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Genetic Population Structure of Walleye (*Sander vitreus*) in Northern Alberta and Application to Species Management

by

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Abstract

Genetic Population Structure of Walleye (*Sander vitreus*) in Northern Alberta and Application to Species Management

Walleye (*Sander vitreus*) is an economically valuable freshwater fish throughout North America. In Alberta, pressure from sport fishing and commercial fishing make effective management and protection of this species crucial to its sustainability. Walleye from 12 Alberta lakes were genetically characterized using 15 microsatellite markers. Each lake contained a genetically distinct walleye subpopulation within a larger population of the river basin in which the lake was situated. Differentiation between subpopulations varied (θ_{ST}=0.05 to 0.29). Patterns of genetic divergence aligned closely with the current hydro-geographical landscape, except where stocking events have occurred. Vicariance and natal philopatry are likely mechanisms maintaining the current genetic structure. The markers detected sufficient genetic variation between most subpopulations to assign an individual fish to a subpopulation of origin. The utility of genetic assignment was illustrated for stocking assessment and forensic enforcement. These genetic data will help to inform management decisions, monitor population status and enforce harvest restrictions for Alberta walleye.

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List of Acronyms

AFWFU	Alberta Fish & Wildlife Forensic Unit
AMOVA	Analysis of Molecular Variance
ASRD	Alberta Sustainable Resource Development
ATB	Lake Athabasca
BCK	Buck Lake
BIS	Bistcho Lake
BVR	Beaver Lake
CAL	Calling Lake
CLUMPP	Cluster Matching Permutation Program
DNA	Deoxyribonucleic Acid
ESU	Evolutionary Significant Unit
FCT	Fawcett Lake
FMIS	Fisheries Management Information System
FWIN	Fall Walleye Index Netting
HRT	Heart Lake
LLB	Lac La Biche
LSL	Lesser Slave Lake
MCMC	Monte Carlo Markov chain
MU	Management Unit
PCR	Polymerase Chain Reaction
PRS	Primrose Lake
RAPD	Random amplified polymorphic DNA
STR	Short tandem repeat
WAB	North Wabasca Lake
WFD	Winefred Lake
WLF	Wolf Lake

Chapter 1: Introduction

Walleye in Alberta

Walleye (*Sander vitreus*) is arguably the most popular sportfish in Alberta (Sullivan 2003) and is also valuable as a commercial resource. In 2005, sportfishing alone contributed an estimated $440 million to the Alberta economy with walleye dominating the catch and harvest province-wide (Park 2007). Walleye are a piscivorous member of the perch family whose natural range covers most of North America east of the Rocky Mountains (Nelson and Paetz 1992). Walleye is found in approximately 240 lakes and rivers throughout Alberta, covering most of the province (Berry 1995). Despite this large range walleye is not abundant in Alberta (Alberta Environmental Protection 1996). Alberta's low number of lakes and high number of active anglers, relative to other prairie provinces, subject the walleye populations to extremely high harvest pressure, resulting in a number of vulnerable and collapsed populations (Sullivan 2003). Alberta fisheries management has allocated many resources to the recovery and protection of walleye in Alberta, including restricted harvesting and extensive stocking. Management and protection of walleye in Alberta is central to keeping both the commercial and sport industries active and profitable. Knowledge regarding the genetic population structure of a species can be integrated into management plans for increased conservation of genetic diversity and detailed tracking of individual movement. The active management plan for walleye in Alberta does not currently incorporate genetic information. Genetic data for walleye populations in Alberta would be useful in evaluating and monitoring the stocking program, enforcing regulations, and creating strategies to conserve the natural genetic diversity.

Population Genetic Analysis

Population genetic analysis is a valuable tool in wildlife research that should be incorporated into species management. Molecular data can address questions concerning genetic diversity, breeding interactions, migratory patterns,

and population structure. Formerly, allozyme and mitochondrial DNA markers were used to elucidate population structure in walleye (Ward et al. 1989; Billington et al. 1992, 1996; Stepien and Faber 1998), but these markers do not give the resolution needed for detailed analysis. Thomas et al. (1999) used random amplified polymorphic DNA (RAPD) analysis to characterize the genetic variation in walleye populations of central Alberta. They correlated genetic information with the stocking history of specific lakes and also created a map of genetic distances between different populations. More recently, broad-scale population structure of walleye in northeastern North America has been characterized using short tandem repeat (STR) microsatellite markers (Stepien et al. 2009). Microsatellites are non-coding regions of nuclear DNA that have repeated simple sequences. Microsatellites are a more informative analytical tool for population genetic analysis because of their high variability. This in turn enables the use of more powerful statistical tests than could be applied with allozymes (Hansen et al. 2001) or RAPD analysis. Genetic population structure of walleye has been investigated in the Great Lakes (Strange and Stepien 2007; Stepien et al. 2009), Minnesota (Eldridge et al. 2002), Quebec (Dupont et al. 2007) and Ohio (White et al. 2005) using microsatellites. Microsatellites are also extremely valuable in a conservation context to determine the impact of stocked fish on wild populations and to show the degree to which current populations are descendants from stocked fish, the native population or immigrants (Hansen et al. 2001). Microsatellite DNA analysis is an appropriate method by which to determine walleye population structure in Alberta and will be useful for species conservation and management.

Genetic Data for Fisheries Management

Genetic variation is integral to determining evolutionary significant units (ESUs) and management units (MUs) for conservation of biological resources (Moritz 1999, 2002). An ESU is the minimal unit of conservation management (Ryder 1986), often a population or set of populations with a distinct evolutionary history. A MU is the ecological component that must be managed to conserve the

larger ESU (Moritz 1999). Molecular data can often be more informative than phenotypic information for defining ESUs because they provide information on population history and allows for inferences about geographical patterning (Moritz 1999). ESUs have been defined for Pacific salmon populations and are considered valuable for the conservation of this resource (McElhany et al. 2000).

Despite the fact that genetic information has been recognized as a key component for managing fisheries (Shaklee 1991), little genetic information is known about the walleye populations in Alberta. The active walleye hatchery and stocking program in Alberta can translocate millions of fry and fingerlings annually (Fisheries Management Information System (FMIS) database). The delineation of ESUs and MUs could be used to evaluate the potential impact of translocations on genetic diversity and conservation strategies. Genetic data could be used to assess and monitor the stocking events. Genetic information for Alberta walleye could be helpful in planning future management actions as well as assessing historical activities. Fisheries management could use knowledge regarding the genetic population structure of the species to assist in the conservation of Alberta walleye populations.

Forensic Application of Genetic Information

Molecular data can also be used for forensic enforcement of management regulations. DNA evidence is highly probative and is used extensively in the field of human forensics, but is also utilized in the investigation of fish and wildlife offences. Terrestrial animals have been the focus of much of the use of DNA in wildlife forensic cases, but it is also applicable to marine and freshwater fishes (Hansen et al. 2001). Traditionally, violations of fishing restrictions are difficult to identify after an offence has taken place. Genetic analysis can be used to identify an offence and link it to a suspect after the incident has occurred. Fishing restrictions in Alberta are usually species specific and directly linked to a specific body of water. Therefore, using the DNA profile to identify the body of water from which a fish was harvested would be useful for enforcement of fishing

regulations. Identification of the population of origin for an individual fish can be accomplished through genetic assignment tests. The probative power of assignment tests in a fisheries forensic context has been shown by Primmer et al. (2000), Withler et al. (2004) and McCusker et al. (2008). The utility of assignment tests for enforcement of fishing regulations of Alberta's walleye will depend on the genetic structure of the populations. The statistical power of the tests relies largely on the genetic differentiation between populations as well as the number of microsatellite markers and the size of the forensic database (Bernatchez and Duchesne 2000; Hansen et al. 2001). Molecular information can also be used forensically to determine the minimum number of individuals present and to match biological material from the site of an offence to a suspect. Having a baseline of molecular data for walleye populations in Alberta could prove useful for enforcement of regulations and prosecution of individuals who violate those restrictions.

Aims and Objectives

The aim of this project is to genetically characterize walleye populations in northern Alberta and illustrate the utility of the data for management and forensic applications. I will use genomic DNA, specifically short tandem repeat (STR) microsatellite markers, to determine the population structure of walleye from twelve lakes in Alberta. I expect that walleye populations from different lakes will be genetically distinct from each other and that the pattern of differentiation will align with geographic separation. I will investigate possible historical and contemporary mechanisms that may have generated and be maintaining the observed population structure. I will discuss how knowledge of the walleye genetic population structure and factors that contribute to it can be useful for defining management units and can aid in the preservation of natural genetic diversity. If the genetic differentiation between walleye populations is significant it could prove valuable for genetic assessment and monitoring of walleye population status. I anticipate that there will be sufficient differentiation between walleye populations that it will be possible to determine the lake of

origin of an individual fish from its DNA profile. I will use a walleye stocking case study and a forensic case to illustrate the potential applications of the genetic data and the possible benefits of incorporating genetic characterization of walleye population structure into the current management plan.

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Chapter 2: Genetic structure of walleye (*Sander vitreus*) in Alberta

Introduction

Genetic characterization of populations is valuable for species conservation and management. Genetic data can be used to estimate population abundance, monitor reintroductions, identify hybridization and resolve population structure (Smith and Wayne 1996). Assessing the genetic connectivity between populations is important in understanding patterns of diversity, migration and breeding. Genetic data are increasingly being used in conservation and management practices world-wide (Pemberton et al. 1996). For example, knowledge regarding the population genetic structure of a species can aid in defining management units, as shown for cod (*Gadus morhua*) (Hutchinson et al. 2001), beluga whales (*Delphinapterus leucas*) (Gladden 1999) and Pacific salmonid species (McElhany et al. 2000). Molecular analysis has also been recommended as a key conservation tool for monitoring trade and differentiating between legal and illegally harvested sturgeon and paddlefish (Acipenseriformes) (Waldman et al. 2008). As DNA analysis has become more discriminating and rapid, genetic analysis of large numbers of individuals in order to investigate population structure is readily available for almost any species. Microsatellites are the current molecular tool of choice when investigating population genetic structure as they are highly informative markers; their large variability is contained in short sequences. Genetic analysis has been especially useful in the area of fisheries conservation and management in identifying migration patterns, estimating population size and elucidating population structure.

