

**FISH CONSUMPTION AND NUTRITIONAL HEALTH
AMONG FIRST NATIONS IN CANADA**

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ABSTRACT

Traditional food is fundamental for the cultural identity, mental and spiritual well-being, and physical and nutritional health of First Nations in Canada. Rapid environment changes including environmental contamination and degradation, climate change, urbanization and industry growth reduce the availability and diversity of traditional foods. This is concomitant with changing lifestyle and an increased prevalence of malnutrition, obesity, diabetes, and cardiovascular diseases. The goal of this thesis is to investigate the roles of traditional fish consumption for First Nations' health in the complex interplays between environmental contaminant exposure, climate change, and food and nutrition security. Data collected from the First Nations Food Nutrition and Environment Study were used. The thesis is comprised of seven chapters presented in three sections.

Section 1 addressed the importance of traditional fish consumption for food and nutritional security among First Nations in Canada. With increased income-related food insecurity, First Nations rely more on traditional foods including fish and participate more in fishing and other traditional practices. Nevertheless, many factors such as climate change, governmental restrictions, hydro and forestry operations continue to reduce the availability of traditional fish and access to traditional food sources, land and waterways.

Section 2 explored the associations between locally-harvested fish consumption, long chain omega-3 fatty acid (n-3 FA) intake and dietary exposure to persistent organic pollutants (POP) with type 2 diabetes in First Nations in Canada. Dietary POP exposure was positively associated with the prevalence of type 2 diabetes whereas fish consumption (n-3 FA) showed protective dose-response associations. Furthermore, we found that relatively high POP exposure from fish may outweigh the protective associations of fish on type 2 diabetes. Therefore, the balance of risks and benefits associated with fish consumption is highly dependent on the regional POP concentrations in fish.

Section 3 entailed studies on the nutritional benefits of seafood consumption and modelling potential impacts of the climate-related decline in seafood abundance on the nutritional quality of adult diets and cardiovascular health among coastal First Nations in British Columbia. We estimated that projected climate change may reduce the intakes of essential nutrients by 21%–31% by 2050 relative to 2000. Moreover, hypothetical substitution of seafood with alternative non-traditional foods would not provide adequate amounts of nutrients. Reduced fish consumption and consequent n-3 FAs intake may increase the risk of cardiovascular diseases in First Nations.

Our findings provide important information for communities, fishery governance, local resource managers and public health professionals to promote traditional food systems, nutritional health, food security, and food sovereignty in Canadian First Nations.

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ABBREVIATIONS

AMDR: Acceptable Macronutrient Distribution Range
AI: Adequate Intake
BC: British Columbia
BMI: body mass index
CNF: Canadian Nutrient File
CVD: cardiovascular diseases
CI: confidence interval
DBEM: Dynamic bioclimate envelope model
DDE: dichlorodiphenyldichloroethylene
DRIs: Dietary Reference Intakes
DHA: docosahexaenoic acid
EPA: eicosapentaenoic acid
EAR: estimated average requirements
FNs: First Nations
FNFNES: First Nations Food Nutrition and Environment Study
FFQ: food frequency questionnaire
HFSSM: Household Food Security Survey Module
MeHg: methyl-mercury
MI: myocardial infarction
n-3 FAs/ n-3 PUFA: omega-3 fatty acids
PCBs: polychlorinated biphenyls
RDA: Recommended Dietary Allowance
RCP: Representative Concentration Pathway
RI: Recommended Intake
SHL: Socio-health-lifestyle questionnaire
TDI: tolerable daily intake
DID: difference in difference method
OR: odds ratio
PCBs: polychlorinated biphenyls
POPs: persistent organic pollutants
SD: Standard deviation
SHL: Socio-health-lifestyle questionnaire
T2D: type 2 diabetes

1 INTRODUCTION

1.1 Background

1.1.1 Indigenous Peoples in Canada

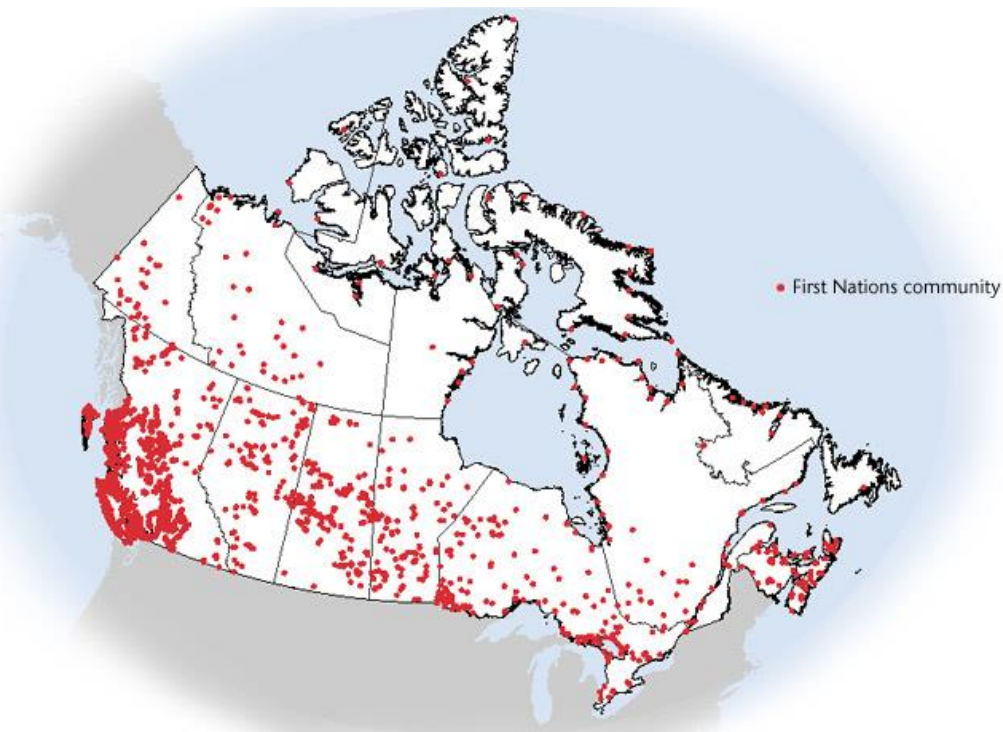
Indigenous Peoples are defined as ethnic groups who are the original inhabitants of a given region and who maintain traditions or other aspects of an early culture, associated with a given area (Douglas 1999). There are approximately 370 million Indigenous People worldwide living in more than 90 countries. While representing about 5% of the total world's population, Indigenous People account for one-third of the world's extremely poor population and recognized as some of the world's vulnerable, disadvantaged and marginalized peoples (APF & OHCHR 2013). Indigenous Peoples possess unique cultures, languages, and histories and have a strong connection to the environment and their traditional lands, the ocean and territories (APF & OHCHR 2013). Many Indigenous People rely on traditional food systems which is essential to maintain their connections with nature, their identity, social cohesion, traditional knowledge and cultural continuity (Kuhnlein, Erasmus, and Spigelski 2009).

In Canada, Indigenous Peoples, also known as Aboriginal peoples, represent the original inhabitants of North America. The Canadian Constitution Action of 1982 (section 35) recognizes three distinct cultural groups of Indigenous people: First Nations ("Indians"), Inuit and Métis. The most recent National Census (2016) indicates that more than 1.67 million people in Canada identified themselves as Aboriginal which accounted for 4.9% of the total population, compared with 4.3% in the 2011 Census and 3.8% in the 2006 Census (Statistics Canada 2017a).

Aboriginal people in Canada are diverse by culture, histories, and homelands with more than 70 Aboriginal languages being spoken across Canada (Statistics Canada 2017c). First Nations represent the greatest share of the Indigenous people in Canada (58.4%) and include individuals

who are members of a First Nation/Indian Band and those who are not, as well as those with and without registered or treaty Indians status under the *Indian Act* (Statistics Canada 2017b). Within the First Nations population, 744,855 (76.2%) have a registered or treaty Indian status with 44.2% living on reserve or in communities on Crown land. Almost one-quarter (24.2%) of First Nations population reside in Ontario while 17.7% - in British Columbia and 13.4% - in Manitoba (Statistics Canada 2017a). The Assembly of First Nations recognizes 634 First Nations/Indian Bands, spread across provinces and territories in Canada. The majorities of First Nations communities are located south of the 60th parallel with one-third (n=203) situated in the province of British Columbia, followed by 126 communities in Ontario and 63 in Manitoba (AFN 2017) (Figure 1).

Figure 1. Map of First Nations communities in Canada



Source: Extracted from Global Forest Watch (canadiangeographic.ca)

1.1.2 First Nations' traditional food systems

For millennia, First Nations in Canada have used their knowledge of the local environment and traditional food systems to live off the land. The traditional food system is defined as “all foods within a particular culture available from local natural resources and culturally accepted” (Kuhnlein and Receveur 1996). Traditional foods are diverse across 20 terrestrial and marine ecozones (www.ecozones.ca) of Canada and include a large variety of fish and marine foods, game, birds, berries, plant and tree foods. Over thousands of years, First Nations people have developed many resource management and food production technologies, including hunting, trapping, foraging, and intensive food production (Deur and Turner 2005). Nowadays, the harvest and consumption of wildlife remains fundamental to First Nations culture and provides them with a range of social, spiritual, physical, economic, and nutritional benefits (Lambden, Receveur, and Kuhnlein 2007).

Fish have always been an essential part of First Nations' culture and diets (Dewailly et al. 2002; Kuhnlein, Fediuk, et al. 2013; Mos et al. 2004; Moss 2016; Proust, Lucas, and Dewailly 2014). Traditionally harvested from the ocean, lakes, ponds and rivers, fish and marine mammals have sustained First Nations, providing them with the ability to survive and thrive in their traditional territories (Brown and Brown 2009). Fish and seafood consumption significantly varied across different ecozones in Canada reflecting THE geographical diversity of species (Chan et al. 2011, 2012, 2014, 2016, 2018). While inland First Nations commonly consume freshwater fish caught from lakes and rivers, coastal communities rely on a great variety of marine sources (saltwater fish, shellfish, seaweeds and marine mammals), with salmon as keystone species (Brown and Brown 2009; Chan et al. 2011, 2012; Fediuk and Thom 2003; Mos et al. 2004). Traditional activities, such as fishing, hand-gathering, spearing, netting, angling and trapping, have been

largely used in traditional ceremonies, festivals and other cultural events, enhancing the First Nations spiritual connections to the land and individual communities (Long 2014). Beyond various cultural benefits, traditional fish and seafood consumption significantly contributes to food and nutrition security by supplying rich sources of high-quality protein, a number of essential vitamins and minerals, and polyunsaturated omega-3 fatty acids (n-3 FAs), such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kuhnlein et al. 2013; Mos et al. 2004).

1.1.3 Food security among First Nations in Canada

Food security (i.e. the state of having reliable access to a sufficient quantity of affordable, nutritious and socially acceptable foods) is an important social determinant of health (World Health Organization 2018). In Canada, the rates of food insecurity are disproportionately higher among First Nations people compared to the non-Indigenous population (Halseth 2015). The First Nations Regional Health Survey revealed that 52.4% of First Nations households living on reserve reported being food insecure with 14.1% having to cut the size of their meals or to skip meals due to a lack of monetary resources (FNIGC 2012). In contrast, 12.3% of Canadian households experienced some level of food insecurity (including marginal food insecurity) with 2.5% of households to be categorized as severely food insecure (Tarasuk, Mitchell, and Dachner 2013).

Food security considerations are unique for First Nations people as their diets are characterized by access to both market foods and traditional foods harvested from the local natural environment (Egeland et al. 2011; Power 2008). Power (2008) proposed “traditional food security” as an additional dimension of food security (Power 2008). Traditional foods are more nutritious and more nutrient dense compared to market foods and remain fundamental to the diet

quality, cultural identity and social health of First Nations (Lambden et al. 2007; Power 2008).

Among First Nations, food insecurity is associated with poor overall health status, mental health issues (family stress, learning impairment, depression, social exclusion) and a weak sense of community belonging (Willows et al. 2011b).

1.1.4 Nutrition transition among First Nations in Canada

Over the last few decades, the First Nations people in Canada have experienced a rapid transition in diets and lifestyle (Halseth 2015; Popkin 2004). This change is characterized by a shift from traditional eating patterns to “western” diets. (Halseth 2015; Johnson-Down and Egeland 2012; Kuhnlein et al. 2004; Mos et al. 2004; Robidoux et al. 2012; Sheehy, Roache, and Sharma 2013). Traditional foods have been gradually replaced with store-bought foods that are usually high in refined carbohydrates and saturated fats (Receveur, Boulay, and Kuhnlein 1997). High-energy and low-nutrient diets coupled with a sedentary lifestyle have led to increasing rates of obesity, malnutrition and other diet-related chronic diseases (Johnson-Down et al. 2015; Johnson-Down and Egeland 2012). Nowadays, heart diseases, cancer and metabolic diseases are among the leading causes of mortality in First Nations adults (Health Canada 2014a) whereas the rates of obesity, type 2 diabetes (T2D) and heart diseases are reaching epidemic proportions (Roland Dyck et al. 2010; FNIGC 2012; Haman et al. 2010; Young et al. 2000).

The decline in traditional food consumption including fish and seafood has stemmed from many social, economic and environmental factors (Batal 2001; Halseth 2015). Colonization is considered as a fundamental underlying driver of the nutrition and lifestyle transition among First Nations (Reading and Wein 2009). The loss of traditional land due to the dispossession and settlement onto reserves, as well as forced attendance of residential schools, have led to erosion of First Nations’ culture, loss of language, reduced access to traditional food sources and

diminished traditional knowledge transition (Adelson 2005; Halseth 2015; Richmond and Ross 2009; Sharma 2010). Socio-economic barriers, such as poverty, unemployment, high cost and limited variety and availability of healthy market foods, as well as high cost of hunting equipment contribute to the nutrition and food insecurity in Indigenous communities (Kuhnlein, Fediuk, et al. 2013; Kuhnlein, Goodman, et al. 2013; Lambden et al. 2006). Under these conditions, many First Nations turn to cheap low-nutrient market foods (Halseth 2015; Kuhnlein, Erasmus, et al. 2013). Other factors such as environmental changes, including rapid urbanization and industry growth, environmental contamination and degradation and climate change have negative impacts on biodiversity and availability of traditional foods (Halseth 2015; Kuhnlein, Fediuk, et al. 2013; Willows 2005).

1.1.5 Environmental changes

Rapid global industrialization over the past 70 years has resulted in pollution of the world's ecosystems. Long-range transport of industrial chemicals from lower latitudes to the northern regions with consequent accumulation and biomagnification of environmental contaminants in food chains presents serious challenges for Indigenous populations who live off the land and for whom consumption of traditional foods are essential to their cultural identity, nutritional health and overall wellbeing (Kuhnlein and Chan 2000; Brian D Laird et al. 2013). Fish and seafood are recognized as the primary pathway of exposure to environmental contaminants, in particular, methylmercury (MeHg) and persistent organic pollutants (POPs) including polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE) (Assembly of First Nations 2007; Kuhnlein and Chan 2000; Brian D Laird et al. 2013; Seabert et al. 2014). People eating locally harvested fish can accumulate high levels of these contaminants in their bodies (Donaldson et al. 2010; Fitzgerald et al. 1999, 2004; Philibert, Schwartz, and Mergler 2009;

Seabert et al. 2014). Exposure to mercury negatively affects growth and development of fetuses and children, immune system and may increase the risk of cardiovascular diseases (CVD), such as myocardial infarction (MI) (Bjørklund et al. 2017; Ha et al. 2017). PCB exposure through fish consumption was associated with impaired cognitive functions in newborns and children, and low birth weight (Ross 2004). Furthermore, POPs are endocrine disrupting chemicals that disturb normal endocrine functions and increase the risk of obesity, diabetes and CVD (Diamanti-Kandarakis et al. 2009).

Climate change is another critical factor affecting traditional food systems. Rising temperature, changes in precipitation patterns, strong winds, thinning ice, melting permafrost and coastal erosion have been well-documented in the Canadian Arctic and southern regions (Ford et al. 2010). These changes impact abundance and availability of wildlife, access to wild food, as well as quality and safety of traditional foods (Guyot and Chan 2006; Rosol, Powell-hellyer, and Chan 2016). Along with other Indigenous people, First Nations are especially vulnerable to the effects of climate change since their close relationship with the land and sea, and their subsistence activities (Ford et al. 2010; Lemelin et al. 2010). Literature documented that climate change affects the distribution, abundance, behaviour and health of wildlife species (Hori 2010; Nancarrow and Chan 2010; Wesche and Chan 2010). Increased water temperature and changes in circulation patterns alter the location and timing of life cycles (such as reproduction and migration), lead to the shift of fish species to northern regions, increase abnormalities and diseases in fish populations, and the loss of keystone species (Chan et al. 2006; Hori 2010; Lemelin et al. 2010; Royer and Herrmann 2011; Thompson et al. 2014). Furthermore, climate change was projected to negatively impact the abundance and catch potential of many marine

species, commercially and culturally important for First Nations communities (Weatherdon et al. 2016).

1.2 Rationale

It is clear that the health and well-being of First Nations are closely linked to foods and diets provided by local food systems. Traditional diets provide a strong foundation for cultural identity, spiritual well-being and sustainable livelihoods. Fish and marine sources are particularly important since they are naturally rich in omega-3 fatty acids, protein, minerals, such as calcium, iron, and phosphorus, trace elements and vitamins, and thus, promote food and nutrition security for First Nations. Historically, coastal communities obtained 90% of their protein from marine sources (Chisholm and Nelson 1983). However, during the last centuries, a dramatic decline in the use and consumption of fish and marine foods in First Nations communities was observed (Butler and Campbell 2004; Colin 2017; Lee, Reyburn, and Carrow 1971; Moss 1993). For salmon alone, estimates suggest that pre-contact per capita annual consumption among Coast Salish communities was from 272 to 316 kg/year (Bennett 1971; Chisholm and Nelson 1983). In contrast, a recent study estimated that Central Coast Salish people consumed salmon on average 10.3 kg/person/year (Mos et al. 2004). Furthermore, a significant decline in salmon and other seafood consumption was observed among younger people (Mos et al. 2004). These data demonstrate a significant dietary transition among First Nations people.

Exposure to environmental contaminants raises a concern about the safety of traditional foods, such as fish and lead to a shift away from traditional lifestyle (Fitzgerald et al. 2004). Whereas locally-harvested fish is the major source of contaminant exposure (Fitzgerald et al. 1999, 2004; Kuhnlein and Chan 2000; Seabert et al. 2014), health implications for First Nations are still unknown. Given the increasing rates of T2D among First Nations and growing body of evidence

that exposure to POP increases the risks of T2D (Fitzgerald et al. 1999, 2004), fish consumption may present a risk for T2D among First Nations (Sharp 2009a). On the other hand, fish provides numerous health benefits including cardioprotective effects and preventing T2D (Chowdhury et al. 2012; Leung Yinko et al. 2014). Therefore, the risks and benefits associated with traditional fish consumption in First Nations need further investigation.

Furthermore, climate change represents significant challenges for First Nations communities. It poses serious threats to biodiversity and access to traditional land and ocean. Many First Nations have already noted the impacts of climate change on species' abundance and distribution, and changes in migration patterns (Chan et al. 2011, 2012, 2014). In coastal communities, climate change has a potential to lead to a decline in keystone species, such as salmon and herring, which are nutritionally, culturally and commercially important for First Nations (Weatherdon et al. 2016). This climate change represents serious implications for food security, nutrition and health of First Nations. In the light of the knowledge that health of First Nations people is vulnerable to environmental contamination, degradation and climate change through impacts on traditional food systems, limited research has examined the links between the environmental changes and First Nations' health.

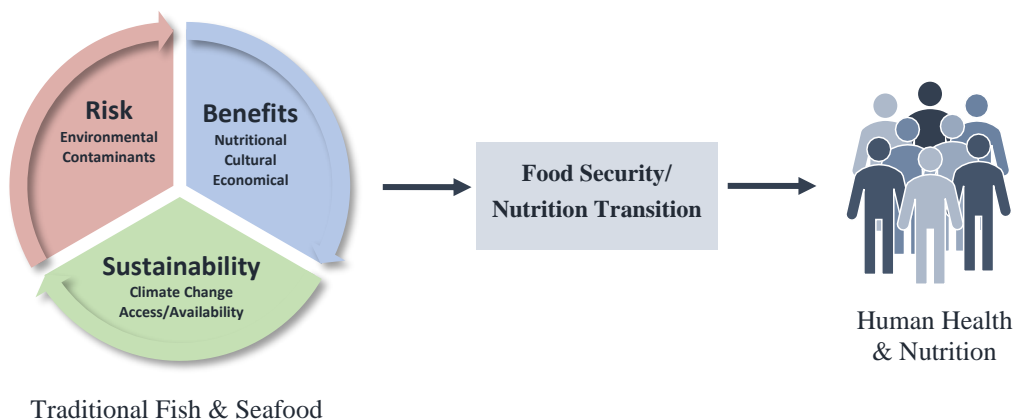
1.3 The Thesis

1.3.1 Thesis Objectives

The overall goal of this thesis is to investigate the roles of traditional fish consumption for First Nations' health in the complex interplays between environmental contaminant exposure, climate change, and food and nutrition security. This thesis attempts to explore how rapid environmental

changes, such as environmental contamination and climate change, combined with socio-economic factors affect diets, food security, nutrition, and health of First Nations in Canada.

Figure 2. Thesis framework: the complex interplay between human health and traditional fish in the context of risk, benefits and sustainability

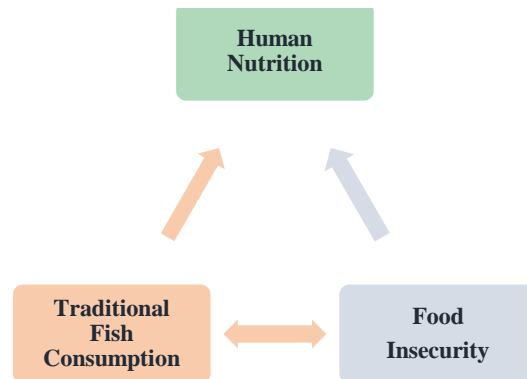


Specific research objectives of the thesis are as follows:

1. To explore the relative importance of fish consumption in the context of food and nutrition security among First Nations in Manitoba and Ontario, Canada

- Investigate the relationships between fish consumption patterns and factors related to food security statuses
- Estimate the contribution of fish to daily nutrient requirements by food security status
- Characterize barriers to the access to and availability of traditional foods including fish

Figure 3. Relationship between traditional fish consumption, food insecurity & human nutrition



2. To perform a risk-benefit assessment of fish consumption with respect to dietary PCB and DDE exposure and n-3 FAs (EPA+DHA) intake with T2D in Manitoba First Nations, Canada

- Describe fish consumption patterns among First Nation adults in Manitoba
- Estimate dietary n-3 FAs intake and PCB and DDE exposure from fish
- Explore the associations between fish consumption, dietary n-3 FAs intake and DDE and PCB exposure with self-reported T2D among First Nations in Manitoba

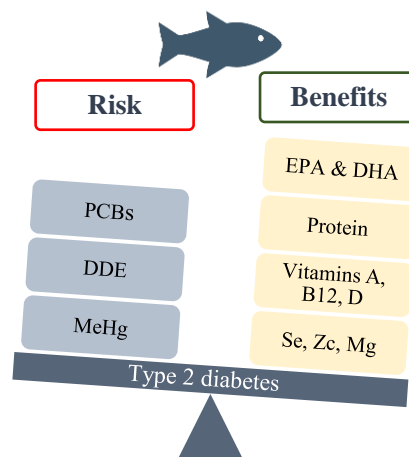
3. To perform a risk-benefit assessment of fish consumption with respect to dietary PCB and DDE exposure and n-3 FAs (EPA+DHA) intake with T2D in Ontario First Nations, Canada

- Describe fish consumption patterns among First Nation adults in Ontario
- Estimate dietary n-3 FAs intake and PCBs and DDE exposure from fish
- Explore the associations between fish consumption, dietary n-3 FAs intake and DDE and PCBs exposure with self-reported T2D among First Nations in Ontario

4. To examine if relatively high exposure to POPs (PCBs and DDE) may outweigh the beneficial associations of fish (n-3 FAs) with the prevalence of T2D in Manitoba and Ontario First Nations, Canada

- Test the relationship between dietary POP (PCBs and DDE) exposure and the prevalence of T2D using a difference in difference (DID) model
- Examine a non-linear relationship between dietary POP intake and prevalence of T2D
- Estimate the thresholds of daily dietary POP exposure that increase the risk of T2D

Figure 4. Risks and benefits of fish consumption in the context of exposure to environmental contaminants and beneficial nutrients

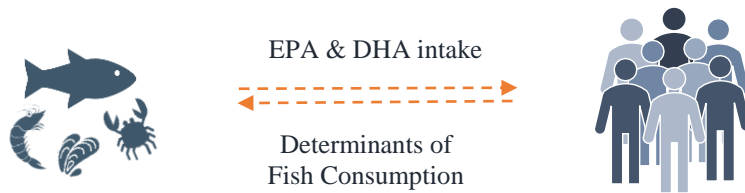


5. To characterize seafood consumption patterns, their nutritional benefits and associated socio-demographic and lifestyle factors among First Nations in British Columbia, Canada

- Describe seafood consumption patterns in First Nations in British Columbia ecozones
- Examine dietary and lifestyle characteristics associated with seafood consumption

- Identify the top 10 most consumed seafood species, their contribution to EPA+DHA intake and dietary exposure to PCBs, DDE, and MeHg

Figure 5. Contribution of fish and seafood consumption to EPA & DHA intake



6. To estimate the potential impacts of the climate-related declines in seafood abundance on the nutritional quality of adult diets among coastal First Nations in British Columbia, Canada

- Model impacts of the climate-related decline of seafood harvest on seafood consumption patterns and nutritional status of coastal First Nations
- Project potential changes in nutrient intakes, after assuming the hypothetical substitution of traditional seafood with alternative non-traditional foods

Figure 6. Linking fishery decline and nutritional health



7. To model the impacts of declined seafood consumption and consequent EPA and DHA intake on the relative risk of myocardial infarction (MI) among coastal First Nations in British Columbia

- Model the combined effects of reduced EPA+DHA intake and MeHg exposure from seafood on the relative risk of MI under lower and upper climate change scenarios

Figure 7. Linking seafood consumption and human health



1.3.2 Research design

This thesis includes epidemiological research involving analysis of data from the First Nations Food Nutrition and Environment Study (FNFNES).

FNFNES was designed to assess traditional food consumption, the exposure to environmental contaminants, total diets and food security status of First Nations people living on reserves, south of the 60th parallel across Canada. This information is needed for the promotion of a healthy environment and healthy foods for First Nations. The FNFNES has been implemented in the eight Assembly of First Nation regions over a 10-year period and is representative of all First Nations for regions south of the 60th parallel. Using a combined ecozone/cultural area framework, the FNFNES collects data from about 100 randomly selected First Nations communities (Figure 8). This strategy was used to ensure that the diversity in ecozones and

cultural areas were represented in the sampling strategy. An ecozone is a large geographical region identified by the distribution patterns of plants, animals, geographical characteristics and climate (www.ecozone.ca). A cultural area is a concept used to define geographic areas within which Indigenous communities share a greater number of traits/cultural affinities than those outside the region (Sturtevant 1978).

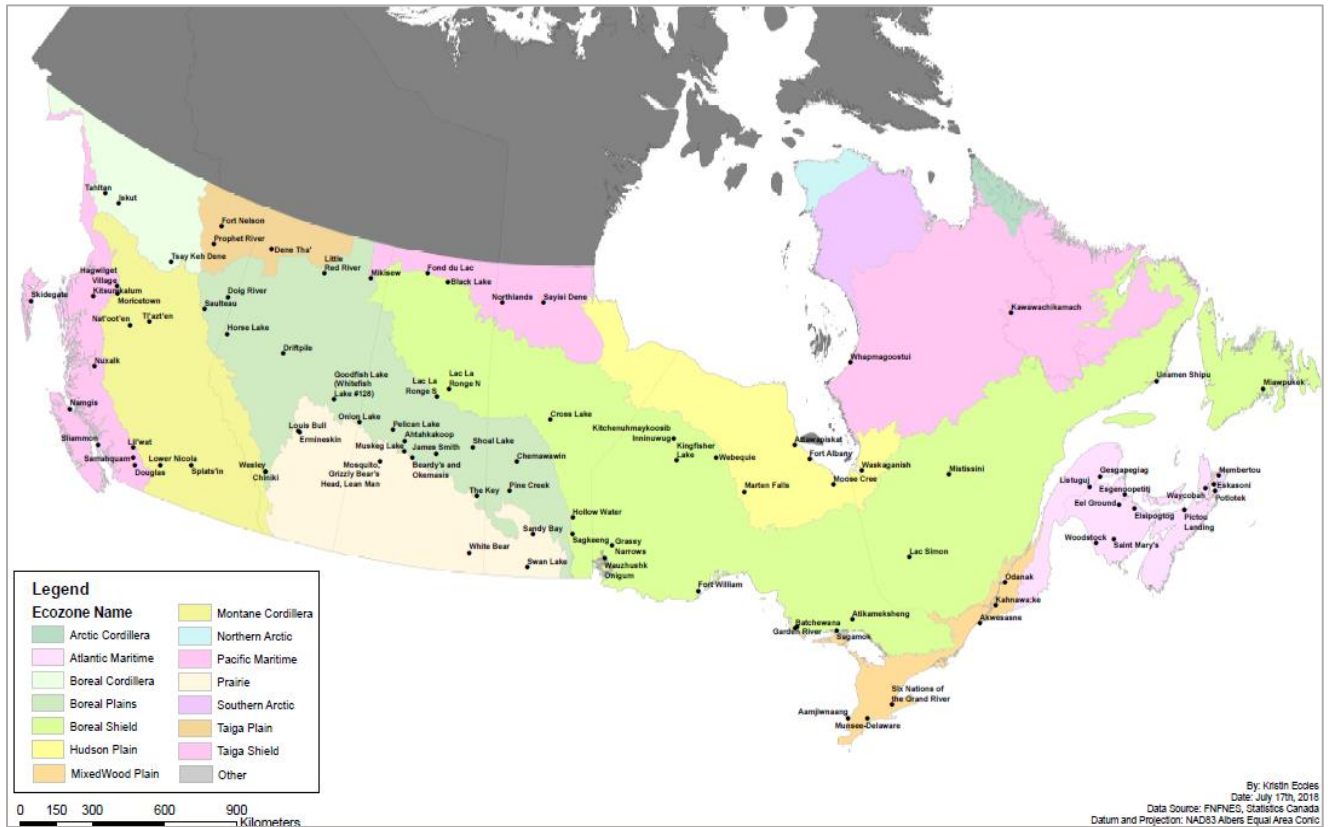
The FNFNES collected comprehensive baseline data on diets (traditional and market foods), health and socio-economic characteristics, and food security. The levels of key environmental chemicals of concern were measured in traditional foods that are commonly consumed by members of participating First Nations communities. Furthermore, hair samples were collected and analyzed for mercury exposure. The study was first undertaken in 21 First Nations communities in British Columbia in 2008 and 2009 (Chan et al. 2011). In 2010, data collection occurred in 9 Manitoba First Nations (Chan et al. 2012). A total of 18 First Nations in Ontario participated in 2011 and 2012 (Chan et al. 2014). In 2013, 10 First Nations from Alberta participated in the study (Chan et al. 2016). In 2014, 11 First Nations in the Atlantic region were surveyed (Chan et al. 2017). A total of 14 First Nations communities were surveyed in Saskatchewan in 2015 (Chan et al. 2018), and 10 First Nations from Quebec participated in 2017. In the current research, data from three provinces: British Columbia, Manitoba and Ontario were analyzed (Figure 8).

1.3.3 Ethical approval

Individual participation in the project was voluntary and based on informed written consent after an oral and written explanation of each project component. This survey was conducted following the “Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans” and in particular Chapter 9 research involving the First Nations, Inuit and Métis Peoples of Canada. The

Ethical Review Boards at the University of Northern British Columbia, the University of Ottawa, the Université de Montréal, and Health Canada approved the study.

Figure 8. Map of participating communities of the First Nation Food Nutrition and Environment Study (2008-2017).



1.3.4 Dissertation Structure

This dissertation is comprised of ten chapters. Seven chapters (chapter 3 – 9) include manuscripts for publications in peer-reviewed academic journals and are presented in three sections. Section 1 and 2 combine research related to fish consumption in Manitoba and Ontario First Nations. In particular, section 1 outlines the importance of traditionally-harvested fish consumption for food and nutrition security for Manitoba and Ontario First Nations. Section 2

focuses on the assessment of risks and benefits of fish consumption regarding exposure to POPs (PCBs and DDE) and beneficial n-3 FAs (EPA+DHA) with T2D in Manitoba and Ontario First Nations. Section 3 includes three studies conducted among British Columbia First Nations. Collectively, they examine determinants of seafood consumption and project the impacts of the climate-related decline of seafood harvest on seafood consumption patterns, nutritional status and cardiovascular health of First Nations in British Columbia.

Chapter 1 presents the background and rationale of the thesis, the overall and specific objectives of this research project as well as an overview of methodology and the structure of the dissertation.

Chapter 2 summarizes the current state of knowledge and place the research within the existing literature on traditional food systems, in particular fish and seafood and the health status of First Nations in Canada.

Section 1: Fish and Food Security in First Nations

This section explores the role of fish consumption for food and nutrition security among First Nations in two provinces, Manitoba and Ontario, in Canada. The manuscript also summarizes barriers to the availability of and access to traditional food including fish and to food harvesting activities.

Chapter 3 presents the manuscript “Importance of fish consumption for the food and nutrition security of First Nations in Manitoba and Ontario, Canada” which has been prepared for the Canadian Journal of Public health.

Section 2. Risk and Benefit assessments of Fish Consumption

This section is comprised of three studies. The first and second studies explore the potential risks and benefits of locally-harvested fish consumption regarding exposure to certain POPs (DDE and PCBs) and beneficial nutrients such as n-3 FAs with T2D in Manitoba and Ontario First Nations. The third manuscript aims to interpret our inconsistent findings on the associations between fish consumption, n-3 FAs and POPs intake with T2D in Manitoba and Ontario. By using the DID model, the study examines if relatively high exposure to POPs may outweigh the protective associations of fish (n-3 FAs) on T2D. In addition, the study estimates the thresholds of daily dietary DDE and PCB exposure that increase the risk of T2D.

Chapter 4 presents the manuscript "Fish consumption is inversely associated with type 2 diabetes in Manitoba First Nation communities" which was published in FACETS.

Chapter 5 presents the manuscript "Association between fish consumption, dietary omega-3 fatty acids and persistent organic pollutants intake, and type 2 diabetes in 18 First Nations in Ontario, Canada", which was published in the Environmental Research journal.

Chapter 6 presents the manuscript "The relationship between persistent organic pollutants exposure and type 2 diabetes among First Nations in Ontario and Manitoba, Canada: difference in difference analysis", which was published in the International Journal of Environmental Research and Public Health.

Section 3. Climate Change and Human Health

This section entailed studies on the nutritional benefits of seafood consumption and modelling potential impacts of the climate-related decline in seafood abundance on the nutritional quality of diets and cardiovascular health among First Nations in British Columbia. This section involves

two datasets: projected scenarios of climate-related change on seafood catch potential for coastal First Nations in British Columbia and dietary data collected by the FNFNES, as well as model formulas and assumptions, extracted from various epidemiology studies.

Chapter 7 presents the manuscript “Seafood consumption patterns, their nutritional benefits and associated socio-demographic and lifestyle factors among First Nations in British Columbia, Canada”, which was published in the Public Health Nutrition journal.

Chapter 8 presents the manuscript “Impacts of the climate-related decline of seafood harvest on nutritional status for coastal First Nations in British Columbia, Canada”, which was accepted for publication in PLOS One.

Chapter 9 presents the manuscript “Potential impacts of reduced seafood consumption on cardiovascular health in First Nations in British Columbia” which was prepared for the Canadian Journal of Public Health.

Chapter 10 summarizes the main findings of the dissertation and discusses the significance and implications of the thesis.

2 LITERATURE REVIEW

2.1 Health disparities experienced by First Nations in Canada

Although the health of First Nations people in Canada has been improved in recent years, disparities in health status remain between First Nations and non-Indigenous population in Canada (Health Canada 2014b; Reading 2009). First Nations continue to experience a lower life expectancy, higher rates of infant mortality and chronic diseases compared with the general population in Canada (Health Canada 2014b; King 2010; Smylie et al. 2010). Other health issues include certain infectious diseases, mental disorders, injuries and drug abuse (Firestone et al. 2015; Health 2012). The majority (59.8%) of First Nations adults have at least one chronic health condition (FNIGC 2018), and three-quarters (74.4%) are overweight or obese (FNIGC 2012, 2018). Among First Nations, the rates of death are two times higher compared to the non-Indigenous population in Canada while diseases of circulatory system, cancer as well as respiratory and metabolic disorders are among the leading causes of mortality (Health Canada 2014b). First Nations continue to experience a disproportionately higher burden of obesity, diabetes and heart diseases (Dyck et al. 2010; FNIGC 2012; Haman et al. 2010; Young et al. 2000).

2.1.1. Cardiovascular diseases

Historically, the rate of CVD among First Nations was lower than that among general Canadians (Reading 2009; Young 2012). Since 1997, heart diseases among First Nations increased drastically and were three times higher compared to the general Canadian population (RHS 1999). The First Nations Regional Health Survey (RHS) 2002/03 reported that older First Nations (aged 50-59 years) had more than a two-fold higher prevalence of self-reported heart disease than the non-Indigenous population in Canada (11.5% vs 5.5%) (FNC 2005). In British

Columbia, Status Indians had rates for ischemic heart diseases 25% higher the rates among other residents in the province (British Columbia Office of the Provincial Health Officer 2009). The RHS (2008/10) found that the prevalence of heart disease among First Nations was 7.6% compared to 5.7% among other Canadians (FNIGC 2012). Data on the rates of MI among First Nations are limited. However, death rates due to MI were estimated to be 25% higher in First Nations men and 55% higher in First Nations women compared to non-Indigenous Canadian population (Statistics Canada 2013; Tjepkema et al. 2012).

2.1.2. Diabetes

Diabetes is one of the most critical health issues among First Nations in Canada. Overall, the prevalence of diabetes among First Nations communities is 3 to 5 times higher than among the non-Indigenous Canadians (Dyck et al. 2010; Young et al. 2000). The First Nations RHS (2008/10) showed that self-reported prevalence of diabetes among on-reserve First Nations was 20.7% (FNIGC 2012). The most recent First Nations RHS (2015/16) revealed that about 19.2% of on-reserve First Nations were diagnosed with diabetes (FNIGC 2018). Data from the FNFNES indicated that prevalence of diabetes among First Nations ranged from 18.1 to 24.3% across different regions in Canada (Chan et al. 2018) but can reach as high as 29% among Quebec's First Nations (INSPQ 2015) and 36% in northern First Nations communities (Imbeault et al. 2011). This stands against 10.3% among off-reserve First Nations, 7.3% among Metis and 5% in the non-Indigenous population (Public Health Agency of Canada, 2011). Type 2 or non-insulin-dependent diabetes (T2D) is the most prevalent type of diabetes which is characterized by insulin resistance, relative insulin deficiency, and subsequent hyperglycemia. The risk of T2D increases with older age, obesity, poor quality diets and lack of physical activity (NCCAH 2012). First Nations tend to be younger at onset, have greater severity of the disease and have more health

complications from T2D (e.g. coronary heart disease, stroke, blindness, kidney failure, and peripheral arterial disease) compared with non-Indigenous Canadians (Dannenbaum et al. 2008; Valera, Dewailly, and Poirier 2011).

2.2. Risk factors for Type 2 Diabetes

The development of T2D is a result of an interaction between environmental and genetic factors. Environmental or lifestyle risk factors of T2D include obesity, sedentary lifestyle, smoking and an unhealthy diet. Obesity, in particular, abdominal adiposity, is a major determinant of the development of T2D. Adipose tissue affects metabolism by secreting hormones and other substances such as leptin, cytokines, adiponectin, proinflammatory substances and nonesterified fatty acids. These substances are involved in the development of insulin resistance and in the impairment of β -cell function (Al-Goblan et al. 2014). Several studies have shown that body mass index (BMI) has a strong relationship with insulin resistance and T2D (Al-Goblan et al. 2014).

Physical activity plays a key role in the prevention and management of T2D. Regular physical activity reduces abdominal adiposity and induces glucose uptake by skeletal muscle, adipose tissue and liver (Den Boer et al. 2013). In addition, exercise increases insulin sensitivity and glucose tolerance as well as improves blood lipid profiles in persons with T2D (Colberg et al. 2010).

Cigarette smoking is another important risk factor for the development of T2D. Several prospective studies confirmed that smoking is associated with increased risk of T2D (Manson et al. 2000). In fact, smokers are 30–40% more likely to develop T2D than non-smokers, and smokers with T2D have higher risks for micro and macrovascular complications (US DHHS 2014). Also, smoking increases inflammation and oxidative stress, it damages β -cell function and

impairs endothelial function (Chang 2012). Since smoking is very prevalent among Indigenous populations, the smoking cessation is important targets for prevention of T2D.

Unhealthy diet also contributes to the development of T2D. A number of epidemiological studies demonstrated positive relations between diets low in fiber and high in simple carbohydrates with T2D (Liu et al. 2000). High intake of saturated fat and processed meat also contributes to the elevated risk of obesity and T2D (Dhingra et al. 2007). In contrast, high fruit and vegetable consumptions showed a preventive effect against T2D (Wu et al. 2015). Over the past decades, Indigenous people in Canada have been undergoing a rapid nutrition transition characterized by a shift away from traditional diets towards store-bought food which is high in energy, fat, and sugars (Kuhnlein et al. 2004). This lifestyle transition has been concomitant with a sedentary lifestyle and is associated with increased prevalence of obesity and T2D (Young et al. 2000).

Besides several modifiable factors, genetic predisposition also contributes to the development of T2D. Several studies have indicated that a family history of diabetes is a strong predictor of diabetes. In fact, the lifetime risk of developing T2D is 40% for individuals who have one parent with T2D and 70% if both parents are affected (Meigs et al. 2000). Moreover, first degree relatives of individuals with T2D are 3 times more likely to develop the disease than individuals without a family history of T2D (Ali 2013). In women, gestational diabetes during pregnancy increases the risk of T2D later in life and elevates the risk of diabetes in offspring. Among Indigenous people, women are more likely to develop T2D compared to men (Dyck et al. 2010).

Ethnicity is considered as a risk factor for T2D which is not correlated with other risk factors. Epidemiological studies suggest that some ethnic groups, such as Hispanics, Asians and North America Indians have a predisposition to T2D (Abate and Chandalia 2003). Neel proposed a “thrifty gene effect” theory which is based on the hypothesis that North America Aboriginal

populations are genetically adapted to starvation conditions by storing nutrients more efficiently when food is available (Neel 1999). This “thrifty gene” was beneficial because Aboriginal individuals lived hunter-gatherer lifestyles, and the access to foods was not stable (Byrne and Nkongolo, 2012). With modern conditions of relatively constant nutrient abundance and a high glycemic load diet, this physiological response results in insulin resistance, hyperglycemia, obesity and diabetes (Young et al. 2000). However, there is still debate concerning the importance of genetic versus other environmental factors associated with susceptibility to T2D (Southam et al. 2009).

2.3 Social determinants of health

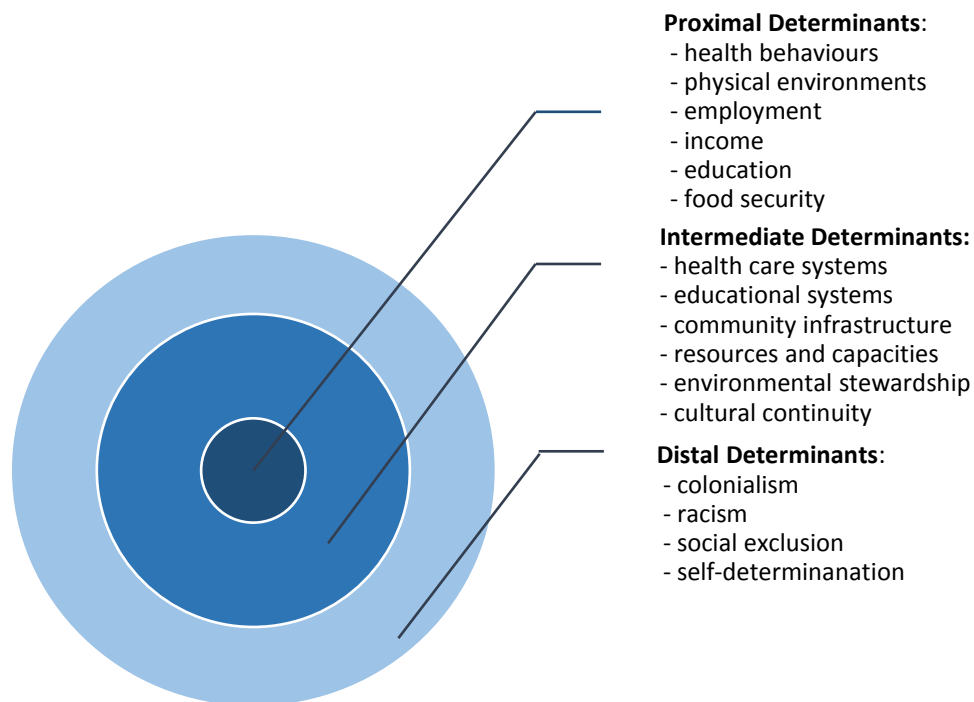
For Indigenous people, holistic understanding of “Health” generally comprises “*physical, mental, spiritual, emotional and social dimensions and not merely the absence of disease or infirmity*” (WHO 2007). The health inequities experienced by First Nations people are determined by multiple factors, including colonization, racism and marginalization (Nesdole et al. 2014). Reading and Wien (2009) grouped various social determinants into three categories: proximal, intermediate, and distal (Reading and Wein 2009). Proximal determinants of health involve conditions that have a direct impact on individual’s physical, emotional, mental or spiritual health and include health behaviours, physical environments, employment and income, education, and food security. Intermediate determinants combined educational and health care systems, community infrastructure and resources, environmental stewardship and cultural continuity, and were defined as those responsible for the proximal determinants. Lastly, distal determinants encompass political, economic and social contexts and have the most profound influence on Indigenous health (Reading and Wein 2009) (Figure 2).

The colonization of Indigenous Peoples is considered as a fundamental underlying health determinant which produced social, political and economic inequalities through the creation of unfavourable intermediate and distal determinants (Adelson 2005). Historically, many Indigenous people were disposed of and relocated from their lands, culture, language and traditional ways of life, which resulted in dramatic and devastating social and cultural changes. Likewise, residential schools, one of the most powerful methods of assimilation, destroyed the connections between children and their families and communities and, thus profoundly impacted their cultural identity, language and the transfer of traditional knowledge (Reading and Wein 2009). Although there are no longer residential schools, the trauma continues to affect First Nations' lives and well-being. According to the RHS (2008-10), almost half (47.3%) of the survivors of residential schools perceived a negative effect on their health and well-being whereas 43% of their children believed that residential schools negatively affected the parenting skills of their parents (FNIGC 2012).

The health of an individual and their families is also impacted by intermediate determinants, such as education and healthcare systems, community infrastructure and resources. Many First Nations especially those living in remote and isolated communities have difficulties accessing health care services because of not available limited local services, inadequate or culturally inappropriate health care services. In addition, low levels of incomes do not allow First Nations to afford the cost of healthcare and services (FNC 2005). Beckett et al. (2017) reported a significant relationship between a self-reported diagnosis of diabetes with a lack of culturally appropriate health care, not having a doctor available in the area, inadequate or a lack of available health care services and facilities in the area among urban First Nations people (Beckett et al. 2018). Furthermore, the insufficient educational system directly impacts an individual's

income, employment and living conditions. A lack of post-secondary education funding from the Canadian government makes education less accessible for First Nations students (Bains 2014). Likewise, limited infrastructure and resource development opportunities significantly contribute to economic insecurity and marginalization (Reading and Wein 2009).

Figure 2. Social determinants of health for Indigenous People (Adapted from Reading & Wien, 2009)



Educational attainment is an important determinate of the health of both children and adults. Education is associated with literacy, awareness about health and nutrition, as well as an ability to make a healthy choice. Additionally, education offers benefits for better employment and

income (National Collaborating Centre for Aboriginal Health (NCCAHA) 2017). Despite some improvement over the last years, there is still a gap in educational attainment between First Nations adults and the general population in Canada. Indeed, 33.7% of First Nations compared to 11.5% of the general Canadians who had less than a high school education while 10.9% of First Nations compared to 26.5% of non-Indigenous Canadians who obtained university degree (FNIGC 2018; National Collaborating Centre for Aboriginal Health (NCCAHA) 2017).

In Canada, the poverty rate is disproportionately higher among First Nations compared to the non-Indigenous people and is directly linked to low educational attainment and high unemployment rates (Reading and Wein 2009). According to the most recent First Nations RHS (2015/16), the rate of unemployment was 31.6% while almost half (48.6%) of younger First Nations (18-29 years) did not work (FNIGC 2018). For comparison, the unemployment rate among the general population in Canada was 7% (FNIGC 2018). Furthermore, 58% of First Nations adults reported an annual income of less than \$20,000 (FNIGC 2012). Among First Nations, low income has been associated with food insecurity which leads to increased rates of obesity and diet-related chronic diseases (Kuhnlein, Erasmus, et al. 2013). Poverty is also linked to poor health status, mental health issues, social exclusion and crime (Kolahdooz et al. 2015; Reading and Wein 2009). Furthermore, financial constraints present barriers to traditional harvesting activities since the high cost of gas and hunting equipment (Chan et al. 2006; Gabrielle 2008).

2.4 Food insecurity among First Nations

Food insecurity (i.e. the inability to afford nutritionally adequate and safe foods) is highly prevalent and recognized as a serious public health issue for First Nations in Canada (Huet, Rosol, and Egeland 2012; Skinner, Hanning, and Tsuji 2013). Food insecurity is directly related to unhealthy diets and associated with decreased consumption of fruits and vegetables, grains

and dairy products, and increased intake of energy from fat and sugary foods (Huet et al. 2012). This results in compromised nutritional status and increased susceptibility to infections and diet-related chronic diseases (such as obesity, diabetes and heart diseases) (Ford 2013; Seligman, Laraia, and Kushel 2009).

There are unique considerations of food security for First Nations. In addition to food insecurity related to monetary access to market food, “traditional food security” is an additional level related to harvesting and consumption of traditional foods (Power 2008). The four dimensions of “traditional food security” are considered as following: food access (i.e. access to traditional food systems through harvesting activities), food availability and supply (i.e. environmental contamination of traditional foods and the impacts of climate change on ecosystems; thus, how these changes may affect the safety, availability and supply of traditional foods), and food utilization (i.e. the levels of traditional knowledge about nutritional quality of traditional foods (Lambden et al. 2007; Power 2008). Therefore, the context of food security for Indigenous people involves both market and traditional food systems (Power 2008).

Several socio-economic and demographic factors contribute to the income-related food insecurity in First Nations communities. Among those, high rates of poverty which are directly linked to the high rates of unemployment, low education attainment and low income remain critical issues in First Nations communities (Reading and Wein 2009). Also, high cost, poor quality, lack of variety and availability of perishable market foods represent additional barriers, especially in remote and isolated communities (Earle 2011; Health 2012; Willows et al. 2008). “Traditional food security” is also affected by several factors. These include the loss of access to traditional lands, environmental contaminants in wildlife, decreased diversity and distribution of species and changes in animal migratory patterns. In addition, the high costs of hunting and

fishing equipment were documented as factors limiting the ability to harvest wild foods (Chan et al. 2012, 2014; Lambden et al. 2006).

2.5 Traditional food use among First Nations

The consumption of traditional foods differs by many factors, such as region, location and size of communities, age, gender and seasonal availability of wildlife. At pre-contact, traditional diets provided large amounts of animal protein and fat, and low amounts of carbohydrates (Haman et al. 2010). Carbon isotope estimates indicate that prehistoric coastal people in British Columbia derived 90% of their energy from marine protein and fat whereas 10% was obtained from gardening (vegetable and fruits) (Chisholm and Nelson 1983). In fact, among the Central Coast Salish people, annual consumption of salmon ranged from 272 to 320 kg per person (Fediuk and Thom 2003). Other studies indicated that annual consumption of marine and land foods among First Nations communities in Canada ranged from 365 to 730 kg per person (Fediuk and Thom 2003). Wein (1990) documented that Métis and First Nations in northern Alberta consumed traditional food about 319 times per year with fish consumed on average 62 times per year (Wein, Sabry, and Evers 1990). Among Yukon First Nations, traditional foods were consumed on average 1.14 times per day providing 17% of total daily energy whereas salmon species were the most consumed fishes (Wein and Freeman 1995). Batal (2004) estimated that among First Nations and Métis communities in the Denendeh and Yukon daily consumption of fish ranged from 184 g to 225 g per person (Batal et al. 2004).

Kuhnlein (2013) documented that an annual rate of salmon consumption among Nuxalk people was approximately 165 kg per family in 1981 and increased up to 424 kg per family following the Nuxalk Food and Nutrition Program (Kuhnlein et al. 2013). However, a dramatic decline in fish (such as salmon and eulachon) and other traditional foods was observed by 2009 (Kuhnlein

2013). Among the James Bay Cree adults in northern Quebec, average daily consumption of fish was 60 grams per day, while heavy fish consumers ate 291 grams of fish per day. Coastal communities consumed a higher amount of fish (78g/d) compared to inland ones (34 g) (Dewailly et al. 2002).

Limited data on traditional food consumption are available for First Nations living in southern regions of Canada. Philibert (2009) reported that a First Nations community in Northern Ontario consumed on average 4.6 fish meals per month (Philibert et al. 2009). The ongoing FNFNES, conducted in 100 First Nations communities south of 60th parallel across Canada, has provided reliable regional information about traditional food consumption patterns among First Nations people living on-reserve (www.fnfnes.ca). In British Columbia, fish consumption was reported by 95% of First Nations with salmon, a keystone species, consumed on average, once per week (Chan et al. 2011). In Manitoba, fish consumption was reported by 83% of respondents with walleye and lake whitefish, the most consumed species, eaten about 11 and 4 times per year, respectively (Chan et al. 2012). In Ontario, the majority (73%) of First Nations reported eating wild fish in the prior year. On average, daily consumption of walleye, lake whitefish and northern pike was 7.2, 2.9 and 1.5 grams per day, respectively (Chan et al. 2014). Overall, research indicates that northern and remote communities consumed more fish and other traditional foods compared with southern ones. In addition, men and older individuals reported higher consumption of traditional foods compared to women, young adults and children (Kuhnlein, Goodman, et al. 2013; Nakano et al. 2005).

2.6 Health benefits of fish and omega-3 fatty acids

Fish is widely promoted as a healthy food choice. The health benefits of fish and seafood are mainly attributed to the content of high-quality protein, vitamins, minerals and n-3 FAs. These nutrients are essential for human health, growth, and development (HLPE 2014).

A body of literature shows that the consumption of fish, rich in n-3 FAs, such as EPA and DHA, provides many health benefits (Shahidi and Ambigaipalan 2018). EPA and DHA intake have favourable effects on neurological and cognitive development in the fetus and infants and may reduce the risk of premature birth (Shahidi and Ambigaipalan 2018). Clinical and epidemiological studies demonstrated protective effects of fish and n-3 FAs against CVD including stroke and MI (Elagizi et al. 2018; Leung Yinko et al. 2014), and lower the risk of sudden cardiac death and cardiovascular mortality (Breslow 2006; Leung Yinko et al. 2014; Shahidi and Ambigaipalan 2018). Epidemiological studies among the Greenland Inuit confirmed that traditional diets rich in marine mammals and fish reduce the incidence of CVD (Bang HO, Dyerberg J 1971). The mechanisms of the EPA and DHA effects include lowering plasma triglycerides, inhibiting platelet aggregation and inflammation, lowering blood pressure, preventing arrhythmias, improving endothelial function and vascular reactivity (Elagizi et al. 2018; McLennan 2014; Shahidi and Ambigaipalan 2018; Yashodhara et al. 2009).

Recent evidence suggests that fish and n-3 FAs may help prevent T2D since their beneficial effects on lipid profile, insulin resistance and inflammation (Chen, Yu, and Shao 2015; Fedor and Kelley 2009; He 2009; Panagiotakos et al. 2007). Also, increased intake of dietary n-3 FAs improves glucose tolerance and metabolic syndrome (Ebbesson et al., 2005; Paquet et al., 2013). Insulin resistance is a pathological condition in which cells fail to respond to the action of insulin in transporting glucose from the bloodstream into muscle and other tissues. Metabolic syndrome

is a clustering of at least three of the five following medical conditions: central obesity, high blood pressure, high blood sugar, high serum triglycerides, and low serum high-density lipoprotein (Ebbesson et al., 2005). Systematic meta-analyses, however, reported geographical differences in the relationship between fish, n-3 FA intake and T2D with protective associations being observed in the populations of Asian countries, and positive associations being observed in the American population (Wallin et al. 2012; Zheng et al. 2012). These discrepancies may be explained by differences in levels of n-3 FAs and contaminants (POPs, MeHg) present in fish (Lee et al. 2014; Lee and Jacobs 2010) which significantly vary by fish species and geographical location (Aguilar, Borrell, and Reijnders 2002). Scientists suggest that relatively high exposure to POPs through fish may outweigh the beneficial effects of n-3 FAs on T2D (Christensen et al. 2016; Domingo 2014; Lee and Jacobs 2010; Wallin et al. 2015)

2.7 Environmental contaminants and their effects on human health

Despite numerous benefits, fish, seafood and marine mammals may be a pathway of exposure to environmental pollutants. Environmental pollutants travel from southern latitudes to the North through the atmosphere and water currents and bioaccumulate and biomagnify in the Arctic food webs. Since predator species are at upper trophic levels of food chains, they accumulate high levels of contaminants in their fatty tissues. Indigenous people including First Nations are particularly vulnerable to environmental chemicals through consumption of wild fish and marine mammals (Laird, Goncharov, and Chan 2013; Seabert et al. 2014). Indeed, elevated exposure to POPs and mercury have been observed in the Inuit whose diets greatly rely on marine mammals (Donaldson et al. 2010; Laird et al. 2013; Oostdam et al. 2005). Among First Nations in Northern Ontario, POPs and mercury blood concentrations were on average 3.5 times higher among those consuming wild foods compared to non-consumers (Seabert et al. 2014). Likewise, blood

concentrations of PCBs were predicted by local fish consumption among Mohawk men and women at Akwesasne (Fitzgerald et al. 1999, 2004). The Grassy Narrows and Wabaseemoong First Nations in Northwest Ontario have been exposed to mercury via consumption of fish contaminated by pulp mill (Takaoka et al. 2014).

2.7.1 Persistent organic pollutants (POPs)

POP exposure exhibits several adverse health effects, including impaired reproductive functions, neurobehavioral deficits, certain types of cancer and hormone-related disorders (Birnbaum 2012; Diamanti-Kandarakis et al. 2009; Ross 2004). Exposure to PCBs can increase the risk of CVD, such as stroke and MI (Bergkvist et al. 2014, 2016). Recently, evidence has linked low-dose chronic POP exposure with obesity, insulin resistance, and T2D (Lee et al. 2014). A number of cross-sectional (Aminov et al. 2016; Lee et al. 2006) and prospective (Lee et al. 2010; Vasiliu et al. 2006) studies confirmed that exposure to PCBs and DDE may increase the risk of T2D.

Systematic review and meta-analyses reported that exposure to certain POPs, such as PCBs and DDE, dioxin and dioxin-like chemicals are associated with T2D (Fakhri et al. 2016; Lee et al. 2014; Ngwa et al. 2015; Taylor et al. 2013). Similarly, research among the Inuit and First Nations in Canada reported positive associations between exposure to certain POPs with T2D (Philibert et al. 2009; Singh and Chan 2017). Pal (2013) observed significantly higher plasma concentrations of POPs in diabetic compared to non-diabetic First Nations adults (Pal et al. 2013).

Several possible biological mechanisms have been proposed to explain the effects of POPs. POPs are well-know endocrine-disrupting chemicals which interfere with the synthesis, secretion, transport and activity of hormones and thus, mimic and disturb hormone functions (Chevalier and Fénichel 2015). PCBs may cause mitochondrial dysfunction via mutations in

mitochondrial DNA and nuclear genes, and through glutathione depletion (Montgomery and Turner 2015; De Tata 2014). Mitochondrial dysfunction, in turn, plays a crucial role in chronic low-grade inflammation and may lead to ectopic fat accumulation in the liver, muscle and pancreas. Also, low-grade inflammation in adipose tissue plays an important role in the development of insulin resistance and T2D (Lee et al. 2014; Montgomery and Turner 2015). Furthermore, PCBs may disrupt the function of pancreatic beta cells (Hectors et al. 2011, 2013; De Tata 2014).

2.7.2 Mercury

Mercury can exist in three forms: elemental mercury, inorganic mercury and organic mercury, e.i. methylmercury (MeHg). The most toxic form is MeHg which affects the central nervous system, particularly in the developing fetus (Bjørklund et al. 2017). Literature shows that prenatal exposure to MeHg is associated with low birth weight, impaired neurodevelopment, growth and development of children (Azevedo et al. 2012; Ha et al. 2017). There is growing evidence that exposure to MeHg increases the risk of metabolic conditions such as obesity, elevated cholesterol and triglyceride levels, high blood pressure, glucose levels, and diabetes (Ha et al. 2017; He et al. 2013). Furthermore, epidemiological studies indicate that chronic exposure to MeHg increases the risk of CVD such as MI (Bjørklund et al. 2017; Ha et al. 2017), through the increases in oxidative stress and the production of free radicals. It also affects heart rate variability and promotes inflammation, hypertension and plaque development (Genchi et al. 2017).

2.7.3 Biomonitoring survey among First Nations

The First Nations Biomonitoring Initiative (FNBI) established baseline information on human exposure to environmental chemicals for on-reserve First Nations people south of the 60th

parallel (Assembly of First Nations 2013). The FNBI was conducted among fifteen First Nations communities across Canada. Among 97 environmental chemicals, PCBs, DDE and MeHg were measured in biospecimens. The FNBI results showed that plasma levels of PCBs and DDE in First Nations were significantly lower than corresponding levels observed in the general population based on the Canadian Health Measure Survey (CHMS). However, the FNBI participants aged 60 - 99 years in the Great Lake ecozone had elevated DDE levels. The blood levels of total mercury among First Nations were comparable to those levels collected by the CHMS (geometric mean (G.M) 0.95 µg/L and 0.83 µg/L, respectively). However, First Nations in the Pacific ecozone and the Great Lake ecozone had significantly higher levels of total mercury (G.M. 1.91 µg/L and 1.07 µg/L) than the CHMS population. The Canadian total blood mercury guidance value is 20 µg/L for the general population and 8 µg/L for children, pregnant women, and women of child-bearing age (Legrand et al. 2010).

