

# Ecology and Food Quality of Fishes in Coastal Rivers of the Far North of Ontario

by

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## Abstract

The Far North of Ontario is home to over 24 000 people in 31 communities, and many of these people rely heavily on wild fish as part of their diet. Six of these communities are situated on the lower reaches of large river systems near the coast of Hudson and James Bays. These large rivers, as well as numerous smaller rivers along the coast, are home to a variety of fish species, including some presumed anadromous (migrating between fresh and salt water) populations, that support important subsistence fisheries. However, little research has been carried out on fish resources of these river systems, and basic information is generally lacking. I examined fish populations from the lower reaches of 14 of these rivers in terms of growth, trophic ecology, life history, and their suitability for human consumption based on mercury and fatty acid content. Stable isotope and fatty acid compositions indicative of predation on marine food sources were most evident in the presumed anadromous species - Brook Trout, Lake Whitefish and Cisco. Compared to inland lakes of the same drainage basins, coastal river populations of Northern Pike and Walleye grew faster and occupied broader trophic niches, suggestive of an indirect marine influence on their diet. In contrast, Lake Whitefish tended to grow more slowly in coastal rivers than in inland lakes. Fish mercury concentrations in coastal rivers have not changed appreciably over the last ~ 30 years. Current fish mercury concentrations did not vary latitudinally among coastal rivers, but were significantly higher than in inland lakes for Walleye, Lake Whitefish and White Sucker. Fillet lipid and essential fatty acid (EPA+DHA) content decreased with increasing latitude in Northern Pike, but not in other species. Both mercury and essential fatty acid concentrations of fish muscle varied among rivers but there did not appear to be spatial concordance between them. Presumed anadromous species - Brook Trout, Lake Whitefish, Cisco – tended to have both the greatest concentrations of essential fatty acids and among the lowest mercury concentrations, making them an especially high quality food source. Walleye generally had the highest muscle mercury concentration and relatively low essential fatty acid content.

**Keywords:** river, freshwater, fish, subarctic, growth, anadromy, food webs, stable isotopes, mercury, fatty acids, food quality

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## **General Introduction**

### **The Far North of Ontario and the Hudson Plains Ecozone**

The Far North of Ontario (approximately north of 51°N), is the most southerly subarctic region in Canada, and the most undeveloped and pristine region of Ontario. This vast area spans 42% of the province and is composed of two ecozones, the Boreal Shield Ecozone (BSE; 46% of the Far North area) in the west, and the Hudson Plains Ecozone (HPE; 54% of the Far North area) in the east. Most of the HPE (~ 80%) is in Ontario with only small portions extending into Manitoba and Quebec. The HPE is the largest wetland complex in North America and third largest wetland complex globally (Far North Science Advisory Panel 2010). The HPE is a formerly submerged marine region which was subsequently covered by the continental ice sheet during the last ice age. As such the present-day HPE is characterized by low lying peatlands overlaying flat-laying sedimentary rock covered by variable depths of glacial till. The compressed nature of the peatlands results in poor drainage and results in a vast network of interconnected fens, muskeg, lakes and rivers that cover >80% of the region. At its northern boundary, the HPE meets the Arctic Archipelago Marine Ecozone (AAME) along the edge of Hudson and James Bays, forming Ontario's northern marine coast (Far North Science Advisory Panel 2010). The long period of ice cover as well as low water temperatures throughout the ice-off season on Hudson and James Bays moisten and cool the air in the HPE resulting in low temperatures and frequent fog. As well, peat-rich soils act as an excellent thermal insulator resulting in the widespread occurrence of permafrost throughout the HPE (Stewart and Lockhart 2005).

## Aquatic ecosystems of the Hudson Plains Ecozone

Large freshwater lakes (>10 ha surface area) comprise only ~8% of the surface waters in the HPE with the majority of the landscape dominated by hydrologically-connected ponds and fens. The interconnected nature of the landscape forms a vast network of freshwater streams and rivers. Five of Ontario's largest rivers (Moose, Albany, Attawapiskat, Winisk and Severn) flow through the HPE and have a mean total discharge of >4500 m<sup>3</sup>/sec (Marshall and Jones 2011). With drainage areas ranging from 35622 - 105857km<sup>2</sup> these rivers span the HPE and all have headwaters originating in the BSE. Many of the rivers in the HPE flow along their natural path, unobstructed from headwaters to coast. However, there are several diversions within the Albany River that feed hydroelectric development in adjacent drainage basins. It is estimated that 17% of the annual discharge for the Albany River has been diverted to Lake Superior and Lake Winnipeg (Browne 2007). As well, 16 hydroelectric generating stations within the Moose River basin generate more than 1000 megawatts of power (Browne 2007).

Due to the connected nature of these river environments, and their relatively large watersheds, fish species presence or absence within a given ecosystem is the result of both local and distant abiotic and biotic features. The upper reaches of these river systems have similar fish communities to the lakes in their headwaters. Common large-bodied species include Walleye (*Sander vitreus*), Northern Pike (*Esox lucius*), White Sucker (*Catostomus commersonii*), Longnose Sucker (*Catostomus catostomus*), Shorthead Redhorse (*Moxostoma macrolepidotum*), Cisco (*Coregonus artedi*) and Lake Whitefish (*Coregonus clupeaformis*), while some sections of river are also home to some of the last strongholds of the provincially-important Lake Sturgeon (*Acipenser fulvescens*). The lower reaches of these

large rivers (mouth to ~ 50 km upstream) have a similar species composition, however some of the large rivers as well as many of the smaller rivers also contain Brook Trout (*Salvelinus fontinalis*). As well, many of the Lake Whitefish, Cisco, and Brook Trout populations in the lower reaches of these rivers are believed to be anadromous, spending the summer months feeding in the rich marine waters along the coast (White 1942; Morin et al. 1982). The numerous smaller rivers closer to the coast have much smaller drainage areas which are completely contained within the HPE, tend to lack Walleye, and have species compositions dominated by Brook Trout, Lake Whitefish and Cisco. However, anecdotal evidence from local fishermen from Fort Severn and Peawanuck have identified increasing numbers of Walleye and Northern Pike in several of the smaller rivers along the Hudson Bay coast in recent decades. Estuarine environments along Ontario's 1200 km marine coast receive nutrient and chemical inputs from both the terrestrial and marine environments and are therefore dynamic and diverse ecosystems both chemically and biologically. The productivity of Hudson Bay and James Bay is low relative to other marine environments at the same latitude (Anderson and Roff 1980). However, the coastal waters, specifically embayments and estuaries, are considered significantly more productive than the pelagic offshore zone and provide ecologically significant refugia for many species of fish, migrating birds and marine mammals (Stewart and Lockhart 2005).

Fish communities within these coastal waters consist of a mixture of marine and anadromous fish that rely on the valuable foraging and nursery habitat in the shallow nutrient-rich water close to shore (Stewart and Lockhart 2005). Due to the marine inputs into their diet, anadromous populations of fish are likely to be both enriched in  $\omega$ -3 fatty acids and lower in methylmercury (MeHg) compared to freshwater conspecifics (Sargent and

Henderson 1995; Swanson et al. 2011a). Fish of these rivers probably also exhibit both species-specific and river-specific differences in contaminant levels and essential fatty acid content. These differences would be due, in part, to differences in life history, age, size and trophic ecology. Therefore, understanding how these fish communities are structured and how growth rates within these systems vary will help identify the factors that influence the food quality of these fishes for local harvesters. Other important characteristics of fluvial systems such as community stability and resilience are linked to the trophic hierarchy within the system (Baxter et al. 2005; Layman et al. 2007). Understanding a system's ability to resist change by identifying populations which are susceptible to extirpation or fragmentation will help inform management planning as this pristine region becomes more developed and experiences the greatest rates of climate change in the province (Far North Science Advisory Panel 2010).

### **Services provided by aquatic ecosystems of the Hudson Plains**

The importance of the HPE cannot be overstated. This vast tract of land represents one of the last pristine environments in the country. The numerous freshwater streams, rivers and fens connect ecosystems while the peatlands sequester atmospheric carbon and bind deposited trace metals to limit their mobility. Home to the most southern populations of Polar Bears (*Ursus maritimus*) in the world as well as provincially-threatened species such as the Lake Sturgeon, Wolverine (*Gulo gulo*) and Woodland Caribou (*Rangifer tarandus caribou*) the HPE and Hudson and James Bay coasts are Ontario's last stronghold for many species. Ontario's coastline is also a globally-significant migratory flyway. The natural funnel shape

of Hudson Bay concentrates flocks of migrating waterfowl and shorebirds into James Bay. Two federally-protected sanctuaries at the mouths of the Moose and Harricanaw Rivers offer rich marshes, extensive eel grass beds, and wide tidal flats to support migrating flocks of geese, ducks and shorebirds.

Freshwater systems throughout the HPE are integral to the sustainability of remote communities that rely on them for fresh drinking water, transportation corridors, and sources of nutritious wild food. One municipality (Moosonee), and seven First Nations communities (Moose Factory, Marten Falls, Fort Albany, Kashechewan, Attawapiskat, Peawanuck, and Fort Severn) are in the HPE. All of these communities are situated on the large river systems, and all but one (Marten Falls) are located on the lower reaches, within 50 km of the marine coast. These communities are located within the traditional territories of the Mushkegowuk Cree and the Nishnawbe-Aski Nations, representing some of the most isolated communities in the province. As a result, many residents rely on traditional sources of food to supplement their diet (Berkes et al. 1994; Hlimi et al. 2012). A survey of 194 Mushkegowuk Cree reported 90% of schoolchildren aged 10-17 consumed wild game and 55% of them were concerned about the environmental contaminants in their wild food. Communities also varied greatly in their reliance on wild game, with a large number of respondents from Moose Factory indicating they consume wild edibles “rarely” and a large number of respondents from Kashechewan and Peawanuck indicating they consume wild edibles “at least once a day” or “at least once a week” (Hlimi et al. 2012). Fish represented nearly 20% of the annual harvest of wild edibles from this region in 1990 with an estimated 133,872 kg of edible fish flesh harvested (Berkes et al. 1994). Whitefishes (*Coregonus* spp.) represented the most commonly harvested fish (42%) followed by Northern Pike (19%), Walleye (17%), suckers

(*Catostomus* spp.) (9%), Brook Trout (6%), Burbot (*Lota lota*) (4%) and Lake Sturgeon (3%). This annual fish harvest had an estimated \$ 1.7 million replacement value in 1990 (equivalent to \$ 2.5 million in 2016) as well as a cultural significance which is beyond measure (Berkes et al. 1994; Statistics Canada 2017). The commercial fishing industry which once existed has been largely replaced by tourism-driven recreational fishing. However, due to poor access, recreational fishing is limited to regions close to communities or to fly-in outpost camps.

### **Stressors on aquatic ecosystems of the Hudson Plains**

The climate of the Hudson Plains Ecozone has remained relatively cool compared to other Arctic regions, largely due to a prolonged ice-cover season on Hudson and James Bays which moderates and cools the surrounding landmass. However, since the mid 1990's rapid and dramatic climate warming has occurred which has affected both physico-chemical, and biological processes in this region's freshwater lakes. Increases in primary productivity and species richness in freshwater planktonic communities throughout the HPE are consistent with warming temperatures and shorter periods of ice cover (Brazeau et al. 2013; Rühland et al. 2013). The recent increase in primary productivity in these lake ecosystems could result in bottom-up changes in food web structure leading to increased fish biomass at higher trophic levels. As well, increased algal productivity observed in HPE lake sediment cores are thought to be increasing in tandem with sediment inorganic mercury (Hg) concentrations. However, inorganic Hg availability does not appear to be limiting MeHg production (Brazeau et al. 2013).



Over the next 30-50 years the HPE is expected to experience some of the most rapid and dramatic changes in climate in the province (Colombo et al. 2007). Most notably, a considerably shorter ice-cover season on Hudson and James Bays is expected to lead to longer, warmer summers with mean temperatures increasing by 2.2 - 2.5 °C, and shorter, warmer winters with mean temperatures increasing by 4.1 - 6.4 °C by 2050 (Far North Science Advisory Panel 2010). The changing climate has led, and will continue to lead, to a loss in permafrost which allows thawed soils to draw water away from the surface, ultimately changing the hydrology of surface waters. Many of the plants and animals in this region have adapted to specific habitats and niches in the ecosystem, and habitat changes accompanying climate change will undoubtedly place stress on them. Also, winter precipitation is expected to increase resulting in a heavier snowpack and larger, more violent, spring freshets (Far North Science Advisory Panel 2010). This change in seasonal distribution of precipitation could also alter the seasonal flow regime of many freshwater fens, streams and lakes which would have a profound effect on aquatic insects, fish and mammals. While there is no immediate course of action to stop the changes brought on by climate change it is important to understand its projected effects on ecosystems to plan for future development and help promote practices which can slow the progression of climate change.

Climate change will also affect the human inhabitants of the HPE. Changes in hydrology such as increased flooding events and longer, drier summers as well as shorter duration of ice cover are likely to affect current transportation networks. Anecdotal reports from local inhabitants of Moosonee and Moose Factory have reported shorter ice-cover seasons which have affected local winter transportation routes (i.e., via snowmobiles) between the two communities as well as large spring flooding events in recent years which

have damaged infrastructure (Peter Kapashesit, MNRF, *pers. comm.*). The development of infrastructure such as rail lines and all-season road networks promises to alleviate some of the stress on communities and promote economic expansion. However, the negative impacts of roads and road construction on biodiversity have been well documented across a variety of ecosystems and taxa (Gunn and Sein 2000; Trombulak and Frissell 2000). Roads create a physical barrier which is difficult and/or dangerous to cross. This in effect, fragments populations and inhibits effective migration therefore limiting reproductive success (Fahrig 2003). Road construction and maintenance practices can also change the physical and chemical properties of the surrounding environment. These changes include sedimentation, changes in run-off direction, as well as the introduction of noise and chemical pollutants (Wood and Armitage 1997; Trombulak and Frissell 2000). An improved transportation corridor also exposes previously inaccessible regions to increased exploitation by hunters, fishermen and poachers, and provides a path of exposure to invasive species (Browne 2007; Kaufman et al. 2009). The current lack of a transportation network is widely regarded as the primary impediment to mineral exploitation in the Far North of Ontario. In 2010 there were 90,579 mining claims in the Far North, up from just 35,386 in 2007. The discovery of rich metalliferous deposits in a region known as the “Ring of Fire”, ~250 km west of Attawapiskat, accounted for the increase in mining claims (Far North Science Advisory Panel 2010). Development of these world-class deposits of chromite, copper, nickel and zinc promise a significant boost to local economic growth. However, mitigation of the environmental footprint left by this development will have to be carefully considered. The mobilization of toxic elements such as Hg, arsenic, zinc, nickel and chromium into aquatic ecosystems could have serious deleterious effects on aquatic taxa (Förstner and Wittmann

2012). The cumulative effects of these stressors need to be the focus of future land use planning if management decisions hope to effectively mitigate their effects. This includes planning for future climate change and how changes in climate may exacerbate the impacts of human development.

### **Research needs for fish and fisheries of the Hudson Plains**

Freshwater rivers and lakes in the Far North of Ontario are relatively poorly studied, and there is a need for more basic and applied research to support management of these ecosystems (McGovern and Vukelich 2009; Marshall and Jones 2011). The difficult access and, until recently, limited public knowledge of the region have left it with limited baseline scientific knowledge to complement the abundant traditional knowledge. A baseline understanding of ecosystem function and status across this region will help document and track changes ahead of further industrial development and climate change. As well, with a high proportion of residents in this region relying on wild edibles, specifically fish, there is a need for updated food quality information. River- and species-specific information on contaminants as well as nutritional value of aquatic resources will enable local fishers to make informed consumption decisions.

# Chapter 1: Understanding ecosystem function through the use of ecological tracers

## 1.1 Introduction

Ontario's high latitude rivers are dynamic ecosystems capable of supporting diverse fish assemblages. The lower reaches of these rivers are unique in Ontario as they receive energy, nutrient and chemical inputs from both the terrestrial and marine environments. Fish communities within these systems are believed to utilize a mixture of life history strategies; freshwater resident species such as Northern Pike and Walleye exist within a community alongside anadromous Brook Trout, Lake Whitefish, and Cisco. Understanding predator-prey interactions and food web structure and the influence of these factors on fish growth and life history in these distinctive systems is central to understanding overall ecosystem function. Ecological tracers provide a quantitative approach to aid in our understanding of the complex relationships within an ecosystem (Dalsgaard et al. 2003; Fry 2006). Stable isotopes of carbon and nitrogen have been used extensively to determine trophic position and habitat use in fish (Peterson and Fry 1987; Boecklen et al. 2011; Layman et al. 2012). More recently, fatty acids have been gaining in popularity as ecological tracers (Dalsgaard et al. 2003; Hebert et al. 2006). Used together these tracers can provide insight into trophic dynamics, habitat use, and life histories of fishes in these unique coastal environments.

