EFFECTS OF CONTAMINANTS AND CLIMATE CHANGE ON THE HEALTH OF WESTERN ARCTIC BELUGA WHALES (*DELPHINAPTERUS LEUCAS*)

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

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Abstract

The Arctic is currently undergoing a period of transformation brought on primarily by climate change. Climate-induced changes in sea ice have led to changes in primary productivity, prey abundance and distribution, and habitat availability. There are concerns that the Arctic could be ice free by 2040, meaning that there is urgency in determining the impacts that climate change will have on the organisms that inhabit the Arctic. Climate change, however, is not the only pressure that marine organisms are facing. Contaminants, such as polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and mercury (Hg) have been identified as contaminants of concern to wildlife in the Arctic. Little is known about the interaction between contaminants and climate change, but concerns have arisen that the two stressors may exacerbate each other. The goal of this thesis is to add to our current knowledge of the impacts of contaminants and climate change on the health of western Arctic beluga whales and to explore the potential interaction between the two stressors. I examined the mRNA expression of 12 genes involved in detoxification of xenobiotics, nutritional stress and metabolism as a proxy for beluga whale health. The relationship between mRNA expression and contaminant burdens, stable isotopes and fatty acid signatures, sea ice levels and body condition metrics were examined in liver and blubber samples taken between 2008 to 2017. A principal component analysis on gene expression resulted in factor 1 explaining 78% percent of the variance for blubber and 90% of the variance for liver. Factor 1 was found to be significantly related to $\delta^{13}C$ in blubber. Analysis of fatty acid profiles using PCA revealed inter-annual clustering of the year with the highest sea ice extent as well as the year with the lowest sea ice extent. Analysis of individual genes revealed that sea ice extent, fatty acids, length, $\delta^{13}C$ and Hg may contribute most to the overall variation in gene expression. Results suggest that physiology of Beaufort Sea beluga whales are affected

by a combination of climate-induced changes in foraging patterns and environmental contaminants.

Lay Summary

The Arctic is currently undergoing a period of rapid transformation brought on by climate change. Large scale changes in sea ice have put stress on animals who rely on the ice for foraging, habitat and reproduction. Animals are also exposed to contaminants, which have been demonstrated to impact the health of many marine organisms, including beluga whales. However, little is known about the potential for these two stressors to interact. In this thesis, I aim to explore the interaction between climate change and contaminants on beluga whale health. I measured the expression of genes that play roles in nutritional health, contaminant detoxification and stress and found that these were related to changes in sea ice, diet and contaminants. My data indicate that there is a relationship between sea ice, contaminant levels and gene expression, suggesting that these factors may be impacting the health of western Arctic beluga whales.

Preface

This project was designed together with Dr. Marie Noël based on her work on beluga whales in the western Arctic. Input on the design was provided by Dr. Peter Ross and Dr. Lisa Loseto.

I wrote all sections of this thesis, with input from my supervisors and supervisory committee. I performed collection of qPCR data, with some assistance from Thomas Dong, a laboratory technician. Guidance and technical support in the lab were given by Dr. Marie Noël. I performed all statistical analysis for this thesis.

Data, including biopsy samples, contaminants data, body measurements, stable isotopes and fatty acids, were collected by third parties. All third parties are acknowledged throughout the methods section of this thesis.

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List of Abbreviations

Acat2 – acetyl coA acetyltransferase 2

Acox1 – acyl coenzyme A oxidase 1

AhR – aryl hydrocarbon receptor

AIC – Akaike information criterion

ATP – adenosine triphosphate

CRP – C-reactive protein

Ct – cycle threshold

Cyp1A1 – cytochrome P450 1A1

DDT - dichlorodiphenyltrichloroethane

DI1 – deiodinase 1

DI2 – deiodinase 2

$\delta^{13}C$ – carbon isotope 13

$\delta^{15}N$ – nitrogen isotope 15

ERα – estrogen receptor alpha

ETC – electron transport chain

Hg - mercury

HP - haptoglobin

Hsp70 – heat shock protein 70

HspA1A – heat shock protein 70 A1A

ILGF1 - insulin-like growth factor 1

MeHg – methyl mercury

NTC – no-template control

PBDE – polybrominated diphenyl ether

PC – positive control

PCA – principal components analysis

PCB – polychlorinated biphenyl

POP – persistent organic pollutant

PPARα – peroxisome proliferator-activated receptor alpha

QA/QC – quality assurance/quality control

ROS – reactive oxygen species

rpL8 – ribosomal protein L8

SD – standard deviation

SIRT2 – sirtuin 2

T3 - triiodothyronine

T4 - thyroxine

TNFα – tumour necrosis factor alpha

TRβ – thyroid receptor beta

UCP2 – uncoupling protein 2

β-actin – beta actin

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Dedication

I dedicate this to my family. Thank you.

Chapter 1: Introduction

1.1 Background

The Arctic environment, located 66.5^{o} north of the equator, is experiencing a period of rapid transformation, brought on primarily through climate change. The Arctic is currently warming at a rate of approximately 0.6^{o}C per decade, which is three times faster than the global average rate (Comiso & Hall, 2014). This rate of warming, combined with factors such as increases in storm surges and decreased albedo, has led to unprecedented rates of sea ice change, including reductions in sea ice thickness and annual sea ice extent (Grannas et al., 2013). The loss of sea ice has the potential to impact the entire Arctic ecosystem, and changes in species abundance and distribution have already been observed at all trophic levels (Loseto et al., 2015; Moore & Reeves, 2018; Moore et al., 2012; Tai et al., 2019).

Earlier sea ice break ups and overall reductions in annual sea ice cover have led to altered timing of phytoplankton blooms, as well as increases in overall primary production (Alava et al., 2017; Reeves et al., 2013). This has direct implications for foraging and distribution of high trophic-level consumers. Warming waters are also expected to cause changes in species makeup, as southern prey species are expected to migrate northward to cooler waters (Tai et al., 2019). In addition to influencing food web dynamics, changes in sea ice are expected to severely impact animals that rely on sea ice for habitat, including walruses and seals that use sea ice for hauling-out and breeding and polar bears that rely on sea ice for hunting (Moore & Reeves, 2018). While it is clear that these animals may be facing impending consequences from reductions in sea ice, the consequences of reduced sea ice extent are less clear for ice-edge associated animals such as narwhals, bowheads and beluga whales that use sea ice for protection from predators, foraging

and navigation (Hauser et al., 2014; Loseto et al., 2015; Moore & Reeves, 2018). There are concerns that the Arctic may experience its first ice-free summer by 2040, and as a result it is critically important to determine the potential risks posed by climate change to marine organisms (Hornby et al., 2016).

The implications of climate change on the Arctic environment alone may be cause for concern, but perhaps even more concerning is that this is not the only pressure this ecosystem is facing. The Arctic is often considered a pristine ecosystem, with a relative lack of anthropogenic activities. However, the lack of industrialization and direct pollution into the Arctic environment does not mean that the Arctic is immune to contaminant exposure. Winds and oceanic currents bring contaminants to the Arctic from more polluted areas of the planet, including Europe, North America and Asia (Noël et al., 2014). Of particular concern are persistent organic pollutants (POPs), such as polychlorinated biphenyls and polybrominated diphenyl ethers (PDBEs), as well as contaminants such as methyl mercury (MeHg), which have been identified as contaminants of concern in the Arctic (Noël et al., 2014). Each of these contaminants has been shown to bioaccumulate in marine mammals, potentially posing a risk to their health (Frouin et al., 2012; Grannas et al., 2013; Loseto et al., 2008; Noël et al., 2014). Contaminant-related health impacts, including changes in mRNA expression, neurochemical markers, hormone concentrations and immunity, have been noted in many Arctic marine mammals such as ringed seals, grey seals, polar bears, and beluga whales (Braathen et al., 2004; Brown et al., 2014; Haave et al., 2003; Levin et al., 2016; Ostertag et al., 2014; Troisi et al., 2020).

As multiple stressors are at play in the Arctic, it is important that we start to look at the combined impact of these stressors on organism health to understand the influence that they may have on marine life. For example, sea ice previously acted as a sink for contaminants in the Arctic, effectively storing contaminants by trapping them in frozen ice. Increases in sea ice melt have led to these stores being released into the Arctic environment (Alava et al., 2017; Grannas et al., 2013). Increases in precipitation are also expected due to climate change, which will increase the intensity of contaminant deposition from the atmosphere (Grannas et al., 2013). Further, prey species are expected to migrate north as the climate warms, and these animals may act as a vector to deliver contaminants from more polluted areas of the globe to the Arctic (Noël et al., 2018; Tai et al., 2019). This phenomenon has already been demonstrated in polar bears, whose change in dietary preference has been associated with increased POP concentrations (McKinney et al., 2013).

Recently, a study looking at the impact of contaminants on beluga whale health found that the mRNA transcript abundance of two genes involved in xenobiotic detoxification, the aryl hydrocarbon receptor (AhR) and cytochrome P450 1A1 (Cyp1A1), were, as expected, correlated with concentrations of PCBs (Noël et al., 2014). Noël et al. (2014) also revealed a surprising finding, whereby genes involved in growth, development and metabolism had increased expression during years with low sea ice extent (Noël et al., 2014). These observations suggest that both contaminants and climate change may be impacting the health of beluga whales. However, these observations were made over only three years (2008 to 2010), so it is not currently possible to draw firm conclusions about the relationship between contaminants and climate change and their influence on the health of beluga whales. In order to more completely

understand this interaction, it is important to extend these observations over a longer time period to adequately assess whether sea ice extent plays a role in gene expression. To this end, this thesis aims to extend the observations that both contaminants and climate change may be impacting the health of beluga whales by studying gene expression in beluga whale tissues from 2008 to 2017 and including additional genes in the analysis that may provide greater insight into the health and nutritional stress of beluga whales in the Arctic.

1.2 Beaufort Sea beluga whales

Beluga whales are a circumpolar, primarily Arctic species, many of which reside in Canadian waters (Choy et al., 2017; Ostertag et al., 2018). They are an important part of the Arctic food web, playing the role of a top predator, and are an important food source for many people who rely on the Arctic Ocean for food. The Beaufort Sea beluga stock is one of the world's largest, with a population of almost 40 000 individuals estimated in 1992 (Allen & Angliss, 2013; Harwood et al., 1996) and although no current population census data are available, the population is considered to be stable (COSEWIC, 2004). This population is migratory and overwinters in the Bering Sea, just outside of the ice-covered Arctic basin (Choy et al., 2017; Hornby et al., 2016; Ostertag et al., 2018). In the spring, the seasonal sea ice starts to melt, allowing a path to open up for the beluga whales to migrate through the Arctic basin to their summer home in the Mackenzie River estuary (Choy et al., 2017; Hornby et al., 2016; Ostertag et al., 2018; Stafford et al., 2018). The sea ice typically reaches its minimum in September and around this time beluga whales start to return towards their wintering grounds before the sea ice starts to build back up in October (Hauser et al., 2016). Climate change may enable the earlier migration of Beaufort Sea beluga whales from their wintering home to their summer home, as

the ice break-up of the Arctic basin has shifted to earlier timing, allowing beluga whales to pass through earlier in the year (Hauser et al., 2016; Hornby et al., 2016).

Beluga whales typically inhabit open water areas close to the ice edge (Hornby et al., 2016). Areas near the ice edge provide important refuge from aquatic predators for small beluga whales and mothers with calves and have an added benefit of attracting high prey densities (Breton-Honeyman et al., 2016; Hornby et al., 2016). Large male beluga whales may venture further from the ice edge into the open water to search for new feeding grounds; however, they will remain near the ice edge when possible (Hauser et al., 2014; Hornby et al., 2016; Loseto et al., 2015). Changes in sea ice may dramatically alter the current habitat selection for beluga whales. For instance, the safety of the ice edge may be pushed further offshore in some areas, making a safe journey to and from their summering grounds more difficult (Breton-Honeyman et al., 2016; Hornby et al., 2016; Loseto et al., 2015). Prey, which aggregate near the ice edge, may also be pushed further offshore (Hornby et al., 2016; Stafford et al., 2018). To make matters worse, predators and competitors have been sighted more frequently in the eastern Arctic, where Beaufort beluga whales reside in the winter, since the reduction in sea ice cover, as they are no longer inhibited from entering the Arctic by their dorsal fins and the cost of thermoregulation is not as high in warmer waters (Clarke et al., 2013; Ferguson et al., 2012; Reeves et al., 2013).

Beluga whales are considered generalist feeders and feed primarily on Arctic cod, as well as Greenland halibut, pacific herring, rainbow smelt, Arctic cisco, saffron cod, salmon, octopus, shrimp and polychaetes (Hornby et al., 2016; Loseto et al., 2009; Loseto et al., 2008). Warming waters are expected to change the abundance of many of these prey species, and changes in sea-

ice may dramatically alter the prey's distribution. However, since beluga whales are generalist feeders, there is hope that they can switch to new prey sources. As waters warm, prey species are expected to migrate northward, and southern species, such as capelin, Atlantic cod and Northern prawns are expected to become abundant (Tai et al., 2019). While this could be seen as beneficial, some concerns come with the increase in non-Arctic species. These species are considered commercially valuable, and fishing may increase in the Arctic (Tai et al., 2019). In addition, a reduction in sea ice cover may open up the fishing season so boats can fish year-round. This could impact beluga whales by leading to a decrease in prey availability as well as an increase in the potential for ship strikes and underwater noise (Niemi et al., 2012; Tai et al., 2019).

1.3 Contaminants

For marine mammals, contaminants that are of most concern are persistent organic pollutants (POPs). Contaminants can be classified as POPs based on three main criteria. First, to be classified as a POP a contaminant must have a long half-life in the marine environment, meaning that it will persist for a long time and therefore is likely to interact with marine life. Second, POPs must be able to bioaccumulate, which typically is common in lipophilic contaminants that accumulate in fat deposits of marine organisms. This is particularly troublesome for marine mammals who have large blubber reserves and are at the top of the food chain. Lastly, POPs must be toxic to marine life.

In the Arctic environment, the most prevalent POPs are PCBs and PBDEs (Allen & Angliss, 2013; Choy et al., 2017; Noël et al., 2014). PCBs and PBDEs represent legacy contaminants and

are no longer in use industrially, however their presence is ubiquitous in the marine environment. Both compounds are similar in structure, containing two phenol rings, however PCBs contain chlorine where PBDEs contain bromine and an ether group. While their structures are similar, PBDEs are generally considered to be less toxic to organisms than PCBs. Planar compounds are typically the most bioavailable, as they mimic natural hormones (Rahman et al., 2001). While not all PCBs are planar, the small atomic size of chlorine allows even non-planar compounds to twist into planar compounds, allowing them to bind to several hormone receptors. In contrast, the increased atomic size of bromine inhibits the ability of PBDEs to twist from non-planar to planar, effectively reducing their bioavailability in comparison to PCBs (Rahman et al., 2001). A study on bioaccumulation of PCBs and PBDEs in the Arctic food web found that trophic magnification factors of PCBs ranged from 2.9 to 11, indicating that PCBs bioaccumulate, whereas PBDEs had trophic magnification factors ranging from 0.7 to 1.6, indicating negligible bioaccumulation (trophic magnification factors over 1 indicate biomagnification; trophic magnification factors under 1 indicate biominification; Kelly et al., 2008).

A third contaminant of concern is mercury, which represents a non-organic contaminant of concern in the Arctic; however, it can still bioaccumulate in the form of methyl mercury (MeHg) and it is toxic at high concentrations (ATSDR, 1999; Frouin et al., 2012; Loseto et al., 2015) (Appendix A, Table A4).

1.3.1 Polychlorinated biphenyls (PCBs)

PCBs are highly lipophilic contaminants, which accumulate in lipid-rich tissues such as blubber (Erickson & Kaley, 2010). PCBs were first described over a century ago and were used

commercially from the 1930s onward (Erickson & Kaley, 2010). PCBs were prized for their fire-resistant properties and for their ability to provide chemical resistance and durability in various products (Erickson & Kaley, 2010; Provost et al., 2007). The primary producer of PCBs was Monsanto in the United States, who marketed PCBs for use in flame retardants, hydraulic fluids, plasticizers, paint additives, adhesives, sealants, neoprene and numerous other products (Erickson & Kaley, 2010).

PCBs were first discovered in wildlife in 1968 by Risebrough et al. (1968), which is when the world was awakening to the dangers of human-made contaminants following the release of Rachel Carson's book, *Silent Spring*, in 1962, which implicated DDT (dichloro-diphenyl-trichloroethane) in the decline of many bird species. Risebrough et al. (1968) noted that DDTs were not the only hydrocarbon of concern and pointed to the evidence that bird eggs with thin shells also had high amounts of PCBs (Risebrough et al., 1968). Even in these early stages of research, Risebrough et al. (1968) raised concern over the potential atmospheric transport of PCBs to less-contaminated areas of the globe, especially through incineration of products containing PCBs.

Shortly after Risebrough's article was published (1968), and with evidence accumulating on the dangers of PCBs to wildlife, Monsanto began to issue warnings to their customers and began to restrict the use of PCBs. In 1970, PCBs were only to be used in closed systems where it was unlikely that they would be released into the environment. In 1977, Monsanto voluntarily ceased production of PCBs and many other companies followed suit (Erickson & Kaley, 2010). By the early 1980s, most companies had stopped manufacturing PCBs. In 2004, a global treaty termed

‘The Stockholm Convention’, based on a 2001 meeting, was put into force with the goal of protecting human health and the environment through addressing the problem of persistent organic pollutants (POPs), including PCBs (UNEP, 2016).

Unfortunately, while the production of PCBs may have ceased, PCBs are persistent in the environment. What originally made PCBs so desirable for use in manufactured goods was their resistance to chemical degradation, thermal degradation and biodegradation (Erickson & Kaley, 2010; Provost et al., 2007), but these are precisely the properties that make PCBs so persistent in the environment. Further, PCBs are still leaching into the environment from products that were manufactured before the 1980s. Landfills are considered to be a major source of PCBs, as many products that contained PCBs were simply thrown out and not properly disposed of. Buildings, bridges and ships still contain vintage paints containing PCBs. When these buildings are dismantled or paints are removed, PCBs are released into the environment (Stuart-Smith & Jepson, 2017). Many studies on the concentrations of PCBs in marine mammals over time have indicated that PCB concentrations in marine mammals have stabilized (Borrell et al., 2010; Hoguet et al., 2013; Isobe et al., 2009; Law et al., 2014; Raach et al., 2011), with very few studies indicating a decline in PCBs following elimination from the market (Law et al., 2010; Ross et al., 2013) reflecting the fact that PCBs are highly persistent in the marine environment.

PCBs have now been found in virtually all areas of the globe, including the relatively pristine Arctic (Noël et al., 2014). In fact, the early paper by Risebrough et al. (1968) noted high concentrations of PCBs in Arctic peregrines. PCBs arrive to the Arctic primarily through atmospheric deposition, with some input from oceanic currents and migratory species (Choy et

al., 2017). These contaminants have been demonstrated to impact marine mammals, including beluga whales, and have been noted as a key contaminant of concern. Indeed, PCBs have been implicated in the altered mRNA expression of a wide variety of genes involved in processes such as cellular metabolism, growth, metabolism and detoxification in ringed seals, juvenile harbour seals, killer whales, beluga whales and even humans (Brown et al., 2014; Buckman et al., 2011; Noël et al., 2017; Simond et al., 2019; Zheng et al., 2017).

1.3.2 Polybrominated diphenyl ethers (PBDEs)

The commercial use of PBDEs started in the 1970s, when they were marketed as alternative flame retardants to PCBs (UNEP, 2010; Environment Canada 2004; Jinhui et al., 2017; EPA, 2009; Abbasi et al., 2019). PBDEs are very similar in structure to PCBs, however they differ by containing an ether group and by containing bromines instead of chlorines (Rahman et al., 2001). Like PBDEs, their flame-retardant properties made them ideal for use in furniture, textiles, polystyrene, polyurethane foams, wire and cable insulation, and electrical parts (Environment Canada, 2004; EPA, 2009; Jinhui et al., 2017; Rahman et al., 2001; UNEP, 2010).

Three main mixtures of PBDE congeners were manufactured and used commercially: penta-brominated diphenyl ethers (pentaBDEs), octa-brominated diphenyl ethers (octaBDEs) and deca-brominated diphenyl ethers (decaBDEs), named to reflect the number of bromine molecules on the majority of the PBDEs in the mixture (Rotander et al., 2012). After several decades of use, PBDEs started to be recognized globally as a threat to wildlife. The European Union (EU) was one of the first to reduce the use of PBDEs. Penta- and octaBDEs were banned in the EU in 2004, and other countries quickly started to follow suit (Rotander et al., 2012). In 2009, penta-

and octa-BDEs officially joined PCBs in being added to the Stockholm Convention's list of substances to reduce and eventually eliminate from the global market (UNEP, 2010; Rotander et al., 2012; Abbasi et al., 2019). DecaBDEs remained on the market for several years, as they were considered to be less persistent, bioaccumulative and toxic (Ross et al., 2009). However, it was soon determined that decaBDEs could be biotransformed into their lesser-brominated penta- and octaBDE counterparts. For example, studies on liver microsomes in polar bears, beluga whales and ringed seals noted that fully brominated decaBDEs were able to degrade into more persistent forms of PBDEs through biotransformation (McKinney et al., 2011). DecaBDEs were banned in Europe in 2008 and were officially added to the Stockholm Convention in 2017 (Abbasi et al., 2019).

While PBDE production has ceased, they are still present in many products that were manufactured before PBDEs were removed from the market (Brown et al., 2018). The phase-out of PBDEs from in-use and waste stocks is projected to finish by 2050, which means continued input of PBDEs into the marine environment for decades to come (Abbasi et al., 2019). However, many studies have indicated that PBDE concentrations have declined in biota since the implementation of PBDE bans. Concentrations of PBDEs in seabird eggs in the Canadian Arctic increased from 1975 to 2003, then declined rapidly (Braune et al., 2015). In British Columbia, PBDE concentrations increased in harbour seal pups from 1984 to 2003, but declined afterwards following some restrictions (Ross et al., 2013). PBDEs increased in ringed seals and polar bears in the Arctic from the 1990s to the early 2000s, before beginning to decline in 2005 (Brown et al., 2018), closely mirroring the gradual decline in production of PBDEs throughout the world.

While similar in properties to PCBs, their adverse health effects in mammals have not been frequently documented. Hall et al. (2003) were one of the only studies to report adverse health effects, noting altered thyroid hormone levels in grey seals. Frouin et al. (2010) also noted effects of PBDEs on immune cells of harbour seals in vitro, however this has not been demonstrated in wild populations. This is likely due to their lower biomagnification properties and the potential for debromination of congeners in organisms to a less toxic state (Kelly et al., 2008).