2.8 Traditional food consumption advisories

When there is a concern about the quality and safety of traditional foods related to potential contaminant exposure, food consumption advisories are issued (McAuley and Knopper 2011). For example, Mohawk First Nations of Akwesasne were advised to limit their fish consumption due to high levels of PCBs (Fitzgerald et al. 1999). Likewise, advisories on fish consumption in the Great Lake areas were posted as a result of concerns about exposure to PCBs and mercury (Health Professionals Task Force 2004; Turyk et al. 2012). Also, recommendations on fish consumption were provided among Cree living in Northern Quebec because of elevated exposure to mercury affected by the La Grande Reservoir Complex (Chevalier et al. 1997). Although food consumption advisories help to reduce the body burden of contaminants, they lead to reduced reliance on traditional foods with consequently increased reliance on store-bought foods and low

intake of essential nutrients. Maintaining of traditional lifestyle is fundamental for health and well-being of First Nations. Consumption of traditional food is associated with better diet quality and nutritional adequacy whereas harvesting activities contribute to physical health. Also, food-sharing practices support social cohesion and spiritual and mental wellness. Given this importance, benefits of traditional food use must be weighed against the risk of contaminant exposure.

2.9 Climate change

Global warming is recognized as the biggest threat of the 21st century. Indigenous people are particularly affected by climate change since their close relationship with the environment and heavy dependence on natural resources for their livelihood and culture (Downing and Cuerrier 2011). First Nations and other Indigenous and northern communities have already noted significant changes in weather patterns and their impacts on abundance and availability of wildlife, access to wild foods, quality and safety of traditional foods (Downing and Cuerrier 2011; Ford et al. 2010; Ford, Smit, and Wandel 2006; Lemmen, Warren, and Resources 2016). Indeed, First Nations communities from the Northwest Territories and Yukon reported rapid changes in water levels, weather patterns, ice dynamics and wildlife distribution (Guyot 2006). Northern Indigenous communities observed significant thinning of the sea- and freshwater ice, reduction in snow cover, coastal erosion, reduction in water levels in rivers and ponds which negatively impact the health and distribution of fish species and other wildlife (Ford et al. 2010; Furgal and Seguin 2006). The Weenusk First Nation at Peawanuck (Northern Ontario) indicated changes in travel routes on water and land, the disappearance of particular insects, bird species, changes in flora and fauna (Lemelin et al. 2010). First Nations communities in the western James Bay region of northern Ontario reported that climate variability and extreme events precipitated

fish die-offs and changes in the timing of harvesting of fish (Hori 2010). Hence, the limited access to subsistence species is likely to reduced traditional food use which, coupled with ongoing nutrition transition, affects food security and nutritional status of Indigenous people (Council of Canadian Academies 2014; Ford et al. 2010; Kuhnlein, Erasmus, et al. 2013).

Climate change poses a significant threat to marine ecosystems. Changes in water temperature, oxygen content, pH levels and other ocean properties directly affect the production and distribution of marine species (Cheung et al. 2013; Lam et al. 2016). Recent research indicates that climate change will lead to the large-scale redistribution of global catch potential with the most apparent changes in the Pacific Ocean (Cheung et al. 2010). This represents a particular challenge to coastal communities including First Nations living in British Columbia whose traditional diets heavily rely on marine sources for food and economy (Cisneros-Montemayor et al. 2016). First Nations communities living along the Pacific coast harvest a wide variety of marine foods (fish, shellfish, and seaweeds) while salmon is a cultural keystone species (Garibaldi and Turner 2004) for their diet (Chan et al. 2011; Mos et al. 2004). These coastal First Nations have developed special skills and technologies to manage their environments and have gained experience to accommodate to environmental changes using traditional ecological knowledge (Butler and Campbell 2004; Campbell and Butler 2010; Turner and Clifton 2009). However, rapid, unpredictable climate change negatively affects their subsistence and commercial fishing practices (Downing and Cuerrier 2011). Recently, researchers projected that the majority of marine species harvested by coastal First Nations will decline in abundance and shift poleward relative to BC's marine environment. The keystone species, such as salmon and herring, are likely to experience the most significant relative impact. (Weatherdon et al. 2016).

Along with the direct impacts on wildlife abundance and distribution, climate change poses risks for harvesting activities. Extreme weather patterns, changes in the timing of freeze-up and breakup of sea ice make harvesting more dangerous and reduce the access to certain hunting areas (CIER 2008; Furgal and Seguin 2006). Strong winds and unsafe travel conditions limit hunting of marine mammals from the open ocean (Ford et al. 2010). Diminished harvesting activities, in turn, lead to cultural changes, loss of language and affect transmission of traditional knowledge about the land, wildlife and harvesting practices with consequent negative implications for food and nutrition security (Centre for Indigenous Environmental Resources (CIER) 2006; Ford et al. 2010; King and Furgal 2014).

Climate change may also affect the levels of environmental pollutants in traditional food systems through influence on long-range transport from southern latitudes to northern regions, affect biodegradation of pollutants, as well as accumulation and biomagnification of contaminants in food chains (Assembly of First Nations 2007). Indeed, levels of contaminants (such as PCBs, pesticides and heavy metals) may increase due to declining water levels (lakes, ponds, rivers) caused by warming temperature (Assembly of First Nations 2007; Kundzewicz et al. 2009).

2.10 Summary

Notwithstanding significant dietary, lifestyle and cultural transformations among First Nations over the last few decades, locally harvested fish and seafood continue to be an integral part of their diets delivering important health and cultural benefits (Kuhnlein al. 2013). Even consumed in small amounts, fish and other wild foods provide significant sources of energy, protein, vitamins, minerals, and n-3 FAs (Gagne et al. 2012; Johnson-Down and Egeland 2012; Kuhnlein and Receveur 1996, 2007; Sheehy et al. 2015) and thus, promote nutritional health of First Nations people. Moreover, many First Nations desire to consume more fish and seafood species

that are available for harvesting (Chan et al. 2011, 2012, 2014; Fediuk and Thom 2003). However, various anthropogenic stressors, such as climate change and environmental contamination, affect traditional food systems and represent a critical challenge to food security and sustainability of harvesting practices (Downing and Cuerrier 2011; Ford et al. 2006; Kuhnlein, Erasmus, et al. 2013; Lemelin et al. 2010). Nevertheless, there is a lack of research characterizing how multiple factors combined affect food security, health and nutrition of First Nations in Canada.

3 IMPORTANCE OF FISH FOR FOOD AND NUTRITION SECURITY AMONG FIRST NATIONS IN CANADA

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ABSTRACT

Fish, harvested from the local environment, is an essential part of culture, diets and nutritional health among Indigenous Peoples in Canada. In this study, we explore the relationships between fish consumption patterns and factors related to food security statuses in Manitoba and Ontario First Nations (FNs). We estimated the contribution of fish to their daily nutrient requirements. Barriers to the availability of traditional foods including fish are also reported. Data collected from the First Nations Food Nutrition and Environment Study, a cross-sectional study, were used. The sample comprised 646 randomly selected adult FNs participants from Manitoba and 1376 from Ontario. Fish consumption was estimated using traditional food frequency questionnaire. Food security status was assessed with the income-related Household Food Security Survey Module. The contribution of fish to protein, omega-3 polyunsaturated fatty acids (n-3 PUFA), vitamins (A, thiamin, riboflavin, niacin, B6, B12, and D), and minerals (magnesium, phosphorus, potassium, selenium and zinc) requirements was assessed using Dietary Reference Intakes (DRIs). Regional differences were observed between fish consumption patterns and food security status. In Ontario, food insecure individuals tend to rely more on wild fish and other traditional foods and to engage in fishing and hunting activities. In Manitoba, financial constraints among food insecure households limit their access to both market and traditional foods. Fish consumption provides good sources of protein, n-3 PUFA, vitamins B12, D, thiamin, niacin, selenium, and phosphorus. However, many factors including high cost of harvesting equipment, governmental restrictions and climate change continue to reduce the access to and availability of fish and other wildlife. Our results show that traditionally harvested fish continue to be vital to the diet and nutritional health of FNs. Improving access to fish and other wildlife has the potential to reduce food insecurity and to promote sustainable livelihood. Future policies and interventions should address socioeconomic determinants of food insecurity, support traditional harvesting activities and sustainable fisheries in FNs communities.

Keywords: First Nations, food security, traditional foods, fish consumption, nutrient intake

1. Introduction

For thousands of years, Indigenous Peoples (First Nations, Metis, and Inuit) in Canada have been using their knowledge of the local environment and traditional food systems to live off the land (Batal et al. 2004; Egeland et al. 2001). Traditional food systems are diverse across geographical regions and include a great variety of fish species, game meat, and plants. The harvest and consumption of wildlife remain fundamental to Indigenous Peoples' culture and provides them with social, spiritual, physical, economic, and nutritional benefits (Lambden et al. 2007). Diets rich in traditional foods prove to be significant sources of energy, protein, and micronutrients [4–6] whereas activities involved in the acquisition, preparation and consumption of traditional foods sustain a spiritual connection with nature, facilitate the transfer of knowledge and contribute to physical fitness and overall well-being of Indigenous Peoples (Egeland et al. 2001; Kuhnlein et al. 2013). There are 977,230 First Nations living in Canada (Statistics Canada 2017b), out of which 744,855 have registered or treaty Indian status and 329,226 were reported to live on reserves, i.e. small communities across Canada (Statistics Canada 2017b). There are 634 unique First Nations/Indian Bands, with 126 located in the province of Ontario and 63 in Manitoba (AFN 2017).

Fish, which is consumed by over 80% of First Nations adults in Canada, has always been an essential part of Indigenous culture and diets (Chan et al. 2011, 2012, 2014, 2016). Fishing activities, including hand-gathering, spearing, netting, angling and trapping, have been largely used in traditional ceremonies, festivals and other cultural events, enhancing the First Nations spiritual connections to the land and individual communities (Long 2014). Beyond various cultural benefits, traditional fish consumption significantly contributes to food and nutrition security by supplying rich sources of high-quality protein, vitamins (such as B12, riboflavin, and

D), several minerals (selenium, zinc, iron and phosphorus) and omega-3 polyunsaturated fatty acids (n-3 PUFA) (Kuhnlein et al. 2013; Mos et al. 2004; Moss 2016).

Over the last few decades, First Nations have been experiencing a rapid nutrition transition when traditional diets have been gradually replaced by market foods that are high in saturated fat, sodium and sugar (Haman et al. 2010; Johnson-Down and Egeland 2012). This nutrition transition has been concomitant with changes in lifestyle practices such as low physical activity and associated with increased prevalence of obesity, diabetes and CVD (Bruce et al. 2010; Riediger et al. 2014; Riediger, Lukianchuk, and Bruce 2015). Several socio-economic factors including poverty, high unemployment rates, high cost and limited variety and availability of healthy market foods (especially in remote areas) contribute to this transition (Willows et al. 2008). Furthermore, rapid environmental changes, such as climate change, urbanization, industry growth, environmental contamination and degradation reduce the availability, diversity and access of Indigenous People to traditional foods. This, in turn, aggravates nutrition transition and food insecurity among First Nations communities (Ford et al. 2010; Kuhnlein and Receveur 1996; Laberge Gaudin et al. 2015; Turner, Mark, and Kuhnlein 2013).

As defined by the World Food Summit in 1996, food security exists “when all people, at all times, have physical and economic access to sufficient, safe and nutritious food that meets their dietary needs and food preferences for an active and healthy life” (FAO 1996). The experience of food insecurity can range from concerns about running out of food before there is more money to buy more (marginal food insecurity), to the inability to afford a balanced diet (moderate food insecurity), to going hungry, missing meals, and in extreme cases, not eating for a whole day because of a lack of food and money for food (severe food insecurity) (Tarasuk et al. 2013).

There are four pillars of income-related food security: food availability, food access, appropriate

food use and stability of supply (World Health Organization 2018). Insufficient food quantity or quality is associated with the consumption of an unhealthy diet and, consequently, the low intake of essential nutrients. This results in nutritional deficiencies and increased susceptibility to diet-related chronic diseases (Ford 2013; Seligman et al. 2009). Among Indigenous populations in Canada, food insecurity (i.e. the inability to afford nutritionally adequate and safe foods) is highly prevalent and recognized as a serious public health issue (Huet et al. 2012; Skinner, Hanning, and Tsuji 2013). Overall, more than half (54.2%) of on-reserve First Nations households in Canada experienced food insecurity, with 14.1% reporting having to cut the size of their meals or skip meals altogether due to a lack of monetary resources (FNIGC 2012). In contrast, 12.3% of Canadian households experienced some level of food insecurity with 5.6% being categorised as moderately food insecure and 2.5% - as severely food insecure (Tarasuk et al. 2013). In First Nations communities, food insecurity is often associated with a compromised diet quality (Egeland et al. 2011), poor general and mental health and a weak sense of community belonging (Willows et al. 2011b).

It is important to note that food security considerations are unique for Indigenous people as their diets are characterized by access to both market foods and traditional foods harvested locally from the “land”. Power (2008) proposed “traditional food security” as an additional dimension of food security (Power Elaine 2008). The four dimensions of traditional food security were defined as follows: food access (i.e. access to traditional foods), food availability and supply (i.e. environmental exposure and the impacts of climate change on ecosystems; how these changes may affect the safety, availability and supply of traditional foods), and food utilization (i.e. knowledge about nutritional quality of tradition foods) (Power Elaine 2008).

Given that traditional foods in particular fish can play an integral role for the sustainable livelihood of Indigenous people, the objective of our study was to investigate the relative importance of fish consumption in the context of food security among First Nations in two provinces, Manitoba and Ontario, Canada. Specifically, we explored the relationships between fish consumption patterns and factors related to food security statuses. We also estimated the contribution of fish to their daily nutrient requirements. Barriers to the availability of traditional food including fish were also characterized and reported.

2. Methodology

2.1. Study population

Data used in this study were derived from the First Nation Food Nutrition and Environment Study (FNFNES). The FNFNES is a 10-year cross-sectional survey (2008-2018) designed to assess diets, food-related exposure to environmental contaminants and the food security status of First Nations living on reserves south of the 60th parallel across Canada (Chan et al. 2012, 2014). More details on the study's design and methodology are available on www.fnfnes.ca. The selection of First Nations communities was performed using a combined ecozone/cultural area framework to ensure the diversity in ecozones and cultural areas (Wiken EB 1986), and it proceeded in three stages. First, communities were randomly sampled using a systematic random selection with probability proportional to the size of the community. Second, 125 households were randomly sampled within each selected community. Third, one adult who met the following criteria was asked to participate in the study: 19 years of age or older, able to provide written informed consent, self-identified as being a First Nation person living on reserve, and whose birthday was next. Sample weights were calculated in order to obtain representative estimates of the total population. Using sample weights minimizes nonresponse bias. The design

weight was adjusted based on the assumption that the responding communities represent both responding and non-responding communities. The Bootstrap method was adopted for the estimation of the sampling error of the estimates produced for this study [1,2].

The current study included data collected from the Manitoba and Ontario regions. In Manitoba, nine First Nations communities across four ecozones: 1 - Prairies/Plains, Prairies/Subarctic, 2 - Boreal Plains, Plains/Subarctic, 3 - Boreal Shield/Subarctic, and 4 - Taiga Shield/Subarctic were surveyed from September to December 2010 (Fig. 1A). In Ontario, eighteen First Nations communities across four Ontario ecozones: 1 - Boreal Shield/ Subarctic, 2 - Boreal Shield/Northeast, 3 - Hudson Plains/Subarctic, 4 - Mixed-wood Plains/Northeast were surveyed from September to December in 2011 and 2012 (Fig. 1B). From a total of 27 communities, eighteen have year-round road access while nine (Manitoba (n=2) and Ontario (n=7)) were remote (fly-in or winter roads only). Participation rates were 82% in Manitoba and 79% in Ontario. In total, 2132 participants were recruited for the study. Individuals that did not complete the Household Food Security Survey Module (HFSSM) were excluded from the analysis. Therefore, the final sample comprised 2022 individuals (646 from Manitoba and 1376 from Ontario) aged 19 years or older.

2.2. Ethics

This survey was conducted following the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans” and in particular Chapter 9 research involving the First Nations, Inuit and Métis Peoples of Canada. Ethical approval was granted by the Research Ethics Boards of Health Canada, the University of Northern British Columbia, the University of Ottawa, and the Université de Montréal.

2.3. Data collection

Data were collected using household interviews conducted “in person” by trained community research assistants. All participating individuals completed the following questionnaires: a dietary 24-hour recall, a traditional food frequency questionnaire (FFQ), a socio/health/lifestyle questionnaire (SHL), and a food security questionnaire (Chan et al. 2012, 2014). The FFQ collected information regarding consumption of all identified locally-harvested traditional foods. The study participants were asked to recall the number of days on which they consumed each traditional food during the four seasons in the past year. Age- and gender-specific portion sizes of each traditional food item were estimated from the 24-hour recall data. In Manitoba, the FFQ consisted of 146 foods including 30 fish products, 23 land mammals, 25 wild birds, 23 wild berries, 4 wild nuts, 29 wild plants, 10 tree foods, and 2 mushrooms. In Ontario, the FFQ included 143 traditional food items: 30 fish species, 21 land mammals, 25 wild birds, 23 wild berries, 4 wild nuts, 29 plants, 9 tree foods, and 2 mushrooms. The FFQ was developed based on a comprehensive list of traditional foods and was representative of each participating community.

The SHL questionnaire collected data on age, sex, weight, height, physical activity level, smoking behaviour, educational attainment, household size, employment status, self-perceived health status, source of income, traditional food gathering activity, access to and availability of traditional foods and factors preventing households from using traditional foods. The access and availability of traditional foods were assessed with the following questions: 1) Does your household like to have more traditional foods? 2) Do you worry that your traditional foods would run out before you could get more (in the last 12 month)? 3) The traditional food that you got

just didn't last, and you couldn't get any more (in the last 12 month). The body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters).

Food security information was collected with the income-related HFSSM adapted from the United States Department of Agriculture (USDA) Food Security Survey Module (Bickel et al. 2000). The HFSSM was used in the CCHS 2.2 questionnaire (Agriculture and Agri-Food Canada 1998) and was further adapted for Aboriginal households (Lawn and Harvey 2004). The module consisted of 18 questions (10 items for adults' status and an additional 8 for households with children) examining the ability of households to afford enough food. Households were classified as "food secure" when no item or only one item in the adult or child scale was affirmed.

Moderately insecure households were defined as respondents affirmed 2-5 questions.

Households that responded affirmatively to six and more questions were categorized as "severely food insecure". Moderately and severely food insecure groups were combined. It is important to note that this questionnaire reflects "household" food security status and not necessarily the status of a particular individual within the household while the FFQ is a tool used with individuals.

2.4. Traditional food consumption

Consumption of traditional foods (grams/day) was estimated by totaling the number of days in the past four seasons when consumption of a particular food item was reported, then multiplied by the age- and gender-specific portion size of the corresponding food (estimated from a 24-hour recall) and divided by 360 days (four seasons of 90 days each). For the purpose of this study, traditional foods consumed by participants were combined into food subgroups: wild fish (fish species), wild game (land mammal species), wild birds (bird species), wild berries, and wild plants (wild nuts, wild plants, tree foods, and mushrooms).

2.5. Estimation of nutrient intakes from fish

Nutrient composition data for fish species reported by First Nations participants were obtained from the Canadian Nutrient File, a national food composition database (2015. Health Canada 2015), taking into account the preparation method (i.e. baked, broiled, boiled, or raw). Nutrients analyzed in this study were: protein, n-3 PUFA, vitamins (A, thiamin (B1), riboflavin (B2), niacin (B3), B6, B12, and D), and minerals (magnesium, phosphorus, potassium, zinc and selenium). The Dietary Reference Intake (DRIs), such as the Recommended Dietary Allowance (RDA) and Adequate Intake (AI) were used to assess the contribution of fish to nutrient requirements (Institute of Medicine 2003). The DRIs is a comprehensive set of nutrient values for healthy populations used for assessing and planning diets. The RDA is the average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%-98%) healthy people (Otten, Jennifer, Hellwig, Jennifer Pitzi 2006). AI is established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy (Otten, Jennifer, Hellwig, Jennifer Pitzi 2006). Pregnant and breastfeeding women (n=59) were excluded from our analyses due to their different nutritional requirements.

2.6 Data analysis

Data management and statistical analyses were performed with STATA statistical software, 14.2 (StataCorp, College Station, Texas, USA). Descriptive statistics included the calculation of means with 95% confidence interval (CI) for continuous variables and proportions for categorical variables. Chi-square and student t-test were performed to assess whether differences between groups were statistically significant. The study participants were divided into two groups by food security status, individually in Manitoba and Ontario. Lifestyle and demographic characteristics and frequency (% consumers) and quantity (grams/person/day) of traditional food

consumption (total and by subgroups) were compared between food secure and food insecure participants. Consumers were defined as individuals reporting consuming more than 0 g/d of a particular traditional food. The percentage contribution of fish to nutrient requirements (DRAs and AI) were calculated according to sex and age groups (Otten et al. 2006; Ross et al. 2011). The mean proportion method was used for the estimation of the percentage contribution of fish to the DRIs (Krebs-smith, Kott, and Guenther 1989). P-values less than 0.05 were considered statistically significant. All statistical analyses were conducted using weighting variables to obtain representative estimates.

3. Results

A total of 2022 individuals (646 from Manitoba and 1376 from Ontario) were included in this study. The mean (\pm standard deviation, SD) age of study participants was 45.1 (\pm 15.4). The proportion of women was 64.7%, and mean BMI was 30.7 (\pm 6.0) kg/m². In total, 35.1% of participants reported being food insecure. Overall, First Nations in Manitoba had a lower level of socioeconomic status compared to First Nations in Ontario. In particular, participants in Manitoba reported higher prevalence of unemployment (43.8% vs 33.6%), higher reliance on social assistance as the main source of income (45.2% vs 26.7%), lower educational attainment (56.5% vs 42.1% less than high school), higher proportion of households with children aged under 18 years (73.8% vs 48.4%) and living in more crowded households (4.4 vs 3.3 persons in a household).

Table 1 summarizes demographic and lifestyle characteristics of participants by food security status in Manitoba and Ontario. The prevalence of food insecurity was higher among Manitoba (38%) compared to Ontario participants (29%). In Manitoba, no statistically significant differences in lifestyle characteristics were found between food secure and food insecure

participants. However, a higher proportion of food insecure individuals lived in households relying on social assistance as the main source of income (54.5% vs 38.7%, respectively) and reported a higher unemployment rate (48.5% vs 40.6%, respectively) compared to food secure participants. In Ontario, food insecure individuals were more likely to be younger, to be current smokers, to be less physically active, and tended to report fair or poor health status compared to food secure respondents. In addition, a higher proportion of food insecure households had children under the age of 18 years (61.4% vs 42.1%) and lived in bigger households (3.8 vs 3.1 people per household, respectively). Furthermore, food insecure individuals in Ontario reported a lower level of education, a higher unemployment rate (43.1 vs 39.0), and a higher reliance on social assistance (46.2% vs 17.45) compared to food secure participants.

Mean consumption and proportion of consumers of traditional foods (total and by subgroups) in Manitoba and Ontario First Nations are presented in Table 2. On average, First Nations in Manitoba consumed 41.5 g/d of traditional foods which was similar to First Nations in Ontario, (42.1g/d). However, Ontario First Nations reported the highest consumption of wild fish (17.1 g/d) followed by wild game (10.5g/d), berries (7.2g/d), wild birds (4.6g/d), and wild plants (0.6g/d). In contrast, First Nations in Manitoba consumed more wild game (17.2g/d) followed by wild fish (9.3g/d), wild birds (7.1g/d), wild berries (6.5g/d), and wild plants (1.9g/d). Overall, wild fish contributed 43% and 22% to the total intake of traditional foods in Ontario and Manitoba, respectively. The percentage of respondents consuming wild fish at least once in the prior year was 82.2% in Manitoba and 72.3% in Ontario (Table 2). Commonly consumed fish species were walleye, lake whitefish, lake trout, northern pike and yellow perch (Chan et al. 2012, 2014).

We compared mean intake (g/d) and percentage of consumers (%) of wild fish and other food subgroups between food secure, moderately and severely food insecure individuals in Manitoba and Ontario First Nations (Figures 2-3). Overall, food secure and insecure individuals from Manitoba did not significantly differ by the frequency and quantity of traditional food consumption. However, the consumption of wild fish and wild birds was significantly lower among severely food insecure respondents (2g/d each) compared to moderately food insecure (11g/d and 7g/d) and food secure respondents (9g/d and 8g/d, respectively) (Figure 2). In contrast, food insecure participants (moderate and severe) in Ontario reported significantly higher consumption of wild fish (23g/d, 20g/d and 14g/d), wild game (15g/d, 14g/d and 8g/d), wild birds (8g/d, 7g/d and 3g/d), and total traditional foods (52g/d, 53g/d, and 35g/d) than food secure subjects. Likewise, the proportions of consumers of traditional foods were higher among food insecure than food secure participants (Figure 3).

The contribution of fish consumption to nutrient requirements by food security status in Manitoba and Ontario are presented in Figures 4 A-B. Nutrients analyzed include protein, n-3 PUFA, vitamins (A, B6, B12, D, niacin, riboflavin, and thiamin), and minerals (magnesium, phosphorus, potassium, zinc and selenium). In Manitoba, there were no statistically significant differences in nutrient intakes between food secure and food insecure participants, except for vitamin B12 (9.7% vs 15.8% of the DRA, respectively) and n-3 PUFA (4.8% vs 8.1% of AI, respectively) (Fig 4A). In contrast, fish contributed higher levels of selected nutrients in Ontario food insecure compared to food secure individuals, including protein, n-3 PUFA, vitamin B12, niacin, phosphorus, and selenium (Fig 4B).

Table 3 summarizes responses on the access to and availability of traditional foods, and food gathering activities in First Nations communities. Overall, 66% of participants in Manitoba and

73% in Ontario reported that their households would like to have more traditional foods. A higher proportion of First Nations in Manitoba compared to Ontario reported that they worry whether their traditional foods (including wild fish) run out before they can get more, (49% and 29%, respectively) ($p=0.01$). Furthermore, a half (49%) of Manitoba respondents and one-third (32%) in Ontario participants reported that their traditional foods did not last, and they could not get any more. When considering food security status, a significantly higher percentage of food insecure individuals in Manitoba (70%) and Ontario (54%) affirmed the former questions.

Traditional gathering activities (i.e. fishing, hunting, setting snares, collecting wild berries and plants) were practiced by 55.8% households in Manitoba and 70.4% in Ontario regions. Fishing was the most common practice reported by 45% of respondents in Manitoba and 55% in Ontario. Overall, greater proportions of food insecure responders engaged in traditional food activities, including fishing, compared to food secure households in both, Manitoba and Ontario (Table 3).

Table 4 summarizes barriers preventing First Nations households from using more traditionally harvested fish and other wildlife (overall and by food security status). Many participants reported the absence of a hunter in their households, lack of equipment and/or transportation, and lack of time for harvesting. In Manitoba, almost one-third of participants (28%) said that their households do not have a hunter. In contrast, individuals in Ontario tended to report the lack of time for harvesting activities (18%). Other constraints that limit traditional gathering activities included governmental restrictions, hydro/forestry operations, and roadways which were reported by 31 - 43% respondents in Manitoba and 19 - 24% in Ontario (Table 4). When considering food security status, a significantly greater percentage of food insecure respondents reported inadequate access to equipment and/or transportation compared to food secure individuals in both Manitoba (19.5% vs 7%) and Ontario (20.0% vs 5%). Also, government

restrictions and forestry operations were perceived as the main barriers to traditional harvesting activities by food insecure individuals in Manitoba (50.9% and 39.1%, respectively).

Furthermore, over half (54%) of First Nations in Manitoba and four out of five (79%) First Nations in Ontario noticed significant climate change in their traditional territories over the last ten years (Chan et al. 2012, 2014). Climate change was perceived to increase the difficulty in getting traditional foods, to affect the animals' usual cycles or patterns, and the growth of traditional foods by one-third of participants (36% in Manitoba and 31% in Ontario).

4. Discussion

In response to the World Food Summit Plan of Action, Canada developed the Action Plan for Food Security to develop economic, social and environmental programs and policies, and to promote national and international food security (Government of Canada 1998). Among Indigenous Peoples, food security was to be addressed by promoting traditional food acquisition, sharing their awareness of traditional foods and actions related to sustainable resource management (including fisheries) and supplementation with high-quality commercial foods (Government of Canada 1998). Several national, provincial and community-based programs, including Nutrition North Canada (NNC) (Government of Canada 2017), were developed to address food insecurity among Indigenous people in Canada. Nevertheless, food insecurity continues to remain a critical public health issue. Overall, more than one out of three households (35%) in Manitoba and Ontario First Nations experience food insecurity which is three times higher compared to the general Canadian population. Concomitant with our findings, several previous studies reported disproportionately higher rates of food insecurity among Indigenous people compared to the general Canadian population (Rosol et al. 2012; Skinner, Hanning, and Tsuji 2014; Tarasuk et al. 2013).

Consumption of fish and other traditional foods was significantly higher among food insecure compared to food secure First Nations adults in Ontario. These findings suggest that individuals with limited availability and access to store-bought foods, especially in isolated communities (i.e. with fly-in or winter roads only), tend to rely more on traditional foods for their subsistence. In fact, a greater proportion of food insecure compared to food secure respondents in Ontario reported engaging in traditional gathering activities. Similar to our findings, seafood consumption was associated with traditional gathering activities, such as fishing, hunting and collecting seafood in First Nations men living on-reserve in British Columbia (Chapter 7). A study among the Nenets people residing in the Arkhangelsk region in Russia reported that fish consumption was positively associated with frequency of fishing and monthly income (Petrenya et al. 2012). On the other hand, participants in Manitoba consumed similar amounts of traditional foods including wild fish regardless of their food security status. Given relatively higher prevalence of food insecurity and lower socioeconomic status among Manitoba than in Ontario First Nations, these results may indicate that financial constraints limit the access of Manitoba First Nations to both market foods and traditional foods. In fact, one out of five (20%) food insecure individuals in Manitoba did not have an adequate access to fishing and hunting equipment, and/or transportation due to the high cost. Lack of a hunter in the family was another important factor mentioned by one-third of participants in Manitoba. Previous research reported that the high cost of harvesting equipment and transportation were primary factors preventing households from acquiring more traditional foods (Chan et al. 2006; Gaudin et al. 2015). Data from the Yukon First Nations, Dene/Métis and Inuit study showed that up to 50% of respondents had inadequate access to fishing and hunting equipment, and up to 46% of participants said they could not afford to go hunting or fishing (Lambden et al. 2006). Among Yukon First Nations,

Dene/Métis, Inuit living in Arctic communities in Canada, only 40 – 45% of women's households had the access to hunting and fishing equipment, with 11% and 29% of women reporting that hunting and fishing, respectively, were too expensive for their families (Gabrielle 2008). Participants from the Dene Nation study indicated that the rising cost of fuel, as well as high cost of hunting and fishing equipment, have been limiting their ability to go out on the land to harvest traditional foods (Kuhnlein et al. 2013).

Our findings show that traditionally harvested fish continues to provide good sources of several nutrients including protein, vitamin B12, D, thiamin, niacin, selenium, phosphorus, and n-3 PUFA. When stratified by food security status, nutrient intakes from fish was significantly greater among food insecure compared to food secure respondents (specifically in Ontario), which reflects higher consumption of fish (by weight) among food insecure households. This confirms the critical role of locally-harvested fish in providing nutritional health and food security in First Nations communities. Previous studies among Indigenous populations have also documented that traditional foods substantially contribute to micronutrient intakes; however, the level of contribution by individual species was not studied (Johnson-Down and Egeland 2010; Kuhnlein et al. 2004; Kuhnlein and Receveur 2007; Sheehy et al. 2015).

We found differences in traditional food consumption patterns across regions with greater reliance on wild fish in Ontario but higher consumption of wild game in Manitoba First Nations. These differences may reflect the diversity of traditional food systems across regions, cultural food preferences, and the impacts of socio-economic and environmental factors. The majority of First Nations in both Manitoba and Ontario would like to have more traditional foods in their diets. Moreover, higher proportions of individuals who experience income-related food insecurity affirmed that they worry that their traditional foods run out before they can get more,

and/or their traditional foods just did not last, and they could not get any more. This indicates that levels of availability for traditional foods fall short of levels of demand by First Nations. Besides financial constraints which diminish the ability to obtain healthy market foods, First Nations experience challenges acquiring traditional foods which, in turn, drastically compromises their sustainability and food security. Our findings are consistent with the results of a survey among Coast Salish people in British Columbia (Fediuk and Thom 2003). This study showed that levels of available traditional foods fall far short of levels desired by almost all respondents who wish to engage in traditional harvesting activities (Fediuk and Thom 2003). The study participants also indicated barriers to harvesting, such as government restrictions, environmental changes, poverty, privatizations and traditional knowledge loss (Fediuk and Thom 2003).

We found that barriers preventing First Nations from the consumption of traditional foods differed in Manitoba and Ontario. Particularly, more First Nations in Manitoba than in Ontario reported governmental restrictions, hydro/forestry operations and roadways as significant constraints to harvesting activities. One-third of respondents from Manitoba observed that these factors decreased their access to and the overall availability of fish species commonly harvested in local areas (Chan et al. 2012). Aside from these barriers, First Nations participants were concerned about climate change, which affects their ability to use the land. Similar to our findings, other studies examining environmental impacts on traditional food systems and food insecurity among Indigenous people reported changes in the timing of fish harvesting, the disappearance of particular wildlife species, changes in travel routes on water and land, and fluctuations in the distributions of particular fauna and flora (Chan et al. 2006; Fediuk and Thom 2003; Hori 2010; Lemelin et al. 2010; Royer and Herrmann 2011; Thompson et al. 2014).

The barriers to traditional fish consumption may have significant implications for human health due to their role in meeting the needs for essential micronutrients in the diet. Although several nutrients (i.e. protein, vitamin B12, niacin) may be obtained from the consumption of alternative traditional foods and/or market foods, the intake of nutrients primarily derived from fish species (such as vitamin D, selenium and n-3 PUFA) would be substantially diminished (Rosol et al. 2016). While n-3 PUFA are well-known for their protective effects against CVD (Mori 2014), vitamin D is essential for maintaining healthy bones and immune function, and selenium reduces the risk of cancer, autoimmune and thyroid diseases (Kulie et al. 2009; Rayman 2012). Low intake of vitamin A, D, calcium, iron and magnesium among Canadian First Nations has been widely reported (Chan et al. 2012, 2014; Delormier and Kuhnlein 1999; Gates et al. 2012; Health Canada 2012a). Furthermore, the high cost, lack of variety and quality of store-bought foods (especially in isolated communities) has been well-documented as barriers to a healthy diet and nutritional security (Chan et al. 2006; Lawn and Harvey 2004; Skinner et al. 2013). For instance, the average cost of a nutritious food basket for a family of four ranges from \$202 to \$327 in different ecozones in Manitoba which is drastically higher when compared to an average cost of \$145 in Winnipeg. Likewise, the average cost of a nutritious food basket ranges from 175\$ to 344\$ in different Ontario ecozones compared to \$205 in Ottawa (Chan et al. 2012, 2014). When healthy and nutritious foods are unaffordable, people rely on less expensive, high-energy and low nutrient-dense market foods. This, in turn, aggravates the on-going nutrition transition characterised by the shift away from traditional diets and the adoption of sedentary lifestyle, and leads to the increased incidence of chronic diseases, such as obesity, type 2 diabetes and heart disease among First Nations people (Johnson-Down et al. 2015; Sheikh et al. 2012; Willows et al. 2011b).

In Northern Canada, several hunter and harvester support programs have been developed to promote traditional harvesting activities. For example, the Nunavut Harvester Support Program in Nunavut, the Inuit Hunting, Fishing and Trapping Support Program in Nunavik and the Community Harvester Assistance Program and the Inuvialuit Harvesters Assistance Program in Northwest Territories provide financial assistance to harvesters in the form of hunting equipment (snowmobiles, boats and all-terrain vehicles) and small supplies (fishing nets, camp stoves, sleeping bags, etc.). In James Bay, The Cree Hunters and Trappers Income Security Program provides an annual income to Cree First Nations who regularly participate in hunting, trapping and fishing activities. In Manitoba, numerous initiatives, such as harvest support programs, traditional food education and nutrition school activities, land-based education programs and communities freezer programs are implemented to support harvesters, to increase access to traditional foods, to teach children and youth hunting skills and to incorporate traditional culture into healthy eating in northern First Nations communities (Food Matters Manitoba 2013). Despite these efforts, more programs are needed to support traditional harvesting practices and to promote traditional food production and consumption in northern and southern First Nations communities in Canada.

Food insecurity in First Nations communities presents a complex challenge and requires a multidimensional approach. Potential strategies, such as increasing access to traditional land and wildlife resources (i.e., protected rights to access lands and to harvest), traditional food subsidy programs directed to offset high cost of hunting equipment, enhanced traditional knowledge transition from Elders to younger community members, traditional foods sharing with community members, as well as transformation of fisheries management would help to promote food sovereignty and sustainable livelihood in First Nations communities.

5. Conclusion

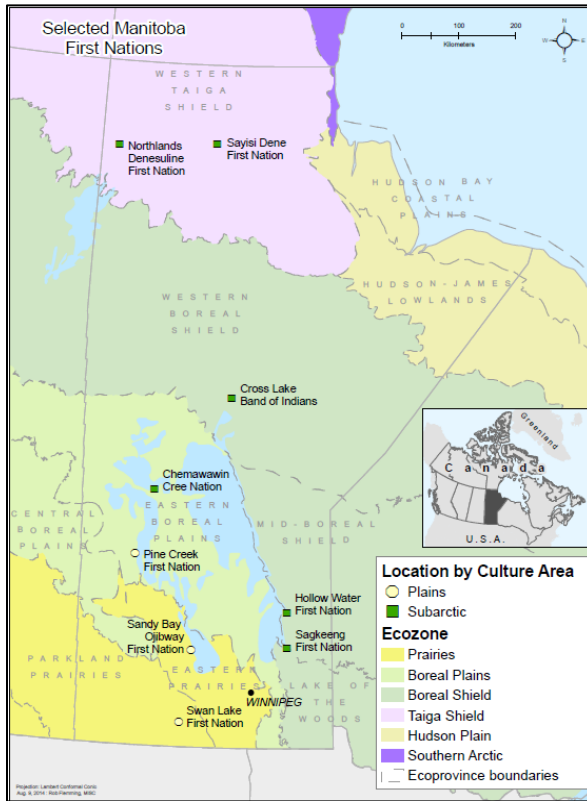
Our findings show that First Nations in Canada continue to experience high rates of food insecurity. Traditional food systems remain important to the contemporary diet of First Nations. Fish consumption provides essential contributions to nutritional health and food security. Several socioeconomic and environmental barriers prevent First Nations from traditional harvesting activities. Improving access to and availability of fish and other wildlife has a potential to reduce food insecurity and to promote sustainable livelihood in First Nations. Future policies and interventions should address socioeconomic determinants of food insecurity, support traditional harvesting activities and sustainable fisheries in First Nations communities.

Acknowledgements

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Figure 1. A-B Maps of participating First Nations communities in Manitoba and Ontario (Chan et al., 2012, 2014)

A.



B.



Table 1. Sociodemographic characteristics of study participants by food security status in Manitoba and Ontario^a (n=2022)

	Manitoba			Ontario		
	food secure	food insecure	P value	food secure	food insecure	P value
N (%)	382 (62)	264 (38)		928 (71)	448 (29)	
Age, years, mean (SD)	42.3 (13.9)	42.4 (14.8)	0.51	48.6 (15.8)	41.9 (14.5)	0.01
Female, n (%)	257 (67.3)	188 (71.8)	0.20	572 (61.6)	289 (64.8)	0.25
BMI, kg/m ² , mean (SD)	30.4 (6.4)	30.0 (6.3)	0.90	31.2 (5.8)	30.1 (5.8)	0.05
Current smokers, n (%)	230 (60.2)	173 (66.0)	0.13	402 (43.3)	289 (64.5)	0.001
Physical inactivity, ^b n (%)	272 (71.2)	197 (75.2)	0.26	584 (62.9)	305 (68.4)	0.04
Health status, n (%)			0.34			0.001
excellent/very good	94 (24.6)	52 (19.7)		262 (28.2)	80 (17.9)	
good	159 (41.6)	115 (43.6)		424 (45.7)	191 (42.8)	
fair/poor	129 (33.8)	98 (37.1)		242 (26.1)	175 (39.2)	
Household size, mean (SD)	4.4 (2.7)	4.4 (2.5)	0.51	3.1 (1.8)	3.8 (2.1)	0.02
Children<18y, n (%)	284 (74.4)	191 (72.9)	0.61	391 (42.1)	274 (61.4)	0.01
Unemployment, n (%)	155 (40.6)	127 (48.5)	0.04	269 (29.0)	192 (43.1)	0.01
Education, n (%)			0.08			0.001
less than high school	208 (54.5)	157 (59.5)		333 (35.8)	246 (54.9)	
high school graduation	115 (30.1)	82 (31.1)		455 (49.0)	165 (36.8)	
vocational training	44 (11.5)	22 (8.3)		69 (7.5)	25 (5.6)	
post-secondary education	15 (3.9)	3 (1.1)		71 (7.7)	12 (2.7)	
Income sources, n (%)			0.001			0.001
wages	181 (47.4)	87 (32.8)		533 (57.3)	180 (40.2)	
social assistance	148 (38.7)	144 (54.5)		161 (17.4)	207 (46.2)	
pension	34 (8.9)	21 (8.0)		196 (21.1)	49 (11.0)	
workers compensation	19 (5.0)	12 (4.5)		38 (4.1)	12 (2.7)	

^a Manitoba & Ontario First Nation Food Nutrition and Environment Study (2008-2012)

^b Physical inactivity includes self-reported sedentary and somewhat active lifestyle

Data are n (%) or mean (SD), unweighted estimates

Table 2. Mean^a consumption (grams/person/day) and percentage of consumers of traditional foods (by subgroup) in Manitoba and Ontario First Nations^b (n=2022)

	Manitoba			Ontario		
	% consumers	mean (g/d)	95%CI	% consumers	mean (g/d)	95%CI
Wild fish ^c	82.2	9.3	0.7-17.7	72.3	17.1	8.8-25.2
Wild game ^d	86.8	17.2	8.4-25.9	67.8	10.5	5.2-15.8
Wild birds	56.4	7.1	0.7-13.9	38.4	4.6	1.7-7.4
Wild berries	67.5	6.5	4.1-9.0	60.0	7.2	4.4-9.9
Wild plants ^e	28.1	1.9	0.3-4.1	35.2	0.6	0.4-08
Total traditional food	94.0	41.5	20.4-62.6	94.5	42.1	25.1-59.1

^a Population mean (consumers and non-consumers), based on the food frequency questionnaire and averaged across seasons, individuals aged ≥ 19 years

^b Manitoba and Ontario First Nation Food Nutrition and Environment Study (2008-2012)

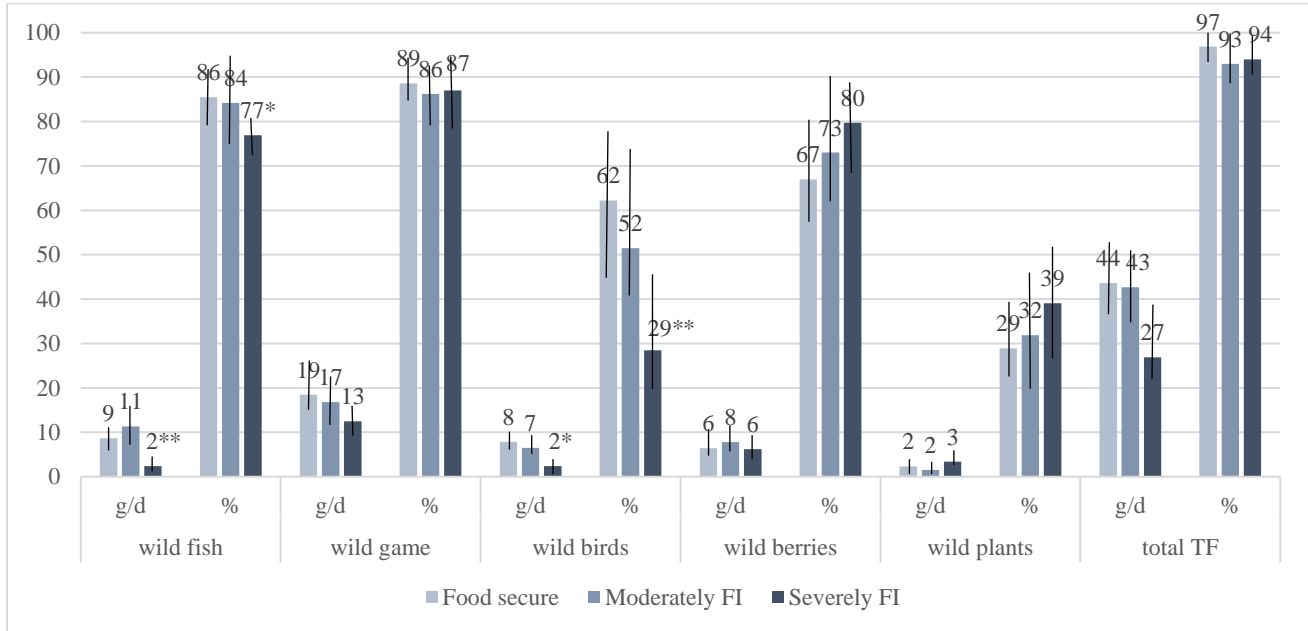
^c Wild fish includes all locally-harvested fish species

^d Wild game includes all land mammal species

^e Wild plants include wild nuts, wild plants, tree foods, and mushroom

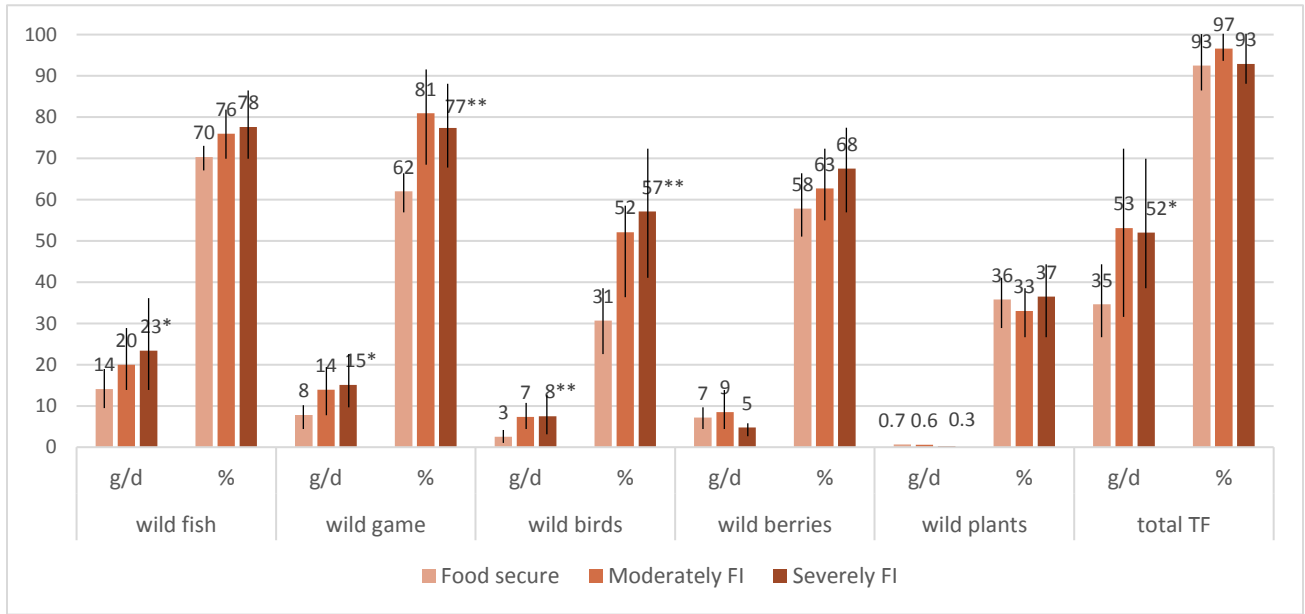
Weighted estimates

Figure 2. Mean^a intake and proportion of consumers of traditional foods (by subgroups) by food security status in Manitoba (n=646)



^a population mean (consumers and non-consumers),
g/d, grams/day/person, data from Manitoba FNFNES, FFQ questionnaire, individuals aged ≥19 years
%, proportion of consumers of respective food group
FI, food insecure
TF, traditional food
* p value < 0.05, ** p value < 0.01

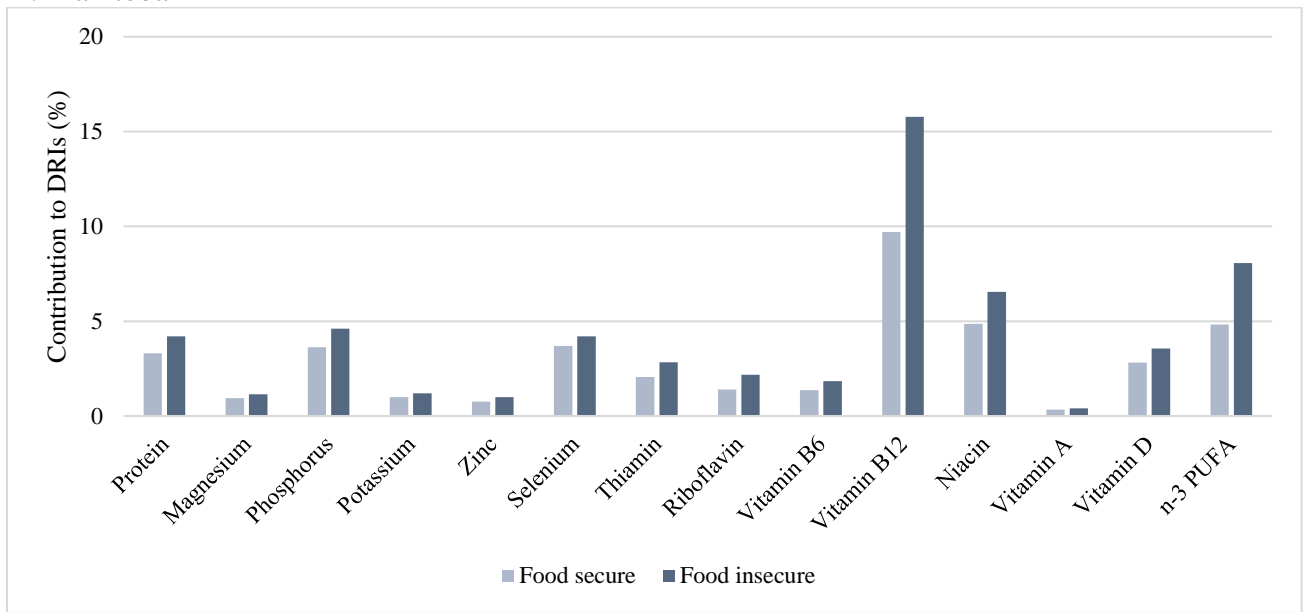
Figure 3. Mean^a intake and proportion of consumers of traditional foods (subgroups) by food security status in Ontario (1376)



^a population mean (consumers and non-consumers),
g/d, grams/day/person, data from Ontario FNFNES, FFQ questionnaire, individuals aged ≥ 19 years
%, proportion of consumers of a respective food group
FI, food insecure
TF, traditional food
* p value < 0.05 , ** p value < 0.01

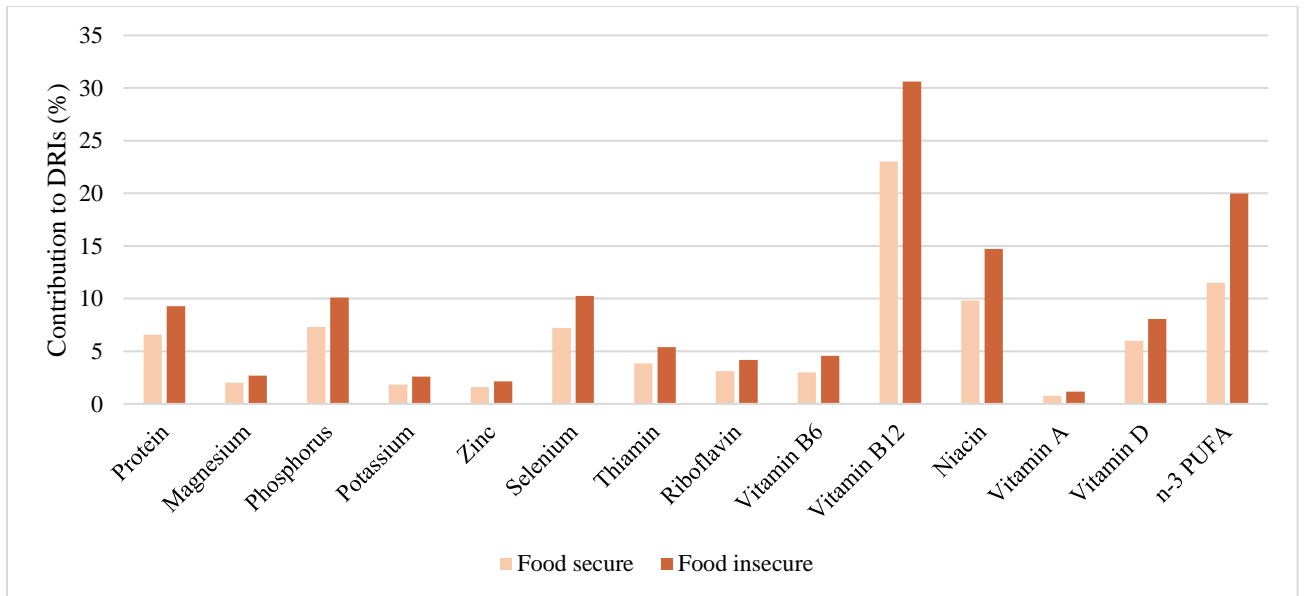
Figure 1 (A, B). Contribution of fish consumption to nutrient requirements (DRIs) by food security status in Manitoba and Ontario First Nations

A. Manitoba



DRI - dietary reference intakes using recommended dietary allowance (RDA) and adequate intake (AI)
Sample includes fish consumers only (n=560)
Weighted estimates

B. Ontario



DRI - dietary reference intakes using recommended dietary allowance (RDA) and adequate intake (AI)
Sample includes fish consumers only (n=1038)
Weighted estimates

Table 3 Responses to questions related to access and availability of traditional foods, and traditional gathering activity by food security status in Manitoba and Ontario

	Manitoba		Ontario	
	Food secure	Food insecure	Food secure	Food insecure
	%	%	%	%
Does your household like to have more traditional foods?	63.1	77.2**	68.2	83.8***
Do you worry that your traditional foods would run out before you could get more?	38.9	62.6***	21.1	46.1***
Traditional food didn't last, and you couldn't get any more	36.7	70.4***	21.5	53.8***
Traditional activity by a participant	43.9	44.1	54.7	59.3**
Traditional activity by someone in a household	47.2	54.7**	49.6	58.5***
Traditional activity by anyone in a household	56.8	62.3	68.2	74.7**
Fishing activity by anyone in a household	45.5	50.8	52.4	61.1***

Traditional activity includes fishing, hunting, setting snares for food, collecting wild plants, seafood or plant a garden

Weighted estimates

** p value<0.1, ** p value<0.05, *** p value<0.01*

Table 4 Barriers preventing households from using more traditional foods in Manitoba and Ontario (overall and by food security status)

	Manitoba			Ontario		
	Total	Food secure	Food insecure	Total	Food secure	Food insecure
Lack of a hunter in the household	28.1	25.2	32.8	11.2 ^{***§}	10.3	13.4
Lack of equipment/transportation	11.7	7.0	19.5 ^{**†}	10.0	5.0	20.0 ^{***†}
Lack of time	10.9	11.2	10.2	18.3 ^{***§}	19.5	15.5
Lack of knowledge	5.0	4.8	5.4	8.9 ^{*§}	8.3	10.4
Government restrictions	46.7	44.1	50.9 ^{*†}	24.3 ^{**§}	25.6	21.1
Hydro operations	38.3	34.3	44.8	18.7 ^{**§}	18.4	19.7
Forestry operations	31.9	27.5	39.1 ^{**†}	24.5	23.9	26.0
Roadways	33.7	29.3	39.7	22.4	23.7	19.3
Climate change ^a	36.0	31.5	39.3	30.5	30.7	30.2

Values are percent (%)

^a Climate change was perceived to decrease the availability of traditional food, increase the difficulty in getting traditional food, affect animals' usual cycles or patterns and growth, and change fish run

* p value < 0.1, ** p value < 0.05, *** p value < 0.01

§, significantly different from Manitoba total population

†, significantly different from a corresponding food secure group

Weighted estimates

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4 FISH CONSUMPTION IS INVERSELY ASSOCIATED WITH TYPE 2 DIABETES IN MANITOBA FIRST NATIONS COMMUNITIES

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Authors' contributions: LM conceived the research question, performed the statistical analyses, interpreted the data and drafted the manuscript. HMC oversaw the research, provided intellectual support, and feedback on the drafted manuscript. HMC, MB, DS, HS, AI, KF, CT were all involved in the design and implementation of the FNFNES survey and provided feedback on the drafted manuscript.

ABSTRACT

Background: Consumption of fish and n-3 fatty acids (n-3 FAs) has been postulated to prevent type 2 diabetes (T2D). **Objective:** To explore the association between self-reported T2D and fish consumption, dietary n-3 FAs and persistent organic pollutants (POP) intake in a regionally representative sample of First Nations (FNs) in Manitoba. **Design:** Data from the cross-sectional First Nations Food Nutrition and Environment Study collected from 706 members of 8 Manitoba FNs in 2010 were analyzed. Household interviews were used to collect social and lifestyle data. The consumption of fish was estimated using a traditional food frequency questionnaire. Fish samples were analyzed for the presence of POPs. Multiple logistic regression models adjusted for potential risk factors for T2D were developed. **Results:** A negative dose-response relationship was found between fish intake and self-reported T2D. Fish consumptions of 2-3 portions per month and ≥ 1 /week were inversely associated with T2D with ORs 0.51 (95% CI:0.28-0.91) and 0.40 (95% CI:0.19-0.82), respectively, compared to no fish intake. Similarly, intake of n-3 FAs was negatively associated with T2D (OR=0.48 (95% CI:0.30-0.77)). Dietary POP intake was not associated with T2D. **Conclusion:** These findings suggest that traditionally harvested fish consumption may have a beneficial effect on T2D in Manitoba First Nations.

Keywords: First Nations, fish consumption, long chain n-3 fatty acids, persistent organic pollutants, type 2 diabetes

1 Introduction

The prevalence of type 2 diabetes (T2D) has been steadily increasing worldwide, substantially contributing to health care costs (Guariguata et al. 2014). In Canada, T2D is one of the most common chronic diseases. According to recent statistics, the prevalence of T2D in Canada has more than doubled between 2000 and 2010 from 1.2 million to 2.4 million, and this trend is projected to continue (Canadian Diabetes Association 2015; Public Health Agency of Canada 2011). In 2015, the prevalence of T2D was 8.9% and estimated to reach 11.4% by 2025 (Canadian Diabetes Association 2015; Public Health Agency of Canada 2011). The Canadian First Nations population is experiencing rates of the epidemic proportion of T2D. The prevalence of T2D in First Nations communities is 3 to 5 times higher compared to that in the general Canadian population (Dannenbaum et al, 2008; Pelletier et al., 2012; Young et al., 2000). Also, an earlier age of diabetes onset, a greater severity of the disease and higher rates of complications of T2D are observed in on-reserve First Nations compared to non-Aboriginal population (Assembly of Manitoba Chiefs (AMC) 2012; First Nations Information Governance Centre 2012). First Nations females experience higher rates of type 2 diabetes than First Nations males, contrary to the pattern observed in the general Canadian population (Health Canada, 2000). This is believed to be because First Nations females have higher rates of obesity than First Nations males, and experience high rates of gestational diabetes which increases the risk of T2D later in life (Millar and Young 2003)

The rapid increase of T2D in First Nations over the last four-five decades has been influenced by a variety of risk factors including genetic, socio-cultural, environmental and lifestyle factors (Young et al. 2000). Historically, the diet of First Nations was based on traditional foods harvested from the local natural environment. This traditional food consisted of wild meat, fish and bird species, plants, and berries acquired by traditional hunting, fishing and gathering,

contributed to dietary intake of essential nutrients as well as physical activity and well-being of First Nations (Jamieson et al. 2012; Kuhnlein and Receveur 2007). First Nations have been undergoing a rapid lifestyle and dietary transitions from traditional high-nutrient diet toward store-bought energy-dense food which are associated with increased rates of obesity and T2D (Kuhnlein et al. 2004).

T2D is associated with numerous complications including retinopathy, neuropathy, kidney and CVD which may lead to disability and mortality (Naqshbandi et al., 2008). A number of modifiable risk factors for T2D are well-established including overweight and obesity, poor diet, sedentary lifestyle, and smoking. Among dietary factors, fish and long-chain omega-3 fatty acids (n-3FAs) eicosapentaenoic acid (EPA) and docosahexaenoic (DHA) have been postulated to prevent T2D. n-3 FAs showed a favorable effect on insulin sensitivity in animal models (Fedor and Kelley 2009). Data from an ecological study demonstrated that the prevalence of T2D was low in regions where fish consumption was high (Nkondjock and Receveur 2003). In addition, evidence of the beneficial effect of fish and n-3FAs on T2D is supported by studies conducted among northern Indigenous populations. Specifically, the Inuit population has lower rates of metabolic and cardiovascular diseases (Bruce et al., 2009) than general Canadians and First Nations. This is attributed to the beneficial effect of traditional food consumption rich in n-3 FAs (Dewailly et al., 2001) which resulted in higher concentrations of EPA and DHA in plasma and serum phospholipids (Proust et al., 2014) Epidemiological studies in general populations have shown inconsistent results on the association between fish consumption, n-3 FAs, and T2D. Several prospective cohort studies reported protective effects of fish and n-3 FAs on the risk of T2D (Patel et al., 2009; Nanri et al., 2011; Villegas et al., 2011) whereas some studies suggest positive (Djoussé et al., 2011; Kaushik et al., 2009) or no associations (van Woudenberg et al. 2009). Meta-analyses reported geographical differences on the associations between fish

consumption, n-3 FAs and the development of T2D: a protective association in populations of Asian countries and a positive association in the American population (Muley et al., 2014).

