

**Using Microsatellite and Mitochondrial DNA Variation to Investigate
Population Structure of Walleye (*Sander vitreus*) in Lake Winnipeg**

by

Stephanie Maxwell Backhouse

A Thesis submitted to the Faculty of Graduate Studies of
The University of Manitoba
in partial fulfilment of the requirements of the degree of

Master of Science

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ABSTRACT

Walleye (*Sander vitreus*) are the main contributors to Manitoba's multi-million dollar commercial fishery. Walleye are known to be philopatric in some systems, providing potential for multiple genetically discrete spawning groups within a system. This study used ten microsatellite loci and one RFLP assay (from the mitochondrial control region) to investigate the degree of genetic differentiation among 13 spawning sites in Lake Winnipeg, and compared these to walleye collected from two hatchery locations and other locations in Manitoba and Ontario, as well as to sauger (*Sauger canadensis*). Little population structure was found within Lake Winnipeg, and slight to moderate genetic differentiation was found between Lake Winnipeg walleye and other sampling locations. Great genetic differentiation was found between walleye and sauger. The lack of genetic differentiation found in Lake Winnipeg possibly indicates a lack of natal philopatry, some philopatry but with sufficient straying to obscure genetic structure, or obscured structure as a result of hatchery stocking.

Acknowledgements

Funding for this project was provided by the Manitoba Fisheries Enhancement Fund (FEF). Additional scholarships were provided by the University of Manitoba Faculty of Science award, and Fish Futures Incorporated. I would like to thank my advisor, Dr. Margaret Docker, for letting me run with this, and always being there when I hit a wall. I would also like to thank the rest of my committee members (Dr. Gary Crow, Dr. Darren Gillis and Dr. Michele Piercey-Normore) for advice and feedback throughout this project. My 'microsatellite' lab mates (Craig McFarlane, Bethany Schroeder and Paulette Penton) were there to help me with analysis (thanks Craig!) and listen to my PCR woes. I would like to thank my family and fiancé, for their support and encouragement, help with sampling, and enthusiasm for my research.

Many people and organizations assisted with sample collection in the project. These include: Manitoba Water Stewardship (Warren Coughlin, Derek Kroeker and Kevin Casper); North-South Consultants (Don MacDonnell and Kevin Ambrose); Whiteshell Hatchery (Don Bilenduke and Kevin Dyck); Swan Creek Hatchery (Wade Biggen and Grant McBain); Manitoba Conservation (Gord Kirbyson, Mervin Sinclair and Rick Lund); Golder Associates (Jonathan Stephens); Bret Backhouse, Harold Disbrowe, Guy Douglas, Norman Guimond, Joel Hunt, Michael James, Ken Kansas, Mark Lowden, Elliot Macdonald, Joe Pope, Doug Watkinson, Frank Young. If I've forgotten anybody in this list, it is not because I am not grateful for all the help provided!

Table of Contents

USING MICROSATELLITE AND MITOCHONDRIAL DNA VARIATION TO INVESTIGATE POPULATION STRUCTURE OF WALLEYE (<i>SANDER VITREUS</i>) IN LAKE WINNIPEG	I
ABSTRACT	I
<i>Acknowledgements</i>	II
<i>List of Tables</i>	V
<i>List of Figures</i>	VI
GENERAL INTRODUCTION	1
Walleye – General Biology	1
<i>Walleye in Manitoba</i>	2
Intraspecific Phenotypic Variation in Fish	2
<i>Colour variation in Walleye</i>	5
<i>Phenotypic Variation in Lake Winnipeg Walleye</i>	6
Philopatry	8
<i>Philopatry in Walleye</i>	9
Genetic Stock Assessment	11
<i>Methods of Stock Assessment</i>	12
<i>Tagging Studies</i>	12
<i>Otoliths</i>	13
<i>Allozymes</i>	14
<i>Mitochondrial DNA</i>	16
<i>Microsatellites</i>	21
Genetic Stock Assessment in Walleye	23
<i>Tagging Studies</i>	24
<i>Otoliths</i>	24
<i>Allozymes</i>	24
<i>Mitochondrial DNA</i>	25
<i>Microsatellites</i>	29
<i>Complementary Methods</i>	31
Study Area – Lake Winnipeg	31
<i>Lake Winnipeg Walleye Fishery</i>	34
<i>Changing Conditions in Lake Winnipeg</i>	35
RESEARCH OBJECTIVES	36
CHAPTER ONE: USING MICROSATELLITE DNA VARIATION TO INVESTIGATE POPULATION STRUCTURE OF WALLEYE (<i>SANDER VITREUS</i>) IN LAKE WINNIPEG AND SURROUNDING AREAS	39
ABSTRACT	39
METHODS	44
<i>Field Collection</i>	44
<i>DNA Extraction, PCR Amplification and Fragment Analysis</i>	46
<i>Data Analysis</i>	48
RESULTS	52
DISCUSSION	58

CHAPTER TWO: USING PCR-RFLP ASSAY OF THE MITOCHONDRIAL CONTROL REGION TO INVESTIGATE BROADSCALE POPULATION STRUCTURE OF WALLEYE (<i>SANDER VITREUS</i>) IN LAKE WINNIPEG AND SURROUNDING AREAS	86
ABSTRACT	86
METHODS	92
<i>Mitochondrial</i>	92
<i>RFLP</i>	93
<i>Data Analysis</i>	94
RESULTS	96
DISCUSSION	98
GENERAL DISCUSSION	110
LITERATURE CITED	123
APPENDIX 1: CALCULATION OF F STATISTICS	142

List of Tables

Table 1: Sampling locations and summary statistics based on nine microsatellite loci.	75
Table 2: Locus name, batch and dye, number of individuals (N), size range, number of alleles (N_a), expected (H_e) and observed (H_o) heterozygosities, motif, primer sequences, and reference of the 10 microsatellite loci used in this study	76
Table 3: Observed and expected heterozygosities (H_o and H_e) and number of alleles (N_a) with sample sizes for nine microsatellite loci from 21 walleye sampling locations and 2 sauger locations.	77
Table 4: Pairwise estimations of F_{ST} values between years at sampling locations (above the diagonal) and genetic differentiation X^2 (below the diagonal).	79
Table 5: Pairwise estimations of F_{ST} values between sampling locations (above the diagonal) and X^2 values (below the diagonal).	81
Table 6: A comparison of levels of genetic diversity (H_o) and the average number of alleles per locus (N_a) as found for walleye in this study and other studies.	83
Table 7: A comparison of genetic differentiation (F_{ST}) values found between Lake Winnipeg walleye spawning sites in this study, between walleye spawning sites in the Laurentian Great Lakes (as determined by other studies), and between spawning sites of smallmouth bass and chum salmon, other philopatric species (as determined by other studies).	84
Table 8: Haplotype designations, fragment sizes and variable nucleotide positions (numbered vertically) in a 715 bp fragment in the control region of walleye from Lake Winnipeg and surrounding areas.	102
Table 9: Pairwise percent sequence divergence for a selected representative of each sequence type in this study.	104
Table 10: Number of samples from each sampling location digested using <i>Tsp45I</i> , resulting haplotypes and haplotype diversity (h).	105
Table 11: Pairwise estimations of differentiation between sampling locations as measured with an exact test, using data generated with a PCR-RFLP (using <i>Tsp45I</i>) of a 715 bp segment of control region DNA in walleye.	106

List of Figures

Figure 1: Lake Winnipeg and surrounding sampling areas, central Canada.....	85
Figure 2: Correlation of pairwise measures of genetic differentiation ($(\theta/(1-\theta))$) and pairwise geographic distances ($\ln(Km)$).....	86
Figure 3: Neighbour Joining phenogram of 21 walleye sampling sites and 2 sauger sampling sites.....	87
Figure 4: Duality diagram of a correspondence analysis using nine microsatellite variables for walleye taken from 13 sampling sites in Lake Winnipeg.....	88
Figure 5: Neighbour Joining phenogram of walleye sequences.....	107
Figure 6: A map of the sampling area depicting the walleye RFLP haplotypes as determined in this study.....	108

GENERAL INTRODUCTION

Walleye – General Biology

Walleye (*Sander vitreus*) are members of the family Percidae, subfamily Luciopercinae (Nelson 2006). They were previously known as *Stizostedion vitreum*, and are closely related to sauger (*Sander canadensis*). Their distribution is limited to freshwater habitats in North America, occurring in Canada from British Columbia to Quebec, north to Northwest Territories, Nunavut and Yukon, and across the American/Canadian border. They are found throughout most of the central and eastern states in the United States and America, and have been widely introduced across and outside of their native range (Scott and Crossman 1973). Walleye are a large bodied piscivorous species reaching average lengths of 30 to 50 cm (Scott and Crossman 1973). They typically have an olive/brown/yellow colouration on their dorsal surface, darker on the head and back, fading on the sides to a pale white or yellow ventral surface. Their eyes are silvery from light reflected from the tapetum lucidum, the light sensitive layer in the eye (Scott and Crossman 1973).

Walleye generally spawn in spring or early summer, after the ice breaks up and when water temperatures reach 6.7 – 8.9°C. Spawning grounds are typically the white water rocky areas below impassable barriers such as falls or dams in rivers, or boulder/coarse graveled shoals in lakes or tributary rivers (Scott and Crossman 1973). Spawning occurs at night, in groups of one to two females and two to six males, and eggs are broadcast into the coarse substrate. Fecundity has been reported at up to 612 000 eggs in an 80 cm female. Males generally mature between two and four years of age, while females

mature later, between three to six years. The maximum age of walleye varies from 10 to 12 years in the southern parts of its distribution, and can be up to 20 years in northern habitats (Scott and Crossman 1973).

Walleye in Manitoba

Walleye are found throughout most of the province of Manitoba, north into the Seal River watershed (Stewart and Watkinson 2004). The Manitoba angling record for walleye is a 99 cm total length fish caught in the Red River in 1997 (Stewart and Watkinson 2004). Numerous spawning sites have been identified within the Lake Winnipeg area, including the Icelandic River, Jackhead River, Fisher River, Dauphin River, Warpath River, Saskatchewan River, Big Black River, Poplar River, Berens River, Bloodvein River, Manigotagan River, Winnipeg River and Red River. However, not all spawning sites have been identified, and the possibility of shoal spawning areas still exists.

Intraspecific Phenotypic Variation in Fish

Pronounced phenotypic variation (e.g., differences in body form, behaviour, or colour) in fish has been observed in a variety of species, particularly in temperate fish species, where colonization of depauperate environments following deglaciation has allowed fish to fill a variety of empty niches (see Schluter and McPhail 1993). Many fish species consist of populations that differ morphologically, ecologically or genetically, but are sympatric for some or all of their lives (Taylor 1999). In some cases, the different morphotypes show complete or partial reproductive isolation (e.g., dwarf and normal lake whitefish, *Coregonus clupeaformis*, see below; Lu and Bernatchez 1999), while in other cases no genetic differentiation between morphs is found, indicating a lack of reproductive isolation (e.g., steelhead and rainbow trout, *Oncorhynchus mykiss*; Docker

and Heath 2003, Olsen et al. 2006, Heath et al. 2008). Such divergent morphotypes are found commonly in salmonids (Taylor 1999), many of which express multiple behavioural types within the same population, possibly as an evolutionary adaptation to a heterogenous environment (Homel et al. 2008). Some examples are discussed below.

Phenotypic differences can result from differing degrees of reproductive isolation between 'types' (Lu and Bernatchez 1999). Sympatric lake whitefish ecotypes (dwarf and normal) differ morphologically in traits related to trophic specialization, with normal whitefish having a planktivorous diet, and dwarf whitefish having a benthivorous diet (Bernatchez et al. 1999). Significant differences have been found between the two morphotypes using meristics, morphological measurements and genetic measures (mitochondrial DNA and microsatellite markers), but the level of genetic divergence is variable between systems (Lu and Bernatchez 1999). The level of genetic divergence between dwarf and normal lake whitefish has been linked to environmental parameters, suggesting that divergence has occurred as a result of increased reproductive isolation in response to increased competition due to reduced habitat and prey availability (Landry et al. 2007). Arctic charr (*Salvelinus alpinus*) also display considerable phenotypic variation, and can exhibit up to four sympatric morphs in postglacial lakes, varying in life history (anadromous or freshwater), trophic level (benthic, planktivorous, piscivorous), colouration, morphology, behaviour and genetic characteristics (Jonsson and Jonsson et al. 2001). Individuals usually spawn with similar morphs, but are capable of interbreeding and producing fertile hybrids (Jonsson and Jonsson et al 2001). Arctic charr are known to be capable of very rapid phenotypic shifts, with evidence of trophic

specialization occurring in as little as 25 years (approximately six generations; Michaud et al. 2008).

Phenotypic differences can also exist without evidence of genetic differentiation. A well documented example is *O. mykiss*, which exists as two divergent morphotypes, steelhead and rainbow trout. Steelhead trout migrate to salt water and return to their natal site to spawn, while rainbow trout migrate only locally within fresh water, if they migrate at all. Morphological, physiological and behavioural differences exist between the two forms, and while there have been some observations of genetic differentiation (e.g., Narum et al. 2004), there appears to be no consistent significant genetic differentiation between them (Docker and Heath 2003, Olsen et al. 2006, Heath et al. 2008). Bull trout (*Salvelinus confluentes*) also exhibit differences in behavioural traits, and often occur as resident and migratory forms in the same stream. The behavioural type of individual fish in the South Fork Walla Walla River (northeast Oregon) was determined through tagging, and microsatellite DNA markers (see below) were used to assess genetic differences between them. Genetic differentiation between the two forms was not statistically significant, indicating a lack of reproductive isolation between them (Homel et al. 2008).

Intraspecific phenotypic variation occurs outside of salmonids as well, and many species are known to adapt morphologically to the prey and habitat type available to them. One such example is the pumpkinseed (*Lepomis gibbosus*), which has been shown to adapt morphologically based on habitat (limnetic versus littoral) and diet (e.g., feeding on zooplankton or gastropods, see Vila-Gisbert et al. 2007). A comparison of pumpkinseeds across a wide geographic range (Spain and North America) found significant differences

in body morphological traits functionally related to prey selection (benthic vs. zooplanktivorous diet; Vila-Gisbert et al. 2007). Significant differences in life history attributes were also observed across a wide geographic range (Spain, England, Canada, Southern USA) encompassing the native and introduced range of pumpkinseed (Fox et al. 2003). The observed variation seen in pumpkinseed is thought to have contributed to their successful colonization of novel habitats, due to the opportunistic life-history strategy they are able to adopt (Fox et al. 2003).

Colour variation in Walleye

Walleye are also known to show phenotypic variation, particularly in terms of colour. The colouration of walleye is highly variable, and changes with habitat or size. In turbid water fish appear paler with less distinct markings, and are more vividly marked in clearer water (Scott and Crossman 1973). Smaller fish (10 to 35 cm) possess dark vertical bands across their back and sides (Scott and Crossman 1973). Colour variants have been found most frequently in the Great Lakes region. A grey-coloured form (called "Hards" in Lake Erie) occur as a result of a blueish tinge to the mucous layer, and are found at various frequencies in most populations. Orange coloured walleye are caught infrequently in Lake Ontario. A blue morph of walleye, once designated as a subspecies (*Sander vitreus glaucus*), was taken historically in Lakes Ontario, Erie and Nipissing. Colouration of this form was slate to steel blue on the dorsal surface, ice blue to silvery on the sides and silvery to white on the ventral surface. COSEWIC lists the blue walleye as becoming extinct in 1965 (http://www.cosewic.gc.ca/eng/sct1/searchdetail_e.cfm). There are still occasional reports of blue walleye being caught, but they have not been found to show significant genetic differentiation from yellow walleye,

and are thought to be different than historic blue walleye (Stepien and Faber 1998).

Phenotypic Variation in Lake Winnipeg Walleye

Lake Winnipeg walleye also show variation in colour (Stewart and Watkinson 2004), as well as other phenotypic and life history traits such as size (Moles et al. 2007), scale outlines (Watkinson and Gillis 2005), and life history characteristics (Lysack 1980, 1986).

In terms of colour variation, the most common colour morph (yellow) has an olive green colouration above, fading to brassy yellow on the sides (Stewart and Watkinson 2004). “Greenbacks” are thought to be an alternate colour morph, and are typified by an emerald colouration of the dorsal surface. They are found in Lake Winnipeg waters, and comprise the majority of walleye entering the Red and Winnipeg rivers in late summer and fall (Stewart and Watkinson 2004). In May 1986, 82 male greenback walleye were captured in a two hour gill net set below St. Andrews dam (Lockport, MB) (Stewart and Watkinson 2004). As males typically arrive at the spawning grounds before females (Scott and Crossman 1973), this could indicate the possibility of spawning segregation between this colour variant and “normal” yellow walleye.

Significant differences in body morphology have been found in the south basin of Lake Winnipeg. Moles et al. (2007) described a “dwarf” type walleye, which exhibited traits typically associated with a benthic polymorphism. The difference in body form was most apparent in the head region, with dwarf individuals characterized by a smaller sloped head, shorter snout, larger eyes and a smaller mouth (Moles et al. 2007). No differences in trophic ecology between the two walleye morphs were found, contrary to expectations,

as dwarf morphological traits are typically associated with benthic feeding (Moles et al. 2007).

Variation in scale shape has also been found in Lake Winnipeg walleye. Watkinson and Gillis (2005) successfully used Fourier and wavelet summaries of scale outlines to discriminate among walleye taken from three locations (Grand Rapids, Matheson Island and Riverton). Significant differences were found between locations, with additional groups potentially present at other spawning sites. Significant age-effects were observed, and the scale shape was not consistent between years at a single spawning location. Scale shape can be influenced by environmental conditions (e.g., water temperature), and different conditions can exist between spawning sites and among years at a single spawning site. The discrimination of walleye from the three distinct spawning aggregations indicates this phenotypic trait may be linked to some degree of population structure at these sites.

Distinct differences in life history characteristics between walleye in the north and south basins of Lake Winnipeg have also been described. Since the establishment of a commercial fishery on Lake Winnipeg in 1883, the fish community has shifted, favouring species with relatively small maximum body size, rapid growth, accelerated maturation, and high fecundity, traits that improve fish survival in a gill net fishery (Lysack and Kristofferson 2004). Walleye in different regions of Lake Winnipeg display variation in terms of growth rate, age at maturity, and mortality rate that reflect the different minimum gill net mesh sizes used in the different areas of the lake (Lysack and Kristofferson 2004). Fish in the south basin, where the commercial fishery uses a smaller

mesh size, have a faster growth rate, a smaller maximum size and an average age of maturity of 4.16 years (Lysack 1980, 1986). Walleye in the north basin, where a larger gill net mesh size is used, have a slower growth rate, a larger maximum size and an average age of maturity of 5.35 years (Lysack 1980, 1986).

Philopatry

Natal homing or spawning site philopatry is a behaviour whereby individuals exhibit a tendency to reproduce at the site where they were born. It is thought that philopatric behaviour offers a benefit in that it ensures individuals return to a spawning site that has proven successful in the past (Rich et al. 2006). Philopatry has been identified in many animal taxa, including migratory birds (Aulsebrook et al. 1992), deer (Purdue et al. 2000), sea turtles (Encalada et al. 1998) and sperm whales (Engelhaupt et al. 2009). Homing has also been observed in many fish species including Atlantic cod (Robichaud and Rose 2001), American shad (Waters et al. 2000), rainbow smelt (Bradbury et al. 2008), and has been extensively studied in salmonids (e.g., Quinn et al. 1999, Candy and Beacham 2000, Bridger et al. 2001, Carruth et al. 2002, Nordeng et al. 2006, Makino et al. 2007, Nordeng 2009; see also review by Dittman and Quinn 1996).

Pacific salmon (*Oncorhynchus* spp.) are well known to travel large distances from their oceanic feeding grounds to their natal stream to spawn. Salmon travel through diverse habitats during this migration, and are thought to rely on a variety of navigation techniques (olfactory cues, position of the sun, polarized light patterns or the magnetic field of the earth, Dittman and Quinn 1996). Homing to an individual's natal site is

generally very precise, resulting in reproductively isolated spawning populations with a high degree of genetic differentiation between them (Dittman and Quinn 1996).

Other fish species also display philopatric behaviour. Adult Atlantic cod (*Gadus morhua*) off the west coast of Scotland were found to return to the same spawning site (return rates of 67 to 97%), with most fish remaining within 100 km of spawning areas year round, indicating resident spawning groups (Wright et al. 2006). Cod spawning off the coast of Newfoundland were found to have homing rates of 39 and 53% in 1999 and 2000, respectively (Robichaud and Rose 2001). Return of an individual to the same spawning site in multiple years occurred in 26% of tagged cod (Robichaud and Rose 2001).

Differences in the nuclear and mitochondrial DNA of American shad (*Alosa sapidissima*) indicated high degrees of homing between four populations (three from the Atlantic coast drainage and one from the Columbia River). The data indicated a 1% level of straying between sites in the Atlantic coast drainage, and differences between the populations indicated that individual river populations should be managed as distinct stocks (Waters et al. 2000). Anadromous rainbow smelt (*Osmerus mordax*) off the coast of Newfoundland were found to return to their natal site with a return rate of 90 – 99% (Bradbury et al. 2008).

Philopatry in Walleye

The homing behaviour of walleye to particular spawning streams or local areas has been extensively documented by tagging studies in multiple lake systems (Eschmeyer 1950, Eschmeyer and Crowe 1955, Forney 1963, Foust and Haynes 2007, Ward et al. 1989). While philopatry in salmonids is usually obligatory, it appears to be more facultative in

walleye (Colby and Nepszy 1981). In fact, Olson and Scidmore (1962) found that homing was not seen in the majority of walleye in Minnesota, and those that exhibited it did so in an irregular fashion. When present, philopatry results in a spatial separation of spawning aggregations across the lake, providing potential for genetic variation among spawning sites and for potentially discrete genetic stocks (see below) within the lake.

Genetic work in the Great Lakes using both mitochondrial control region DNA sequences (Stepien and Faber 1998, Gatt et al. 2002, Stepien 2003) and nuclear markers (Stepien 2003) have revealed genetic differences among spawning site populations consistent with those expected from reduced gene flow as a result of philopatry. Genetic analysis of spawning populations showed similar genotypic frequencies in both sexes, indicating philopatry occurs in both males and females (Stepien and Faber 1998). Walleye have the capability to migrate and move great distances (Crowe 1984, Bickford and Hannigan 2005), making it difficult to predict the spawning stock to which an individual fish belongs from its site of capture. A study by the Manitoba Department of Conservation in 1974 – 75, for example, demonstrated the capabilities of walleye in Lake Winnipeg to move over 200 kilometers within the lake (cited in Watkinson and Gillis 2005). If spawning site philopatry exists in Lake Winnipeg walleye populations, there is potential for numerous genetically distinct stocks to exist. The hydrology of the lake itself (two distinct basins connected by the narrows) increases the likelihood of some degree of separation between spawning populations.

Genetic Stock Assessment

Recognition of population structure is necessary for proper management. From a fisheries perspective, a genetic stock is a group of organisms sharing a gene pool that is sufficiently discrete and identifiable that it warrants its own management strategy (Palmer et al. 2005). Multiple genetic stocks can co-exist in a single water body if their reproduction is separated either temporally or spatially (i.e. spatial separation by natal homing). This co-existence can complicate management strategies in mixed stock fisheries, as each stock must be managed separately with information on the relative abundance, movements, habitat use, spawning sites, and life history traits of each stock taken into consideration (Palmer et al. 2005).

Anthropogenic factors, such as commercial fishing, can reduce the genetic diversity present in a population. Conservation and evolution both rely on genetic diversity within a population, which acts as the heritable basis for evolutionary change (Lande and Shannon 1996). Genetic diversity enables native species to inhabit a variety of environments, by allowing them to adapt to local conditions and withstand perturbations such as exploitation, competition with invasive species, and habitat degradation. Loss of genetic variation can be detrimental to the long term viability and persistence of a population (Lande and Shannon 1996). It is therefore important to identify all stocks within a system, to ensure no single stock is exploited at a level above or below that which is sustainable.

Methods of Stock Assessment

Many characters and methods have been used in an attempt to identify stock structure in exploited fish species, including ecological characteristics, tagging, parasite load, physiological and behavioral traits, morphometrics and meristics, calcified structures, cytogenetics, immunogenetics, blood pigments and molecular techniques (Carvalho and Hauser 1994). Below is a brief summary of some of the more common methods used to detect stock structure in various fish species, and examples of how they have been successfully applied.

Tagging Studies

The presence of stock structure can be detected directly or indirectly through a variety of tagging methods. Radio, acoustic or data storage tags can provide direct measures of movement and habitat use, while passive tags (e.g., numbered or coded tags, fin mutilation, branding, tattooing and chemical or dye batch marks) require mark/recapture studies (Carvalho and Hauser 1994). Recoveries of marked fish over time indicate the range and patterns of movement for individual fish, resulting in the ability to infer the degree of stock separation and mixing. Drawbacks to this methodology are that a large proportion of the population must be tagged, and there are difficulties associated with recapturing sufficient tagged individuals (Carvalho and Hauser 1994).

Using fin-clips and Visual Implant Elastomer (VIE, a passive batch tag), philopatry of anadromous rainbow smelt off the coast of Newfoundland was detected, with 90 to 99% annual spawning site fidelity (Bradbury et al. 2008). Tagging using mark recapture

studies and data transfer tags was also successful in identifying philopatry in adult Atlantic cod off the west coast of Scotland (Wright et al. 2006).

Otoliths

Calcified structures, including scales, otoliths and bones, have been used extensively to determine ages and growth rates in numerous fish species, as well as to identify individuals from specific areas, sub-populations, races or stocks (Campana and Thorrold 2001). Formation patterns of annuli, spacing between annuli (Carvalho and Hauser 1994) and elemental and stable isotope fingerprints within calcified structures (Campana et al. 2000) are some of the methods used. Incorporation of specific trace elements into the growing surface of fish otoliths can reflect the physical and chemical characteristics of the surrounding water (Campana et al. 2000). The chronological properties of otoliths and scales (annual rings) provide opportunities to estimate age, growth and habitat usage on both a daily and yearly scale, in the present as well as in the past (Campana and Thorrold 2001).

Otolith microchemistry has been especially successful in detecting population structure in fish populations. Atlantic silverside (*Menidia menidia*), a ubiquitous marine species found along the coast of North America, undergoes extensive offshore migrations during winter (Clarke et al. 2009). Analysis of chemical signatures in the otoliths of juveniles was successful in assigning individuals to their natal sites with an accuracy of 70% and 77% in 2003 and 2004, respectively. Significant interannual variation in otolith fingerprints was found, indicating that they are year-class specific (Clarke et al. 2009).

Otolith microchemistry can also be applied to multiple species using the same nursery habitats. A study assessing the usage of eight estuarine nursery grounds by five commercially important marine species off the Portuguese coast was successful in assigning juveniles of each species to their natal site with a success rate of between 70.2 to 92% (Vasconcelos et al. 2007). Otolith microchemistry can likewise be successfully applied in freshwater environments. A study assessing the use of adult and nursery habitats in yellow perch (*Perca flavescens*) from Lake Superior was able to differentiate between adult perch residing in bay and wetland habitats with 100% accuracy (Brazner et al. 2004). Assessment of otolith cores in adult perch also identified the presence of multiple nursery sites (Brazner et al. 2004).

Otolith shape has also proved to be a useful technique in the detection of stock structure. Cartesian Fourier analysis of otoliths taken from Atlantic cod (*Gadus morhua*) spawning populations in the northern North Sea and west coast of Scotland was used to successfully differentiate between spawning stocks. Classification rates of >70% were obtained between spawning sites (Galley et al. 2006).

