Genetic population structure of the round whitefish (*Prosopium cylindraceum*) in North America: multiple glacial refugia and regional subdivision

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Thomas David Morgan, candidate for the degree of Master of Science in Biology**,** has presented a thesis titled, ***Genetic population structure of the round whitefish (*Prosopium cylindraceum*) in North America: multiple glacial refugia and regional subdivision,*** in an oral examination held on November 30, 2016. The following committee members have found the thesis acceptable in form and content, and that the candidate demonstrated satisfactory knowledge of the subject material.

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ABSTRACT:

The round whitefish (*Prosopium cylindraceum*) is an understudied species of freshwater fish found throughout northern North America and eastern Eurasia. Little is known about the ecology or population genetics of this species. Round whitefish are sensitive to environmental disturbance and have declined in regions of eastern North America, prompting interest in their conservation and management. Understanding the population genetics and phylogeography of round whitefish will inform planning for this species. I genetically characterized round whitefish from 16 locations across North America, and one site in eastern Russia, using microsatellites, mtDNA sequencing, and thousands of SNP loci using a nextRAD approach. I determined phylogeographic and population genetic relationships across sites in Alaska, Yukon, and Northwest Territories as well as the Laurentian Great Lakes region. Genetic analyses resolved strong delineation between eastern and western populations of round whitefish, indicating that they originated from separate glacial refugia. Analyses of regional relationships highlighted the importance of Lake Huron as a source for round whitefish populations, and Lake Ontario as being disjunct from the other Great Lakes. Populations in Alaska and the Yukon showed evidence of historical gene flow, with contemporary patterns linked to the connectivity of river basins in that region. I conclude that round whitefish population structure exists on multiple spatial scales in North America reflecting the deeper phylogenetic relationships of Pleistocene glacial lineages, and shallower divergences reflecting contemporary connectivity due to hydrology. Management of round whitefish needs to consider these major scales by recognizing separate Designatable Units for eastern and western glacial lineages, and appropriate

Management Units based on contemporary connectivity. Isolated populations in disturbed areas, such as those in Lake Ontario, require particular attention because of the unlikelihood of rescue dispersal. Further study of this species is warranted to determine its status in Canada, and further identify isolated or sensitive populations.

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LIST OF ABBREVIATION AND SYMBOLS

AMOVA	Analysis of Molecular Variance
A_R	Allelic Richness. A measure of genetic diversity defined by the number of alleles at or across loci.
BLAST	Basic Local Alignment Search Tool. Identifies sequences by querying them against a database using sub-regions of high similarity.
BOLD	Barcode of Life Data Systems. A database of publically available DNA barcodes (Cytochrome c oxidase subunit I and others) associated with the International Barcode of Life (iBOL) initiative. Available at boldsystems.org
COI	Cytochrome c oxidase subunit I . A region of the eukaryotic mitochondrial genome coding a principal subunit of cytochrome c oxidase complex. A very common marker in species identification globally; its sequence and mutation rate having been characterized for thousands of species.
DU	Designatable Unit. Intraspecific designation assigned by the Committee on the Status of Endangered Wildlife in Canada when single status designation is thought not to represent the evolutionary diversity of a species.
ESU	Evolutionarily Significant Unit. Intraspecific designation indicating that a population or group represents a significant evolutionary portion of a species.
F_{ST}	Proportion of total genetic variance contained by the subpopulation (S) relative to the total genetic variance (T).
HWE	Hardy-Weinberg equilibrium. The equilibrium state of a locus that , in the absence of evolutionary influence, allele frequencies will remain constant through generations, and conform to Hardy-Weinberg genotype proportions ($p^2 + 2pq + q^2 = 1$).
K	The number of populations, as defined by the program STRUCTURE
MU	Management Unit. A local, demographically independent population managed as a separate unit.
MYA	Million years ago.

P	Proportion of polymorphic loci. The number of loci from the set analyzed that were polymorphic
RADseq	Restriction site-associated DNA sequencing. A series of reduced-representation techniques for sequencing a reproducible and customizable subset of a target's genome using restriction site targets.
SSR	Simple sequence repeats (microsatellites). Region of the genome characterized by short, repeated sequences of DNA (2-6 base pairs). Common marker for genotyping individuals due to their polymorphic nature within populations.
Q	Membership coefficients of individuals or populations as determined by the program STRUCTURE.
YBP	Years before present.

1. GENERAL INTRODUCTION

1.1 Freshwater Fisheries in North America

In North America, freshwater fisheries are culturally and economically important. Aboriginal, commercial, and recreational fisheries collectively harvest an estimated 487,989 tonnes of fish annually (Cooke and Murchie 2013), and the commercial value of freshwater fish harvested in Canada was approximately $63 million dollars (CAD) in 2014 (DFO 2016). The value of recreational freshwater fisheries was estimated to exceed $2 billion dollars annually (CAD; estimated from DFO 2012); however, 39% of freshwater fish are considered imperiled due to impending conservation threats (Jelks et al. 2008). Habitat alteration, invasive species, environmental pollutants, and overfishing have already caused the extirpation and extinction of many species in North America (Miller et al. 1989).

Management of freshwater fish and their habitats is required to prevent extirpations and extinctions, and ensure the long-term persistence of valuable fisheries resources. Freshwater systems are highly impacted by anthropogenic activities, including damming, invasive species, agricultural and industrial pollution, and overexploitation of fish (Dudgeon et al. 2006; Cooke and Murchie 2013; Dudgeon 2014). The accurate assessment and proper management of fisheries is required to prevent overharvesting and subsequent collapse of populations (Begg and Waldman 1999; Cooke and Murchie 2013). Historically, overexploitation of many North American fisheries occurred before any quantitative assessments. This resulted in a "shifted baseline", setting conservation and management goals based on already-impacted population numbers (Humphries and Winemiller 2009). To ensure proper management of fisheries, stocks should be classified

in a holistic manner, incorporating multiple assessment approaches (Begg and Waldman 1999; Taylor and Dizon 1999; Funk et al. 2012).

The correct determination of fish stocks, as large, self-reproducing populations of fish (Hilborn and Walters 1992), is critical to the proper management and conservation of species (Begg and Waldman 1999; Hilborn and Hilborn 2012). Overharvesting of local fish stocks risks their collapse and extirpation, and if minimally fed by migrants these populations would not be expected to regenerate on a contemporary timescale. Discrete stocks represent demographically independent groups with variable ability to self-sustain and respond to environmental and anthropogenic disturbances (Carvalho and Hauser 1994; Hilborn et al. 2003). A precautionary approach suggests treating temporally and spatially distinct spawning groups as discrete until proven otherwise (Stephenson 1999). The mismanagement and overfishing of fish stocks can lead to population collapse (Begg and Waldman 1999; Hilborn and Hilborn 2012), as well as changes in the biological characteristics, life history, and genetic diversity of populations (Kuparinen and Merilä 2007; Pukk et al. 2013; Pinsky and Palumbi 2014). Stock discrimination tools and techniques are therefore integral to determining appropriate spatial scales in the sustainable management of fisheries.

1.2 Genetic Markers in Fisheries Conservation and Management

In fisheries, molecular markers are one of the most effective means of assessing population structure. For example, strong population subdivision has been demonstrated in diadromous species (Wirth and Bernatchez 2001; Beacham et al. 2004; Taylor et al. 2011), those with varied larval dispersal distance (Palumbi 2003), and in those showing

local adaptation (Taylor and Bentzen 1993; Candy et al. 2015; Lemay and Russello 2015). Molecular markers are used to quantify levels of gene flow, migration among populations, levels of genetic diversity within populations, and divergence among populations (Shaklee and Bentzen 1998; Avise 2004; Lowe and Allendorf 2010); and in detecting introgression of non-native genes (Hauser 1991; Amish et al. 2012; Glover et al. 2013). Understanding population subdivision is particularly important where fish may regularly or seasonally migrate between multiple jurisdictions or into international waters, further risking overexploitation (Poff et al. 2003; Dudgeon et al. 2006). Ecosystem-based fisheries management emphasizes multiple species approaches rather than just those of economic importance (Gislason et al. 2000), and the wider utility to preserving biodiversity (Worm et al. 2006). Consequently, there is a current need to examine population structure in a wider variety of fish species.

The incorporation of genetic stocks enables management of groups that may otherwise be difficult to directly observe. The genetic stock concept defines populations as independent groups principally affected by birth and death rates, with minimal influences of immigration and emigration (Carvalho and Hauser 1994; Waples and Gaggiotti 2006; Ovenden et al. 2015). Genetic markers have provided critical insight into the monitoring of fishes for better management, such as in determining quotas, openings, and closures of specific Pacific salmon (*Oncorhynchus*) fisheries according to their management and conservation needs (Shaklee et al. 1999). Genetic markers have been applied in identifying the population structure caused by the natal philopatry (homing) of Pacific salmon species (Shaklee et al. 1999; Larson et al. 2014), the trans-oceanic relationships of Atlantic cod stocks (Hutchinson et al. 2001; Kovach et al. 2010), and the

population structure and monitoring of genetic diversity in Atlantic herring (Larsson et al. 2010; André et al. 2011) and Pacific sardine (Hedgecock et al. 1989). The genetic 'Management Unit' has been developed over the past several decades for the purpose of local conservation and management of species that have multiple stocks or populations (Moritz 1994; Palsbøll et al. 2007; Funk et al. 2012). However, most species have not had their genetic population structure characterized.

Molecular markers can be also used to assess the long-term viability of populations through quantification of genetic diversity. More diverse populations are better able to cope with changing environments and introduced pathogens (Allendorf and Leary, 1986; Ovenden et al. 2015). Preserving existing genetic diversity and connectivity may therefore support population viability over the long term, as populations can better adapt to shifting selection pressures (Mills and Allendorf 1996). The harvest of wild fish stocks has been shown to degrade genetic variability, as well as alter population subdivision, and introduce selective genetic changes (Allendorf et al. 2008; Marty et al. 2015). Therefore, genetic characterization and monitoring of species can enable managers to better implement stocking, translocation, and other management strategies without risking the degradation of existing population structure and genetic diversity.

The molecular tools used to analyze intraspecific genetic relationships have been evolving rapidly over the past several decades. Recently, the most prominent tools in the investigation of population genetics or phylogenetics have been microsatellite-genotyping, sequencing of mitochondrial DNA loci, or genotyping of single-nucleotide polymorphisms. Microsatellites, also known as simple sequence repeats (SSRs), are regions of the nuclear genome that are highly polymorphic for the number of repeated

motifs (usually 2-6 bp; Jarne and Lagoda 1996; O'Connell and Wright 1997). Selecting microsatellite loci that are sufficiently polymorphic within a species allows for genetic characterization of individuals and populations (O'Connell and Wright 1997). Significant divergence in the frequency and/or identity of microsatellite alleles between populations indicates that levels of gene flow and migration are lower than expected, and that these populations are likely separate demographic groups (Slatkin 1987; Waples and Gaggiotti 2006). The delineation of MUs based on these signals of separate demography allows for separate management of contemporary populations. While many definitions exist for delineating 'populations', the demographic cohesion and independent viability of contemporary groups is a key aspect underlying all of them (Booke 1981; McElhany et al. 2000; Waples and Gaggiotti 2006).

Sequencing of mitochondrial DNA has been integral to the study of evolutionary heritage (Moritz 1994; Avise 1995), and has elucidated many previously cryptic evolutionary relationships (Avise et al. 1979a; Avise et al. 1979b; Hebert et al. 2004; Ward et al. 2009). Mitochondrial DNA is maternally-inherited and has a lower effective population size than nuclear ones. This results in mtDNA loci achieving reciprocal monophyly, the coalescence of features within groups, much faster than nuclear loci (Brown et al. 1979; Neigel and Avise 1986; Moritz 1994). Reciprocal monophyly in mtDNA and highly restricted gene flow have been suggested as the main criteria for delineating 'Evolutionarily Significant Units' (ESUs), which indicate historical population structure and deeper genetic distinctiveness than generally applied to define MUs (Dizon et al. 1992; Moritz 1994). In fishes, molecular clocks have been used to better understand significant evolutionary events such as the whole-genome duplication

shared by all salmonids, dated to ~59.1MYA using mitochondrial loci (Crête-Lafrenière et al. 2012). The sequencing of mitochondrial loci and identification of distinct haplotypes has indicated divergence in fishes that isn't apparent through morphological traits (Mandrak and Crossman 1992; Avise 2004). The sequencing of mtDNA allowed the investigation of evolutionary relationships for fishes on timescales insufficient for clear morphological divergence (eg. in the case of recent glaciation events; Hocutt and Wiley 1986; Mandrak and Crossman 1992; April et al. 2013).

Massively parallel ('next-generation') sequencing and reduced-representation library preparation enables simultaneous discovery and genotyping of thousands of markers for non-model species (Shendure and Ji, 2008; Hauser and Seeb 2008; Andrews et al. 2016). Reduced-representation libraries allow for the reliable capture and sequencing of a subset of the genome, enabling researchers to capture thousands of SNPs within individuals, but also tailoring their sequencing effort to maximize the number of individuals that can be genotyped (Andrews et al. 2016). This approach significantly increases the resolution of population genetic analyses while also allowing for analyses of neutral and non-neutral loci, which were previously analyzed on a case-by-case basis. Reduced representation library preparation, such as restriction-site associated DNA sequencing which targets loci adjacent to selected restriction sites (RADseq; Miller et al. 2007; Baird et al. 2008), can be used to both characterize deeper phylogenetic relationships (Emerson et al. 2010; Rubin et al. 2012; Pante et al. 2014), as well as more contemporary measures of population structure (eg. Catchen et al. 2013a; Russello et al. 2015; Rodríguez-Ezpeleta et al. 2016).

