Investigating trophic ecology and dietary niche overlap among morphs of Lake Trout in Lake Superior

by

Justin Michael Hoffmann

A thesis

presented to the University of Waterloo

in fulfillment of the

thesis requirement for the degree of

Master of Science

in

Biology

Waterloo, Ontario, Canada, 2017

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.

I understand that my thesis may be made electronically available to the public.

Abstract

Four morphs of Lake Trout (*Salvelinus namaycush*, Walbaum 1792) have been identified in Lake Superior: leans, siscowets, humpers, and redfins. In this comprehensive study, the trophic ecology of Lake Trout morphs were characterized using stomach content, fatty acid, and stable isotope data. Stomach content results indicated a predominately piscivorous diet for leans, siscowets, and redfins, whereas humper diets were comprised of 50% fish and 50% *Mysis* by mass. Humpers and siscowets were most similar in their dietary fatty acid profiles, whereas redfins had the most distinct dietary fatty acid profile. Results from stable isotope analysis revealed some among-morph differences along a pelagic-profundal consumption gradient ($\delta^{34}S$), but there were no significant differences in trophic position ($\delta^{15}N$) or basal carbon sources among morphs ($\delta^{13}C$). Using the recently developed nicheROVER software package, 4-dimensional trophic niches for each morph were quantified using stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$) and fatty acid profiles (30 dietary fatty acids, condensed to one axis). Humpers had the largest 4-dimensional niche regions of all four morphs, and redfins had the smallest. Pairwise probability of overlap among morphs in these four-dimensional niche regions was determined to be < 50% in most cases. Overall, stomach content results indicate that humpers diets were more planktivorous than the other morphs, consistent with previous research. Results of the niche overlap analysis suggests some degree of generalist feeding for all morphs. Better characterization of seasonal variation in diet using tracers that reflect more recent feeding (e.g., fatty acids, stomach contents, and/or stable isotope analyses performed on tissues that turnover more quickly than muscle) are needed to further elucidate among-morph differences and similarities in diet and trophic ecology.

Acknowledgements

First, I would like to thank my supervisor, Dr. Heidi Swanson for giving a molecular biology student the chance to study ecology, this has been an amazing learning experience and I am sincerely grateful for all that I have learned. I would also like to thank my committee members, Dr. Michael Power and Dr. Andrew Muir for providing constructive feedback and being available for questions when they arose.

Next, I would like to extend thanks to all the individuals who made this project possible. Thanks to Mark Vinson, Charles Bronte, Charles Krueger, and the crew of the R.V. *Kiyi* for assisting with sample collection, analysis of the stomach contents, and for allowing me the opportunity to travel on the ship. Special thanks to Alysse Perreault for performing the morphometric identifications of all the Lake Trout morphs. I would also like to thank Dr. Lisa Loseto and Bruno Rosenburg for facilitating my stay in Manitoba, for teaching me how to perform fatty acid analysis, for analyzing the fatty acids of all my samples, and for being available for any questions while I worked through the methods on my own at home. Lastly, I would like to thank Dr. Bernard Duncker, Dr. Roland Hall, Dr. Mark Servos, and Leslie Bragg for allowing me the use of lab equipment for my fatty acid procedures

A big thank you to all my friends and colleagues in the Swanson lab. Thank you for your mentorship and support throughout this whole process, you helped make this experience a great one. Special thanks to Dr. Leanne Baker for always being available to help with questions, and for giving me a crash course on multivariate statistics, and to all the volunteers in the Swanson lab who helped with lab work, especially Shyann Hang for her help with isotope processing and data entry.

Finally, I would like to thank all my friends and family who supported me through this degree. Thanks to my parents and grandparents for taking the time to ask me how my thesis was going, and for being wonderful listeners when I was lamenting the pains that go along with writing one. I don't know where I would be without all your support and encouragement, and I am grateful for everything. Lastly, thank you to Amanda Soliguin for supporting me through this entire process. You met me when I was starting my Masters, and now you get to see it come to its completion; thank you for helping me get through the time in between.

Funding was provided by the Great Lakes Fishery Commission, and in kind support was provided by the United States Geological Survey.

Table of Contents

List of Figures

List of Tables

List of Equations

Equation 1: Relative Importance Index

$$RI_i = 100 \times \frac{AI_i}{\sum_{i=1}^{n} AI_i}$$

Equation 2: Stable isotope ratio δ notation

$$\delta^j X = \left[\frac{(\frac{^jX}{^iX})\ sample}{(\frac{^jX}{^iX}) standard} - 1 \right] \times 1000$$

Equation 3: Lipid correction model

$$\delta^{13}C_{protein} = \delta^{13}C_{bulk} + [\Delta\delta^{13}C_{bulk} \times (\frac{C:N_{protein} - C:N_{bulk}}{C:N_{bulk}})]$$

Chapter 1

Introduction

1.1 Lake Superior

Lake Superior is the largest of the Laurentian Great Lakes, and the largest lake by surface area in the world, holding an estimated 10% of the world's freshwater (White et al., 2012). It has a mean average water temperature of 3.5 °C, an average depth of 147 m and a maximum depth of 406 m (Horns et al., 2003). Most of the waters of Lake Superior can be classified as offshore; 77% of the total area is represented by waters greater than 80 m deep (Horns et al., 2003; Gorman et al., 2010). Lake Superior oligotrophic, with low concentrations of nutrients and concomitant low net primary productivity (Chraibi et al., 2014; Minor et al., 2014). The Lake Superior region was covered by the Laurentide ice sheet during the Wisconsinan glaciation between 10,000-12,000 years ago, (see Hill, 2007). This glaciation event had major impacts on surrounding land formations, which influenced the distribution and evolutionary history of many species, including Lake Trout (*Salvelinus namaycush*, Walbaum 1792) (Behnke, 1972; Martin & Olver, 1980; Eshenroder et al., 1995).

Lake Superior supports a diverse assemblage of fishes (see Bronte et al., 2003). Native deep water fishes include (but are not limited to) large predators such as Lake Trout and Burbot (*Lota lota*, Linnaeus, 1758), coregonids such as Kiyi (*Coregonus Kiyi*, Koelz, 1921), Cisco (*Coregonus artedi*, Lesueur, 1818), Bloater (*Coregonus hoyi*, Milner, 1874), and Shortjaw (*Coregonus zenithicus*, Jordan and Evermann, 1909), and cottids such as Deepwater Sculpin (*Myoxocephalus thompsonii*, Girard, 1851) and Slimy Sculpin (*Cottus cognatus*, Richardson, 1863). Invertebrates commonly found in fish stomachs include *Mysis*, *Diporeia*, calanoid copepods, *Bythotrephes*, *Daphnia*, and benthic dwelling species such as clams and oligochaetes.

Non-native species have also been introduced to Lake Superior, including (but not limited to) Rainbow Smelt (*Osmerus mordax*, Mitchill, 1814), Alewife (*Alosa pseudoharengus*, Wilson, 1811), and dreissenid mussels.

1.2 A Brief History of Lake Trout Stock Dynamics in Lake Superior

During the 1800s, Lake Superior supported approximately 70 species of fish, including game fishes such as Lake Trout and Lake Whitefish (*Coregonus clupeiformis*, Mitchill, 1818). Commercial fisheries were established in 1830 (Horns et al., 2003), and annual yields between 1900 and 1940 for these fisheries were high, ranging on average between 5-10 million $kg \cdot y^{-1}$ (Christie, 1974). A combination of over fishing, habitat degradation, and parasitism by Sea Lamprey (*Petromyzon marinus*, Linnaeus, 1758), however, eventually led to sharp declines in Lake Trout populations in the mid-twentieth century (Hansen, 1999; Zimmerman & Krueger, 2009; Bunnell et al., 2014). Between the mid-1950s and 1960s, annual yields of Lake Trout declined by 90% in Lake Superior, and similar declines were observed in the other Laurentian Great Lakes (Christie, 1974; Hansen, 1999). By 1960, Lake Trout were considered extirpated in the Laurentian Great Lakes, save for isolated stocks in Lake Superior and Lake Huron (Hansen, 1999).

The Great Lakes Fishery Commission (GLFC) was established in 1955, and was given responsibility for coordinating basin-wide fishery management in the Great Lakes, and developing a program to eliminate sea lamprey populations (Horns et al., 2003). Management actions included fishery catch limits, control of Sea Lamprey populations, and stocking of juvenile Lake Trout (Hansen, 1999). Sea Lamprey populations were culled by targeting larvae with the lampricide 4-nitro-3-(trifluoromethyl) phenol (TFM) (Dawson & Jones, 2009), and by

1962, Sea Lamprey populations were reduced by an estimated 86% (Nieland et al., 2008). Stocking of juvenile Lake Trout began in the 1950s in Lake Superior, with natural reproduction being observed as early as 1960 in Keweenaw Bay (Hansen, 1999). Stocking efforts in Lake Superior ceased in most areas of the Lake by 1993. Exceptions to this included some Western Minnesota, Wisconsin , and Ontario waters; management recommendations for stocking cessation had not been met in these areas (see Schreiner & Schram, 1997; Bronte et al., 2003). Lake Trout were considered rehabilitated in Lake Superior in 1996 (Schreiner & Schram, 1997). Restoration and rehabilitation efforts of Lake Trout stocks in Lake Superior have thus been largely successful, with current population numbers approaching those observed pre-collapse (Negus, 2010; Cline et al., 2013). However, recent evidence suggests that Lake Trout genetic diversity has declined in Lake Superior (Baille et al., 2016).

1.3 Morph Differentiation of Lake Trout

The Wisconsinan glaciation during the Pleistocene Era played a major role in the distribution and evolutionary history of Lake Trout (Behnke, 1972; Martin & Olver, 1980; Eshenroder et al., 1995). Lake Trout are native to post-glacial lakes in North America, but the species has since been introduced worldwide (Behnke, 1972). Lake Trout are omnivorous predators, and consume a variety of fish and invertebrate prey (Martin & Olver, 1980). Lake Trout mature between 4 and 13 years, though this varies depending on prey availability and predator abundance (Martin & Olver, 1980; Elrod et al., 1996). Spawning usually occurs during fall, though Lake Trout have been observed to spawn in all months between June and January, and different stocks of Lake Trout in the same lake may spawn at different times during the year (Eschmeyer, 1955; Martin & Olver, 1980; Hansen et al., 2016).

Intraspecific phenotypic plasticity is well-documented within the genus *Salvelinus* (Martin & Olver, 1980; Muir et al., 2015). Many studies have investigated the ecology of sympatric Arctic Charr (*Salvelinus alpinus*, Linnaeus, 1758) morphs (see Jonsson & Jonsson, 2001; Reist et al., 2013). Morphs are variants of a species that may differ in morphology, feeding ecology, and reproductive spawn times, but are not considered separate species (see Jonsson & Jonsson, 2001; Reist et al., 2013). Indigenous peoples observed considerable phenotypic diversity in populations of Lake Trout in North America as early as the 1850's (see Hansen et al., 2012; Muir et al., 2015). A variety of Lake Trout morphs have since been documented in lakes across North America, including in the Laurentian Great Lakes, Great Bear Lake, Great Slave Lake, Lake Mistassini, and Rush Lake (Rahrer, 1965; Moore & Bronte, 2001; Zimmerman et al., 2006; Zimmerman et al., 2007; Chavarie et al., 2013; Muir et al., 2014, Chavarie et al, 2016).

Morphs of Lake Trout that inhabit the same lake at different depths have been documented in many lakes, including Lake Superior (Muir et al., 2014), Lake Mistassini (Hansen et al., 2012), Great Slave Lake (Zimmerman et al., 2006), and Great Bear Lake (Blackie et al., 2003; Alfonso, 2004, Chavarie et al., 2013). Morphs can further be distinguished by morphometric features such as head shape, fin insertions, and eye location (Muir et al., 2014), as well as other features such as fat content (Eschmeyer & Phillips, 1965; Goetz et al., 2014), diet (Harvey et al., 2003), gill raker structure (Martin & Sanderco, 1967), and spawning time (Eschmeyer, 1955; Hansen et al., 2016).

Recent evidence indicates that there is genetic differentiation among morphs of Lake Trout (Goetz et al., 2010; Perreault-Payette, 2017). Laboratory breeding experiments have shown that leans (a low fat morph) and siscowets (a high fat morph) produce offspring that have intermediate lipid content, suggesting lipid metabolism may be genetically influenced

(Eschmeyer & Phillips, 1965). Other experiments supporting genetic control of lipid metabolism have demonstrated that gene expression related to lipid metabolism and immunity differs between leans and siscowets, though expression of these genes may also be influenced by environmental factors (Goetz et al., 2010). Rearing experiments have also demonstrated that leans and siscowets raised from wild gametes under identical lab conditions maintain morphological differences, indicating that these differences are not likely a result of phenotypic plasticity (Goetz et al., 2010).

Lake Superior supports four Lake Trout morphs: "lean", "siscowet", "humper", and "redfin" (Moore & Bronte, 2001; Bronte & Moore, 2007; Muir et al., 2014). Compared to the other morphs, leans are thinner and more streamlined, have lower tissue lipid content, and are most commonly found in waters < 80m (Harvey & Kitchell, 2000; Harvey et al., 2003; Hansen et al., 2012). Siscowet Lake Trout have high lipid content and are commonly found in waters > 80m deep (Goetz et al., 2014). Humpers have an intermediate lipid content (i.e., between that of siscowets and leans), relatively slower growth rates, smaller total lengths, and inhabit offshore reefs surrounded by mid to deep water > 90m (Rahrer, 1965; Moore & Bronte, 2001; Hansen et al., 2012). Redfins are large, slow growing morphs with high buoyancy, but little else is currently known about this morph (Muir et al., 2014; Hansen et al., 2016).

1.4 Study Rationale

After Lake Trout populations in the Great Lakes collapsed in the 1950s, stocking was used as a management tool in restoration efforts (Hansen, 1999). Lake Superior was the fastest if the Laurentian Great Lakes to recover from the collapse, with stocking efforts beginning in the 1950s (Hansen, 1999) and mostly ceasing by 1993 (Bronte et al., 2003). While there has been

recent evidence of natural reproduction of Lake Trout in Lakes Huron (Riley et al., 2014) and Michigan (Hanson et al., 2013), fisheries managers still depend on stocking to maintain Lake Trout populations (Muir et al., 2012). Stocking efforts have historically focused on the lean morph, as both contemporary commercial and recreational fishers prefer leans because of their low fat content (Eschmeyer & Phillips, 1965; Nieland et al., 2008). More recent management practices, however, have included stocking a variety of morphs to maintain genetic diversity and to maximize habitat use (Kepler et al., 2014). In 2004, the Great Lakes Fishery Commission Lake Erie Committee used a strain of humper morph ("Klondike") from Lake Superior to stock Lake Erie (Markham et al., 2008). The Klondike strain was chosen for stocking purposes because of similarities in habitat between Lake Erie and Lake Michigan to the offshore reef areas of Lake Superior from which the Klondike form originates (Bronte et al., 2008; Markham et al., 2008).

Trophic ecology of humpers and redfins is poorly understood relative to that of siscowet and lean morphs (Hansen et al., 2016). Previous research has shown that although siscowet and lean morphs may overlap in their diet (as inferred by stomach contents) by as much as 50% (Ray et al., 2007), leans prefer to consume Rainbow Smelt and Cisco (Gamble et al., 2011b), whereas siscowet prefer deepwater sculpin and Kiyi (Ray et al., 2007; Gamble et al., 2011a). Stable isotope data have also shown that leans and siscowets differ in $\delta^{15}N$ and $\delta^{13}C$ ratios (Harvey et al., 2003). Seasonal variation in diet has also been observed between these morphs; leans appear to prefer Rainbow Smelt in spring and fall, and coregonids in summer (Gamble et al., 2011b), whereas siscowets prefer Deepwater Sculpin in spring, and coregonids in fall (Gamble et al., 2011a). Little is known about humper diets (Muir et al., 2015), though it appears that humpers retain a planktivorous diet throughout adulthood (Stafford et al., 2014), and thus have a greater

overlap in habitat and resource use with juvenile Lake Trout of siscowet and lean morphs (Zimmerman et al., 2006; Zimmerman et al., 2009). Little is known about the feeding ecology of redfins.

With incomplete knowledge of the trophic ecology and potential trophic overlap between humpers and other morphs, it is difficult to predict how the effects of anthropogenic stressors and invasive species may impact resource partitioning among sympatric morphs. A lack of understanding of resource portioning, and ultimately potential competition among morphs, may impede Lake Trout restoration and management efforts. The objectives of this thesis were: [1] to characterize and compare diets and trophic ecology of sympatric Lake Trout morphs in Lake Superior, and [2] to compare trophic niche overlap among morphs. Specifically, I used stomach content and fatty acid analyses to compare morph diets, and stable isotope analyses to measure differences in trophic position ($\delta^{15}N$), basal carbon sources ($\delta^{13}C$), and pelagic-profundal consumption ($\delta^{34}S$) among morphs. I used stable isotope ratios and fatty acid profiles to estimate trophic niche size for each morph, and the probability of trophic niche overlap was estimated for each pairwise combination of morphs. This research will improve understanding of the ecology of sympatric Lake Trout morphs in Lake Superior by complimenting previous stomach content with longer signals of integrated resource use, and may be used to inform Lake Trout stocking and management practices.

1.5 Description of Chapters

Due to the importance of understanding diet, trophic relationships, and resource partitioning among sympatric morphs of Lake Trout, and the current unavailability of information regarding humper and redfin feeding ecology, I investigated trophic ecology and

niche overlap among morphs of Lake Trout captured in two locations in Lake Superior. To accomplish this, I used a variety of diet tracers, including stomach contents, fatty acids and stable isotope ratios of carbon (C), nitrogen (N), and sulfur (S).

In Chapter two of this thesis, I characterize the trophic ecology morph using stomach content, fatty acid, and stable isotope analysis. For the stomach content analysis, key prey items (at the time of capture) were determined through analyzing biomass and count contributions, and by calculating Relative Importance Index of each prey item found in stomachs (George & Hadley, 1979). Fatty acids identified as indicators of diet were compared among morphs and between sites, and results were used to infer contributions of different prey sources to Lake Trout diets. Stable isotope ratios were examined to identify inter-morph and inter-site differences in trophic position, source contributions of carbon (pelagic vs benthic), and source contributions of sulfur (sedimentary vs planktonic). By combining results of these three analyses, I sought to determine key prey items and identify differences in trophic ecology among morphs.

In chapter three of this thesis, I quantifed probabilistic niche size and niche overlap among Lake Trout morphs. Using the R software nicheROVER (Lysy et al., 2014), the probability of niche overlap among morphs was calculated using stable isotope tracers and fatty acid biomarkers that reflected morph diets. By estimating the probability of niche overlap among morphs, I was able to predict which morphs are most likely to share prey resources.

1.6 General Overview of Methods

1.6.1 Stomach Content Analysis

Stomach content analyses allow for a direct, unambiguous, taxonomically precise examination of prey ingested by a consumer at the time of capture. Prey items collected from

consumer stomachs are typically weighed and counted to determine biomass contributions, which can then be used in a variety of analyses to characterize predator diets. The method of diet characterization I used was the Relative Importance Index (George & Hadley, 1979). This analysis uses percent prey occurrence (%O), percent contribution to total number of prey items (%N), and percent contribution to total mass of prey items (%M) to quantify the Relative Importance Index for each prey item as:

$$RI_i = 100 \times \frac{AI_i}{\sum_{i=1}^{n} AI_i} \quad \text{(Equation 1)}$$

where RI_i is the relative importance of the *i*th prey item, AI_i = (%O + %N + %M) for the *i*th prey item, and *n* is the number of prey items. This method assigns prey items a number between 0 and 100, with relatively more important prey (i.e., prey most commonly found in gut contents) having higher numbers than less important prey.

While stomach content analyses have advantages such as high taxonomic resolution, they are subject to certain biases. Stomach contents only provide a brief snapshot of recently consumed prey, thus temporal trends in diet cannot be determined without multiple sampling campaigns (Couturier et al., 2013). Small, easily digestible prey items may not occur as frequently in stomachs as larger, more difficult to digest prey items, thus certain prey items may be misrepresented in frequency (Couturier et al., 2013). Because of the temporal limitations of stomach content analyses, they are often paired with fatty acid or stable isotope analyses. Fatty acid and stable isotope analyses provide ~2 months (Happel et al., 2016) and 6-12 months (Hesslein et al., 1993) of diet information respectively, and allow for medium- and long-term diet trends to be observed (Kirsch et al., 1998; Post, 2002).

1.6.2 Fatty Acid Analysis

Fatty acid signatures can serve as biomarkers of specific food sources that can be used to infer predator diets (Elsdon, 2010). Fatty acids reflect the average diet of an organism over a ~2 month period of time; fatty acid signatures of juvenile Lake Trout changed to reflect feeding on a specific prey within an 8 week period (Happel et al., 2016). Fatty acid analyses have been used to infer resource partitioning in consumers (Iverson et al., 2001), track seasonal or temporal shifts in consumer diet (Bradshaw et al., 2003), identify differences in feeding based on sex (Beck et al., 2007), and identify habitat source of prey (Henderson & Tocher, 1987). While many fatty acids can be synthesized *de novo* (Stubing & Hagen, 2003), some fatty acids must be obtained from diet. For example, most vertebrates lack enzymes higher than Δ12, inhibiting the synthesis of omega-3 fatty acids longer than 15 carbons (Lanca et al., 2011). If diet is the only possible source for particular fatty acids (i.e., essential fatty acids), it is possible to use them as tracer molecules to obtain information about prey consumption.

Fatty acids that are commonly used as tracers in freshwater environments include polyunsaturated fatty acids (PUFAs), which are synthesized at lower trophic levels by phytoplankton (Arts & Wainman, 1999), and monounsaturated fatty acids (MUFAs), which are synthesized by some species of zooplankton (Dalsgaard et al., 2003) and/or bacteria (Vlaeminck et al., 2006). PUFAs and MUFAs are useful as diet tracers for two reasons. First, specific fatty acids within each group can be indicative of specific groups of phytoplankton or zooplankton. For example, eicosapentaenoic acid (20:5n-3) is dominant in diatoms (Arts & Wainman, 1999), and gondoic acid (20:1n9) and gadoleic acid (20:1n11) are produced by calanoid copepods (Dalsgaard et al., 2003). Other indicator fatty acids are stearidonic acid (18:4n3) for dinoflagellates (Harrington et al., 1970; Budge & Parrish, 1998), and arachidonic acid (20:4n6)

found in red algae (Kirsch et al., 1998). Second, PUFAs and MUFAs are incorporated into consumer tissue relatively unchanged, which can be referred to as dietary routing (Hood-Nowotny et al., 2012). The presence of these FAs in higher organisms such as predatory fish is only possible through consumption, allowing for inferences to be made regarding the flow of specific fatty acids through a food chain.

1.6.3 Stable Isotope Analysis

Food web structure and trophic ecology can be examined through analysis of stable isotope ratios. Ratios of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ are often used in ecology to provide information on consumer trophic position and carbon source, respectively. (Deniro & Epstein, 1981; Post, 2002; Logan & Lutcavage, 2008). Stable isotopes integrate between 6-12 months of diet information, depending on tissue sampled for analysis (Hesslein et al., 1993). The standard delta notation for stable isotope ratios is as follows:

$$\delta^{j}X = \left[\frac{(\frac{^{j}X}{^{i}X})\,sample}{(\frac{^{j}X}{^{i}X})standard} - 1\right] \times 1000 \text{ (Equation 2)}$$

where ^{j}X is the heavier isotope (e.g., ^{15}N), and ^{i}X the lighter isotope (e.g. ^{14}N) in the sample (numerator) and international measurement standard (denominator). The standard for nitrogen is atmospheric nitrogen, for carbon is Vienna PeeDee Belemnite, and for sulfur is Canyon Diablo triolite (see Gonfiantini et al., 1995). Values are reported in parts per mil, denoted by ‰. Relative trophic position is routinely inferred from $\delta^{15}N$ ratio (Peterson & Fry, 1987), whereas carbon and sulfur sources are inferred from $\delta^{13}C$ ratios (France, 1995), and $\delta^{34}S$ ratios (Croisetiere et al., 2009) respectively.

Ratios of stable Carbon isotopes can help differentiate relative reliance on benthic vs pelagic production, as benthic sources of carbon tend to be enriched (i.e. less negative), in $\delta^{13}C$ relative to pelagic sources (France, 1995). This is related to the preference of the photosynthetic enzyme rubisco to utilize the lighter ^{12}C isotope (Keeley & Sandquist, 1992). In aquatic environments with little mixing (i.e., the littoral or benthic zone), CO_2 diffusion is a limiting step in photosynthesis, and primary producers will begin to utilize ^{13}C (Keeley & Sandquist, 1992), which is reflected in a relatively enriched $\delta^{13}C$ ratio. In areas of sufficient mixing (i.e., pelagic zone), CO_2 is more readily available, and primary producers will continue to discriminate against ^{13}C (Keeley & Sandquist, 1992). This results in a relatively depleted $\delta^{13}C$ ratio in the offshore environment. Carbon isotope ratios tend to fractionate (i.e., increase) in fish tissues between 0.4 and 1‰ between trophic levels (Vander Zanden & Rasmussen, 2001; Post, 2002; Sierszen et al., 2014), so $\delta^{13}C$ ratios of higher trophic organisms tend to reflect that of the primary producers at the base of the food chain (Layman et al., 2012).

Carbon isotope ratios are susceptible to bias in animals with high lipid content, as lipids are depleted in $\delta^{13}C$ by 6-7‰ (Kiljunen et al., 2006; Hoffman & Sutton, 2010). Many approaches have been undertaken to correct for the effects of lipids on $\delta^{13}C$ ratios. Mathematical correction models are commonly applied with some success (Mcconnaughey & Mcroy, 1979; Fry, 2002; Kiljunen et al., 2006). However, it has been advised that developing unique models for each system may be the most appropriate way to approach lipid corrections (Logan et al., 2008) because of the specificity in lipid dynamics for each species and tissue used (Hoffman et al., 2015). I used an arithmetic mass balance approach to lipid correction, as described by Hoffman (2010). In brief, this method uses C:N ratios of muscle tissue as a proxy for lipid content (Logan et al., 2008; Hoffman & Sutton, 2010; Hoffman et al., 2015). A subset of lipid-extracted tissue

samples are used to predict $C{:}N_{Extracted}$ and the isotopic depletion factor from lipids ($\Delta\delta^{13}C_{Lipid}$)(Hoffman & Sutton, 2010). Extracted $\delta^{13}C$ values are then predicted using the following equation:

$$\delta^{13}C_{protein} = \delta^{13}C_{bulk} + [\Delta\delta^{13}C_{bulk} \times (\frac{C{:}N_{protein} - C{:}N_{bulk}}{C{:}N_{bulk}})] \text{ (Equation 3)}$$

Where $\delta^{13}C_{protein}$ is the $\delta^{13}C$ ratio of the lipid extracted sample, $\delta^{13}C_{bulk}$ is the $\delta^{13}C$ ratio of the non-extracted sample, $\Delta\delta^{13}C_{bulk}$ is the isotopic depletion factor due to lipids, $C{:}N_{protein}$ is the C:N ratio in the extracted sample, and $C{:}N_{bulk}$ is the C:N ratio in the non-extracted sample.

Stable isotope ratios of nitrogen, $\delta^{15}N$, are used to determine the relative trophic position of consumers (Deniro & Epstein, 1981). The heavier (^{15}N) isotope accumulates in tissue as the ^{14}N isotope is preferentially excreted, and the ratio of heavy to light isotope increases with each trophic transfer (Minagawa & Wada, 1984). Therefore, consumers feeding on lower trophic organisms such as plankton or primary producers will have lower $\delta^{15}N$ ratios than those feeding on higher trophic organisms such as fish or other consumers (Deniro & Epstein, 1981). Nitrogen typically has a trophic fractionation of 3-4‰ per trophic level when averaged across whole food webs (Post, 2002).

Stable isotope ratios of sulphur can be used to determine if food web production is detrital or pelagic in origin (Croisetiere et al., 2009). The pelagic zone is enriched in $\delta^{34}S$ relative to the profundal zone, which is a result of bacterial sulphate reduction in sediments (Croisetiere et al., 2009). Organisms feeding on benthos have been shown to have $\delta^{34}S$ signatures similar to the sediments, while those feeding in the pelagic zone have $\delta^{34}S$ similar to water column sulfate. (Croisetiere et al., 2009; Karube et al., 2012). Sulphur has also been shown to undergo negligible fractionation during trophic transfers (<0.5‰) (McCutchan et al., 2003), so consumers tend to have $\delta^{34}S$ ratios similar to that of the original source of sulphur in the food web.

1.6.4 Niche Overlap

An ecological niche describes the environmental and trophic resources utilized by an organism (Hutchinson, 1957; Newsome et al., 2007). Niche overlap among species is common, as many species share similar resources such as prey or habitat (Rusterholz, 1981; Arlettaz et al., 1997; Hodgson et al., 1997; Hasui et al., 2009). Species can tolerate overlap in niche space as long as they differ in at least one dimension, thus avoiding competitive exclusion (Hutchinson, 1957; May & Macarthur, 1972; Pianka, 1974). Trophic niche space can be quantified using tracer molecules or biomarkers that reflect niche characteristics, such as stable isotopes or fatty acids. Stable isotopes reflect the diet and bases of production for an organism's diet; nitrogen isotopes ($\delta^{15}N$) are used to infer relative trophic position (Deniro & Epstein, 1981), carbon isotope ($\delta^{13}C$) are used to infer origin of food web production (benthic vs pelagic) (Deniro & Epstein, 1978; France, 1995), and sulphur isotopes ($\delta^{34}S$) are used to discriminate between profundal and pelagic sulphate reduction in a food web (see Peterson & Fry, 1987; Croisetiere et al., 2009). Fatty acid biomarkers (e.g., MUFAS and PUFAs) can also be indicators of consumer diets, and therefore reflect an organism's trophic niche. (Budge et al., 2007).

This research focuses on aspects of Lake Trout trophic niche (recognizes that there are other niche dimensions that are not trophic in nature). Trophic niche sizes were estimated for each morph using methods described in Swanson et al., (2015). Niche region and niche overlap between morphs was estimated using a probabilistic method within a Bayesian framework. Isotopes and fatty acids were used as indicators of trophic niche. A sample size of 30 Lake Trout per morph was analyzed using this method, as 30 is the minimum number of samples shown to be necessary for Bayesian comparisons of isotopic niches (Syvaranta et al., 2013).

Chapter 2

Trophic ecology of Lake Trout morphs in Lake Superior

2.1 Introduction

Phenotypic diversity of Lake Trout has been well documented in lakes throughout North America, including Great Bear Lake, Great Slave Lake, Lake Mistassini, Rush Lake, and the Laurentian Great Lakes (see Krueger & Ihssen, 1995; Blackie et al., 2003; Zimmerman et al., 2006; Hansen et al., 2012; Chavarie et al., 2013; Muir et al., 2014; Chavarie et al., 2016c). Lake Trout morphs differ in physical characteristics such as body shape (Muir et al., 2014), fat content (Eschmeyer & Phillips, 1965; Goetz et al., 2014), gill raker structure (Martin & Sanderco, 1967), and spawning time (Eschmeyer, 1955; Hansen et al., 2016). Lake Trout morphs also often vary in their preferred depth range; shallow water morphs commonly exist in sympatry with deepwater morphs (Behnke, 1972). Evidence from previous studies suggests that both habitat use and diet could play a role in the differentiation of phenotypic traits in Lake Trout (e.g., Martin & Sanderco, 1967; Zimmerman et al., 2006; Eshenroder, 2008; Muir et al., 2014; Chavarie et al., 2016b). Recent evidence has shown that depth may be a primary influence on the genetic and phenotypic diversity that is observed among Lake Trout morphs in Lake Superior (Baille et al., 2016). However, another recent study has shown that sympatric shallow water morphs exist in Great Bear Lake (Chavarie et al., 2013); these morphs were differentiated by head and fin characteristics, which have been demonstrated to influence feeding (Proulx & Magnan, 2004; Keeley et al., 2005) and swimming (Webb, 1984), as well as trophic ecology as inferred through stable isotope and fatty acid data (Chavarie et al., 2016b).

Lake Superior is the largest of the Laurentian Great Lakes , with a surface area of 82,100 km^2, average depth of 147 m, and maximum depth of 406 m (see Horns et al., 2003). A majority

of the Lake Superior environment is offshore, with 77% of total area greater than 80 m deep (Horns et al., 2003; Gorman et al., 2010). Lake Superior supports at least four different morphs of Lake Trout: “lean”, “siscowet”, “humper”, and the recently described “redfin” (Eschmeyer, 1957; Muir et al., 2014), though as many as a dozen morphs have been anecdotally described (Goodier, 1981). Leans are streamlined, have relatively low tissue lipid content, and are commonly found in waters < 80m (Thurston, 1962; Harvey et al., 2003; Hansen et al., 2012). Siscowets, the most abundant morph in Lake Superior (Bronte et al., 2003), are large-bodied, have high body lipid content, and are commonly found in waters > 80m deep (Eschmeyer & Phillips, 1965; Sitar et al., 2008; Goetz et al., 2014). Humpers have intermediate lipid content relative to leans and siscowets, relatively slower growth rates, and smaller total lengths; they inhabit offshore reefs surrounded by mid-to deep water > 90m (Rahrer, 1965; Moore & Bronte, 2001; Hansen et al., 2012). Redfins are large, slow-growing morphs with high buoyancy, but little else is currently known about this morph (Muir et al., 2014; Hansen et al., 2016).

The 1950s marked a major collapse in Lake Trout populations throughout the Great Lakes, due to a combination of overfishing and Sea Lamprey parasitization (Hansen, 1999; Zimmerman & Krueger, 2009; Bunnell et al., 2014). Lake Trout of all morphs were extirpated in most of the Great Lakes, except for Lake Superior and some areas of Lake Huron (Christie, 1974; Krueger et al., 1995; Hansen, 1999; Muir et al., 2012). Naturally reproducing populations of Lake Trout in Lake Superior have nearly recovered to pre-collapse levels as a result of sea lamprey control efforts, fishery catch limits, and stocking (Hansen, 1999; Negus, 2010; Cline et al., 2013). Despite similar efforts in the other Great Lakes, stocking is still used to maintain Lake Trout populations in Lakes Erie, Ontario, Michigan, and Huron (Muir et al., 2012). Historically, leans were the primary Lake Trout morph used for stocking (Eschmeyer & Phillips, 1965;

Nieland et al., 2008), but in 2004, a strain of humper originating from Lake Superior (Klondike) was used to stock Lake Erie, and in 2008 this strain was recommended for stocking in Lake Michigan (Bronte et al., 2008; Markham et al., 2008).

Due to the role that humpers play in Lake Trout stocking programs and their poorly understood trophic ecology, it is difficult to predict how humpers interact with other sympatric Lake Trout morphs. The purpose of this research was to compare the trophic ecology of humpers to other Lake Trout morphs (i.e., lean, siscowet, and redfin) in Lake Superior, and determine if prey, basal production sources, and trophic position differed between humpers and the other morphs. Trophic ecology of Lake Trout morphs in Lake Superior was investigated using three tracers of diet and trophic ecology that reflect different temporal integration of prey sources ranges: stomach content, fatty acid, and stable isotope analyses. Analysis of stomach contents allow for a direct examination of prey items with a high degree of taxonomic resolution but very low temporal resolution (stomach contents are often used to provide a 'snapshot' of an organism's diet) (Elsdon, 2010). Indirect tracers, such as fatty acids and stable isotope ratios, integrate a longer time period of fish diets (~2 and ~6-12 months, respectively) but do not have as high degree of taxonomic resolution as stomach contents (Hesslein et al. 1993; Happel et al. 2016).

Feeding ecology of leans and siscowets has been well studied (Harvey et al., 2003; Gamble et al., 2011a; Gamble et al., 2011b; Stafford et al., 2014). Previous research has shown that leans consume mainly pelagic fish, such as Rainbow Smelt and Cisco (Gamble et al., 2011b) whereas primary diet items for siscowets include Deepwater Sculpin and Kiyi (Ray et al., 2007; Gamble et al., 2011a). In contrast to leans, siscowets also display diel vertical migration behaviour, enabling them to capture prey at a variety of depths (Hrabik et al., 2006; Zimmerman

et al., 2009; Gorman et al., 2012). The feeding ecology of the deepwater humper morph is not well characterized, but available data indicate that they maintain a planktivorous diet and have a preference for *Mysis* (Stafford et al., 2014). Feeding ecology of the recently-described redfin is poorly understood. This morph has been shown to demonstrate similarities with siscowets in terms of fat composition and depth range (Muir et al., 2014), which may suggest that the two morphs share similar prey resources, but further research is necessary. Because of the differences in prey consumption between leans and siscowets, and the planktivorous diet of humpers, I hypothesized that trophic and feeding ecology would differ among morphs of Lake Trout in Lake Superior. I predicted that:- i) humper Lake Trout would rely more heavily on *Mysis* than the other morphs, but that there would be prey items in common with siscowets because of their overlap in depth; and, ii) that humpers would occupy a lower trophic position than the other three morphs, consistent with neotenic retention of a juvenile planktivorous diet. The goal of this research was to improve understanding of humper and redfin feeding ecology, and provide fishery managers with information that can be used to inform future Lake Trout re-establishment programs.

2.2Methods

Study Site and Collection Methods

Two sites, Superior Shoal (48° 3'43.54" N, 87° 8'52.57" W) and Stannard Rock (47°12'26.26" N, 87°12'3.82" W) were sampled in Lake Superior during cruises on the R/V *Kiyi* (Figure 2.1). Most fish and invertebrate samples were collected during summer 2013 and 2014, with supplemental prey fishes and invertebrates collected as needed (i.e., to fill in sampling gaps) during summer 2015 and 2016. Superior Shoal and Stannard Rock were selected as study sites because they were known to support humper, lean, and siscowet Lake Trout morphs; when the

study was designed, redfins had not yet been formally described in the literature, and it was not known if they would be present at these two sites.

Lake Trout and a variety of potential prey species, including ciscoes (*C. artedi, C. Kiyi, and C. hoyi*), Deepwater Sculpins, Rainbow Smelt, and invertebrates (*Diporeia*, *Mysis*, *Lepidoptera*, gastropods (snails), bivalves (fingernail clams), and zooplankton) were collected from each site. Other fishes that have been identified as prey for Lake Trout (i.e., Burbot, Slimy Sculpin) (Harvey et al., 2003) were unfortunately not collected in sufficient numbers to be used in analyses. Lake Trout were collected via gill nets in 2013 and 2014. Nets were set over night (between 12 and 24 hours). Three different depth ranges were sampled, 0-50m (ten nets) 50-100m (nine nets) and 100-150m (nine nets); these correspond to the depth ranges thought to be occupied by the morphs. Gill nets were multifilament nylon twine, 183-m long by 1.8-m high with 30.5-m panels ranging from 50.8 to 114.3 mm, in 12.7-mm increments. Prey fishes were collected by trawls performed during summer 2013 and 2015. Tows were made along depth contours at a vessel speed of ~3.5 km/h with a Yankee bottom trawl (11.9 m head rope, 15.5 m footrope, and 2.2 m wing height) with 89-mm, 64-mm, and 13-mm stretched mesh at the mouth, trammel and cod end, respectively. Aquatic invertebrates were collected via surface water neuston trawls in 2013 and 2014 (*Lepidoptera*), performed with a Sea-Gear paired 1-m^2 neuston net with 500-μm mesh. The net was towed 0.5 m below the surface for 10 minutes at 2.5 miles per hour. *Mysis* and zooplankton were collected opportunistically in 2013 and 2014 with a 0.5-m diameter plankton net. Collections in separate years were necessary to collect adequate mass for fatty acid and stable isotope analyses. Vertical zooplankton tows at Superior Shoal were performed from 130 m depth to surface and 60 m depth to surface. Vertical zooplankton tows at Stannard Rock were performed from 60 m to surface and 20 m to surface. Mesh sizes were 500

μm for *Mysis* and 153μm for zooplankton. Upon collection, a subset of zooplankton samples were sorted into two different size fractions, 63-250 μm, and 250-500 μm, and preserved in ethanol for laboratory identification of species composition. The remaining zooplankton were frozen in a small amount of water for fatty acid and stable isotope analysis. The 63-250-μm fraction was determined to be composed mostly of *Diaptomus sp.*, whereas the 250-500-μm fraction was composed mostly of *Limnocalanus marcrurus* (G.O. Sars, 1863). Benthic invertebrate samples were collected in 2013 and 2014 with a standard 0.05-m^2 Ponar dredge, at a depth of 60 m at Superior Shoal and 80 m at Stannard Rock. Sediments were rinsed and sieved with 500-μm mesh using an elutriator.