Microsatellites have been used to study individual movement, evolution, life histories and population structure in many fish species. For example, microsatellites have been used to resolve the stock structure of Pacific salmon species for management purposes (Nelson et al. 2001; Beacham et al. 2003; Beacham et al. 2008; Narum et al. 2008). Araki et al. (2007) used microsatellites

and demographic data to estimate the number of breeders per year and the effective population size per generation for steelhead trout (*Oncorhynchus mykiss*) in the Hood River, Oregon (USA). The effect of supportive breeding programs on natural populations can also be assessed by microsatellite DNA analysis, as shown by Eldridge et al. (2009) and Hansen et al. (2000). Genetic data can be useful in identifying not only the current population structure of a species, but also the underlying contemporary and historical mechanisms that generate and maintain population differentiation.

There are many mechanisms that contribute to the genetic structure of freshwater fish populations. Walleye (*Sander vitreus*) populations in eastern North America exhibit varying levels of genetic differentiation across different habitats at both broad and fine scales (Eldridge et al. 2002; Dupont et al. 2007; Stepien et al. 2009). Broad-scale genetic patterning is chiefly shaped by historical factors, such as glaciation events, and extrinsic factors like geography and climate. Ward et al. (1989) and Billington (1996) showed that western walleye populations most likely originated from Missourian glacial refugia and are distinct from those of the Great Lakes region that recolonised from Atlantic and Mississippian refugia. Recolonizations from glacial refugia and subsequent geographic isolation have clearly shaped the current genetic diversity of eastern walleye populations. Fine-scale genetic patterns are more likely the results of intrinsic factors, such as migration, inbreeding and genetic drift (Strange and Stepien 2007). Natal philopatry is often a mechanism for maintaining population structure and this may create a complex substructure in large lakes or highly connected systems. It is widely accepted that most walleye display spawning site philopatry, foraging and overwintering in large, mixed-stock groups, and then returning to their natal site to spawn in the spring. This natal site fidelity has been hypothesized to have a genetic basis (Stepien and Faber 1998, Strange and Stepien 2007). Geographic isolation also contributes to the contemporary genetic population structure of walleye. As freshwater fish populations are more constrained by geography than many land mammals, these boundaries, as well as

life history traits and evolutionary mechanisms, will contribute to contemporary genetic population structure. Understanding how the genetic population structure relates to the landscape and is influenced by different factors is important in designing management plans that protect natural genetic diversity.

Walleye harvest, through both the sport and commercial fisheries, is a contributor to the Alberta economy; therefore, management and protection of the species is vital for keeping the populations healthy, the harvest sustainable, and the industry profitable. Walleye are the most popular sportfish in Alberta and are under intense harvest pressure throughout the province (Sullivan 2002). Walleye are thought to have populated Alberta lakes at the conclusion of the Pleistocene ice age, between 9000-13000 years ago, after migrating from a single Missourian glacial refugium (Nelson and Paetz 1992). Since that time, extrinsic factors such as change in lake levels and geographic isolation, and intrinsic factors like genetic mutations, selection and genetic drift could potentially have caused genetically distinct populations to evolve. Genetic tagging of individual fish and genetic monitoring of populations could reveal the connectivity between populations and the movement patterns of walleye. Currently, little is known about the movements of walleye throughout the waterways of the province. Radio tagging studies have found that walleye can move hundreds of kilometers along the Athabasca River in a matter of days (RAMP 1998), but it is unknown whether this is a common occurrence. If Alberta walleye exhibit natal philopatry but are highly vagile in non-reproductive times, management strategies may need to adapt in to more effectively protect spawning and foraging walleye populations. Genetic analysis could elucidate walleye movement as well as walleye population structure within Alberta waterways.

My study will characterize the genetic diversity and population structure of walleye in northern Alberta through analysis of microsatellite loci. I initially expect that the genetic diversity of walleye populations in Alberta may be lower than what has been reported for populations in eastern North America (Strange

and Stepien 2007, Stepien et al. 2009). Slower growth rates, recolonization from a single glacial refugium and historical overharvest of a number of populations are all factors that could reduce the natural genetic diversity. Based on previous studies of walleye populations (Thomas et al. 1999, Stepien et al. 2009). I also expect that walleye in Alberta will exhibit a hierarchical population structure in which the genetic differentiation will align with contemporary hydro-geographic features and that genetic distance will correlate with geographic separation. I will test the null hypothesis of panmixia among sample sites against the alternative hypothesis of genetic patterning resulting from geographic isolation. I intend for my results to provide a baseline for comparison with other walleye populations in Alberta and that the genetic data will act as a foundation for future forensic analysis. I will also examine the relevance of my findings for the management of walleye in Alberta.

Methods and Analysis

Sample Localities

I sampled twelve lakes from five major river basins in Alberta based on the walleye population status, management history, sample availability and geographic location (Figure 2-1, Table 2-1). Bistcho Lake is the most northern population and the only sample site in the Liard River basin. The walleye population in Bistcho Lake supports a small commercial fishery and is also used for brood stock in the hatchery program. Lake Athabasca is a large body of water that straddles the Alberta-Saskatchewan border and is the second most northern sample location. It is the catch basin of the largest river system in Alberta (the Athabasca River basin) and is also directly connected to the Peace River system. Lesser Slave, Calling, Fawcett, Heart and Winefred Lakes are the other lacustrine samples sites within the Athabasca River basin. Lesser Slave Lake walleye are also occasionally used as an egg source for the hatchery program. North Wabasca Lake is the only lake in this study located completely within the Peace River basin but it does have a direct connection to the Athabasca delta via the Peace River. There were three sample sites within the Beaver River basin; Primrose, Beaver

and Wolf Lakes. Primrose Lake walleye are also used as brood stock for the hatchery and stocking program. The final sample site was Buck Lake, which is located in the North Saskatchewan River basin and is the walleye population with the highest elevation in Alberta.

Sample Collection

Walleye samples from Lake Athabasca were obtained from a commercial fishery catch and the samples from North Wabasca Lake were collected by angling. All other samples were collected by fisheries biologists as part of Fall Walleye Index Netting (FWIN) sampling. Each sample consisted of a dried fin ray or operculum stored at room temperature. Samples from each lake represented a range of year classes and a mix of sexes.

DNA Profile Development

Genomic DNA was extracted from the dried fin tissue samples using the Kingfisher ML purification system (Thermo Electron Corp.) and the MagExtractor, nucleic acid purification kit (Toyobo Co.). Over 30 different primer sets, developed from walleye or a closely related species such as yellow perch (*Perca flavescens*), were identified from the literature (Borer et al. 1999; Wirth et al. 1999; LeClerc et al. 2000; Eldridge et al. 2002; Cena et al. 2006) as being potentially useful for genotyping Alberta walleye (Table 2-1). Of these loci, 17 have been organized into two multiplexed polymerase chain reactions (PCR). Multiplex 1 consisted of 9 loci (Svi33, Svi18, Svi16, SviL11, SviL8, SviL5, Pfla2, Svi17 and PflaL8) and multiplex 2 consisted of 8 loci (PflaL1, Svi14, Svi2, Svi9, Svi6, SviL7, Svi26 and SviL4). Amplification was carried out in a MJ research PTC-200 DNA Engine ® (MJ Research) using 25ul reactions. Each 25ul reaction contained 12.5ul of 2x QIAGEN Multiplex PCR Master Mix, 0.1 – 2.0uM concentration of each forward and reverse primer in the multiplex, 4ng of genomic DNA, and enough filtered, autoclaved, deionized water to make up the 25ul reaction volume. The PCR conditions used were a “hotstart” of 15 minutes at 95ºC, followed by 30 cycles of a 30s denaturation step (94ºC), a 1.5min annealing step (52ºC for multiplex 1 and 54ºC for multiplex 2), a 1min extension cycle

(72ºC), ending with a final 30min extension cycle (60ºC) and then holding at 22ºC until placed in storage at -20ºC.

The amplified DNA fragments were visualized using an ABI Prism® 310 Genetic Analyzer (Applied Biosystems). The fragments were diluted in Hi-Di™ formamide (Applied Biosystems) and a 400 times dilution of GeneScan® 400HD [ROX] size standard was added to each sample (Applied Biosystems). The data was collected using ABI Prism® 310 Data Collection v.2.0 software (Applied Biosystems). I assigned genotypes using Genemarker v1.85 software (SoftGenetics LLC). Genotypes were reviewed by a second analyst and ambiguous results were either repeated or not included in subsequent analyses.

Preliminary Analysis

I modeled the data set as each geographic sample location representing an individual, panmictic subpopulation within the larger population of the river basin. Each population, subpopulation and locus was tested for Hardy-Weinberg equilibrium and linkage disequilibrium using the Monte Carlo Markov chain (MCMC) method of GENEPOP 4.0.6 (Roussett 2007) with 10000 dememorizations of 3000 batches and 6000 iterations per batch. Non-sequential Bonferroni corrections (Sokal and Rohlf 1995) were applied to adjust the level of significance ($p<0.05$). Populations and subpopulations were also tested for the presence of null alleles using the method of Brookfield (1996) implemented by the program MICRO-CHECKER v.2.2.3 (van Oosterhout et al. 2004). I used the Excel Microsatellite Toolkit (Park 2001) to calculate allele frequencies and check for duplicate samples within each population and subpopulation. The inbreeding coefficients (F_{IS}) and (F_{IT}) and fixation index (F_{ST}) were calculated for each locus across all samples using the program FSTAT (Goudet 1995 2002).

Genetic Diversity and Divergence

Genetic diversity within populations and subpopulations was estimated by observed (H_o) and expected (H_e) heterozygosities, mean number of alleles per locus (M_{NA}), total number of alleles (N_A), inbreeding coefficient (F_{IS}), proportion

of private alleles (P_{PA}) and allelic richness (A_R). Allelic richness was corrected for the smallest sample size (N=43) and a private allele was defined as occurring in only one subpopulation and having a frequency greater than 0.01.

The degree of genetic divergence between subpopulations (F_{ST}) was estimated by $\theta_{ST,}$ (Weir and Cockerham 1984) since it has been shown to have better resolution in fine scale analysis of recently diverged populations than other estimators (Strange and Stepien 2007). The statistical significance of F_{ST} was calculated in FSTAT (Goudet 1995, 2002) with 1000 permutations.