Allozymes

Allozymes are electrophoretically detectable differences in the amino acid composition of enzymes that share a common substrate. The differences are heritable, and can be used as estimators of genetic differentiation, reproductive isolation and mating patterns (Carvalho and Hauser 1994). The mutation rate for allozymes is very low, with a general estimate in the order of 10^{-6} to 10^{-7} mutants per gene transmission, and not all DNA mutations resulting in a change in the amino acid sequence (Nei 1987). It is possible to obtain up to

50 different allozymes (or loci) from a sample with moderate effort (Ihssen et al. 1981). The resolution obtained by allozymes ranges from different genera within a family to differentiation between conspecific populations of fish (Ihssen 1981). When attempting to genetically characterize individual stocks using allozymes, sample sizes of at least 50 individuals and 20 loci are generally required (Ihssen et al. 1981). Allozymes were widely used beginning in the 1970's, but are being replaced by DNA based methods (Liu and Cordes 2004).

Allozymes have been successfully used in detecting genetic diversity and population structure in many freshwater fish species. Allozyme loci (23) were used to estimate intraspecific genetic diversity within ten freshwater fish species in the contact zone between the Rhine/Upper Danube. Sculpins and resident salmonid species were all found to have high levels of differentiation between the Rhine and Upper Danube regions. European barbel (*Barbus barbus*) and other cyprinid species showed high levels of heterogeneity and moderate genetic differentiation between the two regions, while genetic differentiation was found to be very low in three species of anadromous fish (Schreiber et al. 2009).

Allozymes have been successful in detecting intraspecific differentiation in lake whitefish in Lake Winnipeg and surrounding areas. Differences between lake whitefish in northern Lake Winnipeg and Little Playgreen Lake were detected using 36 allozyme loci (eight of which proved informative) (Mavros 1992). These results were comparable to those found in an earlier study (Kristofferson 1978), which found differences in frequencies of glycerol-3-phosphate dehydrogenase (G-3-PDH) between fish from Lake St.

Martin/Dauphin River (frequency of 0.77) and fish from the rest of Lake Winnipeg and Little Playgreen Lake (frequency of 0.46), reflecting the homing movement of some lake whitefish into spawning grounds in Lake St. Martin (Kristofferson 1978).

A drawback to using allozymes to investigate population structure are the tissue types required. Allozyme analysis requires fresh or frozen tissue, typically from organs (such as liver) or muscle, resulting in logistical difficulties in sampling (keeping tissue fresh or frozen, and often lethal sampling procedures).

Mitochondrial DNA

The examination of variation in DNA sequence among organisms allows the identification of a large number of genetic markers. Variation in mitochondrial DNA (mtDNA) has been widely adopted as a tool to perform DNA analysis with fisheries related applications (Billington and Hebert 1991). The use of mtDNA as a molecular marker has many advantages. The high mutation rate (relative to allozymes or nuclear markers) and low effective population size (approximately one quarter that of nuclear DNA, as a result of maternal inheritance; Avise et al. 1987) of mtDNA can make it an effective marker in detecting population structure (Moritz et al. 1987), although in recent years microsatellites have been found to resolve more finescale differences (see below). Differing rates of evolution found in the different regions of the mitochondrial genome (Saccone et al. 1991) allow multiple time scales to be addressed. Typically, the overall rate of mtDNA sequence divergence has been estimated to be 2% per million years (Brown et al. 1979). The conserved regions within the mitochondrial genome allow universal primers to be designed and applied for a variety of organisms without prior

knowledge of their genomes (e.g. Kocher et al. 1989). Mitochondrial DNA is maternally inherited in most organisms and does not recombine (i.e. it represents a single locus) (Carvalho and Hauser 1994). While microsatellites are typically more effective in elucidating finescale population structure, broader scale differences most often detectable using mtDNA may reveal subtle patterns obscured by the microsatellites. The maternal inheritance pattern of mtDNA also allows female specific population structure to be detected, which would be obscured with the bi-parental inheritance system of nuclear DNA (e.g., Engelhaupt et al. 2009).

Variation in mtDNA sequences can easily be investigated through direct sequencing or Restriction Fragment Length Polymorphism (RFLP) assays (either in the entire genome, or specific gene or genes amplified by PCR) (Billington and Hebert 1988). Direct sequencing allows comparison between nucleotide sequences at all sites within the region targeted, and each variable site constitutes a character. RFLP assays, on the other hand, utilize restriction enzymes to cleave the region of interest at particular recognition sites, resulting in variable size fragments if sequence differences result in the gain or loss of these recognition sites. The relatively low cost and high throughput of RFLP assays compared to direct sequencing make them attractive as a method to quickly and easily detect mtDNA haplotypes. However, RFLP assays can only detect differences on a fraction of the gene or gene fragment, while direct sequencing allows information to be obtained at every nucleotide. RFLP assays are most effective when a large number of restriction enzymes are used (resulting in increased coverage of the gene or genome), or when known variable sites (as detected by direct sequencing) are targeted (e.g., Docker and Heath 2002).

Direct sequencing has been successful in resolving population structure in a number of species and systems, both freshwater and marine. Marine systems are often characterized by a large degree of connectivity between habitats, and highly migratory species. The genetic population structure of the flathead mullet (*Mugil cephalus*) was investigated using mtDNA control region sequences. Individuals collected from seven locations in the Pacific north-west were found to belong to two highly divergent lineages, with one lineage found in the East China Sea, and the second lineage in the South China Sea (Jamandre et al. 2009). The high connectivity of marine habitats is also demonstrated by the population structure of albacore (*Thunnus alalunga*) in the northwestern Pacific Ocean. Genetic differences resolved using mtDNA control region sequence variation indicated two distinct clades, although one clade consisted of 98% of the individuals. The clades were found to mix extensively, and constituted a single stock, perhaps due to the highly migratory nature of albacore (Wu et al. 2009).

Variation in mtDNA sequences has also resolved population structure in freshwater systems. For example, phylogenetic analysis using the entire mitochondrial genome of the plains topminnow, *Fundulus sciadicus*, across its distribution resolved two distinct clades distributed across five drainages, with clade one occurring in the Osage, Gasconade and Spring River drainages, and clade two occurring in the Nebraska and Lamine river drainages. Populations among drainages were also found to be genetically distinct from each other, with 93% of the total molecular variance being attributed to among-drainage effects (Li et al. 2009). The wood river sculpin (*Cottus leiopomus*) has a limited range in central Idaho and is considered to be a species of conservation concern

due to its limited distribution. A survey of the genetic diversity of the wood river sculpin revealed significant differences in the mtDNA control region among the three major river sub-basins in which they were captured (Meyer et al. 2008).

The use of PCR-RFLP assays as a tool to investigate genetic diversity present in mtDNA has also been widely applied, especially with respect to population structure in salmonids. PCR-RFLP assays were utilized to assess over 97% of the mtDNA genome in coho salmon (*Oncorhynchus kisutch*). Samples from the northern Pacific Ocean and Bering Sea were resolved into two monophyletic clades, and haplotypes associated with both clades were found to be widely distributed in both basins (Gharrett et al. 2001). This indicates the presence of gene flow, population movements or recolonization following divergence of refugial isolates (Gharrett et al. 2001). Applying RFLP assays to assess genetic diversity of chum salmon (*Oncorhynchus keta*) spawning runs in five rivers in Russia allowed significant differences between the five spawning sites to be resolved (Brykov et al. 2003). When spawning runs in close geographic proximity were sampled (on the same island), no differences were found (Brykov et al. 2001). The lowest diversity in RFLP haplotypes was found in completely artificial stocked populations, while the highest was found in naturally occurring river populations (Brykov et al. 2001).

The use of complementary methods, such as PCR-RFLP assays combined with direct sequencing of mtDNA, can also be useful. A combination of PCR-RFLP assays of the mitochondrial control region and NADH subunit regions, with direct sequencing of three other mitochondrial fragments (tRNA-Ile and NADH-1, tRNA-Leu and 16S rRNA) was used to assess mtDNA variation in Arctic grayling (*Thymallus arcticus*; Redenback and

Taylor 1999). Two haplotypes were detected, characteristic of two refugia during the Wisconsin glacialiation. Populations in British Columbia were thought to have originated from the Bering glacial refuge, while those in Montana were significantly different, and thought to originate from the Upper Missouri or the southwest Alberta glacial refuge. Sequence differences estimated a divergence time of 370 000 years between the two grayling haplotypes (Redenbach and Taylor 1999). Likewise, combined use of mitochondrial and nuclear markers (see below) can be complementary, but may not always provide similar results, due to the increased resolution of microsatellite markers, and different inheritance patterns (see below). When the genetic diversity of Arctic charr (*Salvelinus alpinus*) in central Alpine lakes among the Rhone, Rhine and Danube river systems was assessed using PCR-RFLP assays of two different mtDNA control region sequences and microsatellite loci, very little variation was found with RFLP assays of the two mitochondrial regions with 31 restriction enzymes. In contrast, microsatellite analysis of nuclear DNA using six loci detected high levels of genetic diversity within and among populations (Brunner et al. 1998).

Detection of genetic difference between two ecotypes within a species has also been accomplished through the use of mitochondrial RFLP assays. Kokanee (*Oncorhynchus nerka*) in Okanagan Lake, south-central interior of British Columbia, exhibit two reproductive ecotypes – stream spawners and beach spawners. No significant differences between the two types were found using variation in morphological traits, allozymes or minisatellites. Analysis of mtDNA using RFLP assays revealed significant differences between the two ecotypes, but not between individual populations of each ecotype (Taylor et al. 1997).

The success of stocking has also been assessed using RFLP assays. Stocking success of brown trout (*Salmo trutta*) in a Danish river system was assessed by RFLP mtDNA assays, using seven restriction enzymes. Little differentiation was found between sampled river populations, but significant differences between the hatchery strain and all river samples existed. The haplotype specific to the hatchery strain was almost absent from the stocked river populations, indicating little to no contribution of stocking to the current population (Hansen et al. 1995).

Microsatellites

In recent years, microsatellites have become the molecular marker of choice to investigate stock structure. Microsatellites generally provide greater resolution than other molecular makers in detecting genetic variation among populations, and consist of short (one to six nucleotides) tandem repeats of DNA (Selkoe and Toonen 2006). They vary in length (i.e. the number of repeats, generating multiple alleles), and are typically between 20 and a few hundred repeat units long (Chistiakov et al. 2006). Microsatellites are single locus markers characterized by their multi-allelic nature, co-dominant inheritance and high degree of genome coverage (Balloux and Lugon-Moulin 2002, Chistiakov et al. 2006). The high mutation rates of microsatellites, ranging from 10^{-2} to 10^{-6} mutations per locus per generation, make them extremely useful in detecting changes in the recent past (10 – 100 generations) (Selkoe and Toonen 2006). Their ubiquitous nature throughout the nuclear genome allows multiple loci to be assessed per individual, allowing increased statistical power when comparing individuals and populations (Selkoe and Toonen 2006) and making them useful in identifying stocks and conservation units (Chistiakov et al.

2006). Concerted evolution through molecular drive may promote the divergence of microsatellites even in partially isolated populations, increasing the incidence of population specific markers (Franck et al. 1991). As microsatellites are small in length and amplify efficiently, only a small amount of tissue is required, and samples can usually be collected non-lethally and preserved in alcohol.

The highly mutable microsatellite locus is flanked by conserved regions, allowing for the design of unique oligonucleotides (primers), which define the locus and allow the microsatellite to be amplified using PCR. Differences in the lengths of specific loci (occurring as a result of mutations caused by slippage or proofreading errors during DNA replication) indicate variation between individuals and populations, based on the assumption that all distinct alleles differ in length (Selkoe and Toonen 2006). This is often not the case, as mutations in microsatellites can result in alleles of the same size with different lineages or sequences. This homoplasy is generally a minimal source of bias in populations with a short history or moderate effective population size, with the chance of homoplasy proportionate to the genetic distance between populations and individuals (Selkoe and Toonen 2006).

Microsatellite variation has been successfully applied in the detection of population structure and estimation of the stock composition in mixed stock fisheries in several salmonid species. Population structure has been resolved in sockeye salmon populations of the Nass River, northern British Columbia (Beacham and Wood 1999, six microsatellite loci), and sockeye from coastal lakes of British Columbia (Beacham et al. 2005, 14 microsatellite loci). In both instances regional structuring was detected, and the

differences between stocks were 10 – 13 times greater than inter-annual variation within a stock. Estimation of stock composition within recreational fisheries has also been successful, both with the coho salmon fishery off Vancouver Island (Shaklee et al. 1999, five loci) Chinook salmon (*Oncorhynchus tshawytscha*) along the Pacific Rim (Beacham et al. 2000, 13 loci) and steelhead (Beacham et al. 1999, eight loci). Simulations in each case indicated it was possible to obtain an accurate estimation (greater than 80% of the time) of stock composition in mixed stock fisheries, as well as assign individuals to rivers or regions of origin (Shaklee et al. 1999, Beacham et al. 1999, Beacham et al. 2006).

Population structure in other freshwater fish species has also been resolved using microsatellites. Population structure of smallmouth bass (*Micropterus dolomieu*) was detected in the Great Lakes area using eight microsatellite loci. Significant differences between spawning sites within lakes, between basins within a lake, and between closely spaced sampling sites were detected, although these differences had only a weak correlation to the geographic distance separating them (Stepien et al. 2007). The study also identified marked differences between bass utilizing lake and river spawning habitats.

Genetic Stock Assessment in Walleye

Previous declines of walleye stocks in other commercially fished lakes (e.g., in the Great Lakes region) have demonstrated the sensitivity of walleye to fishing pressure, and have emphasized the importance of proper management of separate stocks to retain genetic diversity and allow sustainable use by commercial, recreational and domestic fisheries. Significant investigation into the stock structure of walleye has been undertaken using

many of the methods discussed above; a summary of the major work done to date will be discussed below.

Tagging Studies

Tagging studies of walleye have provided interesting insight into the population structure present in different lakes. In Claytor Lake, Virginia, radio tagged fish were used to study two distinct populations of walleye found to exhibit different home ranges, spawning movements and spawning grounds, despite the lack of a physical barrier (Palmer et al. 2005). Tagging studies using floy tags on Lake Erie revealed a strong tendency for fish to return each year to the spawning ground they were tagged on (62% of recaptured fish caught at the release site, while an additional 34% were captured within 15 miles of the release site; Wolfert and Van Meter 1978). There was also evidence that walleye stocks from the eastern and western parts of Lake Erie remained geographically separate.

Otoliths

Otolith microchemistry (specifically, the concentration of calcium ions) has been used to distinguish between stocks originating from two separate hatcheries (Missouri State Hatchery and Arkansas Federal Hatchery), as well as a potentially wild stock in the Eleven Point River, northeastern Arkansas (Bickford and Hannigan 2005).

Allozymes

Differentiation of walleye stocks using allozymes has met with limited success. Ward et al. (1989) used allozyme variation to assess twelve populations of walleye from the Great Lakes, and three from northern Manitoba. They screened nine enzyme loci, with five (ADH, MDH-3, IDH-1, Prot-4, Prot-2) displaying appreciable variation (at least two

alleles per locus). However, only one (Prot-4) showed geographic patterning of allele frequencies, which was consistent with earlier research (Uthe and Ryder 1970, Uthe et al. 1966) that showed that this locus is nearly fixed for a single allele (Prot-4¹⁰⁰) in walleye populations west of the Great Lakes and polymorphic for two alleles (Prot-4¹⁰⁰ and Prot-4¹⁶⁰) in Great Lakes walleye populations; Lake Winnipeg has a Prot-4¹⁰⁰ allele frequency of 0.94. Similarly, electrophoresis of extracts from walleye white skeletal muscle revealed six malate dehydrogenase (MDH-3) isozyme phenotypes and geographic patterning of these phenotypes (Clayton et al. 1971). The heritability of these phenotypes was tested in a breeding experiment, and provided evidence for the existence of three non-dominant alleles at a single MDH locus, with allele frequencies varying in walleye from four Canadian locations (Clayton et al. 1971). In particular, the MDH-3⁷⁰ locus occurred in higher frequencies in walleye from Prince Albert National Park in central Saskatchewan than in any other walleye population in western Canada (Clayton et al. 1974). In Claytor Lake, Virginia, MDH allele frequencies have been successfully used to differentiate between stocked, augmented and natural walleye cohorts, indicating that stocked juvenile walleye contributed an average of 67% of year class strength to augmented cohorts (Murphy et al. 1983).

Mitochondrial DNA

Mitochondrial DNA (mtDNA) has been extensively used to investigate the stock structure of walleye, specifically in the Great Lakes. Billington and Hebert (1988) were one of the first to utilize mtDNA for walleye stock assessment, sampling 141 walleye from ten populations in the Great Lakes Basin. Restriction digests of the entire mtDNA genome revealed polymorphisms between fish in six of the 22 restriction enzymes

assessed. Two major groups representing stocks descended from the Atlantic and Mississippian glacial refugia were resolved, but it was impossible to detect finer scale variation (i.e. between spawning sites). When mtDNA (entire genome) variation in 847 walleye sampled from 68 walleye populations across the North American species range was assessed (10 restriction enzymes) by Billington et al. (1992), 34 haplotypes were recognized. Of the 34, 89% of all sampled fish belonged to one of three major haplotypes with a distinct geographical distribution: haplotype 1 (12% of all fish) was dominant in the eastern Great Lakes region; haplotype 4 (58% of all fish) was dominant in the central Great Lakes range and haplotype 10 (19% of all fish) was dominant in the northwest (west of Lake Superior). These three distinct distributions reflect the postglacial colonization of walleye in North America, with haplotype 1 (which corresponds to haplotype A from Ward et al. (1989)) originating in the Atlantic refugium, haplotype 4 (which corresponds to haplotype B from Ward et al. (1989)) from the Mississippi refugium and haplotype 10 (which corresponds to haplotype C from Ward et al. (1989)) from the Missouri refugium. The division of three major phylogenetic assemblages inferred with RFLP assays has been supported by Gatt et al. (2000), who used direct sequencing of a 513 base pair fragment of the control region to detect 17 mtDNA haplotypes in fish from the Great Lakes and Luxapalila Creek (northeastern Mississippi). Gatt et al. (2000) were able to assign walleye from the Great Lakes to one of the three major RFLP haplotypes previously described, and found that southern walleye populations (Luxapalila Creek, Virginia) that were unaffected by glaciations contained more divergent haplotypes (Billington et al. 1992).

In 1996, Billington assessed the usefulness of mtDNA to resolve stock-identification and post-Pleistocene recolonization events. RFLP analysis of the entire mtDNA genome identified a total of 42 walleye haplotypes across North America. These could be divided into five groups with distinct geographic distributions. Three of the groups represent the three glacial refugia in the Pleistocene (Atlantic, Mississippian and Missourian), the fourth group is thought to have resulted from extensive stocking, and the fifth group is a very distinct walleye population found only in the Mobile drainage basin in Alabama and Mississippi (Billington 1996). Walleye from Luxapalila Creek, in the Mobile drainage basin, were found to have a 2.34% sequence divergence compared to walleye from the Mississippi River basin and other northern drainages, compared to a 0.39% sequence divergence between walleye from the Mississippi River basin and other northern drainages (Billington and Strange 1995). Clear broadscale geographic variation (between lake basins and glacial refugia) was discerned using mtDNA haplotypes resolved in walleye from 12 Great Lakes populations and three northern Manitoba populations (Ward et al. 1989). The proportion of total detected genetic diversity attainable to population differentiation (G_{st}) was three to five times greater than that detected with allozymes (Ward et al. 1989).

More recently the genetic and phylogeographic structure of walleye populations at different (including finer) geographical scales within the Great Lakes area (spawning sites, lake basins, lakes and putative glacial refugia) were assessed using mtDNA control region sequences (Stepien and Faber 1998). Phylogeographic structure and significant differences were found at the three broad scales as well as among some of the spawning sites (finer scale). Walleye populations in Lake Erie, Lake St. Clair and Lake Huron

were found to consist of nine mtDNA haplotypes, two of which accounted for 90% of all fish sampled (McParland et al. 1999). Significant genetic differentiation was found between the three sites, with genetic similarity varying relative to geographical proximity (McParland et al. 1999).

Finescale patterning in genetic variability was also observed among spawning sites in Lake Erie (Merker and Woodruff 1996). Gatt et al. (2002) used mtDNA to assess walleye populations in Lake Huron. Significant structuring was found between walleye spawning in eastern Georgian Bay and those spawning in the North Channel. This structure was consistent with the geographic distance between the two sites. Low levels of differentiation were detected between walleye spawning in eastern Georgian Bay and the French River. The majority of fish sampled had a mtDNA haplotype associated with the Mississippian refugium, consistent with theories of postglacial colonization.

However limited numbers of fish did possess haplotypes consistent with the Atlantic and Missourian refugium, indicating partial postglacial colonization by all three refugia (Gatt et al. 2002).

Variation in mtDNA has also been successfully used to examine the relative contributions of fisheries. Gatt et al. (2003) examined the relative contributions of five spawning walleye stocks to three recreational fishing derbies and a commercial fishery in Lake Erie (1995 – 1996). They detected 23 mtDNA haplotypes in 349 walleye collected from the recreational and commercial samples, the results of which suggested the western basin stocks contributed significantly to both the recreational and commercial fishery, but the

contribution of the eastern basin was not significantly different from zero. These results were consistent with mark-recapture data of tagged fish (Gatt et al. 2003).

Microsatellites

Significant work has been done on population structure in walleye, once again focusing on the Great Lakes area. Microsatellites (10 loci) have been used to assess genetic relationships of walleye across North America (Stepien et al. 2009). The authors successfully identified nine to ten primary population groups of walleye across North America, as well as revealing significant differences between spawning locations within lakes and lake basins. BROADSCALE genetic differences corresponded to an isolation by distance model (i.e. genetic similarity between populations decreases exponentially as the geographic distance between them increases), while FINESCALE differences did not (Stepien et al. 2009). General patterns of genetic diversity across the range of North American walleye corresponded to the biogeographical history of isolation by glacial refugia, although more recent gene flow was also detected (Stepien et al. 2009).

Microsatellite DNA markers have been used to determine if discrete stocks (reproductive units) were found in different geographic areas in Lake Erie. Considerable genetic variation was found; however, 99% of variation was found to occur within – as opposed to between – populations, and only one distinct stock in the western end of the lake was found (Wilson and Gatt 2000). The authors interpreted the low divergence to represent high gene flow between spawning sites or a recent shared ancestry. Strange and Stepien (2007) used 10 microsatellite loci to examine genetic variation of walleye in Lake Erie between all 10 major river and reef spawning sites. They found resolution to be

sufficient to distinguish between most spawning sites, but the relationships did not correspond to the physiographic basins or distances between localities.

Microsatellites have also proven to be a useful tool for detecting lineages. Parentage assignment with nine microsatellite loci was used to assess the contributions of the Grand River walleye stock (genetically distinct from all other Lake Erie stocks) to juvenile recruitment in the eastern basin of Lake Erie (MacDougall et al. 2007). It was determined that most Lake Erie young of the year originated from the eastern basin, with little contribution from Grand River. The success of hatchery stocking into the Grand River was also examined using five microsatellite loci. Comparison of these loci between walleye brood stock and juveniles enabled wild-type fish to be distinguished from those descended from hatchery stock (MacDougall et al. 2007). In Lake Superior, walleye populations of Black Bay and Nipigon Bay were analyzed at five microsatellite loci in an attempt to assess the success and relative contributions of previous rehabilitative stocking efforts in Nipigon Bay, as well as to determine the relationship between historical and present populations in Black Bay (Wilson et al. 2007). All four populations examined in Nipigon Bay were found to be genetically distinct, and the present day Black Bay population was found to be closely related to the historical population. Wilson et al. (2007) found microsatellite loci to be highly effective heritable tags for identifying wild-type and descendants of introduced walleye. Microsatellites (nine loci) have likewise been successfully used to determine the parentage of walleye descended from stocked fish in five southern Minnesota lakes (Eldridge et al. 2002).

Finescale variation in walleye population structure within nine pools in the Ohio River was assessed using multiple molecular tools (RFLP analysis of the control region and the 12S rRNA region, allozymes and nuclear microsatellites; White et al. 2005). While variation detected using allozymes and mtDNA was successful in resolving two genetic groups, microsatellite analysis revealed a high degree of significant structuring between walleye populations residing in each of the nine pools.

Complementary Methods

Although microsatellite markers generally provide a higher level of resolution than do mtDNA and allozymes, microsatellites and mtDNA have been used together as complementary genetic markers. Wilson and Gatt (2001) used approximately 715 bp of control region mtDNA and five microsatellite loci to detect genetic variation in walleye taken from various sites in Lake Erie. Microsatellites and control region mtDNA were also used together to determine if walleye from the New York waters of eastern Lake Erie were a distinct stock from those in the Bay of Quinte; however, no significant differences were found between the two locations (Wilson and Mathers 2002). Mitochondrial control region sequences have been used to assess the genetic and phylogeographic structure of walleye populations at different geographical scales within the Great Lakes area (spawning sites, lake basins, lakes and putative glacial refugia) (Stepien and Faber 1998). Phylogeographic structure and significant differences were found at the three broad scales as well as among some of the spawning sites (finer scale).

Study Area – Lake Winnipeg

Lake Winnipeg (located in southeastern Manitoba) is the seventh largest lake in North America, the 11th largest freshwater lake in the world (in terms of surface area; van der

Leeden et al. 1990) and holds world-wide status as a great lake (Torigai et al. 2000). It is the remnant of glacial Lake Agassiz, which covered much of Manitoba as the Laurentide Ice Sheet melted, from 11 000 to 7 800 years ago (Stewart and Watkinson 2004). Lake Agassiz finished draining 7 800 years ago, and the remnants formed the Manitoba Great Lakes Region. Lake Winnipeg began as a large basin in the north, separate from a small lake in the south (7 700 years ago, Matile et al. 1996). As isostatic rebound occurred, the two basins of Lake Winnipeg grew and joined (2 100 years ago), eventually resulting in the present day Lake Winnipeg (Matile et al 1996).

Lake Winnipeg is located between 50° and 54°N latitude, covers an area of approximately 24 000 square kilometers, and its watershed includes four provinces and two states. It is divided into two distinct basins (south and north) connected by the narrows, which is effectively a reversible, fast flowing river. The south basin is smaller and characterized by high temperatures, high turbidity and shallow depths (less than 12 m), as well as a clay/silt bottom (Torigai et al. 2000, Henderson and Last 1998). The narrows has irregular bathymetry, with some locations as deep as 61 m (Todd et al. 1998) and is characterized by lower turbidity, lower temperatures and a coarser silt bottom. The north basin has low turbidity and large algal blooms in summer. It is deeper than the south basin, with average water depths of 16 – 17 m (Torigai et al. 2000, Henderson and Last 1998).

Lake Winnipeg drains a large and varied watershed. The lake lies on the boundary of the Great Plains on the west, and the Canadian Shield on the east (Brunskill 1980). Rivers draining into the lake from the south and west are underlain by sedimentary strata, and

overlain by glacial sediments and fertile chernozemic soils (consisting of a high percentage of organic material, phosphoric acids, phosphorus and ammonia). Vegetation on the west side is characterized by prairie grass, mixed and deciduous forests. Rivers draining into Lake Winnipeg from the east flow over igneous Precambrian shield bedrock covered with thin soils and marked by muskeg swamps and boreal forests (Brunskill 1980). The substantial chemical differences resulting from inflows in different parts of the lake are retained, resulting in markedly different biological, chemical and limnological characteristics in different areas of Lake Winnipeg (Brunskill 1980). The turbulent mixing that occurs in Lake Winnipeg, combined with the relatively shallow depths, results in little to no vertical stratification in the lake. This, along with the short retention time for the entire lake (2.9 – 4.3 years during 1969 – 1974), acts as an important factor in preserving the identity of the separate water masses (Brunskill 1980). The major contributions of annual discharge (water and nutrients) originate from the Red and Winnipeg rivers in the south basin, and the Saskatchewan River in the north basin. The rivers draining shield areas were found to contribute 50% of the flow into Lake Winnipeg, despite the fact that they drain only 18% of the drainage area. This is thought to be a result of increased precipitation, decreased evaporation and low water storage in these areas (Brunskill 1980).

