Resolving Stock Structure of Sauger (*Sander canadensis*) in Manitoba, Canada using Biometric, Isotopic, and Genetic Approaches

by

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ABSTRACT

Many sauger (*Sander canadensis*) populations in Manitoba have declined in numbers and biomass. Fisheries managers have proposed a province-wide sauger management plan to protect and restore sauger populations, but they are uncertain how sauger populations should be defined and to what extent they may interact. In this thesis, I used a multifaceted approach to resolve population structure and identify migratory corridors of sauger in Manitoba. First, I mined biometric data from several long-term monitoring datasets to calculate life history indices for sauger stocks across 29 waterbodies. Sauger growth generally decreased and the age at 50% maturity increased among lakes of increasing latitude. This trend was also observed within Lake Winnipeg, yet the length at 50% maturity remained constant. Sauger grew exceptionally fast in Lake Manitoba and Lake Winnipegosis and matured at an early age. Next, I performed a stable isotope analysis (^{13}C and ^{15}N) of sauger tissue to investigate contemporary sauger migration throughout the Lake Winnipeg watershed. Sauger from Lake Winnipeg, Lake Manitoba, and Lac du Bonnet occupied distinct isotopic niches, and I identified several possible migrants from Lake Manitoba and the Winnipeg River in Lake Winnipeg. Finally, I used microsatellites to assess the genetic health and structure of sauger stocks across Manitoba. Genetic diversity within sample populations was moderate to high, and incidence of inbreeding and hybridization with walleye (*Sander vitreus*) was low. I identified four broad genetic sauger stocks: Lake Winnipeg; Lake Manitoba and Lake Winnipegosis; the Red and Assiniboine Rivers; and the Churchill and Saskatchewan Rivers. Gene flow between Lake Winnipeg and Lake Manitoba stocks is minimal. These findings will assist managers in defining stock management units and optimizing management efforts for sauger populations in Manitoba.

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LIST OF ABBREVIATIONS

Chapter 2

Abbr.	Full Name
A_{50}	Age at 50% maturity
A_{90}	Age at 90% maturity
AIC	Akaike information criterion
ALK	Age-length key
BIC	Bayesian information criterion
CAMP	Coordinated Aquatic Monitoring Program
IDW	Inverse distance weighting
$K_{(growth)}$	Growth coefficient
$K_{(condition)}$	Condition factor
L_∞	Asymptotic average length
L_{50}	Length at 50% maturity
L_{90}	Length at 90% maturity
MGL	Manitoban Great Lakes
NAD83	North American Datum of 1983
SEA	Standard ellipse area
SEAb	Bayesian standard ellipse areas
SEAc	Sample size-corrected standard ellipse area
SIA	Stable isotope analysis
SM	Supplementary Material
t_0	Theoretical age at length 0 (modeling artifact)
TA	Total area
VBGF	von Bertalanffy growth function

Waterbodies/Study Regions

Abbr.	Full Name
LW	Lake Winnipeg
LM	Lake Manitoba
WO	Lake Winnipegosis
RR	Red River
AR	Assiniboine River
WR	Winnipeg River
SR	Saskatchewan River
UNR	Upper Nelson River
LNR	Lower Nelson River
UCR	Upper Churchill River
CRD	Churchill River Diversion

Chapter 3

Abbr.	Full Name
A	Accuracy
AMOVA	Analysis of molecular variance
A_R	Allelic richness
BC	Backcross
BIC	Bayesian information criterion
CAMP	Coordinated Aquatic Monitoring Program
DAPC	Discriminant analysis of principal components
D_{CSE}	Cavalli-Sforza and Edwards' chord distance
DEM	Digital elevation model
dNTP	Deoxynucleotide triphosphate
E	Efficiency
$\bar{F}$	Mean inbreeding coefficient of individuals
F_1	First filial hybrid
F_2	Second filial hybrid
F_{IS}	Inbreeding coefficient
F_{IT}	Individual:total inbreeding coefficient
F_{ST}	Fixation index
H_E	Expected heterozygosity
H_O	Observed heterozygosity
HWE	Hardy-Weinberg equilibrium
IBD	Isolation by distance
K	Hypothesized number of populations within a sample
LD	Linkage disequilibrium
MCMC	Markov chain Monte Carlo
MGL	Manitoban Great Lakes
N_A	Number of alleles
N_e	Effective population size
N_{PA}	Number of private alleles
PC	Principal components
PCR	Polymerase chain reaction
P_{crit}	Minimum allele frequency criteria
P_{ID}	Probability of identity
P_{IDsib}	Probability of identity of siblings
SM	Supplementary material
$\bar{r}_d$	Standardized index of association
Tq	*q*-value threshold
α	Type I error

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Wow. What a journey. When I first started this Master's project back in 2020, I had no idea where it would lead. I also didn't expect this journey to take over three years, but I digress. If I could go back in time, would I do things differently? Probably. Would I rein back my own ambitions and reduce the scope of this project? Perhaps. Would I do it all over again? Definitely.

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XOXO

Chapter 1. General Introduction and Literature Review

1.1 INTRODUCTION

The sauger (*Sander canadensis*) is a predatory freshwater fish native to the Mississippi, Great Lakes, and Nelson-Churchill watersheds (Scott and Crossman 1973). Sauger are a popular sportfish and were once an important species in North America's largest commercial fisheries (Schmalz *et al.* 2011). The International Union for Conservation of Nature (IUCN) lists sauger as a species of Least Concern, citing their wide distribution and large number of subpopulations (NatureServe 2013). However, many sauger populations are in a state of decline (Jaeger *et al.* 2005; Schmalz *et al.* 2011). Several of these populations are undergoing intensive management efforts to prevent their extirpation (NYDEC 2013; WTC 2017; Wyoming Game & Fish Department 2018). This widespread decline of sauger populations suggests that the species is highly vulnerable to ongoing changes in North America's freshwater landscape. Numerous studies have attributed these declines to factors such as habitat degradation (Leach and Nepszy 1976; Ryan *et al.* 2003; Yallaly *et al.* 2014) and fragmentation (Pegg *et al.* 1997; McMahon and Gardner 2001; Jaeger *et al.* 2005), overharvest (McMahon 1999; Seibert *et al.* 2018), and introgression with walleye (*Sander vitreus*) (Regier *et al.* 1969; Bingham *et al.* 2012; Graham *et al.* 2021), though the exact mechanisms of these stressors are only recently being quantified. As such, a fine scale understanding of sauger population dynamics and habitat requirements is needed to identify the most destructive environmental stressors affecting populations today.

Manitoba is home to several threatened sauger populations, including those in Lake Winnipeg, Lake Manitoba and Lake Winnipegosis. Declines in these populations are likely due to commercial overharvest (Lysack 2006; Nicholson 2007; ECCC and MARD 2020), though some

populations may also be experiencing habitat fragmentation and degradation, inbreeding, and introgression with walleye. The ongoing decline of multiple sauger populations in Manitoba highlights the need for a sauger recovery plan. However, there are knowledge gaps regarding the genetic structure and health of sauger populations that must be resolved before a plan can be developed. First, do sauger within the Lake Winnipeg watershed comprise a single genetic population or multiple genetically distinct stocks? If genetic substructure is present, it may be evidence of reproductively isolated sauger stocks with unique management needs. Second, are any sauger populations exhibiting low genetic diversity, inbreeding or introgression with walleye? If so, these populations are at higher risk of extirpation and may not recover without enhancing their gene pool. Finally, is there any evidence of current or historical gene flow between major waterbodies? If this is or was the case, management efforts should focus on preserving or restoring connectivity between lakes and rivers to maintain the genetic integrity of sauger populations.

Topics in this literature review include the current status of sauger populations in North America and common threats to their survival on broad and local scales. I will also cover principles and methodologies of population genetics and their applications in sauger conservation and research.

1.2 SAUGER STATUS IN NORTH AMERICA

The sauger is one of North America's most widespread species, with a current range spanning 30 U.S. states and 5 Canadian provinces (Appendix 1.A) (NatureServe 2021). The sauger's distribution is largely extended by the Mississippi River system, which encompasses about 40% of the U.S. mainland (EPA 2016). The sauger is listed as a species of Least Concern by

the International Union for Conservation of Nature and is not federally protected in the U.S. or Canada (NatureServe 2021). However, many sauger populations are declining throughout their native range. These threatened populations are found in both riverine environments such as the Tennessee River (Ferrell *et al.* 2017), Allegheny River (Loukmas *et al.* 2015) and upper Missouri River (Bingham *et al.* 2012), and in lacustrine environments such as Lake Winnipeg (ECCC and MARD 2020) and the Laurentian Great Lakes (Schmalz *et al.* 2011; Baldwin *et al.* 2018). Sauger are considered extirpated in numerous waterbodies, including the North Platte River in Wyoming (WTC 2017), Lake Champlain in New York and Vermont (NYDEC 2013), and Lake Erie (Baldwin *et al.* 2018; Hartman *et al.* 2019). Sauger disappeared from commercial catches in all the Laurentian Great Lakes by the 1970s (Baldwin *et al.* 2018) and have been scarcely documented since (WTC 2017; iNaturalist). Presently, sauger are classified as Vulnerable in Wyoming and Oklahoma; Imperiled in Montana, Kansas, Mississippi, and Virginia; and Critically Imperiled in Michigan, New York, Vermont, and North Carolina (NatureServe 2021). Alberta, Ontario, and several U.S. states list sauger as a secure but sensitive species, while other states are data deficient (NatureServe 2021). I noticed during my literature search that fish and wildlife publications from several of these 'secure' states still documented declines in sauger abundance and range.

1.2.1 Past, Present, and Future Threats to Sauger Survival

Most sauger population declines have been attributed to habitat degradation (Leach and Nepszy 1976; Ryan *et al.* 2003; Yallaly *et al.* 2014) and fragmentation (Pegg *et al.* 1997; McMahon and Gardner 2001; Jaeger *et al.* 2005), and commercial overharvest (McMahon 1999; Seibert *et al.* 2018). I did note that in many papers and reports, these factors were implicated

based on prior research, statements, or anecdotes rather than by empirical evidence. For example, publications by Regier *et al.* (1969), Hesse (1994), Pegg *et al.* (1997), and Jaeger *et al.* (2005) were frequently referenced in government reports and journal publications. Dam-induced habitat fragmentation was the most cited cause of sauger declines, as several studies have demonstrated that some sauger populations will migrate great upstream distances to return to natal spawning habitat (Pegg *et al.* 1997; Jaeger *et al.* 2005). This explanation is logical for waterbodies such as the Platte River in Wyoming and the Allegheny River in New York, where sauger are absent upstream of large dams (NYDEC 2013; WTC 2017). However, several reports have documented shorter sauger spawning migrations (Siegwarth *et al.* 1993; Schell *et al.* 2004) and thus not all riverine sauger populations are inherently threatened by barriers to fish passage. If anything, the recitation of these few select papers demonstrates the need for additional quantitative research or a comprehensive literature review.

More recently, genetic studies of several declining sauger populations revealed low genetic diversity (Bingham *et al.* 2012; Hartman *et al.* 2019), elevated rates of inbreeding (Hartman *et al.* 2019), and introgression with sympatric walleye (*Sander vitreus*) populations (Bingham *et al.* 2012; Graham *et al.* 2021). The significance of these genetic measures will be further described in Chapter 3 of this thesis. Typically, sauger and walleye remain reproductively isolated due to offset spawning periods, with walleye spawning several weeks before sauger (Scott and Crossman 1973; Becker 1983; Stewart and Watkinson 2004). However, sauger and walleye may interbreed under certain conditions to produce hybrids known as “saugeye”. This can occur naturally if sauger begin spawning at the tail end of the walleye spawn, or when turbid conditions prevent visual discrimination of the other species (Bozek *et al.* 2011).

Hybridization has been found to increase following anthropogenic disturbances such as reservoir development (Graham *et al.* 2021) and stocking or translocation of walleye (White *et al.* 2005; Billington and Sloss 2011). Introgression of sauger populations will decrease the number of genetically pure individuals, create competition with the faster-growing saugeye, and potentially cause outbreeding depression due to backcrossing of sauger and saugeye (Bingham *et al.* 2012).

1.3 SAUGER STATUS IN MANITOBA

Sauger are found in rivers and lakes throughout Manitoba, including Lake Winnipeg, Lake Manitoba, Lake Winnipegosis (Manitoba's "Great Lakes") and many of their tributaries. Sauger have been recorded in all of Manitoba's watersheds except for the Seal River, Thlewiaza River, and Hudson Bay drainage basins (Appendix 1.B) (Stewart and Watkinson 2004). Many of these populations coexist with walleye (Stewart and Watkinson 2004). Sauger have not been sampled in the Whitemouth River sub-basin as of 2004 (Stewart and Watkinson 2004; Milani 2013). Sauger are a source of sustenance for many First Nation communities (Tough 1984; Schmalz *et al.* 2011) and are a popular sportfish in waterbodies such as Wekusko Lake and the Red River (Hunt Fish Manitoba). Sauger were once an important commercial fish in Manitoba's three largest gillnet fisheries—Lake Winnipeg, Lake Manitoba, and Lake Winnipegosis—in both quantity and value (Nicholson 2007). Presently, sauger comprise only a small percentage of commercial production in Manitoba despite being the second-most valuable fish per round kg (Manitoba Sustainable Development 2017; FFMC 2019). This drop in provincial production largely coincides with sauger declines in the Manitoban Great Lakes (ECCC and MARD 2020; EDITNR 2023a).

1.3.1 Lake Winnipeg

Sauger, walleye, and whitefish (*Coregonus clupeaformis*) are Lake Winnipeg's most important commercial species by value and production (Manitoba Sustainable Development 2017; FFMC 2019). They are harvested under a multi-species quota system in which the total allowable harvest is pooled between the three species (Task Force 2011). Sauger production peaked around 1940, with annual yields nearing 5,000,000 kg—over double that of walleye (Task Force 2011). Sauger and walleye yields were roughly equal from the 1940s to the 1990s, after which sauger deliveries slowed and walleye yields soared (Task Force 2011). In the present day, annual walleye production regularly exceeds 3,000,000 kg while sauger deliveries do not match a tenth of that number (ECCC and MARD 2020). Sauger production has declined more than 90% from historical highs, thus meeting the criteria for a commercial collapse (ECCC and MARD 2020). However, commercial sauger deliveries are not a reliable measure of population status in Lake Winnipeg as the rate of production decline is strongly correlated with differences in walleye and sauger prices (Task Force 2011). Commercial fishers estimate that 25-35% of sauger caught in their nets are misreported as walleye (AOFRC 2020). Nevertheless, provincial index netting results suggest that annual sauger mortality has reached unsustainable levels and now exceeds 55% (ECCC and MARD 2020). An independent consulting firm also concluded that Lake Winnipeg sauger are at risk of being overfished and recommended the creation of a sauger management plan (AOFRC 2020).

1.3.2 Lake Manitoba

Sauger are considered commercially extinct in Lake Manitoba (K. Casper, personal communication, September 16, 2020). Sauger production increased significantly following the

creation of a 3” mesh winter perch fishery in 1985 and peaked at over 500,000 kg in 1989 (Lysack 1997; Kroeker 2012). This spike in production was attributed to the reduced mesh size, which increased the capture rate of smaller and younger sauger (Lysack 1997). The elevated rate of harvest proved unsustainable, as the sauger population crashed in the 1990s and has yet to recover (Kroeker 2012; MARD 2021a). Annual sauger production has stabilized at about 2,000-3,000 kg in commercial landings (Manitoba Sustainable Development 2017). The population remains small but seemingly stable as sauger from multiple year classes are still caught in provincial index netting surveys (MARD 2021a; EDITNR 2023a).

1.3.3 Lake Winnipegosis

Sauger are essentially extirpated from Lake Winnipegosis. This population collapsed alongside the rest of the fishery in 1960s and has never recovered (Lysack 2006; Nicholson 2007). Annual production has not exceeded 10,000 kg since 1971 and has only reached 10 kg once in the past decade (MARD, unpublished data). One commercial fisher stated that he has not seen a sauger in 8 years (anonymous, personal communication, September 18, 2020). It is worth noting that Lake Winnipegosis is a 4” gillnet fishery, so all but the largest sauger will generally avoid capture. However, standardized index netting efforts on Lake Winnipegosis have only captured one sauger since the program began in 2008 (CAMP, unpublished dataset). It is uncertain if sauger captured in Lake Winnipegosis are migrants from Lake Manitoba or are from a residual spawning population. The lack of sauger recovery suggests that the population is struggling due to environmental and/or genetic factors.

1.3.4 Assiniboine River

Sauger are found throughout the Assiniboine River and are known by anglers to attain large sizes. Of all the trophy (18+ in) sauger entries in Travel Manitoba's Master Angler program, the Assiniboine River is third in count only to Wekusko Lake and the Red River (Master Angler Record Book). That said, sauger are found in low abundance upstream of the Portage Spillway (Watkinson, unpublished data) and may be cross breeding with walleye—at least 9 of 36 sauger photos available in the Master Angler database appear to be saugeye (Master Angler Record Book). It is therefore possible that the Assiniboine River sauger population is being negatively impacted by habitat fragmentation due to damming or by other environmental factors.

1.3.5 Other Manitoba Waterbodies

Sauger occur in the Winnipeg River, Nelson River, and Churchill River (Stewart and Watkinson 2004). Each of these systems is disrupted by a combined sixteen hydroelectric generating stations, most of are complete barriers to upstream fish passage (Manitoba Hydro 2015). As sauger are primarily a riverine species, these barriers will have likely fragmented some sauger populations (Bozek *et al.* 2011a; Jonagan 2022) and restricted spawning migrations (Bozek *et al.* 2011b). The Nelson River and Churchill River were historically isolated but are now connected by the Churchill River Diversion, which was excavated to increase discharge in the Nelson River for hydroelectric production (MREM 1973). This change in flow regime can lead to increased turbidity, alterations in nutrient cycling, and siltation of spawning habitat (Manitoba Hydro 2015; MARD 2021a). The connection of previously isolated waterbodies is also known to cause outbreeding depression (reduced fitness) in fish populations (Frankham *et al.* 2010).

1.4 POPULATION AND CONSERVATION GENETICS

Conservation genetics is a subfield of population genetics concerned with preserving the genetic integrity of at-risk populations and species (Frankham *et al.* 2010). This field emerged as scientists began recognizing the importance of genetic diversity and fitness in population recovery. Small or declining populations are at higher risk of extinction due to genetic factors such as loss of genetic diversity and inbreeding depression (Frankham *et al.* 2010). Genetic diversity is important as the presence of many phenotypes makes a population more resilient to changes to its environment. If there is less genetic diversity, there is less evolutionary potential (Hamilton 2009). Inbreeding is caustic to population health as deleterious recessive alleles will increase in frequency. This leads to an effect known as inbreeding depression, wherein population fitness is reduced due to poor fish health, reduced fertility, and lower offspring survival (Jobling *et al.* 2014). As in other population genetic disciplines, researchers use molecular markers to characterize the genetic makeup of populations. Commonly used markers include allozymes (enzyme variants with different electrical charges), mitochondrial DNA, microsatellites, and single nucleotide polymorphisms (among-individual variations at a single base position in a DNA sequence; SNPs) (Jobling *et al.* 2014). Microsatellites—tandem repeating DNA sequences of 2-9 nucleotide pairs— are popular in population genetics studies as they are abundant, have a high mutation rate, and regularly occur in non-coding sections of the genome (introns) (Frankham *et al.* 2010). Alleles at these loci are amplified by polymerase chain reaction (PCR) with the use of locus-specific PCR primers. Primers are short, single-stranded DNA sequences that complement the flanking regions of the microsatellite locus. Primers initiate DNA replication under PCR conditions by annealing to single-stranded DNA, whose

complementary sequence is then extended by DNA polymerases (Jobling *et al.* 2014). Researchers will then score these amplified alleles by molecular weight (amplicons) using gel electrophoresis or capillary electrophoresis. Several summary statistics are calculated from these results: allelic richness (A_R), which is the number of alleles within the sample set; allelic frequency, which is how common an allele is found within a population; and observed heterozygosity (Ho), which is the proportion of heterozygous genotypes at a given locus. These parameters are often incorporated into statistics that are tested against a null model. The Hardy-Weinberg equilibrium (HWE) is perhaps the most well-known null model in conservation genetics (Frankham *et al.* 2010). This model states that genotype frequencies in an infinitely large, randomly mating population will remain constant in the absence of a) mutation, b) selection, and c) gene flow (Frankham *et al.* 2010). Observed genotype counts and counts expected under HWE conditions are compared using chi-square statistics. Chi-square statistics are then fitted to a goodness of fit graph, wherein a *p*-value below 0.05 suggests that the sample population is less heterozygous than expected (Hamilton 2009). This indicates that the population is small or is experiencing mutation, selection, gene flow, or non-random mating (Frankham *et al.* 2010). Inbreeding is often measured using Wright's *F*-statistics, that are derived from the inbreeding coefficient (F). The inbreeding coefficient is the probability that two alleles at a given locus are identical by descent (Hamilton 2009). This is calculated as:

$$F = 1 - (H_o/H_e)$$

Where an individual with F = 0 is considered fully outbred, F = 1 is fully inbred, and F = 0.25 means sibling-sibling parenthood (Frankham *et al.* 2010). A comprehensive review of derivations of the inbreeding coefficient can be found in Jobling *et al.* (2014).

I have described only a few key population genetics principles and statistical methods; there are many other analyses that can be run with genetic data. With advances in molecular technology, population genetic studies can now be conducted with thousands of individual markers or with entire DNA sequences. The recent rise in computing power has enabled geneticists to use Bayesian algorithms and increasingly complex models to explain genetic structure and diversity. More comprehensive reviews of these methods have been written by authors such as Excoffier and Heckel (2006) and Casillas and Barbadilla (2017).

1.4.1 Sauger Population Genetics

There are a growing number of studies that have used molecular markers to monitor sauger populations. Objectives have ranged from characterizing stock structure and gene flow (Hartman *et al.* 2019), to quantifying stocking success (Ferrell *et al.* 2017; Bingham *et al.* 2018), to measuring inbreeding and hybridization (Bingham *et al.* 2012; Graham *et al.* 2021). The earliest studies used allozymes as genetic markers. Allozymes proved useful for detecting introgression (Billington *et al.* 1988; White *et al.* 2005) but were limited in their ability to resolve genetic structure (White and Schell 1995; Billington et *al.* 2006). Since 2012, microsatellites have been the molecular marker of choice for most sauger studies. Microsatellites have been used to address several sauger conservation objectives, including measuring rates of hybridization with walleye (Bingham *et al.* 2012; Graham *et al.* 2021), monitoring sauger stocking success (Ferrell *et al.* 2017; Bingham *et al.* 2018), and delineating stock structure (Hartman *et al.* 2019). The body of sauger conservation genetics research remains small but growing.

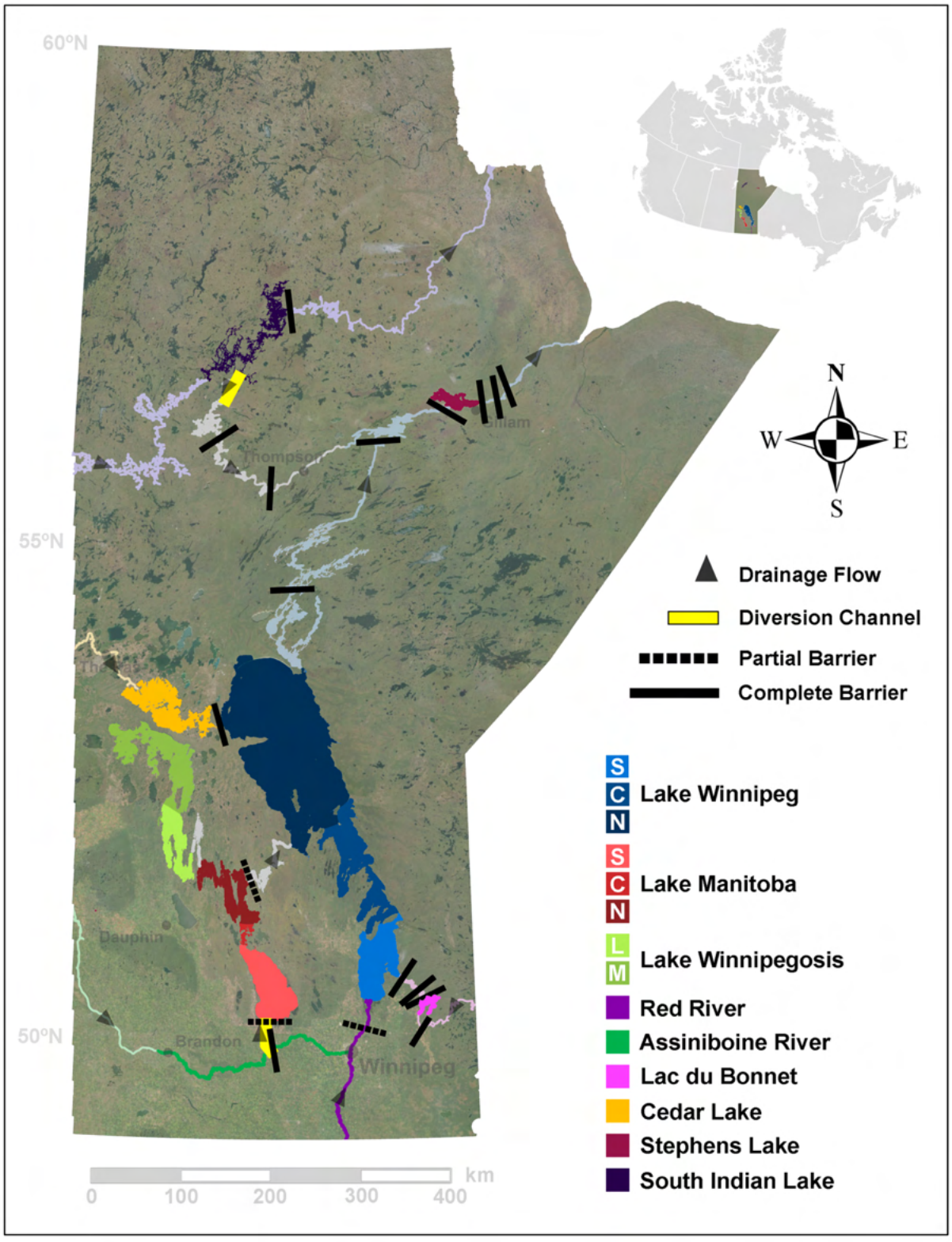


Figure 1.1. Map of the study waterbodies in the Nelson-Churchill watershed. Flow regimes are shown with directional arrows. Barriers to fish passage are represented as solid (complete barrier) or dashed (partial barrier) black bars; waterway diversions are denoted with yellow bars. The Study Regions (CAMP 2017) not reported in the map legend are labeled as follows: Winnipeg River, pink; Saskatchewan River, orange; Upper and Lower Nelson River, blue; Upper Churchill River, purple; and the Churchill River Diversion, grey.

Chapter 2. Resolving population structure and admixture of a freshwater percid, *Sander canadensis*, using life history characteristics and stable isotope analysis

ABSTRACT

The sauger (*Sander canadensis*) is a fish of commercial, recreational, and cultural importance in the province of Manitoba. Sauger stocks have declined in Manitoba's three largest waterbodies, prompting fisheries managers to consider a provincial sauger management plan. However, development of this plan has stalled due to a limited understanding of sauger stock structure and migration within the Nelson-Churchill watershed. In this study, I compared life history characteristics (sex ratios, growth, condition, and length and age at sexual maturity) of sauger across twenty-nine waterbodies to characterize populations as defined at the drainage basin, waterbody, basin, and sample site level. Sauger growth generally decreased and the age at 50% maturity increased with increasing latitude. This trend also occurred within Lake Winnipeg, but the length at 50% maturity remained constant at the basin and site level. Sauger grew quickly in Lake Manitoba and Lake Winnipegosis and matured at an early age; males matured at an abnormally small size. I then performed a stable isotope analysis (^{13}C and ^{15}N) of sauger tissue to evaluate contemporary sauger migration in the Nelson-Churchill watershed. Sauger from Lake Winnipeg, Lake Manitoba, and Lac du Bonnet occupied distinct isotopic niches. I identified possible migrants from Lake Manitoba and the Winnipeg River in Lake Winnipeg, and migration between Lake Winnipeg's south and north basins. These findings will aid managers in defining sauger stocks, describing their migratory behaviours, and tailoring management strategies according to each stock's unique life history.

2.1. INTRODUCTION

The sauger (*Sander canadensis*) is a piscivorous freshwater fish located throughout the Nelson-Churchill watershed in Manitoba, Canada (Scott and Crossman 1973; Stewart and Watkinson 2004). The sauger is highly regarded in Manitoba's commercial fisheries (Franzin *et al.* 2003; Nicholson 2007), with a per-kg market value surpassed only by its sister species, the walleye (*Sander vitreus*) (FFMC 2019). Sauger are a popular sportfish, with many anglers converging on waterbodies such as the Red River, Assiniboine River, and Wekusko Lake in search of a trophy catch (Hunt Fish Manitoba; Manitoba Master Angler Awards). The sauger has long been a staple in the diet of First Nations communities, including those situated along the shores of the 'Manitoban Great Lakes' (MGL): Lake Winnipeg, Lake Manitoba, and Lake Winnipegosis (Tough 1984; Schmalz *et al.* 2011). Thus, the sauger is a species of economic, recreational, and cultural significance in the province of Manitoba.

Today, several of Manitoba's most important sauger stocks are severely depleted. Commercial sauger production in Lake Winnipeg has plummeted since the 1980s, and annual yields are down over ninety percent from historic highs (ECCC and MARD 2020). Recent assessments by both the province and a third-party consultant concluded that Lake Winnipeg sauger stocks are overfished (AOFRC 2020; ECCC and MARD 2020). In Lake Manitoba, commercial sauger landings have declined by over ninety-nine percent since the early 1990s (Lysack 1997; LMRRAC 2003). Although sauger abundance has stabilized in recent years, the Lake Manitoba stock remains small and commercially extirpated (Lysack 1997; MARD 2021a). Sauger have all but vanished from Lake Winnipegosis following a commercial collapse in the 1970s (Lysack 2006), and less than a handful of fish are caught in commercial nets each year

(MARD 2021b; unpublished NRND dataset). Although the status of sauger in other Manitoba waterbodies is less established, many of these waterways have undergone ecosystem alterations that are known to negatively impact sauger populations (CAMP 2017; see below).

Given the generally negative outlook of sauger in Manitoba, fisheries managers have concluded that a province-wide sauger management plan may be necessary to protect and restore Manitoba's sauger populations. Such a plan would be multifaceted and may involve regulatory changes for commercial and recreational fisheries, habitat restoration or enhancement, and stocking programs to fortify sauger populations (Task Force 2011; MARD 2021a; MARD 2021b). However, several knowledge gaps must be addressed before such a plan can be implemented. First, there is no consensus on how sauger stocks should be delineated. Fish stocks in Manitoba have conventionally been defined and managed by waterbody, with each lake or river system assumed to contain its own panmictic populations (Nicholson 2007; G. Klein, personal communication, September 16, 2020). Some recent management efforts have partitioned systems into multiple regulatory zones to account for disproportionate angling pressure, natural breaks in geography, and variations in life history characteristics (ECCC and MARD 2020; NRND 2023, unpublished). For example, minimum commercial mesh sizes differ across Lake Winnipeg to reflect variations in commercial harvest pressure and fish growth and maturity (ECCC and MARD 2020; NRND 2023, unpublished). However, this management regime is scarcely used outside of Lake Winnipeg and does not consider the possibility of fish migration between connected waterbodies (G. Klein, personal communication, September 16, 2020).

Second, there is debate as to what mechanisms are currently contributing to sauger declines in Manitoba. Commercial overfishing is often cited as the leading cause of sauger

declines in the Manitoban Great Lakes (Nicholson 2007; Task Force 2011; MARD 2021a; ECCC and MARD 2020). This is supported by commercial landing records and provincial index netting data, which display a dramatic decline in sauger abundance following increased angling pressure and the use of small mesh gillnets (Lysack 2006; Nicholson 2007; MARD 2021a; EDITNR 2023a). That said, there are other factors that may be negatively impacting sauger populations. The sauger is a migratory fish with a strong affinity for current (Bozek *et al.* 2011a), which makes the species particularly vulnerable to alterations and fragmentation of riverine habitat (Pegg *et al.* 1997; Jaeger *et al.* 2005; NYDEC 2013; Jonagan 2022). The Nelson-Churchill watershed has been heavily modified to facilitate hydroelectric production and flood mitigation, both of which come at the expense of habitat connectivity (Manitoba Hydro 2015). Generating stations in the Nelson River, Winnipeg River, and other tributaries currently act as partial or complete barriers to upstream fish passage (Manitoba Hydro 2015; CAMP 2017). Moreover, some commercial fishers assert that the Fairford River Water Control Structure—designed to regulate water levels on Lake Manitoba—inadvertently caused the demise of Lake Manitoba and Lake Winnipegosis sauger stocks (LMRRAC 2003; Lysack 2006; MARD 2021a). They contest that sauger from Lake Manitoba and Lake Winnipegosis regularly migrated to Lake Winnipeg to spawn and are now unable to return upstream (MARD 2021a). Conversely, channels excavated from the Churchill River to the Nelson River (hydroelectric production; MREM 1973) and the Assiniboine River to Lake Manitoba (flood mitigation; MWC 1980) have created corridors between waterbodies that were previously isolated. Such conditions are known to cause outbreeding depression in fish populations, wherein the unification of two genetically dissimilar populations results in reduced fitness of the new population (Frankham *et al.* 2010). Seasonal discharge from these channels

may also lead to increased turbidity, alterations in nutrient cycling, and siltation of spawning habitat (Manitoba Hydro 2015). Commercial fishers on Lake Manitoba speculate that spring discharge from the Assiniboine River diversion into Lake Manitoba has resulted in siltation of key sauger and walleye spawning sites, thereby reducing annual recruitment (MARD 2021a). Without a clear understanding of what mechanisms are actively contributing to sauger declines in Manitoba, any efforts to rehabilitate these populations may be ineffective.

In this chapter, I present two investigative methods that can aid in defining and characterizing sauger populations; each approach complements existing fisheries research and monitoring programs. First, I will perform a meta-analysis of data collected by two longstanding monitoring programs: the provincial index netting program, which is conducted on the MGL by the provincial Fisheries Branch; and the Coordinated Aquatic Monitoring Program (CAMP), a collaboration between the government and Manitoba Hydro to monitor waterbodies impacted by hydroelectric projects. Both programs collect individual length, weight, sex, maturity, and age data (described hereafter as "biometric" data) to characterize the abundance and health of fish populations in each waterbody (CAMP 2017; ECCC and MARD 2020). Although both programs calculate the same life history statistics for each sample population, no attempt has been made to elucidate broad-scale spatial trends across datasets (G. Klein, personal communication, September 17, 2021). Here, I obtained biometric sauger data from the provincial index netting and CAMP programs to conduct pairwise comparisons of the following life history parameters: sex ratios; growth (length-at-age, von Bertalanffy coefficients); body condition (weight-at-length, *K* condition factor); length at 50% and 90% maturity; and age at 50% and 90% maturity. I then generated heat maps of each parameter to assess for spatial autocorrelation. In theory,

significant parameter differences between these waterbodies could be due to stock structure or environmental variation (Jobling 1997; Begg and Waldman 1999; Cadrin and Secor 2009), while temporal changes within a waterbody may indicate recent selective pressures (Heino and Godø 2002; Cooke *et al.* 2007; Landi *et al.* 2015).

For the second investigative method, I will perform stable isotope analysis using ^{13}C and ^{15}N to describe the stock structure and contemporary migration of sauger within the Lake Winnipeg drainage basin. Stable isotope analysis (SIA) is based on principles of isotopic fractionation, wherein the ratios of stable isotopes in an inorganic or organic material will vary by the biological and geophysical processes used to create that material (Michener and Lajtha 2007). In practice, $\delta^{13}C$ remains relatively static across a food web and reflects the original source of organic carbon (Peterson and Fry 1987), whereas $\delta^{15}N$ values increase incrementally at each trophic level (Post 2002). These isotopes are typically used in fisheries research to describe energy pathways or trophic statuses (Chipps and Garvey 2007; Michener and Lajtha 2007), though they can also be used to identify recent migrants within a population (Hesslein *et al.* 1991; Hobson 1999; Trueman *et al.* 2012). However, this latter application relies on large, consistent differences between the $\delta^{13}C$ and $\delta^{15}N$ values of migrants as compared to the sample population, which is seldom present for fish populations in freshwater systems (Hobson *et al.* 2012; Hoffman 2016). Lake Winnipeg is one of these exceptions. Research conducted by Hobson *et al.* (2012) and Ofukany *et al.* (2014) revealed greater inter-basin variation of $\delta^{13}C$ and $\delta^{15}N$ values in fish muscle tissue than inter-species variation, leading Hobson *et al.* to suggest that these isotopes may be useful in fish movement studies. Thus, I conducted stable isotope analysis using $\delta^{13}C$ and $\delta^{15}N$ to screen for possible sauger migrations within and among

waterbodies in the Lake Winnipeg drainage basin. I collected dorsal muscle tissue from a subsample of sauger netted in Lake Winnipeg, Lake Manitoba, and Lac du Bonnet during index netting and CAMP programs as well as from sauger caught in the Red River and Assiniboine River with rod and reel. The $\delta^{13}C$ and $\delta^{15}N$ profiles derived from these tissues were visualized with boxplots and isotope biplots, and I used non-parametric tests and Bayesian modelling to compare the distributions of these isotope values across waterbodies, basins, and sample sites. Significant differences in $\delta^{13}C$ and $\delta^{15}N$ distributions among sample groups would demonstrate the effectiveness of these isotopes as geolocator tags within the Lake Winnipeg drainage basin. Outliers within a sample group may depict a recent migrant whose origins could be traced to another sample group. If migrant sauger can indeed be identified and traced, this approach will help establish sauger movements and migratory routes. Moreover, the presence of migration between two sample groups with similar life histories would suggest that these groups are part of the same population. In summary, findings in this chapter will provide fisheries managers with a better understanding of sauger stock structure in Manitoba, habitat utilization within the Lake Winnipeg drainage basin, and the potential effects of habitat fragmentation and alterations on contemporary sauger migrations in Manitoba.

2.2. MATERIALS AND METHODS

2.2.1 Biometric Data Analysis

2.2.1.1 Data Collection and Quality Control

Biometric sauger data were compiled from twenty-nine waterbodies using publicly available datasets, historic provincial datasheets, and rod and reel angling (Table 2.1). Data used

were fish ID; sample location and date; weight (g), fork length (mm); maturity (mature “M”, immature “I”); sex (female “F”, male “M”); age (years); and a (*K*) condition factor, where:

$$K = \frac{(weight \times 10^5)}{(fork\ length)^3}$$

Individuals were considered “mature” following the onset of sexual maturity. Sexual maturity was confirmed in the field by visual inspection of the gonads using the guidelines described by Gervais (2017). Sauger ages were estimated by counting the annuli of sagittal otoliths and assigning a confidence index rating with the method described in the CAMP protocol (2017).

Preliminary visualizations of the dataset showed few outliers that would significantly impact statistical tests, thus I only removed outliers that were clear measurement or data entry errors. For example, I removed individuals whose length-weight relationships exceeded the morphological constraints of the species, which I conservatively defined as a *K* condition factor of less than 0.2 and greater than 4.0. To minimize further data loss, individuals with missing biometric data were retained in analyses for which they had the required data and were removed from analyses for which they did not.

Nearly half of the biometric sauger data collected from Lake Winnipegosis existed as summary statistics and required extrapolation. I created rows for all the individuals used to generate these summary statistics and assigned them with their respective ages, maturities, and sexes (n = 77 females, 67 males). I then randomly generated a fork length and weight for each fish by running the NORMINV(RAND()) function in Excel with the mean and standard deviation of each measure. The extrapolated sample set was compared to the individual sample set in R Studio (R Core Team 2023; RStudio Team 2023) using a multiple linear regression (***lm***; *base R*), with weight as the response variable and fork length and sample set as predictor variables. The

sample set was not a significant predictor ($p > 0.05$) of weight-at-length (***summary***; *base R*), so extrapolated and individual fish measurement data were pooled into one sample set.

2.2.1.2 Statistics and Tests

Biometric data were analysed in Excel and within the RStudio development environment using the programming language ***R*** (R Core Team 2023). Sex ratios were calculated in Excel and tested for sex bias using a one-sided binomial test (BINOM.DIST(x_{min},*n*,0.5,TRUE)) and by calculating the criterion binomial (BINOM.INV(*n*,0.5,0.95)). Life history indices were calculated for each sex at the Lake, Basin, and Site population levels where sample size allowed. Statistics were also summarized by Study Regions as defined under CAMP program protocols (2017). When modeling was required to test and compare indices, I evaluated model assumptions such as linearity and homoscedasticity with residual plots (***residualPlots***; *car* ver. 3.102 [Fox and Weisberg 2019]). To test for significant differences between models with bootstrapped support, I developed the custom R function "***compare***" (Supplementary Material 0; SM 0). The ***compare*** function was designed to compare the bootstrapped parameter estimates of two models by calculating the difference between each set of bootstrapped samples. These calculated differences are then converted into two-sided p-values with the following command line:

p-value = 2**min*(*c*(*mean*(*param.diff*>0),mean(*param.diff*<0)))

Thus, the p-value is defined as twice the proportion of bootstrapped sample sets that are greater than or less than (whichever is smaller). Additional scripting within the ***compare*** function enables pairwise comparisons between all model objects that are listed in the command line (see: SM 0).

Table 2.1. Sampling locations, dates, and sample sizes of biometric sauger data, summarized at the lowest available population scale. Sample year schemes are denoted by colons (consecutive sample years) and slashes (break between sample years).

Sampling Location		Latitude	Longitude	Sample Years	Sample Months	Total (Sex)	Weight (g)	Fork Length (mm)	Maturity	Age (years)	*K*
LW	Grand Beach	50.4616	-96.5980	09:21	Jun	6062	6062	6062	6062	5542	6062
	Riverton/Hecla	51.1368	-96.6538	09:21	Jun	5126	5126	5126	5126	5057	5126
	Frog Bay	51.3204	-96.8958	09:21	Jun	4632	4631	4631	4632	4625	4631
	Matheson Is.	51.6938	-97.0511	09:21	Jun	2688	2688	2688	2688	2676	2688
	Dauphin R.	52.0116	-98.0400	10/12:21	Jul	368	368	368	368	365	368
	Grand Rapids	53.2697	-99.2243	09:21	Jul	2166	2162	2161	2166	2155	2161
	Sturgeon Gill	53.4290	-99.0774	21	Jul	311	311	310	311	309	310
	Mossy Bay	53.7326	-98.1026	17:21	Jul	1045	1044	1044	1045	1022	1044
RR	Red River	50.0857	-96.9409	20:21	Sep:Oct	49	49	49	49*	30	49
AR	Assiniboine River	49.9476	-98.3279	20:21	June:Aug	32	32	32	32	28	32
LM	Whitemud	50.2702	-98.5371	09:20	Sep	341	341	341	341*	339	341
	Lundar	50.6710	-98.2406	09:20	Sep	63	63	63	63*	63	63
	Narrows	51.0245	-98.7971	21	Sep	34	34	34	34*	34	34
	Steep Rock	51.4650	-98.8002	09:21	Sep	285	285	285	285*	283	285
	Manipogo	51.6385	-99.5113	09:20	Sep	41	41	41	41*	40	41
WO	Lake Winnipegosis	52.5170	-99.8810	75/80:81/ 91:95/97/16/21	Sep:Oct	328	328	328	328*	326	328
W	Eaglenest Lake	50.3127	-95.2088	13/16/19	Jul	294	288	294	276	290	288
	Pointe du Bois	50.3111	-95.5443	08:21	Jul	2270	2268	2269	2198	2230	2267
R	Lac du Bonnet	50.3748	-95.8932	08:21	Sep	1338	1329	1337	1337*	1313	1328
	Pine Falls	50.5551	-96.1495	17/20	Jun:Jul	45	45	45	41	45	45
SR	Saskatchewan River	53.8300	-101.287	19	Sep	46	46	46	46*	46	46
	Cedar Lake	53.4194	-100.065	15/18:21	Aug:Sep	371	371	370	371*	364	370
	Cormorant Lake	54.2384	-100.655	11/18:21	Aug	74	74	64	74	73	63
UNR	Playgreen Lake	54.1080	-98.1801	18/21	Jun:Jul	82	82	82	82	81	82
	Cross Lake	54.7413	-97.5379	08/17:21	Aug:Sep	81	81	81	81*	50	81
	Sipiwesk Lake	55.0509	-97.7069	17/20	Jun	306	306	306	306	305	306
	Upper Nelson River	55.8498	-96.5851	17/20	Jul	50	50	50	50	49	50
	Setting Lake	54.9561	-98.6638	12:21	Aug:Sep	3296	3294	3289	3296*	3282	3287
LN	Burntwood River	56.1337	-96.7952	17/20	Aug	22	22	22	22	22	22
	Split Lake	56.1793	-96.1749	12/17:21	Aug:Sep	651	651	651	651*	646	651
R	Stephens Lake	56.3756	-95.0978	18/21	Aug:Sep	74	74	74	74*	72	74

Table 2.1. continued

Sampling Location		Latitude	Longitude	Sample Years	Sample Months	Total (Sex)	Weight (g)	Fork Length (mm)	Maturity	Age (years)	*K*
UCR	**Granville Lake**	56.2769	-100.493	17:21	Jul:Aug	886	886	886	886*	849	886
	Opachuanau Lake	56.7220	-99.6034	17/20	Jul:Aug	350	350	350	350*	337	350
	Southern Indian Lake	57.3679	-98.2872	17:21	Jun	742	742	742	742*	740	742
CRD	**Rat Lake**	56.1461	-99.6608	19	Jul	34	34	34	34	34	34
	Notigi Lake	55.9412	-99.3333	09/18/21	Aug	124	124	124	124*	111	124
	Threepoint Lake	55.7008	-98.9499	09/17:21	Aug	423	423	423	423*	358	423
	Footprint Lake	55.7755	-98.9120	19	Aug	58	58	58	58*	58	58
	Wuskwatim Lake	55.5506	-98.5501	21	Aug	146	145	146	146*	144	145
	Apussigamasi Lake	55.8441	-97.6111	09/18/21	Aug	277	277	277	277*	157	277

***** A_{50} and A_{90} estimates were move forward one year to account for fish that did not spawn in the spring but were maturing to spawn for the following year.

Sauger growth was assessed in RStudio using age-length keys and von Bertalanffy growth functions (VBGFs) as described by Ogle (2016). Observed age-length keys were created using the ***xtabs***, ***rowSums*** and ***prop.table*** functions in *base R*, and modeled age-length keys were generated with a multinomial logistic regression (***multinom***; *nnet* ver. 7.3-19 [Ripley and Venables 2002]) and plotted with ***alkPlot*** (*FSA* ver. 0.9.3; Ogle *et al*. 2022). VBGF parameters were estimated for each sample group with the ***nls*** function (*nlstools* ver. 2.0-1; Baty *et al.* 2015) following initiation with ***vbStarts*** (*FSA*) and supported by bootstrapping (***nlsBoot***; *nlstools*). Parameters for each sex were compared between sample groups with the ***compare*** function. The best VBGF subset model for the combined dataset was determined with the Akaike information criterion (***AIC***; *base R*) and the Bayesian information criterion (***BIC***; *base R*). VBGF curves and confidence intervals for each sex within each sample group were plotted with ***ggplot*** (*ggplot2* ver. 3.4.3; Wickham 2016). Finally, I calculated the mean fork length of sauger at age 5 to visualize differences in growth between each sample group at a predetermined age (Johnston *et al.* 2012; MARD 2021a).

Body conditions of sauger in each sample group were described with weight-at-length regressions and the *K* condition factor. Simple weight-length linear regressions (***lm***; *base R*) were constructed for each sample group with the common logarithms of weight and length (***log10***; *base R*), while the influence of sex and sample groups on the total weight-length relationship was tested with a dummy variable regression (***lm****(logW~logL*groups)*; *base R*) and analysis of covariance (***Anova***; *base R*). Differences in regression coefficient estimates between groups were tested by bootstrapping each estimate (***Boot***; *car*) and comparing the bootstrapped samples with the ***compare*** function. Log-transformed and back-transformed weight-length

curves and confidence intervals were plotted with ***ggplot*** (*ggplot2*). The relationship of mean *K* across 20 mm length classes was tested with a general linear regression (***lm***; *base R*) and an ANOVA (***Anova***; *base R*), and homogeneity of the variances within each class were tested with Levene's test (***leveneTest***; *car*). As there were significant differences in mean *K* and distribution variance across most sample groups, I performed a Kruskal-Wallis test (***kruskal.test***; *base R*) followed by a Dunn's test (***dunnTest***; *FSA*). Sample groups displayed similar *K* distributions between 200 mm and 400 mm with no clear trends of stochastic dominance. Thus, *K* values from sauger in the 200 mm to 400 mm length classes were pooled to generate *K* distributions for each sample group. The resulting *K* distributions were then compared with a Kruskal-Wallis test and Dunn's test to assess for differences in condition between sample groups.

I evaluated the length- and age-at-maturity of each sex in each sample group with frequency tables (***prop.table***; *base R*) and logistic regression models (***glm***; *base R*). The proportions of mature individuals were summarized in one year or 20 mm increments, and the first length and age classes that displayed 50% (L_{50}, A_{50}) and 90% (L_{90}, A_{90}) maturity were compiled and exported into a separate table. Proportion tables were preferred when estimating L_{50} and A_{50} for sample populations with small sample sizes or poor logistic regression model fit. Raw maturity data from each sample group were integrated into logistic regression models, and confidence intervals for each coefficient were generated with bootstrapping (***bootCase***, B=1000; *car*). I used these bootstrapped coefficients to calculate a distribution of 50% and 90% maturity estimates from the models of each sample group according to the equation:

$$x = \frac{log(\frac{p}{1-p}) - \alpha}{\beta_1}$$

Where α and β_1 are coefficients 1 and 2 in the logarithmic model, p is the desired proportion of mature individuals, and *x* is the calculated age or length of *p* maturity. I then used the ***compare*** function to test for significant differences in L_{50}, A_{50}, L_{90}, and A_{90} among all sample group pairings. Results for L_{90} and A_{90} were reported in tables but were not described further for brevity. To account for variation in sampling times among waterbodies, age-at-maturity estimates were moved forward one year if fish were sampled in August or later. For example, an initial A_{50} estimate of four years for a population would be increased to five years if sauger were sampled from August 1 onward. This correction was done to account for fish that may not have spawned in the spring (May-June; Stewart and Watkinson 2004) but were maturing to spawn for the following year (G. Klein, personal communication, August 9, 2023). I will address the implications of these corrections in the Discussion section of this chapter. Populations for which this age-at-maturity correction was applied are denoted with asterisks in Table 2.1.

Heat maps displaying spatial variations of sauger sex ratios, growth (length at age 5), condition *K*, L_{50} and A_{50} were produced in ArcGIS Pro. Sample site coordinates and the parameter estimates for each site were exported into ArcGIS Pro and visualized on the NAD83 datum with the 'Display XY Data' function. Gradients for these parameters were generated using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Maps were presented with a Canada Lambert Conformal Conic projection.

2.2.2 Stable Isotope Analysis

2.2.2.1 Data Collection and Quality Control

Dorsal white muscle tissue was sampled in 2020 from a random subsample of sauger collected during provincial index netting and population genetics sampling. Samples were collected from Lake Winnipeg (n = 83), Lake Manitoba (n = 35), Lac du Bonnet (n = 11), the Red River, (n = 14), and the Assiniboine River (n = 5). Tissue samples were dried at 60°C for 48 h, pulverized with a mortar and pestle, and weighed (1.0 ± 0.2 mg) into 5 x 3.5 mm tin capsules. Samples were then combusted and measured using a Delta V mass spectrometer (Thermo-Finnigan, Bremen, Germany) paired with a Costech 4010 elemental analyzer. All samples returned a C:N ratio between 3.0 and 3.4 and analytical error was limited to 0.1 ‰ for both $\delta^{13}C$ and $\delta^{15}N$ values based on repeated runs of an in-house standard (SINLAB, New Brunswick, Canada). Carbon and nitrogen isotope ratios are expressed as the per mil (‰) deviation from international reference materials ($\delta^{13}C$: Vienna Pee Dee Belemnite; $\delta^{15}N$: atmospheric N_2).

2.2.2.2 Statistics and Tests

Isotope data were analysed and visualized with ***R*** in the RStudio environment. Raw stable isotope data were summarized by population stratum (Lake, Basin, Site) and visualized with boxplots and isotope biplots fitted with standard and 95% fitted ellipses (***ggplot***; *ggplot2*). Carbonate and lipid plots (***ggplot***; *ggplot2*) showed no evidence of inorganic carbon or lipid effects (Appendix G, Appendix H), so no corrections were applied to the data. Many sample populations did not exhibit normally distributed $\delta^{13}C$ and $\delta^{15}N$ data according to Shapiro-Wilk tests (***shapiro.tests***; *base R*) and Q-Q plots (***qqnorm***; *base R*), so populations were compared using non-parametric statistics or models assuming non-normal distributions.

I used a combination of frequentist and Bayesian tests to assess inter-population variation of stable isotope distributions. I used Kruskal-Wallis tests (***kruskal.test***; *base R*) to compare the $\delta^{13}C$ and $\delta^{15}N$ distributions at each population stratum. Significant results ($p < 0.05$) were followed up with Dunn's tests with a Bonferroni correction (***dunnTest***; *FSA*) and pairwise Wilcoxon rank sum tests with a continuity correction (***pairwise.wilcox.test***; *base R*). Isotope distributions were also compared among populations by calculating the overlap of maximum likelihood ellipses superimposed on a $\delta^{13}C$–$\delta^{15}N$ isotope biplot, as implemented in *SIBER* (ver. 2.1.9; Jackson *et al.* 2011). With this framework, we can infer that two populations occupy a distinct $\delta^{13}C$–$\delta^{15}N$ isotopic niche when a) there is no overlap between maximum likelihood ellipses, and b) the ellipses have narrow credibility intervals. Maximum likelihood fitted ellipses were generated for each population with ***plotSiberObject*** and measured (expressed as $‰^2$) for total area (TA), standard ellipse area (SEA), and sample size-corrected standard ellipse area (SEAc; Jackson *et al.* 2011) with the ***groupMetricsML*** function. Overlap of the fitted maximum likelihood standard and 95% SEAc ellipses for each population pair were estimated with the ***maxLikOverlap*** function and expressed as a proportion of the non-overlapping area of each ellipses pair. Confidence intervals for each population ellipse were constructed by fitting a Bayesian multivariate normal distribution to the data with the ***siberMVN*** function (parameters: n.iter = 20000, n.burnin = 1000, n.thin = 10, n.chains = 2; priors: R = 1*diag(2), k = 2, tau.mu = 0.001). Posterior ellipse estimates for each population (Bayesian Standard Ellipse Areas; SEAb) were visualized with a highest density regions boxplot (***siberDensityPlot***) with 50%, 75%, and 95% credibility intervals.

2.3. RESULTS

2.3.1 Biometric Data Analysis

2.3.1.1 Sex Ratios

Most sauger populations assessed in this study were female biased. Twenty-five of the twenty-nine waterbody datasets contained more females than males, of which eighteen were significant ($p < 0.05$) (Table 2.2, Table 2.3). Datasets for Cross Lake, Notigi Lake, Sipiwesk Lake, and Stephens Lake contained more males than females; Stephens Lake was significantly male biased ($p < 0.05$; Table 2.3). Thus, only ten (34.5%) of the twenty-nine waterbody datasets contained non-biased sex ratios. Overall, F:M sex ratios ranged from 0.35:1 (26% female; Upper Nelson River) to 8.25:1 (89% female; Cormorant Lake), with a mean ratio of 1.76:1 (57%, *SD* = 13% female) (Figure 2.1).

Further evaluation of the Lake Manitoba and Lake Winnipeg datasets showed variable sex ratios at the Basin and Site level, respectively. Data from Lake Manitoba's south basin were male biased, whereas sauger sampled in the north basin were disproportionately female. The Lake Winnipeg dataset exhibited strong female bias at all sample sites except for in Frog Bay, where there was a small but statistically significant ($p < 0.05$) bias towards male sauger. When summarizing the data by Study Region as defined under CAMP protocols (2017), seven of the ten datasets were significantly female biased when $p < 0.05$ (Table 2.2). Datasets for Lake Manitoba, Lake Winnipegosis, and the Upper Nelson River systems were consistent with unbiased sampling of sexes. More sex bias tests at the Basin and Site level can be viewed in Supplementary Materials 1.3 and 1.4 (SM 1.3; SM 1.4).

Table 2.2. Proportions of female and male sauger sampled in Manitoba waterbodies, summarized by Study Region as defined under CAMP protocols (2017). Sex bias was tested with a one-sided binomial test; the threshold where $p > 0.05$ is defined as the criterion binomial.

Study Region	Total	Females	Males	Proportion Females	F:M	Criterion Binomial	Sex Bias Test
Lake Winnipeg	22398	14061	8337	0.63	1.69	11322	<0.001
Red-Assiniboine R.	81	55	26	0.68	2.12	48	0.001
Lake Manitoba	764	385	379	0.50	1.02	405	**0.428**
Lake Winnipegosis	328	177	151	0.54	1.17	179	**0.084**
Winnipeg River	3947	2246	1701	0.57	1.32	2025	<0.001
Saskatchewan River	417	288	129	0.69	2.23	225	<0.001
Upper Nelson River	519	243	276	0.47	0.88	278	**0.080**
Lower Nelson River	747	431	316	0.58	1.36	396	<0.001
Upper Churchill River	1978	1323	655	0.67	2.02	1026	<0.001
Churchill River Diversion	1062	622	440	0.59	1.41	558	<0.001

Table 2.3. Proportions of female and male sauger sampled in Manitoba waterbodies. Sex bias was tested with a one-sided binomial test; the threshold where $p > 0.05$ is defined as the criterion binomial. Sex data from Lake Winnipeg and Lake Manitoba are presented at the Site and Basin population level, respectively.

