

**HISTORIC AND ANTHROPOGENIC INFLUENCES ON THE GENETIC VARIATION OF
LAKE TROUT (*Salvelinus namaycush*) POPULATIONS
IN THE GREAT LAKES REGION**

A thesis submitted to the Committee on Graduate Studies
in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy
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ABSTRACT

Historic and anthropogenic influences on the genetic variation of lake trout (*Salvelinus namaycush*) populations in the Great Lakes region

Michael A. Halbisen

The practice of supplementing wild populations with captive-raised individuals is often intended to rebuild reduced populations, but has many unintended negative outcomes, including the loss of native genetic variation that may be essential for long-term survival. The lake trout (*Salvelinus namaycush*) populations of the Great Lakes region have been stocked with hatchery strains since the mid-1800s, and provided an ideal system for studying the influence of supplemental stocking on natural genetic variation. Parallel mitochondrial (PCR-RFLP) and microsatellite (11-12 loci) DNA analyses were used to measure the genetic attributes of study populations, and evaluate the genetic impact from stocking. Overall, supplemental stocking had a variable influence on genetic variation relative to influences from postglacial processes and modern-day landscape attributes. Together, the results obtained by these studies provide essential information for refining genetics-based lake trout conservation strategies, and they build a solid foundation for future analyses of adaptive characteristics among populations.

He preferred the hard truth to his dearest illusions, that is the heart of science

- Carl Sagan's commentary on the life of Johannes Kepler,
a medieval theologian and astronomer who demonstrated that
the planetary orbits were not harmonious heavenly circles,
but actually imperfect ellipses

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ABBREVIATIONS

Commonly used

A_R - Allelic richness

DNA - Deoxyribonucleic Acid

ESU - Evolutionary Significant Unit

H_E - Expected heterozygosity

H_O - Observed heterozygosity

KYA - Thousand years ago

mtDNA - Mitochondrial DNA

MU - Management Unit

N_A - Average number of alleles per locus

n_e - Effective number of alleles

N_e - Effective population size

N_p - Number of private alleles

OMNR - Ontario Ministry of Natural Resources

PCR - Polymerase Chain Reaction

q - Admixture coefficient

RFLP - Restriction Fragment Length polymorphism

Population and hatchery strains

BGT - Big Trout Lake

BKG - Boshkung Lake

BPC - Big Porcupine Lake

BRK - Barker Lake

CED - Cedar Lake

CLE - Clean-Clear Lake

DGH - Dog River historical population from 1952

DGU - Dog River

DKS - Dickson Lake

ESS - Esson Lake

FRQ - Farquhar Lake

GIS - Gull Island Shoal

GRC - Grace Lake

HOG - Hogan Lake

HPI - Happy Isle Lake

IRY - Isle Royale

KIO - Lake Kioshkokwi

KL or KIL - Killala Lake

KS or KSG - Kingscote Lake

KTZ - Katzenbach Lake

LAM - Lake LaMuir

LAV - Lake Lavieille
LDG - Lost Dog Lake
LM or LMN - Lake Manitou
LOU - Louisa Lake
MAC - MacDonald Lake
MPI - Michipicoten Island
MLH - Mishibishu hatchery strain
MON - Montreal River
MRQ - Marquette hatchery strain
MSI - Mishi Lake
MSK - Miskwabi Lake
MSL - Mishibishu Lake
OPE - Lake Opeongo
PSD - Parry Sound
RST - Redstone Lake
SL or SLI - Slate Island
SMK - Smoke Lake
TWF - Timberwolf Lake
WPR - White Partridge Lake
XTL - Crystal Lake

Genetic clusters

Chapter 2

H - Haliburton

A - Algonquin

B - Bancroft

Chapter 3

NLS - Northeastern Lake Superior

UGL - Upper Great Lakes

RIV - River-spawner

MSB - Mishibishu

BSN - Basin-spawner

KLC - Killala

UAS - Unassigned

IMC - Inter-model correspondence (not a genetic cluster, but a measure of agreement)

AMB - Ambiguous

ADX - Admixed

Chapter 4

C1 - C25 were the resolved genetic clusters

UAS - Unassigned

ADX - Admixed

GLOSSARY

Admixture - Intermediate state for individuals or populations following the mixture of two genetically divergent sources that precedes eventual homogenization. Can be historical (i.e., glacial) or recent (i.e., following supplementation with divergent hatchery strains). **Admixture coefficients** (q) describe the degree of admixture for individuals.

Allele - A specific instance of a variable character state for a genetic locus sometimes referred to as a haplotype.

Allelic richness (A_R) - A measure of genetic variability that is standardized to sample size.

Allopatry - State of geographical separation that prevents interbreeding between populations.

Allozyme - A protein molecular marker used for early analyses of genetic variation. Allozymes are encoded within an organism's genome, and so indirectly reflect DNA variability. However, Allozyme variation is typically low, in part because they do not reflect "silent" genetic changes where amino acid sequences are not altered.

Anadromous - Pertains to fish that migrate from oceans to reproduce in freshwater; the opposite of **catadromous** fish that migrate from freshwater to reproduce in marine environments.

Biodiversity - Refers to the variety inherent to biological systems. Manifest at many hierarchical levels, including within and among species.

Captive broodstock - Wildlife population segment consisting of individuals raised in captivity, used as a source for supplementing wild populations. For fish, captive broodstocks are maintained in fish hatcheries for one or many successive generations, and serve as convenient sources for gametes that are collected to produce large lots of cultured fish for stocking programs.

Conservation genetics - The study of genetics in the context of conservation, particularly how population and evolutionary processes alter genetic variation among individuals and populations that have been affected by human activities.

Effective number of alleles (n_e), is the number of alleles per locus that if equally frequent would result in the observed homozygosity. This genetic diversity measure is expected to be lower in reproductively isolated populations.

Effective population size (N_e) - Size of an idealized population that loses genetic variation through genetic drift at the same rate as the actual population in question.

Evolutionary Significant Unit (ESU) - An intraspecific conservation genetic unit defined by many, many different criteria (see Fraser and Bernatchez 2001 for a detailed list of ESU definitions). Generally intended to classify a population in terms of genetic distinctiveness relative to other conspecifics, and preserve evolutionary processes that produced the population. **Management Units (MU)** are related to the ESU concept, and provide a category for populations with relatively lower levels of intraspecific divergence.

Exchangeability - Within the context of the Crandall et al. (2000) framework for resolving conservation genetic units, implies no difference between populations. For example, failure to reject the null hypothesis of genetic exchangeability between two populations indicates that they are not divergent from one another, that they have some degree of gene flow between them, and that they should be managed accordingly.

F_{ST} - Wright's fixation index that measures the divergence of subpopulations (pairs or multiple) relative to the population as a whole. Value varies between approximately zero and 1, indicating panmixia (i.e., random mating and no genetic substructure) or complete isolation between populations, respectively. Initially derived by Sewell Wright from path coefficients in pedigreed populations, later estimators of this parameter were calculated from heterozygosities and gene diversities ($F_{ST} = [H_T - H_S]/H_T$), as well allelic probabilities of identity-by-descent (θ_{ST} of Weir & Cockerham). Related to other fixation indices describing the degree of inbreeding within a subpopulation (F_{IS} - commonly

referred to as the inbreeding coefficient), and the degree of inbreeding of individuals relative to the total population (F_{IT}) by the formula $(1-F_{IT}) = (1-F_{ST})(1-F_{IS})$.

Genetic distance - Measure of genetic similarity or dissimilarity between individuals or populations that is calculated from observed allelic or genotypic frequencies.

Genetic diversity - A measure that describes the genetic variability of a population. Common measures include heterozygosity, number of alleles per locus, and allelic richness, among others.

Glacial refuge - Sanctuary area typically located along glacial margins that remained ice-free during the Pleistocene glacial cycles, and served as a source for wildlife dispersal during the recolonization of previously glaciated areas. Commonly cited North American refuges are the Atlantic (eastern), Mississippian (southern), or Beringian (northwestern), refuges, although many other minor refuges probably also existed (e.g., Missourian near the Montana/Alberta border).

Hardy-Weinberg equilibrium - State attained by a (diploid) population where genotypic and allelic proportions remain identical from generation-to-generation. Allows prediction of genotype frequencies (i.e., heterozygosity and homozygosity) from measured population allele frequencies for individual loci. Observed departures from Hardy-Weinberg equilibrium are indicative of underlying population processes (migration, mutation, genetic drift, non-random mating, or natural selection).

Heterozygosity (H_E or H_O) - A measure of genetic variability for an individual or population (see definition of locus for the genetic basis of heterozygosity). **Observed heterozygosities** (H_O) are measured directly but **expected heterozygosities** (H_E) are calculated by use of the Hardy-Weinberg equilibrium principle. This latter value often substituted with Nei's **gene diversity**, which is unbiased estimator of expected heterozygosity.

Individual assignment - Technique that is used to assign individuals to genetic clusters or populations of origin based on their multilocus genotypes.

Interglacial (timeframe) - Time period between glacial cycles when ice-sheets are minimized or absent.

Introgression - Process of integration for exogenous genes into an indigenous population by repeated back crossbreeding between indigenous and exogenous individuals. Can lead to fixation of exogenous genetic material in the native gene pool.

Landscape genetics - The study of how landscape attributes affect fine-scale patterns of genetic variation.

Locus - A single genetic character that corresponds to a physical site within a genome (e.g. on a specific chromosome arm). For **haploids**, each locus has a single character state

(allele or haplotype). For **diploids**, each locus has two character states. If the character states are identical, the individual is a **homozygote**, if they are different, the individual is a **heterozygote**. Character states can also be null (e.g., a null allele may correspond to a chromosomal deletion).

Mantel test - Statistical test used to measure correlations between two or more matrices. Commonly used in landscape genetics to measure correlations between pairwise estimates of genetic and geographical distance to evaluate the hypothesis divergence under the isolation-by-distance model.

Microsatellite DNA - Short sections of hypervariable, repetitive DNA that serve as molecular markers for genetic studies. Microsatellites typically do not code for functional gene elements, and are generally not affected by selective processes unless they are associated with a functional gene locus.

Mitochondrial DNA - Extra-nuclear, organellar DNA that is found in the mitochondria of eukaryotes and encodes for metabolic proteins and RNAs involved in electron transport and oxidative phosphorylation. Evolves faster than allozyme loci, but slower than microsatellites, and is useful for tracking evolutionary change that occurred during postglacial events.

Mitochondrial DNA lineage - Inter- and intraspecific evolutionary lineages established phylogenetic analysis of mitochondrial haplotypes. For species that reside in previously

glaciated regions, **glacial ancestry** inferred from mitochondrial lineages may correspond with glacial events, including cycles of geographical isolation experienced during glacial maxima as well as dispersal and rapid colonization during postglacial periods.

Molecular marker - A heritable genetic component that marks an individual or population, which can be detected using biochemical or molecular biology methods. Common molecular markers include allozymes, mitochondrial DNA, microsatellite DNA, single nucleotide polymorphisms (SNPs), among others.

Phylogeography - The study of how genetic variation corresponds with biogeographical variation, typically on large (i.e., continental or oceanic) spatial scales.

Polymerase Chain Reaction (PCR) - A biochemical reaction that allows an exponential amplification of small quantities of template DNA by use of a thermostable DNA polymerase enzyme, complementary oligonucleotide primer fragments designed to target the template sequence, free nucleotide triphosphates, binding and stabilizing factors, and a machine that repetitively cycles the reaction through several rounds of temperature variation.

Population size bottleneck - Condition of severe population reduction such that genetic variation is likely to be decreased. Can occur as a result of high mortality, or by rapid population expansion following establishment with few founding individuals. Is detectable by use of several genetic statistics including the **M-ratio**, which measures the

ratio of the number of alleles at a locus relative to the range of alleles at a locus (this value is lower in populations with an historical bottleneck), and the observed **gene diversity excess**, which is expected to be higher than the equilibrium value that is calculated from the number of alleles in a recently bottlenecked population.

Periglacial - Pertains to landscape features formed by and immediately adjacent to glaciers (e.g. a periglacial meltwater lake that rims a melting glacial extension would be much smaller than a proglacial lake that could extend for several hundreds of kilometres past a large glacial front). Also often refers to glacial features formed before the Wisconsin glaciation.

Postglacial (timeframe) - Time period following a major glacial cycle. In this thesis, generally refers to the time period immediately after the most recent North American glacial cycle (Wisconsin).

Proglacial - Pertains to landscape features formed in front of a glacier, particularly meltwater streams and lakes.

Restriction Fragment Length Polymorphism (RFLP) - A variable genetic site that can be detected by use of **restriction enzyme** that recognizes and cleaves a specific tandem repeat of nucleotides.

Sanctuary population - Introduced wildlife population established specifically to protect against extinction or extirpation if the original, indigenous population is eliminated. May serve as a source for rehabilitative supplementations or reintroductions.

Supplemental stocking - Generally refers to the practice of supplementing a fish population with hatchery-reared strains to increase production beyond the natural capacity of the system. Supplementation is also used for rehabilitative stocking, however, with the intention of rebuilding reduced populations.

Sympatry - State of shared habitation that does not prevent interbreeding between populations

CHAPTER 1

Population supplementation: genetic threat to freshwater biodiversity or conservation tool?

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Abstract

The practice of supplemental stocking poses one of the greatest risks to freshwater fish biodiversity. When indigenous populations are supplemented with divergent hatchery strains, intraspecific competition and introgressive interbreeding can lead to the reduction or loss of native genetic variation. By these processes, genetic attributes essential for adaptive evolutionary responses and long-term survival may be lost. Lake trout populations of the Great Lakes region have been heavily stocked since regional fish culture practice began over 100 years ago. These stocked populations provide an excellent study system for evaluating the genetic impacts from supplemental stocking on natural genetic diversity and population structure. The overall goals of this thesis work were to measure the genetic attributes of lake trout populations with three different evolutionary histories and contrasting levels of contemporary genetic exchange, in order to evaluate their responses to supplementation with divergent lake trout of Great Lakes origin. An overview of methodological approaches has been provided, as well as a summary of the experimental design for each thesis chapter.

General introduction

Overview

A disproportionately high number of freshwater and anadromous fish species are of conservation concern (Richter et al. 1997), compared with other vertebrate species (Dudgeon et al. 2006). Although freshwater ecosystems represent a small portion of global aquatic habitat (0.01%; Gleick 1996), they provide residence for 40% of the world's fishes (Dudgeon et al. 2006), and are heavily affected by a wide range of human impacts (Cowx 2002; Moritz et al. 2002; Dudgeon et al. 2006).

Human activities associated with freshwater utilization can directly reduce, eliminate, or genetically modify native fish populations. These effects may severely disrupt the natural evolutionary processes (i.e., migration, mutation, selection, and genetic drift) essential for maintenance of biodiversity (Poissant et al. 2005) and ecosystem functionality (Spencer et al. 1991; Moritz et al. 2002). Moritz et al. (2002) grouped the main threats to freshwater fish biodiversity into three main categories: habitat loss and modification, exploitative overharvest, and fish community disruption by introduction of cultured or exotic fish. These threats can act synergistically (Cowx 2002), and all have historically altered natural patterns of genetic variation and reduced inter- and intraspecific biodiversity (Table 1-1).

Widespread supplemental stocking of wild populations with cultured fish has been a common practice since aquaculture began in the late 1800s (Montgomery 2003; Kerr 2006). Although often used to enhance fish production beyond the system's natural

capacity (Evans et al. 1991), supplemental stocking has also been used for rehabilitative purposes (i.e., rehabilitative stocking) to rebuild reduced fish populations (Brannon et al. 2004; Krueger and Ebener 2004). In both cases, however, historically stocked hatchery strains were generally developed from source populations that were easy to access and culture in hatcheries, rather than from sources that were genetically similar to recipient populations (Hindar et al. 1991; Mobrand et al. 2005; Moran et al. 2005; Kerr 2006).

Of the major threats facing freshwater fish, the practice of supplementing populations with divergent hatchery strains has the most dangerous potential to steadily erode native biodiversity without immediate and obvious demographic signals (Wilson and Mandrak 2004). This is because numerically abundant cultured fish that contribute to overall population size may have substantially lower fitness than wild spawning populations (Reisenbichler and Rubin 1999; Araki et al. 2007). In many cases, divergent stocked fish have homogenized native genetic structure over large geographic regions through several generations of introgressive admixture (Guinand et al. 2003; Araguas et al. 2004; Williamson 2005). Even when supplemented fish are reproductively unsuccessful, they may simply replace natives because they can be continually added to native populations (Evans and Willox 1991). Resultant losses of native genetic diversity can have long-term evolutionary consequences, as cryptic local adaptations necessary for long-term survival may be lost (Allendorf et al. 2001; Wilson and Mandrak 2004). These negative effects may become evident relatively soon, however, as ongoing climate change and increasing human activities will undoubtedly further alter already degraded fish habitat (Chu et al. 2005).

In recognition of these issues, contemporary policies towards fish culture and supplemental stocking have largely changed in North America. Greater emphasis is placed on matching source populations for hatchery strains to recipient populations (OMNR 1992; Mobrand et al. 2005), and establishing "conservation hatcheries" for rehabilitation of local fish strains (Flagg and Nash 1999). In some administrative districts, supplementation of native populations has been completely discontinued (Evans and Willox 1991; Kerr 2001). However, substantial challenges remain for identifying native populations because historical, pre-stocking samples or genetic data are rarely available for comparative analysis to contemporary populations of interest. This is an immediate problem as competing commercial and recreational fishing interest groups still consider widespread stocking a useful tool for fish production (Landres et al. 2001; Montgomery 2003).

Population supplementation and lake trout

Lake trout are extremely sensitive to anthropogenic impacts and are considered good indicators of overall freshwater ecosystem health (Gunn and Pitblado 2004). Lake trout are distributed throughout most of the previously glaciated areas of North America, but they are highly adapted to specialized habitats with low salinity (below 10-13‰; Martin and Olver 1980), low temperature (14-18°C; Martin and Olver), high oxygen content (ca. 4 mg/L; Evans 2007), and simple fish communities (Martin and Olver 1980). These habitat requirements limit seasonal use of lake habitat, and generally restrict lake trout movements within and among lakes (Evans 2007).

Lake trout (*Salvelinus namaycush*) populations in the Great Lakes region have been heavily stocked since 1867 (Kerr 2006), and serve as an excellent model system to evaluate the genetic impacts from population supplementation. Hatchery strains used for supplemental stocking have originated from source populations throughout the species range, however, most stocked lake trout strains originated from the Great Lakes (Kerr 2001; OMNR 2003; Page 2005). Large numbers of hatchery-origin lake trout have been stocked into both the Great Lakes (ca. 94 million stocked into Lake Superior since 1950, Hansen et al. 1995) and regional inland lakes (ca. 185 million stocked into inland Ontario lakes since 1880, Kerr 2001). However, it is not certain whether widespread genetic homogenization has occurred in many populations (but see Guinand et al. 2003; Piller et al. 2005), in part because multiple factors affect stocked fish and native fish survival (Evans and Willox 1991; Kerr 2001).

The factors affecting survival of stocked lake trout are well known, and are expected to have limited historical gene flow following stocking events. All of the habitat requirements listed above affect survival of stocked lake trout (Evans and Olver 1995; Powell and Carl 2004), as do additional environmental factors (e.g., lake surface area, bathymetry, productivity, and elevation above sea level; (Evans and Olver 1995), spawning habitat type and availability (Marsden et al. 1995), genetic factors such as origin of hatchery strain (Marsden et al. 1993; Grewe et al. 1994b), and hatchery-related practices (e.g., age of stocked fish at release; Powell and Carl 2004). Since stocked fish compete with native species for limited resources, the presence of an indigenous lake trout population has also been identified as a major factor for supplemental stocking

failures (Martin and Fry 1972; MacLean et al. 1981; Powell et al. 1986; Gunn et al. 1990; Powell and Carl 2004).

Even though supplemented lake trout typically show low survival rates, stocked fish may replace native lake trout if stocking is heavy (Guinand et al. 2003) and native mortality levels are high enough (Evans and Willox 1991). If stocking ceases, and the hatchery-origin lake trout are not adapted for local conditions, reduced or admixed remnant populations may not persist. This series of events can have large-scale repercussions. The elimination or replacement of certain fish species that have evolved an integral or "keystone" role on their communities (Power et al. 1996), such as the former lake trout (Brandt 1986; McDonald et al. 1996), can have catastrophic effects and cause ecosystem collapse (Spencer et al. 1991). Resultant ecological instabilities may persist for long periods of time and compromise rehabilitation programs (Eshenroder et al. 1995; Krueger and Ebener 2004) aimed at rebuilding large-scale, economically valuable commercial and recreational fisheries, such as the lake trout fisheries of the Great Lakes (Hansen 1999). Ecological remediation may even be impossible for some ecosystems, if evolutionarily significant components of species biodiversity are lost and no longer available for return to empty ecological niches. This particular issue has been identified as an important factor in the limited success achieved with rehabilitative lake trout stocking in the lower Great Lakes (Burnham-Curtis et al. 1995; Marsden et al. 1995).

The importance of evolutionary history and population processes for lake trout conservation

Natural patterns of genetic structure and the impacts from lake trout stocking have been extensively investigated in the Great Lakes (Grewe and Hebert 1988; Ihssen et al. 1988; Krueger et al. 1989; Marsden et al. 1993; Page et al. 2003; Page et al. 2004; Page 2005), and to a much lesser extent in the inland lakes of the Great Lakes region (Ihssen et al. 1988; Wilson and Hebert 1996, 1998; Piller et al. 2005). Since these two geographical regions support populations with different fish communities, evolutionary histories, and potential for contemporary gene flow, the long-term evolutionary consequences of historical supplemental stocking with lake trout originating from the Great Lakes will be different (Wilson and Mandrak 2004). Consequently, resolution of regional evolutionary histories, and contemporary genetic exchange that have patterned the genetic structure and diversity is essential for understanding basic lake trout biology, and establishing conservation and management guidelines for contemporary populations.

Comparative phylogeographical analyses (Avice 2000) of freshwater and anadromous fishes of recently glaciated regions (e.g., charrs, Pacific salmon, coregonids, and percids, among others) have shown that northern fish species are characterized by low levels of intraspecific divergence relative to fish species from temperate regions (Bernatchez and Wilson 1998). For example, intraspecific lake whitefish lineages have barely exceeded 1% mitochondrial nucleotide sequence divergence (Bernatchez and Dodson 1991) across their North American ranges (e.g., Canada and Alaska). In comparison, centrarchids in the southeastern United States have shown much higher (ca. 6%) levels of intraspecific mitochondrial divergence over substantially smaller geographical ranges (Bermingham and Avice 1986). These relatively shallow lineages probably evolved during long periods of isolation during the Pleistocene glaciations (ca.

1,650 - 15 KYA; Dawson 1992; Bernatchez and Wilson 1998; Wilson and Mandrak 2004) as massive continental ice-sheets repeatedly advanced and retreated over of northern North America and parts of northern Europe (Dawson 1992), cyclically dividing species distributions and isolating fish populations along glacial margins.

After the most recent glacial cycle ended 15 KYA, many fish species dispersed from multiple glacial refuges (e.g., Beringian in the north, Atlantic in the east and Mississippian in the south, and others) to recolonize current species ranges, and were able to move over large geographical regions through a temporary network of cold, meltwater lakes that disappeared approximately 6 KYA (Bailey and Smith 1981; Underhill 1986; Dyke and Prest 1987; Mandrak and Crossman 1992; Wilson and Hebert 1996; Bernatchez and Wilson 1998; Wilson and Hebert 1998). As a consequence of this dynamic evolutionary history, modern-day fish populations in the north have complex spatial genetic structures in spite of their relatively low degrees of intraspecific divergence. Considering the ability of freshwater and anadromous fishes to rapidly diversify in response to variable environments (Behnke 1972; Schluter 1996; Hendry et al. 2000; Moritz et al. 2002), it seems possible that most northern fish populations are adapted to local conditions and represent unique elements of biodiversity.

During the Pleistocene lake trout diverged into multiple lineages, as inferred by phylogenetic analysis of mitochondrial DNA haplotypes (Grewe and Hebert 1988; Grewe et al. 1990; Wilson and Hebert 1996, 1998). Three major lineages emerged in response to early ice-age events (Mississippian-A, Atlantic/Nahannian-B/D, and Beringian-C). Two of these major lineages were subdivided by later glacial cycles (Atlantic-B, Nahannian-D, Mississippian-C1, Missouri-C2, Beringian-C3). Lake trout descended from

all six of Pleistocene lineages colonized the present-day Great Lakes by use of the historical proglacial lake network (Mandrak and Crossman 1992; Wilson and Hebert 1996, 1998). However, many large geographical areas were colonized by lake trout originating from single glacial refuges with limited dispersal ability (Wilson and Hebert 1998). Although some isolated inland lakes, particularly those in the central species range, were colonized by multiple lineages, many present-day populations proximal to historical glacial margins were not (Wilson and Hebert 1998).

Contemporary waterbodies accessible during the proglacial lake period also had a greater number of colonist species (Underhill 1986), with a broader range of divergent, intraspecific lineages (Bernatchez and Wilson 1998). Since larger lakes provide a greater degree and diversity of habitat, they have supported larger, more diverse aquatic populations since deglaciation (MacArthur and Wilson 1967; Barbour and Brown 1974; Mandrak and Crossman 1992). In lake trout, this diversity with area relationship seems to apply within populations as well: Great Lakes lake trout generally have larger population sizes (Swanson and Swedberg 1980; Reid et al. 2001) and higher genetic diversity (Ihssen et al. 1988) than lake trout in smaller, isolated, inland lakes. They have also been evolving for approximately 6 to 15 KY with a greater diversity of predatory and prey species in more complex aquatic communities (Lawrie et al. 1973; Mandrak and Crossman 1992; Evans and Olver 1995; Coon 1999; Bronte et al. 2003; Dobiesz et al. 2005). Furthermore, since environmental and fish communities attributes are variable among inland lake trout lakes (Evans and Olver 1995; Shuter et al. 1998), ample opportunity has existed for ecological adaptation to local conditions (Behnke 1972; Schluter 1996; Hendry et al. 2000).

Given these contrasting evolutionary histories, facilitated genetic exchange (i.e., supplemental stocking) among the Great Lakes and reproductively isolated inland lake trout populations should have been prevented (Crandall et al. 2000). It is currently unclear to what degree native genetic variation has been homogenized by historical stocking, and whether the long-term evolutionary trajectories of stocked populations have been compromised.

Thesis objectives and research approaches

The general goals of my thesis research were to resolve natural patterns of genetic structure and evaluate the impact from supplemental stocking on lake trout populations with different postglacial evolutionary histories (Figure 1-2). Regional lake trout populations were characterized by fundamentally different postglacial histories and their current ability to exchange migrants, using the following categories: 1) the presence of single glacial lineages in allopatric populations, 2) multiple glacial lineages present in sympatric populations, or 3) multiple glacial lineages present in allopatric populations. These categories are genetically representative of lake trout throughout the species range, and also appropriate for evaluation of other freshwater fish taxa (e.g., other salmonids, coregonids, percids, etc.) with variable degrees of postglacial admixture. A fourth possible category, sympatric populations with shared single postglacial ancestry, was not considered, as most contemporary large lakes that support sympatric lake trout populations are remnants of the dispersal-facilitating proglacial lakes. However, this type of population structure could be relevant for lake trout in the extreme north where

exchange among connected lacustrine populations may not be restricted by temperature or other dispersal-limiting habitat factors.

Molecular techniques are essential tools for evaluating natural population processes and providing information for conservation planning. Molecular marker-based methods are used to resolve evolutionary origins and degrees of divergence among populations (Moritz 1994; Avise 2000), evaluate the levels of contemporary gene flow that pattern uniqueness and natural genetic structure among populations (Wright 1943; Nei 1987; Crandall et al. 2000), discriminate between migrants and resident individuals (Rannala and Mountain 1997; Pritchard et al. 2000; Corander et al. 2004), measure genetic diversity (Frankham et al. 2002), evaluate the effects of landscape variation on genetic attributes (Manel et al. 2003; Storfer et al. 2007; Holderegger and Wagner 2008), estimate effective population sizes to evaluate actual population responses to the erosive effects of genetic drift (Lacy 1987; Waples 2006), assess the effects of genetic drift on captive populations (Wang et al. 2002b; Wang 2005), evaluate the genetic attributes of translocated individuals (Stockwell et al. 1996; Moritz 1999), and measure the degree of unnatural genetic homogenization or hybridization in populations supplemented with divergent sources (Allendorf et al. 2001). More recently, there has been a greater emphasis placed on measurement of quantitative molecular genetic variation (Moran 2002; Stockwell et al. 2003), as most commonly used molecular genetic markers are selectively neutral and therefore provide limited insight into adaptive evolutionary processes. Even so, molecular markers remain a particularly important tool for evaluating evolutionary histories, natural patterns of spatial-temporal genetic exchange,

and estimating the genetic impacts from long-term population supplementation on native species.

Parallel analyses of mitochondrial and microsatellite DNA variation were used to characterize sampled lake trout, as these molecular markers provide many different types of critical information on the biological characteristics of both individuals and populations (Hallerman 2003; Stockwell et al. 2003; Holderegger and Wagner 2008). Mitochondrial DNA evolves slowly (ca. 1% nucleotide sequence divergence per 1 million years; Smith 1992), and is therefore useful for inferential analyses of glacial evolutionary histories. In contrast, microsatellites can evolve relatively quickly (ca. 0.0012 mutations per locus per generation in humans; Weber and Wong 1993), and so they can be highly variable within and among recently divergent populations. Although newer sequence-related technologies are becoming more common for wildlife studies, microsatellites remain a powerful tool for measuring genetic diversity and resolving population structure (Holderegger and Wagner 2008).

In contrast, fragment sequencing has generally replaced older, whole-molecule Restriction Fragment Length Polymorphism (RFLP) technologies for mitochondrial DNA analysis and enabled sophisticated evaluations of evolutionary relationships among variably divergent clades (Brocchieri 2001). When properly designed, however, RFLP surveys can detect high levels of mtDNA variability suitable for phylogenetic analyses (Bernatchez and Danzmann 1993). Additionally, RFLP methodologies are extremely valuable for low-cost, reliable diagnostic applications such as resolving major intraspecific mitochondrial DNA lineages (Piller et al. 2005).

The manuscript chapters that follow this introduction were devoted to evaluating the genetic attributes of lake trout populations in each of the three different population genetic categories defined at the beginning of this subsection. Chapter 2 was designed to expand a conservation genetic approach for evaluating the effects of supplemental stocking on inland lake trout (Piller et al. 2005). Southern Ontario was chosen as a study site for this project because stocked inland populations were more abundant there than the original study (Kerr 2001), regional lake trout populations were allopatric, and they had been colonized by only a single glacial lineage (Mississippian-A; Wilson and Hebert 1996, 1998). Regional (type I) native populations were expected to show high degrees of divergence from one another and Great Lakes lake trout in response to an evolutionary history of postglacial isolation (Ihssen et al. 1988), thereby facilitating a straightforward detection of introgressive admixture with stocked lake trout of Great Lakes ancestry. Even so, analysis was challenging because historical, pre-stocking genetic samples were not available for the study populations. Based on earlier allozyme- and questionnaire-based surveys, it was expected that some stocked lakes would show evidence of introgression, but that individual stocking histories would not be indicative of genetic homogenization with divergent hatchery strains.

Chapter 3 was used to evaluate a long-term rehabilitative stocking program aimed at conserving a unique life-history variant in a type 2 region. River-spawning lake trout were endemic to northeastern Lake Superior and migrated into regional tributaries to reproduce in riverine habitat during the fall spawning season, unlike sympatric basin-spawning lake trout. Their abrupt decline and presumed disappearance was coincident with the catastrophic collapse of all Great Lakes lake trout populations due to commercial

overharvest and sea lamprey (*Petromyzon marinus*) predation (Loftus 1958). Before their disappearance, however, river-spawner gametes had been collected for hatchery rearing, then stocked into the Dog River and separately into an inland lake chain to establish three sanctuary populations (Harrison 1968). Although basin-spawning lake trout were later introduced to these lakes, the sanctuary populations were used to develop a hatchery strain (Mishibishu) that was supplemented into the Dog and Montreal Rivers, along with other hatchery strains of ambiguous origins for nearly twenty years. Hatchery-strain lake trout of varied and often ambiguous origin were also heavily stocked elsewhere in Lake Superior until 1996 (OMNR 1984; Hansen et al. 1995).

The recent reappearance of spawning lake trout in the Dog and Montreal rivers raised the question of whether these fish originated from: 1) a stocking source outside the Great Lakes, 2) a stocking source within the Great Lakes, 3) the sanctuary populations, 4) recovering, indigenous populations, or 5) a mixture of native and hatchery strain individuals. These possible origins were evaluated by comparative genetic analysis of present-day river-spawners, historical samples taken from the Dog River in 1952, the sanctuary populations, the Mishibishu hatchery strain, other surviving Lake Superior and Lake Huron populations, and two divergent inland populations from the Great Lakes region. Similar genetic approaches had been previously used to evaluate contemporary hatchery strains and populations from the Great Lakes in other studies, and showed that although there was evidence for genetic homogenization by historical stocking (Guinand et al. 2003), there remained a low, detectable degree of regional genetic structure (Page et al. 2004).

In Chapter 4, a landscape genetic approach (Manel et al. 2003; Storfer et al. 2007; Holderegger and Wagner 2008) was used to resolve the relative influences from postglacial events, environmental variability, and supplemental stocking on the allopatric populations of Algonquin Park, Ontario. The broad-scale spatial distribution of lake trout genetic diversity is known (Wilson and Hebert 1996, 1998), and large geographical regions throughout the central species range show evidence of varied degrees of postglacial admixture. Fine-scale genetic structure among allopatric populations was expected to be complex in these areas, reflecting dynamic colonization events that were mediated by dispersal through a historical postglacial lake network. Based on regional landscape features and previous mitochondrial DNA surveys (Wilson and Hebert 1996), it was hypothesized that lake trout from multiple glacial lineages had colonized the region (type 3). However, it was expected that key landscape attributes (lake surface area, elevation, conductivity, and mean depth) had modulated genetic diversity and population genetic structure since colonization. Although historical stocking was intermittent and comparatively light (relative to elsewhere in the Great Lakes region), park populations were also evaluated for evidence of introgression with hatchery strains of Great Lakes origin.

Together, the results obtained by these three projects will expand the understanding of how natural processes pattern fine-scale genetic variation in freshwater fishes, and characterize how extensive supplemental stocking has affected the genetic characteristics of native fish populations. This work will also provide much needed genetic information for regional conservation and management of lake trout populations, which will be applicable for genetic management throughout the species range. For

future conservation planning, this information will be important for developing genetic guidelines for identifying conservation units that preserve natural evolutionary processes (Moritz 1994; Moritz et al. 2002), limit genetic exchange among naturally divergent lineages (Fraser and Bernatchez 2001), and reflect possible ecological adaptations (Waples 1991; Crandall et al. 2000), until all the causes for conservation concern can be addressed.

Table 1-1.

Case examples for categorical threats (Moritz et al. 2002) to contemporary freshwater fish biodiversity and their genetic impacts.

Threat	Example	Species	Common	Genetic impacts	Ref.
Habitat loss and modification	Columbia River damming	<i>Oncorhynchus</i> spp.	Pacific salmon	Loss of biodiversity, genetic variation, adaptive potential	1
Exploitation and overharvest	Great Lakes commercial fishing*	<i>Salvelinus namaycush</i>	lake trout	Loss of biodiversity, genetic variation	2,3
Introduction of exotic fish	Rainbow trout introductions to western US streams	<i>Oncorhynchus clarki</i> ssp.	cutthroat trout	Loss of genetic variation by hybridization	4,5
Introduction of cultured fish	Supplemental stocking in European streams	<i>Salmo trutta</i>	brown trout	Homogenization of native genetic diversity	6,7,8

*Effects compounded by coincident introduction of the exotic predatory sea lamprey (*Petromyzon marinus*) and pollution from agricultural, urban, and industrial sources

¹Waples et al. (2008)

²Burnham-Curtis et al. (1995)

³Krueger and Ebner (2004)

⁴Boyer et al. (2008)

⁵Allendorf et al. (2005)

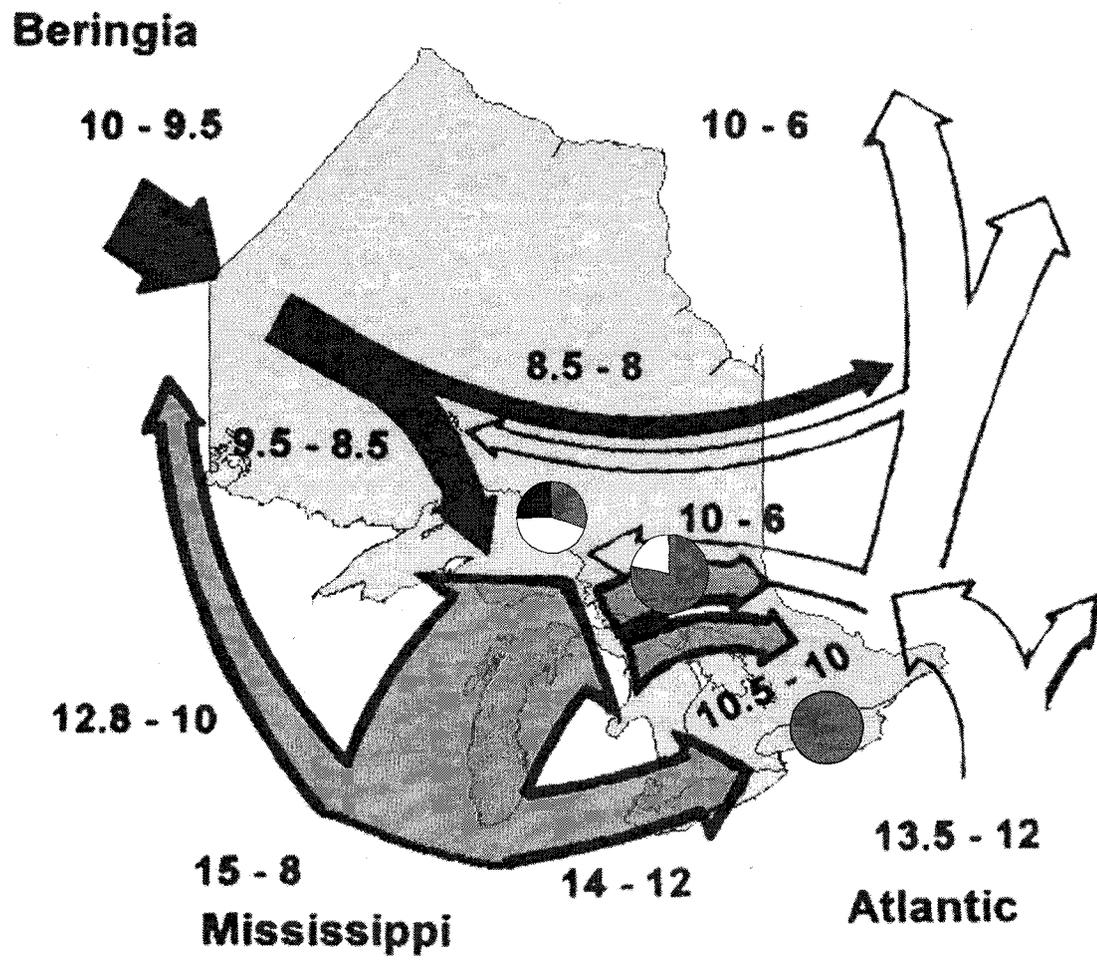
⁶Almodovar et al. (2001)

⁷Araguas et al. (2004)

⁸Marzano et al. (2003)

Figure 1-1

Postglacial dispersal of lake trout (*Salvelinus namaycush*) into the Great Lakes region. Dispersal routes were inferred from the observed spatial distribution of mitochondrial DNA haplotypes sampled in Wilson and Hebert 1996, as well as other genetic and biogeographical studies (Lindsey 1964; Khan and Qadri 1971; Mandrak and Crossman 1992; Grewe et al. 1993). Numbers along the arrows indicate approximate time periods (KYA) for colonization events. Pie charts show mitochondrial haplotype frequencies for regionally representative populations (Wilson and Hebert 1996, 1998). The figure was modified from Wilson and Hebert (1996).



CHAPTER 2**Variable introgression from supplemental stocking in southern Ontario populations
of lake trout (*Salvelinus namaycush*)**

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Abstract

An unintended consequence of fish stocking is genetic homogenization from interbreeding between indigenous populations and genetically distinct hatchery strains. Lake trout populations in southern Ontario have been extensively stocked with hatchery strains originating from Great Lakes sources, but evaluation of introgressive admixture has been challenging without data or samples that precede historical stocking events. We used complementary genetic markers (mitochondrial PCR-RFLP and 12 microsatellite DNA loci) to resolve native and introgressed genetic profiles for lake trout from four unstocked lakes and eight stocked lakes, using samples from an introduced population and source hatchery strains for comparison. We predicted that some inland populations would retain a composite native genetic profile, similar to unstocked populations, whereas introduced and introgressed populations should resemble hatchery sources. Allele frequency-based methods and Bayesian individual assignment techniques gave largely congruent results for inferred population ancestries. Four of the eight stocked lakes included in this analysis exhibited genetic profiles consistent with native ancestry, indicating limited introgressive admixture. The remaining stocked populations, however, showed evidence of introgression and homogenization with genetically distinct stocked fish. Recorded stocking history alone was not indicative of admixture in these populations, suggesting that other genetic, ecological, and/or anthropogenic factors facilitate reproduction between native and stocked fish.

Introduction

Supplemental stocking of wild salmonid populations with hatchery-reared fish has been widely practiced for most of the past century, but has become increasingly controversial in recent years (Hilborn 1992; Brannon et al. 2004; Araki et al. 2007). Members of the salmonid family have been among the most popular fish species for stocking since fish culture began in North America in the 1800s; salmon, trout and charr species collectively support substantial commercial and recreational fisheries (Nehlsen et al. 1991; Epifanio 2000; Post et al. 2002). Since stocked hatchery fish may differ substantially from wild populations in key biological attributes, however, their suitability for enhancing wild populations has been questioned.

Many of these differences have been attributed to unnatural selective pressures and exaggerated genetic drift experienced during captive rearing (Araki et al. 2007). Cultured individuals have shown differences in life-history variation (Leider et al. 1986; Unwin and Glova 1997), morphology (Taylor 1986; Fleming et al. 1994), reproductive behaviours (Berejikian et al. 1997; Jonsson 1997), and genetic backgrounds (Hindar et al. 1991) compared to wild individuals. Hatchery-reared, stocked fish may also show reduced survival (Reisenbichler and McIntyre 1977; Araki et al. 2007), contribute to the loss of wild individuals (Evans and Willox 1991; Nickelson 2003), and reduce or homogenize native intraspecific genetic diversity through interbreeding with evolutionarily divergent indigenous populations (Hindar et al. 1991). As a result, contemporary conservation-oriented hatchery practices are oriented towards limiting potentially negative impacts resultant from captive rearing and domestication (Brannon et

al. 2004; Mobrand et al. 2005). These practices include producing genetically similar fish for supplementing native populations to minimize genetic homogenization among wild populations (Hindar et al. 1991; Allendorf et al. 2001; Wilson and Mandrak 2004).

The lake trout, *Salvelinus namaycush*, has a broad distribution across oligotrophic North American lakes, and has been subjected to intensive stocking for over a century (Evans and Willox 1991; Kerr 2001; Powell and Carl 2004). Even though the presence of an indigenous population limits survival of stocked lake trout (Evans and Olver 1995), indigenous lake trout can be numerically replaced with reproductively unsuccessful stocked lake trout if stocking and mortality rates are high enough. Consequently, Evans and Willox (1991) suggested that supplemental stocking of inland lake trout populations be discontinued in Ontario. The Ontario Ministry of Natural Resources followed this recommendation and phased out supplemental stocking of self-sustaining inland lake trout populations in the 1990s. Provincial inland lakes are now typically stocked with lake trout only for rehabilitation purposes, or to support introduced, "put-grow-take" fisheries that draw angling pressure away from sensitive indigenous lake trout populations (Evans et al. 1991).

It is not clear whether large-scale historical stocking has led to genetic homogenization and detectable introgression in supplemented Ontario lake trout populations. It is estimated that over 100 million hatchery-reared lake trout have been stocked into the inland lakes of Ontario since 1880 (Kerr 2001). Sources for historical stocking are largely unknown and may have included populations from both inland and Great Lakes origins, however, records indicate an early preference for stocking lake trout from the Great Lakes region that continues to the present (Kerr 2001). Provincial

stocking records maintained since 1970 by the Ontario Fisheries Information System (OFIS) show that by the time strain origins were well documented, over 93% of all fish stocked into inland lake trout populations originated from the Great Lakes (Lake Superior, Lake Huron or Lake Ontario), or from regional lake trout populations that shared a common postglacial history with Great Lakes populations (Lake Manitou, Lake Simcoe, or Killala Lake) (Ihssen et al. 1988; Wilson and Hebert 1996, 1998; Stott 1998). Within the province, southern Ontario provides an ideal region to expand the approach used by Piller et al. (2005) for evaluation of hatchery-strain introgression in numerous stocked inland lake trout populations. Since the two supplemented inland populations evaluated in this earlier study showed evidence against introgressive admixture, it is not certain that recorded stocking history is indicative of genetic homogenization resultant from introgression.

Detection of genetic homogenization from interbreeding with stocked fish is relatively straightforward when genetic samples are available from stocking sources and pre-stocking, recipient populations (Araguas et al. 2004; Moran et al. 2005). Since appropriate historical genetic samples are not available for stocked Ontario lake trout populations, an understanding of regional intraspecific genetic diversity is required for the development of population-level genetic profiles. These profiles are used for characterizing populations as indigenous (native) or descendent from stocked fish (introgressed or purely hatchery-ancestry), and can enable the detection of introgression regardless of the origin of the stocked fish, provided the stocking sources and recipient populations are divergent, and thus not too genetically similar (Piller et al. 2005).

The patterning of intraspecific genetic diversity among inland lake trout that resulted from postglacial dispersal facilitates detection of introgression in some native populations (Wilson and Mandrak 2004). Indigenous southern Ontario lake trout populations were largely founded from a single (Mississippian) glacial lineage, and were subsequently isolated from each another and from higher-diversity populations in the Great Lakes region until supplemental stocking began (Wilson and Mandrak 2004). Genetic analysis of lake trout populations in and near the Great Lakes using allozymes, mitochondrial DNA and microsatellite DNA revealed that the habitat-enforced isolation of inland lake trout populations was reflected in their divergence from one another, and from higher genetic diversity lake trout populations from the Great Lakes area (Grewe et al. 1993; Wilson and Hebert 1996, 1998; Stott 1998). While historical patterns of stocking among genetically similar lake trout populations of higher genetic diversity may be difficult to resolve, directional gene flow from regions of higher genetic diversity into divergent, lower genetic diversity populations is readily detectable (Piller et al. 2005).

We predicted that supplemental stocking has had a limited homogenizing effect on native genetic diversity and population structure in southern Ontario. Not only do self-sustaining lake trout populations limit successful establishment of stocked fish (Powell et al. 1986; Gunn et al. 1987; Evans and Olver 1995), but hatchery-reared individuals originating from Great Lakes environments may be less adapted for survival in smaller, ecologically dissimilar inland lakes than native fish that have evolved *in situ* since deglaciation (MacLean et al. 1981; Powell and Carl 2004; Wilson and Mandrak 2004). Consequently, we expected that some stocked populations would be genetically distinct from stocking source populations. Under some scenarios for supplemental

stocking and related exploitation, however, reinforcement of hatchery fish through repeated stocking may result in numerical replacement or erosion of the native population, resulting in genetic homogenization and introgression (Evans and Willox 1991). The results of this analysis provide useful information to management agencies, and provide a more generalized, region-specific methodology for detecting population-level admixture in low diversity lake trout populations throughout the species range.

Methods

Sample collection

Samples for genetic analysis were derived from a number of sources, including directed netting, angling, hatchery monitoring, and through angler creel surveys. Tissue samples varied in composition and individual samples were taken from adipose fin clips, 20 mg caudal fin punches, fish heads collected from ice-fishing creel surveys, or muscle tissue frozen for allozyme analysis. All tissue samples collected for genetic analysis were either immediately frozen or stored in a 95% solution of ethanol until genetic material was extracted.

Three hatchery strains originating from the Great Lakes region were included in this analysis: the Lake Manitou strain, the Slate Islands strain, and the Killala lake strain (Table 2-1). Hatchery strain genetic samples were obtained from the Ontario Ministry of Natural Resources Fish Culture Section. Samples from the Slate Islands strain (Lake Superior) were collected by personnel from the Dorion Fish Culture station (Dorion, ON)

during spawn-collection from the wild population in fall of 2004. Samples from the Lake Manitou strain (Manitoulin Island, Lake Huron) originated from individuals reared at the Blue Jay Creek Fish Culture station (Tehkummah, ON) in 2003. No broodstock is maintained for the Lake Manitou strain; gametes are collected from wild adults annually for fish culture and provincial stocking. Lake trout samples from the Killala Lake strain (Killala Lake, ON) were taken from a third-generation (F_3), captive broodstock (KLHL00HL) bred from north-basin adults; this strain is maintained at the Hills Lake Fish Culture station (Englehart, ON). All of these hatchery strains share a common evolutionary history; their ancestral populations were founded by immigrants from multiple glacial lineages during the postglacial recolonization of the Great Lakes region. Thus, they provide a representative pool of genetic diversity that is broadly characteristic of all documented lake trout strains that have been stocked into inland Ontario lakes (Grewe and Hebert 1988; Stott 1998; Wilson and Hebert 1998). Further information on attributes of these and other provincial hatchery strains is available in the Fish Culture Stocks Catalogue published by the Ontario Ministry of Natural Resources (OMNR 2003).

