Variation in fish fatty acid concentrations among lakes in the Dehcho region of the Northwest Territories

by

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Background: In the subarctic Dehcho region of the Northwest Territories, many remote communities rely on traditional foods, including fish, to supplement more expensive store-bought options. Fish are an excellent source of omega-3 and omega-6 polyunsaturated fatty acids (n-3 and n-6 PUFAs, respectively), essential compounds that can only be obtained through the diet. Long-chain n-3 PUFAs, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are especially important for human health. As the health benefits derived from consuming fish can be diminished by the risk imposed by exposure to contaminants, such as mercury, researchers and communities in the Dehcho region began a collaborative project in 2012 to quantify both fatty acid and mercury concentrations in fish. In the course of this work, it was found that concentrations of fatty acids in fish differed significantly among lakes in the Dehcho region. In freshwater ecosystems, fatty acids are produced by algae and bacteria and transferred up the food chain through consumption. The type and quality of fatty acids produced varies among primary producer taxa, meaning that fatty acid profiles in fish may vary among lakes due to variation in the composition of algal and bacterial communities, which in turn vary in response to abiotic conditions in lakes.
Objectives: As some fish samples were stored for multiple years before processing, the first objective of this study was to determine if there was a relationship between concentrations of fatty acids and storage time at -20°C. After determining which fatty acids were affected by storage time and how they were affected by storage time, the second objective was to update existing fish fatty acid profiles (analysed from samples collected 2013-2015) for the study lakes. The third objective was to determine whether there were differences in concentrations among lakes for several fatty acid groups of interest, including total fatty acids (TFA), n-3 and n-6 PUFAs, DHA, and EPA, and whether observed differences in fish fatty acid profiles could be explained by water chemistry and/or watershed characteristics among lakes.
Methods: A total of 433 fish, including Burbot (*Lota lota*), Cisco (*Coregonus artedi*), Lake Trout (*Salvelinus namaycush*), Longnose Sucker (*Catastomus catastomus*), Lake Whitefish (*Coregonus clupeaformis*), Northern Pike (*Esox lucius*), Walleye (*Sander vitreus*), and White

Sucker (*Catastomus commersoni*) were captured in 10 important subsistence lakes within the Dehcho region between the years of 2013 and 2018. Sampled lakes were located in three different eco-zones, the Hay River Lowlands, the Horn Plateau, and the Northern Alberta Uplands. Fish muscle tissue was frozen on-site and transported back to the University of Waterloo for laboratory analysis of both fatty acid and mercury concentrations. Water samples were collected at each lake to characterise lake chemistry (e.g. major nutrients, ions, dissolved organic carbon, etc.), and these data were compared to an existing dataset on watershed characteristics (e.g. lake area, watershed area, etc.).

Results: In every fish species, DHA concentrations decreased exponentially with increasing storage time, while C:24:0, a saturated fatty acid, increased significantly with increasing storage time. Updated fish fatty acid profiles and mercury concentrations confirmed results found by Reyes et al (2017) and Laird et al (2018); Cisco, Lake Whitefish, Longnose Sucker, and White Sucker are the fish species with the highest fatty acid concentrations and lowest mercury concentrations. Concentrations of all fatty acid groups examined in Northern Pike were statistically different among lakes (TFA, n-3 and n-6 PUFAs, EPA, and DHA), while only some fatty acid groups in Lake Whitefish (TFA, n-6 PUFAs, and DHA) and Walleye (n-3 and n-6 PUFAs) varied significantly among lakes. Significant predictors of concentrations of fish fatty acids included both water chemistry and watershed characteristics, and fell into 3 distinct groups of variables: lake productivity (total phosphorus), indicators of carbon quality (UV_{254}, specific UV absorbance, dissolved organic carbon, and total nitrogen), and catchment influence (chloride concentrations, calcium concentrations, and the ratio of lake perimeter to watershed area). Understanding factors that lead to variation in concentrations of fish fatty acids, both among lakes and because of storage practices, can inform predictions of the nutritional value of fish in other lakes, provide a baseline for assessing ongoing effects of climate-induced change, and allow community members to make informed choices about the fish that they are eating.

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Table of Contents

List of Figures

List of Tables

List of Abbreviations and Symbols

ALA	Alpha-linoleic Acid
APHA	American Public Health Association
BURB	Burbot
CISC	Cisco
$\delta^{13}C$	Delta 13 C; carbon isotopic ratio
$\delta^{15}N$	Delta 15 N; nitrogen isotopic ratio
DHA	Docosahexaenoic Acid
DOC	Dissolved Organic Carbon
EPA	Eicosapentaenoic Acid
GIS	Geographic Information Systems
GNWT	Government of the Northwest Territories
Hg	Mercury
HRL	Hay River Lowlands
HP	Horn Plateau
HUFA	Highly Unsaturated Fatty Acid
LKTR	Lake Trout
LKWH	Lake Whitefish
LNSC	Longnose Sucker
MeHg	Methylmercury
MUFA	Monounsaturated Fatty Acid
NAU	Northern Alberta Uplands
n-3 PUFA	Omega-3 Polyunsaturated Fatty Acid

n-6 PUFA	Omega-6 Polyunsaturated Fatty Acid
NRPK	Northern Pike
PCA	Principal Components Analysis
PC	Principal Component Scores
PUFA	Polyunsaturated Fatty Acid
SDA	Stearidonic Acid
Se	Selenium
SFA	Saturated Fatty Acid
SUVA	Specific UV Absorbance at 254 nm, normalized for DOC concentrations
TFA	Total Fatty Acids
TN	Total Nitrogen
TP	Total Phosphorus
US EPA	United States Environmental Protection Agency
WALL	Walleye
WHSC	White Sucker

Chapter 1. Introduction

1.1 Fish and Human Health

1.1.1 Health Benefits from Fish Consumption

Fish are an important source of food and nutrition for many cultures around the world (Tacon and Metian 2013). In addition to providing essential vitamins and nutrients, consuming fish confers several health benefits, including a reduced risk of heart disease (cardiac arrest and long-term diseases such as congestive heart failure), neurological conditions (such as cognitive decline, anxiety, and depression), and inflammatory diseases (such as inflammatory bowel disease and some forms of arthritis; Arts et al. 2001; Sidhu 2003; Tacon and Metian 2013). Many of the health benefits associated with fish consumption are related to intake of essential polyunsaturated fatty acids (PUFAs), a class of fatty acids that contain at least two double bonds in their carbon chains (Brett and Müller-Navarra 1997; Arts et al. 2001; Taipale et al. 2013). Essential PUFAs are necessary for physiological function, but cannot be produced by consumers *de novo*; consumers must acquire these fatty acids from their diet (Brett and Müller-Navarra 1997). A list of essential PUFAs is presented in Table 1 (Taipale et al. 2013).

Table 1. Polyunsaturated fatty acids considered essential for both animals and humans. Modified from Taipale et al (2013).

	Notation	Common Name	Abbreviation
n-3 PUFAs	18:3ω3	Alpha-linoleic acid	ALA
	18:4ω3	Stearidonic acid	SDA
	20:5ω3	Eicosapentaenoic acid	EPA*
	22:5ω3	Docosapentaenoic acid	DPA*
	22:6ω3	Docosahexaenoic acid	DHA*
n-6 PUFAs	18:2ω6	Linoleic acid	LIN
	18:2ω6	Gamma-linoleic acid	GLA
	20:4ω6	Arachidonic acid	ARA*

*Designates highly unsaturated fatty acids (HUFAs)

Omega-3 polyunsaturated fatty acids (i.e., n-3 PUFAs) are distinguished from omega-6 polyunsaturated fatty acids (n-6 PUFAs) by the location of the first double bond (the 3rd carbon from the methyl end for n-3 PUFAs and the 6th carbon for n-6 PUFAs; Figure 1). Polyunsaturated fatty acids are the precursors for eicosanoids, compounds that mediate inflammation on a cellular level in the human body (Saini and Keum 2018). While n-6 PUFAs produce pro-inflammatory eicosanoids, n-3 PUFAs produce anti-inflammatory eicosanoids (Calder 2006). Although both inflammatory and anti-inflammatory processes are important for health, the typical western diet is thought to be overly enriched in n-6 PUFAs, with a ratio of about 15:1 n-6 to n-3 PUFAs (Simopoulos 2002). Reducing that ratio to 4:1 or 1:1 n-6 to n-3 fatty acids is thought to be ideal for human physiological function, and is associated with decreased risks of cardiovascular disease, obesity, and diabetes, among others (Simopoulos 2002, 2016).

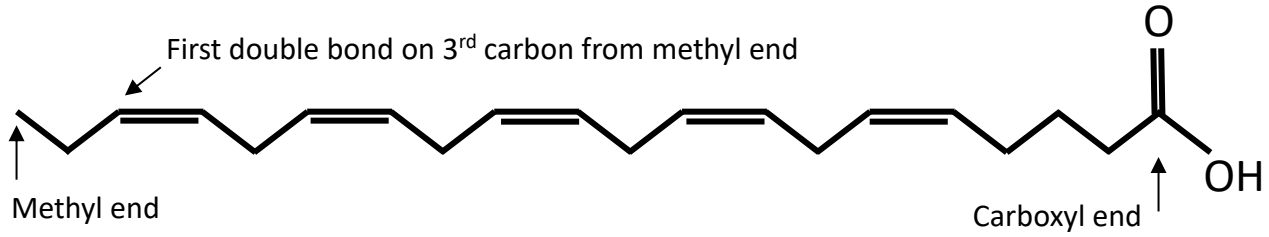

Figure 1. Example of n-3 PUFA structure. Eicosapentaenoic acid, 20:5n3.

One n-3 PUFA, docosahexaenoic acid (DHA), is found in high abundances within the phospholipid membranes in the human brain and retinal tissue in the eye, along with the n-6 PUFA arachidonic acid (ARA; Farooqui 2009). Another n-3 PUFA, eicosapentaenoic acid (EPA), is important for fetal development, growth, and reducing inflammation (Spector 1999; Harris et al. 2009; Swanson et al. 2012). Fatty acids such as EPA and DHA are classified as highly unsaturated fatty acids (HUFAs), which are fatty acids that contain 20 or more carbon atoms and 3 or more double bonds (Calder and Yaqoob 2009). Humans can convert a small amount of the fatty acid Alpha-linoleic acid (ALA) into Stearidonic acid (SDA), then into EPA and finally DHA. However, this process is inefficient, and only a small amount of ALA can be converted into HUFAs in humans (Harris et al. 2009). Hence, the best source of HUFAs for humans, including EPA and DHA, is direct consumption from the diet.

1.1.2 Fish as a Source of Fatty Acids

Fish are an excellent source of fatty acids for humans, and in general fish have higher levels of n-3 PUFAs when compared to other dietary sources, such as plants or nuts (Calder and Yaqoob 2009). However, concentrations of fatty acids vary both within and among fish species (Cardoso et al. 2010). As fattier fish species typically contain more PUFAs than leaner fish species, a consumer's ability to meet the daily recommended doses of EPA and DHA from fish consumption will vary with the type of fish consumed (Tsuchiya et al. 2008; Cardoso et al. 2010).

There can also be intraspecific variation in fatty acid levels in fish among locations (see section 1.2.3), suggesting that the potential nutritional benefits of fish consumption for humans can vary among ecosystems (Ahlgren et al. 1996; Laird et al. 2018). Accurate and precise quantification of the benefits of eating freshwater fish species thus requires a more thorough understanding of variability in fatty acid levels in fish among different water bodies and ecosystems. This is particularly true for northern freshwater ecosystems, where the benefits of eating fish need to be carefully weighed against potential risks from exposure to mercury (Hg) and other contaminants (e.g., AMAP 2011; Reyes et al. 2017; Stow et al. 2017).

1.2 Fatty Acids in Freshwater Ecosystems

1.2.1 Primary Producers

To understand why there might be intraspecific variation in fatty acid concentrations in fish, it is necessary to examine how fatty acids originate in freshwater ecosystems. Fatty acids are produced by phytoplankton and bacteria, primarily as part of organelle lipid bilayers or as energy storage (Thompson 1996; Brett and Müller-Navarra 1997; Guschina and Harwood 2006). Studies on fatty acids in freshwater and other aquatic ecosystems generally focus on the transfer of energy between trophic levels, and the quality of the fatty acids for consumers (Müller-Navarra et al. 2000; Brett et al. 2009). Primary producers with high concentrations of HUFAs are considered to be high food-quality (Brett and Müller-Navarra 1997). HUFA composition in algae and bacteria is largely taxon-specific; whereas dinoflagellates and diatoms produce the highest percentage of long-chain fatty acids, cyanobacteria and bacteria produce little to no long-chain

fatty acids (Galloway and Winder 2015). Lakes with high HUFA-producing algal populations can support larger zooplankton populations compared to those with low HUFA algal populations (Brett and Müller-Navarra 1997).

1.2.2 Zooplankton and Aquatic Animals

For healthy growth, zooplankton need to consume both n-3 and n-6 PUFAs (Persson and Vrede 2006). Like phytoplankton, the nutritional quality of zooplankton for fish or macroinvertebrate consumers is often taxon-specific (Persson and Vrede 2006). For example, calanoid copepods in Swedish lakes contain more DHA than EPA, while cladocerans in the same lakes contain more EPA than DHA (Persson and Vrede 2006). Some copepods may bioconvert EPA into DHA, meaning that composition of the zooplankton community may affect availability of specific fatty acids to higher trophic level consumers, such as fish (Persson and Vrede 2006). Results from recent genetic research suggests that a variety of animals (including some species within phyla cnidaria, rotifera, mollusca, annelida, and arthropoda) can produce PUFAs *de novo* (Kabeya et al. 2018). Thus far, a widespread study has only been carried out on marine animals (Kabeya et al. 2018), but it is possible that related invertebrate taxa in freshwater may also be able to synthesise PUFAs. In summary, the fatty acid composition produced by primary producers has the potential to be heavily modified in the food chain, which impacts availability of fatty acids at higher trophic levels.

1.2.3 Fish

Fatty acids are essential for fish health, and affect growth, metabolism, and reproduction (Sawyer et al. 2016). Fatty acid levels in fish are affected by feeding strategy (e.g., piscivorous, planktivorous), although reported effects vary among studies (Persson and Vrede 2006; Vasconi et al. 2015). As fatty acids are stored in lipids, fattier fish species tend to have higher concentrations of fatty acids than leaner fish species, although results are not entirely consistent (e.g. Tsuchiya et al. 2008; Cardoso et al. 2010). Authors of a study in Austria found that concentrations of total fatty acid levels in fish decreased as trophic level increased, indicating that fatty acids are not biomagnified to higher predators (Kainz et al. 2017). While much research remains to be conducted, the transfer of fatty acids to fish from lower trophic levels is selective and varies among fatty acids. In a lake where ALA (C_{18}) was the most abundant fatty

acid in seston, for example, the proportion of ALA decreased significantly with each trophic level, whereas the proportion of essential fatty acids, such as EPA and DHA, increased with each trophic level, from seston up to a zooplanktivorous fish species (Strandberg et al. 2015).

Fatty acid concentrations in fish can be influenced by local environmental conditions (Ahlgren et al. 1996). Among-lake variability in these conditions may thus help explain intraspecific variation in fish fatty acids. Although the factors that influence fatty acids in ecosystems are complex, taxonomic composition of planktonic and bacterial communities have been found to predict the quality of fatty acids available for consumers (Galloway and Winder 2015). When determining community composition and tracing fatty acids in primary producers is not feasible, some authors have directly related environmental variables to concentrations of fatty acids in fish, making the assumption that abiotic factors indirectly affect fatty acids by changing phytoplankton or zooplankton composition, and thereby fatty acids that are available for higher trophic levels (Gladyshev et al. 2011; Razavi et al. 2014; Taipale et al. 2016a).

In the Great Lakes and smaller surrounding lakes, factors that influence intraspecific variation of combined concentrations of EPA and DHA in fish muscle tissue have been shown to vary among species (Williams et al. 2017). For example, variation in EPA + DHA concentrations was best explained by 'lake' in Lake Trout (*Salvelinus namaycush*; explaining 48% of variation), Northern Pike (*Esox lucius*;78%), and Walleye (*Sander vitreus*; 61%; Williams et al. 2017). EPA + DHA concentrations in all three species also increased with increasing fish length, but the factors that explained additional variation differed among the species (Williams et al. 2017). While variation in fatty acid levels in Northern Pike were best explained by lake and fish size alone, decreasing EPA + DHA concentration with increasing lake latitude and maximum water depth helped to explain variation in Lake Trout, while EPA + DHA concentration in Walleye decreased with increasing lake eutrophication, as measured by the lake trophic state index (Williams et al. 2017).

Strandberg et al. (2016) examined factors driving variation in concentrations of combined EPA and DHA, mercury, and selenium (Se; a micronutrient), in European Perch (*Perca fluviatilis*) in eastern Finland (Strandberg et al. 2016). Lakes included in the study were classified as either

deep and clear, or shallow and humic (containing a large degree of terrestrial dissolved organic matter). A total of 57% of the variation in EPA, DHA, Hg, and Se concentrations was explained by the percentage of peatland in the lake watershed; higher peatland presence was associated with more humic lakes, where European Perch had higher fish mercury concentrations and lower concentrations of EPA + DHA than European Perch in more clear lakes (Strandberg et al. 2016). To a smaller extent, variation in both fatty acid and mercury concentrations was also influenced by lake area, the ratio of shoreline length to lake area, mean lake depth, the proportion of agricultural land in the catchment, and the ratio of organic carbon to nitrogen in lake water (Strandberg et al. 2016). The penetration of light into a water body including increasing amounts of humic, terrestrial carbon can also affect the composition of phytoplankton and bacterial communities, favouring cyanobacteria and decreasing the abundance of PUFA-rich diatoms (de Wit et al. 2016). As such, the nutritional quality of a given fish species (when considering PUFA concentrations) is likely influenced by the abiotic conditions of the local environment (Strandberg et al. 2016).

Not all variation in fish fatty acids can be explained by external factors. One source of intraspecific variation is individual fish genetics. Under experimental conditions, individual Atlantic Salmon (*Salmo salar*) fed an identical diet in a laboratory differed in their levels of EPA and DHA in muscle tissue; the differences were linked to variation in gene expression, with some fish being more capable of converting EPA to DHA than others (Horn et al. 2019). Moreover, n-3 PUFA content of fish muscle tissue is a heritable trait, meaning that fatty acid concentrations can be affected by individual fish genetics (Leaver et al. 2011). Finally, fatty acid profiles in fish can change temporally. Many freshwater fish species undergo ontogenetic shifts in diet, which are especially evident in piscivorous fish species; at younger, smaller life stages, fish tend to consume invertebrates, while older, larger fish consume other fish (Werner and Gilliam 1984; Hayden et al. 2015). As fatty acid intake reflects diet, fatty acid concentrations thus vary with age and size (Kainz et al. 2004). There is also evidence that the incorporation of fatty acids into fish tissue depends on metabolic and growth rates (Robin et al. 2003), which can also change with fish size (Jobling 1983). For fish species that do not have marked ontogenetic shifts in feeding, there can sometimes be a subtle shift in dietary quality of prey due to changing habitat use within a lake (Yang et al. 2018). In some freshwater fish species, there can also be

seasonal changes in fatty acid levels. This likely reflects seasonal shifts in prey availability, and is not observed in all species (Agren et al. 1987; Guler et al. 2008). Finally, some species exhibit a change in fatty acid concentrations at different points in their reproductive cycle, due to changes in dietary habits associated with mating (Kaçar et al. 2016). Thus, fatty acid concentrations in fish muscle tissue can vary both spatially and temporally.

1.3 Local Context

The Dehcho region is located in the southwest corner of the Northwest Territories, Canada. Numerous freshwater lakes are used for commercial and subsistence fishing by the Deh Gah Gotie, Ka'a'gee Tu, Jean Marie River, Pehdzeh Ki, and Liidlii Kue First Nations. Many of these First Nations communities are small and isolated, and therefore food is expensive and logistically challenging to import. To supplement food supplies, most communities rely on traditional foods, including fish, to provide a rich source of nutrients and minerals that can be lacking in affordable store-bought options (Gionet and Roshanafshar 2013).

Fish commonly captured and consumed in Dehcho lakes include Burbot (*Lota;* BURB), Cisco (*Coregonus artedi;* CISC), Lake Trout (LKTR), Longnose Sucker (*Catastomus;* LNSC), Lake Whitefish (*Coregonus clupeaformis:* LKWH), Northern Pike (NRPK), Walleye (WALL), and White Sucker (*Catastomus Nortcommersoni;* WHSC). Burbot, Lake Trout, Northern Pike and Walleye are in general piscivorous species, whereas Cisco, Lake Whitefish, Longnose Sucker, and White Sucker are planktivorous or benthivorous (Scott and Crossman 1973). Lake Whitefish, Lake Trout, Northern Pike, and Walleye in particular are widely consumed and important subsistence fish species in the Dehcho region (Ratelle et al. 2020).

Despite the clear health benefits, there are also risks associated with fish consumption, including exposure to mercury (Lehnherr 2014). Mercury is a contaminant that can enter ecosystems through natural (e.g., volcanoes or forest fires) and/or anthropogenic means (e.g., long-range transport of emissions from coal-burning plants; AMAP 2011). Once in the ecosystem, mercury biomagnifies in the food chain (in the form of methylmercury [MeHg]) to reach highest levels in top predators (AMAP 2011). Humans often target the largest fish for eating, making mercury

exposure a concern (Gionet and Roshanafshar 2013). At high enough concentrations, exposure to MeHg can cause a range of health issues in humans, from affecting normal growth rates in babies at lower levels of exposure to severe mental and physical disabilities at higher levels of exposure (Karagas et al. 2012).

Methylmercury exposure for subsistence fishers is especially concerning in Canada's north, where organisms tend to grow slowly, food chains can be long, and environmental conditions can result in mercury being retained in the environment, particularly in permafrost, for long periods of time (Van Oostdam et al. 2005; Lehnherr 2014; Indigenous and Northern Affairs Canada 2017). In the late 1990s, a series of lakes in the Dehcho region were tested for fish mercury concentrations (Braune et al. 1999; Lockhart et al. 2005). Although the lakes were located in a relatively small geographic area, results were highly variable among lakes; research into the causes of mercury variation is on-going (Heidi Swanson, unpublished data). Mean mercury concentrations in fish were over Health Canada's guideline for commercial sale (0.5 ppm wet weight) in some lakes, and under in other lakes (Braune et al. 1999; Lockhart et al. 2005). As a result, the Government of the Northwest Territories created site-specific fish consumption advisories for several lakes in the Dehcho region (GNWT 2019).

While many people restrict their consumption of fish to lakes without advisories, a recent survey in the Dehcho region found that a sizeable proportion of people catch and consume fish from lakes with advisories (Ratelle et al. 2018). The majority of participants reported frequent consumption of traditional foods, including a yearly average of 1.3 servings of fish per week (Ratelle et al. 2020). Hearing that traditional food may contain contaminants can make people worry, and in some cases cause a shift towards a store-bought diet, demonstrating the need for clear and balanced risk communication (Receveur et al. 1997; Pirkle et al. 2016). Fishing, hunting, and harvesting traditional foods can contribute to making remote communities more food secure (Fieldhouse and Thompson 2012), especially when market food prices are high, and affordable options often lack the vitamins and nutrients that are found in fish and other traditional foods (Kuhnlein et al. 2013). When people do not have access to safe, nutritious food in a large enough quantity to meet their dietary needs, they are considered to be 'food insecure' (Fieldhouse and Thompson 2012). Contrary to what might be expected, food insecurity is

associated with obesity, as well as with long-term health problems, such as heart disease and diabetes (Fieldhouse and Thompson 2012).

In 2012, at the request of community members, a research project was initiated to examine causes of variability in fish mercury concentrations among lakes. Community members and leaders also requested that researchers quantify concentrations of beneficial fatty acids and micronutrients in subsistence fish species. There is evidence to suggest that consuming high levels of fatty acids has a protective effect against the harmful impacts of mercury (Myers et al. 2007; Strain et al. 2015). Conversely, there is also evidence that mercury decreases the beneficial effect of fatty acids on heart disease (Hu et al. 2017). To determine the healthiest fish species for human consumption in the region, "de minimus ratios", or the concentration at which the harmful effects of mercury outweigh the beneficial effects of n-3 fatty acids, were calculated (Tsuchiya et al. 2008; Reyes et al. 2017; Laird et al. 2018). When ratios were calculated for DHA, EPA + DHA, or n-3 PUFAs, Cisco and Lake Whitefish were the only species that exceeded the de minimus ratios for each fatty acid category (Reyes et al. 2017; Laird et al. 2018).

Results from the previous studies indicated that there was among-lake variation in both levels of mercury and levels of fatty acids (Laird et al. 2018). The focus of this research project is to investigate among-lake variability in fatty acid levels in fish, and to determine whether differences are related to among-lake variability in water chemistry and watershed characteristics. Understanding underlying causes of variability in fatty acid levels in fish species in the Dehcho region of the Northwest Territories will be helpful for people who rely on subsistence fishing, and for regulators looking to develop balanced and well-informed consumption guidelines. Ultimately, the results can be used to help people choose the safest, healthiest subsistence fish sources (Reyes et al. 2017; Laird et al. 2018).

1.4 Objectives and Hypotheses

The overall goal of this project is to examine abiotic factors that may explain variation in fish fatty acid levels among lakes in the Dehcho region of the Northwest Territories. To do this, there were four specific objectives:

Objective 1: Determine the effect of storage time on concentrations of fatty acids in flesh samples for each of several fish species, including: Burbot, Cisco, Lake Trout, Lake Whitefish, Longnose Sucker, Northern Pike, Walleye, and White Sucker.

Previous research has shown that concentrations of fatty acids in fish samples stored at temperatures warmer than 80°C can change over time due to degradation, and that the degradation can be species-specific (Nazemroaya et al. 2009; Rudy et al. 2016). As some samples were stored for multiple years at -20°C, I hypothesized that concentrations of some fatty acids would decrease with increasing storage time.

Objective 2: Update existing fish fatty acid profiles, mercury concentrations, and correlations between fatty acid concentrations and mercury concentrations for each fish species listed above.

Although samples from two new lakes were analysed, the majority of new samples related to this objective were included to increase sample sizes for lakes that were studied previously. For lakes that were previously sampled, I assumed that fatty acid profiles would not exhibit differences among sampling years, and consequently that among-species differences would be consistent with results reported by Reyes et al. (2017) and Laird et al. (2018).

Objective 3: Determine whether there is a significant difference in fatty acid concentrations among lakes for each fish species listed above. Specifically, I will investigate among-lake differences in concentrations of:

a) Total fatty acids
b) Total omega-6 PUFAs
c) Total omega-3 PUFAs
d) Eicosapentaenoic Acid (an omega-3 PUFA)
e) Docosahexaenoic Acid (an omega-3 PUFA)

Water chemistry and watershed characteristics can influence the community composition of algae and bacteria, which can affect the types and amounts of fatty acids available to consumers (Müller-Navarra et al. 2000; Brett et al. 2009). As fish were caught in lakes located within distinct eco-regions that vary in water chemistry, watershed characteristics, and structure of biological communities (which can affect trophic ecology), I hypothesized that concentrations of fatty acids in fish would differ among lakes.

Objective 4: After accounting for variation related to biotic factors (e.g., fork length), determine whether variables that reflect water chemistry and watershed size and composition explain among-lake variation in fish fatty acid concentrations for three fish species of particular importance to subsistence fishers: Lake Whitefish, Northern Pike, and Walleye.

If there were significant differences among lakes in the fatty acid groups listed in objective 3, I hypothesized that among-lake differences would be related to abiotic variables that are known to affect fish trophic ecology and/or the ecology of primary producers at the base of the food web (e.g., water chemistry and watershed variables).

Chapter 2. Methodology

2.1 Study Location

The study area includes ten lakes in the Dehcho region of the Northwest Territories (Figure 2). The lakes are located in three eco-regions that have distinct vegetation, soil, and water characteristics (Thie et al. 1982; Wiken 1986). Lakes in the Hay River Lowlands (HRL; Ekali, Sanguez, Gargan, McGill, Tathlina, and Kakisa) are located at relatively lower elevations (mean watershed elevations 274-456 metres above sea level [masl]). Lakes located in the Northern Alberta Uplands (NAU; Trout; 584 masl) and on the Horn Plateau (HP; Big Island, Mustard, and Willow; 704-783 masl) are located at a higher elevation. As the NAU eco-region contains only one study lake, it will be referred to as 'Trout Lake' throughout the text. All study lakes were chosen by local communities as important for subsistence fishing.

2.2 Field Sampling

2.2.1 Fish Sampling

Fish were harvested from ten lakes in the Dehcho region of the Northwest Territories (Big Island, Ekali, Gargan, Kakisa, McGill, Mustard, Sanguez, Tathlina, Trout, and Willow) during August or September between the years of 2013 and 2018. Sampling was conducted with a research permit from the Aurora Research Institute (License # 16046). Of the ten lakes, six currently have site-specific mercury advisories: Ekali (Northern Pike and Walleye), Gargan (Northern Pike), McGill (Northern Pike and Walleye), Sanguez (Northern Pike and Walleye), Tathlina (Northern Pike and Walleye), and Trout (Lake Trout and Walleye) (GNWT 2019). Fish captured included Burbot, Cisco, Lake Whitefish, Lake Trout, Longnose Sucker, Northern Pike, Walleye, and White Sucker. Fish sampling was carried out in accordance with Animal Use Protocol A-18-04 (University of Waterloo Animal Care Committee).

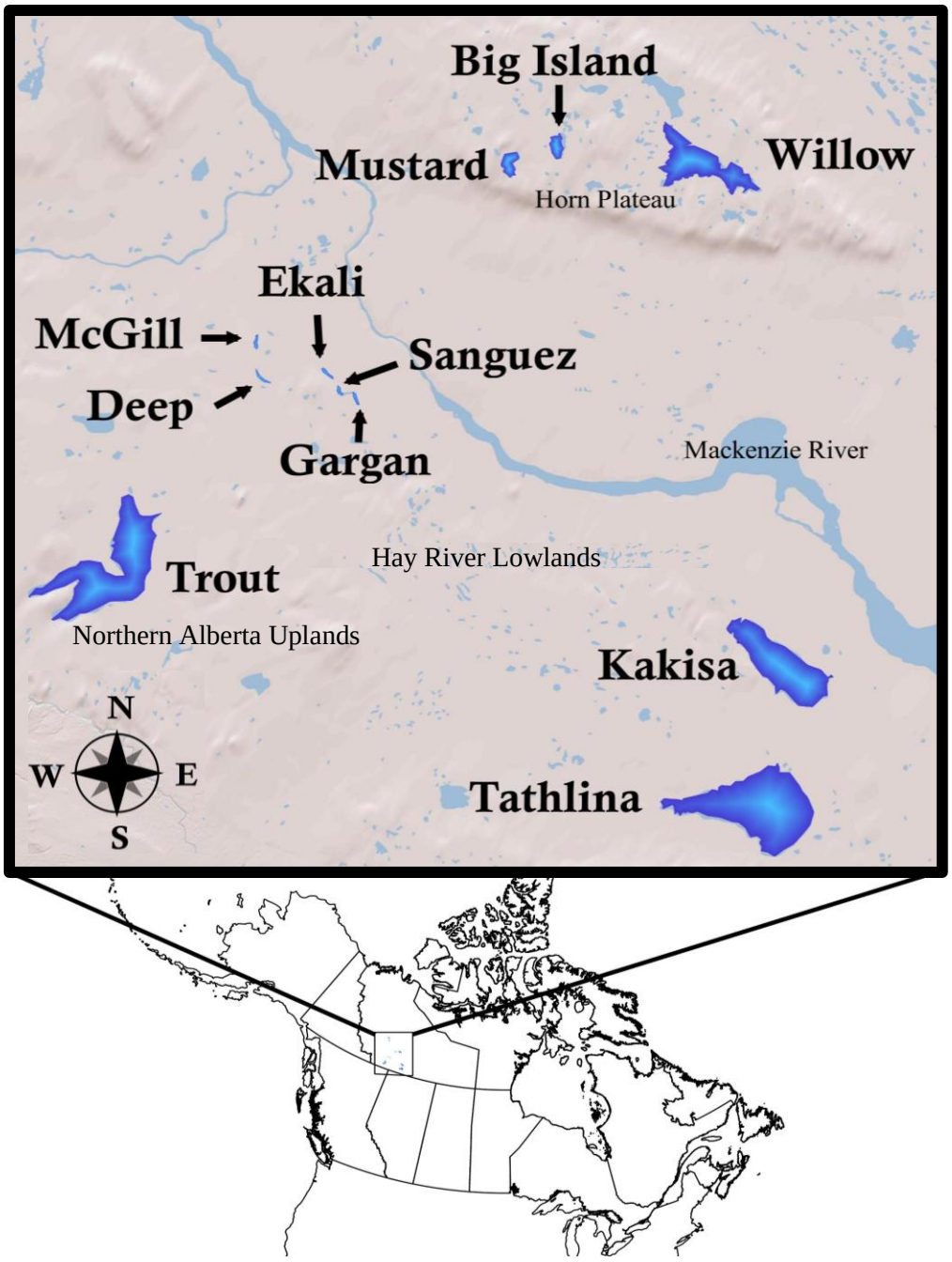

Figure 2. Map of study lakes in the Dehcho region of the Northwest Territories. Lakes are located in three eco-regions, including the Hay River Lowlands (Ekali, Gargan, Kakisa, McGill, Sanguez, and Tathlina), the Horn Plateau (Big Island, Mustard, and Willow), and the Northern Alberta Uplands (Trout).

Experimental gill nets (46m long; mesh sizes ranging from 2 to 14 cm) were set from a boat and soaked for a maximum of 14 hours at depths ranging from 3 to 20 m. Small fish (< 140 mm) were frozen whole immediately after capture in a portable freezer at -20 °C. For larger fish (> 140 mm), data collected for each individual included weight (g), sex, maturity (juvenile or mature), and stomach contents. Measurements of fish length included the fork length, or the distance from the tip of the snout to the fork in the caudal (tail) fin (mm; Cisco, Lake Trout, Lake Whitefish, Longnose Sucker, Norther Pike, Walleye, and White Sucker) or total length, the distance from the tip of the snout to the end of the caudal fin (mm; Burbot; Kahn et al. 2004). Aging structures were collected, including otoliths (all species), opercula (Walleye), cleithra (Northern Pike), and scales (Lake Whitefish). Two muscle samples were taken from behind the head, on each side of the body anterior to the dorsal fin and above the lateral line; one of these samples was used for mercury analysis and the other was used for fatty acid analysis. Skin was removed from the muscle tissue prior to storage in whirl-paks^{TM}. Tissue samples were maintained at a temperature of -20°C before being shipped to the University of Waterloo for processing and analysis. Sanitation measures included thoroughly scrubbing gloves and instruments with water between fish.

2.2.2 Water Sampling

In-situ measurements collected at each lake included pH, conductivity, and temperature (collected with a Professional Plus Handheld YSI meter), as well as Secchi depth. Surface grab samples of water were collected for analyses of water chemistry (detailed below). Dissolved organic carbon (DOC) samples were filtered through muffled quartz filters (nominal pore size 0.45 μm) using a peristaltic pump and were stored in amber glass bottles. All water samples were stored at 4°C before being shipped for laboratory analyses. Water for chlorophyll-a samples was filtered through an ethanol-rinsed 0.45 μm glass fiber filter; filters were frozen and stored at -20°C.

2.3 Laboratory Analyses

2.3.1 Fatty Acids

To prepare samples for analysis, 10g of muscle tissue was pulverized in liquid nitrogen inside a Cryo-Cup Grinder (BioSpec Products, Bartlesville, OK, USA). Samples were stored at -80°C until delivery to the Stark laboratory at the University of Waterloo, where fatty acids were quantified through fatty acid methyl ester (FAME) analysis. Fish fatty acid profiles were quantified using methodology described by Laird et al (2018). Pulverised fish muscle tissue (between 10-30 mg) was homogenised using 3mL of a 2:1 chloroform:methanol solution with ethyl docosatrienoate (ethyl ester, Nu-Chek Prep Inc., Elysian, MN) as the internal standard, and 50 µg/mL butylated hydroxytoluene to decrease oxidation (Metherel and Stark 2015). Approximately 500 µL of 0.2 mol/L $NaHPO_4$ was added to each sample before it was centrifuged. The organic phase was collected and dried using nitrogen (N_2) gas, and then 300 µL of hexane and 1000 µL of 14% BF_3 in methanol were added before samples were warmed on a heating block (kept at 95^{o}C) for one hour. After cooling, 1000 µL of double distilled water and 1000 µL of hexane were added, then samples were vortexed and centrifuged a second time. The hexane layer (containing fatty acid methyl esters) was dried again under N_2 gas, and samples were added into 64 µL of hexane and stored in gas chromatography vials prior to analysis.

