# BIOACCUMULATIVE CONTAMINANTS IN MARINE MAMMALS: UPTAKE AND EFFECTS

by

Marie Noël
Maîtrise, University of La Rochelle, 2004
Diplôme d'Etudes Approfondies, University of Liège, 2006

A Dissertation Submitted in Partial Fulfillment
of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

in the School of Earth and Ocean Sciences

# Supervisory Committee

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# Abstract

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This thesis provides insights into the transport and fate of contaminants of concern (polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and mercury (Hg)), as well as results on the impacts of these compounds on marine mammal health.

Atmospheric transport is known to be a significant pathway for the delivery of contaminants to remote food webs. Air and rain samples were collected from one remote site on the west coast of Vancouver Island, British Columbia (BC), Canada, and from one near-urban site in the Strait of Georgia, BC. While global atmospheric dispersion was observed for the legacy PCBs, 40% of PBDEs detected in BC air appeared to be originating from trans-Pacific transport. It was estimated that 3kg of PCBs and 17kg of PBDEs were deposited every year in the Strait of Georgia.

Once deposited, PCBs, PBDEs and Hg biomagnify up the food chain. Harbour seals are non-migratory and can be used to provide signals of local contaminant sources. They have been extensively used as indicators of PCB and PBDE food web contamination in the BC coastal environment. The collection of over 200 harbour seal fur samples from

various locations around Vancouver Island, BC and Puget Sound, WA, USA helped us pinpoint three sites where Hg levels were significantly higher than our reference site, Bella Bella (Queen Charlotte Strait, Port Renfrew and central Puget Sound). A combination of anthropogenic sources and marine food web processes appeared to influence the delivery of methylmercury (MeHg) to the top of this coastal marine food chain. Our results also suggested that these Hg levels (1.6-46.9 µg/g) could be a concern for the health of these harbour seals.

Genomic techniques were used to generate insights into the implications of contaminant exposure on the health of marine mammals inhabiting industrialized regions (harbour seals from the Northeastern Pacific and Northwestern Atlantic) and remote, supposedly pristine, environment (Arctic beluga whales). In harbour seal blubber, there were positive correlations between the mRNA levels of several genes, including estrogen receptor alpha (*Esr1*), thyroid hormone receptor alpha (*Thra*), and glucocorticoid receptor (*Nr3c1*), and PCB levels. In beluga blubber, aryl hydrocarbon receptor (*Ahr*) and cytochrome P450 (*Cyp1a1*) mRNA levels increased with PCBs, consistent with their role in toxicity.While PCB-related toxic responses were observed in both species, additional factors appeared to be affecting the expression of important genes in beluga. Our results suggested that a shift in beluga diet during periods of low sea ice extent, as evidenced by changes in $\delta^{13}C$ isotope ratios, had a significant impact on mRNA levels coding for genes involved in growth, metabolism and development.

The use of a dual study design to evaluate the long range versus local sources of contaminants highlighted the importance of trans-Pacific transport in the delivery of PBDEs to coastal BC and the occurrence of local Hg sources in this marine environment. However, consistent with previous studies, our results suggested that PCBs remain the top contaminant of concern for marine mammal health. We also raised questions about the potential exacerbation of toxic risks due to PCBs as a consequence of climate changes currently underway in the Arctic.

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## Acknowledgments

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# Chapter 1: Introduction

## 1.1 Background

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), as well as inorganic elements, such as mercury (Hg), are ubiquitous environmental contaminants. Their physicochemical properties allow them to be transported over great distances via environmental processes, deposited, and incorporated into aquatic food webs. PCBs, PBDEs and Hg, in the form of methyl mercury (MeHg), bioaccumulate up the food chain and can induce a variety of short and long term toxic responses. They are therefore a concern for the health of high trophic level predators.

Marine mammals living close to urban and industrialized areas usually have the highest contaminant concentrations. For example, high levels of PCBs have been reported in gray seals (*Halicheorus grypus*) from the Baltic Sea (Sormo et al., 2003), beluga whales (*Delphinapterus leucas*) from the St Lawrence (Muir et al., 1996), and harbour seals from Puget Sound, Washington State (WA), USA (Ross et al., 2004). Killer whales (*Orcinus orca*) from British Columbia (BC), Canada, are considered among the most PCB contaminated cetaceans in the world (Ross et al., 2000). Studies on harbour seal pups from BC and WA have shown that proximity to contaminant sources influences concentrations and patterns and that seals living closer to industrialized areas are exposed to a combination of local and long range sources of contaminants (Ross et al., 2004). In contrast, marine mammals living in the remote Arctic, such as beluga whales, are mainly exposed to long range sources of contaminants being delivered via atmospheric transport, ocean currents and / or riverine discharges. Thus, biota inhabiting the remote Arctic usually exhibit lower contaminant levels. For example, PCB, PBDE and Hg levels are 7-fold, 12-fold and 8-fold lower, respectively, in Arctic beluga whales than levels observed in their

southerly St. Lawrence estuary counterparts (Beland et al., 1993; Hobbs et al., 2003; Raach et al., 2011).

However, regardless of location, from the highly contaminated Baltic Sea or Puget Sound to the remote Arctic, studies have shown that marine mammal health is at risk because of contaminant exposure. After investigating the transport and fate of major contaminants in coastal BC, the present thesis will investigate the impact of contaminants on the health of harbour seals living close to industrialized areas (BC and WA), as well as beluga whales living in the remote Western Canadian Arctic.

## 1.2 Contaminants of concern

### Persistent Organic Pollutants (POPs)

The Stockholm Convention defines persistent organic pollutants as being persistent, bioaccumulative and toxic. In the present thesis, we are investigating the transport and fate of two classes of POPs (PCBs and PBDEs), as well as their potential impact on the health of marine mammals. PCBs, and tetra-, penta-, hexa-, and hepta-BDEs are listed under the Stockholm Convention which requires parties to take measures to eliminate or reduce the release of these contaminants in the environment.

Beginning in 1929, PCBs were used as electrical transformer and capacitor fluids, flame retardants, hydraulic lubricants, sealants, and paints because of their heat resistance and insulating capacity. There are 209 congeners of PCBs with varying degrees of chlorination (Figure 1). They were banned in the 1970s in most industrialized countries resulting in a decrease in their environmental concentrations (Muir et al., 1999).

In contrast, some mixtures of PBDEs are still widely used in plastic housings of electronic equipment such as computers and televisions, as well as in plastic auto parts, lighting panels, electrical connectors and fuses. The textile industry also applies PBDEs to the upholstery of home and office furniture, car, plane and train seating. Similar to PCBs, there are 209 possible congeners depending on their degree of bromination (Figure 1). The major technical PBDE formulations are the penta-, octa- and deca-mixtures. The Deca formulation is a relatively pure mixture composed of approximately 97% of BDE-209. The Octa mixture is mainly composed of BDE-153 while the two dominant congeners in the Penta formulation are BDEs 47 and 99. All three commercial formulations (Penta-, Octa-, and Deca-BDE) are now banned in Europe and Canada. While Penta- and Octa-BDE were removed from the US market at the end of 2004, Deca-BDE remains largely in use although some States have moved to regulate this product and a phase out was planned for the end of 2012. In Asia, there are no regulations on the three PBDE mixtures (http://bsef.com/). Because of European and North American regulations, concentrations of PBDEs are starting to decrease in biota after a couple decades of exponential increase (Elliott et al., 2005; Law et al., 2010; Raach et al., 2011).

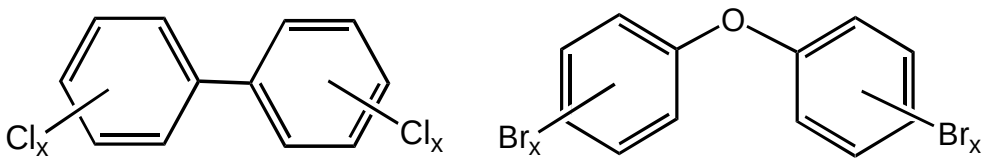


**Figure 1: PCBs and PBDEs have similar chemical structures giving them similar physicochemical properties such as low vapour pressure, hydrophobicity and resistance to acids, bases, light and heat.**

**Mercury**

Mercury is emitted from both natural (~60% of the total Hg atmospheric emissions) (Pirrone et al., 2010) and anthropogenic sources. Natural sources of Hg include volcanoes, forest fires, emissions from surface waters (comprising Hg from anthropogenic sources deposited in the past and being re-emitted), contaminated soils in ancient mining industrial areas or particular geological units rich in mercury. The natural Hg cycle has been enhanced by human activities such that two to three times more Hg is currently cycling through the atmosphere and upper ocean than before the industrial revolution (Pirrone et al., 2010). There are a number of anthropogenic sources of Hg including fossil fuel fired power plants, ferrous and non-ferrous metal smelters, chlor-alakli plants, waste incinerators and small scale gold mining. However, electric power generation facilities using coal are the number one source contributing to more than 50% of the total anthropogenic emissions. In Canada, most European countries, and Japan, there are regulations to limit mercury emissions from coal fired power plants. In December 2011, the US Environmental Protection Agency defined, for the first time, national standards in order to reduce mercury pollution from power plants (www.epa.gov). In Asia, the major emitter of Hg, there are limited regulations currently in place. Asia therefore represents a concern as its contribution is expected to become more significant due to anticipated increases in emissions, particularly in China (Pacyna et al., 2010). On the international level, the Minamata Convention on Mercury was recently agreed on by many nations and will be signed in October 2013. Governments agreed to a global, legally-binding treaty to control and reduce mercury emissions across a range of products, such as thermometers and energy-saving light bulbs. This Convention is also aiming at controlling emissions from mining, cement and coal-fired power sectors (www.unep.org).

## 1.3 POPs and Hg in marine mammals from the Northeastern Pacific and the Western Canadian Arctic

The present thesis focuses on two study animals: harbour seals from the Northeastern Pacific being exposed to a combination of long range and local sources of contaminants and beluga whales from the Western Canadian Arctic exposed mainly to long range global sources.

PCBs and PBDEs have been reported in those two species (Muir et al., 2006; Ross et al., 2004). In harbour seals, PCB and PBDE levels range from 0.3 to 6.9 µg/g lipid weight (lw) and from 0.2 to 0.7 µg/g lw, respectively (Ross et al., 2012). In the Western Canadian Arctic beluga whales, PCB levels range from 0.2 to 8.4 µg/g lw and PBDEs range from 2.1 to 51.6 ng/g lw (Table 1).

While there are no temporal trend data for PCBs and PBDEs in the Western Canadian Arctic beluga whales, PCB levels in harbour seals from BC and WA declined exponentially since their ban in the 1970s. PBDE levels exhibited a different pattern with an exponential increase between 1984 and 2003 followed by a drop in 2009 (Ross et al., 2012).

Hg has been continuously monitored in the Western Canadian Arctic belugas since the early 1980s. Levels increased until the late 1990s, but have been decreasing since then (Loseto, NCP report, in prep). The present levels range from 12.7 to 345.7 µg/g dry weight in muscle (Loseto et al., 2008a) (Table 1). As there are currently no studies on Hg in harbour seals from BC and WA, the present thesis will investigate Hg levels in harbour seal fur and whiskers from these areas.

**Table 1: Comparison table for PCBs, PBDEs and Hg levels in marine mammals inhabiting industrialized areas and the remote Arctic. Species were selected on the basis of their relevance for comparison with the species studied in the present thesis.**

| | Species | Location | Age Class | Contaminant | Tissue | Levels | Reference |
|---|---|---|---|---|---|---|---|
| **Industrialized regions** | Harbour seals | Northeastern Pacific | Pups | PCBs | Blubber | 0.3 - 6.9 µg/g lw | (Ross et al., 2012) |
| | | | | PBDEs | Blubber | 0.2 - 0.7 µg/g lw | |
| | | | Pups | Hg | Fur | 1.6 - 46.9 µg/g dw | Chapter 2 of this thesis |
| | | | Adults | Hg | Fur | 2.5 -17.6 µg/g dw | |
| | Beluga whales | St Lawrence Estuary | Adults | PCBs | Blubber | 2.1 - 28 µg/g lw | (Hobbs et al., 2003) |
| | | | | Hg | Liver | 1.4 - 756 µg/g dw | (Beland et al., 1993) |
| **Arctic** | Harbour seals | Western Hudson Bay | Pups | Hg | Fur | 0.5- 0.7 µg/g ww | (Young et al., 2010) |
| | | | Adults | Hg | Fur | 1.2 - 3.3 µg/g ww | |
| | Beluga whales | Western Canadian Arctic | Adults | PCBs | Blubber | 0.2 -8.4 µg/g lw | (Loseto et al., in prep) |
| | | | | PBDEs | Blubber | 2.1 - 51.6 ng/g lw | |
| | | | Adults | Hg | Muscle | 12.7 - 345.7 µg/g dw | (Loseto et al., 2008b) |
| | | | | Hg | Liver | 0.3 - 66.9 µg/g dw | (Loseto, pers. com.) |

## 1.4 Transport and fate of contaminants in the biosphere

The relatively high PCB, PBDE and Hg concentrations detected in marine mammals inhabiting remote areas (Ikonomou et al., 2002; Loseto et al., 2008a; Muir et al., 2006) indicate that these compounds are readily transported over great distances via environmental processes and are then incorporated into aquatic food webs. The Arctic has previously been described as an important sink for these contaminants (Ariya et al., 2004; Wania et al., 2001). This section provides a brief review of the major processes involved in PCB, PBDE, and Hg transport and fate in the marine environment.

### Persistent organic pollutants (POPs)

Atmospheric transport is the most efficient mechanism by which POPs move in the environment. “Global distillation” has been described as the process by which POPs evaporate in the warmer regions, are atmospherically transported towards the northern regions where they condense and are being deposited (Wania et al., 1995). POPs are transported through a number of hops (repeated cycles of deposition and re-evaporation driven by temperature changes along the path) before reaching their final destination and their ability to travel is highly dependent on their physicochemical properties. Gas/particle partitioning is a key process, as it affects not only the ability of the contaminant to be transported, but its potential for degradation and removal from the atmosphere. Because of their moderate vapour pressure, PCBs and PBDEs are mainly found in the gas phase of the atmosphere (Manchester-Neesvig et al., 1989). It should be noted however that the generally lower vapour pressure of PBDEs results in a higher particle-bound percentage than observed for PCBs (St.Amand et al., 2007).

Atmospheric deposition has been demonstrated to be a significant contributor to aquatic ecosystem contamination. For example, atmospheric PCBs have been estimated to represent 93% of the total PCB inputs to the North Sea (Duce, 1998), and 60-90% of the PCB burden in the Great Lakes may originate from the atmosphere (Eisenreich et al., 1981). Three main processes are responsible for atmospheric deposition of POPs (Cotham et al., 1991):

- rain and snow scavenging of gases and aerosols with particle scavenging usually considered the dominant process (Bidleman, 1988);
- dry particle deposition which is related to the velocity of the particle deposition; and
- gas exchange with water, snow and soil surfaces.

Even though most of PCBs and PBDEs are found in the gas phase, gas deposition is only a minor contributor to the total atmospheric deposition compared to wet deposition (Ter Schure et al., 2004).

The octanol/water partition coefficient ($K_{ow}$) is a key factor in predicting the fate of these chemicals in the marine environment (Mackay et al., 2000; Nfon et al., 2008). Because POPs are highly lipophilic, once deposited in the marine environment, they will partition from water to organic matter. Uptake of POPs at the bottom of the food chain is a passive process driven by a fugacity gradient: rapid adsorption to the phytoplankton and/or zooplankton surface is followed by diffusion through the membrane into the plankton matrix (Del Vento et al., 2002; Swackhammer et al., 1993). Higher up the food chain, bioaccumulation of POPs occurs as the result of a sequence of solvent depletion and solvent switching steps (Macdonald et al., 2002):

- Solvent depletion 1: occurs in the digestive tract when dietary lipids are hydrolysed by digestive enzymes;
- Solvent switching 1: the loss of dietary lipids forces the contaminant to redistribute from lipid to other organic matter;
- Solvent switching 2: the products of lipid hydrolysis diffuse into the cells lining the intestine where triglycerides are resynthesized and form packets called chylomicrons which represent a newly formed solvent for contaminants that are going to diffuse into these cells;
- Solvent depletion 2: the final step in biomagnification happens in every tissue, where all the assimilated lipids are metabolized for energy.

As a result of these processes, high trophic level marine predators usually have high POP burdens.

**Mercury**

Most of the mercury emitted in the atmosphere is elemental Hg ($Hg^o$) accounting for 53% of total mercury. Elemental mercury can remain in the atmosphere for up to two years allowing long range atmospheric transport from industrialized regions to the Arctic. About 5 to 10% of mercury emitted in the industrialized regions is deposited in the Arctic (Pacyna et al., 2010).

Elemental mercury can be oxidized by atmospheric oxidants (e.g. halogens) into HgII which is present in the atmosphere in small amounts in the form of reactive gaseous mercury and particulate mercury. In the Arctic, atmospheric oxidation of elemental mercury is enhanced every spring by a phenomenon called the Atmospheric Mercury Depletion Event (AMDE). This rapid oxidation of $Hg^o$ involves the presence of bromine coming from sea salts associated with seasonal sea ice, ice with a

relatively salt-rich frozen surface (Barrie et al., 1997), or water with light ice cover where pelagic and ice-algal communities produce halogen-containing gases during primary production (Sturges et al., 1992). Because of their solubility in water, low volatility and reactive properties, oxidized forms of mercury have a much shorter residence time in the atmosphere than $Hg^o$ (< two weeks). They are therefore less likely to undergo long range atmospheric transport and will be efficiently deposited (Ariya et al., 2004).

Atmospheric transport and deposition is usually the dominant pathway for the delivery of Hg to the world's oceans, accounting for about 90% (Outridge et al., 2008). However, the Mackenzie river, the largest river discharging to the Beaufort Sea, also plays an important role in the delivery of Hg, mostly in the form of particulate inorganic mercury, to the Western Arctic Ocean (Leitch et al., 2006; Stern et al., 2005).

Once deposited, HgII can be exported to the sediment, transformed into methylmercury (MeHg), or reduced via microbial reduction and /or photoreduction into $Hg^o$ and recycled back to the atmosphere. It has been estimated that 24 to 36% of deposited Hg is photoreduced and ends up back in the atmosphere (Schroeder et al., 1998).

Methylation of HgII is a very important process to consider when it comes to food web contamination. MeHg is both the bioaccumulative and toxic form of Hg. Microorganisms such as methane and sulphate-producing bacteria are key components in the formation of MeHg, both directly through their involvement in methylation-demethylation processes, and indirectly by controlling the availability of HgII through redox transformations (Barkay et al., 2003). Low dissolved oxygen

content, low pH, and high concentrations of organic matter provide optimal environmental conditions for methylation processes. Once in the aquatic environment, MeHg can be demethylated through photodegradation or microbial pathways, phenomena mostly occuring in surface waters, or incorporated into the food web.

Uptake of mercury at the bottom of the food chain occurs by diffusion of mercury complexes ($HgCl_2$ and $CH_3HgCl_2$) through membranes. Both complexes are efficiently retained by microorganisms. HgII binds to the membrane of the diatom, which is excreted rather than absorbed by the predator (e.g. copepod). This results in smaller transfer efficiency for HgII than for MeHg which is associated with the soluble fraction of the diatom that is efficiently assimilated by the copepod. In terms of transfer higher up the food chain, there are still a lot of uncertainties but it appears to partly be the result of the relative solubility of MeHg which allows it to be partly retained in the fatty tissues (Morel et al., 1998). However, the burden of MeHg in fish and top predators is primarily associated with proteins rather than fatty tissues suggesting that MeHg bioaccumulation is explained by factors other than its relative liposolubility. After being taken up by biota, MeHg is bound preferentially to thiol or selenol-containing molecules which are mainly present in cysteine residues of proteins or tripeptide glutathione. As a result, MeHg is found in the body as a complex with amino acid-L- cysteine or reduced glutathione and is able to be transported through amino-acid carrier (Lemes et al., 2011; Zareba et al., 2007)(Zareba et al., 2008, Lemes et al., 2011).

## 1.5 Health risks related to contaminants of concern in marine mammals

### POPs

Organic contaminants are a concern for the health of marine mammals, with observations of impaired reproduction, skeletal lesions, kidney damage, tumors, premature birth and skin lesions in populations inhabiting contaminated areas (Beckmen et al., 1997; Beland et al., 1993; Bergman et al., 2001; Olsson et al., 1994). High PCB concentrations have been linked to decreased immune function in field studies of harbour seals (Mos et al., 2007), bottlenose dolphins (*Tursiops truncatus*) (Lahvis et al., 1995) and polar bears (*Ursus maritimus*) (Lie et al., 2005), as well as captive feeding studies of harbour seals (De Swart et al., 1996). Contaminant-related immunotoxicity has been, in part, blamed for serious outbreaks of infectious disease in marine mammals (Osterhaus et al., 1996). In addition, PCBs have been implicated in the disruption of vitamin A and thyroid hormone systems in harbour seals, which could lead to adverse effects on growth and development (Mos et al., 2007; Tabuchi et al., 2006).

There are fewer studies available on the effects of PBDEs on marine mammal health but, because of their similar physico chemical properties, PBDEs have been shown to exert similar toxicological effects as PCBs. For example, Hall et al. (2003) suggested that PBDEs were altering the thyroid system of young grey seals, thymic atrophy and splenic depletion were associated with high levels of PBDEs in harbour porpoises (*Phocoena phocoena*) (Beineke et al., 2005), and Frouin et al. (2010) showed that PBDEs were altering immune function in harbour seals.

### Mercury

Because of the natural occurrence of heavy metals, marine mammals have been exposed to these elements throughout their evolutionary history and have developed

mechanisms to either control the internal concentrations of certain elements or to mitigate their toxic effects. For instance, cetaceans and pinnipeds have developed a tolerance to mercury based on its association with selenium (Dietz et al., 2000). However, even though they might be able to tolerate higher mercury burdens than terrestrial mammals, mercury, especially in the methylated form, is still a concern for marine mammal health.

Methylmercury is well known for its neurotoxicity which leads to sensory and motor deficits and behavioural impairments. In addition, MeHg is easily transported through the placenta and concentrates in the fetal brain representing a concern for its development (Clarkson, 2002). Liver and kidney damage has also been reported in bottlenose dolphins and polar bears exhibiting high mercury concentrations (Sonne et al., 2007a; Woshner et al., 2008). In addition, in vitro studies on beluga whales and harbour seals showed that mercury exposure could result in immune deficiency (Das et al., 2008; De Guise et al., 1996a).

The use of transcriptomics to investigate marine mammal healthWildlife is affected by a variety of environmental changes such as increase exposure to anthropogenic contaminants, altered habitat, and/or climate change. Such environmental pressures may manifest at different levels of biological organization, including altered population dynamics, behavioural and physiological changes of individual organisms, and with adjustments in molecular biological pathways (Figure 2) (Schirmer et al., 2010). One of the first components of biological response to environmental change includes altered expression of mRNA with a subsequent adjustment in transcriptome profile of a given tissue (Veldhoen et al., 2011) (Figure 2).

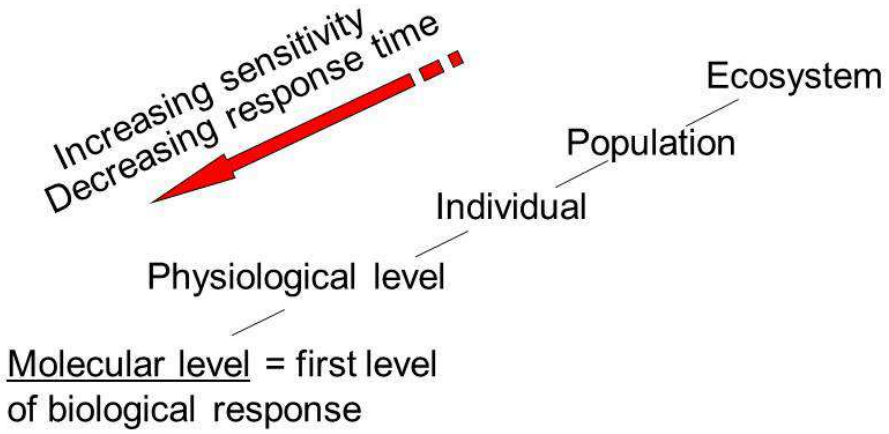


**Figure 2: Toxic effects at the molecular levels can be detected before individual- or population-level effects. Investigating the potential impacts of contaminants of concern (PCBs, PBDEs, and Hg) on the mRNA transcript levels therefore represent the first level of biological response.**

In the past decades, the use of molecular profiling techniques has been increasing in wildlife toxicology. Various techniques exist to evaluate the mRNA transcripts levels but they differ in their level of sensitivity, potential for cross-species use and depth of investigation into the transcriptome (Veldhoen et al., 2012). While DNA arrays allow the measurement of the expression level of a large number of genes simultaneously, it does not provide a precise quantification of mRNA levels.

Highly sensitive techniques such as real time quantitative polymerase chain reaction (QPCR) analysis are the most cost-effective and have the advantage to be adaptable to non-lethal small biopsy samples (Buckman et al., 2011; Mos et al., 2007; Tabuchi et al., 2006; Veldhoen et al., 2012). QPCR is the most sensitive method for the detection and quantification of gene expression. It is particularly effective for low abundance transcripts, for studies where limited tissue sample is available, and for the elucidation of small changes in mRNA transcript levels (Pfaffl et al., 2004). Species-specific DNA primers are designed in order to amplify specific gene transcripts of interest across several orders of magnitude. The high quality data can then be used for relative

quantification of mRNA abundance. This technique determines the changes in mRNA levels of a target gene relative to the level of an internal control mRNA (reference gene) and therefore allows for comparison between samples.

In the present thesis, we will use new genomic techniques to investigate the potential impacts of contaminants (mainly PCBs and Hg) on the health of harbour seals from BC, Quebec, Newfoundland and WA as well as belugas from the Western Canadian Arctic. Marine mammal studies using such techniques are presently limited. Effects of PCBs have been detected at the molecular level in harbour seals and killer whales inhabiting the Northeastern Pacific (Buckman et al., 2011; Mos et al., 2007) as well as in striped dolphins (*Stenella coeruleoalba*) and fin whales (*Balaenoptera physalus*) from the Mediterranean Sea and ringed seals from Svalbard and the Baltic Sea (Fossi et al., 2010; Panti et al., 2011; Routti et al., 2010) (Table 2). To our knowledge, there are no studies looking at the effects of contaminants at the gene expression level in marine mammals inhabiting the Western Canadian Arctic.

**Table 2: Review table on studies investigating the potential impacts of PCBs on the expression of various genes in marine mammals (TRα: thyroid hormone receptor alpha; RAR: retinoic acid receptor alpha; IL-1β: interleukin; 1 beta receptor; DIO1: deiodinase 1; TRβ: thyroid hormone receptor beta; GHR: growth hormone receptor; Cyp1A: cytochrome P450; ERα: estrogen receptor alpha; hsp: heat shock protein; and MT1: metallothinein 1). (↑ : increase in expression ; n/a : non available)**

| Species | Location | PCB levels | Tissue | Effect observed | Reference |
|---|---|---|---|---|---|
| Harbour seals (*Phoca vitulina*) | Northeastern Pacific | 0.6 - 7.2 µg/g lipid weight (lw) | Blubber | ↑TRα | Tabuchi et al., 2006 |
| Harbour seals (*Phoca vitulina*) | Northeastern Pacific | 0.6 - 7.2 µg/g lw | Blubber | ↑RAR | Mos et al., 2007 |
| Ringed seals (*Phoca hispida*) | Baltic Sea, Svalbard | n/a | Blubber | ↑IL-1β, ↑DIO1, ↑TRβ, ↑GHR | Routti et al., 2010 |
| Fin whale (*Balaenoptera physalus*) | Mediterranean Sea, Gulf of California | 1 - 16 µg/g dry weight (dw) | Skin | ↑Cyp1A, ↑ERα | Fossi et al., 2010 |
| Striped dolphin (*Stenella coeruleoalba*) | Mediterranean Basin | n/a | Skin/Blubber | ↑Cyp1A, ↑AhR, ↑hsp70 | Panti et al., 2011 |
| Killer whales (*Orcinus orca*) | Northeastern Pacific | 14.7 - 430 µg/g lw | Blubber | ↑AhR, ↑TRα, ↑ERα, ↑IL-10, ↑MT1 | Buckman et al., 2011 |

## 1.6 Objectives

The main objectives of this thesis are:

*1) to better understand the importance of the global transport and fate of priority contaminants (PCBs, PBDEs, and Hg) in coastal British Columbia using a dual study design (air sampling and seal sampling):*

a) Chapter 2: Contaminant levels and patterns will be investigated in air samples from two stations in coastal British Columbia. This will enable a characterization of the relative importance of 'local' vs 'background' atmospheric contamination, insight into a major mode of spatial distribution of persistent contaminants in the region, and an important input function for marine food webs (deposition). This chapter will focus on PCBs and PBDEs as extensive literature is available concerning atmospheric Hg in the region.

b) Chapter 3: Spatial variations of Hg levels will be investigated in harbour seals from British Columbia, Canada, and Washington State, USA. They are the most abundant marine mammal in the region (~53 000 animals in the Strait of Georgia, BC, and inland waters of WA, USA), are non migratory, high in the food chain and feed on a wide variety of fish and invertebrate species. They will provide us with an integrated signal of local food web contamination and will help us understand Hg exposure at the top of this marine food web. This chapter will focus on Hg levels as extensive literature is available concerning PCBs and PBDEs in this population of harbour seals.

