STABLE ISOTOPES AS INTRINSIC MARKERS OF CONTAMINANT DYNAMICS IN THE LAKE WINNIPEG FOOD WEB

A Thesis Submitted to the College of

Graduate Studies and Research

in Partial Fulfillment of the Requirements

for the Degree of Master of Science

in the Toxicology Graduate Program

University of Saskatchewan

Saskatoon, Saskatchewan, Canada

By

Amy F. A. Ofukany

PERMISSION TO USE

In presenting this thesis in partial fulfilment of the requirements for a Postgraduate degree from the University of Saskatchewan, I agree that the Libraries of this University may make it freely available for inspection. I further agree that permission for copying of this thesis in any manner, in whole or in part, for scholarly purposes may be granted by the professor or professors who supervised my thesis work or, in their absence, by the Head of the Department or the Dean of the College in which my thesis work was done. It is understood that any copying or publication or use of this thesis or parts thereof for financial gain shall not be allowed without my written permission. It is also understood that due recognition shall be given to me and to the University of Saskatchewan in any scholarly use which may be made of any material in my thesis.

Requests for permission to copy or to make other use of material in this thesis in whole or part should be addressed to:

Chair of the Toxicology Graduate Program

Toxicology Centre

University of Saskatchewan

44 Campus Drive

Saskatoon, Saskatchewan, Canada, S7N 5B3

ABSTRACT

Lake Winnipeg, Manitoba, Canada is the tenth largest freshwater lake worldwide and has one of the largest catchment areas of any aquatic system in Canada. Intensive agriculture, livestock production, urban development and industrialization contribute excess nutrients and contaminants to the lake. Although nutrient-driven eutrophication, establishment of invasive fishes, commercial fishing pressures and avian predation may adversely affect fish community structure and function, trophic interactions among Lake Winnipeg's biota have not been intensely examined. Similarly, the mechanisms governing the dietary transfer of mercury (Hg) and trace elements are unknown, despite the fact elevated Hg residues in fish tissues led to a commercial fishing ban in the 1970s. Therefore, the objectives of this study were to use a multi-stable isotope approach (δ^{15}N, δ^{13}C, δ^{34}S and δ^{2}H) to a) identify trophic interactions among fishes and piscivorous birds and b) track the dietary transfer of trace elements.

An intensive field program was undertaken in 2009 and 2010. Muscle samples from fishes and Double-crested Cormorants (*Phalacrocorax auritus*; hereafter "cormorant") were examined isotopically (δ^{15}N, δ^{13}C and δ^{34}S) and the data were used to reconstruct the lake's food web. The effects of trophic position (TP; δ^{15}N), food source (δ^{13}C and δ^{34}S) and organism size on concentrations of aluminum (Al), arsenic (As), cadmium (Cd), copper (Cu), iron (Fe), manganese (Mn), Hg and selenium (Se) in muscle were examined according to an information-theoretic approach. The utility of scale sampling as a non-invasive method for monitoring contaminant concentrations in fillets of commercially-valuable walleye (*Sander vitreus*) was also investigated. Similarly, the concerted analyses of stable isotope ratios (δ^{15}N, δ^{13}C, δ^{34}S and δ^{2}H) and Hg concentrations in cormorant flight feathers were evaluated as a non-destructive method for tracking habitat use and contaminant biotransport. Muscle of fishes and cormorants collected

from Lake Winnipeg's north basin were generally depleted in ^{13}C and ^{34}S but enriched in ^{15}N relative to individuals from the south basin. Stable isotopic mixing models also revealed much of the fish biomass consumed by cormorants (north basin: ≥ 59 %; south basin: ≥ 74 %) consisted of species with little or no direct commercial value. Trace element concentrations in biota were often species-specific; however the influence of TP and pelagic, benthic, or detrital nutrient sources were often evident at fine scales. Biomagnification (south basin) and bioaccumulation (north basin) of Hg were observed within the lake's walleye populations. Fortunately, analysis of walleye scales demonstrated some utility in monitoring fillet-Hg concentrations. Mercury, as well as Cd, Fe, Mn, and Se increased with increasing TP in cormorants nesting on the north basin of Lake Winnipeg. Although the factors driving contaminant concentrations in cormorant muscle differed spatially, concentrations of Cd, Hg and Se in muscle did not differ among adult and hatch-year cormorants, or by nesting location. Measurements of stable isotopes ($\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and $\delta^{2}H$) and Hg in primary feathers revealed cormorants accumulated more Hg from Lake Winnipeg than from winter habitats.

This study is the first to demonstrate the utility of a multi-isotope approach in describing Hg and trace element trophodynamics for the Lake Winnipeg food web. The lake's north and south basins appeared to differ in terms of trophic structure and the mechanisms underlying contaminant transfer. Mercury may be the element of greatest concern for Lake Winnipeg, as biomagnification, bioaccumulation and biotransport of this toxic element have been demonstrated.

ACKNOWLEDGMENTS

I would like to thank my graduate supervisors Drs. Len Wassenaar and Keith Hobson for their guidance. I would also like to extend my gratitude to my committee members Drs. David Janz, Michael Pietrock, Tim Jardine, and to my external examiner, Dr. Chris Somers, for their advice and feedback.

The Lake Winnipeg Research Consortium and the crew of the *MV Namao* provided invaluable logistical and technical support. Mike Maksymchuk, Kevin Casper, Kevin Luprypa, Chelsey Lumb, Logan Queen, Heather Clark, Katie Sheppard and Andrew Olynyk contributed to the field component of this thesis. Thanks to Geoff Koehler, Brenda Stanek, Elise Bird, Mike Kautzman, Xiaofeng Wang and Emily Ritson-Bennett for their analytical expertise and assistance. I am grateful to David Soto, Ana Maria Gonzalez and Steve Van Wilgenburg for their advice. Thanks to all my wonderful friends and colleagues from Lake Winnipeg, Environment Canada and the Toxicology Centre for their support and encouragement. Finally, I would like to thank my family and dearest friends for their love, encouragement and patience.

This research was funded by Environment Canada through the Lake Winnipeg Basin and Red River Initiative, with additional financial support from the University of Saskatchewan and VWR.

TABLE OF CONTENTS

page

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AIC_c	Akaike's Information Criterion corrected for small sample sizes
AIR	Atmospheric nitrogen
ANOVA	Analysis of Variance
BURB	Burbot
BWB	Bowhead Whale Baleen
C_3	Plants using the Rubisco enzyme to fix carbon dioxide
C_4	Plants using phosphoenolpyruvate carboxylase to fix carbon dioxide
CF-IRMS	Continuous-Flow Isotope Ratio Mass Spectrometry
CFS	Chicken Feather Standard
CHS	Cow Hoof Standard
CI	Confidence interval
CISC	Cisco
CPUE	Catch per Unit Effort
CV	Coefficient of variation
CVAFS	Cold Vapour Atomic Fluorescence Spectrometry
DCCO	Double-crested Cormorant
DDT	Dichlorodiphenyltrichloroethane
DIC	Dissolved inorganic carbon
DIN	Dissolved inorganic nitrogen
DORM-2	Dogfish Muscle Certified Reference Material for Trace Elements
DS	Dissolved Sulfate
DW	Dry weight

EMSH	Emerald Shiner
ESRI	Environmental Systems Research Institute
EU	European Union
FL	Fork Length
FRDR	Freshwater drum
FW	Fresh weight
GIS	Geographic Information Systems
GNIP	Global Network of Isotopes in Precipitation
GOLD	Goldeye
Hg_T	Total mercury
HSD	Honestly Significant Difference
HY DCCO	Hatch-year Double-crested Cormorant
IAEA	International Atomic Energy Agency
ICP-MS	Inductively Coupled Mass Spectrometry
K	Number of parameters
LA-ICPMS	Laser Ablation Inductively Coupled Plasma Mass Spectrometry
LKWF	Lake Whitefish
LNSC	Longnose sucker
LoD	Limit of detection
MAE	Model-average estimate
MeHg	Methyl mercury
ML	Maximum Level
MOON	Mooneye

MV	Motor vessel
NNST	Ninespine stickleback
NRPK	Northern pike
NB DCCO	Adult Double-crested Cormorant from the north basin
OMNR	Ontario Ministry of Natural Resources
PCB	Polychlorinated biphenyl
PE	Parameter estimate
POP	Persistent Organic Pollutant
ppm	Parts per million
PRC	Porcine gelatin standard
PUGEL	Bovine gelatin standard
QC	Quality control
R_A	Ratio of the heavy/ light isotope in a sample
R_S	Ratio of the heavy/ light isotope in a standard
RNSM	Rainbow smelt
SAUG	Sauger
SB DCCO	Adult Double-crested Cormorant from the south basin
SD	Standard deviation
SE	Standard error
SIAR	Stable Isotope Analysis in R
SRC	Saskatchewan Research Council
TEF	Trophic enrichment factor
TP	Trophic position

uCI	Unconditional confidence interval
uSE	Unconditional standard error
US EPA	United States Environmental Protection Agency
v/ v	Volume for volume
VCDT	Vienna Canyon Diablo Troilite
VPDB	Vienna Pee Dee Belemnite
VSMOW	Vienna Standard Mean Ocean Water
WALL	Walleye
WHBS	White bass
WHSC	White sucker
ω_i	Akaike weight
YLPR	Yellow perch
YOY	Young of the year
Zoop	Zooplankton

PREFACE

Chapter 1 is a general introduction. Chapters 2 through 6 are formatted as manuscripts intended for publication in scientific journals. As such, there is some repetition of introductory information and methods. Chapter 6 was published in *Environmental Science and Technology* (46: 3263-3272) prior to submission of this M. Sc. thesis.

CHAPTER 1
1.0 GENERAL INTRODUCTION

1.1 Lake Winnipeg

Lake Winnipeg, Manitoba, Canada (53° 17'N, 97° 58'W; Figure 1.1) is the world's tenth largest freshwater lake and together its north and south basins cover an area equal to 23 750 km^2 (Environment Canada and Manitoba Water Stewardship, 2011). The surface area of the north basin is six times larger than that of the south, and these two basins are separated by a channel, which is often referred to as "the narrows" (Patalas and Salki, 1992; Todd et al., 1998). Since the lake extends across approximately four degrees of latitude, it is common for average temperatures to vary by ± 1.8°C between the northern and southernmost points (Environment Canada and Manitoba Water Stewardship, 2011; Patalas and Salki, 1992). Thermal stratification in Lake Winnipeg is rare, as most areas are well-mixed by wind and are relatively shallow, averaging near sixteen and nine meters deep in the north and south basins, respectively (Mayer et al., 2006). This large lake drains an area equivalent to 977 800 km^2 and receives inputs from as far as Minnesota and South Dakota (Pip, 2006; Stewart et al., 2003). The Red and Winnipeg Rivers discharge into the lake's south basin and account for approximately 65 % of total riverine inputs (Environment Canada and Manitoba Water Stewardship, 2011). The Saskatchewan River is another major tributary and flows into the west side of the lake's north basin (Stewart and Watkinson, 2004). These rivers vary greatly in the types of land they serve and in the quality of water they contribute to Lake Winnipeg (Patalas and Salki, 1992). The Nelson River, which drains the north basin of the lake, is the main outflow and feeds into Hudson Bay (Pip, 2006).

1.2 Declining Lake Health

Due to the size and characteristics of Lake Winnipeg's drainage basin, as well as the lake's exploitation by the tourism, fishing and hydroelectric industries, there has been a notable decline

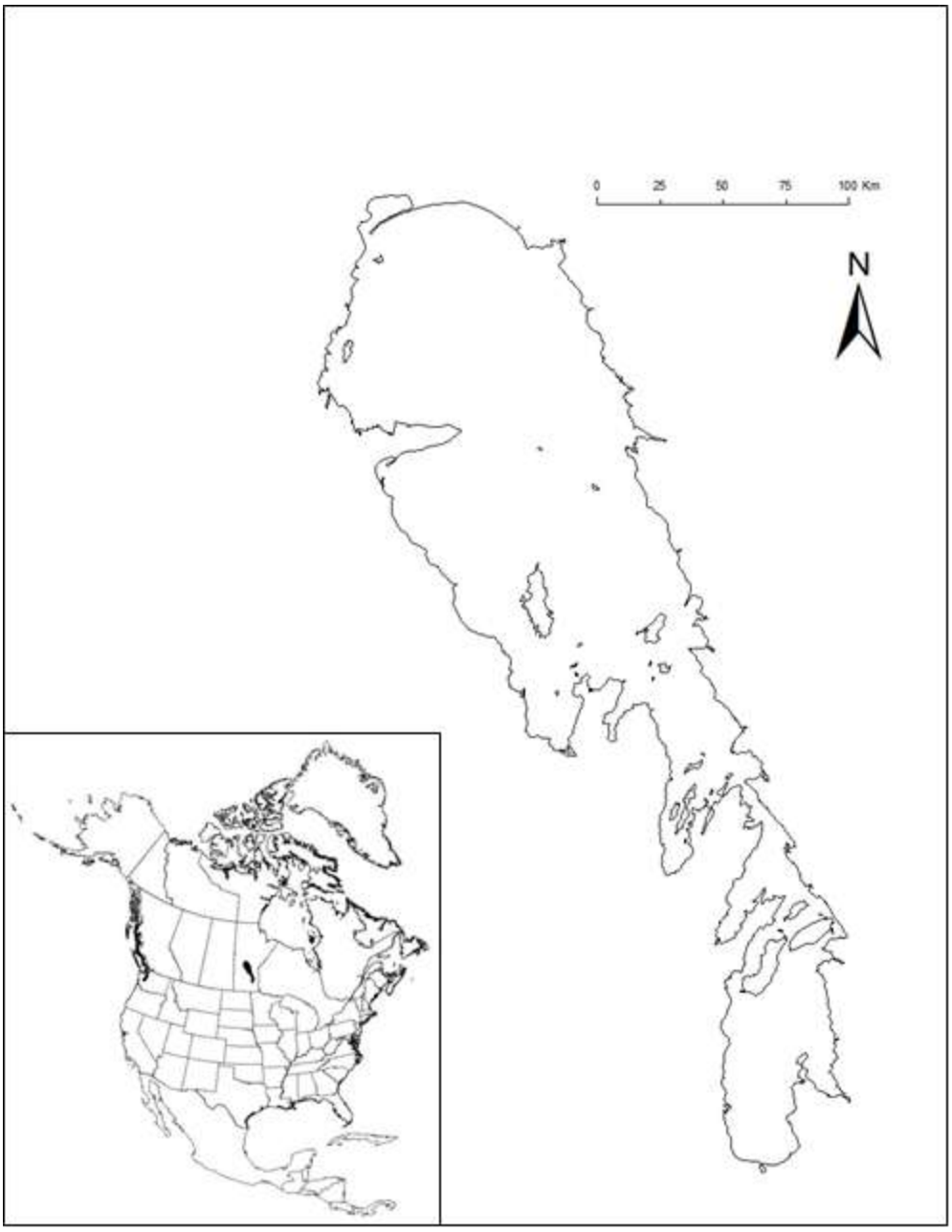


Figure 1.1 Lake Winnipeg, Manitoba, Canada (53° 17'N, 97° 58'W).

in water quality and an increase in public concern for lake health (Pip, 2006; Todd et al., 1998). The catchment area of Lake Winnipeg is populated by nearly six million people and a large proportion of available land is used for agricultural purposes or urban development (Patalas and Salki, 1992; Rawn et al., 2000). It is well known the application of pesticides and herbicides to farmland can have adverse effects on soil and water quality. Intensive livestock operations in the lake's watershed also add excess nutrients, pathogens, salts and veterinary pharmaceuticals to run-off and are contributing to the nutrient enrichment and altered water chemistry in the lake (Kling, 1998; Pip, 2006). However, agriculture is not the only contributor to increased nutrient and contaminant loading into tributaries. Industrial emissions, land development and sewage from municipal sources are also affecting the quality of water entering Lake Winnipeg (Pip, 2006). The Red River is the largest source of nutrients and contaminants, as it receives the greatest inputs from agricultural land and urban centres (Patalas and Salki, 1992). Major flood events have occurred in this river, and with these events, there have been notable increases in the levels of nutrients, organic pesticides and other contaminants reaching Lake Winnipeg from sources such as sewage tanks, landfills and livestock facilities (Pip, 2006; Stewart et al., 2003).

Water and near-shore land use on Lake Winnipeg is also having an effect on water quality and nutrient inputs. The lake's close proximity to urban centres like Winnipeg makes it a convenient destination for recreationists. There are a number of resort communities, as well as more than 10 000 cabins and cottages on the south basin alone, all of which contribute to the nutrients and wastes entering the water (Environment Canada and Manitoba Water Stewardship, 2011; Pip, 2006). Excessive production of garbage, damage to vegetation, and some pollution from boats and recreational vehicles can be expected with increased exploitation by the tourism industry.

Lake Winnipeg also supports Canada's second largest freshwater commercial fishery (Johnston et al., 2010). Over the period of 2008 to 2009, fishery harvests were worth over $27 million dollars (Manitoba Water Stewardship, 2010). Walleye (*Sander vitreus*) represented over 5 million kg (round weight) or 39 % of the total catch over the same period, and accounted for 71 % of total profits (Manitoba Water Stewardship, 2010). Sauger (*Sander canadensis*) and lake whitefish (*Coregonus clupeaformis*) are on average the second and third most valuable fishes harvested commercially from Lake Winnipeg (Manitoba Water Stewardship, 2010). Manitoba's hydroelectric industry is also heavily dependent on Lake Winnipeg, and has regulated the lake's Nelson River outflow for over 30 years (Environment Canada and Manitoba Water Stewardship, 2011; Mayer et al., 2006). There may be subtle food web effects as a result of over-exploitation of commercial species, as well as altered water chemistry and mobilization of harmful compounds resulting from dam construction. Unfortunately, Lake Winnipeg is one of the most poorly studied lakes in the world and few conclusions can be made regarding contaminant levels in the lake, or on the health of its biota (Mayer et al., 2006).

1.3 Biota of Lake Winnipeg

Lake Winnipeg hosts a diverse group of organisms which create a complex system of benthic invertebrates, crustaceans, algae, zooplankton, fishes and water birds. Much information has been collected regarding littoral organisms and planktonic communities in Lake Winnipeg (Kling, 1998; Patalas and Salki, 1992; Pip, 2006), and a small number of studies evaluating the trophic transfer of chlorinated and brominated compounds were also conducted following the record-level 1997 Red River flood (Gewurtz et al., 2006; Law et al., 2006; Stewart et al., 2003). In recent years, the lake's fish community has gained considerably more interest and much of the research has focused on forage fish biomass (Lumb et al., 2011) or population characteristics of walleye and sauger (Johnston et al., 2010; Moles et al., 2010). An isotopic (δ^{15}N and δ^{13}C) food

web study was released in 2010 by Hobson et al. (2010) and is the first major quantitative account of fish community structure in Lake Winnipeg.

1.3.1 Fishes

A variety of fish species inhabit Lake Winnipeg, including several benthivorous fishes such as white sucker (*Catostomus commersonii*), small forage fishes such as emerald shiner (*Notropis atherinoides*) and large predatory species like walleye and northern pike (*Esox lucius*) (Stewart and Watkinson, 2004). Lake whitefish have been harvested for commercial sale since the late 1800's, with walleye and sauger becoming increasingly valuable to the industry since the 1970s (Law et al., 2006). Despite a heavy reliance on the lake's fish population, the food web remains poorly understood relative to that of other North American Great Lakes (Leach and Nepzsy, 1976; Munawar et al., 2005; Schmidt et al., 2009).

Rainbow smelt (*Osmerus mordax*), an invasive species first identified in the Lake Winnipeg system during the early 1990s, have gained considerable attention from researchers and other concerned parties (Stewart and Watkinson, 2004). It is feared this introduced species will have long-term repercussions for forage fishes and upper trophic-level consumers alike. Endemic fishes like ciscoes (*Coregonus artedi*) and emerald shiner share a similar ecological niche with smelt and may therefore suffer from a decrease in available food or habitat (Stewart and Watkinson, 2004). Lake-wide surveys of the pelagic fish assemblage (2002 to 2008) revealed ciscoes and emerald shiner represent the greatest proportion of forage fish biomass in the south basin, while rainbow smelt are the predominant forage fish in the north basin (Lumb et al., 2011). There is evidence smelt may be consuming small life-stages of ciscoes in the north basin, and as a result, are acquiring a slightly higher trophic position, or TP, within the food web (Lumb et al., 2011; Swanson et al., 2006). Increasingly piscivorous behaviour in rainbow smelt may have implications for higher-TP fishes and birds, as the addition of an extra level in the food web

is likely to alter the flow of energy and increase the potential for contaminant biomagnification (Swanson et al., 2003; Swanson et al., 2006). Alternatively, recent investigations which used stable nitrogen isotope data (δ^{15}N) to identify the TP of north basin smelt concluded that smelt do not occupy a position which is elevated over other forage fishes (Gewurtz et al., 2006; Hobson et al., 2010). The inconsistencies in the data would suggest more site-specific studies on this introduced species need to be conducted.

Much of the remaining research over the past ten years has been connected to the lake's fishery and commercially-valuable species. Speers and Gillis (2011) examined the ways in which wave height, water chemistry and other variables influence catch per unit effort (CPUE) for sauger and walleye fishermen in the lake's south basin. Other researchers have noted the presence of growth polymorphism within the lake's walleye population (Moles et al., 2010, 2011) and examined differences in the growth and reproduction of walleye and sauger over a spatio-temporal scale (Johnston et al., 2010).

Although it is generally assumed small-bodied fishes feed toward the base of the food web and large-bodied specimens generally occupy apex positions, there is often overlap or shifts in feeding levels which cannot be accounted for by this assumption (Paradis et al., 2008). These exceptions exist because a fish may integrate diet from several trophic levels and sources, and diet may vary depending on season or fluctuations in prey populations (Kidd et al., 1995). Until the baseline isotopic (δ^{15}N and δ^{13}C) food web study of Hobson et al. (2010), TP was poorly defined for essentially all Lake Winnipeg fishes. The study represents six years (2002-2008) of conditions on the lake, and serves as a benchmark of trophic structure. However, many fish species and size classes were not examined in the 2002-2008 study, and so further refinement of

the Hobson et al. (2010) food web is required in order to gain a comprehensive understanding of interactions between fishes.

1.3.1.1 Species of Interest

The selection of fish species for this investigation was based on a number of factors including commercial value; feeding habits; abundance and availability of specimens; ecological niche, and ease of sample collection. Walleye are a key species for consideration, given they are the most widely exploited and most valuable commercial fish on the lake (Stewart and Watkinson, 2004). This species, along with other large-bodied fishes such as sauger and northern pike were included not only for their value in commercial, recreational and subsistence fishing, but also for their roles in the lake's food web. As young of the year (YOY), these species have a diet similar to that of forage fishes, but within a few weeks to a year, there is a shift toward a mainly piscivorous feeding strategy (Hesslein et al., 1991; Paradis et al., 2008). Adults of these species are expected to occupy a top TP in the lake's food web. Even YOY walleye which were collected between 2002 and 2008 had δ^{15}N values which were elevated over those of forage fishes such as ciscoes, emerald shiner and rainbow smelt. Young walleye and sauger are generally well-represented in beam trawls conducted by Manitoba Fisheries (Lumb et al., 2011), while adult specimens are easily obtained through index netting or cooperation with local fishermen. White bass (*Morone chrysops*), another predatory species, is also of interest because of its status as a recently-established invasive species and ability to consume relatively large volumes of prey fish biomass (Stewart and Watkinson, 2004).

Since the biomass of pelagic fishes with fork lengths (FLs) < 200 mm is comprised primarily of ciscoes, emerald shiner and rainbow smelt, and historical accounts of trophic interactions among these species are not in agreement, their inclusion in food web investigations is well-supported (Hobson et al., 2010; Lumb et al., 2011). Goldeye (*Hiodon alosoides*) are another

pelagic species of interest, because although they only represent approximately 0.1 % of the round weight (kg) commercial product, they serve as a highly-valued food source for walleye (Manitoba Water Stewardship, 2010; Stewart and Watkinson, 2004). Yellow perch (*Perca flavescens*), which are noted more for their value to recreational fishing than commercial sale, are also an important food source for upper level consumers like northern pike and Double-crested Cormorants (*Phalacrocorax auritus*, hereafter "cormorant") (Hobson et al., 1989; Paradis et al., 2008). Similarly, over half the diet of cormorants nesting on Saskatchewan lakes may be comprised of ninespine stickleback biomass (*Pungitius pungitius*) (Doucette et al., 2011), and so it is possible this species serves as a linkage between the fish and cormorant communities on Lake Winnipeg.

White sucker are one of the most widespread fish species in Manitoban waters, and are frequently consumed by predatory fishes and cormorants (Hobson et al., 1989; Stewart and Watkinson, 2004). White and longnose suckers (*Catostomus catostomus*) are characteristically bottom-feeding primary consumers, and as a result, may serve as a conduit between nutrients and contaminants in sediment and higher-TP consumers (Ahlgren, 1996; Stewart and Watkinson, 2004). Benthivorous lake whitefish and troutperch (*Percopsis omiscomaycus*) are also consumed by predatory fish, and whitefish have a long history of exploitation by the commercial fishing industry (Kristofferson and Clayton, 1990). Freshwater drum (*Aplodinotus grunniens*), which may feed on mussels and/ or fishes are also of interest due to their relatively plastic feeding strategy (Stewart and Watkinson, 2004).

Between 1950 and 1954, an extensive survey of the population characteristics and food preferences of burbot (*Lota lota*) was conducted; however, it is unclear whether recent changes

in water chemistry and species composition have affected this predatory bottom-feeder (Hewson, 1955; Stewart and Watkinson, 2004).

1.3.1.2 Fish Tissue Selection

In fishes, dorsal muscle constitutes a relatively large proportion of total body mass and may be a significant dietary source of contaminants for humans and wildlife. As much as 94, 86 and 72 % of the dietary arsenic (As), mercury (Hg) and selenium (Se), respectively, accumulated by fishes is routed to muscle (Ciardullo et al., 2008). Tissues such as fish scales may provide an alternative method for monitoring Hg and other metals in species of ecological or commercial interest (Farrell et al., 2000; Lake et al., 2006). Scales may be useful as a preliminary test to determine which populations are accumulating Hg or other contaminants and if further risk-assessment based tests are required (Lake et al., 2006). Sampling of walleye scales in particular may provide a non-lethal, cost-effective method for monitoring fisheries resources.

1.3.2 Cormorants

There is a great deal of uncertainty regarding the importance of piscivorous water birds to the Lake Winnipeg food web. Cormorants are of special interest due their population size, socio-economic importance and because the feeding habits of this species are poorly characterized for the location. Cormorants arrive on Canadian lakes in April after spending the winter months in the southern United States and Mexico (Dolbeer, 1991; Somers et al., 1993; Trapp et al., 1997). There are a number of established cormorant colonies on Lakes Winnipeg and Winnipegosis, and together these colonies account for the largest breeding population in Canada (OMNR, 2006). Numbers have increased in recent decades and as a result, this bird has received a great deal of negative attention throughout its range, as it is believed to cause reduced catches and profitability of fisheries; losses to aquaculture facilities, and declines in the quality of water resources and recreational property (OMNR, 2006). Many public and corporate groups are in favour of

cormorant population control where there are perceived effects to commercial fishing (Wiese et al., 2008) and many concerned parties have taken matters into their own hands through destruction of birds and nests (Hobson et al., 1989). Although cormorant populations have been studied in Ontario (Johnson et al., 2010; OMNR, 2006), Alberta (Somers et al., 1993), Saskatchewan (Doucette et al., 2011), the southern portion of the prairies (Dolbeer, 1991) and on Manitoba's Lake Winnipegosis (Hobson et al., 1989), there remains a lack of scientific information regarding the feeding habits of birds on Lake Winnipeg. There is potential for this population to consume large quantities of immature walleye and sauger along with small forage species (Hobson, 2009); however, research and reviews conducted in the United States and Canada would suggest cormorants generally do not have a significant negative impact on economically important species (Hobson et al., 1989; Trapp et al., 1997; Wires et al., 2001)

Studies of regions similar to Lake Winnipeg suggest cormorants forage within 10 km of their nest site (OMNR, 2006) and therefore have a propensity to feed on a single body of water (Rudstam et al., 2004). As a result, the isotopic composition of cormorant tissues collected from birds on Lake Winnipeg should be representative of the dietary items available from the lake. Past studies have also demonstrated the cormorant diet is dominated by fish (Caldwell et al., 1999; Ludwig et al., 1989), and is thereby less variable than that of birds which may feed on a wide range of organisms from different taxa. Populations of cormorants in the Laurentian Great Lakes region are known to feed mainly on fishes ≤ 150 mm in length and, in general, the species consumed are of little or no direct commercial value, such as alewife (*Alosa pseudoharengus*), gizzard shad (*Dorsoma cepadianum*), pumpkinseeds (*Lepomis gibossus*), rock bass (*Ambloplites rupestris*), round goby (*Neogobius melanostomus*), rainbow smelt, and white sucker (OMNR, 2006). However, alewife, shads, pumpkinseeds, rock bass, and gobies are poorly represented in

Lake Winnipeg, if they are found there at all. Yellow perch are found in the Laurentian Great Lakes region as well as in Lake Winnipeg, and birds from Lake Huron and Oneida Lake have been known to consume large numbers of juvenile perch each spring (Belyea et al., 1997; Rudstam et al., 2004). The consumption of these young yellow perch, along with juvenile walleye, may have significant implications for adult populations in the years to come. Studies have shown cormorants can consume large numbers of juvenile sport fish between 75 and 125 mm, with young walleye and yellow perch constituting 40 to 81% of the birds' diet in some cases (Belyea et al., 1997; OMNR, 2006; Rudstam et al., 2004). The applicability of these values to Lake Winnipeg is unclear; cormorants are relatively undiscerning when it comes to species consumed and diet appears to vary greatly among locations, depending on availability of prey (Wires et al., 2001). The Ontario Ministry of Natural Resources (OMNR, 2006) acknowledges that there are a number of deficiencies in the data surrounding cormorant diet and more needs to be done in order to evaluate impacts to specific fish populations and age classes. This especially holds true for Lake Winnipeg, as the status of cormorant populations is relatively unknown and the most recent monitoring program in Manitoba was discontinued in 1954 (Belyea et al., 1997; OMNR, 2006). A provincial survey was carried out in the late 1970s and the populations of nearby Lake Winnipegosis were studied in 1987 (Hobson et al., 1989); however, the province has made little effort since then to monitor this species (OMNR, 2006). This would suggest that in order to ascertain the effects of cormorant predation on Lake Winnipeg's food web and the fishing industry, a lake-specific analysis is required. Once the diet and actual impacts of this bird population have been determined, researchers will be able to develop site-specific management guidelines (Trapp et al., 1997).

1.3.2.1 Cormorant Tissue Selection

Cormorants are a migratory bird, and consequently undergo a shift in diet upon reaching Lake Winnipeg in the spring. This change in diet ultimately affects the isotopic ratios in cormorant tissues. In order to ensure isotopic analyses reflect the diet consumed on Lake Winnipeg, specimens must be collected well after the birds' arrival in spring, so that a complete isotopic turnover has occurred in the tissue(s) of interest (Hobson, 2009). Pectoral muscle has a relatively short isotopic turnover rate, usually four to six weeks, and is representative of an individual's body protein pool (Hobson, 2009; Hobson et al., 2011). Therefore, muscle collected between late May and the end of the summer breeding season is likely to represent the Lake Winnipeg food web.

Cormorant feathers may provide a relatively non-invasive indicator of circulating contaminants, as they are only connected to the blood supply during formation, and are "recorders" of contaminant exposure during the two to four week period of feather growth (Caldwell et al., 1999; Chamberlain et al., 1997). However, cormorants undergo a continuous molt, which often stretches well into the winter months, making it difficult to know for certain which feathers were grown on Lake Winnipeg and which represent the wintering grounds (Hebert et al., 2008). Fortunately, stable hydrogen isotopes (δ^2H) have proven useful in assigning tissues of migratory organisms to point of origin. When combined, contaminant and δ^2H analyses of feathers may have utility in tracking annual cycles of diet (Hobson et al., 2009a; Van Wilgenburg and Hobson, 2010) and contaminant accumulation in migratory cormorants.

1.4 Determining Food Web Interactions & Feeding Strategies

Due to uncertainty surrounding food web structure and the socio-economic impacts of certain predator-prey relationships, it is necessary to develop a system for mapping the energy transfer and feeding interactions of Lake Winnipeg's organisms. By measuring stable isotope ratios of

nitrogen ($^{15}N/^{14}N$), carbon ($^{13}C/^{12}C$), sulfur ($^{34}S/^{32}S$) and hydrogen ($^{2}H/^{1}H$) in animal tissues, many researchers have been able to describe food web structure, nutrient flow and food preferences in a number of freshwater and marine systems (Campbell et al., 2005; Croisetiére et al., 2009; Fry, 1988; Hesslein et al., 1991; Soto et al., 2011). Stable isotopes are an excellent tool when working with wildlife, as isotope measurements allow for relatively non-invasive procedures, require less animal handling, do not rely on animal recapture, are present in all life and are reflective of food habits and geographical range (Fry, 2006). Repetitive handling and restraint of wildlife can cause a great deal of stress for animals, and in some cases, may be dangerous for field researchers. Isotope samples, such as scales, feathers and blood may be taken with minimal injury to the animal. The isotopic compositions of such samples are dependent on the rate of tissue renewal and growth, and only represent food components which are assimilated into the specimen's body. As a result, analytical errors associated with incorporated versus excreted dietary compounds can be minimized. Foraging location or range may also be determined by studying the geographical distribution of isotopes in abiotic systems and animal tissues (Fry, 2006; Hobson et al., 2010). Overall, stable isotope measurements provide a more reliable, long-term, quantitative estimate of fish and cormorant diets than stomach, fecal, pellet and/ or regurgitate analyses (Paradis et al., 2008; Seefelt and Gillingham, 2006).

Based on the success of previous research, it is expected stable isotope analyses of fish and cormorant tissues from Lake Winnipeg will provide key information required to determine relative TP, food preference and energy transfer between forage fishes and commercially relevant predators such as walleye. Isotopic information may have added value for fisheries and wildlife managers, as it will be possible to determine which species and sizes of fishes are at the greatest risk for cormorant predation. It is also anticipated food web data may provide

information on the flow of contaminants, as well as energy and nutrients, through biological systems. Stable isotope measurements have proven useful in studies of dietary transfer of toxic compounds or elements (Campbell et al., 2005; Gewurtz et al., 2006; Ikemoto et al., 2008) and may be useful in pinpointing which species are at the greatest risk of exposure and effect.

Isotopic values are expressed in parts per thousand, or per mil (‰) using the δ notation, which refers to the deviation of a sample from a standard value (Fry, 2006).

1.4.1 Nitrogen Isotopes

Stable isotopes of nitrogen, ^{14}N and ^{15}N, are quite useful for determining TP in both marine and freshwater food webs (Hobson et al., 2002; Post, 2002; Vander Zanden and Rasmussen, 1999). The tissues of producers and primary consumers have relatively small $\delta^{15}N$ values, and are always depleted in ^{15}N relative to tissues of top predators (Kelly, 2000). Feeding on tissues with high amounts of ^{15}N will increase the $^{15}N/^{14}N$ ratio in each consecutive consumer. This step-wise increase is fairly consistent (2 to 5 ‰) and can be traced through a food web (Kelly, 2000; Post, 2002). The predictable variation in $\delta^{15}N$ is dependent upon biological processes involving assimilation and excretion of nitrogen from ingested food. Some dietary amino acids are incorporated into tissues, while others are de-aminated and released as wastes (Fry, 2006). Nitrogenous compounds are excreted in the urine (urea/uric acid), and these compounds are comprised largely of the lighter ^{14}N isotope (Kelly, 2000). The ^{15}N isotopes are excreted to a lesser extent, and remain in the animal's tissues, resulting in a larger $\delta^{15}N$ (Kidd et al., 1995).

1.4.2 Carbon Isotopes

Carbon is a major source of energy in both aquatic and terrestrial food webs. Sources of primary production, such as C_3 and C_4 plants, have distinct $\delta^{13}C$ values (Fry, 2006). In a freshwater system, different assemblages of phytoplankton, for example, may have different isotopic profiles based on species composition or spatial and temporal differences in the

dissolved inorganic carbon (DIC) pool (Fry, 1988; Hobson et al., 2010). Littoral sources of carbon also tend to be ^{13}C-enriched relative to pelagic carbon sources (Burgess and Hobson, 2006; Croisetiére et al., 2009). Such unique values change very little as carbon is passed up the food web ($\Delta^{13}C \approx +1$ ‰) allowing researchers to estimate the source of carbon, or food energy, for an individual (Logan et al., 2008; Ricca et al., 2007). Food web models in which δ^{13}C or δ^{34}S (see below) are used to separate energy sources are generally triangular, with many diverse sources spread out at the base and few top predators at the upper point of the triangle, representing the apex of assimilated sources (Hecky and Hesslein, 1995).

1.4.3 Sulfur Isotopes

The most commonly utilized isotopes of sulfur, ^{34}S and ^{32}S, are much like carbon isotopes in that they are useful in identifying energy sources used by organisms. During the transfer of sulfur from the initial source to the top of the food web, little or no metabolic discrimination occurs, and as a result, there is little deviation from the baseline δ^{34}S value, even in top predators (Cucherousset et al., 2011; Hesslein et al., 1991). This characteristic makes δ^{34}S an ideal supplement for δ^{15}N and δ^{13}C studies where it is difficult to determine the feeding habits of low TP consumers with very similar δ^{13}C values (Connolly et al., 2004). The conservation of δ^{34}S values up the food web may also be useful in determining whether the pelagic or sediment-based compartments of Lake Winnipeg are being utilized by fishes or cormorants (Croisetiére et al., 2009). In freshwater lakes, sulfate dissolved within the water column is generally ^{34}S-enriched relative to detritus and sediments (Fry, 1988, 1989). Localized inputs of sulfur-rich compounds, such as chemical fertilizers and pesticides, may create localized alterations in the δ^{34}S values of dissolved or sedimentary sulfate (Fry, 1989). These differences may be detectable in species which feed at the mouths of rivers or near farming operations. Sulfur isotopes have also proven useful in distinguishing the degree to which highly-mobile organisms rely on marine, estuarine,

or freshwater habitats throughout the year (Connolly et al., 2004; Hebert et al., 2008). Sulfur isotope ratios in bird feathers provide an indication of habitat use during the period of feather growth (Hebert et al., 2008; Lott et al., 2003) and are therefore likely to have some utility in linking cormorant feathers with habitat types.

1.4.4 Hydrogen Isotopes

Hydrogen isotope ratios ($^{2}H/^{1}H$) in growing season precipitation exhibit long-term, geographical trends which can be used as a basis for linking tissues of migratory organisms to their point of origin (Hobson et al., 2006; Van Wilgenburg and Hobson, 2010). Apart from isotopic discrimination processes which occur as precipitation is absorbed by plants and incorporated into carbohydrates, there is little change in the $\delta^{2}H$ values with each subsequent step up the food web (Lott et al., 2003). Therefore, based on long-term latitudinal patterns in precipitation, researchers are able to assign migratory birds to breeding, wintering or mid-migration habitats (Clark et al., 2006, 2009; Hobson et al., 2006). Since cormorants do not follow a predictable molt pattern (Hatch and Weseloh, 1999), measurement of $\delta^{2}H$ in cormorant feathers may be useful in matching contaminant concentrations to the appropriate latitude of origin.

1.4.5 Constructing the Lake Winnipeg Food Web

Stable isotope data for a given site, such as Lake Winnipeg, allows for the development of a food web based on energy transfer and isotopic discrimination in that system. Low TP organisms are important in energy source determination and baseline values have been or are currently being developed for Lake Winnipeg and similar systems (Hobson et al., 2010; Tamelander et al., 2009). The isotopic values determined for DIC, dissolved sulfate (DS), benthic invertebrates and plankton, for example, can later be used in the interpretation of isotope values for fishes.

However, the fish-bird component of the lake's food web is the main focus of this study, and therefore planktonic and benthic producers will not be intensely examined.

1.5 Contaminants

Studies regarding concentrations of Persistent Organic Pollutants (POPs) in the Lake Winnipeg food web have been reported in recent years and a number of these consider the distribution of chlorinated or brominated compounds in biota (Gewurtz et al., 2006; Law et al., 2006; Rawn et al., 2000; Stewart et al., 2003; Tomy et al., 2007). Concentrations of organic contaminants, As, cadmium (Cd), Hg, lead (Pb) and Se were also measured in livers and kidneys of Herring Gulls (*Larus argentatus*) collected near the mouth of the Saskatchewan River between 1991 and 1993 (Fox et al., 2002). Additional studies of Hg and trace elements in Lake Winnipeg's biota are rare. This includes scientific data regarding sources and accumulation of metals and metalloids in Lake Winnipeg's fish community or in associated consumers which rely on fish for food. Such information gaps raise questions regarding the health of fish populations, as well as the safety of other wildlife and humans. Organisms which live near contaminant inputs like the Red River, as well as those with long life-spans and higher TPs, may accumulate elevated tissue concentrations of harmful elements (Burger et al., 2008). In order to assess the possible impacts of contaminant loading into Lake Winnipeg and the potential risks to consumers, it is necessary to examine similar systems and historical data. In doing so, a plan for the implementation of a monitoring program and protective guidelines may be developed for Lake Winnipeg.

Other North American lakes, such as the Laurentian Great Lakes, have been of considerable interest in food web and contaminants research, as they are heavily influenced by anthropogenic activities and exploitation of fisheries resources. A large number of studies have also examined the bioaccumulation of Hg in the lakes of Ontario (Swanson et al., 2006; Wren et al., 1983) and

Saskatchewan (Hall et al., 2009), among others. Swanson et al. (2006) reported that slow-growing fish, such as walleye and northern pike, accumulate greater amounts of Hg in their tissues. Therefore, these species may be a greater risk for reproductive failure, altered behaviour and reduced overall fitness where concentrations are great enough to elicit subtle toxic effects without killing the organism (Adout et al., 2007). Even in cases where tissue Hg concentrations may be below the 0.5 ug/g fresh weight (FW) limit for human consumption, there is an increased risk for the ingestion of toxic levels in consumers who rely heavily on fish protein (Lake et al., 2006). A number of studies have examined metal concentrations in tissues of water birds, including Herring Gulls (Fox et al., 2002) and Black-legged Kittiwakes (*Rissa tridactyla*) (Burger et al., 2008). Herring Gulls from the Laurentian Great Lakes display increased tissue contaminant residues with greater proximity to sources of anthropogenic contaminants, as well as increased exposure via diet when feeding at higher TPs (Fox et al., 2002). While elevated trace element concentrations in bird tissues may not reach lethal levels, they may affect fitness and/ or reproductive success (Adout et al., 2007). A similar trend may be predicted for cormorants nesting and feeding on Manitoban lakes. Cormorants are at an increased risk of accumulating toxic levels of contaminants from their diet, especially in highly polluted systems. North American populations of this species were once declining due to high levels of organochlorine contamination, but with increased awareness and stricter guidelines, numbers have recovered throughout their range since the late 1970s (OMNR, 2006; Somers et al., 1993).

Scientific researchers admit there have been few studies which have evaluated short-term contaminant fluxes within the food web of Lake Winnipeg (Stewart et al., 2003). As in other systems previously mentioned, Hg is a metal of great concern. The fishing industry on Lake Winnipeg may be severely impacted by increased contaminant residues in economically

important fishes. In the early 1970s, all commercial fishing activities on the lake were halted for one season after a number of fish species, including walleye, were found to have extremely high concentrations of Hg in their tissues (Environment Canada and Manitoba Water Stewardship, 2011). Since the ban on fishing was removed, few further studies have been conducted. There is opportunity for increased biomagnification of this metal and others when the food web becomes significantly altered. In light of this, the potential implications of species introduction or top-down pressures of commercial fishing deserve further study (Stewart and Watkinson, 2004; Swanson et al., 2003). Other perturbations to the Lake Winnipeg ecosystem may have implications for biomagnification of metals. Recurrent flooding of the Red River is known to alter the composition of the lake's planktonic community and may remobilize organochlorine compounds, increasing bioavailability (Stewart et al., 2003). There is potential for flood events to increase the availability, uptake and trophic transfer of some sediment-associated metals in a similar fashion. Unfortunately, many of these phenomena have not been examined and there are currently few measures in place to effectively monitor the dynamics of trace elements in the Lake Winnipeg system. Without appropriate monitoring, and with the continued deposition of potentially toxic elements into the lake, there is a greater risk for adverse effects to vulnerable and/ or commercially valuable species. A short-term study of the lake's biota and contaminant cycling is required in order to generate current, ecologically relevant data for the lake, while accounting for spatial variability in food availability, water quality and contaminant loading.

1.5.1 Selected Trace Elements

It is well understood that fishes may be exposed to contaminants in the water-column or via ingested prey (Handy, 1992); however, diet is often the primary route of exposure for Hg and many other contaminants (Jardine et al., 2006). Some trace elements, such as As, Cd and Hg have no known physiological function and are toxic to biota. Others, such as copper (Cu), iron

(Fe), manganese (Mn) and Se are considered essential at low concentrations, but may become toxic when tissue concentrations are excessive and homeostatic regulation is sub-optimal (Yang and Swami, 2007). Although the health effects of most trace elements are well known, there is little information regarding their accumulation and food web dynamics in Lake Winnipeg. Monitoring of contaminant concentrations in edible fish tissues is extremely important due to the extent of the lake's fishery (Stewart and Watkinson, 2004) and the potential for adverse effects to piscivorous birds and other fish-eating wildlife. A number of elements have the potential to biomagnify in the food web, leading to increased tissue contaminant concentrations in individuals with higher TPs. Therefore, it is important to monitor these contaminants in order to ensure the integrity of the food web and the protection of vulnerable species. Trace elements examined as part of this study are described below.

1.5.1.1 Aluminum

Elevated concentrations of aluminum (Al), sometimes as large as 1230 µg/g dry weight (DW), have been measured in emergent insects (Scheuhammer, 1987). However in fishes, water-borne exposure via the gill surface is the primary source of Al toxicity, rather than invertebrate consumption (Peakall and Burger, 2003). Aluminum accumulates in water birds to a greater extent than in fishes, and there is potential for disrupted absorption of phosphate from diet and/ or altered calcium homeostasis in birds (Scheuhammer, 1987; Wren et al., 1983). This may have knock-on effects for bone development, growth and muscle strength if Al is efficiently absorbed from ingested food (Scheuhammer, 1987).

1.5.1.2 Arsenic

Compounds containing As have found wide use as pesticides and herbicides (Newman and Unger, 2003). Lake Winnipeg has one of the largest watersheds among all Canadian lakes (Stewart and Watkinson, 2004), with a major portion of the land being used for agriculture. This

raises concern for increased loading of As and other pesticides into the lake. Although biomagnification of this metalloid is unlikely (Campbell et al., 2005; Ikemoto et al., 2008), there is some evidence it may be negatively correlated with δ^{15}N or TP in some systems (Soto et al., 2011).

1.5.1.3 Cadmium

Very little Cd is deposited in aquatic systems via natural processes; however the use of this element in industrial and agricultural applications, along with its release as a by-product of zinc mining, has greatly increased its presence in freshwater lakes (Newman and Unger, 2003; Peakall and Burger, 2003). Anthropogenic Cd may enter water bodies as atmospheric particles or in run-off from farmland fertilized with Cd-rich sewage sludge (Newman and Unger, 2003). There may also be increased risk of exposure to fish and birds which inhabit areas near cottagers who dispense raw sewage directly into Lake Winnipeg. Biomagnification of this metal in fish is poorly substantiated (Peakall and Burger, 2003) and reports suggest Cd concentrations may actually decrease as fish acquire higher TPs (Campbell et al., 2005; Ikemoto et al., 2008). However, high inter-specific variability in Cd concentrations in fishes has been observed (Brumbaugh et al., 2005). It is unclear whether concentrations in the muscle of some, or all, Lake Winnipeg fishes exceed human consumption guidelines, such as the 0.05 µg/g FW limit set by the European Union (EU) or Health Canada's 450 mg Cd/ week recommendation (Campbell et al., 2005). For fishes and birds, sub-lethal toxicity is expected when Cd in food items exceeds 1 ppm, or 1 µg/g FW (Burger and Gochfeld, 2007). Cadmium is known to impair liver and kidney function in a number of wildlife species, and has been linked to metabolic, behavioural and reproductive effects in water birds (Goutner et al., 2001). The distribution of this element in the water and biota of Lake Winnipeg must be studied in order to accurately access the risks to humans and wildlife.

1.5.1.4 Copper

Copper is an essential nutrient required by a number of organisms at low concentrations (Manahan, 2005). However, anthropogenic use of Cu-based compounds may lead to increased levels in aquatic ecosystems. Toxic symptoms occur when concentrations become too high and the body's natural regulatory systems become compromised (Newman and Unger, 2003). Although bioaccumulation of Cu has been documented in Nile tilapia (*Tilapia nilotica*), isotopic food web studies of Arctic and Asian food webs would suggest this element does not biomagnify (Campbell et al., 2005; Ikemoto et al., 2008). In 2001, concentrations of Cu within the water column of Lake Winnipeg's south basin were examined (Pip, 2006). One region had water-Cu concentrations as large as 188 µg/L; however, this is likely attributed to the application of algicidal copper sulfate ($CuSO_4$).

1.5.1.5 Iron

Although Fe is an essential element, it may bioaccumulate in fishes (Rashed, 2001; Yang and Swami, 2007). Consumers in some regions of North America, such as California, may be exposed to Fe concentrations 1.2 to 1.8-fold greater than the United States' recommended daily allowance, based on a single serving (Ruelas-Inzunza and Paez-Osuna, 2007). Since Fe concentrations and levels of organic matter in lake sediments are well-correlated, an examination of Fe and $\delta^{34}S$ in fish tissues may be useful in identifying which species most efficiently remobilize Fe from sedimentary sources (Croisetiére et al., 2009; Wren et al., 1983).

1.5.1.6 Manganese

Manganese is a cofactor for many enzymes in animal tissues, and is therefore an essential dietary element (Ikemoto et al., 2008). A negative correlation between Mn and $\delta^{15}N$, or TP, in fish tissues exists for some species and food webs (Ikemoto et al., 2008), but not others (Campbell et al., 2005). It remains unclear whether Mn concentrations in tissues can be

explained by $\delta^{34}S$ or $\delta^{13}C$ values and therefore energy source. Age is known to have an effect on Mn concentrations in water birds, such as the Common Tern (*Sterna hirundo*) (Burger and Gochfeld, 1994). Burger and Gochfeld (2001) suggest that Mn may be an element of special interest in cormorant species, based on their analysis of Cape Cormorants (*Phalacrocorax capensis*) collected from the Namibian coast of Africa. Cape Cormorants accumulate Mn in feathers, and concentrations may reach levels as much as seven times greater than those measured in Hartlaub's Gulls (*Larus hartlaubii*) collected from the same sites (Burger and Gochfeld, 2001).

1.5.1.7 Mercury

The in-depth study of Hg in marine and freshwater systems (Bodaly et al., 2007; Burger et al., 2008; Campbell et al., 2005; Cizdziel et al., 2003; Jaeger et al., 2009) is warranted by this metal's highly toxic nature. Methyl mercury (MeHg) is highly bioavailable and toxic to aquatic organisms. Over 90 % of the total mercury (Hg_T) burden in fish muscle is comprised of MeHg (Burgess and Hobson, 2006) and essentially all Hg_T in Black-legged Kittiwake and Black Oystercatcher (*Haematopus bachmani*) muscle is MeHg (Burger et al., 2008). Inorganic Hg may enter Lake Winnipeg from the atmosphere (Bodaly et al., 1993) and MeHg influxes may occur as the result of large-scale flooding in tributaries or following hydroelectric development. Microbial activity within tributary or lake sediments converts inorganic Hg into biologically available MeHg (Hall et al., 2009; Wiener et al., 2006). The rate of this conversion increases when terrestrial organic matter becomes submerged and anaerobic conditions develop (Peakall and Burger, 2003).

