EVALUATING METHODS TO DESCRIBE DIETARY PATTERNS OF LAKE MICHIGAN SALMONIDS

by

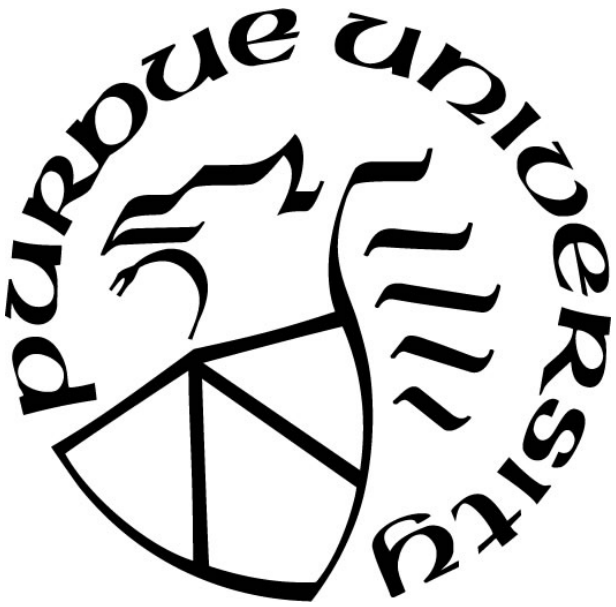
Benjamin Scott Leonhardt

A Thesis

Submitted to the Faculty of Purdue University

In Partial Fulfillment of the Requirements for the degree of

Master of Science



Department of Forestry & Natural Resources

West Lafayette, Indiana

December 2018

THE PURDUE UNIVERSITY GRADUATE SCHOOL
STATEMENT OF COMMITTEE APPROVAL

Dr. Tomas O. Höök, Chair

Department of Forestry & Natural Resources

Dr. Sergiusz J. Czesny

Illinois Natural History Survey

Dr. Paris D. Collingsworth

Department of Forestry & Natural Resources

Approved by:

Dr. Robert Wagner

Head of the Graduate Program

ACKNOWLEDGMENTS

I would like to thank my advisor, Tomas Höök, for all his guidance and support throughout my Master's degree. I also would like to thank my committee members Sergiusz Czesny and Paris Collingsworth for all their help and comments. All current and previous members of the Höök lab including Nick Kalejs, Tim Malinich, Margaret Hutton, Zach Feiner, Mitch Zischke, Carolyn Foley, Patricia Nease, Taylor Senegal, Suse Lagory, Alex Gatch, and Scott Koenigbauer provided valuable help with analysis, comments, and presentation of my research. Thanks to Jay Beugly and Sam Guffey for their assistance with field sampling, equipment, and laboratory work. Thank you also to the undergraduates that helped with field sampling and laboratory processing, including Kylie Schofield, Erika Sherwin, Malena Wolfe, and Sarah Paul. I would also like to thank Ben Turschak, Austin Happel, Harvey Bootsma, Jacques Rinchard, Matt Kornis, and Charles Bronte for their help with arranging collections and processing of samples. Additionally, I would like to thank all those from various state, federal, and tribal agencies who assisted with the collection and providing samples and data, including Michigan DNR, Indiana DNR, Illinois DNR, Wisconsin DNR, Illinois Natural History Survey, USFWS, NOAA, USGS, and Little Traverse Bay Bands of Odawa Indians. Thanks to all the recreational anglers that allowed creel clerks to process and collect data from their catches. I also thanks to my funding source – Great Lakes Fishery Trust. Lastly, this would not for been possible without the love and support of my wonderful wife and my amazing parents. Thank you all.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Author: Leonhardt, Benjamin Scott. MS
Institution: Purdue University
Degree Received: December 2018
Title: Evaluating Methods to Describe Dietary Patterns of Lake Michigan Salmonids
Committee Chair: Tomas O. Höök

Documenting trophic relationships in aquatic ecosystems can facilitate understanding of not only system processes, but also the potential responses of food webs to stressors. Often, trophic studies assume consistent behavior and trophic roles among individuals in a population, but intraspecific diet variation, such as individual specialization, can play a critical role in food web complexity and can promote ecosystem resilience. In Lake Michigan, the introduction of invasive species (e.g., zebra mussel, *Dreissena polymorpha*; quagga mussel, *Dreissena bugensis*; round goby, *Neogobius melanostomus*) and reduced nutrient loading has resulted in changes in nutrient dynamics, system productivity, and community composition over the past two decades. As a result, abundances of many forage fish have declined, including alewife (*Alosa pseudoharengus*) which have historically supported the five dominant salmonid species of Lake Michigan (brown trout, *Salmo trutta*; Chinook salmon, *Oncorhynchus tshawytscha*; Coho salmon, *Oncorhynchus kisutch*; lake trout, *Salvelinus namaycush*; rainbow trout, *Oncorhynchus mykiss*). With these ecosystem changes, there is uncertainty as to the extent of how different species of salmonids will transition to alternative prey items (e.g., round goby). Common methods for examining diet patterns and trophic linkages include stomach content analysis, stable isotope ratios (e.g., $\delta^{13}C$ and $\delta^{15}N$), and fatty acid composition, but these methods vary in temporal resolution and have differential biases. Furthermore,

elucidating agreement of these trophic indicators and whether or not agreement is consistent across species can improve their use in future food web studies. The first research chapter of this thesis investigated the diet complexity of Lake Michigan salmonids by evaluating stomach content composition, diversity, and potential specialized consumption of different alewife lengths. Stomach contents revealed that Chinook salmon almost exclusively consumed alewife and had a lower diet diversity compared to the other four species, which consumed round goby (brown trout and lake trout), aquatic invertebrates (Coho salmon), and terrestrial invertebrates (rainbow trout) in addition to alewife. Although there were clear spatio-temporal and size-related feeding patterns for each species, much of the variation in diet composition and diet diversity was present at the individual-level. Additionally, salmonid species appeared to consume the entire size range of alewife that were available to them and individually specialized on alewife lengths. Due to their reliance on alewife, it is likely that Chinook salmon may be more negatively impacted than other salmonid species if alewife abundance continue to decline in Lake Michigan. The second research chapter assessed the agreement of multiple trophic indicators. Although we found agreement among trophic indicators across the five salmonid species using linear and logistic models, particularly between stomach contents, $\delta^{13}C$, and fatty acid 16:1n-7, there was significant variation in relationships across species, potentially due to variation among salmonids in specific prey items consumed (e.g., alewife and round goby) and species-specific regulation of fatty acids. Additionally, $\delta^{15}N$ estimated from stomach contents using linear mixing models were typically greater relative to observed $\delta^{15}N$, which may suggest small alewife were underrepresented in stomachs of 2016 angler-caught salmonids. Lastly, stomach contents underestimated benthic resource

use by rainbow trout, which may be related to biases associated with fish collection methods and stomach content analysis. Overall, the results of trophic indicator comparisons indicate that caution should be taken when generalizing trophic relationships across species and to consider biases associated with trophic indicators, especially when relying on a single diet metric.

CHAPTER 1. INTRODUCTION

Documenting trophic relationships in aquatic ecosystems can facilitate understanding of not only system processes, but also the potential responses of food webs to stressors. Understanding how basal energy sources flow up the food web (Nakano et al., 1999) and how the relative importance of basal energy sources varies across organisms in an aquatic food web (Driscoll et al., 2015; Turschak & Bootsma, 2015) can provide information on how various parts of the food web may respond to perturbations, such as introductions of new species (e.g., Vander Zanden et al., 1999) and habitat degradation (e.g., Morillo-Velarde et al., 2018). Trophic studies often assume consistent behavior and trophic roles among individuals in a population, even though intraspecific diet variation can significantly contribute to overall complexity and resilience of an ecosystem (Faulks et al., 2015; Feiner et al., in review). For example, niche partitioning among individuals in a population can lead individuals to specialize on a small subset of resources compared to the population's overall resource use (Bolnick et al., 2003), influencing connectivity of food webs and flexibility to environmental disturbances (Layman et al., 2007; Quevedo et al., 2009). Additionally, feeding patterns are known to be influenced by spatio-temporal variation in availability of resources and ontogenetic diet shifts (e.g., Foley et al., 2017; Happel et al., 2017; Happel et al., 2015a, b; Jude et al., 1987; Svanbäck et al., 2015). As a result, food web complexity and ecosystem resilience may vary across spatial and temporal scales and be heavily influenced by intraspecific variability in resource use (Feiner et al., in review).

In the last two decades, Lake Michigan has experienced changes in nutrient dynamics and the relative abundances and composition of aquatic taxa (Bunnel et al., 2017;

Nalepa et al., 2009; Pothoven et al. 2000; Turschak et al., 2014). Several ecosystem-level changes in Lake Michigan occurred coincidental with the introduction of dreissenid mussels (zebra mussel, *Dreissena polymorpha*; quagga mussel, *D. bugensis*) and round goby (*Neogobius melanostomus*). Intense filtering by dreissenid mussels (Hecky et al., 2004; Vanderploeg et al., 2010) and reduced nutrient loading (Dolan & Chapra, 2012) have caused decreased offshore production and oligotrophication of Lake Michigan. This has resulted in the near extirpation of the amphipod *Diporeia*, which was historically a key prey item for many pelagic and profundal fish species (Nalepa et al., 2009). In response, the condition of some fish species, such as alewife (*Alosa pseudoharengus*) and lake whitefish (*Coregonus clupeaformis*; (Madenjian et al., 2003; Pothoven et al., 2004; Pothoven & Madenjian, 2008) has declined due the replacement of energy-rich *Diporeia* with lower energy prey items. Additionally, the biomass of many forage fish species, including alewife, bloater (*Coregonus hoyi*), rainbow smelt (*Osmerus mordax*), deepwater sculpin (*Cottus cognatus*), and slimy sculpin (*Myoxocephalus thompsonii*), have declined since the early 2000's and have shown limited signs of recovery (Bunnel et al., 2017). In contrast to offshore areas, the nearshore area of Lake Michigan has seen increased primary production, a resurgence of benthic algae (*Cladophora*; Auer et al., 2010), and higher densities of invertebrates around nearshore hard and rocky substrates (Pothoven et al., 2000), likely in response to dreissenid mussels sequestering nutrients to nearshore benthic areas and increasing water clarity. The invasive round goby has seemingly benefited from the shunting of nutrients to nearshore areas and now are abundant in the nearshore zone (Foley et al., 2017; Vanderploeg et al. 2002), potentially leading to increased competition for food and cover for native, nearshore benthic species (French & Jude, 2001; Janssen &

Jude, 2001). Collectively, these changes have contributed to the observed increased importance of nearshore trophic pathways, relative to offshore pathways (Turschak et al., 2014).

There is one native (lake trout, *Salvelinus namaycush*) and four nonnative salmonids (brown trout, *Salmo trutta*; Chinook salmon, *Oncorhynchus tshawytscha;* Coho salmon, *Oncorhynchus kisutch*; rainbow trout, *Oncorhynchus mykiss*) that collectively support a valuable recreational fishery in Lake Michigan. Historically, the dominant prey for these salmonids has been the invasive alewife (Jude et al., 1987), but it is unclear how or if different salmonid species will integrate the increasingly more important nearshore trophic pathways into their diet in response to declines in alewife abundance. In the Great Lakes, Chinook salmon and Coho salmon are known to forage almost exclusively on alewife (Happel et al., 2016a; Jacobs et al., 2013; Savitz, 2009; Yuille et al., 2015), but Coho salmon have shown the ability to diversify their diet in response to declines in alewife abundance in Lake Huron (Roseman et al., 2014). On the other hand, Chinook salmon have increased their relative consumption of alewife in Lake Michigan despite declines in alewife abundance (Jacobs et al., 2013). Brown trout, lake trout, and rainbow trout are known to consume an assortment of prey items in the Great Lakes, including round goby (Happel et al. 2017; Happel et al. 2016; Jacobs et al. 2010; Roseman et al. 2014; Tsehaye et al. 2014). In some parts of lakes Michigan, Huron, and Ontario, lake trout have shifted to consume primarily round goby (Colborne et al., 2016; Dietrich et al., 2006; Happel et al., 2016, 2017; Roseman et al., 2014). Additionally in recent years, angler catches of lake trout and rainbow trout have shifted closer to shore and to shallower depths (Simpson et al., 2016), which could reflect an increased reliance on round goby and other nearshore

resources into their diet. Although diets vary across species, they also vary among individuals within a population in response to size-related diet shifts, spatio-temporal variation in prey abundance, and the heterogeneity of Lake Michigan (Happel et al., 2017; Jude et al., 1987; Rybicki & Clapp, 1996). For example, alewife have been shown to be the dominant prey item for lake trout on the western side of Lake Michigan, whereas round gobies contribute more to lake trout diets on the eastern side of the lake (Happel et al., 2017). The resiliency of salmonid species will likely depend upon their ability to consume a high diversity of prey items, but adjust foraging strategies in response to changes in the relative availability of different prey across seasons and regions in Lake Michigan.

Several methods can be used to elucidate trophic relationships in aquatic food webs. Stomach content analysis allows researchers to directly identify taxa that have been consumed over the past 12 to 48 hours, but differences in digestion rates among prey items can bias the importance of some prey items (Brush et al., 2012; Jacobs et al., 2010; MacDonald et al., 1982). To circumvent biases associated with stomach content analysis, researchers have used long-term diet tracers, such as stable isotope ratios (e.g., $\delta^{13}C$ and $\delta^{15}N$; Fry, 2006) and more recently fatty acid compositions (Napolitono 1999), independent of or in conjunction with stomach content analysis. Although these methods have much lower taxonomic resolution, they allow researchers to quantify diet patterns over much longer time scales (i.e., 4-12 weeks for fatty acids, Happel et al., 2015a; 3-4 months for stable isotope ratios, Foley et al., 2017) and represent diet items actually assimilated by a consumer. Additionally, certain stable isotope ratios and fatty acids have been used to distinguish between pelagic and benthic resource use (e.g., DHA, 16:1n-7, $\delta^{13}C$; Happel et al 2015a). Stable isotope ratios and fatty acids are influenced by tissue

turnover (growth rates of predators, temperature, feeding rates; Bendiksen & Jobling, 2003; Brush et al., 2012; Farkas et al., 1980; Hesslein et al., 1993; Kionka & Windell, 1972; Perga & Gerdeaux, 2005), which can make it difficult to interpret trophic pathway used and to estimate the relative importance of prey consumed, particularly in ecosystems with a diverse prey base or when assimilation efficiencies vary across prey.

Researchers consider many assumptions when interpreting stomach contents, stable isotopes ratios, and fatty acid compositions. For example, several fatty acids have been associated with benthic resource use, such as palomitoleic acid (16:1n-7), α-linolenic acid (ALA; 18:3n-3), and eicosapentaenoic acid (EPA; 20:5n-3; Happel et al., 2015a). Based on this association, one might assume individuals who consistently consume benthic prey will have a high abundance of these molecules and will have greater $\delta^{13}C$ values compared to individuals that consume pelagic prey items. Validating these assumptions can provide greater confidence when interpreting stomach contents, stable isotope ratios, and fatty acid compositions. Although some experimental studies have tested the agreement of trophic indicators (Happel et al., 2016b; Hesslein et al., 1993), there have been relatively few field-based studies (Brush et al., 2012; Feiner and Foley et al., in press). Feiner and Foley et al. (in press) documented that assumed relationships between trophic indicators (i.e., stomach contents, stable isotope ratios, fatty acids) were often not consistent across forage fish species in Lake Michigan. Additionally, Brush et al. (2012) found that stable isotopes ratios predicted using linear mixed models based on round goby stomach contents did not accurately reflect observed stable isotope ratios, which was a result of longer digestive rates and limited assimilation of dreissenid mussels into round goby tissue. The lack of agreement of trophic indicators in these studies indicates that caution should be taken when

generalizing trophic relationships within and among species. Moreover, the studies of Brush et al. (2012) and Feiner and Foley et al. (in press) focused on the diets of forage fish (spottail shiner, *Notropis hudsonius*; round goby; yellow perch, *Perca flavescens*) of the Great Lakes, so it is unclear if trophic indicators in taxonomically similar piscivores (e.g., salmonids) of the Great Lakes will have similar inconsistencies.

For this study, we first evaluated the diet complexity of Lake Michigan salmonids through stomach content analysis. We hoped to provide insight into the potential future success of the five salmonids species in a changing Lake Michigan ecosystem by investigating diet composition, diet diversity, and individual specialization of salmonids on alewife lengths. Secondly, we investigated the agreement of stomach contents, stable isotope ratios, and fatty acids across five salmonids species in Lake Michigan. We used linear and logistic models to examine individual-level relationships among stomach contents, stable isotope ratios, and fatty acids. Additionally, we used linear mixed models to investigate if stomach contents of salmonids could accurately predict stable isotope ratios. Elucidating how trophic indicators are related to one another will not only improve their interpretations for salmonids, but may also provide insight into their interpretation and reliability for other aquatic species.

Stomach content analysis revealed that alewife continue to be the dominant prey item across Lake Michigan salmonids. Chinook salmon almost exclusively consumed alewife, whereas other salmonid species consumed additional prey, including round goby (brown trout; lake trout), aquatic invertebrates (Coho salmon), and terrestrial invertebrates (rainbow trout. Ultimately, this resulted in Chinook salmon having a lower diet diversity compared to the other four species. Although there were clear regional, temporal, and size-

related patterns in prey consumption, the majority of variation in diet composition and diet diversity was attributed to the individual-level. This is likely attributed to alewife being consumed consistently across regions, seasons, and size classes and to individual salmonid stomachs being dominated by a single prey type. Lastly, salmonids were found to individually specialize on alewife lengths, but it is unclear if this was a result of long-term prey size specialization or inconsistent distribution of alewife sizes across Lake Michigan and in the water column. Compared to other salmonid species, it is probable that Chinook salmon may struggle in the future due to its strict alewife foraging strategy. Overall, the continued success of Lake Michigan's popular salmonid fishery may depend on the ability of salmonids to forage on prey items other than alewife, such as round goby, so managers should consider trophic interactions of all salmonid species when creating management plans for Lake Michigan salmonids.

Comparisons of trophic indicators revealed several significant relationships that were inconsistent across species, especially between $\delta^{13}C$ and fatty acids. For these models, brown trout and lake trout typically had significant relationships in the same direction, while other species lacked significant relationships. Unlike Chinook salmon, Coho salmon, and rainbow trout, brown trout and lake trout consume both pelagic (i.e., alewife) and benthic resources (i.e., round goby), which may lead to increased contrast of fatty acid composition and $\delta^{13}C$ among individuals and stronger relationships. Although many significant relationships were consistent with *a priori* expectations, several significant models involving fatty acids exhibited relationships in unexpected directions. This brings into question the reliability of certain fatty acids, such as 18:1n-9 and EPA, as accurate measures of trophic pathway use or diet item tracers. In addition to foraging habits,

inconsistencies in relationships across species could indicate that salmonid species are synthesizing and metabolizing fatty acids differently, which may suggest that species-specific interpretations of fatty acids are needed. The comparison of observed $\delta^{13}C$ and $\delta^{15}N$ values from muscle tissue and predicted $\delta^{13}C$ and $\delta^{15}N$ values from linear mixed models based on stomach contents revealed that predicted $\delta^{15}N$ values were commonly greater than observed $\delta^{15}N$ values. We suspect that this is a result of small alewife being under-represented in the stomachs of angler-caught salmonids compared to large alewife. Predicted $\delta^{13}C$ values from stomach contents revealed that benthic resource use is being underestimated in some species, especially in rainbow trout. It appears that stomach contents overestimated the importance of terrestrial insects and underestimated benthic resource use by rainbow trout, which could be due to biases related to stomach content analysis and our fish collection methods. Overall, the results of trophic indicator comparisons show that caution should be taken when generalizing trophic relationships across species and to consider biases associated with trophic indicators, especially when relying on a single diet metric.

CHAPTER 2. DIET COMPLEXITY OF LAKE MICHIGAN SALMONIDS

2.1 Introduction

Trophic studies often describe population trophic connections based on measures of central tendency (e.g., mean diet composition), which assumes consistent behavior and trophic roles among individuals. Intraspecific diet variation can play a vital role in the complexity of an ecosystem (Faulks et al., 2015). Niche partitioning of individuals within a population can lead individuals to specialize on a small subset of resources compared to a population's overall resource use (Bolnick et al., 2003). A population of specialists may function similarly to a population of generalists, in that each population consumes the same range of resources, but the two populations can have different impacts on the connectivity of food webs and flexibility to environmental disturbances (Layman et al., 2007; Quevedo et al., 2009). Not only can individual variation affect diet complexity, but variable environmental factors and behavior, such as seasonal and spatial variation in availability of resources and ontogenetic diet shifts, can influence the use of resources (e.g., Foley et al., 2017; Happel et al., 2017; Happel et al., 2015a, b; Jude et al., 1987; Svanbäck et al., 2015). Given that intraspecific variability in resource use may act as a key component of food web complexity and promote ecosystem resilience, it is useful to describe such variability.

Over the past two decades, the Lake Michigan food web has gone through dramatic changes since the introduction of various invasive species, such as dreissenid mussels (zebra mussel, *Dreissena polymorpha*; quagga mussel, *Dreissena bugensis*) and round goby (*Neogobius melanostomus*). Decreased offshore production and oligotrophication of

Lake Michigan have been attributed to reduced nutrient loading (Dolan & Chapra, 2012) and intense filtering by dreissenid mussels (Hecky et al., 2004; Vanderploeg et al., 2010). Coincidently, there has been a near extirpation of the amphipod *Diporeia* (Nalepa et al., 2009), a historically important prey item for many invertivorous fish in the lake. In the absence of this energy-rich prey item, fishes have used alternative, lower energy prey items, which has resulted in declines in condition for some species, such as alewife (*Alosa pseudoharengus*) and lake whitefish (*Coregonus clupeaformis*; (Madenjian et al., 2003; Pothoven et al., 2004; Pothoven & Madenjian, 2008). In addition, there have been declines in prey fish biomass since the early 2000's, including alewife, bloater (*Coregonus hoyi*), yellow perch (*Perca flavescens*), rainbow smelt (*Osmerus mordax*), slimy sculpin (*Cottus cognatus*), and deepwater sculpin (*Myoxocephalus thompsonii*; Bunnel et al., 2017). In contrast to offshore areas, sequestration of nutrients in nearshore benthic areas by dreissenids and increased water clarity have led to an increase in primary productivity in nearshore areas (Hutton-Stadiq, 2016), the resurgence of benthic algae, (i.e., *Cladophora*; Auer et al., 2010), and high densities of benthic invertebrates (Pothoven et al., 2000) around nearshore hard or rocky substrates. Moreover, high densities of round goby in many nearshore areas (Foley et al., 2017; Vanderploeg et al. 2002), have potentially limited availability of food and cover for other nearshore, benthic fishes (French & Jude, 2001; Janssen & Jude, 2001). Collectively, these changes have potentially contributed to the observed increased importance of nearshore trophic pathways, relative to offshore pathways (Turschak et al., 2014).

Lake Michigan supports a valuable recreational salmonid fishery, which includes one native (lake trout, *Salvelinus namaycush*) and four non-native species (brown trout, *Salmo*

trutta; Chinook salmon, *Oncorhynchus tshawytscha*; Coho salmon, *Oncorhynchus kisutch*; rainbow trout, *Oncorhynchus mykiss*). Historically, invasive alewife has constituted the dominant prey for these salmonids (Jude et al. 1987), but with decreased alewife abundance some species of salmonids may transition to target other prey items (e.g., round goby). Species such as, brown trout, lake trout, and rainbow trout, have displayed diverse diets in the Great Lakes (Happel et al. 2017; Happel et al. 2016; Jacobs et al. 2010; Roseman et al. 2014; Tsehaye et al. 2014) and are known to consume round goby. In fact, there is some evidence that lake trout have shifted to consume primarily round goby in some parts of lakes Michigan, Huron, and Ontario (Colborne et al. 2016; Dietrich et al. 2006; Happel et al. 2017, 2016; Roseman et al. 2014). Furthermore, angler catches of some salmonids in Lake Michigan, like lake trout and rainbow trout, have shifted to shallower and more nearshore habitats in recent years (Simpson et al., 2016) potentially reflecting the importance of round goby and other nearshore resources. Other species, like Chinook salmon and Coho salmon, appear to be less plastic in their diet composition patterns (i.e., consume primarily alewife; Happel et al., 2016a; Jacobs et al., 2013; Savitz, 2009; Yuille et al., 2015), although Coho salmon in Lake Huron have diversified their diet since the 2003 alewife crash to include primarily emerald shiner, round goby, and terrestrial insects (Roseman et al., 2014). In Lake Michigan, Chinook salmon increased their preference for alewife from the mid 1990's to late 2000's even though alewife abundance declined during this time period (Jacobs et al., 2013). Lake Michigan salmonid species appear to differ in their ability to consume a high diversity of prey items, which may have important implications for their flexibility to adjust to changes in the relative availability of different prey.

Lake Michigan provides an opportunity to explore the individual- and population-level diet diversity of multiple top predators and the extent of spatio-temporal variation of diet patterns. Substrate (rocky vs sandy vs mixture; Janssen et al. 2005), drainage-size of rivers (Larson et al., 2013), and land-use (Cloutier et al., 2015) varies considerably across Lake Michigan, which can influence dominant energy pathways and diet patterns across distinct areas of the lake (Foley et al., 2017; Happel et al., 2015a, 2015b, 2017; Feiner et al., in review). For example during 2010, spottail shiner (*Notropis hudsonius*), round goby, and yellow perch collected from southeast Lake Michigan relied more on pelagic energy pathways, whereas those from the southwest relied more on benthic energy pathways (Foley et al., 2017; Happel et al., 2015a, b). These patterns may be associated with the western Lake Michigan coast being characterized by more complex, rocky habitat and high frequencies of upwellings, relative to the eastern coast (Foley et al., 2017; Happel et al., 2015a, b; Feiner et al., in review). Previous work has shown that lake trout diets can vary greatly across different regions of Lake Michigan, with alewife being the dominant prey on the western side of the lake and round gobies comprising greater proportions of diets on the eastern side (Happel et al., 2017). Additionally, the consumption of alewife by salmonids has been shown to change through the seasons with greater proportions of alewife in the diet in the spring, while consumption of other fish, like rainbow smelt and bloater, increases in the summer and fall (Jude et al., 1987; Rybicki & Clapp, 1996).

The diets of fishes can be characterized not only by prey taxa consumed, but also by the diversity of sizes of prey consumed. Lake Michigan salmonids are known to consume a broad length range of alewife (Jacobs et al., 2013; Jude et al., 1987; Rybicki & Clapp, 1996), which could prove beneficial since alewife are known to have highly variable

recruitment (Madenjian et al., 2005). A poor recruitment year could result in a limited range of alewife available (e.g., lack of small alewife), which may lead salmonids to switch to consuming alewife sizes that are most abundant. Warner et al. (2008) found that when the abundance of small alewives was high, age-1 Chinook salmon would switch to consuming small alewives over large alewives. In addition, consuming a broad length range of alewife could allow individuals to specialize on alewife lengths or consume a small subset of alewife lengths compared to the overall population, which has the potential to help reduce competition among salmonids for alewife (Bolnick et al., 2010; Bolnick et al., 2003; McCann et al., 1998; Svanbäck & Bolnick, 2013).

Examining how diet composition, diet diversity, and sizes of alewife consumed by salmonid species varies across Lake Michigan may provide insights to potential future success of salmonids in a changing Lake Michigan ecosystem. To evaluate the diet complexity of Lake Michigan salmonids, we explored 1) the diet patterns of Lake Michigan salmonids in 2015 and 2016, 2) how diet diversity varied across different levels of organization (i.e., individual, region, season, size-class, and year), and 3) quantified individual specialization by salmonids on different alewife lengths. Based off previous studies, we hypothesized that Chinook and Coho salmon would primarily consume alewife and have smaller diet diversities compared to brown trout, lake trout, and rainbow trout, which we expected to consume a wider range of prey, including round goby. Lastly, we expected salmonids to individually specialize on alewife lengths in response to high competition for alewife among individuals.

2.2 Methods

2.2.1 Field Collections

Stomachs of brown trout, Chinook salmon, Coho salmon, lake trout, and rainbow trout were collected from April-November of 2015 and 2016. The vast majority of stomachs were collected from angler-caught fish via the US Fish and Wildlife Service's Great Lakes Mass Marking Program, which is a coordinated tagging program that involves all state, federal, tribal, and provincial agencies that stock salmon and trout in the Great Lakes and its tributaries (C. R. Bronte, USFWS, pers. comm.). In addition, stomachs were collected in annual fishery-independent surveys conducted by Michigan Department of Natural Resources (MDNR), Indiana Department of Natural Resources (INDNR), Wisconsin Department of Natural Resources (WDNR), and Little Traverse Bay Band of Odawa Indians (LTBBOI). Once stomachs were collected they were immediately frozen (-20ºC) until processing. In addition to the collection of stomachs, salmonids were measured for length. To examine spatial variation in salmonid diets, Lake Michigan was divided into four regions: Northeast, Northwest, Southeast, and Southwest (Figure 2.1). Since feeding patterns can be influenced by seasonal variation in prey abundance, stomachs were grouped into two seasons: Early (April-July) and Late (August-November). Salmonids were split into two size classes (<600 mm and ≥600 mm) to account for potential size effects on diets. The goal was to collect up to 20 stomachs for each salmonid species for each region, season, size-class, and year combination. Additionally, lengths of alewife collected in the 2015 and 2016 USGS annual September trawl surveys were obtained for context (B. Bunnell, USGS, pers. comm.).

2.2.2 Stomach Processing

After stomachs were thawed, stomach contents were removed for processing. Individual fish prey items were identified to species (except for sculpins, which were identified to family), weighed to the nearest 0.01 g wet weight, and measured to the nearest 1 mm standard or vertebral length depending on digestion. Highly digested fish prey were identified using cleithras (Traynor et al., 2010) and vertebrae (Elliot et al., 1996). Total lengths of fish prey were estimated from published conversion formulae from standard or vertebral length (Elliot et al., 1996; Knight et al., 1984; Kornis et al., 2012; J. Jonas, MDNR, pers. comm.). We also estimated total lengths of fish from cleithra that were attached to partial vertebrae (Dub & Czesny, 2016). Invertebrate prey were identified to the lowest possible taxonomic level and wet-weighed to the nearest 0.01 g en masse by prey category. We estimated the mean percent composition of diet by weight using only full stomachs and the average wet weight (g) of each prey category per stomach using both full and empty stomachs for each salmonid species (Elliot et al., 1996). Stomach contents were summarized by eleven categories with more rare prey grouped together: alewife, bloater, *Bythotrephes*, *Mysis*, round goby, terrestrial invertebrates, yellow perch, other fish, other, unknown fish, and unidentifiable stomach contents. Other fish were fish prey that rarely appeared in salmonid stomachs, which included rainbow smelt, sculpin, gizzard shad, juvenile lake trout, green sunfish, creek chub, larval fish, and three-spine stickleback. The other category included additional diet items that showed up in relatively few salmonids, namely dreissenid mussels, amphipods, chironomids, and fish eggs. Unknown fish were fish parts (bones, tissue, ect.) that could not be identified to species, whereas unidentifiable stomach contents were stomach contents that were too digested to be identified to any diet category. Unknown fish and unidentifiable stomach contents were not included in further

analyses. Diet items that were not included in diet composition estimations were plastic particles, rocks, vegetation, and fish bait (i.e., earthworms and cocktail shrimp).

2.2.3 Statistical Analyses

2.2.3.1 Variation in Diet Composition

To investigate the variance partitioning of diet composition of Lake Michigan salmonids, we conducted a PerMANOVA with individual proportional diet composition as the response variables and species, region, season, size class, and year as independent variables. A PerMANOVA is a multivariate test, which is analogous to the parametric MANOVA that compares an observed test statistic value (pseudo-F ratio) against recalculated test statistic values from permutations of the data (Happel et al., 2015a). The benefit of PerMANOVA is that the result is not constrained by parametric statistic assumptions or effects of unbalanced sample sizes. We quantified explanatory power (R^2) and p-value for each independent variable. PerMANOVA analyses were conducted separately for each species with region, season, size class, and year as independent variables and diet composition as the response variable. Each PerMANOVA analysis was conducted with Bray-Curtis distances and 999 iterations using the R package vegan (Oksanen et al., 2018; R Core Team 2018).

2.2.3.2 Diversity of Prey Consumed

To quantify the diversity of prey consumed by salmonids, we used the complexity-as-diversity method described by Marion et al. (2015a) and as used by Feiner et al. (in review) to further investigate diet complexity. This method uses Shannon's effective diversity ${}^{q}D$:

$$^{q}D = \left(\sum_{i=1}^{K} p_i^q\right)^{1/(1-q)}$$

where p_i is the proportion of diet item *i* across all K diet items. The parameter *q* allows the index of diversity to be weighted by the relative abundances of each diet item. When *q*=0 the weights are ignored and ^{q}D signifies diet richness. As *q* increases, the relative abundance of diet items has greater influence on diversity. General diversity was represented by q=1, for which the limit is the exponentiated Shannon's diversity. Additionally, we quantified diversity at *q*=3, which highlighted the diversity of abundant diet items. This analysis was conducted separately for each species of salmonids and across all salmonids at five (i.e., region, season, size-class, year, and global) and six nested hierarchical levels (i.e., species, region, season, size-class, year, and global), respectively. We used the group-wise partitioning method of Marion et al (2015a), which averages the diversity for each level across the components of that level (e.g., regional diversity represents the mean diversity across individuals at that region). Bootstrap uncertainties (1000 iterations) were estimated for components at each hierarchical level and the diversity of each component was considered significantly different if they did not have overlapping 95% confidence intervals.

Next, we quantified the partitioning of diversity across levels of organization to determine how excess salmonid diet diversity was partitioned across individuals, species, regions, seasons, size class, and years, again using the group-wise partitioning approach by Marion et al. (2015a) and as done by Feiner et al. (in review). We first estimated the beta diversity, which represents the effective number of diet items that were not observed across an average component of that hierarchical level by subtracting the alpha diversity (i.e., the

effective number of prey items that could be expected to be observed within an average individual or component of a hierarchical level) from the total diversity at each hierarchical level (Feiner et al., in review). With this, we were able to determine which hierarchical level contributed the most to diet diversity. From these estimates, we calculated the proportion that contributed to total diversity by each level (e.g., the proportion of diet diversity observed across regions that would not be observed on average within a region would represent the regional-level contribution). First, we evaluated salmonid-wide partitioning of diversity across individuals, species, regions, seasons, size-classes, within-years, and between-years. Then, we repeated these analyses for each species, separately. We implemented the partitioning at q=0 through q=6 to elucidate how increasing the influence of relative abundances affects diversity partitioning. All diversity analyses were conducted in R (R Core Team 2016) using the R package hierDiversity (Marion et al., 2015b).

Due to multiple factors (e.g., varying digestion rates of prey items), there was a lack of consistent taxonomic resolution for the identification of stomach contents. For example, dreissenid mussels were identified to genus, terrestrial invertebrates to order, and fish were identified to species. Such, different taxonomic resolution can confound interpretations of diet diversity. To evaluate such effects, we conducted our analyses of diet diversity and the partitioning of diversity by grouping stomach contents in three different ways: 1) using abundance based groupings (i.e., categories described above), 2) using only fish prey by species, and 3) groupings based on coarse trophic pathways. For the second grouping method, we included: alewife, bloater, creek chub (*Semotilus atromaculatus*), green sunfish (*Lepomis cyanellus*), larval fish, juvenile lake trout, rainbow smelt (*Osmerus*

mordax), round goby, sculpin (deepwater sculpin and slimy sculpin grouped together), three-spined stickleback (*Gasterosteus aculeatus*), and yellow perch. For the third grouping method, we divided diet items into five groups: pelagic fish, benthic fish, pelagic invertebrates, benthic invertebrates, and terrestrial invertebrates. The pelagic fish group included alewife, bloater, larval fish, and rainbow smelt, whereas the benthic fish category included creek chub, green sunfish, juvenile lake trout, round goby, sculpin, three-spine stickleback, yellow perch, and fish eggs (Turschak et al., 2014). Pelagic invertebrates included *Bythotrephes*, *Mysis*, and dreissenid mussels, whereas benthic invertebrates included amphipods and chironomids (Turschak et al., 2014).

2.2.3.3 Individual Specialization on Alewife Lengths

To describe patterns of alewife lengths consumed by individual salmonids, we used the total niche width (TNW) method developed by Roughgarden (1974). TNW can be defined as all the dietary resources that a population exploits and consists of within individual (WIC) and between individual components (BIC; Roughgarden 1974). The extent of individual specialization can be measured as the proportion of TNW explained by WIC (i.e., WIC/TNW; Bolnick et al., 2002). Values of individual specialization fall between 0 and 1, with smaller values signifying more individual specialization. To test the null distribution that all individuals are sampling equally from the overall distribution of alewife lengths consumed, a Monte Carlo (999 iterations) resampling technique was used to calculate a p-value. The TNW and individual specialization was measured for each species lake-wide and by region, season, and size-class for each year. Since multiple comparisons were completed, we set the significant p-value at 0.01. For these analyses,

only individuals that consumed more than one alewife were included. These analyses were conducted using the R package RInSp (Zaccarelli et al., 2013).

2.3 Results

2.3.1 Within-species Diet Patterns

2.3.1.1 Brown Trout

In 2015 and 2016, a total of 114 and 96 brown trout were collected and analyzed, respectively. Of the identifiable diet items, alewife and round goby represented the majority of diet items by both percent diet composition (calculated as the mean of individual proportional diet composition; alewife: 51% in 2015 and 38% in 2016; round goby: 11% in 2015 and 21% in 2016) and by mean weight per stomach (alewife: 8.6 g in 2015 and 2.1 g in 2016; round goby: 0.6 g in 2015 and 3.1 g in 2016; Figure 2.2). When partitioning variance in diet composition, region ($F_{3, 94} = 2.67$, $R^2 = 0.07$, $P = 0.013$) had the largest explanatory power followed by year ($F_{1, 94} = 4.62$, $R^2 = 0.04$, $P = 0.008$) with 86.6% of the variation being left unexplained. For example in spring of 2016, round gobies were particularly important in diets on the eastern side of Lake Michigan (Figure 2.3). Regardless of how diet contents were grouped (i.e., by abundance, fish species, or coarse trophic groupings) there was minimal diet diversity variation among regions, seasons, size-classes, and years (Figures 2.4, A.2, A.3, and A.4). The bulk of diversity variation for all three diet groupings was partitioned to the individual (10%-42%), regional (23%-51%), and between years (11%-24%; Figures 2.5, A.6. & A.7). Lake-wide individual specialization on alewife lengths did not occur for brown trout in either year likely due to small sample sizes (Table 2.1).