Sampling Location	Total	Females	Males	Proportion Females	F:M	Criterion Binomial	Sex Bias Test
LW SB Grand Beach	6062	4117	1945	0.68	2.12	3095	<0.001
LW SB Riverton/Hecla	5126	3417	1709	0.67	2.00	2622	<0.001
LW CH Frog Bay	4632	2158	2474	0.47	0.87	2372	<0.001
LW CH Matheson Is.	2688	1703	985	0.63	1.73	1387	<0.001
LW NB Dauphin R.	368	280	88	0.76	3.18	200	<0.001
LW NB Grand Rapids	2166	1476	690	0.68	2.14	1121	<0.001
LW NB Sturgeon Gill	311	233	78	0.75	2.99	170	<0.001
LW NB Mossy Bay	1045	677	368	0.65	1.84	549	<0.001
RR Red River	49	34	15	0.69	2.27	30	0.005
AR Assiniboine River	32	21	11	0.66	1.91	21	**0.055**
LM MB South	404	177	227	0.44	0.78	219	0.007
LM MB Narrows	34	19	15	0.56	1.27	22	**0.304**
LM MB North	326	189	137	0.58	1.38	178	0.002
WO Lake Winnipegosis	328	177	151	0.54	1.17	179	**0.084**
WR Eaglenest Lake	294	173	121	0.59	1.43	161	0.001
WR Pointe du Bois	2270	1372	898	0.60	1.53	1174	<0.001
WR Lac du Bonnet	1338	670	668	0.50	1.00	699	**0.489**
WR Pine Falls	45	31	14	0.69	2.21	28	0.008
SR Saskatchewan River	46	31	15	0.67	2.07	29	0.013
SR Cedar Lake	371	257	114	0.69	2.25	201	<0.001
Cormorant Lake	74	66	8	0.89	8.25	44	<0.001
UNR Playgreen Lake	82	51	31	0.62	1.65	48	0.018
UNR Cross Lake	81	40	41	0.49	0.98	48	**0.500**
UNR Sipiwesk Lake	306	139	167	0.45	0.83	167	**0.061**
UNR Upper Nelson River	50	13	37	0.26	0.35	31	<0.001
Setting Lake	3287	1853	1434	0.56	1.29	1691	<0.001
LNR Burntwood River	22	18	4	0.82	4.50	15	0.002
LNR Split Lake	651	385	266	0.59	1.45	346	<0.001
LNR Stephens Lake	74	28	46	0.38	0.61	44	0.024
UCR Granville Lake	886	610	276	0.69	2.21	467	<0.001
UCR Opachuanau Lake	350	185	165	0.53	1.12	190	**0.155**
UCR Southern Indian Lake	742	528	214	0.71	2.47	393	<0.001
CRD Rat Lake	34	20	14	0.59	1.43	22	**0.196**
CRD Notigi Lake	124	61	63	0.49	0.97	71	**0.464**
CRD Threepoint Lake	423	231	192	0.55	1.20	228	0.032
CRD Footprint Lake	58	31	27	0.53	1.15	35	**0.347**
CRD Wuskwatim Lake	146	92	54	0.63	1.70	83	0.001
CRD Apussigamasi Lake	277	187	90	0.68	2.08	152	<0.001

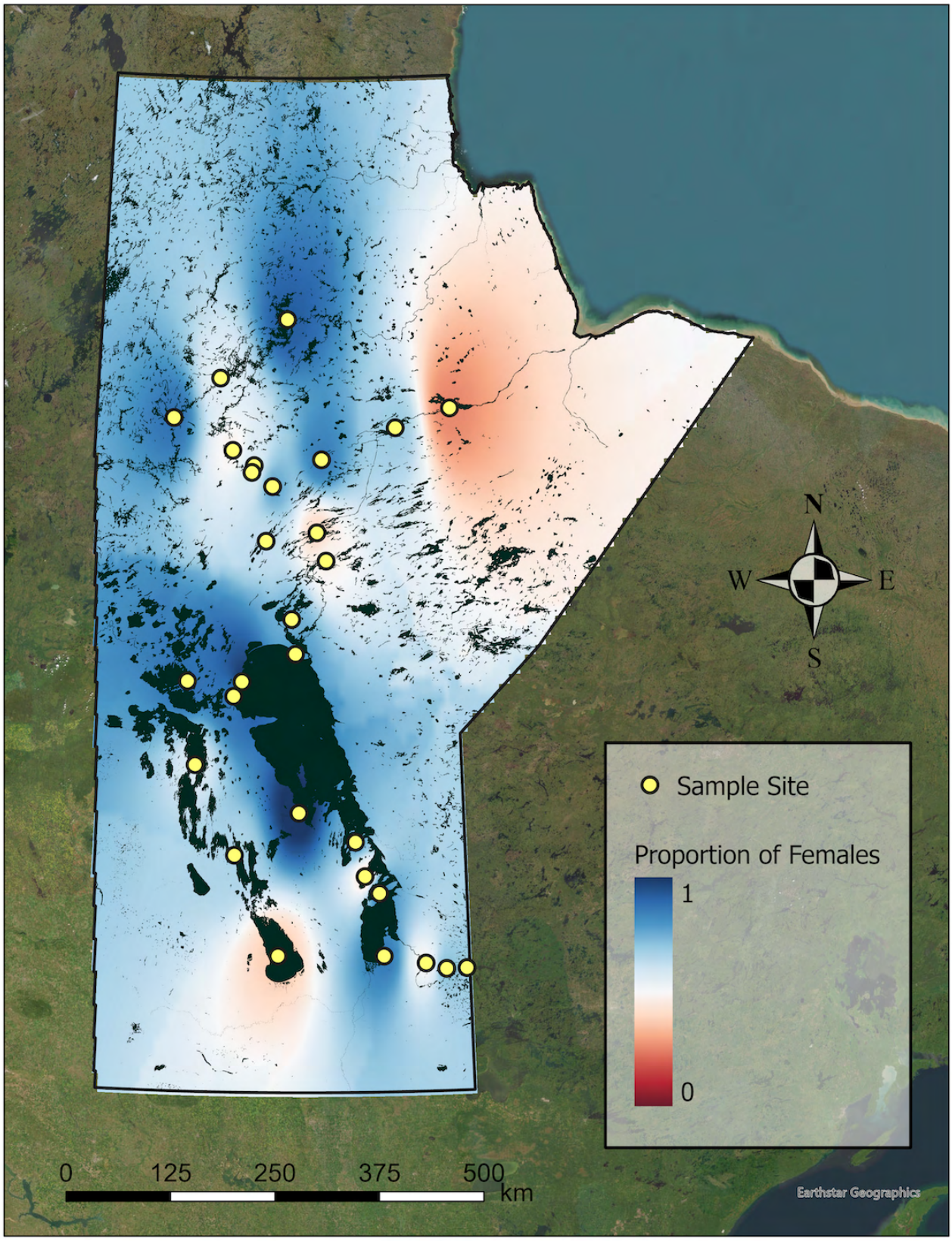


Figure 2.1. Heat map of the proportion of females in sauger populations in Manitoba. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.

2.3.1.2 Growth

Age-length keys (ALKs) and von Bertalanffy growth function (VBGF) parameter estimates consistently varied between sexes, with females showing faster growth than males (Figure 2.2; SM 2-1; SM 2-2). Hence, all growth calculations and tests were performed for each sex separately. The ALK plots generated for each sample population are not presented for brevity but can be reproduced with the *R* script provided in Supplementary Materials 3.

The best fitting VGBF according to the AIC and BIC was the complex model, which estimates L_∞, K, and t_0 parameters for each unique population (Table 2.4). The second-best model had a shared L_∞ parameter and unique K and t_0 coefficients. I used the complex model to estimate VGBF parameters for each sample group, as shown in Table 2.5. Empty cells in Table 2.5 represent missing estimates due to nonconvergence of the VGBF model. Mean estimates of L_∞, K, and t_0 among sample groups were 365 mm (*SD* = 46 mm), 0.34 (*SD* = 0.45), and -1.70 (*SD* = 2.05) for females, and 346 mm (*SD* = 43 mm), 0.28 (*SD* = 0.14), and -1.34 (*SD* = 1.04) for males. Estimate ranges are not described due to variations in model quality but can be seen in Table 2.5 (sample population) and Table 2.6 (Study Region). VBGF plots for a subsample of Lake level populations and for all Lake Winnipeg sites can be seen in Figure 2.3.

VGBF parameter estimates frequently differed among sample groups. Of all possible pairwise comparisons of bootstrapped L_∞, K, and t_0 distributions, 41.4%, 47.0% and 42.5% of the distributions significantly differed ($p < 0.05$) for females, and 43.9%, 37.8%, and 37.2% significantly differed for males, respectively. When comparing within Study Regions, the proportion of significant results generally decreased (see SM 2-3 for all pairwise tests). However, the proportion of the significant results increased within the Winnipeg River region (SM 2-4)

and for females within the Lake Winnipeg system (L_∞ = 46.5%, K = 71.4%, and t_0 = 53.6%). Lake Manitoba and Lake Winnipegosis models presented high growth coefficients and asymptotic lengths for both sexes relative to the sample location and study regions means (Table 2.5, Table 2.56). Growth coefficients (K) were low in the Upper Churchill River and Churchill River Diversion regions for both sexes, as were male asymptotic lengths (Table 2.5, Table 2.6).

VBGF parameters for the Lake Winnipeg sauger population varied between sample sites (Table 2.5). Growth was comparable between the Dauphin River site and sites in Lake Winnipeg's south basin and channel; sauger in the remainder of the north basin were slower growing. Asymptotic average length was comparable across all Lake Winnipeg sites except for Grand Beach and the Dauphin River. Sauger from the Dauphin River site exhibited a higher asymptotic average length for both sexes than other sites, albeit with broad confidence intervals. In contrast, females sampled at Grand Beach expressed the lowest asymptotic average length of all Lake Winnipeg sites with no overlap within their 95% confidence interval (Table 2.5).

Table 2.4. Results of Akaike (AIC) and Bayesian (BIC) information criterion testing of von Bertalanffy growth function subset models (df = degrees of freedom). Model names describe the presence or absence of population-specific parameter estimates (L_∞ = asymptotic average length, K = growth coefficient, t_0 = length-0 modeling artifact), where the absence of a symbol indicates that the parameter is not population-specific.

Model name	df	AIC	df	BIC
fitLKt	109	**325408.8**	109	**326329.8**
fitLK	74	326719	74	327344.2
fitLt	74	326835.5	74	327460.7
fitKt	74	326401.2	74	327026.4
fitL	39	328560.7	39	328890.2
fitK	39	329678.1	39	330007.6
fitt	39	335526.9	39	335856.5
fit0	4	347913.4	4	347947.2

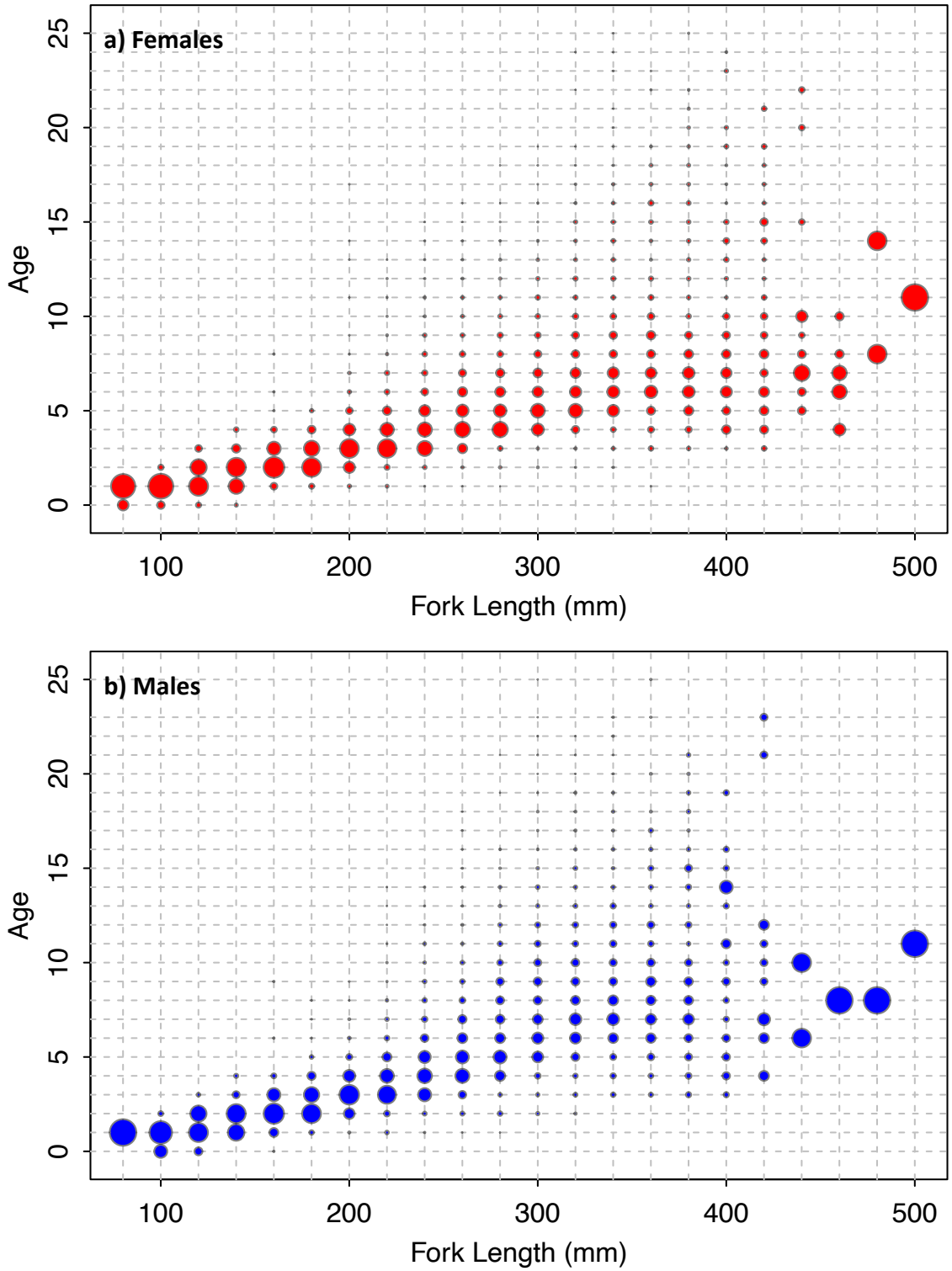


Figure 2.2. Age-length-key plots for **a)** female and **b)** male sauger sampled across twenty-nine Manitoba waterbodies (n = 34550). Circle areas are proportional to the relative contribution of each age class within a 20 mm fork length interval.

Table 2.5. VBGF parameter estimates (L_∞ = asymptotic average length, K = growth coefficient, t_0 = length-0 modeling artifact) with 95% confidence intervals for sauger sample populations in Manitoba, by sex. Estimates for Lake Winnipeg are presented at the Site population level. Empty cells represent missing parameter estimates due to nonconvergence of the VGBF model.

Sampling Location			Female			Male		
			L_∞	K	t_0	L_∞	K	t_0
LW	SB	Grand Beach	335 (331-340)	0.44 (0.41-0.47)	0.2 (0.1, 0.3)	340 (331-352)	0.27 (0.23-0.30)	-1.0 (-1.4, -0.7)
		Riverton/Hecla	371 (365-379)	0.33 (0.30-0.37)	0.0 (-0.2, 0.2)	349 (344-355)	0.33 (0.30-0.36)	-0.2 (-0.4, 0.0)
	CH	Frog Bay	370 (364-376)	0.39 (0.36-0.42)	0.2 (0.1, 0.4)	346 (342-350)	0.34 (0.31-0.36)	-0.4 (-0.7, -0.2)
		Matheson Is.	366 (360-373)	0.42 (0.39-0.46)	0.3 (0.2, 0.4)	347 (340-355)	0.35 (0.31-0.38)	-0.3 (-0.5, -0.1)
	NB	Dauphin R.	405 (379-449)	0.27 (0.19-0.35)	-0.3 (-1.1, 0.3)	376 (343-426)	0.32 (0.21-0.46)	0.1 (-0.7, 0.7)
		Grand Rapids	355 (346-366)	0.33 (0.29-0.37)	0.4 (0.1, 0.6)	356 (341-376)	0.23 (0.19-0.27)	-0.5 (-1.0, -0.2)
		Sturgeon Gill	376 (342-444)	0.18 (0.11-0.26)	-1.0 (-2.3, -0.1)	354 (304-609)	0.17 (0.04-0.31)	-1.8 (-6.3, 0.1)
		Mossy Bay	375 (358-397)	0.21 (0.18-0.25)	-0.5 (-1.0, -0.1)	329 (318-343)	0.28 (0.23-0.32)	-0.3 (-0.6, 0.0)
RR		Red River	313 (307-337)	2.85 (0.67-4.79)	2.2 (0.0, 2.6)	288 (272-318)	0.85 (0.31-1.98)	0.1 (-3.1, 1.2)
AR		Assiniboine River	314 (295-366)	0.51 (0.17-1.34)	-1.7 (-6.4, 0.0)			
LM		Lake Manitoba	445 (428-467)	0.37 (0.30-0.43)	-0.9 (-1.2, -0.6)	401 (389-417)	0.40 (0.34-0.48)	-1.1 (-1.4, -0.8)
WO		Winnipegosis	405 (395-420)	0.55 (0.43-0.68)	0.0 (-0.5, 0.3)	384 (370-406)	0.45 (0.31-0.60)	-0.6 (-1.6, 0.0)
WR		Eaglenest Lake	362 (341-393)	0.24 (0.18-0.30)	-1.6 (-2.2, -1.2)	409 (363-489)	0.15 (0.10-0.21)	-2.2 (-3.0, -1.6)
		Pointe du Bois	370 (363-377)	0.23 (0.22-0.25)	-1.1 (-1.3, -0.9)	344 (336-353)	0.24 (0.22-0.26)	-1.2 (-1.4, -1.0)
		Lac du Bonnet	306 (301-313)	0.38 (0.33-0.44)	-1.4 (-1.9, -1.1)	301 (297-306)	0.35 (0.32-0.38)	-1.5 (-1.8, -1.3)
		Pine Falls	356 (319-443)	0.36 (0.19-0.57)	-0.3 (-1.5, 0.2)	374 (315-604)	0.26 (0.09-0.45)	-0.8 (-2.6, 0.0)
SR		Saskatchewan River	378 (349-449)	0.40 (0.19-0.64)	-0.4 (-2.2, 0.3)	381 (338-847)	0.26 (0.04-0.62)	-1.6 (-7.4, 0.4)
		Cedar Lake	360 (344-385)	0.26 (0.19-0.33)	-1.3 (-2.5, -0.6)	361 (329-534)	0.16 (0.04-0.26)	-4.0 (-11.5, -1.7)
		Cormorant Lake	303 (268-480)	0.19 (0.05-0.41)	-1.5 (-6.8, 0.8)	278 (247-652)	0.35 (0.04-1.19)	2.6 (-3.1, 4.8)
UNR		Playgreen Lake	479 (361-1692)	0.09 (0.01-0.24)	-3.9 (-10.4, -0.6)	310 (274-691)	0.34 (0.04-1.03)	-0.4 (-8.0, 1.7)
		Cross Lake	362 (312-755)	0.19 (0.03-0.47)	-3.1 (-10.9, -0.1)			
		Sipiwesk Lake	403 (375-492)	0.23 (0.11-0.37)	-0.5 (-3.9, 1.0)	418 (376-585)	0.12 (0.04-0.21)	-3.8 (-8.8, -1.4)
		Upper Nelson River	338 (308-580)	0.28 (0.03-1.93)	-1.7 (-20.1, 3.2)	344 (325-541)	0.41 (0.04-1.63)	0.6 (-13.2, 3.2)
		Setting Lake	370 (364-376)	0.14 (0.13-0.16)	-3.2 (-3.6, -2.8)	340 (336-345)	0.17 (0.16-0.18)	-2.9 (-3.2, -2.6)
LNR		Burntwood River	304 (287-341)	0.89 (0.27-1.96)	1.1 (-1.9, 1.7)			
		Split Lake	446 (421-482)	0.13 (0.10-0.15)	-2.9 (-3.7, -2.2)	406 (386-434)	0.15 (0.11-0.18)	-2.5 (-3.5, -1.8)
		Stephens Lake	440 (354-858)	0.11 (0.02-0.22)	-3.6 (-10.2, -0.8)	442 (395-533)	0.12 (0.07-0.18)	-3.0 (-5.3, -1.5)
UCR		Granville Lake	382 (365-413)	0.11 (0.09-0.14)	-4.1 (-5.4, -3.2)	329 (317-346)	0.17 (0.13-0.20)	-2.3 (-3.4, -1.5)
		Opachuanau Lake	314 (293-353)	0.14 (0.08-0.19)	-3.3 (-6.2, -1.8)	281 (273-291)	0.21 (0.17-0.25)	-1.3 (-1.9, -0.7)
		Southern Indian Lake	444 (395-539)	0.06 (0.04-0.08)	-6.1 (-8.7, -4.2)	352 (331-389)	0.10 (0.07-0.13)	-3.8 (-5.9, -2.4)
		Rat Lake	336 (280-674)	0.11 (0.02-0.81)	-7.7 (-22.0, 2.0)	286 (268-307)	0.36 (0.25-0.52)	1.2 (0.4, 1.9)
CRD		Notigi Lake	369 (326-545)	0.12 (0.04-0.20)	-3.3 (-8.4, -0.9)			
		Threepoint Lake	312 (299-330)	0.21 (0.16-0.27)	-1.1 (-2.2, -0.3)	292 (281-308)	0.23 (0.17-0.29)	-1.3 (-2.6, -0.4)
		Footprint Lake	359 (316-771)	0.12 (0.02-0.32)	-5.6 (-18.7, -0.6)	305 (283-519)	0.19 (0.02-0.89)	-5.6 (-35.5, 2.0)
		Wuskwatim Lake	305 (284-350)	0.20 (0.11-0.28)	-2.4 (-4.9, -1.2)			
		Apussigamasi Lake	297 (284-315)	0.27 (0.20-0.34)	-1.0 (-2.1, -0.4)	288 (268-334)	0.26 (0.14-0.40)	-1.6 (-4.2, -0.6)

Table 2.6. Mean (±SD) VBGF parameter estimates (L_∞ = asymptotic average length, K = growth coefficient, t_0 = length-0 modeling artifact) of sauger sample populations in Manitoba, by sex, when grouped by Study Region as defined under CAMP protocols (2017).

Study Region	Female				Male			
	Total groups	mean L_∞	mean K	mean t_0	Total Groups	mean L_∞	mean K	mean t_0
Lake Winnipeg	8	369 (±20)	0.32 (±0.10)	-0.1 (±0.5)	9	350 (±14)	0.29 (±0.06)	-0.6 (±0.6)
Red-Assiniboine R.	2	314 (±1)	1.68 (±1.65)	0.3 (±2.8)	1	288	0.85	0.1
Lake Manitoba	1	445	0.37	-0.9	1	401	0.4	-1.1
Lake Winnipegosis	1	405	0.55	0.0	1	384	0.45	-0.6
Winnipeg River	4	349 (±29)	0.30 (±0.08)	-1.1 (±0.6)	4	357 (±46)	0.25 (±0.08)	-1.4 (±0.6)
Saskatchewan River	2	369 (±13)	0.33 (±0.10)	-0.9 (±0.6)	2	371 (±14)	0.21 (±0.07)	-2.8 (±1.7)
Upper Nelson River	4	396 (±62)	0.20 (±0.08)	-2.3 (±1.5)	3	357 (±55)	0.29 (±0.15)	-1.2 (±2.3)
Lower Nelson River	3	397 (±80)	0.38 (±0.44)	-1.8 (±2.5)	2	424 (±25)	0.14 (±0.02)	-2.8 (±0.4)
Upper Churchill River	3	380 (±65)	0.10 (±0.04)	-4.5 (±1.4)	3	321 (±36)	0.16 (±0.06)	-2.5 (±1.3)
Churchill River Diversion	6	330 (±30)	0.17 (±0.06)	-3.5 (±2.7)	4	293 (±9)	0.26 (±0.07)	-1.8 (±2.8)

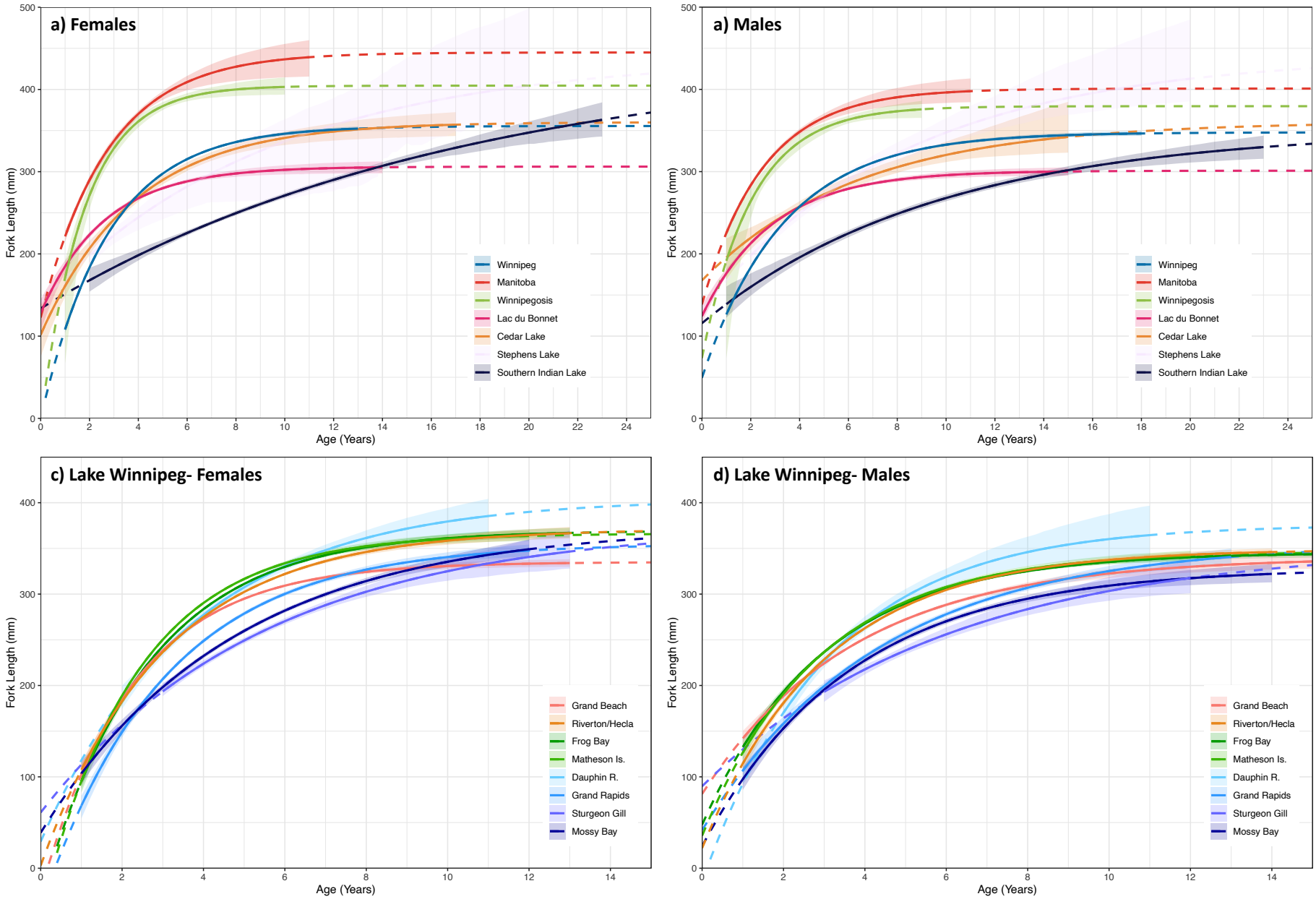


Figure 2.3. VBGF plots for sauger sampled from a subset of Lake level populations (**a**, **b**) and all Lake Winnipeg sample sites (**c**, **d**), separated by sex (**a**/**c** = female, **b**/**d** = male). Dotted lines represent growth projections for unsampled age classes. Ribbons illustrate 95% confidence intervals.

The mean fork lengths of five-year-old sauger were consistent with VBGF modelling across sites except for the Red River, where VBGF parameters were poorly modeled due to small sample size (Table 2.1; Table 2.6). Sauger at age five were largest in Lake Manitoba and Lake Winnipegosis and smallest in waterbodies within the Upper Churchill River and Churchill River Diversion Study Regions (Table 2.7, Figure 2.4). Variations in fork lengths at age five were smaller within Study Regions than among Study Regions except with the Saskatchewan River system; sauger sampled in the Saskatchewan River were markedly larger than Cedar Lake sauger by age five (Table 2.8). The Lower Nelson River and Churchill River systems inhabit a similar latitudinal zone, yet five-year-old sauger from the Lower Nelson River system were closer in size to fish in Lake Winnipeg than in the Churchill River system (Table 2.7). Mean fork lengths of five-year-old sauger appeared to be spatially correlated in the Lake Winnipeg Study Region. Fork lengths increased with latitude for both sexes until the Matheson Island and Dauphin River sites, after which the mean fork length decreased (Table 2.8). This observation and other spatial trends for length-at-age-five sauger are visualized in Figure 2.4 (combined sexes), and Appendix 2.A (females) and 2.B (males).

Table 2.7. Mean fork length (mm ±SD) of five-year-old sauger in Manitoba when grouped by Study Region as defined under CAMP protocols (2017).

Study Region	Total groups	Female FL (mm)	Male FL (mm)	Mean FL (mm)
Lake Winnipeg	8	291 (± 26)	274 (± 24)	283 (± 24)
Red-Assiniboine R.	1	313		
Lake Manitoba	5	402 (± 21)	372 (± 18)	387 (± 19)
Lake Winnipegosis	1	374	353	
Winnipeg River	4	288 (± 17)	265 (± 2)	273 (± 1)
Saskatchewan River	2	318 (± 47)	291 (± 34)	304 (± 40)
Upper Nelson River	4	272 (± 18)	266 (± 5)	269 (± 9)
Lower Nelson River	3	281 (± 17)	259 (± 8)	266 (± 9)
Upper Churchill River	3	224 (± 22)	230 (± 19)	227 (± 18)
Churchill River Diversion	6	239 (± 6)	238 (± 17)	239 (± 11)

Table 2.8. Mean fork length (mm ±SD) of five-year-old sauger sampled in waterbodies across Manitoba. Values for Lake Winnipeg and Lake Manitoba are presented at the Site population level. Empty cells indicate that no five-year-old sauger were sampled in that group.

Sampling Location			Females	Males	Female FL (mm)	Male FL (mm)	Mean FL (mm)
LW	SB	Grand Beach	843	331	292 ±26.9	270 ±26.3	281
		Riverton/Hecla	752	209	304 ±29.3	285 ±23.9	294
	CH	Frog Bay	436	453	317 ±24.3	287 ±22.8	302
		Matheson Island	278	190	322 ±23.0	290 ±24.8	306
	NB	Dauphin River	78	12	306 ±28.3	310 ±27.3	308
		Grand Rapids	403	127	283 ±39.5	259 ±35.0	271
		Sturgeon Gill	91	38	251 ±21.6	238 ±16.1	245
		Mossy Bay	160	61	256 ±23.5	250 ±17.2	253
RR		Red River	4		313 ±15.3		
AR		Assiniboine River					
LM		Whitemud	7	7	411 ±25.3	380 ±12.1	396
		Lundar	2	2	431 ±12.7	390 ±31.1	411
		MB Narrows	2	1	394 ±36.8	380	387
		Steep Rock	32	13	375 ±18.7	342 ±20.4	359
		Manipogo	4	2	396 ±7.8	367 ±1.4	382
WO		Lake Winnipegosis	45	31	374 ±23.0	353 ±21.0	363
WR		Eaglenest Lake	31	13	281 ±24.2	264 ±19.4	272
		Pointe du Bois	160	89	279 ±26.6	264 ±21.1	272
		Lac du Bonnet	110	85	279 ±22.0	268 ±20.4	273
		Pine Falls	6		313 ±21.4		
SR		Saskatchewan River	12	3	351 ±34.0	315 ±5.0	333
		Cedar Lake	52	16	285 ±35.1	267 ±33.6	276
		Cormorant Lake	2		208 ±17.0		
UNR		Playgreen Lake	9	11	262 ±31.2	264 ±23.8	263
		Cross Lake	5	2	298 ±26.9	265 ±33.2	281
		Sipiwesk Lake	10	25	264 ±16.9	273 ±18.8	268
		Upper Nelson River	2	3	262 ±2.8	262 ±38.8	262
		Setting Lake	208	177	263 ±17.5	254 ±13.5	259
LNR		Burntwood River	2		299 ±17.0		
		Split Lake	50	31	279 ±26.8	265 ±17.7	272
		Stephens Lake	8	15	265 ±25.0	253 ±12.6	259
UCR		Granville Lake	80	32	249 ±20.6	238 ±12.0	244
		Opachuanau Lake	18	18	208 ±10.3	208 ±16.8	208
		Southern Indian Lake	21	6	215 ±26.2	244 ±15.9	229
CRD		Rat Lake	1		235		
		Notigi Lake	2	5	245 ±23.3	236 ±22.2	240
		Threepoint Lake	16	18	230 ±18.7	226 ±19.6	228
		Footprint Lake	2	3	247 ±7.8	266 ±24.6	256
		Wuskwatim Lake	8	10	242 ±11.3	226 ±17.5	234
		Apussigamasi Lake	17	12	237 ±20.7	234 ±9.5	236

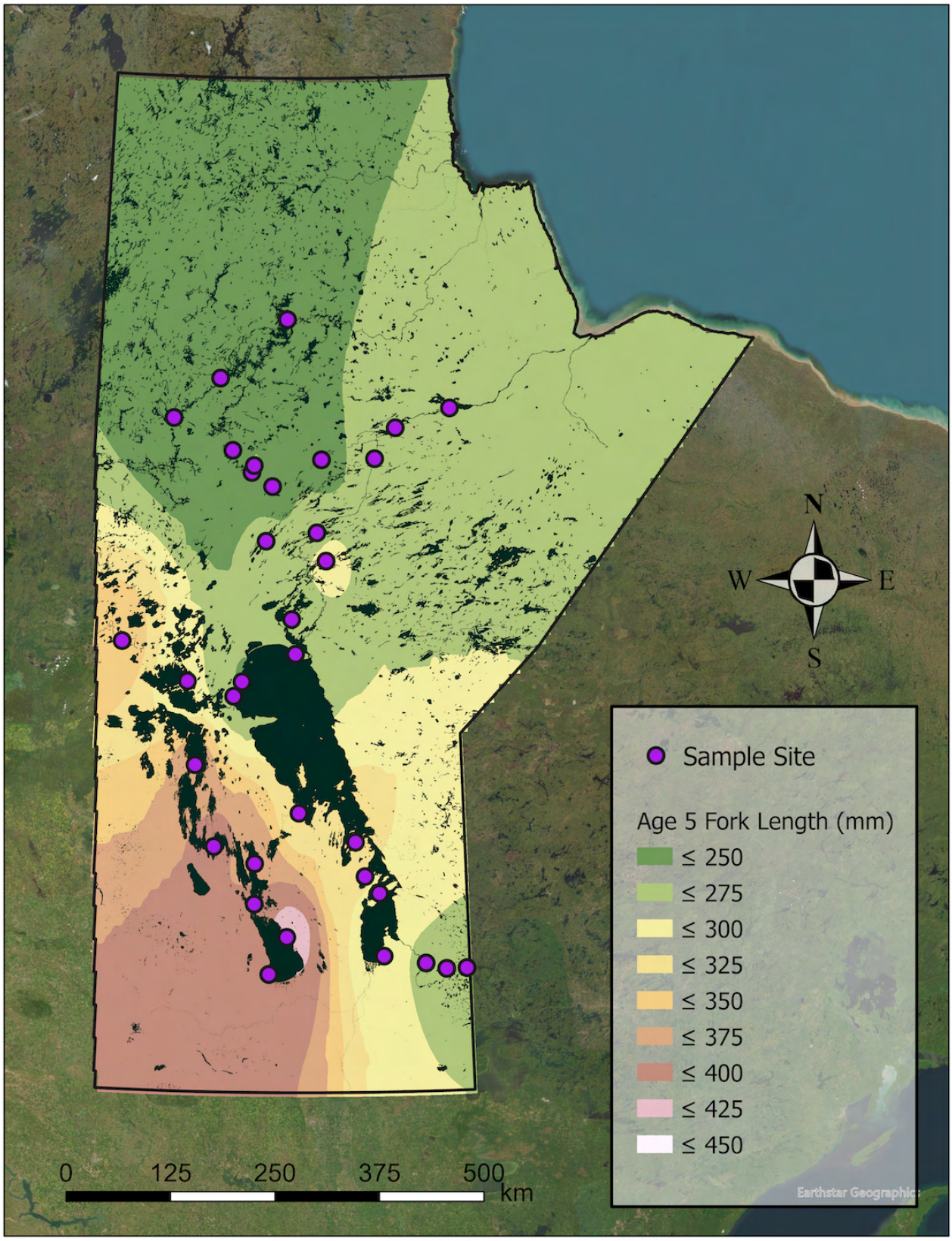


Figure 2.4. Heat map of the mean estimated fork length (mm) of sauger at age 5 in Manitoba waterbodies. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.

2.3.1.3 Body Condition

Female sauger were heavier at a given length than male sauger by a small margin. An analysis of covariance indicated that sex was a significant predictor of weight at length (Table 2.9), and *K* factor distributions significantly differed between sexes according to a Kruskal-Wallis test (Table 2.10). However, weight-at-length plots (Figure 2.6; Appendix 2.C) and *K* boxplots (Figure 2.5) show minimal variation between the sexes, and sex only influenced the intercepts and slopes of population-specific regressions in 33% and 28% of cases, respectively (SM 4-1). Moreover, the estimated weight difference between a 350 mm female and 350 mm male sauger is 3.6 g when using sex-specific weight-at-length regression equations presented in Figure 2.6. Therefore, I pooled male and female data into one dataset for body condition analyses.

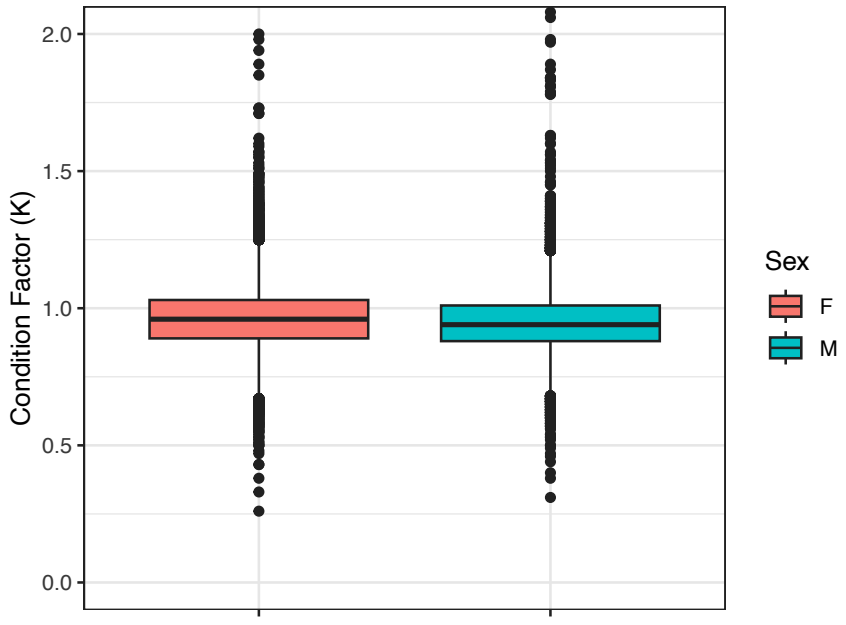


Figure 2.5. Boxplot of condition factor *K* for female (n = 21725) and male (n = 13838) sauger sampled across twenty-nine Manitoba waterbodies.

Table 2.9. Analysis of covariance table for the effects of log_{10} length on log_{10} weight of sauger sampled in Manitoba, with sex as a covariate. Coefficient estimates and 95% confidence intervals are shown in sub-table **b)**.

a)	Sum Sq	Df	F value	Pr(>F)	b)	coef	2.50%	97.50%
logL	2350.68	1	734160.435	<2.20E-16	**(Intercept)**	-4.983	-5.005	-4.961
Sex	0.47	1	145.592	<2.20E-16	**logL**	2.985	2.976	2.994
logL:Sex	0.09	1	27.429	1.64E-07	**SexM**	-0.098	-0.132	-0.064
Residuals	113.86	35559			**logL:SexM**	0.037	0.023	0.051

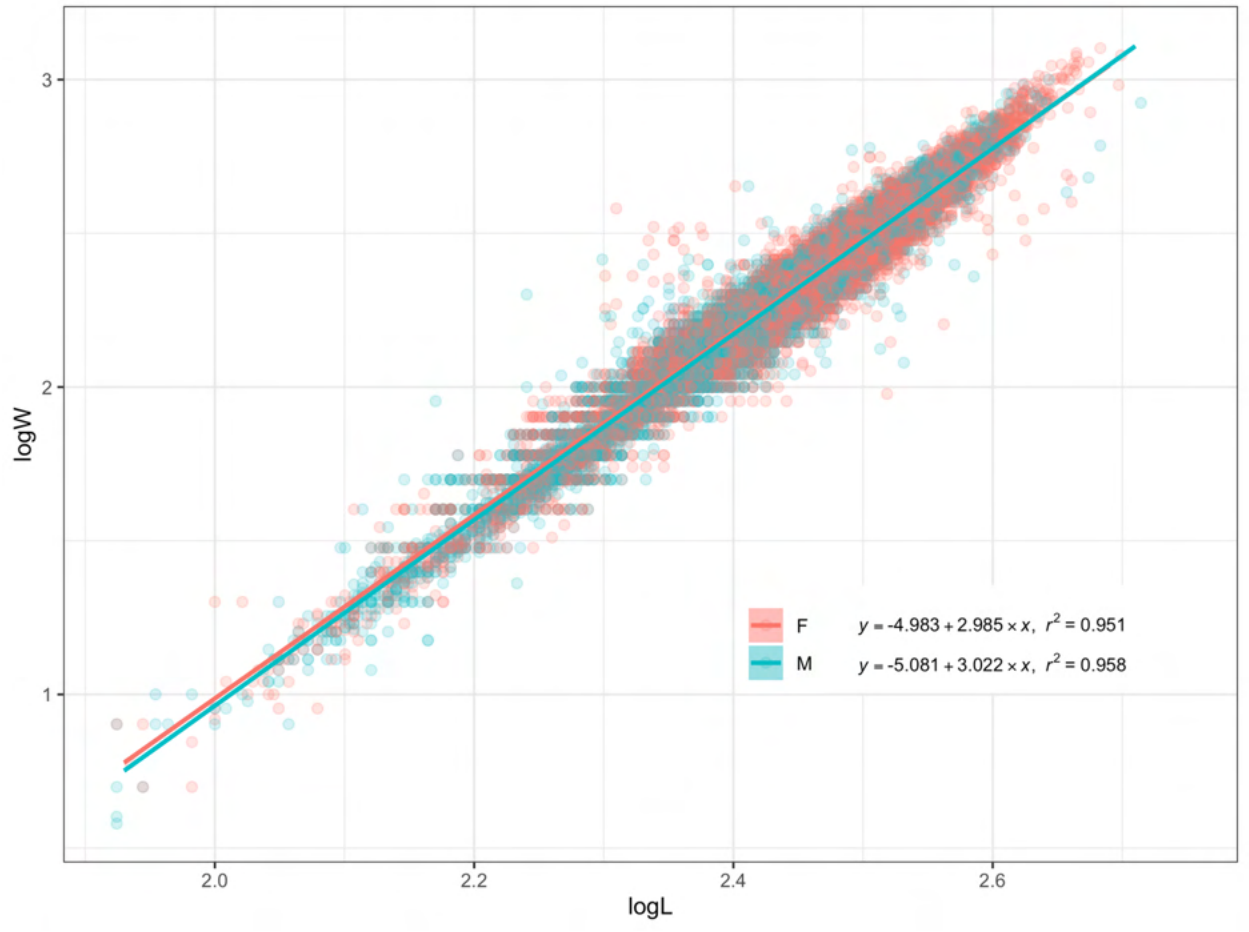


Figure 2.6. Plot of log10 weight (g) at log10 length (mm) of female (n = 21725) and male (n = 13838) sauger captured across twenty-nine waterbodies in Manitoba. The plot is fitted with a simple linear regression for each sex.

The weight-length relationships of sauger varied among sample locations. An analysis of covariance showed that the rate at which weight was added to sauger of increasing lengths differed by sample location (Table 2.10). The intercepts and slopes of population-specific weight-at-length regressions (extracted from the general regression model) can be seen in Table 2.11. Plots for a subset of these regressions, as well as for all Site level populations in Lake Winnipeg, are presented in Figure 2.7. Raw weight-length plots can be seen in Appendices 2.D and 2.E.

Bootstrapped distributions of population-specific intercepts and slopes significantly differed ($p < 0.05$) in 84% and 71% of pairwise comparisons at the Lake population level (SM 4-2, SM 4-3). The proportion of significant results decreased when comparisons were limited to Study Region (SM 4-4), except for in Lake Winnipeg; site-specific regression slopes and intercepts differed in 100% and 93% of these pairwise comparisons. That said, coefficient estimates were more similar within the Lake Winnipeg study region than within most other study regions (Table 2.12), which suggests that statistical significance in these tests is strongly influenced by sample size.

Table 2.10. Analysis of covariance table for the effects of $\log_{10}$ length on $\log_{10}$ weight of sauger sampled in Manitoba, with sample location (sample population) as a covariate.

	Sum Sq	Df	F value	Pr(>F)
logL	2141.07735	1	723982.294	**0**
Location	6.66873824	35	64.4274903	**0**
logL:Location	2.78066721	35	26.8643637	**<0.001**
Residuals	104.959716	35491		

Table 2.11. log_{10}weight—log_{10}length linear regression coefficients and mean (±SD) *K* condition factors for sauger populations in Manitoba. Regression coefficients and mean *K* for Lake Manitoba are presented at the Lake and Site population level, respectively.

Sampling Location			logW-logL Regression		*K* Condition Factor			
			Intercept	Slope	Mean	Q1	Median	Q3
LW	SB	Grand Beach	-4.701	2.866	0.95 ±0.12	0.87	0.94	1.02
		Riverton/Hecla	-4.697	2.870	0.97 ±0.12	0.90	0.96	1.04
	CH	Frog Bay	-4.729	2.880	0.95 ±0.12	0.88	0.94	1.01
		Matheson Is.	-4.858	2.936	0.98 ±0.11	0.90	0.98	1.05
	NB	Dauphin R.	-5.161	3.072	1.06 ±0.13	0.99	1.05	1.12
		Grand Rapids	-5.249	3.099	0.99 ±0.13	0.91	0.99	1.07
		Sturgeon Gill	-5.540	3.218	0.97 ±0.11	0.90	0.96	1.03
		Mossy Bay	-5.046	3.004	0.94 ±0.15	0.86	0.93	1.01
RR		Red River	-6.314	3.522	0.94 ±0.09	0.86	0.95	1.00
AR		Assiniboine River	-6.867	3.753	0.94 ±0.14	0.83	0.91	1.02
LM		Whitemud	-5.456	3.185	1.05 ±0.11	0.97	1.06	1.12
		Lundar			1.05 ±0.10	0.97	1.05	1.09
		MB Narrows			0.97 ±0.09	0.92	0.96	1.03
		Steep Rock			0.98 ±0.08	0.93	0.97	1.03
		Manipogo			0.97 ±0.09	0.92	0.97	1.03
WO		Lake Winnipegosis	-5.137	3.057	1.03 ±0.13	0.95	1.03	1.10
WR		Eaglenest Lake	-5.380	3.145	0.95 ±0.14	0.88	0.94	1.01
		Pointe du Bois	-5.214	3.078	0.96 ±0.14	0.88	0.95	1.03
		Lac du Bonnet	-5.259	3.095	0.94 ±0.10	0.88	0.94	1.00
		Pine Falls	-5.232	3.089	0.97 ±0.09	0.88	0.98	1.04
SR		Saskatchewan River	-5.721	3.282	0.96 ±0.12	0.90	0.97	1.06
		Cedar Lake	-5.026	3.015	1.04 ±0.14	0.96	1.04	1.11
		Cormorant Lake	-3.725	2.427	0.86 ±0.50	0.75	0.80	0.85
UNR		Playgreen Lake	-3.599	2.425	1.04 ±0.31	0.89	0.99	1.07
		Cross Lake	-5.539	3.217	0.98 ±0.10	0.90	0.96	1.05
		Sipiwesk Lake	-4.474	2.788	1.00 ±0.10	0.93	0.99	1.06
		Upper Nelson River	-4.090	2.631	0.99 ±0.14	0.90	0.99	1.02
		Setting Lake	-5.047	3.009	0.95 ±0.11	0.89	0.94	1.00
LNR		Burntwood River	-5.894	3.348	0.92 ±0.12	0.85	0.90	1.01
		Split Lake	-5.017	3.000	0.97 ±0.16	0.89	0.96	1.03
		Stephens Lake	-5.513	3.215	1.02 ±0.15	0.91	1.01	1.09
UCR		Granville Lake	-5.013	2.983	0.89 ±0.10	0.83	0.89	0.94
		Opachuanau Lake	-5.151	3.043	0.91 ±0.13	0.85	0.90	0.94
		Southern Indian Lake	-5.141	3.037	0.90 ±0.11	0.84	0.89	0.95
CRD		Rat Lake	-4.487	2.761	0.86 ±0.08	0.80	0.85	0.92
		Notigi Lake	-5.653	3.254	0.93 ±0.12	0.86	0.91	0.96
		Threepoint Lake	-5.272	3.089	0.88 ±0.10	0.82	0.88	0.94
		Footprint Lake	-4.780	2.892	0.90 ±0.07	0.87	0.91	0.94
		Wuskwatim Lake	-5.419	3.158	0.92 ±0.10	0.87	0.91	0.96
		Apussigamasi Lake	-4.757	2.877	0.89 ±0.08	0.84	0.89	0.93

Table 2.12. log_{10}weight—log_{10}length linear regression coefficients (mean ±SD) and mean *K* (±SD) condition factors for sauger populations in Manitoba when grouped by Study Region as defined under CAMP protocols (2017).

Study Region	Total groups	W:L Regression Intercept	Slope	Condition Mean *K*
Lake Winnipeg	8	-4.997 (±0.306)	2.993 (±0.128)	0.97 (±0.03)
Red-Assiniboine R.	2	-6.590 (±0.391)	3.637 (±0.163)	0.94 (±0.00)
Lake Manitoba	5	-5.456	3.185	1.00 (±0.04)
Lake Winnipegosis	1	-5.137	3.057	1.03
Winnipeg River	4	-5.271 (±0.074)	3.101 (±0.029)	0.95 (±0.01)
Saskatchewan River	2	-5.373 (±0.491)	3.148 (±0.188)	1.00 (±0.05)
Upper Nelson River	4	-4.425 (±0.824)	2.765 (±0.335)	1.00 (±0.02)
Lower Nelson River	3	-5.474 (±0.439)	3.187 (±0.175)	0.97 (±0.05)
Upper Churchill River	3	-5.101 (±0.076)	3.021 (±0.033)	0.90 (±0.01)
Churchill River Diversion	6	-5.061 (±0.452)	3.005 (±0.190)	0.89 (±0.02)

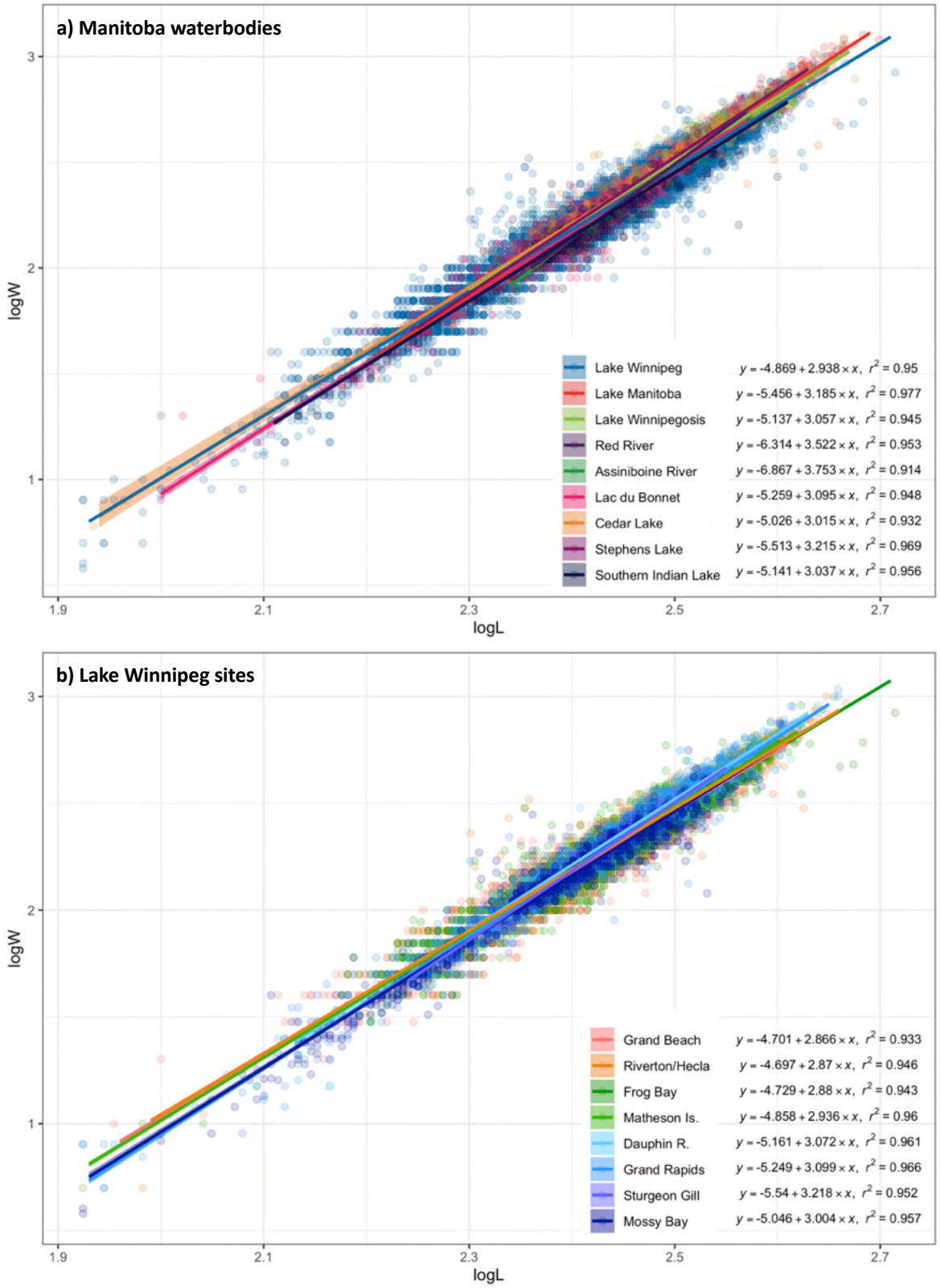


Figure 2.7. Plots of $\log_{10}$ weight (g) at $\log_{10}$ length (mm) for sauger populations in Manitoba as defined by a) Lake level populations, and b) Lake Winnipeg sample sites. Each plot is fitted with a simple linear regression for each sample population.

Variations in the weight-length relationships of sauger populations were magnified when assessed with the *K* condition factor. Mean *K* averaged 0.96 (*SD* = 0.05) across all populations at the Lake level (Table 2.11) and ranged from 0.86 (Cormorant Lake, Rat Lake) to 1.04 (Cedar Lake, Playgreen Lake). Thus, a sauger with a 350 mm fork length would be assigned a weight between 369 g and 446 g depending on the sample population—a difference of 77 g or about 20 percent. Mean *K* was lowest in the Upper Churchill River and Churchill River Diversion study regions (mean *K* = 0.90, 0.89) and highest in Lake Winnipegosis (mean *K* = 1.03; Table 2.12). *K* distributions for Lake level populations and all Site level populations in Lake Winnipeg are presented in Figure 2.8 and Appendix 2.F.

Mean *K* significantly differed ($p < 0.05$) in 48% of pairwise comparisons conducted at the Lake population level with a Holm-adjusted Dunn Kruskal-Wallis multiple comparison test. Mean *K* did not significantly differ between Lake population pairwise comparisons conducted within Study Regions (SM 4-8) except within the Saskatchewan River region (Cedar Lake–Saskatchewan River, $p = 0.03$). In contrast, most pairwise comparisons in Lake Manitoba and Lake Winnipeg were significant at the Site level. Mean *K* did not differ between sites located within Lake Manitoba's south basin (Whitemud, Lundar) or within Lake Manitoba's north basin (Steep Rock, Manipogo) and narrows (Narrows). However, the mean *K*s of sites within the south basin were significantly larger than the mean *K*s of sites within the north basin and narrows (Table 2.11), which constituted 60% of Lake Manitoba comparisons. Mean *K* differed in 86% of the Site level pairwise comparisons conducted within Lake Winnipeg, but I was unable to ascertain if significant results were spatially correlated (SM 4-8). Spatial trends of mean *K* are visualized in Figure 2.9.

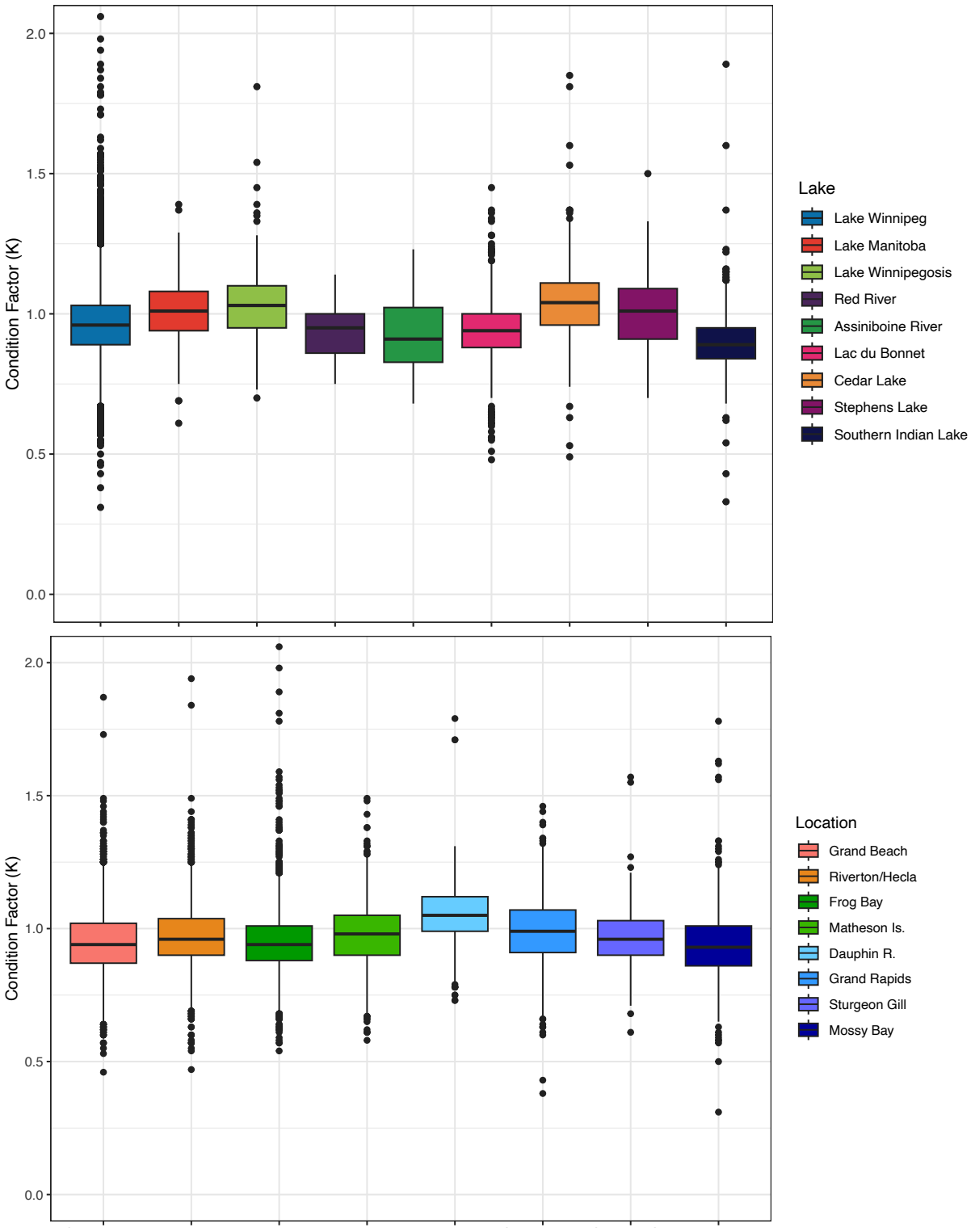


Figure 2.8. Boxplots of condition factor *K* of sauger sampled in a) Lake populations, and b) Site populations within Lake Winnipeg. Coloured boxes represent the interquartile range, whiskers represent K values within 1.5 times the interquartile range, and points represent outliers beyond 1.5 times the interquartile range.