Extracted total lipids were separated with a Varian 3900 gas chromatograph with a DB-FFAP 15 m x 0.1 i.d. x 0.1 µm film thickness, nitroterephthalic acid-modified polyethylene glycol capillary column (J and W Scientific, Agilent Technologies, Mississauga, ON), with hydrogen as the carrier gas. The flame ionization detector was set to 300^{o}C, and 1 µL aliquots of the samples (measured with a Varian CP-8400 Autosampler) were added to the injector at a time. The injector was kept at 250^{o}C and had a 200:1 split ratio. Air and gas flow rates were 300 mL per minute and 45 mL per minute, respectively, and the sample frequency was set to 50 Hz. Samples were heated from an initial temperature of 150^{o}C to 200^{o}C (at a 35^{o}C per minute increase). After 200^{o}C, the temperature increase was slowed to 8^{o}C per minute, up to the final temperature of 245^{o}C. The retention time of each compound was compared to retention times of the external standard (GLC-462, Nu-Check Prep, Elysian, MN, USA) for identification. Duplicates were not run in this round of samples, however past analysis of duplicate and triplicate samples resulted

in a difference between 0.2-7.6% (Laird et al. 2018) . Fatty acid concentrations were reported in mg/100 g (dry weight).

2.3.2 Mercury

In preparation for mercury analysis, fish muscle tissue was freeze-dried with a LabConco FreeZone set to -54°C and 0.014 mBar for 48 hours. Freeze-dried tissue was used for both mercury and stable isotope analysis (see section 2.3.3). After freeze-drying, samples were homogenised with scissors in 20 mL borosilicate scintillation vials and shipped to the Biotron Center for Experimental Climate Change Research (Biotron) at Western University, where samples were processed according to method 7473 described by the U.S. Environmental Protection Agency (US EPA 2007). Total mercury was measured with a Milestone®DMA-80 Direct Mercury Analyzer (Milestone Srl, Italy), using DORM-4 fish protein as a certified reference material. Blanks (empty quartz boats) and reference material were analysed at the beginning of each sample run, and again after every 10^{th} sample. The method detection limit was 0.08 ng/g (dry weight). Sample duplicates had a mean relative percent difference of 4.41 ± 8.80% (n=134), and the mean percent recovery of certified reference material DORM-4 was 98.1 ± 3.70% (n > 42).

2.3.3 Stable Isotope Analysis

Stable isotope ratios of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) can be used to determine dietary carbon source and trophic level of organisms, respectively (Minagawa and Wada 1986; Rounick and Winterboum 1986; Ehleringer et al. 2000). To enable analysis of fish $\delta^{13}C$ and $\delta^{15}N$ stable isotope ratios (relative to the international standards Pee-Dee Belemnite and N_2, respectively), a portion of the freeze-dried fish muscle tissue (0.30-0.35 mg) was transferred into 3.5 mm tin capsules and transported to the University of Waterloo Environmental Isotope Laboratory. Analysis was carried out using a 4010 Elemental Analyzer (Costech Instruments, Italy) and a Delta PLUS XL continuous flow isotope ratio mass spectrometer (Thermo-Finnigan, Germany). In-house reference materials (EIL-72 and 3, JSEC-01), and bovine liver for fish (NIST-1557b) were used for quality control in the sample runs, with the standards making up twenty percent of each run. Reported errors were 0.2‰ or less for $\delta^{13}C$ and 0.3‰ or less for $\delta^{15}N$. The mean

relative percent difference for duplicate samples of $\delta^{13}C$ was 0.372% ± 0.401 (n=123), and 2.21% ± 1.53 (n=123) for $\delta^{15}N$.

2.3.4 Water Chemistry and Fish Age

Analyses of concentrations of chlorophyll a-and nutrients in water were completed at the University of Alberta Biogeochemical Analytical Service Laboratory. Surface water concentrations of total nitrogen (TN) and TP were determined using a Lachat QuickChem QC8500 FIA Automated Ion Analyzer. Total nitrogen was analysed according to US EPA Method 353.2- TN/TDN, while the analysis of total phosphorus followed methodology described in the American Public Health Association (APHA) Method 4500-P-G (TP/TDP). Fluorimetry was used to determine Chlorophyll-a concentrations on a Shimadzu RF-1501 Spectrofluorophotometer, following methods described by Welschmeyer (1994). A Mantech PC-Titration Plus System was used to determine alkalinity, conductivity, and pH, according to methodology described in APHA Titration Method 2320 B (alkalinity), APHA Electrometric Method 4500-H+B (pH), and US Geological Survey (USGS) Method Series 09-A6.6. Concentrations of dissolved organic carbon (DOC) and dissolved inorganic carbon (DIC) were quantified at Western University using an Aurora 1030W (OI Analytical, Texas). All ion concentrations (SO_4^{2-}, NO_3^-, Na^+, K^+, Mg^{2+}, Ca^{2+}) were quantified at the University of Waterloo Biogeochemistry Lab with ion chromatography using a DIONEX ion chromatograph and an AS14 analytical column. Extracted fish otoliths were sent to AAE Tech Services for aging using the crack and burn method.

2.3.5 Excitation-Emission Matrices

The optical properties of dissolved organic matter were quantified using excitation emission matrix (EEMs) data, which combines scans of light emissions at differing excitation wavelengths with fluorescence intensity (Coble 1996). Ultra-violet absorption at 254 nm (UV_{254}) measures the amount of light that is absorbed by aromatic compounds, which in lakes typically represents humic substances, or organic compounds of terrestrial origin (Malcolm 1991; Weishaar et al. 2003). UV_{254} is typically strongly and positively correlated with DOC concentrations, and UV_{254} can be corrected for variability in lake DOC concentrations to calculate a metric known as Specific UV Absorbance ($SUVA_{254}$; Weishaar et al. 2003). Two fluorescence indices (FI2001 and FI2005, which differ in the emission wavelength measured) are based on the ratio of

aromatic compounds to non-aromatic compounds; a value greater than 1.8 indicates that the majority of carbon comes from autochthonous sources (in-lake production), whereas a value less than 1.4 indicates primarily allochthonous carbon (McKnight et al. 2001; Cory and McKnight 2005). Two humification indices (likewise differing in the measured emission wavelength; HIX1999 and HIX2002) were used to compare amounts of humic substances among samples (Zsolnay et al. 1999; Ohno 2002). Finally, the freshness index is based on the ratio of new, autochthonous carbon to older, allochthonous carbon, and is used to help identify carbon source (Parlanti et al. 2000). All EEMs analyses (UV_{254}, $SUVA_{254}$, Fluorescence Indices, Humification Indices, and the Freshness Index) were carried out at Western University using a Spectramax® M2 spectrophotometer.

2.3.6 Watershed Characteristics

Watershed characteristics (watershed area, lake area, etc.) were quantified by MTE Consultants using geographic information systems (GIS) data. GIS data were obtained from several different sources, including CanElevation (HRDM v1.3-ArcticDEM; used for elevation), the Canadian Digital Elevation Model, 1945-2011 (used to compute elevation for Kakisa and Tathlina, which was not available in the ArcticDem dataset), the National Hydro Network (Edition 1.1; measurements of lakes, flow paths and channel length), and the 2010 and 2015 Land Cover of Canada (land cover measurements).

2.4 Statistical Analysis

All statistical analyses were conducted in IBM SPSS Statistics (Version 25) or R Studio (R Studio Version 1.2.1335, R Version 3.6.0; R Team 2019). In addition to the base packages, packages ‘lsmeans’, ‘car’, and ‘ggplot2’ were used for data analysis and graphing. Alpha was set at 0.05, and for analyses when an assumption of normality was made, residuals were assessed visually, and with a Shapiro-Wilk test.

2.4.1 Analysis of Storage Time

As some fish tissue samples were stored for multiple years before processing, an analysis of the effects of storage time was run before any other statistical analysis. Storage times were conservatively calculated as the number of months between the date of collection and the date the FAME data were received from the lab, as the processing date was not available for older

samples. Linear regressions were run between fatty acid concentrations (with individual fish as the replicate) and storage time.

2.4.2 Fatty Acid Profiles

Arithmetic mean fatty acid concentrations (all lakes combined) were calculated for each species (Burbot, Cisco, Lake Trout, Lake Whitefish, Longnose Sucker, Northern Pike, Walleye, and White Sucker). Measured fatty acids included saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), n-6 PUFAs, and n-3 PUFAs. Data previously published by Laird et al. (2018) and new data from this study were combined for analysis. Ranges and mean fatty acid concentrations were calculated for each species and for each fatty acid group of interest, including total fatty acids (TFA; n=433), total n-6 PUFAs (n=433), EPA (n=433), total n-3 PUFAs (n=317), and DHA (n=317). TFA, total n-6 PUFAs, total n-3 PUFAs and EPA were reciprocally transformed prior to analyses to meet assumptions of normality; concentrations of DHA did not require transformation to meet assumptions of the statistical test.

2.4.3 Mercury Analyses

Arithmetic mean mercury concentrations were calculated for each species (all lakes combined), as well as for each individual lake within a species. Data previously published by Laird et al, (2018) and new data from this study were combined for statistical analyses. Spearman rank correlation coefficients were used to examine correlations between concentrations of each fatty acid group and mercury concentrations.

2.4.4 Significant Among-Lake Differences

To determine whether species-specific concentrations of TFA, total n-6 PUFAs, total n-3 PUFAs, EPA, and DHA differed significantly among lakes, one-way ANOVAs were conducted for each species and fatty acid group. A 2-way ANOVA was also performed to determine whether patterns in among-lake variation were consistent among species. As variation in fatty acids among species has been studied in detail in these lakes (Reyes et al. 2017; Laird et al. 2018), quantifying differences among species was not a focus in this analysis.

Before quantifying the abiotic drivers of among-lake variation, it was first necessary to identify and account for variability induced by biotic factors. To investigate biological factors that might

influence fatty acid concentrations in fish, a one-way ANOVA was first conducted for each fatty acid group, with lake as the independent variable. Residuals from this ANOVA were extracted, and the species-specific mean of each fatty acid group (all lakes combined) was added to each calculated residual. This approach removed variability in fatty acid responding variables that was due to 'lake'. The adjusted residuals were regressed against biological variables that might influence concentrations of fatty acids in fish, including $\delta^{13}C$, $\delta^{15}N$, age, and fork length. These analyses were used to determine whether it was necessary to calculate adjusted least-squares means (LS means) for biotic variable(s) before investigating variability induced by abiotic variable(s). All calculated LS means were tested with a Tukey's test to determine whether there were significant differences in fatty acid groups among lakes once biologically induced variation was accounted for.

To examine differences in fatty acid and mercury concentrations among eco-regions, one-way ANOVAs were conducted with three treatment levels: HP lakes, HRL lakes, and Trout Lake. LS means were used where appropriate, for both mercury and fatty acid concentrations. While sample sizes among eco-regions were unequal, the assumption of homogeneity of variance was not violated (Levene's test p-value >0.05).

2.4.5 Principal Component Analyses

Recognising that several interacting variables that represent watershed characteristics and lake biogeochemistry may affect concentrations of fatty acids in fish, several principal components analyses (PCAs) were run to investigate relationships among variables. These included a PCA of watershed characteristics (Table 2) and a PCA of all water chemistry variables (Tables 3 and 4). While water chemistry and watershed characteristics are often related, separate PCAs were conducted because results will be used to assess ultimate and proximate drivers of contaminant: fatty acid ratios in future analyses, and because the methodology for data collection was different between the two; water chemistry parameters were derived from field samples, whereas watershed characteristics were estimated using GIS analyses. Results from initial PCAs indicated that carbon quality could be an important predictor of fatty acid levels in fish, so water chemistry variables were split into two smaller PCAs. One was comprised of excitation-emissions matrix data (EEMs data; Table 3), which indicates carbon quality. The other contained all water

chemistry variables except for EEMs data (Table 4). Lastly, one PCA containing all watershed and water chemistry data was run.

2.4.6 Stepwise Regression

Exploratory linear regressions were run between mean (or adjusted mean) fatty acid concentrations (dependent variables) and measured abiotic variables and principal component (PC) scores (independent variables) to determine significant relationships. Significant variables from these individual analyses were then included in a stepwise regression analysis to determine the best predictor(s) of each fatty acid group for each species.

2.4.7 Analysis of TFA, n-6 PUFAs, and EPA using the restricted dataset

Because among-lake variability in n-3 PUFAs and DHA was analysed using a restricted dataset, the set of lakes included in the analysis was different from the dataset used to analyse TFA, n-6 PUFAs, and EPA (i.e., the restricted dataset did not include the HP lakes). To determine how using different datasets affected results and inferences, a second analysis of TFA, n-6 PUFAs, and EPA was run using only samples stored for less than one year (i.e., analogous to the restricted dataset used for n-3 PUFAs and DHA). As described earlier, a one-way ANOVA was conducted for each species and fatty acid group to determine whether there were differences among lakes using the restricted dataset. After quantifying variation in FAs related to biotic factors (e.g., fork length, $\delta^{15}N$) using methods described above, linear regressions were run between mean (or adjusted mean) fatty acid concentrations and measured abiotic variables and principal component (PC) scores to determine significant relationships. Significant variables from these individual analyses were included in a stepwise regression analysis to determine the best predictor(s) of each fatty acid group for each species. Results and general inferences were then compared between datasets.

2.4.8 Correlations Among Variables

Pearson's R correlation coefficients were used to assess relationships among water chemistry and watershed variables. Results were used to examine relationships among predictor variables to help identify underlying mechanisms, and to explore links among water chemistry, which has to be measured in the field, and watershed characteristics, which can be measured remotely.

Table 2. Measured watershed characteristics included in principal components analysis.

Parameter	Measurement Units	Description
Watershed Area	km^2	-
Lake Area (Sum)	km^2	Sum of all lake areas within the watershed
Lake Perimeter (Sum)	km	Sum of all lake perimeters within the watershed
Lake Perimeter to Watershed Area Ratio (Sum)	-	-
Lake Area to Watershed Area Ratio (Sum)	-	-
Riparian Area (Sum)	km^2	Sum of riparian areas (within 50m of waterbodies) in watershed
Lake Area (Ind)	km^2	Area of individual lake
Lake Perimeter (Ind)	km	Perimeter of individual lake
Lake Perimeter to Watershed Area Ratio (Ind)	-	-
Lake Area to Watershed Area Ratio (Ind)	-	-
Riparian Area (Ind)	km^2	Riparian Areas within 50 m of individual lake
Longest Flow Path	km	The length of the longest flow path in watershed
Longest Flow Path to Watershed Area Ratio	-	-
Total Channel Length (flow)	km	Sum of all channel lengths within the watershed (including lake)
Total Channel Length to Watershed Area Ratio (flow)	-	-
Min Elevation	metres above sea level (masl)	Lowest elevation in watershed
Max Elevation	masl	Highest elevation in watershed
Mean Elevation	masl	Mean elevation in watershed
Max Slope	degrees	Maximum slope found in watershed
Mean Slope	degrees	Mean slope of watershed
Minimum Topographic Wetness Index (Min TWI)	-	Minimum upland area per watershed slope
Maximum Topographic Wetness Index (Max TWI)	-	Maximum upland area per watershed slope
Mean Topographic Wetness Index (Mean TWI)	-	Mean upland area per watershed slope

Table 3. Excitation-emission matrix (EEMs) data included in principal components analysis (Figure E-3, Appendix).

Parameter	Calculation	Description
UV_{254}	$\frac{\text{UV absorbance at 254 nm}}{m}$	Shows the degree of aromatic compounds in a sample- higher aromatic compounds have more humic (terrestrial) carbon
Fluorescence Index (FI 2001)	$\frac{Emission\ at\ 450nm}{Emission\ at\ 500nm}\ at\ excitation\ 370nm$	Ratio of aromatic compounds (found in humic substances) to non-aromatic compounds
Fluorescence Index (FI 2005)	$\frac{Emission\ at\ 470nm}{Emission\ at\ 520nm}\ at\ excitation\ 370nm$	Ratio of aromatic compounds (found in humic substances) to non-aromatic compounds
Freshness	$\frac{Emissions\ at\ 380nm}{Emission\ maximum\ 420-435nm}\ at\ excitation\ 310nm$	The proportion of new dissolved organic matter (DOM; autochthonous carbon) to old dissolved organic matter (allochthonous carbon).
Humification Index (HIX 1999)	$\frac{\text{Area of emissions 435 to 480 nm}}{\text{Area of emissions 300 to 445 nm}}\ at\ excitation\ 354\ nm$	A measure of the extent of humic substances in DOM; made to directly compare among samples.
Humification Index (HIX 2002)	$\frac{\text{Area of emissions 435 to 480 nm}}{\text{Area of emissions 300 to 445 nm}}\ at\ excitation\ 354\ nm$	A measure of the extent of humic substances in DOM; made to directly compare among samples.
Specific UV Absorbance (SUVA)	$\frac{UV_{254}\ m^{-1}}{DOC\ mg/L}$	Determines the degree of aromaticity normalised for the amount of dissolved organic carbon in the water.

Table 4. Water chemistry data included in principal components analysis.

Parameter	Measurement Units
Total Phosphorus (TP)	µg/L
Total Nitrogen (TN)	µg/L
Dissolved Organic Carbon (DOC)	mg/L
Secchi Depth	m
Dissolved Inorganic Carbon (DIC)	mg/L
Chlorophyll-a (*Chl a*)	µg/L
pH	-
Alkalinity	$CaCO_3$ mg/L
Conductivity	uS/cm
Bicarbonate	HCO_3 mg/L
Cl^-	ppm
NO_3^-	mg/L
SO_4^{2-}	mg/L
Sodium (Na^+)	ppm
Potassium Ions (K^+)	ppm
Magnesium (Mg^{2+})	ppm
Calcium (Ca)	ppm

Chapter 3. Results

3.1 Study Samples

Previously published research on lakes in the Dehcho region includes full fatty acid profiles (all lakes combined) for Burbot (n=14), Cisco (n=21), Lake Trout (n=51), Lake Whitefish (n=68), Longnose Sucker (n=19), Northern Pike (n=85), Walleye (n=59), and White Sucker (n=16) (Table 5; Laird et al. 2018; Reyes et al. 2017). Within these studies, lake-specific and species-specific ratios of n-6 to n-3 PUFAs, concentrations of important fatty acid groups (TFA, total PUFAs, total n-3 PUFAs, and EPA+DHA), and ratios of fatty acid concentration to mercury concentration were calculated (Reyes et al. 2017; Laird et al. 2018). In the present study, these data were combined with additional fatty acid data from two newly sampled lakes, Big Island (n=32) and Willow (n=29), and with an additional 39 samples to increase sample sizes from previously studied lakes (Table 6). As samples collected after 2015 were stored for multiple years prior to processing, the effect of storage time on each fatty acid group was investigated prior to any other statistical analysis.

Table 5. Sample sizes by species and lake for previously published data (n=333; Reyes et al. 2017; Laird et al. 2018).

Lake	Burbot	Cisco	Lake Trout	Lake Whitefish	Longnose Sucker	Northern Pike	Walleye	White Sucker
Ekali	-	5	-	11	-	17	17	-
Gargan	-	1	-	16	-	15	-	-
Kakisa	-	8	-	10	7	10	8	7
McGill	-	-	-	9	4	8	7	4
Mustard	5	-	39	-	1	5	-	-
Sanguez	-	1	-	3	-	10	7	-
Tathlina	-	-	-	9	1	10	9	5
Trout	9	6	12	10	6	10	11	-
Total	**14**	**21**	**51**	**68**	**19**	**85**	**59**	**16**

Table 6. Sample sizes by species and lake for all data included in this thesis (n= 433).

Lake	Burbot	Cisco	Lake Trout	Lake Whitefish	Longnose Sucker	Northern Pike	Walleye	White Sucker
Big Island	6	1	4	13	-	8	-	-
Ekali	-	5	-	11	-	16*	15*	-
Gargan	-	1	-	16	-	15	-	-
Kakisa	-	8	-	10	8	10	9	11
McGill	-	-	-	10	4	11	10	4
Mustard	5	-	39	-	1	5	-	-
Sanguez	-	5	-	12*	-	8*	24*	-
Tathlina	-	-	-	11	1	10	10	5
Trout	9	6	11*	10	6	10	11	-
Willow	-	-	5	12	-	12	-	-
Total	**20**	**26**	**59**	**104**	**20**	**105**	**79**	**20**

*A total of nine previously processed samples included in the dataset used by Reyes et al. 2017 and Laird et al. 2018 were excluded from use in this thesis because of data transcription errors that could not be resolved. Affected lakes are marked with an *.

3.2 Effect of Storage Time on Fish Fatty Acids

The fatty acid profiles in every fish species (Burbot, Cisco, Lake Trout, Lake Whitefish, Longnose Sucker, Northern Pike, Walleye, and White Sucker) changed significantly with increasing storage time; a summary of significant relationships is provided in Table 7. DHA decreased exponentially with storage time in Burbot (R^2= 0.86), Cisco (R^2= 0.58), Lake Trout (R^2= 0.52), Lake Whitefish (R^2= 0.75), Longnose Sucker (R^2=0.37), Northern Pike (R^2= 0.70), Walleye (R^2= 0.66), and White Sucker (R^2= 0.74; Figure A-1, Appendix). Fit was assessed with R^2 values. Decreasing DHA concentrations with increasing storage time also resulted in the decrease of several other groups of fatty acids, including total n-3 fatty acids, which decreased significantly with increasing storage time in all species (Linear regression, $F_{\geq 1,18} \geq 14.8$, $p \leq 0.002$; Figure A-2, Appendix). Lignoceric acid, a saturated fatty acid (C 24:0) increased significantly with storage time in every species (Linear regression, $F_{\geq 1,18} \geq 93.7$; $p < 0.002$), which contributed to a signficant overall increase in total saturated fatty acids with longer storage times, although the increase in total saturated fatty acids was only signficant in Burbot, Lake Whitefish, Northern Pike, and Walleye (Linear regression, $F_{\geq 1,18} \geq .83$; $p < 0.002$).

Concentrations of TFA (Figure A-3, Appendix) and n-6 PUFAs (Figure A-4, Appendix) were not significantly related to storage time in any species (Linear regression, $F_{\geq 1,18} \geq 0.008$, $p \geq 0.052$). While concentrations of EPA were significantly related to storage time in Burbot (positive),

Northern Pike (positive), and Lake Trout (negative), EPA concentrations measured at the longest storage times still fell within the range of EPA concentrations found in fish tissue stored for less than one year (Figure A-5, Appendix; Linear regression, $F_{\geq 1,18} \geq 4.66$, $p \leq 0.045$). For this reason, all EPA samples were used in analyses, as were all data for TFA and n-6 PUFAs. However, statistical analyses carried out using DHA or n-3 PUFAs were restricted to samples that were stored for less than one year (Figures A-6 and A-7 respectively), meaning that the sample size decreased to n=317 (from n=433).

There were several other significant results that were of interest because they affect fish nutritional quality, but that did not affect statistics because they were not the focus of any downstream analyses (Table 7). With the decrease of DHA, concentrations of total PUFAs (Linear regression, $F_{\geq 1,18} \geq 8.08$, $p \leq 0.011$) and total HUFAs (Linear regression, $F_{\geq 1,18} \geq 4.87$, $p \leq 0.041$) decrease significantly in every species except for Burbot and Longnose Sucker (Linear regression, $F_{\geq 1,18} \geq 1.77$, $p \geq 0.504$). Since there was no significant relationship between concentrations of n-6 fatty acids and storage time, but concentrations of n-3 fatty acids decrease, the ratio of n-6 fatty acids to n-3 fatty acids increased with storage time in every species (Linear regression, $F_{\geq 1,18} \geq 19.1$, $p \leq 0.001$). Lignoceric acid (C 24:0), a SFA, increased significantly with storage time in every species (Linear regression, $F_{\geq 1,18} \geq 93.7$, $p \leq 0.001$), which contributed to an overall increase in total SFA, although the increase was only significant in Burbot, Lake Whitefish, Northern Pike, and Walleye (Linear regression, $F_{\geq 1,18} \geq 9.84$, $p \leq 0.002$).

Using only data from samples that were stored for less than one year, DHA concentrations in Burbot, Northern Pike, and Walleye were significantly related to storage time (Linear regression, $F_{\geq 1,18} \geq 4.95$, $p \leq 0.029$; Table 8), but the relationships were positive, and thus there was no indication of fatty acid degradation (Figure A-6, Appendix). Likewise, n-3 PUFAs in Longnose Sucker were significantly and positively related to storage time (Figure A-7, Appendix; Linear regression, $F_{1,18} = 5.11$, $p = 0.037$), which also indicated that degradation was not a problem (Table 8). However, Lake Trout total n-3 PUFAs and DHA exhibited significant decreases in concentrations even after restricting data to samples stored for less than one year (Linear regression, $F_{1,48} \geq 18.7$, $p \leq 0.001$) (Table 8). While Lake Trout were not the focus of further

Table 7. P-values obtained from results of linear regressions that investigated relationships between species-specific concentrations of fatty acids and storage time (in months). The direction of the relationship is indicated in brackets. A dash indicates that the relationship was not significant. [1]Indicates groups of interest used in statistical analyses. **The relationship between DHA and storage time (months) in each species was best fit by a model of exponential decay. Non-linear regression does not support p-values, so the relationship was assessed by R^2.

Fatty Acid (mg/100g)	**Burbot** (n=20)	**Cisco** (n=26)	**Lake Trout** (n=59)	**Lake Whitefish** (n=104)	**Longnose Sucker** (n=20)	**Northern Pike** (n=105)	**Walleye** (n=79)	**White Sucker** (n=20)
C 22:0	<0.0001 (+)	-	-	-	-	-	-	-
C 24:0	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)
Total SFAs	<0.0001 (+)	-	-	0.002 (+)	-	<0.0001 (+)	<0.0001 (+)	-
C 12:1	-	0.021 (-)	-	-	-	-	-	-
C 16:1	-	0.048 (+)	-	-	-	-	-	-
C 18:1n-9	-	0.014 (+)	-	-	-	-	-	-
C 20:1n-9	-	0.008 (+)	-	-	-	-	-	-
C 24:1n-9	-	-	-	-	<0.0001 (+)	-	-	-
Total MUFAs	-	0.025 (+)	-	<0.0001 (-)	-	-	-	-
C 22:2n-6	<0.0001 (+)	-	-	-	-	-	-	-
Total n-6 PUFAs[1]	-	-	-	-	-	-	-	-
C 20:5n-3 (EPA) [1]	0.045 (+)	-	0.008 (-)	-	-	0.007 (+)	-	-
C 22:6n-3 (DHA) [1]	** R^2= 0.86 (-)	** R^2= 0.58 (-)	** R^2= 0.52 (-)	** R^2= 0.75 (-)	** R^2= 0.37 (-)	** R^2= 0.70 (-)	** R^2= 0.66 (-)	** R^2= 0.74 (-)
Total n-3 PUFAs[1]	<0.0001 (-)	0.003 (-)	0.006 (-)	<0.0001 (-)	-	<0.0001 (-)	<0.0001 (-)	0.048 (-)
Total PUFAs	-	<0.0001 (-)	<0.0001 (-)	<0.0001 (-)	0.050 (-)	<0.0001 (-)	<0.0001 (-)	0.011 (-)
Total HUFAs	-	<0.0001 (-)	0.004 (-)	<0.0001 (-)	-	<0.0001 (-)	<0.0001 (-)	0.041 (-)
EPA+DHA	<0.0001 (-)	<0.0001 (-)	0.001 (-)	<0.0001 (-)	0.012 (-)	<0.0001 (-)	<0.0001 (-)	0.003 (-)
EPA+DHA+DPA	0.04 (-)	<0.0001 (-)	<0.0001 (-)	-	0.030 (-)	0.008 (-)	0.009 (-)	0.009 (-)
N-6/N-3 Ratio	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)	<0.0001 (+)
TFA[1]	-	-	-	-	-	-	-	-

Table 8. P-values obtained from results of linear regressions that investigated relationships between species-specific concentrations of total PUFAs, DHA, and storage time (in months), with samples restricted to those processed within one year of capture. The direction of the relationship is indicated in brackets. A dash indicates that the relationship was not significant.

Species	N	n-3 PUFAs	DHA
Burbot	14	-	0.029 (+)
Cisco	19	-	-
Lake Trout	50	<0.0001 (-)	<0.0001 (-)
Lake Whitefish	65	-	-
Longnose Sucker	17	-	-
Northern Pike	81	-	0.029 (+)
Walleye	55	-	0.003 (+)
White Sucker	16	0.023 (+)	-

analyses in this thesis, these results suggest that Lake Trout samples analysed in the future might need further restrictions based on storage time.

3.3 Fatty Acid Profiles

Updated fatty acid concentrations for each species (with all lakes pooled) were calculated using arithmetic means unadjusted for fork length. Concentrations were calculated for both data restricted by storage time (Tables B-1 and B-2, Appendix), and using all data regardless of storage time (Tables B-3 and B-4, Appendix). For fatty acid groups not affected by storage time, concentrations of TFA were highest in Lake Trout, Lake Whitefish, and the two species of suckers, lower in Northern Pike, Walleye, and Cisco, and lowest in Burbot, a pattern that was also observed for n-6 PUFAs and EPA, the other two fatty acid groups that were not restricted by storage time (Table 9).

For the fatty acid groups where a restricted dataset was used, and similar to the results for TFA, n-6 PUFAs, and EPA (Table 9), n-3 PUFAs were highest in Lake Trout and White Sucker (Table 10). Mean n-3 PUFAs were slightly lower in Lake Whitefish, Longnose Sucker, and Cisco. While Burbot had the lowest n-3 PUFAs, piscivorous Northern Pike and Walleye also had relatively low n-3 PUFA concentrations. There was a different interspecific pattern for DHA; Lake Trout had the highest DHA concentrations whereas Burbot had the lowest concentrations; the other species exhibited similar ranges at intermediate concentrations (Table 10).

Table 9. Range and mean concentrations (all 10 lakes in the Dehcho region combined) of TFA, n-6 PUFAs, and EPA, ± standard deviation. All samples are included, regardless of storage time, because storage time did not affect these fatty acid groups.

Species	N=433	TFA (mg/100g)		n-6 PUFAs (mg/100g)		EPA (mg/100g)	
		Range	Mean	Range	Mean	Range	Mean
Burbot	20	236-541	361 ± 76	41-111	71 ± 23	12-54	28 ± 10
Cisco	26	510-2400	995 ± 403	58-241	104 ± 43	30-155	64 ± 27
Lake Trout	59	420-13872	2158 ± 2622	58-1322	245 ± 240	21-556	103 ± 96
Lake Whitefish	104	496-8727	1415 ± 1024	58-1095	162 ± 120	39-431	88 ± 46
Longnose Sucker	20	491-4085	1454 ± 1037	60-495	175 ± 117	28-170	77 ± 36
Northern Pike	105	286-1429	525 ± 182	42-201	75 ± 27	17-114	36 ± 14
Walleye	79	331-1693	609 ± 230	45-213	78 ± 30	14-89	35 ± 13
White Sucker	20	444-5004	1464 ± 1212	80-669	199 ± 160	31-341	102 ± 73

Table 10. Range and mean concentrations (all 10 lakes in the Dehcho region combined) of n-3 PUFAs and DHA ± standard deviation. Only samples restricted to less than one year of storage time are included.

Species	N=317	n-3 PUFAs (mg/100g)		DHA (mg/100g)	
		Range	Mean	Range	Mean
Burbot	14	68-149	111 ± 22	45-88	70 ± 12
Cisco	19	187-693	351 ± 123	108-248	167 ± 41
Lake Trout	50	193-4247	671 ± 793	124-1776	318 ± 335
Lake Whitefish	65	211-887	368 ± 130	122-337	185 ± 42
Longnose Sucker	17	170-734	369 ± 148	111-257	171 ± 42
Northern Pike	81	115-432	197 ± 52	81-223	133 ± 27
Walleye	55	112-535	202 ± 60	73-275	137 ± 31
White Sucker	16	172-1064	406 ± 243	120-248	167 ± 36

3.4 Mercury Analyses

Updated (from Reyes et al. 2017, Laird et al. 2018) mercury concentrations (with all lakes pooled) were calculated for each species. Mercury concentrations were highest in piscivorous Northern Pike and Walleye, and intermediate in Lake Trout and Burbot (Table 11). Mean mercury concentrations were lowest in Cisco, but also relatively low in Lake Whitefish, Longnose Sucker, and White Sucker. Lake-specific mean mercury concentrations for Burbot,

Cisco, Lake Trout, and Lake Whitefish can be found in Table C-1, Appendix, whereas lake-specific mean mercury concentrations for Longnose Sucker, Northern Pike, Walleye, and White Sucker can be found in Table C-2, Appendix.

Table 11. Minimum, maximum, and mean mercury concentrations (all 10 lakes in the Dehcho region combined) ± standard deviation, and range of mercury concentrations.

Species	N=433	Mercury (μg/g)	
		Range	Mean
Burbot	20	0.067-0.680	0.232 ± 0.163
Cisco	26	0.034-0.194	0.068 ± 0.042
Lake Trout	59	0.230-0.865	0.250 ± 0.166
Lake Whitefish	104	0.014-0.320	0.100 ± 0.067
Longnose Sucker	20	0.022-0.370	0.130 ± 0.088
Northern Pike	105	0.036-3.12	0.433 ± 0.453
Walleye	79	0.035-1.43	0.504 ± 0.332
White Sucker	20	0.026-0.290	0.134 ± 0.073

Mercury concentrations were related to concentrations (all lakes pooled) of TFA, total n-6 PUFAs, total n-3 PUFAs, EPA, and DHA using Spearman rank correlations (Tables C-3 and C-4, Appendix). For piscivorous, high trophic-level Northern Pike and Walleye, each fatty acid group except for n-6 PUFAs was significantly, negatively correlated with mercury concentrations ($\rho \geq$ -0. 250, $p \leq 0.026$).

For predatory Burbot, planktivorous Cisco, and opportunistic Lake Trout, fatty acid concentrations were generally negatively related to mercury concentrations, but relationships that were significant varied among species. All fatty acid groups except DHA were significantly, negatively correlated with mercury in Burbot ($\rho \geq -0.667$, $p < 0.001$). While all Cisco fatty acid groups appeared to be negatively related to mercury concentrations, the relationship was only significant for EPA and DHA ($\rho \geq$ -0.434, $p \leq 0.027$). Similarly, while all fatty acid groups in Lake Trout appeared to be negatively related to mercury concentration, only EPA was significantly negatively correlated with mercury concentration (ρ= -0.385, p=0.003).

For the non-piscivorous, fattier species, Lake Whitefish, Longnose Sucker, and White Sucker, there were positive relationships between concentrations of fatty acids and concentrations of

mercury. White Sucker had significant, positive correlations between every fatty acid group and mercury concentrations ($\rho \geq 0.463$, $p \leq 0.040$). The other species varied in which fatty acids were significantly correlated with mercury concentration. For Lake Whitefish, concentrations of TFA and total n-6 PUFAs were significantly and positively correlated with mercury concentrations ($\rho \geq 0.199$, $p \leq 0.044$). No other Lake Whitefish total fatty acid groups were significantly related to mercury concentrations. Fatty acid concentrations in Longnose Sucker were not significantly correlated with mercury concentrations ($p > 0.05$).

3.5 Among-lake comparisons of fish fatty acids

Previous research has shown that concentrations of fatty acids differed significantly among lakes in the Dehcho region of the Northwest Territories (n=7 lakes; Laird et al. 2018). To determine whether species-specific concentrations of TFA, n-6 PUFAs, n-3 PUFAs, EPA, and DHA were different among lakes with additional data, one-way ANOVAs were conducted for each species and fatty acid group (Table 12). The interaction term of two-way ANOVAs between lake and species were used to determine whether different species displayed the same patterns of variation in fatty acid concentrations among lakes.

Table 12. P-values obtained from ANOVA results investigating among-lake differences in each fatty acid group. Bolded numbers indicate significant results, and degrees of freedom are listed in brackets.

Species	TFA	n-3 PUFAs	n-6 PUFAs	EPA	DHA
Lake Whitefish	0.103 (8,102)	0.079 (5,64)	**0.024** (8,102)	0.670 (8,102)	**0.033** (5,64)
Northern Pike	**<0.0001** (9,103)	**<0.0001** (7,80)	**<0.0001** (9,103)	**<0.0001** (9,103)	**<0.0001** (7,80)
Walleye	0.101 (5,78)	**0.040** (5,54)	**0.021** (5,78)	0.517 (5,78)	0.108 (5,54)

Concentrations of TFA and EPA were significantly different among lakes in Northern Pike (ANOVA, $F_{8, 98} \geq 11.72$, $p < 0.001$; Figures 3 and 4), whereas these concentrations did not vary among lakes in Lake Whitefish and Walleye (ANOVA, $F_{>5,78} \leq 1.92$, $p \geq 0.101$). Among-lake patterns in concentrations of TFA and EPA did differ among species, as the interaction terms between lake and species in two-way ANOVAs were significant for both TFA and EPA ($F_{13, 280} > 4.10$, $p < 0.001$). Concentrations of both TFA and EPA in Northern Pike appeared to be higher in the HP lakes (Big Island, Willow, and Mustard) and Trout Lake, and lower in the HRL lakes (Gargan, McGill, Sanguez and Tathlina). An exception for both fatty acid groups is Kakisa lake,

which had mean concentrations as high as the HP lakes and Trout Lake, as did Ekali Lake when considering only TFA concentrations. Mean concentrations of TFA in Lake Whitefish displayed the opposite pattern, although the differences were not significant likely due to the high degree of intraspecific variation in each lake. In general, Lake Whitefish had lower concentrations of TFA in the HP lakes and Trout Lake, and higher concentrations in the HRL lakes. The same pattern was not evident in EPA concentrations in Lake Whitefish. There were no discernable patterns in concentrations of TFA or EPA in Walleye.