The risks associated with MeHg are great, as this organo-metal is well-absorbed in the gut tract, capable of passing through the placenta and other protective barriers, and is known to transfer from prey species to higher-TP organisms (Campbell et al., 2005; Peakall and Burger,

2003). Predatory fishes may accumulate large concentrations of MeHg from food, especially when a large amount of biomass is consumed per day and the food web contains a relatively large number of trophic steps (Jardine et al., 2006). The introduction of rainbow smelt into Lake Winnipeg may or may not have implications for Hg concentrations in predatory fishes, depending on whether connections exist between the establishment of smelt, alterations to food web structure and increased exposure in top predators (Stewart and Watkinson, 2004; Swanson et al., 2003). An abnormally high body burden in commercial fishes can be detrimental to human health when safe consumption guidelines are exceeded. Birds such as cormorants, which feed on a wide variety of fishes, are also expected to ingest and absorb a large amount of Hg. This metal may be excreted in feathers, accumulate in soft tissues like organs and muscle, or may be lost by females during egg laying (Bond and Diamond, 2009). Transfer of Hg from mother to young may have subtle implications for specific bird populations or colonies in the future. Maternal transfer of Hg also has implications for human populations, as the unborn fetus may be exposed via the mother's diet (Ubillús et al., 2000).

1.5.1.8 Selenium

Water-borne Se exposure in fishes is secondary to dietary exposure (Hamilton et al., 2002). Selenomethionine is the most common form of Se found in fishes and concentrations accumulated in fish muscle may account for over 70 % of the total body burden (Cabanero et al., 2005; Ciardullo et al., 2008). The relationship between Se and diet tends to vary, depending on location and species. No relationship between Se and δ^{15}N or δ^{13}C values could be identified for the Arctic food web of Baffin Bay's Northwater Polyna (Campbell et al., 2005). Alternatively, Se and δ^{15}N values for organisms collected from the Mekong Delta in South Vietnam were positively correlated and indicative of biomagnification (Ikemoto et al., 2008). Selenium biomagnification also occurs in lakes downstream of a uranium mining facility in Saskatchewan,

Canada, with concentrations in forage fishes outweighing those in plankton (Muscatello et al., 2008). Relative to plankton however, detritus (low δ^{34}S) is a much greater source of Se for lacustrine fishes, especially those which feed at the lake bottom (Hamilton et al., 2002). Diets containing 10 to 13 µg/g of Se (as selenite) may cause renal damage, impaired growth and mortality in some fishes, while muscle Se concentrations exceeding 8 µg/g FW in fishes are likely to induce reproductive failure (Ciardullo et al., 2008; Hamilton et al., 2002).

In fish-eating birds, such as Common Terns and cormorants, Se accumulation in tissues is largely dependent on an individual's age (Burger and Gochfeld, 1994; Sepulveda et al., 1998). Reproductive impairment and developmental deformities stemming from Se accumulation may occur in cormorants (Sepulveda et al., 1998). Mortality in water birds may also occur when Se concentrations in blood exceed levels in the 5 to 14 µg/g FW range (Burgess et al., 2005).

1.6. Research Objectives & Expectations

1.6.1 Research Objectives

The methods employed within this thesis were developed and implemented in order to:

1) Produce isoscapes for inorganic nutrient sources (δ^{13}C and δ^{34}S) and primary consumers (δ^{15}N) at the base of the Lake Winnipeg food web (Chapter 2). This was to form the basis for all subsequent food web isotopic modeling.

2) Model the current trophic structure of the Lake Winnipeg fish community and identify any spatial differences in food web structure and food sources through the use of isotopic (δ^{15}N, δ^{13}C and δ^{34}S) measurements (Chapter 3).

3) Use stable isotope measurements of biota to determine the behavior of trace elements within the Lake Winnipeg fish community (Chapter 3).

4) Evaluate scale sampling as a potential method for monitoring trace element residues in commercially-important walleye (Chapter 4).

5) Characterize the diets of Double-crested Cormorants breeding on the lake (Chapter 5).

6) Use stable isotope ratios for cormorant muscle and feathers to examine the provenance of feather growth and to thus evaluate where during the annual cycle birds were most exposed to contaminants (Chapters 5 and 6).

1.6.2 Expectations

The expected outcome(s) associated with each of the abovementioned research objectives (1.6.1) is/ are described below.

1) It was anticipated the relative distribution of heavy isotopes (^{13}C, ^{34}S and ^{15}N) in baseline nutrient sources ($\delta^{13}C$ and $\delta^{34}S$) and primary consumers ($\delta^{15}N$) would vary spatially within the lake. Spatial patterns were expected to result from a combination of factors, including a) mixing of isotopically distinct riverine nutrient sources, b) gas-exchange (i.e. CO_2) and c) regional differences in nutrient uptake or sequestration by primary producers.

2) Isotopic profiles of Lake Winnipeg fishes ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$) were expected to reflect any regional isotopic variability in the lake's zooplankton assemblage, DIC pool and dissolved sulfate. Since the lake's north and south basins tend to differ in terms of water clarity, temperature and productivity, some fishes, such as sauger and rainbow smelt, have demonstrated a preference for habitats within the south and north basins, respectively. It was anticipated that such regional differences in species abundance or ecological niche would also be reflected in the isotopic structure of the lake's fish communities.

3) Stable isotope ratios ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$) in fish muscle represent assimilated nutrients, and therefore individual diet. It was anticipated then that stable isotope data could be used to identify dietary sources of trace elements and track their behavior in the Lake Winnipeg fish community. Strong positive correlations between trace element concentrations and $\delta^{15}N$ were expected for elements which are generally found to biomagnify (e.g. Hg). Similarly, those

elements more concentrated in benthic or detrital food webs were expected to be positively correlated with $\delta^{13}C$ and negatively correlated with $\delta^{34}S$, respectively.

4) Although scale and muscle tissues of walleye differ in terms of composition and growth, it was anticipated that isotope ratios ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$) and trace element concentrations in paired tissues would be strongly correlated. Therefore, it was postulated that data for walleye scales could be used to predict values in muscle. Subsequently, archived fish scales would provide a non-destructive means of investigating contaminant and dietary patterns through time.

5) This thesis represents the earliest investigation into prey consumption for cormorant populations which nest on Lake Winnipeg, despite the fact this species is often implicated in losses to fisheries and aquaculture facilities. Although this population has access to commercially-valuable species on both the breeding (Lake Winnipeg) and wintering (Gulf of Mexico) grounds, it was anticipated that these fishes would represent only a small proportion of the overall cormorant diet. Forage fishes with little or no direct commercial value were expected to represent the major fraction of the cormorant diet, as these species are abundant (see Lumb et al., 2011) and easily captured by cormorants (i.e. fishes which form schools near the water's surface).

6) Isotopic ratios for cormorant muscle ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$) and primary feathers ($\delta^{2}H$, $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$) were expected to provide an indication of diet and seasonal habitat use. It was reasoned that isotopic data for these two tissues could be used to identify potential dietary sources of Hg and other trace elements, but also demonstrate the importance of trophic position in regulating accumulation.

CHAPTER 2
2.0 DEVELOPMENT OF ISOTOPIC BASELINES (N, C & S) FOR THE LAKE WINNIPEG FOOD WEB

2.1 Introduction

Stable isotope measurements of biota in aquatic systems are useful for identifying nutrient sources and energy transfer pathways; monitoring changes in food web structure following species introduction, and tracking the seasonal movements of higher-order consumers, such as fishes and piscivorous birds (Cucherousset et al., 2011; Post, 2002). Stable isotopes of nitrogen (δ^{15}N) and carbon (δ^{13}C) are also frequently used as a means of tracking the dietary transfer and biomagnification of contaminants within aquatic food webs (Campbell et al., 2005; Gewurtz et al., 2006). The utility of stable isotopes in reconstructing trophic interactions among aquatic organisms lies in the relatively constant degree of dietary discrimination at each trophic step. Stable nitrogen isotope ratios in consumer tissues exhibit a 2 to 5 ‰ increase with each subsequent trophic step, however stable isotope ratios of carbon and sulfur (δ^{34}S) in consumer tissues deviate very little (≈ 1 ‰) from those within the nutrient pool or primary producers (Campbell et al., 2005; Fry, 1988). Therefore, understanding isotopic patterns in baseline nutrients and primary production is fundamental to the successful application of isotopic tracers in aquatic food web studies.

The relative abundance of nitrogen, carbon and sulfur isotopes available to primary producers in freshwater lakes is largely determined by land use within the watershed and environmental cycles (Miyajima et al., 1995; Vander Zanden et al., 2005). Depending on relative inputs and mixing of isotopically distinct sources, the receiving waters may develop spatial isotopic patterns which are then transferred from baseline nutrients to biota (Kline, 1999; Vander Zanden and Rasmussen, 1999). Such spatial variation within and among systems may confound the interpretation of trophic linkages and food sources, depending on the isotope measured (Hobson

et al., 2010; Post, 2002). This becomes particularly challenging for large lakes, where nutrients from separate sources do not mix, resulting in isotopic heterogeneity across regions. Development and implementation of region-specific corrections for isotopic variability in baseline nutrients may be the most effective method for standardizing and comparing food web models within systems that vary spatially in isotopic composition (Post, 2002). The most widely applied correction for normalizing food web data across systems is the derivation of TP, wherein consumer data is adjusted based on dietary discrimination factors and the area-specific δ^{15}N values of primary consumers (Gewurtz et al., 2006; Vander Zanden and Rasmussen, 1999). Other corrections based on isotopic values of dissolved inorganic nitrogen (DIN) and DIC have also been implemented (Croisetiére et al., 2009; Hobson et al., 2010).

Lake Winnipeg, Canada (53° 17'N, 97° 58'W; Figure 2.1) is the world's tenth largest freshwater lake, and drains 977 800 km^2 of urban, agricultural and forested land (Pip, 2006). Nitrogen- and phosphorus-driven eutrophication, introduction of non-native fishes and commercial fishing pressures emphasize the need for isotopic investigations of fish community structure (Environment Canada and Manitoba Water Stewardship, 2011; Hobson et al., 2010). The application of "universal" or "absolute" isotopic niche values to certain fish species or size classes however, may be an especially risky venture when comparing across lake regions. Nutrient loading, particularly from the Red and Winnipeg Rivers to the south, as well as hydroelectric development and eutrophication, may have profound effects on the spatial distribution of nitrogen (δ^{15}N), carbon (δ^{13}C) and sulfur (δ^{34}S) isotopes in primary production (Hobson et al., 2010). Region-to-region variability in the isotopic composition of nutrient sources may confound the interpretation of isotopic data for fishes and other consumers unless

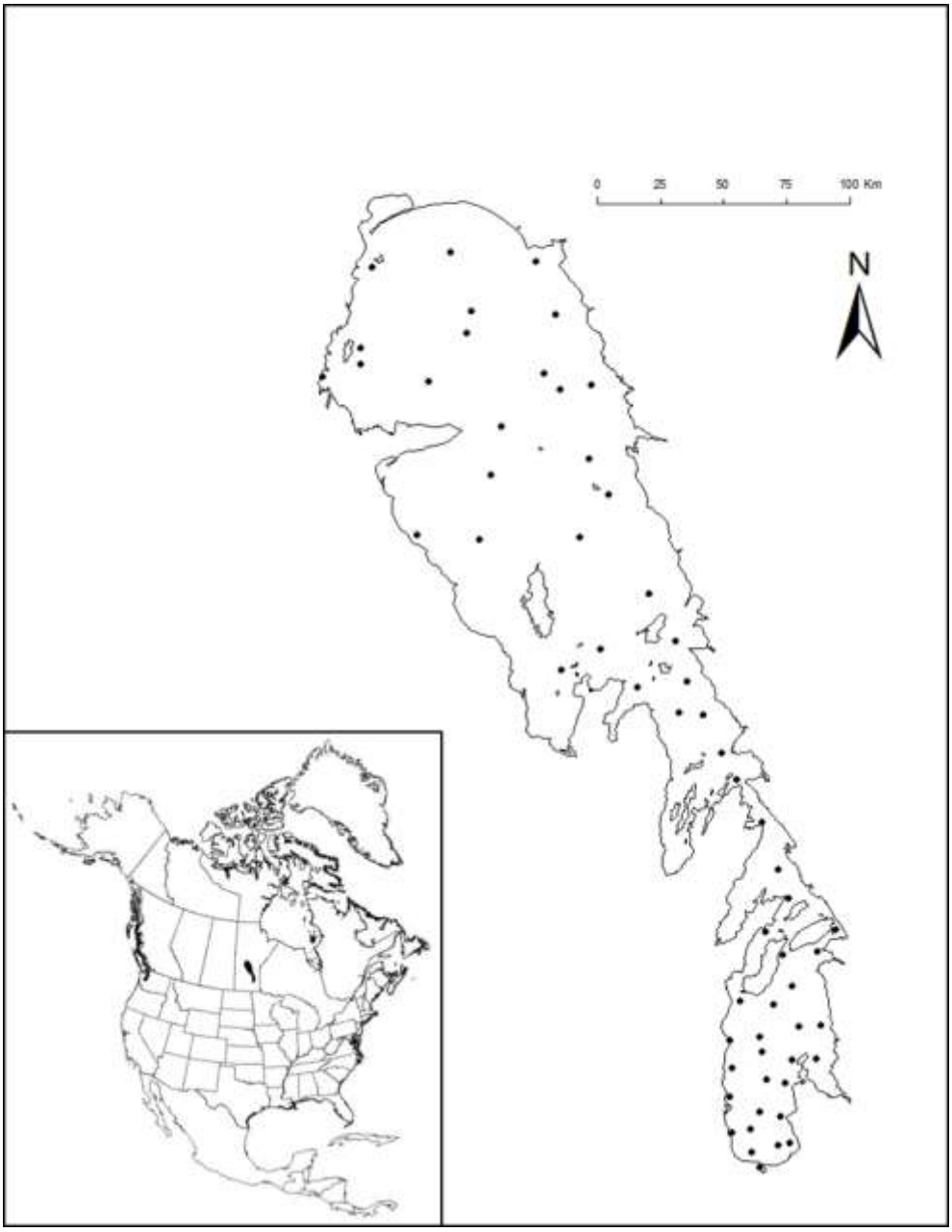


Figure 2.1: Lake Winnipeg, Canada. Sampling stations are marked with black circles.

factors related to the isotopic composition of the nutrient pool can be accounted for (Post, 2002). Therefore, a large-scale analysis of the spatial isotopic structure in Lake Winnipeg is required.

The over-arching objective of this Chapter was to develop lake-wide isotopic baselines from zooplankton ($\delta^{15}N_{Zoop}$), DIC ($\delta^{13}C_{DIC}$) and dissolved sulfate ($\delta^{34}S_{DS}$) data collected from Lake Winnipeg during 2010. It was postulated that this information would be fundamental to any subsequent derivation of TP estimates for fishes and piscivorous birds, as well as identification of food sources and trophic linkages. With the inclusion of spatial nutrient-baseline data in future food web studies, large-scale spatial comparisons between community structure and energy sources may be made with greater confidence. A sample dataset was used to demonstrate how isoscape patterns for energy and nutrient sources at the base of the food web influence the interpretation of fish community structure within the lake.

2.2 Methods

2.2.1 Sample Collection

Zooplankton for $\delta^{15}N_{Zoop}$ analysis, along with water samples for quantification of $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ were collected from a number of research stations across Lake Winnipeg (Figure 2.1) during the summer of 2010. The walleye (*Sander vitreus;* n = 273) data used here was taken from Chapter 3. Walleye were collected from beam trawls and commercial gillnets during 2009 and 2010. Collection, preparation and analysis of fish specimens are fully described in Chapter 3.

2.2.1.1 $\delta^{15}N_{Zoop}$

At each station, a zooplankton net with a 72 µm mesh size and 25-cm diameter opening was lowered to a depth of 1 m off the lake bottom, and then hauled vertically at a rate of approximately 1 m/s (Campbell et al., 2005; Kline, 1999). The contents of each net haul were rinsed into acid-washed polyethylene bottles (Kline, 1999). All bulk zooplankton samples were

stored frozen at -20°C until they could be processed for isotopic ($\delta^{15}N$) analysis (Gorski et al., 2003; Kline, 1999).

2.2.1.2 $\delta^{13}C_{DIC}$

Sterile, glass Exetainer™ vials were used in the collection and preparation of samples for $\delta^{13}C_{DIC}$ assays. In order to prevent any biologically-mediated perturbations to the $^{13}C_{DIC}/^{12}C_{DIC}$ ratio of stored samples, each glass vial was pretreated with 50 µl of a saturated mercuric chloride ($HgCl_2$) solution (Atekwana and Krishnamurthy, 1998; Miyajima et al., 1995). Water samples were retrieved from 1 m below the lake's surface via a series of Niskin bottles which were mounted to an auto-sampling device (Takahashi et al., 1990; Tamelander et al., 2009). At each station, a sterile syringe fitted with a 0.45 µm Luer-Lok™ barrel filter and 22-gauge needle was used to inject 2 ml of water into the appropriate glass vial (Fonyuy and Atekwana, 2008; Salata et al., 2000). Individual samples were wrapped in Parafilm® and refrigerated (4°C) until analysis (Hélie et al., 2002; Miyajima et al., 1995).

2.2.1.2 $\delta^{34}S_{DS}$

Water samples for $\delta^{34}S_{DS}$ determination were collected from 1 m below the surface via an auto-sampling device (Tamelander et al., 2009). Water was immediately filtered through a glass-fibre filter, then a 0.45 µm cellulose acetate membrane (Neumann et al., 2002). Filtered samples were housed in acid-washed polyethylene bottles and held at 4°C until extraction (Blake et al., 2006).

2.2.2 Sample Preparation & Analyses

2.2.2.1 $\delta^{15}N_{Zoop}$

Zooplankton samples were dried at 60°C, then homogenized (Vander Zanden et al., 2005). One milligram (± 0.010 mg) aliquots were weighed into tin capsules (Campbell et al., 2005), then combusted using an elemental analyzer (Eurovector 3000; Milan, Italy) (Hobson et al.,

2010). The N_2 gas generated by the combustion procedure was analyzed via Continuous Flow Isotope Ratio Mass Spectrometry (CF-IRMS) (Wayland and Hobson, 2001). Two in-house standards, Bowhead Whale Baleen (BWB; $\delta^{15}N$ = +14.4 ‰) and bovine gelatin (PUGEL; $\delta^{15}N$ = +5.60 ‰), were run along with the field samples, and had standard deviation (SD) values better than ± 0.2 ‰.

2.2.2.2 $\delta^{13}C_{DIC}$

Carbon isotope ratios in DIC were quantified directly from field collected samples by first flushing the headspace of each Exetainer™ vial with helium for one minute, then adding an 85 % phosphoric acid solution (H_3PO_4) (Atekwana and Fonyuy, 2009). The phosphoric acid was allowed to react with the sample for at least one hour prior to the quantification of $\delta^{13}C$ in the headspace carbon dioxide (CO_2) (Atekwana and Krishnamurthy, 1998). Three in-house isotopic standards, including C-168 ($\delta^{13}C$ = +4.85 ‰), Carerra Marble ($\delta^{13}C$ = +2.05 ‰) and IS-1 ($\delta^{13}C$ = -9.9 ‰), were prepared in Exetainer™ vials and analyzed by CF-IRMS along with the field-collected DIC samples. Within-run precision was better than ± 0.1 ‰.

2.2.2.3 $\delta^{34}S_{DS}$

Prior to commencement of the dissolved sulfate extractions, a dilute hydrochloric acid (HCl) solution was used to adjust the pH of each sample to pH ~4 (Blake et al., 2006). Barium chloride ($BaCl_2$) was then used to precipitate the dissolved sulfate as barium sulfate ($BaSO_4$) (Carmody et al., 1998; Lajtha and Michener, 1994). The precipitate was dried, homogenized and weighed into tin capsules (3.5 ± 0.20 mg) prior to $\delta^{34}S_{DS}$ determination (Revesz et al., 2006). Samples were combusted to form sulfur dioxide (SO_2) gas, which was then analyzed via CF-IRMS (Revesz et al., 2006). International Atomic Energy Agency (IAEA) sulfur reference materials including IAEA-S-1($\delta^{34}S$ = -0.3 ‰), IAEA-S-2 ($\delta^{34}S$ = +22.62 ‰), IAEA-S-3 ($\delta^{34}S$ = -32.49 ‰) were

used along with reagent-grade barium sulfate (Sigma-Aldrich, St. Louis, Missouri; $\delta^{34}S$ = -3.32 ‰) to ensure analytical precision. Replicate measures of standard materials were within ± 0.3 ‰.

All results for $\delta^{15}N_{Zoop}$, $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ were expressed in per mil (‰) relative to atmospheric nitrogen (AIR), Vienna Pee Dee Belemnite (VPDB) and Vienna Canyon Diablo Troilite (VCDT) respectively, where:

$$\delta X = (R_A/R_S - 1) \cdot 1000 \quad (2.1)$$

and X was one of ^{15}N, ^{13}C or ^{34}S, R_A was the isotopic ratio ($^{15}N/^{14}N$, $^{13}C/^{12}C$ or $^{34}S/^{32}S$) of the sample and R_S was the isotope ratio for AIR, VPDB or VCDT, respectively (Fry, 1988).

2.2.3 Statistical Analyses

Station-specific $\delta^{15}N_{Zoop}$, $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ data were converted to lake-wide isoscapes through the use of an exponential semivariogram model and ordinary kriging in ArcGIS 9 (ESRI, Redlands, California; Hobson et al., 2010). Previous studies of Lake Winnipeg suggest the north and south basins are distinct ecological entities in terms of isotopic and community structure (Hobson et al., 2010; Patalas and Salki, 1992). Therefore, a Mann-Whitney *U*-test was used to determine whether the $\delta^{15}N_{Zoop}$, $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ data for the north and south basins came from the same distributions (Dalgaard, 2008).

In order to demonstrate the importance of baseline normalization to the interpretation of food web interactions, isotopic values for walleye muscle were corrected according to:

$$\delta X_{Corrected} = \delta X_{Wall} - \delta X_{Baseline} \quad (2.2)$$

where X was one of ^{15}N, ^{13}C or ^{34}S (Hobson et al., 2010). Mean ± SD uncorrected and baseline-corrected isotope values for walleye muscle were plotted in conventional two-dimensional biplots (Post, 2002; Vander Zanden and Rasmussen, 1999). Statistical analyses were conducted in R Version 2.14.1 (R Development Core Team, 2011) and all isotopic biplots were created in Grapher Version 9 (Golden Software Inc.; Golden, Colorado).

2.3 Results

The distribution of $\delta^{15}N_{Zoop}$, $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ data differed by basin (Table 2.1). From the spatial models presented in Figures 2.2a-c, the most profound basin-specific delineation was seen for $\delta^{15}N_{Zoop}$. The zooplankton assemblage of the south basin was significantly enriched in ^{15}N relative to the north; however the DIC and dissolved sulfate pools at the base of the south basin food web were relatively depleted in ^{13}C and ^{34}S, respectively.

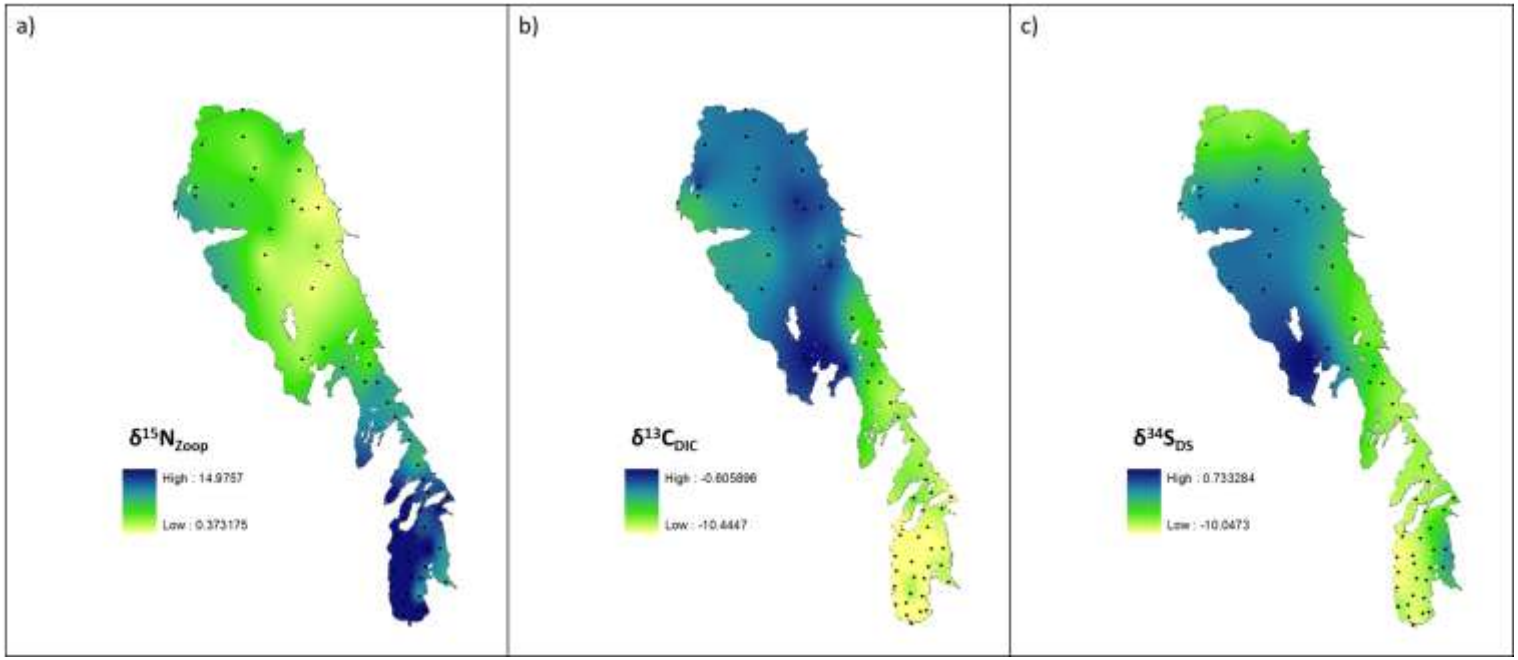
Since the isotopic baselines were different for each basin, the sample walleye dataset was divided accordingly and corrected using the mean values from Table 2.1. Mean ± SD values for each basin, rather than capture-site baseline values, were selected in order to compensate for within-basin fish movements. Mean ± SD walleye data (n = 273) which were baseline corrected according to Equation 2.2 (north basin: $\delta^{15}N = +10.2 \pm 1.3$ ‰, $\delta^{13}C = -21.5 \pm 1.1$ ‰, $\delta^{34}S = -0.7 \pm 1.4$ ‰; south basin: $\delta^{15}N = +5.6 \pm 1.8$ ‰, $\delta^{13}C = -17.4 \pm 1.6$ ‰, $\delta^{34}S = +0.5 \pm 2.1$ ‰) varied substantially from the uncorrected (north basin: $\delta^{15}N = +13.6 \pm 1.4$ ‰, $\delta^{13}C = -25.1 \pm 1.1$ ‰, $\delta^{34}S = -5.3 \pm 1.4$ ‰; south basin: $\delta^{15}N = +15.2 \pm 1.8$ ‰, $\delta^{13}C = -25.8 \pm 1.6$ ‰, $\delta^{34}S = -7.7 \pm 2.1$ ‰,) muscle isotope values (Figures 2.3a-b). This shift in two-dimensional isotopic space differed in both magnitude and direction for the two basins. These findings are corroborated by the earlier work of Hobson et al. (2010), where the spatial variability in $\delta^{15}N$ and $\delta^{13}C$ of the lake's DIN and DIC pools, respectively, as well as the spatial and temporal variability in trophic relationships for fishes < 350 mm (FL), were examined.

2.4 Discussion

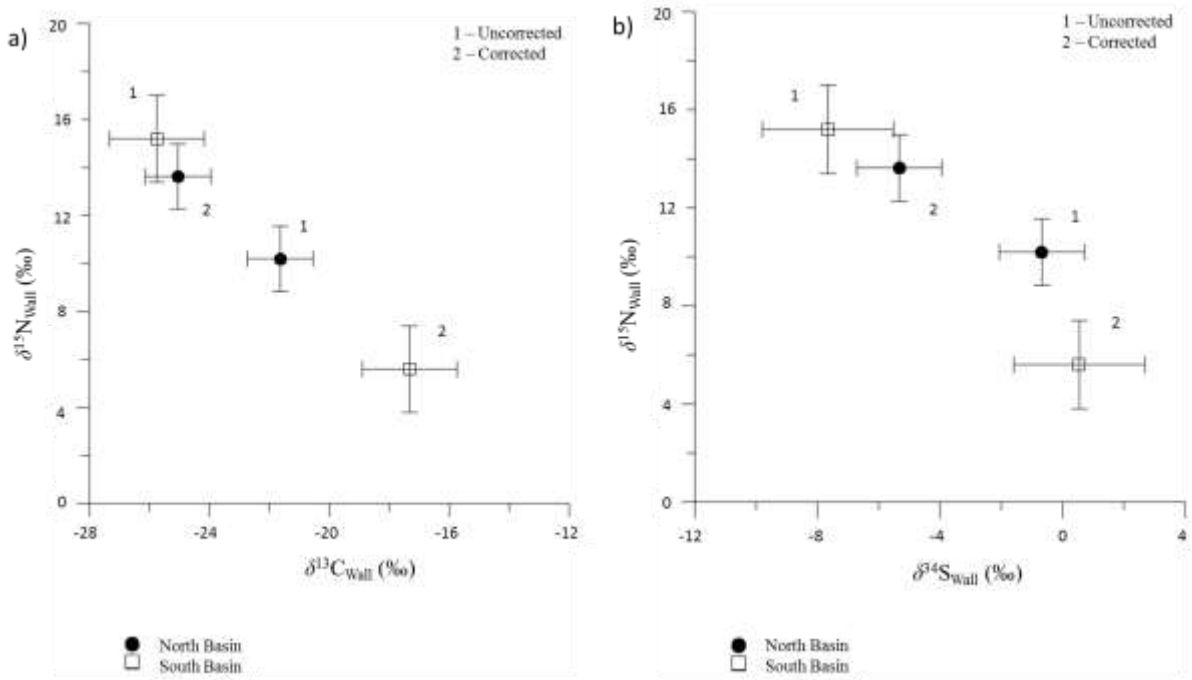
This was among the first extensive descriptions of spatial patterning in baseline stable isotope ratios for any aquatic system, and the findings suggest the diverse isotopic structure in Lake Winnipeg's nitrogen, carbon and sulfate pools had significant consequences for trophic modeling. The incorporation of isotopic baseline data into future food web studies is highly

Table 2.1 Isotopic baselines for the north and south basins of Lake Winnipeg. Basin-specific patterns of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ in zooplankton (Zoop), dissolved inorganic carbon (DIC) and dissolved sulfate (DS) respectively, were compared using a Mann-Whitney *U*-test. Units for mean ± standard deviation (SD) values are per mil (‰).

		North Basin	South Basin		
Baseline	n	Mean ± SD	Mean ± SD	*U*	p-value
$\delta^{15}N_{Zoop}$	58	3.4 ± 1.9	9.6 ± 2.8	23.0	< 0.0001
$\delta^{13}C_{DIC}$	61	-3.4 ± 2.0	-8.4 ± 1.0	917	< 0.0001
$\delta^{34}S_{DS}$	59	-4.7 ± 3.1	-8.2 ± 2.5	697.5	< 0.0001



Figures 2.2a-c: Spatial models for a) $\delta^{15}N_{Zoop}$, b) $\delta^{13}C_{DIC}$ and c) $\delta^{34}S_{DS}$ in Lake Winnipeg. Zooplankton (Zoop), dissolved inorganic carbon (DIC) and dissolved sulfate (DS) samples were collected during summer, 2010. Sampling stations are marked with black circles.



Figures 2.3a-b: Mean ± standard deviation (SD) of a) stable carbon and nitrogen and b) stable sulfur and nitrogen isotope data for walleye (*Sander vitreus*) muscle. "Uncorrected" refers to the raw walleye data from the north and south basins. Subtraction of the appropriate region-specific baseline values (Equation 2.2, Table 2.1) from the raw data produced "Corrected" values for walleye muscle.

recommended for Lake Winnipeg, as the isotopic sources entering the lake are not well-mixed and form a rather heterogeneous foundation for subsequent producer and consumer interactions. Large, significant ($p < 0.0001$) inter-basin differences in nitrogen, carbon and sulfur isotope ratios for zooplankton, DIC and dissolved sulfate, respectively, suggest the north and south basins should be considered separately when investigating food web structure. Cross-basin comparisons of trophic relationships are made possible by the application of a simple isotopic correction model (Equation 2.2).

The significant differences in $\delta^{15}N_{Zoop}$, $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ between the two basins are likely a function of the lake's large size, slow rates of water renewal within the reservoir, nutrient cycling and variable loading of N, C and S from different regions of the watershed. Large volumes of organic matter and nutrients entering the lake's south basin must travel 430 km in order to reach the lake's Nelson River outflow, where damming for hydroelectric purposes has increased the lake's water retention time (Pip, 2006; Todd et al., 1998). As organic materials are slowly transported northward, nutrients may be released during sedimentation and decomposition (Trojanowska et al., 2008), or sequestered as they are assimilated by aquatic organisms (Mayer and Wassenaar, 2012). The higher relative productivity of the north versus south basin is likely related to this availability of nutrients, as well as differences in light and temperature.

The influx of terrestrial organic matter into tributaries during flood events and nitrogenous wastes from municipal sewage and agriculture are thought to be the most significant factors leading to the relative enrichment of zooplankton ^{15}N in the south basin (Trueman and Moore, 2007; Vander Zanden and Rasmussen, 1999). Mayer and Wassenaar (2012) concluded that for Lake Winnipeg's north basin, the influx of high- $\delta^{15}N$ nitrate from the south is limited by biological assimilation, and the relatively small $\delta^{15}N$ values they observed for nitrate in the north

are a function of cyanobacterial N_2fixation and decay. Therefore, it is not surprising that north basin zooplankton were relatively depleted in ^{15}N, given the sources of nitrate available (Trueman and Moore, 2007; Vander Zanden et al., 2005).

While exchange with atmospheric carbon dioxide is an important regulator of the DIC pool (Trojanowska et al., 2008), the bimodal isotopic pattern observed for Lake Winnipeg is likely a function of carbon source in the south basin and productivity in the north basin. Enhanced microbial activity and the subsequent breakdown of organic matter in flooded tributaries may greatly reduce the observed $\delta^{13}C_{DIC}$ of the lake's south basin (Trojanowska et al., 2008; Wachniew, 2006). In the highly productive north basin, photosynthetic primary producers preferentially assimilate ^{12}C, thereby enriching the residual DIC pool (Atekwana and Krishnamurthy, 1998; Gu and Schelske, 1996).

The isoscape for $\delta^{34}S_{DS}$ (Figure 2.2c) shows a great deal of ^{34}S-depleted sulfate entering the south basin via the Red River. Industrialization within the watershed is expected to be the main cause for these inputs (Fry, 1989; Nriagu and Soon, 1985); however factors related to the microbial communities of tributary and lake sediments may play a role (Wayland and Hobson, 2001).

By applying the baseline correction models developed here to the sample walleye dataset, the importance of normalizing consumer isotope profiles to a common baseline was demonstrated. From the raw walleye data, one may have concluded specimens inhabiting the north basin occupied a lower position within the food web than their south basin counterparts. Alternatively, the baseline-corrected values are in better agreement with recent dietary observations, which suggest that frequent consumption of energy-rich rainbow smelt (*Osmerus mordax*) accelerates the growth rates and potentially increases the trophic status of north basin walleye (Environment

Canada and Manitoba Water Stewardship, 2011; Hobson et al., 2010). Smelt are much more abundant in the north basin and represent a substantial portion of the walleye diet (Environment Canada and Manitoba Water Stewardship, 2011). The baseline-correction model used here for walleye has many further applications, including other species-specific comparisons across regions, comparisons between species which are thought to occupy a similar ecological niche and in food web reconstruction. Many of these applications are discussed further in the following Chapters.

CHAPTER 3
3.0 STABLE ISOTOPES (N, C & S) AS INDICATORS OF FISH COMMUNITY STRUCTURE IN LAKE WINNIPEG: IMPLICATIONS FOR MONITORING TROPHODYNAMICS OF MERCURY & OTHER TRACE ELEMENTS

3.1 Introduction

Aquatic ecosystems throughout the world are impacted by agricultural, urban and industrial development. Physical disturbances of aquatic systems, such as the diversion of waterways for irrigation; development of shorelines for residential or recreational purposes, and construction of dams for hydroelectric power generation, may have knock-on effects to aquatic species and communities. For example, destruction of spawning habitat during dam construction may reduce the number of fishes able to reproduce each year, thereby depleting numbers of ecologically valuable predators or prey species (Bocking, 1997). Changes to the relative abundance of nutrients entering lacustrine environments can affect the numbers and nutritional quality of organisms at the base of the food web, thereby affecting the overall condition of upper-level consumers (Hebert et al., 2006). Increased atmospheric deposition and riverine loading of contaminants to lakes, estuaries and oceans has also raised concern for the integrity of aquatic communities (Brumbaugh et al., 2005; Campbell et al., 2005; Ikemoto et al., 2008). In many instances, alterations to trophic interactions among aquatic biota are concomitant with pollution of their habitats (Gewurtz et al., 2006; Ikemoto et al., 2008). Changes in food web structure and trophodynamics may modulate contaminant biomagnification and the rates at which toxic compounds accumulate in biota (Gustin et al., 2005; Jardine et al., 2006). For example, increases in lake productivity following nutrient enrichment and shifts in species diversity or abundance may significantly alter the availability of contaminants as well as the trophic interactions which facilitate their dietary transport (Stewart et al., 2003). Permanent changes in fish community structure may also occur in response to the introduction of non-native organisms or over-

exploitation, and potentially depletion, of predatory fish populations (Bocking, 1997; Cucherousset et al., 2011; Munawar et al., 2005). Such gains or losses of trophic linkages may ultimately decrease or increase the rates at which Hg and other toxic compounds biomagnify through aquatic food webs (Jardine et al., 2006).

The food web of Lake Winnipeg, Canada (53° 17'N, 97° 58'W; Figure 3.1) is subject to a number of environmental and anthropogenic stressors (Environment Canada and Manitoba Water Stewardship, 2011). Floodwaters and run-off from livestock operations, cropland, urban centres and industrial areas within the 977 800 km^2 watershed contribute excess nutrients to the lake, resulting in cultural eutrophication (Environment Canada and Manitoba Water Stewardship, 2011; Pip, 2006). Although much of the lake's nutrient load enters the south basin via the Red River, light and water chemistry parameters within the north basin support algal bloom formation (Pip, 2006). Other perturbations at the base of the food web include changes in the relative abundance of plankton species and establishment of non-native zooplankters (Stewart et al., 2003; Suchy et al., 2010). Populations of non-native fishes, such as common carp (*Cyprinus carpio*), white bass (WHBS; *Morone chrysops*) and rainbow smelt (RNSM; *Osmerus mordax*) have also been established within the lake (Stewart and Watkinson, 2004).

Over the past ten to fifteen years, researchers have attempted to link food web structure and change to contaminant concentrations in Lake Winnipeg's biota. Much of the focus has been on polychlorinated biphenyls (PCBs), flame retardants and organochlorine pesticides (Gewurtz et al., 2006; Law et al., 2006; Stewart et al., 2003; Tomy et al., 2007). After the record-level 1997 Red River flood, a cascade of changes in the lake's nutrient load and planktonic community ultimately led to increased organochlorine concentrations within predatory fishes (Stewart et al., 2003). Biomagnification of PCBs and brominated flame retardants was also confirmed in the

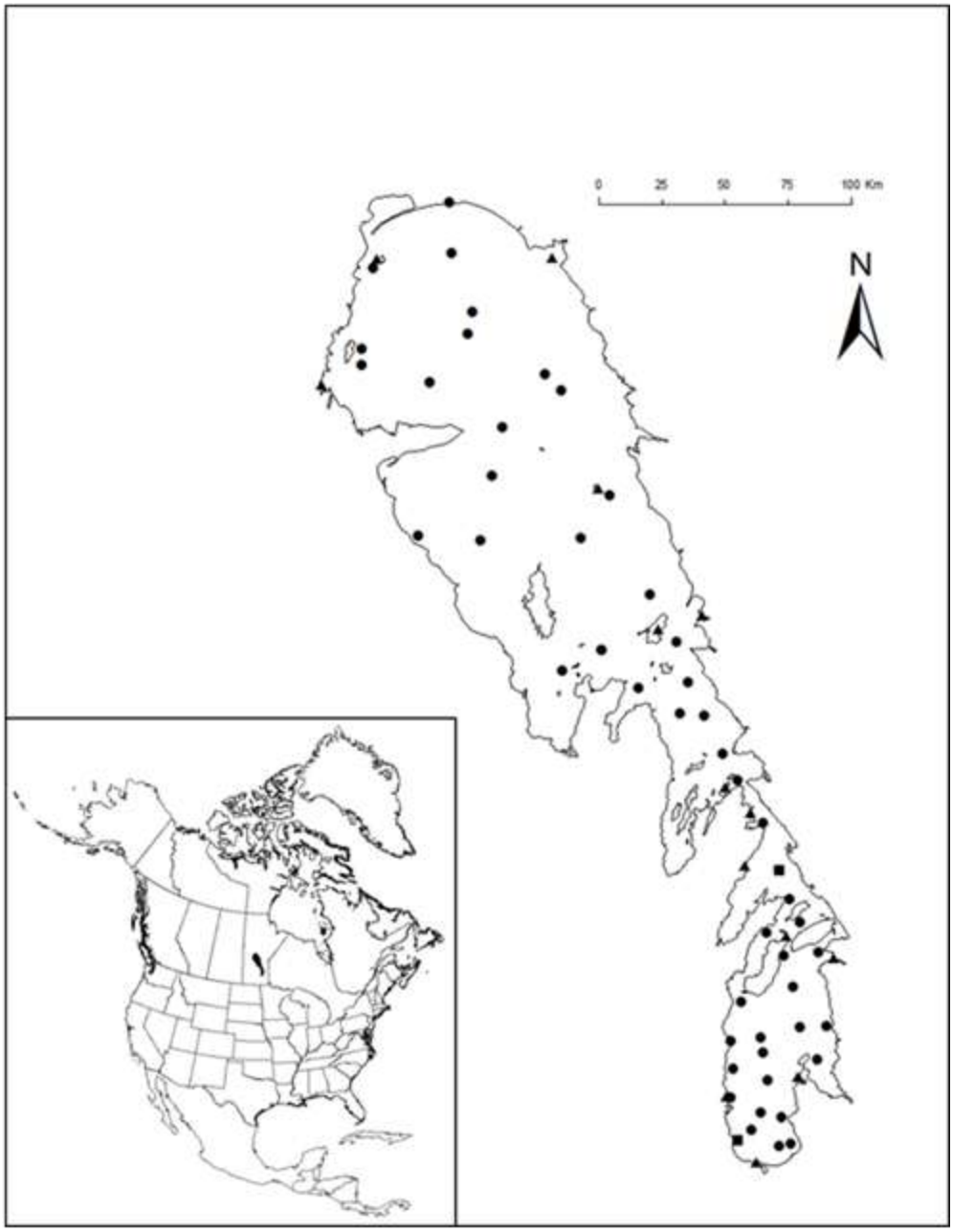


Figure 3.1 Lake Winnipeg, Manitoba, Canada. Fish specimens were collected during the ice-free seasons of 2009 and 2010 (beam trawls: closed circles; gillnets: closed triangles) and the winter of 2009-2010 (gillnets: closed squares).

years following the 1997 flood (Gewurtz et al., 2006; Law et al., 2006). Although the introduction of rainbow smelt is believed to have significantly altered trophic interactions within the lake's north basin, consumption of smelt has not been linked to any apparent increase in total PCB or Hg concentrations in piscivorous fishes (Gewurtz et al., 2006; Swanson et al., 2006). Few other studies of Hg trophodynamics exist for Lake Winnipeg, despite the fact elevated Hg concentrations in northern pike (NRPK; *Esox lucius*), yellow perch (YLPR; *Perca flavescens*) sauger (SAUG; *Sander canadensis*), walleye (WALL; *Sander vitreus*) and freshwater drum (FRDR; *Aplodinotus grunniens*) led to a season-long commercial fishing ban in the 1970s (Environment Canada and Manitoba Water Stewardship, 2011). Many trophic interactions among Lake Winnipeg fishes have not yet been evaluated, and the influence of food web parameters on trace element concentrations in fishes have not been investigated (Environment Canada and Manitoba Water Stewardship, 2011; Hobson et al., 2010).

Trophodynamic interactions among aquatic organisms are often inferred from stable nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) isotope analyses (Hebert et al., 2006; Hobson et al., 2010). With each trophic step, the $^{15}N/^{14}N$ and $^{13}C/^{12}C$ ratios of consumer tissues increase by approximately 2 to 5 ‰ for nitrogen and 1 ‰ for carbon (Fry, 1988; Kelly, 2000). Since $\delta^{15}N$ and $\delta^{13}C$ are reliable measures of TP and food source, respectively, they have added utility in identifying contaminant sources and pathways within aquatic food webs (Ikemoto et al., 2008; Jardine et al., 2006). Stable nitrogen isotope values and TP estimates are used to assess the biomagnification of Hg, trace elements and organic contaminants (Campbell et al., 2005; Gewurtz et al., 2006; Ikemoto et al., 2008). Consumers which obtain energy and contaminants from pelagic versus benthic food sources are also generally separated base on tissue $\delta^{13}C$ values (Burgess and Hobson, 2006).

The objectives of this research were to build upon previous isotopic investigations (δ^{15}N and δ^{13}C) of Lake Winnipeg's food web structure (Hobson et al., 2010) and to use stable isotope ratios in evaluating the trophodynamics of Hg and other trace elements. This study improved upon the previous baseline food web model (Hobson et al., 2010) through the addition of species and a broader examination of fish size classes. Stable sulfur isotope (δ^{34}S) measurements were also used to track food sources, namely sulfate derived from the water column or detritus and sediment-based foods (Croisetiére et al., 2009; Wayland and Hobson, 2001). It was hypothesized that some elements, such as Hg, would be strongly correlated with δ^{15}N, and therefore TP, while others might be related to a species' characteristically pelagic, benthic or detrital feeding habits (δ^{13}C, δ^{34}S).

3.2 Methods

3.2.1 Fish Collections

Fish specimens were collected from Lake Winnipeg during the spring (April-May), summer (June-August) and fall (September-November) of 2009 and 2010. Fish were also collected from the lake's south basin during the winter of 2009-2010. A total of sixteen fish species with variable FLs (mm) were obtained from beam trawls and commercial gillnets (Table A1; Appendix). Beam trawls, which were operated by Manitoba Fisheries personnel, were towed along-side the *MV Namao* research vessel for 30-minute intervals and at a speed of 3.9 km/h (Lumb et al., 2011). Each station in Figure 3.1 (closed circles) represents the ship's position at the end of each trawl. Each of these stations was trawled three times (spring, summer and fall) per year. Commercial gillnets were used during winter of 2009-2010 (south basin only; Figure 3.1; closed squares) and the ice-free season (spring, summer and fall; Figure 3.1; closed triangles) of 2010 only. Gillnets were set in the evening and the catch was removed within eight hours (Johnston et al., 2010). Small specimens were frozen whole; however, for larger

specimens, one or both fillets were removed and placed in polyethylene bags (Bodaly et al., 2007; Hobson et al., 2010). All samples were held at -20°C until they could be processed at the National Hydrology Research Centre (NHRC) laboratory in Saskatoon, Canada.

3.2.2 Sample Dissection

Fish samples were partially thawed and dissected in batches. White muscle was carefully excised from each of the small, whole specimens (Fry, 1988). For the fillet-style samples, a stainless steel scalpel was used to make clean cuts into the dorsal muscle and extract 5 to 10 g of material (Pruell et al., 2003). A subsample of muscle from each specimen was reserved for isotopic assays. Portions of muscle from randomly-selected specimens were also set aside for elemental and/ or Hg analyses.

3.2.3 Isotopic Analyses

The subsamples of fish muscle reserved for isotopic analyses were dried at 60°C and homogenized (Vander Zanden et al., 2005). Nitrogen (δ^{15}N), carbon (δ^{13}C) and sulfur (δ^{34}S) isotope ratios were quantified for separate aliquots of fish tissue (see below). Tissues used in δ^{13}C analyses were lipid extracted with a 2:1 chloroform: methanol solution (Bligh and Dyer, 1959). Since lipid extraction is known to inadvertently remove muscle proteins, thereby confounding the results of δ^{15}N assays (Sotiropoulos et al., 2004), and the effects of extraction on δ^{34}S have not been characterized, tissues used in δ^{15}N and δ^{34}S analyses were not lipid extracted (Hobson et al., 2010).

One (± 0.01) milligram each of untreated (δ^{15}N) and lipid-extracted muscle (δ^{13}C) were weighed into tin capsules for nitrogen and carbon isotope analyses, respectively (Vander Zanden et al., 2005). Sulfur isotope measurements were conducted on 10.00 ± 0.10 mg of dried muscle. All stable isotope ratios (^{15}N/^{14}N, ^{13}C/^{12}C and ^{34}S/^{32}S) were quantified via CF-IRMS (Wayland and Hobson, 2001). Laboratory standards, including BWB (δ^{15}N = +14.4 ‰, δ^{13}C = -18.5 ‰,

$\delta^{34}S$ = +17.5 ‰), PUGEL ($\delta^{15}N$ = +5.6 ‰, $\delta^{13}C$ = -12.6 ‰) and Chicken Feather Standard (CFS; $\delta^{34}S$ = -3.8 ‰), were analyzed along with the fish muscle. Analytical precision was within 0.3 ‰.

All results for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ were expressed in per mil (‰) relative to AIR, VPDB and VCDT, respectively, where:

$$\delta X = (R_A/R_S\text{-}1) \cdot 1000, \quad (3.1)$$

X was one of ^{15}N, ^{34}S or ^{13}C, R_A was the ratio of the heavy to light isotope (example: $^{13}C/^{12}C$) in the sample and R_S was the ratio of AIR, VPDB or VCDT (Fry, 1988).

3.2.4 Elemental Scan

Fish muscle samples reserved for Al, As, Cd, Cu, Fe, Mn and Se analyses were dried at 60°C and homogenized (Vander Zanden et al., 2005). Samples were digested in high purity nitric acid (HNO_3) and 30 % v/v analytical grade hydrogen peroxide (H_2O_2) (Phibbs et al., 2011). Method blanks, DORM-2 (Dogfish Muscle Certified Reference Material for Trace Metals; National Research Council Canada; Ottawa, ON) and duplicate samples were included in each digestion batch at a ratio of one blank, reference and duplicate per every ten field samples. Elemental concentrations were quantified via Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the Toxicology Centre, University of Saskatchewan, Saskatoon, Canada (Phibbs et al., 2011). Acceptable elemental recoveries from DORM-2 and coefficients of variation (CV) on duplicate samples were ± 20 % and ± 15 %, respectively (Brumbaugh et al., 2005). Limits of detection (LoD) for Al, As, Cd, Cu, Fe, Mn and Se were better than 0.9 µg/g, $1.1 \cdot 10^{-3}$ µg/g, $1.8 \cdot 10^{-3}$ µg/g, $5.0 \cdot 10^{-3}$ µg/g, 0.1 µg/g, $1.3 \cdot 10^{-3}$ and $8.0 \cdot 10^{-4}$ µg/g DW, respectively.