Isotopes of carbon and nitrogen are useful as they can be used to differentiate among food sources. Differential rates of metabolism and excretion of isotopically-heavy and light nitrogen isotopes result in greater  $\delta^{15}\text{N}$  ratios at higher trophic levels. Increases of 2.2 - 3.4‰

have been used to distinguish between trophic levels (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 2001). The ratio of isotopically heavy to light carbon isotopes within a consumers tissues are commonly used to distinguish between benthic and pelagic, as well as lentic or lotic sources of primary production and therefore provide information on habitat use (France 1995b; Hecky and Hesslein 1995). The isotopic signatures of prey items are reflected in predictable patterns in consumers and therefore can trace the transfer of nutrients up the food web. However, in systems which experience regular shifts in productivity from migrating prey items, the isotopic signature of predators may reflect both near and distant sources of biological production (Kline Jr et al. 1998).

Brook Trout, Lake Whitefish and Cisco are widely distributed in rivers along the coastline of Hudson and James Bays. In addition to being obligate freshwater spawners, these species may undergo summertime migrations from the rivers to feed in more saline waters along the coast. In analogous systems, this feeding behaviour resulted in isotopic profiles enriched in  $^{13}\text{C}$  and  $^{15}\text{N}$  relative to freshwater residents (Bilby et al. 1996; Kline Jr et al. 1998; MacAvoy et al. 2001). Therefore enriched carbon and nitrogen signatures should also be evident in anadromous fish from the Hudson and James Bay coast.

Similar to isotopic signatures, fatty acid profiles of consumers are largely the product of the fatty acids found in their prey items. Fatty acids exist in different quantities in different environments (Dalsgaard et al. 2003; Colombo et al. 2016), and are selectively retained up the food chain once consumed, thus creating patterns that can be used to distinguish trophic interactions as well as habitat use of consumers (Kainz et al. 2004; Heintz et al. 2010). One very useful signature in northern oceans is that of calanoid copepods of the genus *Calanus*, one of the most abundant groups of zooplankters in higher latitude marine environments. Due

to seasonal changes in food availability these copepods develop large energy reserves in the form of wax esters composed primarily of long chain mono-unsaturated fatty acids (MUFA) and fatty alcohols (Dalsgaard et al. 2003; Colombo et al. 2016). Once consumed, high quantities of these fatty acids and fatty alcohols are incorporated into the flesh of marine planktivorous fish. As well, marine environments are enriched in  $\omega$ -3 fatty acids and have significantly higher  $\omega$ -3/ $\omega$ -6 fatty acid ratios than freshwater environments (Sargent and Henderson 1995). These ratios have been used in conjunction with stable isotopes to discern dietary preference for prey items of marine vs freshwater origin (Henderson and Tocher 1987; Smith et al. 1996; Swanson et al. 2010). I used stable isotopes of both C and N in conjunction with fatty acid composition to track dietary sources and trophic relationships to help provide insight on fish growth and life history.

Riverine environments differ from lacustrine environments in that they lack pelagic zones which largely limits primary production to benthic zones (Wetzel 2001). This is especially true for the lower reaches of my study rivers. As they descend out of the Boreal Shield Ecozone (BSE) onto the relatively flat gradient of the limestone-dominated Hudson Plain Ecozone (HPE) these rivers spread out which reduces water depth. These shallow expanses of flowing water are well mixed and do not stratify like lake environments. As well, temperatures in the lower reaches of these rivers are presumably moderated by the relatively cool waters and prolonged ice cover season of Hudson and James Bays (Stewart and Lockhart 2005). This cooling effect reduces the length of the growing season towards the coast and therefore also affects primary productivity (McKenney et al. 2011). However, these coastal rivers can receive marine nutrient subsidies from migratory fishes, and this may boost productivity (Wipfli et al. 2003). Marine nutrient availability has been shown to increase fish

growth in both anadromous and resident fishes as well as influence the fat content and composition in anadromous species (Swanson et al. 2011b; Gladyshev et al. 2012).

My objective in this chapter is to generate baseline information regarding the structure and ecology of fish communities in these unique but poorly-studied river systems. My emphasis is on growth and trophic ecologies, with the latter analyzed using stable isotopes and fatty acids as ecological tracers. I take a comparative approach both among species, and among populations. Population-level comparisons will be both among the coastal rivers spanning more than 5° in latitude, and between populations in lower reaches of the coastal rivers and those in lake environments in the headwaters of the drainage basins. I will address four key predictions:

i) Climate will have a stronger influence on growth than access to marine-derived nutrients. Therefore, growth of all species will be slower in more northerly coastal river sites than in more southerly headwater lakes due to the colder climate in the former.

ii) Diet breadth, inferred from the observed range in C isotope values, will be lower in coastal river sites than headwater lake sites because of a lower diversity of primary production sources (rivers lack pelagic zones) and less opportunity for specialization along carbon gradients in rivers than lakes.

iii) Food web position, inferred by position in isotopic space relative to populations of White Sucker (*Catostomus commersonii*) will be the same between inland lake and coastal river sites for resident species. However, food web positions of purported anadromous species (e.g., Lake Whitefish) will differ between inland lake and coastal river sites due to the influence of marine food sources in the latter.

iv) Fatty acid composition will be similar among freshwater resident species and different between freshwater and purported anadromous species due to the influence of marine food sources (especially marine calanoid copepods) consumed by the latter.

## **1.2 Methods**

### ***1.2.1 Study area, data acquisition and field sampling***

I examined fish populations from the lower reaches of 14 rivers flowing into Hudson and James Bays with drainage areas ranging from 456 to 105,857 km<sup>2</sup>. These included six very large river systems (drainage areas > 35,000 km<sup>2</sup>) originating in the BSE (Harricanaw, Moose, Albany, Attawapiskat, Winisk, Severn) and eight smaller river systems (drainage areas 456 - 4715 km<sup>2</sup>) wholly contained within the HPE (Sutton, Shagamu, Shell, Goose, Pipowitan, Mintiagan, Tamuna, Majikan) (Fig. 1, Appendix 1). Fish populations in lower reaches of each of the six large river systems were compared with fish populations sampled from upstream lakes within their drainage basins (Table 1).



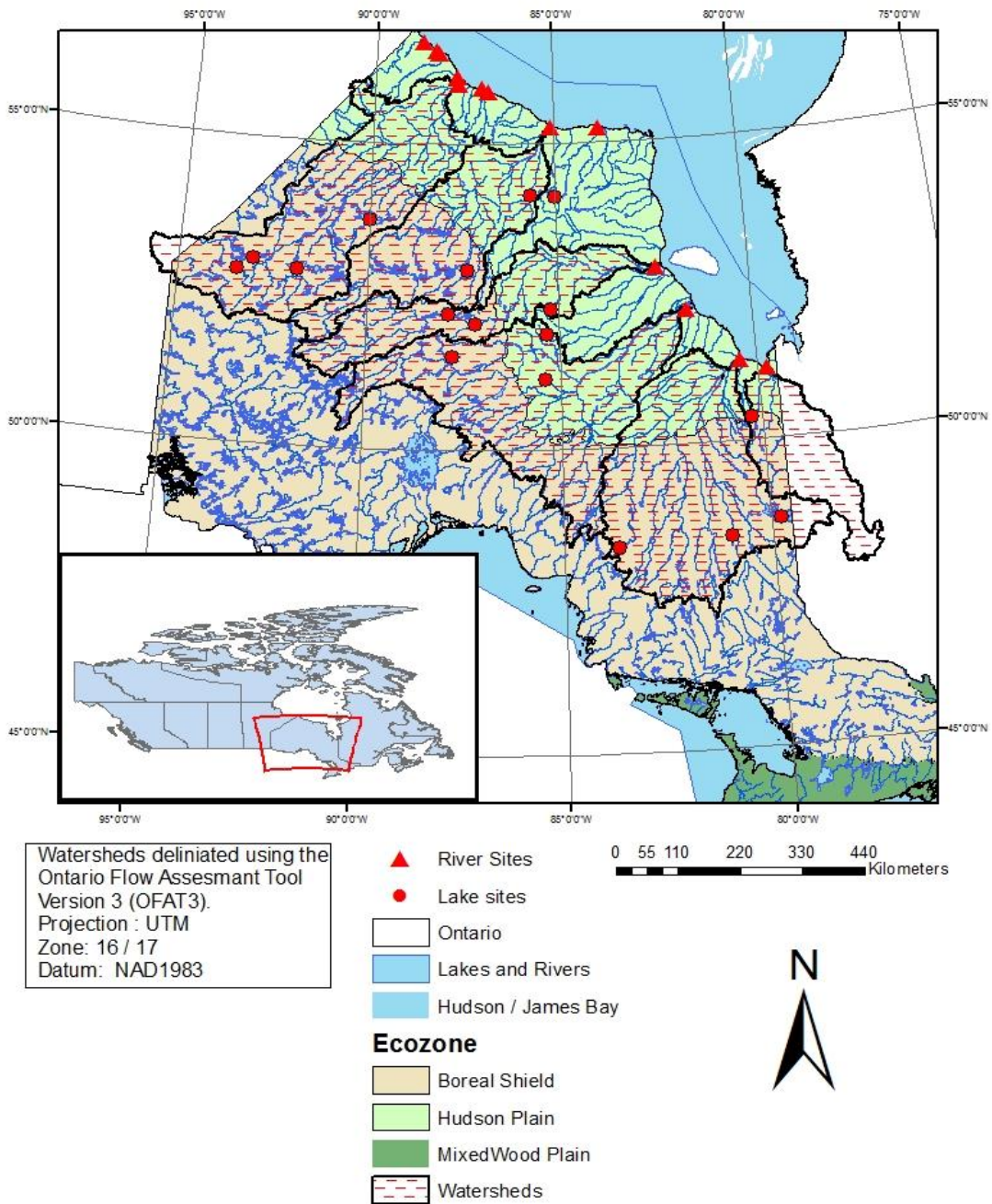


Figure 1. Study area showing major river drainage basins (heavy black outlines) as determined by the Ontario Flow Assessment Tool (OFAT3). Coastal river (▲) and inland lake (●) study sites are indicated. Boreal Shield and Hudson Plains Ecozones delineated by brown and pale green shades, respectively.

Table 1. Headwater lakes (inland sites) used for comparison with lower reaches of mainstem river (coastal sites) for each drainage basin.

<b>River / Drainage Basin</b>	<b>Lake(s)</b>
Harricanaw	Kesagami
Moose	Abitibi, Missinaibi, Night Hawk
Albany	Eabamet, Quantz, Napken
Attawapiskat	Attawapiskat, Beteau, Missisa
Winisk	Winisk, Shamattawa, Spruce
Severn	Big Trout, Nikip, Sandy, North Spirit

I utilized data and archived tissue samples collected from fish communities in coastal rivers and headwater lakes from 2010 to 2014 by Laurentian University, the Ontario Ministry of Natural Resources and Forestry (MNR), and the Ontario Ministry of Environment and Climate Change (MOECC), and I supplemented these with new data and tissues collected from sampling in 2015 and 2016. Sampled fish species included Lake Whitefish, Cisco, Brook Trout, Northern Pike, Walleye, White Sucker and Longnose Sucker. Fish communities differ among the 14 rivers; most notably, Walleye are found only in the larger rivers, and Brook Trout are found primarily in the smaller rivers (Appendix 1). Based on previous research, many of the coastal populations of Lake Whitefish, Cisco and Brook Trout were believed to be anadromous, making migrations into summer feeding sites in the marine nearshore regions of Hudson and James Bays (Dutil and Power 1980; Morin et al. 1982; Lambert and Dodson 1990). The remaining species were presumed to be primarily residents of fresh water.

Sampling was conducted in locations utilized by local fishers, and carried out either solely by local community members, or by field crews composed of local community

members and staff and students of the Cooperative Freshwater Ecology Unit (MNRF, MOECC, and Laurentian University, Sudbury, ON). All fish sampling was conducted using a combination of gill nets and angling, with sampling targets of up to 20 of each species covering a wide size range in each river. The primary gear was multi-mesh benthic gill nets of the Riverine Index Netting (RIN) configuration (28-127 mm stretched mesh size, 0.9 m high, 24.8 m long) (Jones and Yunker 2010). Nets were generally set overnight and all captured fish were identified and tallied.

Fish were processed in the field by recording basic attributes (fork and total lengths, round weight, sex, maturity), and collecting ageing structures (fin rays for suckers, cleithra for Northern Pike, otoliths for all other species). For all fish, a skinless, axial muscle sample (~ 30 g) was removed from the mid-body above the lateral line and placed in a Whirl-Pak plastic bag. For a selected subset of fish (up to 10 per species, per site) a whole, head-to-tail fillet was removed from the opposite side of the fish. Fillets were trimmed to represent the typical portion consumed by fishers (i.e., skin, fins, and rib cage bones removed), wrapped in plastic, and placed in a Ziploc freezer bag. Muscle samples and fillets were transported on ice back to the lab and stored in a freezer at -20 °C. Fin rays were stored in paper envelopes and allowed to air dry, cleithra were cleaned of flesh, stored in paper envelopes and allowed to air dry, and otoliths were rubbed clean, stored in plastic vials and allowed to air dry.

### ***1.2.2 Laboratory analyses***

Fish ages were determined by counting annuli on ageing structures. For Northern Pike, cleithra were examined whole. For Walleye, otoliths were prepared by cracking-and-

burning. For suckers, fin rays were set in epoxy, and thin sections were cut from the proximal end using a jeweller's saw. The sections were then placed on a microscope slide and polished with fine sandpaper. For all other species, otoliths were prepared using the same section-and-polish technique used for fin rays, except that the section was removed through the otolith nucleus, perpendicular to the longitudinal axis. All prepared structures were viewed under a dissecting scope or microscope using reflected light.

Stable isotope analyses were performed on dried and ground muscle. A small piece (~10 g) of the axial muscle sample was cut into fine pieces, placed in a glass vial, and freeze-dried for 7 d (< 0.20 mBar, < - 45 °C). The freeze-dried tissue was then ground to a fine powder and homogenized using a ball mill (Retsch MM 400). Subsamples of the ground muscle were analyzed for C and N isotopes using a Finnigan Mat Delta Plus continuous flow isotope ratio mass spectrometer (CF-IR-MS) coupled to a Thermoquest NC2500 elemental analyzer at the Stable Isotopes in Nature Laboratory (Fredericton, NB, Canada). Duplicates were run every 20 samples and no less than 20% of each run was composed of standard reference material. Reference materials used were Vienna Pee Dee Belemnite for C and Atmospheric Nitrogen for N (Gonfiantini et al. 1995). Isotope ratios were expressed in standard  $\delta$  notation in units of ‰.

Fatty acid analyses were carried out on subsamples of whole fillet. Each frozen fillet was homogenized using a meat grinder in a cold room (<-12°C) and two 5 g aliquots of the homogenate were stored in cryovials at -20°C. Muscle samples were freeze-dried and the % moisture was calculated.

$$Eq\ 1: \quad \frac{\text{fresh weight} - \text{dry weight}}{\text{fresh weight}} \times 100 = \% \text{ moisture}$$

The percent moisture was then used to convert fatty acid mass ratios per dry tissue mass into fatty acid mass ratios per wet tissue mass. Lipids were extracted twice using chloroform and methanol at a 2:1 ratio (Folch et al. 1957). The organic phase / solvent mixture was pooled and exposed to a stream of N<sub>2</sub> gas to evaporate the solvent. Once dryness was achieved hexane was immediately added to the sample. Transmethylation of the fatty acids was achieved using methanolic sulfuric acid (1%) as a catalyst and heating the samples to 90°C for 90 min. 750 µL of Fatty Acid Methyl Ester (FAME) extracted to hexane was analyzed with a gas chromatograph (Shimadzu GC-2010 plus) equipped with a flame ionisation detector. The column used was a SP-2560 (100 m x 0.25 µm, Agilent) with helium used as the carrier gas at an average flow of 1.12 ml min<sup>-1</sup>. Calibration and quantification of FAME was accomplished using tricosanoic acid (23:0, Nu-Chek Prep Inc: catalog# N-23-M). As well, a 37 component FAME mix (Sigma-Aldrich: catalog# 47885-U) was used to identify peaks.

### ***1.2.3 Data management and statistical analyses***

Raw data were initially scrubbed to correct or remove erroneous values presumed to result from human error. This was achieved by visual inspection of bivariate scatterplots of raw data, and residuals analysis. Variables used in subsequent analyses were calculated at the population level (species x location). For the six large river drainage basins, some variables were calculated at both downstream and upstream sites within the basin. In all cases, the downstream sites were the lower reaches of the mainstem rivers, and the upstream sites were headwater lakes contained within their drainage basins (Table 1). These are subsequently referred to as coastal and inland sites, respectively.

Somatic growth was modeled using the von Bertalanffy growth model (VBGM) of the form

$$RWT \sim W_{inf} * [1 - \exp(-K * (t - t_0))]$$

where  $RWT$  is the observed fish round weight (g),  $t$  is the observed fish age (years),  $W_{inf}$  (read W-infinity) is a parameter representing the theoretical maximum weight (or asymptotic weight),  $K$  is the curvature parameter and indicates the rate at which  $RWT$  approaches  $W_{inf}$ , and  $t_0$  is a parameter representing the age at which  $RWT = 0$  (Ricker 1975; Lester et al. 2004). Initial parameter estimates for  $W_{inf}$  and  $K$  were generated following the methods described by Ford (2009) and Walford (1946) while  $t_0$  was initially set at 0. Models were fit using a Levenberg-Marquardt modified non-linear least squares method using the `nlsLM` function from the `minipack.lm` package in R, and used to generate predicted weight at eight years of age. Confidence intervals were generated for the model parameter estimates and predicted weight at age by bootstrapping to 1000 iterations. The precision of the parameter estimates was assessed using these generated confidence intervals. Confidence intervals were also used in conjunction with scatterplots to assess overall model fit. In cases where the VBGM fit was poor a general linear model was applied, and was accepted if the fit was both an improvement from the VBGM and a logical reflection of the data. Age eight was selected as the age of comparison both because the VBGM best represents adult fish rather than juveniles (Lester et al. 2004), and because eight year old fish were represented in most of my sampled populations.