Genetic Clustering and Population Structure

I examined isolation by geographical distance among all subpopulations by comparing genetic divergence ($\theta_{ST}/(1-\theta_{ST})$) with geographic distance (km), as measured by the shortest waterway connection between subpopulations as well as the direct overland distance. Statistical significance of the correlation was tested by a simple Mantel (1967) test with 100,000 MCMC permutations. In addition, I tested this correlation while controlling for the boundaries between river basins using a partial Mantel test with 100,000 MCMC permutations. I also used the partial Mantel test to investigate the relationship between genetic distance and river basin boundaries while controlling for geographic distances. The simple Mantel test was also applied to subpopulations only within the Athabasca River basin.

I evaluated the genetic relationships between populations and subpopulations using several approaches. First I tested for hierarchical partitioning of genetic variation between the Athabasca and Beaver River basins and among subpopulations within these basins using analysis of molecular variance (AMOVA; Excoffier et al. 1992, 2005). I also tested the partitioning of genetic variation among all subpopulations.

I used the Bayesian-based clustering program, STRUCTURE v2.2 (Pritchard et al. 2000), to determine the most likely number of genetic groups within the data set without any prior spatial information. Runs consisted of 500,000 burn-in cycles followed by 500,000 iterations. Ten replicates of K=1 to K=15 were performed, with K representing the number of genetic clusters. I determined the most likely value of K by examining the partitioning of individuals among clusters as well as the log likelihoods and posterior probability values of each run. I then evaluated the consistency among runs and combined all runs at the most likely value of K using CLUMPP (Jakobsson and Rosenberg 2007). The output was displayed in a graphical format using DISTRUCT (Rosenberg et al. 2002).

I also investigated the assignment of individuals to subpopulations using the frequency based method of Paetkau et al. (1995) implemented in GENECLASS2 (Piry et al. 2004). Missing alleles were assigned a frequency of 0.01 and results were compared to the partitioning of individuals by the Bayesian clustering algorithm.

Results

Preliminary Analysis

DNA profiles were analyzed for 1005 walleye from 12 lakes throughout north and central Alberta. Loci Svi14 and Svi16 showed significant linkage disequilibrium over all subpopulations (X^2=infinity, d.f.=28, $p<0.001$); therefore locus Svi16 was discarded from all further analysis. Locus Svi18 exhibited departures from Hardy-Weinberg expectations in a number of subpopulations and was removed from further analysis since the null allele frequencies estimated by MICRO-CHECKER ranged from 0.24 to 0.60. Once loci Svi16 and Svi18 were removed from analysis, all subpopulations conformed to expected Hardy-Weinberg equilibrium after Bonferroni correction ($p<0.05$). When all samples were grouped as a single panmictic population there was significant deviation from Hardy-Weinberg equilibrium after Bonferroni correction ($p<0.05$). I also

observed significant deviation from Hardy-Weinberg equilibrium ($p<0.05$) for the Athabasca River basin and Beaver River basin populations.

Diversity and Divergence

The 15 loci retained for analysis were all highly polymorphic with 5 to 29 alleles per locus (Table 2-2). Each locus was represented by 2 to 5 common alleles. I'm defining a common allele as having a frequency of occurrence greater than 10% across all observed alleles. Observed heterozygosity ranged among subpopulations from 0.470 (North Wabasca) to 0.666 (Primrose) (Table 2-1). Allelic richness also varied among subpopulations, from 4.71 in Bistcho Lake to 6.75 for Lake Athabasca. Buck Lake had the greatest proportion of private alleles (0.085), most of which were at locus Pfla2. Heart Lake and Bistcho Lake were the only two subpopulations that showed no private alleles.

Pairwise tests of genetic divergence using the F_{ST} estimator θ_{ST} of Weir and Cockerham (1984) were all significant ($p<0.01$) (Table 2-3). Buck Lake was the most divergent from other all other sample sites with pairwise comparisons from $\theta_{ST}=0.198$ to $\theta_{ST}=0.290$. On average, divergence within river basins was lower than comparisons between river basins. The lowest divergence was between Lake Athabasca and Lesser Slave Lake ($\theta_{ST}=0.051$). The global θ_{ST} estimate across all sites was 0.133.

Population Structure and Movement Patterns

Genetic divergence was positively correlated with geographic distance between subpopulations but was only significant for waterway distances ($r=0.789$, $p<0.001$) (Figure 2-2, Table 2-4). There was a significant relationship between genetic divergence and river basins when controlling for direct geographic distances ($r=-0.453$, $p<0.001$) but not when controlling for waterway distance ($r=0.103$, $p=0.269$). While genetic divergence and waterway distance were still positively correlated when examining only subpopulations within the Athabasca River basin, the association was not statistically significant ($r=0.261$, $p=0.235$) (Table 2-4).

Partitioning of molecular variation among the Athabasca and Beaver River basins accounted for 3.3% of the total variation and an additional 7.7% of the variation was among subpopulations within these populations (Table 2-5). Variation among all subpopulations accounted for 13.3% of the total genetic variation. The majority of the genetic variation was among individuals (Table 2-5).

The maximum log likelihood of the Bayesian clustering algorithm occurred at K=12, and at K=12 each sample location was the major contributor to a single genetic cluster (Table 2-6, Figure 2-3). At K values greater than twelve no further distinct genetic clusters were identified, rather individual samples begin to demonstrate more admixture. The ten STRUCTURE runs at K=12 had consistent individual genetic cluster membership with pairwise similarity coefficients (H') greater than 0.96 (Figure 2-3).

Frequency based assignment of individuals to subpopulations identified 59 individuals (6%) whose genotype was more likely to arise in a subpopulation other than the one from which they were sampled (Table 2-7). The largest numbers of cross-assigned individuals were from the Lake Athabasca and Lesser Slave Lake subpopulations. No cross-assigned individuals were identified in the Buck, Winefred or North Wabasca subpopulations. Of the 59 cross-assigned individuals, 45 (76%) were between subpopulations in the same river basin. This agreed with the partitioning of individuals between genetic groups via the Bayesian clustering method.

Discussion

Broad-scale Patterns of Population Structure

As expected, walleye populations in Alberta do not comprise a single panmictic population. Populations exhibited deviations from Hardy-Weinberg expectations and some apparent Wahlund effects, supporting the hypothesis of genetic structure among lakes and river basins. The Wahlund (1928) effect is a

reduction of heterozygosity in a population due to genetic substructure. I observed less heterozygosity than expected in Athabasca and Beaver River basin populations (Table 2-1), likely due to the number of distinct genetic subpopulations within these basins. The broad-scale walleye population structure in Alberta appears to align with the contemporary hydro-geographic landscape and the genetic differentiation is significant to the level of individual lakes. This study supports the previous findings of Thomas et al. (1999) that concluded distinct walleye populations exist within different regions of Alberta and that the amount of genetic differentiation indicated some movement of walleye between groups.

I found that each of the twelve lakes included in this study have genetically distinct walleye subpopulations. Subpopulations from the same river basin are more similar than subpopulations from different river basins. Measures of genetic differentiation (θ_{ST}) between geographic subpopulations and populations were similar to those reported for other walleye populations (Strange and Stepien 2007, Stepien et al. 2009). My analyses supported the hypothesis of hierarchical partitioning of genetic variation based on geographic boundaries of river basins and lakes. Analysis of molecular variance for walleye in the Athabasca and Beaver River basins showed that a small percentage of the genetic variation (3.3%) could be attributed to differences between river basin populations with slightly more variation (7.7%) attributable to differentiation among subpopulations within the river basins. This corresponds with the observed broad-scale isolation by distance pattern where waterway distance and river basin boundaries have similar correlations to genetic distance.

Contemporary hydro-geography appears to be a major influence on current walleye genetic patterning. If the walleye population in glacial Lake Agassiz had exhibited a genetic isolation by distance pattern, or if there had been genetically distinct walleye groups within the glacial refugia, then I would expect to see remnants of these patterns in the current population structure. The θ_{ST}'s

between walleye subpopulations of different river basins are similar irrespective of direct overland distance. Lesser Slave and North Wabasca Lakes are separated by just over 100km and have a pairwise θ_{ST} of 0.120. This is similar to the pairwise θ_{ST} of 0.111 between Lesser Slave and Bistcho Lakes that are over 500km apart by direct geographic distance. The lack of a significant correlation between genetic differentiation and direct geographic distance between walleye subpopulations indicates that the current population structure is unlikely to be attributable to historical groupings in glacial refugia or glacial Lake Agassiz. This is analogous with other investigations into walleye population structure (Stepien et al. 2009), but is contrary to what was observed for brook charr (*Salvelinus fontinalis*) populations in Newfoundland, Canada (Poissant et al. 2005). The current hydro-geography of Alberta has likely been relatively stable since the retreat of glacial Lake Agassiz following the end of the Pleistocene glacial period, therefore; populations that have been separated by river basin boundaries are unlikely to have had a natural exchange of migrants allowing processes such as mutation and genetic drift to create genetic differentiation between these populations. The higher θ_{ST} values between subpopulations in different river basins coupled with a significant isolation by waterway distance pattern indicates that these boundaries have been functioning as barriers to gene flow resulting in vicariant populations. The current hydro-geography, especially the boundaries between river basins, appears to be a significant factor in the genetic structure of Alberta's walleye populations.

A small number of individuals (6%) were identified as having a genotype more likely to originate in a subpopulation other than the one from which they were sampled. 45 of the 59 mis-assignments were among subpopulations in the same river basin, indicating potential low levels of migration between connected subpopulations. The remaining 14 mis-assignments were across river basin boundaries. As there is no direct migration route between these lakes, these mis-assignments are likely a result of the common genetic ancestry of the subpopulations. The significant level of divergence between connected

subpopulations, while lower than between non-connected subpopulations, suggests natal philopatry and limited dispersal are functioning along with geographic separation to maintain genetic differentiation.

Athabasca River Basin

Although the individual subpopulations are genetically distinct, walleye subpopulations within the Athabasca River basin do not exhibit a significant isolation by distance pattern. The connectivity between water bodies within a river basin is not solely a function of geographic distance but is also dependent on size, flow rate and geography of the waterway. Fish populations in geographically proximate lakes can be quite isolated from one another if the waterway connection between the two is not conducive to fish movement. This is illustrated within the Athabasca River basin between Lake Athabasca, Lesser Slave and Fawcett Lakes. Although Lesser Slave and Fawcett Lakes are only separated by 109km of river, the connection between Fawcett Lake and the Lesser Slave River is quite shallow and probably only passable by walleye in years of heavy precipitation (M. Sullivan, ASRD, Edmonton, pers. comm.). The pairwise θ_{ST} between Lesser Slave and Fawcett is 0.066. Compare this to Lesser Slave Lake and Lake Athabasca, which are separated by 830km of river but have a pairwise θ_{ST} of 0.051. These two lakes are directly connected by a large waterway and fish movement between the sites has been observed in previous telemetry studies (RAMP 1998). It has been postulated that the Athabasca River may function as a walleye "highway" and fish are moving both up and downstream along this waterway and subsequently joining non-natal populations (M. Sullivan, ASRD, Edmonton, pers. comm.). This hypothesis is supported by the assignment test and Bayesian clustering, which indicated that the Lesser Slave Lake and Lake Athabasca walleye subpopulations had the highest number of cross-assigned and admixed individuals. These individuals are potentially migrants from other walleye subpopulations.