2.3.1.2 Chinook Salmon

In 2015 and 2016, a total of 524 and 314 Chinook salmon stomachs were collected and analyzed, respectively. In both years, alewife was by far the primary prey item for Chinook salmon (proportional diet composition in 2015: 69%, 2016: 73%; mean g/stomach in 2015: 9.2 g, 2016: 9.0 g; Figure 2.2). Much of the diet composition variance was left unexplained (92.7%) with only region ($F_{3, 375} = 5.90$, $R^2 = 0.04$, $P = 0.001$) and season ($F_{1, 375} = 10.06$, $R^2 = 0.02$, $P = 0.001$) having a significant (but minimal explanatory power) effect. There was little difference in diet diversity across regions, seasons, size-classes, and years (Figures 2.4, A.2, A.3, and A.4). However, when abundance was the basis for prey groupings, diet diversity in the spring was significantly less than in the fall (Figure A.4). Regardless of how diet contents were grouped, almost the entirety of diet diversity variation was partitioned towards the individual- (10% - 90%) and regional-level (10% - 50%; (Figures 2.5, A.6., & A.7). Significant lake-wide individual specialization on alewife lengths by Chinook salmon occurred in both years (2015: WIC/TNW = 0.35); 2016: WIC/TNW = 0.21; Table 2.1).

2.3.1.3 Coho Salmon

In 2015 and 2016, a total of 227 and 232 Coho salmon were collected and analyzed in each year, respectively. Overall, Coho salmon fed primarily on alewife (proportional diet composition 2015: 51%, 2016: 54%), but terrestrial invertebrates (2015: 12%; 2016: 3%), *Mysis* (2015: <1%; 2016: 13%), and *Bythotrephes* (2015: 6%; 2016: 17%) made up considerable proportions of Coho salmon stomach contents (Figure 2.2). Though terrestrial invertebrates, *Mysis*, and *Bythotrephes* contributed a large proportion to the mean diet composition of individual Coho salmon, the mean weight of all invertebrates (<1 g in 2015

and 2016) found in Coho salmon stomachs was minimal compared to alewife (2015: 8.7 g; 2016: 10.8 g; Figure 2.2). Terrestrial invertebrates and *Mysis* were primarily consumed by small Coho salmon, whereas *Bythotrephes* were fed on by large Coho salmon (Figure A.1). The diet composition of Coho salmon was explained most by region ($F_{3, 304} = 10.72$, $R^2 = 0.10$, $P = 0.001$) followed by year ($F_{1, 304} = 9.49$, $R^2 = 0.03$, $P = 0.001$), season ($F_{1, 304} = 8.26$, $R^2 = 0.02$, $P = 0.001$), and size-class ($F_{1, 304} = 6.04$, $R^2 = 0.02$, $P = 0.001$), but 83.1% of the variance was unexplained. Coho salmon diet diversity varied little between regions, seasons, size-classes, and years (Figure 2.4, A.2, A.3, & A.4). However, diet diversities calculated for northern regions were generally lower than for southern regions (Figure A.2). Variation of Coho salmon diet diversity was partitioned primarily to the individual- (9%-82%) and regional-levels (16%-56%) with the individual-level being particularly important when stomach contents were grouped by species of fish prey (Figures 2.5, A.6., & A.7). Significant lake-wide individual specialization on alewife lengths occurred in both years (2015: WIC/TNW = 0.16); 2016: WIC/TNW = 0.20; Table 2.1).

2.3.1.4 Lake Trout

In 2015 and 2016, a total of 469 and 484 lake trout were collected and analyzed, respectively. Lake trout diets consisted of primarily alewife (2015: 51% and 8 g/stomach; 2016: 56% and 9.3 g/stomach) and round goby (2015: 32% and 3.4 g/stomach; 2016: 30% and 3.2 g/stomach; Figure 2.2). For lake trout, only region ($F_{3, 474} = 34.40$, $R^2 = 0.17$, $P = 0.001$) and season ($F_{1, 474} = 29.66$, $R^2 = 0.04$, $P = 0.001$) had significant influence on diet composition with 77.6% of the variance being left unexplained. Similar to brown trout, the bulk of round goby consumption occurred in the eastern regions in the spring, whereas alewife was the dominate prey item in the western regions (Figure 2.3). Diet diversity was

consistent across regions, seasons, size-classes, and years (Figure 2.4, A.2, A.3, & A.4). Much of the variation in diet diversity was attributed to individual- (8%-53%), regional- (28%-42%), and seasonal-levels (9%-22%; Figure 5, S.6, & S.7). Significant lake-wide specialization on alewife lengths only occurred in 2016 (WIC/TNW = 0.23; Table 2.1).

2.3.1.5 Rainbow Trout

A total of 291 and 253 rainbow trout were collected and analyzed in 2015 and 2016, respectively. Rainbow trout consumed primarily terrestrial invertebrates (2015: 66%; 2016: 33%) and alewife (2015: 20%; 2016: 37%; Figure 2.2). The mean weight of terrestrial invertebrates (3.4 g) and alewife (3.2 g) found in stomachs were roughly equal in 2015, but in 2016 the mean weight of alewife in stomachs (8.7 g) was much greater than terrestrial invertebrates (1.6 g; Figure 2.2). The variance of rainbow trout diet composition was significant and roughly equally explainable across all variables (year: $F_{1, 381} = 27.55$, $R^2 = 0.06$, $P = 0.001$; season: $F_{1, 381} = 26.14$, $R^2 = 0.05$, $P = 0.001$; size-class: $F_{1, 381} = 21.70$, $R^2 = 0.05$, $P = 0.001$; region: $F_{3, 381} = 6.74$, $R^2 = 0.04$, $P = 0.001$), but 79.9% of the variance was unexplained. There were no regional, seasonal, size-class, or yearly differences in diet diversity (Figure 2.4, A.2, A.3, & A.4). When prey were grouped by fish species, almost the entirety of diet diversity variation was attributed to the individual (15%-83%; Figure A.6), whereas when stomach contents were grouped by abundance and broad trophic categories less diversity was partitioned to the individual (10%-36%) and more diversity was attributed to regional differences (31%-50%; Figures 2.5 & A.7). Significant individual specialization on alewife lengths occurred in 2015 (WIC/TNW = 0.07) and 2016 (WIC/TNW = 0.48; Table 2.1).

2.3.2 Across-species Diet Patterns

2.3.2.1 Diet Composition

When analyzing diet variation across all species, largest contributors in diet composition variation was species ($F_{4, 1652} = 94.69$, $R^2 = 0.17$, $P = 0.001$) followed by region ($F_{3, 1652} = 40.32$, $R^2 = 0.05$, $P = 0.001$), with 73.6% of the variation being unexplained. Season ($F_{1, 1652} = 42.54$, $R^2 = 0.02$, $P = 0.001$), size-class ($F_{1, 1652} = 27.72$, $R^2 = 0.01$, $P = 0.001$), and year ($F_{1, 1652} = 21.88$, $R^2 = 0.01$, $P = 0.001$) had roughly equal explanatory power, but explained little of the variation. Though there were distinct diet patterns across each species, there were some consistent regional and seasonal patterns in the consumption of prey items across the five species. In the spring of 2016, alewife made up larger proportions of the diet in the eastern region compared to the western region for all salmonids, but this trend did not hold in the fall (Figure 2.3). *Mysis* and yellow perch were consumed most in the southern regions in the spring and fall, respectively (Figure 2.3). The consumption of *Bythotrephes* and bloater was most common in the late season. Generally, smaller salmonids consumed more aquatic and terrestrial invertebrates compared to large salmonids (Figure A.1).

2.3.2.2 Diet Diversity

Differences in diet diversity among species depended on how diversity was indexed (i.e., q value) as well as how prey were grouped. Nonetheless, Chinook salmon had consistently relatively lower diet diversities, especially when compared to lake trout and rainbow trout (Figures 2.4, A.3, A.4, & A.5). When only considering fish prey, the diet diversities of Chinook salmon, Coho salmon, and rainbow trout were similar and diet diversities were typically lower than the diet diversities of brown trout and lake trout at

each hierarchical level (Figures 2.4, A.3, A.4, & A.5). At the regional-level, diet diversities were fairly similar across species and regions with diet diversities typically being less than 1.2 (Figure A.3). Though there were significant differences across species, it is unclear how meaningful these results are since diet diversity at the regional-level was so low and differences between species were minimal. Across all the hierarchical levels, diet diversity declined sharply from q=1 to q=3, supporting the general trend of individuals consuming primarily a single type of prey at a time (Figures 2.4, A.2, A.3, A.4, & A.5). When all species were considered, majority of the diversity partitioned to the individual- (5%-56%) and species-level (20%-59%; Figures 2.5, A.6, & A.7).

2.3.2.3 Alewife Lengths Consumed and Individual Specialization

The length distribution of alewives consumed by salmonids varied considerably by year (Figures 2.6 & 2.7). In 2015, the length frequency of consumed alewife was unimodal and dominated by large alewife. The few small alewives that were consumed were found in salmonids collected in the southern portions of the lake in the fall (Figure 2.7). In 2016, the length frequency of consumed alewife was bimodal with the majority of consumed alewife being small alewife less than 120 mm (Figures 2.6 & 2.7). Most small alewives consumed in 2016 were found in salmonids collected in the western regions of the lake (Figure 2.7). Length frequencies of consumed alewives were similar to length frequencies of alewives collected in USGS fall trawls in both years (Figure 2.6). For each salmonid species, the mean alewife length consumed was larger in 2015 (125.9 mm - 138.9 mm) compared to 2016 (95.3 -131.4 mm; Table 2.2). On average, rainbow trout consumed the smallest alewife in both years (2015: 125.9 mm; 2016: 95.3 mm) compared to other salmonid species (Table 2.2; Figure 2.6). In 2015, individual specialization on alewife

lengths was most common in southern regions, in the fall, and by small salmonids (Tables A.1-A.5). In 2016, individual specialization was most common in western regions and was common across all seasons and size-classes (Tables A.1-A.5).

2.4 Discussion

Even though there were differences in consumption patterns among salmonid species, alewife was clearly the dominant prey item for salmonids in Lake Michigan. Chinook salmon almost exclusively consumed alewife, with minimal contributions from other prey, like round goby, yellow perch, bloater, and invertebrates. These observations are consistent with previous studies in lakes Michigan (Jacobs et al., 2013) and Huron (Roseman et al., 2014), in that Chinook salmon are feeding almost exclusively on alewife even though alewife abundances were very depressed (Bunnel et al., 2017; Warner et al., 2017). Coho salmon consumed primarily alewife and aquatic (*Mysis* and *Bythotrephes*) and terrestrial invertebrates with little contribution from round goby, yellow perch and bloater. Although the mean percent diet composition suggests alewife and invertebrates have equal importance in Coho salmon diets, the mean weight of alewife found in Coho stomachs is nearly three times more than all other diet categories combined. This suggests that although Coho salmon have a diverse diet composition, the bulk of their energy is likely derived from alewife compared to other prey items. Rainbow trout were the only species for which terrestrial invertebrates contributed a substantial proportion to the overall diet composition. This is consistent with rainbow trout in Lake Huron where following a decline of alewife abundance terrestrial invertebrates were the dominant prey (Roseman et al., 2014). This could be a result of the combination of two biases associated with relying on stomach analysis and angler-caught fish, respectively: longer digestive rates for terrestrial insects

compared to soft-bodied prey (Kionka & Windell, 1972) and anglers targeting rainbow trout near thermal bars where both rainbow trout and terrestrial invertebrates accumulate (Aultman & Haynes, 1993; Höök et al., 2004; Roseman et al., 2014). Nevertheless, the high importance of terrestrial invertebrates to rainbow trout appears to be a recent phenomenon because terrestrial insects were relatively rare in prior diet studies, especially for larger fish.

Brown trout and lake trout were the primary consumers of round goby. The diets of lake trout are consistent with previous Lake Michigan studies (Happel et al., 2017; Jacobs et al., 2010), showing that lake trout have increased their reliance on round goby with the decline of alewife, which has likely contributed to the resurgence of natural reproduction of lake trout in Lake Michigan (Hanson et al., 2013; Happel et al., 2017). In addition, previous work in Lake Ontario has shown that round goby is contributing substantially more to the diets of lake trout and brown trout compared to the other three species (Happel et al., 2016a; Yuille et al., 2015). Chinook salmon, Coho salmon, and rainbow trout did consume round goby, but round goby contributions to their diet were minimal. In Lake Huron, round goby now make up roughly 10%-15% of Coho salmon and rainbow trout diet by weight (Roseman et al., 2014), which is higher than in Lake Michigan. This may suggest that alewife abundance is sufficiently high in Lake Michigan to allow for continued reliance on alewife for Coho salmon and rainbow trout as their main fish prey. Also, lack of stronger contributions from round goby in salmonid diets could be related to the majority of stomachs being collected from angler-caught fish. Anglers typically target salmonids at the thermocline, which is where alewife commonly inhabit (Brandt et al., 1980). Thus, fishing at the thermocline may increase the likelihood of catching a salmonid with alewife

or other pelagic prey in the stomach rather than benthic prey. For example, round goby tend to contribute more to the diets of lake trout caught in bottom gillnets compared to angler-caught lake trout (J. Jonas, MDNR, pers. comm.). As a result, round goby importance may be underestimated for some salmonids, like Coho salmon and rainbow trout, which are known to forage on round goby in Lake Huron (Roseman et al., 2014). Lastly consistent with the study reputed herein, there is little evidence that Chinook salmon consume round goby in lakes Huron (Roseman et al., 2014) and Ontario (Happel et al., 2016a; Yuille et al., 2015), which points to the potential diet inflexibility of Chinook salmon.

Previous diet studies in Lake Michigan have documented that spatio-temporal patterns in prey consumption of fishes (Foley et al., 2017; Happel et al., 2015a, b), including salmonids (Happel et al., 2017). In general, while we observed relatively low diet variation across regions, some spatial differences were evident. For brown trout in the spring of 2016 and lake trout in the spring of both years, round gobies were more abundant in diets in eastern regions. Happel et al. (2017) documented similar patterns in lake trout collected from Lake Michigan in the spring of 2011. The eastern shoreline has much less complex, sandy habitat compared to the western shoreline (Foley et al., 2017; Happel et al., 2015a, b), which may increase availability and decrease the handling time of round goby compared to the western side. Additionally, salmonids occupy nearshore areas in the spring (Olson et al., 1988), which may lead to the consumption of round gobies at a relatively high rate in the spring compared to late summer and fall. Other consistent spatial patterns included yellow perch and *Mysis* being consumed more in the southern regions, which is consistent with previous work showing higher abundances of these species in

southern regions of Lake Michigan (Beletsky et al., 2007; Feiner et al., in review; Happel et al., 2015; Pothoven et al., 2004). Additionally, increased consumption of *Bythotrephes* in the late season is consistent with relatively higher *Bythotrephes* abundance in late summer and fall (Pothoven et al., 2012).

In 2016, there were strong spatio-temporal trends in the consumption of alewife, which likely arose due to spatial and seasonal differences in their availability as prey. In spring of 2016, alewife consumption was highest in the western regions for all five salmonid species. In past studies, the highest densities of alewife have been found on the western shoreline during the spring (Brandt et al., 1991). Due to high densities of alewife on the western shoreline during the spring, it may not have been energy efficient to forage for other prey species, like round goby. In the fall, this trend did not hold likely due to offshore movements of alewife during the summer (Brandt et al., 1991) and relatively even distribution of alewife across Lake Michigan during late summer and fall of 2016 (Warner et al., 2017). It is unclear why this trend was observed in 2016 and not 2015, even though overall alewife abundance was similar between the two years (Bunnell et al 2017; Warner et al 2017). One difference between the two years was the relatively high abundance of small alewives in 2016 compared to 2015, which were likely yearlings from the 2015 year-class (Bunnell et al., 2017).

Invertebrates are typically an important prey item for juvenile salmonids (Jacobs et al., 2013; Jude et al., 1987), but invertebrates in general contributed very little compared to previous studies. The threshold we used for small salmonids was <600 mm, which is larger than previous studies (<300 mm, Jude et al., 1987; <500 mm, Jacobs et al., 2013). Moreover, since we relied primarily on angler-caught fish and minimum size-limits in Lake

Michigan range from 254 (Michigan, Illinois, and Wisconsin waters; MDNR 2015, 2016; ILDNR 2016; WDNR 2016) to 356 mm (Indiana waters; INDNR 2016), the sizes of salmonids examined may have been too large to see any distinct size-related effects on relative consumption of invertebrates versus fish prey. In addition, the prevalence of small alewife and reduced growth rates of alewife (Madenjian et al., 2003) may increase the availability of small alewives to smaller salmonids. The slower growth of alewife may leave them vulnerable to small salmonids for a longer period of time and may allow small salmonids to shift from invertebrates to alewife at a smaller size. Additionally, there has been a significant decline in the abundance of *Diporeia* (Nalepa et al., 2009), which have previously been shown to be important diet items for juvenile Chinook salmon and lake trout (Jacobs et al., 2013; Madenjian et al., 1998). Small salmonids may have shifted toward increased consumption of fish prey as a consequence of the reduced availability of this once important energy-rich, benthic invertebrate.

At the population level, Chinook salmon on average consumed only one prey category, whereas the other species generally consumed 1-3 prey categories. Chinook salmon are known to select alewife over other prey items, leading to their consistent, relatively low diet diversity. This could be problematic for Chinook salmon if alewife populations were to further decline to similar levels as observed in Lake Huron, whereas brown trout, Coho salmon, lake trout, and rainbow trout appear to be more flexible in their prey consumption. Differences in diet diversity could also be associated with different foraging habitats used by the five species. Previous work has shown that brown trout are associated with nearshore areas with structure (Olson et al., 1988), which could provide better opportunities for feeding on a wider variety of prey, including round goby. The

harvest of lake trout and rainbow trout has shifted closer to shore in recent years (Simpson et al., 2016), which could be reflective of decreased dependence on alewife and greater reliance on nearshore prey items, like round goby or terrestrial invertebrates. In contrast, the combination of Chinook salmon potentially moving farther from shore (Simpson et al ., 2016) and Chinook salmon typically feeding at the thermocline (Olson et al., 1988) may limit the diversity of prey items available to Chinook salmon.

While salmonids expressed variable diet compositions at the population-level, much of the variation in the diet composition and diversity was attributed to the individual, especially for Chinook salmon. Although there were clear regional patterns in diet compositions, the overwhelming importance of alewife through space and time reduced the amount of explained variation in the diet composition. The high mobility of salmonids (Adlerstein et al., 2007, 2008) likely allows them to follow and search for schools of alewife or other preferred prey across the lake, which likely reduces spatial and temporal effects on diet patterns. Additionally, much of the diet diversity variation was observed at the individual-level due to individual salmonids rarely consuming more than one diet category. This could suggest that individuals are specializing on specific prey items, which has been documented in some inland trout species (Bridcut & Giller, 1995; Jirka & Kraft, 2017). Continued long-term specialization could have impacts on the linkage between pelagic and benthic pathways in Lake Michigan (Quevedo et al., 2009) if some individuals are only consuming round goby or alewife. The lack of diet diversity within an individual could also be associated with the patchiness of prey items in the environment, especially if salmonids are foraging pelagically near the thermocline where alewife like to inhabit (Brandt, 1980; Riha et al., 2017). Additionally, abundances of many forage fish species in

2015 and 2016 were relatively low (Bunnell et al 2017; Warner et al 2017), which could make it difficult for salmonids to come across multiple prey items in one foraging event.

Salmonids are known to select alewife over other prey items (Jacobs et al., 2013) and will consume a broad range of alewife lengths (this study; Jude et al., 1987; Jacobs et al., 2013). Our study was novel in that the length distributions of alewife varied dramatically between the two study years. This allowed us to investigate how salmonids respond to a dramatic change in alewife length distributions. In 2015, USGS bottom trawl catches and salmonid stomachs were dominated by large alewife (>110 mm), whereas in 2016, USGS bottom trawls and salmonid stomachs had a bimodal distribution dominated by small alewife (80-110 mm) with fewer large alewife (150-190 mm). The substantial number of small alewives consumed in 2016 are likely yearlings that represent a relatively strong 2015 year-class (Madenjian et al., 2016). In 2016, rainbow trout almost exclusively fed on small alewives (Figure 7) and on average consumed the smallest alewife (95.3 ± 25 mm), which likely reflects both small alewife and rainbow trout occupying higher areas of the water column (Aultman & Haynes, 1993; Brandt, 1980), whereas the other four species fed on similar sized alewife. The 2016 length distribution of consumed alewife showed substantial spatial variation with most small alewives consumed on the western side of Lake Michigan compared to the eastern side where stomachs were dominated by large alewife. It appears that salmonids can be quite plastic to annual and spatial variations in alewife length distributions, which may prove beneficial since high variability in alewife recruitment success (Madenjian et al., 2005) can cause pronounced year-to-year and spatial changes in alewife length distributions.

To the best of our knowledge, elucidating the consumption patterns on different sizes of alewife by salmonids has only been conducted at the population-level (e.g., Jude et al., 1987; Jacobs et al., 2013). Our results show that individual specialization was common for Lake Michigan salmonids in both years; though there was a much broader size range available in 2016 compared to 2015. Individual specialization may help limit competition through reduced niche overlap (Bolnick et al., 2003, 2010; Svanbäck & Bolnick, 2013) and help reduce the intensity of predation (McCann et al., 1998) on a specific size range of alewife. Although individual specialization is commonly associated with competition, it can also occur by an individual feeding in a patchy environment. When foraging in a patchy environment, individual stomach contents can represent the localized prey abundance rather than an individual's preferred prey or the prey abundance over a broad area. For example in 2016, salmonids foraging on the eastern side of the Lake Michigan may have had limited opportunities to feed on both small and large alewife compared to the western side. Young-of-year and adult alewives occupy distinct parts of the water column and do not typically school together (Brandt, 1980), which likely further increases the patchiness of the environment. When a salmonid comes across a school of alewife it may just consume the alewife available in the school whether it contains either small or large alewife. Brown trout, Chinook salmon, and lake trout of Lake Ontario have been shown to forage above, within, and below the thermocline, which overlaps with the different thermal distributions of young-of-year and adult alewife (Olson et al., 1988). This could result in some individuals feeding on young-of-year and others on adult alewife making it appear that individual specialization is occurring.

It is quite clear that salmonids have altered their foraging patterns, though not all in the same way, in response to the dramatic shift in the Lake Michigan ecosystem. Alewife continues to be the dominant prey item for Lake Michigan salmonids, especially for Chinook salmon, but round goby, aquatic invertebrates, and terrestrial insects also contribute significantly to salmonid diets. The continued success of Lake Michigan's popular, valuable salmonid fishery may depend on the ability of salmonids to rely less on alewife and potentially more on abundant invasive round goby and other prey items, so managers should consider the trophic interactions of each salmonid and their prey when trying to understand how ecosystem change will affect salmonids when creating salmonid management plans for Lake Michigan.

Table 2.1. Results of tests for lake-wide individual specialization on alewife lengths by Lake Michigan salmonids in 2015 and 2016. Individual specialization was calculated as WIC/TNW for each species and on individuals that consumed more than one measurable alewife. Values close to 0 indicate size specialization on alewife whereas values close to 1 indicate alewife length generalization. * indicates significant individual specialization.

	2015				
	Brown Trout	Chinook Salmon	Coho Salmon	Lake Trout	Rainbow Trout
WIC	183.74	216.80	187.50	180.22	99.54
TNW	369.15	618.58	1188.63	361.38	1339.97
WIC/TNW	0.50	0.35	0.16	0.50	0.07
P	0.096	0.001*	0.001*	0.012	0.001*
Sample Size	15	85	33	70	18

	2016				
	Brown Trout	Chinook Salmon	Coho Salmon	Lake Trout	Rainbow Trout
WIC	69.34	246.58	250.05	350.24	303.39
TNW	1341.63	1171.39	1271.33	1553.11	628.07
WIC/TNW	0.05	0.21	0.20	0.23	0.48
P	0.013	0.001*	0.001*	0.001*	0.001*
Sample Size	6	70	63	109	53

Table 2.2. Mean length (mm) and standard deviation of alewives consumed by each salmonid species in 2015 and 2016.

	Brown Trout	Chinook Salmon	Coho Salmon	Lake Trout	Rainbow Trout
2015	138.9 ± 14.1	133.6 ± 20.2	132.9 ± 32.1	139.8 ± 13.6	125.9 ± 36.2
2016	131.4 ± 42.5	120.9 ± 36.3	115.2 ± 35.9	118.1 ± 35.3	95.3 ± 25.0

Figure 2.1. Map of Lake Michigan showing the different regions (Northeast=NE; Northwest=NW; Southeast=SE; Southwest=SW) where salmonids were collected. Northeast was defined as fish collected at or between the ports of Ludington and Charlevoix. Northwest was defined as fish collected at or between the ports of Manitowoc and Manistique. Southeast was defined as fish collected at or between the ports of Sheboygan and East Chicago. Southeast was defined as fish collected at or between the ports of Muskegon and Burns Harbor.

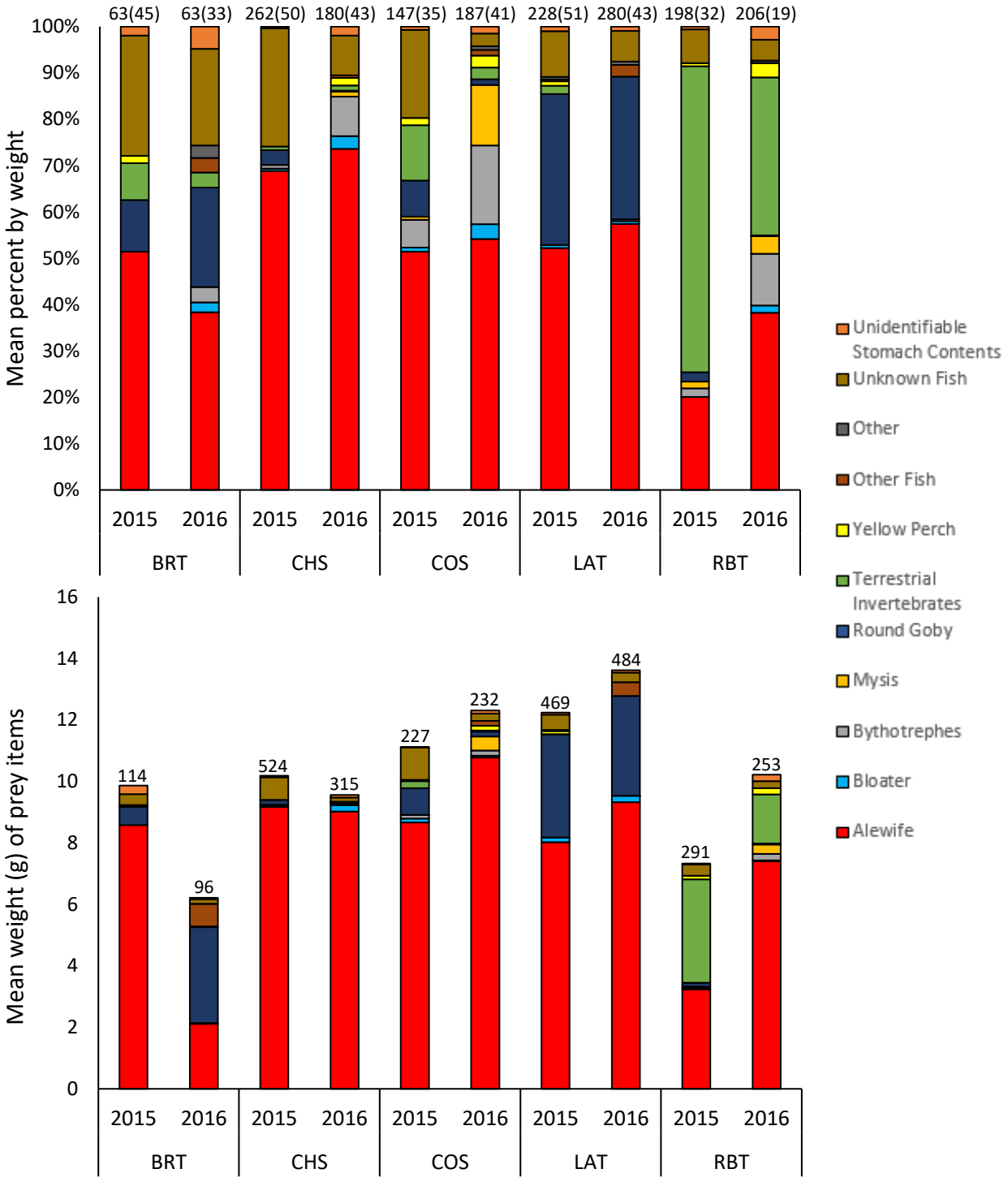


Figure 2.2. Mean percent diet composition by weight (top) for each salmonid species and mean weight of each prey category (bottom) for each salmonid species in 2015 and 2016. Numbers above bars in top figure represent the number of full stomachs and percent that were empty in parentheses and for the bottom figure they represent total number of stomachs analyzed. Salmonid species: BRT=brown trout; CHS=Chinook salmon; COS=Coho salmon; LAT= lake trout; RBT=rainbow trout.

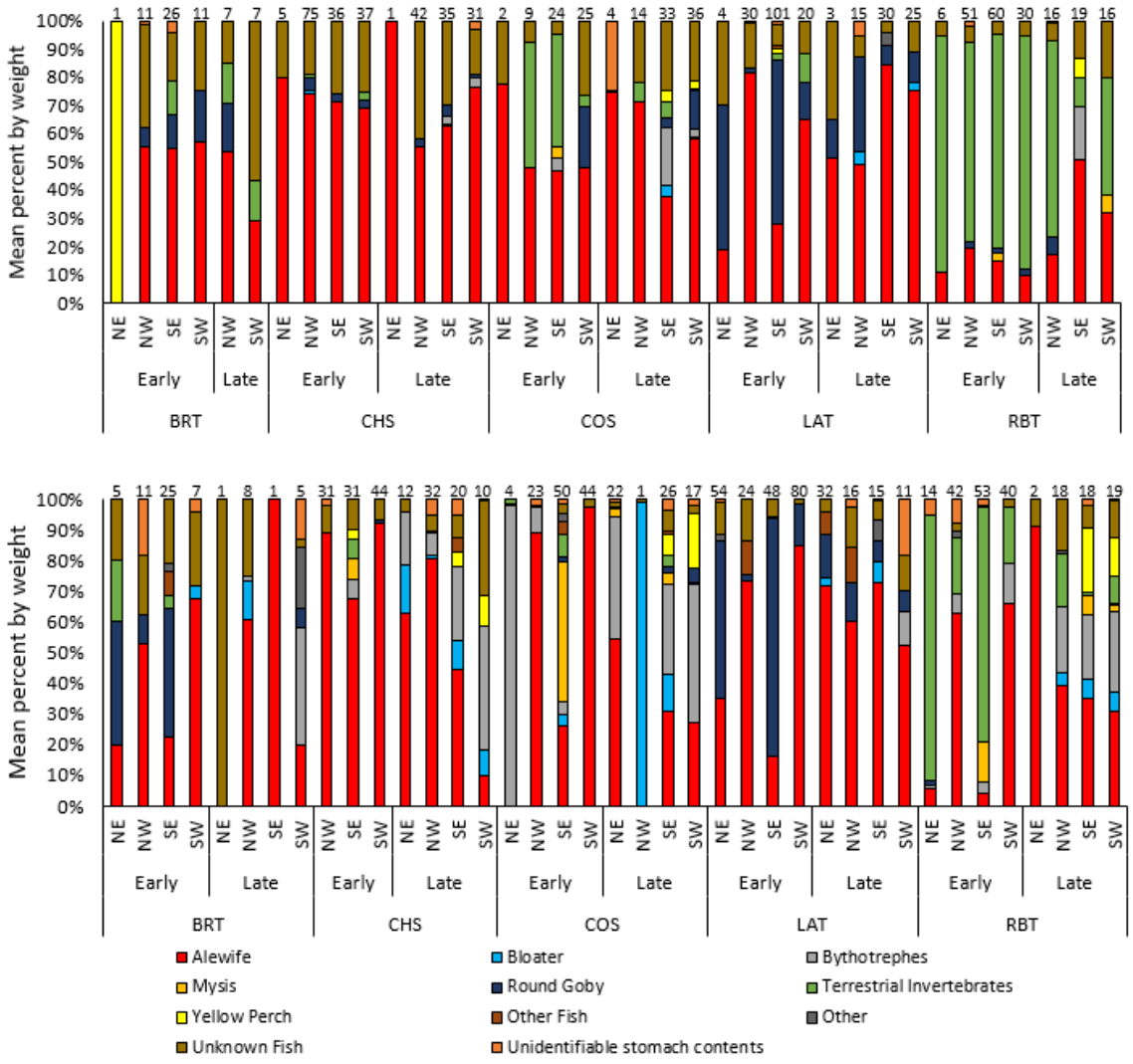


Figure 2.3. Seasonal and regional patterns of mean percent diet composition by weight for Lake Michigan salmonids in 2015 (top) and 2016 (bottom). Numbers above bar represent the total number of full stomachs examined. BRT=brown trout; CHS=Chinook salmon; COS=Coho salmon; LAT= lake trout; RBT=rainbow trout. NE=Northeast; NW=Northwest; SE=Southwest; SW=Southwest.

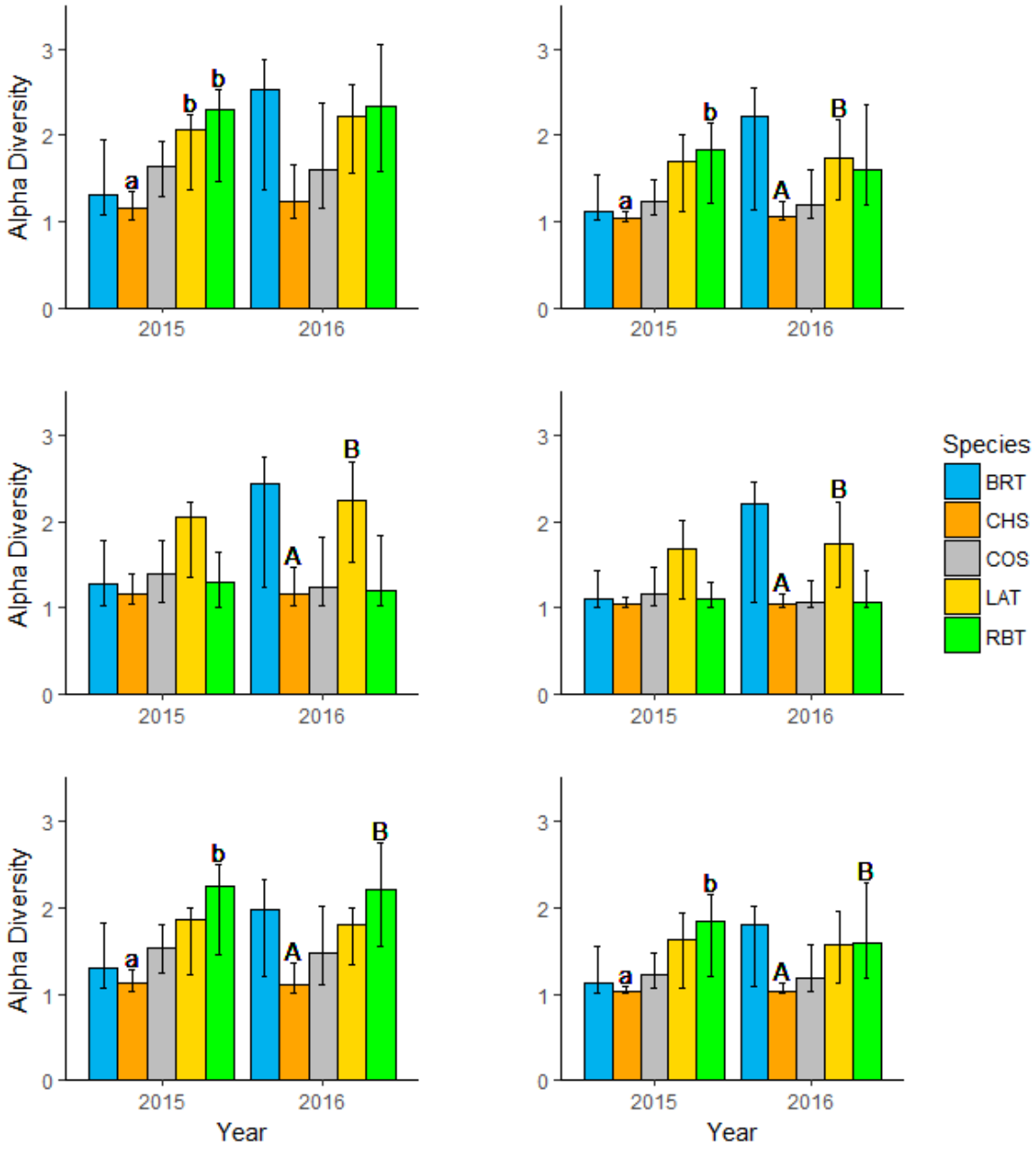


Figure 2.4. Variation in diet diversity of five salmonids in 2015 and 2016 at q=1 (left column) and q=3 (right column) for abundance (top row), fish prey (middle column), and trophic categorizations (bottom column). Error bars represent 95% confidence intervals, where non-overlapping intervals represent significant differences. Upper and lower case letters represent differences across species within each year. BRT=brown trout; CHS=Chinook salmon; COS=Coho salmon; LAT= lake trout; RBT=rainbow trout.

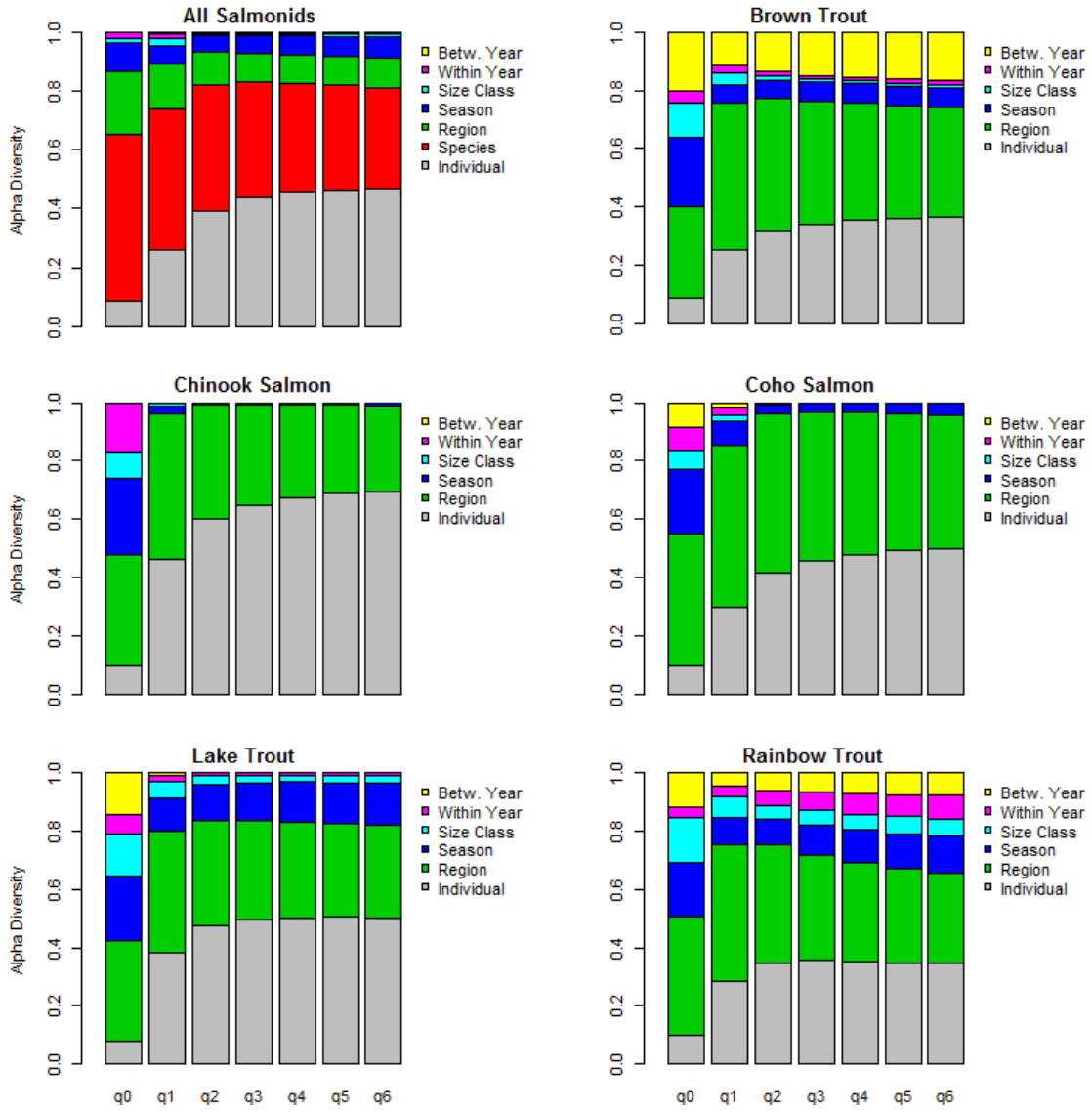


Figure 2.5. Diversity partitioning by proportion of diet items found by weight using the abundance categorizations in all salmonid species combined and each salmonid species individually at q=0 to q=6.