Total length (mm), wet weight (g), sex, and maturity for each Lake Trout were determined upon capture. Dorsal, skinless muscle samples were removed from each Lake Trout for stable isotope and fatty acid analyses. Stomachs were removed from each Lake Trout and frozen. Prey fishes captured in the trawl were identified, and a subsample were measured and weighed before being frozen whole. Total length (mm), wet weight (g), sex and maturity were determined for individual prey fishes in the laboratory prior to use in stable isotope and fatty acid analyses. Invertebrate samples were coarsely sorted (to Order or Family) while on the ship and frozen in scintillation vials. Because determination of morphs is sensitive to fish size (Zimmerman et al., 2006) only Lake Trout > 300 mm in total length were analyzed further.

Assignment of Lake Trout Morphs

Morphometric analysis (Perreault-Payette, 2016) and visual identification (performed by A. Muir, C. Krueger, and C. Bronte) were used to assign each captured Lake Trout (> 300 mm total length) to a morph. Lateral photographs of each fish were used to quantify body and head shape via geometric morphometrics (Muir et al., 2014). These digitized points were analyzed

using the MCLUST R package (Fraley & Raftery, 2009), which assigned individual fish a morph identity based on the head and body models. Three visual assignments per fish were generated by three experienced researchers: Charles Bronte (U.S. Fish and Wildlife Service), Andrew Muir (Great Lakes Fishery Commission), and Charles Krueger (Michigan State University). At least two of the three visual assignments had to agree for a fish to be given a visual identification. The visual identifications were then compared with results of morphometric models. If two of the three of the assignments (visual, head, and body) agreed, the fish was given a morph assignment. If none of the models agreed, the fish was not assigned a morph and excluded from further analysis.

This dual method of morph identification was employed for all morphs except humper, due to their smaller sizes at maturity. Sensitivity of morphometric analysis is decreased for fish less than 430 mm (Zimmerman et al., 2006), and mean size at maturity of humpers is ~450 mm (Hansen et al., 2016); therefore, in the case of humpers, only visual identifications were used. Out of 901 Lake Trout captured, 419 were assigned a morph identification.

Stomach Content Analysis

Stomach content analysis was led by M. Vinson (United States Geological Survey Great Lakes Science Center). Stomachs were weighed, dissected, and the contents were coarsely sorted into taxonomic groups and counted. Each taxonomic group was weighed to determine contribution to total biomass (in grams). Contents were then rinsed, frozen and saved for use in stable isotope analysis (necessary for taxa where adequate directly-sampled organisms were not available). Fish were classified into categories of coregonids, salmonids, sculpins, sticklebacks, smelt, burbot, and unidentified. Invertebrates were classified into categories of *Mysis*, *Diporeia*,

other aquatic, and terrestrial. Unidentified items such as rocks, plastics, and detritus were weighed, but not included in the analysis.

A total of 419 Lake Trout stomachs were analyzed, representing all Lake Trout that received a morph assignment. Stomach contents were analyzed using the Relative Importance Index (George & Hadley, 1979). Percent prey occurrence (%O), percent contribution to total number of prey items (%N), and percent contribution to total mass of prey items (%M) were calculated, and the Relative Importance Index for each prey item was quantified as:

$$RI_i = 100 \times \frac{AI_i}{\sum_{i=1}^{n} AI_i} \quad \text{(Equation 1)}$$

where RI_i is the relative importance of the *i*th prey item, AI_i = (%O + %N + %M) for the *i*th prey item, and n is the number of prey items. This assigns each prey item a percentage between 0 and 100, with relatively more important (i.e., prey most commonly found in gut contents) prey having higher numbers than less important prey.

Fatty Acid Analysis

Lipids were extracted using a modified Folch method (Folch et al., 1957; Budge et al., 2006). Freeze-dried skinless dorsal muscle tissue was used for Lake Trout, whereas freeze-dried whole bodies were used for prey fishes and invertebrates. All samples were homogenized and ground prior to extraction. Approximately 0.2 g of tissue was treated with a 2:1 chloroform-methanol solution containing 0.01% butylated hydroxytoluene (BHT) (v/v/w) and refrigerated overnight. The lipid phase was then separated, dried with anhydrous sodium sulphate and evaporated under nitrogen to obtain total lipid mass. Fatty acid methyl esters (FAMEs) were produced from extracted lipids by transesterification with Hilditch reagent (100:1 parts dry methanol to H_2SO_4 v/v) (Morrison & Smith, 1964). Samples were heated to 100˚C for 1 hour,

back extracted with hexane and dried with anhydrous sodium sulphate. The FAME layer was removed and evaporated under nitrogen until dry and weighed. Finally, FAMEs were diluted in hexane to a concentration of 0.20mg/mL.

FAMEs were analyzed at the Freshwater Institute (Winnipeg, Manitoba). Gas chromatographic (GC) analysis was performed on an Agilent Technologies 7890N GC equipped with a 30-m J&W DB-23 column (0.25-mm I.D; 0.15-μm film thickness). The GC was coupled to a Flame Ionization Detector (FID) operating at 350 ^{o}C. Hydrogen was used as carrier gas flowing at 1.25 mL/min for 14 minutes and ramped to 2.5 mL/min for the remainder of the run. The split/splitless injector was heated to 260 ^{o}C and run in splitless mode with a 50 psi pressure pulse for 1.25 minutes. The oven program was as follows: 60 ^{o}C for 0.66 min; 22.8 ^{o}C/min to 165 ^{o}C with a 2.0 min hold; 4.7 ^{o}C/min to 174 ^{o}C and 7.6 ^{o}C/min to 200 ^{o}C with a 6 min hold. Peaks were quantified using Agilent Technologies ChemStation software. Fatty acid standards were obtained from Supelco (37 component FAME mix) and Nuchek (54 component mix GLC-463). Eighty FAMEs were identified via retention time and known standard mixtures and are reported as percent of total fatty acid. Each fatty acid is described using the shorthand nomenclature of A:Bn-X, where A represents the number of carbon atoms, B the number of double bonds and X the position of the double bond closest to the terminal methyl group. Fatty acids are reported as % total FAME content. A total of 210 Lake Trout samples (30 per morph per site) and 108 prey fishes (15 per species per site, except Rainbow Smelt) were analyzed for fatty acids. Because of mass limitations, Rainbow Smelt, and all invertebrate samples were pooled and analyzed in replicates, ranging from 2-5 reps depending on mass availability (Table 2.1).

Stable Isotope Analysis

Samples from 210 Lake Trout (30 per morph per site, excluding redfins from Stannard Rock; low sample size precluded analysis of redfins at Stannard Rock), and 120 prey fishes (fifteen per species per site) were analyzed for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. Kiyi and Deepwater Sculpin were the only prey fishes collected at Superior Shoal, so analysis of prey fishes was limited to these two species (Table 2.1). Lake Trout dorsal muscle samples (skin off) were freeze dried and ground into a fine powder before being weighed for stable isotope analysis (SIA). The remaining fish species and invertebrates were freeze dried whole and ground prior to weighing. Prey fishes larger than 150 mm were homogenized by blender before freeze drying to ensure even drying and grinding of the tissue. Homogenates of several individual invertebrate samples were pooled together by taxa to ensure adequate sample mass. Samples were analyzed in replicates of 5 per taxa per site for $\delta^{13}C$ and $\delta^{15}N$, and replicates of 3 per taxa per site for $\delta^{34}S$. Due to mass limitations, fingernail clams were not analyzed for $\delta^{34}S$ isotopes at either site. Snails, moths, and clams were not analyzed from Superior Shoal because they could not be collected at that site (Table 2.1).

Ratios of stable carbon and nitrogen isotopes were determined at the University of Waterloo Environmental Isotopes Laboratory (UWEIL) on a 4010 Elemental Analyzer (Costech Instruments) coupled to a Delta XL (Thermo-Fisher) continuous flow isotope ratio mass spectrometer (CFIRMS). Sulfur isotopes were analyzed on a 4010 Elemental Analyzer (Costech Instruments) coupled to an Isochrom (GVInstruments / Micromass UK) CFIRMS. Isotope ratios are reported in δ notation, which is calculated as:

$$\delta^{j}X = \left[\frac{(\frac{^{j}X}{^{i}X})\,sample}{(\frac{^{j}X}{^{i}X})standard} - 1\right] \times 1000 \text{ (Equation 2)}$$

where ^{j}X is the heavier isotope (e.g., ^{15}N), and ^{i}X the lighter isotope (e.g., ^{14}N) in the sample (numerator) and international measurement standard (denominator). Atmospheric nitrogen is the standard for $\delta^{15}N$, Vienna PeeDee Belemnite for $\delta^{13}C$, and Canyon Diablo triolite for $\delta^{34}S$ (see Gonfiantini et al., 1995). All values are reported in parts per mil (‰). Analytical error for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ did not exceed 0.2‰, 0.3‰, or 0.3‰ based on corrections made using an array of international reference material and in-house standards that were calibrated using certified international reference materials (i.e. IAEA-N1 + N2, IAEA-CH3 + CH6, USGS-41 + 41, IAEA-SO-5, IAEA-SO-6, NBS-127, NBS-123, IAEA-S1 to-S3). Of the total sample number analyzed in an analytical run, no less than 20% were Std/Ref materials. Repeatability of samples (one in 10) for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ was 0.2‰, 0.3‰, and 0.3‰.

Lipid Correction Models

As lipids are depleted in $\delta^{13}C$ (Kiljunen et al., 2006; Hoffman & Sutton, 2010), lipid correction models were used to correct Lake Trout and prey $\delta^{13}C$ values for effects of lipid bias. A correction equation (see Appendix I) was developed based on mass balance models presented by Hoffman and Sutton (2010), and applied to individuals of all morphs/species with C:N ratios > 4.0, which was used as the minimum C:N ratio to perform lipid corrections as recommended by Hoffman et al. (2015). Delta^{13}C ratios for *Diporeia,* snails, and clam samples were not corrected for effects of lipid, as low sample mass precluded lipid extractions. Delta^{13}C ratios for Rainbow Smelt were also not corrected for effects of lipid, as all Rainbow Smelt samples had C:N ratios < 4.0. Lipid corrected $\delta^{13}C$ values were estimated as follows:

$$\delta^{13}C_{protein} = \delta^{13}C_{bulk} + [\Delta\delta^{13}C_{bulk} \times (\frac{C{:}N_{protein} - C{:}N_{bulk}}{C{:}N_{bulk}})] \quad \text{(Equation 3)}$$

Where $\delta^{13}C_{protein}$ is the $\delta^{13}C$ ratio of the lipid extracted sample, $\delta^{13}C_{bulk}$ is the $\delta^{13}C$ ratio of the non-extracted sample, $\Delta\delta^{13}C_{bulk}$ is the isotopic depletion factor due to lipids, $C:N_{protein}$ is the C:N ratio in the extracted sample, and $C:N_{bulk}$ is the C:N ratio in the non-extracted sample. $\Delta\delta^{13}C_{bulk}$ and $C:N_{protein}$ were estimated for each morph and species that had $C:N_{bulk} > 4.0$ at each site (see Appendix I for model selection methods). Superior Shoal leans and Stannard Rock humpers each had 2 outliers (leans: $\Delta\delta^{13}C_{bulk}$=-17.8‰, -268.7‰; humpers: $\Delta\delta^{13}C_{bulk}$ = -28.9‰, -30.0‰) that were not included in calculating average $\Delta\delta^{13}C_{bulk}$ or $C:N_{protein}$, as these values were ~2-4 times larger than the literature values reported for $\Delta\delta^{13}C_{bulk}$ of ~7 (Kiljunen et al., 2006; Hoffman & Sutton, 2010). Because there was only one lipid extracted value determined for each pooled invertebrate sample, lipid correction equations were not applied to invertebrates; the measured lipid-extracted $\delta^{13}C$ ratios were used in analyses.

Statistical Analyses

Statistical analyses (significance level α=0.05) were conducted using R software version 3.3.1 (R Core Team, 2016). Sexes were combined for analysis because there were no significant differences between sexes in length (t-test, $t_{1,204}$=-1.117, p=0.266), weight (t-test, $t_{1,204}$=-1.393, p=0.165), isotope ratios (t-test, Carbon: $t_{1,204}$=-0.111, p=0.912, Nitrogen: $t_{1,204}$=-0.282, p=0.779, Sulfur: $t_{1,204}$=1.009, p=0.314) or fatty acid concentrations (Hotelling Test, Hotelling T=0.218, df=1,202, p=0.148). Stable isotope ratios were used to identify differences in trophic ecology among Lake Trout morphs. $\delta^{13}C$ and $\delta^{34}S$ ratios were used to address differences in basal production sources (pelagic-littoral and profundal-pelagic respectively) among Lake Trout morphs. $\delta^{15}N$ ratios were used to investigate differences in trophic position differences among morphs. Stable isotope data were analyzed using general linear models to determine if significant

differences occured among morphs and between sites. After each analysis, residual plots were tested for normality using a Shapiro Wilk test, while Levene's test was used to examine homogeneity of variances.

Linear discriminant analysis (LDA) was performed on fatty acid data (Table 2.2). LDAs were performed three times at each site; the first time used all 70 fatty acids that were quantified, the second used 30 fatty acids that are known dietary biomarkers, and the third used 40 fatty acids that are not used as biomarkers, or are known to be affected by processes other than diet (i.e., metabolism) (Table 2.2). Prior to analysis, fatty acid concentrations were logit transformed (log(p/1-p) to normalize the data, and scaled and centered using a z-score transformation ($z=x-\mu/\sigma$). A Wilk's lambda test was performed to determine which LDA axes were significant. After the LDA that used dietary fatty acids was performed, an ANOVA was conducted to determine if LDA scores from significant axes differed among morphs; morphs with similar fatty acid profiles (as indicated by overlap from the LDA plots) were inferred to have similar diets. Finally, qualitative interpretation of results was accomplished using fatty acid-specific loading scores onto significant axes.

2.3 Results

Stomach Content Analysis

Of the 419 Lake Trout analyzed for stomach contents, siscowets were the most abundant whereas humpers were the least abundant (Table 2.1). Figure 2.2 shows the count and biomass composition of Lake Trout stomachs that were analyzed. Fish contributed the most to total stomach biomass for all morphs except humpers at Stannard Rock. For humpers, *Mysis* contributed the greatest proportion to stomach content biomass (43% and 57% of total stomach

biomass at Superior Shoal and Stannard Rock respectively). *Mysis* contribution (in terms of biomass) was lower for each of the other Lake Trout morphs at both sites. Invertebrates comprised a larger proportion of Lake Trout stomach biomass at Stannard Rock compared to Superior Shoal (Figure 2.2).

The 'fish' category in stomachs was further broken down into species, where possible (Figure 2.3). Coregonids contributed the most to stomach content biomass for leans at both sites, though Deepwater Sculpin were also identified in lean stomachs at Stannard Rock. Coregonids also contributed the most fish biomass to siscowet stomachs at Stannard Rock, with a smaller contribution from Deepwater Sculpin. However, at Superior Shoal, Burbot were the highest biomass contributors to siscowet stomachs, followed by coregonids. At both sites, Deepwater Sculpin were the only identifiable fish found in humper stomachs, and accounted for > 50% of fish biomass in humper stomachs. Burbot and coregonids were the dominant biomass contributors to redfins stomachs, with a small contribution by Deepwater Sculpin. However, as shown by the count data, most of the fish species observed in stomachs were too degraded to be identified; of 128 fish counted in all the Lake Trout stomachs, only 40 were identifiable.

In terms of counts, *Mysis* was the highest contributing prey item for all morphs at both sites, followed by 'other invertebrates' (Figure 2.2). *Diporeia* and fish eggs were the two lowest contributing prey items to both count and biomass, and were rarely observed in Lake Trout stomachs.

Analyses of the Relative importance index indicated that order of importance of prey categories was (most to least) *Mysis*, fish, invertebrates, eggs, and *Diporeia* (Table 2.3). *Mysis* was the most common prey item in the stomachs of all morphs at both sites. Fish prey was slightly less common for humpers than for the other morphs. Invertebrates were more common at

Stannard Rock compared to Superior Shoal (all morphs). *Diporeia* and fish eggs were the least common prey items for all morphs at both sites.

Fatty Acid Analysis

Seventy fatty acids were quantified using GC-FID. Linear discriminant analyses (LDA) were performed separately at each site, using: i) data from all 70 fatty acids; ii) fatty acids known to be tracers of diet (30 fatty acids); and, iii) fatty acids known to reflect metabolism or not known to be tracers of diet (or both; 40 fatty acids) (Table 2.2). Axis loadings for individual fatty acids (up to 10 for each function) can be found in Table 2.4. All raw fatty acid data are presented in Appendix I.

At Superior Shoal, the first two discriminant functions were significant (DF Axis 1 Wilks Lambda=0.018, df=210, $p<0.001$; DF Axis 2 Wilks Lambda=0.112, df=138, p=0.011), and these two functions resulted in good separation of the four known morphs (Figure 2.4a). The first LDA axis explained 56.9% of observed variation in fatty acid signatures, and the second LDA axis explained 26.8% of observed variation. The total misclassification rate (of individuals to the correct morph) for the model was 5.8%. At Stannard Rock, only the first discriminant function was significant (DF Axis 1 Wilks Lambda=0.16, df=140, $p<0.001$; DF Axis 2 Wilks Lambda=0.186, df=69, p=0.059). The first axis explained 70.5% of observed variation, and separated the fish into the three known morphs (Figure 2.4b). The total misclassification rate for this model was 0%.

When only 30 fatty acids that are known dietary biomarkers (Table 2.2) were used, the first discriminant function was significant at Superior Shoal (DF Axis 1 Wilks Lambda=0.253, df=90, p=0.001), explained 61.8% of observed variation, and separated Lake Trout into roughly three groups; the first group contained leans, the second contained humpers and siscowets, and

the third contained redfins (Figure 2.4b). There were significant differences in LD1 scores among morphs (ANOVA, $F_{3,116}$=44.62, p=<0.001). A post-hoc Tukey's test showed that redfins had significantly higher LDA scores compared to the other three morphs, and siscowets and humpers had significantly higher LDA scores compared to leans (Tukey HSD, p=<0.05). Based on this analysis, the diets of humpers and siscowets were most similar, whereas those of lean and redfin were most different. The total misclassification rate for the model was 27.5%.

An LDA on these same 30 fatty acids for Lake Trout from Stannard Rock showed again that the first discriminant function was significant (DF Axis 1 Wilks Lambda=0.272, df=60, p=0.003), accounted for 64.9% of observed variation, and separated the morphs into roughly three groups (no redfins at Stannard Rock). There were significant differences in LD1 scores among morphs (ANOVA, $F_{2,87}$=52.98, p=<0.001). A post-hoc Tukey's test showed leans had significantly higher LDA scores compared to siscowets, and siscowets had significantly higher LDA scores compared to humpers (Tukey HSD, p=<0.05). This analysis thus indicated that the diets of siscowets and leans were most similar, whereas the diets of humpers and leans were most different. The total misclassification rate for this model was 23.3%. (Figure 2.5b).

A third LDA was performed on the fatty acid profiles of Lake Trout morphs to examine how non-dietary fatty acids differ among morphs (Figure 2.4c, 2.5c). These fatty acids helped separate siscowets from humpers at Superior Shoal (Figure 2.4c), and contributed to separation of all three groups at Stannard Rock (Figure 2.5c).

To aid in interpretation of analyses performed using known diet tracers (Figure 2.4b), ordinations of fatty acids with the highest loading scores were plotted with results of Lake Trout LDAs in Figures 2.4d and 2.6d. At Superior Shoal, known markers of bacteria (17:0 and 15:1n6; Table 2.4) and diatoms (16:2n4, 20:5n3; Table 2.4) contributed to the separation of redfins from

leans (Figure 2.4d; Table 2.4). Analyses of fatty acid signatures of prey items revealed that the 20:5n3 marker of diatoms was most concentrated in *Mysis* (Figure 2.4d, Appendix I). The siscowet/humper grouping at Superior Shoal was separated from the lean grouping by the zooplankton markers 18:1n7 and 18:4n3 (Table 2.4) being more closely aligned with siscowets and humpers, and an algal (16:2n6) and an algal/terrestrial (18:3n3) marker being more closely aligned with leans (Table 2.4, Figure 2.4d). The known zooplankton markers 18:1n7 and 18:4n3 were most concentrated in Deepwater Sculpin and zooplankton captured for this study, respectively, and the algal/terrestrial 18:3n3 marker was most concentrated in zooplankton (Figure 2.4d, Appendix I). An algal (20:4n6; most concentrated in *Mysis* in this study) and a bacteria (15:0; most concentrated in zooplankton in this study) marker contributed to separation of the siscowet/humper grouping from the redfin grouping (Table 2.4, Figure 2.4d).

At Stannard Rock, markers of zooplankton (20:1n11, 20:1n9), and diatoms (16:1n7) contributed to the separation of leans from humpers, whereas markers of bacterial (15:0, 17:0), zooplankton (18:4n3), and terrestrial (18:3n3) dietary sources separated siscowets from humpers (Table 2.4, Figure 2.5d). Leans and siscowets were best separated by diatom (16:2n4, 20:5n3), and terrestrial (18:2n6) markers (Table 2.4, Figure 2.5d). Analyses of fatty acid signatures in prey sources showed moths had the highest concentrations of terrestrial markers (18:3n3, 18:2n6) (Figure 2.5d, Table 2.4, Appendix I). The diatom marker 16:1n7 was most concentrated in Deepwater Sculpin whereas, similar to Superior Shoal, the diatom marker 20:5n3 was most concentrated in *Mysis.* The known bacterial 15:0 and 17:0, and the zooplankton marker 18:4n3 were found in highest concentrations in zooplankton.

Stable Isotope Analysis

Carbon Isotopes

A two-way analysis of variance compared lipid corrected (adjusted) $\delta^{13}C$ isotope ratios between sites and among Lake Trout morphs (Table 2.5). Differences in adjusted $\delta^{13}C$ ratios occurred among morphs in the overall ANOVA (ANOVA, $F_{2,177}=3.263$, $p=0.041$), but a post-hoc Tukey's honest significance test showed no pairwise differences (Tukey HSD, $p>0.05$). This seeming incongruity is because the Tukey's HSD test is corrected for multiple comparisons. There were no differences between sites (ANOVA, $F_{1,176}=1.278$, $p=0.260$), and no interactions between morph and site (ANOVA, $F_{2,174}=1.171$, $p=0.183$).

A within site analysis of adjusted $\delta^{13}C$ isotope ratios was also performed for Superior Shoal because the two-way ANOVA did not include redfins (only captured at Superior Shoal),. No significant differences in $\delta^{13}C$ ratios among morphs was detected at Superior Shoal (ANOVA, $F_{3,116}=1.311$, $p=0.274$). This is consistent with the low range of $\delta^{13}C$ ratios among morphs, which was only 0.26 per mil at Superior Shal, and 0.49 per mil at Stannard Rock (Table 2.5).

Nitrogen Isotopes

A two-way analysis of variance compared $\delta^{15}N$ ratios between sites and among Lake Trout morphs (Table 2.5). Delta^{15}N ratios varied between sites (ANOVA, $F_{1,174}=4.092$, $p=0.045$) and among morphs (ANOVA, $F_{2,174}=4.628$, $p=0.011$). The interaction term between morph and site was also significant (ANOVA, $F_{2,174}=5.246$, $p=0.006$). The interaction could indicate that between-site differences were not driven by differences in baseline, as one site was not consistently enriched or depleted in $\delta^{15}N$ compared to the other. A post-hoc Tukey's HSD test

showed that Superior Shoal humpers were enriched in $\delta^{15}N$ compared to Superior Shoal siscowets (Tukey HSD, $p<0.05$) and Stannard Rock leans (Tukey HSD, $p<0.05$). Stannard Rock siscowets were enriched compared to Stannard Rock leans (Tukey HSD, $p<0.05$) (Table 2.5).

Ratios of stable nitrogen isotopes were not corrected for possible differences in baseline between the two sites for a number of reasons. First, there is high variability in $\delta^{15}N$ ratios for small primary consumers with high turnover rates, and an appropriate long-lived (and therefore less sensitive to seasonal variation in $\delta^{15}N$ ratios) primary consumer (e.g., fingernail clams) was not captured at both sites. Second, baseline corrections are appropriate for comparisons where basal $\delta^{15}N$ ratios are highly variable (e.g., Cabana and Rasmussen, 1996). Superior Shoal and Stannard Rock are both oligotrophic environments (in the same lake) with relatively low anthropogenic inputs, and thus these sites should not differ substantially in baseline $\delta^{15}N$. However, to account for possible unaccounted variation in baseline $\delta^{15}N$ ratios between sites,, I ran a mixed effects model on Lake Trout $\delta^{15}N$ ratios where site was a random factor. Delta^{15}N ratios differed among morphs (fixed factor) (ANOVA, $F_{2,176}=4.503$, $p=0.0124$). A post-hoc test showed that humpers were significantly enriched in $\delta^{15}N$ compared to leans (Tukey HSD , $p=>0.05$), but the absolute difference in mean $\delta^{15}N$ was only ~ 0.4 per mil between these two morphs.

Within site analysis of $\delta^{15}N$ isotope ratios (allowed redfins to be included at Superior Shoal) indicated significant differences in $\delta^{15}N$ among morphs at Superior Shoal (Table 2.5) (ANOVA, $F_{3,116}=5.285$, $p=0.002$) and Stannard Rock (Table 2.5) (ANOVA, $F_{2,87}=4.957$, $p=0.009$). At Superior Shoal, humpers (Tukey HSD, $p<0.5$) and redfins were enriched in $\delta^{15}N$ compared to siscowets (Tukey HSD, $p<0.5$). At Stannard Rock a different pattern was found; siscowets were enriched in $\delta^{15}N$ compared to leans (Tukey HSD, $p<0.05$).

Although there were statistically significant differences between sites and morphs in $\delta^{15}N$, absolute variation in $\delta^{15}N$ was low. Mean $\delta^{15}N$ varied from 10.02 in leans from Stannard Rock to 10.84 in redfins from Superior Shoal (Table 2.5). This range (0.82) represents less than a third of a trophic level, and is likely not ecologically relevant.

Sulfur Isotopes

A two-way analysis of variance compared $\delta^{34}S$ ratios between sites and among morphs (Table 2.5). $\delta^{34}S$ differed among morphs (ANOVA, $F_{2,174}$=9.406, p=<0.001), but the interaction between morph and site was also significant (ANOVA, $F_{3,174}$=23.129, p=<0.001). A post hoc Tukey's HSD test showed that leans from Superior Shoal were enriched in $\delta^{34}S$ compared to all other morphs (Tukey HSD, $p<0.05$), whereas humpers from Stannard Rock were enriched relative to siscowets and leans from Stannard Rock (Tukey HSD, $p<0.05$). Sites did not differ in $\delta^{34}S$ (ANOVA, $F_{1,174}$=2.098, p=0.149).

Within site analysis of $\delta^{34}S$ isotope ratios (redfins included from Superior Shoal) indicated differences in $\delta^{34}S$ among morphs at both Superior Shoal (Table 2.5) (ANOVA, $F_{3,116}$=19.004, p=<0.001) and Stannard Rock (Table 2.5) (ANOVA, $F_{2,87}$=11.911, $p<0.001$). Within Superior Shoal, a post-hoc Tukey's test showed leans and redfins were enriched in $\delta^{34}S$ compared to humpers and siscowets (Tukey HSD, p=<0.05). Within Stannard Rock, a post hoc Tukey's test showed humpers were enriched in $\delta^{34}S$ compared to leans and siscowets (Tukey HSD, p=<0.05). The absolute difference in mean $\delta^{34}S$ ratios among morphs and sites, was small, however (max difference was 0.9 per mil), and thus differences are likely not ecologically relevant.

Qualitative Bivariate Analysis of Stable Isotope Data

Stable isotope biplots ($\delta^{15}N$ vs $\delta^{13}C$ and $\delta^{34}S$ vs $\delta^{13}C$) were created for each site and examined qualitatively (Figure 2.6 and 2.7). Particular attention was paid to prey fishes and invertebrate taxa, which were not included in the statistical analyses above. When comparing organisms that were captured at both sites, zooplankton (as expected) had the lowest $\delta^{13}C$ ratios, whereas Lake Trout had the highest $\delta^{13}C$ ratios. However, clams had the lowest $\delta^{13}C$ ratios and snails had the highest $\delta^{13}C$ ratios at Stannard Rock. Note that benthic end member, such as snails, were not sampled at Superior Shoal (Figure 2.6). The range of $\delta^{13}C$ ratios was 3.85 and 3.63 per mil at Superior Shoal and Stannard Rock, respectively, when only organisms that were captured at both sites are included. When snails and clams are included at Stannard Rock, the range of $\delta^{13}C$ ratios was 6.59 per mil (Figure 2.6 and 2.7).

Lake Trout had the highest $\delta^{15}N$ at both sites, indicating, as expected, that they occupy the highest trophic position. However, the range of $\delta^{15}N$ ratios was much higher at Stannard Rock than Superior Shoal (8.54 vs 4.30) (Figure 2.6 and 2.7). This is because the small size fraction of zooplankton (63-250 μm) had much higher $\delta^{15}N$ at Superior Shoal than at Stannard Rock. At Stannard Rock, the community composition of the small zooplankton size fraction was dominated by *Diaptomus sp.,* and the large size fraction was dominated by *Limnocalanus marcrurus*.

The range of $\delta^{34}S$ ratios at Superior Shoal and Stannard Rock was similar when calculated using organisms captured at both sites (2.61 and 2.08 per mil, respectively) (Figure 2.6 and 2.7). In general, *Diporeia* had the highest $\delta^{34}S$ ratios at both sites, whereas zooplankton had the lowest $\delta^{34}S$ ratios. Sediment and water column $\delta^{34}S$ ratios are not yet available, and

without data from these two important endmembers, it is difficult to interpret differences in $\delta^{34}S$ ratios among organisms or between sites.

2.4 Discussion

Stomach Contents and Prey Importance

Results from this study indicate that Lake Trout morphs differ in prey consumption, and these differences vary between sites. Fishes were the dominant prey items for all Lake Trout morphs (excluding humpers) in terms of biomass, ranging between 54%-88%, which is similar to what has been observed for siscowets and leans in previous studies (e.g., Zimmerman et al., 2009; Gamble et al., 2011a; Isaac et al., 2012). Previous stomach content data from Lake Superior (Gamble et al., 2011a) have shown that lean diets are largely comprised of Rainbow Smelt, Cisco, and Slimy Sculpin (Harvey & Kitchell, 2000; Harvey et al., 2003), whereas siscowet diets are dominated by Deepwater Sculpin and coregonids (e.g., Kiyi and Bloater) (Harvey & Kitchell, 2000; Harvey et al., 2003; Gamble et al., 2011a). Lake Trout undergo an ontogenetic shift in feeding at approximately the size of maturity (430 mm) (Zimmerman et al., 2009). Small siscowets, for example, feed primarily on *Mysis*, and switch to feeding on Deepwater Sculpin as they grow larger (Isaac et al., 2012). *Mysis* biomass in stomachs of leans and siscowets > 400mm has been observed to be < 20% (Gamble et al., 2011a; Gamble et al., 2011b; Isaac et al., 2012), which I observed in all morphs except humpers. Humpers are known to maintain foraging behaviour on *Mysis* that is similar to juveniles of all morphs (Stafford et al., 2014), and my observations support this previously-reported neotenic feeding habit. *Mysis* biomass contribution to humper diets was 42% at Superior Shoal and 56% at Stannard Rock, which is similar to what has been observed in juvenile siscowet stomachs (Isaac et al., 2012).

The greater consumption of *Mysis* by humpers suggests their diets are more planktivorous, whereas the other morphs are more piscivorous, indicating that the trophic ecology of humpers differs from the other Lake Trout morphs in Lake Superior.

Further analysis of fishes in Lake Trout stomachs showed that lean diets were dominated by coregonids at both Stannard Rock and Superior Shoal, siscowets consumed mostly coregonids at Stannard Rock and Burbot at Superior Shoal, humpers consumed Deepwater Sculpin at both Stannard Rock and Superior Shoal, and redfins consumed mostly Burbot and coregonids. These observations agree with previous studies of lean and siscowet stomach contents (e.g., Ray et al., 2007; Gamble et al., 2011a; Gamble et al., 2011b), though in contrast to Ray et al., (2007) and Gamble et al., (2011b), no Rainbow Smelt were identified in lean stomachs. The presence of Deepwater Sculpin in humpers stomachs, although not directly observed before, is expected as these two species overlap in depth (Selgeby, 1988). Deepwater Sculpin are also a suitable prey for humpers due to their small size. Humpers are a relatively small Lake Trout morph, therefore, they are likely gape-limited in the size of prey they can consume (Hambright, 1991).

At the time this study was conceived, the redfin morph was not yet formally described in the literature. Evidence from stomach contents suggested that redfins may overlap with siscowets in terms of prey items, as both morphs had coregonids and Burbot in their stomachs. However, a majority of the fish that were detected in stomachs were unidentifiable; the number of fish found in stomachs was 128 whereas the total number of identifiable fish was only 40. Because of these low sample numbers, it is difficult to draw definitive conclusions.

While *Mysis* were differentiated in stomach content analyses, ‘other’ invertebrate taxa were not taxonomically resolved. In general, invertebrate biomass in stomachs of all morphs was

higher at Stannard Rock compared to Superior Shoal. This may reflect differences in prey availability, but further research is necessary to investigate this.

According to the IRI scores, *Mysis* was the most common prey item for all morphs. This was due to the high occurrence of *Mysis*, (found in nearly all stomachs), and high number of individual mysids consumed. Previous studies have shown that Lake Trout will consume forage fish when available (Martin, 1970), but switch to a planktivorous diet if fish are unavailable (Martin, 1966). Fish had the second highest IRI score, reflecting the lower number of fish that were consumed relative to *Mysis*, but their relatively high contribution to biomass. Fish had the lowest IRI in humpers (IRI of 23.94 at Superior Shoal and 24.21 at Stannard Rock), reflecting lower consumption of fish biomass in this morph. *Diporeia* and eggs were rarely consumed by any morphs, and this was reflected in the low IRI scores for both prey items. Low IRI scores for *Diporeia* were expected, as Stannard Rock and Superior Shoal are located in areas of relatively low *Diporeia* density (Auer et al., 2013). Nearshore Lake Trout consume more *Diporeia* than offshore Lake Trout (Gamble et al., 2011a; Gamble et al., 2011b). Stannard Rock is ~45 km offshore and Superior Shoal is ~65km offshore, which means *Diporeia* may be less available at these study compared to previously investigated, more nearshore sites. Lastly, *Diporeia* are more commonly consumed by Lake Trout < 200 mm long (see Isaac et al., 2012), and none of the Lake Trout examined in this study were < 300 mm in length. Fish eggs were also expected to be uncommon in Lake Trout stomachs, as most morphs spawn in the fall, while sampling for this study occurred during summer (Eschmeyer, 1955; Hansen et al., 2016).

While morphs overlapped in terms of consumption of certain prey items (i.e., *Mysis*, unidentified invertebrates), the IRI scores and stomach contents support my hypothesis that trophic ecology (as inferred by stomach contents) differs among morphs. Humpers relied more

heavily on *Mysis*, leans fed more on coregonids, and siscowets and redfins fed on larger prey such as burbot. Feeding ecology may also differ between sites, as evidenced by inter-site differences in stomach contents for siscowets. These differences may reflect variability in prey availability between sites.

Fatty Acids

At Superior Shoal, results from an LDA performed on 30 fatty acids known to be dietary tracers revealed separation of the morphs into three groups: the first group contained leans, the second contained humpers and siscowets, and the third contained redfins. These results suggest that humpers and siscowets have the greatest diet overlap, and support my prediction of humpers having diets that are most similar to siscowets.

Similarities in fatty acid profiles between humpers and siscowets likely reflects overlap in habitat between the two morphs (e.g., Rahrer, 1965; Harvey & Kitchell, 2000), and similarities in prey consumption (Gamble et al., 2011a; this thesis). Interestingly, redfins and leans had the least diet overlap as inferred from fatty acid data, even though redfins and leans have similar depth ranges (Harvey & Kitchell, 2000; Hansen et al., 2016). The minimal fatty acid overlap between redfins and leans was supported by 18:3n3, a fatty acid that can indicate either a terrestrial (Budge & Parrish, 1998; Budge et al., 2001) or algal source (Arts & Wainman, 1999) heavily associating with leans. Redfins also had significantly different fatty acid profiles from humpers and siscowets; trophic ecology of redfin as inferred from fatty acid data thus differed from each of the other morphs. The fatty acids 18:4n3, 20:5n3 15:0, and 15:1n6: zooplankton (Harrington et al., 1970; Budge & Parrish, 1998) diatom (Meziane et al., 2002) and bacterial biomarkers (Volkman et al., 1980; Vestal & White, 1989; Kharlamenko et al., 1995) respectively, were most closely associated with redfins. While this result may suggest a larger contribution of diatom or

zooplankton fatty acids to redfin diets, one issue with bacterial fatty acids is that they can reflect the gut flora of an organism (Iverson et al., 2004). Furthermore, redfins were only collected at Superior Shoal, so without other sites to compare to, it is impossible to determine if these fatty acids are associated with redfins throughout Lake Superior, or if these fatty acids are reflective of habitat use and prey consumption specific to Superior Shoal. Further investigation is required to elucidate dietary-based differences in redfin fatty acids.

Dietary fatty acids at Stannard Rock separated the morphs into three distinct groups. However, unlike Superior Shoal where siscowets and humpers were more similar to each other, siscowets were more similar to leans than humpers at Stannard Rock. This is consistent with the similarities in stomach contents observed between siscowets and leans at Stannard Rock; coregonids, Deepwater Sculpin, and invertebrates were consumed in similar proportions by both leans and siscowets (this thesis). The similarities in fatty acid profiles between siscowets and leans suggest similar diets between siscowets and leans at Stannard Rock. Terrestrial biomarkers were found to associate with both sides of the significant LD1 axis at Stannard Rock. The terrestrial biomarker 18:2n6 was most closely associated with leans at Stannard Rock, and this fatty acid was found in relatively high concentrations in the abundant terrestrial moths that were collected from the surface. On the opposite side of the axis, 18:3n3, which can indicate either terrestrial (Budge & Parrish, 1998; Budge et al., 2001) or algal (Arts & Wainman, 1999) diet sources, was most closely associated with humpers, and was also highly concentrated in terrestrial moths. Terrestrial inputs may play a greater role at Stannard Rock than Superior Shoal because Stannard Rock is shallower (150m compared to 250m) and nearer to shore (45km vs 65km) than Superior Shoal.