Despite its beneficial attributes, fish can be an exposure route to environmental contaminants such as persistent organic pollutants (POP) including polychlorinated biphenyls (PCB) and dichlorodiphenyldichloroethylene (DDE). POPs are lipophilic compounds which persist in the environment and therefore bioaccumulate and biomagnify within living organisms such as fish and mammals (Seabert et al. 2014; Sobek et al. 2010). Several epidemiological studies have reported a positive association between diabetes and POPs including PCBs, DDE and dioxins and dioxin-like chemicals (Codru et al., 2007; Lee et al., 2006; Philibert et al., 2009). These findings are supported by an experimental study which showed a link between exposure to POPs and insulin resistance, visceral obesity and glucose intolerance (Ibrahim et al. 2011). High concentrations of POP in blood have been reported for northern Indigenous populations (Donaldson et al. 2010). Traditional food, in particular, fish, is considered the major source of exposure to POPs (Seabert et al., 2014).

Given the increasing prevalence of T2D and potential exposure to contaminants through fish consumption in First Nations, we aim to describe fish consumption patterns among First Nation adults in four Manitoba ecozones; to estimate n-3 FAs, PCBs, and DDE intake from fish; and to explore the association between fish consumption, dietary DDE, PCBs and n-3 FAs intake, and self-reported T2D in a representative sample of First Nations adults (FNs) living on reserve in Manitoba.

2 Methodology

2.1 Study population

We analyzed data from the First Nations Food Nutrition and Environment Study (FNFNES) (fnfnes.ca). FNFNES is a cross-sectional study designed to assess total diets, food-related

exposure to contaminants, and food security status of First Nations people living on reserves, south of the 60th parallel across Canada (Chan et al. 2012). FNFNES collected data from approximately 100 First Nations communities across Canada. First Nations communities were randomly sampled using a combined ecozone/cultural area framework. An ecozone is a large geographical region identified based on the distribution patterns of plants, animals, geographical characteristics and climate (ecozone.ca). Culture areas is a concept to identify geographic areas within which Indigenous communities shared a greater number of traits/cultural affinities than from those outside the area (Chan et al., 2012). The sampling was done in three stages: primary sampling was performed with a random selection of communities within each ecozone; secondary sampling was conducted with a random sampling of 125 households within each selected community; and tertiary sampling when one adult in each household who was self-identified as being a First Nations person living on reserve aged 19 and older was asked to participate in the study. Sample weights were calculated in order to permit inferences from persons included in the sample to the total population from which they were drawn, and to obtain the tabulations reflect estimates of the population totals. Sample weight allows minimizing biases arising from differences between participating and non-participating persons (Maletta and Aires 2007). The design weight was adjusted based on the assumption that the responding communities represent both responding and non-responding communities. Assuming that non-response is not related to the topic of the study (missing at random), a non-response adjustment factor was calculated within each stratum. The Bootstrap method was adopted for the estimation of the sampling error of the estimates produced for this study (Chan et al., 2012). The current study included data from nine Manitoba First Nations communities across four ecozones: 1- Prairies/Plains, Prairies/Subarctic, 2- Boreal Plains, Plains/Subarctic, 3- Boreal Shield/ Subarctic, 4- Taiga Shield/ Subarctic collected in 2010 (Figure 1). The overall participation rate was 82%.

In total, 706 participants (477 women and 229 men) aged 19 years and over were interviewed for this study.

2.2 Ethics

Individual participation in the project was voluntary and based on informed written consent after an oral and written explanation of each project component. This survey was conducted following the “Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans” and in particular Chapter 9, pertaining to research involving the First Nations, Inuit and Métis Peoples of Canada. The study was approved by the Ethical Review Boards at the University of Northern British Columbia, the University of Ottawa, the Université de Montreal and Health Canada.

2.3 Data collection

Data were collected using an “in-person” household interview. A trained interviewer completed the survey with the participants; the survey was conducted in the participant’s household and included several questionnaires including a 24-hour recall, a traditional food frequency questionnaire (FFQ), and a socio-demographic, health, and lifestyle (SHL) questionnaire. The multi-pass technique with 3 stages was used to record the 24 h recall. In the first stage, a quick list of all foods and beverages consumed during the prior 24 hours was built; in the second, a detailed description of the consumed foods and beverages (brands, amount eaten, etc..) was recorded; in the third, the recall was reviewed (Raper et al., 2004). A subsample of 20% of the participants was invited to complete a second 24 h recall for later analysis using the SIDE SAS (version 9.2) sub-routine to partially adjust for intra-individual variations that allows for a better approximation of the usual diet. To estimate corresponding intake quantities, three-dimensional food and beverage models were used. The FFQ was developed based on a comprehensive list of locally-harvested traditional foods that was representative for each participating community. In Manitoba, the FFQ consisted of 153 traditional food items including 30 fish species. All

participants provided information on traditional food consumption during the four seasons in the past year. Age and gender-specific portion sizes of each traditional food item were determined from the 24 h recall data.

The SHL Questionnaire collected information on socio-demographic characteristics, lifestyle choices, and self-perceived health status. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). BMI categories were considered as follows: normal weight when BMI was $< 25 \text{ kg/m}^2$, overweight was categorized as a BMI of 25 kg/m^2 or higher but less than 30 kg/m^2 , and obesity was categorized as a BMI higher than or equal to 30 kg/m^2 . Physical activity data were self-reported: the study participants were asked to describe their physical activities based on provided descriptions: a) I am usually sitting and do not walk around very much; b) I stand or walk around quite a lot, but I do not have to carry or lift things very often; c) I usually lift or carry light loads or I have to climb stairs or walk up hills often; d) I do heavy work or carry heavy loads.

2.4 Assessment of type 2 diabetes

T2D was defined as a self-reported diagnosis of type 2 diabetes via the SHL questionnaire. In addition, information on the type of diabetes (type 1, 2) and the onset of diabetes (how many years ago individuals were diagnosed with T2D) was collected. In this study, only those participants who reported being diagnosed with type 2 diabetes were coded as cases of T2D (Huerta et al., 2009; Schneider et al., 2012). Participants with type 1 diabetes were grouped with those who did not have diabetes in the analysis.

Self-reported estimates of T2D in the FNFNES survey were validated by comparing the estimates with those reported by the Regional Health Survey (RHS), based on data collected in the similar period of time (2008-2010). The RHS is the only First Nations-governed national health survey in Canada (RHS Manitoba, 2012). The weighted prevalence of diabetes reported

by the FNFNES in Manitoba was 22% which was similar to the 21% reported by RHS in Manitoba (RHS Manitoba, 2012).

2.5 Fish sampling for contaminant content

Fish samples collected for contaminant analyses were representative of all fish species consumed by members in each community. The fish sampling strategy was as follows: each community was to identify the most commonly consumed fish; fish species that are of the most concern from a nutrition and environmental perspective; and those known to accumulate higher concentrations of contaminants (Chan et al., 2012). A total of eight species of fish (bass, perch, pike, sturgeon, suckers, trout, walleye and whitefish) were collected during fall 2010 (September through November). The collected fish samples were analyzed for POPs including total PCBs and DDE at Maxxam Analytics, formerly CANTEST, in Burnaby, British Columbia.

Replicate samples (n=3-5) from each fish species from each community were homogenized to provide a homogeneous pooled sample for subsequent analysis. A total of 41 pooled fish samples were analyzed. If required, a moisture value was determined gravimetrically after drying a portion of the blended sample at 105°C overnight. A modified US EPA 1668C Method was used for extraction. Briefly, six grams of tissue was spiked with C-13 and deuterium labeled extraction internal standard and homogenized in dichloromethane (DCM), followed by soxhlet extraction and filtered through anhydrous sodium sulphate. The extract was evaporated to 6 mL and 5 mL was injected onto the Gel Permeation Chromatography (GPC) column where a fraction of the eluent was collected, concentrated, and solvent exchanged to acetone:hexane (1:1). Further clean-up was performed by eluting this extract through multi-layered silica gel and then alumina column chromatography. The final extract was concentrated, and solvent exchanged to isooctane. The analysis was performed for the DDE and PCBs using GC-MS in Selective Ion Monitoring (SIM) mode with an EI source (Agilent 7890 GC coupled to a 5975-mass spectrometer, Agilent

Technologies, Santa Clara, CA) based on US EPA Method 680. All results were calculated by isotope dilution. Laboratory control samples and blank samples were measured for QA/QC. A total of 27 PCB congeners (PCB-1, 3, 4, 15, 19, 37, 54, 77, 81, 104, 105, 114, 118, 123, 126, 155, 156, 157, 167, 169, 188, 189, 202, 205, 206, 208, and 209) were measured and the sum was used to estimate total PCB concentrations.

2.6 Estimation of fish, dietary POPs (DDE, PCBs), and long-chain omega-3 FAs intake

Daily fish intake (g/d) was estimated using data from the FFQ by summing up the number of days in the past four seasons when fish consumption was reported (each fish species). Then, the total number of days when fish intake was reported was multiplied by the age- and gender-specific portion size of fish (g) estimated from dietary data generated by the 24-hour recalls for each age and sex group, and divided by 360 days (in this study, a year included four seasons of 90 days each).

Total dietary PCBs and DDE intake was calculated as follows: first, the amounts of PCBs and DDE (nanograms) in one gram of each fish species were multiplied by the total amount of each fish species eaten per day (grams); second, the amounts of PCBs and DDE from all fish species consumed per day were summed up and divided by the body weight of each participant (ng/kg of body weight/day).

$$[\Sigma (\text{Fish intake (grams/day)} \times \text{total PCBs/DDE (ng/ gram of fish)})/\text{body weight}]$$

Community-specific data of POPs content in fish species were applied to calculate total PCBs and DDE intake for each participant. Ecozone-specific concentrations of DDE and PCBs content in fish species were used and applied for the communities that were located within a particular ecozone if community-specific data were not available. The validation of dietary assessments was performed through correlation analysis between mercury exposure from traditional food

estimated using the FFQ and mercury concentrations in hair measured in First Nations participants. Dietary mercury intake was correlated with mercury in hair.

The Canadian File of Nutrients (Health Canada, 2014) was used to determine the concentrations of n-3 FAs in all fish species reported by First Nations in Manitoba. In this analysis, n-3FAs means combined EPA and DHA from fish. The data are expressed as mg of EPA+DHA per gram of raw fish. Raw values were used since fish tissue EPA+DHA concentrations may vary according to cooking method. Using the raw values allows the comparison of our results with other studies. The total amount of EPA+DHA consumed by each participant was calculated as follows:

$$[\Sigma(\text{Fish intake (grams/day)} \times \text{EPA + DHA (mg/ gram of fish)})]$$

The most consumed fish species in Manitoba were walleye, northern pike, lake whitefish and lake trout. In order to define fish consumption patterns, the consumption of commonly consumed fish species as well as their contribution to dietary n-3 FAs, DDE, and PCBs intake were described across four ecozones in Manitoba.

2.7 Statistical analyses

Descriptive statistics include the calculation of means with standard deviation (SDs) for continuous variables and proportions for categorical variables. Medians (interquartile range) were calculated for skewed variables. Geometric means (95%CI) were estimated for dietary DDE and PCBs intake. Student t-tests, analysis of variance (ANOVA) and chi-square tests were used to test if differences between groups are statistically significant. Sub-group stratified analysis by gender, age groups and ecozones were performed to describe the study population by diabetes status. Fish consumption was categorized into four groups: no or <1/month, 1/month, 2-3/month, and ≥ 1 /week to examine dose-response relationship between fish and T2D. The portion size of 150 g of fish was considered in this analysis. We chose 150 g since this amount embodies

two servings (of 75 grams each) of fish per week recommended by Canada's Food Guide – First Nations, Inuit, and Métis (Health Canada, 2007). Pearson correlation coefficients were investigated among all continuous predictors. Collinearity was observed between fish intake with n-3 FAs and n-3 FAs with PCBs and DDE.

Simple logistic regression models were performed to explore relationships between an outcome (T2D) and each primary predictor of interest individually (fish intake (categorical), PCBs, DDE (continuous), n-3 FAs (continuous and categorical), as well as all potential confounders (age, gender, BMI, total energy intake, smoking, physical activity, household size (number of people per household), and years of education).

Multiple logistic regression models adjusted for potential covariates were developed in order to investigate the associations between total fish intake, dietary POPs (PCBs and DDE) and n-3 FAs (EPA+DHA) intake, individually and T2D. Independent variables such as DDE, PCBs, and n-3FAs did not fit a normal distribution and were normalized using the natural logarithmic function. POP concentrations below the limit of quantification were imputed with a half limit of detection (LOD) of PCBs and DDE to avoid errors in the analysis. The LOD of DDE is $0.0005\mu\text{g/g}$ and LOD of PCBs is $0.0003\mu\text{g/g}$ (Chan et al., 2012).

Three models were developed to analyze the association between frequency of fish consumption (4 categories) and T2D. Adjustment variables were selected based on well-established risk factors for T2D reported in the literature including age, sex, body mass index (BMI), smoking, physical activity, total energy intake, education, and household size. Covariates were added to the models gradually to evaluate their relative contribution on the association between the predictors of interest and the outcome variable. Model 1 was adjusted for age, gender, and BMI; Model 2 was additionally adjusted for physical activity, total energy intake, smoking, household size, and education; Model 3 was controlled for covariates in Model 2 and DDE/PCBs intake in

order to eliminate their possible effect on T2D. Age, BMI, energy intake, number of people per household, years of education were treated as continuous variables whereas gender, smoking and physical activity were categorical variables. Fat, saturated fat, carbohydrate, fruit and vegetable intake were also considered as covariates but were removed from the models since they did not change the measure of the association between T2D and fish consumption.

To analyze the relationship between log-transformed dietary DDE, PCBs, n-3 FAs intake and T2D, overall and stratified by age group (<45y; ≥45y) logistic regression models were developed. We chose to dichotomize by age of 45 years since it was close to the median age of the study population, across different regions of Canada.

The models were tested for interactions between predictors (x_1 , x_2) by including the product “ $x_1 \times x_2$ ” as an additional predictor in a model containing x_1 and x_2 :

$\log(p(y)/(1-p(y))) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 \times x_2$. No interaction was detected.

Results with a p-value of less than 0.05 were considered statistically significant. All statistical analyses were performed using weighting variables in order to obtain representative estimates at the regional level. JMP version 11 and R software were used to conduct statistical analyses.

Sensitivity analyses were conducted to explore differences in dietary characteristics and physical activity between responders who were recently diagnosed with T2D (≤5 years ago) with those who were diagnosed with T2D for a longer period of time (>5 years) (Supplemental material: table S1). Also, analyses of dietary and lifestyle practice were carried out between participants with and without T2D by dieting status (Supplemental material: table S2).

3 Results

This study included 706 participants (477 women and 229 men). Mean age was 43.4 years ranging from 19 to 96 years. Descriptive characteristics of Manitoba participants by diabetes

status are presented in table 1. The prevalence of diagnosed T2D was 22%. When standardized to the 2015 Canadian population, the prevalence of T2D was 28.4%. Participants with T2D were more likely to be older, male, to have higher BMI, to be less physically active and less educated compared to those without T2D. Some differences in dietary intake were noted between diabetic and non-diabetic participants: individuals with T2D reported significantly lower intakes of total energy, total fat, and carbohydrate. Fruit and vegetable consumption was very low among all Manitoba participants (9.9g/d) and was comparable between diabetic participants (10.2g/d) and non-diabetic ones (9.8g/d).

Overall, 82% of participants reported consumption of fish at least once in the prior year. The mean intake of total fish in the study population was 9.2g/d (median 2.7g/d (range: 0- 230g/d)). Individuals with T2D consumed 11.6g/d of fish while those without T2D reported eating 8.5g/d of fish. The intake of long-chain n-3 FAs (EPA+DHA) was slightly higher in participants with T2D (69mg/d vs. 42g/d, respectively). The mean intake of DDE and PCBs was very low among both groups. Significant differences in fish consumption were found between males and females (16.7g/d vs. 5.2g/d, respectively).

Table 2 presents the characteristics of participants by age groups (<45y vs ≥45y). The prevalence of T2D was higher among older responders (36.8%) compared to younger participants (10%). Mean BMI was comparable between the two groups whereas physical activity was lower in older subjects. Also, individuals aged 45 years and over reported lower total energy, fat and carbohydrates intake, but higher fruit and vegetables, and fish consumption.

Table 3 shows the characteristics of participants by the frequency of fish intake. More frequent fish consumption was associated with lower prevalence of self-reported T2D, older age, being male, and higher physical activity.

Description of fish consumption patterns, long-chain n-3 FAs and DDE/PCBs intake in four Manitoba ecozones (Prairies/Plains, Boreal Plains, Boreal Shield, and Taiga Shield) are summarized in table 4. Overall, the consumption of 30 different types of fish was reported by Manitoba First Nations participants. The most consumed fish species across all Manitoba ecozones were walleye, lake whitefish, northern pike, and lake trout which contributed 78% to the total fish intake. Geographical differences in the amount and type of fish consumed were noted in four ecozones. The highest total fish intake was reported by participants living in the northern ecozone Taiga Shield/Subarctic with a mean intake of 25.6g/d (median, 13.5g/d) compared to only 11.4g/d (median, 3.7g/d) in the Boreal Shield, 5.8g/d (median, 2.2g/d) in the Prairies/Plains, and 5.9g/d (median, 3.3g/d) in the Boreal Plains. Walleye was the most consumed fish species of all fish consumed with the proportion of consumption varying from 60 to 69% across four ecozones. The lowest intake of total fish and the most consumed fish species were reported by participants in the Prairies/Plains ecozone. Lake trout was the primary contributor to DDE and PCBs intake. Nevertheless, lake trout along with lake whitefish were the main sources of EPA and DHA. There were differences in the prevalence of T2D across Manitoba ecozones. In the Taiga Shield, only 5% of individuals reported being diagnosed with T2D whereas in the other three regions, reported prevalence ranged from 18% to 31%. The mean BMI, physical activity and total energy intake were comparable among participants across the four Manitoba ecozones.

Table 5 presents multiple logistic regression analysis of the association between frequency of fish consumption and T2D. Dose-response associations were observed across the frequency of fish intake and T2D. Model 1 was adjusted for age, gender, and BMI only. Model 2 was additionally adjusted for other covariates such as physical activity (categorical), total energy intake (continuous), smoking (yes/no), household size (continuous), and years of education

(continuous). Model 3 was further controlled for DDE/PCBs intake in order to examine whether the relationship between fish intake and T2D was mediated by DDE and PCBs in fish. The results from all the models showed statistically significant inverse associations between fish consumption of 2-3 portions per month with an OR= 0.51 (95% CI: 0.28-0.91) and ≥ 1 /week with an OR= 0.40 (95%CI: 0.19-0.82) and T2D when compared to no consumption or consumption of less than one portion of fish per month.

Multiple logistic regressions of log-transformed DDE, PCBs, and EPA+DHA intakes with T2D overall and stratified by age groups are presented in Table 6. DDE, PCBs, and EPA+DHA variables were not normally distributed, so were normalized using the natural logarithmic function. Two models were developed: Model 1 was adjusted for potential covariates: age, BMI, gender, energy intake, physical activity, smoking, household size, and years of educations. In Model 2, DDE and PCBs were additionally adjusted for EPA+DHA intake whereas EPA+DHA was controlled for DDE/PCBs intake. There were no associations between DDE and PCBs intake with T2D in both overall and stratified by age groups analyses. However, an inverse association between EPA+DHA intake and T2D was found among younger participants in the model controlled for DDE/PCBs intake, and in both models among older individuals. When EPA+DHA intakes were stratified by tertiles (Table 7), the highest intake of EPA+DHA showed statistically significant protective effects on T2D with OR= 0.48 (95%CI: 0.30-0.77) compared to the reference group.

Table 8 presents concentrations of POPs and n-3 FAs (EPA+DHA) in the four most consumed fish species in Manitoba. The concentration of DDE and PCBs was not detected in walleye across the four Manitoba ecozones. In northern pike, PCBs and DDE were detected only in the Boreal Shield. Lake whitefish contained low concentrations of DDE ranging from 0.65 to 1.61ng/g of fish, and PCBs ranging from 0.17 to 0.33ng/g of fish. Lake trout had the highest

POP concentrations across the four regions compared to other species (DDE: 7.65-15.8ng/g; PCBs: 7.41- 11.06ng/g). There were no statistically differences between the four ecozones in PCBs and DDE concentrations in the four most consumed fish species (ANOVA, $p=0.99$ for PCBs, and $p=0.94$ for DDE). In regard to n-3 FAs content, whitefish and lake trout had significantly higher concentrations of EPA+DHA (1.24g/100g and 0.84g/100g, respectively) compared to walleye (0.31g/100g) and northern pike (0.27g/100g of fish).

In addition, we assessed dietary and lifestyle behavior in individuals with and without T2D in order to examine if participants diagnosed with T2D tend to change their diets. The first sensitivity analysis aimed to compare diet and lifestyle practice between participants recently diagnosed with T2D (0-5y) and those who have T2D for a long period of time (>5y) (Supplemental material: table S1). The analysis shows no statistically significant differences in physical activity, macronutrient intakes, and fish consumption between the two groups.

The second sensitivity analysis explored diet and lifestyle in participants with and without T2D which may be associated with self-reported dieting status (Supplemental material: table S2). The analysis revealed that there were no differences in macronutrient intakes between individuals with and without T2D. However, dieting participants had higher mean BMI, reported slightly lower smoking rate, and higher physical activity compared to not-dieting subjects. Fish and n-3 FAs consumptions were comparable between two groups.

4 Discussion

The prevalence of self-reported T2D in Manitoba FNs adults aged 19 years and older was 22%, which is similar to the estimate reported by the Manitoba First Nation RHS (21%) in individuals aged ≥ 18 years (RHS, Manitoba, 2012). These rates are more than three times higher compared to those in the general Canadian population (6.8% in individuals aged ≥ 20 years) (Public Health Agency of Canada 2011). In this study, more males than females reported T2D (26% vs 20%).

The average amount of traditional food consumed by the participant of this study was 45 g/person/day, and 82% of participants reported eating fish (Chan et al. 2012).

In this cross-sectional study, a dose-response relationship between fish intake and self-reported T2D was found. Statistically significant inverse associations between frequency of fish consumption and self-reported T2D were observed in the multivariable-adjusted models when compared to no fish intake or less than one portion per month. Fish intake of one portion per week and more may decrease the OR for T2D by 60%. Similarly, the highest intake of n-3 FAs was associated with a 52% decrease of T2D compared to no n-3 FAs intake. The stronger protective effect of fish consumption on T2D than the effect of n-3 FAs might be attributed to other nutrients contained in fish such as protein and vitamin D which have been reported to decrease the risk of T2D by a favorable effect on glucose metabolism (Pittas et al., 2007). The protective effect of n-3 FAs was also found in the analyses stratified by age groups (>45y vs. \geq 45y), particularly in the models adjusted for DDE and PCBs intakes.

Our findings are consistent with a number of previous population-based prospective cohort studies on the relationships between fish and n-3 FAs on one hand and T2D on the other, conducted among different populations (Patel et al., 2009; Nanri et al., 2011; Villegas et al., 2011). Patel et al. reported that consumption of one or more portion per week decreased the OR for diabetes by about 25% compared to less than one portion per week in analyses adjusted for a number of risk factors for diabetes (Patel et al., 2009). Nanri et al. (2011) found that fish intake in men was significantly associated with a decreased risk of T2D with the ORs of T2D for the highest compared with the lowest quartile of intake were 0.73 (95%CI: 0.54,1.00) for total fish and 0.68 (95%CI: 0.5,0.92) for small and medium fish (Nanri et al., 2011). Villegas et al. (2011) found that the relative risk (RR) for quintiles of fish intake were 1.00, 0.96, 0.84, 0.8, and 0.89 (p=0.003). Recent meta-analyses of prospective studies found that in the overall analysis, fish

consumption and dietary n-3 FAs intake were not associated with lower risk of T2D (Wallin et al., 2012; Wu et al., 2012; Zheng et al., 2012). However, geographical differences in the associations were demonstrated with a protective effect of fish and n-3 FAs on T2D in Asian countries (Nanri et al. 2011; Villegas et al. 2011) and positive associations in US population (Kaushik et al., 2009). This heterogeneity may originate from several sources including genetic differences or gene-diet interactions (Dedoussis et al., 2007; Lee et al., 2011); also, from differences in the type and amount of fish consumed as well as concentrations of contaminants in fish species which were not considered in previous studies (Lee and Jacobs 2010). In fact, Wallin et al. reported that dietary contaminants in fish may influence the relationship between fish and T2D (Wallin et al., 2015). Among the Aboriginal population, limited data on the association between fish and T2D are available. A cross-sectional study in Canadian First Nations communities reported that consumption of whitefish and trout may be beneficial to reduce the risk of diabetes while exposure to contaminants (DDE, PCBs) increased the risk of T2D (Philibert et al. 2009).

The protective associations between n-3 FAs and T2D could reflect beneficial effects on dyslipidemia characterized by low HDL-cholesterol and high triglyceride levels. There is evidence that n-3 FAs can decrease triglyceride levels, improve glucose tolerance and insulin sensitivity (Ebbesson et al. 2005), but have no effect on HDL-cholesterol levels (Hartweg et al., 2009). Tørris et al. reported that fish consumption was associated with a lower risk of metabolic syndrome (Tørris, Molin, and Cvancarova 2014, 2017). Experimental studies reported that fish or fish oil supplements may improve insulin secretion and insulin sensitivity (Akinkuolie et al., 2011).

Dietary DDE and PCBs intake showed no association with T2D in the model controlling for several covariates only; however, there were positive, but not statistically significant associations

in the models controlling for EPA+DHA intake in the total population and older individuals. Several previous studies have suggested a positive association between serum DDE and PCBs concentrations and T2D (Codru et al., 2007; Philibert et al., 2009; Turyk et al., 2009). Statistically significant positive associations between dietary DDE and PCBs intake and T2D were confirmed in Ontario First Nations where dietary POPs exposure was higher than in Manitoba First Nations (Chapter 5). In the current study, the weak association between exposure to DDE and PCBs, and T2D may be explained by very low dietary DDE and PCBs intake via fish; however, it was slightly higher among older participants compared to younger ones since they consumed a higher amount of fish.

The percentage of participants consuming fish differed across Manitoba regions depending on variety and availability of fish species. For example, 90-94% of participants reported eating fish at least once in the prior year in the Boreal Plains, the Plains/Subarctic and the Taiga Shield/Subarctic whereas 80% of responders from the Boreal Shield/Subarctic and only 68% of participants living in the Prairies/Plains, Prairies/Subarctic ecozone did. Even though most of the participants reported eating fish in the prior year, daily mean intake of fish was estimated to be relatively low except in the Taiga Shield/ Subarctic region. Among 30 types of fish walleye, northern pike, lake whitefish, and lake trout were the most consumed species, contributing almost 80% to the total fish intake.

Regional differences in the prevalence of T2D were found across four Manitoba ecozones with the lowest T2D rate being observed in the Taiga Shield/ Subarctic region (5%). Dietary characteristics and lifestyle practice were analyzed and compared between individuals living in the four Manitoba regions. No statistically significant differences in dietary intake were observed; however, participants living in the Taiga Shield/ Subarctic region consumed much more fish compared to those from the other regions (25.6g/d vs 11.4g/d, 5.8g/d, 5.9g/d). In

addition, they reported higher consumption of whitefish and lake trout which contain high levels of n-3 FAs.

The DDE and PCBs concentrations varied between four most consumed fish species. DDE and PCBs were not detectable in walleye and northern pike while lake trout had the highest concentrations of DDE and PCBs. Elevated concentrations of POPs in lake trout were also documented by other studies (McGoldrick et al., 2015). The concentrations of DDE and PCBs in fish species were comparable across four Manitoba ecozones.

This study has several limitations. Since the design of our study is cross-sectional, we cannot state the causal relationship between fish, n-3 FAs and T2D. Second, the reliance on self-reporting for the calculation of the prevalence of T2D could have resulted in an underestimation. We validated our estimates of T2D by comparing them with the self-reports presented by the Regional Health Survey (RHS) which is a representative study for First Nations living on reserve conducted over the same period of time (2008-2010). The RHS collected more comprehensive information on T2D that included data on a kind of treatment used to control diabetes, frequency of checking blood sugar levels, complications of diabetes, whether adopting a healthier lifestyle including (diet and exercise), attendance of a diabetes clinic and getting diabetes education. A study among Cree First Nations living in Northern Quebec reported that 4.5% of participants had undiagnosed diabetes based on glucose levels that indicates diabetes (≥ 7 mmol/L), but no mention of diabetes in their medical charts (Cree Board of Health and Social Services of James Bay 2013). Third, self-reported dietary intake estimates may lead to some degree of measurement error of the intake. Some previous studies reported that overweight and obese individuals tend to underestimate their energy, fat and carbohydrate intake (Heitmann. et al., 1995.), while other scientists reported that both obese and non-obese subjects tend to underreport between-meal snack foods (Poppitt et al. 1998). Bailey et al. 2007 reported that weight status,

education, and smoking status are characteristics consistently associated with underreporting. The authors suggested that these factors should be used to control for in statistical models when examining relationships with diet and health (Bailey et al., 2007). In the current study, the results were controlled for lifestyle confounders including total energy intake, body mass index, physical activity, smoking, education level, and crowding. Other potential covariates such as dietary fat, saturated fat, carbohydrate, fruit and vegetable intake were also considered. However, since they did not change the estimates of the association between T2D and fish consumption, they were not included in the models. Further, lifestyle and dietary habits associated with both fish consumption and type 2 diabetes may influence the results. To examine differences in dietary and lifestyle behaviour between diabetic and non-diabetic individuals, several sensitivity analyses were performed. The analyses revealed that no statistical differences in habits as well as fish consumption, dietary n-3 FAs and POPs intake were found (Supplemental material). Also, physical activity level was self-reported and therefore may not be entirely accurate. Finally, we estimated PCB and DDE exposure from fish consumption, the total PCB and DDE intake may be even higher. Future studies may need to verify this using biomonitoring data. To our knowledge, this is the first study investigating the relationship between fish consumption, dietary intake of n-3 FAs and POPs, and T2D in Manitoba First Nations living on reserve. The strength of our study is a large sample size which is representative of all First Nations adults living on reserve in Manitoba. POPs concentrations in locally-harvested fish were measured in this study. The individual total dietary PCBs and DDE intake was calculated based on community-specific data of POPs content in fish species. Also, we were able to control our results for a number of risk factors for T2D such as age, gender, BMI, smoking, physical activity, energy intake, and education level. Several epidemiological studies reported a positive association between serum levels of n-3 FAs and T2D (Virtanen et al., 2014). We observed the same positive relationship

between dietary n-3 FAs intake and T2D which is useful in developing dietary advisory of fish consumption pattern.

5 Conclusion

The prevalence of self-reported T2D in Manitoba First Nations was 22%. Negative dose-response relationships between fish, long-chain n-3 FAs and T2D were found. Fish consumption of 2-3 portions per month, and one portion per week and more reduced the odds of T2D by about 50% and 60%, respectively compared to no fish or fish consumption less than one portion per month. Similarly, consumption of long-chain n-3 FAs was inversely associated with self-reported T2D. Dietary DDE and PCBs intake was not associated with T2D. The most consumed fish species in the four Manitoba ecozones were walleye, northern pike, lake whitefish and lake trout contributing 78% to the total fish consumption. The concentrations of DDE and PCBs in fish samples were low or not-detectible. Thus, the exposure to POPs from fish was negligible in Manitoba First Nations communities. Lake whitefish and lake trout were the main sources of long-chain n-3 FAs, while lake trout was the primary contributor to the total DDE and PCBs intake. These findings suggest that traditionally harvested fish consumption may have a beneficial effect on T2D in Manitoba First Nations. However, causal relationships between fish, n-3FAs intake and T2D need to be investigated in a prospective study. The results of this study will be useful in developing fish consumption advisories and prevention programs to slow down the increasing rate of T2D in Manitoba First Nations. This study serves as a case study for other regions in Canada and the rest of the world to develop fish consumption advisory. It is important to measure POPs concentrations and n-3 FAs in locally-harvested fish for future studies.

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Table 1. Characteristics of Manitoba First Nations adults by diabetic status (n=706)

	with T2D	without T2D	p value
N, (%)	123 (22)	583 (78)	
Women, n (%)	76 (20)	401 (80)	0.13
Men, n (%)	47 (26)	182 (74)	
Age	52.71 (49.14,56.28)	40.73 (37.04,44.41)	.0001
Age groups, n (%)			.0001
19-30	9 (6.5)	165 (25.9)	
31-50	50 (37.9)	287 (53.3)	
51-70	56 (48.2)	115 (16.8)	
71+	8 (7.5)	16 (3.9)	
BMI, kg/m ²	33.11 (30.52,35.71)	30.03 (29.33,30.73)	.0001
BMI category, n (%)			.0001
<25	7 (8)	142 (22.2)	
25-29.99	31 (25.6)	182 (31.6)	
≥30	85 (66.4)	259 (46.3)	
Physical activity, n (%)			.0007
inactive	45 (35.2)	115 (17.8)	
sedentary	50 (37.2)	307 (53.8)	
moderate	20 (18.6)	104 (18.9)	
vigorous	8 (9.1)	57 (9.5)	
Smoking, n (%)	61 (50)	383 (66)	.0008
Dieting, n (%)	10 (10.1)	50 (7.5)	0.87
Household size	5.71 (4.72,6.69)	5.4 (6.474,6.18)	0.35
Years of education	8.88 (8.26,9.50)	10.08 (9.64,10.51)	.0003
Diploma, n (%)			0.38
Less than high school	72 (62.4)	341 (58.5)	
High school	31 (21.3)	174 (28.3)	
Post-secondary	20 (16.3)	68 (13.3)	
Employment, any (%)	62 (60.8)	323 (61.7)	0.27
Dietary characteristics			
Energy intake (kcal/d)	1807.8(1439.6,1975.9)	2027.9(1818.6,2237.3)	0.020
Fruit/vegetables (g/d)	10.24 (5.48,19.11)	9.81 (6.89,13.95)	0.051
Total fat (g/d)	59.22(33,92.51)	75.78(47.81,106.89)	0.012
Saturated Fat (g/d)	18.68(11.07,28.15)	22.76(14.68,33.94)	0.031
Carbohydrate (g/d)	172.2(107.8,275.1)	223.1(157.8,315.9)	0.020
Protein (g/d)	68 (45-100)	71.08(47.83,100.55)	0.78
Total fish intake (g/d)	11.6 (3.1,26.4)	8.5 (1.7,15.3)	0.06
DDE (ng/kg/d)	0.003 (0.001,0.02)	0.002 (0.001,0.01)	0.09
PCBs (ng/kg/d)	0.001 (0.00,0.01)	0.001 (0.00,0.004)	0.33
EPA+DHA (mg/day)	69.08 (35.11,173.26)	41.9 (61.65,82.27)	0.41

T2D – type 2 diabetes; DDE - dichlorodiphenyldichloroethylene; PCBs - polychlorinated biphenyls; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; values are mean (95%CI), DDE/PCBs – geometric mean; BMI- body mass index; p-values correspond to t-tests for continuous variables and chi-square tests for categorical variables; weighted estimates

Table 2. Characteristics of Manitoba participants by age groups (<45y/≥45y)

	<45	≥45	p value
n	709	717	
T2D (%)	10	36.8	0.0001
Body Mass Index	30.7 (29.9,31.4)	31.4 (30.8,32.1)	0.148
Smoking (%)	60.8	36.7	0.0001
Physical activity (%)			0.0002
inactive	16.1	23.5	
sedentary	40.6	44.9	
moderate	28.4	23.4	
vigorous	14.9	8.2	
Dietary characteristics			
Total energy(kcal/d)	1982(1355,2494)	1730(1193,2210)	0.001
Fruit/vegetables (g/d)	7.3 (5.4,9.8)	14.4 (7.2,29.0)	0.002
Fat (g/d)	84.8 (75.7,93.9)	74.4 (66.2,82.5)	0.002
Protein (g/d)	68.1(45.9,100.4)	73.4(49.4,105.1)	0.55
Carbohydrate (g/d)	244.2(168.8,335.7)	188.5(120.7,275.1)	0.001
Fish intake (g/d)	6.3 (2.5,10.5)	12.7 (1.0,26.3)	0.002
DDE (n/kg/d)	0.002 (0,0.01)	0.004 (0.00,0.03)	0.07
PCBs (n/kg/d)	0.001 (0.0,0.002)	0.002 (0.0,0.01)	0.14
EPA+DHA (mg/d)	32.0 (5.4,58.8)	67.3(15.6,150.4)	0.001

*T2D - type 2 diabetes, DDE-dichlorodiphenyldichloroethylene; PCBs-polychlorinated biphenyls
EPA- eicosapentaenoic acid; DHA - docosahexaenoic acid; values are mean (95%CI),
DDE/PCBs -geometric mean, p-values correspond to t-tests for continuous variables and chi-square
tests for categorical variables; weighted estimates*

Table 3. Characteristics of Manitoba participants by frequency of fish consumption

	0 or <1/mo	1/mo	2-3/mo	≥ 1/week	p value
n	225	232	163	86	
Type 2 Diabetes, %	20	19	15	12	0.35
Age, y	41.0 (34.6,47.3)	45.0 (43.2,46.8)	41.3 (38.7,44.0)	49.9 (41.6,58.2)	0.04
Women, %	82	71	55	44	.0001
Men, %	18	29	45	56	.0001
Body mass index	30.6 (28.8,32.4)	30.9 (30.2,31.6)	31.4 (29.8,33.0)	29.5 (27.6,31.3)	0.08
Smoking, %	68	57	64	63	0.12
Physical activity, %					
sedentary	79	70	74	67	0.001
moderate	12	23	19	14	
vigorous	9	7	7	19	
Total energy (kcal/d)	1931.4 (1614.1,2248.7)	1861.5 (1623.5,2099.5)	2060.2 (1791.9,2328.5)	2046.3 (1643.6,2448.9)	0.81
Fruit/vegetables (g/d)	6.3 (3.6,11.1)	14.3 (5.5,37.0)	13 (5.4,31.5)	9.3 (5.7,15.1)	0.49
Fat (g/d)	76.6 (63.7,89.5)	76.5 (69.3,83.7)	87 (71.9,102.1)	85 (64.1,106.0)	0.18
Protein (g/d)	68.7 (57.9,79.5)	73.4(64.0,82.7)	92.2 (80.5,103.9)	97.3(83.0,111.7)	0.005
Carbohydrate (g/d)	246.5(201.2,291.8)	224.3(183.9,264.8)	232.7(201.2,264.3)	230.9(191.0,270.7)	0.04
Fish intake (g/d)	0.3 (0.2,0.4)	2.6 (2.4,2.9)	9.6 (8.4,10.7)	45.6 (36.0,55.2)	.0001
DDE (n/kg/d)	0.0 (0.0,0.0)	0.003 (0.0,0.032)	0.053 (0.002, 0.71)	1.47 (0.003,7.38)	.0001
PCBs (n/kg/d)	0.0 (0.0,0.0)	0.001 (0.0,0.003)	0.008 (0.001,0.08)	0.69 (0.002,2.39)	.0001
EPA+DHA (mg/d)	1.4 (1.2,1.7)	9.9 (7.8,12.0)	45.6(29.1,62.1)	253 (149.0,356.9)	.0001

DDE - dichlorodiphenyldichloroethylene; PCBs - polychlorinated biphenyls; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; values are mean (95%CI); DDE/PCBs -geometric mean; sedentary physical activity combines inactive and sedentary lifestyle, p-values correspond to ANOVA for continuous variables and chi-square tests for categorical variables; weighted estimates

Table 4. Fish consumption patterns and dietary characteristics by Manitoba ecozones

	Prairies/Plains, Prairies/Subarctic	Boreal Plains, Plains/Subarctic	Boreal Shield/ Subarctic	Taiga Shield/ Subarctic	p value
n	170	187	232	117	
T2D (%)	19.8	31.3	18.6	5.1	.0001
Age, y	42±13.7	43±13.8	43±15.5	40±13.9	0.22
Body mass index	29.8±6.2	31.3±6.7	30.4±6.4	29.1±6.2	0.016
Women (%)	62	71	66	72	0.24
Energy intake (kcal/d)	1884 (1256-3072)	1766 (1273-2349)	1795 (1265-2358)	1642 (1279-2421)	0.16
Smoking (%)	71	59	53	77	.0001
Physical activity (%)					0.57
moderate/vigorous	27	30	27	23	
Fish consumers (%)	68	94	80	92	
Total fish g/d	2.2 (0.14-60.0)	3.3 (0.4-50.0)	3.7 (0.4-81.6)	13.5 (0.5-166.2)	.0001
DDE ng/kg/d	0.001 (0-0.25)	0.001 (0-0.29)	0.001 (0-1.22)	0.46 (0-5.5)	.0001
PCBs ng/kg/d	0.001 (0-0.28)	0.001 (0-0.30)	0.001 (0-1.08)	0.62 (0-7.33)	.0001
EPA+DHA g/d	0.007 (0.001-0.26)	0.01 (0.002-0.19)	0.01 (0.004-0.5)	0.1 (0.005-1.41)	.0001
Walleye consumers %	60	66	69	66	0.28
Walleye g/day	1.9 (0.2-30.2)	2.3 (0.3-43.8)	2.2 (0.3-32.3)	3.2 (0.3-26.6)	0.02
DDE ng/kg/d	0.00	0.00	0.00	0.00	
PCBs ng/kg/d	0.00	0.00	0.00	0.00	
EPA+DHA g/d	0.01 (0.001-0.09)	0.01 (0.001-0.14)	0.001 (0.001-0.1)	0.01 (0.001-0.08)	0.02
Northern Pike consumers %	24	26	29	28	0.74
Northern Pike g/d	1.5 (0.2-32.3)	1.6 (0.1-53.7)	1.6 (0.3-29.0)	2.3 (0.14-27.8)	0.34
DDE ng/kg/d	0.001 (0-0.09)	0.001 (0-0.13)	0.001 (0-0.21)	0.001 (0-0.12)	0.63
PCBs ng/kg/d	0.001 (0-0.04)	0.001 (0-0.05)	0.001 (0-0.08)	0.001 (0-0.05)	0.71
EPA+DHA g/d	0.004 (0.0004-0.09)	0.004 (0.0004-0.16)	0.005 (0.001-0.09)	0.01 (0.0004-0.09)	0.33
Whitefish consumers %	16	28	26	47	.0001
Lake Whitefish g/d	2.05 (0.14-55.9)	1.7 (0.2-88.9)	2.2 (0.14-50.0)	4.1 (0.39-61.2)	0.16
DDE ng/kg/d	0.02 (0-0.73)	0.02 (0-1.43)	0.02 (0-0.67)	0.02 (0-0.95)	0.81
PCBs ng/kg/d	0.001 (0-0.22)	0.001 (0-0.43)	0.001 (0-0.2)	0.001 (0-0.29)	0.42
EPA+DHA g/d	0.03 (0.002-0.88)	0.03 (0.003-1.40)	0.03 (0.002-0.78)	0.06 (0.01-0.96)	0.16
Lake Trout consumers %	8	12	13	33	.0001
Lake Trout g/d	5.4 (0.5-37.1)	2.7 (0.14-23.3)	6.5 (0.14-62.6)	4.3 (0.14-83.4)	0.27
DDE ng/kg/d	0.42 (0.03-6.96)	0.29 (0.01-2.45)	0.73 (0.01-4.73)	0.46 (0.02-6.56)	0.26
PCBs ng/kg/d	0.63 (0.04-3.26)	0.41 (0.01-3.54)	0.85 (0.02-6.83)	0.64 (0.01-9.49)	0.31
EPA+DHA g/d	0.05 (0.004-0.36)	0.03 (0.001-0.23)	0.06 (0.001-0.61)	0.04 (0.001-0.8)	0.27

DDE - Dichlorodiphenyldichloroethylene; PCBs - Polychlorinated biphenyls; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; data are mean ± SD or median (2.5-97.5th percentiles); Fish, DDE, PCBs and EPA+DHA intakes estimated for fish consumers only; ng/kg/d - nanograms per kg body weight per day; p-values correspond to ANOVA for continuous variables and chi-square tests for categorical variables; weighted estimates of T2D prevalence

Table 5. Multiple logistic regression analyses of fish consumption and type 2 diabetes

	no or <1/mo	1/mo	2-3/mo	≥1/week
Model 1	1 (ref)	0.76 (0.25-2.20)	0.51 (0.26-0.97)*	0.49 (0.26-0.91)*
Model 2	1 (ref)	0.77 (0.25-2.32)	0.52 (0.28-0.96)*	0.51 (0.31-0.84)**
Model 3	1 (ref)	0.77 (0.25-2.31)	0.51 (0.28-0.91)*	0.40 (0.19-0.82)**
n	247	191	165	103

Values are ORs (95% CI), Model 1: adjusted for age, gender and BMI; Model 2 - additionally adjusted for physical activity, total energy intake, smoking, household size, and education; Model 3 - adjusted for Model 2 and DDE/PCBs (tertiles);

*- p value < 0.05; **<0.01; portion size is 150grams of fish

Table 6. Logistic regression analyses of log-transformed DDE, PCBs and long-chain n-3 fatty acids intake and type 2 diabetes in total sample and stratified by age groups

	DDE	PCBs	EPA+DHA
Total population			
Model 1	0.97 (0.8-1.1)	0.94 (0.8-1.04)	0.93 (0.8-1.07)
Model 2	1.04 (0.8-1.3)	0.97 (0.8-1.1)	0.87 (0.6-1.18)
<45 y			
Model 1	0.96 (0.78-1.17)	0.90 (0.74-1.28)	0.97 (0.64-1.05)
Model 2	0.99 (0.76-1.29)	0.96 (0.63-1.32)	0.95* (0.59-0.99)
≥45 y			
Model 1	0.98 (0.82-1.16)	0.97 (0.79-1.17)	0.85* (0.63-0.99)
Model 2	1.14 (0.87-1.48)	1.08 (0.86-1.34)	0.75* (0.41-0.92)

Values are OR (95%CI), Model 1 was adjusted for age, BMI, gender, energy intake, physical activity, smoking, household size, education; Model 2: DDE and PCBs were additionally adjusted for EPA+DHA intake; EPA+DHA was adjusted for DDE/PCBs;

ORs: per unit change in regression (log transformed variables); *- p value < 0.05

Table 7. Multiple logistic regression analyses of long-chain n-3 FAs intake (tertiles) and T2D

	Q1	Q2	Q3
Model 1	1(ref)	0.72 (0.28-1.86)	0.49** (0.29-0.83)
Model 2	1(ref)	0.80 (0.46-1.33)	0.53** (0.33-0.84)
Model 3	1(ref)	0.77 (0.28-2.12)	0.48** (0.30-0.77)

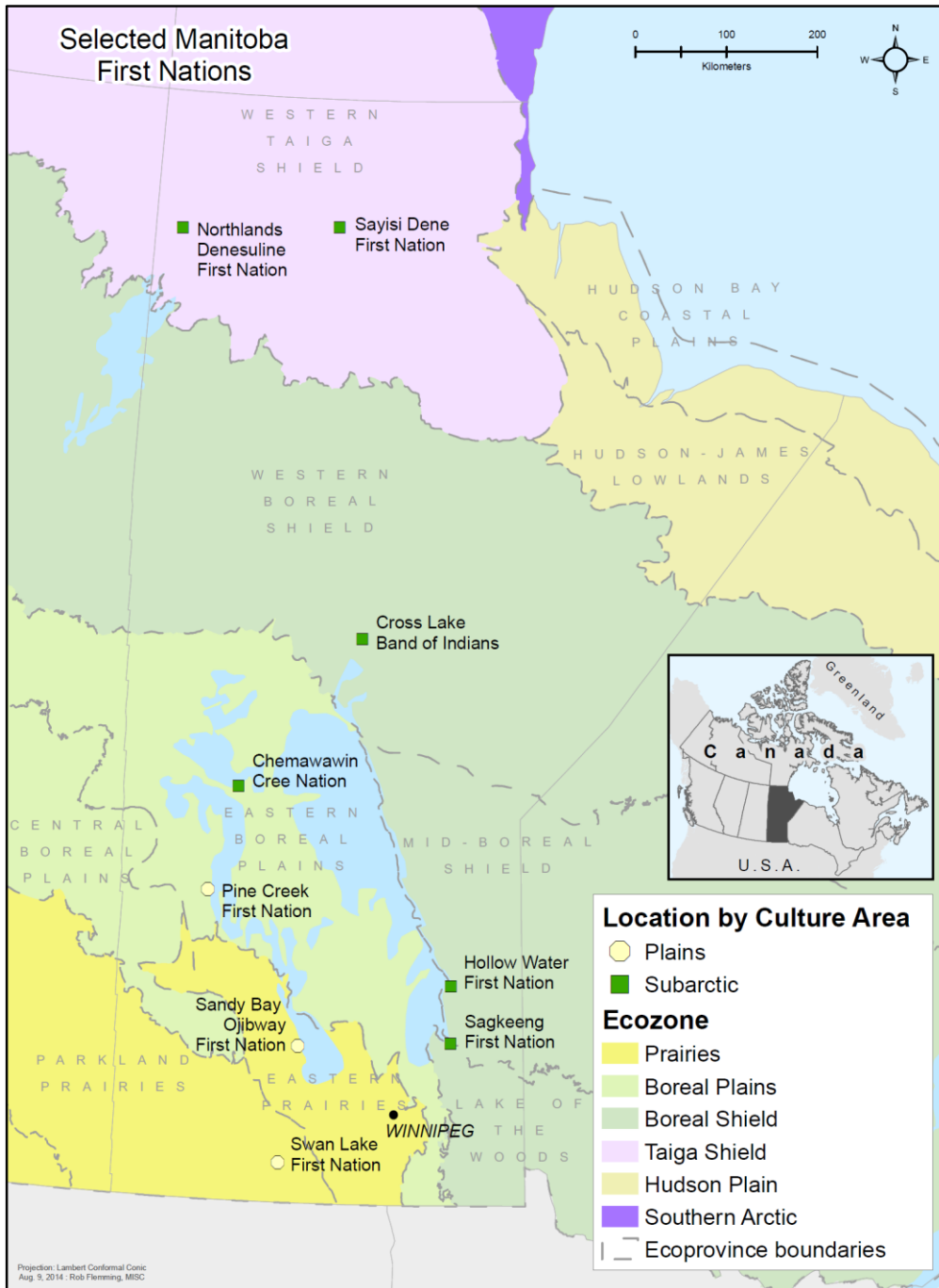
T2D, type 2 diabetes, values are OR (95%CI), Model 1 adjusted for age, BMI, gender; Model 2: further adjusted for energy intake, physical activity, smoking, education and household size; Model 3: adjusted for Model 2 + DDE/PCBs; **- p value < 0.01

Table 8. Concentrations of n-3 FAs and POPs in the most consumed fish species in Manitoba ecozones

Fish species	Ecozones								
	Canada	Prairies/Plains, Prairies/Subarctic		Boreal Plains, Plains/Subarctic		Boreal Shield/ Subarctic		Taiga Shield/ Subarctic	
	EPA+DHA g/100g	DDE ng/g	PCBs ng/g	DDE ng/g	PCBs ng/g	DDE ng/g	PCBs ng/g	DDE ng/g	PCBs ng/g
Walleye/Pickereel	0.31 (0.05)	0	0	0	0	0	0	0	0
Northern Pike	0.27 (0.09)	0	0	0	0	0.89 (0.15)	0.34 (0.12)	0	0
Lake whitefish	1.24 (0.56)	1.34 (0)	0.33 (0)	1.22 (0.71)	0.23 (0.32)	1.61 (0.91)	0.17 (0.33)	0.65 (0.54)	0.20 (0.27)
Lake trout	0.84 (0.14)	11.73 (5.76)	9.24 (2.58)	11.73 (5.76)	9.24 (2.58)	15.8 (2.87)	7.41 (1.29)	7.65 (2.13)	11.06 (2.98)

EPA- eicosapentaenoic acid; DHA-docosahexaenoic acid; EPA+DHA in grams per 100 grams of raw fish; ng/g -nanograms per 1gram of fish; data are mean (SD); 0- non-detectible concentration

Figure 1. Map of participating First Nations communities and four ecozones in Manitoba (Chan et al., 2012)



Supplemental material

Sensitivity analysis 1

Differences in dietary intake and lifestyle practise was explored and compared between participants recently diagnosed with T2D (≤ 5 years ago) and individuals diagnosed with T2D for a long period of time (> 5 y) using data on the onset of T2D. The results presented in Table S1 show that there were no statistically significant differences in dietary characteristics between individuals recently diagnosed with T2D and those having T2D for more than 5 years.

Table S1. Dietary and lifestyle characteristics by onset of type 2 diabetes (n=123)

	Onset of type 2 diabetes		p value
	≤ 5 years	> 5 years	
n	53	70	
Age	50 ± 12	55 ± 12	0.01
Body Mass Index, kg/m ²	34.8 ± 6.7	33.1 ± 6.4	0.92
Dieting, %	10	7.6	0.61
Smoking, %	48.3	53.1	0.51
Physical activity, %			0.07
Inactive	34	34	
Sedentary	46	43	
Moderate	8	20	
Vigorous	12	3	
Dietary characteristics			
Energy intake (kcal/d)	1840 ± 821	1750 ± 940	0.07
Protein (g/d)	82 ± 55	76 ± 48	0.23
Total Fat (g/d)	74 ± 47	63 ± 48	0.08
Saturated fat (g/d)	22 ± 14	21 ± 17	0.81
Carbohydrate (g/d)	210 ± 98	190 ± 110	0.84
Fruit/Vegetables (g/d)	132 ± 198	116 ± 201	0.62
Fish (g/d)	6.5 ± 14	4.4 ± 9.5	0.08

≤ 5 years- diagnosed with type 2 diabetes until 5y; >5 -diagnosed with T2D more than 5 years ago;
Values are mean \pm SD or median (25-75th) percentile

Sensitivity analysis 2

A sensitivity analysis was conducted to examine whether dietary intake and lifestyle behaviour differed between dieting and not dieting participants (Table S2). For this, data on self-reported dieting status (yes/no) on the previous day of an interview were used. Overall, 8% of participants reported to manage their caloric intake on the previous day in order to lose weight. The prevalence of self-reported T2D was lower among dieting participants compared to non-dieting (27.6% vs. 21.7%). No differences in dieting status were found between males and females. However, mean BMI was significantly higher among dieting responders compared to not-dieting ones (33.3 vs. 30.5). Individuals who were on diet reported a lower rate of smoking and tend to be more physically active. Dietary intake including fish and n-3 FAs consumption were similar between dieting and non-dieting participants (Table S2).

Table S2: Dietary characteristics of participants by dieting status (n=706)

	Dieting		p trend
	Yes	No	
Total n, %	8.1	91.9	
Type 2 diabetes, %	21.7	27.6	0.25
Female, %	65.3	63.9	0.64
Age	43.53 (38.33,48.73)	43.36 (39.85,46.88)	0.92
Body Mass Index, kg/m ²	33.32 (31.92,34.72)	30.48 (29.47,31.49)	0.012
Physical activity, %			0.040
inactive	11.4	22.5	
sedentary	41.6	50.8	
moderate	34.9	17.4	
vigorous	12.6	9.2	
Smoking, %	51.7	59.4	0.052
Dietary characteristics			
Energy intake(kcal/d)	2057.8 (1896.9,2218.7)	1948.2 (1712.7,2183.7)	0.31
Fruit & Vegetable (g/d)	10.2 (5.4,19.1)	9.8 (6.9,13.9)	0.21
Total Fat (g/d)	83.5 (74.0,93.1)	79.8 (70.62,88.98)	0.55
Saturated fat (g/d)	25.4 (22.3,28.4)	25.1 (22.6,27.5)	0.89
Carbohydrate (g/d)	255.4 (222.2,288.6)	233.2 (196.7,269.6)	0.25
Protein (g/d)	77.8 (69.5,86.1)	79.4 (67.2,91.7)	0.67
Fish intake (g/d)	10.15 (2.37,17.93)	9.14 (0.40,17.87)	0.74
DDE (ng/kg/d)	00.00,0.02	00.00,0.01	0.21
PCBs (ng/kg/d)	00.00,0.01	00.00,0.00	0.51
EPA+DHA (mg/d)	47.4 (7.6,102.4)	54.6 (3.7,105.5)	0.57

DDE - dichlorodiphenyldichloroethylene, PCBs - polychlorinated biphenyls, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid, values are mean (95%CI), DDE/PCBs -geometric mean, weighted estimates

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5 ASSOCIATION BETWEEN FISH CONSUMPTION, DIETARY OMEGA-3 FATTY ACIDS
AND PERSISTENT ORGANIC POLLUTANTS INTAKE, AND TYPE 2 DIABETES IN 18
FIRST NATIONS IN ONTARIO, CANADA

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ABSTRACT

Background: First Nations (FNs) populations in Canada experience a disproportionately higher rate of obesity and type 2 diabetes (T2D) compared to the general population. Recent data suggest that a high consumption of fish may help prevent T2D. On the other hand, fish might also be a potential source of environmental contaminants which could potentially be a risk factor for T2D. **Objective:** To investigate the potential associations between self-reported T2D and consumption of locally-harvested fish, dietary long-chain omega-3 fatty acids (n-3FAs) and persistent organic pollutants intake among adult FNs living on reserve in Ontario. **Design:** Data from the First Nations Food Nutrition and Environment Study, which included a cross-sectional study of 1429 Ontario FNs adults living in 18 communities across 4 ecozones in 2012 were analyzed. Social and lifestyle data were collected using household interviews. The consumption of locally-harvested fish was estimated using a traditional food frequency questionnaire along with portion size information obtained from 24hr recalls. Fish samples were analyzed for the presence of contaminants including dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs). Dietary intakes of DDE and PCBs were estimated using community-specific levels of DDE/PCBs in fish species. Multiple logistic regression models adjusted for potential covariates including age, gender, body mass index, physical activity, total energy intake, smoking, and education were developed. **Results:** The prevalence of T2D in Ontario FNs was 24.4%. A significant positive association between fish consumption of one portion per week and more and T2D compared to no fish consumption was found (OR=2.5 (95% CI: 1.38-4.58)). Dietary DDE and PCBs intake was positively associated with T2D (OR= 1.09 (95%CI: 1.05-1.75) for DDE and OR=1.07 (95%CI: 1.004-1.27) for PCBs) per unit increase in DDE/PCBs while n-3-FAs intake, adjusted for DDE/PCBs intake, showed an inverse effect against T2D among older individuals (OR=0.86 (95% CI: 0.46-0.99)). **Conclusion:** Our results support previous findings that exposure to DDE and PCBs may increase the risk of T2D. Elevated levels of contaminants in fish may counteract with potentially beneficial effects of n-3FAs from fish consumption. However, the overall health benefits of high consumption of fish with a high n-3 FAs content may outweigh the adverse effect of contaminants.

Keywords: First Nations, fish consumption, type 2 diabetes, persistent organic pollutants, long-chain n-3 fatty acids

1. Introduction

The prevalence of type 2 diabetes (T2D) has dramatically increased worldwide over the last two decades, and it is recognized as one of the most important public health concerns. According to the World Health Organization, the global diabetes prevalence for adults aged 20 years and older was estimated to be 6.6% in 2000 and is predicted to reach 7.7% by 2030 (Wild et al. 2004). In Canada, the First Nations population experiences a disproportionately higher rate of T2D compared to the general Canadian population (Dyck et al., 2010; Young et al., 2000). Age-standardized prevalence of T2D in First Nations was 17.2% compared to 6.8% in general Canadians (Public Health Agency of Canada 2011), ranging from about 7% to 36% in individual First Nation communities (Dannenbaum et al., 2008; Imbeault et al., 2010; Horn et al., 2007). T2D is a multifactorial disease caused by a complex interaction between lifestyle, genetic and environmental factors. Recognized risk factors for T2D are obesity, high-calorie diets, low physical activity and smoking (Byrne et al. 2012; Day & Bailey, 2011; Chang, 2012).

Recent data from the population-based prospective cohort studies have suggested that a high consumption of fish may help prevent T2D (Patel et al., 2009; Rylander et al., 2014). Potential benefits of fish and seafood were attributed to the presence of long chain omega-3 fatty acids (n-3 FAs), specifically eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have shown beneficial effects on multiple risk factors associated with diabetes, such as lipid profile, blood pressure, and inflammation, as well as on coronary heart disease and stroke (He 2009; Panagiotakos et al. 2007). Epidemiological studies on the association between fish consumption, n-3 FAs and T2D have reported controversial results: some studies found a protective effect (Nanri et al., 2011; Nkondjock & Receveur, 2003; Rylander et al, 2014), while others showed a negative effect (Djoussé, 2011; Kaushik, 2009). Meta-analyses on the associations between fish consumption, n-3 FAs and the development of T2D found heterogeneity of the overall summary

estimates based on geographical differences of the studies: an inverse association in population of Asian countries, no association in population of Western countries and a positive relation in US population (Muley et al., 2014; Wallin et al., 2012; Xun & He, 2012; Zheng et al., 2012). Differences in fish consumption patterns may partially explain the inconsistency between the findings. Environmental contaminants present in fish may also influence the association between fish intake and T2D (Lee et al., 2014; A. Wallin et al., 2015).

Fish is a potential source of environmental contaminants, such as persistent organic pollutants (POPs) (Seabert et al. 2014). POPs are toxic substances which persist in the environment, have long half-lives, and therefore bioaccumulate and biomagnify in living organisms such as fish, mammals, predatory birds, and humans (Hardell et al., 2010; Sobek et al., 2010; Vorkamp & Rigét, 2014). This is a concern especially among First Nation populations whose diets rely on locally harvested fish and other wild foods. A study conducted on Mohawk men and women showed that local fish consumption was a major pathway of POPs exposure (Fitzgerald et al., 1999; Fitzgerald et al., 2004). Recently, a number of studies found positive associations between diabetes and POPs such as dioxin-like chemicals, non-dioxin-like polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDE), and other organochlorine pesticides (Codru et al., 2007; Lee et al., 2010; Philibert, Schwartz, & Mergler, 2009; Silverstone et al., 2012a). Pal et al. (2013) found higher POP concentrations in plasma in diabetic compared to non-diabetic Canadian First Nation individuals (Pal et al. 2013). Thus, First Nation people might be exposed to elevated concentrations of POPs from fish consumption which can be risk factors for T2D (Sharp 2009b).

It is clear that there is a need to evaluate the risk-benefit associated with fish consumption with respect to POPs and n-3 FAs intake, and whether it is associated with T2D. The objectives of this study are: to describe fish consumption patterns among First Nation adults in four Ontario

ecozones; to estimate n-3 FAs and PCBs, and DDE intake; and to explore the associations between self-reported T2D and fish consumption, dietary n-3 FAs and POP intake among First Nations living on reserve in Ontario.