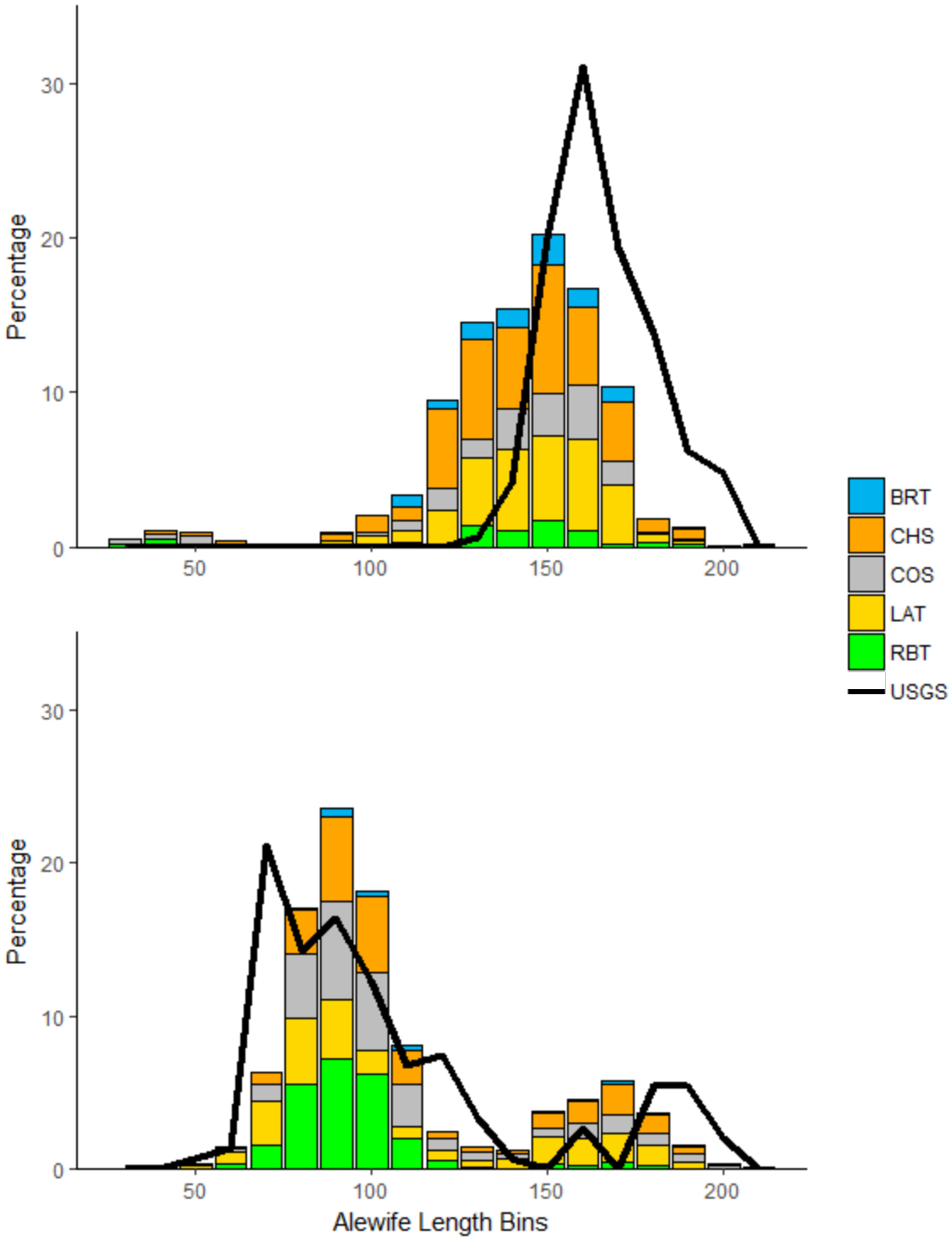


Figure 2.6. Length-frequency distributions (percent of total number) for alewives consumed separated by species in 2015 (top) and 2016 (bottom). Additionally, length frequencies of alewife collected in annual USGS September trawl surveys in 2015 and 2016 are included (black line; B. Bunnell, USGS, pers. comm). BRT=brown trout; CHS=Chinook salmon; COS=Coho salmon; LAT= lake trout; RBT=rainbow trout.

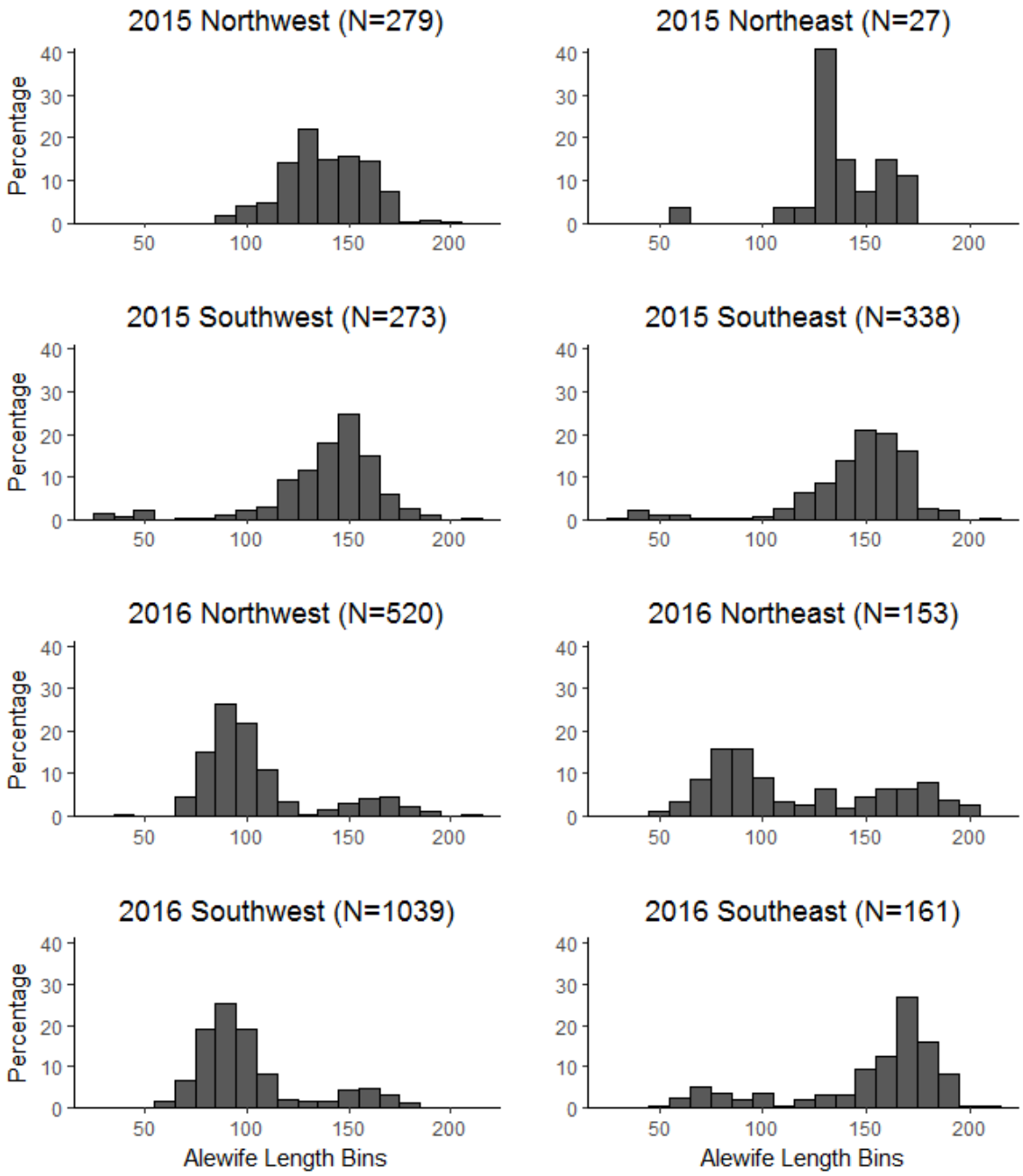


Figure 2.7. Regional length-frequency distributions (percent of total number) for alewives consumed by the five salmonid species in 2015 and 2016. N represents the total number of measurable alewife consumed by salmonids in that region.

CHAPTER 3. COMPARISONS OF THREE TROPHIC INDICATORS IN LAKE MICHIGAN SALMONIDS

3.1 Introduction

Documenting trophic relationships in aquatic ecosystems can facilitate understanding of not only system processes, but also the potential responses of food webs to stressors, such as introductions of new species (e.g., Vander Zanden et al., 1999) and habitat degradation (e.g., Morillo-Velarde et al., 2018). Elucidating trophic relationships can provide insight on how basal energy sources (e.g., pelagic, benthic, or terrestrial; Nakano et al., 1999) transfer up the food web and how the relative importance of energy sources varies across organisms in an ecosystem (Driscoll et al., 2015; Turschak & Bootsma, 2015). Such information could help researchers and managers identify components of the food web that may or may not adjust easily to ecological changes and adapt managing practices to improve ecosystem resilience (McMeans et al., 2016; Valdovinos et al., 2010).

Common methods for assessing trophic relationships in aquatic food webs include stomach content analysis (e.g., Hyslop, 1980), examination of stable isotope ratios, most commonly $\delta^{13}C$ and $\delta^{15}N$ (e.g., Fry, 2006), and more recently analysis of fatty acid composition (e.g., Napolitano 1999). Analyzing stomach contents allows researchers to directly identify taxa that have been consumed, but only represents feeding habits over the past 12 to 48 hours. Moreover, the rate of digestion and assimilation can vary across prey items and between hard and soft tissues, which can cause over- or underestimations of the relative importance of prey items (Brush et al., 2012; Jacobs et al., 2010; Kionka & Windell, 1972; MacDonald et al., 1982). There is evidence that feeding patterns can have high

individual, temporal, and spatial variability (Roswell, et al., 2013), which suggests that to fully understand the trophic relationships of organisms, specimens would need to be collected frequently across broad spatio-temporal scales. To circumvent biases associated with stomach content analysis, long-term diet tracers, such as stable isotope ratios (Fry, 2006) and increasingly fatty acid analysis (Napolitono 1999), have been used independently or in conjunction with stomach content analysis to describe long-term diet patterns.

In aquatic systems, $\delta^{13}C$ can be a reliable indicator of primary energetic sources supporting individual growth, with smaller $\delta^{13}C$ representing pelagic or terrestrial resource use and larger $\delta^{13}C$ corresponding to benthic, nearshore resource use (France, 1995). $\delta^{15}N$ becomes larger with each trophic transfer, so it is a useful measure of trophic position (Vander Zanden et al., 1997). Overall, stable isotopes reflect assimilation of diet items into consumer tissues over the past 3-4 months (e.g., Foley et al., 2017) with tissue turnover rates strongly influencing the exact assimilation time frame indexed by stable isotopes ratios (Brush et al., 2012; Hesslein et al., 1993; Perga & Gerdeaux, 2005). Since stable isotopes of an individual organism potentially reflects an integration of diverse diet items over time, it can be difficult to isolate specific prey items that individuals have been consuming. Although quantification of stable isotope ratios of other potential prey items can help elucidate long-term diet patterns (Turschak & Bootsma, 2015), reconstruction of prey consumed may be challenging especially in systems with diverse prey bases or when assimilation efficiencies vary across prey.

Fatty acid analysis of fish tissues is being increasingly used to identify trophic interactions within aquatic food webs. Long chain fatty acids are a reflection of prey

consumed by individuals over 4 to 12 weeks (Happel et al., 2015a). To understand food web linkages, fatty acids that cannot be synthesized by freshwater fish, such as α-linolenic acid (ALA; 18:3n-3) have been used to infer long-term diet patterns and energy pathway usage (Tocher, 2010). Fatty acid synthesization and metabolism can vary across taxonomic groups, for example freshwater organisms can synthesize their own long-chain polyunsaturated fatty acids, like arachidonic acid (ARA; 20:4n-6), docosahexaenoic acid (DHA; 22:6n-3) and eicosapentaenoic acid (EPA; 20:5n-3), whereas marine organisms are unable to synthesize these same molecules (Tocher, 2003, 2010). Also, internal regulation of fatty acids can be influenced by other factors, such as temperature and feeding rates, which can make the interpretation of trophic pathways based on fatty acid compositions difficult (Bendiksen & Jobling, 2003; Farkas et al., 1980). Indeed, to index potential trophic pathways individual fatty acids are often combined into more comprehensive indices, like the ratio of total omega-3 (n-3) to total omega-6 (n-6) fatty acids or the ratio of oleic acid (18:1n-9) to palomitoleic acid (16:1n-7). The ratio of n-3 to n-6 fatty acids has been used to detect the dominant energetic pathways used by organisms (Happel et al., 2017; Feiner and Foley et al., in press) and the ratio of 18:1n-9 and 16:1n-7 has been used to identify the consumption of alewife vs round goby in Laurentian Great Lakes salmonids (Happel et al., 2017). There is additional evidence suggesting that multivariate approaches (e.g., principal component analysis) to profile a broader suite of fatty acids could help interpret trophic relationships rather than relying on individual fatty acids (Feiner and Foley et al., in press).

The three trophic indicators described above have different potential biases and quantify feeding habits on different time scales. Thus, these diet metrics may not always provide a consistent description of trophic connections and several studies have capitalized

on their potential distinct insights by employing multiple trophic metrics (e.g., Happel et al., 2015a, 2015b, 2017). Nonetheless, one might expect that consistent, distinct feeding patterns could be similarly quantified by all three diet metrics. For example, the monounsaturated fatty acid 16:1n-7 and polyunsaturated fatty acids ALA and EPA are assumed to be associated with benthic resource use due to their association with diatoms, detritus, and bacteria (Happel et al., 2015a). Based on this assumption, one might expect that an individual that consumes benthic prey consistently would not only have a high proportion of benthic prey in its stomach, but would also have tissues with higher amounts of these fatty acids as well as larger $\delta^{13}C$. There have been some experimental studies to test the agreement of trophic indicators (Happel et al., 2016b; Hesslein et al., 1993), but these studies used simplified diet compositions. There have been a few field based studies that have compared the agreement of these diet metrics within individuals (Feiner and Foley et al., in press) and at the population-level (Brush et al., 2012). After conducting correlations between stomach contents, stable isotope ratios, and individual fatty acids across three forage fish species, Feiner and Foley et al (in press) found that assumed relationships between trophic indicators did not always hold, some relationships were in unexpected directions, and relationships were not always consistent across species. Using linear mixed models, Brush et al. (2012) found that predicted stable isotope ratios of round goby (*Neogobius melanostomus*) based on stomach contents did not accurately reflect measured stable isotope ratios. These results suggested that hard-bodied prey, like dreissenid mussels (zebra mussel, *Dreissena polymorpha*; quagga mussel, *Dreissena bugensis*), and soft-bodied prey are likely not assimilated equally into the body tissue and the importance of dreissenid mussels was overestimated based on stomach content analysis.

This lack of agreement of trophic indicators suggests that care needs to be taken when using these trophic indicators to generalize trophic interactions within and across taxonomic groups.

Some field studies that have tested the agreement of trophic indicators have focused on forage fish (round goby; spottail shiner, *Notropis hudsonius*; yellow perch, *Perca flavescens*) of the Laurentian Great Lakes (Brush et al., 2012; Feiner and Foley et al., in press). It is unclear if trophic indicators of taxonomically similar piscivores (brown trout, *Salmo trutta*; Chinook salmon, *Oncorhynchus tshawytscha*; Coho salmon, *Oncorhynchus kisutch*; lake trout, *Salvelinus namaycush*; rainbow trout, *Oncorhynchus mykiss*) of Laurentian Great Lakes will agree or if agreement will be consistent across the five species. Salmonids are of particular interest in the Laurentian Great Lakes, due to a popular recreational fishery reliant on stocking by state and federal agencies. With a shift in the relative importance in the benthic nearshore versus pelagic energy pathways that has occurred in many of the Laurentian Great Lakes (e.g., Lake Michigan; Turschak et al., 2014), which is attributed to invasive dreissenid mussels and round gobies, there is uncertainty as to how salmonids will adjust their foraging habits to account for this shift. There is some evidence that Lake Michigan salmonids are adjusting their foraging habits in different ways to account for this shift (Leonhardt et al., in prep). For example, brown trout and lake trout appear to be relying more on benthic round goby, Coho salmon and rainbow trout are consuming more aquatic and terrestrial invertebrates, and Chinook salmon are continuing to forage exclusively on alewife (*Alosa pseudoharengus*) despite a lake-wide decline in alewife abundance (Leonhardt et al., in prep). This could potentially affect the consistency of agreement of trophic indicators, since these prey types have

distinct stable isotope and fatty acid signatures (Foley et al., 2017; Happel et al., 2015a, b; Turschak et al., 2015) and likely do not assimilate equally into predator tissue (Kionka and Windell, 1972). Lack of consistent agreement across species may suggest that a trophic indicator, such as the relative abundance of an individual fatty acid, may require species-specific interpretations. Additionally, estimating long-term diet tracers (e.g., $\delta^{13}C$ and $\delta^{15}N$) from short-term tracers (e.g., stomach contents) could help identify diet items that are under- or over-represented in stomach contents (Brush et al., 2012). Evaluating the agreement of trophic indicators can improve interpretations of trophic indicators for salmonids of the Laurentian Great Lakes, but may also provide insight on the interpretation of or the reliability certain trophic indicators for other species. To better understand the relationship between diet metrics, the study described herein focused on salmonids collected from Lake Michigan to evaluate the following questions:

1) Are there relationships between stomach contents, stable isotope ratios, and fatty acids and are they consistent across the five salmonid species of Lake Michigan?

2) Using linear mixing models, can stomach contents accurately predict stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) of Lake Michigan salmonids?

3.2 Methods

3.2.1 Fish Collection and Diet Analyses

Five Lake Michigan salmonid species (brown trout, Chinook salmon, Coho salmon, lake trout, and rainbow trout) were collected across the main basin of Lake Michigan from April through November of 2016 (Figure 3.1). Salmonids were collected from anglers by creel clerks with the US Fish and Wildlife Service's Mass Marking Program and through annual fishery independent surveys conducted by the Michigan Department of Natural

Resources, Indiana Department of Natural Resources, Wisconsin Department of Natural Resources, and Little Traverse Bay Band of Odawa Indians. After salmonids were captured, stomachs, dorsal muscle tissue, and belly flap tissue were removed for stomach content, stable isotope, and fatty acid analyses, respectively. Stomachs and dorsal muscle tissue samples were stored at -20ºC and belly flap tissue samples were stored at -80ºC until they could be processed. To acquire stable isotope signatures of potential prey items of salmonids, micromesh gill nets and bottom trawls were used to collect forage fish in the spring and the fall of 2016 throughout Lake Michigan Once captured, forage fish were stored on ice until they could be stored at -80ºC. Due to the importance of terrestrial insects in the diets of rainbow trout (Table 3.1), we also acquired terrestrial insects (Coleoptera, Diptera, and Hymenoptera) from rainbow trout stomachs for stable isotope analysis. Terrestrial insects were stored in -20ºC freezers until processing. Acquiring samples of salmonids across different seasons and regions of Lake Michigan allowed us to assess the relationships between trophic indicators across individuals experiencing a range of environmental conditions, potentially capturing high contrast across trophic metrics and improving our ability to model relationships.

Once stomachs were thawed, stomach contents were removed for processing. Fish prey were identified to species, except for sculpin species which were identified to family. Fish prey that were highly digested were identified using cleithras (Traynor et al., 2010) and vertebrae (Elliot et al., 1996). Published formulae were used to estimate total lengths of fish prey from standard lengths, full vertebral length, and from cleithra attached to partial vertebrae (Dub & Czesny, 2016; Elliot et al., 1996; Knight et al., 1984; Kornis et al., 2012; J. Jonas, MDNR, pers. comm.). Invertebrate prey were identified to the lowest taxonomic

level possible and wet-weighed to the nearest 0.01 g en masse by prey category. The mean percent diet composition of stomachs by weight was calculated for each individual salmonid. All unidentifiable stomach contents were ignored when calculating the mean percent diet composition.

Stable isotope analyses were processed at the University of Wisconsin-Milwaukee School of Freshwater Sciences using the procedures described in detail in Turschak (2013). Briefly, a 2-3 g subsample of salmonid dorsal muscle tissue was homogenized, lyophilized, and again homogenized. For potential prey, the whole fish was homogenized, lyophilized, and again homogenized. For terrestrial insects, each order was homogenized, lyophilized, and again homogenized separately. The dried homogenate of each sample was placed into a tin capsule for stable isotope analysis. An isotope mass spectrometer (DELTA V plus IRMS with universal triple collector, ConFlo IV universal interface, Costech ECS 4010) was used to conduct measurements of carbon and nitrogen isotope concentrations. To ensure instrument calibration, after every 12^{th} sample an acetanilide control was analyzed. Results of the stable isotope analyses are expressed in per mil difference between the isotope ratio of the sample and that of the standard, where $\delta^{13}C$ and $\delta^{15}N = (Rs_{sample}/R_{standard}-1)*1000$, and $R_{sample} = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The standard for C^{12}:C^{13} ratios was PeeDee Belemnite and for ${}^{15}N/{}^{14}N$ was atmospheric nitrogen. $\delta^{13}C$ signatures were lipid-corrected using equations from Turschak (2013).

Fatty acid signatures were quantified at the State University of New York-Brockport using the procedures described in detail in Czesny et al. (2011) and Happel et al. (2015a, b). Briefly, belly flaps were homogenized in a commercial blender, lipids were extracted from the homogenate (Folch et al., 1957), and fatty acids were transmethylated

(Metcalfe & Schmitz, 1961). Fatty acid signatures were identified using a gas chromatography-mass spectrometer (GC/MS Agilent Technologies Inc., Wilmington, DE). Fatty acids were expressed as the relative percentage of fatty acids identified and mg/g of wet sample. Fatty acids that were compared to stable isotope ratios and stomach contents included ALA, ARA, DHA, EPA, 16:1n-7, 18:1n-9, and the ratios of n-3 to n-6 and 16:1n-7 to 18:1n-9, all of which have been suggested to differentiate between benthic and pelagic trophic pathways and/or the relative consumption of alewife versus round goby (Table 3.2).

3.2.2 Individual-level Comparisons

Unlike stomach content analysis, stable isotope and fatty acid analyses were performed on a subset of individuals for each species. Different datasets were created for each paired combination of diet indices, so that direct comparisons can be made within individuals (Table B.1). Due to the use of fatty acids to infer trophic pathway use and past prey consumed (e.g., Happel et al., 2016a) and the inconsistencies that Feiner and Foley et al. (in press) observed for some fatty acids across species, we had fatty acids variables as explanatory variables when compared with other trophic indicators to test if *a priori* expected relationships are valid. Similarly, stable isotope ratios are often used to identify past prey consumed (e.g. Turschak & Bootsma 2015), so stable isotope ratios were explanatory variables when compared to stomach contents. When considering the relationship between fatty acids and stable isotopes, we used linear models with isotopic signature ($\delta^{13}C$ or $\delta^{15}N$) as the response variable, species as the categorical explanatory variable, individual fatty acid proportion as the continuous explanatory variable, and the interaction between species and fatty acid proportion to determine if relationships varied across the salmonid species. For relationships between fatty acids and stomach contents,

we used logistic generalized models with the proportional abundance of stomach contents by weight (alewife or round goby) as the response variable, species as the categorical predictor variable, fatty acid proportion as the continuous predictor variable, and the interaction between species and fatty acid proportion. Lastly, for relationships between stomach contents and stable isotope ratios, we again used logistic generalized models with the proportional abundance of stomach contents by weight (alewife or round goby) as the response variable, species as the categorical predictor variable, stable isotope ratios ($\delta^{13}C$ or $\delta^{15}N$) as the continuous predictor variable, and the interaction between species and stable isotope ratios. Only lake trout and brown trout were included in round goby models since round gobies were a minimal diet component for Chinook salmon, Coho salmon, and rainbow trout (Table 3.1). Additionally, we reran all fatty acids models with fatty acids expressed as mg/g of wet sample in an effort to understand whether the actual concentration of an individual fatty acid will express differential relationships as compared to relative fatty acid abundance.

Similar to Feiner and Foley et al. (in press), we wanted to examine if a more comprehensive, multivariate approach to describe fatty acid composition could better index trophic linkage. Using principle component analysis (PCA), we created orthogonal indicators from compositions of fatty acids across the five salmonid species. We conducted two PCAs with fatty acids expressed in two ways: 1) mean proportion of all fatty acids and 2) mg of individual fatty acid/g of wet sample. We only included fatty acids that had a mean proportional abundance greater than 1% or an abundance greater than 0.01 mg/g of wet sample. Selected axes of each PCA were then used as trophic indictors that were compared to stomach contents and stable isotope ratios following the same modeling

methods described above. Conducting a PCA across all five species allows us to compare PC loadings and relationships with trophic indicators across species, but due to the inclusion of multiple species it can decrease the contrast of PC scores among individuals within a species. To account for this, we conducted PCAs on each species separately and then compared PCA axes to stomach contents (logistic models) and stable isotopes ratios (linear models). These models were similar to the ones described above, except there were no species or interaction terms.

To determine model significance, Chi-squared (logistic models) and F-tests (linear models) were used with Type III sum of squares due to the unbalanced sample sizes across salmonid species (R package car; Fox and Weisburg, 2011). If the interaction term was not significant in a model this term was excluded from the model. When the interaction was significant, significant differences between slopes were identified if their 95% confidence intervals did not overlap (R package lsmeans; Lenth 2016). Conditional R^2 was used to determine explanatory power of generalized linear models (R package MuMIn; Barton 2016). Due to the exploratory nature of this study and large number of models, we did not want to rely solely on a corrected p-value, which could exclude models that could be biologically relevant. Thus, we acknowledged models that had p-values less than 0.05 and 0.001 as meaningful and highly significant, respectively. All statistical analyses were completed in program R (R Core Team 2016).

3.2.3 Linear Mixing Models

For individuals that had both stomach contents and stable isotope ratios analyzed, we used stomach contents to estimate $\delta^{13}C$ and $\delta^{15}N$ for individual salmonids that would then be compared to observed $\delta^{13}C$ and $\delta^{15}N$. These linear mixing models were only

completed using stomach contents and stable isotope ratios because there is little understanding of how to mix stomach contents or fatty acids to predict fatty acid composition or stable isotopes, respectively. We used linear mixing model equations to estimate expected values for $\delta^{13}C$ and $\delta^{15}N$ from proportional abundance of prey items in stomach contents using the following equations (Brush et al., 2012):

$\delta^{15}N_{predator}$ or $\delta^{13}C_{predator} = (P_x)x + (P_y)y + (P_z)z$ ….+ fractionation

where x, y, z, etc. are the $\delta^{15}N$ or $\delta^{13}C$ values for different prey items and P_x represents the proportional abundance of the designated prey item in a stomach. The fractionation used for $\delta^{13}C$ and $\delta^{15}N$ were 0.4‰ and 3.4‰, respectively (Post 2002). The $\delta^{13}C$ and $\delta^{15}N$ estimates were derived from forage fish that were collected from Lake Michigan in 2016 (Table 3.3). Since sculpins were identified to family, we averaged the stable isotope signatures of slimy and deepwater sculpin to obtain overall mean sculpin $\delta^{13}C$ and $\delta^{15}N$. If there were prey items in salmonid stomachs that were not collected from our sampling, we used lake-wide averages from past studies (Turschak 2013; Driscoll et al., 2015; B. Turschak, MDNR personal communication). For isotopic signatures of terrestrial insects, we used the mean $\delta^{13}C$ and $\delta^{15}N$ across all orders analyzed. We had difficulty acquiring stable isotope ratios for some rare prey items (e.g., green sunfish, creek chub and larval fish), so individuals that consumed those prey items were excluded from analyses. Isotopic signatures of alewife and round goby are known to vary across size groups (Foley et al., 2017; Turschak et al., 2015), so they were divided into different size categories. Alewife were separated into three categories (i.e., <100 mm, >100 mm, and unsized alewife), whereas round gobies were separated into four categories (i.e., <60 mm, 60-100 mm, >100 mm, and unsized round goby; Table 3.3). Due to varying degrees of digestion, we were

unable to estimate total lengths of all alewife and round goby identified, so all those without an estimated total length are included in their respective unsized categories. For individuals that had unsized alewife or round goby in their stomachs, the lake-wide mean $\delta^{13}C$ and $\delta^{15}N$ were used to estimate isotopic ratios for that individual (Table 3.3).

Additionally, due to distinct regional isotopic signatures of some prey items (alewife and round goby), which may influence salmonid isotopic signatures, we estimated stable isotope ratios for each individual salmonid using regional (northwest, southwest, southeast, and northeast) stable isotope means of prey items found in stomachs (Table 3.4). For regional mixing models, we only considered regional stable isotopic means for alewife and round goby, as they were the dominant prey items for salmonids and showed spatial isotopic variation. For regional estimations, we used the mean $\delta^{13}C$ and $\delta^{15}N$ for each size class of alewife or round goby from the region the salmonid was collected. For unsized alewife or round goby, salmonids received the overall regional mean alewife or round goby to estimate isotopic signatures. Alewife were not collected in the northeast region of Lake Michigan, so lake-wide means were used for this region. Large round gobies (>100 mm) were not collected in the southwest region of Lake Michigan, so lake-wide mean of large round gobies was used to estimate isotopic signatures. For all other prey items, we again used lake-wide stable isotope ratios because either we did not have regional isotopic data or they lacked isotopic variation across Lake Michigan.

With this information, we wanted to test the agreement of predicted and observed stable isotope ratios at the lake-wide- and regional-levels for each species. Due to predicted stable isotope ratios of each species having nonnormal distributions (Wilk-Shapiro Test), we used nonparametric Wilcoxon Sign Tests for each species to test whether predicted

stable isotope ratios were significantly different from the observed at lake-wide- and regional-levels. The Wilcoxon Sign Test is analogous to a paired t-test, but it is not bound by parametric assumptions. All statistical analyses were completed in program R (R Core Team 2016).

3.3 Results

3.3.1 Principal Component Analyses

PCAs were conducted on the fatty acid compositions of salmonids to investigate if a more comprehensive, multivariate approach can help with indexing trophic linkages from a suite of fatty acids. For the PCA conducted on all salmonids with fatty acids expressed as proportional abundance, PC 1 (41% of variance explained) was negatively associated with DHA and positively associated with 16:1n-7 and 18:1n-9 and PC 2 (28%) was a gradient between fish high in 16:1n-7 to fish high in palmitic acid (16:0; Table 3.5). For PC 2, there was clear separation of salmonids with Chinook salmon having higher positive PC 2 loadings and other species loading more negatively on PC 2, especially lake trout and brown trout (Figure 3.2). PC 3 (12%) was negatively associated with 16:1n-7 and 16:0 and positively associated with 18:2n-6, ALA, and 20:4n-3 (Table 3.5). When PCAs were conducted on each species separately, brown trout, lake trout, and rainbow trout had relatively similar PC 1 (50-59%) loadings which were negatively associated with 16:1n-7 and 18:1n-9 and positively associated with DHA (Table 3.5). In addition, Chinook salmon and Coho salmon had similar PC 1 axes (54% and 61%, respectively), which were negatively associated with DHA and positively associated with 18:1n-9 (Table 3.5). There were much fewer similarities across species for PC 2 (16-22%) and PC 3 (11-12%; Table

3.5). We kept the first three axes for these PCAs since they explained a large proportion of the variance and were easily interpretable.

For all PCAs (all salmonids and species individually) conducted on fatty acids expressed as mg/g of wet sample, PC 1 (83%) was positively associated with all individual fatty acids, but 16:0 and 18:1n-9 had the highest loadings (Table S2). Salmonid-wide PC 2 (7%) was negatively associated with DHA and positively associated with 16:1n-7 and 18:1n-9 (Table S2). Salmonid-wide PC 3 was negatively associated with 16:1n-7, 18:1n-9, and C22 fatty acids and positively with 16:0 (Table B.2). Salmonid-wide PC 3 did a relatively better job than the other PC axes at separating salmonids with lake trout loading negatively and Chinook salmon loading positively (Figure B.1). When PCAs were conducted on each species separately, PC 2 for brown trout and lake trout (4 and 5%, respectively) were negatively associated with 16:1n-7 and positively associated with DHA, whereas for Chinook salmon and Coho salmon PC 2 (7 and 9%, respectively) could be characterized as a gradient of DHA to 16:0 and 18:1n-9 (Table B.2). For rainbow trout, PC 2 (4%) was negatively associated with 18:1n-9 and positively associated with DHA (Table B.2). There were much fewer similarities with PC 3 (1-4%) across species, though 16:0 had high loadings, but direction of loading varied by species (Table B.2). Although, the first two salmonid-wide and species-specific PCA axes explained almost the entirety of the variance, we kept the third axes due to PC 1 axes appearing to be a measure of total lipid content rather than individual fatty acids.

3.3.2 Individual-level Comparisons

3.3.2.1 Stable-Isotope-Fatty Acid

For the majority of significant relationships between stable isotope and individual fatty acids expressed as proportions, there was limited agreement across all species (Table 3.6; Figure 3.3 & 3.4). Nonetheless, brown trout and lake trout tended to have significant slopes in the same direction, whereas Chinook salmon, Coho salmon, and rainbow trout rarely had significant relationships (Figures 3.3 & 3.4). The models that had an interaction effect with p-values <0.001 (highly significant) included relationships between $\delta^{13}C$ and DHA, EPA, and 18:1n-9 (Table 3.6). For the species-specific relationships between $\delta^{13}C$ and DHA, brown trout and lake trout had significant negative slopes (Figure 3.4). $\delta^{13}C$ and EPA exhibited significant positive and negative slopes in brown trout and lake trout, respectively (Figure 3.4), while only lake trout expressed a significant positive relationship between $\delta^{13}C$ and 18:1n-9 (Figure 3.4). There were only two fatty acid-stable isotope ratio relationships that were highly significant and consistent across species: 1) ARA with $\delta^{15}N$, which was a negative, and 2) 16:1n-7 with $\delta^{13}C$, which was positive (Table 3.6; Figure 3.5). The only significant relationship between stable isotope ratios and individual fatty acids expressed as mg/g of wet sample was a consistent positive relationship between 16:1n-7 and $\delta^{13}C$ (Table B.3; Figure B.3).

When fatty acids were combined and expressed as ratios or PCA axes there were several significant relationships with stable isotope ratios (Table 3.7). The ratio of 18:1n-9 to 16:1n-7 and $\delta^{13}C$ had a significant species-specific interaction with brown trout and lake trout having significant negative relationships and other species lacking relationships (Figure 3.4). Additionally, relationships between $\delta^{13}C$ and all three PCA axes with fatty acids expressed as relative abundance exhibited species-specific interactions (Table 3.7).

Brown trout and lake trout exhibited positive slopes, whereas the other species again lacked relationships in the relationship between PC 1 and $\delta^{13}C$ (Figure 3.6). For the relationship between PC 2 and $\delta^{13}C$, brown trout and lake trout had negative slopes and Chinook salmon had a positive slope, whereas Coho salmon and rainbow trout lacked significant slopes (Figure 3.6). All species, except rainbow trout, exhibited significant negative slopes in the relationship between PC 3 and $\delta^{13}C$ (Figure 3.6). Additionally, we observed a consistent negative relationship across all five species in the relationship between PC 2 and $\delta^{15}N$ (Figure 3.5). There were several significant relationships ($p<0.05$) between species-specific PCA axes and stable isotope ratios and we observed three highly significant relationships: 1) positive relationship between PC 1 and $\delta^{15}N$ in Coho salmon, 2) negative relationship between PC 1 and $\delta^{13}C$ in lake trout, and 3) a negative relationship between PC 3 and $\delta^{13}C$ in Chinook salmon (Table 3.8; Figure 3.7).

We observed three highly significant species-specific interactions in relationships between stable isotopes and PCA axes with fatty acids expressed as mg/g of wet sample (Table B.3). For the relationship between PC 2 and $\delta^{13}C$, brown trout and lake trout exhibited positive relationships, whereas the other species lacked relationships (Figure B.3). Brown trout and lake trout exhibited negative slopes, Coho salmon exhibited a positive slope, and Chinook salmon and rainbow trout exhibited no relationship in the relationship between PC 3 and $\delta^{13}C$ (Figure B.3). For the relationship between PC 3 and $\delta^{15}N$, brown trout and lake trout exhibited a negative and positive slope, respectively, while the other three species exhibited no relationships (Figure B.3). Lastly, we observed one highly significant relationship between stable isotope ratios and species-specific PCA axes, which was a negative relationship between PC 2 and $\delta^{13}C$ in lake trout (Figure B.4).

3.3.2.2 Stomach Contents-Other Trophic Indicators

We observed relatively few highly significant relationships between stomach contents and other trophic indicators, but all relationships that were highly significant exhibited consistent relationships across the five species (Tables 3.6 & 3.7). $\delta^{13}C$ and 16:1n-7 expressed as relative abundance were negatively related with proportion of alewife, whereas salmonid-wide PC 3 had a positive relationship with proportion of alewife (Figure 3.5). Additionally, we observed a highly significant relationship between Chinook salmon's species-specific PC 3 and proportion of alewife (Figure 3.7). Lastly, we observed one highly significant relationship between fatty acids expressed as mg/g of wet sample and stomach contents, which was a positive relationship between 16:1n-7 and round goby (Figure B.3).

3.3.2.3 Fatty Acids Expressed as Relative Abundance Versus mg/g of Wet Sample

Overall, we observed more significant relationships with fatty acids expressed as relative abundance compared to mg/g of wet sample (Tables 3.6, 3.7 & B.3). Additionally, when these two methods had significant relationships with the same trophic indicator, relationships were in similar directions (Figures 3.3 & B.2). In response to fewer relationships with fatty acids expressed as mg/g of wet sample and the similarity of relationships between the two methods, the discussion will focus on results involving fatty acids expressed as relative abundance.