Increased genotyping and sequencing capacity has enhanced the ability to characterize groups that have otherwise proven cryptic using traditional techniques (Waples and Gaggiotti 2006; Bradbury et al. 2015; Szulkin et al. 2016). The sequencing of thousands of loci has also resolved phylogenetic relationships for species on timescales that are not well suited for traditional techniques (eg. Emerson et al. 2010; Combosch and Vollmer 2015; Herrera and Shank 2015). Since the development of the original RADseq protocol, various alternative RAD protocols have been developed such as genotyping-by-sequencing (GBS; Elshire et al. 2011), ddRAD (Peterson et al. 2012), 2bRAD (Wang et al. 2012; Guo et al. 2014), ezRAD (Toonen et al. 2013), and nextRAD (see Russello et al. 2015). Each protocol has allowed for further customization of DNA libraries based on the specifics of the study (Andrews et al. 2016). As these techniques continue to develop, ecologists and evolutionary biologists are able to better apply rigorous molecular analyses to the study of intraspecific relationships without significant *a priori* knowledge and development of markers specific to the species.

1.3 Phylogeography and Population Structure of Post-Glacial Fishes

Phylogenetic divergence within a species is used to delineate populations of higher conservation concern through the designation of ESUs (Ryder 1986; Palsbøll et al. 2007; Funk et al. 2012). In Canada, the 'Designatable Unit' is analogous to the ESU, and is based on genetic distinctiveness, natural disjunction, or occupying different eco-geographic regions (COSEWIC 2015). Additionally, recognition of DUs can be assigned based on recognizing the evolutionary significance of populations such that, if lost, an important part of the evolutionary legacy of the species would be affected. Persistent

separation results in the differentiation of intraspecific lineages, which is reflected in reciprocal monophyly of genetic markers. The use of ESUs and DUs has been incorporated into the designation of species of conservation concern, under the Endangered Species Act in the United States (ESA; Waples 1991; Fallon 2007; Funk et al. 2012), and the Species at Risk Act (SARA) in Canada (Funk 2012; COSEWIC 2015). In Canada, assessment of intraspecific groups as DUs has led to legal protection for many regional populations, including the fish species: white sturgeon (*Acipenser transmontanus*), western brook lamprey (*Lampetra richardsoni*), eastern sand darter (*Ammocrypta pellucida*), coastrange sculpin (*Cottus aleuticus*), rainbow smelt (*Osmerus mordax*), and westslope cutthroat trout (*Oncorhynchus clarkii lewisi;* SARA public registry 2016). If assessed at the national level these species may not have qualified for protected status due to relative stability across their range, and threats risking only the extirpation of distinct regional populations. Recognition of DUs has allowed for better conservation and management of many species through finer-scale assessments; the proper assignment of unassessed species into DUs is therefore integral to their proper management.

Pleistocene glaciations, occurring as 20 events lasting ~100,000 years each in the last 2.5 million years (Dawson 2013), likely acted as a 'speciation pump' in North America (Haffer 1969; April et al. 2013). Each glaciation segmented the northern portion of North America and isolated species into separate refugia along the glacier peripheries (Pielou 1991; Ross 2013). This geographic isolation initiated phylogeographic separation into intraspecific glacial lineages in several species, and further facilitated allopatric speciation of already isolated lineages (Bernatchez and Wilson 1998; Avise and Walker

1998). In Nearctic fishes, subsequent secondary contact has since been resolved for many groups through analysis of their morphology, dispersal routes, and genetic relationships (Mandrak and Crossman 1992; April et al. 2013; Mee et al. 2015). These glacial lineages harbour a significant portion of the existing genetic variation for Nearctic fishes, and need to be conserved through the designation of separate DUs.

Species of fish found throughout Canada and the northern United States of America represent lineages that were confined to refugia during the most recent Pleistocene glaciation ~80,000-10,000 YBP (Dawson 2013). These fish subsequently spread to occupy their current ranges as glaciers receded and formed proglacial lakes (McPhail and Lindsey 1970; Mandrak and Crossman 1992; Bernatchez and Wilson 1998). Through analysis of their current ranges, proposed dispersal routes, and genetic variability, fish species have been associated with occupying at least nine separate glacial refugia (Mandrak and Crossman 1992; Ross 2013; Mee et al. 2015). DNA analyses have been applied to analyze the dispersal and secondary contact between certain intraspecific groups (Bernatchez and Wilson 1998; April et al. 2013), and dispersal from multiple refugia has been characterized for species such as lake trout (*Salvelinus namaycush*; Wilson and Hebert 1998), pygmy whitefish (*Prosopium coulterii;* Blanchfield et al. 2014), rainbow trout (*Oncorhyncus mykiss*; Tamkee et al. 2010), arctic grayling (*Thymallus arcticus*; Stamford and Taylor 2004), lake sturgeon (*Acipenser fulvescens*; McDermid et al. 2011), and lake whitefish (*Coregonus clupeaformis*; Foote et al. 1992; Mee et al. 2015). Where deeper genetic differences, such as those from glacial relict groups, indicate discrete and evolutionarily significant populations (COSEWIC 2015), recommendations have been made to assign these groups

as separate DUs. Despite the utility of identifying well delineated groups for their separate conservation and management consideration, many species still haven't been analyzed across their respective ranges for genetic signatures of distinct glacial lineages.

1.4 The Round Whitefish in North America

The round whitefish (*Prosopium cylindraceum*) is a freshwater fish species (Family: Salmonidae; subfamily: Coregoninae) found throughout northern North America and northeastern Eurasia (McPhail and Lindsey 1970; Scott and Crossman 1973). Within North America it occurs as two groups that appear to be geographically disjunct from each other; in the west populations occur from Alaska to northern Manitoba, and in the east from northern Ontario (Lake Nipigon) through to the east coast of Canada and northeastern United States (see Fig. 2; Global Biodiversity Information Facility data).

Round whitefish are elongate, cylindrical in cross-section, and typically 200-300mm in length and less than 1 kg in weight, though some have been observed at 550mm in length and ~2 kg in rare cases (Scott and Crossman 1973; this study). Round whitefish have a short head and pinched snout with one nasal flap (characteristic of the *Prosopium* genus) overhanging a small ventrally positioned mouth (Scott and Crossman 1973). Found mostly in freshwater lakes at depths of 2-35 meters (Rawson 1951), the round whitefish is a benthivore, feeding primarily on aquatic insects and gastropods (Rawson 1951; Normandeau 1969; MacPherson et al. 2010).

Spawning occurs for round whitefish in late November and early December once the water temperature reaches approximately 2-4.5°C, though this varies by region (Normandeau 1969; Scott and Crossman 1973). Round whitefish typically reach sexual

maturity at 178-330mm in length for males and 216-317mm for females (2-6 years; Bailey 1963). Round whitefish lay their eggs in gravelly cobble of lakes or river mouths at an average depth of 3.66 meters (Normandeau 1969). Their eggs then develop under the ice for approximately 140 days before emerging in spring (Normandeau 1969), though this is variable with temperature (Stewart et al. 2007).

Round whitefish were historically harvested in the Great Lakes with a yield up to 230,000 kg per year from Lake Michigan (Mraz 1964), 45,000 kg per year from Lake Huron (Bailey 1963), and an average of 12,000 kg per year in the U.S waters of Lake Superior (Bailey 1963). Targeted commercial harvest for this species decreased due to declining and inconsistent catch (typically less than 4500 kg per year in the Great Lakes; Mraz 1964; Ontario Commercial Fisheries Association data 1994-2013), as well as their smaller size compared to other species (Scott and Crossman 1973). Population sizes for round whitefish are difficult to determine relative to other coregonines in the Great Lakes; however, there is evidence of a decrease in round whitefish numbers in the main basin of Lake Huron (Ebener 2012). Despite its wide distribution throughout Canada as well as Alaska and the northeastern United States, the round whitefish has not been thoroughly studied, and little is known about its ecology or population status outside of the Great Lakes region.

Round whitefish persisted in ice-free refugia during the Wisconsinan glacial maximum, ~80,000-10,000 YBP (McPhail and Lindsey 1970; Dawson 2013). Based on their parasites and likely dispersal routes, round whitefish persisted in multiple glacial refugia: the Beringian in the west (McPhail and Lindsey 1970), and the Mississippian and Atlantic in the east (Mandrak and Crossman 1992). The Great Lakes have been

identified as a potential suture zone where populations from the two eastern refugia meet (April et al. 2013); however, for round whitefish this has yet to be confirmed using molecular markers.

Round whitefish are understudied within their group, with only local evaluations of their populations (e.g., Steinhart et al. 2007; Ebener 2012; Graham et al. 2016). They are thought to be especially sensitive to the adverse effects of invasive species and environmental disturbance (Steinhart et al. 2007; Patrick et al. 2013). Within Lake Huron, round whitefish are in decline in the main basin and parts of Georgian Bay; however, there is limited catch data for this species (Ebener 2012). Round whitefish are listed as Endangered in New York state, where they have been extirpated from the majority of their native lakes by the introduction of non-native species and changes in environmental pH (Steinhart et al. 2007), and critically imperiled due to rarity in Vermont (Vermont Department of Fish and Wildlife 2015) and New Hampshire (Nugent and Carpenter 2015). Despite this apparent sensitivity and uncertain conservation status, round whitefish populations have not been evaluated across most of their range. Thermal pollution can negatively impact diverse fish species, thus the observed sensitivity of round whitefish to thermal pollution has generated interest in using them as an indicator species. Plumes of heated water from nuclear generating stations, such as the Bruce Power site on eastern Lake Huron, have been speculated to be a threat to local spawning species such as round whitefish due to its shortening their embryos' time to hatch (Patrick et al. 2013). An understanding of whitefish population structure relative to nuclear generating stations is therefore of particular interest in order to provide context for their management in relation to the thermal plumes. Graham et al. (2016)

investigated isotopic niche characteristics and genetic population structure of lake and round whitefish adjacent to the Bruce Power site. Results from studying local fish aggregations indicated that if population subdivision for round whitefish exists within Lake Huron, it is at a wider scale than was observable within the 24 km of sampled shoreline. However, to date, Graham et al. (2016) represents the only study of genetic population structure for round whitefish. Wider genetic population structure of round whitefish should be investigated as a key component to understanding this species within the Great Lakes system, as well as across other freshwater systems and watersheds.

1.5 Thesis Objectives

This thesis is an investigation into the population genetics of round whitefish in North America. Round whitefish from 16 sites across North America, and one site in eastern Russia were analyzed using three genetic techniques: microsatellite genotyping, mtDNA sequencing of two loci, and nextRAD sequencing (a reduced-representation library protocol), in order to address the following major objectives:

i. Determine intraspecific phylogenetic relationships for round whitefish in North America.
ii. Relate phylogenetic data for round whitefish to putative glacial refugia for this species during the Wisconsinan glaciation
iii. Characterize the genetic population structure of round whitefish at regional and continental scales.
iv. Analyze contemporary migration rates for round whitefish through regional genetic relationships within Alaska-Yukon and the Laurentian Great Lakes.

Figure 1: The round whitefish (*Prosopium cylindraceum*); Sherman F. Denton. 1892. In: Pennsylvania Report of the State of Commissioners of Fisheries.

Genetic population structure of the round whitefish (*Prosopium cylindraceum*) in North America: multiple markers reveal glacial refugia and regional subdivision

2. INTRODUCTION

The round whitefish (Salmonidae; Coregoninae; *Prosopium cylindraceum*) has become a conservation concern in several regions of North America due to population decline and extirpation. In the northeastern United States several populations have been extirpated and the species is listed as Endangered in New York State (Steinhart et al. 2007), and critically imperiled in Vermont and New Hampshire (Vermont Department of Fish and Wildlife 2015; Nugent and Carpenter 2015). Round whitefish were once targeted for commercial harvest in the Laurentian Great Lakes (Mraz 1964; Bailey 1963); however, declining catch rates and a limited market have caused a reduction in harvest to less than 4,500 kg in Canadian waters (Ontario Commercial Fisheries Association data 1994-2013). Population data for most coregonines is limited in the Great Lakes, but recent evidence suggests a decline of round whitefish in the main basin of Lake Huron (Ebener 2012). Little is known about population trends or conservation status in other areas of the extensive round whitefish range.

Round whitefish are particularly sensitive to environmental and ecological changes. Invasive species and changes in pH are likely responsible for its extirpation from many lakes in New York (Steinhart et al. 2007). In addition, the round whitefish was listed as a valued ecological component and indicator species for environmental monitoring of thermal emissions by the power generating stations on the Canadian portion of the Great Lakes (Ontario Power Generation 2013; Ecometrix 2014). Currently, there are concerns about the effects of thermal emissions on round whitefish

development (Patrick et al. 2013), and associated impacts to local populations near point sources of pollution (Graham et al. 2016). Despite these concerns about the status and sensitivity of round whitefish locally and regionally, the appropriate geographic scale for long-term management of this species is unknown.

Round whitefish populations in North America are likely subdivided on multiple geographic scales relevant to their management. On the continental scale, the range of the species appears to be divided into two large areas that are disjunct from one another (see Fig. 2, Scott and Crossman 1973; Global Biodiversity Information Facility). During previous glacial maxima, fish were isolated within at least nine identified glacial refugia (Mandrak and Crossman 1992; Ross 2013; Mee et al. 2015), from which they propagated after the glaciers receded. The resulting pattern is reflected both in a latitudinal shift of intraspecific genetic variation for freshwater species north of 46° (Bernatchez and Wilson 1998), as well as the detection of distinct glacial lineages using genetic markers (eg. Wilson and Hebert 1998; April et al. 2013; Mee et al. 2015). The delineation of separate glacial lineages is of principal importance in determining conservation priorities for species based on unique evolutionary history (Dizon et al. 1992; Palsbøll et al. 2007; Funk et al. 2012). The disjunct range of round whitefish in North America has led to the hypothesis that they persisted in at least two glacial refugia during the Wisconsinan glaciation: the Beringian in the west, and the Mississippian in the east (McPhail and Lindsey 1970; Scott and Crossman 1973). There is currently a paucity of genetic data to address the hypothesis of separate glacial lineages for round whitefish in North America.