2 Methodology

2.1 Study population

Data from the First Nations Food Nutrition and Environment Study (FNFNES), a 10-year cross-sectional study (2008-2017) were used (Chan et al., 2014). The FNFNES survey was designed to assess diets, the exposure to contaminants, and food security status of First Nations people living on reserves, south of the 60th parallel across Canada. First Nations communities were sampled using a combined ecozone/cultural area framework to ensure that the diversity in ecozones and cultural areas were represented in the sampling strategy. Three stages sampling proceeded: primary sampling was carried out with random sampling of communities within each of eight Assembly of First Nation (AFN) regions of Canada; secondary sampling was conducted with a random sampling of 125 households within each selected community; and tertiary sampling when one randomly selected adult in each household who was self-identified as being a First Nations person living on reserve aged 19 and older was asked to participate in the study. Estimation weights were calculated in order to obtain representative estimates of the total population. Weighting was required to minimize nonresponse bias. The design weight was adjusted based on the assumption that the responding communities represent both responding and non-responding communities. The Bootstrap method was adopted for the estimation of the sampling error of the estimates produced for this study (Chan et al., 2014). The detailed information on the study design and methodology are publicly available online (www.fnfnes.ca). The current study analyzed data from eighteen First Nations communities across four Ontario ecozones: 1- Boreal Shield/ Subarctic, 2- Boreal Shield/Northeast, 3- Hudson Plains/Subarctic,

4- Mixed-wood Plains/ Northeast collected in the fall of 2011 and 2012 (Figure 1). In total, 1429 participants aged 19 years and over were recruited in this study (Chan et al., 2014). The overall participation rate was 79%. To avoid potential misclassification of gestational diabetes, pregnant and breastfeeding women who reported having diabetes were excluded from the analyses. The final sample included 1426 participants (893 women and 533 men).

2.2 Ethics

Individual participation in the project was voluntary and based on informed written consent after an oral and written explanation of each project component. This survey was conducted following the “Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans” and in particular Chapter 9 research involving the First Nations, Inuit and Métis Peoples of Canada. The study was approved by the Ethical Review Boards at the University of Northern British Columbia, the University of Ottawa, the Université de Montréal, and Health Canada.

2.3 Data collection

During the household interviews, the study participants were asked to complete a series of questionnaires that collect information on dietary patterns (a 24-hour recall and a Traditional Food Frequency Questionnaire (FFQ)), and social-demographic, health, and lifestyle data (SHL Questionnaire).

To collect the 24 h recall, the multi-pass technique with 3 stages was used as follows: the first step was to make a quick list of all foods consumed during the prior 24 hours; the second one was to do a detailed description of the consumed foods and beverages (brands, amounts, and amount eaten, etc.); and the third step was to review the recall with the participant to see if anything was missed.(Raper et al., 2004) Three-dimensional food and beverage models were used in order to estimate corresponding quantities of the intakes. The FFQ collected data

regarding consumption of locally-harvested traditional foods during the four seasons in the past year. The questionnaire was developed based on a comprehensive list of traditional foods that was representative of each participating community. In Ontario, the FFQ combined 150 traditional food items, including 30 fish species, 21 land mammals, 26 wild bird species, 22 wild berries, and 48 wild nuts, plants, tree foods, and mushrooms. In this study, only data on the frequency of locally-harvested fish consumption were included.

The SHL Questionnaire included information about age, gender, weight and height (measured or self-reported), physical activity level (sedentary, somewhat active, moderate, vigorous), dieting (to lose weight) on the previous day (yes/no), smoking status (yes/no), household size, source of income (wage, pension, social assistance), education (high school degree, vocational training certificate, Bachelor's degree), employment status (full time, part time, no job), and self-perceived health status (excellent, very good, good, fair, poor). All participants self-reported their level of physical activity based on provided descriptions.: a) I am usually sitting and do not walk around very much; b) I stand or walk around quite a lot, but I do not have to carry or lift things very often; c) I usually lift or carry light loads or I have to climb stairs or walk up hills often; d) I do heavy work or carry heavy loads. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). When available, both measured weights and heights were used in the BMI calculations. Otherwise, self-reported or a combination of self-reported and measured values were used, adjusting the BMI by the addition of the estimated bias value by gender. BMI categories were considered as follows: normal weight when BMI was $< 25 \text{ kg/m}^2$, overweight was categorized as a BMI of 25 kg/m^2 or higher but less than 30 kg/m^2 , and obesity was categorized as a BMI higher than or equal to 30 kg/m^2 .

2.4 Assessment of type 2 diabetes

Data on diabetes were collected through the SHL Questionnaire. The study participants were asked if they have ever been told by a health care provider that they have diabetes. In addition, information on the type of diabetes (type 1, 2) and the onset of diabetes (how many years ago) was collected. In this study, self-reported type 2 diabetes was coded as “yes” if a participant answered to be diagnosed with type 2 diabetes (Huerta et al., 2009; Schneider et al., 2012). All participants who reported having type 1 diabetes were categorized as being non-diabetic for purpose of these analyses. The validity of self-reported T2D estimates from FNFNES survey was analyzed by comparing our estimates with those reported by the Regional Health Survey (RHS) which is the only First Nations-governed national health survey in Canada (FN RHS, 2008). In First Nations in Ontario, the age-standardized prevalence of diabetes reported by the FNFNES was 24%, which was similar to the rate of 21.6% reported by RHS (Phase II, 2008/10) (Appendix (Table 9)). Both surveys found differences in diabetes prevalence between males and females with higher rates in females (Jackson et al., 2014).

2.5 Fish sampling for contaminant content

Fish samples were collected based on the list of commonly consumed fish species in the participating communities and are representative of fish species consumed by members in each community. All fish samples were collected during fall 2011 (September through November). Each fish sample was a composite of tissues from up to 5 different fish. The collected fish samples were analyzed for POPs including total polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE) at Maxxam (2011) and ALS Global (2012), in Burnaby, British Columbia.

All fish samples were homogenized to provide a homogeneous sample for subsequent digestion. If required, a moisture value was determined gravimetrically after drying a portion of the

blended sample at 105°C overnight. Six grams of tissue was homogenized in dichloromethane (DCM) and filtered through anhydrous sodium sulphate. The extract was evaporated to 6 mL and 5 mL was injected onto the Gel Permeation Chromatography (GPC) column where a fraction of the eluent was collected, concentrated, and solvent exchanged to acetone:hexane (1:1). Further clean-up was performed by eluting this extract through PSA columns. The final extract was concentrated, and solvent exchanged to isooctane. The analysis was performed for the DDE and PCBs using GC-MS in Selective Ion Monitoring (SIM) mode with an EI source. Spiked standards and blank samples were measured for QA/QC.

2.6 Estimation of fish, dietary POPs (DDE, PCBs), and long-chain omega-3 FAs intake

Daily fish intake (g/d) for each participant was estimated as follows: firstly, by summing up the number of days in the past four seasons when fish consumption was reported (total and by fish species). Then, mean portion size of fish (g) was estimated from dietary data generated by the 24h recalls for each gender and age group. Finally, the total number of days in the previous year when fish intake was reported was multiplied by mean portion size of fish (g) and divided by 360 days (in this study, a year included four seasons of 90 days each).

Total POPs (total PCBs, DDE) intake was calculated by multiplying the amount of PCBs and DDE (nanograms) in one gram of each fish species by the total amount of each fish species eaten per day (grams), totaling the amount of PCBs and DDE from all fish species consumed per day and dividing the obtained amount by body weight of each participant (ng/kg of body weight/day).

$[\Sigma (\text{Fish intake (grams/day)} \times \text{total PCBs (ng/ gram of fish)})/\text{body weight}]$.

$[\Sigma (\text{Fish intake (grams/day)} \times \text{DDE (ng/ gram of fish)})/\text{body weight}]$.

Community-specific data of POPs content in fish species were applied to calculate total PCBs and DDE intake for each participant. If the community-specific data were not available, ecozone-specific concentrations of POPs content in fish species were calculated and applied for the communities that were located within a particular ecozone. The validation of dietary assessments was performed through correlation analysis between mercury exposure from traditional food estimated using the FFQ and hair mercury levels measured in First Nations participants. Dietary intake of mercury was correlated with hair mercury (Pearson correlation coefficient=0.53).

The concentrations of long-chain n-3 FAs in the various fish species were estimated by using the Canadian Nutrient File (Health Canada, 2014). In this analysis, n-3FAs means long-chain omega-3 fatty acids i.e. combined eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish. The data are expressed as mg EPA+DHA/ gram of raw fish. Raw values were used since fish tissue EPA+DHA concentrations may vary according to cooking method. Also, using the raw values allows comparing our results with other studies. The total amount of n-3 FAs (EPA+DHA) consumed by each participant was calculated as follows:

$$[\Sigma (\text{Fish intake (grams/day)} \times \text{EPA+DHA (mg/ gram of fish)})]$$

Since walleye, lake whitefish, lake trout, and yellow perch were commonly consumed across four ON ecozones, the consumption of these fish species as well as n-3 FAs and POPs intake from these types of fish were also described.

2.7 Statistical analyses

Descriptive statistics include the calculation of proportions for categorical variables and means with standard deviation (SDs) for continuous variables. Geometric means (95%CI) were estimated for dietary DDE and PCBs intake. Medians (interquartile range) were calculated for skewed variables. Student t-tests, analysis of variance (ANOVA), and chi-square tests were used

to test if differences between groups are statistically significant. Sub-group stratified analysis by gender, age groups and ecozones were performed to describe the study population by diabetes status. Fish intake was categorized by frequency of consumption in four groups: no or <1/month, 1/month, 2-3/month, and ≥ 1 /week to examine dose-response relationship between fish and T2D. For this analysis, a portion of fish was considered to be 150 g. We chose 150 g since it represents two servings (of 75 grams each) of fish per week recommended by Canada's Food Guide – First Nations, Inuit, and Métis (Health Canada, 2007). Pearson correlation coefficients were investigated among all continuous predictors. Collinearity was observed between fish intake with n-3 FAs, PCBs and DDE.

Bivariate analyses (simple logistic regression models) were performed between an outcome (T2D) and each primary predictor of interest (fish intake (categorical), PCBs, DDE, n-3FAs (continuous), individually) as well as all potential confounders (age, gender, BMI, total energy intake, smoking, physical activity, household size (number of people per household), education. Multiple logistic regression models adjusted for potential covariates were developed in order to investigate the associations between total fish, dietary POPs (PCBs and DDE) and n-3 FAs (EPA+DHA) intake, individually and T2D. Independent variables that do not fit a normal distribution were normalized using the natural logarithmic function (DDE, PCBs, and n-3FAs (EPA+DHA)). POP concentrations below the limit of quantification were imputed with a half limit of detection (LOD) of PCBs and DDE to avoid errors in the analysis. The LOD of DDE is 0.0005 $\mu\text{g/g}$ and LOD of PCBs is 0.0003 $\mu\text{g/g}$ (Chan et al., 2012).

To investigate the relationship between the frequency of fish consumption (4 categories) and T2D, three models were developed. Control variables were selected based on well-established risk factors for T2D reported in the literature including age, sex, body mass index (BMI), smoking, physical activity, total energy intake, education, and household size. Covariates were

added to the models gradually to evaluate their relative contribution on the association between the predictors of interest and the outcome variable. Model 1 presents crude estimates; Model 2 was adjusted for age, gender, and BMI; Model 3 was additionally adjusted for physical activity, total energy intake, smoking, household size, and education. The following covariates were treated as continuous variables: age, BMI, energy intake, number of people per household, years of education. Gender, smoking and physical activity were categorical variables. Fat, saturated fat, carbohydrate, fruit and vegetable intake were also considered as covariates but were removed from the models since they did not change the measure of the association between T2D and fish consumption.

Overall and stratified by age group (<45y; ≥45y) logistic regression models were developed to analyze the relationship between log-transformed dietary DDE, PCBs, n-3 FAs intake and T2D. We chose to dichotomize by age below or above 45 since 45 years is median age of the study population.

The models were tested for interactions between predictors (x_1 , x_2) by including the product “ $x_1 \times x_2$ ” as an additional predictor in a model containing x_1 and x_2 :

$$\log(p(y)/(1-p(y))) = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_1 \times x_2.$$

Age, gender and BMI were examined as effect modifiers. The covariates did not modify associations of fish consumption, DDE, PCBs, and n-3 FAs and T2D.

Results with a p-value of less than 0.05 were considered statistically significant. All statistical analyses were performed using weighting variables in order to obtain representative estimates at the regional level. JMP version 11 and R software were used to conduct statistical analyses.

Sensitivity analyses were conducted to examine dietary characteristics and physical activity between responders who were recently diagnosed with T2D (≤5 years ago) and those who were

diagnosed with T2D for a longer period of time (>5 years) (Appendix 1: Table A1). Also, analyses of dietary and lifestyle behaviours were carried out between participants with and without T2D by dieting status (Appendix 2: Table A2-3).

3 Results

The study population consisted of 1426 participants (893 women and 533 men) with an average age of 46.4 ± 15 years ranging from 19 to 88 years old. Table 1 shows the characteristics of Ontario First Nations participants by diabetes status. The overall prevalence of self-reported T2D was 24.4% (327 cases out of 1426) and was slightly higher among women (24.6%) compared to men (23.5%). After standardization to the 2015 Canadian population, the prevalence of T2D was 25%. Participants with T2D were older, were more likely to be women, had a higher mean BMI, and were less likely to be physically active. In fact, a larger proportion of diabetes cases (76%) reported an inactive or sedentary lifestyle compared to 58% among participants without T2D. Mean age and BMI were comparable between males and females with and without T2D (data are not shown). The majority of the study participants were overweight and obese (94% with T2D and 85% without T2D). Smoking was highly prevalent among both First Nations men and women reaching 50% but was significantly lower among participants diagnosed with T2D (45.5% vs. 49.7%).

The mean number of people per household was similar between diabetic and non-diabetic participants. Regarding the education level, more participants with T2D didn't complete high school compared to those without T2D (44% vs 35%). Individuals with T2D were more likely to be dieting on the day prior to being interviewed compared to those without T2D (14.8% and 11.1%, respectively). Some differences in nutrient intakes between diabetic and non-diabetic cases were noted. In particular, participants with T2D reported lower saturated fat intake.

Mean fish consumption in the study population was 17 g/d (median 3.4g/d (min-max: 0 - 452g/d)) and was higher among individuals with T2D (mean 25.8g/d, median 4.2 g/d) than without T2D (mean 14.3g/d; median 3.1g/d). The mean DDE and PCBs intakes were significantly higher in diabetic individuals (0.08 ng/kg/d, 0.19 ng/kg/d) compared to non-diabetic (0.03 ng/kg/d, 0.05 ng/kg/d). Similarly, subjects with T2D had a higher mean intake of n-3 FAs (EPA+DHA) – 373.6mg/d vs. 216.4mg/d. Thus, along with fish consumption, average dietary DDE, PCBs and n-3 FAs intakes were higher in participants with T2D.

Table 2 presents the characteristics of the study population by two age groups (<45y vs ≥45y). The prevalence of T2D among older people was 34.5% compared to 13.7% in younger participants. Mean BMI was slightly higher whereas physical inactivity was statistically significantly lower in older participants compared to younger ones. Also, participants aged 45 years and over reported lower total energy and carbohydrates intake, but higher fruit and vegetables. Also, older individuals consumed two times more fish compared to younger participants.

In table 3, the characteristics of the study population by frequency of fish consumption is presented. Higher fish consumption was associated with older age, being male and higher prevalence of self-reported T2D. The BMI, smoking status, level of physical activity and total energy intake were comparable among participants in each of the four fish frequency consumption categories.

Table 4 presents the self-reported prevalence of T2D, fish consumption patterns, dietary n-3 FAs, and DDE, and PCBs intakes across four Ontario ecozones: Boreal Shield/ Subarctic, Boreal Shield/Northeast, Hudson Plains/Subarctic, and Mixed-wood Plains/ Northeast (Figure 1). The frequency of consumption of 30 different fish species was collected from the Ontario

participants. Among all fish species, walleye, lake whitefish, lake trout and yellow perch were commonly consumed contributing, about 70% to the total fish intake.

Overall, 75% of participants reported eating fish over the past year, ranging within ecozones from 54 to 88%. The highest consumption of total fish was reported by participants living in the Boreal Shield/Subarctic ecozone (mean, 31g/d; median, 15g/d) followed by the Hudson Plains (mean, 12.5g/d; median, 6.2g/d), the Boreal Shield/Northeast (mean, 12.2g/d; median, 5.9 g/d), and the Mixed-wood Plain/Northeast (mean, 5g/d; median, 3.5g/d) among fish consumers only. With respect to the commonly consumed fish species, the highest consumption of walleye and whitefish was reported in the Boreal Shield/Subarctic and the Hudson Plains/Subarctic ecozones whereas lake trout was mainly consumed by individuals living in the Boreal Shield/Northeast ecozone. In the Mixed-wood Plains/Northeast ecozone, participants reported eating predominantly walleye and yellow perch. Lake trout was the primary contributor to total POPs intake across all ecozones whereas whitefish and walleye were the main sources of n-3 FAs, especially in the Boreal Shield/Subarctic ecozone.

Logistic regression model (Table 5) presents the association between frequency of fish consumption and T2D. The crude model shows a significant positive association between consumption of one portion of fish per week and more (OR: 2.34 (95%CI: 1.27-4.26) compared to the reference group (no or <1/month). In model 2, the results were adjusted for the following confounders: age, gender, and BMI. Model 3 was additionally controlled for physical activity, total energy intake, smoking, household size, and years of education. We were unable to control the models for DDE, PCBs, and n-3 FAs intake because of collinearity between fish intake with POPs, and with n-3 FAs variables. Overall, fish consumption of ≥ 1 portion per week increased the odds of T2D by about 2.5 times compared to no or <1/mo fish consumption.

Table 6 shows the results from logistic regression models of log-transformed DDE, PCBs and n-3 FAs intakes with T2D (overall and by two age groups). A significant positive association was found between dietary DDE intake and T2D with OR = 1.09 (95% CI: 1.05-1.75) overall and in older participants (OR=1.24 (95% CI: 1.12-2.54)). Similarly, dietary PCB intakes were positively associated with T2D with statistically significant ORs in overall analysis (OR= 1.07 (95% CI: 1.004-1.27) and in individuals aged 45 and over in the model adjusted for n-3 FAs intake (OR= 1.13; 95% CI: 1.001-1.4). Dietary n-3 FAs intake was positively associated with T2D in the model adjusted for the risk factors only. After adjusting for dietary POPs exposure, n-3 FAs intake showed inverse, but not statistically significant effects in the total sample. However, among individuals aged 45 and over, n-3 FAs showed a protective effect against T2D (OR=0.86; 95% CI: 0.46-0.99) after adjusting for POPs intake. The dose-response relationship of dietary POPs and EPA+DHA with T2D presented in Appendix 5 Figures A1, A2, A3.

Average concentrations of n-3 FAs (EPA+DHA), PCBs and DDE in commonly consumed fish species samples by Ontario ecozones are presented in Table A5 (Appendix 4). Overall, the concentrations of both DDE and PCBs were low in walleye across all ecozones. The highest levels of PCBs and DDE were determined to be in lake trout in the Boreal Shield/ Subarctic and Hudson Plains/ Subarctic ecozones. Total PCBs and DDE were also higher in yellow perch in the Boreal Shield/Subarctic, Boreal Shield/Northeast and Hudson Plains/Subarctic than in other species. However, low consumption of this species was reported. In whitefish, the highest concentrations of total PCBs and DDE were found in the Boreal Shield/ Subarctic ecozone.

4 Discussion

The prevalence of T2D in Ontario First Nations living on reserve (24.4%) and was similar to that reported by the First Nation Regional Health Survey (RHS) in Ontario (21.6%) (FNIGC 2012).

The prevalence of self-reported diabetes in the general Canadian population was 6.8% (95% CI:

6.6–7.1%) among individuals aged 20 years and older (Public Health Agency of Canada 2011).

In our study, more women than men reported T2D, which is compatible with other studies among First Nations in Canada (Green et al., 2003; Oster et al., 2011; Riediger et al., 2014).

We found a positive association between dietary DDE and PCBs intakes and self-reported T2D in the whole sample and the sample stratified by age groups (>45y vs. \geq 45y). Dietary n-3 FAs intake (EPA+DHA) showed a protective effect against T2D among older participants after controlling for POPs intake. The consumption of one portion of fish and more per week was positively associated with T2D when compared to the reference group (no fish or <1/month). This association remained significant after adjusting for the confounding factors, although we were unable to control for DDE/PCBs or fatty acids in fish.

Our findings are consistent with previous cross-sectional studies on the relationship between exposure to POPs and T2D conducted in Indigenous communities and general populations (Codru et al., 2007; Lee et al., 2006; Lee et al., 2007; Philibert et al., 2009). These studies investigated serum POPs concentrations in relation to the prevalence of T2D whereas we assessed dietary POPs intake from fish. However, since locally-harvested fish among Indigenous people living on reserve is considered as the main source of exposure to contaminants, dietary intake of POPs might be a good indicator of the exposure. There is also evidence in the literature that frequency of wild food consumption and serum POPs levels in First Nations communities was positively correlated (Seabert et al. 2014). Similar positive correlations between fish consumption and serum POPs levels were also reported by other studies (Duarte-Davidson 1994; Philibert et al., 2009; Turyk et al., 2009).

Our results showed differences in the associations of POPs, n-3 FAs with T2D between younger (>45y) and older participants (\geq 45y). This may be explained by low fish, and consequently, low dietary n-3 FAs and POP intake among individuals aged <45y that resulted in the weak

associations. Since older participants reported higher consumption of fish, the adverse effect of POPs and protective effect of n-3 FAs were more prominent in this age group. Also, the differences could be related to increased risk factors for T2D in older individuals. Turyk et al. reported stronger positive associations of POPs with blood glucose in persons with higher levels of diabetes risk factors (Turyk et al. 2015).

In general, the association between a single chemical and a health outcome is difficult to interpret because of co-exposure to other chemicals that might have similar or opposite effects (Ruzzin 2012) In our study, adjustment for multiple exposures was impossible because of strong correlations between DDE and PCBs due to the same exposure route. In addition, unmeasured contaminants from other sources might also confound the associations between T2D and POPs. Previous studies investigating fish and n-3 FAs consumption in relation to T2D reported conflicting results. A positive association between total and lean fish intake and risk of T2D was reported by van Woudenberg et al. (2009) in a prospective cohort study of participants aged 55 y and older. Similar findings were concluded by Kaushik and Mozaffarian (2009b) in 3 prospective cohorts of adults followed for 14-18y. However, an inverse association between total (white, and oily fish) and shellfish and T2D was found in population-based cohort studies by Nanri et al. (2011b) and Villegas et al. (2011). A protective effect of n-3 FAs (EPA+DHA) against T2D was reported by several studies (Brostow et al. 2011; Paquet et al. 2014; Virtanen et al. 2014). Wallin et al. (2015) found no association between total fish consumption and T2D. However, additional adjustment for PCBs exposure resulted in lower point estimates for fish, but associations remained statistically non-significant (Wallin et al., 2015). Thus, the net effect of fish consumption on T2D may depend on the POPs content in fish.

In our study, a positive relationship between frequency of fish consumption and T2D might be due to the counteraction between the adverse effect of POPs and protective effect of n-3 FAs that

co-exist in fish. In fact, the detrimental effect of POPs usually starts to appear at a lower fish consumption level whereas beneficial effects due to n-3 FAs are often more prominent at a higher fish consumption level (Lee et al. 2014). Since the study participants reported low consumption of fish (only 20% eat \geq one portion (150g) of fish per week that corresponds to Canada's food guide recommendations to eat at least two servings (of 75 grams each) of fish a week), the overall protective effect of n-3 FAs might not be sufficient to outweigh the detrimental effects of POPs.

The fish consumption patterns differed among First Nations depending on the variety and availability of species in the Ontario regions. For example, 80 to 90% of participants reported eating fish at least once in the prior year in the Boreal Shield and the Hudson Plains/ Subarctic ecozones, whereas only half of participants living in the Mixed-wood Plain/ Northeast ecozone did. The highest average intake of total fish was determined to be in the Boreal Shield/Subarctic at 15g/day compared to only about 6g/d in the Boreal Shield/ Northeast and the Hudson Plains/ Subarctic. Walleye was the most commonly eaten fish in Ontario followed by lake whitefish and lake trout. Yellow perch was consumed only by participants in the Boreal Shield/ Northeast (16%) and the Mixed-wood Plain/ Northeast (23%) ecozones. They reported eating yellow perch about 1.7 and 2.4g/d of, respectively.

There were differences in POPs levels in fish species (Table 7). Lake trout had the highest POP concentrations across four regions compared to other species. Walleye, in contrast, had the lowest POPs levels in all regions. Elevated levels of POPs in lake trout were also documented by McGoldrick et al., who reported that concentrations of several POPs continue to dominate the chemical burden of Great Lakes fish (McGoldrick and Murphy 2015). The concentrations of POPs in lake whitefish were low across all Ontario ecozones except for PCBs in Boreal

Shield/Subarctic. With regards to n-3 FAs content, lake trout and whitefish have higher levels of EPA+DHA compared to walleye and yellow perch (Health Canada, 2014).

This study has several limitations. First, a cross-sectional design does not allow the conclusion of the temporality between POPs and T2D. Second, the self-reported prevalence of T2D could have resulted in underdiagnoses since some participants might not be aware of having T2D. To validate our self-reported estimates of diabetes, we compared them with prevalence rates for self-reported diabetes reported by the Regional Health Survey (RHS) which is a representative study for First Nations living on reserve conducted over the similar period of time. The RHS collected more comprehensive information on T2D including kind of treatment used to control diabetes, frequency of checking blood sugar levels, complications of diabetes, whether adopting a healthier lifestyle including (diet and exercise), attendance of a diabetes clinic and getting diabetes education (Table S4). A study among Cree First Nations living in Northern Quebec reported that 4.5% of participants had undiagnosed diabetes based on glucose levels that indicates diabetes (≥ 7 mmol/L), but no mention of diabetes in their medical charts (Cree Board of Health and Social Services of James Bay 2013). Finally, n-3 FAs concentrations in fish species were estimated using the Canadian Nutrient File (Health Canada, 2014). Since there is a considerable variation on reported n-3 FAs in fish species in the literature (Cladis et al., 2014; Pantazopoulos et al., 2013), the potential for error in the estimation of n-3 FAs intake from fish may occur.

In order to examine if participants diagnosed with T2D tend to change their diets, we assessed and compared their dietary and lifestyle behaviour with individuals without T2D. Thus, two sensitivity analyses were conducted. First, dietary intake and lifestyle practices were compared between participants recently diagnosed with T2D (0-5y) and those who have T2D for a long

period of time (>5y) (Table S1). The analysis shows no statistically significant differences in physical activity, macronutrient intakes, and fish consumption between two groups.

The second sensitivity analysis was performed to capture differences in dietary intake and lifestyle behaviour in participants with and without T2D associated with self-reported dieting status (Table S2-3). Overall, dieting individuals had higher mean BMI; they reported a lower smoking rate, lower total energy and carbohydrate intake, and higher fruit and vegetable consumption than non-dieting subjects. Fish and n-3 FAs consumption were comparable between two groups. However, these differences were not statistically significant in further comparison of dietary intakes in dieting and non-dieting participants with and without T2D, individually (Table S4).

This is the first study investigating the relationship of fish consumption, dietary intake of n-3 FAs and POPs, and T2D in a representative sample of First Nations living on reserve across four Ontario ecozones. Our results were adjusted for the main risk factors for T2D such as age, gender, BMI, smoking, physical activity, energy intake, and education.

Most of the other studies reported a correlation between fish consumption and serum POPs levels (Duarte-Davidson 1994; Philibert et al., 2009; Turyk et al., 2009). We found the same relationship between dietary exposure to POPs and T2D. Dietary exposure information is more useful to develop an advisory to limit exposure by changing fish intake pattern.

5 Conclusion

We found positive cross-sectional associations of fish and dietary POPs intake with T2D and a negative association of n-3 FAs with T2D in older individuals. Fish consumption of ≥ 1 portion per week was associated with increased odds of T2D by about 2.5 times compared to no fish consumption. DDE and PCBs dietary intake increased the risk of T2D in both younger and older

participants. Long chain n-3 FAs intake showed a protective effect against T2D in older participants only. Walleye, whitefish, lake trout and yellow perch were commonly consumed fish species across four Ontario ecozones. Lake trout contained the highest levels of POPs whereas lake whitefish was the main contributor of the n-3 FAs (EPA and DHA). Our results support previous findings that exposure to DDE and PCBs may increase the risk of T2D. Elevated levels of contaminants in fish may counteract with potentially beneficial effects of n-3 FAs and fish consumption, and it is important to monitor the levels of POPs in the locally harvested fish. However, the overall health benefits of high consumption of fish rich in n-3 FAs may outweigh the adverse effect of contaminants.

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Figure 1. Map of participating First Nations communities and four ecozones in Ontario (Chan et al., 2014).



Table 1. Characteristics of Ontario First Nations participants by diabetic status (n=1426)

	with T2D	without T2D	p value
N, (%)	327 (24)	1099 (76)	
Women, n (%)	217 (63)	676 (64)	0.1
Men, n (%)	110 (37)	423 (36)	
Age	55.74 (53.33,58.15)	42.96 (41.25,44.68)	.0001*
Age groups, n (%)			
19-30	8 (1.4)	255 (24.3)	.0001*
31-50	119 (36.5)	491 (45.6)	
51-70	155 (45.9)	282 (24.9)	
71+	45 (16.2)	71 (5.2)	
BMI, kg/m ²	33.23 (32.34,34.12)	30.42 (29.80,31.05)	.0001*
BMI category, n (%)			
<25	30 (6.1)	202 (15.0)	.0001*
25-29.99	93 (25.7)	344 (34.5)	
≥30	204 (68.2)	553 (50.5)	
Physical activity, n (%)			
inactive	97 (26.3)	227 (17.8)	0.002*
sedentary	146 (50.1)	455 (40.4)	
moderate	59 (16.0)	284 (29.0)	
vigorous	25 (7.5)	130 (12.8)	
Smoking, n (%)	147 (45.5)	576 (49.7)	0.018*
Dieting, n (%)	48 (14.8)	103 (11.1)	0.006*
Household size	4.18 (3.39,4.96)	4.16 (3.87,4.44)	0.965
Years of education	11.23 (10.36,12.10)	11.87 (11.31,12.44)	0.091
Diploma, n (%)			
Less than high school	159 (44.2)	458 (34.9)	0.003*
High school	117 (42.5)	509 (51.9)	
Post-secondary	50 (13.3)	128 (13.2)	
Employment, any (%)	196 (68.1)	753 (77.5)	0.016*
Dietary characteristics			
Energy intake (kcal/d)	1927 (1763,2091)	2015 (1918,2113)	0.442
Fruit Vegetables (g/d)	22.84 (16.74,31.17)	15.89 (13.34,18.92)	0.421
Total Fat (g/d)	79.11 (72.21,86.01)	80.39 (77.77,83.02)	0.743
Saturated Fat (g/d)	23.73 (21.68,25.77)	26.22 (25.19,27.25)	0.023*
Carbohydrate (g/d)	215.09 (202.03,228.14)	240.89 (220.30,261.49)	0.078
Protein (g/d)	92.43 (77.47,107.38)	84.56 (80.25,88.86)	0.298
Total Fish intake (g/d)	25.75 (13.11,38.40)	14.27 (4.10,24.43)	0.183
DDE (ng/kg/d)	0.08 (0.04,0.16)	0.03 (0.01,0.05)	0.002*
PCBs (ng/kg/d)	0.19 (0.07,0.47)	0.05 (0.03,0.10)	0.025*
EPA+DHA (mg/day)	373.6 (164.8,582.4)	216.4 (69.7,363.1)	0.36

T2D – type 2 diabetes; DDE - dichlorodiphenyldichloroethylene; PCBs - polychlorinated biphenyls; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; values are mean (95%CI); DDE/PCBs – geometric mean; p-values correspond to t-tests for continuous variables and chi-square tests for categorical variables; BMI- body mass index; weighted estimates

Table 2. Characteristics of Ontario participants by age groups (<45y/≥45y)

	<45	≥45	p value
n	709	717	
T2D (%)	13.7	34.5	.0001*
Body mass index, kg/m ²	30.71 (29.97,31.46)	31.48 (30.81,32.15)	0.148
Smoking (%)	60.8	36.7	0.0001*
Physical activity (%)			0.0002*
inactive	16.1	23.5	
sedentary	40.6	44.9	
moderate	28.4	23.4	
vigorous	14.9	8.2	
Dietary characteristics			
Total energy(kcal/d)	2093 (1976,2209)	1897 (1803,1990)	0.016*
Fruit/vegetables (g/d)	11.69 (9.39,14.57)	25.38 (20.62, 31.24)	0.035*
Fat (g/d)	83.68 (79.33,88.03)	76.54 (71.38,81.70)	0.087
Protein (g/d)	84.95 (80.65,89.26)	87.94 (78.71,97.18)	0.522
Carbohydrate (g/d)	252.66 (232.34,272.98)	216.89 (203.86,229.92)	0.0001*
Fish intake (g/d)	10.5 (5.78,15.23)	23.5 (10.43,36.57)	0.012*
DDE (n/kg/d)	0.02 (0.01,0.04)	0.06 (0.03,0.13)	0.0001*
PCBs (n/kg/d)	0.04 (0.02,0.08)	0.13 (0.06,0.28)	0.0001*
EPA+DHA (g/d)	160.1 (91.7,228.4)	347.4 (163.9,531.0)	0.019

T2D - type 2 diabetes, DDE-dichlorodiphenyldichloroethylene; PCBs-polychlorinated biphenyls;

EPA- eicosapentaenoic acid; DHA-docosahexaenoic acid;

Values are mean (95%CI);DDE/PCBs -geometric mean,

p-values correspond to t-tests for continuous variables and chi-square tests for categorical variables; weighted estimates

Table 3. Characteristic of Ontario participants by frequency of fish consumption

	0 or <1/mo	1/mo	2-3/mo	≥ 1/week	p trend
n	508	278	338	302	
Type 2 diabetes, %	20.2	17.3	22.8	37.3	0.02
Age, y	44.1 (42.2,45.9)	43.7 (39.4,48.0)	47.4 (45.0,49.7)	49.9 (47.3,52.6)	.0001
Women, %	72.7	68.9	60.2	45.5	.0001
Men, %	27.3	31.1	39.8	54.5	.0001
Body mass index, kg/m ²	31.5 (30.2,32.8)	30.5 (29.6,31.4)	31.1 (29.7,32.5)	30.9 (29.7,32.1)	0.3
Smoking, %	47.8	50.2	47.3	50.4	0.16
Physical activity, %					
sedentary	65.5	57.2	65.8	58.5	0.05
moderate	25.8	31	19.2	28.8	
vigorous	8.7	11.8	15	12.7	
Total energy(kcal/d)	1917 (1757,2077)	2061(1939,2183)	2071 (1937,2205)	1999 (1768,2229)	0.35
Fruit/vegetables (g/d)	16.6 (11.8,23.4)	21.9 (14.5,33.1)	20.0 (14.0,28.5)	16.9 (6.8,42.0)	0.69
Fat (g/d)	76.7 (71.0,82.4)	85.9 (79.4,92.5)	83.9 (79.1,88.7)	77.6 (68.8,86.3)	0.07
Protein (g/d)	78.3 (72.0,84.4)	86.5 (80.9,92.2)	90.1 (81.8,98.4)	96.6 (82.4,110.8)	0.002
Carbohydrate (g/d)	230 (202,258)	240 (220,260)	241.9 (218,266)	231 (206,256)	0.8
Fish intake (g/d)	0.24 (0.19,0.28)	2.82 (2.64,2.99)	9.07 (8.62,9.51)	64.05 (47.2,80.9)	.0001
DDE (n/kg/d)	0	0.08 (0.05,0.13)	0.26 (0.16,0.44)	1.53 (0.84,2.81)	.0001
PCBs (n/kg/d)	0	0.30 (0.15,0.62)	0.83 (0.41,1.68)	4.8 (2.28,10.10)	.0001
EPA+DHA (mg/d)	3.8 (2.9,4.8)	44.7 (35.8,53.6)	141 (126,155)	948 (707,1189)	.0001

DDE - dichlorodiphenyldichloroethylene;

PCBs - polychlorinated biphenyls; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid;

Values are mean (95%CI); DDE/PCBs -geometric mean;

p-values correspond to ANOVA for continuous variables and chi-square tests for categorical variables;

sedentary physical activity combines inactive and sedentary lifestyle,

weighted estimates

Table 4. Fish consumption patterns and dietary characteristics by Ontario ecozones

	Boreal Shield/ Subarctic	Boreal Shield/ Northeast	Hudson Plains/ Subarctic	Mixed-wood Plain/ Northeast	p trend
n	356	344	266	460	
T2D (%)	23.5	24.8	28.7	24.2	0.46
Age, y	42.6±15.3	47.1±14.9	44.5±15.6	50.0±15.8	.0001
Body mass index, kg/m ²	29.7±5.3	31.3±5.5	30.5±5.5	31.6±6.4	.0001
Women (%)	54	65	65	66	.0024
Energy intake (kcal/d)	1888 (1000-3163)	1826 (958-3357)	1991 (1050-3617)	1780 (927-3150)	.0052
Smoking (%)	55.3	62.9	51.8	32.8	.0001
Fish consumers %	89	84	80	54	
Total fish g/d	15.0 (0.6-228)	5.9 (0.5-106.6)	6.2 (0.5-91.5)	3.5 (0.5-60.2)	.0001
DDE ng/kg/d	0.31 (0.004-9.2)	0.21 (0.007-10.0)	0.16 (0.003-12.03)	0.14 (0.003-10.16)	0.05
PCBs ng/kg/d	1.38 (0.00931.6)	0.32 (0.005-19.6)	0.56 (0.007-34.01)	1.01 (0.001-39.99)	.0020
EPA+DHA g/d	0.25 (0.01-2.9)	0.07 (0.003-1.29)	0.09 (0.004-1.28)	0.04 (0.002-0.79)	.0001
Walleye consumers %	80	56	49	35	
Walleye g/d	7.5 (0.5-100.6)	2.2 (0.5-26.7)	3.3 (0.5-43.1)	2.1 (0.5-26.9)	.0001
DDE ng/kg/d	0.06 (0.003-1.02)	0.08 (0.01-1.22)	0.02 (0.002-0.75)	0.09 (0.007-2.69)	0.1
PCBs ng/kg/d	0.09 (0.002-7.3)	0.13 (0.005-2.19)	0.05 (0.003-6.48)	0.78 (0.05-19.93)	0.023
EPA+DHA g/d	0.18 (0.01-2.33)	0.05 (0.01-0.62)	0.08 (0.01-1.00)	0.05 (0.01-0.62)	.0001
Whitefish consumers %	38	46	15	3	
Lake Whitefish g/d	6.7 (0.5-95.8)	2.03 (0.5-55.7)	3.4 (0.5-43.9)	1.4 (0.5-4.0)	.0001
DDE ng/kg/d	0.14 (0.008-1.90)	0.12 (0.004-3.62)	0.10 (0.01-0.88)	0.07 (0.03-0.31)	0.1
PCBs ng/kg/d	0.13 (0.006-1.91)	0.24 (0.002-9.7)	0.14 (0.02-1.53)	0.17 (0.07-0.78)	0.09
EPA+DHA g/d	0.11 (0.01-1.63)	0.03(0.01-0.94)	0.05 (0.01-0.74)	0.02 (0.01-0.07)	.0001
Lake Trout consumers %	17	32	9	2	
Lake Trout g/d	3.3 (0.5-72.2)	2.1 (0.5-55.2)	2.5 (0.5-61.7)	2.6 (0.5-27.1)	0.2
DDE ng/kg/d	0.49 (0.02-10.08)	0.60 (0.05-4.97)	1.03 (0.14-19.19)	0.89 (0.1-8.35)	.0171
PCBs ng/kg/d	0.51 (0.03-8.42)	0.90 (0.09-9.45)	2.50 (0.35-46.71)	2.12 (0.26-19.96)	.0001
EPA+DHA g/d	0.04 (0.01-0.75)	0.02 (0.01-0.58)	0.03 (0.01-0.64)	0.03 (0.01-0.28)	0.26
Yellow perch consumers %	3	16	1	23	
Yellow perch g/d	1.9 (0.8-9.6)	1.7 (0.5-26.8)	1.1 (0.5-10.1)	2.36 (0.5-34.9)	0.21
DDE ng/kg/d	0.01 (0.002-0.03)	0.08 (0.02-1.57)	0.13 (0.04-1.2)	0.04 (0.01-0.67)	.0001
PCBs ng/kg/d	0.35 (0.13-1.3)	0.12 (0.03-2.47)	1.87 (0.61-16.95)	0.26 (0.05-4.67)	.0001
EPA+DHA g/d	0.01 (0.002-0.03)	0.004 (0.001-0.07)	0.004 (0.001-0.02)	0.01 (0.001-0.09)	0.21

DDE - Dichlorodiphenyldichloroethylene; PCBs - Polychlorinated biphenyls; EPA - eicosapentaenoic acid; DHA - docosahexaenoic acid; data are %, mean ± SD or median (2.5-97.5th percentiles); Fish, DDE, PCBs and EPA+DHA intakes estimated for fish consumers only; ng/kg/d - nanograms per kg body weight per day; p-values correspond to ANOVA for continuous variables and chi-square tests for categorical variables; weighted estimates of T2D

Table 5. Multiple logistic regression analyses of fish consumption and type 2 diabetes

	no or <1/mo	1/mo	2-3/mo	≥1/week	p trend
Model 1	1 (ref)	0.82 (0.49-1.37)	1.16 (0.74-1.79)	2.34 (1.27-4.26)**	0.005
Model 2	1 (ref)	0.92 (0.56-1.48)	1.08 (0.73-1.62)	2.31 (1.32-4.02)**	0.006
Model 3	1 (ref)	0.99 (0.62-1.59)	1.14 (0.73-1.76)	2.50 (1.38-4.58)**	0.008
n	508	278	338	302	

Values are ORs (95%CI); Model 1: crude estimates; Model 2: adjusted for age, gender and BMI; Model 3 - additionally adjusted for physical activity, total energy intake, smoking, household size, and education; ** - p value <0.01; portion size is 150grams of fish

Table 6. Logistic regression analyses of log-transformed DDE, PCBs and long-chain n-3 fatty acids intake and type 2 diabetes in the total sample and stratified by age groups

	DDE	PCBs	EPA+DHA
Total population (n=1426)			
Model 1	1.07* (1.03-1.29)	1.05* (1.003-1.24)	1.12 (0.99-1.20)
Model 2	1.09* (1.05-1.75)	1.07* (1.004-1.27)	0.80 (0.58-1.00)
<45 y (n=709)			
Model 1	1.14 (0.99-1.50)	1.11 (0.99-1.33)	1.14 (0.95-1.35)
Model 2	1.16 (0.78-1.74)	1.12 (0.87-1.26)	0.95 (0.65-1.14)
≥45 y (n=717)			
Model 1	1.15* (1.002-1.3)	1.10 (0.99-1.39)	1.10 (0.93-1.26)
Model 2	1.24* (1.12-2.54)	1.13* (1.001-1.4)	0.86* (0.46-0.99)

Values are OR (95%CI); Model 1 was adjusted for age, BMI, gender, physical activity, smoking, energy intake, household size and education; Model 2: DDE and PCBs were additionally adjusted for EPA+DHA intake; EPA+DHA was adjusted for DDE/PCBs; ORs: per unit change in regression (log transformed variables); * - p value < 0.05

Supplemental material 1

The goal of this analysis was to examine differences in dietary intake of participants recently diagnosed with T2D and those diagnosed with T2D for a long period of time. Using data on the onset of T2D, participants were divided into two groups: 1) those who were diagnosed with T2D until 5 years ago ($\leq 5y$) and those diagnosed with T2D for more than 5 years ago ($> 5y$). Then, dietary and lifestyle behaviours were compared between these groups. The results presented in Table S1 show that there were no statistically significant differences in dietary characteristics between individuals recently diagnosed with T2D and those having T2D for more than 5 years.

Table S1. Dietary and lifestyle characteristics by onset of type 2 diabetes (n=327)

	Onset of type 2 Diabetes		p trend
	≤ 5 years	> 5 years	
n	117	220	
Age	51.2 \pm 12.9	57.0 \pm 13.1	0.0001*
Body Mass Index, kg/m ²	33.1 \pm 5.6	32.6 \pm 6.6	0.6 12
Dieting, %	15	15	0.50
Smoking, %	51.8	44.2	0.05
Physical activity, %			0.061
Inactive	27.4	30.6	
Sedentary	41.1	45.5	
Moderate	23.1	16.7	
Vigorous	8.4	7.2	
Dietary characteristics			
Energy intake (kcal/d)	1949 (1323-2483)	1831 (1240-2176)	0.051
Total Fat (g/d)	71 (51-106)	66 (46-96)	0.061
Saturated fat (g/d)	21 (14-32)	20 (13-29)	0.052
CHO (g/d)	216 (151-270)	190 (135-253)	0.071
Protein (g/d)	77 (57-107)	72(53-104)	0.312
Fruit/Vegetables (g/d)	70 (0-176)	71 (0-213)	0.825
Fish (g/d)	5.0 (1.1-20.0)	4.2 (0-19.8)	0.513

≤ 5 years- diagnosed with type 2 diabetes less than 5 y ago; >5 -diagnosed with T2D more than 5 years ago;
Values are mean \pm SD or median (25-75th) percentile

Supplemental material 2

Using data on self-reported dieting status (yes/no) on the previous day of an interview, a sensitivity analysis was conducted to examine whether dietary intake and lifestyle practices differed between dieting and not dieting participants (Table S2). Overall, about 12% of participants reported limiting their caloric intake on the previous day in order to lose weight. The prevalence of T2D was higher among dieting participants than non-dieting (29.8% vs 23.4%). Women tend to diet more often compared to men. Mean BMI was significantly higher among dieting responders compared to not-dieting ones (34 vs. 30.7). Also, dieting individuals reported lower smoking rate, lower total energy and carbohydrate intake, and higher fruit and vegetable consumption than non-dieting subjects. Fish and n-3 FAs consumption were similar between dieting and non-dieting participants. Also, physical activity and fat, saturated fat and protein intake were comparable.

Further analyses compared dietary intake between dieting and non-dieting individuals with and without T2D. Overall, no statistically significant differences in macronutrients intake between dieting participants with and without T2D, and non-dieting individuals with and without T2D were found. Participants with T2D (both dieting and non-dieting) tended to be less physically active compared to those without T2D. Overall, intakes of macronutrients were comparable between groups with the exception of higher fruit and vegetable consumption reported by dieting subjects without T2D (Table S3).

Table S2: Dietary characteristics of participants by dieting status (n=1426)

	Dieting		p trend
	Yes	No	
Total, %	12	88	
Type 2 diabetes, %	29.8	23.4	0.020*
Female, %	66.2	62.7	0.224
Age	47.86 (44.57,51.15)	45.81 (44.58,47.04)	0.201
Body Mass Index, kg/m ²	34.01 (32.51,35.51)	30.7 (30.20,31.21)	0.0001*
Physical activity, %			0.212
inactive	23.1	19.4	
sedentary	41.1	42.9	
moderate	27.7	25.6	
vigorous	8.1	11.9	
Smoking, %	33.2	50.8	0.030*
Dietary characteristics			
Energy intake(kcal/d)	1865.5 (1609.9,2121.1)	2012.1 (1933.8,2090.4)	0.052*
Fruit & Vegetable (g/d)	33.1 (20.8,52.5)	16.0 (13.6, 18.7)	0.002*
Total Fat (g/d)	74.34 (63.89,84.79)	80.87 (78.22,83.51)	0.172
Saturated fat (g/d)	23.78 (20.41,27.14)	25.87 (24.79,26.95)	0.232
Carbohydrate (g/d)	221.83 (184.40,259.26)	236.4 (218.88,253.91)	0.033*
Protein (g/d)	81.69 (72.76,90.61)	87.11 (81.47,92.75)	0.671
Fish intake (g/d)	21.86 (5.60,38.12)	16.39 (8.69,24.09)	0.254
DDE (ng/kg/d)	0.04 (0.01,0.12)	0.04 (0.02,0.06)	0.163
PCBs (ng/kg/d)	0.08 (0.02,0.28)	0.07 (0.04,0.14)	0.141
EPA+DHA (mg/d)	0.17 (0.04,0.30)	0.11 (0.05,0.17)	0.721

DDE - dichlorodiphenyldichloroethylene, PCBs - polychlorinated biphenyls, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid, values are mean (95%CI), DDE/PCBs -geometric means, weighted estimates

Table S3: Dietary characteristics of Ontario participants by dieting status (n=1426)

	Dieting			Not dieting		
	T2D+	T2D-	p trend	T2D+	T2D-	p trend
Total n, (%)	48	103		279	996	
Female (%)	65.5	66.4	0.851	64	62.3	0.785
Age	56.1 (51.8, 60.2)	44.4 (40.5,60.3)	.0001*	55.6 (53.3,58.1)	42.8 (40.1,44.7)	.0001*
Body Mass Index	36.2 (33.6,38.8)	33.1 (30.9,35.2)	.0001*	32.8 (31.7, 33.6)	30.1 (29.4, 30.8)	.0001*
Physical activity, %			.0269*			.0017*
inactive	31.3	21.4		29.4	20.6	
sedentary	45.8	32.1		44.4	42.7	
moderate	14.6	36.9		18.6	24.7	
vigorous	8.3	9.7		7.5	12.1	
Smoking (%)	40.6	30.1	0.366	46.2	52.1	0.175
Dietary characteristics						
Energy intake(kcal/d)	1558.2 (1328,1788.0)	1996.2 (1669.6,2322.7)	0.077	1991.9 (1822.5,2161.3)	2018.3 (1909.4,2127.3)	0.824
Fruit/Vegetable (g/d)	28.8 (12.6, 66.3)	35.2 (20.0, 62.2)	0.318	21.9 (15.6, 30.7)	14.6 (12.1, 17.6)	0.052
Total Fat (g/d)	60.3 (50.4,70.6)	80.3 (67.2, 93.4)	0.011	82.4 (75.6,89.1)	80.4 (77.6,83.2)	0.604
Saturated fat (g/d)	20.5 (16.1,24.0)	25.5 (21.3,29.8)	0.187	24.4 (22.3, 26.5)	26.2 (25.1,27.5)	0.114
Carbohydrate (g/d)	187.7 (160.3,215.1)	236.3 (186.1,286.6)	0.432	219.8 (205.4,234.3)	241.5 (218.1,264.8)	0.167
Protein (g/d)	70.8 (55.1,86.6)	86.3 (76.6,96.0)	0.156	89.2 (78.2,108.1)	84.3 (79.7, 88.9)	0.713
Fish intake (g/d)	23.5 (8.2,38.8)	21.1 (0.8,41.5)	0.279	26.1 (11.5,40.6)	13.4 (4.3, 22.5)	0.182
DDE (ng/kg/d)	0.08 (0.03, 0.25)	0.04 (0.02, 0.08)	0.093	0.05 (0.04, 0.08)	0.03 (0.02,0.04)	0.449
PCBs (ng/kg/d)	0.15 (0.05, 0.66)	0.08 (0.04, 0.19)	0.039	0.10 (0.06, 0.16)	0.07 (0.05, 0.08)	0.601
EPA+DHA (mg/d)	320.4(146.8, 493.9)	295.4(140.2,592.2)	0.241	382.8(134.5,631.1)	206.5(73.1, 339.8)	0.053

DDE - dichlorodiphenyldichloroethylene, PCBs - polychlorinated biphenyls, EPA - eicosapentaenoic acid, DHA - docosahexaenoic acid, values are mean (95%CI), DDE/PCBs -geometric mean; weighted estimates

Supplemental material 2: Validity of self-reported diabetes data from FNFNES

Since the estimates of diabetes prevalence are based on self-reports, there is a potential for under-reporting as some people may not be aware of having the disease. The validity of self-reported diabetes from FNFNES survey was analyzed by comparison of their estimates on the prevalence of self-reported diabetes with those reported by FN RHS (Phase II, 2008/10) which is the only First Nations-governed national health survey in Canada. RHS collected detailed data on the health and well-being of First Nations adults (aged 18 years and older). The survey sample represented the First Nations population living in First Nations communities in all provinces and territories. In phase II (2008/10) of RHS, 216 communities were included in the study. The communities were randomly selected within each First Nations “sub-region” to provide a representative sample at the regional and national levels. Individual responses were weighted, to represent a proportion of the age group and region.

In total, seven First Nations communities were surveyed by both RHS and FNFNES studies. In the RHS survey, the following information on diabetes was collected: the type of diabetes, kind of treatment used to control diabetes, frequency of checking blood sugar levels, complications of diabetes, whether adopting a healthier lifestyle including (diet and exercise), attendance of a diabetes clinic and getting diabetes education. We compared the estimates of the prevalence of diabetes in Ontario First Nations reported by RHS and FNFNES. The results are presented in Table S4. The FNFNES survey reported similar age-standardized prevalence of diabetes in Ontario First Nations (overall and by gender) compared to the RHS estimates (21.6 vs. 24%). Both surveys reported differences in diabetes prevalence between male and females with higher rates in females.

Table S4. Prevalence of diabetes reported by FN RHS and FNFNES

Prevalence (%)	Ontario	
	RHS (2008/10)	FNFNES (2011/12)
	(18y +)	(19y +)
Total	21.6	24.4
Women	23.6	24.6
Men	19.7	23.5

Supplemental material 4

Table S5. Concentration of n-3 FAs and POPs in the most consumed fish species in Ontario ecozones

Fish species	Ecozones								
	Canada	Boreal Shield / Subarctic		Boreal Shield/ Northeast		Hudson Plains/ Subarctic		Mixed-wood Plain/ Northeast	
	EPA+DHA g/100g	DDE ng/g	PCBs ng/g	DDE ng/g	PCBs ng/g	DDE ng/g	PCBs ng/g	DDE ng/g	PCBs ng/g
Walleye	0.31 (0.05)	1.56 (2.22)	9.04 (10.38)	3.67 (3.26)	6.69 (7.44)	1.23 (2.06)	9.9 (18.48)	4.86 (5.30)	36.23 (27.70)
Lake whitefish	1.24 (0.56)	6.90 (9.95)	22.72 (35.52)	6.38 (5.46)	9.97 (7.49)	2.38 (1.82)	3.31 (1.76)	5.89 (7.21)	14.55 (24.27)
Lake trout	0.84 (0.14)	25.72 (34.05)	77.23 (126.1)	25.55 (24.68)	36.78 (30.25)	31.60 (24.32)	76.89 (64.67)	26.65 (24.32)	63.69 (83.54)
Yellow perch	0.29 (0.05)	3.11 (3.95)	33.17 (57.09)	3.11 (3.95)	33.17 (57.09)	3.11 (3.95)	33.17 (57.09)	1.17 (0.73)	9.80 (5.97)

EPA- eicosapentaenoic acid; DHA-docosahexaenoic acid; EPA+DHA in grams per 100 grams of raw fish; ng/g - nanograms per 1gram of fish; data are mean (SD) - standard deviation

Supplemental material 5

Figure S1. Dose-response relationship between dietary DDE intake (ng/kg/bw) and type 2 diabetes (n=1426)

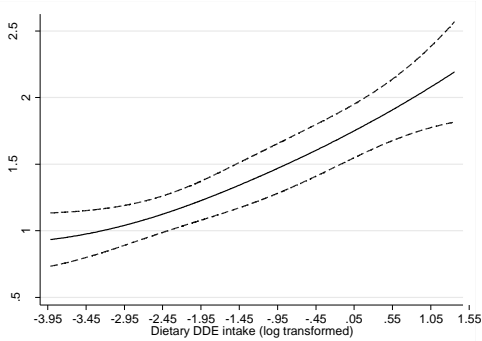


Figure S2. Dose-response relationship between dietary PCBs intake (ng/kg/bw) and type 2 diabetes (n=1426)

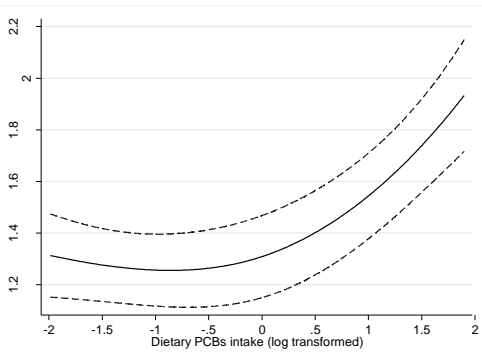
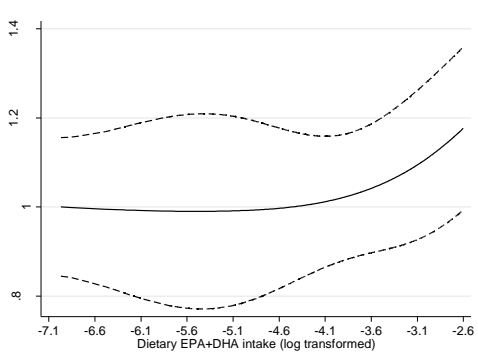


Figure S3. Dose-response relationship between dietary EPA+DHA intake (mg/d) and type 2 diabetes (n=1426)



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6 THE RELATIONSHIP BETWEEN PERSISTENT ORGANIC POLLUTANTS EXPOSURE
AND TYPE 2 DIABETES AMONG FIRST NATIONS IN ONTARIO AND MANITOBA,
CANADA: A DIFFERENCE IN DIFFERENCE ANALYSIS

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ABSTRACT

We previously studied the association between fish consumption and prevalence of T2D in Manitoba and Ontario First Nations (FNs), Canada and found different results. In this study, we used a difference in difference model to combine and compare the results. Dietary and health data from the First Nations Food Nutrition and Environment Study, a cross-sectional study of 706 Manitoba and 1429 Ontario FNs were analyzed. The consumption of fish was estimated using a food frequency questionnaire. Fish samples were analyzed for dichlorodiphenyldichloroethylene (DDE) and polychlorinated biphenyls (PCBs) content. Difference in difference model results showed that POP exposure was positively associated with T2D in a dose-response manner. Stronger positive associations were found among females (OR=14.96 (3.72-60.11)) than in males (OR=2.85 (1.14-8.04)). The breakpoints for DDE and PCBs intake were 2.11ng/kg/d and 1.47ng/kg/d, respectively. Each further 1 ng/kg/d increase in DDE and PCBs intake increased the risk of T2D with ORs 2.29 (1.26-4.17) and 1.44 (1.09-1.89), respectively. Our findings suggest that the balance of risk and benefits associated with fish consumption is highly dependent on the regional POPs concentrations in fish.

Keywords: persistent organic pollutants, type 2 diabetes, fish consumption, difference in difference model, long chain n-3 fatty acids, First Nations

1. Introduction

Type 2 diabetes (T2D) has become increasingly prevalent among Indigenous populations worldwide (Acton et al. 2002; O 'dea 2005; Young et al. 2000). In Canada, the prevalence of T2D among First Nations is 3 to 5 times higher compared to the general population (FNIGC 2012; Public Health Agency of Canada 2011). In addition, T2D has an earlier age of onset, is associated with greater micro- and macrovascular complications and causes higher mortality among First Nations compared to the general Canadian population (FNIGC 2012; Public Health Agency of Canada 2011). Lifestyle factors such as obesity, an unhealthy diet, and lack of physical activity are well-recognized risk factors for T2D. However, other potential risk factors such as an exposure to environmental contaminants may also contribute to the high rates of T2D (Taylor et al. 2013). Epidemiological studies have confirmed positive associations between exposure to certain POPs including polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE) and T2D in general (Everett and Thompson 2012; Lee et al. 2011; Silverstone et al. 2012; Turyk et al. 2009; Turyk et al. 2009; Wu et al. 2013) and among Indigenous populations (Aminov et al. 2016; Aminov, Haase, and Carpenter 2016; Grice et al. 2017; Jorgensen, Borch-Johnsen, and Bjerregaard 2008; Philibert et al. 2009; Singh and Chan 2017). First Nations were reported to be exposed to higher levels of PCBs and DDE compared to the general Canadian population through traditional food, in particular, fish consumption (Seabert et al. 2014). On the other hand, traditional food provides significant nutritional benefits by contributing to the intake of essential nutrients including long chain omega-3 fatty acids (n-3 FAs) (Kuhnlein and Receveur 2007; Sheehy et al. 2015).

Fish consumption is widely promoted because of its beneficial health effects on CVD and mortality (Chowdhury et al. 2012; He 2009; Leung Yinko et al. 2014). Recent evidence suggests

that consumption of fish, rich in n-3 FAs (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) may help prevent T2D since their improved lipid profile, reduces insulin resistance and inflammation (Chen et al. 2015; Fedor and Kelley 2009). Epidemiological studies reported contradicting results on the association between fish, n-3 FAs, and T2D. Some studies found inverse or protective associations (Nanri et al. 2011; Nkondjock and Receveur 2003; Rylander et al. 2014), no association (van Woudenberg et al. 2009) or positive association between fish and n-3 FAs intake, and T2D (Djoussé et al. 2011; Kaushik and Mozaffarian 2009). The discrepancy between the findings on the relationship between fish, n-3 FAs, and T2D may be possibly explained by differences in fish consumption patterns (n-3 FAs content) as well as levels of contaminants present in fish (Lee and Jacobs 2010); however, these important factors were not considered in the previous studies. Wallin et al. found a statistically non-significant inverse association between fish consumption and T2D after adjustment for dietary PCBs and mercury exposure (Wallin et al. 2015). Turyk et al. reported that inverse associations between fish and blood glucose were stronger and statistically significant after adjustment for DDE exposure (Turyk et al. 2015).

We previously reported differences in the association between fish consumption and the prevalence of T2D in First Nations living on reserve in Manitoba and Ontario, Canada. A negative dose-response relationship between the frequency of fish consumption and self-reported T2D was found in First Nations in Manitoba (Chapter 4) (Marushka et al. 2017a) whereas a positive association was observed in First Nations in Ontario (Chapter 5) (Marushka et al. 2017). The availability of traditional food species varies by ecozones and communities, however, the Manitoba and Ontario First Nations generally share similar cultural background and dietary preference (Chan et al. 2012, 2014). Demographic characteristics and other known risk factors

were comparable between First Nations at the provincial level; however, significant differences in dietary POPs exposure from fish consumption were found between Manitoba and Ontario. We hypothesize that the direction of the effect was driven by dietary POP exposure. Due to the relatively higher intake of POPs from fish among Ontario First Nations than in Manitoba First Nations, the adverse effect of POPs may outweigh the protective effects of fish on T2D. Dietary POPs were highly correlated with fish intake in the two groups of First Nations, therefore regression analysis may not fully control and separate their individual effects.

To test our hypothesis, we used a difference in difference (DID) model. The DID model is a statistical method widely used to evaluate the effectiveness of health care policy (Dimick and Ryan 2014). It allows the estimation of causal relationships between policy and an outcome of interest using a series of observational studies (Benmarhnia et al. 2016). The DID is considered a powerful method since it controls for unobserved background confounders that may influence the outcomes and thus, allows to assess the true impact of a predictor of interest (Dimick and Ryan 2014). The DID is also used in a cross-sectional setting (Lee and Kang 2006; Soares 2009). This study aims to examine if dietary exposure to POPs may outweigh the benefits of fish on the prevalence of T2D which helps to interpret our previous inconsistent findings in Manitoba and Ontario First Nations. Furthermore, we estimate the levels of dietary DDE and PCBs exposure which start to increase the risk of T2D.