2) *to investigate the potential impacts of contaminant exposure on marine mammal health*. New genomic techniques were developed and applied and will help generate insight into the implications of contaminant exposure on the health of marine

mammals inhabiting industrialized regions (harbour seals from BC, Quebec, Newfoundland and WA) as well as marine mammals living in a remote, supposedly pristine, environment (Western Canadian Arctic beluga whales).

a) Chapter 4: PCB-associated health effects have been reported in harbour seals from British Columbia, Quebec and Newfoundland, Canada, as well as Washington State, USA (Tabuchi et al., 2006; Mos et al., 2007). In the present thesis, we will deepen the analyses of potential impacts of contaminants at the molecular level by expanding the tool box from an existing three to seven new target genes, giving us additional information on the health of this population of harbour seals.

b) Chapter 5: Beluga whales from the Western Canadian Arctic are exposed to relatively low contaminant concentrations compared to their counterparts living in the St Lawrence Estuary. Their PCB levels are an order of magnitude lower and Hg levels are about five times lower (Hobbs et al., 2003). In the present thesis, we will investigate the potential impact of major contaminants of concern (PCBs, PBDEs and Hg) on the mRNA levels of sixteen target genes. Monitoring the health of this particular population of beluga is important as they remain an important part of healthy diets for many communities in the Inuvialuit Settlement Region.

# Chapter 2: Do trans-Pacific air masses deliver PCBs and PBDEs to coastal British Columbia Canada?

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## 2.1 Introduction

Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), are ubiquitous environmental contaminants. Their physico-chemical properties, including hydrophobicity, moderate vapor pressure and low reactivity, allow transport in the environment, bioaccumulation into food webs and induction of a variety of short and long term toxic responses. A decrease in environmental PCB concentrations has been observed since the ban of this chemical in the 1970s in most industrialized nations (Bignert et al., 1998; Muir et al., 1999). In contrast, levels of PBDEs, chemicals widely used as flame retardants, are increasing rapidly in a variety of biota (Elliott et al., 2005; Lebeuf et al., 2004). All three commercial formulations (Penta-, Octa-, and Deca-BDE) are now banned in Europe, while Penta- and Octa- were removed from the United States (US) and Canadian markets at the end of 2004. Deca-BDE remains largely in use in North America, although Canada and some US states have moved to regulate this product. In Asia, legislation looms for the three PBDE mixtures, but they are still widely used (http://bsef.com/).

Semi-volatile organic compounds, such as PCBs and PBDEs, partition between the gas and particulate phases in air and can undergo long-range atmospheric transport (LRAT). The relatively high PCB and increasing PBDE concentrations detected in marine mammals inhabiting remote areas (Ikonomou et al., 2002; Muir et al., 2006) may indicate that these chemicals are readily transported over great distances via environmental processes and are then subject to incorporation into aquatic food webs. Atmospheric deposition likely plays a significant role in this regard, typically delivering the majority of total PCBs found in many aquatic environments (Duce, 1990). With prevailing winds from the west, the movement of air masses to North America from Asia takes only 2–10 days (Jaffe et al., 1999a; 2003). In this way,

trans-Pacific transport has been implicated in the delivery of Asian dust and particle associated contaminants to the west coast of North America (Jaffe et al., 1999a; McKendry et al., 2001; Primbs et al., 2008).

Atmospheric dispersion of POPs away from sources contaminates remote food webs (Kidd et al., 1998). While the extent of local (North American) sources relative to the 'background' remains unclear, it is increasingly evident that POPs in biota from the Northeastern Pacific Ocean cannot be entirely attributed to local sources. Salmon have been shown to acquire the majority of their POPs during their time in the Pacific Ocean, effectively importing chemicals into coastal waters and terrestrial watersheds, where they are consumed by wildlife, including resident killer whales (*Orcinus orca*) and grizzly bears (*Ursus arctos*) (Christensen et al., 2005; Cullon et al., 2009; Ross, 2000). Since POPs are considered population-level threats to several endangered marine mammal populations in BC (Ross, 2006), a greater distinction between local and global POP concentrations is relevant to the identification and adoption of appropriate mitigative strategies such as national regulations and/or international treaties.

Measuring contaminant concentrations and patterns in air at only one site provides a signal of atmospheric contamination at that site. In this study, we compared and contrasted contaminant concentrations and patterns at two distinct sites, one being near-urban (between Vancouver and Seattle; in the Strait of Georgia where local wind patterns are constrained by regional mountains) and the other being remote (westernmost coast of Vancouver Island exposed to trans-Pacific air masses). We hypothesized that contaminant signals would differ at the two sites, reflecting the influence of prevailing westerly winds at the remote site and local sources at the near-urban site. We collected seasonally-integrated samples of air (vapor and particle) and

water (precipitation) from coastal British Columbia (BC) during a 365-day period in 2004, analyzed samples for up to 275 PCB and PBDE congeners, and compared concentrations, patterns and deposition at the two locations. In this manner, our approach used an integrated method akin to that of passive techniques including the more qualitative or semi-quantitative Polyurethane Foam (PUF) samplers and semipermeable membrane devices (SPMDs) (Harner et al., 2004), while retaining a quantitative approach. Our principal objective was to partially characterize the relative importance of global versus local sources of PCBs and PBDEs in coastal BC, Canada.

## 2.2 Materials and methods

### Sampling sites and techniques

Air (particulate and gas phases) and rain samples were collected continuously for a one-year period at two distinct sites in southern BC, Canada, representing ‘‘remote’’ and ‘‘near-urban’’ locations (Figure 3). The Amphitrite lighthouse at Ucluelet ($48^0$55’12’’N, $125^0$32’24’’W, elevation = 27 m), on the west coast of Vancouver Island, is situated on the far western Pacific edge of Canada, and is influenced by westerly and south-westerly offshore winds. The Canadian Air and Precipitation Monitoring Network (CAPMoN) station on Saturna Island ($48^0$47’24’’N, $123^0$07’48’’W, elevation =178 m) is located in the moderately industrialized Strait of Georgia, between the population centers of Victoria, Vancouver, and Seattle, and is encircled by a variety of industrial and urban sources of contamination (at a distance of 40 km from any known sources).

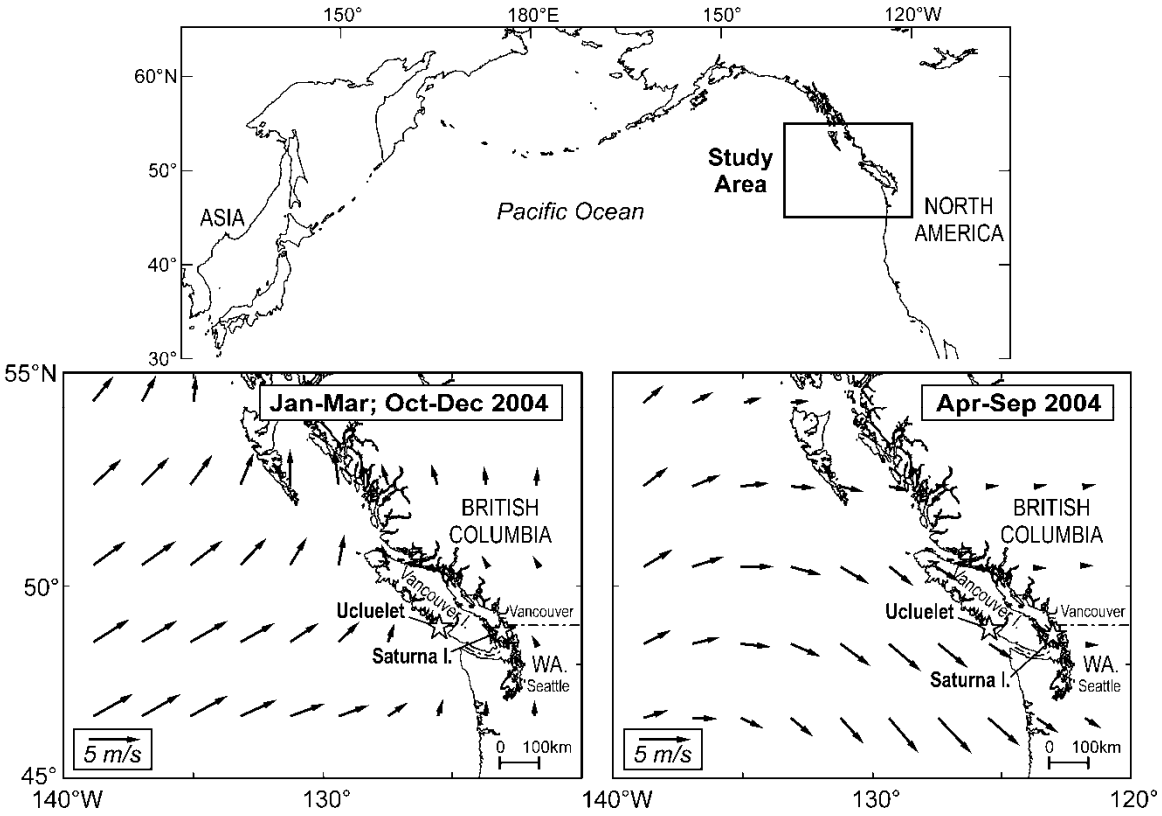


**Figure 3: Air and rain samples were collected at two sites in southern British Columbia: the remote Ucluelet station, on the west coast of Vancouver Island, and the near-urban Saturna Island station. Prevailing winds readily deliver Asian air masses to coastal Bristish Columbia: the two inset maps shown mean NCEP/NCAR reanalysis I (Kistler et al., 2001) 10m winds over 2004 during the cool (January-March and October-December) and warm (April-September) seasons. Data obtained from the NOAA/OAR/ESRL PSD, Boulder, Colorado, USA: http://www.cdc.noaa.gov/.**

High-volume (Hi-Vol) air samplers were modified at the Environmental Technology Centre (Ottawa, ON, Canada) for the National Air Pollution Surveillance Network. Modifications were made, enabling the use of a larger volume motor for larger air samples, and a Roots meter (DI Canada Inc. Toronto, ON, Canada) to accurately determine sample volumes and to correct for any flow reduction due to filter blockage by particulate matter. Teflon coated MIC (Meteorological Instrument Center, Thornhill, ON, Canada) precipitation samplers were provided by Environment

Canada. Sampling procedures were similar to those described in EPA method TO-4 (US EPA, 1999). Two samplers were deployed at each of the two locations for a continuous 365 day period ending January 3rd, 2005. Throughout the year, air samples (gas and particulate phases) were collected on a weekly basis and rain samples were collected on a monthly basis.

PUF (7 cm diameter by 15 cm long)/Amberlite-XAD-2 (PUF-XAD-PUF) plugs were used to capture the gas phase. Before use, PUFs (Tisch Environmental, Cleves, Ohio, USA) were thoroughly Soxhlet-cleaned with pesticide-grade acetone (Caledon laboratories, Georgetown, ON, Canada) for 24 h. The PUFs were then placed in a vacuum desiccator to dry for up to 12 h. XAD-2 (Supelco, Oakville, ON, Canada) was Soxhlet-cleaned with Dichloromethane and rinsed with pesticide-grade methanol (Burdick and Jackson, Muskegon, MI, USA). Quartz fiber filters (QFF) (Whatman QM-A, Clifton, NJ, US), with a pore size of 10 µm, were used to capture the particulate phase. Before use, the filters were baked at 400 $^{0}$C for 4 h. The filters were weighed before and after sampling in order to determine the total suspended particle (TSP) concentrations after equilibrating to air temperature and humidity. Contaminants in unfiltered rain (dissolved + particulate washout) were sampled using pre-cleaned 25 mm x 300 mm XAD-2 resin cartridges. Pre-cleaned glass wool plugs were installed to retain the XAD resin during the sampling process.

One field blank was collected at each site, for each phase and each season, for evidence of possible contamination through handling and transport. Before being deployed in the field, $^{13}$C-labeled PCBs (CB-35, 95, and 153) and PBDEs (BDE-139) were added to PUF and XAD as field surrogates to assess the possible loss of contaminants of interest during the sampling period.

**Sample extraction, cleanup, and analysis**

Samples from the two sites were subject to the same extraction, cleanup, and quantification procedures. Coming back from the field and prior to extraction, all the samples were spiked with $^{13}$C-labeled PCB and PBDE congeners in order to monitor the extraction and cleanup procedure. Extraction was performed during 24 h using large volume Soxhlets and pesticide grade 80:20 toluene:acetone (EMD chemical, Gibbstown, N.J., US). Extracts were reduced in volume (~2 mL) and concentrated by rotary vaporation. They were then combined into four seasonal pools (January–March; April–June; July–September; October–December) for analysis of PCBs and PBDEs. Pools were then filtered through glass fiber filters (GFF- Whatman), and passed through a florisil column. Samples were eluted with 50% DCM (dichloromethane)/hexanes and concentrated under nitrogen stream. A total of 202 PCB and 43 PBDE congeners were quantified by the Regional Contaminant Laboratory of Fisheries and Oceans Canada using high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) as described elsewhere (Ikonomou et al., 2001).

**Data treatment**

In the rest of the paper, air concentrations refer to the sum of the particulate and the gas phase PCB or PBDE concentrations. The particulate and gas phase concentrations were expressed in pg/m$^3$ and the rain concentrations in pg/L. PUF and XAD-2 field recovery values averaged 60.4 ± 18.2 (SEM) % and were within 2–25% of the laboratory surrogate recovery values. All the values were therefore only corrected to laboratory recovery values which were considered well within acceptable ranges (65–112%). In an effort to reduce the impact of the numerous non-detected congeners on the overall total concentrations, the following semiconservative substitutions were

applied: 1) congeners that were not detected in any of the 26 samples were not included in the calculations (7 PCB and 3 PBDE congeners); 2) where congeners were detected in less than 70% of the samples (97 PCB and 27 PBDE congeners), a substitution of half the detection limit was applied; and, 3) where congeners were detected in more than 70% of the samples (39 PCB and 6 PBDE congeners), a detection limit substitution was applied. A total of 63 PCB and 33 PBDE congeners were detected in all samples at all times, for which no substitutions were required.

Detection limits were calculated as three times the chromatogram noise at retention time (Ikonomou et al., 2001) and averaged 0.008 ± 0.002 pg/$m^3$ for all PCB congeners, and 0.01 ± 0.001 pg/$m^3$ for all PBDE congeners. Five of the 24 sample pools (particulate and gas phase winter samples from both sites and the gas phase sample spring from Saturna) revealed a PCB contamination of the procedural blank, constraining our ability to adequately quantify clean signals for some congeners in our true samples. Therefore, we excluded those sample data for congeners that were less than three times the levels reported in the blanks. A total of 38 PCB and 18 PBDE congeners were affected, for which a mean substitution was applied using mean values for those congeners as reported from the other seasons. This represented 18% of the total number of PCB congeners, and 26% of the PBDE congeners, measured. The remaining 82% of PCB congeners measured, and 74% of PBDE congeners, were unaffected, with values passing our QA/QC rules.

Statistical analyses were performed to compare seasonal averages of PCB and PBDE concentrations (gas, particulate, and rain) between the two sites.

The total atmospheric deposition of PCBs and PBDEs (dry particulate, gas and wet) was estimated as follows:

Wet deposition fluxes ($D_r$ in pg/$m^2$/day) were calculated as:

$$D_r = W_i / (A \times t) \quad (1)$$

where $W_i$ is the mass of contaminants in the rain (pg), A is the surface area of the sampler (0.203 $m^2$), and t is the duration of the sampling period (days).

Dry deposition fluxes (kg/ha/year) were estimated as:

$$\text{Dry deposition rate} = V_d \times C \quad (2)$$

where $V_d$ is the dry deposition velocity (cm/s), C is the contaminant concentrations in the particulate or gas phase (µg/$m^3$). The use of a constant deposition velocity value for the calculation of the dry particulate deposition introduced a bias in our estimation of total atmospheric deposition at the two sites. This parameter is highly variable and dependent on environmental features and physical characteristics of both the pollutant and receptor surface (Franz et al., 1998), resulting in a fairly wide range of deposition velocity values reported in the literature. Of the two main PCB deposition velocity values used in previous studies (0.5 cm/s (Leister et al., 1994; Totten et al., 2006) and 0.2 cm/s (Hoff et al., 1996)), we selected the former as it is considered more appropriate for PCBs that bind to particles in air (Franz et al., 1998; Totten et al., 2004). Since no such estimates have been adequately developed for PBDE for aquatic application, we used the deposition velocity value (0.5 cm/s) established for PCBs.

The net flux at the air–water interface is divided into volatilization and absorption. However, in the present study, a one-way gaseous exchange was considered as no water PCB/PBDE data were available to estimate the reverse flow.

The absorptive gas flux ($F_{gas,abs}$) (pg/$m^2$/s) was calculated as:

$$F_{gas;abs} = K_{OL} \times C_{air}/H' \quad (3)$$

where $K_{OL}$ is the overall mass transfer coefficient (m/s); $C_{air}$ is the chemical concentrations in the gas phase (pg/$m_3$); and $H_o$ is the dimensionless Henry's Law

constant [related to Henry's Law constant H ($Pa.m^3/mol$) (Brunner et al., 1990; Xu et al., 2007) as H/RT with R being the ideal gas law constant ($Pa.m^3/mol/K$) and T is the temperature near the air–sea interface (K)]. Details on the calculations are described elsewhere (Eisenreich et al., 1996; Hornbuckle et al., 1994; Totten et al., 2006). The range of $K_{oL}$ values that we calculated are similar to those reported elsewhere (Hornbuckle et al., 1994; Totten et al., 2006).

**Principal components analysis (PCA)**

The stated concentration was used for analytes reported by the laboratory as NDR (non-detectable range; peak detected but confirming ion-ratios outside of the specified range), while undetectable values were replaced by a random number between zero and the limit of detection before PCA. Each contaminant analyzed was evaluated for potential interferences, closeness to the limit of detection and the percentage of undetectable (random value estimated) values before inclusion in the final PCA data set of 104 PCBs and 15 PBDEs. Samples were normalized to the concentration total before PCA to remove artifacts related to concentration differences between samples. 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) and where the average concentration and concentration total were identical for every sample. Data were then auto-scaled before PCA to give every variable equal weight.

### Back trajectories

Back trajectories were generated from our two sampling sites, four times daily (00, 06,12 and 18Z), and at four different elevations (10,100, 500, and 3000 m) for the calendar year 2004. This helped capture temporal and vertical variability of flow both within the local atmospheric boundary layer (representing gas and particulate phases of contaminant transport), as well as near the cloud base (where air parcels with contaminants in rain might originate). A range of two to ten days was previously reported for trans-Pacific transport (Holzer et al., 2003; Jaffe et al., 1999b; Wilkening et al., 2000), but our preliminary results (not shown) reveal that a ten day period for 2004 was more realistic. Ten-day back trajectories were generated using the Canadian Meteorological Center (CMC) trajectory model (D'Amours et al., 2001). Back trajectories were combined into four seasonal clusters that matched the air and rain sampling pool periods. Preliminary results suggested two distinctive trajectory patterns over the sampling year, which led us to pool trajectories over cool (October–March) and warm (April–September) seasons. Cluster analyses were performed on the back trajectories over each of these two seasons to discern the overall trajectory patterns. Cluster mean trajectories and the percentage of total individual trajectories in each cluster using the CMC model were similar to results obtained using the HYSPLIT (HYbrid Single-Particle Lagrangian Integrated Trajectory) model (Draxler et al., 1997) developed at the NOAA Air Resources Laboratory. We present here only the results from the CMC model.

## 2.3 Results and Discussion

During a 365-day period, we operated continuous high-volume air and precipitation samplers at two locations, from which we collected 52 weekly air (vapor and particulate) and 12 monthly rain samples, combined these into four seasonal pools,

and analyzed sample extracts for a total of 275 PCB and PBDE congeners using HRGC/HRMS. The resulting 24 samples analyzed shed light on concentrations and patterns of PCB and PBDE congeners at each site, and also provided us with an opportunity to compare across the two sites. While our study design precluded an assessment of short-term episodic influences, the two-site strategy provided a basis for an evaluation of the nature of PCB and PBDE contamination of air and precipitation in coastal British Columbia, Canada. Throughout the 2004 measurement period, we readily detected PCBs and PBDEs at both the remote Ucluelet station on the westernmost coast of Vancouver Island, and the near-urban Saturna Island station. However, there were noticeable differences in concentrations, patterns, and deposition rates that provide insight into source and transport functions for these contaminants in the northeastern Pacific Ocean.

**PCB and PBDE concentrations, patterns, and partitioning**

Seasonally averaged total PCBs (9.3 ± 0.7 pg/$m^3$ and 8.9 ± 0.7 pg/ $m^3$ for Ucluelet and Saturna, respectively) and PBDEs (13.7 ± 6.1 pg/ $m^3$ and 12.2 ± 6.3 pg/ $m^3$) in air (gas + particulate phases) are similar at both sites (Table 3). These PCB concentrations are lower than those previously reported for rural and urban areas of continental North America and Asia (Lammel et al., 2007; Panshin et al., 1994b), but are in the same range as levels from Mt. Bachelor Observatory, Oregon, US (Primbs et al., 2008). PBDE concentrations in southern BC air samples are lower than the levels from urban areas in Asia or the US (Hoh et al., 2005; Wurl et al., 2005), higher than concentrations reported in remote sites (Strandberg et al., 2001), and similar to levels previously reported over the North Pacific Ocean (Wang et al., 2005).

In rain, the seasonal mean concentrations of PCBs (0.1 ±0.0 ng/L and 4.3 ± 0.9 pg/L for Ucluelet and Saturna, respectively) and PBDEs ± 0.0 ng/L and 14.8 ± 4.4 ng/L) are lower at the remote Ucluelet compared to the near-urban Saturna site (Table 3), consistent with an influence of dilution due to the much higher precipitation at Ucluelet (3270 mm) compared to Saturna (710 mm). When considering the amount of contaminants collected in the rain samples, the total masses of PCBs are similar at both sites, while PBDEs at Saturna exceed the more remote Ucluelet by six times. The higher amount of PBDE in rain at Saturna, even though the air concentrations were similar, can be explained by the higher portion of PBDEs bound to particle at the Saturna site, but can also reflect contamination coming from higher elevation.

These observations support the notion of a somewhat ‘‘uniform’’ distribution of legacy PCBs in air, and an influence of local and current PBDE usage. A concentration gradient of atmospheric PCB and PBDE contamination from urban to rural and remote locations has been observed elsewhere (Shen et al., 2006). PCB levels detected in rain from southern BC are higher than those reported in Atlantic Canada (Brun et al., 1991), but the PCB and PBDE concentrations in our study are comparable to those detected in rain from Sweden (Agrell et al., 2002; Ter Schure et al., 2004).

**Table 3: Seasonal mean temperature, precipitation, and total suspended particles (TSP), as well as PCB and PBDE concentrations in air (gas + particulate) and rain are presented for each site characterizing the near-urban site Saturna Island (Strait of Georgia) and the remote Ucluelet (west coast of Vancouver Island).**

| | | | | | ∑ PCBs | | ∑ PBDEs | |
|---|---|---|---|---|---|---|---|---|
| | | Mean daily Temperature[a] (ºC) | Total Precipitation[a] (mm) | TSP (µg/m$^3$) | air (pg/m$^3$) | rain (pg/L) | air (pg/m$^3$) | rain (pg/L) |
| **Saturna** | Winter | 5.9 ± 0.5 | 199 | 38.1 | 9.9[b] | 4731 | 7.0 | 13016 |
| | Spring | 13.3 ± 0.5 | 67 | 9.5 | 10.2[b] | 6613 | 31.2 | 13923 |
| | Summer | 16.7 ± 0.5 | 159 | 2.8 | 8.1 | 4036 | 4.9 | 5334 |
| | Fall | 8.6 ± 0.5 | 285 | -- | 7.5 | 1842 | 5.7 | 26762 |
| Seasonal mean[c] | | 10.9 ± 1.4 | n.a[d] | 18.7 ± 10.8 | 8.9 ± 0.7 | 4305 ± 985 | 12.2 ± 6.3 | 14759 ± 4441 |
| **Ucluelet** | Winter | 6.9 ± 0.5 | 1037 | 34.2 | 9.2[b] | 124.2 | 14.9 | 172.1 |
| | Spring | 11.5 ± 0.3 | 314 | 16.7 | 10.8 | 148.9 ± 0.2[d] | 30.6 | 87.8 ± 2.5[d] |
| | Summer | 15.0 ± 0.3 | 463 | 10.2 | 9.7 | 119.2 | 4.3 | 58.4 |
| | Fall | 9.1 ± 0.5 | 1456 | -- | 7.3 | 155.9 | 5.1 | 109.3 |
| Seasonal mean[c] | | 10.8 ± 1.1 | n.a[d] | 12.4 ± 4.0 | 9.3 ± 0.7 | 139.4 ± 7.4 | 13.7 ± 6.1 | 103.1 ± 19.1 |

[a]: Values recorded by Environment Canada (www.climate.weatheroffice.ec.gc.ca). Precipitation values include both snow and rain. Our precipitation values (not presented) appeared to be largely underestimated probably because of some loss due to the limited capacity of the sampler (during wet period) and some evaporation process (during warm/dry period). [b] : represents estimation from the remaining seasons. [c] : average ± standard error. [d] : average ± standard error from 2 replicates. e: non applicable. --: non available.

Pincipal component analysis clearly differentiates the different phases based on their PCB and PBDE congener patterns (Figure 4) and convincingly illustrates the partitioning between the vapor, particulate, and aqueous (rain) phases. The first principal component (PC1: 39.3% of the total variance) separates the particulate from the gas and rain phases, while the second component (PC2: 15.1%) separates gas from rain. In the corresponding variable plots, the PBDEs and hepta- to deca-PCB congeners project on the left hand side, indicating a predominance of heavier congeners in the particulate phase. Physico-chemical properties as well as environmental parameters that could be involved in this partitioning process will be further discussed below.

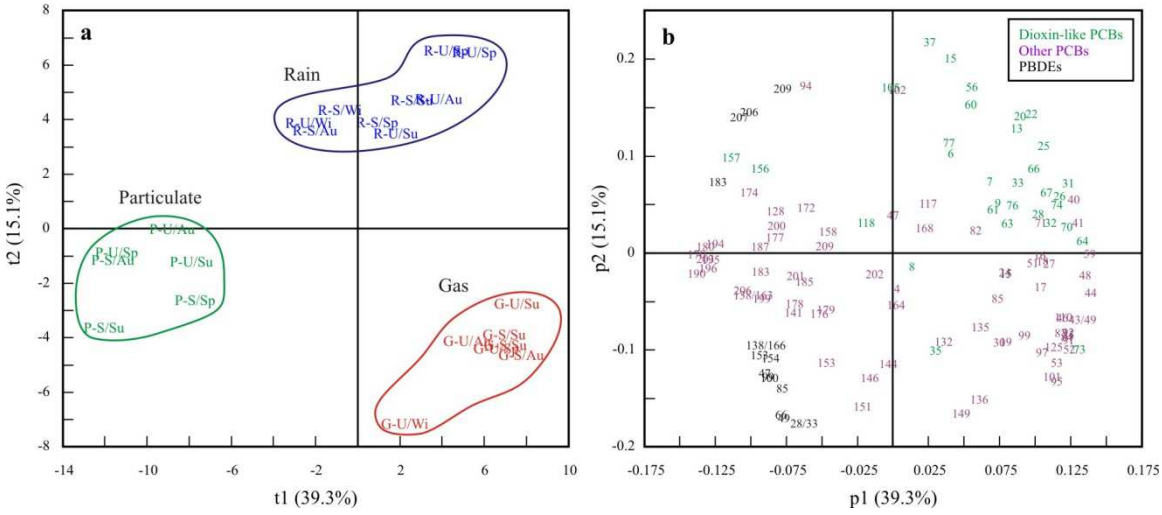


**Figure 4: Principal component analysis (PCA) where the variance accounted for by each principal component is shown in parentheses after the axis label. (a) As shown by the sample score plot (t1 and t2), patterns of polychlorinated biphenyls (PCB) and polybrominated diphenyl ethers (PBDE) congeners differed markedly between the three phases: gas, particulate and rain. Within each phase, there were no clear differences between sites, or between seasons. (b) The PCA loadings plot (p1 and p2) for individual PCB and PBDE congeners. The particulate phase revealed that the heavier halogenated congeners were associated with the particulate phase (R: rain, P: particulate, G: gas phase, Wi: winter, Sp: spring, Su: summer, Au: autumn).**

The seasonal average particulate phase contributions to the total air concentrations are 13 ± 3% and 15 ± 2% for PCBs at Ucluelet and Saturna, respectively, and 31 ± 12% and 49 ± 14% for PBDEs. For both classes of chemicals, the contribution of the particulate phase is similar between sites ($p > 0.05$). The generally lower vapor pressure of PBDEs results in higher particle-bound percentages than observed for PCBs, with the highly brominated congeners such as BDE-209 residing almost entirely in the particulate phase (76 ± 5% and 91 ± 2% for Ucluelet and Saturna, respectively, based on seasonal average). This high binding of BDE-209 to particles is thought to limit the transport of this congener to remote areas (St.Amand et al., 2007). The seasonal average of the percentage of particle bound for the different homologue groups is highly correlated with the log vapor pressure at both sites (Henry's Law constant or H; $p < 0.01$) (Figure 5). The lack of significant differences in slopes between the two sites ($p > 0.05$) suggests similar gas/particle partitioning. This observation is in accordance with the similar environmental parameters, such as temperature and amount of total suspended particles ($p < 0.05$), reported at the remote Ucluelet site and the near-urban Saturna location (Table 3).