3.2.5 Mercury

Fresh rather than dried fish muscle samples were microwave digested (US EPA, 1996) and analyzed for Hg_T (hereafter "Hg") via Cold-Vapor Atomic Fluorescence Spectrometry (CVAFS)

(Baker et al., 2004). Digestions and analyses were conducted at the Saskatchewan Research Council (SRC) Environmental Analytical Laboratory in Saskatoon, Canada. Blanks, reference materials and duplicates were analyzed along with the muscle samples. Recoveries of Hg from DORM-2 were between 85 and 100 %, except for two low recoveries of 75 % and 78 %. The CVs on duplicate samples were within 15 %, and the LoD was 0.01 µg/g FW. Since all other concentrations are presented on a DW basis, percent moisture data for each muscle sample (unpublished data, Environment Canada) was used to convert the FW Hg data to units of µg/g DW.

3.2.6 Statistical Analyses

Baseline $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of nutrients, producers and primary consumers differ between Lake Winnipeg's north and south basins (Chapter 2; Hobson et al., 2010). The spatial isotopic heterogeneity identified for zooplankton ($\delta^{15}N_{Zoop}$), dissolved sulfate ($\delta^{34}S_{DS}$) and DIC ($\delta^{13}C_{DIC}$) may confound the interpretation of isotopic data for higher consumers (Chapter 2; Post, 2002). In order to compensate for this variability, isotope ratios for fish muscle were normalized according to:

$$\delta X_{Corrected} = \delta X_{Fish} - \delta X_{Baseline} \quad (3.2)$$

where X was one of ^{15}N, ^{13}C or ^{34}S, and $\delta X_{Baseline}$ was the mean $\delta^{15}N_{Zoop}$ (north = +3.4 ± 1.9 ‰, n = 31; south = +9.6 ± 2.8 ‰, n = 27), $\delta^{13}C_{DIC}$ (north = -3.4 ± 2.0 ‰, n = 31; south = -8.4 ± 1.0 ‰, n = 30) or $\delta^{34}S_{DS}$ (north = -4.7 ± 3.1 ‰, n = 30; south = -8.2 ± 2.5 ‰, n =29) value for the basin where the fish was captured (Hobson et al., 2010; Post, 2002). Mean $\delta^{15}N_{Zoop}$, $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ values for each basin were used in Equation 3.2 in order to compensate for within-basin fish movements. The potential migration of fishes between the two basins was assumed to be negligible (Hobson et al., 2010). Details regarding the measurement of $\delta^{15}N_{Zoop}$, $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ in zooplankton, DIC and dissolved sulfate respectively, are presented in Chapter 2.

Trophic position (TP) was determined for each species within each basin, and was used to compare $\delta^{15}N$ data across systems. Estimates of TP were calculated from $\delta^{15}N$ values in fish muscle and zooplankton ($\delta^{15}N_{Zoop}$), where zooplankton was assumed to occupy a TP = 2 and all subsequent fish TPs were derived from:

$$TP_{Fish} = 2 + (\delta^{15}N_{Fish} - \delta^{15}N_{Zoop})/3.4\ ‰ \quad (3.3)$$

where $\delta^{15}N_{Fish}$ was the nitrogen isotope value for fish tissue and the dietary isotopic discrimination factor ($\Delta^{15}N$) at each trophic step was 3.4 ‰ (Gewurtz et al., 2006; Jardine et al., 2006).

A Wilcoxon rank sum test with a Bonferroni correction for multiple comparisons was used to determine whether isotopic data for north and south basin fishes came from the same distribution (Dalgaard, 2008; Hobson et al., 2010). A plot of species- and basin-specific $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values was used to examine fish community structure in terms of three-dimensional isotopic space. Concentrations of trace elements were log transformed prior to all statistical analyses (Campbell et al., 2005).

Linear regression models for describing trace element concentrations in fish muscle based on species (community-level only), dietary parameters ($\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$) and/ or fish size (FL) were evaluated according to Akaike's Information Criterion with a correction for small sample sizes (AIC_c) (Anderson, 2008; Mazerolle, 2006). Species was included as a parameter in the community-level models for each basin since contaminant uptake, metabolism, etc. within fishes is often species-dependent (Brumbaugh et al., 2005; Newman and Unger, 2003). Fork length (FL) was included as a potential explanatory factor in community and species-level models since changes in dietary requirements and contaminant bioaccumulation are often related to growth (Newman and Unger, 2003; Stewart and Watkinson, 2004). A null model was also included in

each model-ranking procedure (Irvine et al., 2009). A parsimonious approach to model-selection was taken, since the inclusion of all possible models and/ or models with a large number of parameters (*K*) often leads to unreliable results (Anderson, 2008). Here, the number of models considered in any given AIC_c analysis never exceeded the sample size (n) (Anderson, 2008). The "AICcmodavg" package in R (R Development Core Team, 2011) was used to identify those models which provided the "best" approximation of reality, given the available data (Burnham and Anderson, 2004). Each of the potential models was evaluated based on its ΔAIC_c value, as well as its Akaike weight (ω_i) (Anderson, 2008). Models with $\Delta AIC_c \leq 2.00$ and the top-ranking model ($\Delta AIC_c = 0.00$) were thought to have similar support; however, models with $\Delta AIC_c \geq 10.00$ were considered poor, and therefore unsupported (Mazerolle, 2006; Symonds and Moussalli, 2011). If the top-ranking model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used to identify which parameters elicited a strong effect on the trace element concentration of interest (Burnham and Anderson, 2002). Ranked models which had Akaike weights adding to ≥ 0.95 were included in the model-averaging procedures (Symonds and Moussalli, 2011). A given parameter was identified as having a strong effect on Al, As, Cd, Cu, Fe, Mn, Hg or Se if the 95 % unconditional confidence interval (uCI) around the model-average estimate (MAE) excluded zero (Pluess et al., 2011).

Statistical analyses were conducted in R Version 2.14.1 (R Development Core Team, 2011) and the food web plot was prepared in Grapher Version 9 (Golden Software Inc.; Golden, Colorado).

3.3 Results

3.3.1 Nitrogen Isotopes

Most fishes from the north basin were ^{15}N-enriched relative to their south basin counterparts (Bonferroni, $p \leq 0.05$; Table 3.1, Figure 3.2). North basin sauger ($\delta^{15}N = +10.4 \pm 1.4$ ‰, n = 34)

Table 3.1 Mean ± standard deviation (SD) δ^{15}N, trophic position (TP), δ^{13}C and δ^{34}S for the fishes of Lake Winnipeg's north and south basins. Stable isotope data for muscle is baseline corrected (Equation 3.2). Isotopic units are per mil (‰).

Species	Code		Basin	δ^{15}N	TP	δ^{13}C	δ^{34}S
Burbot	BURB	NA	North	NA	NA	NA	NA
(*Lota lota*)		n = 5	South	6.7 ± 2.2	4.0 ± 0.7	-18.1 ± 0.9	-0.7 ± 1.3
Cisco	CISC	n = 101	North	8.1 ± 1.8	4.4 ± 0.5	-23.1 ± 1.5	-2.1 ± 1.5
(*Coregonus artedi*)		n = 169	South	4.0 ± 1.7	3.2 ± 0.5	-19.1 ± 1.4	0.4 ± 1.5
Emerald Shiner	EMSH	n = 92	North	7.7 ± 1.4	4.3 ± 0.4	-23.4 ± 1.3	-2.7 ± 1.5
(*Notropis atherinoides*)		n = 60	South	3.7 ± 1.5	3.1 ± 0.4	-19.5 ± 0.9	0.0 ± 1.1
Freshwater Drum	FRDR	n = 13	North	7.9 ± 1.0	4.3 ± 0.3	-22.6 ± 0.6	-3.6 ± 1.5
(*Aplodinotus grunniens*)		n = 64	South	4.9 ± 1.7	3.4 ± 0.5	-18.8 ± 1.1	-0.7 ± 2.2
Goldeye	GOLD	NA	North	NA	NA	NA	NA
(*Hiodon alosoides*)		n = 120	South	5.7 ± 1.4	3.7 ± 0.4	-19.8 ± 1.0	-0.1 ± 1.7
Lake Whitefish	LKWF	n = 36	North	8.0 ± 1.6	4.4 ± 0.5	-20.4 ± 1.6	-1.8 ± 1.7
(*Coregonus clupeaformis*)		NA	South	NA	NA	NA	NA
Longnose Sucker	LNSC	n = 7	North	6.7 ± 1.0	4.0 ± 0.3	-21.0 ± 0.8	-2.8 ± 1.4
(*Catostomus catostomus*)		n = 4	South	5.4 ± 0.8	3.6 ± 0.2	-19.1 ± 0.4	-2.7 ± 1.4
Mooneye	MOON	NA	North	NA	NA	NA	NA
(*Hiodon tergisus*)		n = 2	South	5.8 ± 1.6	3.7 ± 0.5	-19.4 ± 0.1	-0.4 ± 0.6
Ninespine Stickleback	NNST	n = 18	North	7.1 ± 2.0	4.1 ± 0.6	-22.9 ± 1.2	0.4 ± 1.4
(*Pungitius pungitius*)		NA	South	NA	NA	NA	NA
Northern Pike	NRPK	n = 11	North	9.5 ± 1.2	4.8 ± 0.3	-21.8 ± 1.8	-0.7 ±1.3
(*Esox lucius*)		n = 20	South	6.8 ± 1.3	4.0 ± 0.4	-18.8 ± 0.7	-0.9 ± 1.4
Rainbow Smelt	RNSM	n = 241	North	8.2 ± 1.3	4.4 ± 0.4	-22.2 ± 0.8	-0.9 ± 1.4
(*Osmerus mordax*)		n = 26	South	5.6 ± 1.9	3.7 ± 0.5	-19.6 ± 0.5	1.7 ± 2.0
Sauger	SAUG	n = 34	North	10.4 ± 1.4	5.0 ± 0.6	-21.9 ± 1.2	-2.6 ± 1.5
(*Sander canadensis*)		n = 111	South	6.5 ± 1.4	3.9 ± 0.4	-17.9 ± 1.6	-0.3 ± 1.5
Troutperch	TRPR	n = 20	North	8.0 ± 0.9	4.4 ± 0.3	-22.1 ± 1.2	-5.3 ± 1.3
(*Percopsis omiscomaycus*)		n = 31	South	4.9 ± 1.2	3.4 ± 0.3	-19.6 ± 0.4	-1.7 ± 2.0
Walleye	WALL	n = 124	North	10.2 ± 1.3	5.0 ± 0.4	-21.5 ± 1.1	-0.7 ± 1.4
(*Sander vitreus*)		n = 152	South	5.6 ± 1.8	3.6 ± 0.5	-17.4 ± 1.6	0.5 ± 2.1
White Bass	WHBS	n = 18	North	8.2 ± 2.2	4.4 ± 0.6	-23.5 ± 1.5	-2.1 ± 1.8
(*Morone chrysops*)		n = 90	South	7.1 ± 1.6	4.1 ± 0.5	-19.3 ± 1.0	0.1 ± 1.7
White Sucker	WHSC	n = 55	North	6.8 ± 1.2	4.0 ± 0.4	-21.4 ± 1.5	-1.0 ± 1.7
(*Catostomus commersonii*)		n = 8	South	4.1 ± 1.6	3.2 ± 0.5	-18.5 ± 1.5	0.7 ± 2.3
Yellow Perch	YLPR	n = 29	North	9.8 ± 2.3	4.9 ± 0.7	-21.4 ± 1.4	-2.4 ± 1.4
(*Perca flavescens*)		n = 56	South	4.9 ± 1.4	3.4 ± 0.4	-18.7 ± 1.4	-0.8 ± 1.7

NA = not available/ not applicable

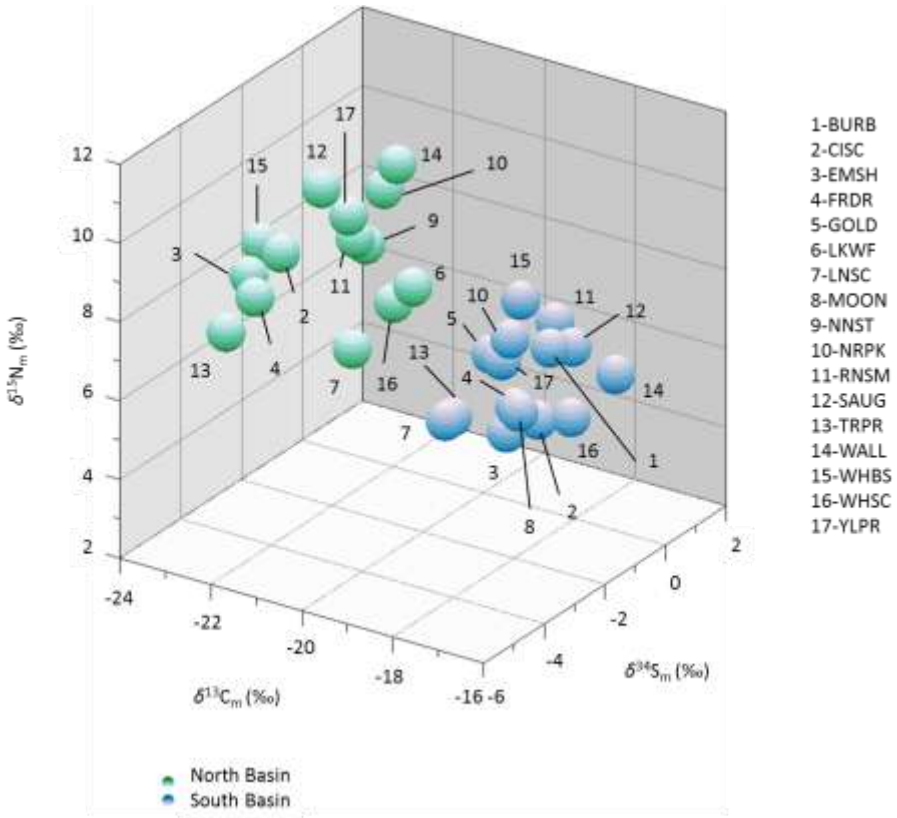


Figure 3.2 Isotopic (δ^{15}N, δ^{13}C and δ^{34}S) food web structure for Lake Winnipeg fishes. Each point represents the mean δ^{15}N, δ^{13}C and δ^{34}S value of a given species within either the north or south basin. Mean ± standard deviation (SD) isotope values can be found in Table 3.1.Species codes: BURB = burbot (*Lota lota*); CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); LNSC = longnose sucker (*Catostomus catostomus*); MOON = mooneye (*Hiodon tergisus*); NNST = ninespine stickleback (*Pungitius pungitius*); NRPK = northern pike (*Esox lucius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*).

and walleye ($\delta^{15}N$ = +10.2 ± 1.3 ‰, n = 124) had similar $\delta^{15}N$ values (Bonferroni, $p \leq 0.05$) and occupied the highest trophic positions (TP_{SAUG} = 5.0 ± 0.6; TP_{WALL} = 5.0 ± 0.4) of any species. Within the south basin fish community, white bass ($\delta^{15}N$ = +7.1 ± 1.6 ‰, n = 90), northern pike ($\delta^{15}N$ = +6.8 ± 1.3 ‰, n = 20) and burbot (BURB; *Lota lota*; $\delta^{15}N$ = +6.7 ± 2.2 ‰, n = 5) occupied the top TPs (TP_{WHBS} = 4.1 ± 0.5; TP_{NRPK} = 4.0 ± 0.4; TP_{BURB} = 4.0 ± 0.7). Longnose sucker (LNSC; *Catostomus catostomus*) were the only species for which $\delta^{15}N$ did not differ across basins ($\delta^{15}N_{North}$ = +6.7 ± 1.0 ‰, n = 7; $\delta^{15}N_{South}$ = +5.4 ± 0.8 ‰, n = 4; p = 0.07). Although they occupied the lowest TP (TP_{LNSC} = 4.0 ± 0.3) of all north basin fishes, longnose sucker had greater $\delta^{15}N$ values than many south basin benthivores and planktivores (Table 3.1; Stewart and Watkinson, 2004). South basin emerald shiner (EMSH; *Notropis atherinoides*; $\delta^{15}N$ = +3.7 ± 1.5 ‰, n = 60) and ciscoes (CISC; *Coregonus artedi*; $\delta^{15}N$ = +4.0 ± 1.7 ‰, n = 169) occupied the lowest TPs identified for all Lake Winnipeg fishes (TP_{EMSH} = 3.1 ± 0.4; TP_{CISC} = 3.2 ± 0.5; Table 3.1, Figure 3.2).

3.3.2 Carbon Isotopes

Dorsal muscle samples from all north basin fishes were significantly depleted in ^{13}C relative to south basin samples (Bonferroni, $p \leq 0.05$; Table 3.1, Figure 3.2). North basin white bass ($\delta^{13}C$ = -23.5 ± 1.5 ‰, n = 18) had the smallest $\delta^{13}C$ values of all species, which would indicate they were the most pelagic fishes in either basin (France, 1995). Carbon isotope values for north basin emerald shiner were also among the smallest ($\delta^{13}C$ = -23.4 ± 1.3 ‰, n = 92). For longnose sucker ($\delta^{13}C$ = -21.0 ± 0.8 ‰, n = 7) and white sucker (WHSC; *Catostomus commersonii*; $\delta^{13}C$ = -21.4 ± 1.5 ‰, n = 55) collected from the north basin, carbon isotope ratios in muscle were representative of relatively benthivorous feeding strategies (Stewart and Watkinson, 2004). Lake whitefish (LKWF; *Coregonus clupeaformis*) also had $\delta^{13}C$ profiles which fell toward the benthic

end of the $\delta^{13}C$ spectrum ($\delta^{13}C$ = -20.4 ± 1.6 ‰, n = 36); however their marginally higher TP (Table 3.1) may have been indicative of some piscivory.

The distribution of south basin fishes along the pelagic-benthic $\delta^{13}C$ gradient was different from that of the north basin. Goldeye (GOLD; *Hiodon alosoides*; $\delta^{13}C$ = -19.8 ± 1.0 ‰, n = 120), rather than white bass or emerald shiner, had the smallest $\delta^{13}C$ values of any south basin species (Table 3.1). Rainbow smelt ($\delta^{13}C$ = -19.6 ± 0.5 ‰, n = 26) and troutperch (TRPR; *Percopsis omiscomaycus*; $\delta^{13}C$ = -19.6 ± 0.4 ‰, n = 31) were also among the species with the smallest $\delta^{13}C$ values. Walleye ($\delta^{13}C$ = -17.4 ± 1.6 ‰, n = 152) collected from the south basin had the greatest $\delta^{13}C$ values of all species (Table 3.1, Figure 3.2), however values for south basin sauger ($\delta^{13}C$ = -17.9 ± 1.6 ‰, n = 111) and burbot ($\delta^{13}C$ = -18.1 ± 0.9 ‰, n = 5) were also among the largest.

3.3.3 Sulfur Isotopes

Stable sulfur isotope values for all but three of the sixteen fish species were significantly lower in the north versus south basin (Bonferroni, $p \leq 0.05$; Table 3.1, Figure 3.2). Longnose sucker from each basin were similar in terms of $\delta^{34}S$ ($\delta^{34}S_{north}$ = -2.8 ± 1.4 ‰, n = 7; $\delta^{34}S_{south}$ = -2.7 ± 1.4 ‰, n = 4; p = 0.93), as were northern pike ($\delta^{34}S_{north}$ = -0.7 ± 1.3 ‰, n = 11; $\delta^{34}S_{south}$ = -0.9 ± 1.4 ‰, n = 20; p = 0.47) and white sucker ($\delta^{34}S_{north}$ = -1.0 ± 1.7 ‰, n = 55; $_{\delta 34Ssouth}$ = +0.7 ± 2.3 ‰, n = 8; p = 0.06). The overlap in $\delta^{34}S$ values ($\delta^{34}S_{overlap} \approx 8.0$ ‰) between the north and south basin fish communities was much greater than that of $\delta^{13}C$ ($\delta^{13}C_{overlap} \approx 4.5$ ‰).

Troutperch collected from the lake's north basin had the smallest $\delta^{34}S$ values of all species ($\delta^{34}S$ = -5.3 ± 1.3 ‰, n = 20), which would indicate that this group was most reliant on detrital sources of sulfate (Croisetiére et al., 2009). Troutperch from the south basin ($\delta^{34}S$ = -1.7 ± 2.0 ‰, n = 31) also had much smaller $\delta^{34}S$ values than other south basin fishes, except for longnose sucker (Table 3.1). The greatest mean sulfur isotope value for north basin fishes was that of ninespine sticklebacks (NNST; *Pungitius pungitius*; $\delta^{34}S$ = +0.4 ± 1.4 ‰, n = 18); however

values for northern pike ($\delta^{34}S$ = -0.7 ± 1.3 ‰, n = 11), walleye ($\delta^{34}S$ = -0.7 ± 1.4 ‰, n = 124), rainbow smelt ($\delta^{34}S$ = -0.9 ± 1.4 ‰; n = 241) and white sucker ($\delta^{34}S$ = -1.0 ± 1.7 ‰, n = 55) were statistically similar (Bonferroni, $p \leq 0.05$). These species were most likely to have rejected detrital sources of sulfate (see below) (Ahlgren, 1996; Croisetiére et al., 2009). Rainbow smelt ($\delta^{34}S$ = 1.7 ± 2.0 ‰, n = 26) had the largest $\delta^{34}S$ values of all south basin fishes (Table 3.1, Figure 3.2).

3.3.4 Trace Element Concentrations

Of all the elements measured in fish muscle, Fe was found at the greatest concentrations, and ranged from 3.06 to 60.43 µg/g DW in south basin walleye and goldeye, respectively. Concentrations of Al were consistently below the LoD, except in one north basin rainbow smelt (Table 3.2; 0.77 µg/g DW, n = 15). Therefore, no models for Al were evaluated. Three and six of the Hg concentrations measured in north basin ciscoes (n = 28) and rainbow smelt (n = 26), respectively, were below the LoD. A single south basin yellow perch (n = 9) also fell below the Hg LoD. These specimens were assigned Hg concentrations equal to LoD/2. All concentrations of As, Cd, Fe, Mn and Se were above their respective LoDs.

3.3.5 Contaminant Trophodynamics

3.3.5.1 Arsenic

Species had the greatest influence on As concentrations within the north basin fish community ($\Delta AIC_c = 0.00$, $\omega_i = 1.00$; Table 3.3). Arsenic concentrations in the muscle of north basin rainbow smelt were significantly greater than concentrations for all other fishes (Bonferroni, $p \leq 0.05$; Table 3.2). The relatively low concentrations of As in walleye and other fishes which likely fed on smelt may have been attributable to poor dietary assimilation or biodilution (Newman and Unger, 2003).

Table 3.2 Mean ± standard deviation (SD) concentrations of Al, As, Cd, Cu, Fe, Mn, Hg and Se measured in white muscle of Lake Winnipeg fishes. Concentrations are expressed in units of µg/g dry weight (DW) and the numbers of samples (n) for each element are included in brackets ().

Code[a]	Basin	Al	As	Cd	Cu
CISC	North	< LoD (13)	0.34 ± 0.12 (16)	$0.04 \pm 3.9 \cdot 10^{-3}$ (7)	0.98 ± 0.35 (21)
	South	< LoD (20)	0.39 ± 0.23 (20)	0.05 ± 0.02 (9)	0.94 ± 0.42 (21)
EMSH	North	< LoD (9)	0.24 ± 0.10 (9)	0.08 ± 0.01 (5)	1.58 ± 0.54 (10)
	South	< LoD (7)	0.26 ± 0.09 (8)	0.07 ± 0.02 (4)	1.73 ± 0.51 (9)
FRDR	North	< LoD (5)	0.49 ± 0.09 (2)	$0.09 \pm 6.0 \cdot 10^{-4}$ (2)	0.45 ± 0.09 (4)
	South	< LoD (7)	0.39 ± 0.16 (3)	0.06 ± 0.03 (3)	0.60 ± 0.32 (6)
GOLD	North	NA	NA	NA	NA
	South	< LoD (20)	0.22 ± 0.13 (18)	0.05 ± 0.01 (15)	1.03 ± 0.58 (20)
LKWF	North	< LoD (9)	0.57 ± 0.32 (4)	0.04 ± 0.01 (5)	0.59 ± 0.33 (8)
	South	NA	NA	NA	NA
LNSC	North	< LoD (4)	0.51 ± 0.22 (4)	0.08 ± 0.01 (4)	1.19 ± 0.44 (4)
	South	NA	NA	NA	NA
NRPK	North	< LoD (9)	0.22 ± 0.02 (5)	$0.08 \pm 4.7 \cdot 10^{-3}$ (2)	0.54 ± 0.30 (6)
	South	< LoD (8)	0.08 ± 0.07 (6)	0.03 (1)	0.51 ± 0.30 (3)
RNSM	North	0.77[b] (15)	0.74 ± 0.14 (21)	0.09 ± 0.04 (10)	1.03 ± 0.31 (24)
	South	NA	NA	NA	NA
SAUG	North	< LoD (7)	0.25 ± 0.04 (5)	NA	0.23 ± 0.12 (2)
	South	< LoD (15)	0.22 ± 0.07 (12)	0.06 ± 0.03 (2)	0.46 ± 0.36 (7)
WALL	North	< LoD (15)	0.45 ± 0.29 (10)	0.11 ± 0.02 (2)	0.58 ± 0.41 (8)
	South	< LoD (19)	0.35 ± 0.13 (14)	0.07 ± 0.05 (2)	0.52 ± 0.40 (9)
WHBS	North	< LoD (3)	0.34 ± 0.04 (2)	0.09 ± 0.01 (2)	0.73 ± 0.12 (3)
	South	< LoD (15)	0.28 ± 0.11 (12)	0.07 ± 0.02 (8)	0.77 ± 0.43 (10)
WHSC	North	< LoD (11)	0.52 ± 0.22 (2)	$0.06 \pm 2.8 \cdot 10^{-3}$ (2)	0.52 ± 0.18 (11)
	South	< LoD (7)	0.39 ± 0.21 (6)	0.05 ± 0.01 (7)	0.86 ± 0.32 (7)
YLPR	North	< LoD (8)	0.07 ± 0.02 (5)	0.05 ± 0.01 (3)	0.35 ± 0.13 (6)
	South	< LoD (12)	0.06 (1)	0.04 (1)	0.26 ± 0.10 (11)

Table 3.2 Continued.

Code[a]	Basin	Fe	Mn	Hg	Se
CISC	North	22.80 ± 12.65 (2)	1.26 (1)	0.13 ± 0.09 (28)	1.27 ± 0.19 (22)
	South	15.41 ± 5.75 (5)	1.08 ± 0.55 (10)	0.20 ± 0.12 (36)	1.29 ± 0.28 (24)
EMSH	North	14.78 ± 8.43 (4)	1.86 ± 0.50 (4)	0.62 ± 0.32 (2)[c]	1.93 ± 0.37 (10)
	South	14.18 ± 1.00 (3)	1.83 ± 0.75 (3)	0.29 ± 0.21 (3)[c]	1.76 ± 0.40 (9)
FRDR	North	NA	0.76 ± 0.15 (5)	NA	2.11 ± 0.21 (5)
	South	13.22 (1)	0.67 ± 0.27 (6)	NA	2.15 ± 0.50 (6)
GOLD	North	NA	NA	NA	NA
	South	25.26 ± 15.11 (10)	0.78 ± 0.31 (15)	0.30 ± 0.15 (23)	2.36 ± 0.62 (24)
LKWF	North	8.95 ± 3.20 (3)	0.95 ± 0.52 (7)	NA	1.45 ± 0.27 (8)
	South	NA	NA	NA	NA
LNSC	North	12.22 ± 2.72 (2)	1.16 ± 0.34 (4)	NA	1.43 ± 0.29 (4)
	South	NA	NA	NA	NA
NRPK	North	8.58 ± 2.83 (3)	1.15 ± 0.64 (6)	0.69 ± 0.43 (5)	1.30 ± 0.36 (9)
	South	8.32 (1)	0.69 ± 0.62 (8)	1.16 ± 0.50 (7)	1.06 ± 0.15 (7)
RNSM	North	11.16 ± 4.52 (7)	0.91 ± 0.03 (7)	0.09 ± 0.07 (26)	1.17 ± 0.21 (24)
	South	NA	NA	NA	NA
SAUG	North	7.48 ± 3.60 (5)	0.42 ± 0.13 (2)	0.97 ± 0.42 (8)	1.25 ± 0.37 (7)
	South	11.09 ± 6.12 (5)	0.40 ± 0.17 (11)	1.10 ± 0.60 (19)	1.49 ± 0.88 (16)
WALL	North	5.66 ± 2.22 (5)	0.46 ± 0.24 (10)	0.78 ± 0.82 (10)	1.23 ± 0.31 (12)
	South	4.36 ± 0.87 (4)	0.43 ± 0.16 (14)	0.76 ± 0.77 (41)	1.29 ± 0.33 (16)
WHBS	North	NA	0.39 ± 0.04 (3)	0.54 (1)	6.55 ± 2.72 (3)
	South	11.86 (1)	0.45 ± 0.13 (14)	1.01 ± 0.37 (11)	2.89 ± 0.51 (15)
WHSC	North	10.23 (1)	1.00 ± 0.64 (11)	0.27 ± 0.13 (7)	1.70 ± 0.47 (11)
	South	11.52 ± 7.74 (6)	1.93 ± 1.28 (6)	0.74 ± 0.50 (5)	1.62 ± 0.56 (7)
YLPR	North	6.17 ± 3.49 (5)	0.94 ± 0.41 (6)	0.87 ± 0.66 (9)	1.62 ± 0.36 (8)
	South	3.57 (1)	0.77 ± 0.17 (10)	0.62 ± 0.41 (9)	2.49 ± 0.35 (12)

[a]CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); LNSC = longnose sucker (*Catostomus catostomus*); NRPK = northern pike (*Esox lucius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

[b]n = 14 are < LoD; value is for the only specimen that was above the LoD

[c]each sample is a composite of two EMSH specimens which were of similar FL and collected from the same station

NA = not available / not applicable

Table 3.3 Top-ranking linear models for describing trace element concentrations in the north and south basin fish communities of Lake Winnipeg. The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Trace element concentrations were log transformed.

Element	Model	*K*	AIC_c	ΔAIC	ω_i
Arsenic	*North Basin*				
	Species	13	98.09	0.00	1.00
	South Basin				
	$\delta^{15}N + \delta^{13}C$	4	270.21	0.00	0.83
Cadmium	*North Basin*				
	Species	12	31.55	0.00	1.00
	South Basin				
	$\delta^{15}N + \delta^{13}C$	4	40.70	0.00	0.41
	$\delta^{15}N$	3	42.10	1.41	0.20
	$\delta^{13}C$	3	42.57	1.87	0.16
Copper	*North Basin*				
	Species	13	130.42	0.00	0.99
	South Basin				
	$\delta^{15}N + \delta^{13}C$	4	145.45	0.00	1.00
Iron	*North Basin*				
	$\delta^{15}N + \delta^{13}C$	4	57.76	0.00	0.38
	South Basin				
	$\delta^{15}N + \delta^{13}C$	4	68.20	0.00	0.31
	$FL + \delta^{13}C$	4	68.27	0.06	0.30
	$\delta^{13}C$	3	69.38	1.17	0.17
Manganese	*North Basin*				
	Species	13	106.33	0.00	0.78
	South Basin				
	$\delta^{34}S + \delta^{13}C$	4	140.85	0.00	0.96
Mercury	*North Basin*				
	Species	11	223.76	0.00	1.00
	South Basin				
	$\delta^{34}S + \delta^{13}C$	4	108.95	0.00	1.00
Selenium	*North Basin*				
	Species	13	-18.02	0.00	1.00
	South Basin				
	Species	12	27.46	0.00	1.00

Table 3.4 Top-ranking linear models for describing As concentrations in fishes based on $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and/ or fork length (FL). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Arsenic data was log transformed.

Model	*K*	AIC_c	ΔAIC	ω_i
North Basin CISC				
FL + $\delta^{34}S$	4	-42.93	0.00	1.00
South Basin CISC				
$\delta^{34}S$	3	19.35	0.00	1.00
North Basin EMSH				
Null model	2	13.48	0.00	0.78
South Basin EMSH				
Null model	2	12.86	0.00	0.76
South Basin GOLD				
$\delta^{34}S$	3	7.17	0.00	1.00
North Basin NRPK				
Null model	2	1.40	0.00	1.00
North Basin NRPK				
Null model	2	27.28	0.00	0.98
North Basin RNSM				
Null model	2	-6.37	0.00	0.42
$\delta^{15}N$	3	-4.91	1.45	0.20
North Basin SAUG				
Null model	2	6.84	0.00	1.00
South Basin SAUG				
$\delta^{34}S$	3	8.30	0.00	0.70
North Basin WALL				
FL	3	14.54	0.00	0.54
Null model	2	16.20	1.67	0.24
South Basin WALL				
$\delta^{15}N$	3	10.99	0.00	0.65
$\delta^{34}S$	3	12.78	1.79	0.27
South Basin WHBS				
FL	3	10.92	0.00	0.79
South Basin WHSC				
Null model	2	18.82	0.00	0.86
North Basin YLPR				
Null model	2	10.07	0.00	1.00

species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); GOLD = goldeye (*Hiodon alosoides*); NRPK = northern pike (*Esox lucius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

When all species from the south basin were examined together (see Table 3.4 for list of species), δ^{15}N and δ^{13}C had the greatest influence on As ($\Delta AIC_c = 0.00$, $\omega_i = 1.00$; Table 3.3). The negative relationship between log(As) and δ^{15}N would suggest that "trophic dilution", or a decrease in concentration with increasing TP (Newman and Unger, 2003; Watanabe et al., 2008), was occurring within the south basin fish guild (MAE = -0.19, unconditional standard error (uSE) = 0.07, 95 % uCI = -0.33, -0.05). The effect of δ^{13}C on As concentrations in the muscle of south basin fishes was weak (MAE = 0.02, uSE = 0.10, 95 % uCI = -0.17, 0.21).

Of the species-specific As models evaluated, the null model often received the greatest support (Table 3.4). For northern pike, sauger and yellow perch collected from the north basin, the null model received 100 % of the support ($\omega_i = 1.00$; Table 3.4). The null model was also well-supported for south basin northern pike ($\omega_i = 0.98$). For north and south basin emerald shiner, the null models received a fair amount of support ($\Delta AIC_c = 0.00$, $\omega_i = 0.76$ to 0.78) and the 95 % uCIs around the MAEs for δ^{15}N, δ^{13}C, δ^{34}S and FL included zero. A similar phenomenon was identified for south basin white sucker, wherein the null model was ranked first ($\Delta AIC_c = 0.00$, $\omega_i = 0.86$) and no strong effects could be identified from the given parameters. Although the null model was ranked as the "best" As model ($\Delta AIC_c = 0.00$, $\omega_i = 0.42$) for north basin rainbow smelt, δ^{15}N was also considered, given its $\Delta AIC_c \leq 2.00$ (Table 3.4). However, model-averaging revealed that δ^{15}N did not have a strong effect on As concentrations in smelt muscle (MAE = -0.01, uSE = 0.03, 95 % uCI = -0.07, 0.05). Stable nitrogen isotope ratios did have a strong negative effect on As concentrations in south basin walleye (MAE = -0.15, uSE = 0.07, 95 % uCI = -0.28, -0.02). For every 1 ‰ increase in δ^{15}N, log(As) decreased by 0.15, indicating that trophic dilution was occurring within this group (Campbell et al., 2005).

Fork length and δ^{34}S dominated the remaining top-models for As; however, strong effects could only be identified in north basin ciscoes, and south basin sauger. Fork length had a strong negative influence on As concentrations in cisco muscle (MAE = -0.02, uSE = 0.00, 95 % uCI = -0.02,-0.02), as did δ^{34}S (MAE = -0.16, uSE = 0.06, 95 % uCI = -0.27, -0.05). Here, the negative average estimate for FL is likely indicative of growth dilution, and the approximately 15 % drop in muscle As with every 1 ‰ increase in δ^{34}S would suggest ciscoes which obtained dietary sulfate from within the water column accumulated less As than fishes feeding near the sediment. Stable sulfur isotope ratios in south basin sauger also had a negative effect on As concentrations in muscle (MAE = -0.06, uSE = 0.02, 95 % uCI = -0.10, -0.02). Although models containing δ^{34}S received 100 % of the available support for south basin ciscoes and goldeye, the influence of δ^{34}S was not strong (CISC: parameter estimate (PE) = -0.02, standard error (SE) = 0.08, 95 % confidence interval (CI) = -0.18, 0.14; GOLD: PE = -0.04, SE = 0.05, 95 % CI = -0.13, 0.05). Similarly, FL did not exert a strong effect on log(As) in north basin walleye (MAE = 0.00, uSE = 0, 95 % uCI = 0, 0) or south basin white bass (MAE = -0.01, uSE = 0.00, 95 % uCI = -0.01, 0.00), despite being amongst the top models.

3.3.5.2 Cadmium

Like As, Cd concentrations in muscle of north basin fishes were species-dependent (ΔAIC_c = 0.00, ω_i = 1.00; Table 3.3). Ciscoes and lake whitefish represented the north basin fishes with the lowest Cd concentrations in muscle. Rainbow smelt and walleye from the north basin accumulated significantly greater amounts of Cd (Bonferroni, $p \leq 0.05$). Concentrations in south basin fishes were quite similar among all species (Table 3.2). Despite their AIC_c ranking, δ^{15}N and δ^{13}C were not strong predictors of log(Cd) (δ^{15}N: MAE = 0.06, uSE = 0.03, 95 % uCI = 0.00, 0.12; δ^{13}C: MAE = 0.00, uSE = 0.05, 95 % uCI = -0.09, 0.09; Table 3.3) for south basin fishes.

The top-ranking model for Cd in north basin ciscoes was indicative of trophic dilution (Newman and Unger, 2003; Watanabe et al., 2008); however, no models containing δ^{15}N were strongly supported for the other groups of fishes examined (Table 3.5). Within north basin ciscoes, log(Cd) decreased by approximately 0.03 for every 1 ‰ increase in δ^{15}N (PE = -0.02, SE = 0.00, 95 % CI = -0.04, -0.02). Sulfur, rather than δ^{15}N, was found to have a strong effect on Cd concentrations in the muscle of south basin ciscoes (MAE = -0.13, uSE = 0.06, 95 % uCI = -0.25, -0.01). This negative effect would suggest that increased reliance on water-column sulfate (relatively large δ^{34}S) in south basin ciscoes was associated with decreased accumulation of Cd in tissues. For the remaining groups of fishes in Table 3.5, either the null model received the most support, or no strong effects (δ^{13}C and FL) could be identified, based on model-averaging across the 95 % confidence set.

3.3.5.3 Copper

The range of Cu concentrations for fishes in each basin were fairly similar (north: 0.12 to 2.29 µg/g DW; south: 0.12 to 2.82 µg/g DW) and few significant differences between paired species could be found (Bonferroni, $p \leq 0.05$). However, concentrations of Cu in emerald shiner from both basins were significantly greater than concentrations in many top-level consumers (Table 3.2). The elevated Cu concentrations in forage fishes rather than top predators were further explained by the top-ranking south basin community model (Table 3.3). Trophic dilution of Cu was occurring (δ^{15}N: PE: -0.15, SE = 0.03, 95 % CI = -0.36, -0.19) and δ^{13}C data suggested that feeding at higher TPs and/ or on increasingly benthic foods resulted in decreased Cu concentrations in muscle (PE = -0.27, SE = 0.04, 95 % CI = -0.36, -0.19). Species was the best-supported parameter at the community-level in the north basin ($\Delta AIC_c = 0.00$, $\omega_i = 0.99$; Table 3.3).

Table 3.5 Top-ranking linear models for describing Cd concentrations in fishes based on $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and/ or fork length (FL). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Cadmium data was log transformed.

Model	*K*	AIC_c	ΔAIC	ω_i
North Basin CISC				
$\delta^{15}N$	3	-10.10	0.00	0.91
South Basin CISC				
Null model	2	12.20	0.00	0.49
$\delta^{34}S$	3	12.66	0.46	0.39
North Basin EMSH				
Null model	2	5.30	0.00	1.00
South Basin GOLD				
Null model	2	0.23	0.00	0.41
FL	3	1.13	0.90	0.26
North Basin LKWF				
$\delta^{13}C$	3	-18.24	0.00	1.00
North Basin RNSM				
Null model	2	17.06	0.00	0.51
South Basin WHBS				
$\delta^{13}C$	3	7.83	0.00	0.94
South Basin WHSC				
Null model	2	8.73	0.00	0.89

Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); RNSM = rainbow smelt (*Osmerus mordax*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*)

Table 3.6 Top-ranking linear models for describing Cu concentrations in fishes based on $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and/ or fork length (FL). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Copper data was log transformed.

Model	***K***	**AIC_c**	**ΔAIC**	**ω_i**
North Basin CISC				
FL + $\delta^{34}S$	4	6.75	0.00	0.92
South Basin CISC				
FL + $\delta^{15}N$	4	7.47	0.00	0.94
North Basin EMSH				
$\delta^{34}S$	3	13.56	0.00	0.45
Null model	2	14.59	1.03	0.27
South Basin EMSH				
Null	2	7.02	0.00	0.47
$\delta^{15}N$	3	8.08	1.06	0.28
South Basin FRDR				
Null model	2	15.95	0.00	0.97
South Basin GOLD				
$\delta^{13}C$	3	21.06	0.00	0.92
North Basin LKWF				
Null model	2	18.23	0.00	0.59
$\delta^{34}S$	3	19.56	1.34	0.30
North Basin NRPK				
FL	3	15.13	0.00	0.39
$\delta^{13}C$	3	15.49	0.37	0.32
Null model	2	15.77	0.65	0.28
North Basin RNSM				
$\delta^{13}C$	3	7.35	0.00	0.55
$\delta^{34}S$	3	8.21	0.85	0.36
South Basin SAUG				
Null	2	21.65	0.00	0.81
North Basin WALL				
FL	3	12.74	0.00	0.89
South Basin WALL				
FL	3	16.40	0.00	0.89
South Basin WHBS				
FL + $\delta^{15}N$	4	16.96	0.00	0.37
Null	2	17.45	0.49	0.29

Table 3.6 Continued.

Model	*K*	AIC_c	ΔAIC	ω_i
North Basin WHSC				
Null	2	20.67	0.00	0.60
South Basin WHSC				
Null model	2	11.47	0.00	0.87
North Basin YLPR				
Null model	2	11.92	0.00	0.61
South Basin YLPR				
Null model	2	15.37	0.00	0.42
$\delta^{15}N$	3	15.92	0.55	0.32

Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); NRPK = northern pike (*Esox lucius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*).

For most of the North and South Basin fishes listed in Table 3.6, species-specific models containing one of $\delta^{15}N$, $\delta^{13}C$ or $\delta^{34}S$ were well-supported based on AIC_c. Support for FL as a tracer of Cu concentrations in fish was consistently weak, considering the 95 % CI around each MAE or PE included zero. For south basin ciscoes, neither FL (PE = 0.00, SE = 0.00, 95 % CI = -0.01, 0.00) or $\delta^{15}N$ (PE = 0.14, SE = 0.07, 95 % CI = -0.01, 0.28) exerted a strong influence on log(Cu). For white bass, a top predator within the south basin (Figure 3.2), a trophic dilution effect was observed. This group exhibited a strong decrease in log(Cu) with increasing $\delta^{15}N$ (MAE = -0.32, uSE = 0.13, 95 % uCI = -0.59, -0.06).

For emerald shiner, lake whitefish and rainbow smelt collected from the lake's north basin, $\delta^{34}S$-based models were ranked among those with $\Delta AIC_c \leq 2.00$; however their respective Akaike weights were relatively small (ω_i = 0.30 to 0.45; Table 3.6). Based on model-averaging, a strong positive relationship between log(Cu) and $\delta^{34}S$ was identified for emerald shiner (MAE = 0.11, uSE = 0.02, 95 % uCI = 0.02, 0.20) and lake whitefish (MAE = 0.30, uSE = 0.14, 95 % uCI = 0.01, 0.58), but not rainbow smelt (MAE = 0.08, uSE = 0.05, 95 % uCI = -0.02, 0.17).

Based on the model-averaging procedure for north basin walleye, $\delta^{13}C$ was identified as having a strong negative effect on log(Cu) in fish muscle (MAE = -0.26, uSE = 0.08, 95 % uCI = -0.41, -0.01). This would suggest that north basin walleye which were feeding at progressively higher TPs ($\Delta^{13}C \approx 1.0$ ‰) or increasingly benthic energy sources accumulated less Cu in muscle tissue. A similar trend was also identified for $\delta^{13}C$ in north basin northern pike (MAE = -0.20, uSE = 0.05, 95 % uCI = -0.30, -0.11) and south basin goldeye (MAE = -0.30, uSE = 0.08, 95 % uCI = -0.42, =0.01). North basin rainbow smelt and yellow perch followed the opposing trend, as a 1 ‰ increase in $\delta^{13}C$ was met with a concomitant 0.14 increase in log(Cu) for smelt (MAE =

0.14, uSE = 0.05, 95 % uCI = 0.04, 0.24) and a 0.23 increase for yellow perch (MAE = 0.23, uSE = 0.07, 95 % uCI = 0.09, 0.36).

3.3.5.4 Iron

Due to a great deal of intra-specific variability, very few significant differences in Fe concentration could be identified among species or basins. However, the distribution of Fe within each of the north and south basin fish communities was quite similar. Concentrations in walleye and yellow perch were the smallest of all the fishes examined, and were significantly lower than concentrations in ciscoes and goldeye from the north and south basins, respectively (Bonferroni, $p \leq 0.05$; Table 3.2). It is not surprising then, that the top-ranking model was the same for the each basin, and received similar support in the north ($\omega_i = 0.38$) and south ($\omega_i = 0.31$; Table 3.3). Model-averaging revealed that between the two basins, $\delta^{13}C$ in the south was the only strong parameter (MAE = -0.21, uSE = 0.09, 95 % uCI = -0.38, -0.03).

In ranking models for use in tracking Fe within species, the null model was consistently identified as having a $\Delta AIC_c \leq 2.00$ (Table 3.7). For south basin white sucker, FL and the null model had similar levels of support ($\omega_i = 0.51$ and $\omega_i = 0.47$, respectively). However, model-averaging revealed that FL did not have a strong effect on Fe concentrations in south basin white suckers (MAE = 0.01, uSE = 0.00, 95 % uCI = 0.00, 0.01). The influence of FL on log(Fe) in south basin goldeye was also weak (MAE = 0.00, uSE = 0.00, 95 % uCI = -0.01, 0.00); however, the relationship between log(Fe) and $\delta^{15}N$ was strong for this group (MAE = -0.22, uSE = 0.10, 95 % uCI = -0.42, -0.01). Rainbow smelt collected from the lake's north basin were the only group for which Fe concentrations in muscle could be linked to food source (Table 3.7). For every 1 ‰ increase in muscle $\delta^{13}C$, log(Fe) decreased by a factor of 0.37 (MAE = -0.37, uSE = 0.13, 95 % uCI = -0.63, -0.10).

Table 3.7 Top-ranking linear models for describing Fe concentrations in fishes based on $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and/or fork length (FL). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Iron data was log transformed.

Model	*K*	AIC_c	ΔAIC	ω_i
South Basin CISC				
Null model	2	34.51	0.00	1.00
South Basin GOLD				
$\delta^{15}N$	3	22.11	0.00	0.30
Null	2	22.12	0.01	0.30
FL	3	22.17	0.05	0.29
North Basin RNSM				
Null model	2	12.25	0.00	0.52
$\delta^{13}C$	3	12.92	0.67	0.37
North Basin SAUG				
Null model	2	15.32	0.00	0.99
South Basin SAUG				
Null model	2	15.04	0.00	1.00
North Basin WALL				
Null model	2	13.43	0.00	1.00
South Basin WHSC				
FL	3	16.24	0.00	0.51
Null	2	16.40	0.16	0.47
North Basin YLPR				
Null model	2	16.20	0.00	1.00

Species codes: CISC = cisco (*Coregonus artedi*); GOLD = goldeye (*Hiodon alosoides*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); WALL = walleye (*Sander vitreus*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*).

3.3.5.5 Manganese

Concentrations of Mn in fish muscle were the smallest for sauger, walleye and white bass (Table 3.2), and no significant differences were found with respect to basin for each of the three species (Bonferroni, $p \leq 0.05$). Concentrations of Mn in the muscle of south basin white sucker were significantly greater than those for walleye, sauger and white bass, and represented the largest values measured for Lake Winnipeg fishes. Although Mn concentrations were lowest in top predators, none of the top models in Table 3.3 were suggestive of strong biodilution effects in either basin. Species was the only supported parameter for the north basin fish guild. In the south basin, Mn concentrations were linked to food source (δ^{13}C and δ^{34}S), with δ^{13}C having a much stronger influence (PE = -0.13, SE = 0.05, 95 % CI = -0.23, -0.02) than δ^{34}S (PE = -0.02, SE = 0.04, 95 % CI = -0.10, 0.06).

The top-ranking species-specific models in Table 3.8 which contained δ^{15}N, δ^{13}C, δ^{34}S or FL were poorly supported relative to the null models, except for that of δ^{13}C + δ^{34}S in south basin goldeye. Model-averaging revealed that δ^{13}C had a strong positive influence on log(Mn) (MAE = 0.21, uSE = 0.09, 95 % uCI = 0.03, 0.38); however the influence of δ^{34}S was weak across the 95 % confidence set of models (MAE = -0.07, uSE = 0.08, 95 % uCI = -0.22, 0.08). For all other fishes, the effects of δ^{15}N, δ^{13}C, δ^{34}S or FL on Mn concentrations were approximately zero, based on their 95 % CIs.

3.3.5.6 Mercury

Mercury (Hg) concentrations in piscivorous fishes, such as sauger, walleye, white bass and northern pike were significantly greater than concentrations measured in forage fishes, such as ciscoes and rainbow smelt (Bonferroni, $p \leq 0.05$; Table 3.2). This would suggest that fish feeding at the top TPs within each of the basins accumulated a significant amount of Hg from their diets over time. However, species was once again the best-supported model for the north

Table 3.8 Top-ranking linear models for describing Mn concentrations in fishes based on $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and/ or fork length (FL). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Manganese data was log transformed.

Model	*K*	AIC_c	ΔAIC	ω_i
South Basin CISC				
Null model	2	19.51	0.00	0.40
$\delta^{15}N$	3	21.03	1.52	0.19
$\delta^{34}S$	3	21.08	1.57	0.18
North Basin FRDR				
Null model	2	5.71	0.00	0.99
South Basin FRDR				
Null model	2	15.37	0.00	0.65
FL	3	16.66	1.28	0.34
South Basin GOLD				
$\delta^{13}C + \delta^{34}S$	4	15.92	0.00	0.62
North Basin LKWF				
Null model	2	18.29	0.00	0.78
North Basin NRPK				
Null model	2	17.77	0.00	0.94
South Basin NRPK				
$\delta^{13}C$	3	8.10	0.00	1.00
North Basin RNSM				
Null model	2	10.34	0.00	0.86
South Basin SAUG				
$\delta^{15}N$	3	14.80	0.00	0.56
North Basin WALL				
FL	3	10.89	0.00	0.93
South Basin WALL				
Null model	2	15.78	0.00	0.59
South Basin WHBS				
FL	3	1.31	0.00	0.95
North Basin WHSC				
Null	2	23.91	0.00	0.65
South Basin WHSC				
Null model	2	18.26	0.00	0.87

Table 3.8 Continued.

Model	*K*	AIC_c	ΔAIC	ω_i
North Basin YLPR				
Null model	2	14.16	0.00	0.94
South Basin YLPR				
Null model	2	3.08	0.00	0.44
FL	3	3.32	0.24	0.39

Species codes: CISC = cisco (*Coregonus artedi*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); NRPK = northern pike (*Esox lucius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*).

basin fish community ($\Delta AIC_c = 0.00$, $\omega_i = 1.00$), and Hg concentrations in south basin fishes appear to be a function of food source ($\delta^{34}S$ and $\delta^{13}C$) rather than TP (Table 3.3). In the south, log(Hg) tended to increase by a factor of 0.20 for every 1 ‰ increase in $\delta^{13}C$ (SE = 0.08, 95 % CI = 0.06, 0.35) and decrease by a factor of 0.17 for every 1 ‰ increase in $\delta^{34}S$ (SE = 0.08, 95 % CI = -0.33, -0.01).