2. Methods

2.1. Manitoba and Ontario First Nations

Data from the First Nations Food Nutrition and Environment Study (FNFNES) were analyzed. FNFNES is a cross-sectional study aimed to assess total diet and exposure to contaminants through traditional food consumption in First Nations adults living on reserves, south of the 60th

parallel across Canada. Detailed information about the study design is available at www.fnfnesc.ca. First Nations communities were randomly selected using a combined ecozone/cultural area framework. Estimation weights were calculated to obtain representative estimates of the total First Nations population. The study was accepted by the Ethical Review Boards at Health Canada, the University of Northern British Columbia, the University of Ottawa and the Université de Montreal. Also, the Assembly of First Nations (AFN) Chiefs-in-Assembly passed resolutions in the support of this research. The current study combined data from First Nations in Ontario and Manitoba. Figures 1A and 1B show the geographic locations of the communities included in the survey. The total sample included 2132 participants (706 from Manitoba and 1426 from Ontario) aged 19 years and over. Participation rates were 82% in Manitoba and 79% in Ontario.

Individual participation in the project was voluntary and based on informed written consent after an oral and written explanation of each project component. This survey was conducted following the “Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans” and in particular Chapter 9, pertaining to research involving the First Nations, Inuit and Métis Peoples of Canada. The study was approved by the Ethical Review Boards at the University of Northern British Columbia, the University of Ottawa, the Université de Montreal and Health Canada.

2.2. Data collection

Household interviews were used to collect dietary data (24-hour recall, a traditional food frequency questionnaire (FFQ)) and demographic characteristics (a socio, health, and lifestyle (SHL) questionnaire). The detailed information has been described previously (Chan et al. 2012, 2014). The traditional FFQ consisted of 153 traditional food items in Manitoba and 150 in Ontario. Traditional food consumption was assessed over four seasons in the past year. The SHL

Questionnaire included data on age, gender, weight and height (measured or self-reported), physical activity, household size, education, and employment status and diagnosis of type 2 diabetes.

2.3. Fish sampling and contaminants analysis

Fish samples collected for contaminant analyses were representative of all fish species consumed by members in each community. Each community identified the most commonly consumed fish species and those that are of the most concern from an environmental perspective. The collected fish samples were analyzed for several POPs including total PCBs and DDE at Maxxam Analytics in Burnaby British Columbia and ALS Global, in Burlington, Ontario.

2.4. Estimation of fish, dietary POPs (DDE, PCBs), and long-chain omega-3 FAs intake

To estimate daily fish intake (g/day), the number of days in the past four seasons when fish consumption was reported were summed up. Then, the total number of days was multiplied by the age- and gender-specific portion size of fish (g) estimated from dietary data generated by the 24-hour recalls for each age and sex group, and divided by 360 days (in this study, a year included four seasons of 90 days each).

Dietary PCBs and DDE intake were estimated by multiplying the amount of PCBs and DDE (ng/g) in each fish species by the total amount (grams) of each fish species consumed per day. Then, the amounts of PCBs and DDE from all fish species were summed up and divided by the body weight of each participant (ng/kg of body weight/day). Community-specific data of POPs content in fish species were applied to estimate total PCBs and DDE intake for each participant. Ecozone contaminant data were used in calculations if no community-specific data were available. Regional data were used if no ecozone data were available.

The validation of dietary assessments was performed through correlation analysis between mercury exposure from traditional food estimated using the FFQ and mercury concentrations in hair measured in First Nations participants. Dietary mercury intake was correlated with mercury in hair (Pearson correlation coefficient = 0.53).

The n-3 FAs concentrations in fish species were derived from the Canadian File of (Statistics Canada 2001). The n-3 FAs concentrations were assumed to be the same for the same fish species in Ontario and Manitoba. In this analysis, n-3 FAs means combined EPA and DHA from fish. The data are expressed as mg of EPA+DHA per gram of raw fish.

2.5. Statistical analyses

We use DID model to test our hypothesis. In the present study, the prevalence of T2D is the outcome of interest. Since a positive dose-response relationship between the frequency of fish consumption and self-reported T2D was previously found in Ontario First Nations, this cohort serves as the treatment group (exposed to POPs through fish consumption) whereas Manitoba First Nations serves as the comparison group (no/low exposure to POPs through fish) (Figure S1). The amount of fish consumption was used as a second source of difference. We explored dose-response relationship by further separating the fish consumers into two doses (medium/high fish consumers).

Preliminary analyses included the calculation of crude and standardized prevalence of T2D, proportions for categorical variables, and means with standard deviations for continuous variables. The direct method was used to calculate the standardized prevalence of T2D, with 2015 Canadian population as the standard population. For this analysis, fish consumption was divided into three categories: <5 g/day, 5-10 g/day, and >10 g/day. Logistic regression was

performed using province, levels of fish intake and their interaction terms, with potential confounders as independent variables. This can be seen in equation 1.

$$\text{Logit}(\text{outcome}) = \alpha + \beta_1 * ON + \beta_2 * F_M + \beta_3 * F_H + \beta_4 * F_{MON} + \beta_5 * F_{HON} + \gamma X + \varepsilon \quad (1)$$

In equation 1, α is the intercept, the exponential form of β values are the odds ratios of each group, γX is a set of control variables, and ε is the model residual. Odds ratios (ORs) of having T2D were calculated for Ontario First Nations and fish consumption categories. The low fish consumer category (<5g/d) served as a reference group. The beta (β) coefficients of main interest were β_4 and β_5 . The β_1 captures the difference in prevalence of T2D between the Ontario and Manitoba First Nations, β_2 and β_3 capture the effect of fish consumption (n-3 FAs) on the prevalence of T2D, β_4 and β_5 , the interaction terms, capture the effect of POPs on the prevalence of T2D. Three underlying assumptions made in this study are: 1) n-3 FAs in fish decrease the risk of T2D, 2) POPs from fish increase the risk of T2D, and 3) Ontario and Manitoba First Nations react similarly to n-3 FAs and POPs.

Covariates were added into the model step by step to show the relative contribution of other risk factors and their influence on the magnitudes of β_4 and β_5 . The control variables included in the final model were age, sex, body mass index (BMI), physical activity, total energy intake, education, and estimated intake of EPA + DHA, total PCBs, and total DDE. Age, BMI, total energy intake, education and estimated intake of EPA+DHA, total PCBs, and total DDE were used as continuous variables. Physical inactivity and gender were used as dummy variables. The total sample size for regression analyses included 2080 participants (1326 females, and 751 males) due to missing values for the control variables.

Segmented logistic regression was fitted to examine if the associations between dietary PCB/DDE intake and prevalence of T2D changed at different doses. The adjusted ORs

associated with each increase of 1 ng/kg/day in dietary PCB and DDE intake were reported. A forward procedure was adopted to show the relative contribution of other risk factors and their influence on PCB/DDE's effect size and the breakpoints. The final sets of covariates include age, sex, smoking, BMI, physical activity, education, total energy intake, and total fish intake. All statistical analyses were performed using weighting variables in order to obtain representative estimates at the regional level. Results with a p-value of less than 0.05 were considered statistically significant. STATA statistical software, 14.2 (StataCorp, College Station, Texas, USA) was used to perform statistical analyses. The segmented logistic regressions were performed with R (R Core Development Team).

3. Results

The study population included 2132 First Nations participants (706 from Manitoba and 1426 from Ontario). Table 1 summarizes demographic characteristics of Ontario and Manitoba First Nations men and women. The crude prevalence of T2D was 22.9% in Ontario participants and 17.4% in Manitoba First Nations. After standardization to the 2015 Canadian population, the prevalence of T2D was higher among Manitoba participants (28.4%) compared to Ontario individuals (25%). The average age of the study sample was lower in Manitoba (42.3y) compared to the Ontario sample (46.5y). The mean BMI was comparable between men and women in both Manitoba and Ontario provinces ranging from 29 to 31kg/m². Physical activity combines moderate and vigorous groups. In Ontario, adults tended to report more physical activity, have higher average total energy intake, and fruit and vegetable consumption than Manitoba adults.

Table 2 presents the average consumption of the top 5 fish species and the concentrations of n-3 FAs and POPs in the fish. The most consumed fish species in both Manitoba and Ontario were

walleye, lake whitefish, lake trout, northern pike and yellow perch. On average, they contributed 79% and 78% to the total fish intake in Manitoba and Ontario, respectively. Total fish consumption and total n-3 FAs intake were higher among Ontario participants than in Manitoba individuals. Besides the top 5 consumed fish species, n-3 FAs concentrations in other fish species were higher in Ontario. In regard to POPs content, all selected fish species had higher concentrations of contaminants in Ontario than in Manitoba. In Ontario, DDE levels in top 5 fish species ranged from 1.85 to 26.64ng/g and from 8.98 to 63.7ng/g of PCBs. In Manitoba, both DDE and PCBs were not detected in walleye and yellow perch and ranged from 0.15 to 11.73ng/g of DDE and from 0.03 to 9.24ng/g of PCBs in the rest of fish species. The average concentration of DDE and PCBs in the sum of top 5 fish species were estimated to be 5 and 36 times higher in Ontario than in Manitoba, respectively. Lake whitefish and lake trout contain the highest levels of n-3 FAs than other commonly consumed fish species (1.24 and 0.73g/100g of fish, respectively).

Table 3 summarizes total fish, dietary n-3 FAs and POPs intake by three categories of fish consumption (<5g/d, 5-10g/d, and >10g/d) in First Nations men and women in Ontario and Manitoba. Men consumed more fish and omega-3 FAs than women did in both Ontario and Manitoba. Dietary exposure to DDE and PCBs, and n-3 FAs intake was significantly higher in Ontario participants compared to Manitoba responders.

The associations between fish consumption, dietary POPs and prevalence of T2D are shown in Table 4. Model 1 shows the crude ORs, model 2 was adjusted for age and gender, and model 3 was further adjusted for BMI, physical activity, total energy intake, smoking, and education. Overall, the Ontario First Nations had a lower prevalence of T2D (OR = 0.53 (95% CI: 0.33-0.87) the Manitoba First Nations. Medium and high consumption of fish was associated with

lower prevalence of T2D, however, the estimates were marginally or not statistically significant. The ORs of the two interaction terms reflect the association between POPs and the prevalence of T2D, after subtracting the association between fish intake (n-3 FAs) and T2D. Dietary POPs were positively associated with T2D. The magnitude of ORs became more prominent after additional adjustment for risk factors across models. In Ontario, the OR in the high fish consumers (3.53 (95%CI: 1.47-8.45)) was almost two times higher than in the medium fish consumers (OR = 2.22 (95%CI: 0.86-5.68)). That translates into a nearly four-fold increase in the prevalence of T2D from low to high POPs exposures. The magnitudes of the association between POPs and T2D outweighed that between fish intake (n-3 FAs) and T2D (Figure 2). The association between frequency of fish consumption, dietary POPs and T2D was also tested using fish consumption as a continuous variable (Supplemental material: Table S1) and resulted in similar conclusions to analyses using categorical fish variables.

Effect estimates were also examined in fully-adjusted sex-stratified models (Table 4). A dose-response relationship for fish consumption was statistically significant in females, but not in males. In females, medium and high fish consumption showed statistically significant negative associations with T2D with OR = 0.29 (0.13-0.62) and OR = 0.16 (95% CI: 0.04-0.61), respectively. This translates into nearly 80% decrease in the prevalence of T2D in high fish consumer compared to low fish consumer females. In males, the point estimate of ORs decreased from 1.45 to 0.99 from medium to high fish consumers but were not statistically significant. Dietary POPs exposure was positively associated with the prevalence of T2D in both First Nations females and males. In females, the magnitude of the association in high fish consumers was about five times higher than that in the medium fish consumers ((OR = 14.96 (95% CI: 3.72-60.11) and OR = 3.08 (95% CI: 1.13-8.42), respectively). This indicates that high exposure to

dietary POPs resulted in a 15-fold increase in the prevalence of T2D compared to the low exposure to dietary POPs in First Nations females. In males, ORs increased from 1.79 (95% CI: 0.27-11.67) in the medium fish consumers to 2.85 (95% CI: 1.14-8.04) in the high fish consumers, in which the estimate was statistically significant. Thus, the effect of dietary POPs exposure on the prevalence of T2D in males was lower than that in females (Figure 2). Gender differences in the association of dietary POPs with the prevalence of T2D in Ontario compared to Manitoba were examined using three-way interaction terms (sex*fish consumption*location), which supported a stronger association of T2D with high vs low fish consumers in Ontario for females compared with males (Supplemental material: Table S2). Associations of T2D with medium vs low fish consumers in Ontario did not differ significantly by sex.

The ORs of T2D associated with each 1 ng/kg/day in dietary PCB/DDE intake are shown in Table 5. Segmented logistic regressions with one breakpoint were fitted, and the identified breakpoints and slopes (i.e. ORs) before and after the breakpoints are shown for PCB and DDE separately. The breakpoint for DDE was around 2.11 ng/kg/day, before which, no significant increase in the prevalence of T2D was found, and after which, each 1 ng/kg/day increase in dietary PCB intake was associated with the OR= 2.29 (95% CI: 1.26-4.17) increase in the prevalence of T2D. The corresponding estimates for PCB were as follow: the breakpoint was 1.47 ng/kg/day, and each further 1 ng/kg/day increase was associated with the OR=1.44 (95% CI: 1.09-1.89) increase in the prevalence of T2D.

Furthermore, we calculated the amounts of daily fish consumption (g/d) containing the concentrations of DDE and PCBs below the estimated breakpoints in Ontario and Manitoba separately (Figure 3-4). The estimates are presented for the top 5 fish species which contributed about 80% to the total fish intake. A body weight of 70 kg was used for the calculations. In

Manitoba, only three fish species are presented since DDE/PCBs were not detected in two out of five most consumed fish species (walleye and yellow perch) (Figures 4). These quantities of fish could be recommended as maximum daily intake in order to prevent exceeding the DDE/PCBs breakpoint exposure. The estimated amounts of daily intake of fish species were lower in Ontario than in Manitoba since significantly higher concentrations of DDE/PCBs in fish species. In Ontario, the estimated max daily fish consumption ranged from 5.5g/d (lake trout) to 79.8g/d (northern pike) with respect to DDE exposure, and from 1.6g/d (lake trout) to 11.4 ((northern pike) with respect to PCBs exposure (Fig. 3). In Manitoba, amounts of lake trout and whitefish that contain DDE/PCBs breakpoint concentrations were 12.6g/d and 116.3g/d regarding DDE, and 11.1g/d and 487.1g/d regarding PCB exposure, respectively. In other fish species (northern pike, walleye and yellow perch), the concentrations of DDE and PCBs were negligible or not detected. Overall, average daily consumptions of the top 5 fish species in Ontario and Manitoba First Nations were below the estimated amounts (Figure 3-4).

The proportions of individuals with total DDE and PCBs intake exceeding the estimated breakpoint exposure were 2.0% and 5.2% in Manitoba, and 9.7% and 27.9% in Ontario, respectively.

4. Discussion

Using the DID analysis, this study examined if relatively high POP exposure from fish may outweigh the protective associations of fish (n-3 FAs) on T2D in Ontario and Manitoba First Nations. Additionally, we examined the non-linear relationship between dietary PCBs and DDE exposure and T2D prevalence and estimated the thresholds of daily dietary DDE and PCB exposure that increase the risk of T2D. The results show that dietary POPs were positively associated with the prevalence of T2D in First Nations living in Ontario. Stronger positive

associations were observed among females compared to males. Higher fish (n-3 FAs) consumption was associated with a lower prevalence of T2D in Manitoba First Nations. When the data were stratified by gender analysis, statistically significant protective associations were found among females, but not in males. The breakpoints for DDE and PCB intake were 2.11 ng/kg/day and 1.47 ng/kg/day, respectively. Each further 1 ng/kg/day increase in dietary DDE/PCB intake increased the risk of T2D with OR = 2.29 (1.26–4.17) for DDE and OR = 1.44 (1.09–1.89) for PCBs, respectively. Based on these estimates, we calculated the approximate amount of fish consumption (by species) that could be recommended as maximum daily intake to prevent exceeding the DDE/PCB breakpoint exposure.

Our findings on the positive relationships between POPs and T2D are consistent with a number of previous cross-sectional studies (Aminov et al. 2016; Everett and Thompson 2012; Lee and Kang 2006; Philibert et al. 2009; Turyk et al. 2015; Turyk et al. 2009). Lee et al. found a strong dose-response relationship between serum concentrations of six POPs including DDE and PCBs, and T2D in the study among the US general population (Lee et al. 2006). In a Native-American population, a significant association between diabetes and serum PCBs (OR = 3.29) and DDE (OR = 6.4) at the highest versus the lowest tertile was observed by (Codru et al. 2007). Similar associations were reported by a study carried out among Inuit population (Singh and Chan 2017) and First Nations in Canada (Philibert et al. 2009). Cross-sectional evidence on the relationship between serum POPs and T2D was also supported by prospective studies (Lee et al. 2010; Turyk et al. 2009; Vasiliu et al. 2006). Former epidemiological studies investigated serum POPs concentrations in relation to prevalence of T2D whereas we assessed dietary exposure to POPs via locally-harvested fish intake. Since traditional food, in particular, fish is considered the main source of exposure to contaminants among Aboriginal population (Seabert et al. 2014), dietary

POPs intake from fish is a good indicator of the exposure. Positive correlations between frequency of wild food consumption and serum POP levels were found in First Nations communities (Fitzgerald et al. 1999; Seabert et al. 2014). Fish consumption positively correlated with serum POP levels in other studies (Duarte-Davidson and Jones 1994; Philibert et al. 2009; Turyk et al. 2009). In the present study, traditionally-harvested fish was estimated to be the main source of DDE and PCBs among all reported traditional foods (Chan et al. 2012, 2014).

In addition to epidemiological findings, experimental studies provide evidence of causal relationship between POPs and insulin resistance (Gray et al. 2013). Recent animal studies observed that chronic exposure to low doses of an environmentally relevant mixture of POPs via salmon oil consumption induced abdominal obesity, dyslipidemia, glucose intolerance, insulin resistance and hepatic steatosis (Ruzzin et al. 2010). The *in vitro* experiment showed that treatment of differentiated adipocytes with nanomolar concentrations of POP mixtures impaired insulin-stimulated glucose uptake (Ibrahim et al. 2011; Ruzzin et al. 2010). Several possible biological mechanisms have been proposed to explain the increased risk of T2D with exposure to POPs. Low-dose chronic exposure to POPs with endocrine-disrupting properties exhibits diabetogenic effect through impairment of glucose and lipid regulations (Neel and Sargis 2011; Newbold et al. 2008). POPs may cause mitochondrial dysfunction via mutations in mitochondrial DNA and in nuclear genes, and through glutathione (GSH) depletion (Montgomery and Turner 2015; De Tata 2014). Mitochondrial dysfunction, in turn, plays a crucial role in chronic low-grade inflammation and may lead to ectopic fat accumulation in liver, muscle, and pancreas. Low-grade inflammation in adipose tissue is suggested to play an important role in the development of insulin resistance and T2D (Montgomery and Turner 2015).

Several epidemiological studies reported that consumption of fish and n-3 FAs may prevent T2D (Nanri et al. 2011; Patel et al. 2009; Villegas et al. 2011), insulin resistance, glucose tolerance and metabolic syndrome (Ebbesson et al. 2005; Paquet et al. 2013). Nevertheless, systematic meta-analyses found geographical differences in the relationship between fish, dietary n-3 FAs intake and T2D (Zheng et al. 2012). Lee et Jacobs (2010) suggested that the direction of the associations between fish consumption and T2D may be driven by concentrations of beneficial nutrients (n-3 FAs) and harmful chemicals present in fish (Lee and Jacobs 2010) which significantly vary by fish species and geographical location (Aguilar et al. 2002). Also, POPs are known to be endocrine disrupting chemicals (Chevalier and Fénichel 2015) with their hormonal effects starting to appear at low doses and diminishing when exposure increases (Welshons et al. 2003). On the other hand, the beneficial effect of fish (n-3 FAs) shows linear dose-response relations. Thus, the beneficial effects of fish on T2D may outweigh harmful effects of POPs in the populations with relatively high fish consumption (Lee et al. 2014). In contrast, in populations with low fish consumption, beneficial effects of fish (n-3 FAs) might not be sufficient to outweigh the detrimental effects of POPs (Lee et al. 2014)

The balance of health benefits and potential risk of fish consumption is still not well understood. Several studies quantified risk and benefits of fish consumption to develop dietary recommendations. Many of those focused on exposure to mercury only (Hu, Laird, and Chan 2017; Laird et al. 2013; Loring, Duffy, and Murray 2010; Wennberg et al. 2012) when other studies considered several POPs (Foran et al. 2005; Sidhu 2003; Sirot, Leblanc, and Margaritis 2012). The relations between environmental chemicals and n-3 FAs with respect to coronary heart diseases and cancer were studied (Mozaffarian 2009). However, limited data are available on the risk and benefit associated with fish or n-3 FAs and POP exposure on T2D. Turyk et al.

(2015) evaluated joint effects of POPs and fish consumption on blood glucose in individuals with and without diabetes. They found that consumption of total and saltwater fish was inversely associated with blood glucose and the associations were more prominent after additional adjustment for DDE exposure. Also, Great Lake sport-caught fish (GLSCF) meals were inversely associated with blood glucose only after adjustment for DDE exposure whereas positive associations of DDE and PCBs with blood glucose were strengthened after controlling for GLSCF meals. The authors emphasized the importance of adjusting for both fish intake and POP exposure in studies of populations consuming contaminated fish (Turyk et al. 2015).

Christensen et al. (2016) examined cross-sectional associations between endocrine disorders (i.e. diabetes), fish consumption habits, and biomarkers among older male anglers in Wisconsin. The authors suggested that effects of fish consumption on risk for endocrine outcomes depend on the balance of the contaminants and nutrients (Christensen et al. 2016). In a population-based cohort study, Wallin et al. (2015) found no association between fish consumption and incidence of T2D. However, after additional adjustment for dietary PCB and mercury exposure, a statistically non-significant inverse association was observed between fish intake and the risk of T2D. The authors suggested that beneficial effect of fish may be attenuated by the detrimental effect of POPs. Therefore, the net effect of fish consumption on T2D may depend on POP content in fish (Wallin et al. 2015).

Sex differences in the relationship between certain POPs and diabetes were observed in other similar studies (Rylander, Rignell-hydbom, and Hagmar 2005; Silverstone et al. 2012; Vasiliu et al. 2006; Wang et al. 2008). Rylander et al. found a strong positive association between diabetes and serum DDE levels in women only, whereas in men, a positive association between PCB-153 and diabetes was observed (Rylander et al. 2005). In a prospective cohort study, serum PCB

levels were positively associated with diabetes in women, but not in men (Vasiliu et al. 2006). In the study carried out in Anniston, Alabama, a city with a history of PCB manufacturing (1929-1971), adjusted ORs for the prevalence of diabetes in the highest versus the lowest quintile of serum PCBs was 2.8 in the total population, and 4.9 for women. No association was observed in men. Similarly, elevated serum DDE levels were associated with diabetes in women, but not in men (Silverstone et al. 2012). The possible explanation of sex differences in the POPs associations with diabetes may be differences in the body fat composition. Women tend to have a higher proportion of body fat than men, with consequent greater accumulation and storage of lipophilic chemicals in adipose tissue. Also, several POPs are well-known endocrine-disrupting chemicals (EDC) affecting the activity of estrogen, a hormone involved in the homeostasis of glucose and lipid metabolism (Neel and Sargis 2011). Gender differences in the relationship between fish consumption and T2D were observed by Villegas et al. (2011). The researchers found an inverse association between fish intake and T2D in women only (Villegas et al. 2011). The discrepancies in our findings reported in the previous studies in two groups of First Nations living in Manitoba and Ontario reflect differences in contaminant levels in fish species (Marushka et al. 2017, 2017a). In fact, the concentrations of total PCBs and DDE in the most consumed fish species were estimated to be significantly higher in Ontario than in Manitoba. Consequently, dietary intake of PCBs and DDE in Ontario First Nations was much higher compared to Manitoba First Nations. Thus, elevated levels of POPs in fish lower beneficial effects of n-3 FAs on T2D. Significant variation in levels of environmental contaminants in fish species between regions and within the same region were reported by other studies (Loring et al. 2010; Neff et al. 2014).

The average concentration of n-3 FAs in all fish species consumed by First Nations was higher in Ontario compared to Manitoba. Thus, there were background differences in n-3 FAs intake between Ontario and Manitoba First Nations with the higher n-3 FAs intake to be estimated in Ontario population. This may suggest that our estimates of the association between POPs and T2D may be underestimated.

The protective effects of fish consumption on T2D were attributed to n-3 FAs based on evidence from epidemiological studies as well as our finding in Ontario and Manitoba First Nations.

Beside n-3 FAs, fish contains other beneficial nutrients such as high-quality protein, vitamin D, and selenium which may also contribute to the protective effect of fish on T2D (Berridge 2017); nevertheless, the results are still inconsistent (Mitri, Muraru, and Pittas 2014; Rayman and Stranges 2013). Low levels of vitamin D were associated with greater insulin resistance, impaired beta-cell function, and greater prevalence of metabolic syndrome in First Nations in Canada (Mansuri et al. 2014) whereas a study among Inuit in Greenland did not support a positive association between vitamin D levels and risk of T2D (Nielsen et al. 2016).

There are several strengths of this study. First, the sample is large and representative of First Nations living on reserve across various ecological zones. Second, POP concentrations in locally-harvested fish were measured in this study. The individual total dietary PCB and DDE intake was calculated based on community-specific data of POPs content in fish species. Third, the difference in difference approach provides more strength in causal inference compared to other statistical methods when using observational study data. Finally, we found a strong dose-response relationship between dietary exposure to POPs and fish intake and T2D.

The study has some limitations. First, the cross-sectional design of the study precludes us from asserting a causal relationship between POPs and T2D. In cross-sectional setting, there is a risk

of inverse causation. To examine if individuals diagnosed with T2D tend to change their diets and lifestyles, we performed sensitivity analyses. First, we compared the dietary intake and lifestyle habits between participants recently diagnosed with T2D (0–5 years) and individuals who had had T2D for a longer period of time (>5 years). The results showed that there were no statistically significant differences in dietary and lifestyle characteristics between the two groups in both Ontario and Manitoba First Nations (Marushka et al. 2017, 2017a). Additionally, using data on self-reported dieting status, we examined whether dieting (i.e., limiting their caloric intake in order to lose weight) and non-dieting individuals with and without T2D differed by macronutrient intakes. This analysis found that macronutrient intakes were comparable between groups of First Nations in Manitoba and Ontario (Marushka et al. 2017, 2017a).

Second, given that data on the prevalence of T2D in the FNFNES were self-reported, we validated the data by comparing our estimates with those estimates reported by the First Nations Regional Health Survey, 2008–2010 (RHS) collected over the similar period of time (FNIGC 2012). The prevalence of diabetes in Manitoba and Ontario First Nations reported by the FNFNES was 22% and 24%, which was similar to the 21% and 21.6% reported by the RHS, respectively (Marushka et al. 2017, 2017a). This evidence suggests that the prevalence rate of T2D reported in this study should be a reasonable estimate.

Third, dietary POP exposure and n-3 FAs intake were calculated from the same questionnaire information on fish intake which may result in collinearity between variables. Dietary POP intake was estimated using community-specific data on POP content in fish species collected locally. The measured POP concentrations significantly vary between fish species and within species sampled from different regions. In contrast, only the n-3 FA concentration reported in the

Canadian Nutrient File for each fish species was used for the estimation. Therefore, the risk of collinearity between POP with EPA+DHA and fish intake should be significantly decreased.

Finally, there are limitations of the DID methods. First, the DID method assumes that, in the absence of the treatment (dietary POP exposure in this study), the average outcomes for the treated and control groups would have followed parallel trends. In this study, the corresponding assumption is that the associations between fish (n-3 FA) intake and T2D are similar in Manitoba and Ontario First Nations. However, we cannot test this assumption due to the cross-sectional nature of the survey. Second, the DID analysis requires the composition of population in the treatment and control groups before and after intervention (high vs. low fish intake in the current study) to be stable. We found that participants from Ontario and Manitoba were not the same in terms of age, gender, and the amounts and species of fish consumed, and we used multivariate regressions to adjust for the effects of these confounding factors.

5. Conclusions

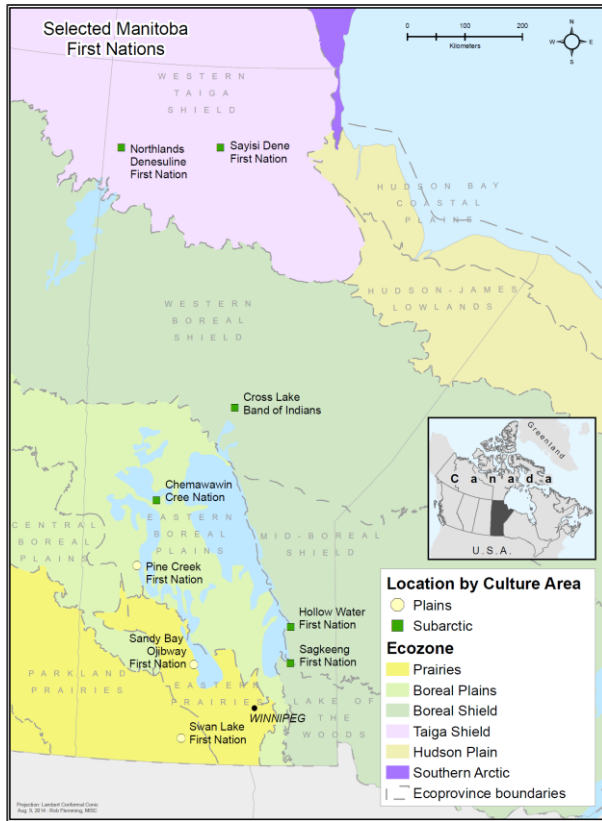
Our findings suggest that high dietary exposure to POPs such as PCBs and DDE may outweigh the beneficial effects of fish on T2D. This helps to explain the inconsistent findings between previous Ontario and Manitoba studies. Gender differences were found with stronger positive associations among females. Furthermore, we were able to estimate the thresholds of daily dietary DDE and PCBs exposure that start to increase the risk of T2D. Potential risks or benefits associated with fish consumption were affected by regional differences in POP concentrations in traditionally-harvested fish. Thus, dietary advice and guidelines should be tailored to reflect the regional differences.

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Figure 1A/B. Map of participating First Nations communities in Manitoba (A) and Ontario (B) (Chan et al. 2012, 2014)

A.



B.

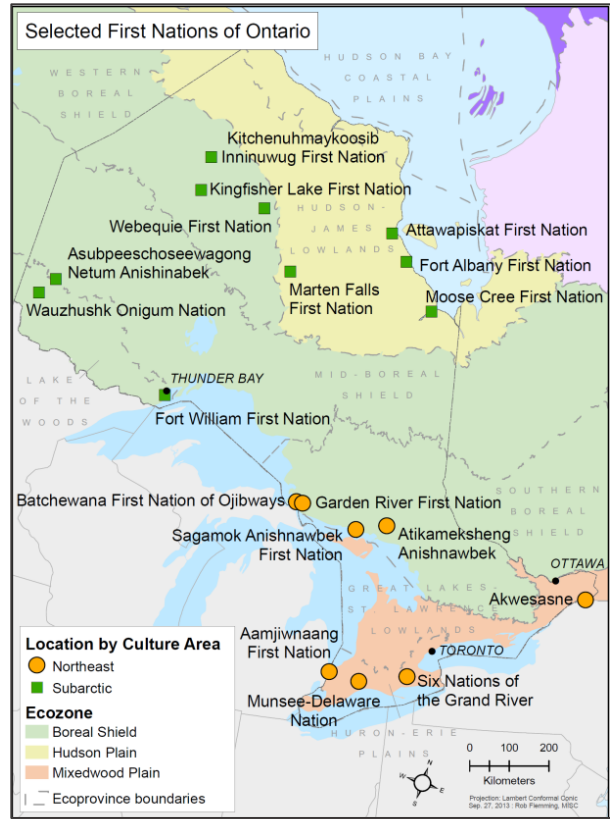


Table 1. Descriptive characteristics of Ontario and Manitoba First Nations participants

	Ontario			Manitoba		
	Total	Male	Female	Total	Male	Female
Sample size	1426	533	893	706	229	477
Type 2 diabetes, n	327	110	217	123	47	76
Type-2 diabetes weighted (%)	24.4	23.5	24.6	22.0	26.0	20.0
Type-2 diabetes standardized (%)	25.0	23.7	25.7	28.4	32.1	26.5
Age, mean (SD)	46.5 (15.8)	47.3 (16.0)	45.9 (15.6)	42.3 (14.4)	43.1 (14.3)	42.0 (14.5)
BMI (kg/m ²), mean (SD)	30.9 (5.9)	30.4 (5.4)	31.1(6.1)	30.3 (6.4)	29.0 (5.8)	30.9 (6.6)
Physical activity, n (%)	498 (34.9)	241 (45.2)	257 (28.8)	189 (26.8)	85 (37.1)	104 (21.8)
Smoking (%)	723 (50.7)	276 (51.8)	447 (50.0)	444 (62.9)	136 (59.4)	308 (64.6)
Years of education, mean (SD)	11.1 (3.8)	10.5 (3.5)	11.5 (3.9)	9.8 (2.5)	9.6 (2.7)	9.9 (2.4)
Total energy (kcal/d)	2042.1 (1026.8)	2344.5 (1222.1)	1861.6 (840.4)	1979.0 (1056.0)	2315.8 (1219.5)	1817.3 (926.5)
Fruit and vegetable intake (g/d)	157.6 (234.6)	141.7 (219.7)	167.1 (242.7)	113.1 (242.8)	88.8 (161.1)	124.8 (272.9)
Household size, mean (SD)	3.4 (2.0)	3.0 (2.0)	3.6 (2.0)	4.4 (2.6)	3.9 (2.8)	4.6 (2.5)

Values are N (%) or mean (SD), unweighted estimates

Notes for weighted prevalence, represent of provincial First Nations only, not comparable between Manitoba and Ontario

Notes for standardized prevalence, standardized to 2015 Canadian population

Physical activity combines moderate and vigorous physical activity

Table 2. EPA+DHA and POPs concentrations in the top 5 fish species in Ontario and Manitoba First Nations

Fish species	Ontario				Manitoba			
	Fish intake	EPA+DHA	DDE	PCBs	Fish intake	EPA+DHA	DDE	PCBs
	g/d	g/100g	ng/g	ng/g	g/d	g/100g	ng/g	ng/g
walleye	5.6 (13.5)	0.31 (0.05)	2.69 (3.36)	14.75 (19.44)	3.7 (9.1)	0.31 (0.05)	-	-
whitefish	2.5 (9.6)	1.24 (0.56)	5.89 (7.21)	14.56 (24.27)	2.0 (8.1)	1.24 (0.56)	1.28 (0.79)	0.21 (0.26)
lake trout	1.1 (5.6)	0.73 (0.14)	26.65 (24.32)	63.69 (83.54)	1.4 (5.9)	0.73 (0.14)	11.73 (5.76)	9.24 (2.58)
northern pike	1.7 (7.5)	0.27 (0.07)	1.85 (1.94)	8.98 (11.65)	1.0 (4.0)	0.27 (0.07)	0.15 (0.31)	0.03(0.10)
yellow perch	0.5 (2.8)	0.25 (0.04)	3.11 (4.18)	33.18 (62.47)	0.2 (1.7)	0.25 (0.04)	-	-
subtotal	11.5 (28.0)	0.56 (0.42)	6.28 (11.82)	22.01 (40.49)	8.4 (18.4)	0.56 (0.42)	1.06 (2.92)	0.59 (2.21)
total	14.7 (34.1)	0.67 (0.48)	10.08 (19.62)	35.21 (68.06)	10.7 (24.5)	0.53 (0.28)	2.05 (4.37)	2.00 (5.40)

FNs - First Nations, values are mean (SD),

"-" - not detected; EPA- eicosapentaenoic acid,

DHA - docosahexaenoic acid, DDE-dichlorodiphenyldichloroethylene, PCBs-polychlorinated biphenyls

Unweighted estimates of fish intake

Table 3. Dietary EPA+DHA and POPs intake by three categories of fish consumption

	<5 g/d		5-10g/d		>10g/d	
	mean	95%CI	mean	95%CI	mean	95%CI
Ontario						
Male						
n	225		86		222	
Total fish intake (g/day)	1.28	0.93-1.64	7.11	6.58-7.63	62.19	41.48-82.89
EPA+DHA (mg/day)	22.04	12.50-31.59	119.87	90.14-149.60	935.09	636.61-1235.36
DDE (ng/kg/day)	0.08	0.04-0.12	0.31	0.13-0.50	3.19	1.60-4.76
PCBs (ng/kg/day)	0.37	0.25-0.49	1.41	0.69-2.12	11.28	7.20-15.37
Female						
n	573		113		207	
Total fish intake (g/day)	0.97	0.83-1.11	6.94	6.78-7.09	39.21	27.49-50.93
EPA+DHA (mg/day)	14.65	12.00-17.31	115.24	100.57-129.90	550.63	398.00-703.28
DDE (ng/kg/day)	0.06	0.04--0.09	0.5	0.26-0.65	3.61	1.39-5.84
PCBs (ng/kg/day)	0.249	0.17-0.32	1.723	1.05-2.39	9.86	4.47-15.24
Manitoba						
Male						
n	104		32		93	
Total fish intake (g/day)	1.61	0.66-2.57	6.9	6.29-7.50	34.4	19.74-49.07
EPA+DHA (mg/day)	6.27	2.58-9.97	28.6	21.45-35.74	195.4	72.73-318.07
DDE (ng/kg/day)	0.012	0.001-0.02	0.02	0.003-0.71	0.63	0.07-1.21
PCBs (ng/kg/day)	0.012	0.002-0.03	0.02	0.005-0.09	0.45	0.09-0.81
Female						
n	346		55		76	
Total fish intake (g/day)	1.31	1.08-1.54	7.02	6.75-7.29	30.85	26.92-34.78
EPA+DHA (mg/day)	5.22	4.00-6.44	31.71	27.65-35.76	183.12	144.41-221.85
DDE (ng/kg/day)	0.004	0.001-0.007	0.06	0.03-0.09	0.34	0.26-0.41
PCBs (ng/kg/day)	0.003	0.0004-0.006	0.07	0.003-0.14	0.26	0.17-0.35

EPA- eicosapentaenoic acid, DHA - docosahexaenoic acid, DDE-dichlorodiphenyldichloroethylene, PCBs-polychlorinated biphenyls; weighted estimates

Table 4. ORs of the association between frequency of fish consumption and dietary POPs exposure with prevalence of type 2 diabetes in Ontario compared to Manitoba First Nations

	Total population			Female	Male
	Model 1	Model 2	Model 3	Model 3	Model 3
T2D in Ontario First Nations	0.53** (0.33 - 0.87)	0.52** (0.30 - 0.91)	0.53* (0.27 - 1.03)	0.64 (0.29 - 1.44)	0.32** (0.12 - 0.82)
Medium fish consumers	0.43** (0.22 - 0.84)	0.58* (0.31 - 1.09)	0.59 (0.29 - 1.18)	0.29*** (0.13 - 0.62)	1.45 (0.46 - 4.56)
High fish consumers	0.89 (0.47 - 1.69)	0.62 (0.32 - 1.19)	0.64 (0.35 - 1.19)	0.16** (0.04 - 0.61)	0.99 (0.57 - 1.71)
Medium fish consumers in Ontario	3.05*** (1.32 - 7.08)	2.12* (0.94 - 4.77)	2.22* (0.86 - 5.68)	3.08** (1.13 - 8.42)	1.79 (0.27 - 11.67)
High fish consumers in Ontario	2.76** (1.25 - 6.09)	3.39*** (1.49- 7.68)	3.53*** (1.47 - 8.45)	14.96*** (372 - 60.11)	2.85** (1.14 - 8.04)
n	2080	2080	2080	1329	751

T2D: type 2 diabetes; low fish consumers: <5 g/d (reference group); medium fish consumers: 5-10g/d; high fish consumers: >10g/d; values are ORs (95% CI) Model 1: crude estimates, Model 2: adjusted for age and gender, Model 3: additionally, adjusted for BMI, total energy intake, physical activity, smoking, education Ontario First Nations served as a treatment group and Manitoba First Nations served as a comparison (control) group

*** p<0.01, ** p<0.05, * p<0.1*

Figure 2. The prevalence of type 2 diabetes by categories of fish intake in Manitoba and Ontario First Nations males and females

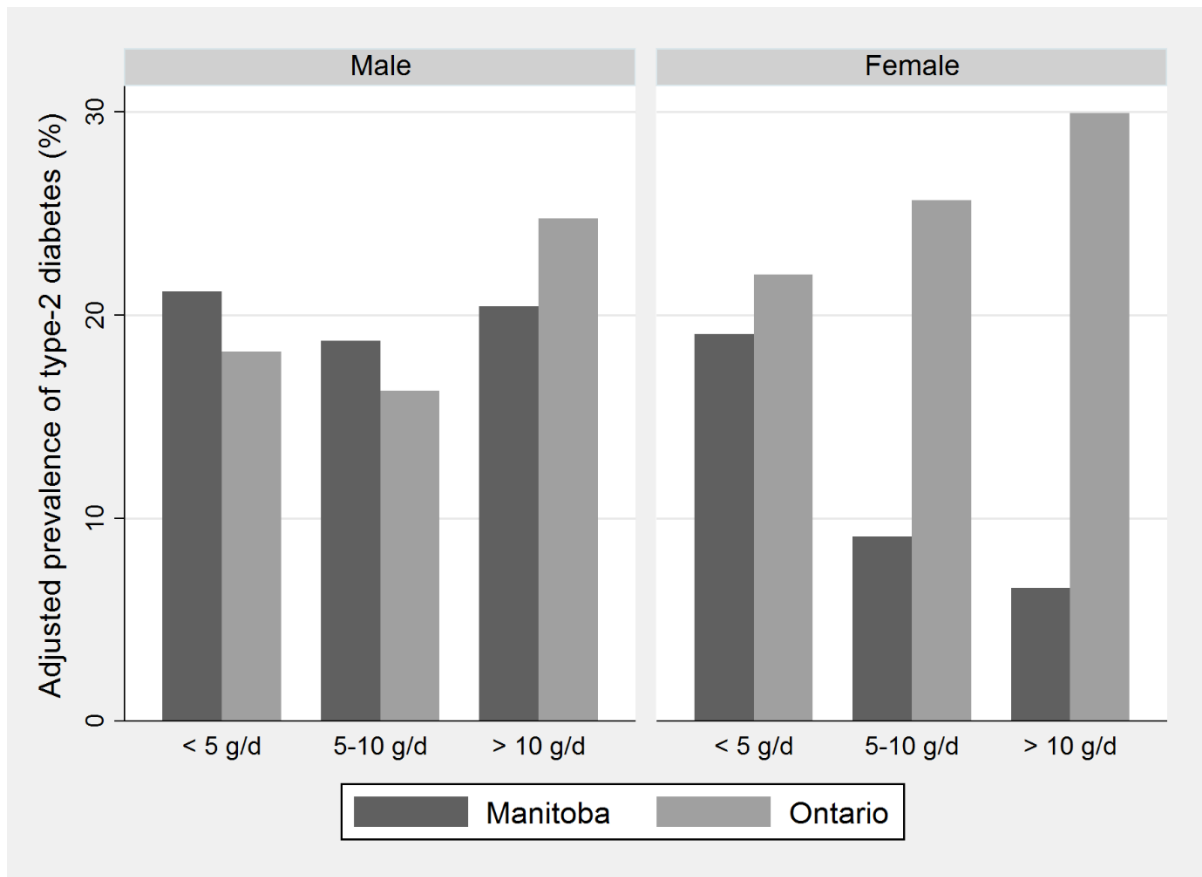


Table 5. Segmented logistic regression of the association between dietary DDE and PCB exposure and T2D in Manitoba and Ontario First Nations*

DDE intake				PCBs intake							
slope 1 (<BP)		BP		slope 2 (>BP)		slope 1 (<BP)		BP		slope 2 (>BP)	
OR	95% CI	ng/kg/d	SE	OR	95% CI	OR	95% CI	ng/kg/d	SE	OR	95% CI
1.03	0.99-1.07	2.11	1.53	2.29	1.26-4.17	1.00	0.96-1.03	1.47	1.95	1.44	1.09-1.89

DDE, dichlorodiphenyldichloroethylene; PCBs, polychlorinated biphenyls; T2D, type 2 diabetes

BP, break point; OR, odds ratio; CI, confidence interval; SE, standard error

OR measures the odds ratio of having T2D per 1ng/kg/day change in DDE/PCB intake from fish

** Model was adjusted for age, gender, body mass index, smoking, physical activity, total energy, education and total fish intake*

Figure 3. Amount of daily fish intake (g/d) with A) DDE levels and B) PCB levels below the estimated breakpoint in Ontario* *DDE breakpoint = 2.11 ng/kg/day; PCBs breakpoint = 1.47 ng/kg/day;*
*A reference body weight = 70 kg

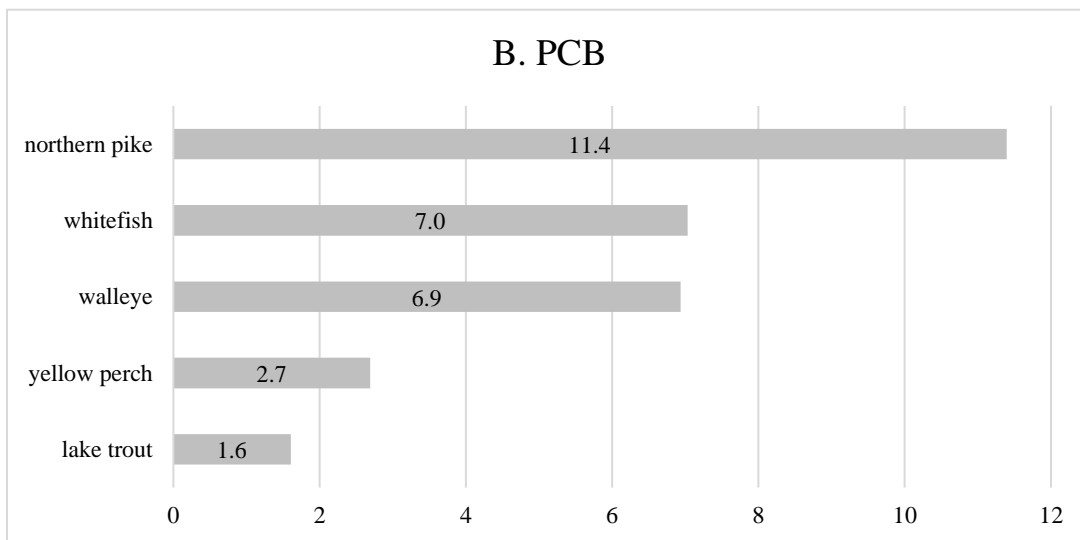
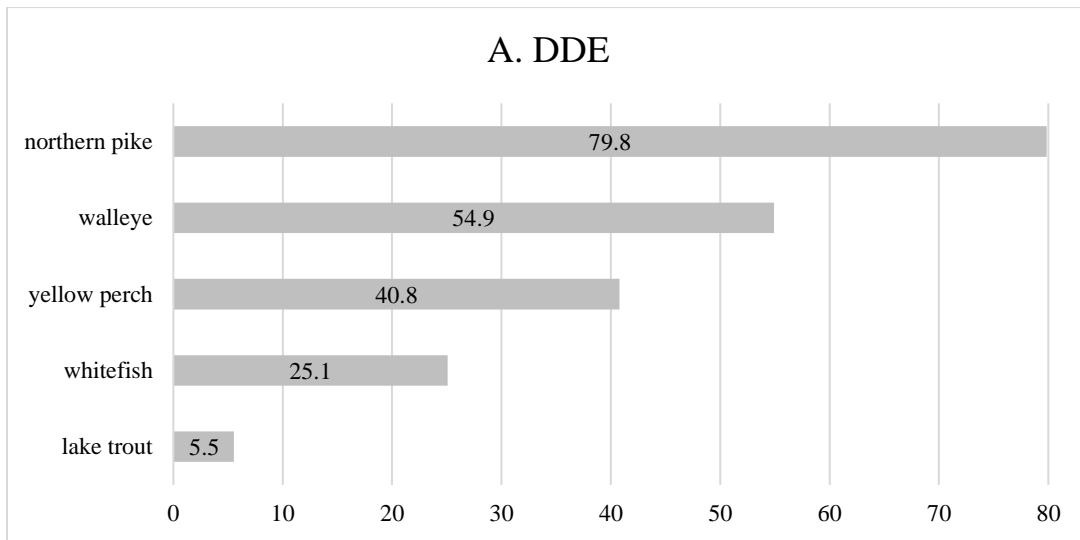
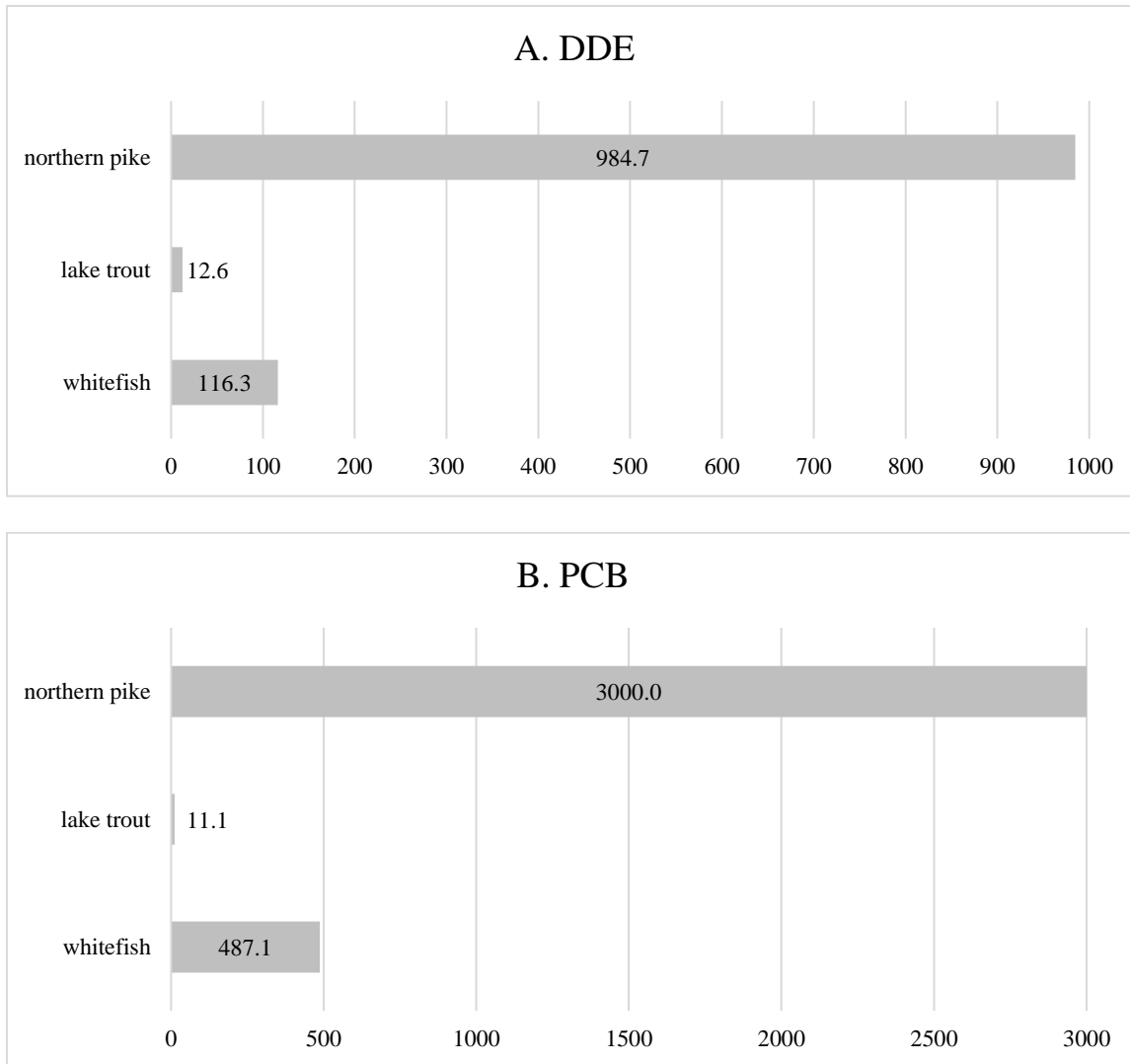
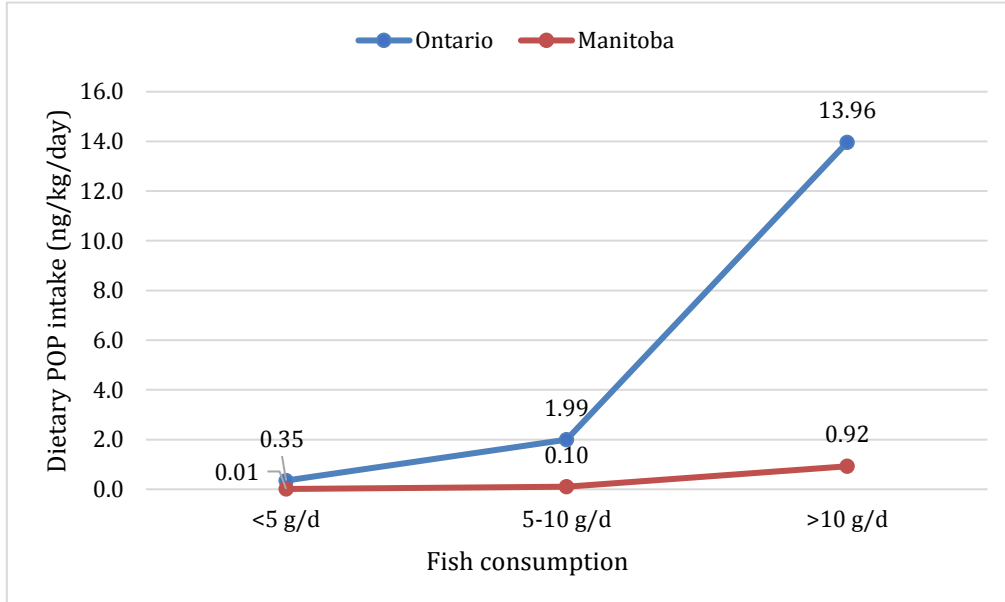


Figure 4. Amount of daily fish intake (g/d) with A) DDE levels and B) PCB levels below the estimated breakpoint in Manitoba* *DDE breakpoint = 2.11 ng/kg/day; PCBs breakpoint = 1.47 ng/kg/day; *A reference body weight = 70 kg*



Supplemental materials

Figure S1. Dietary POP exposure (DDE+PCBs) by fish consumption categories in Ontario and Manitoba First Nations.



Dietary POPs intake from fish in Ontario (blue line) was significantly higher than in Manitoba (orange line) in all fish consumption groups. In the difference in difference model (DID), Ontario serves as a treatment group (exposed to a relatively high POP level through fish consumption) whereas Manitoba serves as the comparison group (no/very low exposure via fish consumption).

Table S1. ORs of the association between fish consumption (continuous) and dietary POPs exposure and prevalence of type 2 diabetes in Ontario and Manitoba First Nations.

Variables	Female	Male
	OR (95%CI)	OR (95%CI)
T2D in Ontario First Nations	0.65 (0.29–1.41)	0.68 (0.37–1.26)
Fish consumption, g/day (continuous)	0.93 ** (0.89–0.97)	1.00 (0.97–1.02)
Fish consumption in Ontario, g/day (continuous)	1.10 ** (1.05–1.15)	1.04 * (1.00–1.08)
n	1329	751

*Model is adjusted for age, gender, BMI, total energy intake, physical activity, smoking, and education; Ontario First Nations served as a treatment group; Manitoba First Nations served as a comparison group; *** p<0.01, ** p<0.05, * p<0.1.*

Table S2. Gender differences of the association between frequency of fish consumption and dietary POPs exposure and prevalence of type 2 diabetes using 3-way interaction.

Variables	OR (95% CI)
T2D in Ontario First Nations	0.38 ** (0.16–0.87)
Medium fish consumers	1.00 (0.53–1.87)
High fish consumers	0.89 (0.47–1.69)
Medium fish consumers in Ontario	1.33 * (0.31–5.81)
High fish consumers in Ontario	2.83 ** (1.00–7.27)
T2D in Ontario (female compared to male)	1.55 (0.57–4.21)
Medium fish consumers (female compared to male)	0.16 *** (0.06–0.45)
High fish consumers (female compared to male)	0.17 *** (0.05–0.54)
Medium fish consumers in Ontario (female compared to male)	2.20 (0.37–12.9)
High fish consumers in Ontario (female compared to male)	4.78 ** (1.11–20.52)

*Low fish consumers: <5 g/d (reference group); medium fish consumers: 5-10g/d; high fish consumers: >10g/d; Model is adjusted for age, BMI, total energy intake, physical activity, smoking, education; Ontario First Nations served as a treatment group; Manitoba First Nations served as a comparison group; males is reference group for gender; *** p<0.01, ** p<0.05, * p<0.1.*

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7 SEAFOOD CONSUMPTION PATTERNS, THEIR NUTRITIONAL BENEFITS AND
ASSOCIATED SOCIO-DEMOGRAPHIC AND LIFESTYLE FACTORS AMONG FIRST
NATIONS IN BRITISH COLUMBIA, CANADA

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Authors' contributions: LM conceived the research question, performed the statistical analyses, interpreted the data and drafted the manuscript. HMC oversaw the research, provided intellectual support, and feedback on the drafted manuscript. HMC, MB, DS, HS, AI, KF, CT were all involved in the design and implementation of the FNFNES survey and provided feedback on the drafted manuscript.

ABSTRACT

Objective: To describe seafood consumption patterns in First Nations (FNs) in British Columbia (BC) and to examine lifestyle characteristics associated with seafood consumption; to identify top 10 most consumed seafood species, their contributions to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) intake and to estimate dietary exposure to methyl-mercury (MeHg), polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE).

Design: Dietary and lifestyle data from the First Nations Food Nutrition and Environment Study, a cross-sectional study of 1103 FNs living in 21 communities across eight ecozones in BC were analyzed. Seafood consumption was estimated using a traditional food frequency questionnaire.

Seafood samples were analyzed for contaminant contents. **Results:** Seafood consumption patterns significantly varied across BC ecozones reflecting geographical diversity of seafood species. The top 10 most consumed species represented 64% of the total seafood consumption, by weight, and contributed 69% to the total EPA+DHA intake. Mean EPA+DHA intake was 660.5mg/d in males and 404.3mg/d in females, and 28% of FNs met the recommended intake (RI) of 500mg/d. Salmon was the most preferred species. Seafood consumption was associated with higher fruit and vegetable consumption, lower smoking rate, and increased physical activity. Dietary exposure to selected contaminants from seafood was negligible. **Conclusion:** In FNs in BC, seafood continues to be an essential part of the contemporary diet. Seafood significantly contributed to reaching the RI of EPA+DHA and was associated with a healthier lifestyle. Given numerous health benefits, seafood should be promoted in FNs. Efforts toward sustainability of fishing should be directed to maintain and improve access to the fisheries for FNs.

Keywords: First Nations, traditional food, seafood consumption, omega-3 fatty acids, lifestyle characteristics, British Columbia

1 Introduction

First Nations (FNs) peoples are original inhabitants of Canada. Today, FNs represent 60.8% of the total Indigenous population and 2.6% of the total Canadian population. More than half (53%) of FNs live on-reserve or in communities while the others (47%) live off reserves (Affairs and Canada 2012). There are 634 unique First Nation communities in Canada (www.afn.ca), one-third of which (203) are in the province of British Columbia (BC) (www.bcafn.ca).

Significant health disparities exist between FNs and non-indigenous Canadians (Adelson 2005; Reading and Wein 2009). FNs people continue to experience shorter life expectancy, a higher rate of mortality, and chronic conditions including obesity, type 2 diabetes and CVD (FNIGC 2012). In BC, the general population has the lowest prevalence of chronic diseases among the Canadian provinces (Fang, Kmetic, and McCarney 2010; Public Health Agency of Canada 2011), however, FNs in BC experience poorer health status and a greater burden from chronic conditions compared to the general population in BC (First Nations Health Authority 2012).

Recent statistics show some improvements including a slowdown in the diabetes prevalence rate recently (Office of the Provincial Health Officer 2015; Update 2012). However, the rates of CVD tend to increase (First Nations Health Authority 2012) and remain a leading cause of death in First Nations adults in BC (Health Canada 2008).

The traditional food systems of Indigenous peoples in Canada are diverse across 12 terrestrial and marine ecozones (www.ecozones.ca) and include a large variety of game, fish, birds, berries and other plant and tree foods. An ecozone is a large geographical region identified based on the distribution patterns of plants, animals, geographical characteristics and climate (www.ecozone.ca). Culture areas is a concept to identify geographic areas within which Indigenous communities shared a greater number of traits/cultural affinities than from those

outside the area (Chan et al. 2011). Over millennia, FNs have developed many resource management and food production technologies, including hunting, trapping, foraging, and intensive food production (clam gardens, estuarine root beds, berry patches, crab-apple orchards, species domestication including sunflower, corn, beans and squash) (Saier and Trevors 2010). Traditional foods are a significant contributor of energy, essential vitamins, minerals, and polyunsaturated fatty acids and is low in saturated fat and carbohydrates (Gagne et al. 2012; Kuhnlein and Receveur 2007; Sheehy et al. 2015) and continue to have important social, health, and cultural benefits (Assembly of First Nations 2007).

Among FNs in BC, particularly in coastal communities, there has always been a far greater reliance on a wide variety of marine foods which are rich in high-quality protein, and several key minerals and vitamins. Furthermore, fish is a major source of essential omega-3 fatty acids (n-3FAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Kuhnlein et al. 2004) which are involved in neurological development, cell membrane functions, immune functions and inflammatory responses (Yashodhara et al. 2009). There is strong evidence of numerous beneficial health effects of n-3FAs including improvement of cardiovascular health (Breslow 2006; He 2009; Leung Yinko et al. 2014), reducing mortality from cardiac causes (Wen, Dai, and Gao 2014), favorable effects on blood pressure, inflammation, and lipid profile (Panagiotakos et al. 2007). Among Indigenous peoples, a lower prevalence of metabolic and CVD has been attributed to high consumption of traditional foods rich in n-3FAs (Bruce, Riediger, and Lix 2014; Dewailly et al. 2001; Jorgensen, Bjeregaard, and Borch-Johnsen 2002). Notwithstanding the benefits of traditional food, including fish, recent research indicates that FNs have been undergoing a nutritional transition characterised by lower consumption of traditional nutrient-dense food and an increased consumption of store-bought food which is high

in energy, fat and sugar (Kuhnlein et al. 2004; Sheehy et al. 2013; Willows 2005). This nutrition transition is concomitant with changing lifestyle practices including the decline in physical activity. The nutrition and lifestyle transitions are driven by various factors preventing FNs from traditional food use including but not limited to government restrictions, decreased harvesting areas, a decline in the abundance and type of traditional food species due to ongoing land privatization, habitat loss, and climate change, as well as household poverty (Chan et al. 2011; Kuhnlein, Fediuk, et al. 2013). Additionally, the human impacts on the local ecosystems have resulted in widespread concern about the risk of contaminant exposure (Kuhnlein and Chan 2000; Brian D Laird et al. 2013) such as polychlorinated biphenyls (PCBs), dichlorodiphenyldichloroethylene (DDE), and heavy metals like methyl-mercury (MeHg) which can have impacts on neurological development, immune and endocrine functions (Ha et al. 2017; Health 2004; Ross 2004).