Most of the inland lake trout populations included in this analysis have been studied to some degree by previous allozyme (Ihssen et al. 1988), mtDNA (Wilson and Hebert 1996, 1998) or microsatellite DNA (Stott 1998) analyses. For comparative purposes, additional populations of known ancestry inferred from both stocking records and genetic background were specifically included in this analysis: Miskwabi Lake supports a known introduced population, and lake trout populations in Macdonald, Clean, Louisa, and Crystal Lakes are known to be unstocked, indigenous populations.

Inland lake trout populations of native or ambiguous ancestry were also selected based on geographic location, lake and population attributes, recorded stocking history, and genetic background from three regions of southern Ontario (Table 2-1): the Haliburton Highlands, the Algonquin Highlands, and the Bancroft region. Lake trout populations selected for genetic analysis in the Haliburton Highlands region originated from Miskwabi Lake (MSK), Boshkung Lake (BKG), Macdonald Lake (MAC), Clean Lake (CLE), Redstone Lake (RST), Grace Lake (GRC), Esson Lake (ESS), Farquhar Lake (FRQ), and Kingscote Lake (KS). Smoke Lake (SMK) and Louisa Lake (LOU) are located in the Algonquin Highlands, while Barker Lake (BRK) and Crystal Lake (XTL) are located in the Bancroft district of southern Ontario. Study lake sizes ranged from 55.3 to 1130.3 ha, with conductivities ranging from 32 to 114 mg/L and mean depths from 4.1 to 23.1 m (Table 2-1) (Gunn et al. 2004). These lake attributes are inclusive of 72% (lake area), 83% (conductivity), and 94% (mean depth) of reported self-sustaining lake trout lakes in southern Ontario (OMNR 1989), and inclusive of 66%, 62%, and 92% of all lake trout lakes in Ontario (Gunn et al. 2004), respectively. Lake area, conductivity, and mean depth are important physical and chemical parameters that are important not only for successful stocking and introductions, but are also predictive for inland lake trout production (Ryder 1965; Shuter et al. 1998) and life-history variation (Shuter et al. 1998). While somewhat less representative of the original lake trout lakes of Ontario, this study lake set is more representative of contemporary populations of conservation concern in southern Ontario.

Inland lake trout samples were collected for genetic analysis from 1998 to 2005 (Table 2-1) either by targeted netting or creel survey. Samples from Miskwabi, Esson,

Farquhar, and Grace Lakes were collected by members of the Minden District Office of the Ontario Ministry of Natural Resources (Minden, ON) as part of an ongoing study of stocking on native lake trout populations during fall, winter and spring creel surveys in 1998, 2001, and 2003. Lake trout samples from Boshkung, Macdonald, Clean, Redstone, Barker, and Crystal Lakes were obtained from the Minden and Bancroft district offices (OMNR) as part of a larger, regional genetic diversity survey performed in 1998, 2001, 2004, and 2005. Genetic samples from Kingscote Lake were collected from spawning adults captured in fall of 1998. Samples from the Smoke and Louisa lake trout populations were collected in summers of 2001 and 2005 by personnel from the Algonquin Fisheries Assessment Unit (Whitney, ON) and the Freshwater Fisheries Cooperative (Sudbury, ON) of the Ontario Ministry of Natural Resources.

DNA extraction and amplification

DNA was extracted from tissue samples through a method detailed in Wilson et al. (2007). In summary, lysis of individual tissue samples (20 mg) was performed in TNES-Urea lysis buffer (10 mM Tris-HCl pH = 7.5, 125 mM NaCl, 5 mM EDTA pH = 8.0, 0.5% SDS, 4 M Urea) supplemented with proteinase K (0.2 mg/mL). The sample DNA was precipitated with sequential addition of a 5 M NaCl and 80% isopropanol solutions, followed by a round of centrifugation. The resultant precipitant was washed with a 70% ethanol solution, centrifuged again, and after evaporation of the ethanol, the sample DNA was resuspended in TE buffer (10 mM Tris-HCl, 1 mM EDTA) (Sambrook et al. 1989).

Approximately 16 ng of DNA was used for each Polymerase Chain Reaction (PCR). Twelve microsatellite loci were amplified by both single locus and multiplex reactions in the following PCR cocktail (10 μ L/reaction): 2 μ L template DNA solution, 1 μ L PCR buffer (Qiagen, Inc.), 1 μ L Q-solution (Qiagen, Inc.), 1 μ L 10 mM dNTPs, 0.05 U *Taq* polymerase (Qiagen, Inc.), and locus-specific, fluorescently-labelled primer pairs (see Appendix Table A-1-1 for annealing temperatures, PCR cycles, and primer sequence references). Primer quantities used for single locus amplifications (per reaction) with dye labels indicated in superscript were: *Sfo18*^{6FAM} (3.8 pmol), *Sfo19*^{HEX} (3.8 pmol), *Sfo23*^{NED} (2.5 pmol), *Oneu14*^{6FAM} (2.5 pmol), *Ots1*^{6FAM} (3 pmol). Primer quantities (per reaction) used for multiplex amplifications: 1 - *Sfo1*^{NED} (2 pmol), *Ssa85*^{6FAM} (2 pmol), *Ogo1a*^{HEX} (5 pmol); 2 - *SfoC88*^{NED} (2 pmol), *SfoC24*^{6FAM} (2 pmol); 3- *SfoD75*^{6FAM} (5 pmol) , *Sfo12*^{HEX} (2 pmol). Amplicons were then pooled, and to each 0.5 μ L individual sample was added 1.0 μ L molecular weight standard (0.22 μ L ROX, 0.78 μ L formamide; Applied Biosystems, Inc.). Pooling was performed in the following manner: pool A - multiplex 1, 2, 3; pool B - *Sfo18*^{6FAM}, *Sfo19*^{HEX}, *Ots1*^{6FAM}; pool C - *Sfo23*^{NED}, *Oneu14*^{6FAM}. Microsatellite amplicons were resolved with an ABI 377 automated DNA sequencer; individual genotypes were visualized and scored with both Genescan and Genotyper software (Applied Biosystems, Inc.), then manually verified by visual inspection of raw gel images. To ensure consistent allele scoring, a standard was included with each machine run, and subsets of eight individuals from each run were re-scored on a common gel for three highly variable loci with large allelic ranges (*Ots1*, *Sfo23*, and *Oneu14*).

Mitochondrial DNA variation was analyzed with the PCR-RFLP method detailed in Piller et al. (2005) for inference of phylogeographic evolutionary history. Briefly, amplicons from the cytochrome *b* and the NADH dehydrogenase (subunits 3 and 4) regions of the lake trout mitochondrial genome were amplified with unlabelled, locus-specific primer pairs, then digested with *Bam*HI (New England Biolabs). Diagnostic restriction enzyme cut sites indicating Mississippian - A, Atlantic/Nahannian - B/D, or Beringian - C lineages (Wilson and Hebert 1996, 1998) were resolved by gel electrophoresis on 1.25% agarose, and visualized by PCR-RFLP fragment staining with SYBR Green dye (Molecular Probes, Inc.).

Population genetic analysis

Microsatellite DNA variation was evaluated by use of both allele frequency based methods and individual assignment tests based on multilocus genotypes. Measures of genetic diversity were estimated with three software packages: FSTAT v. 2.9.3 (Goudet 2001), Microsatellite Toolkit (Park 2001) and GenAlEx (Peakall and Smouse 2006). The genetic diversity measurements included allelic richness (A_R), which is an genetic diversity estimate standardized to sample size (Frankham et al. 2002), the average number of microsatellite alleles per locus (N_A), an unbiased estimator of heterozygosity, the gene diversity (H_E) of Nei (1987), the observed heterozygosity (H_O), and the effective number of alleles (n_e) (Frankham et al. 2002). Monte Carlo analyses were used to test for differences in genetic diversity between populations with categorically higher and lower genetic diversity (Gotelli and Ellison 2004). The absolute value for the difference in

average genetic diversity estimates between categories (D_{AVG}) was compared against a null distribution of differences generated from 10,000 simulations that shuffled diversity values among categories; the test statistic was considered significant if it exceeded 95% of simulated values. Tests for Hardy-Weinberg equilibrium within populations were performed using GenePop v. 3.4 (Raymond and Rousset 1995). Sequential Bonferroni adjustments were used to correct for all multiple pairwise tests; the nominal significance level ($\alpha = 0.05$) was initially divided by k , the total number of pairwise tests (Rice 1989).

Pairwise genetic distances (Nei et al. 1983) were estimated with Populations v. 1.2.28 (Languella 1999); and evaluated with 1000 bootstrap replicates across all loci. The resulting consensus neighbor-joining dendrogram was visualized with TreeView 1.6.6 (Page 2000). Pairwise divergence estimates among populations (F_{ST}) were calculated in FSTAT v. 2.9.3 (Goudet 2001), with corrections for multiple tests as outlined above. Mitochondrial DNA haplotype frequencies were compiled by population, and differences among frequency distributions were compared with the haplotype dataset input option of FSTAT v. 2.9.3 (Goudet 2001).

Multiple different individual assignment methods were evaluated for estimation of population admixture and introgression with hatchery strains. In all of these tests, individuals are assigned to potential source populations on the basis of probabilities calculated from the individual's multilocus microsatellite genotype. Following the approach of Piller et al. (2005) to evaluate admixture in stocked inland lake trout populations, we used the Bayesian assignment method of Rannala and Mountain (1997) as implemented in GeneClass 2.0 (Piry et al. 2004), in tandem with the frequentist exclusion method of Cornuet (1999). Genotype likelihood distributions were generated

through 10,000 simulated genotypes re-sampled from each study population, and were used to either exclude populations of origin or assign individuals based on comparative similarity of individual genotype likelihoods. Populations were rejected as possible origins for individuals at a threshold value ($P < 0.01$ or $P < 0.05$). This approach allowed for individual assignment to a single population of origin or assignment to an admixed group: unresolved Great Lakes (Lake Manitou, Lake Superior, Killala Lake, and Miskwabi Lake), admixed inland (Boshkung, Esson, Farquhar, Grace, Macdonald, Clean, Redstone, Kingscote, Louisa, Smoke, and Barker Lakes), or admixed inland and Great Lakes (Lake Manitou, Lake Superior, Killala Lake, and Miskwabi Lake and any other inland lake population). Thus, individual assignment to an admixed group indicates failure to exclude all but a single population of origin at a threshold value (e.g., $P < 0.01$). For comparison, individuals were also assigned strictly on the maximum probability of belonging to any single reference population (i.e., all other populations were excluded as possible populations of origin). Proportional individual reassignments were also compared to genotype likelihood scores (described by Piry et al. 2004; hereafter abbreviated L/L_{SUM}), which are calculated based on observed genotypes rather than distributions of simulated genotypes. To improve assignment accuracy, two microsatellite loci with extremely low genetic diversity and resolving power (*SfoC88* and *Sfo1*) were excluded from all assignments and probability calculations.

Individual assignments to populations of origin were also assessed using the Bayesian program STRUCTURE 2.1 (Pritchard et. al (2000)). Individuals are assigned to inferred genetic clusters based on membership coefficients (q) calculated from an individual's multilocus genotype. This model-based approach enables genetic clustering

from multiple possible ancestry and allele frequency models, user modulated input parameters, and individual genotypes from study populations. The admixture ancestry model was chosen to allow the possibility that each individual may have mixed ancestry of multiple origins; the Dirichlet parameter for degree of admixture (α) was uniform across all populations and inferred from an initial value of $\alpha_o = 1.0$; similar results were also obtained when α was inferred for each population. Patterns of relatedness resulting from recent immigration or shared ancestry between study populations are accounted for by the correlated allele frequency model; the parameter λ was also constrained to uniformity across all populations, and inferred from an initial value of $\lambda_o = 1.0$. A burn-in period of 200,000 iterations was performed before execution of 200,000 Monte-Carlo Markov Chain repetitions required for population model simulation.

For post-simulation model evaluation and estimation of the optimal number of expected populations (K), we followed the guidelines provided by Pritchard et al. (2000) and Evanno et al. (2005). The number of expected populations was incrementally adjusted from $K = 1$ to 15; five replicate simulations were performed for each population model to evaluate modality of the likelihood distribution ($\Pr[X|K]$). As suggested by Pritchard et al. (2007), we also evaluated substructure within resolved genetic clusters by further simulations using only individuals within each cluster. For sub-cluster analysis the number of expected populations was incrementally adjusted from $K = 1$ to 10. The optimal K value for genetic clusters and sub-cluster groups was estimated by the method of Pritchard et al. (2000), unless computed posterior probabilities for the expected number of populations ($\Pr[K]$) did not clearly indicate a most probable model. For these situations, the method of Evanno et al. (2005) was used to estimate the optimal value for

K. Population-level admixture was inferred from calculated individual membership coefficients (q), which were compared within and among population models and visualized with DISTRUCT, a program for graphical display of STRUCTURE results (Rosenburg 2004).

Genetic profiles for each population were developed by evaluating regional phylogeographic history, genetic diversity, population divergence, population structure, and multilocus genotype individual assignment tests. A regional, southern Ontario native profile corresponded with populations that possessed primarily Mississippian-A mtDNA haplotypes, had low microsatellite DNA diversity, were highly divergent from other inland populations and hatchery strains, and whose individuals reassigned with high probability to their population of origin or a native genetic cluster. Introgressed and introduced populations were expected to have genetic profiles similar to hatchery strains historically stocked into the region; these populations would have multiple mtDNA haplotypes, higher microsatellite genetic diversity, and either assignment to a hatchery-strain genetic cluster or poor individual reassignment to a single genetic cluster or population of origin.

Results

Synthesis of stocking histories

Compiled records indicate that nearly 600,000 lake trout have been stocked into nine of the thirteen inland lakes included in this analysis, but source populations for most

stocked hatchery strains are unknown (Table 2-2). Four lakes have no recorded lake trout stocking history (Macdonald, Clean, Louisa and Crystal Lakes), but the nine stocked lakes have received a range of stocking from as early as 1887 (Redstone Lake) to as recently as 1994 (Barker Lake) (Kerr 2001). Stocking intensity estimates varied from 50.3 (Redstone Lake) to 351.6 lake trout per hectare (Barker Lake). Most recorded fish stocked were fingerlings or juveniles, concordant with the province-wide shift in hatchery practices towards use of later life-stages for inland stocking after the 1930s (Kerr 2001).

Since 1970, 88% of all lake trout stocked into the study lakes originated from Great Lakes populations, or from populations closely related to Great Lakes lake trout (Table 2-2). The origins of the remaining 12% of stocked lake trout were not documented. Three strains have predominantly been stocked in the study lakes since 1970: Lake Manitou, 31%; Lake Superior, 43%; Killala Lake, 13% (Table 2-2; Kerr 2001). The other documented hatchery strains were from similarly diverse sources: Lake Simcoe (4013 individuals stocked into Boshkung Lake in 1985) and Lake Ontario (1000 individuals stocked into Smoke Lake in 1973). The Lake Simcoe lake trout population is genetically similar to populations of Great Lakes origin and divergent from inland lake trout populations in southern Ontario (Grewe et al. 1993; Stott 1998). Lake trout present in Lake Ontario in 1973 would have been a mixture of hatchery strains commonly stocked into Lake Ontario at this time (e.g., Lake Manitou and Lake Superior strains) (Elrod et al. 1995). Since relatively few of these fish were stocked in either lake, their contribution to contemporary populations was expected to be minimal, however, the genetic profiling methodology used in this analysis would allow the detection of potential genetic contributions from these sources. The genetic backgrounds of hatchery-reared

lake trout stocked before 1970 were not well documented, and exact source populations are unknown.

Phylogeographic history

The geographic distribution of mtDNA haplotype frequencies in southern Ontario indicates the presence of both native and introgressed/hatchery strain population profiles (Figure 2-1). Six populations (Macdonald, Clean, Redstone, Crystal, Louisa, and Smoke Lakes) possessed only a single, Mississippian-A haplotype expected of regional native populations, and five populations (Esson, Farquhar, Grace, Kingscote, and Barker Lakes) had an additional Atlantic/Nahannian-B/D lineage present in introduced (Miskwabi Lake), and mixed-ancestry (Boshkung Lake) populations. This second lineage is also present in high proportions in all three hatchery strains (Killala Lake, Manitou and Slate Islands strains). While common in eastern regions of Canada that were colonized by lake trout dispersing directly from the Atlantic refuge, lake trout descendent from the Atlantic/Nahannian-B/D lineage are not expected in southern Ontario outside the maximum extent of the historical pro-glacial lake network (Wilson and Hebert 1998). Lake trout with haplotype C were detected in only one southern Ontario population (Kingscote Lake), but were present in the Slate Islands hatchery strain, as expected from previous mtDNA analyses of Lake Superior lake trout (Grewe and Hebert 1988; Grewe et al. 1993; Wilson and Hebert 1996, 1998; Piller et al. 2005).

Genetic diversity

Properties and genetic diversity statistics of microsatellite loci evaluated in this analysis are summarized in Appendix Table A-1-1. To more accurately estimate divergence among populations, loci with high and low allelic diversity were included. Number of observed alleles ranged from 2 alleles (*SfoC88*) to 33 alleles (*Sfo23*) across all populations. Some allele frequency distributions had one or more common alleles across all populations, while others were extremely variable (Appendix Table A-2-1). No deficit of heterozygotes was detected in 192 pairwise tests of Hardy-Weinberg equilibrium within populations ($\alpha = 0.05$; $k = 192$).

Six traditional estimators of genetic diversity were calculated from microsatellite DNA allele frequency distributions, and compared among study populations (Table 2-3). Ordered distributions of four statistics exhibited a "drop-off" value or discontinuity that clearly partitioned population genetic diversity in terms of a native inland genetic profile (lower genetic diversity) and an introgressed/hatchery strain profile (higher genetic diversity). All four of these statistics (expected heterozygosity - H_E , mean number of alleles per locus - N_A , allelic richness - A_R , and effective number of alleles - n_e) showed significant differences (Monte Carlo analysis; $\alpha = 0.05$) between lower and higher genetic diversity populations. The remaining two statistics (observed heterozygosity - H_O , and number of private alleles - N_P) did not show clear discontinuities across the study populations.

Nei's gene diversity (Nei 1987) provides an unbiased estimator of expected heterozygosity (H_E), a statistic that is expected to be lower in isolated, divergent, inland populations than in populations with recent genetic exchange (Frankham et al. 2002).

Populations with a native inland genetic diversity profile had significantly lower ($D_{\text{AVG}} = 0.191$; $P < 0.001$) gene diversities than higher diversity populations. Values for gene diversity ranged from $H_E = 0.239$ to 0.492 and included eight inland populations (Macdonald, Clean, Redstone, Kingscote, Louisa, Smoke, Barker, and Crystal Lakes) and one hatchery strain (Killala Lake). Two hatchery strains (Lakes Manitou and Superior) and five inland populations (Miskwabi, Boshkung, Grace, Esson and Farquhar Lakes) had an introgressed/hatchery strain diversity profile indicated by higher gene diversities ranging from $H_E = 0.558$ to 0.629 .

Allelic richness (A_R) provides a measure of multi-locus allelic diversity that is standardized among populations of different sample sizes, unlike other commonly used measures of genetic diversity such as the mean number of alleles per locus (N_A). Both measures are expected to be lower in divergent populations whose original genetic diversity has been eroded by genetic drift (Frankham et al. 2002). Both statistics show a concordant partitioning of values: eight populations (Macdonald, Clean, Redstone, Kingscote, Louisa, Smoke, Barker, and Crystal Lakes) had a native inland genetic diversity profile with significantly lower allelic richness ($D_{\text{AVG}} = 2.70$; $P < 0.001$) and mean number of alleles ($D_{\text{AVG}} = 3.00$; $P < 0.001$) than higher diversity populations. Allelic richness in populations with a native profile ranged from $A_R = 2.59$ to 4.73 , and mean number of alleles ranged from $N_A = 2.75$ to 5.42 . All hatchery strains and five inland populations (Miskwabi, Boshkung, Grace, Esson and Farquhar Lakes) showed an introgressed/hatchery strain diversity profile corresponding to a higher range of allelic richness values, $A_R = 6.01$ to 7.40 , and a higher range of values for mean number of alleles, $N_A = 6.67$ to 8.25 .

Genetic drift and mutation should increase the number of private microsatellite alleles (N_p) in isolated, divergent populations descendent from a common source. Surprisingly few private alleles were observed in otherwise lower diversity inland populations (Macdonald, Clean, Redstone, Kingscote, Louisa, Smoke, Barker, and Crystal Lakes; $N_p = 0$ to 1), in contrast to most higher genetic diversity populations (Miskwabi, Boshkung, Esson and Farquhar Lakes and all three hatchery strains; $N_p = 2$ to 4). There was no partitioning discontinuity for this statistic; additionally, the number of private alleles was substantially lower in some populations with otherwise higher genetic diversity (Grace and Killala Lakes; $N_p = 0$).

The final genetic diversity measure evaluated, the effective number of alleles (n_e), is the number of alleles per locus that if equally frequent would result in the observed homozygosity; it is expected to be lower in reproductively isolated populations (Frankham et al. 2002). Calculated values for n_e (averaged across all loci) partitioned the study lake trout populations in the same fashion as allelic richness and the average number of alleles per locus. Populations with a native profile had significantly lower ($D_{AVG} = 1.63$; $P < 0.001$) estimates for effective number of alleles ($n_e = 1.76$ to 2.64), than higher genetic diversity populations ($n_e = 3.34 - 4.72$). For all four discriminatory genetic diversity statistics, the introduced population of Miskwabi Lake partitioned with higher genetic diversity stocked populations, while the unstocked populations of Macdonald, Clean, Louisa, and Crystal Lakes sorted with stocked populations of lower genetic diversity. However, stocked populations typically had higher genetic diversity estimates than the known native populations.

Population differentiation and divergence

Population divergence estimated from mtDNA variation revealed the underlying phylogenetic relationship among inland lake trout populations and hatchery strains of Great Lakes origin (Table 2-4). Inland populations with lower microsatellite DNA genetic diversity showed little or no mitochondrial divergence from one another ($F_{ST} = 0$ to 0.049), and either had pure Mississippian-A ancestry or low frequencies of haplotypes from other lineages. While this low divergence may seem contradictory to the expected profile for divergent, native populations, too few generations have passed since colonization for mitochondrial DNA to diversify (Piller et al. 2005). In contrast, these lower genetic diversity populations showed greater reciprocal divergence based on microsatellite DNA ($F_{ST} = 0.097$ to 0.557) than values observed for populations with higher diversity, reflecting a strong differential in microsatellite-based genetic diversity for trout populations originating from single versus multiple (admixed) glacial lineages (Wilson and Hebert 1996, 1998). Estimates of population divergence varied among most higher genetic diversity populations and ranged from $F_{ST} = 0.057$ to 0.320; however, comparisons within two different subgroups, each containing one hatchery strain as well as higher diversity inland lakes (Lake Manitou, Farquhar and Boshkung Lakes versus Esson, Grace, Miskwabi and Killala Lakes), were substantially lower and ranged from $F_{ST} = 0$ to -0.029.

Comparisons of microsatellite variation among lower diversity inland populations revealed large, significant pairwise F_{ST} estimates, corresponding to a high degree of divergence and concordant with a native genetic profile (Table 2-4). Only three pairwise comparisons among lower diversity populations yielded F_{ST} values comparable to those

between higher diversity populations ($F_{ST} = 0.020$ to 0.127). The three comparisons were among Macdonald, Clean, and Redstone populations; as these lakes are adjacent to one another, it is possible that they may have exchanged migrants during historical periods of high water levels. With the exception of these three lakes, pairwise F_{ST} estimates for lower diversity inland lakes ranged from $F_{ST} = 0.132$ to 0.478 . Comparisons between lower genetic diversity inland populations and higher genetic diversity populations also revealed significant, substantial divergence ranging from $F_{ST} = 0.075$ to 0.372 . In contrast to lower diversity populations, the relatively small pairwise F_{ST} estimates among higher genetic diversity populations, indicative of low levels of divergence, were consistent with predicted profiles for introgressed or introduced populations.

Pairwise genetic distance estimates (D_A ; Nei et al. 1983) among the study populations showed contrasting patterns of divergence among the study populations. Several inland lakes showed clear divergence from all other populations, whereas hatchery sources and several inland populations showed little to no divergence (Figure 2-2). The observed patterns mirrored those observed by Ihssen et al. (1988) based on allozyme data, as well as microsatellite-based divergence for inland populations relative to those with Great Lakes origins Stott (1998). Consistent with F_{ST} -based divergence estimates, low diversity populations (e.g., Louisa Lake) showed the greatest divergence from one another, and from populations of higher genetic diversity (e.g., Miskwabi Lake). Again, the lake trout populations of Macdonald, Clean, and Redstone Lakes showed a closer degree of genetic similarity to one another than all other inland lake populations, concordant with population genetic structure patterned though isolation by

distance. Inland populations with high genetic diversity (e.g., Miskwabi lake) clustered with high diversity hatchery strains (e.g., Lake Manitou), as expected for populations with introgressed ancestry.

Individual assignment tests

One of the evaluated assignment tests (Pritchard et al. 2000) performed well in estimation of admixture in native inland populations, however, the exclusion method of Cornuet et al. (1999) failed to effectively discriminate between introgressed/hatchery strain and native genetic profiles among study populations. Failure to exclude multiple populations of origin at either a threshold of $P < 0.01$ or $P < 0.05$ resulted in apparently spurious assignment of almost all individuals (25% to 100% from each population) to the mixed inland and Great Lakes groups. Reassignment of individuals to populations of origin was low at both threshold values (0% to 35% and 0% to 46%, respectively). Comparative assignment of individuals based strictly on the maximum probability of belonging to any single reference population was less stringent, and gave the higher rates of reassignment among all populations (10% to 83%), but revealed an underlying bias towards assignment of individuals to populations of higher relative genetic diversity. Under these conditions, all divergent, lower genetic diversity inland populations showed moderate reassignment rates of 45% (Crystal and Clean Lakes) to 72% (Smoke Lake), but showed a consistent trend towards incorrect assignment to higher genetic diversity inland populations, which accounted for 50% (Clean Lake) to 86% (Louisa Lake) of all incorrectly assigned individuals.

Assignment based on individual genotype likelihood scores (L/L_{SUM}), in contrast to simulation-based probability calculations, showed a sharply different pattern (Table 2-5): reassignment of individuals to all divergent, low genetic diversity inland populations was high (86% to 100%) except for closely related populations in Macdonald and Clean Lakes (73% and 62% reassignment, but 24% and 31% reciprocal assignment to each other, respectively). Individual likelihood score-based reassignment rates were lowest among inland populations of higher genetic diversity, ranging from 31% (Esson Lake) to 56% (Boshkung Lake). These results suggest that the evolutionary history of post-glacial contact among our study populations, and historical partitioning of genetic variation, limited the effectiveness of the exclusion test for evaluation of inland lake trout population introgression. A less likely interpretation is that all inland populations are highly introgressed, however, this possibility conflicts with all other population genetic analyses performed in this study.

Individual assignment with STRUCTURE (Pritchard et al. 2000) and estimation of the number of expected populations resolved four major genetic clusters ($K = 4$) from all sampled individuals (Figure 2-3). Most individuals from divergent, lower genetic diversity inland populations (94%) consistently reassigned ($q > 0.5$) to one of three native population genetic clusters corresponding to geographical location (Haliburton, Algonquin, or Bancroft genetic clusters). In contrast, most individuals from higher diversity inland populations either assigned to the introgressed/hatchery-strain cluster (86%), which included almost all hatchery-strain individuals. A few individuals from lower diversity populations (3%) and higher diversity populations (6%) did not assign to a single cluster of origin. Nearly all individuals from low diversity inland populations

showed a high degree of membership to one of three native genetic clusters (Table 2-6), which indicated little or no population-level admixture regardless of stocking history. A parallel analysis of population structure, individual assignment, and individual admixture using BAPS 5.1 (Corander and Marttinen 2006) produced similar individual assignment results and identical population profiles (native versus introgressed/hatchery ancestry) for all study populations (data not shown).

For native genetic clusters where measures of interpopulation divergence otherwise indicated regional population structure, further cluster subdivision was evaluated. All three native inland clusters showed evidence of further substructure based on individual assignment tests, corresponding with F_{ST} and genetic distance estimates. Two sub-clusters were resolved from the Haliburton genetic cluster ($K = 2$), corresponding to a Macdonald-Clean Lake sub-cluster distinct from Redstone Lake. The Algonquin genetic cluster was subdivided into three population-specific sub-clusters corresponding to the Kingscote, Smoke, and Louisa Lake populations ($K = 3$). Only two sub-clusters were detected within the Bancroft genetic cluster ($K = 2$), corresponding with samples from Barker Lake and Crystal Lake, respectively.

Genetic substructure was also detected within the "introgressed/hatchery-strain" genetic cluster, with three recognizable sub-clusters ($K = 3$). Lake trout from the Killala Lake (KL) or Lake Superior (Slate Islands - SL) populations each comprised a distinct group, whereas individuals showing high degrees of membership to the third sub-cluster (hatchery subcluster 3) generally originated from higher diversity inland populations (Miskwabi, Boshkung, Esson, Farquhar, and Grace Lakes) or the Lake Manitou population. Individuals from these populations generally showed moderate degrees of

membership to the other two hatchery-strain sub-clusters (average $q = 0.53$ to hatchery sub-cluster 3, $q = 0.11$ to the Killala sub-cluster, and $q = 0.36$ to the Slate Islands sub-cluster). Lack of a population-specific genetic profile indicates that these populations are probably descendent from either admixed hatchery strains or a mix of stocked and native individuals. In the case of the Lake Manitou hatchery strain, this population-level admixture probably indicates introgression with non-native stocked fish; Lake Manitou has been heavily supplemented since 1952 (Evans and Willox 1991).

Discussion

Composite genetic profiles and previous population assessments

The combined genetic analyses showed that resolution of population ancestries was feasible in the absence of historical (pre-stocking) samples or data. Population- and individual-based analyses were generally concordant (Table 2-6). These analyses indicated that half of the study populations with extensive stocking histories still retain a native genetic profile (Redstone, Barker, Smoke, and Kingscote Lakes), genetically distinct and characteristic of unstocked populations (Macdonald, Clean, Louisa, and Crystal Lakes). Dilution or replacement of native genetic diversity was observed, however, in four southern Ontario lake trout populations (Boshkung, Esson, Grace, and Farquhar Lakes). Native genetic diversity in these populations has been homogenized by stocking with exogenous hatchery strains from the Great Lakes region, and they now have a genetic profile characteristic of populations with known hatchery ancestry.

Although the results clearly demonstrated that stocking history alone was not a good predictor of introgression, some trends were evident. The ranges of cumulative stocking intensity overlapped between populations with native (50.3 to 351.6 lake trout/ha) and introgressed/hatchery ancestry (160.3 to 314.8 lake trout/ha). Among the populations with native profiles were the two most intensively stocked lakes (Barker and Kingscote Lakes). Similarly, the range of total number of lake trout supplemented into populations with native profiles (49,507 to 73,136 lake trout stocked) overlapped with populations with hatchery strain/introgressed profiles (35,950 to 114,738 lake trout stocked). In contrast to stocking intensity, however, the two most heavily stocked populations (Boshkung and Farquhar Lakes) showed profiles consistent with introgression after stocking.

Observed distributions of mitochondrial haplotypes and microsatellite genetic diversity were largely consistent with past evaluations of inland lake trout ancestry. In the 1980s, a questionnaire-based evaluation of population status was collected from regional fisheries managers by the Ontario Ministry of Natural Resources, and compiled into an atlas of provincial lake trout lakes (OMNR 1989). Among the study lakes, several populations were identified as indigenous and self-sustaining (Macdonald, Clean, Redstone, Kingscote, Louisa, Smoke, and Crystal Lakes), some were identified as populations bolstered by supplemental stocking (Esson, Farquhar, Grace, Boshkung, and Barker Lakes), and the Miskwabi Lake population was referred to as an introduced population (Table 2-6). With the exception of Barker Lake, these original assessments were predictive and correspondent to composite genetic profiles (native, and introgressed/hatchery strain, respectively).

More recently, in the late 1990's, a limited allozyme-based analysis of population ancestry was performed to discriminate among regional native and stocked-ancestry populations in the Haliburton Highlands. The Miskwabi Lake population was confirmed as an introduced population of higher genetic diversity, and the Boshkung Lake population was identified as a population of mixed hatchery and local native genetic backgrounds (OMNR, unpublished). In contrast to our current analysis, this survey showed low genetic diversity profiles for Esson, Farquhar, and Grace Lakes, otherwise consistent with "native" inland populations. While these results may seem contradictory with the resolved composite genetic profiles, allozyme loci typically have less variation than microsatellite DNA and thus have a higher probability of being shared with hatchery-strains .

Retention of native genetic diversity in supplemented populations

Four of the study populations that had been heavily stocked still retain native genetic profiles. Although Barker Lake has been stocked intensively in the past (351.6 lake trout/ha), its composite population genetic profile is more similar to that of Crystal Lake (an unstocked lake located in the Bancroft area) than introgressed inland populations (e.g., Miskwabi, Esson, Grace and Farquhar Lakes) that show an introgressed/hatchery strain genetic profile. The composite genetic profiles for the supplemented Smoke Lake (91.5 lake trout/ha) and Redstone Lake (50.3 lake trout/ha) populations are also more similar to those from geographically proximal and unstocked populations in Louisa Lake and Macdonald or Clean Lake than to introgressed

populations. It is worth noting that this genetic analysis not only confirms the high degree of divergence and unusual genetic background of the lake trout populations of Redstone, Macdonald and Clean Lakes relative to other populations of the Great Lakes region, but also further demonstrates their divergence from other local lake trout populations of the Haliburton Highlands (Ihssen et al. 1988; Wilson and Hebert 1996; Stott 1998).

In some "native" populations where little evidence exists for introgression, some genetic dilution may have occurred. For instance, the occurrence of two individuals with 'C' mtDNA haplotypes in Kingscote Lake is inconsistent with model of lake trout postglacial colonization (Wilson and Hebert 1998), and probably reflects natural reproduction by historically stocked lake trout. Additionally, STRUCTURE-based individual assignment ($K = 4$) indicated that six of forty-nine individuals sampled from Kingscote lake showed high degrees of membership to the introgressed/hatchery-strain cluster (proportional individual membership to this cluster, $q_{HTH} > 0.80$). The overall genetic characteristics of Kingscote Lake conform to a population-level native genetic profile, however, and are consistent with the retention of native genetic diversity despite substantial historical stocking (342.2 lake trout/ha). In contrast to other introgressed inland populations, the Kingscote lake trout population had lower microsatellite DNA genetic diversity, and was clearly divergent from other inland populations including those with stocked ancestry (Table 2-6). Additionally, most individuals sampled from Kingscote Lake reassigned with high probabilities ($L/L_{SUM} = 0.88$) and showed strong membership to a native Algonquin (ALG) genetic cluster based on multilocus individual assignments ($N = 43/49$ at $q_{ALG} > 0.5$, $N = 36/49$ at $q_{ALG} > 0.9$, $K = 4$). A history of

limited interbreeding with stocked fish is also supported by the observation that both individuals with mtDNA C haplotypes were among the six individuals that had high degrees of membership ($q_{\text{HTH}} > 0.8$) to the introgressed/hatchery-strain genetic cluster.

Limitations of genetic profiling for detecting introgression

The genetic profiling methodology we evaluated is limited in its ability to detect introgression in lake trout populations. For example, even though profiling of microsatellite variation showed that Barker Lake had a "native" genetic profile, it is difficult to be certain that the presence of mitochondrial B/D haplotypes is due to historical stocking. Like the Kingscote Lake population, lake trout from Barker Lake have hatchery-strain mtDNA haplotypes present in low frequencies in the population. This lake has been heavily stocked, but unlike Kingscote Lake, Barker Lake is also situated within the colonization area for lake trout that dispersed from an Atlantic 'B' lineage refuge following glacial retreat (Wilson and Hebert 1996, 1998). Inland lakes north and east of Barker lake, (e.g., Lake Opeongo and Charleston Lake) show low to moderate frequencies of Atlantic mtDNA haplotypes concordant with regional biogeography and postglacial colonization (Wilson and Hebert (1996, 1998). Due to the prevalence of B lineage haplotypes in hatchery populations, however (Grewe and Hebert 1988, Grewe et al. 1993), the possibility of limited hatchery introgression into Barker Lake cannot be ruled out.

Although the use of inland lake trout for stocking has only recently become popular in southern Ontario, detection of interbreeding between divergent donor and

recipient populations is possible with this genetic profiling methodology. However, where some natural genetic exchange exists, resolving power for inter-population discrimination will be reduced, such as was observed for the Macdonald and Clean lake trout populations. Their similarity to Redstone Lake versus other native population groups to the north and east also illustrates the presence of regional genetic structuring among native populations, on a finer scale than is detectable using conventional phylogeographic methods (Ihssen et al. 1988, Wilson and Hebert 1996, 1998). This regional structuring reflects shared ancestry within regional groups, as well as the subsequent divergence among native inland populations due to their reciprocal isolation since post-glacial colonization (Wilson and Mandrak 2004).

The genetic profiling methodology used in this analysis closely paralleled the one developed by Piller et al. (2005), but there was an unexpected challenge encountered by extending their chosen individual assignment method (Cornuet et al. 1999) to a different phylogeographic region. Using the exclusion test, we detected a bias towards incorrect assignment of individuals from low genetic diversity to high genetic diversity populations or population groups (i.e., mixed or unresolved Great Lakes ancestry), even under least stringent conditions (i.e., exclusion of all populations of origin except for the single population of most probable origin), which is described by Cornuet et al. (1999) as a "type E" error. This result was unexpected, as we followed the guidelines for maximizing correct assignment specified by Cornuet et al. (1999) and Manel et al (2002); for populations with divergence estimates of $F_{ST} > 0.1$, recommendations include selecting a Bayesian assignment method (we chose Rannala and Mountain (1997)), excluding loci with low heterozygosities ($H_E < 0.4 - 0.6$), and scoring at least 10

microsatellite loci from approximately 30 to 50 individuals per population. The heterozygosities of four microsatellite loci used in this study were lower than the recommended value ($H_E \sim 0.3$), however, they were retained due to their demonstrated information value in previous assignment and exclusion tests involving lake trout populations (Page et al. 2003; Piller et al. 2005).

The incorrect assignment of almost all individuals to admixed (introgressed/hatchery strain) origins might be erroneously interpreted as the consequence of wide-scale, historical replacement of native genetic diversity through interbreeding and introgression. However, a more probable explanation that is concordant with our data and analyses, is that incorrect assignments of fish from native populations resulted from the frequentist Monte Carlo simulations of genotypes used for exclusion tests. Actual genotypes from lower genetic diversity lake trout populations had allele frequency distributions that were effectively subsets of the actual allele frequency distributions of higher diversity populations. As a consequence of post-colonization isolation, actual genotypes from divergent, low-diversity lake trout populations were rarely generated as simulated genotypes from separate low-diversity populations. However, these actual genotypes were frequently present in genotype distributions simulated from higher diversity population allele frequencies. Higher genetic diversity lake trout populations of the Great Lakes, founded by multiple glacial lineages, effectively contain a substantial proportion of the overall intraspecific variation at microsatellite markers (Ihssen et al. 1988; Wilson and Hebert 1996, 1998). The presence of few private alleles in any of the low diversity inland populations, in the context of discrete microsatellite mutation modes (e.g., stepwise, two-phase and K-alleles models),

suggests that the total number of lake trout microsatellite allelic states is limited, which would further facilitate saturation of genotype states in simulated populations (Estoup and Cornuet 2004). Our results also indicate that drift rather than microsatellite mutation is responsible for population divergence, which is supported by the observed low diversity in native inland populations.

Implications for ecology-based management of inland lake trout populations

It would be valuable to explicitly evaluate the anthropogenic and ecological factors that limit gene flow into stocked lake trout populations (Evans and Willox 1991; Powell and Carl 2004), particularly as recorded stocking intensity does not seem to be predictive of introgression and replacement of native genetic diversity. Although more replicate lakes are needed to evaluate differential genetic backgrounds of stocked fish, lake attributes, and fish communities among stocked populations, some interesting associations were evident in our study lakes. Lake trout populations with composite introgressed genetic profiles resided in lakes that generally had higher conductivities (total dissolved solids > 50 mg/L), greater mean depths (approximately 10 m to 36 m), greater ($N > 9$) or fewer ($N < 7$) number of species, an absence of some early life-stage predators such as burbot (*Lota lota*) and brook trout (*Salvelinus fontinalis*), but the presence of other early-life stage predators also associated with failed introductions (Evans and Olver 1995) including rock bass (*Ambloplites rupestris*) and smallmouth bass (*Micropterus dolomieu*). Despite expectations to the contrary, lake surface area did not show an obvious association with population ancestry. Some of these lake attributes

contrast with those that are known to facilitate stocked fish survival and successful establishment; factors that increase stocking success include lower total dissolved solids (< 50 mg/L), lower species diversity, and limited predation (Evans and Olver 1995; Kerr 2001).

These contrasting factors may indicate some degree of local adaptation in the lake trout populations of southern Ontario. To better characterize the biodiversity of inland lake trout populations relative to the species as a whole, future analyses should incorporate comparative analyses of potentially adaptive differences among populations including life-history, morphological and behavioural traits. In addition, supplemental stocking programs should evaluate the advisability of using allopatric stocking sources with potentially different adaptations, versus augmenting the recruitment success of local populations by some other means. Local-strain stocking programs designed to conserve regional genetic diversity should similarly consider these factors to avoid potentially harmful interruption of adaptive evolutionary processes within local populations, and work to ensure their long-term sustainability (Moran 2002).

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Table 2-1

Attributes of southern Ontario populations, hatchery strains and source lakes of the Great Lakes region evaluated in this analysis showing abbreviation (a superscript (S) on the inland population abbreviation indicates that population has been stocked, and a superscript (I) indicates that population was introduced), category, population type (sample origin), sample size, location, and sampling dates. ¹Data for latitude, longitude, lake sizes, depths, and conductivity: (Lake Superior, Lake Manitou, Killala Lake, Miskwabi Lake) the Ontario Ministry of Natural Resources Aquatic Habitat Inventory Database (AHI) and the Stocks Catalogue (OMNR 2003); (Boshkung Lake, Esson Lake, Farquhar Lake, Grace Lake, Macdonald Lake, Clean Lake, Redstone Lake, Kingscote Lake, Louisa Lake, Smoke Lake, Barker Lake, and Crystal Lake) Gunn et al. (2004).

Population or strain	Abbr.	Category	Sample origin	N	Lat.	Long.	Sampling Dates	Lake size (ha) ¹	Conductivity (mg/L) ¹	Mean Depth (m) ¹
Slate Islands, Lake Superior	SL	Hatchery	Wild	46	48°00'	87°00'	2004	8,210,000	30 - 50	148.3
Lake Manitou, Manitoulin										
Island, Lake Huron	LM	Hatchery	Hatchery	43	45°46'	81°59'	2003	10,800	160 - 167	15
Killala Lake (North Basin)	KL	Hatchery	Hatchery	47	49°05'	86°32'	2000	977	66 - 73	36.7
Miskwabi Lake	MSK	Introduction	Wild	42	45°03'	78°19'	1998 - 2001	263.8	61	19.7
Boshkung Lake	BKG ^S	Inland	Wild	50	45°03'	78°43'	1997 - 1998	715.8	50	23.1
Esson Lake	ESS ^S	Inland	Wild	39	45°01'	78°16'	2001 - 2003	244.8	114	10.8
Farquhar Lake	FRQ ^S	Inland	Wild	42	45°05'	78°12'	2001 - 2003	336.1	86	17.7
Grace Lake	GRC ^S	Inland	Wild	40	45°04'	78°13'	2001 - 2003	226.2	62	15.3
Macdonald Lake	MAC	Inland	Wild	33	45°14'	78°33'	2005	137.8	32	10.8
Clean Lake	CLE	Inland	Wild	29	45°14'	78°31'	2005	160.4	35	14.8
Redstone Lake	RST ^S	Inland	Wild	45	45°10'	78°32'	1998 - 2004	1130.3	38	21.9
Kingscote Lake	KS ^S	Inland	Wild	49	45°12'	78°13'	1998	213.7	38	7.5
Louisa Lake	LOU	Inland	Wild	47	45°28'	78°28'	2001	567	34	17
Smoke Lake	SMK ^S	Inland	Wild	47	45°30'	78°40'	2004	607.1	36	16.2
Barker Lake	BRK ^S	Inland	Wild	50	45°06'	77°22'	2003	140.8	53	4.1
Crystal Lake	XTL	Inland	Wild	49	45°07'	77°27'	1998 - 2003	55.3	32	9.8

Table 2-2

Reported stocking histories of evaluated inland lakes. Lake Superior strains include those originating from the Slate Islands, Michipicoten Islands, Mishibishu Lake, and the Hills Lake (Lake Superior) hatchery strain. Other reported strains stocked sporadically included the Lake Ontario strain (derived from stocked, wild fish; 1000 individuals into Smoke Lake in 1974) and the Lake Simcoe strain (4013 individuals into Boshkung Lake 1985). Percentages for stocking sources are of total fish stocked between 1970-1999. Information sources are as follows: 1 - Ontario Ministry of Natural Resources Fisheries Information Service (OFIS), 2 - Algonquin Provincial Park records, 3 - Kerr (2001) * 1946 is the earliest recorded stocking of Redstone Lake from the OFIS, however an anonymous source listed in Kerr (2001) indicates that Redstone Lake was stocked in 1887 with 50,000 lake trout fry of unknown origin.

Population or strain	Recorded stocking events				Number of individuals by stocking source (1970 - 1999)							Age of stocked fish (% N)			
	Start date	End date	Total stocked	N stocked/ha	Lake			Killala Lake		Other	Unk.	Unk. (pre 1970)	Fry	Fing.	Juv.
					Manitou	Superior	Lake	Lake	Lake						
Miskwabi Lake ¹	1963	1991	45,950	174.2	7,800	25,400	3,000	3,000	3,000	3,000	6,750	3%	30%	100%	
Boshkung Lake ¹	1946	1991	114,738	160.3	6,700	21,400	7,800	4,013	1,750	73,075	3%	30%	64%		
Esson Lake ¹	1962	1990	35,950	146.9	2,900	11,200	10,000	5,200	6,650	93%					
Farquhar Lake ¹	1947	1991	95,730	284.8	45,530	4,800	2,000	43,400	41%	59%					
Grace Lake ¹	1947	1993	71,200	314.8	11,300	20,100	5,000	2,500	32,300	30%	70%				
Macdonald Lake ¹															
Clean Lake ¹															
Redstone Lake ^{1,3}	1946*	1969	56,875	50.3						56,875	61%	39%			
Kingscote Lake ^{1,2}	1925	1985	73,136	342.2		7,511	4,000	9,600	52,025	5%	55%	40%			
Louisa Lake ^{1,2}															
Smoke Lake ^{1,2}	1936	1976	55,575	91.5		12,500	1,000	4,000	38,075	62%	31%				
Barker Lake ¹	1950	1994	49,507	351.6	9,257	12,375	4,200	6,625	17,050	3%	9%	88%			
Crystal Lake ¹															
Totals			598,661		83,487	115,286	36,000	5,013	32,675	326,200	1%	31%	68%		
Percent of Total					31%	42%	13%	2%	12%						

Table 2-3

Genetic diversity statistics for each study population: gene diversity as an unbiased estimator of expected homozygosity (H_E), observed heterozygosity (H_O), allelic richness (A_R), mean number of alleles per locus (N_A), total number of private alleles (N_P), effective number of alleles (averaged across all loci) (n_e). Populations are listed in order of descending value of allelic richness. Values highlighted in bold italics correspond to the "drop-off" value that partitions lower diversity populations from higher genetic diversity populations. A superscript (S) on the inland population name indicates that population has been stocked, a superscript (I) indicates that population was introduced.

Population	H_E	H_O	N_A	A_R	N_P	n_e
Slate Islands	0.629	0.610	8.25	7.40	4	4.72
Farquhar Lake	0.613	0.576	7.67	7.16	3	4.40
Miskwabi Lake ^l	0.593	0.607	7.75	6.95	2	3.89
Esson Lake ^s	0.583	0.549	7.58	6.95	2	3.67
Lake Manitou	0.592	0.599	7.50	6.67	3	3.52
Boshkung Lake ^s	0.560	0.553	7.33	6.44	3	3.84
Grace Lake ^s	0.558	0.520	6.67	6.23		3.34
Killala Lake	0.484	0.443	6.75	6.01		3.49
Kingscote Lake ^s	0.382	0.362	5.42	4.73		2.64
Redstone Lake ^s	0.492	0.498	5.17	4.61	1	2.51
Barker Lake ^s	0.351	0.338	5.08	4.29	1	2.07
Clean Lake	0.481	0.493	4.08	4.08		2.31
Smoke Lake ^s	0.386	0.404	4.58	4.07	1	2.26
Louisa Lake	0.312	0.314	4.50	3.99		2.06
Macdonald Lake	0.460	0.417	3.92	3.80	1	2.22
Crystal Lake	0.239	0.240	2.75	2.59		1.76

Table 2-4

Pairwise estimates of F_{ST} and the haplotype F_{ST} analog of Goudet (2001); non-significant values are italicized ($P \geq 0.05$; $k = 120$). The upper triangular matrix contains estimates based on mtDNA variation, while the lower triangular matrix corresponds to values calculated from microsatellite DNA variation. A superscript (S) on the inland population abbreviation indicates that population has been stocked, a superscript (I) indicates that population was introduced.

