF since k_1 errors cancel out similar k_2 errors. Therefore the model is relatively insensitive to G_V and E_W parameterization error, and a single equation for a variety of species is able to represent ventilation rates and uptake efficiencies. Partitioning properties of the chemical (K_{BW}) play a more important role, which is reasonable because the main role of k_1 and k_2 is to describe the rate of equilibrium partitioning in the organism. Model sensitivity is most affected by k_1 and k_2 for substances taken up from water and food in similar quantities, and/or eliminated by gill ventilation at a rate similar to that for feces egestion, metabolic transformation, and growth dilution combined.

m_O, m_P: PCBs can be exchanged between sediment pore water and organism tissues when the organism spends time in close contact with bottom sediments (i.e., benthic fish and invertebrates). Due to sediment-water disequilibria, concentrations of freely dissolved PCBs in pore water can be greater than those in overlying water (Gobas and Maclean 2003), but the amount of pore water ventilated by benthic fish and invertebrates is often small because of its low oxygen concentration

and food content. Even though little pore water is usually ventilated, it can have a significant effect on the BAF for PCBs with large sediment-water column disequilibria. Organisms with no direct pore water contact have an m_P of 0. For all organisms m_O is equal to 1 - m_P.

$C_{WD,P}$: Freely dissolved pore water PCB concentrations were estimated from bottom sediment PCB concentrations (Kraaij *et al.,* 2002):

$$C_{WD,P} = C_{S,OC} / (10^{(\text{Log Kssw,co})})$$

Where the freely dissolved pore water PCB concentration ($g \cdot L^{-1}$) is $C_{WD,P}$, the organic carbon normalized sediment PCB concentration (g/kg OC) is $C_{S,OC}$, and the organic carbon normalized suspended sediment-water distribution coefficient is log $K_{SSW,CO}$ (log $K_{SSW,CO}$ = log 0.52 · log K_{OW} + 3.02) (Mackintosh *et al.* 2006).

k_D and k_E: The dietary uptake clearance rate constant k_D (kg-food/kg-organism · d) describes the absorption rate of PCBs from the diet via the GIT, and is a function of dietary chemical transfer efficiency (E_D), feeding rate (G_D; $kg \cdot d^{-1}$), and organism weight (W_B; kg) (Gobas 1993):

$$k_D = E_D \cdot G_D / W_B$$

Empirical E_D values for aquatic invertebrates range from 0 to 100% (Bruner *et al.* 1994; Kukkonen and Landrum 1995; Landrum and Poore 1988; Lydy and Landrum 1993; Mayer *et al.* 2001; Morrison *et al.* 1996; Parkerton 1993 ; Wang and Fisher 1999) and from 0 to 90% for fish (Fisk *et al.* 1998; Gobas *et al.* 1988; Gobas *et al.* 1993a; Gobas *et al.,* 1993b; Parkerton 1993). Due to the large variation in empirical data accurate models for dietary uptake rates are difficult to develop, but trends in E_D data can provide guidance. There is often a reduction in dietary uptake efficiency with increasing K_{OW} for high K_{OW} chemicals for invertebrates (Bruner *et al.* 1994; Parkerton 1993) and fish (Gobas *et al.* 1988;Parkerton 1993). Aquatic invertebrates and fish fed continuously have average dietary chemical transfer efficiency (E_D) of ~50% for chemicals with a log K_{OW} ranging from 4 – 6. This is in agreement with a two-phase resistance model for gut-organism exchange,

also found by Gobas *et al.* (1988). PCB congener dietary absorption efficiencies were based on the lipid-water two phase resistance model:

$$E_D = (A_{ED} \cdot K_{OW} + B_{ED})^{-1}$$

Where for zooplankton, invertebrates and fish the constant A_{ED} equals 8.5 (± 1.4) $\cdot 10^{-8}$ and B_{ED} equals 2.0 (±0.6). A general bioenergetic relationship was applied for estimating feeding rates in fish and aquatic invertebrates (Weininger 1978):

$$G_D = 0.022 \cdot W_B^{0.85} \cdot e^{(0.06 \cdot Tw)}$$

Where the mean water temperature (°C) is T_W. Dietary uptake by filter feeding species has a unique mechanism described by:

$$G_D = G_V \cdot C_{SS} \cdot \dot{\sigma}$$

Where feeding rate is a function of gill ventilation rate G_V ($L \cdot d^{-1}$), concentration of suspended solids C_{SS} ($k/\cdot L^{-1}$), and scavenging efficiency of particles from water $\dot{\sigma}$ (%).

PCB elimination by fecal matter egestion was expressed by k_E (d^{-1}), the fecal elimination rate constant (Gobas *et al.* 1993a):

$$k_E = G_F \cdot E_D \cdot K_{GB} / W_B$$

Where the fecal egestion rate is G_F (kg-feces/kg-organism $\cdot$ d) and the PCB partition coefficient between the GIT and organism is K_{GB}. G_F is a function of feeding rate and diet digestibility, which is a function of diet composition:

$$G_F = ((1-\varepsilon_L) \cdot v_{LD}) + (1-\varepsilon_N) \cdot v_{ND} + (1-\varepsilon_W) \cdot v_{WD}) \cdot G_D$$

Where dietary absorption efficiencies of lipid, NLOM and water are ε_L, ε_N and ε_W, respectively. The overall lipid, NLOM and water contents of the diet are v_{LD}, v_{ND}, and v_{WD}, respectively. Absorption efficiencies of lipid and NLOM in fish are approximately 90% and 50%, respectively (Gobas *et al.,* 1999; Nichols *et al.,* 2001).

Invertebrate absorption and assimilation efficiencies vary from 15 - 96% (Berg *et al.* 1996; Gordon 1966; Parkerton 1993; Roditi and Fisher 1999), and generally reflect the organism's dietary matrix (e.g., organic matter quantity and quality) and digestive physiology (e.g., feeding rates and gut retention time). Generally, species with low absorption efficiencies, like worms, consume poor quality sediment or detritus while maintaining high feeding rates to ingest sufficient nutrients. Lipid and non- lipid organic matter absorption efficiencies were set at 75% for aquatic invertebrates.

Zooplankton organic matter assimilation efficiencies range from 55 - 85% (Conover 1966), and are ~85% for carbon and phosphorus (Lehman 1993). We assumed zooplankton lipid and non-lipid organic matter absorption efficiencies were 72%. Water storage capacity has a negligible impact on the mechanism of biomagnification for PCBs, and its assumed absorption efficiency was 55% for zooplankton, invertebrate and fish.

K_{GB}: Is the PCB partition coefficient between the GIT contents and organism, and expresses the effect on phase partitioning properties resulting from digestion after ingestion:

$$K_{GB} = (v_{LG} \cdot K_{OW} + v_{NG} \cdot \beta \cdot K_{OW} + v_{WG}) / (v_{LB} \cdot K_{OW} + v_{NB} \cdot \beta \cdot K_{OW} + v_{WB})$$

Where the lipid (kg lipid/kg digesta ww), NLOM (kg NLOM/kg digesta ww) and water (kg water/kg digesta ww) contents in the gut are v_{LG}, v_{NG}, and v_{WG}, respectively. Summing these fractions (i.e., total digesta) approaches 1 and depends on the absorption efficiency the dietary components:

$$v_{LG} = (1-\varepsilon_L) \cdot v_{LD} / ((1-\varepsilon_L) \cdot v_{LD} + (1-\varepsilon_N) \cdot v_{ND} + (1-\varepsilon_W) \cdot v_{WD})$$
$$v_{NG} = (1-\varepsilon_N) \cdot v_{ND} / ((1-\varepsilon_L) \cdot v_{LD} + (1-\varepsilon_N) \cdot v_{ND} + (1-\varepsilon_W) \cdot v_{WD})$$
$$v_{WG} = (1-\varepsilon_W) \cdot v_{WD} / ((1-\varepsilon_L) \cdot v_{LD} + (1-\varepsilon_N) \cdot v_{ND} + (1-\varepsilon_W) \cdot v_{WD})$$

The bioaccumulation model in equation 2.6 depends on the ratio of k_D and k_E, which is $G_D/(G_F \cdot K_{GB})$, causing the feeding rate G_D (and hence G_F, eq. 2.22) and dietary uptake efficiency E_D model parameterization errors cancel out. If G_D and E_D are not characterized well, the model can still be expected to provide reasonable BAF and BSAF estimates, which is a nice feature because the variability and error in G_D and E_D are usually considerable.

k_G: Growth rates are highly variable among and within species because they are a function of factors such as size, temperature, prey availability, and quality. Reliable growth rate data were not available for most of the species in the food web bioaccumulation model, and instead we used the following generalized growth equations (Thomann 1989), to approximate the growth rate constant k_G (d^{-1}). For zooplankton and invertebrates the equation was:

$$k_G = I_{GR} \cdot W_B^{-0.2}$$

which is representative for temperatures around 10°C, and for fish species the equation was:

$$k_G = F_{GR} \cdot W_B^{-0.2}$$

With an average water temperature of ~15°C, the growth rate coefficient for invertebrates (I_{GR}) is 0.00035 and for fish (F_{GR}) is 0.0007.

k_M: The metabolic transformation rate constant k_M (d^{-1}) is the rate a parent compound is eliminated via metabolic transformation, and depends on the PCB congener and the species in question. Aquatic invertebrates and fish are very poor at metabolizing most PCB congeners, and we assumed k_M was negligible in these species.

A summary of abiotic model state variables is shown in Table F-5.1, while Tables F-5.2 and F-5.3 summarize model state variables for phytoplankton and all other aquatic biota (i.e., zooplankton, invertebrates, and fish), respectively.

Table F-5.1 A summary of abiotic model state variables requiring parameterization in the food web bioaccumulation model.

Definition	Parameter	Units
Mean air temperature	T_A	°C
Mean water temperature	T_W	°C
Dissolved oxygen concentration	DO	mg O_2/L
Practical salinity units	PSU	g/kg
Dissolved organic carbon content – water	OC_{WATER}	kg/L
Particulate organic carbon content – water	POC	kg/L
Concentration of suspended solids – water	C_{SS}	kg/L
Organic carbon content – sediment	$OC_{SEDIMENT}$	%
Chemical concentration – water	C_{WT}	ng/L
Octanol-water partition coefficient	K_{OW}	unitless
Octanol-air partition coefficient	K_{OA}	unitless
Non-lipid organic matter – octanol proportionality constant	β	unitless

Table F-5.2 A summary of biotic state variables that require parameterization in the food web bioaccumulation model for phytoplankton.

Definition	Parameter	Units
Whole body lipid fraction	L	kg/kg
Whole body non-lipid organic carbon fraction	NLOC	kg/kg
Whole body water fraction	WC	kg/kg
Phytoplankton growth rate constant	K_G	1/d
Constant A_P (equation 2.12)	A_P	1/d
Constant B_P (equation 2.12)	B_P	1/d

Table F-5.3 A summary of model state variables that require parameterization in the food web bioaccumulation model for zooplankton, invertebrates, and fish.