Considering the benefits of traditional food and their potential role in promoting better cardiovascular health and in view of the health problems experienced by FNs, the objective of this study was to describe seafood consumption patterns in FNs living in eight BC ecozones and to examine dietary and lifestyle characteristics associated with seafood consumption. In addition, we identified the top 10 most consumed seafood species, their contribution to EPA+DHA intake and dietary exposure to PCBs, DDE, and MeHg. Finally, we estimated the percentage of participants who met dietary EPA+DHA recommendations from total seafood consumption.

2 Methodology

2.1 Study population

The First Nations Food Nutrition and Environment Study (FNFNES) is a 10-year cross-sectional study (2008-2018) designed to assess the quality of total diet combining traditional food and

market food and to provide a national baseline of background levels of environmental contaminants of concern in FN's adults living on reserves, south of the 60th parallel across Canada (www.fnfnes.ca). In this study, data collected from FN's in BC were used (Chan et al. 2011). FN's communities were sampled using a combined ecozone/cultural area framework to ensure that the diversity in ecozones and cultural areas were represented in the sampling strategy. Estimation weights were calculated to obtain representative estimates of the total FN's population. The design weight was adjusted based on the assumption that the responding communities represent both responding and non-responding communities (Chan et al. 2011). The current study analyzed data from 21 FN's communities across eight BC ecozones: 1-Boreal Cordillera/Subarctic; 2- Boreal Plains/Subarctic; 3- Montane Cordillera/Plateau; 4- Montane Cordillera/Subarctic; 5- Montane Cordillera/Subarctic/Northwest Coast; 6- Pacific Maritime/Subarctic/ Northwest Coast; 7- Pacific Maritime/Plateau; 8- Taiga Plains (Figure 1). In total, 1103 participants aged 19 years and over were recruited in this study in the fall of 2008 and 2009. The overall participation rate was 68%.

2.2 Data collection

Household interviews were conducted by trained interviewers. Participants completed a series of questionnaires that collected information on diet (a 24-hour recall and a Traditional Food Frequency Questionnaire (FFQ)), and social-demographic, health, and lifestyle data (SHL Questionnaire). The 24-hour diet recall recorded all foods and beverages including their approximate quantities consumed the previous day using the multi-pass technique with 3 stages (Raper et al. 2004). The quantities consumed were estimated using three-dimensional food and beverage models. The FFQ was used to collect data on locally-harvested traditional foods consumption during the four seasons in the past year. The questionnaire included all identified

traditional foods and was representative of each participating community. In total, the FFQ combined 208 traditional food items including 65 seafood items, 23 land mammals, 26 wild bird species, 53 wild berries, and wild nuts, plants, tree foods, and mushrooms. In this study, only data on seafood consumption were used. For the purpose of this study, the definition of seafood combined all fish species, shellfish, seaweed, and sea mammals reported in this survey.

The SHL questionnaire collected information on age, gender, weight and height (measured or self-reported), physical activity level (sedentary, somewhat active, moderate, vigorous), dieting (to lose weight) on the previous day (yes/no), smoking status (yes/no), years of education, employment status (fulltime, part-time, no job), source of income (wage, social assistance, worker's compensation, pension), household size (number of people per household), self-perceived health status (excellent, very good, good, fair, poor), and traditional food gathering activity (yes/no). Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). When available, both measured weights and heights were used in the BMI calculations, otherwise, self-reported or a combination of self-reported and measured values were used.

Household food security status was accessed using the income-related Household Food Security Survey Module (HFSSM) developed by the USDA (Bickel et al. 2000) and further adapted for Aboriginal communities (Lawn and Harvey 2004). The module consisted of 18 questions (10 adult-referenced items and additional eight child-referenced questions for households with children) asking about the ability of households to afford enough food. Households were considered as "food secure" when no items or only one item in the adult or child scale are affirmed. Households responding affirmatively to two and more questions were categorised as "food insecure".

2.3 Estimation of seafood consumption, dietary MeHg, DDE, PCBs, and EPA+DHA intake

The FFQ was used for estimation of seafood consumption and EPA+DHA intake. This was necessary as species consumption changes across seasons and there was a limited number of participants that had a specific seafood species on the day of the 24-hour recall during the fall data collection period. Usual frequency of intake of each seafood species was calculated from the FFQ. Each participant was asked to estimate their average portion size of each seafood species (grams/serving) from the 24 h recalls with the aid of food models. Average portion size was calculated for each age and sex group. Daily intake of each seafood species (grams/day) was estimated by summing up the number of days in the past four seasons when a particular species was consumed, multiplied by the mean portion size (grams) and divided by 360 days. In this study, a year included four seasons of 90 days each. Total seafood intake for each participant was estimated by summing up the amount of all seafood items consumed per day.

Given that seafood consumption data were derived from the FFQ whereas other dietary variables were estimated using the 24-hour recall (since most of the market foods are eaten on a daily basis), a comparison was made between seafood intake (gram/day and % of consumers) collected from the FFQ and the 24-hour recalls (Table S1).

Dietary PCBs, DDE and MeHg intake for each participant (j) from each seafood species (i) was calculated by multiplying the amount of PCBs, DDE and MeHg (nanograms) in one gram of each species by the total amount of each food items consumed per day (grams) and dividing the obtained amount by the body weight of each participant (ng/kg of body weight/day) (Eq.1). Total dietary PCBs, DDE and MeHg intake were estimated by totaling the amount of each chemical from all fish species consumed per day.

Eq.1

$$MeHg/PCBs/DDE\ intake_{i,j} = Food\ intake_{i,j}(g/d) \times MeHg_i/PCBs_i/DDE_i\ (ng)/body\ weight_j\ (kg)$$

The concentrations of EPA+DHA in seafood species were estimated from the Canadian Nutrient File, a national food composition database (Health Canada 2015), taking into account the preparation method (i.e. baked or broiled, boiled or raw). Daily EPA+DHA intake for each participant (*j*) was estimated by multiplying the amount of EPA+DHA (mg) in one gram of each food item (*i*) by the total amount of each food item consumed per day (grams) (Eq.2).

$$EPA+DHA_{i,j} = Food\ intake_{i,j}(g/d) \times EPA+DHA_i\ (mg/g) \quad Eq.2$$

The validation of dietary assessments was performed by comparing the estimated intake of MeHg from total traditional foods as well as from total fish consumption to the MeHg in the hair sample of the participants. Both estimates of MeHg intake from food were strongly correlated with MeHg in hair (Spearman $r = 0.54$).

2.4 Seafood sampling for contaminant content

Seafood samples were collected based on input from communities so that collected foods are representative of seafood species consumed by members in each community, and those of the most concern from an environmental perspective. The seafood samples were collected during fall 2008. Each fish sample was a composite of tissues from up to 5 different fish. The collected fish samples were analyzed for several toxic chemicals, including MeHg, PCBs and DDE at Maxxam Analytics, formerly CANTEST, in Burnaby, BC. All fish samples (flesh) were homogenized to provide a homogeneous sample for subsequent digestion. If required, a moisture value was determined gravimetrically after drying a portion of the blended sample at 105°C overnight.

DDE and PCBs

Six grams of tissue were homogenized in dichloromethane and filtered through anhydrous sodium sulphate. The extract was evaporated to 6 mL and 5 mL was injected onto the Gel Permeation Chromatography column where a fraction of the eluent was collected, concentrated, and solvent exchanged to acetone:hexane (1:1). Further clean-up was performed by eluting this extract through PSA columns. The final extract was concentrated, and solvent exchanged to isooctane. The analysis was performed for the DDE and PCBs using GC-MS in Selective Ion Monitoring mode with an EI source. Spiked standards and blank samples were measured for QA/QC.

MeHg

Samples were prepared by alkaline digestion. A combination of methanol and potassium hydroxide was used to solubilize MeHg for instrumental analysis. Highly selective and sensitive detection was achieved by Cold Vapour Atomic Fluorescence Spectrometry after pyrolytic decomposition of the GC eluent. The diluted extract was buffered to a pH of 4.5 – 5.0 and treated with Sodium Tetraethyl borate, resulting in ethylation of oxidized mercury species. These volatile ethylated species (as well as elemental mercury) were stripped from the liquid phase with argon gas, retained on Tenaex traps, desorbed back into the sample stream, and separated with a gas chromatography column. Each ethylated mercury species was released from the column of mass into the sample stream, thermally oxidized to elemental mercury, and then detected by cold vapor atomic fluorescence spectrometry.

2.5 Statistical analyses

Proportions (%) and means with 95% confident interval (CI) were calculated to describe seafood consumption in different BC ecozones. Seafood consumption was stratified in tertiles for the

total population, separately for males and females. Other dietary characteristics, such as fruit and vegetable consumption, which appeared frequently in the diet of individuals were calculated using the 24-hour recall data. Mean levels of dietary variables and lifestyle characteristics were compared between participants from three categories of seafood consumption within each gender. Univariate regression was performed to assess if differences were statistically significant. To compare macronutrient intake, nutrient densities per 1000 kcals were estimated by dividing each participant's daily nutrient intake by their total energy intake and multiplied by 1000 kcals. Daily intake of the top 10 most consumed seafood species and their contributions to dietary EPA+DHA and selected contaminant intakes were estimated. Proportions of FNs males and females who met dietary recommendations for EPA+DHA were calculated. Results with a p-value of less than 0.05 were considered statistically significant.

All estimates were weighted in order to obtain representative data at the regional level. All statistical analyses were performed using STATA statistical software, 14.2 (Stata Corp, College Station, Texas, USA).

3 Results

The study population consisted of 1103 participants (398 men and 705 women) living in 21 communities across 8 BC ecozones (Figure 1). Seafood reported by FNs in BC combined the variety of 41 fish, 16 shellfish, 4 seaweed and 4 sea mammal items. Overall, 95% of FNs reported consuming at least one traditionally harvested seafood in the prior year, including fish (94.7%), shellfish (60.0%), seaweed (34.5%), and sea mammals (2.8%). Salmon was the most consumed fish species consumed by 91.2% of FNs in BC. The average and 95%CI intake of total seafood was estimated to be 42.8 (28.3,57.4) g/day. The mean age of the total population was 45.7y (42.7,48.7) and was comparable between males and females (46.1y (43.1,49.1) and 45.5y

(42.4,48.7), respectively). Mean BMI was 29.7kg/m² (28.4,31.1) in both males and females. The smoking rate was higher in males (42%) than females (37.5%). Also, males tended to be more physically active compared to females. About 51% of FNs males reported moderate/vigorous activity while only 38.5% of females did. The total years of education were comparable between males and females FNs.

The consumption of marine foods significantly varied across BC ecozones (Table 1). The highest consumption of total seafood was reported by FNs living in the coastal ecozones of the Pacific Maritime/Subarctic/Northwest Coast (57.8g/day) and the Montane Cordillera/Subarctic/Northwest Coast (31.0g/day) followed by the northern region of the Boreal Cordillera/Subarctic (25.1g/day). In the Pacific Maritime/Plateau and the Montane Cordillera/Plateau, average seafood consumption was 27.3g/day and 26.5g/day, respectively. Likewise, the consumption of total fish, salmon, shellfish and seaweed was determined to be higher among FNs residing in the coastal and northern regions. The lowest intake of total seafood was reported by participants from the Taiga Plain ecozone (8.0g/day). Table 2 shows the proportion and daily intake of different seafood species in consumers only. The highest proportions of FNs who consumed total seafood resided in the Pacific Maritime, the Montane Cordillera/Subarctic/NC, and the Boreal Cordillera/ Subarctic ecozones ranging from 95.7 to 98.4% with the average intake of 26.3–59.1g/day. Among all seafood species, salmon was the most consumed fish, especially among FNs living in the coastal and northern ecozones where salmon contributed about 50% of the total fish consumption. Mean intake of shellfish ranged from about 2 to 13g/day. However, the highest percentage of FNs eating shellfish resided in the Pacific Maritime/Subarctic/Northwest Coast ecozone (81%). Seaweed consumption across regions ranged from 0-9 g/day. The highest percentage of participants consuming seaweed lived in the

Pacific Maritime ecozone (52%) with a mean intake of about 7.2 g/day. The consumption of sea mammals was reported by 3% of FNs in the Pacific Maritime/Subarctic/Northwest Coast ecozone (12.3g/day) and by one participant (1.3%) from the Boreal Cordillera/ Subarctic ecozone (2.1g/day).

Lifestyle and demographic characteristics of FNs males and females by tertiles of fish consumption are presented in table 3. Among both males and females, higher seafood consumers were older, had a higher BMIs and had a lower smoking rate than low seafood consumers. In males, the highest seafood consumption was associated with increased physical activity, traditional food gathering activity, and lower prevalence of food insecurity. In females, physical and traditional gathering activity, as well as food security status, were not associated with seafood consumption. Similarly, self-perceived health status, household size, years of education and sources of income did not differ between seafood consumption groups.

Dietary characteristics of participants are summarized in table 4. Fruit juice significantly contributed to fruit consumption, therefore, solid fruit with and without 100% fruit juice consumption were estimated separately. Among both males and females, individuals with higher fish consumption had higher consumption of both fruit (with and without juice) and vegetables. Overall, males reported higher consumption of fruit than females; however, females consumed more vegetables than males did. Total energy intake increased with fish consumption and was higher among males. The mean percentage of energy from protein, carbohydrates, fat and saturated fat was within the recommended acceptable macronutrient distribution range (AMDR), i.e. protein 10-35%, CHO 45-65%, fat 20-35%, saturated fat <10%. Among females, the proportion of energy from protein significantly decreased with increased fish consumption; whereas in males, the proportion of energy from total fat increased with fish consumption.

Macronutrient and fiber relative intake were not different between fish consumption groups. EPA+DHA intake significantly increased with seafood consumption.

Table 5 summarized the daily intakes of the top 10 most consumed seafood species and their contribution to total EPA+DHA intake. Total salmon combined sockeye, chinook, coho, pink, chum salmon species and salmon eggs, and was the most consumed type of fish. About 91.2% of FNs in BC reported eating any type of salmon with the mean intake of 28.6g/day in men and 16.8g/day in women. Among all salmon species, sockeye was the most popular with a mean intake of 15.3 g/day in men and 8.3g/day in women. Halibut and trout species were the third and fourth most consumed fish reported by about 37% and 41% of all participants. Among shellfish species, crabs and prawn contributed to the top 10 seafood species and were consumed by 25% and 23% of FNs responders, respectively.

Total salmon and top 10 most consumed species represented 46% and 64% of the total seafood intake, respectively, by weight. Salmon was a major source of EPA+DHA in FNs in BC with a mean intake of 392.8 mg/day in men and 238.6 mg/day in women, contributing 59% to the total EPA+DHA intake. The top 10 most consumed seafood species made up 69% of the total dietary intake of EPA+DHA. In general, males consumed more seafood than females did, and therefore, had a significantly higher intake of EPA+DHA.

Table 6 presents the contribution of total seafood to the EPA+DHA intake and the contribution to meeting the dietary recommendation of 500mg/day for the general adult population without CVD which is considered to be sufficient to obtain protective effects for primary prevention of CVD (Academy 2014; American Dietetic Association (ADA) 2007). There is currently, however, no estimated average requirements (EAR) or adequate intake (AI) for EPA+DHA. The proportion of FNs males and females who had EPA+DHA intake >500mg/day was estimated by

gender and age groups since significant differences in seafood and thus, EPA+DHA intake were found. Older participants (>50y) reported significantly higher total seafood consumption compared to the younger individuals, especially among males (47.0g/day, vs 95.0g/day). In younger males and females, total seafood consumption was comparable (32.7g/day and 30.9g/day, respectively). Mean EPA+DHA intake from total seafood exceeded the recommended intake (RI) of 500mg/day in men aged >50y old (Table 4). Overall, the proportion of FNs who met the RI of EPA+DHA \geq 500mg/day from total seafood was 28% and ranged from 22 to 58% in different age and gender groups.

Table 7 summarizes dietary exposure to MeHg, DDE and PCBs from the top 10 seafood species in FNs in BC. Overall, dietary intake of selected contaminants was very low and was far below the established tolerable daily intakes (TDIs) in FNs participants (Canada 2007). Males had higher MeHg, DDE and PCBs intake than females due to their higher seafood consumption.

A comparison of seafood consumption (gram/day and % of consumers) collected with the FFQ and the 24-hour recalls is presented in table S1 (Supplemental material). According to the data collected with the FFQ, significantly higher proportion of individuals reported consuming seafood species compared to the data collected with the 24-hour recall. The consumption of sea mammal was not captured by the 24-hour recall. Overall, the average intake (gram/day) of seafood collected using the FFQ was higher compared to the 24-hour recall.

The concentrations of EPA+DHA (Academy 2014) and selected contaminants (Chan et al. 2011) in the top 10 seafood species are presented in table S2 (Supplemental material).

4 Discussion

Seafood consumption patterns of FNs living on reserve in BC significantly varied across different ecozones reflecting geographical diversity of seafood species. The varieties of fish and

seafood, quantity and frequency consumed depends on geographical location, cultural background and availability of different types of seafood. FN communities living in or near coastal BC ecozones consumed significantly higher amounts of marine foods, including fish species, shellfish and seaweed compared to those living in the interior/inland regions, namely the Boreal Plain/Subarctic, the Montane Cordillera/Plateau and Subarctic, and the Taiga Plains ecozones. On the other hand, freshwater fish species were harvested and consumed by inland FN communities. Salmon represented the most important food, especially in the coastal communities (Jin, Teschke, and Marion 1998; Mos et al. 2004). In fact, salmon species were the most frequently consumed and were the major contributors to the top 10 seafood. Furthermore, salmon alone provided more than one third of the total EPA+DHA intake from total marine foods. The continued heavy reliance and use of salmon reflect its status as a cultural keystone species and favourite food for FNs (Garibaldi and Turner 2004). Crabs and prawn were the most commonly consumed species reported in the shellfish category and were consumed most frequently in the coastal Pacific Maritime ecozone.

Higher fish consumption was associated with other indicators commonly associated with a healthy lifestyle: high fish consumers tended to eat more fruits and vegetables and had a lower smoking rate than the low fish consumers. The increased physical activity with fish consumption in males may indicate that those men who ate more seafood were more likely to be involved in traditional food-gathering activities including hunting, fishing and collecting seafood. Lower protein consumption in female high fish consumers may be explained by the possible reduced intake of other protein sources (such as meat) (Stampfer et al. 2001). Our findings on higher fruit and vegetable consumption, lower smoking rates, and higher physical activity among high fish consumers are in concordance with the findings from other studies and may indicate that fish

consumption is likely an element of a healthier lifestyle (Suominen et al. 2016; Wennberg, Tornevi, et al. 2012). However, those studies were not performed with Indigenous peoples for whom fish harvesting is a traditional activity. A study among Norwegian women revealed that seafood consumption increased with increasing size of the household (Myrland et al. 2000) whereas other studies found that level and frequency of fish consumption were significantly positively correlated with income (Can, Günlü, and Can 2015; Verbeke and Vackier 2005). Regarding education level, prior studies reported a positive (Can et al. 2015; Myrland et al. 2000; Wennberg, Tornevi, et al. 2012) or no association (Verbeke and Vackier 2005) with frequency of fish consumption.

The majority of previous studies investigating determinants of fish/seafood consumption were conducted among general populations. To our knowledge, the only study involving Indigenous population was conducted among the Nenets people residing in Arkhangelsk region in Russia (Petrenya et al. 2012). This study reported that fish consumption was positively associated with monthly income and frequency of fishing (Petrenya et al. 2012).

The prevalence of food insecurity was significantly lower in high seafood consumers compared to low seafood consumers among males whereas no differences were noted in females.

Additional estimations showed that the prevalence of food insecurity was significantly lower among males compared to females (46.6% vs 27.4%, $p=0.04$ (data not shown)) indicating that male participants may underreport the level of food insecurity. In fact, a recent study among the general Canadian population revealed that in married households, higher food insecurity rates were reported when the respondent was female and neither respondent characteristics nor socio-economic factors accounted for the differences (Matheson and McIntyre 2013). These discrepancies were explained by gender-related differences in the perception of food security

status (Jung et al. 2016). Females tend to exhibit greater sensitivity to household needs and well-being of others than men (Matheson and McIntyre 2013). Females are usually responsible for the majority of tasks related to food (food purchasing, processing, and preparation), therefore, they may have better information about food security problems of their households (Ivers and Cullen 2011; Jung et al. 2016). Since the food security questionnaire reflects “household” food security status, it does not reflect the status of a particular individual within the household. In the total sample, there were no differences in the prevalence of food insecurity by seafood consumption categories (data not shown).

Age and gender differences in seafood consumption among FNs in BC were noted. Males and older participants (>50y) tended to eat a greater amount of total seafood compared to females and younger age groups (19-50y). These findings may suggest that younger generations of FNs are more affected by nutrition transitions than older FNs. These dietary and lifestyle changes may have significant health implications for FNs in the future, such as increasing rates of obesity and other chronic conditions (Gagne et al. 2012; Kuhnlein et al. 2004).

In total, 28% of FNs in BC met the RI for EPA+DHA from consumption of seafood whereas 72% of FNs had an intake of EPA+DHA less than 500mg/day. Among older age group (>50y), however, 58% met the RI for EPA+DHA which reflect higher seafood consumption (by weight). Overall, the EPA+DHA intake in BC FNs was much higher than that of the American population. In US adults, mean EPA and DHA intakes were 41mg/day and 72mg/day, respectively (Nordgren et al. 2017; Papanikolaou et al. 2014). In a representative sample of Quebecers, mean intake of EPA+DHA was 291mg/day and 85% of the participants had intakes below the RI (Cunnane and Lucas 2017). Suboptimal intakes of EPA+DHA were also reported in a study among Canadian pregnant women (Friesen and Innis 2009). Among the Inuit from

Nunavik, mean intake of EPA+DHA was 2196 mg/day in males and 2031 mg/day in females which is 3 to 5 times higher than that in the FNs in BC (Dewailly et al. 2001). In the present study, EPA+DHA intake was based solely on fish and seafood consumption and therefore did not include other possible sources, including other types of traditional foods and store-bought foods, whose contribution is likely to be relatively less important.

Numerous health benefits of EPA+DHA have been reported by clinical and epidemiological studies. Increased EPA+DHA intake decrease CVD, sudden cardiac death and stroke (Siriwardhana, Kalupahana, and Moustaid-moussa 2012). In addition, EPA+DHA intake was beneficial for reducing inflammatory disorder, hypertension, insulin resistance and arthritis (Pathways and Events 2011). Recent research demonstrated a potentially favorable effect of EPA+DHA on mental and neurological disorders (Lucas et al. 2009; Zhang et al. 2011).

Furthermore, adequate intake of EPA and DHA during pregnancy and lactation is critical for proper brain development of infants (Yashodhara et al. 2009). However, more intervention trials are needed to better understand the role of EPA and DHA in neurodevelopment (Flock, Harris, and Kris-Etherton 2013). Growing evidence of the importance of EPA+DHA for cardiovascular health and cognitive development led to the recommendations of daily intake of EPA+DHA and fish by national and international health agencies and professional organizations (Flock et al. 2013). Most guidelines recommend from 200 to 1,000mg/day of EPA+DHA with the optimal intake of 500mg/day. The Dietitians of Canada recommend 500 mg/day of long-chain n-3 FAs for general adult population (American Dietetic Association (ADA) 2007). This amount is considered to be sufficient to obtain cardioprotective effects for primary prevention of CVD. Individuals with CVD are recommended to consume 1 g/day of EPA and DHA for secondary prevention of CVD whereas patients with high triglyceride levels are recommended to consume

2 to 4 g/day of EPA and DHA as capsules under a physician's supervision. With regard to fish consumption, the majority of guidelines including the American Heart Association recommend two servings of oily fish per week which provide about 500 mg/day of EPA+DHA (Flock et al. 2013). Nevertheless, in order to establish a DRI for EPA and DHA, more information is needed to define the intakes of EPA and DHA required to reduce the burden of chronic disease (Flock et al. 2013).

The mean intake of MeHg, PCBs and DDE from total seafood did not exceed the TDI for MeHg (0.47 $\mu\text{g}/\text{kg}/\text{day}$ for total population, and 0.2 $\mu\text{g}/\text{kg}/\text{day}$ for women of child bearing age) as well as TDI for PCBs (1 $\mu\text{g}/\text{kg}/\text{day}$) and TDI for DDE (20 $\mu\text{g}/\text{kg}/\text{day}$). Despite the high consumption of seafood, the risk of dietary exposure to selected environmental contaminants was negligible in BC FNs.

Differences in average intake (gram/day) of seafood calculated using the FFQ and the 24-hour recall were found. Overall, the FFQ tended to overestimate seafood consumption compared to the 24-hour recall. The FFQ collected information about seafood intake over the past four seasons, and thus captured all identified seafood species consumed by respondents in the past year. The 24-hour recall gathered information about all foods and beverages consumed by respondents in the past 24 hours. The 24-hour recall is an adequate method to estimate the average usual dietary intakes of a group or a certain population (Shim, Oh, and Kim 2014).

Given that the 24-hour recall was performed on one day in the fall, it may not capture seasonal variability in seafood consumption patterns among FNs individuals. Since the FFQ accounts for the seasonal variability of locally harvested seafood consumption in on-reserve FNs, we preferred over estimating by the FFQ rather than underestimating by the 24-hour recall.

This study has some limitations. The EPA+DHA concentrations in each seafood species were obtained from the Canadian Nutrient File (CNF), considering the preparation method (i.e. baked or broiled, boiled or raw). The CNF includes information for both commercial and wild seafood species. If no data on fatty acid composition for wild seafood species were available, data for commercial seafood species were used. Since there are regional and species variations in fatty acid contents between wild and commercial seafood, the potential for error in the estimation of EPA+DHA intake from seafood may occur. Second, self-reported estimates on seafood consumption may lead to some degree of measurement error of the intake level.

This is the first study that explored fish consumption patterns, estimated dietary EPA+DHA intake, exposure to environmental chemicals from seafood, and examined dietary and lifestyle characteristics associated with seafood consumption in BC FNs. Strengths of this study include a large and representative sample of FNs living on reserve. Also, seafood consumption was assessed using a traditional FFQ that was developed based on previous work conducted with Aboriginal people. The FFQ captures the entire year's intake and is exhaustive when it comes to seafood species. Finally, contaminant concentrations in locally-harvested fish were measured in this study.

5 Conclusion

In BC FNs communities, seafood continues to be an essential part of the contemporary diet, especially among older FNs. Seafood consumption significantly contributed to intakes of EPA+DHA and to reaching the dietary recommendation and was associated with a healthier lifestyle. Seafood species mostly consumed were low in contaminants. Salmon remained the most consumed seafood and an important source of essential nutrients. Given numerous health benefits, seafood consumption should be promoted in BC FNs, especially among the younger

generation to prevent the development of chronic disease. Efforts toward sustainability of fishing should be directed to maintain and improve access to the fisheries for FNs.

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Figure 1. Map of 21 participating First Nations in British Columbia (Chan et al., 2011)



Table 1. Seafood consumption in First Nations in British Columbia by ecozones (n=1103)

		total seafood	total fish	salmon	shellfish	seaweed	sea mammals
	n	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)	Mean (95% CI)
Boreal Cordillera/Subarctic	80	25.1 (23.3,27.1)	23.9 (22.4,25.3)	17.9 (15.7,20.1)	1.0 (0.2,1.6)	0.3 (0.1,0.5)	0.0 (0.0,0.1)
Boreal Plains/Subarctic	122	10.2 (10.2,10.3)	7.8 (7.2,8.4)	4.0 (1.9,6.1)	2.4 (1.9,2.9)	0.0 (0.0,0.02)	0.0 (0.0,0.0)
Montane Cordillera/ Plateau	93	26.5 (6.9,45.9)	23.3 (8.4,38.3)	16.0 (9.8,21.9)	3.1 (1.4,7.7)	0.0 (0.0,0.0)	0.0 (0.0,0.0)
Montane Cordillera/Subarctic	92	8.6 (0.3,18.0)	8.4 (0.2,18.0)	4.1 (4.5,12.6)	0.1 (0.0,0.2)	0.0 (0.0,0.01)	0.0 (0.0,0.0)
Montane Cordillera/Subarctic	128	31.0 (18.2,43.4)	26.3 (22.5,30.3)	21.2 (1.8,24.6)	2.8 (2.4,7.9)	1.8 (1.9,5.5)	0.0 (0.0,0.0)
Pacific Maritime/Subarctic	369	57.8 (36.8,70.3)	41.7 (23.4,60.0)	23.7 (15.1,32.4)	11.4 (7.8,14.9)	4.2 (0.6,7.8)	0.6 (0.2,1.0)
Pacific Maritime/Plateau	117	27.3 (6.9,47.7)	26.2 (5.4,47.2)	18.3 (6.9,29.6)	0.9 (0.2,2.1)	0.1 (0.1,0.3)	0.0 (0.0,0.0)
Taiga Plains	102	8.0 (4.9,11.1)	7.9 (4.3,10.9)	1.7 (0.7,2.7)	0.1 (0.0,0.3)	0.0 (0.0,0.0)	0.0 (0.0,0.0)

Values are mean (95%CI), grams/person/day, all estimates are weighted

Table 2. Seafood consumption in First Nations in British Columbia by ecozones (consumers only)

	total seafood		total fish		salmon		shellfish		seaweed		sea mammals	
	%	mean (95% CI)	%	mean (95% CI)	%	mean (95% CI)	%	mean (95% CI)	%	mean (95% CI)	%	mean (95% CI)
Boreal Cordillera/Subarctic	97.5	26.3 (26.1,26.5)	97.0	25.0 (24.4,25.6)	83.8	21.3 (18.2,24.4)	15	4.0 (1.9,6.2)	8.8	4 (1.1,9.1)	1.3	2.1 (0.04,4.3)
Boreal Plains/Subarctic	84.4	14.2 (8.7,19.9)	84.4	10.8 (7.7,14.4)	56.5	7.0 (3.6,10.5)	27.9	11.1 (4.4,17.8)	1.0	0.5 (0.01,1.0)	0.0	0.0 (0.0,0.0)
Montane Cordillera/ Plateau	92.5	28.7 (10.7,46.8)	91.4	25.7 (12.3,39.1)	83.9	18.5 (13.2,23.8)	18.3	13.8 (1.5,26.1)	0.0	0.0 (0.0,0.0)	0.0	0.0 (0.0,0.0)
Montane Cordillera/Subarctic	83.7	10.4 (0.3,21.1)	83.7	10.3 (2.8,20.1)	51.1	9.1 (1.0,19.2)	2.2	2.1 (0.1,4.1)	2.2	0.3 (0.2,0.9)	0.0	0.0 (0.0,0.0)
Montane Cordillera/Subarctic/NC	97.7	33.0 (23.3,42.6)	97.7	28.1 (27.2,29.1)	89.1	23.6 (21.8,25.5)	17.2	10.9 (8.5,13.3)	13.3	9.1 (4.8,13.3)	0.0	0.0 (0.0,0.0)
Pacific Maritime/Subarctic/NC	98.4	59.1 (39.2,79.3)	97.8	42.7 (25.4,60.1)	96.0	25.2 (16.2,34.2)	81.3	12.6 (7.7,17.6)	52.3	7.2 (5.0,9.4)	3.0	12.3 (5.7,40.2)
Pacific Maritime/Plateau	95.7	30.6 (4.9,56.2)	94.9	30.3 (3.8,56.8)	85.5	21.9 (7.3,36.5)	12.8	7.1 (2.1,12.0)	5.1	2.2 (1.1,5.4)	0.0	0.0 (0.0,0.0)
Taiga Plains	68.6	12.3 (8.0,16.6)	68.6	12.1 (7.8,16.3)	3.0	5.9 (3.3,8.5)	3.9	2.4 (0.2,4.7)	1.0	0.5 (0.3,1.0)	0.0	0.0 (0.0,0.0)

Values are % consumers of respective food items, mean (95%CI) in consumers only, grams/person/day, all estimates are weighted

Table 3. General characteristics of First Nations males and females by tertiles of seafood consumption (n=1103)

	Males						Female							
	Tertile 1		Tertile 2		Tertile 3		p value	Tertile 1		Tertile 2		Tertile 3		p value
	%/mean	95% CI	%/mean	95% CI	%/mean	95% CI		%/mean	95% CI	%/mean	95% CI	%/mean	95% CI	
n	115		136		147			249		235		221		
Age	45.5	40.5,50.5	40.1	37.1,43.1	50.9	44.2,57.7	0.002	42.2	35.6,48.9	45.4	40.9,49.8	48.1	45.1,51.0	0.002
BMI, kg/m ²	27.9	26.7,29.1	30.0	27.1,32.9	30.9	29.0,32.7	0.006	28.1	25.8,30.4	28.1	27.1,29.1	32.4	29.9,35.0	0.006
Smoking	49.4		45.0		36.2		0.050	48.5		33.3		33.9		0.001
Physical activity							0.001							0.681
sedentary	13.0		13.8		5.5			19.3		18.0		20.2		
somewhat active	42.8		45.7		31.4			45.0		42.6		40.2		
moderate	34.1		29.1		39.1			33.2		39.0		35.4		
vigorous	10.2		11.3		24.0			2.5		1.1		4.2		
Health status							0.472							0.727
excellent/very good	38.7		26.6		31.6			20.0		25.2		25.2		
good	32.9		47.2		43.4			39.5		37.7		35.6		
fair & poor	28.4		26.1		25.0			40.5		37.1		39.2		
Dieting	6.8		8.4		13.1		0.242	12.5		13.1		12.3		0.984
Years of education	10.2	9.2,11.1	11.5	10.9,12.1	11.2	10.5,11.7	0.232	10.1	9.3,11.1	11.4	9.6,13.2	10.9	9.7,11.7	0.064
Employment	65.2		58.5		72.3		0.061	74.2		86.0		82.0		0.581
Traditional activity	56.8		68.0		87.8		0.002	49.0		53.7		55.6		0.259
Food insecurity	39.1		30.7		20.0		0.001	34.3		51.4		50.2		0.092
Household size	3.8	3.3,4.4	3.7	3.0,4.3	3.1	2.6,4.5	0.108	4.0	3.6,4.5	4.3	3.5,5.1	4.5	3.8,5.1	0.521
Income sources							0.881							0.485
wages	47.0		49.6		56.9			54.8		69.5		58.0		
social assistance	43.3		32.1		18.5			29.1		21.1		12.9		
workers compensation	2.3		10.8		7.0			5.0		6.4		15.4		
pension	7.4		7.6		17.6			11.2		3.1		13.7		

BMI, body mass index; Traditional activity, any traditional food gathering activity by participants; all estimates are weighted

Table 4. Dietary characteristics of First Nations males and females by tertiles of seafood consumption (n=1103)

	Males						p value	Females						p value
	Tertile 1		Tertile 2		Tertile 3			Tertile 1		Tertile 2		Tertile 3		
	mean	95%CI	mean	95%CI	mean	95%CI		mean	95%CI	mean	95%CI	mean	95%CI	
Total seafood (g/day)*	1.8	1.0,2.5	15.6	12.6,18.6	113.4	72.7,154.1	0.001	2.4	1.8,3.0	16.2	15.6,16.8	81.4	71.1,91.7	0.001
Fish (g/day)*	1.4	0.7,2.2	12.2	8.6,14.8	88.3	55.0,121.6	0.001	2.1	1.6,2.5	12.9	11.5, 14.3	61.1	48.9,73.3	0.001
Salmon (g/day)*	1	0.5,1.6	8.2	6.3,10.2	54.4	33.1,75.5	0.001	1.2	0.8,1.7	7.7	6.2,9.2	35.6	32.9,38.4	0.001
Shellfish (g/day)*	0.5	0.2,0.8	3.3	1.5,5.2	18.5	13.9,23.0	0.001	0.3	0.1,0.5	3.4	1.7,5.0	14.8	8.3,21.2	0.001
Seaweed (g/day)*	0.02	0.03,0.1	0.5	0.2,1.3	6	0.8,11.2	0.001	0.05	0.01,0.1	0.9	0.1,1.9	5.5	1.5,9.5	0.001
Sea mammals (g/day)*	0	0	0	0	1.7	0.4,3.0	0.001	0.0	0.0	0.1	0.2,0.3	0.3	0.01,0.6	0.001
EPA+DHA (mg/day)*	19.9	8.2,31.6	155.2	122.7,187.8	1189.1	767.9,1610.4	0.001	24.6	19.8,29.3	161.3	139.0,183.6	823.4	660.7,986.1	0.001
Fruit/Juice (g/day) †	80.7	1.1,160.2	83	24.0,142.0	190.8	45.6,340.0	0.001	58.6	45.0,72.1	141.5	99.4,183.5	122	96.0,147.9	0.001
Fruit (g/day) †	65.9	4.9,136.7	42.7	24.8, 60.5	120.9	6.1,235.7	0.006	25.1	7.9,42.3	89.5	43.7,135.3	72.1	46.7,97.5	0.001
Vegetables (g/day) †	42	14.8,69.2	74	27.8,120.2	71.1	55.0,87.2	0.059	56.9	43.0,70.8	80.7	53.3,108.0	125.7	97.4,154.0	0.001
Total energy (kcal) †	1739	12,802,197	2262	1695.0,2828.0	2101	1700.0,2503.0	0.004	1543	1426.0,1660.0	1638.0	1462.0,1814.0	2018	1949.0,2087.0	0.001
% Energy from protein†	20.2	15.7,24.7	21.2	12.1,30.3	18.2	16.5,19.9	0.751	18.1	15.7-20.4	16.8	16.0,17.7	17.4	16.2,18.6	0.023
% Energy from CHO†	45.3	41.9,48.6	47.1	40.8,53.4	47.5	43.6,51.2	0.701	48.4	45.5,51.2	50.5	48.5,52.6	47.8	45.8,49.8	0.091
% Energy from fat†	29.3	24.7,33.9	30	27.4,32.5	22.4	29.5,37.3	0.039	33.4	30.9,35.8	32.9	30.1,35.5	34.3	32.3,36.3	0.641
% Energy from sat fat†	9.7	8.3,11.1	9.6	8.2,10.9	10.2	9.3,11.0	0.676	11.1	9.3,12.7	11	9.62, 12.4	10.2	8.8,11.5	0.804
Nutrient density per 1000kcal**														
Protein †	50.6	39.3,61.9	53	30.3,75.5	45.6	41.4,49.9	0.5	45.3	39.4,51.2	42.2	40.1,46.5	43.5	40.6,46.7	0.2
Carbohydrate †	113.1	104.8,121.5	117.9	102.2,133.5	118.7	109.2,128.2	0.7	120.9	113.7,128.1	120.3	117.0,123.5	119.6	114.7,124.5	0.09
Total fat †	32.5	27.4,37.7	33.3	30.5,36.1	37.1	32.7,41.5	0.6	37.1	34.4,39.8	36.5	33.5,39.5	38.1	35.9,40.4	0.6
Saturated fat†	10.8	9.2,12.3	10.7	9.1,12.2	11.3	10.4,12.3	0.6	12.3	10.4,14.2	12.2	10.7,13.8	11.4	9.8,12.9	0.8
Fiber†	6.1	4.0,8.2	6.2	4.6,7.6	7.4	5.7,9.1	0.522	6.8	6.3,7.4	7.3	6.3,8.3	7.5	6.2,8.9	0.421
Mean intake														
Protein (g/day) †	91.3	49.4,133.2	112.5	69.2,155.7	90.1	71.6,108.5	0.323	69	60.9,77.1	63.9	54.3,73.5	92.1	78.6,105.5	0.009
Carbohydrate (g/day) †	196.7	130.5,262.7	265.3	195.1,335.5	252.5	197.2,307.7	0.053	182.3	162.6,201.9	202.8	181.2,224.3	239.7	223.6,255.8	0.001
Total fat (g/day) †	55.8	43.9,67.7	78.1	60.3,95.9	80	63.2,96.7	0.035	58.7	51.1,66.2	62.7	53.3,72.0	76.6	69.6,83.6	0.002
Saturated fat (g/day) †	18.3	15.5,21.1	24.8	17.7,31.8	25.3	19.6,30.8	0.092	19	15.9,22.1	21	18.0,24.0	23.4	19.1,27.7	0.308
Fiber (g/day) †	10.3	6.9,13.7	13.4	9.6,17.1	15	11.1,18.9	0.356	9.9	8.5,11.2	11.7	10.4,12.9	15.6	13.8,17.5	0.001
n	115		136		147			249		235		221		

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; * - estimated using FFQ, † – estimated using 24-hour recalls, values are mean (95%CI), nutrient density: grams/1000kcal, all estimates are weighted, fruit/juice – includes solid fruit and 100% pure fruit juice (fresh, canned, frozen), fruit – includes solid fruit only; vegetables – includes fresh, frozen, canned (excludes potatoes), Acceptable Macronutrient Distribution Range (AMDR): protein 10-35%, CHO 45-65%, fat 20-35%, saturated fat <10%

Table 5. Daily intake of top 10 most consumed seafood species and their contribution to EPA and DHA intake

	% consumers	Men			Women		
		Fish (g/d)	EPA+DHA (mg/d)		Fish (g/d)	EPA+DHA (mg/d)	
		mean (95% CI)	median	mean (95% CI)	mean (95% CI)	median	mean (95% CI)
Sockeye salmon	67.6	15.3 (7.9,22.8)	106.5	188.3 (96.7,279.8)	8.3 (5.3,11.2)	68.7	101.5 (65.6,137.4)
Chinook salmon	38.9	4.8 (0.7,8.9)	70.3	83.5 (12.4,154.4)	3.0 (1.6,4.5)	47.2	52.9 (28.4,777.4)
Halibut	36.7	3.9 (1.5,6.4)	6.8	9.2 (3.4,14.9)	3.7 (1.7,5.7)	6.2	8.6 (4.0,13.3)
Trout, any	40.7	2.7 (1.1,4.1)	14.5	24.3 (10.6,38.1)	1.0 (0.7,1.3)	5.3	9.5 (6.8,12.0)
Coho salmon	28.6	2.7 (1.0,4.3)	22.4	28.3 (10.5,46.0)	1.5 (1.3,1.7)	12.6	16.2 (14.0,18.4)
Salmon eggs	30.9	2.2 (1.3,3.0)	38.7	51.9 (30.7,73.1)	1.8 (0.9,2.7)	32.8	43.5 (21.2,65.7)
Crab	25.1	2.3 (1.0,3.5)	4.1	8.9 (3.8,13.9)	0.9 (0.5,1.4)	2.2	3.6 (1.9,5.4)
Pink salmon	21.4	2.0 (0.2,3.8)	16.8	21.6 (2.7,40.6)	0.9 (0.3,1.5)	5.8	9.7 (3.7,15.8)
Prawn	23.4	1.9 (0.9,2.9)	13.1	20.3 (9.3,31.4)	1.5 (0.2,2.8)	10.2	16.4 (2.4,30.5)
Chum salmon	17.8	1.6 (0.4,2.8)	15.8	19.3 (4.9,33.7)	1.3 (0.3,2.3)	12.3	14.8 (1.5,28.1)
Total salmon	91.2	28.6 (15.2,42.1)	232.7	392.8 (204.9,580.8)	16.8 (13.6,20.0)	139.8	238.6 (185.7,291.5)
Top 10 seafoods	93.3	39.3 (21.8,56.7)	273.8	455.5 (254.0,657.0)	24.0 (19.4,28.5)	147.0	276.8 (228.3,325.2)
Total seafoods	95.0	60.6 (34.4,86.8)	322.4	660.5 (388.5,932.4)	39.0 (28.6,49.5)	187.7	404.3 (276.1,532.4)

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid;

The sample includes fish/seafood consumers only (n=1014), percent (%) consumers of respective food

Total salmon and top 10 most consumed species represented 46% and 64% of total seafood intake, by weight;

Total salmon contributed 59% to EPA+DHA intake among both males and females;

Top 10 most consumed species contributed to EPA+DHA intake 69% among males and 68% among females

Table 6. Contributions of total seafood to EPA+DHA dietary recommendations (n=1103)

	Seafood intake			EPA+DHA		RI	% meeting RI
	n	mean (g/d)	95%CI	mean (mg/d)	95%CI		
males							
19-50	240	32.7	17.6,47.8	232	151.7,312.3	500	23
>50	158	95	30.8,159.1	600.4	150.4,1050.4	500	58
females							
19-50	499	30.9	27.1,34.7	193.8	162.9,224.8	500	22
>50	206	47	25.3,68.7	292.1	165.5,418.6	500	27

*RI, recommended intake for general adult population (Dietitians of Canada, American Heart Association)
 Values are mean (95%CI), food intake (grams/person/day), EPA+DHA (mg/person/day)*

Table 7. Dietary intake of selected contaminants from 10 top fish species in British Columbia

	Male			Female		
	MeHg	DDE	PCBs	MeHg	DDE	PCBs
	ng/kg/d	ng/kg/d	ng/kg/d	ng/kg/d	ng/kg/d	ng/kg/d
Sockeye salmon	7.21 (3.71,10.71)	0.38 (0.19,0.56)	0.07 (0.04,0.10)	4.67 (3.09,6.25)	0.25 (0.16,0.33)	0.45 (0.03,0.06)
Chinook salmon	2.63 (0.37,4.89)	0.18 (0.03,0.34)	0.05 (0.01,0.10)	1.81 (1.04,2.58)	0.13 (0.07,0.18)	0.04 (0.02,0.05)
Halibut	11.28 (3.65,18.92)	0.07 (0.02,0.11)	0.03 (0.01,0.05)	11.42 (5.68,17.14)	0.07 (0.03,0.10)	0.03 (0.02,0.05)
Trout, any	8.45 (3.47,13.44)	0.11 (0.05,0.18)	0.03 (0.01,0.06)	3.43 (2.47,4.39)	0.05 (0.03,0.06)	0.01 (0.01,0.02)
Coho salmon	1.14 (0.41,1.87)	0.10 (0.03,0.16)	0.02 (0.01,0.03)	0.79 (0.67,0.92)	0.07 (0.05,0.08)	0.01 (0.01,0.02)
Salmon eggs	0.05 (0.03,0.08)	0.02 (0.01,0.02)	0.01 (0.00,0.01)	0.05 (0.03,0.07)	0.14 (0.01,0.02)	0.01 (0.00,0.01)
Crab	0.65 (0.25,1.04)	0.01 (0.01,0.02)	0.01 (0.00,0.01)	0.30 (0.14,0.46)	0.01 (0.00,0.02)	0.03 (0.00,0.05)
Pink salmon	0.55 (0.08,1.03)	0.05 (0.01,0.09)	0.01 (0.00,0.01)	0.29 (0.10,0.48)	0.03 (0.01,0.04)	0.03 (0.00,0.01)
Prawn	0.47 (0.22,0.73)	0.01 (0.01,0.02)	0.03 (0.01,0.04)	0.43 (0.07,0.80)	0.01 (0.00,0.01)	0.00 (0.00,0.01)
Chum salmon	0.55 (0.15,0.95)	0.02 (0.01,0.04)	0.01 (0.00,0.01)	0.49 (0.04,0.94)	0.02 (0.01,0.03)	0.01 (0.00,0.03)
Total salmon	12.08 (6.00,18.16)	0.74 (0.34,1.14)	0.15 (0.06,0.24)	8.07 (6.60,9.54)	0.50 (0.40,0.59)	0.10 (0.08,0.12)
Top 10 seafood	32.94 (14.75,51.12)	0.84 (0.33,1.35)	0.26 (0.14,0.38)	23.66 (16.90,30.41)	0.63 (0.51,0.74)	0.18 (0.15,0.21)
Total seafood	53.68 (24.95,82.43)	0.98 (0.48,1.48)	0.30 (0.22,0.41)	34.05 (25.19,421.91)	0.78 (0.38,1.18)	0.21 (0.17,0.26)

DDE, dichlorodiphenyldichloroethylene; PCBs, polychlorinated biphenyls, meHg, methyl-mercury; TDI, tolerable daily intake; Sample includes seafood consumers only (n=1014); values are mean (95%CI), weighted estimates, TDI meHg=0.47 µg/kg/day (total population); 0.2 µg/kg/day – women of child bearing age (19-50y); TDI PCBs = 1 µg/kg/day, TDI DDE = 20 µg/kg/day (1 µg=1000 ng)

Supplemental material

Table S1. Comparison of seafood intake (g/day) collected from FFQ and 24h recall

	FFQ					24h recall				
	total population		consumers only			total population		consumers only		
	mean	95%CI	%	mean	95%CI	mean	95%CI	%	mean	95%CI
total fish	33.9	21.1,46.8	93.8	36.2	23.7, 48.7	23.2	11.1, 35.3	14.3	164.7	143.2, 186.3
salmon	19.6	13.3, 25.8	88.1	22.2	16.2, 28.2	17.9	10.2, 25.7	12.2	146.7	120.1, 173.3
shellfish	7.5	6.1, 8.8	59.6	12.5	8.3, 16.8	10.9	4.5, 26.3	5.4	201.8	102.3,301.2
seaweed	2.5	0.2, 4.8	34.6	7.2	5.1, 9.2	0.06	0.03, 0.09	0.7	7.8	1.5, 9.3
sea mammals	0.3	0.1, 0.5	2.8	12.2	0.1, 29.1	-	-	-	-	-
total seafood	44.2	29.4, 59.1	94.1	47.0	32.6, 61.5	34.1	8.6,59.6	18.3	186.0	152.1, 219.9

14.3% of recalls with seafood intake (n=158)

Table S2. Concentrations of n-3 FAs and selected contaminants in top 10 most consumed fish/seafood

	<u>EPA+DHA</u>	<u>MeHg</u>	<u>DDE</u>	<u>PCBs</u>
	<u>g/100g</u>	<u>ng/g</u>	<u>ng/g</u>	<u>ng/g</u>
Sockeye salmon	1.23 (0.09)	42.63 (12.85)	2.23 (1.45)	0.41 (0.61)
Chinook salmon	1.74 (0.81)	47.00 (18.74)	3.29 (1.87)	0.92 (0.91)
Halibut	0.24 (0.56)	252.00 (80.13)	1.50 (1.54)	0.73 (1.06)
Trout, any	0.94 (0.13)	185.42 (180.23)	4.04 (8.98)	0.47 (0.82)
Coho salmon	1.06 (0.12)	39.50 (15.12)	3.38 (3.51)	0.65 (0.67)
Salmon eggs	2.40 (0.35)	1.54 (2.93)	2.06 (2.35)	0.73 (1.34)
Crab	0.39 (0.15)	61.00 (42.83)	1.60 (3.67)	0.06 (0.14)
Pink salmon	1.08 (0.39)	28.60 (12.83)	2.02 (1.15)	0.28 (0.27)
Prawn	0.28 (0.07)	22.67 (6.50)	0.61 (1.06)	1.39 (2.40)
Chum salmon	1.18 (0.14)	29.67 (7.02)	1.09 (0.76)	0.11 (0.18)
Total salmon average	1.45 (0.52)	31.32 (14.31)	2.67 (1.48)	0.68 (0.55)
Top 10 seafood average	1.05 (0.67)	70.90 (80.80)	2.38 (1.45)	0.67 (0.52)
Total seafood average	0.86 (1.38)	80.14 (124.48)	2.67 (5.38)	0.52 (1.27)

Values are mean (SD), EPA- eicosapentaenoic acid, DHA - docosahexaenoic acid, DDE-dichlorodiphenyldichloroethylene, PCBs-polychlorinated biphenyls, meHg- methyl-mercury

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8 IMPACTS OF CLIMATE-RELATED DECLINE OF SEAFOOD HARVEST ON
NUTRITIONAL STATUS OF COASTAL FIRST NATIONS IN BRITISH COLUMBIA,
CANADA

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Authors' contributions: LM performed the statistical analyses, interpreted the data, and drafted the manuscript. TK created the map, assisted with interpretation of the results and contributed to the writing of the manuscript. CG and HMC assisted with the study design. HMC, MB, KF, TS were all involved in the design and implementation of the FNFNES study and provided feedback on the drafted manuscript. All co-authors discussed the results and contributed to the preparation of the manuscript.

ABSTRACT

Background: Traditional food systems are under pressure from various stressors, including climate change which is projected to negatively alter the abundance of most marine species harvested by coastal First Nations (FNs) in British Columbia (BC). **Objective:** To model the potential impacts of the climate-related declines in seafood production on the nutritional status among coastal BC FNs. In addition, we projected potential changes in nutrient intakes, under different scenarios of substitution where traditional seafood is replaced with alternative non-traditional foods. **Methods:** The study design is a mixed-methods approach that combines two datasets: projected scenarios of climate-related change on seafood catch potential for coastal BC FNs and data derived from the cross-sectional First Nations Food, Nutrition, and Environment Study. The consumption of seafood was estimated using a traditional food frequency questionnaire among 356 FNs adults. The contribution of seafood consumption to protein, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamins (A, B12, D, niacin), and minerals (zinc, selenium and iron) requirements was assessed using Dietary Reference Intakes (DRIs). **Results:** Traditional seafood consumption provided daily recommendations of EPA+DHA (74-184%) and vitamin B12 (84-152%) and substantial levels of niacin (28-55%), selenium (29-55%), vitamin D (15-30%) and protein (14-30%). Projected climate change was estimated to reduce the intakes of essential nutrients by 21% and 31% under ‘strong mitigation’ (Representative Concentration Pathway, RCP 2.6) and ‘business-as-usual’ (RCP 8.5) climate change scenarios, respectively, by the year 2050 relative to 2000. The hypothetical substitution of seafood with selected alternative non-traditional foods does not provide adequate amounts of nutrients, particularly those primarily derived from marine sources, such as EPA+DHA, vitamin B12, vitamin D, and selenium. **Conclusion:** Traditionally-harvested seafood remains fundamental to the contemporary diet and health of coastal BC FNs. Dietary shifts aggravated by climate-related declines in seafood consumption may have significant nutritional and health implications for BC FNs. Strategies to improve access to seafood harvest potential in coastal communities are needed to ensure the nutritional health and overall well-being and to promote food security and food sovereignty in coastal FNs.

Keywords: First Nations, traditional food, nutrient intake, climate change, nutrition transition, food security

1 Introduction

Marine environments support the livelihoods and diets of billions of people worldwide (UN 2017). Fish is a rich source of essential micronutrients (HLPE 2014), with over 4.5 billion people deriving at least 15 % of their average intake of animal protein from fish (Béné et al. 2015). Globally, changes in ocean temperature and acidity are projected to impact marine species distribution, affecting fisheries yields, catch composition, and revenue (Cheung et al. 2010; Cheung, Reygondeau, and Froicher 2016; Lam et al. 2016; Weatherdon et al. 2016). Climate-related declines in fish catch are also projected to mediate knock-on impacts on human health, through the decline of access to critical micronutrients in the diets of seafood-dependent peoples (Golden et al. 2016). Eleven-percent of the current global population is estimated to become vulnerable to poor nutrition from climate-related fisheries declines (Golden et al. 2016). This raises significant equity concerns as the majority of countries that are highly seafood-dependent are also low-income, food-deficient, countries (Kawarazuka 2010). Moreover, at the sub-national level, these risks may be experienced more strongly in certain populations, such as among coastal Indigenous peoples, where fish consumption greatly exceeds national averages (Cisneros-Montemayor et al. 2016), and among whom, significant disparities in socioeconomic and health status exist (Adelson 2005; Anderson et al. 2016).

First Nations (FNs) people are among the original inhabitants of the land and sea that is now considered Canada, which also include Inuit and Métis. The FN population in Canada includes individuals who may or may not be members of a specific FN and hold a registered or treaty Indian status. In the most recent National Census (2016), 1,673,785 or 4.9% of the total population in Canada identified as Indigenous, while 977,230 or 2.8% of the total population identified as First Nation and 744,855 indicated that they have registered or treaty Indian status

(Statistics Canada 2017b). Across Canada, there are 634 unique First Nations/Indian Bands, with one-third (n=203) located in the province of British Columbia (BC) (AFN 2017; BC AFN 2017). Most FNs have tracts of land held by the Crown that fall under the reserve system and are classified as “Indian Reserve” or IRI. Based on the most recent Census, 42% of those with Registered Treaty Status reside on reserve lands (Statistics Canada 2017b).

FNs peoples in Canada sustained themselves for millennia through diverse resource management and food production technologies including hunting, foraging, trapping and intensive food production (clam gardens, estuarine root beds, berry patches, crab-apple orchards, species domestication including sunflower, corn, beans and squash). (Deur and Turner 2005; Groesbeck et al. 2014; Jackley et al. 2016; Kuhnlein et al. 2013; Lepofsky et al. 2015; McKechnie et al. 2014; Mos et al. 2004). These local foods have been collectively called “traditional foods” (Kuhnlein et al. 2013). FNs peoples have known all along that harvesting, processing, sharing and consuming foods traditional to their Nations and territories enhances their mental, emotional, physical and spiritual health and well-being.

Catalyzed by colonial and assimilatory government policies and programs, FNs have been undergoing significant disparities in socioeconomic and health status resulting in a transition in diet and lifestyle during the last century (Fang et al. 2010; Health Canada 2008; Reading and Wein 2009). This nutrition transition, characterized by moving away from healthy traditional foods towards less healthy store-bought foods, has detrimental health effects. FNs people in Canada have lower life expectancy, higher rates of mortality, and a greater burden of chronic diseases (Health Canada 2008, 2014b), relative to the non-Indigenous population of Canada. Cardiovascular disease among BC FNs is almost double (7.9 % vs. 4.8%) the rate among the general BC population (Fang et al. 2010; Office of the Provincial Health Officer 2015), and

many more BC FN's citizens are overweight (36.6 %), obese (36%) or have diabetes (9.9 %) (FNIGC 2012).

FNs in BC are facing high rates of food insecurity (i.e. the inability to afford nutritionally adequate and safe foods). Overall, 41% of BC FN's households on reserve are food insecure (33% are defined as moderately food insecure while 8% are considered to be severely food insecure (skipping or cutting the size of their meals)) (Chan et al. 2011). This is in contrast with the provincial average, where less than ten percent (8.4%) of households in BC (excluding FN's households on reserve) experience food insecurity (Statistics Canada 2010). Food insecurity among Indigenous Peoples in Canada has been associated with a compromised diet quality, low intake of essential nutrients, poorer health, and low mental status (Egeland et al. 2011; Huet et al. 2012; Willows et al. 2011a). Lack of access to traditional foods, and the subsequent erosion of cultural harvesting traditions today can also be attributed to centralized colonial fisheries regulations (Brown and Brown 2009).

Despite significant lifestyle changes, traditional food systems remain fundamental to the culture, livelihood, economy, and health of contemporary FN's (Kuhnlein et al. 2013). Even when consumed in small amounts, traditional foods provide significant sources of energy, protein, essential vitamins, minerals, and long-chain omega-3 fatty acids (n-3 FAs) (Kuhnlein and Receveur 1996, 2007; Sheehy et al. 2015). BC FN's, particularly coastal communities, rely on a wide variety of locally harvested marine foods (fish, shellfish, and seaweeds) and salmon, a cultural keystone species (Garibaldi and Turner 2004) for their diet (Chan et al. 2011; Mos et al. 2004).

Traditional food systems are under pressure from various stressors, including climate change, which is projected to negatively impact the abundance and catch potential of many marine

species harvested by BC FNs communities (Weatherdon et al. 2016). Fisheries declines may result in the decrease in the availability of seafood which is an important source of nutrients for BC FNs, given that their seafood consumption is much higher than the rest of the population in general (Cisneros-Montemayor et al. 2017). Although such decrease in wild-caught seafood availability may be compensated by purchasing food from other sources, previous studies on Indigenous people's diet suggest that such shifts are often towards increased consumption of processed (and other) energy-dense foods that are high in fat, refined sugar, and sodium (Kuhnlein and Receveur, 1996; Charlton et al.,2016). A recent report that assessed Canadian fish stocks suggests that more than 50% of the assessed fish stocks in the Pacific coast are considered to be fully- or over- exploited. Under such a scenario, it is likely that fisheries decline as a result of climate change would further exacerbate the risk of food insecurity and the “nutrition transition” (i.e., the substitution of nutrient-rich traditional foods with store-bought foods of high energy-density but limited nutritional value) among FNs in BC. However, the burden of climate change on seafood consumption and FNs nutritional status has not been quantified. Previous research on the impact of climate change on traditional food systems and adaptation planning has mainly been conducted in the Arctic regions, focusing on Inuit and northern FNs communities (Ford et al. 2010, 2014; Nancarrow and Chan 2010; Rosol et al. 2016). Published research on climate change effects on FNs communities living in southern and coastal regions of Canada is limited. One First Nation, the Gitga'at Nation, located on BC's north coast has been actively engaged in climate related research and its impacts on seasonal harvesting and indicators (Turner and Clifton 2009), incorporating local values and knowledge into creating robust climate change adaptation strategies (Reid et al. 2014).

The objective of this study was to estimate the potential impacts of the climate-related declines in seafood abundance on the nutritional quality of adult diets among coastal FNs in BC, Canada based on a climate model projecting changes in potential fish catch and known FNs dietary data. In addition, we projected potential changes in nutrient intakes, after assuming the hypothetical substitution of traditional seafood with alternative non-traditional foods.

2 Methodology

The study's design, adapted from (Golden et al. 2016), is a mixed-methods approach which integrates methods and data from two traditionally distinct research fields: marine ecology and human nutrition. The approach explores the relationship between changes in fisheries catch potential and human nutrition and aims to assess the impacts of climate-related declines in seafood harvest on human diets and nutrient intakes. To this end, two datasets were used: i) projected scenarios for coastal catch potential under climate change in First Nations fisheries (Weatherdon et al. 2016); and ii) dietary data from the First Nations Food, Nutrition and Environment Study (FNFNES) in BC (Chan et al. 2011). Weatherdon et al. (2016) quantitatively projected impacts of climate-related change on the relative abundance, geographic range distribution and richness of 98 seafood species of commercial and cultural importance to FNs communities in coastal British Columbia. The changes were modeled under two emission scenarios: the low emission 'strong mitigation' (Representative Concentration Pathway (RCP) 2.6) and high emission 'business-as-usual' (RCP 8.5) by 2050 relative to 2000 (Moss et al. 2010). The FNFNES is a cross-sectional survey which was designed to assess traditional food consumption, total diets, food insecurity, and food-related exposure to environmental contaminants in FNs people living on reserves, south of the 60th parallel across Canada. The FNFNES collected data from approximately 100 FNs communities with population on "Indian

Reserves” herein referred to as “on reserve”, across Canada over a 10-year period (2008-2017) and is representative of all Canadian FNs regions south of the 60th parallel (www.fnfnes.ca).