Whereas among-lake differences in concentrations of total fatty acids and EPA were only significant for Northern Pike, there were significant differences in concentrations of n-6 PUFAs among lakes for all three species (ANOVA, $F_{>5,78}>2.34$, $p<0.021$; Table 12; Figure 3). The interaction term between lake and species indicated that among-lake patterns in n-6 PUFAs were also significantly different among species (two-way ANOVA, $F_{13, 285}\geq 2.58$, $p\leq 0.002$). Interestingly, concentrations of n-6 PUFAs in both Lake Whitefish and Walleye were lowest in Trout Lake, while the rest of the lakes had similar mean concentrations. Similarly, concentrations of n-6 PUFAs in Northern Pike were highest in Big Island lake (HP), but there were no clear patterns when considering the rest of the lakes (Figure 5).

Concentrations of n-3 PUFAs differed significantly among lakes for Northern Pike and Walleye (Table 12, Figure 5; ANOVA, $F_{>5,54,}=2.54$, $p\leq 0.04$, but not for Lake Whitefish (ANOVA, $F_{5, 64}=2.16$, $p=0.070$). The interaction term between lake and species indicated that among-lake patterns in n-3 PUFAs were significantly different among Lake Whitefish, Northern Pike, and Walleye (two-way ANOVA, $F_{10, 200}=3.04$, $p=0.001$). Northern Pike and Walleye displayed the same general pattern; mean concentrations of n-6 PUFAs were highest in Trout Lake and lowest in Sanguez Lake, whereas the lakes with intermediate concentrations had similar values.

The concentrations of DHA in Northern Pike and Lake Whitefish differed significantly among lakes (ANOVA, $F_{\geq 5,64}\geq 2.62$, $p\leq 0.033$; Figure 7). DHA concentrations in Walleye did not differ significantly among lakes ($F_{5,54}=13.32$, $p=0.108$). The interaction term between lake and species indicated that among-lake patterns in DHA vary by species (two-way ANOVA, $F_{10, 195}=3.36$, $p<0.001$). Concentrations of DHA in Northern Pike and Walleye were highest in Trout Lake and

lower in the HRL lakes, especially Sanguez, although the difference is not significant in Walleye. There were no clear patterns in variation in Lake Whitefish DHA concentrations (Figure 7).

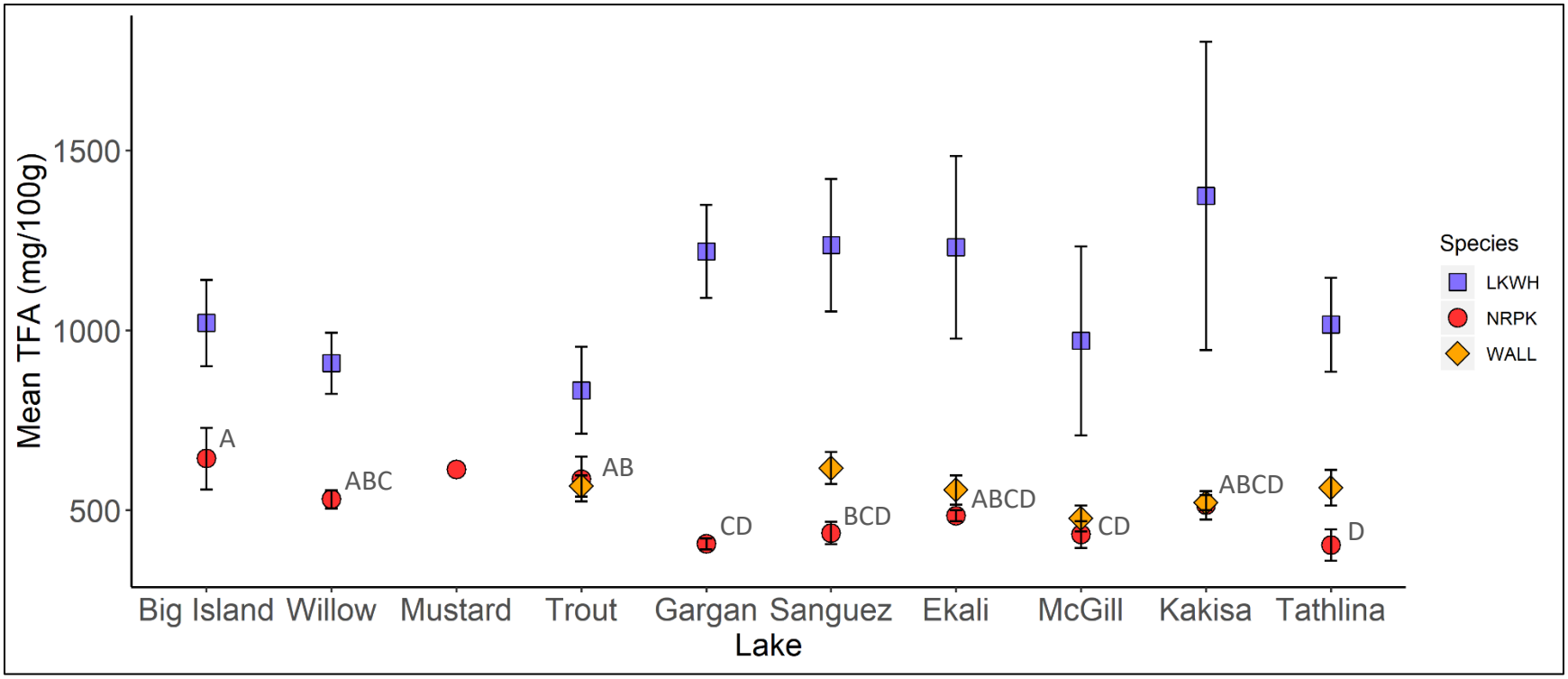

Figure 3. Back-transformed mean TFA concentrations (mg/100g dry weight; ±SE) for three fish species caught in lakes in the Dehcho region of the Northwest Territories. Significant pairwise differences are indicated by letters (Tukey's test, $p<0.05$). Species abbreviations are: LKWH=Lake Whitefish, NRPK= Northern Pike, and WALL= Walleye. Error bars are present where $n>8$; these represent the data included in analyses. Points with no error bars represent data with sample sizes <8.

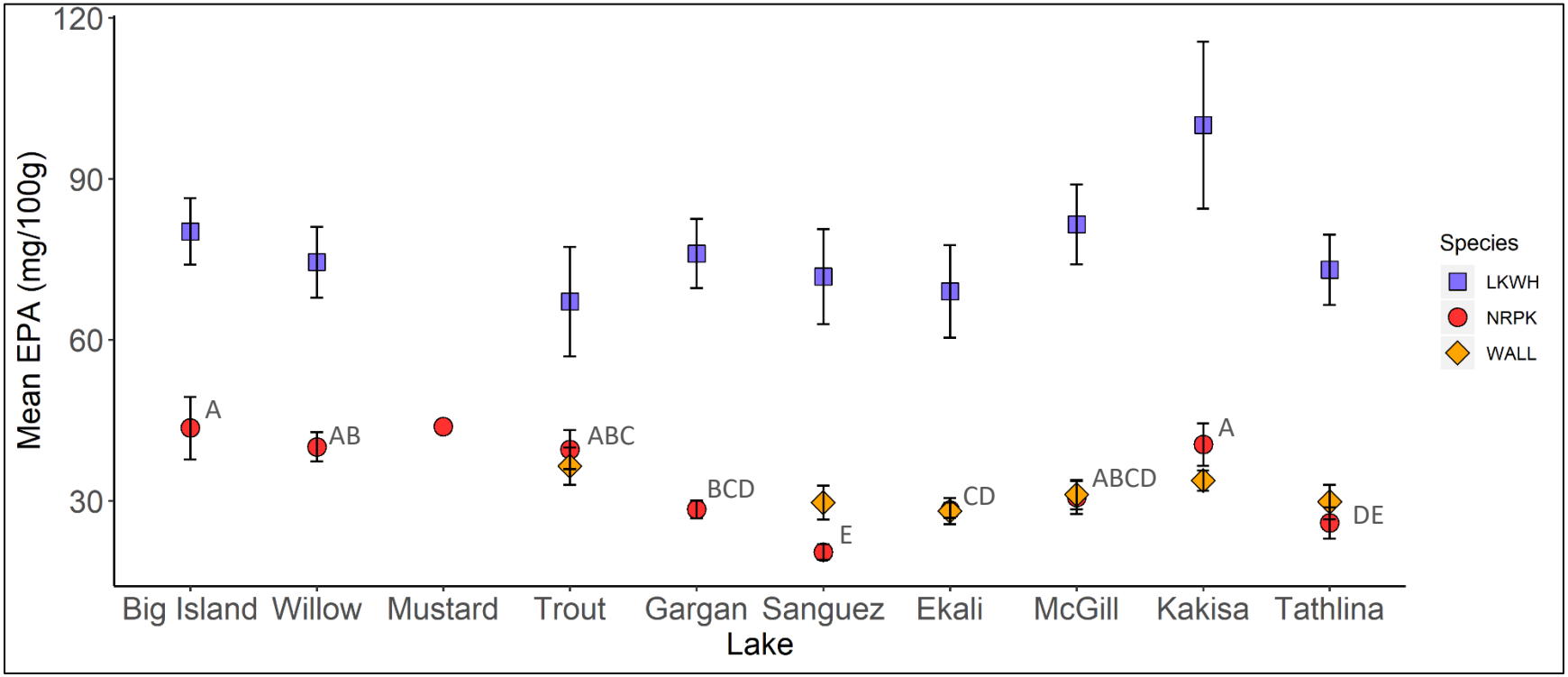

Figure 4. Back-transformed mean EPA concentrations (mg/100g dry weight; ±SE) for three fish species caught in lakes in the Dehcho region of the Northwest Territories. Significant pairwise differences are indicated by letters (Tukey's test, $p<0.05$). Species abbreviations are: LKWH=Lake Whitefish, NRPK= Northern Pike, and WALL= Walleye. Error bars are present where $n>8$; these represent the data included in analyses. Points with no error bars represent data with sample sizes <8.

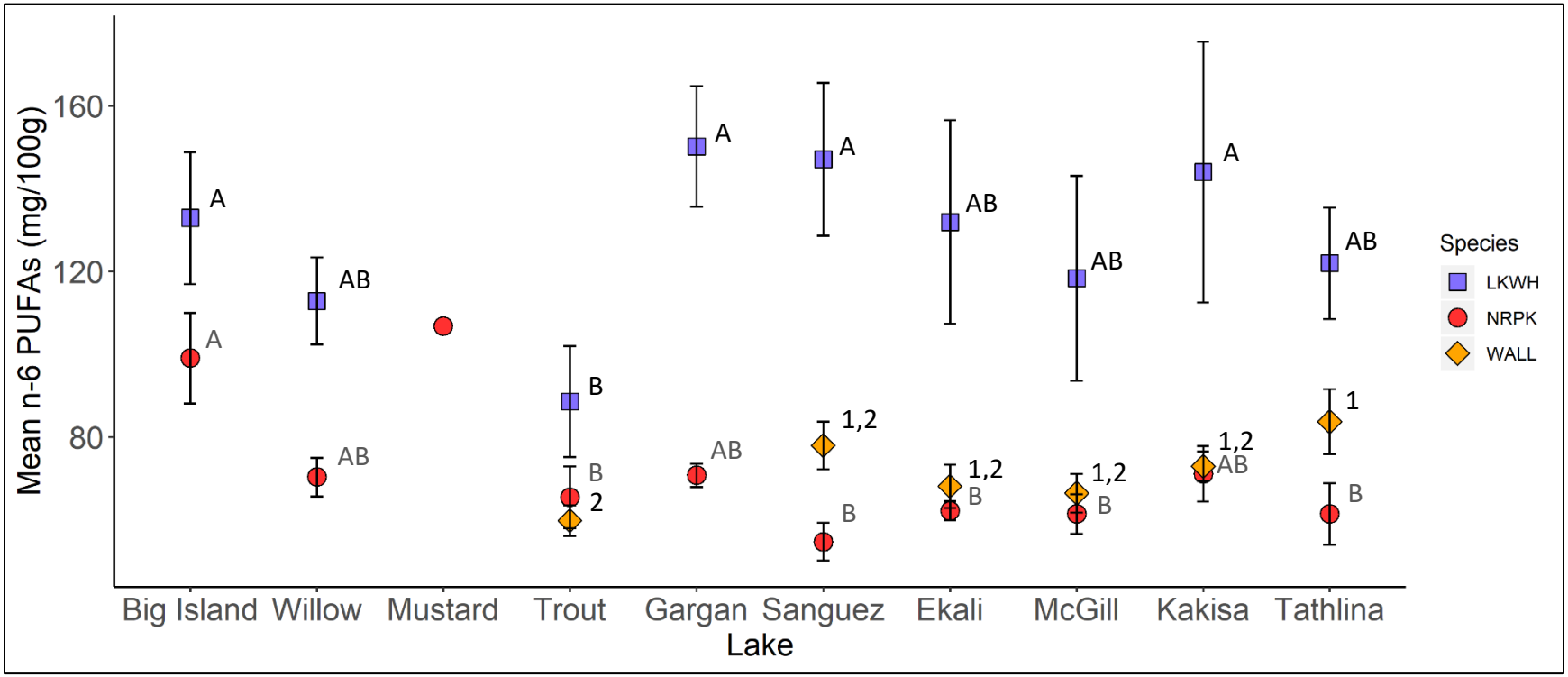

Figure 5. Back-transformed mean n-6 PUFA concentrations (mg/100g dry weight; ±SE) for three fish species caught in lakes in the Dehcho region of the Northwest Territories. Significant pairwise differences (Tukey's test, $p<0.05$) are indicated by letters (LKWH: black; NRPK: grey;) or numbers (WALL). Species abbreviations are: LKWH=Lake Whitefish, NRPK= Northern Pike, and WALL= Walleye. Error bars are present where n>8; these represent the data included in analyses. Points with no error bars represent data with sample sizes <8.

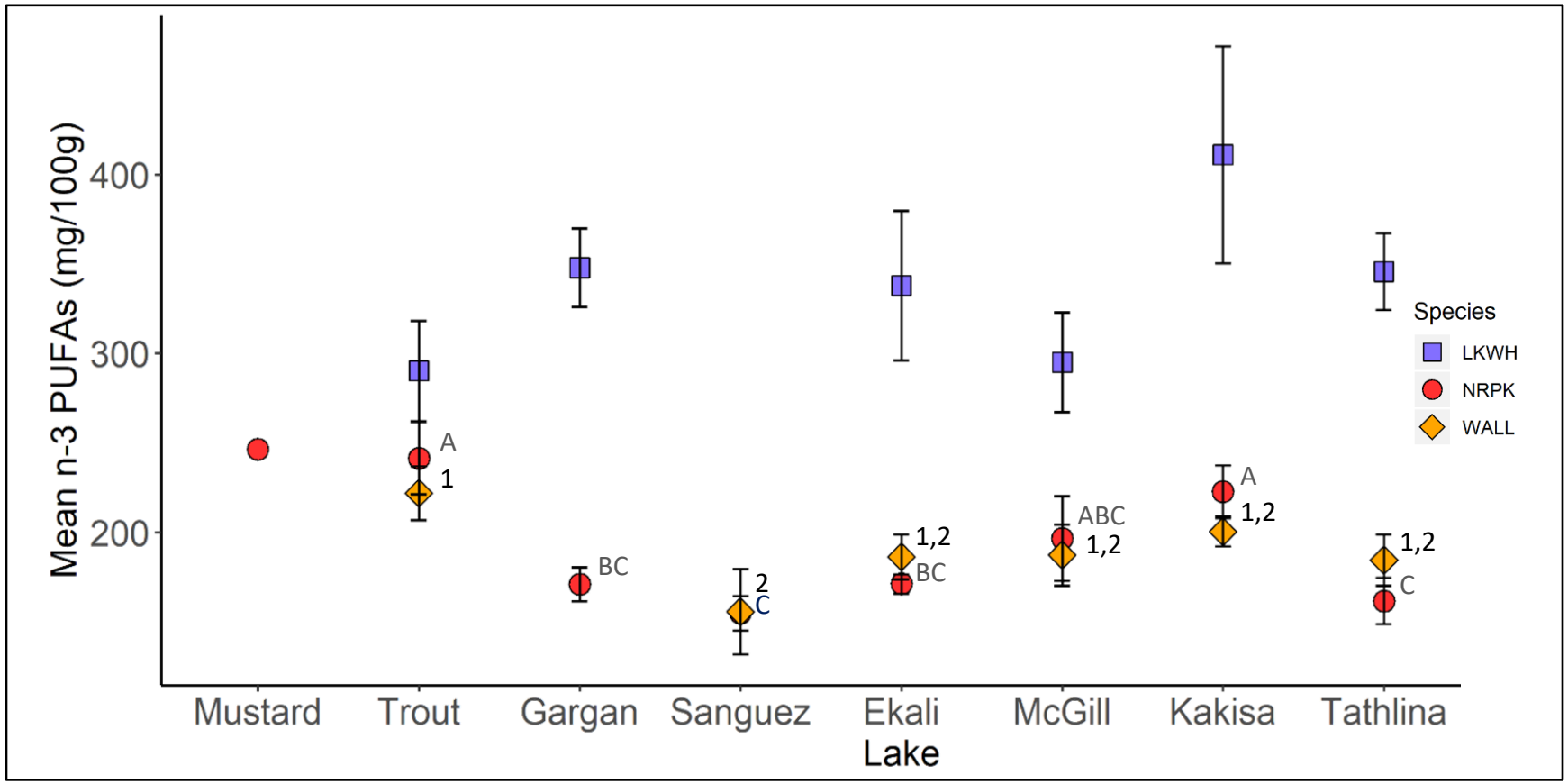

Figure 6. Back-transformed mean total n-3 PUFA concentrations (mg/100g dry weight; ±SE) for three fish species caught in lakes in the Dehcho region of the Northwest Territories. Significant pairwise differences (Tukey's test, $p<0.05$) are indicated by letters (NRPK) or numbers (WALL). Species abbreviations are: LKWH=Lake Whitefish, NRPK= Northern Pike, and WALL= Walleye. Error bars are present where n>8; these represent the data included in analyses. Points with no error bars represent data with sample sizes <8.

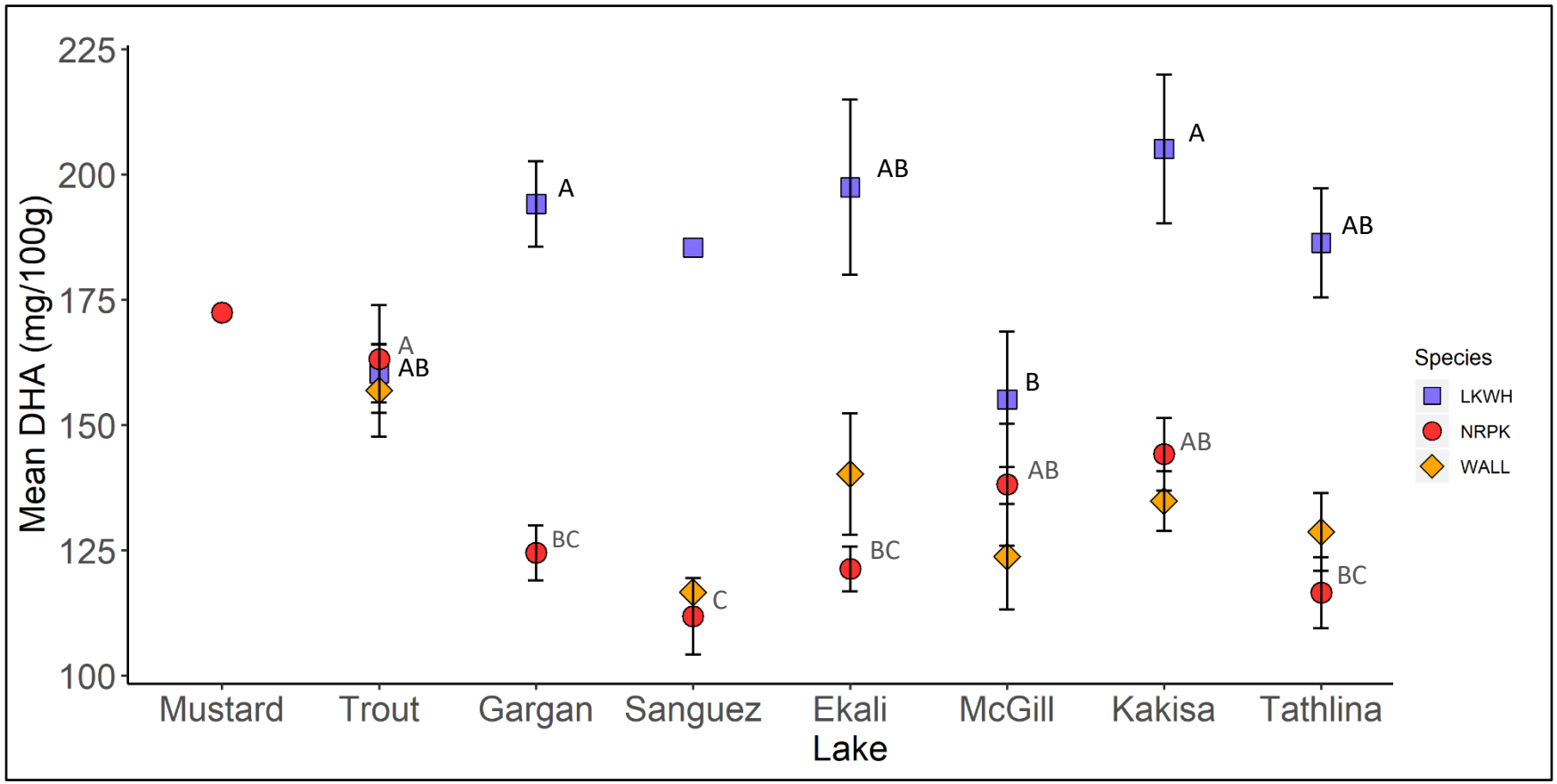

Figure 7. Mean DHA concentrations (mg/100g dry weight; ±SE) for three fish species caught in lakes in the Dehcho region of the Northwest Territories. Significant pairwise differences are indicated by letters (LKWH: black; NRPK: grey; Tukey's test, $p<0.05$). Species abbreviations are: LKWH=Lake Whitefish, NRPK= Northern Pike, and WALL= Walleye. Error bars are present where $n>8$; these represent the data included in analyses. Points with no error bars represent preliminary data on sample sizes <8.

3.6 Abiotic Factors Driving Among-Lake Differences in Fish Fatty Acids

3.6.1 Biological Variables Affecting Fish Fatty Acid Concentrations

For species-specific fatty acid groups where among-lake differences were observed (Table 12), additional analyses were completed to investigate possible abiotic drivers of among-lake variation. Before these analyses were completed, effects of $\delta^{13}C$, $\delta^{15}N$, age, and fork length on fish fatty acid concentrations were investigated to determine whether species-specific means or adjusted means were necessary for subsequent analyses. A summary of biological influences on each fatty acid group in Lake Whitefish, Northern Pike, and Walleye can be found in Table 13, while summaries for other species are located in Table D-1, Appendix. Total fatty acid concentrations in Lake Whitefish were significantly, positively related to fork length (Figure D-1; Appendix; Stepwise regression, $F_{1,100}=7.35$, $p=0.006$). EPA concentrations in Northern Pike and Walleye were significantly, negatively related to fork length (Figure D-2, Appendix; Stepwise regression, $F>_{1,77}\geq 4.548$, $p<0.035$). Concentrations of DHA in Northern Pike were significantly, negatively related to $\log_{10}$ age (Figure D-3, Appendix; Stepwise regression, $F_{1,79}=6.144$, $p=0.015$). Concentrations of n-6 and n-3 PUFAs were not significantly related to any biological predictor ($p>0.05$).

Table 13. Biological variables significantly related to fish fatty acid concentrations. Brackets indicate whether the relationship was positive (+) or negative (-).

Species	TFA	n-3 PUFAs	n-6 PUFAs	EPA	DHA
Lake Whitefish	Fork Length (+)	-	-	-	-
Northern Pike	-	-	-	Fork Length (-)	Log_{10} Age (-)
Walleye	-	-	-	Fork Length (-)	-

Subsequent analyses on abiotic drivers of among-lake differences in species-specific concentrations of fatty acids used unadjusted data (lake-specific arithmetic means) for n-3 PUFAs and n-6 PUFAs (all species), and age-adjusted data for DHA in Northern Pike. Length-adjusted data was used for EPA in Northern Pike and Walleye as well as for TFA in Lake Whitefish. Data were adjusted using analyses of covariance, with lake as a fixed factor,

and the biological variable as a covariate. An interaction term was included. Least-squares means (LS means) for Lake Whitefish TFA and Walleye EPA were calculated at a fork length of 450mm. Northern Pike LS means were calculated at a fork length of 650mm for EPA, and at a log age of 0.75 (the mean age at a fork length of 650mm) for DHA. Standardised sizes were chosen to represent the length of fish typically captured and consumed, and ideally at a size that was captured in every lake so that data did not have to be extrapolated.

Fatty acid groups significantly related to FL or age were re-checked for significant differences among lakes after calculating LS means. While Lake Whitefish TFA was found to have no significant differences when comparing arithmetic means, after calculating LS means, Lake Whitefish TFA were found to be significantly different between Kakisa and McGill lakes (Table D-2, Appendix; Tukey's test <0.05). Northern Pike EPA and DHA (Table D-3, Appendix) and Walleye EPA (Table D-4, Appendix) still displayed significant among-lake differences after LS means were calculated.

3.6.2 Differences among Eco-regions

As among-lake patterns in lakes appeared to be related to eco-region, one-way ANOVAs were used to test whether these patterns were significant. LS means were compared among eco-regions for concentrations of Lake Whitefish TFA, Northern Pike EPA and DHA, Walleye EPA, and mercury for all species. Arithmetic means were used for all other fatty acid groups.

Mean concentrations of n-6 PUFAs and DHA in Lake Whitefish were significantly different among eco-regions (one-way ANOVA, $F_{>1,\,64} \geq 4.04$, $p \leq 0.049$; Figure 8). For both of these fatty acid groups, mean concentrations were highest in the HRL lakes, and significantly lower in Trout Lake. When considering concentrations of n-6 PUFAs, the HP lakes were not significantly different from the HRL lakes; both were higher than concentrations in Trout

Lake. Concentrations of TFA, n-3 PUFAs, and EPA in Lake Whitefish were not significantly different among eco-regions (ANOVA, $F_{\geq 1,64} \geq 0.972$, $p \geq 0.056$; Figure 8).

For Northern Pike, every fatty acid group differed significantly among eco-regions, and in general displayed the opposite pattern from Lake Whitefish. Mean concentrations of TFA, n-3 PUFAs, DHA, and EPA were significantly higher in Trout Lake and lower in the HRL lakes (one-way ANOVA, $F_{\geq 1,80} \geq 12.07$, $p < 0.001$; Figure 9). Concentrations of TFA and EPA in the HP lakes were not significantly different from Trout Lake, meaning that the mean concentrations were higher than the HRL lakes. The exception to this general trend was n-6 PUFAs; mean concentrations were highest in the HP lakes, but significantly lower in the HRL lakes and Trout Lake (ANOVA, $F_{2, 103}=10.5$, $p<0.001$).

Walleye were not captured in the HP lakes, and thus comparisons between eco-regions were limited to lakes in the HRL and Trout Lake. Mean concentrations of n-6 PUFAs were significantly higher in HRL lakes than in Trout Lake (ANOVA, $F_{1, 77}=7.42$, $p=0.008$; Figure 10), whereas mean concentrations of n-3 PUFAs and DHA were higher in Trout Lake and lower in HRL lakes (ANOVA, $F_{1, 54} \geq 6.47$, $p=0.014$). Concentrations of TFA and EPA were not significantly different between eco-regions in Walleye (ANOVA, $F_{\geq 1,78} \geq 0.041$, $p \geq 0.11$).

In contrast to the variation seen in among-lake patterns of fatty acid concentrations, Lake Whitefish, Northern Pike, and Walleye all displayed the same patterns in size-adjusted mean Hg concentrations among eco-regions (Figure 11). For all three species, mean Hg concentrations were highest in the HRL lakes and significantly lower in Trout Lake (ANOVA, $F_{\geq 1, 71} \geq 6.99$, $p \leq 0.001$). For Lake Whitefish and Northern Pike, the HP lakes displayed intermediate concentrations of mercury (Tukey's Test, $p<0.05$; Figure 11).

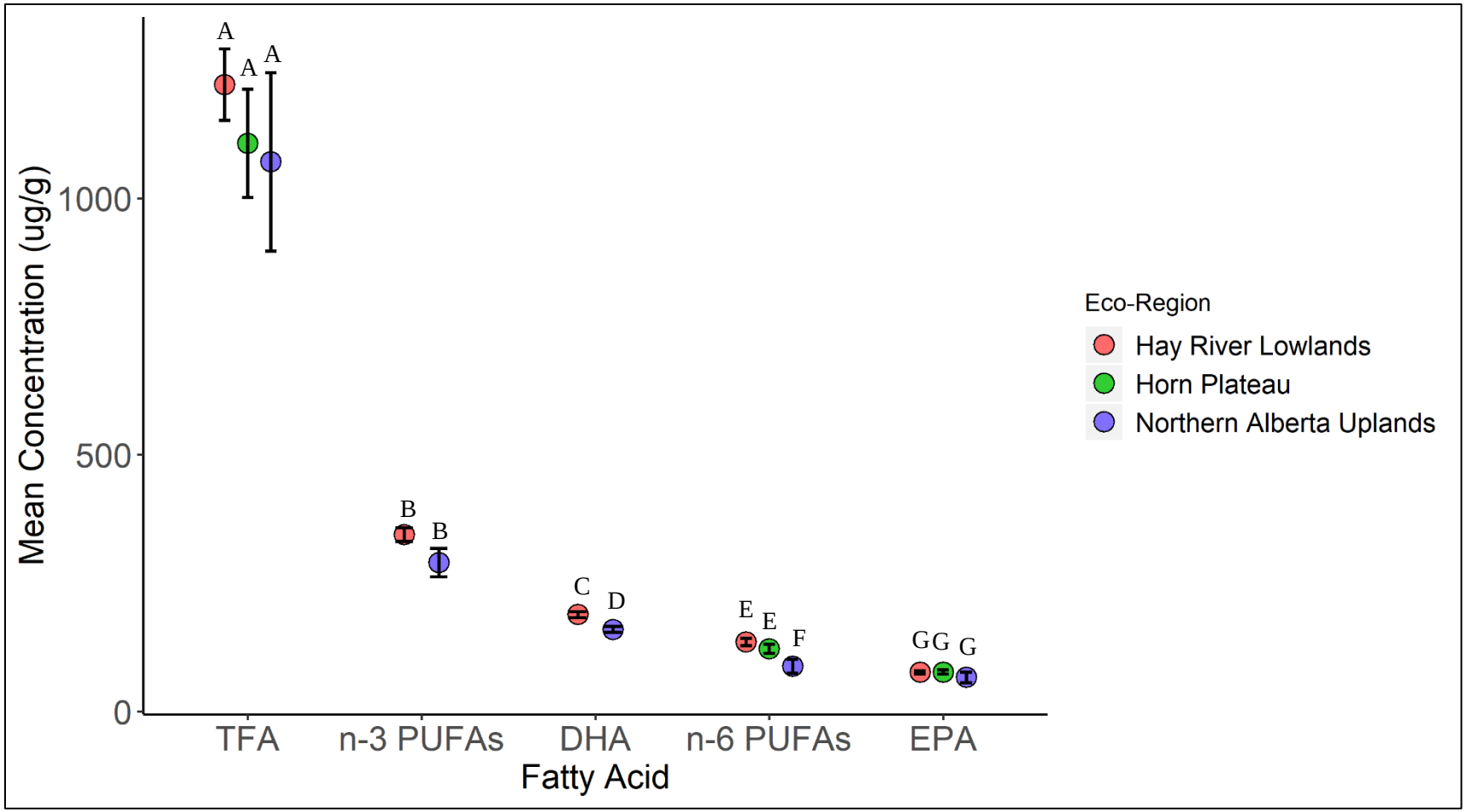

Figure 8. Mean concentrations of TFA, n-3 PUFAs, DHA, n-6 PUFAs, and EPA in Lake Whitefish (mg/100g dry weight; ±SE), comparing mean concentrations of fatty acids in fish caught in lakes located in the HRL, HP, and NAU eco-regions. Significant pairwise differences are indicated by letters (Tukey's test, $p<0.05$). All values were back-transformed, with the exception of DHA. TFA values represent LS means calculated at a standardised size of 450mm.

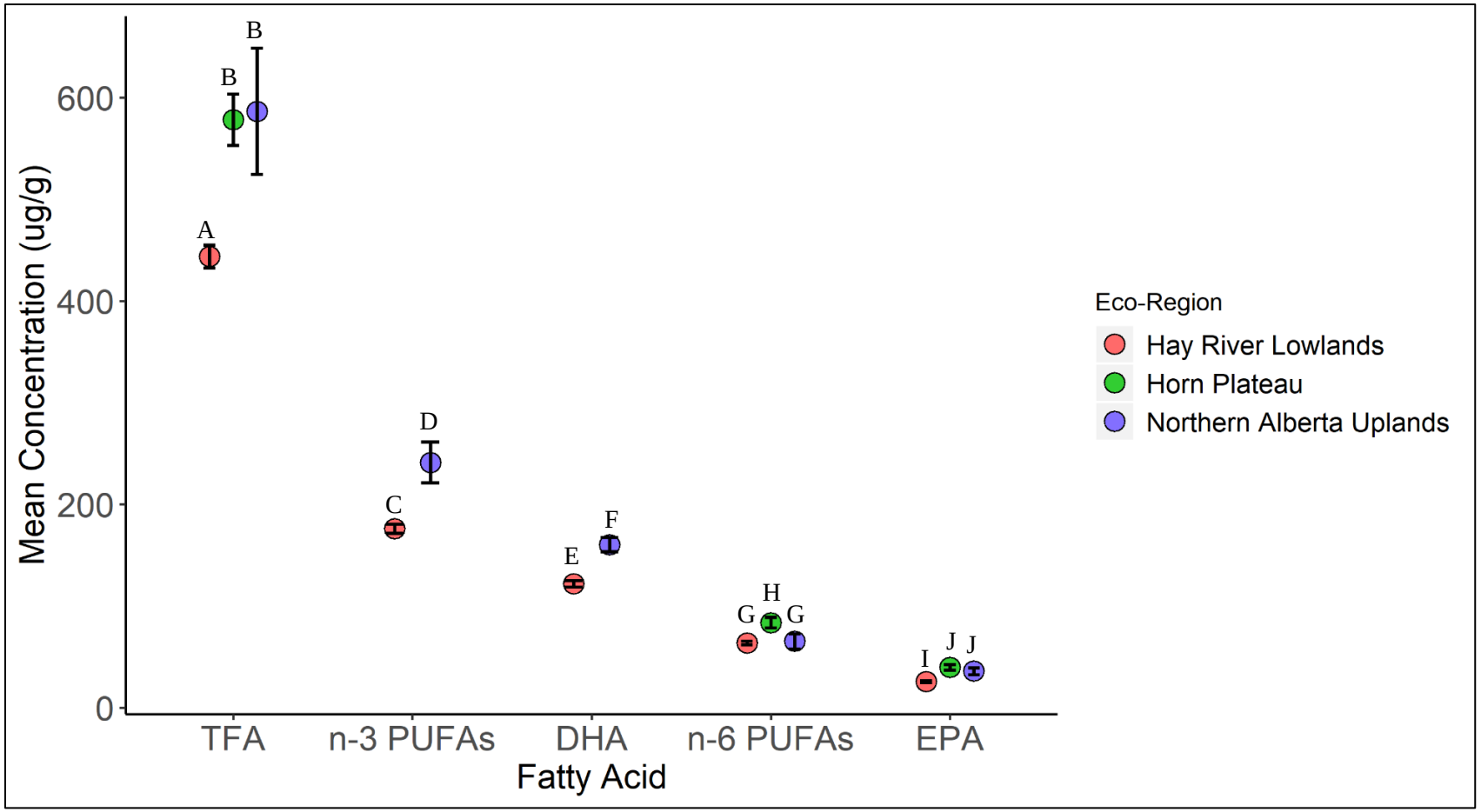

Figure 9. Mean concentrations of TFA, n-3 PUFAs, DHA, n-6 PUFAs, and EPA in Northern Pike (mg/100g dry weight; ±SE), comparing mean concentrations of fatty acids in fish caught in lakes located in the HRL, HP, and NAU eco-regions. Significant pairwise differences are indicated by letters (Tukey's test, $p<0.05$). All values were back-transformed, with the exception of DHA. EPA and DHA values represent LS means calculated at a standardised log age = 0.75 and size of 650mm, respectively.