Definition	Parameter	Units
Wet weight	W	kg
Whole body lipid fraction	L	kg/kg
Whole body non-lipid organic matter fraction	NLOM	kg/kg
Whole body water fraction	WC	kg/kg
Percentage of respired pore water	P_W	%
Invertebrate growth rate coefficient	I_{GR}	unitless
Fish growth rate coefficient	F_{GR}	unitless
Metabolic transformation rate constant	k_M	1/d
Fraction of prey item in diet	P_i	unitless
Lipid absorption efficiency	ε_L	%
NLOM absorption efficiency	ε_N	%
Water absorption efficiency	ε_W	%
Constant A_{EW} (equation 2.10)	A_{EW}	unitless
Constant B_{EW} (equation 2.10)	B_{EW}	unitless
Constant A_{ED} (equation 2.18)	A_{ED}	unitless
Constant B_{ED} (equation 2.18)	B_{ED}	unitless

II. Killer Whale and Steller Sea Lion

The balance between uptake and elimination in the killer whale or Steller sea lion is represented by the following mass balance equation:

$$C_{KW,l} = (k_A C_{AG} + k_D . \Sigma(P_i \cdot C_{D,i})) / (k_O + k_E + k_U + k_G + k_P + k_L + k_M)$$

At steady-state, we can simplify the equation:

$$C_{KW,l} = (k_A C_{AG} + k_D \cdot \Sigma(P_i \cdot C_{D,i})) / (k_O + k_E + k_U + k_G + k_P + k_L + k_M)$$

The lipid-normalized concentration can be used to calculate a whole organism wet weight based concentration in the killer whale C_{KW}:

$$C_{KW} = L_{KW} \cdot C_{S,1}$$

During the year, considerable changes occur in the whole organism's lipid content thus the wet weight concentration is also expected to experience changes of the same magnitude. In the model this is captured by varying L_{KW}. Since killer whales have a high lipid content, non-lipid organic matter does not play a significant role in PCB storage.

The biota-sediment accumulation factor (BSAF; kg dry sediment/kg wet weight) is the ratio of PCB concentrations in the killer whale (C_{KW}) to that in sediments (C_S):

$$BSAF = C_{KW} / C_S$$

BSAFs are a simple method to predict PCB concentrations in killer whales from PCB concentrations in sediments.

Submodels for calculating k_D, k_A, k_O, k_E, k_U, k_G, k_P, and k_L in the killer whale model are described as follows.

k_D and k_E: k_D (kg-food/kg- lipid · d) is the PCB dietary uptake clearance rate constant, which was estimated as a function of dietary chemical transfer efficiency E_D, feeding rate G_D (kg/d), and organism's lipid mass $W_{S,1}$ (kg):

$$k_D = E_D \cdot G_D / W_{S,1}$$

Feeding rates for killer whales were obtained from Hickie *et al*. (2007), and those for Steller sea lion from Winship *et al*. (2006), and Olesiuk (2008). To determine PCB congener dietary absorption efficiencies in male and female killer whales, we used the equation below (based on the lipid-water two-phase resistance model):

$$E_D = (A_{ED} \cdot K_{OW} + B_{ED})^{-1}$$

For killer whales and Steller sea lions the constants A_{ED} and B_{ED} are 1.0 [± 0.17] $\cdot 10^{-9}$ and 1.025 [±0.00125], respectively.

k_E (1/d) is the rate constant for PCB fecal excretion in killer whales and Steller sea lions, and was calculated as:

$$k_E = G_F \cdot E_D \cdot K_{GS,1} / W_{S,1}$$

Where the fecal egestion rate is G_F (kg-feces/kg-organism · d) and the PCB partition coefficient between the GIT and killer whale or Steller sea lion lipids is $K_{GS,1}$. G_F is a function of feeding rate and diet digestibility, which itself is a function of diet composition:

$$G_F = ((1-\varepsilon_L) \cdot v_{LD} + (1-\varepsilon_N) \cdot v_{ND} + (1-\varepsilon_W) \cdot v_{WD}) \cdot G_D$$

Where the dietary absorption efficiencies of lipid, NLOM, and water are ε_L, ε_N, and ε_W, respectively. The overall diet lipid, NLOM, and water contents are v_{LD}, v_{ND}, and v_{WD}, respectively. It was assumed that the absorption efficiencies of lipid and NLOM were approximately 100% and

98%, respectively, for killer whales (Lachmuth *et al*. 2010), and 98% and 75%, respectively for Steller sea lions (Rosen *et al.* 2000; Rosen and Trites 2000).

The PCB partition coefficient $K_{GS,1}$ between the GIT contents and the body lipids of the killer whale and Steller sea lion is calculated as:

$$K_{GB} = (v_{LG} \cdot K_{OW} + v_{NG} \cdot \beta \cdot K_{OW} + v_{WG}) / K_{OW}$$

Where the killer whale or Steller sea lion gut lipid (kg lipid/kg digesta ww), NLOM (kg NLOM/kg digesta ww), and water (kg water/kg digesta ww) contents are v_{LG}, v_{NG}, and v_{WG}, respectively. Summing these fractions (i.e., total digesta) approaches 1 and depends on each diet component's absorption efficiency:

$$v_{LG} = (1-\varepsilon_L) \cdot v_{LD} / ((1-\varepsilon_L) \cdot v_{LD} + (1-\varepsilon_N) \cdot v_{ND} + (1-\varepsilon_W) \cdot v_{WD})$$

$$v_{NG} = (1-\varepsilon_N) \cdot v_{ND} / ((1-\varepsilon_L) \cdot v_{LD} + (1-\varepsilon_N) \cdot v_{ND} + (1-\varepsilon_W) \cdot v_{WD})$$

$$v_{WG} = (1-\varepsilon_W) \cdot v_{WD} / ((1-\varepsilon_L) \cdot v_{LD} + (1-\varepsilon_N) \cdot v_{ND} + (1-\varepsilon_W) \cdot v_{WD})$$

k_A and k_O: The rate of PCB absorption from inhalation is described by k_A (L/kg lipid · d), the inhalation clearance rate constant:

$$k_A = E_A \cdot G_A / W_{S,1}$$

Since inhalation and exhalation both utilize lung ventilation and pulmonary membrane permeation, the PCB elimination rate constant via exhalation k_O (1/d) is related to k_A as:

$$k_O = k_A / K_{S,1A}$$

Where the PCB congener partition coefficient between the killer whale's lipid biomass or Steller sea lion's lipid biomass and air is $K_{S,1A}$ (L/kg, lipid), estimated from the octanol-air partition coefficient (K_{OA}) and the lipid density δ_L (kg/L) as:

$$K_{S,1A} = k_A / k_O = K_{OA} \cdot 1/\delta_L$$

We calculated the urinary excretion rate constant k_U (1/d) as:

$$k_U = G_U / (W_{S,1} \cdot K_{OW} \cdot 1/\delta_L)$$

Where the urinary excretion rate (L/d) is G_U and the octanol-water partition coefficient is K_{OW}.

k_G, k_P, k_L: PCB elimination rate constants in killer whales and Steller sea lions for growth dilution, off-spring, and milk, represent PCB reduction in the lipid biomass of the whale or sea lion that arises from the increase in lipid biomass due to growth, off spring production, and lactation. These rate constants are characterized by the proportional increase in lipid biomass over time:

$$dW_{KW,1} / (W_{KW,1} \cdot dt)$$

$dW_{KW,1}$ represents lipid mass increases attained during a year when calculating k_G, and it describes the calf's lipid mass at birth when assessing k_P. This lipid biomass is produced during the gestation period. $dW_{KW,1}$ describes the lipid mass transferred to the calf in milk during lactation (i.e., the product of lactation rate G_L (L/d) and duration of lactation t_L), when estimating k_L. For simplicity, we calculated the lipid biomass increase in female killer whales or female Steller sea lion by summing lipid masses produced for growth, off-spring production, and lactation and described it as a fraction of the animal's lipid biomass generated over time. Data on reproductive history for female killer whales (i.e. one calf every 5 years) was obtained from Olesiuk *et al*. (1990) and Hickie *et al*. (2007) and for reproductive female Steller sea lions (i.e. one pup per year) from Olesiuk (1998). For other age/sex categories (adult males, subadults and pups), killer whale growth rates were retrieved from Hickie *et al*. (2007), and Kriete *et al*. (1995), while for Steller sea

lions were obtained from growth rate data reported elsewhere (Winship *et al*. 2001; Brandon *et al*. 2005; Winship *et al*. 2006).

k_M: Killer whales can metabolize certain PCB congeners, which can have a significant effect on the magnitude of PCB concentrations attained in the body. Because PCBs can have congener specific metabolic transformation patterns (Boon *et al.* 1987; Boon *et al.* 1994; Boon *et al.* 1997), one can estimate a congener's metabolic transformation relative to a reference congener. PCB 153 is the dominant PCB congener in Harbour seals (Boon *et al.* 1987; Boon *et al.* 1994; Boon *et al.* 1997), and PCB 153 and 138 dominate PCB congeners in resident killer whales of British Columbia (Ross *et al.* 2000). This was included specifically in the Steller sea lion model. However, for the aim of the killer whale model and because information for each PCB congener's metabolic transformation rate constant is scarce, we assumed that metabolic transformation rate constants (k_M) for each PCB congener were 0 /d. Definition of state variables and values for biological parameters for resident killer whales and Steller sea lions are summarized in Table F-5.4 and Table F-5.5, respectively.

Sensitivity Analysis

The *sensitivity analysis* assesses the impact of variability and/or error in the model's state variables (e.g. organism weight, lipid content, temperature etc.) on the model outcome (i.e. the BSAF of total PCBs in wildlife). The sensitivity analysis is useful in determining the effect that errors in model state variables might have on the model outcome. Sensitive variables are variables that have a relatively large impact on the model outcome, i.e. a small change in the value of the variable produces a relatively large change in the model outcome. A less sensitive variable is a variable that causes a relatively small change in model outcome given the same change in the value of the variable. The sensitivity analysis can therefore provide valuable insights into the selection of the parameters that need to be included in the uncertainty analysis. It is important to include sensitive parameters in the uncertainty analysis.

The objective of the sensitivity analysis is to provide insight into the relative importance of the various state variables of the model. This is useful in the analysis of the internal mechanics of the model. It can be used to characterize potential errors in the model and to develop a better

understanding of the relationship between the processes that control the behavior of PCBs in the resident killer whale food web.

The sensitivity analysis was conducted for water and sediment concentrations. The sensitivity analysis then involved changing each model variable (I) at a time by a fixed amount (ΔI). The change (ΔO) that occurred in model outcome (O) was then calculated and the model state variable's sensitivity S was determined as:

$$S = \left(\frac{(\Delta O / O)}{(\Delta I / I)} \right)$$

The quantity S describes the sensitivity of O to changes in I. To calculate the sensitivity of the model input variable's sensitivity, a 10% reduction (i.e., $\Delta I / I$ = -0.1) and 10% increase (i.e., $\Delta I / I$ = +0.1) of the "mean" value used in the model was used. The resulting change in model outcome O was reported for adult male killer whale of the northern resident killer whale critical habitat (i.e., coastal food web) and the outer coast area (i.e., oceanic food web). This provides an illustrative and representative assessment of sensitivity in the food web models.

Table F-5.4 A summary of model state variables that require parameterization in the food web bioaccumulation model for killer whales and Steller sea lions.

Definition	Parameter	Units
Wet weight	W	kg
Whole body lipid fraction	L	kg/kg
Whole body non-lipid organic matter fraction	NLOM	kg/kg
Whole body water fraction	WC	kg/kg
Mean homeotherm temperature	T_H	°C
Growth rate constant	K_G	1/d
Fraction of prey item in diet	P_i	unitless
Lipid absorption efficiency	ε_L	%
NLOM absorption efficiency	ε_N	%
Water absorption efficiency	ε_W	%
Constant A_{ED} (equation 2.34 for killer whales)	A_{ED}	unitless
Constant B_{ED} (equation 2.34 for killer whales)	B_{ED}	unitless
Urine excretion rate	G_U	L/d
Metabolic transformation rate constant	k_M	1/d

Table F-5.5a Overview of values/inputs for species specific model state variables and biological parameter of the PCB food web model for killer whales that require parameterization.