To assess whether growth was different between coastal and inland sites a paired-comparisons approach was used. In cases where data were available from more than one headwater lake within a watershed a mean weight at age eight was calculated among them to

represent the inland site. For each species, paired differences were analyzed with a paired-comparison's t-test for normal data, or a Wilcoxon signed rank test for non-normal data.

Normality was assessed with Shapiro-Wilk tests.

Stable isotope data were used to define food web positions, and calculate trophic niche metrics, using the Stable Isotope Bayesian Ellipses in R (SIBER) package (Jackson et al. 2011). Niche breadth was inferred from carbon range (CR), calculated as the mean difference between maximum and minimum  $\delta^{13}\text{C}$  values within a population (Layman et al. 2012). Niche size of each population was inferred from the standard bivariate ellipse area corrected for sample size (SEAc) (Jackson et al. 2011). The centroid of this ellipse, calculated as the mean of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ , was used to represent the food web position of the population, and 95% confidence ellipses about the means were also calculated. Because environmental isotopic signatures can vary spatially (termed baseline variation, (Cabana and Rasmussen 1996)) I calculated food web positions relative to a single reference species, White Sucker, for each site. Vectors composed of a Euclidean distance value and an angle (degrees) described the relationship between the species. The distance values were log-transformed to meet normality assumptions. The angle values were converted from radians to degrees and were used to imply direction. All variables generated from the stable isotope data were analyzed using a paired-comparisons design, as outlined for growth, above.

Fatty acid (FA) profiles were also used to make inferences regarding feeding behaviour and life history, but in coastal sites only. A total of 31 FAs were identified (Appendix 2) for 184 individual fish (51 Brook Trout, 67 Lake Whitefish, 66 Northern Pike) from six large and five small rivers (Tamuna, Majikan, and Mintiagan were not sampled for fatty acids). Brook Trout, Lake Whitefish and Northern Pike were the primary species of

interest for spatial analyses because they are widely distributed and commonly consumed across the coastal region, and were expected to represent both anadromous and freshwater resident life histories. An additional 37 fish (7 Cisco, 10 White Sucker, 10 Longnose Sucker, 10 Walleye) from the Moose River were also analyzed to expand interspecific comparisons. The Moose River was chosen for interspecific analysis because it was expected to have the highest diversity of fish species.

Fatty acid data were analyzed both as absolute concentrations (expressed on a wet tissue weight basis) and relative concentrations (expressed as proportion of total extracted FAs) to assess spatial and species differences. Interspecific comparisons of omega 3/omega 6 fatty acid ratios were conducted using analysis of variance and Tukey HSD analysis in R. Multivariate analysis was conducted using the PRIMER (Plymouth Routines in Multivariate Ecological Research; PRIMER-E) version 7 software. Due to the presence of zero values FA concentrations were transformed using  $\log(x+1)$  to meet parametric assumptions. An added benefit of using the  $\log(x+1)$  transformation is that less abundant FA will carry more weight in the analysis (Happel et al. 2017). Fatty acid compositions were transformed into a resemblance matrix based on Euclidean distance. Euclidean distances between fatty acid profiles represent known dietary differences well, are easily interpreted, and are compatible with a wide variety of statistical techniques (Happel et al. 2017). From this resemblance matrix data were visualized using a metric multidimensional scaling (MDS) plot. The purpose of MDS is to configure the data, in this case the fatty acid profiles of individual fish, in a multidimensional space according to their relative similarity or dissimilarity to one another. This generates a two-dimensional “map” in which points on the map are separated by a distance which represents the rank order of the matching similarity/dissimilarity taken



from the resemblance matrix (Clarke and Warwick 2001). To test for differences in fatty acid composition between groups the resemblance matrix representing the relative dissimilarity between individual fatty acid profiles was analyzed for each population using a permutational MANOVA (PERMANOVA). PERMANOVA is a resemblance-based permutation method which generates ANOVA-like test statistics from a resemblance matrix using a series of randomly observed permutations.

## **1.3 Results**

### ***1.3.1 Growth***

The von Bertalanffy growth model (VBGM) fit the weight at age data well in most study populations. Mean predicted weights at age eight for the common large-bodied species in the six large drainage basins are summarized in Appendix 3.

Weight at age eight tended to be greater at coastal sites than inland sites for the piscivores, Northern Pike and Walleye, but lower at coastal sites than inland sites for the benthivores (Table 2). These trends were statistically significant for Northern Pike and Lake Whitefish (Table 2). On average, Northern Pike were 31% heavier at coastal sites than at inland sites, and Lake Whitefish were 41% heavier at inland sites than at coastal sites.

Table 2. Results of Wilcoxon signed-rank tests comparing the mean predicted weight at eight years between coastal and inland sites for five of the study species sampled in Far North drainage basins. Predicted weight values are means, across drainage basins,  $\pm 1$  SE. n = number of drainage basins, \* significant at  $P < 0.05$ .

Species	Predicted weight at eight years (g)		n	W	P-value
	Coastal	Inland			
<b>Northern Pike</b>	2753 $\pm$ 239	2106 $\pm$ 99	6	31	0.041*
<b>Lake Whitefish</b>	680 $\pm$ 56	958 $\pm$ 53	4	16	0.029*
<b>White Sucker</b>	826 $\pm$ 161	1158 $\pm$ 67	4	14	0.11
<b>Walleye</b>	810 $\pm$ 73	587 $\pm$ 72	5	21	0.095
<b>Cisco</b>	516 $\pm$ 35	460 $\pm$ 102	2	6	0.70

### *1.3.2 Food web structure based on stable isotope analyses*

Comparisons of niche space, inferred from carbon range (CR) and corrected standard ellipse area (SEAc), indicated that coastal riverine populations of all species tended to have larger niches than inland lacustrine populations, and these differences were statistically significant for Northern Pike and Walleye CR values (Table 3). Northern Pike and Walleye populations from coastal river sites appeared to have more individuals with  $^{13}\text{C}$ -depleted values compared to populations from inland lake sites. Though the mean difference between coastal and inland sites was highest for Lake Whitefish (Table 3), this difference was not statistically significant because of high variability in CR among Lake Whitefish populations.

Table 3. Results of paired comparisons t-tests comparing population  $\delta^{13}\text{C}$  range (CR) between coastal and inland sites for four of the study species sampled in Far North drainage basins. CR values are means, across drainage basins,  $\pm 1$  SE. n = number of drainage basins, \* significant at  $P < 0.05$ .

Species	CR (‰)		df	t	P-value
	Coastal	Inland			
<b>Northern Pike</b>	4.59 $\pm$ 0.92	2.52 $\pm$ 0.60	4	2.84	0.047*
<b>Lake Whitefish</b>	5.87 $\pm$ 2.11	2.84 $\pm$ 0.85	4	1.07	0.34
<b>White Sucker</b>	4.96 $\pm$ 1.14	3.87 $\pm$ 0.88	4	0.68	0.53
<b>Walleye</b>	4.51 $\pm$ 0.53	2.19 $\pm$ 0.54	4	3.63	0.022*

Relative food web positions in isotopic space did not vary greatly between coastal and inland sites. Paired-comparisons of the distances from White Sucker to Lake Whitefish, Northern Pike, and Walleye food web positions between coastal river sites and inland lake sites were not statistically significant (Paired-comparisons t-tests,  $P=0.071$ ,  $P=0.52$ , and  $P=0.28$ , respectively). However, as with niche size, there was considerable variation in Lake Whitefish position among drainage basins. In the Moose River system the angle and length of the vector formed between White Sucker and Lake Whitefish was  $121.2^\circ$  and  $0.63\text{ ‰}$ , respectively, at the inland site (Lake Abitibi), but  $39.1^\circ$  and  $3.27\text{ ‰}$ , respectively, at the coastal site (Fig. 2). This indicates an increase in both the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  dimensions for Lake Whitefish relative to White Sucker moving from inland to coastal sites. Similar patterns were seen in the Albany, Attawapiskat and Severn River systems (Fig. 2), and to a lesser extent in the Winisk River system and indicates both  $^{13}\text{C}$  and  $^{15}\text{N}$  enrichment.

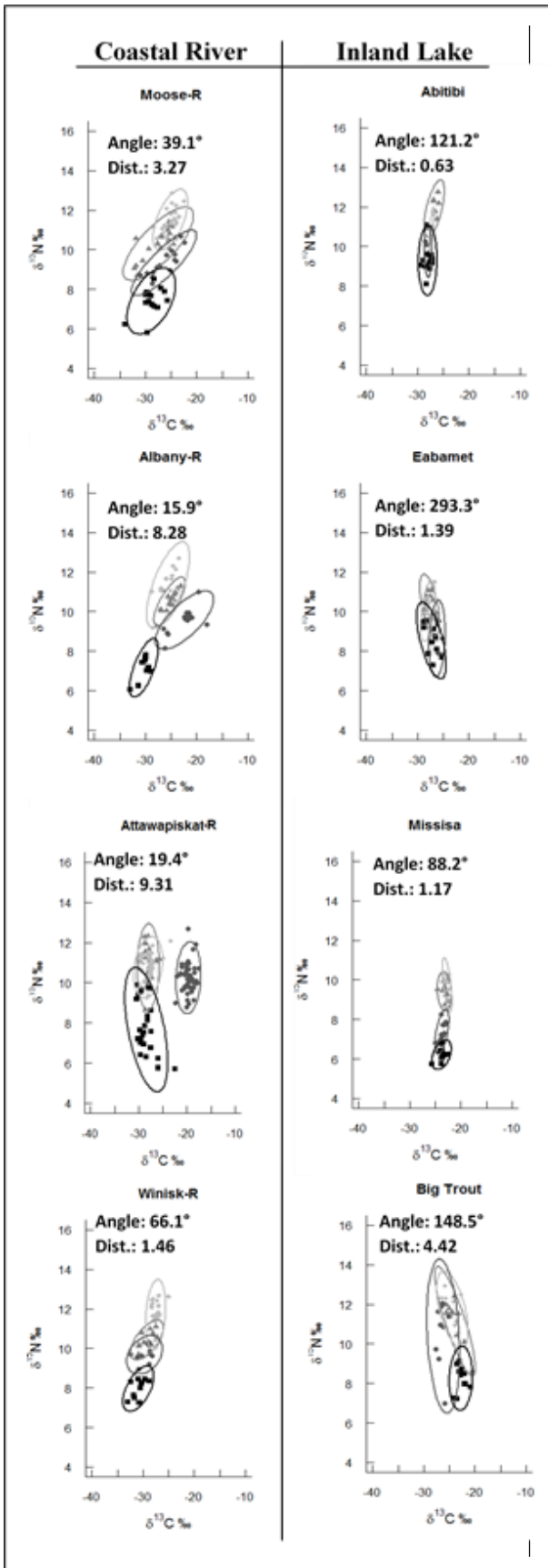


Figure 2. Food web position in isotopic space of individual White Sucker (■), Northern Pike (◐), Walleye (▲) and Lake Whitefish (●) in paired coastal river and inland lake sites. Ellipses are fitted 95% confidence intervals. The angle (degrees) and Euclidean distance (‰) of the vector formed between the centroid of White Sucker and Lake Whitefish populations is expressed for each site.

### ***1.3.3 Food web structure based on fatty acid analyses***

Fish species separated into two distinct groups based on total lipid concentrations of whole fillets. Brook Trout, Lake Whitefish and Cisco had mean total lipid concentrations of 7.61, 5.37, and 3.95 % wet, respectively, and are subsequently referred to as fatty-fleshed species. Northern Pike, Walleye, White Sucker and Longnose Sucker had mean total lipid concentrations of 2.01, 2.25, 2.98, and 1.94 % wet, respectively, and are subsequently referred to as lean-fleshed species.

Interspecific comparisons in the Moose River indicated significant differences in  $\omega$ -3/ $\omega$ -6 fatty acid ratios (ANOVA,  $F_{1,6} = 60.0$ ,  $P < 0.01$ ) (Fig. 3). Post hoc analysis revealed significantly higher  $\omega$ -3/ $\omega$ -6 ratios in Brook Trout than in all other species ( $P < 0.01$ ) as well as higher  $\omega$ -3/ $\omega$ -6 ratios in Cisco than White Sucker ( $P < 0.05$ ) (Fig. 3). Northern Pike and Walleye had the greatest percent of their fatty acids comprised of polyunsaturates (PUFA) at 50.7 % and 43.8 %, respectively, with PUFA in other species representing 33.4 - 39.1 % of total fatty acids. All species had 24.2 - 25.8 % of their fatty acids composed of saturates (SFA). Lake Whitefish and Brook Trout had 40.8 and 40.4 % of their fatty acids, respectively, composed of monounsaturates (MUFA) which was 4.7 - 16.3 % greater than for the other species. However, Lake Whitefish had 5.5 % more of their MUFA composed of short-chained ( $C < 18$ ) MUFA than Brook Trout which have 5.1 % more of their MUFA composed of long-chained ( $C > 18$ ) MUFA. In addition, Lake Whitefish and Brook Trout had a greater percentage of their fatty acids composed of less common types, such as Gondoic acid (20:1n-9), at 1.1% and 3.4%, respectively.

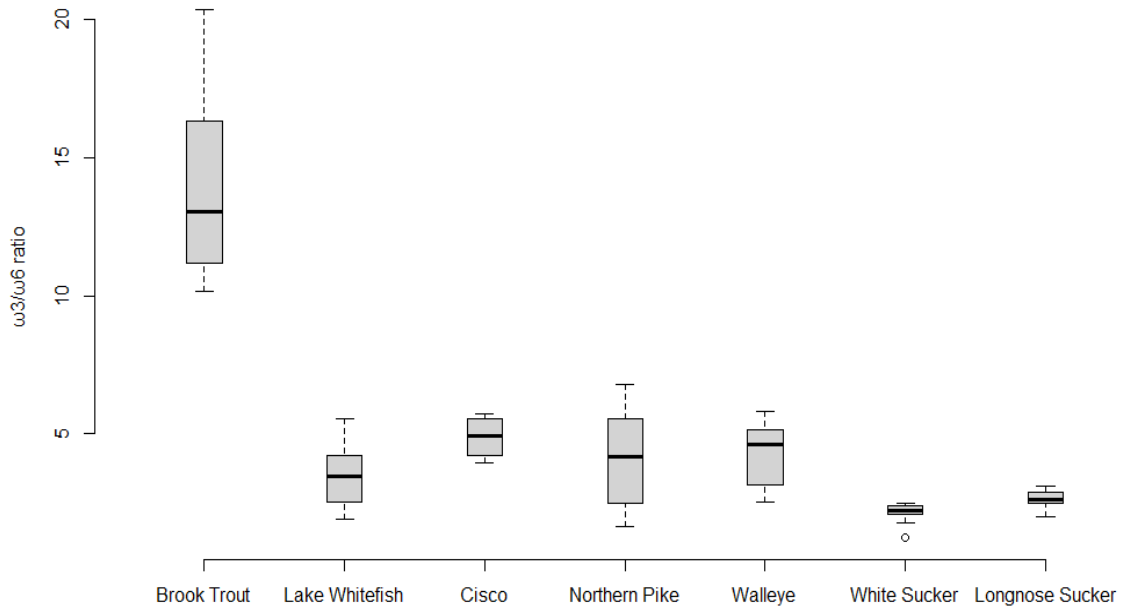


Figure 3.  $\omega_3/\omega_6$  fatty acid ratios in whole fillets of seven fish species sampled from the lower Moose River, ON. Solid bars are medians, boxes are the interquartile range, and whiskers represent maximum and minimum values or  $\pm 1.5 \times$  interquartile range, whichever is less. Data deemed outliers are plotted as points.

Lipid compositions also exhibited considerable spatial variation among coastal rivers, and the pattern of variation appeared to differ among species (Fig. 4). Fillet  $\omega_3/\omega_6$  fatty acid ratios tended to increase from SE to NW among coastal rivers in Lake Whitefish, but decrease along this spatial gradient in Northern Pike (Fig. 4A). Thus,  $\omega_3/\omega_6$  ratios were similar between these species in the SE rivers, but were higher for Lake Whitefish than Northern Pike in the NW rivers (Fig. 4A). Brook Trout  $\omega_3/\omega_6$  fatty acid ratios also appeared to decline from SE to NW, similar to the pattern seen in Northern Pike (Fig. 4B).