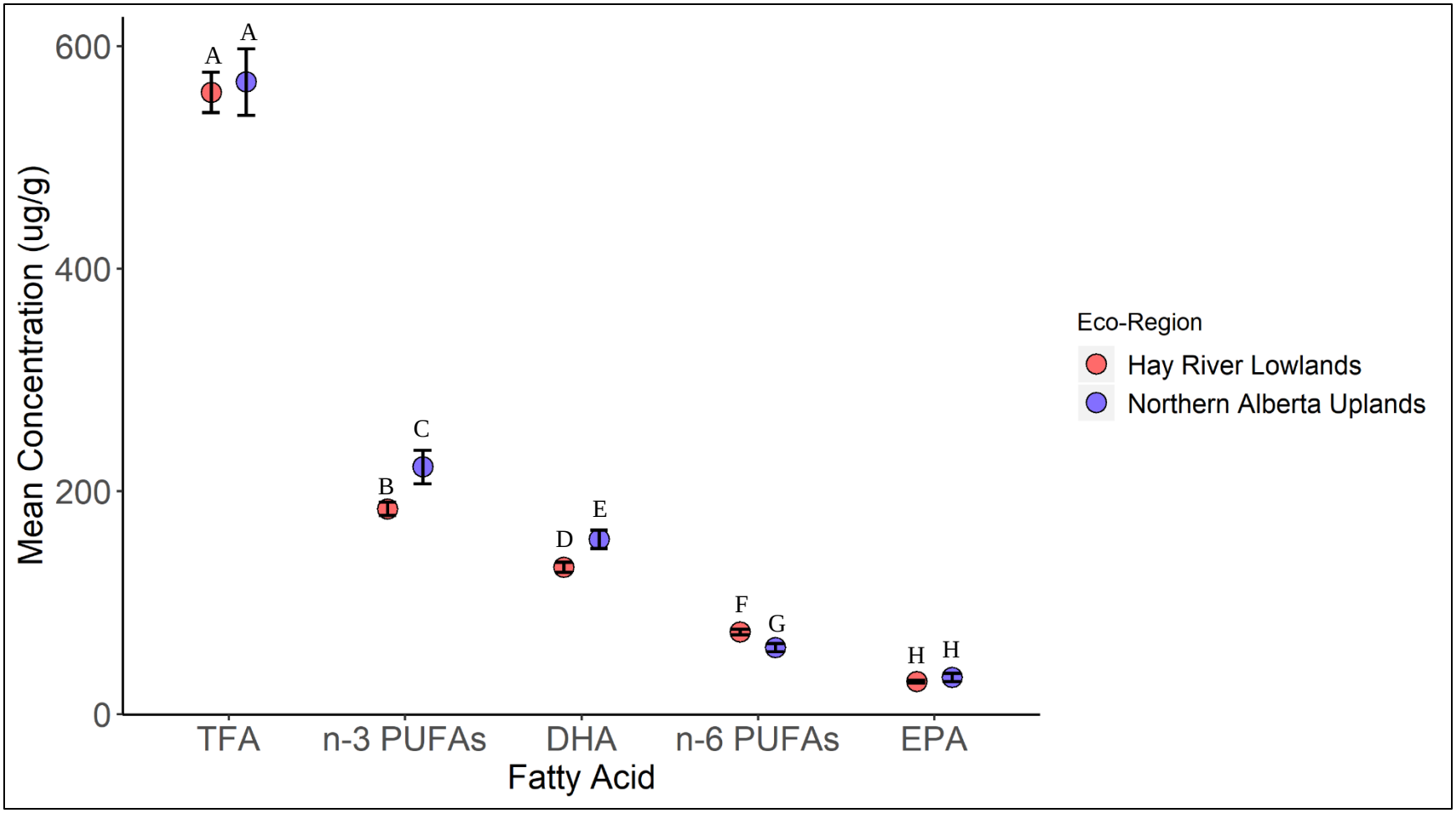

Figure 10. Mean concentrations of TFA, n-3 PUFAs, DHA, n-6 PUFAs, and EPA in Walleye (mg/100g dry weight; ±SE), comparing mean concentrations of fatty acids in fish caught in lakes in the HRL and NAU eco-regions. Significant pairwise differences are indicated by letters (Tukey's test, $p<0.05$). All values were back-transformed, with the exception of DHA. EPA values represent LS means calculated at a standardised size of 450mm.

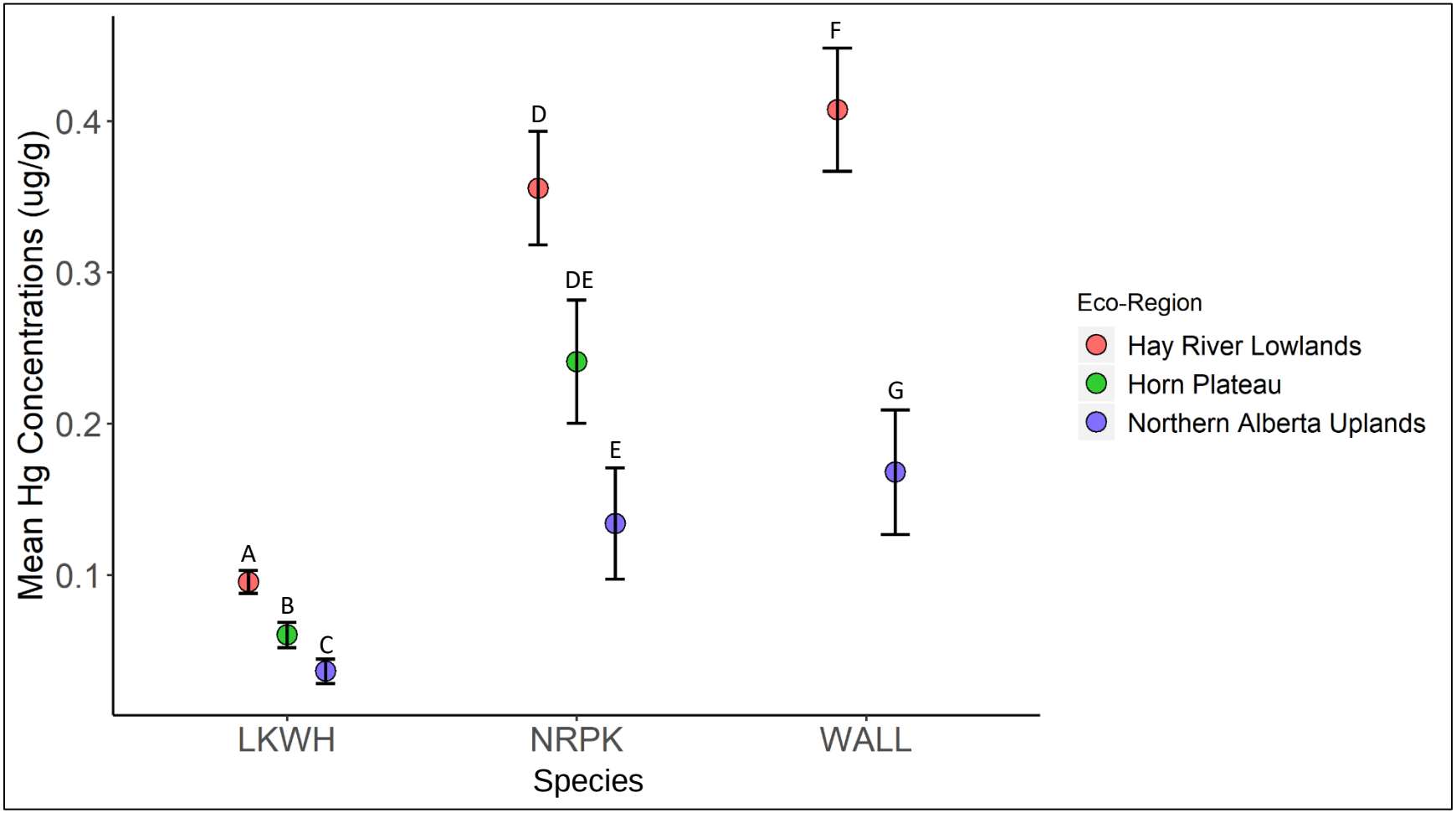

Figure 11. Mean concentrations of Hg (µg/g wet weight; ±SE) in Lake Whitefish, Northern Pike, and Walleye, comparing mean concentrations of mercury in fish caught in lakes in the HRL, HP, and NAU eco-regions. Significant pairwise differences are indicated by letters (Tukey's test, $p<0.05$). Values represent back-transformed LS means, calculated at 450 mm for LKWH and WALL, and 650 mm for NRPK.

3.6.3 Environmental PCAs

Several PCAs were created from the watershed characteristics (Table 14) and measured water chemistry (Table 15) variables of the study lakes. In the PCA including all watershed parameters, PC1 explained 55.18% of the variation, and PC2 explained 31.19% of the variation (Figure E-1, Appendix). PC1 and PC2 combined explained 86.37% of the variation. PC1 was influenced most strongly by total channel length (the longest channel measured in km, not including the flow path through a lake). Lakes with positive loadings (Trout, Tathlina, and Kakisa) had the longest channel lengths, as well as the largest watershed areas and individual lake areas. PC2 separated lakes along a gradient of the ratio between total channel length and watershed area ratio. Lakes with negative loadings (Big Island and Willow) had the highest ratios, or a long channel length relative to the watershed size.

In the PCA of all water chemistry variables, PC1 explained 56.69% of the variation, and PC2 explained 14.27% of the variation (Figure E2, Appendix). Combined, PC1 and PC2 explained 70.96% of the variation. PC1 was influenced most strongly by conductivity and separated the HRL lakes (positive loadings) from the HP lakes and Trout Lake (negative loadings). Lakes with positive loadings had higher conductivity and higher concentrations of ions, such as Ca^{2+}, Na^{+}, Cl^{-}, and bicarbonate. PC2 separated lakes along a gradient of the carbon freshness index. Lakes with negative loadings on PC2 (Willow, Trout, Ekali, Tathlina, and Kakisa) had higher carbon freshness index scores, indicating that there is a greater proportion of fresh, autochthonous carbon in these lakes.

In the PCA of EEMs data, PC1 explained 56.26% of the variation, and PC2 explained 17.15% of the variation (Figure E-3, Appendix). Combined, PC1 and PC2 explained 73.42% of the variation. PC1 was influenced most strongly by the fluorescence index (FI 2005). Whereas all lakes had FI 2005 values that indicated importance of both terrestrial and aquatic sources of carbon, the HP lakes and Trout Lake, as well as Kakisa Lake, had more positive

Table 14. Calculated watershed characteristics from nine study lakes in the Dehcho region.

Parameter	Big Island	Ekali	Gargan	Kakisa	McGill	Sanguez	Tathlina	Trout	Willow
Watershed Area (km)	113	180	73.1	15809	983	107	11606	5893	1249
Lake Area Sum (km^2)	25.8	9.03	3.44	1335	17.1	5.06	923	657	231
Lake Area to Watershed Area Ratio (Sum)	0.229	0.050	0.047	0.084	0.017	0.047	0.080	0.111	0.185
Lake Perimeter Sum (km)	162	71.8	34.3	5654	155	47.9	4732	1519	1347
Lake Perimeter (Sum) to Watershed Area Ratio	1.43	0.400	0.470	0.360	0.160	0.450	0.410	0.260	1.08
Riparian Area Sum (km^2)	19.6	7.08	3.50	849	34.3	5.44	741	369	170
Lake Area Ind (km^2)	18.2	1.88	1.10	336	2.32	1.59	565	500	129
Lake Area to Watershed Area Ratio (Ind)	0.161	0.010	0.015	0.021	0.002	0.015	0.049	0.085	0.103
Lake Perimeter Ind (km)	35.1	12.6	10.0	128	12.4	12.7	161	173	151
Lake Perimeter to Watershed Area Ratio (Ind)	0.311	0.070	0.137	0.008	0.013	0.119	0.014	0.029	0.121
Riparian Area Ind (km^2)	1.67	0.630	0.500	5.17	0.560	0.570	7.85	8.55	6.85
Longest Flow Path (km)	22.8	37.8	21.9	499	104	30.5	442	241	71.6
Longest Flow Path to Watershed Area Ratio	0.200	0.210	0.300	0.030	0.110	0.290	0.040	0.040	0.060
Total Channel Length (km)	222	65.4	31.6	8318	335	50.9	7306	3871	1763
Total Channel Length to Watershed Area Ratio	1.96	0.360	0.430	0.530	0.340	0.480	0.630	0.660	1.41
Min Elevation (masl)	752	226	233	204	209	226	258	291	608
Max Elevation (masl)	841	328	324	888	544	324	888	808	839
Mean Elevation (masl)	783	274	283	409	355	278	457	585	705
Max Slope (degree)	70.9	72.6	72.6	87.8	84.0	72.6	87.8	87.4	87.5
Mean Slope (degree)	2.49	4.36	2.33	4.17	5.22	3.19	4.27	3.34	1.79
Min TWI	-1.06	-1.16	-1.16	-3.27	-2.26	-1.16	-3.27	-3.10	-3.14
Max TWI	12.1	107	107	107	107	107	107	107	12.1
Mean TWI	3.25	3.23	3.73	3.03	2.97	3.49	3.01	3.24	3.59

Table 15. Measured water chemistry variables from nine study lakes in the Dehcho region.

Parameter	Big Island	Ekali	Gargan	Kakisa	McGill	Sanguez	Tathlina	Trout	Willow
Total Phosphorus (µg/L)	10.0	15.0	15.0	60.0	9.00	12.0	13.0	10.0	9.00
Total Nitrogen (µg/L)	282	587	686	523	448	524	678	318	320
Secchi Depth (m)	4.50	2.60	2.00	1.10	2.40	3.25	1.75	1.80	3.15
Chlorophyll-a (µg/L)	3.01	2.79	3.69	4.00	1.00	1.97	0.42	3.10	2.33
pH	7.90	8.10	7.90	8.20	8.00	7.90	8.20	8.10	8.00
Alkalinity ($CaCO_3$ mg/L)	46.1	119	83.9	106	111	104	143	69.8	66
Conductivity (µS/cm)	97.0	239	190	240	238	230	295	146	139
Bicarbonate (HCO_3 mg/L)	56.2	145	102.4	129	136	126	174	85.1	80.5
Cl^- (ppm)	0.091	2.50	1.32	1.29	1.61	2.72	1.62	0.44	0.107
NO_3^- (mg/L)	0.228	-	0.317	0.238	0.176	0.137	0.195	0.275	0.342
SO_4^{2-} (mg/L)	3.56	12.0	11.2	19.4	12.4	12.3	15.0	5.463	6.73
Na^+ (ppm)	1.00	4.50	2.80	5.40	5.30	4.50	6.60	2.50	1.70
K^+ (ppm)	2.60	1.70	1.30	1.80	1.00	2.90	1.80	1.50	2.10
Mg^{2+} (ppm)	5.00	8.90	7.20	9.20	9.30	8.60	10.7	5.40	7.20
Ca^{2+} (ppm)	19.0	46.7	37.9	45.3	44.8	47.2	59.7	29.2	26.3
UV_{254} (absorbance/m)	0.263	0.538	0.717	0.459	0.595	0.524	0.694	0.415	0.284
Dissolved Organic Carbon (µg/L)	10.3	16.0	31.5	16.4	17.0	25.6	20.5	12.7	12.0
Dissolved Inorganic Carbon (µg/L)	10.5	22.1	18.1	23.1	24.5	21.9	30.7	25.7	14.4
FI 2002	1.07	1.14	1.12	1.13	1.13	1.13	1.13	1.09	1.09
FI 2005	1.43	1.45	1.46	1.45	1.47	1.46	1.49	1.43	1.426
Freshness	0.656	0.663	0.634	0.689	0.624	0.65	0.656	0.667	0.684
HIX 1999	0.628	0.655	0.683	0.651	0.711	0.656	0.679	0.639	0.63
HIX 2002	0.386	0.396	0.406	0.394	0.416	0.396	0.405	0.39	0.386
SUVA ($UV_{254}m^{-1}$/DOC mg/L)	2.54	3.37	2.28	2.81	3.49	2.05	3.38	3.27	2.36

loadings on PC1; these lakes had lower FI 2005 indices, which indicates that allochthonous carbon was more important than in the other lakes. However, these lakes also had higher freshness index values, as well as lower DOC concentrations and UV_{254} values, which would suggest higher lake autochthony. PC2 separates lakes along a spectrum of SUVA. Lakes with negative loadings (McGill and Tathlina) have high SUVA values, indicating higher aromatic compounds in the DOM, or more allochthonous carbon.

In the PCA containing water chemistry variables (excluding EEMs data), PC1 explained 66.93% of the variation, and PC2 explained 12.00% of the variation (Figure E-4, Appendix). Combined, PC1 and PC2 explained 78.93% of the variation. PC1 was influenced most strongly by conductivity. Lakes with positive loadings (the HP lakes and Trout Lake, as well as Gargan Lake) had lower conductivity and generally lower ion concentrations. PC2 separated lakes along a gradient of secchi depth; it mostly separated Kakisa Lake (which had the shallowest secchi depth) from the rest of the study lakes, which likely reflected higher primary productivity in Kakisa Lake.

When all watershed and water chemistry variables were combined into a PCA, PC1 explained 44.98% of the variation, and PC2 explained 28.74% of the variation. Combined, PC1 and PC2 explained 73.72% of the variation (Figure E-5, Appendix). PC1 was influenced most strongly by concentration of sodium ions. Consistent with the results of the other PCAs, the HP lakes and Trout Lake, with lower ion concentrations and negative loadings, separated from the HRL lakes (positive loadings). PC2 separated lakes along a gradient of maximum watershed elevation; lakes with positive loadings (Big Island, Willow, Trout, Kakisa, and Tathlina) had a maximum elevation over 800 masl, whereas lakes with negative loadings have maximum elevations under 600 masl.

Based on the concentrations of chlorophyll-a, TP, and TN measured, lakes in the study area can be classified as eutrophic, mesotrophic, and oligotrophic (Burns et al. 2009), although the classification varies depending on which parameter(s) is/are used. When using chlorophyll-a concentrations as the defining metric, McGill, Sanguez, and Tathlina are oligotrophic (Chl-a<2

µg/L), whereas the remaining lakes are mesotrophic (Chl-a 2-5 µg/L; Burns et al. 2009). When considering TP, Kakisa is eutrophic (TP > 20 µg/L), whereas all other lakes are mesotrophic (TP 9-20 µg/L; Burns et al. 2009). Finally, if using TN to classify lakes, Big Island, Trout, and Willow are mesotrophic (157-337 µg/L), while the rest of the lakes are eutrophic (337-725 µg/L; Burns et al. 2009).

3.6.4 Among-lake differences in Lake Whitefish fatty acids

Significant among-lake differences in mean concentrations of TFA in Lake Whitefish were driven by a pairwise difference between McGill Lake and Kakisa Lake (Tukey's test p= 0.029; Figure 12); none of the other lakes were significantly different from each other. McGill Lake is a humic, lower-productivity lake, whereas Kakisa Lake had the highest concentrations of chlorophyll-*a* of any lake sampled (Table 15). Exploratory linear regressions between concentrations of TFA in Lake Whitefish and measured water chemistry and watershed parameters resulted in two significant variables that were included in the stepwise regression: $\log_{10}$ total phosphorus (p=0.001), and PC2 of the PCA including all water chemistry variables (p=0.021). Total fatty acid concentrations in Lake Whitefish were best predicted by lake total phosphorus concentration; mean TFA increased with increasing $\log_{10}$ total phosphorus (Stepwise Regression, $F_{1,8}$=28.2, p=0.001, R^2_{adj}=0.773; Figure 12), indicating that Lake Whitefish had higher concentrations of TFA in lakes with higher nutrient concentrations (and higher primary productivity, as chlorophyll-a and TP were positively correlated; see the PCA of all water chemistry variables in Figure E2, Appendix)**.** It should be noted that if Kakisa Lake is removed from the model, there is still a general positive relationship between TFA and TP, but the relationship is no longer significant.

Exploratory linear regressions between concentrations of omega-6 PUFAs in Lake Whitefish and measured water chemistry and watershed parameters resulted in only one significant variable. N-6 PUFA concentrations in Lake Whitefish were best explained by PC2 of the excitation-emissions matrix data (EEMs data; Stepwise Regression, $F_{1,8}$=28.238, p=0.001, R^2_{adj}=0.773; Figure 13). PC2 separated lakes along a gradient of lake-specific ultraviolet absorbance (SUVA; Figure E-3, Appendix), and n-6 PUFAs in Lake Whitefish increased with increasing PC2 scores,

indicating that Lake Whitefish had higher concentrations of n-6 PUFAs in lakes with lower SUVA (i.e., lakes with higher carbon quality).

Exploratory linear regressions between concentrations of DHA in Lake Whitefish and measured water chemistry and watershed parameters resulted in one significant variable that was included in the stepwise regression: the ratio of lake perimeter (sum of all lake perimeters) to watershed area (p=0.014). DHA concentrations in Lake Whitefish were best explained by the ratio of lake perimeter (the sum of all lake perimeters in a watershed) to watershed area (Stepwise Regression, $F_{1,6}$=13.944, p=0.014, R^2_{adj}=0.679; Figure 14), indicating that DHA is higher in lakes with less catchment inputs.

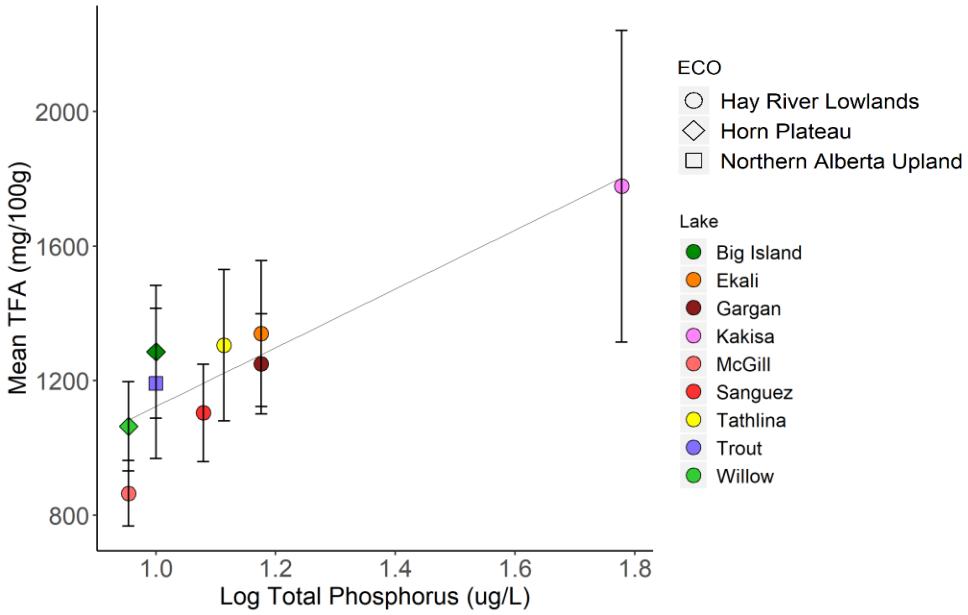

Figure 12. Relationship between back transformed TFA concentrations (mg/100g dry weight; ±SE) in Lake Whitefish tissue and $\log_{10}$ TP concentrations (µg/L) in surface water. Values represent LS means calculated at a standardised FL=450mm.

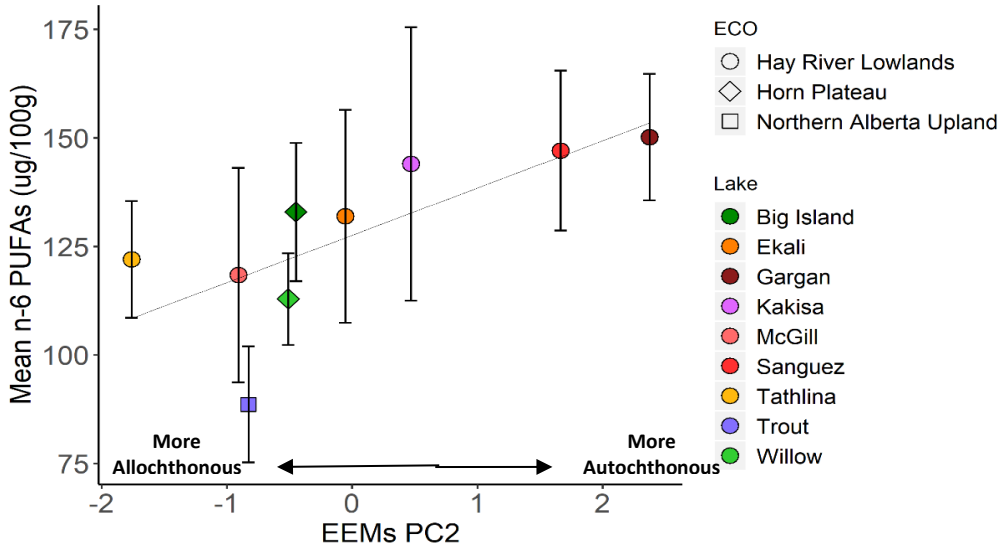

Figure 13. Relationship between mean n-6 PUFA concentrations in Lake Whitefish muscle tissue (mg/100g dry weight; ±SE) and axis 2 (PC2) from the PCA performed on EEMs data. Values represent back-transformed mean n-6 PUFA concentrations.

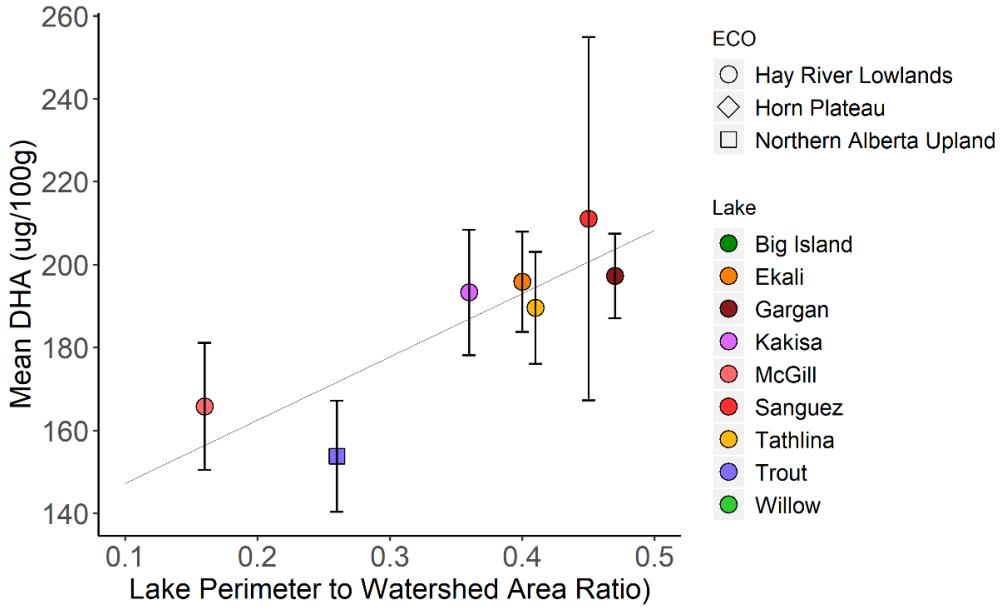

Figure 14. Relationship between mean DHA concentrations in Lake Whitefish muscle tissue (mg/100g dry weight; ±SE) and the ratio of lake perimeter (all lakes in watershed combined) to watershed area.

3.6.5 Among-lake differences in Northern Pike fatty acids

Exploratory linear regressions between TFA concentrations in Northern Pike and measured water chemistry and watershed parameters resulted in a total of 23 significant variables that were included in the stepwise regression (Table 16). The best predictor of TFA in Northern Pike was UV_{254}; TFA decreased significantly as light absorption at 254nm (UV_{254}) increased (Stepwise Selection, $F_{1,8}$=27.988, p=0.001, R^2_{adj}=0.771; Figure 15). This finding indicated that Northern Pike had higher concentrations of TFA in their tissue in lakes that had relatively more labile, likely autochthonous carbon.

Table 16. Watershed and water chemistry parameters significantly related to Northern Pike TFA concentrations.

Stepwise Parameters	Indicator	Direction	p-value
UV_{254}	Carbon Quality	**-**	**0.001**
EEMs PC 1		+	0.001
FI 2005		-	0.002
Log_{10} DOC		-	0.002
TN		-	0.004
HIX 1999		-	0.009
HIX 2002		-	0.010
Log Ratio DOC to Chl-a		-	0.010
All Environmental Parameters PC1	Catchment Input	-	0.004
Ca^{2+}		-	0.007
Conductivity		-	0.008
Mg^{2+}		-	0.012
Bicarb		-	0.014
Alkalinity		-	0.014
Environmental Parameters (No EEMS) PC1		+	0.019
Environmental and Watershed Parameters PC1		-	0.031
Na^{+}		-	0.034
Cl^{-}		-	0.036
Individual Lake Area to Watershed Area Ratio	Watershed Characteristics	+	0.006
Sum of Lake Areas to Watershed Area Ratio		+	0.008
Mean Watershed Elevation		+	0.010
Total Channel Length to Watershed Area		+	0.015
Watershed PC2		-	0.024

Exploratory linear regressions between concentrations of n-6 PUFAs in Northern Pike and measured water chemistry and watershed parameters resulted in a total of three significant variables that were included in the stepwise regression. These included the ratio of lake perimeter (sum of all lakes in watershed) to watershed area (p=0.008), fluorescence index (FI 2001; p=0.012), and the concentration of calcium ions (p=0.026). Concentrations of total n-6 PUFAs in Northern Pike were best predicted by the ratio of lake perimeter to watershed area

($F_{1,6}$=9.964, p=0.025 , R^2_{adj}=0.599; Figure 16); as the ratio increased, so did n-6 PUFA concentrations, indicating that n-6 PUFAs increase with decreasing catchment inputs.

Exploratory linear regressions between concentrations of n-3 PUFAs in Northern Pike and measured water chemistry and watershed parameters resulted in two significant variables that were included in the stepwise regression: chloride concentrations (p=0.033), an indicator of catchment influence, and total nitrogen concentration (p=0.045). Total n-3 PUFA concentrations in Northern Pike were best explained by a negative relationship with lake chloride concentrations (Stepwise Regression, $F_{1,6}$=8.526, p=0.033 , R^2adj=0.630; Figure 17), indicating that Northern Pike had lower concentrations of n-3 PUFAs in lakes that were more influenced by their catchment (i.e., likely more allochthonous).

Exploratory linear regressions between concentrations of EPA in Northern Pike and measured water chemistry and watershed parameters resulted in 19 significant variables that were included in the stepwise regression (Table 17).

Table 17. Watershed and water chemistry parameters significantly related to Northern Pike EPA concentrations.

Stepwise Parameters	Indicator	Direction	p-value
Cl^-	Catchment Input	-	<0.001
Ca^{2+}		-	0.008
All Environmental Parameters PC1		-	0.008
Conductivity		-	0.014
Bicarbonate		-	0.016
Alkalinity		-	0.016
Environmental Parameters (No EEMs) PC1		+	0.021
Mean Watershed Elevation	Watershed Characteristics	+	0.002
Sum of Lake Areas to Watershed Area Ratio		+	0.004
Watershed PC 2		-	0.006
Individual Lake Area to Watershed Area Ratio		+	0.009
Total Channel Length to Watershed Area		+	0.010
Environmental and Watershed Parameters PC2		+	0.036
EEMs PC1	Carbon Quality	+	0.003
UV_{254}		-	0.005
FI 2002		-	0.009
TN		-	0.010
Log DOC		-	0.01767
Log Ratio DOC to Chl-a		-	0.027

Following the same pattern as TFA and n-3 PUFAs, Northern Pike EPA concentrations decreased significantly as chloride concentrations (Figure 18a) and light absorption at 254nm

(UV_{254}; Figure 18b) increased ($F_{2,8}$=41.244, p<0.001 , R^2_{adj}=0.910;). Northern Pike had higher concentrations of EPA in more autochthonous lakes that had less inputs from the catchment.

Exploratory linear regressions between concentrations of DHA in Northern Pike and measured water chemistry and watershed parameters resulted in two significant variables that were included in the stepwise regression: concentrations of chloride (p=0.021) and total nitrogen (p=0.023). Northern Pike DHA concentrations decreased significantly as concentrations of both chloride (Figure 19a) and total nitrogen (Figure 19b) increased ($F_{2,8}$=41.244, p<.001, R^2_{adj}=0.910), indicating that DHA concentrations in Northern Pike were higher in more autochthonous lakes with less catchment influence.

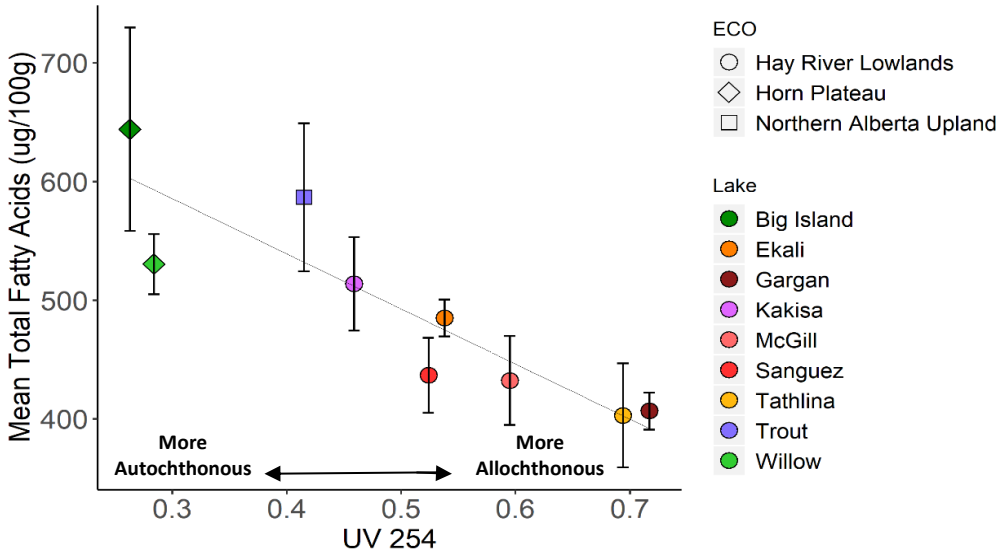

Figure 15. Relationship between TFA concentrations in Northern Pike muscle tissue (mg/100g dry weight; ±SE) and UV_{254} (UV absorbance/m). Values represent back-transformed mean total fatty acid concentrations (±SE).

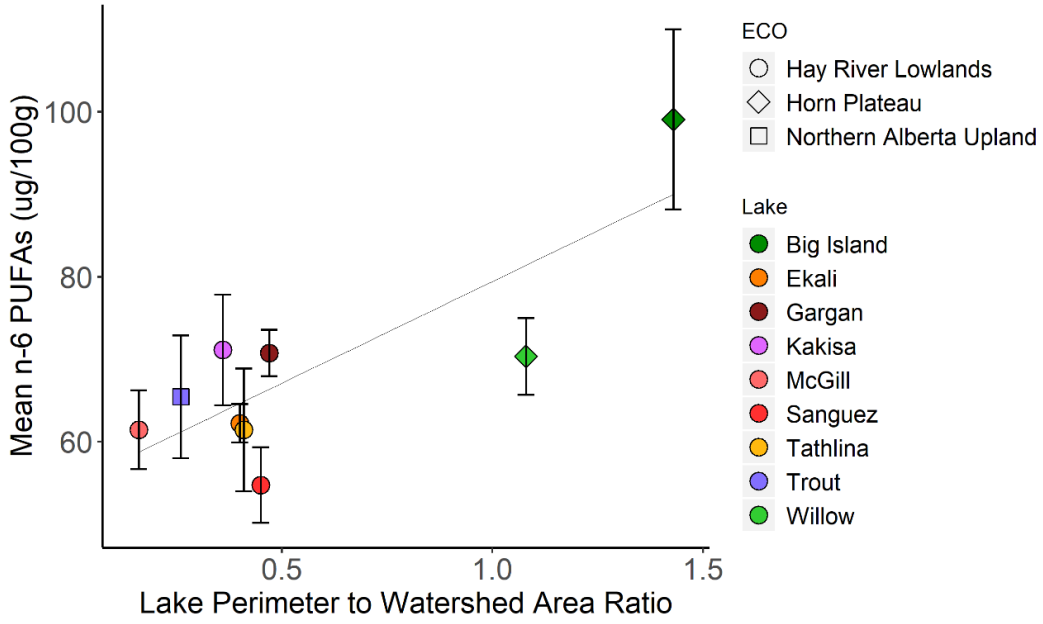

Figure 16. Relationship between n-6 PUFA concentrations in Northern Pike muscle tissue (mg/100g dry weight; ±SE) and the ratio of lake perimeter (sum of all lake perimeters in watershed) to watershed area. Values represent back-transformed mean n-6 PUFA concentrations (±SE).