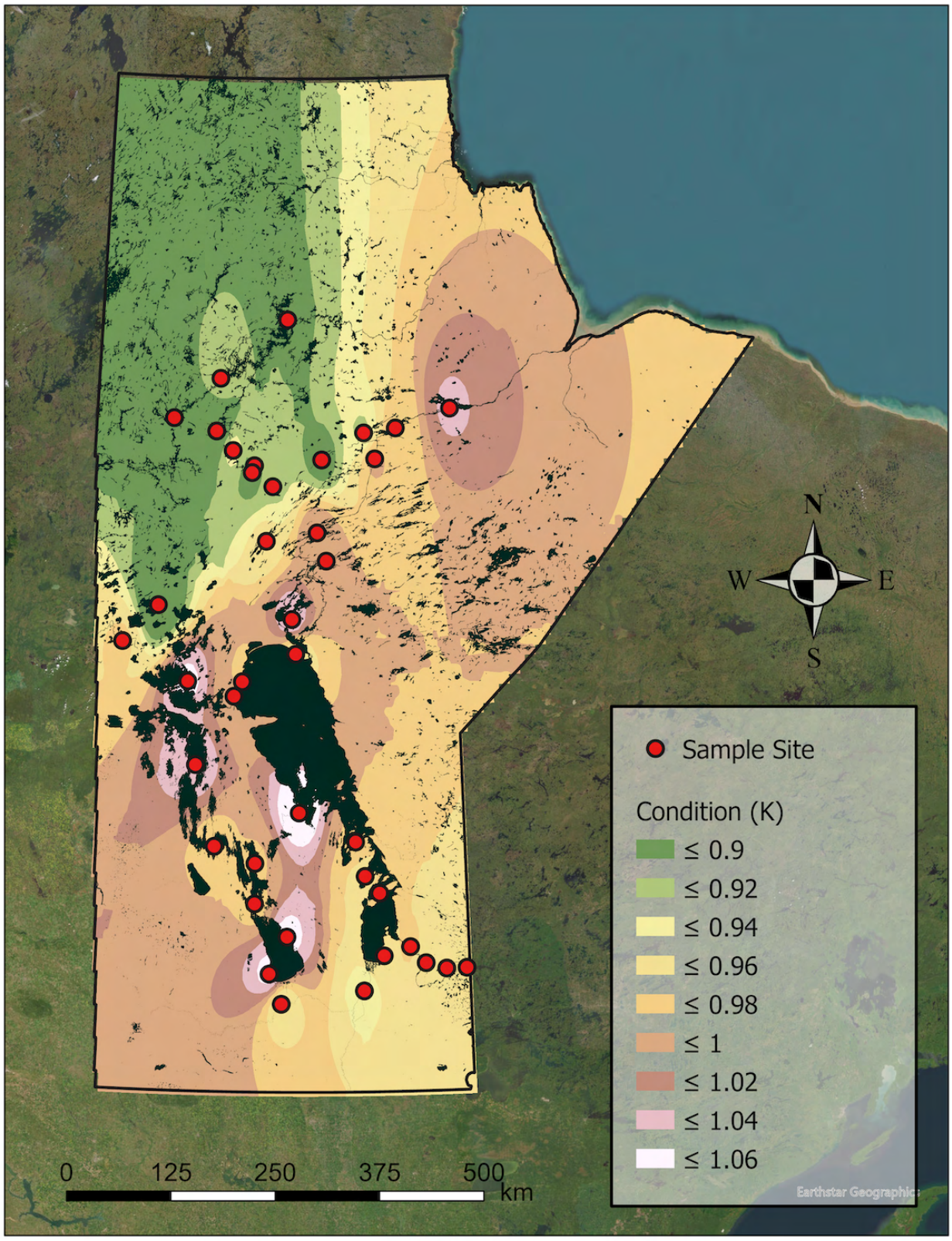


Figure 2.9. Heat map of the mean condition factor *K* of sauger in Manitoba with a fork length between 200 mm and 400 mm. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.

2.3.1.4 Maturity

Male sauger matured at shorter lengths than female sauger in many—but not all—sample populations. At the Lake population level, L_{50} averaged 274 mm (*SD* = 27, range = 233–315 mm) for females and 248 mm (*SD* = 41, range = 163–320 mm) for males using the logistic regression models, and 270 mm (*SD* = 30, range = 220–320 mm) for females and 245 mm (*SD* = 37, range = 180–320 mm) for males using proportion tables. L_{50} could only be calculated for both sexes in 15 of the 29 Lake populations using length-at-maturity logistic regression models (Table 2.14), compared to 24 of 29 populations using proportion tables (Table 2.13). Of these, the L_{50} of male sauger was shorter than females in 10 (66.7%) of populations assessed with logistic regression models and 14 (58.3%) of populations assessed with proportion tables. Male L_{50} was longer than female L_{50} in 33.3% of populations (both methods), with most occurring within the Winnipeg River and Churchill River Diversion Study Regions.

L_{50} estimates derived from logistic regression models frequently differed among sample groups. Bootstrapped L_{50} distributions significantly differed ($p < 0.05$) in 72% of Lake population pairwise comparisons for females and 66% of pairwise comparisons for males (SM 5-7). When comparing within Study Regions, the proportion of significant results for females decreased in all systems except the Saskatchewan River Study Region, but generally increased for males (SM 5-8). That said, many pairwise comparisons were missing due to model nonconvergence, so the frequency of significant results should not be considered reflective of variation within Study Regions. Spatial trends are best represented with heatmaps (Figure 2.10; Figure 2.11). For females, L_{50} was highest in Lake Winnipeg, Lake Manitoba, Lake Winnipegosis, and the Nelson River (~300 mm), and lowest in the Churchill River (~250 mm; Table 2.15, Table 2.16). L_{50} of

females in Cedar Lake and Lac du Bonnet were noticeably smaller than in neighbouring populations. In contrast, L_{50} of male sauger were highest in the Winnipeg River (~290 mm) and lowest in Lake Manitoba and Lake Winnipegosis (~180 mm; Table 2.15, Table 2.16).

Table 2.13. Approximate fork lengths (mm) at which 50% (L_{50}) and 90% (L_{90}) of sauger in a sample population will be sexually mature, according to a proportion table at 20 mm increments. Values for Lake Winnipeg are presented at the Site population level. Empty cells indicate that reasonable L_{50} or L_{90} estimates could not be calculated.

Sampling Location			L_{50} female (mm)	L_{50} male (mm)	L_{50} mean (mm)	L_{90} female (mm)	L_{90} male (mm)	L_{90} mean (mm)
LW	SB	Grand Beach	300	240	270	340	260	300
		Riverton/Hecla	300	240	270	340	280	310
	CH	Frog Bay	300	240	270	320	260	290
		Matheson Is.	300	240	270	340	260	300
	NB	Dauphin R.	300	260	280	360	280	320
		Grand Rapids	300	240	270	380	280	330
		Sturgeon Gill	320	240	280	320	260	290
		Mossy Bay	300	240	270	340	360	350
RR		Red River	280	240	260	300	240	270
AR		Assiniboine River	260	240	250	300	240	270
LM		Lake Manitoba	300	180	240	320	240	280
WO		Lake Winnipegosis	300	180	240	320	220	270
WR		Eaglenest Lake	300	320	310	360	360	360
		Pointe du Bois	300	320	310	380	360	370
		Lac du Bonnet	260	220	240	320	260	290
SR		Saskatchewan River	280	220	250	300	260	280
		Cedar Lake	240	200	220	280	240	260
UNR		Playgreen Lake	300	240	270	340	300	320
		Cross Lake	240	260	250	300		
		Sipiwesk Lake	320	260	290	400	300	350
		Setting Lake	260	220	240	280	260	270
LNR		Split Lake	300	300	300	360	380	370
		Stephens Lake	300	220	260	320	280	300
UCR		Granville Lake	260	260	260	320	300	310
		Opachuanau Lake	220	260	240	280	320	300
		Southern Indian Lake	240	220	230	280	260	270
CRD		Rat Lake	260	280	270	300		
		Notigi Lake	220	240	230	240	280	260
		Threepoint Lake	220	240	230	280	300	290
		Footprint Lake	260	280	270	280	280	280
		Wuskwatim Lake	280			280		
		Apussigamasi Lake	240	220	230	320	260	290

Table 2.14. Approximate fork lengths (mm) at which 50% (L_{50}) and 90% (L_{90}) of sauger in a sample population will be sexually mature, according to a length-at-maturity logistic regression model. Values for Lake Winnipeg are presented at the Site population level. Empty cells indicate that reasonable L_{50} or L_{90} estimates could not be calculated.

Sampling Location			L_{50} female (mm)	L_{50} male (mm)	L_{50} mean (mm)	L_{90} female (mm)	L_{90} male (mm)	L_{90} mean (mm)
LW	SB	Grand Beach	298	234	266	339	269	304
		Riverton/Hecla	299	243	271	332	274	303
	CH	Frog Bay	297	231	264	326	256	291
		Matheson Is.	309	241	275	336	270	303
	NB	Dauphin R.	312	273	293	348	337	343
		Grand Rapids	316	240	278	368	281	325
		Sturgeon Gill	299	233	266	331	252	292
		Mossy Bay	292	257	275	336	335	335
RR		Red River	281			297		
AR		Assiniboine River	272			290		
LM		Lake Manitoba	295	163	229	338	249	293
WO		Lake Winnipegosis	293			328		
WR		Eaglenest Lake	312			365		
		Pointe du Bois	306	320	313	361	377	369
		Lac du Bonnet	251	205	228	306	269	288
SR		Saskatchewan River	288			314		
		Cedar Lake	233			298	281	289
UNR		Playgreen Lake	307	251	279	370	326	348
		Cross Lake	252	204	228	313		
		Sipiwesk Lake	308	259	284	379	314	347
		Upper Nelson River		298			351	
		Setting Lake	255	225	240	286	275	281
LNR		Split Lake	316	327	321	367	408	387
		Stephens Lake		250			299	
UCR		Granville Lake	267	257	262	328	316	322
		Opachuanau Lake	236	266	251	287	329	308
		Southern Indian Lake	240	213	226	274	266	270
CRD		Rat Lake	261			287		
		Notigi Lake		239			276	
		Threepoint Lake	233	240	237	266	288	277
		Footprint Lake	269	274	272	298	294	296
		Wuskwatim Lake	274			314		
		Apussigamasi Lake	250	225	238	306	274	290

Table 2.15. Approximate fork lengths (mm ±SD) at which 50% (L_{50}) and 90% (L_{90}) of sauger in a sample population will be sexually mature, summarized by Study Region as defined under CAMP protocols (2017). Estimates are derived from to a maturity proportion table set in 20 mm increments.

Study Region	Female Groups	Male Groups	L_{50} female (mm)	L_{50} male (mm)	L_{50} mean (mm)	L_{90} female (mm)	L_{90} male (mm)	L_{90} mean (mm)
Lake Winnipeg	8	8	300 (±7)	240 (±7)	270 (±5)	340 (±20)	280 (±34)	310 (±21)
Red-Assiniboine R.	2	2	270 (±14)	240 (±0)	255 (±7)	300 (±0)	240 (±0)	270 (±0)
Lake Manitoba	1	1	300	180	240	320	240	280
Lake Winnipegosis	1	1	300	180	240	320	220	270
Winnipeg River	3	3	290 (±23)	290 (±58)	290 (±40)	350 (±31)	330 (±58)	340 (±44)
Saskatchewan River	2	2	260 (±28)	210 (±14)	235 (±21)	290 (±14)	250 (±14)	270 (±14)
Upper Nelson River	3	3	290 (±42)	250 (±12)	270 (±20)	350 (±50)	300 (±0)	325 (±21)
Lower Nelson River	2	2	300 (±0)	260 (±57)	280 (±28)	340 (±28)	330 (±71)	335 (±49)
Upper Churchill River	3	3	240 (±20)	250 (±23)	245 (±15)	290 (±23)	290 (±31)	290 (±21)
Churchill River Diversion	6	5	250 (±24)	250 (±27)	250 (±22)	280 (±27)	220 (±16)	250 (±56)

Table 2.16. Approximate fork lengths (mm ±SD) at which 50% (L_{50}) and 90% (L_{90}) of sauger in a sample population will be sexually mature, summarized by Study Region as defined under CAMP protocols (2017). Estimates are derived from length-at-maturity logistic regression models. Empty cells indicate that reasonable L_{50} or L_{90} estimates could not be calculated for any population within the Study Region.

Study Region	Female Groups	Male Groups	L_{50} female (mm)	L_{50} male (mm)	L_{50} mean (mm)	L_{90} female (mm)	L_{90} male (mm)	L_{90} mean (mm)
Lake Winnipeg	8	8	303 (±8.5)	244 (±14.3)	273 (±9.2)	339 (±13.3)	284 (±33.5)	312 (±19.8)
Red-Assiniboine R.	2	0	277 (±6.4)			294 (±4.9)		
Lake Manitoba	1	1	295	163	229	338	249	293
Lake Winnipegosis	1	0	293			328		
Winnipeg River	3	2	290 (±33.6)	263 (±81.3)	271 (±60.1)	344 (±33)	323 (±76.4)	329 (±57.3)
Saskatchewan River	2	1	261 (±38.9)			306 (±11.3)	281	289
Upper Nelson River	3	4	289 (±32)	253 (±38.6)	264 (±31)	354 (±35.8)	330 (±18.9)	348 (±0.7)
Lower Nelson River	1	2	316	289 (±54.4)	321	367	354 (±77.1)	387
Upper Churchill River	3	3	248 (±16.9)	245 (±28.4)	246 (±18.4)	296 (±28.2)	304 (±33.3)	300 (±26.9)
Churchill River Diversion	5	4	257 (±16.4)	245 (±20.8)	249 (±19.9)	294 (±18.7)	283 (±9.6)	288 (±9.7)

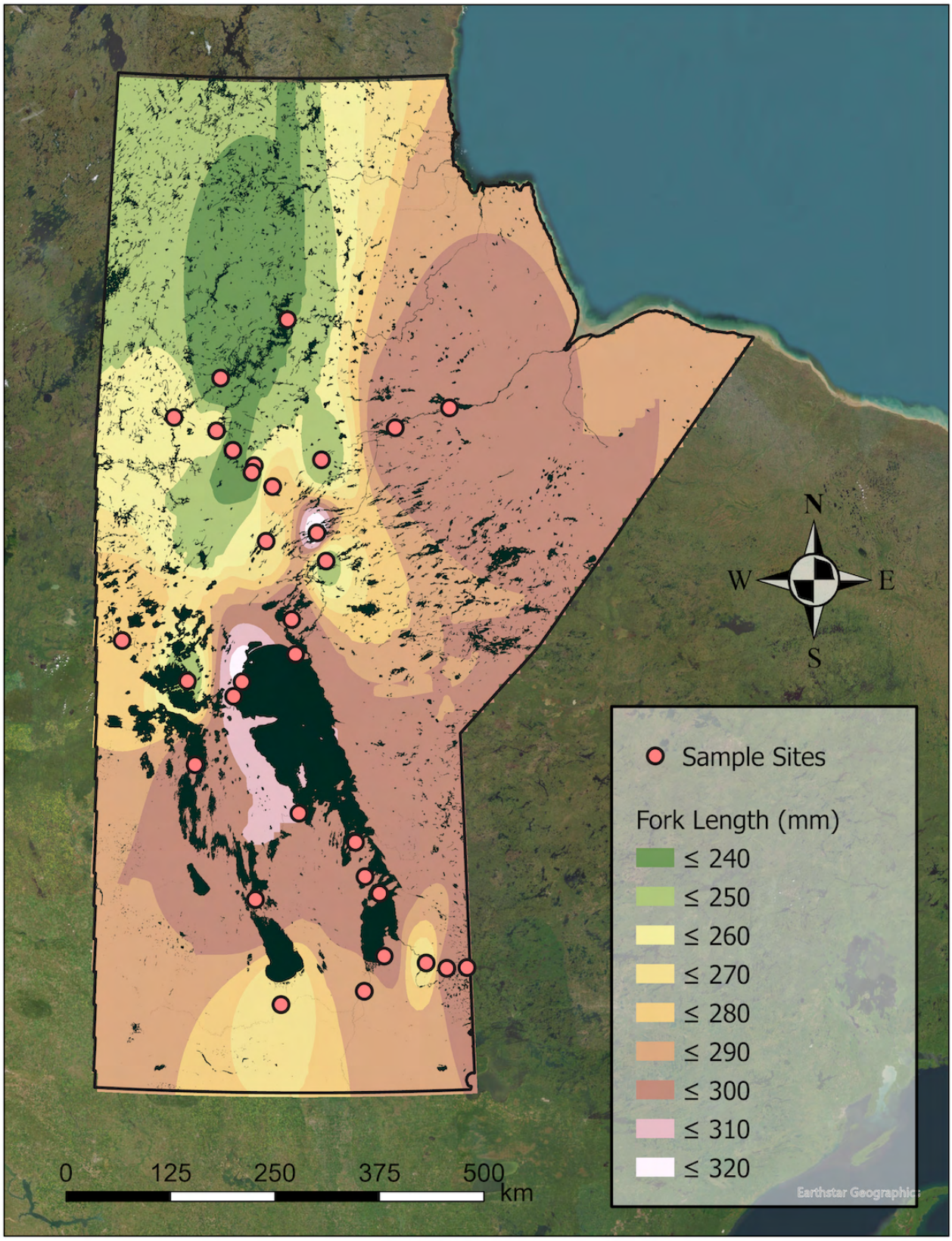


Figure 2.10. Heat map of L_{50} for female sauger in Manitoba waterbodies, as estimated with proportion tables. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.

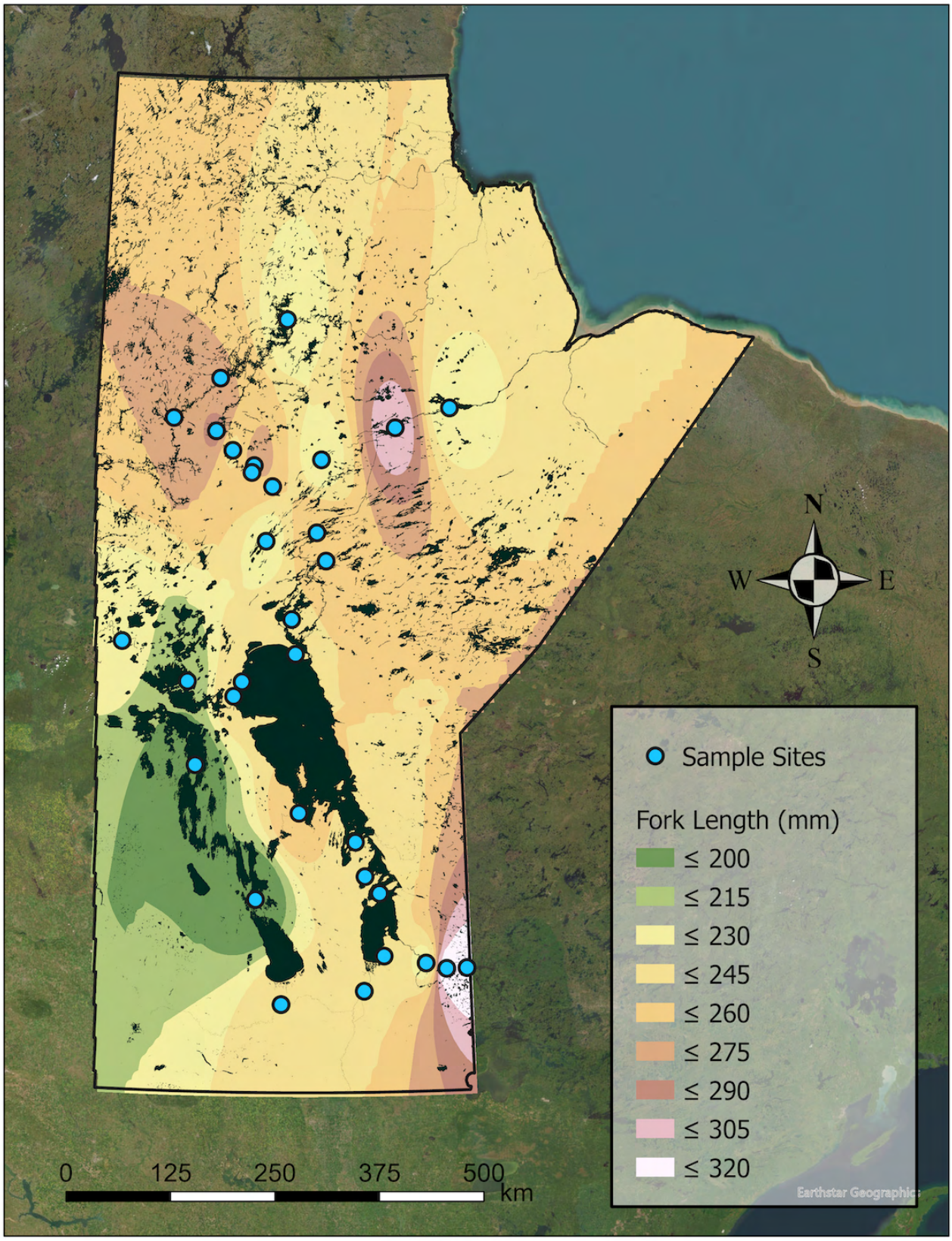


Figure 2.11. Heat map of L_{50} for male sauger in Manitoba waterbodies, as estimated with proportion tables. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.

Ages of maturity were highly variable within and among Study Regions. At the Lake population level, A_{50} averaged 5.72 years (*SD* = 2.17, range = 2.22–10.89 years) for females and 5.71 years (*SD* = 3.23, range = 1.72–11.90 years) using logistic regression models, and 6.0 years (*SD* = 1.24, range = 4–8 years) for females and 5.7 years (*SD* = 2.38, range = 2–13 years) for males using proportion tables. Of the fourteen Lake level populations with modeled A_{50} estimates for both sexes, male sauger matured earlier than females in eight populations (57.1%) and later than females in six populations (42.9%; Table 2.18). Of the twenty-four populations with A_{50} estimates derived from proportion tables, male sauger matured earlier than females in fourteen populations (58.3%), later than females in six populations (25.0%), and at the same age as females in 4 populations (16.7%; Table 2.17). Male sauger matured at a younger age than female sauger at all Site populations within Lake Winnipeg (Table 2.17, Table 2.18).

Bootstrapped A_{50} distributions significantly differed ($p < 0.05$) in 67% of Lake population pairwise comparisons for females and 57% of pairwise comparisons for males (SM 6-8). When comparing within Study Regions, the proportion of significant results decreased in all systems except within Lake Winnipeg (SM 6-9). The increase in significant results within the Lake Winnipeg Study Region may be due to each Site population's narrow confidence intervals, though A_{50} does appear to increase with latitude (Figure 2.12, Figure 2.13). For females, A_{50} was highest in the Churchill River (~7 years) and lowest in Lake Manitoba, Lake Winnipegosis, and the Red-Assiniboine Study Region (~4 years; Table 2.19, Table 2.20). A_{50} was higher in Eaglenest Lake and Point du Bois than neighbouring populations. For males, A_{50} was highest in the Churchill River and Winnipeg River Study Regions (~7–9 years) and lowest in Lake Manitoba and Lake Winnipegosis (~2 years; Table 2.19, Table 2.20). That said, male A_{50} estimates were highly

variable within Study Regions; A_{50} ranged from 6 to 13 in the Upper Churchill River system alone. Thus, A_{50} estimates were better summarised by sample population than by Study Region.

Table 2.17. Approximate ages (years) at which 50% (A_{50}) and 90% (A_{90}) of sauger in a sample population will be sexually mature, according to a maturity proportion table. Values for Lake Winnipeg and Lake Manitoba are presented at the Site population level. Empty cells indicate that reasonable A_{50} or A_{90} estimates could not be calculated.

Sampling Location			A_{50} female (years)	A_{50} male (years)	A_{50} mean (years)	A_{90} female (years)	A_{90} male (years)	A_{90} mean (years)
LW	SB	Grand Beach	5	4	4.5	7	5	6
		Riverton/Hecla	5	4	4.5	6	5	5.5
	CH	Frog Bay	5	3	4	6	4	5
		Matheson Is.	5	4	4.5	6	5	5.5
	NB	Dauphin R.	6	5	5.5	8	5	6.5
		Grand Rapids	7	5	6	10	7	8.5
		Sturgeon Gill	7	5	6	9	7	8
		Mossy Bay	7	5	6	9		
RR		Red River	4	4	4	5	4	4.5
AR		Assiniboine River	4	3	3.5	4	4	4
LM		Lake Manitoba	4	2	3	4	3	3.5
WO		Lake Winnipegosis	4	2	3	5	2	3.5
WR		Eaglenest Lake	7	8	7.5	8	9	8.5
		Pointe du Bois	6	10	8	15	15	15
		Lac du Bonnet	5	4	4.5	6	5	5.5
SR		Saskatchewan River	5	3	4	5	5	5
		Cedar Lake	5	4	4.5	7	5	6
UNR		Playgreen Lake	6	4	5	9	8	8.5
		Cross Lake	5	5	5	8	6	7
		Sipiwesk Lake	6	5	5.5	10	8	9
		Upper Nelson River		6			12	
		Setting Lake	6	5	5.5	8	9	8.5
LNR		Split Lake	7	9	8	12	16	14
		Stephens Lake	7	4	5.5	11	7	9
UCR		Granville Lake	8	8	8	13	11	12
		Opachuanau Lake	7	13	10	10	16	13
		Southern Indian Lake	7	6	6.5	16	12	14
CRD		Rat Lake	7			8		
		Notigi Lake	6	7	6.5	7	9	8
		Threepoint Lake	7	7	7	10	14	12
		Footprint Lake	8	5	6.5	9	9	9
		Wuskwatim Lake	7	8	7.5			
		Apussigamasi Lake	7	6	6.5		10	

Table 2.18. Approximate ages (years) at which 50% (A_{50}) and 90% (A_{90}) of sauger in a sample population will be sexually mature, with 95% confidence intervals. Estimates are derived from age-at-maturity logistic regression models. Values for Lake Winnipeg are presented at the Site population level. Empty cells indicate that reasonable A_{50} or A_{90} estimates could not be calculated.

Sampling Location			A_{50} female (years)	A_{50} male (years)	A_{50} mean (years)	A_{90} female (years)	A_{90} male (years)	A_{90} mean (years)
LW	SB	Grand Beach	5.08 (5.02-5.14)	3.51 (3.45-3.58)	4.30	6.37 (6.25-6.51)	4.60 (4.45-4.74)	5.48
		Riverton/Hecla	4.96 (4.90-5.01)	3.65 (3.56-3.73)	4.30	5.93 (5.81-6.05)	4.82 (4.64-5.01)	5.38
	CH	Frog Bay	4.57 (4.51-4.63)	2.95 (2.86-3.03)	3.76	5.50 (5.37-5.66)	3.95 (3.83-4.07)	4.73
		Matheson Is.	4.68 (4.61-4.75)	3.27 (3.17-3.37)	3.97	5.57 (5.43-5.73)	4.33 (4.15-4.51)	4.95
	NB	Dauphin R.	5.35 (5.11-5.64)	4.58 (3.84-6.00)	4.97	7.03 (6.36-7.91)	7.69 (4.88-13.35)	7.36
		Grand Rapids	6.26 (6.10-6.45)	4.47 (4.32-4.65)	5.37	7.83 (7.48-8.26)	6.23 (5.87-6.60)	7.03
		Sturgeon Gill	7.01 (6.62-7.53)	4.74 (4.15-5.25)	5.87	8.43 (7.61-9.41)	6.80 (5.83-8.50)	7.61
		Mossy Bay	6.57 (6.33-6.83)	5.81 (5.31-6.41)	6.19	8.75 (8.23-9.34)	9.74 (8.42-11.29)	9.24
LM		Lake Manitoba	2.22 (2.11-2.34)	-0.58 (-4.66-0.69)	0.82	2.90 (2.66-3.12)	1.50 (0.41-1.89)	2.20
WO		Lake Winnipegosis	2.59 (2.27-2.91)			3.39 (2.91-3.80)		
WR		Eaglenest Lake	6.69 (6.04-7.56)	9.33 (7.22-12.53)	8.01	9.54 (8.11-11.74)	11.86 (7.97-17.29)	10.70
		Pointe du Bois	7.03 (6.74-7.37)	9.48 (8.73-10.30)	8.25	11.01 (10.29-11.82)	14.19 (12.70-15.91)	12.60
		Lac du Bonnet	3.35 (2.91-3.70)	1.72 (0.93-2.21)	2.54	8.00 (6.84-9.71)	5.17 (4.37-6.15)	6.59
SR		Saskatchewan River	3.54 (3.00-4.32)			4.94 (3.15-8.17)		
		Cedar Lake	2.78 (1.08-3.59)			6.28 (5.11-8.03)	2.09 (-15.96-28.91)	4.18
UNR		Playgreen Lake	7.12 (5.96-8.68)			10.48 (7.95-14.59)		
		Cross Lake	3.08 (0.60-4.63)			6.74 (3.98-11.78)		
		Sipiwesk Lake	6.20 (5.13-6.98)	4.28 (3.1-5.12)	5.24	10.35 (9.04-12.1)	8.25 (6.91-9.86)	9.30
		Upper Nelson River		5.63 (-0.20-8.50)			13.82 (8.78-24.67)	
		Setting Lake	4.86 (4.66-5.04)	3.53 (3.13-3.85)	4.20	7.86 (7.39-8.34)	7.44 (6.88-8.04)	7.65
LNR		Split Lake	7.83 (7.05-8.77)	9.97 (8.52-11.68)	8.90	12.65 (10.86-14.80)	16.65 (13.85-19.97)	14.65
		Stephens Lake	7.77 (5.51-11.33)	4.22 (2.78-5.42)	6.00	9.64 (5.57-13.64)	8.05 (5.90-11.03)	8.84
UCR		Granville Lake	6.83 (6.32-7.35)	7.20 (6.51-7.96)	7.02	13.05 (11.57-14.78)	12.40 (10.74-14.32)	12.73
		Opachuanau Lake	7.38 (6.52-8.31)	11.90 (10.54-13.70)	9.64	13.28 (11.01-15.97)	19.05 (15.91-23.77)	16.17
		Southern Indian Lake	6.30 (5.51-6.94)	4.59 (2.25-5.86)	5.44	13.06 (11.65-14.81)	10.92 (8.92-13.35)	11.99
CRD		Notigi Lake		4.66 (1.88-6.08)			9.11 (6.91-12.52)	
		Threepoint Lake	5.58 (5.11-6.01)	6.39 (5.60-7.27)	5.99	8.17 (7.30-9.10)	11.91 (9.57-15.02)	10.04
		Footprint Lake	6.64 (1.89-8.22)			10.20 (6.11-30.44)		
		Wuskwatim Lake	10.89 (8.37-15.29)			19.34 (13.21-30.67)		
		Apussigamasi Lake	5.95 (-6.86-30.77)	4.93 (3.97-5.84)	5.44	21.90 (-260.87-325.50)	7.6 (5.81-10.04)	14.75

Table 2.19. Approximate ages (years ±SD) at which 50% (A_{50}) and 90% (A_{90}) of sauger in a sample population will be sexually mature, summarized by Study Region as defined under CAMP protocols (2017). Estimates are derived from to a maturity proportion table set in one year increments.

Study Region	Female Groups	Male Groups	A_{50} female (mm)	A_{50} male (mm)	A_{50} mean (mm)	A_{90} female (mm)	A_{90} male (mm)	A_{90} mean (mm)
Lake Winnipeg	8	8	5.88 (±0.99)	4.38 (±0.74)	5.13 (±0.83)	7.63 (±1.6)	5.43 (±1.13)	6.43 (±1.34)
Red-Assiniboine R.	2	2	4 (±0)	3.5 (±0.71)	3.75 (±0.35)	4.5 (±0.71)	4 (±0)	4.25 (±0.35)
Lake Manitoba	1	1	4	2	3	4	3	3.5
Lake Winnipegosis	1	1	4	2	3	5	2	3.5
Winnipeg River	3	3	6 (±1)	7.33 (±3.06)	6.67 (±1.89)	9.67 (±4.73)	9.67 (±5.03)	9.67 (±4.86)
Saskatchewan River	2	2	5 (±0)	3.5 (±0.71)	4.25 (±0.35)	6 (±1.41)	5 (±0)	5.5 (±0.71)
Upper Nelson River	3	4	5.67 (±0.58)	5 (±0.82)	5.17 (±0.29)	9 (±1)	8.5 (±2.52)	8.17 (±1.04)
Lower Nelson River	2	2	7 (±0)	6.5 (±3.54)	6.75 (±1.77)	11.5 (±0.71)	11.5 (±6.36)	11.5 (±3.54)
Upper Churchill River	3	3	7.33 (±0.58)	9 (±3.61)	8.17 (±1.76)	13 (±3)	13 (±2.65)	13 (±1)
Churchill River Diversion	5	4	7 (±0.63)	6.6 (±1.14)	6.8 (±0.45)	8.5 (±1.29)	10.5 (±2.38)	9.67 (±2.08)

Table 2.20. Approximate ages (years ±SD) at which 50% (A_{50}) and 90% (A_{90}) of sauger in a sample population will be sexually mature, summarized by Study Region as defined under CAMP protocols (2017). Estimates are derived from age-at-maturity logistic regression models. Empty cells indicate that reasonable A_{50} or A_{90} estimates could not be calculated for any population within the Study Region.

Study Region	Female Groups	Male Groups	A_{50} female (mm)	A_{50} male (mm)	A_{50} mean (mm)	A_{90} female (mm)	A_{90} male (mm)	A_{90} mean (mm)
Lake Winnipeg	8	8	5.56 (±0.92)	4.12 (±0.94)	4.84 (±0.90)	6.93 (±1.29)	6.02 (±1.99)	6.48 (±1.59)
Red-Assiniboine R.	0	0						
Lake Manitoba	1	1	2.22	-0.58	0.82	2.90	1.50	2.20
Lake Winnipegosis	1	1	2.59			3.39		
Winnipeg River	3	3	5.69 (±2.03)	6.84 (±4.44)	6.27 (±3.23)	9.52 (±1.51)	10.41 (±4.68)	9.97 (±3.07)
Saskatchewan River	2	2	3.16 (±0.54)			5.61 (±0.95)		
Upper Nelson River	3	2	5.47 (±2.12)	4.96 (±0.95)	5.22	9.19 (±2.12)	11.04 (±3.94)	10.12
Lower Nelson River	2	2	7.80 (±0.04)	7.10 (±4.06)	7.45 (±2.05)	11.14 (±2.13)	12.35 (±6.08)	11.75 (±4.11)
Upper Churchill River	3	3	6.83 (±0.54)	7.90 (±3.71)	7.37 (±2.12)	13.13 (±0.13)	14.12 (±4.33)	13.63 (±4.06)
Churchill River Diversion	4	3	7.27 (±2.46)	5.33 (±0.93)	6.30 (±0.39)	14.90 (±6.73)	9.54 (±2.19)	12.22 (±3.33)

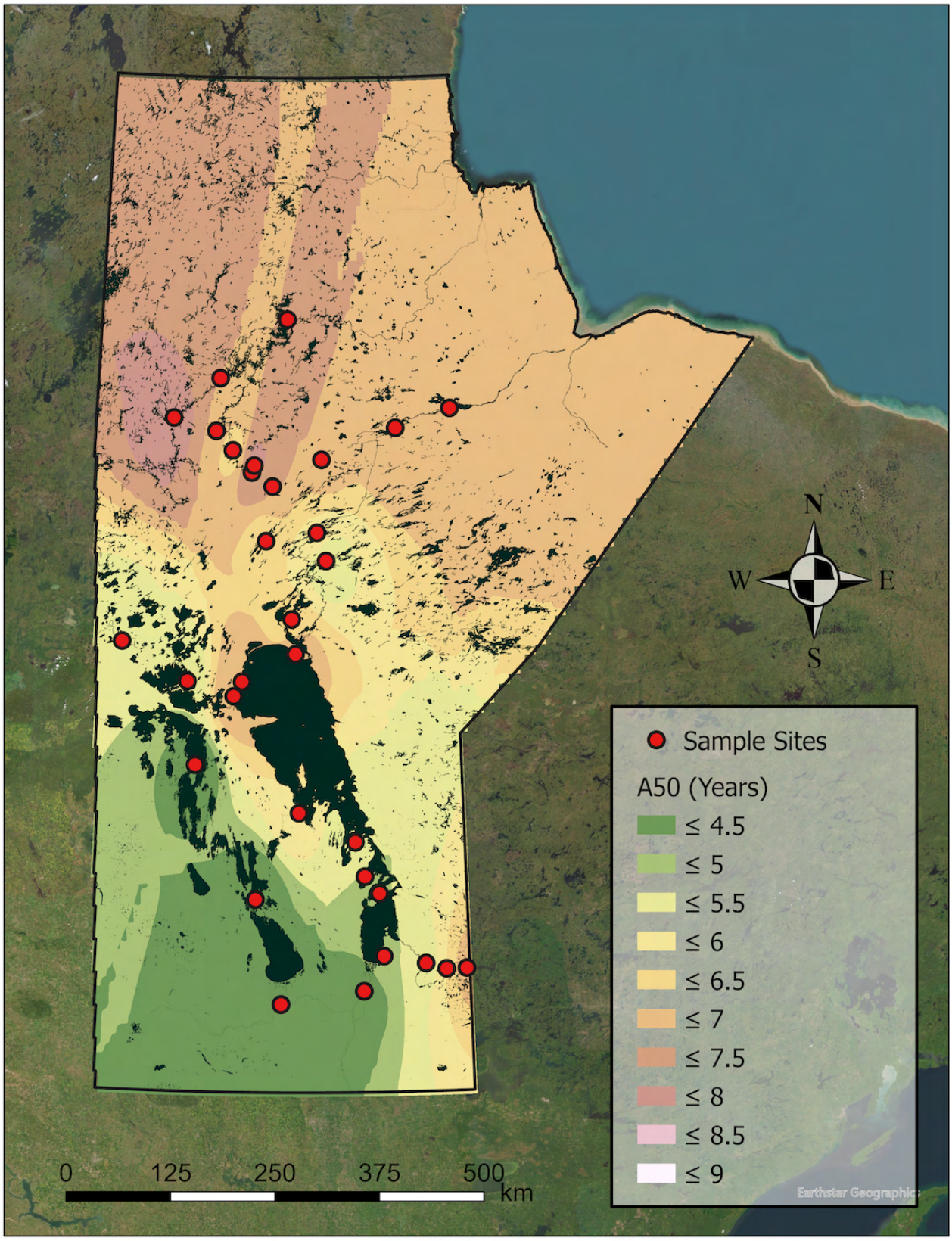


Figure 2.12. Heat map of A_{50} for female sauger in Manitoba waterbodies, as estimated with proportion tables. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.

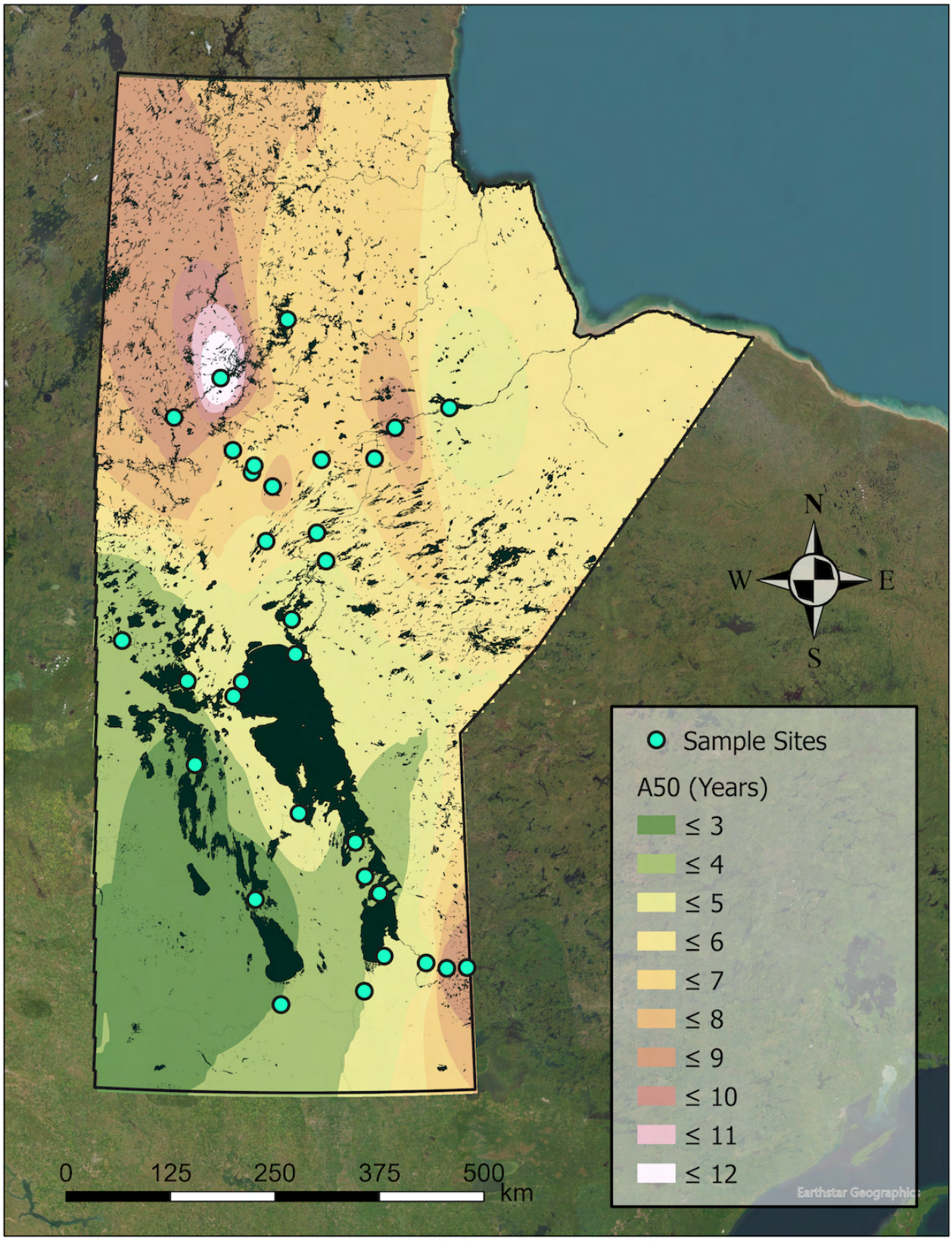


Figure 2.13. Heat map of A_{50} for male sauger in Manitoba waterbodies, as estimated with proportion tables. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.

3.2 Stable Isotope Analysis

Sauger tissues exhibited high $\delta^{13}C$ and $\delta^{15}N$ diversity across isotopic space. Values for $\delta^{13}C$ ranged from -29.7‰ to -22.2‰, with a mean value of -25.7‰ (*SD* = 2.0‰) and a median value of -26.2‰. Mean $\delta^{13}C$ was lowest in Lac du Bonnet (-28.2‰, *SD* = 0.7‰) and highest in Lake Manitoba (-22.9‰, *SD* = 0.2‰). $\delta^{15}N$ values ranged from 9.8‰ to 17.6‰, with mean (*SD* = 1.9‰) and median values of 13.6‰. Mean $\delta^{15}N$ was lowest in Lac du Bonnet (10.9‰, *SD* = 0.7‰) and highest in the Assiniboine River (16.3‰, *SD* = 0.8‰). Within Lake Winnipeg, mean $\delta^{13}C$ values ranged between -27.5‰ (Grand Beach) and -24.8‰ (Dauphin River, Mossy Bay), and mean $\delta^{15}N$ values ranged from 12.5‰ (Mossy Bay) to 15.6‰ (Grand Beach; Appendix 2.I). As latitude increased, sauger tissues from Lake Winnipeg became enriched with ^{13}C and depleted in ^{15}N (Figure 2.14, Figure 2.15; SM 7-4, SM 7-5). Additional summary statistics are presented in Table 2.21 and Appendix 2.J (Lake population level), Table 2.22 and Figure 2.14 (Basin population level), and Appendix 2.I and Figure 2.15 (Site population level).

The Lake Winnipeg Study Region encompassed a particularly broad isotopic niche. Lake Winnipeg sauger occupied a total $\delta^{13}C$–$\delta^{15}N$ area of 19.35‰^{2} and a corrected standard ellipse area (SEAc) of 4.29‰^{2}, as calculated in *SIBER* (Table 2.23, Figure 2.16). In contrast, sauger from other waterbodies covered a combined isotopic area of 6.61‰^{2} and an SEAc of 3.36‰^{2} (Table 2.23, Figure 2.16). All Bayesian standard ellipse area estimates (SEAb) for Lake Winnipeg were larger than SEAbs for other Lake populations at the 95% credibility interval (Figure 2.17). Lake Winnipeg sauger populations also occupied a wider isotopic niche than all other populations at the Basin level. Sauger sampled in Lake Winnipeg's south basin, channel, and north basin yielded $\delta^{13}C$–$\delta^{15}N$ SEAc's of 1.89‰^{2}, 3.81‰^{2}, and 1.50‰^{2}, respectively (Table 2.24, Figure

2.18). The next-largest SEAc—the Red River—covered an area of only 0.81‰^{2} (Table 2.24, Figure 2.18). SEAb estimates for sauger in the Lake Winnipeg channel were always larger than estimates for other waterbodies, and SEAb estimates for Lake Winnipeg's south and north basins only overlapped with sauger populations in the Red River, the Assiniboine River, and Lac du Bonnet at the 95% credibility interval (Figure 2.19). Site level isotopic niche estimates can be found in Appendices 2.K through 2.M.

Most sauger sample populations assessed in this study displayed unique $\delta^{13}C$ and $\delta^{15}N$ isotope distributions. Holm-adjusted Dunn's tests revealed that $\delta^{13}C$ distributions significantly differed ($p < 0.05$) in: 50%, 50%, and 33% of pairwise comparisons at the Lake, Basin, and Site population levels, respectively; and $\delta^{15}N$ distributions differed in 70%, 57%, and 29% of pairwise comparisons at the Lake, Basin, and Site population levels, respectively (SM 7-2). When compared with Wilcoxon rank sum tests, the rate of significant pairwise comparisons increased to 80%, 75%, and 79% for $\delta^{13}C$ distributions, and 90%, 85%, and 78% for $\delta^{15}N$ distributions at the Lake, Basin, and Site population levels (SM 7-3). For both tests, the rate of significant $\delta^{13}C$ and $\delta^{15}N$ comparisons decreased within Study Regions (SM 7-4, SM 7-5). The results of these tests are consistent with the extent of population-wise isotopic niche overlap calculated in *SIBER*. Overlap of the corrected 95% ellipses occurred in 50% of population pairwise comparisons at the Lake level (Table 2.25), 35.7% of comparisons at the Basin level (Table 2.26), and 44.9% of comparisons at the Site level (Appendix 2.N). Of these comparisons, the percentage of overlap between ellipses averaged 16.1% (*SD* = 18.2%), 22.9% (*SD* = 17.4%), and 23.5% (*SD* = 18.2%) at the Lake, Basin, and Site levels, respectively. Isotopic niches of sauger population within Lake Winnipeg Study Region overlapped in all pairwise comparisons at the

Basin level (mean overlap = 24.1%, *SD* = 20.4%) and 93% of comparisons at the Site Level (mean overlap = 28.5%, *SD* = 18.9%). As was observed with independent $\delta^{13}C$ and $\delta^{15}N$ distributions (Figure 2.14, Figure 2.15), isotopic niches in Lake Winnipeg populations shifted along a latitudinal gradient (Figure 2.18, Appendix 2.K). The extent of niche overlap between populations was proportional to geographic distance within Lake Winnipeg, but this was not always the case among other sauger populations. Lac du Bonnet is closest to the Grand Beach sample site in Lake Winnipeg (Figure 1.1), but the isotopic niche of Lac du Bonnet sauger was more similar to sauger sampled at Matheson Island (Appendix 2.K). Assiniboine River sauger were isotopically similar to Red River and Grand Beach sauger and dissimilar to the geographically closer Lundar population (Figure 1.1; Appendix 2.K). That said, the Assiniboine River sample site is closer to the Red River and Grand Beach sites than the Lundar site when measuring by navigable waterway distance (Figure 1.1).

Fourteen sauger tissue samples yielded isotope values outside of their sample population's isotopic niche, as determined with corrected 95% ellipses (Figure 2.16, Figure 2.18, Appendix 2.K). Thirteen of these individuals expressed values closer to another population at the Lake, Basin, or Site level (Table 2.27). Of the eight Lake Winnipeg outliers, one sample was nearest to the Red River isotopic niche, two samples were nearest to the Lac du Bonnet niche, and two samples originated within the Lake Manitoba niche. Red River outliers occupied the Lake Winnipeg or Assiniboine River isotopic niches. Three Lake Manitoba samples had $\delta^{13}C$ and $\delta^{15}N$ values consistent with the Lake Manitoba niche but were outliers in their sample populations at the Basin or Site level. One Lac du Bonnet sample approached the Lac du Bonnet niche at the Lake and Basin levels but occupied the Matheson Island niche at the Site level.

Figure 2.14. Box plots of $\delta^{13}C$ and $\delta^{15}N$ values of sauger dorsal muscle samples, summarized at the Basin population level. Coloured boxes represent the interquartile range, whiskers represent isotope values within 1.5 times the interquartile range, and points represent outliers beyond 1.5 times the interquartile range.

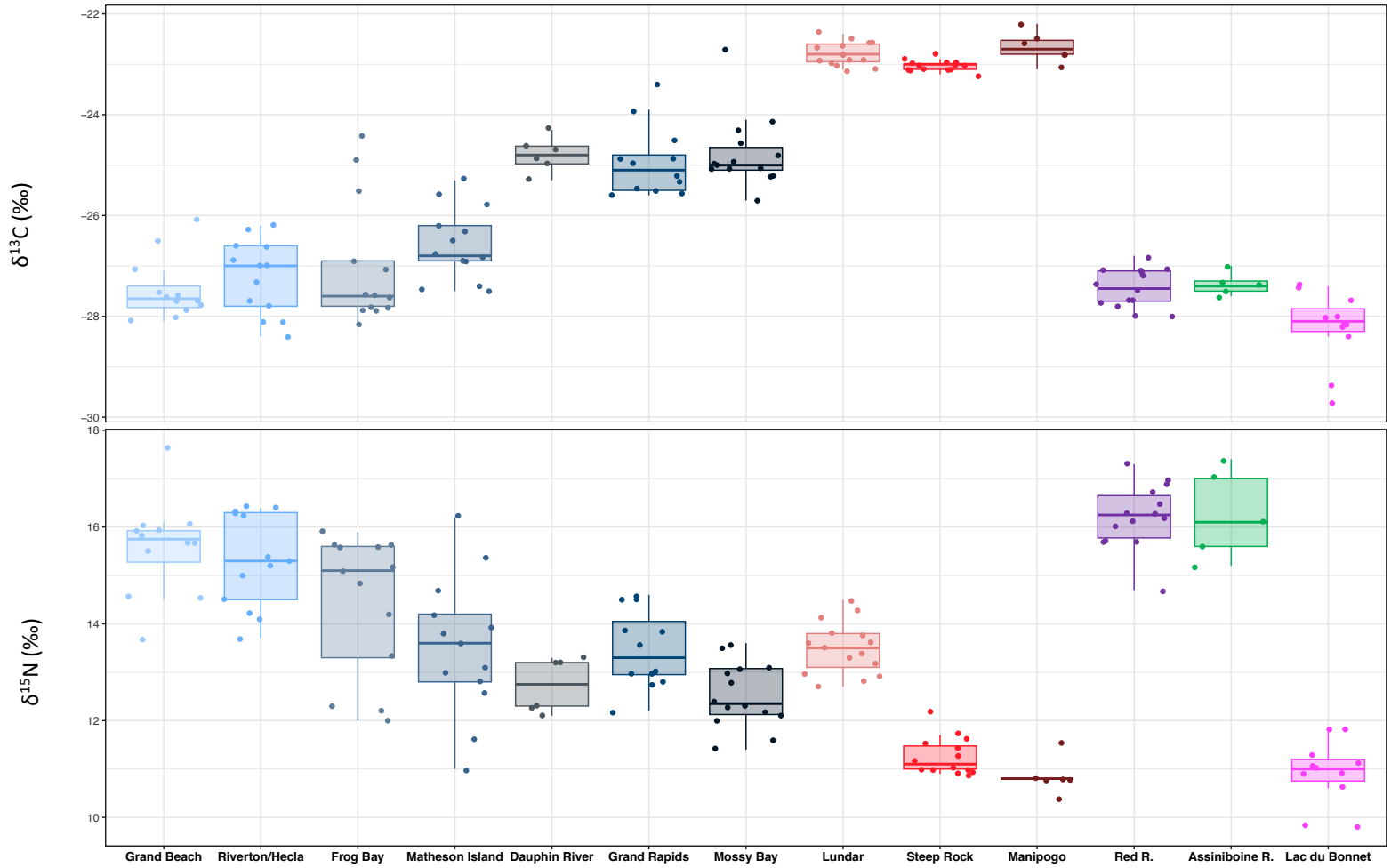


Figure 2.15. Box plots of $\delta^{13}C$ and $\delta^{15}N$ values of sauger dorsal muscle samples, summarized at the Site population level. Coloured boxes represent the interquartile range, whiskers represent isotope values within 1.5 times the interquartile range, and points represent outliers beyond 1.5 times the interquartile range.

Table 2.21. Summary statistics (mean ±SD) for sauger muscle tissue sampled for stable isotope analysis, summarized at the Lake population level.

Lake Population	Samples	Weight (g)	FL (mm)	Age (years)	Prop. Female	Prop. Maturity	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Lake Winnipeg	83	219 (±98)	278 (±47)	5.5 (±2.5)	0.67	0.53	3.2 (±0.1)	-26.2 (±1.4)	14 (±1.5)
Lake Manitoba	35	360 (±167)	322 (±51)	3.4 (±1.5)	0.54	0.89	3.1 (±0.1)	-22.9 (±0.2)	12.1 (±1.3)
Red River	14	283 (±79)	303 (±27)	4.1 (±1.2)	0.86	0.86	3.2 (±0)	-27.4 (±0.4)	16.2 (±0.7)
Assiniboine River	5	138 (±66)	255 (±35)	NA	0.8	0.2	3.1 (±0)	-27.4 (±0.2)	16.3 (±0.8)
Lac du Bonnet	11	171 (±122)	251 (±63)	3.5 (±2.8)	0.82	0.27	3.1 (±0)	-28.2 (±0.7)	10.9 (±0.7)

Table 2.22. Summary statistics (mean ±SD) for sauger muscle tissue sampled for stable isotope analysis, summarized at the Basin population level.

Basin Population	Samples	Weight (g)	FL (mm)	Age (years)	Prop. Female	Prop. Maturity	C:N	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
WPG South	25	216 (±111)	273 (±55)	4.6 (±2.3)	0.88	0.4	3.2 (±0.1)	-27.3 (±0.7)	15.4 (±1)
WPG Channel	26	242 (±111)	289 (±51)	5.7 (±2.7)	0.54	0.73	3.2 (±0)	-26.8 (±1)	14 (±1.5)
WPG North	32	202 (±73)	273 (±35)	6.1 (±2.2)	0.63	0.47	3.2 (±0.1)	-24.8 (±0.6)	12.9 (±0.8)
MB South	15	327 (±228)	301 (±67)	2.5 (±1.6)	0.4	0.73	3.1 (±0.1)	-22.8 (±0.2)	13.5 (±0.5)
MB North	20	385 (±102)	338 (±26)	4.1 (±1.1)	0.65	1	3.1 (±0.1)	-22.9 (±0.2)	11.1 (±0.4)
Red River	14	283 (±79)	303 (±27)	4.1 (±1.2)	0.86	0.86	3.2 (±0)	-27.4 (±0.4)	16.2 (±0.7)
Assiniboine River	5	138 (±66)	255 (±35)	0 (±0)	0.8	0.2	3.1 (±0)	-27.4 (±0.2)	16.3 (±0.8)
Lac du Bonnet	11	171 (±122)	251 (±63)	3.5 (±2.8)	0.82	0.27	3.1 (±0)	-28.2 (±0.7)	10.9 (±0.7)

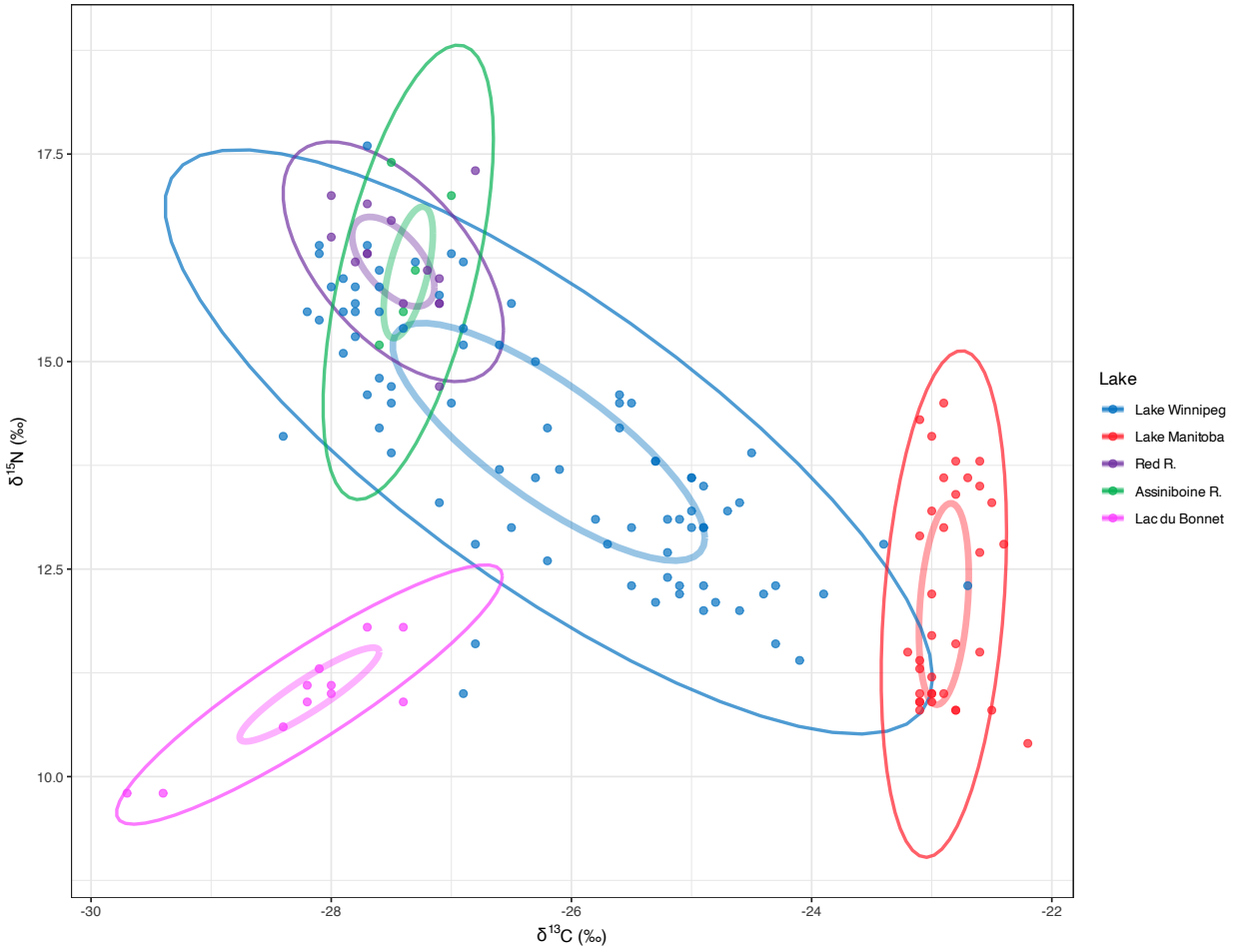


Figure 2.16. Ellipse plot of $\delta^{13}C$ and $\delta^{15}N$ values of sauger dorsal muscle samples, summarized at the Lake population level. Central ellipses represent the standard ellipse area, and outer ellipses represent the 95% ellipse area.