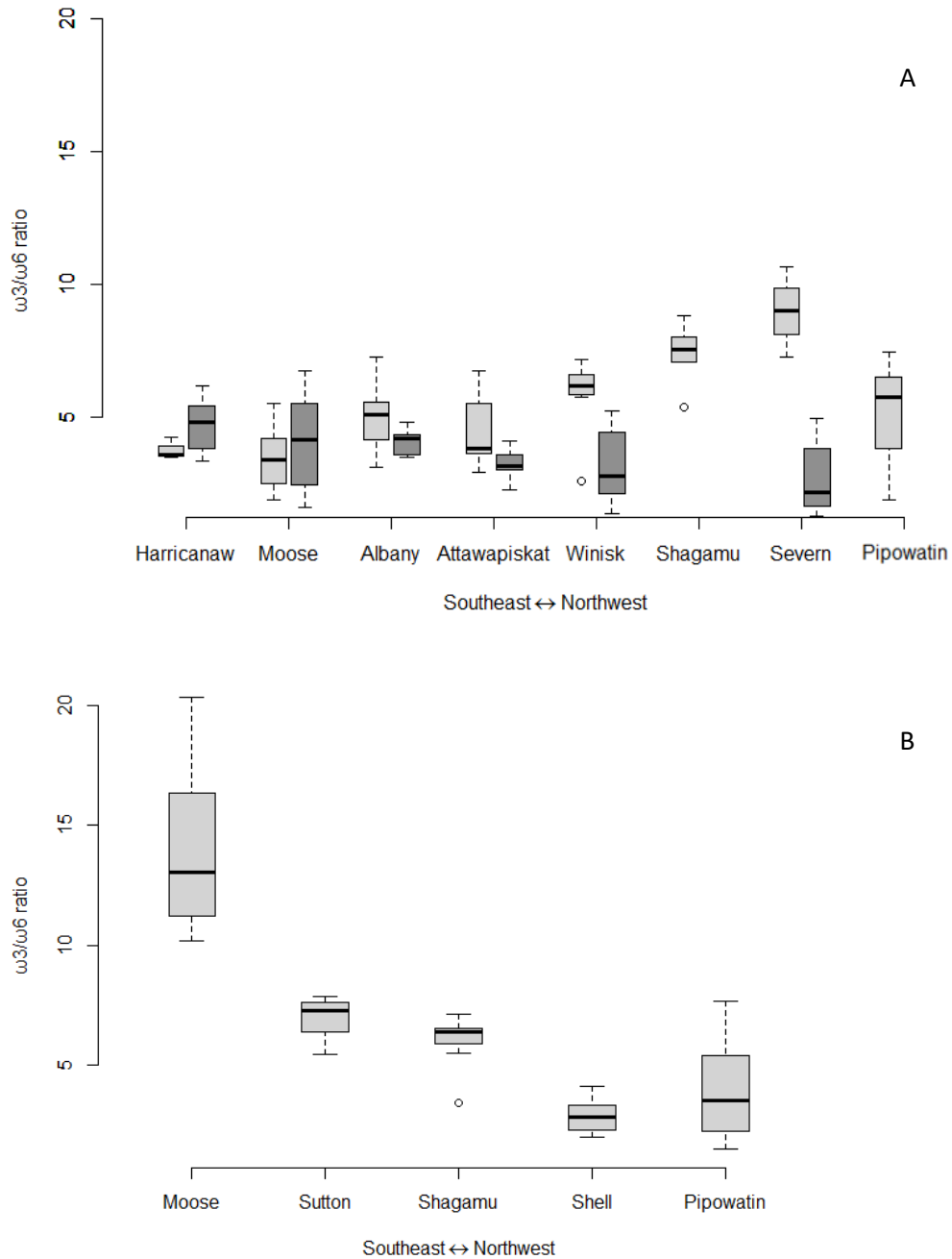


Figure 4. Variation in  $\omega_3/\omega_6$  fatty acid ratios of whole fillets among coastal rivers for A) Lake Whitefish (light grey) and Northern Pike (dark grey), and B) Brook Trout. Solid bars are medians, boxes are the interquartile range, and whiskers represent maximum and minimum values or  $\pm 1.5 \times$  interquartile range, whichever is less. Data deemed outliers are plotted as points.

Species differences were examined further through analysis of complete FA profiles using PERMANOVA. Lake Whitefish and Northern Pike fatty acid compositions differed significantly ( $P < 0.01$ ) in all six river systems where both species were sampled. Lake Whitefish and Brook Trout fatty acid compositions differed significantly in the Shagamu River ( $t = 2.13$ ,  $P = 0.023$ ) but not in the Pipowitan River ( $t = 1.26$ ,  $P = 0.33$ ). Interspecific comparisons of fish sampled from the Moose River revealed significant differences ( $P < 0.05$ ) for all species pairings with the exception of Northern Pike and Walleye, and White Sucker and Longnose Sucker (Table 4).

Table 4. Pairwise species comparison matrix based on PERMANOVA of fillet fatty acid profiles for seven species of fish sampled from the lower Moose River, ON. Resemblance metric for tests was Euclidean distance.

	Brook Trout	Lake Whitefish	Cisco	Northern Pike	Walleye	White Sucker
Lake Whitefish	df=18, t=8.02, p<0.001	.	.	.	.	.
Cisco	df=15, t=4.74, p<0.001	df=15, t=2.20, p<0.05	.	.	.	.
Northern Pike	df=18, t=2.90, p<0.001	df=18, t=5.29, p<0.001	df=15, t=2.81, p<0.001	.	.	.
Walleye	df=18, t=4.04, p<0.001	df=18, t=4.21, p<0.001	df=15, t=1.69, p<0.05	df=18, t=1.65, p=0.052	.	.
White Sucker	df=18, t=8.96, p<0.001	df=18, t=3.67, p<0.001	df=15, t=3.49, p<0.001	df=18, t=5.46, p<0.001	df=18, t=5.10, p<0.001	.
Longnose Sucker	df=18, t=9.45, p<0.001	df=18, t=4.09, p<0.001	df=15, t=3.74, p<0.001	df=18, t=5.86, p<0.001	df=18, t=5.56, p<0.001	df=18, t=1.04, p=0.34

Finally, MDS plots were used to illustrate how differences among species or between different life histories, are associated with particular fatty acids or fatty acid groups in their profiles (Fig. 5). When fish were grouped according to presumed life histories, a noticeable divide was formed with presumed anadromous species on the upper right and freshwater



resident species on the lower left (Fig. 5 A,B). Monounsaturated fatty acids were located on the top right side of the plot in association with purported anadromous fish (Fig. 5 A,B). Polyunsaturated fatty acids, including EPA, DHA, LNA and ARA, were located in the opposite direction towards the lower left in association with fatty acid profiles of freshwater resident fishes (Fig. 5 A,B). Longnose Sucker and White Sucker, both freshwater benthivores, grouped tightly on the bottom right of the plot in association with C18 PUFA or more specifically Vaccenic acid (18:1n-7c) and  $\gamma$ -Linoleic acid (18:3n-6) (Fig. 5 C,D). Palmitoleic acid (16:1n-7c) and Myristic acid (14:0) were located on the right side of the plot in association with Lake Whitefish. Oleic acid (18:1n-9c) and Eurcic acid (22:1n-9) were located on the top right indicating an association with Brook Trout. As well, the relatively less abundant MUFA, Cetoleic acid (22:1n-11) and Gondoic acid were located in the top right of the plot indicating an association with Brook Trout and Lake Whitefish (Fig. 5 D). Two small subsets of Brook Trout separate from the bulk of the Brook Trout samples. One is made up of Moose River fish and is located on the top left of the plot in association with Nervonic acid, similar to other predatory species such as Walleye and Northern Pike (Fig. 5 D). The second subset is composed of five Shagamu River Brook Trout located in the bottom right of the plot and is associated with several odd chained fatty acids. However, it is suspected that the odd fatty acid profiles of these latter Brook Trout may be due to exposure of the fillets to warm temperatures for an extended period of time in the field. Fatty acids are known to metabolize and change at temperatures above -20°C (de Castro et al. 2007).

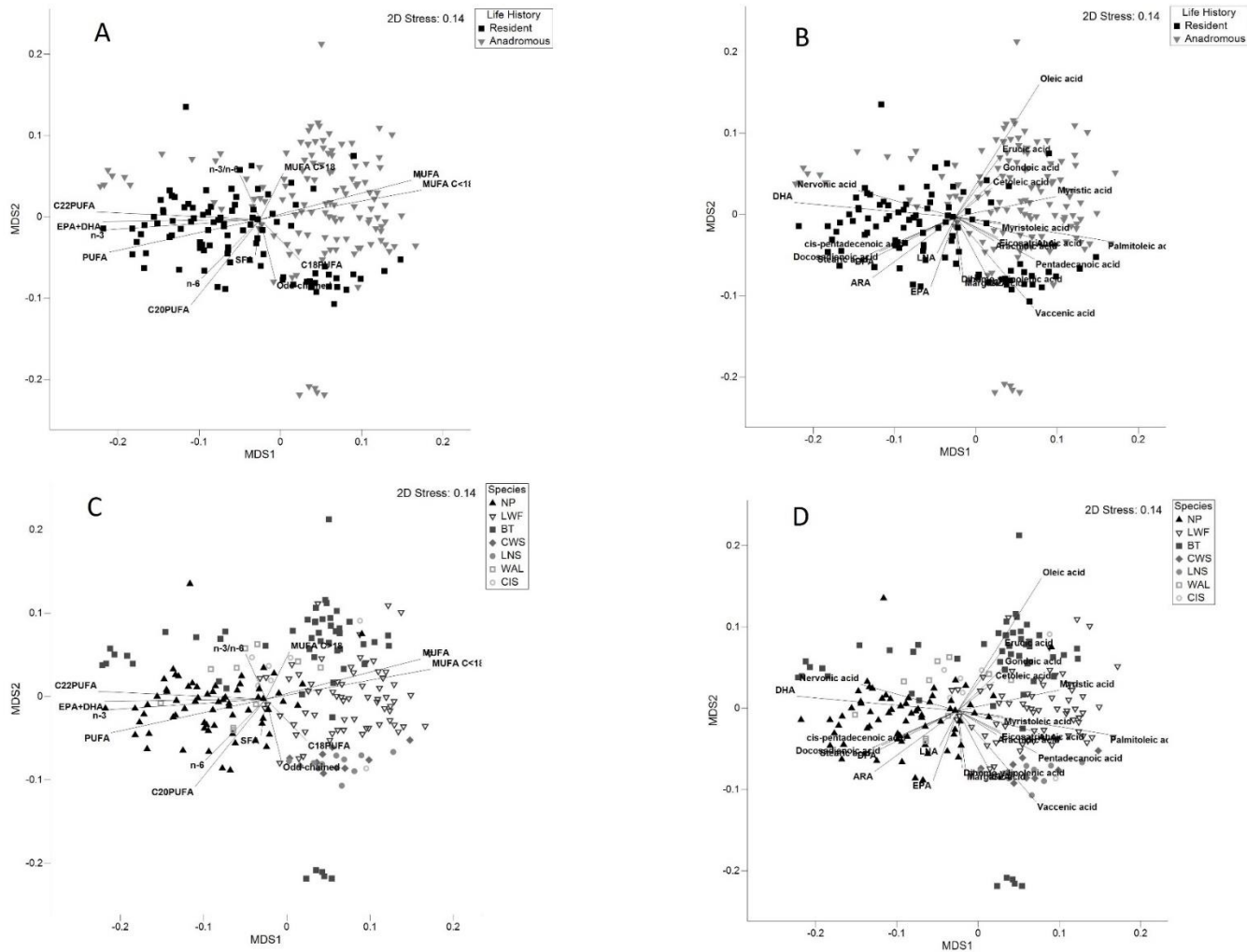


Figure 5. MDS plots based on fillet fatty acid profiles for all individual fish sampled in this study. Fish are categorized according to presumed life history (panels A and B; resident or anadromous) or species (panels C and D; NP = Northern Pike, LWF = Lake Whitefish, BT= Brook Trout, CWS = White Sucker, LNS = Longnose Sucker, WAL= Walleye, CIS = Cisco). Directional vectors represent individual fatty acids (panels B and D; fatty acids with very low abundance were omitted for clarity) or fatty acid groups (panels A and C). Length and orientation of vectors represent relative strength and direction of influence towards particular fish.

## 1.4 Discussion

### *1.4.1 Spatial variation in fish growth*

Fish growth is influenced by a variety of factors including water temperature, food abundance and quality, and the surrounding community structure (Weatherley and Gill 1987). Fish are ectotherms and therefore growth depends on water temperatures. I predicted that, all else being equal, fish growth would be positively related to temperature, and therefore, fish of coastal river populations (mean GDD, 1971-2000, 600-900) would generally grow slower than conspecifics from more southerly, inland lake populations (mean GDD 1971-2000, 750-1050)(Neuheimer and Taggart 2007). This prediction appeared to hold for the benthivorous Lake Whitefish and White Sucker, but the opposite trend was observed in the piscivorous Northern Pike and Walleye, suggesting that temperature is not necessarily the sole limiting factor for growth in all species across the Far North of Ontario.

Differences in food availability or quality could be responsible for the higher than expected growth rates observed in Northern Pike and Walleye from coastal river sites. Could these species be obtaining marine-derived resources at these sites? The migratory behaviour of Northern Pike in the Baltic Sea has been well documented (Muller 1986; Engstedt et al. 2010) but no studies have documented use of marine habitats by North American pike populations. However, anecdotal evidence from northern fishers as well as microchemistry analysis of Northern Pike otoliths from coastal rivers (DeJong 2017) have suggested that some Northern Pike may be feeding in the brackish waters at the mouths of many of these study rivers. Studies have shown that anadromous species have higher lipid content compared to resident conspecifics (Berg and Jonsson 1990; Swanson et al. 2011a; Gladyshev

et al. 2012). In addition, the marine environment has been shown to subsidize nutrient availability in freshwater streams and rivers resulting in increased growth rates of freshwater resident fish (Wipfli et al. 2003). It is therefore possible that predation on anadromous or brackish water prey species is subsidizing Northern Pike and Walleye growth resulting in greater weight at age in coastal river sites.

#### *1.4.2 Stable isotope analysis of food web structure in coastal rivers*

Based on the perceived homogeneity of river ecosystems relative to lake ecosystems, I predicted that niche sizes in general, and niche breadths in particular, would be smaller in coastal river populations than in inland lake populations. I used carbon range (CR) to examine variation in trophic niche breadth among species, and between populations from different sites within drainage basins. Carbon range (CR) quantitatively measures the breadth of primary production sources supporting a population (Layman et al. 2012). Overall, my results were opposite to my prediction; niche breadths tended to be larger in coastal river populations than in inland lake populations for all species examined. Though this trend was only statistically significant for piscivores when examining all drainage basins together, there was good evidence that this trend was also significant for Lake Whitefish in particular drainage basins (Moose, Albany, Attawapiskat), but not others.

A larger niche size or niche breadth indicates greater individual trophic specialization within the population (Araujo et al. 2011). This suggests, that coastal river fish populations with larger CR have a higher diversity of available prey sources (in terms of prey  $\delta^{13}\text{C}$ ) on which to specialize, compared to inland lake populations. The reasons for this may differ

among species. For the piscivores, Northern Pike and Walleye, the larger carbon range in coastal sites compared to inland sites appeared to be due to the presence of individuals with more  $^{13}\text{C}$ -depleted values. This may be related to body composition; lipids are particularly  $^{13}\text{C}$ -depleted and diets rich in lipids have been shown to impart a more negative  $\delta^{13}\text{C}$  signature on consumers (Thompson et al. 2000; Kiljunen et al. 2006). Predation on fatty-fleshed anadromous or brackish water prey at these coastal sites could cause the observed  $^{13}\text{C}$ -depletion in these piscivores. Recent research has suggested that up to 40% of the diet of Northern Pike in lower reaches of the Winisk, Severn and Attawapiskat Rivers is composed of marine-derived nutrients, probably from predation on anadromous prey such as Lake Whitefish and Cisco (DeJong 2017). Alternatively, the larger CR in coastal populations of piscivores may simply indicate that the freshwater habitat of the river offers a much broader diversity in  $\delta^{13}\text{C}$  of prey sources than expected. In contrast to the piscivores, the larger CR of Lake Whitefish at coastal sites compared to inland sites in some drainage basins was associated with the presence of more  $^{13}\text{C}$ -enriched individuals. Enriched carbon isotope signatures are indicative of marine food sources and have been used to differentiate anadromous salmonids from their freshwater conspecifics (McCarthy and Waldron 2000; Ciancio et al. 2008). The higher CR in coastal Lake Whitefish observed in my study could therefore be the result of both freshwater and marine feeding behaviours in coastal sites. Earlier research has noted similar patterns in rivers with both anadromous and freshwater resident fish (Kline Jr et al. 1998; DeJong 2017).

In contrast to niche breadth, I found no overall significant differences in food web positions of the common species between coastal river and inland lake sites. However, there was compelling evidence that Lake Whitefish held quite distinct food web positions in

coastal sites relative to inland sites in some of the drainage basins. In these cases, the Lake Whitefish food web position at coastal sites was at relatively higher  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  compared to inland lake sites. This is consistent with the addition of marine resources to the diet of the coastal population, and similar patterns are evident in comparing freshwater and anadromous individuals in other river ecosystems (France 1995a; McCarthy and Waldron 2000; Ciancio et al. 2008).