South basin walleye were the only group for which $\delta^{15}N$ elicited a strong positive effect on Hg concentrations in muscle (MAE = 0.27, uSE = 0.06, 95 % uCI = 0.15, 0.39; Table 3.9). The approximately 24 % increase in muscle Hg concentrations for every 1 ‰ increase in $\delta^{15}N$ would suggest Hg biomagnification was occurring within south basin walleye. North basin rainbow smelt were the only other group for which $\delta^{15}N$ received any support ($\Delta AIC_c = 0.00$, $\omega_i = 0.69$; Table 3.9); however, model-averaging revealed the influence of $\delta^{15}N$ on log($Hg_)$ was not sufficiently strong (MAE = 0.12, uSE= 0.12, 95 % uCI = -0.11, 0.36). Although models containing $\delta^{34}S$ had the lowest ΔAIC_c values (0.00) and largest Akaike weights ($\omega_i \geq 0.98$; Table 3.9) for north basin ciscoes and south basin goldeye, the 95 % uCI around the slope estimates included zero, indicating the effect was not strong (CISC: MAE = -1.89, uSE = 1.00, 95 % uCI = -3.87, 0.07; GOLD: MAE = 0.05, uSE = 0.05, 95 % uCI = -0.06, 0.16). Strong effects were observed for $\delta^{13}C$ in ciscoes, northern pike and yellow perch collected from the lake's south basin. For every 1 ‰ increase in muscle-$\delta^{13}C$, the value of log(Hg) would increase by a factor of approximately 0.18, 0.95 or 0.63 in ciscoes, northern pike and yellow perch, respectively (CISC: uSE = 0.04, 95 % uCI = 0.10, 0.26; NRPK: uSE = 0.38, 95 % uCI = 0.21, 1.69; YLPR: uSE = 0.10, 95 % uCI = 0.43, 0.83).

For north basin sauger, walleye and yellow perch, and south basin sauger, walleye and white bass, FL was included among the top-ranking models ($\Delta AIC_c \leq 2.00$; Table 3.9). For walleye

Table 3.9 Top-ranking linear models for describing total Hg (Hg_T) concentrations in fishes based on $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and/ or fork length (FL). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Mercury data was log transformed.

Model	*K*	AIC_c	ΔAIC	ω_i
North Basin CISC				
$\delta^{34}S$	3	34.51	0.00	1.00
South Basin CISC				
$\delta^{13}C$	4	42.63	0.00	0.89
South Basin GOLD				
$\delta^{34}S$	3	18.59	0.00	0.98
North Basin NRPK				
Null model	2	18.29	0.00	0.99
South Basin NRPK				
Null model	2	13.87	0.00	0.56
$\delta^{13}C$	3	15.11	1.23	0.30
North Basin RNSM				
$\delta^{15}N$	3	56.16	0.00	0.69
North Basin SAUG				
FL	3	18.69	0.00	0.47
Null model	2	18.80	0.11	0.45
South Basin SAUG				
FL	3	29.01	0.00	0.82
North Basin WALL				
FL	2	22.52	0.00	0.96
South Basin WALL				
FL + $\delta^{15}N$	4	33.43	0.00	1.00
South Basin WHBS				
FL	3	-7.07	0.00	0.99
North Basin WHSC				
Null model	2	15.88	0.00	0.68
South Basin WHSC				
Null model	2	19.30	0.00	1.00
North Basin YLPR				
FL	3	18.47	0.00	0.99
South Basin YLPR				
$\delta^{13}C$	3	21.71	0.00	0.98

Species codes: CISC = cisco (Coregonus artedi); GOLD = goldeye (Hiodon alosoides); NRPK = northern pike (Esox lucius); RNSM = rainbow smelt (Osmerus mordax); SAUG = sauger (Sander canadensis); WALL = walleye (Sander vitreus); WHBS = white bass (Morone chrysops); WHSC = white sucker (Catostomus commersonii); YLPR = yellow perch (Perca flavescens).

collected from the north basin and white bass collected from the south basin, FL had a similar, strong effect on log(Hg) (PE = 0.01, SE = 0.00, 95 % CI = 0.01, 0.02). This would suggest that for every 10 mm of growth in north basin walleye and south basin white bass, Hg concentrations in muscle would increase by approximately 10 %. A similar trend was identified for north basin yellow perch, however the PE was slightly larger, at 0.02 (uSE = 0.00, 95 % uCI = 0.01, 0.02). For all sauger and south basin walleye, the 95 % confidence limits around the MAEs (0.01, uSE = 0.00) and the PE (0.00, SE = 0), respectively, included zero.

3.3.5.7 Selenium

Concentrations of Se in the muscle of north basin white bass were significantly greater than concentrations measured in all other fishes from either basin (Bonferroni, $p \leq 0.05$; Table 3.2). Ciscoes, walleye and rainbow smelt from the north basin and ciscoes and northern pike from the south basin were among the fishes with the smallest muscle Se concentrations. Selenium concentrations in fishes from both basins appear to be species-specific, given that species was the top-ranking model ($AIC_c = 0.00$, $\omega_i = 1.00$) for both communities (Table 3.3).

Selenium biomagnification, as evidence by a strong positive relationship between log(Se) concentrations and $\delta^{15}N$ was identified for freshwater drum and walleye collected from the lake's south basin (Table 3.10). The rate of biomagnification was similar for both of these groups (FRDR: PE = 0.15, SE = 0.03; WALL: PE = 0.14, SE = 0.03). For north basin emerald shiner, an increased reliance on water-column versus sedimentary sources of sulfate led to greater accumulation of Se in muscle, given the strong positive effect of $\delta^{34}S$ (PE = 0.14, SE = 0.03, 95 % CI = 0.09, 0.19). Yellow perch which utilized carbon sources associated with the water-column in the north basin also had greater concentrations of Se in muscle than yellow perch which fed toward the benthic end of the spectrum (MAE = -0.11, uSE = 0.04, 95 % uCI = -0.18, -0.03). An opposing strong effect was observed in south basin goldeye, wherein fish with greater

Table 3.10 Top-ranking linear models for describing Se concentrations in fishes based on $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ and/ or fork length (FL). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Selenium data was log transformed.

Model	*K*	AIC_c	ΔAIC	ω_i
North Basin CISC				
$\delta^{13}C$	3	-18.18	0.00	0.41
Null model	2	-17.99	0.19	0.38
FL	3	-16.43	1.75	0.17
South Basin CISC				
Null model	2	-1.93	0.00	0.46
$\delta^{13}C$	3	-0.91	1.01	0.28
North Basin EMSH				
FL + $\delta^{34}S$	4	5.79	0.00	0.96
South Basin EMSH				
Null model	2	6.13	0.00	0.65
North Basin FRDR				
Null model	2	-0.29	0.00	1.00
South Basin FRDR				
$\delta^{15}N$	3	4.37	0.00	0.77
South Basin GOLD				
$\delta^{13}C$	3	-4.63	0.00	0.95
North Basin LKWF				
Null model	2	1.58	0.00	0.77
North Basin NRPK				
Null model	2	6.99	0.00	0.66
South Basin NRPK				
Null model	2	-2.04	0.00	0.94
North Basin RNSM				
$\delta^{13}C$	3	-11.21	0.00	0.56
Null model	2	-9.59	1.62	0.25
North Basin SAUG				
Null model	2	7.41	0.00	0.85
South Basin SAUG				
$\delta^{13}C$	3	-2.46	0.00	1.00
North Basin WALL				
Null model	2	3.40	0.00	0.62
South Basin WALL				
$\delta^{15}N$	3	-3.94	0.00	0.92

Table 3.10 Continued.

Model	*K*	AIC_c	ΔAIC	ω_i
South Basin WHBS				
$\delta^{15}N$	3	-7.07	0.00	0.64
North Basin WHSC				
Null model	2	6.48	0.00	0.47
$\delta^{15}N$	3	7.70	1.21	0.26
South Basin WHSC				
Null model	2	13.03	0.00	0.86
North Basin YLPR				
$\delta^{13}C$	3	1.21	0.00	0.51
Null model	2	2.45	1.24	0.27
South Basin YLPR				
Null model	2	-8.30	0.00	0.47
$\delta^{34}S$	3	-6.90	1.40	0.23

Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); NRPK = northern pike (*Esox lucius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*).

δ^{13}C values had greater Se concentrations in muscle (PE = 0.12, SE = 0.04, 95 % CI = 0.04, 0.20). Strong effects related to fish size (FL) could only be identified for north basin emerald shiner. Log(Se) concentrations in this group tended to decrease by a factor of 0.05 with every 1 mm increase in FL (SE = 0.01, 95 % CI = -0.07, -0.03).

3.4 Discussion

3.4.1 Food Web

The structures of Lake Winnipeg's north and south basin fish communities exhibit a bimodal pattern, similar to that first described in the Hobson et al. (2010) dual-isotope (δ^{15}N and δ^{13}C) baseline model. In comparing the TPs of yellow perch and northern pike from Lake Winnipeg to those inhabiting Sargent Lake, Isle Royale National Park, Michigan (Gorski et al., 2003), it was discovered that individuals from the south basin of Lake Winnipeg (TP_{YLPR} = 3.4 ± 0.4, n = 56; TP_{NRPK} = 4.0 ± 0.4; n = 20) occupied TPs similar to fishes from Sargent Lake (mean TP_{YLPR} = 3.7, n = 33; mean TP_{NRPK} = 4.3n = 25). North basin perch and pike however, occupied TPs which were much higher (TP_{YLPR} = 4.9 ± 0.7, n = 29; TP_{NRPK} = 4.8 ± 0.3, n = 11) than both south basin and Sargent Lake fishes. This comparison would suggest that the structure of the north rather than south basin food web deviated from that of other lakes which contained perch and pike. Researchers have hypothesized that the establishment of rainbow smelt within the north basin has increased the length of the regional food chain (Swanson et al., 2006). However, Gewurtz et al. (2006) reported that rainbow smelt collected from the north basin during 2000 to 2002 occupy a TP similar to that of emerald shiner and ciscoes. The results for fishes collected in 2009 and 2010 are in good agreement with this earlier report (TP_{RNSM} = 4.4 ± 0.4, n = 241; TP_{CISC} = 4.4 ± 0.5, n = 101; TP_{EMSH} = 4.3 ± 0.4, n = 92).

Of the species examined isotopically between 2002 and 2008, walleye were identified as the top predators in each basin (Hobson et al., 2010). Following the inclusion of additional species

(BURB, NRPK, SAUG) and larger size classes (WALL, WHBS, YLPR) of fishes in the model, it was discovered that for the north basin, sauger, walleye, yellow perch and northern pike occupied TPs which were elevated over all other fishes (Figure 3.2). North basin sauger and northern pike occupied TPs which were approximately one step above their south basin counterparts; however yellow perch and walleye from the north basin appeared to be feeding at a TP ≈ 1.5 steps greater than south basin perch and walleye (Figure 3.2). In the Hobson et al. (2010) baseline isotopic study it was postulated that north basin walleye had larger $\Delta^{15}N$ values relative to south basin walleye, and this was linked to the consumption of protein-rich smelt. Here, the relative positions of rainbow smelt and walleye within the north basin food web would suggest that a substantial portion of the walleye diet was comprised of smelt (Figure 3.2). The existence of this trophic linkage was corroborated by historical stomach content analyses (Gewurtz et al., 2006). Alternatively, the increased $\Delta^{15}N$ for north basin walleye and yellow perch may be the result of increased primary productivity in the north. Eutrophication tends to produce conditions which are favorable to walleye and other percids, with the increased availability and quality of dietary items generally leading to greater nitrogen isotopic discrimination ($\Delta^{15}N$) (Aberle and Malzahn, 2007; Leach et al., 1977). The lack of a similar effect in north basin sauger is likely due to the fact that environmental conditions within the south, rather than north basin, are more favorable for this species (Johnston et al., 2010).

In lakes of northwestern Ontario, pelagic consumers generally have $\delta^{13}C$ values between -38 and -26 ‰, and consumers which are classified as characteristically benthivorous tend to have $\delta^{13}C$ values in the range of -32 to -16 ‰ (France, 1995). Although fishes from the north and south basins of Lake Winnipeg were within the increasingly benthic range of values ($\delta^{13}C$ = -24

to -16 ‰; Figure 3.2) identified for Ontario lakes, the terms "pelagic" and "benthic" were used in the present study to refer to relative position along a $\delta^{13}C$ gradient.

The greatest relative dependence on pelagic (low $\delta^{13}C$) versus benthic (high $\delta^{13}C$) energy sources was observed for white bass and emerald shiner in the lake's north basin (Figure 3.2). The results for white bass were corroborated by the earlier Hobson et al. (2010) isotopic survey of Lake Winnipeg fishes (2002 to 2008), wherein white bass (FL < 350 mm) collected from the north basin were identified as having a relatively pelagic feeding strategy. The fact that emerald shiner were among the fishes with the lowest $\delta^{13}C$ values in each basin was also not surprising, given fish survey data from the 1990s identified emerald shiner as an important pelagic species within the lake (Stewart and Watkinson, 2004). The isotopic data for south basin burbot was also in agreement with historical dietary observations and indicated that the diet of this group has changed very little over the past 60 years. In the early 1950s, Hewson (1955) conducted controlled diet studies on burbot from Lake Winnipeg's south basin and found ciscoes were the species' preferred prey. The isotope values from the current study match extremely well with Hewson's (1955) findings, given that burbot occupy a TP one step ($\Delta^{15}N = 3.4$ ‰) above ciscoes, $\delta^{13}C$ values for burbot muscle are enriched over mean values for ciscoes by ≈ 1 ‰, and the $\delta^{34}S$ profiles of the two species are similar ($\Delta^{34}S \leq 0.5$ ‰).

In recent decades, the density of benthic invertebrates available to benthivores like troutperch, lake whitefish and suckers in Lake Winnipeg has increased substantially (Environment Canada and Manitoba Water Stewardship, 2011). This greater abundance of benthic fauna was reflected in the $\delta^{34}S$ profiles of white sucker. White sucker feed preferentially on benthic prey organisms when these energy sources are plentiful and only switch to a detritus-based feeding strategy when benthic invertebrate densities are low (Ahlgren, 1996). The relationship between

invertebrate density and relative position along the muscle-δ^{34}S gradient was less straightforward for longnose sucker. Longnose sucker may consume plant matter, and the relatively low δ^{34}S values observed for individuals from the south basin may be indicative of an increased reliance on decaying plant materials (Croisetiére et al., 2009; Stewart and Watkinson, 2004). Troutperch collected throughout the lake had muscle-δ^{34}S values which indicated frequent consumption of sediment and detritus-based foods. This species is known to have a benthic feeding strategy, and may be consuming a great deal of sediment along with invertebrate prey (Ahlgren, 1996; Stewart and Watkinson, 2004).

3.4.2 Biomagnification of Trace Elements

None of the models in Table 3.3 provided strong evidence for the biomagnification of As, Cd, Cu, Fe, Mn, Hg or Se in the north and south Basin fish communities. These findings are similar to those of Wren et al. (1983), who found that Cd, Cu, Fe and Mn did not biomagnify through the food web of Tadenac Lake, Ontario. Apparent biomagnification effects were seen at finer scales within Lake Winnipeg, namely within walleye and freshwater drum from the lake's south basin. For walleye, the strong positive effect of δ^{15}N on Se and Hg concentrations would suggest that as walleye became increasingly more piscivorous, they accumulated greater concentrations of these elements in muscle. The same may be true for freshwater drum, where individuals who were more dependent on fish (TL $\geq$ 3) rather than benthic invertebrates (TL $\approx$ 2) obtained greater amounts of Se from their diet (Stewart and Watkinson, 2004). However, the food web model in Figure 3.2 (see also Tables 3.1 & 3.2) would suggest that the potential prey items of walleye and drum were unlikely to have exceeded the 3 µg/g threshold for diet-induced Se toxicity in fishes (Muscatello and Janz, 2009). Therefore, Se biomagnification within walleye and freshwater drum is unlikely to be a major cause for concern. None of the concentrations measured in muscle

approached the United States Environmental Protection Agency's (US EPA) whole-body, chronic limit of 7.91 µg/g DW (US EPA, 2004).

Of the n = 41 south basin walleye analyzed for Hg, four exceeded the 1 µg/g FW recommended guideline for fish-eating wildlife (Ethier et al., 2008) and two exceeded the limit for commercial sale in Canada (Bodaly et al., 2007). The Hg concentrations measured were fairly similar to those reported for walleye in other systems. The mean concentration for south basin walleye (0.76 ± 0.77 µg/g DW, n = 41) was on the lower end of the range reported for specimens of a similar size which had been collected from lakes in Minnesota and Wisconsin (0.85 to 2.61 µg/g DW, n = 79) (Rolfhus et al., 2008).

3.4.3 Trophic Dilution

Trophic dilution, as evidence by a strong negative correlation between contaminant concentrations and δ^{15}N (Watanabe et al., 2008), was a widespread phenomenon within the lake's south basin fish community. In general, the dietary transfer of As and Cu from forage fishes to top-level consumers was not efficient (Newman and Unger, 2003). In fact, As concentrations tended to decrease with increasing TP within south basin walleye, as did Cu within white bass. Trophic dilution of As has been reported for a number of other fishes, such as European catfish (*Silurus glanis*), common carp, rudd (*Scardinius erythrophthalmus*) and roach (*Rutilus rutilus*) collected from the Flix Reservoir in Spain (Soto et al., 2011). A negative effect of δ^{15}N on Fe concentrations in muscle was also identified for south basin goldeye, which would indicate that this species obtained relatively more Fe from the consumption of emergent or terrestrial insects than fish (Stewart and Watkinson, 2004). In the north basin, trophic dilution could only be inferred from δ^{15}N for Cd in ciscoes.

3.4.4 Effects of Fish Size

Yellow perch and walleye from the lake's north basin tended to accumulate greater amounts of Hg in muscle as they increased in size (FL). A similar trend was evident for white bass collected from the lake's south basin. These species tend to grow slower than many small-bodied forage fishes, and may therefore accumulate greater levels of Hg per unit length (Newman and Unger, 2003). Although north basin yellow perch tended to accumulate Hg in muscle, concentrations remained fairly small relative to those measured in perch from other systems. Yellow perch collected from lakes within the Voyageurs National Park, Minnesota had muscle-Hg concentrations ranging from 0.18 µg/g DW to 0.94 µg/g DW (n = 162) (Wiener et al., 2006). Concentrations in north basin yellow perch were at the high end of this range (0.87 ± 0.66 µg/g DW, n = 9); however, the specimens from Lake Winnipeg were substantially larger (158 to 286 mm FL) than those collected from Voyageurs National Park (64 to 66 mm total length) (Wiener et al., 2006).

Growth dilution was only observed for forage fishes within the lake's north basin. Fork length had a strong negative effect on As and Se concentrations in ciscoes and emerald shiner, respectively. The frequent formation of algal blooms within the north basin may be the best explanation for this phenomenon (Pickhardt et al., 2002). Increased algal productivity at the base of the food web has led to increases in fish biomass within the north basin (Environment Canada and Manitoba Water Stewardship, 2011). If the concentrations of As and Se available for uptake by forage fishes were to remain fairly constant, the increase in fish biomass would ultimately decrease the amounts of As and Se per unit of growth in fishes (Gewurtz et al., 2006; Swanson et al., 2006). Growth dilution may be more evident in rapidly-growing forage fishes versus larger, slower growing organisms, since the duration of exposure and accumulation per unit of increased length or weight is much smaller in fast-growing species (Newman and Unger, 2003).

3.4.5 Identifying Sources of Trace Elements in Diet

The influence of $\delta^{34}S$ and $\delta^{13}C$ on trace element concentrations in fish muscle was largely dependent on species for the north basin fish community. Within the south basin however, some generalizations could be made with respect to $\delta^{13}C$ and Cu, Fe, Mn and Hg. Fishes which were increasingly dependent on pelagic carbon sources (low $\delta^{13}C$) generally had greater concentrations of Fe and Mn in muscle. Copper and Hg concentrations tended to increase with increasing dependence on benthic foods (high $\delta^{13}C$). The relative consumption of sedimentary versus dissolved sulfate was also an important factor driving Hg concentrations within the south basin fish guild. South basin ciscoes which fed on increasingly pelagic sources of sulfate and carbon accumulated less Cd and Hg, respectively, than they would have from littoral sources. The same was also true of Hg in south basin pike and yellow perch. The trend identified here for Hg opposed that presented by Ethier et al. (2008), who reported that yellow perch collected from lakes near Clyde Forks, Ontario had muscle Hg concentrations which were negatively correlated with $\delta^{13}C$. Alternatively, the findings presented here are supported by the work of Wren et al. (1983), who found that rainbow smelt and pike which were feeding near the sediment in Tadenac Lake, Ontario accumulated greater concentrations of metals in tissues than relatively pelagic organisms. The mean Hg concentration for northern pike collected from Tadenac Lake was 4.04 µg/g DW (n = 20), assuming the moisture content of pike muscle was 75 % (Gorski et al., 2003; Wren et al., 1983). This is substantially higher than the mean Hg concentration for pike from Lake Winnipeg's south basin (1.16 ± 0.50 µg/g DW, n = 7).

It is important to note that there are some exceptions to the community-wide generalizations mentioned above for south basin fishes. For example, goldeye which fed toward the benthic end of the $\delta^{13}C$ spectrum had lower Cu and greater Mn concentrations in muscle than would be expected, given the community-level model. South basin goldeye were more similar to north

basin fishes in this regard, since Cu concentrations in north basin emerald shiner, lake whitefish, walleye and northern pike tended to be smaller in individuals which obtained dietary energy from the near the sediment, rather than from the water-column. However, rainbow smelt and yellow perch from the same basin tended to exhibit greater concentrations of Cu in muscle with increased reliance on benthic carbon sources.

3.4.6 The Concentration-Diet Disconnect

For many species within both basins, concentrations of As, Cd, Cu, Fe, Mn, Hg and Se could not be strongly, or even weakly, linked to $\delta^{15}N$, $\delta^{13}C$, $\delta^{34}S$ or FL based on AIC_c model-selection. Based on the findings of Campbell et al. (2005), who examined the biomagnification of trace elements through a marine food web in Baffin Bay, a general decline in Cd concentrations with increasing TP was expected. However, trophic dilution of Cd was only observed for north basin ciscoes. Despite the apparent disconnect between Cd and TP in most Lake Winnipeg fishes, the data would suggest concentrations were sufficiently low so as not to pose a significant risk to human consumers. The EU's maximum level (ML) of Cd allowed in commercial fishes is 0.05 µg/g FW (Ciardullo et al., 2008). None of the concentrations measured in fish muscle exceeded this limit (Table 3.2), and the maximum concentration, which was measured in a north basin rainbow smelt, was equal to 0.17 µg/g DW, or approximately 0.03 µg/g FW.

Concentrations of As, Mn, Hg and Se in the muscle of northern pike collected from the north basin could not be linked to dietary parameters or size. However, the mean Hg concentration in pike muscle (mean ± SD = 0.69 ± 0.43 µg/g DW, n = 5) was approximately one-fifth that of pike collected from Tadenac Lake, Ontario (Wren et al., 1983) and slightly below the range of concentrations reported for northern pike collected from 17 lakes throughout Minnesota and Wisconsin (0.79 to 5.15 µg/g DW, n = 401) (Rolfhus et al., 2008). Selenium concentrations in the muscle of north basin pike (Table 3.2) were well below the whole-body guidelines set forth

by the US EPA (US EPA, 2004). However, Se may be a potential problem for white bass populations within Lake Winnipeg. Of the n = 3 north basin white bass for which Se concentrations had been determined, two (4.43 and 5.61 µg/g DW) approached the US EPA threshold of 5.85 µg/g DW, above which further monitoring is recommended (US EPA, 2004). The third specimen had a muscle-Se concentration (9.62 µg/g DW) which exceeded the US EPA's 7.91 µg/g DW draft criterion (US EPA, 2004). Unfortunately, due to the small Se sample size for north basin white bass, potential food web models and diet-related factors which could have contributed to the observed Se concentrations could not be evaluated. Further monitoring of Se concentrations in Lake Winnipeg white bass is highly recommended.

This study was the first extensive investigation into the influence of food web structure and diet on Hg and other trace elements measured in muscle of Lake Winnipeg fishes. Isotopic food web data (δ^{15}N, δ^{13}C and δ^{34}S) indicated fishes in the lake's north basin generally had smaller δ^{13}C and δ^{34}S values, but greater δ^{15}N or TP values, than fishes from the south basin. These findings were in good agreement with those of the 2002-2008 study (Hobson et al., 2010) and further reiterate the earlier suggestion that the north and south basins of Lake Winnipeg be considered as separate systems when examining food web structure. In terms of trace element trophodynamics, species was often the strongest modulator of concentrations at the community-wide level, based on an AIC_c information-theoretic approach. However, the isotopic and size-related parameters driving trace element concentrations within a species tended to differ between basins. Therefore, the north and south basin fish communities should be considered separately in future contaminant trophodynamic studies.

CHAPTER 4
4.0 THE UTILITY OF SCALE SAMPLING IN MONITORING STABLE ISOTOPE RATIOS & TRACE ELEMENT RESIDUES OF COMMERCIALLY-HARVESTED WALLEYE (*SANDER VITREUS*)

4.1 Introduction

Loading of Hg and other metals to freshwater lakes may increase concentrations in the tissues of commercial and sport fishes (Brumbaugh et al., 2005; Gewurtz et al., 2011). This has serious implications for consumer health, as well as the sustainability of recreational and commercial fishing industries. In Canada and the United States, thousands of Hg-related fish consumption advisories are issued each year (US EPA, 2011; Wood and Trip, 2001). In the 1970s, Hg contamination led to moratoriums on the commercial harvest of walleye (*Sander vitreus*) from Lake Erie and Lake Winnipeg (Kristofferson and Clayton, 1990; Leach and Nepzsy, 1976). Although Lake Winnipeg (53° 17'N, 97° 58'W) now supports a ~$20 million/ year commercial fishery, the need for contaminants monitoring continues (Environment Canada and Manitoba Water Stewardship, 2011). Large-scale spring floods within the lake's watershed may increase the influx rate and bioavailability of contaminants entering the reservoir (Stewart et al., 2003). Subtle food-web changes resulting from eutrophication and the introduction of non-native fishes may be affecting the dispersion of contaminants and accumulation of metals in frequently-harvested species (Environment Canada and Manitoba Water Stewardship, 2011; Hobson et al., 2010).

Current programs for monitoring concentrations of Hg and other contaminants in fishes are based on the direct analysis of muscle (Ryba et al., 2008). This fillet-based approach allows regulators to monitor the commercial product and set consumption guidelines for local anglers (Wood and Trip, 2001). Unfortunately, such monitoring programs often require that large numbers of individuals be removed from aquatic systems on an annual basis (Kelly et al., 2006).

The removal of reproductive stock or rare and endangered species may have serious ecological consequences, and loss of fisheries resources may have implications for the regional economy (Baker et al., 2004; Skurdal et al., 1986). These perceived losses associated with lethal fish sampling highlight the need for suitable non-destructive alternatives. For the walleye of Lake Winnipeg, analysis of Hg and other contaminants in scales may provide an attractive surrogate for whole-fillet style sampling. However, before walleye scales can be used in a regional contaminants monitoring program for Lake Winnipeg, the feasibility and utility of this sampling technique require further investigation.

The objective of this study was to determine whether concentrations of Hg and other trace elements in scales of Lake Winnipeg walleye were good predictors of concentrations in muscle (Sinnatamby et al., 2008). Since trophic linkages and food sources regulate contaminant transport to apex predators like walleye (Environment Canada and Manitoba Water Stewardship, 2011), stable isotope ratios (δ^{15}N, δ^{13}C and δ^{34}S) in paired tissues were examined. The influence of fish size, sex and maturity on the relative distribution of contaminants to tissues was also considered (Gewurtz et al., 2011).

4.2 Methods

4.2.1 Field Collections

Beam trawls deployed by Manitoba Fisheries personnel and commercial gillnets were used to collect walleye (n = 34) specimens from Lake Winnipeg during the ice-free season (May - October) of 2010. The beam trawl was towed along-side the *MV Namao* research vessel at a rate of 3.9 km/h for approximately 30 minutes per trawl (Lumb et al., 2011). Each station marked in Figure 4.1 (closed circles) denotes the ship's position at the end of each trawl effort. Gillnets were set overnight at near-shore locations (Figure 4.1; closed triangles) (Johnston et al., 2010). Fork lengths (FLs), which were measured to the nearest mm (Blanco et al., 2009), ranged from

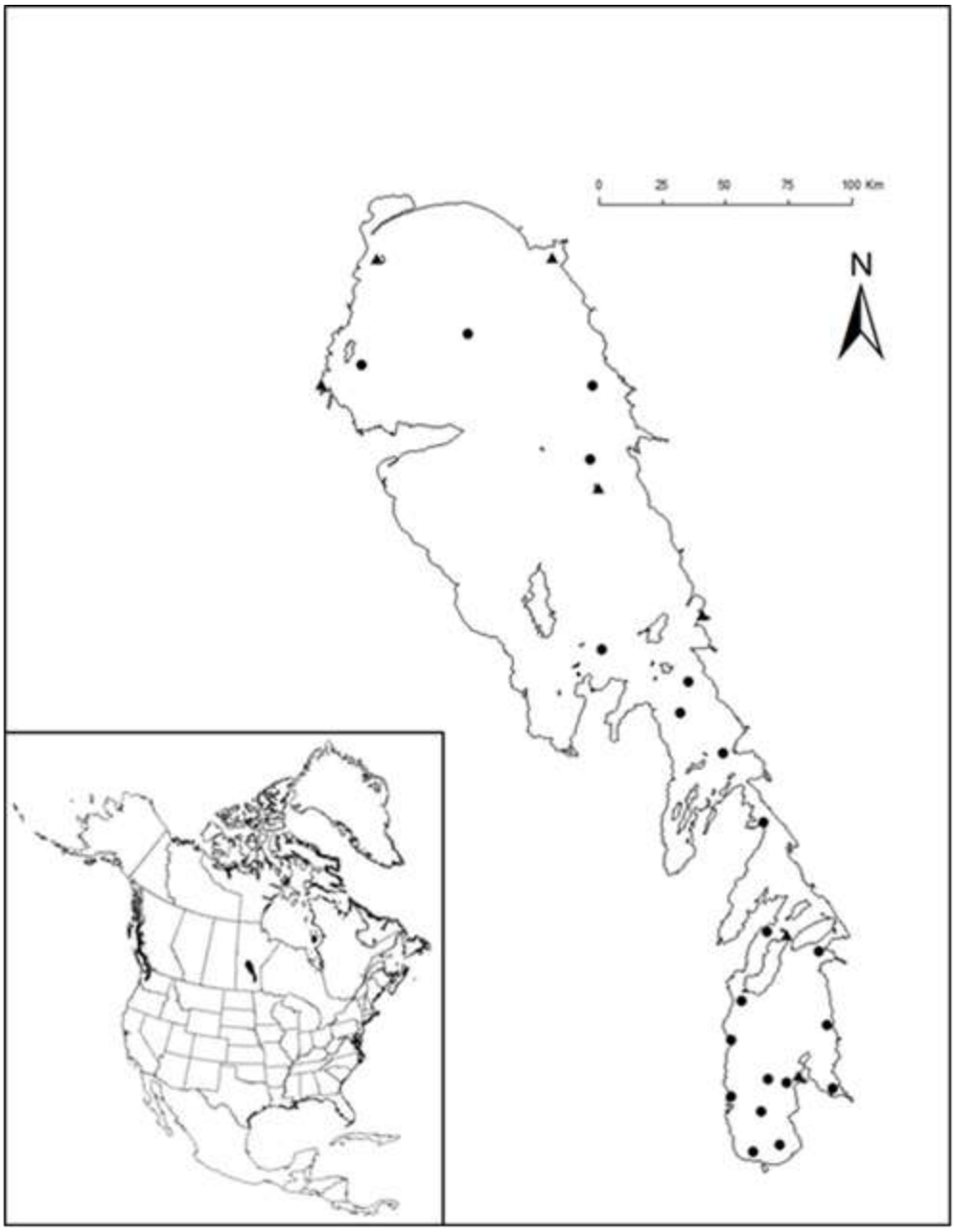


Figure 4.1 Walleye (*Sander vitreus*) collection locations for Lake Winnipeg, Canada. Stations where beam trawls and commercial gillnets were used to capture walleye specimens are marked by black circles and black triangles, respectively.

232 to 492 mm. Sex and maturity were determined through visual examination of the gonads (Moles et al., 2011). One or both fillets, with skin and scales intact, were removed from each fish and stored at -20°C (Fincel et al., 2011; Jardine et al., 2005) until they could be processed at the NHRC laboratory in Saskatoon, Canada.

4.2.2 Preparation of Scales and Muscle Samples

In the laboratory, fillet samples were partially thawed and 5 to 10 g of white muscle was removed by making clean cuts into the inner region of each fillet with a stainless steel scalpel (Fincel et al., 2011; Pruell et al., 2003). The muscle sample was then divided into two portions: one was dried at 60°C and homogenized in preparation for isotopic and elemental analyses (Vander Zanden et al., 2005), and a second FW section was analyzed for Hg (Baker et al., 2004).

Scales were stripped from each fillet and placed in acid-washed polyethylene vials. In order to remove mucus and skin, scales were ultra-sonicated in high purity acetone for at least five minutes, then rinsed multiple times with Milli-Q water (Blanco et al., 2009; Lake et al., 2006). Cleaned samples were dried at 60°C and ground using a stainless steel ball mill which was rinsed with analytical grade 5 % v/v nitric acid (HNO_3) and Milli-Q water between samples (Inamura et al., 2012; Sinnatamby et al., 2007).

4.2.3 Isotopic Analyses

Of the dried sample aliquots reserved for nitrogen, sulfur and carbon isotope analyses, only those muscle samples used in $\delta^{13}C_m$ determination were lipid extracted (Hobson et al., 2010). The chloroform: methanol solution used in removing lipids from tissues may inadvertently affect the nitrogen content of muscle proteins and confound the results of $\delta^{15}N$ analyses (Sotiropoulos et al., 2004). Lipid removal was not required for walleye scales, which consist of collagen and an upper, calcified layer (Hutchinson and Trueman, 2006). One milligram (± 0.010 mg) each of un-extracted muscle ($\delta^{15}N_m$), lipid-extracted muscle ($\delta^{13}C_m$) and homogenized scales ($\delta^{15}N_s$ and

$\delta^{13}C_s$) were weighed into tin capsules (Perga and Gerdeaux, 2003). Sulfur isotope ratios were determined for 3.50 ± 0.02 and 10.00 ± 0.10 mg of scale ($\delta^{34}S_s$) and muscle tissue ($\delta^{34}S_m$), respectively. All $^{15}N/^{14}N$, $^{13}C/^{12}C$ and $^{34}S/^{32}S$ ratios were measured via CF-IRMS (Perga and Gerdeaux, 2003). In-house standards, including BWB ($\delta^{15}N$ = 14.4 ‰, $\delta^{13}C$ = -18.5 ‰, $\delta^{34}S$ = 17.5 ‰), PUGEL ($\delta^{15}N$ = 5.6 ‰, $\delta^{13}C$ = -12.6 ‰) and CFS ($\delta^{34}S$ = -3.8 ‰), were analyzed along with the field samples. Analytical precision was better than ± 0.3 ‰.

All results for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ are expressed in per mil (‰) relative to AIR, VPDB, and VCDT, respectively, where:

$$\delta X = (R_A/R_S - 1) \cdot 1000, \quad (4.1)$$

X was one of ^{15}N, ^{13}C or ^{34}S, R_A was the ratio of the heavy to light isotope (example: $^{13}C/^{12}C$) in the sample and R_S was the ratio of the heavy to light isotope in one of the AIR, VPDB or VCDT standards (Fry, 1988).

4.2.4 Elemental Scan

Laser ablation inductively coupled plasma mass spectrometry (LA-ICPMS) has been used in the successful recovery of Hg and other trace elements from fish scales (Farrell et al., 2000). Here, trace elements in scales were analyzed via ICP-MS; however scales were liquefied, rather than ablated, prior to analysis (Ashoka et al., 2009). Aliquots of dried muscle and scale samples were digested with high purity nitric acid and 30 % v/v analytical-grade hydrogen peroxide (H_2O_2) (Phibbs et al., 2011). Blanks, reference materials, and duplicates were included in each digestion batch at a ratio of one blank, reference and duplicate per ten field samples. The concentrations of Al, As, Cd, Cu, Fe, Mn and Se in paired muscle and scale samples were measured via ICP-MS (Ashoka et al., 2009; Muir et al., 2005). Acceptable elemental recoveries from DORM-2 (National Research Council Canada; Ottawa, ON) and CVs on duplicate samples were ± 20 % and ± 15 %, respectively (Brumbaugh et al., 2005). For the scale samples, only Al,

As and Mn met these criteria. Therefore, the ICP-MS data presented here has been limited to these three elements. Limits of detection (LoD) for Al, As and Mn were better than 0.76 µg/g, $5.2 \cdot 10^{-4}$ µg/g and $1.3 \cdot 10^{-4}$ µg/g DW, respectively.

4.2.5 Mercury

Microwave digestion (US EPA, 1996) and Hg_T (hereafter "Hg") determination via CVAFS (Baker et al., 2004) were conducted at the SRC Environmental Analytical Laboratory in Saskatoon, Canada. Blanks, reference materials, and duplicate samples were included in each analytical batch. The LoD for walleye muscle was 0.01 µg/g FW, and recoveries of DORM-2 were within 85 – 115 %. The LoD for scale samples was 0.05 µg/g DW, and recoveries were 72 %. The CV on duplicate samples was within 13 % for both tissue types. In order to standardize the units of measurement across the study, percent moisture data for each of the muscle samples (unpublished data, Environment Canada) was used to convert the FW Hg data to units of µg/g DW.

4.2.6 Statistical Analyses

All statistical analyses were conducted in R Version 2.14.1 (R Development Core Team, 2011). A Shapiro-Wilk goodness-of-fit test and Levene's test were used to assess the normality and heterogeneity of variance, respectively, for the walleye data (Blanco et al., 2009). Where data transformation was required, log(x) performed the best (Vander Zanden et al., 2005). Although the muscle/ scale isotopic offset ($\delta X_m / \delta X_s$) appears to be species specific, the influence of the regional (i.e. basin-specific) nutrient pool was examined (Blanco et al., 2009; Hobson et al., 2010). A Wilcoxon rank sum test was used to determine if all $\delta X_m / \delta X_s$ for north and south basin walleye came from the same distribution (Dalgaard, 2008).

Linear regression models for predicting $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$ from $\delta^{15}N_s$, $\delta^{13}C_s$, $\delta^{34}S_s$ and other morphological parameters were evaluated with Akaike's Information Criterion corrected

for small sample sizes (AIC_c) (Anderson, 2008; Vander Zanden et al., 2005). Concentrations of Al, As, Mn and Hg in walleye muscle were modeled from scale concentrations, isotopic parameters, FL, sex and maturity. A null model was also included in the analyses (Irvine et al., 2009). The "AICcmodavg" package for R was used to identify the models which provided the "best" approximation of reality, given the data at hand (Burnham and Anderson, 2004). Since analyzing all possible models often leads to illusory results, especially when the number of parameters (*K*) is large (Anderson, 2008), a parsimonious approach to model-fitting was taken. The number of models considered for each response variable never exceeded the sample size (n), and, where possible, the number of parameters per model was maintained at roughly n/10 (Anderson, 2008). The top model ($\Delta AIC_c = 0.00$) and models with $\Delta AIC_c \leq 2.00$ were thought to have similar support (Mazerolle, 2006). Whenever the top-ranking model did not have an Akaike weight (ω_i) ≥ 0.90, model-averaging across a 95 % confidence set of models ($\Sigma\omega_i \geq 0.95$) was used to identify those parameters which had a strong effect (Burnham and Anderson, 2002; Symonds and Moussalli, 2011). A given parameter was identified as having a strong effect on $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$, or Al, As, Mn and Hg in muscle if the 95 % uCI around the MAE excluded zero (Pluess et al., 2011). The results of the model selection procedures were evaluated graphically by plotting the observed versus predicted values (Pineiro et al., 2008). All graphics were created in Grapher Version 9 (Golden Software Inc.; Golden, Colorado).

4.3 Results

4.3.1 Stable Isotopes

Results of the isotopic analyses indicated that scales were generally enriched in ^{13}C and ^{34}S, but depleted in ^{15}N, relative to muscle (Table 4.1). The $\delta X_m / \delta X_s$ offset for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ did not vary significantly with basin (Wilcoxon rank sum test, $p \leq 0.05$; Table A2; Appendix)

and therefore regional differences in the available nitrate, DIC and sulfate pools. Consequently, basin was not considered as a potential parameter in the model-selection procedures.

Since the discriminatory processes governing the distribution of $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values in tissues are not the same, models for $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$ were developed separately (Trueman and Moore, 2007). Overall, models which included sex along with the isotopic data for scales were ranked first ($\Delta AIC_c = 0.00$; Table 4.2). Although models containing FL and maturity performed poorly relative to those which included sex data, none of the top models had $\omega_i \geq 0.90$. Model averaging revealed that $\delta^{15}N_s$, $\delta^{13}C_s$ and $\delta^{34}S_s$ were strongly-supported parameters (Table 4.3); however, sex-based differences were found to be fairly weak, based on the 95 % uCI for each MAE. The uSEs (denoted as SE in Table 4.3) around MAEs were the smallest for nitrogen and the plotted observed versus predicted $\delta^{15}N_m$ values revealed a nearly 1:1 relationship (Figure 4.2a; $r^2 = 0.72$). The predicted $\delta^{13}C_m$ and $\delta^{34}S_m$ values explained much less variation in the observed $\delta^{13}C_m$ and $\delta^{34}S_m$ values (Figures 4.2b-c; $r^2 = 0.47$ and 0.38, respectively) relative to nitrogen. This may have been the result of greater model uncertainty (lower precision), as evidence by the larger SE values for the sulfur and carbon models in Table 4.3.

4.3.2 Trace Elements

Concentrations of Mn and Hg were significantly different between the paired muscle and scale samples; however, no difference was found for As concentrations in the two tissues (Wilcoxon rank sum test; $p \leq 0.05$; Table 4.4). Aluminum concentrations in both tissues were consistently below the LoD. The top-ranking models ($\Delta AIC_c \leq 2.00$) for identifying which parameters best explained muscle As, Mn and Hg are presented in Table 4.5. Mercury was the only element for which the top-ranking model ($\Delta AIC_c = 0.00$) had an Akaike weight ≥ 0.90 ($\omega_i =$

Table 4.1 Mean ± standard deviation (SD) isotope data (in ‰) for scales and muscle of Lake Winnipeg walleye (*Sander vitreus*). Mean ± SD values for walleye samples collected from North Dakota (Fincel et al., 2011) are included in parentheses for comparison.

	$\delta^{15}N$	$\delta^{13}C$	$\delta^{34}S$
	n = 34	n = 34	n = 34
Scales	13.7 ± 1.8 (17.8 ± 2.0)	-23.5 ± 1.4 (-20.6 ± 1.6)	-6.0 ± 1.5
Muscle	14.4 ± 1.7 (17.7 ± 1.4)	-25.2 ± 1.3 (-23.3 ± 1.6)	-6.9 ± 2.5
Pelvic Fin	(17.3 ± 1.7)	(-23.1 ± 1.9)	NA

NA = not available/ not applicable

Table 4.2 Scale-based models which best explain the variation in $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$ for Lake Winnipeg walleye (*Sander vitreus*). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used.

Model	*K*	AIC_c	ΔAIC	ω_i
$\delta^{15}N_m$				
$\delta^{15}N_s$ + Sex	4	89.55	0.00	0.43
$\delta^{15}N_s$	3	89.93	0.38	0.36
$\delta^{34}S_m$				
$\delta^{34}S_s$ + Sex	4	146.86	0.00	0.44
$\delta^{34}S_s$	3	147.45	0.59	0.33
$\delta^{13}C_m$				
$\delta^{13}C_s$ + Sex	4	87.46	0.00	0.67

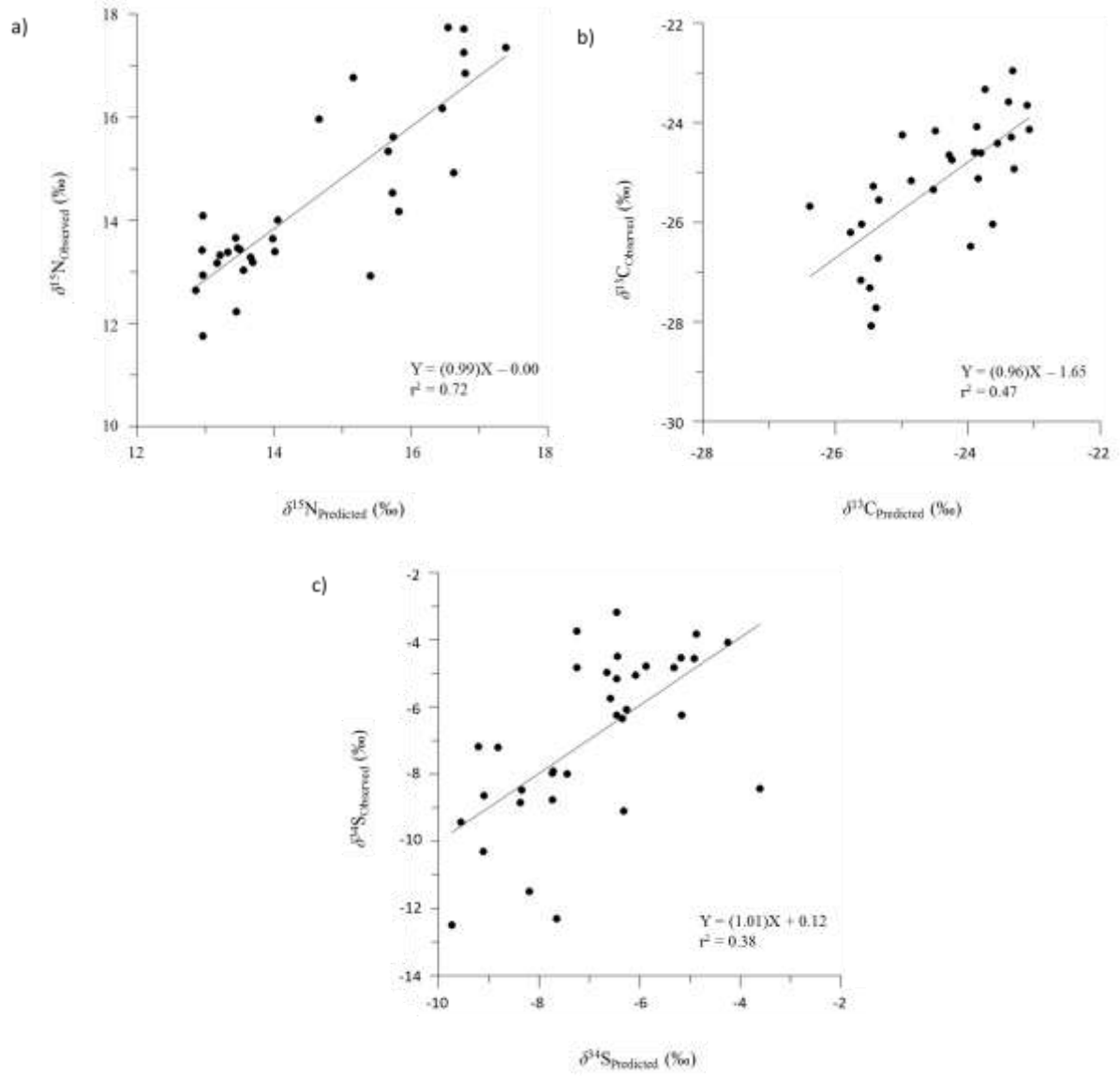
Table 4.3 Parameter estimates for predicting $\delta^{15}N_m$, $\delta^{13}C_m$ and $\delta^{34}S_m$ in walleye muscle based on $\delta^{15}N_s$, $\delta^{13}Cs$ and $\delta^{34}S_s$, respectively, in scales. Parameters were estimated based on simple linear models. Model-averaged parameter estimates (MAE) and corresponding standard errors (SE) were estimated from models with Akaike weights (ω_i) adding to ≥ 0.95.

Model	Intercept(SE)	$\delta^{15}N_s$ (SE)	Sex, Male (SE)
$\delta^{15}N_s$ + Sex	3.01 (1.30)	0.83 (0.10)	0.12 (0.37)[a]
$\delta^{15}N_s$	3.18 (1.27)	0.82 (0.09)	NA
MAE	3.22 (1.37)	0.82 (0.09)	0.12 (0.37)[a]

Model	Intercept(SE)	$\delta^{13}C_s$ (SE)	Sex, Male (SE)
$\delta^{13}C_s$ + Sex	-8.67 (3.23)	0.73 (0.14)	0.77 (0.41)[a]
MAE	-7.87 (3.67)	0.73 (0.15)	0.77 (0.41)[a]

Model	Intercept(SE)	$\delta^{34}S_s$ (SE)	Sex, Male (SE)
$\delta^{34}S_s$ + Sex	-0.90 (1.42)	1.04 (0.25)	0.05 (0.80)[a]
$\delta^{34}S_s$	-0.85 (1.36)	1.05 (0.23)	NA
MAE	-0.97 (1.52)	1.04 (0.24)	0.05 (0.80)[a]

[a]parameter did not have a strong effect.



Figures 4.2a-c Observed versus predicted values for a) δ^{15}N b) δ^{13}C and c) δ^{34}S in walleye (*Sander vitreus*) muscle. Predicted values are based on stable isotope ratios in walleye scales and sex. The line associated with the data-points in each graph represents the linear fit of the model.

0.99). The null model was ranked first for As (Table 4.6), although log([As_s]) and $\delta^{13}C_s$ appeared in models with $\Delta AIC_c \leq 2.00$. Model-averaging revealed log([As_s]) did not have a strong influence on the prediction of log([As_m]) (95 % uCI = -2.29. 1.73), and that $\delta^{13}C_s$ was a weak predictor of log([As_m]) (95 % uCI = 0.00, 0.16; Table 4.6). Similarly, calculation of the MAE for log-transformed Mn data in scales revealed that log([Mn_s]) was a poorly-supported parameter, based on its large uSE (0.25) and wide confidence intervals which overlapped zero (95 % uCI = -0.32, 0.68) .

4.4 Discussion

Consideration of observed versus predicted values for all isotopic (Figures 4.2a-c) and contaminant (Figures 4.3a-c) models revealed that the strongest correlations between scale and muscle parameters existed for $\delta^{15}N$ ($r^2 = 0.72$) and Hg ($r^2 = 0.75$). The ability to predict muscle-$\delta^{15}N$ from walleye scales may be useful in monitoring temporal and spatial variability in the species' TP. The inclusion of sex in all top-ranking isotopic models would suggest the processes governing the relative distribution of nutrients to muscle, scales and gonads were different for male and female walleye (Jardine et al., 2005). Sex has also been identified as a modulator of Hg accumulation for other Canadian walleye populations (Gewurtz et al., 2011); however, no such phenomenon was observed for Lake Winnipeg.

Despite the utility of the Hg data presented here, the application of scale sampling in contaminants monitoring still suffers from a number of methodological and analytical constraints. Isotopic studies or elemental analysis by ICP-MS may require that as much as 1 g of scale material be removed from a single fish (Fincel et al., 2011). Mercury analyses by CVAFS, although extremely reliable (Morita et al., 1995), require approximately 2 g of scale material to achieve a detection limit of 0.05 µg/g (Brenda Stanek, SRC, *personal communication,* 2010).

Table 4.4 Mean ± standard deviation (SD) concentrations of Al, As, Hg and Mn in paired walleye (*Sander vitreus*) scales and muscle. Values are expressed in µg/g dry weight (DW).