PARAMETER	VALUE / INPUT	REFERENCE
SPECIES	**PHYTOPLANKTON / ALGAE**	
Lipid Content (%)	0.12%	Mackintosh *et al.* (2004)
NLOC Content (%)	6.00%	Mackintosh *et al.* (2004)
Growth Rate Constant - k_G (1/day)	8.00E-02	Alpine, A. E. and J. E. Cloern (1988) and Alpine, A. E. and J. E. Cloern (1992)
Aqueous phase resistance constant (Ap) (1/day)	6.00E-05	Arnot and Gobas (2004)
Organic phase resistance constant (Bp) (1/day)	5.50E+00	Arnot and Gobas (2004)
SPECIES	**ZOOPLANKTON - 1**	
Species Name	*Copepoda & sp.*	
Weight (kg)	7.10E-08	Gobas and Wilcockson (2003)
Lipid Content (%)	1.00%	Estimated from Roberts *et al.* (2002)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	9.41E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	85.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	75.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**INVERT - 1**	
Species Name	*Neanthes succinea*	
Weight (kg)	1.10E-04	Gobas and Wilcockson (2003)
Lipid Content (%)	0.75%	Estimated from Roberts *et al.* (2002); and Gobas and Wilcockson (2003)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	20.0%	Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	2.17E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
SPECIES	**INVERT - 2**	
Species Name	*Themisto* sp., *Amphelisca sp*	
Weight (kg)	3.13E-06	Gobas and Arnot (2010)
Lipid Content (%)	1.00%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	4.42E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**INVERT - 3**	
Species Name	*Metacarcinus magister*	
Weight (kg)	2.52E-01	Mackintosh *et al*. (2004)
Lipid Content (%)	8.00%	Mackintosh *et al*. (2004)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	4.61E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**INVERT - 4**	
Species Name	*Mysis sp.*	
Weight (kg)	1.50E-05	Arnot and Gobas (2004)
Lipid Content (%)	1.00%	Estimated
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	3.23E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	75.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
SPECIES	**INVERT - 5**	
Species Name	*Mytilus edulis*	
Weight (kg)	5.00E-03	Mackintosh *et al*. (2004)
Lipid Content (%)	1.30%	Mackintosh *et al*. (2004)
NLOM Content (%)	10.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	5.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	1.01E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**INVERT - 6**	
Species Name	*Crassostrea gigas*	
Weight (kg)	7.00E-03	Mackintosh *et al*. (2004)
Lipid Content (%)	2.1%	Mackintosh *et al*. (2004)
NLOM Content (%)	10.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	3.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	9.44E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**INVERT - 7**	
Species Name	*Harmothoe imbricata*	
Weight (kg)	1.00E-07	Gobas and Wilcockson (2003)
Lipid Content (%)	0.75%	Estimated from Roberts *et al.* (2002); and Gobas and Wilcockson (2003)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	5.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	8.79E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	50.0%	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**INVERT - 8**	
Species Name	*Crangon sp.*	
Weight (kg)	3.72E-04	Gobas and Wilcockson (2003)
Lipid Content (%)	2.00%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	1.70E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 1**	
Species Name	*Shiner surfperch* (*Cytomatogater aggregata*)	
Weight (kg)	1.31E-03	Gobas and Arnot (2010)
Lipid Content (%)	2.0%	Mackintosh *et al.* (2004); Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Mackintosh *et al.* (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	5.28E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 2**	
Species Name	Pollock (*Theragra chalcogramma*)	
Weight (kg)	4.00E-03	
Lipid Content (%)	5.7%	Jeanniard du Dot (2007)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	4.22E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 3**	
Species Name	*Northern anchovy* (*Engraulis mordax*)	
Weight (kg)	3.70E-03	Gobas and Arnot (2010)
Lipid Content (%)	2.0%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	4.29E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 4**	
Species Name	Pacific Herring (*Clupea pallasii*)	
Weight (kg)	7.00E-02	West *et al*. (2008)
Lipid Content (%)	12%	Jeanniard du Dot (2007)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	2.38E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 5**	
Species Name	*Northern anchovy (Engraulis mordax) (>juvenile)*	
Weight (kg)	2.15E-02	Gobas and Arnot (2010)
Lipid Content (%)	2.5%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
Growth Rate Constant - k_G (1/day)	3.02E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 6**	
Species Name	*shiner surfperch* (*Cytomatogater aggregata*) (>juvenile)	
Weight (kg)	5.13E-02	Gobas and Arnot (2010)
Lipid Content (%)	2.6%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	2.54E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 7**	
Species Name	*Pollock (Theragraga chalchogramma) (>juvenile)*	
Weight (kg)	1.406E+00	
Lipid Content (%)	5.7%	Jeanniard du Dot (2007)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	1.31E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 8**	
Species Name	Spotted greenling (*Hexagrammos stelleri*) (>juvenile)	
Weight (kg)	1.26E-01	Mackintosh *et al.* (2004)
Lipid Content (%)	0.6%	Mackintosh *et al.* (2004)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	2.12E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 9**	
Species Name	Plainfin midshipman *(Porichthys notatus*)(>juvenile)	
Weight (kg)	1.30E-01	Gobas and Arnot (2010)
Lipid Content (%)	3.0%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	5.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	2.11E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 10**	
Species Name	Sable fish (*Anoplopoma fimbria*)	
Weight (kg)	5.70E+01	Eschmeyer *et al*. (1983)
Lipid Content (%)	15%	
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	6.24E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 11**	
Species name	Halibut (*Hippoglossus stenolepis*)	

PARAMETER	VALUE / INPUT	REFERENCE
Weight (kg)	2.00+2	Mecklenburg *et al*.(2002)
Lipid Content (%)	11%	
NLOM Content (%)	20%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	4.85E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
SPECIES	**FISH - 12**	
Species name	Chinook salmon (*Oncorhynchus tshawytscha*)	
Weight (kg)	1.09E+01	Cullon *et al*. (2009)
Lipid Content (%)	14.1	Cullon *et al*. (2009)
NLOM Content (%)	20%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
E_D - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - k_G (1/day)	8.69E-04	Derived from Bigler *et al*. (1995)
Metabolic Transformation Rate Constant - k_M (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (ε_L)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (ε_N)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)
	Mammal - 1	
	Adult killer whale (Male)	
Weight (kg)	5.00E+03	Clark *et al*. (2000); Hickie *et al.* (2007)
Lipid Content (%)	26.0%	Ross *et al*. (2000)
NLOM Content (%)	20.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	1.00E-09	Derived from Moser and McLachlan (2002 & 2001).
E_D - Constant B	1.03E+00	Derived from Moser and McLachlan (2002 & 2001)
Lung Respiration Rate (G_V) - (L/day)	7.14E+05	Derived from Hickie *et al.* (1999)
Food Ingestion Rate - (G_D) (kg-food/day)	2.50E+02	Hickie *et al*. (2007); Lachmuth *et al*. (2010)
Growth Rate Constant - k_G (1/day)	1.30E-04	Kriete (1995); Hickie *et al.* (2007)
Urinary Excretion Rate Constant - (G_U) (L/day)	8.50E+00	Estimated from Smith (1936)
Lipid Digestion Efficiency (ε_L)	100.0%	Based on Rosen and Trites

PARAMETER	VALUE / INPUT	REFERENCE
		(2000); Moser and McLachlan (2002 & 2001); Kelly and Gobas (2003)
NLOM Digestion Efficiency (ε_N)	98.0%	Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)
SPECIES	**Mammal - 2**	
Species Name	*Killer whale (Female)*	
Weight (kg)	2.70E+03	Clark *et al*. (2000); Hickie *et al.* (2007)
Lipid Content (%)	22.0%	Ross *et al*. (2000)
NLOM Content (%)	20.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	1.00E-09	Derived from Moser and McLachlan (2002 & 2001)
E_D - Constant B	1.03E+00	Derived from Moser and McLachlan (2002 & 2001)
Lung Respiration Rate (G_V) - (L/day)	4.49E+05	Derived from Hickie *et al.* (1999)
Food Ingestion Rate - (G_D) (kg-food/day)	1.35E+02	Hickie *et al*. (2007); Lachmuth *et al*. (2010)
Growth Rate Constant - k_G (1/day)	8.41E-04	Olesiuk *et al*. (1990); Hickie *et al*. (2007)
Urinary Excretion Rate Constant - (G_U) (L/day)	4.59E-01	Estimated from Smith (1936)
Lactation Rate Constant - (G_L) (L/day)	9.60E-01	Derived from Cottrell *et al.* (2002); Bowen *et al.* (2001) Bowen *et al.* (1992)
Lipid Content Fetus (LFetus) (%)	11.0%	Derived from Cottrell *et al.* (2002), Bowen *et al*. (2001), Bowen *et al.* (1992)
NLOM Content Fetus (NLOMFetus) (%)	26.0%	Arnot and Gobas (2004); Kelly and Gobas (2003)
WC Fetus (WCFetus) (%)	69.0%	Arnot and Gobas (2004); Kelly and Gobas (2003)
Weight - Fetus (Vfetus) (kg)	1.10E+02	Reeves *et al*. (2002)
Lipid Digestion Efficiency (ε_L)	100.0%	Based on Rosen and Trites (2000); Moser and McLachlan (2002 & 2001); Kelly and Gobas (2003)
NLOM Digestion Efficiency (ε_N)	98.0%	Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)
SPECIES	**Mammal - 3**	
Species Name	*Juvenile Killer whale*	
Weight (kg)	1.00E+03	Based on Reeves *et al*. (2002)
Lipid Content (%)	26.0%	Ross *et al*. (2000)
NLOM Content (%)	20.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	1.00E-09	Derived from Moser and McLachlan (2002 & 2001)
E_D - Constant B	1.03E+00	Derived from Moser and McLachlan (2002 & 2001)

PARAMETER	VALUE / INPUT	REFERENCE
Lung Respiration Rate (G_V) - (L/day)	2.13E+05	Derived from Hickie *et al.* (1999)
Food Ingestion Rate - (G_D) (kg-food/day)	5.00E+01	Hickie *et al*. (2007)
Growth Rate Constant - k_G (1/day)	6.30E-04	Kriete (1995); Hickie *et al*. (2007)
Urinary Excretion Rate Constant - (G_U) (L/day)	1.70E+00	Estimated from Smith (1936)
Lipid Digestion Efficiency (ε_L)	100.0%	Based on Rosen and Trites (2000); Moser and McLachlan (2002 & 2001); Kelly and Gobas (2003)
NLOM Digestion Efficiency (ε_N)	98.0%	Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)
SPECIES	**Mammal - 4**	
Species Name	*Killer whale calf*	
Weight (kg)	1.60E+02	Reeves *et al*. (2002)
Lipid Content (%)	25.0%	Based on Ross *et al*. (2000)
NLOM Content (%)	20.0%	Based on Gobas and Arnot (2010)
E_D - Constant A	1.00E-09	Derived from Moser and McLachlan (2002 & 2001)
E_D - Constant B	1.03E+00	Derived from Moser and McLachlan (2002 & 2001)
Lung Respiration Rate (G_V) - (L/day)	3.24E+04	Derived from Hickie *et al.* (1999)
Food Ingestion Rate - (G_D) (kg-food/day)	9.60E+00	Based on Hickie *et al*. (2007)
Growth Rate Constant - k_G (1/day)	3.94E-03	Kriete (1995); Hickie *et al*. (2007)
Urinary Excretion Rate Constant - (G_U) (L/day)	3.56E-01	Estimated from Smith (1936)
Lipid Content Milk (Lmilk) (%)	25.0%	Hickie *et al*. (2007)
NLOM Content Milk (NLOMmilk) (%)	10.0%	Bowen *et al*. (2002)
WC Milk (WCmilk) (%)	65.0%	Bowen *et al*. (1992)
Lipid Digestion Efficiency (ε_L)	100.0%	Based on Rosen and Trites (2000); Moser and McLachlan (2002 & 2001); Kelly and Gobas (2003)
NLOM Digestion Efficiency (ε_N)	95.0%	Based on Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)

Table F-5.5b Overview of values/inputs for species specific model state variables and biological parameter of the PCB food web model for Steller sea lions that require parameterization. This table also includes data for other prey items (i.e. Gonatid squid, lingcod, dove sole, coho and chum salmon) of the resident killer whale's diet.