Population	LM	SL	KL	MSK ¹	BKG ^s	ESS ^s	FRQ ^s	GRC ^s	MAC	CLE	RST ^s	KS ^s	LOU	SMK ^s	BRK ^s	XTL
LM		0.238	0.060	0.102	-0.009	0.123	-0.020	0.122	0.190	0.192	0.219	0.163	0.221	0.221	0.083	0.228
SL	0.035		0.093	0.070	0.320	0.065	0.280	0.057	0.502	0.506	0.541	0.495	0.543	0.543	0.442	0.552
KL	0.124	0.075		-0.017	0.128	-0.010	0.104	-0.017	0.371	0.374	0.407	0.359	0.410	0.410	0.277	0.419
MSK ¹	0.021	0.051	0.117		0.181	-0.021	0.152	-0.028	0.429	0.433	0.468	0.418	0.471	0.471	0.338	0.480
BKG ^s	0.049	0.054	0.091	0.029		0.203	-0.024	0.210	0.119	0.120	0.137	0.097	0.139	0.139	0.032	0.143
ESS ^s	0.005	0.043	0.121	0.005	0.032		0.174	-0.028	0.444	0.447	0.481	0.435	0.484	0.484	0.357	0.492
FRQ ^s	0.016	0.029	0.104	0.009	0.029	0.013		0.178	0.156	0.159	0.187	0.119	0.189	0.189	0.038	0.196
GRC ^s	0.013	0.042	0.097	0.008	0.024	0.002	0.015		0.496	0.501	0.543	0.479	0.547	0.547	0.389	0.557
MAC	0.164	0.203	0.310	0.151	0.173	0.154	0.163	0.171		-	-	0.009	-	-	0.033	-
CLE	0.157	0.199	0.316	0.143	0.166	0.149	0.162	0.165	0.020		-	0.010	-	-	0.034	-
RST ^s	0.163	0.197	0.302	0.143	0.143	0.155	0.158	0.167	0.127	0.088		0.018	-	-	0.046	-
KS ^s	0.160	0.166	0.197	0.106	0.075	0.132	0.118	0.107	0.261	0.265	0.217		0.019	0.019	0.012	0.021
LOU	0.266	0.260	0.304	0.211	0.164	0.247	0.223	0.235	0.326	0.323	0.234	0.184		-	0.046	-
SMK ^s	0.204	0.228	0.294	0.167	0.152	0.179	0.184	0.188	0.273	0.252	0.221	0.242	0.216		0.046	-
BRK ^s	0.183	0.201	0.263	0.184	0.176	0.179	0.199	0.172	0.278	0.293	0.271	0.300	0.312	0.249		0.049
XTL	0.270	0.313	0.372	0.254	0.269	0.272	0.247	0.260	0.478	0.476	0.414	0.346	0.423	0.430	0.419	

Table 2-5

Proportional assignment of individuals to potential populations of origin based on the individual genotype likelihood score (L/L_{SUM}) of Piry et al. (2004). Population abbreviations are defined in the text, a superscript (S) on the inland population abbreviation indicates that population has been stocked. Proportional reassignment is highlighted in bold font.

Assigned population

Source	LM	SL	KL	MSK ¹	BKG ^s	ESS ^s	FRQ ^s	GRC ^s	MAC	CLE	RST ^s	KS ^s	LOU	SMK ^s	BRK ^s	XTL
LM	0.37	0.05		0.07	0.02	0.14	0.16	0.19								
SL		0.72	0.02	0.02		0.11	0.09	0.04								
KL			0.91		0.02			0.04							0.02	
MSK ¹	0.07	0.02		0.40	0.02	0.14	0.17	0.14			0.02					
BKG ^s	0.06	0.04	0.14	0.04	0.56	0.02	0.04	0.06		0.02		0.02				
ESS ^s	0.13	0.03	0.05	0.15		0.31	0.15	0.18								
FRQ ^s	0.12	0.10	0.02	0.12	0.02	0.17	0.38	0.07								
GRC ^s	0.20	0.05		0.23	0.03	0.10	0.08	0.33								
MAC									0.73	0.24	0.03					
CLE							0.03		0.31	0.62	0.03					
RST ^s	0.04			0.02					0.04	0.89						
KS ^s				0.02	0.02	0.04	0.02	0.02				0.88				
LOU													1.00			
SMK ^s	0.02				0.02									0.96		
BRK ^s	0.04		0.02		0.02	0.02	0.02	0.02							0.86	
XTL																1.00

Table 2-6

Composite genetic profiles of inland lake trout populations based on previous analyses, mitochondrial and microsatellite genetic diversity, population divergence, and individual assignment tests. A superscript (S) on the population abbreviation indicates that inland population has been stocked, a superscript (I) indicates that population was introduced. Abbreviations are as follows: occurrence of mitochondrial haplotypes (mtDNA), gene diversity as an unbiased estimator of expected heterozygosity (H_E), allelic richness (A_R), pairwise F_{ST} value (calculated from microsatellite allele frequencies) compared to the Miskwabi Lake population (F_{ST} [vs.MSK]), individual genotype likelihood score of Piry et al. (2004) (L/L_{SUM}), maximum average proportional membership to one of three inland genetic clusters at $K = 4$ using the Bayesian method of Pritchard et al. (2000) (q_{AVG}); genetic cluster indicated by superscript: H-Haliburton, A-Algonquin, B- Bancroft. Composite profiles were developed by comparative analysis of genetic profiles.

Population or strain	Previous status				Genetic Profiling					Composite profile
	Abbr.	Lake trout atlas	Allozyme		A_R	F_{ST} (vs. MSK)	L/L_{SUM}	q_{AVG}		
			mtDNA	survey					H_E	
Miskwabi Lake	MSK ¹	Introduced	Introduced	A, B/D	0.593	6.95	-	0.40	0.07 ^A	Introduced
Boshkung Lake	BKG ^s	Supplemented	Introduced	A, B/D	0.560	6.44	0.029	0.56	0.18 ^H	Introgressed
Esson Lake	ESS ^s	Supplemented	Native	A, B/D	0.583	6.95	0.005	0.31	0.06 ^H	Introgressed
Farquhar Lake	FRQ ^s	Supplemented	Native	A, B/D	0.613	7.16	0.009	0.38	0.07 ^A	Introgressed
Grace Lake	GRC ^s	Supplemented	Native	A, B/D	0.558	6.23	0.008	0.33	0.09 ^B	Introgressed
Macdonald Lake	MAC	Indigenous	Native	A	0.460	3.80	0.151	0.73	0.95 ^H	Native
Clean Lake	CLE	Indigenous	Native	A	0.481	4.08	0.143	0.62	0.94 ^H	Native
Redstone Lake	RST ^s	Indigenous	Native	A	0.492	4.61	0.143	0.89	0.85 ^H	Native
Kingscote Lake	KS ^s	Indigenous	Native	A, C	0.382	4.73	0.106	0.88	0.81 ^A	Native
Louisa Lake	LOU	Indigenous	Native	A	0.312	3.99	0.211	1.00	0.92 ^A	Native
Smoke Lake	SMK ^s	Indigenous	Native	A	0.386	4.07	0.167	0.96	0.91 ^A	Native
Barker Lake	BRK ^s	Supplemented	Native	A	0.351	4.29	0.184	0.86	0.79 ^B	Native
Crystal Lake	XTL	Indigenous	Native	A	0.239	2.59	0.254	1.00	0.97 ^B	Native

Figure 2-1

Mitochondrial DNA variation measured in southern Ontario lake trout populations, hatchery strains, and lakes proximal to the study area. Superscripts for evaluated inland populations indicate whether the population is unstocked (U), has been stocked (S), or was established by introduction of stocked fish (I). Evaluated stocking source populations are underlined and italicized in the inset map. Asterisks indicates populations and hatchery strains assessed in earlier studies: Opeongo, Tim, Lavieille, Seneca, and Charleston Lakes were surveyed in Wilson and Hebert (1996), Lake Simcoe was surveyed by Grewe and Hebert (1988), Grewe et al. (1993), and Stott (1998); the Gull Island Shoal (Lake Superior), Marquette (Lake Superior), and Big Green (Lake Michigan) hatchery strains were evaluated by Piller et al. (2005). Haplotype frequency distributions correspond to mtDNA lineages observed in Grewe and Hebert (1988), Grewe et al. (1993), and Wilson and Hebert (1996, 1998).

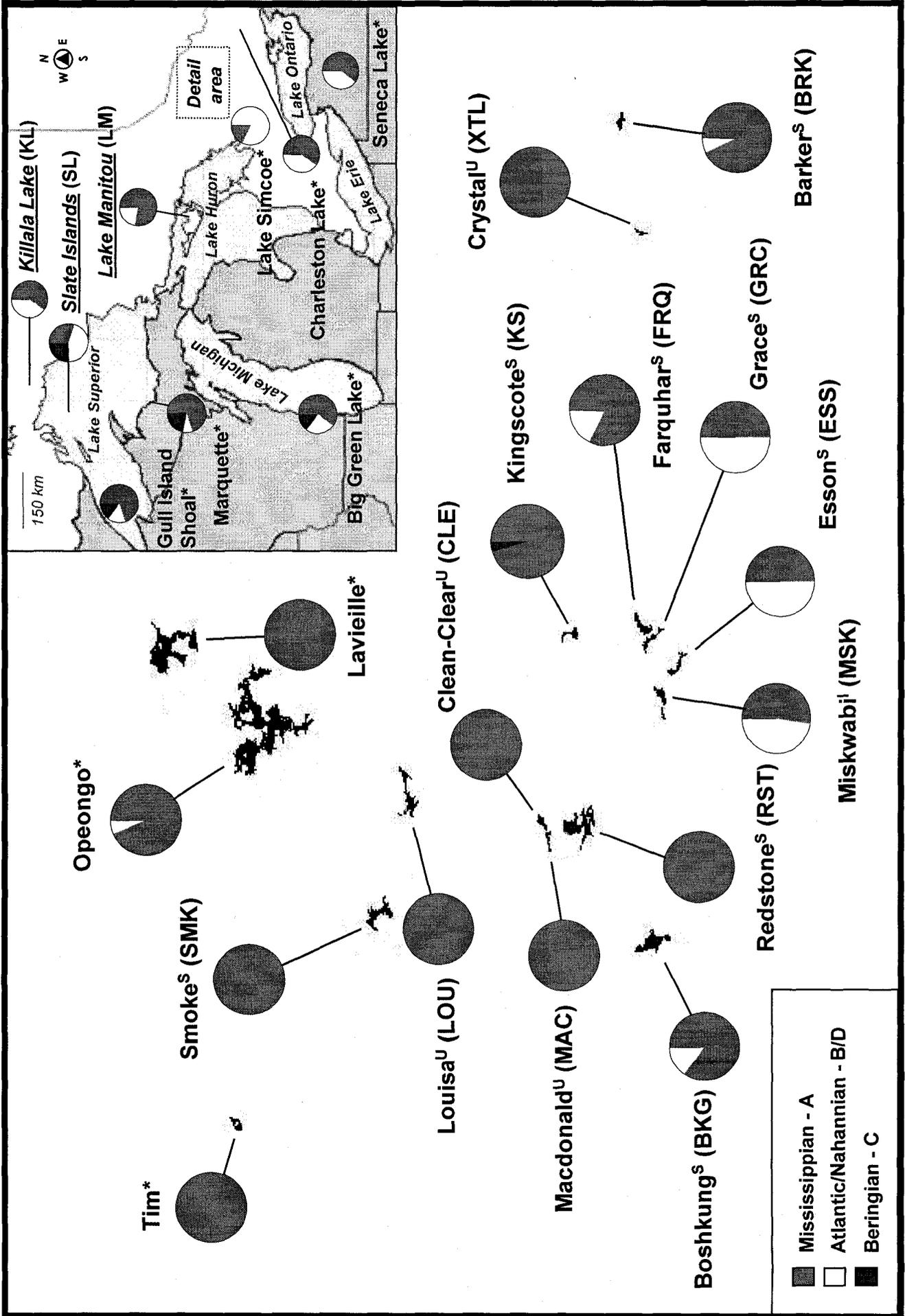
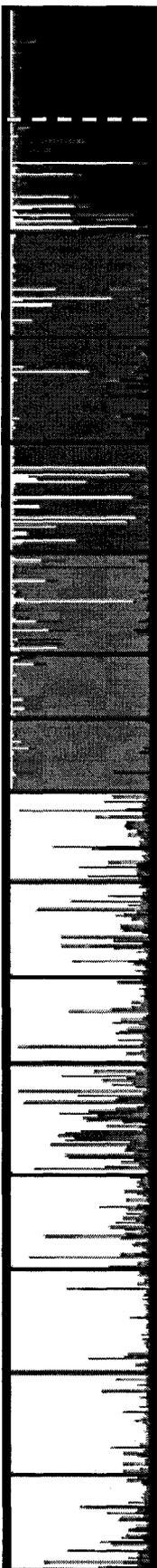


Figure 2-2

Neighbour-joining dendrogram constructed from pairwise genetic distance estimates (D_A ; Nei 1987) for each population. Hatchery-strain source populations are underlined and highlighted by bold italics. Bootstrap replicates greater than 40% are indicated with internal labels. Superscripts for inland populations indicate whether the population has been stocked (S), or was established by introduction of stocked fish (I).

Figure 2-3

Population structure model generated using the Bayesian method of Pritchard et al. (2000) for $K = 4$ populations. Population abbreviations are given in Table 1. Vertical shaded bars correspond to individual membership coefficients (q) for each of the population model genetic clusters. The dashed white line divides individuals that originated from Barker Lake or the Crystal Lake population. Superscripts on the population abbreviation indicate stocked (S) or introduced (I) populations; source populations for stocking are underlined and highlighted by bold italics.



LM SL KL MSK' BKG^s ESS^s FRQ^s GRC^s MAC CLE RST^s KS^s LOU SMK^s BRK^s XTL

CHAPTER 3**Contemporary origins for recovering populations of river-spawning lake trout*****(Salvelinus namaycush)***Michael A. Halbisen¹, Stephen C. Chong², and Chris C. Wilson³

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Halbisen performed experimental design, genetic data collection, analysis, and wrote this manuscript

Chong sampled two populations in 2005 and provided funding

Wilson provided supervision

Abstract

Translocations of captive-reared individuals have been commonly used for population rehabilitation or introductions in response to the loss of wild populations. In the Great Lakes, multiple anthropogenic impacts eliminated a wide range of lake trout (*Salvelinus namaycush*) phenotypic variants between the 1940s and 1960s. This loss of historical phenotypic diversity has been identified as a likely limiting factor for lake trout rehabilitation in the Great Lakes, despite a substantial, long-term international stocking program. Recently, however, an unusual river-spawning life-history variant that was endemic to northeastern Lake Superior, and reproductively isolated from sympatric basin-spawning lake trout, has reappeared at historical, riverine spawning sites. To determine the contemporary origins and genetic distinctiveness of recovering river-spawning lake trout sampled from the Dog and Montreal Rivers, we used a molecular genetic approach to measure both mitochondrial DNA (PCR-RFLP) and microsatellite DNA (12 loci) variation. Together, evaluation of spatial-temporal patterns of genetic variation, microsatellite allele frequencies, and individual genotype-based analyses excluded an allopatric origin for the river-spawning lake trout, and showed that they were genetically distinct from basin-spawning lake trout sampled from Lake Superior and Lake Huron. Furthermore, these analyses showed that the river-spawning lake trout were genetically similar to a hatchery strain (Mishibishu) that was derived from sanctuary populations established with river- and basin-spawning lake trout collected from Lake Superior in the 1950s and 1960s. Although the Dog River lake trout may be a recovering, indigenous population, combined simulations and empirical data indicated

that a hatchery strain origin could not be excluded for either contemporary river-spawning population. These genetic results were then used to evaluate an ecological model for the postglacial evolution of lake trout spawning behaviour, and assess the conservation genetic status of the river-spawning lake trout relative to sympatric basin-spawners.

Introduction

Human activities associated with natural resource utilization have reduced or eliminated indigenous wildlife populations over a diverse range of spatial-temporal scales, necessitating the use of translocations for rehabilitation of affected populations. Translocated individuals are commonly cross-bred and reared in purpose-built facilities (i.e., zoos and fish hatcheries), sanctuary habitats (i.e., game preserves), or both, before they are reintroduced into their formerly occupied ranges or supplemented into depleted wild populations (Griffith et al. 1989; Wolf et al. 1996; Brown and Day 2002). Population genetic processes experienced during and after captive-rearing, however, can alter genetic variation (Lacy 1987; Stockwell et al. 1996; Frankham et al. 2002) and potentially reduce fitness (Lynch and O'Hely 2001; Hufford and Mazer 2003) but see (Wang et al. 2002a; Balloux et al. 2004) through accelerated genetic drift during population size reductions (Robichaux et al. 1997), inbreeding (Van Dierendonck and Wallis De Vries 1996; Wang et al. 2002b), and/or unnatural selective processes (Ford 2002; Araki et al. 2007).

Natural genetic variation can also be reduced or lost in wild populations when mortality rates are high and native individuals are simply replaced by translocated individuals (Evans and Willox 1991). In cases where source populations of ambiguous or divergent origins (i.e., recognized subspecies or intraspecific variants) have been used for translocations in rehabilitative programs (Hedrick 1995; Maehr 1995; Hedrick 2005), "genetic swamping" can occur (Lenormand 2002), where introgressive interbreeding leads to homogenization of native gene pools and ultimately causes the loss of local

genetic adaptations (Allendorf et al. 2001). These negative effects are most acute for rare species or intraspecific variants as genetic alterations from human-mediated translocations may compromise the long-term survival of both captive individuals and reduced, recovering populations.

Hatchery-reared fish from unknown, mixed, or geographically and genetically distinct source populations have been widely used to supplement native freshwater fish populations affected by exploitation, habitat modification and loss, and invasive species introductions (Richter et al. 1997; Cowx 2002). Extensive historical stocking has led to widespread replacement of native populations with non-native fish of hatchery ancestry (Hindar et al. 1991; Hilborn 1992; Brannon et al. 2004; Williamson 2005). However, in many cases stocking with hatchery fish has failed to re-establish local native strains, including those with a specialized or cryptic role in their ecological community (Hansen 1999; Fraser in press). A greater emphasis is now placed on matching source populations to the ecological conditions and genetic background of the recipient or extirpated populations (Seddon and Soorae 1998; Brannon et al. 2004; Mobrand et al. 2005), but the contemporary origins and genetic distinctiveness of many stocked, recovering fish populations is uncertain.

Highly abundant lake trout (*Salvelinus namaycush*) populations were almost entirely eliminated from the entire Great Lakes system between the late 1940s and 1960s (Krueger and Ebener 2004), with indigenous, remnant populations persisting only in Lake Superior and in Georgian Bay of Lake Huron. This large-scale population crash was primarily attributed to the impact from predation by the invasive sea lamprey (*Petromyzon marinus*) and commercial overharvest (Krueger and Ebener 2004).

However, heavy pollution (Baumann 1984), other invasive species (Mills 1993; Riccardi and MacIssac 2000), and impacts from other human activities (Kelso et al. 1996) have also been implicated in the degradation of additional native fish populations in the Great Lakes. The widespread loss of this "keystone" predator had a destabilizing effect on the food webs of the Great Lakes (Krueger and Ebener 2004), and has been implicated in the loss of additional native fish species (Brandt 1986).

Since the crash, a long-term, international program for lake trout rehabilitation in the Great Lakes was implemented to control sea lamprey predation, limit exploitative harvest, and increase lake trout abundance through intensive hatchery strain stocking (Hansen 1999). Although sea lamprey abundance was successfully reduced (Pycha and King 1980), naturally produced lake trout are currently more abundant than stocked individuals only along the inshore waters of Lake Superior (Schreiner and Schram 1997; Krueger and Ebener 2004). There are many possible factors that are still limiting lake trout recovery in the Great Lakes (Krueger et al. 1995), but one major factor that was identified was the limited use of diverse native lake trout forms that originally utilized offshore habitat and alternative spawning sites (Loftus 1958; Brown et al. 1981; Goodier 1981) for historical rehabilitative stocking (Marsden et al. 1995).

During the collapse of lake trout across the Great Lakes, Loftus (1958) documented the coincident decline of a unique lake trout variant that was endemic to northeastern Lake Superior (Goodier 1981). In the fall spawning season, these fish would migrate into regional tributaries and spawn in riverine habitats, unlike other basin-spawning lake trout that utilized spawning beds located at varied depths in the Great Lakes. These river-spawning lake trout showed extremely high spawning-site fidelity:

only a single inter-stream migrant was observed among four streams over a five year long study period (Loftus 1958). By the final year of the study (1956), river-spawning lake trout had been reduced to approximately 3% of their 1952 population size estimate. This decline, and presumed extirpation, was almost entirely attributed to sea lamprey predation, as commercial exploitation was light (ca. 2 - 5% tagged individuals reported captured in commercial nets; Loftus 1958).

Fertilized lake trout eggs were collected from spawning adults present in the Dog and Puckaskwa Rivers in 1956, reared in a fish hatchery, then stocked into the Dog River in 1958 (Loftus 1958). A sanctuary population was also established in an inland lake chain (Mishibishu, Mishi, and Katzenbach Lakes) to help maintain a component of Lake Superior genetic diversity. Other hatchery strains from multiple sources (originating from the sanctuary lakes, elsewhere in the Great Lakes, and other unknown sources) were then used for staggered, intermittent rehabilitative stocking of the Dog (1958 - 1973) and Montreal Rivers (1961 - 1977) (OMNR 1984). During this time period, basin-spawning lake trout were introduced (1961) and became established in the sanctuary lakes (Harrison 1968). Descendants from these river- and basin-spawning lake trout were then used to develop the Mishibishu hatchery strain, which was widely used for stocking into Lake Superior (OMNR 1984). By 1996, when naturally produced lake trout became more abundant than stocked individuals (Schreiner and Schram 1997), over 94 million hatchery-reared lake trout had been stocked into Lake Superior (Hansen et al. 1995). As a great deal of this historical rehabilitative fish stocking utilized source populations from both inside and outside the Great Lakes (Krueger and Ihssen 1995) the origins and

genetic backgrounds of recovering, re-established Superior lake trout populations were generally unknown (Marsden et al. 1993; Grewe et al. 1994b; Page et al. 2003).

Molecular markers have since been used extensively to evaluate population structure, measure genetic diversity, and resolve ancestral origins of recovering lake trout populations in the Great Lakes. Early evaluations of allozyme (Ihssen et al. 1988; Krueger et al. 1989) and mitochondrial DNA variation (Grewe and Hebert 1988; Grewe et al. 1993) provided evidence for weak population structure among basin-spawning lake trout originating from the Great Lakes, the Mishibishu hatchery strain, and the river-spawner sanctuary populations, but were limited in resolving power for measuring inter-strain differences. More recently, however, comparative genetic analysis of historical samples obtained from the upper Great Lakes during the system-wide population crash to samples from the contemporary populations in Lake Superior and Lake Huron revealed that substantial microsatellite genetic diversity had been lost throughout the Great Lakes as a consequence of an historical population size bottleneck (Guinand et al. 2003). Additionally, contemporary lake trout from southern and northwestern Lake Superior showed evidence of recent shared ancestry with a heavily stocked hatchery strain originating from southeastern Lake Superior (Marquette, MI). Partitioning of microsatellite allelic variation among contemporary populations, however, indicated that low but significant levels divergence remained among spatially distributed spawning aggregations in Lake Superior (Page et al. 2004).

The objective of this analysis was to resolve the origins and ancestry of the recovering river-spawning lake trout in Lake Superior by use of a molecular genetic approach. As the precise origins of almost all stocked lake trout were not recorded, it

was unclear whether contemporary populations of river-spawning lake trout had descended from: 1) stocked fish that originated from outside the Great Lakes, 2) a Great Lakes stocking source, 3) stocked fish originating from the sanctuary populations, 4) indigenous individuals, or 5) both native and hatchery strain ancestors.

Contemporary patterns of spatial genetic variation and hierarchical population structure were expected to reflect observed historical patterns of restricted migrant exchange among river-spawners and basin-spawners (Loftus 1958), unless extensive historical stocking with divergent hatchery-strains had homogenized natural genetic structure. A limited ability to discriminate between an indigenous or stocked origin for the Dog River lake trout was expected, however, as they shared recent ancestry with the Mishibishu hatchery strain and the sanctuary populations. Even so, the genetic distinctiveness of all river-spawning lake trout was expected to be proportional to their divergence from sympatric basin-spawning lake trout, and indicative of Great Lakes or allopatric ancestry. In the absence of suitable historical samples from the collapse and early rehabilitation of Lake Superior, forward simulations were used to assess whether historical population size reductions had reduced genetic variation and effective population sizes of the Mishibishu hatchery strain and river-spawners through wild population size bottlenecks or foundation stocking events. We discuss how this genetic analysis has provided a means to evaluate the evolutionary origins of the river-spawning lake trout, and provide recommendations for future conservation strategies based on our results.

Methods

Sample collection

Contemporary genetic samples were obtained from two present-day river-spawning aggregations, the three sanctuary lakes, the Mishibishu hatchery strain broodstock, basin-spawning groups in Lake Superior and Lake Huron, and from a proximal but reproductively isolated lake trout population for comparative analyses of mitochondrial and microsatellite variation. For evaluation of long-term temporal genetic variation, an historical genetic sample taken from lake trout spawning in the Dog River in 1952 was also included. Geographical origins for sampled wild lake trout and hatchery strains are shown in Figure 3-1.

Lake trout samples were collected by two main methods, targeted netting and angling. All lake trout originated from wild collections during the fall spawning season, captive broodstocks, or hatchery-reared individuals bred from adults sampled during fall spawning. River-spawning lake trout were sampled from the Dog and Montreal rivers in 1993, 1994, 2002, and 2005. Archived scale samples were obtained from spawning adults sampled in 1952. Samples were collected from the sanctuary lakes (Mishibishu, Mishi, and Katzenbach Lakes) in fall 2002 as part of ongoing provincial fish culture broodstock propagation activities. In that year, some of these individuals were crossbred with other individuals of the F₂ Mishibishu captive broodstock (1995 year class) to produce the F₃ Mishibishu broodstock (2002 year class), which is currently maintained at the Tarentorus Fish Culture Station by the Ontario Ministry of Natural Resources

(OMNR) in Sault Ste. Marie, Ontario. Samples from both broodstock generations were included in this analysis. Similarly, samples were obtained for the OMNR F₄ Michipicoten Island broodstock (1999 year class) from the Tarentorus Fish Culture Station. Slate Islands lake trout were collected from spawning aggregations in Slate Islands, Lake Superior in 2004, and from an F₂ captive broodstock (1989 year class) maintained at the OMNR Dorion Fish Culture Station in Dorion, Ontario. Samples collected from Parry sound, Lake Huron were obtained in 2004 during captive broodstock replenishment operations by individuals from the Chatsworth Fish Culture Station in Chatsworth, Ontario. Details on sample collection from Lake Manitou, Lake Huron and Killala Lake broodstock are found in Halbisen and Wilson (in press). Remaining samples from other hatcheries and wild sources were provided by jurisdictional agencies (see Piller et al [2005] for details on the Marquette broodstock and Gull Island Shoal, Lake Superior population; Isle Royale samples were provided by the United States Geological Survey Great Lakes Science Centre). To increase sample sizes for the lake trout obtained from the Dog River, Montreal River, Slate Islands, and Mishibishu broodstock, temporal replicates were pooled as they showed no significant differentiation ($P > 0.05$) in terms of microsatellite allele frequencies or a departure from Hardy-Weinberg equilibrium between replicates. Although all pooled mtDNA haplotype frequencies are shown in Table 3-2, temporal replicate frequencies are reported separately in Appendix Table 1-2.

Genetic data collection

Microsatellite and mitochondrial DNA data originating from this study were collected as described by Halbisen and Wilson (in press) and Piller et al. (2005), respectively. Genetic data collection from the historical samples, however, required a modified methodology (see below). For contemporary samples, DNA was extracted and precipitated from individual tissue (approximately 20 mg adipose fin clip, caudal punch or skin) or scale samples (approximately 4-8 scales). Microsatellite alleles were then amplified by PCR for each DNA sample at twelve loci: *Sfo1*, *Sfo12*, *Sfo18*, *Sfo23* (Angers et al. 1995), *SfoC24*, *SfoC88*, *SfoD75* (King, T. L., unpublished), *Scou19* (Taylor et al. 2001), *Oneu14* (Scribner et al. 1996), *Ssa85* (O'Reilly et al. 1996), *Ots1* (Banks et al. 1999), and *Ogo1a* (Olsen et al. 1998). Fluorescently labelled amplicons for each individual were resolved by slab-gel electrophoresis using an ABI 377 (Applied Biosystems) automated sequencer. For samples with partial genotypes collected in earlier studies (Marquette and Gull Island Shoal; Piller et al. 2005), microsatellite variation was measured at four additional loci (*Sfo1*, *SfoC88*, *One14*, and *Ssa85*); additional individuals were also genotyped from the Killala Lake population (Halbisen and Wilson in press) to improve sample size. A PCR-RFLP assay was used to evaluate diagnostic restriction enzyme cut-sites indicative of one of three major mitochondrial lineages: Mississippian - A, Atlantic/Nahannian - B/D, or Beringian - C lineages (Wilson and Hebert 1996, 1998). Resultant restriction fragments were stained with SYBR Green dye (Molecular Probes, Inc.) and visualized on 1.25% agarose gels.

The degraded state of the DNA extracted from the historical 1952 Dog River scale samples necessitated an alternative methodology for genetic data collection. Microsatellite DNA loci were amplified singly, using 35 cycles; reagent concentrations

and annealing temperatures were otherwise identical to those reported in Halbisen and Wilson (in press). Of the twelve loci used evaluated this study, five (*Sfo1*, *SfoC24*, *SfoC88*, *Ssa85*, and *Ogo1a*) produced amplicons, but allele scoring was compromised by weak amplification and extensive PCR artefacts. To minimize errors associated with amplification of degraded DNA from historical samples, reproducibility was ensured by repeated amplification. Only two microsatellite loci (*Ogo1a* and *Sfo1*) were suitable for genetic analysis, as they showed no difference ($P > 0.05$) in allele frequencies or heterozygote deficit, which are diagnostic for allelic dropout, between amplifications. To further minimize potential error, erroneous individual genotypes for these two loci were excluded from analyses of historical genetic variation, as the overall reamplification error rate for was less than 5 % for *Ogo1a* and 7% for *Sfo1*. For comparison, error rates for other loci were as high as 45%.

Additional primers were designed to produce small amplicons (< 200 base pairs) for PCR amplification of mitochondrial DNA extracted from the historical samples. These two primer pairs were targeted to diagnostic restriction enzyme cut-sites (*Bam*HI) identified for lake trout mitochondrial haplotype lineages (Grewe and Hebert 1988; Grewe et al. 1990, 1993; Wilson and Hebert 1996, 1998). The presence of the correct lake trout diagnostic cut-sites was verified directly by sequencing. PCR conditions for mitochondrial DNA amplification were the same as in Halbisen and Wilson (in press), but separate amplifications of the NADH dehydrogenase (F: TCCGCAGTACTAGCCACTAT; R: GAAGGAGGAGGGCAATTT) and the cytochrome *b* (F: RCTCATCCGRAATATCCAC; R: GYCCTCATGGRAGAACGTAG) regions at 35 PCR cycles each were needed for amplification levels suitable for

subsequent restriction enzyme digests. Individual mitochondrial DNA haplotypes were confirmed by repeated amplification for the historical samples.

Estimation of genetic diversity, divergence, and spatial genetic structure

Genetic diversity statistics and distances were estimated with a combination of population genetics software packages. FSTAT v 2.9.3 (Goudet 2001) was used to estimate Nei's (1987) gene diversity (H_E), which is an unbiased estimate of the observed heterozygosity (H_O), the average number of alleles per locus (N_A), allelic richness (A_R), which is an estimate of allelic diversity that is standardized to the number of individual among samples, and Wright's fixation indices (F_{IT} , F_{ST} , F_{IS}) by use of the Weir and Cockerham (1984) estimators (F , θ , f). Randomizations (at least 1000 replicates) were used to generate null distributions for tests of Hardy-Weinberg equilibrium. Alleles were permuted within loci among individuals for tests within samples (F_{IS}), but individual genotypes were permuted for pairwise tests of divergence among sampled populations (F_{ST}). For tests of microsatellite allelic differentiation, P -values were estimated by use of the pairwise genic differentiation option offered in GENEPOP 3.4 (Raymond and Rousset 1995). Subsequent sequential Bonferroni corrections were made for all multiple pairwise tests (Rice 1989). To evaluate hierarchical structure among samples, TreeView 1.6.6 (Page 2000) was used to graph a consensus neighbour-joining tree constructed from bootstrap replicates. Bootstrap replicate trees (1000) were generated across all loci by use of Populations v. 1.2.28 (Languella 1999), and effective recovery of true tree topology under both the infinite alleles model (IAM) or step-wise mutation model

(SMM) for microsatellites (Takezaki and Nei 1996) was facilitated by use of the pairwise chord distance (D_C) of Cavalli-Sforza and Edwards (1967).

Two different but related methodologies were used for spatial-genetic correlational analyses. Mantel tests (Mantel 1967), implemented with GenALEX version 6 (Peakall and Smouse 2006), were used to evaluate correlations between paired $n \times n$ symmetric distance matrices, whose elements corresponded to pairwise estimates of either genetic or geographic distance among all population samples. To investigate individual sample contributions to overall correlations, asymmetric, rowwise distance correlations were also estimated for single population samples (with n pairwise comparisons to other population samples of interest). This was accomplished by modifying the approach of Smouse et al. (1986) by a method proposed by De Vries (1993) for calculation of correlation coefficients for $1 \times n$ (rectangular) distance vectors. Simply stated, Pearson's product-moment correlation coefficient (Zar 1999) was calculated for the n corresponding pairwise distances. As with the Mantel tests, pairwise distance estimations were not independent, so to evaluate the significance of the test-statistics, null distributions were generated by Monte Carlo randomizations (Smouse et al. 1986). Individual genetic distance measurements were shuffled while geographic distance values were held constant to generate 1000 paired replicates. Test statistic values exceeding 95% of resampled values were considered significant. For both types of test, genetic distance matrices of pairwise divergence estimates were transformed ($F_{ST}/1-F_{ST}$) to linearize expected distance correlations under isolation-by-distance model (Rousset 1997) for genetic divergence. Geographical distances were measured as the shortest distance between sites connected by water, and no transformation was applied to

geographical distance matrices since the scaled sampling range was relatively small in measured units (km).

Not all sampled populations were included in these correlational analyses, as the lake trout from the sanctuary lakes and the Mishibishu hatchery strain have a known history of mixture from river- and basin-spawning lake trout aggregations. The Lake Manitou lake trout population was included in these analyses despite its isolation from Lake Huron, as historically both lakes were connected during postglacial high-water periods (Eschman and Karrow 1984; Dyke and Prest 1987), and few indigenous lake trout populations remain in Lake Huron for comparative analysis. Furthermore, models for regional phylogeography indicate that the Lake Manitou population shared an evolutionary history with lake trout from Lake Huron and elsewhere in the upper Great Lakes (Wilson and Hebert 1996, 1998).

Genetic clustering and admixture analysis

Population- and individual-based clustering methods were used to evaluate genetic distinctiveness and admixture among sampled populations. Ordination of multilocus genetic data by principal components analysis (PCA) was performed with PCAGEN (Goudet 1999), which provided an effective means for visualizing relationships among samples based on inter-population allelic correlations (Jones et al. 2005). For comparison, two different individual assignment methods were used to gain inference on genetic distinctiveness and population structure from multilocus genotypes

without requisite information on individual origins. Many available software packages use individual assignment based algorithms for resolving population structure, such as PARTITION (Dawson and Belkhir 2001), and admixture, such as NEWHYBRIDS (Andersen and Thompson 2002), but STRUCTURE (Pritchard et al. 2000), and BAPS (Corander et al. 2003; Corander et al. 2004; Corander and Marttinen 2006) have been shown to work well for resolving pop structure at low levels of differentiation (Latch et al. 2006).

Both individual assignment programs used implement Bayesian statistical approaches for estimating the number of expected populations (K), individual posterior probabilities for membership to resolved genetic clusters, and estimates of individual admixture coefficients (q). STRUCTURE 2.2.2 (Pritchard et al. 2000) is a model-based program that resolves genetic structure by clustering individuals based their multilocus genotypes. The default parameters were used for all model simulations (admixture ancestry model, degree of admixture α inferred from $\alpha_o = 1.0$; correlated allele frequency model, λ inferred from $\lambda_o = 1.0$) to better model the known history of extensive historical stocking in our system, and 100,000 Monte-Carlo Markov Chain repetitions were executed for each simulation after the burn-in period of 100,000 iterations. For each user defined K level ($K = 1$ to 15), five replicate simulations were performed on the whole dataset, excluding the locus-limited genotypes collected from the historical Dog River lake trout. Overall model optimality was evaluated according to the guidelines provided by Pritchard et al. (2007) and Evanno et al. (2005). To further evaluate genetic structure within resolved clusters, a second round of simulations was performed for optimal models identified at $K=2$ and $K=4$ by use of the guidelines

provided by Prichard et al. (2007). Inter-model correspondence values (IMC) were calculated by quantitative comparison of proportional individual assignments to resolved clusters at $K=4$ with correspondent subcluster assignment at $K=2$ (see results section for further details on comparison criteria).

BAPS 5.1 (Corander et al. 2003; Corander et al. 2004; Corander and Marttinen 2006), which uses a different partitioning algorithm than STRUCTURE, was also used to resolve genetic structure from individual genotypes and explicitly test for admixture among lake trout originating from northern and northeastern Lake Superior (Dog River, Montreal River, Michipicoten Island, Slate Islands) and the Mishibishu strain relative to the allopatric population from Killala Lake. Estimates of mixture proportions were obtained by incrementally adjusting K from 1 to 6, with five replicate simulations at each K level, but post-simulation model optimality was evaluated strictly by comparing model likelihood estimates. Admixture proportions for clustered individuals were then estimated after restricting the minimum population size to $N = 3$, as suggested by Latch et al. (2006) to avoid erroneous estimation of admixture coefficients resultant from the overestimation of K . The number of iterations used to estimate admixture coefficients was set at 100, the number of reference individuals sampled from each population was 200, and the number of iterations used to estimate admixture proportions for reference individuals was 10. To improve resolving power, two microsatellite loci with extremely low diversity (*Sfo1* and *SfoC88*) were excluded from all clustering analyses, following Halbisen and Wilson (in press).

Detection of impacts from historical population size reductions

Historical population size reductions can elicit characteristic genetic responses, particularly when size reductions are severe enough to reduce effective population sizes (Garza and Williamson 2001). Since the heterozygote deficiency method for calculating point estimates of effective population size has low precision (Luikart and Cornuet 1999; Wang 2005), estimates were made by use of the linkage disequilibrium method (Hill 1981). A modified version of this method was implemented with LDNE (Waples and Do in press), a software program that enables a bias correction for cases where the sample size is less than the effective population size (Waples 2006). Since the inter-locus correlation of allele frequencies is not independent of allele frequencies, extremely rare or abundant alleles ($P < 0.05$ or $P > 0.95$) were excluded from estimate calculations. Jackknife estimates were used to define 95% confidence intervals since they were more likely to bound the true effective population size (Waples and Do in press) than confidence intervals calculated by the parametric method of Waples (2006). The differential reduction of the number of alleles (k) relative to the allelic range (R) for microsatellite loci is a known response to recent population size reduction, and was evaluated by use of the M-ratio (k/R) of Garza and Williamson (2001). BOTTLENECK (Cornuet and Luikart 1996) was used to determine whether gene diversities (H_E estimates calculated for each sampled population using the method of Nei [1987]), were significantly larger than equilibrium gene diversities (H_{EQ} estimates for each sampled population calculated by a coalescence-based method implemented in BOTTLENECK), as expected for microsatellite loci in a recently reduced populations. Deviations from mutation-drift equilibrium were tested using all three of the available microsatellite

mutation models. Probabilities for individual locus departures from equilibrium were estimated from coalescent simulations, but overall departures were tested with the recommended Wilcoxon rank test, which was more powerful than the sign test for samples of 5-40 individuals, but less than 20 loci. Parametric confidence intervals (95%) for averaged genetic responses were calculated assuming normal distributions for point estimates from each replicate simulation.

The effects of historical population size reductions on the Mishibishu hatchery strain were further investigated by performing a series of forward simulations with BOTTLESIM (Kuo and Janzen 2003). A range of size reductions was simulated by drawing a set number individuals ($N = 10, 30, 50, 70, 90, 200, \text{ and } 500$) from the Mishibishu broodstock sample. For each simulation, equal numbers of males and females were assigned, and ten replicate populations were evaluated. To simulate years of intermittent stocking with juvenile lake trout, as experienced historically by the Dog and Montreal Rivers, individual ages were initially randomly assigned (100% overlap) to age classes (from 1 to 14 years) that reflected historical records of river-spawner population age structure (Loftus 1958). Since no mature individuals (≥ 10 years of age) were produced in simulations that restricted population size to $N < 30$ individuals, an alternate model was used where all individuals were assigned the same age (0% overlap). Following establishment, each randomly mating, replicate population was held to a constant population size over time until the reduction in observed number of alleles (20%) matched that observed difference between the Dog River lake trout and the Mishibishu strain. Each simulation was halted at this variable time point, which provided an estimate of the minimum time required to establish the maximum genetic diversity

differential observed between the river-spawning lake trout, the Mishibishu broodstock, and individual sanctuary populations. Identical samples of $N = 74$ individuals (selected to match the sample size of the Dog River lake trout) were then drawn from each replicate population for measurement of genetic responses, which were averaged across all replicates for each size reduction.

Results

Microsatellite and mitochondrial DNA variation

Microsatellite genetic variation was measured for all contemporary population samples at twelve loci (Table 3-1). Observed genetic diversity for each locus was similar to earlier published analyses in terms of observed number of alleles ($N=2$ for *Sfo1* to $N=35$ for *Sfo23*), allelic size ranges (from 91 base pairs for *SfoC24* to 350 base pairs for *SfoD75*), heterozygosities ($H_E = 0.104$ for *Sfo1* to 0.905 for *Sfo23*; $H_O = 0.104$ for *Sfo1* to 0.912 for *Sfo23*), and allelic distributions among population samples (Page et al. 2003; Page et al. 2004; Piller et al. 2005; Halbisen and Wilson 2008). Pairwise tests of Hardy-Weinberg equilibrium revealed no heterozygote deficits within population samples ($P > 0.05$; $k = 168$), and overall F_{IS} values ranged from -0.01 to 0.07 (Table 3-1).

Contemporary lake trout sampled from wild spawning aggregations and hatchery sources were broadly similar in terms of both microsatellite diversity estimates and mitochondrial haplotype distributions (Table 3-2). Heterozygosity estimates for the river-spawners, Mishibishu hatchery strain, sanctuary populations, and other basin-

spawning lake trout were close in value. Gene diversities for these groups ranged from $H_E = 0.558$ to 0.620 and observed heterozygosities ranged from $H_O = 0.529$ to 0.609 . Both ranges were higher in value than heterozygosity estimates for the reproductively isolated Killala Lake basin-spawners ($H_E = 0.496$; $H_O = 0.462$). Lake trout from the Dog River had the lowest estimates for the average number of alleles ($N_A = 5.83$) and allelic richness ($A_R = 5.36$), two genetic diversity measures that are reduced by increased reproductive isolation. In contrast, estimates of both statistics for Montreal River lake trout ($N_A = 7.17$; $A_R = 6.27$) were similar in value to the Mishibishu hatchery strain and sanctuary lake populations ($N_A = 6.08-7.25$; $A_R = 5.84-6.15$), other Great Lakes basin-spawning population samples ($N_A = 5.83-9.33$; $A_R = 6.10-8.03$), but higher than estimates for basin-spawners sampled from the geographically and reproductively isolated Killala Lake population ($N_A = 6.83$; $A_R = 5.91$). Although estimates for the average number of alleles among the sanctuary populations were lower than the estimate for the Mishibishu strain, their pooled average estimate ($N_A = 7.42$) was closer in value.

The observed spatial distribution of mitochondrial DNA haplotypes was generally consistent with expectations inferred from previous studies of regional phylogeography (Table 3-2) (Wilson and Mandrak 2004). The most abundant mitochondrial haplotype sampled, indicative of Mississippian-A glacial ancestry, was common among both river-spawners and basin-spawners. Haplotypes indicative of postglacial dispersal from other glacial refugia (Atlantic/Nahannian - B/D and Beringian-C) were less common, and unexpectedly absent from the Michipicoten Island basin-spawners. All three major mitochondrial lineages were detected in most of the sanctuary populations and lake trout spawning aggregations sampled from Lake Superior, but the Beringian-C haplotype was

absent from the Mishibishu Lake sample, the Mishibishu hatchery strain, and other lake trout sampled outside Lake Superior. In contrast to patterns of microsatellite DNA variation, there were some differences in mitochondrial haplotype proportions among pooled temporal replicates (Appendix Table 1-2).

All three major mitochondrial lineages were also detected in river-spawning lake trout sampled from the Dog and Montreal Rivers, and there was no significant difference in mitochondrial haplotype proportions among the Montreal River, pre-crash, and post-crash Dog River samples (Fisher's exact test; $P = 0.068$). The presence of multiple mitochondrial lineages shows that the river-spawning lake trout, similar to most regional basin-spawning populations, had a history of postglacial admixture since the formation of the Great Lakes ca. 10-15 KYA (Karrow and Calkin 1984; Wilson and Hebert 1996, 1998), and therefore they were not descendent from a unique (i.e., highly divergent) ancestral lineage.

Comparative analysis of long-term temporal microsatellite variation was limited by the historical degradation of DNA extracted from the 1952 Dog River scale samples. Reliable amplification of microsatellite alleles from these historical samples was possible at only two of the smallest microsatellite loci (*Sfo1* and *Ogo1a*). Pairwise tests of Hardy-Weinberg equilibrium showed that there was no significant deficit of heterozygotes at either locus ($P > 0.05$; $k = 4$) for the temporal replicates. However, these loci did not show enough overall genetic variation to effectively resolve fine-scale spatial-temporal patterns of genetic distinctiveness ($F_{IT} = 0.037$; $F_{ST} = 0.038$; $F_{IS} = -0.002$) among river- and basin-spawning lake trout population samples originating from the Great Lakes.

Analysis of allelic distributions for these two loci demonstrated, however, that the microsatellite allelic variation of the pre-crash river-spawners was comparable to contemporary basin-spawning lake trout in the Great Lakes region. Almost all of the alleles detected in the historical Dog River sample at *Sfo1* ($N = 2$; alleles at 108 and 110 base pairs) and *Ogo1a* ($N = 4$; alleles at 144, 150, 152 and 154 bp), were detected in similar proportions ($Sfo1_{110} > Sfo1_{108}$; $Ogo1a_{150} > Ogo1a_{144} > Ogo1a_{152}$) in all hatchery strain sources and sampled spawning aggregations. However, the number of alleles observed at *Sfo1* in the historical Dog River sample was less than the number observed in the reproductively isolated Killala Lake population ($N = 3$; alleles at 108, 110, and 116 base pairs). The rarer *Sfo1* allele (116 bp) was also detected at similarly low proportions (1%-10%) in six of seven basin-spawning aggregations sampled from the Great Lakes, and in the Mishibishu strain sample ($> 1\%$), but not in contemporary sanctuary lake populations or the river-spawner samples. For *Ogo1a*, one population-specific rare allele (154 bp) was detected in the historical Dog River lake trout sample in low proportion (2%), and another (148 bp) was present only in the Slate Islands sample ($< 1\%$). In comparison, reproductively isolated lake trout descendent from a single glacial lineage (i.e., a highly divergent lineage with no postglacial increase in genetic diversity resultant from secondary admixture), sampled from a remote lake in Algonquin Park, Ontario (Louisa Lake), had fewer alleles at both *Sfo1* ($N = 1$; allele at 110 base pairs) and *Ogo1a* ($N = 3$; 144, 150, and 152 base pairs) (Halbisen and Wilson in press) than the historical Dog River lake trout. Furthermore, allelic proportions for *Ogo1a* in the Louisa Lake sample ($Ogo1a_{144} > Ogo1a_{150} > Ogo1a_{152}$) were dramatically different from the upper Great Lakes samples. These comparisons showed that since the colonization of Lake

Superior, genetic drift had not severely eroded the genetic variation of the pre-crash Dog River lake trout population, even though river-spawners showed a high degree of homing and had small population sizes relative to estimated population size for basin-spawning aggregations (Loftus 1958; Swanson and Swedberg 1980; Reid et al. 2001).