	Al	As	Mn	Hg
	n = 25	n = 16	n = 18	n = 17
Scales	< LoD[a]	0.36 ± 0.22	7.22 ± 2.3	0.06 ± 0.04[b]
Muscle	< LoD	0.34 ±0.09	0.43 ± 0.18	1.05 ±1.26

[a]LOD = limit of detection
[b]n = 9 of the scale Hg concentrations fell below the LoD; these were assigned a value of LoD/2 = 0.025 prior to calculation of the mean ± SD.

Table 4.5 Scale-based models which best explain the variation in muscle As_m, Hg_m and Mn_m for Lake Winnipeg walleye (*Sander vitreus*). The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used.

Model	*K*	AIC_c	ΔAIC	ω_i
As_m				
Null model	2	6.50	0.00	0.34
$Log([As_s]) + \delta^{13}C_s$	4	7.37	0.88	0.22
$Log([As_s])$	3	7.77	1.28	0.18
Mn_m				
Log([Mns]) + Maturity	4	19.20	0.00	0.67
Hg_m				
$Log([Hg_s])$ + FL	4	21.74	0.00	0.99

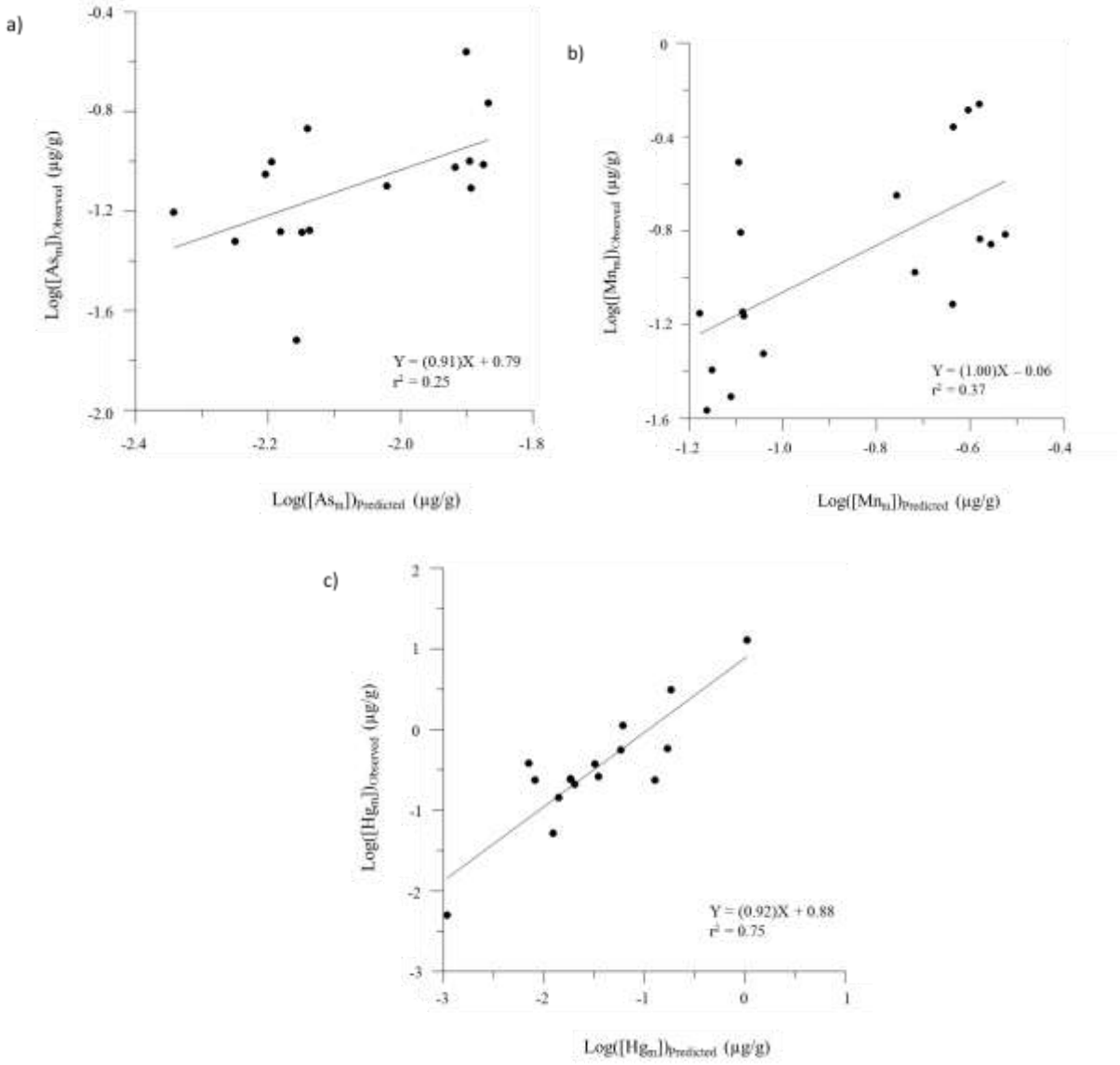
Table 4.6 Parameter estimates for predicting As_m, Hg_m and Mn_m in walleye (*Sander vitreus*) muscle based on concentrations in scales ($[X_s]$) and morphological parameters. Parameters were estimated based on simple linear models and model average estimates (MAEs) were determined from models with Akaike weights (ω_i) adding to ≥ 0.95.

Model	Intercept(SE[a])	Log($[As_s]$) (SE)	$\delta^{13}C_s$ (SE)
Log($[As_s]$) + $\delta^{13}C_s$	0.85 (1.09)	-0.04 (0.10)[b]	0.08 (0.04)
Log($[As_s]$)	-1.24 (0.13)	-0.12 (0.10)[b]	NA
MAE	-0.28 (1.03)	-0.08 (0.11)[b]	0.08 (0.04

Model	Intercept(SE)	Log($[Mn_s]$) (SE)	Maturity, M (SE)
Log($[Mn_s]$) + Maturity	-1.02 (0.50)	0.17 (0.24)[b]	-0.47 (0.16)
MAE	-0.98 (0.48)	0.18 (0.25)[b]	-0.47 (0.16)

Model	Intercept(SE)	Log($[Hg_s]$) (SE)	FL (SE)
Log($[Hg_s]$) + FL[c]	-2.22 (0.62)	0.93 (0.15)	0.01 (0.00)

[a]Standard error
[b]Parameter did not have a strong effect.
[c]FL = fork length (mm)
NA = not available/ not applicable



Figures 4.3a-b: Observed versus predicted values for a) As, b) Mn and c) Hg in walleye (S*ander vitreus*) muscle. Predicted values are based contaminant and/ or isotopic data for fish scales, as well as morphological parameters (see Table 4.6). The line associated with the data-points in each graph represents the linear fit of the model. Note that Hg is the only element for which all parameters in the predictive model had a strong effect.

This equated to approximately half of the scale biomass available from the specimens studied here. Removing such large volumes of scales and overlying mucus from live fish is likely to result in infection and/ or lethality (Tyus et al., 1999). An alternative to sampling live walleye may involve removal of scales from the carcasses as they are processed for commercial sale. The risk of harming live fishes would be negated, yet the highly-prized walleye fillets would remain unadulterated. However, removal of scales during commercial processing, rather than in a clean laboratory, may heighten the risk for sample contamination. Although scales are often cleaned with acetone, water or detergents prior to isotopic and contaminant analyses, no standardized, reliable method has been developed. A combination of acetone and ultra-sonication were effective in cleaning the walleye scales analyzed here, whereas deionized water and hydrogen peroxide are often ineffective or destructive to the sample, respectively (Cervenka et al., 2011; Lake et al., 2006). Finally, the biochemical structure of fish scales was likely to have interfered with the ICP-MS elemental scan conducted here. As much as 60 % (DW) of a scale's biomass may be composed of mineralized calcium phosphate and carbonates, depending on the fish species (Hutchinson and Trueman, 2006; Ventura and Jeppesen, 2010). Scales from Lake Winnipeg walleye were well-calcified (Amy Ofukany, *personal observation*), and may have had a greater calcium content than what could be effectively managed by the ICP-MS instrumentation under normal operating conditions. Even after complete digestion of tissues, high-calcium sample matrices may have suppressed the measurable signal of other elements, or potentially fouled the inner components of the ICP-MS instrument (Holland and Tanner, 2001).

Non-calcified samples, such as muscle biopsies and fin web clips, may be more viable options for the non-destructive monitoring of trace elements in Lake Winnipeg walleye populations

(Fincel et al., 2011). However, the analysis of Hg in walleye scales exhibited some promise as a suitable proxy for monitoring Hg concentrations in fillets.

CHAPTER 5
5.0 A STABLE ISOTOPIC (N, C & S) INVESTIGATION INTO PREY CONSUMPTION AND TRACE ELEMENT CONCENTRATIONS IN DOUBLE-CRESTED CORMORANTS (*PHALACROCORAX AURITUS*) NESTING ON LAKE WINNIPEG, CANADA

5.1 Introduction

Populations of Double-crested Cormorants (*Phalacrocorax auritus*; hereafter "cormorant") throughout North America once declined substantially in response to organochlorine pesticide contamination and human persecution (Ludwig et al., 1996; OMNR, 2006; Somers et al., 1993). However, over the past 40 years numbers have rebounded to near historical levels (OMNR, 2006; Weseloh et al., 2002). Observations of growing cormorant populations on lakes (Doucette et al., 2011; Weseloh et al., 2002), reservoirs (Caldwell et al., 1999), coastal regions (Wires et al., 2001) and aquaculture facilities (Dorr et al., 2004) in recent years has been attributed to a decline in organic pesticide use and increasing densities of potential prey items, such as schooling forage fishes or farmed catfish (*Ictalurus* spp.). Perceived pressures exerted on fish populations by foraging cormorants have garnered considerable interest from managers and the public (Belyea et al., 1997; Trapp et al., 1997). On the species' summer breeding range in the northern United States and southern Canada, much research has focused on populations nesting on the Laurentian Great Lakes (Belyea et al., 1997; Seefelt and Gillingham, 2008), and more recently, in Saskatchewan (Doucette et al., 2011). Consumption of commercially and recreationally-valuable fishes (Fielder, 2008; Hobson, 2009), trophic niche (Doucette et al., 2011; Jones et al., 2010) and effectiveness of population management programs (Fielder, 2010) have been examined in these regions. Hobson et al. (1989) provide an extensive account of prey consumption in cormorants nesting on Lake Winnipegosis, Manitoba during 1987. Cormorants nesting in Manitoba have received little research attention since then, despite the fact Manitoban

lakes, including Winnipegosis and Lake Winnipeg, support the continent's largest known breeding population (Wires et al., 2001).

Another major focus in cormorant research is contaminants monitoring. The relative sensitivity of cormorants to PCBs and other organic chemicals in the environment, such as dichlorodiphenyltrichloroethane (DDT), have made them and ideal indicator species for monitoring contaminant concentrations and effects in aquatic systems (Greichus et al., 1973; Ludwig et al., 1996; Somers et al., 1993). Cormorants have many other characteristics amenable to toxicology-based field studies. This species is largely piscivorous and is often among the top predators within aquatic systems (Hobson, 2009). As such, cormorants serve as integrators of the energy and contaminants available from lower-TP organisms, and may therefore provide a "conservative" estimate of concentrations in fishes. For example, Hg concentrations in the muscle of nestling and adult cormorants collected from South Dakota were 1.3- and 7.8-fold larger, respectively, than concentrations in fishes (Greichus et al., 1973). Cormorant tissues such as blood and feathers (Chapter 6; Burger and Gochfeld, 2001; Caldwell et al., 1999) which can be obtained non-lethally may be useful in that intensive, long-term monitoring programs can be conducted without adverse effects to populations or ecosystem function.

One of the main hurdles in using cormorants to monitor Hg and other contaminants in aquatic systems is connecting concentrations in tissues to diet. Visual examinations of pellets, regurgitated prey items and/ or stomach contents are generally used in diet reconstruction (Seefelt and Gillingham, 2006). However, these samples represent the specimen's most recent meal, and are therefore unlikely to reflect long-term nutrient and contaminant assimilation (Hobson, 2009). Stable isotopes of nitrogen (δ^{15}N), carbon (δ^{13}C) and potentially sulfur (δ^{34}S) are an attractive alternative to other diet analysis methods, as they provide a means of

quantitatively linking the protein pool of consumers to their prey and ultimately to the dietary source of Hg, DDT, etc. (Hall et al., 2009; Rocque and Winker, 2004). The ratio of $^{15}N/^{14}N$, $^{13}C/^{12}C$ and $^{34}S/^{32}S$, in predator (cormorant) tissues should reflect the ratios in prey tissues, plus some diet-to-tissue discrimination factor ($\Delta^{15}N$, $\Delta^{13}C$ and $\Delta^{34}S$) (Caut et al., 2009; Fry, 1988). Stable nitrogen isotopes are most often used to infer TP and identify whether contaminant biomagnification is occurring within a system or species (Campbell et al., 2005; Gewurtz et al., 2006). Contaminant concentrations in tissues can be related to prey or habitat types based on $\delta^{13}C$ and $\delta^{34}S$ (Chapters 3 and 6; Burgess and Hobson, 2006).

This examination of diet and trace element concentrations in cormorants nesting on Lake Winnipeg, Manitoba was encouraged by the information gaps noted by Wires et al. (2001) and the need for a greater understanding of trophic interactions and contaminant dynamics in a system impacted by cultural eutrophication, species introduction and commercial fishing pressures (Environment Canada and Manitoba Water Stewardship, 2011; Manitoba Water Stewardship, 2010; Stewart and Watkinson, 2004). Harvests of walleye (*Sander vitreus*), sauger (*Sander canadensis*) and lake whitefish (*Coregeonus clupeaformis*), from Lake Winnipeg are worth over $20 million/ year (Environment Canada and Manitoba Water Stewardship, 2011). Given the extent of the fishery and the long history of cormorant-fisheries conflicts in North America (Dorr et al., 2010; Hobson et al., 1989; Trapp et al., 1997), identification of fish species most frequently consumed by Lake Winnipeg cormorants was warranted. The potential prey base for Lake Winnipeg birds is rather diverse, with an abundant array of forage fishes, such as ciscoes (*Coregonus artedi*), emerald shiner (*Notropis atherinoides*) and rainbow smelt (*Osmerus mordax*), among others (Hobson et al., 2010; Lumb et al., 2011). Since a large number of alternatives to walleye and sauger are available for consumption, walleye and sauger were

expected to represent a relatively small proportion of the cormorant diet (Doucette et al., 2011; Hobson et al., 1989). The objective was to use a comprehensive multi-isotopic (δ^{15}N, δ^{13}C and δ^{34}S) approach to characterize cormorant diet composition and potentially inform future regulatory and management decisions. Following from this first objective, an assessment of whether or not the isotopic profiles of adult and hatch-hear cormorants could be linked to concentrations of Al, As, Cd, Fe, Mn, Hg and Se in muscle was conducted. Trophodynamics of Hg and other potentially toxic elements are relatively unknown for Lake Winnipeg, and concentrations accumulating in high-TP consumers are not well characterized (but see Chapters 3, 4 and 6, as well as Fox et al., 2002).

5.2 Methods

5.2.1 Sample Collection

Forty adult cormorants were collected from Lake Winnipeg, Manitoba, Canada (53° 17'N, 97° 58'W; Figure 5.1) during May-August 2009 (n = 23) and June-August 2010 (n = 17). A small workboat operated by the crew of the *MV Namao* was used to access each of the lake's known nesting colonies. Adult cormorants which flushed from the colonies as the workboat approached or birds which had been foraging nearby were shot by trained personnel (Doucette et al., 2011). Hatch-year cormorants (n = 12) were also removed from the Black and Devil's Island colonies in the lake's south basin during July-August 2010. Only the Eagle, George and Cox Island colonies were accessed in the north basin during 2010; however, no birds were collected from Cox Island that year, as the colony had been all but wiped out prior to sampling (see Discussion below). All collection activities complied with the appropriate Manitoba Conservation permits (WB09813 and WB11121) and firearms regulations. Whole cormorant carcasses were stored frozen (-20°C) onboard the *MV Namao* until they could be transported to the NHRC laboratory in Saskatoon, Canada.

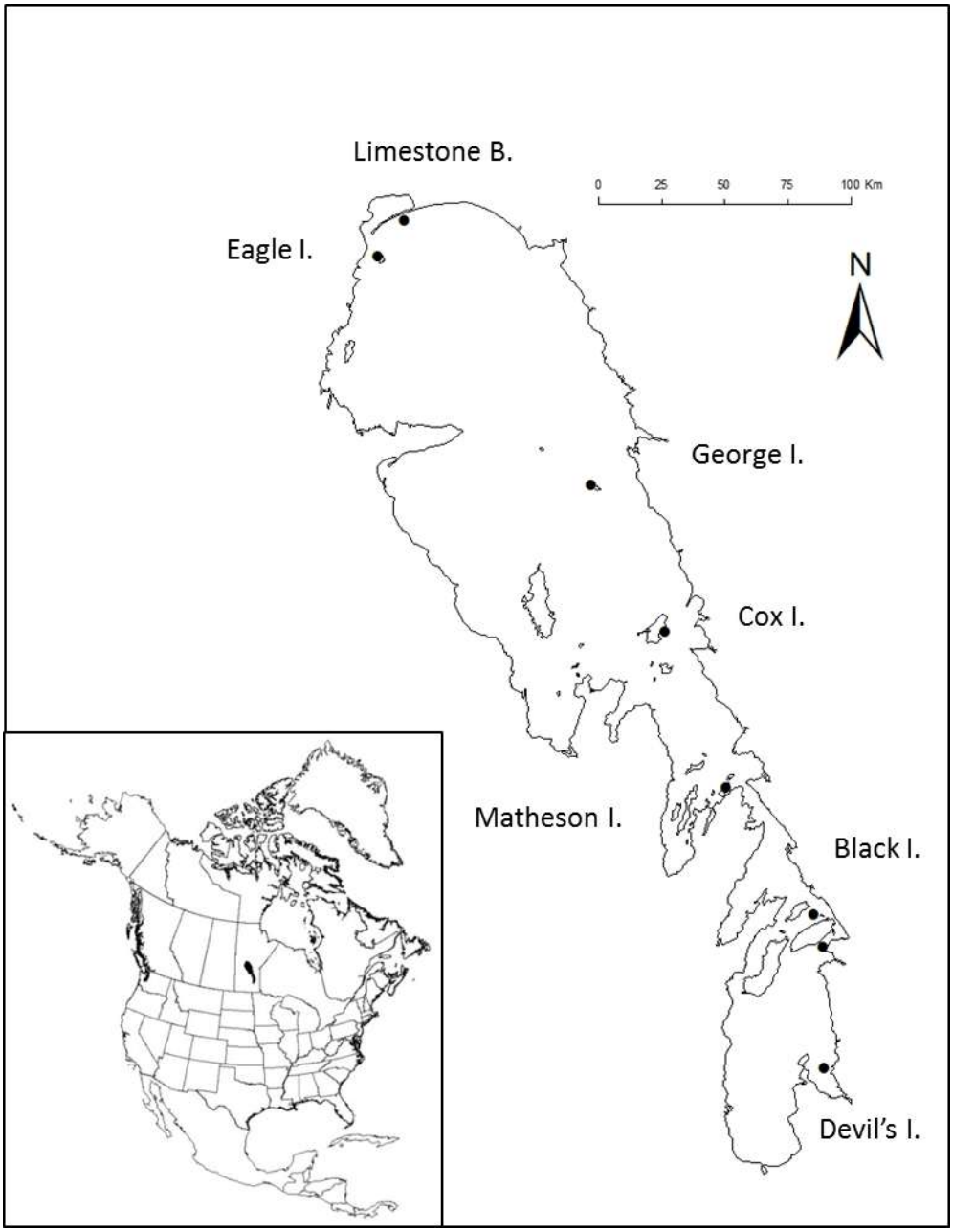


Figure 5.1 Cormorant (*Phalacrocorax auritus*) collection sites on Lake Winnipeg, Canada. Circles represent collection sites for the period of May-August 2009 and June-August 2010. Since some colonies were found on small, un-named islands, regional landmarks (i.e. George Island) were used as colony identifiers.

Isotopic data (δ^{15}N, δ^{13}C and δ^{34}S) for fishes which cormorants may have preyed upon or competed against for dietary resources were obtained as part of another study (Chapter 3; see also Table A3, Appendix). In 2009 and 2010, fishes were obtained from beam trawls which had been towed alongside the *MV Namao* (Lumb et al., 2011). Commercial gillnets were used during 2010 only. Fish samples were stored frozen (-20°C) until the dorsal muscle could be removed and prepared for δ^{15}N, δ^{13}C and δ^{34}S assays. Fish collections and analyses are described in greater detail in Chapter 3.

5.2.2 Cormorant Dissection

Each cormorant specimen was thawed, then the weight (g) and tarsus lengths (mm) were measured (Moreno et al., 2010). After the skin overlying the breast and abdomen had been removed, a stainless steel scalpel was used to make clean cuts into the left pectoral muscle (Hall et al., 2009). Muscle samples from each specimen were divided into three portions: one reserved for isotopic assays (Doucette et al., 2011), a second for trace element determination and a third for Hg analysis. Specimens were sexed based on visual examination of the reproductive organs (Seefelt and Gillingham, 2006).

5.2.3 Isotopic Analyses

Pectoral muscle was dried (60°C) and homogenized prior to isotopic assays (Logan et al., 2008). Since lipids are ^{13}C-depleted relative to carbohydrates or proteins and lipid content may vary substantially among individuals, small aliquots of dried sample were reserved for lipid extraction and δ^{13}C analysis (Post et al., 2007; Sotiropoulos et al., 2004). These aliquots were rinsed multiple times with a 2:1 (v/v) chloroform: methanol mixture, then allowed to dry in a fume hood overnight (Doucette et al., 2010). The results of δ^{15}N analyses may be confounded by the inadvertent removal of muscle proteins during lipid extraction (Doucette et al., 2010; Sotiropoulos et al., 2004), and the effects of chloroform: methanol treatments to δ^{34}S assays have

not been characterized. Therefore, $\delta^{15}N$ and $\delta^{34}S$ isotopic measurements were conducted on un-extracted (bulk) muscle (Doucette et al., 2010; Hobson et al., 2010).

One (± 0.01) and 10.00 (± 0.10) mg of dried, un-extracted muscle were weighed into tin capsules for $\delta^{15}N$ and $\delta^{34}S$ isotope analyses, respectively. Carbon isotope measurements were conducted on 1.00 ± 0.01 mg of lipid-extracted sample (Wayland and Hobson, 2001). Ratios of $^{15}N/^{14}N$, $^{13}C/^{12}C$ and $^{34}S/^{32}S$ in muscle were quantified separately via CF-IRMS (Wayland and Hobson, 2001). Calibrated laboratory standards, including BWB ($\delta^{15}N$ = 14.4 ‰, $\delta^{13}C$ = -18.5 ‰, $\delta^{34}S$ = 17.5 ‰), PUGEL ($\delta^{15}N$ = 5.6 ‰, $\delta^{13}C$ = -12.6 ‰) and CFS ($\delta^{34}S$ = -3.8 ‰), were analyzed along the cormorant muscle. Within-run precision was better than ± 0.2 ‰ for $\delta^{15}N$ and $\delta^{13}C$ and ± 0.3 ‰ for $\delta^{34}S$.

All $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values for cormorant muscle are expressed in per mil (‰) relative to AIR, VPDB and VCDT, respectively, where:

$$\delta X = (R_A/R_S - 1) \cdot 1000, \quad (5.1)$$

X was ^{15}N, ^{13}C and ^{34}S, R_A was the ratio of the heavy to light isotope (example: $^{13}C/^{12}C$) in the sample, and R_S was the ratio in the standard (Fry, 1988; Jones et al., 2010).

5.2.4 Elemental Scan

Cormorant muscle was dried at 60°C and homogenized prior to trace element analyses (Rocque and Winker, 2004). High purity nitric acid (HNO_3; 70 % v/v) and 30 % v/v analytical grade hydrogen peroxide (H_2O_2) were used to digest the dried samples (Phibbs et al., 2011). Each digestion batch included cormorant muscle samples, method blanks, DORM-2 reference material (National Research Council Canada; Ottawa, ON) and duplicate samples at a 10:1:1:1 ratio. Concentrations of Al, As, Cd, Cu, Fe, Mn and Se in muscle were quantified via ICP-MS at the Toxicology Centre, University of Saskatchewan, Saskatoon, Canada (Ashoka et al., 2009; Rocque and Winker, 2004). Recoveries from DORM-2 reference material and CVs on duplicate

samples were accepted when within ± 20 % and ± 15 %, respectively (Padula et al., 2010). Limits of detection (LoD) for Al, As, Cd, Cu, Fe, Mn and Se were better than 0.9 µg/g, $5.2 \cdot 10^{-4}$ µg/g, $1.7 \cdot 10^{-4}$ µg/g, $1.8 \cdot 10^{-3}$ µg/g, 0.1 µg/g, $1.1 \cdot 10^{-3}$ µg/g and $7.2 \cdot 10^{-4}$ µg/g DW, respectively.

5.2.5 Mercury

Samples of fresh rather than dried cormorant muscle were microwave digested (US EPA, 1996) and analyzed for Hg_T (hereafter "Hg") via CVAFS (Hall et al., 2009). Samples were digested and analyzed at the SRC Environmental Analytical Laboratory in Saskatoon, Canada. Blanks, reference material (DORM-2) and duplicates were included in the analyses. Recoveries of Hg from DORM-2 were between 85 and 100 % and the CVs on duplicate samples were within 15 % (Padula et al., 2010). The LoD was 0.01 µg/g FW. In order to maintain consistent units across all elements, percent moisture values for each muscle sample (unpublished data, Environment Canada) were used to convert the FW Hg values to units of µg/g DW.

5.2.6 Statistical Analyses

The isotopic values of zooplankton ($\delta^{15}N_{Zoop}$), DIC ($\delta^{13}C_{DIC}$) and dissolved sulfate ($\delta^{34}S_{DS}$) in Lake Winnipeg exhibit significant spatial heterogeneity (Chapter 2; Hobson et al., 2010). This variation in the baseline $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of the north and south basins may confound the interpretation of isotopic data for fishes and cormorants (Chapter 2; Post, 2002). In order to minimize the effects of baseline variability, isotopic data for cormorant muscle were normalized according to:

$$\delta X_{Corrected} = \delta X_{Fish} - \delta X_{Baseline} \quad (5.2)$$

where X was one of ^{15}N, ^{13}C and ^{34}S, and $\delta X_{Baseline}$ was the mean $\delta^{15}N_{Zoop}$ (north = +3.4 ± 1.9 ‰, n = 31; south = +9.6 ± 2.8 ‰, n = 27), $\delta^{13}C_{DIC}$ (north = -3.4 ± 2.0 ‰, n = 31; south = -8.4 ± 1.0 ‰, n = 30) or $\delta^{34}S_{DS}$ (north = -4.7 ± 3.1 ‰, n = 30; south = -8.2 ± 2.5 ‰, n = 29) value for the basin where a given cormorant was captured. A basin-specific correction was chosen, since

cormorants may feed in an area up to 10 km away from their nest site (Wires et al., 2001) and this type of correction could potentially account for the mobility of cormorants' prey items (see Chapter 3; Hobson et al., 2010). The measurement of $\delta^{15}N_{Zoop}$, $\delta^{13}C_{DIC}$ and $\delta^{34}S_{DS}$ in zooplankton, DIC and dissolved sulfate, respectively, is fully described in Chapter 2.

Cormorant TPs were also estimated on a basin-specific scale, and were used in comparing $\delta^{15}N$ data across systems (Hobson, 2009; Hobson et al., 2010). The equation:

$$TP_{DCCO} = TP_{Fish} + (\delta^{15}N_{DCCO} - \delta^{15}N_{Fish})/ 1.70 ‰ \quad (5.3)$$

was used to calculate the TP of cormorants based on $\delta^{15}N$ values in cormorant muscle ($\delta^{15}N_{DCCO}$), the lowest mean $\delta^{15}N_{Fish}$ and TP_{Fish} of potential prey fishes and a trophic enrichment factor (TEF) of 1.70 ‰ (Caut et al., 2009; Hobson, 2009). Estimates of cormorant TP were based on white sucker (*Catostomus commersonii*; $\delta^{15}N = 6.8 \pm 1.2$ ‰, $TP = 4.0 \pm 0.04$, $n = 55$) in the north basin and emerald shiner ($\delta^{15}N = 3.7 \pm 1.5$, $TP = 3.1 \pm 0.4$, $n = 60$) in the south, as these were the potential prey species with the smallest $\delta^{15}N$ values (Hobson, 2009).

All statistical tests were conducted in R Version 2.14.1 (R Development Core Team, 2011). A Shapiro-Wilk goodness-of-fit test and Levene's test were used to determine whether the cormorant data was normally-distributed and had homogenous variances, respectively (Blanco et al., 2009). The mean (± SD) $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of fishes and cormorants from each basin were used to plot food web structure in terms of three-dimensional isotopic space (Grapher Version 9, Golden Software Inc.; Golden, Colorado).

The Stable Isotope Analysis in R (SIAR) package was used in R (R Development Core Team, 2011) to identify whether a given fish species (source) contributed to the isotopic profile ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$), and therefore diet, of Lake Winnipeg cormorants. First, the fish muscle dataset (Chapter 3, or refer to Table A3, Appendix) was reduced to only those specimens collected

during the ice-free seasons of 2009 and 2010, when cormorants would have been nesting on the lake. Since cormorants are generally physically unable to consume fishes ≥ 400 mm (Doucette et al., 2011), the dataset of potential prey fishes was further limited to individuals with FLs < 400 mm. Species with n < 5 individuals remaining were excluded from the analyses (Hobson, 2009). Since the number of sources (prey species) in the north (n = 12) and south basins (n = 11) far outweighed the number of isotopic tracers, the utility of pooling potential sources was investigated (Phillips and Gregg, 2003). A Wilcoxon rank sum test with a Bonferroni correction for multiple comparisons was used to compare the isotopic profiles of all prey species within each basin (Dalgaard, 2008; Hobson et al., 2010). Where no significant differences in $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ could be found between two species at the $p \leq 0.05$ level, data were pooled into a single source (Phillips and Gregg, 2003).

In order to account for isotopic discrimination between food source and cormorant muscle when developing the SIAR mixing models, TEFs of $\Delta^{15}N = +1.70 \pm 0.43$ ‰, $\Delta^{13}C = 0.92 \pm 0.27$ ‰ and $\Delta^{34}S = 1.9 \pm 1.0$ ‰ were applied (Caut et al., 2009; Moreno et al., 2010). A SD = ± 1.0 was arbitrarily applied to $\Delta^{34}S$, as no value for avian muscle was available from the current literature (Hobson et al., 2011). Mixing models were run using non-informative priors, 1 000 000 iterations, a burn-in of 40 000, thinned by 15 for a final total of 64 000 posterior draws (Bond and Diamond, 2011; Hobson et al., 2011). The proportion of cormorant diet (on a scale of 0 to 100 %) comprised of each potential prey source was estimated from the SIAR models (Doucette et al., 2011). Results are presented in terms of mean contributions (%) and 95 % credibility intervals (Hobson et al., 2011; Moreno et al., 2010).

Akaike's Information Criterion with a correction for small sample sizes (AIC_c) was used to evaluate which dietary ($\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$), morphological (sex, weight, tarsus length) and/ or

locational (nesting colony) parameter(s) provided the "best" explanation for trace element concentrations in cormorant pectoral muscle (Anderson, 2008; Mazerolle, 2006). Simple linear models were contrasted against each other and against a null model (Anderson, 2008; Irvine et al., 2009). Since over-parameterization (large *K*) and/ or analysis of too many models can often lead to unreliable results, the number of parameters per model was maintained at roughly n/10 where possible, and the number of models considered at any one time never exceeded the sample size (n) (Anderson, 2008). The "AICcmodavg" package was used in R to identify the "best" models which could be developed from the dataset (Burnham and Anderson, 2004; R Development Core Team, 2011). Each candidate model was ranked in terms of its ΔAIC_c value and Akaike weight (ω_i; Anderson, 2008). Any model with $\Delta AIC_c \geq 10.00$ was deemed a poor fit and any competing model with $\Delta AIC_c \leq 2.00$ was considered to be a relatively good fit (Mazerolle, 2006; Symonds and Moussalli, 2011). If the top-ranking model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, model-averaging was used to identify which parameter(s) had the strongest influence on the element of interest (Burnham and Anderson, 2002). The overall effect of a given parameter was averaged over a 95 % confidence set of models ($\Sigma\omega_i \geq 0.95$), and if the 95 % uCI around the MAE excluded zero, the parameter was identified as having a strong effect on Al, As, Cd, Cu, Fe, Mn, Hg or Se (Pluess et al., 2011).

Concentrations of Al, As, Cd, Cu, Fe, Mn, Hg and Se in the pectoral muscle of cormorants were log-transformed prior to statistical testing (Campbell et al., 2005).

5.3 Results

5.3.1 Diet

Isotopic profiles of cormorants collected from Lake Winnipeg's north and south basins exhibited a bimodal pattern similar to that identified for baseline nutrients ($\delta^{13}C_{DIC}$, $\delta^{34}S_{DS}$), primary consumers ($\delta^{15}N_{Zoop}$) and fishes (Chapters 2 and 3; Hobson et al., 2010). The mean $\delta^{15}N$,

δ^{13}C and δ^{34}S values of adult cormorants from the north basin (NB DCCO), adults from the south basin (SB DCCO) and hatch-year cormorants (HY DCCO) are plotted in Figure 5.2a, along with mean isotopic values of all fishes collected in 2009 and 2010. Mean (± SD) isotopic values for cormorants and all Lake Winnipeg fishes are also provided in Table 5.1 and Table A3 (Appendix), respectively.

No significant colony-related, annual or seasonal differences in δ^{15}N, δ^{13}C and δ^{34}S were identified within adult or hatch-year cormorants collected from the south basin (ANOVAs, $p \leq 0.05$; *data not shown*). Therefore, one SIAR analysis each was conducted for adults (n = 8) and hatch-year birds (n = 12) from the south. The number of potential sources in the south basin was reduced from n = 12 to n = 8, since δ^{15}N, δ^{13}C and δ^{34}S values of ciscoes, freshwater drum (*Aplodinotus grunniens*) and sauger were statistically indistinguishable from those of emerald shiner, yellow perch (*Perca flavescens*) and walleye, respectively (Bonferroni, $p \leq 0.05$).

In the north basin, δ^{15}N, δ^{13}C and δ^{34}S values for cormorant muscle varied among colonies and between years. Cormorants collected from Eagle Island in 2009 and 2010 had the smallest δ^{15}N values of all north basin cormorants (ANOVA, Tukey HSD; $F_{4, 25} = 13.09$, $p < 0.0001$). Individuals from Eagle Island in 2009 had larger δ^{34}S values than all cormorants collected in 2010 (ANOVA, Tukey HSD; $F_{6, 23} = 8.35$, $p < 0.0001$). Cox Island (2009) birds had δ^{13}C values which were significantly lower than those associated with all other colonies, except Limestone Bay (ANOVA, Tukey HSD; $F_{4, 10} = 17.1$, $p = 0.0002$). Based in these differences, separate mixing models were developing in SIAR for 1) Cox Island (2009), 2) Eagle Island (2009), 3) Eagle Island (2010) and 4) George Island (2009, 2010). Limestone Bay (n = 1) and Matheson Island (n = 1) cormorants fell outside of the isotopic mixing space afforded by potential dietary items (see Discussion below) (Phillips and Koch, 2002), and were therefore excluded from SIAR

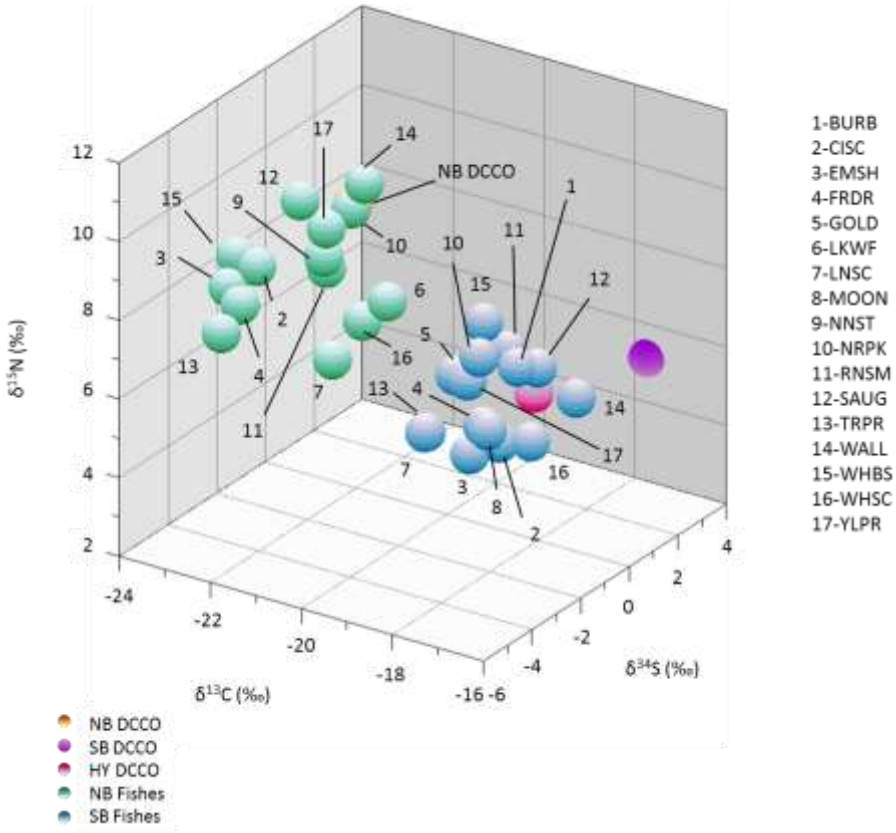


Figure 5.2a Isotopic food web structure for cormorants (*Phalacrocorax auritus*) and fishes collected from Lake Winnipeg during 2009 and 2010. Each point represents the mean δ^{15}N, δ^{13}C and δ^{34}S value of a given species within either the north or south basin. Mean ± standard deviation (SD) isotope values for cormorants and fishes can be found in Tables 5.1 and A3 (Appendix), respectively. Species codes: NB DCCO = north basin cormorants (adult); SB DCCO = south basin cormorants (adult); HY DCCO = hatch-year cormorants; BURB = burbot (*Lota lota*); CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); LNSC = longnose sucker (*Catostomus catostomus*); MOON = mooneye (*Hiodon tergisus*); NNST = ninespine stickleback (*Pungitius pungitius*); NRPK = northern pike (*Esox lucius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catosomus commersonii*); YLPR= yellow perch (*Perca flavescens*).

Table 5.1 Mean ± standard deviation (SD) δ^{15}N, trophic position (TP), δ^{34}S and δ^{13}C for cormorants (*Phalacrocorax auritus*) nesting on Lake Winnipeg. Stable isotope data for muscle is baseline corrected (Equation 5.2). Isotopic units are per mil (‰).

Group	Code		δ^{15}N	TP	δ^{13}C	δ^{34}S
North Basin, Adult	NB DCCO	n = 32	9.4 ± 0.8	5.6 ± 0.5	-21.9 ± 0.7	-0.3 ± 1.6
Cox Island		n = 7	10.1 ± 0.4	5.9 ± 0.2	-22.7 ± 0.4	-1.5 ± 1.8
Eagle Island 2009		n = 3	9.3 ± 0.2	5.5 ± 0.1	-21.5 ± 0.1	2.3 ± 1.0
Eagle Island 2010		n = 10	8.7 ± 0.3	5.1 ± 0.2	-21.4 ± 0.3	-0.6 ± 0.5
George Island		n = 10	9.7 ± 0.7	5.7 ± 0.4	-21.7 ± 0.4	-0.6 ± 1.4
Limestone Bay		n = 1	10.9	6.4	-23.8	1.5
Matheson Island		n = 1	11.0	6.5	-21.7	1.3
South Basin, Adult	SB DCCO	n = 8	5.2 ± 0.9	4.0 ± 0.5	-17.7 ± 1.0	4.0 ± 1.6
South Basin, Hatch-Year	HY DCCO	n = 12	5.5 ± 0.9	4.1 ± 0.5	-18.3 ± 0.4	0.4 ± 1.2

analyses (Parnell et al., 2010). The number of sources in the north basin was reduced by pooling ciscoes with emerald shiner, freshwater drum with yellow perch, and lake whitefish with white sucker, based on similar $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ profiles (Bonferroni, $p \leq 0.05$).

The mean $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values for each of the cormorant sub-groups mentioned above for the north basin are plotted in in Figure 5.2b, along with the mean isotope values for south basin adults, hatch-year birds, and potential food sources. Mean (± SD) $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values for cormorant groupings and potential prey fishes (FL < 400 mm) are given in Tables 5.1 and A4 (Appendix), respectively.

The relative contribution (0 to 100 %) of potential prey fishes to the diet of Lake Winnipeg cormorants varied with location, year and age-class according to the SIAR mixing models (Tables 5.2a-f). In the north basin, troutperch (*Percopsis omiscomaycus*), ciscoes/ emerald shiner and ninespine stickleback (*Pungitius pungitius*) were the fishes most frequently consumed by cormorants. For Cox Island, most potential sources had mean contribution values between 10 to 13 % and 95 % credibility intervals ranging from 0 to 24 % (see Table 5.2a), suggesting that cormorants nesting on this island were likely feeding on a range of prey items. Troutperch and ciscoes/ emerald shiner were the predominant species consumed by Cox Island cormorants. Cormorants collected from George Island in 2009 and 2010 exhibited a similar dependence on ciscoes/emerald shiner (mean = 14 %; 95 % credibility interval = 1, 26 %); however troutperch may have made a slightly larger contribution (mean = 16 %; 95 % credibility interval = 2, 28 %) to their diet (Table 5.2b). Troutperch were also the predominant prey item in the diets of cormorants collected from Eagle Island in 2010 (mean = 21 %, 95 % credibility interval = 10, 32 %; Table 5.2c). In 2009, Eagle Island birds were less reliant on troutperch (Table 5.2d) and exhibited a greater dependence on ninespine stickleback (mean = 14 %, 95 % credibility interval

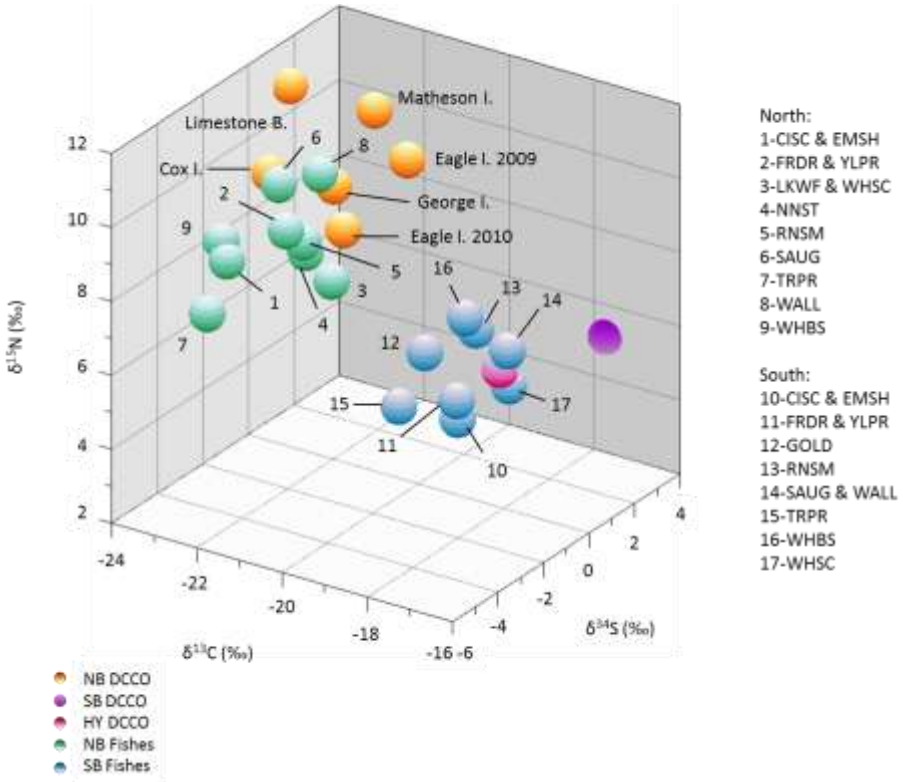


Figure 5.2b Isotopic food web structure for cormorants (*Phalacrocorax auritus*) and their potential prey. Points represent the mean δ^{15}N, δ^{13}C and δ^{34}S values of predator (cormorant, DCCO) groupings and potential prey sources (fishes with fork lengths (FLs) < 400 mm) used in SIAR mixing models. Mean ± standard deviation (SD) isotope values for DCCO groupings and sources can be found in Tables 5.1and A4 (Appendix), respectively. Codes: NB DCCO = north basin cormorants (adult); SB DCCO = south basin cormorants (adult); HY DCCO = hatch-year cormorants; CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); NNST = ninespine stickleback (*Pungitius pungitius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catosomus commersonii*); YLPR= yellow perch (*Perca flavescens*).

Table 5.2a Contribution of prey sources to the overall diet of Cox Island cormorants (*Phalacrocorax auritus*) (2009; n = 7). Contribution estimates are based on a three-isotope (δ^{15}N, δ^{13}C and δ^{34}S) Bayesian mixing model (SIAR) and expressed in terms of percent (0 to 100 %).

Prey Item(s)[a]	Mean (%)	95 % Credibility Interval (%)
CISC & EMSH	13	0, 24
FRDR & YLPR	10	0, 21
LKWF & WHSC	10	0, 21
NNST	11	0, 21
RNSM	11	0, 22
SAUG	11	0, 21
TRPR	13	0, 24
WALL	10	0, 20
WHBS	12	0, 23

[a]Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); LKWF = lake whitefish (*Coregonus clupeaformis*); NNST = ninespine stickleback (*Pungitius pungitius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

Table 5.2b Contribution of prey sources to the overall diet of George Island cormorants (*Phalacrocorax auritus*) (2009 & 2010; n = 10). Contribution estimates are based on a three-isotope (δ^{15}N, δ^{13}C and δ^{34}S) Bayesian mixing model (SIAR) and expressed in terms of percent (0 to 100 %).

Prey Item(s)[a]	Mean (%)	95 % Credibility Interval (%)
CISC & EMSH	14	1, 26
FRDR & YLPR	8	0, 18
LKWF & WHSC	10	0, 20
NNST	13	1, 23
RNSM	12	0, 23
SAUG	7	0, 17
TRPR	16	2, 28
WALL	7	0, 16
WHBS	13	1, 24

[a]Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); LKWF = lake whitefish (*Coregonus clupeaformis*); NNST = ninespine stickleback (*Pungitius pungitius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

Table 5.2c Contribution of prey sources to the overall diet of Eagle Island cormorants (*Phalacrocorax auritus*) (2010; n = 10). Contribution estimates are based on a three-isotope (δ^{15}N, δ^{13}C and δ^{34}S) Bayesian mixing model (SIAR) and expressed in terms of percent (0 to 100 %).

Prey Item(s)[a]	Mean (%)	95 % Credibility Interval (%)
CISC & EMSH	13	1, 24
FRDR & YLPR	10	0, 20
LKWF & WHSC	11	0, 22
NNST	11	0, 21
RNSM	11	0, 22
SAUG	7	0, 17
TRPR	21	10, 32
WALL	6	0, 15
WHBS	11	0, 20

[a]Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); LKWF = lake whitefish (*Coregonus clupeaformis*); NNST = ninespine stickleback (*Pungitius pungitius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

Table 5.2d Contribution of prey sources to the overall diet of Eagle Island cormorants (*Phalacrocorax auritus*) (2009; n = 3). Contribution estimates are based on a three-isotope (δ^{15}N, δ^{13}C and δ^{34}S) Bayesian mixing model (SIAR) and expressed in terms of percent (0 to 100 %).

Prey Item(s)[a]	Mean (%)	95 % Credibility Interval (%)
CISC & EMSH	12	0, 22
FRDR & YLPR	10	0, 20
LKWF & WHSC	12	0, 24
NNST	14	0, 26
RNSM	12	0, 24
SAUG	9	0, 19
TRPR	10	0, 21
WALL	10	0, 20
WHBS	11	0, 22

[a]Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); LKWF = lake whitefish (*Coregonus clupeaformis*); NNST = ninespine stickleback (*Pungitius pungitius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

Table 5.2e Contribution of prey sources to the overall diet of adult cormorants (*Phalacrocorax auritus*; n = 8) collected from Lake Winnipeg's south basin (SB DCCO). Contribution estimates are based on a three-isotope (δ^{15}N, δ^{13}C and δ^{34}S) Bayesian mixing model (SIAR) and expressed in terms of percent (0 to 100 %).

Prey Item(s)[a]	Mean (%)	95 % Credibility Interval (%)
CISC & EMSH	16	0, 32
FRDR & YLPR	13	0, 26
GOLD	9	0, 22
RNSM	11	0, 25
SAUG & WALL	12	0, 26
TRPR	10	0, 22
WHBS	10	0, 22
WHSC	19	0, 37

[a]Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

Table 5.2f Contribution of prey sources to the overall diet of hatch-year cormorants (*Phalacrocorax auritus*; n = 12) collected from Lake Winnipeg's south basin (HY DCCO). Contribution estimates are based on a three-isotope (δ^{15}N, δ^{13}C and δ^{34}S) Bayesian mixing model (SIAR) and expressed in terms of percent (0 to 100 %).

Prey Item(s)[a]	Mean (%)	95 % Credibility Interval (%)
CISC & EMSH	16	0, 30
FRDR & YLPR	15	0, 29
GOLD	11	0, 24
RNSM	7	0, 19
SAUG & WALL	6	0, 15
TRPR	31	9, 56
WHBS	7	0, 18
WHSC	8	0, 19

[a]Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

= 0, 26 %). Diet of south basin adults deviated from that of north basin adults. White sucker (mean = 19 %, 95 % credibility interval = 0, 37 %) were the predominant prey item consumed by cormorants nesting on the Black and Devil's Island colonies (Table 5.2e). The SIAR mixing models for adults and hatch-year cormorants nesting in the south basin suggested a similar dependence on ciscoes/ emerald shiner (Tables 5.2e and 5.2f). It would appear however, that hatch-year cormorants tended to consume troutperch (mean = 31 %, 95 % credibility interval = 9, 56 %) in greater amounts than each of the other sources.

The proportion of walleye and sauger consumed by cormorants in relation to other prey fishes varied depending on colony location, cormorant age and year (Eagle Island). Based on the 95 % credibility interval, less than 15 % of the hatch-year cormorant diet consisted of walleye and sauger (mean = 6 %), so it is unlikely these fishes were key prey items for this age group (Doucette et al., 2011). For adult cormorants, walleye and sauger made the smallest contribution to the diets of birds nesting on the south basin (mean = 12 %, 95 % credibility interval = 0, 26 %; Table 5.2e). Mean estimates of walleye (< 10 %) and sauger (< 11 %) consumption in the north basin appeared to be relatively low, however together these two species represented, on average, 13-22 % of the cormorant diet, depending on location and year (Tables 3a-d).

5.3.2 Trace Element Concentrations

Cormorants were similar to the fishes examined in Chapter 3 in that Fe was the element measured at the greatest concentrations in muscle (Table 5.3). Concentrations of Al in cormorant muscle were consistently below the LoD. Recoveries of Cu from DORM-2 (78 %) and CVs on duplicate samples (CV ≤ 17 %) fell outside the acceptable range for cormorants collected and analyzed in 2010; therefore these values were not included in the statistical analyses. Copper concentrations for cormorants collected and analyzed in 2009 were within acceptable limits, and have been included in Table 5.3.

Table 5.3 Mean ± standard deviation (SD) concentrations of Al, As, Cd, Cu, Fe, Mn, Hg and Se measured in pectoral muscle of cormorants nesting on Lake Winnipeg. Concentrations are expressed in units of µg/g dry weight (DW).