PARAMETER	VALUE / INPUT	REFERENCE
SPECIES	**PHYTOPLANKTON / ALGAE**	
Lipid Content (%)	0.12%	Mackintosh et al. (2004)
NLOC Content (%)	6.00%	Mackintosh et al. (2004)
Growth Rate Constant - kG (1/day)	8.00E-02	Alpine, A. E. and J. E. Cloern (1988) and Alpine, A. E. and J. E. Cloern (1992)
Aqueous phase resistance constant (Ap) (1/day)	6.00E-05	Arnot and Gobas (2004)
Organic phase resistance constant (Bp) (1/day)	5.50E+00	Arnot and Gobas (2004)
SPECIES	**ZOOPLANKTON - 1**	
Species Name	Copepoda & sp.	
Weight (kg)	7.10E-08	Gobas and Wilcockson (2003)
Lipid Content (%)	1.00%	Estimated from Roberts et al. (2002)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	9.41E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	85.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	75.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)
SPECIES	**INVERT - 1**	
Species Name	*Neanthes succinea*	
Weight (kg)	1.10E-04	Gobas and Wilcockson (2003)
Lipid Content (%)	0.75%	Estimated from Roberts et al. (2002); and Gobas and Wilcockson (2003)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	20.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	2.17E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	75.0%	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
NLOM Digestion Efficiency (εN)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	INVERT - 2	
Species Name	*Themisto* sp., *Amphelisca* sp	
Weight (kg)	3.13E-06	Gobas and Arnot (2010)
Lipid Content (%)	1.00%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	4.42E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	INVERT - 3	
Species Name	*Metacarcinus magister*	
Weight (kg)	2.52E-01	Mackintosh *et al*. (2004)
Lipid Content (%)	8.00%	Mackintosh *et al*. (2004)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	4.61E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	INVERT - 4	
Species Name	*Mysis* sp.	
Weight (kg)	1.50E-05	Arnot and Gobas (2004)
Lipid Content (%)	1.00%	Estimated
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	3.23E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
Lipid Digestion Efficiency (εL)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	75.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	INVERT - 5	
Species Name	*Mytilus edulis*	
Weight (kg)	5.00E-03	Mackintosh *et al*. (2004)
Lipid Content (%)	1.30%	Mackintosh *et al*. (2004)
NLOM Content (%)	10.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	5.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	1.01E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	INVERT - 6	
Species Name	*Crassostrea gigas*	
Weight (kg)	7.00E-03	Mackintosh *et al*. (2004)
Lipid Content (%)	2.1%	Mackintosh *et al*. (2004)
NLOM Content (%)	10.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	3.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	9.44E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	INVERT - 7	
Species Name	*Harmothoe imbricata*	
Weight (kg)	1.00E-07	Gobas and Wilcockson (2003)
Lipid Content (%)	0.75%	Estimated from Roberts et al. (2002); and Gobas and Wilcockson (2003)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water(Pw)	5.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	8.79E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
Lipid Digestion Efficiency (εL)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	INVERT - 8	
Species Name	*Crangon* sp.	
Weight (kg)	3.72E-04	Gobas and Wilcockson (2003)
Lipid Content (%)	2.00%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	1.70E-03	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	75.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 1	
Species Name	Shiner surfperch (*Cytomatogater aggregata*)	
Weight (kg)	1.31E-03	Gobas and Arnot (2010)
Lipid Content (%)	2.0%	Mackintosh *et al.* (2004); Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	5.28E-03	Gobas and Arnot (2010)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 2	
Species Name	Pollock (*Theragra chalcogramma*)	
Weight (kg)	4.00E-03	
Lipid Content (%)	5.7%	Jeanniard du Dot (2007)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)

PARAMETER	VALUE / INPUT	REFERENCE
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	4.22E-03	Based on Gobas and Arnot (2010)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 3	
Species Name	Northern anchovy (*Engraulis mordax*)	
Weight (kg)	3.70E-03	Gobas and Arnot (2010)
Lipid Content (%)	2.0%	Gobas and Arnot (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	4.29E-03	Gobas and Arnot (2010)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 4	
Species Name	Pacific Herring (*Clupea pallasii*)	
Weight (kg)	7.00E-02	West *et al*. (2008)
Lipid Content (%)	12%	Jeanniard du Dot (2007)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	2.38E-03	Based on Gobas and Arnot (2010)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 5	
Species Name	Dove sole (*Microstomus pacificus*)	
Weight (kg)	2.45E-01	Choromanski *et al.* (2005)
Lipid Content (%)	8.4%	Bando (2002)

PARAMETER	VALUE / INPUT	REFERENCE
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	1.85E-03	Based on Gobas and Arnot (2010)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 6	
Species Name	Gonatid Squid- (juvenile/adult)	
Weight (kg)	9.70E-02	Based on Hooker *et al*. (2001)
Lipid Content (%)	6.4%	Based on Hooker *et al*. (2001)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	2.23E-03	Based on Gobas and Arnot (2010)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 7	
Species Name	Chum salmon (*Oncorhynchus keta*)	
Weight (kg)	7.50E+00	Ricker (1980); Salo (1991)
Lipid Content (%)	3.5%	Hamilton *et. al.* (2005)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	9.36E-04	Based on Gobas and Arnot (2010)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 8	
Species Name	Lingcod (*Ophiodon elongates*)	

PARAMETER	VALUE / INPUT	REFERENCE
Weight (kg)	3.00E+01	Stock and Meyer (2005)
Lipid Content (%)	1.9%	Duan *et al.* (2010)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	7.09E-04	Based on Gobas and Arnot (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 9	
Species Name	Coho salmon (*Oncorhynchus kisutch)*	
Weight (kg)	1.50E+01	IGFA (2001); Sandercock (1991)
Lipid Content (%)	5.7%	Hamilton *et al.* (2005)
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	5.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	8.15E-04	Based on Gobas and Arnot (2010)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 10	
Species Name	Sable fish (*Anoplopoma fimbria*)	
Weight (kg)	5.70E+01	Eschmeyer *et al*. (1983)
Lipid Content (%)	15%	
NLOM Content (%)	20.0%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	6.24E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

PARAMETER	VALUE / INPUT	REFERENCE
SPECIES	**FISH - 11**	
Species name	Halibut (*Hippoglossus stenolepis*)	
Weight (kg)	2.00+2	Mecklenburg *et al*.(2002)
Lipid Content (%)	11%	
NLOM Content (%)	20%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	4.85E-04	Arnot and Gobas (2004)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	FISH - 12	
Species name	Chinook salmon (*Oncorhynchus tshawytscha*)	
Weight (kg)	1.09E+01	Cullon *et al*. (2009)
Lipid Content (%)	14.1	Cullon *et al*. (2009)
NLOM Content (%)	20%	Arnot and Gobas (2004)
Fraction of Respired Pore Water (Pw)	0.0%	Based on Gobas and Arnot (2010)
ED - Constant A	8.50E-08	Derived from Arnot and Gobas(2004)
ED - Constant B	2.00E+00	Arnot and Gobas (2004)
Growth Rate Constant - kG (1/day)	8.69E-04	Derived from Bigler et al. (1995)
Metabolic Transformation Rate Constant - kM (1/day)	0.00E+00	Arnot and Gobas (2004)
Lipid Digestion Efficiency (εL)	92.0%	Arnot and Gobas (2004)
NLOM Digestion Efficiency (εN)	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (εW)	55.0%	Arnot and Gobas (2004)

SPECIES	Mammal - 1	
Species Name	*Steller sea lion (Male)*	
Weight (kg)	8.00E+02	Olesiuk (2008)
Lipid Content (%)	15.0%	This thesis (see Chapter VI)
NLOM Content (%)	20.0%	Gobas and Arnot (2010)
E_D - Constant A	1.00E-09	Moser and McLachlan (2002 & 2001)
E_D - Constant B	1.03E+00	Moser and McLachlan (2002 & 2001)
Lung Respiration Rate (G_V) - (L/day)	1.81E+05	Derived from Hickie *et al.* (1999)
Food Ingestion Rate - (G_D) (kg-food/day)	3.36E+01	Olesiuk (2008)
Growth Rate Constant - k_G (1/day)	1.95E-04	Winship *et al*. (2001); Winship *et al*. (2006)
Urinary Excretion Rate Constant - (G_U) (L/day)	1.73E+00	Estimated from Smith (1936)

PARAMETER	VALUE/INPUT	REFERENCE
Lipid Digestion Efficiency (ε_L)	98.0%	Based on Rosen and Trites (2000); Moser and McLachlan (2002 & 2001); Kelly and Gobas (2003)
NLOM Digestion Efficiency (ε_N)	75.0%	Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)
SPECIES	**Mammal - 2**	
Species Name	*Steller sea lion (Female)*	
Weight (kg)	3.00E+02	Olesiuk (2008)
Lipid Content (%)	36.0%	This thesis (see Chapter VI)
NLOM Content (%)	20.0%	Gobas and Arnot (2010)
E_D - Constant A	1.00E-09	Derived from Moser and McLachlan (2002 & 2001)
E_D - Constant B	1.03E+00	Derived from Moser and McLachlan (2002 & 2001)
Lung Respiration Rate (G_V) - (L/day)	8.65E+04	Derived from Hickie *et al.* (1999)
Food Ingestion Rate - (G_D) (kg-food/day)	2.01E+01	Olesiuk (2008)
Growth Rate Constant - k_G (1/day)	7.88E-03	Based on Olesiuk (2008)
Urinary Excretion Rate Constant - (G_U) (L/day)	1.03E+00	Estimated from Smith (1936)
Lactation Rate Constant - (G_L) (L/day)	1.84E+00	Derived from Cottrell *et al.* (2002), Bowen *et al*. (2001); Bowen *et al*. (1992)
Lipid Content Fetus (LFetus) (%)	11.0%	Derived from Cottrell *et al.* (2002), Bowen *et al*. (2001); Bowen *et al*. (1992)
NLOM Content Fetus (NLOMFetus) (%)	20.0%	Arnot and Gobas (2004); Kelly and Gobas (2003)
WC Fetus (WCFetus) (%)	69.0%	Gobas and Arnot (2010); Kelly and Gobas (2003)
Weight - Fetus (Vfetus) (kg)	2.00	This thesis (see Chapter VI)
Lipid Digestion Efficiency (ε_L)	98.0%	Based on Rosen and Trites (2000); Moser and McLachlan (2002 & 2001); Kelly and Gobas (2003)
NLOM Digestion Efficiency (ε_N)	75.0%	Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)
SPECIES	**Mammal - 3**	
Species Name	*Juvenile Steller sea lion*	
Weight (kg)	2.12E+01	This thesis (see Chapter VI)
Lipid Content (%)	25.0%	This thesis (see Chapter VI)
NLOM Content (%)	20.0%	Gobas and Arnot (2010)
E_D - Constant A	1.00E-09	Derived from Moser and McLachlan (2002 & 2001)
E_D - Constant B	1.03E+00	Derived from Moser and McLachlan (2002 & 2001)