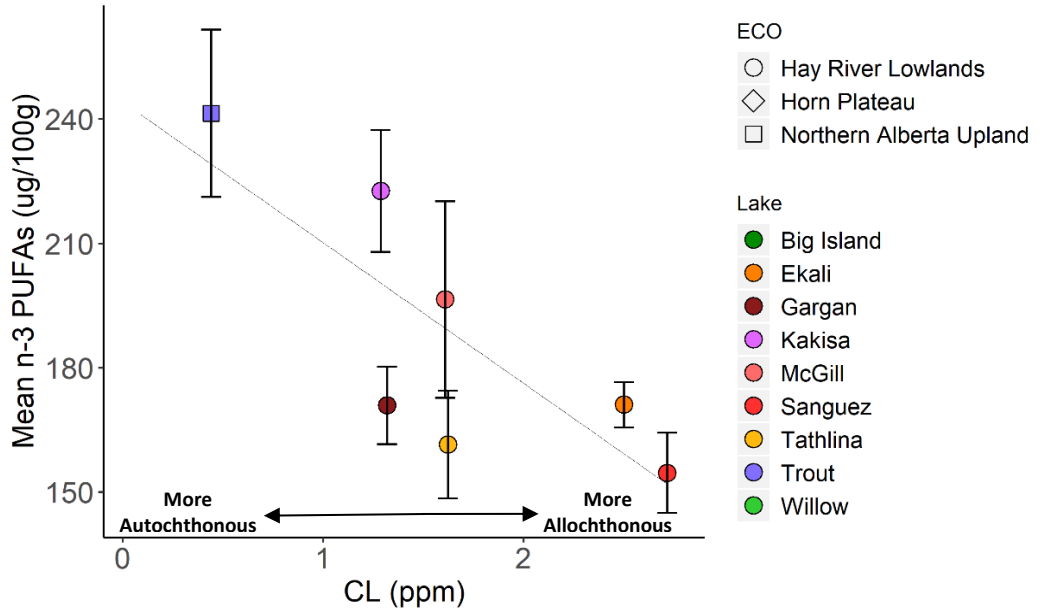

Figure 17. Relationship between n-3 PUFA concentrations in Northern Pike muscle tissue (mg/100g dry weight; ±SE) and lake chloride concentrations (ppm). Values represent back-transformed mean total n-3 PUFA concentrations (±SE).

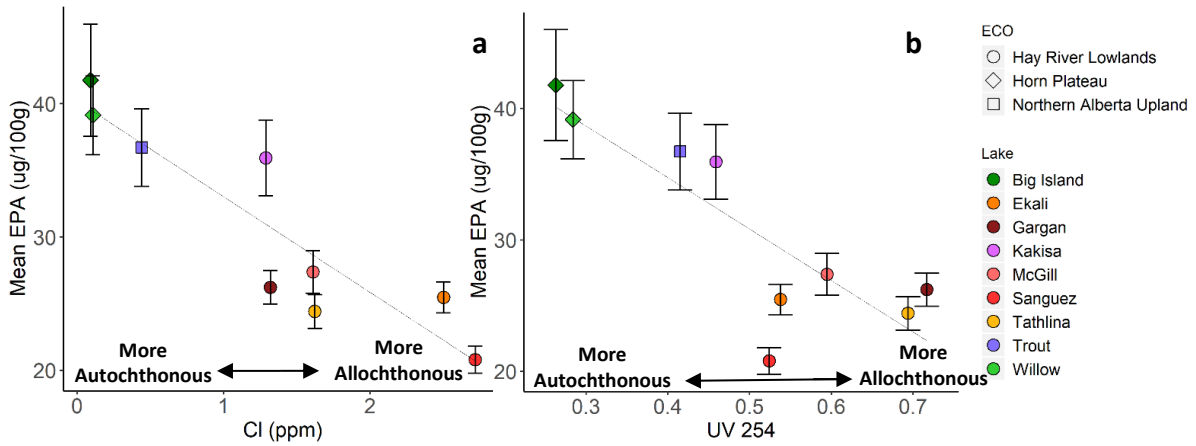

Figure 18. Relationship between EPA concentrations in Northern Pike muscle tissue (mg/100g dry weight; ±SE) and a) lake chloride concentrations (ppm), and b) UV_{254} (UV absorbance/m). Values represent back-transformed LS means calculated at a standardised FL= 650mm (±SE).

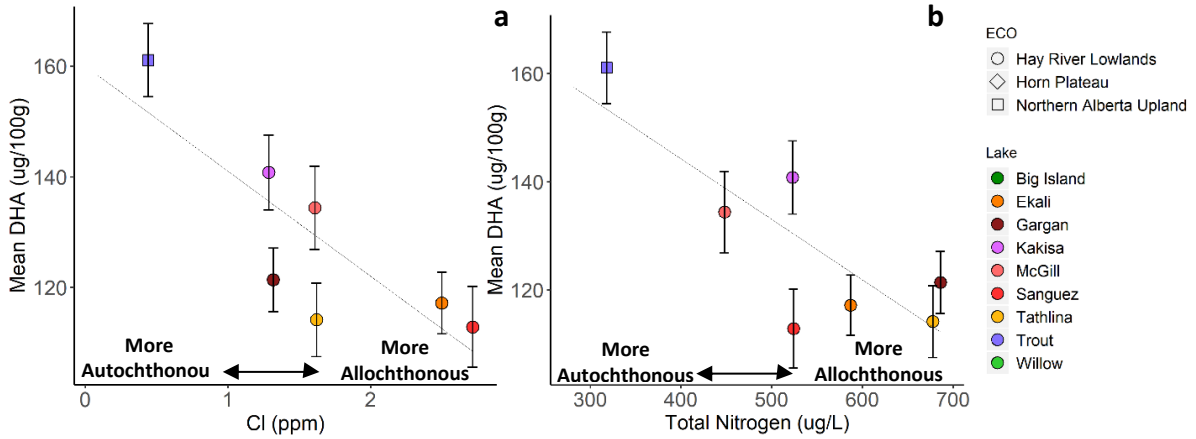

Figure 19. Relationship between DHA concentrations in Northern Pike muscle tissue (mg/100g dry weight; ±SE) and a) lake chloride concentrations (ppm), and b) total nitrogen concentrations (µg/L). Values represent least-squares means calculated at a standardised FL= 650mm.

3.6.6 Among-lake differences in Walleye fatty acids

Exploratory linear regressions between concentrations of n-6 PUFAs in Walleye and measured water chemistry and watershed parameters resulted in two significant parameters that were included in the stepwise regression: concentrations of calcium (p=0.014) and total nitrogen (0.032). The best predictor of n-6 PUFA concentrations in Walleye was calcium; concentrations

of n-6 PUFAs in Walleye increased significantly with increasing lake calcium concentrations (Stepwise Regression, F1,6=16.8, p=0.015, R2 adj=0.760; Figure 20).

Exploratory linear regressions between concentrations of n-3 PUFAs in Walleye and measured water chemistry and watershed parameters resulted in three significant variables that were included in the stepwise regression: concentrations of DOC (p=0.007), chloride (p=0.012), and mean watershed elevation (p=0.049). Concentrations of n-3 PUFAs in Walleye decreased significantly as concentrations of chloride (Figure 21a) and dissolved organic carbon (Figure 21b) increased (Stepwise Regression, $F_{1,6}$=81.217, p=0.002, R^2_{adj}=0.970), indicating that increased catchment inputs and assumed allochthonous carbon resulted in decreased n-3 PUFAs in Walleye.

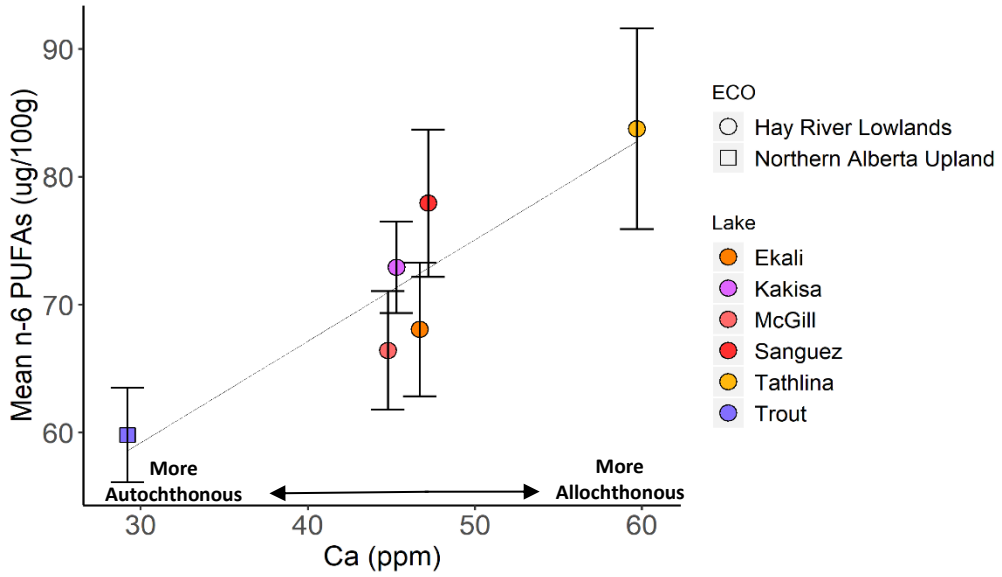

Figure 20. Relationship between mean n-6 PUFA concentrations in Walleye muscle tissue (mg/100g dry weight; ±SE) and lake calcium concentrations (ppm). Values represent back transformed mean n-6 PUFAs (±SE).

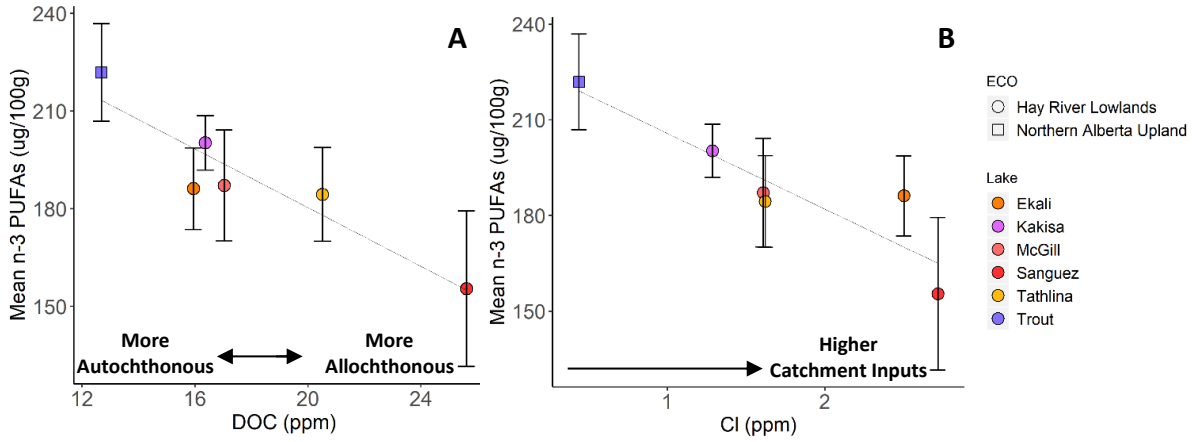

Figure 21. Relationship between n-3 PUFA concentrations in Walleye muscle tissue (mg/100g dry weight; ±SE) and A) lake chloride concentrations (ppm), and B) dissolved organic carbon (ppm). Values represent back-transformed mean n-3 PUFA concentrations (±SE).

A summary of the models that best explain variation in each fatty acid group and species can be found in Table 18.

Table 18. Results of stepwise regressions indicating the best environmental predictors for each fish fatty acid group. Brackets indicate whether the relationship is positive (+) or negative (-). *Indicates the sum of all lake perimeters in the watershed.

Species	Fatty Acid	Best Model	p-val	Adj R2	N Lakes
LKWH	Total FA	Log TP $_{(+)}$	0.001	0.773	9
	n-6 PUFAs	EEMs PC 2	0.026	0.463	9
	DHA	Lake Perimeter to Watershed Area Ratio* $_{(+)}$	0.005	0.655	7
NRPK	Total FA	UV $_{254\ (-)}$	0.001	0.771	9
	n-3 PUFAs	Cl^- $_{(-)}$	0.033	0.556	7
	n-6 PUFAs	Lake Perimeter to Watershed Area Ratio* $_{(+)}$	0.025	0.470	9
	EPA	Cl^- $_{(-)}$ + UV $_{254\ (-)}$	<0.0001	0.910	9
	DHA	Cl^- $_{(-)}$ + TN $_{(-)}$	0.002	0.937	7
Wall	n-3 PUFAs	DOC $_{(-)}$ + Cl^- $_{(-)}$	0.002	0.970	6
	n-6 PUFAs	Ca^{2+} $_{(+)}$	0.015	0.760	6

3.6.7 Predictors of TFA, n-6 PUFAs, and EPA: effects of using the restricted dataset

Using the dataset that was restricted to samples stored for less than one year, concentrations of TFA and EPA did not vary significantly among lakes in Lake Whitefish (ANOVA, $F_{5,64} \leq 1.450$, $p \geq 0.140$), but concentrations of n-6 PUFAs did differ significantly among lakes (ANOVA, $F_{>5,54} \leq 2.244$, $p=0.026$). Concentrations of TFA and EPA were significantly different among

lakes in Northern Pike (ANOVA, $F_{6, 75} \leq 9.709$, $p \leq 0.001$; Table 19), while there was no significant difference in n-6 PUFAs (ANOVA, $F_{6,75} \leq 1.605$, $p=0.159$). Similar to Lake Whitefish, concentrations of TFA in Walleye did not differ significantly among lakes (ANOVA, $F_{>5,54}=0.413$, $p=0.837$), but concentrations of n-6 PUFAs and EPA did differ significantly among lakes (ANOVA, $F_{5,64} \leq 3.743$, $p \leq 0.029$).

In general, among-lake differences in species-fatty acid groups were consistent between the datasets (Table 19). Exceptions included n-6 PUFAs in Northern Pike and EPA in Walleye. Concentrations of n-6 PUFAs in Northern Pike differed significantly among lakes using the full dataset, whereas they did not differ significantly among lakes when using the restricted dataset, likely due to reduced statistical power. By contrast, EPA concentrations in Walleye did not differ significantly among lakes using the larger dataset, but did differ significantly when using samples stored for less than one year. This appeared to reflect high intra-lake variability, likely in Sanguez lake; when some samples were removed from the dataset, among-lake differences became more pronounced.

Table 19. P-values obtained from ANOVA results investigating among-lake differences in each species-fatty acid group. P-values were calculated for both the dataset including all samples, and the dataset restricted to samples stored for less than one year. Bolded numbers indicate significant results, and degrees of freedom are listed in brackets.

		All Data (N=282)	**Samples Stored < One Year (N=193)**
Species	**Fatty Acid**	**Among Lake ANOVA**	**Among Lake ANOVA**
LKWH	Total FA	0.103 (8,102)	0.147 (5,64)
	n-6 PUFAs	**0.024** (8,102)	**0.029** (5,64)
	EPA	0.670 (8,102)	0.140 (5,64)
NRPK	Total FA	**<0.0001** (9,103)	**0.001** (6,75)
	n-6 PUFAs	**<0.0001** (9,103)	0.159 (6,75)
	EPA	**<0.0001** (9,103)	**<0.0001** (6,75)
Wall	Total FA	0.101 (5,78)	0.837 (5,54)
	n-6 PUFAs	**0.021** (5,78)	**<0.0001** (5,54)
	EPA	0.517 (5,78)	**0.006** (5,54)

Consistent with analyses run using the full dataset, TFA concentrations in Lake Whitefish were significantly, positively related to fork length (Stepwise regression, $F_{1,64}=8.811$, $p=0.004$), and TFA concentrations in Northern Pike and Walleye were not significantly related to any

biological predictor (Stepwise regression, $F_{1,54} \geq 1.212$, $p \geq 0.276$). Also consistent with the full dataset, EPA concentrations in Northern Pike and Walleye were significantly, negatively related to fork length (Stepwise regression, $F >_{1,54} \geq 13.150$, $p \leq 0.001$). Concentrations of n-6 PUFAs were not significantly related to any biological predictor (Stepwise regression, $F >_{1,54} \geq 0.598$, $p > 0.442$). LS means were calculated for Lake Whitefish TFA, and EPA in Northern Pike and Walleye at 450mm for Lake Whitefish and Walleye, and 650mm for Northern Pike. Differences among lakes were re-examined using a Tukey's test after calculating LS means. Although EPA concentrations in Walleye were significantly different among lakes when using data unadjusted for fork length, after calculating LS means, there were no significant pairwise differences among lakes. Therefore, analyses of abiotic predictors focused on Northern Pike TFA and EPA concentrations, as well as concentrations of n-6 PUFAs in Lake Whitefish and Walleye.

Results of exploratory linear regressions that were used to relate concentrations of n-6 PUFAs in Lake Whitefish to water chemistry and watershed parameters resulted in two significant variables that were included in the stepwise regression: TN ($p=0.017$), which is correlated with indicators of carbon quality, and mean watershed elevation ($p=0.043$), a watershed variable. The best predictor of n-6 PUFA concentrations in Lake Whitefish was TN. Concentrations of n-6 PUFAs in Lake Whitefish increased significantly as TN increased (Stepwise Regression, $F_{1,5}=9.971$, $p=0.034$, $R^2_{adj}=0.642$).

Exploratory linear regressions between concentrations of TFA in Northern Pike and measured water chemistry and watershed parameters resulted in seven significant variables that were included in the stepwise regression, which included indicators or correlates of carbon amounts and carbon quality: UV_{254} (0.003), FI 2005 (0.006), TN (0.009), PC1 of the EEMs PCA (0.01), DOC (0.021), and the $\log_{10}$ ratio of DOC to Chl-a (0.025), as well as watershed characteristics: PC1 of the PCA including EEMs data and water chemistry data (0.036). The best predictor of TFA concentrations in Northern Pike was UV_{254}. Concentrations of TFA in Northern Pike

decreased significantly as UV_{254} increased (Stepwise Regression, $F_{1,6}$=21.513, p=0.006, R^2_{adj}=0.774).

Exploratory linear regressions between concentrations of EPA in Northern Pike and measured water chemistry and watershed parameters resulted in nine significant variables that were included in the stepwise regression (Table 20). Results of a stepwise regression revealed that the best predictors of EPA concentrations in Northern Pike were PC 2 of the PCA including water chemistry data (but excluding EEMs data) and TN. Concentrations of EPA in Northern Pike decreased significantly with increasing concentrations of TN, and with increasing PC2 scores (associated with secchi depth) from the PCA that was conducted on water chemistry data (Stepwise Regression, $F_{2,6}$=31.672, p=0.004, R^2_{adj}=0.911).

Table 20. Watershed and water chemistry parameters significantly related to Northern Pike EPA concentrations.

Stepwise Parameters	**Indicator**	**p-value**
Cl^-	Catchment Input	0.012
Secchi Depth		0.027
Environmental and Watershed Parameters PC2	Watershed Characteristics	0.027
Longest Flow Path to Watershed Area Ratio		0.043
Max Watershed Slope		0.049
Environmental Parameters (No EEMs) PC1	Carbon Quality	0.008
EEMs PCA PC1		0.042
FI 2005		0.042

Exploratory linear regressions between concentrations of n-6 PUFAs in Walleye and measured water chemistry and watershed parameters resulted in 14 significant variables that were included in the stepwise regression (Table 21). Results of a stepwise regression revealed that concentrations of n-6 PUFAs in Walleye increased significantly as DOC increased (Stepwise Regression, $F_{1,3}$=135.463, p=0.007, R^2_{adj}=0.978).

Table 21. Watershed and water chemistry parameters significantly related to Walleye n-6 PUFA concentrations.

Stepwise Parameters	Indicator	p-value
Na^+	Catchment Input	0.020
All Environmental Parameters PC1		0.021
Ca^{2+}		0.022
Conductivity		0.031
Mg^{2+}		0.042
Bicarbonate		0.043
Alkalinity		0.043
Environmental Parameters (No EEMs) PC1		0.048
Environmental and Watershed Parameters PC1	Watershed Characteristics	0.010
Log DOC	Carbon Quality	0.004
FI 2005		0.015
HIX 1999		0.017
HIX 2002		0.022
UV_{254}		0.048

A comparison of the best predictors for TFA, n-6 PUFAs, and EPA using both datasets (Table 22) reveals similarities in the mechanisms of the predictor variables; all variables fall into categories representing either lake carbon amounts and quality or catchment influence. The only predictor that is the same across both datasets is UV_{254}, which is the best predictor of Northern Pike TFA concentrations. The only new predictor using the restricted dataset is PC2 of the PCA including water chemistry data (but excluding EEMs data), which separates lakes by secchi depth. Two fatty acid groups, Lake Whitefish TFA and Northern Pike n-6 PUFAs, displayed significant differences among lakes when using the full dataset, but were not significantly different among lakes when using samples stored for less than one year.

Table 22. Comparison of the best environmental predictors of TFA, n-6 PUFAs, and EPA using the full dataset and the dataset restricted to samples stored for less than one year. *Indicates that there was no significant difference among lakes after calculating LS means at a standardised fork length.

		All Data (N=282)	Samples Stored < One Year (N=193)
Species	**Fatty Acid**	**Best Predictor**	**Best Predictor**
LKWH	Total FA	Log TP	-*
	n-6 PUFAs	EEMs PC 2	TN
	EPA	-	-
NRPK	Total FA	UV_{254}	UV_{254}
	n-6 PUFAs	Lake Perimeter to Watershed Area Ratio	-
	EPA	$Cl^- + UV_{254}$	Env PC2 + TN
Wall	Total FA	-	-
	n-6 PUFAs	Ca^{2+}	Log DOC
	EPA	-	-*

3.6.8 Correlations Among Variables

There were strong correlations among several variables that best explained among-lake variation in fish fatty acids (measured with Pearson's R correlation coefficients; Table F-1, Appendix). Particularly strong, positive correlations were found between UV_{254} and TN (r =+0.903) and Cl^- and Ca (r=+0.797). However, most of the measured variables were generally highly correlated with each other ($r \geq 0.5$), with two exceptions. EEMs PC2 was strongly, negatively correlated with DOC (r=-0.715) but was not strongly correlated with any of the other top predictors ($r \leq 0.335$), and TP was not strongly correlated with any other top predictor ($r \leq 0.241$; Table F-1, Appendix).

Water chemistry variables found to be the best predictors of fatty acid concentrations (Log TP, EEMs PC2, UV_{254}, Cl^-, TN, DOC, and Ca^{2+}) were also correlated with watershed characteristics, which were measured remotely. Each water chemistry predictor was strongly correlated with at least one watershed variable using Pearson's R correlations. Dissolved organic carbon (r =-0.744), TN (r =-0.797), UV_{254} (r =-0.896), and Cl^- (r =-0.896) were all strongly, negatively correlated with mean elevation. Calcium was highly, positively correlated with the maximum total wetness index (r=+0.757), EEMs PC2 was strongly, positively correlated with the ratio of longest flow path length to watershed area (r=+0.762), and TP was strongly, positively correlated with watershed area (r=+0.745). For PC2 of the PCA containing water chemistry, but excluding EEMs data, a strong, negative correlation was noted with lake area (r=-0.779).

Chapter 4. Discussion

4.1 Effect of Storage Time of Fish Fatty Acids

The length of time that fish tissue was stored at -20°C was found to significantly affect fish fatty acid composition in every fish species examined in this study. Perhaps the most interesting finding is that DHA concentrations decrease exponentially after approximately one year of storage. This decrease, along with an increase in saturated fatty acids over time, is consistent with results of previous studies (Rudy et al. 2016). It is thought that fatty acids degrade through lipid oxidation, a chemical process whereby oxygen acts as an electron acceptor from lipid molecules, causing the lipids to change in structure and degrade (Min and Ahn 2005; Nazemroaya et al. 2009). There are several factors that could create variation between the results shown in this thesis and fatty acid degradation under storage conditions used in homes. Since oxidation in frozen samples is related to the surface area of tissue exposed to air (Nazemroaya et al. 2009), and the samples used in this study were relatively small pieces of fish muscle tissue, it is possible that effects of fatty acid degradation would be less pronounced in stored whole fillets of fish. Data on fish-storing practices in the Dehcho region, such as how the fish were handled before freezing and the temperature of the freezer, both of which can affect fatty acid degradation (Rudy et al. 2016), are not currently available. It is thus possible that concentrations of fatty acids in frozen fish from a given lake or species may be different from reported averages. Specifically, underestimating n-3 PUFA or DHA concentrations is a concern. As fish may be stored in community freezers or in individual home freezers, intake of n-3 PUFAs and DHA may vary among households even when fish are sourced from the same lake.

The effect of storage time on fish fatty acid profiles is known to be highly species-specific (Rudy et al. 2016). It could be useful to determine the conditions under which people typically store their fish and estimate exactly how long it takes for DHA to start to decay for each species; this study has a data gap between one year and 20 months, so it is difficult to estimate when the quality starts to decrease. This would be especially important for Lake Trout, as DHA appeared to begin decaying sooner than the other species, after less than one year of storage time. Understanding when the nutritional quality of fish begins to decrease is especially important because fish mercury concentrations in some fish species (including Northern Pike, Walleye, and

White Sucker) were found to persist with no significant degradation after four years of storage (Peterson et al. 2007). Fish with unchanged mercury concentrations but decreased n-3 PUFAs are not ideal for human health. The Government of the Northwest Territories (GNWT) currently recommends that fish should not be stored in the freezer for more than one year (GNWT 2019). The findings in this study are consistent with that recommendation.

4.2 Fatty Acid and Mercury Concentrations

Mean fatty acid values used for the calculation of full fatty acid profiles (MUFAs, SFAs, PUFAs, and fatty acid groups of interest for Burbot, Cisco, Lake Whitefish, Lake Trout, Longnose Sucker, Northern Pike, Walleye and White Sucker) fell within the range of means previously calculated (Reyes et al. 2017; Laird et al. 2018). Of the three fish species of particular importance to subsistence fishers, Lake Whitefish had the highest concentrations of fatty acids across all measured groups, which is consistent with previous work performed in the region (Reyes et al. 2017; Laird et al. 2018), although they also exhibited the highest variation in fatty acid concentrations within each lake.

Concentrations of TFA, n-6 PUFAs, and DHA differed significantly among lakes in Lake Whitefish. Concentrations of both TFA and DHA displayed the same pattern; they were lowest in McGill Lake, and highest in Kakisa Lake. By contrast, Lake Whitefish n-6 PUFA concentrations were lowest in Trout Lake; the other lakes were not significantly different from each other. In every fatty acid group, with the exception of EPA, Lake Whitefish had significantly higher mean fatty acid concentrations in the HRL lakes, and lower mean concentrations in Trout Lake. Where estimates were available, Lake Whitefish had intermediate fatty acid concentrations in the HP lakes. Interestingly, mercury concentrations follow the same pattern; they are highest in the HRL lakes, and lowest in Trout Lake. While there were significant differences in mercury concentrations among eco-regions, Lake Whitefish had consistently lower mercury concentrations than other species in all lakes (Reyes et al. 2017; Laird et al. 2018).

Northern Pike had consistently lower fatty acid and higher mercury concentrations than Lake Whitefish, which is also consistent with previous research (Reyes et al. 2017; Laird et al. 2018).

In every measured fatty group except for TFA, mean fatty acid concentrations were lowest in Sanguez lake, and most of the other HRL lakes (Gargan, Ekali, and Tathlina) were generally low as well. The exception was Kakisa Lake; Northern Pike in Kakisa Lake had higher mean concentrations of fatty acids when compared to other HRL lakes, and concentrations were not significantly different from the HP lakes and Trout Lake. Differences among eco-regions in Northern Pike were the opposite of observed trends in Lake Whitefish; mean fatty acid concentrations in Northern Pike were significantly higher in the HP lakes and in Trout Lake, and lower in the HRL lakes. Although n-3 PUFA and DHA data from Big Island and Willow lakes had to be excluded due to issues associated with storage time, both of these fatty acid groups follow the same general pattern of the fatty acid groups that included HP lakes; concentrations were significantly higher in Trout Lake, and lower in the HRL lakes. When paired with mean mercury concentrations, Northern Pike have the lowest fatty acid concentrations and highest mercury concentrations in HRL lakes. By contrast, fatty acid concentrations in Northern Pike are highest in Trout lake, and mercury concentrations are significantly lower in Trout Lake than the HP and HRL lakes.

Concentrations of fatty acids in Walleye exhibited similar ranges to Northern Pike. Unlike Northern Pike, only concentrations of n-3 and n-6 PUFAs differed significantly among lakes. Differences among lakes were driven by a single pairwise difference for both n-3 and n-6 PUFAs; all other lakes had intermediate fatty acid concentrations. Concentrations of n-3 PUFAs displayed a similar trend to Northern Pike in that fatty acid concentrations were highest in Trout Lake, and lowest in Sanguez Lake. By contrast, concentrations of n-6 PUFAs are lowest in Trout and highest in Tathlina. Comparisons of Walleye fatty acid concentrations between eco-regions, limited to HRL lakes and Trout Lake, found significant differences in concentrations of n-3 PUFAs, DHA, and EPA. Similar to Northern Pike, concentrations of n-3 PUFAs and DHA were significantly higher in Trout Lake and lower in the HRL lakes. By contrast, concentrations of n-6 PUFAs were significantly higher in the HRL lakes, and lower in Trout Lake.

While patterns in fatty acid concentrations among eco-regions differed among species (Lake Whitefish, Northern Pike, Walleye), the patterns of mercury concentrations among eco-regions

were consistent for all three species; mercury levels were highest in the HRL lakes and lowest in Trout Lake. Previous researchers who have examined variability in fish mercury levels among eco-regions have reported somewhat inconsistent results that may reflect differences in the spatial scale of investigation. On a relatively small spatial scale, a study of mercury levels in yellow perch (*Perca flavescens*), brown bullhead (*Ameiurus nebulosus*), and largemouth bass (*Micropteus salmoides*) in lakes in Massachusetts (USA) found that individual lake characteristics were more important than eco-region for determining fish mercury concentrations (Rose et al. 1999). On a larger spatial scale, researchers who investigated mercury concentrations on the west coast of both Canada and the United States (from California up to Alaska) in many fish species (including all of the study species) reported patterns in mercury concentrations among eco-regions (Eagles-Smith et al. 2016).

At high enough concentrations, mercury in fish muscle tissue can have detrimental effects on fish health (Crump and Trudeau 2009; Scheuhammer et al. 2015), including effects on lipids used in reproduction. Results from studies in bronze featherback (*Notopterus notopterus;* Verma and Tonk 1983), walking catfish (*Clarias batrachus;* Kirubagaran and Joy 1995), and murrel (*Channa punctatus;* Kirubagaran and Joy 1988) have revealed that lipid levels in ovaries can decrease with increasing exposure to inorganic mercury and methylmercury. Results from limited research suggests that toxicological effects on cell tissue and reproduction in fish can occur once concentrations of mercury reach 0.5-1.0 µg Hg/g wet weight (ww; Sandheinrich and Wiener 2011). This range is based on averages from multiple studies and species, which included Northern Pike captured in Michigan, USA, and Germany and Walleye from Washington, USA (Sandheinrich and Wiener 2011). Dillon et al. (2010) reported that the approximate threshold for health effects on fish is 0.3 µg Hg/g wet weight, again using averages from different species; while none of the study species were included in Dillon et al. (2010), there were two species of trout (*Oncorhynhus mykiss* and *Salvelinus fontinalis*).

For the lakes and species included in this study, mean Hg concentrations exceeded the Health Canada guideline of 0.5 µg Hg/g ww for Northern Pike in Sanguez Lake, Walleye in McGill, Sanguez and Tathlina lakes, and White Sucker in Tathlina Lake. All Northern Pike, except those

captured in Mustard and Trout lakes, had mean lake-specific mercury concentrations that exceeded 0.3 µg Hg/g ww. Mean Hg concentrations in Lake Trout from Trout and Willow lakes, and Burbot from Trout Lake, also exceeded 0.3 µg Hg/g ww. Mercury-induced toxicological effects on fish may be especially prevalent in Sanguez lake, where a maximum Hg concentration of 3.12 µg Hg/g ww was recorded for Northern Pike. Only Cisco, Longnose Sucker, Lake Whitefish, and White Sucker have mean mercury concentrations under 0.3 µg Hg/g ww in every lake. Although there is evidence that mercury can affect fish lipids (Dillon et al. 2010; Sandheinrich and Wiener 2011; Scheuhammer et al. 2015), and negative statistical relationships were observed between concentrations of mercury and fish lipids in the Dehcho, it is not yet known whether fish mercury levels are high enough to impact lipids in fishes from the Dehcho region. Further study, including species-specific responses to mercury (Barst et al. 2019), would be necessary to confirm whether mercury is negatively affecting fish health in lakes in the Dehcho region.

Another consistent finding among all three species was low concentrations of n-6 PUFAs in Trout Lake. Finding similar patterns of concentrations among species is interesting because both fatty acids and mercury come from the diet. It is possible that differences stem from how fatty acids and mercury concentrations transfer between trophic levels. While mercury concentrations are known to increase with trophic position (AMAP 2011), the relationship between fatty acid concentrations and fish trophic position is less clear (Kainz et al. 2017). After the effect of lake was removed, there was no relationship between $\delta^{15}N$ and fish fatty acid concentrations. However, there may be a relationship when the effect of lake is included in the model, as the trophic position of fish can change among lakes (Eloranta et al. 2015). A more in-depth exploration of trophic ecology along with an investigation of how biological covariates vary among lakes could be an interesting path for future analysis.

4.3 Abiotic Drivers of Fatty Acid Variation

While each fatty acid group and species had a different variable that best predicted concentrations, the underlying mechanisms driving differences were similar across analyses. For

the 10 species-specific fatty acid groups that differed significantly among lakes, 8 abiotic variables emerged as significant predictors: total phosphorus, EEMs PC2 (most related to SUVA), the ratio of lake perimeter (sum of all lakes in a watershed) to watershed area, UV_{254}, chloride, and concentrations of total nitrogen, dissolved organic carbon, and calcium. These variables can be roughly categorized into three groups based on underlying mechanisms: lake productivity (total phosphorus), carbon quality (EEMs PC2, UV_{254}, TN, and DOC), and lake catchment influence (concentrations of chloride and calcium, and the ratio of lake perimeter to watershed area; Table 23).

Table 23. Summary of abiotic predictors and associated mechanisms. *Indicates the sum of all lake perimeters in the watershed.

Species	Fatty Acid	Predictor Variable	Underlying Mechanism
LKWH	Total FA	Log TP $_{(+)}$	Productivity
	n-6 PUFAs	EEMs PC 2	Carbon Quality
	DHA	Lake Perimeter to Watershed Area Ratio* $_{(+)}$	Catchment Inputs
NRPK	Total FA	UV $_{254\ (-)}$	Carbon Quality
	n-3 PUFAs	Cl^- $_{(-)}$	Catchment Inputs
	n-6 PUFAs	Lake Perimeter to Watershed Area Ratio* $_{(+)}$	Catchment Inputs
	EPA	Cl^- $_{(-)}$ + UV $_{254\ (-)}$	Catchment Inputs/ Carbon Quality
	DHA	Cl^- $_{(-)}$ + TN $_{(-)}$	Catchment Inputs/ Carbon Quality
Wall	n-3 PUFAs	DOC $_{(-)}$ + Cl^- $_{(-)}$	Catchment Inputs/ Carbon Quality
	n-6 PUFAs	Ca $_{(+)}$	Catchment Inputs

Total phosphorus concentration is often positively related to chlorophyll-a concentration in lakes (Jones and Bachman 1976; Stow and Cha 2013), and chlorophyll-a concentration is often used as a proxy for lake productivity (e.g., Maloney 1979). Consistent with the literature, concentrations of total phosphorus in the study lakes were strongly, positively correlated with chlorophyll-a (Table F-1, Appendix). There was a significant and positive relationship between concentrations of TFA in Lake Whitefish, and the concentration of total phosphorus, with differences driven by the significant pairwise difference between Kakisa and McGill lakes. When considering only total phosphorus concentrations, McGill can be considered oligotrophic (TP concentration of 9 µg/L), whereas Kakisa can be considered eutrophic (60 µg/L). The contrast between these two lakes is also evident in chlorophyll a (*chl a*) levels; McGill has a *chl a* concentration of 1 µg/L, whereas Kakisa has a concentration of 4 µg/L. Total phosphorus has previously been used as a proxy for autochthonous carbon produced in lake, although it is considered to be oversimplified

because it accounts for algal, but not microbial sources of in-lake production (Gudasz et al. 2012). Eutrophic lakes are often associated with lower fish fatty acids because of higher relative abundances of cyanobacteria (Taipale et al. 2016b). However, seston analysed for fatty acids had higher concentrations of EPA and DHA in eutrophic lakes than oligotrophic lakes because of the higher number of fatty-acid producing algae (Taipale et al. 2016b). It is possible that eutrophic conditions in Kakisa are not associated with increased cyanobacteria abundances, or that there is greater availability of fatty-acid producers in the food chain for Lake Whitefish in Kakisa. These ideas could be tested by studying the composition of algae and bacteria (fatty-acid producers) and the trophic ecology of Lake Whitefish.