Code[a]	Al	As	Cd	Cu
NB DCCO (n = 32)	< LoD	0.14 ± 0.10	0.10 ± 0.05	20.86 ±1.72[b]
SB DCCO (n = 8)	< LoD	0.09 ± 0.10	0.06 ± 0.03	16.72 ± 5.83
HY DCCO (n = 12)	< LoD	0.07 ± 0.01	0.06 ± 0.01	NA[c]

Table 5.3 Continued.

Code[a]	Fe	Mn	Hg	Se
NB DCCO (n = 32)	247.92 ± 103.35	2.00 ± 0.82	1.79 ± 1.23	1.60 ± 0.63
SB DCCO (n = 8)	232.10 ± 94.18	1.71 ± 0.27	3.78 ± 2.91	1.32 ± 0.38
HY DCCO (n = 12)	161.15 ± 43.33	1.28 ± 0.30	2.00 ± 0.61	1.08 ± 0.18

[a]NB DCCO = north basin cormorants; SB DCCO = south basin cormorants; HY DCCO = hatch-year cormorants
[b]2009 (n = 15) data only; 2010 analytical batch did not pass Quality Control (QC)
[c]NA = not available; analytical batch did not pass QC

The mean rankings of log-transformed Cd, Hg and Se data were not significantly different among adult cormorants from either basin or hatch-year birds at the $p \leq 0.05$ level (See Table 5.3; Kruskal-Wallis test; Cd: $H = 4.51$, 2 df, $p = 0.10$; Hg: $H = 4.44$, 2 df, $p = 0.11$; Se: $H = 5.37$, 2 df, $p = 0.07$). Similarly, log-transformed concentrations of Cu in muscle of north and south basin adults (2009 only) likely came from the same distribution (Mann-Whitney *U*-test; $U = 84$, $p = 0.13$). Mean ranked log(As) values were the greatest in adult cormorants from the north basin, yet they were similar among adult and hatch-year cormorants from the south basin (Kruskal-Wallis test: $H = 16.78$, 2 df, $p = 0.0002$). This was the only element for which a significant difference in concentrations could be found among the north and south basin adults. Log(Fe) and log(Mn) were significantly different among adults and hatch-year birds, with hatch-year individuals having lower mean ranked values than adults (Kruskal-Wallis test; Fe: $H = 11.47$, 2 df, $p = 0.003$; Mn: $H = 13.96$, 2 df, $p = 0.0009$).

5.3.3 Biomagnification (δ^{15}N)

The results of the AIC_c model selection procedures provided evidence that δ^{15}N had a strong positive effect on most elements measured in adult cormorants from the north basin (Cd, Fe, Mn, Hg and Se). This would suggest that cormorants feeding at higher TPs accumulated greater amounts of these elements in muscle (Table A5, Appendix) (Campbell et al., 2005). The strongest effect of δ^{15}N or TP on elemental concentrations was observed for Hg (PE = 0.96, SE = 0.13, 95 % CI = 0.71, 1.21). This would suggest that for every 1 ‰ increase in δ^{15}N, log(Hg) would increase by a factor of 0.96, and µg/g DW Hg concentrations would more than double (2.61 times larger). Every 1 ‰ increase in δ^{15}N was also met with an approximate 1.5-fold (1.46 and 1.47, respectively) increase in Cd and Se concentrations (µg/g DW; Cd: PE = 0.38, SE = 0.10, 95 % CI = 0.18, 0.57; Se: PE = 0.39, SE = 0.07, 95 % CI = 0.26, 0.53). The rate of biomagnification was also similar between Fe and Mn in north basin birds (Fe: PE = 0.28, SE =

0.06, 95 % CI = 0.16, 0.40; Mn: MAE = 0.26, uSE = 0.06, 95 % uCI = 0.13, 0.38) (Burnham and Anderson, 2002; Pluess et al., 2011). In the south basin, δ^{15}N had a strong positive effect on Hg in adult birds only (MAE = 0.50, uSE = 0.23, 95 % uCI = 0.05, 0.95), although the rate of increase in Hg with increasing δ^{15}N was only half that of north basin birds.

5.3.4 Dilution Effects (δ^{15}N)

The only strong negative correlation between δ^{15}N and trace element concentrations in cormorant muscle was for log(Fe) in south basin adults (PE = -0.07, SE = 0.03, 95 % CI = -0.13, -0.02).

5.3.5 Effects of Size & Sex

Weight and sex had little influence on Hg and trace element concentrations in cormorant muscle. A weak positive effect of tarsus length on log(As) was identified for north basin adults, with every 1 mm increase in length resulting in an approximately 2 % increase in As (PE = 0.02, SE = 0.03, 95 % CI = -0.03, 0.07). Weight was included in the top-ranking models for Se in north basin adults and Hg in hatch-year birds (Table A5, Appendix); however, the influence of weight was not strong in either instance (NB DCCO: PE = 0.00, SE = 0.00, 95 % CI = 0.00, 0.00; HY DCCO: MAE = 0.00, uSE = 0.00, 95 % uCI = 0.00, 0.00). Sex did have strong effect on log(Hg) concentrations in HY DCCO however, with males accumulating less Hg in pectoral muscle than females (MAE_{Males} = -0.28, uSE = 0.14, 95 % uCI = -0.55, -0.01).

5.3.6 Influence of Energy Source (δ^{13}C, δ^{34}S)

Few models containing δ^{13}C or δ^{34}S were ranked within the top models ($\Delta AIC_c \leq 2.00$) listed in Table A5 (Appendix). Hatch-year cormorants which obtained dietary carbon from pelagic sources (smaller δ^{13}C), rather than near the sediment (larger δ^{13}C) had greater concentrations of As in muscle (MAE = -0.25, uSE = 0.11, 95 % CI = -0.47, -0.03). Adult cormorants from the north basin which were increasingly reliant on water-column sources of sulfate (larger δ^{34}S

values) (Cucherousset et al., 2011) tended to accumulate more As in muscle (PE = 0.19, SE = 0.06, 95 % CI = 0.07, 0.31).

5.4 Discussion

5.4.1 Diet

The diets of adult cormorants nesting on Lake Winnipeg differed from those of hatch-year birds, and varied among basins and colonies. However, one overriding commonality between adult and hatch-year cormorants, regardless of nesting or feeding location, was that the major dietary fraction likely consisted of fishes with little or no direct commercial value (Tables 5.2a-f). The TPs occupied by adult cormorants collected near Eagle and George Islands in 2009 and 2010 were quite similar to those of cormorants nesting on Canoe, Dore and Last Mountain Lakes in Saskatchewan (TP = 5.0 ± 0.5, n = 106) during 2006 and 2007 (Doucette et al., 2011). These lakes had many fish species in common with Lake Winnipeg, and mixing models revealed that ciscoes and ninespine stickleback were among the most heavily-consumed fishes (Doucette et al., 2011). The proportion of yellow perch in the diet of cormorants from Last Mountain Lake (mean SIAR estimate = 44 %) and in regurgitation samples collected from Lake Winnipegosis in 1987 (27.6 % of biomass) (Hobson et al., 1989) were much larger than estimated for Lake Winnipeg cormorants. The largest mean yellow perch contribution identified from the SIAR mixing models was for hatch-year cormorants in the south basin (Table 5.2f) and the mean estimate (15 %) was only slightly greater than the biomass estimate of 13 % presented by Ludwig et al. (1989), who examined regurgitates of cormorants nesting on Lakes Huron, Michigan and Superior in the late 1980s. Yellow perch consumption by hatch-year cormorants was similar to consumption of ciscoes/ emerald shiner (mean = 16 %, 95 % credibility interval = 0, 30) and lower than that of troutperch (mean = 31 %, 95 % credibility interval = 9, 56; Table 5.2e).

In the south basin, adult (TP = 4.0 ± 0.5, n = 8) and hatch-year (TP = 4.1 ± 0.5, n = 12) cormorants occupied TPs similar to, or only slightly higher than, those of all sauger (TP = 3.9 ± 0.4, n = 111) and walleye (TP = 3.6 ± 0.5, n = 152) collected in 2009 and 2010 (Chapter 3). Sauger and walleye from the lake's north basin occupied a TP of 5.0 (SAUG: SD = ± 0.06, n = 34; WALL: SD = ± 0.4, n = 124), which was slightly lower than that of cormorants from Eagle Island in 2009 (TP = 5.5 ± 0.1, n = 3) and 2010 (TP = 5.1 ± 0.2, n = 10; Table 5.1). However, cormorants from Cox Island, Matheson Island and near Limestone Bay occupied TPs which were nearly one to one-and half steps above walleye and sauger from the same basin (Table 5.1). Although the SIAR analyses for Cox Island birds suggested that together, walleye and sauger (FLs ≤ 400 mm) were marginally important prey items (mean dietary contribution = 10 % and 11 %, respectively; see Table 5.2a), it was possible Cox Island cormorants were feeding on sources which could not be account for in the analyses. The same may have also been true for the Matheson Island and Limestone Bay cormorants, since these individuals had isotopic values which fell outside the mixing space (Phillips and Koch, 2002). Cox Island and Matheson Island are within close proximity to a number of commercial fish packing stations (Manitoba Water Stewardship, 2010), and therefore cormorants may have had access to walleye and sauger tissues which were discarded as waste (Fox et al., 2002). Consumption of processing wastes from high-TP fishes may have elevated the δ^{15}N values measured in cormorant muscle, and as a result, potentially confounded the SIAR data. Also, during the Cox Island colony visit in 2010, not only was there a notable reduction in the number of birds present, but also a large number of fishing vessels were observed within close proximity to the island (A. Ofukany, *personal observation*). It is possible that cormorants were opportunistically consuming fishes trapped in nets (Fox et al., 2002), and as a result, were coming into conflict with local fishermen. With respect to the

cormorant collected from near Limestone Bay, it is possible this specimen was feeding predominantly within the Bay (northwest of the north basin, Figure 5.1), which would have potentially confounded the results of the baseline correction (Equation 5.2).

Little historical dietary data is available for cormorants or other water birds which nest on Lake Winnipeg. Adult Herring Gulls (*Larus argentatus*; n = 19) collected near Grand Rapids (north basin) during 1991 to 1993 occupied a mean TP of 3.7 (Fox et al., 2002). This is substantially lower than the TP derived here for north basin cormorants; however, these two species may not have necessarily consumed the same prey items. If the raw mean $\delta^{13}C$ value for adult gulls (-24.8 ± 0.7 ‰) were to be normalized according to the $\delta^{13}C_{DIC}$ baseline correction used in Equation 5.2, gull tissues would be ^{13}C-enriched ($\delta^{13}C \approx$ -20.5 ‰) relative to cormorants (Table 5.1). It is possible the gull data presented by Fox et al. (2002) is representative of Lake Winnipeg's conditions prior to some of the major environmental (i.e. record floods, changes in flow rate) and food web perturbations which govern the current conditions of the lake (Environment Canada and Manitoba Water Stewardship, 2011).

5.4.1 Mercury & Other Trace Elements

Although the processes which best explained elemental concentrations in cormorant muscle differed according to colony location and bird life-stage (HY versus adult), concentrations of many elements, such as Al (< LoD), Cd, Cu, Hg and Se did not differ between basins or among age groups. It was expected that Hg concentrations in muscle of hatch-year birds would reflect Hg concentrations in south basin fishes (Fox et al., 2002) since the confounding effects of Hg exposure during winter were negated (see Chapter 6). Griechus et al. (1973) found that for hatch-year cormorants inhabiting the Poinsett and Dry Lakes in South Dakota, concentrations of Hg in muscle were 1.3-fold greater than concentrations in fishes. The hatch-year birds examined here shared a TP with burbot (*Lota lota*), northern pike (*Esox lucius*), sauger, and white bass (*Morone*

chrysops). The $\delta^{13}C$ and $\delta^{34}S$ values for this group of birds were most similar to sauger (Table A3, Appendix); however, muscle-Hg of hatch-year cormorants (Table 5.2) were approximately double that of sauger (mean Hg_{SAUG} = 1.10 ± 0.60 µg/g DW, n = 19; Chapter 3). These results were surprising, given the similarities in the isotopic profiles of these species and the fact that much of the Hg consumed by young cormorants is sequestered in newly-formed feathers, rather than muscle (Caldwell et al., 1999). Concentrations of Hg in adult cormorants from both basins were statistically similar to those in young birds and as a group; cormorants had the greatest muscle Hg concentrations of any species examined (see Chapter 3). Unlike hatch-year birds however, Hg concentrations in the muscle of adult cormorants were a function of $\delta^{15}N$, or TP. The 0.50-fold increase in log-transformed Hg with every 1 ‰ increase in $\delta^{15}N$ for adults collected in the south basin was quite similar to the 0.48-fold increase reported for Little Auks (*Alle alle*), Brünnich's Guillemots (*Uria lomvia*), Black-legged Kittiwakes (*Rissa tridactyla*), Northern Fulmars (*Fulmarus glacialis*) and Glaucous Gulls (*Larus hyperboreus*) collected near Kongsfjorden, Svalbard (Jaeger et al., 2009). However, mean concentrations of Hg measured in the muscle of Lake Winnipeg cormorants (Table 5.3) were substantially greater than even the largest concentrations reported for Kongsfjorden seabirds (largest mean ± SE = 1.07 ± 0.20 for Glaucous Gulls; assuming 70 % moisture) (Campbell et al., 2005; Jaeger et al., 2009). When the Hg concentrations of Lake Winnipeg cormorants were compared to those measured in the muscle of a Common Loon (*Gavia immer*; Hg = 5.0 µg/g DW, n = 1), American Coot (*Fulica Americana*; Hg = 1.13 µg/g DW, n = 1) and Herring Gulls (mean Hg = 5.67 µg/g DW, range = 2.2 to 13.33 µg/g DW, n = 5) collected from Tadenac Lake, Ontario, concentrations were fairly similar among hatch-year cormorants and the single coot sample (Table 5.3) (Wren et al., 1983).

Mercury concentrations in all Lake Winnipeg cormorant samples were much smaller than concentrations reported for the loon and Herring Gulls.

The biomagnification effects observed within the north basin cormorant community were unexpected for elements such as Cd and Mn. Trophic position had a negative effect on Cd concentrations in birds, fishes and other organisms collected from the Northwater Polynya, Baffin Bay (Campbell et al., 2005). Bioaccumulation of Cd was reported for seabirds collected from the Barents Sea in 1991 and 1992; however this was attributed to age, rather than trophic niche (Savinov et al., 2003). Although Cd appears to follow a biomagnification-type trend within cormorants collected from Lake Winnipeg's north basin, the mean concentration was an order of magnitude lower than values measured in Little Auks (mean = 1.37 ± 0.33 µg/g DW, n = 10), Black-legged Kittiwakes (mean = 1.53 ± 0.8 µg/g DW, n = 10), Black Guillemots (*Ceppus grille*; mean = 1.40 ± 0.50 µg/g DW, n = 10), Thick-billed Murres (*Uria lomvia*; mean = 1.80 ± 0.87 µg/g DW, n = 10), Ivory Gulls (*Pagophilia eburnean*; mean = 0.63 ± 0.40 µg/g DW, n = 5), Northern Fulmar (mean = 3.90 ± 3.90 µg/g DW, n = 10) and Glaucous Gulls (mean = 0.70 ± 0.53, n = 10) collected from the Northwater Polynya, assuming a moisture content of 70 % (mean = 0.10 ± 0.05 µg/g DW, n = 32; Table 5.2) (Campbell et al., 2005). Concentrations of Mn were not related to δ^{15}N in the same Arctic Polynya food web, or in the food web of Tadenac Lake (Wren et al., 1983). Herring Gulls (n =5) collected from Tadenac Lake had muscle-Mn concentrations which did not differ from concentrations in rainbow smelt, northern pike or other fishes, and ranged from 2.0 to 4.7 µg/g DW. The Mn concentrations measured in the muscle of adult cormorants from the north basin were on the low end of this range (mean = 2.00 ± 0.82 µg/g DW; Table 5.3), and concentrations in adult and hatch-year cormorants from Lake

Winnipeg's south basin were even smaller (mean = 1.71 ± 0.27 µg/g DW and 1.28 ± 0.30 µg/g DW, respectively).

In summary, the findings of this multi-isotopic investigation suggest that on average, walleye and sauger represented a comparatively small proportion of the diet in adult and hatch-year cormorants. It is possible some of the cormorants studied were consuming wastes from commercial processing facilities or preying on netted fish; however, these phenomena need to be investigated more thoroughly. It would appear that although cormorants are a migratory species which can be exposed to concentrations of contaminants throughout the year (see Chapter 6), concentrations of most elements were related to δ^{15}N values in muscle, which would have represented dietary habits over the approximately six week period prior to capture (Hobson, 2009). Concentrations of As in cormorant muscle were lower than those measured in Lake Winnipeg fishes, and Cd, Mn and Se were fairly similar in fish and avian samples. However, concentrations of Hg were elevated in cormorants relative to fishes. Therefore, measurements on cormorant tissues may be a valuable tool for monitoring Hg concentrations in the Lake Winnipeg food web (see Chapter 6).

CHAPTER 6
6.0 CONNECTING BREEDING AND WINTERING HABITATS OF MIGRATORY PISCIVOROUS BIRDS: IMPLICATIONS FOR TRACKING CONTAMINANTS (HG) USING MULTIPLE STABLE ISOTOPES

6.1 Introduction

Interpreting concentrations and origins of contaminants in tissues of migratory animals presents unique challenges in that exposure may occur at breeding, migratory or over-wintering sites (Dietz et al., 2009; Hargreaves et al., 2010). Organic contaminants (Hebert, 1998) and Hg (Bond and Diamond, 2009) accumulate in body tissues and are readily transported by migratory animals from one region to another. In birds, contaminants stored within endogenous energy reserves (e.g. lipids) can be mobilized to eggs, and therefore eggs have been used for monitoring contaminants, especially in colonial water birds (Fox et al., 2002; Hargreaves et al., 2010). However, feathers may provide a more valuable proxy for seasonal Hg exposure in migratory birds, since a) non-lethal sampling methods may be employed; b) feather Hg concentrations are highly correlated with concentrations in blood during the period of feather growth (Burgess et al., 2005), c) the internal Hg concentration becomes fixed once the feather is fully formed (Burger and Gochfeld, 1999) and d) most of the Hg body burden in water birds is sequestered in feathers (Burger and Gochfeld, 1999). Feathers may be an effective mode of Hg biotransport and deposition, as they are often molted at sites far removed from the initial source of Hg exposure. For example, long-lived, piscivorous seabirds may transport marine-sourced Hg to inland colonies, where concentrations in aquatic sediments from feathers, eggs, carcasses and feces may reach toxic levels (Blais et al., 2005, 2007). Alternatively, species such as the Common Loon (Gavia immer), Common Tern (Sterna hirundo) and Roseate Tern (Sterna dougallii) accumulate

more Hg in summer-grown feathers, and may therefore transport Hg from northern breeding sites to the wintering grounds (Burger et al., 1992; Burgess et al., 2005; Nisbet et al., 2002).

Despite compelling reasons to assess where migratory individuals are most exposed to contaminants throughout the year and whether potential exists for the biological transport of contaminants across habitats or regions, it has been extremely difficult to infer the geographical origins of tissue-borne contaminants (Hebert, 1998). The use of stable isotope assays in animal tissues provides a means to track origins of migratory wildlife (Hobson and Wassenaar, 1997; Hobson et al., 2006, 2009a). Stable isotope ratios for muscle, feathers, and so on, represent the accumulation of dietary elements (H, C, N and S) in tissues, as well as any isotopic discriminatory processes which occur during nutrient uptake, distribution, and excretion. For isotopes of carbon and sulfur, there is little to no metabolic isotopic discrimination at each trophic step, and so $\delta^{13}C$ and $\delta^{34}S$ values for consumer tissues show little deviation from those of dietary items (Fry, 1988). Ratios of $^{2}H/^{1}H$ in tissues are also used to link consumers with a specific baseline, typically $\delta^{2}H$ in precipitation; however the effects of metabolic isotopic discrimination must be accounted for (Clark et al., 2006, 2009; Van Wilgenburg and Hobson, 2010). Nitrogen isotope data is used as an indicator of TP rather than as a linkage to baseline values, since metabolic processes result in a 2 to 5 ‰ enrichment in tissue ^{15}N at each trophic step (Kelly, 2000). When combined with contaminant analyses, these multi-isotopic tools may allow for better interpretation of sources and concentrations of dietary contaminant exposure throughout the annual cycle. This approach was tested by examining Hg concentrations in Double-crested Cormorant (*Phalacrocorax auritus*) feathers grown on the species' wintering and breeding grounds.

The Double-crested Cormorant (hereafter "cormorant") is a large, piscivorous water bird found throughout south-central Canada, the United States, and northern Mexico (Hatch and Weseloh, 1999). Populations declined during the 1960s and 70s, likely due to persecution and the widespread application of organochlorine insecticides (Somers et al., 1993); however, with increased legislative protection, reductions in organic pesticide use, and increased food availability, numbers have rebounded to historical levels over the past decades (Hebert et al., 2008; Wires and Cuthbert, 2010). From mid-April into September, the largest breeding populations can be found throughout the north-central interior region of North America, including Lake Winnipeg, Manitoba, and the Laurentian Great Lakes (Hatch and Weseloh, 1999; Wires et al., 2001). Band recovery data suggest birds breeding on Manitoban lakes overwinter near the Gulf of Mexico, with a high proportion of birds concentrating in the Lower Mississippi Valley (Dolbeer, 1991). Predation of commercial freshwater catfish (*Ictalurus punctatus*) within this region has become a source of human-cormorant conflict. Increases in aquaculture extent and productivity throughout Mississippi, Arkansas, Alabama and Louisiana have inadvertently provided cormorants with a high-density alternative food source during the winter, and some reports suggest much of the cormorant population has abandoned natural marine systems in favor of aquaculture resources (Hebert et al., 2008; OMNR, 2006). It is estimated these birds consume as much as 3-7 % of the annual aquaculture production (Dorr et al., 2004; Trapp et al., 1997); however, the real versus perceived financial and ecological impacts have proven difficult to quantify.

Cormorants are of interest not only because of their wide-spread distribution, large population, and socio-economic importance, but also because they have many biological characteristics amenable to isotopic and contaminant studies. Cormorants molt primary feathers

throughout the year (Hatch and Weseloh, 1999) and so feathers from the wintering grounds may be retained well into the breeding season, and vice versa. As a result, a single, annual sampling event on either the breeding or wintering grounds may provide sufficient information for the monitoring of seasonal changes in diet, habitat use, and contaminant accumulation. Here, primary feathers which had been harvested from birds during the 2009 summer breeding season were analyzed for Hg and a suite of stable isotopes (δ^2H, δ^{34}S, δ^{13}C and δ^{15}N). The use of δ^{34}S data in identifying marine versus freshwater food consumption in cormorants was similar to that of Hebert et al. (Hebert et al., 2008), however it was anticipated the weight of evidence from a multi-isotope approach would allow for more powerful inferences than those set forth previously (Hebert et al., 2008). Here, correlations between hydrogen (δ^2H) isotope data for feathers known to be grown in freshwater habitats (i.e. from δ^{34}S data) and latitudinal isoscape patterns in North America were examined in order to link periods of feather growth and feather-Hg concentrations to the wintering grounds or summer breeding sites on Lake Winnipeg (Hobson and Wassenaar, 2008). Additional stable isotope (δ^{13}C and δ^{15}N) analyses of feathers and commercial catfish foods were used in further separating aquaculture-derived and natural freshwater food sources for winter-grown feathers. Carbon isotope data was expected to be useful in tracing the transfer of aquaculture resources through the food web, since commercial catfish foods contain up to 40 % C_4-corn (Robinson et al., 2001), and are therefore significantly enriched in ^{13}C compared to natural (C_3) freshwater foods. This study followed from Yerkes et al. (2008), who used isotopic threshold values to separate potential biomes used by migratory waterfowl. This provided regions in an isotopic solution space that were consistent with a particular biome. The threshold approach was chosen over a mixing model with defined dietary isotopic endpoints (Moore and Semmens, 2008) because birds could potentially use a range of isotopically distinct habitats

within freshwater, marine, and brackish biomes, making it difficult to derive single endpoints with appropriate variance estimates. It was decided this threshold approach could best demonstrate the utility of multi-isotope assays in tracking contaminant origins, given any associations between Hg and broad habitat correlates could be identified.

6.2 Methods

6.2.1 Sample Collection

Twenty-three adult cormorants were collected from various locations on Lake Winnipeg, Manitoba, Canada (53° 17'N, 97° 58'W; Figure 6.1) during May-August 2009. A workboat from the *MV Namao* was used to approach foraging cormorants and to reach remote island colonies. Birds representing each of the known colonies on Lake Winnipeg were shot by trained staff in accordance with applicable permits and firearms regulations. Whole carcasses were frozen (-20°C) onboard until they could be transported to the laboratory in Saskatoon, Canada. Second, fourth, sixth, eighth, and tenth primary feathers (n = 113 feathers) were taken from one wing of each bird (Hebert et al., 2008), since these feathers provided sufficient biomass for analyses. Feather vane material was shorn from the rachis using stainless-steel scissors and then prepared for stable isotope assays. Once the isotopic dataset was complete, the remaining feather material was prepared for Hg analysis. Since stable isotope ratios (δ^2H, δ^{34}S, δ^{13}C and δ^{15}N) and Hg concentrations were quantified for individual feathers, errors associated with pooling of samples potentially grown at different locations (see below) could be eliminated.

6.2.2 Stable Isotopes

Feathers were cleaned with multiple 2:1 (v/v) chloroform: methanol rinses, then dried in a fume hood overnight. Stable isotope ratios of cleaned feather material were determined using an elemental analyzer coupled with CF-IRMS (Hobson et al., 2009). In order to reduce error associated with the exchange of hydrogen between feather material and ambient air for δ^2H

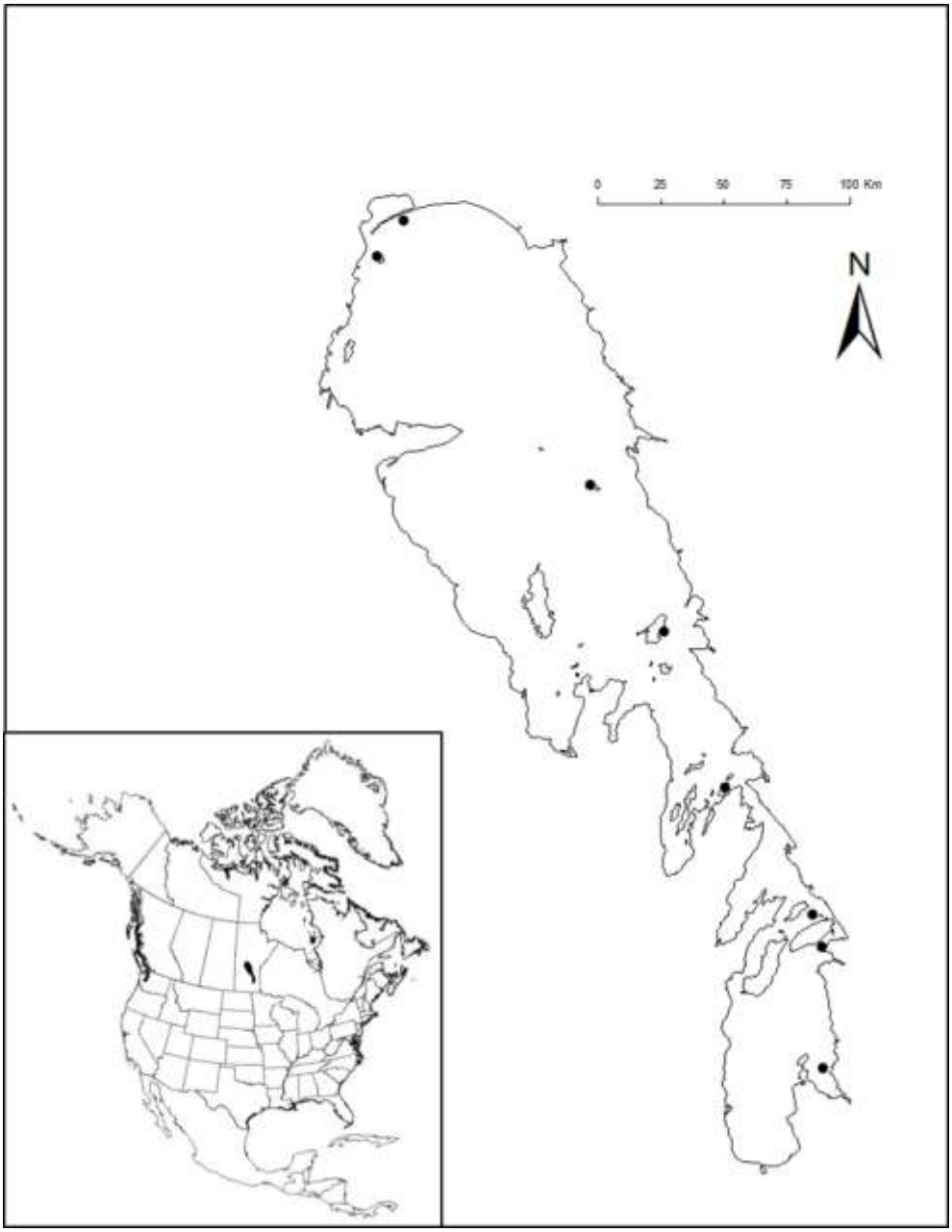


Figure 6.1 Map of Lake Winnipeg with cormorant (*Phalacrocorax auritus*) collection locations. Circles represent collection sites for the period of May-August 2009. The inset shows the position of Lake Winnipeg within North America.

assays, samples and isotopic standards were treated using the comparative equilibration method described by Wassenaar and Hobson (2003). Hydrogen isotope samples (350 µg) were pyrolysed (Wassenaar and Hobson, 2003) and analyzed along with three calibrated, in-house keratin laboratory standards; Cow Hoof Standard (CHS; δ^2H = -187 ‰), BWB (δ^2H = -108 ‰) and CFS (δ^2H = -147.4 ‰), all with SD values better than ± 1.0 ‰ for within-run replicate measurements. For isotopes of sulfur, the BWB and CFS lab standards had calibrated mean δ^{34}S ± SD values of +17.5 ± 0.3 ‰ and -3.8 ± 0.1 ‰, respectively. For carbon and nitrogen isotopes, BWB (δ^{13}C = -18.5 ‰, δ^{15}N = 14.4 ‰) and porcine (PRC) gelatin (δ^{13}C = -13.5 ‰, δ^{15}N = 4.69 ‰) laboratory standards were used, both of which had SD values better than ± 0.2 ‰.

All stable isotope ratios were reported in the delta notation as parts per thousand (‰) deviation from an appropriate international elemental standard according to the equation:

$$\delta X = (R_A / R_S - 1) \cdot 1000 \quad (6.1)$$

where, X was the heavy isotope (^{13}C, ^{15}N, ^{2}H, ^{34}S), R_A was the isotopic ratio (^{13}C/^{12}C, ^{15}N/^{14}N, ^{2}H/^{1}H, ^{34}S/^{32}S) of the sample and R_S was the isotope ratio of the international standard. (δ^{13}C: VPDB; δ^{15}N: AIR; δ^2H: Vienna Standard Mean Ocean Water, [VSMOW]; δ^{34}S: VCDT).

6.2.3 Assigning Feather Origins

A decision system based on a series of isotopic thresholds was used to delineate origins of individual cormorant primary feathers (Figure 6.2). Since the feather δ^2H isoscape used for assigning geographical origins is only valid for the terrestrial/freshwater environment, samples with clear isotopic evidence of marine dietary inputs had to be identified and removed from the δ^2H dataset (Larson and Hobson, 2009; Lott et al., 2003). A δ^{34}S threshold of 10 ‰ was used to separate freshwater (<10 ‰) and marine (>10 ‰) inputs to individual feathers. Feathers falling into the “marine” category (n = 4 feathers [2 birds]) were automatically assigned to the wintering

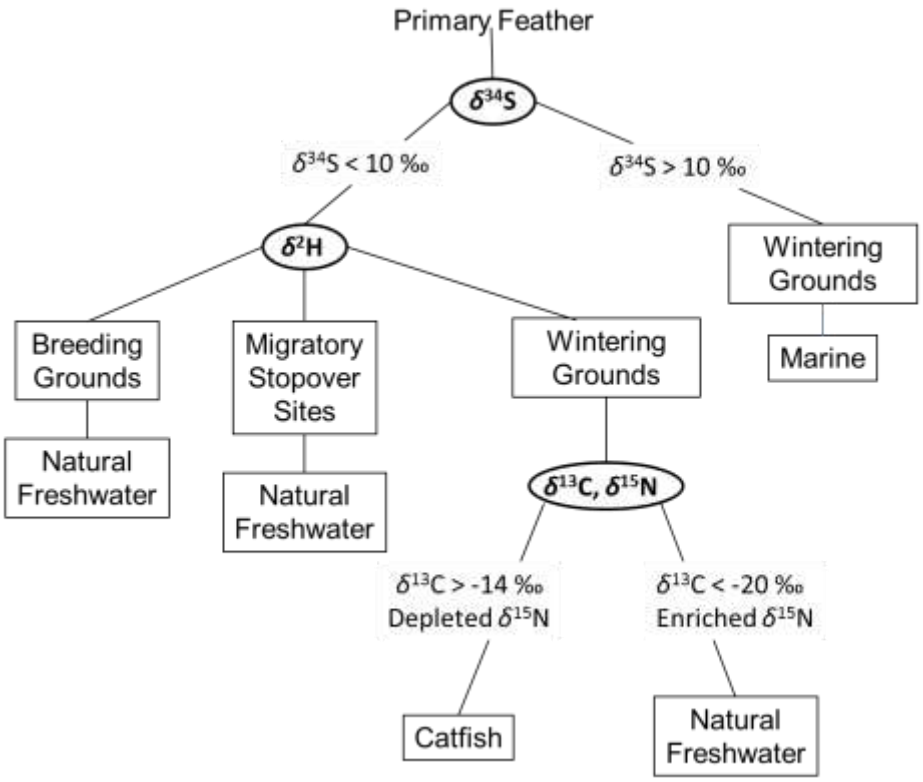


Figure 6.2 Decision-based threshold approach used to delineate origins of feathers collected from adult cormorants (*Phalacrocorax auritus*) nesting on Lake Winnipeg.

grounds. This was based on the isotopic thresholds derived by Lott et al. (2003) for predatory birds inhabiting inland, coastal and marine locations, including those along the Gulf Coast. This approach was further supported by the survey of Fry (2001), wherein fish from the Mississippi ($\delta^{34}S$ = -2.3 ± 0.4 ‰, n = 35) and Atchafalaya ($\delta^{34}S$ = -1.9 ± 0.5 ‰, n = 62) river systems, as well as the brackish waters of Barataria Bay ($\delta^{34}S$ = 9.2 ± 0.8 ‰, n = 19), were examined isotopically.

Geographic Information Systems (GIS) and geostatistical assignment methods were used to link the stable-hydrogen isotope ratio ($\delta^{2}H_f$) of each remaining freshwater feather ($\delta^{34}S < 10$ ‰) to the patterns of hydrogen isotope distribution in growing-season average precipitation ($\delta^{2}H_p$) (Hobson et al., 2009a, b). A precipitation-based isoscape was chosen over models depicting river $\delta^{2}H$ because it was readily accessible and there was close agreement between long-term averaged precipitation $\delta^{2}H$ and river waters in the area of interest (Kendall and Coplen, 2001). Long-term precipitation datasets from the International Atomic Energy Agency's (IAEA) Global Network of Isotopes in Precipitation (GNIP) database were used to create spatially-explicit models and to assign geographical origins of feathers (Bowen et al., 2005; Hobson et al., 2009a). A rastered GIS isoscape (www.waterisotopes.org) for $\delta^{2}H$ in amount-weighted growing season precipitation ($\delta^{2}H_p$) was used as a basis for predicting feather growth location by using a calibration curve determined for known-origin waterfowl across North America (Clark et al., 2006, 2009). The Bowen et al. (2005) $\delta^{2}H_p$ isoscape for North America was delimited to geo-referenced cormorant breeding and wintering ranges (Digital distribution maps of the birds of the western hemisphere version 3.0) in ArcGIS® 9. This map was clipped to exclude the Pacific and Atlantic flyways, as birds from Lake Winnipeg do not typically migrate to the south-western United States or the far Atlantic coast (Dolbeer, 1991; Hobson et al., 2006).

The empirical equation:

$$\delta^2H_f = -21.7\ ‰\ (\pm\ 2.4\ ‰) + 0.96\ (\pm\ 0.02)\ \delta^2H_p \quad (6.2)$$

and the ArcGIS® 9 Spatial Analyst tool were used to transform the Bowen et al. (2005) raster map (δ^2H_p) to a δ^2H_f map of the same spatial scale, where each raster cell (20 km^2) represented a local δ^2H_f value rather than a δ^2H_p value (Clark et al., 2006, 2009). This δ^2H_f isoscape was then imported into the R Version 2.10.1 statistical program (R Core Development Team, 2010) where the rgdal, sp, and raster packages were used to import and export raster data into the R interface; plot 2-D spatial data in map form, and perform spatial statistics on the raster surfaces. When determining whether a raster cell from the δ^2H_f base-map represented a probable point of origin for each non-marine feather (n = 103 feathers [21 birds]), 3:1 odds ratios were calculated and applied (Van Wilgenburg and Hobson, 2010). This odds ratio, while arbitrary, was chosen as the risk of incorrectly assigning a feather's origin was relatively low and analytical error; intra-specific and intra-individual variability, and map resolution could be accounted for (Hobson et al., 2009a; Van Wilgenburg and Hobson, 2010). The trade-off in choice of odds ratio was balancing between spatial resolution of assignments and classification accuracy. The likelihood that a feather was grown within a specific pixel of the δ^2H_f base-map was then classified as being equal to either zero (improbable) or one (highly probable). Maps of probability surfaces for each feather were used to classify samples as having been grown on Lake Winnipeg (n = 30 feathers), migratory stopover sites (n = 39 feathers), or the wintering grounds (n = 30 feathers).

For the non-marine feathers ($\delta^{34}S < 10‰$; n = 34 feathers) which were assigned to the wintering grounds using δ^2H_f, it was determined whether birds had access to natural freshwater or aquaculture foods (Figure 6.2). For $\delta^{13}C$, a threshold of -20 ‰ is suggested to separate marine ($\delta^{13}C > -20$ ‰) from freshwater-feeding birds ($\delta^{13}C < -20$ ‰) (Yerkes et al., 2008). Similarly,

Fry (2001) reported $\delta^{13}C$ values of -28.7 ± 0.6 ‰, -24.9 ± 0.5 ‰ and -22.5 ± 0.6 ‰ for fish from the Mississippi, Atchafalaya and Barataria Bay systems, respectively. Assuming a diet-to-feather discrimination factor of +1.9 ‰ for fish-eating bird primary feathers (Becker et al., 2007), this corresponded to a range of about -26.8 to -20.6 ‰ in cormorant feathers associated with the natural freshwater to brackish habitats, providing confidence in the -20 ‰ $\delta^{13}C$ threshold selected. The limited measurements of aquaculture catfish foods (n = 2) from the Mississippi Delta region in 2011 ($\delta^{13}C$ = -15.0 and -14.3 ‰; $\delta^{34}S$ = 2.7 and 2.6 ‰; $\delta^{15}N$ = 2.5 and 2.9 ‰) confirmed the presence of a C_4 corn-based food web component ($\delta^{13}C$ = -14 to -10 ‰) (Kelly, 2000) and corresponded to expected cormorant feather values of -12.6 ‰ and 2.6 ‰ for $\delta^{13}C$ and $\delta^{34}S$, respectively. Although marine fish from the Gulf of Mexico were expected to have $\delta^{13}C$ values of approximately -16 to -18 ‰ (Bank et al., 2007; Gu et al., 2001), which would correspond to cormorant feather $\delta^{13}C$ values of approximately -14 to -16 ‰, the early identification of marine feeders ($\delta^{34}S$ > 10 ‰) precluded the use of $\delta^{13}C$ data in identifying marine inputs (Figure 6.3). However, in an effort to ensure the most conservative limits were selected, a threshold of $\delta^{13}C$ > -14 ‰ was selected in order to identify cormorants which were heavily, if not completely, reliant on catfish (n = 4 feathers [1 bird]). It was impossible to predict a useful threshold for $\delta^{15}N$ to delineate winter habitat use by cormorants due to the large overlap in reported $\delta^{15}N$ values in freshwater and marine fish (Bank et al., 2007; Fry, 2001; Gu et al., 2001). However, because the aquaculture food web has, on average, fewer trophic steps at which dietary isotopic discrimination can occur compared to marine or freshwater food webs (Burger et al., 2008), feathers from cormorants feeding at catfish ponds were expected to have, on average, lower $\delta^{15}N$ values (Figure 6.4; open circles) than birds feeding in natural systems (Tavares et al., 2009).

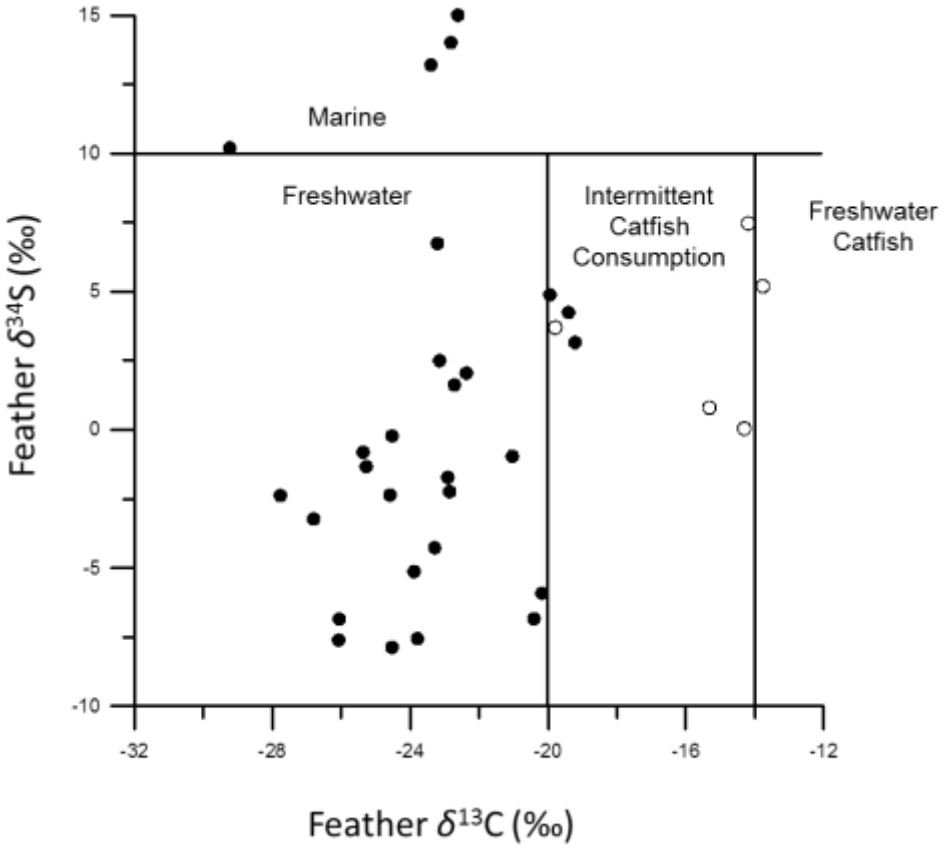


Figure 6.3 δ^{13}C and δ^{34}S bi-plot revealing habitat use associated with feather replacement in the catfish farming states (n = 34 feathers [16 birds]). Feathers with δ^{13}C values > -14 ‰ and δ^{34}S values < +10 ‰ (open circles) represent the introduction of corn based C_4 materials into the food chain for cormorant 2009-1 (*Phalacrocorax auritus*). A threshold of δ^{13}C = -20 ‰ was used to identify feathers from predominantly freshwater habitats.

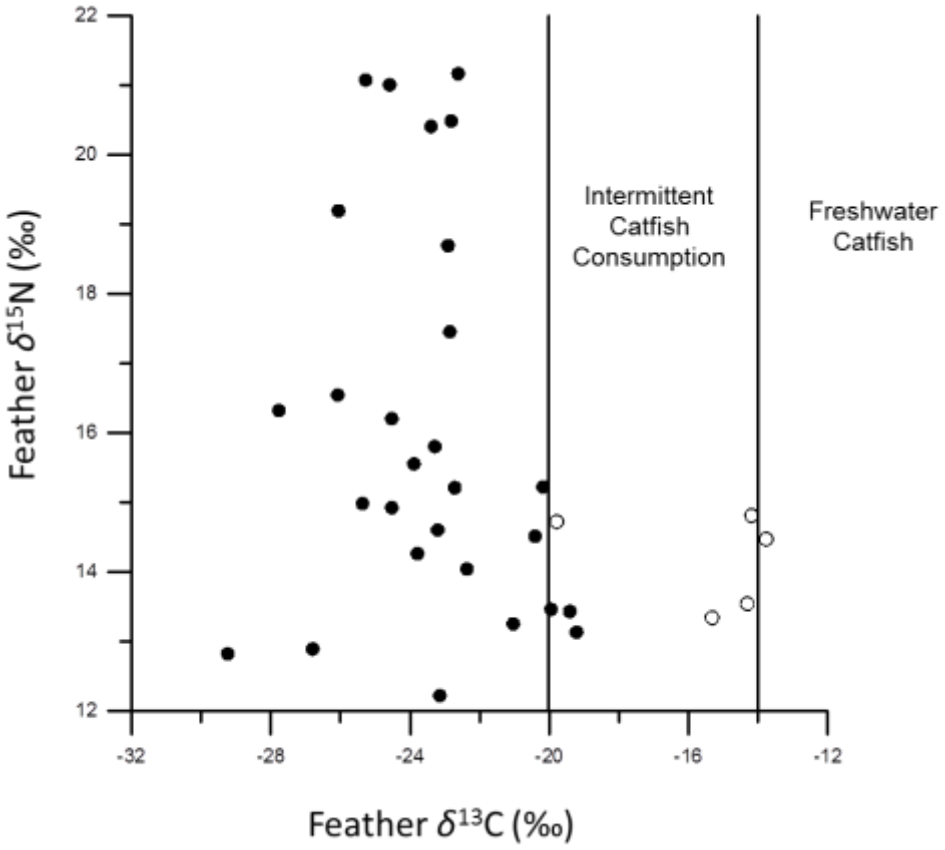


Figure 6.4 δ^{13}C and δ^{15}N bi-plot for cormorant (*Phalacrocorax auritus*) feathers replaced while feeding on freshwater or aquaculture resources within the catfish-farming states (n = 30 feathers [14 birds]). Samples exhibiting positive δ^{13}C values (> -14 ‰) were considered reliant on catfish aquaculture. Samples exceeding or approaching the δ^{13}C threshold were depleted in ^{15}N, as evidence by cormorant 2009-1 (open circles).

6.2.4 Mercury

Feathers which could be isotopically linked to either Lake Winnipeg (n = 20 feathers [12 birds]) or to freshwater and marine feeding regimes on the wintering grounds (n = 19 feathers [13 birds]) were selected for Hg analysis (Table A6, Appendix). Feather materials which had not been consumed during the isotopic assays were washed with high-purity acetone and deionized water to remove potential external Hg contamination (Burger et al., 2008), then microwave digested (US EPA, 1996) and analyzed for Hg_T (hereafter "Hg") (Burger et al., 2008) using CVAFS at the SRC Environmental Analytical Laboratory in Saskatoon, Canada. Detection limits were below 0.05 µg/g FW, and mean recoveries of the standard reference material (DORM-2; National Research Council Canada; Ottawa, ON) were not significantly different from target values at the p = 0.05 level (*t*-test; *t* = 0.39). The CV on duplicate samples was 12 % or better. Multiple feathers from each bird were analyzed for Hg on an individual, rather than pooled basis and six of the cormorants used in this study (Birds 2009-7, 8, 9, 14, 18 and 21) had replaced at least one primary feather at each of the isotopically distinct breeding and wintering locations. All feather Hg data was log transformed prior to statistical analyses. Mercury concentrations in feathers can be affected by order of feather replacement (Furness et al., 1986); with early molted feathers having higher Hg content than later-grown feathers. Since cormorants do not follow a consistent molt pattern (Hatch and Weseloh, 1999), it was impossible to discern whether P1 (primary number one) was molted prior to P2, and so on, during field collections. Nested ANOVAs (Growth Latitude or Habitat Type/Bird/Primary Feather Position) were used in order to account for molt phenology and therefore reduce effects of potential pseudo-replication which may have otherwise influenced comparisons of feather Hg across latitudes or habitat types.

6.3 Results

6.3.1 Stable Sulfur Isotopes and Marine Habitat Use

Feathers from cormorants did not exhibit any sex-based differences (n = 7 males and 16 females) in feather $\delta^{34}S$ (*t*-test; $t = -1.70$, $p = 0.09$), nor were there any detectable differences at the $p = 0.05$ level for $\delta^{34}S$ across the colonies and foraging sites where birds had been collected (ANOVA, Tukey HSD; $F_{7, 107} = 2.17$). All $\delta^{34}S$ values for feathers ($\delta^{34}S$ = -9.4 to +3.0 ‰) assigned to the breeding grounds on Lake Winnipeg fell within the range described for dissolved inorganic sulfate ($\delta^{34}S$ = -12 ‰ to +3.2 ‰; Chapter 2) at the base of the lake's food web, and were significantly lower than $\delta^{34}S$ values associated with feathers ($\delta^{34}S$ = -7.9 ‰ to +15 ‰) from the wintering grounds (*t*-test; $t = 7.54$, $p = <0.0001$). Of the 23 cormorants studied, only Birds 2009-16 and 2009-22 had one and three feathers, respectively, with $\delta^{34}S$ values above +10 ‰ (Figure 6.5). It would appear these individuals were consuming marine foods for extended periods (i.e. sufficient time for a feather to form), however they may have undergone a habitat shift during winter, or the availability of local prey items may have changed, since their remaining feathers had $\delta^{34}S$ values consistent with natural freshwater habitats.