PARAMETER	VALUE/INPUT	REFERENCE
Lung Respiration Rate (G_V) - (L/day)	6.67E+04	Derived from Hickie *et al.* (1999)
Food Ingestion Rate - (G_D) (kg-food/day)	1.48E+01	Winship *et al*. (2006)
Growth Rate Constant - k_G (1/day)	7.39E-04	Based on Winship *et al.* (2001); Winship *et al*. (2006)
Urinary Excretion Rate Constant - (G_U) (L/day)	7.62E-01	Estimated from Smith (1936)
Lipid Digestion Efficiency (ε_L)	98.0%	Based on Rosen and Trites (2000); Moser and McLachlan (2002 & 2001); Kelly and Gobas (2003)
NLOM Digestion Efficiency (ε_N)	75.0%	Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)
SPECIES	**Mammal - 4**	
Species Name	*Steller sea lion pup*	
Weight (kg)	2.30E+01	Reeves *et al.* (2002)
Lipid Content (%)	45.0%	This thesis (see Chapter VI)
NLOM Content (%)	20.0%	Gobas and Arnot (2010)
E_D - Constant A	1.00E-09	Moser and McLachlan (2002 & 2001)
E_D - Constant B	1.03E+00	Derived from Moser and McLachlan (2002 & 2001)
Lung Respiration Rate (G_V) - (L/day)	7.56E+03	Derived from Hickie *et al.* (1999)
Food Ingestion Rate - (G_D) (kg-food/day)	1.84E+00	Winship *et al.* (2006)
Growth Rate Constant - k_G (1/day)	2.09E-02	Brandon *et al*. (2005)
Urinary Excretion Rate Constant - (G_U) (L/day)	5.56E-02	Estimated from Smith (1936)
Lipid Content Milk (Lmilk) (%)	45.0%	Bowen *et al.* (1992)
NLOM Content Milk (NLOMmilk) (%)	10.0%	Bowen *et al*. (1992)
WC Milk (WCmilk) (%)	65.0%	Bowen *et al*. (1992)
Lipid Digestion Efficiency (ε_L)	98.0%	Based on Rosen and Trites (2000); Moser and McLachlan (2002 & 2001); Kelly and Gobas (2003)
NLOM Digestion Efficiency (ε_N)	75.0%	Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)

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Appendix F-6a Sediment and water PCB congener concentrations in the outer coast area included in the model.

PCB congener	Sediment concentration (ng/kg dry weight)	Variability (SD)	Total water concentration (ng/L)
PCB 8	30.67	43.37	1.49E-03
PCB 18	15.10	21.35	5.74E-04
PCB 28	35.04	49.55	8.53E-04
PCB 31	20.14	28.48	4.26E-04
PCB 33	16.60	23.47	4.10E-04
PCB 44	15.33	21.68	3.04E-04
PCB 49	16.38	23.16	2.77E-04
PCB 52	25.12	35.53	4.42E-04
PCB 56	20.13	28.46	3.06E-04
PCB 60	5.75	8.13	7.75E-05
PCB 66	19.56	27.66	3.06E-04
PCB 70	25.16	35.58	3.51E-04
PCB 74	9.96	14.09	1.38E-04
PCB 87	12.32	16.72	1.26E-04
PCB 95	26.15	36.97	3.79E-04
PCB 99	24.84	32.24	2.49E-04
PCB 101	39.38	51.26	4.23E-04
PCB 105	14.50	18.67	8.36E-05
PCB 110	27.11	36.74	2.90E-04
PCB 118	37.95	49.67	2.88E-04
PCB 128	7.62	9.38	4.49E-05
PCB 132	11.84	15.29	9.68E-05
PCB 138	53.96	67.90	1.94E-04
PCB 141	4.34	5.51	2.69E-05
PCB 149	33.32	44.48	2.46E-04
PCB 151	10.46	14.27	7.96E-05
PCB 153	48.48	60.18	2.54E-04
PCB 156	2.69	3.44	1.27E-05
PCB 158	2.60	3.46	1.45E-05
PCB 170	9.80	10.85	3.64E-05
PCB 174	9.11	10.66	3.96E-05
PCB 177	9.13	11.08	4.09E-05
PCB 180	14.55	18.13	5.37E-05
PCB 183	4.95	5.46	1.94E-05
PCB 187	18.91	23.71	7.65E-05
PCB 194	4.56	5.20	8.12E-06
PCB 195	2.10	2.47	5.41E-06
PCB 201	9.26	11.05	2.24E-05

Table F-6b Sediment and water PCB congener concentrations in the Queen Charlotte Strait included in the model.

PCB congener	Sediment concentration (ng/kg dry weight)	Variability (SD)	Total water concentration (ng/L)
PCB 8	30.67	43.37	1.49E-03
PCB 18	15.10	21.35	5.74E-04
PCB 28	35.04	49.55	8.53E-04
PCB 31	20.14	28.48	4.26E-04
PCB 33	16.60	23.47	4.10E-04
PCB 44	15.33	21.68	3.04E-04
PCB 49	16.38	23.16	2.77E-04
PCB 52	25.12	35.53	4.42E-04
PCB 56	20.13	28.46	3.06E-04
PCB 60	5.75	8.13	7.75E-05
PCB 66	19.56	27.66	3.06E-04
PCB 70	25.16	35.58	3.51E-04
PCB 74	9.96	14.09	1.38E-04
PCB 87	12.32	16.72	1.26E-04
PCB 95	26.15	36.97	3.79E-04
PCB 99	24.84	32.24	2.49E-04
PCB 101	39.38	51.26	4.23E-04
PCB 105	14.50	18.67	8.36E-05
PCB 110	27.11	36.74	2.90E-04
PCB 118	37.95	49.67	2.88E-04
PCB 128	7.62	9.38	4.49E-05
PCB 132	11.84	15.29	9.68E-05
PCB 138	53.96	67.90	1.94E-04
PCB 141	4.34	5.51	2.69E-05
PCB 149	33.32	44.48	2.46E-04
PCB 151	10.46	14.27	7.96E-05
PCB 153	48.48	60.18	2.54E-04
PCB 156	2.69	3.44	1.27E-05
PCB 158	2.60	3.46	1.45E-05
PCB 170	9.80	10.85	3.64E-05
PCB 174	9.11	10.66	3.96E-05
PCB 177	9.13	11.08	4.09E-05
PCB 180	14.55	18.13	5.37E-05
PCB 183	4.95	5.46	1.94E-05
PCB 187	18.91	23.71	7.65E-05
PCB 194	4.56	5.20	8.12E-06
PCB 195	2.10	2.47	5.41E-06
PCB 201	9.26	11.05	2.24E-05

Table F-6c Sediment and water PCB congener concentrations in the northern resident killer whale Critical Habitat included in the model.

PCB congener	Sediment concentration (ng/kg dry weight)	Variability (SD)	Total water concentration (ng/L)
PCB 8	9.76	13.80	3.33E-04
PCB 18	4.83	6.83	1.29E-04
PCB 28	20.03	28.33	3.43E-04
PCB 31	8.80	12.45	1.31E-04
PCB 33	6.18	8.74	1.07E-04
PCB 44	7.39	10.45	1.03E-04
PCB 49	6.83	9.66	8.10E-05
PCB 52	11.29	15.97	1.40E-04
PCB 56	12.66	17.90	1.35E-04
PCB 60	4.48	5.46	4.24E-05
PCB 66	14.72	18.38	1.62E-04
PCB 70	16.58	21.40	1.62E-04
PCB 74	6.74	8.96	6.56E-05
PCB 87	9.60	12.58	6.88E-05
PCB 95	14.85	19.98	1.51E-04
PCB 99	18.83	19.66	1.33E-04
PCB 101	24.51	28.76	1.85E-04
PCB 105	11.15	11.73	4.52E-05
PCB 110	17.96	21.13	1.35E-04
PCB 118	30.53	33.56	1.63E-04
PCB 128	6.55	6.21	2.71E-05
PCB 132	7.74	8.16	4.45E-05
PCB 138	42.99	45.86	1.09E-04
PCB 141	2.86	4.05	1.25E-05
PCB 149	20.85	25.80	1.08E-04
PCB 151	6.16	8.56	3.29E-05
PCB 153	32.25	33.23	1.19E-04
PCB 156	1.83	2.52	6.06E-06
PCB 158	1.71	1.99	6.66E-06
PCB 170	6.07	5.48	1.58E-05
PCB 174	7.68	8.40	2.34E-05
PCB 177	6.12	6.73	1.93E-05
PCB 180	10.01	10.06	2.60E-05
PCB 183	4.56	3.75	1.26E-05
PCB 187	14.85	15.95	4.22E-05
PCB 194	2.89	2.78	3.61E-06
PCB 195	2.21	1.14	4.01E-06
PCB 201	6.79	6.66	1.15E-05

Table F-6d Sediment and water PCB congener concentrations in the Strait of Georgia included in the model.

PCB congener		Sediment concentration (ng/kg dry weight)	Variability (SD)	Total water concentration (ng/L)
PCB	**8**	6.88	217.12	6.68E-04
PCB	**18**	6.88	75.99	5.24E-04
PCB	**28**	61.92	54.28	3.01E-03
PCB	**44**	37.84	108.56	1.50E-03
PCB	**49**	32.68	130.27	1.10E-03
PCB	**52**	34.40	173.69	1.21E-03
PCB	**66**	89.45	54.28	2.80E-03
PCB	**74**	22.36	43.42	6.20E-04
PCB	**95**	51.92	74.64	1.51E-03
PCB	**99**	29.24	119.41	5.87E-04
PCB	**101**	76.15	111.33	1.63E-03
PCB	**105**	29.24	119.41	3.37E-04
PCB	**110**	74.19	98.98	1.59E-03
PCB	**118**	86.52	124.39	1.31E-03
PCB	**128**	18.92	119.41	2.23E-04
PCB	**138**	110.81	134.66	7.98E-04
PCB	**149**	61.92	162.84	9.15E-04
PCB	**151**	6.88	303.97	1.05E-04
PCB	**153**	84.29	119.41	8.83E-04
PCB	**156**	3.44	54.28	3.23E-05
PCB	**170**	15.48	271.40	1.15E-04
PCB	**177**	15.48	173.69	1.39E-04
PCB	**180**	24.08	97.70	1.78E-04
PCB	**183**	13.76	0.00	1.08E-04
PCB	**187**	43.00	86.85	3.48E-04
PCB	**194**	5.16	0.000	1.84E-05
PCB	**203**	5.16	0.000	2.42E-05

Table F-6e Sediment and water PCB congener concentrations in the southern resident killer whale Critical Habitat in Canada included in the model.