2.1 Projected effects of climate change on seafood abundance

Climate-related change in the relative abundance of 98 marine fish and invertebrate species of commercial and cultural importance to FNs in coastal British Columbia was projected by 2050 relative to 2000 using a dynamic bioclimate envelope model (DBEM) (Cheung et al. 2016; Weatherdon et al. 2016). Changes in seafood abundance were estimated under two climate change scenarios: RCP 2.6 and 8.5. The basic structure of the DBEM is described elsewhere (Cheung et al. 2016) (Supplementary Information). In brief, the DBEM is a mechanistic species distribution model that simulates changes in the distribution of abundance and catches of fish and invertebrates over time and space, driven by projected changes in ocean conditions (temperature, oxygen, salinity, current, net primary production in both the surface and bottom layers), with consideration of physiological and ecological effects of changes in ocean properties and density-dependent population growth and movement. Changes in ocean conditions under climate change scenarios were projected from the Geophysical Fluid Dynamics Laboratory Earth System Model 2M (Dunne et al. 2013). Projected changes in ocean conditions were estimated on a 0.5° latitude x 0.5° longitude grid of the global oceans on which the DBEM operates (see Cheung et al. (2016) for details). For each species, the carrying capacity in each grid cell is proportional to its habitat suitability, predicted based on ocean variables, bathymetry and habitat types. Movement and dispersal of adults and larvae were modelled through an advection-diffusion-reaction equation. The model simulates changes in relative abundance of a species by a logistic growth function. The model also simulates how changes in temperature and oxygen content affect the growth of individuals (Cheung et al. 2013; Pauly and Cheung 2017).

In total, ninety-eight species comprising marine and diadromous fish, shellfish, and invertebrates harvested by First Nations for food, social, and cultural purposes were selected. The list of species was identified from peer-reviewed literature, government and non-governmental organisations' reports, treaty agreements, and First Nations' reports. Marine mammals, seaweeds and birds were not included in the study (Weatherdon et al. 2016).

2.2 Dietary Data from the FNFNES

A total of 21 FN communities in British Columbia were surveyed between the Fall of 2008 and 2009. FN communities were randomly selected using a combined ecozone/cultural area framework and residency information from on-reserve populations. An ecozone is a large geographical region identified by the distribution patterns of plants, animals, geographical characteristics and climate (ecozone.ca). Cultural area is a concept used to identify geographic areas within which Indigenous communities share a greater number of traits/cultural affinities than those outside the area (Sturtevant 1978). This study uses the data obtained from the six FN communities in the Pacific Maritime ecozone, Subarctic/Northwest coast cultural area, including Kitsumkalum, Hagwilget, Skidegate, Nuxalk, Namgis, and Tla'amin (formerly Sliammon) (Fig 1). The Pacific Maritime ecozone comprises BC marine islands, the land along the Pacific Coast, and the border with Alaska. The participation rate in the Pacific Maritime ecozone was 67%. In total, 369 individuals who self-identify as FN adults living on-reserve were recruited to participate in this study. Pregnant and breastfeeding women (n=13) were excluded from our analyses due to their different nutritional requirements and low sample size. The final sample included 356 individuals. All the data were weighted to obtain representative estimates of the total population by minimizing biases arising from differences between participating and non-participating persons using the Bootstrap method (Chan et al. 2011). Ethical approval was

granted by the Ethical Review Boards at Health Canada, the University of Northern British Columbia, and the Université de Montréal.

Data on age, sex, weight, height, physical activity level, smoking behavior, educational attainment, household size, employment status, self-perceived health status, source of income, body mass index, and traditional food gathering activity were collected. Food security information was obtained using the income-related Household Food Security Survey Module (HFSSM) (Health Canada 2012b) adapted from the United States Department of Agriculture (USDA) Food Security Survey Module (Nord, Coleman-jensen, Alisha Andrews, and Carlson 2010). The HFSSM was further adapted for Aboriginal communities (Lawn and Harvey 2004). Dietary information was collected by a 24-hour diet recall and a Traditional Food Frequency Questionnaire (FFQ) spanning the entire year and collecting information on the frequency of consumption of all traditional foods available for consumption. In total, the FFQ combined 208 traditional food items including 65 seafood species. Seafood species included fish (n=41), shellfish (n=16), seaweed (n=4), and marine mammals (n=4) reported in the survey.

2.3 Estimation of nutrient intakes and nutrient requirements

Each food item reported to be consumed in the dietary intake assessments were matched to the Canadian Nutrient File, a national food composition database (2015. Health Canada 2015) taking into account the preparation method (i.e. baked or broiled, boiled or raw). Nutrients analyzed in this study included: protein, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamins (A, D, B12, and niacin), and minerals (zinc and selenium, and iron).

We used the Dietary Reference Intake (DRIs), in particular, the Recommended Dietary Allowance (RDA), to assess the contribution of seafood consumption to nutrient requirements (Institute of Medicine 2003). The DRIs are a comprehensive set of nutrient values for healthy

populations used for assessing and planning diets. The RDA is the average daily dietary nutrient intake level that is sufficient to meet the nutrient requirements of nearly all (97.5%) healthy individuals in a particular life stage and gender group (Otten, Jennifer, Hellwig, Jennifer Pitzl 2006). The RDAs were available for all nutrients of interest except EPA and DHA. According to the Dietitians of Canada and the American Heart Association, consumption of 500mg/d EPA+DHA is recommended for the general adult population without cardio-heart diseases (CHD) which is considered to be sufficient to obtain protective effects for primary prevention of cardio-vascular diseases (Academy 2014; American Dietetic Association 2007). Thus, the contribution of EPA+DHA intake to the recommended intake (RI) was estimated. Nutrient requirements are the same for those aged 19 to 30 years and those aged 31 to 50 years; for this reason, these age groups were combined. Age groups containing those between 51 and 70 (inclusive) and those over 70 years of age were also combined due to the small sample size among the elderly.

2.4 Statistical analyses

Data management and statistical analyses were performed using STATA statistical software, 14.2 (StataCorp, College Station, Texas, USA). Descriptive statistics included the calculation of means with standard deviations (SD) for continuous variables and proportions for categorical variables. Student t-tests were performed to assess whether differences between groups were statistically significant. The top 20 most consumed seafood species were determined by ranking the mean intake (mean (95% CI)) of food items. The top 20 species represented almost 90% of total seafood intake, by weight. Ranks were performed for the total population, and for men and women, respectively given the significant sex differences in seafood consumption. Mean daily intakes of selected nutrients from the top 20 seafood species were estimated. The percentage

contributions of total seafood consumption to the nutrient requirements (DRAs and RI) were calculated according to sex and age groups (Otten, Hellwig and Pitzzi 2006; Ross et al. 2011). The mean proportion method was used to estimate percentage contribution of each food species to the DRIs (Krebs-smith et al. 1989). All estimates were weighted in order to obtain representative data at the regional level.

2.5 Combining projected climate effects and food use data

Using data on the projected scenarios of seafood harvest decline (Weatherdon et al. 2016), we modeled the potential impact on nutritional adequacy among coastal FNs in BC. Declines in relative species abundance were calculated under the lower (RCP 2.6) and upper (RCP 8.5) scenarios of climate change using 20-year average abundances for 2050 (2041–2060) relative to 2000, within FNs' domestic fishing areas (DFA). The FNs' DFAs are the geographical areas used for harvesting fish and aquatic plants for food, social and ceremonial purposes. The approximate size of each FNs' DFA was derived from the Statement of Intent (SOI) boundaries registered with the BC Treaty Commission as of October 2004, which were converted to 0.5° latitudinal by 0.5° longitudinal grid-cells of the ocean to correspond with the grid system of DBEM. While these boundaries do not signify the full extent of territory previously used by First Nations, particularly with respect to the sharing of resources between communities, they serve to illustrate approximate areas requested by First Nations for food, social, ceremonial, and commercial fishing purposes (Weatherdon et al. 2016).

The average change of all seafood species was calculated as a sum of weighted contributions of all species to total seafood intake (by weight). The obtained average projection of all seafood species was assumed to represent the changes for species with no data. Changes in nutrient intakes and their contributions to the nutrient recommendations were estimated by subtracting

the expected percent change in seafood consumption under RCP 2.6 and 8.5 from the baseline nutrient intakes. In addition, changes in nutrient intakes were projected after substitution (gram-to-gram) of seafood with alternative foods. For this initial stage of exploring potential impacts, three hypothetical scenarios were developed using simplistic replacement of lost seafood with market foods reported to be among the top 10 for protein, market fish and energy in the 24-hr recall results that are: 1) an alternative land protein source (chicken), 2) an alternative marine protein source (canned tuna), and 3) an energy source (bread) (Chan et al. 2011). These foods are not suggested alternatives but simply serve to illustrate possible nutrient intake under hypothetical substitution scenarios.

3 Results

A total of 356 individuals (140 men and 216 women) were included in this study. Demographic and lifestyle characteristics of the study population are summarized in Table 1. The mean age (\pm standard deviation, SD) of participants was 48.4 (\pm 14.0) years old. The mean BMI was 30.9 (\pm 7.1) kg/m², which is classified as obese (BMI \geq 30kg/m²) according to the World Health Organization (WHO, 2000). Smoking cigarettes was reported by 41% among both men and women. The prevalence of unemployment was 33% while 35% of respondents reported food insecurity. Approximately 71% of individuals participated in traditional food gathering, with 34% reporting fishing, and 23% collecting beach food. Overall, coastal BC FNs consumed 56.3 (\pm 72.5) g/d (grams/person/day, g/d) of seafood (unweighted estimates) (Table 1).

The percentage of consumers (total, and by men and women) and mean intake of the top 20 species as well as total seafood consumption among coastal FNs in BC, are presented in Table 2. Salmon and salmon products (salmon eggs) were the most commonly consumed species. Halibut and herring roe were the second and fourth most consumed species, followed by shellfish

(prawn, clam, crab, and shrimp). The proportion of respondents consuming at least one out of the top 20 most consumed seafood species ranged between 21% and 86% and was comparable between men and women (p values >0.05). Mean intake (95% CI) for each of the top 20 seafood species, except herring roe, was higher among males than female. Overall, the top 20 species represented about 87% of men's total seafood intake and 88% of women's intake, by weight.

Overall, 99% of all participants reported eating seafood in the prior year. Total mean seafood consumption was 78.7 (95% CI: 38.2 - 119.2) g/day among men and 51.5 (95% CI: 37.1 - 65.9) g/day among women. Older participants (>50 years of age) reported significantly higher seafood consumption (80.2 (95% CI: 44.5-115.8)) compared to younger individuals (between 19 and 50 years of age) (41.6 (95% CI: 28.6-54.5)) ($p<0.05$) (weighted estimates, data not shown).

Of the 65 seafood items for which dietary consumption data was collected by the FNFNES, projected abundance (biomass) data were available for 29 distinct species (Table 3). When projections were available for sub-species only (i.e., crab, prawn, clam, shrimp, rockfish, trout, mussel, barnacle, scallop, urchin, and oyster), average group level change was calculated based on the arithmetic mean of the projections for its composite species assemblage. Projections were not available for 17 seafood species reported in the dietary surveys, including four seaweed species (laver, kelp, rockweed, sea lettuce), two marine mammals (sea lion, harbour seal), three beach foods (china slipper (gumboot chiton), octopus, sea prune (black chiton)), and eight fish species (cisco, sucker, whitefish, brook trout, kokanee trout, lake trout, rainbow trout, steelhead trout). Overall, seafood species without projections contributed only 6.5% to the total seafood consumption (seaweed contributed 5% whereas other species – 1.5%) Six food items in the consumption data were different parts (organs, fat and eggs) of the species with available projections whereas thirteen species were not consumed by FNs participants in BC. Projected

effects on relative abundance of seafood species are summarized in Table 3. We ranked seafood species according to the percentage decrease in relative abundance by 2050 relative to 2000. Shrimp, herring, chinook and pink salmon were projected to experience the greatest relative impact of climate change under both RCPs (34% to 60% declines). Sockeye salmon, the major contributor to the total seafood consumption, was projected to decline by 10% to 36% by 2050 relative to 2000. Halibut, the second-most consumed seafood, was estimated to decline by about 12% to 13%. The projected decline in abundance of different shellfish species ranged from 8% to 23% under lower and upper scenarios. We estimated that the average climate-related decline of all seafood species by biomass consumed by FNs was 21% and 31% under RCP 2.6 and 8.5, respectively.

In Tables 4A and 4B, we present the current intake of selected nutrients for men and women, respectively. Nutrients analyzed include protein, n-3 FAs (EPA+DHA), vitamins (A, B12, D, and niacin), and minerals (zinc, selenium, and iron) derived from the top 20 types of seafood individually and, combined, as well as all for men and women separately. Overall, males had higher intakes of selected nutrients because they consumed more seafood than females; however, these difference in nutrient intakes were only statistically significant ($p < 0.05$) for EPA+DHA. The contribution of the top 20 types of seafood to total seafood-related nutrient intake ranged from 50% to 92% (men), and 53 to 92% (women) for different nutrients (by weight).

The contributions of the top 10 seafood species to nutrient recommendations for men and women are presented in Figs 2A-2B. Sockeye salmon provided a major contribution to the DRAs and RI. For men and women respectively, sockeye salmon alone provided 44% and 24% of EPA+DHA, 43% and 23% of vitamin B12, 16% and 8.5% of vitamin D, 11% and 7% on niacin, and 12% and 6.5% of selenium. Sockeye salmon contributed less than 10% to the protein, vitamin A, zinc and

iron recommendations. Other salmon sub-species (chinook, coho, chum) and fish eggs (salmon eggs and herring roe) contributed large amounts of EPA+DHA, vitamins B12, D, niacin, and selenium in accordance to their contributions by weight. Halibut was the second-most consumed species yet contributed little to attaining requirements of selected nutrients (0.5% to 6.5%).

Shellfish such as clams and crabs notably contributed to vitamin B12 requirements (19% and 13% in men, and 17% and 6% in women, respectively). Prawns yielded low intake of nutrients, contributing 0.2% to 2% to the recommended nutrient intake (Figs 2A-2B). Intakes of protein, vitamin A, zinc, and iron from the top 10 types of seafood individually, were low among both men and women. However, the top 10 seafood collectively provided 16% and 13% of protein, 4% and 3% of vitamin A, 4.5% and 4.0% of zinc, and 3.9% and 1.9% of iron among men and women, respectively.

The contribution of total seafood consumption to the nutrient recommendations varied by gender and age groups (Fig 3), reflecting differences in seafood consumption (by weight). Among older participants (>50 years of age), total seafood consumption provided approximately two times more nutrients compared to the younger age group (19 to 50 years of age). Likewise, men showed 1.5 to 2 times higher intakes of selected nutrients from seafood than women (Fig 3). Overall, the total seafood supplied substantial levels of nutrients contributing 79-184% to EPA+DHA, 84-152% to vitamin B12, 28 - 55% to niacin, and 29 -55% to selenium recommendations in different gender and age groups. Seafood was found to be an excellent source of vitamin D and protein, providing between 15% and 30%, and 14% and 30%, respectively, of the age/gender-specific recommendations. The contribution of total seafood to vitamin A, zinc, and iron recommendations was low ranging from 4 to 6%, 8 to 12%, and 3% to 13%, respectively, in different age/gender groups (Fig 3).

The impacts of projected climate change on seafood contributions to the DRIs in coastal FNs by age and gender groups are presented in Figures 4A-4B. The weighted average of total seafood decline was estimated to be 21% under lower and 31% under upper climate change scenarios. Consequently, the overall contribution of seafood to the DRIs was modeled to decrease by 21 and 31% by 2050, which would significantly reduce the intake of selected nutrients. The greatest impacts on nutritional adequacy will be observed among individuals that substantially rely on traditional seafood consumption (e.g. men and older age groups), especially for those nutrients that are primarily obtained from marine sources, such as EPA+DHA, vitamin D, selenium and vitamin B12.

We projected changes in nutrient intakes after gram-to-gram substitution of the projected decline in seafood consumption with proposed alternative foods (Supplemental material, table S1). Three hypothetical scenarios were modeled: seafood lost replaced by 1) chicken, 2) canned tuna, and 3) bread. If the chicken were used to replace 21% and 31% of reduced seafood consumption, there were no impacts on protein, niacin, and iron, while only 30% of zinc and selenium, and 50% of vitamin A intake would be replaced. However, the intake of EPA+DHA, vitamin B12, and vitamin D would be decreased by almost 100%. The substitution of traditional seafood with canned tuna would replace the amounts of protein, niacin, selenium, and iron by 100%, and half of the amount of zinc and vitamin B12; nevertheless, EPA+DHA, vitamin A intakes would be substantially reduced compared to that provided by traditional sources of seafood. Finally, if bread was used to replace traditional seafood (gram-to-gram), only iron intake would not be compromised; niacin, zinc, and selenium would be partly covered (commercially enriched) whereas protein, EPA+DHA, vitamins A, B12, and D would not.

4 Discussion

The High Level Panel of Experts (HLPE) on Food Security and Nutrition of the Committee on World Food Security, has the need to develop scenarios to understand the possible impacts of climate change on the food security and nutrition of the most vulnerable zones (HLPE 2014). Canada's Indigenous populations, particularly those residing in rural, remote, and coastal regions, disproportionately experience the effects of climate change (Austin et al. 2015; Ford et al. 2010). Moreover, given their lower socioeconomic status, sociopolitical marginalization, and reduced access to healthful foods, they are particularly vulnerable to the consequences of climate change on food and nutrition security (Furgal and Seguin 2006). By modeling the effect of climate-related declines in seafood abundance on the nutritional adequacy of diets for coastal BC FNs, our results suggest that intakes of essential nutrients will decline by 21% to 31% under lower and upper scenarios of climate change by the year 2050 relative to 2000. Moreover, substitutions of seafood with selected alternative foods, such as chicken, canned tuna and bread, do not provide adequate amounts of selected nutrients.

The modeled scenarios of the impacts of the seafood production decline on nutrient intakes did not account for changes in FNs population size over time. According to the most recent census, the number of FNs people with registered or treaty Indian status rose by 30.8% from 2006 - 2016 (Statistics Canada 2017b). Recent population projections revealed that between 2001 and 2011, the registered Indian population rose at an average annual rate of 2.4%, and was projected to range between 1.4 to 1.8% from 2011 to 2036 (over 25 years) (Statistics Canada 2015). For comparison, the growth rate of the non-Aboriginal population in Canada would average 1.0% annually (Statistics Canada 2015).

Using the FN's population growth rate projection, we estimated the possible increase in numbers of BC FN's by 2050 relative to 2009 (BC FNFNES data collection) as well as the corresponding amounts of seafood needed to supply the growing population.

With an average annual growth rate of 2.4% from 2009 to 2011 and of 1.6% from 2011 to 2050 (Statistics Canada 2015), our sample of 356 individuals will increase to 693 by 2050 (almost double). In 2009, coastal BC FN's consumed about 60g/d per person of seafood; therefore, 21360 g/d of seafood (i.e., $60\text{g/d} \times 356 = 21360$) were needed on a daily basis. By 2050, the same amount of seafood will provide only 30.8 g/d per person (i.e., $21360\text{ g/d}/693 = 30.8\text{ g/d}$) which is about half of the baseline consumption. Thus, two times more seafood will be required to supply coastal FN's population in BC by 2050 relative to 2009. In contrast, seafood harvest potential will decline by 21% - 31% by 2050 relative to 2000.

Under these potential conditions, an average seafood consumption will range between 21.3g/d to 24.3g/d compared to the baseline (i.e. 60g/d). Collectively, the declining seafood harvest and an increased request of seafood will result in nutrient intakes decreased by about 60% - 65% compared to the baseline intake. This again underpins that decline in seafood production will result in severe consequences for nutrition health and food security in coastal FN's in BC. It is important to note that there has been already a steep decline in both the variety and amount of traditional food in the dietary pattern described here (Butler and Campbell 2004; Colin 2017; Moss 1993) which can serve to explain the limited contribution of current seafood intake for protein, vitamin A, zinc and iron. Carbon isotope measurements suggest that pre-contact, adult coastal people obtained 90% of their protein from marine sources (Chisholm and Nelson 1983). Shellfish (a rich source of iron), and other beach foods had a much larger relative contribution (Kuhnlein 1989; Moss 1993) than today where few beaches remain open to harvesting because

of development and contamination (sanitary or biotoxin) and foreshore leases. Other keystone species, like eulachon and eulachon grease (important contributor of vitamin A) are rarely available. For salmon alone, estimates suggest that at pre-contact, annual per capita use, at least among some Coast Salish communities was around 316 kg/year (0.9 kg/capita/day) (Bennett 1971; Chisholm and Nelson 1983). This serves to highlight, while it is difficult to know with certainty what portion was used for purposes other than food, that current seafood use and its relative contributions of nutrients is at a critically low level. Climate change, while likely to have a further impact on the seafood contribution to protein and vitamin D may have relatively minor changes on nutrients such as iron, zinc and vitamin A, as cornerstone species such as bivalves and eulachon are already at minimal levels in the diet.

Micronutrients (vitamins and minerals) are essential for human health, growth, and development. Micronutrient deficiencies have profound health effects throughout the life-course. In childhood, malnutrition leads to impaired growth and cognitive development and infectious diseases (Mosby et al., 2017). During pregnancy, undernutrition increases the risk of stillbirths, pre-term births, complications of labour and decreased offspring birth weight (Victoria et al., 2008).

Deficiencies in micronutrients are also associated with numerous health disorders and related chronic disease, such as anemia (vitamin B12, iron), osteoporosis and osteomalacia (vitamin D, calcium), cardiovascular diseases and diabetes (EPA and DHA, selenium, vitamin D), night blindness (vitamin A), pellagra (niacin), impaired thyroid and immune function, and growth retardation (zinc) (Eckhardt 2006; Hoeft, Weber, and Eggersdorfer 2012; Tulchinsky 2010).

Locally-harvested seafood continues to provide a significant source of selected nutrients, such as protein, vitamins (A, B12, niacin, D), minerals (zinc, selenium, and iron) and n-3 FAs for coastal BC FNs. Seafood alone supplies daily recommendations of EPA+DHA and vitamin B12, as well

as substantial levels of other nutrients. Given low or insufficient intake of selected nutrients in the total diet of BC FNs (Chan et al. 2011), seafood consumption plays a critical role in preventing nutrient deficiencies. As such, nutritional health of coastal BC FNs is highly vulnerable to any declines in seafood consumption – including declines related to changing climate and species abundance. Along the effects on diet quality, the decline in seafood consumption has detrimental impacts on mental health as well as cultural practices, language, self-determination and social cohesion.

Among a wide variety of seafood species reported to be consumed by BC FNs, salmon was the most frequently consumed, which reflects its relative abundance in BC coastal ecosystems (Johannes and Council 2007) as well as its status as a cultural keystone species and preferred food for BC FNs (Garibaldi and Turner 2004). Salmon species (collectively) supplied over 70% of EPA+DHA recommendation, 50% of vitamin B12, 20% of vitamin D, 18% of niacin, 17% of selenium, and 10% of protein requirements. The diversity of salmon species is an important dimension of food security and cultural stability for BC FNs. Greater population and species diversity of salmon is associated with more stable catch and longer fishing seasons (Nesbitt and Moore 2016). In recent years, however, salmon returns have been lower than predicted, and the lowest in the past 120 years due, in part, to the warming ocean temperature (Morton Brian 2016). With climate change affecting salmon abundance, protecting and increasing diversity among salmon populations may help provide a buffer against food insecurity among BC FNs (Nesbitt and Moore 2016). Climate change adaptation strategies should incorporate Indigenous traditional ecological knowledge (Williams et al., 2013).

Food insecurity is an important social determinant of health for FNs and is directly related to low incomes, high costs of nutritious store-bought foods, and constraints in accessing traditional

foods (Kuhnlein et al. 2013; Teresa et al. 2012; Willows 2005). According to the 2008-2009 BC FNFNES, 75% of respondents observed that climate change was affecting the availability of traditional foods for harvest, while almost half the respondents reported that climate change decreased the availability of traditional foods in their households (Chan et al. 2011). Aside from climate change, there are other barriers to traditional food consumption. Although Canadian courts have established that food, social and ceremonial fisheries of Indigenous Peoples have priority over all other uses of the resource (Harris and Millerd 2010), government restrictions, fleet rationalization programs, commercial and recreational harvest allocation, and other industrial activities such as forestry, hydroelectricity, mining, farming and oil/gas industries were noted by BC FNs as potential barriers to traditional food consumption (Chan et al. 2011). Over two-thirds (68%) of FNs in BC said that all barriers, when combined, decreased access to salmon and other fish species (Chan et al. 2011).

We inevitably had to simplify the complex interactions between climate change, ecological responses, and fisheries governance impacts on fisheries and nutritional health; these assumptions can be further refined in the future to provide more detailed projections of climate change effects on FNs' food security. Firstly, the coarse resolution of the earth system model used by DBEM renders fine scale interpretation of the DBEM outputs uncertain. Thus, some of the meso-scale processes and features that may determine the fine scale distribution, changes in ecosystem productivity or abundance could not be presented in the model projection. Secondly, we only used one earth system model (GFDL ESM2M). Inter-model comparison of different earth system model outputs suggests that projected effects of climate change using GFDL ESM2M are likely to be more conservative relative to projections using other earth system models (Weatherdon et al. 2016). Thirdly, we assumed a linear relationship between changes in

species abundance and seafood availability for FNs. However, responses of FN fishing activities, including shifts in fishing grounds, fishing effort, gear modification and changes in targeted species may also affect seafood availability under climate change. Also, changes in the exploitation status of fish stocks, fishing effort of BC FN as well as other commercial and recreational fisheries, and the allocation of resources to different fisheries would affect the availability of fish to BC FN directly (Baum and Fuller 2016). This study assumes that these are all kept relatively unchanged in the future which is a main source of scenario uncertainty (Cheung et al. 2016). Overall, our analysis and conclusions on the broad-scale trends and comparison between climate change scenarios should be robust to the above assumptions. Future studies could include alternative fishing and governance scenarios to further explore these critical dimensions of access to traditional foods. Furthermore, traditional ecological knowledge which are essential in identifying strategies for climate change adaptation and the implementation of sustainable land-management principles, should be also incorporated.

Our model results show that decreases in nutrient intakes as a result of the potential decline in seafood harvest cannot be easily replaced by market foods such as chicken, canned tuna or bread. It is important to note that the results of this modeling exercise are intended to be used by local resources managers and public health professionals as a starting point to develop adaptation plans for climate change only. There are many underlying factors such as the distribution of seafood among members in the communities, food preferences of individuals, price elasticities and access to alternative foods, etc. that will affect the relationship between fish harvest and intake. This information, as well as local data on alternative food choices, need to be collected for more realistic and relevant models. Another limitation of this exercise is the insufficient data or lack of information for sensitive sub-populations such as pregnant/lactating women and

children. Since these sub-populations are the most nutritionally vulnerable, they will likely be most affected by the impacts of declining availability of seafood.

5 Conclusion

Traditionally-harvested seafood remains fundamental to the contemporary diet of coastal BC FNs and provides substantial levels of essential nutrients. Dietary shifts aggravated by climate-related declines in seafood consumption may have significant nutritional and health implications for BC FNs. It is important to note that the traditional diet of coastal FN communities has already been declining as a result of social and environmental changes and has led to the inadequate intakes of important nutrients. For example, from the Fraser River to northern BC, eulachon which was formerly a staple for coastal communities, and is known to be an excellent source of vitamin A, has almost disappeared. Similarly, bivalves, an excellent source of protein and iron, has diminished substantively. These major changes account for some of the limited changes in iron and vitamin A seen from the climate change modelling. Therefore, strategies to improve seafood harvest potential and access rights to coastal communities are needed to ensure nutritional health and overall well-being, and to promote food security and food sovereignty in coastal FNs communities. In particular, more rigorous adaptations scenarios and a transformation in fisheries governance, involving participatory methodologies in incorporating traditional ecological knowledge, livelihood objectives and perspectives, are needed to address the nutritional burden facing FNs' diets.

Acknowledgments

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Figure 1. Map of participating First Nations communities in British Columbia

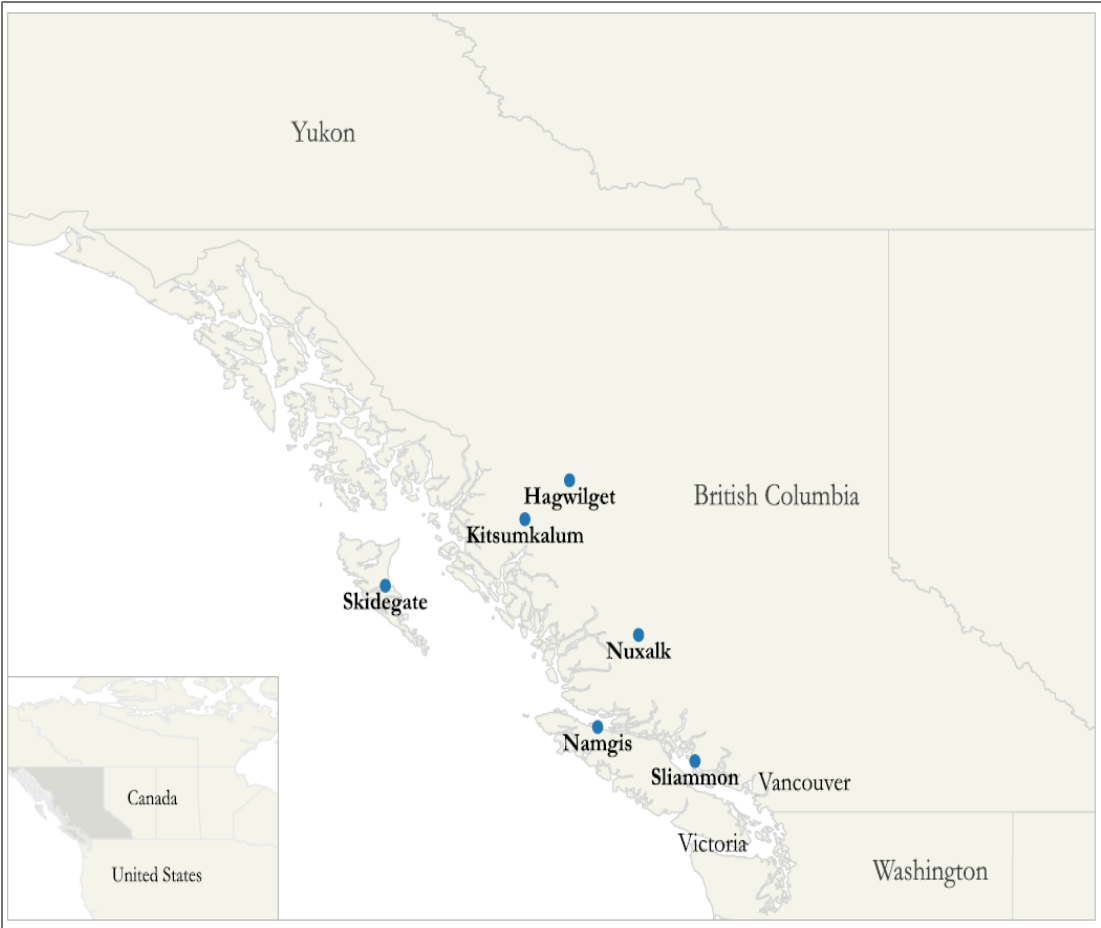


Table 1. Descriptive characteristics of coastal First Nations in British Columbia

	mean/n	SD/%
n	356	100
Age, year (mean, SD)	48.4	14
Female (n, %)	216	61
Smoking (n, %)	145	41
Dieting (n, %)	44	12
BMI (kg/m ²) (mean, SD)	30.9	7.1
Physical inactivity (n, %)	66	18
Health status (n, %)		
excellent/very good	99	28
good	141	39
fair/poor	116	33
Years of education (mean, SD)	11.1	2.7
Household size (no. of people) (mean, SD)	3.1	1.9
Unemployment (n, %)	116	33
Food insecurity (n, %)	126	35
Income sources (n, %)		
wages	193	54
social assistance	99	27
workers compensation	15	4
pension	49	14
Traditional activity (n, %)		
any	251	71
fishing	121	34
collecting seafood	82	23
Seafood consumption (g/d) (mean, SD)	56.3	72.5
Seafood consumption (19-50y) (mean, SD)	52.0	58.2
Seafood consumption (50+y) (mean, SD)	62.1	87.7

*SD, Standard Deviation; %, percent; n, number; unweighted estimates
Dieting (on the day before the interview) in order to lose weight
Traditional activity, traditional food gathering activity by participants
Food insecurity combines moderate and severe food insecurity
Pregnant and lactating women were excluded (n=13)*

Table 2. Top 20 most consumed seafood species in coastal First Nations in British Columbia, ranked by from greatest to least mean intake (total)^a

Seafood	Total			Men			Women		
	%	mean	95% CI	%	mean	95% CI	%	mean	95% CI
Sockeye salmon	85	12.20	6.4, 18.0	85	18.0	6.5, 29.4	86	9.6	5.7, 13.6
Halibut	82	5.81	2.9, 8.7	82	6.4	2.5, 10.2	83	5.6	2.8, 8.3
Chinook salmon	57	3.95	1.3, 6.6	62	5.8	1.8, 11.9	54	3.1	1.5, 4.7
Herring roe	62	3.01	2.1, 3.0	52	2.0	1.3, 6.6	67	3.4	1.5, 2.4
Coho salmon	54	2.54	0.5, 5.5	55	3.9	0.9, 3.1	53	1.9	0.5, 6.4
Prawn	53	2.24	0.2, 4.4	55	2.9	1.1, 4.6	51	2.0	0.3, 4.3
Clam	67	2.20	1.0, 3.3	66	2.3	1.8, 2.8	68	2.1	0.6, 3.5
Salmon egg	41	2.11	0.1, 4.2	44	2.5	0.5, 4.6	38	1.9	0.2, 4.0
Chum salmon	42	2.10	0.5, 3.1	46	2.6	1.1, 4.7	38	1.9	0.4, 2.4
Crab	59	1.82	1.6, 1.9	59	2.9	1.0, 1.9	59	1.4	1.7, 2.1
Shrimp	46	1.80	1.7, 1.9	33	1.5	1.0, 1.9	52	2.0	1.7, 2.2
Eulachon grease	58	1.64	0.2, 3.1	45	1.5	0.3, 2.7	64	1.7	0.1, 3.3
Pink salmon	33	1.56	0.8, 2.3	36	2.8	0.1, 5.5	32	1.0	0.0, 2.0
Rockfish	40	1.54	1.3, 1.8	47	3.1	1.3, 4.8	37	0.9	0.6, 1.1
Ling cod	29	1.45	0.7, 2.2	42	1.2	0.8, 1.6	22	1.6	0.6, 2.4
Eulachon	53	1.34	0.4, 2.3	43	1.9	0.8, 2.9	58	1.1	0.2, 2.0
Black cod	28	1.03	0.1, 2.0	34	1.2	0.1, 2.2	25	1.0	0.0, 1.9
Pacific cod	28	1.01	0.4, 1.6	25	1.4	0.3, 2.5	29	0.8	0.4, 1.2
Basket cockle	50	0.93	0.6, 1.3	44	1.0	0.4, 1.5	53	0.9	0.5, 1.3
Trout, any	25	0.76	0.1, 1.4	21	1.7	0.6, 4.1	26	0.3	0.2, 0.5
Top 20 combined	98	52.5	37.9, 67.2	98	68.6	37.0, 100.2	99	45.4	35.0, 55.9
Total seafood	99	59.9	40.3, 79.4	98	78.7	38.2, 119.2	99	51.5	37.1, 65.9

^a - Data from the First Nation Food, Nutrition and Environment Study in British Columbia (2008-09), FFQ questionnaire, Individuals aged 19 years+

% - percent consumers of a respective food; mean intake (g/person/day) of each food item based of the sample of 351 (out of 356) consumers of seafood;

Top 20 seafood species represented 87% (males), and 88% (females) of total seafood intake, by weight; weighted estimates

Table 3. Projected changes in relative abundance of seafood species under lower (RCP 2.6) and upper (RCP 8.5) scenario of climate change in coastal First Nations in British Columbia by 2050 relative to 2000*

Seafood	Difference (%)	
	lower	upper
Shrimp	46.1	64.1
Herring	31.8	48.7
Chinook salmon	47.8	46.8
Pink salmon	40.3	44.1
Eulachon	26.4	37.6
Sockeye salmon	10.2	36.2
Pacific cod	12.6	35.0
Starry flounder/English sole	21.6	28.9
Dolly varden trout	10.8	28.1
Sea urchin	13.6	25.9
Mussel	10.6	23.9
Trout, any	10.1	22.4
Prawn	12.4	18.1
Oyster	17.8	18.3
Cutthroat trout	9.4	16.6
Coho salmon	8.8	15.2
Sea cucumber	12.8	14.9
Abalone	10.5	13.9
Crab	12.8	9.7
Rockfish	7.9	9.2
Halibut	12.3	13.0
Chum salmon	9.6	12.1
Scallops	8.0	11.2
Basket cockle	12.6	11.1
Barnacle	11.7	10.8
Clams	9.3	4.9
Black cod	10.8	9.2
Ling cod	8.7	7.3
Kelp greenling	7.7	+2.2

* Lower and upper scenarios of climate change represent the low and high greenhouse gas emission scenarios based on evidence of latitudinal and regional trends. Declines in relative abundance were projected by 2050 (relative to 2000) for seafood species within British Columbia's marine environment under both scenarios of climate change (Weatherdon et al., 2016)

Table 4A. Mean daily intake of selected nutrients by First Nations men, derived from the top 20 types of seafood^a

Seafood	Protein g	EPA+DHA mg	vitamin D µg	vitamin A µg RAE*	vitamin B12 µg	Niacin mg NE ^{''}	Zinc mg	Selenium µg	Iron mg
Sockeye salmon	4.57	221.14	2.36	12.41	1.02	1.74	0.09	6.56	0.09
Halibut	1.43	15.24	0.30	1.52	0.08	0.50	0.03	3.52	0.01
Chinook salmon	1.50	101.51	0.75	8.69	0.17	0.87	0.03	2.73	0.05
Herring roe	0.45	47.19	0.24	1.63	0.16	0.04	0.02	0.81	0.01
Coho salmon	0.92	41.47	0.44	2.00	0.20	0.48	0.02	1.49	0.02
Prawn	0.50	5.02	0.00	1.77	0.02	0.05	0.04	1.08	0.01
Clam	0.59	6.61	0.00	3.98	0.45	0.19	0.06	1.49	0.07
Salmon eggs	0.69	60.98	0.00	0.00	0.00	0.14	0.02	0.00	0.02
Chum salmon	0.55	29.99	0.17	0.46	0.11	0.18	0.03	1.11	0.02
Crab	0.64	11.36	0.00	0.89	0.30	0.10	0.16	1.37	0.01
Shrimp	0.33	4.09	0.00	1.31	0.02	0.04	0.02	0.72	0.00
Eulachon grease	0.00	15.03	0.00	0.32	0.00	0.00	0.00	0.00	0.00
Pink salmon	0.64	29.88	0.40	0.55	0.14	0.32	0.03	1.10	0.02
Rockfish	0.68	10.58	0.14	0.15	0.05	0.24	0.01	2.34	0.01
Ling cod	0.28	3.21	0.00	0.21	0.05	0.08	0.01	0.57	0.01
Eulachon	0.28	58.61	0.00	0.17	0.00	0.08	0.00	0.00	0.02
Black cod	0.20	20.91	0.00	1.19	0.02	0.10	0.00	0.55	0.02
Pacific cod	0.27	2.27	0.01	0.03	0.03	0.07	0.01	0.40	0.00
Basket cockle	0.26	2.84	0.00	1.71	0.20	0.08	0.03	0.64	0.00
Trout, any	0.45	15.90	0.08	0.32	0.13	0.18	0.01	0.28	0.03
Top 20 combined	15.21	703.83	4.01	39.32	3.15	5.87	0.85	26.74	0.43
Total seafood	17.80	817.83	4.49	47.83	3.63	7.55	1.21	28.92	0.86
% top 20 to total seafood	85%	86%	89%	82%	87%	78%	70%	92%	50%

^a - Data from the First Nation Food, Nutrition and Environment Study in British Columbia (2008-09), FFQ questionnaire, Individuals aged 19 years+

* - Retinol activity equivalents, RAE; '' - Niacin equivalents, weighted estimates

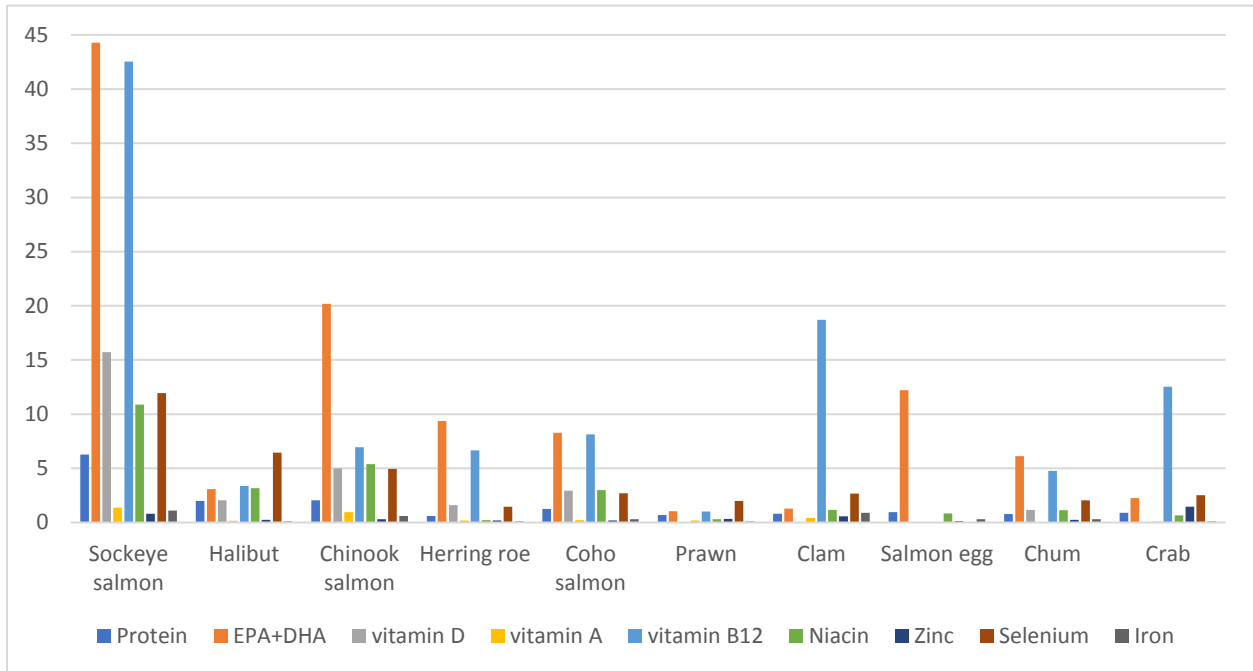
Table 4B. Mean daily intake of selected nutrients by First Nations women, derived from the top 20 types of seafood^a

Seafood	Protein g	EPA+DHA mg	vitamin D µg	vitamin A µg RAE*	vitamin B12 µg	Niacin mg NE"	Zinc mg	Selenium µg	Iron mg
Sockeye salmon	2.45	118.46	1.26	6.65	0.55	0.93	0.05	3.52	0.05
Halibut	1.25	13.36	0.27	1.34	0.07	0.44	0.02	3.08	0.01
Chinook salmon	0.80	54.26	0.40	4.65	0.09	0.46	0.02	1.46	0.03
Herring roe	0.77	80.62	0.42	2.79	0.28	0.06	0.03	1.39	0.02
Coho salmon	0.45	20.52	0.22	0.99	0.10	0.24	0.01	0.74	0.01
Prawn	0.34	3.45	0.00	1.22	0.02	0.03	0.02	0.74	0.00
Clam	0.55	6.10	0.00	3.67	0.42	0.17	0.06	1.37	0.06
Salmon eggs	0.52	46.14	0.00	0.00	0.00	0.10	0.01	0.00	0.01
Chum salmon	0.41	22.30	0.13	0.34	0.08	0.13	0.02	0.82	0.01
Crab	0.30	5.33	0.00	0.42	0.14	0.05	0.07	0.64	0.01
Shrimp	0.44	5.47	0.00	1.76	0.03	0.05	0.03	0.97	0.01
Eulachon grease	0.00	17.03	0.00	0.36	0.00	0.00	0.00	0.00	0.00
Pink salmon	0.24	11.01	0.15	0.20	0.05	0.12	0.01	0.40	0.01
Rockfish	0.19	2.96	0.04	0.04	0.01	0.07	0.00	0.65	0.00
Ling cod	0.35	4.08	0.00	0.26	0.06	0.10	0.01	0.73	0.01
Eulachon	0.16	34.13	0.00	0.10	0.00	0.05	0.00	0.00	0.01
Black cod	0.17	17.87	0.00	1.02	0.01	0.08	0.00	0.47	0.02
Pacific cod	0.15	1.32	0.00	0.02	0.02	0.04	0.00	0.23	0.00
Basket cockle	0.23	2.57	0.00	1.55	0.18	0.07	0.02	0.58	0.00
Trout, any	0.09	3.25	0.02	0.07	0.03	0.04	0.00	0.06	0.01
Top 20 combined	9.87	470.21	2.17	27.43	2.01	3.89	0.51	16.60	0.28
Total seafood	12.17	511.35	2.43	31.69	2.25	4.93	0.71	17.99	0.53
% top 20 to total seafood	81%	92%	89%	87%	89%	79%	72%	92%	53%

^a - Data from the First Nation Food, Nutrition and Environment Study in British Columbia (2008-09), FFQ questionnaire, Individuals aged 19 years+

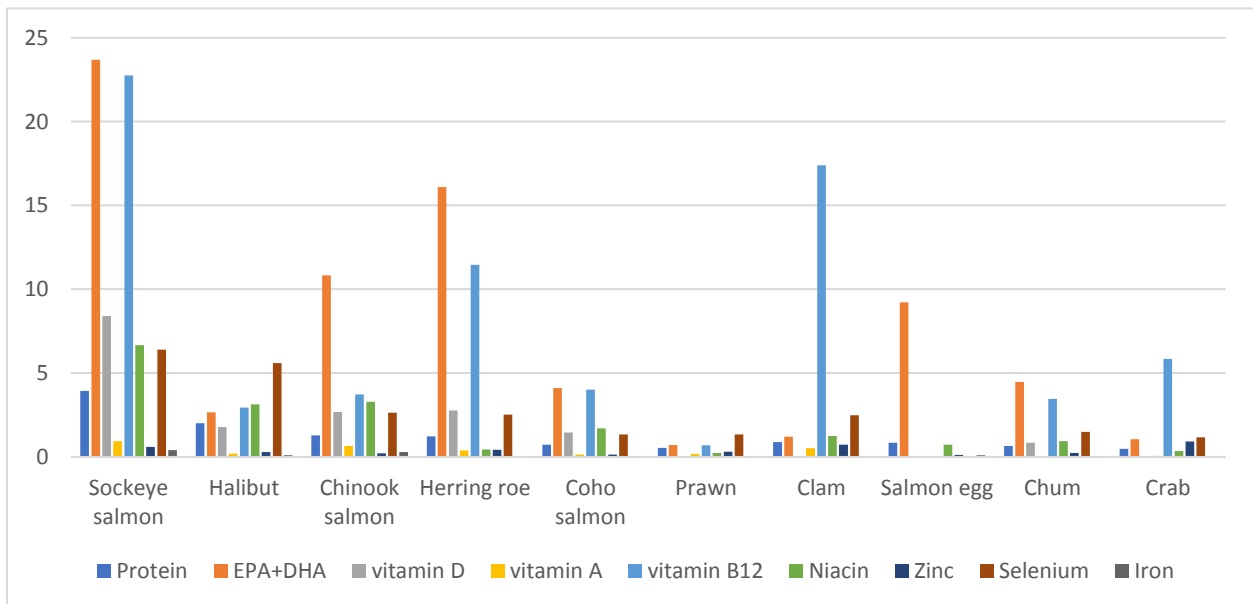
* - Retinol activity equivalents, RAE; " - Niacin equivalents, weighted estimates

Figure 2A. Percentage contribution of top 10 most consumed seafood species to nutrient requirements (DRIs) in men.



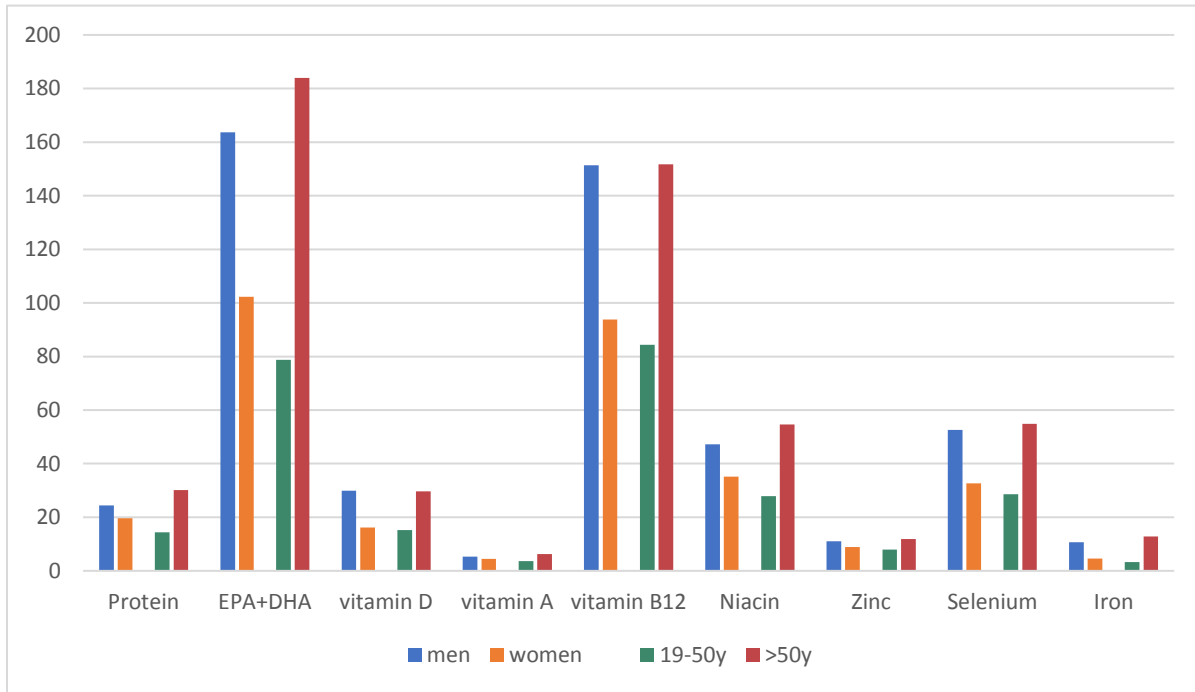
DRI - dietary reference intakes using recommended dietary allowance (RDA) and recommended intake (RI) for EPA+DHA

Figure 2B. Percentage contribution of top 10 most consumed seafood species to nutrient requirements (DRIs) in women.



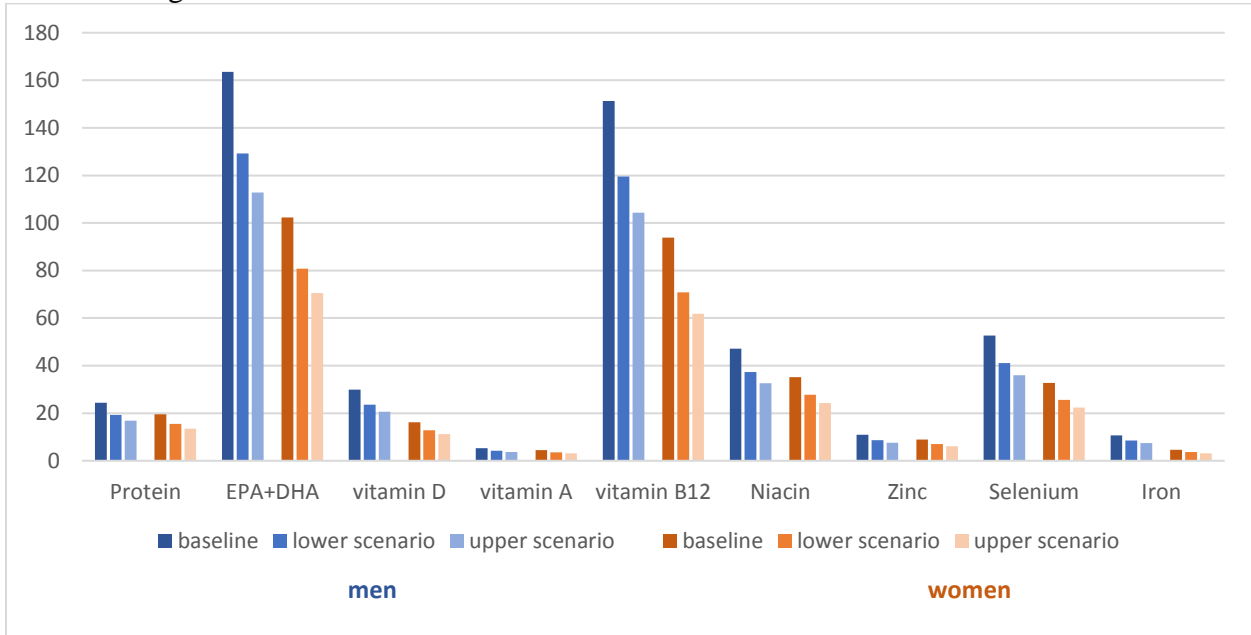
DRI - dietary reference intakes using recommended dietary allowance (RDA) and recommended intake (RI) for EPA+DHA

Figure 3. Percentage contribution of total seafood intake to nutrient requirements (DRIs) by gender and age groups.



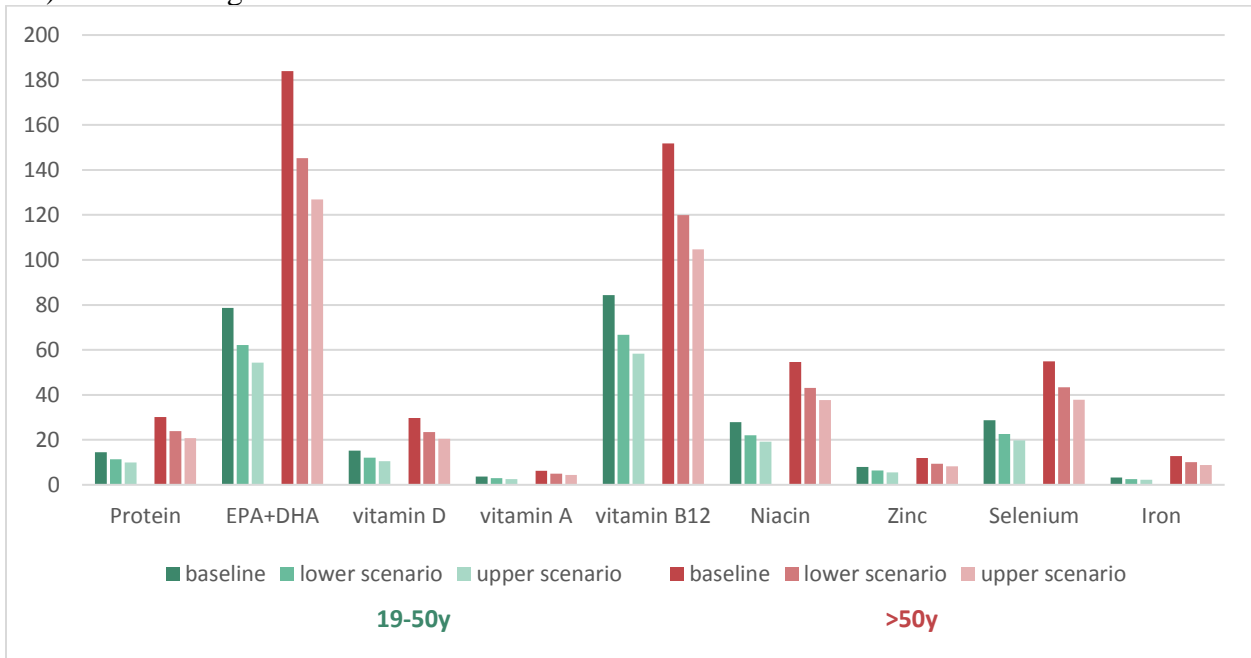
DRI - dietary reference intakes using recommended dietary allowance (RDA) and recommended intake (RI) for EPA+DHA

Figure 4A. Baseline and projected percentage contributions to the DRI from total seafood in First Nations by gender under ‘strong mitigation’ (RCP 2.6) and ‘business-as-usual’ (RCP 8.5) climate change scenarios.



DRI - dietary reference intakes using recommended dietary allowance (RDA) and recommended intake (RI) for EPA+DHA

Figure 4B. Baseline and projected percentage contributions to the DRIs from total seafood in First Nations by age groups under ‘strong mitigation’ (RCP 2.6) and ‘business-as-usual’ (RCP 8.5) climate change scenarios.



DRI - dietary reference intakes using recommended dietary allowance (RDA) and recommended intake (RI) for EPA+DHA

Supplemental material

Table S1. Projected changes in nutrient intakes after substitution by potential alternative foods (chicken, canned tuba, and bread)¹

	Protein	EPA+DHA	Vitamin D	Vitamin A	Vitamin B12	Niacin	Zinc	Selenium	Iron
	g	mg	µg	µg RAE*	µg	mg NE ^{''}	mg	µg	mg
baseline nutrient intake ²	13.90	598.72	3.07	36.65	2.61	5.74	0.87	21.17	0.63
lower scenario ⁶	10.98	472.99	2.42	28.95	2.06	4.53	0.68	16.72	0.5
upper scenario ⁶	9.59	413.12	2.12	25.29	1.80	3.96	0.60	14.61	0.43
replaced by chicken ³									
lower scenario ⁶	14.74	472.99	2.44	32.48	2.10	6.85	0.81	19.84	0.63
upper scenario ⁶	15.14	413.12	2.13	30.50	1.86	7.38	0.79	19.20	0.63
replaced by canned tuna ⁴									
lower scenario ⁶	14.20	473.02	2.57	28.95	2.43	6.80	0.78	26.85	0.69
upper scenario ⁶	14.34	413.17	2.34	25.29	2.35	7.31	0.74	29.56	0.72
replaced by bread ⁵									
lower scenario ⁶	12.14	472.99	2.46	28.95	2.06	5.29	0.79	20.62	0.96
upper scenario ⁶	11.29	413.12	2.17	25.29	1.80	5.08	0.76	20.35	1.11

¹ - Average daily intake of nutrients, estimated base on gram-to-gram replacement by alternative foods

² - Baseline nutrient intakes from seafood based on the food frequency questionnaire of the FNFNES

³ - Chicken: nutrient content is based on most popular preparation method (chicken breast meat and skin roasted)

⁴ - Canned tuna: nutrient content is based on most reported type of canned tuna (tuna, light, canned in water, drained, unsalted)

⁵ - Bread: nutrient content is based on most reported type of bread (bread, white, commercial)

⁶ - decline in seafood consumption under lower (21%) and upper (31%) scenarios of climate change

* - Retinol activity equivalent, RAE, '' - Niacin equivalent

Bolded are amount of nutrients not substituted by alternative foods

Table S2. Nutrient content of top 20 most consumed seafood species (Canadian Nutrient File, Health Canada, 2015)

Seafood	Protein g/100g	EPA+DHA mg/100g	vit D µg/100g	vit A µg*/100g	vit B12 µg/100g	Niacin mg"/100g	Zinc mg/100g	Selenium µg/100g	Iron mg/100g
Sockeye salmon	25.4	1230	13.1	69.0	5.7	9.7	0.5	36.5	0.5
Halibut	22.5	240	4.8	24.0	1.3	7.9	0.4	55.4	0.2
Chinook salmon	25.7	1740	12.9	149.0	2.9	14.9	0.6	46.8	0.9
Herring roe	22.3	2340	12.1	81.0	8.0	1.8	1.0	40.3	0.6
Coho salmon	23.5	1060	11.3	51.0	5.0	12.3	0.6	38.0	0.6
Prawn	17.4	176	0.1	62.0	0.9	1.7	1.2	37.8	0.2
Clam	25.6	284	0.1	171.0	19.5	8.1	2.7	64.0	2.8
Salmon eggs	27.0	2400	0.0	0.0	0.0	5.4	0.6	0.0	0.7
Chum salmon	21.4	1175	6.7	18.0	4.4	7.0	1.0	43.3	0.7
Crab	22.3	394	0.0	31.0	10.4	3.6	5.5	47.6	0.4
Shrimp	22.8	280	0.1	90.0	1.7	2.7	1.6	49.5	0.3
Eulachon grease	0.0	1000	0.0	21.0	0.0	0.0	0.0	0.0	0.0
Pink salmon	23.1	1077	14.5	20.0	5.0	11.6	1.0	39.5	0.8
Rockfish	22.2	345	4.6	5.0	1.6	7.8	0.4	76.2	0.4
Ling cod	22.6	263	0.0	17.0	4.2	6.5	0.6	46.8	0.4
Eulachon	14.6	3100	0.0	9.0	0.0	4.2	0.0	0.0	0.9
Black cod	17.2	1787	0.0	102.0	1.4	8.3	0.4	46.8	1.6
Pacific cod	18.7	160	0.6	2.0	2.3	5.2	0.4	28.0	0.2
Basket cockle	25.6	284	0.1	171.0	19.5	8.1	2.7	64.0	0.4
Trout, any	26.6	936	5.0	19.0	7.5	10.7	0.9	16.2	1.9

* - µg RAE, retinol activity equivalent, "– mg NE, niacin equivalent

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9 POTENTIAL IMPACTS OF REDUCED SEAFOOD CONSUMPTION ON
CARDIOVASCULAR HEALTH AMONG COASTAL FIRST NATIONS IN BRITISH
COLUMBIA

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ABSTRACT

Background: Climate change was projected to reduce seafood consumption and intake of essential nutrients, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), among coastal First Nations (FNs) in British Columbia (BC). This may represent serious implications for cardiovascular health of FNs. **Objective:** To model the impacts of reduced seafood consumption and consequent EPA+DHA intake on the relative risk (RR) of myocardial infarction (MI) among FNs adults in BC. Specifically, we will project the combined effects of reduced EPA+DHA and methylmercury (MeHg) intake from seafood on the RR of MI.