Table 2.23. Estimates of $\delta^{13}C$ x $\delta^{15}N$ niche size ($‰^2$) of sauger dorsal muscle samples at the Lake population level, expressed as total area (TA), standard ellipse area (SEA), and sample size corrected standard ellipse area (SEAc).

	Lake Winnipeg	Lake Manitoba	Red River	Assiniboine River	Lac du Bonnet
TA ($‰^2$)	19.35	2.74	1.74	0.63	1.5
SEA ($‰^2$)	4.23	0.95	0.75	0.6	0.69
SEAc ($‰^2$)	4.29	0.98	0.81	0.8	0.77

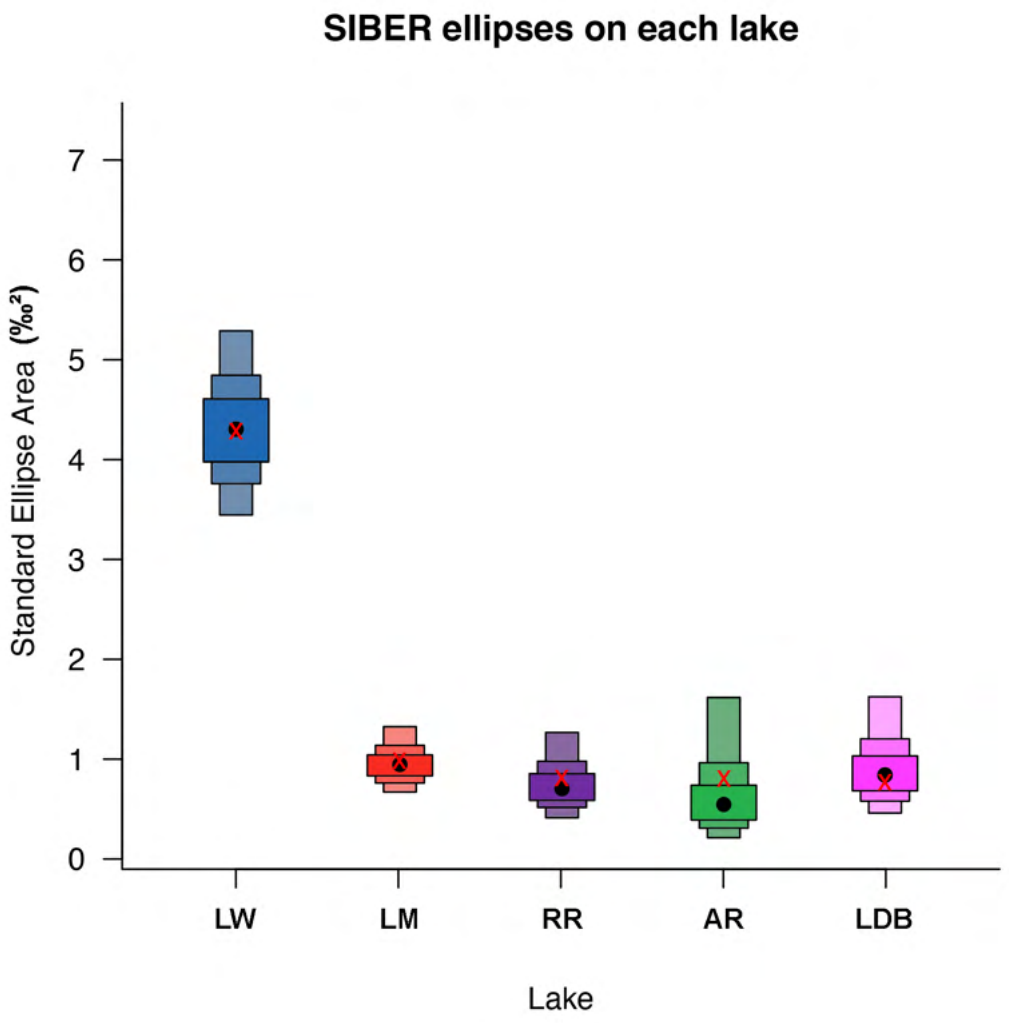


Figure 2.17. Distributions of $\delta^{13}C$ x $\delta^{15}N$ Bayesian standard ellipse area estimates of sauger dorsal muscle tissue, as calculated at the Lake population level. Gradations denote 50%, 75%, and 95% credibility intervals. Point estimates of corrected standard ellipse areas (maximum likelihood) are presented as red crosses. LW = Lake Winnipeg, LM = Lake Manitoba, RR = Red River, AR = Assiniboine River, LDB = Lac du Bonnet.

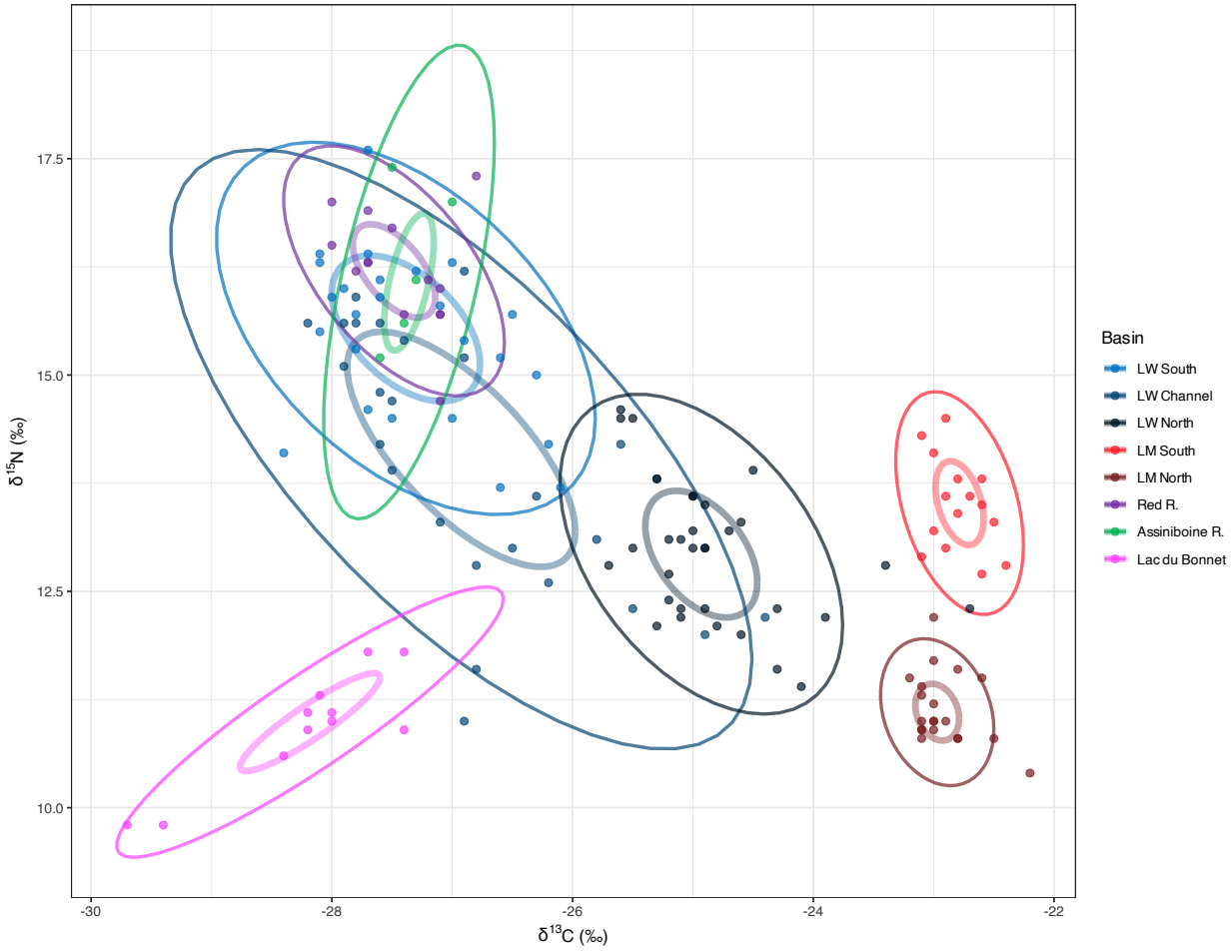


Figure 2.18. Ellipse plot of $\delta^{13}C$ and $\delta^{15}N$ values of sauger dorsal muscle samples, summarized at the Basin population level. Central ellipses represent the standard ellipse area, and outer ellipses represent the 95% ellipse area.

Table 2.24. Estimates of $\delta^{13}C$ x $\delta^{15}N$ niche size ($‰^2$) of sauger dorsal muscle samples at the Basin population level, expressed as total area (TA), standard ellipse area (SEA), and sample size corrected standard ellipse area (SEAc).

	LW South	LW Channel	LW North	LM South	LM North	Red River	Assiniboine R.	Lac du Bonnet
TA ($‰^2$)	5.43	10.16	4.95	0.83	0.84	1.74	0.63	1.5
SEA ($‰^2$)	1.81	3.66	1.45	0.34	0.3	0.75	0.6	0.69
SEAc ($‰^2$)	1.89	3.81	1.5	0.37	0.32	0.81	0.8	0.77

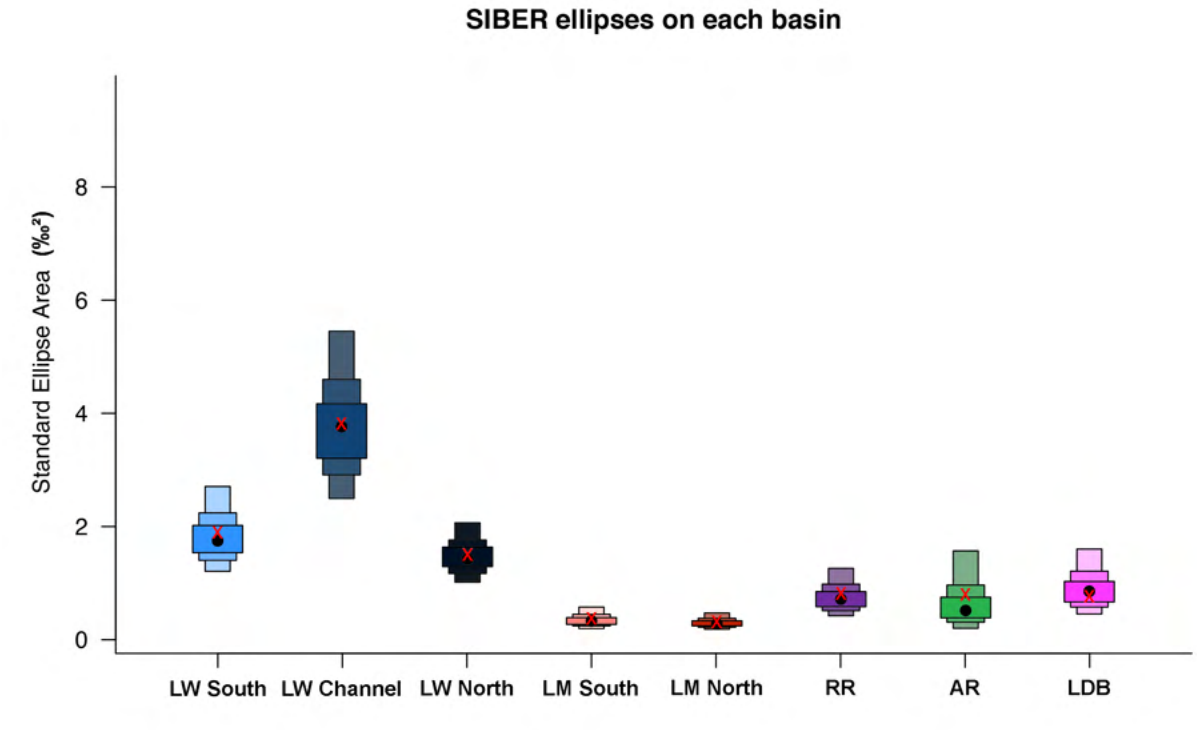


Figure 2.19. Distributions of $\delta^{13}C$ x $\delta^{15}N$ Bayesian standard ellipse area estimates of sauger dorsal muscle tissue, as calculated at the Basin population level. Gradations denote 50%, 75%, and 95% credibility intervals. Point estimates of corrected standard ellipse areas (maximum likelihood) are presented as red crosses. LW = Lake Winnipeg, LM = Lake Manitoba, RR = Red River, AR = Assiniboine River, LDB = Lac du Bonnet.

Table 2.25. Overlap (%) of $\delta^{13}C$ x $\delta^{15}N$ niches of sauger dorsal muscle tissue at the Lake population level, as estimated with sample size corrected standard ellipses (SEAc) and 95% ellipses. Overlap of the SEAc's are presented above the diagonal and overlap of the 95% ellipses are presented below the diagonal.

	Lake Winnipeg	Red River	Assiniboine River	Lake Manitoba	Lac du Bonnet
Lake Winnipeg		0	1.1	0	0
Red R	16.6		45.3	0	0
Assiniboine River	12.8	46.7		0	0
Lake Manitoba	3.5	0	0		0
Lac du Bonnet	1.1	0	0	0	

Table 2.26. Overlap (%) of $\delta^{13}C$ x $\delta^{15}N$ niches of sauger dorsal muscle tissue at the Basin population level, as estimated with sample size corrected standard ellipses (SEAc) and 95% ellipses. Overlap of the SEAc's are presented above the diagonal and overlap of the 95% ellipses are presented below the diagonal.

	LW South	LW Channel	LW North	Red River	Assiniboine R.	LM South	LM North	Lac du Bonnet
LW South		16.8	0	21.7	18.7	0	0	0
LW Channel	45.8		0	0	1.4	0	0	0
LW North	5.2	21.3		0	0	0	0	0
Red River	41.3	18.5	0		45.3	0	0	0
Assiniboine R.	30.4	14.2	0	46.7		0	0	0
LM South	0	0	0	0	0		0	0
LM North	0	0	1.7	0	0	0		0
Lac du Bonnet	0	3.6	0	0	0	0	0	

Table 2.27. Sauger tissue samples with isotopic values outside of their sample population's $\delta^{13}C$–$\delta^{15}N$ isotopic niche, as estimated with sample size corrected 95% ellipses. The closest-fitting isotopic niches for each sample are presented at the Lake, Basin, and Site population levels. Niche assignments that differ from the samples' expected isotopic niche are highlighted in red.

Individual	Sample Site	$\delta^{13}C$	$\delta^{15}N$	Nearest Lake	Nearest Basin	Nearest Site
GB 43	Grand Beach	-27.7	17.6	Red River	Red River	Red River
GB 52	Grand Beach	-26.1	13.7	Lake Winnipeg	LW South	Riverton/Hecla
RV 104	Riverton/Hecla	-28.4	14.1	Lake Winnipeg	LW Channel	Matheson Is.
FB 97	Frog Bay	-24.4	12.2	Lake Winnipeg	LW North	Mossy Bay
MI 34	Matheson Is.	-26.8	11.6	Lac du Bonnet	LW Channel	Matheson Is.
MI 98	Matheson Is.	-26.9	11.0	Lac du Bonnet	Lac du Bonnet	Matheson Is.
GR 3	Grand Rapids	-23.4	12.8	Lake Manitoba	LM South	Grand Rapids
MB 192	Mossy Bay	-22.7	12.3	Lake Manitoba	LM South	Lundar
SR 9	Steep Rock	-23.0	12.2	Lake Manitoba	LM South	Steep Rock
SR 125	Steep Rock	-22.8	11.6	Lake Manitoba	LM North	Manipogo
MP 34	Manipogo	-22.2	10.4	Lake Manitoba	LM North	Manipogo
LR 4	Red River	-26.8	17.3	Assiniboine R.	Assiniboine R.	Assiniboine R.
LR 5	Red River	-27.1	14.7	Lake Winnipeg	LW South	Riverton/Hecla
LB 138	Lac du Bonnet	-27.4	10.9	Lac du Bonnet	Lac du Bonnet	Matheson Is.

2.4. DISCUSSION

2.4.1 Sex Ratios

Most sauger populations in this study appeared to be female biased. I propose several possible mechanisms for this phenomenon. First, the sampling gear used during index netting and CAMP monitoring may preferentially select for females. Index nets for these programs are comprised of mesh sizes of increasing diameter (38 mm to 152 mm) to capture fish of various sizes and body types (EDITNR 2023a). Catch rates for sauger generally peak at the 76 mm mesh, after which only the largest sauger are selected (G. Klein 2023, unpublished data). Since female sauger grow faster than males (Figure 2.2), these larger mesh sizes may be capturing more females and thereby introducing sex bias into the dataset. I summarized the master dataset by mesh size *post hoc* to test this theory and found that female bias persisted to a similar extent at all mesh sizes. That said, most fish in the dataset were sampled before or after peak gonadal maturation, during which females may have a greater weight-at-length than males (Hickman *et al.* 1989); this merits further investigation. Alternatively, female sauger may be more vulnerable to capture during spawning events. Yet, male sauger are thought to be more active during the spawn (Barton and Barry 2011), as has been observed in walleye (Bade *et al.* 2019; Smith *et al.* 2021). Moreover, sex ratios at sauger spawning grounds typically favour males (Ellis and Giles 1965; Scott and Crossman 1973; Bozek *et al.* 2011), making female bias less likely. Recent studies have reported sex-based segregation of walleye during the spring and fall migration events (Bade *et al.* 2019; Smith *et al.* 2021; McKee *et al.* 2022; Schall *et al.* 2023). Male walleye were more likely to emigrate than females in several of these populations (Smith *et al.* 2021; Schall *et al.* 2023) including in South Indian Lake, Manitoba (Bodaly 1980). If this is also the case

in sauger, it could explain the high proportion of males in the Lake Winnipeg Channel and Upper Nelson River (potential migratory corridors), and in Stephens Lake and the Churchill River Diversion (range expansion). Finally, the data may truly reflect female-dominant sauger populations. This explanation is well-supported in literature, as foundational research on sauger populations also report female bias ranging from 55% to 71% (Carlander 1950; Vanicek 1964; Priegal 1969; Nelson 1974). An early study on Lake of the Woods—a waterbody in the Winnipeg River system—reported that 66% of captured sauger were female (Carlander 1950). The cause of female bias in sauger populations is unknown, though higher young-of-year mortality in males is a possible mechanism (Hassler 1958). Thus, the observed variation of sex ratios among waterbodies may be due to intrinsic differences in sauger life histories. Sex ratios may also be altered by selective pressures such as commercial fishing, particularly in depressed stocks. Female sauger are disproportionately caught in Lake Manitoba's winter fishery due to the use of large mesh, which could artificially decrease the proportion of females within the population (G. Klein, personal communication, August 9, 2023). Regardless, variations in sauger sex ratios should be considered as potential differences in sauger life histories.

2.4.2 Growth and Body Condition

Annual sauger growth generally decreased as the latitude of the sample population increased, as visualized with VBGF plots (Figure 2.3) and length-at-age heatmaps (Figure 2.4, Appendix 2.A, Appendix 2.B). However, there is substantial variation in sauger growth that is not explained by latitude. In Lake Winnipeg, sauger growth increased with latitude up until the Dauphin River (Site) before decreasing further into the north basin. Notably, female sauger from Grand Beach (Site) yielded a high *K* growth coefficient but were short at age five, and male

sauger growth was low by both metrics. In Lake Manitoba, five-year-old sauger in the south basin were markedly larger than north basin sauger relative to the change in latitude (Table 2.8). Sauger in Lac du Bonnet returned a higher *K* growth coefficient than other waterbodies in the Winnipeg River but were similar in length by age five.

Unlike growth, trends in sauger body condition do not follow a latitudinal gradient. Condition was more similar among sauger populations within waterbodies and Study Regions, but this relationship was also variable. Condition factor *K* was mostly consistent among sample populations within the Red-Assiniboine River, Winnipeg River, Upper Nelson River, and Churchill River Study Regions. Mean *K* condition was also uniform within Lake Winnipeg except at the Dauphin River site, where mean *K* condition was noticeably higher (Figure 2.9). In contrast, mean *K* condition was very high at the south basin sites in Lake Manitoba ($K = 1.05$) compared to the channel and north basin sites ($K = 0.97$). In the Lower Nelson River Study Region, body condition increased from upstream to downstream.

Previous research on sauger life histories show a strong negative correlation between sauger growth rates and latitude, specifically growing degree days (Colby and Nepszy 1981; Carlander 1997; Braaten and Guy 2002). Fish achieve maximal growth efficiency at their thermal optimum (Beitinger and Fitzpatrick 1979; Jobling 2009), which for sauger is about 22°C (Hokanson 1977; Hasnain *et al.* 2010). These temperatures are less frequent at higher latitudes, and sauger growth decreases as a result (Neuheimer and Taggart 2007; Bozek *et al.* 2011). This phenomenon likely explains the latitudinal trend in sauger growth in Manitoba. Still, there are significant variations in sauger growth that are not explained by latitude. Growth estimates for many sample populations—including the Red River, Assiniboine River, and Upper Nelson River—

may be local outliers due to poor VBGF model fit or small sample sizes (see Table 2.1, Table 2.5, Table 2.8). Sauger growth in other waterbodies may be influenced by prey availability and quality, which can vary according to system productivity (Nelson 1974), population density (Lynch *et al.* 1982; Maceina *et al.* 1998), and competition with sympatric walleye populations (Staggs and Otis 1996; Bellgraph *et al.* 2008; Sheppard *et al.* 2018). This is supported by the body condition results, which show a strong relationship between condition factor *K* and length-at-age-five (Figure 2.4, Figure 2.9). For example, both mean *K* condition and length-at-age-five are highest in Lake Manitoba and Lake Winnipegosis, and lowest in the Churchill River study regions. Likewise, mean *K* condition and length-at-age-five were both higher in Lake Manitoba's south basin than in the north basin and were higher in the Lake Winnipeg channel sites and the Dauphin River site than the rest of Lake Winnipeg. Previous research on Lake Winnipeg sauger also observed reduced body condition in the south basin due to low mesenteric fat levels (Sheppard *et al.* 2018). Despite this, sauger biomass is higher in the south basin than the north basin (Sheppard *et al.* 2018; ECCC and MARD 2020). Moreover, walleye appear to have a nutritive stronghold on the south basin, feeding on energy-dense emerald shiners (*Notropis atherinoides*) compared to the trout-perch (*Percopsis omiscomaycus*) typically consumed by sauger (Sheppard *et al.* 2018).

I must note that the growth and condition estimates I have presented are influenced by the date at which sauger were sampled. Sauger populations sampled in the fall experienced a longer growing period than populations sampled in the summer, which would skew growth estimates upward. Similarly, post-spawn sauger would likely weigh less than pre-spawn sauger or fish sampled later in the fall (Bozek *et al.* 2011). That said, I am confident that the broad-scale

trends I have described are not influenced by sampling date. For example, four-year-old sauger in Lake Manitoba and Lake Winnipegosis were still longer than five-year-old sauger in every other population (SM 2-5, SM 2-6), and body condition was lowest in the Churchill River system despite most sampling occurring in late fall. Nevertheless, these factors should be considered in future analyses, either with models such as seasonally-adjusted von Bertalanffy functions (Cloern and Nichols 1978; Somers 1988), or with added sampling regimes at different times of the year.

2.4.3 Maturity

Spatial trends in length-at-maturity differed between female and male sauger. For females, L_{50} was approximately 300 mm throughout Lake Winnipeg, Lake Manitoba, Lake Winnipegosis, and most of the Nelson River, and closer to 275 mm in the Red and Assiniboine rivers. Female L_{50} was also about 300 mm in Eaglenest Lake and Pointe du Bois in the Winnipeg River, but shorter in Lac du Bonnet. Most populations (Lake) within the Churchill River system yielded L_{50} estimates in the 250 mm range. For male sauger, L_{50} was in the 240 to 260 mm range in the Red and Assiniboine Rivers, the Upper Nelson and most of Lake Winnipeg, though estimates were slightly higher at the Dauphin River site. In contrast, male sauger in Lake Manitoba and Lake Winnipegosis achieved a 50% maturity rate before reaching 200 mm. L_{50} was only slightly larger in the Saskatchewan River system. Male L_{50} also approached 200 mm in Lac du Bonnet yet exceeded 300 mm in the upper portions of the Winnipeg River Study Region (Table 2.13). L_{50} also surpassed 300 mm in Split Lake, which was significantly larger than other estimates within the Nelson River system. Male L_{50} estimates were variable within the Upper Churchill River and Churchill River Diversion regions but were generally equal to or higher than

female L_{50} estimates. I could not find any exhaustive reviews on sauger length-at-maturity outside of Manitoba, but the range of L_{50} estimates in this study were within the range of L_{50} estimates in populations outside of Manitoba (Nelson 1974; Bozek *et al.* 2011; Hartman *et al.* 2019; Pritt *et al.* 2019). Factors influencing sauger length-at-maturity are poorly understood, but larger body sizes are likely selected in females to maximize egg capacity and overall fecundity (Bozak *et al.* 2011; G. Klein 2023, unpublished data).

Age-at-maturity estimates generally increased with latitude for both sexes. A_{50} increased with rising latitude in the Nelson River system and in Lake Winnipeg, though males sampled in the Lake Winnipeg channel did mature earlier than males sampled in the south basin (Table 2.18). This latitudinal trend seems to extend up into the Churchill River study regions, albeit with less variation between the sexes—males matured as late or later than females (Table 2.18). A_{50} was large in Eaglenest Lake and Pointe du Bois, with males maturing much later than in Lac du Bonnet and other neighbouring sample populations. As with growth, age-at-maturity is known to increase with latitude and growing degree days. This suggests that sexual maturity is driven by body size, wherein sauger will mature at a later age in order to achieve a higher total length (Geoff Klein 2023, unpublished data).

As with growth and condition estimates, spatial trends in length- and age- at maturity are skewed by the sample date of each population. Specifically, an “immature” sauger captured in the spring may have become a “mature” sauger if it was sampled in the fall. Sexual maturation in sauger becomes noticeable by midsummer, during which spermatogenic activity increases and vitellogenic oocytes begin to develop (Barton and Barry 2011; G. Klein, personal communication, August 9, 2023). To account for this seasonal discrepancy, I pushed age-at-

maturity estimates of sample populations forward one year if fish were sampled in August or later. However, this correction falsely assumes that all individuals in the A_{50} cohort would have been identified as "immature" prior to August 1, thus skewing A_{50} estimates upward. Conversely, length-at-maturity estimates were uncorrected and did not account for seasonal growth of mature individuals sampled in the fall, or the sampling of "mature" individuals in the fall that were maturing for their first spawn the following year. The first variable would skew L_{50} estimates upward, and the latter would skew L_{50} estimates downward; I am unsure which factor would have a greater effect on L_{50} estimates. Nevertheless, these sampling biases should not have a major effect on the broader spatial trends observed. For example, A_{50} was lowest in Lake Manitoba and Lake Winnipegosis and highest in the Upper Churchill River whether or not a seasonal correction was applied (Table 2.19; Table 2.20). Similarly, L_{50} was smallest in males in Lake Manitoba and Lake Winnipegosis, and smallest in females within the Churchill River system, even after an extra year worth of growth was added to these estimates (Table 2.15; Table 2.16). A_{50} and L_{50} were also much lower in Lac du Bonnet than in other Winnipeg River waterbodies regardless if the correction was applied (Table 2.13; Table 2.17). Therefore, all of the largest differences in A_{50} and L_{50} among sample populations should be considered biologically relevant irrespective of sampling date.

2.4.4 Stable Isotope Analysis

There was virtually no isotopic overlap between Lake Winnipeg, Lake Manitoba, and Lac du Bonnet sauger, and individuals from these populations were easily identified in isotopic space (Figure 2.16). Isotopic niches for sauger from the Red River and Assiniboine River were mostly similar to the south basin of Lake Winnipeg, but there was no niche overlap between the

Red and Assiniboine rivers and the north basin. At the Basin population level, there was strong separation between the south and north basins in Lake Manitoba, and between the south and north basins in Lake Winnipeg. In Lake Winnipeg, sauger tissues became enriched in ^{13}C and depleted in ^{15}N with increasing latitude. This latitudinal trend has been observed in other fish species in Lake Winnipeg (Ofukany *et al.* 2014) and has been directly attributed to inter-basin variation in baseline ^{13}C and ^{15}N ratios (Hobson *et al.* 2012). Hobson *et al.* (2012) partially credited these inter-basin variations to the extensive blue-green algae blooms in Lake Winnipeg's north basin (ECCC 2020 and MARD 2020), which lower $\delta^{15}N$ values and reduce algal fractionation of ^{13}C (Hobson *et al.* 2012). I would also suggest that $\delta^{15}N$ values are initially enriched in the south basin by Red River discharge, which is high in $^{15}N_{NO3}$ (Mayer and Wassenaar 2012), then depleted by other riverine inputs as water moves northward. This would explain the isotopic overlap between sauger in the south basin of Lake Winnipeg and the Red and Assiniboine Rivers. Conversely, differences in isotopic niches between waterbodies are likely due to differences in the $\delta^{13}C$ and $\delta^{15}N$ values of their respective carbon and nitrogen sources (Chipps and Garvey 2007).

I identified thirteen sauger that could be recent migrants based on their isotopic signatures (Table 2.27). I would deem the migratory statuses of the individuals sampled in the Red River, Grand Beach, and Riverton/Hecla as inconclusive due to the high isotopic overlap among these sample populations. The same applies for any sauger from the south or north basins of Lake Winnipeg that could be re-assigned to the channel (basin) population. The isotopic niche for sauger sampled in the Lake Winnipeg channel is broad, and it is difficult to know if this is due solely to resident sauger populations or by the added presence of fish

migrating between the north and south basins. The latter is supported by ridgeline plots I generated *post hoc*, which showed a bimodal distribution with isotope values resembling the south basin, and to a lesser extent, the north basin. Inter-basin migration may also occur in Lake Manitoba, as one sauger yielded $\delta^{13}C$ and $\delta^{15}N$ values more compatible with the opposite basin from which it was sampled. Most notably, two sauger sampled from Lake Winnipeg were nearest to the Lac du Bonnet niche, and two samples originated within the Lake Manitoba niche. Using models developed by Thomas and Crowther (2014) and Vander Zanden *et al.* (2015), I estimate that sauger muscle tissue would experience 50% turnover of ^{13}C and ^{15}N after two to three months of somatic growth. Given the date these fish were sampled (June) and the lack of isotopic turnover observed in the samples, we can infer that these fish migrated from the Winnipeg River and Lake Manitoba into Lake Winnipeg in the spring.

Note: Applications of these data patterns and findings to fisheries management are discussed in Chapter 4 of this thesis.

Chapter 3. Applied population genetics in the management of a freshwater percid, *Sander canadensis*, in Manitoba, Canada

ABSTRACT

Sauger (*Sander canadensis*) populations in Manitoba are declining, and fisheries managers are determined to implement a sauger management plan in response. However, there is limited understanding of the genetic health and structure of sauger populations in Manitoba. In this study, I used twelve microsatellite loci to resolve genetic diversity and structure of sauger (n = 872) across nine Manitoba waterbodies in the Nelson-Churchill watershed. Five waterbodies contained walleye–sauger hybrids (n = 13), though hybridization rates were otherwise low. Genetic diversity was highest in Lake Winnipeg and lowest in Lake Manitoba and Lake Winnipegosis, but inbreeding coefficients were low at all sample sites. Effective population size (N_e) was highest in Lake Winnipeg and was also high in Stephens Lake. Genetic divergence was moderate between most waterbodies, but low between Lake Winnipeg and the Red River, Assiniboine River, Lac du Bonnet, and Stephens Lake (F_{st} = 0.007–0.015). I could not resolve genetic stock structure within Lake Winnipeg, but there were genetic signatures from sauger populations in neighbouring waterbodies. I identified four broad genetic sauger stocks: Lake Winnipeg; Lake Manitoba and Lake Winnipegosis; the Red and Assiniboine Rivers; and the Churchill and Saskatchewan Rivers. Sauger in Lake Manitoba and Lake Winnipegosis were genetically indistinguishable. Gene flow between Lake Winnipeg and Lake Manitoba stocks is minimal. These findings will assist managers in defining management units based on genetic structure and selecting surrogate stocks for future stocking programs.

3.1. INTRODUCTION

Fisheries management is undergoing a critical paradigm shift (Beamish and Rothschild 2009). Decades of rising commercial pressure and fisheries collapses have forced managers to reconsider the validity of simple, deterministic management models (Caddy 1999; Travis *et al.* 2014). Through years of trial and error, fisheries boards now recommend a precautionary, ecosystem-based approach to fisheries management (Hilborn *et al.* 2001; Hilborn 2012; King *et al.* 2015). Stock assessments have perhaps seen the greatest reappraisal with this shift (Begg *et al.* 1999; Maunder and Punt 2013). Classic stock assessment models—which use commercial landing data, measures of abundance and life history characteristics to describe the state of a fish population (Methot 2009)—are often violated in real-life scenarios (Travis *et al.* 2014; Maunder and Piner 2015), highlighting their poor representation of complex ecological processes. To combat this issue, managers have begun taking a more holistic approach to stock assessment by integrating factors such as climate change (Bryndum-Buchholz *et al.* 2021), habitat trends (Brown *et al.* 2018) and ecosystem dynamics (Mangel and Levin 2005; Townsend *et al.* 2019) into their assessment models. As such, there is a constant search for new classes of data that can improve the accuracy and predictive power of stock assessments in a practical, cost-effective manner (Degnbol *et al.* 2006; Cowan *et al.* 2012; Maunder and Punt 2013; Lorenzen *et al.* 2016).

With advances in molecular technology, population genetics is now an accessible and versatile tool for assessing fish stocks (Ferguson 1994; Ward 2000; Chistiakov *et al.* 2006). Nuclear microsatellites (simple sequence repeats, SSRs; short tandem repeats, STRs) are especially popular in population genetics research due their high mutation rate and abundance

in non-coding portions of the genome (Jarne and Lagoda 1996; Chistiakov *et al.* 2006). This ensures that each study locus is highly polymorphic and that allelic variation between populations is due to reproductive isolation rather than selective pressures (Oliveira *et al.* 2006). Microsatellites are commonly used to delineate genetically distinct fish stocks (Abdul-Muneer 2014), which can aid in defining biologically relevant stock management units (Palsbøll *et al.* 2007). Similarly, microsatellite-derived measures of historic and contemporary gene flow are valuable in identifying important migratory pathways and minimizing habitat fragmentation (Stepien *et al.* 2009; Heilveil and Stockwell 2017). Microsatellite studies have played a central role in popularizing the 'conservation genetics' concept, wherein population genetics principles are applied in conservation efforts to maximize their success (Frankham *et al.* 2010). Managers now recognize that small fish populations are vulnerable to genetic threats such as inbreeding depression, introgressive hybridization, and an overall loss of evolutionary potential (Ryman 1991; Hallerman 2003). Thus, special care is taken to maximize genetic diversity, minimize inbreeding depression and exclude introgressed individuals in captive breeding and stocking programs (Vrijenhoek 1998; Frankham *et al.* 2010).

Although population genetics research is demonstrably useful in informing fisheries management decisions, it is rarely used as a routine management tool (Vernesi *et al.* 2008; Holderegger *et al.* 2019). Fisheries managers often cite high project costs, a lack of genetics experience, and skepticism that genetics research is actionable as reasons for not using genetic data in fisheries management (Taylor *et al.* 2017; Holderegger *et al.* 2019). Moreover, rapid advances in genomic technologies have led some to believe that genetics studies using fewer molecular markers are obsolete (Ouborg *et al.* 2010; Allendorf 2017). These assumptions could

not be further from the truth. Classic population genetic analyses can be an informative, cost-effective stock assessment tool with actionable management implications (Hodel *et al.* 2016; Puckett 2017). This chapter serves as a case study to demonstrate how populations genetics can address many fisheries management objectives from a single dataset.

The sauger (*Sander canadensis*) is a freshwater percid fish found throughout the Nelson-Churchill watershed in Manitoba, Canada (Scott and Crossman 1973; Stewart and Watkinson 2004). Sauger once rivalled walleye (*Sander vitreus*) and whitefish (*Coregonus clupeaformis*) as a commercial species in the Nelson-Churchill watershed, including Manitoba's Great Lake (MGL) fisheries: Lake Winnipeg, Lake Manitoba, and Lake Winnipegosis (Franzin *et al.* 2003; Nicholson 2007). In the present day, these sauger stocks are in variable states of decline. In Lake Winnipeg, commercial sauger production has decreased over 90 percent from historical highs, and annual mortality has now reached unsustainable levels (ECCC and MARD 2020). Sauger stocks in Lake Manitoba are commercially collapsed, with production plummeting from excesses of 500,000 kg in the 1980s to under 5000 kg by the end of the century (Lysack 1997; Kroeker 2012). Sauger are effectively extirpated from Lake Winnipegosis (Lysack 2006), and only one sauger has been captured in index netting surveys over the past decade (NRND, unpublished dataset). Provincial managers and independent consultants have both concluded that a sauger-specific management plan is necessary to prevent a permanent collapse of commercial sauger stocks in Manitoba (Task Force 2011; AOFRC 2020; ECCC and MARD 2020; G. Klein, personal communication, September 16, 2020).

Recent attempts by the province to develop a sauger management plan have been hindered by several knowledge gaps. First, it is unknown if sauger in each lake comprise a

genetically homogenous metapopulation or a series of distinct, reproductively isolated breeding populations. While Manitoba's index netting survey dataset is robust and facilitates accurate cohort tracking, the gillnets serve as a 'catch-all' for MGL species and are set independent of spawning location (LWQRTF 2011; K. Casper, personal communication, June 2, 2020). It is therefore uncertain whether observed phenotypic variations within and between MGL waterbodies (see Chapter 2) are due to environmental factors or by the presence of separate stocks with unique life histories. If the latter is true, stocks with larger fish or lower productivity are at a greater risk of depletion under current commercial regulations (Manitoba Fishery Regulations 1987).

Second, the effects of habitat alteration and fragmentation on provincial sauger stocks are poorly understood. The Nelson-Churchill drainage basin is disrupted by sixteen hydroelectric generating stations, most of which act as complete barriers to upstream fish passage (Manitoba Hydro 2015). As sauger are primarily a riverine species, these barriers have likely fragmented sauger populations (Bozek *et al.* 2011a; Jonagan 2022). The generating stations may also restrict sauger spawning migrations, which are known to exceed 300 km in other river systems (Bozek *et al.* 2011b). Some commercial fishers assert that a water control structure on the Fairford River caused the demise of Lake Manitoba and Lake Winnipegosis sauger stocks (LMRRAC 2003; Lysack 2006), arguing that sauger moving downstream to spawn in Lake Winnipeg are unable to return upstream. Although the presence of a fish ladder makes this argument unlikely (Katopodis 1992), such claims cannot be discredited without a better understanding of sauger movement across MGL waterbodies.

A key objective of the provincial sauger management plan is to reintroduce sauger in Lake Winnipegosis through a dedicated hatchery program. Similar stocking efforts have seen the resurgence of walleye stocks in Lake Winnipegosis (MARD 2021a) and eastern Manitoba (D. Kroeker, personal communication, September 23, 2020), and fisheries staff are eager to replicate this success with sauger. Managers have narrowed down a list of surrogate stock candidates but have requested a genetic assessment of these stocks before finalizing their decision. Ideally, the donor population should display a high degree of genetic variation and be acclimated to an ecosystem resembling Lake Winnipegosis. These criteria will improve survival rates by minimizing inbreeding depression within the hatchery (Kincaid 1983) and reducing young-of-year mortality and outbreeding depression outside of it (Frankham *et al.* 2011; Huff *et al.* 2011). However, I recommend that provincial managers also assess the risk of introgressive hybridization between sauger and walleye before committing to a sauger stocking program. Sauger and walleye can produce hybrid "saugeye" in instances of high turbidity and spawning period overlap (Billington and Sloss 2011), a phenomenon supplemental stocking has been shown to exacerbate (Fiss *et al.* 1997; Koigi 2004; White *et al.* 2005). Interspecific hybridization has contributed to fish extinctions in North America (Miller *et al.* 1989; Todesco *et al.* 2016) and is thought to have played a role in the extirpation of sauger from the Laurentian Great Lakes (Regier *et al.* 1969; Johnston 1977). Fishers from Lake Winnipegosis also describe a period in the late 1970s when they struggled to distinguish sauger from walleye (G. Parker, personal communication, November 5, 2021), which may indicate that sauger in Lake Winnipegosis experienced a similar fate. If sauger are especially vulnerable to introgression in Lake

Winnipegosis, managers should identify the root causes of these sauger-walleye interactions and devise possible steps to minimize them.

In this study, I used microsatellites to address gaps in the body of knowledge for Manitoba sauger that conventional stock assessments have not resolved. Over 850 sauger were sampled across nine waterbodies and genotyped at twelve microsatellite loci to assess the genetic diversity, health, and structure of sauger populations in the Nelson-Churchill watershed. First, sauger genotypes were screened for walleye alleles to assess the relative occurrence of natural introgression in Manitoba waterbodies. Then, the status of sauger in each waterbody was described with summary statistics, inbreeding coefficients, and N_e (effective population size) estimates. Population structure was resolved using differentiation statistics, clustering algorithms and ordination methods. Bayesian algorithms were used to estimate contemporary and historic gene flow, with a disequilibrium-based approach taken to estimate recent migration. The relationships between spatial distance, geographic barriers, and population divergence were explored with landscape genetics analyses. Findings will greatly improve provincial managers' understanding of sauger stocks in Manitoba and advance the development of their sauger management plan. It is my hope that this study will demonstrate the relevance of population genetics in fisheries management and embolden fisheries managers to integrate population genetics into their own stock assessment framework.

3.2. MATERIALS AND METHODS

3.2.1 Sample Collection

Sauger (n = 872) and walleye (n = 68) were collected across nine Manitoba waterbodies (Figure 1.1) between 2020 and 2022 by gillnetting or rod-and-reel angling. Historic Lake Winnipegosis scale samples used in this study were collected as aging structures in 1980 and 1981 by provincial fisheries staff. Coordinates are exact for collection sites sampled by me or by staff from the Manitoba Fisheries Branch and North/South Consultants, and approximated for sites sampled by commercial fishers (Table 3.1; Table 3.2).

Fish provided from index netting and Coordinated Aquatic Monitoring Programs (CAMP) had already been lethally sampled for sex and maturity determination as well as for aging structures. These fish were often in contact with each other during field sampling, so I elected to use liver tissue for genetic samples to minimize the risk of cross-contamination. Liver tissue was collected from each fish and preserved in 95% ethanol stored at -20°C. Genomic DNA was extracted from liver tissue with MicroGEM's *prep*GEM Universal reagent kit using the manufacturer's Solid Tissue protocol. DNA was extracted from historic scale samples at a separate lab bench using the phenol-free lysis buffer method described by Li *et al.* (2013). DNA yield was determined with a NanoDrop™ One Spectrophotometer (Thermo Scientific) and standardized to 50 ng/μL with deionized water. DNA quality was assessed on a 1% agarose gel.

Table 3.1. Summary genetic statistics for sauger populations in Manitoba, as defined at the Lake population level. N_A = number of alleles; N_{PA} = number of private alleles; A_R = mean allelic richness; H_O = mean observed heterozygosity; H_E = mean expected heterozygosity; F_{ST} = fixation index; F_{IS} = inbreeding coefficient; $\bar{r}_d$ = standardized index of association.

	Sampling Location	Latitude	Longitude	Samples	N_A	N_{PA}	*A_R	H_O	H_E	F_{ST}	F_{IS}	$\bar{r}_d$
	Lake Winnipeg	52.9319	-98.1064	321	199	29	6.79	0.68	0.68	0.015 (-0.010-0.038)	0.010 (-0.018-0.026)	0.0039
	Red River	50.0857	-96.9409	46	122	1	6.24	0.60	0.63	0.092 (0.039-0.168)	0.029 (-0.009-0.111)	-0.0100
	Assiniboine River	49.9476	-98.3279	31	110	1	6.36	0.68	0.67	0.029 (-0.045-0.073)	-0.007 (-0.055-0.054)	0.0344
	Lake Manitoba	51.1459	-98.8078	190	158	10	6.32	0.67	0.68	0.022 (-0.021-0.070)	0.006 (-0.018-0.035)	0.0106
	Lake Winnipegosis	52.6793	-99.8683	145	148	5	6.21	0.68	0.68	0.023 (-0.018-0.069)	0.004 (-0.031-0.042)	0.0083
	Lac du Bonnet	50.3748	-95.8932	25	108	2	6.73	0.68	0.69	0.012 (-0.088-0.08)	-0.013 (-0.070-0.084)	0.0123
	Cedar Lake	53.4194	-100.0648	30	111	1	6.35	0.59	0.64	0.078 (0.03-0.137)	0.143 (0.032-0.152)	0.0024
	Stephens Lake	56.3756	-95.0978	28	114	3	7.12	0.68	0.69	0.007 (-0.037-0.051)	0.056 (-0.057-0.086)	0.0331
	South Indian Lake	57.3679	-98.2872	24	98	3	6.61	0.66	0.65	0.062 (0.013-0.128)	-0.009 (-0.048-0.030)	0.0292

*Calculated from 22 rarefied samples

Table 3.2. Summary genetic statistics for sauger populations in Manitoba, as defined at the Site population level. N_A = number of alleles; N_{PA} = number of private alleles; A_R = mean allelic richness; H_O = mean observed heterozygosity; H_E = mean expected heterozygosity; F_{ST} = fixation index; F_{IS} = inbreeding coefficient; $\bar{r}_d$ = standardized index of association.

L/B/S	Sampling Location	Latitude	Longitude	Samples	N_A	N_{PA}	*A_R	H_O	H_E	F_{ST}	F_{IS}	$\bar{r}_d$
LW SB	Grand Beach	50.4616	-96.5980	48	125	0	6.58	0.67	0.68	0.027 (-0.011-0.062)	0.015 (-0.042-0.067)	0.0009
LW SB	Riverton/Hecla	51.1368	-96.6538	50	132	1	6.63	0.69	0.66	0.042 (0.013-0.08)	-0.025 (-0.066--0.007)	-0.0110
LW CH	Frog Bay	51.3204	-96.8958	48	125	3	6.64	0.7	0.69	0.012 (-0.01-0.034)	-0.032 (-0.054-0.008)	0.0027
LW CH	Matheson Is.	51.6938	-97.0511	47	125	4	6.52	0.66	0.67	0.032 (-0.006-0.073)	0.019 (-0.022-0.076)	-0.0057
LW NB	Dauphin R.	52.0116	-98.0400	17	95	0	6.77	0.67	0.69	0.012 (-0.025-0.05)	0.025 (-0.030-0.090)	0.0402
LW NB	Poplar R.	53.0140	-97.3685	31	130	6	7.28	0.7	0.72	-0.032 (-0.11-0.011)	0.010 (-0.045-0.069)	0.0347
LW NB	Grand Rapids	53.2697	-99.2243	49	135	1	6.79	0.67	0.68	0.017 (-0.007-0.039)	0.049 (-0.017-0.068)	0.0044
LW NB	Mossy Bay	53.7326	-98.1026	31	118	4	6.70	0.71	0.69	0.01 (-0.036-0.039)	-0.002 (-0.098-0.040)	-0.0191
RR	Red R.	50.0857	-96.9409	46	122	1	6.24	0.6	0.63	0.092 (0.039-0.168)	0.029 (-0.009-0.111)	-0.0096
AR	Assiniboine R.	49.9476	-98.3279	31	110	1	6.36	0.68	0.67	0.029 (-0.045-0.073)	-0.007 (-0.055-0.054)	0.0344
LM SB	Whitemud	50.2940	-98.5346	31	107	2	6.52	0.67	0.68	0.022 (-0.012-0.071)	0.057 (-0.031-0.075)	0.0253
LM SB	St. Laurent	50.3609	-98.0382	30	104	2	6.23	0.69	0.68	0.022 (-0.001-0.047)	-0.017 (-0.059-0.037)	0.0046
LM SB	Lundar	50.6653	-98.2371	16	83	0	6.18	0.71	0.66	0.043 (-0.008-0.096)	-0.070 (-0.154-0.017)	0.0059
LM NW	Narrows	51.1459	-98.8078	48	111	2	6.21	0.66	0.68	0.02 (-0.005-0.053)	0.024 (-0.013-0.078)	0.0103
LM NB	Steep Rock	51.4367	-98.8196	50	124	1	6.43	0.67	0.69	0.01 (-0.025-0.043)	0.012 (-0.026-0.076)	0.0096
LM NB	Manipogo	51.5890	-99.5399	15	85	1	6.39	0.67	0.67	0.04 (-0.013-0.101)	-0.013 (-0.066-0.044)	0.0429
WO LOW	Lower Winnipegosis	51.6691	-99.8449	88	136	0	6.24	0.68	0.68	0.025 (-0.008-0.06)	0.003 (-0.050-0.055)	0.0090
WO MID	Mid Winnipegosis	52.6691	-99.9930	57	122	3	6.14	0.67	0.68	0.024 (-0.017-0.061)	0.007 (-0.037-0.060)	0.0013
LDB	Lac du Bonnet	50.3748	-95.8932	25	108	2	6.73	0.68	0.69	0.012 (-0.088-0.08)	-0.013 (-0.070-0.084)	0.0123
CED	Cedar Lake	53.4194	-100.0648	30	111	1	6.35	0.59	0.64	0.078 (0.03-0.137)	0.143 (0.032-0.152)	0.0024
ST	Stephens L.	56.3756	-95.0978	28	114	3	7.12	0.68	0.69	0.007 (-0.037-0.051)	0.056 (-0.057-0.086)	0.0331
SIL	South Indian L.	57.3679	-98.2872	24	98	3	6.62	0.66	0.65	0.062 (0.013-0.128)	-0.009 (-0.048-0.030)	0.0292

*Calculated from 22 rarefied samples

3.2.2 Molecular Protocol

3.2.2.1 Microsatellite Amplification and Genotyping

Sixteen percid-specific microsatellite loci previously used in sauger studies were screened for amplification and reproducibility (Table 3.3; *Svi* L10, Wirth *et al.* 1999; *Pfla L1*, Leclerc *et al.* 2000; YP113, Li *et al.* 2007; *MSL-1*, Kohlmann and Kersten 2008). Forward primers were fluorescently labeled (HEX, 6-FAM, PET or NED; Applied Biosystems) at the 5'-end, and a 'PIG' tail (Brownstein *et al.* 1996) was added to the 3'-end of the reverse primer to regulate adenylation. Singleplexes were performed for each locus in a 15 μL reaction containing 1x PCR buffer, 2 mM $MgCl_2$, 0.2 mM each dNTP, 0.5U Hot Start *Taq* DNA polymerase (Thermo Fisher), 0.1–0.5 μM of each forward and reverse primer, 50 ng genomic DNA, and deionized water. Samples were denatured at 95°C for 2 min, followed by 35 cycles of denaturation (95°C, 30 s), annealing (45–62°C, 30 s) and extension (95°C, 1 min), and a final extension of 72°C for 10 min. Reactions were run over a range of primer concentrations and annealing temperatures and screened on a 3% agarose gel to optimize PCR conditions. *Svi* L10 and *MSL-1* weakly amplified with Hot Start *Taq* polymerase and did not amplify with high-fidelity, proofreading *Taq* polymerase (Thermo Fisher). The *Pfla L1* and YP113 loci yielded irreproducible stuttering. Consequently, *Svi* L10, *MSL-1*, *Pfla L1* and YP113 were removed from the study.

Multiplexes of the 12 selected microsatellites were designed in MultiplexManager (Holleley and Geerts 2009) and experimentally optimized by varying reagent and primer concentrations (Table 3.3). Optimized multiplexes were performed in a 15 μL reaction with the same reagent concentrations and PCR cycles described for the singleplex reactions, and PCR products were stored at -20°C prior to genotyping. PCR products were diluted 5x with deionized

water, and 2 μL of the dilution was mixed with 13 μL of HiDi formamide (Applied Biosystems) and 0.2 μL of GeneScan 500 LIZ size standard (Applied Biosystems). Samples were then denatured at 95°C for 5 min and chilled on ice for another 5 min. The denatured products were electrophoretically separated with a 3730xl capillary sequencer (Thermo Fisher; TCAG, Toronto) and electropherogram outputs were scored with the Microsatellite Analysis application in Thermo Fisher Cloud. Reproducibility of allelic data was confirmed by re-amplifying and re-scoring the loci of five sauger from each collection site (22 sites; n = 110).

Table 3.3. Summary of PCR multiplex parameters and microsatellite characteristics. Multiplex 1 was amplified with two separate PCR reactions whose products were then pooled (1a = 15 μL, 1b = 7 μL) into one sample.

Locus	Multiplex #	Fluorescent Label	Primer conc. (μM)	Annealing Temp (°C)	Repeat motif	Reference
***Svi* L6**	1a	HEX	0.15	46	$(AC)_{17}$	Wirth *et al.* 1999
Svi18	1b	NED	0.10	55	$(AC)_{11}$	Borer *et al.* 1999
YP41*	1b	NED	0.15	55	$(TCTT)_{11}$	Li *et al.* 2007
MSL-2****	1b	6-FAM	0.30	55	2/4 bp complex	Kohlmann and Kersten 2008
YP60*	1a	HEX	0.40	46	$(AGAA)_{10}$	Li *et al.* 2007
Svi33	2	PET	0.20	57	$(AC)_{14}$	Borer *et al.* 1999
Svi4*	2	HEX	0.20	57	$(AC)_{16}$	Borer *et al.* 1999
Svi20**	2	6-FAM	0.20	57	$(AC)_{20}$	Eldridge 2002
Svi2*	2	HEX	0.40	57	$(AC)_{18}$	Eldridge 2002
***Svi* L8**	3	PET	0.40	52	$(TG)_{22}$	Wirth *et al.* 1999
Svi26	3	NED	0.25	52	2 bp complex	Eldridge 2002
Svi7*	3	HEX	0.30	52	$(AC)_{14}$	Eldridge 2002

*Diagnostic for sauger/walleye **Not PIG-tailed (Brownstein *et al.* 1996) due to primer dimerization

3.2.2.2 Quality Control

Allele scores generated within the Microsatellite Analysis application were visually verified and then tested for stuttering and scoring errors with Micro-Checker (ver. 2.2.3; van Oosterhout *et al.* 2004). Individuals missing data at four or more loci were removed from the sample pool. The remaining percentage of missing data at each locus per collection site was visualized with the ***info_table*** function in *poppr* (ver. 2.9.3; Kamvar *et al.* 2014).

To assess the individual exclusion power of this study's microsatellite panel, I calculated the probability of identity (P_{ID}) and probability of identity of siblings (P_{IDsib}) with GenAlEx (ver. 6.503; Peakall and Smouse 2012) and generated a genotype accumulation curve with ***genotype_curve*** (*poppr*).

3.2.3 Genetic Analysis

3.2.3.1 Defining Populations for Genetic Analyses

Many population genetic analyses rely on population-wise allele frequencies. To conduct these analyses, researchers must define populations based on *a priori* knowledge or *a posteriori* clustering models. As there is evidence of sauger stock structure within and among Manitoba waterbodies (see Magsino 2011 and Chapter 2 of this thesis), I will predefine populations according to Lake, Basin, and collection site (Site). I will also report results for genetic groups inferred with the clustering methods described in methods section *3.2.3.4*. Thus, all population-specific analyses described in the methods below were performed on populations defined at the Lake, Basin, Site, and genetic cluster levels.

3.2.3.2 Introgression with Walleye

Two Bayesian clustering programs, STRUCTURE (ver. 2.3.4; Pritchard *et al.* 2000) and NEWHYBRIDS (ver. 1.1 beta; Anderson and Thompson 2002), were used to screen for introgression between sauger and walleye. All twelve microsatellite loci were considered in this screening process. First, the statistical performance and optimal *q*-value threshold of each program was evaluated by simulating hybrid individuals of known admixture. Sauger and walleye genotypes were analyzed using an initial run in NEWHYBRIDS (see settings below) and filtered for fish with a > 0.995 probability of being a pure sauger (n = 671) or walleye (n = 40). Allele frequencies from this refined dataset were then used to simulate 500 of each parent species, 100 F_1 hybrids, 100 F_2 hybrids, and 100 of each F_1 x parent backcross in HYBRIDLAB (ver. 1.1; Nielsen *et al.* 2006). Simulated individuals were analyzed in STRUCTURE and NEWHYBRIDS (see settings below) to test each program's performance with the following measures:

1. Efficiency (*E*): the proportion of simulated hybrids correctly identified as hybrids (Vähä and Primmer 2006)
2. Accuracy (*A*): the proportion of identified hybrids that were truly simulated hybrids (Vähä and Primmer 2006)
3. Type I error (α): the proportion of simulated purebreds incorrectly identified as hybrids.

The optimal *q*-value threshold for each program was identified with the equation:

$$(E*A*(1-\alpha))$$

with the *q*-value producing the largest number selected as the class assignment threshold.

The complete genotype dataset was then re-run through STRUCTURE and NEWHYBRIDS. For STRUCTURE, I used an admixture model with K = 2 to represent the parent species, with 10 runs of 100,000 burn-ins followed by 100,000 retained replicates each. For NEWHYBRIDS, I assigned Jeffreys-like priors for the mixing proportions and allele frequencies and specified six hybrid categories: pure sauger; pure walleye; F_1; F_2 (F_1 x F_1); BC_1 (F_1 x pure sauger); and BC_2 (F_1 x pure walleye). NEWHYBRIDS was run 10 times with 10,000 burn-ins followed by 50,000 MCMC sweeps. For each program, mean *q*-values from the 10 runs were calculated and compared to the optimal *q*-value thresholds established in the simulation experiment. Sauger with *q*-values below the thresholds of either program were considered introgressed and were removed from downstream analysis.

3.2.3.3 Assumption Checking and Summary Statistics

All loci and each locus per population were tested for Hardy-Weinberg equilibrium (HWE) conformance with a χ^2 goodness-of-fit test and Guo and Thompson's (1992) exact test with Monte Carlo sampling (B = 1000), as implemented in the ***hw.test()*** function from *pegas* (Paradis 2010; Table 3.3). Pairwise linkage disequilibrium (LD) was tested for each locus and locus per population with ***pair.ia*** (*poppr*) and Genepop (ver. 4.7.5; Rousset 2008). Putative locations for each locus within the sauger genome were determined by aligning primer sequences to a congeneric Pikeperch genome (*Sander lucioperca*; GenBank accession: GCA_008315115.2) with BLAST (NCBI). Loci found within 5 Mb of each other on the same chromosome were deemed more susceptible to linkage disequilibrium (Mohlke *et al.* 2001; Slatkin 2008; Rexroad and Vallejo 2009).