While there were differences between sites in how fatty acids loaded onto LDA axes, many similarities were observed. A majority of the highest loading fatty acids were biomarkers for zooplankton (Harrington et al., 1970; Budge & Parrish, 1998), diatoms (Dunstan et al., 1994; Kharlamenko et al., 1995), algae (Kirsch et al., 1998), or bacteria (Meziane et al., 2002), and the same category of biomarker (e.g., diatom markers) would often load onto both sides of an LDA axis simultaneously. This makes it difficult to infer contributions of specific prey types from the fatty acid profiles of the Lake Trout morphs; morphs were often associated with biomarkers indicating similar sources. This is not unexpected, as fatty acids can provide evidence for the organism that produced them, but they cannot determine if a predator assimilated a fatty acid through direct consumption of the original organism, or if the predator consumed other prey that had consumed the original organism (Budge et al., 2006). The offshore Lake Superior food web has many connections, with species such as *Mysis* acting as integrators of many basal food sources, and as an important food source for many fishes (Ahrenstorff et al., 2011; Gamble et al., 2011a; Gamble et al., 2011b; Sierszen et al., 2011). *Mysis* consume a variety of zooplankton (Grossnickle, 1982; Johannsson et al., 2001), which are known to produce their own unique fatty acid biomarkers, in addition to containing biomarkers of their prey such as algae and diatoms (Brett et al., 2009). *Mysis* is therefore able to provide fatty acids produced by primary producers and zooplankton to virtually all other levels of the food web. It is thus not surprising the same category of biomarker would load onto both sides of an LDA axis; many of the highest loading fatty acids that are known biomarkers of particular taxa (e.g., 18:4n3 for zooplankton, 16:2n4 and 20:5n3 for diatoms) (Harrington et al., 1970; Dunstan et al., 1994; Kharlamenko et al., 1995; Budge & Parrish, 1998) were present in *Mysis*, and *Mysis* was found in the stomachs of all Lake Trout morphs at both sites.

Even though many of the fatty acids present in Lake Trout morphs can be explained by the consumption of *Mysis,* differences in fatty acids among morphs (e.g., terrestrial inputs) showed that humper fatty acid profiles were similar to siscowets at Superior Shoal, and differed from leans and redfins. In contrast, leans and siscowets had similar fatty acid profiles at Stannard Rock, whereas humpers were most different in fatty acid profile. These observed differences in dietary-based overlap morphs between sites could reflect site-specific differences in prey availability, but further research is necessary.

Non-dietary fatty acids contributed to separation among morphs at both sites, but was most obvious at Superior Shoal, where the morphs were clustered quite closely using dietary fatty acids, but separated into more discrete groups when non-dietary fatty acids were included in the analysis. Differences in fatty acids among morphs may be driven by factors other than diet, such as metabolism (Cook, 1991; Tocher, 2003; Budge et al., 2006; Iverson, 2009), and a number of previous studies have demonstrated metabolic differences among morphs (Eschmeyer & Phillips, 1965; Goetz et al., 2010; Goetz et al., 2014). Recent evidence suggests that different morphs at the same site are more closely related to each other than the same morph at different sites (Baille et al., 2016). For example, different fatty acids loaded at each site (e.g.,12:0, 13:1, 20:0, 21:3n5 were unique to Stannard Rock), which could indicate that differences in genetic control of enzymes involved with lipid synthesis and circulation (i.e., acyl-CoA desaturase, lipoproteins) differs between sites. Overall, fatty acid results suggest subtle differences in diet among morphs, differences in metabolism among morphs, and differences in both diet and physiology within morphs but between sites.

Stable Isotopes

Earlier studies comparing $\delta^{13}C$ ratios between siscowets and leans demonstrated that juveniles of both morphs have similar $\delta^{13}C$ ratios, but that the ratios diverge as morphs mature; large siscowets have more depleted $\delta^{13}C$ ratios (Harvey et al., 2003) than leans (Omara et al., 2015). In this study, however, all Lake Trout morphs had similar lipid-corrected $\delta^{13}C$ ratios. It is possible that inter-morph patterns are dissimilar among studies because of different approaches to lipid correction models. Harvey et al. (2003) developed their own lipid correction model, whereas Omara et al. (2015) utilized the Post et al. (2007) method of lipid correction. Hoffmann et al. (2015) used the same mass-balance lipid corrections performed in this study, showed that leans and siscowets from Lake Superior had very similar $\delta^{13}C$ ratios (absolute difference of ~0.4‰). All of the morphs analyzed in this study had average $\delta^{13}C$ ratios within 0.5‰ of each other. The trophic fractionation of carbon is variable and can range between 0.4 and 1‰ (Vander Zanden & Rasmussen, 2001; Post, 2002; Sierszen et al., 2014). If data were interpreted using Post's (Post, 2002) values of 0.4‰, then some morphs may be feeding on different carbon sources. However, the lack of statistically significant differences among the morphs, coupled with previous studies in Lake Superior using 1‰ as the trophic fractionation of $\delta^{13}C$ (Sierszen et al., 2014) suggest that it is most likely that the differences in $\delta^{13}C$ observed among the Lake Trout morphs are not ecologically significant. Because both Superior Shoal and Stannard Rock are offshore sites, this result is perhaps not surprising. Another possible explanation for minimal differences in $\delta^{13}C$ among these morphs arises from the fact that large, mobile generalist predators, such as Lake Trout, often consume prey from a variety of environments (McMeans et al., 2013), which may result in similar $\delta^{13}C$ ratios, but not as a result of feeding at the same basal carbon source. Results similar to these have been recently observed in Great Bear Lake

(Chavarie et al., 2016a), where Lake Trout morphs were more differentiated by $\delta^{15}N$ ratios, with little differences in $\delta^{13}C$ ratios among morphs.

Delta^{15}N ratios differed among morphs, but the absolute differences (~0.6‰) were small. At Stannard Rock, siscowets had the highest $\delta^{15}N$, followed by humpers and leans. These results are consistent with previously observed trends where siscowets tend to be enriched in $\delta^{15}N$ compared to leans (Harvey & Kitchell, 2000; Harvey et al., 2003; Omara et al., 2015). In contrast, humpers and redfins had the highest $\delta^{15}N$ at Superior Shoal, followed by leans and siscowets. This was contrary to my hypothesis. Slightly elevated $\delta^{15}N$ in humpers at Superior Shoal could reflect feeding on Deepwater Sculpin, which was the only identifiable fish found in humper stomachs at both sites. Deepwater Sculpin are relatively enriched in $\delta^{15}N$ (Schmidt et al., 2009; Zimmerman et al., 2009; Omara et al., 2015), had higher $\delta^{15}N$ at Superior Shoal compared to Stannard Rock, and overlap in depth range with humpers (Rahrer, 1965; Selgeby, 1988). The relatively slow growth of humpers could also have caused higher $\delta^{15}N$ than expected (Rahrer, 1965; Hansen et al., 2016). An inverse relationship between $\delta^{15}N$ enrichment and growth rate has been observed in several fishes (Trueman et al., 2005).

Redfins are the largest and oldest of the four morphs (Hansen et al., 2016). Enrichment of $\delta^{15}N$ has been observed to occur with size (e.g., Hobson & Welch, 1995; Kwak & Zedler, 1997; Lindsay et al., 1998) and age (Overman & Parrish, 2001) **)**, which could explain the slightly elevated $\delta^{15}N$ ratios relative to leans and siscowets at Superior Shoal. However, although inter-morph differences in $\delta^{15}N$ ratios were significant, average $\delta^{15}N$ ratios were within 1.0‰ among all morphs at both sites. The trophic fractionation of nitrogen is approximately 3.4‰ (Vander Zanden & Rasmussen, 2001; Post, 2002), and it is therefore likely that all the Lake Trout morphs are feeding at a similar trophic level.

Delta $\delta^{34}S$ ratios differed significantly among morphs at both sites. At Superior Shoal, leans were the most enriched, followed by redfins, humpers and siscowets. The trophic fractionation of Sulphur is < 0.5‰ (see Schmidt et al., 2015). Observed differences in $\delta^{34}S$ among morphs (range of $\delta^{34}S$ ~0.75‰) at Superior Shoal suggest that leans may be relying more on pelagic inputs compared to humpers and siscowets, which may be feeding on basal resources from the profundal zone. Similarities in $\delta^{34}S$ ratios between humpers and siscowets support my prediction of humpers and siscowets sharing similar prey resources. These results are supported by fatty acid data from this study, which showed that leans had distinct fatty acid profiles from humpers and siscowets. Humpers, siscowets, and redfins were also associated with more bacterial biomarkers than leans, which would be expected in morphs consuming more profundal resources (Croisetiere et al., 2009; Karube et al., 2012).

In contrast to the results at Superior Shoal, humpers at Stannard Rock were most enriched in $\delta^{34}S$ ratios, followed by siscowets and leans. Like Superior Shoal, these results are also supported by fatty acid data from this study, which showed that leans and siscowets had more similar fatty acid profiles at this site. Unlike what was observed at Superior Shoal, the differences in $\delta^{34}S$ ratios between humpers and siscowets at Stannard Rock is contrary to my prediction of humpers and siscowets sharing similar prey resources. However, the difference in $\delta^{34}S$ between humpers and leans is ~0.4‰, which indicates that there is no difference in sulphur source between the morphs. Humpers and leans were also associated with the same number of bacterial fatty acids, suggesting similar reliance on profundal resources. Differences in $\delta^{34}S$ ratios between sites could reflect the fact that Stannard Rock is much shallower and closer to shore than Superior Shoal. This means that there may not be as much spatial separation between pelagic and profundal sources of production, and all three morphs may consume inputs from

both sources. The differences in $\delta^{34}S$ ratios among Lake Trout morphs at Superior Shoal does provide some support that there is a weak pelagic-profundal gradient of resource use among morphs. However, because analysis of $\delta^{34}S$ ratios of the water column and sediments have not yet been completed, it is difficult to infer what may be driving the different $\delta^{34}S$ ratios in Lake Trout morphs between sites.

Qualitative Food Web Analysis

The food webs of Superior Shoal and Stannard Rock were quite similar; ranges of $\delta^{13}C$, $\delta^{15}N$ and $\delta^{34}S$ and relative positioning of organisms were consistent among sites, with the exception of the 63-250-µm size fraction of zooplankton. Zooplankton of this size were enriched by ~4‰ at Superior Shoal compared to Stannard Rock, which reflects about ~1 trophic level. At Stannard Rock, the 63-250-µm size fraction contained mostly *Diaptomus sp.*, and the 250-500-µm fraction contained predominately *Limnocalanus marcrurus*. Temporal and spatial variability in zooplankton $\delta^{13}C$ and $\delta^{15}N$ ratios are well documented (e.g., Cabana and Rasmussen, 1996; Matthews and Mazumder, 2005; Syvaranta et al., 2006). Since the zooplankton sampling took place in two different years, this may have influenced the $\delta^{13}C$ and $\delta^{15}N$ ratios reported in this study. However, other possible factors may have influenced the observed $\delta^{13}C$ and $\delta^{15}N$ ratios. The *Limnocalanus* dominated fraction was more enriched in $\delta^{15}N$ compared to the *Diaptomus* dominated fraction, which is consistent with previous studies (Jackson et al., 2013). The size fractions from Superior Shoal were unfortunately not identified prior to isotope analysis, but both fractions had similar $\delta^{15}N$, suggesting that the 63-250-µm size fraction at Superior Shoal may have been predominately *Limnocalanus.* It is also possible that the small size fraction was enriched due to the depth it was collected, as enrichment in

zooplankton $\delta^{15}N$ ratios with depth has been demonstrated elsewhere (Johannsson et al., 2001; Koppelmann et al., 2009). Further research is necessary to examine between site differences in zooplankton community composition and isotope ratios.

Similarities in isotope ratios among prey species, as well as their relative position in the food webs, suggest that most species analyzed could be consumed by Lake Trout morphs. Consistent with the stomach content results from this study, the isotope ratios of *Diporeia*, snails, clams, moths, and zooplankton suggest that these species are unlikely to be consumed in large proportions by Lake Trout morphs. *Mysis*, Deepwater Sculpin, and coregonids (i.e., Kiyi, Bloater, Cisco) were the most common prey items found in Lake Trout stomachs, but because all three coregonid species have similar isotope ratios (likely because of similarities in diet (*Mysis*, calanoid copepods, and *Daphnia*) (Gamble et al., 2011a; Isaac et al., 2012), evaluating specific prey/proportions of prey that the Lake Trout morphs are consuming cannot be achieved using stable isotopes alone.

Conclusion

Subtle differences in stomach content, fatty acid profiles, and stable isotope ratios among the Lake Superior Lake Trout morphs were observed, and support my hypothesis that feeding ecology differs among morphs. While temporally limited, stomach contents indicated that humpers receive 40%-50% of their total biomass from *Mysis*, reflecting a planktivorous diet for humpers, which contrasted the piscivorous diet of the other three morphs. Fatty acid profiles of dietary fatty acids showed overlap between humpers and siscowets, suggesting diet similarities between these two morphs. However, siscowets fatty acid profiles were more similar to leans at Stannard Rock, suggesting that variability between sites may also influence morph diets. Stable

isotope ratios of C, N, and S were very similar among Lake Trout morphs. This was contrary to my prediction that humpers would occupy a lower trophic level than the other morphs; all four Lake Trout morphs in Lake Superior appear to occupy a similar trophic level. The only stable isotope that suggested differences was $\delta^{34}S$, but further examination is required to explain the observed differences in $\delta^{34}S$ ratios between sites, and if these differences reflect dietary differences among morphs. Overall, it appears that humper feeding ecology differs from the other three morphs of Lake Trout in Lake Superior in terms of reliance on *Mysis*, though based on fatty acid profiles they appear to share similarities in prey items with siscowets (at least at Superior Shoal). This provides support for my prediction that humpers would have different diets from the other Lake Trout morphs, but have some prey items in common with the siscowets.

The findings of this study highlight the need for multiple sampling campaigns throughout the year; stomach contents only show a brief snapshot of diet, so long-term trends are not detectable without multiple sampling efforts. Stable isotope analyses performed on muscle tissue are reflective of ~1 year or more of dietary inputs, depending on growth rates (Hesslein et al., 1993), and do not indicate small scale seasonal differences. Stable isotope analysis conducted on tissues with different turnover rates (e.g., liver, blood) may also be useful in more accurately determining subtle temporal differences in diets among morphs. Fatty acids integrate roughly 2 months of diet information (Happel et al., 2016), so multiple sampling campaigns would also likely be necessary to capture small seasonal dietary changes.

Future directions of study include examining if there are ontogenetic differences in feeding in redfins and humpers, as this has been demonstrated for leans and siscowets (Zimmerman et al., 2009). DNA analysis and enzyme studies of humpers and redfins could also be performed, which may help explain metabolic differences among morphs. Another avenue of

future research is applying quantitative fatty acid signature analysis (QFASA) to Lake Trout morphs. This technique quantifies the contribution of fatty acids from prey items (Iverson et al., 2004), and may be useful in determining if some of the fatty acids that were not used as dietary biomarkers actually play a role in indicating differences in diet. However, QFASA requires calibrations of fatty acid concentrations through controlled feeding studies (Iverson et al., 2004), and thus further laboratory work would be necessary before this can be accomplished.

2.5 Figures and Tables

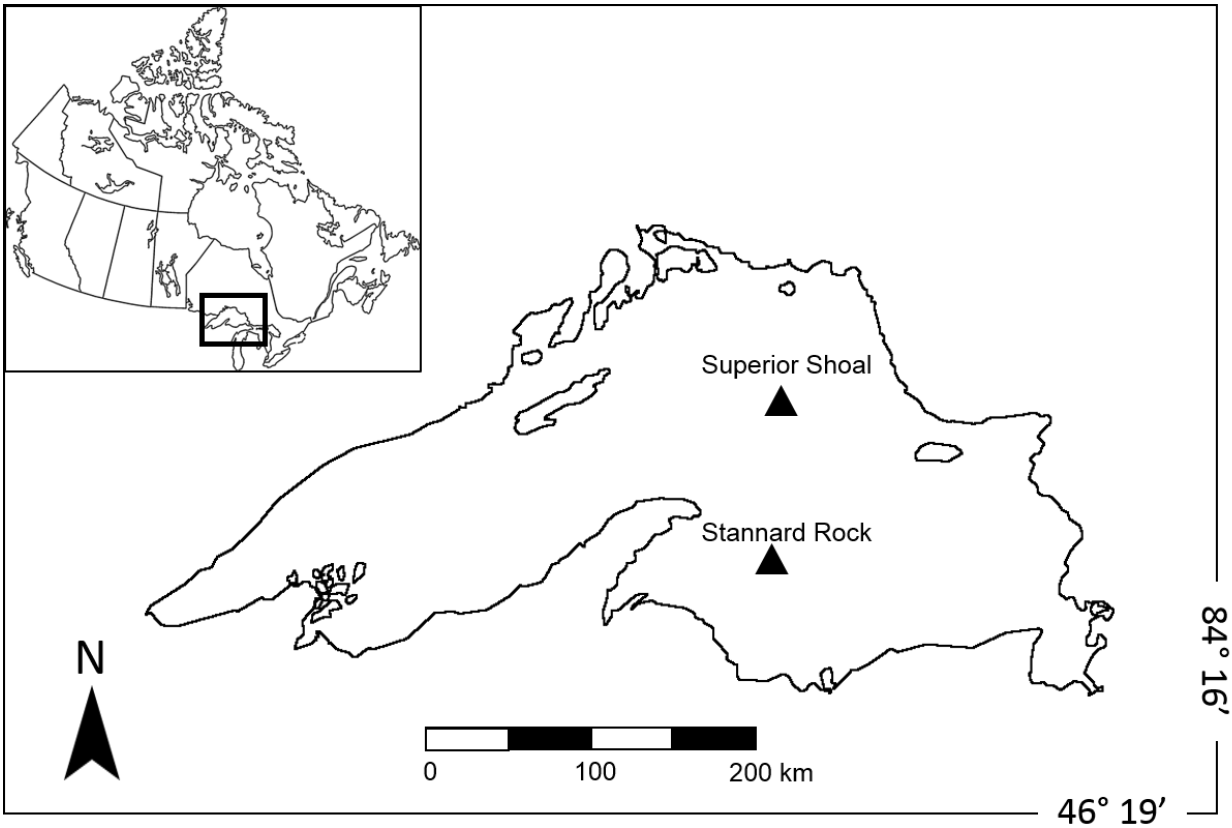

Figure 2.1. Location of sampling sites in Lake Superior.

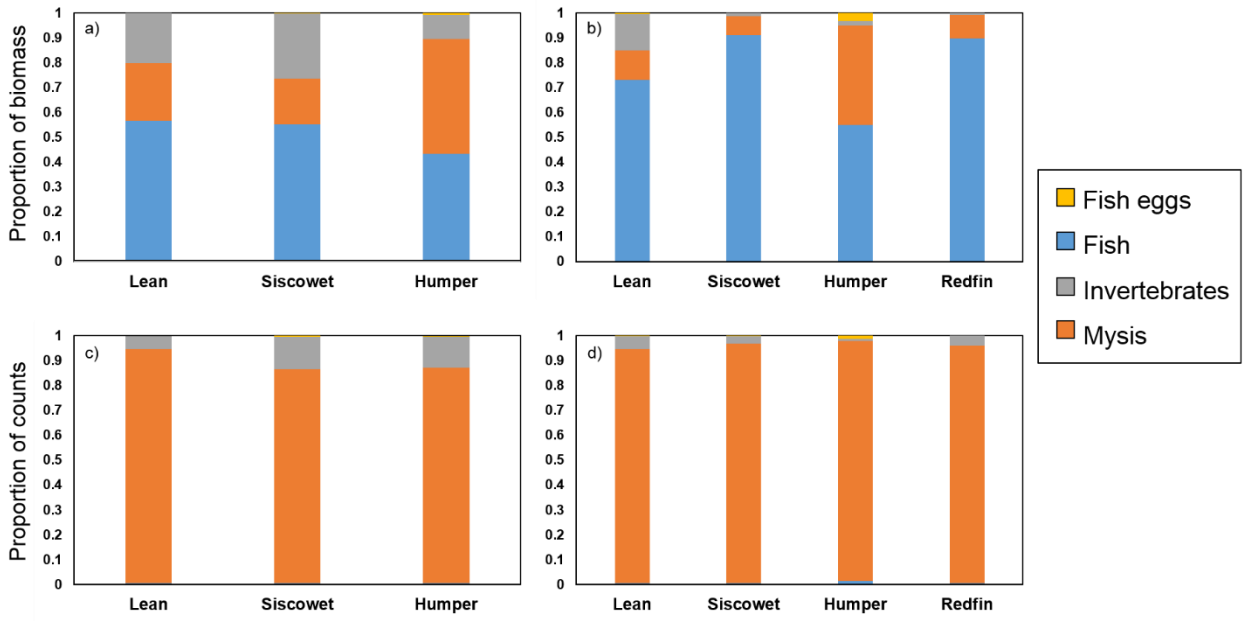

Figure 2.2. Stomach content biomass and counts for morphs of Lake Trout captured at Stannard Rock (a,c) and Superior Shoal (b,d). With the exception of humpers at Stannard Rock (a), fish accounted for >50% of biomass in Lake Trout stomachs at both sites and in all morphs. Numerically, *Mysis* was the most abundant prey consumed (c,d). *Mysis* contributed more biomass to stomach contents of humpers compared to the other morphs (a,b).

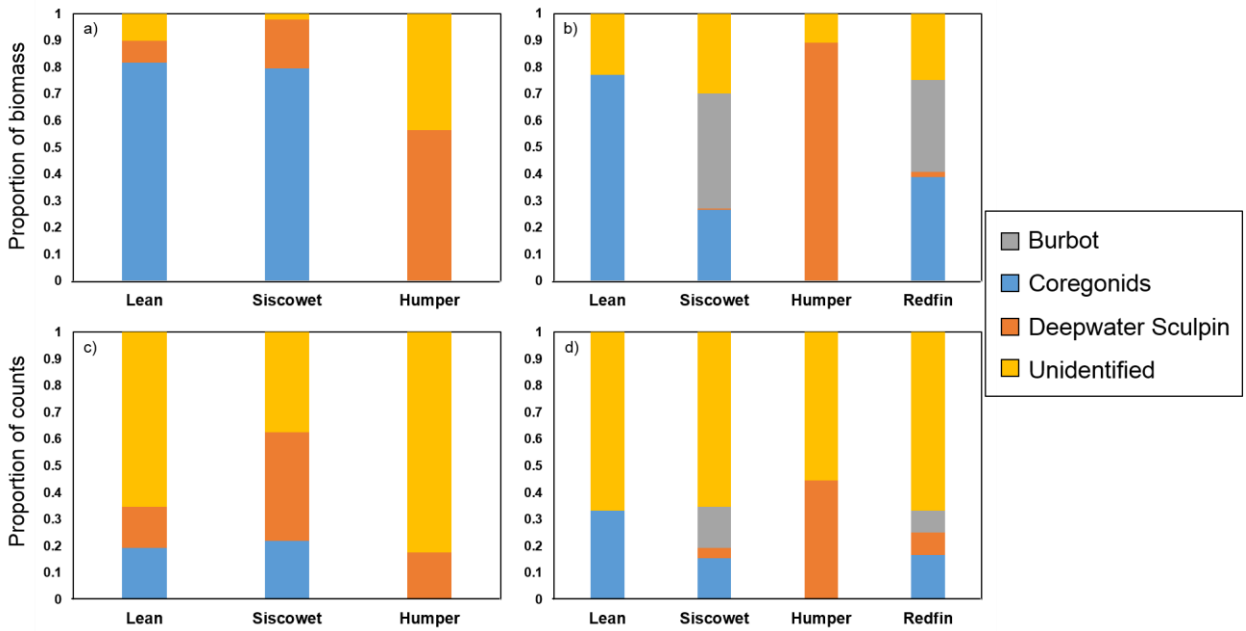

Figure 2.3. Composition of fish in Lake Trout stomachs captured at Stannard Rock (a,c) and Superior Shoal (b,d). Many of the fish remains in stomachs could not be identified (c,d). Coregonids contributed high proportions of biomass to the stomachs of leans and siscowets captured at Stannard Rock whereas Deepwater Sculpin contributed the greatest proportion of biomass to the stomachs of humpers collected at Stannard Rock (a). At Superior Shoal, coregonids contributed the greatest biomass to lean stomachs, whereas Deepwater Sculpin contributed the greatest biomass to humper stomachs (b); biomass in siscowet and redfin stomachs represented contributions from both coregonids and Burbot (b).

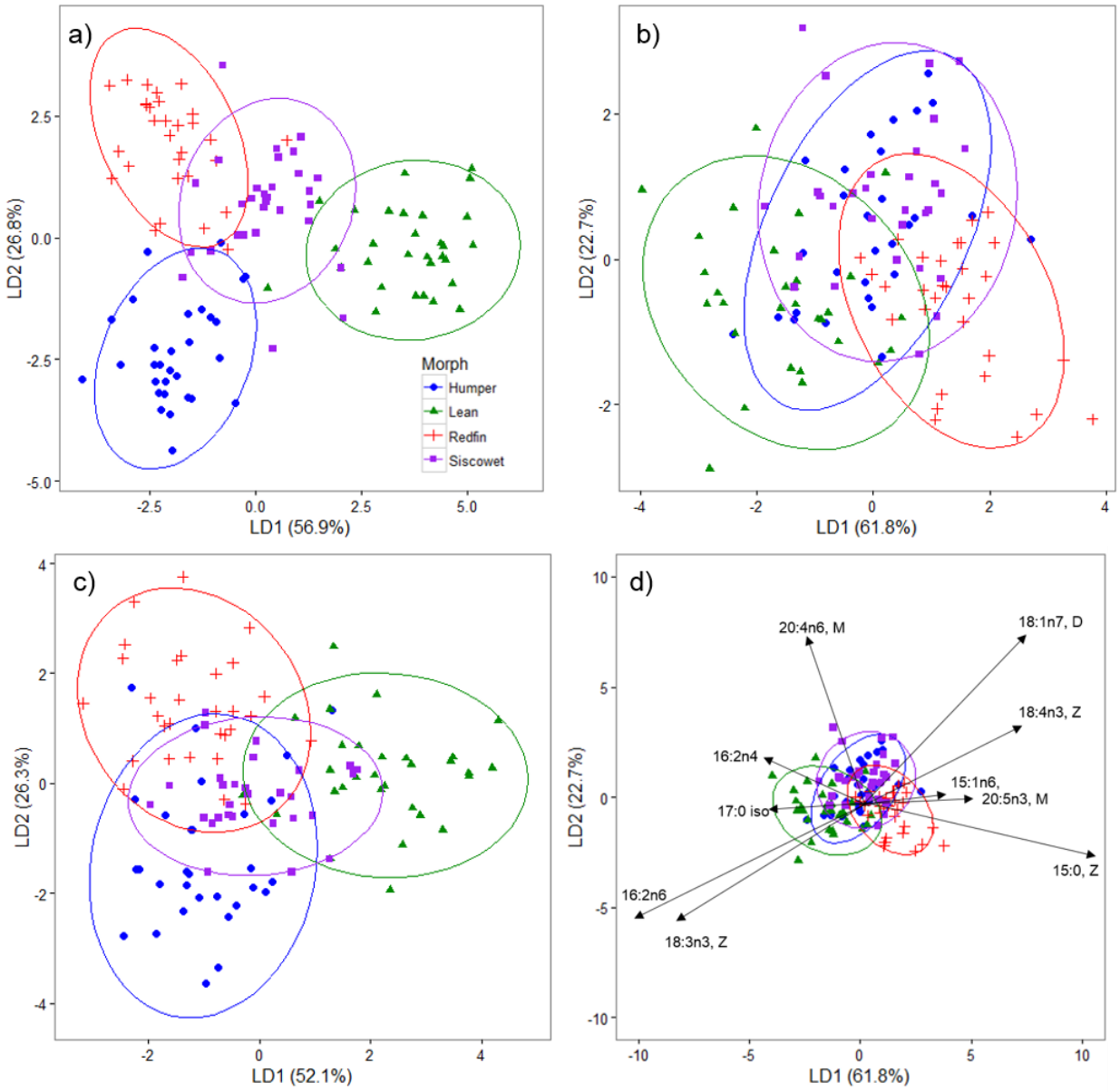

Figure 2.4. Linear discriminant function plot of fatty acid data from the four Lake Trout morphs from Superior Shoal. Refer to text for statistics associated with each LDA. In the LDA with all 70 fatty acids (a), the first two discriminant functions explained 83.7% of the variation. In the LDA of 30 dietary fatty acids, only the first discriminant function was significant, explaining 61.8% of the variation. When dietary fatty acids were excluded (c), three discriminant functions were significant (only the first two are presented here). The 5 highest-loading fatty acids for each side of the significant axis from the LDA with dietary fatty acids (b) are presented in (d). Letters beside fatty acids represent prey organisms from this study that had the highest concentration of that fatty acid. Letter codes: D= Deepwater Sculpin, M= *Mysis*, Z= Zooplankton. Only organisms with >1% concentration of a fatty acid are shown in (d).

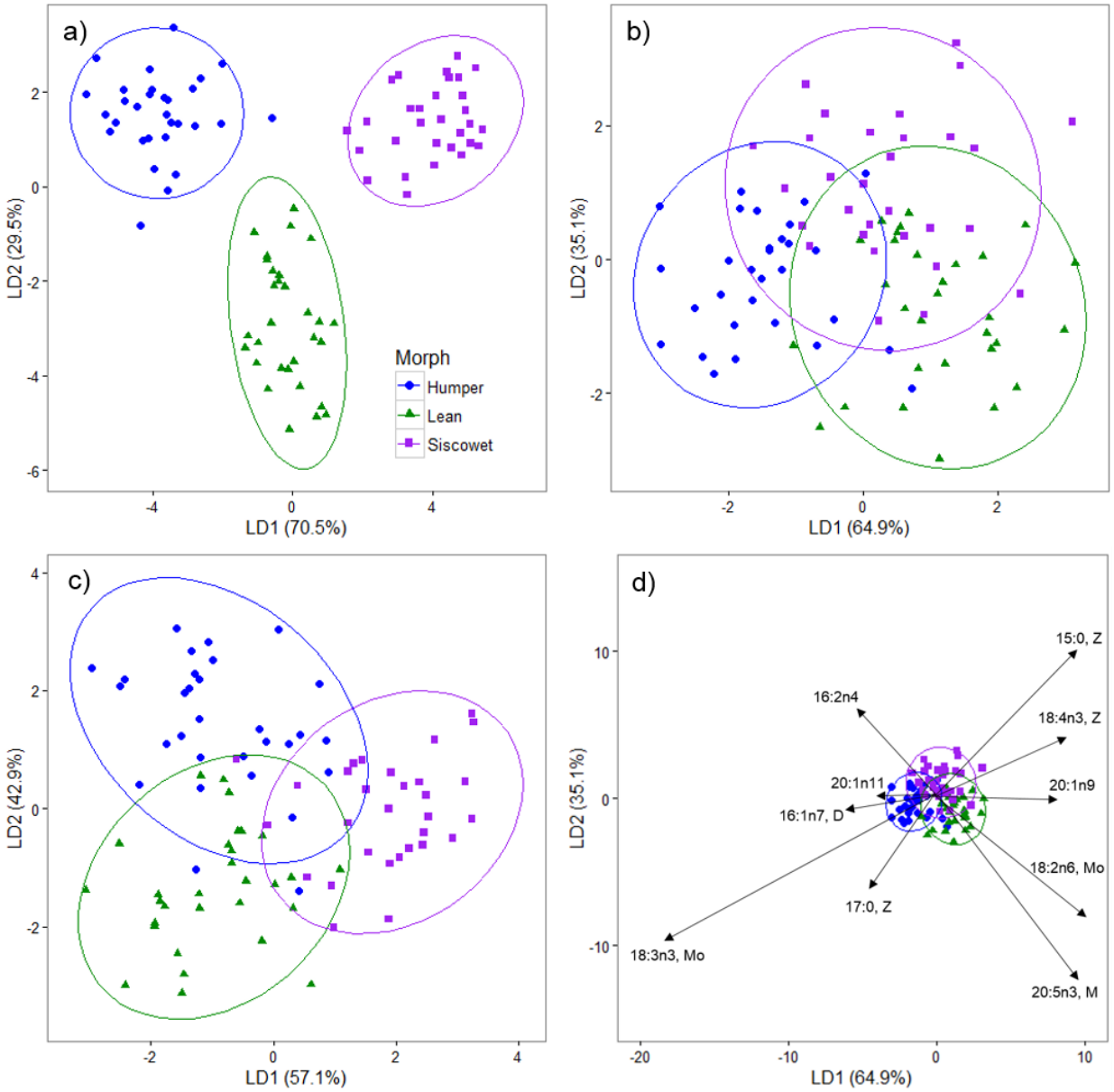

Figure 2.5. Linear discriminant function plots of the three Lake Trout morphs from Stannard Rock. Refer to text for statistics associated with each LDA. In both the LDA using all 70 fatty acids (a), and the LDA with 30 dietary fatty acids (b), only the first axes were significant. When dietary fatty acids were excluded, both discriminant axes were significant. The 5 highest-loading fatty acids for each side of the significant axis from the analysis of dietary fatty acids (b) are presented in (d). Letters beside fatty acids represent prey organisms from this study that had the highest concentration of that fatty acid. Letter codes: D= Deepwater Sculpin, M= *Mysis*, Mo= Moths, Z= Zooplankton. Only organisms with >1% concentration of a fatty acid are shown in (d).

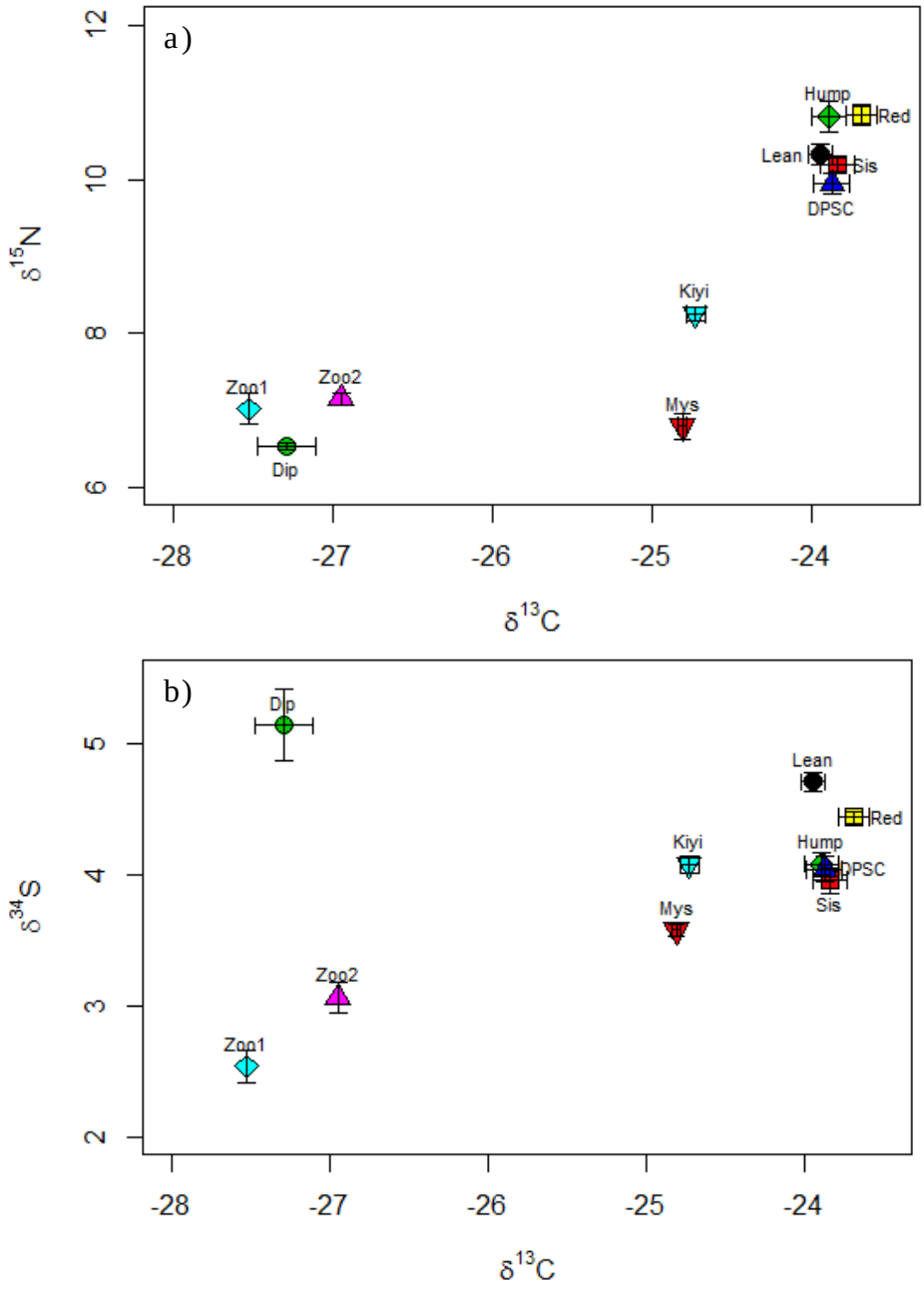

Figure 2.6. Superior Shoal food web bi-plots depict A) $\delta^{13}C$ and $\delta^{15}N$ ratios, and B) $\delta^{13}C$ and $\delta^{34}S$ ratios. Values are plotted as average isotope ratio measured in ‰ ± 1 standard error. Species codes are as follows: Hump=Humper, Lean=Lean, Sis=Siscowet, Red=Redfin, DPSC=Deepwater Sculpin, Kiyi=Kiyi, Mys=*Mysis*, Dip=*Diporeia*, Zoo1= Zooplankton 63-250 µm, Zoo2=Zooplankton 250-500 µm. Ranges for isotope ratios were $\delta^{13}C$=3.85, $\delta^{15}N$=4.30, and $\delta^{34}S$=2.61.