The biology of Lake Winnipeg has come into the spotlight due to its commercial, industrial, hydroelectric, municipal and recreational importance to the province of Manitoba (Waybrant and Shauver 1979). The lake also supports a large amount of tourism in the province of Manitoba, generating over \$100 million per year (Todd et al. 1998). The recognition of the lake's importance has resulted in a surge of research

activity on the lake, and the formation of the Lake Winnipeg Research Consortium in 1998, which undertakes a large amount of research on Lake Winnipeg.

Some research has been done on the population structure of lake whitefish in Lake Winnipeg. Using morphological characteristics, allozymes and stable isotopes, some differentiation between lake whitefish groups was detected. Significant differences in morphological characteristics were detected between fish from five sampling sites (Mavros 1992), and allozymes were able to resolve genetic differences between fish in northern Lake Winnipeg, Lake St. Martin and Little Playgreen Lake (Kristofferson 1978, Mavros 1992).

Lake Winnipeg Walleye Fishery

Lake Winnipeg supports a large and varied walleye fishery. Commercial fishing in Manitoba is a valuable industry, generating \$29 million annually in direct income for fishermen, and an excess of \$60 million in value added processed fish products sold by the Freshwater Fish Marketing Corporation, a federal crown corporation established in 1969 (Manitoba Water Stewardship 2006). From 1996 to 2006, walleye composed the greatest portion of Manitoba's commercial fishery, both by weight (averaging 29% of the total landed weight of all species) and by value (averaging \$18 million per year) (Manitoba Water Stewardship 2006, more recent values unavailable). Of all the lakes sustaining a commercial fishery in Manitoba, Lake Winnipeg is the largest and most profitable, contributing over 40% of the total province-wide walleye production and 61% of the total walleye harvest's landed value. The first commercial fishermen began fishing the waters of Lake Winnipeg in 1883 (Lysack and Kristofferson 2004), and commercial

fishing has grown in economic value since then, with the lake now supporting an average of 1 036 licensed fishermen and hired help annually (Manitoba Water Stewardship 2006).

Recreational and subsistence or domestic fisheries also play a large role in the walleye fisheries of Lake Winnipeg. In 2005 there were a total of 166 433 licensed adult anglers in Manitoba, with a total expenditure of \$106.3 million on activities and supplies directly related to fishing (Manitoba Water Stewardship 2005). Walleye accounted for 51% of the total 2005 catch (approximately 12 million fish; Manitoba Water Stewardship 2005); however, there is no information on the proportion of this catch taken from Lake Winnipeg. Lake Winnipeg supports a large tourism industry, with shallow, nutrient rich waters that provide ideal habitat for recreational species and make it a world-renowned walleye fishing destination. Residents of the communities on Lake Winnipeg and surrounding areas also engage in recreational angling as a pastime. Subsistence fisheries on Lake Winnipeg exist primarily in the northern communities, including Norway House First Nations, Grand Rapids Reserve, Berens River Band, Bloodvein Reserve, Brokenhead Reserve, Jackhead Reserve, Little Black River Reserve and Poplar River Band. These communities remain traditional in many ways, and utilize the Lake Winnipeg walleye fishery for sustenance and income.

Changing Conditions in Lake Winnipeg

Walleye may be adversely affected by current changing conditions (e.g., increased eutrophication and number of invasive species) in Lake Winnipeg. Invasive species such as rainbow smelt and Asian tapeworm (*Bothriocephalus acheilognathi*) are affecting the food base of walleye in Lake Winnipeg, making it potentially unstable. Rainbow smelt,

an important prey species in the north basin, demonstrate a typical invader “boom and bust” life history (Stewart and Watkinson 2004), resulting in a temporally unstable prey base. Asian tapeworms have been found to infect emerald shiners (*Notropis atherinoides*), another important prey species for adult walleye, especially in the south basin (Choudhury et al. 2006). The effects of this parasite on emerald shiner populations are as yet unknown, but may result in reduced numbers of this important prey species. Lake Winnipeg’s water demand and rate of eutrophication are both increasing as development increases (Patalas and Salki 1992), and the effects these demands have on the biology of the lake are largely unknown. These trophic instabilities and increased eutrophication, combined with continued exploitation by commercial, recreational and domestic fisheries, may result in increased stress on walleye in Lake Winnipeg.

RESEARCH OBJECTIVES

This study utilizes nuclear (and measured by microsatellites) and mitochondrial DNA variation to investigate the genetic diversity of walleye in Lake Winnipeg, quantify the degree of genetic differentiation among several spawning and sampling sites in Lake Winnipeg, and compare genetic diversity between Lake Winnipeg walleye and walleye from hatchery sites that have been stocked into Lake Winnipeg. While microsatellite markers have been found to provide greater resolution in several previous studies (e.g., Stepien and Strange 2007, Wilson and Gatt 2007, Stepien et al. 2009; see above), the inclusion of maternally inherited mtDNA as a complementary method may allow inference of female population structure.

The quantification of genetic variation detected with microsatellite and mitochondrial techniques will be used to determine the degree of genetic diversity present in Lake Winnipeg walleye as a whole. Overall genetic diversity will be compared to that found in walleye in other systems (mainly the Laurentian Great Lakes). Microsatellite and mitochondrial DNA variation will also be used to determine if population structure is present among walleye sampling locations in Lake Winnipeg. If genetic variation within a site (among individuals and among sampling years) is significantly less than between sampling sites, genetic stock structure is present in Lake Winnipeg walleye. If significant population structure is found, the genetic and geographic distance between sampling sites and between regions (i.e. the north and south basins) will also be compared, to determine if Lake Winnipeg walleye fit an isolation by distance model. The history of the establishment of walleye in Lake Winnipeg following glaciation will also be investigated by comparing mtDNA haplotypes found in the lake to three mtDNA haplotypes previously described as representing three glacial refugia (Ward et al. 1989, Billington et al. 1992, Gatt et al. 2000).

If significant walleye population structure is present in Lake Winnipeg, it is possible to apply stock composition analysis techniques to actual mixed-stock fishery samples (obtained from commercial processing plants) in order to gain insight into the relative abundance and contribution to the fishery of each distinct spawning group. Use of a site-specific "gene tag" would also allow inferences into the movement patterns, home ranges, life history characteristics and habitat use of each spawning group to be made.

Knowledge of the population structure and biology of walleye in Lake Winnipeg is necessary in order to effectively manage the multi-use fishery on the lake, ensuring

stocks are not under or over utilized. Effective management will ensure the Lake Winnipeg walleye fishery remains a sustainable resource.

CHAPTER ONE: USING MICROSATELLITE DNA VARIATION TO INVESTIGATE POPULATION STRUCTURE OF WALLEYE (*SANDER VITREUS*) IN LAKE WINNIPEG AND SURROUNDING AREAS

ABSTRACT

Walleye (*Sander vitreus*) are the main contributor to Manitoba's multi-million dollar commercial fishery, with Lake Winnipeg providing the most profitable catch. In some systems, walleye have been found to return to the same location to spawn each spring, a behaviour known as natal homing or philopatry. This spatial segregation of gene flow provides potential for multiple genetically discrete spawning groups to exist throughout the lake. The existence of multiple genetic groups in Lake Winnipeg has been suggested previously by significantly different scale shapes between populations, as well as different size morphs, colours and life histories at different locations throughout the lake. This study used ten microsatellite loci to determine the degree of genetic variation present among 13 spawning sites in Lake Winnipeg, and compared these to walleye collected from two hatchery locations, other locations in northern and eastern Manitoba, Lake of the Woods and the Laurentian Great Lakes. Genetic diversity between walleye and sauger (*Sander canadensis*), a closely related species, was also compared. Little population structure was found within Lake Winnipeg, although Grand Rapids and the Icelandic River showed slight significant differentiation from other Lake Winnipeg sampling locations ($F_{st} = 0.0066$ to 0.0457). Great genetic differentiation was found between walleye and sauger ($F_{st} = 0.1346$ to 0.2693). Slight to moderate genetic differentiation was found between Lake Winnipeg walleye and sampling locations outside of Lake Winnipeg ($F_{st} = 0.0037$ to 0.1054). The lack of genetic differentiation found in Lake Winnipeg possibly indicates a lack of natal philopatry, some philopatry but

with sufficient straying to obscure genetic structure, or obscured structure as a result of historical and current stocking.

INTRODUCTION

Walleye (*Sander vitreus*) are a large bodied piscivorous fish species reaching average lengths of over 50 cm. They generally spawn in spring or early summer, when water temperatures reach 6.7 – 8.9°C. Spawning grounds are typically the white water rocky areas below impassable barriers such as falls or dams in rivers, or boulder/coarse graveled shoals in lakes (Scott and Crossman 1973). Numerous spawning sites have been identified within the Lake Winnipeg area, including the Icelandic River, Hecla Bar, Jackhead River, Dauphin River, Warpath River, Saskatchewan River, Big Black River, Poplar River, Berens River, Bloodvein River, Winnipeg River, Red River and Netley Creek (Manitoba Conservation, personal communication; Figure 1). Lake Winnipeg walleye show considerable phenotypic variation, displaying differences in body morphology (including “dwarf” walleye, Moles et al. 2007), colour (“greenbacks”, Stewart and Watkinson 2004) and life history characteristics such as growth rate, age at maturity and life span (Lysack 1980, 1986, Lysack and Kristofferson 2004). Walleye in Lake Winnipeg have also been separated using Fourier and wavelet analysis of their scale shapes (Watkinson and Gillis 2005).

Natal homing or spawning site philopatry is a behaviour whereby individuals exhibit a tendency to reproduce at the site where they were born. The homing behaviour of walleye to particular spawning streams or local areas has been extensively documented by tagging studies in multiple lake systems (Eschmeyer 1950, Eschmeyer and Crowe 1955, Forney 1963, Foust and Haynes 2007, Ward et al. 1989). While philopatry in salmonids is usually obligatory, it appears to be more facultative in walleye (Colby and Nepszy 1981). In fact, Olson and Scidmore (1962) found that homing was not seen in the majority of walleye in Minnesota, and those that exhibited it did so in an irregular fashion.

Genetic work in the Great Lakes using both mitochondrial sequences (Stepien and Faber 1998, Gatt et al. 2002, Stepien 2003) and nuclear DNA variation (Strange and Stepien 2007, Stepien et al. 2009) has revealed genetic differences among walleye spawning sites consistent with those expected from reduced gene flow as a result of philopatry. When present, philopatry results in a spatial separation of spawning aggregations across the lake, providing potential for genetic variation to develop over several generations, and for potentially discrete genetic stocks to form within the lake. If spawning site philopatry exists in Lake Winnipeg walleye populations, there is potential for numerous genetically distinct stocks to exist.

From a fisheries perspective, a genetic stock is a group of organisms sharing a gene pool that is sufficiently discrete and identifiable that it warrants its own management strategy (Palmer et al. 2005). Multiple genetic stocks can co-exist in a single water body if their reproduction is separated either temporally or spatially (i.e. spatial separation by natal homing). This co-existence can complicate management strategies in mixed stock fisheries, as each stock must be managed separately with information on the relative abundance, movements, habitat use, spawning sites, and life history traits of each stock taken into consideration (Palmer et al. 2005). There is a large commercial, recreational and domestic walleye fishery on Lake Winnipeg, and proper management of this multi-use fishery requires increased knowledge of the population structure within the lake to ensure no stocks are being under or over utilized.

Anthropogenic factors, including commercial fishing, can reduce the genetic diversity present in a population. Conservation and evolution both rely on genetic diversity within a population, which acts as the heritable basis for evolutionary change (Lande and Shannon 1996). Genetic diversity enables native species to inhabit a variety

of environments, by allowing them to adapt to local conditions and withstand perturbations such as exploitation, competition with invasive species, and habitat degradation. Loss of genetic variation can be detrimental to the long term viability and persistence of a population (Lande and Shannon 1996).

The varied morphological and life history characteristics found in Lake Winnipeg walleye, along with diverse spawning locations, suggests the possibility of separate genetic stocks. Recognition of population structure is necessary for proper management. Previous declines of walleye stocks in other commercially fished lakes (e.g., in the Great Lakes region) have demonstrated the sensitivity of walleye to fishing pressure, and have emphasized the importance of proper management of separate stocks to retain genetic diversity and allow sustainable use by commercial, recreational and domestic fisheries.

Microsatellites are generally able to resolve finescale genetic differences among populations, and consist of short (one to six nucleotides) tandem repeats of DNA (Selkoe and Toonen 2006). Microsatellites are single locus markers characterized by their multi-allelic nature, co-dominant inheritance and high degree of genome coverage (Balloux and Lugon-Moulin 2002, Chistiakov et al. 2006). The high mutation rates of microsatellites, ranging from 10^{-2} to 10^{-6} mutations per locus per generation, make them extremely useful in detecting changes in the recent past (10 – 100 generations) (Selkoe and Toonen 2006). Their ubiquitous nature throughout the genome allows multiple loci to be assessed per individual, allowing increased statistical power when comparing individuals and populations (Selkoe and Toonen 2006) and making them useful in identifying stocks and conservation units (Chistiakov et al. 2006). In walleye, microsatellites have been successful in detecting population structure between reef and river spawning sites in Lake Erie (Strange and Stepien 2007); detecting lineages (MacDougall et al. 2007, Wilson et al.

2007); determining the success of hatchery stocking (MacDougall et al. 2007, Eldridge et al. 2002); determining relationships between physical lake parameters and genetic diversity (Cena et al. 2006); and detecting genetic differences within nine pools in the Ohio River (White et al. 2005). However, some studies have found no genetic variation between walleye spawning sites using microsatellites (e.g., Wilson and Mathers 2002)

Using ten microsatellite loci, the genetic population structure of walleye from 13 spawning sites within Lake Winnipeg will be investigated. Genetic comparisons of walleye from Lake Winnipeg, hatcheries from which walleye have been stocked into Lake Winnipeg, and numerous sites outside of Lake Winnipeg will also be assessed. This study will be the first such investigation into genetic diversity and population structure of Lake Winnipeg walleye. This information is required for effective and sustainable management of the Lake Winnipeg walleye fishery.

METHODS

Field Collection

Samples were collected from 13 spawning locations in Lake Winnipeg during spring spawning periods in the years 2007 to 2009. Spring sampling occurred at known spawning locations, at times when water temperatures and lake conditions were ideal for walleye spawning and accessible by boat. Samples were also taken from two hatchery sites during spawn collection (Swan Creek Hatchery, Swan Creek, Lake Manitoba and Whiteshell Hatchery, Falcon Creek, Falcon Lake). Fish from both of these hatcheries

have historically been stocked into Lake Winnipeg. Additional samples from sites outside of Lake Winnipeg were also obtained, in order to compare genetic diversity within the lake to sites outside of the lake (Figure 1, Table 1).

Sampling locations are summarized in Table 1. In 2007, initial sampling occurred at five Lake Winnipeg sites (Netley Creek, Winnipeg River, Icelandic River, Grand Rapids (Saskatchewan River), Hecla Bar), one hatchery site (Swan Creek) and four other sites outside of Lake Winnipeg (Pointe Du Bois, MB; Nelson River, MB; Bay of Quinte, ON and Black Sturgeon River, ON. Sauger (*Sander canadensis*), a closely related species, were sampled in 2007 at Pointe Du Bois, MB and Nelson River, MB. In spring 2008, 11 recognized Lake Winnipeg spawning sites were sampled (Netley Creek, Icelandic River, Hecla Bar, Jackhead Creek, Sturgeon Bay, Grand Rapids (Saskatchewan River), Big Black River, Poplar River, Berens River, Bloodvein River and Winnipeg River).

Samples were also collected from both hatchery sites (Swan Creek and Falcon Creek), and obtained from fish caught while angling at Lake of the Woods, ON. The Red River (MB) was sampled in fall 2008. Four Lake Winnipeg sites were sampled in spring 2009 (Hecla Bar, Warpath River, Grand Rapids (Saskatchewan River) and Winnipeg River). Samples were again obtained from hatchery sites at Swan Creek and Falcon Creek, as well as from Paint Lake, Northern MB.

Accepted sample sizes for microsatellite studies are approximately 50 individuals (e.g., Cena et al. 2006). While we attempted to catch 50 fish at each site, it was not always possible; total numbers sampled at each site ranged from 7 to 52 (mode = 50) and are summarized in Table 1. Fish were collected by short set gill nets or hoop nets during

spawning periods, or samples were provided by First Nations fishermen fishing in the area. Red River samples were collected by electrofishing, and Paint Lake and Lake of the Woods samples by angling. Fin clips and scale samples were collected non-lethally, after which fish were re-released at or near to the point of capture. In 2007 fin clips and scales were dried in scale envelopes, however, problems with DNA degradation in fin clips stored in this manner prompted us to store all future fin clips in 95% ETOH. Scales were dried and stored in scale envelopes. Length (nearest mm) and weight (nearest gram) measurements were taken at most sampling locations, and sex and maturity was recorded for fish that were visibly running.

DNA Extraction, PCR Amplification and Fragment Analysis

Genomic DNA was extracted from either fin tissue or scales, using the Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol with the exception of adding 10 µL of 20 mg/ml proteinase K (Invitrogen) to 500 µL of nuclei lysis solution.

A total of 27 microsatellite loci have been developed and successfully utilized for walleye (Borer et al. 1999, Wirth et al. 1999, Cena et al. 2006, Wilson and Mathers 2002). From these, ten were selected for this study (Svi L5, Svi L6, Svi L7, Svi L8 and Svi L10; Wirth et al. 1999 and Svi 4, Svi 6, Svi 17, Svi 18 and Svi 33; Borer et al. 1999; Table 2) based on the simplicity of the repeat sequence and success of amplification in prior studies (Wilson and Mathers 2002, Cena et al. 2006). After surveying approximately 500 walleye, one locus (Svi L10) was dropped from the analysis after showing evidence of a

null allele (i.e. one that can no longer be detected because of a mutation at one or both primer annealing sites).

DNA from the remaining nine walleye-specific loci was amplified using Polymerase Chain Reaction (PCR). Each 10 uL reaction contained sterile deionized water, 1X PCR buffer (20 mM Tris-HCL pH 8.4, 50mM KCl; Invitrogen), 2.1 mM MgCl₂, 0.08 mM each dNTP, 0.176 mM each forward and reverse primer, 0.008 units *Taq* polymerase (Invitrogen) and 0.52 uL genomic DNA (approximately 50 nanograms). Reactions were initially denatured at 94°C for 90 seconds. Amplification of all fragments was carried out in 35 cycles: denaturation at 94°C for 30 seconds, primer annealing at a primer specific temperature for 30 seconds (Table 2) and extension at 72°C for 30 seconds. Reactions underwent a final extension at 72°C for 5 minutes, before being held at 4°C indefinitely. PCR products were amplified using 5'-end-labelled primers labelled with dyes 6-FAM or HEX (Sigma Life Science) or NED or PET (Applied Biosystems) and a "PIG" tail on the reverse (3'-) primer to regulate adenylation (Brownstein et al. 1996). Amplified dye-labelled PCR products of the nine loci were pooled in two groups (Table 2), and run on a 3130 Genetic Analyzer (Applied Biosystems) alongside GeneScan-600 LIZ size standard (Applied Biosystems), to determine lengths of loci (size fractioning). Fragment lengths were scored using GeneMapper v.4.0 (Applied Biosystems), and verified by manual scoring. A negative control and two positive controls were included in each run to ensure consistent results. Numbers of samples run from each sampling location are summarized in Table 1. Dilution series trials examining the effects of DNA concentration on amplification of microsatellites were conducted, and it was determined that DNA concentration did not change the determined sizes of alleles for individuals.

Data Analysis

The genetic structure of each spawning location was characterized based on the number of alleles present at each microsatellite locus as well as the observed degrees of heterozygosity. Populations were tested for the presence of null alleles, scoring error and allele dropout using the program Microchecker 2.2.3 (Van Oosterhout et al. 2004). After examining approximately 500 fish, it was determined that locus Svi L10 demonstrated a null allele, and was dropped from subsequent analysis. Nei's (1978) unbiased estimate of expected heterozygosity (H_e), observed heterozygosity (H_o), and allele frequencies were calculated for each population, and each locus within each population, using Tools for Populations Genetic Analyses (TFPGA) software (Miller 1997). Conformity of each population, as well as each locus within each population, to Hardy-Weinberg Equilibrium (HWE) was tested using a Monte Carlo exact test for goodness of fit (20 000 permutations) in TFPGA (Miller 1997), and adjusted for significance using the sequential Bonferroni correction (Rice 1989) to account for multiple simultaneous tests. The inbreeding coefficient (F_{IS} , see Appendix 1) was calculated for each sampling location using GENEPOP 4 (Raymond and Rousset 1995a). This is a measure of the extent of genetic inbreeding within a subpopulation, and ranges from -1.0 (all individuals heterozygous) to +1.0 (all individuals homozygous). The number and proportion of private alleles (alleles that are specific to a single sampling location, and not shared between locations) at each sampling location was also calculated.

The program FSTAT (Goudet 2001) was used to estimate pairwise genetic differentiation indices (F_{ST} , the amount of significant genetic differentiation in allele frequencies among populations, see Appendix 1) using Weir and Cockerham's (1984) θ statistic, a

maximum-likelihood based estimator of F_{ST} . The significance of each pairwise comparison was tested with a G-statistic (Raymond and Rousset 1995b), which weights loci according to their allelic diversity (Goudet 1995), and compared to a sequential Bonferroni corrected p-value (Rice 1989). F_{ST} values were calculated for samples collected in a single year for locations that were sampled in multiple years, as well as for all years pooled together. Additional χ^2 (X^2) tests examining the heterogeneity of pairwise genotypic frequencies were performed according to the procedure described by Goudet et al. (1996) and implemented in GENEPOP 4 (Raymond and Rousset 1995a), using an exact nonparametric procedure with probabilities estimated by Monte Carlo Markov Chain (MCMC) simulations. Probability values were adjusted using the sequential Bonferroni correction. This method is not affected by sample sizes or dependent on a normal distribution (Raymond and Rousset 1995b, Rousset 2008).

Examination of the hierarchical patterning of genetic variation (% variance) among groups of walleye sampling locations, among individual locations within a group, between individuals within sampling locations, and among individuals was evaluated using Analysis of MOlecul VARIation (AMOVA; Excoffier et al. 1992) as implemented in ARLEQUIN (Excoffier et al. 2005, v3.11 by Excoffier 2007). Tests were performed using nine microsatellite loci, with all samples and various groupings, and with only Lake Winnipeg samples, with various groupings.

A regression analysis was performed to test if a correlation was present between the genetic and geographic differences between sampling sites. For each pairwise comparison, the geographic distance was estimated using Topo Canada v2 software in

MapSource v. 6.13.6 (Garmin International, Kansas). Distances between sampling sites within Lake Winnipeg were estimated by connecting the shortest water route between the two sites, while sampling sites outside of Lake Winnipeg were estimated by the shortest straight line distance between the two points. Scatter plots of the natural log of the geographic distance ($\ln(\text{Km})$) and genetic distance (pairwise F_{ST} values, plotted as $(F_{ST}/(1 - F_{ST}))$) between three groups of pairwise comparisons of sampling locations (all walleye sampling locations, all Lake Winnipeg walleye sampling locations, and all sampling locations outside of Lake Winnipeg) were graphed using SigmaPlot v. 10 (Systat Software, Germany). Linear regression analysis was performed independently for each grouping, and the slope and associated r^2 values were used to determine if the points could be described by linear analysis. A Mantel (1967) test was also performed for all walleye sampling locations using the program ISOLDE (as used in Genepop, Raymond and Rousset 1995a) to test for correlations between geographic distance and genetic differentiation.

A neighbour joining cluster analysis (which does not assume lineages evolve at the same rate, Hillis et al. 1996) was performed with all individuals and loci, using Cavalli-Sforza and Edwards' (1967) chord distance, and bootstrapped for 1000 permutations (to generate confidence intervals) in Populations 1.2.14 (O. Langella, Centre National de la Recherche Scientifique, Laboratoire Populations, Génétique et Evolution, Gif sur Yvette, France, available at <http://www.cnrs-gif.fr/pge/bioinfo/populations>). The resulting phenogram was visualized with Treeview 1.6.6 (Page 1996). Cavalli-Sforza and Edwards' (1967) chord distance was chosen as it requires no assumptions of constant population size or

mutation rates, and is generally preferred when determining relationships among closely related populations (Anders and Bernatchez 1998).

Allele frequencies of all Lake Winnipeg sampling locations at all loci were used as variables to cluster the locations spatially using correspondence analysis as implemented in R using the package ade4 (Dray and Dufour 2007). Correspondence analysis uses X^2 distances to judge proximity among sampling locations (Lebart et al. 1984). The first two major factors were plotted on a two dimensional duality diagram displaying all 13 Lake Winnipeg sampling locations.

The effective population size (N_e) of a population is equivalent to the number of breeding individuals required to produce the same dispersal of allele frequencies under random genetic drift, or the same amount of inbreeding (Wright 1931). It is generally smaller than the actual population size, typically around 1/10. The effective population size of the entire Lake Winnipeg walleye population, as well as the effective population sizes of the Grand Rapids and Icelandic River sampling locations within Lake Winnipeg, were estimated using the program ONeSAMP (Tallmon et al. 2008; <http://genomics.jun.alaska.edu/asp>). ONeSAMP is a web-based program that uses summary statistics and approximate Bayesian computation (50 000 simulations) to estimate N_e from a single temporal sample of microsatellite data. This program requires estimates of the maximum and minimum estimated N_e (priors for Bayesian methodology); however, results appear to be robust to changes in the values used for the prior (Maudet et al. 2002). To test the effect of priors on the entire Lake Winnipeg dataset, values of 500 and 10 000 were used for one simulation (maximum and minimum estimated N_e ,

respectively), and 1 000 and 5 000 for a second simulation. When samples from Grand Rapids and Icelandic River were run, the second simulation used values of 50 and 1 000. Computational limitations specific to this program limit the maximum N_e calculated to 10 000, which is far below the expected N_e for the entire Lake Winnipeg population, and therefore results from the Lake Winnipeg estimation are not expected to accurately reflect the actual N_e of the Lake Winnipeg walleye population.

RESULTS

In total, 1387 walleye and 13 sauger were genotyped at nine loci, with a missing data rate of 0.4%. Using Microchecker 2.2.3 (Van Oosterhout et al. 2004), it was determined that Svi L10 demonstrates a null allele (and was dropped from subsequent analysis).

Conformity of each sampling location, and each locus at each sampling location, to Hardy-Weinberg Equilibrium was tested; results are summarized in Table 1 and 3. Eight out of 23 sampling locations showed significant deviation from Hardy Weinberg Equilibrium after sequential Bonferroni correction. The deviations may be attributable to Wahlund effects (the reduction of heterozygosity in a population due to subpopulation structure; Waples and Teel 1990), cryptic population structure or inbreeding effects (Castric et al. 2002). Number of alleles, allele size ranges, observed and expected heterozygosities, the fixation index (F_{IS}) and the number and proportion of private alleles were determined for each population and each locus within each population using GENEPOP 4 (Raymond and Rousset 1995a); results are shown for all samples in this study as well as all Lake Winnipeg samples pooled (Table 1 and 3). Average observed

heterozygosity (H_o , across all loci) for Lake Winnipeg was 0.66, and the average number of alleles per locus (N_a) was 13.9.