Allele rarefaction curves were generated for each population with ADZE-1.0 (Szpiech *et al.* 2008) to estimate allelic sampling coverage. Summary statistics were calculated for each locus and each locus per population with ***basic.stats*** (*hierfstat*; Goudet 2005), ***summary*** (*adegenet*; Jombart 2008) and ***locus_table*** (*poppr*) (Table 3.1; Table 3.2), and allele frequencies and private alleles were compiled with ***popgenreport*** (*PopGenReport*; Adamack and Gruber 2014). Mean inbreeding ($\bar{F}$) was estimated for individuals with the ***inbreeding*** function in *adegenet* and F_{IS} was generated for each population along with a 95% confidence interval (nboot = 1000) with ***boot.ppfis*** in *hierfstat*. Effective population size (N_e) was estimated for each population in NeEstimator (ver. 2.1; Do *et al.* 2014) using Waples' (2006) linkage disequilibrium method with a minimum allele frequency (P_{crit}) of 0.02.

3.2.3.4 Genetic Differentiation and Population Structure

Population structure was assessed with genetic differentiation and distance indices, Bayesian clustering and an ordination approach. F-statistic estimates (*sensu* Weir and Cockerham 1984) were calculated for each locus and each population across loci with the ***wc*** function in *hierfstat* and supported with confidence intervals generated with ***boot.vc*** (nboot = 1000). Pairwise population F_{ST} estimates and 95% confidence intervals were generated with ***boot.ppfst*** (nboot = 1000) in *hierfstat*; confidence intervals containing only positive F_{ST} values indicate significant genetic differentiation between a population pair. To account for computational biases associated with Wright's F-statistics (Pearse and Crandall 2004; Jost *et al.* 2018; Alcala and Rosenberg 2019), estimates and confidence intervals were also calculated for Nei's (1973) G_{ST}, Hedrick's (2005) G''_{ST}, ϕ'_{ST} (Meirmans and Hedrick 2011), and Jost's D (Jost 2008) with the ***diff_stats*** and ***summarise_bootstrap*** functions in *mmod* (Winter 2012).

To visualize the evolutionary relationship between each population, I generated neighbour-joining trees with Edwards' (1971) genetic distance with bootstrapped support (***aboot***, sample = 1000; *poppr*). The resulting distance matrices were then used to compute the proportion and significance of genetic variation at each population stratum (Lake, Basin, Site) with an analysis of molecular variance (AMOVA; ***poppr.amova***, method = "pegas"; *poppr*) followed by the random permutation test described by Excoffier *et al.* (1992; ***randtest***; *poppr*).

I inferred the number of genetic sauger stocks (*K*) sampled within the study area with STRUCTURE (ver. 2.3.4; Pritchard *et al.* 2000) and a discriminant analysis of principal components (DAPC; Jombart *et al.* 2010). STRUCTURE was run using an admixture ancestry model with no population or location priors, with *K-values* ranging from 1 (panmixia) to 22 (number of sample sites). Each *K-value* was assessed with 10 trials of 100,000 burn-ins and 500,000 MCMC replications. STRUCTURE outputs were collated with Structure Harvester (ver. 0.6.94; Earl and vonHoldt 2012) and Evanno *et al.*'s (2005) ΔK statistic was used to identify the likeliest number of *K* groups. Results from the replicate trials were combined and visualized with CLUMPAK (Kopelman *et al.* 2015). DAPCs were run for *a priori* population clusters (Lake, Basin, or Site) as well as *a posteriori* genetic clusters derived with K-means clustering as implemented in *adegenet*. The optimal *K-value* for location-independent clusters was determined with the ***find.clusters*** function, wherein I retained the *K-value* positioned within the elbow of the BIC curve for the DAPC. The number of principal components (PCs) retained for each DAPC was validated with the ***xvalDapc*** function in *pegas* (n.pca.max = 200, n.rep = 1000). Relationships between clusters were displayed using a scatter plot with an integrated minimum spanning tree, and individual membership probabilities derived from the DAPCs were visualized with

compoplot (*adegenet*). The relative contributions of alleles to each discriminant function were plotted with the ***loadingplot*** function (*adegenet*).

3.2.3.5 Gene Flow and Divergence Mechanisms

Contemporary migration rates among populations were estimated in BayesAss (ver. 3.04; Wilson and Rannala 2003) using 10 runs of 10 million iterations (including 1 million burn-in iterations) and a sampling interval of 100. Mixing parameters were initialized at Δm = 0.05, Δa = 0.30 and Δf = 0.2 to maintain acceptance rates between 20 and 60 percent, which is considered optimal for MCMC mixing (Wilson and Rannala 2003). I then verified MCMC convergence for each run by plotting trace file outputs in the R software environment with a script adapted from Meirmans (2014). The run with the lowest Bayesian deviance was considered the best fitting model (Faubet *et al.* 2007; Meirmans 2014).

Next, I used geospatial methods to examine potential interactions between population divergence and the geography of the study area. I tested the isolation by distance (IBD) hypothesis with a Mantel test (***mantel.randtest***; *ade4* (Dufour 2007)) consisting of an Edwards' genetic distance matrix and a Euclidean geographic distance matrix. These matrices were then visualized using scatterplots with a kernel density surface overlay (***kde2d***; *MASS* (Venables and Ripley 2002), wherein clustering within the plot would suggest the existence of additional geographic barriers. Finally, I drew geographic lines between neighbouring sauger populations with high genetic differentiation using Monmonier's algorithm (Monmonier 1973), as implemented in *adegenet* (***optimize.monmonier***, ***monmonier***). These lines were overlaid on topographical and hydrological maps (Manitoba Government *b*) to identify possible geographic barriers to gene flow.

3.3. RESULTS

3.3.1 Quality Control

Assembled sauger genotypes were accurate and robust as demonstrated by allele score reproducibility (see Appendix 3.A for an example of an electropherogram output file). Of the 110 sauger samples re-amplified and re-scored, 102 (92.7%) matched the alleles of the initial samples across all 12 loci. Of the eight individuals that did not have a 100% allele match, two exhibited inter-well contamination in multiplex 3 (confirmed with a third PCR run), two had different alleles at the *MSL-2* locus, and four individuals expressed a second allele at the YP60 locus where only one had been previously amplified.

Assessment of the genetic data in Micro-Checker revealed no evidence of large allele dropout and only one significant result for scoring error due to stuttering (YP41; Lake Winnipeg, North Basin). However, there was significant evidence for null alleles at the Lake, Basin, and Site level, particularly at the YP60 locus. Instances of null alleles outside of the YP60 locus were sporadic and there were no other obvious trends across loci or populations (see Appendix 3.B).

Nineteen sauger and eight walleye were missing data at four or more loci and were consequently removed from the dataset. Data loss among the remaining 853 sauger and 60 walleye averaged 1.99% and 2.36% across loci, respectively, with data loss being most prevalent at the YP60 and Svi2 loci (Figure 3.1).

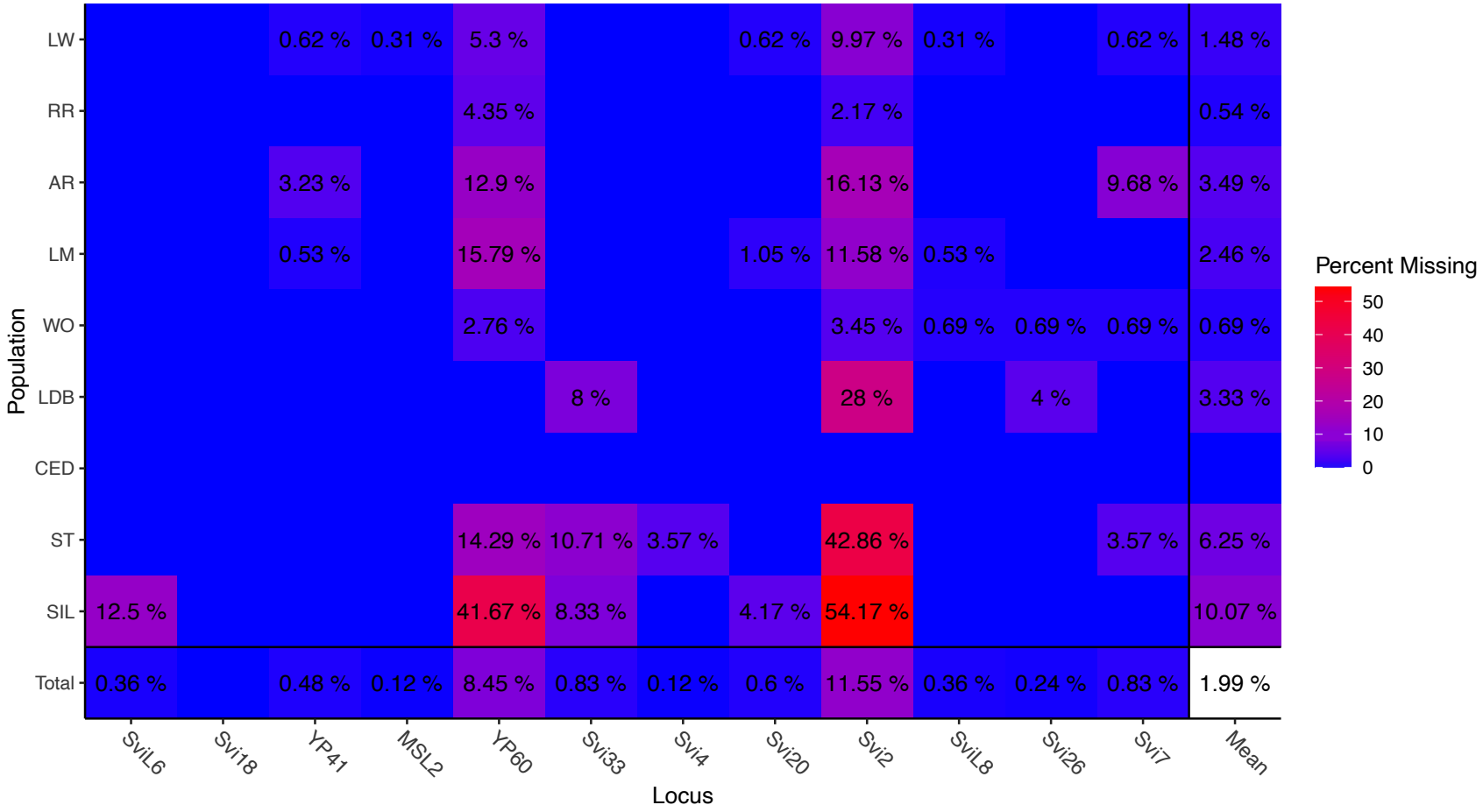


Figure 3.1. Frequencies of missing allelic data per locus per Lake. Missing data is defined as the absence of alleles at a locus.

Individual genotypes in this study could be differentiated with a high degree of certainty. The genotype accumulation curve showed complete separation of individual genotypes when any nine loci were sampled (Figure 3.2). Mean estimates of P_{ID} and P_{IDsib} were similar across population strata (Lake: P_{ID} = 2.2E-12, P_{IDsib} = 5.8E-05; Basin: P_{ID} = 1.6E-12, P_{IDsib} = 5.3E-05; Site: P_{ID} = 1.8E-12, P_{IDsib} = 5.3E-05). These estimates are equivalent to a 1-in-457 billion to 1-in-629 billion chance of unrelated individuals having an identical multilocus genotype, or a 1-in-17,000 to 1-in-19,000 chance of siblings sharing the same multilocus genotype.

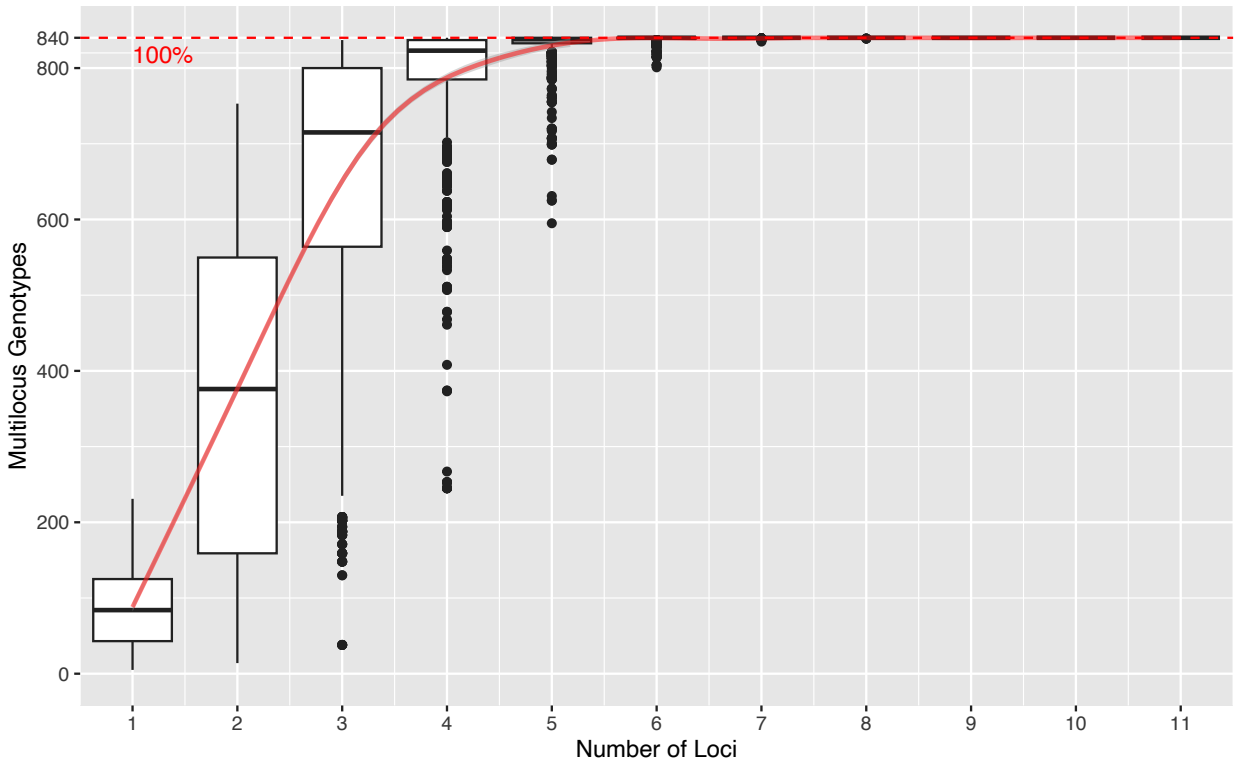


Figure 3.2. A genotype accumulation curve of 840 sauger genotypes. Distributions for each number of loci were generated by randomly sampling combinations of loci (n = 1000).

3.2 Genetic Analysis

3.2.1 Introgression with Walleye

Simulated sauger-walleye hybrids were efficiently and accurately differentiated from purebred individuals in STRUCTURE and NEWHYBRIDS. In STRUCTURE, simulated purebred sauger and walleye expressed *q*-values ranging from 0.956 to 0.995 (mean = 0.993; *SD* = 0.003) and 0.975 to 0.995 (mean = 0.992, *SD* = 0.002), respectively. For introgressed fish, sauger-specific *q*-values ranged from 0.387 to 0.632 among F_1 hybrids (mean = 0.495, *SD* = 0.063), 0.202 to 0.746 among F_2 hybrids (mean = 0.490, *SD* = 0.108), 0.520 to 0.945 among sauger backcrosses (mean = 0.743, *SD* = 0.082), and 0.090 to 0.442 among walleye backcrosses (mean = 0.256, *SD* = 0.087). Thus, all simulated individuals were correctly assigned as either purebred or hybrids in STRUCTURE using a *q*-value threshold (*Tq*) of 0.95 (Table 3.4).

Simulated individuals were also reliably assigned as purebreds or hybrids in NEWHYBRIDS, though performance decreased when assigning introgressed individuals to specific hybrid classes. Using a *Tq* of 0.5, all simulated sauger (range = 0.804-1.000, mean = 0.999, *SD* = 0.009) and walleye (range = 0.950-1.000, mean = 0.998, *SD* = 0.003) were correctly assigned as purebreds, and 396 of the 400 introgressed individuals were assigned into any of the four hybrid categories; one hybrid was misclassified as a pure sauger and three hybrids did not meet the *q*-value threshold for any classification. Moreover, all first generation saugeye were successfully classified as F_1 hybrids (range = 0.587-0.995, mean = 0.948, *SD* = 0.052). NEWHYBRIDS was less accurate when distinguishing between second generation hybrids, as sixteen F_2 hybrids were misassigned as F_1 hybrids (1), backcrossed walleye (11) or backcrossed

sauger (4); five backcrossed sauger were misassigned as F_1 hybrids (1), F_2 hybrids (2) or pure sauger (1); and four backcrossed walleye were misassigned as F_1 (2) or F_2 (2) hybrids (Table 3.5).

Of the 853 sauger and 60 walleye genotypes tested for introgression in STRUCTURE, seventeen sauger (2.0%) and two walleye (3.3%) were identified as hybrids (*q*-value > 0.95). However, four of the eighteen putative sauger hybrids were barely below the *q*-value threshold in STRUCTURE (0.944, 0.941, 0.941, 0.939) and were assigned as pure sauger in NEWHYBRIDS; these individuals were retained in the study as pure sauger. One field-assigned sauger (WO8094) and one field-assigned walleye (wallWPG-8) were confirmed to be the opposite species in STRUCTURE in NEWHYBRIDS and were re-assigned accordingly. In total, 13 of 853 sauger (1.5%) and two of 60 walleye (3.3%) were classified as introgressed fish (Table 3.6) and removed from the dataset; 840 sauger were retained for downstream analyses.

Table 3.4. Measures of the performance of STRUCTURE in assigning simulated purebred sauger (n = 500) and walleye (n = 500) and walleye-sauger hybrids (F_1 = 100; F_2 = 100; BC_{sauger} = 100; $BC_{walleye}$ = 100) to their respective classes at different *q*-value thresholds. Compound performance is defined by the equation ($E^*A^*(1-\alpha)$).

	Percentage of correct assignment (pure vs. hybrid)						Performance Index			
***q*-value**	**Pure Sauger**	**Pure Walleye**	**F_1 Hybrids**	**F_2 Hybrids**	**Backcross Sauger**	**Backcross Walleye**	**Efficiency (*E*)**	**Accuracy (*A*)**	**Type I error (α)**	**Compound performance**
0.96	99.6	100	100	100	100	100	1.000	0.995	0.002	0.993
0.95	100	100	100	100	100	100	1.000	1.000	0	**1.000**
0.94	100	100	100	100	98	100	0.995	1.000	0	0.995
0.93	100	100	100	100	98	100	0.995	1.000	0	0.995
0.9	100	100	100	100	97	96	0.9825	1.000	0	0.983
0.85	100	100	100	100	91	86	0.8825	1.000	0	0.883

Table 3.5. Measures of the performance of NEWHYBRIDS in assigning simulated purebred sauger (n = 500) and walleye (n = 500) and walleye-sauger hybrids (F_1 = 100; F_2 = 100; BC_{sauger} = 100; $BC_{walleye}$ = 100) to their respective classes at different *q*-value thresholds. Compound performance is defined by the equation ($E^*A^*(1-\alpha)$).

	Percentage of correct assignment (exact purebred or hybrid class)						Performance Index			
***q*-value**	**Pure Sauger**	**Pure Walleye**	**F_1 Hybrids**	**F_2 Hybrids**	**Backcross Sauger**	**Backcross Walleye**	**Efficiency (*E*)**	**Accuracy (*A*)**	**Type I error (α)**	**Compound performance**
0.9	99.8	100	90	73	63	63	0.608	1.000	0	0.608
0.8	100	100	99	77	84	82	0.888	1.000	0	0.888
0.7	100	100	99	77	87	89	0.915	1.000	0	0.915
0.6	100	100	99	79	91	94	0.945	1.000	0	0.945
0.5	100	100	100	83	95	95	0.99	1.000	0	0.99

Table 3.6. Individuals that were classified as introgressed or misidentified in STRUCTURE (*Tq* = 0.95) and NEWHYBRIDS (*Tq* = 0.5), and their likeliest hybrid status as assigned in NEWHYBRIDS.

Individual	Site	Field Assignment	STRUCTURE	NEWHYBRIDS Assignment		Genetic Assignment
GB176	Grand Beach (LW)	Sauger	0.424	F_1	0.894	F_1 saugeye
GB216	Grand Beach (LW)	Sauger	0.5003	F_1	0.926	F_1 saugeye
FB51	Frog Bay (LW)	Sauger	0.8505	F_2	0.248	Unassigned hybrid
FB92-2021	Frog Bay (LW)	Sauger	0.6404	F_2	0.856	F_2 saugeye
MI47	Matheson Island (LW)	Sauger	0.8669	BCsauger	0.564	Sauger backcross
DR291	Dauphin River (LW)	Sauger	0.8926	BCsauger	0.423	Unassigned hybrid
LR42	Red River	Sauger	0.7137	BCsauger	0.688	Sauger backcross
LR50	Red River	Sauger	0.716	BCsauger	0.522	Sauger backcross
WO8116	Lower Winnipegosis	Sauger	0.408	F_2	1	F_2 saugeye
WO8094	Lower Winnipegosis	Sauger	0.002	Walleye	1	Walleye
WO8097	Middle Winnipegosis	Sauger	0.8646	F_2	0.237	Unassigned hybrid
WO8128	Middle Winnipegosis	Sauger	0.9451	BCsauger	0.944	Sauger backcross
LDB-127	Lac du Bonnet	Sauger	0.8485	BCsauger	0.475	Unassigned hybrid
ST-41	Stephens Lake	Sauger	0.8871	BCsauger	0.704	Sauger backcross
wallWPG-8	Poplar River (LW)	Walleye	0.0207	Sauger	0.994	Sauger
wallWO-7	Lower Winnipegosis	Walleye	0.4508	F_2	0.999	F_2 saugeye
wallSP-4	Stephens Lake	Walleye	0.4809	F_1	0.974	F_1 saugeye

3.3.2.2 Assumption Checking and Summary Statistics

All loci assessed in this study were polymorphic and contained between 3 and 35 alleles across all samples (mean = 18.9, *SD* = 10.4; Table 3.7). Eight of the twelve microsatellite loci observed in this study did not conform to Hardy-Weinberg Equilibrium (HWE) expectations when tested across all individuals (Table 3.7). However, the incidence of HWE non-conformance generally decreased when individuals were partitioned into increasingly specific sample populations (Table 3.8; Appendix 3.C). No loci were in HWE across populations using a χ^2 test, and three loci significantly differed within 25 percent or more populations at the Basin level (4/14 populations; Svi7, Svi26, *MSL-2*). Only Svi33 and *Svi* L6 were in HWE within all populations according to an exact test, and only YP60 violated HWE assumptions in 25 percent or more populations (Lake: 3/9 populations; Basin: 4/14 populations). No loci exceeded a non-conformance threshold of 25 percent at any population level following a Bonferroni correction, and no locus consistently deviated from HWE expectations across populations and population strata (Appendix 3.C). Therefore, all twelve loci were retained for analyses assuming HWE of loci.

Table 3.7. Summary genetic statistics and divergence indices for the microsatellite loci used in this study. Loci were tested for Hardy-Weinberg equilibrium (HWE) conformance with a χ2 goodness-of-fit test and Guo and Thompson's (1992) exact test with Monte Carlo sampling (B = 1000). N_A = number of alleles; H_E = expected heterozygosity; H_O = observed heterozygosity; F_{IT} = individual:total population inbreeding coefficient; F_{IS} = individual:subpopulation inbreeding coefficient; F_{ST} = fixation index; subsequent columns are of F_{ST} analogues.

Locus	Size Range	N_A	H_E	H_O	HWE χ^2 test	HWE exact test	F_{IT}	F_{IS}	F_{ST}	G_{ST}	G''_{ST}	Jost's D	ϕ'_{ST}
***Svi* L6**[2]	109-121	7	0.49	0.49	0.9999	0.698	0.011	-0.024	0.017	0.017	0.035	0.017	0.038
Svi18[1]	126-132	4	0.2	0.19	0.0489	0.02	0.048	0.036	0.023	0.023	0.030	0.006	0.035
YP41[4]	171-179	3	0.11	0.1	0.010	0.028	0.083	0.083	0.010	0.011	0.013	0.001	0.017
MSL-2[5]	149-223	25	0.78	0.8	8.53E-7	0.021	-0.024	-0.040	0.014	0.013	0.062	0.049	0.071
YP60[4]	180-320	35	0.94	0.85	0	0	0.092	0.082	0.015	0.017	0.227	0.213	0.206
Svi33[1]	97-149	27	0.86	0.83	0.9970	0.113	0.035	-0.0002	0.023	0.023	0.153	0.132	0.169
Svi4[1]	109-151	20	0.82	0.81	0.9999	0.11	0.017	-0.020	0.032	0.032	0.157	0.128	0.188
Svi20[3]	155-207	24	0.89	0.89	0.9928	0.747	-0.006	-0.022	0.020	0.020	0.161	0.143	0.136
Svi2[3]	218-276	23	0.88	0.84	0	0	0.050	0.034	0.022	0.023	0.182	0.162	0.167
***Svi* L8**[2]	128-144	9	0.77	0.73	0.0023	0.001	0.060	0.012	0.049	0.049	0.194	0.150	0.253
Svi26[3]	149-203	26	0.72	0.69	0.0002	0	0.052	0.025	0.028	0.029	0.101	0.073	0.135
Svi7[3]	176-222	24	0.85	0.81	0.0193	0.012	0.045	-0.007	0.043	0.043	0.244	0.209	0.262

[1]Borer *et al.* 1999; [2]Wirth *et al.* 1999; [3]Eldridge 2002; [4]Li *et al.* 2007; [5]Kohlmann and Kersten 2008

There was limited but inconclusive evidence of linkage disequilibrium (LD) among loci. Microsatellites were mapped across eight chromosomes within the Pikeperch genome (Figure 3.3), with three chromosomes containing multiple microsatellites (Chr. 1: YP60, Svi2, *MSL-2*; Chr. 3: Svi26, Svi20; Chr. 20: YP41, Svi18). Notably, YP60 and Svi2 were separated by 5.075 Mb on chromosome 1, while YP41 and Svi18 were mapped within 4.770 Mb of each other on the chromosome 20 assembly. G-tests for LD between these locus pairings were not significant (Fisher's method, Genepop; Appendix 3.D). One locus pairing, *MSL-2* and Svi20, yielded significant G-test results in Genepop (p = 0.0438). Although these loci are located on the distal end of their respective chromosomes, they are not found on the same chromosome within the Pikeperch genome.

Pairwise $\bar{r}_d$ was low across most combinations of loci (-0.041 to 0.070) but elevated between Svi2 and YP60 (0.286; Figure 3.4). However, G-tests for this locus pairing do not show evidence of LD (p = 0.995). Although evidence of LD between Svi2 and YP60 was inconclusive, the YP60 locus was ultimately dropped from all analyses that required LD estimates for population delineation.

Table 3.8. Proportion of locus-population combinations that did not conform to Hardy-Weinberg Equilibrium expectations based on a chi-square test or Guo and Thompson's (1992) exact test. Proportions are also shown after a Bonferroni correction, where the revised p-value for each test is equal to α/n.

Population Stratum	χ^2 test	χ^2 test- Bonferroni cor.	Exact test	Exact test- Bonferroni cor.
Lake (n = 108)	0.120	0.065	0.093	0.009
Basin (n = 168)	0.143	0.071	0.107	0.012
Site (n = 264)	0.114	0.038	0.076	0

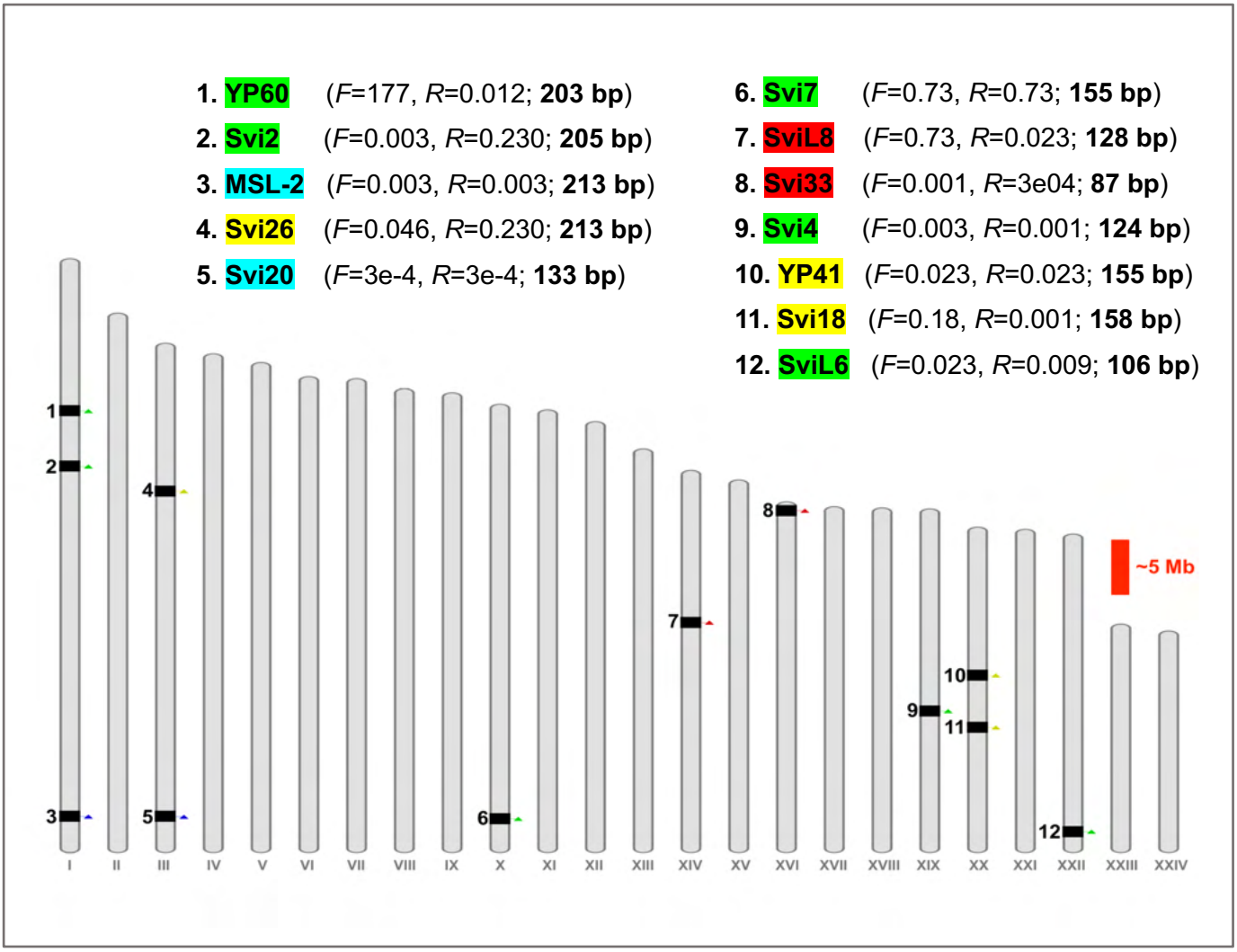


Figure 3.3. Microsatellite positions transposed on a Pikeperch genome (*Sander lucioperca*; GenBank accession: GCA_008315115.2). Putative primer locations were identified with BLAST (NBCI). Allele sizes, E values for forward (*F*) and reverse (*R*) primers, and the fluorescent tag used on the forward primer (green = HEX, blue = 6-FAM, yellow = NED, red = PET) are presented for each microsatellite. Chromosomes were visualized with the *RIdeogram* package in R.

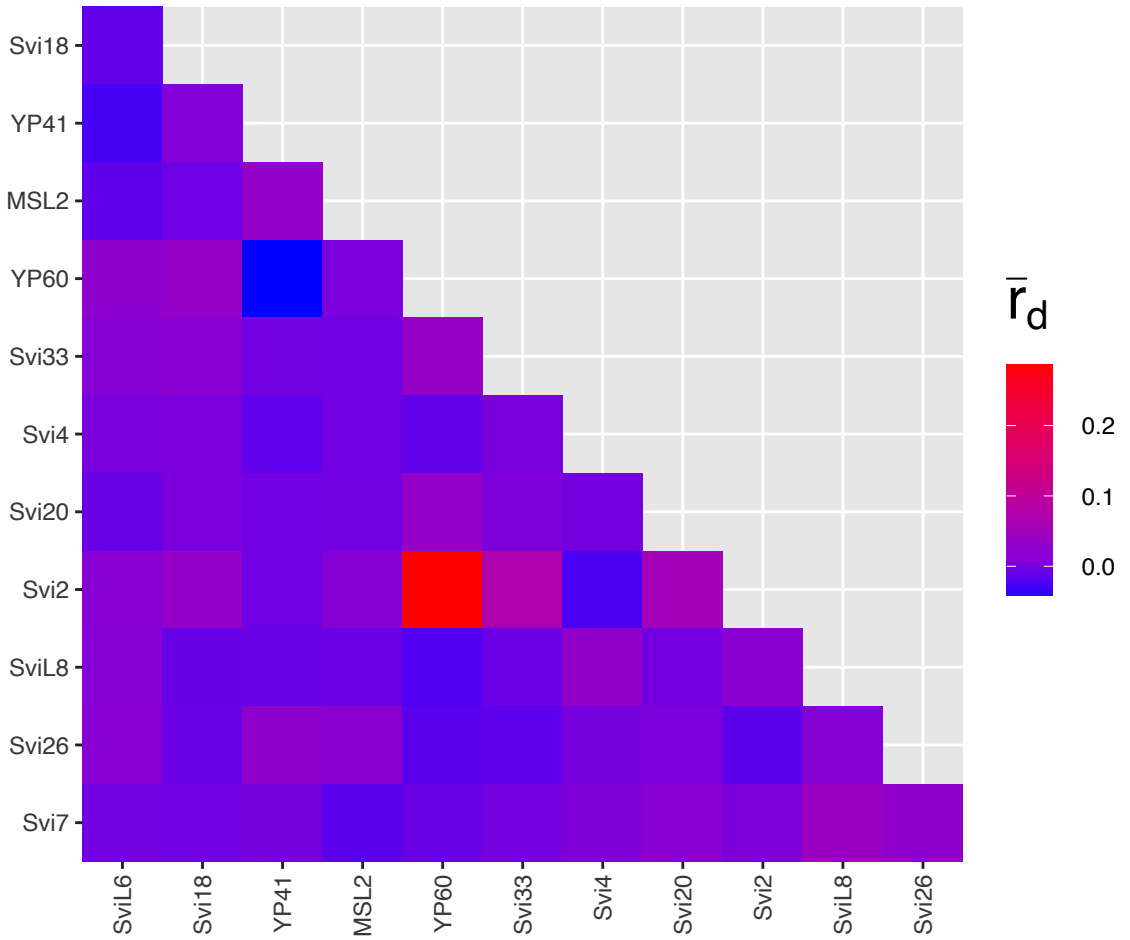


Figure 3.4. Pairwise $\bar{r}$d among the twelve microsatellite loci analyzed in this study, calculated across all individuals in the sample set (n = 840).

All measures of genetic diversity differed between *a priori* sauger populations within each population stratum. Mean allelic richness ranged from 6.21 to 7.12 alleles based on 22 rarified samples, with Stephens Lake exhibiting the highest allelic richness and Lake Winnipegosis yielding the fewest rarefied alleles (Table 3.1; Table 3.2; Appendix 3.E). These results were validated with an allele rarefaction curve comprised of all individuals in the dataset (Figure 3.5; Appendix 3.F). As such, there was no clear trend between waterbody size and allelic richness. Lake Winnipeg and Lake Manitoba populations also showed variations in allelic richness at the Basin and Site level, with higher richness observed in the northernmost populations (Table 3.2; Appendix 3.E). Populations in Lake Winnipeg and Lake Manitoba contained the highest number of private alleles, though this may be a result of sample size.

Mean observed and expected heterozygosity (H_O and H_E) was similar across most Lake level sauger populations, with mean H_O and H_E ranges of 0.66 to 0.68 and 0.65 to 0.69, respectively (Table 3.1). In contrast, sauger genotypes from Cedar Lake exhibited mean H_O and H_E frequencies of 0.59 and 0.64, and individuals from the Red River yielded H_O and H_E frequencies averaging 0.60 and 0.63 (Table 3.1). These reduced frequencies indicate a heterozygote deficiency in the Cedar Lake and Red River populations that may be attributed to allele fixation or inbreeding.

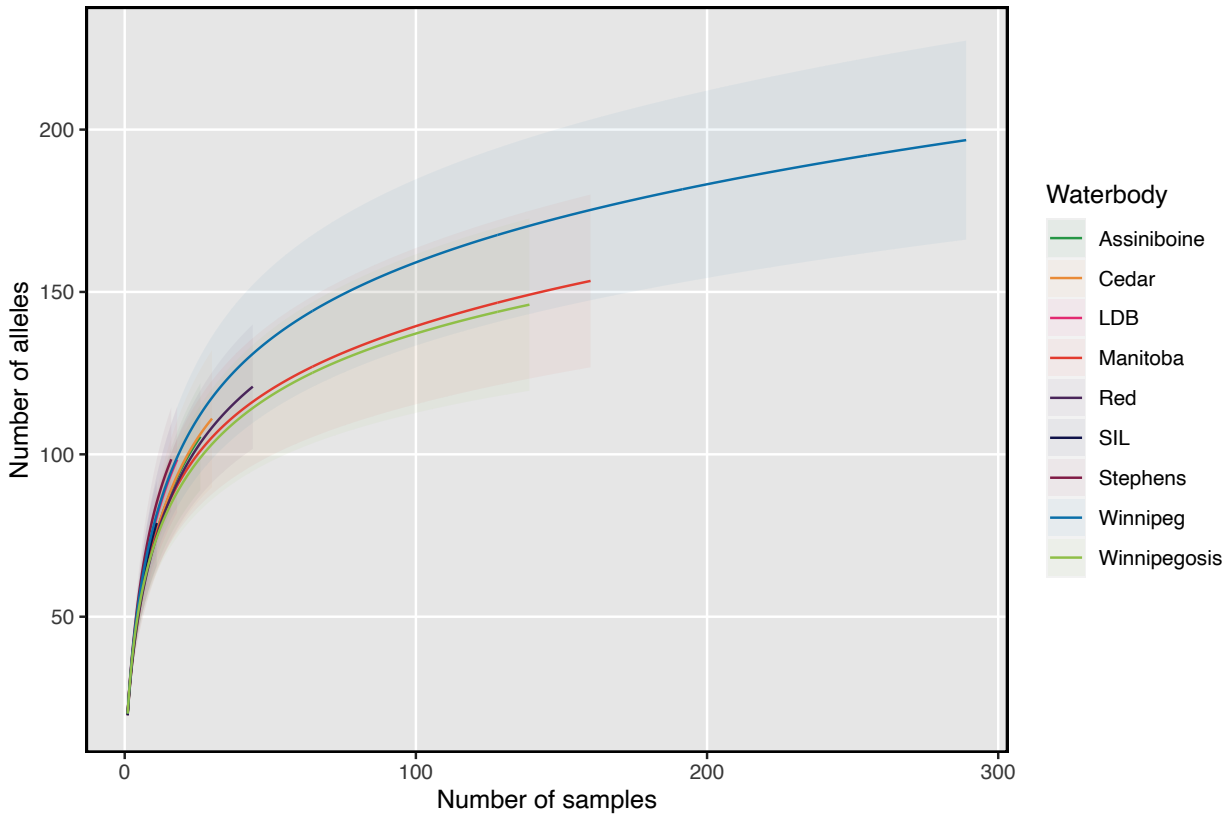


Figure 3.5. Allele rarefaction curves, consisting of twelve microsatellite loci, for sauger populations defined at the Lake population level. Allele rarefaction curves for populations defined at the Basin and Site level can be found in Appendix F.

There was little or no evidence of persistent inbreeding within any sample population. Most population F_{IS} estimates were low and not statistically significant as negative estimates were present in the accompanying 95% confidence intervals (Table 3.1; Table 3.2; Appendix 3.E). Cedar Lake was the only sauger population with a significant F_{IS} estimate at any population stratum (F_{IS}= 0.143, 95% CI = 0.032-0.152). Moreover, few genotypes returned high mean inbreeding coefficient ($\bar{F}$) estimates, and those that did comprised only a small percentage of the parent population (Figure 3.6).

Attempts to estimate population size (N_e) were largely ineffective at all population levels. N_e estimates could not be calculated for the Red River, Lake Manitoba, Lake Winnipegosis, and Lac du Bonnet (Table 3.9). Estimates for the remaining waterbodies were accompanied by broad confidence intervals, with most containing an upper limit of infinity. Adding the YP60 locus back into N_e calculations did not dramatically alter N_e estimates. However, shifts in the minimum allele frequency permitted in calculations resulted in multiple N_e estimates increasing or decreasing severalfold. For these reasons, the N_e estimates presented in Table 3.9 should be viewed with reservation.

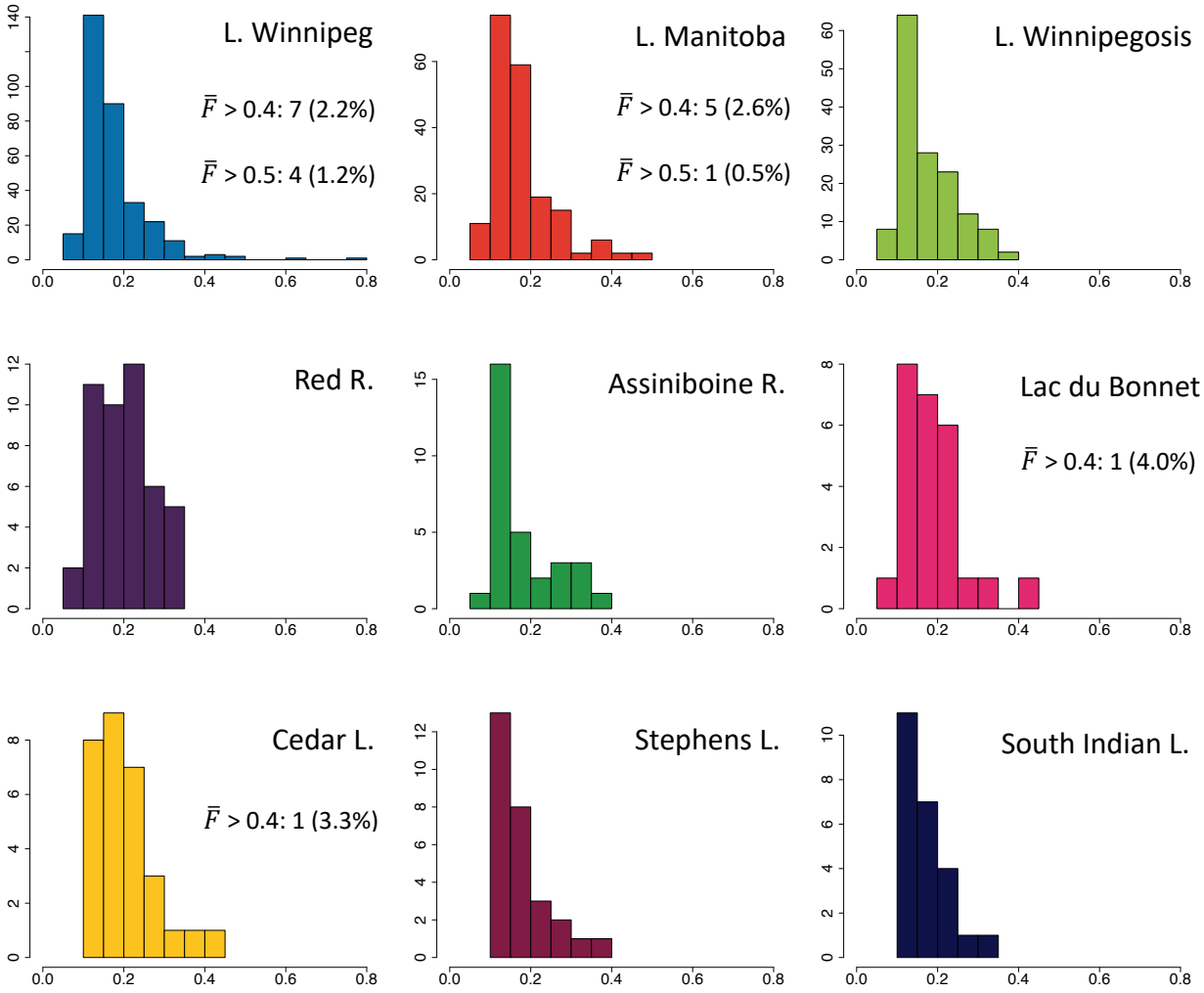


Figure 3.6. Distribution of individual inbreeding coefficient ($\bar{F}$) estimates within Manitoba sauger populations, as defined at the Lake population level.

Table 3.9. Estimates of effective population size (N_e), with 95% confidence intervals, for populations defined at **a)** the Lake level and **b)** the lowest available population stratum within each waterbody. N_e was estimated using Waples' (2006) linkage disequilibrium method with a minimum allele frequency (P_{crit}) of 0.02. An N_e estimate of infinity indicates that N_e could not be calculated for a given population.

a)

Population (Lake)	N_e
L. Winnipeg	3213 (1730-18155)
L. Manitoba	∞ (1010-∞)
L. Winnipegosis	∞ (803-∞)
Red R.	∞ (251-∞)
Assiniboine R.	108 (60-384)
Lac du Bonnet	∞ (156-∞)
Cedar L.	526 (119-∞)
Stephens L.	1973 (128-∞)
South Indian L.	704 (76-∞)

b)

Population (Basin/Site)	N_e
Grand Beach	366 (159-∞)
Riverton/Hecla	661 (233-∞)
Frog Bay	835 (215-∞)
Matheson Is.	1437 (207-∞)
Dauphin R.	∞ (86-∞)
Poplar R.	802 (127-∞)
Grand Rapids	482 (177-∞)
Mossy Bay	1003 (121-∞)
Whitemud	183 (84-∞)
St. Laurent	194 (65-∞)
Lundar	∞ (115-∞)
Narrows	541 (177-∞)
Steep Rock	568 (184-∞)
Manipogo	∞ (97-∞)
Lower Winnipegosis	∞ (632-∞)
Mid Winnipegosis	∞ (547-∞)

3.3.3.3 Genetic Differentiation and Population Structure

The differentiating power of each locus varied according to the statistic being calculated. Global estimates of F_{ST} (Weir and Cockerham's θ statistic; identified hereafter as F_{ST}) and G_{ST} were primarily driven by the *Svi* L8, Svi7 and Svi4 loci (Table 3.7). For global estimates of statistics that correct for allelic diversity—including the G''_{ST}, D, and ϕ'_{ST} statistics—YP60, Svi2 and Svi33 had improved differentiating power (Table 3.7). Despite these variations, relationships between differentiation statistics were strongly correlated across loci (Appendix 3.G).

Most population-specific differentiation estimates were statistically insignificant when compared to the pooled dataset. Under these conditions, F_{ST} estimates were only significant for the Red River, Cedar Lake, and South Indian Lake sauger populations (Lake level; Table 3.1), and the Riverton/Hecla population (Site level; Table 3.2). In contrast, pairwise F_{ST} comparisons among populations were often significant at the Lake, Basin, and Site population strata. Pairwise F_{ST} ranged from 0.0031 (Red River–Assiniboine River) to 0.0643 (Red River–South Indian Lake) at the Lake level; only the Red River–Assiniboine River pairing was not statistically significant (Table 3.10). Significant pairwise F_{ST} estimates were also prevalent at the Basin and Site levels (Appendix 3.H; Appendix 3.I), but many pairwise estimates were not significant among neighbouring populations.

At the Basin level, F_{ST} estimates did not significantly differ between *a priori* populations within Lake Manitoba or within Lake Winnipegosis, or between the following pairings: Assiniboine River–Red River; Assiniboine River–Winnipeg South; Winnipeg Channel–Winnipeg North; Winnipeg Channel–Stephens Lake; and Manitoba Narrows and Middle Winnipegosis (Appendix 3.H).

Likewise, populations could not be readily distinguished within Lake Manitoba or Lake Winnipegosis at the Site level (Appendix 3.I). F_{ST} estimates were also insignificant for the Lower Winnipegosis–Manitoba Lundar, Middle Winnipegosis–Manitoba Lundar, Middle Winnipegosis–Manitoba Narrows, and Middle Winnipegosis–Manitoba Manipogo population pairings. Within Lake Winnipeg, the Riverton population could be distinguished from the populations in Poplar River and Mossy Bay, and the Grand Beach population was significantly different from all except the Dauphin River population; all other Lake Winnipeg pairings yielded confidence intervals with negative F_{ST} estimates. The Stephens Lake population did not significantly differ from the Frog Bay, Matheson Island, Dauphin River, Poplar River and Mossy Bay populations within Lake Winnipeg (Appendix 3.I).

Table 3.10. Population pairwise F_{ST} (θ statistic) as defined at the Lake level. Point estimates are above the diagonal line, and 95% confidence intervals are below the diagonal line. Non-significant results (i.e., negative F_{ST} estimates are present within the confidence interval) are highlighted in red.

	L. Winnipeg	Red R.	Assiniboine R.	L. Manitoba	L. Winnipegosis	Lac du Bonnet	Cedar L.	Stephens L.	S. Indian L.
L. Winnipeg		0.0189	0.0109	0.0309	0.0377	0.0151	0.0305	0.0072	0.0347
Red R.	(0.0096-0.0272)		0.0031	0.0475	0.0527	0.0394	0.0481	0.0367	0.0643
Assiniboine R.	(0.0036-0.0183)	**(-0.0035-0.0148)**		0.0349	0.0392	0.0154	0.0406	0.0291	0.0555
L. Manitoba	(0.0226-0.0410)	(0.0213-0.0756)	(0.0216-0.0493)		0.0074	0.0468	0.0520	0.0425	0.0606
L. Winnipegosis	(0.0210-0.0567)	(0.0170-0.0957)	(0.0164-0.0636)	(0.0036-0.0117)		0.0493	0.0597	0.0464	0.0608
Lac du Bonnet	(0.0060-0.0277)	(0.0175-0.0648)	(0.0056-0.0249)	(0.0299-0.0689)	(0.0303-0.0740)		0.0518	0.0257	0.0535
Cedar L.	(0.0166-0.0433)	(0.0272-0.0713)	(0.0242-0.0534)	(0.0355-0.0641)	(0.0400-0.0752)	(0.0369-0.0666)		0.0318	0.0365
Stephens L.	(0.0002-0.0146)	(0.0181-0.0570)	(0.0099-0.0509)	(0.0257-0.0628)	(0.0239-0.0709)	(0.0081-0.0490)	(0.0041-0.0658)		0.0253
S. Indian L.	(0.0171-0.0487)	(0.0302-0.1019)	(0.0314-0.0781)	(0.0409-0.0798)	(0.0350-0.0845)	(0.0244-0.0847)	(0.0178-0.0566)	(0.0115-0.0378)	

The neighbour-joining tree generated with Cavalli-Sforza and Edwards' chord distance (D_{CSE}) closely paralleled the pairwise F_{ST} estimates described above (Figure 3.7). South Indian Lake and Cedar Lake sauger exhibited the greatest genetic distances among all pre-defined population, with high bootstrap support (100% and 82.4%, respectively). All other populations were split into two clades, one containing Lake Manitoba and Lake Winnipegosis, and the other encompassing populations found within Lake Winnipeg, the Red River, the Assiniboine River, Stephens Lake and Lac du Bonnet. Populations assigned at the Site level were also clustered with populations found within the same waterbody with the exceptions of Grand Beach and Dauphin River in Lake Winnipeg, and Lundar and Manipogo in Lake Manitoba (Figure 3.7).

There was no clear trend between population proximity and genetic similarity at the Basin and Site levels. For example, the Frog Bay and Matheson Island populations—both located in the Channel region of Lake Winnipeg—would be grouped based on proximity. Instead, D_{CSE} calculations group the Frog Bay and Matheson Island populations with the Grand Rapids (North Basin) and Riverton/Hecla (South Basin) populations, respectively. This finding is supported by an analysis of molecular variance (AMOVA) and random permutation test as variation was significant among individuals (94.7% variance; $p < 0.0001$) and Lakes (4.8% variance; $p < 0.0001$), but not significant among Basins within Lakes (0.2% variance; $p = 0.9990$) or among Sites within Basins (0.3% variance; $p = 0.0819$). However, variation was significant among Sites within Lakes (0.4% variance; $p = 0.0060$) when the Basin level was excluded from the AMOVA (Lakes = 6.0% variance; Individuals = 93.6% variance).

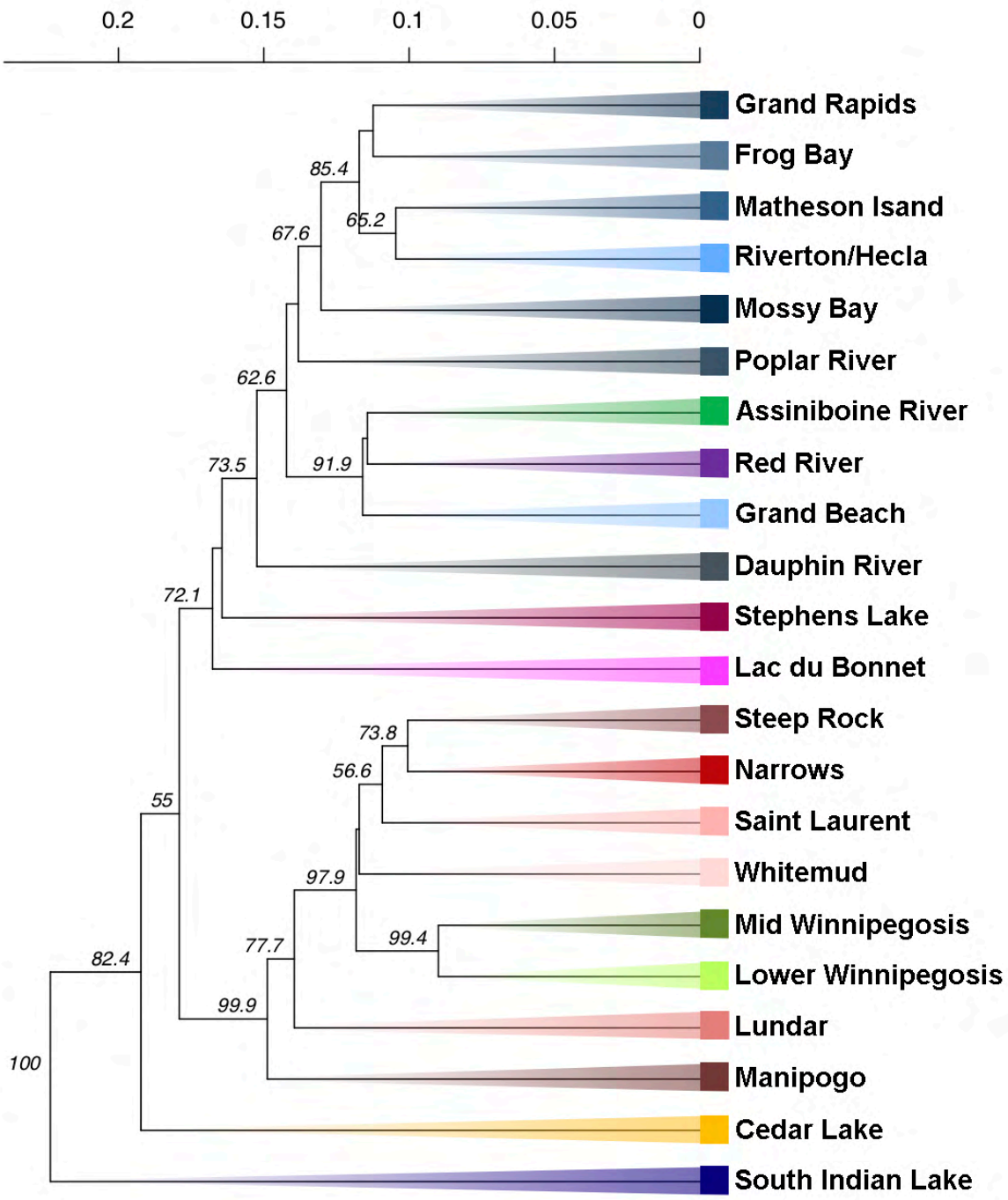


Figure 3.7. Neighbour-joining tree depicting the relationship and degree of differentiation between sauger populations in Manitoba. The tree was generated using Cavalli-Sforza and Edwards' chord distance (DCSE) with bootstrapped support (n = 1000).

Bayesian analysis with STRUCTURE identified $K = 4$ and $K = 7$ as the likeliest number of genetically distinct sauger groups (ΔK statistic; Figure 3.8). Under the $K = 4$ model, genetically distinct sauger stocks were distributed between Lake Winnipeg and Lac du Bonnet (Group 1, Blue), the Red River and Assiniboine River (Group 2, Green), Lake Manitoba and Lake Winnipegosis (Group 3, Red), and Cedar Lake and South Indian Lake (Group 4, Purple) (Figure 3.9). The Stephens Lake sauger population appeared to be an admixture of Group 1 and Group 4 (Figure 3.9). Admixture could also be seen at finer population scales. Sauger from Grand Beach yielded high membership coefficients favoring the Group 2 stock, and the Grand Rapids population had a pronounced Group 4 signature. A Group 3 signature may be present within the Dauphin River population, although a larger sample size is necessary to validate this observation.

The $K = 7$ model was noisy and did not elucidate any genetic stock structure beyond what was described with the $K = 4$ model (Figure 3.9). That said, the volatile membership coefficients observed within the Stephens Lake, Grand Beach, and Grand Rapids sauger populations in the $K = 4$ model were also retained in the $K = 7$ model, which further supports the presence of admixture.

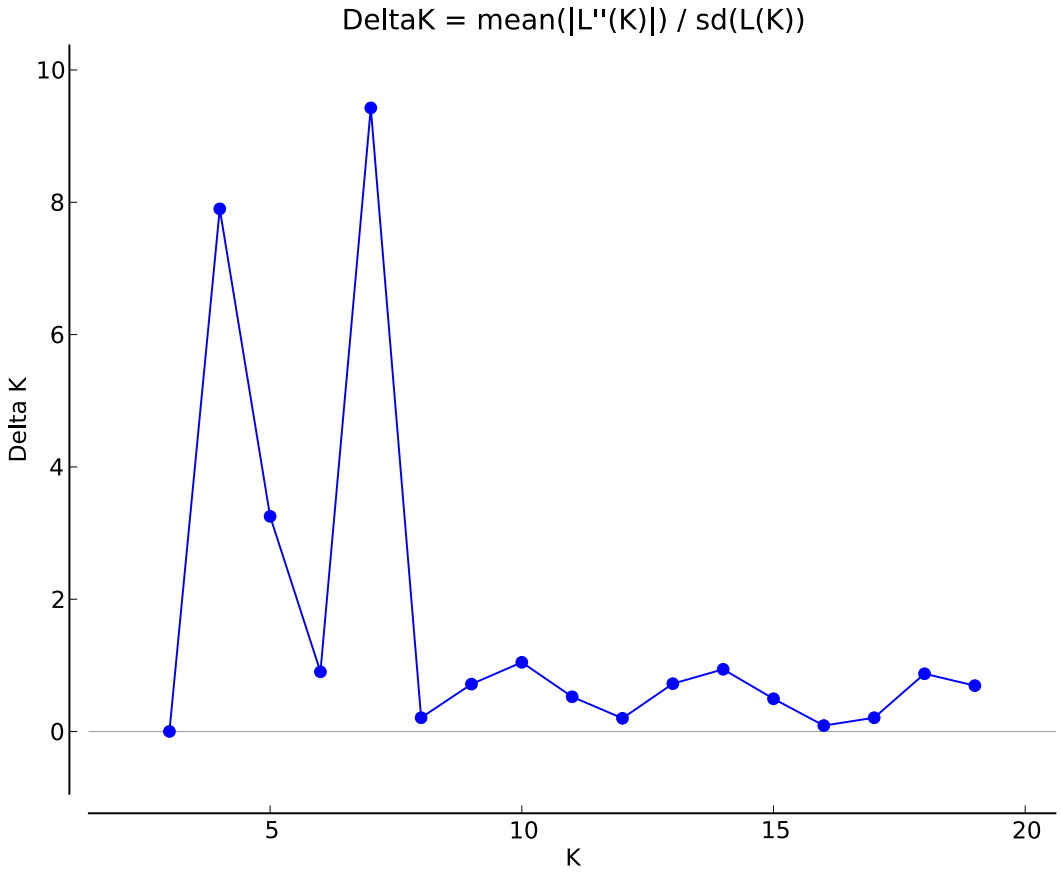


Figure 3.8. Likeliest number of genetically distinct sauger populations using Evanno *et al.*'s (2005) ΔK criterion, as implemented in Structure Harvester (Earl and vonHoldt 2012).