1.3.3 Mercury

Mercury (Hg) is a ubiquitous contaminant of concern. It is present in the environment in three forms: elemental Hg (unbound, pure Hg), inorganic Hg (Hg bound to non-carbon sources, such as chlorine, sulfur or oxygen) and organic mercury (Hg bound with carbon, e.g. methyl mercury (MeHg)) (ATSDR, 1999). Hg undergoes natural cycling in the environment and is released from rocks and soils through processes such as erosion and volcanic activity. However, anthropogenically-driven Hg cycling has increased the availability of Hg to the environment (ATSDR, 1999; Lu et al., 2001). Anthropogenic Hg is mined as cinnabar ore, which is a form of mercuric sulfide. This ore is refined by heating it to temperatures above 538°C, which vapourizes the Hg, allowing it to be separated and cooled into pure, liquid Hg (ATSDR, 1999). Hg has many commercial uses, including extracting gold from ore, producing chlorine gas, dental fillings, fungicides and electrical switches (Alava et al., 2017; ATSDR, 1999). Before Hg was considered a toxic substance, it was even used for purposes such as laxatives, teething powders and spiritual herbal remedies (ATSDR, 1999). It is estimated that approximately ⅓ to ⅔ of the total mercury released into the environment is through anthropogenic processes (ATSDR, 1999). Most mercury, released by both natural and anthropogenic means, is released as an elemental Hg

vapour or as inorganic Hg, which can undergo long-range atmospheric transport to remote areas of the globe, such as the Arctic (ATSDR, 1999; Frouin et al., 2012). Both long range transport and river inputs are suspected to be the largest contributors of mercury to the Arctic (Kirk et al., 2012).

After making its way into the environment, elemental Hg is transformed into the biologically available form of Hg, MeHg, by organisms such as bacteria, fungi and phytoplankton (ATSDR, 1999). Inorganic Hg and elemental Hg do not have the same bioaccumulative properties as MeHg (ATSDR, 1999; Frouin et al., 2012; Loseto et al., 2015; Scheuhammer et al., 2015). MeHg can then enter the food chain and bioaccumulate in top level consumers, such as beluga whales (Loseto et al., 2015). Once in an organism, MeHg can either be transported throughout the body, or it can be demethylated in the liver into inorganic Hg (Frouin et al., 2010; Loseto et al., 2015). It is speculated that this inorganic Hg may be able to be detoxified by binding to selenium, however the precise mechanism for this is unclear (Frouin et al., 2012). MeHg has been implicated in a variety of toxicological effects, such as lymphocyte proliferation, metallothionein induction and nephrotoxicity, however its most notable toxicological impact may be as a neurotoxin (Alava et al., 2017; ATSDR, 1999; Frouin et al., 2012; Kirk et al., 2012). Mercury concentrations in the brain were found to be correlated with altered neurochemical biomarkers and mRNA expression of genes involved in neurochemical pathways in beluga whales (Ostertag et al., 2014).

Awareness of the negative environmental impacts of mercury spurred many initiatives in Canada, including regulations through the Environmental Protection Agency in 1999, a Risk

Management Strategy on Mercury in 2010, and a set of strict regulations titled 'Products Containing Mercury' in 2014 (Government of Canada, 2017; Sullivan et al., 2019). The regulations under Products Containing Mercury prohibited the manufacture and import of many products containing mercury, except for several types of fluorescent lamps (Government of Canada, 2017). Canadian regulations lead to the decrease of Hg emissions by over 90% over the past 40 years (Government of Canada, 2017).

In addition to Canada's own initiatives, a global initiative has sought to reduce mercury emissions. The Minamata Convention on Mercury was held in 2013 by the United Nations to address human and environmental health concerns around anthropogenic mercury emissions and to provide a framework to reduce these emissions globally (United Nations, 2013). Regulations put forth by the Minamata Convention required member countries to provide a national plan for controlling and reducing mercury emissions, placing emission limit values, using best available techniques and environmental practices to control emissions and providing alternative measures to reduce emissions (United Nations, 2013). The Government of Canada produced its national plan in 2017 and intends to address all remaining guidelines set forward by the Minamata Convention (Government of Canada, 2017).

Hg levels have been closely monitored in Beaufort Sea beluga whales and have been observed to increase since the 1990s (Choy et al., 2016; Ostertag et al., 2014). From the 1980's to 2000, total Hg in beluga whales showed a steady increase. However, after 2000, these trends either reversed or stabilized, depending on the size of the animal (Loseto et al., 2015). Large males had an observed decrease in total Hg, whereas other animals remained stable (Loseto et al., 2015). This

indicates a potential change in dietary exposure, as environmental Hg levels increased over the same period (Loseto et al., 2015), which could be linked to climate-driven changes in feeding patterns. In addition to changes in dietary exposure to Hg, concerns have been raised that the potential immunosuppressant roles of Hg may pose additional risk for a beluga's ability to fight infection in the face of climate change (Frouin et al., 2012).

1.4 Health

Both contaminants and climate change may have an impact on the health of beluga whales. Impacts on health may be reflected by changes in nutrition, which can be measured using stable isotope analyses, fatty acid analyses and body condition, as well as changes at the molecular level, which can be measured using mRNA expression profiles of key genes involved processes such as nutritional health, detoxification pathways, growth, stress and metabolism. This study will use a combination of mRNA expression profiling, fatty acid analysis, stable isotope analysis and body condition metrics to assess beluga whale health.

1.4.1 Genes

1.4.1.1 Leptin

Leptin is a hormone produced primarily by white adipocytes whose main role is to send signals from fat cells to the brain to communicate energy stores (Houseknecht et al., 1998; Oswal & Yeo, 2010). Leptin is produced in response to a variety of transcription factors, such as insulin and cortisol, in response to changes in energy levels (Houseknecht et al., 1998; Oswal & Yeo, 2010; Yu et al., 2011). The amount of leptin released by white adipocytes is correlated to the amount of fat contained in the body: white adipocytes will release high levels of leptin when

energy stores are adequate or high, where low levels of leptin will be released to alert the body to low energy stores (Houseknecht et al., 1998; Oswal & Yeo, 2010; Yu et al., 2011). Low levels of leptin result in a reduction of the incorporation of nutrients into fat depots, instead using the nutrients for energy (Yu et al., 2011). This system of regulation leads to a fairly stable adipose mass (Ferrante et al., 2014; Oswal & Yeo, 2010; Yu et al., 2011). For marine mammals, proper functioning of the leptin signaling pathway is of particular importance because these animals depend on their lipid reserves for both thermoregulation in cold waters and as an energy storage system for times of deficit (Castelli et al., 2014; Mashburn & Atkinson, 2008; Yu et al., 2011). Unfortunately, due to their lipophilic nature, lipophilic contaminants such as PCBs tend to accumulate in lipid-rich compartments, such as the adipose-rich blubber layers in marine mammals, which could impact a major source of leptin production (Ferrante et al., 2014; Provost et al., 2007). PCBs and other aromatic compounds have been associated with both increased leptin levels and reduced leptin signaling (Howell & Mangum, 2011; Provost et al., 2007). By looking at the expression of the leptin gene, we may be able to gain insight into both nutritional status and impacts of contaminants in beluga whales.

1.4.1.2 Adiponectin

Adiponectin is a hormone secreted by adipose tissue and released into circulation. Its main roles appear to be to control energy homeostasis, sensitize the body to insulin, inhibit inflammatory responses and reduce the formation of atherosclerotic plaques (Ahl et al., 2015; Cui et al., 2011; Howell & Mangum, 2011; Kadowaki et al., 2006; Stefan & Stumvoll, 2002; Taxvig et al., 2012; Yamauchi et al., 2014). Adiponectin levels are inversely related to the mass of adipose tissue in the body, meaning that obese individuals tend to have low levels of adiponectin, where lean

individuals have high levels (Ahl et al., 2015; Cui et al., 2011; Howell & Mangum, 2011; Kadowaki et al., 2006; Stefan & Stumvoll, 2002; Taxvig et al., 2012). In humans, higher levels of adiponectin were also found in metabolically healthy individuals compared to unhealthy ones (Ahl et al., 2015). Expression of the adiponectin gene may be reflected by both changes in feeding patterns, such as those we expect to see with changes in sea ice levels, and with exposure to contaminants. Changes in diet have been associated with increases in adiponectin levels in dolphins (Sobolesky et al., 2016). In seals, circulating adiponectin decreased between suckling and weaning in pups (Bennett et al., 2015). In terms of contaminant exposure, adipose is high in lipid content and is therefore an attractive destination and storage facility for lipophilic contaminants; however, this may pose a risk for proper hormone release by adipose tissue (Howell & Mangum, 2011). Indeed, PCBs have been associated with increased adiponectin gene expression and release of adiponectin (Howell & Mangum, 2011; Taxvig et al., 2012).

1.4.1.3 Thyroid receptor beta

Thyroid receptor beta (TRβ) is involved in the regulation of basal metabolism, growth and development (Maia et al., 2011; Martinez et al., 2013; Tabuchi et al., 2006). It is activated primarily by its two ligands, triiodothyronine (T3) and sometimes thyroxine (T4) (Maia et al., 2011; Tabuchi et al., 2006). These hormones differ only in the amount of iodine they contain in their outer rings (3 or 4, respectively), however T3 is the active form of the hormone (Maia et al., 2011; Tabuchi et al., 2006). Xenobiotics are able to interfere directly with the signalling of the thyroid receptor (Cheek et al., 1999). This is due to the fact that thyroid hormones structurally resemble several contaminants, including PCBs and PBDEs, since they all contain two phenol rings with halogens attached (e.g. chlorine, bromine, iodine) (Cheek et al., 1999). In particular,

hydroxylated PCBs show a particular affinity for the thyroid hormone receptors, and strongly compete with thyroid hormones for a chance to bind (Cheek et al., 1999). Therefore, these contaminants can directly interact with both thyroid binding proteins in the blood and with thyroid receptors, causing both up- and down-regulation of downstream products (Cheek et al., 1999). Studies in harbour seal pups noted significantly lower T4 levels in PCB-contaminated pups than in pups from less contaminated sites (Tabuchi et al., 2006). Ringed seals in areas with high concentrations of POPs were found to have higher mRNA expression of TRβ than in areas with low POPs (Routti et al., 2010). TRβ gene expression has also been shown to be both up- and down-regulated in response to mercury exposure in fish (Fu et al., 2017; Li et al., 2014). Further, reduced thyroid hormone metabolism has been noted in food deprivation studies in marine mammals, which indicates that we may see alterations TRβ expression not only due to xenobiotics, but also due to nutritional stress (Martinez et al., 2013).

1.4.1.4 Deiodinases 1 and 2

Deiodinases 1 and 2 (DI1 and DI2) are proteins responsible for removing one iodine from T4, turning it into an active T3 molecule (Bianco & Kim, 2006; Maia et al., 2011; Martinez et al., 2013; Tabuchi et al., 2006). Upregulation or downregulation of DI1 and DI2 receptors can therefore limit the amount of T3 available to TRβ, which is essentially a way of limiting or increasing TRβ activities (Bianco & Kim, 2006; Maia et al., 2011; Martinez et al., 2013; Tabuchi et al., 2006). While the thyroid hormone and its receptor help to modulate metabolism, the measurements of these genes may not fully represent the cellular metabolism of the cell (Martinez et al., 2013). Therefore, evaluating the expression of genes involved in the thyroid receptor pathway may give us additional insight into the activity of TRβ. Additionally, a

disconnect between DI1 and DI2 activity versus TRβ activity may indicate that genes are not being appropriately expressed. There is also some evidence that deiodinases may be able reflect exposure to contaminants, as ringed seals in areas with high concentrations of POPs had higher expression of DI1 than seals in areas with low concentrations of POPs (Routti et al., 2010).

1.4.1.5 Uncoupling protein 2

The uncoupling protein 2 (UCP2) plays an important role in reducing the generation of reactive oxygen species (ROS) during the electron transport chain (ETC) in mitochondria, protecting the cell from oxidative stress (Diano & Horvath, 2012; Donadelli et al., 2014). The protonmotive force generated during the electron transport chain is necessary to generate adenosine triphosphate (ATP); however, an excessive force can lead to an increase in ROS production, which is damaging to the cell (Brand & Esteves, 2005, Donadelli et al., 2014). UCP2 may be responsible for uncoupling the protonmotive force slightly (Brand & Esteves, 2005; Donadelli et al., 2014). This would allow for ATP to be formed while reducing the potential for ROS production (Brand & Esteves, 2005). However, it has also been established that an increase in fatty acids, for example during fasting or over-eating, will lead to an increase in UCP2 transcription (Brand & Esteves, 2005; Thompson & Kim, 2004; Toda & Diano, 2014). Indeed, fatty acids may be a ligand for the activation of UCP2 (Brand & Esteves, 2005). The precise mechanism for this remains elusive, however this may act as a good indicator of change in nutritional status. Fasting, high-fat diets and suckling of newborn pups all result in an increase in plasma fatty acids, as well as an increase in UCP2 expression in white adipose tissues (Thompson & Kim, 2004). An increase in UCP2 gene expression was observed in fasting

elephant seals, which may contribute to regulation of lipid metabolism and oxidative stress (Martinez et al., 2013).

1.4.1.6 Estrogen receptor alpha

Estrogen receptors, including estrogen receptor alpha (ERα) play a key role in development, growth and reproductive growth and maintenance (Matthews et al., 2000). Downstream impacts of ERα include roles in sperm maturation and function, fertility, and in the growth and development of reproductive tissues (Levin 2001, Matthews et al., 2000; Miyagawa, 2014). Unfortunately, ERα's ligand-binding domain must be slightly larger than necessary in order to accommodate its primary ligand, E2, which allows for a wide variety of compounds to bind, including contaminants (Heldring et al., 2007; Katzenellenbogen & Katzenellenbogen, 2000). PCBs with low levels of chlorination have highly estrogenic properties and have been observed to have both estrogenic and antiestrogenic activities (Ma & Sassoon, 2006; Zhang et al., 2014). There are concerns that these estrogen-mimicking compounds may have serious consequences for the reproductive success of animals such as marine mammals (Heldring et al., 2007; Larkin et al., 2003; Pinto et al., 2007).

1.4.1.7 Haptoglobin

Haptoglobin (HP) is a positive acute phase protein that acts as an antioxidant by binding free hemoglobin that is released into circulation in events such as intravascular hemolysis, protecting the body from reactive oxygen species that could be formed from unbound iron (Nielsen & Moestrup, 2009; Sadrzadeh & Bozorgmehr, 2004). HP also effectively protects the kidney from free hemoglobin, which unbound is small enough to pass through the kidney and cause renal

damage (Sadrzadeh & Bozorgmehr, 2004; van Vlierberghe et al., 2004). Haptoglobin is typically elevated in the serum during bacterial or viral infections, inflammation, tissue injury and environmental stress, and can therefore act as a useful indicator of health (Eurell et al., 1991; Gånheim et al., 2007; Murray et al., 2014). Indeed, farming industries are using elevated haptoglobin levels in farm animals, including pigs and cows, as early indicators of poor health (Eurell et al., 1991; Gånheim et al., 2007; Murray et al., 2014).

1.4.1.8 Heat shock protein 70 A1A

The heat shock protein 70 (Hsp70) family of proteins aid in the degradation of unstable and misfolded proteins, prevent the dissolution of vital protein complexes, and fold and refold proteins, among other cellular chores (Daugaard et al., 2007; Najafizadeh et al., 2015; Song et al., 2006; Tsan & Gao, 2009). When an organism encounters a stress, such as high temperatures, environmental pollutants, infection, injury, oxidative stress, ultraviolet, irradiation and parasitism, among others, heat shock protein levels are elevated in response (Bierkens et al., 1998; McCallister et al., 2015; Song et al., 2006). These proteins play a protective role, protecting proteins in the cell from denaturation and facilitating repair and are considered a key component of the cellular stress response (Song et al., 2006). The heat shock protein 70, isoform 1A1 (HspA1A) is a key member of the Hsp70 family. It is primarily produced in response to stressors, including mercury and organochlorines, and is therefore a good indicator of potential exposure to stress (Hegde et al., 2009; McCallister et al., 2015; Najafizadeh et al., 2015; Nargesi et al., 2016).

1.4.1.9 Tumour necrosis factor alpha

Tumour necrosis factor alpha (TNFα) is a cytokine produced by the immune system, primarily through activated macrophages, but that also can be produced by lymphoid cells, mast cells, endothelial cells, fibroblasts and neuronal tissue (Baud & Karin, 2001; Chen & Goeddel, 2002; Wajant et al., 2003). TNFα is involved in the body's response to stressors such as inflammation, apoptosis, bacterial and viral invasion, infection, trauma and environmental pressures (Baud & Karin, 2001; Chen & Goeddel, 2002; Leong & Karsan, 2000). TNFα gets its name from its apparent anti-tumoural activity that was described when TNFα was first discovered, but this is likely from its induction of apoptosis in tumour target cells (Wajant et al., 2003). Improper production of TNFα has been implicated in human disease, such as sepsis, diabetes, cancer, osteoporosis and autoimmune diseases (Chen & Goeddel, 2002). In addition, maintenance of TNFα concentrations in the body is extremely important, as high levels may cause shock and low levels may cause wasting syndrome (Wajant et al., 2003). What is concerning is that a previous study on PCB toxicity in adipocytes found that PCBs perturbed this delicate balance and were associated with an increase in transcription of TNFα (Ferrante et al., 2014). With this in mind, and recalling its role in the immune response, TNFα may be an excellent candidate for monitoring health in contaminant-exposed organisms.

1.4.1.10 Insulin-like growth factor 1

Growth factors are important in the regulation of both cell reproduction and cell death (Baserga, 2000; Pollak, 2000). The insulin-like growth factor 1 (ILGF1), specifically, is a largely regulatory molecule and induces mitosis in cells, as well as playing roles in the promotion of cell size, sending anti-apoptotic signals, inducing differentiation in some cells, and playing a role in

the transformation of cells (Baserga, 2000; Chitnis et al., 2008; Pollak, 2000). ILGF1s are required for normal growth and development and removal of ILGF1 genes has been shown to result in both infertility and abnormal embryonic growth, including dwarfism and impaired postnatal growth (Adams et al., 2000; Stewart & Rotwein, 1996). One study on the neurotoxicity of PBDEs found that ILGF1 may actually be able to protect cells from oxidative stress and cell apoptosis related to intense PBDE exposure (Bai et al., 2017), so it may play an important role in PBDE-exposed Arctic animals such as beluga whales.

1.4.1.11 Aryl hydrocarbon receptor

The aryl hydrocarbon receptor (AhR) is a mediator of the response to xenobiotics, playing a role in the regulation of cytochrome P450 1A1, which is responsible for metabolizing many xenobiotics (Denison & Nagy, 2003; Mulero-Navarro & Fernandez-Salguero, 2016; Nguyen & Bradfield, 2008; Rowlands & Gustafsson, 1997). Upon encountering a xenobiotic, including halogenated aromatic hydrocarbons, polyaromatic hydrocarbons (PAHs), and natural ligands, the AhR will trigger a cascade in order to metabolize the xenobiotic (Denison & Nagy, 2003; Nguyen & Bradfield, 2008). However, in some instances, metabolism of a compound can actually make the compound more toxic, as is the case for chlorinated PAHs, such as PCBs, which are known to exert toxicity through binding to the AhR (Nguyen & Bradfield, 2008). This pathway makes the AhR a key receptor in the study of the impact of contaminants (e.g. Noël et al., 2014).

1.4.2 Feeding ecology

It is difficult to determine diet in free-ranging marine mammals. Many marine mammals are rarely observed feeding, and those studies that are able to obtain observational data on marine mammal feeding are limited. Daylight hours and geographical barriers such as ice sheets may limit the opportunities for observation, and those observations that can be made are limited to prey items captured in the upper surface level of the ocean (Herman et al., 2005). Stomach content analyses have been used to determine prey items but are biased towards prey that do not break down easily in stomach acids (Herman et al., 2005; Loseto et al., 2008). Therefore, techniques such as stable isotope analysis and fatty acid analysis are used in order to get an understanding of an organism's diet. Both techniques are able to reflect a longer time span, instead of a snapshot in time like stomach content or observational analyses (Dahl et al., 2000). Additionally, these techniques are able to be used to draw comparisons between years, allowing us to determine if diet has changed over time.

1.4.2.1 Stable isotopes

Stable isotopes are variations of the same element that have an identical number of protons and electrons, meaning they are capable of making the same chemical bonds; however, they differ in the number of neutrons (Ben-David & Flaherty, 2012; West et al., 2006). Carbon and nitrogen are present in the marine environment in their normal chemical states, ^{12}C and ^{14}N, as well as in their isotopic forms, ^{13}C and ^{15}N, which both contain an extra neutron and are therefore slightly heavier (Ben-David & Flaherty, 2012). These ratios of ^{12}C:^{13}C and ^{14}N:^{15}N are able to tell us information about feeding patterns as stable isotope ratios in consumers reflect those in their prey (Dahl et al., 2003; Herman et al., 2005).

The ratio of ^{12}C:^{13}C provides information about the sources of carbon entering the food chain, as many sources have distinct ratios (Søreide et al., 2006). Onshore and nearshore carbon signatures are lower in ^{13}C than offshore signatures, so an increase in ^{13}C would infer that an organism is feeding further from shore than normal (Dahl et al., 2003; Roth et al., 2002). A previous study on beluga whale foraging was able to use carbon isotopes to determine that smaller whales fed closer to shore than larger whales (Loseto et al., 2008). This tool can help us to determine if foraging locations are changing with reductions in sea ice cover.

The ratio of ^{14}N:^{15}N tells us different information than carbon isotopes. ^{15}N is retained more easily than ^{14}N up the food chain, and therefore an increase in ^{15}N is reflective of feeding at a higher trophic position (Dahl et al., 2003). A consumer tends to be enriched in ^{15}N by 3-4 ‰ relative to its diet (Søreide et al., 2006). We can therefore use this tool to determine whether animals are feeding at different trophic levels, which can infer changes in diet (Søreide et al., 2006; West et al., 2006).

1.4.2.2 Fatty acids

There is very little synthesis or modification of fatty acids in the food web. Many fatty acids are synthesized at the bottom of the food chain, for example in phytoplankton, and are transferred up the food chain with very little alteration (Budge et al., 2007; Dahl et al., 2000; Grahl-Nielsen et al., 2003). Each organism within the food web exhibits their own unique fatty acid signature based on their prey, and we can use this knowledge to compare signatures between predators and potential prey items to determine the predator's diet (Grahl-Nielsen et al., 2003; Thiemann et al.,

2008). On a broader scale, changes in fatty acid signatures over time can be used to infer changes in foraging patterns (Thiemann et al., 2008). A previous study on beluga whale foraging patterns was able to use fatty acids to determine that length was related to beluga whale fatty acid profiles, reflecting the fact that larger belugas feed in different locations than smaller belugas (Loseto et al., 2008). This invaluable tool can allow us to determine if beluga whales have exhibited changes in prey items over time, which will help us to determine if climate change has impacted foraging patterns.

1.5 Goal of this study

The goal of this study is to provide more information about the potential interacting effects of contaminants and climate change on the health of beluga whales. To address this goal, I used body condition, contaminant levels, muscle stable isotope ratios, blubber fatty acid composition and the expression of genes involved in endocrine function, metabolism and contaminant detoxification in blubber and liver and beluga whales over a 9-year period (2008-2017). The specific goals of this study are two-fold:

1) Explore, develop and utilize new tools for evaluating beluga whale health.

This was accomplished by exploring the use of novel genes in beluga whales that play roles in the acute stress response, nutritional stress, growth and development.