Evaluation of potential allopatric origins

Basin-spawning lake trout that originated from Lake Superior have shown greater genetic similarity to one another than to lake trout from other Great Lakes (Krueger et al. 1989; Marsden et al. 1989; Marsden et al. 1993; Guinand et al. 2003) and isolated populations outside the Great Lakes basin (Ihssen et al. 1988; Piller et al. 2005; Halbisen and Wilson in press). River-spawning lake trout native to Lake Superior were expected to share this pattern of genetic dissimilarity with allopatric populations, and a genetic similarity to sympatric lake trout proportional to reproductive isolation since postglacial colonization.

Ordination by principal components analysis (PCA) provided a useful exploratory method for evaluating patterns of genetic similarity in terms of correlated multilocus allele frequencies among samples, and resolved three significant axes of variation (PC1 = 30%; PC2 = 23%; PC3 = 14%). The principal component scores for each population sample were plotted along the first two principle axes as shown in Figure 2. Members of the three resolved genetic clusters were roughly distributed along a diagonal line, orthogonal to a bisector oriented towards the Killala Lake population (i.e., the dotted grey line in Figure 2), in the following order: river-spawning lake trout, sanctuary lake trout

populations and the Mishibishu hatchery strain, Lake Superior lake trout, and lake trout from Lake Huron. River-spawning lake trout clustered separately from basin-spawning lake trout from Lake Superior, Lake Huron, the Mishibishu hatchery strain, and populations of the sanctuary lakes. However, the river-spawners from the Dog and Montreal rivers clustered together, and more closely to all basin-spawning populations evaluated relative to the Killala Lake population, which is geographically proximal to but physically isolated from Lake Superior. Two other genetic clusters were resolved from the basin-spawning populations that originated from the Great Lakes. The first cluster included lake trout from Lake Superior and Lake Huron, and the second included lake trout sampled from the Mishibishu broodstock and the sanctuary populations. This ordination pattern showed that the contemporary river-spawners were relatively similar to lake trout that originated from the Great Lakes (i.e., Lake Superior), as that they are not as genetically distinct as a population (i.e., in Killala Lake) that was founded at approximately the same time as those of the Great Lakes but has been reproductively isolated since colonization (Wilson and Hebert 1996, 1998).

Tests of correlation between geographic and genetic distance among river and basin spawning lake trout revealed several concordant patterns. There was no significant correlation (Mantel test; $r = -0.182$; $P = 0.37$) between geographical (km) and genetic distance ($F_{ST}/ 1-F_{ST}$) matrices among basin-spawning population samples originating from Lake Superior sites (Michipicoten Island, Slate Islands, Isle Royale, Marquette MI, and Gull Island Shoal), although there was a significant positive correlation (Mantel test; $r = 0.571$; $P < 0.05$) between distance matrices for lake trout originating from the Dog and Montreal rivers, Lake Superior, and Lake Huron. To better compare the distance

correlations between river-spawner and basin-spawners, a secondary test was performed by statistical comparison of asymmetric matrices that excluded distance estimates among basin-spawning populations (Figure 3). The correlation between geographical and genetic distance remained positive for both the Dog River ($r = 0.722$; $P < 0.05$) and Montreal River comparisons ($r = 0.603$; $P = 0.064$), although the latter correlation was not significant. In contrast, there was no significant correlation ($r = 0.261$; $P = 0.245$) between distance matrices for pairwise comparisons between the Killala Lake population and sampled sites for river-spawners, Lake Superior, and Lake Huron populations. Together, these correlated patterns of spatial and genetic variation support Lake Superior as the origin for contemporary river-spawning lake trout, over another genetically similar (e.g., Great Lakes) or divergent (e.g., Killala Lake) source.

A more detailed evaluation of pairwise divergence estimates (Table 3-3) indicated that river-spawning lake trout were weakly but significantly different ($P < 0.01$; $k = 91$) from one another ($F_{ST} = 0.025$), from the Mishibishu strain (Dog River- $F_{ST} = 0.032$; Montreal River- $F_{ST} = 0.019$), the sanctuary populations ($F_{ST} = 0.023$ to 0.049), and the basin-spawning lake trout from Lake Superior ($F_{ST} = 0.033$ to 0.066). River-spawning lake trout were more divergent, however, from basin-spawning lake trout of Lake Huron ($F_{ST} = 0.063$ to 0.084) and the Killala Lake population ($F_{ST} = 0.097$ to 0.114). Estimates of differentiation among the sanctuary populations and the Mishibishu hatchery strain were lower and not significant ($F_{ST} \leq 0.017$), and also were lower than significant estimates between these samples and Lake Superior basin-spawners ($F_{ST} = 0.029$ to 0.054). Pairwise estimates of differentiation among basin-spawning aggregations within Lake Superior ($F_{ST} = 0.009$ to 0.035) and Lake Huron ($F_{ST} = 0.041$) were low relative to

estimates between Lake Superior and Lake Huron ($F_{ST} = 0.041$ to 0.079), and estimates between Great Lakes basin-spawners and the Killala lake population ($F_{ST} = 0.069$ to 0.102), but consistent with broader evaluations of hierarchical population structure within and among the Great Lakes (Ihssen et al. 1988; Krueger et al. 1989; Guinand et al. 2003; Page et al. 2004). These results showed that the weakly divergent river-spawners, the Mishibishu hatchery strain, and the sanctuary lake populations were more divergent from the Lake Huron and Killala Lake basin-spawners than from the Lake Superior basin-spawners, again consistent with a Lake Superior origin for the river-spawners, the Mishibishu hatchery strain, and the sanctuary populations.

Evaluation of potential sympatric origins in the Great Lakes

Individual assignment with STRUCTURE (Pritchard et al. 2000) revealed that lake trout that originated from northeastern Lake Superior and the sanctuary lakes had a distinctive genetic background relative to other sampled basin-spawners (Table 4). Post-simulation analysis identified two probable population models, one at $K = 2$ (Evanno et al. 2005), and another at $K = 4$ (Pritchard et al. 2007). In the first model ($K = 2$) a northeastern Lake Superior cluster (NLS) was resolved and populated primarily with individuals sampled from the Dog and Montreal Rivers, the Mishibishu hatchery strain, the sanctuary lakes, and the Michipicoten Island broodstock (but also included 26% of individuals sampled from the Marquette broodstock that originated from southeastern Lake Superior). Basin-spawning individuals sampled elsewhere in the upper Great Lakes

region (Lake Superior, Lake Huron, and Killala Lake) assigned in high proportions to the second genetic cluster (UGL).

In the second model ($K = 4$) the majority of individuals from both river-spawning aggregations assigned to a single genetic cluster (RIV), which also included fewer individuals sampled from the Mishibishu hatchery strain, the sanctuary lakes, and low proportions of basin-spawners primarily originating from eastern Lake Superior (Table 4). Most of the remaining individuals sampled from the Mishibishu broodstock and the sanctuary lake populations assigned to a second cluster (MSB), although this cluster included some individuals sampled from the Dog and Montreal rivers, as well as a low proportion of individuals from all of the other Great Lakes basin-spawning aggregations except from Gull Island Shoal, which is located in western Lake Superior. Basin-spawning lake trout sampled from Lake Superior and Lake Huron represented the majority of individuals that assigned to a third genetic cluster (BSN), with low proportional representation from all sample origins but the Dog River. The final resolved cluster was almost entirely comprised of individuals sampled from the Killala Lake population (KLC), which reflected their divergence relative to lake trout of Great Lakes origin. Unassigned individuals (UAS) at $K = 4$ showed low degrees of membership any single cluster ($q_{MAX} < 0.5$), and were present at low to moderate frequencies in all samples except those from the Dog River and the Killala Lake population.

Evaluation of genetic structure within each of the resolved clusters was performed according to guidelines suggested by Pritchard et al. (2007) but revealed further substructure only within the first model ($K = 2$). The northeastern Lake Superior (NLS) and upper Great Lakes (UGL) clusters were each subdivided into two separate

subclusters. Inter-model correspondence (IMC) was calculated as the proportional assignment of individuals to one of the four subclusters at $K=2$ relative to correspondent individual assignment to one of the genetic clusters at $K=4$. For example, individuals originating from the Dog River were assigned almost entirely (73/74) to a single genetic cluster (NLS) at $K=2$, then to one of two subclusters of origin (subcluster 1: 59/73) and (subcluster 2: 14/73) resolved from the NLS cluster. All of the individuals assigned the first subcluster also shared corresponding membership to a single cluster (RIV) resolved for the $K=4$ population model. Furthermore, the remaining members of the first subcluster originated almost entirely from either the Montreal River or Mishibishu hatchery strain samples. Of the 14 individuals assigned to the second subcluster, 6 showed correspondent cluster membership (MSB) at $K=4$, but 8 assigned to a different (RIV) genetic cluster. The single individual (1/74) that assigned to the UGL cluster at $K=2$ showed correspondent membership to the KLH cluster resolved at $K=4$. Thus the inter-model correspondence for the Dog River individuals was reasonably high (66/74 correspondent assignments to resolved genetic units comprised primarily of individuals with river-spawner origins).

Overall, other correspondence values were also high and ranged from 67% for the lake trout sampled from the Mishibishu hatchery strain (a similar value was also observed for the Michipicoten Island lake trout) to 96% for individuals sampled from the Killala Lake population. However, the low to moderate proportion of unassigned individuals at $K=4$, and individual admixture coefficient profiles in both models, were indicative of admixture among some the weakly divergent sanctuary populations, basin-spawning populations, and the Montreal River lake trout (see below). Even so, both population

models identified northeastern Lake Superior or the sanctuary lakes as the most probable origin for the contemporary river-spawners relative to the other basin-spawning sources evaluated in the Great Lakes.

Hierarchical clustering based on pairwise genetic distance estimates (D_C ; Cavalli-Sforza and Edwards 1967) resolved weak population structure within the groups identified by individual assignment analysis (Figure 4). Bootstrap support exceeded 50% for the branch point connecting the Dog and Montreal river-spawner samples, and for internal nodes connecting the Mishibishu strain and sampled sanctuary lake populations. Low levels of bootstrap support were observed for branch points connecting the basin-spawners sampled from northeastern Lake Superior, Lake Huron (Parry Sound), and the Killala Lake population. Although seemingly contrary to expectations based on the other reported genetic analyses (i.e., pairwise F_{ST} estimates and genetic clustering), this clustering pattern simply reflected the higher degree of genetic similarity among samples taken from the Mishibishu broodstock and sanctuary populations, and also between river-spawner sampling sites, relative to samples from other basin-spawning lake trout in the Great Lakes region. Comparatively, the hierarchical clustering highlighted the genetic distinctiveness of the Dog River lake trout in a similar fashion to both individual assignment and PCA, but indicated a somewhat greater degree of distinctiveness for the Montreal River lake trout from the sanctuary lake populations relative to the STRUCTURE clustering results.

Estimation of admixture proportions

Comparative admixture analyses were focused on individuals sampled from river-spawning aggregations (Dog and Montreal rivers), from proximal basin-spawners in Lake Superior (Michipicoten Island broodstock and the Slate Islands), and the Mishibishu broodstock (as a genetic representative of the sanctuary populations) as extensive molecular genetics-based analyses of phylogeography (Grewe and Hebert 1988; Grewe et al. 1993; Wilson and Hebert 1996, 1998), genetic diversity (Piller et al. 2005; Halbisen and Wilson in press), hierarchical population structure (Ihssen et al. 1988; Krueger et al. 1989; Guinand et al. 2003), and sample origins (Marsden et al. 1989; Marsden and Krueger 1991; Marsden et al. 1993; Grewe et al. 1994a; Grewe et al. 1994b; Guinand et al. 2003; Page et al. 2003; Page et al. 2004) have been performed for the other Great Lakes populations and reported elsewhere. Admixture profiles for these population samples were compared to the divergent Killala Lake population, which had been stocked in the past but whose native population remained reproductively segregated from the introduced, and established, lake trout (Ihssen et al. 1988).

Analysis of recent inter-strain admixture with STRUCTURE was limited by inter-sample divergence, which was below the threshold necessary ($F_{ST} \sim 0.21$ for 12 loci) for highly accurate (e.g., $q > 0.95$) identification of admixed individuals (Vaha and Primmer 2006). However, analysis of ordered individual admixture coefficients (q) provided an alternative method for evaluating admixture among samples (Hansen 2001; Susnik et al. 2004), and revealed two contrasting assignment profiles for sampled strains at $K = 4$ as shown in Figure 5. The admixture profile for the Dog River lake trout was similar to the profile for lake trout from Killala Lake, and for both samples, ordered admixture coefficients increased rapidly towards large asymptotic values (e.g., $q > 0.85$). This

admixture profile was also observed for the Slate Islands lake trout but, in contrast, a different profile was observed for samples taken from the Montreal River, the Mishibishu broodstock, and the Michipicoten Island broodstock. For these samples, ordered q -values were sigmoidally distributed because few sampled individuals showed high assignment probabilities to a single genetic cluster. The distribution of admixture coefficients for the Montreal River lake trout was more similar in profile to that of the Mishibishu hatchery strain, which was historically established with river- and basin-spawning lake trout, and the Michipicoten Island broodstock, which originated from a region where multiple lake trout strains had been heavily stocked in the past (OMNR 1984).

For comparison, BAPS 5.1 (Corander et al. 2003; Corander et al. 2004; Corander and Marttinen 2006) was also used to evaluate inter-strain genetic structure and explicitly test for admixture (Table 5). Admixture tests were similarly limited by low inter-strain divergence, but proportional individual assignment at the most probable number of populations ($K = 5$) was comparable to STRUCTURE assignments at $K = 4$ (Table 4). There were no unassigned individuals as almost all individuals showed high proportional membership ($q > 0.99$) to one of four resolved genetic clusters, but a few individuals from almost all populations assigned to a fifth cluster of ambiguous structural relevance (AMB). The only individual showing significant admixture ($P < 0.05$) was sampled from the Dog River. However, almost all individuals that originated from the Dog River assigned to a single cluster (RIV), as did most individuals that originated from the Slate Islands (BSN), the Michipicoten Island broodstock (BSN) and Killala Lake (KLC). Varied degrees of assignment to multiple clusters were observed for individuals sampled from both the Montreal River and Mishibishu broodstock, however, which were

consistent with a recent history of mixture or admixture with weakly divergent basin-spawning lake trout.

Evaluation of impacts from historical population size reductions

Evaluation of indicative genetic responses provided some evidence for historical population size reductions among representative sampled populations used for admixture analysis (Table 6). Inter-locus M -ratios were averaged across all loci for each sample (Garza and Williamson 2001). Values ranged between $M = 0.7$, the threshold value below which is indicative of an historical population bottleneck, and $M = 0.8$, the value above which historical populations are considered stable, for the sampled populations evaluated (the river-spawners, the Mishibishu hatchery strain, northeastern Lake Superior basin-spawners, and the Killala Lake population). The lowest M -ratio estimates were observed for the Michipicoten Island lake trout, which may have reflected a statistical sensitivity to a relatively lower sample size ($N = 47$), or alternatively an increase in allelic range (R) relative to the observed number of alleles (k) resultant from somewhat recent admixture, as genetic diversity estimates for these lake trout were comparable to other Lake Superior basin-spawners.

Contemporary river-spawning lake trout showed a significant gene diversity excess relative to the other lake trout evaluated, indicative of a historical population size reduction, only under the recommended model for microsatellite evolution (TPM), implemented by use of the BOTTLENECK program (Cornuet and Luikart 1996). However, the departure from equilibrium for Montreal River lake trout was slight (0

individual loci out of equilibrium, but an overall H_E excess by the Wilcoxon Sign-Rank Test, $P = 0.046$) relative to those sampled from the Dog River (4 individual loci out of equilibrium; Wilcoxon sign-rank test; $P < 0.01$). Comparative evaluation of the BOTTLENECK output from different microsatellite mutation models suggests that tests under the infinite alleles model (IAM) were overly sensitive for evaluating significance of gene diversity excess (i.e., potentially prone to Type I error), as all samples showed significant gene diversity excess (Wilcoxon sign-rank test, $P = 0.046$, $P < 0.05$), and a proportionally larger number of loci were out of equilibrium for each sample evaluated, relative to the significant number of loci detected under the TPM. In contrast, tests under the stepwise mutation model (SMM) seemed less sensitive to gene diversity excess (i.e., potentially prone to Type II error), as no samples showed a significant departure from equilibrium.

Genetic responses from simulated population size reductions demonstrated that a founder effect could account for the observed levels of divergence, genetic diversity, and the effective population size of the contemporary river-spawning lake trout within a realistic time frame for re-establishment with stocked individuals of sanctuary lake ancestry (Figure 6). Low but significant divergence estimates relative to the Mishibishu hatchery strain ($F_{ST} = 0.013$ to 0.025 ; $P < 0.01$) were calculated for most simulated populations, which were reduced in size to a value between $N = 30$ to 500 individuals, but all estimates were lower than the observed divergence between the contemporary Dog River and Mishibishu strain lake trout ($F_{ST} = 0.032$). Only the most severe population size reduction ($N = 10$), which caused a severe drop in genetic diversity (65%), generated a comparable divergence level.

Ranges for the indicators of historical population size reductions overlapped in value for almost all simulations. M -ratio estimates were similar in value ($M \sim 0.7$) to those calculated for actual population samples, and all were less than $M = 0.8$, but none were significantly different from one another, including the estimate for the most severely reduced simulated population. A significant gene diversity excess was detected for all simulated populations, but generally the average number of loci in disequilibrium (~ 2 loci) was closer in value to that observed for the Mishibishu broodstock (2 loci) than the contemporary Dog River (4 loci) or Montreal River (0 loci) lake trout, except for the most severely reduced simulated population (4.5 loci).

Effective population sizes were reduced in most simulations ($N = 10$ to 90) relative to the effective population size of the Mishibishu broodstock, but for less severe size reductions ($N = 200$ and 500), the effective population size was similar in value or increased relative to the Mishibishu broodstock. This effect was probably a response to reduced residual linkage disequilibrium resulting from historical admixture, as the Mishibishu hatchery strain was derived from the historically mixed sanctuary populations of river- and basin-spawning lake trout. With the exception of the estimate for the Slate Islands lake trout, all other effective population size estimates were similar in value. It is worth noting, however, that the lowest estimates were for two of the three sampled populations that showed some evidence of admixture (the Michipicoten Islands and Mishibishu lake trout), and for the reproductively isolated Killala Lake population.

Discussion

Genetic distinctiveness and contemporary origins for river-spawning lake trout populations

The river-spawning lake trout from the Dog and Montreal Rivers generally showed a significant degree of genetic distinctiveness from basin-spawning lake trout of Lake Superior, Lake Huron, and Killala Lake, but they showed a lesser degree of dissimilarity from one another, the Mishibishu hatchery strain, and the sanctuary populations. The genetic similarity between the river-spawners and lake trout originating from the sanctuary lakes could be attributed to the historical use of Dog River lake trout as one of the known sources for establishment of the sanctuary populations (Loftus 1958; Harrison 1968). Analysis of historical samples from the Dog River was limited, but indicated that this historical population was more similar in genetic variation to regional basin-spawning populations than a highly divergent, reproductively isolated lake trout population (Louisa Lake) from the Great Lakes region. The weak divergence and apparent admixture among samples taken from the Montreal River and the Mishibishu broodstock, however, was indicative of a more recent shared ancestry, presumably mediated through intermittent post-crash stocking events.

Comparisons of gene diversity excess indicated that the river-spawning lake trout may have experienced an historical population size bottleneck, either through founder effects or a native population size reduction, in contrast to comparisons of averaged inter-locus M -ratios. However, all calculated M -ratio values were similar in range ($M \sim 0.6$ to 0.9) to estimates for historical and contemporary Great Lakes lake trout (Guinand et al. 2003). For comparison, the M -ratio estimate for the divergent, allopatric Louisa Lake population was marginally larger ($M = 0.82; \text{VAR}_M = 0.05$), as 3 of 12 loci were fixed for

a single allele ($k = R$) (Halbisen and Wilson in press); inter-locus variability for M -ratio estimates were also comparable to those observed in other species (Garza and Williamson 2001). Genetic diversity estimates indicated that the sanctuary lake populations probably did not experience a secondary, post-crash population size bottleneck when founded with captive-reared individuals, as their genetic diversity (and that of the Mishibishu broodstock) was comparable to Lake Superior basin-spawning lake trout. Furthermore, the Mishibishu hatchery strain was essentially genetically indistinguishable from the weakly structured sanctuary populations.

Forward simulations revealed that the observed divergence levels, genetic diversity and effective population size estimates for both the Dog River and Montreal River lake trout were within probable expectations for a recently established population of Mishibishu strain ancestry, but did not eliminate the possibility that either of the river-spawners descended from a remnant indigenous population. These simulations also showed that simulated genotype resampling (which models admixture reduction through higher levels of random mating during population growth) had an elevating effect on effective population sizes when actual population sizes exceeded effective population size estimates. This might account for the reduced N_e estimate for the Mishibishu broodstock relative to the estimate for the contemporary Dog River lake trout, if the latter were descended from a small number of stocked individuals of sanctuary lake ancestry. The contemporary genetic characteristics and simulated population responses indicate that if the Dog River lake trout descended from stocked individuals of sanctuary lake ancestry, however, then establishment would probably have occurred early, with few

individuals, followed by a period of population growth where little or no genetic exchange occurred with other indigenous or stocked individuals.

Basin-spawning lake trout and hatchery strains

Generally, the other basin-spawning lake trout sampled from the Great Lakes, the hatchery strains, and Killala Lake showed genetic characteristics consistent with earlier, published assessments of mitochondrial variation, genetic diversity, population structure, inter-strain admixture, and historical population size reductions (Ihssen et al. 1988; Page et al. 2003; Page et al. 2004; Piller et al. 2005; Halbisen and Wilson in press). However, an unexpected genetic profile was resolved for the broodstock established with lake trout sampled from spawning shoals near Michipicoten Island. In contrast to the lake trout sampled from the Slate Islands and the Killala Lake population, the Michipicoten Islands lake trout showed evidence of recent admixture (i.e., with stocked lake trout), as well as mitochondrial DNA haplotype distribution (only Mississippian-A ancestry) uncharacteristic of Great Lakes basin-spawners (Grewe and Hebert 1988; Grewe et al. 1993; Wilson and Hebert 1996, 1998).

These genetic characteristics may have inadvertently resulted from fish culture practices, although provincial guidelines for broodstock establishment and captive rearing are designed to both capture a representative sample of native genetic diversity and prevent the loss of allelic diversity during multigenerational cross-breeding (Ferguson et al. 1991). There were no significant differences in terms of allelic and genotypic frequencies between the samples taken from the 1989 F₂ Slate Islands broodstock (1989)

and the wild population sample (2004), or between the F₂ (1995) and F₃ (2005) Mishibishu broodstock samples. However, there were notable temporal fluctuations in mitochondrial DNA haplotype frequencies for both (as well as for pooled, wild river-spawner samples) which may reflect unknown selective factors encountered during captive rearing, or accelerated drift (i.e., stochastic change) owing to the reduced effective population size (1/4 of nuclear DNA) of mitochondrial DNA.

A revised model for the evolution and recovery of river-spawning lake trout

These genetic results were used to evaluate an earlier model for the evolution of river-spawning behaviour in the lake trout of Lake Superior (Eshenroder et al. 1995; Marsden et al. 1995). The authors identified postglacial isostatic rebound as the primary agent for evolution of river-spawning lake trout from basin-spawning lake trout. This rebound model indicated that as contemporary river-spawning sites were lifted from Lake Superior, basin-spawning lake trout that initially colonized Lake Superior (ca. 10 KYA) continued homing to their natal spawning beds. That is, the river-spawning behaviour evolved after colonization of Lake Superior. Since the pre-crash river-spawning lake trout showed definitive evidence of postglacial admixture, it is clear that their ancestors must have originated from multiple glacial refugia. It is possible that river-spawning behaviour arose (or was retained from stream-spawning ancestors) independently among multiple highly divergent, geographically isolated lineages during or before the last glacial cycle (ca. 100 - 10 KYA), and that the ancestral river-spawning lake trout were always spatially segregated from basin-spawning lake trout. However, it is more

parsimonious that their unusual behaviour evolved (or was retained) only once. If the latter scenario is correct, then a single glacial lineage of ancestral river-spawning lake trout could not have existed, as spatial segregation would have kept them reproductively isolated from other glacial lineages, which clearly contributed to present day river-spawners. Thus, the ancestors of the river-spawners would necessarily have interbred with basin-spawning lake trout, supporting the notion that the reproductively segregated river-spawners evolved from basin-spawning lake trout after the formation of Lake Superior.

While the rebound model provides a plausible mechanism for the establishment of river-spawning lake trout populations, it is less suitable for explaining the long-term reinforcement and evolution of river-spawning behaviour. Unlike the river-spawning lake trout, basin-spawners typically showed weak homing tendencies during the spawning season (MacLean et al. 1981; McAughey and Gunn 1995; Kapuscinski et al. 2005) relative to many other philopatric salmonids (Neville et al. 2006b); also reviewed in (Balon 1980; Stabell 1984; Quinn 2005). It is difficult to reconcile this dramatic difference in spawning site fidelity unless there was some adaptive advantage for maintaining the river-spawning behaviour, or relative disadvantage for a reversion to basin-spawning behaviour.

Two key characteristic differences between the river-spawning and basin-spawning lake trout (Loftus 1958) have potential adaptive value. River-spawning lake trout were known to have an earlier spawning period in the wild (Goodier 1981) and shorter time to hatching under hatchery conditions (Loftus 1958) relative to basin-spawning lake trout originating from similar latitudes in Lake Superior. Both attributes

could facilitate early life-stage survival during peak seasonal stream flows, when high mortality could result as a consequence of egg dislocation or sedimentation (Claramunt et al. 2005), particularly as lake trout are broadcast-spawners that are adapted to complete lifecycles in freshwater lakes and not flowing streams (Behnke 1972; Wilson and Mandrak 2004). Furthermore, there is some evidence that early-life stage predation on lake trout decreases on shallower spawning beds (Claramunt et al. 2005; Jonas et al. 2006), and also in alternative spawning habitats (i.e., extreme deepwater locations; (Janssen 2006), although Loftus (1958) documented widespread egg predation in streams by round whitefish (*Prosopium cylindraceum*) during the river-spawning season. Finally, it is worth noting that the contemporary Montreal River lake trout are genetically similar to the Dog River lake trout, the Mishibishu hatchery strain, and the sanctuary populations, relative to expectations based on historical observations of reproductive isolation (Loftus 1958). If this degree of genetic similarity indicates that they were purely derived from stocked fish, then their recent re-establishment would imply the testable possibility there is some degree of heritability to the river-spawning behaviour.

It is not certain whether observed patterns of genetic distinctiveness and similarity between the contemporary Dog and Montreal River lake trout, and between the river-spawners and the Mishibishu strain/sanctuary populations resulted from divergence since rehabilitative re-establishment, or the divergence of the reproductively isolated sanctuary populations relative to recovering, indigenous river-spawning populations. However, early rehabilitative stocking into the Dog and Montreal Rivers (OMNR 1984) with lake trout bred from river-spawners (Loftus 1958; Harrison 1968), shortly after the dramatic reduction of sea lamprey abundance in Lake Superior (Hansen et al. 1995), may have

buffered reductive impacts from demographic stochasticity for recovering indigenous populations. Further stocking events into the main basin of Lake Superior with lake trout derived from the sanctuary populations (i.e., earlier generations of the Mishibishu hatchery strain) may have also facilitated numerical population recovery, provided the river-spawning behaviour has some heritability (and that hatchery strays of basin-spawning ancestry had little or no reproductive success in streams, as expected. It is certain though that the successful establishment of the sanctuary populations provided a wild, genetically diverse, self-sustaining source of lake trout with a Lake Superior genetic background for historical rehabilitative stocking efforts.

Defining genetic units to facilitate conservation and management

The Adaptive Evolutionary Conservation (AEC) approach draws from the numerous available methods to provide a cohesive conceptual framework for defining intraspecific conservation units (Fraser and Bernatchez 2001). Emphasis is placed on synthesis of available ecological and genetic information for developing conservation strategies, rather than relying on single, restrictive definitions for conservation units. Within this framework, the river-spawning lake trout of Lake Superior do not meet the one of most stringent definitions of an Evolutionarily Significant Unit (ESU), as they do not show reciprocal monophyly for mitochondrial DNA haplotypes (Moritz 1994) relative to Lake Superior basin-spawning lake trout. They and the sanctuary populations do, however, show significant divergence in terms of microsatellite DNA variation from basin-spawning lake trout indicating somewhat restricted gene flow from other lineages

within the hierarchical organization of the species (Fraser and Bernatchez 2001), and based on their unusual biology they represent an important component in the evolutionary history of the species (Waples 1991).

The AEC framework also advocates comparative use of Crandall et al.'s (2000) more generalized, hypothesis-testing methodology for assessing conservation status, through which river-spawning lake trout show recent evidence of both ecological and genetic distinctiveness relative to the basin-spawners. Provided the river-spawners have descended from the early basin-spawning colonists of Lake Superior, as supported by the rebound model and patterns of mitochondrial and microsatellite DNA variation, then historical gene flow occurred between both lake trout varieties (accept historical genetic exchangeability). By default they both would then have historically shared identical functions in the same ecological system as part of the same interbreeding ancestral population (accept historical ecological exchangeability). Since then however, river-spawning lake trout have become genetically distinct from basin-spawning lake trout (reject recent genetic exchangeability). Furthermore, the river spawning lake trout have shown highly segregated spawning behaviour (Loftus 1958), as well as other characteristic differences in spawning-related traits, relative to the basin-spawning lake trout of Lake Superior (reject recent ecological exchangeability). This four-fold pattern of exchangeability falls under Crandall et al.'s (2000) case (5c) for population distinctiveness categories, suggesting recent ecological distinction and that the river-spawners should be managed as separate populations from the basin-spawning lake trout.

Currently there is no formal emphasis on differential management for the river-spawning lake trout relative to basin-spawning lake trout in Lake Superior, in spite of

their unique biological attributes, habitat requirements, and genetic characteristics. Since extensive sea lamprey control measures have reduced lamprey populations in the Great lakes, thus undermining the major cause for the disappearance of native river-spawning lake trout populations, newer conservation issues have emerged. Of immediate conservation concern are indiscriminate commercial and recreational fisheries (Hansen 1999) that have the immediate potential to limit or reverse population recovery as river-spawning populations were historically small (ca. 1500 spawning adults; Loftus 1958) relative to typical basin-spawning aggregations (ca. 10,000+; Swanson and Swedburg 1980; Reid et al 2001). Additionally, although the Dog River is afforded some protection from human activities by its relatively remote location, the Montreal River is bridged by a major highway and regulated by a hydroelectric facility that discharges immediately upstream from the only available river-spawning lake trout habitat. Finally, nothing is currently known about impact of other invasive species (Riccardi and MacIssac 2000; Fitzsimons et al. 2006) that are potential early life-stage predators (Janssen et al. 2007) on the recovering river-spawning lake trout populations.

To address these issues, future evaluations should focus on providing estimates for inter-stream migration rates and spawning-population sizes, continuing further assessment of fine-scale regional population genetic structure, quantifying potential adaptive differences among populations, and determining how more recent anthropogenic impacts are affecting contemporary river-spawning lake trout. This level of information is essential for status assessment by conservation-oriented agencies (e.g., COSEWIC), and for determining whether further steps (e.g., additional translocations of lake trout of

river-spawning origin) are necessary to maintain or enhance the recovery of river-spawning lake trout in Lake Superior.

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Table 3-1

Genetic diversity attributes for all spawning aggregations and captive broodstocks sampled. Assigned numbers (No.) correspond either to sampling sites for wild populations, populations of origin for broodstocks (Marquette strain), or location of fish culture station (Mishibishu strain). Abbreviations are as follows: number of sampled individuals (N), gene diversity (H_E), observed heterozygosity (H_O), average number of alleles per locus (N_A), allelic richness (A_R), and Wright's fixation index (F_{IS}). Conventions for mitochondrial haplotype designations follow Wilson and Hebert (1998), and correspond to the following glacial lineages: Mississippian (A), Atlantic/Nahannian (B/D), Beringian (C). Mitochondrial DNA haplotype frequencies for Gull Island Shoal and Marquette were reported in Piller et al. (2005); both mitochondrial haplotype frequencies and microsatellite genetic diversity estimates for Lake Manitou were reported in Halbisen and Wilson (2008). *Captive broodstock. ^SSource for hatchery broodstock. [§]Differential mitochondrial DNA distributions for temporally pooled samples are given in the text.

No.	Spawning aggregation or captive broodstock (*)	N	Microsatellite DNA diversity					Mitochondrial DNA haplotypes ^s			
			H _E	H _O	N _A	A _R	F _{IS}	A	B/D	C	
1	Dog River	74	0.590	0.570	5.83	5.36	0.03	0.75	0.07	0.18	
2	Montreal River	78	0.603	0.577	7.17	6.27	0.04	0.79	0.12	0.09	
3	Mishibishu Lake	42	0.558	0.574	6.25	5.84	-0.03	0.85	0.15	0	
4	Mishi Lake	34	0.590	0.585	6.08	5.84	0.01	0.58	0.35	0.08	
5	Katzenbach Lake	36	0.601	0.599	6.42	6.15	0.00	0.51	0.42	0.07	
6	Mishibishu strain, Tarentorus hatchery*	87	0.589	0.596	7.25	6.08	-0.01	0.54	0.46	0	
8	Michipicoten Island, Lake Superior ^{S,*}	45	0.594	0.600	6.67	6.10	-0.01	1	0	0	
7	Slate Islands, Lake Superior ^S	87	0.620	0.609	9.33	7.53	0.02	0.42	0.45	0.13	
9	Isle Royale, Lake Superior ^S	42	0.591	0.565	7.83	6.98	0.04	0.6	0.2	0.2	
10	Marquette, Lake Superior*	27	0.585	0.593	7.17	7.17	-0.01	0.68	0.24	0.08	
11	Gull Island Shoal, Lake Superior ^S	32	0.586	0.562	8.42	8.03	0.04	0.7	0.14	0.16	
12	Parry Sound, Lake Huron ^S	42	0.559	0.529	6.50	5.98	0.06	0.56	0.44	0	
13	Lake Manitou, Lake Huron ^S	42	0.595	0.599	7.50	6.72	-0.01	0.79	0.21	0	
14	Killala Lake ^S	72	0.496	0.462	6.83	5.91	0.07	0.59	0.41	0	
	Dog River historical samples	61	-	-	-	-	-	0.87	0.1	0.03	

Table 3-2

Summary of microsatellite allelic variation observed for the twelve loci evaluated in contemporary spawning aggregations and hatchery strains, showing number of observed alleles, allelic size ranges, gene diversity (H_E), observed heterozygosity (H_O), and Hardy-Weinberg equilibrium within populations (F_{IS}).

Locus	No. of alleles	Size range (bp)		H_E	H_O	F_{IS}
		Min	Max			
<i>Ogo1a</i>	4	144	152	0.475	0.469	0.013
<i>Ots1</i>	29	203	263	0.832	0.835	-0.004
<i>Scou19</i>	12	160	180	0.629	0.602	0.043
<i>Sfo1</i>	3	108	116	0.104	0.104	0.000
<i>Sfo12</i>	4	253	259	0.206	0.201	0.024
<i>Sfo18</i>	12	166	192	0.500	0.503	-0.006
<i>SfoC24</i>	5	91	111	0.509	0.465	0.086
<i>SfoC88</i>	2	174	177	0.410	0.399	0.027
<i>SfoD75</i>	21	258	350	0.856	0.844	0.014
<i>Ssa85</i>	5	126	138	0.522	0.513	0.017
<i>Oneu14</i>	27	206	248	0.828	0.818	0.012
<i>Sfo23</i>	35	169	247	0.905	0.912	-0.008
Total or overall value	159	-	-	0.565	0.555	0.018

Table 3-3

Pairwise estimates of population divergence (F_{ST}) based on 12 microsatellite loci are given in the lower triangular matrix. Values are significant ($P < 0.01$; $k = 91$) unless highlighted by italics. The abbreviation LS refers to a Lake Superior origin, while LH refers to a Lake Huron origin.

Population	Abbr.	DGU	MON	MSL	MSI	KTZ	MLH	SLI	MPI	IRY	MRQ	GIS	PSD	LMN	KIL
Dog River	DGU	-													
Montreal River	MON	0.025	-												
Mishibishu Lake	MSL	0.049	0.030	-											
Mishi Lake	MSI	0.033	0.027	0.017	-										
Katzenbach Lake	KTZ	0.035	0.023	0.017	-0.003	-									
Mishibishu strain	MLH	0.032	0.019	0.012	0.006	0.002	-								
Slate Islands, LS	SLI	0.054	0.035	0.038	0.047	0.037	0.032	-							
Michipicoten Isl., LS	MPI	0.035	0.033	0.047	0.041	0.029	0.031	0.029	-						
Isle Royale, LS	IRY	0.066	0.062	0.036	0.054	0.042	0.044	0.017	0.033	-					
Marquette, LS	MRQ	0.041	0.033	0.043	0.046	0.039	0.041	0.025	0.036	0.035	-				
Gull Island Shoal, LS	GIS	0.046	0.037	0.029	0.041	0.031	0.032	0.009	0.025	0.005	0.015	-			
Parry Sound, LH	PSD	0.083	0.084	0.049	0.058	0.050	0.046	0.045	0.052	0.036	0.079	0.050	-		
Lake Manitou, LH	LMN	0.074	0.063	0.059	0.065	0.065	0.051	0.041	0.045	0.049	0.076	0.055	0.041	-	
Killala Lake	KIL	0.112	0.097	0.055	0.083	0.076	0.070	0.069	0.102	0.076	0.070	0.071	0.093	0.123	-

Table 3-4

Individual assignment based on admixture coefficients estimated by STRUCTURE. Proportional assignment at $K = 2$ revealed the genetic distinctiveness of lake trout originating from northeastern Lake Superior (NLS) relative to those originating from elsewhere in the upper Great Lakes Region (UGL). Proportional assignment at $K = 4$ resolved four genetic clusters. Individuals that assigned to the river-spawner cluster (RIV) were primarily sampled from river-spawning aggregations, the Mishibishu hatchery strain, or the sanctuary lakes. The majority of individuals that assigned to the Mishibishu cluster (MSB), were sampled from either the Mishibishu hatchery strain or from the sanctuary lake populations. The basin-spawner cluster (BSN) was comprised primarily of individuals sampled from basin-spawning aggregations in the Great Lakes, and the Killala cluster (KLC) was almost entirely made up of individuals sampled from the Killala Lake population. Individuals that did not assign to a single genetic cluster ($q < 0.5$) were designated as unassigned (UAS). The final column (IMC) shows proportional inter-model correspondence between individual assignments at $K = 4$, and subcluster assignments at $K = 2$ (see text for details on subcluster assignment criteria).

Origin of individuals	Proportional assignment									
	K = 2					K = 4				
	NLS	UGL	RIV	MSB	BSN	KLC	UAS	IMC		
Dog River	0.99	0.01	0.91	0.08	-	0.01	-	0.89		
Montreal River	0.83	0.17	0.45	0.23	0.12	0.04	0.17	0.79		
Mishibishu Lake	0.67	0.33	0.17	0.60	0.02	0.10	0.12	0.67		
Mishi Lake	0.79	0.21	0.12	0.65	0.03	0.09	0.12	0.76		
Katzenbach Lake	0.83	0.17	0.14	0.67	0.08	-	0.11	0.78		
Mishibishu strain	0.74	0.26	0.17	0.62	0.01	0.08	0.11	0.74		
Michipicoten Island, Lake Superior	0.56	0.44	0.16	0.20	0.49	-	0.16	0.67		
Slate Islands, Lake Superior	0.06	0.94	0.01	0.07	0.79	0.05	0.08	0.87		
Isle Royale, Lake Superior	0.14	0.86	0.02	0.02	0.83	-	0.12	0.81		
Marquette, Lake Superior	0.26	0.74	0.30	0.04	0.33	0.11	0.22	0.70		
Gull Island Shoal, Lake Superior	0.13	0.88	-	-	0.88	0.03	0.09	0.88		
Parry Sound, Lake Huron	0.07	0.93	-	0.14	0.71	-	0.14	0.74		
Lake Manitou, Lake Huron	0.07	0.93	-	0.10	0.81	0.05	0.05	0.83		
Killala Lake	-	1.00	-	-	0.01	0.97	0.01	0.96		

Table 3-5

Individual assignment and admixture tests among northeastern Lake Superior populations and stocked hatchery strains by use of BAPS 5.1. Assigned genetic clusters abbreviations are so-named for the same reasons detailed in the STRUCTURE analysis, with two exceptions: individuals that assigned in low proportions from all samples to a single ambiguous cluster (AMB), and proportion of individuals showing significant ($P < 0.05$) admixture (ADX) as determined by the method of Corander and Marttinen (2006).

Sample origin	Assigned genetic cluster					
	RIV	MSB	BSN	KLC	AMB	ADX
Dog River	0.91	0.03	-	-	0.05	0.01
Montreal River	0.55	0.19	0.21	0.04	0.01	-
Mishibishu strain	0.28	0.59	0.10	0.01	0.02	-
Michipicoten Island	0.18	0.07	0.73	-	0.02	-
Slate Islands	0.01	0.03	0.86	0.02	0.07	-
Killala Lake	-	0.07	0.07	0.86	-	-

Table 3-6

Evaluation of the effects from historical population size reductions on inter-locus genetic diversity. Average inter-locus M -ratios (M) and inter-locus variances (VAR_M) results are shown; $M < 0.7$ is indicative of an historical population bottleneck, while $M > 0.8$ indicates an historically stable population. For each microsatellite mutation model (IAM - infinite alleles model, TPM - two-phase model, SMM - stepwise mutation model) the number of loci with significant ($P < 0.05$) gene diversity excess is given. Significant departures from equilibrium over all loci (Wilcoxon sign-rank test) are indicated ($P < 0.05^*$, $P < 0.01^{**}$).

Sample origin	M-ratio		Bottleneck		
	M	VAR_M	IAM	TPM	SMM
Dog River	0.730	0.059	5**	4**	1
Montreal River	0.772	0.038	2**	0*	1
Mishibishu strain	0.755	0.032	3**	2	2
Michipicoten Island	0.714	0.041	3**	2	1
Slate Islands	0.781	0.028	2**	0	2
Killala Lake	0.750	0.025	1*	0	1

Figure 3-1

Sampling locations for river-spawning aggregations (1-Dog River, 2-Montreal River), sanctuary lake populations (3-Mishibishu Lake, 4-Mishi Lake, 5-Katzenbach Lake), the Tarentorus Fish Culture Station (6), which maintains the captive broodstock for the Mishibishu hatchery strain, basin-spawning aggregations sampled from the Great Lakes (7-Slate Islands, 8-Michipicoten Island, 9-Isle Royale, 10-Marquette, 11-Gull Island Shoal, 12-Parry Sound, 13-Lake Manitou), and the basin-spawning population of Killala Lake (14). The numbered sites for the Marquette (Page et al. 2004) and Michipicoten Island hatchery strains indicate the geographical origins of their broodstock founders. Names for all of the numbered sites are listed in Table 1.

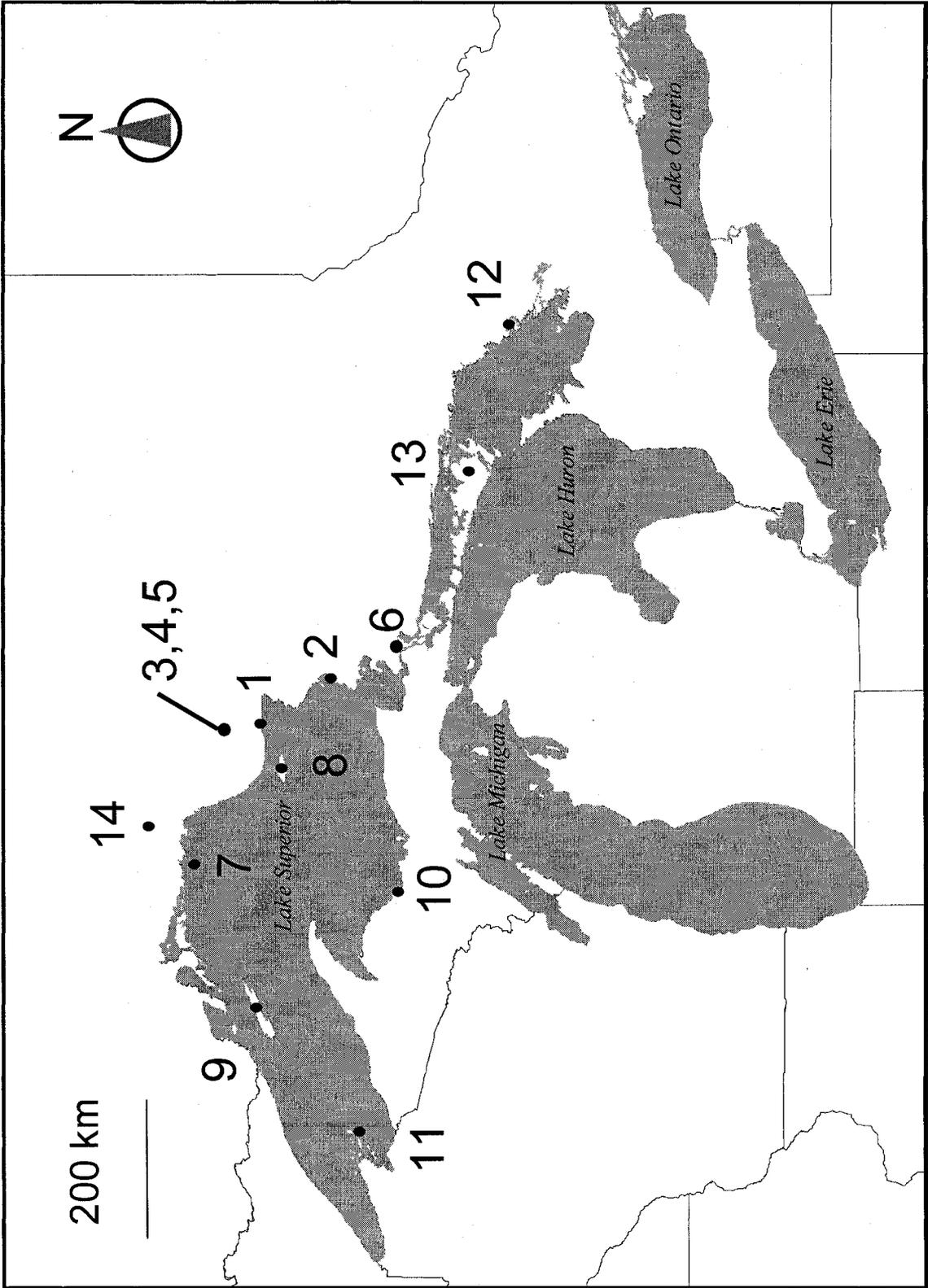


Figure 3-2

Principal components analysis (PCA) of multilocus allelic co-variance among population samples shows patterns of genetic similarity correspondent to plotted locations on the graph. Tick marks along both axes indicate 0.2 units from the origin. Names for numbered populations are given in Table 1. The dotted grey line shows radius for circle that includes all river-spawners (\diamond), the Mishibishu hatchery strain and sanctuary populations (\blacksquare), and basin-spawners from the Great Lakes (\blacktriangle), relative to the allopatric Killala lake population (\bullet). Circles were hand-drawn to group populations by origin.

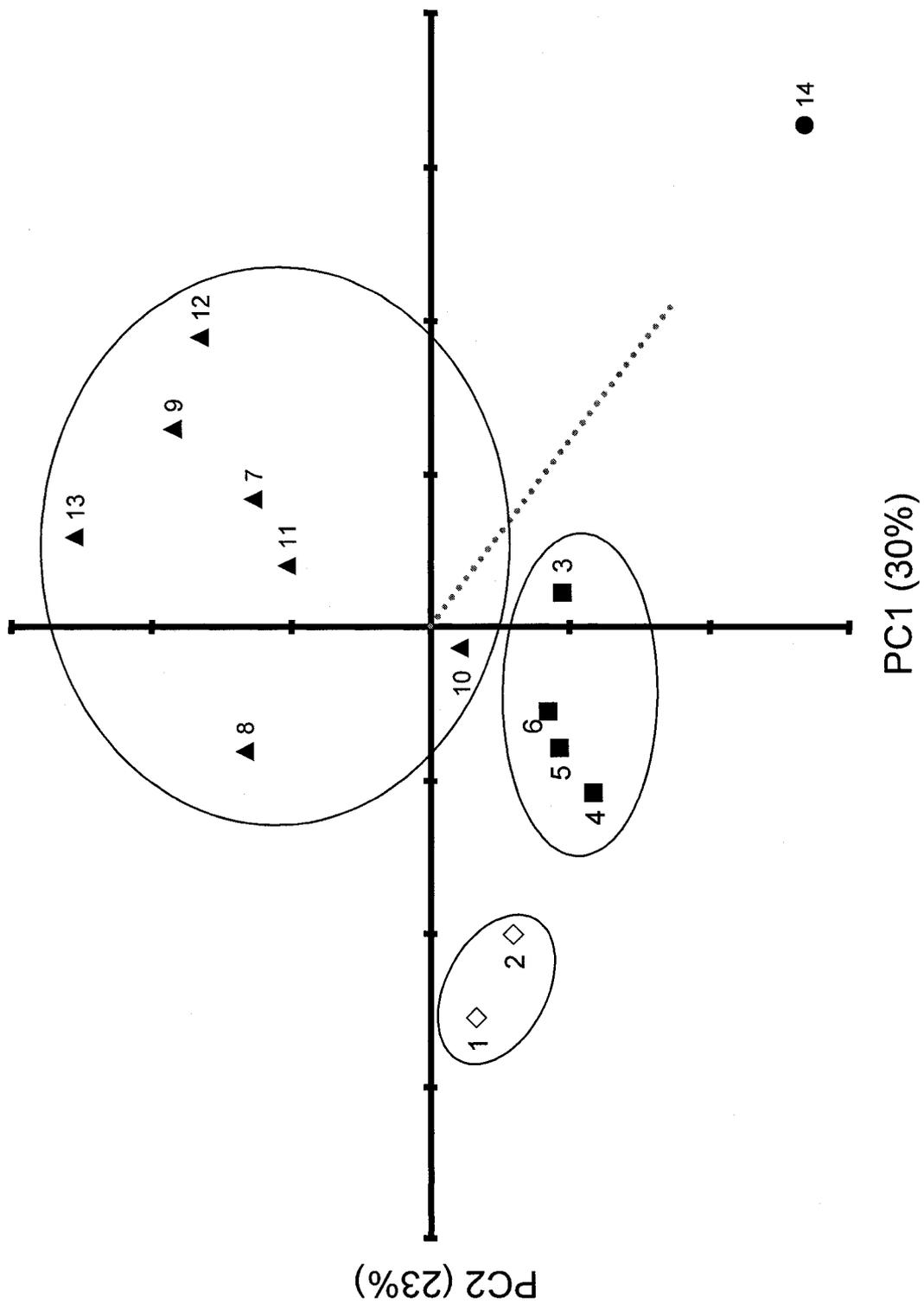


Figure 3-3.