Based on seasonal averages, correlations between the air/rain concentration ratio and the particle-bound percentage were observed for both PCBs ($r^2 = 0.41$ and 0.70 for Ucluelet and Saturna, respectively, $p < 0.05$) and PBDEs ($r^2 = 0.62$ and 0.56, $p < 0.05$). There is no correlation between the gas/rain concentration ratio and Henry's Law constant for PCBs and PBDEs at both sites, further demonstrating the limited contribution of the gas phase to rain associated contaminants.

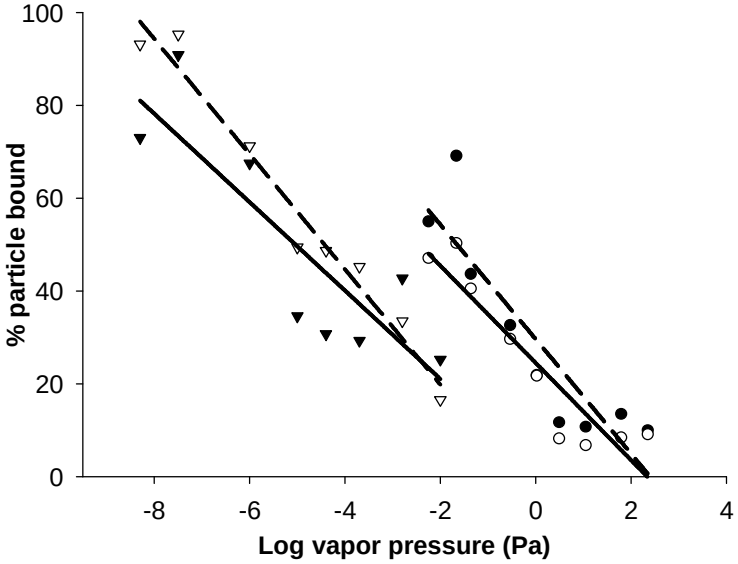


**Figure 5: There are no significant differences in the PCB and PBDE gas-particle partitioning between the remote Ucluelet site (-) and the near-urban site Saturna Island (---) consistent with the similar environmental parameters (temperature, amount of total suspended particles) reported at each site. (Vapor pressures are from (Falconer et al., 1994; Xu et al., 2007)).**

Based on the average for the four seasons, PCB homologue group patterns in air and rain are similar (Figure 6). Tri and tetra-CBs comprise between 21 and 30% of the total PCB concentrations at each site and dominate the profiles, as reported in studies from other parts of the world (Panshin et al., 1994a; Totten et al., 2004; Yeo et al., 2004). Di-CBs contribute between 14 and 23% of the total and the heavier homologue groups comprise decreasing contribution with increasing molecular weight. The pattern of PCBs in air is similar at the two sites, but a lighter pattern is evident in rain at the remote Ucluelet site. The contribution of Di-CBs is higher at Ucluelet, whereas rain at Saturna has significantly higher contributions of the heavier homologue groups (penta, nona, and deca-CBs). Lighter congeners CB-8, 18, 28, 31, and 33 are the dominant congeners (see top six congeners in Table 4), consistent with previous studies conducted in other remote locations (Panshin et al., 1994a; Shen et al., 2006).

The contribution of these lighter PCB congeners to PPCBs is higher at the remote Ucluelet site (30–40%) compared to the near-urban Saturna site (25–35%).

At both sites, the seasonal average reveals that tetra-, penta-, and deca-BDE homologue groups dominate the PBDEs in air and rain samples, but the pattern differs between these two matrices. In air, tetra- and penta-BDE groups dominate the composition of PBDEs (75 ± 7% and 67 ± 8% of ∑PBDEs at Ucluelet and Saturna, respectively), while deca-BDE dominates the composition in rain (61 ± 4% and 78 ± 6% of ∑PBDEs). This dominance of Deca-BDE in rain is consistent with the high percentage of BDE-209 bound to particles and the high contribution of particle scavenging by precipitation (Hirai et al., 2004). In both matrices, PBDE homologue group pattern appears lighter at the remote Ucluelet site, although this was only significant in rain samples (Figure 6). In terms of congeners, BDE-47, 99, 100 and 209 are dominant in air, representing 80 ± 8% of ∑PBDEs at Ucluelet and 81 ± 13% at Saturna (Table 4). In rain, BDE-47, 99 and 209 accounted for 81 ± 6% and 90 ± 9% of the total PBDEs at Ucluelet and Saturna, respectively, while BDE-100 does not appear in the top six (Table 4). Significant contributions of BDE-209 to PBDE contamination of air have been reported in urban and remote sites (Gouin et al., 2006; Hoh et al., 2005).

**Table 4: Mean annual concentrations of total PCB and PBDE concentrations, as well as their six dominant congeners, in the gas phase, particulate phase (pg/m$^3$) and rain (pg/L) at the remote Ucluelet and the near-urban Saturna Island are presented.**

| | RAIN | | | | PARTICULATE PHASE | | | | GAS PHASE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ucluelet | | Saturna | | Ucluelet | | Saturna | | Ucluelet | | Saturna | |
| ∑PBDEs | 103.07 ± 19.09 | | 14758.83 ± 4440.77 | | 2.30 ± 0.10 | | 3.62 ± 0.34 | | 11.41 ± 6.13 | | 7.10 ± 5.05 | |
| | 209 | 65.09 ± 14.50 | 209 | 12263.69 ± 4501.96 | 209 | 0.71 ± 0.26 | 209 | 1.17 ± 0.06 | 99 | 4.82 ± 2.78 | 99 | 3.07 ± 2.39 |
| | 47 | 10.57± 1.36 | 99 | 532.62 ± 74.27 | 47 | 0.51 ± 0.10 | 99 | 0.83 ± 0.17 | 47 | 3.50 ± 1.76 | 47 | 2.20 ± 1.52 |
| | 99 | 7.86 ± 1.48 | 47 | 499.32 ± 98.21 | 99 | 0.42 ± 0.12 | 47 | 0.71 ± 0.09 | 100 | 1.01 ± 0.57 | 100 | 0.59 ± 0.45 |
| | 3 | 5.61 ± 2.23 | 206 | 415.42 ± 100.18 | 3 | 0.27 ± 0.07 | 100 | 0.16 ± 0.03 | 153 | 0.45 ± 0.27 | 153 | 0.28 ± 0.22 |
| | 207 | 2.76 ± 1.25 | 207 | 305.59 ± 64.51 | 100 | 0.09 ± 0.02 | 3 | 0.13 ± 0.03 | 85 | 0.41 ± 0.24 | 154 | 0.23 ± 0.18 |
| | 206 | 2.35 ± 1.04 | 3 | 220.81 ± 73.01 | 207 | 0.06 ± 0.01 | 207 | 0.10 ± 0.02 | 209 | 0.20 ± 0.05 | 209 | 0.11 ± 0.03 |
| ∑ *Top 6* | | *94.24 ±21.86* | | *14237.45 ±4912.14* | | *2.05 ±0 .58* | | *3.11 ±0.40* | | *10.39 ±5.67* | | *6.48 ±4.78* |
| ∑ PCBs | 139.42 ± 7.39 | | 4305.36 ± 985.35 | | 1.12 ± 0.27 | | 1.06 ± 0.22 | | 8.14 ± 1.29 | | 7.08 ± 0.34 | |
| | 8 | 13.91 ± 2.56 | 31 | 214.31 ± 46.68 | 8 | 0.06 ± 0.01 | 8 | 0.07 ± 0.02 | 11 | 0.79 ± 0.16 | 8 | 0.31 ± 0.00 |
| | 28 | 8.14 ± 1.05 | 28 | 203.27 ± 46.75 | 31 | 0.05 ± 0.02 | 31 | 0.05 ± 0.01 | 18 | 0.43 ± 0.06 | 95 | 0.27 ± 0.02 |
| | 31 | 8.09 ± 0.96 | 33 | 199.86 ± 45.24 | 18 | 0.05 ± 0.02 | 18 | 0.05 ± 0.01 | 31 | 0.39 ± 0.07 | 33 | 0.24 ± 0.03 |
| | 18 | 6.81 ± 0.65 | 8 | 153.27 ± 56.43 | 4 | 0.03 ± 0.01 | 28 | 0.04 ± 0.01 | 8 | 0.36 ± 0.06 | 44 | 0.21 ± 0.02 |
| | 33 | 6.70 ± 1.01 | 18 | 149.34 ± 31.24 | 16 | 0.03 ± 0.01 | 33 | 0.04 ± 0.01 | 28 | 0.36 ± 0.06 | 43/49 | 0.18 ± 0.02 |
| | 15 | 5.88 ± 0.47 | 70 | 146.80 ± 32.73 | 52/73 | 0.02 ± 0.01 | 4 | 0.04 ± 0.01 | 52/73 | 0.36 ± 0.05 | 16 | 0.17 ± 0.02 |
| ∑ *Top 6* | | *49.53 ±6.7* | | *1066.85 ±259.07* | | *0.24 ±0.01* | | *0.29 ±0.01* | | *2.69 ±0.07* | | *1.38 ±0.01* |

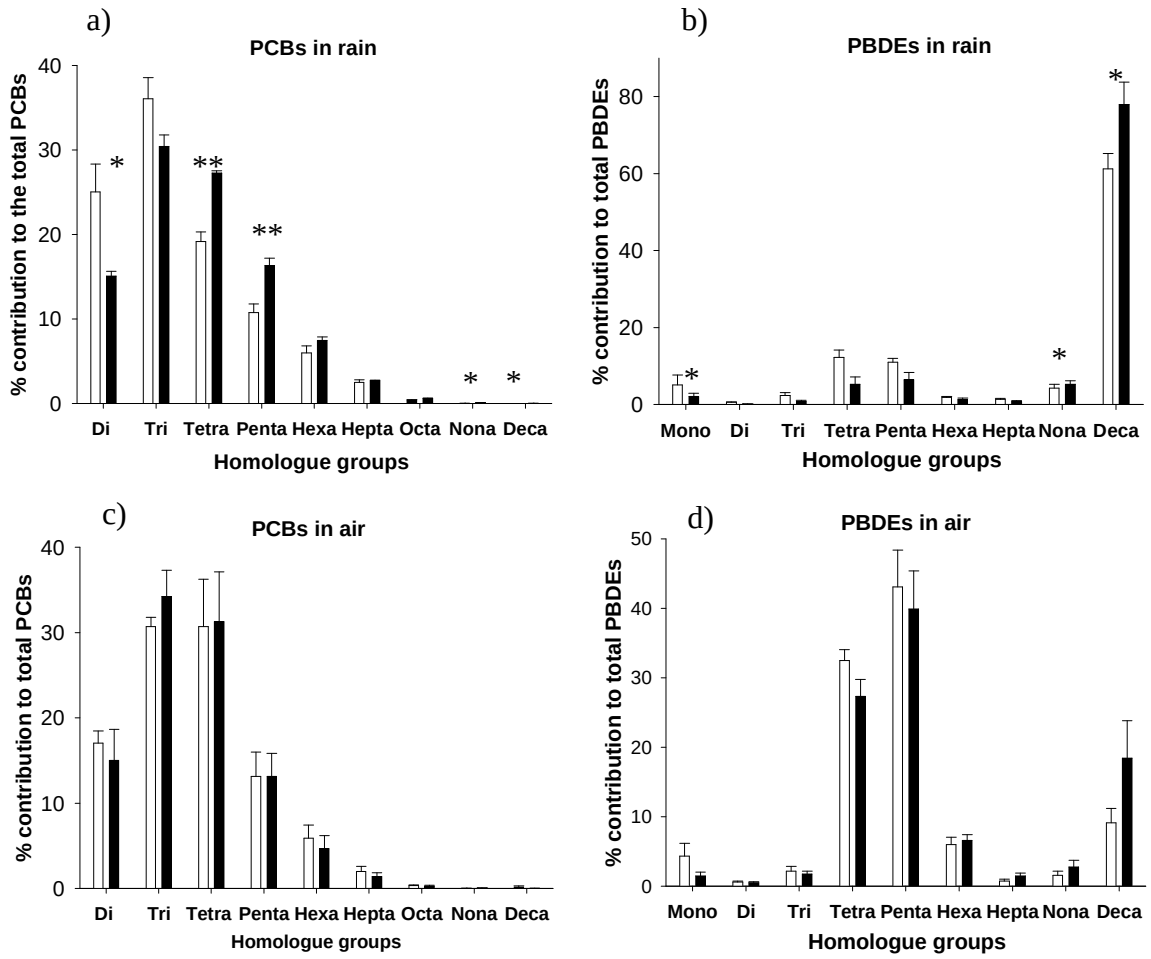


**Figure 6: PCB (a,c) and PBDE (b,d) homologue group patterns in rain and air (particulate + gas) reveals lighter signature for both chemical classes at the remote Ucluelet (white) compared to the near-urban Saturna Island (black) consistent with long-range atmospheric transport of less halogenated PCBs and PBDEs. Differences between sites: * = $p < 0.05$; ** = $p < 0.01$.**

### Seasonal variation in PCBs and PBDEs

Along coastal BC, two prevailing wind patterns occur during the year, reflecting the dominant influence of two large-scale atmospheric circulation features over the Northeast Pacific: the Aleutian Low in winter and the North Pacific High in summer. During the cool season, the effect of eastward-tracking storms into the Gulf of Alaska give rise to the Aleutian Low pressure system over the Northeast Pacific and prevailing winds generally blow from the south/south-west along coastal BC. During

the warm season, the Aleutian Low weakens, with a decreased frequency and intensity of storm systems. At the same time, the North Pacific High pressure system to the south strengthens, and resulting winds along coastal BC originate from the west/north-west (Lange; 2003) (Figure 3). Offshore, and over much of the North Pacific, both the cool and warm season circulation patterns favour a westerly atmospheric flow in the mid-to lower troposphere (below 5 km), regardless of season. Our ten-day back trajectory analyses (Figure 7) reflect this dominant flow from the west, with little seasonal variation in wind direction along the BC coast.

We present only the back-trajectory results for the Ucluelet site, as the two sampling sites revealed similar results. This is consistent with the large-scale atmospheric flow from the west, although nearer the west coast of North America prevailing wind directions are more variable due to the influence of topographic features such as coastal mountains and inland waterways. In a region such as the Strait of Georgia, the complex behaviour of local atmospheric circulation patterns is due to the interaction between the large-scale flow and local circulation regimes, such as topographically-steered along-channel flow, upslope-downslope winds, and land-sea breezes (Lange, 2003). Since this complex circulation regime is on a similar or finer scale than that of regional atmospheric models, they would be of limited value in our current study.

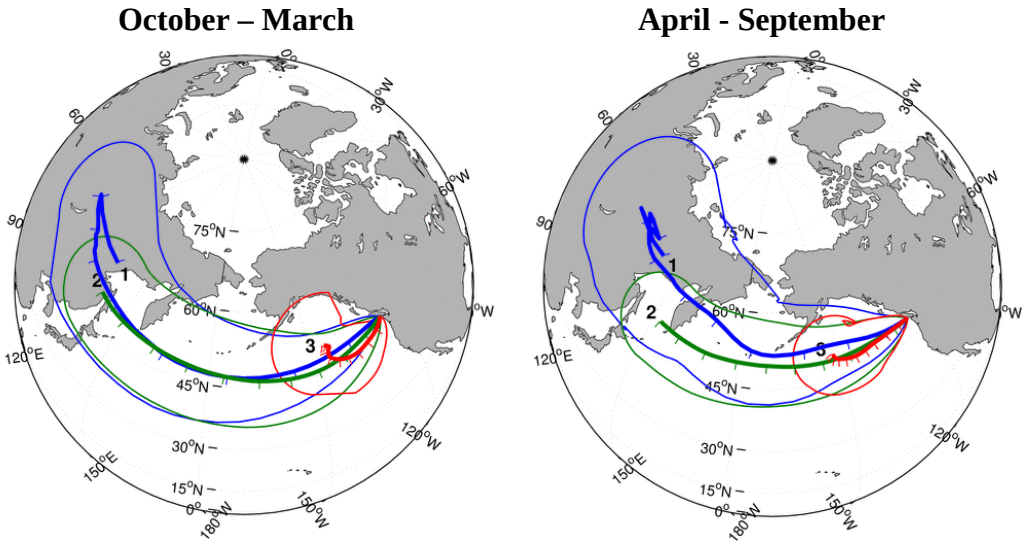


**Figure 7: Six-hourly, 10-day, back trajectories from Ucluelet were calculated over 2004 using the Canadian Meteorological Center trajectory model. Trajectories were clustered over the cool (January-March) and warm (April-September) seasons. The mean trajectory for each of the three clusters is shown and each cluster is enclosed by en envelope indicating +/- 0.5 standard deviation. Cluster results are similar between stations (Saturna Island not shown) and seasons. The short distance trajectory cluster (cluster 3) reflects low-level (~ 1km) short-range transport air masses from the northwest and southwest. The remaining two clusters reflect long-range eastward transport from eastern Asia (predominantly Russia / China), one characterizing high altitude (~ 5 km) flow (cluster 1) and a small percentage of the total clusters; and the other one representing lower altitude (~ 2 km) flow and almost half of the total number of trajectories (cluster 2).**

At both air sampling sites, PCB concentrations in air appear relatively stable throughout the year, but the highest levels of both PCBs (10.8 pg/m3 and 10.2 pg/m3 for Ucluelet and Saturna, respectively) and PBDEs (30.6 pg/ $m^3$ and 31.2 pg/ $m^3$ for Ucluelet and Saturna, respectively) were observed in spring at both sites (Table 3). Spring is the most favorable time for the delivery of air pollutants from the west to the coast of North America (Jaffe et al., 1999b; Wilkening et al., 2000). While similar inter-seasonal PCB and PBDE patterns would suggest similar sources and pathways

throughout the year, the highest concentrations reported in spring, especially for PBDEs, could indicate increased delivery from trans-Pacific air mass movement during this season, consistent with the findings of others (Holzer et al., 2003). Episodic sampling and analysis would help better discern the influence of seasonal weather systems.

A lack of correlation between temperature and PCB or PBDE concentrations in air (results not shown) may be explained by the narrow range of annual temperatures in the temperate coastal environment of BC as well as by our limited sample size.

### Global versus local sources of PCBs and PBDEs in southern BC

Since atmospheric deposition represents a significant route of entry of contaminants into aquatic ecosystems (Duce, 1990), we estimated the deposition to the water surface adjacent to each sampling station. For PCBs and PBDEs, the wet and particulate deposition (50.5 ± 8.2% and 39.7 ± 3.5%, respectively, on average at both sites for all seasons) dominate the total atmospheric deposition, followed by gas deposition (9.8 ± 6.2%). As reported elsewhere (Cetin et al., 2007; Holsen et al., 1991; Venier et al., 2008), dry deposition was dominated by particulate washout, despite the majority of PCBs and PBDEs being found in the gas phase. While the gas phase contaminants are deposited by diffusion, particulate contaminants are deposited mostly by gravitational settling resulting in a much higher deposition velocity (Holsen et al., 1991). Using the values recorded at Saturna, we estimated the total atmospheric inputs of PCBs and PBDEs to the Strait of Georgia (8900 $km^2$) at 3.5 ± 0.7 kg/year and 17.1 ± 6.5 kg/year, respectively, highlighting the increasing dominance of the PBDEs as environmental contaminants.

A comparison of total deposition rates at the remote west coast site and the near-urban site provides a means of estimating the contribution of a global PCB and PBDE ‘background’’ (namely, those PCBs and PBDEs derived from long-range atmospheric transport) in southern BC air. The similar PCB deposition rates at both sites (4.4 mg/ha/year and 3.9 mg/ha/year for Ucluelet and Saturna, respectively) underscore a relatively uniform geographical ‘‘background’’ for this legacy compound. On the other hand, the much higher PBDE deposition rate at our near-urban site (19.1 mg/ha/year) strongly suggests a local (North American) influence for this at the time still used flame retardant (Figure 8). Despite this signal, we did detect PBDEs at the remote Ucluelet site (8.1 mg/ha/year), on the outer west coast of Vancouver Island, where they amounted to 42% of the rates calculated for the near-urban Saturna site. In conducting over 12,000 ten-day back trajectories, we found that 40% originated over Asia. Prevailing winds from the west are therefore consistent with our observed difference in PBDE deposition between the two sites, with these two lines of evidence supporting the notion that non-North American sources account for a significant percentage of the PBDEs in coastal BC air.

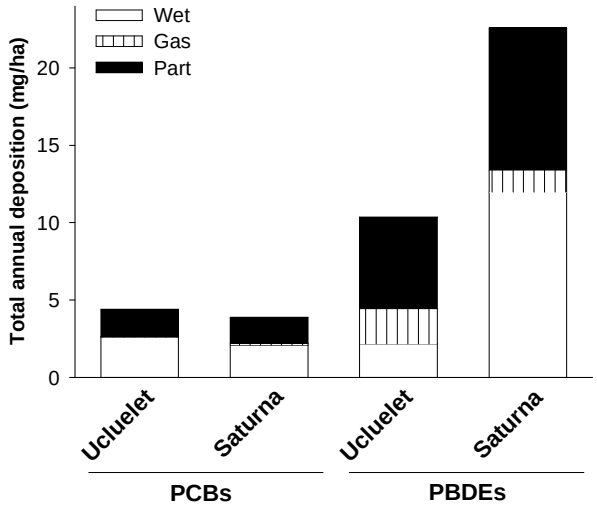


**Figure 8: Annual PCB deposition (wet + particulate + gaseous) is similar at both the remote and near-urban sites, reflecting the relatively uniform environmental dispersion of this legacy chemical. In contrast, Saturna Island receives higher amounts of PBDEs than the remote Ucluelet reflecting the influence of local sources for this currently-used flame retardant. Nonetheless, the detection of PBDEs at the Ucluelet station can be traced back via prevailing winds to the Asian continent.**

## 2.4 Conclusions

While PCBs remain the persistent contaminant of concern in aquatic biota from BC, PBDEs are increasingly seen as an emerging threat to marine mammals, including killer whales (Ross, 2006). The rapid movement of westerly air masses across the Pacific Ocean provides a mechanism for the ready delivery of pollutants to North America from a burgeoning Asian economic zone (Jaffe et al., 1999b; Wilkening et al., 2000). Moreover, PBDE concentrations in Asian air are likely to increase in part as a result of extensive electronic waste recycling sites; 80% of the North American ‘e-waste’ is exported to Asia for recycling, dumping and/or open burning (Wong et al., 2007). Our observation of what appears to be a notable, trans-Pacific contribution to BC for the commonly used PBDEs highlights the need for global regulatory scrutiny (Ross et al., 2009), such as has been afforded to other POPs by the

Stockholm Convention. The application of high-resolution regional atmospheric models, combined with additional, episode-oriented sampling and congener-specific contaminant analyses, should contribute to a better understanding of this mechanism in coastal British Columbia.

# Chapter 3: Harbour seal fur and whiskers: insights into mercury exposure at the top of a coastal northeastern Pacific food web.

## 3.1 Introduction

Mercury (Hg), in the form of toxic methylmercury (MeHg), can bioaccumulate up the food chain and has the ability to induce a variety of short and long term toxic responses in marine top predators. Significant Hg concentrations reported in various species of marine mammals around the world (Beckmen et al., 2002; Brookens et al., 2008; Loseto et al., 2008b) have been linked to neurotoxicity and immunotoxicity (Basu et al., 2009; Frouin et al., 2011).

Globally, mercury is emitted from both natural (~60% of the total Hg atmospheric emissions, most of which includes re-emissions from past deposition) (Pirrone et al., 2010) and anthropogenic sources. Human activities during the past two centuries have augmented the Hg cycle such that two to three times more Hg is currently cycling through the biosphere than in pre-industrial times(Pirrone et al., 2010). Among a variety of anthropogenic mercury sources, which have included fossil fuel fired power plants, ferrous and non-ferrous metal smelters or waste incinerators, the leading emission, accounting for about 40% of total anthropogenic emissions, has been artisanal small scale gold mining activities (www.unep.org). However, electric power generation facilities are the number one source contributing to more than 50% of the total anthropogenic emissions (Pirrone et al., 2010). In Canada, most European countries, and Japan, there are regulations to limit mercury emissions from coal fired power plants. In December 2011, the US Environmental Protection Agency defined, for the first time, national standards in order to reduce mercury pollution from power plants (www.epa.gov). In Asia, the major emitter of Hg, there is limited regulations currently in place representing a concern as its contribution is expected to become more significant due to anticipated increases in emissions, particularly in China (Pacyna et al., 2010). On the international level, the Minamata Convention was recently agreed on by many nations and will be signed in October 2013. Governments

agreed to a global, legally-bonding treaty to control and reduce mercury emissions across a range of products, such as thermometers and energy-saving light bulbs. This Convention is also aiming at controlling emissions from mining, cement and coal-fired power sectors (www.unep.org).

With prevailing winds from the west delivering air masses from Asia to North America in two to ten days (Jaffe et al., 1999b; Jaffe et al., 2003), it has been estimated that Asian emissions contribute between 15 and 24% of the total Hg deposition over the western United States (US) (Seigneur et al., 2004; Strode et al., 2008). In addition to long-range transport, a long history of local Hg contamination in the Salish Sea commenced in the late 1800s (Johannessen et al., 2005). The most recent significant contamination period (1965-1975) was associated with a chlor-alkali plant at the head of Howe Sound, which may have been discharging in its effluent as much as 20kg.$d^{-1}$ of inorganic Hg (Thompson et al., 1980).

Harbour seals are the most abundant marine mammals in the transboundary waters of British Columbia (BC), Canada, and Washington State (WA), USA. There are about 39 000 harbour seals inhabiting the Strait of Georgia (BC) (Olesiuk, 2009) and between 13 000 and 14 000 in the inland waters of WA (Jeffries et al., 2003). They are relatively non migratory and feed on a wide variety of fish and invertebrate species providing us with an integrated signal of local food web contamination. There are a number of studies on persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs), in this population of harbour seals and their food web that revealed spatial variations in contamination. For example, harbour seals from WA have been reported to be seven times more PCB contaminated than the ones from BC (Ross et al., 2004; Ross et al., 2012). In contrast, little is known about Hg levels in this population of harbour seals.

Hair provides a stable medium that has been used as a non-invasive way to monitor Hg exposure in pinnipeds (Brookens et al., 2008). In addition, Hg is stored in the hair as it grows, providing a history of accumulation over time (Legrand et al., 2004; Rodushkin et al., 2003; Stadlbauer et al., 2005). As opposed to human hair, which grows continuously over time, harbour seal hair grows rapidly over a short period of time after the annual moult therefore preventing temporal trend analyses of mercury accumulation. Harbour seal whiskers, on the other hand, grow throughout the year (Greaves et al., 2004; Hirons et al., 2001). Here, we use a combination of hair and whisker samples from live-captured harbour seals to investigate 1) the factors affecting Hg accumulation in harbour seals; 2) spatial variations of Hg at the top of the coastal BC and WA marine food web; 3) Hg accumulation along harbour seal pup whiskers; and 4) determine whether current Hg levels are of concern for this population.

## 3.2 Materials and Methods

### Sampling

A total of 167 harbour seal pups (82 males; 85 females), 14 juveniles (8 males; 6 females) and 28 adults (14 males; 14 females) were live captured at ten sites in British Columbia, Canada, and Washington State, USA, between 2003 and 2010 (Figure 9). An average of 7 ± 2 seals were collected at each site. Harbour seals were caught using two techniques. At rocky sites, individual seals were captured using salmon landing nets. At sandy haul out sites, multiple seals were captured at once using a rapidly deployed beach seine net (Jeffries et al., 1993). Body weight, length, girth (only for pups) and sex were determined. Fur was collected using an electric razor following cleaning of the site with deionized water. Whiskers (one per

individual) were cut as close to the face as possible and were collected for 10 pups captured in Puget Sound.

Capture stress and holding time were minimized. All procedures were carried out under the auspices of the respective animal care committees and scientific research permits for researchers in British Columbia (Fisheries and Oceans Canada Animal Care Committee with guidelines from the Canadian Council on Animal Care; Scientific Research Permit) and Washington State (U.S. Marine Mammal Protection Act Permit 835).