2) Explore factors, and the relationships between factors, that influence the health of beluga whales in the Beaufort Sea from 2008-2017.

This was accomplished by exploring relationships between gene expression, body condition, stable isotopes, fatty acids, sea ice extent and contaminants.

My hypothesis was that beluga whale gene expression is influenced by climate change and contaminants. I hypothesized that climate change would influence beluga whale fatty acids and stable isotopes, through changes in prey distribution and type, which in turn might influence the expression of genes involved in nutrition. I also hypothesized that contaminants would be related to gene expression, and that this relationship would be particularly evident for PCBs, which have already been demonstrated to have effects on gene expression in beluga whales (Noël et al., 2014). My hope is that this thesis will add valuable insight into the potential roles of both climate change and contaminants on beluga whale health.

Chapter 2: Interacting effects of contaminants and climate change on the health of western Arctic beluga whales

2.1 Introduction

Beluga whales (*Delphinapterus leucas*) are a culturally and ecologically valuable Arctic species, many of which reside in Canadian waters (Choy et al., 2017; Ostertag et al., 2018). These animals are situated at the top of the food web and are a valuable resource to the Inuvialuit, who rely on beluga whales as a source of sustenance. The Beaufort Sea population is one of the largest beluga whale populations, with an estimated 40,000 individuals (Allen & Angliss, 2013; COSEWIC, 2004; Harwood et al., 1996). This population migrates from their wintering grounds in the Bering Sea to their summer home in the Mackenzie River Estuary each year, following the retreat of the sea ice (Choy et al., 2017; Hornby et al., 2016; Ostertag et al., 2018; Stafford et al., 2018). Sea ice plays an important role in the life of a beluga whale. Not only does the distribution of sea ice dictate the habitat available to beluga whales, it also acts as habitat for prey and a refuge from predators such as killer whales, whose dorsal fins restrict them from swimming under sea ice (Breton-Honeyman et al., 2016; Hauser et al., 2018; Hornby et al., 2016; Smythe et al., 2018). However, Arctic sea ice is undergoing a period of rapid and unprecedented transformation, brought on primarily by climate change, and the implications for beluga whales remain unclear.

It is estimated that the Arctic is warming at a rate of approximately 0.6ºC per decade, which is three times faster than the global average rate (Comiso & Hall, 2014). Combined with other factors such as increases in storm surges and decreased albedo, this rate of warming has led to

drastic changes in sea ice distribution, which has the potential to impact the entire Arctic ecosystem (Grannas et al., 2013; Loseto et al., 2015; Moore & Reeves, 2018; Reeves et al., 2013). Indeed, changes in distribution and abundance have been noted for species at all trophic levels (Loseto et al., 2015; Moore & Reeves, 2018; Reeves et al., 2013; Tai et al., 2019), highlighting the fact that the Arctic is undergoing a period of transformation. It is likely that beluga whales will be facing altered habitat, increases in competition and predation, and changes in prey distribution, abundance and species (Moore & Reeves, 2018; Ostertag et al., 2018). However, these changes are not the only concern associated with climate change. There is concern that reductions in sea ice cover may lead to an increase in oil and gas exploration in the Arctic, increases in shipping traffic and therefore underwater noise, increases in tourism and increases in fishing pressures (Reeves et al., 2013). In addition to increases in anthropogenic activities in the Arctic, there may also be increased or altered exposure to contaminants associated with climate change.

While the Arctic may not seem a likely place for evaluating the impacts of contaminants, its remote location does not make it immune to contaminant exposure. Atmospheric and oceanic currents bring contaminants to the Arctic from more industrialized areas of the globe, including Europe, North America and Asia (Noël et al., 2018). Contaminants that have been highlighted as a concern in the Arctic include polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and methyl mercury (MeHg). All three of these contaminants have negative impacts on mammal physiology, and all are known to bioaccumulate in measurable amounts in Arctic marine mammals (Frouin et al., 2012; Grannas et al., 2013; Loseto et al., 2008; Noël et al., 2014). For example, PCB-related changes in the expression profiles of thyroid hormone

receptors have been noted in harbour seals. Since thyroid receptors play a key role in the regulation of metabolism, it is vital that proper thyroid hormone regulation and expression is not impacted (Tabuchi et al., 2006). Changes in gene expression due to contaminants have also been observed in killer whales (*Orcinus orca*), harbour porpoises (*Phocoena phocoena*), ringed seals (*Pusa hispada*), sperm whales (*Physeter microcephalus*) and grey seals (*Halichoerus grypus*) (Brown et al., 2014; Buckman et al., 2011; Jepson et al., 2005).

Recently, a study evaluated the correlations between PCBs and gene expression in Beaufort Sea beluga whales. They found that the mRNA transcript abundance of two genes involved in xenobiotic detoxification, the aryl hydrocarbon receptor (AhR) and cytochrome P450 1A1 (Cyp1A1), were correlated with PCB concentrations (Noël et al., 2014). Upon evaluating further patterns, the study found that several genes involved in growth, development and metabolism had higher expression in years of low sea ice extent (Noël et al., 2014). Sea ice has previously acted as a sink for contaminants in the Arctic but increases in sea ice melt have released many of these stores (Alava et al., 2017; Grannas et al., 2013). Increases in precipitation due to climate change are expected to increase the intensity of atmospheric contaminant deposition (Grannas et al., 2013). In addition, prey species are expected to migrate north as the climate warms, and these organisms may act as a vector to deliver contaminants from more industrialized areas of the globe (Alava et al., 2017; Noël et al., 2014; Tai et al., 2019). It is imperative that we begin to understand the potential for climate change and contaminants to interact to impact the health of beluga whales.

The goal of this study is to provide more information about the effects of contaminants and climate change on the health of beluga whales. To this end, we will explore, develop and utilize new tools for evaluating beluga whale health, and we will use these tools, along with existing tools, to explore factors, and the relationships between factors, that influence the health of beluga whales in the Beaufort Sea from 2008-2017.

To this extent, we evaluated the expression of twelve key genes involved in nutritional health, detoxification pathways and stress. These genes were chosen to represent indicators of beluga whale health under changing Arctic conditions and contaminant exposure. Further metrics, such as body condition, contaminant burdens, stable isotopes, fatty acids and sea ice extent, were included in the analysis to assess potential patterns associated with gene expression. These analyses will give valuable insight into the potential interaction between climate change and contaminants on beluga whale health in a changing Arctic.

2.2 Methods

2.2.1 Sample collection

Blubber, liver and muscle samples were obtained from beluga whales that were harvested at Hendrickson Island, in the shallow Mackenzie Delta near the community of Tuktoyaktuk, Northwest Territories, Canada, each summer over the period of 2008-2017. Samples were collected in collaboration with the annual traditional harvest by Inuvialuit hunters. Inuvialuit hunters do not typically target female beluga whales, so only a few females were available for analysis. Since females offload some of their contaminant load to their offspring during gestation and lactation, females and males have very different contaminant loads (Ross et al., 2000).

Therefore, we decided to remove females from analysis to allow for stronger comparisons to be drawn between years.

Body condition metrics were taken in the field. Length, girth and blubber thickness were recorded for each whale. Blubber thickness was determined on the beluga whales' ventral side, just posterior to the arm fin, to ensure consistency and accuracy of depth (e.g. Noël et al., 2014). Jaws were removed and sent to the Freshwater Institute (Winnipeg, MB, Canada) for age determination using teeth (details on this method are described below).

Blubber samples for organic contaminants (PCBs, PBDEs) were cut from each beluga and immediately frozen in liquid nitrogen for transport to the lab where they were stored at -80 ^{o}C until analysis. Muscle samples for determination of mercury content were collected from the same beluga whales and immediately stored in a -20^{o}C freezer until analysis.

Blubber and liver samples were also collected in the field for mRNA extraction. Samples were cut and were immediately placed into RNALater Stabilization Solution (Invitrogen, Life Technologies Corporation, Carlsbad, CA, USA) within two hours post-mortem to ensure that RNA integrity was maintained (Noël et al., 2014). Samples were stored on ice during sampling and subsequently stored at -20^{o}C within 24 hours until further analysis.

In the lab, blubber for mRNA extraction was sectioned into inner and outer layers. For the purposes of this analysis, only inner blubber layers were used, as inner blubber is more metabolically active than outer blubber layers (Koopman et al., 2002).

2.2.2 RNA extraction

Approximately 20 mg of blubber or liver tissue for each individual was obtained and blotted on a KimWipe (Kimberly-Clark Professional, Roswell, GA, USA) to remove excess fat and RNALater solution. Samples were immediately placed into a 2 ml tube with 700 µl QIAzol Lysis Reagent (QIAGEN, Austin, TX, USA) and a 3 mm tungsten-carbide bead. The tissue was then homogenized in a Retcsh MM400 mixer mill (Thermo Fisher Scientific, Ottawa, ON, Canada) at 20 Hz for 2 minutes, then placed on ice for 2 minutes. This step was repeated three times. Samples were then placed in a centrifuge at 4ºC for 10 minutes at 13,000 rpm. After centrifugation, the aqueous top layer was pipetted off of the QIAzol phenol layer and placed into 140 µl chloroform. Samples were vortexed for 15 seconds, then incubated at room temperature for 2-3 minutes. Samples were then centrifuged at 4ºC for 15 minutes at 13,000 rpm.

The aqueous top layer was pipetted off and placed into 350 µl isopropanol (Fisher Chemical, Life Technologies Corporation, Carlsbad, CA, USA). For blubber samples, 1 µl of glycogen (20 mg/ml) (Roche Diagnostics, Laval, QC, Canada) was added to facilitate precipitation and increase RNA recovery rates. Samples were mixed thoroughly using a Fisher Vortex Genie (Thermofisher Scientific, Ottawa, ON, Canada) and left for 10 minutes at room temperature before centrifugation at 4ºC for 15 minutes at 14,000 rpm. Following centrifugation, the liquid was pipetted out of the tube, leaving only the precipitated pellet behind. The pellet was then resuspended in 700 µl of 70% ethanol (Reagent Alcohol, Fisher Chemical, Life Technologies Corporation, Carlsbad, CA, USA) and mixed using a Fisher Vortex Genie (Thermofisher Scientific, Ottawa, ON). It was then centrifuged for 5 minutes at 7500 rpm at 4ºC. The ethanol

was removed with a pipette and the pellet was then re-suspended in 23 µl Invitrogen UltraPure DEPC-treated water, and the mixture was mixed using the Vortex Genie (Thermofisher Scientific, Ottawa, ON, Canada). The mixture was briefly centrifuged to ensure that all material was at the bottom of the tube, then the solution was placed into a water bath at 55°C for 10 minutes. The tube was then briefly centrifuged to ensure all condensation was at the bottom of the tube.

Total RNA concentration was determined on a 1 µl aliquot via spectrophotometry using BioTek Gen5 software with a Take 3 Micro-Volume Plate attachment (BioTek, Winooski, VT, USA). Remaining RNA samples were stored at -80°C until thawed for cDNA synthesis.

Total RNA was converted to cDNA using the Applied Biosystems High-Capacity cDNA Reverse Transcription kit, following manufacturer's instructions (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA). To this end, RNA samples were thawed on ice and subsequently diluted to roughly 100 ng/µl. 10 µl RNA was then added to 10 µl High-Capacity cDNA Reverse Transcription solution. Mixtures were then lightly centrifuged to ensure that all liquids were mixed. Samples were then placed into a thermocycler (Realplex4 Eppendorf thermocycler, Eppendorf, Westbury, NY, USA) and were cycled at 25°C for 10 minutes, 37°C for 120 minutes, and 85°C for 5 minutes. Resulting cDNA samples were diluted 20x prior to use and were stored at -20°C.

2.2.3 Qualitative real-time polymerase chain reaction

Quantitative real time polymerase chain reaction (qPCR) assays were run on an Applied Biosystems StepOnePlus real time PCR system (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA) for all analysis of gene expression, except for AhR, which was assessed using an Applied Biosystems 7500 real time PCR system (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA, USA). Housekeeping genes rpL8 and β-actin were run in quadruplicate on both machines so that samples could be normalized to genes run on the same machine. Primer sequences and sources are listed in Table A3 (Appendix A).

In theory, housekeeping genes are invariant control genes used to normalize samples. In practice, however, common housekeeping genes have shown to be variable among tissues and in different conditions. Therefore, it is suggested to use multiple housekeeping genes in combination by taking the geometric mean. As such, we used both rpL8 and β-actin as housekeeping genes, which have been successfully used as housekeeping genes in marine mammals including beluga whales and harbour seals (Noël et al., 2014; Tabuchi et al., 2006).

Samples for all primer pairs, with the exception of AhR, were run using Applied Biosystems SYBR Green PowerUp Master Mix on a fast cycle. Samples were prepared according to the manufacturer's suggestions, with a total of 15 µl per well consisting of 2 µl cDNA, 7.5 µl Master Mix, 0.5 µl primer pairs (0.002 mol/L) and 5 µl Invitrogen UltraPure DEPC-treated water. The thermocycle program for this kit consisted of an initial activation step at 95°C for 20 seconds. This was followed by 40 cycles of denaturation at 95°C for 3 seconds, annealing/elongation at 60°C for 30 seconds. Following amplification, a melt profile was established to ensure that

amplified products had the expected thermal melt profiles and to ensure the absence of primer-dimer products.

AhR was not successfully amplified using SYBR Green PowerUp Master Mix, so an alternative kit, QuantiTect SYBR Green by Qiagen, was used. Each well consisted of 1 µl sample, 12.5 µl QuantiTect mixture, 0.625 µl primer pairs and 10.875 µl Invitrogen Ultra-Pure DEPC-treated water. We followed the manufacturer's suggested cycling conditions, which consisted of a 15-minute initial enzyme activation at 95°C, followed by 40 cycles of denaturation for 15s at 94°C, annealing for 30s at 50°C, and extension for 30s at 72°C. A thermal melt profile was created at the end.

Regardless of machine or master mix used, all samples were run in quadruplicate using no-template controls (NTC) and positive controls (PC) when possible. NTCs consisted of a no-cDNA sample to assess for any contamination in reagents. In cases where NTCs showed amplification, plates were discarded and re-run. PCs contained a standard mix of cDNA samples and were used for all runs to compare machine efficiency between runs. In cases where PCs showed more than 2-fold difference from the mean of all positive controls, plates were similarly discarded and re-run. A subset of samples was also run for each gene with no-DNA samples to assess the possibility of genomic contamination. The results of this indicate that this was not an issue.

2.2.4 Treatment of qPCR data

Arithmetic means and standard deviations (SD) were computed for all cycle thresholds for quadruplicate samples of target and housekeeping genes. If quadruplicates showed more than +/- 0.5 SD from the mean, the sample that deviated from the mean most was removed. In cases where removing one to two samples did not reduce the SD to less than +/- 0.5 from the mean, the entire sample group was removed from analysis. In some cases, quadruplicate samples separated into two distinct sets of values. If the standard deviation for these quadruplicates was more than +/- 0.5, the pair with the lowest standard deviation was retained for analysis.

Data were normalized to a combination of housekeeping genes, as studies have indicated that no genes are constitutively expressed in all cell types, nor in all conditions (Anderson et al., 2004). As this is a wild population of beluga whales, we cannot assume that all encountered the same conditions and one gene will be suitable for normalization for all samples. To this end, two common housekeeping genes, rpL8 and β-actin, were used in conjunction to normalize samples. First, arithmetic means were found for the quadruplicate cycle thresholds for each housekeeping gene. Next, these values were used to find the geometric mean of the cycle thresholds (Pfaffl et al., 2004), denoted as cycle threshold of the housekeeping genes from this point forward.

As an additional internal control measure, and to fulfill the requirements of the $2^{-\Delta\Delta Ct}$ method, the cycle threshold of the beluga that had a contaminant load nearest to the average PCB contaminant load of all belugas was found (e.g. Noël et al., 2014). The resulting values were used to calculate $2^{-\Delta\Delta Ct}$ values for each individual for each target gene, as per the following formula:

$$2^{-\Delta\Delta Ct} = 2\text{^}(-(Ct_{TG} - Ct_{HK}) - (Ct_{HK} - Ct_{MC})),$$

where Ct_{TG} is the mean cycle threshold of the target gene, Ct_{HK} is the cycle threshold of the housekeeping genes and Ct_{MC} is the cycle threshold of the beluga with an average contaminant load. *Ct* values were used for all subsequent statistical analysis.

2.2.5 Quality assurance/quality control of new primers

New primer sequences were sought out in the literature that had been previously used in cetaceans and pinnipeds (Appendix A, Table A3). New primers assessed for this study included deiodinase 1, deiodinase 2, peroxisome proliferator-activated receptor alpha, tumour necrosis factor alpha, haptoglobin, sirtuin 2, acyl coenzyme A oxidase 1, uncoupling protein 2, acetyl coA acetyltransferase 2 and C-reactive protein.

The compatibility of these primers with beluga whale blubber and liver samples was assessed following a three-tier quality assurance/quality control (QA/QC) procedure, as described in Veldhoen et al. (2011). This method has been used previously to determine the suitability of target genes for beluga whale tissues (Noël et al., 2014). This allowed us to satisfy all requirements of the comparative Ct ($2^{-\Delta\Delta Ct}$) method (Bustin et al., 2009; Dorak, 2006). Tier 1 included a gel-electrophoresis run to determine the size of the amplicon. Tier 2 included direct DNA sequencing of the amplicon. Tier 3 comprised of a standard curve analysis, performed for each new gene-specific primer pair against a 2-fold serially diluted cDNA template, in other words, 2- to 256-fold dilution. Tier 3 assesses whether the efficiency of all primers is similar to that of the normalizer gene, rpL8. To this end, ΔCt values of the target gene to the normalizer gene were plotted against the log of the dilution series. The plots yielded a linear relationship

with a slope of <0.1, indicating that efficiency assumptions of the $2^{-\Delta\Delta Ct}$ method were met. In addition, partway through analysis, the beluga whale genome was published (Jones et al., 2017), so all primer sequences were confirmed using GenBank and were determined to be correct.

2.2.6 PCBs and PBDEs

Blubber samples were assessed for both PCB and PBDE concentrations. Samples collected between 2008-2012 were analyzed at the Freshwater Institute (Winnipeg, MB, Canada). Samples collected after 2012 were analyzed at ALS Environmental (Winnipeg, MB, Canada) and Axys Analytical (Sidney, BC, Canada). Details on extraction procedures can be found elsewhere (Alava et al., 2009; Stern et al., 2005). In brief, samples were homogenized and spiked with a surrogate internal standard containing 10 ^{13}C-labelled PCBs and 10 ^{13}C-labelled PBDEs (Cambridge Isotope Laboratory, Andover, MA, USA) as per the isotope dilution method. Spiked samples were mixed with 20 g sodium sulphate and were dried for 12-24 hours, then were transferred to an extraction column and were extracted with dichloromethane/hexane (1:1, v/v). The extracts were dried, and the residue was weighed to determine lipid content. The residue was then resuspended in dichloromethane/hexane and cleaned up using silica gel chromatography. PCB and PBDE concentrations were determined on an aliquot of the resulting hexane fraction via high resolution gas chromatography/high resolution mass spectrometry.

QA/QC measures were taken to ensure accuracy of results. To this end, one sample consisting of a standard reference material (NIST 1945 whale blubber SRM), two procedural blanks containing pure lipid (triolein), and one replicate sample were run with every batch of ten samples.

2.2.7 Mercury

Mercury content was analyzed for each sample at the Freshwater Institute (Winnipeg, MB, Canada) following previously established methods (Loseto et al., 2015). Muscle samples were used for mercury analysis because 97-100% of total mercury in muscle is composed of MeHg and muscle is more reflective of dietary sources of Hg than other tissues (Loseto et al., 2008). Approximately 0.15 g of beluga muscle sample was analyzed by heating samples to 90^{o}C with a hydrochloric/nitric acid digestion mixture. The resulting mixture was analyzed using cold vapour atomic absorption spectrometry (CVAAS) using a 0.005 μg/g detection limit. Certified Reference Materials (CRM 2976, TORT-2, DOLT-2) were run in duplicate with every run. Loseto et al. (2008) noted that 99% of total Hg present in beluga muscle was MeHg, so I will assume that total Hg results are fairly representative of MeHg for the remainder of this thesis.

2.2.8 Stable isotopes

Muscle samples were freeze-dried and homogenized for the determination of carbon and nitrogen stable isotopes. A chloroform/methanol extraction was employed to remove lipids for carbon isotope determination, as lipids are typically depleted in carbon isotope samples relative to proteins, biasing results (Loseto et al., 2015). Analysis of the resulting samples was performed using continuous flow, ion ratio mass spectrometry at the University of Winnipeg Isotope Laboratory (MB, Canada). Both carbon and nitrogen isotope results are presented using the standard delta (δ) notation (units of *per mil* (‰)), which represent deviations from a standard:

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

where R represents the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio. The carbon standard used was Vienna PeeDee Belemnite (VPDB), and IAEN-N1 (IAEA, Vienna, Austria) was used as a standard for nitrogen.

2.2.9 Fatty acids

Fatty acid analyses were performed at the Freshwater Institute (Winnipeg, MB, Canada). Details on extraction procedures are described elsewhere (Loseto et al., 2009). Briefly, lipids were extracted from 0.5 g of the inner blubber layer using a 2:1 chloroform:methanol mixture. In order to minimize oxidation, 0.01% butylated hydroxytoluene (BHT) (v/v/w) was added. The lipid phase was collected and subsequently washed and filtered through anhydrous sodium sulphate. The mixture was evaporated under nitrogen to determine total lipid weight. Fatty acid methyl esters were then prepared using the resulting lipids through transesterification with the Hilditch reagent (0.5 N H_2SO_4 in methanol). The resulting fatty acid methyl ester samples were analyzed using gas chromatography with a mass spectrometer detector. Fatty acid peaks are expressed as a mass percent of the total fatty acids identified for each year.

2.2.10 Age estimates

Ages were determined at the Freshwater Institute (Winnipeg, MB, Canada) by slicing thin sections of a tooth and measuring dentine growth groups, or growth lines. Each dentine growth group corresponds to one year of growth, allowing for a reliable age estimate (Stewart et al., 2006).

2.2.11 Sea ice

Percentage sea ice values were obtained from the Sea Ice Index through the National Snow and Ice Data Center (Fetterer et al., 2017). The percentage sea ice extent in the Beaufort Sea in June was used for all analyses, as the sea ice extent in June limits the start of the Beaufort Sea beluga population's annual migration.

2.2.12 Statistical analysis

All statistical analyses were performed in R (R Core Team, 2013). For all data analysis, blubber and liver samples were considered separately.

Ct values for all target genes, determined from the $2^{-\Delta\Delta Ct}$ method, were assessed for normality and homogeneity by fitting a linear model to the data for each target and using the plot() function in R to visually assess the data. For all target genes, outliers were identified and data were non-normally distributed. Data for all target genes were therefore log-transformed to fit the assumption of normality and homogeneity of variance.