Graph illustrating correlations between geographic distance (km) and genetic distance ($\log_e F_{ST}/1 - F_{ST}$) for river-spawning lake trout (\diamond - Dog River, \blacklozenge - Montreal River) or the Killalala lake population (\bullet) and basin-spawning lake trout sampled from the Great Lakes. Dog River ($r = 0.722$; $P < 0.05$), Montreal River ($r = 0.603$; $P = 0.064$), and the Killalala Lake population ($r = 0.261$; $P = 0.245$).

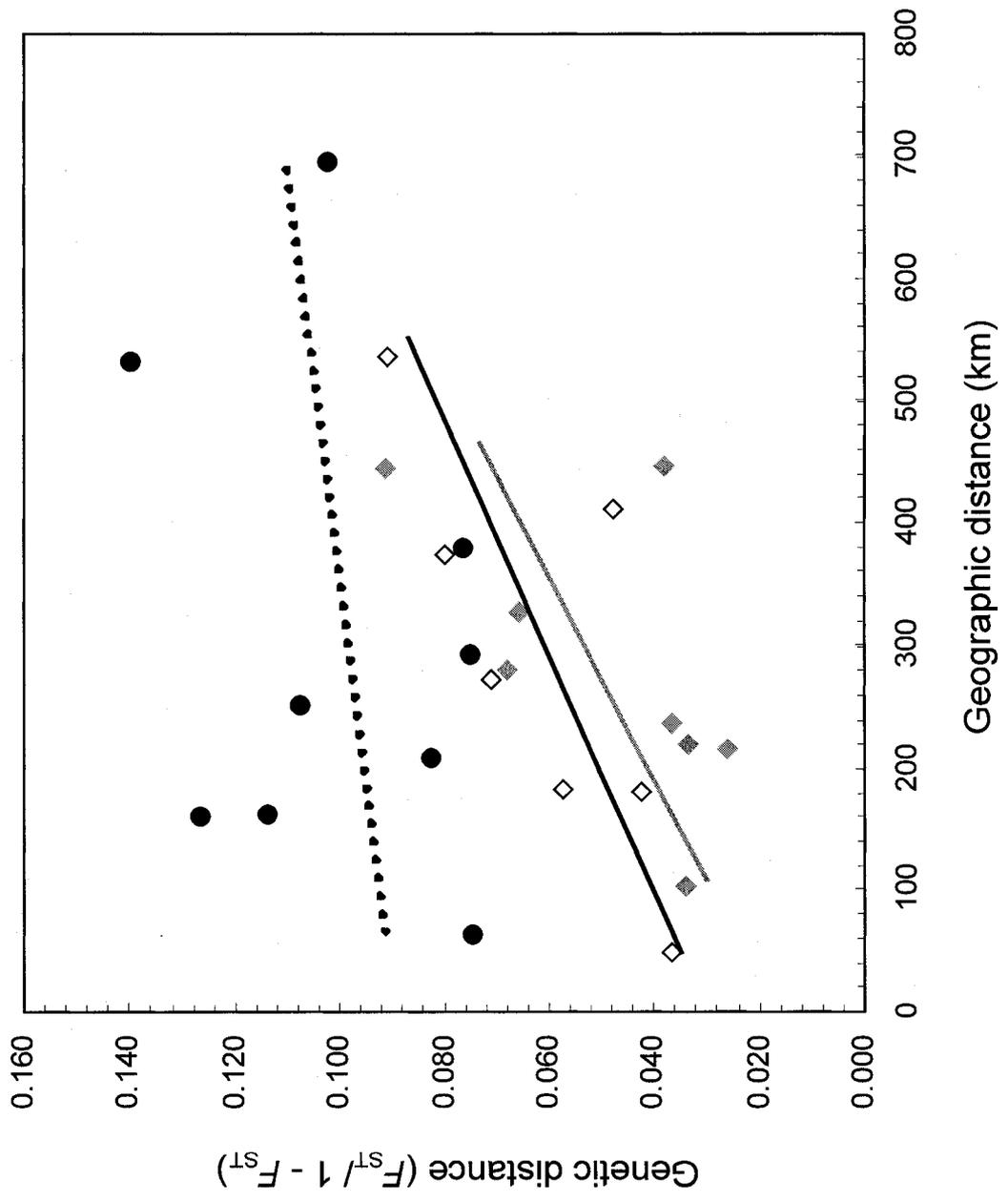


Figure 3-4.

Unrooted, neighbour-joining dendrogram based on pairwise genetic distance (D_C) estimates showing hierarchical population structure among sampled river-spawning sites, sanctuary lake populations, the Mishibishu hatchery strain, and northeastern Lake Superior populations relative to lake trout populations from Parry Sound (Lake Huron) and Killala Lake. Bootstrap support was generated from 1,000 bootstrap replicates and is shown for nodes with values exceeding 50%. The dotted line shows approximate overlapping membership to genetic clusters resolved with STRUCTURE at $K = 4$.

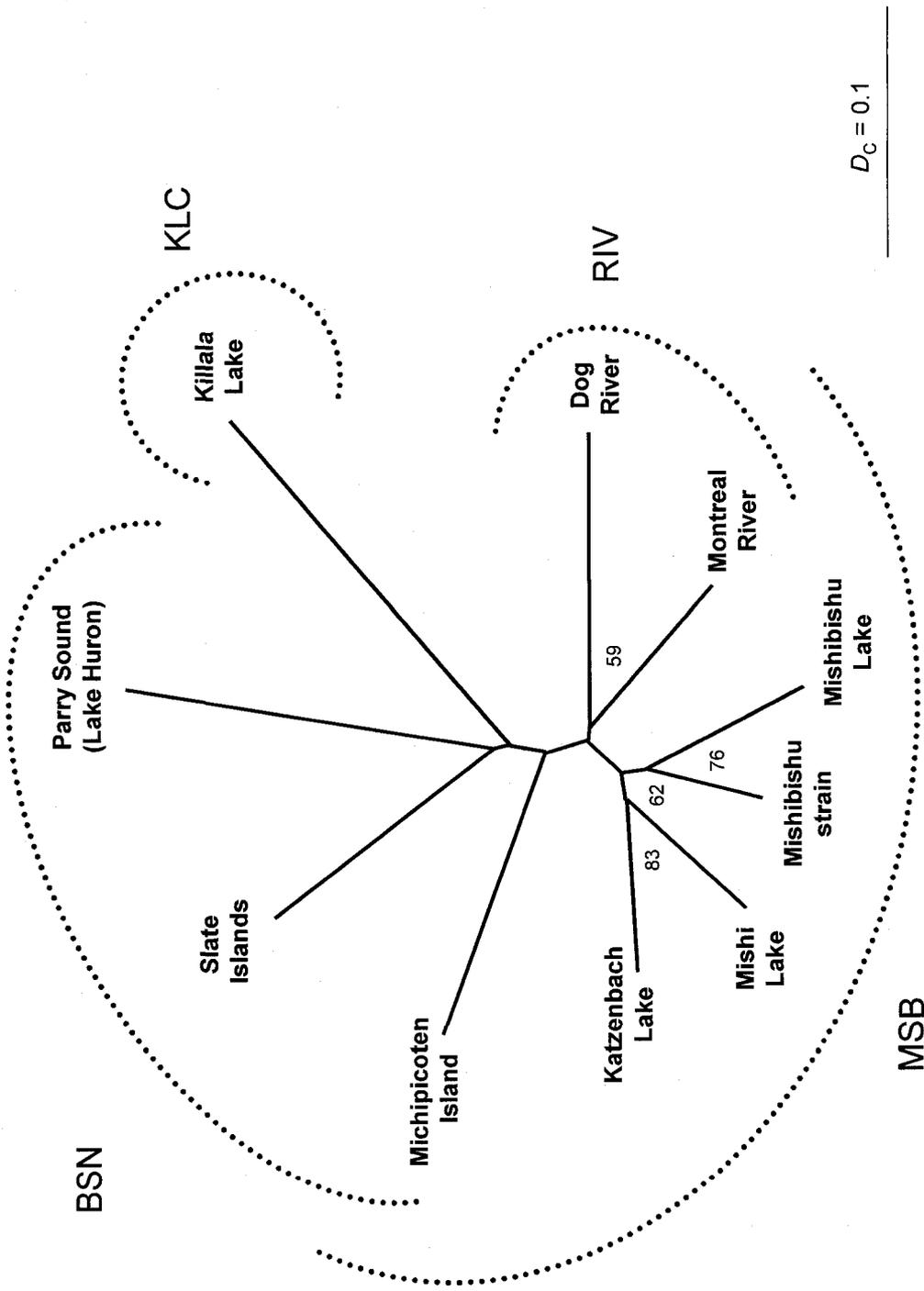


Figure 3-5.

Ordered distributions of individual admixture coefficients estimated with STRUCTURE ($K = 4$) for river-spawners, the Mishibishu hatchery strain, and northeastern Lake Superior population samples. Parenthesis indicate majority membership of individuals within each sample, and vertical lines within each graph show 95% confidence intervals for admixture coefficient estimates (q).

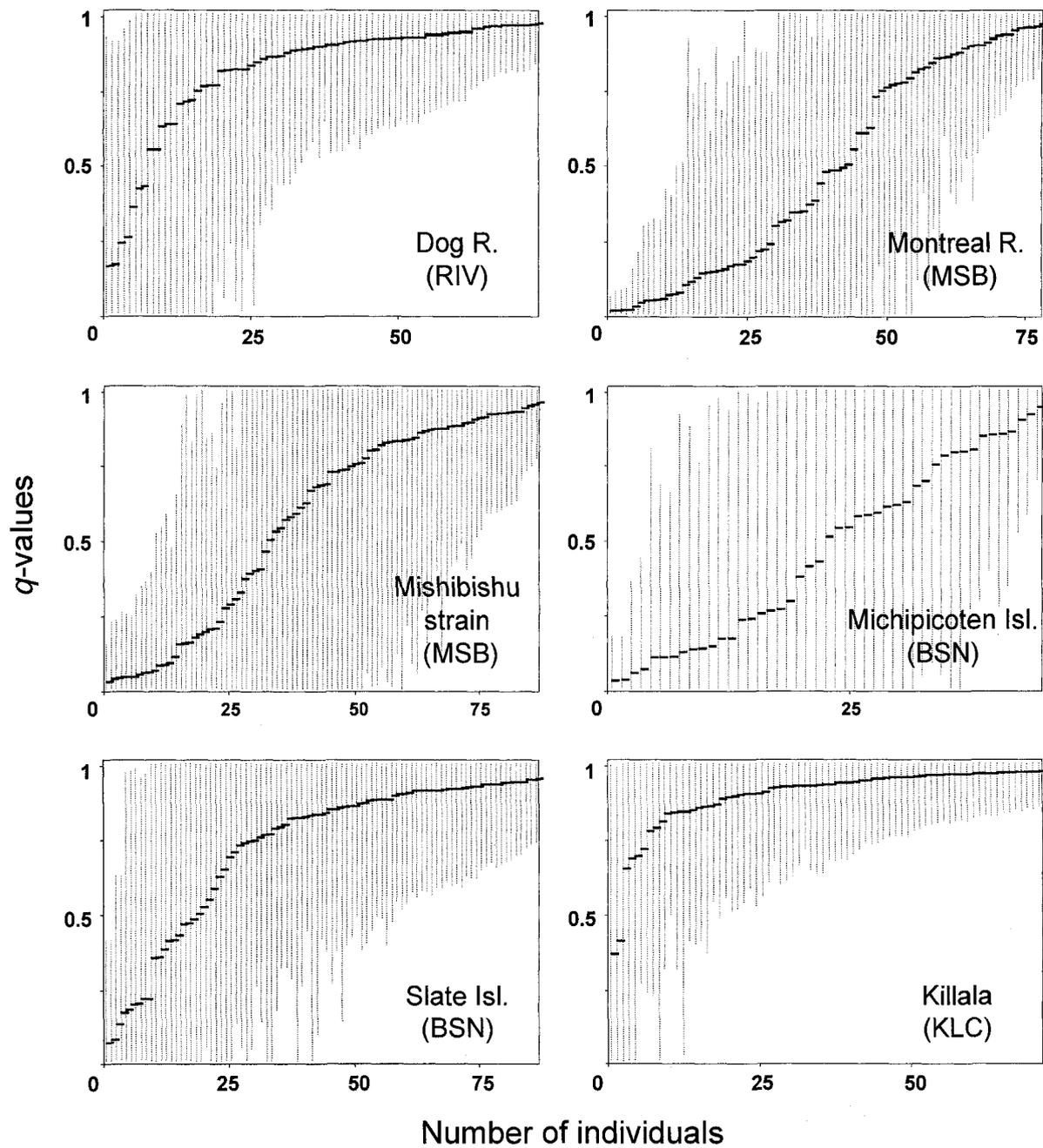
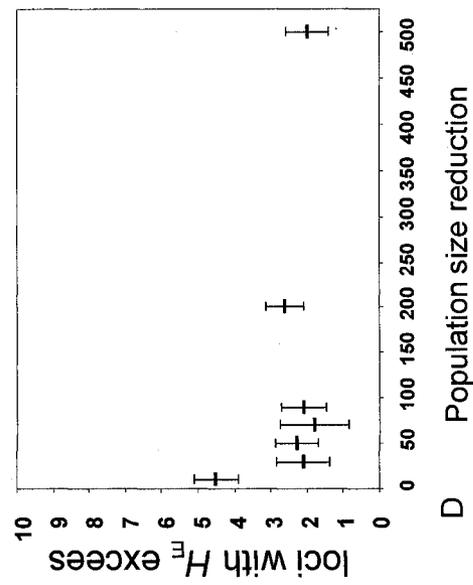
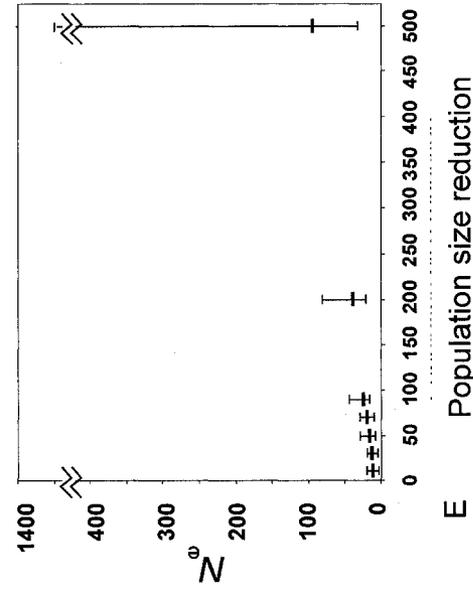
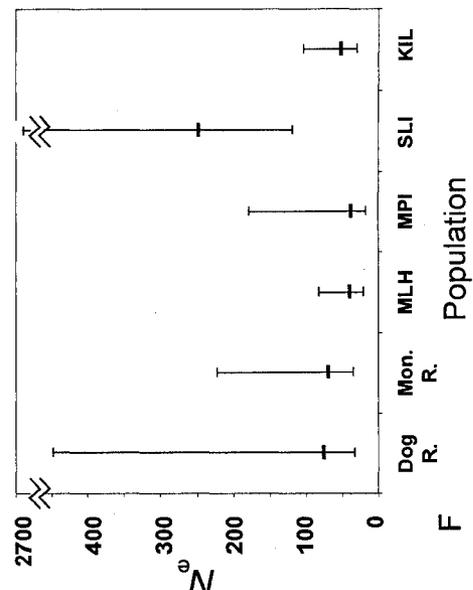
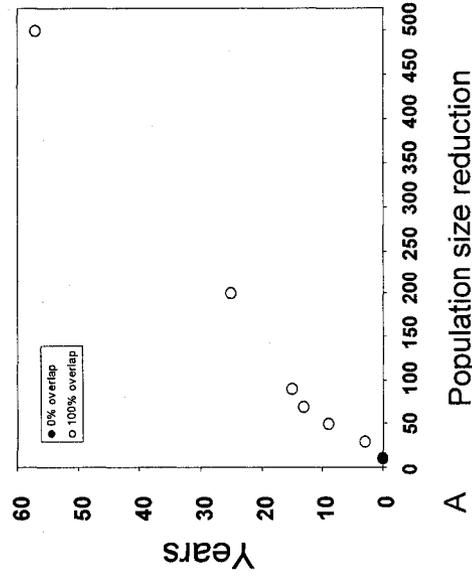
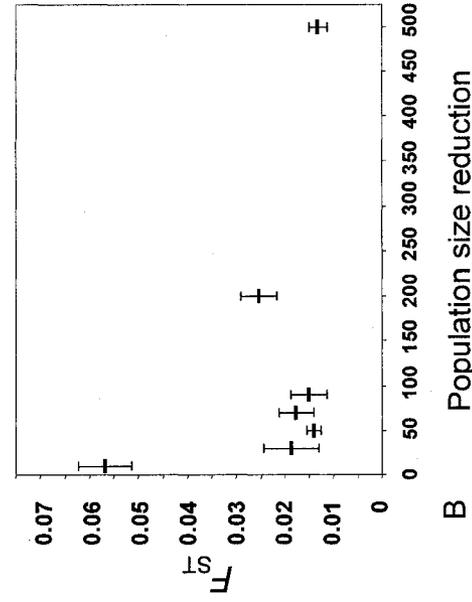
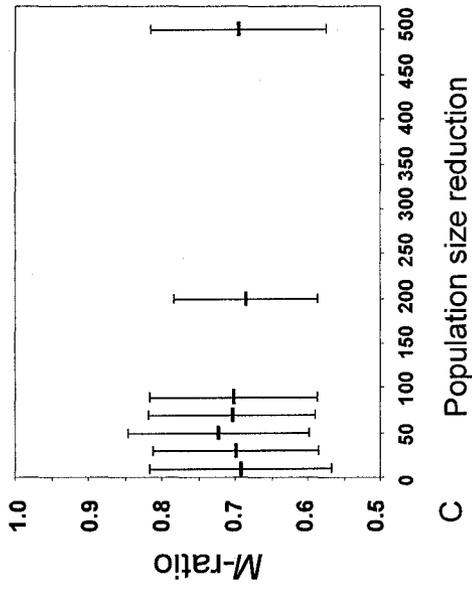


Figure 3-6

Genetic responses to simulated population size reduction for individuals sampled from the Mishibishu hatchery broodstock. Error bars indicate 95% confidence intervals except for (E). A. Time in years until the average number of alleles was reduced by 20%. B. Average divergence estimates (F_{ST}) for simulated populations relative to the Mishibishu hatchery strain. C. Average M-ratios for simulated populations. D. Average number of loci with a significant gene diversity excess. E. Average effective population sizes for simulated populations; error bars show the maximum and minimum values for the 95% confidence interval estimates for all replicates at each population size reduction. F. Effective population size estimates for actual population samples (Dog-Dog River, Mon-Montreal River, MLH-Mishibishu hatchery strain, MPI-Michipicoten Island, SLI-Slate Island, KIL-Killala Lake).



A

B

C

D

E

F

CHAPTER 4

The landscape genetics of inland lake trout (*Salvelinus namaycush*): resolving the relative influences of postglacial dispersal, contemporary environmental attributes, and population supplementation on spatial genetic variation

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Halbisen performed experimental design, genetic data collection, analysis, and wrote this manuscript

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Wilson provided supervision

Abstract

A landscape genetics approach was used to resolve how postglacial dispersal, modern-day landscape attributes, and supplemental stocking had affected fine-scale spatial genetic variation among cryptically structured inland lake trout populations. It was predicted that landscape features that influenced colonization (proximity to postglacial dispersal routes, lake surface area, and elevation), life-history attributes and population size (area and conductivity), and lake productivity (conductivity and mean lake depth) would affect the genetic attributes of regional lake trout populations. Individual- and population-level landscape genetic analyses were used to evaluate how genetic characteristics had been influenced by spatial-temporal landscape variability and supplementation with divergent hatchery strains. The regional distribution of mitochondrial DNA haplotypes (PCR-RFLP) and AMOVA-based hierarchical partitioning of microsatellite DNA (11 loci) variation indicated that a degree of modern genetic structure was due to a transient postglacial landscape. However, all sampled populations were genetically distinct from one another and exhibited substantial inter-population divergences ($F_{ST} = 0.061-0.381$) since postglacial colonization. Genetic diversity estimates were positively correlated with lake area, and negatively correlated with elevation, but generally not associated with lake productivity indicators (conductivity, and mean lake depth). Similarly, both lake area and elevation were associated with estimates of population divergence and distinctiveness, which were evaluated by ordination (principal coordinates analysis), full and partial Mantel tests, and a logistic regression model-based Bayesian analysis (GESTE). Statistical comparisons of

diversity and divergence estimates between unstocked and stocked populations revealed a little if any impact from population supplementation. Together, these analyses indicated that both founding events and landscape attributes that influenced population sizes had patterned contemporary population genetic structure.

Introduction

Variable landscapes profoundly influence spatial genetic variation in wildlife populations by affecting fundamental evolutionary processes. An understanding of the relationship between landscape variability and population genetic structure is not only critical for resolving evolutionary history (Slatkin 1987), but is also essential for conservation planning in managed populations (Crandall et al. 2000; Palsboll et al. 2007). Landscape features such as elevation changes or habitat discontinuities can act as barriers to migration, thereby modulating gene flow and promoting diversification among subdivided populations (Castric et al. 2001; Giordano et al. 2007). Many ecological attributes that affect population size and genetic diversity also reflect landscape variability (e.g., habitat type or quality; Edwards et al. 2004). These attributes can be important factors for local adaptation as well (Neville et al. 2006a; Dionne et al. 2008). Evaluation of associations between landscape and genetic variability is particularly challenging for species of commercial or recreational interest, however, as relatively recent human activities can obscure natural patterns of genetic variation (Araguas et al. 2004; Williamson 2005).

Traditional phylogeographic approaches have been used to investigate the correspondence between geographical and genetic variation on broad spatial-temporal scales (Avice 2000). More recently, however, the field of landscape genetics (Manel et al. 2003; Storfer et al. 2007) has emerged as a framework for understanding how landscape attributes affect fine-scale genetic structure over shorter time periods. Although correlation-based methods such as Mantel tests for isolation-by-distance;

(Smouse et al 1986; Rousett 1997) have been used extensively for this purpose (Storfer et al. 2007), the integration of newer population genetic techniques (for reviews see (Manel et al. 2005; Excoffier and Heckel 2006) with methods used in landscape ecology (e.g., ordination on multivariate datasets; (Gotelli and Ellison 2004) has facilitated population analyses for a broad range of species in terrestrial (Spear et al. 2005; Coulon et al. 2006) and aquatic habitats (Jorgensen et al. 2005; Leclerc et al. 2008). Landscape genetic approaches are ideal for resolving the relative contributions from present and previous landscapes (Poissant et al. 2005), natural landscape features (Angers et al. 1999; Castric et al. 2001), and human impacts (Edwards et al. 2004) on genetic structure.

Studies of freshwater and anadromous fishes have contributed substantially to understanding how historical, large-scale landscape changes affected evolutionary processes during the Pleistocene glaciations (ca. 1650 - 15 KYA; Dawson 1992; Bernatchez and Wilson 1998). Bio- and phylogeographic analyses revealed that repeated cycles of glacial advance and retreat isolated many fish populations for long periods of time, but also allowed for brief periods of dispersal and recolonization (Bailey and Smith 1981; Crossman and McAllister 1986; Dyke and Prest 1987; Wilson and Mandrak 2004). For some species and refugial populations, postglacial dispersal was limited (Underhill 1986; Bernatchez and Dodson 1991; Mandrak and Crossman 1992; Danzmann et al. 1998). For others, however, long-range dispersal and postglacial, inter-refugial mixing (i.e., secondary contact) was facilitated by temporary connections across otherwise impassable barriers (Wilson and Hebert 1996, 1998; Turgeon and Bernatchez 2001). In some cases these historical landscapes, and associated evolutionary processes, have had a

stronger relative impact on contemporary genetic structure than more recent hydrological connections over fine spatial scales (Danzmann and Ihssen 1995; Poissant et al. 2005).

The lake trout (*Salvelinus namaycush*) is a freshwater fish species whose native distribution is almost entirely restricted to oligotrophic, boreal shield lakes in previously glaciated regions of North America (Lindsey 1964; Martin and Olver 1980). Evolutionary relationships inferred from mitochondrial DNA haplotypes (Grewe et al. 1993; Wilson and Hebert 1996, 1998) indicated that the species diversified into three major evolutionary lineages during the Pleistocene (Mississippian-A, Atlantic/Nahannian-B/D, and Beringian-C). These lineage splits were considered to be responses to repeated isolations in refuges that persisted through glacial maxima (Wilson and Hebert 1996, 1998; Wilson and Mandrak 2004).

The most recent postglacial colonization of the species range (ca. 15 to 6 KYA) resulted in a differential spatial distribution of mitochondrial DNA genetic diversity (Wilson and Hebert 1996, 1998; Wilson and Mandrak 2004). The prehistoric founders of contemporary populations present in much of the central species range dispersed through a large proglacial network of meltwater lakes (Dyke and Prest 1987). These colonists experienced a transient periods of secondary contact and intermixing with others from allopatric refugia, and so gave rise to present-day populations with multiple mtDNA haplotypes. In contrast, lake trout populations outside of the maximum extent of this lake network typically did not experience a high degree of secondary postglacial contact and are generally descended from single glacial lineages (Wilson and Hebert 1996, 1998; Wilson and Mandrak 2004).

Areas of extensive secondary postglacial admixture are ideal study regions from a landscape genetics perspective because they are characterized by complex evolutionary histories and temporally variable landscape attributes. Four key lines of evidence indicated that the Algonquin Provincial Park area of southern Ontario was a region of postglacial admixture for lake trout. First, the remains of a temporary postglacial drainage channel from the proglacial Great Lakes, known as the Fossmill outflow (Karrow and Calkin 1984; Mandrak and Crossman 1992; Danzmann and Ihssen 1995), are present in the northern portion of the park. Second, there was a bimodal distribution of key glacial-marine relict species (Martin and Chapman 1965) which was positively associated with regional features that were previously part of a proglacial lake network (Mandrak and Crossman 1992). Third, other fish species distributions in the park were indicative of colonization through one or more aquatic dispersal routes (Mandrak and Crossman 1992). Fourth, the presence of multiple mtDNA lineages in proximal lake trout populations (Atlantic-B and Mississippian-C; Wilson and Hebert 1996, 1998) and regional brook charr populations (*Salvelinus fontinalis*; Acadian-B1, Atlantic-B2, and Atlantic-A; Danzmann and Ihssen 1995) revealed that regional fish populations had been colonized by multiple glacial lineages.

The Fossmill outflow was a transient landscape feature (ca 10.8 - 10 KYA; Dyke and Prest 1987) but it had a substantial, long-term impact on regional aquatic communities. This historical waterway facilitated early establishment of cold-water species (e.g., charrs and coregonids; Scott and Crossman 1973; Mandrak and Crossman 2003) in regional lakes and streams (for a detailed description of local postglacial events see Danzmann and Ihssen 1995). By comparison, nearby waterbodies were colonized by

a more diverse assemblage of freshwater fish species (including percids and centrarchids). For many of these colonists, contemporary migrant exchange may have weakened population structure and erased the effects of historical landscape variation on fine-scale genetic patterns. However, lake trout have highly restrictive habitat requirements (Martin and Olver 1980; but see (Evans 2007), and so are expected to show genetic structure that reflects both historical and contemporary landscape attributes.

A landscape genetic approach was used to resolve the relative influence of postglacial hydrological connectivity and contemporary landscape variability on lake trout mitochondrial and microsatellite variation. It was expected that if Atlantic/Nahannian-B/D mtDNA haplotypes were detected in lake trout populations, they would be proximal to the historical Fossmill drainage, and associated with the known distribution of glacial-marine relict species, an indicator of long-range dispersal through the proglacial lake network. We hypothesized that microsatellite DNA diversity would show correspondence with key landscape features that reduce the number founding species (elevation, area) and potentially limit long-term population sizes (lake area, lake productivity). For similar reasons, we also hypothesized that genetic drift would be accelerated in smaller and/or higher elevation lakes and thus they would hold more highly divergent populations. Contemporary migrant exchange was not expected to be an important factor for spatial genetic variation, and so we did not expect to see an association between inter-population genetic and geographical distances (i.e., isolation-by-distance), or other genetic structuring related to contemporary watershed connectivity.

Overall, we expected spatial genetic structure would conform to one of three alternate possibilities based on the relative influence of historical and contemporary

landscape features: 1) all populations would be highly divergent from one another, with some degree of correspondence to contemporary landscape attributes, 2) more than one genetic cluster of highly divergent populations would be resolved, and be associated with postglacial landscape features, or 3) a more complex genetic structure would be resolved, resulting from a combination of dynamic postglacial events and recent human-mediated gene flow. To address the latter possibility, the relative impact from lake trout stocking activities was also evaluated, since many regional lake trout populations have been supplemented with higher diversity but divergent hatchery strains (Evans and Willox 1991; Halbisen and Wilson 2008).

Methods

Sample collection

The lake trout obtained for this study ($N = 730$) were collected by targeted netting or angling from twenty-seven inland lakes in the Algonquin Park region of southern Ontario between 1999 and 2006 (Table 4-1; Figure 4-1). For comparative analysis, samples were also obtained from the provincial Manitou hatchery strain ($N=43$), which originated from an island in the Great Lakes (Lake Huron), has been commonly used for regional supplemental stocking (Halbisen and Wilson 2008). A non-invasive genetic sample (ca. 20 mg) was taken from each individual by removing approximately 20 mg of fin tissue. Fin clip samples were then either air-dried or preserved in 95% ethanol for long-term storage prior to genetic analysis.

Landscape attributes for the sampled inland lakes (Table 4-1) were obtained from a revised version of the Ontario Ministry of Natural Resources (OMNR) Aquatic Habitat Inventory (AHI). Sampled lakes were situated in four different contemporary stream drainages (designated as tertiary watersheds in the OMNR-AHI) that ultimately flowed into the St. Lawrence River system (Figure 4-2). Three sampled lakes (Kioshkokwi Lake, Cedar Lake, and Radiant Lake) were located within the original main channel of the Fossmill outflow (Figure 4-1). The remaining lakes were distributed at higher elevations (320-457m above sea level) that spanned the 381m (1250 ft) contour above which no indicator glacial-marine relict species were detected (copepods - *Senecella calanoides* and *Limnocalanus macrurus*, amphipods - *Mysis relicta* [now *Mysis diluviana*] and *Pontoporeia affinis*; Martin and Chapman 1965). Bathymetric attributes were variable among lakes; surface areas ranged from 26 to 5154 ha, and mean depth ranged from 5.2 to 17 m. However, all lakes had low conductivity (TDS- total dissolved solids; 17-53 mg/L) were low and relatively similar as the Algonquin Highlands are situated on Precambrian Shield bedrock (Gunn and Pitblado 2004). These three latter attributes are known to affect lake carrying capacity (surface area; Shuter et al. 1998), productivity (conductivity and mean depth; Ryder 1965), and lake trout life-history attributes (surface area and conductivity; Shuter et al. 1998). Estimation of the total number of fish species in each lake was based on criteria used by Evans and Olver (1995) for evaluation of fish community structure in inland populations: only species present at greater than 5% abundance in native lake trout lakes were included.

Information on lake trout stocking was obtained in part from the OMNR FISHNET database, which was based on provincial records collected intermittently

between 1935 and 1996. Additional stocking records were maintained by the park's administrative staff, and were included in estimates of the total number lake trout stocked in each lake. Excluding Lake Opeongo, 97% of recorded stocking events utilized hatchery strains that originated from the Great Lakes (Lake Manitou and Lake Superior). Lake Opeongo was stocked with a larger variety of strains that originated from the Great Lakes region and Algonquin Park, including Lake Ontario, Lake Simcoe, Lake Lavieille, and with fish collected from Lake Opeongo itself. Since lake trout from the Great Lakes share a common evolutionary history, and have shown similar genetic characteristics relative to native southern Ontario inland lake trout populations (Halbisen and Wilson in press), the Lake Manitou population was included as a contemporary Great Lakes population for comparative genetic analyses. Stocking effort was calculated as the stocking intensity (i.e., total stocked per lake [no.] divided by lake area [ha]) divided by the total period of time the study lakes were stocked (61 years), in order to standardize stocking effect estimates among lakes.

Genetic data collection

Explicit methods for DNA extraction and microsatellite amplification by PCR followed those in Thesis Chapter 2 (Halbisen and Wilson in press). Eleven microsatellite loci were used for this study: *Sfo1*, *Sfo12*, *Sfo23* (Angers et al. 1995), *SfoC24*, *SfoC88*, *SfoD75* (King, T. L., unpublished), *Scou19* (Taylor et al. 2001), *Oneu14* (Scribner et al. 1996), *Ssa85* (O'Reilly et al. 1996), *Ots1* (Banks et al. 1999), and *Ogo1a* (Olsen et al. 1998). Amplified, fluorescently labelled microsatellite alleles were resolved on an ABI

3730 48-capillary automated sequencer (Applied Biosystems) using POP-7 polymer. Collected allelic scores were standardized with previously published microsatellite data (Halbisen and Wilson in press) that was collected with an ABI 377 sequencer (Applied Biosystems) by analysis of an identical sample set on both sequencers.

Mitochondrial DNA haplotypes were detected by use of a PCR-RFLP assay (see Chapter 2). Two regions of the mitochondrial genome were targeted for amplification, as both had diagnostic polymorphisms for resolving major glacial lake trout lineages (Piller et al. 2005). A *Bam*HI restriction site in the cytochrome-*b* amplicon was diagnostic for Atlantic/Nahannian - B/D haplotypes, and second site in the NADH dehydrogenase amplicon resolved Mississippian - A and Atlantic/Nahannian - B/D, from the Beringian - C lineage (Wilson and Hebert 1996, 1998). Digested amplicons were visualized by SYBR Green Dye (Molecular Probes) staining and agarose-gel electrophoresis.

Genetic clustering techniques

Two individual-based methods were used to estimate genetic distinctiveness among sampled populations. Bayesian Analysis of Population Structure (BAPS) version 5.1 (Corander et al. 2003; Corander et al. 2004; Corander and Marttinen 2006) is a model-based software program that estimates the number of genetic clusters (*K*) from a set of individual multi-locus genotypes, and explicitly tests for inter-cluster admixture among individuals. BAPS has previously produced very similar or identical results to those obtained from STRUCTURE software (Pritchard et al. 2000) when used to resolve lake trout genetic structure (Halbisen and Wilson in press), but uses a much more

efficient computational algorithm for individual-assignment based genetic clustering (Latch et al. 2006). To determine whether individuals sampled from the inland lakes ($N=730$) were genetically similar to contemporary Great Lakes lake trout, individuals from the Lake Manitou population ($N=43$) were also included in this analysis. Mixture proportions for the expected number of populations (i.e., genetic clusters or K) were estimated by performing five replicate simulations for each population model, where the number of expected populations was adjusted from $K=1$ to $K=30$. After the most probable model was selected ($K=25$), individual admixture proportions were calculated and tested. As suggested by Latch et al. (2006), resolved clusters with $N=3$ or fewer members were not included in estimation of admixture proportions. Values for the other model parameters needed for admixture estimation were set according to the author's recommendations: 100 iterations were performed for calculation of individual admixture coefficients, but 10 iterations were used for estimation of admixture coefficients for reference individuals (200 sampled per population). *Sfo1* and *SfoC88* were excluded from individual genotypes to as have extremely low polymorphism (Halbisen and Wilson in press).

Ordination by principal coordinates analysis was used to evaluate the degree of genetic similarity among all individuals sampled from all inland lakes ($N=730$). GENALEX version 6 (Peakall and Smouse 2006) was used to extract the first six principal coordinate axes from a genetic covariance matrix, which was initially converted from individual-by-individual genetic distance matrix (Peakall et al. 1995; Smouse and Peakall 1999). The extracted principal components were then used as response variables

for evaluation of the impact of landscape attributes and population supplementation on regional patterns of genetic distinctiveness (see below).

Population-level divergence was examined by hierarchical clustering, and was restricted to populations where 25 or more individuals were sampled, based on results obtained by individual-assignment tests (see below). Pairwise genetic distances (D_C ; Cavalli-Sforza and Edwards 1967) were estimated with Populations v. 1.2.28 (Languella 1999), as this chord distance provides a useful measure for reconstructing accurate phylogenies from microsatellite genetic data (Takezaki and Nei 1996). Populations were clustered by constructing a neighbour-joining tree based on the pairwise genetic distances. To estimate bootstrap support for each branch point, multiple rounds of resampling across all loci were executed to generate 1000 additional replicate trees, from which a single consensus tree was constructed.

Estimation of genetic diversity statistics and population differentiation

Genetic diversity estimates were also calculated for population-level samples. Heterozygosities (H_E - Nei's standardized estimate of heterozygosity, or gene diversity [Nei 1987]; H_O - observed heterozygosity), standardized allelic richness (A_R), the average number of alleles per locus (N_A), estimates of Hardy-Weinberg equilibrium within populations (F_{IS}), and pairwise estimates of population differentiation (F_{ST} - Hardy-Weinberg equilibrium among populations) were calculated with FSTAT version 2.9.3.2 (Goudet 2001). Tests of Hardy-Weinberg equilibrium were performed by comparing statistical estimates to null distributions generated by random permutations (at least 1000)

of alleles among individual loci (F_{IS}), or multi-locus genotypes among individuals (F_{ST}). All pairwise tests were corrected by sequential Bonferroni adjustments ($\alpha = 0.05$; (Rice 1989).

AMOVA

Hierarchical analysis of molecular variance (AMOVA) was performed with ARLEQUIN version 3.1 (Excoffier et al. 2005) to evaluate whether spatial genetic structure was better explained by historical (i.e. postglacial) or contemporary watershed connectivity. Fixation indices and variance partitioning were compared for population-level samples grouped by elevation category (above or below the indicative 381m contour), to reflect proximity to the Fossmill outflow, and for the same samples grouped by river drainage, to reflect current hydrological connections. The statistical significance of the different covariance components associated with each genetic structure level was calculated by component-specific permutations (1000 permutations per test; Excoffier et al. 1992).

Comparisons of lake attributes and genetic characteristics

Since there was no distributional expectation for genetic diversity estimates, nonparametric tests (Spearman rank-order correlations; [Spearman 1904]) were used to measure correlations between genetic diversity and the key landscape attributes (elevation, surface area, conductivity, and mean depth) with STATISTICA version 7.0

(StatSoft). Similarly, correlations between extracted principal coordinates, which reflected genetic distinctiveness among sampled populations, were tested in the same manner.

Mantel tests (Mantel 1967) were used to evaluate the correlations between pairwise genetic and geographic distance matrices among populations with GENALEX version 6 (Peakall and Smouse 2006). Separate transformations were applied to both distance measures to linearize correlations between genetic distance ($F_{ST}/1-F_{ST}$), and straight-line geographical distance (none for the one-dimensional stepping stone model; $\log_e[\text{km}]$ for the two-dimensional stepping-stone model) under the isolation-by-distance model of divergence (Rousset 1997). Null distributions for test statistics (correlation coefficients) were generated by randomization (1000 replicates).

To test associations between genetic distance ($F_{ST}/1-F_{ST}$) and key landscape attributes, both full and partial Mantel tests (Smouse et al. 1986) were conducted with FSTAT version 2.9.3.2 (Goudet 2001). Simple differences were calculated between each population pair for these attributes, and then \log_e -transformed to improve normality. Since there is some controversy over the use of randomization-based methods for generating null distributions in partial Mantel tests (Raufaste and Rousset 2001; Castellano and Balleto 2002; Rousset 2002) regression models were compared with a model selection method (AIC- Akaike's Information Criterion; [Akaike 1973]), that relies on an alternative information-theoretic approach (Burnham and Anderson 2002). Briefly, AIC scores (AIC) were calculated from the residual sum of squares error and the number of parameters used (K) for each regression model. These scores were then corrected (AIC_C) for small sample size bias ($N/K < 40$) according to suggestions made by Hurvich

and Tsai (Hurvich and Tsai 1989). Model selection was performed by comparison of Akaike weights (w_i), which are relative values that were calculated from the differences between the individual, corrected scores and the minimum AIC_C value; larger weight values indicated better regression models (for an explicit example of this methodology see Roach et al. (2001).

Associations between landscape attributes and population differentiation (F_{ST}) were further evaluated with the GESTE software package (Foll and Gaggiotti 2006). This program uses a hierarchical Bayesian approach that estimates how environmental factors affect genetic structure with a series of predictive logistic regression models, and iteratively calculates the posterior probabilities for each model to facilitate subsequent model selection. Since this implementation is limited to a maximum of two predictive variables for each model series, the four key landscape attributes were evaluated in pairs. Default values were used for initialization of the Monte Carlo Markov Chain and parameterization of the proposal distributions, with the exception that 2000 additional burn ins rounds were included. Following Leclerc et al. (2008), three replicate runs were completed for each pair of landscape variables to evaluate the accuracy of the posterior probability estimates.

Analysis of population supplementation

Genetic diversity statistics, landscape attributes, extracted principal coordinates, and pairwise divergence estimates (F_{ST} estimates versus Lake Manitou) were compared between unstocked and stocked populations, and evaluated for correlations with stocking

effort in stocked lakes. A non-parametric test was chosen (Mann-Whitney U test; Mann and Whitney (1947) for the majority of two-sample comparisons to provide a somewhat conservative estimate of stocking effect, since sample sizes were generally small and unequal between groups, and also to avoid erroneous assumptions about variable distributions (Zar 1999). Spearman rank-order correlations were chosen for testing the associations with stocking effort for the same reasons. Randomization-based tests were used for comparisons of population differentiation (F_{ST} versus Lake Manitou), as the pairwise estimates were not strictly independent of one another. A simple difference in average F_{ST} values (D_{AVG}) between stocked and unstocked lakes was calculated and used as a test statistic. The null distribution for this test statistic was generated by Monte Carlo-based resampling (1000 replicates). The same randomization procedure was used to generate a null distribution for the testing the statistical significance of the correlation between population differentiation and stocking effort.

Results

Descriptive statistics for microsatellite loci

The microsatellite loci that were evaluated showed variable polymorphism (3 to 37 alleles observed per locus), similar to previous studies of inland lake trout (Chapters 2 and 3, see also Piller et al. 2005). Population-level estimates of gene diversity (H_E) ranged from 0.298 (Big Porcupine Lake) to 0.583 (Lavieille Lake) (Appendix Table A-1-3). The average number of alleles per locus ranged from 2.36 (Timberwolf Lake) to 6.73

(Lake Kioshkokwi), and standardized allelic richness ranged from 2.26 (Timberwolf Lake) to 5.93 (Lake Kioshkokwi). Overall F_{IS} estimates ranged from -0.08 (Lake LaMuir) to 0.08 (Big Porcupine Lake), and there was no significant ($P < 0.05$) heterozygote deficit or excess detected in 187 pairwise tests (population by locus) of departure from Hardy-Weinberg equilibrium.

Genetic clustering

The mixture module of BAPS 5.1 (Corander et al. 2003; Corander et al. 2004; Corander and Marttinen 2006) resolved 25 genetic clusters by individual assignment of 773 individuals sampled from 27 inland lakes and the Lake Manitou hatchery strain (Table 4-2). Most resolved clusters (14/25 clusters) were lake-specific as almost all individuals sampled from any single lake shared membership to the same genetic cluster. Lake-specific clusters were not observed in cases where fewer than 15 individuals were sampled from a single lake (9/28 lakes), which indicated a probable sample-size limitation for detection of discrete genetic units. Two clusters had fewer than four members (C24 and C25) which probably reflected the tendency of the BAPS algorithm to overestimate K unless clusters with 3 individuals or fewer are excluded (Latch et al. 2006). For lakes where more than 20 individuals were sampled (19/28 lakes) proportional assignment to a single cluster was high (74-100%), except for Lake Manitou (47%), Cedar Lake (58%), and Lake Kioshkokwi (38%). Individuals from these three lakes generally shared low to moderate degrees of membership to the same genetic clusters (C1, C2, C3, C11, C18, C19 and C21), however, individuals sampled from 8

other lakes ($N > 20$ individuals sampled) also showed somewhat similar degrees of membership to these genetic clusters. Few individuals showed evidence of inter-cluster admixture, but those individuals showing either a low probability of assignment ($q < 0.5$) to a single resolved cluster or showing significant admixture ($P < 0.05$; Corander and Marttinen 2006) were designated as unassigned (UAS) or admixed (ADX), respectively.

Evaluation of hierarchical structure among lake trout populations indicated weak evidence of population genetic structure correspondent to geographical region (Figure 4-3). Pairwise genetic distance estimates (D_C ; Cavalli-Sforza and Edwards 1967) ranged in value from $D_C = 0.22$ to 0.51 among all population pairs, but bootstrap support was low (less than 40%) for all but two branch points of the consensus tree that was built from 1,000 replicate joining-joining trees (Figure 4-3). However, this clustering pattern indicated some degree of genetic similarity among the lake trout from Lake Manitou, Cedar Lake, and Lake Kioshkokwi Lake relative to other sampled populations, particularly the population samples from more southern populations (i.e., Smoke Lake, Timberwolf Lake, Kingscote Lake, Big Porcupine Lake, and Louisa Lake). By comparison, no strong association between contemporary watershed and hierarchical genetic structure was evident. As different measures of genetic distance may reflect different aspects of evolutionary history, consensus trees were also constructed from two other pairwise estimates of genetic distance: D_A (Nei et al. 1983) and D_S (Nei 1972). The resultant patterns of hierarchical structure reflected high degrees of divergence and were almost identical to those detailed above (data not shown).

Genetic variation and landscape features

In contrast to microsatellite DNA-based genetic clustering, the non-uniform spatial distribution of mitochondrial DNA (mtDNA) haplotypes and showed an obvious pattern of glacial lineage segregation, which indicated some degree of spatial population genetic structure (Figure 4-4). Although regionally common Mississippian-A haplotypes were detected in almost all sampled lakes, Atlantic/Nahannian-B/D mitochondrial DNA (mtDNA) haplotypes (hereafter referred to as Atlantic-B) were also found in eight inland lake trout populations (Cedar Lake, Lake Kioskokwi, White Partridge Lake, Hogan Lake, Lake Opeongo, Radiant Lake, Gilmour Lake and Little Dickson Lake). These less common haplotypes may have originated from stocked fish of Great Lakes origin (i.e., Lake Manitou) as five of the eight lakes (Table 4-1) were historically stocked. However, the almost mutually exclusive spatial partitioning of mtDNA haplotype lineages among regional populations suggested that the observed haplotype distribution could also have naturally resulted from a much earlier postglacial colonization by lake trout of Atlantic-B glacial ancestry through the Fossmill outflow (Figure 4-4). There was a notable association between mtDNA haplotype and glacial-marine relict species distributions, as six the eight lakes where Atlantic-B haplotypes were detected are situated below the 381 m elevation contour (Table 4-1). Finally, the two Beringian-C haplotypes detected in Kingscote Lake were not informative for resolving regional patterns of postglacial dispersal as they had previously been attributed to stocked fish of hatchery strain origin (Wilson and Hebert 1998; Halbisen and Wilson in press).

Hierarchical analysis of molecular variance (AMOVA) showed that grouping by historical hydrological association (above or below the 381m elevation contour)

explained a rather small proportion of the overall variance ($F_{CT} = 0.019$; $P = 0.06$; Table 4-3). Comparative hierarchical partitioning by contemporary drainage, however, provided an even less suitable grouping ($F_{CT} = -0.011$; $P = 0.59$), however. In both cases, partitioning of molecular variance among populations within groups (elevation: $F_{SC} = 0.199$; watershed $F_{SC} = 0.202$; $P < 0.0001$), and among individuals within populations (elevation: $F_{SC} = 0.214$; watershed $F_{SC} = 0.211$; $P < 0.0001$) accounted for almost all of the proportional variation (approximately 20% and 80%, respectively).