Within river basin subpopulation structure is defined more by habitat connectivity than geographic distance. Natal philopatry is also a mechanism working to maintain the genetic divergence between connected lacustrine subpopulations. The walleye behaviour of dispersing to forage and overwinter but returning to natal sites to spawn maintains and creates genetic differentiation between spawning groups. The genetic pattern of walleye subpopulations in Alberta is likely the result of behaviour and contemporary environmental barriers to gene flow. This is congruent with what is previously described for other walleye populations in North America (Stepien et al. 2009).

Genetic Diversity of Alberta Walleye Populations

This study incorporated more loci than prior investigations of walleye population structure. Loci Svi14 and Svi16 showed highly significant linkage across all populations and subpopulations in my study; therefore, I only used data from locus Svi14. The only previously reported study that included both locus Svi14 and locus Svi16 was that of Eldridge et al. (2002), but Svi14 was only characterized for a small sample number and not included in the full analysis. It is possible that the primers for these two loci are actually targeting the same region of DNA. Locus SviL8 was reported to have null alleles in studies in the Great Lakes region. This was not evident in Alberta walleye populations, but I did notice the consistent presence of weak alleles at this locus. I did find that locus Svi18 showed significant evidence for null alleles which was not noted in other studies. I observed fewer alleles per locus than detected in Great Lakes walleye populations (Stepien et al. 2009) for loci that my studies had in common even though I had slightly larger sample sizes. This could be a result of lower overall diversity.

Alberta walleye appear to have less microsatellite diversity than populations sampled in Quebec (Dupont et al. 2007) and the Great Lakes (Strange and Stepien 2007; Stepien et al. 2009). Since different markers were used for population characterization it is difficult to determine if the lower diversity is a

result of the study design or an accurate reflection of differences between the populations. The recent study of Stepien et al. (2009) included walleye populations from the Great Lakes and Hudson Bay drainages and found that the populations from the Hudson Bay drainage exhibited lower diversity and significant genetic differentiation from Great Lakes walleye populations. The walleye populations west of the Great Lakes region most likely originated from the same glacial refugia (Ward et al. 1989; Billington 1996) and therefore would share a genetic history that would influence the current diversity. This lower diversity for walleye populations outside of the Great Lakes watershed could be attributed to populations originating from different glacial refugia. Alternately, perhaps the larger total population numbers in Great Lakes walleye have helped protect those populations from historical bottlenecks and loss of heterozygosity. Lower diversity in Alberta walleye populations could also be due to environmental conditions. Alberta is at the northern and western limit of walleye natural range. The colder climate results in fish having longer generation times, lower productivity and populations may not have reached the same levels of divergence as fish that are living in warmer, more productive climates with a more rapid generation turnover.

Correspondence to Population Structure in Other Fishes

The rainbow trout (*Oncorhynchus mykiss*) is the only other fish species in Alberta that has been genetically characterized, and this species has been extensively supplemented by stocking (Taylor et al. 2007). In the absence of comparable data from sympatric freshwater fish species in Alberta, I do not know whether other lake fish species exhibit similar genetic differentiation patterns. Yellow perch (*Perca flavescens*; Miller 2003) and smallmouth bass (*Micropterus dolomieu*; Stepien et al. 2007) within the Great Lakes region exhibit population structure similar to that of walleye due to common isolation in glacial refugia, similar recolonization patterns and subsequent vicariance. Additionally, colonization patterns from glacial refugia, contemporary hydro-geography and natal site fidelity are integral mechanisms defining lake sturgeon (*Acipenser*

fulvescens) population structure in the Great Lakes (Welsh et al. 2008). Due to parallel biogeographic histories and shared evolutionary mechanisms, I would expect other freshwater fish species in Alberta to exhibit population structure similar to what I have observed in walleye.

Relevance for Conservation and Management of Walleye in Alberta

My findings are relevant for the management of the Alberta walleye fishery and stocking program. While each lake is currently treated as an independent management unit the walleye stocking program gives little consideration to genetic diversity when deciding on hatchery stock sources and recipient lakes. In order to maintain as much natural genetic diversity as possible, walleye stocks should not be transferred across river basin boundaries and hatchery brood stock should be selected in order to be genetically similar to the native population of the recipient lake. Knowing the genotypes of distinct fish populations will facilitate tracking the success of stocked and natural fish populations in the same water body (Eldridge et al. 2002). Results of this study can be used to refine management units and to select appropriate brood stock for hatchery-based population supplementation or reintroduction.

Genetic data for local walleye subpopulations has the potential to be a valuable tool for fisheries enforcement. The combination of microsatellite data and powerful statistical software will allow for greater enforcement of harvest restrictions through DNA profile comparisons and population assignment. Wildlife officers will have the ability to link an individual fish to a subpopulation of origin after being removed from the body of water; eliminating the need to apprehend resource violators at the site of their offence to obtain a conviction. The ability to use DNA analysis as an enforcement tool may also reduce the amount of illegal harvest of walleye through deterrent effects.

Genetic data for walleye in Alberta should aid in fish management by: 1) providing information that will guide decisions designed to conserve genetic

diversity, 2) protect genetically distinct populations of walleye, 3) aid in the monitoring of stocking programs and 4) detect and assist in convicting individuals who illegally take and/or traffic in walleye.

Table 2-1 Genetic diversity of walleye populations and subpopulations based on 15 microsatellite loci. N, number of individual fish; H_o, observed and H_e, expected heterozygosity; F_{IS}, deviation from Hardy-Weinberg expectations (Weir and Cockerham 1984); N_A, number of alleles; P_{PA}, proportion of private alleles; A^R, allelic richness. Italic type indicates means and totals for regions. * indicates significance at $p<0.05$ after Bonferroni corrections.

Locality	Lat °N	Long °W	N	H_O	H_E	F_{IS}	N_A	P_{PA}	A_R
Athabasca Basin			*514*	*0.592*	*0.638*	*0.072**	*217*	*0.046*	*8.97*
1 Lake Athabasca	59.18	109.37	89	0.576	0.588	0.021	162	0.019	6.75
2 Calling Lake	55.21	113.19	102	0.655	0.633	-0.034	133	0.015	5.82
3 Fawcett Lake	55.32	113.83	76	0.522	0.510	-0.023	106	0.009	4.94
4 Heart Lake	55.02	111.50	80	0.612	0.645	0.050	108	0.000	5.47
5 Lesser Slave Lake	55.45	115.43	104	0.623	0.606	-0.029	151	0.013	6.14
6 Winefred Lake	55.50	110.58	63	0.510	0.548	0.059	108	0.019	5.50
Beaver Basin			*269*	*0.617*	*0.651*	*0.053**	*188*	*0.074*	*8.46*
7 Beaver Lake	54.72	111.62	89	0.586	0.587	0.002	122	0.025	5.51
8 Primrose Lake	54.99	109.88	93	0.666	0.664	-0.004	155	0.058	6.48
9 Wolf Lake	54.68	110.95	87	0.592	0.601	0.015	117	0.017	5.29
North Saskatchewan Basin									
10 Buck Lake	53.00	114.75	92	0.581	0.598	0.029*	118	0.085	5.35
Liard Basin									
11 Bistcho Lake	59.73	118.80	87	0.491	0.499	0.017	106	0.000	*4.71*
Peace Basin									
12 North Wabasca Lake	55.95	113.83	43	0.470	0.468	-0.005	95	0.011	4.93
All sites			*1005*	*0.583*	*0.669*	*0.128**	*274*	*0.128*	*7.29*

Table 2-2 Diversity characteristics of 17 microsatellite loci in Alberta walleye. NA, number of alleles; size range in base pairs; F_{IS}, genetic variation within subpopulations; F_{IT}, genetic variation in the total sample; and F_{ST}, genetic divergence among subpopulations; as measured by θ (Weir and Cockerham 1984). * indicates loci that were removed from subsequent analysis.

Locus	N_A	size range	F_{IS}	F_{IT}	F_{ST}	Source
Svi33	9	77-98	0.066	0.231	0.177	Borer et al. (1999)
Svi18*	5	117-128	0.261	0.356	0.129	Borer et al. (1999)
Svi16*	21	175-269	-0.007	0.075	0.069	Eldridge et al. (2002)
SviL11	28	307-388	-0.002	0.188	0.190	Wirth et al. (1999)
SviL8	16	121-150	0.040	0.136	0.100	Wirth et al. (1999)
SviL5	14	182-206	-0.006	0.152	0.157	Wirth et al. (1999)
Pfla2	29	241-289	0.028	0.216	0.193	Cena et al. (2006)
Svi17	7	101-117	-0.017	0.081	0.096	Borer et al. (1999)
PflaL8	29	137-227	0.006	0.094	0.088	LeClerc et al. (2000)
PflaL1	19	84-138	-0.030	0.128	0.154	LeClerc et al. (2000)
Svi14	20	156-209	-0.002	0.080	0.082	Eldridge et al. (2002)
Svi2	10	248-284	0.013	0.191	0.180	Cena et al. (2006)
Svi9	19	327-376	0.019	0.079	0.062	Cena et al. (2006)
Svi6	14	115-149	-0.010	0.143	0.151	Borer et al. (1999)
SviL7	20	181-222	0.014	0.065	0.051	Wirth et al. (1999)
Svi26	26	135-195	-0.007	0.155	0.161	Borer et al. (1999)
SviL4	14	200-239	-0.001	0.181	0.182	Wirth et al. (1999)
Total			*0.018*	*0.148*	*0.133*	

Table 2-1 Pairwise θ_{ST} (Weir & Cockerham 1984) comparisons between Alberta walleye subpopulations. All θ_{ST} values were significant at $p<0.01$ after 1000 permutations.