Regionally, within both the eastern and western portions of their range round whitefish occupy multiple watersheds that are variable in connectivity, and likely the

amount of corresponding gene flow. For example, the upper Great Lakes are contiguous and hydrologically connected, enabling fish movement, but many inland lakes occupied by round whitefish are completely isolated from one another. These hydrological features that affect connectivity and gene flow should be reflected in the resulting level of genetic differentiation among fish in different bodies of water (Pringle 2003; Waples and Gaggiotti 2006). Molecular tools have been instrumental in quantifying levels of connectivity between populations, as well as detecting the biogeographic relationships of glacial lineages (eg. Bernatchez and Wilson 1998; April et al. 2013); however, for round whitefish there has yet to be any analyses of genetic population structure beyond very local fine-scale, applications (see Graham et al. 2016).

The use of different molecular markers allows for varying perspectives on population structure and phylogeography. Microsatellites are well suited for characterizing contemporary population connectivity and genetic diversity, and have been a significant contributor to studies of population genetics for the past several decades (Avise 2004; Schwartz et al. 2007; Dudgeon et al. 2012). Mitochondrial DNA (mtDNA) SNPs achieve reciprocal monophyly more quickly than in nuclear DNA due to their lower effective population size (Neigel and Avise 1986); consequently, mtDNA loci are one of the principal delineators of Evolutionarily Significant Units (ESUs; Ryder 1986; Palsbøll et al. 2007; Funk et al. 2012). The analysis of mtDNA sequences has been instrumental in the demonstration of distinct glacial lineages for North American freshwater fishes such as lake trout (Wilson and Hebert 1998), pygmy whitefish (Blanchfield et al. 2014), rainbow trout (Tamkee et al. 2010), arctic grayling (Stamford

and Taylor 2004), lake sturgeon (McDermid et al. 2011), and lake whitefish (Mee et al. 2015).

More recently, the advent of massively parallel ('next-generation') sequencing has enabled genotyping of tens of thousands of loci in non-model species (Shendure and Ji, 2008; Nielsen et al. 2011). The subsequent development of reduced-representation libraries such as restriction-site associated DNA sequencing (RADseq; Miller et al. 2007; Baird et al. 2008) and others such as nextRAD (Russello et al. 2015), have enabled the customization of sequencing such that depth and number of loci sampled can be tailored to the individual study (Andrews et al. 2016). These approaches have been applied to the study of phylogenetic relationships (eg. Emerson et al. 2010; Rubin et al. 2012; Pante et al. 2014), as well as genetic diversity and connectivity (eg. Catchen et al. 2013a; Russello et al. 2015; Rodríguez-Ezpeleta et al. 2016). Together, the application of these molecular techniques enables thorough characterization of population structure on both contemporary and deeper timescales with unprecedented resolution. However, round whitefish remain poorly studied relative to other postglacial fishes, and there is a current need to understand their population structure on multiple spatial and temporal scales.

Here I present the first broad-scale study of North American round whitefish population genetics and phylogeography. I applied microsatellite-genotyping, mtDNA loci, and nextRAD sequencing to address the following major objectives: (1) characterize intraspecific phylogenetic relationships for round whitefish in North America; (2) relate phylogenetic data for round whitefish to putative glacial refugia during the Wisconsinan glaciation; and (3) characterize the genetic population structure of round whitefish at local, regional, and continental scales using traditional and next

generation techniques. The overall aim was to provide as much perspective on the population structure for this species as possible. In addition, I chose to emphasize analyses of fish from the Laurentian Great Lakes due to the high impact of invasive species and environmental disturbance on the region, as well as its economic importance as the largest freshwater fishery in the world (Kohler and Hubert 1999). This work will provide valuable insight into the future management of round whitefish.

3. MATERIALS AND METHODS

3.1 Sample Collection and DNA Isolation

I obtained samples of round whitefish tissue for DNA extraction opportunistically from sampling done by various groups (Table 1). Tissue samples (pectoral fin clip, adipose fin clip, or muscle stored in 95% ethanol, or lysis buffer) were obtained for round whitefish from various sites across North America and one site in eastern Russia. From the western portion of the round whitefish range, the samples included fish from six different watersheds. The north Alaska site was north of the Brooks Range within the north slope watershed that drains into the Arctic Ocean, and the south Alaska sites were within the Nushagak River Basin. Bennett and Little Salmon Lakes are part of the Yukon River Basin, while Simpson Lake, the Rat River sites, and Great Bear Lake are within watersheds of the Mackenzie River system (the Liard, Peel, and Great Bear Lake watersheds, respectively; Benke and Cushing 2005). In the eastern part of the range, the samples include round whitefish caught in each of the Laurentian Great Lakes, Lake Nipigon, and one site in Labrador, Canada (Fig. 2).

Genomic DNA was extracted from 414 samples of round whitefish tissue following the manufacturer's guidelines (Genomic DNA Isolation Kit, Norgen Biotek Corp., Ontario, Canada). However, I extended lysis to 12-14 hours at 55°C and performed the optional step of treatment with RNase A (Qiagen Inc., Ontario, Canada). DNA concentration was determined using a Qubit 2.0 Fluorometer (Life Technologies Inc., Ontario, Canada). Subsets of the 414-sample collection were then analyzed using various molecular techniques as indicated (Table 1).

3.2 Mitochondrial DNA Sequencing

Portions of the control region (D-loop) and COI barcode region were PCR-amplified for 124 round whitefish individuals representing 7-12 samples from each site, with the exceptions of Russia (n=3) and the Rat River site in the Yukon (n=4). The Lake Michigan – Door County fish were excluded from this analysis due to the close proximity between this location and the Lake Michigan – Milwaukee site. Loci were amplified as described in Delling et al. (2014; D-loop) and Ward et al. (2005; COI), resulting in amplicons of approximately 400 bp and 655 bp respectively. PCR products were purified using MinElute PCR Purification Kits (Qiagen Inc., Ontario, Canada) and Sanger-sequenced commercially (University of Calgary Core DNA Services; See Table 1 for sample details).

Table 1: Source information for round whitefish samples from 16 sites across North America and one site in eastern Russia. Information includes site name, GPS coordinates, number of total samples (N), the year collected, the number of individuals included in mtDNA analyses (mtDNA), microsatellite analyses (microsatellites), nextRAD analyses (NextRAD), and additional notes on source including catalog information for museum specimens.

Location	GPS coordinates	N	Year	MtDNA	Microsatellites	NextRAD	Source
North Alaska (N-AK)	N70°00'43", W153°09'11"	8	2014	8	8	8	University of Alaska Museum Ichthyology collection – catalog number 8068
South Alaska (S-AK)	N59°19'16", W156°19'07"	16	2014	8	15	16	University of Alaska Museum Ichthyology collection – catalog number 9114 & 9136
Yukon – Bennett Lake (YK-Ben)	N60°03'23", W156°33'50" N60°04'20", W134°52'26"	20	2014	9	18	8	Environment Yukon – Jul 2014
Yukon – Little Salmon Lake (YK-LSa)	N62°11'10", W134°42'05"	20	2015	7	19	3	Environment Yukon – Jul-Aug 2015
Yukon – Simpson Lake (YK-Sim)	N60°43'28", W129°14'35"	20	2014	8	19	10	Environment Yukon – Jun 2014
Lake Huron (LHU)	N44°23'56", W81°31'51"	61	2010-2012	9	60	27	This study
	N44°22'31", W81°33'21"						
	N44°21'22", W81°35'10"						
	N44°20'25", W81°35'32"						
	N44°17'51", W81°36'33"						
	N44°16'54", W81°36'18"						
	N44°15'44" W81°36'52"						
	N44°23'17", W81°31'57"						
Northern Georgian Bay (NGB)	N45°56'58", W81°30'10"	20	2014	8	19	11	RL Eberts (This study)
Southern Georgian Bay (SGB)	N44°34'19", W80°04'41"	27	2014	7	27	9	RL Eberts (This study)
	N44°31'00", W80°06'18"						
Lake Ontario (LON)	N43°51'53", W78°44'10"	62	2012, 2014	7	60	32	7 sites within 4 km of this point
	N43°48'08", W79°03'15"						6 sites within 1 km of this point This study Dec 2012 and Ontario Ministry of Natural Resources and Forestry – Nov-Dec 2014
Lake Michigan – Milwaukee (LMI-M)	N42°59'11", W87°49'25"	36	2015	8	36	16	Wisconsin Department of Natural Resources – Jun 2015
Lake Michigan - Door County (LMI-D)	N45°06'20", W87°02'46"	30	2015	0	30	0	Wisconsin Department of Natural Resources – Nov 2015
	N45°00'18", W87°07'00"						
	N44°54'08", W87°11'46"						
Lake Nipigon (LNI)	N50°00'24", W88°54'29"	15	2015	8	14	15	Ontario Ministry of Natural Resources and Forestry – Sept 2015
	N49°53'24", W88°57'57"						
Lake Superior (LSU)	N48°23'45", W89°02'02"	41	2015	12	40	16	Sites within 120 km of each other. Tested for differentiation between sites before being combined; Ontario Ministry of Natural Resources and Forestry – Sept 2015
	N48°50'05", W88°06'19"						
	N48°44'36", W86°28'53"						

Labrador – T-Bone Lake (LAB)	N56°09'10", W63°56'21"	25	2010	9	25	16	Dalhousie University – from T-Bone Lake. The system is described in McCracken et al. 2013
Russia – Taniorer River (RUS)	N66°09'17", E175°45'44"	3	2005	3	0	3	Swedish Museum of Natural History Ichthyology collection – catalog numbers NRM52850, NRM57539, NRM57540
Northwest Territories – Rat River (NTRR)	N67°44'57", W136°17'10" N68°17'56", W136°21'20"	5		4	0	0	Department of Fisheries and Oceans
Great Bear Lake (GBL)	N65°08`08", W123°14'40"	10		9	0	0	Department of Fisheries and Oceans

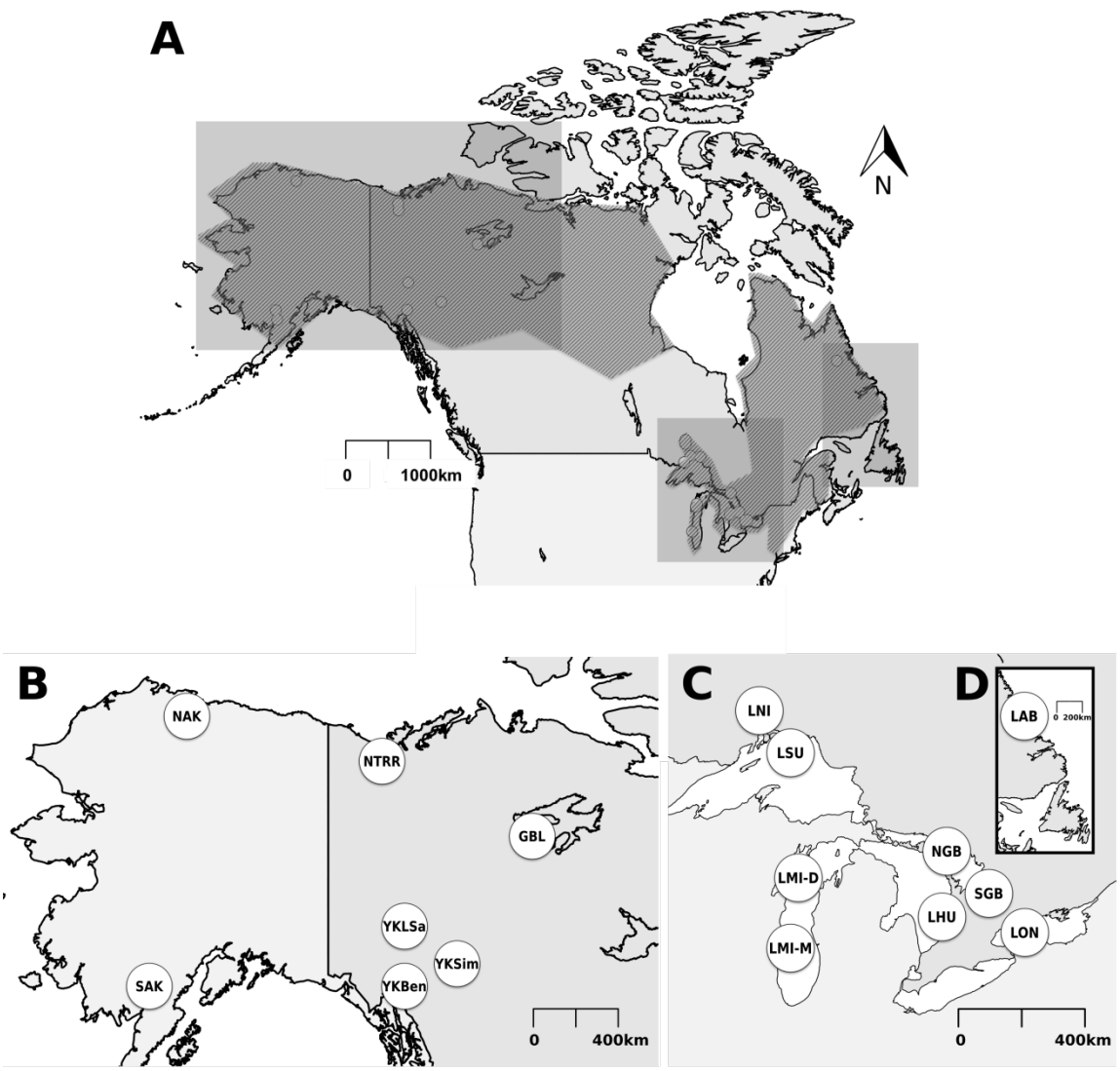

A
N
0
1000km
B
NAK
NTRR
GBL
YKLSa
YKSim
YKBen
SAK
0
400km
C
D
LNI
LSU
LAB
0 200km
NGB
LMI-D
SGB
LHU
LON
LMI-M
0
400km

Figure 2: Map of round whitefish range (dark grey; Global Biodiversity Information Facility data) and sampling sites in A: North America, B: Alaska and Yukon regions, C: Great Lakes region, and D: Labrador. Shaded boxes in A indicate areas enlarged in B, C, and D. Site abbreviations NAK = north Alaska, SAK = south Alaska, YKBen = Bennett Lake, YKLSa = Little Salmon Lake, YKSim = Simpson Lake, LHU = Lake Huron main basin, NGB = north Georgian Bay, SGB = south Georgian Bay, LON = Lake Ontario, LMI = Lake Michigan, LNI = Lake Nipigon, LSU = Lake Superior, LAB = Labrador.