Unfortunately, many target genes had missing data due to low amplification of target genes. In cases where target genes had more than 30% missing data, data were not used in any multivariate analysis. This was the case for adiponectin in liver (54% missing), as well as the aryl hydrocarbon receptor in blubber (54% missing) and in liver (39% missing). In cases where more than 70% of the *Ct* data was present, the package Amelia II in R (Honaker et al., 2011) was used to fill in missing data. Amelia II is designed to use multiple imputations to input missing data for multivariate methods, including principal components analysis (Honaker et al., 2011). Amelia II

uses expectation-maximum with bootstrapping to estimate the missing data points based on maximum-likelihood. Limits to the imputed data were set as 0 and infinity.

To determine whether the expression of genes showed any relationships with each other, and to determine what variables influenced total gene expression, I employed a principal component analysis (PCA). *Ct* values for all target genes were entered into the PCA using the function prcomp() in R. Screeplots and cumulative proportions plots were assessed to determine the relative contributions of each principal component to the data. A varimax rotation was then performed in order to better visualize the data, as recommended by Noël et al., (2014). The first principal component (factor 1) for both blubber and liver was subsequently plotted against all environment, body condition, contaminant and stable isotope variables in order to determine if the principal components could describe any of the variation in the data. A Bonferroni p-value correction was applied to allow for multiple comparisons of factor 1:

$$p=\alpha/n$$

where α is the original p-value (0.05) and n is the number of comparisons made.

Fatty acids were reduced in dimensions by a PCA. To this end, the same functions and assessments were performed on fatty acids data as gene transcript data. Data were log-transformed to prepare for analysis. Resulting principal components, or factors, were explored for their relationships between environment, body condition, contaminant and stable isotope variables. Further, factors 1 and 2 were plotted against gene expression principal components to investigate possible associations. A Bonferroni correction was applied to allow for multiple comparisons of factors 1 and 2.

Next, individual genes were assessed for any relationships with environmental data, body condition, contaminants and the principal components obtained from fatty acid data. An incomplete data set precluded the inclusion of stable isotopes in these analyses, so they were considered separately and will be discussed shortly. To this end, I used the Akaike Information Criterion (AIC) using the stepAIC function in the MASS package in R (Venables & Ripley, 2002). Parameters included in the analysis were length, total PCBs, total mercury, total PBDEs, sea ice extent and factors 1 and 2 obtained through the principal component analysis of fatty acids. Length was used as a proxy for body condition as length, girth, blubber thickness and age are expected to be highly correlated.

In order to assess if there was a relationship between stable isotopes and gene expression across years, a linear model was built using the function lm() in R.

2.3 Results

For the remainder of this thesis, I will use the term 'explanatory variables' to refer collectively to sea ice extent, body condition metrics (girth, length, blubber thickness, age), contaminants (total PCBs, total PBDEs, total Hg), stable isotopes ($\delta^{13}C$ and $\delta^{15}N$) and fatty acids.

2.3.1 Explanatory variables

Over the period of 2008 to 2017 when beluga whales were sampled, there was significant variation in age, PCBs, $\delta^{13}C$ and $\delta^{15}N$ between years (one-way ANOVA, Appendix A, Table A1; Table A2) but no significant variation between years for blubber thickness, length, girth, total PBDEs, or total Hg. PCBs and PBDEs were highest in 2014, which was a year of high sea ice extent (Appendix A, Table A1; Table A2). Hg was highest in 2013 and 2014, both of which were also periods of high sea ice extent (Appendix A, Table A1; Table A2). However, contaminants were not similarly high in 2009, which was the year with the highest sea ice extent over the study period.

In order to reduce fatty acids data for subsequent analysis with gene expression, a PCA was performed. Factor 1 of the principal component analysis performed on the fatty acid profile of individual beluga whales explained 34.0% of the variance, where factor 2 explained 12.6% of the variance. The PCA revealed some interannual clustering between individuals (Figure 2.3.1). 2009 and 2017 showed distinct separation from other years. 2009 had the highest sea ice extent in the Beaufort Sea in June for the study period (95.9%), where 2017 had the lowest sea ice extent over the study period (76.4%). Contributions of each fatty acid to principal components are listed in the Appendix (Table A5).

To determine if fatty acid profiles of beluga whales were related to any other explanatory variables, I plotted factors 1 and 2 of the PCA against the explanatory variables. The Bonferroni correction for multiple comparisons resulted in a p-value of 0.005. Factor 1 was found to be related to sea ice ($p<0.005$, $R^2=0.29$; Figure 2.3.2). Factor 2 was related to all three body condition metrics: blubber thickness ($p<0.05$, $R^2=0.32$; Figure 2.3.3), length ($p<0.05$, $R^2=0.10$) and girth ($p<0.005$, $R^2=0.22$).

2.3.2 Relationships between total gene expression and explanatory variables

To determine whether there were any relationships between the expression of the genes of interest, a PCA was performed on the expression of all genes for individual whales for blubber and liver separately.

The PCA for blubber gene expression did not reveal any distinct clustering patterns (Figure 2.3.4). Factor 1 accounted for 78% of the variance, whereas factor 2 accounted for 11% of the variance. Next, factor 1 from the PCA on blubber gene expression was plotted against explanatory variables to see if these variables could explain any variation in gene expression. The application of a Bonferroni correction led to a p-value of 0.0045 for this analysis. Factor 2 is not discussed here, as factor 1 accounted for such a high percentage of the variance in gene expression. Some interesting patterns emerged between factor 1 and the explanatory variables; however, these patterns were not significant after the Bonferroni correction. Percentage of sea ice extent in June, which is the determining factor in the timing of the Beaufort Sea beluga population's annual migration towards the Mackenzie River Estuary, total mercury (THg) and

total polychlorinated biphenyls (PCBs) all displayed a non-significant negative relationship with factor 1 (Figure 2.3.5). In contrast, beluga length and $\delta^{13}C$ values displayed positive relationships with Factor 1, however only the relationship with $\delta^{13}C$ was found to be significant after a Bonferroni correction ($p<0.0045$).

Similar to the PCA performed for blubber transcripts, liver transcripts did not reveal any distinct clustering patterns (Figure 2.3.6). Factor 1 accounted for 90% of the variance and factor 2 accounted for 2.7% of the variance. As in blubber, I only explored relationships between factor 1 loadings and explanatory variables due to the high amount of variance explained by factor 1. Like blubber, June sea ice extent was related to factor 1, but this was not statistically significant after a Bonferroni correction. This indicates that climate, which influences sea ice extent, may play a role in the gene expression of beluga whales. However, no other variables exhibited strong relationships with factor 1 of the liver transcript profiles.

Factors 1 and 2 obtained from the PCA performed on fatty acids were plotted against factor 1 obtained from the PCAs performed on both blubber and liver transcripts. No significant relationships emerged.

2.3.3 Relationships between individual genes and explanatory variables

AIC was used to determine which variables (percent sea ice extent in June in the Beaufort Sea, factor 1 of the PCA for fatty acids, factor 2 of the PCA for fatty acids, beluga body length, total PCBs, total PBDEs and total Hg) contributed most to explaining the variance in the expression of each gene for both blubber and liver. Results are presented in Table 2.3.1. Factors 1 and 2 of the

PCA for fatty acids, sea ice and length seemed to contribute most to the expression of many genes. In order to reduce the number of variables in the analysis and to avoid confounding variables, length was the only body condition metric used, as length is most indicative of beluga whale habitat and foraging patterns (Loseto et al., 2008).

As stable isotopes could not be included in the AIC analysis, stable isotopes were plotted against gene expression results for all genes, in both blubber and liver. $\delta^{13}C$ was found to have significant relationships with HP, ERα, DI1, DI2, TNFα, UCP2, HspA1A and ILGF1 in blubber (Figure 2.3.8), as well as HP, ERα, DI2, TNFα and TRβ in liver (Figure 2.3.9). All genes were negatively correlated with $\delta^{13}C$. Similar patterns were conserved among all genes, where individuals from 2008 and 2010 tended towards the bottom right corner (lowest gene expression and highest $\delta^{13}C$ values), where individuals from 2013, 2015 and 2017 tended towards the top left corner (highest gene expression and lowest $\delta^{13}C$ values). Other years were distributed between the two groupings, forming a negative relationship between gene expression and $\delta^{13}C$. No significant correlations were found when $\delta^{15}N$ was plotted against gene expression.

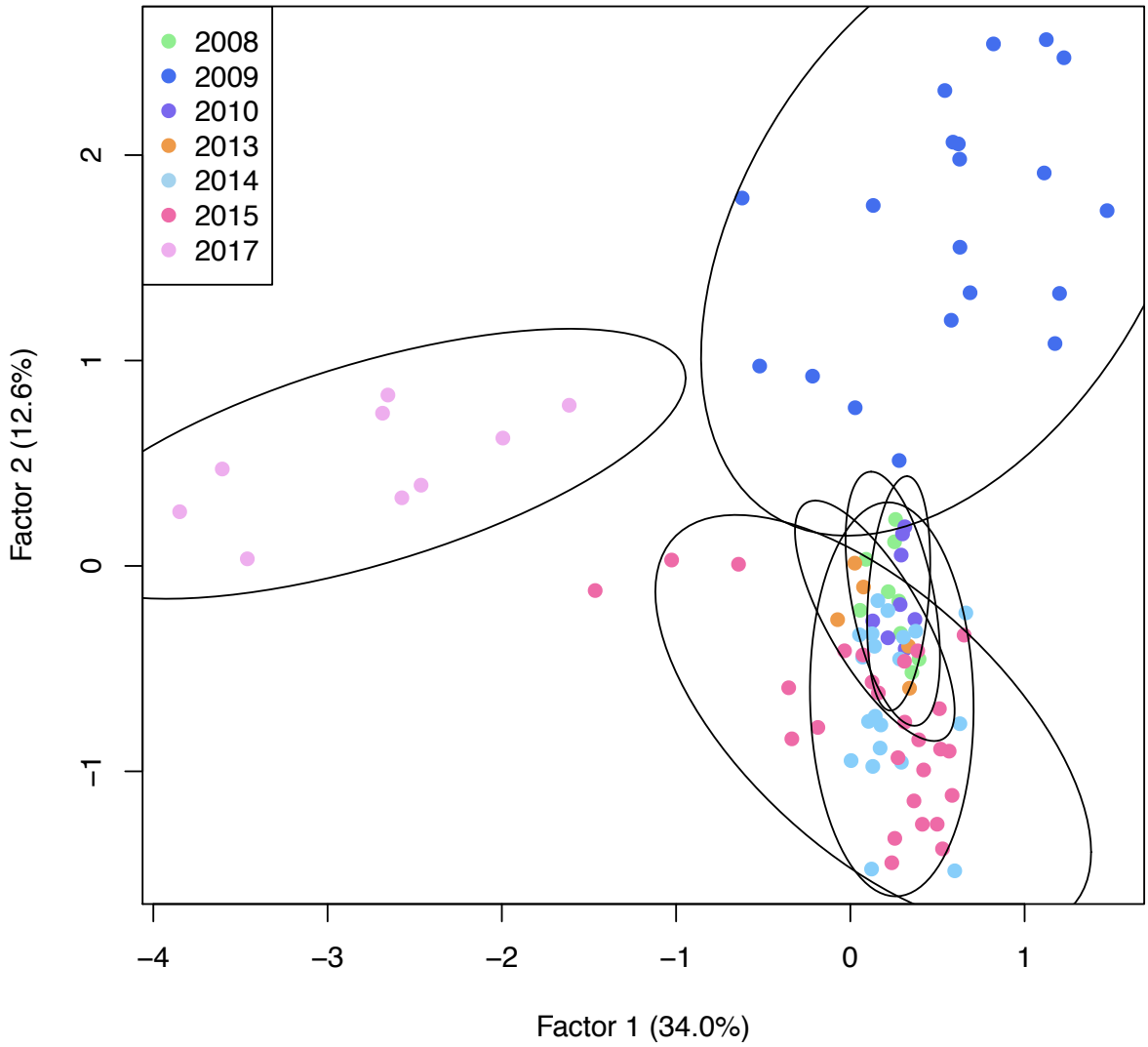

Figure 2.3.1 A principal component analysis (PCA) was performed for fatty acids across all years. Fatty acids were represented as percentages. The PCA reveals interannual clustering, with 2009 and 2017 separating from the rest of the data. 2009 has the highest sea ice extent over the study period, where 2017 has the lowest sea ice over the study period. However, other years have similarly high and low sea ice extents and do not show the same distinct clustering, indicating that other factors may influence fatty acids.

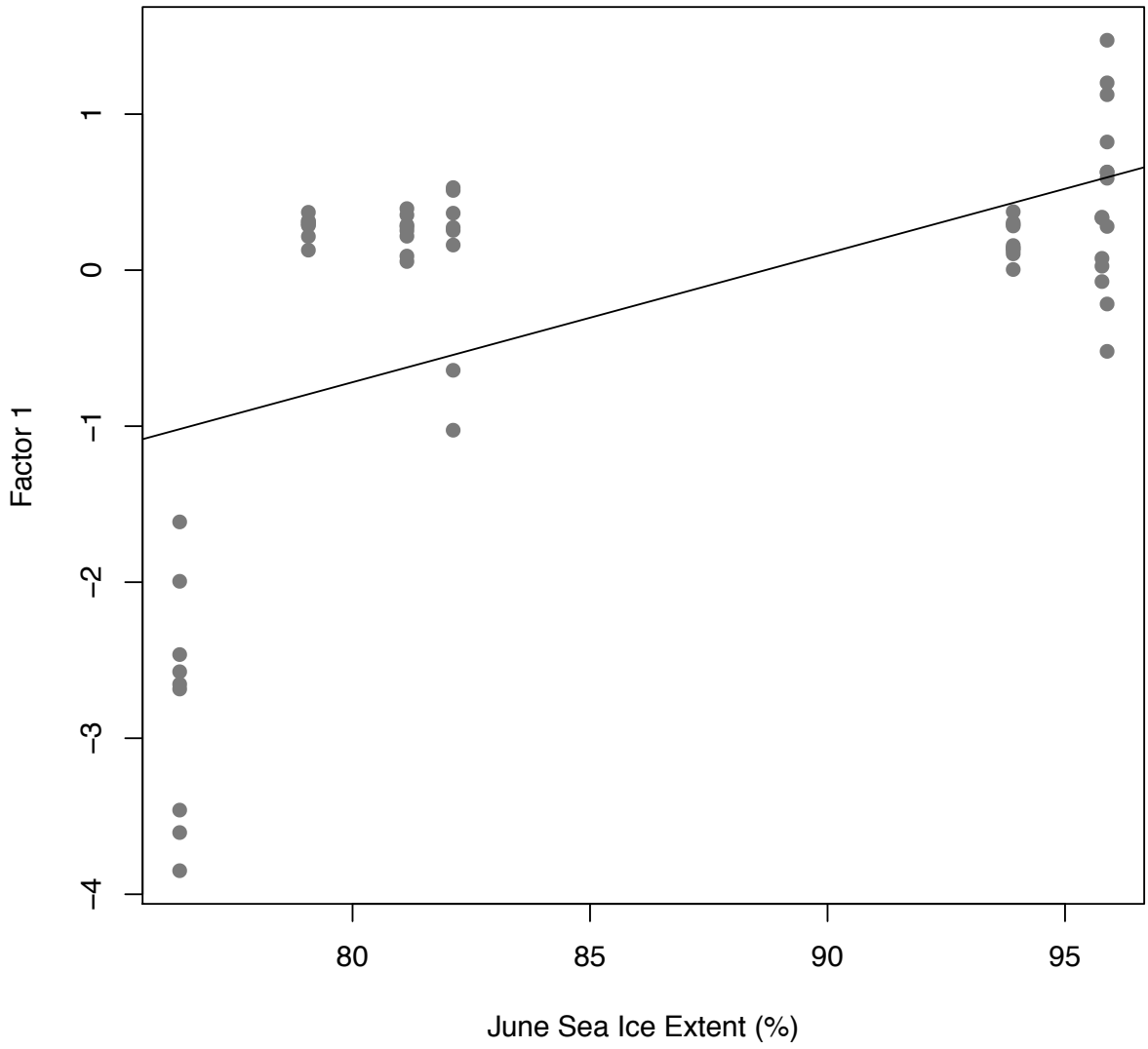

Figure 2.3.2 Factor 1 obtained through a principal component analysis for fatty acids across all years versus percent June sea ice extent in the Beaufort Sea reveals a significant relationship ($p<0.005$, $R^2=0.29$).

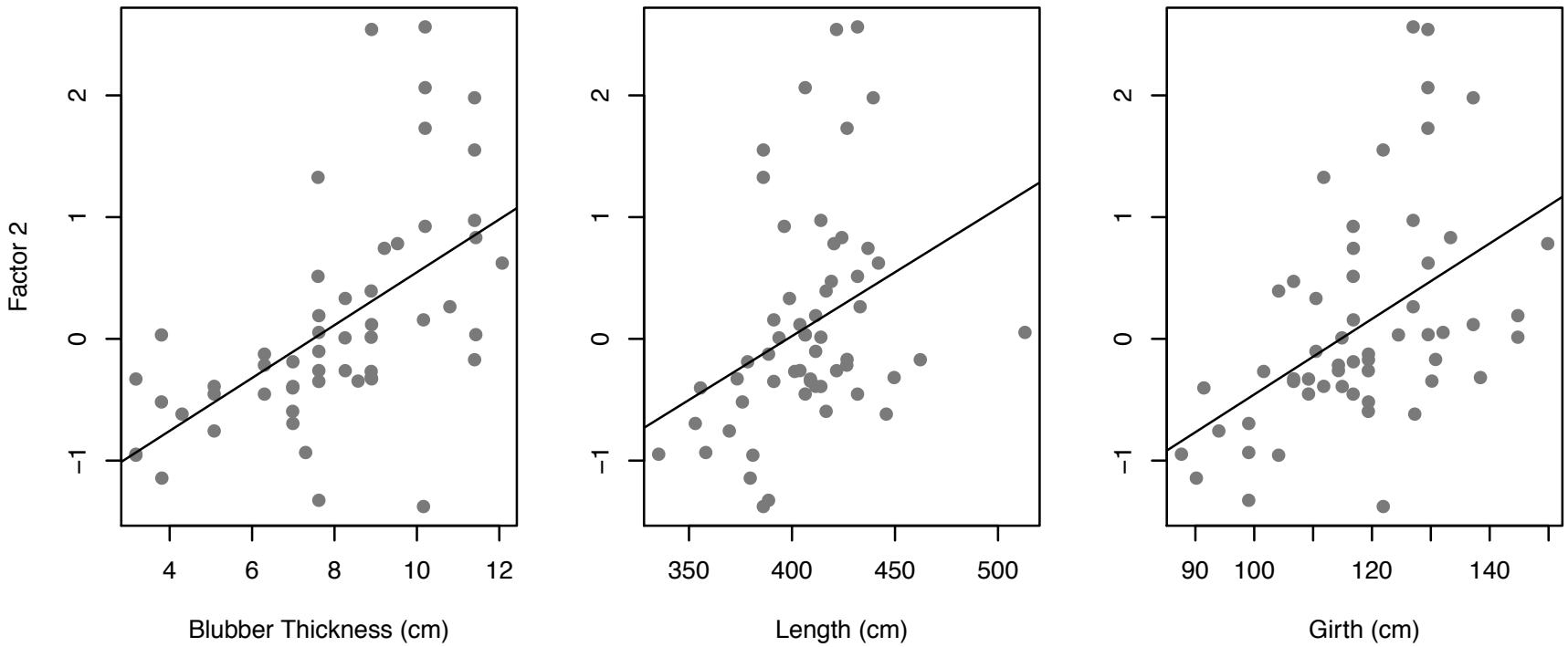

Figure 2.3.3 Factor 2 of the principal component analysis performed on fatty acids across all years versus blubber thickness ($p<0.005$, $R^2=0.032$), length ($p<0.005$, $R^2=0.10$) and girth ($p<0.005$, $R^2=0.22$) of beluga whales reveal significant relationships.

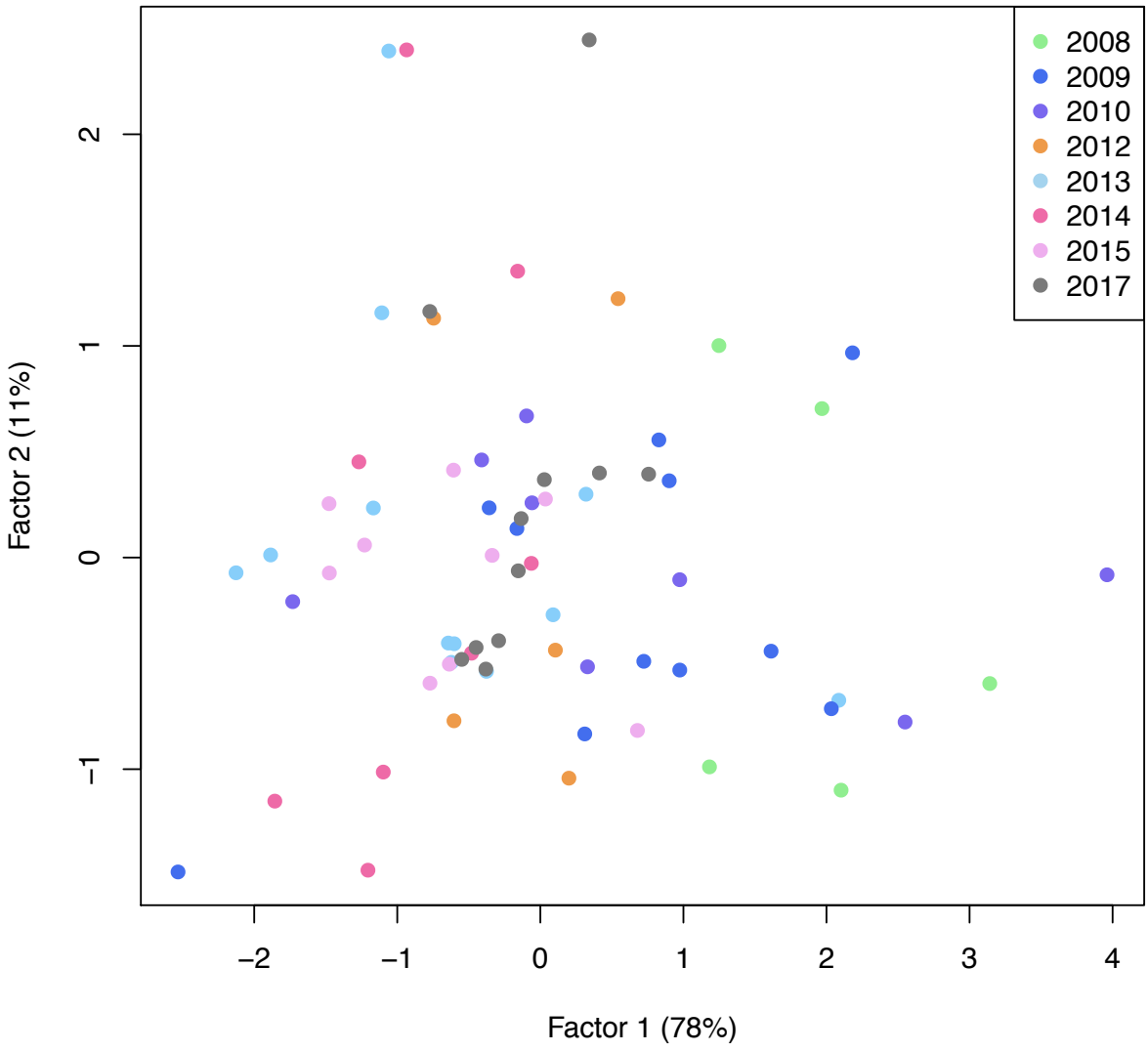

Figure 2.3.4 Results of the principal component analysis (PCA) performed on the gene expression found for all genes, with the exception of AhR due to low amplification, in blubber. Individuals are represented by dots. No distinct clustering patterns emerged.