	ATB	CAL	FCT	HRT	LSL	WFD	BVR	PRS	WLF	BCK	BIS
Athabasca (ATB)											
Calling (CAL)	0.101										
Fawcett (FCT)	0.114	0.067									
Heart (HRT)	0.102	0.053	0.104								
Lesser Slave (LSL)	0.051	0.071	0.066	0.083							
Winefred (WFD)	0.073	0.092	0.110	0.095	0.103						
Beaver (BVR)	0.124	0.094	0.145	0.072	0.129	0.101					
Primrose (PRS)	0.110	0.081	0.125	0.064	0.114	0.115	0.086				
Wolf (WLF)	0.131	0.086	0.148	0.072	0.134	0.110	0.059	0.070			
Buck (BCK)	0.218	0.198	0.262	0.211	0.216	0.219	0.206	0.206	0.208		
Bistcho (BIS)	0.103	0.117	0.140	0.144	0.111	0.147	0.162	0.144	0.179	0.274	
North Wabasca (WAB)	0.090	0.164	0.176	0.147	0.120	0.146	0.184	0.160	0.178	0.290	0.184

Table 2-2 Simple and partial Mantel (1964) tests of the relationships between genetic distance ($\theta_{ST}/1-\theta_{ST}$), waterway distance (km), direct overland geographic distance (km) and river basin boundaries. Mantel tests were run with 100,000 permutations.

Samples	Simple Mantel /Partial Mantel Test	r	p
All populations	$F_{ST}/(1-F_{ST})$ vs. waterway distance	0.789	<0.001
	$F_{ST}/(1-F_{ST})$ vs. direct distance	0.321	0.141
	$F_{ST}/(1-F_{ST})$ vs. basin	-0.500	<0.001
	$F_{ST}/(1-F_{ST})$ vs. waterway distance, controlling basin	0.708	0.013
	$F_{ST}/(1-F_{ST})$ vs. direct distance, controlling basin	0.223	0.175
	$F_{ST}/(1-F_{ST})$ vs. basin, controlling waterway distance	0.103	0.269
	$F_{ST}/(1-F_{ST})$ vs. basin, controlling direct distance	-0.453	<0.001
Athabasca River Basin	$F_{ST}/(1-F_{ST})$ vs. waterway distance	0.261	0.235
	$F_{ST}/(1-F_{ST})$ vs. direct distance	0.125	0.307

Table 2-3 Genetic variance within and among populations and subpopulations partitioned by analysis of molecular variance (AMOVA; Excoffier et al. 2005). Tests were among river basins containing more than one subpopulation and among all walleye subpopulations.

Groups compared	Number of Groups	Components	Percentage of Variation	P value
River Basin	2	Among populations	3.3	<0.001
(Athabasca and Beaver)		Among subpopulations	7.7	<0.001
		Among individuals within subpopulations	0.2	0.326
		Within individuals	88.8	<0.001
All Lakes	1	Among subpopulations	13.3	<0.001
		Among individuals within subpopulations	0.5	0.114
		Within individuals	86.2	<0.001

Table 2-4 Proportioning of individuals between K=12 genetic clusters identified by the Bayesian STRUCTURE analysis. Columns represent genetic clusters and rows indicate percentage of individuals from each sample location identified as belonging to a genetic cluster averaged over 10 STRUCTURE runs (H'=0.979; CLUMPP, Rosenberg et al. 2002). Bold type denotes dominant cluster membership.

	Genetic Cluster											
	1	2	3	4	5	6	7	8	9	10	11	12
Lake Athabasca	0.052	0.027	0.019	0.044	0.018	0.010	0.012	0.036	0.056	0.026	0.029	**0.672**
Calling Lake	0.032	0.040	0.017	0.014	0.020	0.009	0.020	0.025	0.021	**0.737**	0.047	0.018
Fawcett Lake	0.031	0.017	0.015	0.013	0.016	0.006	0.010	0.022	0.022	0.064	**0.761**	0.024
Heart Lake	0.025	**0.748**	0.024	0.023	0.035	0.006	0.014	0.017	0.015	0.042	0.032	0.019
Lesser Slave Lake	**0.654**	0.024	0.015	0.027	0.013	0.010	0.011	0.038	0.024	0.035	0.077	0.072
Winefred Lake	0.015	0.014	0.015	0.015	0.021	0.012	0.013	0.026	**0.787**	0.023	0.028	0.033
Buck Lake	0.007	0.008	0.007	0.006	0.006	**0.914**	0.009	0.008	0.008	0.010	0.008	0.009
Bistcho Lake	0.017	0.010	0.012	0.010	0.010	0.005	0.010	**0.841**	0.017	0.017	0.028	0.024
Beaver Lake	0.016	0.020	0.018	0.010	0.031	0.012	**0.777**	0.021	0.018	0.034	0.024	0.020
Primrose Lake	0.018	0.026	**0.773**	0.013	0.038	0.007	0.022	0.021	0.016	0.026	0.019	0.021
Wolf Lake	0.025	0.023	0.029	0.016	**0.735**	0.010	0.058	0.015	0.019	0.027	0.025	0.018
North Wabasca Lake	0.010	0.011	0.008	**0.870**	0.006	0.005	0.006	0.023	0.013	0.012	0.015	0.022

Table 2-5 Assignment of individuals to subpopulations based on the frequency method of Paetkau et al. (1995). Missing alleles were assigned a frequency of 0.01. Individuals assigned to the subpopulation from which they were collected are indicated in bold along the diagonal of the table. 59 individuals were assigned to a subpopulation other than where they were collected. Mis-assignments within river basins are within the dotted lines (green = Athabasca River basin, blue = Beaver River basin).

		Assigned to											
		ATB	**CAL**	**FCT**	**HRT**	**LSL**	**WFD**	**BVR**	**PRS**	**WLF**	**BCK**	**BIS**	**WAB**
Sampled from	**Athabasca (ATB)**	**71**	2	0	2	3	6	0	0	0	0	3	2
	Calling (CAL)	0	**93**	3	4	0	0	0	1	0	0	0	1
	Fawcett (FCT)	0	3	**71**	2	0	0	0	0	0	0	0	0
	Heart (HRT)	0	2	0	**75**	0	0	0	0	2	0	0	1
	Lesser Slave (LSL)	1	0	9	0	**92**	0	0	0	0	0	1	1
	Winefred (WFD)	0	0	0	0	0	**63**	0	0	0	0	0	0
	Beaver (BVR)	0	0	0	1	0	0	**86**	0	2	0	0	0
	Primrose (PRS)	0	0	0	0	0	0	1	**89**	3	0	0	0
	Wolf (WLF)	0	0	0	0	0	0	2	0	**85**	0	0	0
	Buck (BCK)	0	0	0	0	0	0	0	0	0	**92**	0	0
	Bistcho (BIS)	0	0	1	0	0	0	0	0	0	0	**86**	0
	North Wabasca (WAB)	0	0	0	0	0	0	0	0	0	0	0	**43**

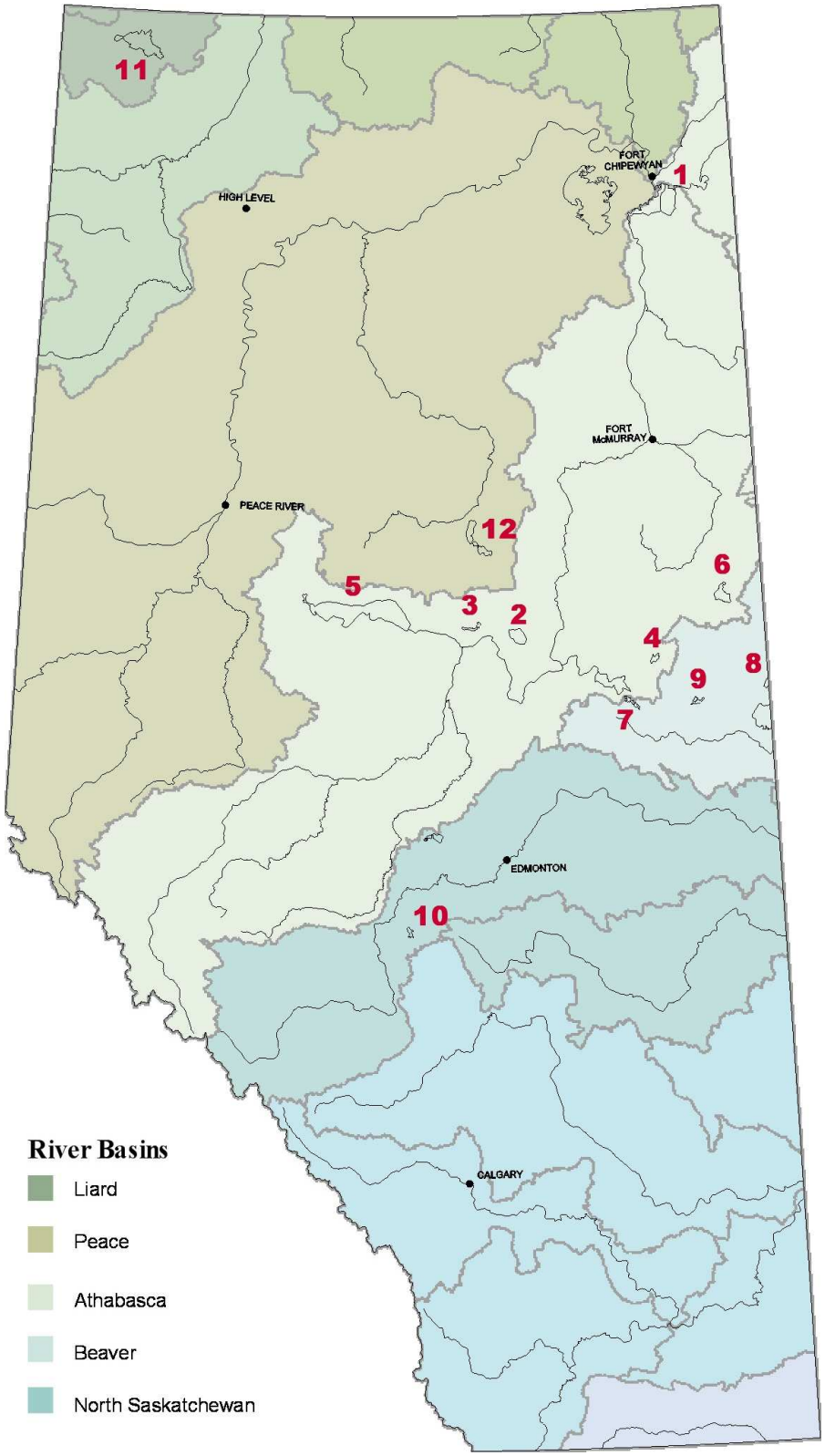

Figure 2-1 Sample collection locations for walleye. Sample locations are indicated by numbers (Table 1). River basins are indicated by shading.