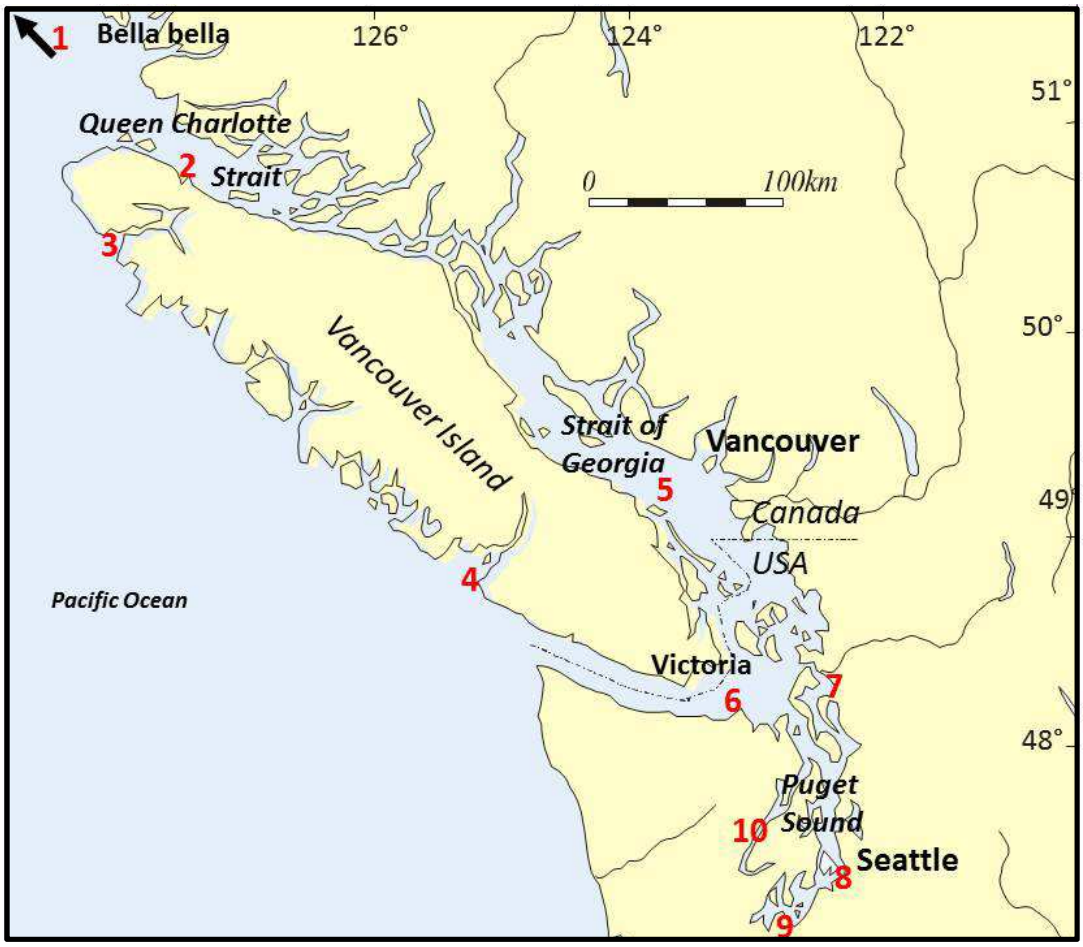


**Figure 9: A total of 209 seals were live-captured at various sites in British Columbia, Canada, and Washington State, USA, between 2003 and 2010 (1: Bella bella; 2: Queen Charlotte Strait; 3: Quatsino Sound; 4: Port Renfrew; 5: Strait of Georgia; 6: Juan de Fuca Strait; 7: Skagit Bay; 8: Central Sound; 9: South Sound; 10: Hood Canal).**

**Total mercury (THg) analyses in harbour seal hair and whiskers**

To remove any external contamination, all hair sub-samples were rinsed with acetone / de-ionized water / acetone and left to dry at room temperature. They were then stored in a dessicator until analysis. An average of 1.7 ± 0.7 mg of hair was analysed for THg using a thermal decomposition Zeeman atomic absorption spectrometer RA-915+ coupled with a PYRO-915 attachment (Lumex, St. Petersburg, Russia). The detection limit was 0.002 µg/g dry weight. We used a customized high sensitivity version of the methods developed previously by Sholupov et al. (2004).

Two standards were used: a sediment standard NIST 2709 (National Institute of Standards and Technology, Gaithersburg, USA), and a human hair standard NIES 13 (National Institute for Environmental Studies, Ibaraki, Japan). As NIST 2709 appeared to be a more homogeneous standard, it was used to make the calibration curve. One NIST 2709 and one NIES 13 standard was run every six samples to ensure that there was no deviation from the calibration curve. Each of the 209 seal hair samples was run in triplicate.

Mercury levels along pup whiskers were analyzed using laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS). Whiskers were first mounted on a glass slide using double-sided tape. LA-ICPMS analyses were conducted following the protocol outlined in Sanborn and Telmer (2003). The LA-ICPMS system used was a Thermo X-Series 2 Quadrupole ICP-MS coupled to a New Wave UP-213 UV laser ablation system. The laser system operates at a wavelength of 213 nm and a maximum energy output of 3mJ. All whiskers were ablated at a spot size of 65 µm with an output frequency of 20 Hz and 65% power. Line scans along the middle line of the whiskers were completed by tacking the laser along the whisker at 100 µm/s. Background intensities were collected for 30s prior to running the laser. In addition, a

pressed synthetic calcium carbonate pellet standard reference material MACS-3 (US Geological Survey) was analyzed, both at the beginning and at the end of each run, in order to complete an external drift correction to compensate for any changes in machine sensitivity.

The determination of THg in hair and whiskers is representative of MeHg concentrations as previous studies have shown that more than 90% of mercury present in hair is in the methylated form (Kehrig et al., 1998; Voegborlo et al., 2010).

**Stable isotope analyses**

All hair sub-samples were washed with 2:1 chloroform:methanol three times to remove any surface contamination. Each hair sample was then freeze-dried at -50°C for 24 to 48 hours and then stored in a dessicator until analysis. Subsamples of approximately 0.9 ± 0.09 mg were placed in tin capsules. Stable isotope measurements were carried out at the Biogeochemistry Facility (School of Earth and Ocean Sciences, University of Victoria, BC) using a Fisons NA 1500 Elemental Analyser-Isotope Ratio Mass-Selective (Milano, Italy) interface to a FinniganMAT 252 Isotope Ratio Mass Spectrometer (Bremen, Germany). Results are reported in parts per mil (‰):

$$\delta X = [(R_{Sample} / R_{Standard}) - 1] \times 1000$$

where $\delta X$ is $\delta^{13}C$ (‰ vs PDB) or $\delta^{15}N$ (‰ vs air $N_2$), and R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio, respectively. Carbon and nitrogen measurements were made relative to run of acetanilide (an in-house standard with known isotope ratios) and blanks. Replicates were analyzed in every batch to evaluate variations within samples, variations over time and variations between sample racks. Isotopic values were adjusted.

**Data treatment**

Each fur sample was analyzed in triplicates for THg. In every case, variation amongst triplicates was < 10% which is considered within the instrument / scale / user average precision and therefore the average for the three replicates will be used.

Preliminary results on Hg levels in harbour seal pup hair revealed no significant differences among sites from the same geographic region. Samples were therefore pooled as follows: *Strait of Georgia* includes Hornby Island, Quadra Island and Vancouver; *Juan de Fuca Strait* includes Sidney, Victoria and Smith Island.

The 209 harbour seals sampled were grouped into three different age classes: (1) pups that were between 4 and 6 weeks old, (2) juveniles that were under 4 years old for males and 5 years old for females and (3) adults which included seals above 4 and 5 years old for males and females, respectively. Adult and juvenile harbour seals were only captured in Southern Puget Sound. Because of the spatial variations observed in Hg levels in harbour seal pup fur, only pups from southern Puget Sound will be used for comparison with adults and juveniles.

Normality and homogeneity of variances were tested for Hg levels (expressed on a dry weight basis) using the Kolmogorov-Smirnov test and Levene's test, respectively (SPSS, IBM Corporation, Armonk, NY, USA). If the data did not meet the assumption of normality and homogeneity of variances, they were log-transformed. Analyses of variance (ANOVA) followed by a Dunnett's test were performed to determine differences in Hg levels from our reference site, Bella Bella. ANOVAs were also used to determine possible differences in Hg levels among age classes and a t-test was used to investigate differences between sexes.

LA-ICPMS data collection and data reduction were completed using Thermo Electron PlasmaLab Software 2003, Version 2.6.1 (Thermo Fisher Scientific Inc.). The "fully quantitative analysis" option was chosen. Similar to previous studies

investigating Hg variations along hair strands, we used sulfur as an internal standard in order to reduce the influence of variations in the rate of ablation on the data. Sulfur amounts to 5% of the element concentrations in hair and its concentration has been reported to be stable among hair and along hair strands (Rodushkin et al., 2003; Stadlbauer et al., 2005).

The laser was run across the whisker at 100 µm/s. A 100 µm length corresponds to a whisker growth of approximately 3h, on the basis of an average whisker growth rate for newly grown whisker of 0.78 mm/day (Zhao et al., 2004). In order to get approximately a daily signal, an average was generated every eight data points. Low standard deviations within these subgroupings ensured a representative average.

Hg variations along harbour seal whiskers were assessed by segmented linear regressions using the program SegReg (downloaded from http://www.waterlog.info/segreg.htm) which selects the best-fitting break-points and linear regression functions for a given data set. The selection for best fit is based on significance and maximal explanation of variation (Oosterbaan; 2005).

## 3.3 Results and discussion

### Influence of age group, sex, and other biological variables on Hg accumulation in harbour seals

Adult harbour seals (8.3 ± 0.8 µg/g) had higher mercury levels than juveniles (4.5 ± 0.5 µg/g; $p = 0.001$) and pups (5.3 ± 0.3 µg/g; $p = 0.007$) (Figure 10) consistent with previous studies reporting an increase of mercury with age (Aubail et al., 2011; Brookens et al., 2007; Skaare et al., 1994). An increase of Hg concentrations with age has been attributed to bioaccumulation as well as changes in diet over time. Juvenile seals usually have different diving and foraging behavior than adults resulting in a different diet usually comprised of a higher proportion of smaller fish (Lesage et al.,

2001; Young et al., 2010). In the present, $\delta^{15}N$ and $\delta^{13}C$ data indicated no significant difference in feeding ecology between juveniles and adults probably reflecting the relatively low sample size for the two age classes.

Influence of sex was investigated for each age class. No sex differences were reported for adults (8.7 ± 1.4 and 8.1 ± 1.3 µg/g for males and females, respectively), juveniles (4.6 ± 0.6 and 4.4 ± 0.8 µg/g, respectively) or pups (5.4 ± 0.3 and 6.1 ± 0.8 µg/g, respectively) (Figure 10). This finding differs from previous studies reporting gender differences in Hg levels in adult pinnipeds as a result of diet differences between males and females as well as the offload of Hg from the female to the pup via gestation and lactation (Brookens et al., 2007; Skaare et al., 1994). While age was not available for adult seals, we can speculate that the lack of significant difference in Hg levels between males and females is the result of a fairly young age resulting in no diet differences and no gestation / lactation effect.

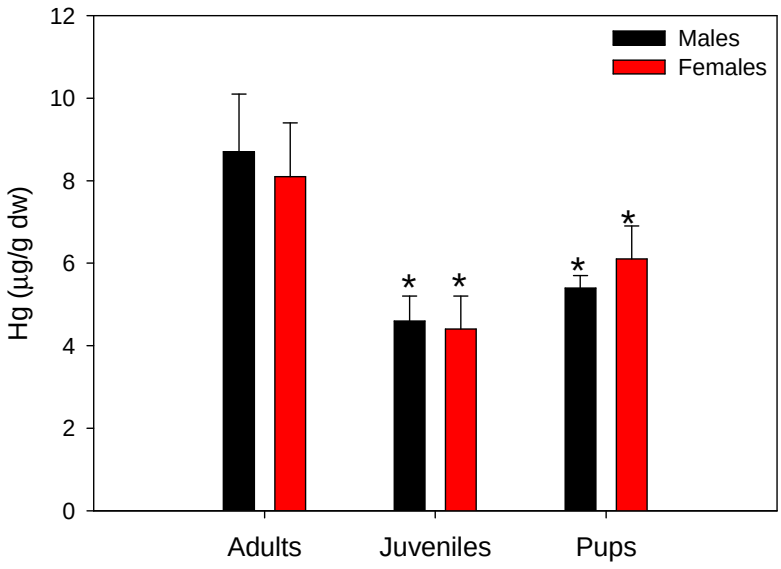


**Figure 10: Adult harbour seals had significantly higher Hg levels than juveniles and pups ($p<0.001$). There were no differences between males and females for any of the age group.**

The influence of weight, length and feeding ecology (inferred from $\delta^{15}N$ and $\delta^{13}C$) was investigated using Pearson correlation coefficients as well as stepwise regressions. As opposed previous studies reporting an increase of Hg with age and length in adult seals (Aubail et al., 2011; Brookens et al., 2007), there were no correlations between Hg and weight or length probably reflecting the small sample size for this age class. In juveniles, weight, length and $\delta^{15}N$ were all positively correlated with Hg ($r^2$=0.37, $r^2$=0.49 and $r^2$=0.37, respectively). Stepwise regression, however, revealed that $\delta^{15}N$ was the main factor explaining most of the variance in Hg levels observed in juvenile seals probably reflecting the wide range of diet in this particular age class (Table 5). With their rather limited diving and foraging abilities, weaned pups usually feed on a higher proportion of crustaceans and/or small fish which are lower in the food chain. As they grow older, the pup/juvenile diet is likely to resemble more and more a typical adult diet comprised of Pacific herring (*Clupea pallasii*) and/or hake (*Merluccius productus*) which are higher in the food chain (Lance et al., 2007; Olesiuk et al., 1990) and mercury in known to bioaccumulate in the food chain (Kainz et al., 2006). In pups, weight and length were positively correlated with Hg levels ($r^2$=0.16 and $r^2$=0.12, respectively) and the stepwise regression revealed that weight was the most important factor (Table 5). As weight can be used as a surrogate for age (Cottrell et al., 2002), these results suggest an increase of Hg in hair with the duration of lactation similar to that was found in Faroe Island infants (Grandjean et al., 1995a).

**Table 5: Pearson correlation coefficients between Hg and the different biological variables (weight, length, $\delta^{15}N$, $\delta^{13}C$) (*: $p < 0.05$; **: $p < 0.001$). Stepwise regressions revealed that $\delta^{15}N$ was the main parameter explaining Hg in juvenile harbour seals and weight explained most of the variations of Hg observed in pups (underlined in the table).**

| | Adults | Juveniles | Pups |
|---|---|---|---|
| Weight | 0.072 | **0.373*** | **0.157**** |
| Length | 0.248 | **0.494**** | **0.117**** |
| $\delta^{15}N$ | 0.028 | **0.370**** | 0.028 |
| $\delta^{13}C$ | 0.127 | 0.124 | 0.017 |

**Mercury levels in harbour seal pup hair revealed spatial variations.**

A total 167 harbour seal pups were sampled at various sites in BC and WA. THg concentrations in fur ranged from 1.6 to 46.9 µg/g. Pups from Port Renfrew had the highest levels of Hg in fur with concentrations reaching up to 46.9 µg/g (average ± standard error = 25.0 ± 3.3 µg/g), followed by pups from Queen Charlotte Strait (11.5 ± 1.8 µg/g) and then those sampled in central Puget Sound (11.1 ± 1.7 µg/g). Pups from those three sites had similar concentrations ($p = 0.383$) which were significantly higher than those reported in pups from our reference site, Bella Bella (4.5 ± 0.5 µg/g) (Table 6). The three locations exhibiting higher Hg concentration in seal hair can partly be explained by the fact that pups from those sites were among the heaviest and Hg levels appeared to increase significantly with weight ($r^2 = 0.15$; $p < 0.001$).

**Table 6: 167 harbour seal pup hair samples were collected at various sites in British Columbia, Canada, and Washington State, USA, between 2003 and 2010. Compared to our reference site, Bella Bella, harbour seal pups from Queen Charlotte Sound, Port Renfrew and Central Puget Sound had significantly higher mercury levels ($p < 0.05$). (n/a: non available)**

| Site | N | weight (kg) | Hg (µg/g) | p-value (difference from reference site) |
|---|---|---|---|---|
| Bella bella | 4 | n/a | 4.5 ± 0.5 | reference site |
| Queen Charlotte Sound | 6 | 24.3 ± 2.2 | 11.5 ± 3.2 | **0.045** |
| Quatsino Sound | 6 | 21.3 ± 2.3 | 6.0 ± 0.4 | 0.896 |
| Port Renfrew | 5 | 25.7 ± 2.3 | 24.4 ± 5.7 | **0.000** |
| Strait of Georgia | 71 | 16.3 ± 0.4 | 4.9 ± 0.3 | 0.997 |
| Juan de Fuca Strait | 39 | 18.9 ± 0.7 | 5.6 ± 0.4 | 0.988 |
| Skagit Bay | 6 | 21.6 ± 0.6 | 4.5 ± 0.4 | 0.999 |
| Central Puget Sound | 3 | 21.5 ± 0.5 | 11.1 ± 2.1 | **0.046** |
| South Puget Sound | 19 | 21.1 ± 0.7 | 5.7 ± 0.4 | 0.753 |
| Hood Canal | 8 | 22.4 ± 0.7 | 3.5 ± 0.3 | 1.000 |

The high Hg levels observed in Port Renfrew might seem surprising given the absence of any documented sources of contamination nearby. However, there is likely another process – upwelling – that delivers MeHg-enriched water to shelf's shallow water along the west coast of Vancouver. This process, already implicated in cadmium enrichments observed in mussels (*Mytilus edulis*) (Bruland et al., 1978; Lares et al., 1997), supplies nutrient-rich water from depth in the NE Pacific Ocean. It has recently been shown that zones of nutrient regeneration in the ocean, including the North Pacific, are associated with higher concentrations of MeHg (Sunderland et al., 2009). Upwelling in spring, a common occurrence along the coast including near Port Renfrew, would therefore provide the means both to stimulate primary production and introduce higher MeHg at the bottom of the food web. A similar process of MeHg enrichment has been proposed for the California coast where upwelled water

delivered dimethylmercury (DMeHg) to surface waters, where it was converted to the bioaccumulative MeHg (Conaway et al., 2009).

Queen Charlotte Strait is, likewise, the recipient of upwelled, nutrient-rich water and therefore may receive enrichments of MeHg in the foodweb in the same way as does the Port Renfrew area. Locally, this region also has a high concentration of fish farms. Farms release organic carbon to nearby water and sediments, thus producing conditions conducive to methylation of THg. Local organic enrichments from farms therefore has been proposed as a plausible mechanism to explain the higher levels of Hg in demersal rockfish near fish farms than in fish farther away (DeBruyn et al., 2006). Provided that seals obtain a significant component of their diet from areas proximal to such farms, this could explain part or all of the higher Hg levels observed in harbour seal pups.

The third hot spot, Central Puget Sound, likely reflects local contamination from a highly-populated drainage basin, which includes the city of Seattle. A study on the loading of contaminants into Puget Sound revealed that the Elliott Bay study area had the greatest unit area loading rate for Hg (Herrera Environmental Consultants et al., 2008). In addition, rockfish collected from Elliott Bay had the greatest Hg concentrations when compared to non-urban rockfish from Puget Sound (West et al., 1995).

Our results therefore suggest that both anthropogenic loadings and natural processes likely contribute to the wide range of MeHg concentrations observed at the top of this coastal marine food web.

**Whisker analyses: insight into transplacental and lactational transfer of Hg**

LA-ICPMS has been extensively used to evaluate Hg along strands of human hair strands (Legrand et al., 2004; Stadlbauer et al., 2005). Here, we provide the first examination of the potential for whiskers to provide temporal records of Hg accumulation in harbour seals. Preliminary results from whiskers collected from several stranded seals revealed minimal variation in Hg profiles amongst whiskers collected from the same individual (not shown) suggesting that single whiskers well represent the Hg profile of a given seal.

To infer Hg accumulation as a function of time from the Hg profile along the whiskers, several assumptions have been made based on the literature. First, we assume a constant growth rate as shown by. Zhao et al. (2004) who determined an average rate of 0.78mm/day for newly grown whiskers irrespective of whether the seal was captive or living in the wild. This growth rate implies an average whisker age of 116 ± 2 days (~ 4 months). Given a gestation time of nine months (not including the delayed implantation period), the sampled whiskers therefore represent approximately the second half of fetus development. Second, as the whiskers were cut as close to the face as possible, the signal from the root was missing representing approximately 13 days based on a growth rate of 0.78mm/day and an average root length of 1cm. Finally, the delay between uptake of Hg from the diet into circulating blood and its manifestation in the whisker has to be taken into account. Based on several hair studies in human and mice, it appears that there is an average lag of 10 days before circulating Hg in blood can be detected in hair (Cernichiari et al., 1995; Harnly et al., 1997; Zareba et al., 2007). With these assumptions in mind, we have to recognize that the mercury signal obtained from the cut whiskers is representative of

Hg accumulation that started 4 months prior to sampling and ended 20 to 25 days prior to sampling (referred to as t=0 in the rest of the chapter).

The average Hg levels for the whole whiskers were highly correlated with Hg levels in fur ($r^2 = 0.86$; $p < 0.001$). However, significant Hg variations were observed along the whisker. Hg profiles were similar among pup whiskers and were characterized by stable levels towards the tip of the whisker followed by two periods of increase at different rates (Figure 11). A few adult seal whiskers were analyzed for comparison and exhibited different patterns suggesting that the pattern observed in Figure 11 is unique to the pup stage.

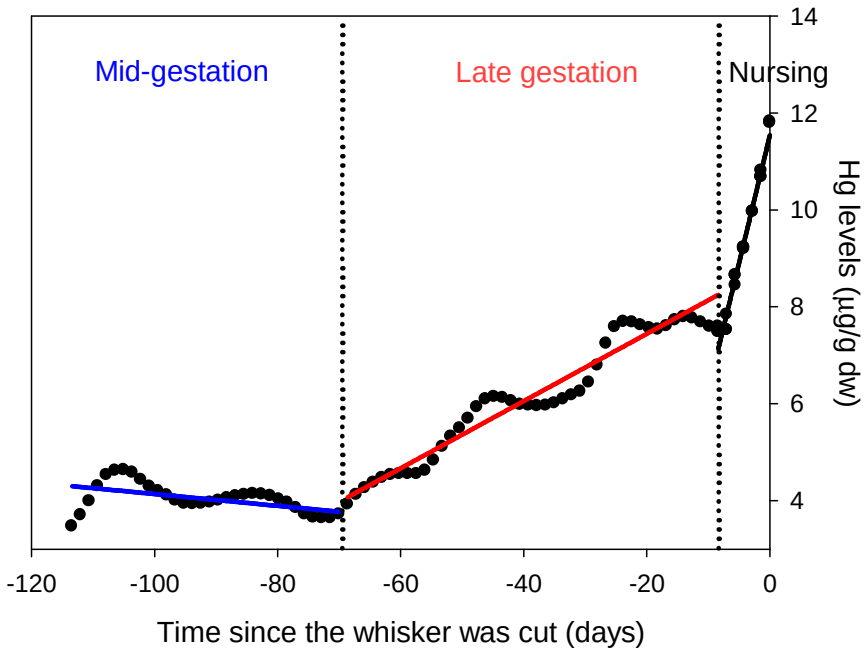


**Figure 11: Changes in Hg levels along one harbour seal pup whisker revealed two breakpoints suggesting strong differences in Hg transfer from the mother to the pup between mid-gestation, late gestation, and early nursing. Breakpoints were assessed by segmented linear regression analyses.**

Breakpoint analyses distinguished two breakpoints for seven out of the nine whiskers analyzed (Figure 11; Table 7; Appendix 1). The first breakpoint was on average 11.6 ± 1.3 days from the base of the cut whisker and the second breakpoint was on average 58.2 ± 6.7 days from the base. There was a positive correlation between the time of the first breakpoint and the age of the pup at t=0 ($r^2 = 0.66$; $p = 0.027$) suggesting that the first breakpoint could be representative of the time of the birth. Two whiskers only had one breakpoint probably reflecting the younger age of those animals and / or the fact that these whiskers might not have been cut as close to the face as possible due to the movement of the animal during sampling.

Based on these two breakpoints and the relationship with the age of the pup, we suggest that Hg profiles along whiskers are representative of three mains periods: mid-gestation from the tip of the whisker to 58.2 ± 6.7 days from the base; late gestation from 58.2 ± 6.7 to 11.6 ± 1.3 days from the base; and lactation from 11.6 ± 1.3 days to the base of the cut whisker (Figure 11). The average Hg levels for each period were significantly different ($p < 0.001$) with an increase from mid-gestation (4.7 ± 0.8 µg/g) to late gestation (6.6 ± 1.3 µg/g) and lactation (8.1 ± 1.3 µg/g) (Table 7).

**Table 7: SegReg revealed two breakpoints for seven out of the nine whiskers analysed. Analyses of Hg levels along pup whiskers revealed that Hg levels in late gestation and early nursing were significantly higher than the one measured during mid-gestation ($p<0.05$).**

| | Age of the seal at t=0 (days) | Breakpoint 1 (days) | Breakpoint 2 (days) | Mid gestation | Late gestation | Nursing |
|---|---|---|---|---|---|---|
| PV09-41 | 9 | 13 | 70 | 9.2 ± 0.9 | 10.5 ± 0.2 | 14.8 ± 1.1 |
| PV09-23 | 10 | 15 | 76 | 4.3 ± 0.1 | 5.5 ± 0.1 | 6.9 ± 0.2 |
| PV08-23 | 17 | 16 | 77 | 4.3 ± 0.1 | 5.9 ± 0.1 | 7.8 ± 0.2 |
| PV09-42 | 7 | 8 | 40 | 3.3 ± 0.1 | 3.8 ± 0.1 | 6.3 ± 0.5 |
| PV09-31 | 6 | 8 | 65 | 4.0 ± 0.1 | 6.2 ± 0.2 | 9.3 ± 0.6 |
| PV09-37 | 9 | 13 | 35 | 2.7 ± 0.1 | 3.9 ± 0.1 | 4.5 ± 0.1 |
| PV09-21 | 7 | 8 | 45 | 2.8 ± 0.1 | 4.4 ± 0.2 | 6.7 ± 0.1 |
| PV08-24 | 1 | 23 | n/a | 3.5 ± 0.1 | 4.1 ± 0.3 | n/a |
| PV09-26 | 3 | 46 | n/a | 8.1 ± 0.1 | 15.1 ± 0.8 | n/a |

Transplacental transfer of Hg is well known. The increase observed here between mid and late gestation is consistent with studies showing an increased Hg transfer from the mother to the fetus associated with increased blood flow at the end of the gestation period in rats, hamsters, and guinea pigs (Inouye et al., 1988; Nordenhall et al., 1995; Yoshida et al., 2002). MeHg is efficiently transferred to the fetus through amino acid carriers while inorganic Hg is likely to be trapped in placental tissues. Such transplacental transfer of Hg is known to affect placental oxygen consumption, hormonal secretion and membrane fluidity but it can also induce immunotoxic effects, still observable later in life (Gundacker et al., 2012; Silva et al., 2005).

Lactation is an important period during which female harbour seals transfer high fat content milk to their pups. Unlike females from other phocid species, female harbour seals forage regularly during lactation (Boness et al., 1994). However, because of the limited pup diving ability and high risk of lost or predation during the first week of

lactation, foraging trips are limited during the first week of lactation as evidenced by empty stomachs found in females (Bowen et al., 1992, 1999). While food provides most of the energy to support lactation, catabolism of maternal tissues is also an important source of energy exported in milk, especially during the first week of lactation (Bowen et al., 2001). Females lose 80% of their stored fat in the first 19 days of lactation (Bowen et al., 1992).

While milk provides energy, nutrients, as well as immunoprotective components that are essential for the growth, development, and immunity of the pups, it also delivers contaminants such as Hg. In humans for example, studies have reported mercury levels in milk being about 30% of the levels found in the mother's blood (Oskarsson et al., 1996). Mercury in marine mammal milk has only been reported in a few studies looking at harp seals (*Pagophilus groenlandicus*) (Wagemann et al., 1988), northern elephant seals (*Mirounga angustirostris*) (Habran et al., 2011) and grey seals (Habran et al., 2012).

The whisker record implies higher Hg concentrations in harbour seal pups during early lactation compared to those observed in the fetus likely reflecting the intake of Hg through the ingestion of milk. The high levels of mercury in the milk could result from catabolism of Hg rich female tissues in harbour seal mothers exhibiting partial fasting. Human and rodent studies have suggested that increased or stable Hg levels in neonates are the result of Hg intake through the milk as well as limited ability of the newborn to demethylate and eliminate Hg (Grandjean et al., 1995b; Sundberg et al., 1991).The present results also revealed an increase oin Hg levels in fur with increasing pup weight supporting the evidence of increasing Hg levels in the pup with increasing lactation duration. Even though limited data is available concerning feeding ecology of female harbour seals during lactation, the Hg increase observed

here could also be resulting from the female consuming higher mercury contaminated prey during lactation. In contrast, Habran et al. (2012) suggested that transplacental transport is playing a greater role than milk in the delivery of Hg to the elephant seal (*Mirounga angustirostris*) pup based on decreasing Hg concentrations in pup blood between early and late lactation. However, this species exhibit different reproductive behaviour where the female is not feeding at all during lactation.

While our whisker analyses indicate that both transplacental and lactational transfers are important, more studies are needed. In particular, a bigger sample size associated with better knowledge of the whisker growth rate would help having a more accurate understanding of the Hg signal along the whisker.

### What are the potential consequences of Hg exposure for this population of harbour seals?

Marine mammals have been exposed to metals in the environment throughout their evolutionary history and have therefore developed mechanisms either to control the internal concentrations of certain element or to mitigate their toxic effects. For instance, cetaceans and pinnipeds have developed a tolerance to Hg based on its association with selenium (Dietz et al., 2000). However, even though marine mammals might be able to tolerate higher Hg burdens than terrestrial mammals, Hg, especially in the methylated form, remains a concern for marine mammal health.

While MeHg is well known for its neurotoxicity leading to sensory, motor deficits and behavioural impairments (Clarkson, 2002), studies have also linked high Hg levels with liver and kidney damages in bottlenose dolphins (*Tursiops truncatus*) and polar bears (*Ursus maritimus*) (Sonne et al., 2007a; Woshner et al., 2002). Mercury exposure has also been linked to deleterious effects on hepatic, renal, endocrine and hematological parameters in bottlenose dolphins from the Eastern coast of Florida

(Schaefer et al., 2011). Finally, *in vitro* studies on beluga whales (*Delphinapterus leucas*) and harbour seals showed that Hg exposure could result in immune deficiency (Das et al., 2008; De Guise et al., 1996b; Frouin et al., 2011).