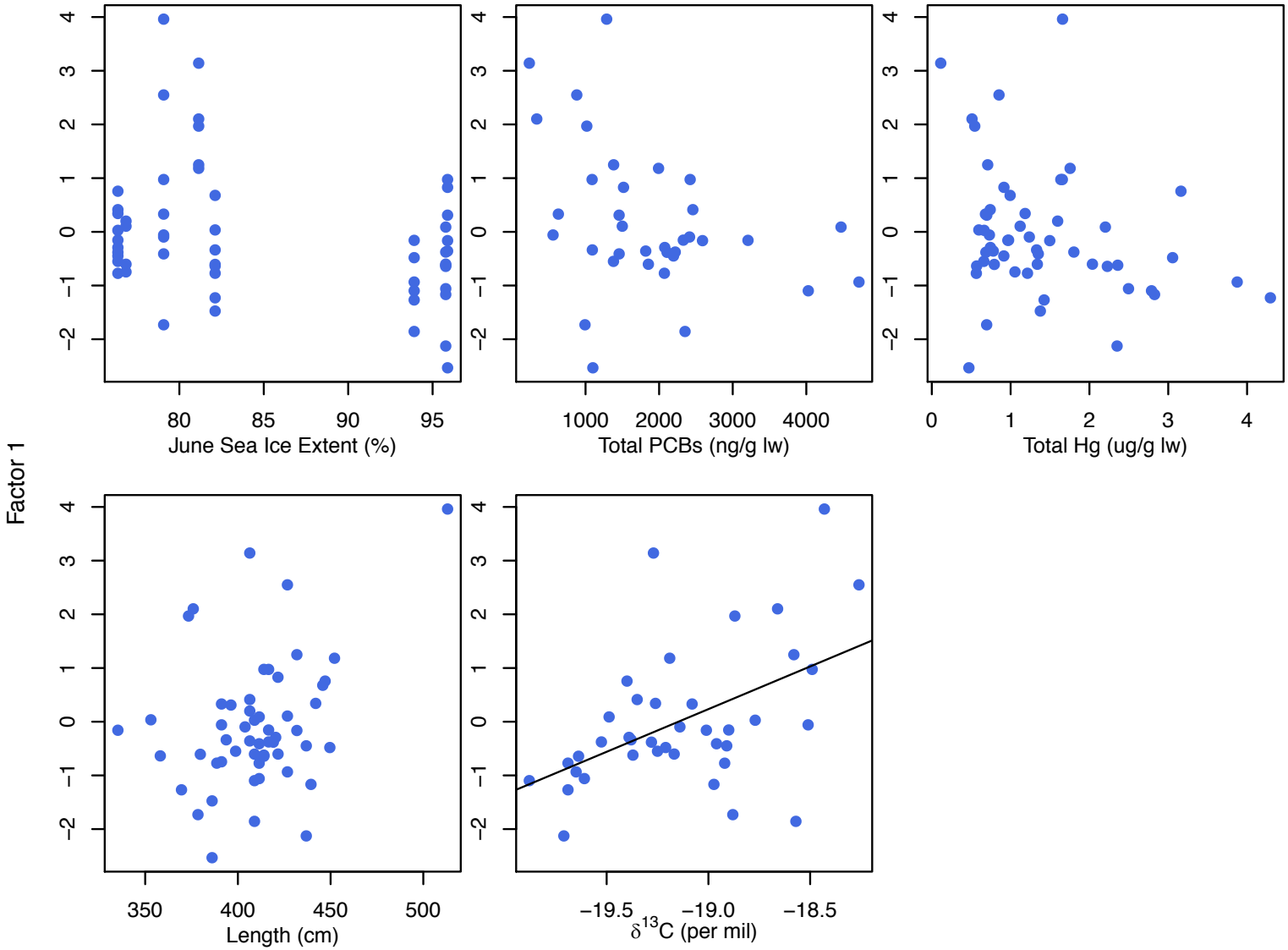
Factor 1
June Sea Ice Extent (%)
Total PCBs (ng/g lw)
Total Hg (ug/g lw)
Length (cm)
δ13C (per mil)

Figure 2.3.5 Factor 1, determined from a principal component analysis of blubber gene expression profiles, revealed relationships between several of the explanatory variables. After a Bonferroni correction, only $\delta^{13}C$ ($p<0.0045$, $R^2=0.2206$) displayed a significant positive correlation with factor 1. All other relationships were non-significant. Percent June sea ice extent (which determines the timing of the commencement of the Beaufort Sea beluga migration; $p=0.0162$, $R^2=0.0873$), total Hg (ug/g lw; $p=0.0459$, $R^2=0.05663$), and total PCBs (ng/g lw; $p=0.0226$, $R^2=0.1219$) displayed non-significant negative relationships with factor 1. Length of each beluga (cm; $p=0.0185$, $R^2=0.0849$) displayed a non-significant positive correlation with factor 1.

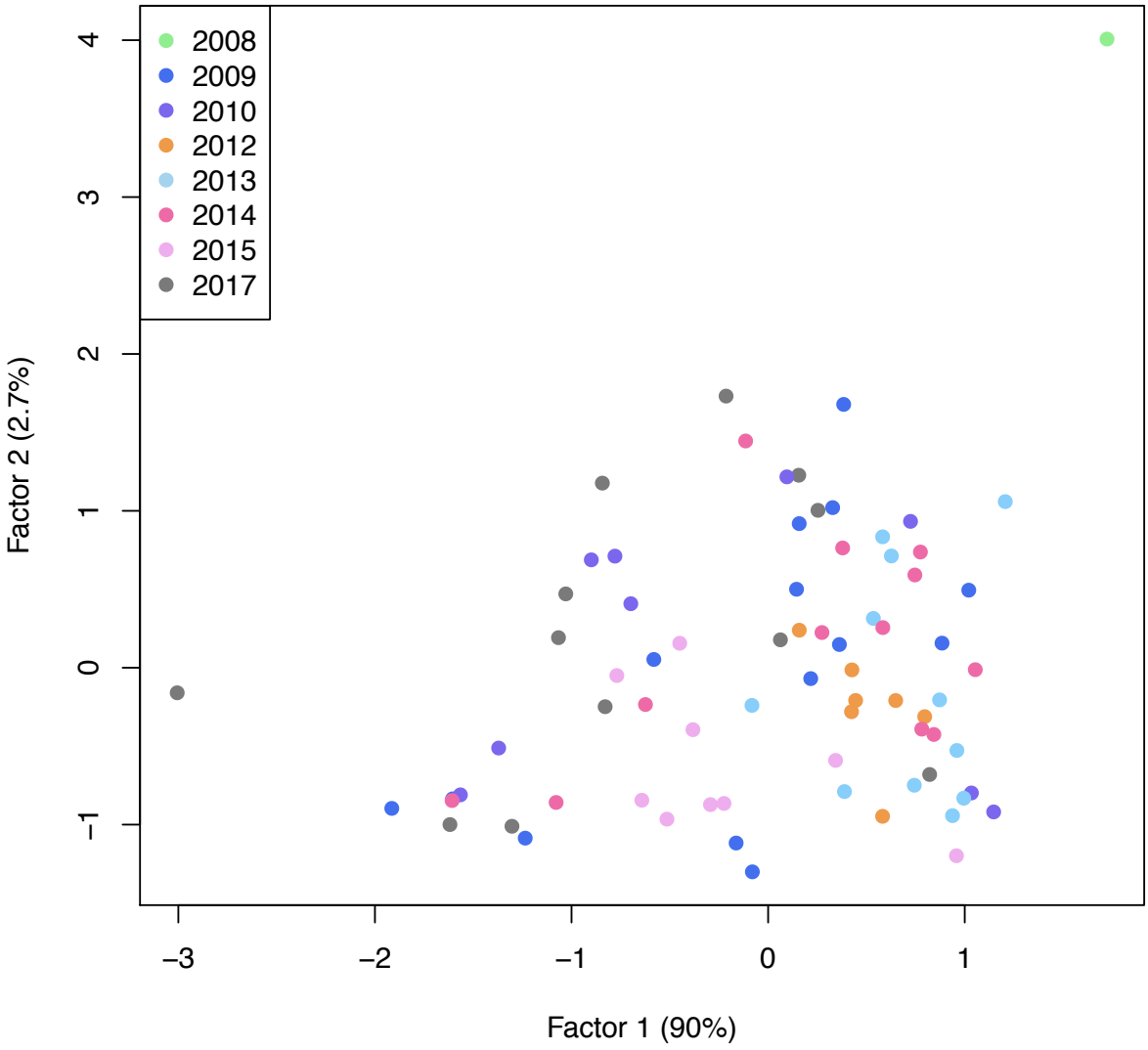

Figure 2.3.6 The principal component analysis (PCA) performed on gene expression for all genes with the exception of AhR and Adiponectin which both had low expression, for liver. Dots represent individual beluga whales. No distinct clustering patterns emerged during this analysis.

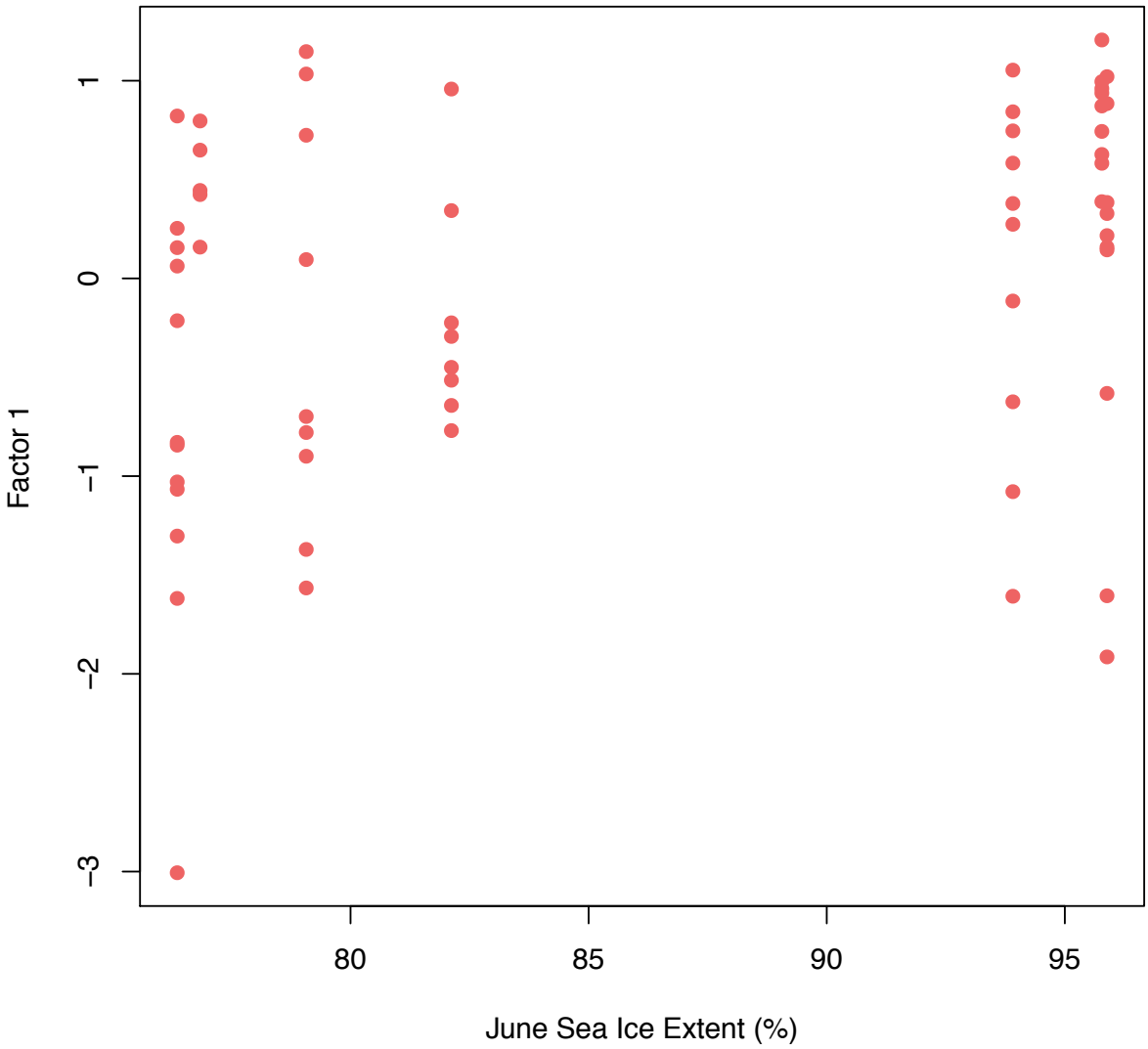
Factor 1
1
0
−1
−2
−3
80
85
90
95
June Sea Ice Extent (%)

Figure 2.3.7 Factor 1, determined from a PCA of liver gene expression profiles, revealed a non-significant relationship with June sea ice extent in the Beaufort Sea after a Bonferroni correction ($p=0.016$, $R^2=0.077$).

Table 2.3.1 Results of the Akaike Information Criterion (AIC) performed to determine which variables explained most of the variance for each gene. Due to a small sample size, second order AIC_c was calculated (AIC_c=AIC + (2K(K+1/n-K-1), where K is the total number of parameters and n is sample size. ΔAIC_c is the difference AIC-AIC_{min}. W_i indicates the relative weight of each parameter compared to the rest of the models, calculated as $W_i=(\exp(-1/2*\Delta AIC_c)/\Sigma(\exp(-1/2*\Delta AIC_c)$, where the bottom term represents the sum of all model weights. The model chosen is 'best' is represented below. In cases where the model chosen as 'best' was not different than the intercept (represented as N/A under predictors), the second 'best' model is also shown.

Gene	Tissue	Predictors	p	R^2	AIC	AIC_c	ΔAIC_c	W_i
AhR	Blubber	Sea ice, PC1 fatty acids, PC2 fatty acids	**p<0.05**	0.77	177.22	214.55	0	0.43
	Liver	N/A	N/A	N/A	294.34	302.96	0	0.23
		Sea ice	p>0.05	0.038	294.45	303.07	0.11	0.22
ERα	Blubber	Length, Hg	**p<0.05**	0.25	296.14	301.74	0	0.24
	Liver	Length, PCBs, Hg	**p<0.05**	0.21	500.59	504.59	0	0.27
TRβ	Blubber	Sea ice, length, PCBs, PC1 and PC2 fatty acids	p>0.05	0.12	335.24	343.86	0	0.24
	Liver	N/A	N/A	N/A	590.12	595.21	0	0.25
		Length	p>0.05	0.016	590.6	595.69	0.48	0.20
DI1	Blubber	PC2 fatty acids	p>0.05	0.090	386.23	392.82	0	0.22

	Liver	PC2 fatty acids	p>0.05	0.077	286.11	202.004	0	0.27
DI2	Blubber	N/A	N/A	N/A	402.23	407.83	0	0.27
		Length	p>0.05	-0.000028	402.18	408.78	0.95	0.17
	Liver	Sea ice, length, PC1 fatty acids	**p<0.05**	0.24	443.15	448.75	0	0.35
UCP2	Blubber	Hg, PC1 fatty acids	**p<0.05**	0.16	241.65	247.25	0	0.28
	Liver	Length, PC2 fatty acids	p>0.05	0.089	515.03	519.18	0	0.25
HP	Blubber	Hg	**p<0.05**	0.14	394.36	400.25	0	0.35
	Liver	PC2 fatty acids	p>0.05	0.066	162.18	166.18	0	0.25
HspA1A	Blubber	Sea ice, length, Hg	**p<0.05**	0.64	348.63	354.85	0	0.35
ILGF1	Blubber	N/A	N/A	N/A	292.98	298.58	0	0.27
		Length	p>0.05	0.00029	293.91	299.51	0.93	0.17
TNFα	Blubber	N/A	N/A	N/A	434.75	440.35	0	0.26
		Length	p>0.05	0.0032	435.61	441.21	0.86	0.17
	Liver	Sea ice, length, Hg	p>0.05	0.092	528.31	532.31	0	0.20

Adipo	Blubber	N/A	N/A	N/A	196.58	201.91	0	0.25
		Sea ice	p>0.05	0.022	196.87	202.20	0.29	0.22
	Liver	Length	p>0.05	0.19	310.72	329.39	0	0.27
Leptin	Blubber	PC1 fatty acids	p>0.05	0.050	562.68	569.27	0	0.27

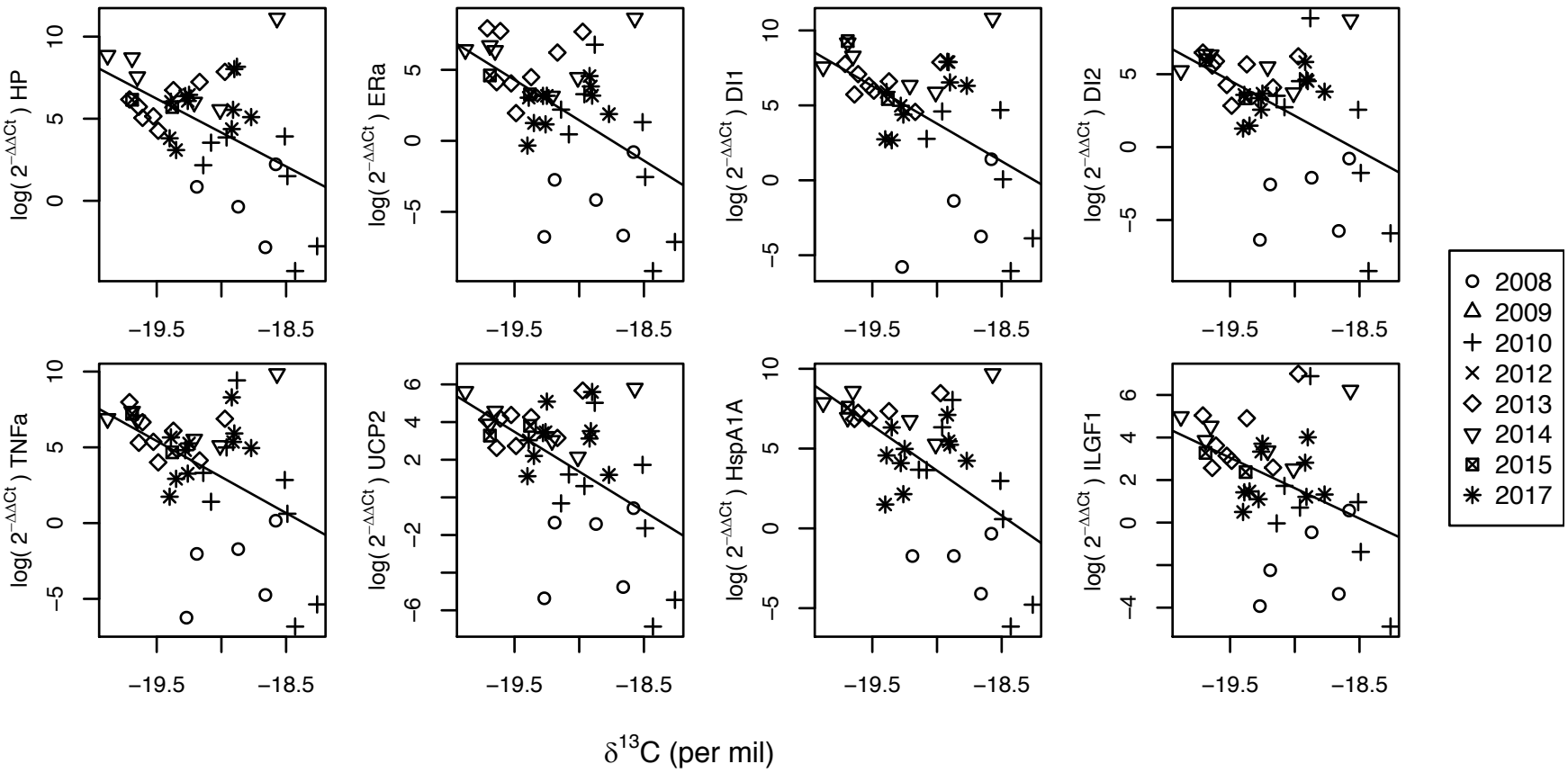

Figure 2.3.8 Expression of each gene in blubber was logged and plotted against $\delta^{13}C$. Relationships were evaluated using a linear model. All relationships displayed were determined to be significant: HP ($p=0.00102$, $R^2=0.2414$), ERα ($p=0.000654$, $R^2=0.2528$), DI1 ($p=0.00195$, $R^2=0.233$), DI2 ($p=0.0022$, TNFα

(p=0.00309, R^2=0.192), UCP2 (p=0.000556, R^2=0.2589), HspA1A (p=0.000378, R^2=0.3093), and ILGF1 (p=0.0107, R^2=0.1446). This indicates a negative relationship between gene expression and $\delta^{13}C$.