Dissolved organic carbon can be separated into newer, labile carbon that is easy for biota to break down (high quality carbon) and older, recalcitrant carbon that is more difficult for biota to break down (low quality carbon; Ostapenia et al. 2009). Variables indicating the degree of carbon quality included PC2 from the PCA conducted on EEMS data, which separated lakes by SUVA, UV_{254}, and concentrations of TN and DOC. The characteristics of UV_{254} and EEMs PC2 directly indicate the amount of terrestrial dissolved organic matter (DOM) in a lake; a higher proportion of humic substances in the lake DOM, and therefore a higher UV_{254} and $SUVA_{254}$, is inferred to reflect higher levels of allochthonous, recalcitrant carbon (Malcolm 1991; Weishaar et al. 2003). Total nitrogen is often linked to productivity, or autochthonous production (Trommer et al. 2019). However, TN in the study lakes was not strongly correlated with chlorophyll-a concentrations (Table F-1, Appendix). Given the strong correlation between UV_{254} and TN, the main source is likely the catchment, indicating higher catchment inputs. Likewise, while DOC concentrations in lakes includes carbon of both allochthonous and autochthonous origins, in this study DOC was highly correlated with UV_{254}, TN, and EEMs PC2 (Pearson's $R \geq 0.715$; Table F-1, Appendix), suggesting that lakes with higher DOC have relatively higher amounts of recalcitrant, likely allochthonous inputs of terrestrially derived organic matter.

Concentrations of chloride and calcium are inferred to indicate catchment influence in this study. Chloride enters a lake through runoff from the catchment, but is relatively inert once in the lake ecosystem because it is not taken up by organisms (Appelo and Postma 2005). Ions and salts,

which influence water conductivity, can be used as a proxy of water residence time, with higher concentrations indicating that lake water has lower turnover (They et al. 2017). Previous work in the study lakes using hydrogen and oxygen stable isotopes as hydrological tracers has determined a preliminary order of water residence time in the study lakes. The order of shortest residence times to longest is: McGill, Sanguez, Ekali, Mustard, Gargan, Big Island, Willow, Tathlina, and Kakisa (Heidi Swanson, Unpublished Data). However, chloride and calcium ions, when arranged in ascending order, do not fit this pattern. Alternatively, chloride concentrations can be higher because there is more terrestrially derived matter entering the lakes (Lockwood et al. 1995). Calcium, which is highly correlated with chloride (Table F-1, Appendix), also enters lakes through weathering in the catchment (Lockwood et al. 1995). Therefore, it is likely that chloride and calcium concentrations can be used as a measure of catchment input. The ratio of lake perimeter (the sum of all lake perimeters) to watershed area is equally negatively correlated with UV_{254} and Cl^- (Table F-1, Appendix), meaning that this variable likely indicates degree of allochthony vs autochthony and catchment influence.

Most variation in Lake Whitefish, Northern Pike, and Walleye fatty acid concentrations was explained by variables that reflect carbon quality (explaining variation in LKWH TFA, n-6 PUFAs and DHA, NRPK TFA, EPA, and DHA, and WALL n-3 PUFAs) and catchment influence (explaining variation in NRPK n-3 PUFAs and DHA, and WALL n-3 and n-6 PUFAs). The differences in terms of which predictor variable was significant is likely due to small sample sizes (i.e., only ten lakes - with a larger sample size, there would likely be more consistency). One measure of lake allochthony or autochthony is the fluorescence index, where values less than 1.4 indicate primarily terrestrial sources of DOM in a lake, and values greater than 1.8 indicate primarily in-lake sources of autochthonous DOM (Cory and McKnight 2005). Values in all study lakes fell somewhere between 1.4 and 1.8, suggesting that both allochthonous and autochthonous carbon are important. As such, this thesis compares the relative degree of lake

allochthony among study lakes while recognising that none can be called fully allochthonous or autochthonous.

The analysis aimed at determining the best environmental predictors of fish fatty acids was limited by the different sample sizes and lakes included in the analysis of TFA, n-6 PUFAs, and EPA, as compared to n-3 PUFAs and DHA. The main difference between the two datasets was the exclusion of the HP lakes in the restricted dataset. This was unfortunate, but unavoidable. Encouragingly, when analyses for TFA, n-6 PUFAs, and EPA were re-run using only samples stored for less than one year (i.e., mimicking the restricted dataset), the majority of predictors for all fatty acid groups in both the full and restricted dataset were indicators of either lake catchment inputs or carbon quality. This suggests that the underlying mechanisms that drive variability in fish fatty acid levels are consistent, regardless of whether the HP lakes are included or not. The only 'new' predictor using the smaller dataset was for EPA in Northern Pike; EPA levels in Northern Pike using the restricted dataset were best explained by PC2 scores of the PCA that included only water chemistry (but excluded EEMs data). This PCA separated lakes along a gradient of secchi depth. Since secchi depth was highly and positively correlated with the concentration of chloride in a lake, it is likely related to lake catchment inputs, where lakes with greater secchi depth (i.e., with clearer water) have less inputs from the catchment (Hakanson 1995). Based on the comparison of datasets, it is likely that the smaller dataset used for n-3 PUFAs and DHA still lends useful insights into environmental variables that contribute to among-lake variation in concentrations of n-3 PUFAs and DHA.

4.3 Allochthony vs Autochthony: Climate Change and Contaminants

The majority of the variables that best predicted fatty acids in fish are inferred to reflect catchment influence and the lake carbon quality. Relationships between lake colour (where humic, terrestrial-influence lakes are darker coloured) and fish fatty acids have been demonstrated in several previous studies (e.g. Strandberg et al. 2016; Taipale et al. 2016). In each study, more humic lakes were associated with lower concentrations of EPA and DHA in fish muscle tissue (Strandberg et al. 2016; Taipale et al. 2016). In addition to quantifying concentrations of fatty acids in fish, one study in lakes in Finland cultured the major phytoplankton taxa found in the study lakes, determined the average fatty acid concentrations

and profiles for each taxa, and then collected plankton community composition samples from the study lakes to estimate fatty acids found in lake seston (Taipale et al. 2016). Both EPA and DHA in fish muscle tissue were negatively correlated with the percentage of cyanobacteria and green algae in the lakes, and these taxa were found at a higher relative abundance in lakes with higher total phosphorus (TP in studied lakes ranged from 1.5 - 51 µg/L) and higher dissolved organic carbon (4-24 mg/L; Taipale et al. 2016). Decreasing fish fatty acids in eutrophic lakes with high TP has been demonstrated in other studies as well, with TP concentrations ranging from 9 to 81 µg/L and 12 to 87 µg/L (Ahlgren et al. 1996; Razavi et al. 2014).

The results from Strandberg et al (2016) and Taipale et al (2016) are consistent with the results for Northern Pike and Walleye presented here; increasing terrestrial carbon was associated with lower n-3 PUFAs. Lake Whitefish in Dehcho lakes do not fit the pattern of decreasing fatty acids with increasing eutrophication, however; TFA were highest in Kakisa Lake, which can be classified as eutrophic when considering TP (Kakisa TP: 60 µg/L), although when considering chlorophyll-a, Kakisa can be considered mesotrophic (4 µg/L; Dodds 2002). Moreover, Lake Whitefish display increasing DHA concentrations with increasing allochthonous carbon. While this study did not directly compare bacterial and phytoplankton communities to fish fatty acid concentrations, the factors that emerged as important (allochthonous vs autochthonous DOC, TP, and ions) are known to affect phytoplankton composition. In an analysis of 1558 lakes across Europe, changes in the dominant genera or species were associated most strongly with water colour, then lake alkalinity, and then TP (Maileht et al. 2013). The biggest difference between Lake Whitefish and Northern Pike and Walleye is diet; Lake Whitefish are primarily benthivorous, although they have been found to consume small fish or plankton in some areas, while Northern Pike and Walleye are primarily piscivorous (Scott and Crossman 1973). It is possible that the different patterns are related to diet or trophic ecology. Changes due to diet could be tested by examining fatty acid concentrations of prey found in fish stomachs. Trophic ecology could be examined by relating the effect of $\delta^{15}N$ on fatty acid concentrations for each species when the effect of lake is included, as changing levels of allochthonous carbon may affect benthic and pelagic food chains differently (Jansson et al. 2007; Kivilä et al. 2019).

The effect of changing lake colour on primary producer composition as well as fish fatty acids is important to understand, as the general trend of 'brownification', or increasing inputs of dark-coloured, terrestrial humic substances into lakes, is a documented effect of climate change affecting lakes across the northern hemisphere (de Wit et al. 2016). There are multiple mechanisms responsible for observed increases of terrestrially derived DOC in lakes. Warming temperatures in Northern regions have led to higher rainfall than has been typical of the recent past; with increased rainfall, there is increased transport of humic substances into lakes (de Wit et al. 2016). Other evidence links increased DOC in European and North American lakes to a decrease in deposition of atmospheric anthropogenic sulfur and chloride; decreasing sulfur changes the acidity of soils in catchments, making some organic matter in soils more soluble (Monteith et al. 2007). However, the impact of sulfur on subarctic lakes specifically is debated (Monteith et al. 2007; Couture et al. 2012; de Wit et al. 2016). There is conflicting evidence about which lakes are more likely to be affected by brownification; some data suggest that lake browning is more likely to occur in lakes with smaller catchments, whereas a study from Northern Europe suggests that brownification will affect lakes of all catchment sizes equally (de Wit et al. 2016). Northern lakes specifically are thought to be more at risk of browning if they have a higher percentage of wetlands in their catchments (Creed et al. 2018).

Study lakes in the Dehcho have a high amount of variability in the percentage of wetlands in their catchment, ranging from less than one percent (McGill) to 62% (Ekali; Heidi Swanson, Unpublished Data). As such, brownification could vary in intensity across Dehcho lakes. While a simplification, the results of this study suggest that if lakes in the Northwest Territories experience increasing lake brownification, Northern Pike and Walleye may decrease in nutritional quality (as measured by n-3 PUFAs) due to greater inputs of allochthonous carbon, whereas nutritional quality of Lake Whitefish concentrations may be less affected (e.g., neither EPA nor n-3 PUFAs vary significantly among lakes with differing carbon quality). Future studies to determine whether Dehcho lakes are being affected by brownification, and whether brownifcation affects fish fatty acid profiles (if changes are occurring), would be valuable.

Aside from possible changes to primary producer composition, increased concentrations of humic acids decrease visibility, because more light is scattered or absorbed (De Robertis et al. 2003). Reduced light can limit the primary producer biomass, potentially offsetting the possibility of increased biomass due to greater nutrient availability (Creed et al. 2018), which could decrease abundance of fatty acid-producing taxa (Bourassa and Cattaneo 2000), and therefore the pool of fatty acids available for higher trophic levels. There is also evidence, however, to suggest that some algae produce greater concentrations of EPA under low-light conditions (Guschina and Harwood 2006), making the effects on fatty acids in seston complex and difficult to predict.

Fish can be directly impacted by reduced visibility in lakes due to increases of terrestrial organic matter (De Robertis et al. 2003). Under experimental conditions mimicking increased turbidity, visual hunters exhibited decreased hunting success, while planktivorous fishes were not as strongly affected (De Robertis et al. 2003). Northern Pike is a visual hunter; decreased success in hunting could lead to a further reduction in already low concentrations of fatty acids. Furthermore, there is some evidence in the Dehcho that mercury concentrations in Northern Pike is related to water clarity (measured by secchi depth; Heidi Swanson, unpublished data). In clear lakes in the HRL, Northern Pike hunt farther offshore, and $\delta^{13}C$ values are consistent with pelagic food webs; these fish have higher mercury concentrations (Heidi Swanson, unpublished data). A decrease in visibility due to diminished light penetration into lakes from greater inputs of dissolved organic matter could change these patterns. In addition, increasing amounts of allochthonous carbon are known to increase methylmercury concentrations in zooplankton and fish (Taipale et al. 2016; Poste et al. 2019). Legacy mercury in the subarctic is often stored in snow and permafrost, so that there are seasonal increases in mercury inputs into lakes with melting snow and freshet in the spring (AMAP 2011). On a broader time scale, melting permafrost is thought to have the potential to release more mercury into lakes (AMAP 2011). As such, having a baseline of both fish fatty acid and fish mercury concentrations in the Dehcho is important, and this work contributes to that baseline.

4.4 Implications for Human Health

Analyses of risks and benefits of consuming wild-caught fish in the Dehcho region must include consideration of socioeconomic and cultural factors. In many Indigenous cultures, traditional foods are more than just a source of food; they are part of traditional values and a system of well-being that includes mental, physical, and spiritual health (Kuhnlein et al. 2013; Lemire et al. 2015). Thus, there are benefits to fish consumption beyond the nutritional value (Kuhnlein et al. 2013). Recent results from food frequency questionnaire (FFQs) data collected from the Dehcho region suggests that traditional foods are widely consumed across all demographics (Ratelle et al. 2020). In a survey of 125 people from the region, participants reported consuming Lake Whitefish (94% of participants), Northern Pike (60%), and Walleye (51%) on a regular basis, on average 1.7 times per week for Lake Whitefish and 1.3 times per week for Northern Pike and Walleye (Ratelle et al. 2020). Thus, studying these three species is important, as is understanding what makes some fish healthier (from a food quality point of view) than others, especially when balanced against the possibility of mercury contamination. Encouragingly, the majority of survey participants in the Dehcho region that were tested for mercury levels had blood and hair mercury concentrations well below health-based guidance values (Ratelle et al. 2018; Ratelle et al. 2020). The same participants had average concentrations of fatty acids in blood plasma that were similar to average concentrations found across Canada (Ratelle et al. 2018; Stark et al 2016). Authors of these studies thus concluded that the current pattern of traditional food consumption (as reported in 2016-2018) does not result in an elevated risk of mercury exposure (Ratelle et al. 2018; Ratelle et al. 2020).

The work presented here is valuable for determining underlying causes of variation in concentrations of fatty acids in several food fish species. Consistent with previous research and current health recommendations from the GNWT, Lake Whitefish were found to be a healthy option and a good source of EPA and DHA in any lake and at any fish size (GNWT 2016; Reyes et al. 2017; Laird et al. 2018). While Northern Pike and Walleye had lower concentrations of all fatty acid groups, both generally had higher fatty acid concentrations in lakes with less allochthonous carbon, which in this study were the HP lakes and Trout Lake. Northern Pike and Walleye may be more nutritionally dense at smaller sizes; both species had higher concentrations

of EPA at smaller sizes, as well as lower concentrations of mercury (Laird et al. 2018). Differences were not large, however; Northern Pike that were 400 mm or smaller (fork length) had an average EPA concentration of 42 mg/100g, while Northern Pike larger than 700 mm (fork length) had an average EPA concentration of 35 mg/100g. Results for Walleye were similar to those observed for Northern Pike. Concentrations of DHA in Northern Pike also decreased as fish age increased. The information presented on differences in fatty acid concentrations among species, lakes, and eco-regions can help inform decision-makers and harvesters on the safest, healthiest sources of fish in the Dehcho region.

4.5 Future Directions

4.5.1 Additional Factors Affecting Fish Fatty Acids

It should be noted that statements and findings regarding which fish are healthiest for human consumption reported in this thesis do not take into account how the fish are prepared after being caught. The analysis of fatty acid composition is based on uncooked fish muscle tissue. There is evidence to suggest that the manner of cooking fish can change the fatty acid profiles, although the effects recorded vary by study (e.g. Agren and Hanninen 1993; Cieślik et al. 2018; Kaya et al. 2008; Neff et al. 2014). Concentrations of n-3 PUFAs increased in fillets of Northern Pike from Finland that were boiled, baked, and microwaved, although the degree of increase was less than in the other species in the study, Rainbow Trout (*Oncorhynchus mykiss*) and Vendace (*Coregonus albula*; Agren and Hanninen 1993). Frying did not significantly change n-3 PUFA concentrations, but concentrations of n-6 PUFAs increased significantly, a trend that was thought to be explained by the addition of oil rich in n-6 PUFAs (Agren and Hanninen 1993). However, baking, broiling and frying did not significantly change concentrations of n-3 or n-6 PUFAs in Lake Trout, Walleye, and White Sucker from the Great Lakes (Neff et al. 2014). The process of smoking Northern Pike from lakes in Poland decreased all concentrations of n-3 and n-6 PUFAs, while saturated fatty acids increased, an effect found in smoked sturgeon (*Huso huso*) from Turkey as well (Kaya et al. 2008; Cieślik et al. 2018). In addition to uncertainty caused by cooking method, people in the Dehcho region report eating other parts of the fish, including the liver, heads, eggs, and ‘fish-pipe’, or esophagus (GNWT 2011; Ratelle et al. 2020). We do not currently have estimates of fatty acid or mercury concentrations for non-muscle tissues. As such,

quantifying fatty acids in fish from the Dehcho region prepared in different ways (and using a variety of tissues) would be an interesting and useful future step.

While we can estimate the starting nutritional quality of Lake Whitefish, Northern Pike, or Walleye in the studied lakes, there are a myriad of factors that can affect how fatty acids are incorporated into the human body. There is some evidence that the incorporation of fish fatty acids into the human body depends on diverse personal factors, such as age and body mass index (Sands et al. 2005). Work in the Dehcho region indicated that older individuals reported more frequent fish consumption (Ratelle et al. 2020). As such, general recommendations for fish consumption may need to be tailored to specific demographics.

The availability of fatty acids for aquatic and then human consumers begins with lake abiotic conditions. Ratios of dissolved organic carbon from allochthonous vs autochthonous sources are known to fluctuate with season, temporarily increasing in allochthonous carbon when snowmelt leads to increased runoff, and increasing in autochthonous carbon during algal blooms (Gabor et al. 2014). Likewise, shifts in algae and bacteria composition over seasons and the corresponding dietary shifts in predators due to available prey can alter fatty acid composition of invertebrates and fish (Persson and Vrede 2006). As such, it would be interesting to examine seasonal changes in concentrations of fish fatty acids. While fish consumption in the Dehcho region is highest during the summer, the period studied in this thesis, fish are still a commonly harvested and consumed food source in the winter (Ratelle et al. 2020). If samples from all seasons could be combined with the quantification of fatty acids in fish that had low sample sizes in this study (Burbot, Cisco, Lake Trout, Longnose Sucker, and White Sucker), as well as fish cooked using different methods and alternate fish body parts, estimations of fatty acid availability for humans in the Dehcho could become more precise.

4.5.2 Predicting Fish Fatty Acid Concentrations

One of the most important findings of this study is that the best predictors of fatty acids are highly correlated with watershed characteristics (Table F-1, Appendix). Watershed characteristics can be quantified using land classification data and GIS software, and together with the results presented here, may enable at least approximate estimations of levels of fatty

acids in Lake Whitefish, Northern Pike, and Walleye in unstudied Dehcho region lakes. Since field research is often logistically difficult and very expensive to perform, having a way to predict concentrations of fatty acids without sampling would be an excellent way to prioritize lakes for further study and monitoring.

While the value of being able to predict fish fatty acids in lakes we have not studied is clear, at the moment its value is limited by the small number of lakes in the data set. By the end of the larger, on-going project, fish fatty acids will be quantified in at least three more lakes in the Dehcho region (Fish, Greasy, and Deep). It will be interesting to see if these lakes follow the same patterns of fatty acid concentrations, as two (Fish and Greasy) are located in a different ecozone that thus far does not have any fatty acids quantified. Ultimately, these results could be used to help people choose the safest, healthiest subsistence fish sources.

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Appendix A. Storage Time Analyses

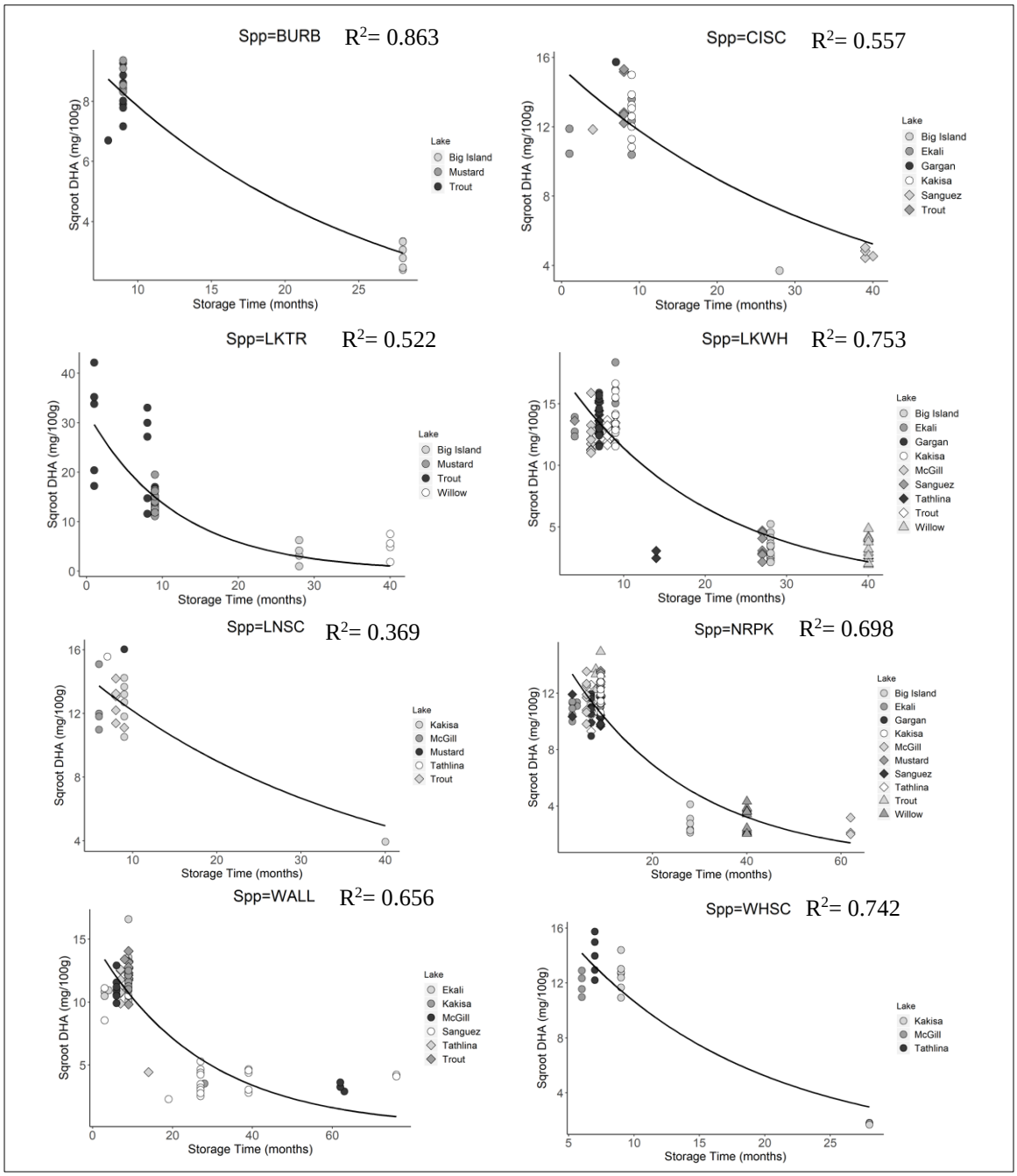

Figure A-1. DHA (square-root transformed) vs. storage time (months), fit to an exponential curve. Abbreviations: BURB= Burbot, CISC= Cisco, LKTR= Lake Trout, LNSC= Longnose Sucker, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walley, and WHSC= White Sucker.

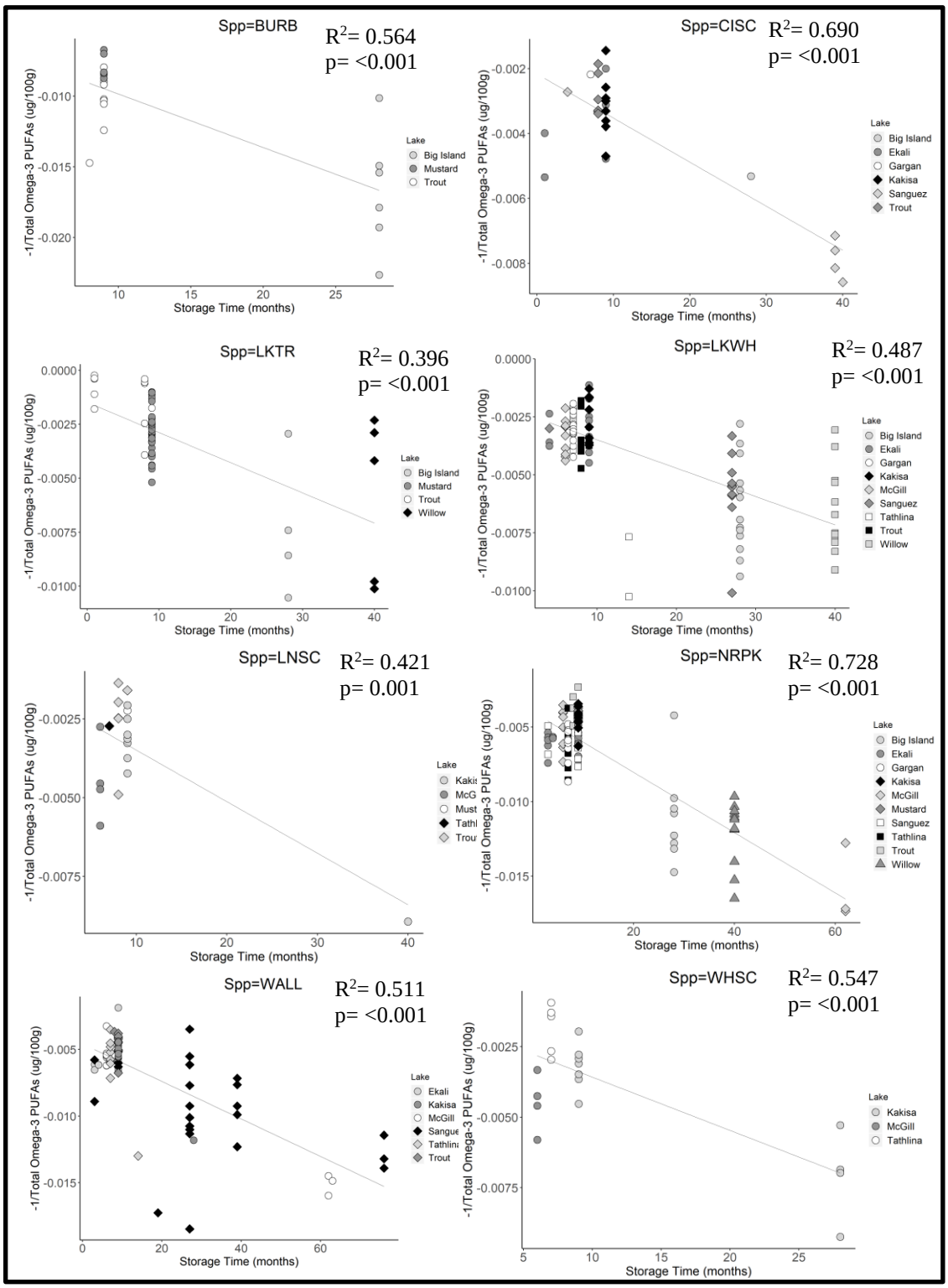

Figure A-2. Total n-3 PUFAs (reciprocally transformed) vs. storage time (months). BURB= Burbot, CISC= Cisco, LKTR= Lake Trout, LNSC= Longnose Sucker, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walley, and WHSC= White Sucker.

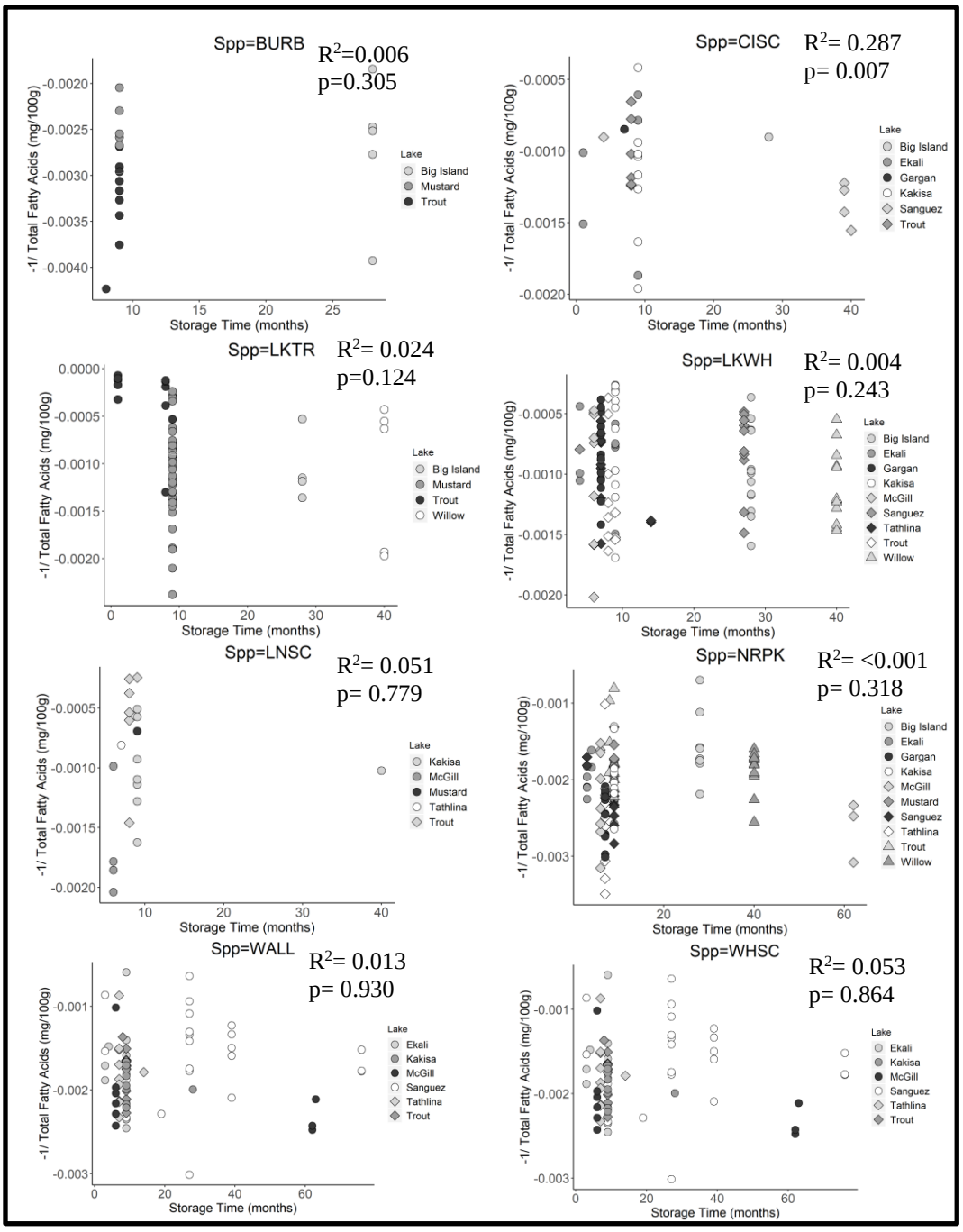

Figure A-3. TFA (reciprocally transformed) vs. storage time (months). BURB= Burbot, CISC= Cisco, LKTR= Lake Trout, LNSC= Longnose Sucker, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walley, and WHSC= White Sucker.

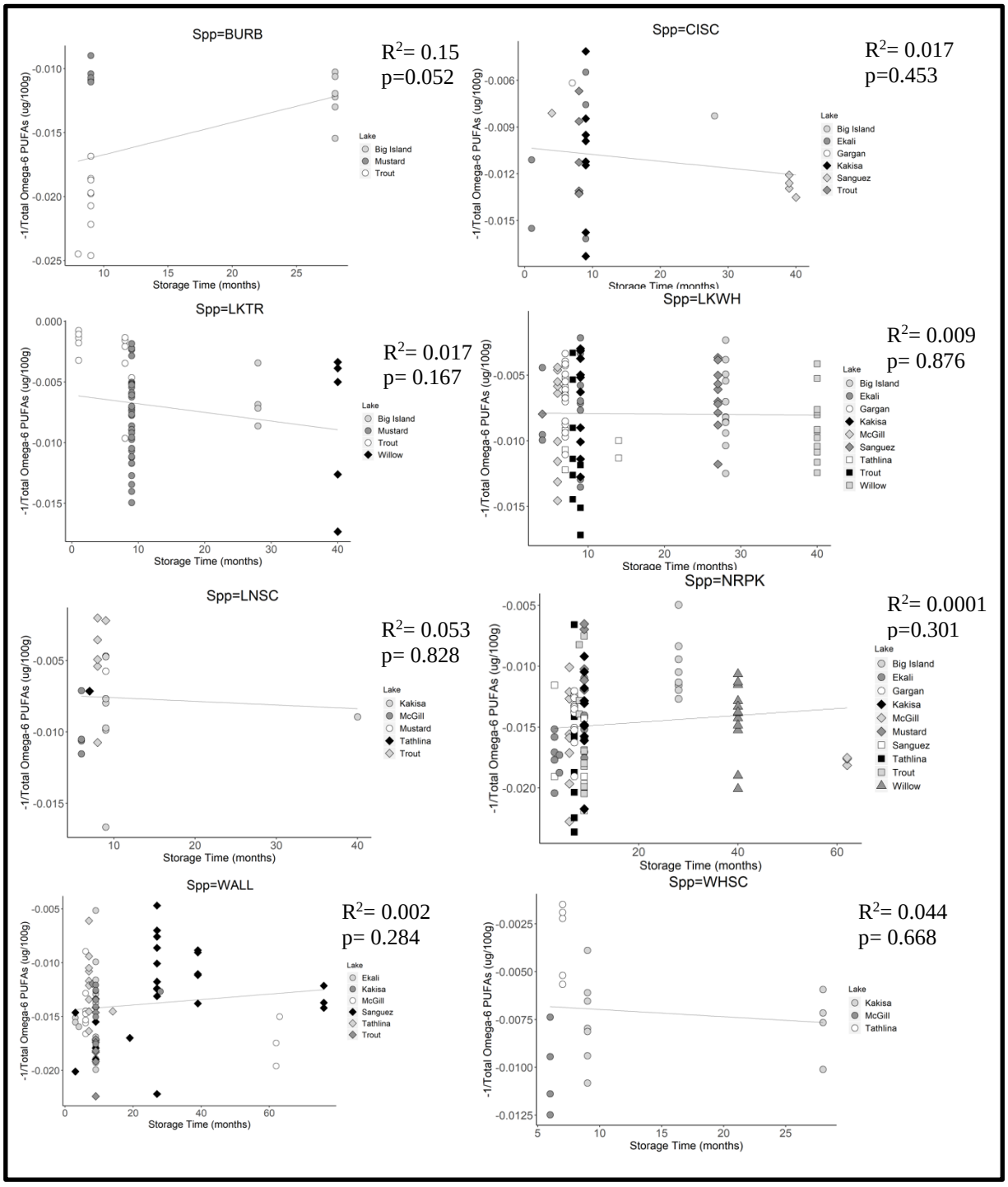

Figure A-4. n-6 PUFAs (reciprocally transformed) vs. storage time (months). BURB= Burbot, CISC= Cisco, LKTR= Lake Trout, LNSC= Longnose Sucker, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walley, and WHSC= White Sucker.