#### ***1.4.3 Fatty acid analysis of food web structure in coastal rivers***

Lipids serve key structural and energy storage roles, and both the quantity and quality of lipids found in living organisms can provide important ecological information (Adams 1999). I observed significant variation in both the total lipid content and composition in muscle samples of coastal river fishes. Total lipid content was significantly different between “fatty-fleshed” Brook Trout, Lake Whitefish and Cisco and “lean-fleshed” Northern Pike, Walleye and suckers. Fatty species tend to store the majority their body lipids in their skeletal muscle tissues, whereas lean species store the majority of their body lipids in organ (e.g., liver) and mesenteric tissues (Henderson and Tocher 1987; Weatherley and Gill 1987; Jobling et al. 1998). In addition to having a higher total lipid content, the fatty-fleshed species sampled in this study also had significantly greater absolute concentrations of PUFA, including the nutritionally essential EPA and DHA, a common characteristic of anadromous species (Gladyshev et al. 2012). In contrast, lean-fleshed species had greater relative concentrations of PUFA; for example, PUFA composed >50% of the total fatty acids in Northern Pike muscle. The essential nature of many PUFAs often leads to their selective retention through the metabolism of precursor lipids; this often results in greater ratios of

essential relative to non-essential fatty acids in lean-fleshed species (Sargent et al. 2002). This is an example of the different interpretations that may arise when analyzing fatty acid compositions from absolute and relative perspectives. The choice of approach needs to be based on the objectives of the analysis.

Differences in habitat use and life history affect diet and therefore lipid composition of consumers. I found that muscle lipid composition, based on the whole fatty acid profile, was highly species-specific; only comparisons between Northern Pike and Walleye, and between Longnose Sucker and White Sucker were found to be not significant within the Moose River. MDS analysis illustrated these differences by highlighting the relative importance of different fatty acids to an individual's overall lipid composition. Life history strategies presumed to be anadromous (Lake Whitefish, Brook Trout, Cisco) and freshwater resident (Northern Pike, Walleye, suckers) formed two fairly distinct clusters in multivariate FA space. Concentrations of  $\omega$ -6-PUFA, specifically 20:4n-6 (arachidonic acid; ARA) and 18:2n-6 (linoleic acid; LNA) partially characterized freshwater resident species. Several comparative studies have shown that these fatty acids exist in higher concentrations in freshwater animals than in their marine counterparts (Muje et al. 1989; Linko et al. 1992; Bell et al. 1994). In contrast, MUFA characterize anadromous populations. Two long-chain (LC) MUFA of particular interest, 20:1n-9 (Gondoic Acid) and 22:1n-11 (Cetoleic Acid), normally account for only small proportions of the total FA content. But, these LC-MUFA are unique because they are characteristic of the wax esters found in marine copepods of the genus *Calanus* (Clarke et al. 1987). These copepods dominate marine zooplankton communities in the northern hemisphere and are important prey for planktivorous fish. Once consumed copepod lipids are passed up conservatively to each successive trophic level

(Clarke et al. 1987). Therefore, high proportions of these LC-MUFA fatty acids have been used as a biomarker of marine habitation (Dalsgaard et al. 2003; Graeve et al. 2005).

Differences in feeding strategy were also evident at a finer scale in my FA analyses. Lake Whitefish and Brook Trout had statistically significant differences in their FA profiles, and this is related, in part, to the relative accumulation of long (>18) and short (<18) chain MUFA. Lake Whitefish populations were characterized by high mass fractions of 16:1n-7 (palmitoleic acid), one of the primary products of the desaturation of SFAs and widely distributed in nature. However, among aquatic primary producers it is much less widely distributed; 16:1n-7 is often the most prominent constituent of the lipids of diatoms (Napolitano et al. 1994). High quantities of palmitoleic acid were also identified in a variety of arctic benthos suggesting a benthic or planktonic feeding strategy in Lake Whitefish. In contrast, high quantities of 24:1n-9 (nervonic acid) have been associated with a diet rich in fish oils and could be indicative of piscivory (Parrish 2009). The strong association I observed between nervonic acid and Walleye, Northern Pike and a subset of Brook Trout from the Moose River is consistent with piscivory. The effect of trophic position on the lipid composition of fishes is well documented (Dalsgaard et al. 2003; Alfaro et al. 2006). With each successive trophic transfer lipids are metabolized by the consumer, but some like 20:1n-9 (gondoic acid) remain relatively stable as they are passed up the food chain making them more reliable biomarkers.

Ratios of fatty acids can also provide researchers with a relatively robust biomarker. I found that Brook Trout, Lake Whitefish and Cisco tend to have greater  $\omega$ -3/ $\omega$ -6 fatty acid ratios than Northern Pike, Walleye, White Sucker and Longnose Sucker in coastal rivers. This ratio has been used to assess the relative contribution of marine versus freshwater fatty



acids to the diet, with ratios of 0.5-3.8, and 4.7-14.4 reported for freshwater and marine fish, respectively (Henderson and Tocher 1987). A more recent analysis of globally distributed freshwater and marine fish populations indicates that mean  $\omega$ -3/ $\omega$ -6 ratios for marine and freshwater fish fatty acids are 10.9 and 2.6, respectively (Colombo et al. 2016). In my study, Brook Trout had the highest mean ratio ( $6.7 \pm 4.2$ ,  $n = 51$ ) however, variability was quite high (range 1.5 - 20.4) suggesting a mix of life history strategies between and within populations. Similarly, the  $\omega$ -3/ $\omega$ -6 ratio for Lake Whitefish populations in these coastal rivers ( $5.5 \pm 2.2$ ,  $n = 67$ ) was also quite variable, suggesting mixed individual life history strategies within and/or between populations.

#### ***1.4.4 Summary and conclusions***

Isotopic and fatty acid signatures from Brook Trout, Lake Whitefish, Cisco, Northern Pike and Walleye indicate that marine sources of productivity have been incorporated into the diet of many of these fishes to varying degrees. Fatty acid profiles were found to be highly species-specific and grouped fish by species, presumed life history and feeding behaviour. Evidence of Brook Trout and Lake Whitefish predation on marine calanoid copepods as well as high  $\omega$ -3/ $\omega$ -6 ratios are consistent with an anadromous life history. Walleye and Northern Pike from the lower reaches of these rivers exhibited greater growth and diet breadth than their inland lacustrine conspecifics, possibly as a result of predation on migrating anadromous prey species such as Brook Trout, Lake Whitefish and Cisco. Lake Whitefish growth was however greater in fish from inland lake systems, perhaps due to the longer growing season in the more southern lake ecosystems. Feeding behavior inferred from isotope and fatty acid signatures was variable among rivers, between locations in the

drainage basins, and both between and within species. Coastal populations of Lake Whitefish and Brook Trout exhibited evidence for a variety of feeding behaviours, with many exhibiting signatures associated with the consumption of planktivorous prey or direct planktivorous feeding. Additionally there was evidence of piscivorous behaviour in Moose River Brook Trout and benthic feeding in Lake Whitefish.

## **Chapter 2: Food quality of wild fish inferred from mercury concentrations and essential fatty acid content**

### **2.1 Introduction**

Fish are an excellent dietary source of nutritionally essential amino acids, minerals, vitamins and lipids, specifically omega-3 long-chain polyunsaturated fatty acids ( $\omega$ -3 LC-PUFA). Pioneering studies of Greenland Inuit in the 1970's revealed lower incidence of coronary heart disease and other cardiovascular outcomes among a population with a diet rich in  $\omega$ -3 PUFA (Bang et al. 1976; Kromann and Green 1980). Since then, several studies have identified two  $\omega$ -3 LC-PUFA, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), as the key nutrients responsible for many of the cardio-protective effects associated with consuming fish (Sargent and Henderson 1995; Sargent 1997). Both EPA and DHA are incorporated into cell membrane phospholipids and influence a wide variety of bodily functions including inflammation, immune response, blood clotting, and vasoconstriction (Calder 2015b,a). There is also evidence to suggest that adequate concentrations of  $\omega$ -3 LC-PUFA (and especially DHA) are essential for optimal neurological development in infants and young children (Richardson and Puri 2002; Helland et al. 2003; Stevens et al. 2003; Lauritzen et al. 2004). There are some terrestrial plants (e.g. canola) that are a source of the EPA and DHA precursor,  $\alpha$ -linolenic acid (ALA; 18:3n-3). However, the conversion rate of ALA into EPA and DHA is low in humans (<1% ALA to DHA) making fish and other seafood the primary dietary source of EPA and DHA (Williams and Burdge 2006).

In addition to being essential to many physiological processes in the human body,  $\omega$ -3 fatty acids also play important roles in the health of fish and other vertebrates. Similar to humans, fish require  $\omega$ -3 PUFA's for the maintenance and permeability of cell membranes as well as acting as precursors for highly bioactive compounds known as eicosanoids (Sargent et al. 1999). Lipid mobilization and utilization plays an especially important role in overwinter survival, stress response, reproduction and juvenile success in fish (Adams 1999). Due to the mobilization of particular lipids to meet a variety of physiological demands the lipid composition of fish varies from season to season. These effects are most evident around spawning season and after overwinter starvation (Jeziarska et al. 1982; Guler et al. 2007; Guler et al. 2008).

Consuming fish has also been shown to be a significant route of exposure to environmental contaminants such as mercury (Hg), persistent organic pollutants, and other trace metals in both wildlife and humans (Svensson et al. 1992; McDowell et al. 2004; Castro-González and Méndez-Armenta 2008; Strandberg et al. 2017). The human dietary exposure to these contaminants is highly variable among waterbodies, species, and age and size classes of fish (Ontario Ministry of the Environment and Climate Change (OMOEC) 2015). The most widespread and studied contaminant in aquatic systems of northern Ontario is Hg, a highly reactive metal emitted from both natural and anthropogenic sources. Natural sources include volcanoes and geothermal vents while anthropogenic sources include fossil fuel burning, waste incineration, gold mining, ferrous and non-ferrous metal smelting, and cement manufacturing (Pirrone et al. 2010). Elemental mercury ( $\text{Hg}^0$ ) can be transported long distances through the atmosphere before being oxidized to  $\text{Hg}^{2+}$  and deposited onto the land and water with precipitation. In aquatic systems anaerobic microbes can convert  $\text{Hg}^{2+}$  into

the organic form, methylmercury (MeHg), which is much more bioavailable and toxic. Because MeHg is more easily absorbed than excreted by organisms, concentrations tend to increase over time in consumer tissues, a process called bioaccumulation (Keating et al. 1997). Bioaccumulated MeHg can pass through the mammalian placenta, and across the blood-brain barrier leading to neurological impairment of the fetus. Young or unborn children are most at risk; fetal exposure to very high levels of MeHg through maternal diets of contaminated seafood can result in cases of severe mental dysfunction and sometimes diseases such as cerebral palsy (Eto 1997; Gochfeld 2003). High MeHg exposure has also been associated with several deleterious cardiovascular outcomes such as greater incidence of myocardial infarction, high blood pressure, irregular heart rate and atherosclerosis (Rissanen et al. 2000; Salonen et al. 2000; Choi et al. 2009). However, there is some evidence that the nutritional benefits of consuming fish may outweigh these risks from Hg exposure (Guallar et al. 2002; Virtanen et al. 2005). Clearly, the food quality of fish needs to be considered both in terms of the health benefits of important nutrients and the deleterious effects of contaminants, and recent research on subsistence diets has been moving in this direction (Seabert et al. 2014).

The coastal rivers of the Hudson Plains Ecozone support fish populations that are important food sources for northern First Nations communities, but the food quality of these fishes has not been closely examined. There has been no study, to my knowledge, examining fatty acid contents of fishes in these waters. There has also not been an extensive study of fish Hg concentrations across these rivers for many years (McCrea et al. 1984), and fish Hg data for most rivers is either out-dated or lacking (McGovern and Vukelich 2009). Though rivers of the Hudson Plains are far from point sources of Hg emissions, anthropogenic Hg is

still a significant source of inorganic Hg in the region (Brazeau et al. 2013). A current and comprehensive study of fish Hg levels in these rivers is needed to update and refine fish consumption guidelines, provide baseline information on environmental contaminant levels ahead of projected climate change and anthropogenic development in the region, and allow us to test predictions about the factors contributing to spatio-temporal variation in fish Hg levels. Combining such a survey with analysis of essential fatty acid content of the same fish could allow further refinement of consumption recommendations through a cost-benefit approach.

Both fatty acid and Hg contents in fishes of the Hudson Plains are expected to vary at multiple scales – among species, among waterbodies, and over time. My Chapter 1 results demonstrated that fatty acid compositions of coastal river fish vary among fish species, and possibly among rivers, and it is well-known that fish Hg concentrations vary widely among species and lakes of northern Ontario (Tang et al. 2013). However, it is unknown how fatty acid and Hg contents of fish co-vary with each other. In terms of interspecific patterns, some co-variation is expected. The group of fish that I identified in Chapter 1 as lean-fleshed includes piscivores known to have high Hg levels (e.g., Walleye, Northern Pike), whereas the group I identified as fatty-fleshed includes only low Hg species (e.g., Lake Whitefish, Cisco). Furthermore, anadromous fish generally have lower Hg (Swanson et al. 2011a) and higher essential fat content (Gladyshev et al. 2012) than resident freshwater fish. In terms of temporal variation, there is no historic fish fatty acid data from northern rivers to test for temporal changes, but some limited historic fish Hg data do exist. Given recent declines in atmospheric deposition of Hg in North America (Prestbo and Gay 2009), a similar decline

may be expected in fish of northern rivers, though recent temporal analyses in Ontario lakes have shown conflicting trends (Bhavsar et al. 2010; Tang et al. 2013).

In terms of spatial co-variation of fish fatty acid and Hg levels, the patterns may be more complex. The coastal rivers of the Hudson Plains in Ontario span over five degrees in latitude (Appendix 1), and environmental variation over this wide spatial scale could contribute to variation in lipid and Hg contents of native fishes in diverse ways. First, atmospheric Hg deposition varies latitudinally and may decline from SE to NW (Muir et al. 2009). Second, the cooling climate from SE to NW along the coast may be associated with decreasing fish growth, and growth dilution effect, hence increasing Hg concentrations at a given size (Karimi et al. 2007). Cooler climates may also be associated with higher EPA+DHA contents of fish flesh (Arts et al. 2015). Third, there is a decline from SE to NW in human habitation, landscape disturbance (agriculture, forestry, mining), water regulation, and hydroelectric development in the upper drainage basins of these rivers. Water chemistry and MeHg production can be affected by such disturbances, particularly water diversion and impoundment for hydroelectric development (Kelly et al. 1997). Finally, there is a SE to NW increase in salinity of the nearshore coastal zones of Hudson and James Bays (Stewart and Lockhart 2005), with major rivers creating local pockets of lower salinity water (Prinsenberg 1988). Osmoregulatory responses to changes in salinity can affect the EPA+DHA contents of an organism's flesh (Chen et al. 2014), and thus the salinity of water utilized by anadromous fish may influence their lipid composition and food quality.

The objective of this chapter is to explore patterns in the spatio-temporal variability of both Hg concentrations and fatty acid contents of fishes in coastal rivers of the Far North

of Ontario, and determine how fish consumption decisions based on Hg may influence essential fatty intake from eating these fish. I had five key predictions:

i) Mercury concentrations in fishes of Far North coastal rivers will have declined since the late 1970's due to reductions in atmospheric Hg deposition.

ii) Mercury concentrations in fishes of Far North coastal rivers will be highest in the SE and lowest in the NW extent of my study area following decreasing gradients of human development and atmospheric Hg deposition with increasing latitude.

iii) Fish Hg concentrations will vary between coastal rivers and inland headwater lakes according to differences in fish growth rates; fish Hg concentrations will be higher where growth rates are lower (testing of growth dilution hypothesis).

iv) Essential fatty acid contents (specifically EPA+DHA) of anadromous species will be lowest in the SE and highest in the NW following the marine salinity gradient along the coast, but there will be no spatial gradient in non-anadromous species.

v) In all rivers, anadromous species of fish will provide the consumer with the highest food quality due to their combination of lower Hg concentration and higher EPA+DHA content.



## **2.2 Methods**

### ***2.2.1 Study area, data acquisition and field sampling***

The study area for this research was the drainage basins of 14 coastal rivers in the Hudson Plains Ecozone of northern Ontario, as described in Chapter 1. I utilized data and archived tissue samples collected from fish communities in lower reaches of these rivers and in headwater lakes from 1978 to 2014 by the Ontario Ministry of Natural Resources and Forestry (MNR), Laurentian University, and the Ontario Ministry of Environment and Climate Change (MOECC), and supplemented these data and tissues with my own sampling program in 2015 and 2016. Fish species included those most commonly harvested by local fishers; Lake Whitefish (*Coregonus clupeaformis*), Cisco (*Coregonus artedii*), Brook Trout (*Salvelinus fontinalis*), Northern Pike (*Esox lucius*), Walleye (*Sander vitreus*), as well as two other common native benthivores, White Sucker (*Catostomus commersonii*) and Longnose Sucker (*Catostomus catostomus*). Fish collection and field processing are outlined in Chapter 1, section 1.2.1.