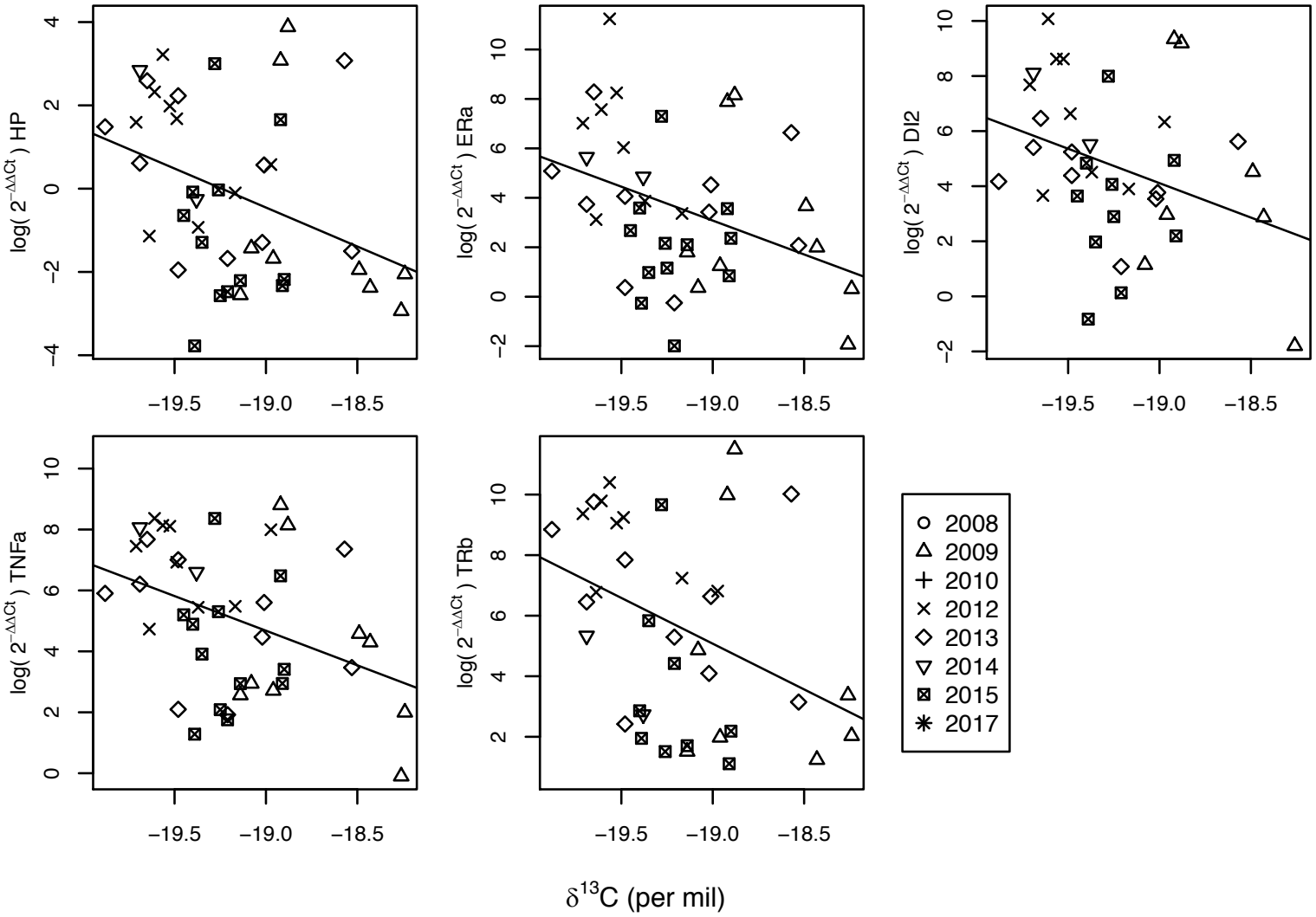
log(2^−ΔΔCt) HP
log(2^−ΔΔCt) ERa
log(2^−ΔΔCt) DI2
log(2^−ΔΔCt) TNFa
log(2^−ΔΔCt) TRb
−19.5
−19.0
−18.5
δ13C (per mil)
2008
2009
2010
2012
2013
2014
2015
2017

Figure 2.3.9 Expression of each gene in liver was logged and plotted against $\delta^{13}C$. Relationships were evaluated using a linear model. All relationships displayed were determined to be significant: HP ($p=0.0188$, $R^2=0.2414$), ERα ($p=0.0169$, $R^2=0.1157$), ($p=0.0409$, $R^2=0.08881$), TNFα ($p=0.0113$, $R^2=0.1288$), and TRβ ($p=0.0202$, $R^2=0.1202$). This indicates a negative relationship between gene expression and $\delta^{13}C$.

2.4 Discussion

2.4.1 Impacts of climate change on gene expression

The results of this study indicate that interannual variation in nutritional status may be responsible for wide-spread changes in gene expression in both blubber and liver. When factor 1 from the PCA performed on gene expression for blubber was plotted against $\delta^{13}C$, a significant relationship was detected ($p<0.0045$; Figure 2.3.5). $\delta^{13}C$ was the only explanatory variable that displayed a significant relationship with a principle component from either blubber or liver after a Bonferroni correction. Carbon isotope signatures are typically reflective of changes in diet from a more land-based source, which is heavier in ^{12}C, to a more marine-based source, which is heavier in ^{13}C. The use of $\delta^{13}C$ can therefore be used to assess whether organisms are feeding closer to shore or offshore (Choy et al., 2016; Dahl et al., 2003; Søreide et al., 2006). For beluga whales in the Arctic, this tool can be used to suggest whether beluga whales are foraging further offshore in periods of low sea ice extent. The relationship between factor 1 of total gene expression in blubber and $\delta^{13}C$ indicates that a change in foraging behaviour in beluga whales influenced the total gene expression in blubber.

The relationship between $\delta^{13}C$ and gene expression was also reflected in many individual genes in both blubber and liver. HP, ERα, DI1, DI2, TNFα, UCP2, HspA1A and ILGF1 all showed significant relationships with $\delta^{13}C$ in blubber (Figure 2.3.8). In liver, HP, ERα, DI2, TNFα and TRβ all showed significant relationships with $\delta^{13}C$ (Figure 2.3.9). For all genes that displayed a significant relationship, in both blubber and liver, the relationship was negative, indicating that a decrease in gene expression is associated with an increase in $\delta^{13}C$. The most likely explanation

for an enrichment in $\delta^{13}C$ values would be a decrease in sea ice, which would compel beluga whales to feed further offshore. Indeed, 2008 and 2010 have reasonably low sea ice extents (81% and 79%, respectively; Appendix A, Table A1) and consistently displayed the lowest gene expression in blubber associated with a ^{13}C-enriched $\delta^{13}C$ ratio (Figure 2.3.8). Liver gene expression for 2010 was also the lowest and was associated with a ^{13}C-enriched $\delta^{13}C$ ratio (Figure 2.3.9). Further, blubber gene expression was consistently highest in 2014, associated with ^{13}C-depleted $\delta^{13}C$ values over the study period and a high sea ice extent (93%; Appendix A, Table A1). These observations are indicative of feeding further offshore, which would be consistent with reduced sea ice extent. However, other years do not follow the same pattern of reduced sea ice extent coupled with enriched $\delta^{13}C$. If we explore the results from the AIC and relate them to $\delta^{13}C$ results, we see that those genes that showed relationships with $\delta^{13}C$ are not best explained by June sea ice extent in the Beaufort Sea. Only HspA1A in blubber, and DI2 and TNFα in liver, showed relationships with $\delta^{13}C$ and were best explained by sea ice (Table 2.3.1, Figures 2.3.8 & 2.3.9).

Several explanations may exist for the disconnect between the influence of sea ice extent and $\delta^{13}C$ on gene expression. One study noted that $\delta^{13}C$ ratios were notably different in ice algae versus phytoplankton (Søreide et al., 2006). Ice algae was 2-10 ‰ enriched in ^{13}C compared to phytoplankton, which could account for some of the differences seen, as beluga whales tend to feed closest to the ice edge, regardless of sea ice extent (Choy et al., 2016; Loseto et al., 2008; Søreide et al., 2006). Another explanation could be that the incorporation of isotopes into the tissues of consumers takes at minimum several weeks, which could allow for considerable variation depending on time of sampling and exact sea ice conditions (Boecklen et al., 2011).

None of the genes considered exhibited a clear relationship with $\delta^{15}N$. Two possible explanations could exist for this. $\delta^{15}N$ isotopes are typically an indicator of trophic feeding level, with higher $\delta^{15}N$ ratios indicating that the consumer is feeding higher in the trophic level (Boecklen et al., 2011; Dahl et al., 2003). One explanation for the lack of relationships between gene expression and $\delta^{15}N$ is that beluga whales may not be feeding at significantly different trophic levels, and therefore we would not see a change in gene expression reflected. A second explanation could be that the expression of the genes considered in this study is not influenced by trophic feeding level.

PCA analysis of fatty acids revealed the separation of 2009, which was the year with the highest sea ice during the study period (95%), and 2017, which was the year of the lowest sea ice extent over the study period (76%), into two distinct clusters, separate from the rest of the represented years (Figure 2.3.1). Most fatty acids are obtained through diet, so any changes in fatty acid signatures may be indicative of altered feeding regimes (Choy et al., 2017). This indicates that climate change may be a driver behind fatty acid profiles in beluga whales, as fatty acid signatures are expected to be different in periods of high and low sea ice due to altered prey availability. Evidence for the relationship between fatty acids and climate change is further demonstrated by Figure 2.3.2, which shows a correlation between factor 1 of the fatty acid PCA and sea ice extent. These results suggest that sea ice may be a driver of changes in feeding composition; however, if sea ice were the only factor contributing to changes in fatty acid

composition between years, I would expect that other years with similarly high or low sea ice extent would separate out, as well.

2.4.2 Impacts of contaminants on gene expression

AIC results for individual genes show some evidence that contaminants may be playing a role in the expression of individual genes (Table 2.3.1). Several genes in blubber, including ERα, UCP2, HP, HspA1A, as well as TNFα in both blubber and liver, included total Hg in their best-fit models. HP expression in blubber was of particular interest in these results, as it was best explained by Hg alone, where the other genes were explained by a combination of variables. Many of these genes, including HP, HspA1A and TNFα, are involved in the acute phase response. While I was not able to find many associations in the literature between these genes and mercury exposure, Hsp70 transcripts have been reported to increase in response to toxic assaults by mercury in mammals and plants (Aït-Aïssa et al., 2000; Sánchez Reus et al., 2003; Zhang et al., 2017). In addition, a study on gene responses to methyl mercury in zebrafish showed overall transcriptional changes in genes involved in the acute inflammatory response (Ho et al., 2013).

Two genes, TRβ in blubber and ERα in liver, were explained by a combination of PCBs and other variables. No other genes were explained by PCBs (Table 2.3.1). Both TRβ and ERα have been studied extensively for their relationships with PCB exposure. For example, PCBs have been associated with a decrease in thyroid receptor activity in harbour seal pups and ringed seals (Routti et al., 2010; Tabuchi et al., 2006). PCBs have been noted to have both estrogenic and anti-estrogenic effects on ERα (Ma & Sassoon, 2006; Zhang et al., 2017). Indeed, they have been

associated with altered ERα mRNA expression in killer whales, harbour seals and beluga whales (Buckman et al., 2011; Noël et al., 2017; Simond et al., 2019).

2.4.3 Impacts of contaminants and climate change on gene expression

Based on the results from this analysis, some subtle indicators in the analysis point to a potential interaction between climate change and contaminants. For example, PCBs, PBDEs and Hg were all highest in 2014, which was a period of high sea ice extent (Appendix A, Table A1). Hg was also high in 2013, which was another year of high sea ice extent. However, other years of high sea ice extent do not have similarly high contaminant concentrations.

Further small indicators of the interaction between contaminants and climate change lie in the AIC results (Table 2.3.1). Several genes, including TRβ, UCP2 and HspA1A in blubber, as well as TNFα in liver, were best explained by a combination of contaminants, body length, sea ice extent and fatty acids. This indicates that both contaminants and climate change may be a driver behind the expression patterns of these genes; however, it is difficult to conclude whether contaminants and climate change are interacting to influence gene expression patterns or acting individually.

Neither contaminants nor climate change were able to fully explain all of the variation in gene expression alone, so it is conceivable that the two may interact or exacerbate each other. It is expected that the altered immunity, metabolism and physiology associated with exposure to contaminants may make marine mammals more susceptible to climate change (Alava et al., 2017). Conversely, there is concern that a loss of blubber due to changes in prey availability

could release contaminants from lipid layers, causing further harm (de Swart et al., 1996). This study was not able to provide firm evidence that contaminants and climate change are interacting to influence gene expression of beluga whales, however impending changes in the Arctic environment are still cause for concern. It is still largely uncertain as to how prey abundance and distribution will change, whether delivery of contaminants to the Arctic will be altered, and how beluga habitat distribution will be impacted by climate change. It is therefore important that we continue to monitor these species for signs of potential stress due to contaminants and climate change.

Chapter 3: General Discussion and Conclusions

3.1 Concluding remarks

The aim of this thesis was to explore the effects of contaminants and climate change on the health of western Arctic beluga whales. To achieve this goal, gene expression of various genes involved in xenobiotic detoxification, metabolism, stress response and nutritional health were measured. Gene expression values were compared across years and were related to possible explanatory variables that could give us a better understanding of how genes might be influenced by both climate change and contaminants. Measures of the influence of climate change included sea ice extent, as well as nutritional indicators that are likely to be influenced by sea ice extent: $\delta^{13}C$, $\delta^{15}N$, fatty acid data, and body condition metrics. While both $\delta^{13}C$ and $\delta^{15}N$ have been related to contaminants and gene expression in western Arctic beluga whales, the inclusion of fatty acid data was novel.

The PCA performed on fatty acids revealed the separation of the year with the highest sea ice extent over the study period and the year with the lowest sea ice extent (Figure 2.3.1), which is reflected in a significant relationship between fatty acids and sea ice extent (Figure 2.3.2). This suggests alterations in feeding patterns related to climate change. Carbon isotopes were found to be related to factor 1 of a PCA on gene expression in blubber. When exploring the relationship between the expression of individual genes and $\delta^{13}C$, I found that several genes were correlated with $\delta^{13}C$. These results indicate that changes in feeding patterns, represented by carbon isotopes, may influence the expression of genes. However, AIC results indicated that both contaminants and climate change may best explain the observed changes in gene expression.

Measures of the influence of contaminants included analyzing total concentrations of the top three contaminants of concern in the Arctic: PCBs, PBDEs and Hg. These measures were taken over a period of almost a decade, providing a larger time frame than previous work in order to demonstrate long-term trends. PCBs and Hg were both found to be related to the expression of several genes studied in an AIC analysis, indicating that contaminants may be playing a role in the health of beluga whales (Table 2.3.1)

In terms of determining the interaction of contaminants and climate change on the health of western Arctic beluga whales, I believe that this is a difficult conclusion to draw based on the available data. This thesis is able to demonstrate that both contaminants and climate change are likely influencing the expression of genes, and literature suggests that exposure to contaminants is likely to be influenced by changes in sea ice distribution. This would likely be due to several different factors. One factor is that prey species distribution is expected to move northward, bringing new species to the Arctic from the south (Ostertag et al., 2018). In the case of migratory prey species, this may bring new sources of contaminants to the Arctic (Ostertag et al., 2018). Another factor is that changes in prey species distribution and abundance may lead to beluga whales feeding at different trophic levels than normal. This would lead to a potential increase or decrease in exposure to toxins (Ostertag et al., 2018), which is reflected in fatty acid data.

This study was based on results from a previous study by Noël et al. (2014) who showed that altered gene expression of 11 genes involved in growth, metabolism and development was related to changes in sea ice extent; however, this study was conducted over a period of three years (2008-2010) and therefore it is difficult to draw broad conclusions about the relationship

between climate change and gene expression. PCA analysis of gene expression did not show strong associations with sea ice as in Noël et al. (2014), but it did show some associations with ^{13}C stable isotopes, indicating that climate may indeed be a driver behind changes in gene expression. Noël et al. (2014) also noted that two genes involved in detoxification of contaminants, Cyp1A1 and AhR, were related to PCB concentrations in beluga whales. Unfortunately, low concentrations of RNA did not allow for amplification of Cyp1A1. AhR was not amplified in all samples, so its inclusion in the PCA was not possible. Therefore, we cannot determine whether this pattern continued across an extended study period (2008-2017).

It is incredibly important that we continue to monitor this population of beluga whales. The monitoring of this population has been possible for 30 years through collaboration with the community of Tuktoyaktuk and the Department of Fisheries and Oceans, which presents a key opportunity to include scientists in the evaluation of beluga whale health. Not only are beluga whales a key part of the Arctic ecosystem, playing a role at the top of the food chain, but they are also strong indicators of the health of the Arctic food web. As top predators, they bioaccumulate pollutants that are likely to be represented throughout the Arctic food web. Finally, these creatures are an important subsistence and cultural resource for the Inuvialuit people of the Arctic. The health of the Inuvialuit is directly linked to the health of beluga whales, so monitoring this population is vital (Ostertag et al., 2018).

3.2 Future directions

Several paths could be taken to continue exploring the impacts of contaminants and climate change on the health of beluga whales. One such path includes the assembly of the beluga whale

transcriptome using RNASeq. The transcriptome of ringed seals was recently assembled, and several novel genes were found to be related to PCB exposure (Brown et al., 2017). This would be an invaluable tool in pinpointing genes that could be serve as biomarkers of health in relation to contaminants and climate change. While selecting genes that have previously been identified as biomarkers is a cost-efficient and useful tool to determine health impacts, RNASeq could provide additional insight.

This study also pointed to the limitations in using stable isotopes and fatty acids to infer changes in foraging patterns. While changes in foraging grounds are likely to occur with changes in sea ice, this will not necessarily be identifiable in isotope or fatty acid signatures, as prey may also be changing. It would be useful to incorporate a tagging study or aerial monitoring in order to get a better understanding of beluga whale migration patterns during periods of low and high sea ice. In addition, this would provide more information about population numbers, as the most recent population surveys were performed in 1992 (Allen & Angliss, 2013; COSEWIC, 2004; Harwood et al., 1996).

Another path that would be useful to take would be modelling changes in populations of beluga whales in a changing Arctic environment. Models have been built to predict changes in fish composition in various climate change scenarios in the Arctic (Tai et al., 2019). By incorporating this knowledge with habitat and feeding preferences of beluga whales, we may be able to predict the prey species that beluga whales may favour in the future, and potential areas that beluga whales may target as feeding grounds. This may help us in identifying key prey species that should be targeted for monitoring and conservation. It may also help identify areas of high

beluga whale abundance in the future, both of which will become increasingly important as the Arctic opens up to increased fishing and vessel traffic. While it is important to monitor beluga whale health and determine potential impacts of climate change and contaminants, it is also important to look forward and predict the way in which beluga whales might respond to stressors so that we can help them before they become in danger.

In terms of improving methods from this current study, it may be worth trying to amplify both the target and reference gene in the same well. This has been recommended for genes in tissues of low expression, such as our blubber samples. With such a low starting number of cDNA, there are concerns that even accurate pipetting will not allow for the same starting material between two samples. By amplifying both the target and reference gene in one well, we could ensure that both target and reference genes had exactly the same amount of starting material (cDNA), which would allow for more accurate readings of PCR results (Livak & Schmittgen, 2001).

Bibliography

1. Minamata Convention on mercury. in *United Nations Minamata Convention on Mercury* (2013).

2. Abbasi, G., Li, L. & Breivik, K. Global historical stocks and emissions of PBDEs. *Environ. Sci. Technol.* **53**, 6330–6340 (2019).

3. Adams, T. E., Epa, V. C., Garrett, T. P. J. & Ward, C. W. Structure and function of the type 1 insulin-like growth factor receptor. *Cell. Mol. Life Sci.* **57**, 1050–1093 (2000).

4. Ahl, S. *et al.* Adiponectin levels differentiate metabolically healthy vs unhealthy among obese and nonobese white individuals. *J. Clin. Endocrinol. Metab.* **100**, 4172–4180 (2015).

5. Aït-Aïssa, S., Porcher, J. M., Arrigo, A. P. & Lambré, C. Activation of the hsp70 promoter by environmental inorganic and organic chemicals: relationships with cytotoxicity and lipophilicity. *Toxicology* **145**, 147–157 (2000).

6. Alava, J. J. *et al.* Polychlorinated biphenyls and polybrominated diphenyl ethers in Galapagos sea lions (Zalophus wollebaeki). *Environ. Toxicol. Chem.* **28**, 2271–2282 (2009).

7. Alava, J. J., Cheung, W. W. L., Ross, P. S. & Sumaila, U. R. Climate change-contaminant interactions in marine food webs: toward a conceptual framework. *Glob. Chang. Biol.* **23**, (2017).

8. Allen, B. & Angliss, R. Alaska Marine Mammal Stock Assessments, 2012. *NOAA Tech. Memo.* 282 (2013). doi:10.7289/V5NS0RTS

9. Anderson, C. L., Jensen, J. L. & Ørntoft, T. F. Normalization of real-time quantitative reverse transcription-PCR data: a model-based variance estimation approach to identify genes suited for normalization, applied to bladder and colon cancer data sets. *Cancer Res.* **64**, 5245–5250 (2004).

10. ATSDR. Toxicological profile for mercury. *U.S. Department of Health and Human Services* (1999). doi:10.1104/pp.17.01785

11. Bai, X. *et al.* The protective effects of insulin-like growth factor-1 on neurochemical phenotypes of dorsal root ganglion neurons with BDE-209-induced neurotoxicity in vitro. *Toxicol. Ind. Health* **33**, 250–264 (2017).

12. Baserga, R. The contradictions of the insulin-like growth factor 1 receptor. *Oncogene* **19**, 5574–5581 (2000).

13. Baud, V. & Karin, M. Signal transduction by tumor necrosis factor and its relatives. *Trends Cell Biol.* **11**, 372–377 (2001).

14. Ben-David, M. & Flaherty, E. A. Stable isotopes in mammalian research: a beginner's guide. *J. Mammal.* **93**, 312–328 (2012).

15. Bennett, K. A. *et al.* Adiponectin and insulin in gray seals during suckling and fasting: relationship with nutritional state and body mass during nursing in mothers and pups. *Physiol. Biochem. Zool.* **88**, 295–310 (2015).

16. Bianco, A. C. & Kim, B. W. Deiodinases: implications of the local control of thyroid hormone action. *J. Clin. Invest.* **116**, 2571–2579 (2006).

17. Bierkens, J., Maes, J. & Vander Plaetse, F. Dose-dependent induction of heat shock protein 70 synthesis in Raphidocelis subcapitata following exposure environmental pollutants. *Environ. Pollut.* **101**, 91–97 (1998).

18. Boecklen, W. J., Yarnes, C. T., Cook, B. A. & James, A. C. On the use of stable isotopes in trophic ecology. *Annu. Rev. Ecol. Evol. Syst.* **42**, 411–440 (2011).

19. Borrell, A., Garcia-Solà, A., Aguilar, A., García, N. A. & Crespo, E. A. Organochlorine residues in south american sea lions, Otaria flavescens (Shaw, 1800): bioaccumulation and time trends. *Bull. Environ. Contam. Toxicol.* **84**, 731–737 (2010).

20. Braathen, M. *et al.* Relationships between PCBs and thyroid hormones and retinol in female and male polar bears. *Environ. Health Perspect.* **112**, 826–833 (2004).

21. Braune, B. M., Letcher, R. J., Gaston, A. J. & Mallory, M. L. Trends of polybrominated diphenyl ethers and hexabromocyclododecane in eggs of Canadian Arctic seabirds reflect changing use patterns. *Environ. Res.* **142**, 651–661 (2015).

22. Breton-Honeyman, K., Hammill, M. O., Furgal, C. M. & Hickie, B. Inuit Knowledge of beluga whale (Delphinapterus leucas) foraging ecology in Nunavik (Arctic Quebec), Canada. *Can. J. Zool.* **94**, 713–726 (2016).

23. Brown, T. M., Macdonald, R. W., Muir, D. C. G. & Letcher, R. J. The distribution and trends of persistent organic pollutants and mercury in marine mammals from Canada's Eastern Arctic. *Sci. Total Environ.* **618**, 500–517 (2018).

24. Brown, T. M. *et al.* PCB related effects thresholds as derived through gene transcript profiles in locally contaminated ringed seals (Pusa hispida). *Environ. Sci. Technol.* **48**, 12952–12961 (2014).

25. Buckman, A. H. *et al.* PCB-associated changes in mRNA expression in killer whales (Orcinus orca) from the NE Pacific Ocean. *Environ. Sci. Technol.* **45**, 10194–10202 (2011).