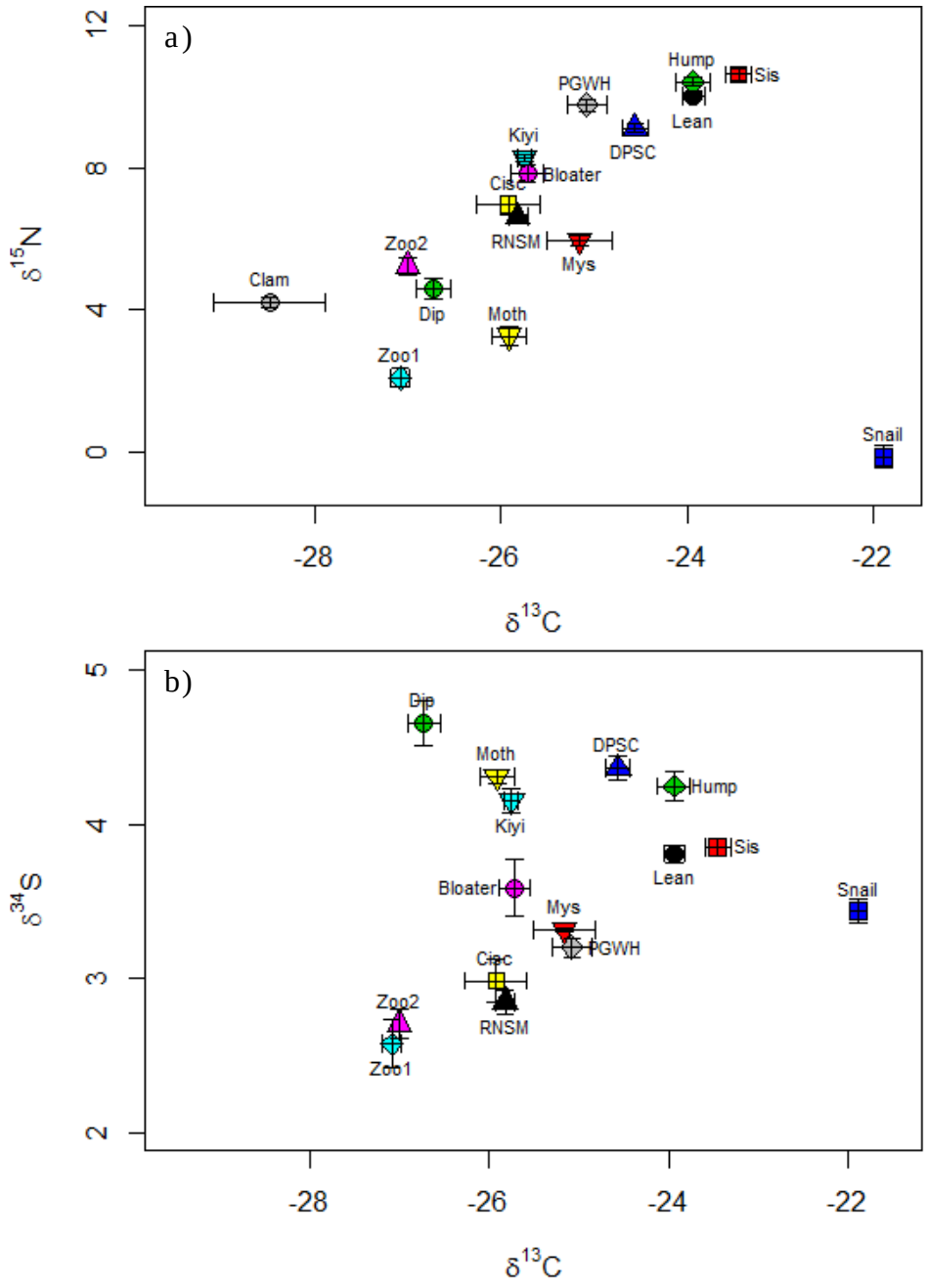

Figure 2.7. Stannard Rock food web bi-plots depict A) $\delta^{13}C$ and $\delta^{15}N$ ratios, and B) $\delta^{13}C$ and $\delta^{34}S$ ratios. Values are plotted as average isotope ratio measured in ‰ ± 1 standard error. Species codes are as follows: Hump=Humper, Lean=Lean, Sis=Siscowet, DPSC=Deepwater Sculpin, Kiyi=Kiyi, Bloater=Bloater, Cisc=Cisco, RNSM=Rainbow Smelt, PGWH=Pygmy Whitefish Mys=*Mysis*, Dip=*Diporeia*, Zoo1= Zooplankton 63-250 µm, Zoo2=Zooplankton 250-500 µm, Clam=Clam, Snail=Snails, Moth=Moths. Ranges for isotope ratios were $\delta^{13}C$=3.63, $\delta^{15}N$=8.54, and $\delta^{34}S$=2.08. To facilitate comparisons, organisms collected at Stannard Rock that were not collected at Superior Shoal were excluded from isotope range calculations.

Table 2.1. Taxa- and site-specific sample sizes analyzed for stable isotopes and fatty acids, and total number of Lake Trout stomachs used for stomach content analysis. A number in brackets indicates that replicates were composed of composite samples of several individuals.

Sample	$\delta^{13}C$ (bulk)	$\delta^{13}C$ (extracted)	$\delta^{15}N$	$\delta^{34}S$	Fatty Acids	Stomach Contents
Superior Shoal						
Lean	30	15	30	30	30	38
Siscowet	30	15	30	30	30	131
Humper	30	15	30	30	30	31
Redfin	30	30	30	30	30	60
Kiyi	15	8	15	15	15	
Deepwater Sculpin	15	8	15	15	15	
Mysis	(5)	(1)	(5)	(3)	(2)	
Diporeia	(5)	0	(5)	(3)	0	
Zooplankton	(10)	(2)	(10)	(6)	(2)	
Stannard Rock						
Lean	30	15	30	30	30	41
Siscowet	30	15	30	30	30	85
Humper	30	15	30	30	30	33
Kiyi	15	8	15	15	15	
Deepwater Sculpin	15	8	15	15	15	
Bloater	15	6	15	15	15	
Cisco	15	8	15	15	15	
Rainbow Smelt	15	0	15	15	(3)	
Pygmy Whitefish	15	8	15	15	15	
Mysis	(5)	(1)	(5)	(3)	(3)	
Diporeia	(5)	0	(5)	(3)	0	
Zooplankton	(10)	(2)	(10)	(6)	(3)	
Clam	(5)	0	(5)	0	0	
Snail	(5)	0	(5)	(3)	0	
Moth	(5)	(1)	(5)	(3)	(3)	

Table 2.2. Fatty acids analyzed as indicators of trophic resources use. Fatty acids were separated into known dietary biomarkers (n=30), and those that are either not currently known to be used as dietary markers, or that are known to reflect metabolism (n=40). References for studies using fatty acids are included where available.

Dietary Fatty Acids		
Fatty Acid	**Indicates**	**Reference**
15:0	Bacteria	(Meziane et al., 2002)
15:0 iso	Bacterial	(Meziane et al., 2002)
15:1n:8	Bacteria	(Volkman et al., 1980; Vestal & White, 1989)
15:1n6	Bacteria	(Volkman et al., 1980; Vestal & White, 1989)
16:1n7	Bacteria/Diatom	(Kharlamenko et al., 1995; Dalsgaard et al., 2003)
16:2n4	Diatom	(Dunstan et al., 1994)
16:2n6	Algal	(Dunstan et al., 1992)
17:0	Bacteria	(Meziane et al., 2002)
17:1	Bacteria	(Meziane et al., 2002)
17:0 iso	Bacteria	(Meziane et al., 2002)
16:4n1	Diatom	(Dunstan et al., 1994)
16:4n3	Algal	(Kelly & Scheibling, 2012)
18:1n9	Zooplankton	(Kattner & Hagen, 2009)
18:1n7	Bacteria/Diatom	(Kharlamenko et al., 1995; Dalsgaard et al., 2003)
18:2n6	Terrestrial	(Budge & Parrish, 1998; Budge et al., 2001)
18:3n3	Terrestrial/Algal	(Budge & Parrish, 1998; Arts & Wainman, 1999; Budge et al., 2001)
18:4n3	Zooplankton	(Harrington et al., 1970; Budge & Parrish, 1998)
20:1n11	Zooplankton	(Dalsgaard et al., 2003; Iverson, 2009)
20:1n9	Zooplankton	(Dalsgaard et al., 2003)
20:4n6	Algal	(Kirsch et al., 1998)
20:5n3	Diatom	(Kharlamenko et al., 1995)
20:2 NMI D1	Bivalves	(Joseph, 1982)
C20:2 NMI D2	Bivalves	(Joseph, 1982)
20:3 NMI T	Bivalves	(Joseph, 1982)
22:1n11	Zooplankton	(Hagen et al., 1993)
22:1n9	Zooplankton	(Hagen et al., 1993)
22:5n6	Zooplankton	(Ahlgren et al., 2009)
22:6n3	Diatom	(Cook, 1991)
22:2 NMI D1	Bivalves	(Joseph, 1982)
22:2 NMI D2	Bivalves	(Joseph, 1982)
Non Dietary Indicators		
Fatty Acid	**Production**	**Reference**
12:0	Metabolism	(Iverson et al., 2004)
12:1		

13:1		
14:0	Metabolism	(Iverson, 2009)
14:0 iso		
14:1n9		
14:1n7		
14:1n5	Metabolism	(Iverson et al., 2004)
14:0 ante		
16:0	Metabolism	(Iverson, 2009)
16:0 iso		
16:1n11	Metabolism	(Iverson et al., 2004)
16:1n9	Metabolism	(Iverson et al., 2004)
16:1n5		
18:0	Metabolism	(Iverson, 2009)
7Me 16:0		
16:3n4		
18:1n5		
18:2d5, 11		
18:2n7		
18:2n4		
18:3n6	Metabolism	(Tocher et al., 2006)
18:3n4		
18:3n1		
18:4n1		
20:0		
20:1n7		
20:2n9	Metabolism	(Cook, 1991)
20:2n6		
20:3n6	Metabolism	(Cook, 1991)
20:3n3		
20:4n3	Metabolism	(Cook, 1991)
22:0		
22:1n7		
22:2n6		
21:5n3		
22:3n3		
22:4n3		
22:5n3	Metabolism	(Cook, 1991)
24:1n9		

Table 2.3 Site- and morph-specific relative importance indices for each prey category. Values range from 0 to 100; higher numbers represent prey items that are relatively more common in stomachs. *Mysis* was the most important prey item for all morphs at both sites, and fish were the second most important prey item for all morphs at both sites. For each morph, invertebrates were relatively more important at Stannard Rock than at Superior Shoal.

Morph	Site	Fish	Mysis	Diporeia	Invertebrates	Eggs
Lean	Superior Shoal	28.49	54.81	0	15.30	1.40
	Stannard Rock	28.59	51.17	0.88	19.36	0
Siscowet	Superior Shoal	34.30	53.34	0.34	11.34	0.69
	Stannard Rock	26.13	47.68	0.54	23.88	1.78
Humper	Superior Shoal	24.21	62.55	0	7.02	6.22
	Stannard Rock	23.94	50.39	2.63	21.09	1.95
Redfin	Superior Shoal	33.05	54.57	0	12.39	0

Table 2.4. Fatty acid loading scores for LDAs conducted at Stannard Rock and Superior Shoal. Only axes that were significant (as determined by Wilk's lambda, up to the first 2 axes) are reported. When two axes were significant, a fatty acid was listed only under the axis for which it scored highest. 'Score' refers to the unstandardized canonical discriminant function coefficient. Negative scores indicate loadings on the negative side of the axis, and positive scores indicate loadings on the positive side of the axis. Magnitude of the score reflects the overall effect the fatty acid had on the discriminant function. Indicator is the organism or physiological process a fatty acid is associated with. For dietary LDAs, biomarker names in italics indicate organisms from this study that had the highest concentration of a given fatty acid. Asterisks (*****) beside an italicized name indicate a fatty acid concentration less than 1.0% of total fatty acids. Only the 5 highest (or fewer) scoring fatty acids for each side of an axis are presented.

LDA		**LD1**			**LD 2**	
Superior Shoal	**Fatty Acid**	**Score**	**Indicator**	**Fatty Acid**	**Score**	**Indicator**
All fatty acids(n=70)	17:0	30.19	Bacteria	18:1n7	34.86	Bacteria
	18:2n6	22.58	Terrestrial	16:0	23.71	Algal
	16:2n4	19.35	Diatom	20:0	10.55	
	18:4n3	17.05	Zooplankton	16:1n11	8.35	Metabolism
	16:1n5	16.58		17:0 iso	5.70	Bacterial
	18:3n6	-13.67	Metabolism	20:1n7	-5.11	Zooplankton
	17:1	-13.77	Bacteria	16:1n7	-7.14	Bacteria
	14:0 anteiso	-13.97		18:2n4	-11.68	
	18:3n3	-19.73	Terrestrial	16:2n6	-11.74	Algal
	15:0	-43.25	Bacteria	22.5n3	-12.01	Metabolism
Non dietary/ unknown if dietary fatty Acids (n=40)	20:3n3	9.49		7Me 16:0	6.49	
	16:1n5	7.87		16:1n11	4.88	Metabolism
	18:3n4	7.15		14:0 iso	-2.47	
	16:1n9	6.49	Metabolism	16:0 iso	-3.67	
	18:0	6.48	Metabolism			
	18:1n5	-4.43				
	18:2Δ5,11	-4.61				
	20:1n7	-4.78				
	22:5n3	-7.97	Metabolism			
	14:1n7	-8.76				
Known dietary fatty acids (n=30)	15:0	13.86	Bacteria *Zooplankton*			
	18:1n7	7.92	Bacteria/ Diatom, *Deepwater Sculpin*			
	18:4n3	7.57	Zooplankton, *Zooplankton*			
	20:5n3	6.23	Diatom, *Mysis*			
	15:1n6	4.24	Bacteria, *Kiyi**			
	20:4n6	-2.61	Algal, *Mysis*			
	17:0 iso	-4.49	Bacteria, *Kiyi**			
	16:2n4	-5.06	Diatom, *Deepwater Sculpin**			
	18:3n3	-7.48	Terrestrial or Algal, *Zooplankton*			
	16:2n6	-10.99	Algal, *Deepwater Sculpin**			

Stannard Rock	**LD1**			**LD 2**		
	Fatty Acid	**Score**	**Biomarker**	**Fatty Acid**	**Score**	**Biomarker**
All fatty acids(n=70)	15:0	65.72	Bacteria			
	21:5n3	60.90				
	18:0	57.75	Metabolism			
	16:1n9	43.3225	Metabolism			
	18:2n4	40.9829				
	20:0	-41.41				
	18:3n3	-51.34	Terrestrial			
	16:0	-55.19	Metabolism			
	17:0	-58.05	Bacteria			
	14:0	-71.85	Metabolism			
Non dietary/ unknown if dietary fatty Acids (n=40)	21:5n3	22.33		14:0	20.18	Metabolism
	18:0	17.37	Metabolism	18:3n4	14.87	
	13:1	13.41		16:1n5	13.42	
	22:5n3	12.52	Metabolism	22:4n3	8.87	
	12:0	11.45	Metabolism	18:1n5	-8.27	
	20:0	-8.63		140: anteiso	-8.45	
	22:3n3	-24.60		24:1n9	-9.54	
	16:0	-36.05	Metabolism	20:4n3	-19.93	Metabolism
Known dietary fatty acids (n=30)	15:0	10.27	Bacterial, *Zooplankton*			
	18:2n6	10.26	Terrestrial, *Moths*			
	20:5n3	9.83	Diatom, *Mysis*			
	18:4n3	9.22	Zooplankton, *Zooplankton*			
	20:1n9	9.02	Zooplankton, *Cisco**			
	20:1n11	-4.43	Zooplankton, *Moths**			
	17:0	-4.46	Bacteria, *Zooplankton*			
	16:2n4	-5.87	Diatom, *Mysis**			
	16:1n7	-6.94	Diatom/Bacteria, *Deepwater Sculpin*			
	18:3n3	-17.66	Terrestrial or Algal, *Moths*			

Table 2.5. Mean ± SD isotope ratios for each site and morph of Lake Trout. $\delta^{13}C_{Adjusted}$ represent carbon isotope ratios after lipid correction was applied. $\delta^{15}N$ and $\delta^{34}S$ ratios differed among morphs within sites (ANOVA, $F_{\geq 2,87} \geq 4.957$, $p \leq 0.009$). Letters indicate significant pairwise differences (Tukey's HSD <0.05) among morphs within the same site. In a two-way ANOVA, significant differences among morphs and between sites in average $\delta^{13}C_{Adjusted}$, $\delta^{15}N$, and $\delta^{34}S$ ratios were observed (ANOVA, $F_{\geq 2,174} \geq 3.263$, $p \leq 0.041$). Despite these statistical differences, absolute differences in isotope ratios were very minor, and given the uncertainty around fractionation factors, are likely not ecologically relevant.

Sample	**$\delta^{13}C_{Adjusted}$ (mean±SD)**	**δ15N (mean±SD)**	**δ34S (mean±SD)**
Superior Shoal			
Lean	-23.94±0.44	10.32±0.75 *a,b*	4.71±0.40 *a*
Siscowet	-23.84±0.58	10.19±0.52 *b*	3.96±0.54 *b*
Humper	-23.89±0.61	10.81±1.06 *a*	4.09±0.47 *b*
Redfin	-23.68±0.52	10.84±0.75 *a*	4.44±0.26 *a*
Stannard Rock			
Lean	-23.93±0.64	10.02±0.64 *b*	3.81±0.30 *b*
Siscowet	-23.45±0.79	10.63±0.92 *a*	3.85±0.30 *b*
Humper	-23.94±0.99	10.41±0.67 *a*	4.25±0.51 *a*
Site	**$\delta^{13}C_{Adjusted}$ Range**	**δ15N Range**	**δ34S Range**
Superior Shoal	0.26	0.65	0.75
Stannard Rock	0.49	0.61	0.44

Chapter 3

Niche overlap among Lake Trout morphs in Lake Superior

3.1 Introduction

Ecological niches can be defined as the environmental and trophic resources utilized by organisms (see Newsome et al., 2007). Hutchinson (1957) proposed that a niche is an *n*-dimensional space, where each environmental and biological component that comprises the niche is represented by a dimension (Hutchinson, 1957). Niche overlap is commonly observed in nature as many species share resources, such as prey or habitat (Rusterholz, 1981; Arlettaz et al., 1997; Hodgson et al., 1997; Hasui et al., 2009). It has been proposed that species can tolerate overlap in niche space, differences in at least one dimension prevent competitive exclusion (Hutchinson, 1957; May & Macarthur, 1972; Pianka, 1974).

One way to quantify the trophic dimensions of an organism's niche is through the use of stable isotope ratios. Stable isotope ratios are routinely used as tracers of diet and habitat use. In freshwater systems, nitrogen isotope ratios ($\delta^{15}N$) are used to infer relative trophic position (Deniro & Epstein, 1981), carbon isotope ratios ($\delta^{13}C$) are used to discriminate between benthic and pelagic basal food sources (Deniro & Epstein, 1978; France, 1995), and sulphur isotopes ($\delta^{34}S$) are used to discriminate between pelagic and profundal food sources (see Peterson & Fry, 1987; Croisetiere et al., 2009). Isotopic niches have been used as proxies for ecological niches in many previous studies (e.g., Bearhop et al., 2004; Newsome et al., 2007; Jackson et al., 2011). Fatty acids can also be used to quantify trophic niche, as some fatty acids, particularly long chain monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids can be indicative of diet (see Arts & Wainman, 1999; Bradshaw et al., 2003; Dalsgaard et al., 2003). Most vertebrates lack the

necessary enzymes to produce MUFAs and PUFAs *de novo* (see Arts & Wainman, 1999; Hastings et al., 2001) and must obtain them through consumption. Certain fatty acids are biomarkers for the organisms that produce them; for example, 20:5n3 is dominant in diatoms, and 18:4n3 is high dinoflagellates (Harrington et al., 1970; Budge & Parrish, 1998).

While resource partitioning among species is ubiquitously observed in nature, resource partitioning within species is also common (Bolnick et al., 2002). There is a high degree of intraspecific phenotypic diversity among populations within the *Salvelinus* genus (see Martin & Olver, 1980; Jonsson & Jonsson, 2001; Reist et al., 2013). The Lake Trout, *Salvelinus namaycush*, exhibits phenotypic variation in many lake habitats in North America (see Krueger & Ihssen, 1995; Blackie et al., 2003; Zimmerman et al., 2006; Hansen et al., 2012; Chavarie et al., 2013; Muir et al., 2014). Different phenotypic variants, or morphs, of Lake Trout exist in sympatry, and display differences in characteristics such as body shape (Muir et al., 2014), fat content (Eschmeyer & Phillips, 1965; Goetz et al., 2014), gill raker structure (Martin & Sanderco, 1967), spawning time (Eschmeyer, 1955; Hansen et al., 2016), feeding (Harvey et al., 2003; Ray et al., 2007; Gamble et al., 2011a) and habitat use (Behnke, 1972). Four Lake Trout morphs inhabit Lake Superior: leans, siscowets, humpers, and redfins (Muir et al., 2014). Each morph occupies a specific but overlapping depth range; leans occupy the shallowest depth range of the four morphs, and are commonly found in waters< 80m, (e.g., Harvey et al., 2003; Hansen et al., 2012). Siscowets are the most abundant morph in Lake Superior (Bronte et al., 2003), and are commonly found in waters > 80m deep (Sitar et al., 2008; Goetz et al., 2014). Siscowets display diel vertical migration (DVM) behaviour, which allows them to feed in the pelagic and profundal zone (Moore & Bronte, 2001; Gorman et al., 2012a, 2012b). Humpers are the smallest and slowest growing of the four morphs, and inhabit offshore reefs surrounded by mid to deep

water > 90m (Rahrer, 1965; Hansen et al., 2012). Redfins are highly buoyant morphs that are found between 50-100 m (potentially overlapping in depth with leans, siscowets, and humpers), but little else is currently known about this morph (Muir et al., 2014; Hansen et al., 2016).

In addition to spatial overlap among morphs in lake habitat use, prey consumption also overlaps. Coregonid species (i.e., Kiyi, Cisco, Shortjaw, Bloater), Deepwater Sculpin, and *Mysis* are prey items commonly consumed by the Lake Trout morphs in Lake Superior (Gamble et al., 2011a; Gamble et al., 2011b). Diet overlap and resource partitioning between lean and siscowet Lake Trout have been investigated in Lake Superior, in response to concerns that the more abundant siscowets may compete for resources with lean Lake Trout (Harvey et al., 2003). Evidence from both direct diet data and stable isotopes suggested little dietary overlap between these two morphs (Harvey & Kitchell, 2000; Harvey et al., 2003; Ray et al., 2007; Gamble et al., 2011b). Dietary overlap and resource partitioning have not been quantified between humper or redfin and the other morphs.

As humper have been used in some recent stocking efforts (Markham et al., 2008), quantifying trophic niche overlap between humpers and the other morphs is of interest for predicting potential competitive interactions and resiliency to stressors. The purpose of this research was to quantify trophic niche overlap (as determined by stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and fatty acid profiles) among the four morphs of Lake Trout in Lake Superior, and determine if the humper morph occupies a different trophic niche compared to other morphs. Because there is some overlap in prey consumption among morphs, I hypothesized that there would be some trophic niche overlap among Lake Trout morphs, but that the degree of overlap would vary between morphs. Specifically, I predicted that humpers would occupy a different niche from the other Lake Trout morphs, and that their niche region would be the smaller than

the other morphs, reflecting their consumption of primarily *Mysis* and small bodied fishes. I also predicted that siscowets would have the largest niche region of the four Lake Trout morphs (reflecting DVM behaviour)

Quantifying niche space and trophic overlap of Lake Trout morphs is critical for inferring inter-morph partitioning of resources. By understanding the resource needs and degree of overlap among morphs, predictions about stocking success can be made when a specific morph or set of morphs are introduced into a new environment.

3.2 Methods

Study Site and Collection Methods

Two sites, Superior Shoal (48° 3'43.54" N, 87° 8'52.57" W) and Stannard Rock (47°12'26.26" N, 87°12'3.82" W) were sampled in Lake Superior during cruises on the R/V *Kiyi* (Figure 2.1). Superior Shoal and Stannard Rock were selected as study sites because they were known to support humper, lean, and siscowet Lake Trout morphs; when the study was designed, redfins had not yet been formally described in the literature, and it was not known if they would be present at these two sites.

Lake Trout were collected via gill nets in 2013 and 2014. Nets were set over night (between 12 and 24 hours). Three different depth ranges were sampled, 0-50m (ten nets) 50-100m (nine nets) and 100-150m (nine nets); these correspond to the depth ranges thought to be occupied by the morphs. Gill nets were multifilament nylon twine, 183-m long by 1.8-m high with 30.5-m panels ranging from 50.8 to 114.3 mm, in 12.7-mm increments. Total length (mm), wet weight (g), sex, and maturity for each Lake Trout were determined upon capture. Dorsal, skinless muscle samples were removed from each Lake Trout for stable isotope and fatty acid

analyses. Because determination of morphs is sensitive to fish size (Zimmerman et al., 2006) only Lake Trout > 300mm in total length were analyzed further.

Assignment of Lake Trout Morphs

Morphometric analysis (Perreault-Payette, 2016) and visual identification (performed by A. Muir, C. Krueger, and C. Bronte) were used to assign each captured Lake Trout (> 300 mm total length) to a morph. Lateral photographs of each fish were used to quantify size-free body and head shape via geometric morphometrics (Muir et al., 2014). These digitized points were analyzed using the MCLUST R package (Fraley & Raftery, 2009), which assigned individual fish a morph identity based on the head and body models. Three visual assignments per fish were generated by three experienced researchers: Charles Bronte (U.S. Fish and Wildlife Service), Andrew Muir (Great Lakes Fishery Commission), and Charles Krueger (Michigan State University). At least two of the three visual assignments had to agree for a fish to be given a visual identification. The visual identifications were then compared with results of morphometric models. If two of three of the assignments (visual, head, and body) agreed, the fish was given that morph assignment. If none of the models agreed, the fish was not assigned a morph and excluded from further analysis.

This dual method of morph identification was employed for all morphs except humper, due to their smaller sizes at maturity. Sensitivity of morphometric analysis is decreased for fish less than 430 mm (Zimmerman et al., 2006), and mean size at maturity of humpers is ~450 mm (Hansen et al., 2016); therefore, in the case of humpers, only visual identifications were used. Out of 901 Lake Trout captured, 419 were assigned a morph identification.

Fatty Acid Analysis

Lipids were extracted using a modified Folch method (Folch et al., 1957; Budge et al., 2006). Freeze-dried skinless dorsal muscle tissue was used for Lake Trout. Approximately 0.2 g of tissue was treated with a 2:1 chloroform-methanol solution containing 0.01% butylated hydroxytoluene (BHT) (v/v/w) and refrigerated overnight. The lipid phase was then separated, dried with anhydrous sodium sulphate and evaporated under nitrogen to obtain total lipid mass. Fatty acid methyl esters (FAMEs) were produced from extracted lipids by transesterification with Hilditch reagent (100:1 parts dry methanol to H_2SO_4 v/v) (Morrison & Smith, 1964). Samples were heated to 100°C for 1 hour, back extracted with hexane and dried with anhydrous sodium sulphate. The FAME layer was removed and evaporated under nitrogen until dry and weighed. Finally, FAMEs were diluted in hexane to a concentration of 0.20mg/mL.

FAMEs were analyzed at the Freshwater Institute (Winnipeg, Manitoba). Gas chromatographic (GC) analysis was performed on an Agilent Technologies 7890N GC equipped with a 30-m J&W DB-23 column (0.25-mm I.D; 0.15-µm film thickness). The GC was coupled to a Flame Ionization Detector (FID) operating at 350 ºC. Hydrogen was used as carrier gas flowing at 1.25 mL/min for 14 minutes and ramped to 2.5 mL/min for the remainder of the run. The split/splitless injector was heated to 260 ºC and run in splitless mode with a 50 psi pressure pulse for 1.25 minutes. The oven program was as follows: 60 ºC for 0.66 min; 22.8 ºC/min to 165 ºC with a 2.0 min hold; 4.7 ºC/min to 174 ºC and 7.6 ºC/min to 200 ºC with a 6 min hold. Peaks were quantified using Agilent Technologies ChemStation software. Fatty acid standards were obtained from Supelco (37 component FAME mix) and Nuchek (54 component mix GLC-463). Eighty FAMEs were identified via retention time and known standard mixtures and are reported as percent of total fatty acid. Each fatty acid is described using the shorthand

nomenclature of A:Bn-X, where A represents the number of carbon atoms, B the number of double bonds and X the position of the double bond closest to the terminal methyl group. Fatty acids are reported as % total FAME content. A total of 210 Lake Trout samples (30 per morph per site) were analyzed for fatty acids.

Stable Isotope Analysis

Samples from 210 Lake Trout (30 per morph per site, excluding redfins from Stannard Rock; low sample size precluded analysis of redfins at Stannard Rock), and 120 prey fishes (fifteen per species per site) were analyzed for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$. Lake Trout dorsal muscle samples (skin off) were freeze dried and ground into a fine powder before being weighed for stable isotope analysis (SIA).

Ratios of stable carbon and nitrogen isotopes were determined at the University of Waterloo Environmental Isotopes Laboratory (UWEIL) on a 4010 Elemental Analyzer (Costech Instruments) coupled to a Delta XL (Thermo-Fisher) continuous flow isotope ratio mass spectrometer (CFIRMS). Sulfur isotopes were analyzed on a 4010 Elemental Analyzer (Costech Instruments) coupled to an Isochrom (GVInstruments / Micromass UK) CFIRMS. Isotope ratios are reported in δ notation, which is calculated as:

$$\delta^{j}X = \left[\frac{(\frac{^{j}X}{^{i}X})\, sample}{(\frac{^{j}X}{^{i}X}) standard} - 1 \right] \times 1000 \text{ (Equation 2)}$$

where ^{j}X is the heavier isotope (e.g., ^{15}N), and ^{i}X the lighter isotope (e.g., ^{14}N) in the sample (numerator) and international measurement standard (denominator). Atmospheric nitrogen is the standard for $\delta^{15}N$, Vienna PeeDee Belemnite for $\delta^{13}C$, and Canyon Diablo triolite for $\delta^{34}S$ (see Gonfiantini et al., 1995). All values are reported in parts per mil (‰). Analytical error for $\delta^{13}C$,

$\delta^{15}N$, and $\delta^{34}S$ did not exceed 0.2‰, 0.3‰, or 0.3‰ based on corrections made using an array of international reference material and in-house standards that were calibrated using certified international reference materials (i.e. IAEA-N1 + N2, IAEA-CH3 + CH6, USGS-41 + 41, IAEA-SO-5, IAEA-SO-6, NBS-127, NBS-123, IAEA-S1 to-S3). Of the total sample number analyzed in an analytical run, no less than 20% were Std/Ref materials. Repeatability of samples (one in 10) for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ was 0.2‰, 0.3‰, and 0.3‰.

Lipid Correction Models

As lipids have been shown to be depleted in $\delta^{13}C$ (Kiljunen et al., 2006; Hoffman & Sutton, 2010), lipid correction models were used to correct Lake Trout $\delta^{13}C$ values for effects of lipid bias. A correction equation (see Appendix I) was developed based on mass balance models presented by Hoffman and Sutton (2010), and applied to individuals of all morphs with C:N ratios > 4.0, which was used as the minimum C:N ratio to perform lipid corrections as recommended by Hoffman et al. (2015). Lipid corrected $\delta^{13}C$ values were estimated as follows:

$$\delta^{13}C_{protein} = \delta^{13}C_{bulk} + [\Delta\delta^{13}C_{bulk} \times (\frac{C{:}N_{protein} - C{:}N_{bulk}}{C{:}N_{bulk}})] \text{ (Equation 3)}$$

Where $\delta^{13}C_{protein}$ is the $\delta^{13}C$ ratio of the lipid extracted sample, $\delta^{13}C_{bulk}$ is the $\delta^{13}C$ ratio of the non-extracted sample, $\Delta\delta^{13}C_{bulk}$ is the isotopic depletion factor due to lipids, $C{:}N_{protein}$ is the C:N ratio in the extracted sample, and $C{:}N_{bulk}$ is the C:N ratio in the non-extracted sample. $\Delta\delta^{13}C_{bulk}$ and $C{:}N_{protein}$ were estimated for each morph and species that had $C{:}N_{bulk} > 4.0$ at each site (see Appendix I for model selection methods). Superior Shoal leans and Stannard Rock humpers each had 2 outliers (leans: $\Delta\delta^{13}C_{bulk}$=-17.8‰, -268.7‰; humpers: $\Delta\delta^{13}C_{bulk}$ = -28.9‰, -30.0‰) that

were not included in calculating average $\Delta\delta^{13}C_{bulk}$ or $C:N_{protein}$, as these values were ~2-4 times larger than the literature values reported for $\Delta\delta^{13}C_{bulk}$ of ~7 (Kiljunen et al., 2006; Hoffman & Sutton, 2010).

Statistical Analysis

Statistical analyses were conducted using R software version 3.3.1 (R Core Team, 2016). Analyses were performed separately for each site. A number of statistical techniques aimed at quantifying ecological niche size have been developed in recent years. (e.g., Jackson et al., 2011; Syvaranta et al., 2013; Swanson et al., 2015; Rossman et al., 2016). One technique, called NicheROVER (Lysy et al., 2014) was developed using a Bayesian framework in the statistical program R and quantifies probabilistic pairwise n-dimensional overlap in niche space among any number of species (Swanson et al., 2015). I used stable isotope ratios and fatty acid biomarkers in NicheROVER to quantify trophic niche (hereafter referred to as niche) size and overlap among the four morphs of Lake Trout in Lake Superior, and to determine if humpers occupy a different niche compared to the other morphs. The three stable isotope ratios were selected for inclusion in the analysis as they reflect the diets ($\delta^{15}N$) and origin of production sources ($\delta^{13}C$, $\delta^{34}S$) for Lake Trout morphs (e.g., Deniro & Epstein, 1981; France, 1995; Croisetiere et al., 2009). Fatty acids were selected based on their use as dietary biomarkers (Chapter 2, this thesis). Using linear discriminant analysis, 30 fatty acids known to reflect diet were compared among Lake Trout morphs (Chapter 2, this thesis). The LDA scores for each morph from the first (and significant) axis (LD1) were used with stable isotope data to quantify niches and niche overlap for each pairwise combination of morphs. Prior to analysis, these four niche dimensions were z-score transformed, to ensure all four dimensions were on the same scale.

Data were determined to reasonably satisfy multivariate normal assumptions through analysis of a Chi-Square Q-Q plot, and the default uninformative prior was used for all calculations (Lunn et al., 2013; Swanson et al., 2015). A sample size of 30 Lake Trout per morph was used in the analysis (each sample was represented by $\delta^{13}C$, $\delta^{34}S$, $\delta^{15}N$ ratios, and an LD1 score); previous research has shown that 30 is the minimum number of samples necessary for Bayesian comparisons of isotopic niches (Syvaranta et al., 2013). The four-dimensional 95% probabilistic niche region was quantified, along with probabilities of pairwise overlap.

3.3 Results

Probabilistic (95%) niche regions were determined for each morph at each site. At both sites, humpers had the largest niche region, and siscowets and leans had similarly sized niche regions (Figure 3.2). Redfins had the smallest niche region at Superior Shoal. Niche regions can be visualized for each dimension from the density (diagonal) plots in Figure 3.3 and 3.4.

Consistent with the results of Chapter 2, LD1 scores from dietary fatty acid data differentiated among morphs more than stable isotope ratios. Of the three isotopes, the largest differences among morphs were observed in $\delta^{34}S$, though substantial overlap among morphs in all three isotopes was observed (Figure 3.3, Figure 3.4).

At Superior Shoal, pairwise overlap between morphs was most likely to occur with humpers, with a 70% probability of a redfin falling within the humper niche, and an 83% probability of a siscowet falling within the humper niche (Table 3.1, Figure 3.5). The probability of finding a lean within the niche of humper was only 53%, and the niches of humper and lean were mainly differentiated by fatty acid signatures and $\delta^{34}S$ ratios (Table 3.1, Figure 3.3). Interestingly, the lowest probability of niche overlap was between the redfins and leans; there

was only a 9% probability of finding a lean within the redfin niche, and a 25% probability of finding a redfin within the lean niche (Table 3.1; Figure 3.5). The niches of redfins and leans were mostly differentiated by fatty acid signatures (LD1) (Figure 3.3).

Pairwise overlap at Stannard Rock was most likely to occur between leans and siscowets (Table 3.1; Figure 3.6); whereas leans and siscowets were differentiated by fatty acid signatures (LD1) at Superior Shoal, LD1 scores were very similar between these two morphs at Stannard Rock (Figure 3.4). The lowest probability of niche overlap at Stannard Rock occurred between humpers and leans; there was a 23% probability of finding a humper in the lean niche, and a 32% probability of finding a lean in the humper niche (Table 3.1, Figure 3.6). The niches of humpers and leans were differentiated by LD1, but also by $\delta^{34}S$ ratios and $\delta^{15}N$ ratios (Figure 3.4). However, if pairwise comparisons involving redfin are ignored from Superior Shoal, the lowest probability of overlap was also that of humpers onto leans (Table 3.1).

3.4 Discussion

Humpers had the largest niche regions of all morphs at both Superior Shoal and Stannard Rock. This was contrary to my prediction, as I expected humpers would have the smallest niche region, and siscowets would have the largest. Because humpers appear to feed primarily on *Mysis* and small bodied prey fishes such as Deepwater Sculpin (Stafford et al., 2014; this thesis), I expected humpers would have a smaller niche compared to vertically migrating siscowets that consume prey across a variety of habitats (Hrabik et al., 2006; Zimmerman et al., 2009; Gorman et al., 2012a). However, stomach content analysis from humpers in this study revealed that humper diets are roughly 50% fish and 50% *Mysis* (Chapter 2, this thesis). Due to the limitations of morphometric identification, a majority of the Lake Trout examined in this study were >400

mm total length, roughly the size when Lake Trout shift to a primarily piscivorous diet (Zimmerman et al., 2009; Isaac et al., 2012). Stomach content results were consistent with a piscivorous diet in adult Lake Trout, as stomach contents from lean, siscowets, and redfin stomachs were predominately fish (by mass) (Chapter 2, this thesis). Therefore, the larger niche observed in humpers could be a result of humpers feeding on both piscivorous and planktivorous resources, opposed to primarily piscivorous resources like the other three morphs (at least by mass). Siscowets and leans had similar sized niches at both sites, and redfins had the smallest niches of all four Lake Trout morphs, indicating that redfins may be more specialized in their prey consumption than the other three morphs.

Interestingly, niche size was similar between sites for all Lake Trout morphs, even though niche overlap was observed to differ between sites. Pianka (1974) hypothesized that a greater amount of niche overlap could suggest less competition among organisms when resources are plentiful. This has been supported by studies showing niche overlap among sympatric species, and even within species (Santos-Carvallo et al., 2015; Vluet et al., 2015; Yang et al., 2016). As the niche size of Lake Trout morphs doesn't appear to change in response to differences in niche overlap, this suggests morphs are tolerant of some degree of overlap with each other, possibly because prey resources are not limiting. However, without measurements of prey density at each site, conclusions about the effects of niche overlap on the niche size of Lake Trout morphs cannot be drawn at this time.

I observed site-specific differences in niche overlap among Lake Trout morphs, as there was higher probability of overlap between siscowets and leans at Stannard Rock compared to Superior Shoal. At Stannard Rock, there was >50% probability of siscowets overlapping onto the niche of leans, and >70% probability of leans overlapping onto the niche of siscowets. These

results from Stannard Rock are contrary to previous studies, where investigators observed differences between lean and siscowet diets, and low dietary overlap between these two morphs (Harvey et al., 2003; Ray et al., 2007; Gamble et al., 2011a; Gamble et al., 2011b). There are some differences between previous studies and this study that may help explain this discrepancy. Ray et al., (2007) examined spring diets of leans and siscowets in Lake Superior, and Gamble et al., (2011b) specifically examined nearshore sites in Lake Superior with average depths ~40m. It is possible that competition between morphs was greater in these studies, and overlap therefore smaller, because prey was more limiting in spring and in shallow habitats, but this requires further investigation. I observed similar prey items in the stomach contents of leans and siscowets at Stannard Rock (e.g., *Mysis*, coregonids, Deepwater Sculpin) (Chapter 2, this thesis), as well as similarities in fatty acids profiles and stable isotope ratios (Chapter 2, this thesis). In particular, $\delta^{34}S$ ratios were much more similar between these two morphs at Stannard Rock than at Superior Shoal. This is likely a result of the habitat differences between the two sites; Stannard Rock is 20km closer to shore, and 100m shallower than Superior Shoal. It is possible that spatial overlap between the two morphs is greater at Stannard Rock than at Superior Shoal, which suggests that they may be more likely to consume similar resources, and suggests that niche overlap among morphs is likely driven by a combination of prey availability, and habitat availability and heterogeneity (e.g., range of depths).