3.3.3 Lake-wide and Regional Agreement of Observed and Predicted Stable Isotope Ratios

The agreement of lake-wide observed $\delta^{13}C$ and $\delta^{15}N$ values and predicted $\delta^{13}C$ and $\delta^{15}N$ values from linear mixing models were variable across the five salmonid species

(Table 3.9; Figure 3.8). Brown trout and lake trout lake-wide observed and predicted stable isotope ratios were not significantly different from each other (Table 3.9). Lake-wide predicted $\delta^{13}C$ values of Chinook salmon were significantly greater than observed $\delta^{13}C$ values, whereas for rainbow trout predicted $\delta^{13}C$ values were significantly less than observed $\delta^{13}C$ values (Table 3.9; Figure 3.8). Lake-wide predicted $\delta^{15}N$ values of Chinook and Coho salmon were significantly greater than observed $\delta^{15}N$ values (Table 3.9; Figure 3.8).

Agreement of observed and predicted $\delta^{13}C$ and $\delta^{15}N$ values were not only variable across species, but also regionally in Lake Michigan (Table 3.10; Figure 3.8). There were no significant differences within regions between observed and predicted $\delta^{13}C$ in brown trout and Coho salmon (Table 3.10). Chinook salmon predicted $\delta^{13}C$ values were significantly larger than observed $\delta^{13}C$ values in the northwest (Table 3.10; Figure 3.8). Lake trout predicted $\delta^{13}C$ values were significantly less than observed $\delta^{13}C$ values in the eastern half of the lake, whereas rainbow trout predicted $\delta^{13}C$ values were significantly less than observed $\delta^{13}C$ values in all regions, except the northeast (Table 3.10; Figure 3.8). When differences occurred between observed and predicted $\delta^{15}N$ values, predicted $\delta^{15}N$ values were significantly greater than observed $\delta^{15}N$ values, except for lake trout in southwest (Table 3.10; Figure 3.8). There were no significant differences between observed and predicted $\delta^{15}N$ values in rainbow trout (Table 3.10).

3.4 Discussion

Analysis of stomach contents (Hyslop, 1980), stable isotopes ratios (Fry, 2006), and fatty acids (Napolitano 1999) are common methods for identifying trophic relationships within a wide range of aquatic ecosystems. These trophic indices each represent different

timescales and have their own biases (e.g., Brush et al., 2012; Happel et al., 2015b; MacDonald et al., 1982), but nonetheless one might expect that distinct, consistent feeding patterns could be similarly quantified by all three diet metrics. Observing consistent trends across these trophic indicators in multiple species could improve generalizations of trophic relationships. However, across multiple ecosystems there is evidence that these trophic indicators do not always agree and agreement can be inconsistent across species (Brush et al., 2012; Dethier et al., 2013; Feiner and Foley et al., in press). During this study, we observed that trophic indicators agreed with each other in expected directions, but there were several instances where agreement was inconsistent across species or not in *a priori* expected directions. Additionally, we observed mixed results when using linear mixed models based on stomach contents to predict stable isotope ratios across species and spatially across Lake Michigan. These results provide additional evidence that caution should be taken when using these diet indicators to generalize trophic relationships across multiple taxa.

Similar to $\delta^{13}C$, fatty acids are often used to infer energy pathway supporting consumer growth, for example relatively high concentrations of ALA and DHA may indicate reliance on benthic and pelagic pathways, respectively (e.g., Czesny et al., 2011; Happel et al., 2015a, b). However, models between fatty acids and $\delta^{13}C$ were not consistent across species. We observed strong species-specific interactions between $\delta^{13}C$ and fatty acid variables such as DHA, EPA, 18:1n-9, ratio of 18:1n-9 to 16:1n-7, and salmonid-wide PCA axes. For many of these models, brown trout and/or lake trout expressed significant $\delta^{13}C$-fatty acid relationships, often in the same direction, whereas Chinook salmon, Coho salmon, and rainbow trout typically lacked significant relationships. Lack of agreement in

our results is similar to Feiner and Foley et al. (in press), who also observed several species-specific interactions between stable isotopes and fatty acids within forage fish (i.e., round goby, spottail shiner, and yellow perch). Inconsistent relationships across salmonid species may be influenced by species-specific diet patterns. Brown trout and lake trout consume both pelagic alewife and benthic round goby (Leonhardt et al., in prep; Happel et al., 2017), which have distinct fatty acid compositions (Czesny et al., 2011; Happel et al., 2016a) and $\delta^{13}C$ (Turschak & Bootsma, 2015; Table 3). This could lead to more contrast in fatty acid compositions and $\delta^{13}C$ among individuals and may result in stronger and similar relationships between brown trout and lake trout. For example, $\delta^{13}C$ of brown trout and lake trout in fatty acid-stable isotope models had larger standard deviations of $\delta^{13}C$ (1.22 and 1.02, respectively), compared to Chinook salmon, Coho salmon, and rainbow trout (0.86, 0.61, and 0.59, respectively). Additionally, $\delta^{13}C$ of alewife collected in 2016 had smaller standard deviations (0.53) compared to round goby (2.14). The high importance of alewife and minimal importance of benthic prey items in stomachs of Chinook salmon, Coho salmon, and rainbow trout could restrict the variation of fatty acid abundance and $\delta^{13}C$ among individuals, which could be why we observed few significant slopes for these species.

Although fatty acids are acquired through consuming prey, some fatty acids are metabolized *in vivo*, which is influenced by environmental conditions (e.g., temperature; lack of prey; Bowden et al., 1996; Farkas et al., 1980; Hsieh et al., 2003; Kiessling et al., 1990). Thermal preferences vary across the five salmonids with lake trout preferring the coldest temperatures (8-10ºC; Bergstedt et al., 2003; Olson et al., 1988; Stewart et al., 1983) and brown trout, Chinook salmon, Coho salmon, and rainbow trout preferring warmer

temperatures (13-16 ºC (Larsson, 2005; Magnuson et al., 1990; Olson et al., 1988; Rand et al., 1993). In response to cold temperatures, fish maintain homeoviscous fluidity in cell membranes by retaining unsaturated fatty acids (Bowden et al., 1996; Hsieh et al., 2003). With lake trout occupying cooler temperatures, lake trout may regulate their fatty acids differently than species occupying warmer temperatures, which may lead to species-specific differences in fatty acid regulation. In addition to temperature, fish have been shown to internally regulate their fatty acids differently in response to feeding rates (Kiessling et al., 1990). Chinook salmon are known to select alewife over other prey items even though alewife abundance has declined to extremely low levels relative to past years (Jacobs et al., 2013). Chinook salmon and others may go for longer periods between feedings due to their dependence on alewife, which may lead to additional differences among individuals and species in the regulation of fatty acids. Additionally, fatty acid compositions can vary between sexes of fish due differences in mobilization of lipids into gonads by males and females (Henderson et al., 1984), which could have potentially affected the relationships we observed. However, it is unlikely that sex of salmonids affected our relationships as we saw no highly significant sex-specific relationships within each of the salmonid species (Tables B.6-B.10).

Many of the introduced salmonid strains (brown trout, Chinook salmon, Coho salmon, and rainbow trout) in Lake Michigan originated from anadromous (i.e., migrating up rivers from the ocean to spawn) populations in their native range (Crawford 2001; Keller et al., 1990). Juvenile salmonids exhibit the ability to use C18 precursors to synthesize long chain C20 and C22 fatty acids while in freshwater (Tocher, 2003), and once ocean migrations begin they exhibit a fatty acid composition indicative of a marine lifestyle (high

EPA and DHA, low 18:2n-6; (Gong & Farrell, 1990; Haliloğlu et al., 2004; Li & Yamada, 1992; Sheridan et al., 1985). Although previous work has shown that salmonids are able to retain the ability to synthesize essential fatty acids in freshwater (Betancor et al., 2016; Li & Yamada, 1992), these studies did not compare across species. If there is variability in the ability of anadromous salmonids to adjust their elongase activity in response to spending adulthood in freshwater, this could lead to differences in fatty acid regulation among species. Investigating differences in the activity of elongases in the landlocked salmonids of the Great Lakes could help further elucidate the relationships observed and improve interpretations of fatty acid compositions.

For the most part when fatty acid-stable isotope ratio relationships were significant, relationships were in *a priori* expected directions. For brown trout and lake trout, the $\delta^{13}C$ and DHA relationships were significantly negative, which is consistent with previous assumptions that DHA is associated with pelagic resource use (e.g., Czesny et al., 2011). Also, $\delta^{13}C$ and 18:1n-9/16:1n-7 had negative slopes in brown trout and lake trout. Similarly, 18:1n-9/16:1n-7 has previously been used as a measure of alewife to round goby consumption in Laurentian Great Lakes salmonids (Happel et al., 2016), and for brown trout and lake trout 18:1n-9/16:1n-7 was a significant measure of $\delta^{13}C$. In addition, we documented a consistent positive relationship between $\delta^{13}C$ and 16:1n-7 across the five species of salmonids, which further supports that 16:1n-7 is associated with benthic resource use (e.g., Czesny et al., 2011; Foley et al., 2017). We also observed a consistent negative relationship between ARA and $\delta^{15}N$ across the five salmonids species. In addition to benthic resource use, ARA has been associated with subsidizing diets with terrestrial resources (Ahlgren et al., 2009). Terrestrial insects were consumed primarily by smaller

salmonids (<600 mm; Leonhardt et al., in prep), which may explain the negative relationship with $\delta^{15}N$.

Additionally, significant relationships between stable isotopes and salmonid-wide and species-specific PCA axes were, for the most part, in expected directions. For example, $\delta^{13}C$ and salmonid-wide PC 2, which could be interpreted as a gradient of benthic (negative loadings of 16:1n-7; Czesny et al., 2011; Foley et al., 2017) to pelagic resource use (positive loadings of 16:0; Czesny et al., 2011), had negative relationships in brown trout and lake trout. Additionally, $\delta^{13}C$ and salmonid-wide PC 3, which represented a similar benthic (negative loadings of 16:1n-7; Czesny et al., 2011; Foley et al., 2017) to pelagic gradient (positive loadings of 18:2n-6; Czesny et al., 2011) exhibited negative relationships in all species, except rainbow trout. There were also significant relationships with species-specific PCA axes that were in expected directions, such as Coho salmon having a positive relationship between $\delta^{15}N$ and its PC 1, which was interpreted as invertivory (negative loadings of DHA and 16:0; Czesny et al., 2011) to piscivory (positive loadings of 18:1n-9; Czesny et al., 2011). These results provide additional evidence that using a more holistic approach for investigating fatty acid composition, like PCA, instead of relying solely on individual fatty acids can help improve interpretations (Feiner and Foley et al., in press).

We observed several significant relationships between fatty acids and stable isotopes, but not all relationships were in *a priori* expected directions. We documented positive relationships between $\delta^{13}C$ and 18:1n-9 and $\delta^{13}C$ and salmonid-wide PC 1 in lake trout and both brown trout and lake trout, respectively. PC 1 was negatively associated with DHA and positively associated with 16:1n-7 and 18:1n-9. Additionally, we observed a negative relationship between $\delta^{13}C$ and Chinook salmon's species-specific PC 3, which

loaded negatively towards 18:1n-9. These relationships were unanticipated since 18:1n-9 is associated with alewife, which have relatively small $\delta^{13}C$. Happel et al. (2017) documented that 18:1n-9 increased with size even though there was evidence that large lake trout appeared to rely more on benthic resources compared to smaller lake trout. The combination of 18:1n-9 being absorbed more readily into the tissue compared to longer chain fatty acids in salmonids (C20 and 22; Tocher, 2003) and round goby also having relatively high amounts of 18:1n-9 (Foley et al., 2017; Happel et al., 2016a) may result in these positive relationships in lake trout and brown trout. In addition, 18:1n-9 is elongated from 18:0 (Tocher, 2003) and it appears that fish lack the ability to elongate or desaturate 18:1n-9 into other fatty acids. With 18:1n-9 representing an endpoint in fatty acid elongation and having positive relationships with $\delta^{13}C$, it may not be the best indicator of alewife consumption. Additionally, we observed contrasting results between brown trout and lake trout in the relationship between $\delta^{13}C$ and EPA. Brown trout had a significant positive relationship, whereas lake trout had a significant negative relationship even though they exhibit similar diet patterns across space and time (Leonhardt et al., in prep). With EPA being a precursor for DHA (Tocher 2003), it is possible that EPA concentrations are regulated differently between the two species, so EPA may not be a reliable indicator of trophic pathway use that can be generalized for these species.

Although we observed several significant relationships between stomach contents and other trophic indicators, few were highly significant. This is inconsistent with Feiner and Foley et al. (in press) who documented several highly significant relationships between stomach contents, stable isotope ratios, and fatty acids in round goby, spottail shiner, and yellow perch. Lack of stronger relationships could be related to spatio-temporal diet

patterns of salmonids and integration times for fatty acids and stable isotopes into salmonid tissue. Lake Michigan salmonids are known to have seasonally-variable feeding habits, for example brown trout and lake trout of Lake Michigan tend to consume more round goby in the spring compared to late summer and early fall (Leonhardt et al., in prep). Additionally, salmonids are larger and grow at slower rates than round goby, yellow perch, and spottail shiner, so more time is needed for fatty acids and stable isotopes of consumed prey to fully integrate into tissues (Happel et al., 2016b; Hesslein et al., 1993). Due to longer integration times, fatty acid compositions and stable isotope ratios may not accurately reflect the stomach contents of salmonids at time of collection (Perga & Gerdeaux, 2005).

Although, we found few highly significant consistent relationships between $\delta^{13}C$ and stomach contents and fatty acids, we found that $\delta^{13}C$ could be a reliable predictor of alewife and round goby consumption in Lake Michigan salmonids. We saw that individuals that consumed less alewife had a higher proportional abundance of 16:1n-7 and greater $\delta^{13}C$. In addition, we saw a significant positive relationship (although not highly significant) between round goby consumption and 16:1n-7 and $\delta^{13}C$ in brown trout and lake trout. These results are consistent with round gobies having a higher proportional abundance of 16:1n-7 and greater $\delta^{13}C$ compared to alewife (Czesny et al., 2011; Foley et al., 2017; Turschak & Bootsma, 2015). Additionally, we observed a positive relationship between alewife consumption and Chinook salmon's species-specific PC 3, which was somewhat unexpected because 18:1n-9 was loading negatively on PC 3. As mentioned before, this may be additional evidence that 18:1n-9 may not be the best indicator of alewife consumption for salmonids.

Lake-wide and regional comparisons of predicted $\delta^{13}C$ and $\delta^{15}N$ values from linear mixed models based on stomach contents and observed $\delta^{13}C$ and $\delta^{15}N$ values showed mixed results for agreement of these trophic indicators. Generally, predicted $\delta^{15}N$ values were significantly greater than observed $\delta^{15}N$ values, especially in Chinook and Coho salmon, and in the northwest region for all species except rainbow trout. Overall, we suspect that greater predicted $\delta^{15}N$ values are a result of large alewife being over-represented in the stomach contents of salmonids. Small alewife and *Bythotrephes* have similar $\delta^{13}C$ values as large alewife, but have smaller $\delta^{15}N$ values (Table 3), which could indicate that the importance of either small alewife, *Bythotrephes* or both are being underestimated in stomachs, especially for Chinook salmon, Coho salmon, and for salmonids collected in the northwest region. Small alewife were much more abundant than large alewife in 2016 (Bunnell et al., 2017) and the length frequency of alewife consumed by salmonids in 2016 was dominated by small alewife (Leonhardt et al., in prep). So, it is possible that salmonids could be consuming more small alewife than is being observed in stomachs.

Predicted $\delta^{13}C$ values of lake trout were significantly less than observed $\delta^{13}C$ values in the eastern half of the lake, which indicates that benthic resource use is being under-represented. In the northeast, it appears that round goby and/or rainbow smelt could be underestimated in the diets of lake trout as they both have more benthic signatures than alewife and are common prey items for lake trout in northern areas of Lake Michigan (Happel et al., 2017; Leonhardt et al., in prep). In contrast in the southeast, the results suggest that round goby may be over-represented in the diets of lake trout, which is surprising since round goby now dominate the diets of lake trout in the southeast (Happel

et al., 2017; Leonhardt et al., in prep). This is likely a result of medium (60-100 mm) and large round goby (>100 mm) having smaller $\delta^{13}C$ values compared to round goby in other regions of Lake Michigan and alewife in the southeast region (Table 10; Foley et al., 2017). Although, small round goby in the southeast (<60 mm) and rainbow smelt have similar $\delta^{13}C$ values as lake trout in the southeast, it is unlikely that they are being under-represented since they have relatively large $\delta^{15}N$ values (Table B.5). It is quite clear that lake trout are using benthic pathways in the southeast region, but based on stomach content analyses and 2016 prey fish collections it is difficult to determine the origin.

Relative to the other salmonid species, rainbow trout stomach contents did a poor job of estimating $\delta^{13}C$. The predicted $\delta^{13}C$ values of rainbow trout were significantly less than observed $\delta^{13}C$ values, which indicates that stomach contents are underestimating the importance of benthic resource (i.e., round goby) use by rainbow trout and overestimating alewife and terrestrial insects. Although round goby were uncommon in stomachs of Lake Michigan rainbow trout, round goby now make up nearly 15% of the diet composition by weight in Lake Huron rainbow trout (Roseman et al., 2014). So, it is not unlikely that rainbow trout could be consuming more benthic food items in Lake Michigan, like round goby or yellow perch, which would result in larger $\delta^{13}C$ values. Additionally in recent years, the catch rates of rainbow trout have shifted to shallower and more nearshore habitats in Lake Michigan (Simpson et al., 2016), which further supports the idea that rainbow trout are using more nearshore, benthic resources than might be expected from stomach contents.

Disagreement between predicted and observed stable isotope ratios could be due to biases associated with stomach content analysis and methods used to collect salmonids. There are differing rates of digestion and assimilation of soft-bodied prey (e.g., fish prey)

and hard-bodied prey (e.g., terrestrial insects; Kionka & Windell, 1972), which may lead to either over- or underestimates of particular prey items in stomachs. For example, terrestrial insects were an important diet component in rainbow trout stomachs. The high importance of terrestrial insects may be related to chitin in insect exoskeletons taking longer to digest than soft-bodied prey (Kionka & Windell, 1972). Terrestrial insects that were collected in 2016 had smaller $\delta^{13}C$ relative to other prey items, so the high importance of terrestrial insects in rainbow trout stomachs likely caused predicted $\delta^{13}C$ values to be less than observed $\delta^{13}C$ values. Additionally, our study was dependent on acquiring salmonid stomachs and tissue samples from primarily recreational anglers, which do not have homogenous effort across the lake and may concentrate their effort to relatively small areas of Lake Michigan (Simpson et al., 2016). For example, rainbow trout are attracted to thermal bars that can accumulate terrestrial insects (Aultman & Haynes, 1993; Höök et al., 2004), so anglers targeting rainbow trout may target these small areas of the lake exclusively. Also, many anglers target salmonids at the thermocline, which is where alewife tend to congregate (Olson et al., 1988). Salmonids, like rainbow trout, caught at the thermocline may have an increased chance of having alewife rather than round goby or other benthic prey items in their stomach. The combination of slow digestion of terrestrial insects and anglers fishing for rainbow trout near thermal bars and at the thermocline likely leads to the over-representation of terrestrial insects and alewife in the stomachs of rainbow trout resulting in predicted $\delta^{13}C$ values being less than observed $\delta^{13}C$ values. Lastly, small and large alewife do not typically school together with small alewife preferring waters above the thermocline and large alewife below the thermocline (Brandt et al., 1980). Additionally, salmonids have similar thermal tolerances as large alewife (Brandt et al.,

1980). This likely influences the size of alewife found in angler-caught salmonid stomachs. Salmonids targeted at the bottom of the thermocline may have an increased chance of having large alewife in the stomach despite small alewife being much more abundant (Bunnell et al., 2017).

By examining agreement of trophic indicators, we found inconsistent agreement across the five salmonid species. Disagreements of relationships between trophic indicators among the five species reveal that there may be species-specific differences in the regulation of fatty acids, which is not typically considered when interpreting fatty acid compositions for these species. Additionally, disagreement of predicted $\delta^{13}C$ and $\delta^{15}N$ values from stomach contents and observed $\delta^{13}C$ and $\delta^{15}N$ values may be attributed to biases associated with digestion rates and fish collection methods. But, this also revealed that stomach contents are underestimating the importance of benthic resource use, particularly in rainbow trout. These results encourage the continued use of multiple trophic indicators and to keep in mind biases when inferring diet patterns of not only Laurentian Great Lakes salmonids, but other fish species as well.

Table 3.1. Mean proportional lake-wide diet composition by weight of each diet category for salmonids with both stomach contents and stable isotope ratios analyzed in 2016. Not included are unidentifiable stomach contents and prey that lack known stable isotope ratios in Lake Michigan. BRT=brown trout; CHS=Chinook salmon; COS=Coho salmon; LAT=lake trout; RBT=rainbow trout.

Prey categories	BRT	CHS	COS	LAT	RBT
Alewife <100 mm	0.281	0.282	0.160	0.153	0.202
Alewife >100 mm	0.108	0.326	0.298	0.398	0.118
Alewife unsized	0.107	0.119	0.085	0.093	0.120
Amphipod	0.033	0	0	0	0
Bloater	0.067	0.057	0.051	0.019	0.034
Bythotrephes	0.074	0.143	0.223	0.019	0.118
Chironomidae	0	0	0	0	0.000
Dreissenidae	0.049	0	0	0.019	0
Mysis	0	0.015	0.059	0.000	0.024
Rainbow Smelt	0	0.015	0	0.019	0
Round Goby < 60 mm	0.059	0	0	0	0
Round Goby 60-100 mm	0.082	0	0.009	0.145	0.006
Round Goby >100 mm	0.070	0	0.012	0.089	0
Round Goby unsized	0.038	0	0.001	0.025	0
Sculpin	0	0	0	0.019	0
Terrestrial Insect	0.033	0.015	0.039	0	0.334
Three-spine stickleback	0	0	0.017	0	0
Yellow Perch	0	0.028	0.047	0	0.043

Table 3.2. Components of three trophic measures that were compared within individual salmonids and expected relationships.

	Components used in analyses	Expected to be positively correlated with
Fatty acids	α-linolenic acid (ALA)	Benthic reliance[a,b,f]
	Arachidonic acid (ARA)	Benthic reliance[f]
	Docosahexaenoic acid (DHA)	Pelagic reliance[a,b,c,e]
	Eicosapentaenoic acid (EPA)	Benthic reliance[a,d]
	n-3/n-6	Benthic reliance[d]
	Palmitoleic acid (16:1n-7)	Benthic reliance[a,b,c]; proportion round goby[g,h]
	Oleic acid (18:1n-9)	Proportion alewife[e,g]
	18:1n-9/16:1n-7	Proportion alewife[g]; inverse proportion round goby[g]
Stable Isotopes	$\delta^{13}C$	Benthic reliance[i]
	$\delta^{15}N$	Trophic level[j]
Stomach contents	Proportion alewife	18:1n-9[e,g], pelagic reliance
	Proportion round goby	16:1n-7[e,g,h]; benthic reliance

[a]Happel et al., 2015a
[b]Happel et al., 2015b
[c]Foley et al., 2016
[d]Feiner and Foley et al., in press
[e]Czesny et al., 2011
[f]Paterson et al., 2014
[g]Happel et al., 2016
[h]Happel et al., 2017
[i]Post 2002
[j]Fry 2006

Table 3.3. Lake-wide mean $\delta^{13}C$ and $\delta^{15}N$ of Lake Michigan salmonid prey items used in linear mixing models to estimate $\delta^{13}C$ and $\delta^{15}N$ from stomach contents of salmonids.

Prey Item	$\delta^{13}C$	$\delta^{15}N$
Alewife <100 mm[a]	-24.3	7.1
Alewife >100 mm[a]	-24.6	9.7
Alewife unsized*[a]	-24.4	8.2
Amphipods[b]	-16.9	3.5
Bloater[a]	-25.6	9.8
Bythotrephes[c]	-24.4	5.7
Chironomidae[d]	-18.3	5.1
Dreissenidae[b]	-27.0	8.9
Mysis[b]	-24.4	10.1
Rainbow Smelt[a]	-23.2	10.3
Round Goby < 60 mm[a]	-21.6	8.6
Round Goby 60-100 mm[a]	-22.2	8.8
Round Goby >100 mm[a]	-23.2	8.7
Round Goby unsized*[a]	-22.3	8.7
Sculpins[a]	-24.1	11.3
Terrestrial Insect[a]	-26.4	4.9
Three-spine Stickleback[b]	-25.2	10.3
Yellow Perch <100[a]	-21.3	9.5

*Mean across all sizes
[a] based on 2016 collection
[b] from Turschak 2014
[c] from Driscoll et al., 2015

Table 3.4. Regional mean $\delta^{13}C$ and $\delta^{15}N$ of alewife size groups and round goby used in linear mixing models to estimate $\delta^{13}C$ and $\delta^{15}N$ for salmonids in each region of Lake Michigan.

Prey Item	Region	$\delta^{13}C$	$\delta^{15}N$
Alewife <100 mm[a]	Northwest	-24.2	7.7
	Southwest	-24.7	8.2
	Southeast	-23.9	5.3
	Northeast**	-24.3	7.1
Alewife >100 mm[a]	Northwest	-24.8	11.9
	Southwest	-24.6	8.8
	Southeast	-24.1	6.1
	Northeast**	-24.6	9.7
Alewife unsized*[a]	Northwest	-24.5	9.8
	Southwest	-24.7	8.5
	Southeast	-24.0	5.5
	Northeast**	-24.4	8.2
Round Goby < 60 mm[a]	Northwest	-20.9	8.2
	Southwest	-19.4	8.7
	Southeast	-23.6	9.8
	Northeast	-20.5	7.3
Round Goby 60-100 mm[a]	Northwest	-20.7	9.1
	Southwest	-21.0	8.4
	Southeast	-24.4	9.3
	Northeast	-21.2	8.2
Round Goby >100 mm[a]	Northwest	-21.0	9.3
	Southwest**	-23.2	8.7
	Southeast	-25.6	8.8
	Northeast	-22.7	8.3
Round Goby unsized*[a]	Northwest	-20.8	9.1
	Southwest	-20.2	8.6
	Southeast	-24.5	9.3
	Northeast	-21.5	7.9

*Mean across all sizes in that region

**lake-wide mean for that size group

[a] based on 2016 collection

Table 3.5. Loadings of PCA analyses completed on all salmonids and separately for each species using fatty acids expressed as relative abundance.

	All Salmonids			Brown Trout			Chinook Salmon		
Fatty Acid	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
C14:0	0.050	0.177	0.076	0.098	0.113	0.061	0.082	0.122	0.120
C16:0	-0.087	0.697	-0.493	-0.066	0.722	0.215	-0.094	0.618	-0.369
C16:1n-7	0.333	-0.423	-0.593	-0.666	0.253	0.000	0.149	0.094	0.090
C18:0	-0.049	0.269	-0.178	-0.104	0.143	0.022	-0.090	0.065	-0.292
C18:1n-9	0.633	-0.041	0.126	-0.391	-0.518	0.561	0.609	-0.443	-0.544
C18:1n-7	0.107	-0.149	-0.128	-0.270	-0.151	-0.788	0.072	0.041	0.079
C18:2n-6	0.051	0.164	0.367	0.230	-0.056	0.000	0.164	0.002	0.137
C18:3n-3	0.027	0.105	0.215	0.170	0.000	0.005	0.117	0.123	0.179
C18:4n-3	0.010	0.010	0.058	0.059	0.016	0.006	0.046	0.092	0.089
C20:1	0.050	0.007	0.106	0.009	-0.135	0.022	0.055	-0.074	0.060
C20:2n-6	-0.015	0.009	0.119	0.080	-0.044	0.006	0.011	-0.018	0.056
C20:4n-6	-0.078	-0.069	-0.049	0.019	-0.035	-0.003	-0.077	0.014	-0.066
C20:3n-3	-0.021	0.002	0.116	0.097	-0.032	-0.007	0.011	0.008	0.095
C20:4n-3	-0.024	0.013	0.258	0.200	-0.053	0.008	0.050	0.033	0.203
C20:5n-3	-0.140	-0.173	-0.167	-0.001	0.091	-0.074	-0.151	0.201	0.042
C22:5n-6	-0.090	-0.100	0.065	0.089	-0.092	0.010	-0.077	-0.080	-0.006
C22:5n-3	-0.100	-0.224	0.089	0.002	-0.207	-0.079	-0.186	-0.445	0.431
C22:6n-3	-0.643	-0.274	-0.067	0.395	0.041	0.033	-0.678	-0.342	-0.383
Prop. Variance	0.41	0.28	0.12	0.53	0.16	0.12	0.54	0.18	0.11

Table 3.5 continued

	Coho Salmon			Lake Trout			Rainbow Trout		
Fatty Acid	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
C14:0	0.084	0.025	0.197	0.017	0.035	0.271	0.023	0.118	0.223
C16:0	-0.422	-0.585	0.385	-0.076	-0.560	0.653	-0.102	0.859	0.232
C16:1n-7	0.108	-0.140	0.125	-0.508	-0.491	-0.394	-0.289	-0.054	0.182
C18:0	-0.215	-0.317	-0.048	-0.081	-0.158	-0.036	-0.041	0.161	-0.185
C18:1n-9	0.535	-0.543	-0.551	-0.615	0.581	0.163	-0.780	-0.076	-0.443
C18:1n-7	0.074	0.042	0.128	-0.072	-0.070	-0.089	-0.036	-0.097	0.157
C18:2n-6	0.178	0.162	0.051	0.135	0.187	0.146	-0.102	-0.109	0.219
C18:3n-3	0.070	0.114	0.090	0.162	0.082	0.140	0.029	-0.051	0.161
C18:4n-3	0.002	0.031	0.038	0.096	0.019	0.073	0.053	-0.018	0.070
C20:1	0.106	0.048	0.030	-0.041	0.090	-0.061	-0.026	-0.107	-0.057
C20:2n-6	0.062	0.113	0.041	0.052	0.047	0.013	0.038	-0.079	0.009
C20:4n-6	-0.102	0.010	-0.046	0.004	0.038	-0.120	0.109	-0.035	-0.033
C20:3n-3	0.061	0.121	0.075	0.083	0.030	0.030	0.047	-0.072	0.044
C20:4n-3	0.131	0.233	0.090	0.166	0.141	0.093	0.138	-0.142	0.098
C20:5n-3	-0.185	0.024	0.093	0.105	-0.018	-0.008	0.187	-0.049	0.128
C22:5n-6	0.001	0.081	-0.085	0.047	0.029	-0.125	0.095	-0.073	-0.094
C22:5n-3	0.083	0.263	-0.003	0.019	-0.017	-0.397	0.178	-0.284	-0.022
C22:6n-3	-0.584	0.201	-0.655	0.490	-0.036	-0.246	0.412	0.239	-0.698
Prop. Variance	0.61	0.17	0.12	0.59	0.16	0.09	0.50	0.22	0.11

Table 3.6. Results of pairwise comparisons between fatty acids and stable isotopes or stomach contents. Fatty acid-stable isotope regressions were linear models using F-statistics and fatty acid-diet content regressions were logistic GLMs using χ^2. Models with p-values less than 0.001 are bolded and those that are between 0.05 and 0.001 are italicized. Interaction terms were removed from models if p-values were greater than 0.05. Fatty acids are expressed as relative abundance.

Fatty Acid	Response	Species	Species P	FA	FA P	Interaction	Interaction P	R2
ALA	*$\delta^{13}C$*	*5.20*	*<0.0001*	*13.56*	*0.0003*	*3.30*	*0.0115*	*0.40*
ALA	*$\delta^{15}N$*	12.47	*<0.0001*	5.17	0.0237	4.37	0.0019	*0.61*
ALA	*Alewife*	*31.93*	*<0.0001*	*6.75*	*0.0094*	-	-	*0.13*
ALA	*Round goby*	*0.82*	*0.3657*	*3.95*	*0.0469*	-	-	*0.04*
ARA	$\delta^{13}C$	17.41	<0.0001	3.31	0.0699	-	-	0.22
ARA	**$\delta^{15}N$**	**108.84**	**<0.0001**	**15.564**	**0.0001**	-	-	**0.61**
ARA	*Alewife*	*33.05*	*<0.0001*	*6.40*	*0.0115*	-	-	*0.12*
ARA	*Round goby*	0.28	0.5948	0.43	0.5120	-	-	0.01
DHA	**$\delta^{13}C$**	**15.24**	**<0.0001**	**17.97**	**<0.0001**	**8.65**	**<0.0001**	**0.3**
DHA	*$\delta^{15}N$*	*102.03*	*<0.0001*	*6.64*	*0.0105*	-	-	*0.6*
DHA	*Alewife*	*22.81*	*0.0001*	*5.87*	*0.0154*	*13.93*	*0.0075*	*0.14*
DHA	*Round goby*	*1.37*	*0.2415*	*6.07*	*0.0137*	-	-	*0.06*
EPA	**$\delta^{13}C$**	**7.48**	***<0.0001***	**7.73**	**0.0058**	**5.41**	**0.0003**	**0.26**
EPA	*$\delta^{15}N$*	*2.36*	*0.0536*	*3.25*	*0.0728*	*3.24*	*0.0129*	*0.61*
EPA	*Alewife*	33.35	<0.0001	0.12	0.7243	-	-	0.11
EPA	*Round goby*	0.27	0.603	0.75	0.3852	-	-	0.01
n-3/n-6	*$\delta^{13}C$*	*5.20*	*0.0005*	*2.11*	*0.1476*	*3.44*	*0.0092*	*0.25*
n-3/n-6	*$\delta^{15}N$*	*1.78*	*0.13211*	*3.34*	*0.0685*	*2.74*	*0.0289*	*0.60*
n-3/n-6	Alewife	43.02	<0.0001	0.29	0.5915	-	-	0.12
n-3/n-6	*Round goby*	0.79	0.3733	0.15	0.6957	-	-	0.01

Table 3.6 continued

16:1n-7	**$\delta^{13}C$**	**11.06**	**<0.0001**	**37.28**	**<0.0001**	-	-	**0.31**
16:1n-7	$\delta^{15}N$	45.85	*<0.0001*	3.41	0.0657	-	-	0.59
16:1n-7	**Alewife**	**41.21**	**<0.0001**	**13.53**	**0.0002**	-	-	**0.17**
16:1n-7	*Round goby*	*0.01*	*0.9190*	*8.66*	*0.0033*	-	-	*0.08*
18:1n-9	**$\delta^{13}C$**	**3.57**	**0.0074**	**1.78**	**0.1836**	**4.87**	**<0.0001**	**0.27**
18:1n-9	$\delta^{15}N$	93.26	*<0.0001*	2.42	0.1206	-	-	0.59
18:1n-9	*Alewife*	*49.37*	*<0.0001*	*4.51*	*0.0336*	-	-	*0.12*
18:1n-9	*Round goby*	*<0.01*	*0.9715*	*5.56*	*0.0184*	-	-	*0.05*
18:1n-9/16:1n-7	**$\delta^{13}C$**	**5.28**	**<0.0001**	**13.19**	**<0.0001**	**4.82**	**<0.0001**	**0.30**
18:1n-9/16:1n-7	*$\delta^{15}N$*	*5.92*	*0.0001*	*13.57*	*0.0003*	*3.16*	*0.0147*	*0.60*
18:1n-9/16:1n-7	*Alewife*	*14.45*	*0.0060*	*7.29*	*0.0069*	*11.60*	*0.0206*	*0.15*
18:1n-9/16:1n-7	*Round goby*	*<0.01*	*0.9544*	*8.37*	*0.0038*	-	-	*0.08*

Table 3.7. Results of pairwise comparisons between stable isotopes and stomach contents or salmonid-wide principal components with fatty acids expressed as relative abundance. Models including stomach contents were logistic GLMs using $\chi 2$, whereas PCA axes-stable isotope ratios comparisons were linear models using F-statistics models. Models with p-values less than 0.001 are bolded and those between 0.05 and 0.001 are italicized. Interaction terms were removed from models if p-values were greater than 0.05.

Explanatory	Response	Species	Species P	FA	FA P	Interaction	Interaction P	R2
$\delta^{13}C$	**Alewife**	**7.19**	**0.1260**	**12.19**	**0.0005**	-	-	**0.11**
$\delta^{13}C$	Round goby	0.01	0.9382	3.75	0.0529	-	-	0.06
$\delta^{15}N$	*Alewife*	*10.38*	*0.0345*	*4.15*	*0.0417*	*11.35*	*0.0229*	*0.15*
$\delta^{15}N$	*Round goby*	*8.05*	*0.0046*	*5.44*	*0.0197*	*8.00*	*0.0047*	*0.03*
PC1	*Alewife*	*31.38*	*<0.0001*	*10.27*	*0.0014*	*12.38*	*0.0147*	*0.15*
PC1	*Round goby*	*0.08*	*0.7707*	*8.12*	*0.0044*	-	-	*0.08*
PC1	**$\delta^{13}C$**	**15.65**	**<0.0001**	**12.91**	**0.0004**	**8.67**	**<0.0001**	**0.31**
PC1	*$\delta^{15}N$*	*81.28*	*<0.0001*	*4.53*	*0.0343*	*2.64*	*0.0344*	*0.61*
PC2	Alewife	28.70	<0.0001	0.58	0.4456	-	-	0.13
PC2	Round goby	0.07	0.7877	1.88	0.1702	-	-	0.02
PC2	**$\delta^{13}C$**	**7.95**	**<0.0001**	**29.16**	**<0.0001**	**9.75**	**<0.0001**	**0.31**
PC2	**$\delta^{15}N$**	**49.13**	**<0.0001**	**12.661**	**0.0004**	-	-	**0.61**
PC3	**Alewife**	**46.35**	**<0.0001**	**24.11**	**<0.0001**	-	-	**0.17**
PC3	*Round goby*	*1.14*	*0.2850*	*9.96*	*0.0016*	-	-	*0.09*
PC3	**$\delta^{13}C$**	**106.59**	**<0.0001**	**7.38**	**0.0070**	**9.1727**	**<0.0001**	**0.35**
PC3	*$\delta^{15}N$*	*15.82*	*<0.0001*	*6.32*	*0.0125*	*3.0967*	*0.01624*	0.64

Table 3.8. Results of pairwise comparisons between species-specific fatty acid PCA axes and stable isotopes and stomach contents with fatty acids expressed as relative abundance. Models with stable isotope ratios as response variables were linear models using F-statistics and models involving stomach contents were logistic GLMs using χ^2. Models with p-values less than 0.001 are bolded and those that were between 0.05 and 0.001 are italicized.