26. Buckman, A. H. *et al.* PCB-associated changes in mRNA expression in killer whales (Orcinus orca) from the NE Pacific Ocean. *Environ. Sci. Technol.* **45**, 10194–10202 (2011).

27. Budge, S. M., Springer, A. M., Iverson, S. J. & Sheffield, G. Fatty acid biomarkers reveal niche separation in an Arctic benthic food web. *Mar. Ecol. Prog. Ser.* **336**, 305–309 (2007).

28. Bustin, S. A. *et al.* The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clin. Chem.* **55**, 611–622 (2009).

29. Castelli, M. G., Rusten, M., Goksøyr, A. & Routti, H. mRNA expression of genes regulating lipid metabolism in ringed seals (Pusa hispida) from differently polluted areas. *Aquat. Toxicol.* **146**, 239–246 (2014).

30. Cheek, A. O., Kow, K., Chen, J. & Mclachlan, J. A. Potential mechanisms of thyroid disruption in humans: interaction of organochlorine compounds with thyroid receptor, transthyretin, and thyroid-binding globulin. *Exp. Heal. Perspect.* **107**, 273–278 (1999).

31. Chen, G. & Goeddel, D. V. TNF-R1 signaling : a beautiful pathway. *Science (80-.).* **296**, 1634–1635 (2002).

32. Chitnis, M. M., Yuen, J. S. P., Protheroe, A. S., Pollak, M. & Macaulay, V. M. The type 1 insulin-like growth factor receptor pathway. *Clin. Cancer Res.* **14**, 6364–6370 (2008).

33. Choy, E. S., Rosenberg, B., Roth, J. D. & Loseto, L. L. Inter-annual variation in environmental factors affect the prey and body condition of beluga whales in the eastern Beaufort Sea. *Mar. Ecol. Prog. Ser.* **579**, 213–225 (2017).

34. Choy, E. S., Roth, J. D. & Loseto, L. L. Lipid removal and acidification affect nitrogen and carbon stable isotope ratios of beluga whales (Delphinapterus leucas) and their potential prey species in the Beaufort Sea ecosystem. *Mar. Biol.* **163**, (2016).

35. Clarke, J. *et al.* Subarctic cetaceans in the southern Chukchi Sea. *Oceanography* **26**, 136–149 (2013).

36. Comiso, J. C. & Hall, D. K. Climate trends in the Arctic as observed from space. *WIREs Clim. Chang.* **5**, 389–409 (2014).

37. Committee on the Status of Endangered Wildlife in Canada. *COSEWIC assessment and update status report on the beluga whale Delphinapterus leucas in Canada*. (2004).

38. Cui, J., Panse, S. & Falkner, B. The role of adiponectin in metabolic and vascular disease: a review. *Clin. Nephrol.* **75**, 26–33 (2011).

39. Dahl, T. M. *et al.* Lipids and stable isotopes in common eider, black-legged kittiwake and northern fulmar: Atrophic study from an Arctic fjord. *Mar. Ecol. Prog. Ser.* **256**, 257–269 (2003).

40. Dahl, T. M. *et al.* Fatty acid composition of the blubber in white whales (Delphinapterus leucas). *Polar Biol.* **23**, 401–409 (2000).

41. Daugaard, M., Rohde, M. & Jäättelä, M. The heat shock protein 70 family: Highly homologous proteins with overlapping and distinct functions. *FEBS Lett.* **581**, 3702–3710 (2007).

42. de Swart, R. L., Ross, P. S., Vos, J. G. & Osterhaus, A. D. M. E. Impaired immunity in harbour seals (Phoca vitulina) exposed to bioaccumulated environmental contaminants: review of a long-term feeding study. *Environ. Health Perspect.* **104**, 823–828 (1996).

43. Denison, M. S. & Nagy, S. R. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu. Rev. Pharmacol. Toxicol.* **43**, 309–334 (2003).

44. Diano, S. & Horvath, T. L. Mitochondrial uncoupling protein 2 (UCP2) in glucose and lipid metabolism. *Trends Mol. Med.* **18**, 52–58 (2012).

45. Donadelli, M., Dando, I., Fiorini, C. & Palmieri, M. UCP2, a mitochondrial protein regulated at multiple levels. *Cell. Mol. Life Sci.* **71**, 1171–1190 (2014).

46. Dorak, M. T. *Real-Time PCR (Advanced Methods Series)*. (2006).

47. Environment Canada. *Canadian Environmental Protection Act, 1999. Environmental screening assessment report on polybrominated diphenyl ethers (PBDEs)*. (2004).

48. EPA. *Polybrominated diphenyl ethers (PBDEs) action plan*. *United States Environmental Protection Agency* (2009).

49. Erickson, M. D. & Kaley, R. G. Applications of polychlorinated biphenyls. *Environ. Sci. Pollut. Res.* (2010). doi:10.1007/s11356-010-0392-1

50. Eurell, T. E., Bane, D. P., Hall, W. F. & Schaeffer, D. J. Serum haptoglobin concentration as an indicator of weight gain in pigs. *Can. J. Vet. Res.* **56**, 6–9 (1991).

51. Ferguson, S. H., Kingsley, M. C. S. & Higdon, J. W. Killer whale (Orcinus orca) predation in a multi-prey system. *Popul. Ecol.* **54**, 31–41 (2012).

52. Ferrante, M. C. *et al.* Polychlorinated biphenyls (PCB 101, PCB 153 and PCB 180) alter leptin signaling and lipid metabolism in differentiated 3T3-L1 adipocytes. *Toxicol. Appl. Pharmacol.* **279**, 401–408 (2014).

53. Fetterer, F., Knowles, K., Meier, W. N., Savoie, M. & Windnagel, A. K. Sea Ice Index, Version 3 [Beaufort Sea Ice Index]. *National Snow and Ice Data Center* (2017). Available at: https://doi.org/10.7265/N5K072F8.

54. Frouin, H., Loseto, L. L., Stern, G. A., Haulena, M. & Ross, P. S. Mercury toxicity in beluga whale lymphocytes: limited effects of selenium protection. *Aquat. Toxicol.* **109**, 185–193 (2012).

55. Frouin, H., Lebeuf, M., Hammill, M., Masson, S. & Fournier, M. Effects of individual polybrominated diphenyl ether (PBDE) congeners on harbour seal immune cells in vitro. *Mar. Pollut. Bull.* **60**, 291–298 (2010).

56. Fu, D., Leef, M., Nowak, B. & Bridle, A. Thyroid hormone related gene transcription in southern sand flathead (Platycephalus bassensis) is associated with environmental mercury and arsenic exposure. *Ecotoxicology* **26**, 600–612 (2017).

57. Gånheim, C., Alenius, S. & Persson Waller, K. Acute phase proteins as indicators of calf herd health. *Vet. J.* **173**, 645–651 (2007).

58. Government of Canada. Canadian measures to implement the Minamata Convention on mercury. in (2017).

59. Grahl-Nielsen, O. *et al.* Fatty acid composition of the adipose tissue of polar bears and of their prey: ringed seals, bearded seals and harp seals. *Mar. Ecol. Prog. Ser.* **265**, 275–282 (2003).

60. Grannas, A. M. *et al.* The role of the global cryosphere in the fate of organic contaminants. *Atmos. Chem. Phys.* **13**, 3271–3305 (2013).

61. Haave, M. *et al.* Polychlorinated biphenyls and reproductive hormones in female polar bears at Svalbard. *Environ. Health Perspect.* **111**, 431–436 (2003).

62. Hall, A. J., Kalantzi, O. I. & Thomas, G. O. Polybrominated diphenyl ethers (PBDEs) in grey seals during their first year of life - are they thyroid hormone endocrine disrupters? *Environ. Pollut.* **126**, 29–37 (2003).

63. Hall, A. J. *et al.* Individual-based model framework to assess population consequences of polychlorinated biphenyl exposure in bottlenose dolphins. *Environ. Health Perspect.* **114**, 60–64 (2006).

64. Harwood, L. A., Innes, S., Norton, P. & Kingsley, M. C. S. Distribution and abundance of beluga whales in the Mackenzie estuary, southeast Beaufort Sea, and west Amundsen Gulf during late July 1992. *Can. J. Fish. Aquat. Sci.* **53**, 2262–2273 (1996).

65. Hauser, D. D. W. *et al.* Decadal shifts in autumn migration timing by Pacific Arctic beluga whales are related to delayed annual sea ice formation. *Glob. Chang. Biol.* (2016). doi:10.1111/gcb.13564

66. Hauser, D. D. W., Laidre, K. L., Suydam, R. S. & Richard, P. R. Population-specific home ranges and migration timing of Pacific Arctic beluga whales (Delphinapterus leucas). *Polar Biol.* **37**, 1171–1183 (2014).

67. Hegde, P. S., Jackson, J. R. & Schroeck, J. R. HSPA1A as a marker for sensitivity to KSP inhibitors. (2009).

68. Heldring, N. *et al.* Estrogen receptors: how do they signal and what are their targets. *Physiol. Rev.* **87**, 905–931 (2007).

69. Herman, D. P. *et al.* Feeding ecology of eastern North Pacific killer whales Orcinus orca from fatty acid, stable isotope, and organochlorine analyses of blubber biopsies. *Mar. Ecol. Prog. Ser.* **302**, 275–291 (2005).

70. Ho, N. Y. *et al.* Gene responses in the central nervous system of zebrafish embryos exposed to the neurotoxicant methyl mercury. *Environ. Sci. Technol.* **47**, 3316–3325 (2013).

71. Hoguet, J. *et al.* Spatial and temporal trends of persistent organic pollutants and mercury in beluga whales (Delphinapterus leucas) from Alaska. *Sci. Total Environ.* **449**, 285–294 (2013).

72. Honaker, J., King, G. & Blackwell, M. Amelia II: a program for missing data. 1–47 (2011).

73. Hornby, C. A., Hoover, C., Iacozza, J., Barber, D. G. & Loseto, L. L. Spring conditions and habitat use of beluga whales (Delphinapterus leucas) during arrival to the Mackenzie River Estuary. *Polar Biol.* **39**, 2319–2334 (2016).

74. Houseknecht, K. L., Baile, C. A., Matteri, R. L. & Spurlock, M. E. The biology of leptin: a review. *J. Anim. Sci.* **76**, 1405–1420 (1998).

75. Howell, G. I. & Mangum, L. Exposure to bioaccumulative organochlorine compounds alters adipogenesis, fatty acid uptake, and adipokine production in NIH3T3-L1 cells. *Toxicol. Vitr.* **25**, 394–402 (2011).

76. Isobe, T. *et al.* Organohalogen contaminants in striped dolphins (Stenella coeruleoalba) from Japan: present contamination status, body distribution and temporal trends (1978-2003). *Mar. Pollut. Bull.* **58**, 396–401 (2009).

77. Jepson, P. D. *et al.* Relationships Between Polychlorinated Biphenyls and Health Status in Harbor Porpoises (Phocoena phocoena) Stranded in the United Kingdom. *Environ. Toxicol. Chem.* **24**, 238–248 (2005).

78. Jinhui, L., Yuan, C. & Wenjing, X. Polybrominated diphenyl ethers in articles: a review of its applications and legislation. *Environ. Sci. Pollut. Res.* **24**, 4312–4321 (2017).

79. Jo, S. *et al.* Estimation of the biological half-life of methylmercury using a population toxicokinetic model. *Int. J. Environ. Res. Public Health* **12**, 9054–9067 (2015).

80. Jones, S. *et al.* The genome of the beluga whale (Delphinapterus leucas). *Genes (Basel).* **8**, 378 (2017).

81. Kadowaki, T. *et al.* Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest.* **116**, (2006).

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

83. Katzenellenbogen, B. S. & Katzenellenbogen, J. A. Estrogen receptor transcription and transactivation - estrogen receptor alpha and estrogen receptor beta: regulation by selective estrogen receptor modulators and importance in breast cancer. *Breast Cancer Res.* **2**, 335–344 (2000).

84. Kehrig, H. A. *et al.* Different species of mercury in the livers of tropical dolphins. *Anal. Lett.* **41**, 1691–1699 (2008).

85. Kelly, B. C., Ikonomou, M. G., Blair, J. D. & Gobas, F. A. P. C. Bioaccumulation behaviour of polybrominated diphenyl ethers (PBDEs) in a Canadian Arctic marine food web. *Sci. Total Environ.* **401**, 60–72 (2008).

86. Kirk, J. *et al.* Mercury in Arctic marine ecosystems: sources, pathways, and exposure. *Environ. Res.* **119**, 64–87 (2012).

87. Koopman, H. N., Pabst, D. A., McLellan, W. A., Dillaman, R. M. & Read, A. J. Changes in blubber distribution and morphology associated with starvation in the harbor porpoise (Phocoena phocoena): evidence for regional differences in blubber structure and funtion. *Physiol. Biochem. Zool.* **75**, 498–512 (2002).

88. Krey, A., Ostertag, S. K. & Chan, H. M. Assessment of neurotoxic effects of mercury in beluga whales (Delphinapterus leucas), ringed seals (Pusa hispida), and polar bears (Ursus maritimus) from the Canadian Arctic. *Sci. Total Environ.* **509**–**510**, 237–247 (2015).

89. Larkin, P., Sabo-Attwood, T., Kelso, J. & Denslow, N. D. Analysis of gene expression profiles in largemouth bass exposed to 17-β-Estradiol and to anthropogenic contaminants that behave as estrogens. *Ecotoxicology* **12**, 463–468 (2003).

90. Law, R. J. An overview of time trends in organic contaminant concentrations in marine mammals: going up or down? *Mar. Pollut. Bull.* **82**, 7–10 (2014).

91. Law, R. J. *et al.* Chlorobiphenyls in the blubber of harbour porpoises (Phocoena phocoena) from the UK: levels and trends 1991-2005. *Mar. Pollut. Bull.* **60**, 470–473 (2010).

92. Leong, K. G. & Karsan, A. Signaling pathways mediated by tumor necrosis factor alpha. *Histol. Histopathol.* (2000).

93. Levin, E. R. Cell localization, physiology, and nongenomic actions of estrogen receptors. *J. Appl. Physiol.* **91**, 1860–1867 (2001).

94. Levin, M. *et al.* Immunomodulatory effects of exposure to polychlorinated biphenyls and perfluoroalkyl acids in East Greenland ringed seals (Pusa hispida). *Environ. Res.* **151**, 244–250 (2016).

95. Li, Z. H. *et al.* Alteration of thyroid hormone levels and related gene expression in Chinese rare minnow larvae exposed to mercury chloride. *Environ. Toxicol. Pharmacol.* **38**, 325–331 (2014).

96. Livak, K. J. & Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCT method. *Methods* **25**, 402–408 (2001).

97. Loseto, L. L. *et al.* Summer diet of beluga whales inferred by fatty acid analysis of the eastern Beaufort Sea food web. *J. Exp. Mar. Bio. Ecol.* **374**, 12–18 (2009).

98. Loseto, L. L., Stern, G. A. & Ferguson, S. H. Size and biomagnification: how habitat selection explains beluga mercury levels. *Environ. Sci. Technol.* **42**, 3982–3988 (2008).

99. Loseto, L. L., Stern, G. A. & Macdonald, R. W. Distant drivers or local signals: Where do mercury trends in western Arctic belugas originate? *Sci. Total Environ.* **509**–**510**, 226–236 (2015).

100. Lu, J. Y. *et al.* Magnification of atmospheric mercury deposition to polar regions in springtime: the link to tropospheric ozone depletion chemistry. *Geophys. Res. Lett.* **28**, 3219–3222 (2001).

101. Ma, R. & Sassoon, D. A. PCBs exert an estrogenic effect through repression of the Wnt7a signaling pathway in the female reproductive tract. *Environ. Health Perspect.* **114**, 898–904 (2006).

102. Maia, A. L., Goemann, I. M., Meyer, E. L. S. & Wajner, S. M. Type 1 iodothyronine deiodinase in human physiology and disease. *J. Endocrinol.* **209**, 283–297 (2011).

103. Martinez, B. *et al.* Prolonged food deprivation increases mRNA expression of deiodinase 1 and 2, and thyroid hormone receptor -1 in a fasting-adapted mammal. *J. Exp. Biol.* **216**, 4647–4654 (2013).

104. Mashburn, K. L. & Atkinson, S. Variability in leptin and adrenal response in juvenile Steller sea lions (Eumetopias jubatus) to adrenocorticotropic hormone (ACTH) in different seasons. *Gen. Comp. Endocrinol.* **155**, 352–358 (2008).

105. Matthews, J., Celius, T., Halgren, R. & Zacharewski, T. Differential estrogen receptor binding of estrogenic substances: a species comparison. *J. Steroid Biochem. Mol. Biol.* **74**, 223–234 (2000).

106. McCallister, C., Siracusa, M. C., Shirazi, F., Chalkia, D. & Nikolaidis, N. Functional diversification and specialization of cytosolic 70-kDa heat shock proteins. *Sci. Rep.* **5**, (2015).

107. Mckinney, M. A. *et al.* Comparative hepatic microsomal biotransformation of selected PBDEs, including decabromodiphenyl ether, and decabromodiphenyl ethane flame retardants in Arctic marine-feeding mammals. *Environ. Toxicol. Chem.* **30**, 1506–1514 (2011).

108. McKinney, M. A. *et al.* Global change effects on the long-term feeding ecology and contaminant exposures of East Greenland polar bears. *Glob. Chang. Biol.* **19**, 2360–2372 (2013).

109. Miyagawa, S. *et al.* Differing species responsiveness of estrogenic contaminants in fish is conferred by the ligand binding domain of the estrogen receptor. *Environ. Sci. Technol.* **48**, 5254–5263 (2014).

110. Moore, S. E. & Reeves, R. R. Tracking arctic marine mammal resilience in an era of rapid ecosystem alteration. *PLoS Biol.* **16**, (2018).

111. Moore, S. E. *et al.* A new framework for assessing the effects of anthropogenic sound on marine mammals in a rapidly changing Arctic. *Bioscience* **62**, 289–295 (2012).

112. Mos, L., Cameron, M., Jeffries, S. J., Koop, B. F. & Ross, P. S. Risk-based analysis of polychlorinated biphenyl toxicity in harbor seals. *Integr. Environ. Assess. Manag.* **6**, 631–640 (2010).

113. Mos, L. *et al.* Chemical and biological pollution contribute to the immunilogical profiles of free-ranging harbor seals. *Environ. Toxicol. Chem.* **25**, 3110–3117 (2006).

114. Mulero-Navarro, S. & Fernandez-Salguero, P. M. New trends in aryl hydrocarbon receptor biology. *Front. Cell Dev. Biol.* **4**, (2016).

115. Murray, C. F. *et al.* Associations of serum haptoglobin in newborn dairy calves with health, growth, and mortality up to 4 months of age. *J. Dairy Sci.* **97**, 7844–7855 (2014).

116. Najafizadeh, S. R. *et al.* Analysis of serum heat shock protein 70 (HSPA1A) concentrations for diagnosis and disease activity monitoring in patients with rheumatoid arthritis. *Cell Stress Chaperones* **20**, 537–543 (2015).

117. Nargesi, A. A. *et al.* The lost correlation between heat shock protein 70 (HSPA1A) and plasminogen activator inhibitor-1 in patients with type 2 diabetes and albuminuria. *Cell Stress Chaperones* **21**, 361–365 (2016).

118. Nguyen, L. P. & Bradfield, C. A. The search for endogenous activators of the aryl hydrocarbon receptor. *Chem. Res. Toxicol.* **21**, 102–116 (2008).

119. Nielsen, M. J. & Moestrup, S. K. Receptor targeting of hemoglobin mediated by the haptoglobins: roles beyond heme scavenging. *Blood* **114**, 764–771 (2009).

120. Niemi, A. *et al. State of the ocean report for the Beaufort Sea Large Ocean Management Area*. (2012).

121. Noël, M., Loseto, L. L. & Stern, G. Legacy contaminants in the Eastern Beaufort Sea beluga whales (Delphinapterus leucas): are temporal trends reflecting regulations? *Arct. Sci.* **14**, (2018).

122. Noël, M. *et al.* Polychlorinated biphenyl-related alterations of the expression of essential genes in harbour seals (Phoca vitulina) from coastal sites in Canada and the United States. *Arch. Environ. Contam. Toxicol.* **73**, 310–321 (2017).

123. Noël, M. *et al.* Mercury accumulation in harbour seals from the northeastern Pacific Ocean: The role of transplacental transfer, lactation, age and location. *Arch. Environ. Contam. Toxicol.* **70**, 56–66 (2016).

124. Noël, M. *et al.* PCBs are associated with altered gene transcript profiles in Arctic beluga whales (Delphinapterus leucas). *Environ. Sci. Technol.* **48**, 2942–2951 (2014).

125. Ostertag, S. K. *et al.* 'That's how we know they're healthy': the inclusion of traditional ecological knowledge in beluga health monitoring in the Inuvialuit Settlement Region. *Arct. Sci.* **4**, 292–320 (2018).

126. Ostertag, S. K., Shaw, A. C., Basu, N. & Chan, H. M. Molecular and neurochemical biomarkers in Arctic beluga whales (Delphinapterus leucas) were correlated to brain mercury and selenium concentrations. *Environ. Sci. Technol.* **48**, 11551–11559 (2014).

127. Oswal, A. & Yeo, G. Leptin and the control of body weight: A review of its diverse central targets, signaling mechanisms, and role in the pathogenesis of obesity. *Obesity* **18**, 221–229 (2010).

128. Pfaffl, M. W., Tichopad, A., Prgomet, C. & Neuvians, T. P. Determination of stable housekeeping genes, differentially regulated target genes and sample integrity: BestKeeper - Excel-based tool using pair-wise correlations. *Biotechnol. Lett.* **26**, 509–515 (2004).

129. Pinto, B. *et al.* Monitoring of polychlorinated biphenyl contamination and estrogenic activity in water, commercial feed and farmed seafood. *Environ. Monit. Assess.* **144**, 445–453 (2007).

130. Pollak, M. Insulin-like growth factor physiology and cancer risk. *Eur. J. Cancer* **36**, 1224–1228 (2000).

131. Provost, T., Kennedy, M., Castracane, V. D. & Meserve, L. A. The effects of polychlorinated biphenyl on circulating leptin and thyroid hormone status in Sprague-Dawley rats, Rattus norvegicus. *Ohio J. Sci.* **107**, 19–22 (2007).

132. R Core Team. A language and environment for statistical computations. (2013).

133. Raach, M., Lebeuf, M. & Pelletier, É. PBDEs and PCBs in the liver of the St Lawrence Estuary beluga (Delphinapterus leucas): a comparison of levels and temporal trends with the blubber. *J. Environ. Monit.* **13**, 649–656 (2011).

134. Rahman, F., Langford, K. H., Scrimshaw, M. D. & Lester, J. N. Polybrominated diphenyl ether (PBDE) flame retardants. *Sci. Total Environ.* **275**, 1–17 (2001).

135. Reeves, R. R. *et al.* Distribution of endemic cetaceans in relation to hydrocarbon development and commercial shipping in a warming Arctic. *Mar. Policy* **44**, 375–389 (2013).