Several thresholds have been determined for Hg toxicity in various species. For purpose of comparison, we will limit the review to thresholds that are based on mercury concentrations in hair. The highest threshold values are 20 µg/g for neurological effects in terrestrial mammals and 30 µg/g for neurological effects in mink (*Mustela visori*) and river otters (*Lontra canadensis*) (Basu et al., 2007; Thompson; 1996). In humans, the US Environmental Protection Agency defines 1 µg/g as the no observed effect level (NOEL). Recently, a low value of 5.4 µg/g was determined as a threshold to avoid biocheminal alterations in polar bear brains (Basu et al., 2009).

If we compare our harbour seal Hg levels with the most conservative threshold available for wildlife, 33% of pups, 25% of juveniles and 59% of adults had levels exceeding the threshold for biochemical alterations in the brain. In addition, it is important to notice that those values were determined for adults and pups are usually considered to be more at risk as Hg can affect systems essential in growth, metabolism and development. In addition, pups usually have higher percentage of the toxic MeHg than adults (Dehn et al., 2005). For example, Brookens et al (2007) reported 100% MeHg in the liver of a 4-month old fetus, 65% in a full term fetus and only 5 to 10% in adult harbour seals reflecting the low ability of young animals to detoxify and demethylate. The present results thus suggest that Hg can represent a risk for the health of this population of harbour seals.

## 3.4 Conclusions

Harbour seals are exposed to a mixture of contaminants including mercury but also persistent organic pollutants such as PCBs and PBDEs. Recently, Ross et al. (2012) estimated that 100% of harbour seal pups sampled in WA had PCB levels that surpassed threshold for health effects. While PCB associated health risks are well known in these seals (Mos et al., 2010), this is the first report of mercury levels in this population. While consequences of mercury exposure for the health of this population of harbour seals are unclear, the present study shed some light on the Hg levels observed at the top of this marine food web as well as important biological variables influencing its bioaccumulation such as diet but also transplacental / lactational mother-pup transfer.

# Chapter 4: PCB-related alterations of the expression of essential genes in harbour seals (*Phoca vitulina*) from the Northeastern Pacific and Northwestern Atlantic

## 4.1 Introduction

Wildlife species may be affected by a variety of changes in environmental conditions such as increased prevalence of anthropogenic chemical contaminants and/or altered climate. Such environmental pressures may manifest at different levels of the marine biota, including altered population dynamics, behavioural and physiological changes of individual organisms, and with adjustments in molecular biological pathways. Persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), and mercury (Hg) are widely distributed in the marine environment. They bioaccumulate up the food chain and induce a variety of acute and long-term toxic responses in marine top predators (Tabuchi et al., 2006; Tanabe et al., 1994).

A component of the biological response to environmental change can include altered expression of mRNA with a subsequent adjustment in transcriptome profile of a given tissue. Highly sensitive techniques such as quantitative polymerase chain reaction (QPCR) analysis are able to detect the altered abundance of select mRNA profiles which could provide indications of a potential change in health status (Veldhoen et al., 2011). Using a QPCR assay, correlations between altered mRNA levels and PCB concentrations have been established in both harbour seals and killer whales (*Orcinus orca*) residing in the Northeastern Pacific (Buckman et al., 2011; Mos et al., 2007), Mediterranean striped dolphins (*Stenella coeruleoalba*) (Panti et al., 2011), fin whales (*Balaenoptera physalus*) from the Mediterranean and Gulf of Mexico (Fossi et al., 2010) and ringed seals (*Phoca hispida*) from Svalbard and the Baltic Sea (Routti et al., 2010).

Long-lived, high trophic level harbour seals (*Phoca vitulina*) are considered to be an important indicator species of the marine environment and their wide latitudinal distribution along the coastline of both Atlantic and Pacific North America provides a

unique opportunity to investigate the potential impacts of major contaminants of concern (e.g. PCBs, PBDEs and Hg) on wildlife. Harbour seals are relatively non-migratory and feed on a variety of fish and invertebrate species providing an integrated signal of local food web contamination. A number of studies have been conducted on wild and captive harbour seals increasing our understanding of the relationship between contaminant exposure and changes in physiology, endocrinology and immunology (Ross, 2000). High PCB concentrations have been linked to decreased immune function in field-based and captive feeding studies of harbour seals (Mos et al., 2007; Ross et al., 1995). Exposure to PCBs has also been implicated in the disruption of vitamin A and thyroid hormone (TH) regulated pathways in this marine species with the potential for adverse effects on growth and development (Mos et al., 2007; Tabuchi et al., 2006). Finally, PBDEs and Hg have been shown to alter harbour seal immune function (Das et al., 2008; Frouin et al., 2010).

In the present study, we collected samples from four North American coastal regions including British Columbia (BC), Newfoundland and Quebec, Canada and Washington State (WA), USA. On both coasts, sampling was performed both at locations close to industrialized areas as well as in regions of low human population in order to provide a range of contaminant levels. We developed a harbour seal QPCR primer set directed towards seven gene transcripts that were selected based on their ability to provide information on health status and/or response to organic contaminant exposure in order to investigate the relationship of mRNA abundance profiles with the exposure of major contaminants of concern (PCBS, PBDEs and Hg).

## 4.2 Materials and Methods

### Tissue sampling

Fifty-four harbour seal pups were live captured at multiple sites in Canada (BC, Newfoundland and Quebec) as well as in WA, USA between 2006 and 2009 (Figure 12). An average of 5 ± 2 seals was collected at each site. On the west coast of North America, harbour seals were caught using two techniques. At rocky sites, individual seals were captured using landing net. At sandy haul out sites, multiple seals were captured at once using a rapidly deployed beach seine net (Jeffries et al., 1993). On the east coast, harbour seals were captured in the water using a dip net and an inflatable boat. They were then transferred to a bigger boat where all handling took place. Body weight, length, girth and sex were determined.

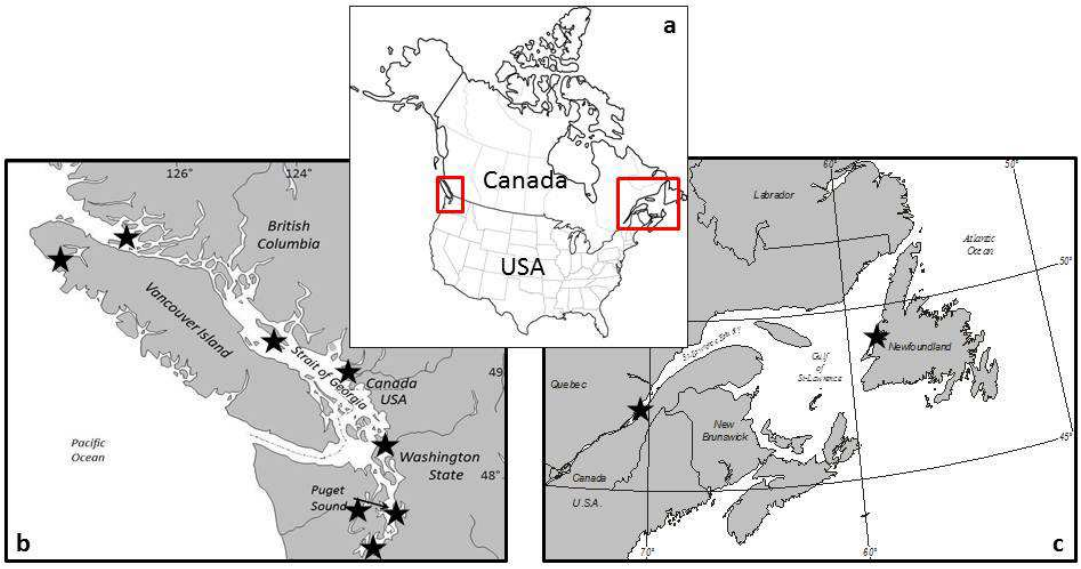


**Figure 12: Maps denoting sampling sites for harbour seals along the Northeastern Pacific coast (British Columbia, Canada, and Washington State, USA) and the Northwestern Atlantic coast of North America (Newfoundland and Quebec, Canada). Stars indicate capture sites.**

The biopsy site (left side of the animal on the pelvic region) was shaved with an electric razor. Hair samples were collected for Hg analyses and stored at room temperature. Blubber/skin biopsies were obtained using sterile 3.5 and 8.0 mm biopsy punches. In the field, the 8.0-mm biopsy was transferred immediately into liquid nitrogen and subsequently stored at -80°C in the laboratory prior to analysis of organic contaminant. The 3.5-mm biopsy was rinsed with buffered saline solution and placed immediately in RNA*later* tissue preservation solution (Life Technologies Inc., Burlington, ON) and maintained at 4°C for 24 hours prior to storage at -20°C.

Capture stress and holding time were minimized. All procedures were carried out under the auspices of the respective animal care committees and scientific research permits for researchers in BC and QC (Fisheries and Oceans Canada Animal Care Committee with guidelines from the Canadian Council on Animal Care; Scientific Research Permit) and WA (U.S. Marine Mammal Protection Act Permit 835).

**PCB and PBDE quantification**

Each frozen 8 mm blubber/skin biopsy was cut vertically and the ~2 mm upper skin layer was removed. A portion of each blubber sample (100 mg to 300 mg wet weight) was used for quantifying PCBs and PBDEs at the Fisheries and Oceans Canada LEACA (Laboratory of Excellence in Aquatic Chemical Analysis, Institute of Ocean Sciences, Sidney, BC, Canada). The 54 seal pup blubber biopsies were organized into batches, each containing a replicate sample for quality assurance (QA) purposes. Other QA samples, including a Standard Reference Material (NIST 1945 whale blubber SRM) and two procedural blanks containing pure lipid (triolein) to imitate the behaviour of real extracts, were also included in each batch. The procedural blanks, along with weighed amounts of each seal biopsy, replicate and SRM sample, were

spiked with a mixture of surrogate internal standards containing 30 $^{13}$C-labeled PCBs and 10 $^{13}$C-labeled PBDEs obtained from Cambridge Isotope Laboratories (Andover, MA, USA), to enable precise and accurate quantification using the isotope dilution method (Ikonomou et al., 2001).

Blubber samples were ground with anhydrous sodium sulphate. Using dichloromethane/hexane (1:1 ratio), the samples were extracted from a glass column. The extracts were then evaporated to dryness and weighed. Total lipid concentrations were determined gravimetrically. The residues were resuspended in dichloromethane/hexane (1:1), and analyzed using high resolution gas chromatography and high resolution mass spectrometry (HRGC-HRMS). Details of the chromatography and mass spectrometry conditions, the criteria used for chemical identification and quantification, the quality assurance and quality control practices can be found in Ikonomou *et al*. (2001).

**Mercury analyses**

To remove any external contamination, all hair sub-samples were rinsed with acetone / de-ionized water / acetone and left to dry at room temperature. They were then stored in a dessicator until analysis. An average of 1.7 ± 0.7 mg of hair was analysed for THg using a thermal decomposition Zeeman atomic absorption spectrometer RA-915+ coupled with a PYRO-915 attachment (Lumex, St. Petersburg, Russia). The detection limit was 0.002 µg/g dry weight. Details on the methods and instrumentation can be found elsewhere (Sholupov et al., 2004).

Two standards were used: a sediment standard NIST 2709 (National Institute of Standards and Technology, Gaithersburg, USA), and a human hair standard NIES 13 (National Institute for Environmental Studies, Ibaraki, Japan). As NIST 2709

appeared to be a more homogeneous standard, it was used to make the calibration curve. One NIST 2709 and one NIES 13 standard was run every six samples to ensure that there was no deviation from the calibration curve. Each hair sample was run in triplicate.

### Total RNA isolation and cDNA synthesis

Because a possible stratification in transcriptome content can exist across blubber layers that may influence mRNA abundance analyses (Tabuchi et al., 2006), the 3.5 mm blubber/skin biopsy preserved in RNA*later* was divided into outer blubber (5 mm thick portion of blubber closest to the skin), inner blubber (5 mm thick portion of blubber below the outer blubber), and skin. Detailed procedures on RNA extraction and cDNA synthesis are described elsewhere (Buckman et al., 2011; Veldhoen et al., 2011). Briefly, tissues were homogenized in a 1.5 mL microcentrifuge tube with 700 µL of TRIzol reagent (Invitrogen, Burlington, ON, Canada) and a 3 mm diameter tungsten-carbide bead using a Retsch MM400 mixer mill (Thermo Fischer Scientific, Ottawa, ON, Canada). Samples were homogenized in two three minute intervals, at a frequency of 20 Hz. An additional 3 minutes of mixing was performed for skin samples and any other blubber sample that was difficult to fully homogenize. All samples were cooled on ice between homogenization intervals. For the blubber extractions, after phase separation, 20 µg of glycogen (Roche Diagnostics, Laval, QC, Canada) was added to each retained aqueous phase prior to alcohol-mediated precipitation. Precipitated total RNA was resuspended in 20 µL diethyl pyrocarbonate-treated distilled/deionized water (DEPC dd$H_2O$) and stored at -80°C. Spectrophotometry was used to determine the RNA concentration of each sample. One microgram of RNA was used to generate cDNA using the High Capacity cDNA

Reverse Transcription kit, as described by the manufacturer (Applied Biosystems, Carlsbad, CA, USA). Prepared cDNA samples were diluted 20-fold using DEPC $ddH_2O$ prior to QPCR analysis.

**QPCR analysis**

Seven target gene transcripts were originally selected based on their ability to provide information on health and/or biological response to organic contaminant exposure. These include thyroid hormone receptors alpha (*Thra*), estrogen receptor alpha (*Esr1*), aryl hydrocarbon receptor (*Ahr*), glucocorticoid receptor (*Nr3c1*), heat shock protein 70 (*Hspa1*), peroxisome proliferator-activated receptor gamma (*Nr1c3*), and vitamin D receptor (*Nr1l1*). Primers were designed using Primer Premier Version 5 (Premier Biosoft, Palo Alto, CA, USA) and purchased from Integrated DNA Technologies (Coralville, IA, USA) (Appendix 2). Each gene-specific primer pair was assessed for their ability to amplify a single targeted DNA amplicon using a three-tier quality control process described elsewhere (Veldhoen et al., 2011). Three additional genes were selected as potential normalizer genes for correction of experimental variance: ribosomal protein L8 (*Rpl8*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), and cytoplasmic beta actin (*Actb*). The sequence information for each of the amplicons has been deposited to Genbank (Appendix 2) or has been published previously (Tabuchi et al., 2006). Gene specific primer pairs were tested for incorporation into the QPCR assay on blubber and skin cDNA separately to ensure that all quality control criteria were met for each tissue since differences in transcriptome composition could influence primer performance (Appendix 2). Limited tissue biopsy samples were available from harbour seals resident along the

Atlantic coast of North America (Newfoundland and Quebec) and, therefore, mRNA abundance analysis included only skin for seals from these sites.

Quantitative DNA amplification reactions (15 µL) were performed on a Realplex4 thermocycler (Eppendorf, Westbury, NY, USA). Reaction components were as described previously (Veldhoen et al., 2011) and included 2 µL of 20-fold diluted cDNA sample. The thermocycle program for most gene targets included an initial enzyme activation step at 95°C (9 min), followed by 40 cycles of 95°C denaturation (15 sec), 60°C annealing (30 sec), and 72°C elongation (45 sec). The annealing temperature for *Hspa1* was 62°C. For each sample, quadruplicate reactions were performed and amplification specificity determined by evaluation of thermodenaturation profiles performed at the end of each QPCR run. Additional no cDNA template and no amplification controls were included in each run to confirm reagent performance. Inter-run variation was assessed using a universal standard control as described previously (Veldhoen et al., 2011).

**Data analyses**

For each sample, QPCR-derived replicate cycle threshold (Ct) data were averaged and normalized to the geometric mean Ct value of the normalizer gene transcripts. Suitability of normalizer application to the data derived for each tissue was established using Reffinder (http://leonxie.com/reference.gene.php?type=reference) and based upon invariance between sample groups with no contribution to Ct values made by biological metrics or contaminant levels examined. The geometric mean of all three normalizer transcripts (*Rpl8, Gapdh*, and *Actb*) was employed for blubber. *Rpl8* and *Actb* were used as normalizers for skin. Relative fold change in mRNA

abundance were subsequently determined using the comparative ΔΔCt method (Livak et al., 2001).

Normality and homogeneity of variances were tested for the relative mRNA abundance data and total PCB levels (expressed on a lipid weight basis) using the Kolmogorov-Smirnov test and Levene's test, respectively (SPSS, IBM Corporation, Armonk, NY, USA). If the data did not meet the assumption of normality and homogeneity of variances, they were log-transformed.

Linear regressions were used to investigate the potential influence of contaminants of concern (PCBs, PBDEs and Hg) as well as biological variables (weight, length, girth) on individual mRNA levels measured in blubber and skin. In addition, the variable or combination of variables (weight, length, girth, total PCBs, total PBDEs, Hg) best describing the mRNA level of each gene was selected using the Akaike information criterion (AIC) (SYSTAT, Chicago, IL, USA). The AIC method ranks models based on their overall statistical support. Lowest AIC models are most supported and AIC weights ($W_i$) provide information on the relative support of each model. Backward stepwise regressions were used in combination to AIC.

## 4.3 Results and discussion

### Molecular endpoints of harbour seal health

Previous incorporation of QPCR-derived transcriptome endpoints that investigated the biological impacts on harbour seal of environmental exposure to contaminants employed a limited number of gene transcripts including *Thra*, thyroid hormone receptor beta (*Thrb*), and retinoic acid receptor alpha (*Rara*). We have expanded the QPCR assay to include evaluation of six additional gene transcripts (*Esr1*, *Ahr*, *Hspa1*, *Nr1c3*, *Nr3c1*, and *Nr1l1*) that can provide more information as to the

biological status of this important marine sentinel species. The isolated cDNA regions represented ≥ 94% sequence identity compared to the relevant dog sequences.

Blubber was divided into inner and outer layer as this tissue is known to be stratified in terms of fatty acid composition, fat soluble hormone levels, as well as chemical contaminant levels. The inner blubber layer is more involved in the storage and mobilization of lipids therefore comprising more metabolically active adipocyte cells. In contrast, the outer blubber layer has been described as less biologically active and primarily functions in insulation and buoyancy (Strandberg et al., 2008). In addition, higher organochlorine concentrations were measured in the inner blubber of yearling harbour porpoises (Tilbury et al., 1997). For these reasons, earlier investigations have reported inner blubber as being more appropriate than outer blubber to investigate the interrelationship of contaminant exposure and mRNA abundance profiles (Tabuchi et al., 2006). Our results show a positive correlation in gene transcript profiles between harbour seal inner and outer blubber (Table 8) and, therefore, we elected to focus on the mRNA abundance profiles derived for inner blubber as they relate to tissue levels of contaminants.

Overall, there were limited correlations in mRNA abundance profiles between skin and the inner or outer blubber (Table 8). The exception being *Nr1c3* and *Nr3c1* mRNA levels in skin displaying similarity with transcript levels measured in inner blubber ($r^2 = 0.77$, $p = 0.024$; $r^2 = 0.80$, $p = 0.01$, respectively). While a strong correlation in mRNA abundance profiles between skin and blubber were reported in killer whales from the Northeastern Pacific (Buckman et al., 2011), limited correlations were observed in the Beaufort Sea beluga whales (M. Noël, University of Victoria,BC, Canada, personal communication) further highlighting specificity that exists across different species and tissues.

**Table 8: Pearson correlation analysis of mRNA abundance values obtained for harbour seal inner blubber, outer blubber and skin samples. (*: p<0.05; n/a, not applicable when tissue-specific quantification of mRNA abundance was not possible)**

| | Inner blubber/ Outer blubber | Inner blubber/ Skin | Outer blubber/ Skin |
|---|---|---|---|
| *Esr1* | 0.49* | 0.04 | 0.17 |
| *Ahr* | n/a | n/a | n/a |
| *Hspa1* | 0.91* | -0.34 | 0.18 |
| *Nr1c3* | 0.97* | 0.77 | 0.24 |
| *Nr3c1* | 0.67* | 0.80 | 0.33 |
| *Nr1l1* | n/a | n/a | n/a |
| *Thra* | 0.80* | n/a | n/a |
| *Thrb* | n/a | n/a | n/a |

### Association between PCB concentrations and gene transcript profiles in blubber and skin

Significant positive correlations were noted between the abundance of three gene transcripts investigated in inner blubber and PCB levels measured in blubber (*Esr1*: $r^2 = 0.12$, $p = 0.038$; *Thra*: $r^2 = 0.16$, $p = 0.028$; and *Nr3c1*: $r^2 = 0.12$, $p = 0.049$; Figure 13). A similar positive association was found for *Esr1*, *Thra*, and *Nr3c1* mRNA abundance ($r^2 = 0.24$, $p = 0.041$; $r^2 = 0.37$, $p = 0.004$ and $r^2 = 0.26$, $p = 0.032$, respectively, not shown) with PBDE levels in blubber. No correlation was found between any gene transcript examined and Hg levels. While a PBDE-related effect on the blubber transcriptome cannot be ruled out, there was a strong correlation between PCB and PBDE levels in harbour seal blubber ($r^2 = 0.51$, $p < 0.001$) suggesting that

the relationships between *Esr1, Thra, Nr3c1* mRNA levels and PBDEs are likely confounded by the relationship between the two contaminants. In the present study, PBDE levels in seals were on average 5 times lower than that of PCBs. To our knowledge, there are no reports of direct PBDE-associated influence on mRNA transcript abundance in wildlife. Because of their similar structure and physico-chemical properties, PCBs and PBDEs are often reported to have similar toxic mechanisms of action. However, PBDEs have been identified as less toxic than PCBs (Hallgren et al., 2002; Hallgren et al., 2001) and, under controlled laboratory-based exposures, correlations between *Thra* mRNA levels as well as other transcripts involved in thyroid metabolism and PBDEs have been established (Lema et al., 2008; Szabo et al., 2009). A recent bald eagle (*Haliaeetus leucocephalus)*study from BC also reported an impact of PCBs on circulating thyroid hormone and retinol but no impact of PBDEs (Cesh et al., 2010). PCBs are one class of contaminants; they co-correlate with other POPs and may exhibit additive, synergistic and or antagonistic effects. In this manner, while PBDEs may contribute to the effects observed, the contribution of the PCBs is very likely to dominate. For example, in their study, Mos et al. (2010) ranked 13 organic contaminant based on their relative toxicity using comparative risk quotient and found that PCBs presented the greatest risk to the health of harbour seals.

In skin, data was available for harbour seal pups sampled both on the west and east North American coasts. There were no significant differences between the mRNA abundance levels of the seven target genes between the two coasts ($p>0.05$ for all genes; Figure 14); data were therefore pooled together to investigate the potential influence of PCBs, PBDEs and Hg. Similar to inner blubber, univariate analyses revealed no correlations between skin mRNA levels and weight, length or girth of the

animal sampled. However, analysis of the relationship between contaminant levels measured in blubber and transcript abundance in harbour seal skin provided results different from that observed in inner blubber. While no apparent relationship was observed for PBDEs or Hg levels and mRNA profiles, there were significant negative correlations between the abundance of three mRNA transcripts in skin and PCB concentrations measured in blubber (*Esr1*: $r^2 = 0.21$, $p = 0.021$, *Nr3c1:* $r^2 = 0.22$, $p = 0.033$ and *Hspa1*: $r^2 = 0.39$, $p < 0.001$).

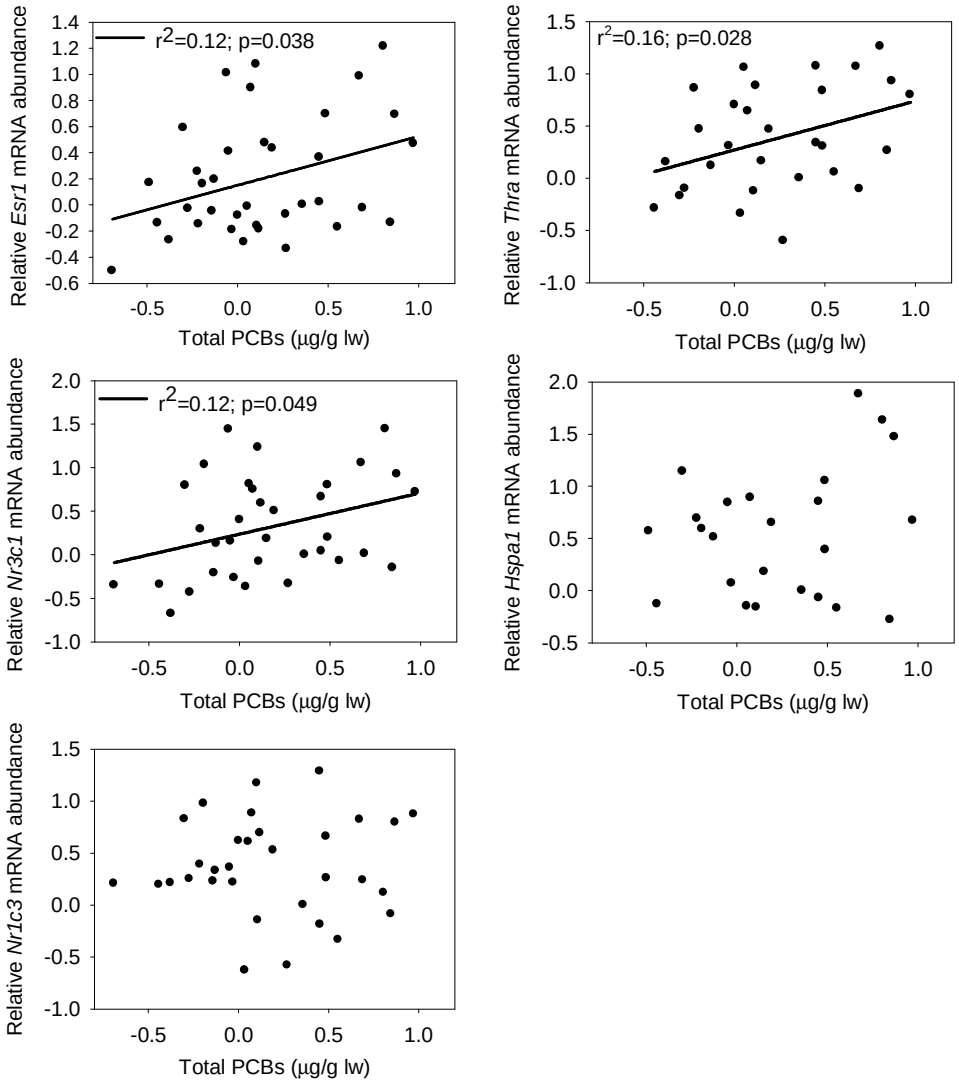


**Figure 13: Relationship between blubber relative mRNA abundance (log transformed) of five target genes and total PCB concentrations (log transformed). These analyses reveal an increase of *Esr1, Thra, Nr3c1* mRNA levels with increasing PCBs in harbour seal pups from the Northeastern Pacific coast of North America.**

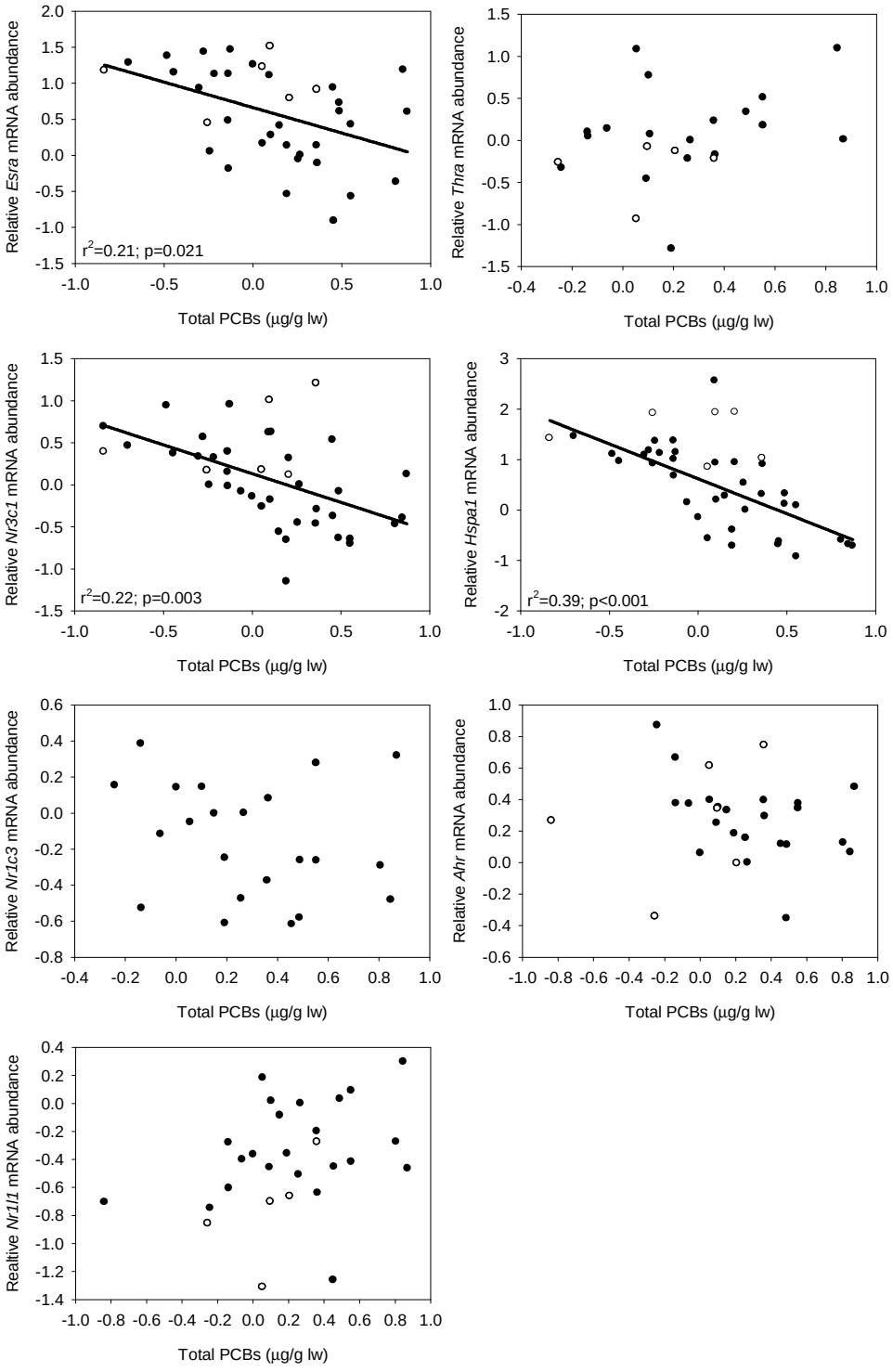


**Figure 14: Relationship between skin relative mRNA abundance (log transformed) of five target genes and total PCB concentrations (log transformed). These analyses reveal a decrease in *Esr1*, *Nr3c1* and *Hspa1* mRNA levels with increasing PCBs in harbour seal skin from both the Northeastern Pacific coast (closed circle) and the Northwestern Atlantic coast (open circle) of North America. Data for *Nr1c3* mRNA from Northwestern Atlantic coast animals were below the detection limit and therefore not included in the analysis.**