		Chinook Salmon			Coho Salmon			Rainbow Trout		
Explanatory	Response	FA	FA P	R^2	FA	FA P	R^2	FA	FA P	R^2
PC 1	Alewife	0.35	0.5523	0.00	1.37	0.2421	0.01	0.43	0.5131	0.01
PC 1	$\delta^{13}C$	0.17	0.6795	-0.01	*7.22*	*0.0095*	*0.10*	2.63	0.1133	0.04
PC 1	$\delta^{15}N$	4.39	0.0398	0.04	**13.50**	**0.0005**	**0.18**	1.28	0.2646	0.01
PC 2	Alewife	0.54	0.4624	0.01	10.20	0.0014	0.11	1.72	0.1903	0.02
PC 2	$\delta^{13}C$	0.51	0.4792	-0.01	1.05	0.3094	0.00	0.44	0.5115	-0.02
PC 2	$\delta^{15}N$	*8.21*	*0.0055*	*0.09*	3.55	0.0649	0.04	0.41	0.5255	-0.02
PC 3	Alewife	**15.36**	**0.0004**	**0.19**	3.61	0.0576	0.04	0.09	0.7608	0.00
PC 3	$\delta^{13}C$	**17.52**	**0.0001**	**0.19**	1.17	0.2843	0.00	1.85	0.1827	0.02
PC 3	$\delta^{15}N$	*7.21*	*0.0090*	*0.08*	0.00	0.9476	-0.02	*4.62*	*0.0384*	*0.09*

Table 3.8 continued

		Brown Trout			Lake Trout		
Explanatory	Response	FA	FA P	R^2	FA	FA P	R^2
PC 1	Alewife	*10.55*	*0.0012*	*0.36*	3.73	0.0534	0.04
PC 1	Round Goby	*7.04*	*0.0080*	*0.21*	*3.94*	*0.0471*	*0.05*
PC 1	$\delta^{13}C$	*8.48*	*0.0056*	*0.14*	**38.29**	**<0.0001**	**0.37**
PC 1	$\delta^{15}N$	*9.51*	*0.0035*	*0.16*	0.65	0.4219	-0.01
PC 2	Alewife	0.00	0.9638	0.00	0.07	0.7971	0.00
PC 2	Round Goby	0.10	0.7513	0.00	0.59	0.4439	0.01
PC 2	$\delta^{13}C$	3.82	0.0571	0.06	*5.07*	*0.0278*	*0.06*
PC 2	$\delta^{15}N$	0.57	0.4544	-0.01	0.03	0.8684	-0.02
PC 3	Alewife	0.36	0.5465	0.01	0.10	0.7496	0.00
PC 3	Round Goby	0.29	0.5872	0.01	0.09	0.7674	0.00
PC 3	$\delta^{13}C$	*8.37*	*0.0059*	*0.14*	*4.38*	*0.0404*	*0.04*
PC 3	$\delta^{15}N$	0.86	0.3590	0.00	0.08	0.7846	-0.01

Table 3.9. Observed and predicted lake-wide $\delta^{13}C$ and $\delta^{15}N$ mean and standard error of Lake Michigan salmonids collected in 2016. Bolded predicted isotopic signatures indicate that they were significantly different from observed based on Wilcoxon Sign Tests.

	$\delta^{13}C$				$\delta^{15}N$			
Species	Observed	Predicted	V	P	Observed	Predicted	V	P
Brown Trout	-23.41 ± 0.23	-23.50 ± 0.33	207	0.612	11.19 ± 0.10	11.25 ± 0.27	275	0.3931
Chinook Salmon	-24.5 ± 0.11	**-24.02 ± 0.08**	**1668**	**0.001**	10.72 ± 0.09	**11.53 ± 0.18**	**1765**	**<0.0001**
Coho Salmon	-24.0 ± 0.08	-24.0 ± 0.11	819	0.482	10.13 ± 0.08	**11.46 ± 0.22**	**1552**	**<0.0001**
Lake Trout	-23.28 ± 0.13	-23.62 ± 0.14	504	0.0929	12.22 ± 0.10	12.26 ± 0.14	746	0.6069
Rainbow Trout	-23.43 ± 0.09	**-24.55 ± 0.18**	**81**	**<0.0001**	10.38 ± 0.08	10.30 ± 0.26	358	0.8634

Note: Expected values are based on the proportion of diet items by weight in stomachs and their respective stable isotope values; observed values are empirical data.

Table 3.10. Observed and predicted regional $\delta^{13}C$ and $\delta^{15}N$ mean and standard error of Lake Michigan salmonids collected in 2016. Bolded predicted isotopic signatures indicate that they were significantly different from observed based on Wilcoxon Sign Tests.

		$\delta^{13}C$				$\delta^{15}N$			
Species	Region	Observed	Predicted	V	P	Observed	Predicted	V	P
Brown Trout	Northwest	-23.81 ± 0.27	-23.85 ± 0.39	27	1	11.16 ± 0.11	**13.05 ± 0.48**	**53**	**0.0059**
	Southwest	-23.03 ± 0.46	-24.22 ± 0.45	10	0.0830	10.90 ± 0.19	11.34 ± 0.41	39	0.2754
	Southeast	-22.76 ± 0.60	-22.82 ± 1.29	6	0.4375	11.31 ± 0.28	11.15 ± 1.06	11	1
	Northeast	-24.36 ± 0.30	-23.01 ± 1.19	8	0.375	11.80 ± 0.21	10.50 ± 0.78	0	0.125
Chinook Salmon	Northwest	-24.94 ± 0.18	**-24.03 ± 0.05**	**210**	**0.0004**	10.78 ± 0.11	**12.63 ± 0.36**	**222**	**<0.0001**
	Southwest	-24.11 ± 0.20	-24.08 ± 0.18	96	0.7562	10.80 ± 0.20	11.26± 0.31	149	0.1054
	Southeast	-24.15 ± 0.19	-23.74 ± 0.22	108	0.1454	10.46 ± 0.19	10.01 ± 0.39	51	0.2435
	Northeast	-25.00 ± 0.14	-24.40 ± 0.16	39	0.0547	10.86 ± 0.18	**12.38 ± 0.45**	**42**	**0.0195**
Coho Salmon	Northwest	-24.23 ± 0.12	-24.23 ± 0.12	38	0.7002	10.25 ± 0.11	**13.11 ± 0.60**	**64**	**0.0029**
	Southwest	-23.74 ± 0.14	-23.76 ± 0.28	53	0.2842	10.16 ± 0.20	**11.69 ± 0.26**	**147**	**0.0002**
	Southeast	-23.85 ± 0.11	-24.15 ± 0.23	55	0.1964	9.97 ± 0.13	10.91 ± 0.47	123	0.1084
	Northeast	-24.32 ± 0.22	-24.03 ± 0.03	69	0.3258	10.19 ± 0.17	10.15 ± 0.46	49	0.8552
Lake Trout	Northwest	-23.42 ± 0.23	-23.92 ± 0.15	28	0.2439	12.53 ± 0.19	**13.95 ± 0.41**	**83**	**0.0061**
	Southwest	-23.49 ± 0.25	-23.76 ± 0.47	45	0.4212	12.29 ± 0.15	**11.78 ± 0.20**	**24**	**0.0413**
	Southeast	-23.11 ± 0.26	**-24.18 ± 0.19**	**15**	**0.0021**	11.68 ± 0.13	11.33 ± 0.40	59	0.4307
	Northeast	-23.00 ± 0.39	**-23.49 ± 0.54**	**1**	**0.0313**	12.82 ± 0.27	11.71 ± 0.40	5	0.1563
Rainbow Trout	Northwest	-23.58 ± 0.18	**-24.55 ± 0.25**	**9**	**0.0161**	10.23 ± 0.12	11.15 ± 0.70	53	0.3013
	Southwest	-23.52 ± 0.14	**-24.47 ± 0.18**	**1**	**0.0039**	10.45 ± 0.11	10.73 ± 0.43	32	0.6953
	Southeast	-23.33 ± 0.20	**-24.40 ± 0.55**	**6**	**0.0273**	10.43 ± 0.22	10.24 ± 0.53	25	0.8457
	Northeast	-23.16 ± 0.19	-25.21 ± 0.47	1	0.0625	10.44 ± 0.14	9.56 ± 0.80	4	0.2188

Note: Expected values are based on the proportion of diet items by weight in stomachs and their respective stable isotope values; observed values are empirical data.

Figure 3.1. Map of Lake Michigan showing the different regions (Northeast=NE; Northwest=NW; Southeast=SE; Southwest=SW) where salmonids and prey fish were collected. Northeast was defined as fish collected at or between the ports of Ludington and Charlevoix. Northwest was defined as fish collected at or between the ports of Manitowoc and Manistique. Southeast was defined as fish collected at or between the ports of Sheboygan and East Chicago. Southeast was defined as fish collected at or between the ports of Muskegon and Burns Harbor.

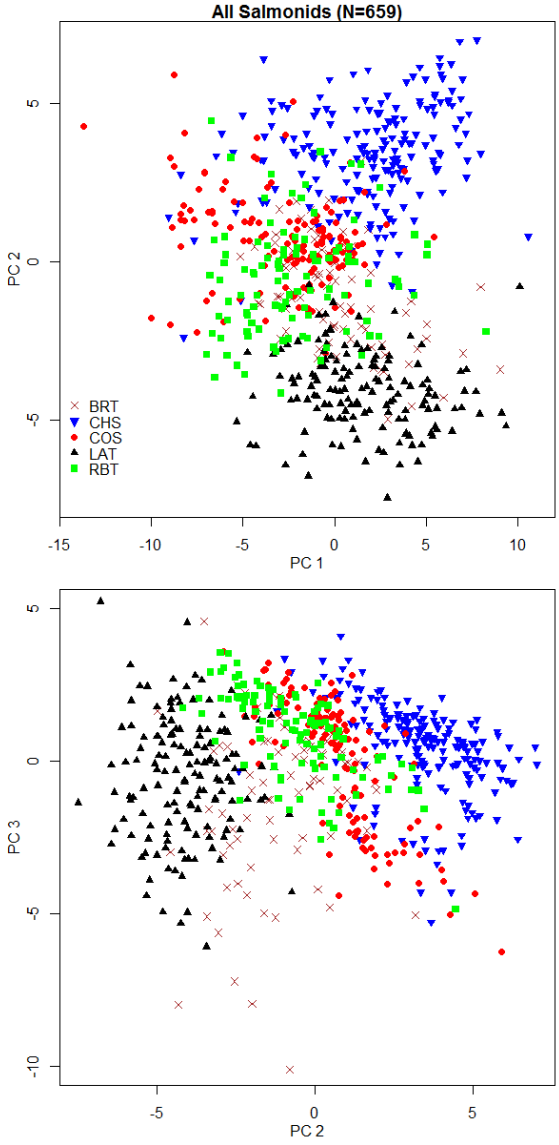
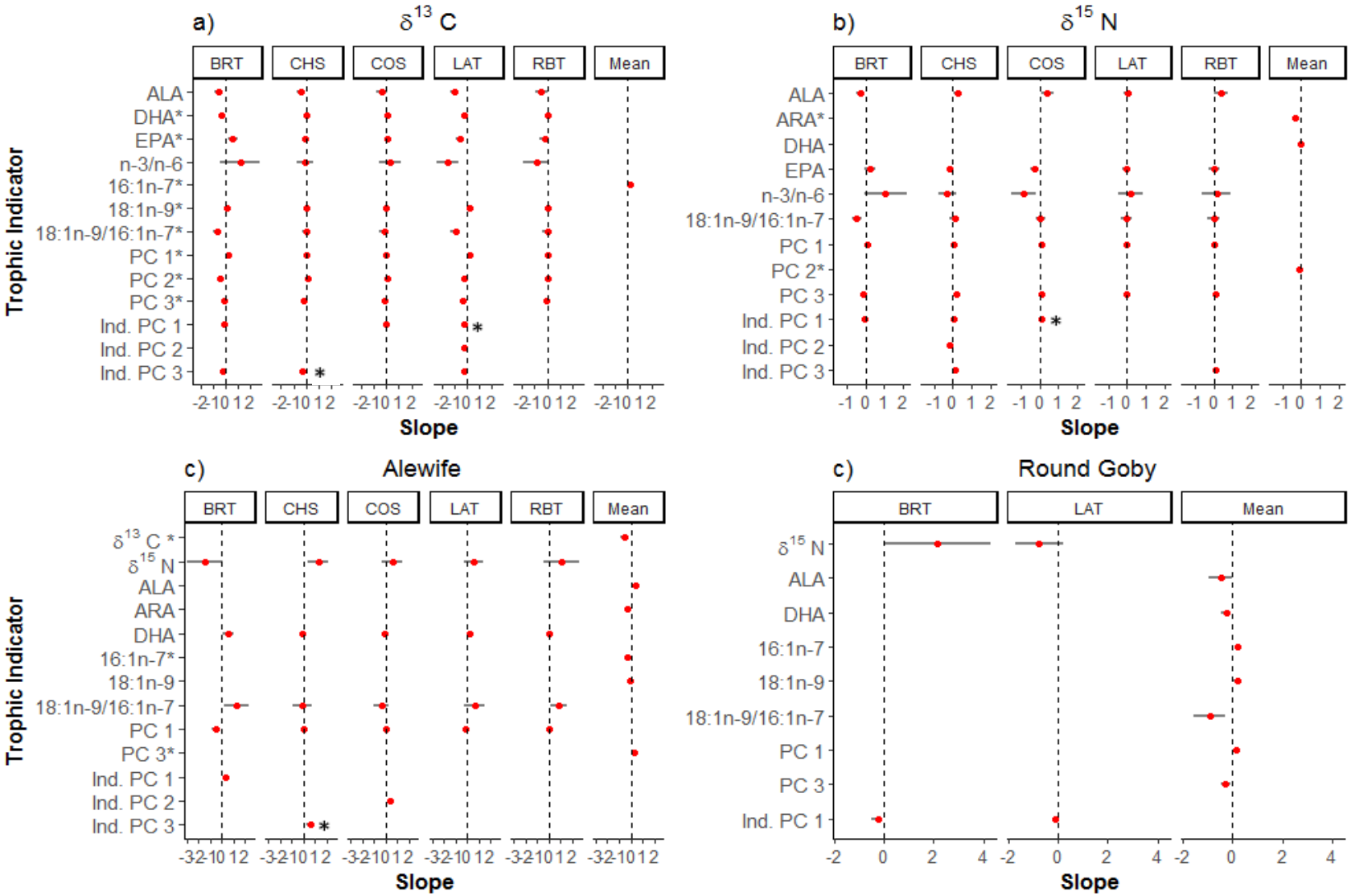


Figure 3.2. Principal component (PC) axes 1, 2, and 3 of all salmonids of fatty acids with a relative abundance greater than 1.0% of total fatty acids. PC 1 appears to be a gradient of invertebrate (DHA) to fish (18:1n-9 and 16:1n-7), whereas PC 2 represents benthic (negative loadings for 16:1n-7) to pelagic resource use (positive loadings for 16:0). For PC 3, 16:1n-7 and 16:0 loaded negatively, whereas 18:2n-6, ALA, and 20:4n-3 loaded positively.

Figure 3.3. Mean slopes (red points) and 95% confidence intervals (grey bars) of significant relationships between fatty acids, salmonid-wide PC axes, and species-specific PC axes and (a) δ13C, and (b) δ15N. The bottom figures represent significant relationships between fatty acids and stable isotope ratios to proportion by weight of (c) alewife and (d) round goby in salmonid stomachs. When models had significant species-specific interactions, the mean slope and confidence intervals are shown for each species. For significant models with no interaction effect, the mean model slope and 95% confidence interval is shown. The dashed line represents a slope of zero. * represents models with p values less than 0.001. These models used fatty acids expressed as proportional abundance. BRT=brown trout; CHS=Chinook salmon; COS=Coho Salmon; LAT=lake trout; RBT=rainbow trout.


a)
δ13 C
b)
δ15 N
BRT
CHS
COS
LAT
RBT
Mean
Trophic Indicator
ALA
DHA*
EPA*
n-3/n-6
16:1n-7*
18:1n-9*
18:1n-9/16:1n-7*
PC 1*
PC 2*
PC 3*
Ind. PC 1
Ind. PC 2
Ind. PC 3
ARA*
EPA
18:1n-9/16:1n-7
PC 1
PC 2*
PC 3
Slope
c)
Alewife
Round Goby
δ13 C *
δ15 N
ARA
16:1n-7*
18:1n-9
18:1n-9/16:1n-7
16:1n-7

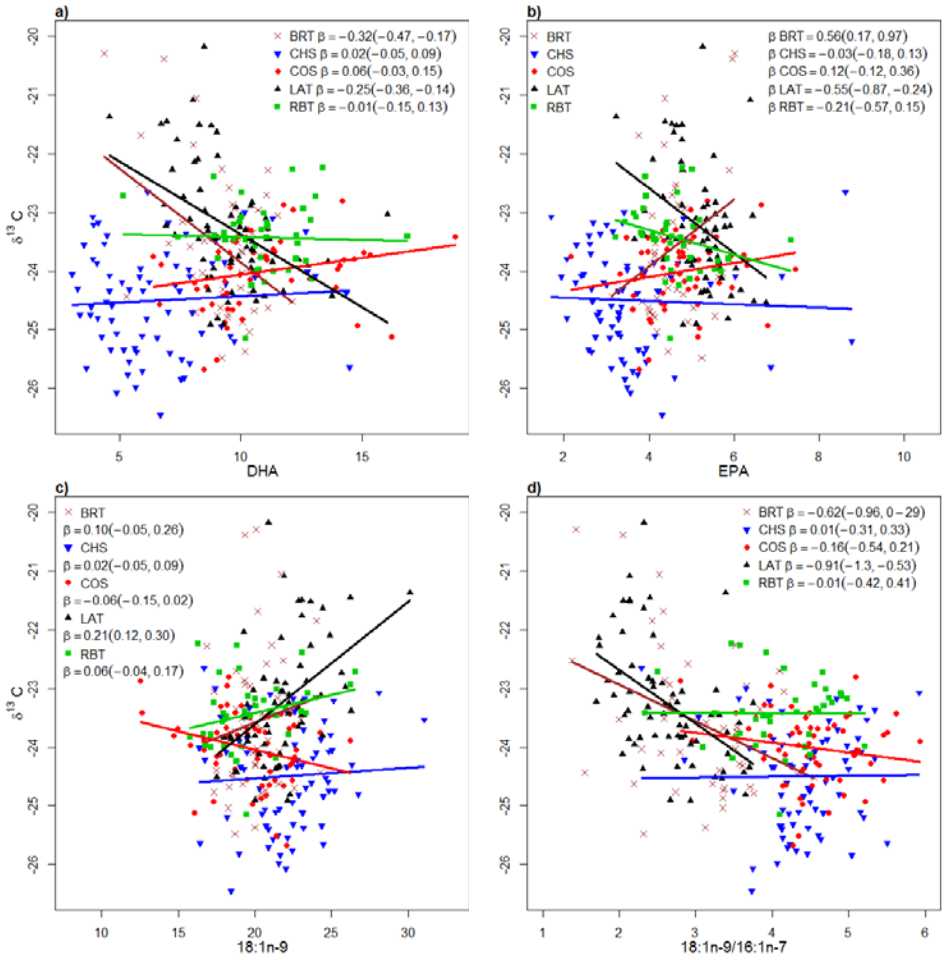
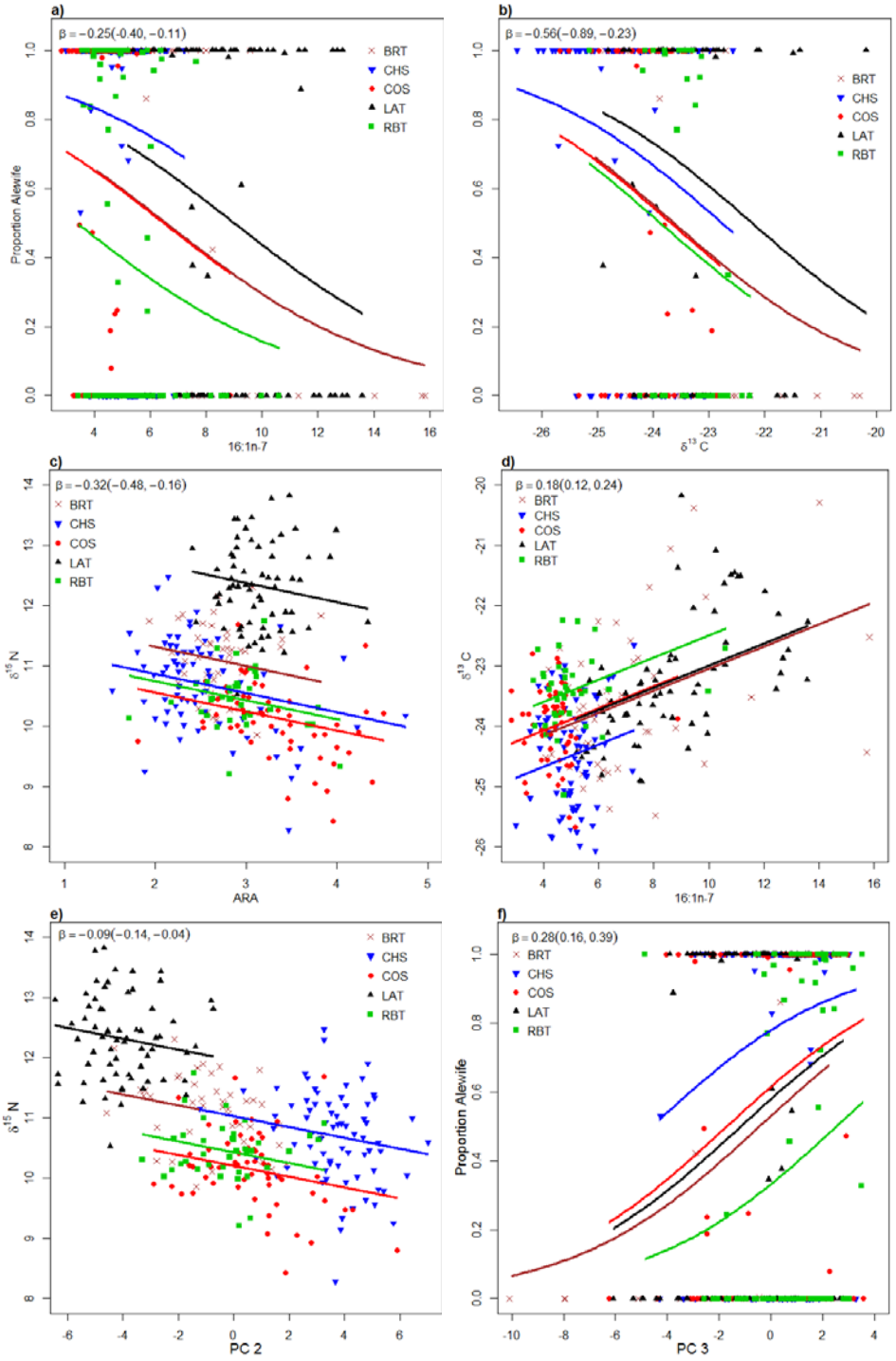


Figure 3.4. Relationships between $\delta^{13}C$ and fatty acids with highly significant interactions ($p<0.001$), including DHA (a), EPA (b), 18:1n-9 (c), and 18:1n-9/16:1n-7 (d). Points and lines represent individual fish and relationships for each species of salmonid, respectively. Species-specific slopes (β) and 95% confidence intervals are given for each species. These models used fatty acids expressed as relative abundance. BRT=brown trout; CHS=Chinook salmon; COS=Coho Salmon; LAT=lake trout; RBT=rainbow trout.

Figure 3.5. Highly significant relationships ($p<0.001$) between stomach contents, stable isotope ratios, fatty acids, and salmonid-wide PCA axes that did not have interaction effects. Negative relationships existed between $\delta^{13}C$ and alewife consumption (a), 16:1n-7 and alewife consumption (b), ARA and $\delta^{15}N$ (c), and PC 2 and $\delta^{15}N$ (e). Positive relationships existed between 16:1n-7 and $\delta^{13}C$ (d) and alewife consumption and PC 3 (f). Mean slope and 95% confidence intervals are provided. Fatty acids were expressed as relative abundance. BRT=brown trout; CHS=Chinook salmon; COS=Coho Salmon; LAT=lake trout; RBT=rainbow trout.


a)
β = −0.25(−0.40, −0.11)
BRT
CHS
COS
LAT
RBT
Proportion Alewife
16:1n-7
b)
β = −0.56(−0.89, −0.23)
δ13 C
c)
β = −0.32(−0.48, −0.16)
δ15 N
ARA
d)
β = 0.18(0.12, 0.24)
δ13 C
16:1n-7
e)
β = −0.09(−0.14, −0.04)
δ15 N
PC 2
f)
β = 0.28(0.16, 0.39)
Proportion Alewife
PC 3

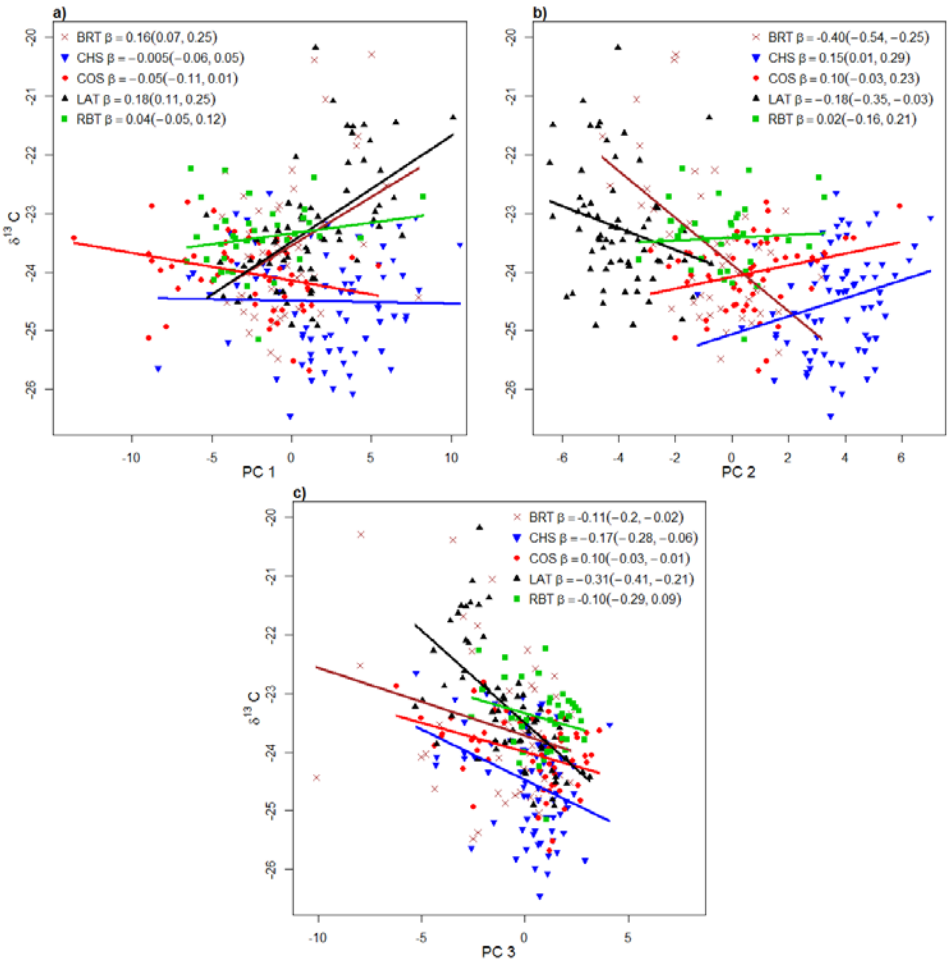


Figure 3.6. Relationships between $\delta^{13}C$ and salmonid-wide PC axes with highly significant interactions ($p<0.001$). PC 1 was only positively correlated with $\delta^{13}C$ in brown trout and lake trout (a). PC 2 was negatively correlated with $\delta^{13}C$ in brown trout and lake trout, but positively correlated in Chinook salmon (b). PC 3 was negatively correlated with $\delta^{13}C$ in all species, except rainbow trout (c). Points and lines represent individual fish and relationships for each species of salmonid, respectively. Species-specific slopes (β) and 95% confidence intervals are given for each species. These models used fatty acids expressed as relative abundance. BRT=brown trout; CHS=Chinook salmon; COS=Coho Salmon; LAT=lake trout; RBT=rainbow trout.

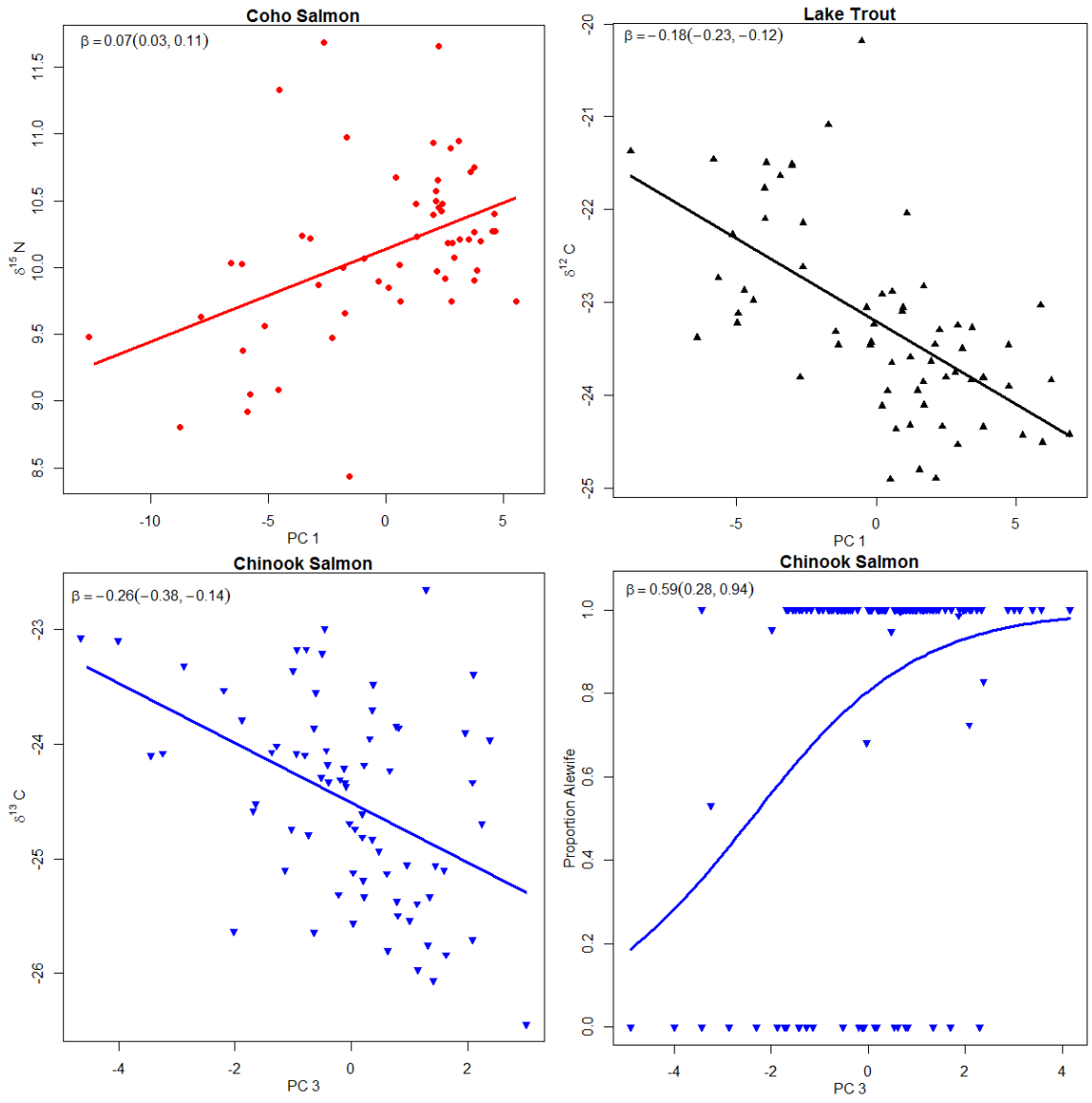


Figure 3.7. Highly significant ($p<0.001$) relationships between species-specific PCA axes and stable isotopes and stomach contents. There was a significant positive relationship between $\delta^{15}N$ and PC 1 in Coho salmon and a significant negative relationship between $\delta^{13}C$ and PC 1 in lake trout. Additionally in Chinook salmon, we observed a negative and positive relationship between PC 3 and $\delta^{13}C$ and alewife consumption, respectively. The proportional abundance of fatty acids were used to conduct species-specific PCA axes. Mean slopes and 95% confidence intervals are provided.

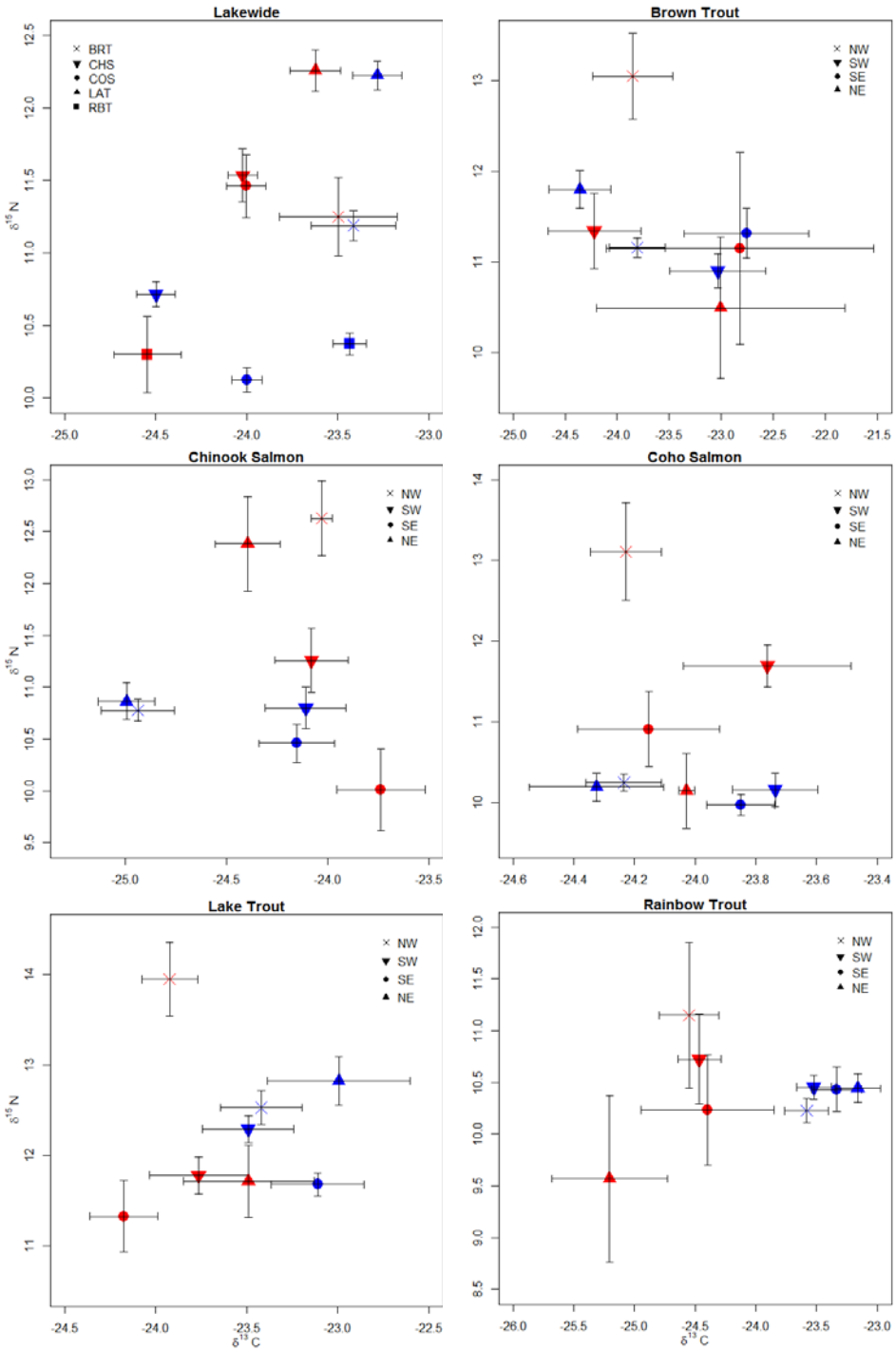


Figure 3.8. The lake-wide and regional mean and standard errors of observed (blue) and predicted (red) $\delta^{13}C$ and $\delta^{15}N$ of Lake Michigan salmonids in 2016. Lake-wide estimations used lake-wide $\delta^{13}C$ and $\delta^{15}N$ means of prey. Regional estimations used regional $\delta^{13}C$ and $\delta^{15}N$ means of alewife and round goby. BRT=brown trout; CHS=Chinook salmon; COS=Coho Salmon; LAT=lake trout; RBT=rainbow trout. NW=Northwest; SW=Southwest; SE=Southeast; NE=Northeast.

REFERENCES

Adlerstein, S. A., Rutherford, E. S., Claramunt, R. M., Clapp, D. F., & Clevenger, J. A. (2008). Seasonal movements of Chinook salmon in Lake Michigan based on tag recoveries from recreational fisheries and catch rates in gill-net assessments. *Transactions of the American Fisheries Society*, *137*(3), 736–750. https://doi.org/10.1577/T07-122.1

Adlerstein, S. A., Rutherford, E. S., Clevenger, J. A., Johnson, J. E., Clapp, D. F., & Woldt, A. P. (2007). Lake trout movements in U.S. waters of Lake Huron interpreted from coded wire tag recoveries in recreational fisheries. *Journal of Great Lakes Research*, *33*(1), 186–201. https://doi.org/10.3394/0380-1330(2007)33[186:LTMIUW]2.0.CO;2

Ahlgren, G., T. Vrede, and W. Goedkoop. (2009). Fatty acid ratios in freshwater fish, zooplankton and zoobenthos – are there specific optima?, p. 147–178. *In* M. Kainz, M.T. Brett, and M.T. Arts [eds.], Lipids in Aquatic Ecosystems. Springer New York.