Using FSTAT (Goudet 2001), θ values (an unbiased estimator of F_{ST}) were calculated for pairwise comparisons of each sampling year at each sampling location, and adjusted for Type I error using the sequential Bonferroni correction (Rice 1989). F-statistics and associated p-values are summarized in Table 4. A lack of significant differentiation between years at a single sampling location was found for all comparisons, with the exception of slight differentiation between Swan Creek samples taken in 2007 and 2009.

Pairwise comparisons of genotypic differentiation between sampling years at each sampling location were also made using X^2 values (Table 4). A lack of significant differentiation between years at a single sampling location was found for all comparisons, with the exception of Swan Creek samples taken in 2007 and 2009. These results are congruent with those found using θ , and as a result, samples from each sampling location have been pooled for all years for all subsequent analysis.

A general guideline for F_{ST} values is as follows: $0 < F_{ST} < 0.05$ indicates little genetic differentiation, $0.05 < F_{ST} < 0.15$ indicates moderate differentiation and $0.15 < F_{ST} < 0.25$ indicates great differentiation (Hedrick 2005). These values provide guidelines for use with all taxa, and should be applied with caution as divergence levels are often taxon specific. Pairwise comparisons of θ within Lake Winnipeg samples show a lack of significant differentiation between most Lake Winnipeg sites (-0.0024 – 0.0243, Table 5). Negative values of θ are an artifact of the estimation procedure, and occur for ten of 78

pairwise comparisons within Lake Winnipeg. Negative values indicate values that are not significantly different from zero, and are interpreted as a lack of significant differentiation between populations. The exceptions to the lack of genetic structure within Lake Winnipeg are samples taken from Grand Rapids, which show consistent slight significant ($p = 0.05$) genetic differentiation from all other Lake Winnipeg sampling sites (0.0066 – 0.475) and the Icelandic River, which shows slight significant ($p = 0.05$) differentiation from all Lake Winnipeg sampling (0.0086 – 0.0275) sites except for Big Black and Poplar Rivers. Netley Creek shows slight significant ($p = 0.05$) variation from seven of twelve comparisons within Lake Winnipeg (0.0086 – 0.457).

The pairwise θ values for sauger collected in the Nelson River and at Pointe Du Bois show great differentiation ($p = 0.05$) from all walleye sampled at most locations (0.1548 – 0.2693). The exceptions are moderate differentiation ($p = 0.05$) observed between Nelson River sauger and Bay of Quinte walleye (0.1346), and no significant differentiation found between Pointe Du Bois sauger and Pointe Du Bois walleye (0.2033, $p > 0.05$). Walleye caught in the Black Sturgeon River and the Bay of Quinte are moderately different ($p = 0.05$) from all other walleye sampling sites (0.0514 – 0.1171), with the exception of Netley Creek, which is only slightly ($p = 0.05$) genetically different (0.0349). With few exceptions, all other pairwise comparisons outside of Lake Winnipeg show slight to moderate genetic differentiation. No significant differentiation was found between Pointe Du Bois walleye and most Lake Winnipeg sites, or Lake of the Woods. No significant differences were found between Nelson River walleye and seven of the twenty comparisons of walleye sampling sites.

Genotypic differentiation between all sampling locations was compared using X^2 values (Table 5). Patterns seen are largely congruent to those observed using θ , although significant differences are detected in more (11 additional) of the comparisons. A value of infinity was assigned when the X^2 value exceeded the capacity of the program to calculate ($p < 0.0001$). Differences from the results seen with θ occurred at Red River, Netley Creek, Icelandic River, Hecla Bar and Sturgeon Bay (within Lake Winnipeg), and Pointe Du Bois and Nelson River outside of Lake Winnipeg. Significant differences are observed between Netley Creek and most other Lake Winnipeg sites (10 out of 13 comparisons, X^2 range from 37.51 to infinity) and between the Icelandic River and all Lake Winnipeg sites except for Poplar River (X^2 range from 36.63 to infinity). Pointe Du Bois samples differ from nine out of twenty comparisons (X^2 range from 30.16 to infinity), and Nelson River samples differ from sixteen out of twenty comparisons (X^2 range from 30.68 to infinity).

Analysis of the hierarchical patterning of genetic variation between groups of sampling locations revealed a small percentage of variation explained by population structure. When AMOVA was run on all walleye samples, with all Lake Winnipeg samples pooled, only 2.63% ($p < 0.01$) of variance occurred among sampling groups, while 95.18% ($p < 0.01$) occurred among individuals within sampling groups. Similar results were obtained when AMOVA was run with two separate groupings: 1) all Lake Winnipeg walleye 2) Lake Winnipeg north and south basins pooled separately. Variation among groups in this scenario explained 2.00% ($p < 0.01$) of the total variation, while variation within individuals accounted for 95.91% ($p < 0.01$) of the total variation. When variation within Lake Winnipeg alone was examined, pooling north basin and south basin samples

separately resulted in 0.09% ($p < 0.01$) of the variation occurring among groups, and 97.59% ($p < 0.01$) of the variation occurring among individuals. Other groupings consistently resulted in low percentages of variation occurring among groups, and percentages consistently greater than 97% occurring among individuals.

Linear regression analysis of the genetic ($F_{ST}/(1-F_{ST})$) and geographical ($\ln(Km)$) distances between walleye sampling locations resulted in an r^2 value of 0.43 ($p < 0.001$) for all walleye sampling locations, 0.08 ($p < 0.05$) for all Lake Winnipeg sampling locations, and 0.36 ($p < 0.001$) for all sampling locations outside of Lake Winnipeg (Figure 2). Genetic distance is weakly correlated to geographic distance inside of Lake Winnipeg, but when sampling locations outside of Lake Winnipeg are included, a strong correlation between genetic and geographic distance occurs. A strong correlation between genetic and geographic distance in walleye sampled outside of Lake Winnipeg also occurs. The Mantel test for all walleye sampling locations, which assessed all pairwise genetic distances ($F_{ST}/(1-F_{ST})$) against the natural logarithm of geographic distance, shows a positive association, indicating broadscale correspondence to a genetic isolation by geographical distance hypothesis when all walleye sampling locations are included ($p < 0.00000$; $y = -0.0864 + 0.0205x$).

A neighbour joining (NJ, Figure 3) phenogram was constructed using the Cavalli-Sforza and Edwards' (1967) chord distance in Populations 1.2.14. Bootstrap values lower than 50 were removed. The NJ phenogram shows high bootstrap support (100) for a strong genetic difference between walleye and sauger, as well as high support (91 for both nodes) for differences between Great Lakes walleye and all other walleye samples. Northern

samples (Paint Lake and Nelson River) cluster together with a bootstrap value of 74, as do the eastern samples (Falcon Creek and Lake of the Woods), with a bootstrap value of 72. The Pointe Du Bois samples cluster with the other eastern samples, with low bootstrap support. The Lake Winnipeg and Swan Creek walleye are scattered throughout the rest of the phenogram. The Big Black and Poplar rivers, which are close together geographically, cluster together with moderate bootstrap support (58).

The duality diagram resulting from the correspondence analysis of all Lake Winnipeg walleye samples (Figure 4) shows a clear distinction between Grand Rapids walleye (positioned in the top right quadrat), sampling locations on the west side of the South basin (Red River, Netley Creek and Icelandic River; positioned in the top left quadrat), and all other Lake Winnipeg sampling locations (positioned below the x axis around the centerline). Two axes were kept for the analysis and graphed on the duality diagram. The first axis (horizontal) explains 21.5% of the variance in all Lake Winnipeg walleye sampling locations, while the second axis (vertical) explains 12.3% of the variance.

The effective population size (N_e as estimated by the program ONeSAMP) of Lake Winnipeg using values of 500 and 10 000 as the minimum and maximum estimated priors, respectively, had a mean of 2932.8 and a median of 2825.1. The lower and upper 95% confidence levels were 2084.5 and 5456.9, respectively. When the lower and upper estimated priors were changed to 1 000 and 5 000, estimates of N_e had a mean of 778.2 and a median of 774.2, with the lower and upper 95% confidence intervals of 691.4 and 934.2, respectively. As mentioned previously, the computational limitations of this program have a maximum N_e of 10 000, which is well below the actual expected value

for Lake Winnipeg (estimated at 1 million fish, based on the surface area of Lake Winnipeg; Baccante and Colby 1996), and may have resulted in skewed results. When N_e for the Icelandic River was calculated using priors of 500 and 10 000 as the minimum and maximum estimated priors respectively, a mean of 92.8 and a median of 93.0 was found. The lower and upper 95% confidence levels were 86.2 and 104.3, respectively. When the lower and upper estimated priors were changed to 50 and 1 000, estimates of N_e were 164.9 and 163.9. The lower and upper 95% confidence levels were 134.0 and 290.7, respectively. When N_e for Grand Rapids was calculated using priors of 500 and 10 000 as the minimum and maximum estimated priors respectively, a mean of 104.4 and a median of 104.3 was calculated. The lower and upper 95% confidence levels were 95.6 and 117.8, respectively. When the lower and upper estimated priors were changed to 50 and 1 000, estimates of the N_e were 365.2 and 363.0. The lower and upper 95% confidence levels were 279.0 and 619.1, respectively.

DISCUSSION

Overall, Lake Winnipeg walleye (with all sampling locations pooled) have a level of genetic diversity comparable to walleye in other large lakes. Average observed heterozygosity (H_o , across all loci) for Lake Winnipeg was 0.66, and the average number of alleles per locus (N_a) was 13.9. These values are similar to those found in the Laurentian Great Lakes and other systems (Table 6, $H_o = 0.613 - 0.735$, $N_a = 7.1 - 11.9$) using ten microsatellite loci (Cena et al. 2006, Dupont et al. 2007, Stepien et al. 2009). A study by Stepien and Strange (2007) looked at the entire North American range of walleye. Using ten loci, they found an average observed heterozygosity of 0.698 and an

average number of alleles per locus of 13.9. These values are all comparable to those determined for Lake Winnipeg in this study. In contrast, very low heterozygosity was found in an endangered freshwater goby species (*Rhinogobius* sp. BB) found on Okinawa Island, Japan ($H_o = 0.263$ and 0.281 , $N_a = 3.6$ and 2.0) (Ohara et al. 2005). Walleye in Lake Winnipeg appear to have heterozygosity levels comparable to levels found in other large lake populations (i.e. Laurentian Great Lakes) also under similar pressures (such as commercial fisheries, eutrophication and invasive species).

Little or no genetic differentiation was found within (i.e. temporally) or among (i.e. spatially) sampling sites in Lake Winnipeg. Some Lake Winnipeg locations that are geographically close together cluster together genetically in the NJ tree, such as Big Black River and Poplar River, with a bootstrap value of 58 (Figure 3). Pairwise measures of genetic distance also identify low genetic differentiation between these two locations. Two Lake Winnipeg sampling locations were significantly different from most other Lake Winnipeg sampling locations. Grand Rapids samples showed slight genetic differentiation from all other Lake Winnipeg sites (F_{ST} from $0.0066 - 0.0457$), while the Icelandic River samples showed slight genetic differentiation from all but two Lake Winnipeg sites (F_{ST} from $0.0086 - 0.0275$). Stocking has occurred at both of these locations, and may account for the genetic differences observed. Manitoba Hydro stocks walleye annually at Grand Rapids, and in excess of 70 million larvae and 23 million eggs have been stocked since 1997 (Kroeker and MacDonell 1998, Kroeker 1999, MacDonell and Cooley 2000, Eddy and MacDonell 2000, MacDonell and Graveline 2002, MacDonell and Johnson 2003, Funk and MacDonell 2004, MacDonell and Funk 2005, MacDonell et al. 2006, Richardson and Neufeld 2007, Ambrose and MacDonell 2008,

Ambrose and MacDonell 2009). All of the fish stocked in Grand Rapids were taken from creeks on Lake Manitoba (including Swan Creek hatchery), as well as creeks in the Snow Lake area. The significant numbers of fish stocked at Grand Rapids from sites outside of Lake Winnipeg may account for the slight but significant genetic differentiation detected there now. A private hatchery operated out of Riverton (on the Icelandic River) for a time; however, records of the years it operated, the numbers stocked and the source of the fish stocked cannot be found. The significant genetic differences between walleye taken from Grand Rapids and the rest of Lake Winnipeg may reflect the large geographic separation between Grand Rapids and the rest of the Lake Winnipeg sites. It could also reflect the glacial history of Lake Winnipeg. While Lake Winnipeg began to form 7 700 years ago, a connection between the north and south basins did not form until 4 000 years ago (Matile et al. 1996). This isolation could have resulted in the genetic differences seen between Grand Rapids and the rest of the Lake Winnipeg walleye sampling locations. However, if this were the case, a difference between all north basin and south basin spawning locations isolated from each other would be expected, unless there was sufficient gene flow from the south basin only to the more southerly north basin sites after the connection between the two basins formed.

Most methods of calculating effective population size require samples from two periods in time, separated by a generation (Waples 1991). This was not possible with the data in this study, and so a Bayesian method which requires only one temporal sample was used (Tallmon et al. 2008). The program ONEsAMP was used to calculate the effective population size for Lake Winnipeg walleye as a whole, as well as at Grand Rapids and the Icelandic River. Estimates of N_e varied greatly (95% confidence intervals did not

overlap) with changing values of the upper and lower estimated priors. Other studies that have calculated N_e using the Bayesian methods utilized in the program ONeSAMP have found that changing the values of the priors did not significantly alter the estimates, and that the 95% confidence intervals overlapped (e.g., European white stork, Shephard et al. 2009; Amazonian frogs, Funk et al. 2009; Maudet et al. 2002). The program ONeSAMP is limited computationally to a maximum estimated prior of 10 000. This value is far below the expected N_e for the entire Lake Winnipeg population, as well as the Grand Rapids and Icelandic River populations, and therefore results from the Lake Winnipeg estimation are not expected to accurately reflect the actual N_e of these locations. It is possible that two sample methods of estimating N_e would be more accurate, but such methods were not possible with the data and samples in this study.

Genetic differences between Lake Winnipeg walleye and samples taken outside of the lake (Pointe Du Bois, Falcon Creek, Swan Creek, Lake of the Woods, Paint Lake, Nelson River and Great Lakes) were greater than those between spawning sites within Lake Winnipeg (Table 5). The northern samples (Paint Lake and Nelson River) showed greater similarity to each other than to most other sites, as did the eastern samples (Falcon Creek, Pointe Du Bois and Lake of the Woods). The Great Lakes samples (Bay of Quinte and Black Sturgeon River) are very divergent from all other sampling sites and each other. The significant genetic differences seen between sauger and all walleye samples were greater than any intraspecific differences found between walleye samples. These results indicate that the microsatellites and methods used in this study were able to detect genetic differentiation between species (walleye and sauger) as well as within a

species (Lake Winnipeg walleye vs. walleye from other locations), and that within species differentiation increased with geographical separation.

Measures of genetic differentiation found within Lake Winnipeg walleye are lower than those found in other walleye studies (Table 7). Strange and Stepien (2007) looked at walleye sampled from the western, central and eastern basins of Lake Erie, and found significant F_{ST} values among sites within each basin, ranging from 0.004 to 0.063 (using ten loci). The geographic distance between spawning sites found to be genetically distinct ranged from approximately 25 to 600 kilometers, distances comparable to those found between spawning sites in this study. In the Bay of Quinte (Lake Ontario), no significant levels of genetic differentiation were found (using five loci, F_{ST} ranged from 0.0006 to 0.0098), except for West Lake, which was significantly different from all other Bay of Quinte spawning sites (F_{ST} ranged from 0.0141 to 0.0264) (Wilson and Gatt 2000). The majority of the spawning sites in Wilson and Gatt's (2000) study were found within 10 kilometers of each other, with the exception of West Lake, which is outside of the Bay of Quinte by approximately 130 kilometers (by water). A recent study comparing genetic diversity of walleye across their entire North American range (using ten loci) found significant pairwise F_{ST} values among sites within the individual Great Lakes to range from 0.008 to 0.067 (Stepien et al. 2009). The numbers, as well as the individual loci, used by Stepien et al. (2009) were comparable to those used in the above studies, as were the geographic distances separating spawning sites. The low levels of genetic differentiation found in Lake Winnipeg walleye in this study could be a result of a lack of natal homing, straying between spawning sites or fidelity of walleye to a non-natal site.

Likewise, measures of genetic differentiation found for walleye in this study are generally lower than those found for other species thought to be philopatric (Table 7). Smallmouth bass, *Micropterus dolomieu*, in Lake Erie have basin specific significant θ_{ST} values (a value analogous to F_{ST}) of 0.021 – 0.034 within the west basin, 0.054 – 0.178 within the central basin and 0.020 – 0.118 within the east basin; significant values between basins ranged from 0.011 to 0.200 (Stepien et al. 2007). Microsatellites resolved 16 regional stocks for chum salmon (*Oncorhynchus keta*) in British Columbia, and significant F_{ST} values between stocks ranged from 0.009 (between the central and north coast) to 0.036 (between Fraser River and Taku River; Beacham et al. 2008). Pacific salmon are known to be highly philopatric with a large level of local adaptation, and this is reflected by the high level of genetic differentiation between stocks. Beacham et al. (2008) found that genetic differentiation between stocks of chum salmon as determined by 14 microsatellite loci was sufficient to provide finescale resolution of stock composition in British Columbia coastal fisheries.

In systems where spawning sites show significant genetic differentiation from each other (high pairwise F_{ST} values), it is possible to use genetic differences as a ‘genetic tag’ for mixed stock analysis of commercial fisheries. This has been especially useful in salmonid fisheries on the west coast, where test fisheries in spawning rivers determine the abundance of spawning stocks before they reach the commercial fishery, allowing fishery limits that reflect the actual abundance of a specific stock at that time. Simulated mixed stock assessments of sockeye salmon (*Oncorhynchus nerka*) in the Nass River, British Columbia (using six microsatellite loci) was successful in estimating the contributing proportion of specific spawning groups, and the estimates were found to be in close

agreement with actual proportions as determined by direct tagging of fish in the test fishery (greater than 60% accuracy when genetically similar groups were combined, the highest contributing stock was correctly assigned with 87.3% accuracy; Beacham and Wood 1999). A similar simulation of sockeye salmon in coastal lakes in British Columbia (using 14 microsatellite loci) was able to determine the proportions of each contributing stock with a mean error of less than 7%, and often less than 1% (Beacham et al. 2005). Mixed stock analysis of Chinook salmon (*Oncorhynchus tshawytscha*) from 325 localities from Russia to California was able to assign individuals to their population of origin with up to 90% accuracy (average 84%) using 13 microsatellite loci (Beacham et al. 2006). The ability to perform mixed stock analysis using genetic tags can allow accurate estimates of contribution, providing genetic differentiation is sufficient to distinguish between each contributing stock. This analysis can prevent under and over utilization of distinct stocks in a mixed stock commercial fishery, and is important for effective management. The lack of genetic differentiation found in Lake Winnipeg walleye means performing genetic mixed stock assessment on walleye captured in the commercial fishery is not possible using these loci.

There are several possible explanations for the lack of population structure detected in Lake Winnipeg walleye. While it is possible that population structure is present in Lake Winnipeg walleye, it is very unlikely that the neutral markers used in this study would be unable to detect it. The loci chosen were selected because of their successful application in other studies (Wilson and Gatt 2000, Eldridge et al. 2002, Wilson and Mathers 2002, White et al. 2005, Cena et al. 2006, Dupont et al. 2007, Strange and Stepien 2007, Wilson et al. 2007, Stepien et al. 2009), where they were able to detect a reduction in gene flow

associated with population structure. Within this study, the loci used were able to detect genetic differentiation within walleye in Lake Winnipeg and other locations, indicating the effectiveness of the methods and loci in detecting a reduction of gene flow. Great interspecific genetic differentiation was also found between walleye and sauger. These results indicate that the lack of population structure found within Lake Winnipeg is not an artifact of the loci used. Genetic differences among different phenotypes and life history types might be detectable using functional loci such as the Major Histocompatibility Complex (MHC) – where selection on certain traits may have resulted in differentiation at these loci even with continued gene flow (see General Discussion) – but the microsatellites used in this study have been sufficient to show reduced gene flow among allopatric walleye in this study and among spawning sites within a lake in other studies.

Likewise, the lack of genetic differentiation found is also not likely a result of the analytical methods used. Two methods were used for comparison of pairwise tests of allelic heterogeneity between years at a single sampling location and between all sampling locations in this study: Weir and Cockerham's (1984) unbiased theta (θ) estimator of F-statistics, and an exact nonparametric procedure using X^2 values (Raymond and Rousset 1995b). These two methods are based on different assumptions. Weir and Cockerham's (1984) unbiased estimator θ is based on the assumption that all populations are statistically independent, with no mutation, migration or selection (Weir and Cockerham 1984). It also assumes the same sample size is maintained for all populations and all generations (Nei 1986), however has been found to not be affected by differing samples sizes (Ruzzante 1998). In comparison, the X^2 method is not biased by

rare alleles, low or unequal sample sizes, and is not dependant on a normal distribution (Raymond and Rousset 1995b).

Sampling sites that were sampled in additional years were pooled together. Within the eight sites sampled in multiple years, only one significant difference within a sampling site was found. A significant difference between sampling years (using both θ and X^2 methods) was detected between 2007 and 2009 samples at Swan Creek. Samples at Swan Creek were collected by the same people, at the same sampling location and using the same protocol at each of these sites. Trap nets are set up pre-spawn, and checked regularly, so samples were collected from the start of the spawning run each year. The genetic differences between spawners in these two years could reflect a year class change in the spawning individuals arriving first, or a random change, with a different group arriving at the spawning site first.

Application of both tests of significant pairwise genetic differentiation (θ and X^2) to the microsatellite data in this study yielded results that were largely congruent. The X^2 test assigned significance to 11 pairwise comparisons of sampling locations that were not found to be significantly different using F-statistics. Of the 11 comparisons with conflicting results between the two methods, 8 involved sampling locations with low sample sizes ($n = 18 - 30$), and one involved a sampling location with uneven sample sizes between years (Winnipeg River, $n = 50$ in 2007, $n = 35$ in 2008). It is possible that the assumption of equal sample sizes required by θ was not met in these samples, preventing an accurate conclusion. However the findings by Ruzzante (1998) indicate that uneven sample sizes should not increase the sampling variance of θ .

Consequently, it is unlikely that the methods used in this study resulted in reduced resolution of population structure in Lake Winnipeg. The allopatric differences seen between Lake Winnipeg walleye and walleye outside of Lake Winnipeg indicate the effectiveness of these methods in detecting reduced gene flow. Therefore, the lack of significant population structure in Lake Winnipeg walleye is presumably the result of biological characteristics of the population. These characteristics could include a population approaching panmixia (i.e. a genetically homogenous population with no population structure and no fidelity to natal sites), some degree of philopatry but with a sufficient amount of straying between spawning sites, or fidelity to a site other than the natal site.

Genetic differences within a population can arise as a result of isolated reproductive groups. If walleye in Lake Winnipeg do not home to the same spawning site each year, a lack of genetic differentiation between spawning sites would be expected. Homing behaviour in walleye appears to be facultative, and has been detected in some studies (Eschemeyer 1950, Eschmeyer and Crowe 1955, Wolfert and Van Meter 1978, Van Vooren 1978, Ward et al. 1989, Gatt et al. 2002, Stepien and Faber 1998) and not in others (Olson and Scidmore 1962, Colby and Nepszy 1981). If homing is a facultative behaviour in Lake Winnipeg, genetic differences between spawning locations would not be expected.

The lack of genetic structure found between spawning sites in Lake Winnipeg could also be explained by some degree of straying or a lack of obligatory philopatry. The number

of strays or migrants per generation required to break down genetic structure can be very small (Waples 1998). The detection of stock structure is very sensitive to gene flow, with a rate of gene flow as little as 1% being sufficient to homogenize the gene pool (Carvalho and Hauser 1994). A study on American shad (*Alosa sapidissima*) population structure found that a straying rate of 1% was sufficient to promote a substantial level of gene flow between discrete populations despite strong philopatric behaviour, when population sizes were large (Waters et al. 2002). In the context of large population sizes, a 1% straying rate can represent a large number of breeding migrants or strays each generation; therefore, the lack of detectable genetic structure could be a result of only a small proportion of individuals straying each generation. In some tagging studies, a large degree of male mediated gene flow has been found to occur in walleye. Females appeared to return to the same spawning site each year (Regier et al. 1969, Spangler et al 1977), while males appeared to be more promiscuous with a higher level of straying (Billington and Hebert 1988). These sex dependent differences in philopatry could result in a sufficient amount of gene flow to obscure population structure. The degree of straying in Lake Winnipeg cannot be determined from these results alone. The microsatellite results indicate only that sufficient gene flow occurs between spawning sites to prevent genetic differences at neutral markers from accumulating; this could mean a relatively small number of individuals straying each generation or limited philopatry to begin with. If male mediated gene flow is obscuring the population structure of walleye in Lake Winnipeg, future investigation using maternally inherited mtDNA markers may resolve any structure remaining (Engelhaupt et al. 2009; see Chapter 2).

The homing behaviour of walleye to particular spawning streams or local areas has been extensively documented by tagging studies in multiple lake systems (Eschmeyer 1950, Eschmeyer and Crowe 1955, Forney 1963, Foust and Haynes 2007, Ward et al. 1989). Tagging studies provide no direct evidence that the homing behaviour is to an individual's natal site; however, it is widely assumed that this is the case (Spangler et al. 1977, Colby and Nepszy 1981). Olsen et al. (1978) presented an alternate hypothesis that homing (or rather site fidelity) in walleye is not a natally imprinted behaviour, but rather learned as an adult. They argued that the dispersal of eggs and fry away from the natal area by wind and river currents, presumably beyond where imprinting would occur, precludes any natally induced behaviour. While it is unknown how many eggs drift away from the spawning site, it is true that the larval stages of walleye do not remain on the spawning grounds for a long period of time (Horrall 1981). Neither is it known how early the olfactory system develops in larval walleye, and whether it is developed enough for olfactory imprinting to occur before larval walleye drift away from the spawning grounds (Horrall 1981). Horrall (1981) postulated that if larvae form schools or aggregations that remain together, it is possible that individuals may imprint to stock specific pheromones of their group as they are transported away from the spawning grounds. Residual pheromonal odours on the spawning grounds, or odours from older walleye arriving earliest on the spawning grounds, may create odour trails guiding new recruits to their natal grounds. This behaviour is seen in white bass (*Morone chrysops*; Bodaly 1980). It is also possible that site fidelity is a behaviour learned by adults, and new recruits follow older walleye when they first reach sexual maturity. The site fidelity of walleye would be influenced by physical characteristics of the environment and strengthened over time by repeated migrations to the same site (Olsen et al. 1978).

If walleye return to a site other than their natal site, genetic discreteness of the spawning sites would depend on their remoteness to each other. The degree of mixing of eggs and larval walleye from different spawning grounds would depend on the proximity of the spawning grounds, and the strength of the wind and water currents to which they were exposed. It is possible that more remote sites or sites with lower exposure to wind and river currents may show some degree of genetic differentiation, while other sites that mix more extensively may not differ. If site fidelity in walleye is an adult learned behaviour, it could explain why genetic studies do not consistently detect significant differences between spawning grounds, even though site fidelity is regularly detected using tagging studies.