Population-level genetic diversity measures estimated from microsatellite DNA variation showed contrasting correlations with key landscape attributes (Figure 4-5; Appendix Table 4-1). All three diversity estimators, (gene diversity [H_E], the average number of alleles per locus [N_A], and standardized allelic richness [A_R]), were negatively correlated with elevation (m) and positively correlated with lake surface area (ha). All of these correlations were significant (Spearman rank-order correlation; elevation: $r < -0.61$; area $r > 0.64$; $P < 0.05$), except for the weaker correlation between N_A and elevation (Spearman rank-order correlation; $r < -0.44$; $P > 0.05$). None of the genetic diversity estimators were significantly correlated with lake conductivity (Spearman rank-order correlation; TDS-total dissolved solids: $0 < r < 0.17$; $P > 0.05$) or mean depth (Spearman rank-order correlation; m; $0 < r < 0.34$; $P > 0.05$). For comparison, no significant inter-correlations were observed (Spearman rank-order correlation; $-0.37 < r < 0.27$; $P > 0.05$) between any of the key landscape attributes for all of the evaluated inland lakes ($N = 28$), including lake surface area and elevation.

Divergence, genetic distinctiveness, and landscape features

Mantel tests based on microsatellite allele frequencies showed no evidence for divergence associated with geographical proximity among sampled inland populations through the isolation-by-distance model (Rousset 1997). There was no significant correlation between genetic distance ($F_{ST}/1-F_{ST}$) and geographic distance (km or $\log_e[\text{km}]$) under the linear, one-dimensional (Mantel test; $r = 0.003$; $P = 0.48$) or the two-dimensional (Mantel test; $r = 0.029$; $P = 0.40$) stepping-stone models. A more detailed analysis of pairwise estimates of population differentiation (i.e., the genetic distance estimates) indicated that all calculated F_{ST} values, which ranged from 0.061 to 0.381 (Table 4-4), were significant ($P < 0.01$; $k = 136$). However, the weakest divergence estimates more commonly occurred between samples from the largest inland lakes (greater than ~ 1000 ha) situated in or near the Fossmill outflow channel.

The effects of lake area, elevation, conductivity, and mean lake depth on inter-population divergence were evaluated by model selection (AIC - Akaike's Information Criteria) on a series of regression models generated with partial and full Mantel tests (Table 4-5). Matrices of \log_e -transformed, pairwise differences were calculated for each of these variable attributes and tested, both individually and in combinations, for their effect on genetic distance ($F_{ST}/1-F_{ST}$). The difference in lake surface area alone was identified as the best predictive model for divergence relative to the other six models tested ($AIC_C = -557.05$; $w_i = 0.68$). The negative correlation between pairwise differences in lake surface areas and genetic distance estimates (Mantel test; $r = -0.38$; $P < 0.005$) indicated that populations residing in lakes with little difference in surface area (i.e., comparisons among the smallest lakes) showed a greater degree of genetic

divergence from one another relative to other populations (i.e., comparisons of any lake against medium or large lakes) (Figure 4-6).

Both lake surface area and elevation were identified as important factors for population divergence, however, by the hierarchical Bayesian method implemented in GESTE (Foll and Gaggiotti 2006; Table 4-6). In contrast to results obtained from Mantel tests, the most probable models considering both variables together suggested that elevation ($P = 0.34$) and surface area ($P = 0.30$) had similar impacts on population divergence. Elevation or lake surface area alone was identified as the major variable of influence for the other models involving these two variables separately. The null model (no effect from either model variable) was the most probable ($P = 0.75$) when only conductivity and mean lake depth were considered as predictors of population divergence.

These same four key landscape attributes showed varied degrees of correlation with the first six principal coordinates that were extracted from a converted, pairwise, individual-by-individual genetic distance matrix (Peakall and Smouse 2006) (Table 4-7). Significant correlations ($P < 0.05$) were detected between all six principal coordinate axes, which reflected patterns of genetic distinctiveness among all sampled individuals, and both elevation, and lake surface area. The strongest correlations, however, existed between elevation and PCO3 (Spearman rank order correlation; $r = 0.56$; $P < 0.05$), and between lake surface area and PCO1 (Spearman rank order correlation; $r = 0.40$; $P < 0.05$) (Figure 4-6). These two principal coordinate axes accounted for 18% and 24% of the variation explained by the first six axes output by the GENALEX program, respectively. Correlations between the coordinate axes and both lake conductivity and

mean lake depth were generally weaker ($-0.14 < r < 0.26$), and although some were significant ($P < 0.05$), these trends indicated a stronger influence from elevation and lake surface area on the degree of genetic distinctiveness among sampled lake trout.

Impacts from population supplementation

The impact from supplemental stocking on genetic diversity, genetic distinctiveness and population divergence was assessed using two different approaches. There was no significant difference (Mann-Whitney U tests; all $P > 0.05$) in any of the population genetic diversity estimates (H_E , N_A , or A_R) between unstocked ($N = 10$) and stocked ($N = 6$) lake trout populations. The only significant correlations detected were between stocking effort and H_E (Spearman rank-order correlation; $r = -0.83$; $P < 0.05$), and between stocking effort and N_A (Spearman rank order correlation; $r = -0.83$; $P < 0.05$), as the correlation between stocking effort and A_R was slightly weaker (Spearman rank-order correlation; $r = -0.70$; $P = 0.07$). Together, the minimal categorical differences in genetic diversity statistics, as well as the negative correlation of stocking effort with all three estimators, indicated that the stocked study lakes did not show diversity measures characteristic of populations that have been homogenized by introgressive admixture with stocked hatchery strains (Halbisen and Wilson in press).

Key landscape attributes were also evaluated for all sampled lakes to determine whether local supplemental stocking was historically biased by lake surface area, elevation, conductivity, mean lake depth, or total fish species present. Although median values for most of these attributes (excluding elevation) were larger in stocked lakes, only

conductivity was significantly different ($P < 0.05$) between unstocked ($N = 16$) and stocked lakes ($N = 11$) (Mann-Whitney U test; $Z = 2.17$; $P = 0.03$), as the median conductivity of unstocked lakes (median = 28 mg/L; range: 17-46 mg/L) was slightly lower than stocked lakes (median = 30 mg/L; range: 27-53 mg/L). Correlations between stocking effort and these landscape attributes were relatively weak and not significant (Spearman rank-order correlations; $-0.32 < r < 0.15$; $P \geq 0.35$), with the exception of the negative lake surface area correlation. This correlation was relatively stronger but not significant (Spearman rank order correlations; $r = -0.57$; $P = 0.07$), indicating a weak bias towards heavier stocking into the smaller study lakes.

Supplemental stocking impact on genetic distinctiveness and population divergence was evaluated by further categorical comparisons and correlational analyses. Small, significant differences (Mann-Whitney U tests; all $P < 0.05$) were detected between all unstocked ($N = 437$) and stocked lakes ($N = 293$) for three of the six extracted principal coordinate axes (PCO1, PCO4, and PCO6). Three of the six principal coordinates (PCO3, PCO4, and PCO5) also showed relatively weak ($r = 0.41, -0.14,$ and 0.19 , respectively) but significant correlations ($P < 0.05$) with stocking effort in stocked lakes. In contrast pairwise, population-level divergence estimates between inland lake populations and the Lake Manitou population (i.e., F_{ST} versus Lake Manitou) were not significantly different (Monte Carlo analysis; $D_{AVG} = 0.042$; $P = 0.14$) between unstocked ($N=10$) and stocked populations ($N=6$). Divergence estimates for unstocked populations (median F_{ST} versus Lake Manitou = 0.129; range: 0.079-0.266) were higher than stocked populations (median F_{ST} versus Lake Manitou = 0.171; range: 0.061-0.221), however. Finally, a positive correlation was detected between pairwise divergence

estimates and stocking effort (Spearman rank order correlation; $r = 0.70$; $P = 0.06$), which indicated that the more heavily supplemented study populations were still more divergent than populations receiving less supplementation, presumably due to in part to the weak, negative correlation between stocking effort and lake surface area (i.e., divergent populations resided in smaller lakes that were stocked more heavily).

Discussion

Relative importance of postglacial and modern landscapes

Natural population processes associated with a dynamic, regional postglacial history and variable contemporary landscape features had differential impacts on sampled inland lake trout populations. Divergence following colonization was high among all populations, supporting the first overall expectation that present-day landscape attributes had stronger impact on genetic structure than postglacial or human-mediated events. However, both the segregated distribution of Atlantic-B mtDNA haplotypes near the Fossmill outflow channel, and the weak but detectable hierarchical partitioning of microsatellite variation indicated that the transient postglacial landscape had a role in patterning spatial genetic variation. Although not completely separable, evidence of human impacts through supplemental stocking was weaker, and indicated little if any genetic impact relative to other studies involving stocked lake trout populations (Chapters 2 and 3, Guinand et al. 2003; Page 2005). Together, the combined landscape genetic

analyses indicated that the sampled lakes support allopatric populations with effectively no genetic exchange within or among contemporary drainages.

Lake surface area and elevation had the strongest effects on the genetic attributes of sampled populations. Lake trout from smaller lakes showed lower levels of genetic diversity and were more divergent from other populations. Smaller lakes probably received fewer founding individuals during colonization, which would have limited initial genetic diversity levels. This conclusion is supported by previous theoretical studies (MacArthur and Wilson 1967) that predict fewer founders will colonize smaller "island" habitats, and empirical analyses that have shown lake surface area is positively correlated with the number of fish species present (Barbour and Brown 1974; Minns 1989). Genetic drift is also accelerated in small populations (Frankham et al. 2002), and since lake surface area is proportional to carrying capacity (Shuter et al. 1998), lake trout population size in these lakes may have been constrained since postglacial colonization. Alternatively, if small lakes hosted dense populations of small lake trout maturation may have occurred earlier (Winemiller 2005), which would have shortened generation times and accelerated genetic drift. Higher elevation populations also had lower levels of diversity and higher degrees of divergence, again presumably because fewer founders had opportunities for colonization of higher altitude lakes (Minns 1989).

Contrary to expectations, two of the indicators for lake productivity, conductivity (TDS- total dissolved solids; Shuter et al 1998) and lake depth (Ryder 1965) showed no strong association with genetic diversity, and only a weak association with genetic divergence, as indicated by correlations between extracted principal coordinate axes and all four landscape features. A weak positive influence of conductivity on genetic distance

estimates ($F_{ST}/1-F_{ST}$) was also revealed by model selection on multiple linear regression models from Mantel tests, but not supported by other logistic regression model probabilities estimated with GESTE. It is possible that value ranges for both of these productivity indicators were below thresholds necessary for detection of their effects, as all of the study lakes were relatively deep and generally showed low conductivities. For comparison, lake area had a stronger (ca. 3-fold) effect on lake trout production (i.e., equilibrium yield with fishing mortality) relative to conductivity (ca. 1.6-fold) within the context of a life-history based model for lake trout productivity that included a broader sample of Ontario lake trout populations (Shuter et al. 1998).

A model for regional postglacial colonization

The spatial distribution of mitochondrial haplotypes and hierarchical partitioning of genetic variance was consistent with at least two regional postglacial colonization events (Figure 4-7). The first wave of colonization began sometime after 14-12 KYA (Wilson and Hebert 1998), but probably did not reach the Algonquin Park area until glaciers retreated north of the area between 11.6 and 11 KYA (Danzmann and Ihssen 1995; Mandrak and Crossman 2003). Lake trout could have used multiple means to access newly formed lakes from the south and west during this period of glacial recession (Dyke and Prest 1987; Mandrak and Crossman 1992). Historical waterway connections included turbid periglacial lakes that formed along melting glacial margins and cold stream networks that drained to larger outflows from the early, proglacial Great Lakes to the west (Dyke and Prest 1987; Wilson and Mandrak 2004). These first lake trout

colonists presumably would have originated from a Mississippian refuge because the saline Champlain Sea served as a large-scale barrier to lake trout dispersal from eastern refugia between 11 and 9.8 KYA (Khan and Qadri 1971; Underhill 1986; Wilson and Hebert 1996). It is thus rather surprising then that the Fossmill outflow, which existed between 10.8 and 10 KYA and drained into the Champlain Sea (Wilson and Hebert 1996), could have facilitated a second, independent round of colonization for lake trout from an Atlantic refuge.

It is certain that other freshwater fish species that colonized lakes in Algonquin Park originated from eastern glacial refuges. Both the fallfish (*Semolitus corporal*); (Scott and Crossman 1973) and the round whitefish (*Prosopium cylindraceum*) are common in park lakes, and both have range-wide distributions consistent with eastern postglacial origins. Phylogeographic analysis showed that the northern and southern areas of the park were colonized by brook charr from two different eastern glacial refuges (Acadian and Atlantic; Danzmann and Ihssen 1995; Danzmann et al. 1998), presumably during two successive postglacial colonization events. In contrast to the lake trout, however, higher temperature and salinity tolerances for brook charr (Power 1980) may have enabled a longer window for colonization of northern park areas by use of alternate routes including the Champlain Sea (Danzmann and Ihssen 1995).

It is also clear that species that dispersed over large geographical distances through the proglacial lake network used the Fossmill outflow for colonization of the park. The regional distribution of glacial-marine relict species (Martin and Chapman 1965) in local lakes below the 381m (1250ft) elevation contour was interpreted as evidence of regional colonization by aquatic species through an historical proglacial lake

network. Of the four crustacean species evaluated by Martin and Chapman (1965), the amphipod *Mysis diluviana* is the most common relict species in the park. *M. diluviana* shares a wide North American distribution with the lake trout, but could not disperse by swimming over great distances, and therefore required large-scale hydrological events for dispersal through proglacial lakes after the Wisconsin glaciation (Vainola et al. 1994; Audzijonyte and Vainola 2006; Dooh et al. 2006). Another relict species found in the Fossmill outflow channel, the deepwater sculpin (*Myoxocephalus quadricornis*; Cedar Lake), requires highly specialized habitat (extremely deep, cold lakes; Scott and Crossman 1973) and so would not have migrated into the park through shallow meltwater streams.

The presence of Atlantic-B haplotypes in proximity to the Fossmill outflow channel indicated that some lake trout may have immigrated from an Atlantic refuge into the proglacial Great Lakes before the Champlain Sea disappeared. The absence of these haplotypes from Ontario's inland lakes to south of Algonquin Park suggested that Atlantic lineage lake trout could not utilize Lake Algonquin's earlier west-to-east drainages (i.e., the Petawawa River; Danzmann and Ihssen 1995) for inland colonization. It is not clear what would have prevented Atlantic lake trout from using these dispersal opportunities, particularly as they could have migrated into Lake Algonquin through the Kirkfield outlet from proglacial lake Iroquois as early as 12-11 KYA (Wilson and Hebert 1996). It is possible that the first Atlantic lake trout colonists to reach proglacial Lake Algonquin could have used an unknown, transitory dispersal route to circumvent the Champlain Sea. However, if this was the case, it seems evident that they did not have full access to the upper proglacial Great Lakes before formation of the Fossmill outflow,

perhaps as a consequence of sequestration in a transitional 'staging' area until approximately 10.8-10 KYA.

A more parsimonious model for regional colonization suggests that Atlantic lake trout were able to cross the Champlain Sea and migrate upstream through the Fossmill outflow, in spite of their low salinity tolerance and the difficulty of swimming against strong outflow currents (Figure 4-7). Fossil invertebrate associations indicated that the salinity of the Champlain Sea was non-uniform, and lower in shallower depths (Rodrigues 1988). Isotopic analyses of fossil molluscs (Hillaire-Marcel 1988) also showed that there was salinity gradient in the Champlain Sea, and that it decreased exponentially from 20-30‰ at depths greater than 50m to 4-20‰ at surface water levels. Surface salinities in this range could have been permissive for lake trout dispersal, as lake trout can tolerate salinity levels below 10-13‰ (Martin and Olver 1980). Large-scale migration events may have been limited, however, as this historical gradient was spatially variable (Hillaire-Marcel 1988; Rodrigues 1988) and resulted from inflows of low-salinity glacial meltwater from many different sources (Sharpe 1988). In contrast, strong currents from high discharge volumes in the Fossmill outflow may not have been particularly restrictive for lake trout movements, as they are strong swimmers (Martin and Olver 1980; Gunn and Pitblado 2004) unlike other aquatic species whose dispersal ability could be reduced by similar hydrological conditions (Bodaly and Lindsey 1977; Lindsay and McPhail 1986). Together these historical conditions, and the broader spatial distribution of Atlantic mitochondrial haplotypes in eastern North America, support the possibility that the Champlain Sea provided a direct route for lake trout colonization of Algonquin Park lakes (Wilson and Hebert 1996, 1998).

Supplemental stocking and native genetic characteristics

Supplemental stocking with Great Lakes hatchery strains was historically a common practice in Ontario. However, this practice has been phased out as more recent studies showed that stocking pre-existing populations is ineffective (Martin and Fry 1972; MacLean et al. 1981; Gunn et al. 1990; Powell and Carl 2004), and detrimental to native inland lake trout (Evans and Willox 1991). Indigenous lake trout can be rapidly replaced with hatchery-reared individuals, whose presence can lead to introgressive admixture and homogenization of native gene pools. These negative effects can be amplified by other human activities, as stocked inland lakes are typically subjected to higher degrees of recreational harvest (Evans and Olver 1995).

None of the stocked populations in Algonquin Park showed genetic characteristics consistent with widespread introgression and admixture with hatchery strain lake trout, in contrast to other stocked populations in southern Ontario (Halbisen and Wilson in press). This is probably because stocked lake trout survival was low, as indicated by long-term studies of stocking success in Lake Opeongo (Martin and Fry 1972; MacLean et al. 1981). Even so, it is possible that some stocked fish have contributed to the genetic variation of park populations.

Patterns of lower proportional individual re-assignment indicated potential for weak population structure or recent introgressive admixture, following historical hatchery strain stocking, within both Cedar Lake and Lake Kioshkokwi. There were multiple lines of evidence against these possibilities, however. Neither population showed a significant

heterozygote deficit, which indicated that there was no recent admixture or population sub-structure. Both populations were also significantly differentiated (F_{ST}) from one another and the Great Lakes hatchery strain (i.e., Lake Manitou).

Historical stocking was relatively light for these lakes, and less intensive than for other stocked populations with an established native genetic profile (e.g., Smoke Lake and Kingscote Lake; [Halbisen and Wilson in press]). Even in these relatively heavily stocked populations the genetic contribution from historical stocking seemed to be limited to a few individuals that showed genetic similarities to individuals of Great Lakes origin. For comparison, introgressed inland populations elsewhere in southern Ontario were essentially indistinguishable from Great Lakes lake trout (Halbisen and Wilson 2008). In consideration of these post-stocking genetic patterns, it seems more plausible that the moderate genetic similarity of the Cedar and Kioshkokwi populations to each another and the population from the Great Lakes (Manitou strain) was due to postglacial colonization rather than recent hatchery-strain stocking.

It is clear that the lake trout populations proximal to the Fossmill outflow (Cedar Lake, Lake Kioshkokwi, Gilmour Lake, White Partridge Lake, Hogan Lake, Radiant Lake, and Little Dickson Lake) are genetically distinct from other native populations in southern Ontario of Mississippian-A glacial ancestry. Consequently these populations would serve as inappropriate sources for most local-strain rehabilitative stocking programs in southern Ontario. Strong consideration should also be given towards matching the ecological attributes of donor and recipient populations during rehabilitative stocking to increase the chances that stocked fish share evolved traits essential for long-term survival (OMNR 2002; DFO 2003). For future studies, it will be important to

consider the effects of exploitation, which is typically associated with inland supplemental stocking (Evans and Willox 1991), on quantitative genetic variation as this human-mediated activity has known negative impacts on adaptive characteristics (Moran 2002; Kuparinen and Merila 2007).

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Table 4-1

Attributes for sampled lake trout populations showing sample sizes (N), location, drainages, sampling dates, lake attributes (surface area, elevation above sea level, conductivity, and mean depth), elevation category above or below the 381m elevation contour indicating presence or absence of glacial-marine relict species (GMR), and stocking history (Stk.- the population is unstocked [0] or has been stocked [1], Stk. No. - number of lake trout stocked, and Stk. Eff - stocking effort; defined in the methods section).

No.	Origin	N	Lat.	Long.	Drainage	Sampling dates	Area (ha)	Elev. (m)	Cond. (mg/L)	Mean depth (m)	GMR	Stk.	Stk. no.	Stk.
													(x1000)	Off.
Samples included for population-level analyses														
1	Lake Manitou (Great Lakes)	43	45°46'	81°59'	Lake Huron	2003	10800	176	160	15	-	1	100+	-
2	Cedar Lake	36	46°01'	78°28'	Petawawa	2006	2543	307	30	14.1	1	1	21.25	0.137
3	Lake Kioshkokwi	47	46°04'	78°53'	Amable du Fond	2006	1127	303	46	12.5	1	1	9.25	0.135
4	Lavieille Lake	43	45°52'	78°14'	Petawawa	2006	2426	386	27	14.4	0	0		
5	Dickson Lake	25	45°46'	78°12'	Petawawa	2005	975	396	28	5.5	0	0		
6	White Partridge Lake	37	45°50'	78°06'	Petawawa	2007	574	352	34	15.3	1	0		
7	Hogan Lake	31	45°52'	78°29'	Petawawa	2005-2006	1303	381	30	6.7	1	0		
8	Lake LaMuir	36	45°49'	78°35'	Petawawa	2006	757	401	28	10.4	0	0		
9	Big Trout Lake	40	45°45'	78°37'	Petawawa	2006	1519	403	22	8.3	0	0		
10	Happy Isle Lake	34	45°44'	78°30'	Madawaska	2006	536	439	23	13	0	0		
11	Opeongo Lake	46	45°42'	78°22'	Madawaska	2003	5154	404	28	14.6	0	1	171.71	0.546
12	Kingscote Lake	49	45°12'	78°13'	Madawaska	1999	214	421	29	7.5	0	1	88.65	6.791
13	Louisa Lake	47	45°28'	78°28'	Madawaska	2001	489	441	24	17	0	0		
14	Big Porcupine Lake	37	45°27'	78°36'	Oxtongue	2005	235	488	46	7.5	0	0		
15	Timberwolf Lake	46	45°40'	78°48'	Petawawa	2005	168	427	17	7	0	0		
16	Smoke Lake	47	45°30'	78°40'	Oxtongue	2004-2005	607	422	28	16.2	0	1	71.08	1.920
17	Lost Dog Lake	26	45°57'	79°05'	Amable du Fond	1999	50	385	29	11.1	0	1	6.20	2.033

Table 4-1., cont.

No.	Origin	N	Lat.	Long.	Drainage	Sampling dates	Area (ha)	Elev. (m)	Cond. (mg/L)	Mean depth (m)	GMR	Stk. (x1000)	Stk. no.	Stk. Off.
Samples included for individual-level analyses														
18	Radiant Lake	1	45°59'	78°17'	Petawawa	2006	643	279	35	7.7	1	1	1	
19	Gilmour Lake	7	46°04'	78°29'	Petawawa	2006	165	320	40	7.6	1	1	10.00	0.255
20	Little Dickson Lake	21	45°48'	78°10'	Petawawa	2006	118	415	24	7.3	0	0	4.00	0.397
21	Big Crow Lake	8	45°49'	78°26'	Petawawa	2006	440	407	32	8.2	0	0		
22	Burnt Island Lake	8	45°38'	78°38'	Oxtongue	2006	854	427	53	10.8	0	1		
23	Ragged Lake	11	45°28'	78°38'	Oxtongue	2006	629	436	27	5.8	0	1	6.80	0.131
24	Catfish Lake	9	45°56'	78°33'	Petawawa	2006	641	387	27	5.2	0	0	4.00	0.104
25	Blue Lake	7	45°46'	78°40'	Petawawa	2006	44	400	26	9.4	0	0		
26	Merchant Lake	10	45°46'	78°31'	Petawawa	2006	412	438	28	8.9	0	0		
27	Pen Lake	15	45°27'	78°22'	Madawaska	2006	379	402	33	9.2	0	0		
28	Delano Lake	6	45°30'	78°35'	Madawaska	2006	26	457	41	6.6	0	0	30.50	1.319

Table 4-2

Proportional assignment of all sampled individuals ($N=773$ total) to genetic clusters resolved with BAPS 5.1. Bold values indicate the clusters with highest proportion of assigned individuals for each population of origin.

Lake of origin	N	Assigned genetic cluster													
		C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14
Lake Manitou (Gr. Lakes)	43	0.47	0.02	0.16	-	-	-	-	-	-	-	-	-	-	-
Cedar Lake	36	0.06	0.58	-	-	-	-	-	-	-	0.03	-	-	-	-
Lake Kioskokwi	47	-	0.04	0.38	0.02	-	-	-	-	-	0.04	-	-	0.02	-
Lavieille Lake	43	-	-	-	0.74	-	-	-	-	-	0.02	-	-	-	-
Dickson Lake	25	-	-	-	-	0.96	-	-	0.04	-	-	-	-	-	-
White Partridge Lake	37	0.03	-	-	-	-	0.84	-	-	-	-	-	-	-	-
Hogan Lake	31	-	-	-	-	-	-	0.94	0.03	-	-	-	-	-	-
Lake LaMuir	36	-	-	-	-	-	-	1.00	-	-	-	-	-	-	-
Big Trout Lake	40	0.05	0.05	-	-	-	-	-	0.78	-	0.03	-	-	-	-
Happy Isle Lake	34	-	-	-	-	-	-	-	-	0.88	-	-	-	-	-
Opeongo Lake	46	0.09	-	-	-	-	0.02	-	-	-	0.83	-	-	-	-
Kingscote Lake	49	0.06	0.04	-	-	-	-	-	-	-	0.04	0.82	-	-	-
Louisa Lake	47	-	0.02	-	-	-	-	-	-	-	-	-	0.91	0.04	-
Big Porcupine Lake	37	-	-	-	-	-	-	-	-	-	-	-	-	1.00	-
Timberwolf Lake	46	-	-	-	-	-	-	-	-	-	-	-	-	0.04	-
Smoke Lake	47	-	-	-	-	-	-	-	-	-	0.04	-	-	-	-
Lost Dog Lake	26	-	0.04	-	-	-	-	-	-	-	-	-	-	-	-
Radiant Lake	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gilmour Lake	7	-	-	-	-	-	-	0.29	-	-	0.14	-	-	-	-
Little Dickson Lake	21	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Big Crow Lake	8	-	-	-	-	-	-	-	-	-	0.25	-	-	-	-
Burnt Island Lake	8	-	-	-	0.13	-	-	-	-	-	-	-	-	-	-
Ragged Lake	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catfish Lake	9	-	-	0.11	-	-	-	-	-	-	0.11	-	-	-	-
Blue Lake	7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Merchant Lake	10	-	0.90	0.10	-	-	-	-	-	-	-	-	-	-	-
Pen Lake	15	-	-	-	-	-	-	-	-	-	-	0.07	-	-	-
Delano Lake	6	-	-	-	-	-	-	-	-	-	-	0.50	-	-	-

Table 4-2. cont.

Lake of origin	Assigned genetic cluster													
	C15	C16	C17	C18	C19	C20	C21	C22	C23	C24	C25	UAS	ADX	
Lake Manitou (Gr. Lakes)	43	-	-	0.07	0.07	-	0.09	-	-	0.07	-	0.05	-	
Cedar Lake	36	-	-	0.22	-	-	0.11	-	-	-	-	-	-	
Lake Kioskokwi	47	-	-	0.09	0.36	-	0.02	-	-	-	-	-	0.02	
Lavielle Lake	43	-	-	-	-	-	0.19	-	-	-	-	0.02	0.02	
Dickson Lake	25	-	-	-	-	-	-	-	-	-	-	-	-	
White Partridge Lake	37	-	-	0.08	-	-	-	-	-	-	-	0.03	0.03	
Hogan Lake	31	-	-	-	-	-	-	-	-	-	-	0.03	-	
Lake LaMuir	36	-	-	-	-	-	-	-	-	-	-	-	-	
Big Trout Lake	40	-	-	0.05	-	-	-	-	-	-	0.03	0.03	-	
Happy Isle Lake	34	-	-	0.03	0.06	-	-	-	-	-	-	-	0.03	
Opeongo Lake	46	-	-	0.02	-	-	-	-	-	-	-	0.04	-	
Kingscote Lake	49	-	-	0.04	-	-	-	-	-	-	-	-	-	
Louisa Lake	47	-	-	-	-	-	-	-	-	-	-	-	0.02	
Big Porcupine Lake	37	-	-	-	-	-	-	-	-	-	-	-	-	
Timberwolf Lake	46	0.96	-	-	-	-	-	-	-	-	-	-	-	
Smoke Lake	47	-	0.89	-	-	-	0.02	-	-	-	-	0.02	0.02	
Lost Dog Lake	26	-	0.96	-	-	-	-	-	-	-	-	-	-	
Radiant Lake	1	-	-	1.00	-	-	-	-	-	-	-	-	-	
Gilmour Lake	7	-	-	-	0.57	-	-	-	-	-	-	-	-	
Little Dickson Lake	21	-	-	-	-	1.00	-	-	-	-	-	-	-	
Big Crow Lake	8	-	-	-	-	-	0.75	-	-	-	-	-	-	
Burnt Island Lake	8	-	-	-	-	-	0.88	-	-	-	-	-	-	
Ragged Lake	11	-	0.64	-	-	-	0.18	-	-	-	-	0.09	0.09	
Catfish Lake	9	-	-	0.78	-	-	-	-	-	-	-	-	-	
Blue Lake	7	-	-	-	-	-	-	1.00	-	-	-	-	-	
Merchant Lake	10	-	-	-	-	-	-	-	-	-	-	-	-	
Pen Lake	15	-	-	-	-	-	0.07	-	0.87	-	-	-	-	
Delano Lake	6	-	-	-	-	-	0.17	-	-	-	-	0.17	0.17	

Table 4-3

Hierarchical analysis of microsatellite molecular genetic variance (AMOVA) for populations grouped by postglacial (Elevation category) or recent (Drainage) hydrological associations. Drainage categories are given in Table 1, as well as elevation categories (GMR; above or below the 381m elevation contour)

Grouping	Source of variation	df	Variance component	% Variation	Fixation index	<i>P</i>
Elevation category	Among groups	1	0.060	1.92	$F_{CT} = 0.019$	0.06
	Among pops within groups	14	0.605	19.51	$F_{SC} = 0.199$	< 0.0001
Drainage	Within populations	1238	2.436	78.57	$F_{ST} = 0.214$	< 0.0001
	Among groups	3	-0.035	-1.15	$F_{CT} = -0.011$	0.79
	Among pops within groups	12	0.653	21.39	$F_{SC} = 0.202$	< 0.0001
	Within populations	1238	2.436	79.76	$F_{ST} = 0.211$	< 0.0001

Table 4-4

Pairwise population divergence estimates (F_{ST}) for population samples. All values are significant ($P=0.01$; $k=136$).

Population	Abbr.	LMN	CED	KIO	LAV	DKS	WPR	HOG	LAM	BGT	HPI	OPE	KSG	LOU	BPC	TWF	SMK	LDG
Lake Manitou (Great Lakes)	LMN	-																
Cedar Lake	CED	0.061	-															
Lake Kioskokwi	KIO	0.085	0.114	-														
Lavieille Lake	LAV	0.095	0.131	0.122	-													
Dickson Lake	DKS	0.167	0.166	0.172	0.159	-												
White Partridge Lake	WPR	0.116	0.125	0.120	0.197	0.201	-											
Hogan Lake	HOG	0.175	0.163	0.125	0.173	0.185	0.170	-										
Lake LaMuir	LAM	0.189	0.184	0.173	0.226	0.276	0.257	0.258	-									
Big Trout Lake	BGT	0.079	0.092	0.126	0.137	0.159	0.111	0.175	0.223	-								
Happy Isle Lake	HPI	0.121	0.165	0.217	0.152	0.237	0.224	0.257	0.275	0.156	-							
Opeongo Lake	OPE	0.072	0.113	0.128	0.084	0.096	0.176	0.166	0.193	0.112	0.124	-						
Kingscote Lake	KSG	0.140	0.112	0.124	0.193	0.236	0.219	0.248	0.147	0.168	0.237	0.164	-					
Louisa Lake	LOU	0.255	0.214	0.188	0.233	0.224	0.258	0.188	0.278	0.214	0.310	0.219	0.188	-				
Big Porcupine Lake	BPC	0.247	0.164	0.221	0.255	0.263	0.276	0.262	0.270	0.233	0.297	0.221	0.154	0.166	-			
Timberwolf Lake	TWF	0.266	0.288	0.186	0.254	0.278	0.308	0.278	0.307	0.269	0.381	0.272	0.209	0.176	0.324	-		
Smoke Lake	SMK	0.194	0.209	0.227	0.142	0.226	0.288	0.226	0.300	0.192	0.212	0.127	0.248	0.222	0.268	0.334	-	
Lost Dog Lake	LDG	0.221	0.203	0.201	0.204	0.215	0.234	0.116	0.307	0.227	0.285	0.190	0.285	0.233	0.262	0.345	0.230	-

Table 4-5

Model selection on regression models using residuals from full and partial Mantel tests. Abbreviations are as follows: number of parameters including intercept and error term (K), full or partial correlation coefficient (Corr), sum-of-squares error for each regression model (SSE), corrected AIC score (AIC_C), difference between model and minimum AIC_C (Δ_i), relative Akaike weight for each model (w_i). P -values calculated for partial Mantel models were omitted owing to difficulties in accurately estimating probabilities using randomization-based tests (Raufaste and Roussett 2002).

Model	K	Model r^2	P	Corr.	SSE	AIC _c	Δ_i	w_i
Surface area	3	14.66	0.0001	-0.38	1.055	-561.86	0.00	0.68
Conductivity	3	1.48	0.18	0.12	1.218	-544.63	17.23	0.00
Mean Depth	3	0.27	0.57	0.05	1.233	-543.16	18.70	0.00
Elevation	3	0.05	0.81	-0.02	1.236	-542.90	18.96	0.00
Surface area + Conductivity	4	14.83	-	-0.38 0.04	1.053	-559.95	1.90	0.26
Elevation + Surface area + Conductivity + Mean Depth	6	15.85	-	-0.02 -0.38 0.06 0.09	1.040	-557.00	4.85	0.06

Table 4-6

Summarized output from GESTE (Foll and Gaggiotti 2006). Posterior probabilities are shown for all possible models; bold italics indicate the most probable models for each series. Inclusion of the variables (V1, V2), the regression constant (C), or the interaction term (I) into each model is indicated; the null model uses only the constant.

		Posterior probabilities by model								
Variable 1	Variable 2	1	2	3	4	5	6	7	8	9
		C	VI	C+VI	V2	C+V2	VI+V2	C+VI+V2	VI+V2+I	ALL
Area	Elevation	0.22	0	0.30	0	0.34	0	0.14	0	0.01
Area	Conductivity	0.33	0	0.57	0	0.04	0	0.06	0	0.01
Area	Mean Depth	0.14	0	0.74	0	0.03	0	0.06	0	0.03
Elevation	Conductivity	0.36	0	0.55	0	0.05	0	0.03	0	0
Elevation	Mean Depth	0.28	0	0.61	0	0.06	0	0.06	0	0
Conductivity	Mean Depth	0.75	0	0.15	0	0.08	0	0.01	0	0

Table 4-7

Spearman rank-order correlations showing associations between extracted principal coordinates (genetic distinctiveness) and landscape attributes ($*P < 0.05$). Percent cumulative variance (% Cum.) is for the first six principal axes only.

PCo	% Cum.	Elevation (m)	Surface area (ha)	Cond. (mg/L)	Mean depth (m)
1	24%	-0.09*	0.40*	0.01	0.22*
2	44%	-0.13*	0.16*	-0.05	0.25*
3	62%	0.56*	-0.31*	-0.02	0.04
4	79%	-0.11*	0.20*	-0.14*	0.06
5	91%	0.37*	-0.10*	0.13*	0.15*
6	100%	0.19*	-0.11*	0.26*	-0.10*

Table 4-8

Statistical comparisons using genetic diversity estimates, landscape attributes, principal coordinates, and population differentiation between unstocked and stocked lakes.

Correlations in right-hand columns apply to stocked lakes only.

	Unstocked			Stocked			Stocked		
	Median	Range	Median	Range	Z	P	r	P	
Genetic diversity statistic (Population-level samples)	(N = 10)	(N = 6)	(N = 6)				(N = 6)		
H_E	0.473	0.298-0.583	0.471	0.388-0.575	0.87	0.39	-0.83	0.04	
N_A	4.86	2.36-6.45	5.86	4.45-6.73	1.36	0.18	-0.83	0.04	
A_R	4.44	2.26-5.72	5.12	4.06-5.93	1.41	0.16	-0.77	0.07	
Landscape attribute (All samples)	(N = 16)	(N = 11)	(N = 11)				(N = 11)		
Elevation (m)	405	352-488	402	279-436	-1.46	0.15	-0.02	0.96	
Surface Area (m)	513	26-2426	629	50-5154	0.69	0.49	-0.57	0.07	
TDS (mg/L)	28	17-46	30	27-53	2.17	0.03	-0.32	0.35	
Mean Depth (m)	8.3	5.2-17	10.8	5.8-16.2	1.11	0.27	0.15	0.67	
Total fish species (no.)	6.5	3-14	8	4-21	0.74	0.46	-0.28	0.40	
Ordination (Principle coordinate)	(N = 437)	(N = 293)	(N = 293)				(N = 293)		
PCo1	-0.025	-0.418-0.435	0.021	-0.278-0.381	3.11	<0.005	-0.06	0.30	
PCo2	-0.002	-0.384-0.373	0.022	-0.409-0.348	1.10	0.27	-0.06	0.29	
PCo3	0.012	-0.462-0.331	-0.003	-0.416-0.319	0.16	0.87	0.41	<0.0001	
PCo4	-0.024	-0.339-0.388	0.018	-0.32-0.302	3.18	<0.005	-0.14	0.02	
PCo5	0.018	-0.276-0.371	0.000	-0.293-0.285	-1.16	0.25	0.19	<0.005	
PCo6	0.018	-0.316-0.237	0.005	-0.31-0.187	-2.39	0.02	-0.02	0.76	
Pairwise divergence estimates (versus Lake Manitou)	Average	Range	Average	Range	D_{AVG}	P	r	P	
F_{ST}	(N = 10)	(N = 6)	(N = 6)				(N = 6)		
	0.171	0.079-0.266	0.129	0.061-0.221	0.042	0.14	0.70	0.06	

Figure 4-1.

Sampling locations of the lake trout populations examined in this study. The shaded square in the inset map shows the approximate position of the sampling area relative to the Laurentian Great Lakes. Lake names for numbered sampling locations are given in Table 1. Lakes 3, 19, 2, and 18 lie along the approximate location of main channel for the Fossmill outflow, and lead to the proglacial outwash plain which is the large, dark triangular area in the southwestern part of the park.

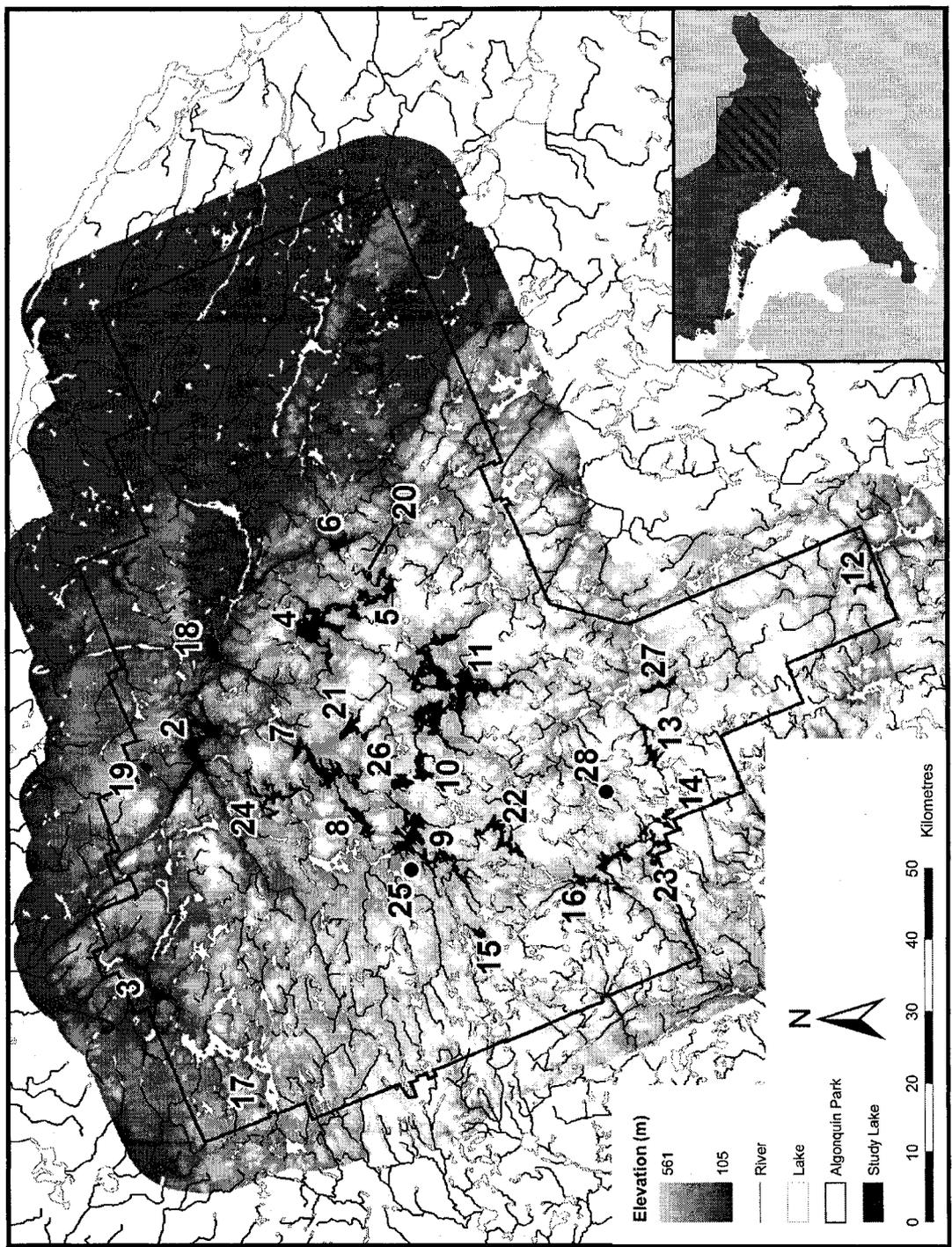


Figure 4-2

Major regional drainages for the study area. Bold arrows show approximate flow directions.

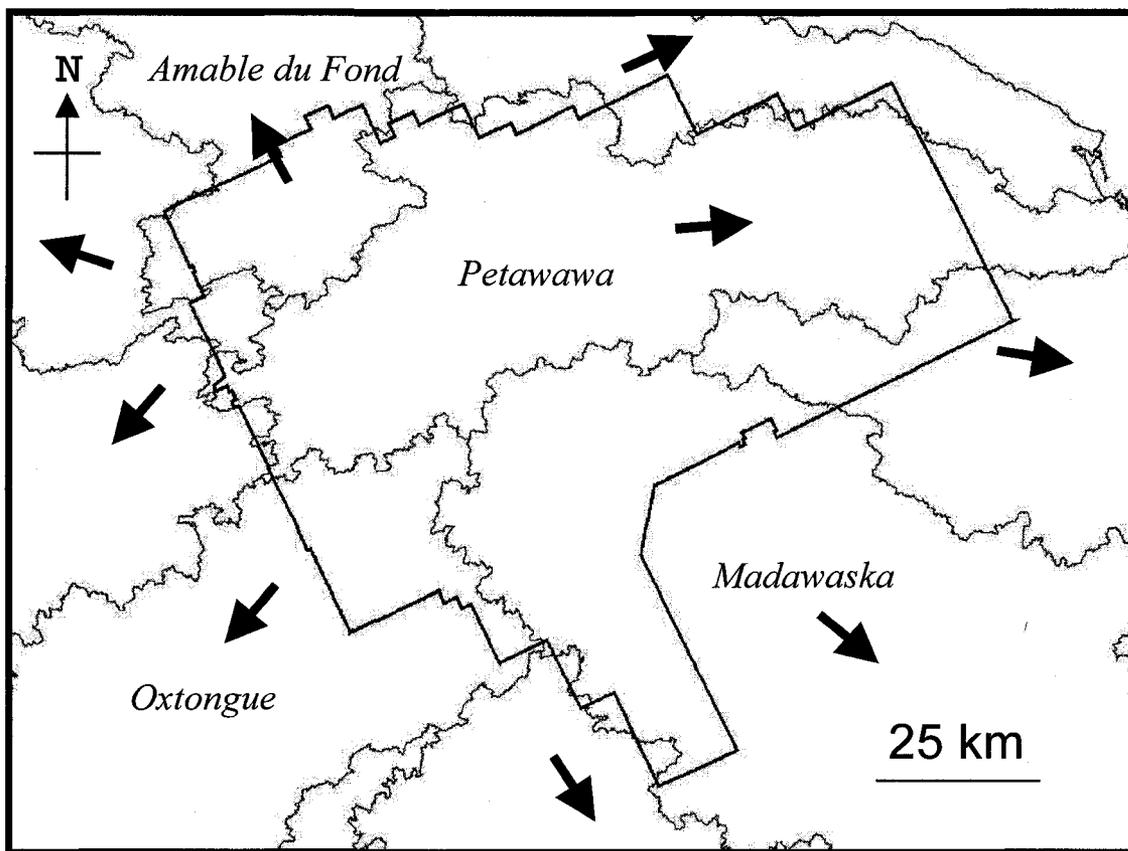


Figure 4-3

Consensus neighbour-joining dendrogram indicating genetic divergence among population samples based on Cavalli-Sforza and Edward's chord distance (D_C ; 1967). Bootstrap confidence estimates are shown for nodes with greater than 50% bootstrap support. Drainages (tertiary watershed designations) are given under lake names in italic.

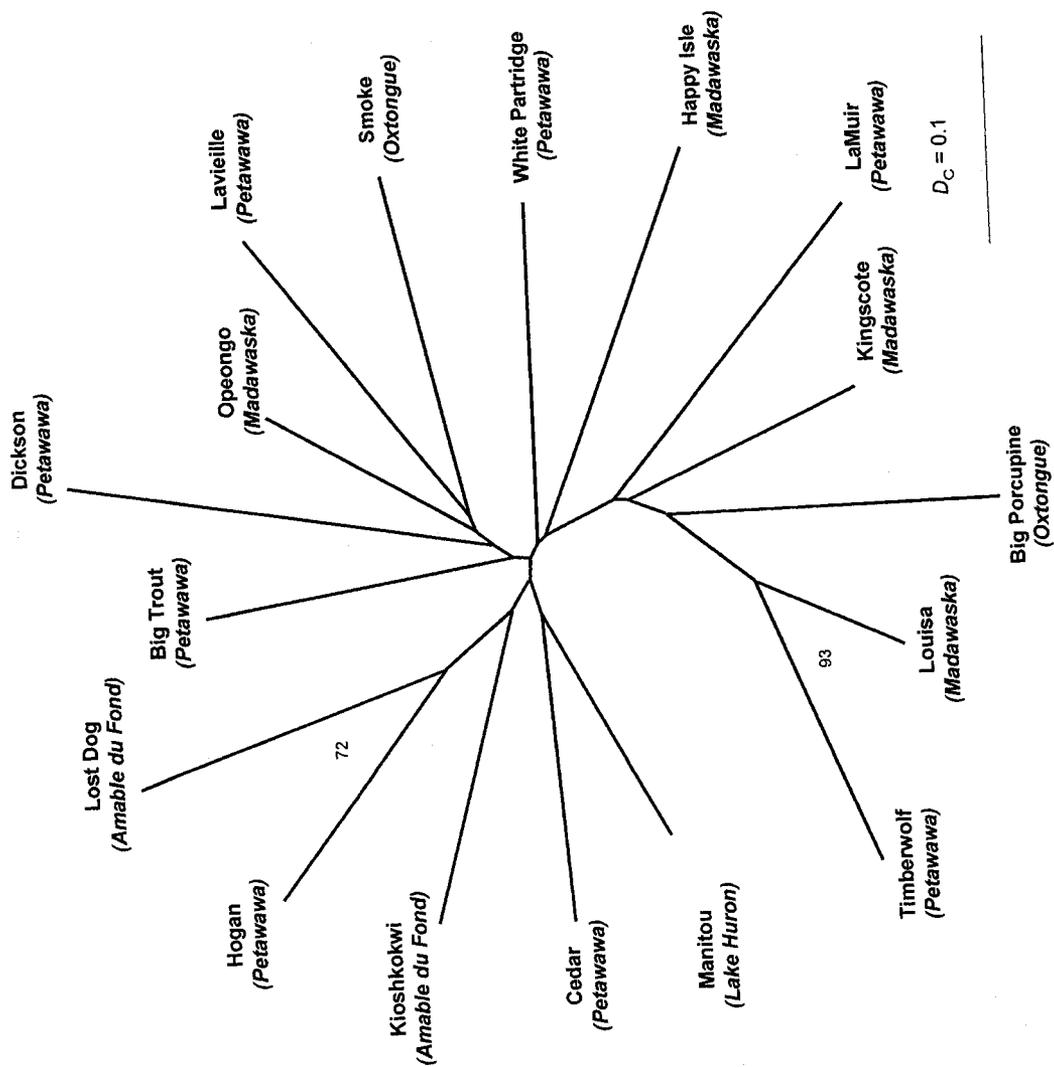


Figure 4-4

Spatial distribution of mitochondrial DNA haplotypes sampled from regional populations. Circle areas are proportional to sample size, except for Radiant Lake (*), where only a single individual was sampled. The dotted line corresponds to the approximate level of the 381m (1250 ft) contour above which no glacial relict species were detected by Martin and Chapman (1965). Mitochondrial haplotype distributions for Louisa Lake, Smoke Lake, and Kingscote Lake were originally reported in Halbisen and Wilson (in press). Designations for mitochondrial haplotypes follow Grewe et al. (1993).