Auer, M. T., Tomlinson, L. M., Higgins, S. N., Malkin, S. Y., Howell, E. T., & Bootsma, H. A. (2010). Great Lakes Cladophora in the 21st century: same algae-different ecosystem. *Journal of Great Lakes Research*, *36*(2), 248–255. https://doi.org/10.1016/j.jglr.2010.03.001

Aultman, D. C., & Haynes, J. M. (1993). Spring thermal fronts and salmonine sport catches in Lake Ontario. *North American Journal of Fisheries Management*, *13*(3), 502–510. https://doi.org/10.1577/1548-8675(1993)013<0502:stfass>2.3.co;2

Barton, K. (2018). MuMIn: Multi-Model Inference. R package version 1.40.4. https://CRAN.R-project.org/package=MuMIn

Beletsky, D., Mason, D. M., Schwab, D. J., Rutherford, E. S., Janssen, J., Clapp, D. F., & Dettmers, J. M. (2007). Biophysical model oflarval yellow perch advection and settlement in Lake Michigan. *Journal of Great Lakes Research*, *33*(4), 842–866. https://doi.org/10.3394/0380-1330(2007)33[842:BMOLYP]2.0.CO;2

Bendiksen, E. Å., & Jobling, M. (2003). Effects of temperature and feed composition on essential fatty acid (n-3 and n-6) retention in Atlantic salmon (*Salmo salar L.*) parr. *Fish Physiology and Biochemistry*, *29*, 133–140. https://doi.org/10.1023/B:FISH.0000035937.68098.83

Bergstedt, R. A., Argyle, R. L., Seelye, J. G., Scribner, K. T., & Curtis, G. L. (2003). In Situ Determination of the Annual Thermal Habitat Use by Lake Trout (*Salvelinus namaycush*) in Lake Huron. *Journal of Great Lakes Research*, *29*(Supplement 1), 347–361. https://doi.org/10.1016/S0380-1330(03)70499-7

Betancor, M. B., Olsen, R. E., Solstorm, D., Skulstad, O. F., & Tocher, D. R. (2016). Assessment of a land-locked Atlantic salmon (*Salmo salar L.*) population as a potential genetic resource with a focus on long-chain polyunsaturated fatty acid biosynthesis. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids*, *1861*(3), 227–238. https://doi.org/10.1016/j.bbalip.2015.12.015

Bolnick, D. I., Ingram, T., Stutz, W. E., Snowberg, L. K., Lau, O. L., & Paull, J. S. (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1689), 1789–1797. https://doi.org/10.1098/rspb.2010.0018

Bolnick, D. I., Svanbäck, R., Fordyce, J. a, Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: incidence and implications of individual specialization. *American Naturalist*, *161*(1), 1–28. https://doi.org/10.1086/343878

Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M., & Svanbäck, R. (2002). Measuring individual-level resource specialization. *Ecology*, *83*(10), 2936–2941. https://doi.org/10.1890/0012-9658(2002)083[2936:MILRS]2.0.CO;2

Brandt, S. B. (1980). Spatial segregation of adult and young-of-the-year alewives across a thermocline in Lake Michigan. *Transactions of the American Fisheries Society*, *109*(5), 469–478. https://doi.org/10.1577/1548-8659(1980)109<469

Brandt, S. B., Mason, D. M., Patrick, E. V., Argyle, R. L., Wells, L., Unger, P. A., & Stewart, D. J. (1991). Acoustic measures of the abundance and size of pelagic planktivores in Lake Michigan. *Canadian Journal of Fisheries and Aquatic Sciences*, *48*(5), 894–908. https://doi.org/10.1139/f91-106

Bridcut, E. E., & Giller, P. S. (1995). Diet variability and foraging strategies in brown trout (*Salmo trutta*): an analysis from subpopulations to individuals. *Canadian Journal of Fisheries and Aquatic Sciences*, *52*(12), 2543–2552. https://doi.org/10.1139/f95-845

Brush, J. M., Fisk, A. T., Hussey, N. E., & Johnson, T. B. (2012). Spatial and seasonal variability in the diet of round goby (*Neogobius melanostomus*) stable isotopes indicate that stomach contents overestimate the importance of dreissenids. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*, 573–586.

Bunnell, D.B., Madenjian, C.P., Desorcie, T.J., Kostich, M.J., Armenio, P., & Adams, J. V. (2017). Status and trends of prey fish populations in Lake Michigan, 2016. Lake Michigan Fishery Commission Meeting, USGS, Ypsilanti, MI.

Cloutier, D. D., Alm, E. W., & McLellan, S. L. (2015). Influence of land use, nutrients, and geography on microbial communities and fecal indicator abundance at Lake Michigan beaches. *Applied and Environmental Microbiology*, *81*(15), 4904–4913. https://doi.org/10.1128/AEM.00233-15

Colborne, S. F., Rush, S. A., Paterson, G., Johnson, T. B., Lantry, B. F., & Fisk, A. T. (2016). Estimates of lake trout (*Salvelinus namaycush*) diet in Lake Ontario using two and three isotope mixing models. *Journal of Great Lakes Research*, *42*(3), 695–702. https://doi.org/10.1016/j.jglr.2016.03.010

Crawford, S.S. (2001). Salmonine introductions to the Laurentian Great Lakes: an historical review and evaluation of ecological effects. *Canadian Special Publication of Fisheries and Aquatic Sciences.* p. 132-205.

Czesny, S. J., Rinchard, J., Hanson, S. D., Dettmers, J. M., Dabrowski, K., & Smith, R. (2011). Fatty acid signatures of Lake Michigan prey fish and invertebrates: among-species differences and spatiotemporal variability. *Canadian Journal of Fisheries and Aquatic Sciences*, *68*(7), 1211–1230. https://doi.org/10.1139/f2011-048

Dethier, M. N., Sosik, E., Galloway, A. W. E., Duggins, D. O., & Simenstad, C. A. (2013). Addressing assumptions: variation in stable isotopes and fatty acids of marine macrophytes can confound conclusions of food web studies. *Marine Ecology Progress Series, 478*, 1–14. https://doi.org/10.3354/meps10310

Dietrich, J. P., Morrison, B. J., & Hoyle, J. A. (2006). Alternative ecological pathways in the Eastern Lake Ontario food web—round goby in the diet of lake trout. *Journal of Great Lakes Research, 32*(2), 395–400. https://doi.org/10.3394/0380-1330(2006)32[395:AEPITE]2.0.CO;2

Dolan, D. M., & Chapra, S. C. (2012). Great Lakes total phosphorus revisited: 1. Loading analysis and update (1994-2008). *Journal of Great Lakes Research, 38*(4), 730–740. https://doi.org/10.1016/j.jglr.2012.10.001

Driscoll, Z. G., Bootsma, H. A., & Christiansen, E. (2015). Zooplankton trophic structure in Lake Michigan as revealed by stable carbon and nitrogen isotopes. *Journal of Great Lakes Research, 41*, 104–114. https://doi.org/10.1016/j.jglr.2015.04.012

Dub, J. D. & Czesny, S. J. (2016). Yellow perch population assessment in southwestern Lake Michigan, Indiana Natural History Survey Technical Report 2015 (43).

Elliott, R. F., P. J. Peeters, M. P. Ebener, R. W. Rybicki, P. J. Schneeberger, R. J. Hess, J. T. Francis, G. W. Eck, and C. P. Madenjian. 1996. Conducting diet studies of Lake Michigan piscivores: a protocol. U.S. Fish and Wildlife Service, Report 96-3, Ann Arbor, Michigan.

Farkas, T., Csengeri, I., Majoros, F., & Oláh, J. (1980). Metabolism of fatty acids in fish. III. Combined effect of environmental temperature and diet on formation and deposition of fatty acids in the carp, *Cyprinus carpio* Linnaeus 1758. *Aquaculture, 20*(1), 29–40. https://doi.org/10.1016/0044-8486(80)90059-9

Faulks, L., Svanbäck, R., Ragnarsson-stabo, H., & Eklöv, P. (2015). Intraspecific niche aariation drives abundance-occupancy relationships in freshwater fish communities. *The American Naturalist, 186*(2). https://doi.org/10.1086/682004

Feiner, Z.S., C.J. Foley, H.A. Bootsma, S.J. Czesny, J. Janssen, J. Rinchard, and T.O. Höök. In press. Species identity matters when interpreting trophic markers in aquatic food webs. *PLOS ONE*.

Folch, J., Lees, M., & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem*. https://doi.org/10.1007/s10858-011-9570-9

Foley, C. J., Henebry, M. L., Happel, A., Bootsma, H. A., Czesny, S. J., Janssen, J., Jude, D. A., & Höök, T. O. (2017). Patterns of integration of invasive round goby (*Neogobius melanostomus*) into a nearshore freshwater food web. *Food Webs*, *10*, 26–38. https://doi.org/10.1016/j.fooweb.2016.10.001

Fox J. & Weisberg, S. (2011). An {R} companion to applied regression, Second Edition. Thousand Oaks CA: Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion

France, R. L. (1995). Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnology and Oceanography*, *40*(7), 1310–1313. https://doi.org/10.4319/lo.1995.40.7.1310

French, J. R. P., & Jude, D. J. (2001). Diets and diet overlap of nonindigenous gobies and small benthic native fishes co-inhabiting the St. Clair River, Michigan. *Journal of Great Lakes Research*, *27*(3), 300–311. https://doi.org/10.1016/S0380-1330(01)70645-4

Fry, B. (2006). *Stable Isotope Ecology*. Springer.

Gong, B., & Farrell, A. (1990). Comparison of blood and muscle levels of unsaturated fatty acids in parr and mature Coho salmon. *Comp.Biochem.Physiol.*, *96B*(3), 483–486.

Haliloğlu, H. I., Bayir, A., Sirkecioğlu, A. N., Aras, N. M., & Atamanalp, M. (2004). Comparison of fatty acid composition in some tissues of rainbow trout (*Oncorhynchus mykiss*) living in seawater and freshwater. *Food Chemistry*, *86*(1), 55–59. https://doi.org/10.1016/j.foodchem.2003.08.028

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. *North American Journal of Fisheries Management*, *33*(1), 186–191. https://doi.org/10.1080/02755947.2012.754804

Happel, A., Creque, S., Rinchard, J., Höök, T., Bootsma, H., Janssen, J., Jude, D., & Czesny, S. (2015a). Exploring yellow perch diets in Lake Michigan through stomach content, fatty acids, and stable isotope ratios. *Journal of Great Lakes Research, 41,* 172–178. https://doi.org/10.1016/j.jglr.2015.03.025

Happel, A., Jonas, J. L., McKenna, P. R., Rinchard, J., He, J. X., & Czesny, S. J. (2017). Spatial variability of lake trout diets in Lakes Huron and Michigan revealed by stomach content and fatty acid profiles. *Canadian Journal of Fisheries and Aquatic Sciences*, 1–11. https://doi.org/10.1139/cjfas-2016-0202

Happel, A., Lafountain, J., Creque, S., Rinchard, J., Höök, T., Bootsma, H., Janssen, J., Jude, J., & Czesny, S. (2015b). Spatio-temporal description of spottail shiner (*Notropis hudsonius*) fatty acid profiles in Lake Michigan's southern basin. *Journal of Great Lakes Research*, *41*, 179–184. https://doi.org/10.1016/j.jglr.2015.04.013

Happel, A., Pattridge, R., Walsh, M., & Rinchard, J. (2016a). Assessing diet compositions of Lake Ontario predators using fatty acid profiles of prey fishes. *Journal of Great Lakes Research,* 43(5), 838-845. https://doi.org/10.1016/j.jglr.2016.12.008

Happel, A., Stratton, L., Kolb, C., Hays, C., Rinchard, J., & Czesny, S. (2016b). Evaluating quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding experiments. *Canadian Journal of Fisheries and Aquatic Sciences*, *73*(8), 1222–1229. https://doi.org/10.1139/cjfas-2015-0328

Hecky, R. E., Smith, R. E., Barton, D. R., Guildford, S. J., Taylor, W. D., Charlton, M. N., & Howell, T. (2004). The nearshore phosphorus shunt : a consequence of ecosystem engineering by dreissenids in the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *61*(7), 1285–1293. https://doi.org/10.1139/F04-065

Henderson, R., Sargent, J., & Hopkins, C. (1984). Changes in the content and fatty acid composition of lipid in an isolated population of the capelin *Mallotus villosus* during sexual maturation and spawning. *Marine Biology*, 78(3), 255-263.

Hesslein, R. H., Hallard, K. A., & Rarnlal, P. (1993). Replacement of sulfur, carbon, nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by $\delta^{34}S$, $\delta^{13}C$, and $\delta^{15}N$. *Canadian Journal of Fisheries and Aquatic Sciences, 50*, 2071–2076.

Höök, T. O., Rutherford, E. S., Brines, S. J., Schwab, D. J., & McCormick, M. J. (2004). Relationship between surface water temperature and steelhead distributions in Lake Michigan. *North American Journal of Fisheries Management, 24*(1), 211–221. https://doi.org/10.1577/M02-159

Hutton-Stadiq, M. (2016). Lower food web dynamics of Lakes Michigan and Huron: spatial and temporal responses to recent oligotrophication. Purdue University, West Lafayette, Indiana, USA.

Hyslop, E. J. (1980). Stomach contents analysis—a review of methods and their application. *Journal of Fish Biology*, *17*(4), 411–429. https://doi.org/10.1111/j.1095-8649.1980.tb02775.x

Illinois DNR (2016). 2016 Illinois Fishing Information. Retrieved from https://www.ifishillinois.org/regulations/2016_Fishing_Guide.pdf

Indiana DNR (2015). Indiana Fishing Regulation Guide 2015-2016. Retrieved from http://www.eregulations.com/wp-content/uploads/2015/02/15INFW_LR.pdf

Jacobs, G. R., Madenjian, C. P., Bunnell, D. B., & Holuszko, J. D. (2010). Diet of lake trout and burbot in northern Lake Michigan during spring: Evidence of ecological interaction. *Journal of Great Lakes Research, 36*(2), 312–317. https://doi.org/10.1016/j.jglr.2010.02.007

Jacobs, G. R., Madenjian, C. P., Bunnell, D. B., Warner, D. M., & Claramunt, R. M. (2013). Chinook salmon foraging patterns in a changing Lake Michigan. *Transactions of the American Fisheries Society*, *142*(2), 362–372. https://doi.org/10.1080/00028487.2012.739981

Janssen, J., & Jude, D. J. (2001). Recruitment failure of mottled sculpin *Cottus bairdi* in Calumet Harbor, southern Lake Michigan, induced by the newly introduced round goby *Neogobius melanostomus*. *Journal of Great Lakes Research*, *27*(3), 319–328. https://doi.org/10.1016/S0380-1330(01)70647-8

Jirka, K. J., & Kraft, C. E. (2017). Diet niche width and individual specialization of brook trout in Adirondack Lakes. *Transactions of the American Fisheries Society*, *146*(4), 716–731. https://doi.org/10.1080/00028487.2017.1290680

Jude, D. J., Tesar, F. J., Deboe, S. F., & Miller, T. J. (1987). Diet and selection of major prey species by Lake Michigan USA salmonines 1973-1982. *Transactions of the American Fisheries Society*, *116*(5), 677–691. https://doi.org/10.1577/1548-8659(1987)116<677

Keller, M., Smith, K.D., & Rybicki, R.W. (1990). Review of salmon and trout management in Lake Michigan. Michigan Department of Natural Resources, Fisheries Report 14, Lansing.

Kiessling, A., Johansson, L., & Kiessling, K. H. (1990). Effects of starvation on rainbow trout muscle. *Acta Agriculturae Scandinavica*, *40*(3), 309–324. https://doi.org/10.1080/00015129009438565

Kionka, B. C., & Windell, J. T. (1972). Differential movement of digestible and indigestible food fractions in rainbow trout, *Salmo gairdneri*. *Transactions of the American Fisheries Society*, *101*(1), 112–115. https://doi.org/10.1577/1548-8659(1972)101<112:dmodai>2.0.co;2

Knight, R. L., Margraf, F. J., & Carline, R. F. (1984). Piscivory by walleyes and yellow perch in western Lake Erie. *Transactions of the American Fisheries Society*, *113*(January), 667–693. https://doi.org/10.1577/1548-8659(1984)113<677

Kornis, M. S., Mercado-Silva, N., & vander Zanden, M. J. (2012). Twenty years of invasion: A review of round goby *Neogobius melanostomus* biology, spread and ecological implications. *Journal of Fish Biology*, 80(2), 235-285. https://doi.org/10.1111/j.1095-8649.2011.03157.x

Larson, J. H., Trebitz, A. S., Steinman, A. D., Wiley, M. J., Mazur, M. C., Pebbles, V., Braun, H. A., & Seelbach, P. W. (2013). Great Lakes rivermouth ecosystems: Scientific synthesis and management implications. *Journal of Great Lakes Research*, *39*(3), 513–524. https://doi.org/10.1016/j.jglr.2013.06.002

Larsson, S. (2005). Thermal preference of Arctic charr, *Salvelinus alpinus*, and brown trout, *Salmo trutta* - Implications for their niche segregation. *Environmental Biology of Fishes*, *73*(1), 89–96. https://doi.org/10.1007/s10641-004-5353-4

Layman, C. A., Quattrochi, J. P., Peyer, C. M., & Allgeier, J. E. (2007). Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecology Letters*, *10*(10), 937–944. https://doi.org/10.1111/j.1461-0248.2007.01087.x

Lenth, R. V. (2016). Least-squares means: The R package lsmeans. *Journal of Statistical Software*, 69(1), 1-33. doi:10.18637/jss.v069.i01

Li, H. O., & Yamada, J. (1992). Changes of the fatty acid composition in smolts of Masu salmon (*Oncorhynchus masou*), associated with desmoltification and sea-water transfer. *Comp.Biochem.Physiol.[A].*, *103*(1), 221–226.

MacDonald, J. S., Waiwood, K. J., & Green, R. H. (1982). Rates of digestion of different prey in Atlantic cod (*Gadus morhua*), Ocean Pout (*Macrozoarces americanus*), Winter Flounder (*Pseudopleuronectes amerkanus*), and American Plaice (*Hippoglossoides platessoides*). *Canadian Journal of Fisheries and Aquatic Sciences*, *39*(5), 651–659.

Madenjian, C.P., Bunnell, D.B., Desorcie, T.J., Kostich, M.J., Chriscinske, M. A., & Adams, J. V. (2016). Status and trends of prey fish populations in Lake Michigan, 2015. Lake Michigan Fishery Commission Meeting, USGS, Milwaukee, WI.

Madenjian, C. P., DeSorcie, T. J., & Stedman, R. M. (1998). Ontogenic and spatial patterns in diet and growth of lake trout in Lake Michigan. *Transactions of the American Fisheries Society*, *127*, 236–252.

Madenjian, C. P., Holuszko, J. D., & Desorcie, T. J. (2003). Growth and condition of alewives in Lake Michigan, 1984–2001. *Transactions of the American Fisheries Society*, *132*(6), 1104–1116. https://doi.org/10.1577/T02-133

Madenjian, C. P., Höök, T. O., Rutherford, E. S., Mason, D. M., Croley II, T. E., Szalai, E. B., & Bence, J. R. (2005). Recruitment variability of alewives in Lake Michigan. *Transactions of the American Fisheries Society*, *134*(June), 218–230. https://doi.org/10.1577/FT03-222.1

Magnuson, J. J., Meisner, J. D., & Hill, D. K. (1990). Potential changes in the thermal habitat of Great Lakes fish after global climate warming. *Transactions of the American Fisheries Society*, *119*(September 2013), 254–264. https://doi.org/10.1577/1548-8659(1990)119<0254

Marion, A. Z., Fordyce, J., & Fitzpatrick, B. (2015). hierDiversity: hierarchical multiplicative partitioning of complex phenitypes. R Package Version 0.1, 10p.

Marion, Z. H., Fordyce, J. A., & Fitzpatrick, B. M. (2015). Extending the concept of diversity partitioning to characterize phenotypic complexity. *The American Naturalist*, *186*(3), 348–361. https://doi.org/10.1086/682369

McCann, K., Hastings, A., & Huxel, G. R. (1998). Weak trophic interactions and the balance of nature. *Nature*, *395*(6704), 794–798. https://doi.org/10.1038/27427

McMeans, B. C., McCann, K. S., Tunney, T. D., Fisk, A. T., Muir, A. M., Lester, N., Shuter, B., & Rooney, N. (2016). The adaptive capacity of lake food webs: from individuals to ecosystems. *Ecological Monographs*, *86*(1), 4–19.

Metcalfe, L. D., & Schmitz, A. A. (1961). The rapid preparation of fatty acid esters for gas chromatographic analysis. *Analytical Chemistry*, *33*(3), 363–364. https://doi.org/10.1021/ac60171a016

Michigan DNR (2015). 2015 Michigan Fishing Guide. Retrieved from https://www.michigan.gov/documents/dnr/2015-MIFishingGuide-April10_486679_7.pdf

Michigan DNR (2016). 2016-2017 Michigan Fishing Guide. Retrieved from https://www.michigan.gov/documents/dnr/2016-2017MIFishingGuide_515573_7.pdf

Morillo-Velarde, P. S., Briones-Fourzán, P., Álvarez-Filip, L., Aguíñiga-García, S., Sánchez-González, A., & Lozano-Álvarez, E. (2018). Habitat degradation alters trophic pathways but not food chain length on shallow Caribbean coral reefs. *Scientific Reports*, *8*(1), 4109. https://doi.org/10.1038/s41598-018-22463-x

Nakano, S., Miyasaka, H., & Kuhara, N. (1999). Terrestrial - aquatic linkages: riparain arthropod inputs alter trophic cascades in a stream food web. *Ecology*, *80*(7), 2435–2441. https://doi.org/10.1890/0012-9658(1999)080[2435:TALRAI]2.0.CO;2

Nalepa, T. F., Fanslow, D. L., & Lang, G. A. (2009). Transformation of the offshore benthic community in Lake Michigan: Recent shift from the native amphipod *Diporeia* spp. to the invasive mussel Dreissena *rostriformis bugensis*. *Freshwater Biology*, *54*(3), 466–479. https://doi.org/10.1111/j.1365-2427.2008.02123.x

Napolitano, G. E. 1999. Fatty acids as trophic and chemical markers in freshwater ecosystems, p. 21–44. In Lipids in Freshwater Ecosystems. Springer, New York, NY.

Oksanen, A. J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., & Wagner, H. (2017). vegan: community ecology package. R package version 2.4-2. https://CRAN.R-project.org/package=vegan.

Olson, R. A., Winter, J. D., Nettles, D. C., & Haynes, J. M. (1988). Resource partitioning in summer by salmonids in south-central Lake Ontario. *Transactions of the American Fisheries Society*, *117*(6), 552–559. https://doi.org/10.1577/1548-8659(1988)117<0552

Perga, M. E., & Gerdeaux, D. (2005). “Are fish what they eat” all year round? *Oecologia*, *144*(4), 598–606. https://doi.org/10.1007/s00442-005-0069-5

Pothoven, S. A., Fahnenstiel, G. L., & Vanderploeg, H. A. (2004). Spatial distribution, biomass and population dynamics of Mysis relicta in Lake Michigan. *Hydrobiologia*, *522*(1–3), 291–299. https://doi.org/10.1023/B:HYDR.0000029982.52263.c0

Pothoven, S. A., & Madenjian, C. P. (2008). Changes in consumption by alewives and lake whitefish after dreissenid mussel invasions in Lakes Michigan and Huron. *North American Journal of Fisheries Management*, *28*(June), 308–320. https://doi.org/10.1577/M07-022.1

Pothoven, S. A., Nalepa, T. F., & Brandt, S. B. (2000). Age-0 and age-1 yellow perch diet in southeastern Lake Michigan. *Journal of Great Lakes Research*, *26*(2), 235–239. https://doi.org/10.1016/S0380-1330(00)70689-7

Pothoven, S. A., Vanderploeg, H. A., Höök, T. O., Ludsin, S. A., & Sprules, G. (2012). Hypoxia modifies planktivore–zooplankton interactions in Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*(12), 2018–2028. https://doi.org/10.1139/cjfas-2012-0144

Quevedo, M., Svanbäck, R., & Eklöav, P. (2009). Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology*, *90*(8), 2263–2274. https://doi.org/10.1890/07-1580.1

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

Rand, P. S., Stewart, D. J., Seelbach, P. W., Jones, M. L., & Wedge, L. R. (1993). Modeling steelhead population energetics in Lakes Michigan and Ontario. *Transactions of the American Fisheries Society*, *122*(February 2014), 977–1001. https://doi.org/10.1577/1548-8659(1993)122<0977:MSPEIL>2.3.CO;2

Riha, M., Walsh, M. G., Connerton, M. J., Holden, J., Weidel, B. C., Sullivan, P. J., Holda, T. J., & Rudstam, L. G. (2017). Vertical distribution of alewife in the Lake Ontario offshore: Implications for resource use. *Journal of Great Lakes Research*, *43*(5), 823–837. https://doi.org/10.1016/j.jglr.2017.07.007

Roseman, E. F., Schaeffer, J. S., Bright, E., & Fielder, D. G. (2014). Angler-caught piscivore diets reflect fish community changes in Lake Huron. *Transactions of the American Fisheries Society*, *143*(6), 1419–1433. https://doi.org/10.1080/00028487.2014.945659

Roswell, C. R., Pothoven, S. A., & Höök, T. O. (2013). Spatio-temporal, ontogenetic and interindividual variation of age-0 diets in a population of yellow perch. *Ecology of Freshwater Fish*, *22*(3), 479–493. https://doi.org/10.1111/eff.12041

Roughgarden, J. (1974). Niche Width : Biogeographic patterns among *Anolis* lizard populations. *The American Naturalist*, *108*(962), 429–442.

Rybicki, R. W., & Clapp, D. F. (1996). Diet of Chinook salmon in eastern lake diet of Chinook salmon in Eastern Lake Michigan , 1991-93. *Michigan Department of Natural Resources, Fisheries Research Report No. 2027*.

Savitz, J. (2009). Diets of Lake Michigan salmon and maximum size of alewife prey. *Journal of Freshwater Ecology*, *24*(4), 563–566. https://doi.org/10.1080/02705060.2009.9664333

Sheridan, M. A., Allen, W. V., & Kerstetter, T. H. (1985). Changes in the fatty acid composition of steelhead trout, *Salmo gairdnerii richardson*, associated with parr-smolt transformation. *Comparative Biochemistry and Physiology -- Part B: Biochemistry And*, *80*(4), 671–676. https://doi.org/10.1016/0305-0491(85)90444-4

Simpson, N. T., Honsey, A., Rutherford, E. S., & Höök, T. O. (2016). Spatial shifts in salmonine harvest, harvest rate, and effort by charter boat anglers in Lake Michigan, 1992-2012. *Journal of Great Lakes Research*, *42*(5), 1109–1117. https://doi.org/10.1016/j.jglr.2016.07.030

Stewart, D. J., Weininger, D., Rottiers, D. V., & Edsall, T. A. (1983). An energetics model for lake trout, *Salvelinus namaycush* : Application to the Lake Michigan population. *Canadian Journal of Fisheries and Aquatic Sciences*, *40*(6), 681–698. https://doi.org/10.1139/f83-091

Svanbäck, R., & Bolnick, D. I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society of London*. Biological Sciences, 274(1611), 839-844.

Svanbäck, R., Quevedo, M., Olsson, J., & Eklöv, P. (2015). Individuals in food webs: the relationships between trophic position, omnivory and among-individual diet variation. *Oecologia*, *178*(1), 103–114. https://doi.org/10.1007/s00442-014-3203-4

Tocher, D. R. (2003). Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews in Fisheries Science*, *11*(2), 107–184. https://doi.org/10.1080/713610925

Tocher, D. R. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research*, *41*(5), 717–732. https://doi.org/10.1111/j.1365-2109.2008.02150.x

Traynor, D., Moerke, A., & Greil, R. (2010). Identification of Michigan fishes using cleithra. *Great Lakes Fishery Commission Miscellaneous Publication.* 2010-02.

Tsehaye, I., Jones, M. L., Brenden, T. O., Bence, J. R., Randall, M., & Claramunt, R. M. (2014). Changes in the salmonine community of Lake Michigan and their implications for predator – prey balance. *Transactions of the American Fisheries Society*, *143*(October), 420–437. https://doi.org/10.1080/00028487.2013.862176

Turschak, B. A. (2013). Changes in the Lake Michigan trophic structure as revealed by stable isotopes. University of Wisconsin-Milwaukee. Milwaukee, Wisconsin, USA.

Turschak, B. A., & Bootsma, H. A. (2015). Lake Michigan trophic structure as revealed by stable C and N isotopes. *Journal of Great Lakes Research*, *41*, 185–196. https://doi.org/10.1016/j.jglr.2015.04.004

Turschak, B. A., Bunnell, D., Czesny, S., Höök, T. O., Janssen, J., Warner, D., & Bootsma, H. A. (2014). Nearshore energy subsidies support Lake Michigan fishes and invertebrates following major changes in food web structure. *Ecology*, *95*(5), 1243–1252. https://doi.org/10.1890/13-0329.1

Valdovinos, F. S., Ramos-Jiliberto, R., Garay-Narváez, L., Urbani, P., & Dunne, J. A. (2010). Consequences of adaptive behaviour for the structure and dynamics of food webs. *Ecology Letters*, *13*(12), 1546–1559. https://doi.org/10.1111/j.1461-0248.2010.01535.x

Vander Zanden, M. J., Cabana, G., & Rasmussen, J. B. (1997). Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios δ^{15}N and literature dietary data. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(5), 1142–1158. https://doi.org/10.1139/cjfas-54-5-1142

Vander Zanden, M. J., Casselman, J. M., & Rasmussen, J. B. (1999). Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature*, *401*(6752), 464–467. https://doi.org/10.1038/46762

Vanderploeg, H. A., Liebig, J. R., Nalepa, T. F., Fahnenstiel, G. L., & Pothoven, S. A. (2010). Dreissena and the disappearance of the spring phytoplankton bloom in Lake Michigan. *Journal of Great Lakes Research*, *36*, 50–59. https://doi.org/10.1016/j.jglr.2010.04.005

Vanderploeg, H. A., Nalepa, T. F., Jude, D. J., Mills, E. L., Holeck, K. T., Liebig, J. R., Grigorovich, I. A., & Ojaveer, H. (2002). Dispersal and emerging ecological impacts of Ponto-Caspian species in the Laurentian Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *59*(7), 1209–1228. https://doi.org/10.1139/f02-087

Warner, D. M., Kiley, C. S., Claramunt, R. M., & Clapp, D. F. (2008). The influence of alewife year-class strength on prey selection and abundance of age-1 Chinook salmon in Lake Michigan. *Transactions of the American Fisheries Society*, *137*(6), 1683–1700. https://doi.org/10.1577/T07-130.1

Warner, D.M., Claramunt R.M., Hanson D., Descorcie, T., O'Brien T.P., Armenio, P., Ogilvie L., & Donner, K. (2017). Status of pelagic prey fish in Lake Michigan, 2016. Lake Michigan Fishery Commission Meeting, USGS, Ypsilanti, MI.

Wisconsin DNR (2016). Guide to Wisconsin Hook and Line: Fishing Regulations 2016-2017. Retrieved from https://dnr.wi.gov/topic/fishing/documents/regulations/fishregs1617web.pdf

Yuille, M. J., Fisk, A. T., Stewart, T., & Johnson, T. B. (2015). Evaluation of Lake Ontario salmonid niche space overlap using stable isotopes. *Journal of Great Lakes Research*, *41*(3), 934–940. https://doi.org/10.1016/j.jglr.2015.05.011

Zaccarelli, N., Bolnick, D. I., & Mancinelli, G. (2013). RInSp: An r package for the analysis of individual specialization in resource use. *Methods in Ecology and Evolution*, *4*(11), 1018–1023. https://doi.org/10.1111/2041-210X.12079

APPENDIX A. CHAPTER 2 SUPPLEMENTARY MATERIAL

Appendix Table A.1. Results of tests for individual specialization on alewife lengths by brown trout in 2015 and 2016. Test for individual specialization was done separately by region, size-class, and season. Individual specialization was calculated as WIC/TNW for each species and on individuals that consumed more than one measurable alewife. Values close to 0 indicate size specialization on alewife whereas values close to 1 indicate alewife length generalization. * indicates significant individual specialization.

2015

	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	-	54.94	409.89	177.15	55.04	248.09	203.40	55.93
TNW	-	109.12	558.63	288.50	241.11	368.46	413.43	61.05
WIC/TNW	-	0.50	0.73	0.61	0.23	0.67	0.49	0.92
p-value	-	0.174	0.2	0.391	0.005*	0.519	0.077	0.721
number	-	3	2	10	5	10	13	2

2016

	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	-	99.71	-	-	99.71	38.98	69.34	-
TNW	-	100.47	-	-	100.47	2255.40	1341.63	-
WIC/TNW	-	0.99	-	-	0.99	0.02	0.05	-
p-value	-	0.969	-	-	0.971	0.056	0.971	-
number	-	3	-	-	3	3	6	-

Appendix Table A.2. Results of tests for individual specialization on alewife lengths by Chinook salmon in 2015 and 2016. Test for individual specialization was done separately by region, size-class, and season. Individual specialization was calculated as WIC/TNW for each species and on individuals that consumed more than one measurable alewife. Values close to 0 indicate size specialization on alewife whereas values close to 1 indicate alewife length generalization. * indicates significant individual specialization.

2015

	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	248.01	166.43	310.05	181.55	172.95	227.76	182.63	257.10
TNW	450.57	374.99	522.78	1124.91	983.88	500.13	386.58	890.42
WIC/TNW	0.55	0.44	0.59	0.16	0.18	0.46	0.47	0.29
p-value	0.092	0.015	0.24	0.001*	0.001*	0.001*	0.012	0.001*
number	3	36	26	20	17	68	46	39

2016

	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	415.66	297.49	182.02	223.75	165.91	300.35	168.09	560.53
TNW	512.25	696.47	573.30	1191.92	643.84	1432.50	1006.77	1658.75
WIC/TNW	0.81	0.43	0.32	0.19	0.26	0.21	0.17	0.34
p-value	0.571	0.002*	0.001*	0.002*	0.001*	0.001*	0.001*	0.002*
number	3	29	27	11	28	42	56	14

Appendix Table A.3. Results of tests for individual specialization on alewife lengths by Coho salmon in 2015 and 2016. Test for individual specialization was done separately by region, size-class, and season. Individual specialization was calculated as WIC/TNW for each species and on individuals that consumed more than one measurable alewife. Values close to 0 indicate size specialization on alewife whereas values close to 1 indicate alewife length generalization. * indicates significant individual specialization.

2015

	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	-	142.99	191.29	209.57	94.36	286.45	141.22	210.64
TNW	-	248.84	1707.82	396.37	1583.47	718.24	262.28	1629.92
WIC/TNW	-	0.57	0.11	0.53	0.06	0.40	0.54	0.13
p-value	-	0.464	0.001*	0.151	0.001*	0.031	0.21	0.001*
number	-	6	18	9	17	16	11	22

2016

	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	61.17	189.25	222.71	480.18	137.84	310.26	212.73	447.82
TNW	104.00	570.99	620.42	993.14	934.58	1382.54	880.91	975.93
WIC/TNW	0.59	0.33	0.36	0.48	0.15	0.22	0.24	0.46
p-value	0.306	0.003*	0.001*	0.145	0.001*	0.001*	0.001*	0.09
number	3	11	39	10	22	41	53	10

Appendix Table A.4. Results of tests for individual specialization on alewife lengths by lake trout in 2015 and 2016. Test for individual specialization was done separately by region, size-class, and season. Individual specialization was calculated as WIC/TNW for each species and on individuals that consumed more than one measurable alewife. Values close to 0 indicate size specialization on alewife whereas values close to 1 indicate alewife length generalization. * indicates significant individual specialization.

2015

	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	-	219.40	222.16	126.21	178.49	182.67	206.62	138.19
TNW	-	415.96	298.94	350.54	453.01	318.99	419.98	235.91
WIC/TNW	-	0.53	0.74	0.36	0.39	0.57	0.49	0.59
p-value	-	0.191	0.908	0.002*	0.022	0.151	0.031	0.309
number	-	20	19	30	22	47	43	27

2016

	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	369.18	291.82	395.00	193.08	382.98	339.47	340.77	382.09
TNW	1662.10	1202.00	1297.55	1008.49	1792.92	1472.48	1444.73	1384.99
WIC/TNW	0.22	0.24	0.30	0.19	0.21	0.23	0.24	0.28
p-value	0.001*	0.001*	0.001*	0.011	0.001*	0.001*	0.001*	0.001*
number	19	21	58	11	27	82	84	25

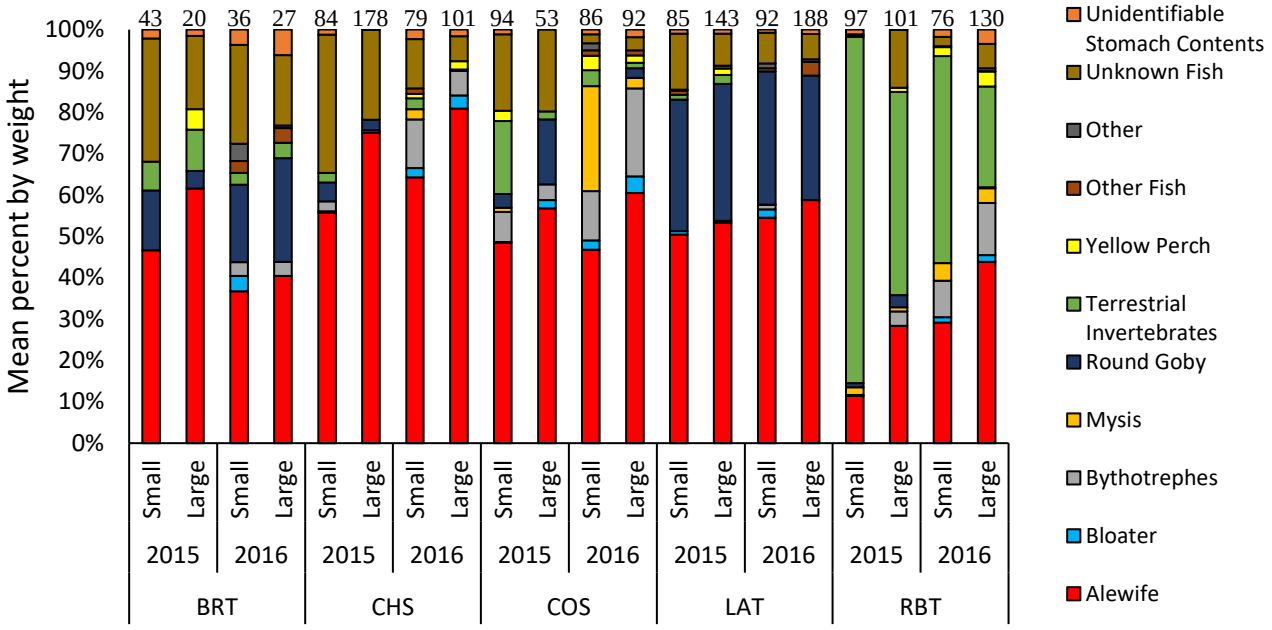
Appendix Table A.5. Results of tests for individual specialization on alewife lengths by rainbow trout in 2015 and 2016. Test for individual specialization was done separately by region, size-class, and season. Individual specialization was calculated as WIC/TNW for each species and on individuals that consumed more than one measurable alewife. Values close to 0 indicate size specialization on alewife whereas values close to 1 indicate alewife length generalization. * indicates significant individual specialization.