Siscowet niches had the highest probability of overlapping onto humper niches at both Superior Shoal and Stannard Rock, and humper niches had the highest probability of overlapping onto siscowet niches at Superior Shoal. The relatively high degree of niche overlap between these two morphs at Superior Shoal is likely a result of common prey consumption (i.e., Deepwater Sculpin and *Mysis*), and spatial overlap in depth (Rahrer, 1965; Harvey & Kitchell,

2000), and is consistent with my prediction of humpers and siscowets having overlapping niches. At Stannard Rock, leans had the highest probability of overlapping onto the niche of siscowets. This could be a reflection of the fact that depth ranges occupied by each morph likely differ with available depths, and thus between sites.

Redfins and leans at Superior Shoal had the lowest probabilities of niche overlap out of all the morphs. Even though redfins spatially overlap with leans (Muir et al., 2014; Hansen et al., 2016), and had similar stable isotope ratios, the niche overlap between these two morphs was extremely small. Stomach contents revealed similarities between redfins and siscowets diets (Chapter 2, this thesis), though most fish present in stomachs were not identifiable. The largest differences between leans and redfins were in dietary fatty acid signatures (Chapter 2, this thesis). As adequate sample size for redfins was only achieved at Superior Shoal, it is impossible to determine if this trend would be consistent among sites, or if minimal overlap between redfins and leans is specific to Superior Shoal.

Lean Lake Trout were used exclusively to stock the Great Lakes until the initial introduction of a humper strain ("Klondike") to Lake Erie in 2004 (Markham et al., 2008; Muir et al., 2012). The use of Klondikes in Lake Erie have led to the consideration of stocking alternative morphs (e.g., humper) in other Great Lakes by fishery managers (Bronte et al., 2008; Muir et al., 2012). My observations suggest that humpers may be more appropriate candidates than siscowets for stocking in areas already occupied by leans. Humpers showed the lowest probability of overlapping onto the niche space of leans at both sites (excluding redfins from Superior Shoal). However, these results showed overlap probabilities between adult humpers and adults leans. As has been demonstrated, a large proportion of humper diet is composed of *Mysis* (Stafford et al., 2014; this thesis), which is similar to that of juvenile Lake Trout of all morphs

(Zimmerman et al., 2009; Isaac et al., 2012; Stafford et al., 2014). This suggests that humpers may have increased niche overlap with juvenile Lake Trout morphs instead of adults. Further research is necessary, as increased competition with juveniles could impede stocking efforts.

These estimates of niche size and overlap may serve as a benchmark for future research, as a variety of factors may change or influence how Lake Trout morphs partition resources. A recent survey of Lake Superior by the USGS found declines in coregonid, Rainbow Smelt, and Lake Trout biomass (Gorman et al., 2013). These declines seem to be related – prey fishes have been declining since the 1990's, and Lake Trout biomass began to decline in the 2000's, most likely as a response to decreased prey availability (Gorman et al., 2013). If prey fishes continue to decline, it may drive increased competition among Lake Trout morphs for prey resources, resulting in declines of less adapted morphs. Contrary to previous studies (Harvey et al., 2003; Ray et al., 2007; Gamble et al., 2011b), there was no evidence of Rainbow Smelt consumption (a dominant prey item) by leans, (Harvey et al., 2003; Ray et al., 2007; Gamble et al., 2011b). Declines in Rainbow Smelt abundance may be forcing leans to consume other prey, which may be why lean diets were very similar to siscowets at Stannard Rock, and why niche overlap was greater between leans and siscowets at Stannard Rock compared to Superior Shoal. However, further research is necessary, as it is possible leans are still consuming Rainbow Smelt, but they were not detected in stomachs. Another factor that could affect niche overlap among morphs is climate change. Lake Superior has been steadily warming since the 1980s, and the preferred thermal habitat for leans has been expanding, while the preferred thermal habitat for siscowets has been declining (Cline et al., 2013). This suggests that habitat overlap among morphs may increase as climate change continues, which may increase competition among morphs for resources.

Conclusion

The trophic niches of Lake Trout morphs in Lake Superior were shown to overlap, suggesting that, as expected, none of the Lake Trout morphs occupy a completely separate trophic niche. This likely reflects the generalist trophic ecology of Lake Trout feeding; data to date indicate that all morphs previously studied consume a variety of prey (Harvey et al., 2003; Ray et al., 2007; Gamble et al., 2011a; Gamble et al., 2011b, Chapter 2, this thesis). The amount of pairwise niche overlap varied among morphs, however, with certain morphs exhibiting greater niche overlap than others. This was consistent with my predictions. Contrary to my predictions, humpers had the largest niche regions, possibly reflecting heavy reliance on *Mysis*, whereas redfins had the narrowest niche regions, suggesting that redfins are the most specialized morphs. The least amount of niche overlap was observed between leans and redfins at Superior Shoal, and the most overlap was observed between humpers and siscowets at Superior Shoal, and leans and siscowets at Stannard Rock. Pairwise overlap in trophic niches varied between sites.

Knowledge of niche overlap among morphs can be informative to policy makers for Lake Trout management practices. Previous studies have demonstrated minimal trophic overlap between leans and siscowets, as they consume different prey items and differ in stable isotope ratios (Bronte et al., 2003; Harvey et al., 2003; Ray et al., 2007; Gamble et al., 2011a; Gamble et al., 2011b). However, my observations show that the trophic overlap among leans and siscowets differed between sites, and that niche overlap between leans and siscowets can be substantial. It appears that it may be appropriate for managers to consider site-specific stocking programs, as it is clear that the amount of niche overlap between morphs can vary between sites. Humpers, however, had low niche overlap with leans at both Superior Shoal and Stannard Rock, which suggests humpers may be appropriate to stock concurrently with leans regardless of habitat

conditions, and may help future stocking programs maximize the genetic diversity of Lake Trout populations in the other Great Lakes. Future research should examine niche overlap at other sites in Lake Superior (e.g., Isle Royale), prey density, seasonal variability in diet, and seasonal variability in habitat use to investigate variability in niche overlap among sites; this information may aid in informing future Lake Trout management decisions.

3.5 Figures and Tables

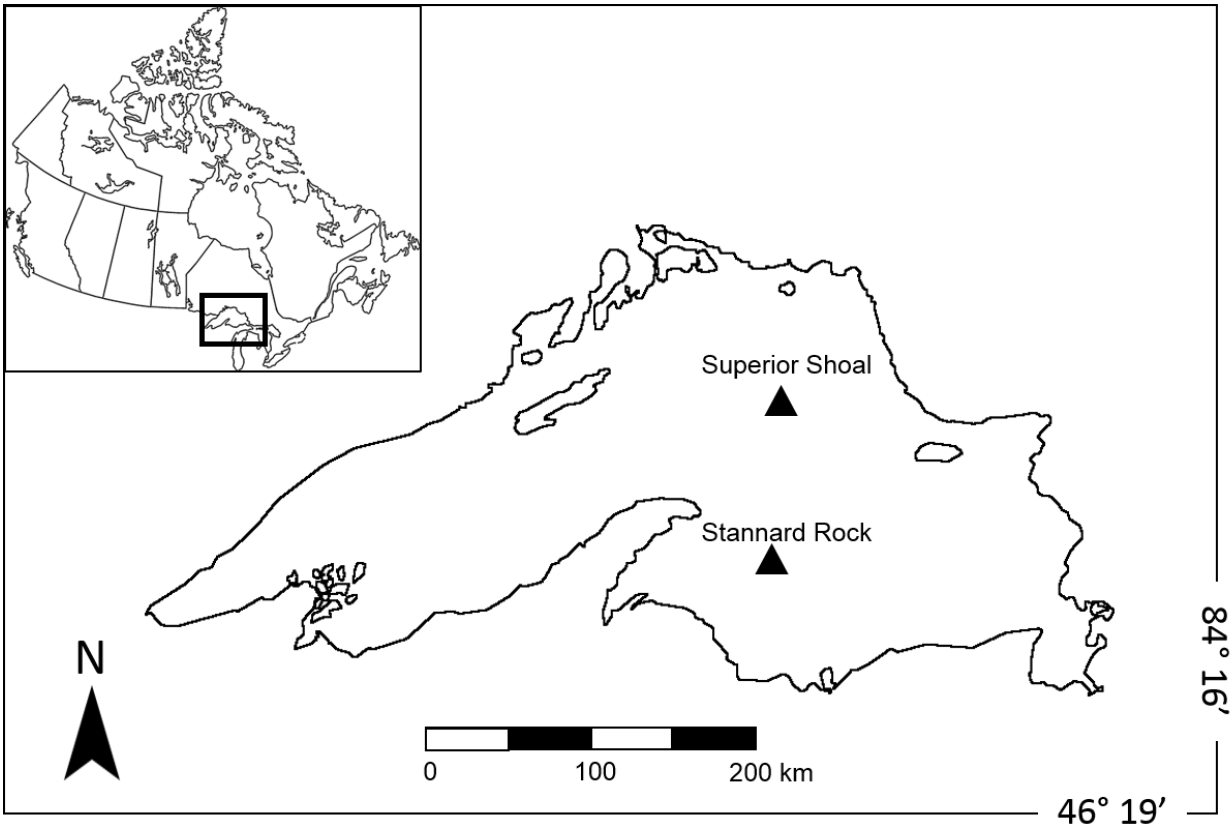

Figure 3.1. Location of sampling sites in Lake Superior.

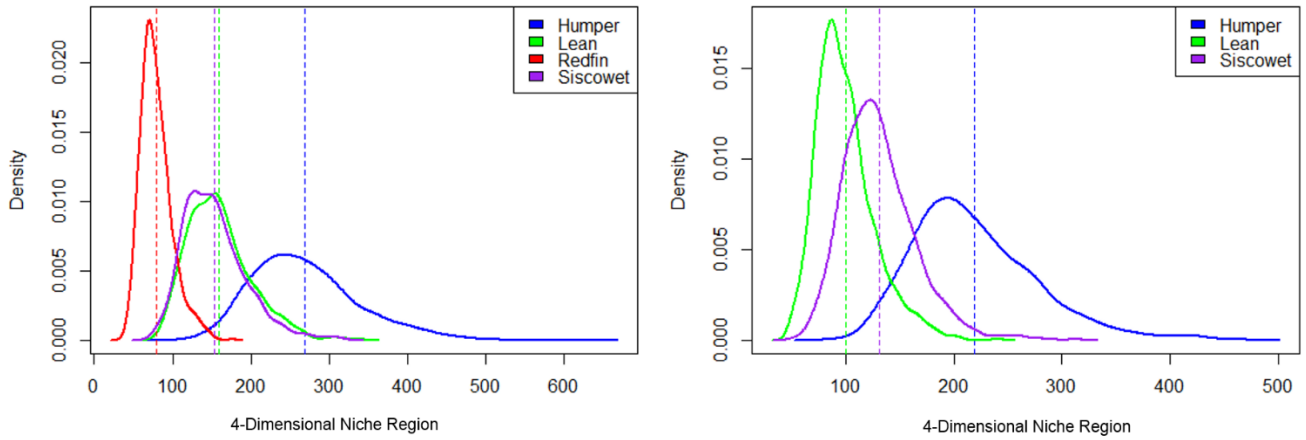

Figure 3.2. Probabilistic 95% niche region of morphs from a) Superior Shoal, and b) Stannard Rock. Results indicate humpers had the largest niche region, siscowets and leans had similar size niche regions, and redfins had the smallest niche regions.

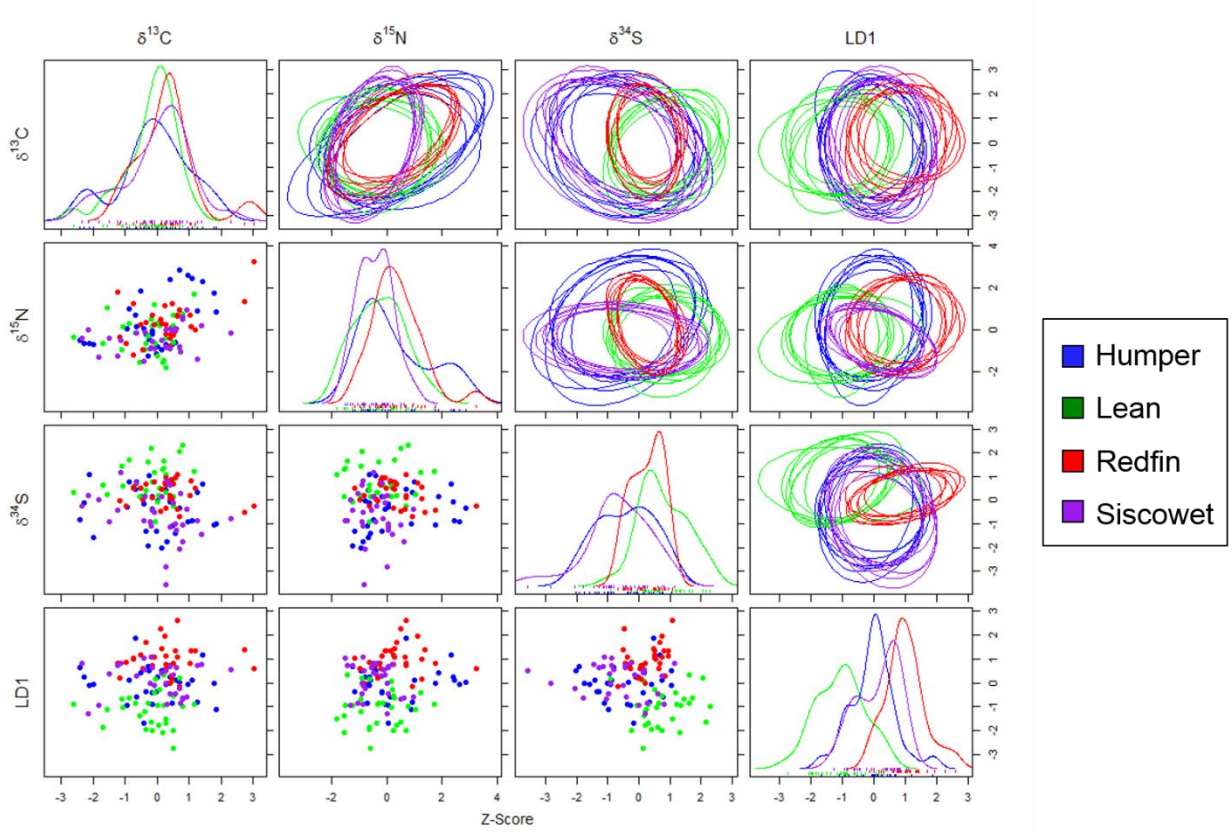

Figure 3.3. 7 randomly drawn elliptical projections of the 95% niche regions for each morph and niche dimension (stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and fatty acid profiles, LD 1) at Superior Shoal. Data was converted to a z-score prior to analyses. Ellipses (above the diagonal) represent two-dimensional projections of niche regions for each morph. Also presented are two-dimensional scatter plots (below the diagonal), and one-dimensional density plots (on the diagonal). While there was substantial overlap in each dimension of niche space, LD 1 (fatty acids) showed the least overlap among morphs.

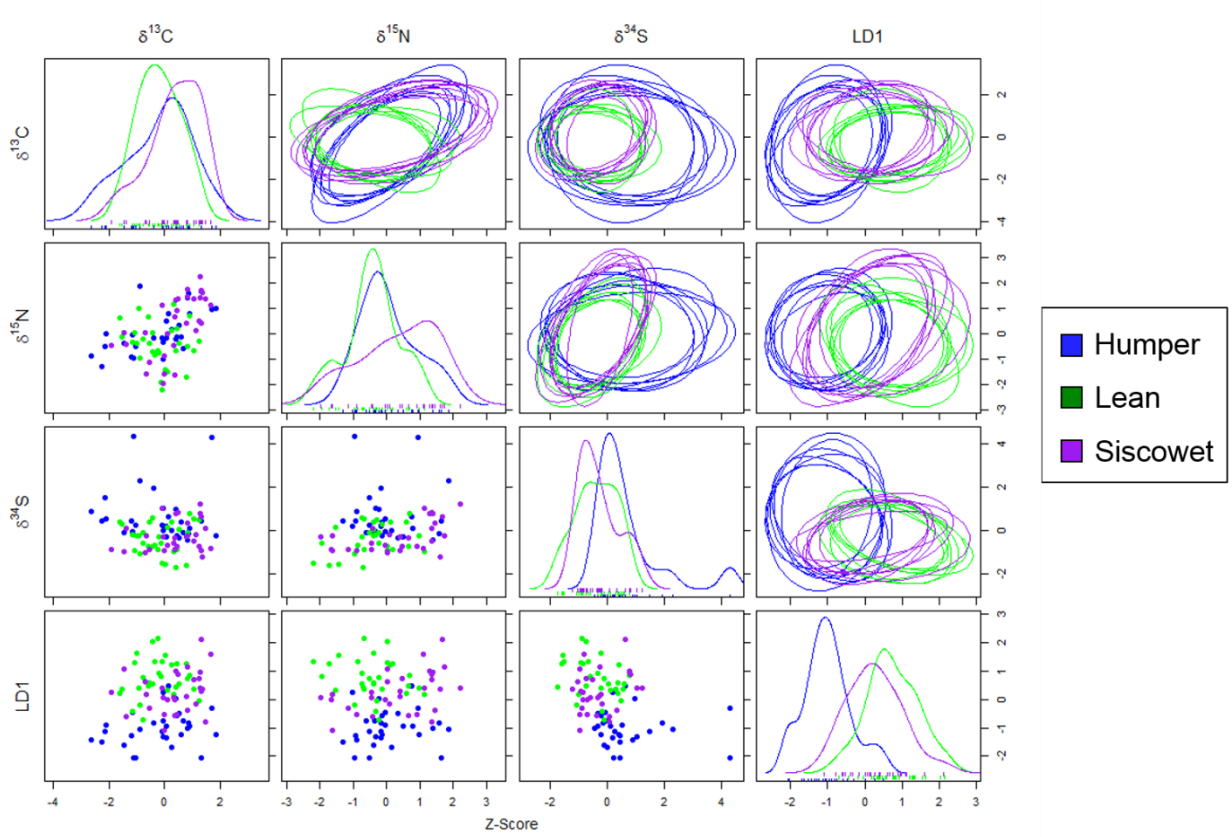

Figure 3.4. 7 randomly drawn elliptical projections of the 95% niche regions for each morph and niche dimension (stable isotope ratios of $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$, and fatty acid profiles, LD 1) at Stannard Rock. Data was converted to a z-score prior to analyses. Ellipses (above the diagonal) represent two-dimensional projections of niche regions for each morph. Also presented are two-dimensional scatter plots (below the diagonal), and one-dimensional density plots (on the diagonal). While there was substantial overlap in each dimension of niche space, LD 1 (fatty acids) showed the least overlap among morphs.

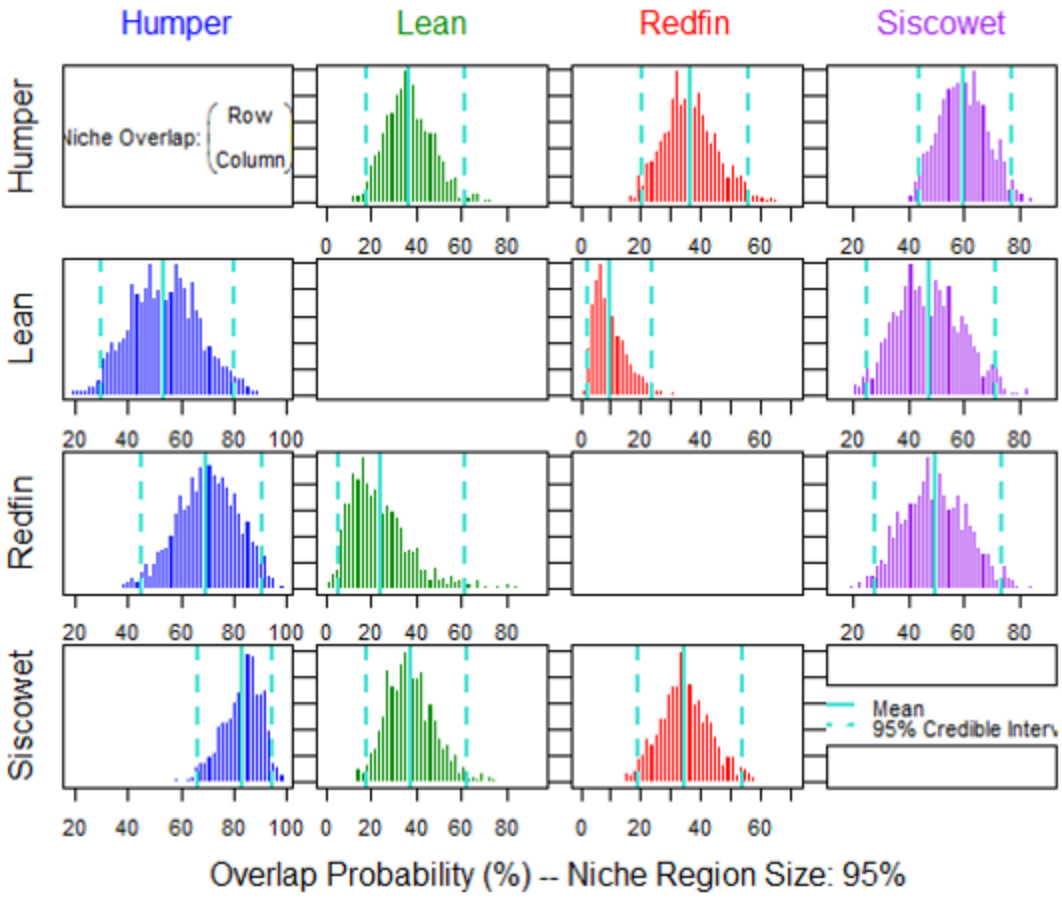

Figure 3.5. Posterior distribution of the probabilistic niche overlap metric (%) for the 95% niche regions of Lake Trout morphs from Superior Shoal. Plots show the overlap probability of morph A (row) onto the niche of morph B (column). Solid blue lines show the mean overlap probability, while dashed blue lines indicate the 95% credible interval.

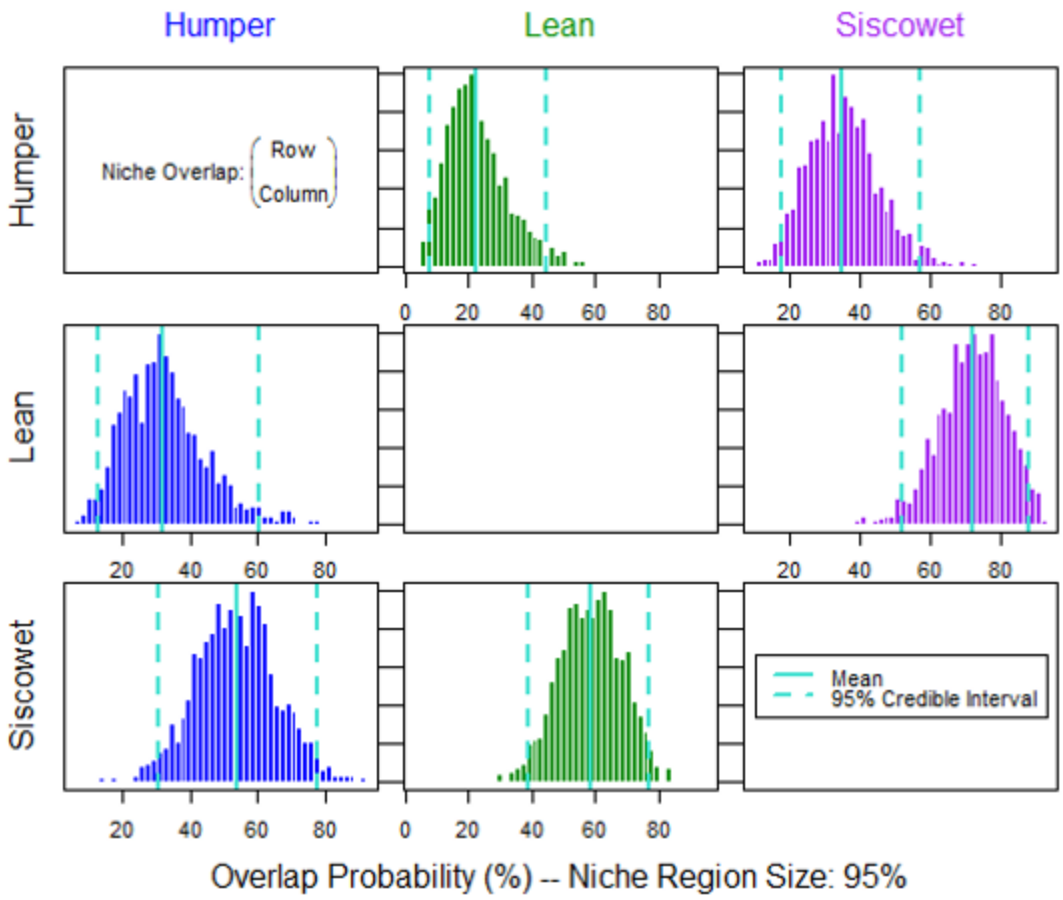

Figure 3.6. Posterior distribution of the probabilistic niche overlap metric (%) for the 95% niche regions of Lake Trout morphs from Stannard Rock. Plots show the overlap probability of morph A (row) onto the niche of morph B (column). Solid blue lines show the mean overlap probability, while dashed blue lines indicate the 95% credible interval.

Table 3.1. Summary of niche overlap among Lake Superior Lake Trout morphs. Presented are the mean 95% 4-dimensional niche region sizes of each morph, the mean overlap probability of 95% niche regions (probability of morph A overlapping with morph B), and 95% credible intervals.

Morph B	95% niche region size	Morph A	FA and SI mean overlap probability [credible interval]
Superior Shoal			
Humper	269.40	Lean	53.52[30.46, 79.79]
		Redfin	70.34[45.72, 91.77]
		Siscowet	83.04[67.30, 94.93]
Lean	159.79	Humper	37.02[18.19, 61.07]
		Redfin	25.39[5.67, 59.42]
		Siscowet	38.11[19.40, 61.54]
Redfin	79.46	Humper	36.76[20.16, 56.29]
		Lean	9.83[2.24, 24.44]
		Siscowet	34.89[18.56, 56.68]
Siscowet	153.39	Humper	59.63[42.50, 77.13]
		Lean	48.31[27.29, 72.34]
		Redfin	49.75[27.41, 73.41]
Stannard Rock			
Humper	219.32	Lean	32.01[12.57, 60.79]
		Siscowet	53.43[31.87, 76.79]
Lean	99.80	Humper	22.71[8.12, 43.33]
		Siscowet	58.91[40.31, 78.24]
Siscowet	130.86	Humper	34.90[17.22, 56.91]
		Lean	71.59[51.15, 88.34]

Chapter 4

General Conclusions and Future Areas of Study

4.1 Conclusion

This research sought to characterize and compare the diets of humper Lake Trout relative to other Lake Trout morphs in Lake Superior and quantify the size and probability of trophic niche overlap among morphs to determine if humpers occupy a different trophic niche than the other morphs. Using both direct (i.e., stomach content analysis) and indirect (i.e., stable isotope, fatty acid analyses) methods, diets of Lake Trout morphs were characterized at two sites within Lake Superior: Superior Shoal and Stannard Rock. Stomach content analyses revealed similarities in the diets of all Lake Trout morphs, with prey such as *Mysis*, coregonids, and Deepwater sculpin appearing in lean, siscowet, and redfin stomachs. Deepwater sculpin was the only identifiable fish found in humper stomachs, suggesting this might be a dominant prey fish for humpers. *Mysis* was identified as a common prey item for all morphs, but accounted for a much larger biomass contribution to humper stomachs than to stomachs of other morphs. This agrees with the findings of previous authors who have reported that humpers have planktivorous diets (Stafford et al., 2014), though my findings show that their diets are almost equal parts fish and *Mysis*. Fatty acid analyses demonstrated differences in both dietary and non-dietary fatty acids among morphs, and provided novel insight into humper and redfin fatty acids in Lake Superior; redfin diets were the most different from the other three morphs, and humper diets were most similar to siscowets at Superior Shoal, but differed at Stannard Rock. This is consistent with previous literature, which shows that Lake Trout morphs differ both in diet (Harvey et al., 2003; Ray et al., 2007; Gamble et al., 2011a; Gamble et al., 2011b) and metabolism (Eschmeyer & Phillips, 1965; Goetz et al., 2010; Goetz et al., 2014), and is consistent with stomach content observations showing some differences among morph diets

(Chapter 2, this thesis). Stable isotope analyses demonstrated that Lake Trout morphs do not differ in relative trophic position or carbon source. Sulfur stable isotopes may suggest possible differences among morphs in profundal inputs, though further investigation is required. These results suggest that Lake Trout morphs do not greatly differ in diet, which contradicts the stomach content and fatty acid results. However, stable isotopes integrate diet information on a long temporal scale (6-12 months) (Hesslein et al., 1993), so seasonal variations in diet might not be detectable with this method. Therefore, Lake Trout morph diets may be similar over the course of a year, but seasonally different from each other.

Trophic niche overlap analyses demonstrated that niche overlap occurs among Lake Trout morphs, but this overlap is usually < 50%. There was evidence of site-specific differences in resource partitioning (inferred by probabilities of niche overlap) among Lake Trout morphs, as pairwise niche overlap between morphs differed between sites. This was likely due to a combination of factors, such as habitat depth that varied between sites, and suggests that resource partitioning between morphs is not fixed, and that morph feeding strategies are likely influenced by both prey availability and their physical environment (e.g., depth).

This research contributes to the understanding of Lake Trout ecology in Lake Superior, and may be useful for informing Lake Trout management practices. Leans and humpers are used as stocking fish for the other four Great Lakes (Erie, Huron, Michigan, Huron) (Muir et al., 2012). My findings showed humpers had < 40% probability of overlap onto the trophic niche of leans, which suggests competition between these two morphs is likely to be low. While more research is required, these results suggest concurrent stocking of leans and humpers is not likely to impede the stocking successes of these morphs. However, as these niche overlap models were

performed on adult Lake Trout, future research should focus on examining possible niche overlap of adult humpers with juvenile Lake Trout of other morphs.

My findings showed site-specific differences in niche overlap among siscowets and leans. Siscowets had a 38% probability of overlap onto lean niches at Superior Shoal, compared to 58% probability of overlap onto lean niches at Stannard Rock. This suggests managers should consider a site-specific approach to Lake Trout management, as certain environments may facilitate greater niche overlap among morphs than others. While more research is needed to determine the potential drivers of site-specific differences in niche overlap, it is likely a result of prey availability or habitat heterogeneity. Redfins were identified as having the lowest niche overlap with leans, suggesting redfins may also be a suitable candidate for concurrent stocking with leans. However, the extent to which niche overlap varies between leans and redfins among seasons and between sites is unknown, and further research is required before the possibility of stocking redfins can be properly evaluated.

My research also highlights the importance of *Mysis* as a prey item to all four Lake Trout morphs examined in this study. Previous studies have shown the importance of *Mysis* to many species of fishes, as *Mysis* integrates many basal resources (Ahrenstorff et al., 2011; Sierszen et al., 2011). This is consistent with my fatty acid results, which indicate *Mysis* is a source of many essential fatty acids for Lake Trout morphs. Therefore, managers may be interested in monitoring *Mysis* populations, especially in areas of heavy Lake Trout stocking, as they appear to be a vital resource to not only Lake Trout, but many other fish species (Ahrenstorff et al., 2011; Gamble et al., 2011a; Gamble et al., 2011b; Sierszen et al., 2011).

Future research could focus on examining seasonal differences in Lake Trout diets.

One drawback of stomach content analysis is the lack of temporal resolution, and fatty acids integrate roughly 2 months of diet information (Happel et al., 2016). Thus, my observations reflect the summer diets of Lake Trout morphs. Since the diets of leans and siscowets vary among seasons (Ray et al., 2007; Gamble et al., 2011a; Gamble et al., 2011b), it is likely that redfins and humpers may experience seasonal changes in diet as well. Therefore, future research examining the fall and spring fatty acid profiles of these Lake Trout morphs, or tissues with higher turnover rates of fatty acids (see Budge et al., 2006) would contribute to further understanding dietary differences among Lake Trout morphs.

References

Chapter 1 References

Alfonso, N.R. (2004). Evidence for two morphotypes of lake charr, Salvelinus namaycush, from Great Bear Lake, Northwest Territories, Canada. *Environ Biol Fish, 71*(1), 21-32.

Arlettaz, R., Perrin, N., & Hausser, J. (1997). Trophic resource partitioning and competition betweenthe two sibling bat species *Myotis myotis* and *Myotis blythii*. *J Anim Ecol, 66*(6), 897-911.

Arts, M.T., & Wainman, B. (1999). Lipids in freshwater ecosystems. New York: Springer.

Baille, S.M., Muir, A.M., Scribner, K., Bentzen, P., & Krueger, C.C. (2016). Loss of genetic diversity and reduction of genetic distance among lake trout Salvelinus namaycush ecomorphs, Lake Superior 1959 to 2013. *J Great Lakes Res, 42*(2), 204.

Beck, C.A., Iverson, S.J., Bowen, W.D., & Blanchard, W. (2007). Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: evidence from quantitative fatty acid signature analysis. *J Anim Ecol, 76*(3), 490-502.

Behnke, R.J. (1972). Systematics of Salmonid Fishes of Recently Glaciated Lakes. *J Fish Res Board Can, 29*(6), 639-671.

Blackie, C.T., Weese, D.J., & Noakes, D.L.G. (2003). Evidence for resource polymorphism in the lake charr (Salvelinus namaycush) population of Great Bear Lake, Northwest Territories, Canada. *Ecoscience, 10*(4), 509-514.

Bradshaw, C.J., Hindell, M.A., Best, N.J., Phillips, K.L., Wilson, G., & Nichols, P.D. (2003). You are what you eat: describing the foraging ecology of southern elephant seals (Mirounga leonina) using blubber fatty acids. *Proc Biol Sci, 270*(1521), 1283-1292.

Bronte, C.R., Ebener, M.P., Schreiner, D.R., DeVault, D.S., Petzold, M.M., Jensen, D.A., Richards, C., & Lozano, S.J. (2003). Fish community change in lake superior, 1970-2000. *Can J Fish Aquat Sci, 60*(12), 1552-1574.

Bronte, C.R., Krueger, C.C., Holey, M.E., Toneys, M.L., Eshenroder, R.L., & Jonas, J.L. (2008). A guide for the rehabilitation of lake trout in Lake Michigan. *Great Lakes Fish Comm Misc Publ, 2008-01*, Available from http://www.glfc.org/pubs/pub.htm#misc.

Bronte, C.R., & Moore, S.A. (2007). Morphological variation of siscowet lake trout in Lake Superior. *T Am Fish Soc, 136*(2), 509-517.

Budge, S.M., & Parrish, C.C. (1998). Lipid biogeochemistry of planton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. *Org Geochem, 29*(5-7), 1547-1559.

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

Bunnell, D.B., Barbiero, R.P., Ludsin, S.A., Madenjian, C.P., Warren, G.J., Dolan, D.M., Brenden, T.O., Briland, R., Gorman, O.T., He, J.X., Johengen, T.H., Lantry, B.F., Lesht, B.M., Nalepa, T.F., Riley, S.C., Riseng, C.M., Treska, T.J., Tsehaye, I., Walsh, M.G., Warner, D.M., & Weidel, B.C. (2014). Changing ecosystem dynamics in the Laurentian Great Lakes: bottom-up and top-down regulation. *Bioscience, 64*(1), 26-39.

Chavarie, L., Howland, K.L., & Tonn, W.M. (2013). Sympatric Polymorphism in Lake Trout: The Coexistence of Multiple Shallow-Water Morphotypes in Great Bear Lake. *T Am Fish Soc, 142*(3), 814-823.

Chavarie, L., Muir, A.M., Zimmerman, M.S., Baille, S.M., Hansen, M.J., Nate, N.A., Yule, D.L., Middel, T., Bentzen, P., & Krueger, C.C. (2016). Challenge to the model of lake charr evolution: shallow-and deep-water morphs exist within a small postglacial lake. *Biol J Linnean Soc*. http://dx.doi.org/10.1111/bij.12913

Chraibi, V.L.S., Kireta, A.R., Reavie, E.D., Cai, M.J., & Brown, T.N. (2014). A paleolimnological assessment of human impacts on Lake Superior. *J Great Lakes Res, 40*(4), 886-897.

Christie, W.J. (1974). Changes in Fish Species Composition of Great Lakes. *J Fish Res Board Can, 31*(5), 827-854.

Cline, T.J., Bennington, V., & Kitchell, J.F. (2013). Climate change expands the spatial extent and duration of preferred thermal habitat for Lake Superior fishes. *PloS one, 8*(4), e62279.

Couturier, L.I., Rohner, C.A., Richardson, A.J., Marshall, A.D., Jaine, F.R., Bennett, M.B., Townsend, K.A., Weeks, S.J., & Nichols, P.D. (2013). Stable isotope and signature fatty acid analyses suggest reef manta rays feed on demersal zooplankton. *PloS one, 8*(10), e77152.

Croisetiere, L., Hare, L., Tessier, A., & Cabana, G. (2009). Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. *Freshwater Biol, 54*(5), 1006-1015.

Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol, 46*, 225-340.

Dawson, H.A., & Jones, M.L. (2009). Factors affecting recruitment dynamics of Great Lakes sea lamprey (Petromyzon marinus) populations. *J Great Lakes Res, 35*(3), 353-360.

Deniro, M.J., & Epstein, S. (1978). Influence of Diet on Distribution of Carbon Isotopes in Animals. *Geochim Cosmochim Ac, 42*(5), 495-506.

Deniro, M.J., & Epstein, S. (1981). Influence of Diet on the Distribution of Nitrogen Isotopes in Animals. *Geochim Cosmochim Ac, 45*(3), 341-351.

Elrod, J.H., OGorman, R., & Schneider, C.P. (1996). Bathythermal distribution, maturity, and growth of lake trout strains stocked in US waters of Lake Ontario, 1978-1993. *J Great Lakes Res, 22*(3), 722-743.

Elsdon, T.S. (2010). Unraveling diet and feeding histories of fish using fatty acids as natural tracers. *J Exp Mar Biol Ecol, 386*(1-2), 61-68.

Eschmeyer, P.H. (1955). The reproduction of Lake Trout in southern Lake Superior. *T Am Fish Soc, 84*(1), 47-74.

Eschmeyer, P.H., & Phillips, A.M. (1965). Fat Content of Flesh of Siscowets and Lake Trout from Lake Superior. *T Am Fish Soc, 94*(1), 62-&.

Eshenroder, R.L., Crossman, E.J., Meffe, G.K., Olver, C.H., & Pister, E.P. (1995). Lake trout rehabilitation in the Great Lakes: An evolutionary, ecological, and ethical perspective. *J Great Lakes Res, 21*, 518-529.

France, R.L. (1995). Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnol Oceanogr, 40*(7), 1310-1313.

Fry, B. (2002). Stable isotopic indicators of habitat use by Mississippi River fish. *J N Am Benthol Soc, 21*(4), 676-685.