In conclusion, this study successfully used microsatellite variation at nine loci in 1387 walleye and 13 sauger to assess genetic differentiation between 21 walleye sampling sites and two sauger sampling sites. Overall genetic diversity within Lake Winnipeg is comparable to that found in other walleye studies (Cena et al. 2006, Dupont et al. 2007, Stepien et al. 2009). Significant genetic differentiation was detected between species (sauger and walleye), and slight to moderate genetic differentiation was detected between broadscale walleye sampling sites (different lakes). Some degree of geographic patterning was observed, with northern samples (Nelson River and Paint Lake) showing genetic similarity, as well as eastern samples (Falcon Lake and Lake of the Woods). Little indication of population structure was detected among the 13 spawning sites sampled in Lake Winnipeg, with Grand Rapids and Icelandic River being the only two sites showing consistent slight genetic differentiation from other sites within Lake

Winnipeg. The observed differentiation at these two sites may be a result of stocking that occurs or has occurred historically at these two locations, or the historic separation of the north and south basins in Lake Winnipeg until 2 200 years ago (Matile et al. 1996). The lack of genetic differentiation found between spawning sites in Lake Winnipeg is not comparable to the genetic differences found between spawning sites in the Great Lakes and elsewhere (Stepien and Faber 1998, Strange and Stepien 2007, Stepien et al. 2009). The lack of population structure observed in Lake Winnipeg could indicate that the population is approaching panmixia, that there is some degree of philopatry but sufficient straying to obscure population structure, that walleye are 'homing' in subsequent years to a site other than their natal site, or that population structure exists but was not detected with the markers used. While neutral markers, such as microsatellites, have been successful in detecting a lack of gene flow indicating population structure in other walleye systems, such a lack of gene flow was not found in this study. The use of functional loci, such as the Major Histocompatibility Complex (MHC), may be able to resolve differences not detectable with neutral markers (see General Discussion). Non-genetic methods, such as tagging studies or otolith microchemistry, may also resolve population structure not detected with microsatellites due to straying or fidelity to a non-natal site, or determine if such straying or fidelity to a non-natal site is indeed occurring (See General Discussion). The lack of genetic differences found is not necessarily evidence for panmixia in the Lake Winnipeg walleye population, and does not necessarily mean that there is no genetic basis for the phenotypic differences observed (i.e. in terms of colour, body morph, or life history characteristics). It does mean, however, that these microsatellite markers cannot be used as genetic tags for performing stock assessments on the commercial fishery.

Table 1: Sampling locations and summary statistics based on nine microsatellite loci (N , sample size; H_o , Observed Heterozygosity; H_e , Expected Heterozygosity; F_{IS} , (as measured by θ_{IS}), deviation from Hardy-Weinberg (H-W) proportions, with positive values indicating a heterozygote deficiency and negative values denoting a heterozygote excess (Weir and Cockerham 1984); N_a , number of alleles; N_{pa} , number of private alleles; P_{pa} , Proportion of private alleles). Significant deviation from Hardy Weinberg equilibrium is denoted with an *.

	Lat	Long	Sampling Date	N	H_o	H_e	F_{IS}	N_a	N_{pa}	P_{pa}
<i>Sander vitreus</i>										
Lake Winnipeg				932	0.66	0.68	0.0268	125	18	0.60
South Basin	50.85067	-96.66039		444	0.6676	0.6913	0.0344	113	6	0.19
Red River	50.26713	-96.84915	Oct '08	34	0.6601	0.7011	0.05921	68	0	0.00
Netley Creek	50.28751	-96.93267	Apr '07; Apr'08	25	0.7111*	0.7328	0.03018	64	0	0.00
Icelandic River	51.01553	-96.97872	Apr '07; Apr'08	85	0.6614	0.6822	0.03065	75	1	0.03
Hecla Bar	51.14495	-96.62131	May '07; May '08; May '09	150	0.6819*	0.682	-0.00165	93	3	0.10
Winnipeg River	50.62190	-96.30654	Apr '07; May '08; May '09	150	0.6511*	0.6818	0.03644	89	2	0.06
North Basin	52.88087	-97.94223		488	0.6584	0.6699	0.0170	106	6	0.19
Jackhead Creek	51.91262	-97.34203	May '08	50	0.7134	0.6763	-0.09487	70	0	0.00
Sturgeon Bay	51.98655	-97.91488	May '08	36	0.6327	0.6498	0.02672	60	1	0.03
Warpath River	52.23506	-98.15606	Jun '09	50	0.6506	0.6526	0.00091	69	0	0.00
Grand Rapids	53.18481	-99.26697	May '07; May '08; Jun '09	152	0.6129*	0.6346	0.03390	76	0	0.00
Big Black River	53.17038	-97.42607	Jun '08	50	0.6822	0.6926	0.01512	72	1	0.03
Poplar River	53.01403	-97.36851	Jun '08	50	0.6978*	0.6981	0.00052	65	0	0.00
Berens River	52.34633	-97.02297	May '08	50	0.713	0.6944	-0.02968	71	1	0.03
Bloodvein River	51.78771	-96.70889	Jun '08	50	0.6527	0.6804	0.03815	73	2	0.06
Hatchery Sites										
Swan Creek	50.62551	-98.13229	Apr '07; May '08; May '09	150	0.6613*	0.6456	-0.03978	83	3	0.10
Falcon Creek	49.67758	-95.31303	Apr '08; May '09	83	0.6267*	0.6559	0.04029	68	0	0.00
Northern Sites										
Paint Lake	55.50238	-97.93220	Jun '09	50	0.6444	0.6741	0.04445	63	1	0.03
Nelson River	55.84583	-96.59581	Jun '07	30	0.6926	0.6823	-0.01535	64	1	0.03
Eastern Sites										
Pointe Du Bois	50.30050	-95.55055	Aug '07	18	0.6605	0.685	0.03680	54	0	0.00
Lake of the Woods	49.50478	-94.48294	Jul '08; Jul '09	66	0.6479	0.6954	0.06718	72	2	0.06
Bay of Quinte	44.12855	-76.82494	2005	24	0.7778	0.7921	0.01842	83	5	0.16
Black Sturgeon River	48.86150	-88.36967	2006; Apr '07	36	0.679	0.6728	-0.0093	63	1	0.03
<i>Sander canadensis</i>										
Nelson River Sauger	55.84583	-96.59581	Jun '07	8	0.5833*	0.7065	0.18447	42	4	0.13
Pointe Du Bois Sauger	50.30050	-95.55055	Aug '07	5	0.5778	0.6519	0.12605	50	3	0.10

Table 2: Locus name, batch (1 or 2, for pooling) and dye, number of individuals (N), size range, number of alleles (N_a), expected (H_e) and observed (H_o) heterozygosities, motif, primer sequences, and reference of the 10 microsatellite loci being used in this study. Number of alleles (N_a), and expected (H_e) and observed (H_o) heterozygosities are shown for all samples in this study as well as all samples from Lake Winnipeg. Significant deviation from Hardy Weinberg equilibrium is denoted with an *.

Locus	Batch	n	Size Range	All Samples			Lake Winnipeg			Motif	Forward Primer (5'-3')	Reverse Primer (5'-3')	Reference
				N_a	H_e	H_o	N_a	H_e	H_o				
Svi L5	1 (Hex)	1401	182-206	18	0.81	0.71*	13	0.81	0.71	(CA) ₁₈	CATATCCTACTGTAGTATGG	CAAATCCCATTACACCCAC	Wirth et al. 1999, Cena et al. 2006
Svi 4	1 (Hex)	1399	105-119	17	0.72	0.71	8	0.72	0.72*	(AC) ₁₆	ACAAATGCGGGCTGCTGTTT	GATCGCGGCACAGATGTATTG	Borer et al. 1999
Svi 17	1 (Ned)	1401	102-116	12	0.45	0.43	11	0.43	0.44*	(AC) ₁₃	GCGCACTCTCGCATAGGCCCTG	CGTTAAAGTCCTTGAAACC	Borer et al. 1999, Cena et al. 2006
Svi 18	1 (Pet)	1401	120-132	7	0.58	0.50*	7	0.58	0.52	(AC) ₁₁	GATCTGTAACTCCAGCGTG	CTTAAGCTGCTCAGCATCCAGG	Borer et al. 1999
Svi L6	2 (Hex)	1401	107-131	14	0.50	0.51	14	0.48	0.50	(AC) ₁₇	AGAGGAAGAAGAGGTATC	TTAAAGGGTAAGTCCACTG	Wirth et al. 1999
Svi L7	2 (Ned)	1388	188-222	26	0.86	0.87	23	0.86	0.87*	(TG) ₂₂	GATGTGCATACATTTACTCC	GCTTTAATCTGCTGAGAAC	Wirth et al. 1999, Cena et al. 2006
Svi L8	2 (Fam)	1397	118-146	20	0.83	0.79*	17	0.82	0.79*	(TG) ₂₂	GCTTATACGTCGTTCTTATG	ATGGAGAAGCAAGTTGAG	Wirth et al. 1999, Cena et al. 2006
Svi 6	2 (Pet)	1375	113-151	20	0.81	0.75*	18	0.80	0.76	(AC) ₁₉	CATATTATGTAGAGTGCAGACCC	TGAGCTTCACCTCATATTCC	Borer et al. 1999
Svi 33	2 (Ned)	1401	075-119	20	0.67	0.67*	14	0.64	0.66	(AC) ₁₄	CAGGACTGCTGTGTATAGACTTG	GATATAGCTTTCTGCTGGGGTC	Borer et al. 1999

Table 3: Observed and expected heterozygosities (H_o and H_e) and number of alleles (N_a) with sample sizes (N) for nine microsatellite loci from 21 walleye sampling locations and 2 sauger locations. The size range of each locus (number of base pairs) is indicated by values in parentheses. Significant departures from HWE (after Bonferroni correction) are indicated by asterisks (*). Sampling locations are represented as follows: WRR – Red River, WNC – Netley Creek, WIR – Icelandic River, WHB – Hecla Bar, JHC – Jackhead Creek, WSB – Sturgeon Bay, WWP – Warpath River, WGR – Grand Rapids, BBR – Big Black River, POP – Poplar River, BER – Berens River, BLV – Bloodvein River, WWR – Winnipeg River, MSC – Swan Creek, PDB – Pointe Du Bois, FC – Falcon Creek, LOW – Lake of the Woods, BQ – Bay of Quinte, BSR – Black Sturgeon River, PL – Paint Lake, NR – Nelson River, PDBS – Pointe Du Bois Sauger, NRS – Nelson River Sauger.

Locus		WRR	WNC	WIR	WHB	JHC	WSB	WWP	WGR	BBR	POP	BER	BLV	WWR	MSC	PDB	FC	LOW	BQ	BSR	PL	NR	NRS	PDBS
Svi L5 (182 - 206 bp)	H_o	0.76	0.80	0.65	0.75	0.76	0.72	0.72	0.77	0.68	0.76	0.78	0.66	0.61*	0.63	0.67	0.73	0.62	0.83	0.89	0.82	0.70	0.40	0.75
	H_e	0.83	0.82	0.76	0.81	0.75	0.78	0.81	0.82	0.79	0.81	0.82	0.77	0.81	0.68	0.80	0.74	0.81	0.88	0.84	0.76	0.83	0.71	0.84
	N_a	9	8	9	10	8	8	9	9	8	9	10	8	9	11	7	9	8	13	10	9	9	4	8
	N	34	25	85	150	50	36	50	152	50	50	50	50	150	150	18	82	66	24	36	50	30	5	8
Svi 4 (105 - 119 bp)	H_o	0.59	0.72	0.74	0.73	0.71	0.58	0.78	0.74	0.68	0.74	0.70	0.76	0.71	0.74	0.56	0.68	0.73	0.75	0.78	0.64	0.67	1.00	0.88
	H_e	0.72	0.71	0.73	0.71	0.67	0.63	0.70	0.74	0.68	0.73	0.70	0.70	0.71	0.69	0.70	0.72	0.72	0.73	0.73	0.71	0.73	0.98	0.82
	N_a	5	4	5	7	4	4	5	7	5	4	5	4	7	5	4	6	7	6	5	4	5	9	7
	N	34	25	85	150	49	36	49	152	50	50	50	50	150	150	18	82	66	24	36	50	30	5	8
Svi 17 (102 - 116 bp)	H_o	0.59	0.44	0.47	0.47	0.44*	0.44	0.42	0.30	0.52	0.58	0.44	0.46	0.41	0.41	0.67	0.33	0.44	0.71	0.47	0.34	0.47	0.20	0.25
	H_e	0.57	0.53	0.44	0.45	0.53	0.38	0.38	0.31	0.48	0.50	0.47	0.42	0.42	0.42	0.61	0.36	0.50	0.78	0.61	0.38	0.43	0.20	0.68
	N_a	5	4	4	7	5	3	5	6	4	4	5	5	6	6	5	5	7	7	5	4	6	2	4
	N	34	25	85	150	50	36	50	152	50	50	50	50	149	150	18	83	66	24	36	50	30	5	8
Svi 18 (120 - 132 bp)	H_o	0.44	0.72	0.55	0.49*	0.53	0.36	0.46	0.47	52.00	0.62	0.66	0.58	0.49	0.39*	0.61	0.49	0.55	0.71	0.61	0.36*	0.47	0.60	0.13
	H_e	0.57	0.67	0.61	0.59	0.53	0.49	0.55	0.52	0.58	0.62	0.61	0.62	0.55	0.51	0.62	0.56	0.63	0.68	0.67	0.55	0.48	0.47	0.34
	N_a	5	5	4	4	3	4	4	4	5	4	5	5	5	5	3	4	5	4	4	3	3	2	3
	N	34	25	85	150	49	36	50	152	50	50	50	50	150	150	18	83	66	24	36	50	30	5	8

Table 3: Continued.

Locus		WRR	WNC	WIR	WHB	JHC	WSB	WWP	WGR	BBR	POP	BER	BLV	WWR	MSC	PDB	FC	LOW	BQ	BSR	PL	NR	NRS	PDBS
Svi 33 (89 - 103 bp)	Ho	0.59*	0.60	0.71	0.73	0.78*	0.56	0.52	0.63	0.60	0.50*	0.76	0.66	0.67	0.75	0.56	0.60	0.64	0.92	0.72	0.64	0.80	0.80	63.00
	He	0.63	0.67	0.69	0.65	0.63	0.59	0.55	0.59	0.68	0.62	0.65	0.63	0.65	0.64	0.67	0.58	0.67	0.83	0.69	0.67	0.71	0.89	0.83
	N _a	7	5	8	12	6	5	6	5	6	5	6	5	7	8	5	6	7	10	6	8	6	6	5
	N	34	25	85	150	50	36	50	152	50	50	50	50	149	150	18	83	66	24	36	50	30	5	8
Svi L6 (107 - 131 bp)	H _o	0.47	0.69	0.52	0.44	0.62*	0.50	0.40	0.43	0.66	0.50	0.58	0.36	0.57	0.59*	0.22	0.52	0.40	0.71	0.53	56.00	0.53	0.20	0.38
	He	0.50	0.68	0.51	0.44	0.48	0.48	0.43	0.43	0.53	0.55	0.50	0.44	0.51	0.52	0.29	0.53	0.50	0.76	0.48	0.56	0.50	0.20	0.49
	N _a	3	7	8	8	5	4	6	5	7	5	7	6	7	6	2	5	4	10	5	5	3	2	3
	N	34	25	85	150	50	36	50	151	50	50	50	50	150	150	18	83	66	24	36	50	30	5	8
Svi L7 (188 - 222 bp)	H _o	0.88	0.84	0.82	0.93*	0.85	0.92	0.94	0.78	0.90	0.92	0.84	0.84	0.89	0.88	0.94	0.82	0.88	0.79	0.87	0.84	0.90	0.80	0.88
	He	0.90	0.91	0.86	0.87	0.87	0.86	0.88	0.80	0.87	0.85	0.85	0.88	0.87	0.79	0.89	0.75	0.82	0.83	0.86	0.83	0.85	0.96	0.87
	N _a	16	13	16	18	15	11	15	15	16	12	14	16	21	17	12	11	15	11	13	13	14	8	7
	N	34	25	85	150	48	36	50	152	50	50	50	50	149	139	18	83	66	24	36	50	30	5	8
Svi L8 (118 - 146 bp)	H _o	0.76	0.72	0.73	0.81	0.92	0.86	0.84	0.74	0.82	0.84	0.82	0.84	0.76	0.82	0.89	0.73	0.71	0.88	0.78	0.78	0.87	0.60	1.00
	He	0.76	0.82	0.76	0.83	0.83	0.81	0.81	0.81	0.82	0.80	0.85	0.83	0.81	0.81	0.78	0.83	0.78	0.88	0.81	0.83	0.81	0.84	0.83
	N _a	8	9	11	14	13	9	10	13	11	10	11	11	13	11	8	11	10	12	9	11	10	6	7
	N	34	25	85	150	48	36	50	152	50	50	50	50	147	150	18	83	66	24	36	50	30	5	8
Svi 6 (113 - 151 bp)	H _o	0.85	0.88*	0.77	0.80	0.81	0.75	0.78	0.64	0.76	0.82	0.84	0.71	0.76*	0.73	0.83	0.73	0.88	0.71*	0.33	0.82	0.83	0.60	0.38*
	He	0.83	0.79	0.79	0.80	0.77	0.83	0.77	0.70	0.79	0.81	0.81	0.84	0.80	0.76	0.82	0.82	0.83	0.77	0.36	0.79	0.81	0.62	0.68
	N _a	10	9	10	13	11	12	9	12	10	12	8	13	14	14	8	11	9	10	6	6	8	3	6
	N	34	25	85	148	41	36	50	152	50	50	49	49	145	143	18	83	65	24	35	50	30	5	8
TOTAL	H _o	0.66	0.71*	0.66	0.68*	0.71	0.63	0.65	0.61*	0.68	0.70*	0.71	0.65	0.65*	0.66*	0.66	0.63*	0.65	0.78	0.68	0.64	0.68	0.58*	0.58
	He	0.70	0.73	0.68	0.68	0.68	0.65	0.65	0.63	0.69	0.70	0.69	0.68	0.68	0.65	0.69	0.66	0.70	0.79	0.67	0.67	0.67	0.65	0.71

Table 4: Pairwise estimations of F_{ST} values between years at sampling locations (above the diagonal) and genetic differentiation X^2 (below the diagonal). Significant divergence between groups after Bonferroni correction, $p < 0.05$, is represented by an asterisk. Sampling locations are represented as follows: WNC – Netley Creek, WIR – Icelandic River, WHB – Hecla Bar, WGR – Grand Rapids, WWR – Winnipeg River, MSC – Swan Creek, FC – Falcon Creek, LOW – Lake of the Woods. Suffixes for each sampling location (07, 08, 09) indicate the sampling year (2007, 2008, 2009). A value of infinity was assigned when the X^2 value exceeded the capacity of the program to calculate ($p < 0.0001$).

	WNC '07	WNC '08	WIR '07	WIR '08	WHB '07	WHB '08	WHB '09	WGR '07	WGR '08	WGR '09
WNC '07		0.0059	0.0237	0.0206*	0.0207	0.0251*	0.0277	0.0518*	0.0461*	0.0518*
WNC '08	28.12		0.0200	0.0047	0.0068	0.0089	0.0150	0.0416	0.0326	0.0402
WIR '07	56.63*	51.31*		0.0034	0.0191*	0.0168*	0.0162	0.0338*	0.0299*	0.0381*
WIR '08	Inf*	28.15	25.16		0.0104	0.0124*	0.0066	0.0261*	0.0195*	0.0235*
WHB '07	40.67	24.84	51.63*	Inf*		0.0032	0.0027	0.0209*	0.0141*	0.0169
WHB '08	54.89*	30.81	Inf*	Inf*	21.37		0.0000	0.0192*	0.0161*	0.0168*
WHB '09	Inf*	33.94	42.22*	31.67	20.88	24.49		0.0129*	0.0113*	0.0102*
WGR '07	78.08*	Inf*	98.86*	Inf*	Inf*	70.79*	Inf*		0.0010	-0.0044
WGR '08	82.61*	44.34*	Inf*	Inf*	57.01*	61.54*	63.36*	17.46		-0.0028
WGR '09	82.95*	48.49*	Inf*	Inf*	47.37*	56.73*	51.18*	12.62	15.99	
WWR '07	50.67*	32.91	69.34*	39.87	41.62	30.36	37.53	Inf*	Inf*	52.13*
WWR '08	52.42*	25.34	Inf*	Inf*	23.75	28.3	29.89	67.76*	Inf*	47.89*
WWR '09	60.23*	31.85	64.38*	50.42*	25.63	29.41	26.8	52.29*	60.72*	50.45*
MSC '07	74.50*	60.91*	69.44*	70.53*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*
MSC '08	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*
MSC '09	Inf*	Inf*	Inf*	109.86*	Inf*	97.77*	Inf*	Inf*	Inf*	Inf*
FC '08	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*
FC '09	80.50*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*
LOW '07	68.16*	31.72	Inf*	Inf*	Inf*	74.65*	68.57*	71.24*	73.32*	68.42*
LOW '08	55.91*	36.99	Inf*	Inf*	43.83*	61.91*	Inf*	52.62*	58.68*	57.34*

Table 4: Continued.

	WWR '07	WWR '08	WWR '09	MSC '07	MSC '08	MSC '09	FC '08	FC '09	LOW '07	LOW '08
WNC '07	0.0278	0.0205	0.0383*	0.0333*	0.0884*	0.0544*	0.0646*	0.0529*	0.0484*	0.0265*
WNC '08	0.0056	0.0006	0.0140	0.0297	0.0762*	0.0424*	0.0468*	0.0437*	0.0253	0.0147
WIR '07	0.0152*	0.0215*	0.0230*	0.0207*	0.0789*	0.0336*	0.0762*	0.0750*	0.0433*	0.0256*
WIR '08	0.0073	0.0131	0.0178*	0.0235*	0.0717*	0.0383*	0.0671*	0.0617*	0.0323*	0.0203*
WHB '07	0.0103	0.0033	0.0106	0.0291*	0.0600*	0.0345*	0.0644*	0.0541*	0.0313*	0.0075*
WHB '08	0.0053	0.0019	0.0088	0.0202*	0.0634*	0.0313*	0.0665*	0.0514*	0.0322*	0.0136*
WHB '09	0.0044	0.0019	0.0067	0.0231*	0.0574*	0.0305*	0.0673*	0.0533*	0.0334*	0.0173*
WGR '07	0.0115*	0.0127*	0.0131*	0.0311*	0.0785*	0.0464*	0.0563*	0.0505*	0.0345*	0.0143*
WGR '08	0.0132*	0.0149	0.0214*	0.0346*	0.0816*	0.0489*	0.0733*	0.0639*	0.0392*	0.0168*
WGR '09	0.0130	0.0131*	0.0181*	0.0346*	0.0842*	0.0501*	0.0657*	0.0559*	0.0396*	0.0182*
WWR '07		0.0018	0.0025	0.0070*	0.0494*	0.0151*	0.0366*	0.0310*	0.0117	0.0057
WWR '08	29.73		-0.0004	0.0131*	0.0438*	0.0274*	0.0409*	0.0344*	0.0132*	0.0053*
WWR '09	25.77	12.21		0.0102*	0.0390*	0.0210*	0.0308*	0.0293*	0.0119	0.0035
MSC '07	47.16*	65.67*	52.19*		0.0488*	0.0096	0.0367*	0.0370*	0.0163*	0.0112*
MSC '08	Inf*	Inf*	Inf*	Inf*		0.0386*	0.0796*	0.0838*	0.0716*	0.0556*
MSC '09	50.08*	Inf*	53.50*	34.95	Inf*		0.0508*	0.0457*	0.0380*	0.0253*
FC '08	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*		0.0009	0.0266*	0.0263*
FC '09	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	19.32		0.0228*	0.0243*
LOW '07	31.04	45.34*	40.06	Inf*	Inf*	Inf*	63.75*	54.82*		0.0082
LOW '08	35.8	Inf*	36.64	54.03*	Inf*	68.95*	Inf*	70.10*	22.15	

Table 5: Pairwise estimations of F_{ST} values between sampling locations (above the diagonal) and X^2 values (below the diagonal). Significant divergence between groups after Bonferroni correction, $p < 0.05$, is represented by an asterisk. Lake Winnipeg locations are highlighted in grey. Sampling locations are represented as follows: WRR – Red River, WNC – Netley Creek, WIR – Icelandic River, WHB – Hecla Bar, JHC – Jackhead Creek, WSB – Sturgeon Bay, WWP – Warpath River, WGR – Grand Rapids, BBR – Big Black River, POP – Poplar River, BER – Berens River, BLV – Bloodvein River, WWR – Winnipeg River, MSC – Swan Creek, PDB – Pointe Du Bois, FC – Falcon Creek, LOW – Lake of the Woods, BQ – Bay of Quinte, BSR – Black Sturgeon River, PL – Paint Lake, NR – Nelson River, PDBS – Pointe Du Bois Sauger, NRS – Nelson River Sauger. Values of Inf, denoted as infinite by GENEPop ($p < 0.00001$), with the infinite designation occurring when the value exceeds the maximum specified by the program.

	WRR	WNC	WIR	WHB	JHC	WSB	WWP	WGR	BBR	POP	BER	BLV	WWR
WRR		0.0127	0.0086*	0.0040	0.0035	0.0022	0.0125	0.0222*	-0.0001	-0.0045	0.0052	0.0039	0.0069*
WNC	42.11*		0.0163*	0.0181*	0.0243	0.0329*	0.0412*	0.0457*	0.0173	0.0075	0.0240*	0.0215	0.0210*
WIR	47.34*	Inf*		0.0114*	0.0142*	0.0129*	0.0172*	0.0275*	0.0093	0.0051	0.0153*	0.0120*	0.0145*
WHB	37.29	58.81*	Inf*		0.0044	0.0025	0.0047	0.0154*	0.0019	0.0019	0.0002	-0.0004	0.0048
JHC	30.78	52.71*	Inf*	16.29		0.0042	0.0095	0.0232*	-0.0012	0.0017	0.0029	0.0022	0.0053
WSB	27.22	69.67*	56.69*	23.51	24.02		0.0026	0.0132*	-0.0024	0.0042	0.0008	-0.0036	0.0004
WWP	47.02*	69.39*	Inf*	25.85	30.24	23.76		0.0066*	0.0061	0.0100	0.0026	0.0035	0.0091
WGR	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	67.20*		0.0172*	0.0194*	0.0121*	0.0145*	0.0148*
BBR	25.34	37.51	Inf*	25.71	14.42	15.12	20.75	Inf*		-0.0014	-0.0018	-0.0015	-0.0013
POP	25.08	35.59	36.63	18.20	19.22	28.72	27.78	Inf*	10.65		0.0006	0.0020	0.0056
BER	35.68	60.56*	76.10*	20.30	21.30	23.55	23.93	Inf*	16.96	21.84		-0.0017	0.0006
BLV	22.89	43.61*	Inf*	19.59	15.43	13.64	15.61	Inf*	11.65	22.60	15.51		0.0009
WWR	49.26*	55.32*	Inf*	56.03*	31.49	26.26	41.24	Inf*	12.41	30.91	36.56	22.30	
MSC	Inf*	Inf*	Inf*	Inf*	Inf*	47.79*	Inf*	Inf*	Inf*	Inf*	Inf*	67.54*	Inf*
PDB	37.37	34.29	53.42*	26.17	31.76	32.07	21.62	41.39*	21.56	30.18	18.91	20.58	30.16
FC	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*
LOW	Inf*	67.60*	Inf*	Inf*	Inf*	57.80*	Inf*	Inf*	Inf*	Inf*	53.65*	44.39*	Inf*
BQ	Inf*	89.38*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*
BSR	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*
PL	82.21*	Inf*	Inf*	Inf*	83.49*	52.40*	88.39*	Inf*	71.90*	61.56*	60.23*	Inf*	Inf*
NR	49.96*	65.23*	Inf*	53.56*	49.47*	36.11	69.65*	Inf*	39.92	48.78*	30.68	40.04	Inf*
PDBS	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*
NRS	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*

Table 5: Continued.