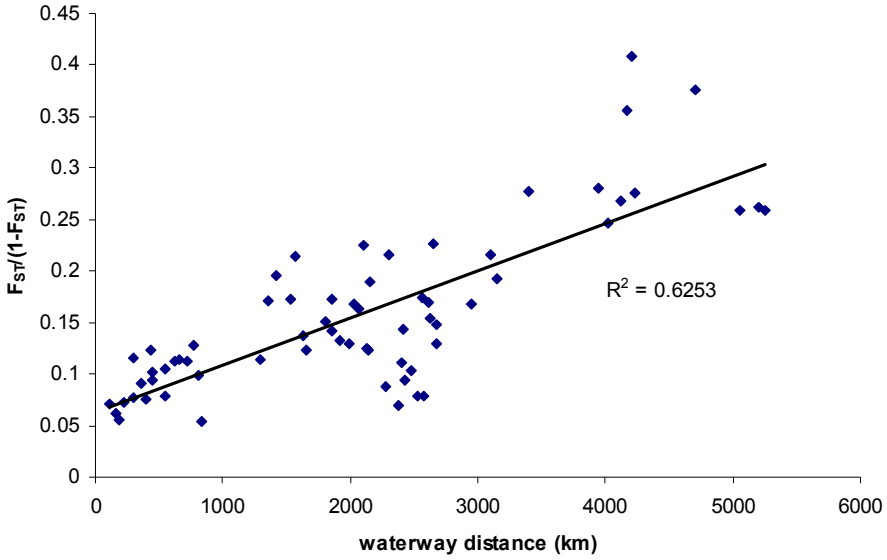

Figure 2-2 Genetic distance ($\theta_{ST}/1-\theta_{ST}$) increases with geographic waterway distance (km) between Alberta walleye sample locations (Mantel test; $p<0.001$).

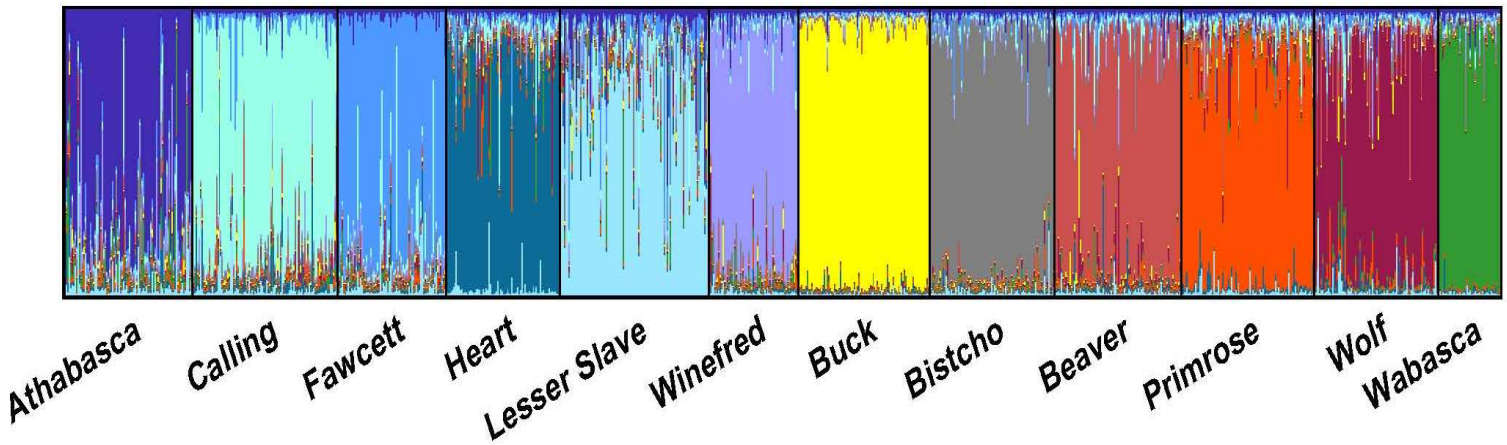

Figure 2-3 Estimated population structure of walleye from Bayesian STRUCTURE analysis at K=12. Each individual is represented by a single vertical line which is partitioned into coloured segments representing the individuals cluster membership. Ten STRUCTURE runs with 500,000 burn-in cycles followed by 500,000 iterations were combined (H'=0.967).

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Chapter 3: Genetic assessment of walleye (*Sander vitreus*) stocking in Lac La Biche, Alberta

Introduction

Walleye (*Sander vitreus*) are one of the most popular commercial and sportfish in Alberta; therefore, it is not surprising that they are also one of the most intensively managed species in the province (Berry 1995). Alberta implemented an active management plan for walleye in 1996 that implied a shift in philosophy from maximizing harvest to maintaining healthy, sustainable populations that can function both ecologically and economically (Sullivan 2003). Active management programs for recreational fisheries should include four major components; (1) clear goals and objectives, (2) regulations, (3) assessment procedures and (4) enforcement (Pereira and Hansen 2003). Each of these management components can include a myriad of activities that require resources in terms of time, money and expertise. Establishing and implementing a pertinent and successful active management plan depends on accurate population, life history and harvest information for the fishery of interest.

Genetic data is available for some walleye populations in central Alberta (Thomas et al. 1999) but this information is not currently included in the active management of the species in Alberta. Genetic data can be a valuable contributor to the four major components of an active management plan. It can assist in describing the natural diversity of the species, which will aid in defining goals, objectives, management units and creating regulations (Chapter 2). Genetic data can be applied in a forensic context to enforce existing regulations (Chapter 4). Genetic information can also be a valuable tool for assessment and monitoring of species and management activities (Schwartz et al. 2007; Nichols and Williams 2006), including translocations and stocking.

The walleye hatchery and stocking program has been formally operating in Alberta since 1986 (with occasional documented walleye stocking activities occurring since 1926) and is a publicly popular component of the current active management strategy (Sullivan 2008a). The purpose of the hatchery and stocking program in Alberta is to supplement vulnerable or collapsed populations as well as to introduce walleye into water bodies that do not have natural populations (Berry 1992). The ultimate goal is to restore or create self-sustaining fisheries that maintain the natural genetic biodiversity (Berry 1992; Sullivan 2008a). The rearing and stocking of fish is an expensive and time-consuming activity. The annual cost of rearing and stocking walleye in Alberta for 2007 was $211,956, not including infrastructure costs (Copeland 2007). Despite the amount of resources invested in the walleye stocking program in Alberta, over the last 20 years there has been limited quantitative assessment of the survival and reproductive contribution of the hatchery reared and stocked walleye. Past assessments have been hindered by an inability to differentiate between stocked and native walleye (Sullivan 2008a). The Alberta walleye program strategy for the 1990s (Berry 1992) stated that it "will emphasize evaluation of walleye stocking, so that future efforts can benefit from a sound knowledge of successes and failures", but there is little empirical data available regarding the evaluation of walleye stocking in Alberta. The recent microsatellite genetic characterization of the major walleye populations in Alberta (Chapter 2) presents an opportunity to quantitatively evaluate the success of the walleye stocking program.

Evaluation of the stocking program is important in order to identify successes and failures and to allow for adjustment of the stocking activities so that resources are not wasted or used inefficiently. Successful assessment of the stocking program would entail the identification of the presence or absence of each year of stocking introduction, as well as the presence of fish resulting from natural reproduction. Ongoing monitoring of the program would allow for an estimation of survival of stocked fish and their contribution to the harvest and reproducing population. Historically, evaluation of walleye stocking programs

have used test netting, alternate year stockings and yields to estimate the survival of the stocked fish and their contribution to year classes and harvest (Laarman 1978). In many of these cases it would be difficult separate stocked and native fish from the same year class.

Genetic tagging is a reliable, non-invasive method that can be applied as both an evaluation and monitoring tool for stocking events. A number of genetic tagging methods have been used to evaluate walleye stocking programs in North America since the mid 1990s. Jennings and Philipp (1992) used allozymes to compare the relative stocking success of walleye fry versus walleye fingerlings. Allozymes were also used to evaluate the success of stocking walleye in Dauphin Lake, Manitoba and to estimate the contribution to the commercial harvest (Mathias et al. 1992). Billington et al. (1992) suggested that mitochondrial DNA variation would be a useful genetic tag for assessing the success of stocked populations and Henry and Barkley (2008) employed this method in the Eleven Point River in Arkansas. While allozymes and mitochondrial haplotypes offer genetic information, microsatellites are more discriminating markers that allow for fine scale population analysis and parentage assignment. These markers have been used to determine the relative survival of stocked and resident walleye populations in Minnesota (Eldridge et al. 2002) and to assess the success of walleye rehabilitation stocking in Nipigon Bay, Lake Superior (Wilson et al. 2007). Genetic data is also being used to aid the management of various freshwater fish species globally. The rehabilitation of brook trout (*Salvelinus fontinalis)* in Lake Superior (Sloss et al. 2008) and the impact of stocking on northern pike (*Esox lucius*) populations in Denmark (Larsen et al. 2005) have both been monitored and assessed via microsatellite markers. In cases where the natural or resident population has a different genetic signature than that of the stock source population(s), assessment and monitoring can be achieved easily and non-invasively via genetic sampling.

Quantitative assessment of the survival and contribution of stocked walleye in Alberta has been limited to comparison between stocked and non-stocked lakes (Sullivan 2008a) and some broad-scale conclusions based on RAPD DNA analysis (Thomas et al. 1999). Correlative assessment, case histories and conclusions based on a single year of stocking are not sufficient evaluations of a program (Jennings and Philipp 1992). The current stocking program in Alberta is well suited for genetic assessment. The two principle source subpopulations are highly genetically differentiated from each other and from many of the lakes in which they are being stocked (Chapter 2), thereby allowing for identification of the subpopulation of origin for individual fish via genetic analysis. Lac La Biche has been identified by Sullivan (2008b) as a lake on which provincial stocking efforts should be focused; therefore, it is an excellent lake to use as a case study for genetic assessment of stocking activities. The walleye subpopulation of Lac La Biche collapsed in the 1960s and despite walleye harvest regulations and extensive stocking activities the subpopulation has not recovered to a self-sustaining level. An assessment of the stocking program to date would be useful in informing management decisions for the future rehabilitation of the Lac La Biche walleye subpopulation.