PCB congener		Sediment concentration (ng/kg dry weight)	Variability (SD)	Total water concentration (ng/L)
PCB	**8**	3.32	46.02	3.22E-04
PCB	**18**	3.32	16.11	2.53E-04
PCB	**28**	29.88	11.51	1.45E-03
PCB	**44**	18.26	23.01	7.25E-04
PCB	**49**	15.77	27.61	5.32E-04
PCB	**52**	16.60	36.82	5.84E-04
PCB	**66**	43.16	11.51	1.35E-03
PCB	**74**	10.79	9.20	2.99E-04
PCB	**95**	26.00	17.93	7.54E-04
PCB	**99**	14.11	25.31	2.83E-04
PCB	**101**	38.50	27.35	8.27E-04
PCB	**105**	14.11	25.31	1.63E-04
PCB	**110**	38.83	23.54	8.30E-04
PCB	**118**	45.00	33.36	6.84E-04
PCB	**128**	9.13	25.31	1.08E-04
PCB	**138**	56.67	36.90	4.08E-04
PCB	**149**	29.88	34.52	4.42E-04
PCB	**151**	3.32	64.43	5.05E-05
PCB	**153**	40.67	25.31	4.26E-04
PCB	**156**	1.66	11.51	1.56E-05
PCB	**170**	7.47	57.53	5.55E-05
PCB	**177**	7.47	36.82	6.69E-05
PCB	**180**	11.62	20.71	8.58E-05
PCB	**183**	6.64	0.00	5.21E-05
PCB	**187**	20.75	18.41	1.68E-04
PCB	**194**	2.49	0.00	8.88E-06
PCB	**203**	2.49	0.00	1.17E-05

Table F-6f Sediment and water PCB congener concentrations in the southern resident killer whale Critical Habitat in the USA (summer core & Juan de Fuca Strait) included in the model.

PCB congener		Sediment concentration (ng/kg dry weight)	Variability (SD)	Total water concentration (ng/L)
PCB	**8**	1.60E+02		2.04E-02
PCB	**18**	1.60E+02		1.60E-02
PCB	**28**	1.70E+02		1.09E-02
PCB	**44**	2.30E+02		1.20E-02
PCB	**49**	1.42E+02	1.42E+02	6.32E-03
PCB	**52**	3.20E+02	1.41E+01	1.48E-02
PCB	**66**	1.60E+02		6.59E-03
PCB	**74**	1.33E+02	4.75E+01	4.87E-03
PCB	**95**			0.00E+00
PCB	**99**	1.27E+02		3.36E-03
PCB	**101**	3.35E+02	3.54E+01	9.46E-03
PCB	**105**	2.90E+02		4.40E-03
PCB	**110**	3.10E+02		8.72E-03
PCB	**118**	3.55E+02	2.12E+01	7.10E-03
PCB	**128**	2.40E+02		3.72E-03
PCB	**138**	3.63E+02	1.16E+02	3.44E-03
PCB	**149**	2.69E+02	1.78E+02	5.24E-03
PCB	**151**	2.99E+01		6.00E-04
PCB	**153**	3.67E+02	1.31E+02	5.05E-03
PCB	**156**	1.50E+01	5.93E+01	1.85E-04
PCB	**170**	6.75E+02	1.34E+02	6.60E-03
PCB	**177**	6.73E+01		7.94E-04
PCB	**180**	6.50E+02		6.31E-03
PCB	**183**	5.99E+01		6.19E-04
PCB	**187**	3.85E+02	9.19E+01	4.10E-03
PCB	**194**	2.24E+01		1.05E-04
PCB	**203**	2.24E+01		1.39E-04

Table F-6g Sediment and water PCB congener concentrations in the southern resident killer whale Critical Habitat in the USA (Puget Sound) included in the model.

PCB congener	Sediment concentration (ng/kg dry weight)	Variability (SD)	Total water concentration (ng/L)
PCB 8	2.90E+02	1.00E+00	2.43E-02
PCB 18	5.52E+02	1.15E+00	3.62E-02
PCB 28	3.36E+03	5.52E+00	1.41E-01
PCB 44	6.09E+03	2.13E+01	2.09E-01
PCB 49	1.54E+03	1.60E+01	4.48E-02
PCB 52	3.29E+03	4.55E+00	1.00E-01
PCB 66	3.53E+03	2.23E+01	9.54E-02
PCB 74	1.05E+03	5.33E+00	2.52E-02
PCB 95	0.00E+00	0.00E+00	0.00E+00
PCB 99	1.38E+03	1.47E+01	2.39E-02
PCB 101	4.30E+03	1.14E+01	7.96E-02
PCB 105	8.34E+03	1.33E+01	8.29E-02
PCB 110	6.70E+02	1.00E+00	1.24E-02
PCB 118	2.76E+03	1.02E+01	3.61E-02
PCB 128	4.17E+03	1.82E+01	4.24E-02
PCB 138	4.15E+03	1.13E+01	2.58E-02
PCB 149	2.92E+03	2.00E+01	3.72E-02
PCB 151	3.24E+02	3.73E+01	4.26E-03
PCB 153	3.97E+03	1.47E+01	3.59E-02
PCB 156	1.62E+02	6.66E+00	1.31E-03
PCB 170	4.88E+03	1.35E+01	3.12E-02
PCB 177	7.29E+02	2.13E+01	5.63E-03
PCB 180	4.66E+03	2.48E+01	2.96E-02
PCB 183	6.48E+02	0.00E+00	4.39E-03
PCB 187	1.01E+04	1.61E+01	7.07E-02
PCB 194	2.43E+02	0.00E+00	7.47E-04
PCB 203	2.43E+02	0.00E+00	9.84E-04

Table F-7a Data for PCB congener concentrations predicted in male killer whale with the PCB food web bioaccumulation model using initial diet and updated diet compositions for the northern resident killer whale Critical Habitat.

PCB congeners	NRKW Critical Habitat (male)			
	Initial Diet	Updated Diet	Initial Diet	Updated Diet
	PCB (µg/kg lipid)	PCB (µg/kg lipid)	Log PCB (µg/kg lipid)	Log PCB (µg/kg lipid)
8	3.19.E+01	2.91.E+01	1.50	1.46
18	2.29.E+01	2.10.E+01	1.36	1.32
28	2.15.E+02	1.98.E+02	2.33	2.30
31	1.16.E+02	1.07.E+02	2.07	2.03
33	6.66.E+01	6.13.E+01	1.82	1.79
44	1.12.E+02	1.03.E+02	2.05	2.01
49	1.23.E+02	1.13.E+02	2.09	2.05
52	1.94.E+02	1.78.E+02	2.29	2.25
56	2.66.E+02	2.43.E+02	2.42	2.39
60	1.08.E+02	9.83.E+01	2.03	1.99
66	3.02.E+02	2.76.E+02	2.48	2.44
70	3.84.E+02	3.49.E+02	2.58	2.54
74	1.57.E+02	1.43.E+02	2.20	2.16
87	3.01.E+02	2.70.E+02	2.48	2.43
95	3.31.E+02	3.02.E+02	2.52	2.48
99	5.98.E+02	5.36.E+02	2.78	2.73
101	7.32.E+02	6.59.E+02	2.86	2.82
105	4.63.E+02	3.99.E+02	2.67	2.60
110	5.41.E+02	4.87.E+02	2.73	2.69
118	1.17.E+03	1.03.E+03	3.07	3.01
128	2.75.E+02	2.38.E+02	2.44	2.38
132	2.86.E+02	2.53.E+02	2.46	2.40
138	1.56.E+03	1.29.E+03	3.19	3.11
141	1.19.E+02	1.03.E+02	2.08	2.01
149	8.14.E+02	7.16.E+02	2.91	2.86
151	2.37.E+02	2.09.E+02	2.37	2.32
153	1.36.E+03	1.16.E+03	3.13	3.07
156	7.63.E+01	6.47.E+01	1.88	1.81
158	7.20.E+01	6.20.E+01	1.86	1.79
170	2.31.E+02	1.92.E+02	2.36	2.28
174	3.17.E+02	2.67.E+02	2.50	2.43
177	2.56.E+02	2.16.E+02	2.41	2.33
180	3.79.E+02	3.14.E+02	2.58	2.50
183	1.80.E+02	1.50.E+02	2.25	2.18
187	5.95.E+02	4.98.E+02	2.77	2.70
194	3.68.E+01	2.92.E+01	1.57	1.47
195	5.81.E+01	4.68.E+01	1.76	1.67
201	1.61.E+02	1.29.E+02	2.21	2.11
Mean	**3.48E+02**	**3.04E+02**	**2.34**	**2.29**
SD	**3.59E+02**	**3.07E+02**	**0.44**	**0.44**

Table F-7b Data for PCB congener concentrations predicted in female killer whale with the PCB food web bioaccumulation model using initial diet and updated diet compositions for the northern resident killer whale Critical Habitat.

PCB congeners	NRKW Critical Habitat (female)			
	Initial Diet	Updated Diet	Initial Diet	Updated Diet
	PCB (µg/kg lipid)	PCB (µg/kg lipid)	Log PCB (µg/kg lipid)	Log PCB (µg/kg lipid)
8	6.07E+00	5.53E+00	0.78	0.74
18	4.38E+00	4.02E+00	0.64	0.60
28	3.60E+01	3.32E+01	1.56	1.52
31	1.91E+01	1.76E+01	1.28	1.25
33	1.09E+01	1.01E+01	1.04	1.00
44	1.76E+01	1.62E+01	1.25	1.21
49	1.97E+01	1.81E+01	1.30	1.26
52	3.11E+01	2.85E+01	1.49	1.46
56	4.15E+01	3.79E+01	1.62	1.58
60	1.68E+01	1.52E+01	1.22	1.18
66	4.68E+01	4.28E+01	1.67	1.63
70	5.97E+01	5.44E+01	1.78	1.74
74	2.44E+01	2.23E+01	1.39	1.35
87	4.66E+01	4.19E+01	1.67	1.62
95	5.15E+01	4.70E+01	1.71	1.67
99	9.27E+01	8.31E+01	1.97	1.92
101	1.14E+02	1.03E+02	2.06	2.01
105	7.16E+01	6.17E+01	1.85	1.79
110	8.39E+01	7.55E+01	1.92	1.88
118	1.80E+02	1.59E+02	2.26	2.20
128	4.24E+01	3.67E+01	1.63	1.56
132	4.42E+01	3.92E+01	1.65	1.59
138	2.40E+02	1.99E+02	2.38	2.30
141	1.84E+01	1.60E+01	1.26	1.20
149	1.26E+02	1.11E+02	2.10	2.04
151	3.66E+01	3.23E+01	1.56	1.51
153	2.10E+02	1.80E+02	2.32	2.26
156	1.18E+01	9.98E+00	1.07	1.00
158	1.11E+01	9.56E+00	1.05	0.98
170	3.55E+01	2.95E+01	1.55	1.47
174	4.89E+01	4.12E+01	1.69	1.62
177	3.94E+01	3.33E+01	1.60	1.52
180	5.84E+01	4.85E+01	1.77	1.69
183	2.77E+01	2.31E+01	1.44	1.36
187	9.17E+01	7.68E+01	1.96	1.89
194	5.64E+00	4.49E+00	0.75	0.65
195	8.94E+00	7.20E+00	0.95	0.86
201	2.47E+01	1.98E+01	1.39	1.30
Mean	**5.41E+01**	**4.72E+01**	**1.54**	**1.48**
SD	**5.54E+01**	**4.73E+01**	**0.43**	**0.43**

Table F-7c Data for PCB congener concentrations predicted in male killer whale with the PCB food web bioaccumulation model using initial diet and updated diet compositions for the outer coast area.