**Table 9: Akaike information criterion (AIC) analyses of variables associated with mRNA abundance profiles. PCBs was the best variable explaining variation in the mRNA levels of genes involved in growth, metabolism, reproduction and development. (*: $p < 0.05$). [a] AICc = second order Akaike information criteria (AIC ) $n\log(\sigma 2) + 2K$) bias adjusted AIC for small sample size = AIC + ($2K(K + 1/(n - K - 1)$ where $K$ is the total number of estimated regression parameters including $\sigma 2$ (no intercept) and $n$ is sample size. [b] $\Delta_i$ = AIC differences computed as $AIC_i$-$AIC_{min}$. [c] $w_i = \exp(-1/2\Delta_i)/\Sigma\exp(-1/2\Delta_r)$. Data are only presented for model with $\Delta_i AIC_c$ below 2 which are considered the most important.**

| Gene | Function | Tissue | Predictors | $r^2$ | p-value | AIC | $AIC_c$ [a] | $\Delta AIC_c$ [b] | $w_i$ [c] |
|---|---|---|---|---|---|---|---|---|---|
| *Esr1* | Growth, cell differentiation, development, reproduction | Blubber | PCBs | 0.24 | 0.097 | 30.1 | 34.7 | 0 | 0.86 |
| | | Skin | PCBs, girth | 0.29 | 0.102 | 44.53 | 46.88 | 0.99 | 0.32 |
| | | Skin | PCBs | 0.38 | < **0.001**** | 43.23 | 45.89 | 0 | 0.52 |
| *Thra* | Development, cell differentiation, metabolism | Blubber | PCBs, PBDEs | 0.36 | 0.082 | 17.13 | 21.58 | 0 | 0.9 |
| | | Skin | PCBs, length | 0.26 | 0.222 | 11.89 | 13.57 | 0 | 0.32 |
| *Nr3c1* | Lipid metabolism, maintenance of growth, response to stress | Blubber | PCBs, length, Hg | 0.13 | 0.11 | 37.25 | 40.11 | 1.6 | 0.27 |
| | | Blubber | PCBs, length | 0.24 | **0.024**** | 36.91 | 38.51 | 0 | 0.6 |
| | | Skin | PCBs | 0.62 | **0.002**** | 10.89 | 13.97 | 0 | 0.73 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Hsp1l* | Protect cells from environmental stress conditions | Blubber | PCBs | 0.12 | 0.268 | 48.31 | 49.91 | 0 | 0.66 |
| | | Skin | PCBs | 0.76 | < **0.001**** | 47.82 | 50.17 | 0 | 0.73 |
| *Nr1c3* | Cell differentiation, development, metabolism (lipids, proteins) | Blubber | Hg | 0.18 | 0.222 | 33.85 | 35.45 | 0 | 0.8 |
| | | Skin | PCBs, weight | 0.1 | 0.938 | 33.25 | 36.11 | 0 | 0.77 |
| *Ahr* | Induction of metabolizing enzymes | Skin | PCBs, weight, length, Hg | 0.1 | 0.452 | 26.78 | 30.31 | 1.2 | 0.22 |
| | | Skin | PCBs, weight, length | 0.1 | 0.32 | 27.21 | 29.43 | 0.3 | 0.36 |
| | | Skin | PCBs, weight | 0.15 | 0.238 | 25.71 | 29.11 | 0 | 0.4 |
| *Nr111* | Regulation of calcium transport, immune system | Skin | PCBs, girth | 0.53 | **0.022**** | 5.09 | 8.73 | 0 | 0.89 |

Best fit models (AIC) indicated that, amongst the variables tested, PCBs are explaining the variation in *Esr1*, *Nr3c1*, *Hspa1*, and *Nr1l1* mRNA levels in either or both blubber and skin depending upon the transcript (Table 9). With length only being a significant contributor in the final model for *Nr3c1* and girth for *Nr1l1*, biological variables appeared to play a minor role in the variation of mRNA levels of the target genes investigated. These univariate and multivariate results strongly suggest that PCB contamination is an important factor explaining some of the variation in mRNA levels observed in these harbour seals.

Estrogen receptor alpha is activated by the hormone 17β-estradiol and functions as a transcription regulatory factor that coordinates expression of genes involved in cell differentiation and proliferation, organogenesis, and reproductive development (Bonefeld-Jorgensen et al., 2001). PCBs and their hydroxy-metabolites having both estrogenic and anti-estrogenic properties can disrupt estrogen signalling pathways at multiple levels. For example, PCBs are known to interfere with enzymes involved in the metabolism of 17β-estradiol (Kester et al., 2000) while also disrupting the ligand-mediated ER binding to estrogen-response elements within gene promoter regions (Bonefeld-Jorgensen et al., 2001). Similar to our present findings in blubber, association of increased PCB levels and *Esr1* mRNA levels was observed in the blubber of known-age killer whales from the Northeastern Pacific (Buckman et al., 2011). A positive relationship was also reported in fin whales from the Mediterranean Sea and Gulf of California (Fossi et al., 2010). However, the absence of age data in the latter study did not allow for the examination of age as a potential confounding factor. Such abnormal alterations of *Esr1* transcript levels could potentially interfere with sexual development and behaviour. Studies have also shown that increased

expression of ER may influence transcription of AhR-regulated genes as a result of functional crosstalk between the two receptors (Matthews et al., 2007).

THs play a central role in development, growth and metabolism in all vertebrate species through binding to their nuclear receptors, TRα and TRβ (Wu et al., 2000). PCBs have the ability to interfere with TH physiology at multiple levels including hormone synthesis, circulatory transport, and hormone removal via metabolism (Jugan et al., 2010; Zoeller, 2005). Decreased circulating TH have been related to PCB exposure in various species of marine mammals, such as harbour seals from the Northeastern Pacific (Tabuchi et al., 2006) and polar bears (*Ursus maritmus*) (Braathen et al., 2004). At the molecular level, PCBs are able to affect TR activity and subsequent regulation of TH-responsive gene expression (Zoeller, 2005). Similar to the present blubber results, PCB-associated increase in *Thra* mRNA levels was observed in the blubber of killer whales and harbour seals from the Northeastern Pacific (Buckman et al., 2011; Tabuchi et al., 2006). Given the important role of the blubber layer for thermoregulation and buoyancy in marine mammals, it was suggested that disruption of the TH signalling pathway might affect metabolism within adipocytes therefore compromising the integrity of this vital tissue (Tabuchi et al., 2006).

One of the primary adaptive physiological responses to physical and chemical stressors is the stimulation of the hypothalamic pituitary adrenal (HPA) axis and the neuroendocrine system resulting in the release of glucocorticoids, such as cortisol. Through binding to its receptor, cortisol is involved in the regulation of genes involved in lipid metabolism, growth, development and immune response (Norris, 2000; Zimmer et al., 2009). Several studies have shown that PCBs and other organochlorines can affect the HPA axis through disruption of corticosteroidogenesis

leading to a decrease in circulating cortisol as observed in experimental studies (Machala et al., 1998) as well as wild polar bears from Svalbard (Oskam et al., 2004). To our knowledge, contaminant-related variation of *Nr3c1* mRNA transcript levels has not been reported in any other marine mammal species. However, similar to our findings in blubber, an increase in *Nr3c1* mRNA levels was observed in mice after exposure to TCDD (Abbott, 1995). Such alterations in *Nr3c1* mRNA levels and therefore glucocorticoid signaling pathway might impair the ability of individuals to efficiently respond to various stresses by affecting brain function and the immune system (Odermatt et al., 2006). While the current cross-species information on this observed relationship is limited, Aluru et al. (2004) reported a decrease in *Nr3c1* mRNA expression in Arctic char brain as a result of stress induced by high PCB levels. At present, the potential biological mechanisms of action involved remain unclear.

While there were no correlations detected *Nr1c3* mRNA levels and PCBs, we did find a strong relationship between *Hspa1* transcripts and this dominant contaminant (Figure 13,14 and Table 9). The nature of this correlation was tissue-specific. A very strong negative correlation was observed between skin *Hspa1* mRNA levels and PCB levels (Figure 13,14 and Table 9) whereas no correlation existed in blubber. This contrasts with a recent study showing an increase in *Hspa1* transcript levels in striped dolphin skin slices exposed to a mixture of organochlorine compounds, PBDEs, and polycyclic aromatic hydrocarbons (PAHs). Although age was not available to investigate the possible relationship with *Hspal* mRNA levels, a similar higher expression of *Hspa1* was noted in the blubber of more contaminated male fin whales (Fossi et al., 2010) and suggested an influence of organic contaminants on the expression of this stress-related protein.

### Tissue-specific response to contaminant exposure

As noted previously, our current observations suggest that a genomic response to PCB exposure in harbour seal is tissue specific. In the literature, evidence of tissue-specific response to PCB exposure is mainly available for hormone levels. However, extensive rodent studies on *Thra* mRNA levels showed that the effects of PCB exposure were strongly correlated with *Cyp1a1* mRNA levels and concluded that tissue-specific metabolism resulting in different congener patterns as well as different metabolites level is an important component of the action of PCBs on TR mRNA levels (Giera et al., 2011; Yeung et al., 2003). While different congener patterns have previously been reported in various tissues of harbour seals from the Northwestern Atlantic (Shaw et al., 2012), it is unlikely that they would explain the divergent results observed here for *Esr1*, *Thra*, *Nr3c1*, and *Hspa1*. This highlights the need for caution when interpreting transcriptomics results and comparing contaminant-associated effects between studies. Further studies are therefore needed to evaluate tissue-specific response to PCBs at the mRNA levels.

## 4.4 Conclusions

PCBs are highest ranked in terms of concentrations as well as risk to the health of marine mammals which is in agreement with the present results. PCB levels ranged from 0.15 to 7.5 µg/g and 45% of pups surpassed the 1.3 µg/g threshold determined for endocrine disruption (Mos et al., 2010). Our results revealed correlations between PCB levels and gene transcripts involved in such diverse biological processes as reproduction, growth, development, stress response and metabolism. Even though consequences at the population level remain unknown, our results reveal changes at the molecular level that could reflect the physiology of individual animals and their impaired ability to respond to additional environmental or anthropogenic stressors.

The risk appeared to be higher for individuals living in industrialized enclosed water basins, such as Puget Sound and the St. Lawrence Estuary, where the highest PCB levels were reported.

# Chapter 5: When threats converge: do both PCBs and climate change alter gene transcript profiles in Arctic beluga whales (*Delphinapterus leucas*)?

## 5.1 Introduction

The Arctic has been described as an important sink for persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCBs) and polybrominated diphenyl ethers (PBDEs), and for mercury (Hg) (Ariya et al., 2004; Wania et al., 2001). Once deposited, PCBs, PBDEs and Hg (in the form of methylmercury; MeHg), biomagnify in Arctic food webs such that relatively high levels have been detected in polar bears (*Ursus maritimus*), beluga whales (*Delphinapterus leucas*), and ringed seals (*Phoca hispida*) (Dietz et al., 2004; Ikonomou et al., 2002; Loseto et al., 2008b; Muir et al., 2006).

POPs and some metals can disrupt key biological processes necessary for normal development and physiological homeostasis in animals. PCBs, PBDEs and Hg have been linked to effects on the endocrine and immune systems of several marine mammal populations inhabiting contaminated areas (Beineke et al., 2007; Das et al., 2008; Frouin et al., 2010; Frouin et al., 2011; Hall et al., 2003; Lahvis et al., 1995; Mos et al., 2007; Schaefer et al., 2011). Recently, PCB exposure has been associated with changes in the mRNA levels of various genes in harbor seals (*Phoca vitulina*) and killer whales (*Orcinus orca*) from the Northeastern Pacific (Buckman et al., 2011; Mos et al., 2007), as well as in striped dolphins (*Stenella coeruleoalba*) from the Mediterranean Sea (Fossi et al., 2010).

While levels of POPs in Arctic wildlife are typically lower than those reported in marine top predators inhabiting more industrialized areas, several studies have shown that Arctic marine mammal health can be at risk due to contaminant exposure. For example, PCBs have been implicated in endocrine disruption, reproductive impairment and immunotoxicity in polar bears (Braathen et al., 2004; Lie et al., 2005; Sonne et al., 2004). A link between thyroid hormone disruption and certain PCB and PBDE congeners was suggested in beluga whales from Svalbard (Villanger et al.,

2011). Finally, damage to liver, kidney, and neurological function has been noted in polar bears with elevated Hg concentrations (Basu et al., 2009; Sonne et al., 2007a).

In addition to the incursion of anthropogenic contaminants, the Arctic has been undergoing significant climate-related changes with a 12.4% loss of September sea ice extent over the last decade (Stroeve et al., 2012). Reduced sea ice extent and changes in temperatures are likely to impact contaminant pathways, and thereby affect delivery, partitioning, transformation, and degradation of POPs (Macdonald, 2005; Macdonald et al., 2005; Stern et al., 2011). Changes in primary production have been attributed to altered sea ice extent and thickness (Mundy et al., 2009; Perovich et al., 2008; Tremblay et al., 2011) and could consequently impact food web structure and exposure to biomagnifying contaminants by high trophic level biota, including beluga whales. The distribution and feeding ecology of beluga whales are intricately linked to ice in the Arctic as these small cetaceans track the seasonal movement of ice and rely heavily on ice edge-associated food webs for prey (Loseto et al., 2006). As such, any change to sea ice extent or its dependent food web is expected to result in changes in the movement, behavior, and/or feeding ecology of beluga whales, all of which may affect contaminant-related exposure and health risks.

In the present study, we examine beluga whales harvested by Inuvialuit hunters in the Beaufort Sea between 2008 and 2010 and evaluate the relationship between the presence of contaminants of concern in Arctic food webs (PCBs, PBDEs and Hg) and hepatic and blubber abundance profiles of mRNAs encoding proteins that are essential for regulating growth, metabolism, development, and xenobiotic detoxification.

## 5.2 Materials and methods

### Sample collection

All tissues were sampled from beluga whales in collaboration with the annual harvest by Inuvialuit hunters at a field station on Hendrickson Island near the community of Tuktoyaktuk, Northwest Territories, Canada (Figure 15). A total of 43 males were sampled between 2008 and 2010 (n=20 in 2008; n=13 in 2009 and n=10 in 2010). Inuvialuit hunters typically select medium to larger sized males, such that the number of samples obtained from females (n = 4, 7, and 0 in 2008, 2009, and 2010, respectively) precluded a proper evaluation of the influence of gender. We therefore discuss only data derived from males.

Blubber and skin samples collected for organic contaminant analyses (PCBs and PBDEs) were immediately flash frozen in liquid nitrogen for transport. Samples were subsequently transferred to a -80°C freezer at Fisheries and Oceans Canada in Sidney, BC, Canada. For determination of mercury (Hg) content, muscle samples were collected and stored at -20°C until further analyses.

For measurement of mRNA abundance profiles, approximately 1g each of blubber and liver were preserved directly in RNA*later* tissue preservation solution as per the manufacturer's instructions (Applied Biosystems, Foster City, CA, USA) and stored at -20°C until isolation of total RNA.

All post mortem samples were collected within two hours of harvesting. A serial sampling of liver and blubber tissues from three whales at the field station revealed no impact on data quality within this collection period providing confidence about the quality and comparability of our sample set (results not shown).

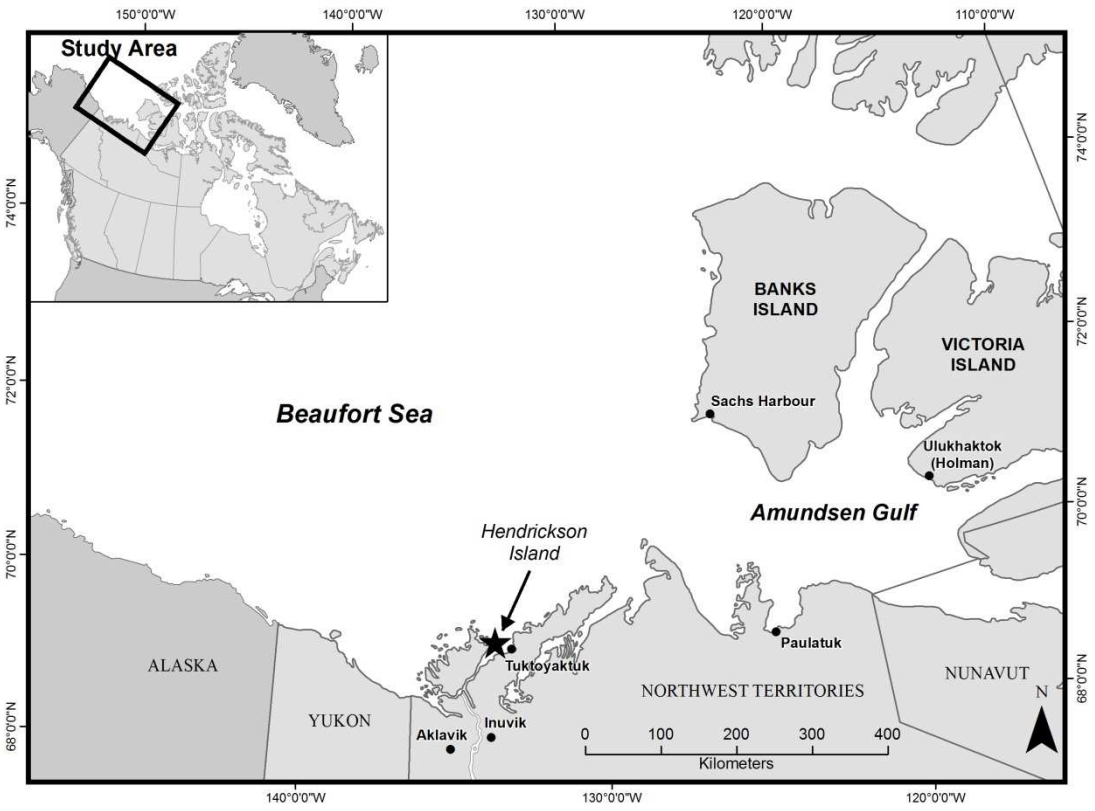


**Figure 15: Beluga tissues from 43 beluga whales harvested by Inuvialuit hunters in the Western Canadian Arctic were collected near Hendrickson Island between 2008 and 2010.**

### RNA isolation and cDNA synthesis

Blubber samples were divided into inner, middle, and outer blubber based on differences in color and texture. Detailed procedures on total RNA extraction and cDNA synthesis are described elsewhere (Buckman et al., 2011; Veldhoen et al., 2001). Briefly, tissues were homogenized in a 1.5 mL microcentrifuge tube with 700 µL of TRIzol reagent as recommended by the manufacturer's protocol (Life Technologies Inc., Burlington, ON, Canada) and a 3 mm diameter tungsten-carbide bead using a Retsch MM400 mixer mill (Thermo Fisher Scientific, Ottawa, ON, Canada). Samples were homogenized in two 3 minute intervals at a frequency of 20 Hz with a brief sample cooling on ice and rotation of the shaking rack between

intervals. For the blubber, 20 µg of glycogen (Roche Diagnostics, Laval, QC, Canada) was added to each sample after phase separation. Isolated total RNA was resuspended in diethyl pyrocarbonate-treated distilled, deionized water (DEPC dd$H_2O$) (20 µL for blubber samples and 50 µL for liver samples) and stored at -80°C. RNA concentrations were confirmed through spectrophotometry and 1 µg of each sample used to produce cDNA with the High Capacity cDNA reverse transcription kit as described by the manufacturer (Applied Biosystems, Carlsbad, CA, USA). Each cDNA sample was diluted 40-fold with PCR-grade water prior to evaluation using the quantitative real time polymerase chain reaction (QPCR) assay.

**Quantitative real time polymerase chain reaction (QPCR) assay**

Thirteen gene transcripts were selected based on their ability to provide information on animal health and/or response to contaminant exposure. These include thyroid hormone receptors alpha and beta (*Thra, Thrb*), estrogen receptor alpha (*Esr1*), retinoid X receptor alpha (*Rxra*), aryl hydrocarbon receptor (*Ahr*), cytochrome P450 1A1 (*Cyp1a1*), glucocorticoid receptor (*Nr3c1*), heat shock protein 70-like (*Hspa1l*), metallothionein 1 (*Mt1*), leptin (*Lep*), adiponectin (*Adipoq*), vitamin D receptor (*Nr1l1*), peroxisome proliferator-activated receptor gamma (*Nr1c3*), and insulin like growth factor receptor 1 (*Igf1*). Three additional transcripts were selected as potential normalizers for correction of experimental variance: ribosomal protein L8 (*Rpl8*), glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*), and cytoplasmic beta actin (*Actb*).

Gene-specific primers were obtained from Integrated DNA Technologies (Coralville, IA, USA) and assessed for their ability to amplify a single specific DNA amplicon using beluga whale cDNA prepared from the two tissues. A three-tier

quality control (QC) process outlined in detail elsewhere (Veldhoen et al., 2011) confirmed the targeted expressed gene sequence and satisfied the criteria for use of the comparative ΔΔCt quantification method (Veldhoen et al., 2011). Information on the QPCR primers is outlined in Appendix 3 and the associated beluga whale expressed gene sequences and cloning primers were deposited in NCBI GenBank or are presented in Appendix 4.

The QPCR data presented herein are derived from the blubber and liver. In blubber, all primers, except those for *Cyp1a1*, passed the quality control tests. Inner and outer blubber mRNA levels correlated with each other for all gene transcripts (Appendix 5), we only present the data from the inner blubber in the present study. Previous studies have reported the inner blubber to be more appropriate than outer blubber to investigate the association of contaminant concentrations (Buckman et al., 2011; Tabuchi et al., 2006) and changes in mRNA levels likely related to increased vascularisation and metabolic activity in this region (Strandberg et al., 2008; Wilson et al., 2007). Two additional gene transcripts, *Ahr* and *Cyp1a1*, involved in detoxification were also analyzed in the liver.

QPCR reactions (15 µL) were performed on a Realplex4 Eppendorf thermocycler (Eppendorf, Westbury, NY, USA). The thermocycle program for all transcripts included an initial enzyme activation step at 95°C (9 min), followed by 40 cycles of 95°C denaturation (15 sec), 60°C (or 55°C for *Rxra*) annealing (30 sec), and 72°C elongation (45 sec). Quadruplicate reactions were performed for each sample and QPCR target specificity was determined by inclusion of no template and no amplification reaction controls as well as through evaluation of each post-reaction thermodenaturation profile. Inter-run variation was assessed as described previously (Veldhoen et al., 2011). The geometric mean of all three normalizer gene transcripts

was invariant between groups and not affected by biological variables or contaminant concentrations under investigation. Additional suitability of the normalizers was established using Reffinder (http://www.leonxie.com/referencegene.php?type=reference). Replicate data was averaged and normalized to the geometric mean and relative mRNA abundance (fold change) values generated using the comparative (ΔΔCt) method (Livak et al., 2001).

**Contaminant analyses**

A portion of each blubber sample (500 mg to 1g wet weight) was analyzed at the Laboratory of Expertise for Aquatic Chemical Analysis (LEACA) at the Institute of Ocean Sciences (Sidney, BC, Canada) for congener-specific PCB and PBDE determination. For quality assurance/quality control (QA/QC) purposes, a standard reference material (NIST 1945 whale blubber SRM), two procedural blanks containing pure lipid (triolein) to imitate the behavior of tissue extracts, and one replicate sample were run for each batch of 10 samples. The procedural blanks, along with approximately 600 mg amounts of each beluga blubber sample, replicates and SRM samples were spiked with a mixture of surrogate internal standard containing ten $^{13}$C-labeled PCBs and ten $^{13}$C-labeled PBDEs obtained from Cambridge Isotope Laboratories (Andover, MA, USA) to enable precise and accurate quantification using the isotope dilution method. Details on sample clean-up, instrument-based analysis, quantification protocols, criteria used for congener identification and QA/QC control measures undertaken for the high resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) analysis of all the analytes of interest are described elsewhere (Ikonomou et al., 2001).

Beluga muscle samples of approximately 0.15g were analyzed for total Hg. Samples were digested with a hydrochloric/nitric acid mixture heated to 90ºC. The digest was analyzed by cold vapor atomic absorption spectrometry (CVAAS) at the Freshwater Institute, Winnipeg, MB, Canada. Certified Reference Materials (CRM 2976, TORT-2, DOLT-2) were analyzed in duplicate in every run. Details on the procedures and QA/QC can be found elsewhere (Armstrong et al., 1971; Loseto et al., 2008b).

**Stable isotope analyses**

Isotope determination was performed on dried homogenized beluga liver samples. Lipids were removed for the carbon isotope determination using a chloroform/methanol extraction and then dried for analysis. Carbon and nitrogen isotopic analyses were performed using continuous flow, ion-ratio, mass spectrometry (CF-IRMS) (University of Winnipeg Isotope Laboratory, MB, Canada). Details on the procedures as well as QA/QC methods can be found elsewhere (Loseto et al., 2008b). Carbon and nitrogen 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:

$$\delta_{sample}\ ‰ = [(R_{sample} / R_{standard}) - 1] \times 1000$$

where R is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the sample and the standard. The standards used for carbon and nitrogen analyses were Vienna PeeDee Belemnite (VPDB) and IAEN-N1 (IAEA, Vienna, Austria), respectively.

**Data analyses**

Normality and homogeneity of variances were evaluated using the Kolmogorov-Smirnov test and Levene's test, respectively (SPSS, IBM Corporation, Armonk, NY, USA) for relative mRNA abundance, total PCB and PBDE concentration (expressed on a lipid weight basis), and mercury concentration data sets. If the assumption of normality and homogeneity of variances were not met, data were log-transformed. Pearson correlation was determined between inner and outer blubber samples using SPSS.

Principal component analysis (PCA) was used to evaluate mRNA abundance patterns among individual belugas (Pirouette, Infometrix, Bothell, WA, USA). Transcript fold change values were transformed and autoscaled before PCA. This centered log ratio procedure was used to avoid negative biased associated with normalized data, as described elsewhere (Ross et al., 2004). We examined which of the biological variables best explained variation in beluga mRNA transcript levels by regressing the biological variables with the PCA from axis one and two (SPSS).

The variable or combination of variables (year, age, length, total PCBs, total PBDEs, Hg, $\delta^{15}N$, $\delta^{13}C$) best describing the mRNA level of each gene was selected using the lowest Akaike information criterion (AIC) (SYSTAT, Chicago, IL, USA). The AIC method ranks models based on their overall statistical support. Lowest AIC models are most supported and AIC weights provide information on the relative support of each model. Backward stepwise regressions were used in combination to AIC.

## 5.3 Results and discussion

### Morphometrics and contaminant levels in beluga

While body length, girth and blubber thickness did not differ among sampling years, 2010 beluga were younger than animals sampled in 2008 and 2009 ($p = 0.030$) (Table 10). PCB (0.68 – 8.36 µg/g lw) and Hg concentrations (0.47 – 2.79 µg/g dw) did not differ among study years. Inter-annual differences were observed for stable isotopes measured in liver, with $\delta^{13}C$ being higher in 2008 and 2010 compared to 2009 ($p < 0.001$). Finally, PBDE levels in 2009 whales were higher than in those sampled in 2008 ($p = 0.049$). These PCB, PBDE and Hg levels are 7-fold, 12-fold and 8-fold lower, respectively, than levels observed in the southerly St. Lawrence estuary beluga whales (Beland et al., 1993; Hobbs et al., 2003; Raach et al., 2011), wherein reproductive impairment and a high incidence of tumors are thought to underlie a lack of population recovery (Beland et al., 1993). The present results collectively indicate that inter-annual differences in beluga feeding ecology occur in the Arctic, with consequential implications for uptake of dietary contaminants. The influence of habitat selection on contaminant exposure in Beaufort Sea beluga is evaluated in more detail elsewhere (Loseto et al., 2008a; Loseto et al., 2012).