3.3 Microsatellite Genotyping

Round whitefish were genotyped at 11 microsatellite loci previously developed for this species (O'Bryhim et al. 2013; Graham et al. 2016; Details in Table A1). I genotyped individuals from 14 sites that had DNA for 8 or more fish (n=390). Samples were included for five sites in the western range (the Yukon and Alaska), eight sites in the Great Lakes region, and one site in Labrador. Microsatellite loci were amplified as described in Graham et al. (2016), then size-fractionated using a Beckman Coulter GenomeLab GeXP Genetic Analysis System with a 400 bp internal size standard (Beckman Coulter, Mississauga, ON). Genotypes were determined using GENEMARKER 2.20 software (Softgenetics, State College, PA) with a bin-width of one nucleotide.

Round whitefish genotype data were assessed for scoring errors and null alleles (Micro-Checker v2.2.3; Van Oosterhout et al. 2004), and for conformation to Hardy-Weinberg Equilibrium (HWE) within each of the 14 presumptive populations (Genepop v4.3; Rousset 2008). A sequential Bonferonni correction was applied to account for multiple HWE tests. Individuals with complete data for at least seven loci were retained in all subsequent analyses. Prwi56 and Prwi72 showed evidence of null alleles; these loci were excluded from subsequent analyses. The nine remaining loci conformed to HWE for all populations and were retained for subsequent analyses; all 390 individuals had data for at least seven of nine loci.

3.4 NextRAD Sequencing

Genomic DNA for 190 round whitefish samples from 14 sites was converted into nextRAD genotyping-by-sequencing libraries (SNPsaurus, LLC) as in Russello et al. (2015). Sites included in the analysis were those that had high quality DNA for >8 individuals, with the exceptions of Little Salmon Lake in the Yukon and Russia (n=3 in both cases). Briefly, genomic DNA was first fragmented with Nextera reagent (Illumina, Inc), which also ligates short adapter sequences to the ends of the fragments. The Nextera reaction was scaled for fragmenting 7 ng of genomic DNA, although 15.75-17.5 ng of genomic DNA was used for input to compensate for degraded DNA in the samples. Fragmented DNA was then amplified, with one of the primers matching the adapter and extending 9 nucleotides into the genomic DNA with the selective sequence GTGTAGAGC. Thus, only fragments starting with a sequence that can be hybridized by the selective sequence of the primer will be efficiently amplified. PCR amplification was done at 73°C for 26 cycles. The nextRAD libraries were sequenced on a HiSeq 2500 with 100-bp reads (University of Oregon).

NextRAD data were uploaded to an online Galaxy analysis platform. FASTQ files were first processed using Trimmomatic (Bolger et al. 2014); Nextera adaptors were trimmed, long reads were trimmed to 100bp, and low quality/short reads (<100bp) reads were removed from subsequent analyses. The remaining reads were analyzed using FastQC (Galaxy version 0.64; Andrews 2010) and individuals containing <100,000 reads were removed. FastQC analysis indicated eight samples with low read numbers, six from Lake Ontario, and one each from Lake Huron and Lake Nipigon sites. These samples

were excluded from subsequent analyses leaving 182 samples with a mean read number of 1,031,989 +/- 452,088.

Sequences for all individuals used for phylogenetics and population structure (see below) were analyzed using Stacks 1.41 (Catchen et al. 2013b). Putative loci were assembled into 'stacks' using *ustacks* with a minimum stack depth (-m) of 3, and 2 mismatches allowed between stacks (-M). A catalogue of the loci was then constructed for all individuals using *cstacks* allowing a mismatch (-n) of 3 between sample tags. Sets of stacks were then searched against the catalogue using *sstacks*.

3.5 Phylogenetic Analyses

MtDNA

Trace files were compiled and edited in CodonCode Aligner v.6.0.2 (CodonCode Corporation, Centerville, MA, USA), then trimmed for quality and concatenated in MEGA6 (Tamura et al. 2013). Unique haplotypes were identified in the final alignment and concatenated sequences were run through Modeltest in Paup4.0a147 (Swofford 2002) to determine the best model of sequence evolution.

Phylogenetic tree building analysis of mtDNA was first conducted using a maximum-likelihood approach with the Russian samples designated as the outgroup. ML trees were inferred using the GTRCAT model in the PTHREADS version of RAxML v8.2.8 (Stamatakis 2014), using the default bootstrap procedure with robustness tested using 1000 replicates. A Bayesian search of tree space was also conducted using MrBayes v.3.2.6 (Ronquist et al. 2012) and seeded with the RAxML tree. I used four chains under the HKY+I model until after 7×10^6 generations the standard deviation of

split frequencies fell below 0.01. Haplotypes for the concatenated sequences were determined and mapped back to their putative population. COI haplotypes were compared to round whitefish sequences already on the International Barcode of Life Database (BOLD) using the BLAST tool (Ratnasingham and Hebert 2007).

NextRAD

For phylogenetic analyses of deeper relationships, a consensus sequence (with IUPAC ambiguity codes) was exported for each population using *Populations* in full-sequence Phylip format. To account for unequal sample sizes, consensus sequences were based on the three samples from each site with the most raw reads after Trimmomatic filtering. Loci present in 50% of individuals within a population, in at least 12 of the 14 presumptive populations, and with a minimum read depth of 3x were exported. The matrix of RAD loci was used to determine phylogeographic patterns among locations. Maximum likelihood and Bayesian trees were constructed using the PTHREADS version of RAxML v7.2.7 (Stamatakis 2014) and MrBayes v3.1.2 on CIPRES (Miller et al. 2010) following the same procedure as for the 2 mitochondrial loci. The samples from Russia were used as an outgroup around which the tree was rooted.

3.6 Population Structure

Microsatellites

Continental and regional population structure were analyzed using three types of analyses. First, a distance matrix was created for all sites using an AMOVA (Excoffier et al. 1992) to calculate pairwise F_{ST} between presumptive populations (Weir and

Cockerham 1984; GENODIVE; Meirmans and Van Tienderen 2004; Table 2). Measures of genetic diversity were determined for each population (GENODIVE), including a measure of allelic richness rarefied to the smallest sample size of eight individuals (HP-Rare; Kalinowski 2005).

Population structure was further analyzed using discriminant analysis of principal components (DAPC; Adegenet; Jombart 2008, Jombart and Ahmed 2011). Ellipses were generated hierarchically for all sites, Alaska and Yukon sites, and Great Lakes sites, using one third of principal components (85, 26, and 63 respectively); group delineations were then determined through visual examination of their separation along the first and second principal axes.

Contemporary migration rates were determined for sites in Alaska and the Yukon, and the Great Lakes region using the program BAYESASS 3.0 (Wilson and Rannala 2003). I ran BAYESASS separately on the Alaska and Yukon sites, and the Great Lakes sites using 5×10^6 iterations with a burn-in of 1×10^6 and sampling every 2000 iterations; mixing parameters were adjusted to $f = 0.35$ and $a = 0.35$ to maintain optimal values between 0.20 and 0.60 (Wilson and Rannala 2003).

The Bayesian clustering program STRUCTURE was run on all samples to determine the most likely number of genetic groups (Pritchard et al. 2000). The analysis was run 10 times with K ranging from 1 to 16 to account for the maximum number of populations (14) and additional unanticipated sub-structure. Each run consisted of a burn-in of 100,000 followed by 100,000 MCMC steps. STRUCTURE was then run hierarchically on the western sites (Alaska and the Yukon) with K ranging from 1 to 7,

the Great Lakes sites and Labrador with K ranging from 1 to 11, and the Great Lakes sites on their own with K ranging from 1 to 10.

After each STRUCTURE run, the most likely number of genetic groups was determined using the methods of Evanno et al. (2005) in the program STRUCTURE HARVESTER v0.6.94 (Earl and vonHoldt 2012). Results of all 10 runs were combined for the most likely K value using the Greedy algorithm of CLUMPP (Jakobsson and Rosenberg 2007), and membership coefficients for individuals were visualized using the program DISTRUCT (Rosenberg 2004).

NextRAD

Single-SNP genotypes for loci present in 50% of individuals, found in at least 3 of 14 populations, with a minor allele frequency of 0.05, and with a read depth of at least 6x were used for population structure analyses. These analyses retained the relative frequencies of SNPs within populations and allowed for finer-scale population metrics. Loci were outputted and checked in PLINK1.07 (Purcell et al. 2007) for conformation to Hardy-Weinberg equilibrium ($P<0.05$) in the 13 sites with >8 samples. Loci that didn't conform to HWE in two or more populations were used to create an exclusion list for *Populations* module of *Stacks*, and reduced datasets were re-exported in STRUCTURE format, as well as to calculate F_{ST} values using the *Populations* p-value fst correction of 0.05. This ensured I captured allele frequency data for neutral loci in each comparison, and with reasonable confidence in calling homozygous and heterozygous individuals where present. Hierarchical runs of STRUCTURE were implemented using STRAUTO1.0 (Chhatre et al. 2016) to determine the number of genetic groups at

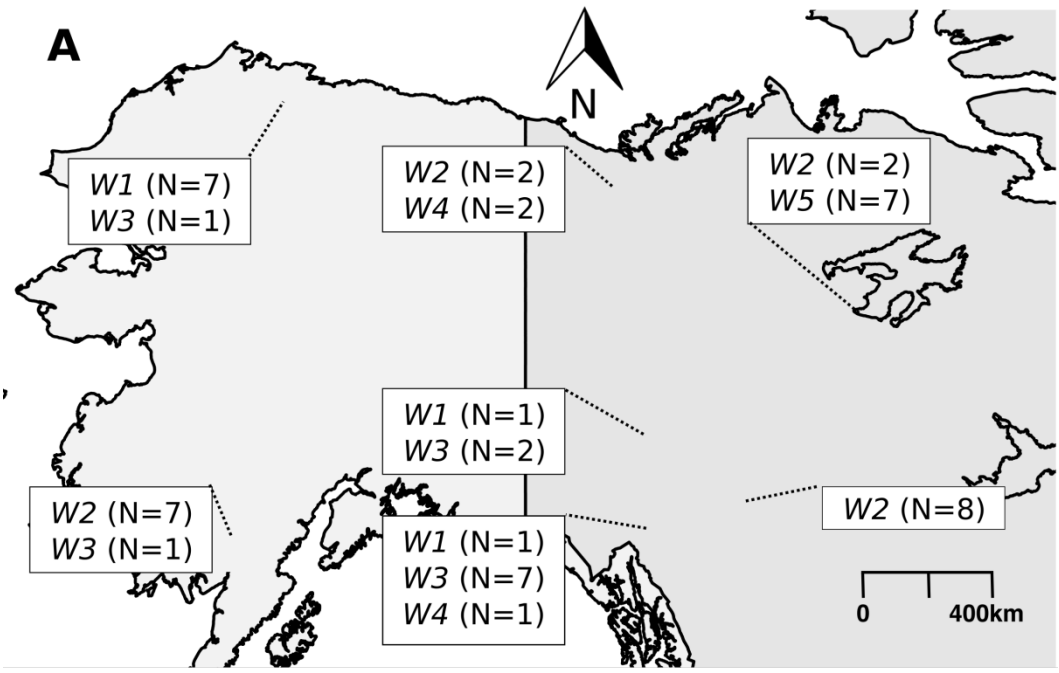
continental and regional levels until no further substructure was detected. Each analysis was run 10 times with a burn-in of 100,000 followed by 100,000 MCMC steps. K was tested for values ranging from 1 to 15 for all sites, then 1 to 8 for western sites, 1 to 10 for eastern sites, and 1 to 10 for the Great Lakes. The most likely number of genetic groups was determined as described above for microsatellite loci.