136. Risebrough, R. W., Rieche, P., Herman, S. G., Peakall, D. B. & Kirven, M. N. Polychlorinated biphenyls in the global ecosystem. *Nature* **220**, 1098–1102 (1968).

137. Ross, P. S. Marine mammals as sentinels in risk assessment. *Hum. Ecol. Risk Assess. An Int. J.* **6**, 29–46 (2000).

138. Ross, P. S. *et al.* Large and growing environmental reservoirs of Deca-BDE present an emerging health risk for fish and marine mammals. *Mar. Pollut. Bull.* **58**, 7–10 (2009).

139. Ross, P. S., De Swart, R. L., Van Loveren, H., Osterhaus, A. D. M. E. & Vos, J. G. The immunotoxicity of environmental contaminants to marine wildlife: A review. *Annu. Rev. Fish Dis.* **6**, 151–165 (1996).

140. Ross, P. S. *et al.* Declining concentrations of persistent PCBs, PBDEs, PCDEs, and PCNs in harbor seals (phoca vitulina) from the salish sea. *Prog. Oceanogr.* **115**, 160–170 (2013).

141. Ross, P. S. *et al.* Declining concentrations of persistent PCBs, PBDEs, PCDEs, and PCNs in harbor seals (phoca vitulina) from the salish sea. *Prog. Oceanogr.* **115**, 160–170 (2013).

142. Rotander, A. *et al.* Polybrominated diphenyl ethers (PBDEs) in marine mammals from Arctic and North Atlantic regions, 1986-2009. *Environ. Int.* **40**, 102–109 (2012).

143. Roth, J. D. Temporal variability in arctic fox diet as reflected in stable-carbon isotopes; the importance of sea ice. *Oecologia* **133**, 70–77 (2002).

144. Routti, H. *et al.* Comparative endocrine disruptive effects of contaminants in ringed seals (Phoca hispida) from Svalbard and the Baltic Sea. *Comp. Biochem. Physiol. - Part C* **152**, 306–312 (2010).

145. Routti, H. *et al.* Hormone, vitamin and contaminant status during the moulting/fasting period in ringed seals (Pusa [Phoca] hispida) from Svalbard. *Comp. Biochem. Physiol. - A Mol. Integr. Physiol.* **155**, 70–76 (2010).

146. Rowlands, J. C. & Gustafsson, J.-A. Aryl hydrocarbon receptor-mediated signal transduction. *Crit. Rev. Toxicol.* **27**, 109–134 (1997).

147. Sadrzadeh, S. M. & Bozorgmehr, J. Haptoglobin phenotypes in health and disorders. *Am. J. Clin. Pathol.* **121**, 97–104 (2004).

148. Sánchez Reus, I., Bando, I., Andrés, D. & Cascales, M. Relationship between expression of HSP70 and metallothionein and oxidative stress during mercury chloride induced acute liver injury in rats. *J. Biochem. Mol. Toxicol.* **17**, 161–168 (2003).

149. Scheuhammer, A. *et al.* Recent progress on our understanding of the biological effects of mercury in fish and wildlife in the Canadian Arctic. *Sci. Total Environ.* **509–510**, 91–103 (2015).

150. Simond, A. E. *et al.* Associations between organohalogen exposure and thyroid- and steroid-related gene responses in St. Lawrence Estuary belugas and minke whales. *Mar. Pollut. Bull.* **145**, 174–184 (2019).

151. Smythe, T. A. *et al.* Temporal Trends of Brominated and Fluorinated Contaminants in Canadian Arctic Beluga (Delphinapterus leucas). *Arct. Sci.* **404**, In press (2018).

152. Sobolesky, P. M., Harrell, T. S., Parry, C., Venn-Watson, S. & Janech, M. G. Feeding a modified fish diet to bottlenose dolphins leads to an increase in serum adiponectin and sphingolipids. *Front. Endocrinol. (Lausanne).* 7–33 (2016). doi:10.3389/fendo.2016.00033

153. Song, L. *et al.* The cDNA cloning and mRNA expression of heat shock protein 70 gene in the haemocytes of bay scallop (Argopecten irradians, Lamarck 1819) responding to bacteria challenge and naphthalin stress. *Fish Shellfish Immunol.* **21**, 335–345 (2006).

154. Søreide, J. E., Hop, H., Carroll, M. L., Falk-Petersen, S. & Hegseth, E. N. Seasonal food web structures and sympagic-pelagic coupling in the European Arctic revealed by stable isotopes and a two-source food web model. *Prog. Oceanogr.* **71**, 59–87 (2006).

155. Squadrone, S. *et al.* Analysis of mercury and methylmercury concentrations, and selenium: Mercury molar ratios for a toxicological assessment of sperm whales (Physeter macrocephalus) in the most recent stranding event along the Adriatic coast (Southern Italy, Mediterranean Sea. *Chemosphere* **138**, 633–641 (2015).

156. Stafford, K. M. *et al.* Beluga whales in the western Beaufort Sea: current state of knowledge on timing, distribution, habitat use and environmental drivers. *Deep. Res. Part II Top. Stud. Oceanogr.* **152**, 182–194 (2018).

157. Stefan, N. & Stumvoll, M. Adiponectin-its role in metabolism and beyond. *Horm. Metab. Res.* **34**, 469–474 (2002).

158. Stern, G. A. *et al.* Spatial trends and factors affecting variation of organochlorine contaminants levels in Canadian Arctic beluga (Delphinapterus leucas). *Sci. Total Environ.* **351**–**352**, 344–368 (2005).

159. Stewart, C. E. H. & Rotwein, P. Growth, differentiation, and survival: multiple physiological functions for insulin-like growth factors. *Physiol. Rev.* **76**, 1005–1026 (1996).

160. Stuart-Smith, S. & Jepson, P. D. Persistent threats need persistent counteraction: responding to PCB pollution in marine mammals. *Mar. Policy* **84**, 69–75 (2017).

161. Sullivan, B. G. Tracking Canadian mercury emissions from products containing mercury. *Proceedings of the Air and Waste Management Association's Annual Conference and Exhibition, AWMA* (2019).

162. Tabuchi, M. *et al.* PCB-related alteration of thyroid hormones and thyroid hormone receptor gene expression in free-ranging harbor seals (Phoca vitulina). *Environ. Health Perspect.* **114**, 1024–1031 (2006).

163. Tai, T. C., Steiner, N. S., Hoover, C., Cheung, W. W. L. & Sumaila, U. R. Evaluating present and future potential of arctic fisheries in Canada. *Mar. Policy* **108**, (2019).

164. Taxvig, C. *et al.* Differential effects of environmental chemicals and food contaminants on adipogenesis, biomarker release and PPARγ activation. *Mol. Cell. Endocrinol.* **361**, 106–115 (2012).

165. Thiemann, G. W., Iverson, S. J. & Stirling, I. Variation in blubber fatty acid composition among marine mammals in the Canadian Arctic. *Mar. Mammal Sci.* **24**, 91–111 (2008).

166. Thompson, M. P. & Kim, D. Links between fatty acids and expression of UCP2 and UCP3 mRNAs. *FEBS Lett.* **568**, 4–9 (2004).

167. Toda, C. & Diano, S. Mitochondrial UCP2 in the central regulation of metabolism. *Best Pract. Res. Clin. Endocrinol. Metab.* **28**, 757–764 (2014).

168. Troisi, G. M., Barton, S. J., Liori, O. & Nyman, M. Polychlorinated biphenyls (PCBs) and sex hormone concentrations in ringed and grey seals: a possible link to endocrine disruption? *Arch. Environ. Contam. Toxicol.* (2020). doi:10.1007/s00244-020-00716-z

169. Tsan, M.-F. & Gao, B. Heat shock proteins and immune system. *J. Leukoc. Biol.* **85**, 905–910 (2009).

170. UNEP. *Consolidated assessment of efforts made toward the elimination of polychlorinated biphenyls*. (2016).

171. UNEP. Draft paper on reductive debromination of bromo-aromatics. in *Stockholm Convention on Persistent Organic Pollutants* 1–39 (2010).

172. Van Vlierberghe, H., Langlois, M. & Delanghe, J. Haptoglobin polymorphisms and iron homeostasis in health and in disease. *Clin. Chim. Acta* **345**, 35–42 (2004).

173. Veldhoen, N. *et al.* Relationship between mRNA biomarker candidates and location near a marine municipal wastewater outfall in the benthic indicator species Modiolus modiolus (L.). *Aquat. Toxicol.* **105**, 119–126 (2011).

174. Venables, W. N. & Ripley, B. D. Modern Applied Statistics with S. (2002).

175. Wajant, H., Pfizenmaier, K. & Scheurich, P. Tumor necrosis factor signaling. *Cell Death Differ.* **10**, 45–65 (2003).

176. West, J. B., Bowen, G. J., Cerling, T. E. & Ehleringer, J. R. Stable isotopes as one of nature's ecological recorders. *Trends Ecol. Evol.* **21**, 408–414 (2006).

177. Yamauchi, T., Iwabu, M., Okada-Iwabu, M. & Kadowaki, T. Adiponectin receptors: A review of their structure, function and how they work. *Best Pract. Res. Clin. Endocrinol. Metab.* **28**, 15–23 (2014).

178. Yu, L. *et al.* Evidence for positive selection on the leptin gene in cetacea and pinnipedia. *PLoS One* **6**, (2011).

179. Zhang, Q. *et al.* Characterization of estrogen receptor α activities in polychlorinated biphenyls by in vitro dual-luciferase reporter gene assay. *Environ. Pollut.* **189**, 169–175 (2014).

180. Zhang, Z., Boelen, A., Bisschop, P. H., Kalsbeek, A. & Fliers, E. Hypothalamic effects of thyroid hormone. *Mol. Cell. Endocrinol.* **458**, 143–148 (2017).

181. Zheng, J. *et al.* Disruption of thyroid hormone (TH) levels and TH-regulated gene expression by polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), and hydroxylated PCBs in e-waste recycling workers. *Environ. Int.* **102**, 138–144 (2017).

Appendix A

Table A1 Average of each variable presented with standard deviation for each year. June sea ice extent was measured in the Beaufort Sea. Sample size for each variable (N) is presented in brackets. An ANOVA was run for each variable to determine if there were differences between years, with the exception of sea ice.

Year (N)	Age	Blubber thickness (cm)	Length (cm)	Girth (cm)	PCBs (ng/g lw)	PBDEs (ng/g lw)	Hg (ug/g)	δ ^{13}C(‰)	δ^{15}N(‰)	June sea ice (%)
2008 (13)	32.3 ± 15.0 (11)	8.69 ± 2.95 (13)	416.4 ± 26.64 (13)	119.3 ± 9.46 (13)	1140.2 ± 646.01 (13)	13.7 ± 5.56 (5)	0.95 ± 0.46 (13)	-18.7 ± 0.45 (12)	16.9 ± 0.53 (12)	81.1
2009 (10)	29.6 ± 9.1 (10)	9.91 ± 1.44 (10)	414.0 ± 19.52 (10)	124.7 ± 7.71 (10)	1498.9 ± 638.7 (10)	20.3 ± 6.57 (10)	0.87 ± 0.39 (10)	n/a (0)	n/a (0)	95.9
2010 (10)	24.3 ± 4.6 (10)	8.38 ± 1.33 (10)	408.9 ± 41.79 (10)	118.6 ± 16.20 (10)	1122.9 ± 527.61 (10)	15.1 ± 6.72 (10)	1.01 ± 0.43 (10)	-18.7 ± 0.34 (10)	17.2 ± 0.46 (10)	79.1
2012 (4)	23.5 ± 5.1 (4)	7.14 ± 0.61 (4)	408.3 ± 14.57 (4)	119.7 ± 7.79 (4)	1676.6 ± 250.65 (2)	12.4 ± 4.31 (2)	1.23 ± 0.24 (5)	n/a (0)	n/a (0)	76.8
2013 (9)	33.7 ± 7.1 (9)	7.33 ± 1.11 (9)	421.6 ± 10.92 (9)	119.2 ± 10.44 (9)	3345.4 ± 1593.43 (2)	17.0 ± 4.16 (6)	2.18 ± 0.43 (9)	-19.4 ± 0.24 (9)	17.7 ± 0.29 (9)	95.8
2014 (9)	27.3 ± 7.3 (9)	5.51 ± 2.37 (8)	400.1 ± 33.6 (9)	113.2 ± 17.23 (9)	3300.8 ± 913.51	25.1 ± 5.31 (4)	2.11 ± 1.13 (8)	-19.2 ± 0.49 (9)	17.4 ± 0.46 (9)	93.9

					(6)					
2015 (7)	22.1 ± 5.2 (7)	6.91 ± 2.21 (7)	386.4 ± 30.38 (7)	107.3 ± 13.95 (7)	1581.7 ± 692.55 (2)	n/a (0)	1.40 ± 1.21 (7)	-19.5 ± 0.22 (2)	17.0 ± 0.43 (2)	82.1
2017 (13)	N/A (0)	10.24 ± 1.25 (11)	423.6 ± 15.39 (13)	121.5 ± 14.33 (13)	2423.0 ± 1188.57 (8)	17.3 ± 7.04 (8)	1.05 ± 0.70 (13)	-19.2 ± 0.22 (13)	17.4 ± 0.29 (13)	76.3
All years	28.7 ± 8.89	8.3 ± 2.32	411.9 ± 27.6	118.4 ± 13.12	1869.7 ± 1034.00	17.51 ± 7.11	1.3 ± 0.75	-19.0 ± 0.47	17.3 ± 0.47	--
ANOVA	p=<0.05, R^2=0.2	p>0.05, R^2=0.0013	p>0.05, R^2=-0.013	p>0.05, R^2=-0.0021	p<0.05, R^2=0.21	p>0.05, R^2=-0.015	p<0.05, R^2=0.40	p<0.05, R^2=0.28	p<0.05, R^2=0.12	--

Table A2 Results from a post-hoc Tukey's test run on all variables that showed significant differences after an ANOVA (Table A2). If a variable showed a significant difference between two years ($p<0.05$), it is listed for the corresponding years below.

	2008	**2009**	**2010**	**2012**	**2013**	**2014**	**2015**
2008							
2009	--						
2010	Age	--					
2012	--	--	--				
2013	^{13}C, ^{15}N, Hg	Hg	PCBs, ^{13}C, Hg	--			
2014	PCBs, ^{13}C, Hg	PCBs, Hg	PCBs, ^{13}C, Hg	--	--		
2015	Age, ^{13}C	--	^{13}C	--	Hg	Hg	
2017	^{13}C, ^{15}N	--	PCBs, ^{13}C	--	Hg	Hg	--

Table A3 Primer sequences used in qPCR experiments, obtained through previous studies on marine mammals (referenced below). Primers originally evaluated by Noël et al. (2014) for beluga whales were not re-evaluated using quality assurance/quality control procedures (QA/QC). All new primers that had not previously been established in beluga whales were assessed following a three-tiered QA/QC procedure (detailed in the methods section). Tier 1 serial dilution slopes are presented (blubber slope & liver slope). Outliers and extreme values were removed in some cases. All new primers were assigned either a 'pass', and were used for further analysis, or a 'fail' and were not included in data collection. The quality assurance/quality control tier at which each gene failed is noted (QA/QC was performed from backwards from Tier 3 to Tier 1). In addition, the beluga whale genome was sequenced partway through analysis, and all primers were confirmed as correct in GenBank ((Martinez et al., 2013)).

Primer	Forward sequence	Reverse sequence	Animal	Reference	Amplicon length	GenBank Accession #	Blubber slope	Liver slope	QA/QC
rpL8	GCGGACGGAG TTGTTCAT	TTTGTCTCAGG GTTGTGGG	Beluga	(Noël et al., 2014)	219	111165130			
βactin	CCTGGACTTCG AGCAGGAG	GCACCGTGTTG GCATAGAG	Beluga	(Noël et al., 2014)	236	111170344			
ERα	CCGAGCCCACT CTTGATT	CCTCTTTGCCC AGTTGAT	Beluga	(Noël et al., 2014)	213	111183804			
Adipon ectin	ATTCCCATTCG CTTTACC	AGGAGCACAGA GCCAGAG	Beluga	(Noël et al., 2014)	227	111176597			
Leptin	AGTCCAGGAT GACACCAA	CCAAACCAGTG ACCCTCT	Beluga	(Noël et al., 2014)	104	111182801			
ILGF1	TTTATTTCAAC	TACATCTCCAG	Beluga	(Noël et al.,	112	111187347			

	AAGCCCACG	CCTCCTCA		2014)					
Hsp70	ACAGGCAAGG CTAACAAG	GCATAGGATTC TAAGGCATTTT	Beluga	(Noël et al., 2014)	155	111178087			
AhR	TCGAATGCACGC TTAGTT	TTGCCTTGGTAG CAGAAT	Beluga	(Noël et al., 2014)	241	111178528			
TRβ	AGATCCATCGGT CACAAG	CCACCTTCTGGG CGTTT	Beluga	(Noël et al., 2014)	170	111164774			
				New Primers					
HP	CTGGCAGGCTAA GATGGTTT	GTCAGCAGCCAT TGTTCATT	Harbour porpoise	(Muller et al., 2013)	75	AY919335	0.09	-0.05	Pass
DI1	GATGGCTGGGCT TTTAAGAAC	ACCCTTGTAGAG GATCCTGC	Elephant seal	(Spitz et al., 2015)	N/A	111175838	0.07	-0.06	Pass
DI2	AGCGTAGACTTG CTGATCAC	CGAGTGGACTTG GAGCGGCT	Elephant seal	(Spitz et al., 2015)	N/A	111176195	0.06	-0.009	Pass
UCP2	GGCATTGGGAGC CGCCTCC	CAGTGCTTTGGT ATCTCCGGCC	Elephant seal	(Muller et al., 2013)	N/A	111184847	-0.07	-0.03	Pass
TNFα	GGCTGAACACAT ATGCCAAC	TGAAGAGGACCT GGGAGTAGA	Harbour porpoise	(Muller et al., 2013)	111	AY919337	-0.02	-0.06	Pass
SIRT2	CACTACTTCATC CGCCTGCT	GCGTGTCTATGT TCTGCGTG	Sea lion	(Spitz et al., 2015)	302	111180885	N/A	N/A	Failed Tiers 1

									and 2
Acox1	GTGGCCGACTGTGGTGGACA	AGTGGCAGTGTGCCTCGCTTG	Ringed seal	(Castelli et al., 2014)	N/A	111182805	N/A	N/A	Failed Tier 3
Acat2	TGCTAATCGAGGACTCACACC	TGGAAGGCTCCACACCTACT	Sea lion	(Castelli et al., 2014)	282	111167584	N/A	N/A	Failed Tier 3
PPARα	TATCACAGACACGCTCTCACCAGC	TTCAGCCGGATGGTTCGCCG	Ringed seal	(Muller et al., 2013)	N/A	111186471	N/A	N/A	Failed Tier 3
CRP	TTCTCGTATGCCACCAAGAG	TTCAGACCCACCCACTGTAA	Harbour porpoise	(Muller et al., 2013)	192	AY919336, 111167155	N/A	N/A	Failed Tier 3

Table A4 log(K_{OW}) (octanol-water coefficient), half-life in biota, estimated toxicity thresholds and examples of concentrations in free-ranging wildlife for PCBs, PBDEs and MeHg. It is important to note that log(K_{OW}) and half-life is represented for only a subset of PBDEs and PCBs, as calculating these values for every congener is difficult.

Contaminant	Range of log(K_{OW})	Half-life (biota)	Toxicity thresholds	Example concentrations
PCB	4.63-8.20[1]	Up to 1000 days[3]	1.3 mg/kg lw (harbour seals)[6]	0.40-6.58 mg/kg lw (harbour seals)[11]
	7.72-8.82[2]		10 mg/kg lw (bottlenose dophins)[7]	37.4 mg/kg lw (killer whales)[12]
			17 mg/kg lw (harbour seals)[8]	0.77-3.98 mg/kg lw (harbour seals)[13]
				0.10 mg/kg lw (sea lions)[14]
PBDE	5-10[3]	Up to 500 days[3]	1.5 mg/kg lw (harbour seals)[9]	0.49-1.05 mg/kg lw (harbour seals)[13]
				0.68-6.24 mg/kg lw (harbour seals)[6]
				Not detectable (sea lions)[14]
MeHg	1.7-2.5[4]	80 days (humans)[5]	4 mg/kg lw (beluga whales)[10]	200 mg/kg lw THg (sperm whale; liver)[15]
				0.84-87.92 mg/kg dw (dolphins; liver)[16]
				0.0053 mg/kg (harbour seals; hair)[17]

[1](P. S. Ross et al., 2000); [2](Peter S. Ross et al., 2013); [3](Juan J. Alava et al., 2009); [4](Squadrone et al., 2015); [5](Jo et al., 2015); [6](Mos et al., 2010); [7](Hall et al., 2006); [8](Peter S. Ross et al., 1996); [9](Hall et al., 2003); [10](Krey et al., 2015); [11](Mos et al., 2006); [12](P. S. Ross et al., 2000); [13](Peter S. Ross et al., 2013); [14](Juan J. Alava et al., 2009); [15](Squadrone et al., 2015); [16](Kehrig et al., 2008); [17](Noël et al., 2016)

Table A5 Relative contributions of each fatty acid to principle components 1 and 2 of a principle component analysis performed on all fatty acids.

Fatty acid	Contribution to PC1	Contribution to PC2
12:00	9.44	18.42
13:00	10.09	0.08
14:00	0.01	0.04
14:1n9	0.13	1.62
14:1n7	0.61	0.86
14:1n5	0.30	0.72
15:0 iso	1.06	19.05
15:00	0.16	0.04
15:1n8	14.58	11.87
15:1n6	11.25	3.11
16:0 iso	0.01	1.24
16:00	0.00	0.14
16:1n11	0.03	0.16
16:1n9	0.19	0.56
16:1n7	0.01	0.00
16:1n5	0.09	0.01
16:2n6	0.42	1.07
17:0 iso	0.11	0.09
16:2n4	1.12	1.52
17:00	0.10	0.13
16:3n4	0.66	0.35

17:01	1.00	0.75
16:4n3	5.53	1.19
16:4n1	0.44	0.00
18:00	0.02	0.00
18:1n11	0.01	0.05
18:1n9	0.03	0.10
18:1n7	0.01	0.06
18:1n5	0.03	0.02
18:3n6	1.74	8.27
18:3n4	1.41	1.32
18:3n3	0.54	1.04
18:3n1	2.16	1.13
18:4n3	3.16	0.02
18:4n1	3.08	0.34
20:00	0.40	1.02
20:1n11	0.00	0.68
20:1n9	0.02	2.19
20:1n7	0.00	0.65
20:2n9	0.08	1.26
20:2n6	0.01	0.00
20:3n6	0.17	0.02
20:4n6	0.29	0.21
20:3n3	2.48	2.27
20:4n3	0.20	0.00

20:5n3	0.10	0.05
22:1n11	0.01	0.81
22:1n9	0.02	0.81
22:1n7	0.04	0.28
22:2n6	12.08	0.55
21:5n3	0.20	3.42
22:4n6	6.59	9.82
22:5n6	1.05	0.01
22:4n3	6.72	0.50
22:5n3	0.01	0.00
22:6n3	0.00	0.04