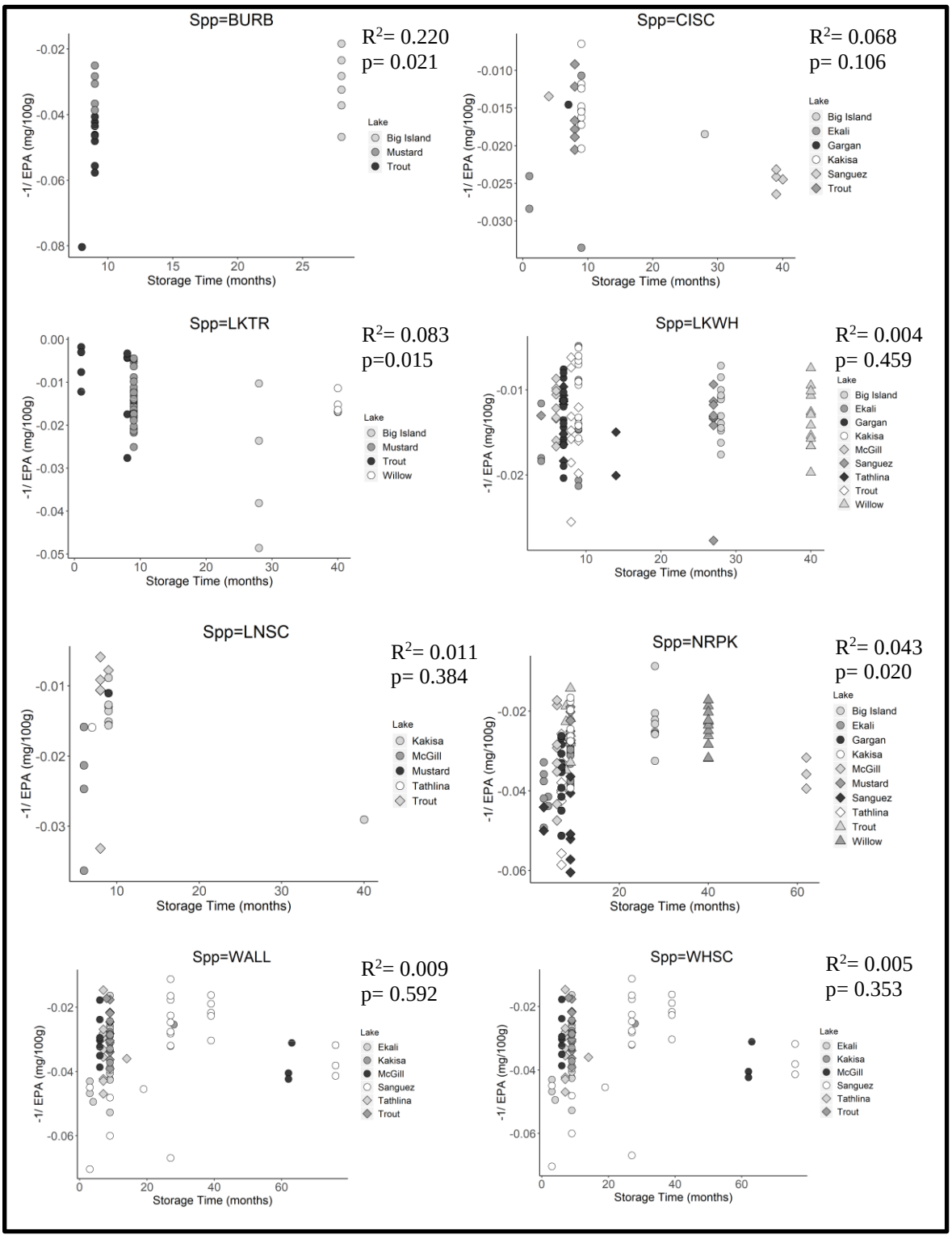

Figure A-5. EPA (reciprocally transformed) vs. storage time (months). BURB= Burbot, CISC= Cisco, LKTR= Lake Trout, LNSC= Longnose Sucker, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walley, and WHSC= White Sucker.

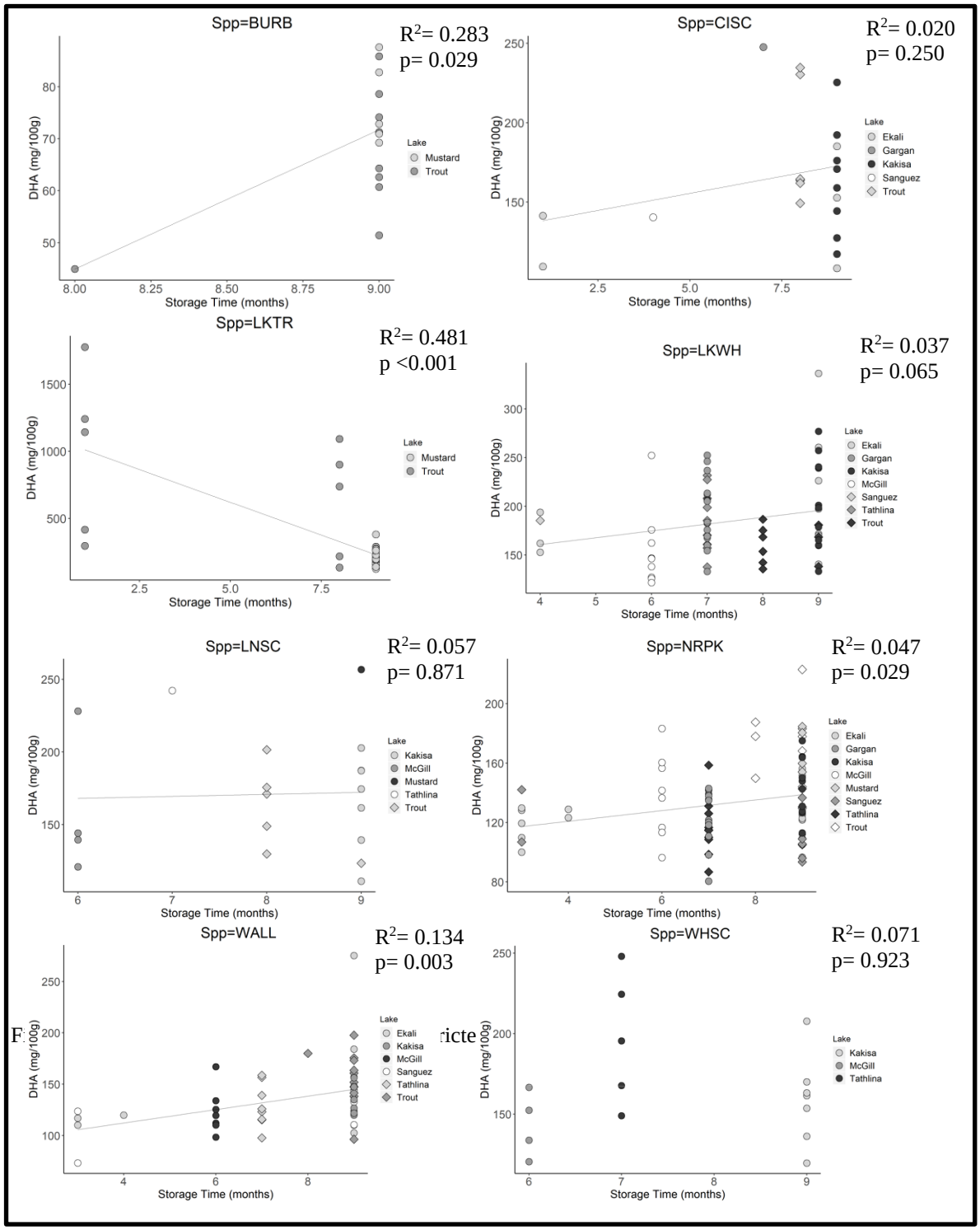

Figure A-6. DHA vs. storage time (months), restricted to samples stored for less than one year. BURB= Burbot, CISC= Cisco, LKTR= Lake Trout, LNSC= Longnose Sucker, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walley, and WHSC= White Sucker.

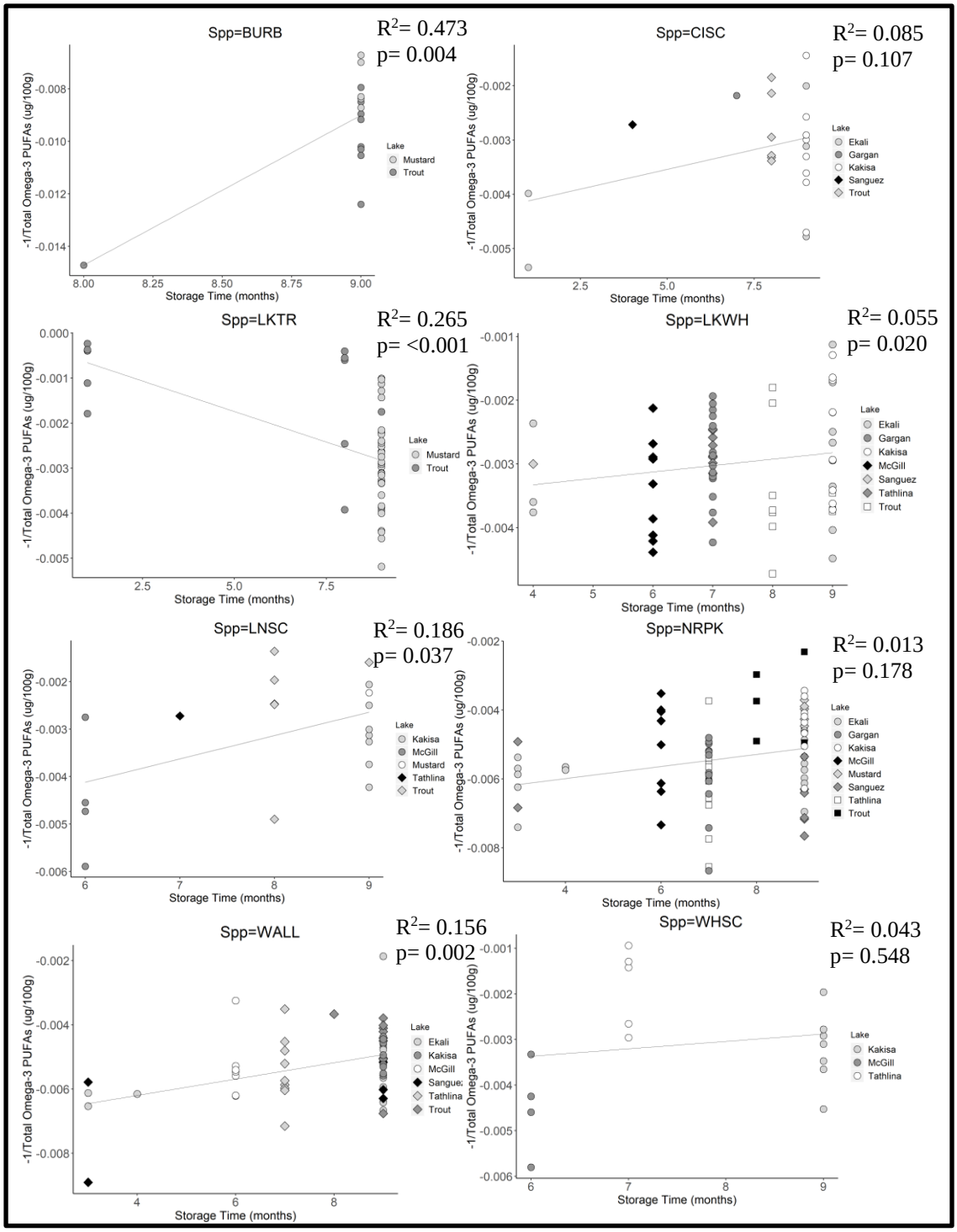

Figure A-7. Total n-3 PUFAs (reciprocally transformed) vs. storage time (months), restricted to samples stored for less than one year. BURB= Burbot, CISC= Cisco, LKTR= Lake Trout, LNSC= Longnose Sucker, LKWH= Lake Whitefish, NRPK= Northern Pike, WALL= Walley, and WHSC= White Sucker.

Appendix B . Full Fatty Acid Profiles

Table B-1. Fatty Acid Profiles: Arithmetic mean (± standard deviation) of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs). *Indicates that only samples stored for less than one year were used to calculate means.

Fatty Acid (mg/100g)	Burbot (n=20)	Cisco (n=26)	Lake Trout (n=59)	Lake Whitefish (n=104)	Longnose Sucker (n=20)	Northern Pike (n=105)	Walleye (n=79)	White Sucker (n=20)
C 10:0	0.04 ± 0.05	0.19 ± 0.27	0.34 ± 0.44	0.19 ± 0.23	0.11 ± 0.07	0.18 ± 0.26	0.21 ± 0.42	0.20 ± 0.15
C 12:0	0.25 ± 0.19	4.82 ± 13.7	2.67 ± 4.10	5.84 ± 8.48	1.61 ± 2.01	0.71 ± 0.96	2.90 ± 7.40	3.05 ± 6.13
C 14:0	2.30 ± 0.89	36.6 ± 31.2	53.3 ± 78.1	25.1 ± 33.9	43.4 ± 60.7	5.56 ± 4.97	7.45 ± 6.76	24.7 ± 35.6
C 16:0	80.0 ± 13.5	205 ± 78.3	358 ± 434	274 ± 173	263 ± 168	103 ± 34.5	133 ± 44.4	233 ± 152
C 17:0	1.75 ± 0.89	9.16 ± 6.10	13.2 ± 18.7	9.61 ± 14.9	8.33 ± 6.26	2.44 ± 1.81	3.77 ± 2.57	8.62 ± 6.53
C 18:0	28.8 ± 13.5	64.8 ± 34.2	107 ± 118	69.7 ± 42.5	70.3 ± 30.0	39.6 ± 22.3	49.7 ± 49.1	63.4 ± 24.9
C 20:0	0.77 ± 0.47	2.73 ± 1.58	5.17 ± 6.76	5.75 ± 6.74	3.24 ± 2.23	1.20 ± 1.19	1.77 ± 1.91	3.65 ± 2.59
C 22:0	0.31* ± 0.12	1.85 ± 1.12	1.91 ± 2.69	2.61 ± 2.21	1.34 ± 0.45	0.67 ± 0.64	1.17 ± 1.36	1.83 ± 0.57
C 24:0 *	0.29 ± 0.14	1.05* ± 0.77	0.85* ± 0.88	1.06* ± 0.57	0.79* ± 0.31	0.78* ± 0.58	0.78* ± 0.38	1.14* ± 0.30
Total SFAs *	**118* ± 14.5**	**345* ± 144**	**589* ± 700**	**409* ± 211**	**399* ± 270**	**155* ± 57.5**	**207* ± 107**	**363* ± 243**
C 12:1	0.05 ± 0.08	0.61* ± 0.61	0.92 ± 1.00	0.56 ± 0.83	0.78 ± 0.76	0.16 ± 0.31	0.12 ± 0.21	0.68 ± 0.81
C 14:1	0.07 ± 0.89	1.36 ± 1.73	2.54 ± 3.52	1.21 ± 1.84	2.26 ± 3.06	0.26 ± 0.48	0.34 ± 0.47	2.23 ± 3.83
C 16:1	5.54 ± 3.34	0.0* ± 0.0	194 ± 268	129 ± 138	247 ± 295	14.5 ± 22.6	20.4 ± 21.0	237 ± 288
C 18:1n-7	14.7 ± 6.59	46.4 ± 32.0	114 ± 154	80.5 ± 72.4	100 ± 80.0	14.9 ± 14.0	20.1 ± 12.5	112 ± 102
C 18:1n-9	28.7 ± 8.22	101* ± 52.0	396 ± 573	232 ± 244	158 ± 140	44.7 ± 26.8	55.7 ± 44.1	177 ± 209
C 20:1n-9	1.11 ± 0.61	3.56* ± 2.11	17.9 ± 25.4	10.7 ± 14.1	8.58 ± 7.43	1.21 ± 0.94	1.56 ± 1.79	6.44 ± 7.67
C 22:1n-9	0.60 ± 0.29	2.03 ± 1.22	3.58 ± 4.04	2.69 ± 2.67	1.50 ± 0.56	0.83 ± 0.59	1.08 ± 0.87	2.02 ± 1.02
C 24:1n-9	3.63 ± 2.25	4.54 ± 1.78	8.94 ± 9.78	4.85 ± 3.96	1.98* ± 0.68	3.08 ± 1.55	4.95 ± 2.13	1.51 ± 0.38
Total MUFAs	**54.4 ± 19.7**	**235* ± 140**	**739 ± 1024**	**462 ± 467**	**521 ± 522**	**79.7 ± 61.2**	**104 ± 79.2**	**539 ± 612**

* Indicates groups that were restricted due to a significant increase or decrease with storage time. An * on the name of the fatty acid (far left column) indicates that restrictions applied to every species; an * on a number indicates that it only applies to that fish species. Restricted sample sizes are as follows: Burbot (n=14), Cisco (n=21), Lake Trout (n=50), Lake Whitefish (n=66), Longnose Sucker (n=19), Northern Pike (n=81), Walleye (n= 55), and White Sucker (n=16).

Table B-2. Fatty Acid Profile: Arithmetic mean (± standard deviation) of n-6 PUFAs (omega-6 PUFAs), n-3 PUFAs (omega-3 PUFAs), and important fatty acid groups. *Indicates that only samples stored for less than one year were used to calculate means.

Fatty Acid (mg/100g)	Burbot (n=20)	Cisco (n=26)	Lake Trout (n=59)	Lake Whitefish (n=104)	Longnose Sucker (n=20)	Northern Pike (n=105)	Walleye (n=79)	White Sucker (n=20)
C 18:2n-6	5.63 ± 2.71	33.2 ± 20.3	79.5 ± 101	50.2 ± 58.6	81.9 ± 98.0	14.5 ± 8.76	12.5 ± 10.1	62.8 ± 69.4
C 18:3n-6	0.75 ± 0.39	2.55 ± 2.12	4.73 ± 5.66	4.09 ± 4.37	4.62 ± 5.26	0.96 ± 1.12	1.07 ± 0.88	7.96 ± 13.6
C 20:2n-6	1.57 ± 0.87	3.41 ± 2.19	14.5 ± 18.4	7.80 ± 9.78	5.21 ± 3.20	2.60 ± 1.75	1.90 ± 1.72	6.00 ± 5.97
C 20:3n-6	1.06 ± 0.32	2.65 ± 1.26	10.6 ± 11.2	4.76 ± 3.28	7.11 ± 5.69	1.34 ± 0.96	1.53 ± 1.10	6.72 ± 5.61
C 20:4n-6	47.2 ± 15.2	37.0 ± 13.2	80.8 ± 52.1	68.4 ± 29.8	63.2 ± 18.1	40.7 ± 16.1	42.3 ± 12.9	96.2 ± 57.9
C 22:2n-6	0.0* ± 0.0	1.35 ± 1.08	3.27 ± 5.00	1.83 ± 1.89	0.87 ± 0.68	0.93 ± 0.85	0.96 ± 1.02	1.00 ± 0.57
C 22:4n-6	2.02 ± 0.98	3.98 ± 2.13	18.9 ± 18.9	8.46 ± 6.78	3.56 ± 1.86	2.33 ± 1.26	3.63 ± 2.18	6.85 ± 5.96
C 22:5n-6	12.6 ± 4.13	19.5 ± 7.37	32.6 ± 38.6	16.7 ± 12.7	8.55 ± 3.16	11.4 ± 3.84	14.6 ± 6.67	11.7 ± 7.25
Total n-6 PUFAs	**71.3 ± 22.7**	**104 ± 42.7**	**245 ± 240**	**162 ± 120**	**175 ± 117**	**74.8 ± 27.4**	**78.4 ± 29.7**	**199 ± 160**
C 18:3n-3	2.48 ± 1.16	41.7 ± 28.9	70.9 ± 116	39.7 ± 44.99	58.9 ± 68.0	8.12 ± 7.58	8.63 ± 7.97	46.7 ± 52.6
C 18:4n-3	0.99 ± 0.35	22.4 ± 18.2	23.8 ± 39.8	13.8 ± 27.3	9.04 ± 9.49	2.86 ± 3.28	3.49 ± 2.85	18.5 ± 33.4
C 20:3n-3	1.27 ± 0.76	4.73 ± 2.35	11.7 ± 23.9	5.39 ± 6.11	4.91 ± 2.85	1.67 ± 1.23	2.50 ± 2.39	4.51 ± 3.28
C 20:4n-3	2.19 ± 0.47	15.4 ± 8.11	39.1 ± 76.8	8.47 ± 13.1	9.34 ± 8.33	3.36 ± 2.50	5.02 ± 5.88	7.44 ± 7.05
C 20:5n-3 (EPA)	27.8 ± 9.97	63.7 ± 26.6	103 ± 95.8	88.0 ± 46.2	76.6 ± 35.6	35.7 ± 14.0	34.8 ± 13.2	102 ± 73.3
C 22:5n-3 (DPA)	9.99 ± 3.69	22.6 ± 9.94	77.7 ± 86.7	31.8 ± 19.0	34.1 ± 15.1	15.8 ± 6.73	17.3 ± 10.2	40.7 ± 28.0
C 22:6n-3 (DHA) *	69.8* ± 12.3	167* ± 40.6	318* ± 335	185* ± 42.3	171* ± 41.9	133* ± 27.3	137* ± 31.0	167* ± 36.1
Total n-3 PUFAs *	**111* ± 22.0**	**351* ± 123**	**671* ± 793**	**368* ± 130**	**369* ± 148**	**197* ± 51.7**	**202* ± 60.1**	**406* ± 243**
Total PUFAs *	**177* ± 44.2**	**458* ± 167**	**928* ± 1043**	**524* ± 204**	**547* ± 262**	**268* ± 69.2**	**275* ± 80.7**	**622* ± 418**
Total HUFAs *	**166* ± 41.6**	**344* ± 99.6**	**722* ± 767**	**410* ± 125**	**382* ± 103**	**239* ± 53.6**	**248* ± 62.4**	**463* ± 238**
EPA+DHA *	**94.4* ± 18.6**	**235* ± 61.3**	**429* ± 430**	**272* ± 69.7**	**250* ± 62.5**	**166* ± 36.1**	**170* ± 39.1**	**277* ± 114**
EPA+DHA+DPA *	**104* ± 21.6**	**259* ± 70.6**	**511* ± 520**	**301* ± 81.2**	**284* ± 75.7**	**181* ± 41.4**	**185* ± 45.8**	**320* ± 145**
N-6/N-3 Ratio *	**0.59* ± 0.14**	**0.30* ± 0.05**	**0.40* ± 0.10**	**0.41* ± 0.10**	**0.46* ± 0.12**	**0.37* ± 0.08**	**0.37* ± 0.08**	**0.49* ± 0.09**
TFA	**361 ± 75.7**	**996 ± 403**	**2159 ± 2622**	**1415 ± 1024**	**1454 ± 1037**	**525 ± 182**	**610 ± 230**	**1464 ± 1212**

* Indicates groups that were restricted due to a significant increase or decrease with storage time. An * on the name of the fatty acid (far left column) indicates that restrictions applied to every species; an * on a number indicates that it only applies to that fish species. Restricted sample sizes are as follows: Burbot (n=14), Cisco (n=21), Lake Trout (n=50), Lake Whitefish (n=66), Longnose Sucker (n=19), Northern Pike (n=81), Walleye (n= 55), and White Sucker (n=16).

Table B-3. Fatty Acid Profiles: Arithmetic mean (± standard deviation) of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs; using all data, including samples stored for greater than one year). *Indicates data that is significantly affected by storage time.

Fatty Acid (mg/100g)	Burbot (n=20)	Cisco (n=26)	Lake Trout (n=59)	Lake Whitefish (n=104)	Longnose Sucker (n=20)	Northern Pike (n=105)	Walleye (n=79)	White Sucker (n=20)
C 10:0	0.04 ± 0.05	0.19 ± 0.27	0.34 ± 0.44	0.19 ± 0.23	0.11 ± 0.07	0.18 ± 0.26	0.21 ± 0.42	0.20 ± 0.15
C 12:0	0.25 ± 0.19	4.82 ± 13.7	2.67 ± 4.10	5.84 ± 8.48	1.61 ± 2.01	0.71 ± 0.96	2.90 ± 7.40	3.05 ± 6.13
C 14:0	2.30 ± 0.89	36.6 ± 31.2	53.3 ± 78.1	25.1 ± 33.9	43.4 ± 60.7	5.56 ± 4.97	7.45 ± 6.76	24.7 ± 35.6
C 16:0	80.0 ± 13.5	205 ± 78.3	358 ± 434	274 ± 173	263 ± 168	103 ± 34.5	133 ± 44.4	233 ± 152
C 17:0	1.75 ± 0.89	9.16 ± 6.10	13.2 ± 18.7	9.61 ± 14.9	8.33 ± 6.26	2.44 ± 1.81	3.77 ± 2.57	8.62 ± 6.53
C 18:0	28.8 ± 13.5	64.8 ± 34.2	107 ± 118	69.7 ± 42.5	70.3 ± 30.0	39.6 ± 22.3	49.7 ± 49.1	63.4 ± 24.9
C 20:0	0.77 ± 0.47	2.73 ± 1.58	5.17 ± 6.76	5.75 ± 6.74	3.24 ± 2.23	1.20 ± 1.19	1.77 ± 1.91	3.65 ± 2.59
C 22:0	0.62* ± 0.53	1.85 ± 1.12	1.91 ± 2.69	2.61 ± 2.21	1.34 ± 0.45	0.67 ± 0.64	1.17 ± 1.36	1.83 ± 0.57
C 24:0*	24.5* ± 39.4	51.4* ± 109	32.*3 ± 87.7	81.3* ± 113	10.9* ± 45.1	41.0* ± 78.3	55.1* ± 85.9	32.1* ± 64.5
Total SFAs*	139* ± 41.2	377* ± 151	575* ± 651	475* ± 269	402* ± 264	194* ± 96.2	255* ± 130	372* ± 219
C 12:1	0.05 ± 0.08	0.50* ± 0.59	0.92 ± 1.00	0.56 ± 0.83	0.78 ± 0.76	0.16 ± 0.31	0.12 ± 0.21	0.68 ± 0.81
C 14:1	0.07 ± 0.89	1.36 ± 1.73	2.54 ± 3.52	1.21 ± 1.84	2.26 ± 3.06	0.26 ± 0.48	0.34 ± 0.47	2.23 ± 3.83
C 16:1	5.54 ± 3.34	58.0* ± 53.5	194 ± 268	129 ± 138	247 ± 295	14.5 ± 22.6	20.4 ± 21.0	237 ± 288
C 18:1n-7	14.7 ± 6.59	46.4 ± 32.0	114 ± 154	80.5 ± 72.4	100 ± 80.0	14.9 ± 14.0	20.1 ± 12.5	112 ± 102
C 18:1n-9	28.7 ± 8.22	88.3* ± 53.6	396 ± 573	232 ± 244	158 ± 140	44.7 ± 26.8	55.7 ± 44.1	177 ± 209
C 20:1n-9	1.11 ± 0.61	3.03* ± 2.19	17.9 ± 25.4	10.7 ± 14.1	8.58 ± 7.43	1.21 ± 0.94	1.56 ± 1.79	6.44 ± 7.67
C 22:1n-9	0.60 ± 0.29	2.03 ± 1.22	3.58 ± 4.04	2.69 ± 2.67	1.50 ± 0.56	0.83 ± 0.59	1.08 ± 0.87	2.02 ± 1.02
C 24:1n-9	3.63 ± 2.25	4.54 ± 1.78	8.94 ± 9.78	4.85 ± 3.96	1.98* ± 0.68	3.08 ± 1.55	4.95 ± 2.13	1.51 ± 0.38
Total MUFAs	**54.4 ± 19.7**	**235* ± 140**	**739 ± 1024**	**462 ± 467**	**521 ± 522**	**79.7 ± 61.2**	**104 ± 79.2**	**539 ± 612**

Table B-4. Fatty Acid Profiles: Arithmetic mean (± standard deviation) of n-6 PUFAs (omega-6 PUFAs), n-3 PUFAs (omega-3 PUFAs), and important fatty acid groups (using all data, including samples stored for greater than one year). *Indicates data that is significantly affected by storage time.

Fatty Acid (mg/100g)	Burbot (n=20)	Cisco (n=26)	Lake Trout (n=59)	Lake Whitefish (n=104)	Longnose Sucker (n=20)	Northern Pike (n=105)	Walleye (n=79)	White Sucker (n=20)
C 18:2n-6	5.63 ± 2.71	33.2 ± 20.3	79.5 ± 101	50.2 ± 58.6	81.9 ± 98.0	14.5 ± 8.76	12.5 ± 10.1	62.8 ± 69.4
C 18:3n-6	0.75 ± 0.39	2.55 ± 2.12	4.73 ± 5.66	4.09 ± 4.37	4.62 ± 5.26	0.96 ± 1.12	1.07 ± 0.88	7.96 ± 13.6
C 20:2n-6	1.57 ± 0.87	3.41 ± 2.19	14.5 ± 18.4	7.80 ± 9.78	5.21 ± 3.20	2.60 ± 1.75	1.90 ± 1.72	6.00 ± 5.97
C 20:3n-6	1.06 ± 0.32	2.65 ± 1.26	10.6 ± 11.2	4.76 ± 3.28	7.11 ± 5.69	1.34 ± 0.96	1.53 ± 1.10	6.72 ± 5.61
C 20:4n-6	47.2 ± 15.2	37.0 ± 13.2	80.8 ± 52.1	68.4 ± 29.8	63.2 ± 18.1	40.7 ± 16.1	42.3 ± 12.9	96.2 ± 57.9
C 22:2n-6	1.35* ± 0.37	1.35 ± 1.08	3.27 ± 5.00	1.83 ± 1.89	0.87 ± 0.68	0.93 ± 0.85	0.96 ± 1.02	1.00 ± 0.57
C 22:4n-6	2.02 ± 0.98	3.98 ± 2.13	18.9 ± 18.9	8.46 ± 6.78	3.56 ± 1.86	2.33 ± 1.26	3.63 ± 2.18	6.85 ± 5.96
C 22:5n-6	12.6 ± 4.13	19.5 ± 7.37	32.6 ± 38.6	16.7 ± 12.7	8.55 ± 3.16	11.4 ± 3.84	14.6 ± 6.67	11.7 ± 7.25
Total n-6 PUFAs	**71.3 ± 22.7**	**104 ± 42.7**	**245 ± 240**	**162 ± 120**	**175 ± 117**	**74.8 ± 27.4**	**78.4 ± 29.7**	**199 ± 160**
C 18:3n-3	2.48 ± 1.16	41.7 ± 28.9	70.9 ± 116	39.7 ± 44.99	58.9 ± 68.0	8.12 ± 7.58	8.63 ± 7.97	46.7 ± 52.6
C 18:4n-3	0.99 ± 0.35	22.4 ± 18.2	23.8 ± 39.8	13.8 ± 27.3	9.04 ± 9.49	2.86 ± 3.28	3.49 ± 2.85	18.5 ± 33.4
C 20:3n-3	1.27 ± 0.76	4.73 ± 2.35	11.7 ± 23.9	5.39 ± 6.11	4.91 ± 2.85	1.67 ± 1.23	2.50 ± 2.39	4.51 ± 3.28
C 20:4n-3	2.19 ± 0.47	15.4 ± 8.11	39.1 ± 76.8	8.47 ± 13.1	9.34 ± 8.33	3.36 ± 2.50	5.02 ± 5.88	7.44 ± 7.05
C 20:5n-3 (EPA)	27.8 ± 9.97	63.7 ± 26.6	103 ± 95.8	88.0 ± 46.2	76.6 ± 35.6	35.7 ± 14.0	34.8 ± 13.2	102 ± 73.3
C 22:5n-3 (DPA)	9.99 ± 3.69	22.6 ± 9.94	77.7 ± 86.7	31.8 ± 19.0	34.1 ± 15.1	15.8 ± 6.73	17.3 ± 10.2	40.7 ± 28.0
C 22:6n-3 (DHA)*	51.4* ± 30.5	138* ± 69.1	272* ± 327	127* ± 101	163* ± 53.6	108* ± 63.5	99.5* ± 62.2	134* ± 74.5
Total n-3 PUFAs*	**96.6* ± 30.2**	**310* ± 139**	**601* ± 749**	**316* ± 224**	**356* ± 155**	**176* ± 74.8**	**172* ± 72.7**	**354* ± 241**
Total PUFAs*	**168* ± 42.0**	**414* ± 176**	**846* ± 982**	**478* ± 334**	**531* ± 265**	**251* ± 91.1**	**251* ± 89.8**	**554* ± 397**
Total HUFAs*	**155* ± 40.2**	**306* ± 119**	**649* ± 727**	**358* ± 201**	**371* ± 112**	**220* ± 80.6**	**219* ± 74.4**	**410* ± 238**
EPA+DHA*	**79.2* ± 29.0**	**202* ± 88.0**	**376* ± 416**	**215* ± 132**	**240* ± 75.4**	**144* ± 66.4**	**134* ± 64.1**	**236* ± 131**
EPA+DHA+DPA*	**104* ± 21.6**	**259* ± 70.6**	**512* ± 520**	**315* ± 138**	**284* ± 75.6**	**185* ± 55.2**	**185* ± 45.8**	**320* ± 144**
N-6/N-3 Ratio*	**40.9* ± 63.9**	**12.1* ± 24.8**	**14.0* ± 33.6**	**29.9* ± 40.7**	**5.42* ± 22.2**	**20.9* ± 39.9**	**27.1* ± 41.1**	**18.7* ± 37.5**
TFA	**361 ± 75.7**	**996 ± 403**	**2159 ± 2622**	**1415 ± 1024**	**1454 ± 1037**	**525 ± 182**	**610 ± 230**	**1464 ± 1212**

Appendix C. Correlations with Mercury

Table C-1. Range and arithmetic mean mercury concentrations (± standard deviation) for Burbot, Cisco, Lake Trout, and Lake Whitefish. Means have not been adjusted for fish length. Numbers in brackets represent number of samples.

Lake	Burbot		Cisco		Lake Trout		Lake Whitefish	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Big Island	0.07-0.68	0.21 ± 0.24 (6)	-	0.05 (1)	0.35-0.87	0.08 ± 0.22 (4)	0.02-0.17	0.08 ± 0.05 (13)
Ekali	-	-	0.07-0.19	0.11 ± 0.06 (5)	-	-	0.06-0.13	0.08 ± 0.02 (11)
Gargan	-	-	-	0.08 (1)	-	-	0.05-0.25	0.13 ± 0.06 (16)
Kakisa	-	-	0.04-0.05	0.04 ± 0.003 (8)	-	-	0.02-0.08	0.04 ± 0.02 (10)
McGill	-	-	-	-	-	-	0.09-0.32	0.17 ± 0.08 (9)
Mustard	0.09-0.13	0.11 ± 0.02 (5)	-	-	0.08-0.51	0.19 ± 0.08 (39)	-	-
Sanguez	-	-	0.08-0.13	0.10 ± 0.02 (5)	-	-	0.07-0.26	0.17 ± 0.05 (12)
Tathlina	-	-	-	-	-	-	0.04-0.13	0.09 ± 0.03 (11)
Trout	0.23-0.55	0.32 ± 0.10 (9)	0.03-0.04	0.04 ± 0.002 (6)	0.21-0.64	0.32 ± 0.15 (11)	0.03-0.06	0.04 ± 0.01 (10)
Willow	-	-	-	-	0.02-0.60	0.33 ± 0.28 (5)	0.01-0.27	0.09 ± 0.09 (12)
Total	0.07-0.68	0.23 ± 0.16 (20)	0.03-0.19	0.07 ± 0.04 (26)	0.20-0.86	0.25 ± 0.17 (59)	0.01-0.32	0.10 ± 0.07 (104)

Table C-2. Range and arithmetic mean mercury concentrations (± standard deviation) for Longnose Sucker, Northern Pike, Walleye, and White Sucker. Means have not been adjusted for fish length. Numbers in brackets represent number of samples.

Lake	Longnose Sucker		Northern Pike		Walleye		White Sucker	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Big Island	-	-	0.24-0.49	0.32 ± 0.08 (8)	-	-	-	-
Ekali	-	-	0.13-1.10	0.42 ± 0.29 (16)	0.10-0.46	0.28 ± 0.10 (15)	-	-
Gargan	-	-	0.16-1.24	0.41 ± 0.32 (15)	-	-	-	-
Kakisa	0.02-0.16	0.08 ± 0.04 (8)	0.04-0.91	0.30 ± 0.27 (10)	0.11-0.67	0.26 ± 0.19 (9)	0.03-0.15	0.09 ± 0.04 (11)
McGill	0.12-0.37	0.25 ± 0.13 (4)	0.12-1.43	0.46 ± 0.36 (11)	0.51-1.16	0.94 ± 0.23 (10)	0.13-0.19	0.17 ± 0.28 (4)
Mustard	-	0.15 (1)	0.08-0.26	0.16 ± 0.07 (5)	-	-	-	-
Sanguez	-	-	0.29-3.12	1.39 ± 0.91 (8)	0.13-1.43	0.62 ± 0.30 (24)	-	-
Tathlina	-	0.19 (1)	0.1-0.99	0.37 ± 0.31 (10)	0.15-0.99	0.58 ± 0.27 (10)	0.11-0.29	0.13± 0.07 (5)
Trout	0.09-0.13	0.10 ± 0.01 (10)	0.07-0.29	0.15 ± 0.08 (10)	0.04-0.85	0.29 ± 0.31 (11)	-	-
Willow	-	-	0.12-1.06	0.39 ± 0.30 (12)	-	-	-	-
Total	0.02-0.37	0.13 ± 0.09 (20)	0.04-3.12	0.43 ± 0.45 (105)	0.04-1.43	0.50 ± 0.33 (79)	0.01-0.29	0.13 ± 0.07 (20)

Table C-3. Spearman rank correlations between TFA[1] vs. mercury, n-6 PUFAs[2] vs. mercury, and EPA[3] vs. mercury for all species (all samples; N=433).