4. RESULTS

4.1 Phylogenetic Analyses

MtDNA

Edited sequences for D-loop and COI yielded 354 and 578bp respectively for a concatenated sequence of 932bp. Phylogenetic analyses using the concatenated sequences yielded 11 haplotypes for RWF across North America and two haplotypes for samples from eastern Russia (Fig. 3; Table A1). Five haplotypes were exclusively found in Alaska, Yukon, and Northwest Territories sites (designated *W1, W2, W3, W4* and *W5;* n = 10, 19, 14, 3, and 7 respectively). Six haplotypes were found exclusively in the Great Lakes and Labrador sites (designated *E1-E6*). In the east, the *E3* and *E6* haplotypes represented the majority of samples (n= 30 and 31 respectively of 68 total). Tree building analyses were unable to resolve the relationships between most haplotypes with high degrees of confidence; however, Russian haplotypes were strongly assigned to the outgroup, and within North America there was moderate support for *E1*, *E2*, *E3*, and *E4* sharing a lineage, and strong support for *E5* and *E6* being from a shared lineage more closely associated with *W1, W2*, and *W3* (Fig. 4). COI sequences were identified through


A
N
W1 (N=7)
W3 (N=1)
W2 (N=2)
W4 (N=2)
W2 (N=2)
W5 (N=7)
W1 (N=1)
W3 (N=2)
W2 (N=7)
W3 (N=1)
W2 (N=8)
W1 (N=1)
W3 (N=7)
W4 (N=1)
0
400km

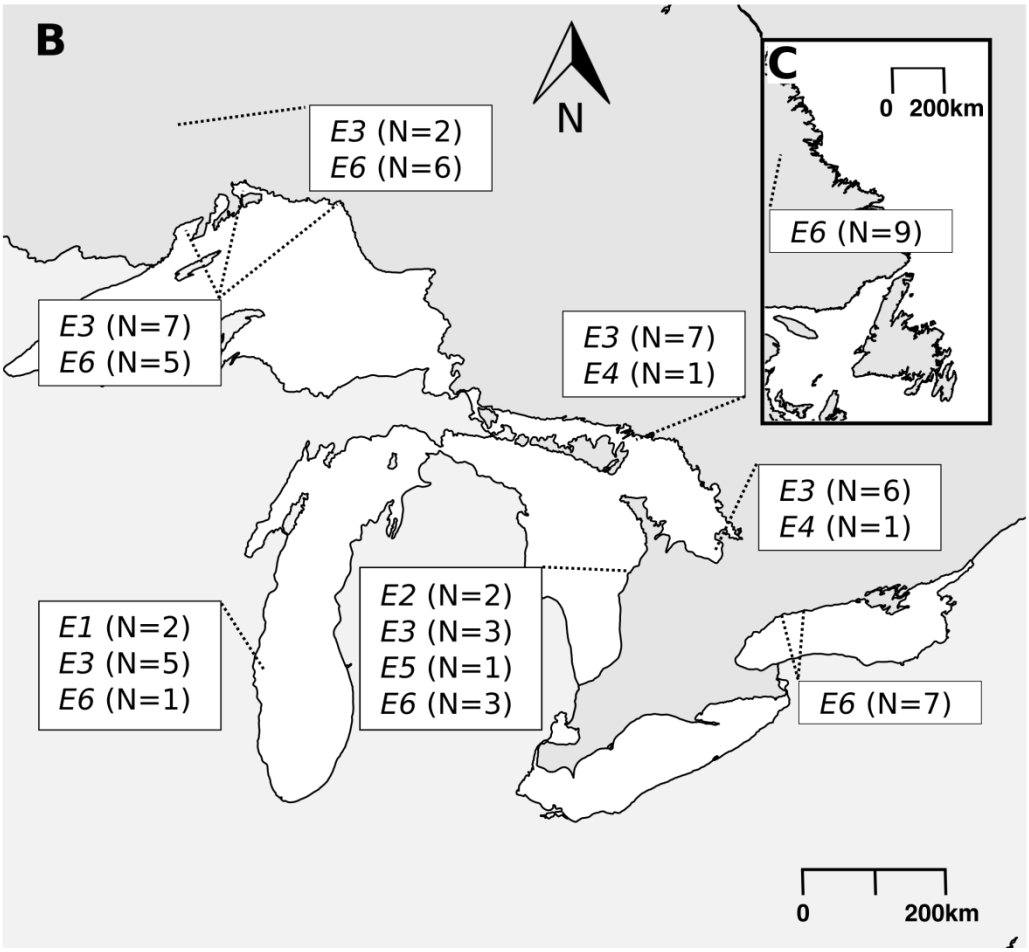

B
N
C
0 200km
E3 (N=2)
E6 (N=6)
E6 (N=9)
E3 (N=7)
E6 (N=5)
E3 (N=7)
E4 (N=1)
E3 (N=6)
E4 (N=1)
E2 (N=2)
E3 (N=3)
E5 (N=1)
E6 (N=3)
E1 (N=2)
E3 (N=5)
E6 (N=1)
E6 (N=7)
0
200km

Figure 3: Haplotype presence for concatenated D-loop-COI sequences (354 and 578 nucleotides) for A) Seven sites in the Alaska and Yukon regions, B) 7 sites in the Laurentian Great Lakes region, and C) one site in Labrador. W1-W5 = western haplotypes, E1-E6 = eastern haplotypes.

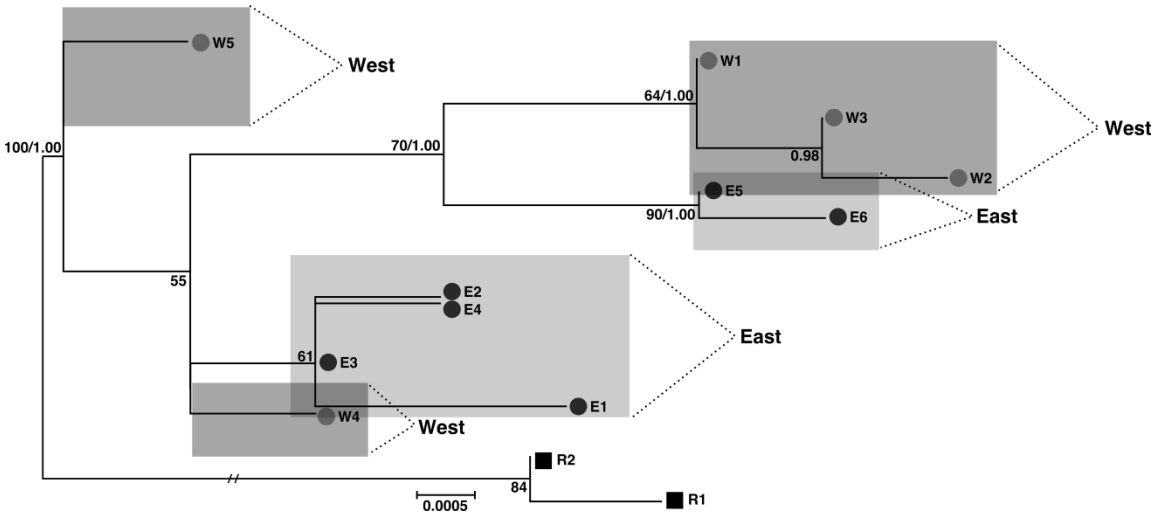


Figure 4: Phylogenetic tree for concatenated D-loop-COI sequences (354 and 578 nucleotides) for round whitefish from 15 sites in North America and one site in eastern Russia. E1-E6 = eastern haplotypes, W1-W5 =western haplotypes, R1-R2 = Russian haplotypes. Node supports shown for Maximum-Likelihood bootstrap (>50) and Bayesian values (>0.90). Scale bar showing number of substitutions per base pair.

BLAST as closest to round whitefish for all samples. North American round whitefish returned a >99.5% match to sequences already on BOLD while round whitefish from Russia returned a top hit of 98.25% sequence similarity to round whitefish.

NextRAD

Stacks analyses yielded a matrix of 4918 loci for phylogenetic analysis. Relationships among major regions resolved with high support (Fig. 5); the eastern sites were all highly differentiated from the western and Russian sites (ML-bootstrap = 100, Bayesian = 1.00). There was also high support (Bayesian = 1.00) for western sites being distinct from the Russian site. The branch lengths between the western sites and the Great Lakes were 0.00112 substitutions/site compared to 0.00041 substitutions/site between the Great Lakes and Labrador (2.7X difference). Whereas, the Russian group, separated from the east by a branch length of 0.00421 substitutions/site, and the west by 0.00367 substitutions/site (3.8X and 3.3X the distance from each other). Within the western region individual sites resolved with moderate support in the maximum likelihood analysis (ML > 60) and with moderate to high support in the Bayesian analysis (>0.80). In the east there was strong support separating Labrador from all the Great Lakes sites (ML = 75, Bayesian = 1.00). Lake Nipigon and Lake Superior resolved as their own group from the other Great Lakes (ML = 94, Bayesian = 1.00), and there was moderate to strong support for Lake Ontario being separate from Lake Huron, Lake Michigan, and Georgian Bay (ML >50; Bayesian = 1.00). Further moderate to high

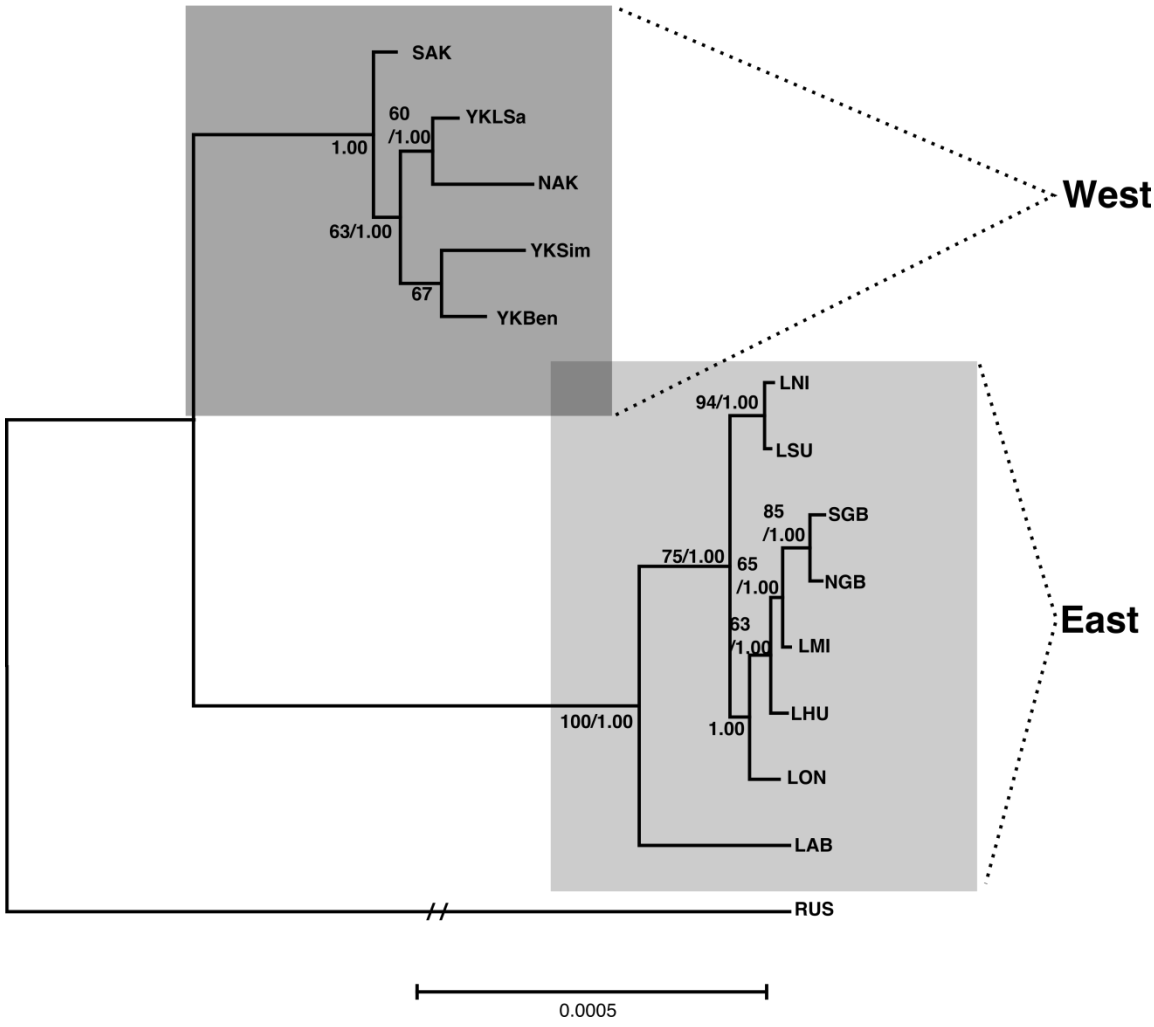

SAK
60
/1.00
YKLSa
1.00
NAK
63/1.00
YKSim
67
YKBen
West
LNI
94/1.00
LSU
85
/1.00
SGB
75/1.00
65
/1.00
NGB
63
/1.00
LMI
East
100/1.00
LHU
1.00
LON
LAB
RUS
0.0005

Figure 5: Phylogenetic tree for 4918 loci from 13 sites in North America and one site in eastern Russia. Node supports shown for Maximum-Likelihood bootstrap values (>50) and Bayesian support (>0.90). NAK = north Alaska, SAK = south Alaska, YKBen = Bennett Lake, YKLSa = Little Salmon Lake, YKSim = Simpson Lake, LHU = Lake Huron main basin, NGB = north Georgian Bay, SGB = south Georgian Bay, LON = Lake Ontario, LMI = Lake Michigan, LNI = Lake Nipigon, LSU = Lake Superior, LAB = Labrador, RUS = Russia. Scale bar showing number of substitutions per base pair.

support delineated relationships within Lake Huron, Lake Michigan, and Georgian Bay sites (ML > 60; Bayesian = 1.00; Fig. 5).

4.2 Population Structure

Microsatellites

AMOVA indicated significant differentiation for 26 of the 28 pair-wise comparisons between sites. There was non-significant differentiation between fish from Lake Michigan Door County and those from Lake Michigan Milwaukee, as well as Lake Huron – main basin and northern Georgian Bay (Table 2). Within the Great Lakes F_{ST} values ranged from 0.020 to 0.108, whereas in the Alaska-Yukon region F_{ST} values ranged from 0.049 to 0.283. The nature of the sampling distribution did not permit formal testing of isolation by distance; however, the highest values of F_{ST} observed were for comparisons among the most geographically distant populations. Simpson Lake in Yukon Territory and T-Bone Lake in Labrador had a F_{ST} value of 0.416, Simpson Lake and south Georgian Bay 0.321, and south Georgian Bay and Labrador 0.336.

In the west, southern Alaska and Yukon sites showed similar allelic richness (average of 5.0 - 5.1 alleles per locus after rarefaction; Table 2). Northern Alaska and Simpson Lake had lower values indicating less genetic diversity (3.6 and 3.4 average alleles per locus respectively). Measures of allelic richness were fairly uniform across the Great Lakes region (4.4 - 4.8 average alleles per locus), whereas T-Bone Lake in Labrador had the lowest measure at 2.8 average alleles per locus.

The total variance retained for the DAPC of all sites, Alaska-Yukon, and the Great Lakes was 89.6%, 73.3%, and 87.6% respectively (Fig. 6). The DAPC of all sites

Table 2: Allelic richness (A_R), observed heterozygosity (H_O), and AMOVA F_{ST} values (below diagonal) and p-values (above diagonal) for round whitefish at nine microsatellite loci representing 14 sites across North America. Insignificant values after sequential Bonferronni correction are indicated with NS.