2015

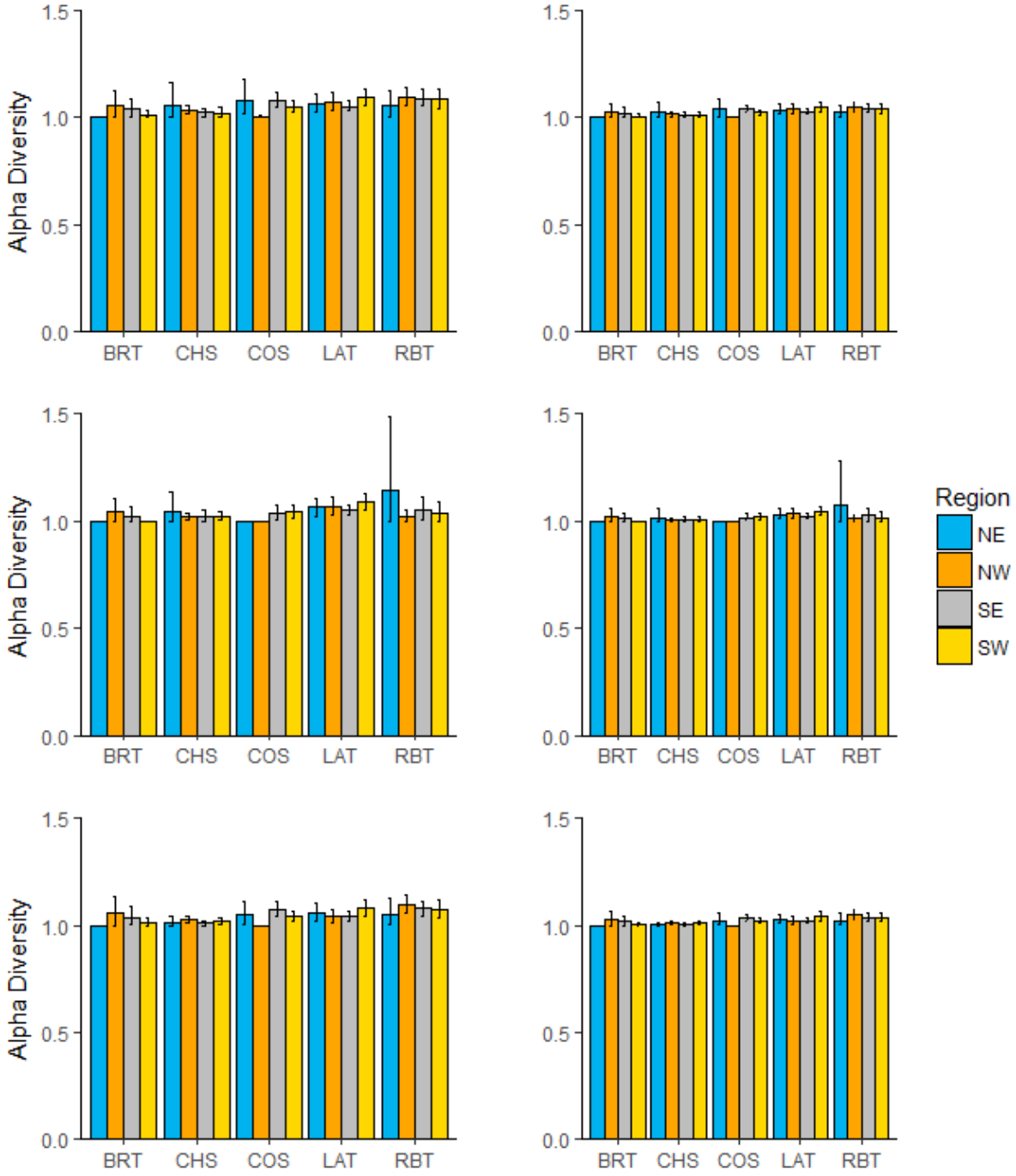
	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	-	121.20	74.89	107.71	82.63	108.00	129.84	51.93
TNW	-	395.11	198.99	2599.87	2414.78	338.38	364.66	2562.79
WIC/TNW	-	0.31	0.38	0.04	0.03	0.32	0.36	0.02
p-value	-	0.075	0.217	0.002*	0.001*	0.018	0.035	0.002*
number	-	6	4	7	6	12	11	7

2016

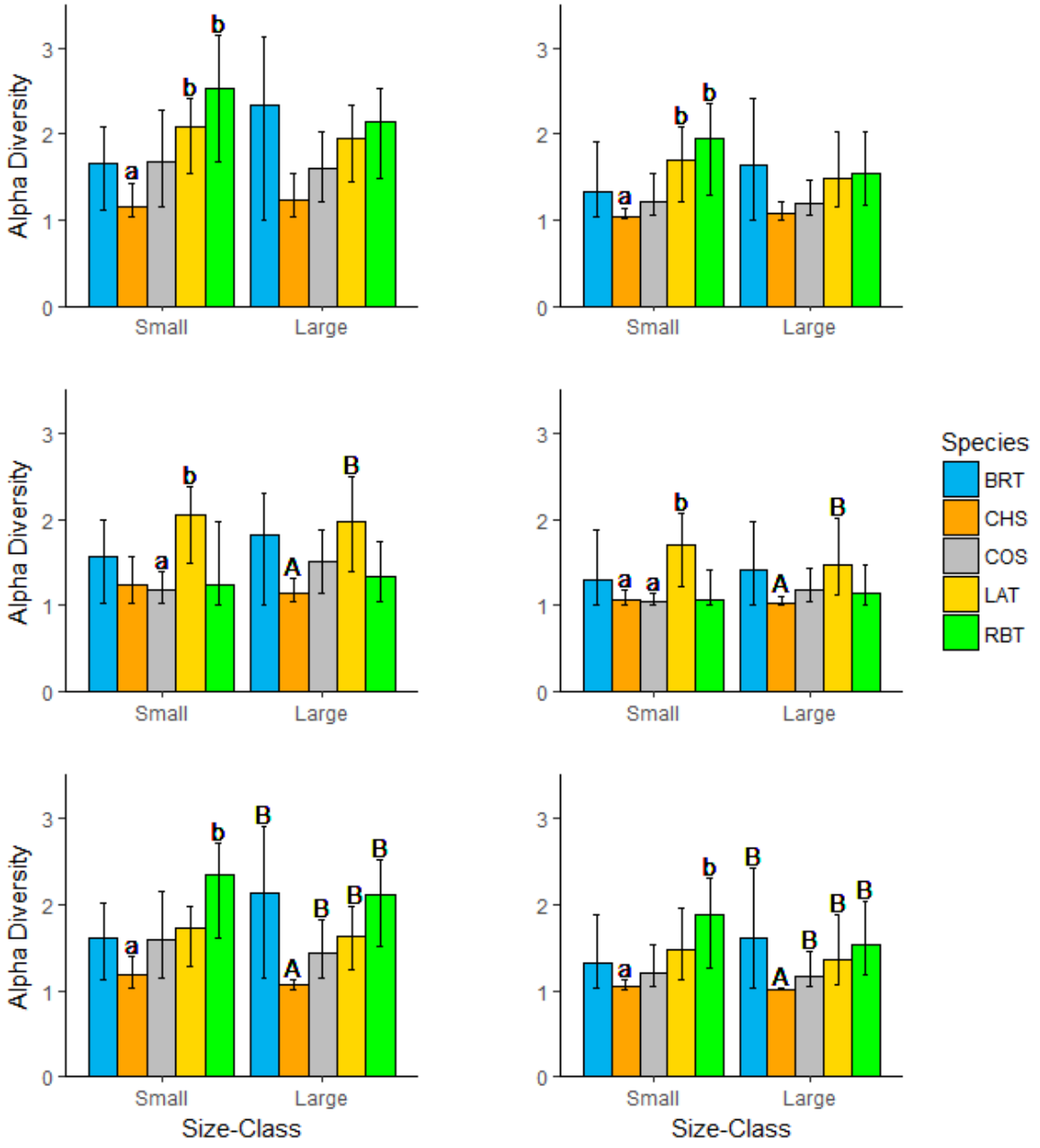
	Region				Size-class		Season	
	NE	NW	SW	SE	Small	Large	Early	Late
WIC	361.52	344.67	241.88	563.60	123.27	374.49	263.62	527.10
TNW	864.47	546.45	371.06	997.86	199.78	762.49	380.44	2016.52
WIC/TNW	0.42	0.63	0.65	0.56	0.62	0.49	0.69	0.26
p-value	0.01*	0.072	0.028	0.221	0.032	0.001*	0.045	0.008*
number	2	20	28	3	15	38	45	8



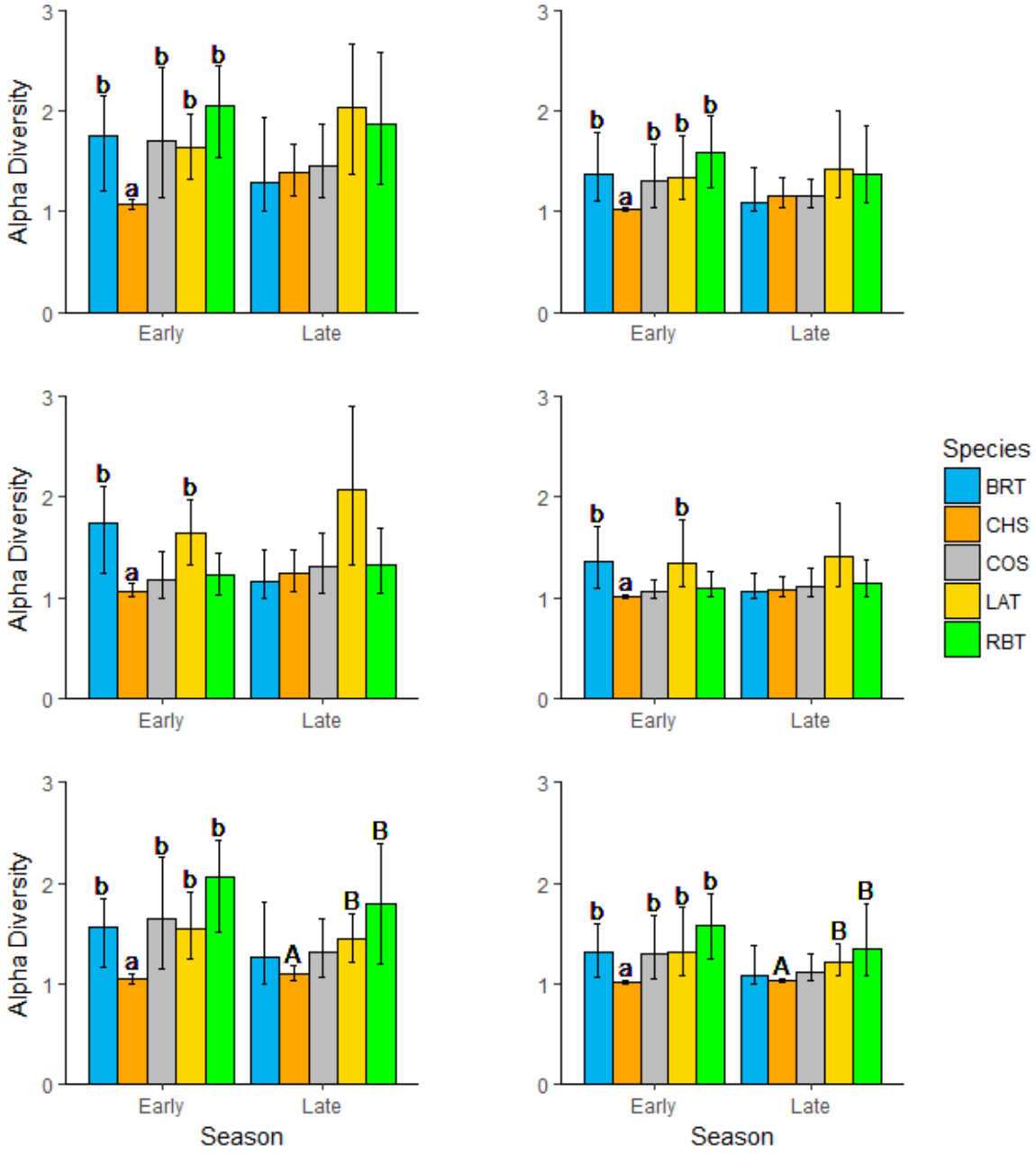
Appendix Figure A.1. Mean percent diet composition by weight for small (<600 mm) and large (≥600 mm) salmonids in 2015 and 2016. Numbers above bars represent the number of full stomachs analyzed. Salmonid species: BRT=brown trout; CHS=Chinook salmon; COS=Coho salmon; LAT= lake trout; RBT=rainbow trout.



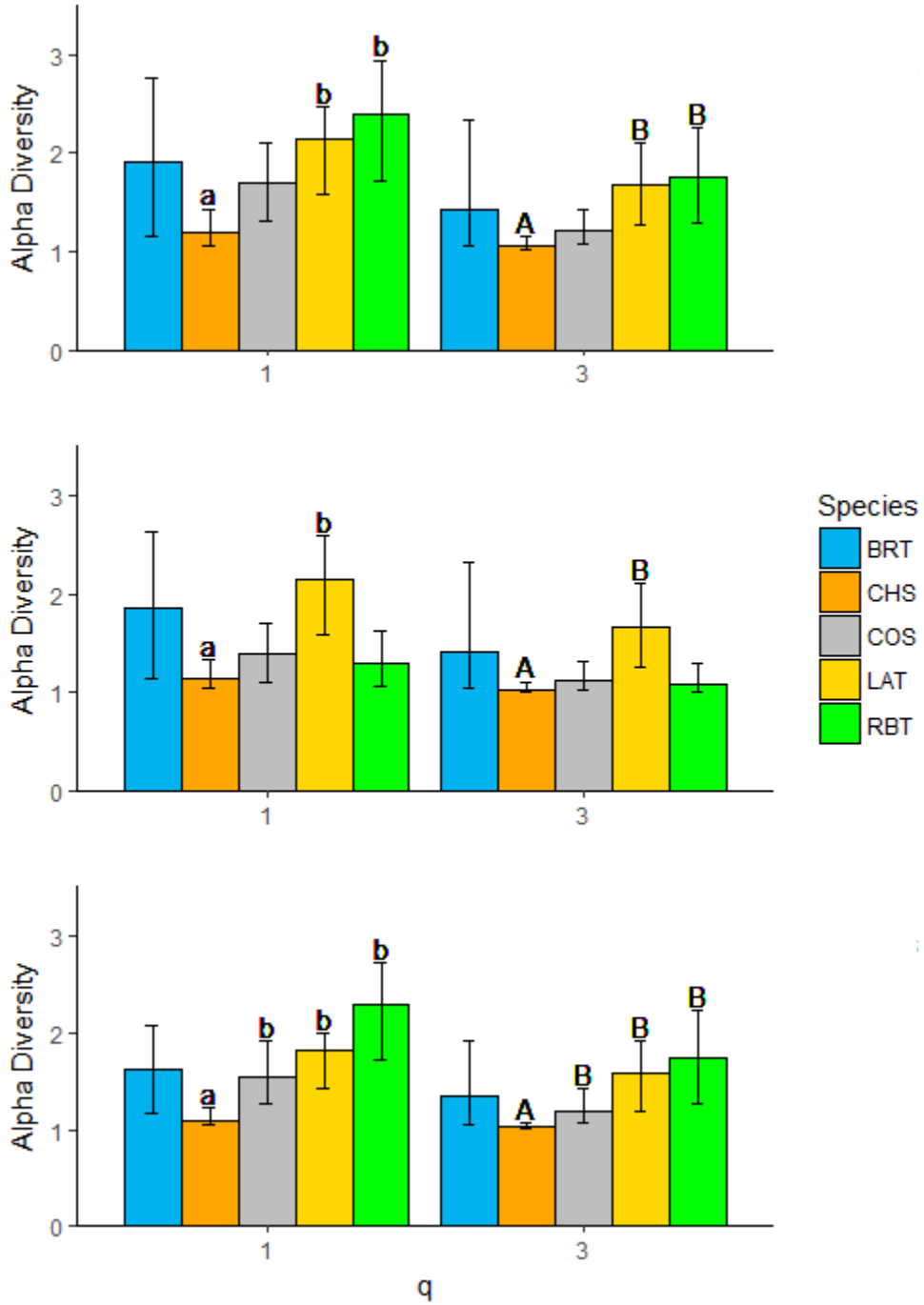
Appendix Figure A.2. Variation in regional diet diversity of salmonids at q=1 (left column) and q=3 (right column) for the abundance (top), fish prey (middle), and trophic categorizations (bottom). Region is the bottom level of organization, so alpha diversity represents the mean diet diversity of individuals in each region for each species. Error bars represent 95% confidence intervals, where non-overlapping intervals represent significant differences. BRT=brown trout; CHS=Chinook salmon; COS=Coho salmon; LAT= lake trout; RBT=rainbow trout.



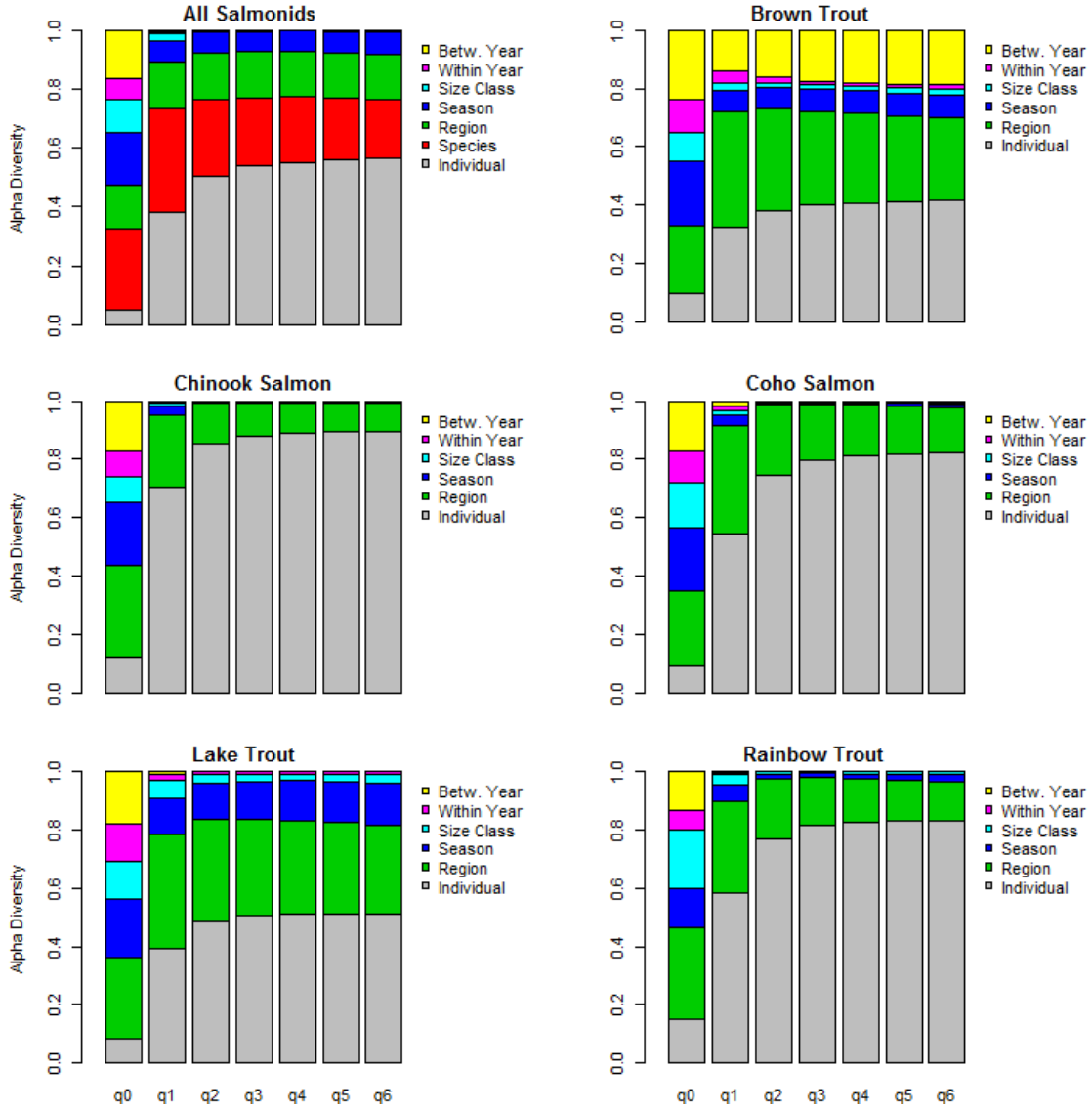
Appendix Figure A.3. Variation in diet diversity of small and large salmonids at q=1 (left column) and q=3 (right column) for the abundance (top), fish prey (middle), and trophic categorizations (bottom). Error bars represent 95% confidence intervals, where non-overlapping intervals represent significant differences.



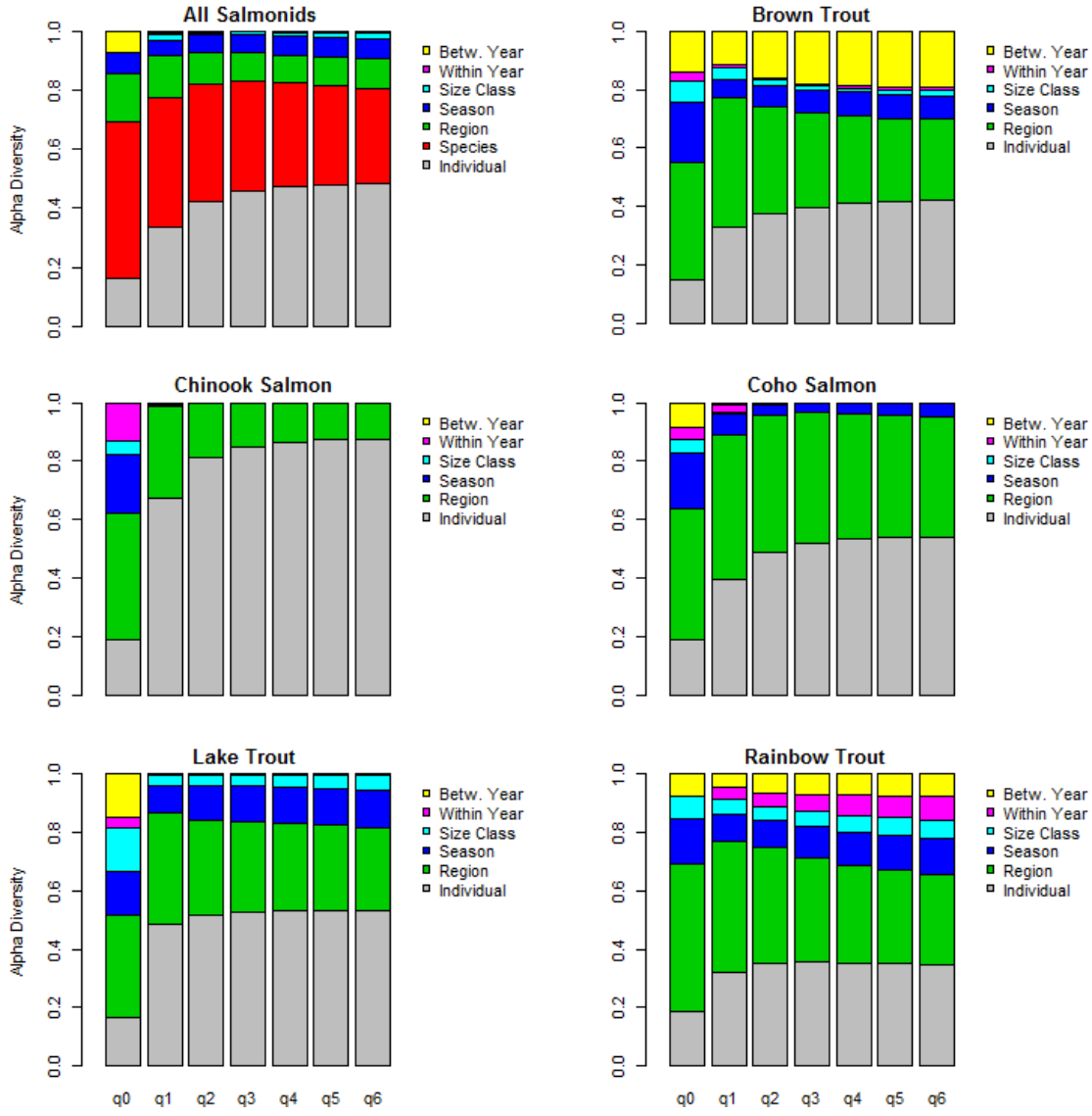
Appendix Figure A.4. Variation in diet diversity of salmonids in spring and fall at q=1 (top) and q=3 (bottom). Error bars represent 95% confidence intervals, where non-overlapping intervals represent significant differences.



Appendix Figure A.5. Overall diet diversity of salmonids at q=1 (gray bars) and q=3 (white bars) by abundance (top, fish prey (middle), and trophic categorizations (bottom). Error bars represent 95% confidence intervals, where non-overlapping intervals represent significant differences.



Appendix Figure A.6. Diversity partitioning by proportion of diet items by weight using just fish consumed in all salmonid species combined and each salmonid species separately at q=0 to q=6.



Appendix Figure A.7. Diversity partitioning by proportion of diet items by weight grouped into coarse trophic categorizations for salmonids as a whole and each salmonid species separately at q=0 to q=6.

APPENDIX B. CHAPTER 3 SUPPLEMENTARY MATERIAL

Appendix Table B.1. Sample sizes of Lake Michigan salmonid species by region of capture whose trophic indicators were compared. BRT=brown trout, CHS=Chinook salmon, COS=Coho salmon, LAT=lake trout, RBT=rainbow trout. NE=Northeast; NW=Northwest; SE=Southeast; SW=Southwest.

		Sample Sizes				
Individual-level Comparisons	Region	BRT	CHS	COS	LAT	RBT
Fatty Acids-Stable Isotopes	NE	7	15	12	9	8
	NW	16	21	13	18	10
	SE	11	17	16	21	10
	SW	12	20	16	17	10
Fatty Acids-Stomach Contents	NE	4	12	23	16	12
	NW	12	48	21	20	28
	SE	15	39	34	40	27
	SW	10	34	39	20	30
Stable Isotopes-Stomach Contents	NE	4	9	14	7	6
	NW	10	21	11	14	12
	SE	7	18	18	17	10
	SW	10	20	17	15	10
Linear Mixed Models						
Stomach contents-Stable Isotopes	NE	4	9	14	7	6
	NW	10	21	11	13	12
	SE	6	17	18	17	10
	SW	10	20	17	15	10

Appendix Table B.2. Loadings of PCA analyses completed on all salmonids and separately for each species using fatty acids expressed as mg of fatty acid/g of wet sample

	All Salmonids			Brown Trout			Chinook Salmon		
Fatty Acid	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
C14:0	0.112	0.034	0.172	0.128	0.100	0.076	0.128	0.048	0.115
C16:0	0.540	-0.185	0.576	0.569	-0.067	0.688	0.553	-0.276	0.646
C16.1n.9	0.020	0.002	0.012	0.017	0.016	0.000	0.020	0.013	0.002
C16:1n-7	0.186	0.289	-0.497	0.251	-0.662	-0.152	0.155	0.105	0.090
C18:0	0.146	-0.085	0.232	0.161	-0.133	0.251	0.148	-0.158	0.048
C18:1n-9	0.691	0.415	-0.209	0.618	0.016	-0.595	0.721	0.351	-0.486
C18:1n-7	0.149	0.061	-0.162	0.178	-0.181	-0.058	0.140	0.022	0.008
C18:2n-6	0.153	0.019	0.153	0.126	0.288	-0.060	0.180	0.109	-0.026
C18:3n-3	0.102	0.008	0.112	0.094	0.204	-0.057	0.122	0.084	0.108
C18:4n-3	0.038	0.006	0.015	0.034	0.063	-0.029	0.043	0.041	0.095
C20:1	0.069	0.025	0.003	0.064	0.053	-0.109	0.069	0.030	-0.082
C20:2n-6	0.034	-0.028	0.013	0.026	0.104	-0.031	0.032	-0.001	-0.019
C20:4n-6	0.085	-0.106	-0.086	0.084	0.044	-0.047	0.063	-0.102	0.004
C20:3n-3	0.029	-0.030	0.012	0.023	0.122	-0.023	0.030	-0.002	0.011
C20:4n-3	0.071	-0.045	0.036	0.079	0.249	-0.073	0.074	0.027	0.026
C20:5n-3	0.125	-0.175	-0.206	0.156	-0.038	-0.013	0.075	-0.171	0.193
C22.4n.6	0.026	-0.033	-0.076	0.022	0.033	-0.059	0.014	-0.044	-0.041
C22:5n-6	0.048	-0.120	-0.106	0.045	0.130	-0.075	0.027	-0.109	-0.084
C22:5n-3	0.089	-0.145	-0.216	0.103	0.064	-0.166	0.050	-0.184	-0.341
C22:6n-3	0.244	-0.784	-0.341	0.274	0.502	0.110	0.132	-0.808	-0.363
Prop. Variance	0.83	0.07	0.05	0.92	0.04	0.01	0.87	0.07	0.03

	Coho Salmon			Lake Trout			Rainbow Trout		
Fatty Acid	PC1	PC2	PC3	PC1	PC2	PC3	PC1	PC2	PC3
C14:0	0.108	0.119	0.012	0.080	0.092	0.055	0.087	0.087	0.034
C16:0	0.560	-0.307	0.558	0.454	0.036	-0.605	0.503	0.254	-0.567
C16.1n.9	0.020	0.012	0.005	0.017	0.017	0.027	0.018	0.001	0.025
C16:1n-7	0.119	0.132	0.082	0.324	-0.466	-0.343	0.153	-0.279	-0.141
C18:0	0.152	-0.192	0.279	0.108	-0.100	-0.188	0.155	0.049	-0.274
C18:1n-9	0.615	0.555	-0.019	0.730	-0.152	0.600	0.631	-0.605	0.072
C18:1n-7	0.138	0.102	-0.058	0.177	0.019	-0.064	0.140	-0.089	0.129
C18:2n-6	0.140	0.212	-0.210	0.101	0.268	0.173	0.173	-0.088	0.277
C18:3n-3	0.092	0.098	-0.097	0.065	0.255	0.060	0.115	0.062	0.200
C18:4n-3	0.032	0.014	-0.020	0.029	0.141	0.011	0.036	0.063	0.099
C20:1	0.067	0.123	-0.057	0.059	0.011	0.090	0.078	-0.074	0.106
C20:2n-6	0.042	0.067	-0.118	0.019	0.080	0.042	0.044	0.006	0.129
C20:4n-6	0.103	-0.082	-0.029	0.087	0.051	0.024	0.104	0.112	0.127
C20:3n-3	0.037	0.070	-0.097	0.014	0.106	0.016	0.043	0.016	0.124
C20:4n-3	0.081	0.154	-0.206	0.036	0.242	0.108	0.100	0.088	0.316
C20:5n-3	0.153	-0.142	0.000	0.146	0.184	-0.076	0.164	0.186	0.221
C22.4n.6	0.030	0.010	-0.059	0.033	-0.007	0.005	0.047	0.000	0.098
C22:5n-6	0.071	-0.003	-0.146	0.047	0.083	0.003	0.076	0.082	0.138
C22:5n-3	0.122	0.110	-0.303	0.094	0.083	-0.010	0.144	0.091	0.437
C22:6n-3	0.377	-0.614	-0.600	0.212	0.675	-0.225	0.379	0.619	0.077
Prop. Variance	0.85	0.09	0.03	0.92	0.05	0.02	0.92	0.04	0.01

Appendix Table B.3. Results of pairwise comparisons between fatty acids, fatty acid PCA axes, stable isotopes, and stomach contents. For these models, fatty acids were expressed as mg of fatty acid/g of wet sample. Models with stable isotope ratios as response variables were linear models using F-statistics, whereas models involving stomach contents were logistic GLMs using χ^2. Models with p-values less than 0.001 are bolded and those that were between 0.05 and 0.001 are italicized. Interaction terms were removed from models if p-values were greater than 0.05.

Explanatory	Response	Species	Species P	FA	FA P	Interaction	Interaction P	R^2
ALA	*$\delta^{13}C$*	*7.96*	*<0.0001*	*2.71*	*0.1009*	*2.44*	*0.0473*	*0.25*
ALA	$\delta^{15}N$	97.92	<0.0001	0.02	0.8752	-	-	0.59
ALA	Alewife	37.76	<0.0001	0.26	0.6099	-	-	0.11
ALA	Round goby	0.23	0.6326	1.28	0.2585	-	-	0.02
ARA	$\delta^{13}C$	20.67	*<0.0001*	1.41	0.2355	-	-	0.22
ARA	*$\delta^{15}N$*	*101.94*	*<0.0001*	*8.51*	*0.0038*	-	-	*0.60*
ARA	Alewife	45.97	<0.0001	2.39	0.1220	-	-	0.11
ARA	*Round goby*	*0.11*	*0.7439*	*6.93*	*0.0085*	-	-	*0.07*
DHA	$\delta^{13}C$	20.26	<0.0001	0.16	0.6916	-	-	0.21
DHA	*$\delta^{15}N$*	*99.24*	*<0.0001*	*6.14*	*0.0138*	-	-	*0.60*
DHA	Alewife	43.56	<0.0001	0.49	0.4839	-	-	0.11
DHA	Round goby	0.08	0.7811	2.04	0.1533	-	-	0.02
EPA	$\delta^{13}C$	20.18	<0.0001	0.02	0.8800	-	-	0.21
EPA	*$\delta^{15}N$*	*104.53*	*<0.0001*	*7.25*	*0.0075*	-	-	*0.60*
EPA	Alewife	44.26	<0.0001	0.46	0.4986	-	-	0.11
EPA	*Round goby*	*0.06*	*0.8010*	*6.73*	*0.0095*	-	-	*0.07*
16:1n-7	*$\delta^{13}C$*	*19.00*	<0.0001	*9.96*	*0.0018*	-	-	*0.24*
16:1n-7	*$\delta^{15}N$*	*31.59*	<0.0001	*3.28*	*0.0712*	*2.45*	*0.0463*	*0.60*
16:1n-7	*Alewife*	*12.16*	*0.0162*	*4.92*	*0.0266*	*11.09*	*0.0256*	*0.15*
16:1n-7	**Round goby**	**0.29**	**0.5898**	**12.35**	**0.0004**	-	-	**0.16**
18:1n-9	$\delta^{13}C$	20.11	0.0000	1.23	0.2688	-	-	0.22
18:1n-9	$\delta^{15}N$	100.72	0.0000	0.73	0.3929	-	-	0.59
18:1n-9	Alewife	46.13	<0.0001	1.20	0.2725	-	-	0.11
18:1n-9	*Round goby*	*0.09*	*0.7663*	*8.01*	*0.0046*	-	-	*0.09*
PC1	$\delta^{13}C$	19.78	<0.0001	1.14	0.2864	-	-	0.22
PC1	$\delta^{15}N$	99.42	<0.0001	2.02	0.1567	-	-	0.59
PC1	Alewife	46.13	<0.0001	1.22	0.2697	-	-	0.11
PC1	*Round goby*	*0.03*	*0.8651*	*7.63*	*0.0057*	-	-	*0.08*
PC2	**$\delta^{13}C$**	**13.31**	**<0.0001**	**13.73**	**0.0003**	**7.49**	**<0.0001**	**0.29**
PC2	*$\delta^{15}N$*	*81.38*	*<0.0001*	*4.27*	*0.0397*	*3.09*	*0.0165*	*0.61*
PC2	Alewife	44.24	<0.0001	0.31	0.5784	-	-	0.11
PC2	*Round goby*	*0.24*	*0.6227*	*6.78*	*0.0092*	-	-	*0.07*
PC3	**$\delta^{13}C$**	**8.62**	**<0.0001**	**22.87**	**<0.0001**	**7.40**	**<0.0001**	**0.29**
PC3	**$\delta^{15}N$**	**52.01**	**<0.0001**	**7.62**	**0.0062**	**4.80**	**0.0009**	**0.61**
PC3	*Alewife*	*23.38*	*0.0001*	*3.58*	*0.0586*	*9.70*	*0.0457*	*0.11*
PC3	*Round goby*	*1.38*	*0.2401*	*10.65*	*0.0011*	-	-	*0.12*

Appendix Table B.4. Results of pairwise comparisons between species-specific fatty acid PCA axes and stable isotopes and stomach contents with fatty acids expressed as mg/ g of wet sample. Models with stable isotope ratios as response variables were linear models using F-statistics and models involving stomach contents were logistic GLMs using χ^2. Models with p-values less than 0.001 are bolded and those that were between 0.05 and 0.001 are italicized.

		Chinook Salmon			Coho Salmon			Rainbow Trout		
Explanatory	Response	FA	FA P	R^2	FA	FA P	R^2	FA	FA P	R^2
PC 1	Alewife	1.45	0.2289	0.02	2.88	0.0896	0.03	0.29	0.5872	0.00
PC 1	$\delta^{13}C$	2.07	0.1551	0.01	1.55	0.2179	0.01	0.69	0.4111	0.03
PC 1	$\delta^{15}N$	0.23	0.6322	-0.01	0.63	0.4303	-0.01	1.16	0.2890	0.00
PC 2	Alewife	1.43	0.2311	0.02	3.36	0.0667	0.03	0.29	0.5928	0.00
PC 2	$\delta^{13}C$	1.52	0.2219	0.01	*6.99*	*0.0107*	*0.10*	0.93	0.3414	0.03
PC 2	$\delta^{15}N$	3.52	0.0646	0.03	*11.23*	*0.0015*	*0.15*	1.09	0.3030	0.00
PC 3	Alewife	0.16	0.6933	0.00	*4.30*	*0.0381*	*0.05*	*4.83*	*0.0280*	*0.06*
PC 3	$\delta^{13}C$	0.31	0.5815	-0.01	0.99	0.3230	0.00	0.93	0.3403	0.00
PC 3	$\delta^{15}N$	*6.16*	*0.0154*	*0.07*	*4.61*	*0.0362*	*0.06*	*4.98*	*0.0319*	*0.10*

		Brown Trout			Lake Trout		
Explanatory	Response	FA	FA P	R^2	FA	FA P	R^2
PC 1	Alewife	2.09	0.1484	0.06	1.63	0.2021	0.02
PC 1	Round Goby	1.34	0.2465	0.04	*6.55*	*0.0105*	*0.10*
PC 1	$\delta^{13}C$	0.01	0.9352	-0.02	0.68	0.4111	0.00
PC 1	$\delta^{15}N$	0.12	0.7264	-0.02	*5.83*	*0.0186*	*0.07*
PC 2	Alewife	3.491	0.0617	0.13	3.32	0.0686	0.04
PC 2	Round Goby	*5.13*	*0.0235*	*0.18*	2.23	0.1354	0.03
PC 2	$\delta^{13}C$	*7.76*	*0.0078*	*0.13*	**24.58**	**<0.0001**	**0.27**
PC 2	$\delta^{15}N$	*8.46*	*0.0057*	*0.14*	0.21	0.6475	-0.01
PC 3	Alewife	2.59	0.1076	0.08	0.03	0.8709	0.00
PC 3	Round Goby	3.46	0.0628	0.10	0.41	0.5222	0.01
PC 3	$\delta^{13}C$	*8.66*	*0.0052*	*0.15*	0.71	0.4032	0.00
PC 3	$\delta^{15}N$	2.81	0.1006	0.04	0.03	0.8690	-0.02

Appendix Table B.5. Mean proportional regional diet composition by weight of each diet category for salmonids with both stomach contents and stable isotope ratios analyzed in 2016. Not included are unidentifiable stomach contents and prey that lacked known stable isotope ratios in Lake Michigan. NW=Northwest; SW=Southwest; SE=Southeast; NE=Northeast.