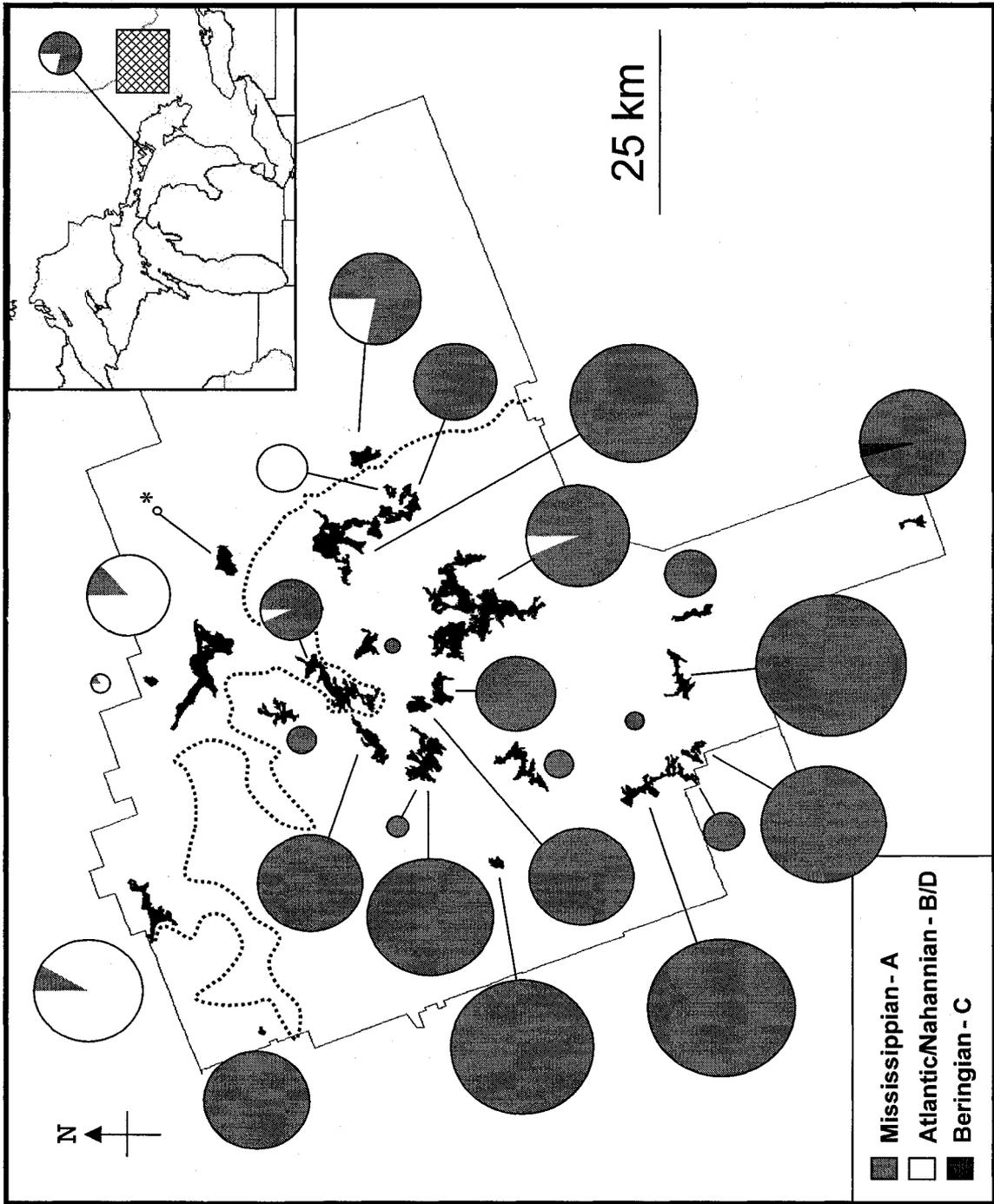


Figure 4-5

Graphs showing correlations between landscape features (elevation, lake surface area, conductivity by total dissolved solids [TDS], and mean depth) and genetic diversity estimates (gene diversity [H_E], average number of alleles per locus [N_A], and allelic richness - [A_R]) from inland lake population samples. Transformed values (\log_e) were plotted for some landscape attributes to linearize bivariate plots, but do not affect rank-order correlation coefficients.

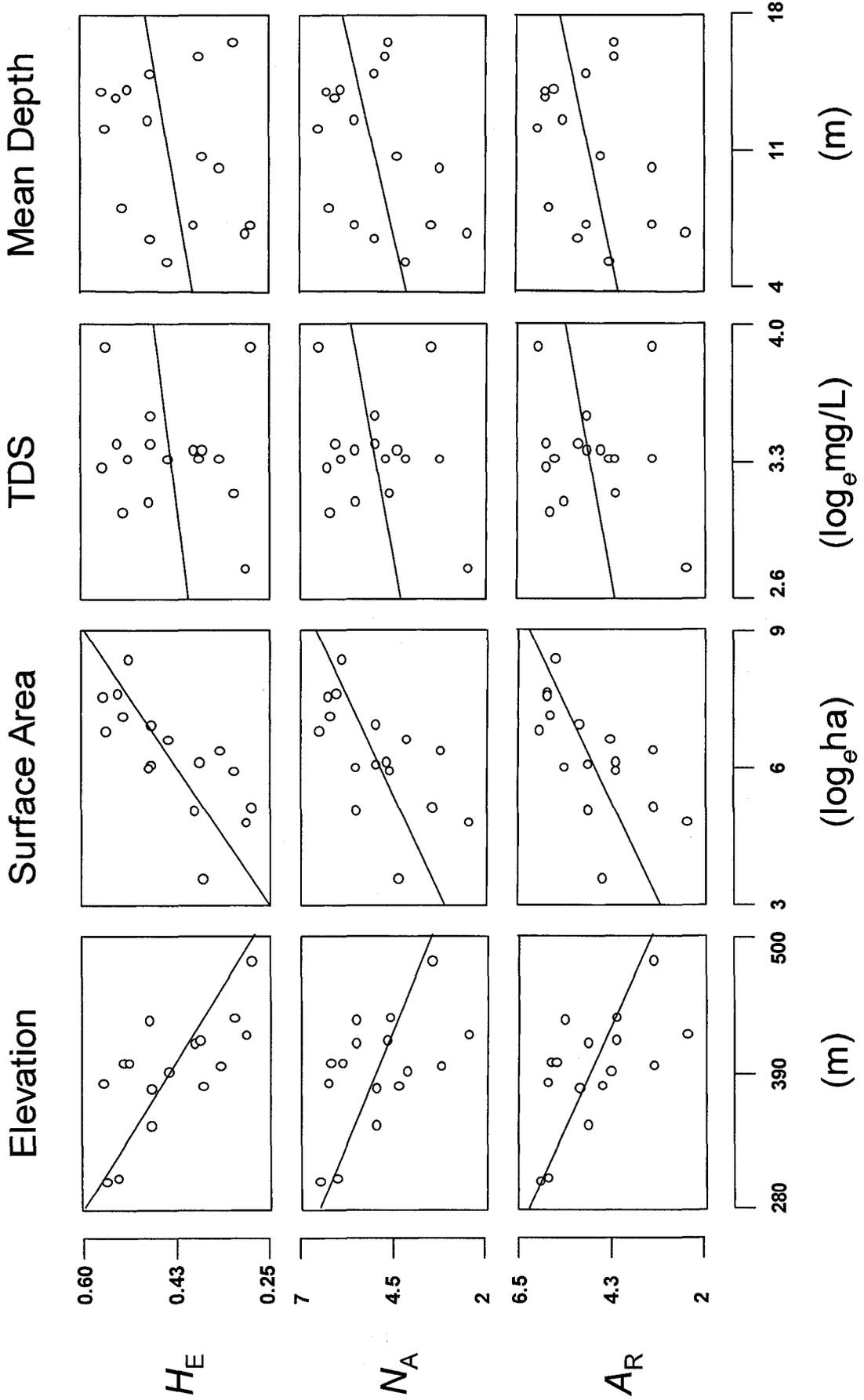


Figure 4-6

Correlation between differences in lake surface area and genetic distances revealed by a partial Mantel test for population-level samples.

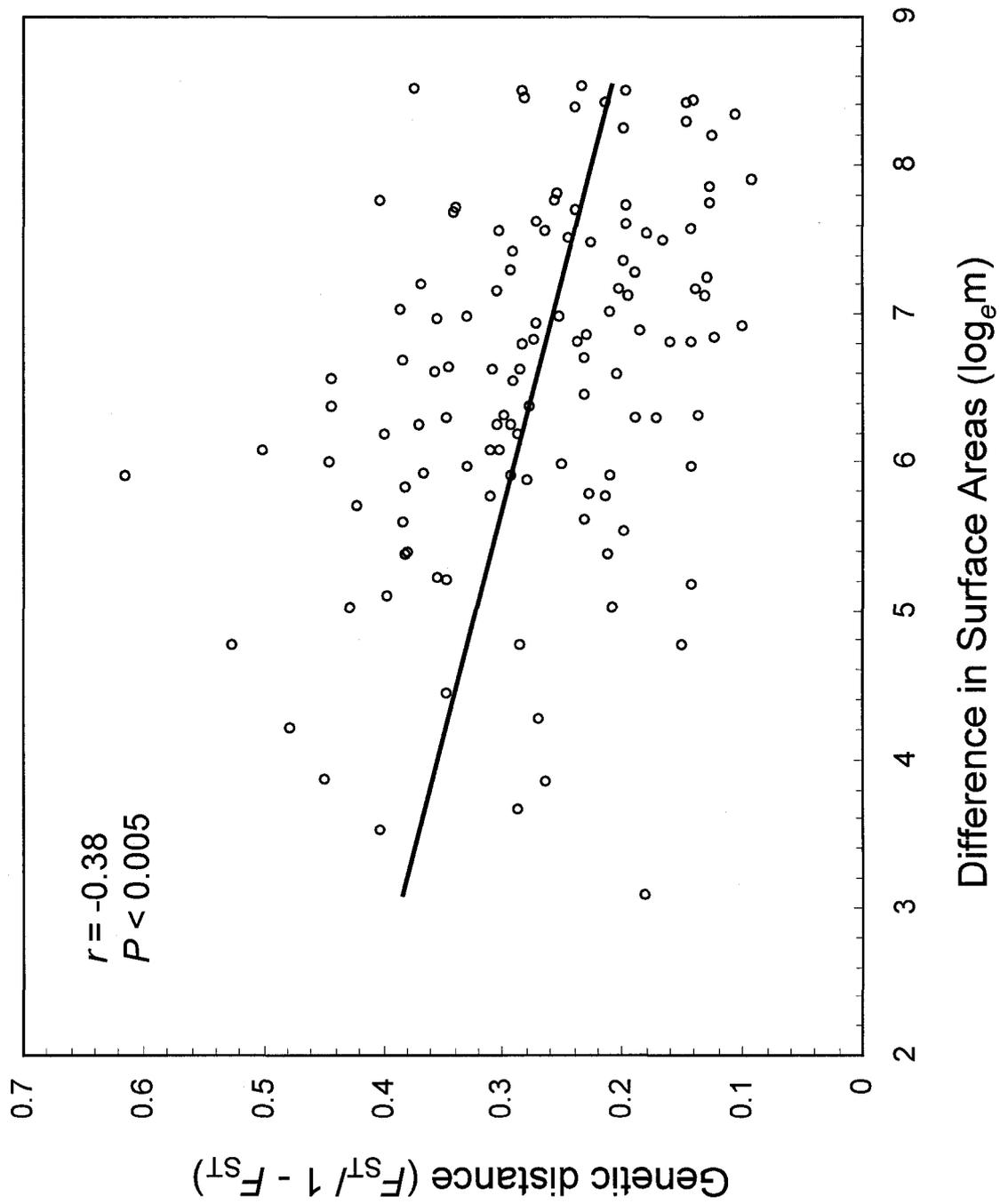
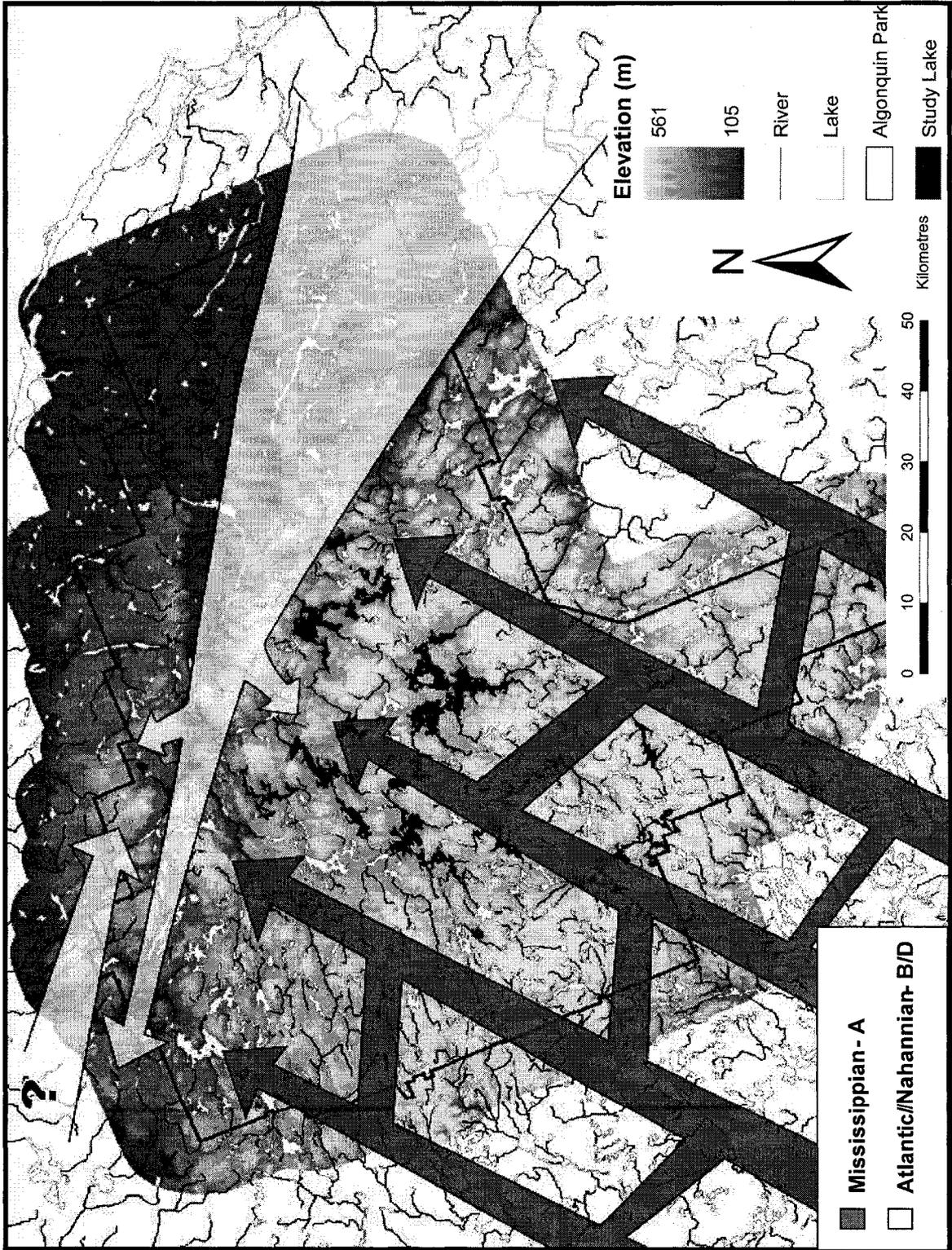


Figure 4-7

A model for regional postglacial colonization. Early colonists that dispersed from a Mississippian refuge used a network of meltwater streams to access southern and western park areas. A second round of colonization from an Atlantic refuge was enabled by the Fossmill outflow, which drained proglacial Lake Algonquin in the west. Multiple lines of evidence suggest that these lake trout migrated directly across the Champlain Sea (located to the southeast of the park), then upstream into the regional lakes. It is less probable that they used an alternative northwestern dispersal route through the proglacial Great Lakes.



CHAPTER 5**Towards a broader role for evolutionary processes and population genetics in lake
trout conservation and management**

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Abstract

Molecular genetic analyses revealed that supplemental stocking had a variable impact on the genetic attributes of stocked populations. These findings, along with resolved patterns of natural genetic variation, suggest that genetics-based lake trout management strategies need to be updated to reflect contemporary conservation genetic concepts. A practical example of how this may be accomplished is given by a comparative synthesis of previous population assessments, composite genetic profiles, and modern genetic criteria used for defining conservation units. For future analyses, possible directions towards the evaluation of adaptive genetic variation are discussed. A special-case example is given for how these studies may be initiated with existing information for lake trout populations of the Great Lakes region.

General Discussion

Summarized conclusions

Each chapter of this thesis showed that supplemental stocking with divergent hatchery strains of Great Lakes origin had variable genetic impacts. Molecular genetic analyses revealed that some inland populations have been completely homogenized by hatchery-strain stocking, whereas others appeared to retain natural genetic diversity and population structure, even after substantial hatchery strain supplementation. For some populations, it was not possible to completely partition stocking effect on genetic variation from natural evolutionary processes. Generally, however, stocking history was not strongly indicative of hatchery-strain genetic contribution into native populations, which suggests that other biological, environmental, and anthropogenic factors play a role.

Stocked lake trout populations in southern Ontario showed varied degrees of introgression with stocked hatchery strains, as expected from previous studies of stocked lake trout survival (Gunn et al. 1990; Evans and Willox 1991; Evans and Olver 1995; Powell and Carl 2004) and genetic impact (Piller et al. 2005). Genetic profiling provided a convenient method for characterizing local populations in terms of inferred ancestry (native, introgressed, or introduced), but was not concordant with all previous population assessments. Furthermore, detection of historical gene flow between evolutionarily related populations (i.e., McDonald Lake, Clean Lake, and Redstone Lake), and also

from divergent Great Lakes populations was limited by natural levels of divergence and the postglacial partitioning of intraspecific genetic variation, respectively.

Although several lines of genetic evidence indicated that the river-spawning lake trout from the Dog and Montreal Rivers originated from an indigenous Great Lakes source, it was not possible to exclude the possibility of hatchery-strain ancestry for both populations. However, the river-spawners were more genetically similar to a hatchery strain (the Ontario Ministry of Natural Resources [OMNR] Mishibishu broodstock) derived from sanctuary populations that were initially established with individuals from the Dog River, than to other Great Lakes hatchery strains and basin-spawning wild populations. Comparative analysis of mitochondrial haplotype distributions, microsatellite-based genetic structure, and ecological attributes of the historical river-spawning populations (Loftus 1958; Goodier 1981) indicated that the river-spawners had probably evolved since postglacial colonization of Lake Superior from ancestral basin-spawning lake trout. This evolutionary model, and divergence levels that indicated a degree of reproductive isolation between the basin- and river-spawning lake trout, suggested that separate management strategies would be necessary for further conservation efforts.

Genetic diversity and population genetic structure for the inland lake trout populations from Algonquin Park, Ontario primarily reflected historical demographic responses to key landscape attributes. Lake surface area and elevation above sea level had the strongest effects as they controlled the number of postglacial founders and limited possible population sizes. Indicators of lake productivity had little or no detectable effect on genetic variation. There was reasonable evidence from the spatial

distribution of mitochondrial DNA haplotypes and the hierarchical partitioning of microsatellite variation that two divergent glacial lineages colonized regional lakes through separate postglacial dispersal routes (Danzmann and Ihssen 1995; Wilson and Hebert 1996). There was no indication of contemporary genetic exchange among proximal lakes within present-day watersheds, however. Finally, statistical comparisons showed little evidence for extensive introgression in regional populations, but were limited by the natural genetic similarity of some populations to lake trout from the Great Lakes.

Together, these results are essential for modifying contemporary provincial management policy to better reflect the realities of lake trout evolutionary history and contemporary genetic structure. Some genetics-oriented management strategies are in place in Ontario, but they would be improved by incorporating modern conservation genetic criteria into supplemental stocking assessments. For demonstration, an example of how to accomplish this with composite genetic profiles from my study populations is provided in Appendix 3. In future genetic analyses, a greater priority should be placed on measuring adaptive genetic variation in wild populations. I have also provided a special-case example of how this may be accomplished with existing information on population attributes and stocking histories, and suggest future directions for continuing this research in the following sections.

Lake trout and contemporary fisheries management strategies in Ontario

Lake trout have long been an important resource fish for the commercial and recreational fisheries in Ontario and the Great Lakes region. As once abundant populations had been become noticeably reduced (Ontario Game and Fish Commission 1892; Hansen 1999), however, more restrictive management, invasive species control, and supplemental stocking was required for to maintain fishable lake trout populations (Evans 1912; Pycha and King 1975; Hansen 1999; Lester et al. 2003). During most of the 20th century, lake trout fisheries were often managed for maximal fish production by targeting harvest levels at the maximum possible level of sustainable yield (MSY; Lester et al. 2003). Stocking was an important part of many MSY-based management strategies, because actual harvest levels often exceeded natural lake trout production (Evans and Willox 1991; Evans et al. 1991; Lester et al. 2003). As sustainability-based management strategies became more popular (OMNR 1992; Shuter et al. 1998; Lester et al. 2003), and empirical studies had demonstrated that supplemental stocking was not a sustainable practice for producing more lake trout (Evans and Willox 1991), management attitudes changed. This conceptual transition was facilitated by the demonstration that supplemental stocking could also lead to the rapid replacement of indigenous, self-sustaining lake trout populations that were potentially better suited to local conditions than stocked hatchery fish (Evans and Willox 1991).

Inland lake trout management in Ontario is now implemented within an "ecological framework for recreational fisheries management" that should better emphasize biological information in the regulation of regional fish populations (OMNR 2005a; OMNR 2008). Region-specific Fisheries Management Zones (FMZs) have been designated for both inland watersheds and for the Ontario waters of the Great Lakes.

However, additional international regulations (e.g., through the Great Lakes Fisheries Commission) have governed lake trout management policies on commercial fishing in the Great Lakes (for historical management perspectives and policy reviews of see [Hansen 1999] and ([Brown et al. 1999])). Contemporary management strategies for inland and Great Lakes populations also reflect an awareness of the negative impacts from exotic species introductions. It is worth noting though, that control of the predatory sea lamprey (*Petromyzon marinus*) has played a larger, critical role in shaping international harvest policies for Great Lakes lake trout than for inland populations.

The management zone framework was derived from earlier approaches for sustainable fisheries management (see above), but is focused primarily on a reducing regulatory complexity by managing ecologically similar fish populations over large geographical regions as common units. Some exceptional cases within FMZs are allowed separate management strategies, such as sanctuary fish populations. However, the overall strategy of the FMZ system still does not rely strongly enough on well-established indicators of fishery development (fish biomass and fishing mortality) to assess the state of the fishery (healthy, overexploited early, overexploited late, and degraded, recovering; Lester et al. 2003; OMNR 2008). The FMZ system also does not widely employ alternatives to traditional catch limits (i.e. population-level harvest quotas; (Lester et al. 2003; Olver et al. 2004; OMNR 2008) that are essential for adaptive management strategies that enable rapid responses to size reductions in populations of conservation concern. These oversights may be particularly problematic for the long-term success of developing, community-based stocking programs that seek to rehabilitate reduced native lake trout populations with local source strains (e.g., local programs in the

southern Ontario region include those initiated by the Haliburton Fish Hatchery and the North Hastings Community Fish Hatchery), as public access is required for any lake stocked in partnership with the OMNR (OMNR 2002).

Although provincial stocking policy restricts the range of governmental stocking (Kerr 2006), and discourages population supplementation with genetically divergent and ecological dissimilar lake trout strains (OMNR1992; 2002; 2003), guidelines for genetically divergent hatchery strain stocking remain permissive at the regional (OMNR 2002) and federal levels (DFO 2003). For example, Ontario's provincial stocking policy states that "hatchery-reared fish *shall not* be stocked any water where they may compete or hybridize with fish species that are designated as threatened, endangered, or a species of concern, [emphasis added] " however, only "*consideration* must be given to potential genetic effects that stocked fish might have on native and naturalized species [emphasis added]" (OMNR 2002). Similarly, the federal Department of Fisheries and Oceans identifies one of three major concerns for aquatic introductions and transfers as "genetic changes that will lessen the possibility of local populations to survive, [emphasis added] " but indicates that while "some resource managers have developed policies which recognize this concern by recommending that donor stocks for transfers closely match the stocks in the receiving waters" admits that only "*a trend* exists also towards adopting a conservative approach in approving transfers between distant locations [emphasis added]" (DFO 2003).

At both regulatory levels, some additional assessment requirements do further limit potential genetic impacts from supplemental stocking (e.g. within-watershed limitations on source populations for local-strain broodstock development; [OMNR]

2002). Even so, the adaptive nature of these permissive guidelines, which is intended to reflect other cultural, social, and economic perspectives, could be modified into a more rigorous and explicit assessment framework by incorporating modern conservation genetic guidelines. For a broader audience, guidelines on conservation of intraspecific genetic variation pertaining to population supplementation could also be more explicitly incorporated into existing biodiversity strategies (OMNR 2005b).

Conservation genetic strategies for future lake trout management

A broad range of different molecular marker-based methodologies are currently accepted for the identification of intraspecific conservation units (for review see Fraser and Bernatchez 2001). Even so, some controversy over their use remains (Riddle and Hafner 1999; Kelt and Brown 2000). Of these methods, two main, complementary approaches provide a relatively robust, comparative set of genetic criteria for evaluation of most wildlife populations. The first approach relies on the concept of the Evolutionary Significant Unit (ESU) to characterize relevant sub-species units. One of the earliest ESU definitions was developed for discrimination of salmon stocks on the Pacific coast (Waples 1991). This generalist approach required that an ESU "must be substantially reproductively isolated from other conspecific population units" and "must represent an important component in the evolutionary legacy of the species." Although this ESU concept is useful across a wide range of taxa, it was not considered specific enough for strict classifications by some.

In response to this perceived shortcoming, a different set of ESU concepts were developed that reflected earlier phylogeographic approaches for resolution of genetically relevant sub-species units (Avice et al. 1987; Dizon et al. 1992). Moritz (1994) combined and modified these early concepts, and restricted ESUs to populations with reciprocal monophyly for mitochondrial lineages (i.e., single haplotypes within units), and significant divergence in nuclear allele frequencies (Fraser and Bernatchez 2001). While useful for species characterized by simple evolutionary histories with straightforward, branching lineages, this ESU definition is unsuitable for fish populations with complex evolutionary histories, such as those in previously glaciated regions of North America and Europe. To address these limitations, Moritz (Moritz 1994) also provided a complementary Management Unit (MU) category, which classified groups with more recently restricted gene flow as "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles". Even though this MU modification broadened the applicability of Moritz's phylogenetic ESU concept, others chose to develop an alternative approach, independent of earlier ESU concepts, to more explicitly evaluate the relative importance of adaptive evolutionary processes.

Crandall et al. (2000) devised a hypothesis-testing framework for evaluating genetic and ecological exchangeability in terms of historical and recent time frames. In this system, the null hypothesis of genetic exchangeability is rejected when two or more populations show evidence for genetic distinctiveness or divergence resultant from recent genetic exchange. Similarly, the null ecological exchangeability hypothesis is rejected when two or more populations show evidence for population differentiation, in terms of

acquired adaptive traits or preferential habitat use among other possibilities. While the genetic criteria emphasize the importance of gene flow and historical isolation in population differentiation, the ecological criteria are expected to better reflect adaptations to local ecological conditions. Although this framework is extremely useful, the subjectivity of the time frame categories (how recent? how historical?) has been questioned (Fraser and Bernatchez 2001).

To provide an example of how to assess the genetic status of Ontario's lake trout populations with these conservation genetic approaches, a comparative analysis of previous population assessments, current genetic profiles (from the combined genetic data presented in this thesis), and assigned conservation genetic status based on ESU and exchangeability concepts was performed Appendix Table A-3-1). This information was then compiled to synthesize a set of population-specific conservation and management recommendations.

Several trends are evident in the compiled information. The questionnaire-based status designations from the Lake Trout Atlas (OMNR 1989) correspond extremely well to categories inferred from the composite genetic profiles, but not quite as well to allozyme-based categorizations (see Chapter 2 for further discussion of this trend). Many stocked populations did not show evidence of introgression, however, some inland populations with evolutionary histories of postglacial colonization from the proglacial lake network (Cedar Lake, Lake Kioshkokwi, potentially Lake Opeongo and Boshkung Lake) showed genetic similarities to Great Lakes lake trout and thus somewhat intermediate genetic profiles. A similar issue arose with the strict categorization of the Dog River and Montreal River lake trout, as both are genetically distinct from basin-

spawning Great Lakes lake trout, but both share a high degree of similarity to sanctuary lake populations of mixed river-and basin-spawner ancestry.

The two comparative ESU concepts provided contrasting designations for population status. Both ESU designations were generally concordant for native, allopatric populations with single glacial ancestries, and as expected the Moritz (1994) ESU criteria were so restrictive as to be of no use for native populations with mixed mtDNA lineages. Although native inland populations show divergence levels consistent with reproductive isolation, reside in a variable landscape, often have differences in life-history attributes (Shuter et al. 1998), and are morphologically variable, there is no definitive evidence that *each* represents a significant component in the evolutionary legacy of the species. Thus, according to Waples' guidelines I suggest that inland lake trout populations can be grouped into ESUs along regional postglacial evolutionary histories corresponding to single glacial lineages or multiple lineages if they share a common evolutionary history such as the colonists from the Fossmill outflow in Algonquin Park. In contrast, evaluation based strictly on the patterns of significant divergence detected at nuclear loci (i.e., microsatellites) indicated that all native inland populations would be discrete MUs according to the criteria of Moritz (1994). It is worth noting as well that only the more generalized Waples (1991) ESU definition allows for interpretive analysis of the conservation status for introgressed (e.g. Boshkung Lake) and introduced populations (e.g., the sanctuary Mishibishu lake chain), although Moritz does recognize that other factors may impart conservation value to a particular population (Moritz 1994).

The Crandall et al. (2000) approach was the most broadly applicable for all evaluated populations, including a straightforward assessment of introduced and introgressed populations. However, this approach was limited for classifications where information on ecological distinctiveness was limited (e.g., Cedar Lake and Lake Kioshkokwi). Evaluation of genetic exchangeability (i.e., gene flow) between inland and Great Lakes lake trout was based on observed mitochondrial DNA haplotypes (historical) and microsatellite-based measures of divergence levels (recent). Since inland lakes as a group typically are ecologically dissimilar (i.e., lower habitat volume, simpler fish communities, etc.) to the Great Lakes, recent ecological exchangeability was rejected for native inland populations, but accepted for introgressed or introduced populations derived from hatchery-strain fish that evolved earlier in the Great Lakes. In comparison, historical ecological exchangeability was accepted for native populations colonized by multiple glacial lineages that, like the colonists of the early Great Lakes, dispersed through a common proglacial lake network. It is certain, however, that a more quantitative evaluation of potentially adaptive (i.e., ecologically relevant) traits, such as those listed above, would provide a better framework for evaluating populations in the context of ecological exchangeability.

When considered together, the four possible modes of exchangeability classify populations in terms of a numerical value for population distinctiveness (the exchangeability category in Appendix Table A-3-1), whose magnitude is lowest (1) for the most highly differentiated pairs (two highly divergent species), and lowest (8) for two samples from a single population. Most native inland populations fell into category 2, which indicated treatment as separate species as the suggested management action. This

designation, partially a response to the methodological limitations indicated above, also indicated the relatively subjective nature of the historical categorization. However, the implications of this classification were still valid, as historical reproductive isolation (between 15 KYA until stocking began) that led to high degrees of divergence from regional populations should be maintained for each separate population. Introduced and introgressed populations were classified as either category 5c or 6, for which the recommended management action for these populations was to treat as separate populations, but allow for gene flow consistent with current population structure. The river-spawning lake trout had a category 5a classification that suggested management as separate populations and reflected their recent ecological distinction from Great Lakes basin-spawners.

My specific recommendations for lake trout and conservation management were based on these evaluations and fell into one of four main categories: 1) Manage population as a put-grow-take lake, 2) Population is genetically distinct; do not supplement unless for rehabilitative purposes (see general guidelines below for more detail), 3) Manage as sanctuary population, or 4) Requires further assessment (Appendix Table A-3-1). This final category was included because some inland populations that may be genetically similar to Great Lakes lake trout as a result of earlier, pre-stocking evolutionary histories should be evaluated for adaptive differences to determine whether their evolutionary trajectories have been affected by stocking.

More generally, this status evaluation indicates that the following conservation genetic guidelines should be broadly employed for future population management and regional supplemental stocking programs: 1) Given the genetic distinctiveness of most

inland lake trout populations, even after substantial stocking, all put-grow-take stocking activities should cease in all lakes that formerly held indigenous (i.e. non-introduced) lake trout until conservation genetic evaluations can be undertaken, 2) If supplemental stocking is needed to rehabilitate a reduced inland population, then stocked fish *must* be derived from the target population, unless, 3) If too few or no individuals are available from the target populations, then a source population with a similar glacial evolutionary history (as indicated by mtDNA analysis) from a regional, ecologically similar lake (OMNR 2002; 2003) could be used, but 4) that such a selection should not be restricted to within the same watershed (OMNR 2002), as present-day, regional lake trout genetic structure reflects proglacial events and genetic similarities resultant from dynamic landscapes that existed before contemporary watersheds formed. Clearly, each genetically distinct population that was identified by molecular markers also has the potential have evolved a diversity of specialized adaptations.

A simplified approach to evaluate conservation genetic status for inland lake trout

The genetic results reported in this thesis suggest that an alternative approach may also be suitable for evaluating the conservation genetic status of inland lake trout populations. Simply stated, *all* contemporary indigenous inland lake trout populations should be considered unique, whether they have been supplemented or not. It is clear that many inland populations that were heavily stocked with hatchery fish (essentially Great Lakes lake trout) have retained native genetic variation and naturally high degrees of divergence from other inland and Great Lakes populations (Chapters 1 and 3). These

genetic characteristics reflect the diversifying effects of reproductive isolation resultant from natural gene flow limitations among inland populations. Additionally, these attributes also indicate that there was little or no interbreeding between native and hatchery-strain lake trout that evolved under two different postglacial evolutionary histories. Consequently, these results strongly suggest that inland lake trout have accumulated heritable adaptive differences as they have evolved in their local environments over the past 15,000 years.

Even in cases where hatchery-strain stocking has led to extensive genetic homogenization, key biological and genetic attributes with potential adaptive value (e.g., small body size [Winemiller 2005], early ages at maturity Pazzia [2002], and locally common mtDNA haplotypes [Ashford and Danzmann 2001]) may still persist. From a practical standpoint, evidence for local adaptation should be given serious consideration when developing management policy that may alter local lake environments. The evidence outlined above also provides a strong argument against the continued supplementation of once-indigenous inland lake trout populations for recreational fishing opportunities. A critical next step for lake trout conservation and management, then, is for future genetic analyses to focus on measuring these potentially adaptive differences, and tracking adaptive responses to captive rearing, particularly those that reduce fitness in the wild (Araki et al. 2007), for rehabilitative stocking programs.

Insights on adaptation from neutral marker genetic studies and future research directions

The long-term evolutionary potential of populations adapted to local environmental conditions ultimately depends on their ability to evolve and retain adaptive genetic variation (Moran 2002; Holderegger et al. 2006). There is some evidence from survival studies following stocking experiments that have indicated that lake trout can adapt to local lake conditions (Plosila 1977; Maclean et al. 1991). Efforts to measure heritable and potentially adaptive genetic variation in lake trout have been historically limited, however, by their long generation time (age at maturity ca. 7-10+ years; Martin and Olver 1980). Even so, measured genetic differences in spawn timing (Bill Sloan and Cheryl Murphy, unpublished data), early developmental rate (Horns 1985; McDermid et al. 2007), later growth rate and age at maturity (McDermid et al. 2007), fat content (Eshmeyer and Phillips 1965), swim bladder gas retention (Ihssen 1973), and other traits (Krueger and Ihssen 1995) have been observed by use of hatchery-based, "common garden" experiments. These traditional experiments are generally designed to constrain confounding effects in a controlled environment to measure the proportion of observed, quantitative trait variance (V_P) that is attributable to quantitative genetic variance (for narrow-sense heritability, the additive genetic component V_A), relative to variance from environmental factors (V_E), and gene-by-environment interactions ($V_{G \times E}$). Newer linkage-mapping and sequencing-based technologies have enabled powerful molecular genomic analyses of quantitative trait variation for inbred model organisms (the fruit fly, *Drosophila melanogaster*; Carroll 2000), as well as for cultured fish (salmonids; Leder et al. 2006) and wild populations (sticklebacks; Colosimo et al. 2004; Cresko et al. 2004). Access to these genomic approaches remain limited, however, because they are still

extremely costly and require extensive labour to provide essential partial or whole-genome sequences.

Although neutral molecular markers generally do not provide direct insight into adaptive evolutionary processes, under certain circumstances they can reveal associations between specific genetic backgrounds and measurable traits. For example, although there is no direct evidence that river-spawning behaviour is heritable in lake trout, there is an association between a distinctive genetic background, relative to basin-spawning populations, and a highly unusual reproductive behaviour that promotes assortative mating (see Chapter 3 for details).

Similarly, high diversity lake trout populations that originate from the Great Lakes have evolved in more diverse and complex fish communities than most smaller, inland lake trout lakes as a result of associated postglacial events (Barbour and Brown 1974; Evans and Olver 1995; Coon 1999). Although this higher level of neutral genetic diversity does not indicate that they have greater adaptive potential (i.e., heritable, additive genetic variation), their genetic attributes provide a convenient marker for evaluating whether stocked lake trout from Great Lakes have successfully interbred with, or replaced, local populations in stocked inland lakes with naturally lower genetic variation.

Evidence of introgression or numerical replacement in stocked, native populations of species poor lakes could be accounted for by the absence of colonization-inhibiting predators (Evans and Olver 1995; Powell and Carl 2004). However, evidence of gene flow into inland populations with naturally richer fish communities, which should inhibit stocked fish survival, could indicate some degree of adaptive advantage for stocked lake

trout whose ancestors evolved in the species-rich Great Lakes. Furthermore, evidence of restricted gene flow in intermediate fish communities could indicate a selective advantage for locally adapted inland populations and/or a selective disadvantage for stocked lake trout.

A comparative analysis of population designations from the OMNR Lake Trout Atlas (1989), OMNR-OFIS stocking histories, and the presence of 20 select fish species identified by Evans and Olver (1995) as early life stage predators (13), species associated with failed introductions (3), or successful colonizations (4) from the OMNR Aquatic Habitat Inventory provides some preliminary support for the possibilities that both Great Lakes and inland lake trout populations have evolved adaptive differences in response to fish communities since postglacial colonization. Of the 233 indigenous inland lake trout populations in southern Ontario that had been stocked (according to OFIS records), the OMNR Lake Trout Atlas (1989) identified 79 as self-sustaining native populations, but listed 154 populations that were partially or completely maintained by non-native (i.e. Great Lakes origin) hatchery strain supplementation. Although there are a number of confounding, associated factors to consider for future analyses (e.g., habitat variables, detailed stocking histories, and other human impacts), these two categories are expected to represent populations where post-stocking hatchery fish survival is low, or high, respectively, particularly as these categories have shown some concordance with post-stocking ancestral status (native or introgressed) as diagnosed by microsatellite marker analyses in this thesis. Consequently, if there was no effect from the total number of species on supplemental stocking success, then each species category (defined by the number of species present) should have approximately equal proportions of self-

sustaining (34%) and maintained (66%) populations. Conversely, if there is an effect from the fish community composition, a trend towards unequal categorical partitioning should be present.

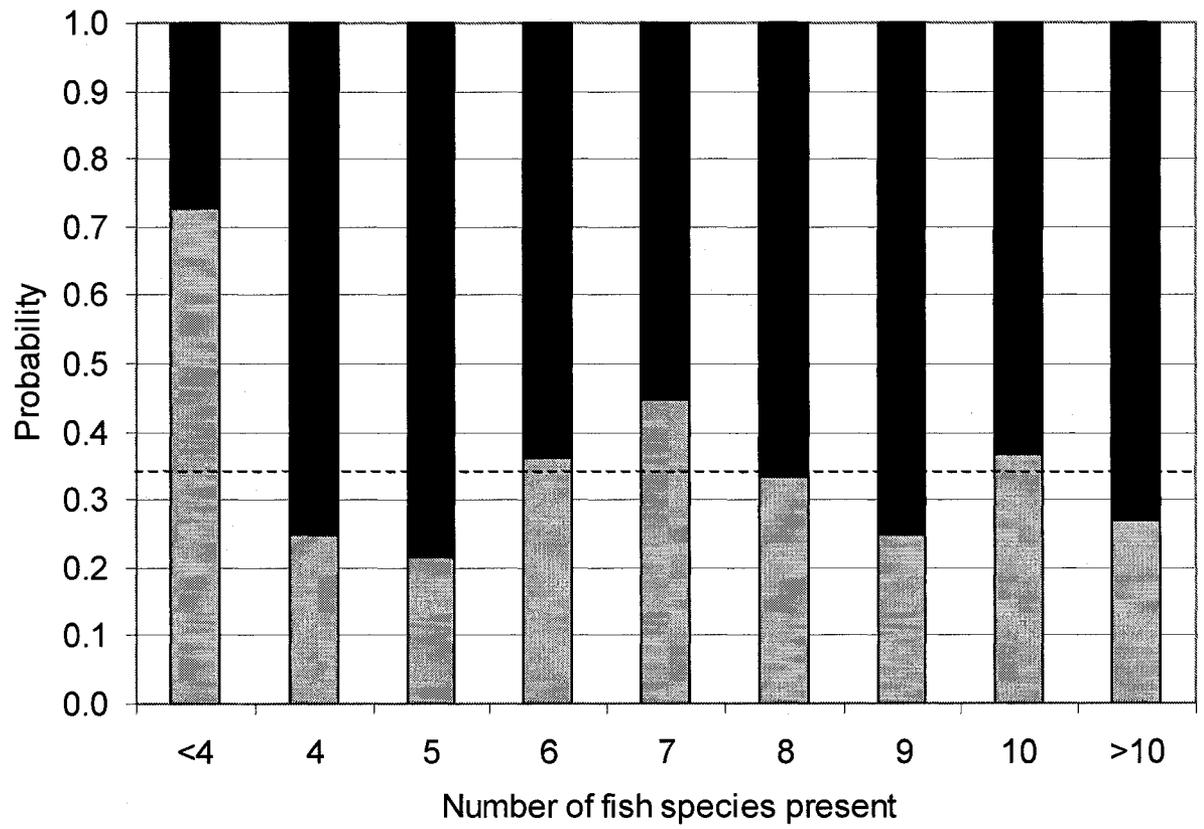
The results of this categorical comparison indicated that the fish community composition of indigenous lake trout lakes does seem to have a weak but detectable effect on estimated stocked lake trout "survival" (Figure 1). Post-stocking "survival" showed a weak dependence on fish community composition (Chi-square test; $\chi^2 = 13.05$; $df = 8$; $P = 0.11$), when the populations were categorized so that there was no species category with fewer than 4 replicate populations. A greater proportion of lakes (73%) with extremely simple fish communities (less than 4 select species) supported indigenous, self-sustaining populations after supplemental stocking than expected (34%), contrary to predictions based on earlier stocking experiments (Evans and Olver 1995; Powell and Carl 2004). However, trends towards lower "survival" for stocked fish were evident in stocked lakes with either few (4-6) or many (8+) select fish species. Stocked fish showed lowest estimated "survival" in lakes with seven select species present, as 45% of the indigenous, stocked populations in this category remained self-sustaining.

These trends suggest the exciting possibility that native inland lake trout may be adapted to extremely simple fish communities and may have a selective advantage over stocked Great Lakes lake trout. The apparent advantage that the stocked lake trout may have in species-rich inland lakes seems to be limited, however, and lower "survival" in intermediate fish communities could be attributed to either a selective advantage for natives or a selective disadvantage for stocked fish. Further studies are planned to address these possibilities by direct evaluation long-term survival and reproductive

success of stocked lake trout of Great Lakes origin in inland populations by analysis of past genetic contributions with microsatellite DNA techniques. These studies, and the population genetic results obtained during execution of this thesis, will provide baseline genetic characterizations essential for future analyses of adaptive genetic variation among lake trout and other freshwater fish species of conservation concern.

Figure 5-1

Probability that post-stocking supplementation will be required for population sustainability, based on the number of fish species present in stocked, indigenous lake trout populations in southern Ontario ($N = 233$). Categorization was performed to eliminate categories with less than four lake replicates. Proportional probabilities for were calculated as 1) the number of stocked, but self-sustaining, indigenous populations (gray bars; category N1, N2, N4, or N6 from the OMNR Lake Trout Atlas), or 2) the number of stocked, indigenous populations maintained by supplemental stocking with non-native hatchery strains (black bars; category N3, N5 from the OMNR Lake Trout Atlas), divided by the total number of stocked populations within each category. The dotted line shows the overall expected probability (34%) for no fish community composition effect.



APPENDIX 1**Manuscript appendix tables and figures**

Appendix Table A-1-1

Summary of microsatellite genetic diversity observed in Chapter 2 lake trout populations, PCR parameters, and primer sequences: (H_E) expected heterozygosity, (H_O) observed heterozygosity, (T_M) annealing temperature.

Locus	Number of alleles	H_E	H_O	Size range (bp)		T_M (°C)	PCR cycles	Primer references
				Min	Max			
<i>Ogo1a</i>	3	0.350	0.332	144	152	52	30	Olsen et al. 1998
<i>Ots1</i>	25	0.634	0.633	211	263	56	30	Banks et al. 1999
<i>Scou19</i>	11	0.532	0.511	152	180	52	30	Taylor et al. 2001
<i>Sfo1</i>	4	0.086	0.090	108	116	52	30	Angers et al. 1996
<i>Sfo12</i>	4	0.228	0.217	253	259	52	30	Angers et al. 1996
<i>Sfo18</i>	13	0.389	0.348	164	192	52	30	Angers et al. 1996
<i>SfoC24</i>	4	0.332	0.325	99	111	52	30	King, T.L., unpublished (GENBANK accession code: AY168187)
<i>SfoC88</i>	3	0.396	0.403	174	180	52	30	King, T.L., unpublished (GENBANK accession code: AY168192)
<i>SfoD75</i>	18	0.804	0.769	274	356	52	30	King, T.L., unpublished (GENBANK accession code: AY168197)
<i>Ssa85</i>	5	0.429	0.426	126	140	52	30	O'Reilly et al. 1996
<i>Oneu14</i>	17	0.742	0.729	208	242	52	30	Scribner et al. 1996
<i>Sfo23</i>	33	0.864	0.858	171	237	54	32	Angers et al. 1996
Total	140							

Appendix Table A-1-2

Mitochondrial DNA haplotypes for pooled temporal samples used in Chapter 3.

Conventions for mitochondrial haplotype designations are given in the text.

Pooled sample	Origin	<i>N</i>	Mitochondrial DNA haplotypes		
			A	B/D	C
Slate Islands, Lake Superior	broodstock (1989)	41	0.55	0.45	0.00
	wild (2004)	46	0.30	0.44	0.26
Mishibishu hatchery strain	broodstock (1995)	46	0.38	0.62	0.00
	broodstock (2002)	41	0.67	0.33	0.00
Dog River	wild (1993,1994)	16	0.86	0.00	0.14
	wild (2002, 2005)	58	0.72	0.09	0.19
Montreal River	wild (1994)	48	0.85	0.05	0.10
	wild (2002, 2005)	30	0.70	0.22	0.07

Appendix Table A-1-3

Population genetic diversity attributes for Chapter 4 study populations. Abbreviations are as follows: Nei's standardized estimate of heterozygosity (H_E), or gene diversity (Nei 1987), observed heterozygosity (H_O), the average number of alleles per locus (N_A), standardized allelic richness (A_R), and Hardy-Weinberg equilibrium within populations (F_{IS}).