Gamble, A.E., Hrabik, T.R., Stockwell, J.D., & Yule, D.L. (2011a). Trophic connections in Lake Superior Part I: The offshore fish community. *J Great Lakes Res, 37*(3), 541-549.

Gamble, A.E., Hrabik, T.R., Yule, D.L., & Stockwell, J.D. (2011b). Trophic connections in Lake Superior Part II: The nearshore fish community. *J Great Lakes Res, 37*(3), 550-560.
George, E.L., & Hadley, W.F. (1979). Food and habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieui*) young of the year. *T Am Fish Soc, 108*(3), 253-261.
Goetz, F., Jasonowicz, A., Johnson, R., Biga, P., Fischer, G., & Sitar, S. (2014). Physiological differences between lean and siscowet lake trout morphotypes: Are these metabolotypes? *Can J Fish Aquat Sci, 71*(3), 427-435.
Goetz, F., Rosauer, D., Sitar, S., Goetz, G., Simchick, C., Roberts, S., Johnson, R., Murphy, C., Bronte, C.R., & Mackenzie, S. (2010). A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (Salvelinus namaycush). *Mol Ecol, 19*, 176-196.
Gonfiantini, R., Stichler, W., & Rozanski, K. (1995). Standards and intercomparison materials distributed by the international atomic energy agency for stable isotope measurements *Reference and intercomparison materials for stable isotopes of light elements* (pp. 13-29). Vienna, Austria: International Atomic Energy Angency.
Gorman, O.T., Ebener, M.P., & Vinson, M.R. (2010). The State of Lake Superior in 2005. *Great Lakes Fish Comm Spec Publ, 10-01*.
Hansen, M.J. (1999). Lake trout in the Great Lakes: basinwide stock collapse and binational restoration. In W. W. Taylor & C. P. Ferreri (Eds.), *Great Lakes fisheries policy and management* (pp. 417–454). East Lansing, Mich: Michigan State University Press.
Hansen, M.J., Nate, N.A., Krueger, C.C., Zimmerman, M.S., Kruckman, H.G., & Taylor, W.W. (2012). Age, growth, survival, and maturity of lake trout morphotypes in Lake Mistassini, Quebec. *T Am Fish Soc, 141*(6), 1492-1503.
Hansen, M.J., Nate, N.A., Muir, A.M., Bronte, C.R., Zimmerman, M.S., & Krueger, C.C. (2016). Life history variation among four lake trout morphs at Isle Royale, Lake Superior. *J Great Lakes Res, 42*(2), 421-432.
Hanson, S.D., Holey, M.E., Treska, T.J., Bronte, C.R., & Eggebraaten, T.H. (2013). Evidence of Wild Juvenile Lake Trout Recruitment in Western Lake Michigan. *N Am J Fish Manage, 33*(1), 186-191.
Happel, A., Stratton, L., Pattridge, R., Rinchard, J., & Czesny, S. (2016). Fatty-acid profiles of juvenile lake trout reflect experimental diets consisting of natural prey. *Freshwater Biol, 61*(9), 1466-1476.
Harrington, G.W., Beach, D.H., Dunham, J.E., & Holz Jr., G.G. (1970). The polyunsaturated fatty acids of marine dinoflagellates. *J Eukaryot Microbiol, 17*(2), 213-219.
Harvey, C.J., & Kitchell, J.F. (2000). A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. *Can J Fish Aquat Sci, 57*(7), 1395-1403.
Harvey, C.J., Schram, S.T., & Kitchell, J.F. (2003). Trophic relationships among lean and siscowet lake trout in Lake Superior. *T Am Fish Soc, 132*(2), 219-228.
Hasui, E., da Mota Gomes, V.S., Kiefer, M.C., Tamashiro, J., & Silva, W.R. (2009). Spatial and seasonal variation in niche partitioning between blue manakin (*Chiroxiphia caudata*) and greenish schiffornis (*Schiffornis virescens*) in southeastern Brazil. *Stud Neotrop Fauna E, 44*(3), 149-159.
Henderson, R.J., & Tocher, D.R. (1987). The lipid composition and biochemistry of freshwater fish. *Progress in lipid research, 26*(4), 281-347.

Hesslein, R.H., Hallard, K.A., & Ramlal, P. (1993). Replacement of sulfur, carbon, and nitrogen, in tissue of growing broad whitefish (Coregonus nasus) in response to a change in diet traced by δ34S, δ13C, and δ15N. *Can J Fish Aquat Sci, 50,* 2071-2076.

Hill, C.L. (2007). Late glacial landscape ecology in Central North America. *Geoarchaeology, 22*(1), 15-47.

Hodgson, J.R., He, X., Schindler, D.E., & Kitchell, J.F. (1997). Diet overlap in a piscivore community. *Ecol Freshw Fish, 6*(3), 144-149.

Hoffman, J.C., Sierszen, M.E., & Cotter, A.M. (2015). Fish tissue lipid-C:N relationships for correcting C-13 values and estimating lipid content in aquatic food-web studies. *Rapid Commun Mass Sp, 29*(21), 2069-2077.

Hoffman, J.C., & Sutton, T.T. (2010). Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep-Sea Res Pt I, 57*(8), 956-964.

Hood-Nowotny, R., Schwarzinger, B., Schwarzinger, C., Soliban, S., Madakacherry, O., Aigner, M., Watzka, M., & Gilles, J. (2012). An analysis of diet quality, how it controls fatty acid profiles, isotope signatures and stoichiometry in the malaria mosquito *Anopheles arabiensis*. *PloS one, 7*(10).

Horns, W.H., Bronte, C.R., Busiahn, T.R., Ebener, M.P., Eshenroder, R.L., Gorenflo, T., Kmiecik, N., Mattes, W., Peck, J.W., Petzold, M., & Schreiner, D.R. (2003). Fish-community objectives for Lake Superior. *Great Lakes Fish Comm Spec Publ, 03-01*, 0-78.

Hutchinson, G.E. (1957). Population studies - animal ecology and demography - concluding remarks. *Cold Spring Harb Sym, 22*, 415-427.

Iverson, S.J., Lang, S.L.C., & Cooper, M.H. (2001). Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids, 36*(11), 1283-1287.

Jonsson, B., & Jonsson, N. (2001). Polymorphism and speciation in Arctic charr. *J Fish Biol, 58*(3), 605-638.

Karube, Z., Okada, N., & Tayasu, I. (2012). Sulfur stable isotope signature identifies the source of reduced sulfur in benthic communities in macrophyte zones of Lake Biwa, Japan. *Limnology, 13*(3), 269-280.

Keeley, J.E., & Sandquist, D.R. (1992). Carbon - Fresh-Water Plants. *Plant Cell Environ, 15*(9), 1021-1035.

Kepler, M.V., Wagner, T., & Sweka, J.A. (2014). Comparative Bioenergetics Modeling of Two Lake Trout Morphotypes. *T Am Fish Soc, 143*(6), 1592-1604.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R.I. (2006). A revised model for lipid-normalizing delta C-13 values from aquatic organisms, with implications for isotope mixing models. *J Appl Ecol, 43*(6), 1213-1222.

Kirsch, P.E., Iverson, S.J., Bowen, W.D., Kerr, S.R., & Ackman, R.G. (1998). Dietary effects on the fatty acid signature of whole Atlantic cod (Gadus morhua). *Can J Fish Aquat Sci, 55*(6), 1378-1386.

Lanca, M.J., Rosado, C., Machado, M., Ferreira, R., Alves-Pereira, I., Quintella, B.R., & Almeida, P.R. (2011). Can muscle fatty acid signature be used to distinguish diets during the marine trophic phase of sea lamprey (Petromyzon marinus, L.)? *Comp Biochem Phys B, 159*(1), 26-39.

Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L.A., Post, D.M., & Bearhop, S.

(2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol Rev, 87*(3), 545-562.
Logan, J.M., Jardine, T.D., Miller, T.J., Bunn, S.E., Cunjak, R.A., & Lutcavage, M.E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. *J Anim Ecol, 77*(4), 838-846.
Logan, J.M., & Lutcavage, M.E. (2008). A comparison of carbon and nitrogen stable isotope ratios of fish tissues following lipid extractions with non-polar and traditional chloroform/methanol solvent systems. *Rapid Commun Mass Sp, 22*(7), 1081-1086.
Markham, J.L., Cook, A., MacDougall, T., Witzel, L., Kayle, K., Murray, M., Fodale, M., Trometer, E., Neave, F., Fitzsimons, J., Francis, J., & Stapanian, M. (2008). A strategic plan for the rehabilitation of lake trout in Lake Erie, 2008-2020. *Great Lakes Fish Comm Misc Publ, 2008-02*, Available from http://www.glfc.org/pubs/pub.htm#misc.
Martin, N.V., & Olver, C.H. (1980). The lake charr, *Salvelinus namaycush*. In E. K. Balon (Ed.), *Charrs: Salmonid Fishes of the Genus Salvelinus* (pp. 205-277). Hague, Netherlands: Dr. W. Junk Publishers.
Martin, N.V., & Sanderco, F.K. (1967). Pyloric Caeca and Gill Raker Development in Lake Trout Salvelinus Namaycush in Algonquin Park Ontario. *J Fish Res Board Can, 24*(5), 965-&.
May, R.M., & Macarthur, R.H. (1972). Niche Overlap as a Function of Environmental Variability. *P Natl Acad Sci USA, 69*(5), 1109-+.
Mcconnaughey, T., & Mcroy, C.P. (1979). Food-Web Structure and the Fractionation of Carbon Isotopes in the Bering Sea. *Mar Biol, 53*(3), 257-262.
McCutchan, J.H., Lewis, W.M., Kendall, C., & McGrath, C.C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos, 102*(2), 378-390.
Minagawa, M., & Wada, E. (1984). Stepwise enrichment of N-15 along food-chains - further evidence and the relation between Delta-N-15 and animal age. *Geochim Cosmochim Ac, 48*(5), 1135-1140.
Minor, E.C., Forsman, B., & Guildford, S.J. (2014). The effect of a flood pulse on the water column of western Lake Superior, USA. *J Great Lakes Res, 40*(2), 455-462.
Moore, S.A., & Bronte, C.R. (2001). Delineation of sympatric morphotypes of lake trout in Lake Superior. *T Am Fish Soc, 130*(6), 1233-1240.
Muir, A.M., Bronte, C.R., Zimmerman, M.S., Quinlan, H.R., Glase, J.D., & Krueger, C.C. (2014). Ecomorphological diversity of lake trout at Isle Royale, Lake Superior. *T Am Fish Soc, 143*(4), 972-987.
Muir, A.M., Hansen, M.J., Bronte, C.R., & Krueger, C.C. (2015). If Arctic charr *Salvelinus alpinus* is 'the most diverse vertebrate', what is the lake charr *Salvelinus namaycush*? *Fish Fish, 17*, 1194-1207.
Muir, A.M., Krueger, C.C., & Hansen, M.J. (2012). Re-establishing lake trout in the Laurentian Great Lakes: past, present, and future. In Taylor WW, Lynch AJ & L. NJ (Eds.), *Great Lakes fisheries policy and management: a binational perspective* (2 ed., pp. 533-588). Michigan State University Press, East Lansing.
Negus, M.T. (2010). Contribution of lake trout stocked as fry to an adult population in Lake Superior. *J Great Lakes Res, 36*(2), 380-386.
Newsome, S.D., del Rio, C.M., Bearhop, S., & Phillips, D.L. (2007). A niche for isotopic ecology. *Front Ecol Environ, 5*(8), 429-436.

Nieland, J.L., Hansen, M.J., Seider, M.J., & Deroba, J.J. (2008). Modeling the sustainability of lake trout fisheries in eastern Wisconsin waters of Lake Superior. *Fish Res, 94*(3), 304-314.

Perreault-Payette A., Muir A.M., Goetz F., Perrier C., Normandeau E., Sirois P., Bernatchez L. (2017) Investigating the extent of parallelism in morphological and genomic divergence among Lake Trout ecotypes in Lake Superior. *Mol Ecol*, In press.

Peterson, B.J., & Fry, B. (1987). Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst, 18*, 293-320.

Pianka, E.R. (1974). Niche Overlap and Diffuse Competition. *P Natl Acad Sci USA, 71*(5), 2141-2145.

Post, D.M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology, 83*(3), 703-718.

Rahrer, J.F. (1965). Age, growth, maturity, and fecundity of humper lake trout, Isle Royale, Lake Superior. *T Am Fish Soc, 94*(1), 75-83.

Ray, B.A., Hrabik, T.R., Ebener, M.P., Gorman, O.T., Schreiner, D.R., Schram, S.T., Sitar, S.P., Mattes, W.P., & Bronte, C.R. (2007). Diet and prey selection by Lake Superior lake trout during spring, 1986–2001. *J Great Lakes Res, 33*(1), 104-113.

Reist, J.D., Power, M., & Dempson, J.B. (2013). Arctic charr (Salvelinus alpinus): a case study of the importance of understanding biodiversity and taxonomic issues in northern fishes. *Biodiversity, 14*(1), 45-56.

Riley, S.C., Binder, T.R., Wattrus, N.J., Faust, M.D., Janssen, J., Menzies, J., Marsden, J.E., Ebener, M.P., Bronte, C.R., He, J.X., Tucker, T.R., Hansen, M.J., Thompson, H.T., Muir, A.M., & Krueger, C.C. (2014). Lake trout in northern Lake Huron spawn on submerged drumlins. *J Great Lakes Res, 40*(2), 415-420.

Rusterholz, K.A. (1981). Niche overlap among foliage-gleaning birds: support for Pianka's niche overlap hypothesis. *Am Nat, 117*(3), 395-399.

Saarnisto, M. (1974). The deglaciation history of the Lake Superior region and its climatic implications. *Quat Res, 4*(3), 316-339.

Schreiner, D.R., & Schram, S.T. (1997). Lake trout rehabilitation in Lake Superior - Maintaining our progress. *Fisheries, 22*(7), 12-14.

Sierszen, M.E., Hrabik, T.R., Stockwell, J.D., Cotter, A.M., Hoffman, J.C., & Yule, D.L. (2014). Depth gradients in food-web processes linking habitats in large lakes: Lake Superior as an exemplar ecosystem. *Freshwater Biol, 59*(10), 2122-2136.

Stafford, C.P., McPhee, M.V., Eby, L.A., & Allendorf, F.W. (2014). Introduced lake trout exhibit life history and morphological divergence with depth. *Can J Fish Aquat Sci, 71*(1), 10-20.

Stubing, D., & Hagen, W. (2003). Fatty acid biomarker ratios - suitable trophic indicators in Antarctic euphausiids? *Polar Biol, 26*(12), 774-782.

Vander Zanden, M.J., & Rasmussen, J.B. (2001). Variation in d15N and d13C trophic fractionation: Implications for aquatic food web studies. *Limnol Oceanogr, 46*(8), 2061-2066.

Vlaeminck, B., Fievez, V., Cabrita, A.R.J., Fonseca, A.J.M., & Dewhurst, R.J. (2006). Factors affecting odd- and branched-chain fatty acids in milk: A review. *Anim Feed Sci Tech, 131*(3-4), 389-417.

White, B., Austin, J., & Matsumoto, K. (2012). A three-dimensional model of Lake Superior with ice and biogeochemistry. *J Great Lakes Res, 38*(1), 61-71.

Zimmerman, M.S., & Krueger, C.C. (2009). An ecosystem perspective on re-establishing native deepwater fishes in the Laurentian Great Lakes. *N Am J Fish Manage, 29*(5), 1352-1371.
Zimmerman, M.S., Krueger, C.C., & Eshenroder, R.L. (2006). Phenotypic diversity of lake trout in Great Slave Lake: Differences in morphology, buoyancy, and habitat depth. *T Am Fish Soc, 135*(4), 1056-1067.
Zimmerman, M.S., Krueger, C.C., & Eshenroder, R.L. (2007). Morphological and ecological differences between shallow- and deep-water lake trout in Lake Mistassini, Quebec. *J Great Lakes Res, 33*(1), 156-169.
Zimmerman, M.S., Schmidt, S.N., Krueger, C.C., Vander Zanden, M.J., & Eshenroder, R.L. (2009). Ontogenetic niche shifts and resource partitioning of lake trout morphotypes. *Can J Fish Aquat Sci, 66*(6), 1007-1018.

Chapter 2 References

Ahlgren, G., Vrede, T., & Goedkoop, W. (2009). Fatty acid ratios in freshwater fish, zooplankton and zoobenthos - are there specific optima? In M. T. Arts, M. T. Brett & M. J. Kainz (Eds.), *Lipids in Aquatic Ecosystems* (pp. 147-178). New York, NY: Springer.
Ahrenstorff, T.D., Hrabik, T.R., Stockwell, J.D., Yule, D.L., & Sass, G.G. (2011). Seasonally dynamic diel vertical migrations of *Mysis diluviana*, coregonine fishes, and siscowet Lake Trout in the pelagia of Western Lake Superior. *T Am Fish Soc, 140*(6), 1504-1520.
Arts, M.T., & Wainman, B. (1999). Lipids in freshwater ecosystems. New York: Springer.
Auer, M.T., Auer, N.A., Urban, N.R., & Auer, T. (2013). Distribution of the Amphipod Diporeia in Lake Superior: The Ring of Fire. *J Great Lakes Res, 39*(1), 33-46.
Baille, S.M., Muir, A.M., Scribner, K., Bentzen, P., & Krueger, C.C. (2016). Loss of genetic diversity and reduction of genetic distance among lake trout Salvelinus namaycush ecomorphs, Lake Superior 1959 to 2013. *J Great Lakes Res, 42*(2), 204.
Behnke, R.J. (1972). Systematics of Salmonid Fishes of Recently Glaciated Lakes. *J Fish Res Board Can, 29*(6), 639-671.
Blackie, C.T., Weese, D.J., & Noakes, D.L.G. (2003). Evidence for resource polymorphism in the lake charr (Salvelinus namaycush) population of Great Bear Lake, Northwest Territories, Canada. *Ecoscience, 10*(4), 509-514.
Brett, M.T., Muller-Navarra, D.C., & Persson, J. (2009). Crustacean zooplankton fatty acid composition In M. T. Arts, M. T. Brett & M. J. Kainz (Eds.), *Lipids in aquatic ecosystems* (pp. 115-146). New York, NY: Springer.
Bronte, C.R., Ebener, M.P., Schreiner, D.R., DeVault, D.S., Petzold, M.M., Jensen, D.A., Richards, C., & Lozano, S.J. (2003). Fish community change in lake superior, 1970-2000. *Can J Fish Aquat Sci, 60*(12), 1552-1574.
Bronte, C.R., Krueger, C.C., Holey, M.E., Toneys, M.L., Eshenroder, R.L., & Jonas, J.L. (2008). A guide for the rehabilitation of lake trout in Lake Michigan. *Great Lakes Fish Comm Misc Publ, 2008-01*, Available from http://www.glfc.org/pubs/pub.htm#misc.
Budge, S.M., Iverson, S.J., & Koopman, H.N. (2006). Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Mar Mammal Sci, 22*(4), 759-801.

Budge, S.M., & Parrish, C.C. (1998). Lipid biogeochemistry of planton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. *Org Geochem, 29*(5-7), 1547-1559.

Budge, S.M., Parrish, C.C., & Mckenzie, C.H. (2001). Fatty acid composition of phytoplankton, settling particulate matter, and sediments at a shelterd bivalve aquaculture site. *Mar Chem, 76*, 285-303.

Bunnell, D.B., Barbiero, R.P., Ludsin, S.A., Madenjian, C.P., Warren, G.J., Dolan, D.M., Brenden, T.O., Briland, R., Gorman, O.T., He, J.X., Johengen, T.H., Lantry, B.F., Lesht, B.M., Nalepa, T.F., Riley, S.C., Riseng, C.M., Treska, T.J., Tsehaye, I., Walsh, M.G., Warner, D.M., & Weidel, B.C. (2014). Changing ecosystem dynamics in the Laurentian Great Lakes: bottom-up and top-down regulation. *Bioscience, 64*(1), 26-39.

Cabana, G., & Rasmussen, J.B. (1996). Comparison of aquatic food chains using nitrogen isotopes. *P Natl Acad Sci USA, 93*(20), 10844-10847.

Chavarie, L., Harford, W.J., Howland, K.L., Fitzsimons, J., Muir, A.M., Krueger, C.C., & Tonn, W.M. (2016a). Multiple generalist morphs of Lake Trout: Avoiding constraints on the evolution of intraspecific divergence? *Ecol Evol, 6*(21), 7727-7741.

Chavarie, L., Howland, K.L., Gallagher, C., & Tonn, W.M. (2016b). Fatty acid signatures and stomach contents of four sympatric Lake Trout: assessment of trophic patterns among morphotypes in Great Bear Lake. *Ecol Freshw Fish, 25*(1), 109-124.

Chavarie, L., Howland, K.L., & Tonn, W.M. (2013). Sympatric Polymorphism in Lake Trout: The Coexistence of Multiple Shallow-Water Morphotypes in Great Bear Lake. *T Am Fish Soc, 142*(3), 814-823.

Chavarie, L., Muir, A.M., Zimmerman, M.S., Baille, S.M., Hansen, M.J., Nate, N.A., Yule, D.L., Middel, T., Bentzen, P., & Krueger, C.C. (2016c). Challenge to the model of lake charr evolution: shallow-and deep-water morphs exist within a small postglacial lake. *Biol J Linnean Soc*. http://dx.doi.org/10.1111/bij.12913

Christie, W.J. (1974). Changes in Fish Species Composition of Great Lakes. *J Fish Res Board Can, 31*(5), 827-854.

Cline, T.J., Bennington, V., & Kitchell, J.F. (2013). Climate change expands the spatial extent and duration of preferred thermal habitat for Lake Superior fishes. *PloS one, 8*(4), e62279.

Cook, H.W. (1991). Fatty acid desaturation and chain elongation in eucaryotes. In D. E. Vance & J. Vance (Eds.), *Biochemistry of lipids, lipoproteins and membranes* (pp. 141-169). New York, NY: Elsevier Science.

Croisetiere, L., Hare, L., Tessier, A., & Cabana, G. (2009). Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. *Freshwater Biol, 54*(5), 1006-1015.

Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol, 46*, 225-340.

Dunstan, G.A., Volkman, J.K., Barrett, S.M., Leroi, J.M., & Jeffrey, S.W. (1994). Essential Polyunsaturated Fatty-Acids from 14 Species of Diatom (Bacillariophyceae). *Phytochemistry, 35*(1), 155-161.

Dunstan, G.A., Volkman, J.K., Jeffrey, S.W., & Barret, S.M. (1992). Biochemical composition of microalgae from the green algal classes Chlorophyceae and Prasinophyceae. 2. Lipid classes and fatty acids. *J Exp Mar Biol Ecol, 161*(1), 115-134.

Elsdon, T.S. (2010). Unraveling diet and feeding histories of fish using fatty acids as natural tracers. *J Exp Mar Biol Ecol, 386*(1-2), 61-68.
Eschmeyer, P.H. (1955). The reproduction of Lake Trout in southern Lake Superior. *T Am Fish Soc, 84*(1), 47-74.
Eschmeyer, P.H. (1957). The Lake Trout (*Salvelinus namaycush*) *US Fish and Wildl Serv, Fish Leaflet 441*, 0-11.
Eschmeyer, P.H., & Phillips, A.M. (1965). Fat Content of Flesh of Siscowets and Lake Trout from Lake Superior. *T Am Fish Soc, 94*(1), 62-&.
Eshenroder, R.L. (2008). Differentiation of deep-water lake charr *Salvelinus namaycush* in North American lakes. *Environ Biol Fish, 83*(1), 77-90.
Folch, J., Lees, M., & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem, 226*(1), 497-509.
Fraley, C., & Raftery, A.E. (2009). MCLUST version 3 for R: normal mixture modeling and model-based clustering. University of Washington, Technical Report 504, Seattle.
Gamble, A.E., Hrabik, T.R., Stockwell, J.D., & Yule, D.L. (2011a). Trophic connections in Lake Superior Part I: The offshore fish community. *J Great Lakes Res, 37*(3), 541-549.
Gamble, A.E., Hrabik, T.R., Yule, D.L., & Stockwell, J.D. (2011b). Trophic connections in Lake Superior Part II: The nearshore fish community. *J Great Lakes Res, 37*(3), 550-560.
George, E.L., & Hadley, W.F. (1979). Food and habitat partitioning between rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieui*) young of the year. *T Am Fish Soc, 108*(3), 253-261.
Goodier, J.L. (1981). Native Lake Trout (Salvelinus namaycush) stocks in the Canadian waters of Lake Superior prior to 1955. *Can J Fish Aquat Sci, 38*, 1724-1737.
Goetz, F., Jasonowicz, A., Johnson, R., Biga, P., Fischer, G., & Sitar, S. (2014). Physiological differences between lean and siscowet lake trout morphotypes: Are these metabolotypes? *Can J Fish Aquat Sci, 71*(3), 427-435.
Goetz, F., Rosauer, D., Sitar, S., Goetz, G., Simchick, C., Roberts, S., Johnson, R., Murphy, C., Bronte, C.R., & Mackenzie, S. (2010). A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (Salvelinus namaycush). *Mol Ecol, 19*, 176-196.
Gonfiantini, R., Stichler, W., & Rozanski, K. (1995). Standards and intercomparison materials distributed by the international atomic energy agency for stable isotope measurements *Reference and intercomparison materials for stable isotopes of light elements* (pp. 13-29). Vienna, Austria: International Atomic Energy Angency.
Gorman, O.T., Ebener, M.P., & Vinson, M.R. (2010). The State of Lake Superior in 2005. *Great Lakes Fish Comm Spec Publ, 10-01*.
Gorman, O.T., Yule, D.L., & Stockwell, J.D. (2012). Habitat use by fishes of Lake Superior. I. Diel patterns of habitat use in nearshore and offshore waters of the Apostle Islands region. *Aquat Ecosyst Health, 15*(3), 333-354.
Grossnickle, N.E. (1982). Feeding-Habits of Mysis-Relicta - an Overview. *Hydrobiologia, 93*(1-2), 101-107.
Hagen, W., Kattner, G., & Graeve, M. (1993). Calanoides acutus and Calan us propinquus, Antarctic copepods with different lipid storage modes via wax esters or triacylglycerols *Mar Ecol Prog Ser, 97*, 135-142.
Hambright, K.D. (1991). Experimental-Analysis of Prey Selection by Largemouth Bass - Role of Predator Mouth Width and Prey Body Depth. *T Am Fish Soc, 120*(4), 500-508.

Hansen, M.J. (1999). Lake trout in the Great Lakes: basinwide stock collapse and binational restoration. In W. W. Taylor & C. P. Ferreri (Eds.), *Great Lakes fisheries policy and management* (pp. 417–454). East Lansing, Mich: Michigan State University Press.

Hansen, M.J., Nate, N.A., Krueger, C.C., Zimmerman, M.S., Kruckman, H.G., & Taylor, W.W. (2012). Age, growth, survival, and maturity of lake trout morphotypes in Lake Mistassini, Quebec. *T Am Fish Soc, 141*(6), 1492-1503.

Hansen, M.J., Nate, N.A., Muir, A.M., Bronte, C.R., Zimmerman, M.S., & Krueger, C.C. (2016). Life history variation among four lake trout morphs at Isle Royale, Lake Superior. *J Great Lakes Res, 42*(2), 421-432.

Happel, A., Stratton, L., Pattridge, R., Rinchard, J., & Czesny, S. (2016). Fatty-acid profiles of juvenile lake trout reflect experimental diets consisting of natural prey. *Freshwater Biol, 61*(9), 1466-1476.

Harrington, G.W., Beach, D.H., Dunham, J.E., & Holz Jr., G.G. (1970). The polyunsaturated fatty acids of marine dinoflagellates. *J Eukaryot Microbiol, 17*(2), 213-219.

Harvey, C.J., & Kitchell, J.F. (2000). A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. *Can J Fish Aquat Sci, 57*(7), 1395-1403.

Harvey, C.J., Schram, S.T., & Kitchell, J.F. (2003). Trophic relationships among lean and siscowet lake trout in Lake Superior. *T Am Fish Soc, 132*(2), 219-228.

Hesslein, R.H., Hallard, K.A., & Ramlal, P. (1993). Replacement of sulfur, carbon, and nitrogen, in tissue of growing broad whitefish (Coregonus nasus) in response to a change in diet traced by δ34S, δ13C, and δ15N. *Can J Fish Aquat Sci, 50*, 2071-2076.

Hobson, K.A., & Welch, H.E. (1995). Cannibalism and Trophic Structure in a High Arctic Lake - Insights from Stable-Isotope Analysis. *Can J Fish Aquat Sci, 52*(6), 1195-1201.

Hoffman, J.C., Sierszen, M.E., & Cotter, A.M. (2015). Fish tissue lipid-C:N relationships for correcting C-13 values and estimating lipid content in aquatic food-web studies. *Rapid Commun Mass Sp, 29*(21), 2069-2077.

Hoffman, J.C., & Sutton, T.T. (2010). Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep-Sea Res Pt I, 57*(8), 956-964.

Horns, W.H., Bronte, C.R., Busiahn, T.R., Ebener, M.P., Eshenroder, R.L., Gorenflo, T., Kmiecik, N., Mattes, W., Peck, J.W., Petzold, M., & Schreiner, D.R. (2003). Fish-community objectives for Lake Superior. *Great Lakes Fish Comm Spec Publ, 03-01*, 0-78.

Hrabik, T.R., Jensen, O.P., Martell, S.J.D., Walters, C.J., & Kitchell, J.F. (2006). Diel vertical migration in the Lake Superior pelagic community. I. Changes in vertical migration of coregonids in response to varying predation risk. *Can J Fish Aquat Sci, 63*(10), 2286-2295.

Isaac, E.J., Hrabik, T.R., Stockwell, J.D., & Gamble, A.E. (2012). Prey selection by the Lake Superior fish community. *J Great Lakes Res, 38*(2), 326-335.

Iverson, S.J. (2009). Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In M. T. Arts, M. T. Brett & M. J. Kainz (Eds.), *Lipids in aquatic ecosystems* (pp. 281-308). New York, NY: Springer.

Iverson, S.J., Field, C., Bowen, W.D., & Blanchard, W. (2004). Quantitative fatty acid signature analysis: A new method of estimating predator diets. *Ecol Monogr, 74*(2), 211-235.

Jackson, E.W., Doubek, J.P., Schaeffer, J.S., & Lehman, J.T. (2013). Historical and recent biomass and food web relations of Limnocalanus in Lake Huron. *J Great Lakes Res, 39*(3), 404-408.

Johannsson, O.E., Leggett, M.F., Rudstam, L.G., Servos, M.R., Mohammadian, M.A., Gal, G., Dermott, R.M., & Hesslein, R.H. (2001). Diet of Mysis relicta in Lake Ontario as revealed by stable isotope and gut content analysis. *Can J Fish Aquat Sci, 58*(10), 1975-1986.

Joseph, J.D. (1982). Lipid composition of marine and estuarine invertebrates. Part II: Mollusca *Progress in lipid research, 21*(2), 109-153.

Karube, Z., Okada, N., & Tayasu, I. (2012). Sulfur stable isotope signature identifies the source of reduced sulfur in benthic communities in macrophyte zones of Lake Biwa, Japan. *Limnology, 13*(3), 269-280.

Kattner, G., & Hagen, W. (2009). Lipids in marine copepods: latitudinal characteristics and perspective to global warming. In M. T. Arts, M. T. Brett & M. J. Kainz (Eds.), *Lipids in aquatic ecosystems* (pp. 257-280). New York, NY.: Springer.

Keeley, E.R., Parkinson, E.A., & Taylor, E.B. (2005). Ecotypic differentiation of native rainbow trout (Oncorhynchus mykiss) populations from British Columbia. *Can J Fish Aquat Sci, 62*(7), 1523-1539.

Kelly, J.R., & Scheibling, R.E. (2012). Fatty acids as dietary tracers in benthic food webs. *Mar Ecol Prog Ser, 446*, 1-22.

Kharlamenko, V.I., Zhukova, N.V., Khotimchenko, S.V., Svetashev, V.I., & Kamenev, G.M. (1995). Fatty acids as markers of food sources in a shallow-water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). *Mar Ecol Prog Ser, 120*, 231-241.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R.I. (2006). A revised model for lipid-normalizing delta C-13 values from aquatic organisms, with implications for isotope mixing models. *J Appl Ecol, 43*(6), 1213-1222.

Kirsch, P.E., Iverson, S.J., Bowen, W.D., Kerr, S.R., & Ackman, R.G. (1998). Dietary effects on the fatty acid signature of whole Atlantic cod (Gadus morhua). *Can J Fish Aquat Sci, 55*(6), 1378-1386.

Koppelmann, R., Bottger-Schnack, R., Mobius, J., & Weikert, H. (2009). Trophic relationships of zooplankton in the eastern Mediterranean based on stable isotope measurements. *J Plankton Res, 31*(6), 669-686.

Krueger, C.C., & Ihssen, P.E. (1995). Review of genetics of lake trout in the great lakes: History, molecular genetics, physiology, strain comparisons, and restoration management. *J Great Lakes Res, 21*, 348-363.

Krueger, C.C., Jones, M.L., & Taylor, W.W. (1995). Restoration of lake trout in the Great Lakes: Challenges and strategies for future management. *J Great Lakes Res, 21*, 547-558.

Kwak, T.J., & Zedler, J.B. (1997). Food web analysis of southern California coastal wetlands using multiple stable isotopes. *Oecologia, 110*(2), 262-277.

Lindsay, D.J., Minagawa, M., Mitani, I., & Kawaguchi, K. (1998). Trophic shift in the Japanese anchovy Engraulis japonicus in its early life history stages as detected by stable isotope ratios in Sagami Bay, Central Japan. *Fisheries Sci, 64*(3), 403-410.

Markham, J.L., Cook, A., MacDougall, T., Witzel, L., Kayle, K., Murray, M., Fodale, M., Trometer, E., Neave, F., Fitzsimons, J., Francis, J., & Stapanian, M. (2008). A strategic plan for the rehabilitation of lake trout in Lake Erie, 2008-2020. *Great Lakes Fish Comm Misc Publ, 2008-02*, Available from http://www.glfc.org/pubs/pub.htm#misc.

Martin, N.V. (1966). The significance of food habits in the biology, exploitation, and management of Algonquin Park, Ontario, Lake Trout. *T Am Fish Soc, 95*(4), 415-422.

Martin, N.V. (1970). Long-term effects of diet on the biology of Lake Trout an the fishery in Lake Opeongo, Ontario. *J Fish Res Board Can, 27*(1), 125-146.
Martin, N.V., & Sanderco, F.K. (1967). Pyloric Caeca and Gill Raker Development in Lake Trout Salvelinus Namaycush in Algonquin Park Ontario. *J Fish Res Board Can, 24*(5), 965-&.
Matthews, B., & Mazumder, A. (2005). Temporal variation in body composition (C:N) helps explain seasonal patterns of zooplankton delta C-13. *Freshwater Biol, 50*(3), 502-515.
McMeans, B.C., Rooney, N., Arts, M.T., & Fisk, A.T. (2013). Food web structure of a coastal Arctic marine ecosystem and implications for stability. *Mar Ecol Prog Ser, 482*, 17-28.
Meziane, T., Sanabe, M.C., & Tsuchiya, M. (2002). Role of fiddler crabs of a subtropical intertidal flat on the fate of sedimentary fatty acids. *J Exp Mar Biol Ecol, 270*(2), 191-201.
Moore, S.A., & Bronte, C.R. (2001). Delineation of sympatric morphotypes of lake trout in Lake Superior. *T Am Fish Soc, 130*(6), 1233-1240.
Morrison, W.R., & Smith, L.M. (1964). Preparation of Fatty Acid Methyl Esters + Dimethylacetals from Lipids with Boron Fluoride-Methanol. *J Lipid Res, 5*(4), 600-&.
Muir, A.M., Bronte, C.R., Zimmerman, M.S., Quinlan, H.R., Glase, J.D., & Krueger, C.C. (2014). Ecomorphological diversity of lake trout at Isle Royale, Lake Superior. *T Am Fish Soc, 143*(4), 972-987.
Muir, A.M., Krueger, C.C., & Hansen, M.J. (2012). Re-establishing lake trout in the Laurentian Great Lakes: past, present, and future. In Taylor WW, Lynch AJ & L. NJ (Eds.), *Great Lakes fisheries policy and management: a binational perspective* (2 ed., pp. 533-588). Michigan State University Press, East Lansing.
Negus, M.T. (2010). Contribution of lake trout stocked as fry to an adult population in Lake Superior. *J Great Lakes Res, 36*(2), 380-386.
Nieland, J.L., Hansen, M.J., Seider, M.J., & Deroba, J.J. (2008). Modeling the sustainability of lake trout fisheries in eastern Wisconsin waters of Lake Superior. *Fish Res, 94*(3), 304-314.
Omara, M., Crimmins, B.S., Back, R.C., Hopke, P.K., Chang, F.C., & Holsen, T.M. (2015). Mercury biomagnification and contemporary food web dynamics in lakes Superior and Huron. *J Great Lakes Res, 41*(2), 473-483.
Overman, N.C., & Parrish, D.L. (2001). Stable isotope composition of walleye: N-15 accumulation with age and area-specific differences in delta C-13. *Can J Fish Aquat Sci, 58*(6), 1253-1260.
Perreault-Payette, A. (2016). *Génomique des populations et association génotypephénotypedes écotypes de touladi du lac Supérieur.* Unpublished Master's thesis, Universite Laval, Quebec City, Quebec.
Post, D.M. (2002). Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology, 83*(3), 703-718.
Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., & Montana, C.G. (2007). Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia, 152*(1), 179-189.
Proulx, R., & Magnan, P. (2004). Contribution of phenotypic plasticity and heredity to the trophic polymorphism of lacustrine brook charr (Salvelinus fontinalis M.). *Evol Ecol Res, 6*(4), 503-522.