Species	Lake	N	Hg vs. TFA	Hg vs n-6 PUFAs	Hg vs. EPA
Burbot	Big Island	6	-.829*	-1.000**	-.943**
	Mustard	5	-0.800	-0.300	-0.100
	Trout	9	-0.310	-0.293	-0.435
	Total	**20**	**-.897****	**-.822****	**-.883****
Cisco	Big Island	1	-	-	-
	Ekali	5	0.100	0.100	0.100
	Gargan	1	-	-	-
	Kakisa	8	-.714*	-.738*	-0.429
	Sanguez	5	0.872	.975**	0.359
	Trout	6	0.058	0.319	0.319
	Total	**26**	**-0.211**	**-0.076**	**-.434***
Lake Trout	Big Island	6	-0.800	-0.800	-0.400
	Mustard	39	-.556**	-.531**	-.729**
	Trout	11	-.918**	-.945**	-.927**
	Willow	5	0.700	0.600	0.500
	Total	**61**	**0.013**	**0.058**	**-.385****
Lake Whitefish	Big Island	13	.570*	0.474	0.058
	Ekali	11	0.318	0.318	0.291
	Gargan	16	0.041	-0.153	-0.265
	Kakisa	10	0.091	0.212	-0.018
	McGill	9	-0.250	-0.050	-0.033
	Sanguez	11	0.000	0.078	0.269
	Tathlina	11	-0.191	-0.273	-0.255
	Trout	10	-0.212	-0.164	-0.479
	Willow	12	0.140	0.368	-0.238
	Total	**103**	**.199***	**.231***	**-0.040**
Longnose Sucker	Kakisa	8	0.647	0.240	0.359
	McGill	4	-0.200	0.400	-0.200
	Mustard	1	-	-	-
	Tathlina	1	-	-	-
	Trout	6	-0.429	-0.314	-0.314
	Total	**20**	**-0.116**	**-0.038**	**-0.315**
Northern Pike	Big Island	8	0.095	-0.024	-0.190
	Ekali	16	0.215	-.618*	-.768**
	Gargan	14	-0.431	-0.222	-.660*
	Kakisa	10	-0.212	0.212	-0.382
	McGill	11	-.700*	-0.318	-.727*
	Mustard	5	-0.400	-0.400	-0.400
	Sanguez	8	-0.405	-0.024	-0.429
	Tathlina	10	-0.042	-0.115	0.188
	Trout	10	-0.079	0.115	-0.406
	Willow	12	-0.081	.806**	0.147
	Total	**99**	**-.316****	**-0.150**	**-.559****

[1]Total fatty acids, [2]omega-6 polyunsaturated fatty acids, [3]eicosapentaenoic acid

Table C-3. Continued from above

Species	Lake	N	Hg vs. TFA	Hg vs n-6 PUFAs	Hg vs. EPA
Walleye	Ekali	15	-0.061	-0.264	-0.400
	Kakisa	9	-0.117	-0.100	-0.600
	McGill	10	-0.128	-0.176	-0.456
	Sanguez	24	-0.379	-0.311	-.430*
	Tathlina	10	-0.418	-0.103	-0.370
	Trout	11	-0.473	-.645*	-0.536
	Total	**79**	**-.250***	**-0.084**	**-.319****
White Sucker	Kakisa	11	.683*	0.533	.697*
	McGill	4	0.632	0.316	0.632
	Tathlina	5	0.359	0.359	0.359
	Total	**20**	**.508***	**.482***	**.463***

Table C-4. Spearman rank correlations between total n-3 PUFAs (omega-3 polyunsaturated fatty acids) vs. mercury, and DHA (docosahexaenoic acid) vs. mercury all species (samples stored for less than one year; N=317).

Species	Lake	N	Hg vs. n-3 PUFAs	Hg vs. DHA
Burbot	Mustard	5	-0.600	-0.700
	Trout	9	-0.310	-0.117
	Total	**14**	**-.667****	**-0.469**
Cisco	Ekali	5	-0.300	-0.700
	Gargan	1	-	-
	Kakisa	8	-.714*	-0.571
	Sanguez	5	-	.975**
	Trout	6	0.580	0.696
	Total	**19**	**-0.436**	**-.576****
Lake Trout	Mustard	39	-.631**	-.404*
	Trout	11	-.927**	-0.550
	Total	**50**	**-0.195**	**0.079**
Lake Whitefish	Ekali	11	0.209	0.200
	Gargan	16	-0.488	-.538*
	Kakisa	10	0.006	0.103
	McGill	9	0.000	-0.167
	Tathlina	9	0.017	-0.217
	Trout	10	-0.333	0.067
	Total	**65**	**-0.081**	**-0.205**
Longnose Sucker	Kakisa	7	0.750	0.750
	McGill	4	-0.200	-0.200
	Trout	6	-0.429	0.314
	Total	**17**	**-0.157**	**0.124**
Northern Pike	Ekali	16	-0.371	-.574*
	Gargan	14	-.675**	-.691**
	Kakisa	10	-0.467	-0.479
	McGill	8	-.738*	-0.643
	Mustard	5	-.900*	-.900*
	Sanguez	8	-0.238	0.048
	Tathlina	10	-0.055	0.079
	Trout	10	-0.067	0.200
	Total	**81**	**-.583****	**-.555****
Walleye	Ekali	15	-0.457	-0.496
	Kakisa	8	-0.643	-.881**
	McGill	7	0.144	0.054
	Sanguez	5	-0.300	0.200
	Tathlina	9	-0.467	-0.450
	Trout	11	-0.436	-0.336
	Total	**55**	**-.455****	**-.694****
White Sucker	Kakisa	7	.893**	0.679
	McGill	4	0.316	0.316
	Tathlina	5	0.359	0.359
	Total	**16**	**.543***	**.548***

Appendix D. Biological Influences on Fatty Acids

Table D-1. Biological predictors of TFA[1], n-3 PUFAs[2], n-6 PUFAs[3], EPA[4], and DHA[5] for all species.

Species	TFA	n-3 PUFAs	n-6 PUFAs	EPA	DHA
Burbot	-	-	-	-	-
Cisco	-	-	-	-	-
Lake Trout	-	-	-	-	-
Lake Whitefish	Fork Length (+)	-	-	-	-
Longnose Sucker	-	-	-	-	-
Northern Pike	-	-	-	Fork Length (-)	Log Age (-)
Walleye	-	-	-	Fork Length (-)	-
White Sucker	Fork Length (+)	Fork Length (+)	-	-	-

[1]Total fatty acids, [2]omega-3 polyunsaturated fatty acids, [3]omega-6 polyunsaturated fatty acids, [4]eicosapentaenoioc acid, and [5]docosahexaenoic acid

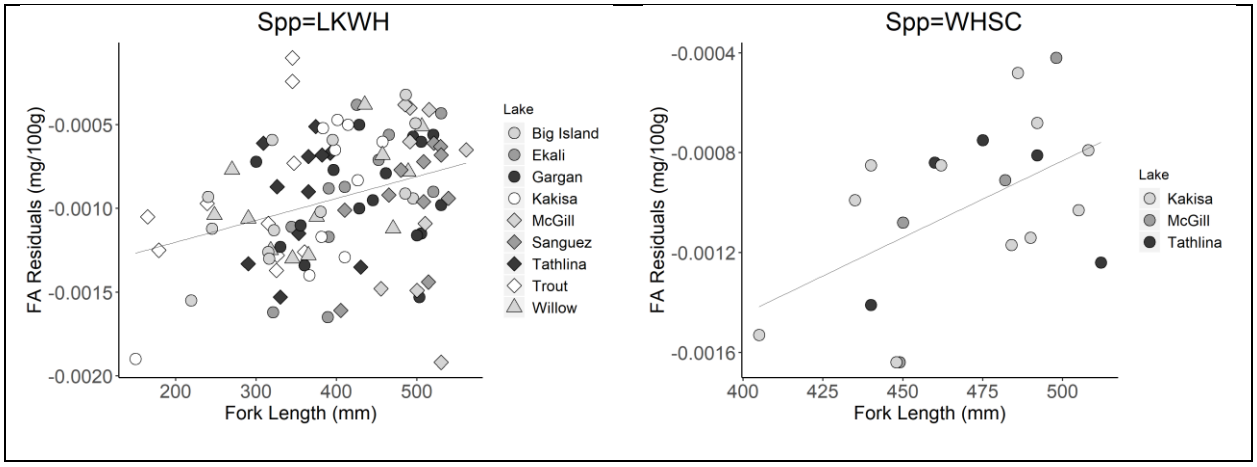

Figure D-1. Regressions between TFA and fork length (mm). Abbreviations are FA= Total fatty acids, LKWH= Lake Whitefish, and WHSC= White Sucker.

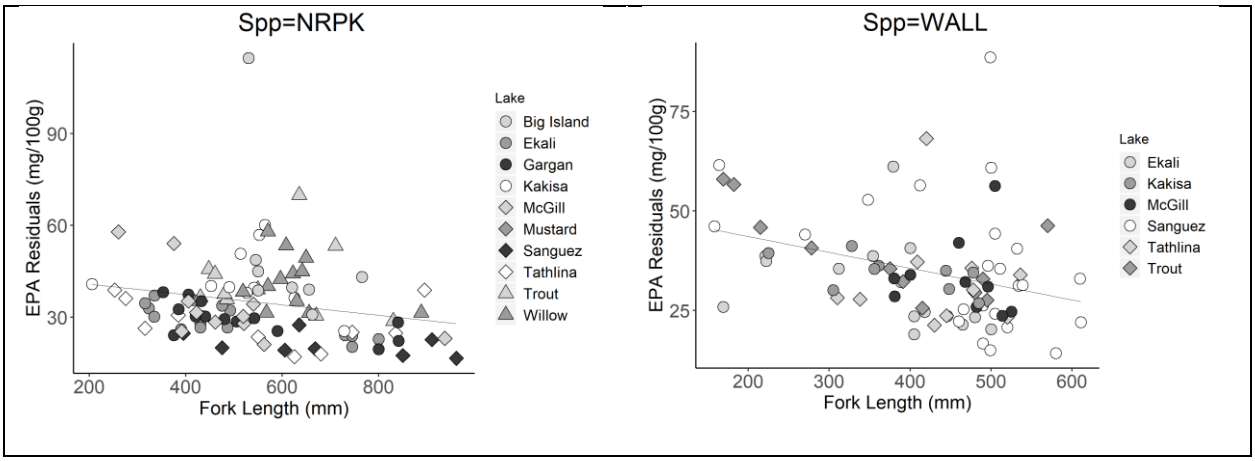

Figure D-2. Regression between EPA and fork length (mm). Abbreviations are EPA= Eicosapentaenoic Acid, NRPK= Northern Pike, and WALL= Walleye.

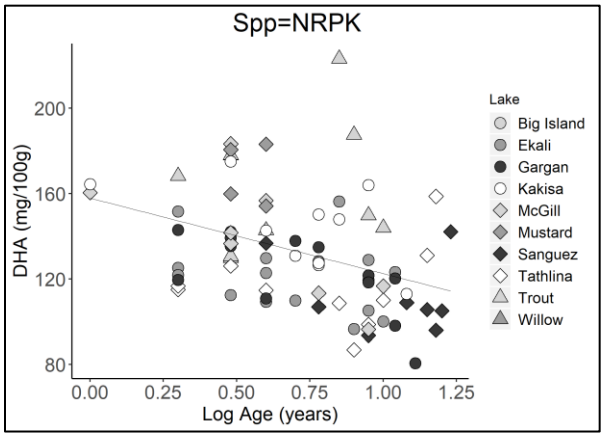

Figure D-3. Northern Pike DHA vs Log Age (Years). Abbreviations are DHA= Docosahexaenoic Acid, and NRPK= Northern Pike.

Table D-2. P-values from Tukey's test highlighting pairwise comparisons among lakes for Lake Whitefish total fatty acids (TFA) *Indicates a significant pairwise difference.

Lake Comparisons		Tukey's p-val
Big Island:	Ekali	1
Big Island:	Gargan	1
Big Island:	Kakisa	0.8846
Big Island:	McGill	0.4093
Big Island:	Sanguez	0.9968
Big Island:	Tathlina	1
Big Island:	Trout	1
Big Island:	Willow	0.9687
Ekali:	Gargan	1
Ekali:	Kakisa	0.9623
Ekali:	McGill	0.2644
Ekali:	Sanguez	0.9833
Ekali:	Tathlina	1
Ekali:	Trout	0.9998
Ekali:	Willow	0.9343
Gargan:	Kakisa	0.7992
Gargan:	McGill	0.3347
Gargan:	Sanguez	0.998
Gargan:	Tathlina	1
Gargan:	Trout	1
Gargan:	Willow	0.9854
Kakisa:	McGill	0.0297*
Kakisa:	Sanguez	0.5082
Kakisa:	Tathlina	0.9293
Kakisa:	Trout	0.764
Kakisa:	Willow	0.2659
McGill:	Sanguez	0.8272
McGill:	Tathlina	0.4203
McGill:	Trout	0.7944
McGill:	Willow	0.937
Sanguez:	Tathlina	0.9956
Sanguez:	Trout	1
Sanguez:	Willow	1
Tathlina:	Trout	0.9999
Tathlina:	Willow	0.9637
Trout:	Willow	0.9994

Table D-3. P-values from Tukey's test highlighting pairwise comparisons among lakes for Northern Pike EPA and DHA. *Indicates a significant pairwise difference.

NRPK	EPA		NRPK	DHA	
Lake Comparisons		Tukey's p-val	Lake Comparisons		Tukey's p-val
Big Island:	Ekali	<.0001*	Ekali:	Gargan	0.9992
Big Island:	Gargan	0.0001*	Ekali:	Kakisa	0.0999
Big Island:	Kakisa	0.9411	Ekali:	McGill	0.5344
Big Island:	McGill	0.0026*	Ekali:	Sanguez	0.9998
Big Island:	Sanguez	<.0001*	Ekali:	Tathlina	1
Big Island:	Tathlina	<.0001*	Ekali:	Trout	<.0001*
Big Island:	Trout	0.9769	Gargan:	Kakisa	0.3192
Big Island:	Willow	0.9998	Gargan:	McGill	0.8423
Ekali:	Gargan	0.9999	Gargan:	Sanguez	0.984
Ekali:	Kakisa	0.0011*	Gargan:	Tathlina	0.9894
Ekali:	McGill	0.9767	Gargan:	Trout	0.0004*
Ekali:	Sanguez	0.0753	Kakisa:	McGill	0.9978
Ekali:	Tathlina	0.9992	Kakisa:	Sanguez	0.1142
Ekali:	Trout	0.0005*	Kakisa:	Tathlina	0.089
Ekali:	Willow	<.0001*	Kakisa:	Trout	0.3667
Gargan:	Kakisa	0.0066*	McGill:	Sanguez	0.4651
Gargan:	McGill	0.9995	McGill:	Tathlina	0.4436
Gargan:	Sanguez	0.029*	McGill:	Trout	0.1327
Gargan:	Tathlina	0.9795	Sanguez:	Tathlina	1
Gargan:	Trout	0.0032*	Sanguez:	Trout	0.0002*
Gargan:	Willow	0.0002*	Tathlina:	Trout	0.0001*
Kakisa:	McGill	0.0661			
Kakisa:	Sanguez	<.0001*			
Kakisa:	Tathlina	0.0006*			
Kakisa:	Trout	1			
Kakisa:	Willow	0.9963			
McGill:	Sanguez	0.0106*			
McGill:	Tathlina	0.8232			
McGill:	Trout	0.0396*			
McGill:	Willow	0.0043*			
Sanguez:	Tathlina	0.3645			
Sanguez:	Trout	<.0001*			
Sanguez:	Willow	<.0001*			
Tathlina:	Trout	0.0003*			
Tathlina:	Willow	<.0001*			
Trout:	Willow	0.9995			

Table D-4. P-values from Tukey's test highlighting pairwise comparisons among lakes for Walleye EPA.

Lake Comparisons		Tukey's p-val
Ekali:	Kakisa	0.687
Ekali:	McGill	0.5323
Ekali:	Sanguez	0.524
Ekali:	Tathlina	0.8639
Ekali:	Trout	0.3799
Kakisa:	McGill	0.9999
Kakisa:	Sanguez	1
Kakisa:	Tathlina	0.9996
Kakisa:	Trout	0.9993
McGill:	Sanguez	0.9995
McGill:	Tathlina	0.9939
McGill:	Trout	1
Sanguez:	Tathlina	0.9995
Sanguez:	Trout	0.997
Tathlina:	Trout	0.9833

Appendix E. Environmental PCAs

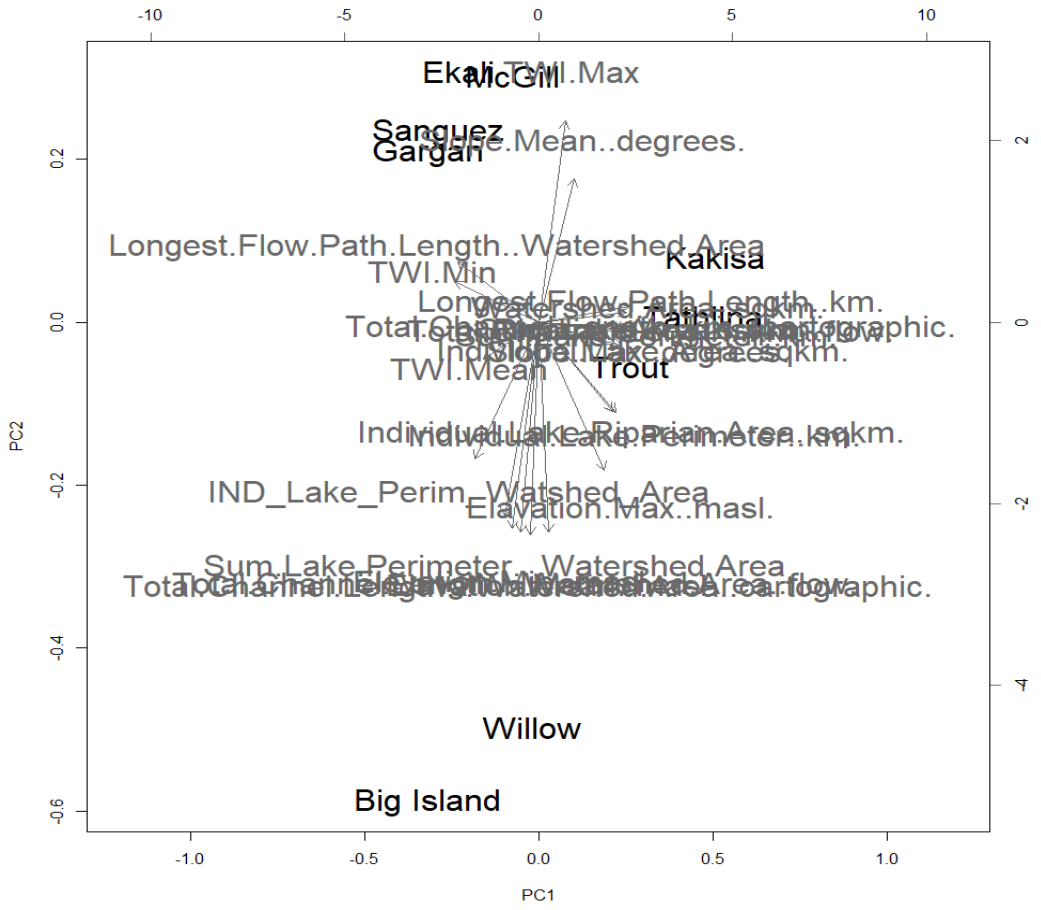

Figure E-1. PCA of all watershed parameters. PC1 explains 55.18% of the variation, and PC2 explains 31.19%. PC1 and PC2 combined explain 86.37% of the variation. PC1 is influenced most strongly by total channel length (the longest channel measured in km, not including the flow path through a lake; positive loadings) as well as the minimum topographic wetness index. PC2 separates lakes along a spectrum of total channel length to watershed area ratio (negative loadings) as well as the maximum topographic wetness index (positive loadings).

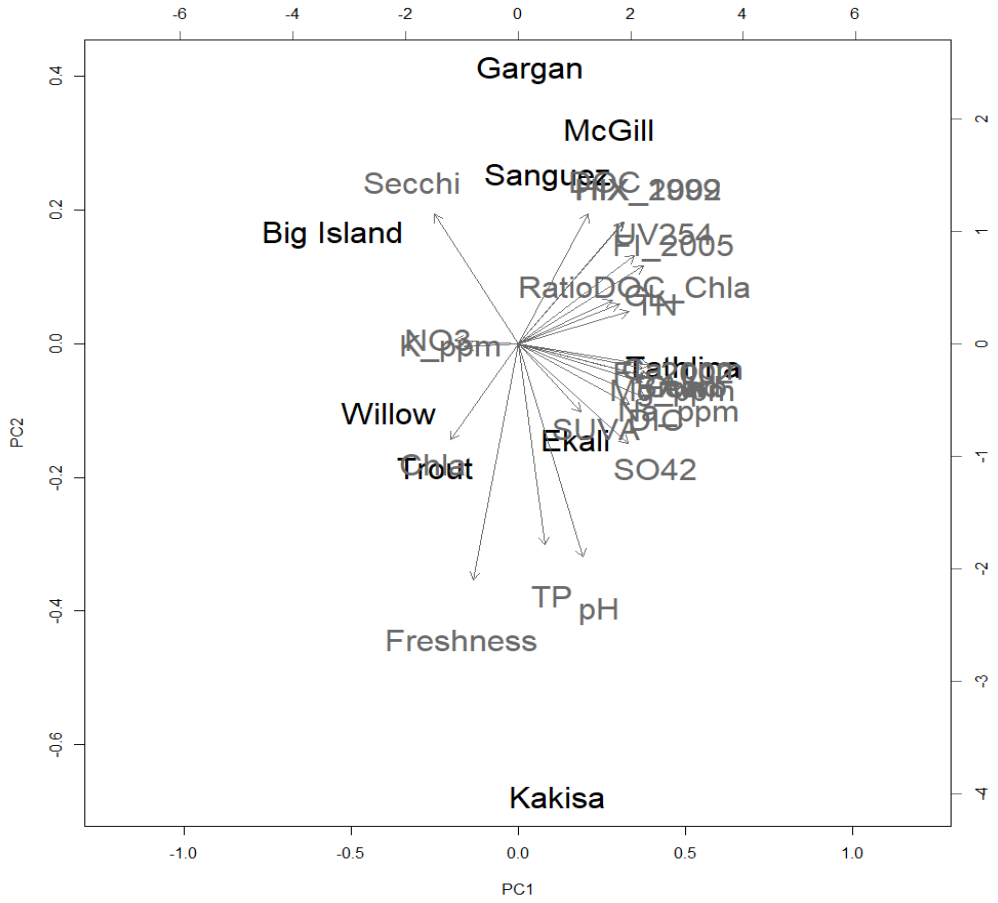

Figure E-2. PCA of all water chemistry variables. PC1 explains 56.69% of the variation, and PC2 explains 14.27%. PC1 and PC2 combined explain 70.96% of the variation. PC1 is influenced most strongly by conductivity (positive loadings) and to a lesser extent by secchi depth (negative loadings). PC2 separates lakes along a spectrum of the carbon freshness index (negative loadings) as well as dissolved organic carbon (positive loadings).

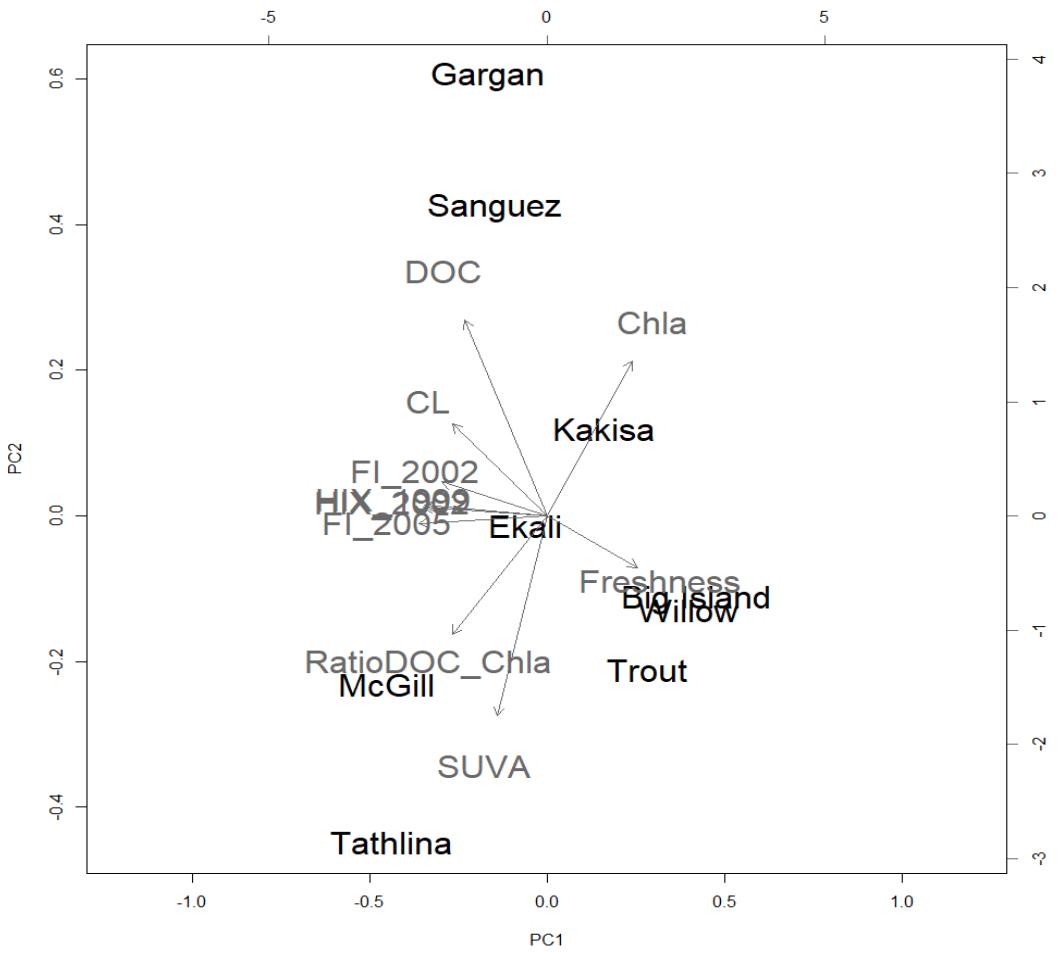

Figure E-3. PCA of all EEMs data. PC1 explains 56.26% of the variation, and PC2 explains 17.15%. PC1 and PC2 combined explain 73.42% of the variation. PC1 is influenced most strongly by lake fluorescence index (negative loadings) as well as the carbon freshness (positive loadings). PC2 separates lakes along a spectrum of specific UV absorbance (SUVA; negative loadings) as well as dissolved organic carbon (positive loadings).

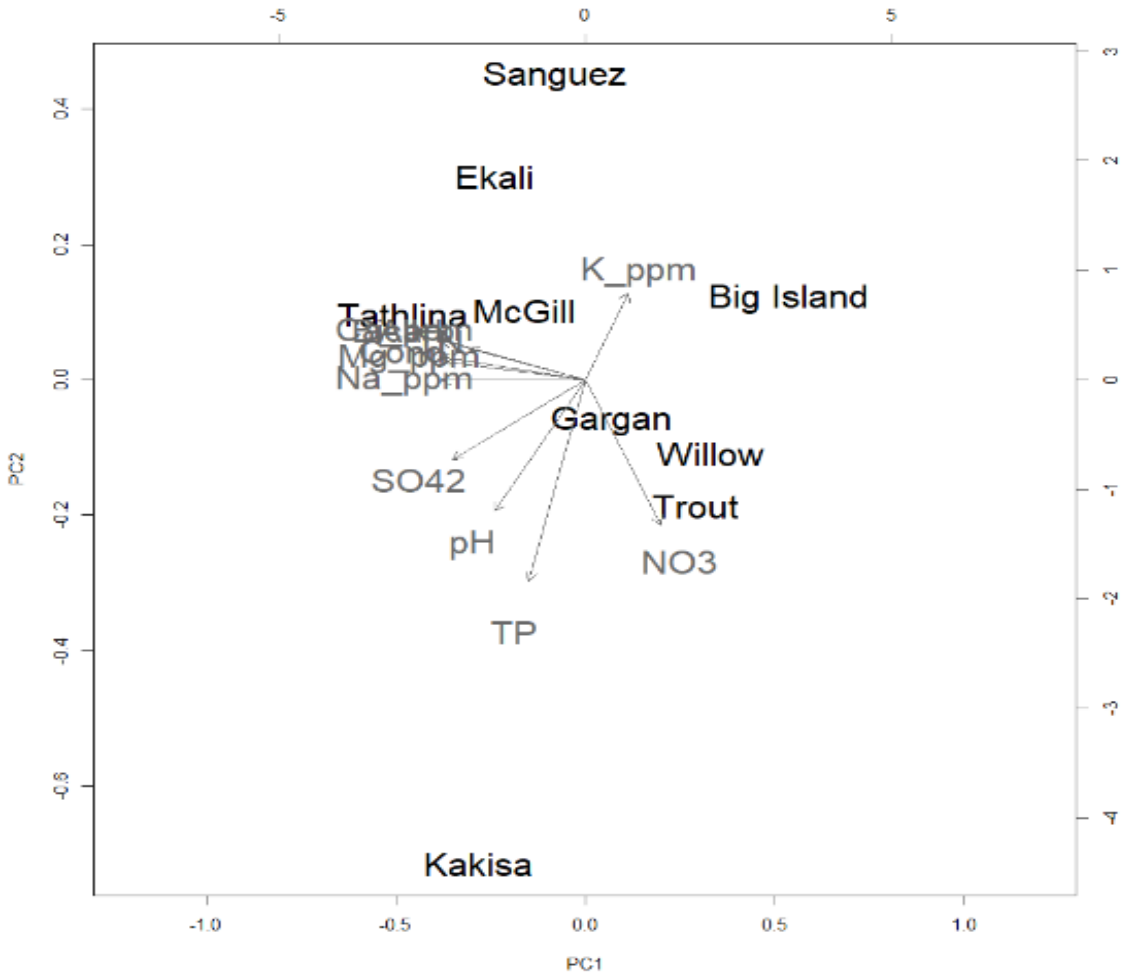

Figure E-4. PCA of all environmental variables (excluding EEMs data). PC1 explains 66.93% of the variation, and PC2 explains 12.00%. PC1 and PC2 combined explain 78.93% of the variation. PC1 is influenced most strongly by conductivity (positive loadings) as well as secchi depth (negative loadings). PC2 also separates lakes along a spectrum of secchi depth (negative loadings) as well as nitrate (positive loadings).

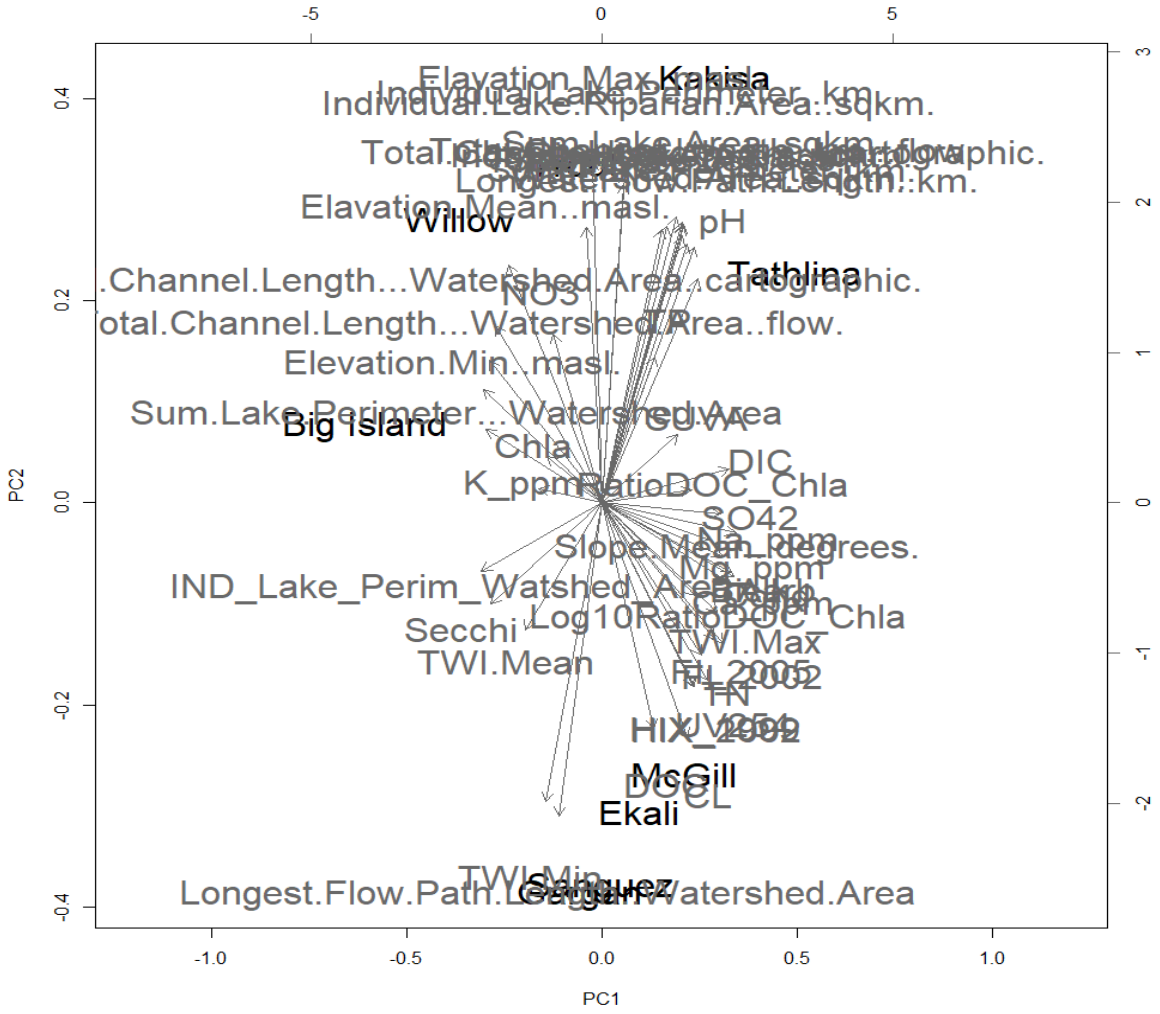

Figure E-5. PCA of combined environmental and watershed variables. PC1 explains 44.98% of the variation, and PC2 explains 28.74%. PC1 and PC2 combined explain 73.72% of the variation.PC1 is influenced most strongly by sodium ions (positive loadings) as well as the ratio of lake perimeter to watershed area (calculated for each individual lake; negative loadings). PC2 separates lakes along a spectrum of maximum watershed elevation (positive loadings) as well as the ratio of the longest flow path length to watershed area (negative loadings).

Appendix F. Correlation Analyses

Table F-1. Pearson's Correlation Coefficients among select parameters.

	DOC	**TP**	**TN**	**CL**	**Ca**	**UV254**	**EEMs PC2**	**Lake Perimeter (Sum) to Watershed Area Ratio (LP to WA)**	Min Elevation	Max Elevation	Mean Elevation	Chl-a	Lake Area
DOC	1.000												
TP	0.006	1.000											
TN	**0.793**	0.018	1.000										
CL	**0.575**	-0.094	**0.698**	1.000									
Ca_ppm	**0.502**	-0.016	**0.815**	**0.797**	1.000								
UV254	**0.798**	-0.122	**0.903**	**0.646**	**0.789**	1.000							
EEMs PC2	**0.715**	-0.043	0.350	0.335	-0.035	0.233	1.000						
LP to WA	-0.419	-0.060	**-0.534**	**-0.609**	**-0.706**	**-0.709**	-0.060	1.000					
Min Elevation	**-0.582**	0.037	**-0.702**	**-0.743**	**-0.792**	**-0.789**	-0.246	**0.963**	1.000				
Max Elevation	**-0.656**	-0.121	-0.467	**-0.711**	-0.255	-0.491	**-0.672**	0.339	0.459	1.000			
Mean Elevation	**-0.744**	-0.026	**-0.797**	**-0.896**	**-0.749**	**-0.805**	**-0.503**	**0.769**	0.892	0.763	1.000		
Chl-a	-0.014	**0.858**	-0.148	-0.272	-0.495	-0.318	**0.656**	0.175	-	0.065	-0.053	1.000	
Water Chemistry (no EEMs) PC 2	0.292	**-0.75**	0.189	0.703	0.285	0.325	0.044	0.193	0.077	-0.672	-0.479	**-0.574**	**-0.779**