	MSC	PDB	FC	LOW	BQ	BSR	PL	NR	PDBS	NRS
WRR	0.0254*	0.0113	0.0613*	0.0236*	0.0662*	0.0552*	0.0225*	0.0136*	0.1998*	0.1626*
WNC	0.0421*	0.0247	0.0540*	0.0271*	0.0349*	0.0514*	0.0330*	0.0263*	0.1939*	0.1548*
WIR	0.0329*	0.0239*	0.0683*	0.0250*	0.0694*	0.0728*	0.0278*	0.0194*	0.2217*	0.1865*
WHB	0.0272*	0.0048	0.0596*	0.0174*	0.0724*	0.0620*	0.0219*	0.0119*	0.2132*	0.1727*
JHC	0.0185*	0.0127	0.0594*	0.0220*	0.0755*	0.0676*	0.0278*	0.0182*	0.2310*	0.1893*
WSB	0.0127	0.0098	0.0581*	0.0161*	0.0965*	0.0676*	0.0151*	0.0078	0.2381*	0.1980*
WWP	0.0338*	0.0095	0.0724*	0.0218*	0.0884*	0.0758*	0.0299*	0.0206*	0.2408*	0.2036*
WGR	0.0439*	0.0138	0.0624*	0.0231*	0.1054*	0.0888*	0.0206*	0.0174*	0.2488*	0.2125*
BBR	0.0111*	0.0032	0.0449*	0.0094*	0.0726*	0.0593*	0.0155*	0.0056	0.2182*	0.1775*
POP	0.0193*	0.0101	0.0552*	0.0191*	0.0602*	0.0577*	0.0176*	0.0128	0.2154*	0.1757*
BER	0.0177*	0.0000	0.0405*	0.0089*	0.0764*	0.0643*	0.0137*	0.0039	0.2198*	0.1800*
BLV	0.0152*	0.0005	0.0491*	0.0097*	0.0720*	0.0614*	0.0157*	0.0055	0.2146*	0.1756*
WWR	0.0137*	0.0054	0.0335*	0.0052*	0.0783*	0.0626*	0.0111*	0.0037*	0.2277*	0.1867*
MSC		0.0258	0.0443*	0.0216*	0.1092*	0.0774*	0.0266*	0.0206*	0.2652*	0.2262*
PDB	55.91*		0.0437*	0.0019	0.0657*	0.0694*	0.0226*	0.0063	0.2033	0.1669*
FC	Inf*	Inf*		0.0231*	0.1171*	0.1039*	0.0349*	0.0242*	0.2693*	0.2232*
LOW	Inf*	15.65	Inf*		0.0731*	0.0772*	0.0137*	0.0053*	0.2189*	0.1831*
BQ	Inf*	Inf*	Inf*	Inf*		0.0713*	0.0949*	0.0830*	0.1695*	0.1346*
BSR	Inf*	Inf*	Inf*	Inf*	Inf*		0.0863*	0.0772*	0.2192*	0.1941*
PL	Inf*	51.32*	Inf*	Inf*	Inf*	Inf*		0.0009	0.2359*	0.1909*
NR	Inf*	27.46	Inf**	52.63*	Inf*	Inf*	27.26		0.2231*	0.1750*
PDBS	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*		0.0049
NRS	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	Inf*	14.26	

Table 6: A comparison of levels of genetic diversity (H_o) and the average number of alleles per locus (N_a) as found for walleye in this study and other studies.

Lake	H_o	N_a	Reference
Lake Winnipeg	0.671	10.2	This Study
Lake Superior	0.686	7.2	Stepien et al. 2009
Lake Ontario	0.69	9.8	
Lake Huron	0.709	9.6	
Lake Erie	0.711	11.9	
Lake Michigan	0.72	7.1	
Lake Mistassini, QC	0.613	7.6	Dupont et al. 2007
NA range of walleye	0.698	13.9	Stepien and Strange 2007

Table 7: A comparison of genetic differentiation (F_{ST}) values found between Lake Winnipeg walleye spawning sites in this study, between walleye spawning sites in the Laurentian Great Lakes (as determined by other studies), and between spawning sites of smallmouth bass and chum salmon, other philopatric species (as determined by other studies).

Species	Location	Comparison	Range of F_{ST}	Reference
<i>Sander vitreus</i> (walleye)	Lake Winnipeg	All sites (except WGR and WIR)	-0.0001 - 0.0215	This study
		WGR	0.0066 - 0.0457	
		WIR	0.0051 - 0.0275	
	Lake Erie	western basin	0.004 - 0.063	Strange and Stepien 2007
		eastern basin	0.029 - 0.052	
		between basins	0.011 - 0.063	
	Bay of Quinte	West Lake	0.0141 - 0.0264	Wilson and Gatt 2000
		all other sites (no significant difference)	0.0006 - 0.0098	
<i>Micropterus dolomieu</i> (smallmouth bass)	Lake Erie	west basin	0.021 - 0.034	Stepien et al. 2007
		central basin	0.054 - 0.178	
		east basin	0.020 - 0.118	
<i>Oncorhynchus keta</i> (chum salmon)	British Columbia	between central and north coast	0.009	Beacham et al. 2008
		between Fraser and Taku Rivers	0.036	

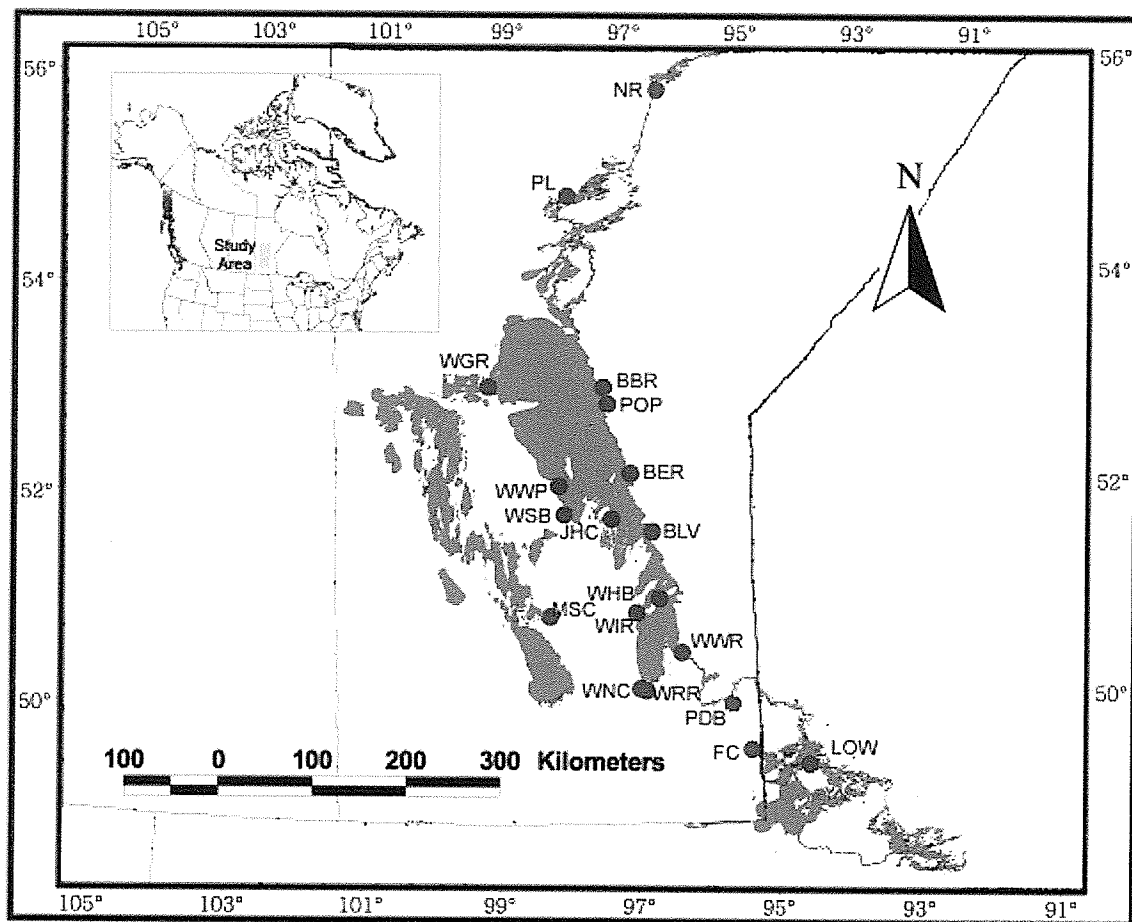


Figure 1: Lake Winnipeg and surrounding sampling areas, central Canada. Sampling sites are as follows: WRR – Red River; WNC – Netley Creek; WIR – Icelandic River; MSC – Swan Creek; WHB – Hecla Bar; JHC – Jackhead Creek; WSB – Sturgeon Bay; WWP – Warpath River; WGR – Grand Rapids; PL – Paint Lake; NR – Nelson River; BBR – Big Black River; POP – Poplar River; BER – Berens River; BLV – Bloodvein River; WWR – Winnipeg River; PDB – Pointe Du Bois; FC – Falcon Creek; LOW – Lake of the Woods. Sampling sites on the Black Sturgeon River (BSR) and Bay of Quinte (BQ) are not shown on this map.

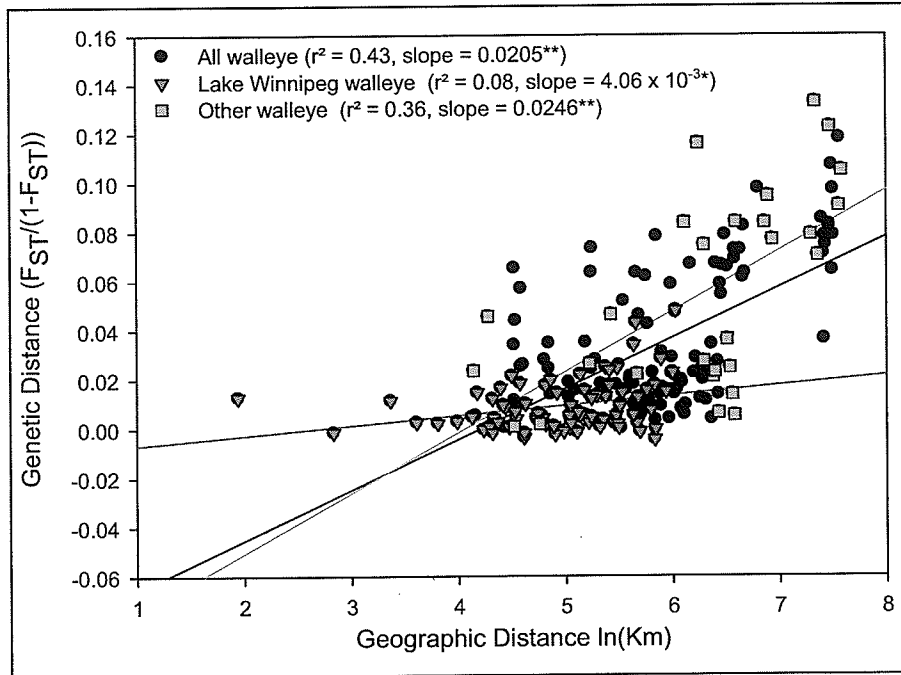


Figure 2: Correlation of pairwise measures of genetic differentiation ($(\theta/(1-\theta))$) and pairwise geographic distances ($\ln(Km)$). Distances were measured from the centroid of the populations within the lake. Comparisons are made with all walleye, all Lake Winnipeg walleye, and all walleye outside of Lake Winnipeg. There is a significant relationship between genetic and geographic distance for all comparisons (* = $p < 0.05$, ** $p < 0.001$).

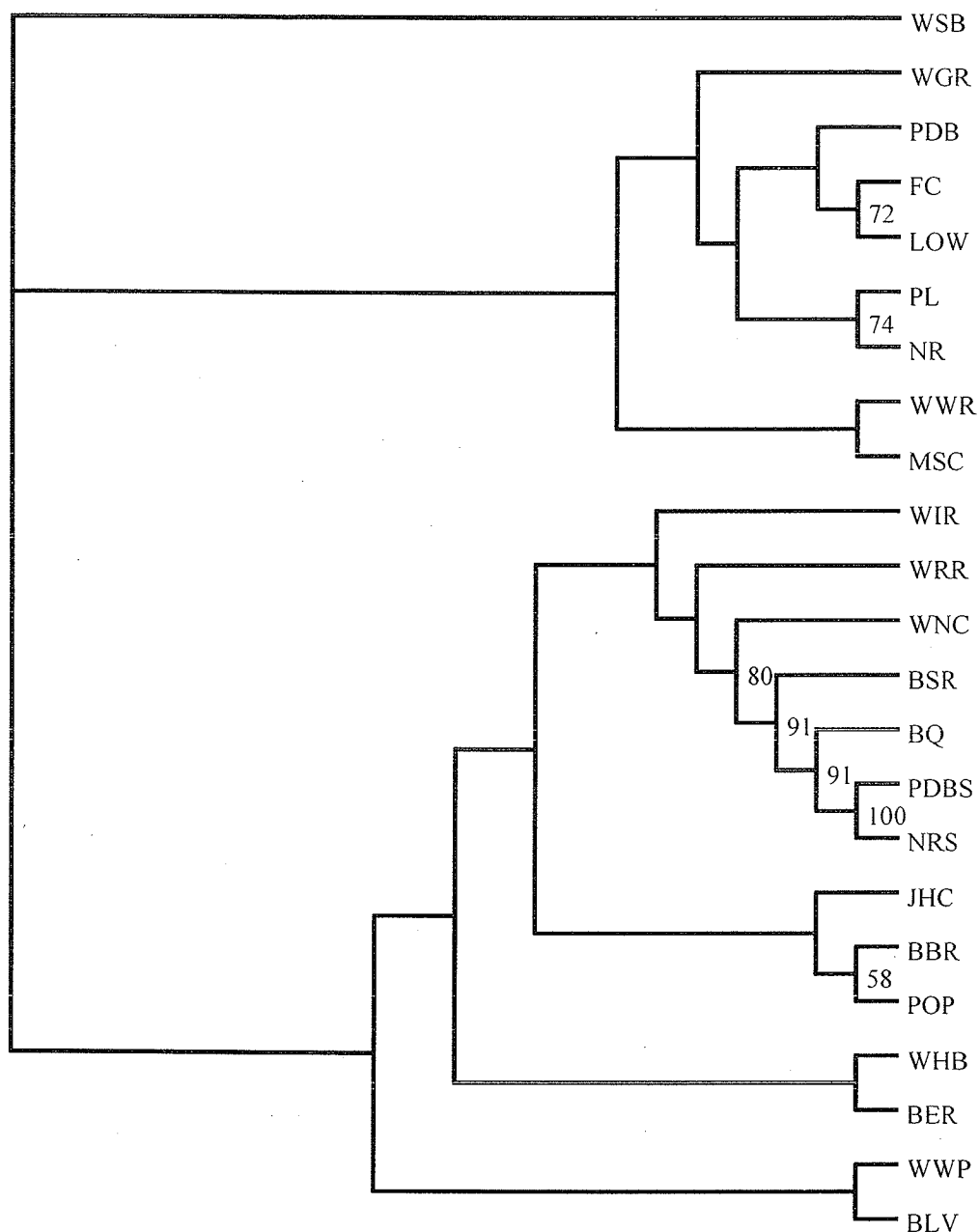


Figure 3: Neighbour Joining phenogram of 21 walleye sampling sites and 2 sauger sampling sites, using nine microsatellite loci and based on Cavalli-Sforza & Edwards (1967) chord-distance (Dce). Genotypic data were bootstrapped over loci with replacement and 2000 replicates. Bootstrap values less than 50 have been removed. WRR – Red River, WNC – Netley Creek, WIR – Icelandic River, WHB – Hecla Bar, JHC – Jackhead Creek, WSB – Sturgeon Bay, WWP – Warpath River, WGR – Grand Rapids, BBR – Big Black River, POP – Poplar River, BER – Berens River, BLV – Bloodvein River, WWR – Winnipeg River, MSC – Swan Creek, PDB – Pointe Du Bois, FC – Falcon Creek, LOW – Lake of the Woods, BQ – Bay of Quinte, BSR – Black Sturgeon River, PL – Paint Lake, NR – Nelson River, PDBS – Pointe Du Bois Sauger, NRS – Nelson River Sauger.

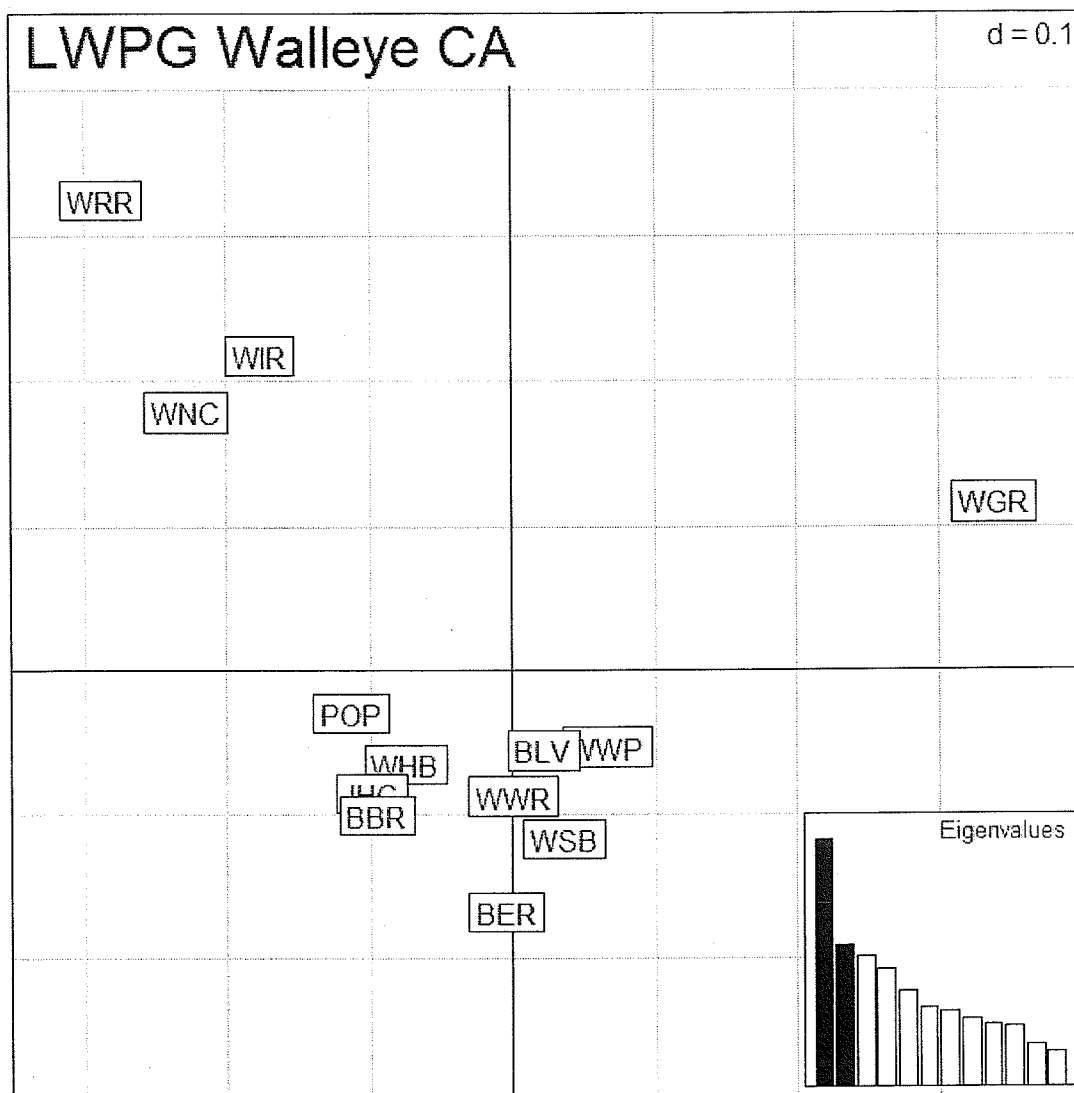


Figure 4: Duality diagram of a correspondence analysis using nine microsatellite variables for walleye taken from 13 sampling sites in Lake Winnipeg. The width of each grid square is equal to 0.1. The bar chart in the lower right corner represents the eigenvalues and the two axes represented on the plot are denoted by the black shading. The first axis (horizontal) explains 21.5% of the variance in all Lake Winnipeg walleye sampling locations, while the second axis (vertical) explains 12.3% of the variance. Sampling locations are represented as follows: WRR – Red River, WNC – Netley Creek, WIR – Icelandic River, WHB – Hecla Bar, JHC – Jackhead Creek, WSB – Sturgeon Bay, WWP – Warpath River, WGR – Grand Rapids, BBR – Big Black River, POP – Poplar River, BER – Berens River, BLV – Bloodvein River, WWR – Winnipeg River.

CHAPTER TWO: USING PCR-RFLP ASSAY OF THE MITOCHONDRIAL CONTROL REGION TO INVESTIGATE BROADSCALE POPULATION STRUCTURE OF WALLEYE (*SANDER VITREUS*) IN LAKE WINNIPEG AND SURROUNDING AREAS

ABSTRACT

In some systems, walleye (*Sander vitreus*) have been found to return to the same location to spawn each spring (a behaviour known as natal homing or philopatry). Some evidence suggests male mediated gene flow exceeds that of females, potentially obscuring genetic differences between spawning sites that could accumulate as a result of philopatry. The maternal inheritance pattern of mitochondrial DNA (mtDNA) allows isolation of the gene flow of females, providing an opportunity to identify population structure persisting as a result of female philopatry. In this study I developed a Polymerase Chain Reaction – Restriction Fragment Length Polymorphism (PCR-RFLP) assay with one restriction enzyme (identified as targeting a variable site in Lake Winnipeg walleye) to assess population structure and genetic diversity in 193 walleye from Lake Winnipeg and surrounding areas. Five haplotypes were resolved, two of which (haplotypes 1 and 3) accounted for 96% of all fish. Sequence divergence between the five haplotypes ranged from 0.8 to 3.6%. Exact tests and AMOVA detected no significant population structure, indicating uniform dispersal of the two haplotypes and a lack of strong philopatry in female walleye; however the data allows no strong conclusions to be drawn regarding population structure or homing behaviour in Lake Winnipeg walleye. Three of the haplotypes identified in this study corresponded to three major mitochondrial haplotypes previously identified in walleye, thought to correspond to three glacial refugia.

Haplotypes 1 and 3 corresponded to previous haplotypes thought to represent the Missourian and Mississippi refugia respectively, indicating colonization of Lake Winnipeg from two glacial refugia, or repeated colonizations from a single refugium. Haplotype 5 in this study was found only in the Bay of Quinte, and corresponds to the Atlantic refugium. Overall haplotype diversity was comparable to that in other studies in the Laurentian Great Lakes area.

INTRODUCTION

Variation in mitochondrial DNA (mtDNA) has been widely adopted as a tool to perform genetic analysis with fisheries related applications (Billington and Hebert 1991). The high mutation rate and low effective population size of mtDNA (relative to nuclear DNA) can make it an effective marker in detecting population structure (Moritz et al. 1987).

Although the use of microsatellites is becoming much more prevalent, as they are generally able to provide greater resolution in intraspecific population structure (Selkoe and Toonen 2006), the maternal inheritance of mtDNA provides unique opportunities compared to the bi-parental inheritance observed in nuclear DNA (Carvalho and Hauser 1994). In philopatric species, or in species where male-mediated gene flow exceeds that of females (as has been postulated for walleye, Billington and Hebert 1988), gene frequency divergence in nuclear DNA can be suppressed, while the maternal inheritance pattern of mtDNA allows genetic divergence to accumulate in mtDNA. The fact that mtDNA is generally inherited as a single locus (i.e. without recombination) means that it maintains an intact record of evolutionary history, and can be informative in situations where secondary contact or gene flow obscures population structure (Billington and Hebert 1991). Estimates of genetic divergence in mtDNA can be determined by comparison of nucleotide sequences obtained through direct sequencing, or by comparison of congruence in fragment patterns obtained by digestion with restriction enzymes (Restriction Fragment Length Polymorphisms, RFLPs).

Direct sequencing allows comparison between nucleotide sequences, with each variable site constituting a character. It has been successful in resolving population structure among geographic locations in a number of species and systems (e.g., flathead mullet,

Mulgil cephalus (Jamander et al. 2009); plains topminnow, *Fundulus sciadicus* (Li et al. 2009); anchovy, *Engraulis encrasicolus* (Magoulas et al. 2006); Arctic charr, *Salvelinus alpinus* (Brunner et al. 2001)). The maternal inheritance pattern of mtDNA can be especially useful in some organisms. Sperm whales (*Physeter macrocephalus*) are a species in which females show an increased level of philopatry compared to males. Control region sequence data in this species were successful in detecting significant differences between sperm whales in the Gulf of Mexico, the western North Atlantic, the Mediterranean Sea and the North Sea. The same populations could not be differentiated using 16 polymorphic microsatellite loci (Engelhaupt et al. 2009).

Mitochondrial control region sequences have also been used to assess the genetic and phylogeographic structure of walleye populations at different geographical scales within the Great Lakes area (spawning sites, lake basins, lakes and putative glacial refugia) (Stepien and Faber 1998). Phylogeographic structure and significant differences were found at these three broad scales as well as among some of the spawning sites (finer scale).

Another way to examine nucleotide sequence (either in the entire genome, or a certain gene amplified by Polymerase Chain Reaction, PCR) is through RFLP assays. This method utilizes restriction enzymes to cleave the region of interest at particular recognition sites, resulting in variable size fragments if sequence differences result in the gain or loss of these recognition sites. The relatively low cost and high throughput of RFLP assays compared to direct sequencing make them attractive as a method to quickly and easily detect mtDNA haplotypes. However, RFLP assays can only detect differences

on a fraction of the **gene** or gene fragment, while direct sequencing allows information to be obtained at every **nucleotide**. RFLP assays are most effective when a large number of restriction enzymes are used (resulting in increased coverage of the gene or genome), or when known variable sites (as detected by direct sequencing) are targeted (e.g., Docker and Heath 2002). The use of RFLP assays as a tool to investigate genetic diversity present in mtDNA has been widely applied to resolve population structure in lamprey (Docker et al. 2007, Goodman et al. 2008) and salmonids (coho Salmon, *Oncorhynchus kisutch* (Gharrett et al. 2001); chum salmon, *Oncorhynchus keta* (Brykov et al. 2003); kokanee, *Oncorhynchus nerka* (Taylor et al. 1997) and brown trout, *Salmo trutta* (Hansen et al. 1995)).

RFLP assays have also been used to assess genetic population structure in walleye. RFLP assays on the entire mitochondrial genome were successful in detecting three major walleye haplotypes which differed from each other by a maximum percent sequence divergence of 0.39% (Ward et al. 1989, six restriction enzymes; Billington et al. 1992, ten restriction enzymes; Billington and Strange 1995, 23 restriction enzymes). Each of the three haplotypes possessed a distinct geographical distribution, with haplotype A being predominant in the eastern Great Lakes region, haplotype B predominant in the central range and haplotype C predominant in the northwest (west of Lake Superior). These three distinct distributions reflect the postglacial colonization of walleye in North America, with haplotype A originating in the Atlantic refugium, haplotype B from the Mississippi refugium and haplotype C from the Missouri refugium (initially referred to as haplotypes 1 (Atlantic), 4 (Mississippi) and 10 (Missouri) by Billington et al. 1992). The division of three major phylogenetic assemblages, as inferred

with RFLP assays, has been supported by Gatt et al. (2000), who used direct sequencing of a 513 base pair fragment of the control region to detect 17 mtDNA haplotypes in fish from the Great Lakes and Luxapalila Creek (northeastern Mississippi), which could be assigned to one of the three major RFLP haplotypes previously described (Table 8).