	Outer Coast (male)			
PCB congeners	**Initial Diet**	**Updated Diet**	**Initial Diet**	**Updated Diet**
	PCB (µg/kg lipid)	**PCB (µg/kg lipid)**	**Log PCB (µg/kg lipid)**	**Log PCB (µg/kg lipid)**
8	2.24E+02	1.47E+02	2.351	2.168
18	1.68E+02	1.05E+02	2.226	2.022
28	9.74E+02	5.49E+02	2.989	2.739
31	7.07E+02	3.85E+02	2.849	2.586
33	4.61E+02	2.60E+02	2.664	2.416
44	6.26E+02	3.36E+02	2.797	2.527
49	8.15E+02	4.23E+02	2.911	2.627
52	1.19E+03	6.21E+02	3.074	2.793
56	1.19E+03	6.04E+02	3.074	2.781
60	3.95E+02	1.97E+02	2.596	2.294
66	1.12E+03	5.74E+02	3.050	2.759
70	1.65E+03	8.28E+02	3.217	2.918
74	6.60E+02	3.31E+02	2.820	2.520
87	1.12E+03	5.39E+02	3.049	2.732
95	1.65E+03	8.30E+02	3.216	2.919
99	2.29E+03	1.10E+03	3.360	3.042
101	3.40E+03	1.65E+03	3.532	3.217
105	1.67E+03	7.96E+02	3.223	2.901
110	2.36E+03	1.14E+03	3.374	3.058
118	4.17E+03	1.98E+03	3.620	3.296
128	8.93E+02	4.24E+02	2.951	2.628
132	1.27E+03	6.02E+02	3.104	2.780
138	4.69E+03	2.38E+03	3.671	3.376
141	5.08E+02	2.41E+02	2.706	2.382
149	3.75E+03	1.77E+03	3.574	3.249
151	1.16E+03	5.50E+02	3.065	2.741
153	5.57E+03	2.67E+03	3.746	3.426
156	2.96E+02	1.43E+02	2.471	2.157
158	3.03E+02	1.45E+02	2.482	2.160
170	9.09E+02	4.56E+02	2.959	2.659
174	9.75E+02	4.76E+02	2.989	2.678
177	9.97E+02	4.85E+02	2.999	2.685
180	1.34E+03	6.74E+02	3.128	2.829
183	4.87E+02	2.41E+02	2.687	2.383
187	1.91E+03	9.44E+02	3.282	2.975
194	9.74E+01	6.20E+01	1.989	1.792
195	1.14E+02	6.25E+01	2.056	1.796
201	4.37E+02	2.45E+02	2.640	2.390
Mean	**1.38E+03**	**6.83E+02**	**2.96E+00**	**2.67E+00**
SD	**1.32.E+03**	**6.32.E+02**	**4.24.E-01**	**4.02.E-01**

Table F-7d Data for PCB congener concentrations predicted in female killer whale with the PCB food web bioaccumulation model using initial diet and updated diet compositions for the outer coast area.

	Outer Coast (Female)			
PCB congeners	**Initial Diet**	**Updated Diet**	**Initial Diet**	**Updated Diet**
	PCB µg/kg lipid	**PCB µg/kg lipid**	**Log PCB µg/kg lipid**	**Log PCB µg/kg lipid**
8	5.01E+01	3.29E+01	1.700	1.517
18	3.78E+01	2.36E+01	1.577	1.373
28	1.91E+02	1.08E+02	2.282	2.032
31	1.37E+02	7.45E+01	2.136	1.872
33	8.90E+01	5.03E+01	1.949	1.701
44	1.16E+02	6.21E+01	2.063	1.793
49	1.54E+02	7.98E+01	2.187	1.902
52	2.23E+02	1.17E+02	2.348	2.067
56	2.17E+02	1.11E+02	2.337	2.044
60	7.18E+01	3.58E+01	1.856	1.554
66	2.04E+02	1.04E+02	2.310	2.019
70	3.02E+02	1.51E+02	2.480	2.180
74	1.20E+02	6.03E+01	2.080	1.781
87	2.04E+02	9.81E+01	2.309	1.992
95	3.01E+02	1.52E+02	2.478	2.181
99	4.16E+02	2.00E+02	2.620	2.302
101	6.22E+02	3.01E+02	2.794	2.479
105	3.03E+02	1.44E+02	2.481	2.159
110	4.31E+02	2.08E+02	2.634	2.319
118	7.58E+02	3.59E+02	2.880	2.555
128	1.62E+02	7.69E+01	2.209	1.886
132	2.30E+02	1.09E+02	2.363	2.039
138	8.50E+02	4.31E+02	2.929	2.634
141	9.21E+01	4.36E+01	1.964	1.640
149	6.80E+02	3.22E+02	2.833	2.507
151	2.11E+02	9.98E+01	2.324	1.999
153	1.01E+03	4.84E+02	3.005	2.685
156	5.36E+01	2.60E+01	1.729	1.414
158	5.49E+01	2.62E+01	1.740	1.418
170	1.65E+02	8.25E+01	2.216	1.916
174	1.77E+02	8.62E+01	2.247	1.935
177	1.81E+02	8.77E+01	2.257	1.943
180	2.43E+02	1.22E+02	2.386	2.086
183	8.81E+01	4.37E+01	1.945	1.640
187	3.46E+02	1.71E+02	2.539	2.233
194	1.75E+01	1.12E+01	1.244	1.048
195	2.05E+01	1.13E+01	1.313	1.053
201	7.89E+01	4.43E+01	1.897	1.647
Mean	**2.53E+02**	**1.25E+02**	**2.23E+00**	**1.94E+00**
SD	**2.38.E+02**	**1.14.E+02**	**4.17.E-01**	**3.96.E-01**

Table F-8a Predicted PCB congener concentrations (ng/kg wet weight) of fish-diet items for resident killer whales to represent the outer coast area included in the model.

PCB congener	Chinook salmon	Halibut	Sablefish
	Predicted concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)
PCB 8	256.59	163.17	170.93
PCB 18	194.36	118.61	120.95
PCB 28	949.46	528.87	511.08
PCB 31	676.46	366.15	347.89
PCB 33	439.85	245.73	237.87
PCB 44	565.69	302.35	285.15
PCB 49	757.08	393.01	364.08
PCB 52	1097.84	573.97	534.09
PCB 56	1063.70	543.22	497.98
PCB 60	351.34	176.51	160.02
PCB 66	996.86	511.28	470.02
PCB 70	1476.98	745.24	677.66
PCB 74	588.84	296.88	269.82
PCB 87	997.74	488.63	433.98
PCB 95	1471.27	745.65	680.35
PCB 99	2039.47	997.99	885.54
PCB 101	3051.29	1498.84	1335.48
PCB 105	1485.20	742.18	645.98
PCB 110	2108.34	1035.39	922.30
PCB 118	3714.63	1814.14	1588.99
PCB 128	793.65	394.16	343.06
PCB 132	1128.72	549.19	482.28
PCB 138	4182.10	2306.99	2017.09
PCB 141	451.48	222.95	194.21
PCB 149	3332.52	1625.64	1422.28
PCB 151	1033.33	503.52	440.97
PCB 153	4956.14	2502.96	2176.19
PCB 156	263.19	135.58	117.90
PCB 158	269.40	134.83	117.27
PCB 170	808.86	439.62	383.69
PCB 174	866.40	451.94	393.09
PCB 177	885.78	458.96	399.06
PCB 180	1193.27	649.73	567.17
PCB 183	432.47	231.40	201.65
PCB 187	1699.95	902.43	785.87
PCB 194	87.65	63.59	59.00
PCB 195	101.40	62.27	55.38
PCB 201	389.85	246.03	220.13
Total	47159.14	24169.61	21516.45

Table F-8b Predicted PCB congener concentrations (ng/kg wet weight) of fish-diet items for resident killer whales to represent the Queen Charlotte Strait included in the model.

PCB congener	Chinook salmon	Halibut	Sablefish
	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/·kg wet weight)	Predicted Concentration (ng/kg wet weight)
PCB 8	182.59	159.35	170.93
PCB 18	130.98	114.85	120.95
PCB 28	575.30	503.23	511.08
PCB 31	397.59	346.53	347.89
PCB 33	267.37	233.95	237.87
PCB 44	328.22	285.47	285.15
PCB 49	426.72	369.03	364.08
PCB 52	623.10	539.69	534.09
PCB 56	590.26	508.46	497.98
PCB 60	192.05	164.68	160.02
PCB 66	555.42	478.97	470.02
PCB 70	810.50	695.90	677.66
PCB 74	322.90	277.19	269.82
PCB 87	533.69	453.37	433.98
PCB 95	810.69	696.93	680.35
PCB 99	1090.26	925.79	885.54
PCB 101	1635.94	1391.77	1335.48
PCB 105	814.92	688.16	645.98
PCB 110	1130.15	961.36	922.30
PCB 118	1988.27	1679.58	1588.99
PCB 128	432.88	365.24	343.06
PCB 132	601.59	508.43	482.28
PCB 138	2534.03	2159.74	2017.09
PCB 141	244.78	206.49	194.21
PCB 149	1782.56	1504.65	1422.28
PCB 151	551.96	466.05	440.97
PCB 153	2749.95	2322.84	2176.19
PCB 156	148.99	126.07	117.90
PCB 158	148.10	125.02	117.27
PCB 170	483.11	410.94	383.69
PCB 174	496.78	420.72	393.09
PCB 177	504.50	426.97	399.06
PCB 180	714.00	607.46	567.17
PCB 183	254.33	215.97	201.65
PCB 187	991.89	841.57	785.87
PCB 194	69.42	61.01	59.00
PCB 195	68.33	58.89	55.38
PCB 201	269.91	233.28	220.13
Total	26454.03	22535.61	21516.45

Table F-8c Predicted PCB congener concentrations (ng/kg wet weight) of fish-diet items for resident killer whales to represent the northern resident killer whale Critical Habitat included in the model.

PCB congener	Chinook salmon	Halibut	Sablefish
	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)
PCB 8	36.13	31.57	34.71
PCB 18	26.17	23.06	24.85
PCB 28	207.14	183.43	190.13
PCB 31	109.71	96.98	99.25
PCB 33	62.66	55.50	57.60
PCB 44	99.94	88.22	89.77
PCB 49	112.57	98.95	99.26
PCB 52	177.15	155.90	156.96
PCB 56	235.08	205.92	204.79
PCB 60	94.84	82.70	81.48
PCB 66	264.80	232.18	231.46
PCB 70	338.38	295.47	291.85
PCB 74	138.37	120.80	119.26
PCB 87	264.23	228.01	220.53
PCB 95	291.60	254.93	252.55
PCB 99	524.87	452.68	437.43
PCB 101	646.28	558.70	541.99
PCB 105	406.58	346.58	327.49
PCB 110	475.28	410.81	398.43
PCB 118	1022.47	874.88	834.13
PCB 128	240.87	205.21	194.02
PCB 132	250.68	214.77	205.41
PCB 138	1373.83	1174.67	1104.90
PCB 141	104.33	88.92	84.20
PCB 149	713.62	609.92	580.90
PCB 151	207.56	177.52	169.27
PCB 153	1194.92	1017.52	959.47
PCB 156	66.97	57.05	53.70
PCB 158	63.15	53.78	50.77
PCB 170	202.94	173.22	162.87
PCB 174	278.57	237.22	223.11
PCB 177	224.36	191.00	179.69
PCB 180	333.23	284.46	267.47
PCB 183	157.87	134.62	126.56
PCB 187	522.45	445.30	418.65
PCB 194	32.95	28.85	28.19
PCB 195	51.37	44.24	41.95
PCB 201	142.33	122.85	116.93
Total	11696.22	10058.40	9661.99

Table F-8d. Predicted PCB congener concentrations (ng/kg wet weight) of fish-diet items for resident killer whales to represent the Strait of Georgia included in the model.