### ***2.2.2 Laboratory analysis***

Skinless, dorsal muscle samples collected during fish processing were each divided into a smaller (~ 10 g) portion and a larger (~ 20 g) portion. The larger portion was stored at -20 °C, then homogenized, subsampled and analyzed for total mercury concentration ([THg]) by the Laboratory Services Branch of the Ontario Ministry of Environment and Climate Change (MOECC) (Etobicoke, ON). Analysis was by hot-block digestion followed by cold vapour-flameless atomic absorption spectroscopy (CV-FAAS) according to protocol

HGBIO-E3057 (Ontario Ministry of the Environment (OMOE) 2006). Mercury analysis methods in this laboratory have remained consistent from the 1970s to the present. Results were expressed as  $\mu\text{g g}^{-1}$  (ppm) on a wet mass basis. The smaller portion of muscle sample was freeze-dried and ground in preparation for stable isotope analysis, as described in Chapter 1 (Section 1.2.2). For a subset of the fish analyzed for stable isotope composition, an aliquot (1 – 3 mg) of this dried and ground muscle was also analyzed for total mercury content by Atomic Absorption Spectroscopy (AAS) on a Milestone direct mercury analyser (DMA-80) at the BioTron Analytical Services Laboratory (Western University, London, ON). Total Hg concentrations were calculated as  $\mu\text{g g}^{-1}$  (ppm) then converted to a wet mass basis using mean moisture content (%) values for each species generated during the fatty acid analysis of fish muscle tissue (Chapter 1, Section 1.2.2). Wet mass concentrations were used for all subsequent analyses. Laboratory analysis for fatty acid mass and compositions of whole fillets is outlined in Chapter 1 (Section 1.2.2).

### ***2.2.3 Statistical analyses***

Spatio-temporal analyses of fish [THg] were carried out exclusively with data generated by the MOECC laboratory; available fish [THg] data spans nearly 40 years (1978-2017). Data were selected for two time periods - historical (1978-1989) and modern (2010-2017) - for Lake Whitefish, Northern Pike, Walleye, White Sucker and Brook Trout samples from the coastal sites (lower reaches) of the study rivers, and where available, for these same species from inland sites in headwater lakes of the drainage basins (Chapter 1, Section 1.2.1, Table 1). For each population (species x site) and time period a power series regression using a non-linear least squares model of [THg] vs total length (TLEN) was used to estimate mean

predicted [THg] at 459, 557, 478, 437, and 456 mm for Lake Whitefish, Northern Pike, Walleye, White Sucker, and Brook Trout, respectively. These TLEN values correspond to predicted mean length at 1 kg, and thus, the mean predicted [THg] values are for a standard 1 kg fish. Subsequent spatio-temporal analyses used these predicted [THg] at 1 kg values. The one exception was for spatial analysis of Brook Trout where mean predicted [THg] was estimated at a body size of 500 g (TLEN = 366 mm) because size distributions for this species were generally lower across rivers.

Analyses using these predicted [THg] values proceeded as follows. Temporal analysis was carried out for each species using only coastal site data, and took a two-point, paired-comparisons (modern minus historic) approach with either a paired-comparisons t-test for normal data or a Wilcoxon signed-rank test for non-normal data (Tang et al. 2013). Normality was assessed by the Shapiro-Wilk test. Subsequent spatial analyses used only modern (2010-2017) [THg] data and were carried out in two steps. First, I tested for a latitudinal effect on [THg] using data for coastal sites only. Mean predicted [THg] at 1 kg (or 500 g for Brook Trout) was regressed against latitude for each species. Second, I tested for a drainage basin position effect (coastal vs inland sites) using a linear mixed effects model of the form;

$$\text{Model 1: } \text{Predicted [Hg]} = \text{Position}(\text{fixed effect}) + \text{Species}(\text{fixed effect}) + \\ \text{Drainage Basin}(\text{random effect}) + \text{Species} * \text{Position}(\text{interaction term})$$

The significance of the species\*position interaction term was determined using a likelihood ratio approach whereby an analysis of variance (ANOVA) was conducted between Model 1 and an identical model without the interaction term. The interaction term was found to be

significant indicating the position effect was not the same for each species. Therefore mixed effects models (Model 2) were fit for each species individually.

*Model 2: Predicted [Hg] = Position(fixed effect) + Drainage Basin (random effect)*

In each case the significance of the position effect was determined using a likelihood estimate whereby an ANOVA between Model 2 and an identical model with the fixed effect position removed determined the significance of the drainage basin position for each species.

Whole fillet total lipid and fatty acid concentration data were available only for recent sampling of coastal sites. I found no consistent, significant relationships between lipid variables and body size, and therefore, calculated unadjusted means for each population (species x site). I first examined latitudinal variation in each of total lipid and EPA+DHA concentrations (wet mass basis) of whole fillets using the linear model:

*Model 3:  $Y = \text{Species (fixed effect)} + \text{Latitude (fixed effect)} + \text{Species} * \text{Latitude (Interaction)}$*

However, in both cases, the interaction term was determined to be significant using the likelihood estimate approach, indicating that the latitude effect differed among species. Therefore, I reduced the models to simple regression models for each species.

Fatty acid and [THg] data were combined in a cost-benefit analysis of consuming fish from each population. Mercury data from the MOECC were used, except for fish populations from the Moose River, where [THg] data from the Western Biotron Lab were used. In all cases, only [THg] data from modern sampling (2010-2017) were used. Predicted mean [THg] was estimated at 50 mm intervals from 200 to 700 mm total length using a power series regression for each fish population. Predicted values were compared with benchmark values

for both the general human population (all > 15 years old, except women of child-bearing age) and sensitive human population (all < 15 years old, plus women of child-bearing age). Benchmark values are recommended maximum meals per month of fish of different [THg], assuming all Hg in fish is MeHg. They are calculated by MOECC using Health Canada's tolerable daily intake values (TDI) for MeHg (Bhavsar et al. 2011) which are currently 0.47 and 0.20 ug MeHg kg body mass<sup>-1</sup> day<sup>-1</sup> for the general population and sensitive population, respectively. Using the benchmark values (Appendix 4), and the predicted mean [THg] values I calculated the maximum number of meals (227 g serving) that could be consumed per month. The maximum monthly intake of EPA+DHA was generated by applying the population mean EPA+DHA content in one 227 g meal and extrapolating using the maximum number of meals per month. The recommended dose of EPA+DHA differs among different organizations worldwide. A range of values from 250-1000 mg day<sup>-1</sup> of EPA+DHA have been recommended for a variety of possible positive outcomes. The World Health Organization as well as numerous studies on cardio-vascular disease (CVD) prevention recommend 500 mg day<sup>-1</sup> of EPA+DHA (Kris-Etherton et al. 2009). I therefore chose 500 mg day<sup>-1</sup> of EPA+DHA as the recommended dose and used this to generate the percentage of the recommended monthly intake of EPA+DHA that would be obtained from consuming the maximum number of meals allowed per month based on mercury guidelines. Values generated for fish which had total length values outside the range of total lengths present in the data set were omitted.

## 2.3 Results

### 2.3.1 Temporal trends in mercury concentrations

A comparison of historical and modern fish [THg] at coastal river sites indicated a declining trend over time in all species. However, mean declines over this ~ 30 year period were small (e.g., 0.01 ppm for Lake Whitefish, 0.06 ppm for Northern Pike) and none of these trends were statistically significant (Table 5).

Table 5. Results of paired-comparisons t-tests of mean predicted muscle [THg](ppm wet) at 1 kg for the time periods 1978-1989 (H = historic) and 2010 -2017 (M = modern) for four fish species sampled from coastal rivers of the Far North of Ontario. All data from MOECC Fish Contaminant Monitoring Program. n = number of populations sampled.

Species	Trend	t	n	P-value
Lake Whitefish	M < H	-0.76	6	0.48
Northern Pike	M < H	-0.70	7	0.51
Walleye	M < H	-1.03	3	0.41
White Sucker	M < H	-1.72	5	0.16

### 2.3.2 Spatial trends in mercury and essential fatty acids

Regression analysis of mean predicted muscle [THg] against latitude indicated a declining trend moving northwards for most species, but an increasing trend for White Sucker (Table 6). However, all of these trends were slight and not statistically significant (Table 6).

Table 6. Analysis of variance results from regressions of mean predicted muscle [THg] at 1 kg vs latitude for five species of fish sampled from coastal rivers in the Far North of Ontario. All data from MOECC Fish Contaminant Monitoring Program.

<b>Species</b>	<b>Slope</b>	<b>F</b>	<b>df</b>	<b>P-value</b>
<b>Northern Pike</b>	-0.009	0.21	4	0.67
<b>Lake Whitefish</b>	-0.001	0.004	4	0.95
<b>White Sucker</b>	0.019	0.40	4	0.53
<b>Walleye</b>	-0.036	0.40	4	0.56
<b>Brook Trout</b>	-0.002	0.37	6	0.57

The results of likelihood analysis to test for drainage basin position effects indicated significantly greater predicted [THg] at 1 kg at coastal river sites than at inland lake sites for Lake Whitefish (Chi Sq = 6.06, P = 0.014), White Sucker (Chi Sq= 4.86, P= 0.028), and Walleye (Chi Sq= 5.40, P= 0.020) but not Northern Pike.

Whole fillet lipid content declined from south to north for Northern Pike (regression analysis, slope = -4.56, F = 7.48, df = 5, P= 0.041). Similarly, fillet EPA+DHA content showed a northward decline for Northern Pike (Fig. 6, Table 7). In contrast, these variables both tended to increase with latitude in Lake Whitefish and Brook Trout but the trends were not statistically significant (Fig. 6, Table 7). In general, there did not appear to be any indication of concordance between muscle mercury and EPA+DHA contents across rivers for any of the species examined (Fig. 6).

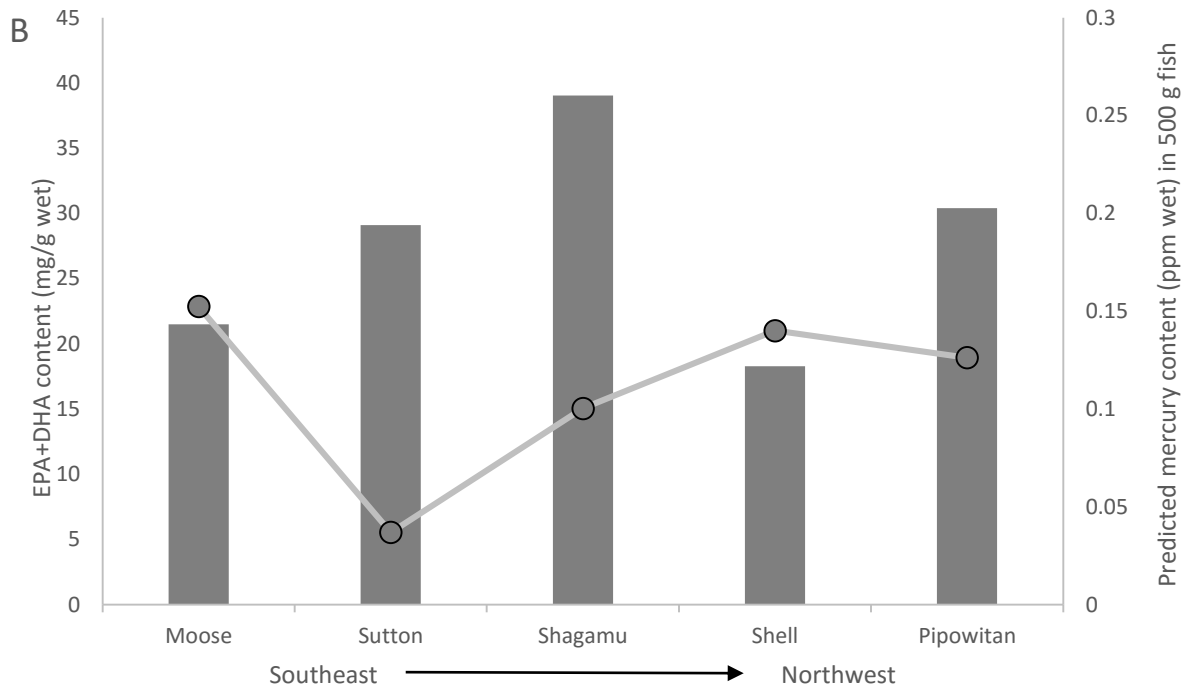
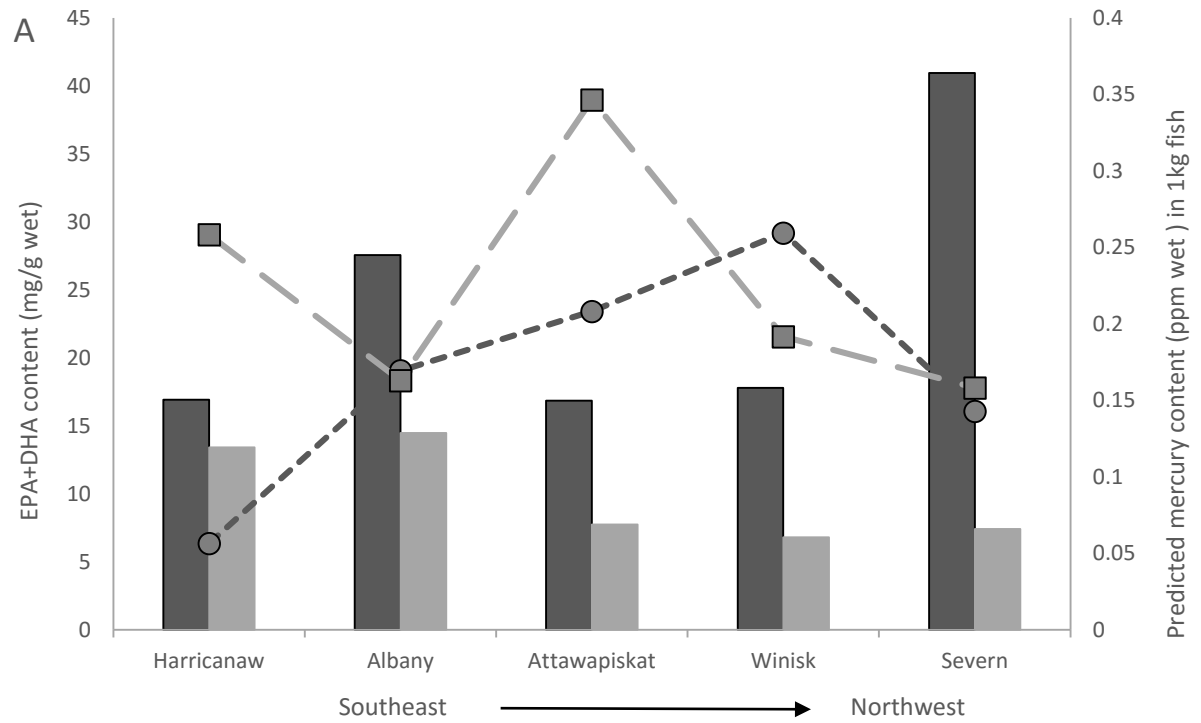


Figure 6. Mean essential fatty acid (EPA+DHA; bars) and mercury (symbols and lines) contents in fish from Far North coastal rivers. A) Northern Pike (light grey bars, and line joining squares), and Lake Whitefish (dark grey bars, and line joining circles). B) Brook Trout. All mercury data from MOECC Fish Contaminant Monitoring Program.



Table 7. Results of linear regressions of mean muscle EPA+DHA content vs latitude for three species of fish sampled from Far North coastal rivers. \* significant at  $P < 0.05$

Species	Slope	F	df	P-value
Northern Pike	-1.74	10.5	5	0.023*
Lake Whitefish	1.97	1.49	5	0.277
Brook Trout	1.70	0.890	4	0.399

### 2.3.3 Interspecific comparison of mercury and essential fatty acid concentrations

As expected, fish [THg] showed interspecific variation. In the Moose River, Walleye had the highest, and Brook Trout and Lake Whitefish had the lowest mean predicted muscle [THg] at 1 kg (Fig. 7A). As noted in Chapter 1 (Section 1.3.3), when fish from all rivers were combined, they roughly divided into two groups based on mean fillet lipid content – fatty-fleshed species ( $> 3.9\%$  WW) and lean-fleshed species ( $< 3\%$  WW). When just the Moose River fish were examined, the ranking differed somewhat, most notably Brook Trout had much lower than expected fillet total lipid (Fig. 7B), relative to the other Brook Trout populations. For all fish combined, total EPA+DHA content was positively correlated with total lipid content ( $r = 0.57$ ,  $df = 211$ ). However, the total EPA+DHA vs total lipid correlation was less distinct among species in the Moose River (Fig. 7B), suggesting that it may be driven more by intraspecific and/or among-population variation. Omega-3 fatty acid content was highly variable among co-habiting species in the Moose River (Fig. 6B). The highest concentrations of EPA+DHA were found in Cisco ( $36.7 \pm 4.2$  mg g wet<sup>-1</sup>), Brook Trout ( $21.5 \pm 3.3$  mg g wet<sup>-1</sup>) and Lake Whitefish ( $19.6 \pm 2.6$  mg g wet<sup>-1</sup>).