This study will characterize the current genetic composition of the walleye subpopulation in Lac La Biche using microsatellite markers and population assignment in order to evaluate the contribution of stocked walleye. This will determine the relative contribution of the stocked walleye to the current Lac La Biche subpopulation as well as illustrate the utility of genetic analysis for assessment and monitoring of management activities. It is expected that most walleye samples from Lac La Biche will be pure strains from one of the three donor lakes (Primrose, Lesser Slave and Bistcho) used for recent stocking events. However, there is a possibility of some fish being hybrids of any combination of previously introduced strains, or of a remnant (i.e. native Lac La Biche) subpopulation. Due to the geographic proximity and waterway connection, I expect that Heart Lake walleye would be the most similar to a potential Lac La

Biche remnant subpopulation Therefore in the absence of a known native Lac La Biche walleye subpopulation, Heart Lake will be included as a reference subpopulation for the native strain.

Methods and Analysis

Sample Localities

Lac La Biche is an eutrophic, 234 km^2 lake located in the Eastern Alberta Plains and is part of the Athabasca River basin (Figure 3-1). The lake is managed for commercial, sport, and subsistence fisheries. The dominant fish species currently in the lake are lake whitefish (*Coregonus clupeaformis)*, cisco (*Coregonus artedii)*, and northern pike. Lac La Biche was once one of the largest walleye fisheries in Alberta (Sullivan 2003) but the subpopulation collapsed through the 1950s and 60s. The Lac La Biche walleye subpopulation collapse was so severe that it was difficult to even get an estimate of the number of walleye remaining (Sullivan 2003). Since walleye are a top predator its effective removal from a lake can have an effect on the entire ecosystem. The collapse of the walleye subpopulation in Lac La Biche resulted in an increase of minnows, fish-eating birds and algae, reducing the lake value for many users (Sullivan 2008b).

The two principle source subpopulations for the walleye hatchery in Alberta are Bistcho and Primrose Lakes (Table 3-1). These two lakes are comparable in size and elevation, but different in location and average temperature. Bistcho Lake is located in the northwest corner of Alberta, approximately 675 km northwest of Lac La Biche (Figure 3-1). It is part of the Liard River basin and is one of the larger lakes in the province. The majority of Primrose Lake is in the province of Saskatchewan, about 150 km east of Lac La Biche (Figure 3-1) and is located in the Beaver River basin. Hatchery raised individuals from a Lesser Slave Lake walleye egg source have also been stocked into Lac La Biche (Table 3-1). Lesser Slave Lake is an eutrophic, 1160 km^2 body of water located within the Athabasca River basin. Lesser Slave Lake is approximately 260 km northwest of Lac La Biche. The two lakes are connected

via a combination of the Lesser Slave, Athabasca and La Biche Rivers. The walleye subpopulations from these three lakes are genetically distinct from each other and from the Heart Lake walleye subpopulation (Chapter 2). Heart Lake is located northeast of Lac La Biche, within the Athabasca River basin. The two lakes are connected by an approximately 60 km stretch of the Owl and Piche rivers. The walleye subpopulation in Heart Lake is currently in a collapsed state and there is thought to be little natural movement of walleye between Heart Lake and Lac La Biche (C. Davis, ASRD, Lac La Biche, pers. comm.)

Sample Collection and Preparation

Walleye samples were collected from Lac La Biche (Figure 3-1) by fisheries biologists as part of Fall Walleye Index Netting (FWIN) sampling. The majority of the samples from Lac La Biche were collected in September of 2007, with a few samples collected in 1984, 2003, 2005 and 2006. All samples consisted of a dried fin ray stored at room temperature in a sample collection envelope. A total of 301 samples from Lac La Biche were prepared for DNA extraction. An approximately 0.5cm^2 piece of the distal end of the fin was minced using scissors directly into a microcentrifuge tube. An experienced biologist aged the samples in order to determine the year class of the individual.

DNA Profile Development

The Lac La Biche walleye samples were treated in the same manner as described for the walleye samples in Chapter 2. Samples from Heart Lake, Primrose Lake, Lesser Slave Lake and Bistcho Lake have been previously collected and analyzed (Chapter 2).

Preliminary Analysis

I first assumed that the Lac La Biche samples represent a single, panmictic breeding subpopulation. Each locus in the Lac La Biche subpopulation was tested for Hardy-Weinberg equilibrium and linkage disequilibrium using the Monte Carlo Markov chain (MCMC) method of GENEPOP 4.0.6 (Rousset 2007) with 10000 dememorizations of 3000 batches and 6000 iterations per batch. Non-

sequential Bonferroni corrections (Sokal and Rohlf 1995) were applied to adjust the level of significance. The Lac La Biche sample set was also tested for the presence of null alleles using the method of Brookfield (1996) as implemented in MICRO-CHECKER v.2.2.3 (van Oosterhout et al. 2004).

Genetic Variation

Genetic diversity within the Lac La Biche walleye subpopulation was estimated by observed (H_o) and expected (H_e) heterozygosities, mean number of alleles per locus (M_{NA}), allelic richness (A_R) and number of private alleles (N_{PA}). Allelic richness was calculated as the average number of alleles per locus corrected for minimum sample size (N=81) and a private allele was defined as having a frequency of 0.01 or greater in only one subpopulation. F_{IS} estimates from GENEPOP 4.0.6 were used to quantify within subpopulation variation. These diversity statistics were compared to those from the four reference walleye subpopulations (Chapter 2).

Genetic Distance

The degree of genetic divergence between subpopulations (F_{ST}) was estimated by θ_{ST} of Weir and Cockerham (1984). I estimated pairwise F_{ST} comparisons between Lac La Biche and the reference subpopulations in FSTAT (Goudet 1995, 2002). The significance of the pairwise F_{ST} comparisons was calculated after 1000 permutations and assessed after Bonferroni correction ($p<0.05$).

Genetic Clustering and Population Assignment

To confirm that the reference dataset of Heart, Bistcho, Lesser Slave and Primrose Lake walleye represented all the most likely contributors to the Lac La Biche subpopulation I used the exclusion method within GENECLASS2 (Piry et al. 2004) to determine if any of the Lac La Biche samples would be excluded from all four reference subpopulations. I used the partial Bayesian analysis of Rannala and Mountain (1997) in conjunction with the probability computation of Paetkau et al. (2004) for the exclusion test. Simulations were run with 100,000

individuals and the threshold for the probability of exclusion set to 0.001. Excluded samples were not included in further genetic clustering and subpopulation assignment.

I used STRUCTURE 2.2 (Pritchard et al. 2000) to perform Bayesian clustering of genotypes. STRUCTURE 2.2 tests the likelihood of various numbers of genetic clusters (K) from the data with no prior information about sample location. Runs consisted of 500,000 burn-in cycles and 500,000 iterations of the Markov chain, with the allele frequency correlation parameter (λ) predetermined and set as λ =0.413. I ran ten replicates of K=1 to K=9 for the Lac La Biche samples combined with the reference subpopulations from Bistcho, Primrose, Lesser Slave and Heart Lakes. The most likely value of K was determined by agreement between the peaking of the log likelihoods and the change in ΔK following the procedure of Evanno et al. (2005).

The partitioning of individuals between genetic clusters was used to assign individuals from Lac La Biche to a subpopulation of origin. The genetic clusters were identified by the principle contributing subpopulations. Individuals were assigned to the cluster in which they had the highest percentage of belonging. Individuals with greater than 0.20 belonging to more than one cluster were designated as originating from a mixed genetic background.

Assignments were linked to available age data for individual fish in order to investigate possible patterns in assignment between year classes. I used a two-tailed Chi-squared test to investigate relative survival of different hatchery sources stocked in the same year. I compared the observed number of individuals that assigned to each subpopulation of origin for the 2006 and 2007 year classes with the number of fish that would be expected based on the proportions of individuals stocked from each source.

Results

Preliminary Analysis

Of the 301 Lac La Biche samples prepared for DNA extraction, only one sample did not produce a genetic profile suitable for further analysis.

As previous investigations showed significant linkage between loci Svi14 and Svi16 in all subpopulations (Chapter 2), locus Svi16 was not included in any analysis. Locus Svi18 was also removed from analysis due to previous indications of a high frequency of null alleles at this locus (Chapter 2).

Loci pairs SviL11 and PflaL1, SviL5 and Svi2, and PflaL8 and Svi2 all displayed a significant ($p<0.05$) level of linkage within the Lac La Biche subpopulation after non-sequential Bonferroni correction. Locus Svi2 in the Lac La Biche sample set displayed an excess of homozygotes and possible null alleles at this locus with a estimated frequency of 0.0498 (Brookfield 1996). The Lac La Biche walleye subpopulation exhibited significant deviation from Hardy-Weinberg equilibrium after non-sequential Bonferroni correction ($p<0.05$) (Table 3-2).

Genetic Variation

Compared to the reference walleye subpopulations Lac La Biche had the highest mean number of alleles per locus and allelic richness but was similar to Primrose Lake in observed and expected heterozygosities (Table 3-2). Only the Lesser Slave Lake walleye subpopulation exhibited private alleles at a frequency greater than 0.01; although, there were a number of private alleles at lower frequencies in all subpopulations. The F_{IS} value (0.024) for Lac La Biche was significant at $p<0.05$ indicating a deviation from Hardy-Weinberg expectations and potential inbreeding (Table 3-2).

Genetic Distance

All pairwise F_{ST} comparisons were significant after Bonferroni correction ($p<0.05$), except for that of Lac La Biche compared to Primrose Lake (Table 3-3). This comparison indicated almost no genetic differentiation between these two subpopulations ($F_{ST} = -0.001$). All four reference subpopulations showed a moderate level of genetic differentiation from each other.

Genetic Clustering and Assignment

One sample was excluded from all reference subpopulations ($p<0.001$) and given an unknown source designation (Table 3-5). This sample was from the 2006 year class.