	Genetic Variation		Pairwise F_{ST}													
Site	**A_R**	**H_O**	**NAK**	**SAK**	**YKBen**	**YKLSa**	**YKSim**	**LHU**	**NGB**	**SGB**	**LON**	**LMI-M**	**LMI-D**	**LNI**	**LSU**	**LAB**
NAK	3.60	0.611	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SAK	5.01	0.828	0.176	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
YKBen	5.07	0.802	0.189	0.093	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
YKLSa	4.97	0.794	0.17	0.049	0.067	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
YKSim	3.37	0.608	0.283	0.192	0.167	0.174	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LHU	4.44	0.721	0.226	0.172	0.172	0.175	0.27	-	0.0021^{NS}	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
NGB	4.36	0.765	0.25	0.18	0.179	0.196	0.283	0.014^{NS}	-	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
SGB	4.00	0.705	0.295	0.208	0.221	0.225	0.321	0.049	0.034	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LON	4.76	0.793	0.211	0.141	0.15	0.15	0.242	0.052	0.054	0.1	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
LMI-M	4.68	0.824	0.214	0.152	0.157	0.162	0.255	0.02	0.026	0.068	0.041	-	0.2413^{NS}	<0.0001	<0.0001	<0.0001
LMI-D	4.64	0.722	0.233	0.153	0.155	0.163	0.264	0.025	0.037	0.075	0.044	0.002^{NS}	-	<0.0001	<0.0001	<0.0001
LNI	4.29	0.765	0.289	0.192	0.2	0.202	0.305	0.066	0.072	0.108	0.056	0.07	0.083	-	<0.0001	<0.0001
LSU	4.28	0.806	0.262	0.192	0.199	0.204	0.286	0.037	0.034	0.071	0.048	0.035	0.049	0.032	-	<0.0001
LAB	2.79	0.489	0.404	0.332	0.303	0.336	0.416	0.237	0.302	0.336	0.226	0.246	0.25	0.296	0.271	-

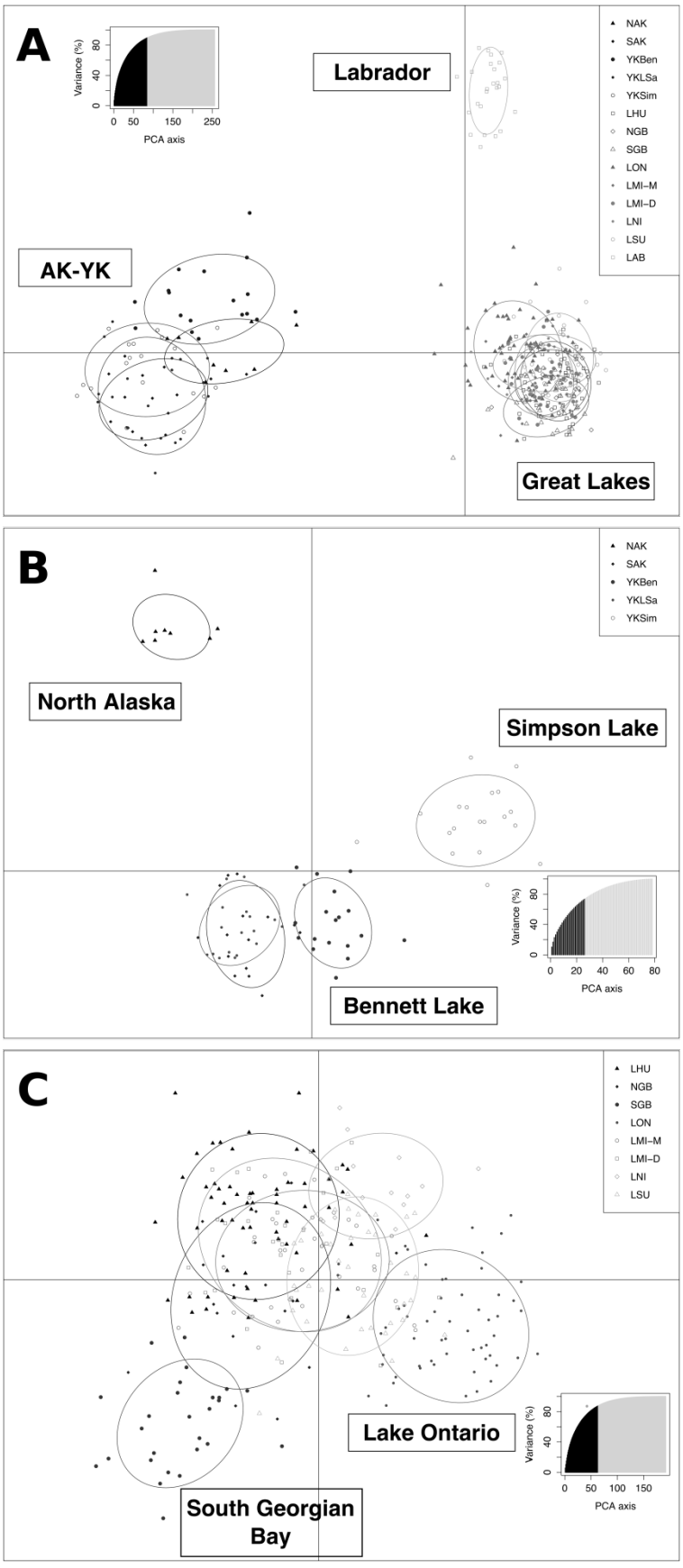

A
Labrador
AK-YK
Great Lakes
Variance (%)
0
40
80
0
50
150
250
PCA axis
NAK
SAK
YKBen
YKLSa
YKSim
LHU
NGB
SGB
LON
LMI–M
LMI–D
LNI
LSU
LAB
B
North Alaska
Simpson Lake
Bennett Lake
Variance (%)
0
40
80
0
20
40
60
80
PCA axis
NAK
SAK
YKBen
YKLSa
YKSim
C
Lake Ontario
South Georgian Bay
Variance (%)
0
40
80
0
50
100
150
PCA axis
LHU
NGB
SGB
LON
LMI–M
LMI–D
LNI
LSU

Figure 6: Discriminant analysis of principal components for nine round whitefish microsatellite loci within A) 13 sites across North America B) Five sites in Alaska and Yukon regions and C) Seven sites in the Laurentian Great Lakes region. NAK = north Alaska, SAK = south Alaska, YKBen = Bennett Lake, YKLSa = Little Salmon Lake, YKSim = Simpson Lake, LHU = Lake Huron main basin, NGB = north Georgian Bay, SGB = south Georgian Bay, LON = Lake Ontario, LMI = Lake Michigan, LNI = Lake Nipigon, LSU = Lake Superior, LAB = Labrador.

resolved the eastern and western regions well along the first axis, and also separated Labrador from the Great Lakes along the second axis (Fig. 6A). The subsequent DAPCs were for only the western sites (Fig. 6B) and only the Great Lakes sites (Fig. 6C) and once again resolved obvious groups along the two principal axes. For the western sites DAPC ellipses indicate four groups, with north Alaska and Simpson Lake-Yukon separating most distinctly. Much less separation was apparent between south Alaska, Bennett Lake-Yukon, and Little Salmon Lake-Yukon. In the Great Lakes, there was little evidence for clear separation among groups from different sites. However, fish from Lake Ontario and southern Georgian Bay sites showed some evidence of being distinct from the other locations sampled.

STRUCTURE analysis of microsatellites returned the same patterns as observed in DAPC. Analysis of all samples returned a most likely number of genetic groups of K=2, corresponding to eastern and western regions (Q>0.95; Fig. 7A). Subsequent hierarchical runs of STRUCTURE on the Alaska-Yukon region, and Great Lakes and Labrador regions returned most likely values of K=3 in both cases. The three clusters identified in the west corresponded to north Alaska, Simpson Lake in the Yukon (Q=0.984 and 0.956 respectively), and the three other western sites as one cluster (Q>0.95; Fig. 7B). The three clusters identified in the east delineated T-Bone Lake in Labrador (Q=0.976), and two clusters in the Great Lakes. There was weaker cluster assignment within the Great Lakes; Lake Huron, northern Georgian Bay, and southern Georgian Bay all assigned most closely to cluster 1 (Q=0.813, 0.808, and 0.923 respectively), whereas Lake Nipigon and Lake Ontario assigned to cluster 2 (Q=0.851 and 0.906 respectively; see Fig. 7C). Lake Michigan and Lake Superior sites didn't

assign with high confidence to either of the two Great Lakes clusters (Q<0.70). Further hierarchical runs on subgroups in both the east and west returned most likely values of K=1 indicating no further population structure.

In the west, BAYESASS analysis detected recent migration between Little Salmon Lake-Yukon and the south Alaska sites of 0.2526 (SD +/- 0.039; Fig. 8A), but no other sites. Within the Great Lakes, connectivity with recent migration was detected for Lake Huron to Lake Superior, Lake Huron to Lake Michigan and northern Georgian Bay, and migration from Lake Superior to Lake Nipigon (Fig. 8B).

NextRAD

After filtering SNPs for conformation to HWE and only retaining the first SNP from each locus, an output of 8835 SNP loci was retained for population structure analyses. Measures of genetic variation and F_{ST} values for the nextRAD loci showed similar relationships to those identified using microsatellites, with one notable exception being Labrador, which showed a closer relationship to the Great Lakes than indicated by microsatellites (Table 3). Great Lakes sites were less differentiated from Labrador (F_{ST} = 0.109 to 0.144) than western sites were to Labrador (F_{ST} = 0.294 to 0.375). Within the Great Lakes F_{ST} values ranged from 0.012 to 0.037, whereas in the Alaska-Yukon region F_{ST} values ranged from 0.022 to 0.150. Samples from the Russian sites showed the highest differentiation from all North American sites (F_{ST} = 0.234 to 0.423), with the exception of south Alaska (F_{ST} = 0.154).

STRUCTURE analysis returned a most likely value K=2, once again corresponding to eastern (Q>0.94) and western sites (Q>0.97), with samples from Russia

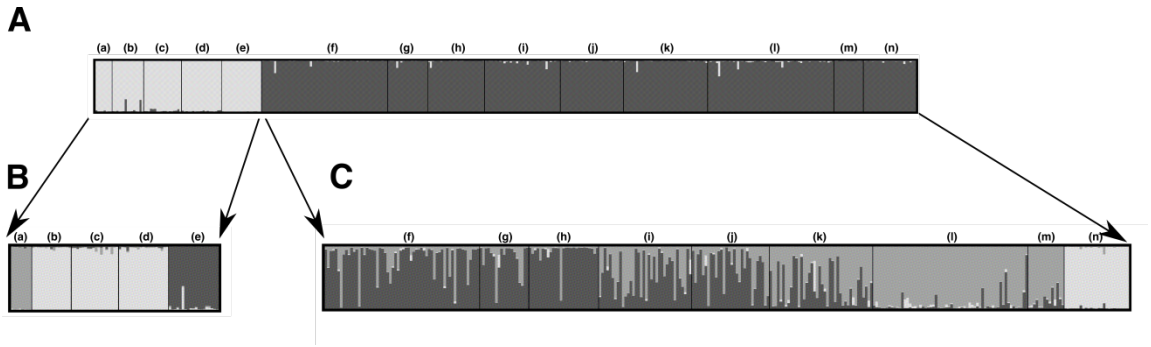
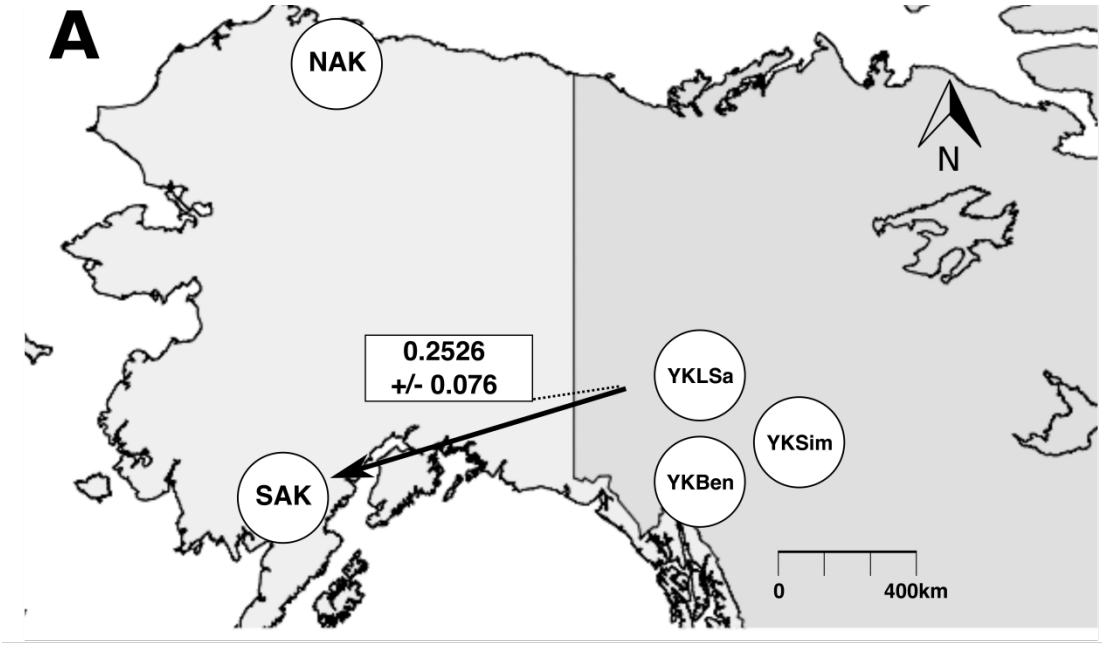


Figure 7: DISTRUCT plots showing hierarchical population structure of round whitefish in A) North America (K=2), B) Alaska and Yukon (K=3), and C) the Laurentian Great Lakes and Labrador (K=3) using nine microsatellite loci. (a) North Alaska, (b) South Alaska, (c) Bennett Lake, (d) Little Salmon Lake, (e) Simpson Lake, (f) Lake Huron, (g) North Georgian Bay, (h) South Georgian Bay, (i) Lake Michigan – Milwaukee, (j) Lake Michigan – Door County, (k) Lake Superior, (l) Lake Ontario, (m) Lake Nipigon, (n) Labrador.