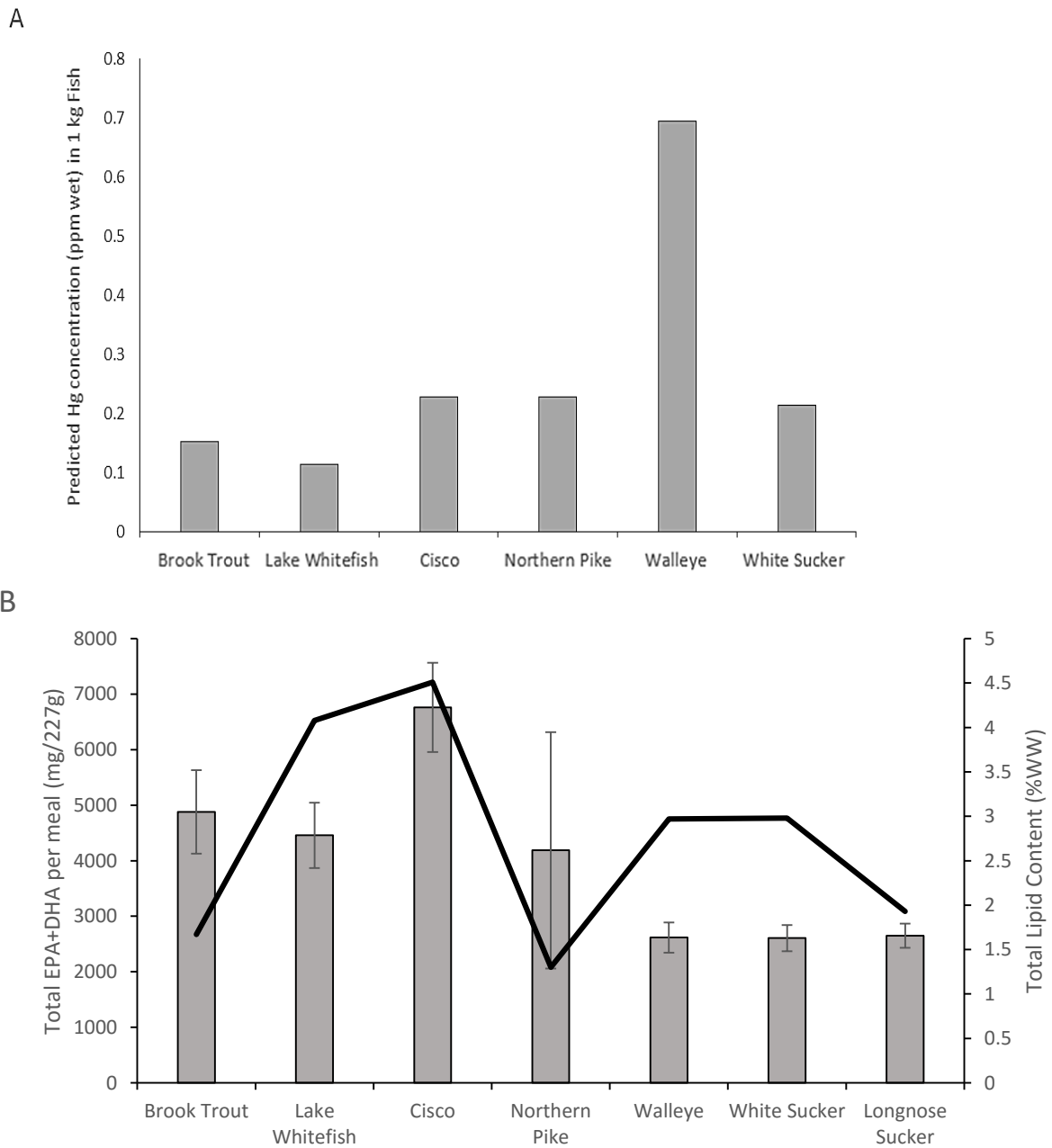


Figure 7. Interspecific comparisons of mercury and essential fat content in seven species of fish sampled from the lower Moose River, ON, 2015-2016. A) Mean predicted muscle [THg] at 1 kg. Mercury analysis by Western University BioTron Lab. B) Mean ( $\pm$  SE) EPA+DHA content per meal (227 g) (bars), and mean total lipid concentration of fillet (line). n = 7-10 fish per species.

Concentrations of  $\omega$ -3 fatty acids were also relatively high in Northern Pike, but also quite variable ( $18.5 \pm 9.4$  mg g wet<sup>-1</sup>). Walleye, White Sucker and Longnose Sucker had the lowest essential fat content with  $13.6 \pm 1.4$ ,  $16.1 \pm 1.6$  and  $16.3 \pm 1.3$  mg g wet<sup>-1</sup>, respectively.

### ***2.3.4 Cost-Benefit analysis***

The recommended maximum number of meals per month based on [THg] is highly river- and species-specific for both general and sensitive population groups. Within the Moose River, the most restrictive limits are placed on Walleye while the most lenient restrictions are placed on Brook Trout, Lake Whitefish and Cisco (Table 8). In all fish species from all rivers the realized intake of EPA+DHA decreased with increasing fish length due to the increased restrictions placed on larger fish with higher [THg] (Table 8; Appendix 5). Within the Moose River the maximum number of meals per month for the general population for all species and lengths allow for the recommended daily intake (RDI) of 500 mg day<sup>-1</sup> of EPA+DHA with the exception of Walleye over 400 mm (Table 8). Across all rivers and lengths both Brook Trout and Lake Whitefish meal restrictions allow the RDI of EPA+DHA to be met for both the general and the sensitive populations. As well, northern fishers in the general population can meet the RDI of EPA+DHA, within mercury-imposed maximum meal limits, by consuming Northern Pike from all rivers except the Attawapiskat (93% of RDI for > 600 mm TLEN; Appendix 5). Greater restrictions on the number of meals for the sensitive population reduce the dose of EPA+DHA below recommended levels in larger Northern Pike from all rivers with the exception of the Moose River. Meal recommendations generated for all other rivers are presented in Appendix 5.

Table 8. Fish consumption recommendations for the Moose River. Maximum number of meals (227 g) per month, based on MOECC benchmarks, and the percentage of the recommended dose of EPA+DHA (500 mg day<sup>-1</sup>) achievable when consuming the maximum number of meals for both general and sensitive populations.

Moose River	Total Length (mm)										
	200	250	300	350	400	450	500	550	600	650	700
<b>Brook Trout</b>											
General Population (meals)	.	.	.	.	32	32	16	16	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	.	1027	1027	513	513	.	.	.
Sensitive Population (meals)	.	.	.	.	12	12	8	8	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	.	385	385	257	257	.	.	.
<b>Lake Whitefish</b>											
General Population (meals)	.	.	32	32	16	16	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	938	938	469	469	.	.	.	.	.
Sensitive Population (meals)	.	.	16	16	8	8	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	469	469	234	234	.	.	.	.	.
<b>Cisco</b>											
General Population (meals)	.	.	32	32	.	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	1423	1423	.	.	.	.	.	.	.
Sensitive Population (meals)	.	.	16	16	.	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	711	711	.	.	.	.	.	.	.
<b>Northern Pike</b>											
General Population (meals)	.	.	32	32	32	16	16	16	16	16	12
Monthly dose EPA+DHA(%)	.	.	881	881	881	441	441	441	441	441	331
Sensitive Population (meals)	.	.	16	16	12	12	8	8	4	4	4
Monthly dose EPA+DHA(%)	.	.	441	441	331	331	220	220	110	110	110
<b>Walleye</b>											
General Population (meals)	.	.	16	12	8	4	4	4	.	.	.
Monthly dose EPA+DHA(%)	.	.	275	207	138	69	69	69	.	.	.
Sensitive Population (meals)	.	.	4	4	4	0	0	0	.	.	.
Monthly dose EPA+DHA(%)	.	.	69	69	69	0	0	0	.	.	.
<b>White sucker</b>											
General Population (meals)	.	.	32	32	16	16	16	12	.	.	.
Monthly dose EPA+DHA(%)	.	.	548	548	274	274	274	206	.	.	.
Sensitive Population (meals)	.	.	16	12	8	8	4	4	.	.	.
Monthly dose EPA+DHA(%)	.	.	274	206	137	137	69	69	.	.	.
<b>Longnose Sucker</b>											
General Population (meals)	.	.	32	32	16	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	557	557	279	.	.	.	.	.	.
Sensitive Population (meals)	.	.	16	12	8	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	279	209	139	.	.	.	.	.	.

## 2.4 Discussion

### *2.4.1 Temporal trends in fish mercury contamination*

I noted a slight decline in fish [THg] between historical (1978-1989) and modern (2010-2017) time periods in coastal rivers of the Far North but these trends were weak and not statistically significant. North American atmospheric Hg concentrations have decreased in recent decades due to reductions in fossil fuel and coal-fired utility emissions (Mohapatra et al. 2007; Zhang et al. 2016), and I predicted that temporal trends in fish [THg] in coastal rivers may reflect these trends. But, analysis of fish Hg trends over recent decades in other northern regions has shown conflicting trends (Monson 2009; Simonin et al. 2009; Bhavsar et al. 2010; Tang et al. 2013). This suggests that temporal changes in fish Hg may vary regionally, and/or that atmospheric deposition of inorganic Hg is not currently limiting to fish Hg levels. Atmospheric Hg can travel long distances due to its slow rate of oxidation, however, the deposition rate generally decreases with increasing distance from point sources (Muir et al. 2009). Recent sediment core analysis of lakes in the Hudson Plains indicated that waterbodies in the region still receive significant atmospheric Hg deposition, despite their remote location, but that these inorganic inputs are probably not currently limiting MeHg production (Brazeau et al. 2013).

There are several confounding factors that complicate the interpretation of my temporal analysis results. First, Hg is a global pollutant and while North American atmospheric Hg is declining, Russian and Chinese atmospheric Hg emissions have remained the same or increased (Pirrone et al. 1996; Pirrone et al. 2010). As well, changes in climate over the past several decades may have a profound influence on Hg bioavailability. Increased

primary production in lakes in the HPE as a result of climate warming may increase Hg bioavailability (Rühland et al. 2013). Brazeau et al. (2013) identified a positive relationship between sediment organic matter and MeHg in sediment cores from HPE lakes, and hypothesized that algal scavenging in the water column may be transporting inorganic Hg to methylation sites on the sediment surface. This would imply of course that increased productivity due to a warming climate could increase MeHg production, and increased bioaccumulation in fish. In contrast, increased fish growth has been shown to dilute (biodilution) the concentration of MeHg in aquatic organisms (Karimi et al. 2007; Ward et al. 2010). It is possible that increases in primary productivity and growing season (GDD), both driven by climate change, also resulted in increased fish growth which may have counteracted the effects of increased algal scavenging on MeHg production.

#### ***2.4.2 Spatial trends in fish mercury and essential fatty acid concentrations***

I predicted a latitudinal decline in fish [THg] at coastal river sites, following the expected geographic pattern of declining atmospheric Hg deposition, and the observed geographic pattern of decreasing human disturbance to the drainage basins. In general, I found that Hg levels did decrease along this gradient, for most species, but the decline was slight and not significant. An additional consideration is that differences in species diversity among rivers may influence food web complexity and Hg biomagnification. In the Far North, the larger and more southern study rivers have higher fish diversity than the smaller or more northern study rivers (Browne 2007). Increases in food web diversity can potentially lengthen food chains, thus increasing Hg biomagnification to top predators. This effect has been demonstrated in Lake Trout (*Salvelinus namaycush*) lakes of the Boreal Shield (Cabana

et al. 1994). However, despite these various factors that could potentially promote a south to north gradient in fish Hg levels, I found the latitudinal trend to be quite weak.

Mercury concentrations in fish can show substantial regional and even waterbody-specific variation. I found that [THg] was higher at coastal river sites than at inland lake sites for some fish species, but not others. I predicted that coastal vs inland patterns in fish [THg] would mirror those seen in fish growth, following the growth dilution hypothesis. My results partly supported this prediction; Lake Whitefish grew significantly more slowly and had higher [THg] at coastal sites, whereas Northern Pike grew significantly faster and had lower (though not significantly) [THg] at coastal sites. The observed variability in fish Hg patterns is due to the complex pathways by which inorganic Hg is made bioavailable through microbial methylation, and the subsequent bioaccumulation and biomagnification processes as MeHg moves through the food web. As discussed above, increased algal production related to climate warming can influence Hg bioavailability by altering methylation (Brazeau et al. 2013; Rühland et al. 2013). Regional differences in climate between the relatively cooler coastal sites and warmer inland lake sites could influence algal productivity and MeHg production, but could also influence fish growth and biodilution. The counteracting effects of these two mechanisms may be partially responsible for the variability observed in fish [THg] between coastal river and inland lake sites.

Methylmercury production is also linked to a variety of other environmental conditions in waterbodies and their drainage basins (Hintelmann 2010) and landscape variability could have influenced my coastal vs inland comparison. A recent landscape assessment of variation in fish [THg] across northern Ontario lakes concluded that water chemistry and drainage basin characteristics accounted for more of the observed variation

than climate or food web structure (Sumner 2016). My coastal sites were all located within the peatland-dominated Hudson Plains Ecozone while my inland sites included both lakes on the Hudson Plains and lakes on the granite-rich Boreal Shield Ecozone. Mercury concentrations in European Perch (*Perca fluviatilis*) from humic lakes with catchments containing a large proportion of peatland were significantly greater than conspecifics from clear-water lakes (Strandberg et al. 2016). Peatland sediments are high in organic carbon and studies indicate that peatlands can have high peat and pore water MeHg concentrations (Branfireun et al. 1999), possibly due to the reduction of sulfates by anaerobic bacteria along the peat-water interface (Heyes et al. 2000). However, recent findings indicate that peatlands in the HPE may not contain high concentrations of sulfate and MeHg production may be limited by sulfate availability. Experimental introductions of sulfates into a string fen in the HPE resulted in elevated pore water [MeHg] downstream of the introduction site (McCarter et al. 2017).

I predicted that muscle lipid and combined EPA+DHA concentrations of anadromous fishes would show a latitudinal increase from south to north following the temperature and salinity gradient along the Hudson and James Bay coasts, but that no such pattern would exist for non-anadromous species. Salinity tolerance has been identified as an important factor influencing fish migrations between freshwater and seawater (McCormick 1994; Jørgensen et al. 2010) and probably influences the patterns of migration of fish species from my study rivers. Though I did observe positive latitudinal trends in lipids for Brook Trout and Lake Whitefish, as predicted, none of these trends were significant.

Interestingly, I did observe a significant declining trend northward for both muscle total lipid and EPA+DHA concentrations in one of the presumed non-anadromous species,



Northern Pike. Tidal action and fish migration may subsidize productivity in the lower reaches of all the study rivers, and marine food sources tend to be higher in  $\omega$ -3 LC-PUFA such as EPA and DHA (Sargent and Henderson 1995; Li et al. 2011; Gladyshev et al. 2012). Thus, anadromous fishes tend to incorporate greater total quantities of  $\omega$ -3 LC-PUFA into their flesh than their freshwater resident conspecifics (Gladyshev et al. 2012). In North America, Northern Pike are considered a freshwater species and, to my knowledge, there are no documented cases of marine habitat use. However, anecdotal reports from local fishers indicate that Northern Pike may use estuarine zones near the mouths of several of my study rivers, and recent otolith microchemistry analysis of Northern Pike from these rivers appears to confirm this (DeJong 2017). In contrast, Northern Pike populations in the Baltic Sea region of northern Europe routinely use marine habitats (Muller 1986; Engstedt et al. 2010). The salinity of the Baltic Sea is relatively low (6-7 ‰), and salinities above 11 -13 ‰ are probably lethal to juvenile Northern Pike (Jacobsen et al. 2007; Jørgensen et al. 2010), though adults may be able to tolerate up to 18 ‰ (Jørgensen et al. 2010). Lower salinities in southern James Bay could allow Northern Pike from southern rivers to exploit the estuarine regions more extensively than those from northern rivers entering the more saline Hudson Bay. Alternatively, small-bodied fish of freshwater origin could be exploiting these brackish water regions and transporting marine nutrients back into the freshwater environments. Predation on prey items which have fed in marine or brackish water could impart an elevated total lipid content with increased marine-derived EPA and DHA.

### ***2.4.3 Cost-Benefit analysis and implications for food quality***

Based on my stable isotope and fatty acid analyses, Lake Whitefish and Brook Trout populations in both large and small rivers across the Hudson Plains showed evidence of anadromous behaviour whereas Northern Pike, Walleye, and White and Longnose Suckers exhibited a more freshwater resident life history (Chapter 1). Previous studies have demonstrated that anadromous fishes possess lower [THg] as well as higher total lipid contents than landlocked, freshwater fishes (Swanson et al. 2011a; Gladyshev et al. 2012). This makes them an important source of high quality food for subsistence fisheries. In coastal rivers of the Hudson Plains, I found that presumed anadromous species tended to have lower muscle Hg content and greater total EPA+DHA than freshwater resident species, consistent with my predictions. However, the comparison of anadromous and freshwater resident fishes in this study is confounded by inherent differences among them. Total lipid shows a strong positive correlation with EPA+DHA concentration indicating total EPA+DHA content is dependent on overall fish fattiness. This is likely the result of physiological differences in fat storage locations between “fatty-fleshed” and “lean-fleshed” species (see Chapter 1, Section 1.4.3). Other studies have implicated differences in total fat content as the driving force resulting in the difference in EPA+DHA content between anadromous and freshwater fish of the same species (Ahlgren et al. 1994; Gladyshev et al. 2012). Regardless of lipid origin, the fatty Lake Whitefish, Brook Trout and Cisco had the most beneficial fatty acid profiles of fish from the coastal rivers. In addition, these species all had relatively low muscle Hg concentrations.