6.3.2 Isotopes and Latitude of Origin

The feather $\delta^{2}H$ isoscape used for assigning geographical origins is only valid for the terrestrial/freshwater environment, and so removal of samples with clear isotopic evidence of marine dietary inputs was required. Therefore, the two "marine-influenced" birds which had at least one feather- $\delta^{34}S$ value above the +10 ‰ threshold were excluded from the $\delta^{2}H_f$ dataset used to infer origins (Figure 6.5). Assignments for all remaining feathers (n = 103 feathers [21 birds]) revealed a broad latitudinal distribution of feather molt, with feathers being classified as having been grown a) on Lake Winnipeg ($\delta^{2}H_f$ = -100.0 to -85.5 ‰; n = 30 feathers), b) at mid-

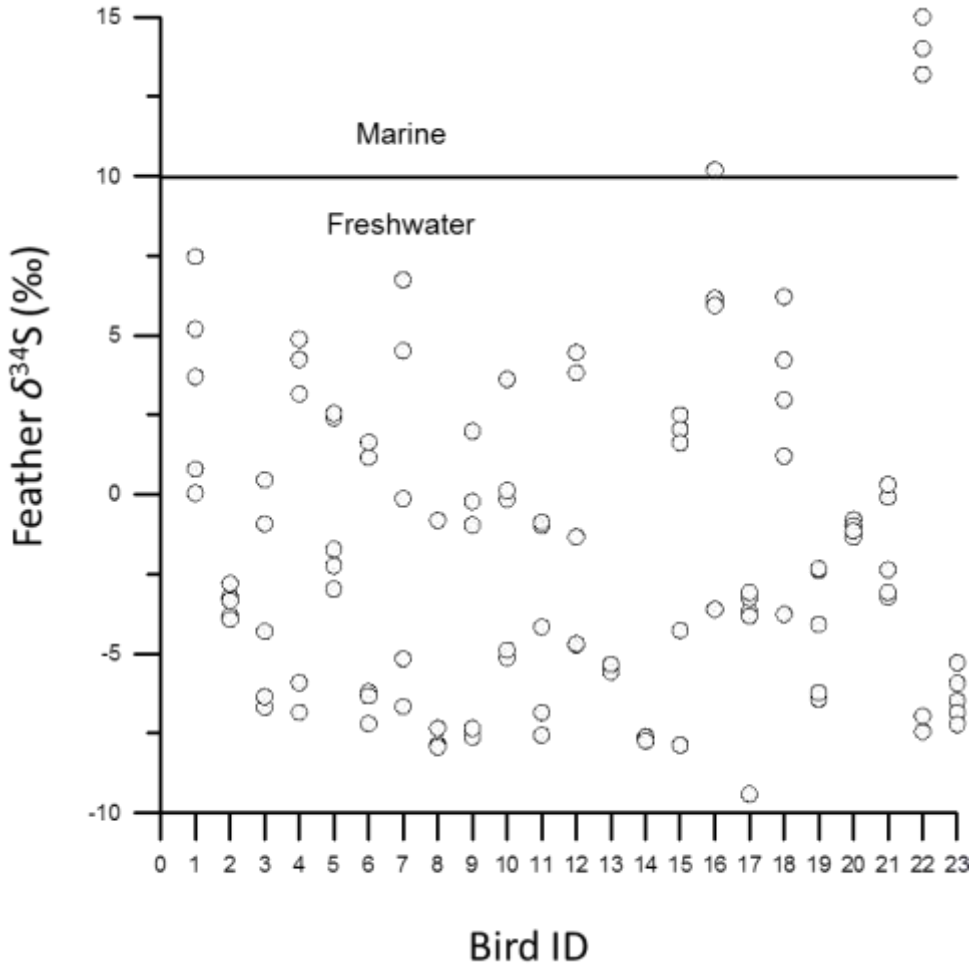


Figure 6.5 δ^{34}S values of primary feathers (n = 113) by bird [n = 23]. Each point represents the δ^{34}S value determined for one of the second, fourth, sixth, eighth, or tenth primary feathers. Feathers with δ^{34}S values above +10 ‰ were assumed to be indicative of marine habitat use during the period of feather growth.

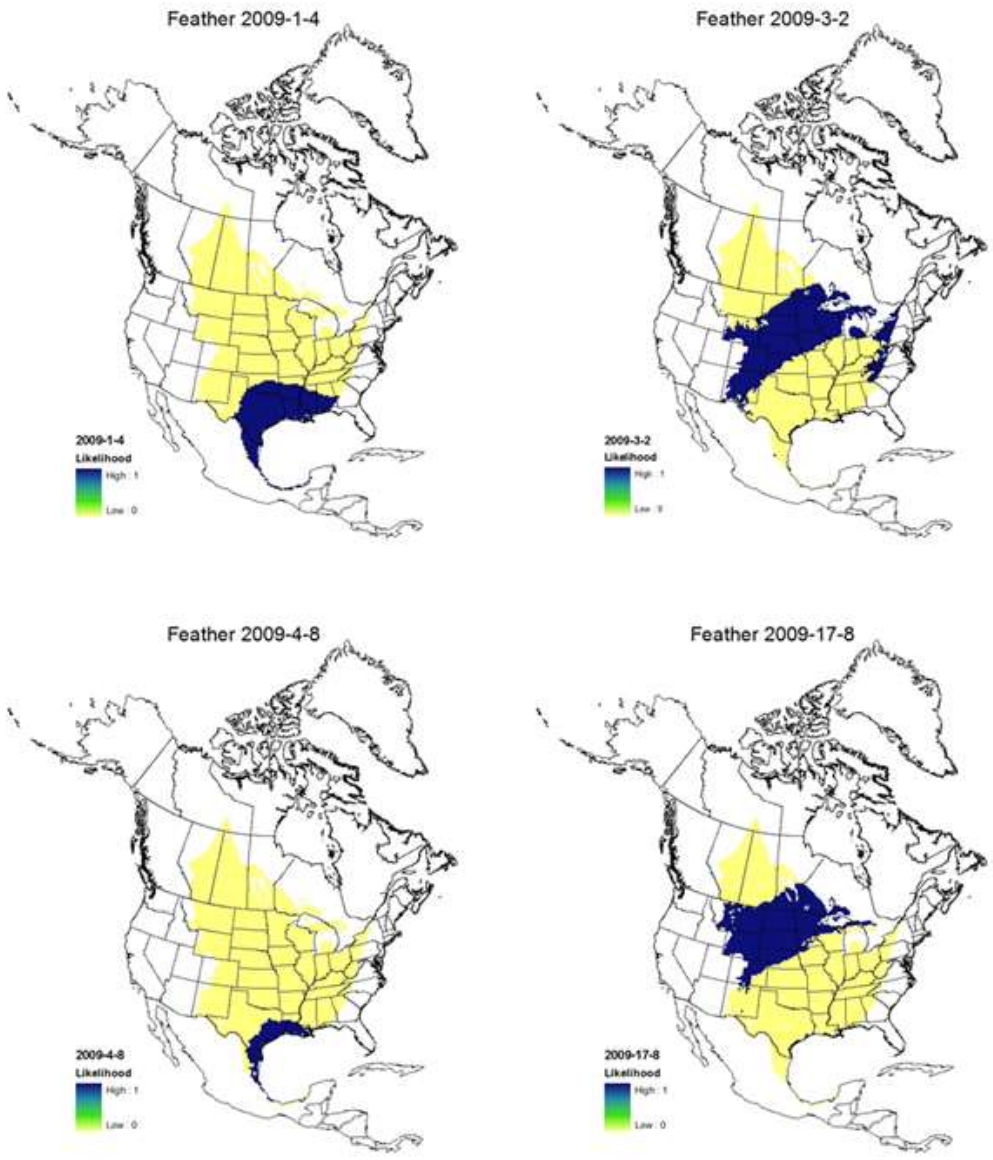


Figure 6.6 Example GIS output used in assigning latitudinal origins of individual cormorant (*Phalacrocorax auritus*) primaries. Estimates of feather growth locations were based on a δ^2H_f base-map where each raster cell represented a possible point of origin. Assignments were based on 3:1 odds ratios, with each map (n = 103; n = 4 shown) depicting the areas of likely (1; dark blue) or unlikely (0; yellow) origin within the species range. Each assignment was identified by the year, bird number, then primary number (example: 2009-1-4).

latitude stopover sites (δ^2H_f = -84.0 to -52.1 ‰; n = 39 feathers), or c) on the wintering grounds (δ^2H_f = -60.6 to 3.1 ‰; n = 30 feathers; Figure 6.6).

6.3.3 Defining Use of Aquaculture Resources

Of the non-marine feathers (n = 30 feathers [14 birds]) associated with the wintering grounds, only one bird (2009-1) had feather $\delta^{13}C$ and $\delta^{34}S$ ($\delta^{34}S$ < 10 ‰) values which suggested a heavy reliance on C_4 aquaculture resources during feather growth (Figure 6.3; open circles). A single feather from this specimen exceeded the $\delta^{13}C$ > -14 ‰ threshold ($\delta^{13}C$ = -13.8 ‰) used to predict near complete reliance on catfish consumption. However, the remaining primary feathers from the same bird ($\delta^{13}C$ = -19.8, -15.3, -14.3, and -14.2 ‰) approached the $\delta^{13}C$ = -14 ‰ threshold for C_4-based food webs. This would suggest Bird 2009-1 was largely reliant on aquaculture-raised catfish over an extended period of feather replacement. All feathers (n = 5) for Bird 2009-4 had $\delta^{13}C$ values around -20 ‰ (mean ± SD = -19.8 ± 0.5 ‰), and were also relatively depleted in ^{15}N (mean ± SD = +14.0 ± 0.9 ‰; Figure 6.4). These feathers which are slightly more enriched with ^{13}C, and depleted in ^{15}N (lower trophic status) are likely representative of a diet based on natural freshwater systems, with occasional, but consistent predation of catfish over time.

6.3.4 Mercury Concentrations in Feathers

Feathers assigned to Lake Winnipeg (n = 20 feathers [12 birds]) using δ^2H_f data had significantly higher Hg concentrations than feathers grown on the wintering grounds (n = 19 [13 birds]) (ANOVA, $F_{1, 33}$ = 5.47, p = 0.03; Table A7, Appendix). Differences in Hg concentrations were examined for winter-grown feathers based on marine or freshwater classification criteria; however, no significant difference in Hg burden was found among feathers grown on marine or freshwater habitats after controlling for individual variability (ANOVA, $F_{1, 13}$ = 0.42, p = 0.53).

6.4 Discussion

6.4.1 Mercury & Habitat

The application of multiple isotopic assays to feathers from migratory Double-crested Cormorants provided new insights into how isotopic tools can be used to characterize the habitat-driven accumulation of Hg in growing tissues, and provided a new means of tracking contaminant origins. Hydrogen isotope data provided a means of assigning feathers to breeding and wintering grounds, and subsets of individuals that were likely dependent on marine foods or catfish aquaculture could be identified through the use of δ^{34}S and δ^{13}C measurements, respectively. The isotopic and Hg data suggested that cormorants accumulated the highest concentrations of Hg in their feathers while nesting on Lake Winnipeg (mean ± SD = 4.26 ± 1.47 µg/g FW) rather than on the wintering grounds (mean ± SD = 3.19 ± 1.64 µg/g FW). Feeding at freshwater lakes, rather than in marine, brackish or riverine ecosystems, has been linked to greater Hg concentrations in feathers of Common Terns (Nisbet et al., 2002) and blood of Belted Kingfishers (*Ceryle alcyon*) and Bald Eagles (*Haliaeetus leucocephalus*) (Evers et al., 2005). Inland lakes generally have slower cycles of water renewal and conditions conducive to the bacterial methylation of Hg (Evers et al., 2005). The bioavailability of Hg within Lake Winnipeg may be further amplified by enhanced methylation during large-scale spring floods and longer contaminant retention times resulting from lake impoundment (Gewurtz et al., 2006; Pip, 2006). Recent changes in local food web structure resulting from species introduction and eutrophication (Gewurtz et al., 2006; Hobson et al., 2010) may also be accelerating contaminant biomagnification within the system. Historically, Hg in Lake Winnipeg's predatory fish populations has reached concentrations great enough to warrant bans on commercial fishing (Kristofferson and Clayton, 1990). Mean muscle Hg concentrations of adult walleye (*Sander vitreus*) captured in 2009 and 2010 (mean ± SD = 0.77 ± 0.73 µg/g DW, or 0.17 ± 0.16 µg/g FW;

n = 51; Chapter 2) approached the recommended guideline (0.2 µg/g FW) for frequent fish consumption, and two individuals (0.65 & 1.1 µg/g FW) exceeded the 0.5 µg/g FW limit set for commercial fish in Canada (Bodaly et al., 2007). It was not surprising then, given the piscivorous nature of both adult walleye and cormorants, that birds would accumulate significantly more Hg in tissues while feeding in a system where other apex predators have high concentrations in tissues (Bodaly et al., 2007). Mercury data for local walleye may or may not provide an approximation of the Hg concentrations cormorants are exposed to while nesting on Lake Winnipeg.

A suite of isotopes (δ^{34}S, δ^{13}C and δ^{15}N) was used to demonstrate the utility of identifying potential sources or origins of Hg. In order to adequately assess Hg accumulation and biomagnification rates associated with natural (marine and freshwater) versus aquaculture-based feeding regimes on the wintering grounds, further investigations with larger sample sizes are encouraged. Studies similar to that of Elliot et al. (2009), wherein contaminant exposure and accumulation were compared with varying degrees of marine food consumption in bald eagles, may be useful in identifying any temporal trends related to diet/habitat shifts and subsequent changes in Hg exposure for cormorants or other species.

6.4.2 Diet & Habitat

When comparing the ratio of marine: freshwater assigned feathers to those reported for populations of cormorants breeding on Lake Erie in 1997 and 2006, Hebert et al. (2008) noted a trend toward decreased marine inputs over time. Based on δ^{34}S values in feathers, Hebert et al. (2008) found 57 % (n = 23) of birds collected from Lake Erie in 1997, and 37 % (n = 30) of birds captured in 2006 utilized marine habitats in winter, while only 9 % (n =23) from Lake Winnipeg in 2009 were consistently using marine food sources at some time during feather regrowth. Hebert et al. (2008) suggested winter diet for freshwater-feeding cormorants likely consisted of

farmed catfish; however the data presented here showed that this was highly unlikely for Lake Winnipeg cormorant populations because a high level of farmed catfish consumption would have led to more positive δ^{13}C values than was observed. Moreover, it is well known that this species exploits a wide range of non-aquaculture freshwater habitats on the wintering grounds (Wires and Cuthbert, 2010). Hebert et al. (2008) based their delineations on the use of a single isotope (δ^{34}S) measurement, which is precarious because cormorants so delineated had feather δ^{34}S values consistent with natural freshwater, brackish and aquaculture feeding (Fry, 2001, this study). Thus, although 96 % of all feathers (n = 113) and 12 % of winter-grown feathers (n = 34) from cormorants sampled on Lake Winnipeg [n = 23 birds] used freshwater or mixed freshwater-aquaculture foods throughout the period of primary feather growth, the δ^{13}C and δ^{15}N data revealed that only one of the 113 feathers tested indicated a clear, complete reliance on catfish aquaculture resources, with three other feathers from the same bird exhibiting signs of a partial or intermittent reliance on farmed foods. This suggested either sufficient isotopic overlap to preclude any strong conclusions about cormorant use of aquaculture resources, or a much more conservative reliance on aquaculture than expected for Lake Erie populations (2008). The latter argument may be supported by radio telemetry studies, which have revealed that cormorant predation at commercial catfish farms is often associated with specific roost sites, rather than the population as a whole (Dorr et al., 2004). Perhaps the Lake Erie population of cormorants investigated by Hebert et al. (2008) should be re-analyzed using the multi-isotope approach outlined here.

An acknowledged weakness in any threshold approach to delineating use by consumers of isotopically unique biomes is that the approach necessarily becomes arbitrary near the threshold values. Thus cormorants with very similar isotope values can be assigned to different biomes. In

addition, there are some local situations where isotopes will not follow the rules applied. For example, while marine biomes are overwhelmingly enriched in ^{34}S compared to freshwater systems, stagnant freshwater marshes with bacterial SO_4^{2-} reduction under anaerobic conditions can also have comparatively high $\delta^{34}S$ values (Krouse et al., 1991). Such situations can result in "marine" food web $\delta^{34}S$ values but freshwater $\delta^{13}C$ values. Freshwater systems close to oceans can also receive sulfates from marine aerosols that can in turn raise freshwater food web $\delta^{34}S$ values beyond the marine threshold (Zazzo et al., 2011). The two cormorants (2009-16 and 2009-22) identified as having feathers above the marine $\delta^{34}S$ value (> 10 ‰) had $\delta^{13}C$ values which were within the freshwater threshold (< 20 ‰). Nonetheless, at the scales involved with birds wintering over vast areas, like those examined here, the multi-isotope threshold approach presented a powerful tool in ecotoxicological investigations. Moreover, $\delta^{34}S$ values provided a convenient means of screening individuals to be assigned to terrestrial/ freshwater origin using derived feather $\delta^{2}H$ isoscapes (Lott et al., 2003).

Future studies that evaluate multi-isotope assays of catfish from a broad survey of aquaculture farms on the wintering grounds of cormorants are encouraged. The establishment of aquaculture isotopic dietary endpoints together with the refinement of those isotopic endpoints associated with natural freshwater, brackish and marine prey will inform Bayesian isotopic mixing models that can provide quantitative estimates of diet and habitat use in this species and where contaminant exposure occurs (e.g. Moore and Semmens, 2008). In the meantime, researchers are encouraged to use the approach of delineating multi-dimensional isotopic space that is consistent with diets in known biomes. The combination of multiple isotopic assays and contaminant concentrations in feathers that are so assigned to origins represents a major advance in how feather contaminant data can be interpreted.

CHAPTER 7
7.0 GENERAL DISCUSSION & CONCLUSIONS

7.1 General Discussion

The current conditions of Lake Winnipeg and the integrity of its biotic communities are governed by a number of anthropogenic and environmental stressors. These include land-use practices within the watershed; biological invasions, and exploitation by the hydroelectric, tourism and commercial fishing industries. The perceived deterioration of the Lake Winnipeg ecosystem has garnered considerably more attention from the public, regulators and researchers in recent years (Environment Canada and Manitoba Water Stewardship, 2011; Gewurtz et al., 2006; Hobson et al., 2010; and others). Concerns over food web perturbations, avian predation and the sustainability of the lake's fishery warranted an intensive investigation into the structure and function of the lake's food web. Similarly, increased loading of contaminants from the 977 800 km^2 watershed; altered dietary availability of POPs and Hg following flood events, and historical commercial fishing bans highlight the need for up-to-date contaminants data (Environment Canada and Manitoba Water Stewardship, 2011; Stewart et al., 2003). The purpose of this thesis was to characterize the trophic interactions and energy sources used by the fishes and Double-crested Cormorants (*Phalacrocorax auritus*; hereafter "cormorant") of Lake Winnipeg, and to then apply this food web data in tracking the trophodynamic transfer of Hg and other trace elements. The utility of non-destructive sampling methods (scales, feathers) for identifying dietary niches and in monitoring contaminant body burdens was also evaluated.

7.1.1 Fishes

7.1.1.1 Trophic Interactions & Food Sources

Overall, the baseline-corrected $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ profiles of fishes differed between the north and south basins and exhibited a pattern much like that observed for $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ in

zooplankton, DIC and dissolved sulfate, respectively (Chapter 2). Fishes from the north basin generally had larger $\delta^{15}N$ values and smaller $\delta^{13}C$ and $\delta^{34}S$ values than southern fishes. These findings are corroborated by an earlier examination of DIN ($\delta^{15}N$), DIC ($\delta^{13}C$) and small pelagic fishes collected during 2002-2008 (Hobson et al., 2010).

The isotopic food web data presented in Chapter 3 suggested populations of recently-established rainbow smelt (*Osmerus mordax*) within the lake's north basin did not occupy a TP which was elevated over native forage fishes, such as emerald shiner (*Notropis atherinoides*) and ciscoes (*Coregonus artedi*; $TP_{RNSM} = 4.4 \pm 0.4$, n = 241; $TP_{CISC} = 4.4 \pm 0.5$, n = 101; $TP_{EMSH} = 4.3 \pm 0.4$, n = 92). Therefore, it was unlikely rainbow smelt increased the number of trophic steps between baseline energy sources and top-level consumers. However, the establishment of rainbow smelt within the lake's north basin may have affected endemic fishes through other mechanisms. In the south basin, ciscoes and emerald shiner represent the largest fraction of forage fish biomass, but in the north, smelt are the most prevalent small-bodied fishes (Lumb et al., 2011). Although north basin rainbow smelt, ciscoes and emerald shiner occupied a fairly similar TP, the $\delta^{13}C$ and $\delta^{34}S$ data suggested smelt tended to use different energy sources, and occupied a niche most similar to ninespine sticklebacks (*Pungitius pungitius*). Therefore, it was unclear whether smelt altered the feeding habits of other low-TP fishes, and if so, by how much. Gewurtz et al., (2006) reported that smelt have displaced emerald shiner as the main prey item consumed by north basin walleye (*Sander vitreus*). Although this thesis would suggest smelt did not represent an additional step in the food chain leading up to walleye, there is some evidence fishes which feed on smelt grow faster and develop larger $\Delta^{15}N$ values (Hobson et al., 2010). Although this phenomenon would have accounted for the approximately 1.5 step difference in TPs of walleye from the north and south basins, it did not explain the same 1.5 step difference in

yellow perch (*Perca flavescens*) TP across the two basins. Yellow perch had $\delta^{13}C$ and $\delta^{34}S$ values which were suggestive of a heavier reliance on benthic and detrital energy sources than what would have been afforded by smelt consumption. Yellow perch are opportunistic, and may feed on plankton, invertebrates and/ or fishes (Stewart and Watkinson, 2004); therefore, a comprehensive mixing model for perch diet could not be developed based on the dataset. However, an exploratory mixing model analysis in SIAR (see Chapter 5 for methods; *data not shown*) which was limited to potential fish prey only, revealed rainbow smelt were likely to have represented only a small fraction of the yellow perch diet (mean = 8 %, 95 % credibility interval = 0, 20 %). It must be stressed that planktonic and invertebrate prey items were missing from the yellow perch mixing model, and that further confirmatory investigations of yellow perch diet are required.

An alternative to the smelt-based hypothesis is that the relatively large upward shifts in north basin walleye and yellow perch TPs may have been representative of eutrophication and increasingly favorable conditions for percids within the north basin (Aberle and Malzahn, 2007; Leach et al., 1997). An in-depth examination of the interactions among percids (sauger, walleye and yellow perch), rainbow smelt and algal blooms (i. e. density, frequency of formation) within each of the lake's basins may be of interest in future research.

7.1.1.2 Trace Elements & Diet

Based on an AIC_c information-theoretic approach, species was often the strongest factor influencing trace element concentrations in fish muscle. However, some general trends were identified for the north and south basin fish communities, as well as within certain species. Within the south basin, the sources of carbon and sulfur utilized by fishes had the strongest influence on Hg concentrations in muscle. Individuals which were increasingly dependent on littoral or detrital food sources accumulated more Hg from their diets. Therefore, further studies

which examine sources and mixing of carbon and sulfur inputs in the south basin may be useful in identifying the most significant sources of bioavailable Hg within the lake.

For some species, feeding at higher TPs (south basin walleye) or developing larger body sizes (north basin walleye and yellow perch, south basin white bass [*Morone chrysops*]) translated into elevated Hg body burdens. Selenium concentrations in south basin walleye and freshwater drum also tended to increase with increasing TP. Within the north basin, white bass accumulated Se in muscle; however, due to the small sample size (n = 3) examined in Chapter 3, potential sources or mechanisms related to this phenomenon could not be identified.

Unlike Hg and Se, both As and Cu tended to decrease with increasing TP in fishes collected from the lake's south basin. Although δ^{15}N was not identified as having a strong negative influence on As concentrations in north basin fishes, rainbow smelt accumulated significantly more As in muscle than top-level predators.

Concentrations of some elements, such as Cd, Fe, and Mn in fish muscle were poorly explained by stable isotope ratios and/ or fish size. Although no relationship between concentrations of these three elements and TP was observed for fishes of other systems such as Tadenac Lake, Ontario, it was anticipated the energy sources used by fishes would be informative (Wren et al., 1983). This was not so. Ciscoes were the only species for which Cd could be related to isotopic values measured in muscle. In the north basin, Cd concentrations were negatively correlated with δ^{15}N. In the south, food source had a much stronger effect on Cd concentration in cisco muscle, with fishes accumulating more Cd from detrital versus water-column-associated sources of sulfate. A great deal of intraspecific variability was observed for Fe; however, percids had the lowest concentrations in muscle.

7.1.1.3 Fish Scales in Biomonitoring

Analysis of stable isotope ratios and trace metals in fish scales had limited use in monitoring the diet and fillet-contaminant residues of Lake Winnipeg walleye. Stable nitrogen isotopes in scales were fairly good predictors of $^{15}N/\ ^{14}N$ ratios in muscle ($r^2 = 0.72$, n = 34); however, $\delta^{13}C$ and $\delta^{34}S$ values for scales were less reliable indicators of corresponding values n muscle ($r^2 =$ 0.47 and 0.38, respectively). Analysis of $\delta^{15}N$ in archived scales could potentially provide a record of changing walleye TP over periods coinciding with species introduction and the onset of eutrophication; however this information would be of limited use, since energy sources could not be identified with confidence (Perga and Gerdeux, 2003). Reliable quantification of trace element concentrations in scales was not easily achieved through acid digestion and analysis by ICP-MS (Holland and Tanner, 2001). Therefore, other methods, such as LA-ICPMS are recommended should researchers pursue further monitoring of trace metals in scales of Lake Winnipeg fishes (Farrell et al., 2000). Concentrations of As, Mn and Hg could be reliably measured in fish scales, and were later contrasted with concentrations in corresponding muscle samples. Arsenic concentrations in paired scales and fillets were not significantly different, and values in scales were poor predictors of concentrations in muscle ($r^2 = 0.25$, n = 16). Similarly, Mn concentrations in scales were not well-correlated with muscle values ($r^2 = 0.25$, n = 18). Mercury was different from As and Mn in that concentrations in scales showed some utility in estimating concentrations in fillets ($r^2 = 0.75$, n = 17). However, the use of scale-Hg, rather than direct measurement of fillet-Hg, in monitoring programs should be approached with extreme caution. Concentrations measured in walleye scales were an order of magnitude smaller than concentrations measured in corresponding fillet samples. Therefore, measurement of Hg concentrations in fish scales was only predictive, but did not provide a “protective” indicator of Hg in muscle (Darafsh et al., 2008; Farrell et al., 2000).

Based on the findings of this thesis, further investigations into the utility of fish scales for monitoring diet and/ or trace metal concentrations in Lake Winnipeg fishes are not encouraged. Rather, researchers should consider analyses of soft tissues, such as muscle or fin webs (Baker et al., 2004; Ryba et al., 2008). Under most circumstances, these two sampling methods are unlikely to cause serious lethality or reductions in fish health, and muscle biopsies have an added advantage in that the fillet is sampled directly (Gremillion et al., 2005; Ryba et al., 2008).

7.1.2 Cormorants (*Phalacrocorax auritus*)

7.1.2.1 Fish Consumption on Lake Winnipeg

This thesis is the first stable isotopic (δ^{15}N, δ^{13}C and δ^{34}S) investigation of diet composition and prey preference in adult or hatch-year cormorants collected from Lake Winnipeg. It was hypothesized that since the potential prey base for Lake Winnipeg cormorants consists of a diverse array of fish species and an abundance of ciscoes, emerald shiner and rainbow smelt are found within the lake, walleye and sauger (*Sander canadensis*) were unlikely to represent a substantial proportion of the cormorant diet (Doucette et al., 2011; Lumb et al., 2011). For hatch-year cormorants, sauger and walleye represented on average 6 % of overall diet (95 % credibility interval = 0, 15 %). Adult cormorants from the south basin consumed a comparatively larger proportion of young (FLs < 400 mm) walleye and sauger (mean = 12 %); however, these fish species constituted < 26 % of cormorant diet 95 % of the time. It is possible that some cormorants, namely those from the Cox and Matheson Islands in the North basin, were consuming fish wastes from nearby processing stations (Fox et al., 2002; Manitoba Water Stewardship, 2010). These birds had elevated muscle-δ^{15}N values which may have been attributed to ingestion of discarded walleye and sauger carcasses. However, further investigations are required in order to confirm or disprove this this theory. Overall, troutperch (*Percopsis omiscomaycus*), ciscoes, emerald shiner and ninespine stickleback were the most

frequently-consumed fishes in the north basin. For adult and hatch-year birds collected from the south basin, white sucker (*Catostomus commersonii*; mean = 19 %, 95 % credibility interval = 0, 37 %) and troutperch (mean = 31 %, 95 % credibility interval = 9, 56 %), respectively, were the species which made the largest contributions to total diet.

7.1.2.2 Annual Cycles of Diet

Stable isotopic analyses of cormorant muscle and feathers suggested that overall; cormorants nesting on Lake Winnipeg during the summer breeding season did not have diets which consisted predominantly of commercially-valuable fish biomass. During the summer breeding season on Lake Winnipeg, smaller life-stages of percids constituted anywhere from 0 % to, at most, 41 % (Cox Island) of the cormorant diet. Primary feathers which had been grown on freshwater sites within the population's wintering range (δ^{34}S, δ^{2}H) were further delineated as having been formed while individuals were dependent on either farmed or natural freshwater foods (δ^{13}C, δ^{15}N). The results revealed that while on the on wintering grounds, few birds were heavily reliant on valuable aquaculture resources. Therefore, it appeared that throughout the annual cycle, most cormorants from Lake Winnipeg tended to have diets which contained a relatively small to moderate proportion of economically-valuable fish biomass. These findings may not have a widespread application, since other cormorant populations may have a larger relative dependence on commercial fisheries resources (Dorr et al., 2004); however it is hoped the information presented in this thesis will better inform fisheries managers and regulators in Manitoba.

7.1.2.3 Trace Elements and Diet

Adult cormorants tended to accumulate Fe and Mn to a greater extent than hatch-year birds; however, concentrations of Cd, Hg and Se did not differ between hatch-year and adult cormorants, or even among the different colonies examined. In terms of Hg and trace element

body burdens of hatch-year cormorants, there was little within-group variability, which was not surprising given concentrations measured in tissues represented a single season of dietary exposure on Lake Winnipeg (Fox et al., 2002; Somers et al., 1993). Overall, concentrations measured in the muscle of adult birds were more variable, and were likely influenced by factors such as age, molt, and exposure to contaminants while over-wintering near the Gulf of Mexico (Bond and Diamond, 2009; Burger et al., 1992).However, the use of δ^2H in assigning feather-Hg body burdens to either Lake Winnipeg or the over-wintering grounds revealed birds accumulated significantly more Hg in feathers while nesting and feeding on Lake Winnipeg. Depuration of Hg into molted feathers and eggs may have reduced the body burden of Hg, and potentially Cd and Se, in adult cormorants, thereby drawing down the concentrations in muscle. Hatch-year birds may have retained a relatively large Hg load in muscle after distribution to rapidly forming feathers ceased (Burger and Gochfeld, 1999; Caldwell et al., 1999). Hatch-year birds from the south basin of Lake Winnipeg had muscle Hg concentrations (mean ± SD = 2.00 ± 0.61 µg/g DW, n = 12) which were more than double the concentrations measured in hatch-year birds collected from the Elephant Butte (mean ± SD = 0.57 ± 0.37 µg/g DW, n = 12) and Caballo reservoirs (mean ± SD = 0.60 ± 0.03 µg/g DW, n = 7) of New Mexico (Caldwell et al., 1999). These findings further reiterate the need for further investigations into Hg biotransport and accumulation in the fishes and birds of Lake Winnipeg.

7.2 Conclusions

This thesis provides the most comprehensive account of isotopic food web structure and trace element trophodynamics for the fishes and cormorants of Lake Winnipeg. A multi-isotope approach (δ^{15}N, δ^{13}C and δ^{34}S) proved effective in tracking the dietary transfer of energy through the Lake's food web and in identifying the sources and pathways of most trace elements measured in muscle. Lake Winnipeg was identified as an isotopically heterogeneous system, as

evidence by the bimodal pattern observed for the zooplankton, DIC, dissolved sulfate, fishes and cormorants collected from the north and south basins. The energy sources and trophic linkages underlying trophic transfer of Hg and other trace elements also differed by basin. Therefore, it is recommended that the north and south basins of Lake Winnipeg be considered separately in future dietary and/ or contaminant studies.

Chapter 6 of this thesis represents the earliest application of δ^2H analyses and geostatistical assignment methods to assigning contaminant concentrations in tissues of migratory animals to their latitude of origin. These findings demonstrated the collection and analysis of cormorant primary feathers has utility in tracking both year-round habitat use and accumulation of Hg. As such, cormorant feathers are likely to provide a valuable investigative or monitoring tool for regulators and researchers.

LIST OF REFERENCES

Aberle, N., Malzahn, A. M., 2007. Interspecific and nutrient-dependent variations in stable isotope fractionation: Experimental studies simulating pelagic multitrophic systems. Oecologia 154(2), 291-303.

Adout, A., Hawlena, D., Maman, R., Paz-Tal, O., Karpas, Z., 2007. Determination of trace elements in pigeon and raven feathers by ICPMS. Int. J. Mass Spectrom. 267, 109-116.

Ahlgren, M. O., 1996. Selective ingestion of detritus by a north temperate omnivorous fish, the juvenile white sucker, *Catostomus commersonii*. Environ. Biol. Fishes 46, 375-381.

Anderson, D. R., 2008. Model based inference in the life sciences: A primer on evidence. Springer, New York. 184 pp.

Aranda, P. R., Gil, R. A., Moyano, S., De Vito, I., Martinez, L. D., 2009. Slurry sampling in serum blood for mercury determination by CV-AFS. J. Hazard. Mater. 161, 1399-1403.

Ashoka, S., Peake, B. M., Bremner, G., Hageman, K. J., Reid, M. R., 2009. Comparison of digestion methods for ICP-MS determination of trace elements in fish tissues. Anal. Chim. Acta 653, 191-199.

Atekwana, E. A., Fonyuy, E. W., 2009. Dissolved inorganic carbon concentrations and stable carbon isotope ratios in streams polluted by variable amounts of acid mine drainage. J. Hydrol. 372, 136-148.

Atekwana, E. A., Krishnamurthy, R.V., 1998. Seasonal variations of dissolved inorganic carbon and $\delta^{13}C$ of surface waters: application of a modified gas evolution technique. J. Hydrol. 205, 265-278.

Baker, R. F., Blanchfield, P. J., Paterson, M. J., Flett, R. J., Wesson, L., 2004. Evaluation of nonlethal methods for the analysis of mercury in fish tissue. Trans. Am. Fish. Soc. 133, 568-576.

Bank, M. S., Chesney, E., Shine, J. P., Maage, A., Beissinger, S. R., 2007. Mercury bioaccumulation and trophic transfer in sympatric snapper species from the Gulf of Mexico. Ecol. Appl. 17, 2100-2110.

Becker, B. H., Neman, S.H., Inglis, S., Beissinger, S. R., 2007. Diet-feather stable isotope ($\delta^{15}N$ and $\delta^{13}C$) fractionation in common murres and other seabirds. Condor 109(2), 451-456.

Belyea, G. Y., Maruca, S. L., Diana, J. S., Schneeberger, P. J., Scott, S. J., Clark, R. D. Jr., Ludwig, J. P., Summer, C. I. L., 1997. Impact of Double-crested Cormorant predation on the yellow perch population in the Les Cheneaux Islands of Michigan. Symposium on Double-crested Cormorants: Population status and management issues in the midwest, pp. 47-59.

Blais, J. M., Kimpe, L. E., McMahon, D., Keatley, B. E., Mallory, M. L., Douglas, M. S. V., Smol, J. P., 2005. Arctic seabirds transport marine-derived contaminants. Science 309, 445.

Blais, J. M., Macdonald, R. W., Mackay, D., Webster, E., Harvey, C., Smol, J. P., 2007. Biologically mediated transport of contaminants to aquatic systems. Environ. Sci. Technol. 41(4), 1075-1084.

Blake, R. E., Surkov, A.V., Bottcher, M. E., Ferdelman, T. G., Jorgensen, B. B., 2006. Oxygen isotope composition of dissolved sulfate in deep-sea sediments: Eastern equatorial Pacific Ocean. In Jorgensen, B. B., D'Hondt, S. L., Miller, D. J. (Eds.), Proc. ODP Sci. Results 201, 1-23.

Blanco, A., Deudero, S., Box, A., 2009. Muscle and scale isotopic offset of three fish species in the Mediterranean Sea: *Dentex dentex*, *Argyrosomus regius* and *Xyrichtys novacula*. Rapid Commun. Mass Spectrom. 23, 2321-2328.

Bligh, E. G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37(8), 911-917.

Bocking, S., 1997. Fishing the inland seas: Great Lakes research, fisheries management, and environmental policy in Ontario. Environ. Hist. 2(1), 52-73.

Bodaly, R. A., Jansen, W. A., Majewski, A. R., Fudge, R. J. P., Strange, N. E., Derksen, A. J., Green, D. J., 2007. Postimpoundment time course of increased mercury concentrations in fish in hydroelectric reservoirs of northern Manitoba, Canada. Arch. Environ. Contam. Toxicol. 53, 379-389.

Bodaly, R. A., Rudd, J. W. M., Fudge, J. P., Kelly, C. A., 1993. Mercury concentrations in fish related to size of remote Canadian Shield lakes. Can. J. Fish. Aquat. Sci. 50(5), 980-987.

Bond, A. L., Diamond, A. W., 2009. Mercury concentrations in seabird tissues from Machias Seal Island, New Brunswick, Canada. Sci. Total Environ. 407, 4340-4347.

Bond, A. L., Diamond, A. W., 2011. Recent Bayesian stable-isotope mixing models are highly sensitive to variation in discrimination factors. Ecol. Appl. 21(4), 1017-1023.

Bowen, G. J., Wassenaar, L. I., Hobson, K. A., 2005. Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143, 337-348.

Brumbaugh, W. G., Schmitt, C. J., May, T. W., 2005. Concentrations of cadmium, lead and zinc in fish from mining-influenced waters of Northeastern Oklahoma: Sampling of blood, carcass, and liver for aquatic biomonitoring. Arch. Environ. Contam. Toxicol. 49, 76-88.

Burger, J., Gochfeld, M., 1994. Heavy metal and selenium levels in feathers of known-aged common terns (*Sterna hirundo*). Arch. Environ. Contam. Toxicol. 26(3), 351-355.

Burger, J., Gochfeld, M., 1999. Interspecific and locational differences in heavy metal levels in four species of birds near Sidney, Australia. Environ. Monit. Assess. 58, 105-119.

Burger, J., Gochfeld, M., 2001. Metal levels in feathers of cormorants, flamingos and gulls from the coast of Namibia in Southern Africa. Environ. Monit. Assess. 69, 195-203.

Burger, J., Gochfeld, M., 2007. Metals and radionuclides in birds and eggs from Amchitka and Kiska Islands in the Bering Sea/ Pacific Ocean ecosystem. Environ. Monit. Assess. 127, 105-117.

Burger, J., Gochfeld, M., Sullivan, K., Irons, D., McKnight, A., 2008. Arsenic, cadmium, chromium, lead, manganese, mercury, and selenium in feathers of Black-legged Kittiwake (*Rissa tridactyla*) and Black Oystercatcher (*Haematopus bachmani*) from Prince William Sound, Alaska. Sci. Total Environ. 398, 20-25.

Burger, J., Nisbet, I. C., Gochfeld, M., 1992. Metal levels in regrown feathers: Assessment of contamination on the wintering and breeding grounds in the same individuals. J. Toxicol. Environ. Health 37(3), 363-374.

Burgess, N. M., Evers, D. C., Kaplan, J. D., 2005. Mercury and other contaminants in common loons breeding in Atlantic Canada. Ecotoxicology 14, 241-252.

Burgess, N. M., Hobson, K. A., 2006. Bioaccumulation of mercury in yellow perch (*Perca flavescens*) and common loons (*Gavia immer*) in relation to lake chemistry in Atlantic Canada. Hydrobiologia 567, 275-282.

Burnham, K. P., Anderson, D. R., 2002. Model selection and multimodel inference: A practical information-theoretic approach, Second Edition. Springer-Verlag, New York, pp. 149-205.

Burnham, K. P., Anderson, D. R., 2004. Multimodel inference: Understanding AIC and BIC in model selection. Soc. Methods & Res. 33, 261-304.

Cabanero, A. I., Carvalho, C., Madrid, Y., Batoreu, C., Camara, C., 2005. Quantification and speciation of mercury and selenium in fish samples of high consumption in Spain and Portugal. Biol. Trace Elem. Res. 103, 17-35.

Caldwell, C. A., Arnold, M. A., Gould, W. R., 1999. Mercury distribution in blood, tissues, and feathers of double-crested cormorant nestlings from arid-lands reservoirs in south central New Mexico. Arch. Environ. Contam. Toxicol. 36, 456-461.

Campbell, L. M., Norstrom, R. J., Hobson, K. A., Muir. D. C. G., Backus, S., Fisk, A. T., 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). Sci. Total Environ. 351-352, 247-263.

Carmody, R. W., Plummer, L.N., Busenberg, E., Coplen, T.B., 1998. Methods for collection of dissolved sulfate and sulfide and analysis of their sulfur isotopic composition: U. S. Geological Survey Open-File Report 97-234.

Caut, S., Angulo, E., Courchamp, F., 2009. Variation in discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$): The effect of diet isotopic values and applications for diet reconstruction. J. Appl. Ecol. 46, 443-453.

Cervenka, R., Bednarik A., Komarek, J., 2011. The relationship between the mercury concentration in fish muscles and scales/ fins and its significance. Cent. Eur. J. Chem. 9(8), 1109-1118.

Chamberlain, C. P., Blum, J. D., Holmes, R. T., Feng, X., Sherry, T. W., Graves, G. R., 1997. The use of isotope tracers for identifying populations of migratory birds. Oecologia 109, 132-141.

Ciardullo, S., Aureli, F., Coni, E., Guandalini, E., Iosi, F., Raggi, A., Rufo, G., Cubadda, F., 2008. Bioaccumulation potential of dietary arsenic, cadmium, lead, mercury, and selenium in organs and tissues of rainbow trout (*Oncorhyncus mykiss*) as a function of fish growth. J. Agric. Food Chem. 56, 2442-2451.

Cizdziel, J., Hinners, T., Cross, C., Pollard, J., 2003. Distribution of mercury in the tissues of five species of freshwater fish from Lake Mead, USA. J. Environ. Monit. 5, 802-807.

Clark, R. G., Hobson, K. A., Wassenaar, L. I., 2006. Geographic variation in the isotopic (δD, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) composition of feathers and claws from lesser scaup and northern pintail: Implications for studies of migratory connectivity. Can. J. Zool. 84(10), 1395-1401.

Clark, R. G., Hobson, K. A., Wassenaar, L. I., 2009. Corrigendum - Geographic variation in the isotopic (δD, $\delta^{13}C$, $\delta^{15}N$, $\delta^{34}S$) composition of feathers and claws from lesser scaup and northern pintail: Implications for studies of migratory connectivity. Can. J. Zool. 87, 553-554.

Connolly, R. M., Guest, M. A., Melville, A. J., Oakes, J. M., 2004. Sulfur stable isotopes separate producers in marine food-web analysis. Oecologia 138, 161-167.

Croisetiére, L., Hare, L., Tessier, A., Cabana, G., 2009. Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. Freshwater Biology 54, 1006-1015.

Cucherousset, J., Bouletreau, S., Martino, A., Roussel, J.-M., Santoul, F., 2011. Using stable isotope analysis to determine the ecological effects of non-native fishes. Fisheries Manag. Ecol. 1-9.

Dalgaard, P., 2008. Introductory statistics with R, Second Edition. Springer, New York, pp. 95-107.

Darafsh, F., Maschinchian, A., Fatemi, M., Jamili, S., 2008. Study of the application of fish scale as bioindicator of heavy metal pollution (Pb, Zn) in the *Cyprinus carpio* of the Caspian Sea. Res. J. Environ. Sci. 2(6), 438-444.

Dietz, R., Outridge, P. M., Hobson, K. A., 2009. Anthropogenic contributions to mercury levels in present-day Arctic animals - A review. Sci. Total Environ. 407, 6120-6131.

Digital distribution maps of the birds of the western hemisphere version 3.0; http://natureserve.org/getData/birdMaps.jsp. Accessed March 9, 2010.

Dolbeer, R. A., 1991. Migration patterns of double-crested cormorants east of the Rocky Mountains J. Field Ornithol. 62(1), 83-93.

Dorr, B., King, D. T., Tobin, M. E., Harrel, J. B., Smith, P. L., 2004. Double-crested Cormorant movements in relation to aquaculture in eastern Mississippi and western Alabama. Waterbirds 27(2), 147-154.

Dorr, B. S., Moerke, A., Bur, M., Bassett, C., Aderman, T., Traynor, D., Singleton, R. D., Butchko, P. H., Taylor, J. D. II., 2010. Evaluation of harassment of migrating double-crested cormorants to limit depredation on selected sport fisheries in Michigan. J. Great Lakes Res. 36, 215-223.

Doucette, J. L., Wissel, B., Somers, C. M., 2010. Effects of lipid extraction and lipid normalization on stable carbon and nitrogen isotope ratios in double-crested cormorants: Implications for food web studies. Waterbirds 33(3), 273-284.

Doucette, J. L., Wissel, B., Somers, C. M., 2011. Cormorant-fisheries conflicts: Stable isotopes reveal a consistent niche for avian piscivores. Ecol. Appl. 21(8), 2987-3001.

Elliot, K. H., Cesh, L. S., Dooley, J. A., Letcher, R. J., Elliot, J. E., 2009. PCBs and DDE, but not PBDEs, increase with trophic level and marine input in nestling bald eagles. Sci. Total Environ. 407, 3867-3875.

Environment Canada and Manitoba Water Stewardship, 2011. State of Lake Winnipeg: 1999 to 2007 technical report. Public Works & Government Services of Canada, Winnipeg, Manitoba. 209 pp.

Ethier, A. L. M., Sheuhammer, A. M., Bond, D. E., 2008. Correlates of mercury in fish from lakes near Clyde Forks, Ontario, Canada. Environ. Pollut. 154, 89-97.

Evers, D. C., Burgess, N. M., Champoux, L., Hoskins, B., Major, A., Goodale, W. M., Taylor, R. J., Poppenga, R., Daigle, T., 2005. Patterns and interpretation of mercury exposure in

freshwater avian communities in northeastern North America. Ecotoxicology 14, 193-221.

Farrell, A. P., Hodaly, A. H., Wang, S., 2000. Metal analysis of scales taken from arctic grayling. Arch. Environ. Contam. Toxicol. 39, 515-522.

Fielder, D. G., 2008. Examination of factors contributing to the decline of the yellow perch population and fishery in Les Cheneaux Islands, Lake Huron, with emphasis on the role of double-crested cormorants. J. Great Lakes Res. 34, 506-523.

Fielder, D. G., 2010. Response of yellow perch in Les Cheneaux Islands, Lake Huron to declining numbers of double-crested cormorants stemming from control activities. J. Great Lakes Res. 36, 207-214.

Fincel, M. J., Vandehey, J. A., Chipps, S. R., 2011. Non-lethal sampling of walleye for stable isotope analysis: a comparision of three tissues. Fish. Manag. Ecol. 1-10.

Fonyuy, E. W., Atekwana, E. A., 2008. Effects of acid mine drainage on dissolved inorganic carbon and stable carbon isotopes in receiving streams. Appl. Geochem. 23, 743-764.

Fox, G. A., Grasman, K. A., Hobson, K. A., Williams, K., Jeffrey, D., Hanbridge, B., 2002. Contaminant residues in tissues of adult and prefledged herring gulls from the Great Lakes in relation to diet in the early 1990s. J. Great Lakes Res. 28(4), 643-663.

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

Fry, B., 1988. Food web structure on George Bank from stable C, N and S isotopic compositions Limnol. Oceanogr. 33(5), 1182-1190.

Fry, B., 1989. Sulfate fertilization and changes in stable isotopic composition of lake sediments. In Rundel, P. W., Ehleringer, J. R., Nagy, K. A. (Eds.), Stable isotopes in ecological research. Springer-Verlag, New York, pp. 445-453.

Fry, B., 2001. Stable isotopic indicators of habitat use by Mississippi River fish. J. N. Am. Benthol. Soc. 21, 676-685.

Fry, B., 2006. Stable Isotope Ecology. Springer, New York.308 pp.

Furness, R. W., Muirhead, S. J., Woodburn, M., 1986. Using bird feathers to measure mercury in the environment - relationships between mercury content and molt. Mar. Pollut. Bull. 17, 27-30.

Gewurtz, S., Bhavsar, S. P., Fletcher, R., 2011. Influence of fish size and sex on mercury/ PCB concentration: Importance for fish consumption advisories. Environ. Int. 37, 425-434.

Gewurtz, S. B., Gandhi, N., Stern, G. A., Franzin, W. G., Rosenberg, B., Diamond, M. L., 2006. Dynamics of PCBs in the foodweb of Lake Winnipeg. J. Great Lakes Res. 32, 712-727.

Gorski, P. R., Cleckner, L. B., Hurley, J. P., Sierszen, M. E., Armstrong, D. E., 2003. Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. Sci. Total Environ. 304, 327-348.

Goutner, V., Papagiannis, I., Kalfakakou, V., 2001. Lead and cadmium in eggs of colonially nesting waterbirds of different position in the food chain of Greek wetlands of international importance. Sci. Total Environ. 267, 169-176.

Greichus, Y. A., Greichus, A., Emerick, R. J., 1973. Insecticides, polychlorinated biphenyls and mercury in wild cormorants, pelicans, their eggs, food and environment. Bull. Environ. Contam. Toxicol. 9(6), 321-328.

Gremillion, P. T., Cizdziel, J. V., Cody, N. R., 2005. Caudal fin mercury as a non-lethal predictor of fish muscle mercury. Environ. Chem. 2, 96-99.

Gu, B., Schelske, C. L., 1996. Temporal and spatial variations in phytoplankton carbon isotopes in a polymictic subtropical lake. J. Plankton Res. 18(11), 2081-2092.

Gu, B., Schell, D. M., Fraser, T., Hoyer, M., Chapman, F.A., 2001. Stable carbon isotope evidence for reduced feeding of Gulf of Mexico sturgeon during their prolonged river residence period. Estuarine Coastal Shelf Sci. 53, 275-280.

Gustin, M. S., Saito, L., Peacock, M., 2005. Anthropogenic impacts on mercury concentrations and nitrogen and carbon isotope ratios in fish muscle tissue of the Truckee River watershed, Nevada, USA. Sci. Total Environ. 347, 282-294.

Hall, B. D., Baron, L. A., Somers, C. M., 2009. Mercury concentrations in surface water and harvested waterfowl from the Prairie Pothole region of Saskatchewan. Environ. Sci. Technol. 43, 8759-8766.

Hamilton, S. J., Holley, K. M., Buhl, K. J., Bullard, F. A., Weston, L. K., McDonald, S. F., 2002. Impact of selenium and other trace metals on the endangered razorback sucker. Environ. Toxicol. 17, 297-323.

Handy, R. D., 1992. The assessment of episodic metal pollution II. The effects of cadmium and copper enriched diets on tissue contaminant analysis in rainbow trout (*Oncorhynchus mykiss*). Arch. Environ. Contam. Toxicol. 22, 82-87.

Hargreaves, A. L., Whiteside, D. P., Gilchirst, G., 2010. Concentrations of 17 elements, including mercury, and their relationship to fitness measures in arctic shorebirds and their eggs. Sci. Total Environ. 408, 3153-3161.

Hatch, J. J., Weseloh, D. V., 1999. Double-crested Cormorant (*Phalacrocorax auritus*). The birds of North America online. Accessed March 27, 2011.

Hebert, C. E., 1998. Winter severity affects migration and contaminant accumulation in northern Great Lakes herring gulls. Ecol. Appl. 8(3), 669-679.

Hebert, C. E., Arts, M. T., Weseloh, D. V. C., 2006. Ecological tracers can quantify food web structure and change. Environ. Science Technol. 40(18), 5618-5623.

Hebert, C. E., Bur, M., Sherman, D., Shutt, J. L., 2008. Sulfur isotopes link overwinter habitat use and breeding condition in double-crested cormorants. Ecol. Appl. 18(3), 561-567.

Hecky, R. E., Hesslein, R. H., 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. J. N. Am. Benthol. Soc. 14(4), 631-653.

Hélie, J. F., Hillaire-Marcel, C., Rondeau, B., 2002. Seasonal changes in the sources and fluxes of dissolved inorganic carbon through the St. Lawrence River - isotopic and chemical constraints. Chem. Geol. 186, 117-138.

Hesslein, R. H., Capel, M. J., Fox, D. E., Hallard, K. A., 1991. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the Lower Mackenzie River Basin, Canada. Can. J. Fish. Aquat. Sci. 48, 2258-2265.

Hewson, L. C., 1955. Age, maturity, spawning, and food of burbot, *Lota lota*, in Lake Winnipeg. J. Fish. Res. Board Can. 12(6), 930-940.

Hobson, K. A., 2009. Trophic interactions between cormorants and fisheries: Towards a more quantitative approach using stable isotopes. Waterbirds 32(4), 481-490.

Hobson, K. A., Fisk, A., Karnovsky, N., Holst, M., Gagnon, J.-M., Fortier, M., 2002. A stable isotope (δ^{13}C, δ^{15}N) model for the North Water food web: Implications for evaluating trophodynamics and the flow of energy and contaminants. Deep Sea Research II, 49, 5131-5150.