A
NAK
N
0.2526
+/- 0.076
YKLSa
YKSim
YKBen
SAK
0
400km

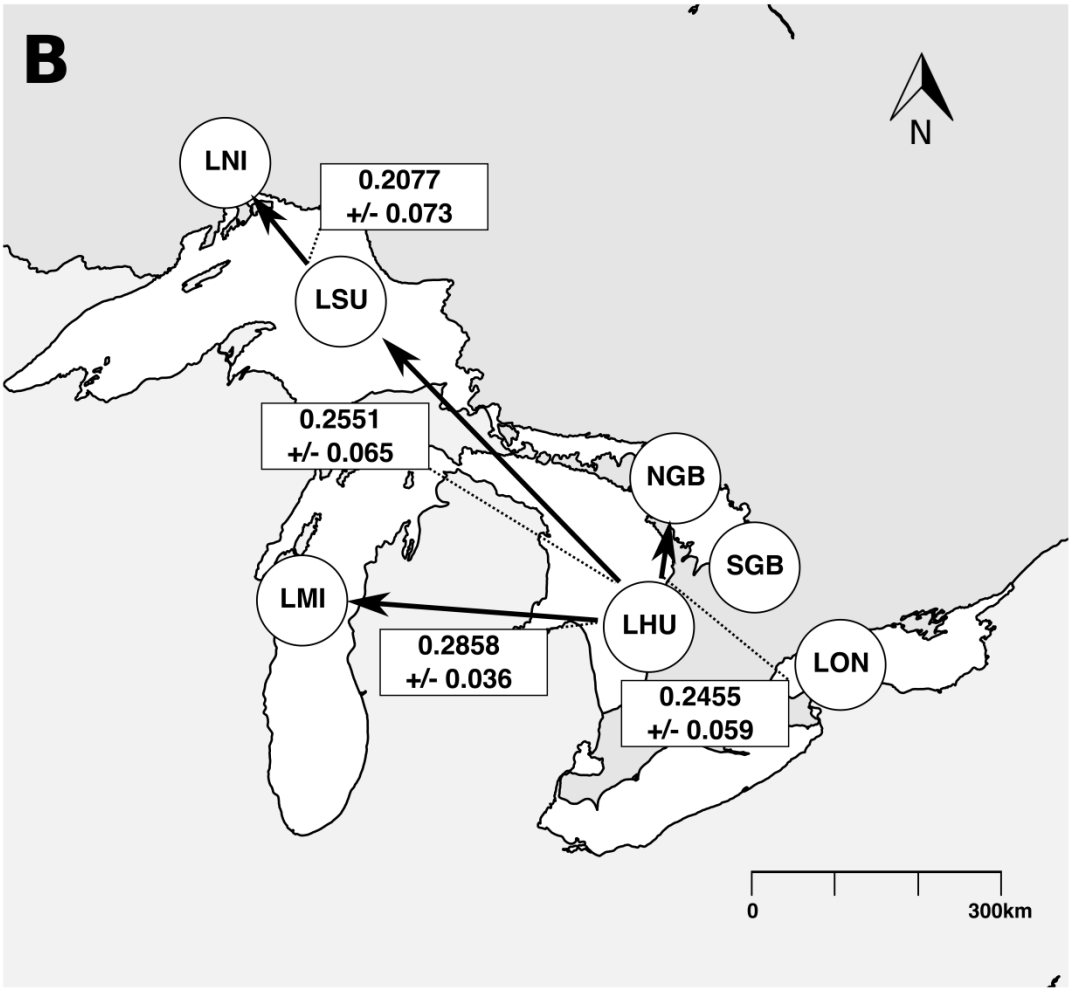

B
N
LNI
0.2077
+/- 0.073
LSU
0.2551
+/- 0.065
NGB
SGB
LMI
LHU
0.2858
+/- 0.036
LON
0.2455
+/- 0.059
0
300km

Figure 8: Contemporary migrations rates as determined using the program BAYESASS for five sites in Alaska-Yukon and seven sites in the Laurentian Great Lakes region. Values are shown only for migration rates with 95% confidence not overlapping with zero. NAK = north Alaska, SAK = south Alaska, YKBen = Bennett Lake, YKLSa = Little Salmon Lake, YKSim = Simpson Lake, LHU = Lake Huron main basin, NGB = north Georgian Bay, SGB = south Georgian Bay, LON = Lake Ontario, LMI = Lake Michigan, LNI = Lake Nipigon, LSU = Lake Superior.

Table 3: Proportion of polymorphic loci (P), Observed heterozygosity (H_O), expected heterozygosity (H_E), F_{IS}, and pairwise F_{ST} values for round whitefish at 8835 neutral single nucleotide polymorphisms representing 13 sites across North America and one site in eastern Russia.

	Genetic Variation				Pairwise F_{ST}												
Site	**P**	**H_O**	**H_E**	**Fis**	**NAK**	**SAK**	**YKBen**	**YKLSa**	**YKSim**	**LHU**	**NGB**	**SGB**	**LMI-M**	**LSU**	**LON**	**LNI**	**LAB**
NAK	0.8668	0.2001	0.1672	-0.0196													
SAK	0.8401	0.2116	0.2089	0.0429	0.072												
YKBen	0.8409	0.1402	0.2083	0.2082	0.097	0.043											
YKLSa	0.8625	0.1869	0.1650	0.0430	0.074	0.025	0.022										
YKSim	0.8542	0.1872	0.1875	0.0511	0.150	0.075	0.076	0.053									
LHU	0.8254	0.2379	0.2262	0.0315	0.269	0.229	0.234	0.188	0.264								
NGB	0.8338	0.1895	0.2167	0.1171	0.284	0.235	0.231	0.193	0.272	0.016							
SGB	0.8414	0.2116	0.2051	0.0402	0.299	0.241	0.238	0.198	0.284	0.017	0.012						
LMI-M	0.8266	0.2295	0.2251	0.0436	0.266	0.231	0.225	0.192	0.262	0.014	0.017	0.020					
LSU	0.8188	0.2853	0.2313	-0.0639	0.277	0.233	0.226	0.194	0.264	0.020	0.020	0.021	0.021				
LON	0.8183	0.2732	0.2335	-0.0414	0.273	0.231	0.229	0.191	0.266	0.031	0.033	0.037	0.031	0.033			
LNI	0.8224	0.2874	0.2256	-0.0818	0.275	0.226	0.243	0.214	0.271	0.026	0.029	0.036	0.026	0.012	0.033		
LAB	0.8720	0.2134	0.1584	-0.0873	0.375	0.295	0.309	0.294	0.353	0.112	0.136	0.139	0.122	0.127	0.109	0.144	
RUS	0.9281	0.0899	0.0857	0.0318	0.314	0.154	0.238	0.253	0.284	0.295	0.330	0.354	0.308	0.286	0.234	0.413	0.423

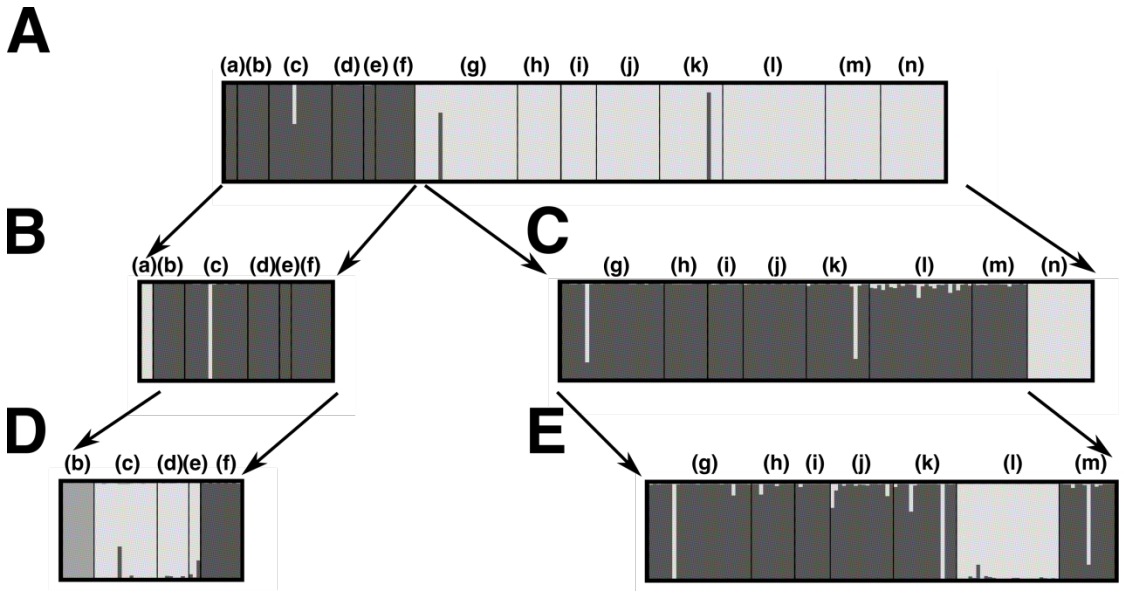


Figure 9: DISTRUCT plots showing hierarchical population structure of round whitefish in A) North America and Russia (K = 2), B) Russia, Alaska and Yukon (K = 2), C) the Laurentian Great Lakes and Labrador (K = 2), D) Alaska and Yukon (K = 3), and E) the Laurentian Great Lakes (K = 2) for 8835 SNP loci. (a) Russia – Taniorer River, (b) North Alaska, (c) South Alaska (d) Bennett Lake, (e) Little Salmon Lake, (f) Simpson Lake, (g) Lake Huron, (h) North Georgian Bay, (i) South Georgian Bay, (j) Lake Michigan – Milwaukee, (k) Lake Superior, (l) Lake Ontario, (m) Lake Nipigon, (n) Labrador.

clustering more closely with western samples (Q=1.00; Fig. 9A). Subsequent hierarchical runs returned K=2 separating Russia (Q=1.00) from all Alaska-Yukon sites (Q>0.93; Fig. 9B), and K=3 within Alaska-Yukon corresponding to north Alaska (Q=1.00), Simpson Lake (Q=1.00), and the other three western sites (Little Salmon Lake, Bennett Lake, and south Alaska; Q>0.92; Fig. 9D). Within the east, STRUCTURE indicated a most likely value of K=2 corresponding to the Great Lakes (Q> 0.94) and Labrador (Q=1.00; Fig. 9C). Analysis within the Great Lakes indicated a most likely value of K=2 within the Great Lakes with subdivision of Lake Ontario (Q=0.99) from the other Great Lakes (Q>0.91; Fig 8E).

5. DISCUSSION

5.1 Phylogeography of Round Whitefish

Eastern and western round whitefish have distinct lineages based on several genetic marker types. This is reflected in the mitochondrial haplotypes forming distinct clades in phylogenetic analyses, as well as a shared haplotypes between the Great Lakes and Labrador (the east-west extent of the eastern range). However, these clades were resolved into five groups that did obviously represent east and west, indicating a need for higher resolution analyses. NextRAD loci provided the requisite number of SNPs required to observe glacial lineages, and separated the eastern and western groups with high confidence (bootstrap =100) into separate clades. Analysis of SNP loci showed much deeper phylogenetic differences between the Great Lakes and western sites than

the Great Lakes and Labrador site, also supporting separate glacial lineages for fish from the eastern vs. western portions of the range. These results for round whitefish are consistent with other studies of mitochondrial loci in postglacial fishes, such as lake trout (Wilson and Hebert 1998), pygmy whitefish (Blanchfield et al. 2014), rainbow trout (Tamkee et al. 2010), arctic grayling (Stamford and Taylor 2004), lake sturgeon (McDermid et al. 2011), and lake whitefish (Mee et al. 2015), which have led to suggestions for separate DUs based on genetic distinctiveness. Further supporting the disjunction in round whitefish, there is also some evidence of regional meristic differences between eastern and western round whitefish (McPhail and Lindsey 1970; Scott and Crossman 1973), although its relevance is uncertain (Lindsey et al. 1981). The presence of separate glacial lineages for round whitefish will be important in considering the appropriate scale for management across North America.

The occurrence of the *W1*, *W2*, *W3*, and *W4* mtDNA haplotypes across the western region is consistent with a common lineage for all seven sites. Tree building analyses of mtDNA were unable to resolve a clear relationship between western haplotypes; however, this lineage most likely originated from the Beringian refugium (McPhail and Lindsey 1970; Ross 2013). This is supported by the distinct clade formed for the five western sites in the tree analysis of nextRAD loci. The Beringian lineage of round whitefish likely spread to the Mackenzie River Basin via proglacial lakes that formed in northern and southern Yukon, similar to how other fish species migrated to the Peel River and Liard River watersheds (Foote et al. 1992; Wilson and Hebert 1998; Stamford and Taylor 2004). Interestingly, the *W5* haplotype was only detected in fish from Great Bear Lake. Aquatic systems around Great Bear Lake are a potential mixing

zone for lake whitefish from the Nahanni, Beringian, and Mississippian glacial refugia (Foote et al. 1992; Mee et al. 2015). Wider sampling of round whitefish from other western watersheds is necessary to determine whether there are any previously unidentified glacial lineages represented. Previous studies of postglacial fish have identified Nahanni lineages within the lower Liard River system for other fish species (Foote et al. 1992; Stamford and Taylor 2004); however, round whitefish from this region have not been previously characterized. Post-glacial migration of round whitefish from the Nahanni refugium may explain why the *W5* haplotype was only observed in Great Bear Lake, but further sampling from within the lower Liard system is necessary to confirm this.

Key areas of the round whitefish range should be assessed to better characterize post-glacial migration and the range disjunction between the east and west. Genetic analysis of round whitefish from within the Churchill and Keewatin River Basins will confirm the hypothesized migration routes from the Beringian refugium following glacial retreat. The analyses of western round whitefish represent four of the most western watersheds in North America, and only the most western reaches of the Mackenzie River Basin. The recent discovery of populations of pygmy whitefish in northern Ontario (Blanchfield et al. 2014), extending their range approximately 320 km, highlights an example where apparent geographic range disjunctions in other species have been mischaracterized. Genetic characterization of round whitefish at the extents of the known western range will inform the glacial lineages of populations in this region. Sequencing of additional mtDNA loci may also resolve western haplotypes that didn't strongly assign within clades (*W4* and *W5*).