Rahrer, J.F. (1965). Age, growth, maturity, and fecundity of humper lake trout, Isle Royale, Lake Superior. *T Am Fish Soc, 94*(1), 75-83.
Ray, B.A., Hrabik, T.R., Ebener, M.P., Gorman, O.T., Schreiner, D.R., Schram, S.T., Sitar, S.P., Mattes, W.P., & Bronte, C.R. (2007). Diet and prey selection by Lake Superior lake trout during spring, 1986–2001. *J Great Lakes Res, 33*(1), 104-113.
Schmidt, H.L., Robins, R.J., & Werner, R.A. (2015). Multi-factorial in vivo stable isotope fractionation: causes, correlations, consequences and applications. *Isot Environ Healt S, 51*(1), 155-199.
Schmidt, S.N., Vander Zanden, M.J., & Kitchell, J.F. (2009). Long-term food web change in Lake Superior. *Can J Fish Aquat Sci, 66*(12), 2118-2129.
Selgeby, J.H. (1988). Comparative biology of the sculpins of Lake Superior. *J Great Lakes Res, 14*(1), 44-51.
Sierszen, M.E., Hrabik, T.R., Stockwell, J.D., Cotter, A.M., Hoffman, J.C., & Yule, D.L. (2014). Depth gradients in food-web processes linking habitats in large lakes: Lake Superior as an exemplar ecosystem. *Freshwater Biol, 59*(10), 2122-2136.
Sierszen, M.E., Kelly, J.R., Corry, T.D., Scharold, J.V., & Yurista, P.M. (2011). Benthic and pelagic contributions to Mysis nutrition across Lake Superior. *Can J Fish Aquat Sci, 68*(6), 1051-1063.
Sitar, S.P., Morales, H.M., Mata, M.T., Bastar, B.B., Dupras, D.M., Kleaver, G.D., & Rathbun, K.D. (2008). Survey of siscowet lake trout at their maximum depth in Lake Superior. *J Great Lakes Res, 34*(2), 276-286.
Stafford, C.P., McPhee, M.V., Eby, L.A., & Allendorf, F.W. (2014). Introduced lake trout exhibit life history and morphological divergence with depth. *Can J Fish Aquat Sci, 71*(1), 10-20.
Syvaranta, J., Hamalainen, H., & Jones, R.I. (2006). Within-lake variability in carbon and nitrogen stable isotope signatures. *Freshwater Biol, 51*(6), 1090-1102.
R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. Retrieved from https://www.R-project.org/
Thurston, C.E. (1962). Physical Characteristics and Chemical Composition of 2 Subspecies of Lake Trout. *J Fish Res Board Can, 19*(1), 39-44.
Tocher, D.R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Rev Fish Sci, 11*(2), 107-184.
Tocher, D.R., Dick, J.R., MacGlaughlin, P., & Bell, J.G. (2006). Effect of diets enriched in Δ6 desaturated fatty acids (18:3n − 6 and 18:4n − 3), on growth, fatty acid composition and highly unsaturated fatty acid synthesis in two populations of Arctic charr (Salvelinus alpinus L.). *Comp Biochem Physiol B Biochem Mol Biol, 144*(2), 245-253.
Trueman, C.N., McGill, R.A.R., & Guyard, P.H. (2005). The effect of growth rate on tissue-diet isotopic spacing in rapidly growing animals. An experimental study with Atlantic salmon (Salmo salar). *Rapid Commun Mass Sp, 19*(22), 3239-3247.
Vander Zanden, M.J., & Rasmussen, J.B. (2001). Variation in d15N and d13C trophic fractionation: Implications for aquatic food web studies. *Limnol Oceanogr, 46*(8), 2061-2066.
Vestal, J.R., & White, D.C. (1989). Lipid analysis in microbial ecology. *Bioscience, 39*(8), 535-541.
Volkman, J.K., Johns, R.B., Gillan, F.T., & Perry, G.J. (1980). Microbial lipids of an intertidal sediment—I. Fatty acids and hydrocarbons. *Geochim Cosmochim Ac, 44*(8), 1133-1143.

Webb, P.W. (1984). Body Form, Locomotion and Foraging in Aquatic Vertebrates. *Am Zool, 24*(1), 107-120.
Zimmerman, M.S., & Krueger, C.C. (2009). An ecosystem perspective on re-establishing native deepwater fishes in the Laurentian Great Lakes. *N Am J Fish Manage, 29*(5), 1352-1371.
Zimmerman, M.S., Krueger, C.C., & Eshenroder, R.L. (2006). Phenotypic diversity of lake trout in Great Slave Lake: Differences in morphology, buoyancy, and habitat depth. *T Am Fish Soc, 135*(4), 1056-1067.
Zimmerman, M.S., Schmidt, S.N., Krueger, C.C., Vander Zanden, M.J., & Eshenroder, R.L. (2009). Ontogenetic niche shifts and resource partitioning of lake trout morphotypes. *Can J Fish Aquat Sci, 66*(6), 1007-1018.

Chapter 3 References

Arlettaz, R., Perrin, N., & Hausser, J. (1997). Trophic resource partitioning and competition betweenthe two sibling bat species *Myotis myotis* and *Myotis blythii*. *J Anim Ecol, 66*(6), 897-911.
Arts, M.T., & Wainman, B. (1999). Lipids in freshwater ecosystems. New York: Springer.
Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., & Macleod, H. (2004). Determining trophic niche width: a novel approach using stable isotope analysis. *J Anim Ecol, 73*(5), 1007-1012.
Behnke, R.J. (1972). Systematics of Salmonid Fishes of Recently Glaciated Lakes. *J Fish Res Board Can, 29*(6), 639-671.
Blackie, C.T., Weese, D.J., & Noakes, D.L.G. (2003). Evidence for resource polymorphism in the lake charr (Salvelinus namaycush) population of Great Bear Lake, Northwest Territories, Canada. *Ecoscience, 10*(4), 509-514.
Bolnick, D.I., Yang, L.H., Fordyce, J.A., Davis, J.M., & Svanback, R. (2002). Measuring individual-level resource specialization. *Ecology, 83*(10), 2936-2941.
Bradshaw, C.J., Hindell, M.A., Best, N.J., Phillips, K.L., Wilson, G., & Nichols, P.D. (2003). You are what you eat: describing the foraging ecology of southern elephant seals (Mirounga leonina) using blubber fatty acids. *Proc Biol Sci, 270*(1521), 1283-1292.
Bronte, C.R., Ebener, M.P., Schreiner, D.R., DeVault, D.S., Petzold, M.M., Jensen, D.A., Richards, C., & Lozano, S.J. (2003). Fish community change in lake superior, 1970-2000. *Can J Fish Aquat Sci, 60*(12), 1552-1574.
Bronte, C.R., Krueger, C.C., Holey, M.E., Toneys, M.L., Eshenroder, R.L., & Jonas, J.L. (2008). A guide for the rehabilitation of lake trout in Lake Michigan. *Great Lakes Fish Comm Misc Publ, 2008-01*, Available from http://www.glfc.org/pubs/pub.htm#misc.
Budge, S.M., Iverson, S.J., & Koopman, H.N. (2006). Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Mar Mammal Sci, 22*(4), 759-801.
Budge, S.M., & Parrish, C.C. (1998). Lipid biogeochemistry of planton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. *Org Geochem, 29*(5-7), 1547-1559.
Chavarie, L., Howland, K.L., & Tonn, W.M. (2013). Sympatric Polymorphism in Lake Trout: The Coexistence of Multiple Shallow-Water Morphotypes in Great Bear Lake. *T Am Fish Soc, 142*(3), 814-823.

Cline, T.J., Bennington, V., & Kitchell, J.F. (2013). Climate change expands the spatial extent and duration of preferred thermal habitat for Lake Superior fishes. *PloS one, 8*(4), e62279.

Croisetiere, L., Hare, L., Tessier, A., & Cabana, G. (2009). Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. *Freshwater Biol, 54*(5), 1006-1015.

Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., & Hagen, W. (2003). Fatty acid trophic markers in the pelagic marine environment. *Adv Mar Biol, 46*, 225-340.

Deniro, M.J., & Epstein, S. (1978). Influence of Diet on Distribution of Carbon Isotopes in Animals. *Geochim Cosmochim Ac, 42*(5), 495-506.

Deniro, M.J., & Epstein, S. (1981). Influence of Diet on the Distribution of Nitrogen Isotopes in Animals. *Geochim Cosmochim Ac, 45*(3), 341-351.

Eschmeyer, P.H. (1955). The reproduction of Lake Trout in southern Lake Superior. *T Am Fish Soc, 84*(1), 47-74.

Eschmeyer, P.H., & Phillips, A.M. (1965). Fat Content of Flesh of Siscowets and Lake Trout from Lake Superior. *T Am Fish Soc, 94*(1), 62-&.

Folch, J., Lees, M., & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem, 226*(1), 497-509.

Fraley, C., & Raftery, A.E. (2009). MCLUST version 3 for R: normal mixture modeling and model-based clustering. University of Washington, Technical Report 504, Seattle.

France, R.L. (1995). Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnol Oceanogr, 40*(7), 1310-1313.

Gamble, A.E., Hrabik, T.R., Stockwell, J.D., & Yule, D.L. (2011a). Trophic connections in Lake Superior Part I: The offshore fish community. *J Great Lakes Res, 37*(3), 541-549.

Gamble, A.E., Hrabik, T.R., Yule, D.L., & Stockwell, J.D. (2011b). Trophic connections in Lake Superior Part II: The nearshore fish community. *J Great Lakes Res, 37*(3), 550-560.

Goetz, F., Jasonowicz, A., Johnson, R., Biga, P., Fischer, G., & Sitar, S. (2014). Physiological differences between lean and siscowet lake trout morphotypes: Are these metabolotypes? *Can J Fish Aquat Sci, 71*(3), 427-435.

Gonfiantini, R., Stichler, W., & Rozanski, K. (1995). Standards and intercomparison materials distributed by the international atomic energy agency for stable isotope measurements *Reference and intercomparison materials for stable isotopes of light elements* (pp. 13-29). Vienna, Austria: International Atomic Energy Angency.

Gorman, O.T., Evrard, L.M., Cholwek, G.A., & Vinson, M.R. (2013). Status and trends in the fish community of Lake Superior, 2012. U.S. Geological Survey, Washington, D.C.

Gorman, O.T., Yule, D.L., & Stockwell, J.D. (2012a). Habitat use by fishes of Lake Superior. I. Diel patterns of habitat use in nearshore and offshore waters of the Apostle Islands region. *Aquat Ecosyst Health, 15*(3), 333-354.

Gorman, O.T., Yule, D.L., & Stockwell, J.D. (2012b). Habitat use by fishes of Lake Superior. II. Consequences of diel habitat use for habitat linkages and habitat coupling in nearshore and offshore waters. *Aquat Ecosyst Health, 15*(3), 355-368.

Hansen, M.J., Nate, N.A., Krueger, C.C., Zimmerman, M.S., Kruckman, H.G., & Taylor, W.W. (2012). Age, growth, survival, and maturity of lake trout morphotypes in Lake Mistassini, Quebec. *T Am Fish Soc, 141*(6), 1492-1503.

Hansen, M.J., Nate, N.A., Muir, A.M., Bronte, C.R., Zimmerman, M.S., & Krueger, C.C. (2016). Life history variation among four lake trout morphs at Isle Royale, Lake Superior. *J Great Lakes Res, 42*(2), 421-432.

Harrington, G.W., Beach, D.H., Dunham, J.E., & Holz Jr., G.G. (1970). The polyunsaturated fatty acids of marine dinoflagellates. *J Eukaryot Microbiol, 17*(2), 213-219.

Harvey, C.J., & Kitchell, J.F. (2000). A stable isotope evaluation of the structure and spatial heterogeneity of a Lake Superior food web. *Can J Fish Aquat Sci, 57*(7), 1395-1403.

Harvey, C.J., Schram, S.T., & Kitchell, J.F. (2003). Trophic relationships among lean and siscowet lake trout in Lake Superior. *T Am Fish Soc, 132*(2), 219-228.

Hastings, N., Agaba, M., Tocher, D.R., Leaver, M.J., Dick, J.R., Sargent, J.R., & Teale, A.J. (2001). A vertebrate fatty acid desaturase with Delta 5 and Delta 6 activities. *P Natl Acad Sci USA, 98*(25), 14304-14309.

Hasui, E., da Mota Gomes, V.S., Kiefer, M.C., Tamashiro, J., & Silva, W.R. (2009). Spatial and seasonal variation in niche partitioning between blue manakin (*Chiroxiphia caudata*) and greenish schiffornis (*Schiffornis virescens*) in southeastern Brazil. *Stud Neotrop Fauna E, 44*(3), 149-159.

Hodgson, J.R., He, X., Schindler, D.E., & Kitchell, J.F. (1997). Diet overlap in a piscivore community. *Ecol Freshw Fish, 6*(3), 144-149.

Hoffman, J.C., Sierszen, M.E., & Cotter, A.M. (2015). Fish tissue lipid-C:N relationships for correcting C-13 values and estimating lipid content in aquatic food-web studies. *Rapid Commun Mass Sp, 29*(21), 2069-2077.

Hoffman, J.C., & Sutton, T.T. (2010). Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep-Sea Res Pt I, 57*(8), 956-964.

Hrabik, T.R., Jensen, O.P., Martell, S.J.D., Walters, C.J., & Kitchell, J.F. (2006). Diel vertical migration in the Lake Superior pelagic community. I. Changes in vertical migration of coregonids in response to varying predation risk. *Can J Fish Aquat Sci, 63*(10), 2286-2295.

Hutchinson, G.E. (1957). Population studies - animal ecology and demography - concluding remarks. *Cold Spring Harb Sym, 22*, 415-427.

Isaac, E.J., Hrabik, T.R., Stockwell, J.D., & Gamble, A.E. (2012). Prey selection by the Lake Superior fish community. *J Great Lakes Res, 38*(2), 326-335.

Jackson, A.L., Inger, R., Parnell, A.C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *J Anim Ecol, 80*(3), 595-602.

Jonsson, B., & Jonsson, N. (2001). Polymorphism and speciation in Arctic charr. *J Fish Biol, 58*(3), 605-638.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R.I. (2006). A revised model for lipid-normalizing delta C-13 values from aquatic organisms, with implications for isotope mixing models. *J Appl Ecol, 43*(6), 1213-1222.

Krueger, C.C., & Ihssen, P.E. (1995). Review of genetics of lake trout in the great lakes: History, molecular genetics, physiology, strain comparisons, and restoration management. *J Great Lakes Res, 21*, 348-363.

Lunn, D., Jackson, C., Best, N., Thomas, A., & Spiegelhalter, D. (2013). The BUGS book: a practical introduction to Bayesian analysis. Boca Raton, FL: CRC Press.

Lysy, M., Stasko, A.D., & Swanson, H.K. (2014). nicheROVER: (Niche) (R)egion and Niche (Over)lap Metrics for Multidimensional Ecological Niches (Version 1.0). Retrieved from https://cran.r-project.org/web/packages/nicheROVER/index.html.

Markham, J.L., Cook, A., MacDougall, T., Witzel, L., Kayle, K., Murray, M., Fodale, M., Trometer, E., Neave, F., Fitzsimons, J., Francis, J., & Stapanian, M. (2008). A strategic plan for the rehabilitation of lake trout in Lake Erie, 2008-2020. *Great Lakes Fish Comm Misc Publ, 2008-02*, Available from http://www.glfc.org/pubs/pub.htm#misc.

Martin, N.V., & Olver, C.H. (1980). The lake charr, *Salvelinus namaycush*. In E. K. Balon (Ed.), *Charrs: Salmonid Fishes of the Genus Salvelinus* (pp. 205-277). Hague, Netherlands: Dr. W. Junk Publishers.

Martin, N.V., & Sanderco, F.K. (1967). Pyloric Caeca and Gill Raker Development in Lake Trout Salvelinus Namaycush in Algonquin Park Ontario. *J Fish Res Board Can, 24*(5), 965-&.

May, R.M., & Macarthur, R.H. (1972). Niche Overlap as a Function of Environmental Variability. *P Natl Acad Sci USA, 69*(5), 1109-+.

Moore, S.A., & Bronte, C.R. (2001). Delineation of sympatric morphotypes of lake trout in Lake Superior. *T Am Fish Soc, 130*(6), 1233-1240.

Morrison, W.R., & Smith, L.M. (1964). Preparation of Fatty Acid Methyl Esters + Dimethylacetals from Lipids with Boron Fluoride-Methanol. *J Lipid Res, 5*(4), 600-&.

Muir, A.M., Bronte, C.R., Zimmerman, M.S., Quinlan, H.R., Glase, J.D., & Krueger, C.C. (2014). Ecomorphological diversity of lake trout at Isle Royale, Lake Superior. *T Am Fish Soc, 143*(4), 972-987.

Muir, A.M., Krueger, C.C., & Hansen, M.J. (2012). Re-establishing lake trout in the Laurentian Great Lakes: past, present, and future. In Taylor WW, Lynch AJ & L. NJ (Eds.), *Great Lakes fisheries policy and management: a binational perspective* (2 ed., pp. 533-588). Michigan State University Press, East Lansing.

Newsome, S.D., del Rio, C.M., Bearhop, S., & Phillips, D.L. (2007). A niche for isotopic ecology. *Front Ecol Environ, 5*(8), 429-436.

Perreault-Payette, A. (2016). *Génomique des populations et association génotypephénotypedes écotypes de touladi du lac Supérieur.* Unpublished Master's thesis, Universite Laval, Quebec City, Quebec.

Peterson, B.J., & Fry, B. (1987). Stable Isotopes in Ecosystem Studies. *Annu Rev Ecol Syst, 18*, 293-320.

Pianka, E.R. (1974). Niche Overlap and Diffuse Competition. *P Natl Acad Sci USA, 71*(5), 2141-2145.

R Core Team. (2016). R: A language and environment for statistical computing. R Foundation for Statistical Computing,Vienna, Austria. Retrieved from https://www.R-project.org/

Rahrer, J.F. (1965). Age, growth, maturity, and fecundity of humper lake trout, Isle Royale, Lake Superior. *T Am Fish Soc, 94*(1), 75-83.

Ray, B.A., Hrabik, T.R., Ebener, M.P., Gorman, O.T., Schreiner, D.R., Schram, S.T., Sitar, S.P., Mattes, W.P., & Bronte, C.R. (2007). Diet and prey selection by Lake Superior lake trout during spring, 1986–2001. *J Great Lakes Res, 33*(1), 104-113.

Reist, J.D., Power, M., & Dempson, J.B. (2013). Arctic charr (Salvelinus alpinus): a case study of the importance of understanding biodiversity and taxonomic issues in northern fishes. *Biodiversity, 14*(1), 45-56.

Rossman, S., Ostrom, P.H., Gordon, F., & Zipkin, E.F. (2016). Beyond carbon and nitrogen: guidelines for estimating three-dimensional isotopic niche space. *Ecol Evol, 6*(8), 2405-2413.

Rusterholz, K.A. (1981). Niche overlap among foliage-gleaning birds: support for Pianka's niche overlap hypothesis. *Am Nat, 117*(3), 395-399.

Sitar, S.P., Morales, H.M., Mata, M.T., Bastar, B.B., Dupras, D.M., Kleaver, G.D., & Rathbun, K.D. (2008). Survey of siscowet lake trout at their maximum depth in Lake Superior. *J Great Lakes Res, 34*(2), 276-286.

Stafford, C.P., McPhee, M.V., Eby, L.A., & Allendorf, F.W. (2014). Introduced lake trout exhibit life history and morphological divergence with depth. *Can J Fish Aquat Sci, 71*(1), 10-20.

Swanson, H.K., Lysy, M., Power, M., Stasko, A.D., Johnson, J.D., & Reist, J.D. (2015). A new probabilistic method for quantifying n-dimensional ecological niches and niche overlap. *Ecology, 96*(2), 318-324.

Syvaranta, J., Lensu, A., Marjomaki, T.J., Oksanen, S., & Jones, R.I. (2013). An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. *PloS one, 8*(2).

Zimmerman, M.S., Krueger, C.C., & Eshenroder, R.L. (2006). Phenotypic diversity of lake trout in Great Slave Lake: Differences in morphology, buoyancy, and habitat depth. *T Am Fish Soc, 135*(4), 1056-1067.

Zimmerman, M.S., Schmidt, S.N., Krueger, C.C., Vander Zanden, M.J., & Eshenroder, R.L. (2009). Ontogenetic niche shifts and resource partitioning of lake trout morphotypes. *Can J Fish Aquat Sci, 66*(6), 1007-1018.

Chapter 4 References

Ahrenstorff, T.D., Hrabik, T.R., Stockwell, J.D., Yule, D.L., & Sass, G.G. (2011). Seasonally dynamic diel vertical migrations of *Mysis diluviana*, coregonine fishes, and siscowet Lake Trout in the pelagia of Western Lake Superior. *T Am Fish Soc, 140*(6), 1504-1520.

Budge, S.M., Iverson, S.J., & Koopman, H.N. (2006). Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Mar Mammal Sci, 22*(4), 759-801.

Eschmeyer, P.H., & Phillips, A.M. (1965). Fat Content of Flesh of Siscowets and Lake Trout from Lake Superior. *T Am Fish Soc, 94*(1), 62-&.

Gamble, A.E., Hrabik, T.R., Stockwell, J.D., & Yule, D.L. (2011a). Trophic connections in Lake Superior Part I: The offshore fish community. *J Great Lakes Res, 37*(3), 541-549.

Gamble, A.E., Hrabik, T.R., Yule, D.L., & Stockwell, J.D. (2011b). Trophic connections in Lake Superior Part II: The nearshore fish community. *J Great Lakes Res, 37*(3), 550-560.

Goetz, F., Jasonowicz, A., Johnson, R., Biga, P., Fischer, G., & Sitar, S. (2014). Physiological differences between lean and siscowet lake trout morphotypes: Are these metabolotypes? *Can J Fish Aquat Sci, 71*(3), 427-435.

Goetz, F., Rosauer, D., Sitar, S., Goetz, G., Simchick, C., Roberts, S., Johnson, R., Murphy, C., Bronte, C.R., & Mackenzie, S. (2010). A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (Salvelinus namaycush). *Mol Ecol, 19*, 176-196.

Happel, A., Stratton, L., Pattridge, R., Rinchard, J., & Czesny, S. (2016). Fatty-acid profiles of juvenile lake trout reflect experimental diets consisting of natural prey. *Freshwater Biol, 61*(9), 1466-1476.

Harvey, C.J., Schram, S.T., & Kitchell, J.F. (2003). Trophic relationships among lean and siscowet lake trout in Lake Superior. *T Am Fish Soc, 132*(2), 219-228.

Hesslein, R.H., Hallard, K.A., & Ramlal, P. (1993). Replacement of sulfur, carbon, and nitrogen, in tissue of growing broad whitefish (Coregonus nasus) in response to a change in diet traced by δ34S, δ13C, and δ15N. *Can J Fish Aquat Sci, 50*, 2071-2076.

Muir, A.M., Krueger, C.C., & Hansen, M.J. (2012). Re-establishing lake trout in the Laurentian Great Lakes: past, present, and future. In Taylor WW, Lynch AJ & L. NJ (Eds.), *Great Lakes fisheries policy and management: a binational perspective* (2 ed., pp. 533-588). Michigan State University Press, East Lansing.

Ray, B.A., Hrabik, T.R., Ebener, M.P., Gorman, O.T., Schreiner, D.R., Schram, S.T., Sitar, S.P., Mattes, W.P., & Bronte, C.R. (2007). Diet and prey selection by Lake Superior lake trout during spring, 1986–2001. *J Great Lakes Res, 33*(1), 104-113.

Sierszen, M.E., Kelly, J.R., Corry, T.D., Scharold, J.V., & Yurista, P.M. (2011). Benthic and pelagic contributions to Mysis nutrition across Lake Superior. *Can J Fish Aquat Sci, 68*(6), 1051-1063.

Stafford, C.P., McPhee, M.V., Eby, L.A., & Allendorf, F.W. (2014). Introduced lake trout exhibit life history and morphological divergence with depth. *Can J Fish Aquat Sci, 71*(1), 10-20.

Appendix References

Budge, S.M., Iverson, S.J., & Koopman, H.N. (2006). Studying trophic ecology in marine ecosystems using fatty acids: A primer on analysis and interpretation. *Mar Mammal Sci, 22*(4), 759-801.

Fagan, K.A., Koops, M.A., Arts, M.T., & Power, M. (2011). Assessing the utility of C:N ratios for predicting lipid content in fishes. *Can J Fish Aquat Sci, 68*(2), 374-385.

Folch, J., Lees, M., & Stanley, G.H.S. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem, 226*(1), 497-509.

Hoffman, J.C., Sierszen, M.E., & Cotter, A.M. (2015). Fish tissue lipid-C:N relationships for correcting C-13 values and estimating lipid content in aquatic food-web studies. *Rapid Commun Mass Sp, 29*(21), 2069-2077.

Hoffman, J.C., & Sutton, T.T. (2010). Lipid correction for carbon stable isotope analysis of deep-sea fishes. *Deep-Sea Res Pt I, 57*(8), 956-964.

Kiljunen, M., Grey, J., Sinisalo, T., Harrod, C., Immonen, H., & Jones, R.I. (2006). A revised model for lipid-normalizing delta C-13 values from aquatic organisms, with implications for isotope mixing models. *J Appl Ecol, 43*(6), 1213-1222.

Mintenbeck, K., Brey, T., Jacob, U., Knust, R., & Struck, U. (2008). How to account for the lipid effect on carbon stable-isotope ratio (delta C-13): sample treatment effects and model bias. *J Fish Biol, 72*(4), 815-830.

Appendix

A.1 Lipid Correction Models

As lipids have been shown to be depleted in $\delta^{13}C$ (Kiljunen et al., 2006; Hoffman & Sutton, 2010), lipid correction models were used to correct Lake Trout and prey $\delta^{13}C$ values for effects of lipid bias. A subset of 120 Lake Trout (30 per morph), 54 prey fishes (8 per species per site, except Bloaters, where n=6), and 7 pooled invertebrate samples (*Mysis*, zooplankton, and moths) were lipid extracted using a modified Folch method (Folch et al., 1957; Budge et al., 2006), and $\delta^{13}C_{extracted}$ ratios were estimated based on mass balance models presented by Hoffman and Sutton (2010). Dorsal muscle was used for Lake Trout lipid extractions, whereas whole bodies were homogenized and extracted for prey fishes and invertebrates. Samples of *Diporeia*, snails, clams, and Rainbow Smelt were not subject to lipid extraction due to lack of sample mass. Rainbow Smelt also had C:N ratios < 4.0, which was used as the minimum C:N ratio to perform lipid corrections, as recommended by Hoffman et al. (2015). Lipid corrected $\delta^{13}C$ values were estimated as follows:

$$\delta^{13}C_{protein} = \delta^{13}C_{bulk} + [\Delta\delta^{13}C_{bulk} \times (\frac{C{:}N_{protein} - C{:}N_{bulk}}{C{:}N_{bulk}})] \quad \text{(Equation 3)}$$

Where $\delta^{13}C_{protein}$ is the $\delta^{13}C$ ratio of the lipid extracted sample, $\delta^{13}C_{bulk}$ is the $\delta^{13}C$ ratio of the non-extracted sample, $\Delta\delta^{13}C_{bulk}$ is the isotopic depletion factor due to lipids, $C{:}N_{protein}$ is the C:N ratio in the extracted sample, and $C{:}N_{bulk}$ is the C:N ratio in the non-extracted sample.

While established models have been demonstrated to sufficiently predict lipid corrected values (Hoffman et al., 2015), in some instances it may be more appropriate to develop models for a specific system or population, as general models may not accurately predict lipid

corrections for every species (Mintenbeck et al., 2008; Fagan et al., 2011). Four different models were evaluated for lipid correction. The first model used established values for $\Delta\delta^{13}C_{bulk}$ and $C:N_{protein}$ of -6.5 ‰ and 3.5 respectively and was applied to all species, which Hoffman et al., (2015) used for Lake Trout in Western Lake Superior. The second model involved predicting $\Delta\delta^{13}C_{bulk}$ and $C:N_{protein}$ for each morph/species at each site using average values of $\Delta\delta^{13}C_{bulk}$ and $C:N_{protein}$ from the extracted samples. The third model used the established values of $\Delta\delta^{13}C_{bulk}$ and $C:N_{protein}$ of -6.5 ‰ and 3.5 respectively for all morphs/species on samples with $C:N_{bulk}$ > 4.0. The fourth model estimated $\Delta\delta^{13}C_{bulk}$ and $C:N_{protein}$ for each morph/species from averages of the extracted individuals that had $C:N_{bulk}$ > 4.0 for each morph at each site. Superior Shoal leans and Stannard Rock humpers each had 2 outliers (leans: $\Delta\delta^{13}C_{bulk}$=-17.8‰, -268.7‰; humpers: $\Delta\delta^{13}C_{bulk}$ = -28.9‰, -30.0‰) that were not included in calculating average $\Delta\delta^{13}C_{bulk}$ or $C:N_{protein}$, as these values were ~2-4 times larger than the literature values reported for $\Delta\delta^{13}C_{bulk}$ of ~7 (Kiljunen et al., 2006; Hoffman & Sutton, 2010)

Figure A-1 illustrates the correction models compared to measured values. Model error and bias was quantified using methods described by Hoffmann and Sutton (2010). Model 4 had the lowest residual error and mean bias, followed by Model 2, Model 1, and finally Model 3 (Table A-1). Using established literature values for $\Delta\delta^{13}C_{bulk}$ and $C:N_{protein}$ was not appropriate to estimate lipid-corrected $\delta^{13}C$ ratios in this system, as redfins from Superior Shoal and all three morphs from Stannard Rock had average $\Delta\delta^{13}C_{bulk}$ values that varied from the established value of -6.5‰ (Table A-2). All Lake Trout morphs also had $C:N_{protein}$ values lower than the established literature value of 3.5 (Table A-2) (Hoffman et al., 2015), suggesting 3.5 is not the most appropriate estimate of $C:N_{protein}$ for Lake Trout at these sites. $\Delta\delta^{13}C_{bulk}$ values appeared to be more similar among morphs within the same site, as Stannard Rock morphs had more

negative $\Delta\delta^{13}C_{bulk}$ values than their Superior Shoal counterparts. Because of the observed variability in $\Delta\delta^{13}C_{bulk}$ and $C:N_{protein}$ between sites, among morphs/species, and between these data and literature estimates, literature values were not used for further analyses. Instead, Model 4 was used to generate lipid-corrected $\delta^{13}C$ values that were then used in analyses of isotope data.

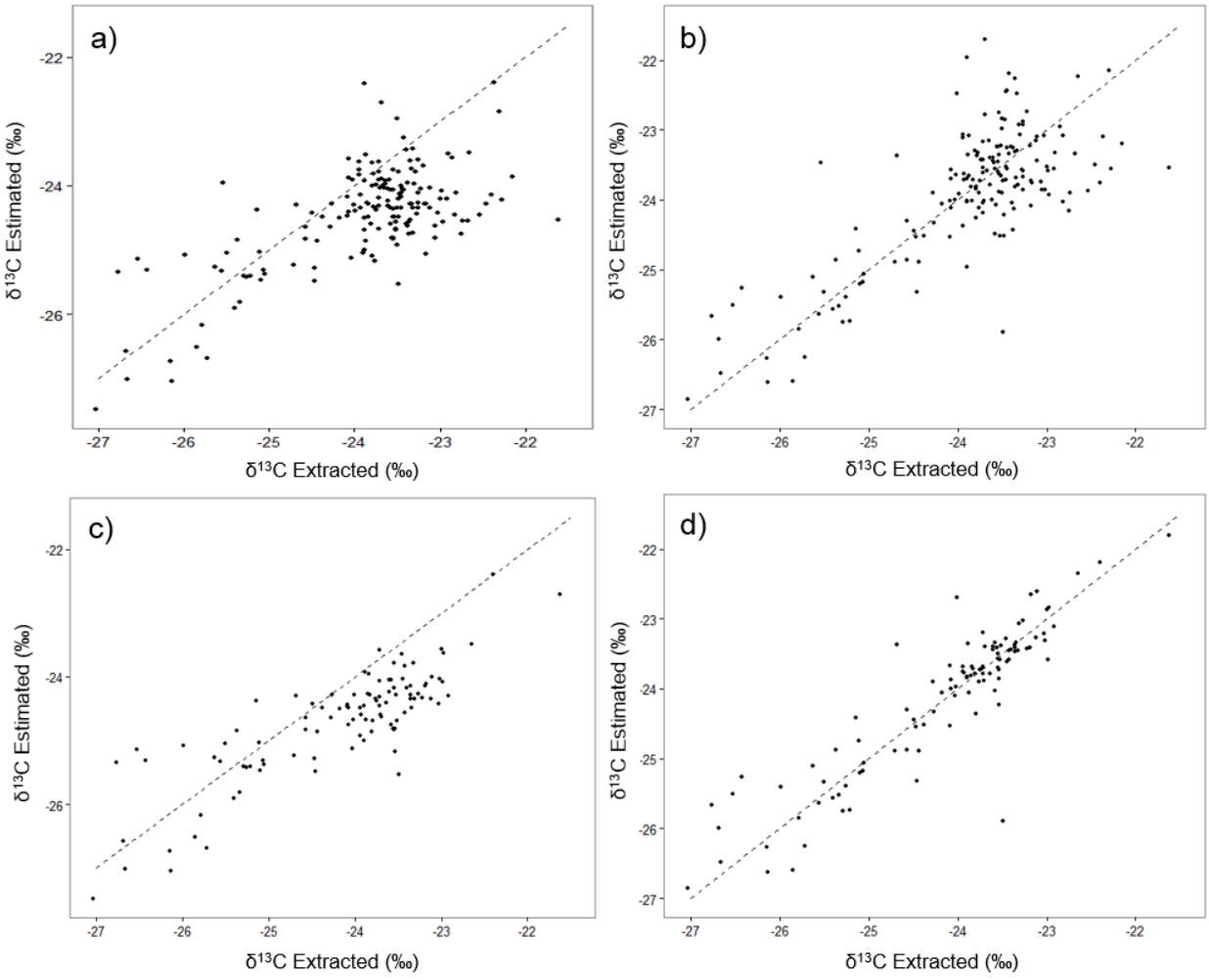

Figure A-1 Measured vs estimated lipid-corrected $\delta^{13}C$ values. Modelled values were plotted against measured values for a) Model 1, b) Model 2, c) Model 3, and d) Model 4. See text for a description model parameterization. The dotted line represents the 1:1 line. Model 4 was selected as the model to correct $\delta^{13}C$ values throughout the thesis.

A.2 Supplementary Figures and Tables

Table A-1. Model error summaries. Residual error was calculated as the average absolute difference between $\delta^{13}C_{extracted}$ and $\delta^{13}C_{estimated}$ (±1 S.D). Bias was calculated as the average difference between $\delta^{13}C_{extracted}$ and $\delta^{13}C_{estimated}$

Model	Residual Error (SD)	95% CI	Range of Residuals	RSS	Mean Bias	n
1	0.71(0.42)	0.65-1.36	0.01-2.04	117.66	0.59	174
2	0.32(0.35)	0.26-0.37	0.01-2.39	38.48	0.02	174
3	0.68(0.41)	0.61-0.76	0.01-2.04	69.37	0.53	106
4	0.30(0.35)	0.23-0.37	0.00-2.39	22.16	-0.02	106

Table A-2. Parameter estimates for Model 2 and Model 4. Model 4 averages were calculated from only those fish with $C:N_{bulk}$ ratios >4.0.

Morph	Model 2			Model 4		
Superior Shoal	*n*	$\Delta\delta^{13}C_{bulk}$	$C:N_{protein}$	*n*	$\Delta\delta^{13}C_{bulk}$	$C:N_{protein}$
Lean	13	-6.84	3.18	6	-7.08	3.19
Siscowet	15	-6.28	3.13	7	-7.33	3.17
Humper	15	-6.94	3.17	10	-7.57	3.21
Redfin	30	-4.11	3.15	10	-6.11	3.19
Kiyi	8	-6.63	3.60	8	-6.63	3.60
Deepwater Sculpin	8	-8.83	3.52	8	-8.83	3.52
Stannard Rock						
Lean	15	-9.69	3.09	3	-8.29	3.13
Siscowet	15	-8.70	3.17	11	-8.43	3.19
Humper	13	-8.70	3.13	11	-8.41	3.14
Kiyi	8	-6.60	3.89	8	-6.60	3.89
Deepwater Sculpin	8	-7.89	3.34	2	-6.75	3.37
Bloater	6	-7.20	3.78	6	-7.20	3.78
Cisco	8	-8.57	3.43	8	-8.57	3.43
Pygmy Whitefish	8	-7.18	3.46	8	-7.18	3.46

Table A-3. Gill net sets performed in Lake Superior, 2013-2014. Lake Trout were captured between August 7-11 in 2013 and August 4-5 in 2014. Superior Shoal was not netted during the 2014 effort.

Site	Set	Year	Start Latitude (N)	Start Longitude (W)	Min Depth (m)	Max Depth (m)	Duration
Stannard Rock	LSSR13-11	2013	47 10.852	87 14.921	26	32	13 h 47 min
Stannard Rock	LSSR13-12	2013	47 11.340	87 15.145	116	136	13 h 58 min
Stannard Rock	LSSR13-13	2013	47 12.057	87 14.002	59	76	14h 25 min
Stannard Rock	LSSR13-14	2013	47 10.417	87 13.293	22	44	20 h 58 min
Stannard Rock	LSSR13-15	2013	47 10.713	87 14.293	35	42	21 h 12min
Stannard Rock	LSSR13-16	2013	47 10.187	87 11.634	124	129	21 h 20 min
Stannard Rock	LSSR13-17	2013	47 11.073	87 11.636	119	132	21 h 45 min
Superior Shoal	LSSS13-1	2013	48 01.772	87 07.205	123	144	15 h 10 min
Superior Shoal	LSSS13-10	2013	48 05.742	87 07.484	69	83	18h 29 min
Superior Shoal	LSSS13-2	2013	48 02.201	87 06.321	47	66	15 h 27 min
Superior Shoal	LSSS13-3	2013	48 02.460	87 05.919	32	35	16 h
Superior Shoal	LSSS13-4	2013	48 02.949	87 07.973	129	148	15 h 50 min
Superior Shoal	LSSS13-5	2013	48 03.134	87 07.227	41	77	16 h 14 min
Superior Shoal	LSSS13-6	2013	48 03.223	87 07 050	18	41	17 h 48 min
Superior Shoal	LSSS13-7	2013	48 03.582	87 12.344	104	142	16 h 52 min
Superior Shoal	LSSS13-8	2013	48 03.582	87 13.920	19	35	17 h 27 min
Superior Shoal	LSSS13-9	2013	48 03.113	87 11.729	136	155	18 h 17 min
Stannard Rock	300	2014	47 14.114	87 08.383	113	124	17 h 5 min
Stannard Rock	301	2014	47 14.720	87 08.813	76	88	17 h 45 min
Stannard Rock	302	2014	47 12.081	87 08.813	59	76	18 h 30 min
Stannard Rock	303	2014	47 11.656	87 13.983	33	36	18 h 40 min
Stannard Rock	304	2014	47 10.875	87 14.922	28	33	19 h 30 min
Stannard Rock	305	2014	47 11.354	87 15.131	117	137	18 h 25 min
Stannard Rock	306	2014	47 13.961	87 08.049	135	145	23 h
Stannard Rock	307	2014	47 14.166	87 09.566	50	82	22 h 45 min
Stannard Rock	308	2014	47 14.166	87 09.566	28	32	19 h 30 min
Stannard Rock	309	2014	47 10.901	87 14.890	29	31	19 h 20 min
Stannard Rock	310	2014	47 11.183	87 14.715	55	86	19 h 50 min
Stannard Rock	311	2014	Not Listed	Not Listed	61	94	19 h 45 min

Table A-4. Trawling efforts in Lake Superior, 2013 and 2015. Prey fishes were collected between August 9-10 in 2013, and June 4-6, July 12 in 2015. Trawling was not performed in 2014. Superior Shoal was only trawled in 2013.