As expected, all species and populations of fish that I sampled from the coastal rivers had positive relationships between muscle [THg] and body size, and thus, recommended

consumption limits decreased with increasing size category. In general the greatest number of meals per month are achievable in small to moderate sized (< 450 mm TLEN) Lake Whitefish, Cisco and Brook Trout. However, Brook Trout populations appear to be much less widespread than Cisco and Lake Whitefish populations across the coast, and are often limited to smaller, less accessible river systems (Browne 2007). The combination of high EPA+DHA, low [THg] and the widespread accessibility of Lake Whitefish and Cisco make them an excellent source of food, and when available, Brook Trout are also an excellent source of food. All three of these species can provide a potential consumer from both the general and sensitive populations with the recommended dose of EPA+DHA. Higher doses of up to 1000 mg day<sup>-1</sup> are also achievable for the general population with these species (Kris-Etherton et al. 2009).

Northern Pike can also be a quality source of food, especially at smaller sizes (< 600 mm TLEN). Moderate quantities of EPA+DHA and only moderately restrictive recommended meals per month allow for the consumption of the recommended dose of 500 mg day<sup>-1</sup> of EPA+DHA for the general population. Lower tolerable intakes for the sensitive population make large Northern Pike less likely to provide the recommended dose of EPA+DHA. Northern Pike and Walleye are two of the most widespread fish species in the HPE and are readily available to most local fishers (Browne 2007). At smaller sizes (< 400 mm TLEN) Walleye have relatively low [THg] and can provide a potential consumer from the general population with the recommended dose of EPA and DHA. This, combined with their widespread availability, makes smaller Walleye a good source of food for the general population. However, a lower number of meals per month in larger Walleye and a relatively low essential fat content make them less desirable at larger sizes. As well, lower

recommended intake for members of the sensitive population greatly restricts the EPA+DHA dose that can be obtained by this group.

White and Longnose Suckers are less commonly considered “food fish” however both had muscle [THg] similar to Northern Pike and Cisco of the same size and therefore have the potential to provide the general population with a high number of meals per month at small to moderate sizes (< 450 mm TLEN). These low restrictions on the number of meals per month allow members of both the general and sensitive populations to achieve the recommended dose of EPA+DHA. Both White and Longnose Suckers are widespread across the HPE, usually with one or the other present in higher abundance (Browne 2007).

#### ***2.4.4 Summary and conclusions***

This research represents the first extensive study of food quality in riverine fishes of the Hudson Plains of northern Ontario. Despite declining atmospheric deposition, fish Hg concentrations in these coastal rivers have not changed significantly over the past ~ 30 years, suggesting that inorganic Hg availability may not be limiting. Fish [THg] in the coastal rivers did not show any significant latitudinal trend across the region for any species, but was significantly higher than in more southerly headwater lakes for Lake Whitefish, Walleye and White Sucker. Fillet lipid and essential fatty acid content decreased with increasing latitude in Northern Pike, but not in other species. Both Hg and essential fatty acid concentrations of fish muscle varied among rivers but there did not appear to be spatial concordance between them. The three presumed anadromous species - Brook Trout, Lake Whitefish, Cisco - tended to have both the greatest concentrations of essential fatty acids and among the lowest

[THg], making them an especially high quality food source. Northern Pike and White and Longnose Suckers can also meet or exceed RDI of EPA+DHA within mercury-imposed consumption limits, with the exception of very large Northern Pike. Walleye generally have the highest muscle [THg] and relatively low essential fatty acid content. Small Walleye (< 400 mm TLEN) can provide the general population with the recommended dose of EPA+DHA however greater restrictions on the number of recommended meals per month for the sensitive population do not provide the consumer the recommended doses of EPA+DHA.

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## Appendices

Appendix 1. River and stream characteristics at mouth, including latitude (Lat) and longitude (Long) in decimal degrees (DD), growing degree days (GDD, cumulative, above 5 °C), mean annual discharge (m<sup>3</sup> / sec), and drainage basin area (km<sup>2</sup>) as measured by the Ontario Flow Assessment Tool web application (OFAT 3). Species sampled: LWF- Lake Whitefish, NP- Northern Pike, WALL- Walleye, CIS- Cisco, WS- White Sucker, LNS- Longnose Sucker, BT- Brook Trout.

River / Stream	Lat (DD N)	Long (DD W)	GDD	Q (m <sup>3</sup> /sec)	Area (km <sup>2</sup> )	Fish Species Sampled
<b><i>James Bay Rivers</i></b>						
<b>Harricanaw</b>	51.17	-79.75	1203	358	35622	LWF, NP, WALL, CIS, LNS, WS
<b>Moose</b>	51.33	-80.40	1148	1176	105857	LWF, NP, WALL, CIS, LNS, WS, BT
<b>Albany</b>	52.28	-81.52	1149	1076	105292	LWF, NP, WALL, CIS, LNS, WS
<b>Attawapiskat</b>	52.95	-82.30	1086	491	49630	LWF, NP, WALL, CIS, LNS, WS
<b><i>Hudson Bay Rivers</i></b>						
<b>Sutton</b>	55.25	-83.75	902	39.6	4715	BT
<b>Winisk</b>	55.28	-85.08	853	495	64889	LWF, NP, WALL, CIS, LNS, WS

<b>Shagamu</b>	55.87	-86.78	931	15.6	1914	LWF, CIS, BT
<b>Shell</b>	55.92	-86.97	936	7.9	974	BT
<b>Goose</b>	55.95	-87.37	937	16.4	2042	NP, BT
<b>Severn</b>	56.03	-87.60	931	804	99896	LWF, NP, WALL, CIS, LNS, WS
<b>Pipowitan</b>	56.13	-87.63	926	10.3	1284	LWF, BT
<b>Majikun</b>	56.50	-88.15	889	10.1	456	BT
<b>Tamuna</b>	56.52	-88.25	883	9.8	603	BT
<b>Mintiagan</b>	56.68	-88.63	862	10.1	675	BT

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Appendix 2. Fatty acid methyl esters identified using Flame Ionization Gas Chromatography. Fatty acid classes; saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA).

Structure	Scientific Name	Common Name	Class
14:0	Tetradecanoic acid	Myristic acid	SFA
14:1	cis-9-tetradecanoic acid	Myristoleic acid	MUFA
i15:0	iso-Pentadecanoic acid	-	SFA
a15:0	anteiso-Pentadecanoic acid	-	SFA
15:0	Pentadecanoic acid	Pentadecanoic acid	SFA
15:1	cis-10-pentadecenoic acid	-	MUFA
16:0	Hexadecanoic acid	Palmitic acid	SFA
16:1n-9	9-cis-hexadecenoic acid	-	MUFA
16:1n-7c	9-hexadecenoic acid	Palmitoleic acid	MUFA
17:0	Heptadecanoic acid	Margaric acid	SFA
17:1	cis-10-heptadecanoic acid	-	MUFA
18:0	Octadecanoic acid	Stearic acid	SFA
18:1n-9c	cis-9-octadecenoic acid	Oleic acid	MUFA
18:1n-7c	11-octadecenoic acid	Vaccenic acid	MUFA
19:0	Nonadecanoic acid	Nonadecylic acid	SFA
18:2n-6c	cis-9,12-octadecadienoic acid	Linoleic acid (LNA)	PUFA
20:0	Eicosanoic acid	Arachidic acid	SFA
18:3n-6	9,12,15-octadecatrienoic acid	$\gamma$ -Linoleic acid (GLA)	PUFA
20:1n-9	cis-11-eicosenoic acid	Gondoic acid	MUFA
18:3n-3	9,12,15-octadecatrienoic acid	$\alpha$ -Linoleic acid (ALA)	PUFA
del-19:0	cis-9,10-methylene-octadecanoate	-	SFA
20:1n-11	cis-11-docosenoic acid	Gadoleic acid	MUFA
20:2n-6	cis-11,14-Eicosadienoic acid	Eicosadienoic acid	PUFA
22:0	Docosanoic acid	Behenic acid	SFA
20:3n-6	cis-8,11,14-eicosatrienoic acid	Dihomo- $\gamma$ -linolenic acid	PUFA
22:1n-11	cis-11-docosenoic acid	Cetoleic acid	MUFA
22:1n-9	13-docosenoic acid	Erucic acid	MUFA
20:3n-3	11,14,17-eicosatrienoic acid	Eicosatrienoic acid	PUFA
20:4n-6	5,8,11,14-eicosatetraenoic acid	Arachidonic acid (ARA)	PUFA
22:2n-6	cis-13,16-docosadienoic acid	Docosadienoic acid	PUFA
24:0	Tetracosanoic acid	Lignoceric acid	SFA
20:5n-3	5,8,11,14,17-eicosapentaenoic acid	Eicosapentaenoic acid (EPA)	PUFA
24:1n-9	15-tetracosanoic acid	Nervonic acid	MUFA
22:4n-6	7,10,13,16-docosatetraenoic acid	Docosatetraenoic acid	PUFA
22:5n-6	4,7,10,13,16-docosapentaenoic acid	Docosapentaenoic acid (DPA)	PUFA
22:5n-3	7,10,13,16,19-docosapentaenoic acid	Docosapentaenoic acid (DPA)	PUFA
22:6n-3	4,7,10,13,16,19-docosahexaenoic acid	Docosahexaenoic acid (DHA)	PUFA

Appendix 3. Mean predicted weight (g) at eight years old, estimated from fitted von Bertalanffy growth models for populations of Northern Pike, Walleye, Lake Whitefish, and White Sucker from each of two locations (Coastal = lower reach of mainstem river, Inland = lakes in upper drainage basin) within six Far North drainage basins.

<b>Drainage Basin</b>	<b>Species</b>	<b>Coastal</b>	<b>Inland</b>
<b>Harricanaw</b>	Northern Pike	3141	2272
<b>Harricanaw</b>	Walleye	1027	495
<b>Moose</b>	Lake Whitefish	735	902
<b>Moose</b>	Northern Pike	1782	2160
<b>Moose</b>	White Sucker	1029	1332
<b>Moose</b>	Walleye	597	689
<b>Albany</b>	Lake Whitefish	653	1221
<b>Albany</b>	Northern Pike	2313	2303
<b>Albany</b>	White Sucker	1137	1191
<b>Albany</b>	Walleye	703	490
<b>Attawapiskat</b>	Lake Whitefish	782	1005
<b>Attawapiskat</b>	Northern Pike	2799	1727
<b>Attawapiskat</b>	White Sucker	1044	896
<b>Attawapiskat</b>	Walleye	700	428
<b>Winisk</b>	Lake Whitefish	682	1493
<b>Winisk</b>	Northern Pike	2883	1633
<b>Winisk</b>	Walleye	932	782
<b>Severn</b>	Lake Whitefish	523	1060
<b>Severn</b>	Northern Pike	3129	1827
<b>Severn</b>	White Sucker	421	990

Appendix 4. Benchmark recommended maximum number of meals per month of fish with varying total mercury concentrations ([THg]) for the general human population (all > 15 years old, except women of child-bearing age) and sensitive human population (all < 15 years old, plus women of child-bearing age). Values from MOECC, based on Health Canada total daily intake recommendations, and body mass of 33 kg for < 15 years old, and 70 kg for > 15 years old. Calculations assume all mercury is in the form of methylmercury.

<b>General Population</b>		<b>Sensitive Population</b>	
[THg] (ppm wet)	Meals per Month	[THg] (ppm wet)	Meals per Month
<b>&lt;0.15</b>	32	<b>&lt;0.06</b>	32
<b>0.149-0.29</b>	16	<b>0.06-0.119</b>	16
<b>0.3-0.39</b>	12	<b>0.12-0.159</b>	12
<b>0.4-0.59</b>	8	<b>0.16-0.249</b>	8
<b>0.6-1.19</b>	4	<b>0.25-0.49</b>	4
<b>1.2-1.79</b>	2	<b>&gt;0.5</b>	0
<b>&gt;1.8</b>	0		

Appendix 5. Fish consumption recommendations for coastal rivers of the Far North of Ontario. Maximum number of meals (227 g) per month, based on MOECC benchmarks, and the percentage of the recommended dose of EPA+DHA (500 mg day<sup>-1</sup>) achievable when consuming the maximum number of meals for both general and sensitive populations.

River	Total Length (mm)										
	200	250	300	350	400	450	500	550	600	650	700
<b>Albany River</b>											
<b>Northern Pike</b>											
General Population	.	.	.	.	.	.	.	.	16	16	12
Monthly dose EPA+DHA(%)	.	.	.	.	.	.	.	.	346	346	259
Sensitive Population	.	.	.	.	.	.	.	.	8	4	4
Monthly dose EPA+DHA(%)	.	.	.	.	.	.	.	.	172	86	86
<b>Lake Whitefish</b>											
General Population	.	.	32	32	32	16	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	1317	1317	1317	658	.	.	.	.	.
Sensitive Population	.	.	16	12	12	8	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	658	494	494	329	.	.	.	.	.
<b>Attawapiskat</b>											
<b>Lake Whitefish</b>											
General Population	.	.	32	32	16	16	16	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	805	805	403	403	403	.	.	.	.
Sensitive Population	.	.	12	12	8	8	8	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	301	805	201	201	201	.	.	.	.
<b>Northern Pike</b>											
General Population	.	.	32	16	16	16	16	12	12	8	8
Monthly dose EPA+DHA(%)	.	.	370	185	185	185	185	139	139	93	93
Sensitive Population	.	.	12	8	8	8	4	4	4	4	4
Monthly dose EPA+DHA(%)	.	.	139	93	93	93	46	46	46	46	46
<b>Harricanaw</b>											
<b>Lake Whitefish</b>											
General Population	.	32	32	32	.	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	808	808	808	.	.	.	.	.	.	.
Sensitive Population	.	16	16	16	.	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	404	404	404	.	.	.	.	.	.	.

<b>Northern Pike</b>											
General Population	.	.	.	32	32	16	16	16	16	12	12
Monthly dose EPA+DHA(%)	.	.	.	642	642	321	321	321	321	240	240
Sensitive Population	.	.	.	16	12	8	8	8	4	4	4
Monthly dose EPA+DHA(%)	.	.	.	321	240	160	160	160	80	80	80
<b>Pipowitan</b>	<b>200</b>	<b>250</b>	<b>300</b>	<b>350</b>	<b>400</b>	<b>450</b>	<b>500</b>	<b>550</b>	<b>600</b>	<b>650</b>	<b>700</b>
<b>Brook Trout</b>											
General Population	.	.	32	32	32	32	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	1452	1452	1452	1452	.	.	.	.	.
Sensitive Population	.	.	16	16	16	12	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	726	726	726	545	.	.	.	.	.
<b>Lake Whitefish</b>											
General Population	.	.	.	32	32	32	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	838	838	838	.	.	.	.	.
Sensitive Population	.	.	.	32	16	16	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	838	419	419	.	.	.	.	.
<b>Severn</b>	<b>200</b>	<b>250</b>	<b>300</b>	<b>350</b>	<b>400</b>	<b>450</b>	<b>500</b>	<b>550</b>	<b>600</b>	<b>650</b>	<b>700</b>
<b>Northern Pike</b>											
General Population	.	.	.	.	32	32	32	32	32	16	16
Monthly dose EPA+DHA(%)	.	.	.	.	354	354	354	354	354	177	177
Sensitive Population	.	.	.	.	16	16	12	12	8	8	8
Monthly dose EPA+DHA(%)	.	.	.	.	177	177	133	133	89	89	89
<b>Lake Whitefish</b>											
General Population	.	.	.	32	32	32	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	1957	1957	1957	.	.	.	.	.
Sensitive Population	.	.	.	16	16	12	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	978	978	734	.	.	.	.	.
<b>Shagamu</b>	<b>200</b>	<b>250</b>	<b>300</b>	<b>350</b>	<b>400</b>	<b>450</b>	<b>500</b>	<b>550</b>	<b>600</b>	<b>650</b>	<b>700</b>
<b>Lake Whitefish</b>											
General Population	.	.	.	.	32	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	.	1075	.	.	.	.	.	.
Sensitive Population	.	.	.	.	16	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	.	537	.	.	.	.	.	.
<b>Brook Trout</b>											



General Population	.	.	.	32	32	32	32	32	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	1864	1864	1864	1864	1864	.	.	.
Sensitive Population	.	.	.	16	16	16	16	16	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	932	932	932	932	932	.	.	.
<b>Shell</b>	<b>200</b>	<b>250</b>	<b>300</b>	<b>350</b>	<b>400</b>	<b>450</b>	<b>500</b>	<b>550</b>	<b>600</b>	<b>650</b>	<b>700</b>
<b>Brook Trout</b>											
General Population	.	.	.	32	32	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	873	873	.	.	.	.	.	.
Sensitive Population	.	.	.	16	12	.	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	437	327	.	.	.	.	.	.
<b>Winisk</b>	<b>200</b>	<b>250</b>	<b>300</b>	<b>350</b>	<b>400</b>	<b>450</b>	<b>500</b>	<b>550</b>	<b>600</b>	<b>650</b>	<b>700</b>
<b>Lake Whitefish</b>											
General Population	.	.	.	32	32	16	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	850	850	425	.	.	.	.	.
Sensitive Population	.	.	.	16	12	8	.	.	.	.	.
Monthly dose EPA+DHA(%)	.	.	.	425	319	213	.	.	.	.	.
<b>Northern Pike</b>											
General Population	.	.	.	.	32	32	32	16	16	16	12
Monthly dose EPA+DHA(%)	.	.	.	.	326	326	326	163	163	163	122
Sensitive Population	.	.	.	.	16	16	12	8	8	4	4
Monthly dose EPA+DHA(%)	.	.	.	.	163	163	122	82	82	41	41