PCB congener	Chinook salmon	Halibut	Sablefish
	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)
PCB 8	41.39	56.23	61.54
PCB 18	62.79	83.45	89.31
PCB 28	1190.00	1476.59	1519.86
PCB 44	1000.14	1196.96	1210.71
PCB 49	1095.11	1274.27	1271.69
PCB 52	1086.22	1272.91	1274.66
PCB 66	3328.87	3824.96	3794.30
PCB 74	978.24	1102.41	1083.38
PCB 95	2148.53	2437.96	2403.95
PCB 99	1858.47	2004.18	1926.83
PCB 101	4518.64	4912.57	4742.78
PCB 105	2649.00	2717.52	2539.73
PCB 110	4421.59	4804.56	4637.33
PCB 118	6935.58	7268.20	6879.67
PCB 128	1723.50	1771.03	1656.85
PCB 138	9150.42	9146.44	8428.93
PCB 149	5085.64	5311.84	5023.48
PCB 151	554.28	580.48	549.78
PCB 153	7833.47	7987.70	7437.34
PCB 156	318.23	322.44	299.07
PCB 170	1333.72	1335.18	1230.89
PCB 177	1442.24	1457.63	1349.98
PCB 180	2066.68	2068.32	1906.56
PCB 183	1222.85	1227.50	1132.88
PCB 187	3874.90	3895.47	3597.67
PCB 194	161.51	157.86	148.64
PCB 203	274.00	269.39	249.14
Total	66355.99	69964.02	66446.94

Table F-8e Predicted PCB congener concentrations (ng/kg wet weight) of fish-diet items for resident killer whales to represent the southern resident killer whale Critical Habitat in Canada included in the model.

PCB congener	Chinook salmon	Halibut	Sablefish
	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)
PCB 8	19.97	27.13	29.69
PCB 18	30.30	40.26	43.09
PCB 28	574.16	712.44	733.32
PCB 44	482.55	577.52	584.15
PCB 49	528.38	614.82	613.58
PCB 52	524.09	614.16	615.01
PCB 66	1606.14	1845.50	1830.70
PCB 74	471.99	531.90	522.72
PCB 95	1076.03	1220.98	1203.94
PCB 99	896.69	966.99	929.67
PCB 101	2284.49	2483.65	2397.81
PCB 105	1278.11	1311.17	1225.39
PCB 110	2314.31	2514.76	2427.23
PCB 118	3607.09	3780.08	3578.01
PCB 128	831.57	854.50	799.41
PCB 138	4679.34	4677.31	4310.39
PCB 149	2453.79	2562.96	2423.82
PCB 151	267.48	280.16	265.32
PCB 153	3779.55	3853.97	3588.43
PCB 156	153.54	155.57	144.30
PCB 170	643.50	644.21	593.89
PCB 177	695.86	703.29	651.35
PCB 180	997.15	997.94	919.89
PCB 183	590.01	592.25	546.60
PCB 187	1869.59	1879.51	1735.83
PCB 194	77.93	76.17	71.72
PCB 203	132.20	129.98	120.21
Total	32865.80	34649.15	32905.44

Table F-8f Predicted PCB congener concentrations (ng/kg wet weight) of fish-diet items for resident killer whales to represent the southern resident killer whale Critical Habitat in the USA (summer core & Juan de Fuca Strait) included in the model.

PCB congener	Chinook salmon	Halibut	Sablefish
	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)
PCB 8	1.01.E+03	2.07E+03	2.24E+03
PCB 18	1.61.E+03	3.12E+03	3.30E+03
PCB 28	3.87.E+03	6.67E+03	6.81E+03
PCB 44	7.41.E+03	1.21E+04	1.22E+04
PCB 49	5.93.E+03	9.36E+03	9.29E+03
PCB 52	1.25.E+04	1.99E+04	1.98E+04
PCB 66	7.48.E+03	1.16E+04	1.15E+04
PCB 74	7.44.E+03	1.13E+04	1.10E+04
PCB 95	0.00.E+00	0.00E+00	0.00E+00
PCB 99	1.07.E+04	1.54E+04	1.47E+04
PCB 101	2.61.E+04	3.79E+04	3.65E+04
PCB 105	3.59.E+04	4.82E+04	4.48E+04
PCB 110	2.42.E+04	3.52E+04	3.39E+04
PCB 118	3.84.E+04	5.33E+04	5.02E+04
PCB 128	2.98.E+04	4.01E+04	3.73E+04
PCB 138	4.14.E+04	5.20E+04	4.74E+04
PCB 149	2.99.E+04	4.13E+04	3.89E+04
PCB 151	3.25.E+03	4.51E+03	4.26E+03
PCB 153	4.66.E+04	6.19E+04	5.72E+04
PCB 156	1.90.E+03	2.48E+03	2.28E+03
PCB 170	7.99.E+04	1.01E+05	9.22E+04
PCB 177	8.60.E+03	1.12E+04	1.03E+04
PCB 180	7.67.E+04	9.71E+04	8.84E+04
PCB 183	7.31.E+03	9.33E+03	8.52E+03
PCB 187	4.76.E+04	6.11E+04	5.58E+04
PCB 194	1.01.E+03	1.14E+03	1.04E+03
PCB 203	1.67.E+03	1.97E+03	1.78E+03
Total	5.58.E+05	7.51.E+05	7.02.E+05

Table F-8g Predicted PCB congener concentrations (ng/kg wet weight) of fish-diet items for resident killer whales to represent the southern resident killer whale Critical Habitat in the USA (Puget Sound) included in the model.

PCB congener	Chinook salmon	Halibut	Sablefish
	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)	Predicted Concentration (ng/kg wet weight)
PCB 8	1.41E+03	2.74E+03	2.96E+03
PCB 18	4.18E+03	7.64E+03	8.04E+03
PCB 28	5.35E+04	8.72E+04	8.82E+04
PCB 44	1.32E+05	2.05E+05	2.03E+05
PCB 49	4.15E+04	6.26E+04	6.14E+04
PCB 52	8.41E+04	1.28E+05	1.26E+05
PCB 66	1.05E+05	1.56E+05	1.52E+05
PCB 74	3.64E+04	5.28E+04	5.11E+04
PCB 95	0.00E+00	0.00E+00	0.00E+00
PCB 99	6.68E+04	9.24E+04	8.79E+04
PCB 101	1.96E+05	2.74E+05	2.61E+05
PCB 105	5.52E+05	7.10E+05	6.64E+05
PCB 110	3.06E+04	4.28E+04	4.08E+04
PCB 118	1.64E+05	2.19E+05	2.06E+05
PCB 128	2.77E+05	3.58E+05	3.35E+05
PCB 138	2.46E+05	2.95E+05	2.74E+05
PCB 149	1.77E+05	2.35E+05	2.22E+05
PCB 151	1.94E+04	2.58E+04	2.43E+04
PCB 153	2.68E+05	3.41E+05	3.18E+05
PCB 156	1.08E+04	1.36E+04	1.26E+04
PCB 170	3.01E+05	3.64E+05	3.38E+05
PCB 177	4.89E+04	6.09E+04	5.67E+04
PCB 180	2.86E+05	3.45E+05	3.21E+05
PCB 183	4.13E+04	5.04E+04	4.69E+04
PCB 187	6.56E+05	8.04E+05	7.48E+05
PCB 194	5.29E+03	5.63E+03	5.40E+03
PCB 203	9.12E+03	1.02E+04	9.56E+03
Total	3.81E+06	4.95E+06	4.67E+06

Table F-8h Predicted PCB congener concentrations (ng/kg wet weight) of Pacific herring to represent the levels of the major diet item for Steller Sea lion in the Strait of Georgia included in the model.

PCB congener	Pacific herring
	Predicted Concentration (ng/kg wet weight)
PCB 8	2.41E+01
PCB 18	3.09E+01
PCB 28	4.45E+02
PCB 44	3.37E+02
PCB 49	3.44E+02
PCB 52	3.47E+02
PCB 66	1.02E+03
PCB 74	2.87E+02
PCB 95	6.38E+02
PCB 99	5.05E+02
PCB 101	1.24E+03
PCB 105	7.06E+02
PCB 110	1.21E+03
PCB 118	1.83E+03
PCB 128	4.57E+02
PCB 138	2.73E+03
PCB 149	1.34E+03
PCB 151	1.46E+02
PCB 153	2.11E+03
PCB 156	8.72E+01
PCB 170	3.89E+02
PCB 177	3.97E+02
PCB 180	6.05E+02
PCB 183	3.50E+02
PCB 187	1.10E+03
PCB 194	7.85E+01
PCB 203	1.03E+02
Total	1.89E+04

Table F-9 Data for PCB congener concentrations predicted in Steller sea lions (male and female) with the PCB food web bioaccumulation model using a diet composition, including 80% herring, 6.7% Chinook salmon, 6.7% chum salmon and 6.7% coho salmon. PCB concentrations (wet weight) were lipid normalized using lipid contents in blubber reported for males (15%) and females (36%).

PCB congener		Steller Sea Lion			
		male	female	male	female
		(µg/kg wet weight)	(µg/kg wet weight)	(µg/kg lipid)	(µg/kg lipid)
PCB	**8**	5.22E-02	6.07E-02	3.48E-01	1.69E-01
PCB	**18**	7.02E-02	8.15E-02	4.68E-01	2.26E-01
PCB	**28**	1.10E+00	1.27E+00	7.33E+00	3.54E+00
PCB	**44**	8.61E-01	9.97E-01	5.74E+00	2.77E+00
PCB	**49**	8.98E-01	1.04E+00	5.99E+00	2.89E+00
PCB	**52**	8.13E+00	2.94E+00	5.42E+01	8.18E+00
PCB	**66**	5.53E+00	4.95E+00	3.68E+01	1.38E+01
PCB	**74**	6.94E+00	2.50E+00	4.63E+01	6.94E+00
PCB	**95**	4.93E+00	3.75E+00	3.28E+01	1.04E+01
PCB	**99**	2.02E+01	4.92E+00	1.35E+02	1.37E+01
PCB	**101**	2.15E+01	1.10E+01	1.43E+02	3.06E+01
PCB	**105**	1.90E+00	2.20E+00	1.27E+01	6.10E+00
PCB	**110**	9.58E+00	7.28E+00	6.39E+01	2.02E+01
PCB	**118**	1.14E+01	9.78E+00	7.61E+01	2.72E+01
PCB	**128**	1.95E+01	4.46E+00	1.30E+02	1.24E+01
PCB	**138**	1.09E+02	2.48E+01	7.28E+02	6.88E+01
PCB	**149**	1.46E+01	9.29E+00	9.74E+01	2.58E+01
PCB	**151**	1.66E-01	2.29E-01	1.11E+00	6.36E-01
PCB	**153**	8.90E+01	2.03E+01	5.94E+02	5.64E+01
PCB	**156**	1.13E+00	6.29E-01	7.52E+00	1.75E+00
PCB	**170**	1.57E+01	3.57E+00	1.05E+02	9.92E+00
PCB	**177**	1.44E+01	3.69E+00	9.61E+01	1.02E+01
PCB	**180**	2.44E+01	5.54E+00	1.63E+02	1.54E+01
PCB	**183**	1.43E+01	3.25E+00	9.52E+01	9.01E+00
PCB	**187**	4.19E+01	1.01E+01	2.80E+02	2.81E+01
PCB	**194**	2.66E+00	5.91E-01	1.77E+01	1.64E+00
PCB	**203**	3.63E+00	8.42E-01	2.42E+01	2.34E+00
Total		**4.44E+02**	**1.40E+02**	**2.96E+03**	**3.89E+02**