**Table 10: Forty-three beluga males were sampled in collaboration with Inuvialuit hunters. Inter-annual differences were observed for age, PBDEs, $\delta^{15}N$ and $\delta^{13}C$.**

| | 2008 (A) | 2009 (B) | 2010 (C) | Tukey's |
|---|---|---|---|---|
| Length (cm) | 398 ± 28 | 405 ± 42 | 404 ± 36 | p = 0.368 |
| Girth (cm) | 113 ± 16 | 120 ± 13 | 118 ± 16 | p =0.706 |
| Age (years) | 34 ± 12 | 34 ± 11 | 24 ± 4 | p = 0.030 (AC; BC) |
| Blubber thickness (cm) | 8.6 ± 0.6 | 9.9 ± 0.4 | 8.7 ± 0.5 | p = 0.704 |
| PCBs (µg/g lw) | 3.4 ± 1.7 | 3.6 ± 1.5 | 3.2 ± 1.3 | p = 0.368 |
| PBDEs (ng/g lw) | 17.1 ± 1.6 | 27.3 ± 3.8 | 21.2 ± 2.8 | p = 0.049 (AB) |
| Hg (µg/g dw) | 1.3 ± 0.13 | 1.1 ± 0.2 | 1.1 ± 0.1 | p = 0.355 |
| $\delta^{15}N$ (‰) | 16.9 ± 0.4 | 16.1 ± 0.4 | 18.2 ± 0.4 | p = 0.010 (BC) |
| $\delta^{13}C$ (‰) | -20.5 ± 0.9 | -19.2 ± 0.3 | -20.2 ± 0.3 | p < 0.001 (AB; BC) |

### PCB-related changes in mRNA abundance

While beluga whales are exposed to a complex mixture of persistent environmental contaminants, PCBs, PBDEs, and Hg are considered among the top contaminant threats to high trophic level marine mammals in the northern hemisphere (Mos et al., 2010). Therefore, we explore the association between these priority contaminants and mRNA abundance profiles determined in select beluga tissues.

A significant correlation was observed between liver *Ahr* and *Cyp1a1* mRNA levels (Figure 16a; $r^2 = 0.62$; $p < 0.001$), consistent with their inter-relationship with respect to biological responses to xenobiotic exposure and previous studies in other species (Mimura et al., 2003). The abundance of *Ahr* (2008 and 2009: $r^2 = 0.18$; $p = 0.045$; 2010: n.s.) and *Cyp1a1* (2008 and 2009: $r^2 = 0.20$; $p < 0.001$, 2010: $r^2 = 0.20$; $p = 0.049$) mRNA was positively correlated with PCB concentrations in beluga (Figure 16b, c). *Ahr* mRNA levels in 2010 beluga were not correlated with PCB levels, perhaps reflecting the small sample size for that particular year. An increase in

blubber *Ahr* and *Cyp1a1* mRNA levels in relation to PCB exposure was also recently observed in highly contaminated killer whales from the Northeastern Pacific Ocean, where age and sex were not contributory variables (Buckman et al., 2011). While animal age was not known, positive relationships between *Ahr* and *Cyp1a1* mRNAs *versus* PCB concentrations have been established in both striped dolphins from the Mediterranean (Panti et al., 2011) and fin whales (*Balaenoptera physalus*) from the Mediterranean and Gulf of California (Fossi et al., 2010).

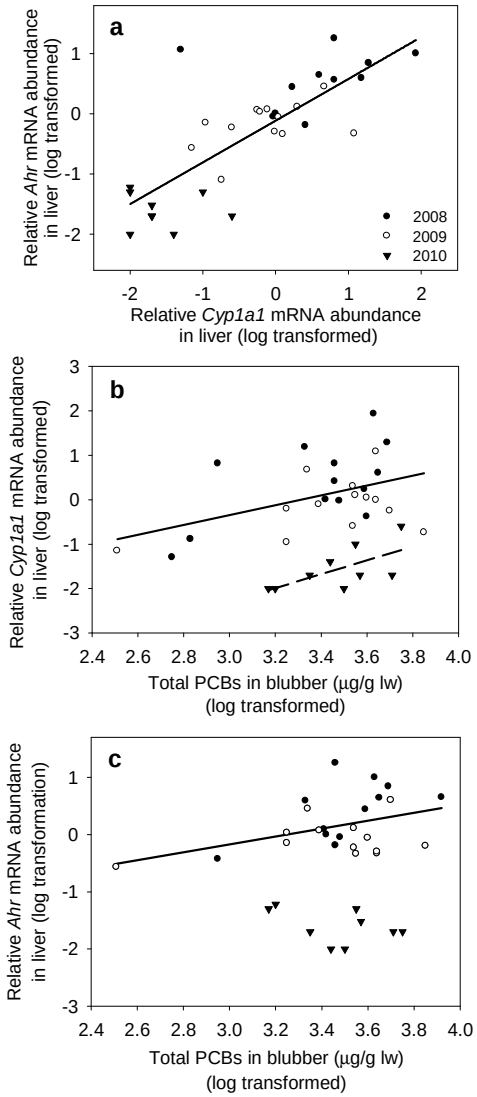


**Figure 16: Relationship between *Cyp1a1* and *Ahr* mRNA levels and total PCBs: (a) *Cyp1a1* and *Ahr* mRNA levels in the liver of male beluga whales were closely interrelated ($r^2 = 0.62$; $p < 0.01$); (b) *Cyp1a1* transcripts correlated with total PCB concentrations (solid line: 2008+2009; $r^2 = 0.20$, $p < 0.001$; dash line: 2010: $r^2 = 0.43$, $p = 0.049$); and (c) *Ahr* mRNA levels were correlated with total PCB concentrations (solid line: 2008+2009: $r^2 = 0.18$, $p = 0.045$; 2010: n.s). Whales sampled in 2010 had lower *Cyp1a1* and *Ahr* transcript levels possibly due to their younger age.**

The high affinity of beluga AHR protein for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and the relatively high levels of hepatic CYP1A1 protein observed in Arctic beluga may indicate a sensitivity of this species to dioxin-like compounds, including planar PCBs (Jensen et al., 2001; Wilson et al., 2005). The highly significant correlation between *Ahr* and *Cyp1a1* mRNA levels and PCB concentrations in beluga in the present study suggests that an increase in mRNA abundance can be detected at contaminant levels that are substantially lower than those reported for southerly marine mammal populations in which adverse effects have been noted (Hobbs et al., 2003). This underscores the sensitivity of transcriptome-based endpoints in detecting toxic injury and/or response to contaminant exposure.

The lines depicting the relationships between hepatic *Ahr* and *Cyp1a1* mRNA levels and PCB concentrations differed between 2010 whales and the 2008 and 2009 whales (Figure 16b, c), possibly reflecting the younger age of these animals (Table 10). Beluga *Ahr* and *Cyp1a1* transcript abundance increased with age ($r^2 = 0.20$ ; $p = 0.015$ and $r^2 = 0.27$ ; $p = 0.002$, respectively; data not shown) in a manner similar to that observed in Baikal seals (*Pusa sibirica*) (Kim et al., 2005). Age-correcting the *Ahr* and *Cyp1a1* mRNA levels eliminated the inter-annual differences (described in Figure 16b and c), with results collectively revealing positive correlations between *Ahr* and *Cyp1a1* mRNAs *versus* PCBs, regardless of age.

The abundance profiles measured for other gene transcripts in beluga blubber exhibited no relationship with PCBs for any study year. These included transcripts encoding proteins involved in key biological processes such as hormone signalling pathways, regulation of growth and metabolism, and development (*Thra*, *Thrb*, *Esr1*, *Nr3c1*, *Pparg*, *Adipoq*, *Lep*, *Igf1* and *Rxra*). This observation for arctic beluga contrasts results from more contaminated marine mammals, where, for example, *Thra*

and/or *Thrb* transcripts correlated with PCB concentrations in harbor seals (Tabuchi et al., 2006) and killer whales from the Northeastern Pacific Ocean (Buckman et al., 2011), and ringed seals from the Baltic Sea (Routti et al., 2010). Additionally, higher *Esr1* mRNA levels with increasing PCBs have been observed in both the Northeastern Pacific killer whales (Buckman et al., 2011) and the Mediterranean and Gulf of California fin whales (Fossi et al., 2010).

No association between any of the gene transcripts measured and PBDE concentrations were detected in beluga blubber. To our knowledge, there are no reports of direct PBDE-associated influence on mRNA transcript abundance in wildlife. Because of their similar structure and physico-chemical properties, PCBs and PBDEs are often reported to have similar toxic mechanisms of action. However, PBDEs have been identified as less toxic than PCBs (Hallgren et al., 2002; Hallgren et al., 2001) and, under controlled laboratory-based exposures, correlations between *Thra* mRNA levels as well as other transcripts involved in thyroid metabolism and PBDEs have been established (Lema et al., 2008; Szabo et al., 2009).

Elevated metallothionein is often associated with metal exposure (Ikemoto et al., 2004). No consistent associations were observed between *Mt1* mRNA levels and total Hg (or PCBs and PBDEs), perhaps reflecting the relatively low levels of this metal in our study animals. MeHg biomagnifies in food webs along with PCBs, perhaps explaining the positive correlation observed between these two compounds in beluga ($r^2 = 0.82$; $p < 0.001$). Buckman *et al* (2011) reported an increase in the *Mt1* mRNA levels with increasing PCB levels in killer whales, although Hg was not measured and contaminant concentrations were one to two orders of magnitude higher than for beluga assessed in the present work. Sled-dogs fed whale blubber containing high

levels of Hg and PCBs also exhibited an increase in *Mt1* mRNA levels (Sonne et al., 2007b).

While only three major contaminants of concern (PCBs, PBDEs, and Hg) were measured in the present study, it is important to recognize that these whales are exposed to a highly complex mixture of contaminants (Muir et al., 1999). There is evidence suggesting that the Mackenzie River delta, where the belugas were sampled, has relatively high levels of polycyclic aromatic hydrocarbons (PAHs) (Yunker and Macdonald, 1998). Even though these chemicals are also able to bind to AHR, they have a relatively low half-life and limited ability to bioaccumulate compared to PCBs (Gray, 2002). In addition, as evidence by the empty stomachs observed in all the whales sampled, these belugas are not known to feed directly in the Mackenzie delta area, having just arrived from their Bering Sea in-migration. While the contribution of other persistent contaminants cannot be ruled out, a weight of evidence indicates that PCBs represent the dominant POP of concern in marine mammals (Mos et al., 2010), and remains the likely driver of our observations.

**PCBs alone do not explain all gene transcript responses**

While univariate statistical approaches provide evidence of PCB-related increases in two hepatic gene transcripts (*Ahr* and *Cyp1a1*), PCA provides additional insight into the factors affecting the health of beluga whales. Seventy-six percent of the variance in mRNA transcript expression profiles in beluga was explained by the first two PCA factors (PC1: 56%; PC2: 20%) (Figure 17a, b). Inter–annual clustering was observed along both axes, suggesting that differences among years explained some of the gene expression pattern. Beluga sampled in 2009 clustered on the left side of PC1 while most of the beluga sampled in 2008 and 2010 fell on the right side (Figure 17a).

Beluga sampled in 2010 were clustered towards the lower end of PC2, contrasting those sampled in 2008 and 2009 that clustered towards the top of the plot. PC1 correlated with $\delta^{13}C$ and PBDE concentrations ($r^2 = 0.17$; $p = 0.007$; $r^2 = 0.13$; $p = 0.012$, respectively; Figure 17c, d). PC2 appeared to be explained by a single factor, the age of the beluga ($r^2 = 0.15$; $p = 0.022$; Figure 17e), with 2010 whales being younger than those sampled in 2008 and 2009.

The PCA variables plot of mRNA transcripts revealed a clear divergence between the two toxicology-related mRNA transcripts (*Ahr* in both liver and blubber, and liver *Cyp1a1)* and the other 11 blubber gene transcripts (*Thra, Thrb, Esr1, Nr3c1, Mt1, Hspa1l, Nr1c3, Adipoq, Lep, Igf1* and *Rxra*) (Figure 17b). This is consistent with the highly specific roles of *Ahr* and *Cyp1a1* in detoxification processes in mammals (White et al., 2000). The 11 non-toxicology-related gene transcripts were all higher in 2008 and, to a lesser extent, in 2010. The relatively lower $\delta^{13}C$ values in whales sampled in 2008 and 2010 help explain the multivariate patterns observed here, and are consistent with inter-annual differences in feeding behavior suggested here and in a parallel study (Loseto et al., in prep).

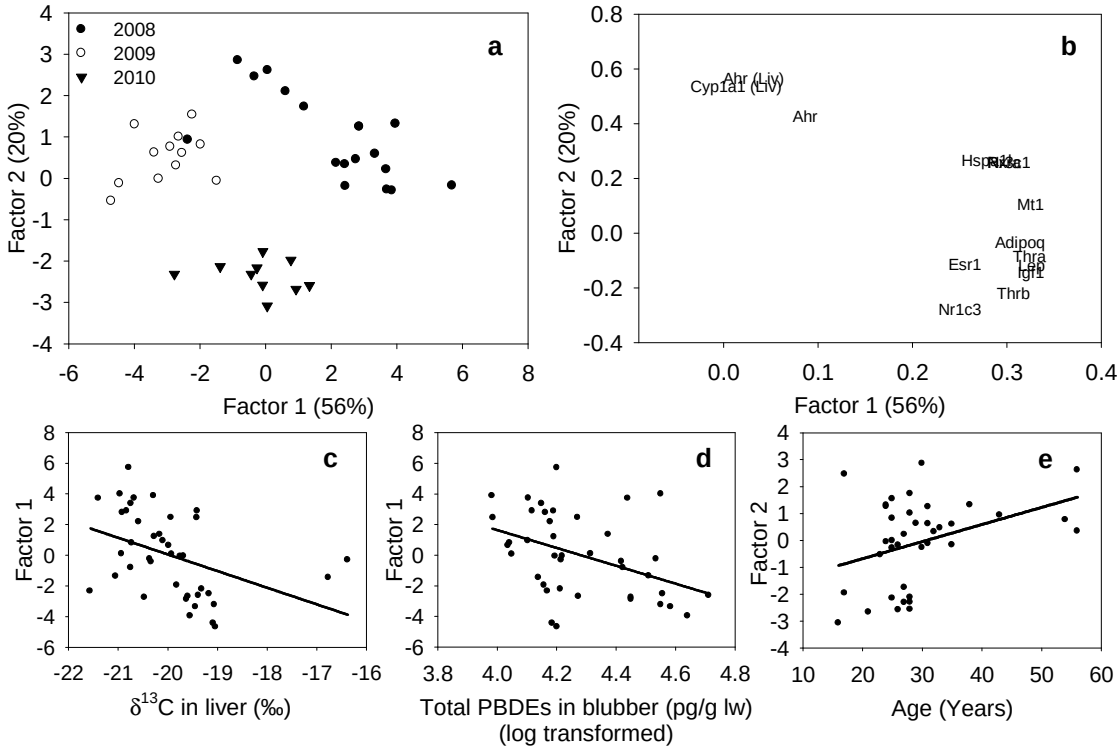


**Figure 17: (a) Principal Component Analysis (PCA) was performed on all mRNA transcripts in inner blubber as well as *Ahr* and *Cyp1a1* in liver. It revealed inter-annual differences in mRNA transcript abundance in male beluga whales. The 13 gene transcripts involved in the PCA are shown in (b). Factor 1 was plotted against (c) $\delta^{13}C$ in liver or (d) total PBDEs in blubber. Factor 1 was negatively correlated with $\delta^{13}C$ ($r^2 = 0.17$; $p = 0.007$) and PBDEs ($r^2 = 0.13$; $p = 0.012$), pointing to inter-annual differences in diet. (e) Factor 2 was positively correlated with age ($r^2 = 0.15$; $p = 0.022$), although clustering by year was still evident.**

Best fit models confirmed that year sampled, $\delta^{13}C$ ratio, and PBDE concentration explain the variation in *Thra*, *Thrb*, *Nr3c1*, *Mt1*, *Hspa1l*, *Nr1c3*, *Adipoq*, *Lep*, *Igf1* and *Rxra* mRNA abundance. The $\delta^{13}C$ ratio contributed to the final model for 10 out of 11 target gene transcripts, while sampling year and PBDE level contributed to the final model for 8 out of 11 target mRNAs (Table 11). These results build on the univariate and multivariate observations above, and suggest that inter-annual differences in feeding ecology explain some of the variation in beluga *Thra*, *Thrb*, *Nr3c1*, *Mt1*,

*Hspa1l*, *Nr1c3*, *Adipoq*, *Lep*, *Igf1* and *Rxra* mRNA transcript profiles. Given the low concentrations of PBDEs in these beluga whales, we suspect that the relationship between PBDEs and the mRNA abundance of this battery of genes is not due to toxicity, but rather reflects a dietary shift to prey containing somewhat higher PBDE levels in 2009. This is supported by the lack of univariate correlations between the levels of individual mRNA and PBDE concentrations, as well as the coincident changes in $\delta^{13}C$.

**Table 11: Results from the Akaike information criterion (AIC) analyses. Year and PBDEs were the best variables explaining variations in the mRNA levels of genes involved in growth, metabolism and development. [a] AICc = second order Akaike information criteria (AIC ) *n*log ($\sigma$2) + 2*K*) bias adjusted AIC for small sample size = AIC + (2*K*(*K* + 1/(*n* - *K* - 1) where *K* is the total number of estimated regression parameters including $\sigma$2 (no intercept) and *n* is sample size. [b] $\Delta_i$ = AIC differences computed as $AIC_i$-$AIC_{min}$. [c] $w_i = exp(-1/2\Delta_i)/\Sigma exp(-1/2\Delta_r)$. Data are only presented for model with $\Delta_i AIC_c$ below 2 which are considered the most important.**

| Gene | Major roles | Predictors | $r^2$ | p-value | AIC | $AIC_c$ | $\Delta AIC_c$ | $w_i$ |
|---|---|---|---|---|---|---|---|---|
| *Thra* | Development, cell differentiation, metabolism | year, PBDEs, $\delta^{13}C$ | 0.73 | <0.001 | 16.52 | 21.9 | 0 | 0.71 |
| *Thrb* | Development, cell differentiation, metabolism | PBDEs, $\delta^{13}C$, Hg | 0.68 | <0.001 | 43.4 | 45.5 | 0 | 0.58 |
| *Esr1* | Growth, cell differentiation, development, reproduction | Year, PBDEs, $\delta^{13}C$ | 0.53 | <0.001 | 46.6 | 49.5 | 1.3 | 0.27 |
| | | Year, PBDEs | 0.63 | <0.001 | 46.2 | 48.2 | 0 | 0.52 |
| *Ahr* | Induction of metabolizing enzyme | PCBs, age, PBDEs | 0.42 | <0.001 | 84.4 | 86.5 | 1.5 | 0.25 |
| | | PCBs, age | 0.48 | <0.001 | 83.7 | 85 | 0 | 0.53 |
| *Cyp1a1* | Metabolism of lipophilic subtsances such as dioxin-like compounds | PBDEs, Hg, PCBs, Age, year | 0.54 | 0.012 | 57.2 | 61.9 | 1.5 | 0.29 |
| | | PBDEs, Hg, Age, PCBs | 0.63 | <0.001 | 50.7 | 60.4 | 0 | 0.61 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Nr3c1* | Lipid metabolism, maintenance of growth, response to stress | year, PBDEs, $\delta^{13}C$, PCBs | 0.52 | <0.001 | 67.5 | 70.7 | 1.3 | 0.3 |
| | | year, PBDEs, $\delta^{13}C$ | 0.67 | <0.001 | 67.2 | 69.4 | 0 | 0.57 |
| *Mt1* | Cellular metal homeostasis, scavenging of oxygen radicals | Year, $\delta^{13}C$ | 0.42 | <0.001 | 37.1 | 40.6 | 0 | 0.76 |
| *Hspa1l* | Protect cells from environmental stress conditions | Year, $\delta^{13}C$, PBDEs | 0.2 | 0.056 | 54.1 | 57.2 | 2 | 0.35 |
| | | Year, $\delta^{13}C$ | 0.23 | 0.043 | 53.8 | 56.2 | 0.5 | 0.44 |
| *Nr1c3* | Cell differentiation, development, metabolism (lipids, proteins) | PBDEs, $\delta^{13}C$,PCBs | 0.53 | 0.042 | 55.4 | 58.1 | 2 | 0.24 |
| | | PBDEs, $\delta^{13}C$ | 0.67 | 0.013 | 54.3 | 56.1 | 0 | 0.65 |
| *Adipoq* | Glucose regulation, fatty acid catabolism | Year, $\delta^{13}C$, Hg, age, PBDEs | 0.56 | <0.001 | 97.6 | 98.7 | 0.6 | 0.36 |
| | | Year, $\delta^{13}C$, Hg, age | 0.65 | <0.001 | 97.4 | 98.1 | 0 | 0.48 |
| *Lep* | Lipid metabolism | Year, PBDEs, $\delta^{13}C$, PCBs | 0.64 | <0.001 | 71.5 | 74.5 | 1.4 | 0.28 |
| | | Year, PBDEs, $\delta^{13}C$ | 0.68 | <0.001 | 71.1 | 73.1 | 0 | 0.56 |
| *Igf1* | Protein and lipid metabolism | Year, PBDEs, $\delta^{13}C$, PCBs | 0.69 | <0.001 | 67 | 68.2 | 0.6 | 0.34 |
| | | Year, PBDEs, $\delta^{13}C$ | 0.72 | <0.001 | 66.9 | 67.6 | 0 | 0.45 |
| *Rxra* | Cell proliferation and differentiation, immune system | Year, PBDEs, $\delta^{13}C$ | 0.89 | <0.001 | 30.4 | 35.9 | 0 | 0.75 |

While there is no information on the effects of changes in diet and altered abundance of gene transcripts associated with growth, metabolism and development in marine mammals, there is growing evidence of such relationships in studies of laboratory animals. Nutritional changes, including altered food intake and/or changes in the types of nutrients consumed, have affected the synthesis and metabolism of hormones and the expression of hormone receptors (Dauncey et al., 2001). For example, *Pparg, Adipoq, Lep, Igf1, Thra, Thrb, Nr3c1*, and *Hspal* mRNA levels in rodents were altered following changes to diet, including shifts in fatty acid or caloric intake, as well as with fasting–refeeding cycles. These changes in expression of mRNA transcripts and encoded protein subsequently affected development, energy metabolism, body weight, and nutrient utilization (Bertram et al., 1999; Dauncey et al., 2001; Flier et al., 2000; Kadowaki et al., 2005; Lin et al., 1997; Schlesinger, 1990). These observations from controlled feeding studies of mammals suggest that changes in mRNA transcript levels in beluga whales may indeed occur as a result of changes in feeding ecology.

In western Hudson's Bay, changes in sea ice extent has been linked to changes in distribution and feeding ecology of polar bears and beluga whales (Gaden and Stern, 2010; McKinney et al., 2009). Beaufort Sea beluga whales rely heavily on sea ice edge-associated prey such as Arctic cod (*Boreogadus saida*) (Loseto et al., 2009; Loseto et al., 2008b). Sea-ice associated changes in the diet of this population of beluga have been described, with whales feeding more offshore and/or more pelagically during years of low sea ice extent (Loseto et al., 2012). Stroeve et al. (2012) reported a rapid loss of ice in June 2008 and 2010 in the Beaufort Sea. The average percent ice coverage in the Mackenzie River delta during the month of June was 19% and 16% lower in 2008 and 2010, respectively, compared to 2009 when ice

extent was close to historical averages (Canadian Ice Services; http://www.ec.gc.ca/glaces-ice, Appendix 6). These changes to sea ice conditions coincide with the lower $\delta^{13}C$ ratios and higher mRNA levels for genes related to growth, metabolism and development in 2008 and 2010 beluga but not those from 2009.

## 5.4 Conclusions

While the full implications of change in mRNA status remain unclear, there exists strong links between exposure to planar hydrocarbons and changes in *Ahr* and *Cyp1a1* expression across a wide range of fauna. An extensive weight-of-evidence also exists which documents PCB-related effects to the health of marine mammals inhabiting industrialized regions. This includes increased susceptibility to disease, higher incidence of cancer, reproductive impairment, and developmental abnormalities (Helle et al., 1976; Ross, 2000; Ylitalo et al., 2005).

The present study provides strong evidence of PCB-related increases in *Ahr* and *Cyp1a1* mRNA abundance in free-ranging beluga whales and also suggests that the inter-annual levels of additional gene transcripts involved in development, growth, and metabolic homeostasis are altered under different sea ice extent profiles. This portends potentially troubling health risks to long-lived beluga whales associated with a rapidly changing climate in the Arctic. The degree to which these results reflect a shift in diet, or a stress response to changes in feeding ecology, is unclear. Nevertheless, our results suggest that the effects of PCBs on the health of beluga whales may be modulated under different climate regimes. Altered health of beluga exposed to POPs in a rapidly changing arctic environment could have important

consequences for the aboriginal communities that have relied on this species as an important food source for hundreds of years.

# Chapter 6: Conclusions

Persistent organic pollutants (POPs) and methylmercury (MeHg) are present in every environmental compartment including air, water, sediment and biota. They can therefore be defined as multi-media compounds. This characteristic represents a challenge when trying to predict and understand their environmental fate and transport.

While Hg is emitted from both natural and anthropogenic sources and has been present in the environment for centuries, POPs are man-made chemicals. The first POP was manufactured 80 years ago but it is only 30 to 40 years ago that their toxicity, bioaccumulation ability and persistence became apparent. Since the 1970s, there have been an increasing number of research programs monitoring POPs and Hg in air, water, sediment and biota increasing our understanding on transport, trends and potential health effects of these compounds.

The present thesis filled some gaps and furthered our knowledge on the transport and fate of these major contaminants of concern (PCBs, PBDEs and Hg) in the coastal British Columbia (BC) environment. It also provided new information on the potential impacts of these compounds on the health of marine mammals inhabiting industrialized areas as well as remote locations.

## 6.1 What is the contribution of long-range versus local sources of PCBs, PBDEs and Hg in coastal BC, Canada?

### Atmospheric investigation

Atmospheric transport is the most efficient mechanism by which POPs move in the environment and are delivered to remote location. Atmospheric PCBs have been estimated to represent 93% of the total PCB inputs to the North Sea (Duce, 1998), and 60 to 90% of the PCB burden in the Great Lakes may originate from the atmosphere (Eisenrich et al., 1981). Similarly, atmospheric Hg deposition accounts for up to 90%

of the total Hg delivery to the world's ocean (Outridge et al., 2008). Understanding atmospheric transport of these compounds is therefore important to further our understanding of contaminants in aquatic ecosystems.

With Asia currently being the major emitter of Hg and prevailing winds from the west delivering air masses to North America from Asia in only 2 to 10 days (Jaffe et al., 1999; 2003), several studies have investigated the trans-Pacific transport of Hg. It has been estimated that Asian emissions contribute between 15 and 24% of mercury deposition over the western US (Seigneur et al., 2004; Strode et al., 2008) (Figure 18). On the other hand, no estimation of the relative importance of trans-Pacific transport on the delivery of PCBs and PBDEs to the West Coast of North America is available.

In chapter 1, we demonstrated that the legacy PCBs are globally dispersed in the atmosphere as opposed to the still widely used PBDEs for which a local BC signal was clearly evident (Figure 18). The ten day back trajectories helped determine the source of air masses reaching BC during our sampling year (2004) reflecting a dominant flow from the west, with little seasonal variation in wind direction. Together, these results suggested that 40% of PBDEs detected in BC air originated from trans-Pacific transport. In terms of deposition, we estimated the total atmospheric inputs of PCBs and PBDEs to the coastal BC marine environment at 3.5 ± 0.7 kg/year and 17.1 ± 6.5 kg/year, respectively, highlighting the increasing dominance of PBDEs as environmental contaminants in this coastal ecosystem. This represents approximately 50% and 35% of total PCB and PBDE inputs to the Strait of Georgia, respectively, with municipal effluents being the second most significant contributor (R. Macdonald, personal communication).

The significant intercontinental transport of contaminants from Asia to the west coast of North America combined with a burgeoning Asian economic zone raises concerns for the BC coastal environment. It also highlights the importance of international treaties such as the Convention on Long Range Transboundary Air Pollution whose primary goal is to control and reduce the damage to human health and the environment caused by trans-boundary air pollution. Within this Convention, two protocols, one on persistent organic pollutants and one on heavy metals, were ratified by 31 countries and entered into force in 2003 (http://www.unece.org). However, it is important to notice that Asian countries, significant contributors to atmospheric contamination, did not take part in these regulations. The lack of participation of major emitting countries in these intergovernmental regulations might impede the large-scale efficiency of current treaties.