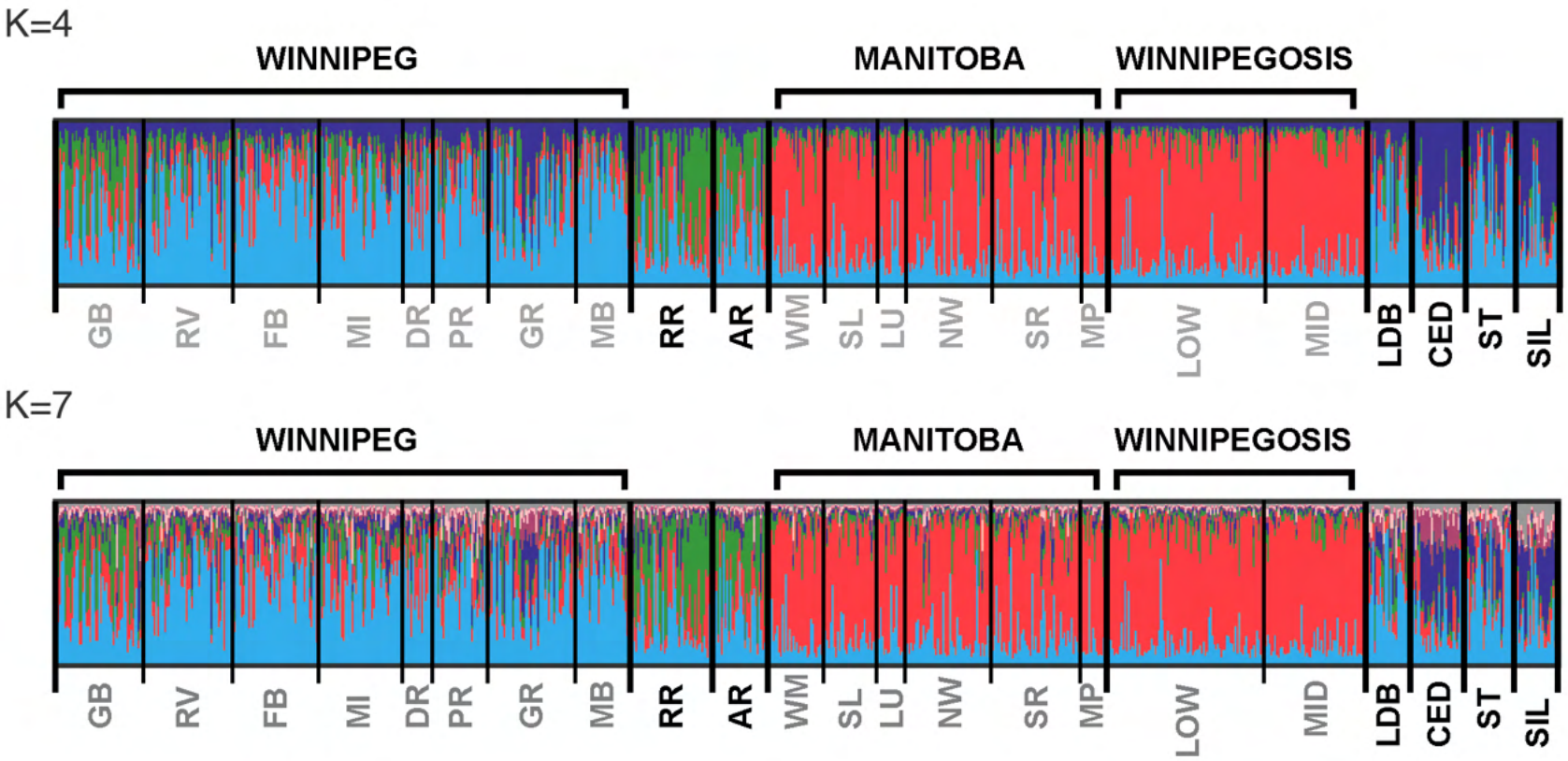


Figure 3.9. Estimated population structure of sauger (n = 840) following STRUCTURE analysis where *K* = 4 and *K* = 7. Waterbodies are written in black and collection sites (i.e. Site level populations) are denoted in gray. Each vertical line represents an individual sauger and the colours within each line represent admixture, as expressed with a posterior membership coefficient.

Ordination of individuals using DAPC supports the inter-population relationships described with the neighbour-joining tree and STRUCTURE analysis. Individuals assigned to *a priori* populations remained loosely but visibly clustered in DAPCs performed at the Site (92 PCs retained, 94.8% of variance; Figure 3.10), Basin (92 PCs retained, 94.8% of variance; Appendix 3.J), and Lake (96 PCs retained, 95.4% of variance; Appendix 3.K) population strata. The plotting of central coordinates for each population revealed clustering of populations within and among Lake Manitoba and Lake Winnipegosis, Lake Winnipeg and Lac du Bonnet, and the Red River and Assiniboine River. At the Site level, the Grand Beach population within Lake Winnipeg was clustered with the Red River and Assiniboine River populations (Figure 3.10). The Cedar Lake and South Indian Lake populations were isolated from these other clusters, while the Stephen Lake population presented as an intermediate between Lake Winnipeg and South Indian Lake. Collectively, the central coordinates of the *a priori* populations on the DAPC scatterplot closely resemble the spatial coordinates of these same populations as viewed on a map (Figure 1.1).

Using the *K*-means clustering procedure described by Jombart *et al.* (2010), I determined the optimal number of population clusters to be $K = 6$ at the Site, Basin, and Lake population levels (Appendix 3.L). However, reassignment of individuals into these *a posteriori* clusters was uninformative as each reassigned population appeared to be an arbitrary admixture of individuals from both near and distant waterbodies (Appendix 3.L:3.O). Thus, I concluded that the *a priori* populations were a better descriptor of population structure than the *a posteriori* populations proposed using DAPC and *K*-means clustering.

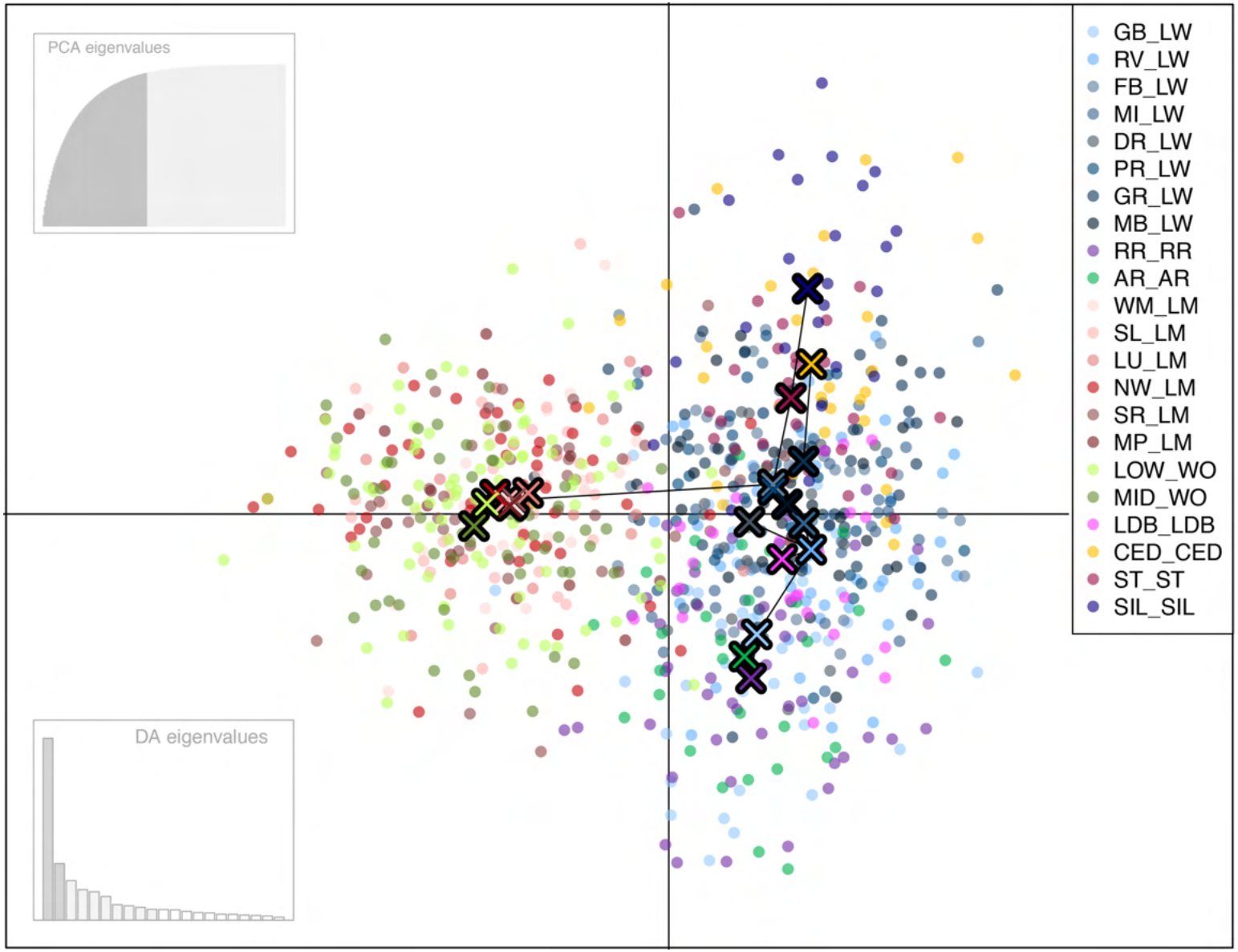


Figure 3.10. Discriminant analysis of principal components (DAPC) of sauger genotypes (n = 840) as assigned at the Site population level. Mean values for each population are represented with an "X" symbol, and connecting lines depict a minimum spanning tree. Ninety-two principal components were retained based on cross-validation testing. The first and second discriminant functions are represented on the x and y axis, respectively.

3.3.3.4 Gene Flow and Divergence Mechanisms

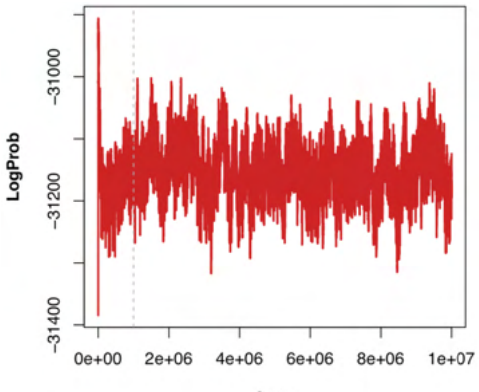
Several BayesAss models were run to account for the population structure described in results section *3.3.3.3*. In all models, sauger from Lake Manitoba and Lake Winnipegosis were considered one population, as were sauger from the Red River and the Assiniboine River. The first model included the above populations as well as the Lake Winnipeg, Stephens Lake, and South Indian Lake sauger populations. The second model substituted the South Indian Lake population with the Cedar Lake population. The final model was comprised of the Lake Winnipeg, Red River–Assiniboine River, and Lake Manitoba–Lake Winnipegosis populations. Migration estimates and log-probability plots for the best run of each model can be seen in Figure 3.11.

The first model failed to converge during the burn-in period of all ten runs (mean log likelihood = -31164; mean Bayesian deviance = 62330), and the proportion of non-migrants approached 66% or 100% in all five populations. The proportion of non-migrant sauger per generation was projected to be 0.974 (*SD* = 0.012) in Lake Winnipeg, 0.981 (*SD* = 0.007) in Lake Manitoba–Lake Winnipegosis, and 0.705 (*SD* = 0.036) in the Red River–Assiniboine River with a 0.268 (*SD* = 0.037) contribution from Lake Winnipeg. The model projected that 28.5% (+/- 4.2%) of Stephens Lake sauger and 24.7% (+/- 8.7%) of South Indian Lake sauger were Lake Winnipeg migrants, even though South Indian Lake is not directly accessible from Lake Winnipeg.

Similarly, no runs of the second model achieved convergence within the burn-in period (mean log likelihood = -31393; mean Bayesian deviance = 62798). The suggested proportions of non-migrant sauger in Lake Winnipeg, Lake Manitoba–Lake Winnipegosis, and the Red River–Assiniboine River were comparable to those proposed in the first model (Figure 3.11),

and 28.2% (+/- 4.4%) of sauger in Stephens Lake were projected to be Lake Winnipeg migrants. The model also predicted that 29.1% (+/- 4.0%) of sauger in Cedar Lake would be Lake Winnipeg migrants per generation despite the presence of natural and man-made barriers prohibiting upstream fish migration.

The model containing only the Lake Winnipeg, Lake Manitoba–Lake Winnipegosis and Red River–Assiniboine River populations converged in all ten runs (mean log likelihood = -29280, mean Bayesian deviance = 58568) with minimal variation of migration rate estimates. In Lake Winnipeg, migration rate estimates ranged between 0.006 (*SD* = 0.005) and 0.007 (*SD* = 0.005) from the Lake Manitoba–Lake Winnipegosis population, and 0.0010 (*SD* = 0.0010) and 0.0011 (*SD* = 0.0010) from the Red River–Assiniboine River population. In Lake Manitoba, the likeliest migration rates were 0.016 (*SD* = 0.007) or 0.017 (*SD* = 0.008) from the Lake Winnipeg population and 0.010 (*SD* = 0.010) from the Red River–Assiniboine River population. The projected contributions of the Lake Winnipeg and Lake Manitoba–Lake Winnipegosis populations to the Red River–Assiniboine River population were 0.317 (*SD* = 0.010) and 0.013 (*SD* = 0.010) across all ten runs, respectively.

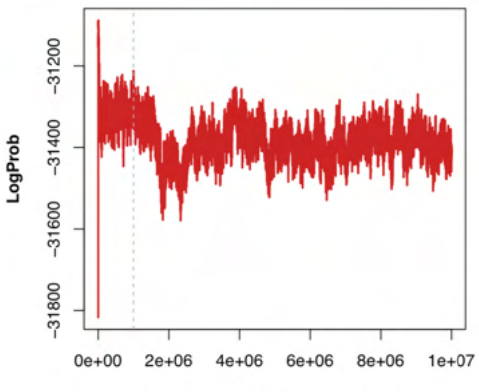


MODEL 1

```
1->AR-RR 2->MB-WPO 4->SIL 3->Stephens 0->Winnipeg

Migration Rates:

m[0][0]: 0.9738(0.0115) m[0][1]: 0.0153(0.0100) m[0][2]: 0.0081(0.0056) m[0][3]: 0.0010(0.0010) m[0][4]: 0.0018(0.0016)
m[1][0]: 0.2677(0.0374) m[1][1]: 0.7048(0.0358) m[1][2]: 0.0189(0.0142) m[1][3]: 0.0041(0.0040) m[1][4]: 0.0045(0.0044)
m[2][0]: 0.0154(0.0067) m[2][1]: 0.0019(0.0018) m[2][2]: 0.9807(0.0070) m[2][3]: 0.0010(0.0010) m[2][4]: 0.0011(0.0011)
m[3][0]: 0.2851(0.0216) m[3][1]: 0.0108(0.0104) m[3][2]: 0.0158(0.0138) m[3][3]: 0.6768(0.0098) m[3][4]: 0.0116(0.0111)
m[4][0]: 0.2473(0.0443) m[4][1]: 0.0195(0.0159) m[4][2]: 0.0167(0.0158) m[4][3]: 0.0115(0.0112) m[4][4]: 0.7050(0.0381)
```

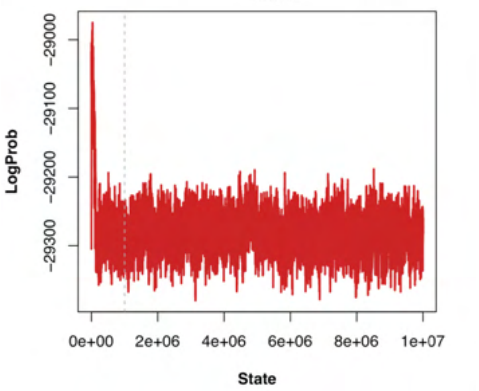


MODEL 2

```
1->AR-RR 3->Cedar 2->MB-WPO 4->Stephens 0->Winnipeg

Migration Rates:

m[0][0]: 0.9523(0.0209) m[0][1]: 0.0368(0.0200) m[0][2]: 0.0088(0.0058) m[0][3]: 0.0011(0.0011) m[0][4]: 0.0010(0.0010)
m[1][0]: 0.2101(0.0493) m[1][1]: 0.7648(0.0480) m[1][2]: 0.0169(0.0140) m[1][3]: 0.0041(0.0041) m[1][4]: 0.0041(0.0040)
m[2][0]: 0.0143(0.0065) m[2][1]: 0.0027(0.0028) m[2][2]: 0.9810(0.0070) m[2][3]: 0.0010(0.0010) m[2][4]: 0.0010(0.0009)
m[3][0]: 0.2905(0.0205) m[3][1]: 0.0104(0.0101) m[3][2]: 0.0124(0.0119) m[3][3]: 0.6771(0.0107) m[3][4]: 0.0096(0.0093)
m[4][0]: 0.2823(0.0222) m[4][1]: 0.0117(0.0111) m[4][2]: 0.0189(0.0152) m[4][3]: 0.0103(0.0099) m[4][4]: 0.6768(0.0098)
```



MODEL 3

```
1->AR-RR 2->MB-WPO 0->Winnipeg

Migration Rates:

m[0][0]: 0.9922(0.0047) m[0][1]: 0.0010(0.0010) m[0][2]: 0.0067(0.0046)
m[1][0]: 0.3166(0.0104) m[1][1]: 0.6708(0.0041) m[1][2]: 0.0126(0.0096)
m[2][0]: 0.0168(0.0075) m[2][1]: 0.0010(0.0010) m[2][2]: 0.9822(0.0075)
```

Figure 3.11. Log-probability tracer plots and migration rate estimates from the best run (lowest Bayesian deviance) of each BayeAss model. Model 1 consists of the Lake Winnipeg, Red River–Assiniboine River, Lake Manitoba–Lake Winnipegosis, Stephens Lake and South Indian Lake sauger populations. Model 2 replaces South Indian Lake with Cedar Lake. Model 3 excludes Stephens Lake, South Indian Lake, and Cedar Lake populations.

The degree of genetic differentiation observed between sauger populations is consistent with an isolation by distance (IBD) model of population divergence. There was a significant relationship between the genetic and geographic distances of sauger populations (Lake level: $p = 0.003$; Basin level: $p < 0.001$; Site level: $p < 0.001$), individual gillnets ($p = 0.031$), and individual sauger ($p<0.001$) based on Mantel tests with 1000 randomized permutations (Figure 3.12). Scatterplots of these IBD models (Figure 3.13) showed that the correlation between genetic and geographic distance was positive: as geographic distance between populations or individuals increased, so too did their genetic distance. Kernel density surface overlays for each scatterplot suggest that the IBD relationships between populations were mostly clinal (Figure 3.13). However, patches outside of the largest kernel density clouds may also suggest the presence of other population divergence mechanisms.

Monmonier's algorithm generated a single extended boundary within the study area that, when superimposed onto a Digital Elevation Model (DEM) of Manitoba (Figure 3.14), may depict physical barriers to gene flow. The boundary was drawn across the networks connecting the Cedar Lake sauger population to adjacent populations in Lake Winnipeg and Lake Winnipegosis. This section of the boundary resembles the Pas Moraine ridge formation separating Cedar Lake from Lake Winnipegosis (EDITNR 2023b; Figure 3.14). The boundary then separates sauger populations in Lake Winnipeg from populations in Lake Winnipegosis, which roughly aligns with the fluted till plain seen between the Pas Moraine and the Hodgson Plain landforms (EDITNR 2023b; Figure 3.14). Finally, the generated boundary intersects between Lake Winnipegosis and Lake Manitoba and separates the Manitoba-Manipogo population from the rest of the sauger populations within Lake Manitoba.

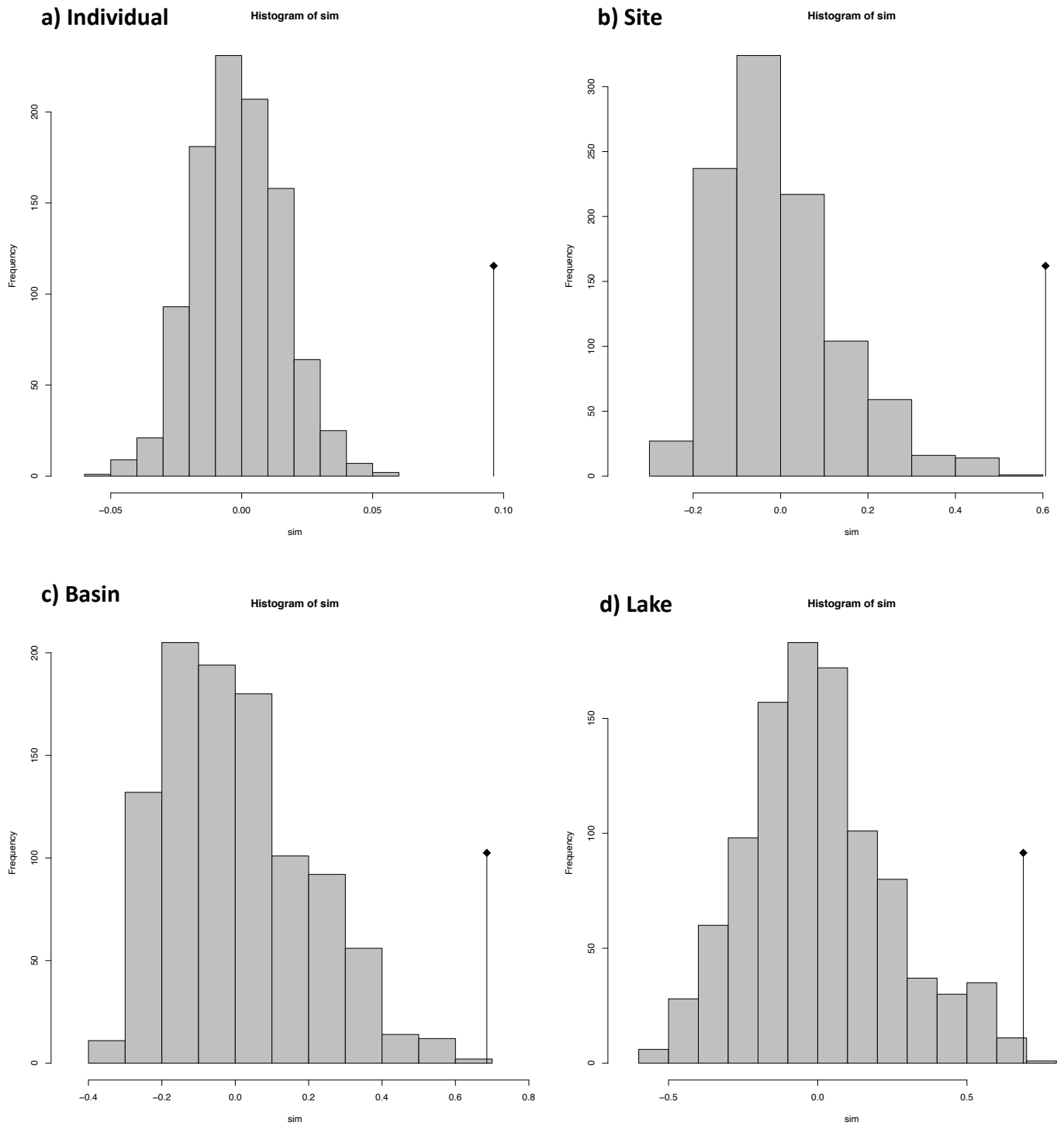


Figure 3.12. The correlation between genetic and geographic distance within the dataset (black line and diamond) compared to values generated using a Mantel test with random permutation (N = 1000). Histograms are based on tests of a) individuals and populations at the b) Site, c) Basin, and d) Lake population strata.

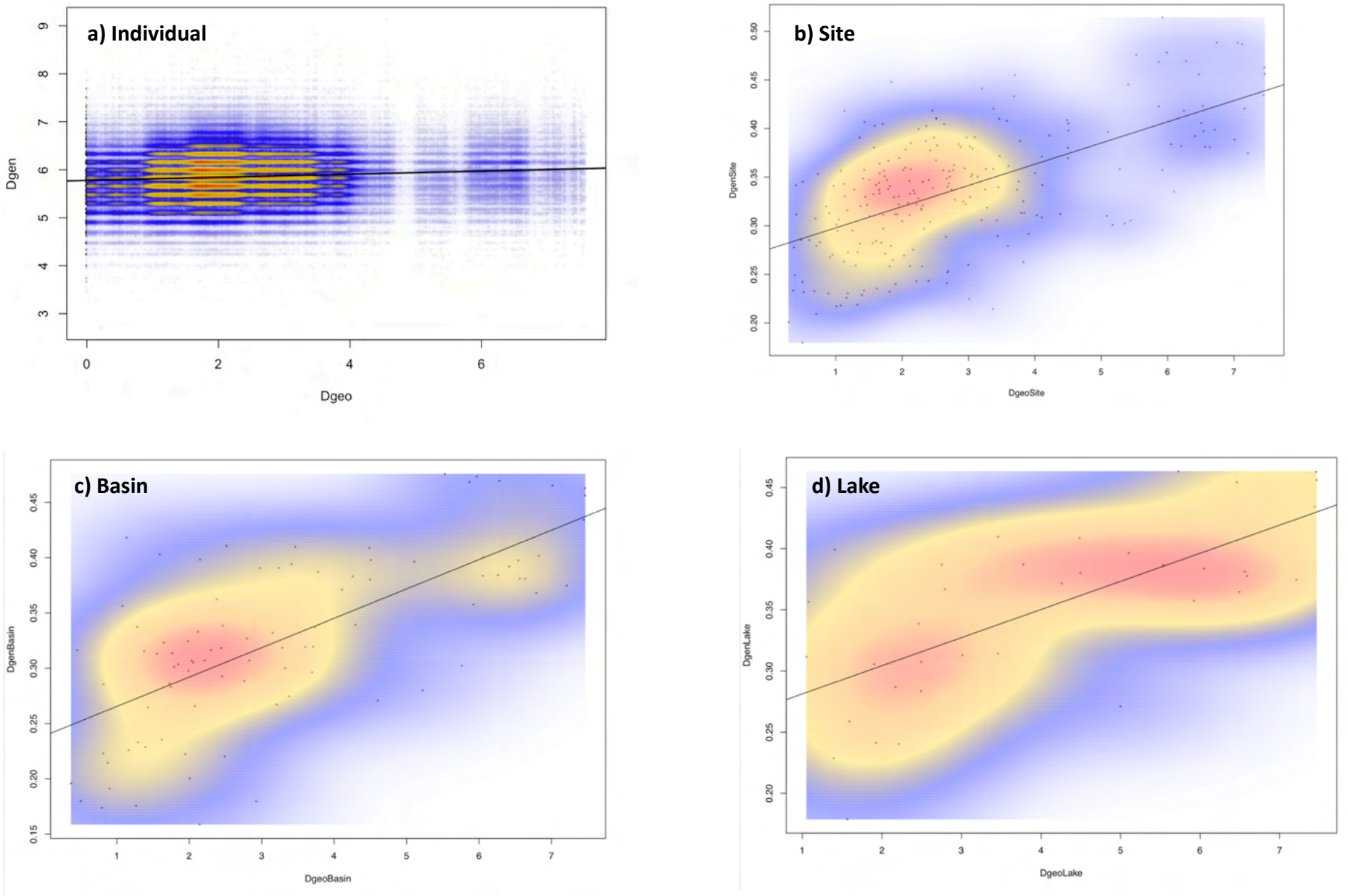


Figure 3.13. Isolation-by-distance (IBD) scatterplots showing the relationship between geographic distance (x-axis) and Euclidean genetic distance (y-axis) of **a)** individuals and populations at the **b)** Site, **c)** Basin, and **d)** Lake population strata. Point density for each scatterplot is visualized using a kernel density surface overlay.

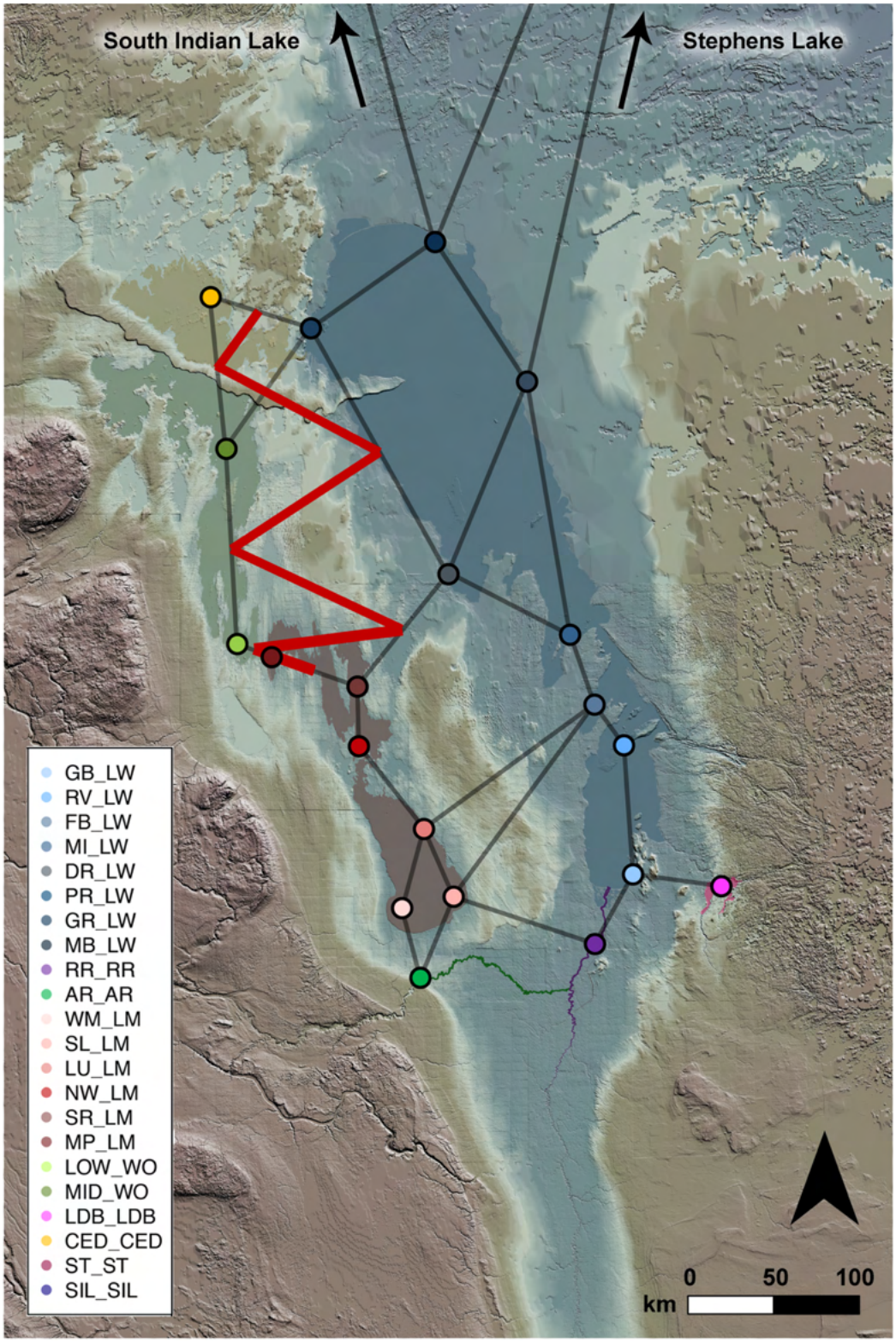


Figure 3.14. Genetic boundaries (red) between sauger populations as defined at the Site population stratum. Boundaries were generated using Monmonier's algorithm as implemented in *adegenet* (Jombart *et al.* 2010). Connection networks (gray lines) were computed with a Gabriel graph algorithm. Networks and boundaries were superimposed over a Digital Elevation Model (DEM) map of Manitoba, which is publicly available at: https://www.manitoba.ca/iem/geo/demsm/index.html

3.4. DISCUSSION

3.4.1 Introgression with Walleye

Fifteen walleye-sauger hybrids were sampled in five waterbodies, but introgression was otherwise low (1.6% across all waterbodies). Only three of these individuals were identified as F_1 sauger, with the remaining fish assigned as backcrosses or hybrids of unknown descent. Hybridization naturally occurs in systems where sauger and walleye coexist, with reported frequencies ranging from 0% to as high as 39% (Billington and Sloss 2011). In Lake Diefenbaker and Tobin Lake—two reservoirs in the Saskatchewan River system—introgressive hybridization rates were as high as 12% (Graham *et al.* 2021). Several factors are known to increase the frequency of sauger-walleye hybridization. Sauger and walleye both spawn in late spring over rocky substrate, with sauger typically spawning slightly later than walleye (Stewart and Watkinson 2004; Barton and Barry 2011). In some instances, the start of the sauger spawn overlaps with the end of the walleye spawn, which increases the likelihood of sauger-walleye interactions (Billington and Sloss 2011). Hybridization is also thought to increase at higher turbidities, wherein walleye and sauger may struggle to visually discern between species (Billington 1997). This could explain the elevated rates of introgression observed at the Red River (4.3% frequency) and Grand Beach (4% frequency) sites, whose water is especially turbid (ECCC and MARD 2020). This is also consistent with my observations on the Master Angler website as a sizeable proportion of trophy sauger caught on the Red River and Assiniboine River appeared to be saugeye (Master Angler Record Book). Hybridization also occurs more frequently when either species is first introduced into a system (White *et al.* 2005; Billington and Sloss 2011; Bingham *et al.* 2012). This may account for the introgression observed

in Stephens Lake (5.9% frequency) as sauger have only recently appeared in this reach to a meaningful extent (CAMP 2023, unpublished data). Researchers have suggested that sauger were extirpated from the Laurentian Great Lakes due to introgression with walleye (see Chapter 1, section 1.2), wherein remnant sauger stocks were "hybridized out of existence" (Regier *et al.* 1969; Johnston 1977). Testimony from commercial fishers seemed to also suggest this scenario took place in Lake Winnipegosis (G. Parker, personal communication, November 5, 2021). However, the rate of introgression was not significantly higher in Lake Winnipeg sauger in 1980 and 1981 (2.7% frequency) than in other contemporary sauger populations.

3.4.2 Genetic Diversity

Genetic diversity of sauger populations was unrelated to the size of the sample waterbody. Allelic richness was highest in Stephens Lake and Lake Winnipeg, and lowest in Lake Manitoba and Lake Winnipegosis (Table 3.2, Figure 3.5). Low allelic richness is usually associated with small population sizes or genetic bottlenecks (Frankham *et al.* 2010), so the lack of genetic diversity in Lake Manitoba and Lake Winnipegosis—two of Manitoba's largest waterbodies—may seem counterintuitive at first glance. However, this result may depict the loss of genetic diversity that likely occurred during repeated collapses of sauger stocks in these waterbodies (Hamilton 2009). The allelic richness observed in Stephens Lake, on the other hand, is confounding. Stephens Lake is located at the northernmost extent of the sauger's natural range, and sauger were not abundant there until the turn of the century (CAMP 2023, unpublished data); I would expect genetic diversity to be low due to the founder effect (Frankham *et al.* 2020). I can think of only two scenarios in which the observed allelic richness is possible. First,

mean allelic richness may be skewed by small sample sizes. Allelic data loss in the Stephens Lake samples was high (6.25%), so only 22 sauger were used when calculating A_R. Thus, it is possible that allelic richness estimates would equilibrate at a lower value with increasing sample size. When allele discovery is mapped with an allele rarefaction curve; however (Figure 3.5), this scenario seems unlikely. Second, this result may represent admixture of two genetically dissimilar sauger populations. Historically, sauger populations in the Churchill River and Nelson River watersheds were reproductively isolated. With the creation of the Churchill River Diversion (MREM 1973), these populations are now able to interact. In this regard, Stephens Lake may contain sauger with origins in both the Nelson River and the Churchill River.

There was little evidence of persistent inbreeding in any of the sauger populations I sampled. While a heterozygote deficiency was observed in the Red River and Cedar Lake sample populations, only one sauger in Cedar Lake had an inbreeding coefficient ($\bar{F}$) greater than 0.4 (3.3% frequency). Individuals with $\bar{F}$ < 0.4 were also documented in Lake Winnipeg (2.2% frequency), Lake Manitoba (2.6% frequency), and Lac du Bonnet (4.0% frequency), though most sauger in these waterbodies had a coefficient at or below 0.1 (Figure 3.6). No sauger in Lake Winnipegosis had an inbreeding coefficient greater than 0.4. This was surprising, as these sauger samples were collected in the early 1980s when sauger stocks were already depleted (Lysack 2006; Nicholson 2007). By comparison, historic Lake Erie sauger samples tested by Hartman *et al.* (2019) had elevated inbreeding coefficients relative to extant sauger populations. Inbreeding is often one of the final steps in the extirpation of a population or species (Höglund 2009; Jobling *et al.* 2014), but inbreeding was not a factor in the Lake Winnipegosis sauger population as late as 1981.

3.4.3 Gene Flow and Population Structure

Genetic differentiation indices, neighbour-joining trees and population assignment algorithms all depicted sauger population structure similarly. Sample populations within Lake Winnipeg were genetically similar to each other, as were sample populations within Lake Manitoba (Appendix 3.H, Appendix 3.I). Sauger in South Indian Lake were genetically distant from other populations in the study area, which coincides with longstanding geographical isolation between the Churchill River and Nelson River watersheds (MREM 1973). Conversely, there were several pairs of Lake level sauger populations that actually comprised a single genetic stock. The Assiniboine River and Red River sample sites were about 150 km apart by waterway distance, yet sauger sampled at these locations were genetically indistinguishable (Table 3.10). Likewise, historic Lake Winnipegosis sauger and contemporary Lake Manitoba sauger could not be differentiated (Figure 3.9, Figure 3.10). STRUCTURE outputs superficially suggest that Cedar Lake and South Indian Lake may comprise a single genetic stock (Figure 3.9), but pairwise F_{ST} estimates between these two waterbodies were high (Table 3.10); I suspect this result is an artifact of data loss and small sample sizes. There were also instances of unexpected or excessive population admixture. Sauger sampled in Grand Beach were more similar to the Red River population than other Lake Winnipeg (Site) populations: 58.3% of Grand Beach sauger were assigned to the Red-Assiniboine stock, 75% of which had a posterior membership coefficient higher than 0.5. Moreover, sauger in Stephens Lake appeared to display genetic signatures from both Lake Winnipeg (*q*-membership > 0.5 = 39.3%) and South Indian Lake (*q*-membership > 0.5 = 42.9%; Figure 3.9, Figure 3.10). This finding supports my suggestion that gene flow is occurring between the Churchill River and Nelson River through the Churchill River

Diversion. Genetic substructure existed at all *a priori* population strata to varying degrees, but all population structure was derived from at least four primary genetic sauger stocks (K = 4): Lake Winnipeg; Lake Manitoba and Lake Winnipegosis; the Red and Assiniboine rivers; and the Churchill and Saskatchewan rivers. That said, I would not go so far as to declare these groups as the only distinct genetic stocks in the study area. Rather, I was only able to resolve four distinct genetic stocks with this study's microsatellite suite and sample sizes. Five of the twelve microsatellite loci showed little divergence among sample populations (Table 3.7), and STRUCTURE results were noisy and lacked precision at the individual level (Figure 3.9). I suspect that additional stock structure would be elucidated with increased sample size and a greater number of microsatellites.

Genetic differentiation of sauger populations was strongly correlated with geographic distance. DAPC scatterplots (Figure 3.10, Appendix 3.J, Appendix 3.K) closely resembled the spatial distribution of sample sites in the study area (Figure 1.1) despite the analysis being completely aspatial (Jombart 2010). Mantel tests confirmed a significant relationship between the genetic and geographic distances of sauger populations at all *a priori* population levels (Figure 3.12), and isolation-by-distance (IBD) scatterplots confirmed that this relationship was positive (Figure 3.13). This is also consistent membership coefficients computed in STRUCTURE when K = 4. The Grand Beach sample site in Lake Winnipeg is in close vicinity to the Red River sample site, and most sauger from Grand Beach displayed genetic signatures from the broader Red–Assiniboine genetic stock (Figure 3.9). Similarly, many sauger from Grand Rapids showed admixture with the neighbouring Cedar Lake population (membership > 0.5 = 34.7; Figure 3.9). There also appeared to be signatures of the Manitoba–Winnipegosis genetic stock within the

Dauphin River sample population, but none of the sauger at this site were assigned as Lake Manitoba fish.

While the isolation-by-distance model explains most genetic distance and gene flow observations, there is evidence for genetic barriers within the study area. IBD scatterplots displayed clusters above the clinal trendlines (Figure 3.13), which suggests the presence of populations with higher genetic differentiation than expected relative to geographic distance (Jombert 2008). Monmonier's algorithim also revealed abrupt breaks in genetic distance when visualized with a local distances plot (Appendix 3.P), which is consistent with physical barriers to gene flow (Manni *et al.* 2004). When plotted over geographic space, Monmonier's algorithm identified immediate barriers to gene flow between Lake Winnipeg, Lake Winnipegosis, and Cedar Lake (Figure 3.14). These geographic lines correspond with ridge formations separating these waterbodies including the Pas Moraine, which separates Cedar Lake from Lake Winnipegosis, and the fluted till plain between the Pas Moraine and Hodgson Plain, which separates Lake Winnipeg from Lake Winnipegosis (EDITNR 2023b). Although these three waterbodies are in close geographic proximity, the high degree of genetic differentiation between sauger populations suggests that the surrounding landforms have effectively restricted gene flow (Frankham *et al.* 2004; Manni *et al.* 2004; Holderegger and Wagner 2006).

The most surprising results in this study may be the lack of historical and contemporary gene flow between Lake Manitoba and Lake Winnipeg. BayesAss models predicted that fewer than 2% of sauger in Lake Manitoba were Lake Winnipeg migrants, and that less than 1% of sauger sampled in Lake Winnipeg had Lake Manitoba origins (Figure 3.11). In contrast, BayesAss models predicted that 31.7% of sauger in the Red and Assiniboine rivers originated from Lake

Winnipeg. Superficially, this seems to support the argument that the Fairford River water control structure is obstructing fish movements between Lake Winnipeg and Lake Manitoba (LMRRAC 2003; Lysack 2006). However, the high degree of genetic differentiation between sauger in Lake Winnipeg and Lake Manitoba suggests that gene flow between these waterbodies was negligible long before th Fairford River water control structure was built (LMRRAC 2003). Genetic differentiation between Lake Winnipeg and Lake Manitoba (F_{ST} = 0.0309) was higher than every other pairwise Lake Winnipeg comparison except South Indian Lake (F_{ST} = 0.0347; Table 3.10). DAPC showed strong genetic separation between Lake Winnipeg and Lake Manitoba samples at the first discriminant function (Figure 3.10), and the Lake Winnipeg and Lake Manitoba–Lake Winnipeg stocks were also the first genetic clusters identified by STRUCTURE when *K* = 2. Little gene flow is required to mitigate population differentiation (ex. the "one-migrant-per-generation" principle; Mills and Allendorf 1996; Frankham *et. al.* 2004). For context, interactions between sauger in Lake Winnipeg and Cedar Lake should be rare due to the steep gradient between waterbodies (Brunskill *et al.* 1980) and the presence of the Grand Rapids Generating Station. Yet, sauger sampled in Grand Rapids were genetically similar to sauger in Cedar Lake, and some individuals were identified as Cedar Lake sauger (Figure 3.9). Thus, admixture between the Cedar Lake and Grand Rapids sauger populations appears to be maintained by incidental spillover of Cedar Lake sauger into Lake Winnipeg. The lack of admixture between Lake Manitoba and Lake Winnipeg would therefore suggest that the Fairford River was never a major migratory corridor for sauger. Moreover, the high level of genetic differentiation between these waterbodies indicates that the Lake Manitoba sauger population diverged from the Lake Winnipeg population around the same

time as Cedar Lake and South Indian Lake. This is likely to have occurred when these waterbodies become geographically isolated, which palaeogeographers attribute to the recession of glacial Lake Agassiz around 8000 years ago (McMartin 2000; Hillaire-Marcel *et al.* 2008).

Note: Applications of these data patterns and findings to fisheries management are discussed in Chapter 4 of this thesis.

Chapter 4: General Discussion

4.1 Synthesis

The overarching goal of this thesis was to resolve and describe the population structure of sauger in Manitoba. I used life history indices, stable isotopes and population genetics to achieve this goal. Although each method would have delineated sauger populations on its own to some extent, this multidisciplinary approach gives fisheries managers the ability to define sauger stocks and management units from both a demographic and genetic perspective. I have consolidated the insights gained from each approach below, summarized by waterbody or Study Region.

The population structure of sauger in Lake Winnipeg is difficult to define. The genetic substructure I did observe is subtle and consistent with the isolation-by-distance model, but most sauger from Lake Winnipeg appear to have originated from a single genetic stock. The genetic stock structure of sauger in Lake Winnipeg may best be described as panmictic, with localized genetic signatures from connected systems such as the Red River and Cedar Lake. However, sauger collected in Grand Beach were more similar to Red River sauger than to other Lake Winnipeg sauger and may therefore belong to the Red–Assiniboine genetic stock. Body condition and length-at-maturity are constant for both sexes, but growth generally decreases and age-at-maturity increases at higher latitudes. Sauger in Grand Beach were significantly smaller at age five than at other sample sites. Because fish in Grand Beach are genetically similar to Red and Assiniboine rivers sauger, which display low asymptotic lengths, it is possible that this smaller size is actually phenotypic in nature. Stable isotope analysis showed that sauger tissues became enriched in ^{13}C and depleted in ^{15}N with increasing latitudes, and that

sauger residing in the south basin occupied a different isotopic niche than sauger in the north basin. Sauger sampled in the channel region displayed a wide but bimodal range of $\delta^{13}C$ and $\delta^{15}N$ values, which suggests that sauger in the south and north basins use the channel as either a migratory corridor or a congregation area during the spawning season.

Sauger in Lake Manitoba and Lake Winnipegosis are effectively the same stock, both demographically and genetically. Sauger in these waterbodies grow quickly and have good body condition, and mature at an early age; growth and body condition is higher in the south basin of Lake Manitoba than the north basin. Female sauger achieve 50% sexual maturity by age four and at fork length of about 300 mm, and 50% of males are mature by age two with a fork length of 180 mm. Sauger in Lake Manitoba and Lake Winnipegosis originate from a single broad genetic stock, which has likely not interacted with the Lake Winnipeg genetic stock to any meaningful extent throughout modern history. A small amount of migration still occurs, however, as evidenced by slight genetic admixture near the Dauphin River and the capture of two sauger in Lake Winnipeg with isotopic signatures from Lake Manitoba.

Life history indices suggest that sauger in the Winnipeg River system are comprised of at least two unique stocks. Sauger grew faster in Lac du Bonnet than in Eaglenest Lake and Point du Bois but had a shorter asymptotic length; fish were similar in size by age five. Body condition was similar in all waterbodies. L_{50} was small in Lac du Bonnet and large in Eaglenest Lake and Point du Bois, and A_{50} was late in the latter two waterbodies. Interestingly, male sauger in Eaglenest Lake and Point du Bois matured at longer lengths and at older ages than females. Stable isotope analysis suggests that sauger may occasionally migrate from the Winnipeg River

to Lake Winnipeg, but I was unable to discern between sauger from Lake Winnipeg and the Winnipeg River using my current microsatellite suite.

The two sample populations I assessed in the Saskatchewan River system also showed differing life histories. Sauger in the Saskatchewan River had lower body conditions than sauger in Cedar Lake but grew faster and matured at longer lengths. L_{50} in Cedar Lake was small relative to Lake Winnipeg, and males matured at an earlier age. Sauger in Cedar Lake are genetically distinct from other sample populations in this study, though incidental admixture does occur where Cedar Lake empties into Lake Winnipeg.

Sauger life histories in the Nelson River generally presented as a latitudinal extension of Lake Winnipeg. For the most part, growth and length-at-age-five was comparable to Lake Winnipeg's north basin, as were the average lengths and ages at 50% maturity. However, three populations deviated from this trend. Females in Cross Lake matured at smaller sizes and an early age. Males in Split Lake matured late and at larger sizes, whereas males in Stephens Lake matured early compared to other Nelson River populations. Surprisingly, population genetic analyses revealed that the sauger population in Stephens Lake may have origins in both Lake Winnipeg and the Churchill River.

The life histories of sauger in the Churchill River can be summarized with two words: "slow" and "small". Sauger in this system grow slowly, have low body conditions, and mature at a late age. Female and male reach sexual maturity at a similar length, with an L_{50} equal or slightly larger than the L_{50} of males in Lake Winnipeg. These observations also apply to sauger sampled in the Churchill River Diversion. Sauger in South Indian Lake are genetically distinct

from all other sample populations in this study but are least differentiated with the sauger in Stephens Lake.

4.2 Management Implications

The demographic and genetic population structures I described in this thesis will assist fisheries managers in defining functional stock and management units for sauger in Manitoba. I will use the results from Lake Winnipeg to illustrate how these findings can help resolve functional stock structure. Sauger in the north basin, channel, and Riverton/Hecla sites are comprised of a single, panmictic genetic stock and show minimal genetic divergence. Stable isotope analysis also revealed that a portion of Lake Winnipeg sauger will migrate between basins shortly before the spawn. Together, these findings suggest that sauger migrate freely between basins and that the observed variations in life histories are likely due to the environmental factors or recent selective pressures. In contrast, sauger from Grand Beach are genetically similar to sauger in the Red River which, along with the Assiniboine River, comprise a separate genetic stock altogether. This suggests that sauger in the lower portion of Lake Winnipeg's south basin interact more with sauger in the Red River than in the rest of Lake Winnipeg, and that the observed differences in life histories may be phenotypic in nature. Moreover, sauger stocks in the lower reaches of Lake Winnipeg's south basin will rely heavily on immigrant subsidies from the Red River in the event of a fisheries collapse.

Fisheries managers should find the life history section of this thesis both useful and affirming when establishing commercial and recreational angling regulations, especially when optimizing mesh and slot sizes. One of the biggest contributors to fisheries collapses is the overharvest of juvenile fishes; if cohorts are unable to recruit into adulthood, the fishery will fail

from the bottom up (Beamish and Rothschild 2009). It is therefore critical to set mesh and slot size limits that will protect juvenile sauger until they can spawn for one or several seasons (Methot 2009; Task Force 2011). My results show that sauger populations across Manitoba grow and mature at different rates, and that a "one size fits all" approach will not address the management concerns of all populations. If 3.5" mesh was permitted in both Lake Winnipeg and Lake Manitoba, for example, the number of spawning opportunities for sauger in Lake Manitoba would be greatly limited. Thus, fisheries managers can use my research to tailor their sauger management strategies according to the life histories of their target populations.

My stable isotope analysis results suggest that the channel area of Lake Winnipeg may serve as an important migratory corridor or spawning zone for sauger in the south and north basins. The spaces between Hecla Island, Black Island and the mainland are pinch points that all sauger in the south basin must pass before entering the Lake Winnipeg channel, and I have personally seen these areas inundated with commercial nets in the spring. As the commercial season initially opens when 80% of walleye have spawned (Task Force 2011), it is likely that most of the sauger captured in these nets have not yet spawned. Managers should take this into account when planning for future commercial seasons. Conversely, stable isotope and population genetic analyses both demonstrate that sauger rarely migrate between Lake Manitoba and Lake Winnipeg. The high genetic distance between Lake Manitoba and Lake Winnipeg sauger also indicates that gene flow between these waterbodies has been minimal for many millennia. It is therefore unlikely that the Fairford River water control structure has or ever had any major effect on the Lake Manitoba sauger population.

Fisheries managers have considered developing a stocking program to restore sauger numbers in Lake Winnipegosis. In fact, this thesis was created in part to identify sauger populations that could act a genetically compatible surrogate stock. My research shows that Lake Manitoba would be the best source for donor sauger for this stocking program, as the genetic stocks in Lake Manitoba and historical Lake Winnipegosis are one and the same. Unfortunately, this finding brings with it more questions than answers. If sauger regularly travelled between these waterbodies in the past, why hasn't the Lake Winnipegosis population re-established on its own? The question managers should ask now is not what surrogate sauger stock is compatible with Lake Winnipegosis, but why Lake Winnipegosis may no longer be compatible for sauger.

4.3 Future Work

My thesis served both to answer pertinent sauger management questions as well as to lay the groundwork for future research of sauger in Manitoba. As such, I leave behind many unanswered questions that deserve to be explored. The life histories I described in this thesis were the summation of many years of data, and it would be valuable to break these data down further in search of any temporal trends. Some of the indices I calculated should be re-run to account for differences in sampling dates, either with corrective models or supplemental sampling at other times in the year. I also described possible mechanisms for variations in sauger life histories, including commercial fishing pressure, prey availability, and growing degree days; more research is needed for such connections to be made. These connections will likely prove critical in explaining the life history variations within Lake Winnipeg and Lake Manitoba as well as the lack of sauger recovery in Lake Winnipegosis.

The stable isotope analysis section of my thesis was effectively a proof-of-concept. In it, I demonstrated that stable isotopes can be used as geolocator tags to screen for migrant sauger in the Lake Winnipeg watershed. Carbon and nitrogen isotopes are not frequently used in migration studies due to their involvement in the food web (Hobson and Wassenaar 2019), and thus more conventional isotopes such as ^{2}H and ^{87}Sr may be better suited to this context. If ^{13}C and ^{15}N continue to be used, the collection of isotopic baselines would assist in generating isoscapes and even mixing models. Further research should be conducted to realize the potential and limitations of this methodology.

Although the population genetics analyses I conducted in this study were informative, there remains room for improvement. My methods were effective in describing population structure and gene flow at a Lake, Basin, and Site population level, but not at an individual level. Specifically, I was unable to reliably assign individuals to their respective sample populations without *a priori* knowledge of their origins. More microsatellite loci and increased sampling efforts would be needed to achieve this level of resolution. Likewise, more genetic samples are required to confirm the presence of gene flow from the Churchill River into the Lower Nelson River. Lastly, my selection of microsatellite loci has made it possible to perform a meta-analysis of the genetic stock structure of sauger across North America. Each of the loci used in this study have been used in previous genetic sauger studies with compatible methods and reporting. This includes research conducted on sauger populations as far east as Lake Erie, Ontario (Hartman *et al.* 2019) and as far south as the Arkansas River, Arkansas (Jonagan 2022). The potential to synthesize over a decade of genetics research would make for an excellent research project, or perhaps, for some poor, starry-eyed student out there… a Master's thesis.

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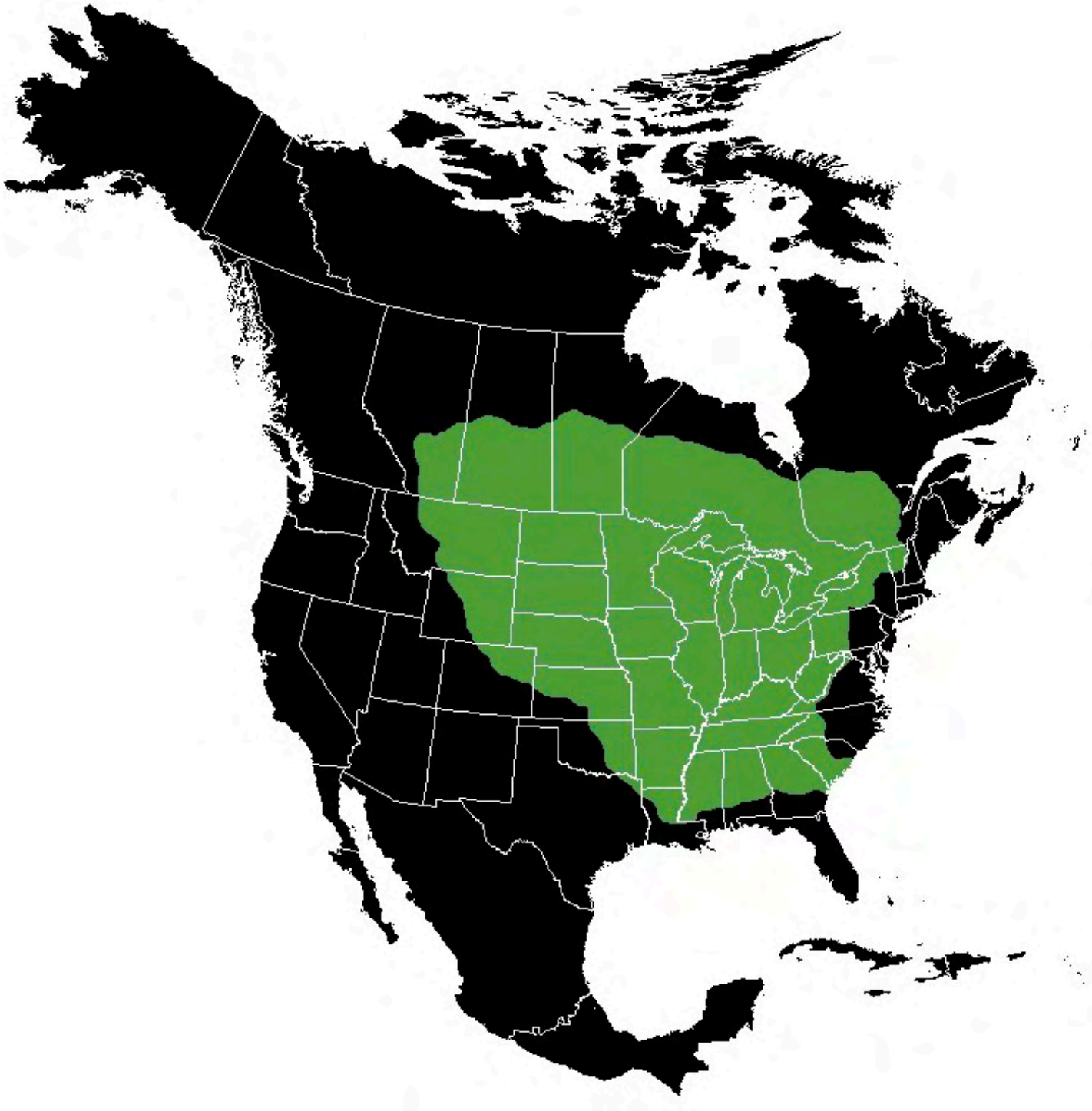
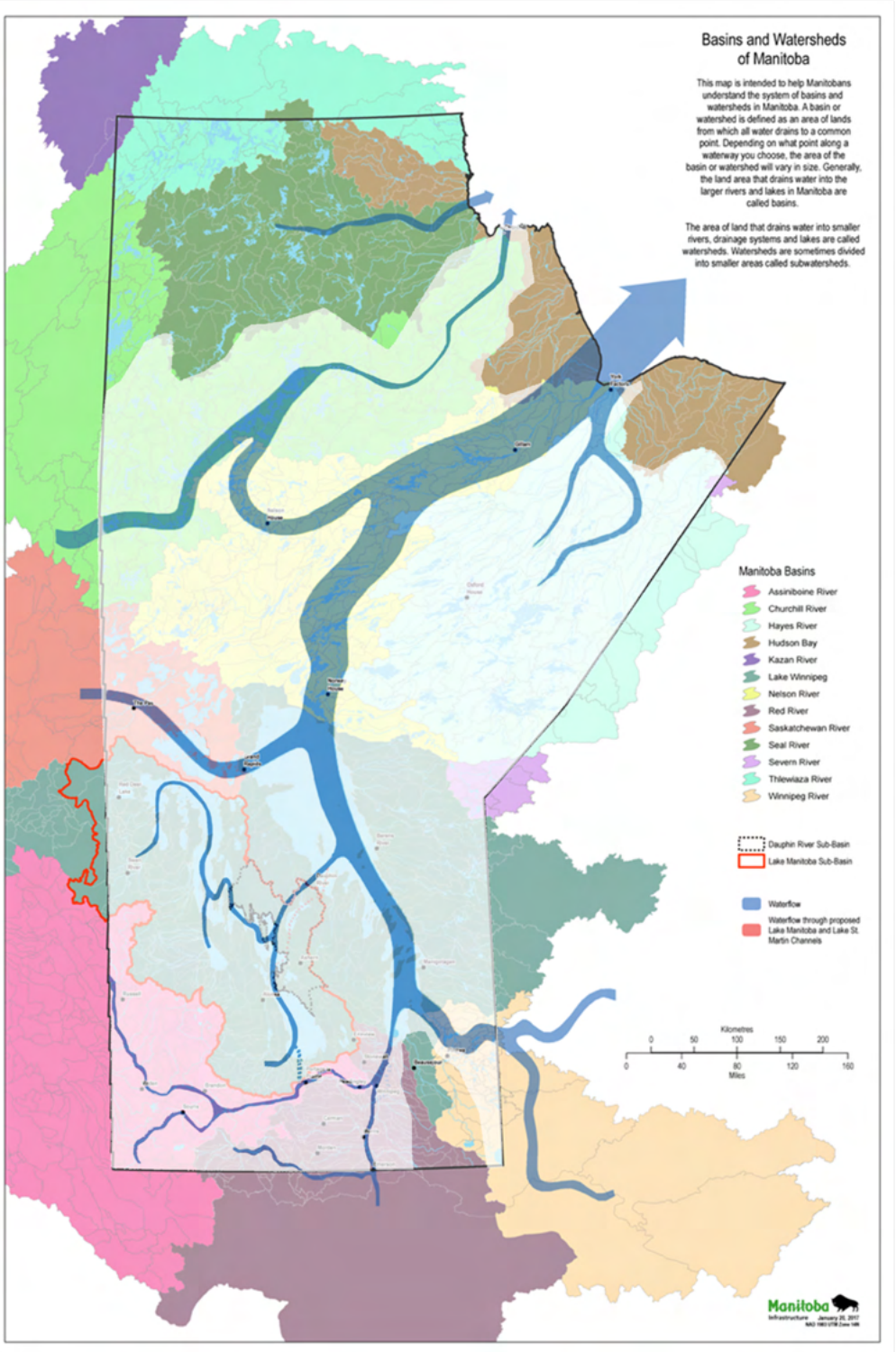
Appendix: Chapter 1

Appendix 1.A. Distribution of sauger (*Sander canadensis*) in North America, adapted from Scott and Crossman (1973). Note that the sauger's range has likely shifted since the source publication, as is the case in Manitoba.