	Brown Trout				Chinook Salmon				Coho Salmon			
Prey categories	NW	SW	SE	NE	NW	SW	SE	NE	NW	SW	SE	NE
Alewife <100 mm	0.346	0.396	0	0.250	0.559	0.286	0.083	0	0.316	0.256	0.069	0.035
Alewife >100 mm	0.323	0	0	0	0.329	0.165	0.446	0.447	0.453	0.283	0.285	0.213
Alewife unsized	0.117	0.104	0.167	0	0.090	0.198	0.031	0.178	0.049	0.178	0.029	0.073
Amphipod	0	0	0.167	0	0	0	0	0	0	0	0	0
Bloater	0.100	0.100	0	0	0	0.050	0.028	0.258	0.091	0.003	0.111	0
Bythotrephes	0.014	0.208	0	0	0.021	0.250	0.182	0.117	0.091	0.114	0.056	0.673
Chironomidae	0	0	0	0	0	0	0	0	0	0	0	0
Dreissenidae	0	0.100	0.079	0	0	0	0	0	0	0	0	0
Mysis	0	0	0	0	0	0	0.059	0	0	0	0.195	0.001
Rainbow Smelt	0	0	0	0	0	0	0.059	0	0	0	0	0
Round Goby < 60 mm	0	0	0.296	0	0	0	0	0	0	0	0	0
Round Goby 60-100 mm	0	0.092	0	0.381	0	0	0	0	0	0	0	0
Round Goby >100 mm	0.100	0	0.125	0.085	0	0	0	0	0	0.041	0.029	0
Round Goby unsized	0	0	0.167	0.033	0	0	0	0	0	0.003	0	0
Sculpin	0	0	0	0	0	0	0	0	0	0	0	0
Terrestrial Insect	0	0	0	0.250	0	0	0.059	0	0	0.003	0.125	0.005
Three-spine stickleback	0	0	0	0	0	0	0	0	0	0	0.056	0
Yellow Perch	0	0	0	0	0	0.050	0.053	0	0	0.118	0.045	0

Appendix Table B.5 continued

	Lake Trout				Rainbow trout			
Prey categories	NW	SW	SE	NE	NW	SW	SE	NE
Alewife <100 mm	0.214	0.208	0	0.290	0.282	0.387	0.035	0.010
Alewife >100 mm	0.510	0.369	0.385	0.286	0.178	0.070	0.094	0.118
Alewife unsized	0.084	0.156	0.026	0.139	0.124	0.203	0.005	0.167
Amphipod	0	0	0	0	0	0	0	0
Bloater	0	0	0.059	0	0.021	0	0.106	0
Bythotrephes	0	0.067	0	0	0.140	0.200	0.077	0.006
Chironomidae	0	0	0	0	0	0.001	0	0
Dreissenidae	0	0	0.059	0	0	0	0	0
Mysis	0.001	0	0	0	0	0	0.093	0
Rainbow Smelt	0.078	0	0	0	0	0	0	0
Round Goby < 60 mm	0	0	0	0	0	0	0	0
Round Goby 60-100 mm	0.035	0.067	0.345	0.031	0	0	0	0
Round Goby >100 mm	0	0.124	0.059	0.254	0	0	0	0.037
Round Goby unsized	0	0.010	0.067	0.001	0	0	0	0
Sculpin	0.077	0	0	0	0	0	0	0
Terrestrial Insect	0	0	0	0	0.256	0.139	0.426	0.662
Three-spine stickleback	0	0	0	0	0	0	0	0
Yellow Perch	0	0	0	0	0	0	0.165	0

Appendix Table B.6. Results of pairwise comparisons between fatty acids and stable isotopes or stomach contents for brown trout with sex, fatty acid, and their interaction as response variables. Fatty acid-stable isotope regressions were linear models using F-statistics and fatty acid-diet content regressions were logistic GLMs using χ^2. Models with interaction p-values between 0.05 and 0.001 are italicized. Interaction terms were removed from models if p-values were greater than 0.05. Fatty acids are expressed as relative abundance. Models only included individuals that had sex identified.

Fatty Acid	Response	Sex	Sex P	FA	FA P	Interaction	Interaction P	R^2
ALA	*$\delta^{13}C$*	*5.39*	*0.0253*	*0.02*	*0.8756*	*5.97*	*0.0189*	*0.22*
ALA	$\delta^{15}N$	1.95	0.1701	6.25	0.0164	-	-	0.17
ALA	Alewife	0.66	0.4181	1.73	0.1887	-	-	0.09
ALA	Round goby	0.28	0.5970	2.67	0.1022	-	-	0.11
ARA	$\delta^{13}C$	0.24	0.6238	0.02	0.8981	-	-	0.01
ARA	$\delta^{15}N$	2.79	0.1026	0.16	0.6958	-	-	0.06
ARA	Alewife	0.92	0.3369	0.50	0.4811	-	-	0.05
ARA	Round goby	1.10	0.2940	0.92	0.3363	-	-	0.06
DHA	$\delta^{13}C$	0.25	0.6218	10.39	0.0025	-	-	0.20
DHA	$\delta^{15}N$	2.91	0.0957	5.91	0.0194	-	-	0.17
DHA	Alewife	1.40	0.2361	6.43	0.0112	-	-	0.24
DHA	Round goby	0.92	0.3371	2.71	0.0995	-	-	0.11
EPA	$\delta^{13}C$	1.47	0.2316	6.28	0.0162	-	-	0.13
EPA	$\delta^{15}N$	5.88	0.0197	6.77	0.0127	-	-	0.18
EPA	*Alewife*	*10.14*	*0.0015*	*3.71*	*0.0542*	*9.37*	*0.0022*	*0.35*
EPA	Round goby	0.81	0.3681	0.01	0.9185	-	-	0.03
n-3/n-6	$\delta^{13}C$	0.18	0.6761	1.06	0.3081	-	-	0.03
n-3/n-6	$\delta^{15}N$	2.48	0.1229	3.98	0.0524	-	-	0.13
n-3/n-6	Alewife	0.81	0.3680	3.54	0.0599	-	-	0.14
n-3/n-6	Round goby	0.82	0.3655	0.00	0.9779	-	-	0.03
16:1n-7	$\delta^{13}C$	0.00	0.9871	6.22	0.0167	-	-	0.13
16:1n-7	$\delta^{15}N$	1.27	0.2671	12.39	0.0011	-	-	0.26
16:1n-7	Alewife	0.30	0.5862	8.97	0.0027	-	-	0.43
16:1n-7	Round goby	0.17	0.6817	4.95	0.0260	-	-	0.18
18:1n-9	$\delta^{13}C$	0.05	0.8191	0.68	0.4147	-	-	0.02
18:1n-9	$\delta^{15}N$	2.12	0.1526	0.13	0.7194	-	-	0.06
18:1n-9	Alewife	0.61	0.4337	6.28	0.0122	-	-	0.27
18:1n-9	Round goby	0.16	0.6934	5.40	0.0201	-	-	0.20
18:1n-9/16:1n-7	$\delta^{13}C$	0.01	0.9367	7.19	0.0105	-	-	0.14
18:1n-9/16:1n-7	$\delta^{15}N$	1.50	0.2283	22.58	<0.0001	-	-	0.38
18:1n-9/16:1n-7	Alewife	0.43	0.5120	7.51	0.0061	-	-	0.26
18:1n-9/16:1n-7	Round goby	0.23	0.6348	5.16	0.0231	-	-	0.19
PC 1	$\delta^{13}C$	0.01	0.9217	7.94	0.0074	-	-	0.16
PC 1	$\delta^{15}N$	1.77	0.1903	8.13	0.0067	-	-	0.20
PC 1	Alewife	0.50	0.4787	10.26	0.0014	-	-	0.41
PC 1	Round goby	0.27	0.6043	6.49	0.0109	-	-	0.22
PC 2	$\delta^{13}C$	0.03	0.8733	3.47	0.0695	-	-	0.08
PC 2	$\delta^{15}N$	3.28	0.0774	1.19	0.2820	-	-	0.08
PC 2	Alewife	1.15	0.2827	0.00	0.9660	-	-	0.03
PC 2	Round goby	0.83	0.3624	0.10	0.7501	-	-	0.03
PC 3	$\delta^{13}C$	0.91	0.3461	8.91	0.0047	-	-	0.17
PC 3	$\delta^{15}N$	3.25	0.0788	1.39	0.2446	-	-	0.09
PC 3	*Alewife*	*1.03*	*0.3104*	*5.86*	*0.0155*	*9.25*	*0.0024*	*0.53*
PC 3	*Round goby*	*0.48*	*0.4885*	*3.76*	*0.0524*	*5.28*	*0.0216*	*0.20*

Appendix Table B.7. Results of pairwise comparisons between fatty acids and stable isotopes or stomach contents for Chinook salmon with sex, fatty acid, and their interaction as response variables. Fatty acid-stable isotope regressions were linear models using F-statistics and fatty acid-diet content regressions were logistic GLMs using χ^2. Models with interaction p-values between 0.05 and 0.001 are italicized. Interaction terms were removed from models if p-values were greater than 0.05. Fatty acids are expressed as relative abundance. Models only included individuals that had sex identified.

Fatty Acid	Response	Sex	Sex P	FA	FA P	Interaction	Interaction P	R^2
ALA	$\delta^{13}C$	1.90	0.1724	8.43	0.0050	-	-	0.13
ALA	$\delta^{15}N$	1.37	0.2458	4.18	0.0448	-	-	0.07
ALA	Alewife	5.72	0.0168	3.19	0.0742	-	-	0.10
ARA	*$\delta^{13}C$*	*6.98*	*0.0103*	*6.62*	*0.0123*	*5.99*	*0.0170*	*0.11*
ARA	$\delta^{15}N$	0.80	0.3739	8.75	0.0043	-	-	0.13
ARA	Alewife	6.86	0.0088	7.45	0.0063	-	-	*0.14*
DHA	*$\delta^{13}C$*	*6.39*	*0.0139*	*4.01*	*0.0494*	*5.05*	*0.0279*	*0.09*
DHA	$\delta^{15}N$	0.67	0.4144	2.53	0.1161	-	-	0.05
DHA	Alewife	6.57	0.0104	4.97	0.0258	-	-	*0.12*
EPA	$\delta^{13}C$	2.42	0.1241	3.14	0.0808	-	-	0.06
EPA	$\delta^{15}N$	0.83	0.3667	0.82	0.3675	-	-	0.03
EPA	Alewife	5.19	0.0227	0.69	0.4046	-	-	*0.07*
n-3/n-6	$\delta^{13}C$	6.23	0.0150	1.13	0.2914	5.22	0.0255	0.10
n-3/n-6	$\delta^{15}N$	0.92	0.3399	0.15	0.7039	-	-	0.02
n-3/n-6	Alewife	4.33	0.0375	0.12	0.7316	-	-	0.06
16:1n-7	*$\delta^{13}C$*	*6.91*	*0.0106*	*4.27*	*0.0426*	*8.14*	*0.0058*	*0.12*
16:1n-7	$\delta^{15}N$	0.63	0.4285	2.61	0.1111	-	-	0.05
16:1n-7	Alewife	5.08	0.0242	0.44	0.5077	-	-	0.06
18:1n-9	$\delta^{13}C$	1.94	0.1680	1.40	0.2402	-	-	0.04
18:1n-9	$\delta^{15}N$	0.87	0.3530	1.14	0.2895	-	-	0.03
18:1n-9	Alewife	4.65	0.0311	0.01	0.9248	-	-	0.06
18:1n-9/16:1n-7	$\delta^{13}C$	1.28	0.2612	0.93	0.3391	-	-	0.03
18:1n-9/16:1n-7	$\delta^{15}N$	1.02	0.3151	0.48	0.4904	-	-	0.02
18:1n-9/16:1n-7	Alewife	4.99	0.0255	0.50	0.4812	-	-	0.07
PC 1	*$\delta^{13}C$*	*0.91*	*0.3431*	*3.22*	*0.0772*	*5.49*	*0.0222*	*0.09*
PC 1	$\delta^{15}N$	0.66	0.4178	2.76	0.1015	-	-	0.05
PC 1	Alewife	5.72	0.0168	1.87	0.1718	-	-	0.08
PC 2	$\delta^{13}C$	1.51	0.2241	0.01	0.9222	-	-	0.02
PC 2	$\delta^{15}N$	1.16	0.2851	4.43	0.0391	-	-	0.08
PC 2	Alewife	4.67	0.0307	0.49	0.4825	-	-	0.07
PC 3	**$\delta^{13}C$**	2.09	0.1527	22.34	<0.0001	-	-	0.26
PC 3	*$\delta^{15}N$*	1.41	0.2387	10.89	0.0015	-	-	0.15
PC 3	*Alewife*	5.6549	0.0174	16.27	<0.0001	-	-	0.05

Appendix Table B.8. Results of pairwise comparisons between fatty acids and stable isotopes or stomach contents for Coho salmon with sex, fatty acid, and their interaction as response variables. Fatty acid-stable isotope regressions were linear models using F-statistics and fatty acid-diet content regressions were logistic GLMs using χ^2. Models with interaction p-values between 0.05 and 0.001 are italicized. Interaction terms were removed from models if p-values were greater than 0.05. Fatty acids are expressed as relative abundance. Models only included individuals that had sex identified.

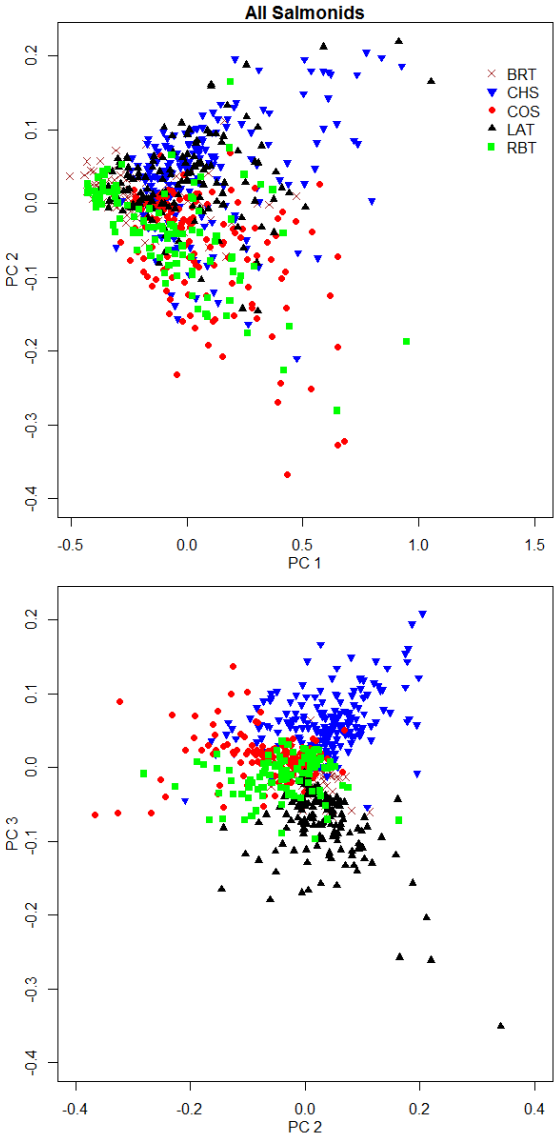
Fatty Acid	Response	Sex	Sex P	FA	FA P	Interaction	Interaction P	R^2
ALA	$\delta^{13}C$	0.05	0.8221	5.58	0.0221	-	-	0.10
ALA	$\delta^{15}N$	0.03	0.8716	5.10	0.0283	-	-	0.09
ALA	Alewife	0.08	0.7718	3.79	0.0517	-	-	0.04
ARA	$\delta^{13}C$	*0.28*	0.6021	*5.68*	0.0210	-	-	0.10
ARA	*$\delta^{15}N$*	*8.27*	*0.0059*	*0.20*	*0.6602*	*8.62*	*0.0051*	*0.24*
ARA	*Alewife*	*4.98*	*0.0257*	*0.13*	*0.7213*	*5.00*	*0.0253*	*0.11*
DHA	$\delta^{13}C$	0.15	0.6973	1.80	0.1864	-	-	0.04
DHA	*$\delta^{15}N$*	*4.72*	*0.0347*	*0.06*	*0.8099*	*4.95*	*0.0307*	*0.18*
DHA	Alewife	0.05	0.8277	1.28	0.2572	-	-	0.01
EPA	$\delta^{13}C$	0.08	0.7808	0.65	0.4245	-	-	0.02
EPA	*$\delta^{15}N$*	*7.81*	*0.0074*	*0.30*	*0.5872*	*7.46*	*0.0088*	*0.30*
EPA	*Alewife*	*3.93*	*0.0474*	*1.82*	*0.1778*	*3.96*	*0.0466*	*0.04*
n-3/n-6	$\delta^{13}C$	0.13	0.7198	0.10	0.7529	-	-	0.01
n-3/n-6	$\delta^{15}N$	0.15	0.6988	7.67	0.0079	-	-	0.13
n-3/n-6	*Alewife*	*3.93*	*0.0474*	*2.29*	*0.1301*	*3.92*	*0.0478*	*0.05*
16:1n-7	$\delta^{13}C$	0.17	0.6781	0.01	0.9267	-	-	<0.01
16:1n-7	$\delta^{15}N$	0.01	0.9422	0.85	0.3596	-	-	0.02
16:1n-7	Alewife	0.02	0.8995	0.14	0.7103	-	-	<0.01
18:1n-9	$\delta^{13}C$	0.07	0.7956	2.65	0.1099	-	-	0.05
18:1n-9	$\delta^{15}N$	0.03	0.8666	4.26	0.0443	-	-	0.07
18:1n-9	Alewife	0.00	0.9816	1.88	0.1699	-	-	0.02
18:1n-9/16:1n-7	$\delta^{13}C$	0.07	0.7977	1.93	0.1707	-	-	0.04
18:1n-9/16:1n-7	$\delta^{15}N$	<0.01	0.9773	0.30	0.5836	-	-	0.01
18:1n-9/16:1n-7	Alewife	0.01	0.9164	0.92	0.3371	-	-	0.01
PC 1	$\delta^{13}C$	0.01	0.9365	5.08	0.0287	-	-	0.09
PC 1	*$\delta^{15}N$*	*0.24*	*0.6282*	*0.73*	*0.3969*	*4.53*	*0.0384*	*0.26*
PC 1	Alewife	0.08	0.7740	1.28	0.2578	-	-	0.01
PC 2	$\delta^{13}C$	0.02	0.9020	1.83	0.1824	-	-	0.02
PC 2	$\delta^{15}N$	0.13	0.7219	3.42	0.0704	-	-	0.06
PC 2	Alewife	0.05	0.8153	12.37	0.0004	-	-	0.15
PC 3	$\delta^{13}C$	0.02	0.8826	1.23	0.2718	-	-	0.03
PC 3	$\delta^{15}N$	<0.01	0.9647	0.11	0.7360	-	-	0.00
PC 3	Alewife	<0.01	0.9961	4.24	0.0394	-	-	0.05

Appendix Table B.9. Results of pairwise comparisons between fatty acids and stable isotopes or stomach contents for lake trout with sex, fatty acid, and their interaction as response variables. Fatty acid-stable isotope regressions were linear models using F-statistics and fatty acid-diet content regressions were logistic GLMs using χ^2. Models with interaction p-values between 0.05 and 0.001 are italicized. Interaction terms were removed from models if p-values were greater than 0.05. Fatty acids are expressed as relative abundance. Models only included individuals that had sex identified.

Fatty Acid	Response	Sex	Sex P	FA	FA P	Interaction	Interaction P	R^2
ALA	*$\delta^{13}C$*	*6.86*	*0.0120*	*37.03*	*<0.0001*	*5.44*	*0.0243*	*0.55*
ALA	$\delta^{15}N$	1.92	0.1722	1.09	0.3011	-	-	0.05
ALA	Alewife	1.47	0.2248	0.05	0.8217	-	-	0.03
ALA	Round goby	0.97	0.3241	0.10	0.7461	-	-	0.02
ARA	$\delta^{13}C$	5.73	0.0207	0.06	0.8120	-	-	0.11
ARA	$\delta^{15}N$	1.31	0.2575	0.06	0.8135	-	-	0.03
ARA	Alewife	1.34	0.2464	0.27	0.6032	-	-	0.03
ARA	Round goby	0.85	0.3558	0.29	0.5922	-	-	0.02
DHA	*$\delta^{13}C$*	*5.98*	*0.0184*	*14.73*	*0.0004*	*5.03*	*0.0298*	*0.34*
DHA	$\delta^{15}N$	2.64	0.1107	2.56	0.1161	-	-	0.08
DHA	Alewife	1.26	0.2616	0.27	0.6031	-	-	0.03
DHA	Round goby	0.84	0.3603	0.12	0.7297	-	-	0.02
EPA	$\delta^{13}C$	7.56	0.0084	8.32	0.0059	-	-	0.24
EPA	$\delta^{15}N$	1.24	0.2702	1.19	0.2810	-	-	0.05
EPA	Alewife	1.40	0.2372	0.16	0.6924	-	-	0.03
EPA	Round goby	0.85	0.3566	1.06	0.3025	-	-	0.04
n-3/n-6	$\delta^{13}C$	6.06	0.0176	4.68	0.0356	-	-	0.18
n-3/n-6	$\delta^{15}N$	1.58	0.2154	3.79	0.0577	-	-	0.10
n-3/n-6	*Alewife*	*3.85*	*0.0498*	*1.02*	*0.3122*	*4.41*	*0.0358*	*0.11*
n-3/n-6	*Round goby*	*3.70*	*0.0544*	*1.74*	*0.1866*	*4.17*	*0.0412*	*0.09*
16:1n-7	*$\delta^{13}C$*	*1.63*	*0.2085*	*29.67*	*<0.0001*	*4.08*	*0.0492*	*0.51*
16:1n-7	$\delta^{15}N$	1.35	0.2518	0.01	0.9325	-	-	0.03
16:1n-7	Alewife	1.50	0.2201	0.26	0.6077	-	-	0.03
16:1n-7	Round goby	0.95	0.3287	0.02	0.8834	-	-	0.02
18:1n-9	$\delta^{13}C$	6.40	0.0148	7.46	0.0089	-	-	0.22
18:1n-9	$\delta^{15}N$	1.50	0.2267	2.75	0.1040	-	-	0.08
18:1n-9	Alewife	1.73	0.1890	0.83	0.3621	-	-	0.04
18:1n-9	Round goby	1.13	0.2874	0.59	0.4422	-	-	0.03
18:1n-9/16:1n-7	$\delta^{13}C$	6.21	0.0163	29.32	<0.0001	-	-	0.44
18:1n-9/16:1n-7	$\delta^{15}N$	1.24	0.2719	0.16	0.6909	-	-	0.03
18:1n-9/16:1n-7	Alewife	1.41	0.2343	0.14	0.7102	-	-	0.03
18:1n-9/16:1n-7	Round goby	0.92	0.3377	0.10	0.7500	-	-	0.02
PC 1	*$\delta^{13}C$*	*5.61*	*0.0221*	*28.60*	*<0.0001*	*7.41*	*0.0091*	*0.48*
PC 1	$\delta^{15}N$	1.82	0.1840	1.54	0.2212	-	-	0.06
PC 1	Alewife	1.50	0.2214	0.50	0.4805	-	-	0.03
PC 1	Round goby	0.97	0.3252	0.24	0.6223	-	-	0.02
PC 2	$\delta^{13}C$	4.04	0.0501	8.37	0.0058	-	-	0.24
PC 2	$\delta^{15}N$	1.01	0.3192	0.50	0.4816	-	-	0.04
PC 2	Alewife	1.54	0.2150	0.15	0.7006	-	-	0.03
PC 2	Round goby	0.97	0.3248	0.03	0.8623	-	-	0.02
PC 3	$\delta^{13}C$	8.41	0.0057	13.45	0.0006	-	-	0.30
PC 3	$\delta^{15}N$	1.39	0.2440	0.21	0.6523	-	-	0.03
PC 3	*Alewife*	*0.80*	*0.3708*	*1.77*	*0.1828*	*4.21*	*0.0401*	*0.10*
PC 3	*Round goby*	*0.40*	*0.5296*	*3.92*	*0.0478*	*5.42*	*0.0199*	*0.12*

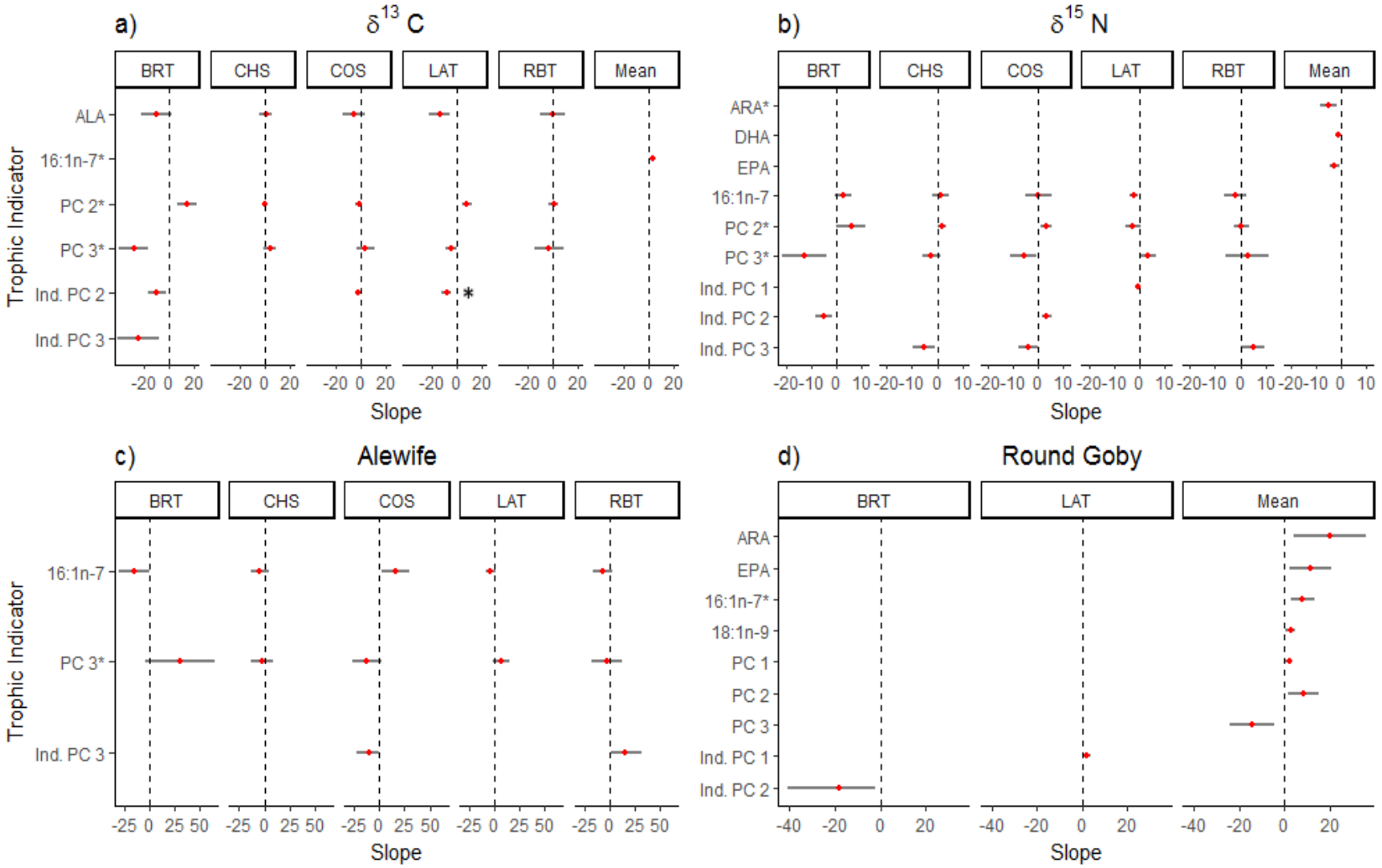
Appendix Table B.10. Results of pairwise comparisons between fatty acids and stable isotopes or stomach contents for rainbow trout with sex, fatty acid, and their interaction as response variables. Fatty acid-stable isotope regressions were linear models using F-statistics and fatty acid-diet content regressions were logistic GLMs using χ^2. Models with interaction p-values between 0.05 and 0.001 are italicized. Interaction terms were removed from models if p-values were greater than 0.05. Fatty acids are expressed as relative abundance. Models only included individuals that had sex identified.

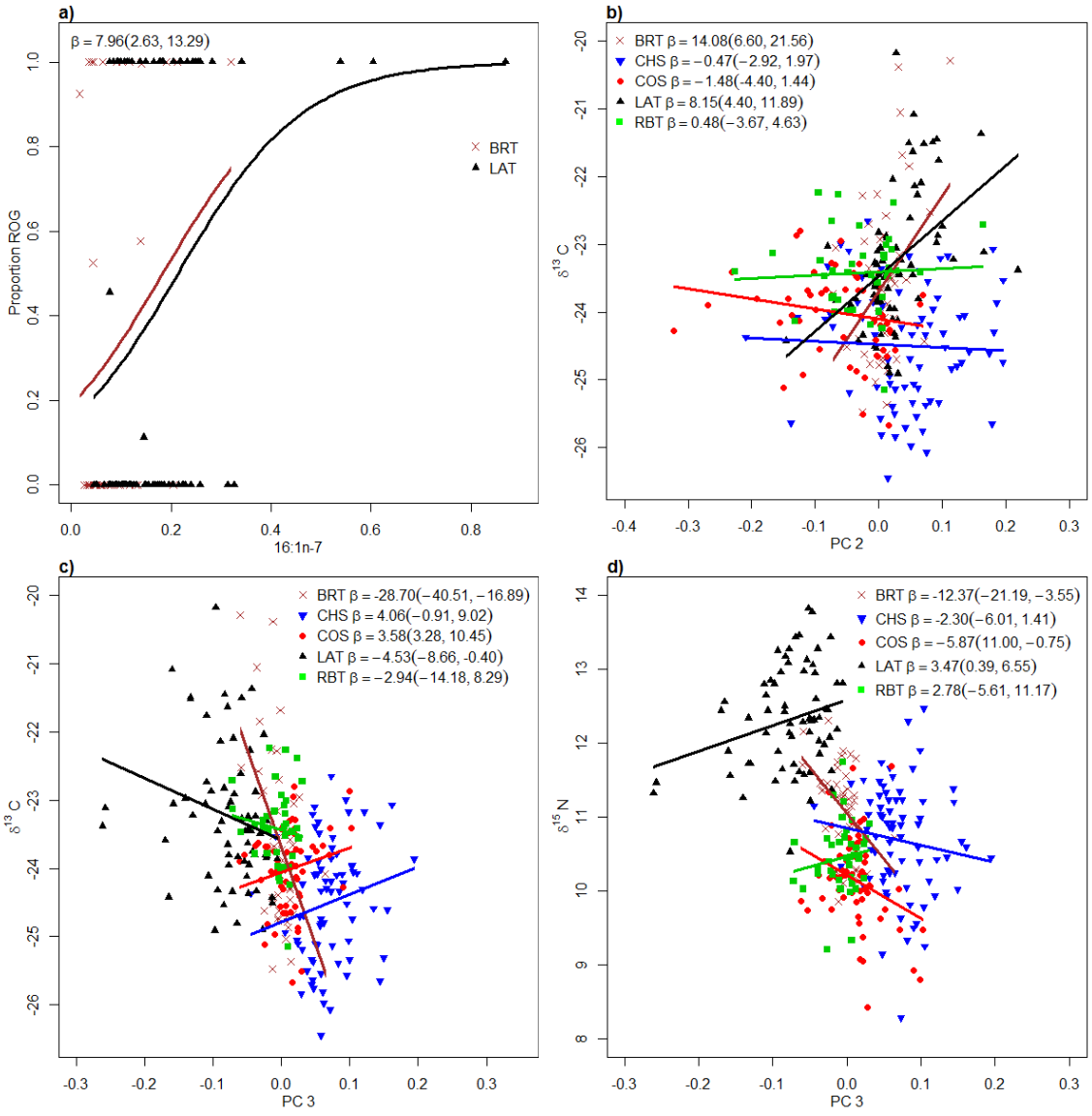
Fatty Acid	Response	Sex	Sex P	FA	FA P	Interaction	Interaction P	R^2
ALA	$\delta^{13}C$	1.07	0.3088	7.68	0.0091	-	-	0.20
ALA	$\delta^{15}N$	0.20	0.6595	7.43	0.0102	-	-	0.18
ALA	Alewife	2.33	0.1270	1.54	0.2143	-	-	0.04
ARA	$\delta^{13}C$	1.01	0.3221	0.13	0.7214	-	-	0.03
ARA	$\delta^{15}N$	0.26	0.6118	0.76	0.3890	-	-	0.03
ARA	Alewife	2.22	0.1362	0.65	0.4193	-	-	0.03
DHA	$\delta^{13}C$	1.04	0.3149	0.08	0.7766	-	-	0.03
DHA	$\delta^{15}N$	0.42	0.5238	0.37	0.5447	-	-	0.02
DHA	Alewife	1.87	0.1716	0.00	0.9982	-	-	0.02
EPA	$\delta^{13}C$	0.99	0.3268	2.61	0.1154	-	-	0.09
EPA	$\delta^{15}N$	0.20	0.6565	0.05	0.8216	-	-	0.01
EPA	Alewife	1.79	0.1813	0.04	0.8489	-	-	0.02
n-3/n-6	$\delta^{13}C$	0.14	0.7063	4.85	0.0348	-	-	0.15
n-3/n-6	$\delta^{15}N$	0.10	0.7514	0.16	0.6896	-	-	0.01
n-3/n-6	Alewife	1.90	0.1681	0.28	0.5956	-	-	0.03
16:1n-7	$\delta^{13}C$	0.53	0.4697	0.70	0.4079	-	-	0.05
16:1n-7	$\delta^{15}N$	0.05	0.8165	0.59	0.4493	-	-	0.02
16:1n-7	Alewife	1.70	0.1929	5.10	0.0239	-	-	0.10
18:1n-9	$\delta^{13}C$	0.38	0.5431	1.98	0.1692	-	-	0.08
18:1n-9	$\delta^{15}N$	0.00	0.9463	2.21	0.1468	-	-	0.06
18:1n-9	Alewife	1.81	0.1790	0.41	0.5235	-	-	0.03
18:1n-9/16:1n-7	$\delta^{13}C$	0.93	0.3420	0.00	0.9727	-	-	0.03
18:1n-9/16:1n-7	$\delta^{15}N$	0.22	0.6418	0.02	0.8857	-	-	0.01
18:1n-9/16:1n-7	Alewife	1.69	0.1941	4.93	0.0264	-	-	0.09
PC 1	$\delta^{13}C$	0.30	0.5902	1.63	0.2104	-	-	0.07
PC 1	$\delta^{15}N$	0.01	0.9407	1.27	0.2675	-	-	0.04
PC 1	Alewife	1.73	0.1881	0.73	0.3921	-	-	0.03
PC 2	$\delta^{13}C$	1.13	0.2955	0.86	0.3611	-	-	0.05
PC 2	$\delta^{15}N$	0.26	0.6129	0.54	0.4673	-	-	0.02
PC 2	Alewife	1.30	0.2546	0.83	0.3621	-	-	0.03
PC 3	$\delta^{13}C$	1.59	0.2164	2.18	0.1489	-	-	0.08
PC 3	$\delta^{15}N$	0.76	0.3890	4.98	0.0326	-	-	0.13
PC 3	Alewife	1.91	0.1669	0.25	0.6142	-	-	0.03



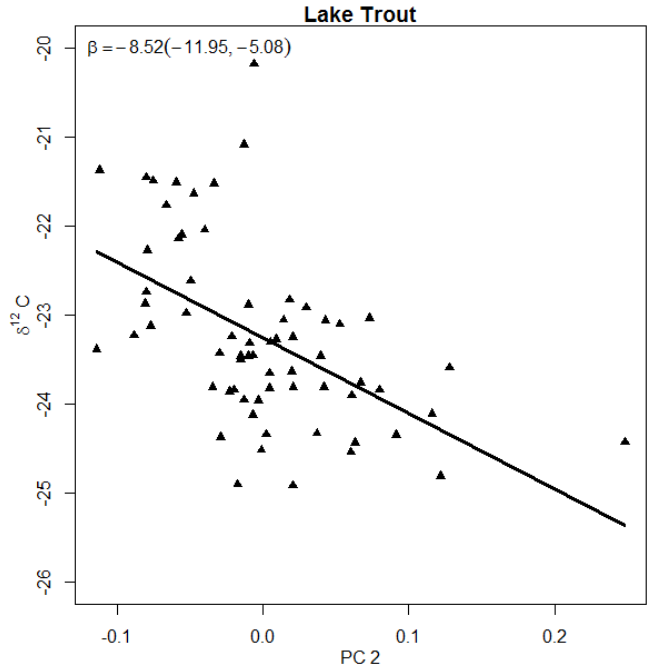
Appendix Figure B.1. Principal component (PC) axes 1, 2, and 3 of all salmonids of fatty acids with a relative abundance greater than 0.01 mg/g of sample quantified. For PC 1, all fatty acids loaded positively, which indicates that this is likely a measure of lipid content. PC 2 had negative loadings of DHA and positive loadings of 16:1n-7 and 18:1n-9. PC 3 had negative loadings of 16:1n-7, DHA, and 18:1n-9 and positive loadings of 16:0.

Appendix Figure B.2. Mean slopes (red points) and 95% confidence intervals (grey bars) of significant relationships between fatty acids, salmonid-wide PC axes, and species-specific PC axes and (a) $\delta^{13}C$, and (b) $\delta^{15}N$. The bottom figures represent significant relationships between fatty acids and stable isotope ratios to proportion by weight of (c) alewife and (d) round goby in salmonid stomachs. When models had significant species-specific interactions, the mean slope and confidence intervals are shown for each species. For significant models with no interaction effect, the mean model slope and 95% confidence interval is shown. The dashed line represents a slope of zero. * represents models with p values less than 0.001. These models used fatty acids expressed as mg/ g of wet sample. BRT=brown trout; CHS=Chinook salmon; COS=Coho Salmon; LAT=lake trout; RBT=rainbow trout.


a)
δ13 C
BRT
CHS
COS
LAT
RBT
Mean
ALA
16:1n-7*
PC 2*
PC 3*
Ind. PC 2
Ind. PC 3
Trophic Indicator
-20 0 20
Slope
b)
δ15 N
ARA*
DHA
EPA
16:1n-7
PC 2*
PC 3*
Ind. PC 1
Ind. PC 2
Ind. PC 3
-20-10 0 10
Slope
c)
Alewife
16:1n-7
PC 3*
Ind. PC 3
-25 0 25 50
Slope
d)
Round Goby
ARA
EPA
16:1n-7*
18:1n-9
PC 1
PC 2
PC 3
Ind. PC 1
Ind. PC 2
-40 -20 0 20
Slope



Appendix Figure B.3. Highly significant ($p<0.001$) relationships between fatty acids expressed as mg of FA/g of wet sample and stable isotopes and stomach contents. There were consistent negative relationships between 16:1n-7 and round goby across brown trout and lake trout (a). PC 2 was positively correlated with $\delta^{13}C$ in brown trout and lake trout, but not in other species (b). PC 3 was negatively correlated with $\delta^{13}C$ in brown trout and lake trout, but positively correlated in Coho salmon (c). Additionally, PC 3 was negatively correlated with $\delta^{15}N$ in brown trout, but positively correlated in lake trout (d). Mean slope and 95% confidence intervals are provided when there were no species-specific relationships. For significant interactions, species-specific slopes and 95% confidence intervals are provided for each species. BRT=brown trout; CHS=Chinook salmon; COS=Coho Salmon; LAT=lake trout; RBT=rainbow trout.



Appendix Figure B.4. Highly significant ($p<0.001$) negative relationship between $\delta^{13}C$ and PC 2 in lake trout with fatty acids expressed as mg/ g of wet sample. Mean slope and 95% confidence intervals are provided.