Hobson, K. A., Knapton, R. W., Lysack, W., 1989. Population, diet and reproductive success of Double-crested Cormorants breeding on Lake Winnipegosis, Manitoba, in 1987. Colonial Waterbirds 12(2), 191-197.

Hobson, K. A., Lormee, H., Van Wilgenburg, S. L., Wassenaar, L. I., Boutin, J. M., 2009*a*. Stable isotopes (δD) delineate the origins and migratory connectivity of harvested animals: The case of European woodpigeons. J. Appl. Ecol. 46, 572-581.

Hobson, K. A., Ofukany, A., Soto, D. X., Wassenaar, L. I., 2010. An isotopic baseline (δ^{13}C, δ^{15}N) for fishes of Lake Winnipeg: Implications for investigating impacts of eutrophication and invasive species J. Great Lakes Res. doi: 10.1016/jglr.2010.11.008.

Hobson, K. A., Sharp, C. M., Jefferies, R. L., Rockwell, R. F., Abraham, K. F., 2011. Nutrient allocation strategies to eggs by lesser snow geese (*Chen caerulescens*) at a sub-Arctic colony. The Auk 128(1), 156-165.

Hobson, K. A., Van Wilgenburg, S. L., Larson, K., Wassenaar, L. I., 2009*b*. A feather hydrogen isoscape for Mexico. J. Geochem. Explor. 102, 63-70.

Hobson, K. A., Van Wilgenburg, S., Wassenaar, L. I., 2006. Using stable hydrogen isotope analysis of feathers to delineate origins of harvested sandhill cranes in the central flyway of North America. Waterbirds 29(2), 137-147.

Hobson, K. A., Wassenaar, L. I., 1997. Linking breeding and wintering grounds of neotropical migrant songbirds using stable hydrogen isotopic analysis of feathers. Oecologia 109(1), 142-148.

Hobson, K. A., Wassenaar, L. I. (Eds.), 2008. Tracking animal migration using stable isotopes. Elselvier, San Diego. 160 pp.

Holland, G., Tanner, S. D., 2001. Plasma source mass spectrometry: The new millennium. Royal Society of Chemistry, Cambridge. 412 pp.

Hutchinson, J. J., Trueman, C. N., 2006. Stable isotope analysis of collagen in fish scales: Limitations set by scale architecture. J. Fish Biol. 69, 1874-1880.

Ikemoto, T., Tu, N. P. C., Okuda, N., Iwata, A., Omori, K., Tanabe, S., Tuyen, B. C., Takeuchi, I., 2008. Biomagnification of trace elements in the aquatic food web in the Mekong Delta, South Vietnam using stable carbon and nitrogen isotope analysis. Arch. Environ. Contam. Toxicol. 54, 504-515.

Inamura, O., Zhang, J., Minagawa, M., 2012. δ^{13}C and δ^{15}N values in scales of *Micropterus salmoides* largemouth bass as a freshwater environmental indicator. Rapid Commun. in Mass Spectrom. 26, 17-24.

Irvine, R. L., Oussoren, T., Baxter, J. S., Schmidt, D. C., 2009. The effects of flow reduction rates on fish stranding in British Columbia, Canada. River Res. Applic. 25, 405-415.

Jaeger, I., Hop, H., Gabrielsen, G. W., 2009. Biomagnification of mercury in selected species from an Arctic marine food web in Svalbard. Sci. Total Environ. 407, 4744-4751.

Jardine, T. D., Gray, M. A., McWilliam, S. M., Cunjak, R. A., 2005. Stable isotope variability in tissues of temperate stream fishes. Trans. Am. Fish. Soc. 134(5), 1103-1110.

Jardine, T. D., Kidd, K. A., Fisk, A. T., 2006. Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. Environ. Sci. Technol. 40(24), 7501-7511.

Johnson, J. H., Ross, R. M., McCullough, R. D., Mathers, A., 2010. Diet shift of double-crested cormorants in eastern Lake Ontario associated with the expansion of the invasive round goby. J. Great Lakes Res. 36, 242-247.

Johnston, T. A., Lysack, W., Leggett, W. C., 2010. Abundance, growth and life history characteristics of sympatric walleye (*Sander vitreus*) and sauger (*S. canadensis*) in Lake Winnipeg, Manitoba. J. Great Lakes Res.doi: 10.1016/jglr.2010.06.009.

Jones, A. W., Dalton, C. M., Stowe, E. S., Post, D. M., 2010. Contribution of declining anadromous fishes to the reproductive investment of a common piscivorous seabird, the double-crested cormorant (*Phalocrocorax auritus*). The Auk 127(3), 696-703.

Kelly, J. F., 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. Can. J. Zool. 78, 1-27.

Kelly, M. H., Hagar, W. G., Jardine, T. D., Cunjak, R. J., 2006. Nonlethal sampling of sunfish and slimy sculpin for stable isotope analysis: How scale and fin tissue compare with muscle tissue. N. Am. J. Fish. Manage. 26, 921-925.

Kendall, C., Coplen, T., 2001. Distribution of oxygen-18 and deuterium in river waters across the United States. Hydrol. Processes 15, 1363-1393.

Kidd, K. A., Schindler, D. W., Hesslein, R. H., Muir, D. C. G., 1995. Correlation between stable nitrogen isotope ratios and concentrations of organochlorines in biota from a freshwater food web. Sci. Total Environ. 160/161, 381-390.

Kline, T. C. J., 1999. Temporal and spatial variability of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ in pelagic biota of Prince William Sound, Alaska. Can. J. Fish. Aquat. Sci. 58(1), 94-117.

Kling, H. J., 1998. A summary of past and recent plankton of Lake Winnipeg, Canada using algal fossil remains. J. Paleolimnol. 19, 297-307.

Kristofferson, A. H., Clayton, J. W., 1990. Subpopulation status of lake whitefish (*Coregonus clupeaformis*) in Lake Winnipeg. Can. J. Fish. Aquat. Sci. 47, 1484-1494.

Krouse, H. R., Stewart, J. W. B., Grinenko, V. A., 1991. Pedosphere and biosphere. In Krouse, H. R., Grinenko, V. A. (Eds.), Stable isotopes: Natural and anthropogenic sulphur in the environment. John Wiley and Sons, New York, pp. 267-306.

Lajtha, K., Michener, R. H. (Eds.), 1994. Stable isotopes in ecology and environmental science. Blackwell, London.

Lake, J. L., Ryba, S. A., Serbst, J. R., Libby, A. D., 2006. Mercury in fish scales as an assessment method for predicting muscle tissue mercury concentrations in largemouth bass. Arch. Environ. Contam. Toxicol. 50, 539-544.

Larson, K. W., Hobson, K. A., 2009. Assignment to breeding and wintering grounds using stable isotopes: A comment on lessons learned by Rocque et al. J. Ornithol. 150, 709-712.

Law, K., Halldorson, T., Danell, R., Stern, G., Gewurtz, S., Alaee, M., Marvin, C., Whittle, M., Tomy, G., 2006. Bioaccumulation and trophic transfer of some brominated flame retardants in a Lake Winnipeg (Canada) food web. Environ. Toxicol. Chem. 25(8), 2177-2186.

Leach, J. H., Johnson, M. G., Kelso, J. R. M., Hartmann, J., Numann, W., Entz, B., 1977. Responses of percid fishes and their habitats to eutrophication. J. Fish. Res. Board Can. 34(10), 1959-1963.

Leach, J. H., Nepzsy, S. J., 1976. The fish community in Lake Erie. J. Fish. Res. Board Can. 33, 622-638.

Logan, J. M., Jardine, T. D., Miller, T. J., Bunn, S. E., Cunjak, R. A., Lutcavage, M. E., 2008. Lipid corrections in carbon and nitrogen stable isotope analyses: Comparison of chemical extraction and modelling methods. J. Anim. Ecol. 77, 838-846.

Lott, C. A., Meehan, T. D., Heath, J. A., 2003. Estimating the latitudinal origins of migratory birds using hydrogen and sulfur stable isotopes in feathers: Influence of marine prey base. Oecologia 134, 505-510.

Ludwig, J. P., Hull, C. N., Ludwig, M. E., Auman, H. J., 1989. Food habits and feeding ecology of nestling Double-crested Cormorants in the upper Great Lakes, 1986-1989. Jack-Pine Wr. 67, 115-126.

Ludwig, J. P., Kurita-Matsuba, H., Auman, H. J., Ludwig, M. E., Summer, C. L., Giesy, J. P., Tillitt, D. E., Jones, P. D., 1996. Deformities, PCBs, and TCDD-Equivalents in Double-crested Cormorants (*Phalacrocorax auritus*) and Caspian Terns (*Hydroprogne caspia*) of the Upper Great Lakes 1986-1991: Testing a cause-effect hypothesis. J. Great Lakes Res. 22(2), 172-197.

Lumb, C. E., Franzin, W. G., Watkinson, D. A., 2011. Temporal and spatial patterns in pelagic trawl fish catches in Lake Winnipeg. J. Great Lakes Res. doi: 10.1016/jglr.2011.07.008.

Manahan, S. E., 2005. Environmental chemistry. CRC Press, Boca Raton, pp. 507-541.

Manitoba Water Stewardship, 2010. A profile of Manitoba's commercial fishery. Manitoba Conservation and Water Stewardship. http://www.gov.mb.ca/waterstewardship/fisheries/commercial/history.pdf. Accessed March 11, 2012.

Mayer, T., Simpson, S. L., Thorleifson, L. H., Lockhart, W. L., Wilkinson, P., 2006. Phosphorus geochemistry of recent sediments in the South Basin of Lake Winnipeg. Aquat. Ecosyst. Health Manage. 9(3), 307-318.

Mayer, B., Wassenaar, L. I., 2012. Isotopic characterization of nitrate sources and transformations in Lake Winnipeg and its contributing rivers, Manitoba, Canada. J. Great Lakes Res. doi: 10.1016/j.jglr.2012.02.004.

Mazerolle, M. J., 2006. Improving data analysis in herpetology: Using Akaike's Information Criterion (AIC) to assess the strength of biological hypotheses. Amphibia-Reptilia 27, 169-180.

Miyajima, T., Yamada, Y., Hanba, Y. T., Yoshii, K., Koitabashi, T., Wada, E., 1995. Determining the stable isotope ratio of total dissolved inorganic carbon in lake water by GC/C/IRMS. Limnol. Oceanogr. 40(5), 994-1000.

Moles, M. D., Johnston, T. A., Robinson, B. W., Bernard, A. M. Wilson, C. C., Wiegand, M. D., Leggett, W. C., 2011. Reproductive divergence between growth forms of Lake Winnipeg walleye (*Sander vitreus*). Ecol. Freshw. Fish. 20, 52-66.

Moles, M. D., Robinson, B. W., Johnston, T. A., Cunjak, R.A., Jardine, T. D., Casselman, J.M., Leggett, W.C., 2010. Morphological and trophic differentiation of growth morphotypes of walleye (*Sander vitreus*) from Lake Winnipeg, Canada. Can. J. Zool. 88, 950-960.

Moore, J. W., Semmens, B. X., 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecol. Lett. 11, 470-480.

Moreno, R., Jover, L., Munilla, I., Velando, A., Sanpera, C., 2010. A three-isotope approach to disentangling the diet of a generalist consumer: The yellow-legged gull in northwest Spain. Mar. Biol. 157, 545-553.

Morita, H., Tanaka, H., Shimomura, S., 1995. Atomic fluorescence spectrometry of mercury: Principles and developments. Spectrochim. Acta, Part B 50(1), 69-84.

Muir, D., Wang, X., Bright, D., Lockhart, L., Kock, G., 2005. Spatial and temporal trends of mercury and other metals in landlocked char from the lakes in the Canadian Arctic archipelago. Sci. Total Environ. 351-352, 464-478.

Munawar, M., Munawar, I. F., Mandrak, N. E., Fitzpatrick, M., Dermott, R., Leach, J., 2005. An overview of the impact of non-indigenous species on the food web integrity of North American Great Lakes: Lake Erie example. Aquat. Ecosyst. Health Manage. 8(4), 375-395.

Muscatello, J. R., Belknap, A. M., Janz, D. M., 2008. Accumulation of selenium in aquatic systems downstream of a uranium mining operation in northern Saskatchewan, Canada. Environ. Pollut. 156, 387-393.

Muscatello, J. R., Janz, D. M., 2009. Selenium accumulation in aquatic biota downstream of a uranium mining and milling operation. Sci. Total Environ. 407, 1318-1325.

Neumann, T., Heiser, U., Leosson, M. A., Kersten, M., 2002. Early diagenetic processes during Mn-carbonate formation: Evidence from the isotopic composition of authigenic Ca-rhodochrosites of the Baltic Sea. Geochim. Cosmochim. Acta 66(5), 867-879.

Newman, M. C., Unger, M. A., 2003. Fundamentals of ecotoxicology, Second Edition. Lewis Publishers, Boca Raton. 458 pp.

Nisbet, I. C. T., Montoya, J. P., Burger, J., Hatch, J. J., 2002. Use of stable isotopes to investigate individual differences in diets and mercury exposures among common terns *Sterna hirundo* in breeding and wintering grounds. Mar. Ecol. Prog. Ser. 242, 267-274.

Nriagu, J. O., Soon, Y. K., 1985. Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. Geochim. Cosmochim. Acta 49, 823-834.

Ontario Ministry of Natural Resources (OMNR), 2006. Review of the status and management of double-crested cormorants in Ontario. Fish and Wildlife Branch. Wildlife section. Peterborough, Ontario, pp. 1-76.

Padula, V., Burger, J., Newman, S. H., Elbin, S., Jeitner, C., 2010. Metals in feathers of black-crowned night-heron (*Nycticorax nycticorax*) chicks from the New York harbor estuary. Arch. Environ. Contam. Toxicol. 59, 157-165.

Paradis, Y., Bertolo, A., Magnan, P., 2008. What do the empty stomachs of northern pike (*Esox lucius*) reveal? Insights from carbon (δ^{13}C) and nitrogen (δ^{15}N) stable isotopes. Environ. Biol. Fishes 83, 441-448.

Parnell, A. C., Inger, R., Bearhop, S., Jackson, A. L., 2010. Source partitioning using stable isotopes: Coping with too much variation. PLoS ONE, doi: 10.1371/journal.pone.0009672.

Patalas, K., Salki, A., 1992. Crustacean plankton in Lake Winnipeg: Variation in space and time as a function of lake morphology, geology, and climate. Can. J. Fish. Aquat. Sci. 49, 1035-1059.

Peakall, D., Burger, J., 2003. Methodologies for assessing exposure to metals: Speciation, bioavailability of metals, and ecological host factors. Ecotoxicol. Environ. Saf. 56, 110-121.

Perga, M. E., Gerdeaux, D., 2003. Using the δ^{13}C and δ^{15}N of whitefish scales for retrospective ecological studies: Changes in isotope signatures during the restoration of Lake Geneva, 1980-2001. J. Fish. Biol. 63, 1197-1207.

Phibbs, J., Wiramanaden, C. I. E., Hauck, D., Pickering, I. J., Liber, K., Janz, D. M., 2011. Selenium uptake and speciation in wild and caged fish downstream of a metal mining and milling discharge. Ecotoxicol. Environ. Saf. 74, 1139-1150.

Phillips, D. L., Gregg, J. W., 2003. Source partitioning using stable istopes: Coping with too many sources. Oecologia 136, 261-269.

Phillips, D. L., Koch, P. L., 2002. Incorporating concentration dependence in stable isotope mixing models. Oecologia 130, 114-125.

Pickhardt, P. C., Folt, C. L., Chen, C. Y., Klaue, B., Blum, J. D., 2002. Algal blooms reduce the uptake of toxic methylmercury in freshwater food webs. Proc. Natl. Acad. Sci. U. S. A. 99(7), 4419-4423.

Pineiro, G., Perelman, S., Guershman, J. P., Paruelo, J. M., 2008. How to evaluate models: Observed vs. predicted, or predicted vs. observed? Ecol. Modell. 216, 316-322.

Pip, E., 2006. Littoral mollusc communities and water quality in southern Lake Winnipeg, Manitoba, Canada. Biodiversity Conserv. 15, 3637-3652.

Pluess, T., Cannon, R., Jarosik, V., Pergl, J., Pysek, P., Bacher, S., 2011. When are eradication campaigns successful? A test of common assumptions. Biol. Invasions 1-14.

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

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., Montaña, C. G., 2007. Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152(1), 179-189.

Pruell, R. J., Taplin, B. K., Cicchelli, K., 2003. Stable isotope ratios in archived striped bass scales suggest changes in trophic structure. Fisheries Manag. Ecol. 10, 329-336.

R Development Core Team, 2010. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. ISBN 3-900051-07-0, http://www.R-project.org.

R Development Core Team, 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna. ISBN 3-900051-07-0, http://www.R-project.org.

Rashed, M. N., 2001. Monitoring of environmental heavy metals in fish from Nasser Lake. Environ. Int. 27, 27-33.

Rawn, D. F. K., Muir, D. C. G., Savoie, D. A., Rosenberg, G. B., Lockhart, W. L., Wilkinson, P., 2000. Historical deposition of PCBs and Organochlorine Pesticides to Lake Winnipeg (Canada). J. Great Lakes Res. 26(1), 3-17.

Revesz, K., Qi, H., 2006. Determination of the $\delta(^{34}S/^{32}S)$ of low-concentration sulfate in water. In Revesz, K., Coplen, T. B. (Eds.), Methods of the Reston Stable Isotope Laboratory:

Reston, Virginia, U. S. Geological Survey, Techniques and Methods, book 10, sec. C. pp. 1-35.

Ricca, M. A., Miles, A. K., Anthony, R. G., Deng, X., Hung, S. S. O., 2007. Effect of lipid extraction on analyses of stable carbon and stable nitrogen isotopes in coastal organisms of the Aleutian archipelago. Can. J. Zool. 85(1), 40-48.

Robinson, E. H., Li, M. H., Manning, B. B., 2001. A practical guide to nutrition, feeds and feeding of catfish (Second Revision) Mississippi Agricultural & Forestry Experiment Station, Mississippi State University, Starkville, Mississippi, pp. 1-44.

Rocque, D. A., Winker, K., 2004. Biomonitoring of contaminants in birds from two trophic levels in the North Pacific. Environ. Toxicol. Chem. 23(3), 759-766.

Rolfhus, K. R., Sandheinrich M. B., Wiener, J. G., Bailey, S. W., Thoreson, K. A., Hammerschmidt, C. R., 2008. Analysis of fin clips as a nonlethal method for monitoring mercury in fish. Environ. Sci. Technol. 42, 871-877.

Rudstam, R. G., Van De Valk, A. J., Adams, C. M., Coleman, J. T. H., Forney, J. L., Richmond, M. E., 2004. Cormorant predation and the population dynamics of walleye and yellow perch on Oneida Lake. Ecol. Appl. 14(1), 149-163.

Ruelas-Inzunza, J., Paez-Osuna, F., 2007. Essential and toxic metals in nine fish species for human consumption from two coastal lagoons in the Eastern Gulf of California. J. Environ. Sci. Health, Part A: Environ. Sci. Eng. 42(10), 1411-1416.

Ryba, S. A., Lake, J. L., Serbst, J. R., Libby, A. D., Ayvazian, S., 2008. Assessment of caudal fin clip as a non-lethal technique for predicting muscle tissue mercury concentrations in largemouth bass. Environ. Chem. 5, 200-203.

Salata, G. G., Roelke, L. A., Cifuentes, L. A., 2000. A rapid and precise method for measuring stable carbon isotope ratios of dissolved inorganic carbon Mar. Chem. 69, 153-161.

Savinov, V. M., Gabrielsen, G. W., Savinova, T. N., 2003. Cadmium, zinc, copper, arsenic, selenium and mercury in seabirds from Barents Sea: Levels, inter-specific and geographical differences. Sci. Total Environ. 306, 133-158.

Scheuhammer, A. M., 1987. The chronic toxicity of aluminum, cadmium, mercury, and lead in birds: A review. Environ. Pollut. 46, 263-295.

Schmidt, S. N., Vander Zanden, M. J., Kitchell, J. F., 2009. Long-term food web change in Lake Superior. Can. J. Fish. Aquat. Sci. 66(12), 2118-2129.

Seefelt, N. E., Gillingham, J. C., 2006. A comparison of three methods to investigate the diet of breeding Double-crested Cormorants (*Phalocrocorax auritus*) in the Beaver Archipelago, northern Lake Michigan. Hydrobiologia 567, 57-67.

Seefelt, N. E., Gillingham, J. C., 2008. Bioenergetics and prey consumption of breeding Double-crested Cormorants in the Beaver Archipelago, Northern Lake Michigan. J. of Great Lakes Res. 34, 122-133.

Sepulveda, M. S., Poppenga, R. H., Arrecis, J. J., Quinn, L. B., 1998. Concentrations of mercury and selenium in tissues of Double-crested Cormorants (*Phalacrocorax auritus*) from southern Florida. Colon. Waterbird 21(1), 35-42.

Sinnatamby, R. N., Bowman, J. E., Dempson, J. B., Power, M., 2007. An assessment of de-calcification procedures for δ^{13}C and δ^{15}N analysis of yellow perch, walleye and Atlantic salmon scales. J. Fish Biol. 70, 1630-1635.

Sinnatamby, R. N., Dempson, J. B., Power, M., 2008. A comparison of muscle- and scale-derived δ^{13}C and δ^{15}N across three life-history stages of Atlantic salmon, *Salmo salar*. Rapid Communications in Mass Spectrometry, 22, 2773-2778.

Skurdal, J., Skogheim, O. K., Qvenild, T., 1986. Indicative value of mercury concentration in adipose fin of brown trout, *Salmo trutta* L. J. Fish Bio. 29, 515-517.

Somers, J. D., Goski, B. C., Barbeau, J. M., Barrett, M. W., 1993. Accumulation of organochlorine contaminants in Double-crested Cormorants. Environ. Pollut. 80, 17-23.

Sotiropoulos, M. A., Tonn, W. M., Wassenaar, L. I., 2004. Effects of lipid extraction on stable carbon and nitrogen isotope analyses of fish tissues: Potential consequences for food web studies. Ecol. Freshw. Fish 13, 155-160.

Soto, D. X., Wassenaar, L. I., Hobson, K. A., Catalan, J., 2011. Effects of size and diet on stable hydrogen isotope values (δD) in fish: Implications for tracing origins of individuals and their food sources. Can. J. Fish. Aquat. Sci. 68(11), 2011-2019.

Speers, J., Gillis, D., 2011. Catch and effort variation in the commercial gillnet fishery of Lake Winnipeg, Canada, in relation to environmental factors. J. Great Lakes Res. doi: 10.1016/j.jglr.2011.05.006.

Stewart, K. W., Watkinson, D. A., 2004. The Freshwater Fishes of Manitoba. University of Manitoba Press, Winnipeg.pp. 1-278.

Stewart, R. A., Stern, G. A., Lockhart, W. L., Kidd, K. A., Salki, A. G., Stainton, M. P., Koczanski, K., Rosenberg, G. B., Savoie, D. A., Billeck, B. N., Wilkinson, P., Muir, D. C. G., 2003. Assessing trends in organochlorine concentrations in Lake Winnipeg fish following the 1997 Red River flood. J. Great Lakes Res. 29(2), 332-354.

Suchy, K. D., Salki, A., Hann, B. J., 2010. Investigating the invasion of the nonindigenous zooplankter, *Eubosmina coregoni*, in Lake Winnipeg, Manitoba, Canada. J. Great Lakes Res. 36, 159-166.

Swanson, H. K., Johnston, T. A., Leggett, W. C., Bodaly, R. A., Doucett, R. R., Cunjak, R. A., 2003. Trophic positions and mercury bioaccumulation in rainbow smelt (*Osmerus mordax*) and native forage fishes in northwestern Ontario lakes. Ecosystems 6, 289-299.

Swanson, H. K., Johnston, T. A., Schindler, D. W., Bodaly, R. A., Whittle, D. M., 2006. Mercury bioaccumulation in forage fish communities invaded by rainbow smelt (*Osmerus mordax*). Environ. Sci. Technol. 40, 1439-1446.

Symonds, M. R. E., Moussalli, A., 2011. A brief guide to model selection, multimodel inference and model averaging in behavioural ecology using Akaike's information criterion. Behav. Ecol. Sociobiol. 65, 13-21.

Takahashi, K., Yoshioka, T., Wada, E., Sakamoto, M., 1990. Temporal variations in carbon isotope ratios of phytoplankton in a eutrophic lake. J. Plankton Res. 12(4), 799-808.

Tamelander, T., Kivimäe, C., Bellerby, R. G. J., Renaud, P. E., Kristiansen, S., 2009. Base-line variations in stable isotope values in an Arctic marine ecosystem: Effects of carbon and nitrogen uptake by phytoplankton. Hydrobiologia 630, 63-73.

Tavares, P. C., McGill, R., Newton, J., Pereira, E., Duarte, A., Furness, R. W., 2009. Relationship between carbon sources, trophic level and mercury exposure in generalist shorebirds revealed by stable isotope ratios in chicks. Waterbirds 32(2), 311-321.

Todd, B. J., Lewis, C. F. M., Thorleifson, L. H., Nielsen, E., Last, W. M., 1998. Paleolimnology of Lake Winnipeg: Introduction and overview. J. Paleolimnol. 19, 211-213.

Tomy, G. T., Pleskach, K., Ismail, N., Whittle, D. M., Helm, P. A., Sverko, E., Zaruk, D. Marvin, C. H., 2007. Isomers of dechlorane plus in Lake Winnipeg and Lake Ontario food webs. Environ. Sci. Technol. 41, 2249-2254.

Trapp, J. L., Lewis, S. J., Pence, D. M., 1997. Double-crested Cormorant impacts on sport fish: Literature review, agency survey, and strategies. U. S. D. A. National Wildlife Research Center symposia, symposium on Double-crested Cormorants; population status and management issues in the midwest. University of Nebraska, Lincoln. Pp. 87-96.

Trojanowska, A., Lewicka-Szczebak, D., Jedrysek, M.-O., Kurasiewicz, M., Wassenaar, L. I., Izydorczyk, K., 2008. Diurnal variations in the photosynthesis-respiration activity of a cyanobacterial bloom in a freshwater dam reservoir: An isotopic study. Isot. Environ. Health Stud. 44(2), 163-175.

Trueman, C. N., Moore, A., 2007. Use of the stable isotope composition of fish scales for monitoring aquatic systems. In Dawson, T. E., Siegwolf, R. T. W. (Eds.), Stable isotopes as indicators of ecological change. Elselvier, London, pp. 145-162.

Tyus, H. M., Starnes, W. C., Karp, C. A., Saunders, J. F. III., 1999. Effects of invasive tissue colleciton on rainbow trout, razorback sucker, and bonytail chub. N. Am. J. Fish. Manage. 19(3), 848-855.

Ubillús, F., Alegría, A., Barberá, R., Farré, R., Lagarda, M. J., 2000. Methylmercury and inorganic mercury determination in fish by cold vapour generation atomic absorption spectrometry. Food Chem. 71, 529-533.

United States Environmental Protection Agency (US EPA), 1996. Method 3052: Microwave assisted acid digestion of siliceous and organically based matrices. United States Government Printing Office, Washington, pp. 1-20.

United States Environmental Protection Agency (US EPA), 2004. Draft aquatic life water quality criteria for selenium. EPA-822-D-04-001. Office of Water, Office of Science and Technology, Washington, pp 1-82.

United States Environmental Protection Agency (US EPA), 2011. 2010 Biennial National Listing of Fish Advisories. EPA-820-F-11-014. http://www.epa.gov/fishadvisories/advisories/tech2010.pdf. Accessed November 26, 2011.

Van Wilgenburg, S. L., Hobson, K. A., 2010. Combining stable isotope (δD) and band recovery data to improve probabilistic assignment of migratory birds to origin. Ecol. Appl. 21(4), 1340-1351.

Vander Zanden, M. J., Vadeboncoeur, Y., Diebel, M. W., Jeppesen, E., 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. Environ. Sci. Technol. 39, 7509-7515.

Vander Zanden, M., Rasmussen, J. B., 1999. Primary consumer δ^{13}C and δ^{15}N and the trophic position of aquatic consumers. Ecology 80, 1395-1404.

Ventura, M., Jeppesen, E., 2010. Evaluating the need for acid treatment prior to δ^{13}C and δ^{15}N analysis of freshwater fish scales: Effects of varying scale mineral content, lake productivity and CO_2 concentration. Hydrobiologia 644, 245-259.

Wachniew, P., 2006. Isotopic composition of dissolved inorganic carbon in a large polluted river: The Vistula, Poland. Chem. Geol. 233, 293-308.

Wassenaar, L. I., Hobson, K. A., 2003. Comparative equilibration and online technique for determination of non-exchangeable hydrogen of keratins for use in animal migration studies. Isot. Environ. Health Stud. 39(3), 211-217.

Watanabe, K., Monaghan, M. T., Takemon, Y., Omura, T., 2008. Biodilution of heavy metals in a stream macroinvertebrate food web: Evidence from stable isotope analysis. Sci. Total Environ. 394, 57-67.

Waterisotopes.org. http://wateriso.eas.purdue.edu/waterisotopes/pages/data_access/ArcGrids.html. Accessed March 9, 2010.

Wayland, M., Hobson, K. A., 2001. Stable carbon, nitrogen, and sulfur isotope ratios in riparian food webs on rivers receiving sewage and pulp-mill effluents. Can. J. Zool. 79, 5-15.

Weseloh, D. V. C., Pekarik, C., Havelka, T., Barrett, G., Reid, J., 2002. Population trends and colony locations of Double-crested Cormorants in the Canadian Great Lakes and immediately adjacent areas, 1990-2000: A manager's guide. J. Great Lakes Res. 28(2), 125-144.

Wiener, J. G., Knights, B. C., Sandheinrich, M. B., Jeremiason, J. D., Brigham, M. E., Engstrom, D. R., Woodruff, L. G., Cannon, W. F., Balogh, S. J., 2006. Mercury in soils, lakes, and fish in Voyageurs National Park (Minnesota): Importance of atmospheric deposition and ecosystem factors. Environ. Sci. Technol. 40, 6261-6268.

Wiese, F. K., Parrish, J. K., Thompson, C. W., Maranto, C., 2008. Ecosystem-based management of predator-prey relationships: Piscivorous birds and salmonids. Ecol. Appl. 18(3), 681-700.

Wires, L. R., Cuthbert, F. J., 2010. Characteristics of Double-crested Cormorant colonies in the U. S. Great Lakes island landscape. J. Great Lakes Res. 36, 232-241.

Wires, L. R., Cuthbert, F. J., Trexel, D. R., Joshi, A. R., 2001. Status of the Double-crested Cormorant (*Phalacrocorax auritus*) in North America. Final Report to United States Fish and Wildlife Service (USFWS). 359 pp.

Wood, M.-E., Trip, L., 2001. Examining fish consumption advisories related to mercury contamination in Canada. http://www.ec.gc.ca/mercure-mercury/default.asp?lang=En&xml=BA8EA930-01C7-47A4-988F-949B9062A05C#2.3. Accessed January 24, 2012.

Wren, C. D., Maccrimmon, H. R., Loescher, B. R., 1983. Examination of bioaccumulation and biomagnification of metals in a Precambrian Shield lake. Water, Air Soil Pollut. 19, 277-291.

Yang, K. X., Swami, K., 2007. Determination of metals in marine species by microwave digestion and inductively coupled plasma mass spectrometry analysis. Spectrochim. Acta Part B 62, 1177-1181.

Yerkes, T., Hobson, K. A., Wassenaar, L. I., Macleod, R., Coluccy, J. M., 2008. Stable isotopes (δD, δ^{13}C, δ^{15}N) reveal associations among geographic location and condition of Alaskan northern pintails. J. Wildl. Manage. 72, 715-725.

Yong, C., 2000. Speciation and analysis of mercury, arsenic, and selenium by atomic fluorescence spectrometry. Trends Anal. Chem. 19(1), 62-66.

Zazzo, A., Monahan, F. J., Moloney, A. P., Green, S., Schmidt, O., 2011. Sulphur isotopes in animal hair track distance to sea. Rap. Comm. Mass. Spect. 25, 2371-2378.

SOURCES OF PERSONAL COMMUNICATION

Brenda Stanek
SRC Environmental Analytical Laboratories
102-422 Downey Road
Saskatoon, SK S7N 4N1

APPENDIX
SUPPLEMENTARY TABLES

Table A1 Fork lengths (FL; mm) of fishes collected from Lake Winnipeg during 2009 and 2010.

Species	Code		Basin	Min FL	Max FL	Mean ± SD
Burbot	BURB	NA	North	NA	NA	NA
(Lota lota)		n = 5	South	362	696	563 ± 124
Cisco	CISC	n = 101	North	46	258	127 ± 45
(*Coregonus artedi*)		n = 169	South	54	413	171 ± 72
Emerald Shiner	EMSH	n = 92	North	33	85	63 ± 11
(*Notropis atherinoides*)		n = 60	South	33	86	66 ± 11
Freshwater Drum	FRDR	n = 13	North	50	460	256 ± 151
(*Aplodinotus grunniens*)		n = 64	South	21	514	288 ± 75
Goldeye	GOLD	NA	North	NA	NA	NA
(*Hiodon alosoides*)		n = 120	South	58	313	181 ± 81
Lake Whitefish	LKWF	n = 36	North	84	520	320 ± 122
(*Coregonus clupeaformis*)		NA	South	NA	NA	NA
Longnose Sucker	LNSC	n = 7	North	332	449	409 ± 38
(*Catostomus catostomus*)		n = 4	South	330	382	356 ± 25
Mooneye	MOON	NA	North	NA	NA	NA
(*Hiodon tergisus*)		n = 2	South	57	70	64 ± 9
Ninespine Stickleback	NNST	n = 18	North	32	55	44 ± 6
(*Pungitius pungitius*)		NA	South	NA	NA	NA
Northern Pike	NRPK	n = 11	North	340	692	537 ± 115
(*Esox lucius*)		n = 20	South	79	784	591 ± 162
Rainbow Smelt	RNSM	n = 241	North	39	206	91 ± 22
(*Osmerus mordax*)		n = 26	South	35	118	64 ± 16
Sauger	SAUG	n = 34	North	172	406	311 ± 64
(*Sander canadensis*)		n = 111	South	38	422	287 ± 60
Troutperch	TRPR	n = 20	North	50	110	75 ± 15
(*Percopsis omiscomaycus*)		n = 31	South	36	100	61 ± 18
Walleye	WALL	n = 124	North	52	552	363 ± 85
(*Sander vitreus*)		n = 152	South	25	664	311 ± 121
White Bass	WHBS	n = 18	North	25	350	107 ± 103
(*Morone chrysops*)		n = 90	South	30	432	234 ± 101

Table A1 Continued.

Species	Code		Basin	Min FL	Max FL	Mean ± SD
White Sucker	WHSC	n = 55	North	262	530	387 ± 61
(*Catostomus commersonii*)		n = 8	South	272	531	369 ± 101
Yellow Perch	YLPR	n = 29	North	31	304	199 ± 77
(*Perca flavescens*)		n = 56	South	37	256	154 ± 72

NA = not available/ not applicable

Table A2 $\delta X_m / \delta X_s$ ratios for north (n = 18) and south (n = 16) basin walleye (*Sander vitreus*). A Wilcoxon rank sum test (*W*) was used to examine basin-specific differences in muscle: scale isotopic ratios. For Lake Winnipeg walleye, the relative distribution of ^{15}N, ^{13}C and ^{34}S to muscle and scales does not vary with basin.

	North Basin	South Basin		
Ratio	Mean ± SD[a]	Mean ± SD	*W*	p-value
$\delta^{15}N_m / \delta^{15}N_s$	1.0 ± 0.1	1.1 ± 0.1	138	0.72
$\delta^{34}S_m / \delta^{34}S_s$	1.2 ± 0.6	1.3 ± 0.3	99	0.12
$\delta^{13}C_m / \delta^{13}C_s$	1.1 ± 0.3	1.1 ± 0.1	123	0.66

[a]SD = standard deviation

Table A3 Mean ± standard deviation (SD) δ^{15}N, trophic position (TP), δ^{13}C and δ^{34}S for the fishes of Lake Winnipeg's north and south basins. Stable isotope data for muscle is baseline corrected (Equation 3.2). Isotopic units are per mil (‰).

Species	Code		Basin	δ^{15}N	TP	δ^{13}C	δ^{34}S
Burbot	BURB	NA	North	NA	NA	NA	NA
(*Lota lota*)		n = 5	South	6.7 ± 2.2	4.0 ± 0.7	-18.1 ± 0.9	-0.7 ± 1.3
Cisco	CISC	n = 101	North	8.1 ± 1.8	4.4 ± 0.5	-23.1 ± 1.5	-2.1 ± 1.5
(*Coregonus artedi*)		n = 169	South	4.0 ± 1.7	3.2 ± 0.5	-19.1 ± 1.4	0.4 ± 1.5
Emerald Shiner	EMSH	n = 92	North	7.7 ± 1.4	4.3 ± 0.4	-23.4 ± 1.3	-2.7 ± 1.5
(*Notropis atherinoides*)		n = 60	South	3.7 ± 1.5	3.1 ± 0.4	-19.5 ± 0.9	0.0 ± 1.1
Freshwater Drum	FRDR	n = 13	North	7.9 ± 1.0	4.3 ± 0.3	-22.6 ± 0.6	-3.6 ± 1.5
(*Aplodinotus grunniens*)		n = 64	South	4.9 ± 1.7	3.4 ± 0.5	-18.8 ± 1.1	-0.7 ± 2.2
Goldeye	GOLD	NA	North	NA	NA	NA	NA
(*Hiodon alosoides*)		n = 120	South	5.7 ± 1.4	3.7 ± 0.4	-19.8 ± 1.0	-0.1 ± 1.7
Lake Whitefish	LKWF	n = 36	North	8.0 ± 1.6	4.4 ± 0.5	-20.4 ± 1.6	-1.8 ± 1.7
(*Coregonus clupeaformis*)		NA	South	NA	NA	NA	NA
Longnose Sucker	LNSC	n = 7	North	6.7 ± 1.0	4.0 ± 0.3	-21.0 ± 0.8	-2.8 ± 1.4
(*Catostomus catostomus*)		n = 4	South	5.4 ± 0.8	3.6 ± 0.2	-19.1 ± 0.4	-2.7 ± 1.4
Mooneye	MOON	NA	North	NA	NA	NA	NA
(*Hiodon tergisus*)		n = 2	South	5.8 ± 1.6	3.7 ± 0.5	-19.4 ± 0.1	-0.4 ± 0.6
Ninespine Stickleback	NNST	n = 18	North	7.1 ± 2.0	4.1 ± 0.6	-22.9 ± 1.2	0.4 ± 1.4
(*Pungitius pungitius*)		NA	South	NA	NA	NA	NA
Northern Pike	NRPK	n = 11	North	9.5 ± 1.2	4.8 ± 0.3	-21.8 ± 1.8	-0.7 ±1.3
(*Esox lucius*)		n = 20	South	6.8 ± 1.3	4.0 ± 0.4	-18.8 ± 0.7	-0.9 ± 1.4
Rainbow Smelt	RNSM	n = 241	North	8.2 ± 1.3	4.4 ± 0.4	-22.2 ± 0.8	-0.9 ± 1.4
(*Osmerus mordax*)		n = 26	South	5.6 ± 1.9	3.7 ± 0.5	-19.6 ± 0.5	1.7 ± 2.0
Sauger	SAUG	n = 34	North	10.4 ± 1.4	5.0 ± 0.6	-21.9 ± 1.2	-2.6 ± 1.5
(*Sander canadensis*)		n = 111	South	6.5 ± 1.4	3.9 ± 0.4	-17.9 ± 1.6	-0.3 ± 1.5
Troutperch	TRPR	n = 20	North	8.0 ± 0.9	4.4 ± 0.3	-22.1 ± 1.2	-5.3 ± 1.3
(*Percopsis omiscomaycus*)		n = 31	South	4.9 ± 1.2	3.4 ± 0.3	-19.6 ± 0.4	-1.7 ± 2.0
Walleye	WALL	n = 124	North	10.2 ± 1.3	5.0 ± 0.4	-21.5 ± 1.1	-0.7 ± 1.4
(*Sander vitreus*)		n = 152	South	5.6 ± 1.8	3.6 ± 0.5	-17.4 ± 1.6	0.5 ± 2.1
White Bass	WHBS	n = 18	North	8.2 ± 2.2	4.4 ± 0.6	-23.5 ± 1.5	-2.1 ± 1.8
(*Morone chrysops*)		n = 90	South	7.1 ± 1.6	4.1 ± 0.5	-19.3 ± 1.0	0.1 ± 1.7
White Sucker	WHSC	n = 55	North	6.8 ± 1.2	4.0 ± 0.4	-21.4 ± 1.5	-1.0 ± 1.7
(*Catostomus commersonii*)		n = 8	South	4.1 ± 1.6	3.2 ± 0.5	-18.5 ± 1.5	0.7 ± 2.3
Yellow Perch	YLPR	n = 29	North	9.8 ± 2.3	4.9 ± 0.7	-21.4 ± 1.4	-2.4 ± 1.4
(*Perca flavescens*)		n = 56	South	4.9 ± 1.4	3.4 ± 0.4	-18.7 ± 1.4	-0.8 ± 1.7

NA = not available/ not applicable

Table A4 Mean ± standard deviation (SD) $\delta^{15}N$, $\delta^{13}C$ and $\delta^{34}S$ values of fishes used as potential food sources in dietary mixing models. Data was limited to specimens collected during the ice-free seasons of 2009 and 2010, as well as specimens with fork lengths (FLs) < 400 mm. All values have been baseline corrected according to Equation 5.2. Isotopic units are ‰.

Basin	Source Code[a]		$\delta^{15}N$	$\delta^{13}C$	$\delta^{34}S$
North	CISC & EMSH	n = 193	7.9 ± 1.6	-23.2 ± 1.5	-2.4 ± 1.5
	FRDR & YLPR	n = 40	9.3 ± 2.1	-21.7 ± 1.4	-2.7 ± 1.5
	LKWF & WHSC	n = 55	7.4 ± 1.5	-21.5 ± 1.6	-1.0 ± 1.9
	NNST	n = 18	7.1 ± 2.0	-22.9 ± 1.2	0.4 ± 1.3
	RNSM	n = 241	8.2 ± 1.3	-22.2 ± 0.8	-0.9 ± 1.4
	SAUG	n = 32	10.5 ± 1.4	-21.9 ± 1.2	-2.5 ± 1.5
	TRPR	n = 20	8.0 ± 0.9	-22.1 ± 1.2	-5.3 ± 1.3
	WALL	n = 76	10.1 ± 1.4	-21.8 ± 1.1	-0.9 ± 1.6
	WHBS	n = 18	8.2 ± 2.2	-23.5 ± 1.5	-2.1 ± 1.8
South	CISC & EMSH	n = 217	3.9 ± 1.7	-19.2 ± 1.3	0.3 ± 1.4
	FRDR & YLPR	n = 113	5.0 ± 1.6	-18.7 ± 1.2	-0.7 ± 1.9
	GOLD	n = 111	5.6 ± 1.3	-19.8 ± 1.0	-0.1 ± 1.7
	RNSM	n = 26	5.6 ± 1.9	-19.6 ± 0.5	1.7 ± 2.0
	WALL & SAUG	n = 209	6.3 ± 1.6	-17.9 ± 1.6	-0.1 ± 1.7
	TRPR	n = 31	4.9 ± 1.2	-19.6 ± 0.4	-1.7 ± 2.0
	WHBS	n = 57	6.5 ± 1.4	-19.2 ± 1.2	0.6 ± 1.8
	WHSC	n = 5	4.4 ± 1.8	-18.8 ± 1.6	1.7 ± 2.2

[a]Species codes: CISC = cisco (*Coregonus artedi*); EMSH = emerald shiner (*Notropis atherinoides*); FRDR = freshwater drum (*Aplodinotus grunniens*); GOLD = goldeye (*Hiodon alosoides*); LKWF = lake whitefish (*Coregonus clupeaformis*); NNST = ninespine stickleback (*Pungitius pungitius*); RNSM = rainbow smelt (*Osmerus mordax*); SAUG = sauger (*Sander canadensis*); TRPR = troutperch (*Percopsis omiscomaycus*); WALL = walleye (*Sander vitreus*); WHBS = white bass (*Morone chrysops*); WHSC = white sucker (*Catostomus commersonii*); YLPR = yellow perch (*Perca flavescens*)

Table A5 Top-ranking linear models for describing trace element concentrations in the pectoral muscle of cormorants (*Phalacrocorax auritus*) nesting on Lake Winnipeg. The number of parameters (*K*), AIC_c, ΔAIC_c and Akaike weight (ω_i) are given for each of the top-ranking models ($\Delta AIC_c \leq 2.00$). Where the "best" model ($\Delta AIC_c = 0.00$) did not have $\omega_i \geq 0.90$, a model-averaging approach was used. Trace element concentrations were log transformed.

Element	Group[a]	Model	*K*	AIC_c	ΔAIC	ω_i
As	*NB DCCO*	Tarsus length + $\delta^{34}S$	4	44.03	0.00	0.92
	SB DCCO	Null model	2	25.00	0.00	0.58
		$\delta^{13}C$	3	26.86	1.86	0.23
	HY DCCO	$\delta^{13}C$	3	-7.75	0.00	0.44
		Null model	2	-6.56	1.20	0.24
Cd	*NB DCCO*	$\delta^{15}N$	3	27.50	0.00	0.95
	SB DCCO	Null model	2	15.39	0.00	0.91
	HY DCCO	Null model	2	-11.84	0.00	0.51
Cu	*NB DCCO*	Null model	2	-28.97	0.00	0.58
		$\delta^{13}C$	3	-27.08	1.89	0.23
	SB DCCO	Null model	2	13.71	0.00	0.65
Fe	*NB DCCO*	$\delta^{15}N$	3	12.84	0.00	0.94
	SB DCCO	$\delta^{15}N$	3	0.11	0.00	1.00
	HY DCCO	Sex	3	4.92	0.00	0.52
		Null model	2	6.78	1.86	0.21

Table A5 Continued.

Element	Group	Model	*K*	AIC_c	ΔAIC	ω_i
Mn	*NB DCCO*	$\delta^{15}N$	3	5.55	0.00	0.87
	SB DCCO	$\delta^{15}N$	3	1.29	0.00	0.55
		Null model	2	1.89	0.60	0.41
	HY DCCO	Null model	2	5.76	0.00	0.47
Hg	*NB DCCO*	$\delta^{15}N$	3	56.65	0.00	1.00
	SB DCCO	$\delta^{15}N$	3	22.45	0.00	0.54
		Null model	2	23.39	0.95	0.34
	HY DCCO	Weight	3	5.06	0.00	0.42
		Sex	3	6.33	1.27	0.22
		Null	2	6.81	1.75	0.18
Se	*NB DCCO*	$\delta^{15}N$ + Weight	4	9.73	0.00	0.91
	SB DCCO	Null model	2	7.51	0.00	0.82
	HY DCCO	Null model	2	-5.72	0.00	0.36
		$\delta^{34}S$	3	-5.31	0.41	0.29

[a]Codes: NB DCCO = north basin cormorants (adult); SB DCCO = south basin cormorants (adult); HY DCCO = hatch-year cormorants

Table A6 Geospatial classification for cormorant (*Phalacrocorax auritus*) primary feathers collected in 2009. Feathers were classified as having been grown on Lake Winnipeg (summer) or the Gulf Coast (winter) based on δ^2H and δ^{34}S. Freshwater feathers from the wintering grounds were further classified into natural or aquaculture-based habitats based on δ^{13}C and δ^{15}N data. Each feather is identified by its primary number (2, 4, 6, 8, or 10) and those selected for Hg analyses are underlined.

	Lake Winnipeg		Gulf Coast & Mississippi Valley	
Bird Number		Marine	Freshwater	Aquaculture
2009-1			2, 4, 8, 10	6
2009-3	6, 8, 10			
2009-4[a]			2, 4, 6, 8, 10	
2009-5			6, 10	
2009-6	2, 8, 10			
2009-7	10		8	
2009-8	2, 8		4	
2009-9	2		4, 10	
2009-10			6	
2009-11			4, 8	
2009-12	10			
2009-13	2, 4, 6, 8, 10			
2009-14	2, 4, 6, 8		10	
2009-15			2, 4, 6, 8, 10	
2009-16		8		
2009-17	4, 8			
2009-18	6		8	
2009-19			4	
2009-20			10	
2009-21	2, 8		4, 6	
2009-22		2, 6, 10		
2009-23	2, 4, 6, 8, 10			
	n = 30 (20)	n = 4 (3)	n = 29 (16)	n = 1

[a]Feather values for cormorant 2009-4 (mean ± standard deviation [SD] = -19.83 ± 0.51 ‰), likely represent a diet predominantly natural freshwater diet, with infrequent predation of catfish.

Table A7 Total mercury (Hg; µg/g fresh weight [FW]) in primary feathers collected from cormorants (*Phalacrocorax auritus*) breeding on Lake Winnipeg, Canada in 2009. In order to minimize variability in the Hg data, feathers (primary 2, 4, 6, 8 or 10) were analyzed on an individual, rather than pooled, basis. The mean ± standard deviation (SD) Hg concentrations for feathers grown on Lake Winnipeg (n = 20) or the wintering grounds (n = 19) were 4.26 ± 1.47 and 3.19 ± 1.64 µg/g fresh weight (FW), respectively.

Bird Number	Feather Number	Growth Location	Habitat Type	Hg (µg/g FW)
2009-1	2	Gulf Coast	Freshwater	2.00
	4	Gulf Coast	Freshwater	7.50
2009-3	6	Lake Winnipeg	Freshwater	6.20
	10	Lake Winnipeg	Freshwater	3.10
2009-4	2	Gulf Coast	Freshwater	4.00
2009-5	6	Gulf Coast	Freshwater	3.30
	10	Gulf Coast	Freshwater	2.40
2009-6	8	Lake Winnipeg	Freshwater	5.40
	10	Lake Winnipeg	Freshwater	1.90
2009-7	8	Gulf Coast	Freshwater	1.50
	10	Lake Winnipeg	Freshwater	2.40
2009-8	2	Lake Winnipeg	Freshwater	3.10
	4	Gulf Coast	Freshwater	4.40
	8	Lake Winnipeg	Freshwater	4.20
2009-9	2	Lake Winnipeg	Freshwater	6.60
	4	Gulf Coast	Freshwater	2.70
	10	Gulf Coast	Freshwater	2.30
2009-11	4	Gulf Coast	Freshwater	1.60
	8	Gulf Coast	Freshwater	1.80

Table A7 Continued.

Bird Number	Feather Number	Growth Location	Habitat Type	Hg (µg/g FW)
2009-12	10	Lake Winnipeg	Freshwater	5.20
2009-13	2	Lake Winnipeg	Freshwater	6.10
	4	Lake Winnipeg	Freshwater	4.40
2009-14	6	Lake Winnipeg	Freshwater	5.40
	8	Lake Winnipeg	Freshwater	5.10
	10	Gulf Coast	Freshwater	5.30
2009-15	2	Gulf Coast	Freshwater	4.80
	4	Gulf Coast	Freshwater	4.80
2009-16	8	Gulf Coast	Marine	3.80
2009-17	4	Lake Winnipeg	Freshwater	5.60
	8	Lake Winnipeg	Freshwater	3.90
2009-18	6	Lake Winnipeg	Freshwater	1.90
	8	Gulf Coast	Freshwater	3.10
2009-21	2	Lake Winnipeg	Freshwater	3.00
	6	Gulf Coast	Freshwater	1.40
	8	Lake Winnipeg	Freshwater	2.90
2009-22	2	Gulf Coast	Marine	1.30
	6	Gulf Coast	Marine	2.70
2009-23	2	Lake Winnipeg	Freshwater	5.30
	4	Lake Winnipeg	Freshwater	3.40