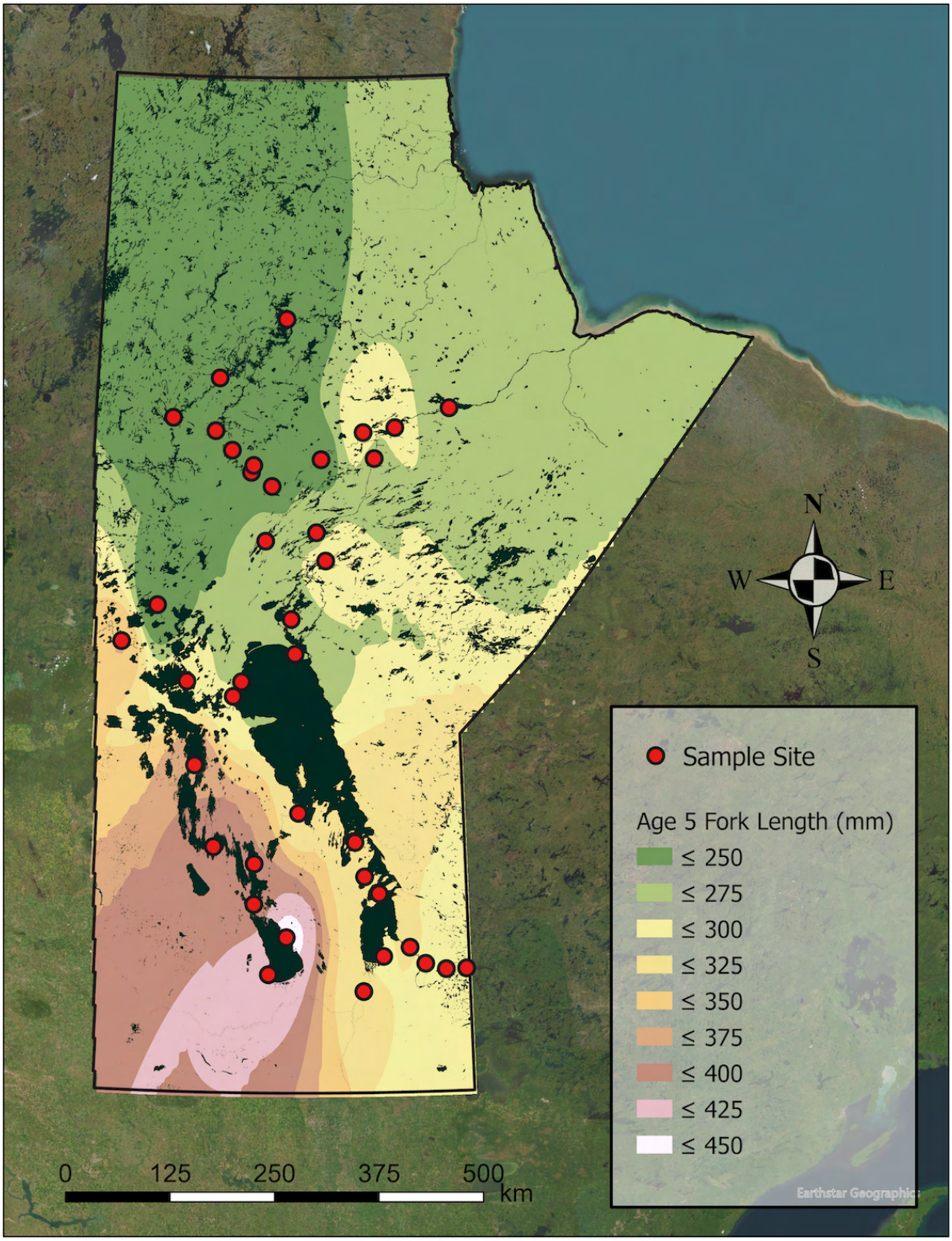


Appendix 1.B Watersheds and water flow in Manitoba, from Manitoba Infrastructure (2017). The range of sauger in Manitoba is superimposed in white and is adapted from Stewart and Watkinson (2004).

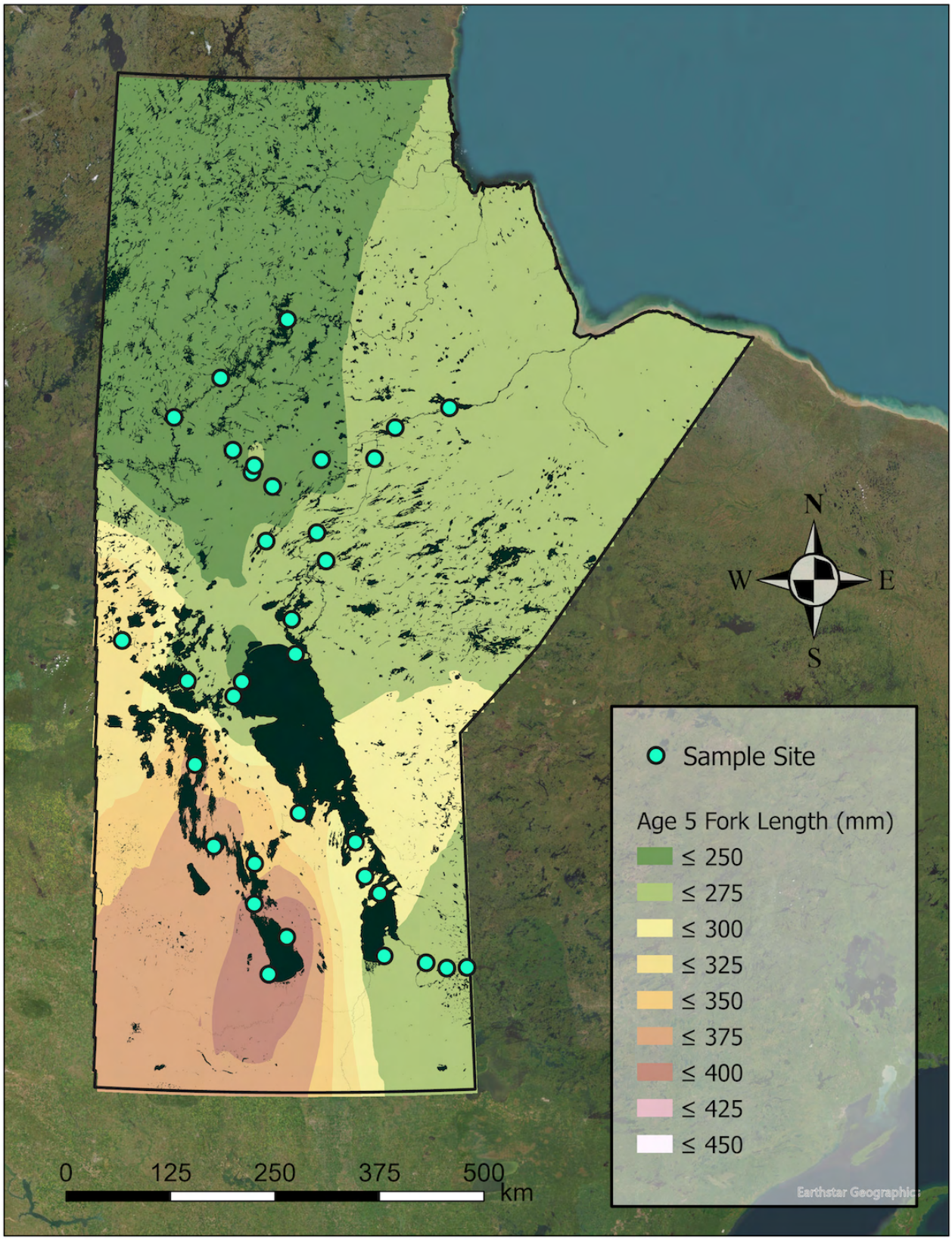


Appendix: Chapter 2

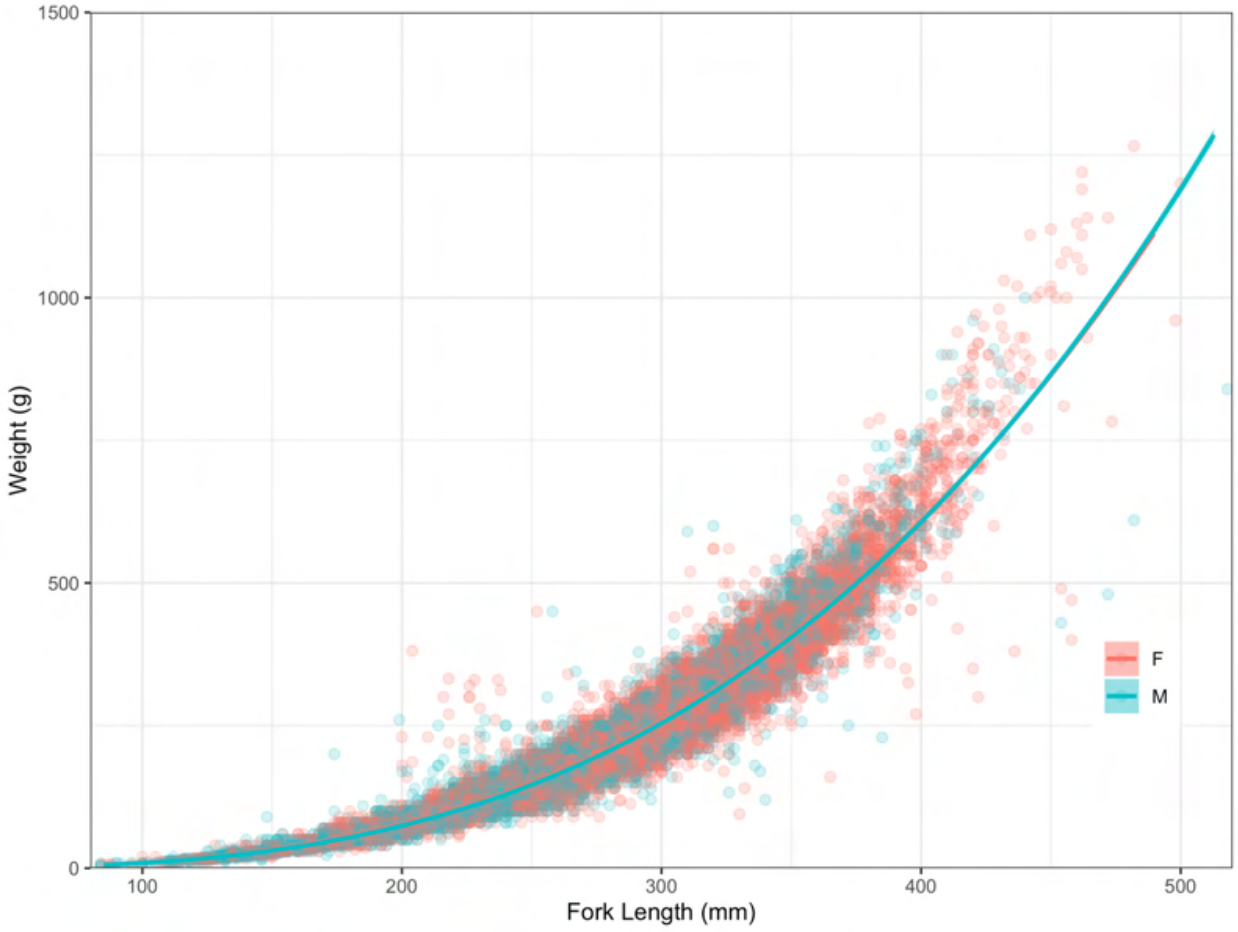
Appendix 2.A. Heat map of the estimated fork length (mm) of **female** sauger at age 5 in Manitoba waterbodies. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.



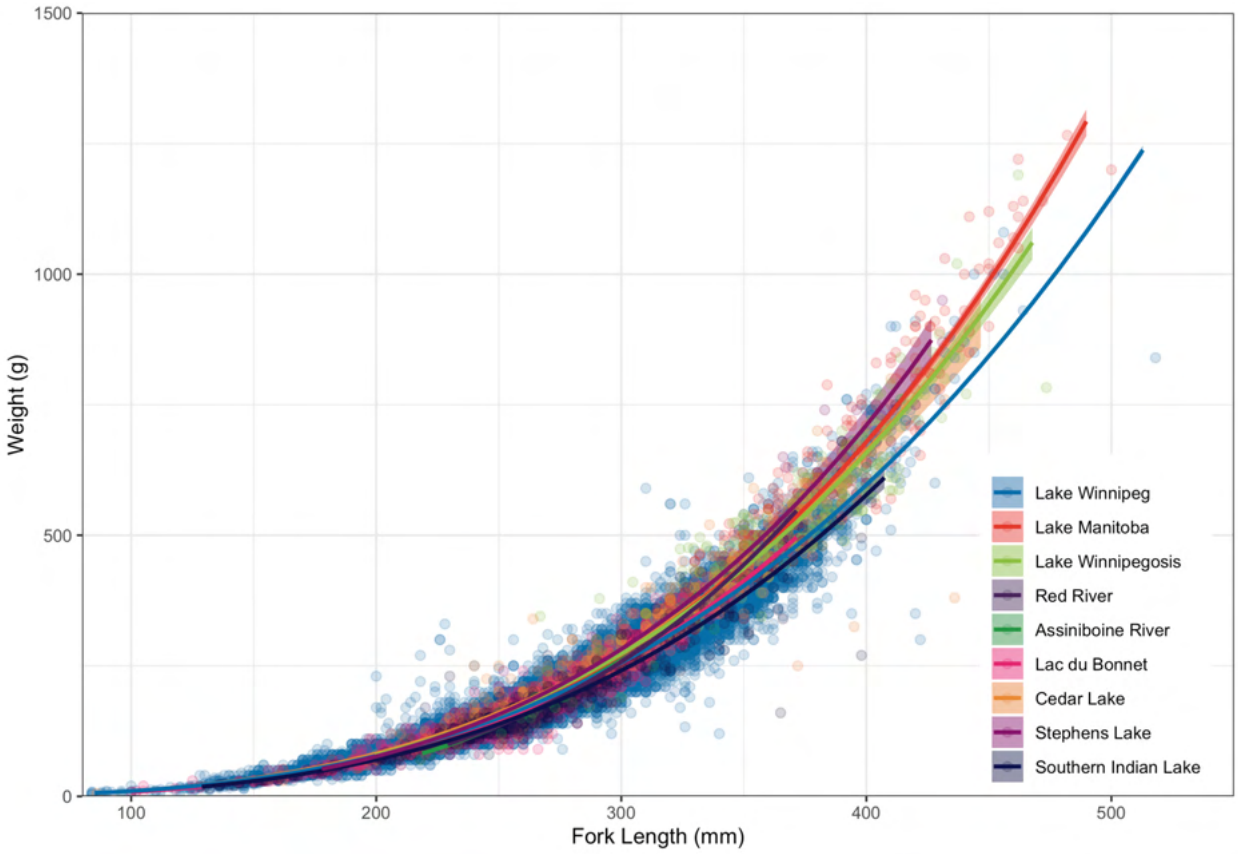
Appendix 2.B. Heat map of the estimated fork length (mm) of **male** sauger at age 5 in Manitoba waterbodies. Gradients were generated in ArcGIS Pro from sample sites (points) using the Inverse Distance Weighting (IDW) tool with an output cell size of 0.1, a power exponent of 3, and a variable search radius incorporating the 6 nearest points. Data are visualized on the NAD83 datum with a Canada Lambert Conformal Conic projection.



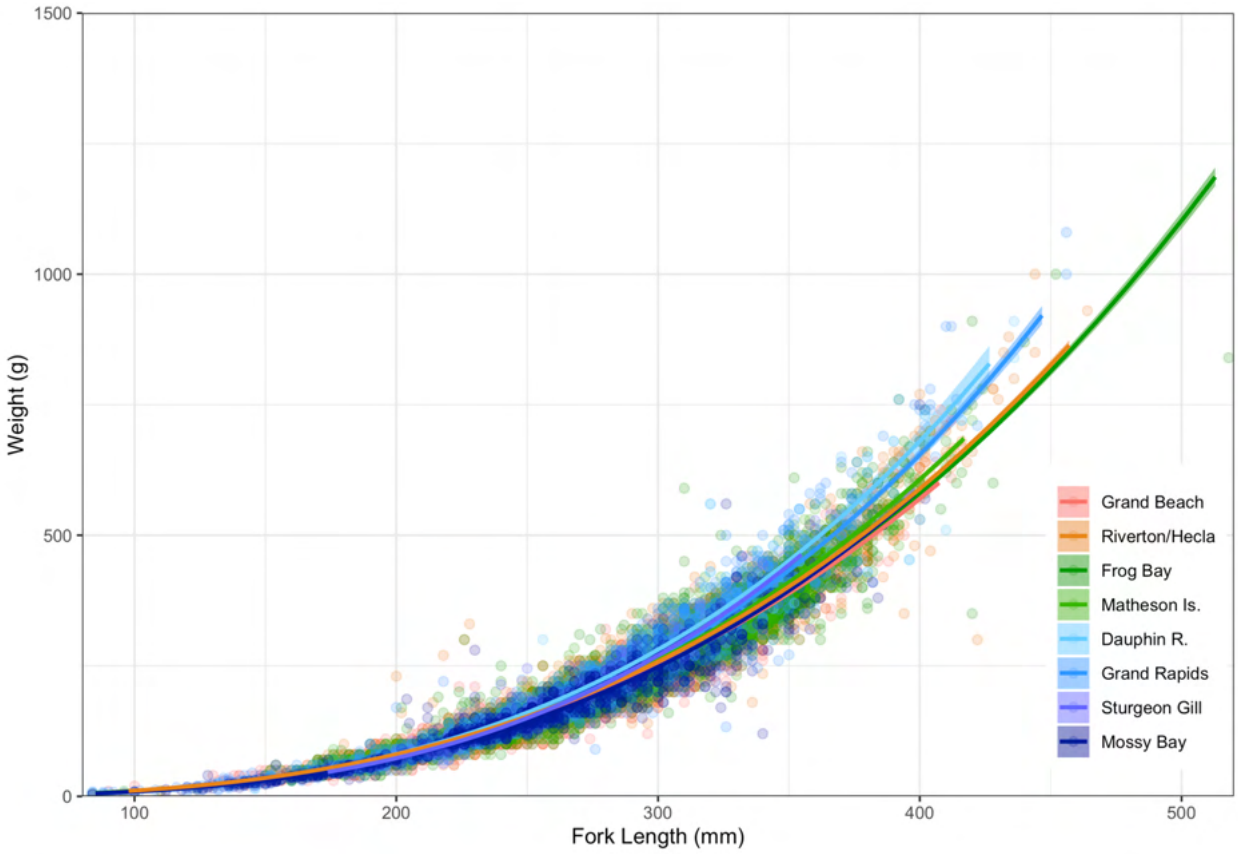
Appendix 2.C. Weight-at-length plot of female (n = 21725) and male (n = 13838) sauger captured across twenty-nine waterbodies in Manitoba. The plot is fitted with a back-transformed simple linear regression models for each sex.



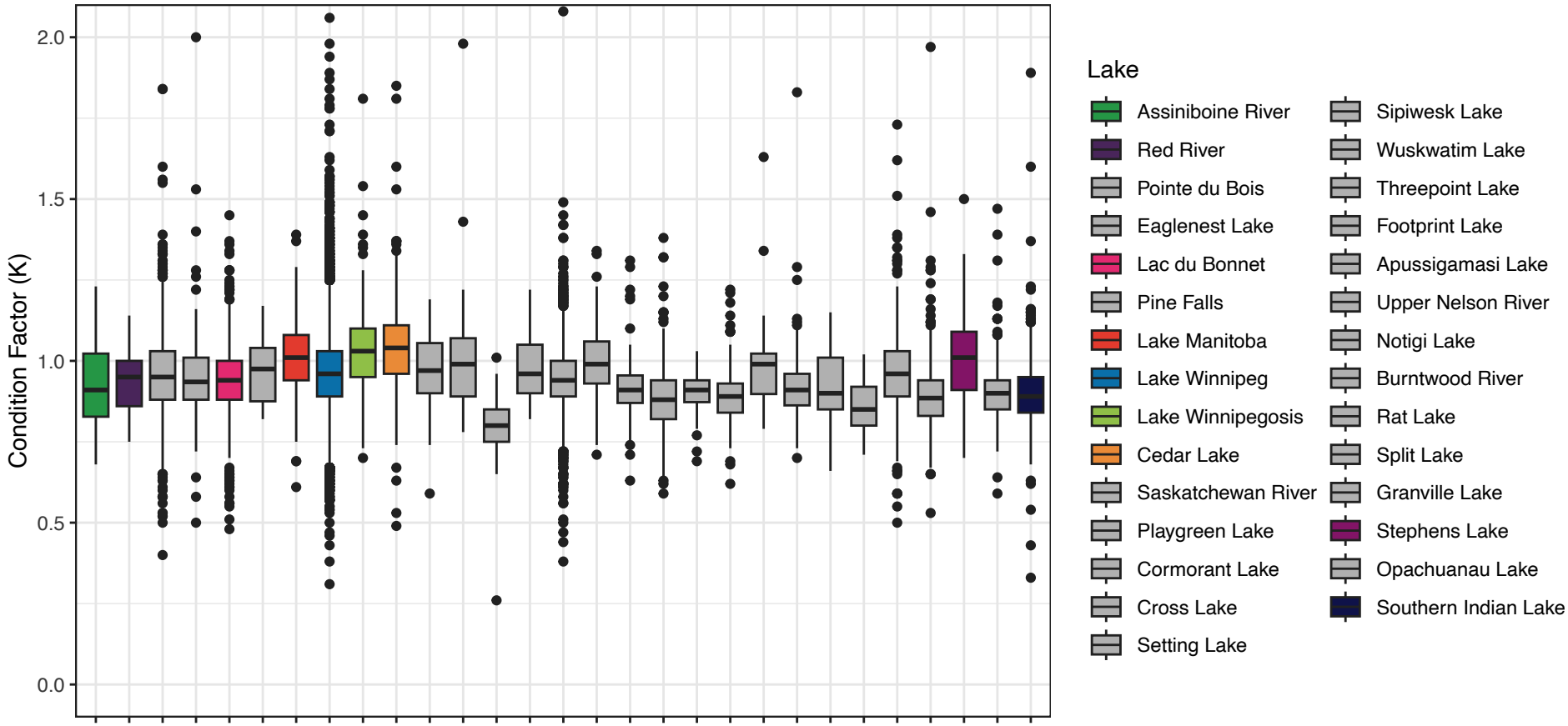
Appendix 2.D. Weight-at-length plot of sauger populations in Manitoba as defined at the Lake population level. The plot is fitted with back-transformed simple linear regression models for each sample population.



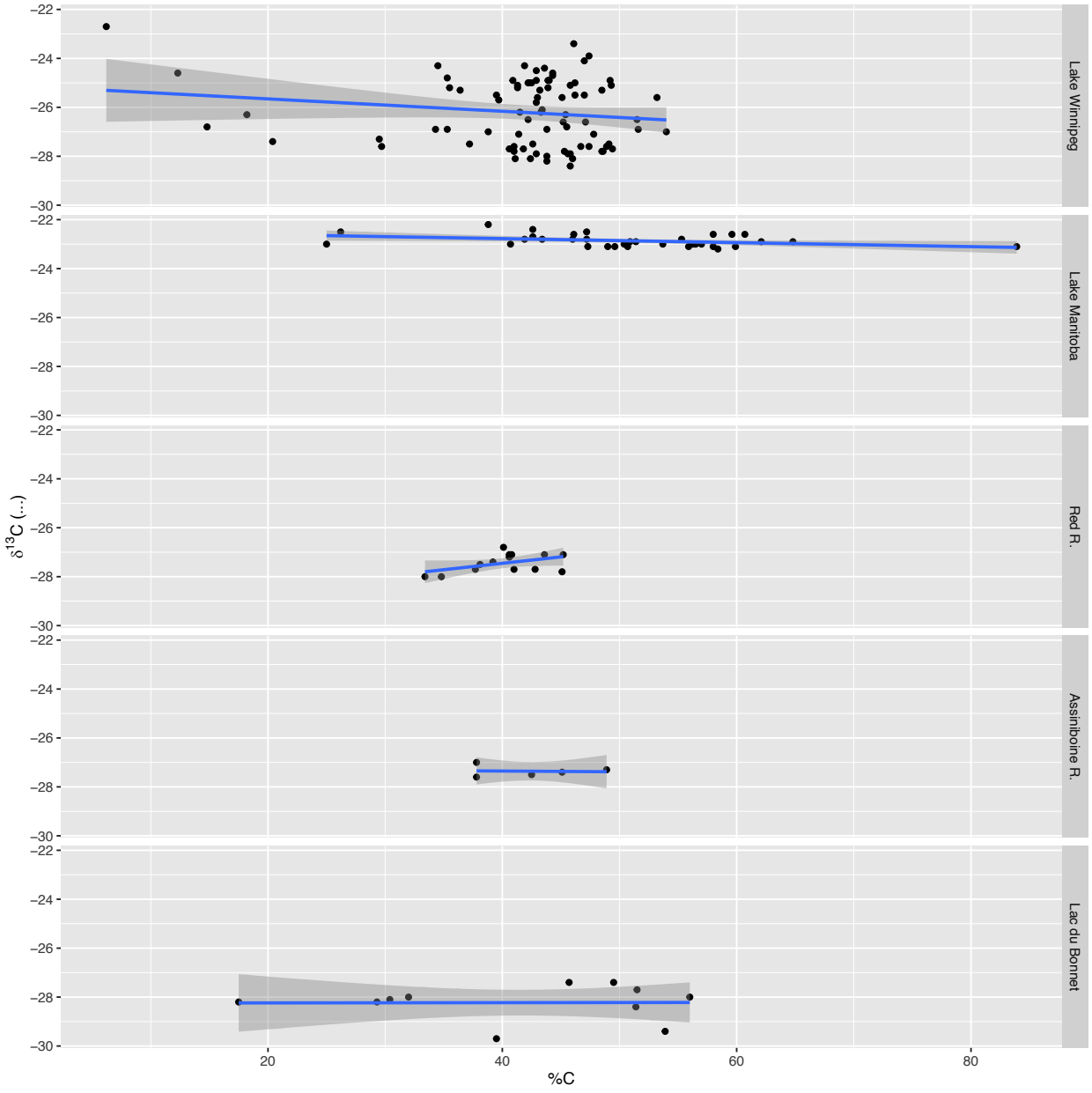
Appendix 2.E. Weight-at-length plot of sauger populations in Lake Winnipeg as defined at the Site population level. The plot is fitted with back-transformed simple linear regression models for each sample population.



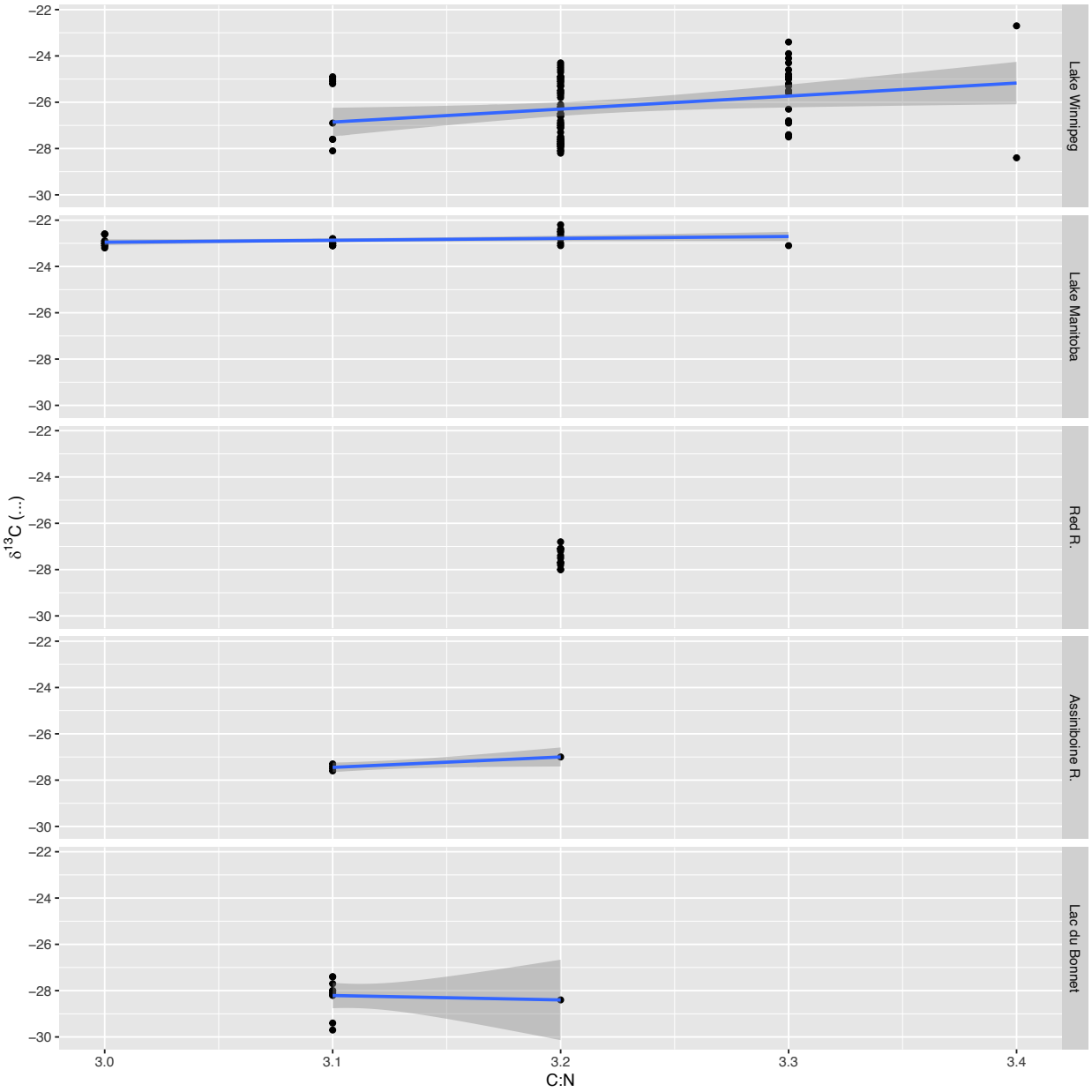
Appendix 2.F. Boxplots of condition factor *K* of sauger populations in Manitoba as defined at the Lake population level. Coloured boxes represent the interquartile range, whiskers represent *K* values within 1.5 times the interquartile range, and points represent outliers beyond 1.5 times the interquartile range. Populations are sorted by increasing latitude.



Appendix 2.G. Carbonate plots showing the effects of %C on $\delta^{13}C$ values of sauger dorsal muscle samples, sorted at the Lake population level. A positive relationship between %C and $\delta^{13}C$ indicates that inorganic carbon is present in the samples Plots are fitted with a simple linear regression.



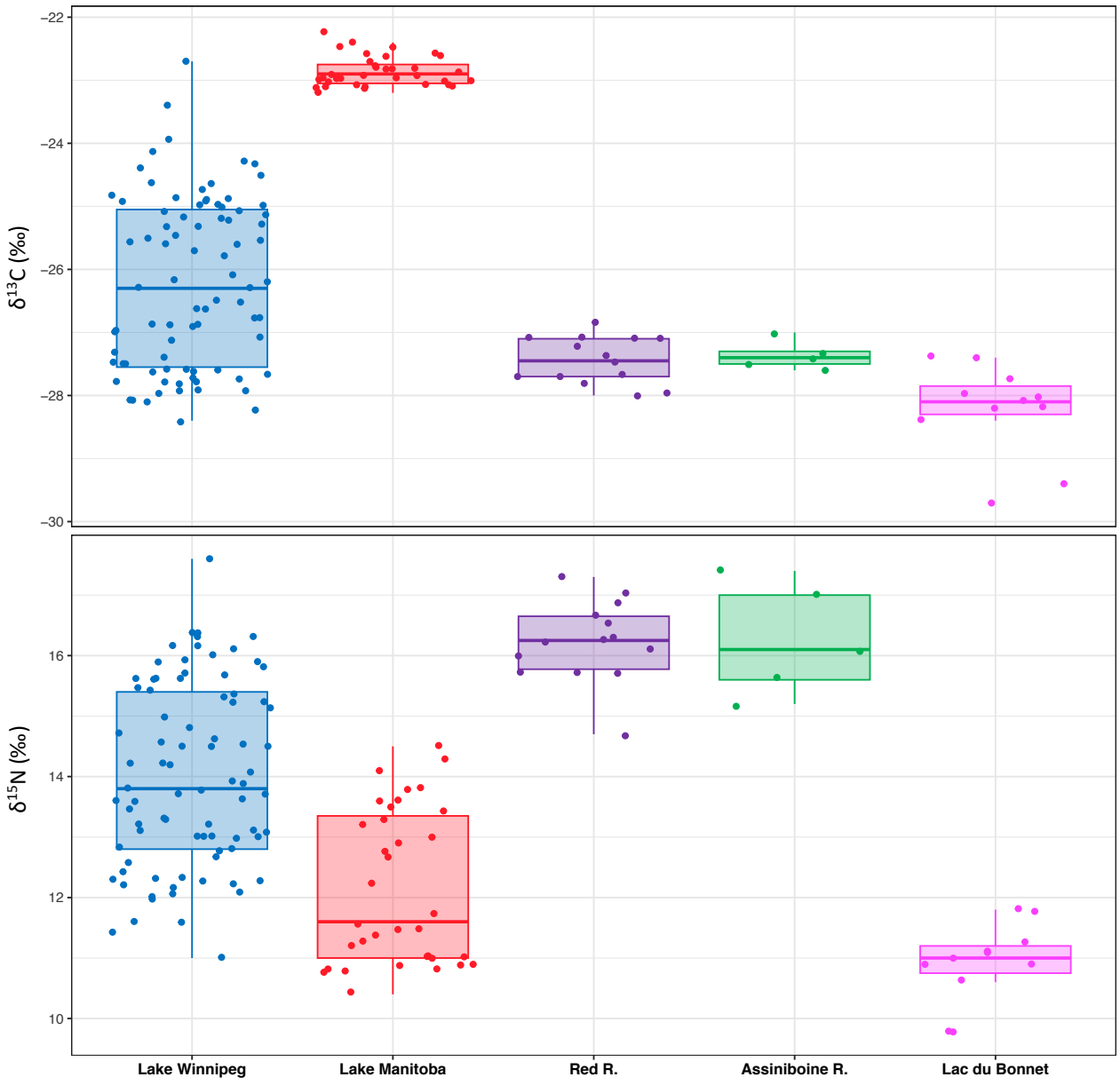
Appendix 2.H. Lipid plots showing the effects of C:N ratios and $\delta^{13}C$ values of sauger dorsal muscle samples, sorted at the Lake population level. Higher C:N ratios indicate a greater presence of lipids in the muscle tissue. Plots are fitted with a simple linear regression.



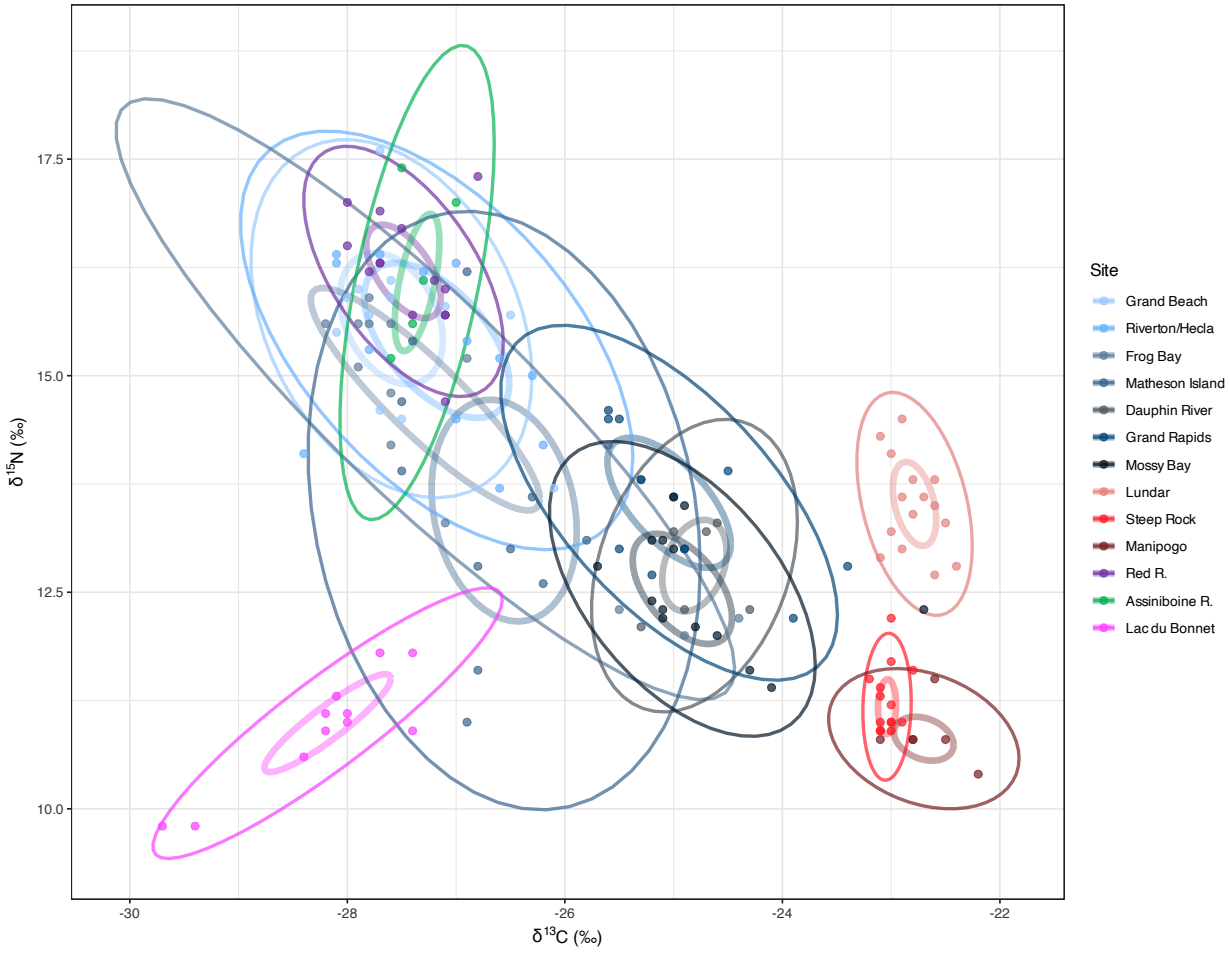
Appendix 2.I. Summary statistics (mean ±SD) for sauger muscle tissue sampled for stable isotope analysis, summarized at the Site population level.

Site Population	Samples	Weight (g)	Fork Length (mm)	Age (years)	Prop. Female	Prop. Maturity	C:N Ratio	$\delta^{13}C$ (‰)	δ^{15N} (‰)
Grand Beach	12	186 (±109)	260 (±63)	4.2 (±2.2)	0.75	0.42	3.2 (±0)	-27.5 (±0.6)	15.6 (±1)
Riverton/Hecla	13	244 (±109)	284 (±46)	5.1 (±2.5)	1	0.38	3.2 (±0.1)	-27.2 (±0.7)	15.3 (±1)
Frog Bay	13	242 (±101)	294 (±42)	6.2 (±2.2)	0.31	0.92	3.2 (±0)	-27 (±1.3)	14.4 (±1.5)
Matheson Island	13	243 (±124)	284 (±59)	5.3 (±3.1)	0.77	0.54	3.2 (±0)	-26.6 (±0.7)	13.5 (±1.4)
Dauphin River	6	231 (±83)	282 (±37)	5.2 (±1.2)	0.67	0.67	3.2 (±0)	-24.8 (±0.3)	12.7 (±0.6)
Grand Rapids	12	219 (±72)	278 (±33)	6.3 (±1.6)	0.67	0.5	3.3 (±0)	-24.9 (±0.7)	13.5 (±0.8)
Mossy Bay	14	175 (±65)	265 (±37)	6.2 (±3)	0.57	0.36	3.2 (±0.1)	-24.8 (±0.7)	12.5 (±0.7)
Lundar	15	327 (±228)	301 (±67)	2.5 (±1.6)	0.4	0.73	3.1 (±0.1)	-22.8 (±0.2)	13.5 (±0.5)
Steep Rock	14	386 (±109)	337 (±28)	4.2 (±1.1)	0.64	1	3.1 (±0)	-23 (±0.1)	11.3 (±0.4)
Manipogo	6	380 (±92)	342 (±24)	3.7 (±0.8)	0.67	1	3.2 (±0)	-22.7 (±0.3)	10.9 (±0.4)
Red River	14	283 (±79)	303 (±27)	4.1 (±1.2)	0.86	0.86	3.2 (±0)	-27.4 (±0.4)	16.2 (±0.7)
Assiniboine River	5	138 (±66)	255 (±35)	0 (±0)	0.8	0.2	3.1 (±0)	-27.4 (±0.2)	16.3 (±0.8)
Lac du Bonnet	11	171 (±122)	251 (±63)	3.5 (±2.8)	0.82	0.27	3.1 (±0)	-28.2 (±0.7)	10.9 (±0.7)

Appendix 2.J. Box plots of $\delta^{13}C$ and $\delta^{15}N$ values of sauger dorsal muscle samples, summarized at the Lake population level. Coloured boxes represent the interquartile range, whiskers represent isotope values within 1.5 times the interquartile range, and points represent outliers beyond 1.5 times the interquartile range.



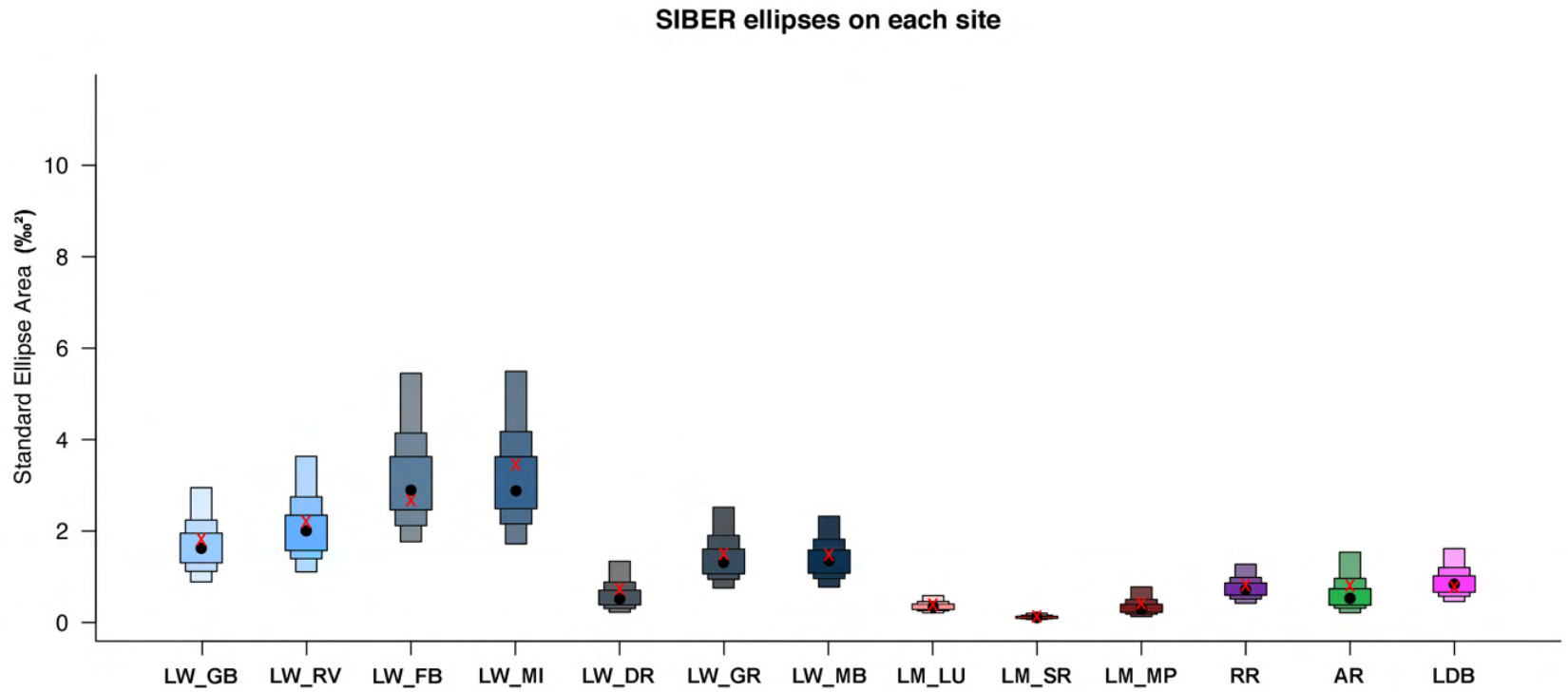
Appendix 2.K. Ellipse plot of $\delta^{13}C$ and $\delta^{15}N$ values of sauger dorsal muscle samples, summarized at the Site population level. Central ellipses represent the standard ellipse area, and outer ellipses represent the 95% ellipse area.



Appendix 2.L. Estimates of $\delta^{13}C$ x $\delta^{15}N$ niche size (‰^{2}) at the Site population level, expressed as total area (TA), standard ellipse area (SEA), and sample size corrected standard ellipse area (SEAc).

	Grand Beach	Riverton / Hecla	Frog Bay	Matheson Island	Dauphin River	Grand Rapids	Mossy Bay	Lundar	Steep Rock	Manipogo	Red River	Assiniboine River	Lac du Bonnet
TA (‰^{2})	3.82	4.39	4.91	6.10	0.74	2.69	3.13	0.83	0.32	0.42	1.74	0.63	1.50
SEA (‰^{2})	1.65	2.02	2.45	3.16	0.59	1.36	1.37	0.34	0.12	0.33	0.75	0.60	0.69
SEAc (‰^{2})	1.82	2.21	2.67	3.45	0.74	1.49	1.48	0.37	0.13	0.42	0.81	0.80	0.77

Appendix 2.M. Distributions of $\delta^{13}C$ x $\delta^{15}N$ Bayesian standard ellipse area estimates of sauger dorsal muscle tissue, as calculated at the Site population level. Gradations denote 50%, 75%, and 95% credibility intervals. Point estimates of corrected standard ellipse areas (maximum likelihood) are presented as red crosses. LW = Lake Winnipeg, LM = Lake Manitoba, RR = Red River, AR = Assiniboine River, LDB = Lac du Bonnet.

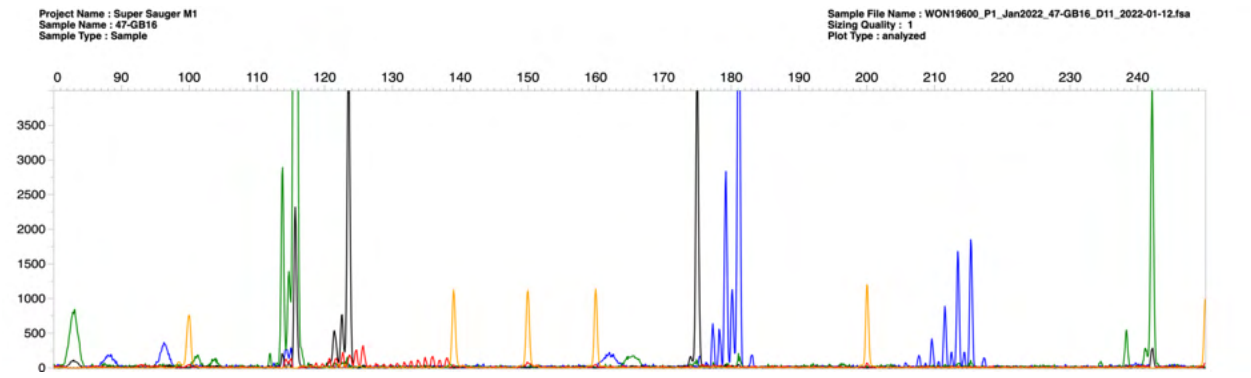


Appendix 2.N. Overlap (%) of $\delta^{13}C$ x $\delta^{15}N$ niches of sauger at the Site population level, as estimated with sample size corrected standard ellipses (SEAc) and 95% ellipses. Overlap of the SEAc's are presented above the diagonal and overlap of the 95% ellipses are presented below the diagonal.

	Grand Beach	Riverton / Hecla	Frog Bay	Matheson Island	Dauphin River	Grand Rapids	Mossy Bay	Red River	Assiniboine River	Lundar	Steep Rock	Manipogo	Lac du Bonnet
GB-LW		61	20.4	1.4	0	0	0	26.7	20.8	0	0	0	0
RV-LW	75.9		26.3	7.7	0	0	0	16.9	15.6	0	0	0	0
FB-LW	41.6	45.6		31.6	0	0	0	0	2	0	0	0	0
MI-LW	30.8	41.1	37.1		0	0	0	0	0	0	0	0	0
DR-LW	0	0	15.6	9.5		18.9	40.7	0	0	0	0	0	0
GR-LW	7.1	13.3	19.6	19.4	36.5		12.8	0	0	0	0	0	0
MB-LW	2.1	5.8	28.4	16.9	45.8	49		0	0	0	0	0	0
RR	44.4	35.5	15.5	15	0	0.5	0		45.3	0	0	0	0
AR	32.2	26.9	13.6	14.7	0	0	0	46.7		0	0	0	0
LU-LM	0	0	0	0	0	0	0	0	0		0	0	0
SR-LM	0	0	0	0	0	0	2.6	0	0	0		1.9	0
MP-LM	0	0	0	0	0	0	2.6	0	0	0	24.5		0
LDB	0	0	0.5	6.9	0	0	0.1	0	0	0	0	0	

Appendix: Chapter 3

Appendix 3.A. Electropherogram output of Multiplex 1 for individual GB16 (Grand Beach). Alleles were scored as follows: *Svi* L6 (green) = 117/117; Svi18 (black) = 124/124; YP41 (black) = 175/175; *MSL-2* (blue) = 181/215; YP60 (green) = 242/242. *Pfla L1* (red) was omitted from the study. The electropherogram output file was visualized and scored with the Microsatellite Analysis application in Thermo Fisher Cloud.



Appendix 3.B. MICRO-CHECKER test results for null alleles (1), large allele dropout (2), and stuttering (3) at three levels of population stratification. Cells in yellow indicate a significant deviation from HWE but not over all homozygote classes (Fisher's test), and cells in red indicate a significant result across homozygote classes. Greyed-out rows in the BASIN and SITE tables represent populations that cannot be delineated beyond the previous population stratum.

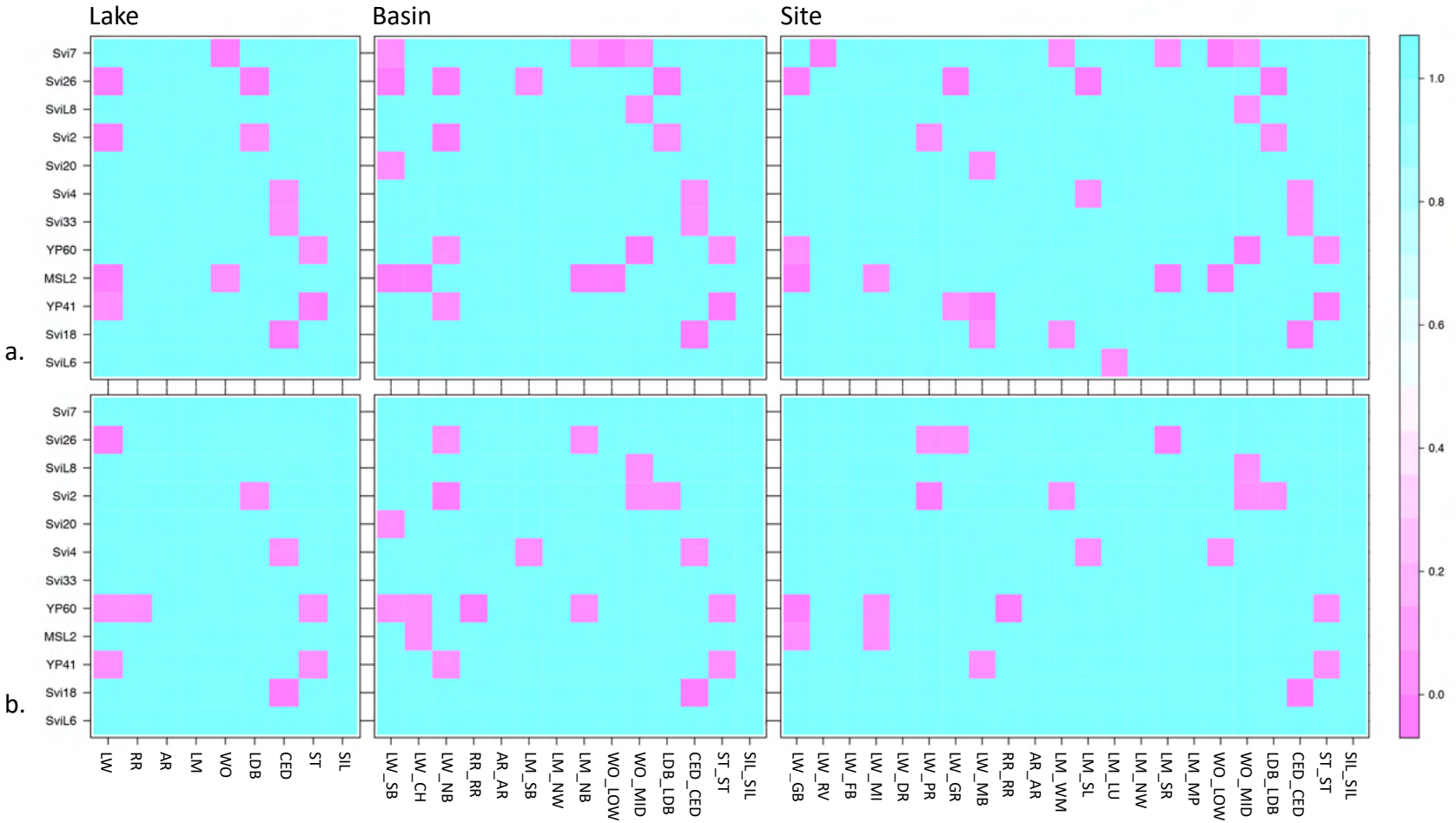
LAKE	SviL6	Svi18	YP41	MSL2	YP60	Svi33	Svi4	Svi20	Svi2	SviL8	Svi26	Svi7
Winnipeg			1		1							
Red					1							
Assiniboine					1							
Manitoba					1						1	
Winnipegosis						1						1
LDB					1				1			
Cedar		1										
Stephens			1		1							
SIL												

Appendix 3.B cont.

BASIN	SviL6	Svi18	YP41	MSL2	YP60	Svi33	Svi4	Svi20	Svi2	SviL8	Svi26	Svi7
Winnipeg S					1							
Winnipeg C					1							
Winnipeg N			1,3						1			
Red					1							
Assiniboine					1							
Manitoba S												
MB NW												
Manitoba N											1	
WPO LOW						1						1
WPO MID									1			
LDB					1				1			
Cedar MID												
Cedar E												
Stephens			1		1							
SIL												

SITE	SviL6	Svi18	YP41	MSL2	YP60	Svi33	Svi4	Svi20	Svi2	SviL8	Svi26	Svi7
Grand B.					1							
Riverton/H.												
Frog B.												
Matheson					1							
Dauphin R.												
Poplar R.					1							
Grand Rap.												
Mossy B.			1									
Red					1							
Assiniboine					1							
Whitemud												
St. Laurent												
Lundar												
MB NW												
Steep Rock												
Manipogo												
WPO LOW						1						1
WPO MID									1			
LDB					1				1			
Cedar MID												
Cedar E												
Stephens			1		1							
SIL												

Appendix 3.C. Conformance of each locus per population to Hardy-Weinberg Equilibrium (HWE) expectations at the Lake, Basin, and Site levels. HWE was tested using **a)** a χ^2 test and **b)** Guo and Thompson's (1992) exact test. Significant deviations from HWE expectations ($p < 0.05$) are designated in pink.



Appendix 3.D. Pairwise global tests (Fisher's method) of linkage disequilibrium across loci. Global tests are derived from G-tests using the MCMC method of Raymond & Rousset (1995), as implemented in Genepop. Significant tests ($p < 0.05$) are bolded in red.