The studies described above (Ward et al. 1989 and Billington et al. 1992) included some samples from Manitoba in their assessment of genetic diversity in North American walleye. Ward et al. (1989) used six endonucleases to resolve genetic differences in the entire mtDNA genome in walleye taken from the Great Lakes including 32 from northern Manitoba (Issett Lake and Missi Falls, South Indian Lake). Haplotype C (Missouri) was predominant in the Manitoba samples, with contributions from haplotype B (Mississippi) found at two of the three sites. Billington et al. (1992) used ten restriction endonucleases, and also included 76 walleye samples from Manitoba (South Indian Lake, Missi Falls, Issett Lake, Dauphin Lake, Lake Manitoba, Lake Winnipeg and Falcon Lake). They also found haplotype C (Missouri) to be predominant in Manitoba, with contributions from haplotype B (Mississippi) as well as other haplotypes (in small numbers).

In this study, a 715 base pair fragment from the control region of the walleye mitochondrial genome was sequenced to detect variable sites. A PCR-RFLP assay was then developed to target the variable sites using one enzyme (*Tsp45I*), resolving a total of five haplotypes in walleye. Variation detected with this RFLP assay was used to determine if there were significant differences in the distribution of haplotypes between spawning sites or lake basins. While little population structure was detected using microsatellites (which generally provide better resolution, see Chapter 1), the maternal

inheritance of mtDNA makes it useful in detecting population structure if female philopatry is strong. Haplotypes detected in this study were also compared to three previously determined walleye haplotypes representing lineages thought to have resulted from three separate glacial refugia, and compared to previous glacial history studies which included Manitoba walleye. Mitochondrial DNA variation detected in this study may help resolve population structure of walleye in Lake Winnipeg, as well as determining the colonization pattern of walleye into Lake Winnipeg from glacial refugia.

METHODS

Mitochondrial

Walleye were collected from 13 spawning sites within Lake Winnipeg as well as additional sites in Manitoba and Ontario (Chapter 1, Table 1, Figure 1). Genomic DNA was extracted from either fin tissue or scales using the Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol with the exception of adding 10 µL of 20 mg/ml proteinase K (Invitrogen) to 500 µL of nuclei lysis solution.

Approximately 715 base pairs of the mitochondrial control region were amplified by Polymerase Chain Reaction (PCR) using primers LN20 (5'-GTCCCTCACCTTCAATAACCG-3') and HW1 (5'-ACCACTAGCACCCAAAGCTA-3') from Gatt et al. (2000). Each 25 µL reaction contained sterile deionized water, 1X manufacturers buffer (20 mM Tris-HCl pH 8.4, 50mM KCl), 1.65 mM MgCl₂, 0.02 mM each dNTP, 0.504 uM forward (LN20) and reverse (HW1) primer, 0.6 units *Taq* polymerase (Invitrogen) and 1.00 µL genomic DNA (approximately 100 nanograms). Reactions were initially denatured at

94°C for 1 minute. Amplification was carried out in 30 cycles: denaturation at 92°C for 1 minute, primer annealing at 52°C for 1 minute and extension at 72°C for 1 minute. Reactions underwent a final extension at 72°C for 2 minutes, before being held at 4°C indefinitely. PCR products were purified by precipitation with isopropanol and sodium acetate, and used as a template for the AB PRISM® Big-Dye™ Terminator (v3.1, Applied Biosystems) sequencing reaction, using the reverse primer (HW1). Sequences were read on the AB 3130 Genetic Analyzer (Applied Biosystems) automatic sequencer. Resulting electropherograms (AB files) were viewed and manually checked in Chromas Lite v2.01 (Conor McCarthy, Griffith University, Australia), before being aligned using ClustalW (<http://www.ebi.ac.uk/Tools/clustalw2/index.html>; Larkin et al. 2007).

RFLP

Control region sequences from 34 individuals were screened for differences in restriction enzyme recognition sites using the program Restriction Mapper (available at www.restrictionmapper.org). Among these 34 individuals there were 16 haplotypes; many of these showed only minor variation and could be divided into 7 major sequence haplotypes (Table 9, Figure 5). After screening 15 restriction enzymes, two (*Tsp45I* and *MaeIII*) produced fragment sizes capable of distinguishing among four of the 7 major haplotypes. Restriction patterns in the four haplotypes (as predicted by the program Restriction Mapper) produced by the enzyme *Tsp45I* (New England Biolabs) resulted in fragment sizes that were easily distinguishable on an agarose gel, while restriction patterns resulting from *MaeIII* consisted of fragment sizes that would be more difficult to distinguish. *Tsp45I* (recognition sequence 5' – GTSAC – 3'; S = C or G) was selected for screening of walleye samples in this study.

Control region PCR product (amplified as above) from 193 walleye (Table 10, Figure 1) was digested in 10 uL reactions containing sterile deionized water, 1X appropriate buffer, 1X BSA, 2 units *Tsp45I* and 7 uL control region PCR product. Reactions were digested at 60°C for 60 minutes. Digested product was run on a 1.8% agarose gel, and visualized using ethidium bromide staining and ultraviolet (UV) transillumination. In addition to the four haplotypes (1 – 4) that were identified in the original screening, a fifth walleye restriction pattern (haplotype 5) was detected among the additional walleye screened (Table 8 and 10). Five sauger included in the analysis produced a sixth pattern (no cuts, haplotype 0) that will not be discussed in this study. All individuals sequenced for the original screening produced the same haplotypes (as resolved on the agarose gel) that were predicted by the program Restriction Mapper. The control region of some individuals showing the new restriction patterns (0 and 5) were sequenced to confirm the predicted gain or loss of the recognition sites (Table 8). Individuals unable to be assigned to a haplotype with 100% confidence (due to poor resolution in gel, smearing between fragments or unsuccessful PCR) were re-run, and sequenced if resolution remained unclear; all of these individuals were assigned to one of the five identified walleye haplotypes.

Data Analysis

Percent sequence divergence between the 16 haplotypes identified by sequencing was calculated, and a neighbour joining phylogeny representing the relationship between them was constructed using 500 bootstrap replicates in the program *MEGA* version 4 (Tamura, Dudley, Nei, and Kumar 2007).

The frequency of haplotypes at each sample location was determined, and haplotype diversity (h) was calculated for each sampling location according to Nei and Tajima (1981). In randomly mating diploid organisms, h is comparable to observed heterozygosity as a measure of genetic heterogeneity (Nei and Tajima 1981). Haplotype diversity (h) ranges from 0 (fixed for a single haplotype) to 1 (equal frequency of all haplotypes).

An exact test for differences in allele frequencies between sampling locations, and between pooled Lake Winnipeg samples and all other sampling locations was completed (20 000 permutations, Raymond and Rousset 1995b) using TFGA 1.3. Significance levels were adjusted according to the Bonferroni correction (Rice 1989) to correct for type I sampling errors.

Examination of the hierarchical patterning of genetic variation (% variance) among groups of sampling locations, among individual locations within a group, among individuals within sampling locations, and among individuals was evaluated using AMOVA (Excoffier et al. 1992) as implemented in ARLEQUIN (Excoffier et al. 2005, v3.11 by Excoffier 2007) using RFLP haplotypes. Tests were performed with all Lake Winnipeg samples pooled and with Lake Winnipeg samples pooled into north and south basins.

RESULTS

The percent sequence divergence between the five walleye haplotypes ranged from 0.8 to 3.6% (Table 9, Figure 5). Haplotypes 3 and 5 were the most similar (0.8 – 2.2% sequence divergence), while haplotypes 4 and 5 had the greatest divergence (3.0 – 3.6% sequence divergence). The percent sequence divergence between the three main haplotypes are also similar, with haplotype 1 differing from haplotype 3 by 0.8 – 2.6%, haplotype 1 differing from haplotype 5 by 1.0 – 2.6%, and haplotype 3 differing from haplotype 5 by 0.8 – 2.2%.

In total, 193 walleye were genotyped using the restriction enzyme *Tsp451* (New England Biolabs), with a total of 5 haplotypes found (Tables 8 and 10). Two of the six haplotypes (haplotypes 1 and 3) accounted for the majority (186 walleye or 96% of fish) of all genotypes found. Haplotypes 2 and 4 were found in two and one individuals, respectively, all in Lake Winnipeg. Haplotype 5 was found in four individuals, all from the Bay of Quinte, Lake Ontario. Four restriction sites occurred in total, and all haplotypes differed from one another by a single variable restriction site (Table 8). Cut patterns characterizing the five haplotypes, and frequencies of haplotypes found at each sampling location, can be found in Table 8 and 10. A map representing the distribution of the different haplotype frequencies can be found in Figure 6.

Haplotype diversity (h , Nei and Tajima 1981) was calculated for each sampling location, the north and south basins of Lake Winnipeg, and Lake Winnipeg as a whole (Table 10). Haplotype diversity is a method of determining genetic diversity in RFLP type data,

while standardizing for sample size and number. Values of h ranged from 0.3030 (Sturgeon Bay) to 0.6444 (Bloodvein River), with a mean of 0.5148. The south and north basins of Lake Winnipeg had a haplotype diversity of 0.5319 and 0.5130, respectively, while Lake Winnipeg as a whole had a haplotype diversity of 0.5173. Outside of Lake Winnipeg, values of h ranged from 0.3556 (Lake of the Woods and Paint Lake) to 0.5714 (Black Sturgeon River).

Pairwise exact tests for differences in allele frequency were performed for all sampling locations separately, and with all Lake Winnipeg samples pooled (Table 11). When Lake Winnipeg sampling sites were compared separately, no significant differences were found. When Lake Winnipeg samples were pooled, Bay of Quinte walleye were found to be significantly different from walleye in Lake Winnipeg and Paint Lake ($p = 0.0000$ to 0.0008), all other pairwise comparisons showed no significant differentiation.

Analysis of the hierarchical patterning of genetic variation between groups of sampling locations revealed a small percentage of variation explained by population structure. When AMOVA was run on all RFLP walleye samples, with Lake Winnipeg samples pooled into north and south basins and all other sampling locations grouped separately, 6% ($p < 0.1$) of variance occurred among regions, 0% of variance occurred among sampling locations, and 94% ($p < 0.1$) occurred within sampling locations. Similar results were obtained when all Lake Winnipeg sites were pooled together; 8% ($p < 0.1$) of variance occurred among regions, 0% of variance occurred among sampling locations, and 92% ($p < 0.1$) occurred within sampling locations. When variation within Lake Winnipeg alone was looked at, pooling north basin and south basin samples separately

resulted in 0% ($p < 0.01$) of the variation occurring among groups, and 100% ($p < 0.01$) of the variation occurring among individuals. Other groupings consistently resulted in among group variation not significantly different from zero.

DISCUSSION

Genetic diversity of Lake Winnipeg walleye as determined by this PCR-RFLP study is comparable to that found in other studies. Prior examination of haplotype diversity in fish species using RFLPs have commonly found a few dominant haplotypes and a larger number of rare haplotypes, which are their mutational derivatives (e.g., Billington and Hebert 1991). A similar situation was found in Lake Winnipeg, where two haplotypes (1 and 3) made up 96% all walleye sampled. A similar predominance of one or two haplotypes has occurred in other studies. McParland et al. (1999) surveyed 1009 walleye (with six restriction enzymes) from the Lake Huron – Lake Erie corridor, resolving nine haplotypes in total, two of which accounted for 90% of all fish sampled. Gatt et al. (2002) surveyed 433 walleye (with eight restriction enzymes) from Lake Huron and culture ponds, resolving nine haplotypes, two of which accounted for 84% of all samples. A similar study on Lake Erie with 449 walleye resolved 14 haplotypes, again with two accounting for 90% of all fish sampled (Gatt et al. 2003).

The index (h) of Nei and Tajima (1981) for quantification of haplotype diversity standardizes for sample number, allowing comparisons to be drawn across studies. Values for haplotype diversity (h) in Lake Winnipeg as found by this study, using one restriction enzyme, ranged from 0.3030 to 0.6444, with a value of 0.5173 for Lake Winnipeg as a whole. These values are comparable to those for walleye in other systems.

Spawning populations of walleye in the Lake Erie – Lake Huron corridor had values of h ranging from 0.057 to 0.626, with an average of 0.4465 for eight spawning locations (McParland et al. 1999). Gatt et al. (2002) compared walleye from spawning sites in eastern Lake Huron to pond-reared walleye used for stocking. They considered walleye from Eastern Georgian Bay to have low haplotype diversity (0.275 – 0.509) compared to the North Channel region of Lake Huron (0.661 – 0.781). Cultured fish had the lowest values of h , ranging from 0.181 to 0.451 (mean 0.288). Another study looking at the contribution of various spawning stocks to the commercial and recreational walleye fisheries in eastern Lake Erie found haplotype diversity to be quite high, ranging from 0.72 to 0.94 (Gatt et al. 2003). While some of the values found in the current study are lower than those found in eastern Lake Erie, the mean for Lake Winnipeg is comparable to that found in Lake Huron and the Lake Erie – Lake Huron corridor. This indicates that genetic diversity within Lake Winnipeg is comparable to that found in other large lake walleye populations experiencing similar pressures (e.g., commercial fishing, invasive species and eutrophication).

No population structure was resolved in Lake Winnipeg walleye using the five control region haplotypes detected with the PCR-RFLP assay in this study. Of these five haplotypes, four (haplotypes 1 – 4) were found in Lake Winnipeg walleye, and two (haplotypes 1 and 3) accounted for over 96% of the Lake Winnipeg fish surveyed. There were no significant differences in relative frequency of the two dominant haplotypes within Lake Winnipeg. This indicates uniform dispersal of the two types, with no support for strong philopatric behaviour in female walleye. Distribution or frequency of the haplotypes in Lake Winnipeg did not resolve any population structure between

sampling locations. Haplotype frequencies at the two hatchery locations (Falcon Creek and Swan Creek) were similar to those in the north and south basins of Lake Winnipeg, with observed frequencies of 0.36 – 0.43 and 0.56 – 0.64 for haplotypes 1 and 3, respectively. While the similar frequencies observed may indicate some degree of success experienced by stocking efforts from Falcon Creek and Swan Creek into Lake Winnipeg, or a lack of significant differences between Lake Winnipeg and hatchery populations to begin with, the low numbers of individuals surveyed (Table 10) do not allow any firm conclusions.

Of the five walleye haplotypes resolved in this study, three (haplotypes 1, 3 and 5) correspond to the three major haplotypes reported in previous studies across the North American range of walleye (Ward et al. 1989, Billington et al. 1992., Gatt et al. 2000). Haplotype 1 represents individuals associated with haplotype C (Missourian refugium), haplotype 3 with haplotype B (Mississippi refugium) and haplotype 5 with haplotype A (Atlantic refugium). In this study, haplotype five was found only in the Bay of Quinte, which is consistent with the distribution of haplotype A (Eastern Great Lakes Region). Haplotypes 1 and 3 were both present at all other walleye sampling sites (with the exception of an absence of haplotype 1 in the Bay of Quinte samples). Previous studies (Ward et al. 1989 and Billington et al. 1992) included limited numbers of walleye from Manitoba, all of which were found to possess haplotype B or C (with haplotype C occurring at higher frequencies). This study also found haplotype C at slightly higher frequencies in all Lake Winnipeg samples (56%). The increased numbers of walleye assessed in this study detected an additional two haplotypes in Lake Winnipeg, although in very small numbers (< 3% of fish).

If Lake Winnipeg was colonized by fish from a single glacial refugium, we would expect to find only one predominant haplotype in Lake Winnipeg walleye. The presence of two haplotypes occurring at near equal frequencies indicates that Lake Winnipeg may have been colonized by walleye residing in two different glacial refugia. Ward et al. (1989) found haplotype C to predominate in walleye from South Indian Lake, MB and western Lake Superior, and postulated the colonization of these areas from the Mississippi and Missourian glacial refuges. Sauger utilized both the Mississippi and Missourian refugium (Crossman and McAllister 1986) during the Wisconsinan glacial period, and it appears likely that walleye also utilized both refugia.

Alternatively, it could be that haplotypes B and C represent repeated colonizations from a single refugium. Ward et al. (1989) found haplotypes B and C to be separated by only a single restriction site difference, while clonal groups A and B were separated by a minimum of two site changes. High genetic similarity between haplotypes indicates a more recent common ancestral haplotype, so it may be that haplotypes B and C represent recently diverged groups originating from repeated colonizations of walleye from the Mississippi refugium. The three haplotypes in this study (1, 3, 5) that correspond with the three refugia all differed from each other by a single restriction site. The percent sequence divergence between them is also similar, and average comparisons between haplotypes range from 1.06 to 1.09%. Typically, the overall rate of mtDNA sequence divergence has been estimated to be 2% per million years (Brown et al. 1979), a value which has been used to estimate divergence times in a variety of fish taxa (see review by Billington and Hebert 1991). The similar divergence between the three main haplotypes

may support the theory that Lake Winnipeg was colonized by two glacial refugia, as we would expect a lower divergence between haplotypes 1 and 3 if they had originated from the same refugium.

In conclusion, this study utilized PCR-RFLP assays on a portion of the control region to detect five walleye haplotypes in 193 individuals from Lake Winnipeg and surrounding areas. Haplotype diversity was similar to that found in other studies in the Laurentian Great Lakes area. The lack of population structure found in Lake Winnipeg indicates a uniform dispersal of the two main haplotypes, with no indication of strong philopatric behaviour in females. The lack of finescale population structure resolved using this method supports the theory that microsatellites are generally able to provide greater resolution in detecting population structure. Three of the mtDNA haplotypes resolved in this study corresponded to haplotypes previously described as representing three glacial refugia (Ward et al. 1989, Billington et al. 1992, Gatt et al. 2000), with haplotype 1 and 3 (corresponding to haplotypes C and B from Ward et al. 1989, and Billington et al. 1992), thought to represent the Missouri and Mississippi refugia, respectively, and haplotype 5 (corresponding to haplotype A from Ward et al. 1989, and Billington et al. 1992), representing the Atlantic refugium. The geographic distribution of the three haplotypes in this study also corresponded to that found in previous studies. Divergence times between the three haplotypes indicate that Lake Winnipeg was most likely colonized by walleye from both the Missouri and Mississippi refugia.

Table 8: Haplotype designations, fragment sizes and variable nucleotide positions (numbered vertically) in a 715 bp fragment in the control region of walleye from Lake Winnipeg and surrounding areas. Dots indicate the same nucleotide as in haplotype 1, dashes indicate a deletion. Shaded areas indicate the presence of a variable nucleotide in the *Tsp45I* recognition sequence. An additional recognition sequence was found 74 nucleotides before the sequence included in this table. This recognition sequence was found in only one individual (WIR1), the sole representative of haplotype 4. Additional sequence (upstream of that included in this table, where the additional recognition sequence is found) was not obtained for all individuals sequenced, and so was not included in this table. Sauger were not cut with the enzyme *Tsp45I* (haplotype 0), and are not included in this table. Haplotypes (mtDNA and RFLP) from Gatt et al. (2000) have been assigned to one of the haplotypes found by this study (with the exception of four haplotypes (L, Q and T that were not cut with *Tsp45I* used in this study, and N, which formed a new haplotype (fragment sizes 419, 241, 55)). The three RFLP haplotypes correspond to three possible glacial refugia: A – Atlantic, B – Mississippi, C – Missouri.

Haplotype	Fragment Sizes	5	6	9	10	11	15	16	17	20	21	22	28	36	69	77	95	132	169	194	202	204	205	209	210
1	419, 187, 109	T	T	C	G	C	-	T	T	C	G	C	G	G	-	A	T	A	G	T	G	G	T	A	C
		.	.	.	.	.	A	.	.	.	.	.	A		.	.		.	.	.		.	.	.	.
		.	.	.	.	.	.	.	.	.	.	.	.		.	.		.	A	.		.	.	.	.
		.	.	.	.	.	.	.	.	.	.	.	.		.	.		.	.	.		.	.	.	.
		.	.	.	.	.	.	.	.	.	.	.	.		.	.		.	.	.		.	.	.	.
		.	.	.	.	.	.	.	.	.	.	.	.		.	.		.	.	.		.	.	.	.
2	364, 187, 109, 55	.	.	.	.	.	.	.	.	.	.	.	.		.	.		.	.	.	T	A	C	.	T
		.	.	.	.	.	.	.	.	.	.	.	.		.	.		.	.	A	T	A	C	.	T
3	419, 296	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.		G	A	.		.	.	G	.
		.	.	.	.	.	.	.	.	.	.	.	.	C	.	G		.	.	.		.	.	.	.
		.	.	.	.	.	.	.	.	.	.	.	.	C	.	.		G	A	.		.	.	G	.
		.	.	.	.	.	.	.	.	.	.	.	.	C	.	.		G	A	.		.	.	G	.
4	419, 110, 109, 77	C	A	T	A	A	A	C	A	T	A	A	A		A	.		.	.	.		.	.	.	.
5	419, 187, 55, 54	.	.	.	.	.	.	.	.	.	.	.	.		.	.	C	G	A	.		.	.	G	.
		.	.	.	.	.	.	.	.	.	.	.	.		.	.	C	G	A	.		.	.	G	.
		.	.	.	.	.	.	.	.	.	.	.	.		.	.	C	G	A	.		.	.	G	.
		.	.	.	.	.	.	.	.	.	.	.	.		.	.	C	G	A	.		.	.	G	.

Table 8: Continued.

213	351	369	373	382	383	405	455	459	461	462	463	465	466	468	470	471	473	479	487	Gatt's Haplotypes	Glaial Refugium
G	C	G	A	A	-	A	G	T	A	G	T	A	T	C	C	T	T	A	C	C and G	C Missouri
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	A	T	.		
.	.	.	.	.	.	.	.	.	.	A	.	C	.	.	.	.	.	.	.		
.	.	C	.	.	.	.	.	A	C	.	G	.	.	.	-	A	.	.	.		NONE
T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.		
.	T	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	D-F, H-K, M,O,P,S	B Mississippi
.	.	.	T	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.		
.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T		
.	T	.	.	.	.	T	A	.	.	.	.	.	.	.	.	.	.	.	.		
.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	NONE	A Atlantic
.	.	.	.	T	A	.	A	.	.	.	.	.	.	A	.	.	.	.	.	A,B,R	
.	.	.	.	.	.	.	A	A	.	A	C	.	G	.	.	.	-	.	.		
.	.	.	.	.	.	.	A	A	.	A	.	C	G	.	.	.	-	.	.		
.	.	.	.	.	C	.	A	.	.	A	.	.	.	.	.	.	.	.	.		

Table 9: Pairwise percent sequence divergence for a selected representative of each sequence type in this study. Corresponding RFLP haplotypes (1 – 5) and the individual from which the sequence was obtained (A – E) are also listed. Comparisons within a single RFLP haplotype are shaded.

	1-A	1-B	1-C	1-D	1-E	2-A	2-B	3-A	3-B	3-C	3-D	4-A	5-A	5-B	5-C	5-D
1-A		0.2	0.4	0.8	1.4	1.4	1.2	1.0	1.2	1.0	1.0	2.0	1.6	1.6	1.0	1.2
1-B			0.2	0.6	1.2	1.2	1.0	1.2	1.4	1.2	0.8	1.8	1.8	1.8	1.2	1.4
1-C				0.8	1.4	1.4	1.2	1.4	1.6	1.4	1.0	2.0	2.0	2.0	1.4	1.6
1-D					1.4	1.8	1.6	1.8	2.0	1.8	1.4	2.0	2.0	1.6	1.4	2.0
1-E						2.4	2.2	2.4	2.6	2.4	2.0	2.6	2.4	2.6	2.4	2.6
2-A							0.2	2.4	2.6	2.4	2.0	3.0	3.0	3.0	2.4	2.6
2-B								2.2	2.4	2.2	1.8	2.8	2.8	2.8	2.2	2.4
3-A									0.6	0.4	1.6	3.0	1.8	1.8	1.2	1.4
3-B										0.2	1.4	3.2	1.6	1.6	1.0	1.2
3-C											1.2	3.0	1.4	1.4	0.8	1.0
3-D												2.6	2.2	2.2	1.6	1.8
4-A													3.6	3.6	3.0	3.2
5-A														0.4	0.6	1.2
5-B															0.6	1.2
5-C																0.6
5-D																

Table 10: Number of samples from each sampling location digested using *Tsp45I*, resulting haplotypes and haplotype diversity (h). Sampling locations are represented as follows: WRR – Red River, WNC – Netley Creek, WIR – Icelandic River, WHB – Hecla Bar, JHC – Jackhead Creek, WSB – Sturgeon Bay, WGR – Grand Rapids, BBR – Big Black River, POP – Poplar River, BER – Berens River, BLV – Bloodvein River, WWR – Winnipeg River, MSC – Swan Creek, FC – Falcon Creek, PDB – Pointe Du Bois, LOW – Lake of the Woods, PL – Paint Lake, NR – Nelson River, BQ – Bay of Quinte, BSR – Black Sturgeon River, PDBS – Pointe Du Bois Sauger, SBLWPG – south basin Lake Winnipeg, NBLWPG – north basin Lake Winnipeg, LWPG – Lake Winnipeg.

Sample	N	Haplotype						h
		0	1	2	3	4	5	
WRR	10	0	3	0	7	0	0	0.4667
WNC	12	0	5	1	6	0	0	0.6212
WIR	11	0	5	0	5	1	0	0.6364
WHB	10	0	4	0	6	0	0	0.5333
JHC	9	0	6	0	3	0	0	0.5000
WSB	12	0	2	0	10	0	0	0.3030
WGR	9	0	5	0	4	0	0	0.5556
BBR	10	0	5	0	5	0	0	0.5556
POP	10	0	3	0	7	0	0	0.4667
BER	10	0	4	0	6	0	0	0.5333
BLV	10	0	5	1	4	0	0	0.6444
WWR	10	0	4	0	6	0	0	0.5333
MSC	11	0	4	0	7	0	0	0.5091
FC	10	0	4	0	6	0	0	0.5333
PDB	6	0	4	0	2	0	0	0.5333
LOW	10	0	2	0	8	0	0	0.3556
PL	10	0	8	0	2	0	0	0.3556
NR	9	0	4	0	5	0	0	0.5556
BQ	6	0	0	0	2	0	4	0.5333
BSR	8	0	4	0	4	0	0	0.5714
PDBS	5	5	0	0	0	0	0	0.0000
SBLWPG	53	0	21	1	30	1	0	0.5319
NBLWPG	70	0	30	1	39	0	0	0.5130
LWPG	123	0	51	2	69	1	0	0.5173
TOTAL	198	5	81	2	105	1	4	0.5530

Table 11: Pairwise estimations of differentiation between sampling locations as measured with an exact test, using data generated with a PCR-RFLP (using *Tsp45I*) of a 715 bp segment of control region DNA in walleye. Comparisons with Lake Winnipeg samples pooled are above the diagonal, and comparisons with Lake Winnipeg sites individually are below the diagonal. Significant divergence between groups pooled, after Bonferroni correction, $p < 0.05$, is represented by an asterisk. Sampling locations are represented as follows: LWPG – Lake Winnipeg, WRR – Red River, WNC – Netley Creek, WIR – Icelandic River, WHB – Hecla Bar, JHC – Jackhead Creek, WSB – Sturgeon Bay, WGR – Grand Rapids, BBR – Big Black River, POP – Poplar River, BER – Berens River, BLV – Bloodvein River, WWR – Winnipeg River, MSC – Swan Creek, PDB – Pointe Du Bois, FC – Falcon Creek, LOW – Lake of the Woods, PL – Paint Lake, NR – Nelson River, BQ – Bay of Quinte, BSR – Black Sturgeon River, PDBS – Pointe Du Bois Sauer.