Methods: Data were derived from the cross-sectional 2008-09 BC First Nations Food, Nutrition, and Environment Study. Seafood consumption was estimated with a food frequency questionnaire among 369 FNs adults. EPA+DHA intake was calculated using the Canadian Nutrient File. Hair Hg concentrations were measured in FNs. Model formulas and assumptions were extracted from various epidemiology studies. **Results:** Declined seafood consumption was projected to increase the RR of MI by 1.9% and 2.6% in men and by 1.3% and 1.8% in women under lower and upper climate change scenarios, respectively, by 2050 relative to 2009. The greatest impact was observed among older individuals 50 years of age and older with the risk of MI to be increased by 4.5% and 6.5%. Furthermore, the projected increase in MI cases will result in an extra Can\$ 45.7 million and Can\$ 96.3 million healthcare cost under lower and upper climate change scenarios, respectively. **Conclusion:** Reduced seafood consumption may have serious implications for cardiovascular health among coastal FNs in BC. Therefore, effective strategies to improve seafood harvest potential and access to seafood for coastal FNs communities are needed to promote cardiovascular health in FNs and reduce the risk of CVD.

Keywords: seafood consumption, n-3 fatty acids, myocardial infarction, First Nations diets

1 Introduction

First Nations peoples are original inhabitants of Canada which also include Métis and Inuit. There is a great diversity of First Nations culture, languages, traditions and histories across Canada. The First Nations population include those with and without registered or treaty Indian status under *the Indian Act* and may or may not be members of a First Nation/Indian Band. In 2016, there were 977,230 of First Nations which represented 58.4% of the total Indigenous population in Canada (Statistics Canada 2017b). In British Columbia (BC), 172,520 identified as First Nations with 125,635 indicated that they have registered or treaty Indian status and 40.1% live on reserve (Statistics Canada 2017b). The Assembly of First Nations recognizes 634 First Nations communities in Canada (www.afn.ca) while 203 are located within the province of BC (www.bcafn.ca).

First Nations experience significant socio-economic and health disparities compared to the non-Indigenous population in Canada (Reading and Wein 2009). They continue to face serious health challenges including shorter life expectancy, high rates of mortality and chronic diseases, including obesity, type 2 diabetes and cardiovascular diseases (CVD) (FNIGC 2012; Reading and Wein 2009). In British Columbia, the rate of CVD has increased and remains a leading cause of death among First Nation adults (First Nations Health Authority 2012; Health Canada 2014b; Office of the Provincial Health Officer 2015; Reading 2015). CVD are conditions which combine all diseases of the heart and/or blood vessels, including ischemic heart diseases (IHD) (acute myocardial infarction (MI)), cerebrovascular disease (stroke), heart failure, congenital heart disease, inflammatory, rheumatic and hypertensive heart diseases (WHO, WHF and WSO, 2014). IHD is the most common type of CVD and is characterized by narrowed blood vessels by

plaque buildup in the wall of arteries which restricts or blocks blood flow to the heart. It can cause angina (chest pain) and MI (WHO, WHF and WSO, 2014).

Traditional food systems (i.e. all culturally acceptable foods within a particular local, natural environment) are essential for cultural identity, spiritual and mental health and the social well-being of First Nations (Egeland et al. 2001). Hunting and harvesting activities contribute to physical fitness whereas traditional foods provide an abundance of essential nutrients (Assembly of First Nations 2007; Kuhnlein and Receveur 2007; Sheehy et al. 2015). In British Columbia, traditional diets of First Nations include a rich diversity of marine foods, such as fish, shellfish, seaweeds and marine mammals (Chan et al. 2011; Mos et al. 2004) which supply significant sources of protein, micronutrients and essential polyunsaturated omega-3 fatty acid (n-3 FAs) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Sheehy et al. 2015).

Health effects of fish and dietary n-3 FAs have been intensively investigated since the first study in 1970s demonstrating an inverse relationship between n-3 FAs and IHD among Greenland Inuit (Bang and Dyerberg 1971). Following this observation, several clinical and epidemiological studies continue to demonstrate protective effects of fish and dietary n-3 FAs intake against CVD, such as stroke and MI (Elagizi et al. 2018; Leung Yinko et al. 2014), as well as reduced cardiac death and total mortality (Breslow 2006; Leung Yinko et al. 2014; Shahidi and Ambigaipalan 2018). The mechanisms by which EPA and DHA reduce cardiac death include lowering plasma triglycerides, inhibiting platelet aggregation and inflammation, lowering blood pressure, preventing arrhythmias, and improving endothelial function and vascular reactivity (McLennan 2014; Shahidi and Ambigaipalan 2018; Yashodhara et al. 2009).

Although dietary n-3 FAs confer a wide range of cardiovascular benefits, they can be diminished by adverse effects of methyl-mercury (MeHg) present in fish (Laird et al. 2013; Lipfert and

Sullivan 2006). Indeed, consumption of traditionally harvested fish and marine mammals are the major pathway of MeHg exposure among Indigenous people (Laird et al. 2013). The negative effects of MeHg on nervous system, growth and development of fetuses and children, and immune function are well-established (Bjørklund et al. 2017; Ha et al. 2017). Recent evidence also indicates that chronic exposure to MeHg increases the risk of CVD including MI (Genchi et al. 2017). The mechanism by which MeHg produces toxic effects on the cardiovascular system is not fully elucidated yet but is believed to increase oxidative stress, increase the production of free radicals, affect heart rate variability, and promote inflammation, hypertension, and plaque development (Genchi et al. 2017). The intake of EPA+DHA and exposure to MeHg via seafood depends on the concentrations of EPA+DHA and MeHg which greatly vary between species. The most common biomarkers of human exposure to MeHg are whole blood Hg, hair Hg and toenail Hg concentrations. The n-3 FA status are commonly characterised by dietary intake assessments and biomarkers, such as the concentrations of EPA and DHA in plasma and red blood cells (Serra-Majem et al. 2012).

During the last few decades, First Nations people have been undergoing a dietary transition that has resulted in lower consumption of traditional foods, greater reliance on nutrient-poor market foods (Halseth 2015), and increasingly sedentary lifestyle. Several social, economic and environmental factors, such as poverty, food insecurity, governmental restrictions and the concern about the risk of environmental exposure are contributing to the diet and lifestyle transition (Chan et al. 2011; Kuhnlein, Fediuk, et al. 2013). Furthermore, climate change presents a significant stressor to traditional food systems (Ford et al. 2010). A number of studies have documented that climate change affects the timing of fish harvesting, alters travel routes of water and land and decrease the abundance and availability of wildlife species (Hori 2010;

Lemelin et al. 2010). Weatherdon et al. (2016) estimated that climate change may decrease the abundance and distribution of the majority of marine species harvested by coastal First Nations in British Columbia (Weatherdon et al. 2016) which, in turn, was projected to reduce the intake of essential nutrients including EPA+DHA by 21% under lower (Representative Concentration Pathway [RCP] 2.6) and 31% under higher (RCP 8.5) climate change scenarios, by 2050 relative to 2000 (Chapter 8). Since current seafood consumption contributes to reaching daily intake of EPA+ DHA (Chapter 7 and 8) which is recommended for primary prevention of CVD (Academy 2014), the reduced consumption of seafood and associated reduction of n-3 fatty acids (FA) intake may have significant implications for cardiovascular health of coastal First Nations in British Columbia.

The objective of this study was to model the effects of projected declines in seafood consumption on the relative risk (RR) of MI among coastal First Nations in British Columbia under lower and upper climate change scenarios (i.e., 21% and 31%). Specifically, we will model the combined effects of reduced EPA+DHA and methylmercury (MeHg) intake from seafood on the RR of MI.

2 Methodology

2.1 Population and study design

The First Nations Food Nutrition and Environment Study (FNFNES) is a representative 10-year cross-sectional survey which was designed to assess traditional food consumption, total diets and food-related exposure to environmental contaminants in First Nations people living on reserves, south of the 60th parallel across Canada (www.fnfnes.ca). In British Columbia, 21 First Nations communities were randomly selected using a combined ecozone/cultural area framework (Chan et al. 2011). An ecozone is a large geographical region identified based on the distribution

patterns of plants, animals, geographical characteristics and climate (www.ecozone.ca). Culture area refers to a geographic area within which Indigenous communities shared a greater number of traits/cultural affinities than from those outside the area (Sturtevant WC 1978). The current study analyzed data from the six First Nations communities in the Pacific Maritime ecozone, Subarctic/Northwest Coast cultural area (Figure 1). In total, 369 participants aged 19 years and older who self-identify as First Nations living on-reserve, were recruited in this study in the fall of 2008 and 2009. The participation rate in the Pacific Maritime ecozone was 67%. All data were weighted to obtain representative estimates of the total First Nations population. The design weights were adjusted based on the assumption that the responding communities represent both responding and non-responding communities (Chan et al. 2011). The study was approved by the Ethical Review Boards at Health Canada, the University of Northern British Columbia, the University of Ottawa and the Université de Montréal.

2.2 Data collection

All data were collected using household interviews by trained interviewers. The study participants completed a 24-hour diet recall and a Traditional Food Frequency Questionnaire (FFQ). The FFQ collected information on the frequency of consumption of all available traditional foods during the four seasons in the prior year and was representative for each participating community. Overall, 208 traditional food items were included in the FFQ. Seafood species comprised 65 food items, including fish (n=41), shellfish (n=16), seaweed (n=4), and marine mammals (n=4). The social-demographic, health, and lifestyle data questionnaire (SHL) collected information on age, sex, weight, height, physical activity level and smoking status. Body mass index (BMI) was calculated as weight (in kilograms) divided by the square of height (in meters). BMI categories were defined as follows: underweight when BMI was $<18.5 \text{ kg/m}^3$,

normal weight when BMI was from 18kg/m³ to 25 kg/m², overweight was categorized as a BMI of 25 kg/m² or higher but less than 30 kg/m², and obesity was defined as a BMI equal to or higher than 30 kg/m².

2.3 Estimation of seafood, EPA, DHA and MeHg intake

The FFQ was used to estimate the frequency of consumption of each seafood item over the prior year while mean portion size of the corresponding food was calculated from dietary data generated by the 24-hour recalls for each gender and age group. Daily intake of each seafood species (grams/day) was estimated by totaling the number of days in the past four seasons when a particular species was consumed, multiplied by the mean portion size and divided by 360 days (a year included four seasons of 90 days each). Total intake of seafood was calculated as the sum of all seafood species consumed per day. The Canadian Nutrient File (Health Canada 2015), a national food composition database, was used to estimate daily intake of EPA+DHA from each seafood item taking into account the preparation method (i.e. baked or broiled, boiled or raw). The total EPA+DHA intake was calculated using Equation 1:

$$EPA + DHA \text{ intake} = \sum_i^{65} \text{seafood}_i (g/d) \times (EPA + DHA)_i (g/g) \quad \text{Eq. 1}$$

The intake of MeHg from seafood was calculated by multiplying the concentration of MeHg (micrograms per gram) in each species by the total amount of each seafood item consumed per day (grams). The total MeHg intake was estimated with the Equation 2:

$$MeHg \text{ intake} = \sum_i^{65} \text{seafood}_i (g/d) \times MeHg_i (\mu g/g) \quad \text{Eq. 2}$$

Mercury analysis in hair (Hair-Hg)

Each hair bundle was cut into 1 cm segments, starting from the scalp end. Three segments were analyzed to provide the level of mercury in participants' hair for approximately the last three

months. Segmented hair samples were chemically treated to release ionic mercury species which were further selectively reduced to elemental mercury. The latter is concentrated as its amalgam using gold traps. The mercury was then thermally desorbed from the gold traps into argon gas stream, and concentration of mercury vapors was measured with a UV-detector at 254 nm wavelength using Cold Vapor Atomic Fluorescence Spectrophotometer (CVAFS). Selective reduction of the ionic mercury species allows measurement of total or inorganic mercury. The limit of quantitation was 0.06 ppm (or $\mu\text{g/g}$) for total and 0.02 ppm (or $\mu\text{g/g}$) for inorganic mercury in hair. Hair samples were collected in the fall of 2008-2009 and analyzed in the First Nations and Inuit Health Branch (FNIHB) Laboratory (Chan et al. 2011).

Mercury analysis in seafood

Seafood species sampled for contaminant analyses were selected as follow: 1) commonly consumed species in each participating community, 2) those species that are of the greatest concern from an environmental perspective, 3) and those seafood species known to accumulate higher concentrations of contaminants (Chan et al. 2011). Each seafood sample was a composite of tissues from up to 5 different animals or seaweeds. The collected seafood samples were analyzed for several chemicals including MeHg at Maxxam Analytics, formerly CANTEST (Burnaby, BC).

Samples were prepared by alkaline digestion. A combination of methanol and potassium hydroxide was used to solubilize MeHg for instrumental analysis. Highly selective and sensitive detection was achieved by Cold Vapour Atomic Fluorescence Spectrometry after pyrolytic decomposition of the GC eluent. The diluted extract was buffered to a pH of 4.5 – 5.0 and treated with Sodium Tetraethyl borate, resulting in ethylation of oxidized mercury species. These volatile ethylated species (as well as elemental mercury) were stripped from the liquid phase

with argon gas, retained on Tenaex traps, desorbed back into the sample stream, and separated with a gas chromatography column. Each ethylated mercury species was released from the column of mass into the sample stream, thermally oxidized to elemental mercury, and then detected by cold vapor atomic fluorescence spectrometry.

2.4 Statistical analyses

Data management and analysis were performed using STATA statistical software, 14.2 (Stata Corp, College Station, Texas, USA). Descriptive statistics included the calculation of means and 95% confident intervals (95% CI) for continuous variables and proportions (%) for categorical variables. Geometric means (95% CI) were calculated for hair Hg concentrations. Student's t-tests and χ^2 tests were used to assess if differences between groups were statistically significant. Results with a p-value of less than 0.05 were considered statistically significant. The intakes of total and top 10 most consumed seafood species, as well as their contribution to the EPA+DHA and MeHg intake, were estimated for gender and age groups.

Plasma EPA+DHA and Hair-Hg change

We evaluated the potential risk of the projected reduced seafood consumption (i.e., 21% and 31%) by comparing the corresponding changes in plasma EPA+DHA and hair-Hg concentrations with the baseline statuses (which reflect current seafood intake). First, we estimated current EPA+DHA intake from seafood (reported by the BC FNFNES, 2008-09); then, we modeled changes in plasma EPA+DHA concentrations due to the projected decline in seafood intake (i.e. 21% and 31%) using equation 3 developed by Patterson et al. (2015); finally, we subtracted the estimated changes from the baseline EPA+DHA concentrations representing the current intake of EPA+DHA. The baseline concentrations of EPA+ DHA in plasma phospholipids were imputed from James Bay Cree First Nations (3.51% for men, 3.53% for women, 2.95% for 18-34y, 3.84%

for 35-49y, and 5.36% by wt of total fatty acids for $\geq 50y$ (geometric mean)) which reported similar fish consumption patterns (Dewailly et al. 2002) compared to the study participants (60g/day). The baseline EPA+DHA concentrations in the study participants and the modeled changes were within the range of the original paper.

$$\text{Plasma EPA + DHA change} = 2.67 \times \text{EPA + DHA intake (g/d)} + 3.49 \quad \text{Eq. 3}$$

Changes in hair Hg concentrations due to the projected decline in seafood intake were modeled as follows: first, we estimated current MeHg intake from seafood (reported by the BC FNFNES, 2008-09); then, we calculated changes in hair-Hg due to the projected decline in seafood intake (e.i. 21% and 31%) using a compartment model (Equation 4) (Legrand et al. 2010; World Health Organization 1990); lastly, we subtracted the estimated hair-Hg changes from the baseline hair-Hg concentrations (measured in the BC FNFNES, 2008-09). The following assumptions were made for the Equation 4: 95% of the MeHg intake is absorbed, 5% of the absorbed amount goes to the blood compartment, the average blood volume for survey participants is 5 L, and the elimination constant is 0.01 per day (World Health Organization 1990).

$$\text{Hair - Hg } (\mu\text{g/g}) = 0.95 \times 0.7 \times \text{MeHg intake } (\mu\text{g/day}) \times 250/1000 \quad \text{Eq. 4}$$

The relative risk of MI under projected seafood intake scenarios

The RR of MI under the projected scenarios of decline in seafood intake and consequent EPA and DHA intake among coastal First Nations (Chapter 8) was estimated using a logistic model developed by Wennberg, et al. (2012). This model describes the combined effect of EPA+DHA and MeHg exposure on the RR of MI and is adjusted for confounding factors (Wennberg et al. 2012).

In the following model (Equation 5), RR_{MI} represents relative risk of MI, *baseline* EPA+DHA and MeHg biomarkers correspond to the current seafood consumption (reported by the BC FNFNES, 2008-09), *projected* EPA+DHA and MeHg estimates represent biomarkers after subtraction of 21% and 31% of EPA+DHA and MeHg intake. We assume that the conversion factor for plasma EPA+DHA to serum EPA+DHA is 1:1.

Eq. 5

$$\begin{aligned} \ln(RR_{MI}) = & -0.127 \times \left[\frac{(\text{Serum EPA} + \text{DHA}_{\text{projected}})^3}{100} - \frac{(\text{Serum EPA} + \text{DHA}_{\text{baseline}})^3}{100} \right] \\ & + 0.096 \times \left[\frac{(\text{Hair} - \text{Hg}_{\text{projected}})^2}{10} - \frac{(\text{Hair} - \text{Hg}_{\text{baseline}})^2}{10} \right] \end{aligned}$$

With this model, we estimated: 1) the effects of projected 21% and 31% decrease in total intake of seafood and consequent EPA+DHA and MeHg intake on the RR of MI, 2) the individual effects of the top 10 most consumer seafood species using species' specific decline data (Chapter 8). The RR of MI was estimated for gender and age groups.

Projection of MI cases and related healthcare cost

Finally, we projected the potential increase in cases of MI and a corresponding extra healthcare cost by 2050 compared to 2009. The following assumptions were made: 1) the baseline prevalence on MI in the study population was calculated using data on the prevalence of IHD among on-reserve First Nations collected by the First Nations Regional Health Survey in British Columbia over the same period of time (2008-10) (6.40%) (FNIGC 2012) and the prevalence of IHD and MI in the general Canadian population in 2008-10 (8.36% and 1.91%, respectively) (Agency Public Health 2018); 2) data on First Nations population in the Pacific Maritime ecozone were derived from the Census 2011 ($n = 33,877$) (Chan et al. 2011), 3) annual growth

rate among on-reserve First Nations population was projected to be 2.4% from 2009 to 2011 and 1.6% from 2011 to 2050 (Statistics Canada 2015), 4) the incidence rate on MI was based on the data from the Canadian Chronic Disease Surveillance System (CCDSS) for general Canadians (2.24 per 1000 (age-adjusted)) (Agency Public Health 2018), 5) average healthcare cost for one patient with MI was derived from a study among general population in Alberta (Can\$12,935 per one case) (Tran et al. 2017), and 6) an average annual inflation rates in Canada was assumed to be 2% (Statista 2018).

3 Results

This study included 369 participants (140 men and 229 women) with an average age of 47.6 (± 14.3) ranging from 21 to 90 years old. Descriptive characteristics and hair-Hg concentrations of the coastal First Nations in British Columbia are presented in Table 1. Mean age was higher among men than women. Average BMI and the prevalence of obesity were comparable between men and women but significantly higher in older compared to the younger age group. Men and younger participants tend to be more physically active than women and older individuals. The prevalence of smoking was higher among younger individuals but was comparable between men and women. The concentration of Hg in hair was relatively higher in men and older age group compared to women and younger individuals. However, average hair Hg levels were considerably below the established Health Canada mercury guideline across gender and age groups (Legrand et al. 2010).

Table 2A and B summarizes mean daily intake of the most frequently consumed seafood species and corresponding EPA+DHA and MeHg exposure by gender and age groups. The average daily intake of seafood was 78g/day in men and 50g/day in women. Older participants reported significantly higher seafood consumption compared to younger individuals with the mean intake

of 34.8g/day, 45.7g/day and 78.2g/day among 19-34y, 35-49y and ≥ 50 y age groups, respectively. Mean EPA+DHA intake from seafood was higher in men than women (0.81g/day and 0.50g/day, respectively). Likewise, older individuals (≥ 50 y) consumed more EPA+DHA (0.81g/day) than the middle age group (35-50y) (0.49g/day) and younger individuals (19-34y) (0.29g/day). The average daily intake of MeHg from seafood was 5.10 μ g/day in men and 3.14 μ g/day in women. Among older participants (≥ 50 years), the average daily MeHg intake was 5.02 μ g/day which is almost two times higher than in younger age groups (2.60 μ g/day in 19-34y and 2.64 μ g/day in 35-49y). Overall, daily intake of MeHg in First Nations was below the established Tolerable Daily Intake (TDI) of 0.47 μ g/kg bw/day (Legrand et al. 2010)

The top 10 most consumed species contributed about 65% to the total seafood consumption, 71% to the EPA+DHA intake and 67% to the MeHg intake. Salmon species (i.e., sockeye, chinook, coho and salmon eggs) were the most consumed type of fish. Salmon was also the main source of EPA+DHA and the second contributor to the MeHg intake. Halibut, the second most consumed fish, was the major source of MeHg and contributed 31% and 47% to the total MeHg intake in men and women, respectively. However, halibut provided a low intake of EPA+DHA (Table S1). Herring roe was the fourth most consumed species and provided a good source of EPA+DHA. Shellfish, such as prawn, clam and crab, contributed little to either EPA+DHA or MeHg. Overall, the intake of MeHg from seafood in the study participants was very low and was far below the established tolerable daily intakes (TDI) (Canada 2007). Men and older respondents had relatively higher MeHg exposure than women and younger individuals due to the relatively higher seafood consumption.

The modeled effects of the projected decline in seafood consumption on the RR of MI among coastal First Nations are presented in Table 3. We reported the combined effects of reduced

EPA+DHA and MeHg intake under lower and upper climate change scenarios (i.e., 21% and 31%). Reduced seafood intake was estimated to increase the RR of MI by 1.9% and 2.6% in men and 1.3% and 1.8% in women under lower and upper climate change scenarios, respectively, by 2050 relative to 2009. When considering age groups, the most prominent adverse effects were observed in older participants (aged 50 years and older) with the risk of MI to be increased by 4.5% under lower and 6.5% under upper scenarios of climate change.

Table 4 presents the RR of MI due to projected decline of the top 10 most consumed seafood species, individually, considering species' specific data on the decline (Table S2). The most pronounced adverse effects on the RR of MI were observed for salmon species and herring with the RR of MI ranged from 0.2 to 0.7% under lower and from 0.2 to 1.0% under upper climate change scenarios. These species were projected to experience the greatest relative impact from climate change (Table S2). In addition, their contributed relatively high sources of EPA+DHA. The individual effects of reduced halibut and shellfish species were not significant due to their relatively low daily consumption and low EPA+DHA levels.

Considering the projected growth of First Nations population by 2050 and the incidence of MI, we estimated that potential increase in MI to be 2,286 and 4,819 cases in British Columbia First Nations due to the 21% and 31% decline in seafood consumption, respectively. This will result in an extra healthcare cost of Can\$ 45.7 million and Can\$ 96.3 million under lower and upper climate change scenarios, respectively, by 2050 relative to 2009.

4 Discussion

Although CVD has been decreasing in Canada (Agency Public Health 2018), First Nations continue to experience a growing burden of CVD morbidity and mortality (Reading 2015). Historically, the mortality from CVD in First Nations was lower than that among the general

Canadians (Young 2012). Since 1997, heart diseases in First Nations increased drastically and were three times higher compared to the general Canadian population (Young et al. 2004). The First Nations Regional Health Survey (RHS) 2002/03 found that overall prevalence of self-reported heart disease was slightly higher in First Nations than in the non-Indigenous people in Canada (7.6% vs 5.6%) whereas among older individuals (50-59 years), the rate of heart disease was more than two times greater than in the general population in Canada (11.5% vs 5.5%) (The First Nations Information Governance Centre 2005). In British Columbia, Status Indians had rates for IHD 25% higher than the rates among other residents in the province (British Columbia Office of the Provincial Health Officer 2009). In 2008/10, the RHS reported that 5.7% of First Nations in Canada (FNIGC 2012) and 6.4% of First Nations in British Columbia were diagnosed with heart disease (First Nations Health Authority 2012). When data on the prevalence of MI in First Nations are limited (Reading 2015), the mortality rates due to MI were estimated to be 25% higher in First Nations men and 55% higher in First Nations women compared to the non-Indigenous population (Tjepkema et al. 2012).

In this study, we modeled effects of the climate-related decline in seafood consumption and consequent dietary EPA+DHA intake on the RR of MI among coastal First Nations in British Columbia. Our results show that reduced EPA+DHA intake may increase the RR of MI by 1.9% and 2.6% in men and by 1.3% and 1.8% in women under the lower and upper climate change scenarios, respectively, by 2050 relative to 2009. The greatest impact was observed among older individuals 50 years of age and over with the risk of MI to be increased by 4.5% and 6.5% under two climate change scenarios. When considering individual seafood, the most prominent adverse effects on the RR of MI was estimated for salmon species, such as sockeye (0.4% to 1.0%) and chinook (0.5% to 0.7%) since their greater contribution to the EPA+DHA intake and higher than

the average decline in the species' production. Indeed, salmon was the most consumed species which reflects its status as a cultural keystone and favourite food for coastal First Nations. On the other hand, salmon species are among those that are the most sensitive to climate change. For instance, the abundance of chinook salmon was projected to decline by 47.8% and 46.8% under lower and upper climate change scenarios, respectively (Weatherdon et al. 2016).

Furthermore, the projected increase in MI cases will result in an extra Can\$ 45.7 million and Can\$ 96.3 million healthcare cost under lower and upper climate change scenarios, respectively.

Traditional seafood continues to play an essential role for coastal First Nations providing significant sources of n-3 FAs. In the present study, seafood supplied 0.81g/day and 0.50g/day of EPA+DHA in men and women, respectively and 38% (44% of men and 34% of women) of individuals met the recommended intake (RI) of 0.5g/day which is considered to be sufficient to obtain protective effects for primary prevention of CVD (Academy 2014). Overall, n-3 FAs intake in coastal First Nations in BC was lower than in the Canadian Inuit people whose traditional diets mainly composed of fish and marine mammals, rich in n-3 FAs. According to Hu et al. (2017), traditional diets provided about 2.3g and 1.5g daily for men and women, respectively (Hu et al. 2017). Likewise, Dewailly et al. (2001) reported that the Inuit of Nunavik consumed on average 2.1g/day of EPA+DHA (Dewailly et al. 2001). Indeed, low rates of IHD in the Inuit population were attributed to the high consumption of n-3 FAs (Bang and Dyerberg 1971; Dewailly et al. 2001). At the same time, Inuit people are exposed to relatively higher levels of MeHg through consumption of marine foods which diminish the cardioprotective effect of EPA+DHA (Lipfert and Sullivan 2006). Hu et al. (2017) estimated that seafood accounted for about 38 µg/day intake of MeHg for Inuit men and 25 µg/day for Inuit women (Hu et al. 2017). In contrast, First Nations in British Columbia had MeHg intake of about 5.10µg/day in men and

3.14µg/day in women which is 7.5 to 8 times lower than among the Inuit population. Compared to inland First Nations living in Manitoba and Ontario (Chapter 4 and 5), coastal First Nations in British Columbia consumed more n-3 FAs, which reflects differences in geographical diversity and availability of species. In the current study, younger individuals consumed less seafood and consequently had a lower intake of EPA+DHA than older participants. These findings are consistent with previous studies among Indigenous people (Dewailly et al. 2001; Moss et al., 2003; Hu et al. 2017) and indicate an ongoing nutrition transition characterized by the shift away from traditional diets towards nutrient-poor market foods.

First Nations continue to experience a disproportional burden of food insecurity (i.e. the inability to afford nutritionally adequate and safe foods) with prevalence rates greatly exceeding those of the non-Indigenous population (Chan et al. 2011; Tarasuk 2012). Indeed, 41% of on-reserve First Nations in British Columbia reported living in food-insecure households compared to 8.4% in the general population in British Columbia (Chan et al. 2011; Statistics Canada 2010). Food insecurity is directly related to unhealthy diets reflecting a low intake of essential nutrients and an increased intake of energy from sugar and fat foods, which in turn, increases the risk of CVD. In fact, several studies have demonstrated that trans-fats increase the “bad” LDL-cholesterol and decrease the “good” HDL-cholesterol levels. In addition, trans-fats promote inflammation, increase abdominal fat and decrease the health of the endothelium which has metabolic consequences including obesity, diabetes and CVD (de Souza et al. 2015). Several factors, such as limited income, high cost and insufficient availability and access to affordable, healthy and nutritious market food, contribute to food insecurity (Willows 2005).

Furthermore, traditional food systems undergo significant pressures from various environmental factors including climate change which negatively impacts the availability, diversity and access

to traditional foods. Indigenous people, especially remote and coastal communities, are particularly vulnerable to climate change impacts as they live off the land and the ocean and have limited resources and abilities to adapt to changing conditions. Most of the previous research on the impact of climate change on traditional food systems were performed among Inuit and Northern First Nations in the Canadian Arctic (Ford et al. 2010; Nancarrow and Chan 2010; Rosol et al. 2016). The main effects of climate change documented included: warming temperature, increased precipitation, unpredictable weather patterns, strong winds, coastal erosion and alteration in sea-ice dynamics (Ford et al. 2010; Rosol et al. 2016). These factors have been reported to affect the availability, distribution and health of wildlife species with consequent serious implications for food security in Indigenous communities (Ford et al. 2010; Rosol et al. 2016). Among First Nations in British Columbia, the majority (75%) of participants observed that climate change decreased the availability of traditional foods for harvest through declining abundances, altered growth and migration patterns, and observed diseases in animals (Chan et al. 2011). In addition to climate change, First Nations noted other constraints to traditional food consumption including government restrictions and industrial activities, such as forestry, hydroelectricity, mining, farming and oil/gas industries. These barriers combined were perceived by 68% of First Nations in British Columbia to decrease the access to salmon, shellfish and other fish species (Chan et al. 2011).

As to our knowledge, this is the first study which predicts the potential impacts of the climate-related decline in seafood consumption and consequent reduced EPA+DHA intake on the RR of MI among coastal First Nations. The main strengths of this study included a representative sample of coastal First Nations in British Columbia, comprehensive traditional food frequency

questionnaires over the prior year to estimate seafood consumption and empirical analysis of mercury concentrations in seafood species and the hair of the study participants.

There are several uncertainties in this study. The strength of the associations between EPA+DHA, MeHg and MI among First Nations in British Columbia may not be the same as observed by Wennberg and colleagues. The robustness of the relative risk estimates was tested by varying the effect size of EPA+DHA and MeHg in the previous study among Inuit in Canada (Hu et al. 2017). Secondly, the baseline plasma EPA+DHA concentrations were not measured in the study participants but were imputed from the James Bay Cree First Nations. This may introduce bias for the relative risk estimate. However, the concern can be kept minimal since the objective of the present study was to estimate the potential RR change in MI due to seafood decline scenarios, which depends on the modeled change in plasma EPA+DHA rather than the baseline value itself. Finally, the MI incidence and cost related to MI were adopted from the general Canadian population, which may under- or over-estimate the situation for coastal First Nations in British Columbia.

5 Conclusion

Our findings show that potential decline in seafood consumption may have serious implications for cardiovascular health among coastal First Nations in British Columbia. The greatest impacts were observed among men and older individuals 50 years of age and older since their greater reliance on seafood consumption. Furthermore, the projected increase in MI cases will result in a significant extra healthcare cost. This study highlights the urgent need for effective strategies to improve seafood harvest potential and access to seafood for coastal First Nations, which would promote nutritional and cardiovascular health among First Nations. Transformations in fishery management and the implementation of mitigation and adaptation programs involving

Indigenous knowledge and experience would support sustainable fishery in First Nations communities. In addition, programs and initiatives to improve the availability and affordability of high-quality market food are recommended to improve the quality of diets and reduce the risk of chronic disease including CVD.

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Figure 1. Map of participating First Nations communities in British Columbia

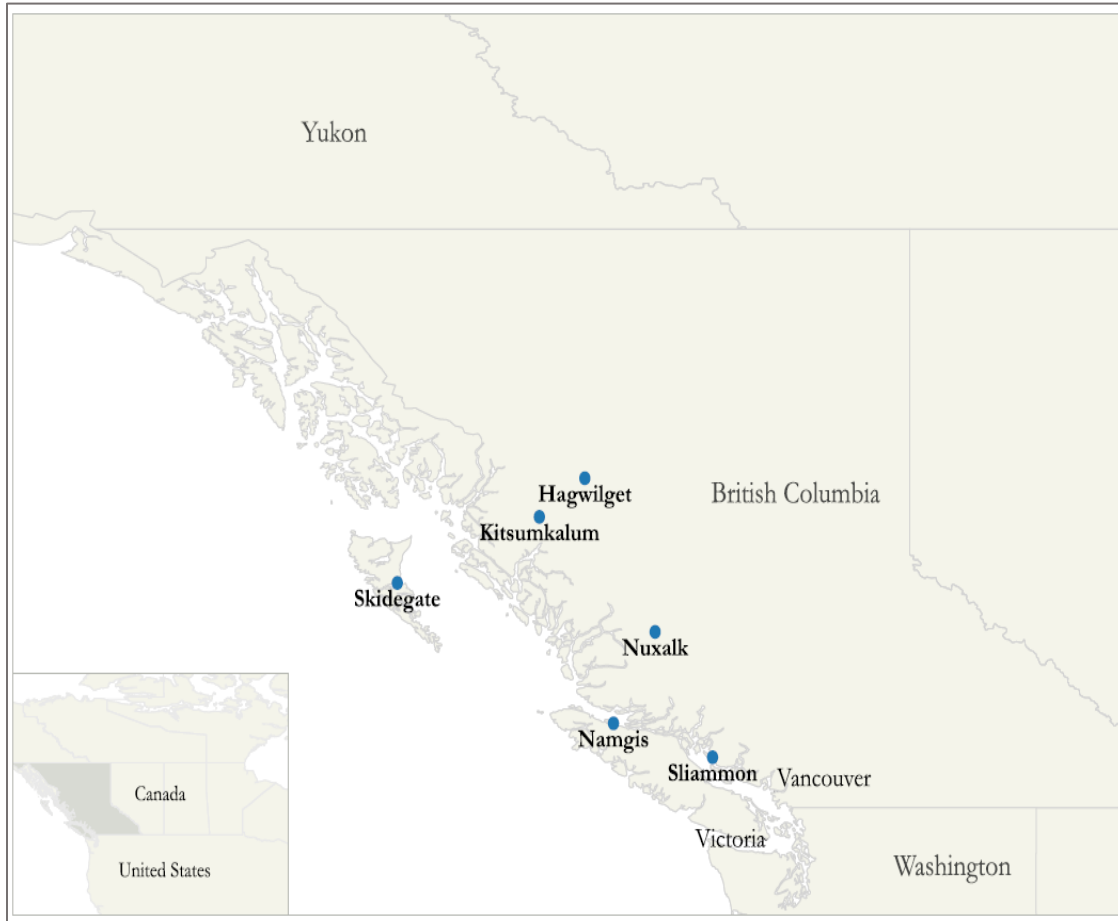


Table 1. Descriptive characteristics of coastal First Nations in British Columbia

	Sex		p value	Age groups			p value
	Men	Women		19-34y	35-49y	50+y	
n	140	229		75	130	164	
Age, y	49.8 (47.4, 52.2)	46.3 (44.4, 48.1)	0.02	28.4 (27.5, 29.3)	42.2 (41.4, 42.9)	60.8 (59.5, 62.1)	0.001
BMI, kg/m ²	30.7 (29.6, 31.8)	31.0 (29.7, 32.2)		27.7 (26.2, 29.2)	31.1 (29.5, 32.8)	31.7 (29.5, 32.8)	0.001
Hair-Hg, µg/g	0.38 (0.22, 0.67)	0.35 (0.25, 0.48)		0.23 (0.16, 0.34)	0.37 (0.25, 0.55)	0.55 (0.21, 1.47)	0.001
Weight, %							0.001
Normal weight	15.8	22.0		27.0	20.8	15.4	
Overweight	33.0	30.5		43.8	31.3	27.7	
Obesity	51.2	47.5		29.2	47.9	56.9	
Physical activity, %			0.01				0.001
Sedentary	11.4	22.7		14.7	18.5	20.1	
Somewhat active	38.6	39.3		34.7	33.9	45.1	
Moderately active	34.3	32.8		37.3	33.1	31.7	
Highly active	15.7	5.2		13.3	14.6	3.1	
Smoking, %	40.7	40.6		50.7	41.5	35.4	0.010

Values are mean (95%CI) or %

BMI, body mass index, overweight was defined as BMI 25.00-29.99 kg/m², obesity was defined as BMI > 30.00 kg/m²

Hair-Hg, amount of mercury in hair (geometric mean), baseline levels (measured in BC FNFNES 2008/09)

Baseline concentrations of EPA+ DHA in plasma phospholipids were inputted from James Bay Cree First Nations which have similar seafood consumption patterns (60.0g/d): 3.51 (3.37, 3.66) for men, 3.53 (3.40, 3.66) for women, 2.95 (2.87, 3.04) for 18-34y, 3.84 (3.65, 4.05) for 35-49y, and 5.36 (5.05, 5.69) for ≥50y (geometric mean (95%CI)) (Dewailly et al. 2002)

Table 2A. Intake of top 10 seafood species and their contribution to EPA+DHA and MeHg intake in BC First Nations men and women

	Men			Women		
	Food intake g/d (95% CI)	EPA+DHA g/d (95% CI)	MeHg µg/d (95% CI)	Food intake g/d (95% CI)	EPA+DHA g/d (95% CI)	MeHg µg/d (95% CI)
Sockeye salmon	18.0 (6.5, 29.4)	0.22 (0.08, 0.36)	0.77 (0.30, 1.30)	9.6 (5.7, 13.6)	0.12 (0.07, 0.17)	0.41 (0.23, 0.58)
Halibut	6.4 (2.5, 10.2)	0.01 (0.01, 0.02)	1.60 (0.64, 2.55)	5.6 (2.8, 8.3)	0.01 (0.01, 0.02)	1.49 (0.74, 2.22)
Chinook salmon	5.8 (1.8, 11.9)	0.10 (0.00, 0.21)	0.27 (0.01, 0.55)	3.1 (1.5, 4.7)	0.05 (0.02, 0.08)	0.14 (0.06, 0.22)
Herring roe	2.0 (1.3, 6.6)	0.05 (0.02, 0.07)	0.00 (0.00, 0.00)	3.4 (1.5, 2.4)	0.08 (0.01, 0.15)	0.00 (0.00, 0.00)
Coho salmon	3.9 (0.9, 3.1)	0.04 (0.01, 0.07)	0.15 (0.05, 0.26)	1.9 (0.5, 6.4)	0.02 (0.02, 0.03)	0.08 (0.06, 0.10)
Prawn	2.9 (1.1, 4.6)	0.01 (0.00, 0.01)	0.06 (0.02, 0.10)	2.0 (0.3, 4.3)	0.00 (0.00, 0.01)	0.05 (0.01, 0.09)
Clam	2.3 (1.8, 2.8)	0.01 (0.01, 0.01)	0.01 (0.01, 0.01)	2.1 (0.6, 3.5)	0.01 (0.00, 0.01)	0.01 (0.002, 0.02)
Salmon egg	2.5 (0.5, 4.6)	0.06 (0.03, 0.09)	0.00 (0.00, 0.00)	1.9 (0.2, 4.0)	0.05 (0.02, 0.08)	0.00 (0.00, 0.00)
Chum	2.6 (1.1, 4.7)	0.03 (0.01, 0.05)	0.08 (0.02, 0.14)	1.9 (0.4, 2.4)	0.02 (0.00, 0.05)	0.06 (0.01, 0.11)
Crab	2.9 (1.0, 1.9)	0.01 (0.00, 0.02)	0.09 (0.04, 0.14)	1.4 (1.7, 2.1)	0.01 (0.00, 0.01)	0.03 (0.01, 0.06)
Top 10 combined	49.3 (26.6, 72.1)	0.54 (0.24, 0.84)	3.03 (1.30, 4.76)	32.8 (24.1, 44.4)	0.37 (0.22, 0.51)	2.27 (1.35, 3.18)
Total seafood	78.0 (37.8, 118.2)	0.81 (0.40, 1.21)	5.10 (2.20, 8.00)	50.1 (35.9, 64.2)	0.50 (0.31, 0.69)	3.14 (2.07, 4.21)

Table 2B. Intake of top 10 seafood species and their contribution to EPA+DHA and MeHg intake in BC First Nations by age groups

	19-34 y			35-49 y			≥50 y		
	Food intake g/d (95% CI)	EPA+DHA g/d (95% CI)	MeHg µg/d (95% CI)	Food intake g/d (95% CI)	EPA+DHA g/d (95% CI)	MeHg µg/d (95% CI)	Food intake g/d (95% CI)	EPA+DHA g/d (95% CI)	MeHg µg/d (95% CI)
Sockeye salmon	9.5 (4.7, 14.4)	0.12 (0.06, 0.18)	0.41 (0.20, 0.61)	10.3 (6.1, 14.6)	0.13 (0.07, 0.18)	0.44 (0.26, 0.63)	14.4 (6.2, 22.5)	0.18 (0.08, 0.28)	0.61 (0.27, 0.96)
Halibut	4.5 (1.6, 7.4)	0.01 (0.00, 0.02)	1.14 (0.41, 1.87)	4.3 (3.0, 5.6)	0.01 (0.01, 0.01)	1.08 (0.76, 1.41)	7.5 (2.6, 12.4)	0.02 (0.01, 0.03)	2.01 (0.63, 3.38)
Chinook salmon	1.0 (0.3, 1.7)	0.02 (0.01, 0.03)	0.05 (0.02, 0.08)	2.6 (2.0, 3.2)	0.04 (0.03, 0.05)	0.12 (0.09, 0.15)	6.1 (0.8, 11.5)	0.11 (0.01, 0.20)	0.29 (0.04, 0.54)
Herring roe	0.6 (0.0, 1.2)	0.01 (0.00, 0.03)	0.00 (0.00, 0.00)	2.2 (1.4, 2.9)	0.05 (0.03, 0.07)	0.00 (0.00, 0.00)	4.5 (0.3, 8.8)	0.11 (0.01, 0.21)	0.00 (0.00, 0.00)
Coho salmon	1.0 (0.4, 1.6)	0.01 (0.00, 0.02)	0.04 (0.02, 0.06)	1.7 (0.5, 2.9)	0.02 (0.01, 0.03)	0.07 (0.02, 0.12)	3.9 (2.5, 5.2)	0.04 (0.03, 0.05)	0.15 (0.10, 0.20)
Prawn	3.6 (0.7, 6.5)	0.01 (0.00, 0.01)	0.09 (0.01, 0.17)	1.7 (0.3, 3.1)	0.00 (0.00, 0.01)	0.04 (0.01, 0.07)	2.1 (0.1, 4.3)	0.00 (0.00, 0.01)	0.05 (0.00, 0.10)
Clam	1.2 (0.6, 1.8)	0.00 (0.00, 0.01)	0.00 (0.00, 0.01)	2.1 (1.2, 3.1)	0.01 (0.00, 0.01)	0.01 (0.00, 0.01)	2.6 (0.5, 4.7)	0.01 (0.00, 0.01)	0.01 (0.00, 0.02)
Salmon egg	0.5 (0.1, 0.8)	0.01 (0.00, 0.02)	0.00 (0.00, 0.00)	1.1 (0.3, 1.8)	0.03 (0.01, 0.04)	0.00 (0.00, 0.00)	3.6 (0.9, 6.3)	0.09 (0.02, 0.15)	0.00 (0.00, 0.00)
Chum	1.6 (0.2, 3.0)	0.02 (0.00, 0.04)	0.05 (0.01, 0.09)	2.0 (-1.0, 5.0)	0.02 (0.01, 0.06)	0.06 (-0.03, 0.15)	2.5 (0.1, 4.8)	0.03 (0.00, 0.06)	0.07 (0.00, 0.14)
Crab	2.5 (0.2, 4.7)	0.01 (0.00, 0.02)	0.06 (0.01, 0.12)	1.4 (0.8, 2.0)	0.01 (0.00, 0.01)	0.04 (0.02, 0.05)	1.9 (0.4, 3.3)	0.01 (0.00, 0.01)	0.06 (0.02, 0.09)
Top 10 combined	26.1 (13.4, 38.8)	0.22 (0.13, 0.32)	1.84 (0.80, 2.88)	29.4 (21.2, 37.5)	0.31 (0.24, 0.39)	1.86 (1.34, 2.37)	49.0 (27.8, 70.3)	0.58 (2.43, 0.92)	3.25 (1.34, 5.15)
Total seafood	34.8 (16.8, 52.7)	0.29 (0.14, 0.44)	2.60 (1.35, 3.84)	45.7 (37.9, 53.6)	0.49 (0.36, 0.54)	2.64 (2.06, 3.23)	78.2 (43.3, 113.1)	0.81 (0.43, 1.20)	5.02 (2.25, 7.78)

Table 3. Percent increase in relative risk of myocardial infarction due to projected 21% and 31% decline in seafood consumption among coastal First Nations in British Columbia*

	21% decline	31% decline
	RR (95%CI)	RR (95%CI)
Males	1.019 (1.004, 1.032)	1.026 (1.008, 1.040)
Females	1.013 (1.003, 1.023)	1.018 (1.007, 1.029)
19-34	1.004 (0.999, 1.009)	1.006 (1.001, 1.033)
35-49	1.012 (1.000, 1.027)	1.018 (1.007, 1.033)
50+	1.045 (1.001, 1.089)	1.065 (1.004, 1.125)

*, Relative risk of MI was estimated using a model developed by Wennberg et al. 2012

Table 4. Relative risk of myocardial infarction due to projected decline of top 10 most consumed seafood species (individually) among coastal First Nations in British Columbia

	Males		Females	
	lower decline*	upper decline*	lower decline*	upper decline*
	RR (95% CI)	RR (95% CI)	RR (95% CI)	RR (95% CI)
Sockeye salmon	1.004 (1.000, 1.009)	1.010 (1.000, 1.020)	1.003 (1.000, 1.010)	1.007 (1.001, 1.015)
Halibut	1.001 (0.996, 1.010)	1.001 (0.995, 1.010)	1.002 (1.000, 1.008)	1.002 (1.001, 1.008)
Chinook salmon	1.007 (1.000, 1.015)	1.007 (1.000, 1.024)	1.005 (1.000, 1.013)	1.005 (1.000, 1.013)
Herring	1.003 (0.998, 1.008)	1.004 (0.997, 1.015)	1.005 (1.000, 1.014)	1.006 (1.000, 1.017)
Coho salmon	1.003 (0.999, 1.009)	1.002 (1.000, 1.024)	1.002 (1.000, 1.009)	1.002 (0.996, 1.009)
Prawn	1.001 (0.995, 1.011)	1.001 (0.995, 1.011)	1.002 (1.000, 1.008)	1.002 (0.996, 1.008)
Clams	1.001 (0.995, 1.011)	1.001 (0.995, 1.011)	1.002 (0.996, 1.008)	1.002 (0.996, 1.008)
Salmon eggs	1.003 (0.999, 1.013)	1.003 (1.000, 1.024)	1.003 (1.000, 1.010)	1.004 (1.000, 1.011)
Chum salmon	1.002 (0.999, 1.011)	1.002 (1.000, 1.024)	1.002 (0.996, 1.009)	1.002 (0.999, 1.009)
Crab	1.001 (0.995, 1.011)	1.001 (0.995, 1.011)	1.002 (0.996, 1.008)	1.002 (0.998, 1.008)

* Lower and upper declines represent projected species' specific estimates (Table S2)

Supplemental material

Table S1. EPA, DHA and MeHg concentrations in top 10 most consumed seafood species

	EPA g/100g	DHA g/100g	MeHg µg/100g
Sockeye salmon	0.42 (0.26)	0.81 (0.46)	4.26 (1.29)
Halibut	0.08 (0.01)	0.16 (0.02)	25.20 (8.01)
Chinook salmon	1.01 (0.02)	0.73 (0.13)	4.70 (1.87)
Herring roe	0.98 (0.09)	1.36 (0.07)	0.00 (0.00)
Coho salmon	0.40 (0.08)	0.66 (0.09)	3.95 (1.51)
Prawn	0.09 (0.04)	0.09 (0.04)	2.27 (0.65)
Clam	0.14 (0.05)	0.15 (0.05)	0.39 (0.46)
Salmon eggs	1.10 (0.06)	1.30 (0.03)	0.00 (0.00)
Chum salmon	0.47 (0.14)	0.70 (0.15)	2.97 (0.70)
Crab	0.28 (0.02)	0.11 (0.01)	6.10 (4.28)
Top 10 average	0.50 (0.39)	0.61 (0.48)	5.00 (7.40)
Total seafood average	0.35 (0.53)	0.50 (0.85)	8.01 (12.41)

EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; MeHg, methyl-mercury
Values are mean (SD)

Table S2. Projected changes in relative abundance of top 10 seafood species under lower (RCP 2.6) and upper (RCP 8.5) scenario of climate change in coastal First Nations in British Columbia by 2050 relative to 2000*

Seafood	Projected decline (%)	
	lower	upper
Sockeye salmon	10.2	36.2
Halibut	12.3	13.0
Chinook salmon	47.8	46.8
Herring	31.8	48.7
Coho salmon	8.8	15.2
Prawn	12.4	18.1
Clams	9.3	4.9
Salmon eggs	23.3	30.9
Chum salmon	9.6	12.1
Crab	12.8	9.7

* Lower and upper scenarios of climate change represent the low and high greenhouse gas emission scenarios based on evidence of latitudinal and regional trends. Declines in relative abundance were projected by 2050 (relative to 2000) for seafood species within British Columbia's marine environment under both scenarios of climate change (Weatherdon et al., 2016)

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10 DISCUSSION & CONCLUSION

10.1 Summary of Thesis Findings

Food security and dietary adequacy remain challenging among First Nations communities.

Climate change and environmental contamination of traditional foods compromise food security and health of First Nations who continue to rely on traditional fish and seafood as a fundamental source of essential nutrients, as well as cultural and economic bases.

In Section 1, I attempted to explore the role of traditional fish consumption for food security among First Nations in Manitoba and Ontario. This research revealed that traditionally-harvested fish remain to be vital to the diet and nutritional health of First Nations. The majority (72 to 82%) of First Nations continues to consume locally-harvested fish which provides good sources of essential nutrients, such as protein, n-3 FAs, vitamin B12, D, thiamin, niacin, selenium and phosphorus. Food insecure individuals tend to consume more fish compared to food secure First Nations. This suggests that individuals who experience limited monetary-related access to market foods tend to rely more on wild fish and other traditional foods for their subsistence. Many food-insecure individuals would like to have more traditional foods; however, the high cost of harvesting equipment, as well as governmental restrictions and climate change, reduce the access to and availability of fish and other wildlife. This, in turn, drastically compromise their sustainability and food security. Findings from this study showed that improving access to fish and other wildlife has the potential to promote food security and sustainable livelihoods in First Nations communities.

In Section 2 (Chapter 4 – 5), I explored the potential risks and benefits of consumption of locally-harvested fish regarding exposure to DDE and PCBs and beneficial nutrients such as n-3

FAs with T2D in Manitoba and Ontario First Nations. Omega-3 FAs are well-known for their beneficial health effects on cardiovascular diseases and potential protective effects against T2D. Unfortunately, fish is also a major pathway of exposure to environmental contaminants, such as PCBs and DDE, which can increase the risk of T2D. First Nations people experience a disproportionate burden of T2D with the prevalence of T2D to be 3–5 times higher compared to the general Canadian population (Young et al. 2000). Our study among First Nations in Manitoba demonstrated that consumption of traditional fish and dietary n-3 FAs intake may have a beneficial effect on T2D. Indeed, fish consumption of 2–3 portions per month and one portion per week and more were inversely associated with T2D with OR values of 0.51 and 0.40, respectively, compared with no fish intake. Likewise, intake of n-3 FAs was negatively associated with T2D (OR value of 0.48). In contrast, among Ontario First Nations, fish consumption of one portion per week and more showed positive associations with T2D (OR value of 2.5). In addition, dietary PCBs and DDE exposure was positively associated with T2D (OR values of 1.09 and 1.07, respectively) whereas intake of EPA+DHA showed an inverse association after controlling for DDE and PCB intake (OR value of 0.86).

While Manitoba and Ontario First Nations generally share similar fish consumption patterns, the levels on POP (PCBs and DDE) in the most consumed species, such as walleye, whitefish, lake trout, northern pike and yellow perch, were significantly higher in Ontario compared to Manitoba. Consequently, dietary POP intake from fish was relatively higher in Ontario First Nations. The inconsistent findings on the associations between fish consumption and T2D initiated a follow-up study aiming to investigate if differences in dietary POP exposure may drive the direction of the associations. By using the DID model, which allows estimation of the causal relationship between exposure and the outcome of interest with a cross-sectional setting,

the study confirmed that elevated levels of POPs in fish may outweigh beneficial associations between fish and n-3 FAs with T2D. Furthermore, I estimated that the risk of T2D starts to increase at dietary exposure to 2.11 ng/kg/day of DDE and 1.47 ng/kg/day of PCBs. Based on these estimates, I calculated approximate amounts of fish consumption (by species) that could be recommended as the maximum daily intake to prevent exceeding the DDE/PCB breakpoint exposure. Collectively, this research demonstrates that effects of locally-harvested fish on T2D may depend on the balance of contaminants (PCBs, DDE) and beneficial nutrients (n-3 FAs) in fish. Therefore, it is important to monitor the levels of POPs in the locally harvested fish.

In Section 3 (chapters 7- 9), I addressed several questions about seafood consumption patterns and its nutritional values for First Nations in British Columbia, as well as potential impacts of climate change on nutritional quality of diets and cardiovascular health. In British Columbia, the variety of fish and seafood consumption significantly varies across ecozones and cultural areas (from 12 to 81.3g/d) reflecting geographical location and availability of different types of seafood. Among 65 different fish and seafood species, salmon was both the most frequently reported fish (consumed by over 91% of participants) and the species consumed in the highest amounts (5.9 to 23.6g/d) (depending on ecozone/cultural area). Heavy reliance on salmon reflects its status as a cultural keystone species and favourite food for First Nations (Garibaldi and Turner 2004). The top 10 most consumed fish species (salmon, halibut, trout, crabs and prawns) combined represented 64% of the total seafood consumption. On average, total seafood supplied 660.5mg/d of EPA+DHA in males and 404.3mg/d – in females, which significantly contributed to reaching the dietary recommendations for EPA+DHA intake (500mg/d) for primary prevention of CVD (22% to 58%, depending on sex and age group, met the RI). Moreover, seafood consumption was associated with a healthier lifestyle, i.e. higher fruit and

vegetable consumption, lower smoking rates and increased physical activity. These findings highlight that seafood remains essential to the contemporary diets, health and well-being of First Nations in British Columbia.

However, traditional food systems experience pressure from various stressors. In particular, climate change was projected to reduce the abundance and availability of marine species (Cheung et al. 2010). This raises questions about impacts on the sustainability of First Nations' fisheries which is fundamental to their culture, economy and nutrition. There is already documented a dramatic decline in fish and seafood consumption compared to historical use (Butler and Campbell 2004; Colin 2017; Lee, Reyburn, and Carrow 1971; Moss 1993). Chapters 8 and 9 jointly attempted to investigate the implications of climate change on nutrient intakes and cardiovascular health of coastal First Nations living in the Pacific Maritime ecozone.

In chapter 8, I modelled impacts of the climate-related decline in seafood harvest, projected by Weatherdon et al. (2016), on diets and nutrient intakes. Among coastal First Nations, locally-harvested seafood consumption provided daily recommendations of EPA+DHA (74-184%) and vitamin B12 (84-152%) and substantial levels of niacin (28-55%), selenium (29-55%), vitamin D (15-30%) and protein (14-30%). On average, climate change was projected to reduce seafood consumption and consequently, intake of essential nutrients, by 21% to 31% under lower and upper climate change scenarios by the year 2050 relative to 2000. Keystone species, including five Pacific salmon species (in particular, sockeye, chinook and pink), herring and shrimps, were projected to experience the greatest relative impacts (from 34% to 60% declines). Furthermore, it was estimated that projected reduced seafood consumption cannot be easily replaced by market foods, such as chicken, canned tuna and bread, since they do not provide adequate amounts of nutrients, particularly those primarily derived from marine sources, such as EPA+DHA, vitamin

B12, vitamin D, and selenium. This raises an urgent need in strategies directed toward improving access to seafood harvest potential in coastal communities to ensure nutritional health in coastal First Nations.

The follow-up study (chapter 9) examines the importance of seafood for the cardiovascular health of coastal First Nations. CVD including MI represents a public health issue among First Nations in Canada. This research modelled the effects of reduced EPA+DHA intake on the risk of MI taking into account coexisting exposure to MeHg from seafood. It was estimated that climate-related decline in seafood consumption and consequent reduced EPA+DHA intake may increase the risk of MI by 1.9% and 2.6% among men and by 1.3% and 1.8% among women under lower and upper climate change scenarios. Older individuals (50 years of age and older), who heavily rely on traditional seafood, will experience the greatest impact with the risk of MI to be increased by 4.5% and 6.5% under two climate change scenarios. Furthermore, the predicted increase in MI cases will result in an extra Can\$ 45.7 million and Can\$ 96.3 million healthcare cost (under lower and upper climate change scenarios, respectively). This research underpins that effective strategies are needed to improve seafood harvest potential and access to seafood for coastal First Nations communities to promote nutritional and cardiovascular health as well as food security and food sovereignty.

10.2 Research Contribution

This dissertation attempted to explore the roles of traditional fish consumption in the complex interplays between the environmental contaminant exposure, climate change, food security, nutrition and the health of First Nations communities across Canada. This research is among a very limited body of literature to address questions on how multiple stressors (environmental contamination and climate change) combined with socio-economic factors affect diets, food

security, nutrition, and health of First Nations population in Canada. First Nations are highly vulnerable to food insecurity which is a predisposing factor for poor health and nutrition. Several studies examined determinants of food insecurity among Indigenous populations (Domingo 2016; Skinner et al. 2013; Teresa et al. 2012); however, limited research provided insights into the role of traditional fish and other wildlife for food and nutritional security among First Nations communities in Canada. The findings point to the continued importance of adequate access to natural resources to cope with food insecurity and to maintain the traditional ways of life among First Nations.

This dissertation also provides an understanding of the potential risks and benefits of locally-harvested fish, which are rich in essential nutrients including n-3 FAs but may also represent a risk due to POP exposure. This research adds to global understandings of the role of fish consumption, n-3 FAs and dietary POPs intake in the prevention of T2D. In particular, it contributes to the recent findings (Lee and Jacobs 2010; Turyk et al. 2015; Wallin et al. 2015) that beneficial effects of fish may be attenuated by the detrimental effects of POPs on T2D. Furthermore, the estimated thresholds of daily dietary DDE and PCB exposure which start to increase the risk of T2D will help to develop fish intake recommendations. These findings can be utilized by public health agencies and local health authorities to develop dietary advisories and intervention programs to slow the increasing rate of T2D among First Nations in Canada.

Finally, section 3 integrates two distinct fields of marine ecology and nutritional epidemiology to explore the relationship between changes in fisheries catch potential and human health and nutrition (Golden et al. 2016). This study, for the first time, projected the impacts of the climate-related decline in seafood harvest on nutrient intakes and the risk of heart diseases among coastal First Nations in British Columbia. Although many factors, such distribution of seafood among

members in the communities, food preferences, the access to alternative foods and changes in fishing activities, were not taken into considerations, the results of this modelling are useful for local resources managers and public health professionals as a starting point to develop adaptation plans for climate change.

10.3 Future research

First of all, there is a need for longitudinal studies examining the relationship between exposure to environmental contaminants and health outcomes among First Nations and other Indigenous people. Since the present research is based on a cross-sectional survey of First Nations, causal inferences on the relationship between POP exposure and T2D cannot be stated. In addition, the analyses of this work studied the associations between exposure to a single chemical (individual POP) and T2D. However, humans are exposed simultaneously to more than one chemical which may have different mechanisms of toxicity. There is a need in studies exploring interactions between multiple POP exposures and their joined effects on the health of First Nations. Therefore, future studies should focus on examining health effects of chemical mixtures (Monosson 2005). Also, ongoing monitoring of contaminant burden and disease outcomes among First Nations population is essential.

There is a lack of longitudinal studies examining the links between diets, nutrition and health transition among First Nations and other Indigenous populations. While global biodiversity loss is well documented (Butchart et al. 2010; Dudgeon et al. 2015), there is no research studying consequences of wildlife species decline for food and nutrition security as well as the health of Indigenous peoples like the First Nations in Canada who live off the land. Also, there has been little documentation on the impacts of climate change on traditional food systems and adaptation planning in First Nations communities living in southern regions. Previous research on climate

change effects was mainly conducted in the Arctic regions (Ford et al. 2010, 2014; Nancarrow and Chan 2010; Rosol, Powell-hellyer, and Chan 2016).

Furthermore, our model on the effects of climate change assumes a linear relationship between changes in species abundance and seafood availability for First Nations. Future projections should deliberate responses of First Nations fishing activities (such as shifts in fishing grounds, fishing effort, gear modification and changes in targeted species) and governance scenarios to further explore these critical dimensions of the access to traditional foods.

Finally, along with effects on traditional food systems, climate change may also influence the bioaccumulation of contaminants in food chains with potential implications on the quality of traditional foods consumed by Indigenous people as well as contaminant exposures (Alava et al. 2017; AMAP 2015). Therefore, biomonitoring of environmental contaminants is recommended.

10.4 Conclusion

First Nations continue to experience food insecurity which, along with dietary and lifestyle transition, have serious consequences for their health and well-being. Traditional fish and seafood provide fundamental sources of essential nutrients, along with social and cultural benefits for First Nations. However, various anthropogenic stressors, such as climate change and environmental contamination, affect traditional food systems and represent barriers to traditional food consumption. This has detrimental effects on diet quality, nutritional status and the health of First Nations. Given the central role of fish and other wildlife for food security, health and nutrition of First Nations, strategies are needed for improving access to the land.

Potential risks and benefits associated with fish consumption depend on the regional POP concentrations in traditionally-harvested fish. Therefore, risk communication strategies should be

designed at the regional level. Public health agencies and local health authorities need to develop dietary advisories and guidelines in order to lower the risk of contaminant exposure and to maximize nutritional benefits. Also, POP levels in locally-harvested fish should be monitored.

Maintaining traditional lifestyles and diets is complex and required a multidisciplinary approach which considers the relationship between risk, benefits and sustainability (access and availability). This dissertation provides important information for communities, fishery governance, local resource managers and public health professionals to promote traditional food systems, nutritional health, food security, and food sovereignty in Canadian First Nations.

10.5 Bibliography (Introduction, Literature Review, Discussion)

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