Locus Pairing	χ^2	df	p-value
SviL6 & Svi18	41.72	44	0.570
SviL6 & YP41	18.83	40	0.998
Svi18 & YP41	19.65	40	0.997
SviL6 & MSL-2	37.29	44	0.753
Svi18 & MSL-2	34.25	44	0.854
YP41 & MSL-2	39.28	40	0.502
SviL6 & YP60	19.80	38	0.993
Svi18 & YP60	15.27	38	1.000
YP41 & YP60	14.52	36	0.999
MSL-2 & YP60	12.24	38	1.000
SviL6 & Svi33	46.96	44	0.352
Svi18 & Svi33	25.06	44	0.990
YP41 & Svi33	34.63	40	0.710
MSL-2 & Svi33	17.75	44	1.000
YP60 & Svi33	10.59	38	1.000
SviL6 & Svi4	38.25	44	0.715
Svi18 & Svi4	35.11	44	0.829
YP41 & Svi4	37.44	40	0.586
MSL-2 & Svi4	34.06	44	0.860
YP60 & Svi4	17.39	38	0.998
Svi33 & Svi4	25.44	44	0.989
SviL6 & Svi20	36.69	44	0.775
Svi18 & Svi20	41.19	44	0.593
YP41 & Svi20	32.25	40	0.803
MSL-2 & Svi20	61.22	44	**0.044**
YP60 & Svi20	14.73	38	1.000
Svi33 & Svi20	27.99	44	0.971
Svi4 & Svi20	21.52	44	0.998
SviL6 & Svi2	48.46	40	0.169
Svi18 & Svi2	23.70	40	0.981
YP41 & Svi2	22.22	36	0.965
MSL-2 & Svi2	46.05	49	0.236
YP60 & Svi2	15.17	32	0.995
Svi33 & Svi2	13.71	40	1.000

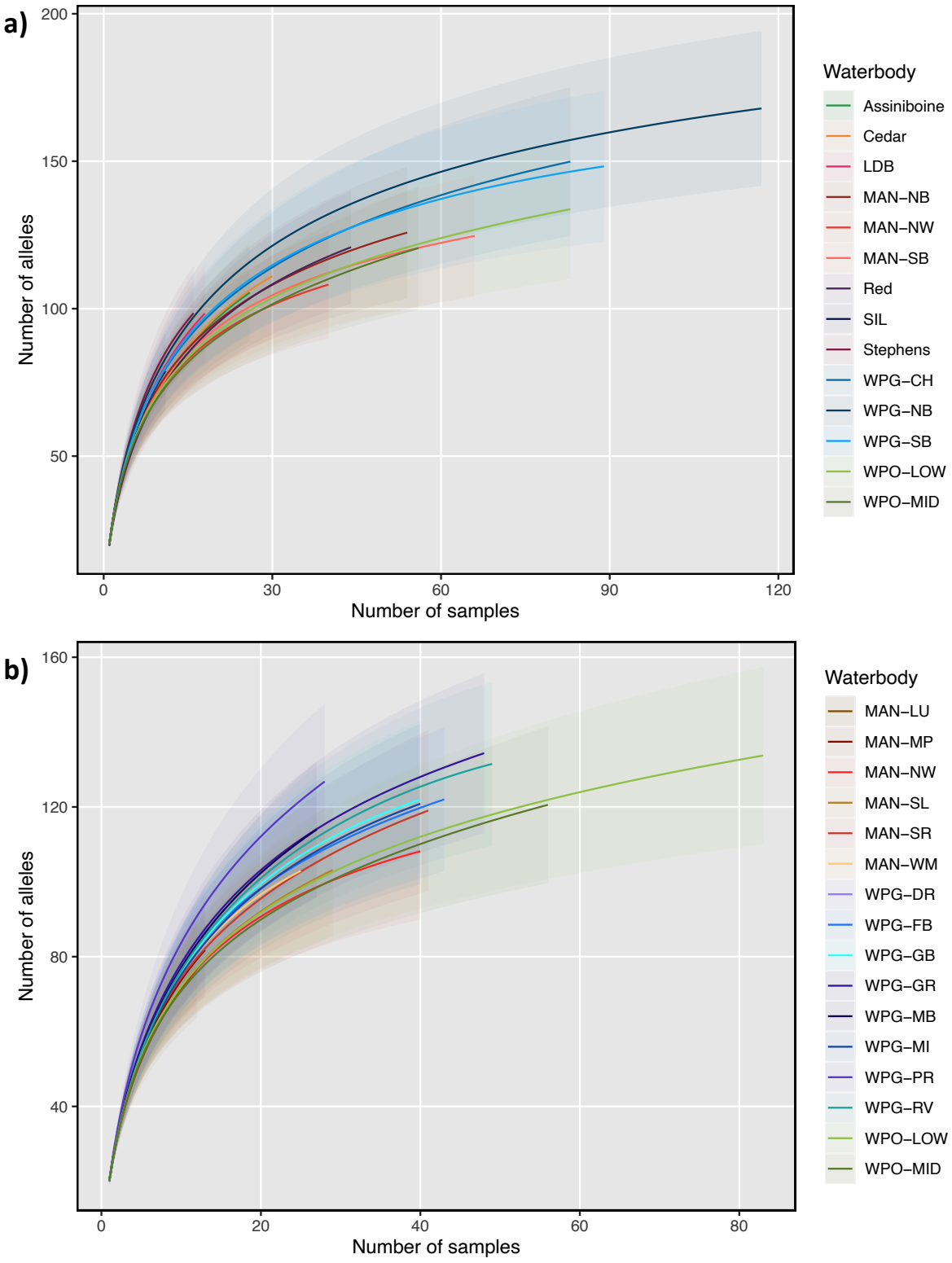
Appendix 3.D cont.

Locus Pairing	χ^2	df	p-value
Svi4 & Svi2	24.90	40	0.970
Svi20 & Svi2	26.93	40	0.943
SviL6 & SviL8	46.17	44	0.383
Svi18 & SviL8	26.94	44	0.980
YP41 & SviL8	25.06	40	0.969
MSL-2 & SviL8	33.24	44	0.882
YP60 & SviL8	19.06	38	0.996
Svi33 & SviL8	37.88	44	0.730
Svi4 & SviL8	31.84	44	0.914
Svi20 & SviL8	24.85	44	0.991
Svi2 & SviL8	33.36	40	0.762
SviL6 & Svi26	54.50	44	0.133
Svi18 & Svi26	44.39	44	0.455
YP41 & Svi26	54.49	40	0.063
MSL-2 & Svi26	40.37	44	0.628
YP60 & Svi26	25.53	38	0.939
Svi33 & Svi26	12.50	44	1.000
Svi4 & Svi26	32.49	44	0.900
Svi20 & Svi26	20.88	44	0.999
Svi2 & Svi26	32.54	40	0.793
SviL8 & Svi26	43.80	44	0.480
SviL6 & Svi7	59.34	44	0.061
Svi18 & Svi7	34.70	44	0.841
YP41 & Svi7	44.86	40	0.275
MSL-2 & Svi7	59.24	44	0.062
YP60 & Svi7	8.61	38	1.000
Svi33 & Svi7	23.30	44	0.996
Svi4 & Svi7	24.08	44	0.994
Svi20 & Svi7	42.79	44	0.523
Svi2 & Svi7	15.80	40	1.000
SviL8 & Svi7	33.41	44	0.878
Svi26 & Svi7	43.46	44	0.495

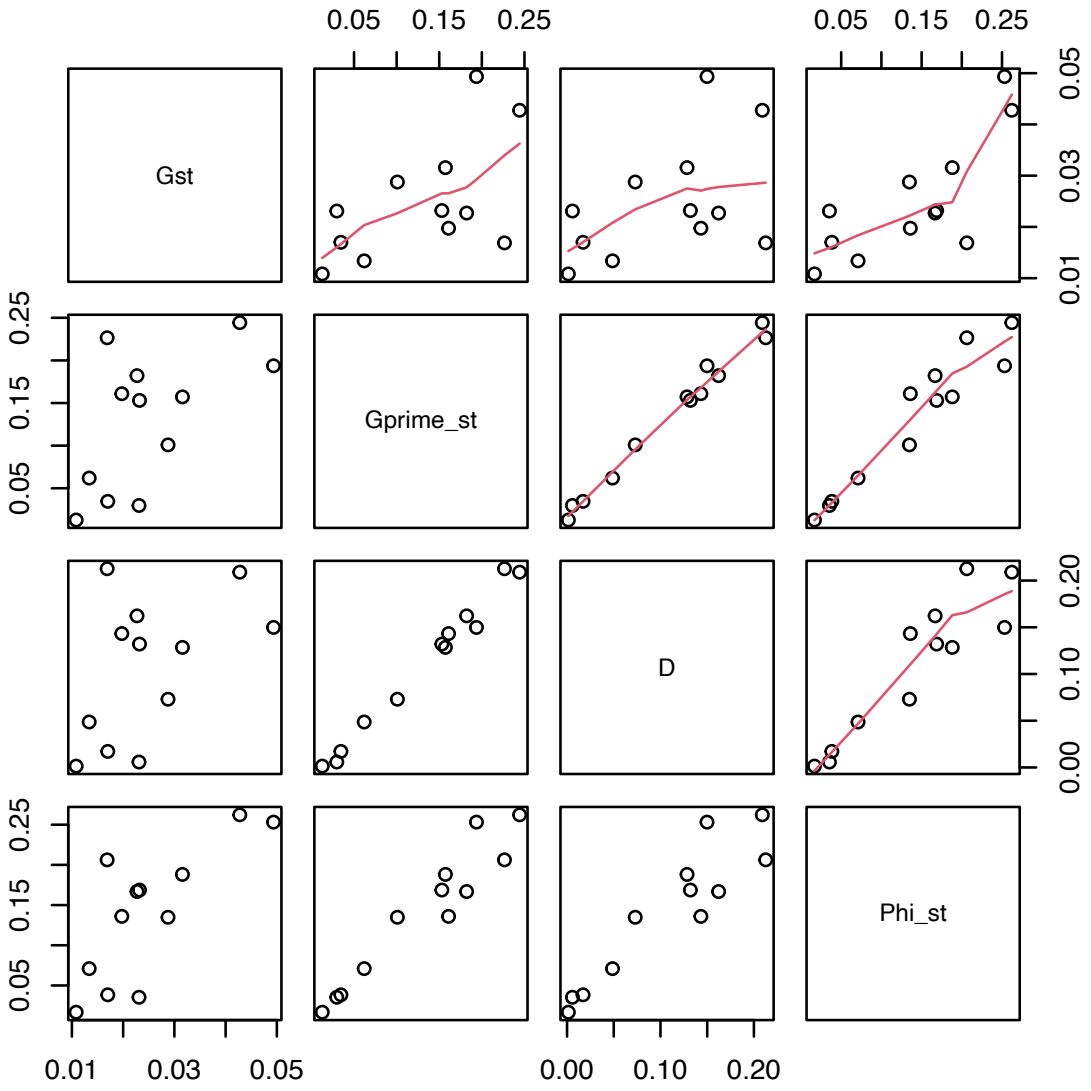
Appendix 3.E. Summary genetic statistics for sauger populations in Manitoba, as defined at the Basin population level. N_A = number of alleles; N_{PA} = number of private alleles; A_R = mean allelic richness; H_O = mean observed heterozygosity; H_E = mean expected heterozygosity; F_{ST} = fixation index; F_{IS} = inbreeding coefficient; $\bar{r}_d$ = standardized index of association.

	Sampling Location	Latitude	Longitude	N	N_A	$*A_R$	N_{PA}	H_O	H_E	F_{ST}	F_{IS}	$\bar{r}_d$
LW	**Winnipeg South**	50.8344	-96.6726	98	149	6.66	1	0.68	0.67	0.034 (0.000-0.067)	-0.003 (-0.041-0.024)	-0.0044
LW	**Winnipeg Channel**	51.6384	-96.7387	95	154	6.64	10	0.68	0.68	0.024 (-0.005-0.053)	-0.007 (-0.032-0.032)	-0.0036
LW	**Winnipeg North**	52.9319	-98.1064	128	170	6.93	13	0.69	0.69	0.002 (-0.032-0.025)	0.022 (-0.017-0.040)	0.0125
RR	**Red R.**	51.6938	-97.0511	46	122	6.24	1	0.60	0.63	0.092 (0.039-0.168)	0.029 (-0.009-0.111)	-0.0096
AR	**Assiniboine R.**	52.0116	-98.0400	31	110	6.36	1	0.68	0.67	0.029 (-0.045-0.073)	-0.007 (-0.055-0.054)	0.0344
LM	**Manitoba South**	50.5485	-98.3580	77	128	6.31	4	0.68	0.68	0.028 (-0.005-0.065)	-0.002 (-0.038-0.021)	0.0114
LM	**Manitoba Narrows**	51.1459	-98.8078	48	111	6.21	2	0.66	0.68	0.021 (-0.009-0.054)	0.024 (-0.009-0.078)	0.0103
LM	**Manitoba North**	51.4503	-99.0449	65	130	6.42	2	0.67	0.68	0.017 (-0.022-0.057)	0.006 (-0.016-0.055)	0.0101
WO	**Lower Winnipegosis**	51.6691	-99.8449	88	136	6.24	0	0.68	0.68	0.025 (-0.008-0.06)	0.003 (-0.050-0.055)	0.0090
WO	**Mid Winnipegosis**	52.6691	-99.9930	57	122	6.14	3	0.67	0.68	0.024 (-0.017-0.061)	0.007 (-0.037-0.060)	0.0013
LDB	**Lac du Bonnet**	50.3748	-95.8932	25	108	6.73	2	0.68	0.69	0.012 (-0.088-0.08)	-0.013 (-0.070-0.084)	0.0123
CED	**Cedar L.**	53.4194	-100.0648	30	111	6.35	1	0.59	0.64	0.078 (0.03-0.137)	0.143 (0.032-0.152)	0.0024
ST	**Stephens L.**	56.3756	-95.0978	28	114	7.12	3	0.68	0.69	0.007 (-0.037-0.051)	0.056 (-0.057-0.086)	0.0330
SIL	**South Indian L.**	57.3679	-98.2872	24	98	6.62	3	0.66	0.65	0.062 (0.013-0.128)	-0.009 (-0.048-0.030)	0.0292

Appendix 3.F. Allele rarefaction curves, consisting of twelve microsatellite loci, for sauger populations defined at **a)** the Basin population level, and **b)** the Site level for populations in Lake Winnipeg, Lake Manitoba, and Lake Winnipegosis.



Appendix 3.G. Pairwise comparisons of allele frequency-corrected (G''_{ST}, D, and ϕ'_{ST}) and uncorrected G_{ST}) differentiation statistics across the twelve microsatellite loci used in this study. The red lines depict the correlation between statistics across increasingly powerful loci.



Appendix 3.H. Population pairwise F_{ST} (θ statistic) as defined at the Basin level. Point estimates are above the diagonal line, and 95% confidence intervals are below the diagonal line. Non-significant results (i.e., negative F_{ST} estimates are present within the confidence interval) are highlighted in red.

	Winnipeg SB	Winnipeg CH	Winnipeg NB	Red R.	Assiniboine R.	Manitoba SB	Manitoba NW
Winnipeg SB		0.0046	0.0046	0.0091	0.0023	0.0350	0.0348
Winnipeg CH	(0.0014-0.0083)		-0.0003	0.0235	0.0163	0.0320	0.0306
Winnipeg NB	(0.0017-0.0080)	**(-0.0015-0.0013)**		0.0252	0.0158	0.0307	0.0298
Red R.	(0.0040-0.0141)	(0.0116-0.0349)	(0.0132-0.0360)		0.0031	0.0493	0.0492
Assiniboine R.	**(-0.0022-0.0079)**	(0.0063-0.0276)	(0.0078-0.0227)	**(-0.0034-0.0139)**		0.0356	0.0357
Manitoba SB	(0.0262-0.0472)	(0.0220-0.0419)	(0.0231-0.0384)	(0.0244-0.0749)	(0.0232-0.0488)		-0.0016
Manitoba NW	(0.0201-0.0573)	(0.0161-0.0477)	(0.0162-0.0452)	(0.0190-0.0872)	(0.0201-0.0562)	**(-0.0038-0.0003)**	
Manitoba NB	(0.0219-0.0430)	(0.0183-0.0406)	(0.0207-0.0380)	(0.0201-0.0665)	(0.0197-0.0446)	**(-0.0045-0.0020)**	**(-0.0048-0.0007)**
Winnipegosis LOW	(0.0204-0.0650)	(0.0190-0.0578)	(0.0197-0.0519)	(0.0161-0.0906)	(0.0161-0.0643)	(0.0040-0.0145)	(0.0006-0.0103)
Winnipegosis MID	(0.0222-0.0651)	(0.0199-0.0606)	(0.0201-0.0553)	(0.0216-0.0931)	(0.0193-0.0632)	(0.0029-0.0139)	**(-0.0013-0.0080)**
Lac du Bonnet	(0.0029-0.0273)	(0.0101-0.0313)	(0.0061-0.0276)	(0.0177-0.0656)	(0.0057-0.0252)	(0.0279-0.0636)	(0.0277-0.0741)
Cedar L.	(0.0225-0.0483)	(0.0133-0.0504)	(0.0140-0.0433)	(0.0272-0.0702)	(0.0248-0.0523)	(0.0360-0.0670)	(0.0351-0.0672)
Stephens L.	(0.0005-0.0241)	**(-0.0016-0.0125)**	(0.0009-0.0114)	(0.0183-0.0554)	(0.0096-0.0504)	(0.0270-0.0625)	(0.0244-0.0672)
South Indian L.	(0.0198-0.0574)	(0.0193-0.0502)	(0.0163-0.0460)	(0.0305-0.1020)	(0.0307-0.0775)	(0.0422-0.0826)	(0.0356-0.0791)

	Manitoba NB	Winnipegosis LOW	Winnipegosis MID	Lac du Bonnet	Cedar L.	Stephens L.	South Indian L.
Winnipeg SB	0.0312	0.0412	0.0425	0.0130	0.0353	0.0143	0.0405
Winnipeg CH	0.0290	0.0389	0.0408	0.0195	0.0312	0.0047	0.0355
Winnipeg NB	0.0290	0.0364	0.0381	0.0157	0.0286	0.0058	0.0321
Red R.	0.0425	0.0519	0.0556	0.0394	0.0481	0.0367	0.0643
Assiniboine R.	0.0320	0.0392	0.0409	0.0154	0.0406	0.0291	0.0555
Manitoba SB	-0.0014	0.0093	0.0076	0.0430	0.0540	0.0419	0.0628
Manitoba NW	-0.0023	0.0054	0.0033	0.0485	0.0522	0.0440	0.0576
Manitoba NB		0.0084	0.0083	0.0485	0.0477	0.0404	0.0589
Winnipegosis LOW	(0.0029-0.0139)		0.0027	0.0492	0.0557	0.0456	0.0603
Winnipegosis MID	(0.0029-0.0144)	**(-0.0016-0.0083)**		0.0510	0.0672	0.0491	0.0631
Lac du Bonnet	(0.0301-0.0704)	(0.0302-0.0712)	(0.0275-0.0773)		0.0518	0.0257	0.0535
Cedar	(0.0300-0.0646)	(0.0356-0.0752)	(0.0472-0.0837)	(0.0370-0.0656)		0.0318	0.0365
Stephens L.	(0.0232-0.0612)	(0.0231-0.0698)	(0.0248-0.0739)	(0.0082-0.0485)	(0.0027-0.0648)		0.0253
South Indian L.	(0.0392-0.0783)	(0.0344-0.0824)	(0.0362-0.0869)	(0.0245-0.0810)	(0.0177-0.0557)	(0.0116-0.0384)	

Appendix 3.I. Population pairwise F_{ST} (θ statistic) as defined at the Site level. Point estimates are above the diagonal line, and 95% confidence intervals are below the diagonal line. Non-significant results (i.e., negative F_{ST} estimates are present within the confidence interval) are highlighted in red.

	GB_LW	RV_LW	FB_LW	MI_LW	DR_LW	PR_LW	GR_LW
GB_LW		0.0042	0.0108	0.0094	0.0043	0.0082	0.0119
RV_LW	(0.0008-0.0076)		0.0034	-0.0014	-0.0024	0.0054	0.0022
FB_LW	(0.0057-0.0163)	**(-0.0010-0.0076)**		-0.0009	-0.0018	0.0013	-0.0014
MI_LW	(0.0014-0.0213)	**(-0.0041-0.0022)**	**(-0.0039-0.0017)**		-0.0004	0.0040	-0.0002
DR_LW	**(-0.0037-0.0143)**	**(-0.0074-0.0049)**	**(-0.0051-0.0017)**	**(-0.0074-0.0108)**		0.0009	0.0006
PR_LW	(0.0017-0.0156)	(0.0011-0.0107)	**(-0.0024-0.0058)**	**(-0.0014-0.0118)**	**(-0.0061-0.0081)**		0.0025
GR_LW	(0.0057-0.0193)	**(-0.0018-0.0075)**	**(-0.0038-0.0006)**	**(-0.0032-0.0035)**	**(-0.0059-0.0067)**	**(-0.0016-0.0077)**	
MB_LW	(0.0056-0.0193)	(0.0004-0.0084)	**(-0.0058-0.0051)**	**(-0.0019-0.0044)**	**(-0.0067-0.0012)**	**(-0.0003-0.0093)**	**(-0.0026-0.0058)**
RR_RR	**(-0.0002-0.0084)**	(0.0069-0.0248)	(0.0136-0.0347)	(0.0104-0.0381)	(0.0056-0.0409)	(0.0100-0.0391)	(0.0105-0.0433)
AR_AR	**(-0.0053-0.0042)**	(0.0011-0.0152)	(0.0090-0.0268)	(0.0028-0.0308)	(0.0007-0.0248)	(0.0032-0.0244)	(0.0084-0.0223)
WM_LM	(0.0199-0.0544)	(0.0246-0.0649)	(0.0169-0.0427)	(0.0221-0.0568)	(0.0122-0.0360)	(0.0225-0.0566)	(0.0221-0.0492)
SL_LM	(0.0193-0.0400)	(0.0224-0.0476)	(0.0119-0.0335)	(0.0221-0.0423)	(0.0049-0.0309)	(0.0154-0.0400)	(0.0188-0.0371)
LU_LM	(0.0163-0.0649)	(0.0185-0.0582)	(0.0167-0.0428)	(0.0174-0.0622)	(0.0079-0.0343)	(0.0168-0.0524)	(0.0142-0.0429)
NW_LM	(0.0180-0.0569)	(0.0189-0.0653)	(0.0132-0.0420)	(0.0169-0.0558)	(0.0096-0.0320)	(0.0160-0.0547)	(0.0155-0.0473)
SR_LM	(0.0170-0.0402)	(0.0209-0.0454)	(0.0129-0.0327)	(0.0175-0.0417)	(0.0115-0.0247)	(0.0175-0.0374)	(0.0159-0.0360)
MP_LM	(0.0159-0.0644)	(0.0221-0.0725)	(0.0205-0.0580)	(0.0227-0.0675)	(0.0132-0.0487)	(0.0241-0.0741)	(0.0232-0.0614)
LOW_WO	(0.0167-0.0663)	(0.0196-0.0714)	(0.0183-0.0535)	(0.0181-0.0654)	(0.0128-0.0370)	(0.0232-0.0595)	(0.0170-0.0560)
MID_WO	(0.0184-0.0667)	(0.0225-0.0734)	(0.0197-0.0583)	(0.0188-0.0652)	(0.0117-0.0417)	(0.0216-0.0637)	(0.0203-0.0579)
LDB_LDB	(0.0048-0.0299)	(0.0015-0.0277)	(0.0096-0.0325)	(0.0105-0.0297)	(0.0019-0.0240)	(0.0038-0.0242)	(0.0064-0.0335)
CED_CED	(0.0256-0.0501)	(0.0164-0.0517)	(0.0122-0.0453)	(0.0109-0.0554)	(0.0064-0.0544)	(0.0140-0.0474)	(0.0065-0.0341)
ST_ST	(0.0080-0.0352)	(0.0021-0.0187)	**(-0.0040-0.0100)**	**(-0.0028-0.0139)**	**(-0.0052-0.0148)**	**(-0.0020-0.0159)**	(0.0006-0.0166)
SIL_SIL	(0.0204-0.0672)	(0.0201-0.0576)	(0.0200-0.0501)	(0.0198-0.0534)	(0.0143-0.0414)	(0.0086-0.0519)	(0.0163-0.0536)

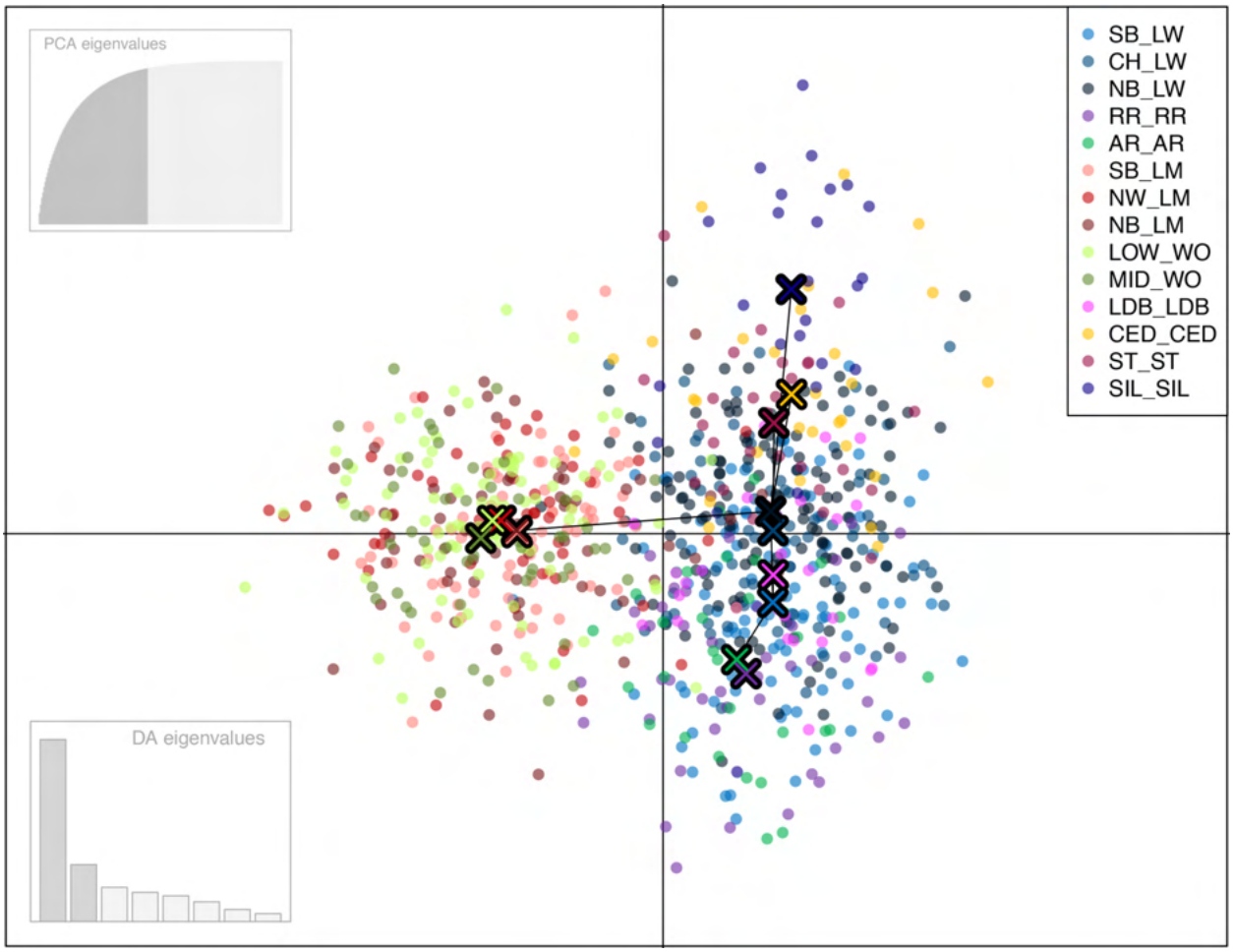
Appendix 3.I cont.

	MB_LW	RR_RR	AR_AR	WM_LM	SL_LM	LU_LM	NW_LM
GB_LW	0.0126	0.0043	-0.0011	0.0348	0.0296	0.0402	0.0333
RV_LW	0.0044	0.0158	0.0076	0.0428	0.0343	0.0353	0.0383
FB_LW	-0.0006	0.0237	0.0179	0.0290	0.0228	0.0307	0.0268
MI_LW	0.0012	0.0228	0.0143	0.0383	0.0327	0.0391	0.0340
DR_LW	-0.0029	0.0205	0.0121	0.0240	0.0184	0.0228	0.0207
PR_LW	0.0052	0.0224	0.0134	0.0381	0.0278	0.0346	0.0334
GR_LW	0.0021	0.0251	0.0159	0.0354	0.0290	0.0296	0.0308
MB_LW		0.0345	0.0236	0.0365	0.0294	0.0359	0.0324
RR_RR	(0.0233-0.0464)		0.0031	0.0496	0.0437	0.0586	0.0492
AR_AR	(0.0123-0.0345)	**(-0.0036-0.0155)**		0.0374	0.0313	0.0394	0.0357
WM_LM	(0.0220-0.0535)	(0.0254-0.0831)	(0.0219-0.056)		-0.0017	0.0039	-0.0027
SL_LM	(0.0163-0.0418)	(0.0211-0.0713)	(0.0190-0.0431)	**(-0.0058-0.0033)**		-0.0020	-0.0031
LU_LM	(0.0196-0.0506)	(0.0225-0.1046)	(0.0187-0.0609)	**(-0.0033-0.0108)**	**(-0.0088-0.0043)**		0.0018
NW_LM	(0.0172-0.0505)	(0.0200-0.0931)	(0.0196-0.0587)	**(-0.0051-[-]0.0006)**	**(-0.0063-[-]0.0005)**	**(-0.0063-0.0084)**	
SR_LM	(0.0198-0.0402)	(0.0205-0.0655)	(0.0198-0.0424)	**(-0.0076-0.0000)**	**(-0.0058-0.0014)**	**(-0.0076-0.0124)**	**(-0.0052-0.0011)**
MP_LM	(0.0248-0.0667)	(0.0166-0.0898)	(0.0155-0.0611)	**(-0.0140-0.0125)**	**(-0.0088-0.0077)**	**(-0.0086-0.0285)**	**(-0.0072-0.0076)**
LOW_WO	(0.0216-0.0628)	(0.0174-0.0946)	(0.0151-0.0662)	(0.0044-0.0147)	(0.0022-0.0174)	**(-0.0011-0.0174)**	(0.0008-0.0105)
MID_WO	(0.0211-0.0627)	(0.0214-0.0981)	(0.0194-0.0647)	(0.0020-0.0175)	(0.0002-0.0088)	**(-0.0025-0.0213)**	**(-0.0008-0.0081)**
LDB_LDB	(0.0094-0.0358)	(0.0165-0.0678)	(0.0053-0.0258)	(0.0283-0.0786)	(0.0251-0.0570)	(0.0159-0.0591)	(0.0280-0.0754)
CED_CED	(0.0162-0.0617)	(0.0281-0.0705)	(0.0236-0.0531)	(0.0363-0.0692)	(0.0293-0.0665)	(0.0323-0.0768)	(0.0348-0.0667)
ST_ST	**(-0.0026-0.0112)**	(0.0184-0.0573)	(0.0101-0.0511)	(0.0219-0.0733)	(0.0203-0.0530)	(0.0293-0.0609)	(0.0241-0.0687)
SIL_SIL	(0.0216-0.0521)	(0.0312-0.1026)	(0.0316-0.0786)	(0.0472-0.0935)	(0.0365-0.0770)	(0.0322-0.0955)	(0.0362-0.0819)

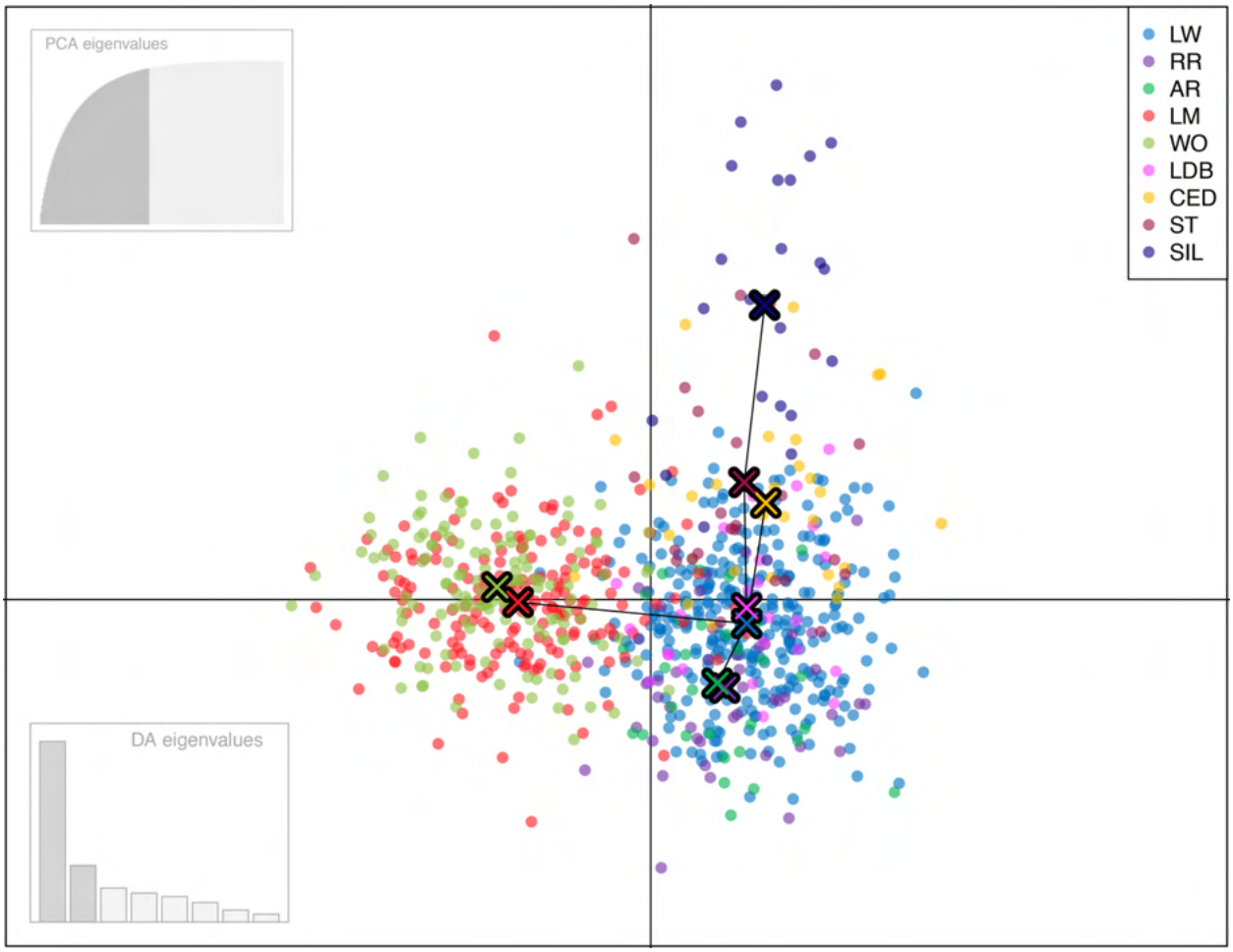
Appendix 3.I cont.

	SR_LM	MP_LM	LOW_WO	MID_WO	LDB_LDB	CED_CED	ST_ST	SIL_SIL
GB_LW	0.0275	0.0383	0.0402	0.0404	0.0161	0.0397	0.0205	0.0439
RV_LW	0.0324	0.0443	0.0443	0.0466	0.0120	0.0334	0.0103	0.0393
FB_LW	0.0222	0.0375	0.0362	0.0395	0.0195	0.0293	0.0027	0.0341
MI_LW	0.0291	0.0433	0.0411	0.0417	0.0190	0.0326	0.0063	0.0366
DR_LW	0.0186	0.0289	0.0243	0.0268	0.0098	0.0302	0.0045	0.0289
PR_LW	0.0266	0.0469	0.0395	0.0410	0.0142	0.0311	0.0068	0.0284
GR_LW	0.0264	0.0411	0.0369	0.0397	0.0177	0.0207	0.0081	0.0344
MB_LW	0.0307	0.0437	0.0424	0.0419	0.0202	0.0406	0.0044	0.0380
RR_RR	0.0411	0.0489	0.0519	0.0556	0.0394	0.0481	0.0367	0.0643
AR_AR	0.0314	0.0355	0.0392	0.0409	0.0154	0.0406	0.0291	0.0555
WM_LM	-0.0039	-0.0016	0.0093	0.0088	0.0494	0.0550	0.0452	0.0691
SL_LM	-0.0021	-0.0014	0.0093	0.0045	0.0397	0.0505	0.0351	0.0562
LU_LM	0.0031	0.0091	0.0089	0.0100	0.0351	0.0569	0.0462	0.0623
NW_LM	-0.0025	-0.0005	0.0054	0.0033	0.0485	0.0522	0.0440	0.0576
SR_LM		0.0017	0.0072	0.0085	0.0437	0.0465	0.0366	0.0569
MP_LM	**(-0.0077-0.0125)**		0.0140	0.0093	0.0657	0.0532	0.0542	0.0673
LOW_WO	(0.0023-0.0121)	(0.0007-0.0323)		0.0027	0.0492	0.0557	0.0456	0.0603
MID_WO	(0.0039-0.0145)	**(-0.0042-0.0292)**	**(-0.0018-0.0085)**		0.0510	0.0672	0.0491	0.0631
LDB_LDB	(0.0277-0.0621)	(0.0331-0.1100)	(0.0303-0.0734)	(0.0282-0.0779)		0.0518	0.0257	0.0535
CED_CED	(0.0284-0.0626)	(0.0278-0.0749)	(0.0347-0.0757)	(0.0460-0.0832)	(0.0372-0.0663)		0.0318	0.0365
ST_ST	(0.0202-0.0563)	(0.0298-0.0841)	(0.0230-0.0724)	(0.0249-0.0751)	(0.0081-0.0479)	(0.0012-0.0661)		0.0253
SIL_SIL	(0.0391-0.0760)	(0.0386-0.0981)	(0.0337-0.0844)	(0.0341-0.0904)	(0.0255-0.0838)	(0.0175-0.0573)	(0.0123-0.0385)	

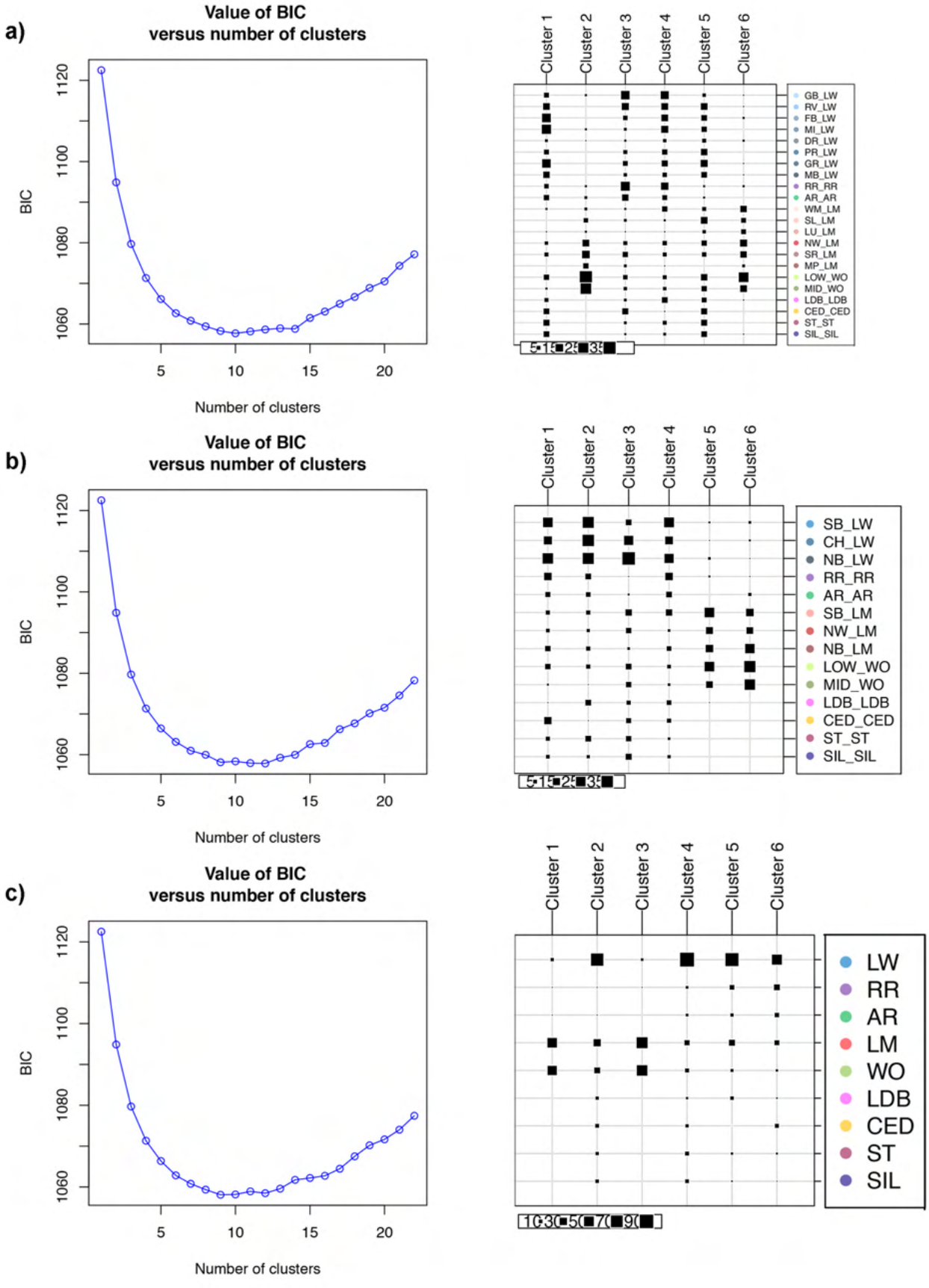
Appendix 3.J. Discriminant analysis of principal components (DAPC) of sauger genotypes (n = 840) as assigned at the Basin population level. Mean values for each population are represented with an "X" symbol, and connecting lines depict a minimum spanning tree. Ninety-two principal components were retained based on cross-validation testing. The first and second discriminant functions are represented on the x and y axis, respectively.



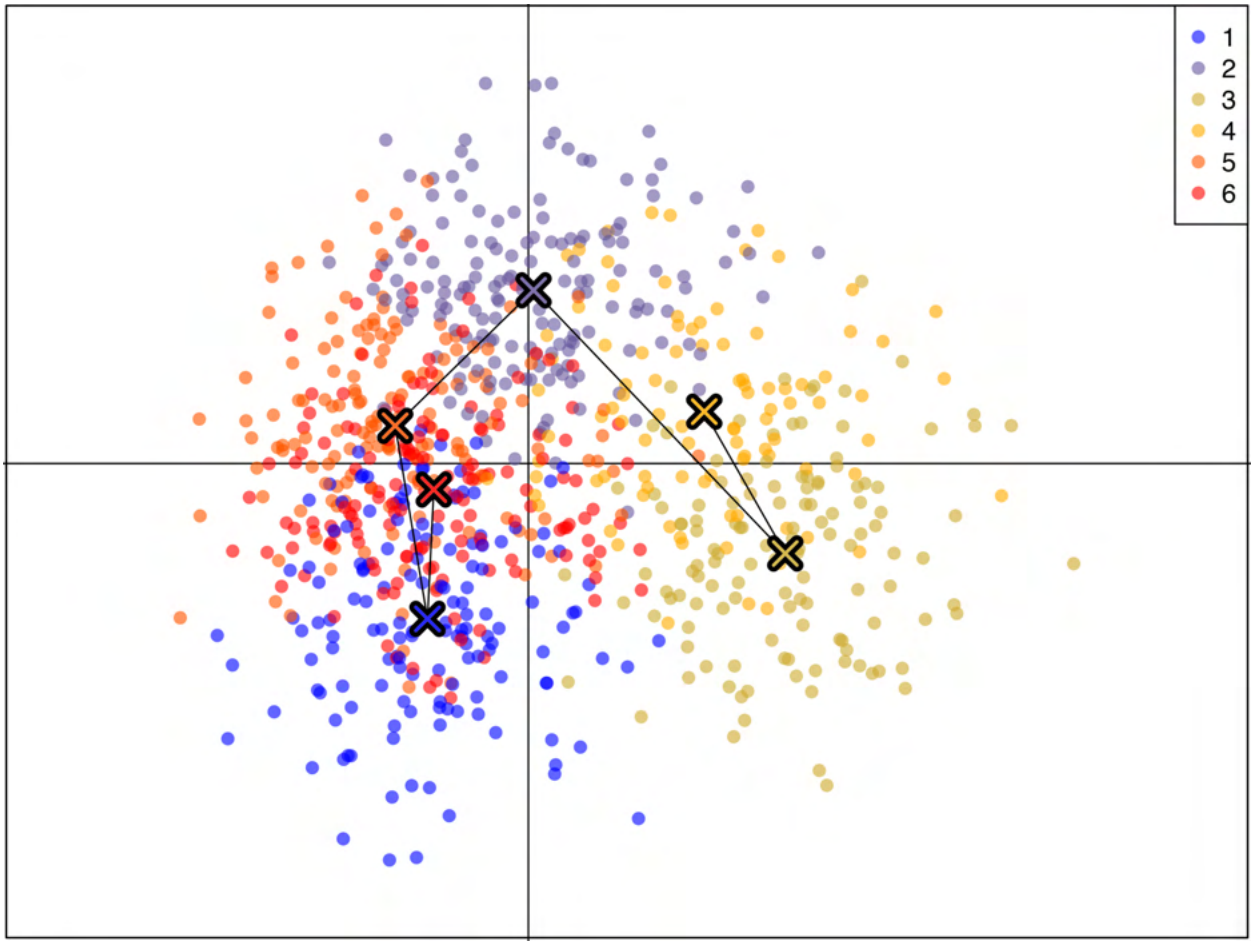
Appendix 3.K. Discriminant analysis of principal components (DAPC) of sauger genotypes (n = 840) as assigned at the Lake population level. Mean values for each population are represented with an "X" symbol, and connecting lines depict a minimum spanning tree. Ninety-six principal components were retained based on cross-validation testing. The first and second discriminant functions are represented on the x and y axis, respectively.



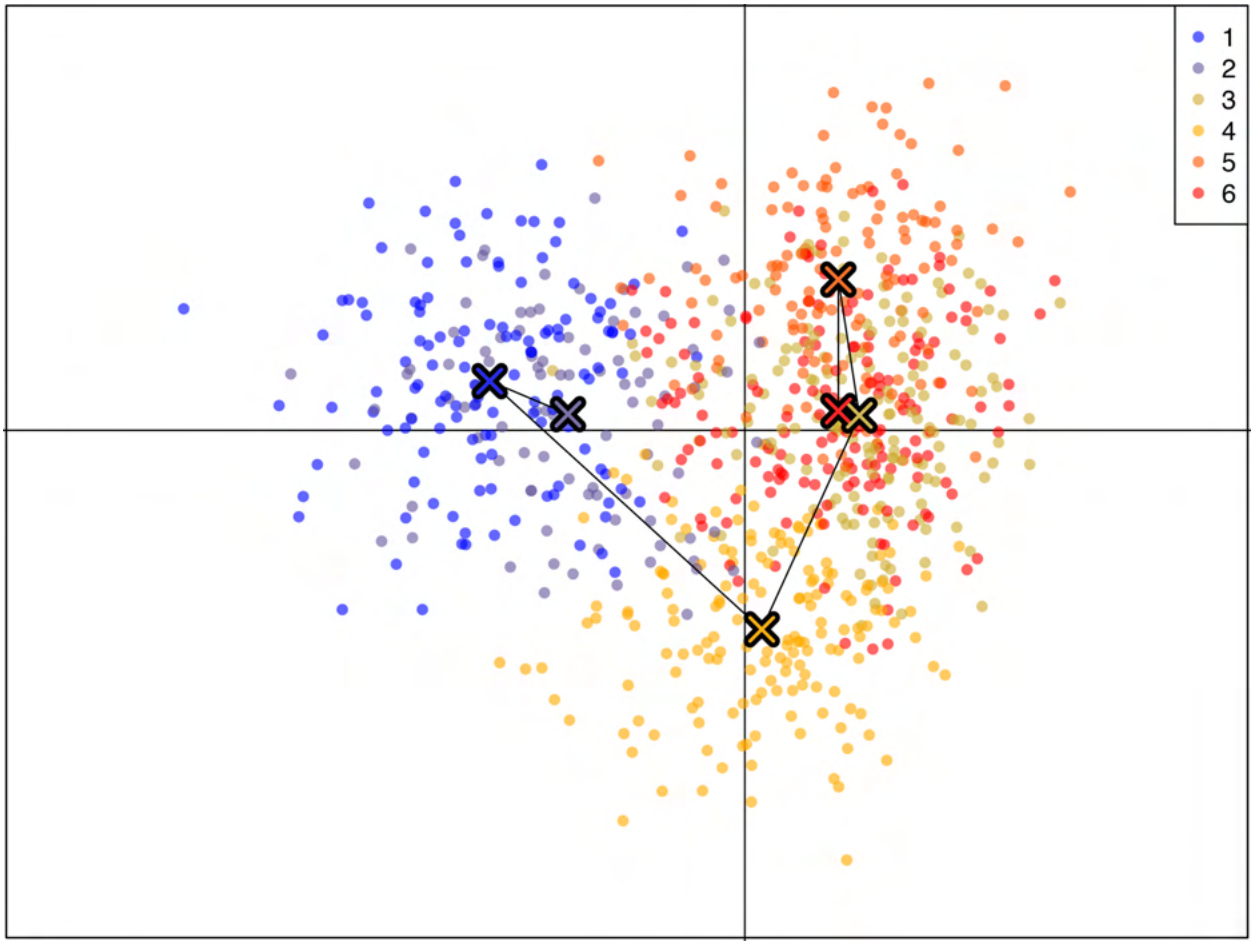
Appendix 3.L. BIC curve for increasing values of *K*, and *a posteriori* cluster assignment as derived from *K*-means clustering at the **a)** Site, **b)** Basin, and **c)** Lake population levels.



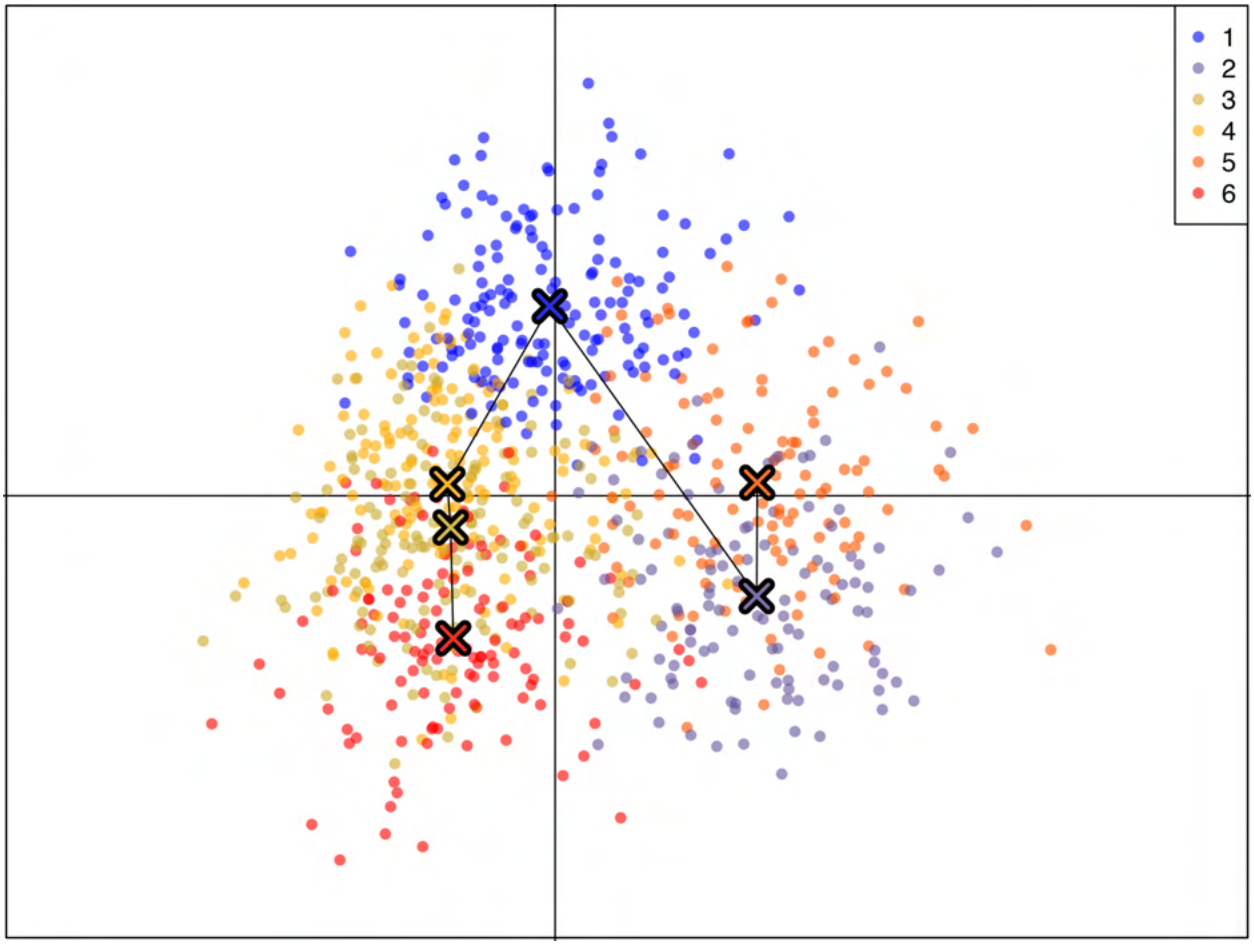
Appendix 3.M. Discriminant analysis of principal components (DAPC) of sauger genotypes (n = 840) based on *K*-means clustering (*K* = 6), as reassigned at the Site population level. Mean values for each population are represented with an "X" symbol, and connecting lines depict a minimum spanning tree. Ninety-two principal components were retained based on cross-validation testing. The first and second discriminant functions are represented on the x and y axis, respectively.



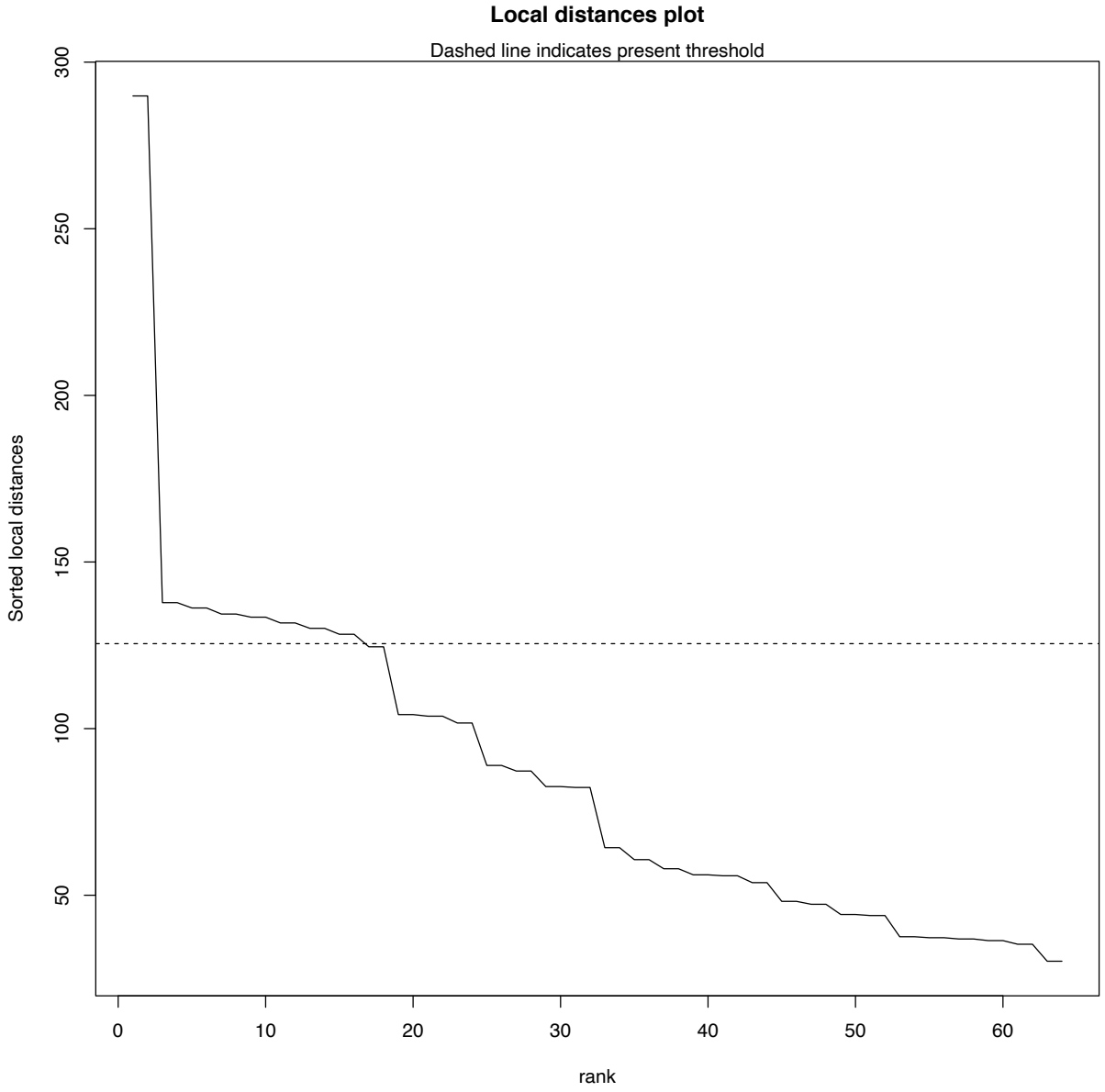
Appendix 3.N. Discriminant analysis of principal components (DAPC) of sauger genotypes (n = 840) based on *K*-means clustering (*K* = 6), as reassigned at the Basin population level. Mean values for each population are represented with an "X" symbol, and connecting lines depict a minimum spanning tree. Ninety-two principal components were retained based on cross-validation testing. The first and second discriminant functions are represented on the x and y axis, respectively.



Appendix 3.O. Discriminant analysis of principal components (DAPC) of sauger genotypes (n = 840) based on *K*-means clustering (*K* = 6), as reassigned at the Lake population level. Mean values for each population are represented with an "X" symbol, and connecting lines depict a minimum spanning tree. Ninety-six principal components were retained based on cross-validation testing. The first and second discriminant functions are represented on the x and y axis, respectively.



Appendix 3.P. Local distances plot computed with the Monmonier algorithm. The solid black line depicts the genetic distance between population pairs as ranked along the x-axis. The dotted line represents the threshold prior to the second distinct genetic break over Euclidean geographic distance.



Supplementary Materials

Supplementary Materials, datasets, and program outputs are available upon request. I can be contacted at the following email address:

wong.caleb.0708@gmail.com

For all inquiries, please include your name, affiliation, the material(s) of interest, and the purpose of your request in the body of your email.