	WRR	WNC	WIR	WHB	JHC	WSB	WGR	BBR	POP	BER	BLV	WWR	MSC	PDB	FC	LOW	PL	NR	BQ	BSR
WRR																				
WNC	0.8199										LWPG		0.8227	0.4907	1.0000	0.3444	0.1666	1.0000	0.0000*	1.0000
WIR	0.5125	1.0000									MSC			0.3334	1.0000	0.6353	0.0824	1.0000	0.0138	0.6564
WHB	1.0000	1.0000	1.0000								PDB				0.6036	0.1224	0.6046	0.6065	0.0369	0.6296
JHC	0.1845	0.5242	0.8036	0.3763							FC					0.6323	0.1689	1.0000	0.0130	1.0000
WSB	0.6231	0.1903	0.1096	0.3553	0.0316						LOW									
WGR	0.3714	1.0000	1.0000	0.6514	1.0000	0.1526					PL									
BBR	0.6541	1.0000	1.0000	1.0000	0.6565	0.1618	1.0000				NR									
POP	1.0000	0.8120	0.4985	1.0000	0.1785	0.6235	0.3627	0.6548			BQ									
BER	1.0000	1.0000	1.0000	1.0000	0.3640	0.3424	0.6603	1.0000	1.0000		BSR									
BLV	0.3609	1.0000	1.0000	0.6563	1.0000	0.1117	1.0000	1.0000	0.3649	0.6631										
WWR	1.0000	1.0000	1.0000	1.0000	0.3604	0.3471	0.6555	1.0000	1.0000	1.0000	0.6419									
MSC	1.0000	1.0000	0.6545	1.0000	0.3631	0.3688	0.6488	0.6790	1.0000	1.0000	0.5182	1.0000								
PDB	0.2966	0.7456	0.7561	0.6147	1.0000	0.1037	1.0000	0.6251	0.3115	0.6086	1.0000	0.6051	0.3234							
FC	1.0000	1.0000	1.0000	1.0000	0.3728	0.3520	0.6497	1.0000	1.0000	1.0000	0.6621	1.0000	1.0000	0.6153						
LOW	1.0000	0.2636	0.2501	0.6263	0.0661	1.0000	0.1773	0.3519	1.0000	0.6310	0.1793	0.6275	0.6269	0.1237	0.6290					
PL	0.0664	0.1303	0.2672	0.1806	0.6336	0.0086	0.3441	0.3498	0.0724	0.1648	0.3519	0.1622	0.0770	0.6005	0.1684	0.0253				
NR	0.6494	1.0000	1.0000	1.0000	0.6399	0.3424	1.0000	1.0000	0.6556	1.0000	1.0000	1.0000	1.0000	0.6020	1.0000	0.3496	0.1659			
BQ	0.0100	0.0061	0.0084	0.0145	0.0048	0.0068	0.0056	0.0087	0.0101	0.0122	0.0073	0.0123	0.0131	0.0394	0.0125	0.0143	0.0024	0.0141		
BSR	0.6307	1.0000	1.0000	1.0000	0.6367	0.1663	1.0000	1.0000	0.6266	1.0000	1.0000	1.0000	0.6584	0.6345	1.0000	0.3121	0.3317	1.0000	0.0159	

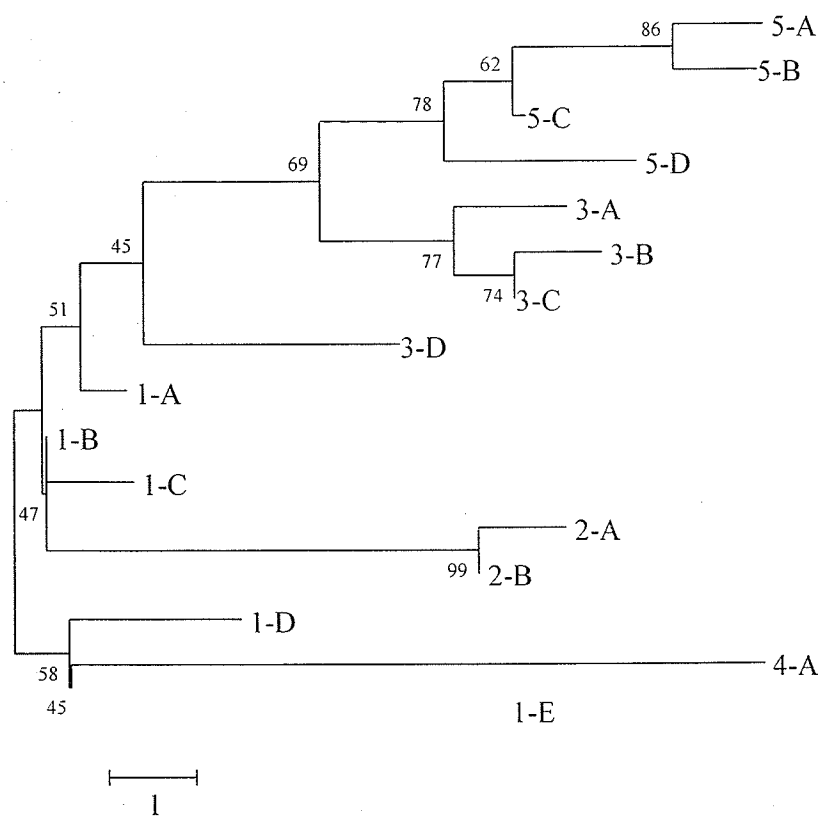


Figure 6: Neighbour Joining phenogram of walleye sequences (a single representative of each sequence type), resolved into five walleye mtDNA RFLP haplotypes (1 – 5) and individuals (A – E). Sequence data was bootstrapped with replacement and 500 replicates.

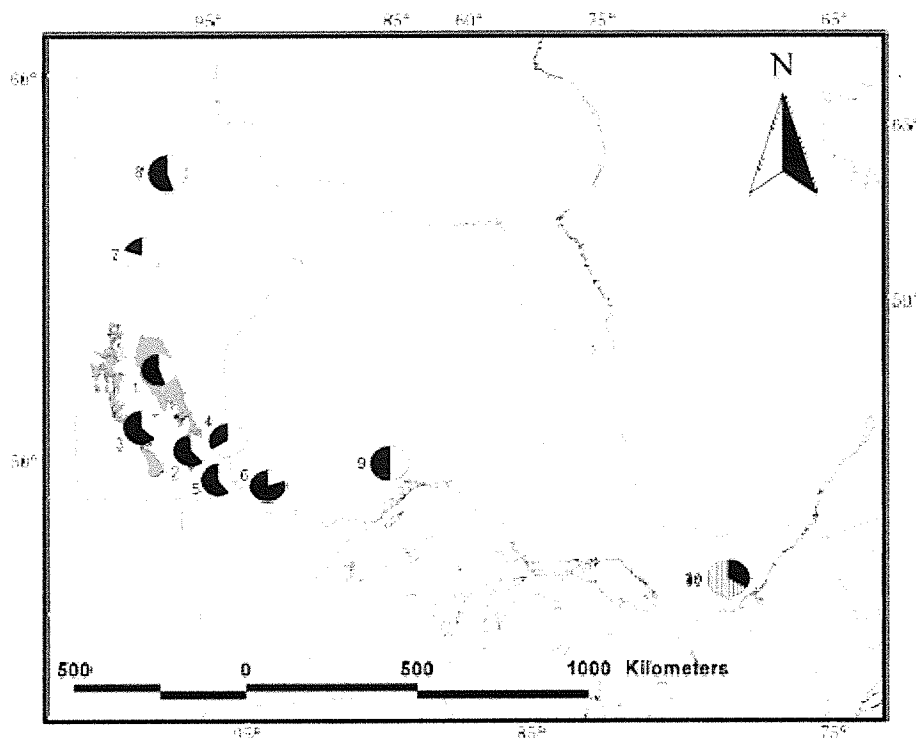


Figure 6: A map of the sampling area depicting the walleye RFLP haplotypes as determined in this study. Numbers represent sampling locations as follows: 1 - north basin Lake Winnipeg, 2 - south basin Lake Winnipeg, 3 - Swan Creek, 4 - Pointe Du Bois, 5 - Falcon Creek, 6 - Lake of the Woods, 7 - Paint Lake, 8 - Nelson River, 9 - Black Sturgeon River and 10 - Bay of Quinte. Black shading represents haplotype 3 (corresponding to the Mississippi refugium), white shading represents haplotype 1 (corresponding to the Missouri refugium) and bars represent haplotype 5 (corresponding to the Atlantic refugium). Haplotype 2 is found in one individual in the north basin, and haplotypes 2 and 4 are found in 1 individual each in the south basin.

GENERAL DISCUSSION

Overall genetic variation found in Lake Winnipeg walleye, using both mitochondrial and nuclear markers, is comparable to that found in other walleye and fish populations.

Genetic diversity can also be measured in terms of heterozygosity. In randomly mating diploid organisms, H_o (observed heterozygosity, microsatellite data) and h (haplotype diversity, RFLP data) are comparable as measures of genetic diversity (Nei and Tajima 1981). Average H_o for Lake Winnipeg was 0.66, and h was 0.52, values which are comparable to each other as well as values found in other studies in the Great Lakes ($H_o = 0.686 - 0.735$ (Stepien et al. 2009), $h = 0.4465 - 0.828$ (McParland et al. 1999, Gatt et al. 2003)). Two dominant RFLP haplotypes were found in Lake Winnipeg, which correspond to haplotypes previously described as originating from the Mississippi and Missouri glacial refugia, as well as haplotypes previously assigned to walleye from Lake Winnipeg and other locations in Manitoba (Ward et al. 1989, Billington et al. 1992, Gatt et al. 2000). Percent sequence divergence between the three main haplotypes (1, 3 and 5) in this study averaged 1.08%, and sequences for haplotypes 1 and 3 (corresponding to haplotypes C and B; Ward et al. 1989, and Billington et al. 1992, Gatt et al. 2000) diverged by 1.09%. Sequence divergence calculated for the same region using Great Lakes sequence data from Gatt et al. (2000) was lower, with an average percent sequence divergence of 0.41%, and haplotypes B and C differed by 0.40%.

The level of genetic diversity found in a system is typically influenced by the evolutionary history of a species, the population history of a species, and the level of environmental heterogeneity (Booy et al. 2000). A previous study assessed genetic

diversity in 46 walleye populations across Ontario (11 microsatellite loci), and found strong correlations between genetic diversity and lake parameters such as lake surface area, growing degree days and number of species (Cena et al. 2006). The similar values of heterozygosity in walleye as determined by microsatellites and mtDNA PCR-RFLPs indicate levels of genetic diversity on a par with those found in the Laurentian Great Lakes, while sequence data indicate Lake Winnipeg walleye show greater genetic diversity than those in the Laurentian Great Lakes. As the Laurentian Great Lakes system formed 9 000 to 14 000 years ago (Bailey and Smith 1981), in comparison to Lake Winnipeg, which began forming 7 700 years ago (Matile et al. 1996), the similar genetic diversity found in the two systems contradicts the longer evolutionary history of the Laurentian system. Allozyme work done on lake whitefish in Lake Winnipeg found values of H_o ranging from 0.106 to 0.148 (Mavros 1992). These values are on the high end of the scale seen in salmonids (0.015 – 0.14), and are also higher than values seen in lake whitefish in the Great Lakes (Imhof 1980, Casselman et al. 1981, Ihssen et al. 1981b). Mavros (1992) hypothesizes this to be a result either of increased environmental heterogeneity found in the outlet lake systems in Lake Winnipeg and Little Playgreen Lake, which are more complex than those found in simple large lakes, or simply that lake whitefish in Lake Winnipeg have higher fitness and greater genetic diversity than those in the Great Lakes. It is possible that environmental heterogeneity in Lake Winnipeg has resulted in increased genetic diversity in Lake Winnipeg walleye. The diverse phenotypes of walleye found in Lake Winnipeg (i.e. colour, body morph, life history characteristics) or the stocking history of the lake may have also played a role. It is also possible that declines of walleye stocks in the Laurentian Great Lakes due to fishing

pressure and eutrophication have caused a slight population bottleneck, decreasing the overall diversity.

Both nuclear and microsatellite markers resolved little to no population structure in Lake Winnipeg, with no evidence of reproductive isolation among spawning locations. If significant genetic differentiation among sites was found, it would indicate a reduction of, or barrier to, gene flow among spawning locations. The fact that the nine microsatellite loci used in this study were successfully able to distinguish Lake Winnipeg walleye from walleye sampled in other Manitoba locations (Paint Lake and northern Nelson River), as well as Lake of the Woods ON and the Great Lakes, indicates that the lack of genetic differentiation detected within Lake Winnipeg is not a result of poor resolution by microsatellite markers. This lack of differentiation has several possible interpretations (as discussed in Chapter 1). These include a population approaching panmixia (a genetically homogenous population with no homing instincts), some degree of philopatry but sufficient straying between sites to obscure population structure, or site fidelity of individuals to a site other than their natal site. Other potentially confounding influences could include the type of marker used (neutral vs. functional loci), or the long term effects of stocking (discussed below). Time is required for an accumulation of genetic differentiation between spawning sites; therefore other methods that can determine whether contemporary gene flow is occurring may help differentiate between these interpretations.

Lake Winnipeg has received supplementary stocking over the years. While records on numbers and origins of stocked fry are scarce, the majority have been stocked from the Grand Rapids fish hatchery. A private hatchery based out of Riverton (Icelandic River) was in operation for a short time period, but has since ceased stocking. Slight genetic structure seen in F_{ST} values from Icelandic River walleye (Table 5) may reflect this stocking history, but more information on the numbers, sources and locations of walleye stocked at this location is needed before this can be explored further. Grand Rapids Hatchery obtains walleye fry from Swan Creek Hatchery on Lake Manitoba, Lunda (MB), which collects spawning stock from various creeks on Lake Manitoba. Spawning stock has also been collected from the Snow Lake Area. Swan Creek Hatchery has historically received large amounts of male gametes and fertilized female gametes from the Whiteshell fish hatchery on Falcon Creek. Records indicating rough numbers of gametes are available from 1994 onwards (Don Bilenduke, Manitoba Water Stewardship, pers. comm.), but there are no records of where these mixed origin fish were stocked. It is possible the large numbers of fish stocked into Lake Winnipeg (numbers ranging from 3 million to 26.5 million annually from 1970 – 1983 (Lysack 1986), and over 70 million larvae plus additional eggs at Grand Rapids in the past 12 years (Kroeker and MacDonell 1998, Kroeker 1999, MacDonell and Cooley 2000, Eddy and MacDonell 2000, MacDonell and Graveline 2002, MacDonell and Johnson 2003, Funk and MacDonell 2004, MacDonell and Funk 2005, MacDonell et al. 2006, Richardson and Neufeld 2007, Ambrose and MacDonell 2008, Ambrose and MacDonell 2009)) have partially obscured the native stock structure. The significantly different F_{ST} values reflecting slight genetic

structure between Grand Rapids walleye and all other Lake Winnipeg walleye (Table 5) may be due to this stocking.

The lack of differentiation found between walleye spawning locations using neutral markers does not necessarily mean there is no genetic basis to phenotypic differences observed. Colour variation has been documented in walleye, with the most common colour morph (yellow) possessing an olive green colouration above, fading to brassy yellow on the sides (Stewart and Watkinson 2004). “Greenbacks” are the most common alternate colour morph, and are typified by an emerald colouration of the dorsal surface and a lack of yellow pigment. They are thought to occur in Lake Winnipeg waters, comprising the majority of walleye entering the Red and Winnipeg rivers in late summer and fall (Stewart and Watkinson 2004). Pigment cells responsible for colouration patterns can be influenced by many things, including light, stress and turbidity (Price et al. 2008). While fish with a bright emerald colouration were caught in this study, no fish lacking yellow pigment were found in any of the samples taken in Lake Winnipeg, including fish collected from the Red River in the fall of 2008 (where greenbacks are typically targeted by anglers). The Red and Winnipeg rivers did not show significant genetic differences from most of the other sampling sites within Lake Winnipeg, with the exception of Grand Rapids and Icelandic River. No firm conclusions can be drawn on the status of greenback walleye within Lake Winnipeg, but the lack of genetic structure found within the lake suggests that the greenback phenotype is a result of intraspecific phenotypic variation (see General Introduction), and not restricted to a reproductively isolated stock. Greenback colouration may be the result of a diet or habitat shift in larger

individuals, as greenbacks are typically mature fish, which may be supported by the collection of 82 male greenback walleye below St. Andrews dam (Lockport, MB) in May 1986 (Stewart and Watkinson 2004). The fact that this collection was exclusively male could either indicate the beginning of the spawning run (males typically arrive at the spawning grounds first, Scott and Crossman 1973) or that “greenback” colouration is a physiological trait expressed in large fish (or large males exclusively) due to a shift in diet or habitat. However, it is possible that there is a genetic basis for greenback colouration, which could be resolved using functional loci (see below).

This study did not specifically try to correlate phenotypic differences with genotype, but was limited to quantifying gene flow among locations, many of which showed phenotypic differences (e.g., life history characteristics between north and south basins, colour differences or body morph differences). The results suggest that the phenotypic differences found within Lake Winnipeg walleye do not occur within reproductively isolated stocks. A lack of genetic differentiation between divergent morphotypes has been found in some species (e.g., steelhead and rainbow trout; Docker and Heath 2003, Olsen et al. 2006, Heath et al. 2008); however, genetic differentiation is found between morphotypes in others (e.g., dwarf and normal lake whitefish, *Coregonus clupeaformis*; Lu and Bernatchez 1999).

The lack of genetic population structure found in Lake Winnipeg walleye is mirrored in other species. Multiple studies examining genetic diversity of lake whitefish (*Coregonus clupeaformis*) in Lake Winnipeg and surrounding areas have also found very little

structure. Differences in frequencies of glycerol-3-phosphate dehydrogenase (G-3-PDH) were detected between fish from Lake St. Martin/Dauphin River (frequency of 0.77) and fish from the rest of Lake Winnipeg and Little Playgreen Lake (frequency of 0.46), reflecting the homing movement of some lake whitefish into spawning grounds in Lake St. Martin (Kristofferson 1978, Kristofferson and Clayton 1990). Differences between lake whitefish caught throughout the rest of Lake Winnipeg were not found. Allozymes (36 loci, eight of which were informative) were successful in differentiating between lake whitefish in northern Lake Winnipeg and Little Playgreen Lake (Mavros 1992), but not between other sampling sites within Lake Winnipeg. Analysis of nucleotide sequence from 312 base pairs in the control region of mtDNA also found very little variation within lake whitefish captured in Little Playgreen Lake and surrounding areas. While a total of eight haplotypes were found, 222 of 234 fish examined possessed a single haplotype (Bodaly et al. 1996).

While little genetic population structure has been resolved for lake whitefish in and around Lake Winnipeg, other techniques have proven more successful. A study looking at morphometric and meristic differences in Lake Winnipeg lake whitefish resulted in five divisions: Lake St. Martin/Dauphin River, Traverse Bay, Berens/Poplar/Big Black Rivers, Grand Rapids and Little Playgreen Lake (Kristofferson 1978, Kristofferson and Clayton 1990). A second study by Mavros (1992) used morphology/meristics and isotope differences to resolve differences between lake whitefish from five sites in Lake Winnipeg (Traverse Bay, Grand Rapids, Little Playgreen Lake, Berens River and Big Black River). Fish were successfully differentiated (with an accuracy of 89.8 – 95.7%) on the basis of morphology and meristics, with the highest discrimination resulting from

measurements of head depth, body depth, preorbital length, postorbital length and trunk length (Mavros 1992). Preliminary work using stable isotopes (C, S and N) was successful in differentiating between lake whitefish caught from Berens River, Big Black River and Little Playgreen Lake using one or more isotopes; Traverse Bay and Grand Rapids samples showed some overlap in all three elements (Mavros 1992). Previous tagging studies of lake whitefish on Lake Winnipeg also indicated some degree of population structure within the lake (Kennedy 1954, Pollard 1973).

The detection of population structure in Lake Winnipeg lake whitefish through physical or phenotypic differences (without detectable genetic differences) is comparable to the lack of genetic differences found in Lake Winnipeg walleye, despite the presence of phenotypic differences. Phenotypic differences in colouration, body shape and morph and life history characteristics in walleye are known to occur among locations in Lake Winnipeg (e.g., north vs. south basins), but there was no evidence of reproductive isolation among locations using nuclear DNA microsatellites or PCR-RFLP of mtDNA in this study. Significant genetic differentiation among locations would be indicative of barriers to gene flow; however, the lack of such genetic differences found in this study has several possible interpretations (approaching panmixia, some philopatry with sufficient straying to obscure population structure, fidelity to a non-natal site, effects of stocking or recent shared ancestry). There have been many studies where stock separation determined by tagging, morphometrics, population characteristics or parasite load could not be confirmed with genetic measures (cod and herring, Smith et al. 1990; Atlantic mackeral and Atlantic bluefin tuna, Brown et al. 1987). While differences

indicate genetic isolation and population structure, a lack of differences cannot be simply interpreted, and do not prove a lack of genetic identity. Other methods (such as the use of functional loci, tagging studies or otolith microchemistry) may be able to determine if contemporary gene flow is occurring.

The low level of genetic population structure found in Lake Winnipeg walleye does suggest gene flow, but is not conclusive evidence of no population structure, or that there is no genetic basis to the different phenotypes observed in the lake (i.e. greenbacks, life history characteristics, dwarf walleye). Neutral markers (i.e. those under low selection pressure), such as nuclear microsatellites, have been successful in detecting population structure in many other walleye systems, as well as in many other philopatric species (see General Introduction). The high mutation rates characteristic of microsatellites make them particularly useful for detecting recent reductions in gene flow with very high resolution. However, this resolution can be affected by gene flow between spawning groups caused by straying. Functional markers (genes under selection) are genes that encode traits under selection, and have been shown to be highly powerful in resolving population structure (e.g., Dixon et al. 1996, Heath et al. 2008). Within Lake Winnipeg, examination of genes involved in phenotypic traits such as growth, life history characteristics and body morphology (dwarf and normal type) may prove to be informative in detecting a genetic basis for the observed phenotypic differences. However, a large number of genes are most likely involved in such traits and these genes have not yet been identified. However, genes such as the Major Histocompatibility Complex (MHC), involved in the immune response to pathogens (Stet et al. 2003), are

highly polymorphic and have been found to be under strong selection (e.g., Heath et al. 2008). As a result, they are thought to diversify in response to selection pressure exerted by local pathogens, making them a powerful potential marker for resolution of population structure. The different conditions (pathogens, prey items, habitat and fishing pressure) between basins in Lake Winnipeg may result in different sets of MHC alleles in the walleye that spawn there. Walleye have four copies of the MHC class I genes (Fujiki et al. 2001), one copy of the MHC class II alpha gene and at least three copies of the MHC class II beta gene per individual (Dixon et al. unpublished, from Johnson et al. unpublished manuscript). While MHC genes have been used to distinguish walleye stocks (Krausse 2002), their high levels of diversity can result in differentiation at the level of individuals as opposed to stocks or populations (Johnson et al. 2002 unpublished). Assays have been used to assess differentiation in walleye MHC genes (e.g., Fujiki et al. 2001), but are a more time consuming methodology. The development of a PCR-RFLP (cf. Docker and Heath 2002, see above) to explore differentiation between walleye MHC alleles in Lake Winnipeg would allow genetic differences related to immune function to be assessed.

Non-genetic methods (such as tagging studies or otolith microchemistry) may also prove useful in detecting population structure in Lake Winnipeg walleye that was not detectable with microsatellite and mtDNA variation, particularly if the lack of resolved population structure is due partly to straying, or fidelity of walleye to a non-natal site. Tagging studies have proven effective in detecting population structure in other systems (rainbow smelt, Bradbury et al. 2008; Atlantic cod, Wright et al. 2006), but require large numbers

of tags deployed, as well as a large sampling effort to recapture sufficient numbers of tagged individuals. Tagging using passive tags such as floy tags or VIE batch tags would be an effective way to determine if walleye in Lake Winnipeg home to the same site to spawn each year, but the sampling effort required to tag and recapture sufficient numbers at each spawning site each year would be tremendous. Determining if walleye are returning to their natal site or an alternate site would also be difficult, as larval walleye would be too small to retain a tag through to adulthood.

Lake Winnipeg covers a large and varied landscape. The substantial chemical differences resulting from inflows in different parts of the lake are retained, resulting in markedly different biological, chemical and limnological characteristics in different areas of Lake Winnipeg (Brunskill 1980). Lake whitefish from different areas of the lake have been successfully differentiated on the basis of stable isotope analysis (Mavros 1992). If larval walleye reside in rivers for a sufficient amount of time to develop an otolith core, it is possible that the natal site of individuals could be identified. Comparison of isotope signatures from otolith cores and subsequent rings could determine if walleye are returning to their natal site or an alternate site to spawn. Hatchery cultured walleye have been successfully aged based on daily otolith increments for ages of 14 – 42 days (although large variations in estimated age occurred between readers and between sequential readings; Parrish et al. 1994), indicating that daily increments are of a sufficient size for aging, and perhaps elemental analysis. Daily otolith increments in hatchery reared larval European lake whitefish were found to be easily identifiable at temperatures of 8°C and higher, but more difficult to distinguish at temperatures of 4 or

6°C (Klink and Eckmann 1992). As it would only be necessary to sample the otolith core during the time larval walleye reside in the spawning site, it is possible that an elemental signature could be obtained.

In conclusion, this study successfully used nine microsatellite loci to resolve genetic differences between species (sauger and walleye) and between broadscale sampling sites (different lakes); however, genetic differences within Lake Winnipeg were limited. Similar resolution was obtained using mtDNA PCR-RFLPs, that is, descendants from two presumed glacial refugia were detected, but there were no finescale differences in haplotype frequencies. These results indicate Lake Winnipeg walleye are not strongly philopatric. Levels of genetic diversity within Lake Winnipeg, using both nuclear and mtDNA variation, are comparable to those found in walleye in other systems, indicating that levels of genetic diversity within the lake are sufficient to allow Lake Winnipeg walleye populations to adapt to local conditions and perturbations (Lande and Shannon 1996). Previous studies on lake whitefish in Lake Winnipeg also failed to resolve population structure using allozymes and mtDNA sequence, but were successful in differentiating spawning groups using morphology and meristics (Kristofferson 1978, Kristofferson and Clayton 1990, Mavros 1992, Bodaly et al. 1996). This indicates that the use of other markers, such as functional loci (e.g., MHC) or non-genetic methods (e.g., tagging studies or otolith microchemistry) may provide further insight into Lake Winnipeg walleye population structure, and allow us to determine if the Lake Winnipeg walleye population is weakly philopatric, not philopatric at all, or displays fidelity to a non-natal site. The nuclear and mitochondrial markers used in this study are unable to be

applied as stock specific tags for assessment of a mixed-stock walleye, due to the lack of genetic differentiation detected between spawning locations.

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APPENDIX 1: CALCULATION OF F STATISTICS

H_I = mean observed heterozygosity per individual within subpopulations

H_S = mean expected heterozygosity within random mating subpopulations

H_T = expected heterozygosity in random mating total population

Inbreeding coefficient – the mean reduction in heterozygosity of an individual due to non-random mating within a subpopulation

$$F_{IS} = (H_S - H_I) / H_S$$

Fixation index – the mean reduction in heterozygosity of a subpopulation (relative to the total population) due to genetic drift among subpopulations

$$F_{ST} = (H_T - H_S) / H_T$$

Overall fixation index – the mean reduction in heterozygosity of an individual relative to the total population

$$F_{IT} = (H_T - H_I) / H_T$$

The relationship between the three F-statistics is:

$$(1 - F_{IT}) = (1 - F_{IS})(1 - F_{ST})$$

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