No.	Origin	H_E	H_O	N_A	A_R	F_{IS}
1	Lake Manitou (Lake Huron)	0.596	0.601	7.73	6.67	-0.01
2	Cedar Lake	0.556	0.586	6.18	5.74	-0.06
3	Lake Kioshkokwi	0.575	0.574	6.73	5.93	0.00
4	Lavieille Lake	0.583	0.557	6.45	5.72	0.04
5	Dickson Lake	0.458	0.465	4.18	4.16	-0.02
6	White Partridge Lake	0.490	0.496	5.09	4.72	-0.01
7	Hogan Lake	0.489	0.473	5.09	4.96	0.03
8	Lake LaMuir	0.357	0.386	3.18	3.11	-0.08
9	Big Trout Lake	0.543	0.548	6.36	5.61	-0.01
10	Happy Isle Lake	0.495	0.509	5.64	5.27	-0.03
11	Opeongo Lake	0.535	0.544	6.09	5.48	-0.02
12	Kingscote Lake	0.407	0.386	5.64	4.76	0.05
13	Louisa Lake	0.329	0.335	4.64	4.00	-0.02
14	Big Porcupine Lake	0.298	0.274	3.45	3.10	0.08
15	Timberwolf Lake	0.310	0.308	2.36	2.26	0.01
16	Smoke Lake	0.399	0.421	4.73	4.06	-0.06
17	Lost Dog Lake	0.388	0.395	4.45	4.37	-0.02

APPENDIX 2**Population allele frequency distributions**

Appendix Table A-2-1.

Chapter 2 microsatellite allele frequency distributions and sample sizes (N) for characterized lake trout populations and hatchery strains: Miskwabi Lake (MSK), Boshkung Lake (BKG), MacDonald Lake (MAC), Clean-Clear Lake (CLE), Redstone Lake (RST), Grace Lake (GRC), Esson Lake (ESS), Farquhar Lake (FRQ), Kingscote Lake (KS), Smoke Lake (SMK), Louisa Lake (LOU), Barker Lake (BRK), and Crystal Lake (XTL). Stocking source hatchery strains are indicated in highlighted bold italics: Lake Manitou (LM), Slate Island (SL) and Killala Lake (KL).

116	0.023	0.076	0.053	0.012	0.080	0	0.024	0	0	0	0.488	0	0	0	0	0
N	43	46	47	42	50	39	41	40	33	29	43	48	47	47	50	49

Sfo12	253	0.116	0.272	0.021	0.095	0.030	0.077	0.073	0.064	0	0	0.010	0	0.011	0	0
	255	0.070	0.054	0	0.060	0.020	0.038	0.110	0.051	0.455	0.603	0.409	0	0	0	0.020
	257	0.767	0.674	0.979	0.845	0.920	0.885	0.817	0.885	0.545	0.397	0.591	0.990	1	0.989	0.980
	259	0.047	0	0	0	0.030	0	0	0	0	0	0	0	0	0	0
N	43	46	47	42	50	39	41	39	33	29	44	49	47	47	50	49

Sfo18	164	0	0	0	0	0.163	0	0	0	0	0	0	0	0	0	0.010
	166	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.010
	168	0	0	0	0	0	0	0	0	0	0	0	0	0	0.117	0
	172	0.476	0.500	1	0.655	0.755	0.592	0.475	0.713	0.203	0.304	0.467	0.949	0.936	0.862	0.949
	174	0.024	0	0	0.012	0	0.039	0.025	0	0	0	0	0	0.053	0	0
	176	0	0	0	0	0	0	0.038	0	0	0	0	0	0	0	0
	180	0	0	0	0	0	0	0	0	0.016	0	0	0	0.011	0	0
	182	0.476	0.304	0	0.250	0.071	0.316	0.313	0.2	0.297	0.429	0.533	0.031	0	0.021	0.031
	184	0.012	0.033	0	0.083	0	0.026	0.050	0.038	0	0	0	0	0	0	0
	186	0	0	0	0	0.010	0	0.038	0	0.422	0.268	0	0	0	0	0
	188	0.012	0.152	0	0	0	0.026	0.063	0.050	0	0	0	0.020	0	0	0
	190	0	0	0	0	0	0	0	0	0.063	0	0	0	0	0	0
	192	0	0.011	0	0	0	0	0	0	0	0	0	0	0	0	0
N	42	46	47	42	49	38	40	40	40	32	28	45	49	47	47	48

SfoC24	99	0.024	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	102	0.143	0.272	0.413	0.238	0.190	0.192	0.131	0.145	0	0.103	0.089	0	0	0	0

	43	46	46	40	40	42	39	42	40	33	27	44	49	46	44	50	48
242	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>N</i>	43	46	46	40	40	42	39	42	40	33	27	44	49	46	44	50	48
<i>Sfo23</i>																	
171	0	0	0	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0
175	0	0.011	0	0	0	0.027	0	0.013	0	0	0	0	0	0	0	0	0
177	0	0	0	0	0	0	0	0	0	0	0.025	0	0	0	0	0	0
179	0	0	0.012	0.012	0	0.014	0	0	0	0	0	0	0	0	0	0	0
181	0.012	0.011	0	0.012	0	0.064	0	0	0	0	0	0	0	0.024	0	0	0
183	0.024	0.011	0.012	0	0.033	0	0	0.026	0	0	0.275	0	0	0	0	0	0
185	0	0.011	0.128	0.012	0.033	0.014	0	0.013	0.045	0.019	0	0	0	0	0	0.020	0
187	0.357	0.054	0.035	0.262	0.098	0.324	0.128	0.316	0.182	0.222	0.100	0.071	0	0	0.131	0.031	0
189	0.024	0.011	0.023	0.060	0.011	0.108	0.103	0.092	0.015	0.019	0	0	0.011	0.012	0	0	0
191	0.060	0.011	0	0.083	0.152	0.054	0.115	0.092	0	0.056	0.025	0.143	0.011	0.036	0	0	0
193	0	0.109	0	0.036	0.065	0.014	0.038	0	0	0	0	0	0	0	0	0	0
195	0.012	0	0.093	0.048	0.033	0.027	0	0.026	0.379	0.37	0.063	0	0	0	0	0	0
197	0.012	0.033	0	0	0.033	0.027	0.013	0.026	0	0	0.100	0.010	0	0	0	0	0
199	0.024	0.033	0	0	0	0.014	0	0	0.106	0.019	0	0	0	0.012	0	0	0
201	0	0.043	0.012	0.024	0	0.014	0	0	0.015	0.019	0	0	0.051	0	0	0.031	0
203	0.012	0.011	0	0	0.011	0.014	0.026	0	0	0.019	0	0	0.01	0.011	0.036	0.010	0.011
205	0	0.043	0.023	0.024	0.033	0	0	0.013	0	0	0.025	0.041	0.043	0.452	0.041	0	0
207	0.024	0.033	0.023	0.060	0	0	0.051	0.013	0	0	0	0.051	0.085	0.012	0.296	0.120	0
209	0	0.033	0.070	0.012	0.141	0.041	0.026	0.013	0.03	0.093	0.013	0.224	0.043	0.179	0.112	0.033	0
211	0.060	0.098	0.023	0.012	0.043	0.014	0.038	0.039	0.076	0.019	0	0.051	0.032	0.083	0.306	0.022	0
213	0.012	0.043	0.047	0.036	0.011	0.014	0.013	0.039	0.152	0.130	0.063	0.071	0.043	0	0.041	0.228	0
215	0.071	0.098	0.047	0.083	0.065	0.054	0.051	0.092	0	0.019	0.013	0.092	0.340	0	0.082	0.141	0
217	0.071	0.065	0.105	0.036	0.076	0.041	0.077	0.092	0	0	0.075	0.122	0.032	0.012	0	0.033	0

219	0.048	0.043	0.035	0.024	0.043	0.014	0.064	0	0	0	0.163	0.031	0.223	0	0.010	0.217
221	0.024	0.022	0.198	0.036	0.033	0.068	0.064	0.026	0	0	0.025	0	0.032	0.012	0.010	0.109
223	0.024	0.054	0.012	0.012	0.043	0.027	0.077	0	0	0	0.025	0.020	0.085	0	0.010	0.033
225	0.012	0.065	0.035	0.012	0.022	0.041	0.026	0.039	0	0	0	0	0.011	0	0	0.054
227	0.036	0.011	0.023	0.048	0.011	0.014	0	0.026	0	0	0.013	0	0	0	0	0
229	0.036	0	0.047	0.024	0	0	0.013	0	0	0	0	0	0	0	0	0
231	0	0	0	0.012	0	0.014	0	0	0	0	0	0	0	0	0	0
233	0.024	0.043	0	0	0	0.014	0.013	0	0	0	0	0	0	0	0	0
235	0	0	0	0.012	0	0	0	0	0	0	0	0	0	0	0	0
237	0.024	0	0	0	0.011	0	0	0	0	0	0	0.010	0	0	0	0
N	42	46	43	42	46	37	39	38	33	27	40	49	47	42	49	46

Appendix Table A-2-2

Chapter 3 microsatellite allele frequency distributions and sample sizes (*N*) for characterized lake trout populations and hatchery strains: Dog River (DGU), Dog River historical population from 1952 (DGH), Montreal River (MON), Mishibishu Lake (MSL), Mishi Lake (MSI), Katzenbach Lake (KTZ), Mishibishu hatchery strain (MLH), Slate Islands (SLI), Michipicoten Island (MPI), Isle Royale (IRY), Marquette hatchery strain (MRQ), Gull Island Shoal (GIS), Parry Sound (PSD), Lake Manitou (LMN; same source as LM in Chapter 1), Killala Lake (KIL), Louisa Lake (LOU).

Populations

Locus	Allele or N	DGU	DGH	MON	MSL	MSI	KTZ	MLH	SLI	MPI	IRY	MRQ	GIS	PSD	LMN	KIL	LOU
<i>Ogo1a</i>	144	0.270	0.132	0.175	0.207	0.348	0.368	0.270	0.086	0.278	0.095	0.037	0.047	0.298	0.179	0.160	0.967
	148	0	0	0	0	0	0	0	0.006	0	0	0	0	0	0	0	0
	150	0.601	0.745	0.714	0.659	0.561	0.559	0.580	0.764	0.489	0.583	0.833	0.781	0.464	0.512	0.792	0.025
	152	0.128	0.104	0.110	0.134	0.091	0.074	0.149	0.144	0.233	0.321	0.130	0.172	0.238	0.310	0.049	0.008
	154	0	0.019	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<i>N</i>		74	53	77	41	33	34	87	87	45	42	27	32	42	42	72	61
<i>Ots1</i>	203	0	0	0	0	0	0	0	0	0.011	0	0	0	0	0	0	0
	211	0	0	0	0	0	0	0	0	0	0	0	0	0	0.012	0	0
	215	0	0	0	0	0	0	0	0	0.011	0	0	0	0	0	0	0
	217	0	0	0	0	0	0	0.006	0	0	0	0	0	0	0	0	0
	219	0.236	0.135	0.063	0.206	0.278	0.167	0.198	0.364	0.333	0.426	0.359	0.012	0	0.076	0	0
	220	0	0	0	0	0	0	0	0	0	0.019	0	0	0	0	0	0
	221	0.095	0.077	0.038	0.044	0.042	0.029	0.029	0.057	0.036	0.056	0.031	0	0	0	0	0.041
	222	0	0	0	0	0	0	0	0	0	0.019	0	0	0	0	0	0
223	0.054	0.026	0.063	0.044	0.028	0.04	0.081	0	0.048	0.019	0.031	0.024	0.024	0.024	0	0.008	
225	0.128	0.179	0.213	0.191	0.194	0.276	0.122	0.080	0.190	0.056	0.141	0.369	0.183	0.028	0.68	0.049	
227	0	0.032	0.025	0	0	0.011	0.052	0.023	0	0.016	0	0.134	0.049	0.098	0.049	0.098	

229	0	0.006	0.013	0	0	0.006	0.029	0	0.012	0	0.078	0.024	0.049	0.007	0.008
231	0.041	0.058	0.138	0.088	0.042	0.075	0.076	0.045	0.143	0.037	0.109	0.119	0.280	0.007	0.148
233	0	0	0.013	0	0.014	0	0.052	0.045	0.036	0.037	0	0.036	0.061	0.007	0.008
235	0.007	0.026	0.088	0.074	0.028	0.046	0.035	0.193	0.012	0	0.047	0.060	0.024	0.021	0
237	0	0.006	0	0	0	0	0.006	0.011	0.012	0	0	0	0.012	0.208	0
239	0	0	0	0	0	0	0.006	0	0.036	0.056	0.016	0	0	0.028	0
241	0	0	0	0	0	0	0.012	0.057	0	0	0	0	0	0	0
243	0	0	0	0	0	0	0.023	0	0.024	0.111	0	0	0	0	0
245	0	0.026	0.025	0.029	0.042	0.057	0.029	0.091	0.012	0.056	0.047	0.036	0.012	0.076	0
247	0	0.006	0	0	0	0	0.006	0	0	0	0.016	0.083	0.037	0	0
249	0	0.013	0	0	0	0	0.023	0.011	0.012	0	0.031	0.048	0	0.007	0
251	0.108	0.045	0.025	0.015	0.042	0.029	0.023	0	0	0	0	0	0.012	0.250	0
253	0.108	0.167	0.013	0.074	0.042	0.034	0	0	0	0.056	0	0	0	0.028	0
255	0.122	0.154	0.225	0.162	0.139	0.132	0.14	0	0.036	0.019	0.063	0.143	0.073	0.097	0
257	0.074	0.045	0.038	0.059	0.097	0.057	0.017	0	0.024	0	0	0.036	0.085	0.042	0.008
259	0.027	0	0.025	0.015	0.014	0.034	0.035	0	0.012	0.037	0	0.012	0	0.021	0
261	0	0	0	0	0	0	0.006	0	0.012	0	0.016	0	0	0.049	0
263	0	0	0	0	0	0	0	0	0.012	0	0	0	0	0	0
N	74	0	78	40	34	36	87	86	44	42	27	32	41	72	61

Scou19	160	0.333	0.301	0.119	0.118	0.139	0.206	0.126	0.182	0.060	0.056	0.188	0	0.024	0	0
	162	0.083	0	0	0	0	0	0.006	0	0	0	0	0	0	0	0.008
	164	0	0	0	0	0	0.006	0.046	0	0	0.037	0.016	0	0.012	0	0
	166	0	0	0	0	0	0	0.029	0	0.012	0	0	0.012	0	0	0

168	0	0.013	0	0	0.006	0.011	0	0	0.031	0	0	0	0	0	
170	0.285	0.327	0.31	0.471	0.375	0.335	0.247	0.284	0.202	0.389	0.266	0.244	0.262	0.222	0.869
172	0	0	0	0	0.042	0.006	0.023	0	0.071	0.019	0.016	0.049	0.012	0	0.008
174	0.167	0.218	0.476	0.324	0.389	0.347	0.466	0.455	0.536	0.463	0.406	0.671	0.595	0.708	0.082
176	0	0.019	0	0	0	0	0.011	0	0.071	0.019	0.047	0.024	0.06	0.063	0.033
177	0	0	0	0	0	0	0	0	0	0	0.016	0	0	0	0
178	0.132	0.122	0.095	0.088	0.056	0.094	0.017	0.08	0.048	0.019	0.016	0	0.036	0.007	0
180	0	0	0	0	0	0	0.017	0	0	0	0	0	0	0	0
N	72	0	78	42	34	36	85	87	44	42	32	41	42	72	61

Sfo1	108	0.020	0.084	0.058	0.036	0.029	0.069	0.017	0.006	0.033	0.071	0	0.016	0	0.083	0	
	110	0.980	0.920	0.942	0.964	0.971	0.931	0.977	0.925	0.967	0.869	0.907	0.919	0.988	0.976	0.854	1
	116	0	0	0	0	0	0	0.006	0.069	0	0.060	0.093	0.065	0.012	0.024	0.063	0
N	74	56	77	42	34	36	87	87	87	45	42	27	31	42	42	72	60

Sfo12	253	0	0.090	0.024	0.088	0.086	0.082	0.082	0.264	0.100	0.131	0.037	0.065	0.036	0.119	0.021	0
	255	0.034	0.071	0.048	0.074	0.057	0.018	0.046	0	0.036	0.037	0.037	0.065	0.024	0.071	0	0
	257	0.966	0.840	0.929	0.838	0.857	0.900	0.690	0.690	0.900	0.833	0.926	0.871	0.940	0.762	0.979	1
	259	0	0	0	0	0	0	0	0	0	0	0	0	0	0.048	0	0
N	74	0	78	42	34	35	85	87	45	42	42	27	31	42	42	72	61

Sfo18	166	0.061	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	172	0.453	0.434	0.726	0.636	0.583	0.651	0.540	0.456	0.607	0.607	0.519	0.563	0.561	0.476	0.993	0.951
	174	0	0.033	0.06	0.061	0.125	0.081	0.017	0	0.012	0	0	0	0.024	0.024	0	0.041

176	0	0	0.024	0.015	0.028	0.029	0	0	0.012	0.037	0.016	0.037	0	0.007	0	
178	0	0	0	0	0	0	0	0	0	0	0	0.146	0	0	0	
180	0	0	0	0.015	0	0	0	0.011	0	0	0	0	0	0	0.008	
182	0.486	0.487	0.190	0.258	0.208	0.233	0.259	0.489	0.143	0.333	0.281	0	0.476	0	0	
184	0	0	0	0	0	0	0.023	0	0	0	0.031	0.134	0.012	0	0	
186	0	0	0	0	0	0	0	0.022	0.012	0	0	0	0	0	0	
187	0	0	0	0	0	0	0	0	0	0.056	0	0	0	0	0	
188	0	0.046	0	0.015	0.056	0.006	0.155	0.022	0.202	0.056	0.109	0.098	0.012	0	0	
192	0	0	0	0	0	0	0.006	0	0.012	0	0	0	0	0	0	
N	74	0	76	42	33	36	87	87	45	42	27	32	41	41	72	61

SfoC24	91	0	0	0	0	0	0	0	0	0	0.037	0	0	0	0	0
	99	0	0	0	0	0	0	0	0	0	0	0	0.024	0	0	0
	102	0.270	0.468	0.417	0.545	0.458	0.437	0.201	0.178	0.095	0.370	0.234	0.134	0.146	0.396	0
	105	0.480	0.391	0.452	0.364	0.389	0.471	0.684	0.678	0.762	0.463	0.688	0.744	0.683	0.514	0.992
	111	0.250	0.141	0.131	0.091	0.153	0.092	0.115	0.144	0.143	0.130	0.063	0.122	0.146	0.090	0.008
	112	0	0	0	0	0	0	0	0	0	0	0.016	0	0	0	0
N	74	0	78	42	33	36	87	87	45	42	27	32	41	41	72	61

SfoC88	174	0.622	0.590	0.786	0.662	0.625	0.529	0.603	0.600	0.810	0.704	0.797	0.595	0.440	0.750	0.992
	177	0.378	0.410	0.214	0.338	0.375	0.471	0.397	0.400	0.190	0.296	0.203	0.405	0.560	0.250	0.008
N	74	78	42	34	36	87	87	87	45	42	27	32	42	42	72	61

SfoD75	258	0	0	0	0	0	0	0.011	0	0	0	0	0	0	0	0
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134	0.590	0.625	0.607	0.606	0.708	0.678	0.586	0.591	0.583	0.556	0.597	0.548	0.405	0.750	0.558
136	0.042	0.079	0.012	0.015	0	0.006	0.109	0.068	0.107	0.185	0.097	0	0.012	0.056	0
138	0.340	0.270	0.333	0.364	0.236	0.264	0.259	0.318	0.286	0.185	0.274	0.452	0.583	0.160	0.442
N	72	76	42	33	36	87	87	44	42	27	31	42	42	72	60
<i>Oneu14</i>															
206	0	0	0	0	0	0	0	0	0.012	0	0	0	0	0	0
209	0	0	0	0	0	0	0	0	0	0	0.016	0	0	0	0
212	0	0	0	0	0	0	0.006	0	0	0	0	0	0	0	0
214	0	0.007	0	0	0	0	0	0.056	0	0	0.016	0.012	0	0	0.008
216	0	0.007	0	0	0	0	0	0	0	0	0.016	0	0.083	0	0
217	0	0	0	0	0	0	0	0	0	0	0.016	0	0	0	0
218	0	0	0	0	0	0	0.012	0.022	0	0	0.031	0	0.024	0.063	0
219	0	0	0	0	0	0	0	0	0	0.019	0	0	0	0	0
220	0	0	0	0	0	0	0.041	0.044	0.024	0.037	0.016	0	0.024	0.021	0.008
222	0.036	0.047	0.024	0.031	0.030	0.029	0.088	0.033	0.048	0.056	0.031	0.012	0.119	0.229	0
223	0	0	0	0	0	0	0	0	0	0.019	0.016	0	0	0	0
224	0.143	0.033	0.036	0.125	0.076	0.075	0.076	0.089	0.036	0.111	0.047	0.357	0.095	0.194	0
225	0	0	0	0	0	0	0	0	0	0	0.016	0	0	0	0
226	0.007	0.060	0.071	0.156	0.152	0.098	0.088	0.011	0.143	0.037	0.109	0.036	0.083	0.021	0
227	0	0	0	0	0	0	0	0	0	0	0.031	0	0	0	0
228	0.093	0.413	0.369	0.188	0.303	0.287	0.294	0.267	0.286	0.222	0.281	0.274	0.250	0.222	0.033
229	0	0	0	0	0	0	0	0	0	0.019	0.031	0	0	0	0
230	0.221	0.120	0.155	0.313	0.303	0.207	0.112	0.133	0.190	0.056	0.141	0.167	0.179	0.021	0.075
232	0.314	0.113	0.143	0.094	0.061	0.121	0.076	0	0.107	0.185	0.094	0.083	0.095	0.007	0.375

203	0.108	0	0	0	0	0.006	0.011	0	0	0.048	0.036	0.012	0	0.008
205	0.142	0.091	0.024	0.029	0.042	0.058	0.034	0.111	0	0.037	0.032	0.036	0	0.014
207	0	0	0	0	0	0.017	0.011	0	0.019	0.016	0.036	0.024	0.014	0.066
209	0.128	0.039	0	0	0.028	0.012	0.046	0.011	0.083	0.056	0.065	0.012	0	0.116
211	0	0.058	0.024	0.029	0	0.006	0.103	0.033	0.024	0.074	0.048	0.107	0.061	0.022
213	0.027	0.045	0.012	0	0	0.017	0.034	0.044	0.071	0.167	0.065	0.048	0.012	0.029
215	0.034	0.019	0.012	0	0.069	0.029	0.103	0.022	0.083	0.167	0.145	0.131	0.061	0.344
217	0.020	0.091	0.107	0	0.083	0.058	0.069	0.078	0.095	0.019	0.129	0.048	0.073	0.094
219	0.068	0.149	0	0.015	0.083	0.041	0.057	0.156	0.071	0.222	0.032	0.024	0.049	0.23
221	0.081	0.078	0.083	0.221	0.083	0.11	0.034	0.033	0.06	0.056	0.016	0.071	0.024	0.188
223	0.068	0.084	0	0.088	0.042	0.047	0.046	0.078	0.012	0.019	0	0	0.024	0.107
225	0.007	0	0	0	0.014	0.012	0.034	0	0	0.037	0.016	0	0.012	0.008
227	0.014	0.006	0	0	0.014	0	0.017	0	0.036	0	0	0.024	0.037	0.014
229	0	0	0.024	0.029	0.042	0.017	0	0.089	0.012	0	0	0.024	0.037	0.051
231	0	0	0	0	0	0.006	0	0	0	0	0	0	0	0
233	0	0	0	0	0	0	0.063	0.011	0	0.016	0	0.024	0	0
235	0	0	0	0	0.014	0	0.017	0	0	0	0	0	0	0
237	0	0	0	0	0	0.006	0.011	0	0	0.016	0.012	0.024	0	0
239	0	0	0.024	0.015	0	0.041	0.006	0	0	0	0.012	0	0	0
241	0	0	0	0	0.014	0	0	0	0	0	0	0	0	0
247	0	0	0	0	0	0	0	0	0	0.019	0	0	0	0
N	74	0	77	42	34	36	86	87	45	42	27	31	41	61

Appendix Table A-2-3

Chapter 4 microsatellite allele frequency distributions and sample sizes (N) for characterized lake trout populations and hatchery strains: Cedar Lake (CED), Lake Kioshkokwi (KIO), Lake Lavieille (LAV), Dickson Lake (DKS) White Partridge Lake (WPR), Hogan Lake (HOG), Lake LaMuir (LAM), Big Trout Lake (BGT). Happy Isle Lake (HPI), Lake Opeongo (OPE), Kingscote Lake (KSG), Louisa Lake (LOU) Big Porcupine Lake (BPC), Timberwolf Lake (TWF), Smoke Lake (SMK), and Lost Dog Lake (LDG).

Locus	Allele or N	LMN	CED	KIO	LAV	DKS	WPR	HOG	LAM	BGT	HPI	OPE	KSG	LOU	BPC	TWF	SMK	LDG
<i>Ogola</i>	144	0.174	0.111	0.553	0.791	0.700	0.189	0.855	0.375	0.338	0.409	0.641	0.367	0.957	0.662	1	0.979	0.962
	148	0	0	0.053	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	150	0.512	0.722	0.170	0.209	0	0.216	0.081	0.139	0.238	0.394	0.185	0.561	0.032	0.338	0	0.011	0.038
	152	0.314	0.167	0.223	0	0.3	0.595	0.065	0.486	0.425	0.197	0.174	0.071	0.011	0	0	0.011	0
N		43	36	47	43	25	37	31	36	40	33	46	49	47	37	46	47	26
<i>Ots1</i>	211	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	217	0	0	0	0	0	0	0.016	0	0	0	0	0	0	0	0	0	0
	219	0	0	0	0	0	0	0	0	0.05	0	0	0.010	0	0	0	0	0
	221	0	0	0	0	0	0	0	0	0.013	0.015	0	0.031	0.032	0	0	0	0
	223	0.024	0	0	0	0	0	0	0	0	0.015	0	0.02	0	0	0	0	0
	225	0.190	0.361	0.479	0.244	0.04	0.149	0.048	0.764	0.488	0.353	0.174	0.847	0.713	0.986	0.717	0.170	0
	227	0.131	0	0.043	0	0.62	0.149	0.032	0	0.138	0.250	0.554	0.031	0.106	0.014	0.076	0.021	0
	229	0.048	0.014	0	0.07	0.14	0.014	0	0	0.113	0	0.022	0	0	0	0	0.319	0
	231	0.274	0.222	0.106	0.012	0.1	0	0.161	0.236	0.013	0.176	0.022	0.031	0.138	0	0.207	0	0
	233	0.06	0	0.011	0.023	0.1	0	0	0	0	0.132	0	0	0.011	0	0	0.032	0
	235	0.024	0.056	0	0	0	0	0	0	0	0	0.022	0.02	0	0	0	0	0.019
	237	0.012	0.028	0.011	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	239	0	0	0	0.035	0	0.108	0	0	0	0	0	0	0	0	0	0	0

178	0.035	0.125	0.021	0.14	0	0	0	0	0.015	0	0	0	0	0	0	0	0	0
180	0	0.111	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
N	43	36	47	43	25	37	31	36	40	34	46	49	47	37	46	47	46	26

Sfo1	108	0	0	0.058	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	110	0.977	0.958	0.942	1	1	0.952	1	1	1	1	1	1	1	1	1	1	0.981
	116	0.023	0.042	0.064	0	0	0.048	0	0	0	0	0	0	0	0	0	0	0.019
N	43	36	47	43	25	36	31	36	40	34	45	48	47	37	46	47	46	26

Sfo12	245	0	0	0	0	0	0	0	0	0	0.033	0	0	0	0	0	0	0
	251	0	0	0	0	0.028	0.033	0	0	0.061	0	0	0	0	0	0	0	0
	253	0.116	0	0.309	0.023	0	0.264	0.2	0	0.545	0.011	0.01	0	0	0	0.011	0.135	0
	255	0.070	0.114	0.085	0.058	0	0.042	0	0.038	0	0.054	0	0	0	0	0	0	0
	257	0.767	0.886	0.606	0.698	1	0.667	0.767	1	0.963	0.394	0.902	0.99	1	1	0.989	0.865	0
	259	0.047	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	261	0	0	0	0.221	0	0	0	0	0	0	0	0	0	0	0	0	0
N	43	35	47	43	25	36	30	36	40	33	46	49	47	37	46	47	46	26

SfoC24	99	0.024	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	102	0.143	0.181	0.234	0.128	0.06	0.068	0.242	0.167	0.15	0.044	0.12	0	0	0	0	0	0.115
	105	0.69	0.681	0.532	0.581	0.64	0.932	0.548	0.833	0.713	0.956	0.609	0.99	1	1	0.926	0.846	0
	111	0.143	0.139	0.234	0.291	0.3	0	0.21	0	0.138	0	0.272	0.01	0	0	0.074	0.038	0
N	42	36	47	43	25	37	31	36	40	34	46	49	47	37	46	47	46	26

<i>SfoC88</i>	174	0.442	0.750	0.936	0.465	0.920	0.792	0.919	1	0.436	0.227	0.489	0.939	0.989	1	1	0.404	0.865
	177	0.558	0.250	0.064	0.535	0.080	0.208	0.081	0	0.564	0.773	0.511	0.061	0.011	0	0	0.596	0.135
<i>N</i>		43	36	47	43	25	36	31	36	39	33	46	49	47	37	46	47	26
<i>SfoD75</i>	274	0	0	0	0	0	0	0	0	0	0	0	0.061	0	0	0	0	0
	282	0.023	0	0.043	0	0	0	0	0	0	0	0	0	0.021	0	0.022	0	0.038
	286	0.035	0	0	0	0	0	0	0	0	0	0	0	0.287	0	0.413	0.074	0
	290	0.023	0.014	0.128	0.116	0.200	0.014	0.097	0	0.013	0	0.043	0.010	0.362	0	0.391	0.138	0.077
	294	0.093	0.042	0	0.047	0.460	0.095	0	0	0.275	0.059	0.076	0.02	0.021	0	0.174	0.011	0.038
	298	0.279	0.250	0.106	0.326	0.30	0.446	0.065	0	0.463	0.441	0.217	0.031	0.074	0.095	0	0.021	0
	302	0.128	0.250	0.053	0.174	0.040	0.216	0.468	0.736	0.100	0.162	0.217	0.112	0.128	0.189	0	0.181	0.5
	306	0.163	0.014	0.266	0.163	0	0.189	0.274	0.125	0.013	0.059	0.207	0.031	0.043	0	0	0.096	0.212
	310	0.128	0.153	0.117	0.128	0	0.027	0.097	0.139	0.075	0.059	0.098	0.020	0	0.230	0	0.191	0.096
	314	0.081	0.208	0.096	0.035	0	0.014	0	0	0.063	0.015	0.076	0.255	0.011	0.351	0	0.255	0.019
	318	0.012	0.056	0.191	0	0	0	0	0	0	0.044	0.065	0.245	0.053	0.108	0	0.032	0
	322	0.012	0.014	0	0.012	0	0	0	0	0	0.029	0	0.143	0	0	0	0	0.019
	326	0	0	0	0	0	0	0	0	0	0.088	0	0.071	0	0.027	0	0	0
	330	0.012	0	0	0	0	0	0	0	0	0.015	0	0	0	0	0	0	0
	334	0	0	0	0	0	0	0	0	0	0.015	0	0	0	0	0	0	0
	338	0.012	0	0	0	0	0	0	0	0	0.015	0	0	0	0	0	0	0
<i>N</i>		43	36	47	43	25	37	31	36	40	34	46	49	47	37	46	47	26

<i>Ssa85</i>	134	0.419	0.458	0.628	0.209	0.3	0.851	0.742	0.056	0.613	0.044	0.163	0.286	0.554	0.324	0.576	0.207	0.519
	136	0.012	0	0	0	0	0	0	0	0	0	0	0.01	0	0.014	0	0	0
	138	0.570	0.542	0.351	0.791	0.7	0.149	0.258	0.944	0.225	0.956	0.837	0.694	0.446	0.662	0.424	0.793	0.481
	140	0	0	0	0	0	0	0	0	0.038	0	0	0.01	0	0	0	0	0
	142	0	0	0.021	0	0	0	0	0	0.125	0	0	0	0	0	0	0	0
<i>N</i>	43	36	47	43	25	37	31	36	40	34	46	49	46	46	37	46	46	26

<i>Oneu14</i>	206	0	0.056	0	0	0	0	0	0.069	0.325	0.103	0	0	0	0	0	0	0
	208	0	0.028	0	0	0	0	0	0	0	0.088	0	0	0	0	0	0	0
	210	0	0	0	0	0	0	0	0	0	0.074	0	0	0	0	0	0	0
	212	0	0	0	0.012	0.167	0	0	0	0	0.015	0.012	0	0	0	0	0	0
	214	0	0.028	0	0.012	0	0.014	0	0	0	0.015	0	0	0.011	0	0	0	0.019
	216	0.081	0	0	0	0.021	0.014	0	0	0	0.206	0.083	0.01	0	0	0	0	0
	218	0.023	0	0	0.012	0	0.071	0	0	0.138	0.25	0	0	0	0	0	0	0.019
	220	0.023	0	0	0.012	0	0	0.145	0.028	0.05	0.059	0.012	0	0.011	0	0.37	0	0.038
	222	0.128	0.083	0.13	0.244	0.188	0.071	0	0	0.225	0.029	0.012	0.153	0	0	0	0	0.019
	224	0.093	0.153	0.12	0.116	0.125	0.029	0.065	0	0.125	0.029	0.286	0.184	0	0	0	0.25	0.231
	226	0.081	0.153	0.022	0.081	0.063	0.014	0	0	0.013	0.029	0.06	0.112	0	0.111	0.011	0.057	0
	228	0.244	0.306	0.207	0.174	0.292	0.471	0.016	0.056	0.013	0.044	0.298	0.061	0.043	0.542	0	0.034	0.154
	230	0.174	0.097	0.141	0.105	0	0.100	0.355	0.194	0.013	0.029	0.024	0.010	0.054	0.014	0	0	0.5
	232	0.105	0	0.185	0.023	0.125	0.043	0.081	0.097	0.025	0.029	0.119	0.398	0.38	0.194	0.554	0.023	0.019
	234	0.035	0.097	0.087	0.186	0.021	0.057	0.113	0	0.038	0	0	0.031	0.304	0.125	0	0.58	0
	236	0	0	0.022	0.012	0	0.086	0.065	0.042	0.038	0	0.083	0.02	0.098	0.014	0.065	0.023	0

238	0	0	0.065	0	0	0.029	0.048	0.250	0	0	0	0.02	0.065	0	0	0.034	0
240	0	0	0.022	0.012	0	0	0.065	0.028	0	0	0.012	0	0.033	0	0	0	0
242	0.012	0	0	0	0	0	0.048	0.236	0	0	0	0	0	0	0	0	0
<i>N</i>	43	36	46	43	24	35	31	36	40	34	42	49	46	36	46	44	26
<i>Sfo23</i>	171	0	0	0	0	0	0	0	0	0	0.011	0	0	0	0	0	0
175	0	0	0.011	0	0	0	0	0	0	0	0	0	0	0	0	0	0
177	0	0	0	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0
181	0.012	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.024	0
183	0.024	0.069	0.074	0.037	0	0	0	0	0	0.273	0	0	0	0	0	0	0
185	0	0	0.021	0	0	0	0	0	0.013	0	0	0	0	0	0	0	0
187	0.357	0	0.181	0.122	0	0	0	0	0.038	0.03	0.200	0.071	0	0	0	0.131	0.080
189	0.024	0.069	0	0.012	0	0	0	0	0.026	0.015	0.044	0	0.011	0	0	0.012	0
191	0.060	0.028	0	0	0	0	0	0	0	0.03	0.033	0.143	0.011	0	0	0.036	0.020
193	0	0	0.011	0	0.020	0	0	0	0.077	0	0.022	0	0	0	0	0	0
195	0.012	0.028	0.074	0	0.020	0.028	0	0	0	0	0.056	0	0	0	0	0	0
197	0.012	0.250	0.011	0	0.040	0	0	0	0.038	0	0.011	0.010	0	0	0	0	0
199	0.024	0.097	0.021	0.024	0	0	0	0.029	0	0	0.022	0	0	0	0	0.012	0
201	0	0.014	0	0	0	0.028	0	0	0.013	0	0	0.051	0	0	0	0	0
203	0.012	0	0.011	0.024	0.080	0.042	0.016	0	0.026	0	0.111	0.01	0.011	0	0	0.036	0
205	0	0.028	0.021	0.134	0.060	0.069	0.032	0	0.013	0.015	0.056	0.041	0.043	0	0	0.452	0
207	0.024	0.014	0.181	0.037	0.060	0.014	0.032	0.171	0.115	0.045	0.056	0.051	0.085	0	0.012	0.012	0
209	0	0.056	0.021	0	0	0.111	0.048	0.414	0.192	0.182	0.111	0.224	0.043	0	0	0.179	0

211	0.060	0	0.043	0.098	0	0.210	0	0.013	0.152	0.089	0.051	0.032	0	0	0.083	0
213	0.012	0.014	0.043	0.098	0.020	0.306	0.113	0.129	0.103	0.091	0.071	0.043	0	0	0	0
215	0.071	0.097	0.106	0.159	0	0.153	0.048	0.086	0.026	0.045	0.092	0.340	0	0.209	0	0
217	0.071	0.056	0.032	0.037	0.020	0.181	0.097	0.129	0.064	0.045	0.122	0.032	0.068	0.523	0.012	0.040
219	0.048	0.042	0.032	0.073	0.020	0.028	0	0.029	0.077	0	0.031	0.223	0	0.256	0	0
221	0.024	0.028	0	0	0.080	0.028	0.032	0.014	0.013	0	0	0.032	0.176	0	0.012	0.040
223	0.024	0.069	0.032	0.037	0.060	0.014	0.032	0	0.026	0	0.02	0.085	0	0	0	0
225	0.012	0	0.021	0.073	0.240	0	0.081	0	0	0.076	0	0.011	0	0	0	0.020
227	0.036	0	0.032	0.024	0.100	0	0.145	0	0.013	0	0	0	0.243	0	0	0.420
229	0.036	0.014	0	0	0.120	0	0	0	0.013	0	0.011	0	0	0	0	0.060
231	0	0.028	0.011	0	0.060	0	0.113	0	0	0	0	0	0.014	0	0	0.060
233	0.024	0	0	0	0	0	0	0	0.051	0	0.011	0	0.189	0	0	0.260
235	0	0	0.011	0	0	0	0	0	0.038	0	0	0	0.027	0	0	0
237	0.024	0	0	0	0	0	0	0	0.013	0	0.01	0	0.014	0	0	0
239	0	0	0	0	0	0	0	0	0	0	0	0	0.149	0	0	0
247	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0	0	0
251	0	0	0	0	0	0	0	0	0	0	0	0	0.014	0	0	0
253	0	0	0	0	0	0	0	0	0	0	0	0	0.068	0	0	0
257	0	0	0	0	0	0	0	0	0	0	0	0	0.027	0	0	0
N	42	36	47	41	25	36	31	35	39	33	49	47	37	43	42	25

APPENDIX 3

Synthesis of conservation genetic designations for evaluated study populations

Appendix Table A-3-1

A comparative synthesis of previous status, composite genetic profiles, conservation genetic designations, and management recommendations given for the inland and river-spawning lake trout populations evaluated in this thesis. Previous status designations are from three sources. The Ontario Ministry of Natural Resources (OMNR) Lake Trout Atlas (OMNR 1989) was assembled from a questionnaire-based survey administered to regional managers in 1987. This survey identified populations that were considered indigenous and self-sustaining (category N1, N2, N4, or N6), maintained by supplementation with non-native hatchery strains (category N3 or N5), introduced (category I1 or I2), or of unknown status (category E, L, O, or U) for categorization. The river-spawner populations were not evaluated during compilation of the Lake Trout Atlas, but are currently considered extirpated by the OMNR. Several populations had been previously characterized by allozyme analysis (Ihssen et al. 1988; OMNR, unpublished data), and based on their genetic attributes they were classified as native, introgressed, or introduced populations. Stocking records obtained from the OMNR Ontario Fisheries Information System (OFIS) and regional fisheries managers indicated whether populations had been previously stocked. Composite genetic profiles were compiled from measured population-level microsatellite and mitochondrial DNA attributes, and categorized using the same status designations for the allozyme-based classification. The glacial lineages present in each population are indicated; mitochondrial haplotype designations follow (Grewe et al. 1993; Wilson and Hebert 1996, 1998). Evolutionary Significant Unit (ESU) designations follow criteria specified by Waples (1991) or Moritz (1994). For the former, native populations were grouped

according to regional hierarchical genetic structure: pure glacial ancestry (Mississippian-A), regionally mixed glacial ancestry (AB-ALG, a proglacial admixed lineage of Mississippian-A and Atlantic-B colonists in the Algonquin park region), and river-spawner ancestry (R). For the latter, ESU designations are strictly based on pure glacial lineages (Mississippian-A only), but numbered MU's correspond to populations showing significant divergence at nuclear loci. Exchangeability criteria are given by Crandall et al. (2000), and were used to compare the genetic distinctiveness of inland lake trout populations to Great Lakes populations. The null hypothesis of recent genetic exchangeability was rejected if populations were genetically similar in terms of microsatellite-based genetic diversity levels, divergence estimates, and genetic clustering. The null hypothesis of historical genetic exchangeability was rejected if populations shared a common evolutionary history, as indicated by the presence of mtDNA lineages. Recent ecological exchangeability was rejected if compared populations did not recently share similar habitat attributes and fish communities. Historical ecological exchangeability was rejected if indigenous populations were descendent from colonists that did not disperse through the proglacial lake network. Exchangeability categories are based on compiled exchangeabilities, and detailed in Crandall et al (2000): 1-2 treat as separate species, 3-4, 5a, 5b, treat as separate populations, 5c-7, treat as separate populations, but allow gene flow consistent with current genetic structure, 8- manage as single population. Summarized management recommendations are based on the compilation of past and present assessments. Asterisks indicate designations for populations with categorically intermediate genetic attributes.

Population	Thesis chapter	Previous status		
		LT Atlas	Allozyme	Stocked?
Miskwabi Lake	2	Introduced	Introduced	YES
Boshkung Lake	2	Supplemented	Introgressed	YES
Esson Lake	2	Supplemented	Native	YES
Farquhar Lake	2	Supplemented	Native	YES
Grace Lake	2	Supplemented	Native	YES
Macdonald Lake	2	Indigenous	Native	NO
Clean Lake	2	Indigenous	Native	NO
Redstone Lake	2	Indigenous	Native	YES
Kingscote Lake	2	Indigenous	Native	YES
Louisa Lake	2	Indigenous		NO
Smoke Lake	2	Indigenous		YES
Barker Lake	2	Supplemented		YES
Crystal Lake	2	Indigenous		NO
Dog River	3	<i>(extirpated)</i>		YES
Montreal River	3	<i>(extirpated)</i>		YES
Mishibishu Lake	3	Introduced	Introduced	YES
Mishi Lake	3	Introduced	Introduced	YES
Katzenbach Lake	3	Introduced	Introduced	YES
Cedar Lake	4	Indigenous		YES
Lake Kioshkokwi	4	Indigenous		YES
Lavieille Lake	4	Indigenous		NO
Dickson Lake	4	Indigenous		NO
White Partridge Lake	4	Indigenous		NO
Hogan Lake	4	Indigenous		NO
Lake LaMuir	4	Indigenous		NO
Big Trout Lake	4	Indigenous		NO
Happy Isle Lake	4	Indigenous		NO
Opeongo Lake	4	Indigenous	Native*	YES
Louisa Lake	4	Indigenous		NO
Big Porcupine Lake	4	Indigenous		NO
Timberwolf Lake	4	Indigenous		NO
Lost Dog Lake	4	Indigenous		YES

Population	Composite profile	mtDNA	Evolutionary Significant Unit		
			Waples (ESU)	Moritz (ESU)	Moritz (MU)
Miskwabi Lake	Introduced	A, B/D	NO	-	-
Boshkung Lake	Introgressed*	A, B/D	?	-	-
Esson Lake	Introgressed	A, B/D	NO	-	-
Farquhar Lake	Introgressed	A, B/D	NO	-	-
Grace Lake	Introgressed	A, B/D	NO	-	-
Macdonald Lake	Native	A	A	A	A1
Clean Lake	Native	A	A	A	A2
Redstone Lake	Native	A	A	A	A3
Kingscote Lake	Native	A,C	A	-	-
Louisa Lake	Native	A	A	A	A5
Smoke Lake	Native	A	A	A	A6
Barker Lake	Native	A	A	A	A7
Crystal Lake	Native	A	A	A	A8
Dog River	Native*	A,B/D,C	R	NO	R1
Montreal River	Native*	A,B/D,C	R	NO	NO
Mishibishu Lake	Introduced	A,B/D	?	-	-
Mishi Lake	Introduced	A,B/D,C	?	-	-
Katzenbach Lake	Introduced	A,B/D,C	?	-	-
Cedar Lake	Native*	A,B/D	AB-ALG	NO	AB1
Lake Kioshkokwi	Native*	A,B/D	AB-ALG	NO	AB2
Lavieille Lake	Native	A	A	A	A9
Dickson Lake	Native	A	A	A	A10
White Partridge Lake	Native	A,B/D	AB-ALG	NO	AB3
Hogan Lake	Native	A,B/D	AB-ALG	NO	AB4
Lake LaMuir	Native	A	A	A	A11
Big Trout Lake	Native	A	A	A	A12
Happy Isle Lake	Native	A	A	A	A13
Opeongo Lake	Native*	A,B/D	AB-ALG	NO	AB5
Louisa Lake	Native	A	A	A	A14
Big Porcupine Lake	Native	A	A	A	A15
Timberwolf Lake	Native	A	A	A	A16
Lost Dog Lake	Native	A	A	A	A17

Exchangeability with Great Lakes populations					
Population	Recent genetic	Historical genetic	Recent ecological	Historical ecological	Exch. Category
Miskwabi Lake	Accept	Accept	Reject	Accept	6
Boshkung Lake	Accept	?	Accept	?	?
Esson Lake	Accept	Reject	Accept	Reject	5c
Farquhar Lake	Accept	Reject	Accept	Reject	5c
Grace Lake	Accept	Reject	Accept	Reject	5c
Macdonald Lake	Reject	Reject	Reject	Accept	2
Clean Lake	Reject	Reject	Reject	Accept	2
Redstone Lake	Reject	Reject	Reject	Accept	2
Kingscote Lake	Reject	Reject	Reject	Accept	2
Louisa Lake	Reject	Reject	Reject	Accept	2
Smoke Lake	Reject	Reject	Reject	Accept	2
Barker Lake	Reject	Reject	Reject	Accept	2
Crystal Lake	Reject	Reject	Reject	Accept	2
Dog River	Reject	Accept	Reject	Accept	5a
Montreal River	Reject	Accept	Reject	Accept	5a
Mishibishu Lake	Accept	Accept	Reject	Accept	6
Mishi Lake	Accept	Accept	Reject	Accept	6
Katzenbach Lake	Accept	Accept	Reject	Accept	6
Cedar Lake	Reject	Accept	?	Accept	?
Lake Kioshkokwi	Reject	Accept	?	Accept	?
Lavieille Lake	Reject	Reject	Reject	Accept	2
Dickson Lake	Reject	Reject	Reject	Accept	2
White Partridge Lake	Reject	Reject	Reject	Accept	2
Hogan Lake	Reject	Reject	Reject	Accept	2
Lake LaMuir	Reject	Reject	Reject	Accept	2
Big Trout Lake	Reject	Reject	Reject	Accept	2
Happy Isle Lake	Reject	Reject	Reject	Accept	2
Opeongo Lake	Reject	Accept	?	Accept	?
Louisa Lake	Reject	Reject	Reject	Accept	2
Big Porcupine Lake	Reject	Reject	Reject	Accept	2
Timberwolf Lake	Reject	Reject	Reject	Accept	2
Lost Dog Lake	Reject	Reject	Reject	Accept	2

Population	Recommendations for conservation and management
Miskwabi Lake	Manage as put-grow-take.
Boshkung Lake	Manage as put-grow-take but may require further assessment.
Esson Lake	Manage as put-grow-take.
Farquhar Lake	Manage as put-grow-take.
Grace Lake	Manage as put-grow-take.
Macdonald Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Clean Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Redstone Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Kingscote Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Louisa Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Smoke Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Barker Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Crystal Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Dog River	Genetically distinct; do not supplement; develop stream-specific strain for rehabilitative stocking, if unavailable use only sanctuary lake population.
Montreal River	Genetically distinct; do not supplement; develop stream-specific strain for rehabilitative stocking, if unavailable use only sanctuary lake population.
Mishibishu Lake	Manage as sanctuary populations.
Mishi Lake	Manage as sanctuary populations.
Katzenbach Lake	Manage as sanctuary populations.
Cedar Lake	Requires further assessment.

Population, cont.	Recommendations for conservation and management, cont.
Lake Kioshkokwi	Requires further assessment.
Lavieille Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Dickson Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
White Partridge Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A and B ancestry population from lake with similar ecological attributes.
Hogan Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A and B ancestry population from lake with similar ecological attributes.
Lake LaMuir	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Big Trout Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Happy Isle Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Opeongo Lake	Requires further assessment.
Louisa Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Big Porcupine Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Timberwolf Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.
Lost Dog Lake	Genetically distinct; do not supplement; develop lake-specific strain for rehabilitative stocking, if unavailable use only regional A ancestry population from lake with similar ecological attributes.

APPENDIX 4

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From: Aaron Lerner
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