The mtDNA haplotypes and the nextRAD tree analysis, for eastern sites indicated a common lineage of round whitefish within all of the Great Lakes and Labrador. The *E6* haplotype was found throughout the Great Lakes and was the only haplotype detected in Labrador. The maternal-inheritance of mtDNA loci allows for the tracing of lineage that may be obscured in other markers by secondary contact and recombination of nuclear markers (Avise 2004). Past analyses of Great Lakes fishes have identified the region as a likely 'suture zone' for glacial lineages from the Mississippian and Atlantic refugia (Mandrak and Crossman 1992; April et al. 2013); two lineages were detected in the mtDNA phylogenetic analysis, consistent with this theory. The *E5* and *E6* haplotypes were strongly assigned to their own group, within a group with *W1, W2* and *W3*; whereas, *E1*, *E2*, *E3*, and *E4* strongly assigned to their own separate group (Fig. 4). Wider genetic characterization of populations east of the Great Lakes will determine the eastern extent of the other haplotypes observed in the Great Lakes, and the likely refugial origins of observed haplotypes. With the noted decline of round whitefish in the northeastern United States (Steinhart 2007; Vermont Department of Fish and Wildlife 2015; Nugent and Carpenter 2015) and the Great Lakes (Ebener 2012), characterizing genetic diversity and glacial lineages will be important to preserving long-term viability for the species in this part of their range.

5.2 Regional Population Structure

Alaska and Yukon Region

Round whitefish in the western sites showed greater regional differentiation than in the Great Lakes. Within the Alaska and Yukon sites, I detected a pattern of gene flow

consistent with the more fragmented hydrologic connectivity of the sampled watersheds using both microsatellites and nextRAD SNPs (Benke and Cushing 2005). Hydrologic connectivity facilitates contemporary gene flow; proper understanding of gene flow within and between watersheds is therefore integral to informed management (Pringle 2003; Waples and Gaggiotti 2006). The gene flow and subdivision of round whitefish populations in Alaska-Yukon will allow for the determination of genetic stocks across the US-Canada border, and allow informed management between jurisdictions (Poff et al. 2003; Ban et al. 2013). The genetic analyses of western sites resolved the relationship of round whitefish populations from within five sites from four watersheds. Evidence from both microsatellite and nextRAD SNP analyses indicated a connection between Nugashek River Basin populations and Yukon River Basin populations that is not present for the site north of the Brooks Range, or the site in eastern Yukon, which are both isolated from the Yukon River by mountain ranges. The subdivisions I have identified support the notion that round whitefish should be considered multiple MUs in western North America, that are not principally determined by geographic distance. These MUs will have to be managed across the jurisdictions of both Canada and the US.

I detected strong differentiation of Simpson Lake round whitefish, in eastern Yukon, and those north of the Brooks Range in north Alaska, in both the microsatellite and nextRAD SNP analyses. Simpson Lake is separated from the Yukon River Basin by the Cassiar and Selwyn Mountains. Following glaciation, fish species migrated from the Beringian refugium east to the Mackenzie River Basin and other northern watersheds. Migration was facilitated through the formation of proglacial lakes in northern and southern Yukon that connected the Yukon River Basin and the Mackenzie River Basin

(Lindsey et al. 1981; Pielou 1991). These routes of dispersal have been genetically characterized in several other species such as lake whitefish (Foote et al. 1992), lake trout (Wilson and Hebert 1998), and Arctic grayling (Stamford and Taylor 2004). The Simpson Lake population of round whitefish is isolated from others further west in Alaska and Yukon Territory, and should be managed in a separate MU. The assessment of Simpson Lake round whitefish relative to other Liard River populations should be prioritized to determine its regional status.

Further characterization of the Liard system round whitefish will determine the degree of isolation of the Simpson Lake population relative to others in the Mackenzie River Basin. Additionally, these populations should be investigated to assess whether there is secondary contact with any lower-Liard glacial lineages of round whitefish, because this is the predicted location of the Nahanni refugium (Foote 1992; Wilson and Hebert 1998; Stamford and Taylor 2004). Similar to the results from Simpson Lake, round whitefish north of the Brooks Range are distinct from the rest of the sites in Alaska and Yukon. This differentiation is likely due to topographical barriers (mountain range) limiting migration from more southern populations. These isolated populations of round whitefish exist close to the northern and southern periphery of the western range, and therefore should be thoroughly investigated for proper management. Isolation may limit the possibility of dispersal, including potential rescue by migrants in the event of major population decline.

Analysis of contemporary migration detected a greater isolation of western round whitefish populations relative to sites in the Great Lakes. Little Salmon Lake in Yukon contributed migrants to the south Alaska sites. This is consistent with the hydrological

connectivity of these sites, and in line with the known limitations of BAYESASS in detecting weak relationships (Meirmans 2014). The Bennett Lake site, though closely associated to south Alaska and Little Salmon Lake through microsatellite STRUCTURE analysis, was not detected to exchange contemporary migrants with these sites. This relationship was also observed in the DAPC analysis of microsatellite data, which showed a close but non-overlapping relationship, between Bennett Lake and south Alaska/Little Salmon Lake ellipses. Because the sites and samples were obtained opportunistically, these sites may not be suited to BAYESASS analysis, and may be connected through migration with several intermediate populations. BAYESASS analysis will require more thorough sampling of round whitefish populations to characterize migration between the more stratified populations of round whitefish in northwestern North America.

The Laurentian Great Lakes Region

The microsatellite and nextRAD SNP analyses identified levels of subdivision and gene flow consistent with the hydrological connectivity of the Great Lakes. I observed significant genetic differentiation of round whitefish between Lake Michigan, Lake Superior, Lake Nipigon, Lake Ontario, Lake Huron, and Georgian Bay using both microsatellites and nextRAD SNPs. This subdivision was weak to moderate between the contiguous Great Lakes, with consistently higher levels of differentiation for southern Georgian Bay, and Lake Ontario. The analyses indicate significant genetic differences on the level of each lake, consistent with previous studies on species such as lake whitefish (VanDeHey et al. 2009; Bernard et al. 2009; Stott et al. 2010), smallmouth bass

(*Microterus dolomieu*; Stepien et al. 2007), walleye (*Sander vitreus*; Stepien et al. 2009; Haponski and Stepien 2014), and yellow perch (*Perca flavacens*; Sullivan and Stepien 2014; Kocovsky et al. 2013). DAPC of microsatellite genotypes, STRUCTURE analysis of nextRAD SNPs, and tree analysis of nextRAD loci strongly supported that Lake Ontario is isolated relative to the other Great Lakes. In addition, there was moderate support in the STRUCTURE analysis of microsatellite genotypes, and higher relative F_{ST} for both microsatellites and nextRAD SNPs. Lake Ontario is likely disjunct from the other Great Lakes due to absence of round whitefish in Lake Erie, which lacks suitable habitat as the shallowest and warmest Great Lake and tends to support warm-water species (Leach and Nepszy 1976). Monitoring of round whitefish in each lake is necessary to ensure persistence of the species within the Great Lakes, and to be aware of any further declines; as have been observed in Lake Huron and Georgian Bay (Ebener 2012). Considering indications of their genetic differentiation, Lake Ontario round whitefish populations should also be considered separately within the Great Lakes.

Analysis of contemporary migration using microsatellites further highlights the potential importance of Lake Huron and Lake Ontario to the genetic diversity of round whitefish in the Great Lakes. Lake Huron contributes to round whitefish populations in Lakes Michigan, Superior, and northern Georgian Bay, as well as indirectly to Lake Nipigon through migrants from Lake Superior. The noted decline of round whitefish in Lake Huron and northern Georgian Bay may therefore have a wider impact on populations within the rest of the Great Lakes (Ebener 2012). Lake Ontario does not appear to exchange migrants with the other Great Lakes, consistent with a disjunction of round whitefish populations in the Great Lakes. This further supports that Lake Ontario

should therefore be considered separately as an important and distinct unit for conserving round whitefish genetic diversity in the Great Lakes. The recent declines of round whitefish in Lake Huron and Georgian Bay should be considered with the additional understanding that they may supplement or contribute migrants in the other Great Lakes.

5.3 Russian Round Whitefish

Round whitefish from Russia were highly differentiated from those in North America. COI sequences for North American round whitefish were >99.5% similar to others already on BOLD; however, Russian individuals returned a match of only 98.25%. This difference of 1.75% is substantially higher than the average intraspecific difference of 0.73% (SE 0.053) for other North American freshwater fish (April et al. 2011). NextRAD tree analyses also indicated strong differentiation of the Russian site from North American populations with branch lengths >3.3X longer than the differences observed within North American sites. These two analyses support that Russian round whitefish may warrant designation as a separate ESU or species, especially considering the yet-uncharacterized genetic diversity of populations further west in Russia. Round whitefish are the only species from the genus *Prosopium* found outside of North America, and have likely been isolated from North American populations since the Wisconsinan glaciations (McPhail and Lindsey 1970). Further investigation into the Eurasian populations of round whitefish will improve understanding of coregonine postglacial migration and contemporary connectivity globally.

5.4 Marker Comparison

NextRAD analysis resolved important relationships with more clarity than microsatellite genotyping or mtDNA sequencing. While all analyses corroborated the same relationships, I saw varied resolution and statistical support. For example, I was able to resolve the eastern and western groups of round whitefish with high confidence using phylogenetic analysis of nextRAD data, whereas the concatenated mtDNA loci did not have sufficient statistical power to clearly delineate these relationships. In addition, the resolution of distinct round whitefish populations in Labrador and Lake Ontario was much more strongly supported based on nextRAD data than for either the microsatellite or mtDNA markers. NextRAD and other reduced-representation techniques have the potential to characterize intraspecific relationships with higher resolution than traditional techniques. As library-preparation and sequencing costs decrease, computational power increases, and bioinformatic pipelines incorporate additional tools, genomic datasets are becoming more feasible to work with for conservation and management applications. However, their wider application is still in its infancy, and requires continued development and dissemination outside of academia (Narum et al. 2013; Schafer et al. 2015; Garner et al. 2016; Schafer et al. 2016). Application of reduced-representation library techniques (such as nextRAD) will provide valuable information in managing these species in North America, and should be further implemented in the study and management of postglacial fishes.

6. GENERAL CONCLUSION

Appropriate management of round whitefish requires recognition and designation of any significant genetic demarcations at different spatial scales. The genetic relationships I have identified here support the existence of east and west round whitefish representing separate lineages associated with distinct Wisconsinan glacial refugia, thereby qualifying round whitefish as at least two DUs in North America. However, the extent of migration and any sub-lineages within the eastern and western ranges need to be further refined. Within each region I have identified genetic population structure that could prove instrumental to ongoing monitoring and management programs in the Laurentian Great Lakes and northwestern North America. I suggest as a priority the assessment of round whitefish status in each Great Lake separately, with Lake Ontario and Lake Huron of principal importance to management of regional stocks. Wider assessment across the North American range of the round whitefish range is necessary, including the inland lakes of Ontario and Quebec in the east, as well as the Churchill River and Keewatin River Basins in the west. Wider sampling of round whitefish is necessary to determine the contribution of isolation by distance to population subdivision in such a widespread species (Wright 1943), while an understanding of adaptation in round whitefish will inform managers of the ability of the species to persist through environmental disturbances. I also presented the first genetic comparison of Russian round whitefish to North American round whitefish, and show that Russian fish are more deeply diverged than is typical for North American freshwater fishes. Assessment of Russian round whitefish for additional genetic differences and any

morphological distinctiveness will inform its designation as a separate ESU, or whether it should be considered a separate species.

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8. APPENDICES

8.1 Appendix Tables

Table A1: PCR conditions and primers for 11 round whitefish microsatellite loci. TD65 = touchdown annealing temperature starting at 65ºC and lowering to 55ºC as described in Graham et al. 2016.

Locus	Annealing temp (°C)	Primer sequence 5' – 3'	Repeat motif	Reference
Prwi6	TD65	F: CGTCCCTCTCCTCCACACC	AGTG	O'Bryhim et al. 2013
		R: ACCTCCTCATTAACCCAACCC		
Prwi15	TD65	F: ACTGCCTATCTCGACGCTCC	AAAG	O'Bryhim et al. 2013
		R: CCTGTGATGTTGTTGTCAAGGG		
Prwi24	TD65	F: TTAATACTGCAGATCAGTATCACCC	AAAG	O'Bryhim et al. 2013
		R: GATGAGCACTGCAGAACATAGC		
Prwi25	TD65	F: TCTCGCTTGTCACTCTCATTAGG	TGCC	O'Bryhim et al. 2013
		R: GCCAAATAAATCTGCTCTCAGC		
Prwi27	TD65	F: CACTTTATTGAGTAATTGAACGGAGCTCTG	TCTG	O'Bryhim et al. 2013
		R: GGAAAGGGATGTAAACCACAGC		
Prwi28	TD65	F: GCTGAGGCTAACTCCCTTGC	TCTG	O'Bryhim et al. 2013
		R: GGCCCTGGGATAAAGATAAACC		
Prwi55	TD65	F: TCATTATTACTGACACAGATAGACGG	TCTG	Graham et al. 2016
		R: CAGATTAATCAGATACTGCTAGCCC		
Prwi56	TD65	F: GGCTCTGGCTGCTTTCTAGC	AAAG	Graham et al. 2016
		R: CATGAACCCTCTGCGAACC		
Prwi60	TD65	F: ACTTCTATACAGTCATCATCTGCCC	TGCC	Graham et al. 2016
		R: GCAATTTCATAAATGCCTGCC		
Prwi65	TD65	F: TCATTAACCTACAGCTATTACAGAGGC	TCTG	Graham et al. 2016
		R: GGTCTGTAGCTGTCGGGC		
Prwi72	TD65	F: GGCTGACACAGTAAGAGGGC	TCTG	Graham et al. 2016
		R: TTGGTGTGATGCAATACAGTAGC		