Site	Location	Year	Start Latitude (N)	Start Longitude (W)	Start Depth (m)	End Depth (m)	Duration (min)
Superior Shoal	LSSS13-11	2013	48 04.719	87 18.035	232	228	20
Stannard Rock	055	2013	47 09.783	87 13.795	88	98	20
Stannard Rock	Not Listed	2013	47 09.453	87 11.311	120	150	20
Stannard Rock	82	2015	46 58.743	88 23.734	17	67	20
Stannard Rock	84	2015	46 53.546	88 19.247	119	140	20
Stannard Rock	101	2015	47 22.812	87 48.651	20	36	20
Stannard Rock	158	2015	46 56.292	88 08.284	15	54	20
Stannard Rock	142	2015	46 51.161	87 43.754	18	62	20
Stannard Rock	196	2015	46 46.664	87 33.662	29	72	20
Stannard Rock	88	2015	46 31.351	86 55.428	29	85	20
Stannard Rock	209	2015	46 31.661	86 42.921	30	95	20
Stannard Rock	2150	2015	47 08.492	87 23.349	136	132	20
Stannard Rock	2154	2015	47 04.342	87 09.927	182	182	20

Table A-5. Relative fatty acid concentrations for species at A) Superior Shoal and B) Stannard Rock. Fatty acids are measured as percentages ± 1 standard error. Species codes are as follows: Hump=Humper, Lean=Lean, Sis=Siscowet, DPSC=Deepwater Sculpin, Kiyi=Kiyi, Bloater=Bloater, Cisc=Cisco, RNSM=Rainbow Smelt, PGWH=Pygmy Whitefish Mys=*Mysis*, Dip=*Diporeia*, Zoo1= Zooplankton 63-250 µm, Zoo2=Zooplankton 250-500 µm, Clam=Clam, Snail=Snails, Moth=Moths

2.10a. Superior Shoal Fatty Acids

Fatty Acid	Lean	Sis	Hump	Red	DPSC	Kiyi	Mys	Zoop1	Zoop2
12:0	0.099 ± 0.013	0.113 ± 0.010	0.141 ± 0.012	0.105 ± 0.009	0.175 ± 0.010	0.297 ± 0.012	0.101 ± 0.001	0.051	0.051
12:1	0.125 ± 0.029	0.024 ± 0.008	0.053 ± 0.020	0.010 ± 0.001	0.031 ± 0.002	0.033 ± 0.001	0.000	0.026	0.032
13:1	0.046 ± 0.004	0.054 ± 0.004	0.057 ± 0.004	0.050 ± 0.004	0.085 ± 0.005	0.105 ± 0.005	0.044 ± 0.002	0.067	0.066
14:0	2.834 ± 0.240	3.196 ± 0.206	3.263 ± 0.217	2.533 ± 0.246	5.403 ± 0.211	6.586 ± 0.251	1.935 ± 0.015	2.302	2.054
14:1n9	0.038 ± 0.003	0.029 ± 0.002	0.035 ± 0.003	0.021 ± 0.002	0.048 ± 0.002	0.049 ± 0.002	0.022	0.104	0.098
14:1n7	0.110 ± 0.013	0.060 ± 0.004	0.069 ± 0.009	0.039 ± 0.002	0.060 ± 0.002	0.054 ± 0.003	0.024 ± 0.002	0.054	0.051
14:1n5	0.066 ± 0.006	0.076 ± 0.006	0.085 ± 0.007	0.055 ± 0.006	0.157 ± 0.017	0.126 ± 0.003	0.047	0.033	0.034
14:0 iso	0.268 ± 0.024	0.318 ± 0.021	0.319 ± 0.026	0.247 ± 0.022	0.622 ± 0.023	0.598 ± 0.020	0.175 ± 0.002	0.197	0.200
14:0 ante	0.122 ± 0.011	0.143 ± 0.010	0.146 ± 0.009	0.108 ± 0.011	0.238 ± 0.011	0.257 ± 0.008	0.073	0.100	0.094
15:0	0.336 ± 0.015	0.371 ± 0.013	0.384 ± 0.020	0.329 ± 0.015	0.617 ± 0.025	0.656 ± 0.020	0.383	4.761	5.040
15:1n:8	0.007 ± 0.002	0.007 ± 0.001	0.006 ± 0.001	0.003 ± 0.001	0.011 ± 0.001	0.009 ± 0.003	0.000	0.000	0.000
15:1n6	0.061 ± 0.006	0.037 ± 0.006	0.029 ± 0.003	0.025 ± 0.003	0.042 ± 0.004	0.070 ± 0.009	0.015 ± 0.003	0.000	0.000
15:0 iso	0.204 ± 0.032	0.199 ± 0.028	0.210 ± 0.038	0.183 ± 0.022	0.332 ± 0.059	0.528 ± 0.036	0.122 ± 0.002	0.308	0.356
16:0	15.900 ± 0.438	15.849 ± 0.492	16.350 ± 0.638	16.289 ± 0.483	18.468 ± 0.694	23.167 ± 0.386	17.998 ± 0.025	6.466	6.213
16:1n11	0.220 ± 0.014	0.254 ± 0.019	0.273 ± 0.030	0.197 ± 0.010	0.364 ± 0.016	0.377 ± 0.032	0.224 ± 0.009	0.478	0.548
16:1n9	0.511 ± 0.028	0.575 ± 0.026	0.619 ± 0.041	0.531 ± 0.043	0.939 ± 0.031	0.706 ± 0.022	0.242 ± 0.001	0.454	0.435
16:1n7	6.230 ± 0.495	7.938 ± 0.546	8.101 ± 0.584	5.733 ± 0.542	10.617 ± 0.351	11.581 ± 0.290	4.233 ± 0.022	3.803	3.986
16:1n5	0.256 ± 0.013	0.287 ± 0.010	0.343 ± 0.028	0.244 ± 0.011	0.764 ± 0.030	0.416 ± 0.011	0.260 ± 0.003	0.320	0.305
16:2n6	0.262 ± 0.012	0.296 ± 0.011	0.334 ± 0.021	0.283 ± 0.012	0.492 ± 0.026	0.469 ± 0.012	0.363 ± 0.010	0.153	0.128
16:0 iso	0.034 ± 0.003	0.036 ± 0.002	0.043 ± 0.003	0.033 ± 0.002	0.096 ± 0.007	0.031 ± 0.001	0.021 ± 0.002	0.038	0.059
7Me 16:0	0.109 ± 0.006	0.116 ± 0.006	0.129 ± 0.006	0.098 ± 0.005	0.255 ± 0.008	0.144 ± 0.007	0.093 ± 0.001	0.230	0.238
16:2n4	0.167 ± 0.016	0.215 ± 0.019	0.215 ± 0.015	0.167 ± 0.016	0.411 ± 0.029	0.170 ± 0.013	0.271 ± 0.011	0.208	0.247

17:0	0.257 ± 0.010	0.259 ± 0.008	0.271 ± 0.013	0.291 ± 0.015	0.332 ± 0.016	0.416 ± 0.012	0.364 ± 0.004	10.638	11.468
16:3n4	0.076 ± 0.009	0.107 ± 0.010	0.094 ± 0.014	0.064 ± 0.007	0.162 ± 0.016	0.151 ± 0.016	0.227 ± 0.006	0.130	0.179
17:1	0.259 ± 0.016	0.306 ± 0.016	0.298 ± 0.017	0.244 ± 0.018	0.501 ± 0.008	0.403 ± 0.011	0.132 ± 0.001	0.124	0.150
16:4n3	0.029 ± 0.003	0.030 ± 0.002	0.056 ± 0.019	0.026 ± 0.001	0.062 ± 0.002	0.038 ± 0.002	0.024 ± 0.001	0.106	0.120
17:0 iso	0.206 ± 0.009	0.218 ± 0.008	0.201 ± 0.008	0.209 ± 0.008	0.227 ± 0.008	0.321 ± 0.008	0.201 ± 0.002	0.062	0.074
16:4n1	0.040 ± 0.011	0.033 ± 0.003	0.030 ± 0.003	0.028 ± 0.003	0.061 ± 0.003	0.057 ± 0.005	0.077 ± 0.002	0.071	0.097
18:0	3.137 ± 0.154	2.817 ± 0.143	2.999 ± 0.174	3.450 ± 0.171	2.627 ± 0.139	3.608 ± 0.087	1.703 ± 0.031	1.012	0.977
18:1n9	20.461 ± 1.412	21.080 ± 1.602	20.557 ± 1.408	19.389 ± 1.544	21.758 ± 0.393	30.950 ± 0.458	14.235 ± 0.011	5.565	6.064
18:1n7	3.917 ± 0.242	4.344 ± 0.239	4.529 ± 0.312	3.739 ± 0.244	6.715 ± 0.153	5.492 ± 0.119	2.900 ± 0.005	2.981	3.082
18:1n5	0.228 ± 0.015	0.255 ± 0.015	0.290 ± 0.028	0.215 ± 0.013	0.535 ± 0.023	0.319 ± 0.008	0.213 ± 0.001	0.112	0.119
18:2d5, 11	0.070 ± 0.002	0.070 ± 0.002	0.087 ± 0.006	0.071 ± 0.003	0.107 ± 0.007	0.102 ± 0.004	0.048 ± 0.011	0.017	0.019
18:2n7	0.037 ± 0.003	0.048 ± 0.003	0.049 ± 0.002	0.042 ± 0.002	0.045 ± 0.002	0.035 ± 0.002	0.034 ± 0.004	0.066	0.077
18:2n6	2.687 ± 0.167	3.062 ± 0.177	3.129 ± 0.131	2.603 ± 0.161	3.568 ± 0.211	1.987 ± 0.127	3.242 ± 0.009	3.912	3.952
18:2n4	0.130 ± 0.009	0.143 ± 0.010	0.146 ± 0.007	0.116 ± 0.008	0.203 ± 0.011	0.096 ± 0.008	0.134 ± 0.007	0.293	0.323
18:3n6	0.116 ± 0.010	0.135 ± 0.010	0.136 ± 0.008	0.107 ± 0.010	0.212 ± 0.017	0.057 ± 0.009	0.185 ± 0.003	0.311	0.290
18:3n4	0.144 ± 0.008	0.172 ± 0.009	0.161 ± 0.005	0.144 ± 0.009	0.155 ± 0.003	0.158 ± 0.006	0.133 ± 0.002	0.228	0.234
18:3n3	1.533 ± 0.120	1.696 ± 0.126	1.769 ± 0.106	1.361 ± 0.108	1.785 ± 0.155	0.564 ± 0.082	1.418 ± 0.007	3.424	3.414
18:3n1	0.031 ± 0.003	0.031 ± 0.003	0.039 ± 0.004	0.028 ± 0.002	0.062 ± 0.004	0.047 ± 0.004	0.028 ± 0.003	0.314	0.340
18:4n3	0.631 ± 0.059	0.720 ± 0.055	0.770 ± 0.055	0.554 ± 0.053	0.828 ± 0.070	0.233 ± 0.035	0.615	3.456	3.273
18:4n1	0.022 ± 0.007	0.018 ± 0.005	0.018 ± 0.005	0.017 ± 0.005	0.033 ± 0.005	0.060 ± 0.005	0.000	0.000	0.062
20:0	0.092 ± 0.005	0.098 ± 0.005	0.116 ± 0.011	0.106 ± 0.011	0.130 ± 0.003	0.218 ± 0.006	0.138 ± 0.005	0.140	0.120
20:1n11	0.096 ± 0.010	0.102 ± 0.007	0.095 ± 0.005	0.093 ± 0.009	0.126 ± 0.008	0.160 ± 0.001	0.043	0.000	0.000
20:1n9	1.406 ± 0.107	1.592 ± 0.087	1.517 ± 0.093	1.347 ± 0.107	1.074 ± 0.032	2.080 ± 0.072	1.473 ± 0.013	1.055	1.098
20:1n7	0.340 ± 0.025	0.389 ± 0.024	0.365 ± 0.019	0.331 ± 0.023	0.321 ± 0.010	0.491 ± 0.013	0.235 ± 0.008	0.278	0.316
20:2 NMI D1	0.015 ± 0.003	0.023 ± 0.005	0.028 ± 0.010	0.016 ± 0.003	0.049 ± 0.002	0.055 ± 0.002	0.012 ± 0.012	0.000	0.000
20:2n9	0.036 ± 0.006	0.046 ± 0.010	0.066 ± 0.032	0.037 ± 0.004	0.065 ± 0.009	0.092 ± 0.005	0.029	0.040	0.045
C20:2 NMI D2	0.022 ± 0.003	0.030 ± 0.005	0.029 ± 0.004	0.041 ± 0.007	0.124 ± 0.065	0.109 ± 0.008	0.018 ± 0.018	0.037	0.000
20:2n6	1.949 ± 0.252	2.113 ± 0.125	2.165 ± 0.172	1.865 ± 0.113	0.660 ± 0.036	0.629 ± 0.058	2.149 ± 0.233	1.863	1.980

20:3 NMI T	0.002 ± 0.001	0.003 ± 0.001	0.003 ± 0.001	0.005 ± 0.002	0.000	0.000	0.057 ± 0.007	0.000	0.000
20:3n6	0.331 ± 0.022	0.356 ± 0.026	0.328 ± 0.017	0.328 ± 0.020	0.228 ± 0.022	0.135 ± 0.023	0.169 ± 0.013	0.272	0.274
20:4n6	3.510 ± 0.306	3.001 ± 0.294	3.034 ± 0.201	3.772 ± 0.289	2.836 ± 0.279	0.466 ± 0.094	4.621 ± 0.033	2.736	1.983
20:3n3	0.612 ± 0.042	0.652 ± 0.046	0.650 ± 0.036	0.563 ± 0.035	0.394 ± 0.030	0.186 ± 0.032	0.819 ± 0.013	2.940	2.475
20:4n3	1.423 ± 0.107	1.544 ± 0.125	1.422 ± 0.089	1.276 ± 0.097	0.676 ± 0.062	0.220 ± 0.053	0.883 ± 0.010	3.664	3.498
20:5n3	5.307 ± 0.432	4.815 ± 0.482	4.781 ± 0.332	5.576 ± 0.461	5.800 ± 0.557	0.647 ± 0.150	16.492 ± 0.099	9.530	9.847
22:0	0.058 ± 0.008	0.043 ± 0.003	0.075 ± 0.016	0.071 ± 0.013	0.050 ± 0.007	0.117 ± 0.009	0.112 ± 0.016	0.202	0.353
22:1n11	0.039 ± 0.012	0.057 ± 0.018	0.051 ± 0.014	0.040 ± 0.013	0.013 ± 0.005	0.094 ± 0.027	0.000	0.000	0.000
22:1n9	0.237 ± 0.016	0.277 ± 0.014	0.261 ± 0.018	0.232 ± 0.018	0.288 ± 0.014	0.384 ± 0.024	0.387 ± 0.008	0.242	0.250
22:1n7	0.045 ± 0.005	0.063 ± 0.004	0.062 ± 0.012	0.048 ± 0.005	0.076 ± 0.004	0.117 ± 0.005	0.132 ± 0.005	0.236	0.218
22:2 NMI D1	0.006 ± 0.003	0.002 ± 0.001	0.013 ± 0.008	0.004 ± 0.002	0.000	0.002 ± 0.001	0.000	0.000	0.000
22:2 NMI D2	0.029 ± 0.004	0.030 ± 0.003	0.040 ± 0.003	0.017 ± 0.003	0.020 ± 0.005	0.013 ± 0.004	0.000	0.000	0.000
22:2n6	0.139 ± 0.011	0.168 ± 0.015	0.136 ± 0.009	0.126 ± 0.010	0.073 ± 0.005	0.114 ± 0.011	0.061 ± 0.007	0.736	0.786
21:5n3	0.131 ± 0.010	0.137 ± 0.011	0.132 ± 0.009	0.125 ± 0.009	0.080 ± 0.009	0.005 ± 0.005	0.102 ± 0.002	0.293	0.291
22:3n3	0.550 ± 0.037	0.540 ± 0.044	0.499 ± 0.030	0.561 ± 0.035	0.126 ± 0.009	0.084 ± 0.016	0.132 ± 0.002	0.753	0.758
22:5n6	1.594 ± 0.126	1.397 ± 0.116	1.292 ± 0.105	1.714 ± 0.129	0.620 ± 0.051	0.157 ± 0.037	1.344 ± 0.016	2.928	2.801
22:4n3	0.340 ± 0.034	0.428 ± 0.048	0.340 ± 0.024	0.309 ± 0.032	0.105 ± 0.014	0.054 ± 0.020	0.205 ± 0.013	3.966	3.817
22:5n3	2.938 ± 0.196	2.947 ± 0.233	2.639 ± 0.178	3.044 ± 0.185	0.518 ± 0.036	0.215 ± 0.057	0.507 ± 0.016	2.304	2.253
22:6n3	16.296 ± 1.760	12.727 ± 1.521	12.579 ± 1.382	17.840 ± 1.975	4.671 ± 0.382	0.909 ± 0.193	16.961 ± 0.173	11.157	10.594
24:1n9	0.433 ± 0.033	0.676 ± 0.233	0.437 ± 0.024	0.537 ± 0.033	0.673 ± 0.053	0.724 ± 0.040	0.358 ± 0.009	1.655	1.695

2.10b. Stannard Rock Fatty Acids

Fatty Acid	Lean	Sis	Hump	Bloater	Kiyi	DPSC	PGWH	Cisc	Mys	Zoop1	Zoop2	Moth	RNSM
12:0	0.115 ± 0.009	0.126 ± 0.010	0.159 ± 0.009	0.230 ± 0.016	0.277 ± 0.012	0.141 ± 0.014	0.079 ± 0.007	0.099 ± 0.026	0.163 ± 0.007	0.039	0.045	0.150 ± 0.002	0.068 ± 0.030
12:1	0.010 ± 0.001	0.014 ± 0.001	0.016 ± 0.001	0.024 ± 0.002	0.027 ± 0.001	0.030 ± 0.004	0.031 ± 0.005	0.027 ± 0.003	0.011 ± 0.002	0.018 ± 0.001	0.028	0.000	0.006 ± 0.006
13:1	0.062 ± 0.002	0.063 ± 0.003	0.075 ± 0.003	0.086 ± 0.008	0.083 ± 0.004	0.084 ± 0.011	0.075 ± 0.009	0.095 ± 0.013	0.042 ± 0.006	0.070	0.068	0.015 ± 0.001	0.036 ± 0.011
14:0	2.851 ± 0.138	3.150 ± 0.122	3.323 ± 0.111	5.914 ± 0.580	5.451 ± 0.276	3.559 ± 0.277	4.692 ± 0.275	6.348 ± 0.474	2.477 ± 0.331	3.026 ± 0.445	3.999	0.973 ± 0.007	2.622 ± 0.451
14:1n9	0.025 ± 0.002	0.028 ± 0.002	0.032 ± 0.001	0.043 ± 0.001	0.047 ± 0.002	0.047 ± 0.004	0.021 ± 0.002	0.031 ± 0.004	0.036 ± 0.008	0.066 ± 0.008	0.049	0.028	0.026 ± 0.008

14:1n7	0.042 ± 0.001	0.047 ± 0.003	0.046 ± 0.001	0.036 ± 0.003	0.040 ± 0.004	0.072 ± 0.008	0.042 ± 0.003	0.050 ± 0.005	0.031 ± 0.007	0.043 ± 0.002	0.020	0.014 ± 0.001	0.023 ± 0.004
14:1n5	0.066 ± 0.004	0.083 ± 0.003	0.077 ± 0.003	0.098 ± 0.004	0.101 ± 0.005	0.118 ± 0.017	0.085 ± 0.008	0.075 ± 0.008	0.051 ± 0.013	0.029	0.043	0.034 ± 0.002	0.048 ± 0.009
14:0 iso	0.227 ± 0.024	0.306 ± 0.024	0.344 ± 0.021	0.569 ± 0.061	0.504 ± 0.019	0.638 ± 0.063	0.555 ± 0.031	0.752 ± 0.077	0.214 ± 0.013	0.237 ± 0.030	0.356	0.023 ± 0.002	0.259 ± 0.026
14:0 ante	0.123 ± 0.007	0.150 ± 0.007	0.159 ± 0.006	0.239 ± 0.028	0.216 ± 0.009	0.239 ± 0.026	0.270 ± 0.020	0.329 ± 0.033	0.101 ± 0.12	0.135 ± 0.016	0.199	0.055 ± 0.002	0.067 ± 0.008
15:0	0.319 ± 0.012	0.352 ± 0.015	0.385 ± 0.011	0.594 ± 0.053	0.545 ± 0.020	0.511 ± 0.024	0.649 ± 0.037	0.826 ± 0.072	0.323 ± 0.062	3.856 ± 0.822	1.875	0.163 ± 0.001	0.379 ± 0.019
15:1n:8	0.003 ± 0.001	0.008 ± 0.003	0.006 ± 0.001	0.006 ± 0.003	0.003 ± 0.002	0.004 ± 0.001	0.008 ± 0.002	0.013 ± 0.004	0.005 ± 0.005	0.000	0.000	0.007 ± 0.004	0.004 ± 0.004
15:1n6	0.016 ± 0.002	0.019 ± 0.005	0.016 ± 0.001	0.030 ± 0.007	0.041 ± 0.010	0.031 ± 0.003	0.053 ± 0.005	0.053 ± 0.009	0.014 ± 0.002	0.007 ± 0.007	0.014	0.009 ± 0.002	0.004 ± 0.004
15:0 iso	0.109 ± 0.008	0.159 ± 0.017	0.164 ± 0.007	0.315 ± 0.051	0.282 ± 0.038	0.258 ± 0.026	0.415 ± 0.046	0.552 ± 0.084	0.147 ± 0.014	0.323 ± 0.078	0.199	0.053 ± 0.001	0.138 ± 0.005
16:0	14.910 ± 0.302	14.414 ± 0.441	14.906 ± 0.338	20.156 ± 1.345	19.071 ± 0.685	17.847 ± 0.394	26.438 ± 1.301	26.966 ± 2.553	14.297 ± 3.481	9.251 ± 1.844	13.570	31.814 ± 0.043	18.994 ± 0.208
16:1n11	0.212 ± 0.011	0.215 ± 0.011	0.233 ± 0.010	0.335 ± 0.042	0.309 ± 0.017	0.373 ± 0.017	0.508 ± 0.042	0.552 ± 0.060	0.224 ± 0.017	0.388 ± 0.020	0.187	0.185 ± 0.010	0.366 ± 0.011
16:1n9	0.532 ± 0.021	0.638 ± 0.024	0.593 ± 0.024	0.436 ± 0.013	0.498 ± 0.027	0.934 ± 0.017	0.512 ± 0.025	0.620 ± 0.045	0.305 ± 0.042	0.341 ± 0.056	0.337	0.401 ± 0.006	0.385 ± 0.038
16:1n7	7.643 ± 0.354	9.023 ± 0.204	7.957 ± 0.310	9.805 ± 0.236	10.555 ± 0.382	11.248 ± 0.885	9.579 ± 0.497	7.845 ± 0.774	7.757 ± 1.208	3.726 ± 0.073	4.386	2.615 ± 0.033	5.638 ± 0.704
16:1n5	0.297 ± 0.015	0.351 ± 0.019	0.335 ± 0.014	0.412 ± 0.025	0.361 ± 0.028	0.864 ± 0.062	0.582 ± 0.059	0.538 ± 0.044	0.359 ± 0.045	0.328 ± 0.016	0.425	0.142 ± 0.002	0.371 ± 0.021
16:2n6	0.267 ± 0.011	0.325 ± 0.013	0.342 ± 0.009	0.396 ± 0.027	0.371 ± 0.016	0.529 ± 0.031	0.560 ± 0.037	0.740 ± 0.081	0.280 ± 0.062	0.240 ± 0.021	0.329	0.031 ± 0.004	0.386 ± 0.029
16:0 iso	0.039 ± 0.002	0.051 ± 0.003	0.049 ± 0.003	0.036 ± 0.003	0.040 ± 0.002	0.092 ± 0.004	0.032 ± 0.002	0.033 ± 0.003	0.039 ± 0.010	0.031 ± 0.004	0.022	0.043	0.030 ± 0.003
7Me 16:0	0.125 ± 0.006	0.143 ± 0.006	0.131 ± 0.006	0.209 ± 0.008	0.204 ± 0.008	0.319 ± 0.010	0.199 ± 0.012	0.322 ± 0.027	0.137 ± 0.021	0.160 ± 0.029	0.153	0.019 ± 0.002	0.294 ± 0.022
16:2n4	0.276 ± 0.018	0.304 ± 0.013	0.322 ± 0.016	0.380 ± 0.029	0.394 ± 0.029	0.433 ± 0.038	0.194 ± 0.031	0.199 ± 0.035	0.456 ± 0.040	0.188 ± 0.009	0.226	0.030 ± 0.002	0.398 ± 0.054
17:0	0.221 ± 0.008	0.232 ± 0.010	0.271 ± 0.007	0.329 ± 0.028	0.297 ± 0.014	0.353 ± 0.011	0.515 ± 0.035	0.666 ± 0.064	0.243 ± 0.065	8.406 ± 1.979	3.945	0.203 ± 0.004	0.251 ± 0.013
16:3n4	0.111 ± 0.009	0.119 ± 0.006	0.123 ± 0.007	0.214 ± 0.018	0.167 ± 0.013	0.150 ± 0.011	0.198 ± 0.019	0.174 ± 0.019	0.246 ± 0.026	0.104 ± 0.003	0.152	0.022 ± 0.002	0.204 ± 0.024
17:1	0.268 ± 0.010	0.334 ± 0.011	0.313 ± 0.011	0.300 ± 0.008	0.329 ± 0.008	0.420 ± 0.023	0.356 ± 0.013	0.264 ± 0.013	0.189 ± 0.028	0.119 ± 0.015	0.122	0.115 ± 0.001	0.203 ± 0.016
16:4n3	0.026 ± 0.001	0.033 ± 0.002	0.031 ± 0.001	0.032 ± 0.002	0.034 ± 0.002	0.072 ± 0.005	0.057 ± 0.005	0.049 ± 0.004	0.041 ± 0.009	0.087 ± 0.022	0.039	0.000	0.034 ± 0.001
17:0 iso	0.165 ± 0.006	0.181 ± 0.008	0.196 ± 0.005	0.249 ± 0.019	0.233 ± 0.011	0.196 ± 0.006	0.297 ± 0.022	0.423 ± 0.043	0.189 ± 0.017	0.124 ± 0.026	0.212	0.073 ± 0.004	0.162 ± 0.007
16:4n1	0.043 ± 0.005	0.042 ± 0.005	0.050 ± 0.004	0.107 ± 0.014	0.097 ± 0.011	0.062 ± 0.006	0.070 ± 0.013	0.194 ± 0.029	0.205 ± 0.006	0.049 ± 0.003	0.079	0.000	0.119 ± 0.015
18:0	2.826 ± 0.084	2.675 ± 0.108	2.679 ± 0.099	2.972 ± 0.237	2.726 ± 0.132	3.611 ± 0.193	4.405 ± 0.231	5.168 ± 0.381	1.191 ± 0.355	1.643 ± 0.328	2.336	2.695 ± 0.044	2.493 ± 0.019
18:1n9	20.729 ± 0.799	22.927 ± 0.683	20.650 ± 0.986	21.766 ± 0.616	25.360 ± 1.049	18.722 ± 0.618	19.336 ± 0.548	15.881 ± 1.567	13.333 ± 1.202	5.654 ± 0.189	5.952	24.898 ± 12.218	9.037 ± 1.536
18:1n7	3.989 ± 0.096	4.694 ± 0.130	4.307 ± 0.120	4.938 ± 0.303	4.773 ± 0.175	5.763 ± 0.149	6.676 ± 0.294	5.564 ± 0.273	3.002 ± 0.201	2.936 ± 0.005	3.169	0.441 ± 0.051	3.011 ± 0.073
18:1n5	0.252 ± 0.012	0.337 ± 0.018	0.320 ± 0.020	0.309 ± 0.022	0.280 ± 0.007	0.591 ± 0.040	0.436 ± 0.024	0.331 ± 0.019	0.186 ± 0.026	0.122 ± 0.015	0.155	0.040 ± 0.002	0.160 ± 0.011
18:2d5, 11	0.069 ± 0.003	0.084 ± 0.004	0.082 ± 0.005	0.075 ± 0.006	0.080 ± 0.006	0.128 ± 0.008	0.102 ± 0.008	0.097 ± 0.007	0.033 ± 0.017	0.017 ± 0.001	0.000	0.076 ± 0.002	0.047 ± 0.005

18:2n7	0.045 ± 0.001	0.050 ± 0.001	0.047 ± 0.001	0.045 ± 0.002	0.050 ± 0.003	0.048 ± 0.002	0.048 ± 0.003	0.066 ± 0.007	0.044 ± 0.001	0.060 ± 0.009	0.066	0.031 ± 0.003	0.051 ± 0.006
18:2n6	3.193 ± 0.129	3.596 ± 0.107	3.722 ± 0.099	3.029 ± 0.199	3.188 ± 0.175	3.652 ± 0.096	2.502 ± 0.154	3.348 ± 0.259	3.371 ± 0.499	3.861 ± 0.085	4.682	7.641 ± 0.062	3.621 ± 0.171
18:2n4	0.162 ± 0.004	0.182 ± 0.004	0.172 ± 0.005	0.202 ± 0.009	0.189 ± 0.013	0.142 ± 0.006	0.213 ± 0.016	0.202 ± 0.017	0.164 ± 0.012	0.232 ± 0.019	0.244	0.075 ± 0.005	0.174 ± 0.027
18:3n6	0.165 ± 0.009	0.182 ± 0.007	0.201 ± 0.008	0.192 ± 0.021	0.184 ± 0.021	0.207 ± 0.010	0.096 ± 0.014	0.100 ± 0.017	0.310 ± 0.046	0.284 ± 0.014	0.255	0.193 ± 0.002	0.200 ± 0.017
18:3n4	0.192 ± 0.007	0.215 ± 0.004	0.208 ± 0.005	0.209 ± 0.006	0.210 ± 0.007	0.163 ± 0.009	0.216 ± 0.010	0.223 ± 0.009	0.144 ± 0.019	0.164 ± 0.017	0.216	0.000	0.159 ± 0.028
18:3n3	1.786 ± 0.067	1.945 ± 0.078	2.046 ± 0.073	1.798 ± 0.184	1.707 ± 0.181	1.481 ± 0.065	0.990 ± 0.119	1.288 ± 0.167	2.312 ± 0.428	3.223 ± 0.107	4.012	8.329 ± 0.133	2.223 ± 0.143
18:3n1	0.041 ± 0.002	0.038 ± 0.002	0.037 ± 0.001	0.061 ± 0.006	0.065 ± 0.005	0.087 ± 0.006	0.073 ± 0.007	0.133 ± 0.013	0.034 ± 0.006	0.164 ± 0.061	0.055	0.303 ± 0.003	0.112 ± 0.011
18:4n3	0.828 ± 0.046	0.885 ± 0.050	1.009 ± 0.057	1.182 ± 0.128	1.024 ± 0.137	0.676 ± 0.048	0.583 ± 0.081	0.599 ± 0.096	1.568 ± 0.228	3.404 ± 0.187	4.157	0.179 ± 0.003	1.417 ± 0.043
18:4n1	0.018 ± 0.002	0.021 ± 0.003	0.017 ± 0.002	0.048 ± 0.003	0.049 ± 0.005	0.030 ± 0.005	0.040 ± 0.003	0.036 ± 0.004	0.012 ± 0.006	0.058 ± 0.021	0.089	0.000	0.030 ± 0.015
20:0	0.134 ± 0.013	0.166 ± 0.013	0.187 ± 0.014	0.215 ± 0.012	0.182 ± 0.006	0.143 ± 0.003	0.179 ± 0.015	0.387 ± 0.030	0.135 ± 0.016	0.176 ± 0.003	0.246	0.448 ± 0.004	0.126 ± 0.005
20:1n11	0.096 ± 0.007	0.097 ± 0.004	0.106 ± 0.005	0.119 ± 0.007	0.137 ± 0.009	0.135 ± 0.022	0.084 ± 0.010	0.120 ± 0.030	0.071 ± 0.020	0.000	0.000	0.218 ± 0.005	0.048 ± 0.027
20:1n9	1.355 ± 0.067	1.439 ± 0.054	1.517 ± 0.47	1.485 ± 0.052	1.819 ± 0.055	0.988 ± 0.035	0.869 ± 0.023	1.453 ± 0.121	1.299 ± 0.104	0.947 ± 0.126	0.790	0.060 ± 0.003	0.578 ± 0.117
20:1n7	0.334 ± 0.020	0.370 ± 0.012	0.393 ± 0.011	0.499 ± 0.048	0.452 ± 0.015	0.271 ± 0.013	0.471 ± 0.029	0.761 ± 0.080	0.266 ± 0.030	0.252 ± 0.018	0.279	0.052 ± 0.003	0.230 ± 0.026
20:2 NMI D1	0.014 ± 0.003	0.019 ± 0.004	0.017 ± 0.002	0.042 ± 0.002	0.039 ± 0.004	0.052 ± 0.003	0.039 ± 0.002	0.047 ± 0.007	0.000	0.000	0.000	0.065 ± 0.002	0.005 ± 0.005
20:2n9	0.033 ± 0.003	0.038 ± 0.006	0.035 ± 0.002	0.048 ± 0.007	0.045 ± 0.006	0.051 ± 0.003	0.080 ± 0.008	0.085 ± 0.008	0.027 ± 0.003	0.041 ± 0.011	0.064	0.000	0.034 ± 0.004
C20:2 NMI D2	0.032 ± 0.004	0.030 ± 0.003	0.027 ± 0.003	0.023 ± 0.008	0.036 ± 0.012	0.043 ± 0.010	0.072 ± 0.008	0.049 ± 0.005	0.000	0.021 ± 0.021	0.017	0.017 ± 0.008	0.000
20:2n6	2.042 ± 0.121	2.138 ± 0.152	2.401 ± 0.114	1.198 ± 0.131	1.164 ± 0.082	0.631 ± 0.020	0.644 ± 0.046	0.983 ± 0.063	1.817 ± 0.114	2.090 ± 0.298	1.343	0.165 ± 0.035	1.574 ± 0.502
20:3 NMI T	0.001 ± 0.001	0.002 ± 0.001	0.003 ± 0.001	0.006 ± 0.003	0.002 ± 0.002	0.000	0.019 ± 0.005	0.060 ± 0.013	0.031 ± 0.008	0.000	0.000	0.421 ± 0.009	0.012 ± 0.012
20:3n6	0.412 ± 0.015	0.401 ± 0.020	0.429 ± 0.011	0.261 ± 0.025	0.266 ± 0.024	0.500 ± 0.084	0.191 ± 0.025	0.258 ± 0.031	0.194 ± 0.004	0.257 ± 0.013	0.229	0.025 ± 0.025	0.268 ± 0.019
20:4n6	3.485 ± 0.122	3.145 ± 0.122	3.497 ± 0.104	1.620 ± 0.229	1.559 ± 0.187	4.064 ± 0.383	1.250 ± 0.213	0.999 ± 0.178	4.349 ± 0.140	2.151 ± 0.010	2.240	0.588 ± 0.019	3.591 ± 0.301
20:3n3	0.657 ± 0.021	0.700 ± 0.034	0.744 ± 0.033	0.649 ± 0.061	0.601 ± 0.065	0.310 ± 0.015	0.477 ± 0.077	0.504 ± 0.057	0.878 ± 0.005	2.059 ± 0.434	1.554	0.084 ± 0.002	0.831 ± 0.122
20:4n3	1.690 ± 0.068	1.755 ± 0.089	1.816 ± 0.066	1.353 ± 0.173	1.237 ± 0.170	0.505 ± 0.032	0.915 ± 0.182	0.942 ± 0.157	1.222 ± 0.033	3.346 ± 0.365	2.883	0.150 ± 0.006	1.856 ± 0.364
20:5n3	5.338 ± 0.226	5.022 ± 0.245	5.592 ± 0.178	4.154 ± 0.571	3.448 ± 0.495	6.581 ± 0.525	2.783 ± 0.493	2.002 ± 0.349	16.129 ± 0.403	10.033 ± 0.819	10.540	1.747 ± 0.042	9.268 ± 0.134
22:0	0.126 ± 0.023	0.134 ± 0.018	0.156 ± 0.022	0.125 ± 0.007	0.105 ± 0.004	0.083 ± 0.010	0.099 ± 0.008	0.226 ± 0.016	0.087 ± 0.003	0.248 ± 0.029	0.205	0.221 ± 0.024	0.127 ± 0.020
22:1n11	0.034 ± 0.004	0.038 ± 0.006	0.036 ± 0.008	0.027 ± 0.007	0.018 ± 0.008	0.020 ± 0.013	0.003 ± 0.003	0.024 ± 0.012	0.000	0.000	0.000	0.050 ± 0.025	0.000
22:1n9	0.228 ± 0.010	0.241 ± 0.011	0.257 ± 0.011	0.357 ± 0.023	0.386 ± 0.019	0.266 ± 0.015	0.251 ± 0.021	0.520 ± 0.035	0.217 ± 0.014	0.223 ± 0.006	0.258	0.045 ± 0.009	0.195 ± 0.008
22:1n7	0.051 ± 0.004	0.052 ± 0.004	0.060 ± 0.004	0.135 ± 0.014	0.114 ± 0.004	0.083 ± 0.005	0.145 ± 0.013	0.245 ± 0.030	0.092 ± 0.012	0.210 ± 0.018	0.231	0.014 ± 0.014	0.076 ± 0.016
22:2 NMI D1	0.011 ± 0.003	0.014 ± 0.003	0.017 ± 0.003	0.001 ± 0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

22:2 NMID2	0.030 ± 0.002	0.026 ± 0.002	0.033 ± 0.003	0.029 ± 0.006	0.027 ± 0.008	0.025 ± 0.004	0.000	0.009 ± 0.005	0.073 ± 0.041	0.000	0.000	0.000	0.000
22:2n6	0.167 ± 0.009	0.174 ± 0.010	0.187 ± 0.008	0.245 ± 0.031	0.188 ± 0.011	0.061 ± 0.003	0.144 ± 0.014	0.370 ± 0.053	0.082 ± 0.004	0.602 ± 0.128	0.586	0.000	0.148 ± 0.018
21:5n3	0.161 ± 0.005	0.166 ± 0.007	0.170 ± 0.006	0.121 ± 0.017	0.103 ± 0.016	0.071 ± 0.006	0.087 ± 0.019	0.050 ± 0.012	0.168 ± 0.010	0.287 ± 0.028	0.253	0.000	0.205 ± 0.007
22:3n3	0.637 ± 0.024	0.667 ± 0.034	0.645 ± 0.027	0.282 ± 0.035	0.272 ± 0.031	0.178 ± 0.019	0.137 ± 0.023	0.183 ± 0.038	0.173 ± 0.003	0.613 ± 0.116	0.330	0.026 ± 0.001	0.299 ± 0.005
22:5n6	1.422 ± 0.090	1.262 ± 0.052	1.390 ± 0.077	0.741 ± 0.137	0.393 ± 0.145	0.772 ± 0.078	0.249 ± 0.111	0.634 ± 0.207	1.473 ± 0.020	2.982 ± 0.075	2.555	0.000	2.702 ± 0.277
22:4n3	0.434 ± 0.029	0.450 ± 0.039	0.474 ± 0.027	0.461 ± 0.045	0.385 ± 0.052	0.071 ± 0.008	0.309 ± 0.052	0.498 ± 0.105	0.255 ± 0.008	3.322 ± 0.675	1.660	0.026 ± 0.002	0.728 ± 0.186
22:5n3	3.043 ± 0.073	2.984 ± 0.139	3.097 ± 0.076	1.282 ± 0.182	1.129 ± 0.156	0.673 ± 0.064	1.039 ± 0.210	0.826 ± 0.179	0.616 ± 0.046	1.886 ± 0.289	1.184	0.114 ± 0.002	1.780 ± 0.335
22:6n3	14.104 ± 1.183	9.232 ± 0.745	10.055 ± 0.954	5.084 ± 0.637	4.225 ± 0.570	7.481 ± 0.960	5.411 ± 1.002	4.004 ± 0.659	15.714 ± 0.478	13.466 ± 2.200	14.381	0.000	20.324 ± 1.337
24:1n9	0.422 ± 0.015	0.380 ± 0.019	0.423 ± 0.017	0.775 ± 0.095	0.715 ± 0.055	0.996 ± 0.076	0.844 ± 0.098	1.576 ± 0.150	0.348 ± 0.024	1.576 ± 0.007	1.705	0.065 ± 0.008	0.743 ± 0.041