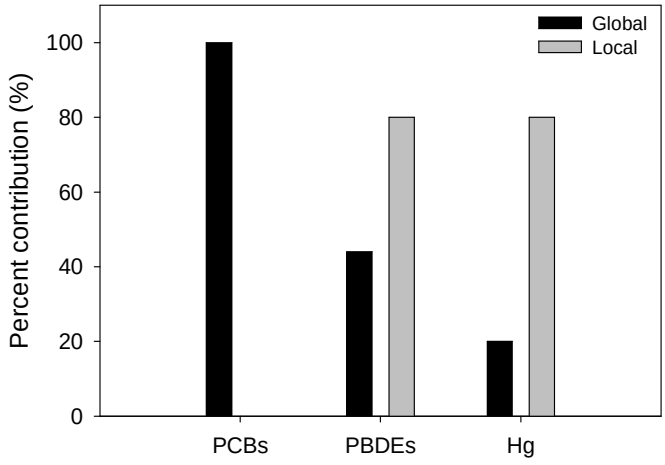


**Figure 18: While long range sources (trans-Pacific transport) are dominant for the legacy PCBs, local sources of PBDEs and Hg on the west coast of North America are significant. (Hg data are adapted from Seigneur et al., 2004; Strode et al., 2008)**

**Harbour seal investigation**

While atmospheric sampling shed some light on the relative importance of large-scale background *versus* local sources of contaminants in BC highlighting PBDE trans-Pacific transport, harbour seals (*Phoca vitulina*) can help determine local contaminant sources on a more regional scale. Indeed, these long-lived, high trophic level marine mammals are relatively non-migratory, feed on a wide variety of fish and invertebrate species therefore providing a signal of local food web contamination.

There are a number of studies on PCBs and PBDEs in the Northeastern Pacific harbour seals and their food web revealing spatial variations in contamination. For example, harbour seals from Washington State (WA), USA, have been reported to be seven times more PCB contaminated than the ones from BC (Ross et al., 2004, 2012).

In Chapter 2, we reported, for the first time, Hg levels in harbour seal pups collected from various sites along the BC / WA coast. Total Hg concentrations in pups ranged from 1.6 to 46.9 µg/g and revealed three hot spots for Hg (Queen Charlotte Strait, Port Renfrew, and Central Puget Sound). Our results suggested that a combination of anthropogenic sources and marine food web processes influence the delivery of MeHg to the top of this coastal marine food chain.

Together with previous POP results, our study confirmed that, on a regional scale, Puget Sound can be defined as an area of concern. Indeed, because of the important inputs from industrial and domestic activities and the physical properties of the basin such as lower sedimentation rate and higher flushing time than in the Strait of Georgia (Macdonald and Crecelius, 1994), harbour seals inhabiting these waters have elevated levels of PCBs, PBDEs, and Hg (Ross et al. 2004, 2012).

## 6.2 What risks do contaminants represent for the health of harbour seals inhabiting the industrialized BC/WA coast?

Wildlife toxicology is a challenging field. Using the actual species of interest is sometimes challenging and extrapolation from one species to another using comparative physiology is usually needed. Over the past decades, numerous studies on captive and wild harbour seals have increased our understanding of their behaviour, physiology, endocrinology, immunology, toxicology (Ross, 2000) and helped develop thresholds for health effects in marine mammals. They can therefore be considered the "laboratory rat" of the ocean.

Thresholds based on contaminant levels have been established to assess their potential for deleterious health effects on wildlife population and provide guidelines for conservation and risk management. Previous studies found that 100% of harbour seal pups sampled in WA had PCB levels that surpassed threshold for alterations of immune and endocrine functions (Ross et al., 2012). The results presented in chapter 3 revealed that 33% of harbour seal pups from WA had Hg levels exceeding the threshold for biochemical alterations in the brain.

In Chapter 4, we used genomics techniques to provide insight, at the molecular level, into the health of harbour seals from the west (BC and WA) and east (Quebec, Newfoundland) coasts of North America. We expanded the tool box previously used for this species from three genes (Mos et al., 2007; Tabuchi et al., 2006) to seven. Best-fit models revealed that the dominant POP (PCBs) was the main factor explaining variation of mRNA transcript profile in blubber and skin. However, while there were positive correlations between PCB concentrations and the mRNA levels of estrogen receptor alpha (*Esr1*: $r^2$=0.12, p=0.038), thyroid hormone receptor alpha (*Thra*: $r^2$=0.16; p=0.028), and glucocorticoid receptor (*Nr3c1*: $r^2$=0.12; p=0.049) in

blubber, negative relationships were observed for *Esr1* ($r^2$=0.21, p=0.021), *Nr3c1* ($r^2$=0.22, p=0.003) and heat shock protein 70 (*Hspa1*: $r^2$=0.39, p=0.000) in skin. The divergent results between blubber and skin might reflect different ability of various tissues to uptake and metabolize PCBs and highlight the need for caution when interpreting transcriptomics results. While the population-level consequences are unclear, these results suggested that PCB-associated alterations of the mRNA levels of these genes may lead to adverse effects on growth and development as well as deleterious consequences on metabolism and the immune and reproductive systems.

Approximately 53 000 harbour seals inhabit the Strait of Georgia, BC, and Puget Sound, WA, USA, and this estimate has been stable for the past several years suggesting a healthy population (Jeffries et al., 2003; Olesiuk, 2009). However, the changes observed here at the molecular level indicate that harbour seal physiology is affected by contaminant exposure which might weaken their ability to respond to other environmental stressors and / or diseases. Evidence from the past supports the fact that the highest risk for marine top predator populations occur when contaminant exposure and stress from other environmental parameters converge. For example, in the late 1980s, 20 000 harbour seals and several hundred grey seals (*Halichoerus grypus*) died in Northern Europe as a result of the introduction of a morbillivirus into these immunologically naïve populations. Many studies were undertaken leading to a "weight of evidence" suggesting that dioxin-like PCBs may have contributed to these mass mortalities by affecting immune functions (Ross, 2002) and the ability of these populations to respond to the virus.

### 6.3 What risks do contaminants represent for the health of beluga whales (*Delphinapterus leucas*) inhabiting the remote Arctic?

In the Arctic, local sources of contaminants are rare and long range sources are therefore the most important. Indeed, PCBs, PBDEs and Hg are efficiently delivered to the Arctic through long-range atmospheric transport. While ocean currents and river discharge represent 46% of total mercury input to the Western Arctic Ocean, atmospheric deposition accounts for 50% (Outridge et al., 2008). The long-lived, high trophic level beluga whales are inherently associated with the ice edge where they feed on a variety of prey species, including arctic cod (*Boreogadus saida*). While levels of contaminants in the Western Canadian Arctic beluga population have been monitored for decades, there are no published data on the health of these belugas.

In Chapter 5, we showed that the Beaufort Sea beluga whales may be responding to the exposure of PCBs with increased *Ahr* and *Cyp1A1* mRNA levels, two genes involved in detoxification. While this is consistent with what has been observed in highly contaminated marine mammals such as the northeastern Pacific killer whales (*Orcinus orca*) (Buckman et al., 2010), it was surprising and concerning to detect significant response to PCBs in this population of beluga exhibiting PCB levels an order of magnitude lower than those measured in their St Lawrence counterparts (Hobbs et al., 2003).

It also appeared that contaminants alone did not explain the variations observed in gene expression profiles. Higher mRNA levels of genes involved in growth, metabolism and development were observed in years where whales exhibited low $\delta^{13}C$ (2008 and 2010) and these changes were coincident with low sea ice years. Our results therefore suggested that sea ice-associated changes in diet might have an impact on beluga physiology impacting important genes.

Recently, studies on polar bears (*Ursus maritimus*) and ringed seals (*Phoca hispida*) reported an association between the annual sea ice break up date and changes in feeding ecology leading to changes in contaminant burdens (McKinney et al., 2009; Gaden et al., 2012). For the first time we suggested that, in addition to changes in feeding ecology and contaminant load, climate change, and in particular decrease in sea ice extent, might impact beluga health at the molecular level. Such findings raise important questions about the potential exacerbation of toxic risks due to POPs as a consequence of large scale climate changes currently underway in the Arctic.

Beluga whales have been an important part of the traditional diet of most communities in the Inuvialuit Settlement Region along the Beaufort Sea coast for hundreds of years. Any changes not only to the health of the beluga population but also to the contaminant loads carried by these belugas might represent a concern for the health of Inuvialuit communities. Other studies have been evaluating human health risks associated with the consumption of traditional foods in northern communities (Van Oostdam et al., 2005).

### 6.4 What does the future hold?

The marine environment is under various anthropogenic-related stressors including contaminants, loss of natural habitat, oil and gas exploration and climate change. Warming of the climate is undeniable as evidenced by increased average air and ocean temperatures, rising sea level and widespread melting of snow and ice (Figure 19; ipcc, 2007). While the magnitude of climate-associated changes is regionally variable, changes are occurring on a global scale.

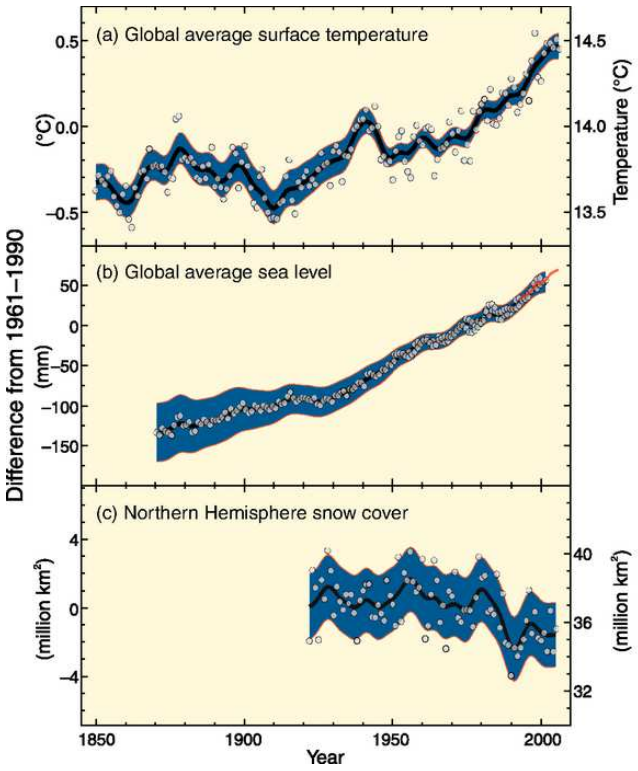


**Figure 19: Observed changes in global surface temperature (a), global average sea level (b) and Northern Hemisphere snow cover (c) (From www.ipcc.ch, 2007).**

In southern BC, ocean warming of 0.5 to 1$^{o}$C has been observed during the last 50 years (Environment Canada, 2006). In their study, Johanessen and Macdonald (2009) projected alterations in geochemical cycles (inorganic / organic carbon, nutrients), decreased pH and oxygen concentrations for the Strait of Georgia. They also reported decreased and earlier peaks for zooplankton species potentially affecting marine food webs upon which harbour seal depends.

In the Arctic, changes are occurring faster than anywhere else (Jones and Moberg, 2003). On top of the global loss of sea ice over the past few decades (12.4% over the past decade (Stroeve et al., 2012), the fraction of thin first-year ice increased from 38% of the total ice cover in the mid-1980s to 64% in the spring 2010 with a peak to 72% in the spring 2008 (Stroeve et al., 2012). As sea ice extent is decreasing, the amount of solar irradiance penetrating the water column is increasing. Throughout the

record low 2007 melt season, Perovich et al. (2008) estimated anomalies of 500% in solar heat input to the Beaufort and Chukchi Sea upper ocean compared to 1979 – 2005 averages. Such increases in light availability associated with increased nutrient supply from wind mixing and shelf upwelling have the potential to impact primary production therefore impacting organisms higher up in the food chain.

It is therefore clear that, regardless of location, changes are occurring within marine ecosystems leading to structural changes in food web species composition. Climate change might therefore have direct effects associated with the loss of sea ice (or habitat), changes in air and water temperatures but also indirect effects related to alterations of pathogens transmission, changes in food availability and / or quality and changes in contaminant exposure (Burek et al., 2008; Kovacs et al., 2011). Such alterations would, in turn, affect the health of marine top predators (Ross, 2002)(Figure 20).

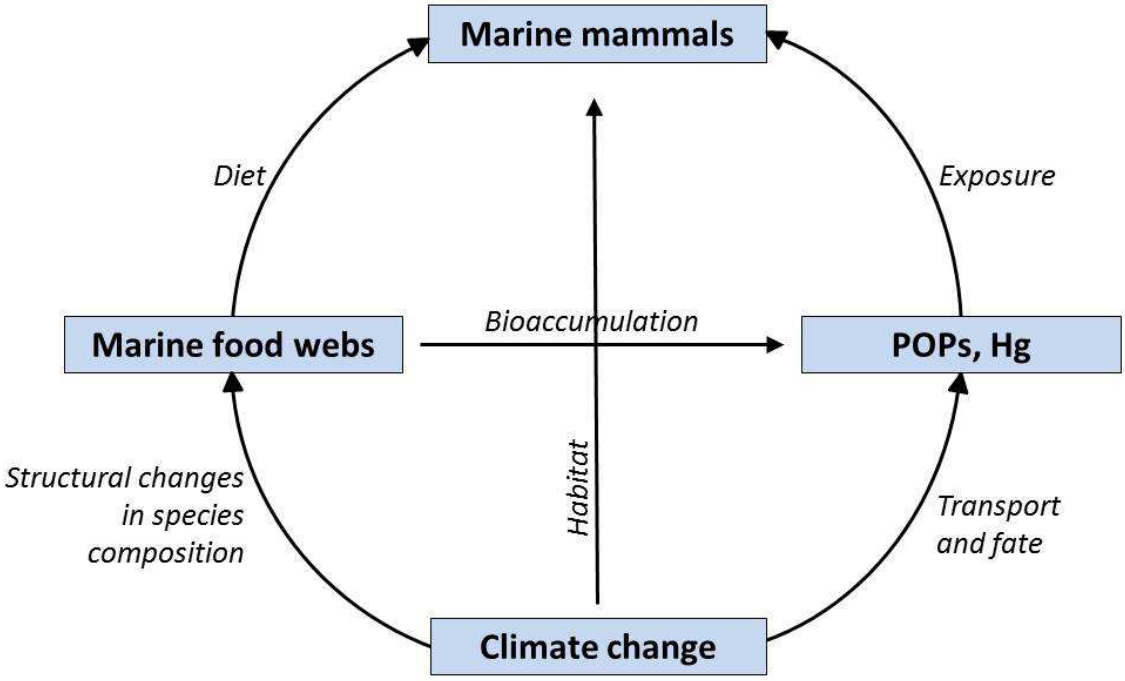


**Figure 20: While climate change can impact marine mammals directly through habitat change and/or loss, it can also have indirect impacts by affecting marine food webs and transport and fate of contaminants.**

Recently, a decline in sea ice has been linked to nutritionally stressed polar bears resulting in lower reproductive success and reduced body size (Regehr et al., 2010; Rode et al., 2010). On the other hand, a longer feeding season due to a decrease in sea ice was linked to an improvement of the body condition of the West Greenland harbour porpoises (*Phocoena phocoena*) (Heide-Jorgensen et al., 2012). The results presented in Chapter 5 provided preliminary evidence that sea ice-associated changes in diet might induce changes in beluga physiology affecting mRNA levels of genes involved in growth, development and metabolism.

While knowledge on POPs and Hg considerably increased during the past 50 years, the large-scale climate changes currently occurring add another level of complexity when trying to understand the transport and fate of these compounds (Macdonald et al., 2003), their behaviour in marine food webs and their potential impacts on marine mammal health.

The present thesis showed that while local sources are important to take into account when it comes to currently in use compounds such as PBDEs and Hg, global dispersion is a significant factor affecting the distribution of legacy contaminants (PCBs) and affecting contamination of remote location. While efficient regulations have successfully resulted in the decrease of PCBs in the environment, results presented in chapter 4 and 5 confirmed that it remains number one in terms of potential threats for the health of marine mammals (Mos et al., 2010). Finally, we highlighted the importance of integrating climate and contaminant research in order to better understand their potential combined impacts on marine food webs and marine mammal health.

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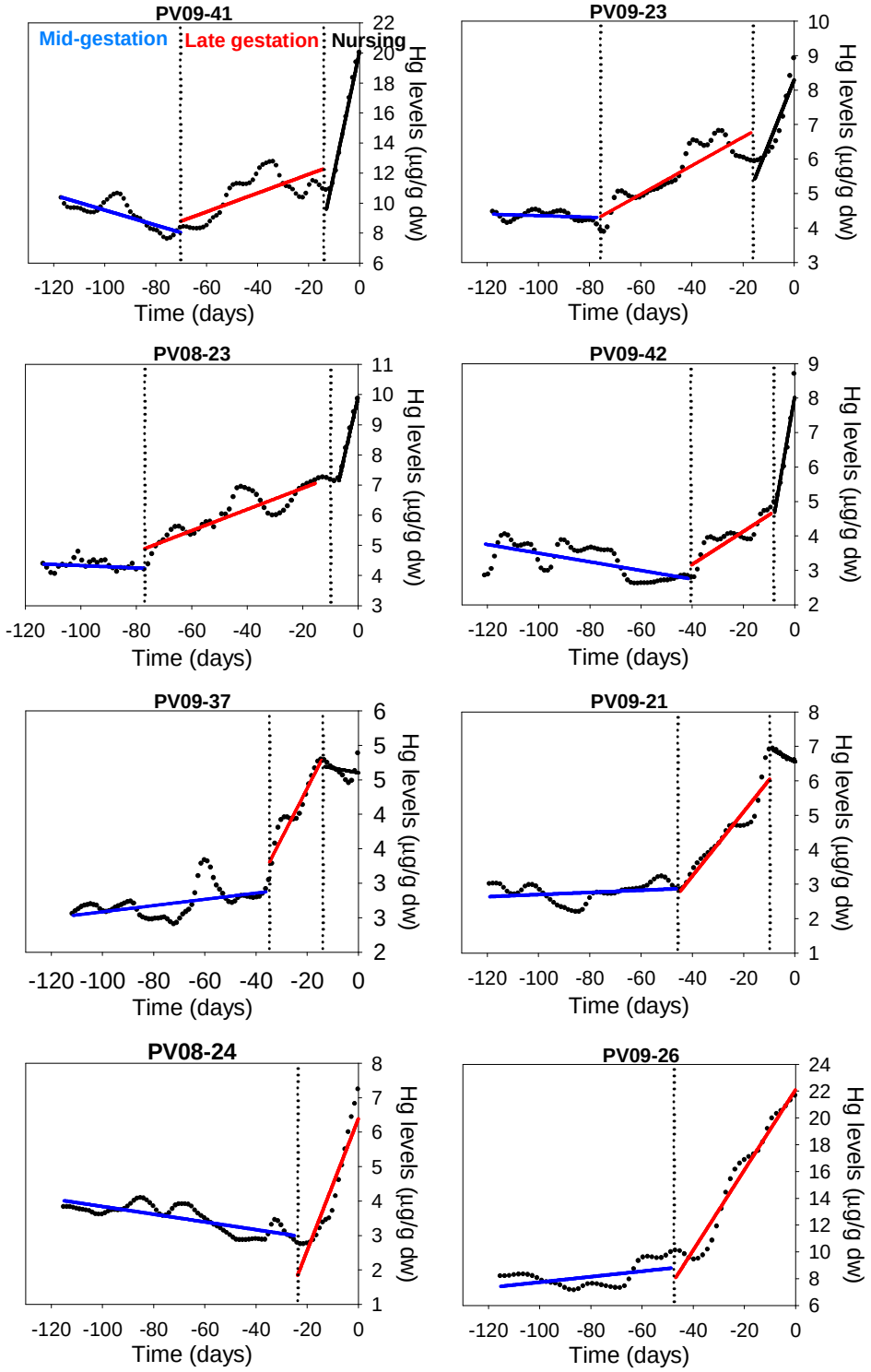
# Appendix

**Appendix 1: Changes in Hg levels along individual seal pup whiskers. Trends during mid-gestation (blue line), late gestation (red line) and lactation (black line) are presented.**



**Appendix 2: Gene-specific primers for QPCR analysis of mRNA abundance in harbour seal tissues.**

| Gene Transcript | Genbank Accession Number | Primer Name | Primer Sequence | Amplicon Size (bp) | ΔΔCt Criteria[a] | |
|---|---|---|---|---|---|---|
| | | | | | Blubber | Skin |
| *Rpl8*[b] | In progress | UL8up | GGTGTGGCTATGAATCCTGT | 126 | n/a | n/a |
| | | L8-2dn | ACGACGAGCAGCAATAAGAC | | | |
| *Gapdh* | | ORQ15u | ATCCCGCCAACATCAAAT | 493 | 0.14 | 0.2[c] |
| | | ORQ15d | AGATCCACGACGGACACG | | | |
| *Actb* | | ORQ16u | CCTGGACTTCGAGCAGGAG | 236 | 0.04 | 0.11 |
| | | ORQ16d | GCACCGTGTTGGCATAGAG | | | |
| *Esr1* | | ORQ2up | CCGAGCCCACTCTTGATT | 213 | 0.04 | 0.03 |
| | | ORQ2dn | CCTCTTTGCCCAGTTGAT | | | |
| *Ahr* | | HIQ3up | ACCCACTGCTTGTGATGC | 308 | 0.49[c] | 0.03 |
| | | HIQ3dn | TTCGCTTTCGTAAATGYTCT | | | |
| *Hspa1* | | ORQ7up | ATGACGCGGGACAACAAC | 392 | 0.13 | 0.09 |
| | | ORQ7dn | GAAATCACCTCCTGGCACTT | | | |
| *Nr1c3* | | ORQ11u | GCCTTCCAACTCCCTCAT | 398 | 0.02 | 0.02 |
| | | ORQ11d | TCGCCTTTGCTTTCGTCA | | | |
| *Nr3c1* | | ORQ12u | GCCCAGTTTATTGTCAGG | 139 | 0.01 | 0.03 |
| | | ORQ12d | TGTTGAGAAAGGGATGCT | | | |
| *Nr1l1* | | ORQ13u | GCGTTCCAACCAGTCCTT | 294 | 0.62[c] | 0.04 |

| | | | | | |
|---|---|---|---|---|---|
| | ORQ13d | TGGCAGCAGCGGATGTAG | | | |
| *Thra* | PV19 | CGACGGAAGGAGGAAATG | 231 | 0.11 | 0.12 |
| | PV20 | GATCTTGGTAAACTCGCTGAA | | | |

[a]The difference of test minus *Rpl8* Ct is plotted against $\log_2$ cDNA concentration (2-fold cDNA dilution series). Slope absolute values less than or equal to 0.1 were considered suitable for gene-specific QPCR analysis within a given tissue type; n/a, not applicable.

[b]Transcript abundance of *Rpl8* was used as the comparator in relative primer efficiency calculations.

[c]Slope values did not satisfy ΔΔCt criteria and the indicated primer sets were not used in that tissue.

**Appendix 3: QPCR primers used for assessment of mRNA abundance in beluga whale (*Delphinapterus leucas*).**

| Gene Transcript | Abbreviation | NCBI Genbank Accession No. | Primer Name | Primer Sequence | DNA Amplicon Size (bp) |
|---|---|---|---|---|---|
| Ribosomal protein L8 | *Rpl8* | KC145562 | ORQ14 up | GCGGACGGAGTTGTTCAT | 219 |
| | | | ORQ14 dn | TTTGTCTCAGGGTTGTGGG | |
| Glyceraldehyde3-phosphate dehydrogenase | *Gapdh* | KC145563 | ORQ15 up | ATCCCGCCAACATCAAAT | 493 |
| | | | ORQ15 dn | AGATCCACGACGGACACG | |
| Cytoplasmic β Actin | *Actb* | KC145564 | ORQ16 up | CCTGGACTTCGAGCAGGAG | 236 |
| | | | ORQ16 dn | GCACCGTGTTGGCATAGAG | |
| Thyroid hormone receptor α | *Thra* | KC145565 | ORQ17 up | GCCCATCGCAGCACAAAT | 317 |
| | | | ORQ17 dn | TCCCCGCTCAGTGTCAGG | |
| Thyroid hormone receptor β | *Thrb* | KC145566 | ORQ18 up | AGATCCATCGGTCACAAG | 170 |
| | | | ORQ18 dn | CCACCTTCTGGGGCGTTT | |
| Estrogen receptor α | *Esr1* | NS | ORQ2 up | CCGAGCCCACTCTTGATT | 213 |
| | | | ORQ2 dn | CCTCTTTGCCCAGTTGAT | |
| Vitamin D receptor | *Nr1l1* | KC145561 | ORQ13 up | GCGTTCCAACCAGTCCTT | 294 |
| | | | ORQ13 dn | TGGCAGCAGCGGATGTAG | |
| Aryl hydrocarbon receptor | *Ahr* | KC145555 | ORQ3 up | TCGAATGCACGCTTAGTT | 241 |
| | | | ORQ3 dn | TTGCCTTGGTAGCAGAAT | |
| Cytochrome P450 1a1 | *Cyp1a1* | KC145556 | ORQ6A | ACAGCCTGATTGAGCACT | 293 |
| | | | ORQ6A | AAAGAGGAATGTCGGAAG | |
| Glucocorticoid receptor | *Nr3c1* | KC145560 | ORQ12 up | GCCCAGTTTATTGTCAGG | 139 |

| | | | | | |
|---|---|---|---|---|---|
| | | | ORQ12 dn | TGTTGAGAAAGGGATGCT | |
| Metallothionein 1 | *Mt1* | NS | ORQ5 up | ATGGACCCCAACTGCTCC | 153 |
| | | | ORQ5 dn | TTTGCAGACGCAGCCCTG | |
| Heat shock 70kDa protein 1-like | *Hspa1l* | KC145557 | LEQ7 up | ACAGGCAAGGCTAACAAG | 155 |
| | | | LEQ7 dn | GCATAGGATTCTAAGGCATTT | |
| Peroxisome proliferator-activated receptor | *Nr1c3* | KC145559 | ORQ11 up | GCCTTCCAACTCCCTCAT | 398 |
| | | | ORQ11 dn | TCGCCTTTGCTTTCGTCA | |
| Adiponectin | *Adipoq* | KC145569 | ORQ21 up | ATTCCCATTCGCTTTACC | 227 |
| | | | ORQ21 dn | AGGAGCACAGAGCCAGAG | |
| Leptin | *Lep* | KC145570 | ORQ22 up | AGTCCAGGATGACACCAA | 104 |
| | | | ORQ22 dn | CCAAACCAGTGACCCTCT | |
| Insulin-like growth factor 1 | *Igf1* | KC145572 | ORQ25 up | TTTATTTCAACAAGCCCACG | 112 |
| | | | ORQ25 dn | TACATCTCCAGCCTCCTCA | |
| Retinoid X receptor α | *Rxra* | KC145568 | ORQ20 up | GCTGGTGTCCAAGRTGCG | 249 |
| | | | ORQ20 dn | TGTTCCAGGCACTTGAGGC | |

**Appendix 4: Isolated *Delphinapterus leucas* expressed gene sequences not submitted to NCBI GenBank.**

| Gene Transcript | Cloning PCR Primers and Plasmid Clones | cDNA Sequence | Conceptual Protein Sequence |
|---|---|---|---|
| estrogen receptor alpha (*Esr1*) | ORQ2up; 5'-CCGAGCCCACTCTTGATT-3'<br>ORQ2dn; 5'-CCTCTTTGCCCAGTTGAT-3'<br>Clone; LEU2.1 | Base pairs; 177<br>AAACACACTAAGAAGAACAGC<br>CCTGTCTTGTCCCTGACAGCCG<br>ATCAGATGATCAGTGCCTTGCT<br>GGAGGCTGAGCCCCCCATAATC<br>TACTCCGAATACGATCCTACCA<br>GACCCTTCAGTGAGGCCTCAAT<br>GATGGGCTTGCTGACCAGCCTT<br>GCAGACAGGGAGCTGGTCCAC<br>ATG | Frame; +1<br>KHTKKNSPVLSLTADQMI<br>SALLEAEPPIIYSEYDPTRP<br>FSEASMMGLLTSLADREL<br>VHM |
| Metallothionein 1 (*Mt1*) | IOS9; 5'-ATGGACCCCAACTGCTCC-3'<br>IOS10; 5'-TTTGCAGAYGCAGCCCTG-3'<br>Clone; LEU5.1 | Base pairs; 117<br>TGCACCGCAGGCGGATCCTGCA<br>CGTGTGCCGGCTCCTGCAAATG<br>CAAAGACTGCAAATGCACCTCC<br>TGCAAGAAGAGCTGCTGCTCCT<br>GCTGCCCTCCGGGCTGCGCCAA<br>GTGTGCC | Frame; +2<br>APQADPARVPAPANAKT<br>ANAPPARRAAAPAALRA<br>APSV |

**Appendix 5: Pearson correlation analysis of mRNA abundance values obtained from inner and outer blubber samples from beluga whales.**

| Gene Transcript | Pearson coefficent |
|---|---|
| *Thra* | 0.51** |
| *Thrb* | 0.33* |
| *Esr1* | 0.21* |
| *Ahr* | 0.12* |
| *Cyp1a1* | NA |
| *Nr3c1* | 0.43** |
| *Mt1* | 0.24** |
| *Hspa1l* | NA |
| *Pparg* | 0.29* |
| *Adipoq* | 0.10* |
| *Lep* | 0.48** |
| *Igf1* | 0.66** |
| *Rxra* | 0.45** |

Significance is noted by '**' for $p<0.05$ and '*'for $p<0.10$. NA= non applicable because mRNA transcripts were not quantifiable in the outer blubber.

**Appendix 6: Percent sea ice coverage for the month of June in the Mackenzie River delta during our three sampling years revealed lower sea ice extent in 2008 and 2010 compared to 2009 (Data from Canadian Ice Services; http://www.ec.gc.ca/glaces-ice).**