The peaking of the log likelihoods and ΔK both indicated that K=4 was the most likely number of genetic clusters for the sample set of Lac La Biche walleye and the four reference subpopulations (Figure 3-2). Each reference subpopulation was the major contributor to a single genetic cluster and there was no grouping of any of the reference subpopulation samples with clusters other than those containing samples from the same geographic location (Figure 3-3, Table 3-4). At K=4, the majority of the Lac La Biche samples grouped with the Primrose Lake walleye genetic cluster. A small number of the Lac La Biche samples grouped with the Bistcho, Lesser Slave and Heart Lake clusters (Figure 3-3). While the analysis indicated four genetic clusters as being most likely, there was also an indication of possible substructure at two genetic clusters (Figure 3-2). The structure at K=2 placed the Lac La Biche and Primrose Lake subpopulations in one genetic cluster and the Bistcho, Heart and Lesser Slave Lake subpopulations in the other cluster (data not shown).

The majority of Lac La Biche samples assigned to Primrose Lake as their subpopulation of origin (262 of 300), while 18 Lac La Biche samples assigned to Bistcho Lake, 5 to Heart Lake, 3 to Lesser Slave Lake and 11 samples were designated as having a mixed genetic background (Table 3-5). The age

distribution of the Lac La Biche sample set was heavily weighted towards younger fish. The age 0 samples corresponded to those that would have been stocked and/or born in 2007. The genotypic assignment of this year class showed an approximate 40%-60% split between Bistcho and Primrose strains. From the 2006 year class (age 1 designation) the majority of the fish were assigned to the Primrose Lake strain. The five samples that assigned to the Heart Lake subpopulation were from the 2003, 1997 and pre-1985 year classes (Table 3-5). The 11 samples that were designated as having a mixed genetic background were also distributed across a range of year classes with the majority (6 of 11) designated as age 1 (Table 3-5). Primrose Lake was indicated as a contributor to the genetic pool of all but one of these mixed origin samples, with the other part of the mixture being from Heart Lake (6 individuals), Lesser Slave Lake (3 individuals) or Bistcho Lake (1 individual) source. The single walleye of mixed origin with no Primrose Lake contribution was from the pre-1985 collection and indicated a mixed genetic contribution from Heart and Lesser Slave Lakes.

I found no difference in survival rates between the Lesser Slave and Primrose Lake hatchery stock sources in the 2006 stocking events relative to the proportion of individuals stocked from each source (X^2 =0.126, d.f.=1, $p>0.05$). There was also no significant difference in survival rates of the Bistcho and Primrose hatchery stock sources in the 2007 stocking events (X^2=0.245, d.f.=1, $p>0.05$) (Table 3-6).

Discussion

Population structure of Lac La Biche walleye

Lac La Biche walleye are not a single, panmictic subpopulation in Hardy-Weinberg equilibrium. The deviation from Hardy-Weinberg expectations for the Lac La Biche sample set as well as the indication of possible linkage between some loci pairs is likely due to a Wahlund effect. There was no evidence for linkage of these loci in other, non-stocked walleye subpopulations in Alberta (Chapter 2). The apparent linkage between loci and the deviation from Hardy-

Weinberg expectations is likely a direct consequence of subpopulation admixture resulting in genetic substructure of walleye within Lac La Biche. The age distribution of the walleye sampled from Lac La Biche leaned heavily to younger fish and therefore mostly of one or two year classes. This is likely an accurate representation of the actual age distribution of the walleye subpopulation in Lac La Biche (C. Davis, ASRD, Lac La Biche, pers. comm.). While the high genetic diversity may seem at odds with the suspected inbreeding the number of different stock sources introduced into Lac La Biche could explain this as well as the lack of private alleles in the Lac La Biche walleye sample. Given that the subpopulation age distribution indicated limited natural reproduction of either remnant or previously stocked walleye and there is only one known spawning location for walleye in Lac La Biche (C. Davis, ASRD, Lac La Biche, pers. comm.), it is unlikely that natural reproduction is causing the inbreeding effect. A likely cause is a Wahlund effect from the mixed genetic stocks introduced into the lake. The Wahlund (1928) effect is a reduction of heterozygosity in a population due to genetic substructure. The current walleye subpopulation in Lac La Biche appears to be a mix of the fish that have been hatchery reared and introduced via stocking, with the most recent stocking events comprising the majority of the subpopulation.

The Lac La Biche walleye samples, as a group, were not genetically distinct from the Primrose Lake walleye subpopulation, implying that the offspring from the Primrose Lake hatchery brood stock were the principle contributors to the Lac La Biche walleye subpopulation. The individual assignments support this, as 262 of the 300 fish sampled were likely of a Primrose Lake origin. The presence of fish that assigned to a Primrose Lake origin for the years in which no stocking occurred in Lac La Biche suggests that a number of the hatchery raised walleye are surviving to reproductive age and naturally contributing to the subpopulation. Also, a number of the individuals that were assigned a mixed genetic background indicated that Primrose Lake walleye were

likely a contributor to the mixture. This is another indication that there is natural reproduction involving stocked walleye in Lac La Biche.

Eighteen Lac La Biche walleye assigned to a Bistcho Lake origin. All of these individuals were from the 2007 year class. These are most likely individuals from the most recent stocking event and not naturally produced offspring as there is only one indication of Lac La Biche walleye assigning to the Bistcho Lake subpopulation outside of this year class and that is the partial assignment of an individual with an admixed genetic signature. Walleye of a Bistcho Lake origin were stocked into Lac La Biche in 1992 and 1994 and the lack of older individuals assigned to a Bistcho Lake origin in the current Lac La Biche subpopulation implies that the previous stocking of hatchery walleye from Bistcho brood stock did not make a significant contribution to the current subpopulation.

Lesser Slave Lake walleye have also been used as hatchery brood stock and subsequently stocked into Lac La Biche. Three samples from Lac La Biche assigned to a Lesser Slave Lake origin as well as partial assignment of four mixed genetic background individuals. The Lesser Slave Lake assigned individuals were all of year classes that made them possible hatchery reared stock or offspring of hatchery reared fish. Lesser Slave Lake is the only hatchery stock source that is within the same river basin as Lac La Biche and the two lakes are connected by relatively large waterways, therefore; it would be possible for fish to naturally migrate from one lake to the other. If walleye were regularly migrating between Lesser Slave Lake and Lac La Biche then I would have expected to see more Lesser Slave assigned fish in Lac La Biche as well as some Lac La Biche assigned fish in the Lesser Slave Lake population. I also would expect to see these individuals in distributed somewhat evenly among year classes. This was not the case, suggesting infrequent or nil movement of individuals between the two lakes. It is more likely that walleye which were assigned to a Lesser Slave Lake origin are from the hatchery reared stock.

Lac La Biche has a closer geographic relationship to the Heart Lake walleye subpopulation than to any other subpopulation in the Athabasca River basin. As geographically connected walleye populations tend to share more genetic similarities (Billington et al. 1996; Cena et al. 2006; Strange and Stepien 2007), this leads to the supposition that Heart Lake walleye may be the closest genetic representation of native Lac La Biche walleye. Three of the four walleye samples from Lac La Biche that were collected prior to 1985 were assigned to Heart Lake and the fourth sample was assigned a mixed genetic background of Heart Lake and Lesser Slave Lake, supporting the hypothesis that Heart Lake walleye are likely akin to the native Lac La Biche subpopulation. The assignment of more recent samples and younger individuals to the Heart Lake subpopulation may be an indication that some of the original Lac La Biche walleye subpopulation remain and are reproducing or that there is some natural walleye movement between Heart Lake and Lac La Biche.

One individual was excluded as originating from any of the reference subpopulations. The individual was heterozygous at 14 of the 15 loci genotyped with a number of low frequency alleles (data not shown). This individual could be an immigrant from another walleye subpopulation, a remnant from a Lac La Biche subpopulation that is not genetically similar to the Heart Lake subpopulation, an individual from an unknown stock source, or simply an individual with a rare genotype.

Assessment of Stocking Activities

There are a multitude of factors that influence the success of a single stocking event, such as environment, predation and availability of food, therefore; the outcomes of individual stocking events are highly variable (Sullivan 2008a). Due to the large number of uncontrollable variables influencing the success of stocking events it is difficult to compare the results between different years. During the 1980s and 1990s there were 13 separate stocking events in Lac

La Biche, each from a single source hatchery brood stock. The majority of the stocked fish were from a Primrose Lake source. The low number of older fish collected from Lac La Biche for this study indicates that there was very limited survival of fish from these stocking events or that the allowable walleye harvest in Lac La Biche is selecting heavily for older fish. While I can't directly compare the survival of one year to another due to uncontrollable variables, I can look at the overall trends to draw some conclusions about the success of these stocking events.

Of the 38 fish in this study that could possibly have originated from the stocking events prior to 2006, more than half of them assigned to a Primrose Lake origin. There are no fish that assigned to a Bistcho Lake origin and only two that assigned to a Lesser Slave Lake origin. From this I can conclude that the 1990 event had very limited success (or these fish are natural migrants) and the1992 and 1994 stocking events had no success (or so limited that it is undetected in this study). Alternately, it is possible that these stocking events were successful and that the mature fish were subsequently harvested prior to the 2007 FWIN sampling. There is evidence that some fish of a Primrose Lake origin that were stocked from 1995-1999 have survived and are reproducing; as evidenced by the Primrose assigned fish for year classes in which there were no stocking events (2000-2005). The stocking of fish into Lac La Biche through the 1980s and 1990s appears to have had a low level of success by establishing a small subpopulation of Primrose Lake origin fish that are reproducing, but the numbers do not seem sufficient to reach the goal of a self-sustaining population.

While assessment of the stocking events during the 1980s and 1990s showed a greater survival of Primrose origin fish, it is impossible to tell if this is due to a superior stock source, environmental conditions, or simply larger numbers. The two most recent stocking events in Lac La Biche occurred in 2006 and 2007. These events are distinctive in that fish from two different hatchery sources were introduced into Lac La Biche at the same time, and in the case of the

2007 event, in similar numbers. The two recent events gave us an opportunity to assess not only the overall survival and success of the stocking event, but also the relative survival of fish from different stock sources.

The number of Primrose Lake origin fish in the 2007 year class is higher than what would be expected based on the number of stocked individuals. The number of Bistcho Lake fish is lower than expected. While it appears as if the Primrose origin fish had a slightly higher survival rate in the most recent stocking event the numbers are not statistically significant when compared to the expected numbers based on the proportion of fish stocked (Table 3-6). Also, when taking into account that some the Primrose assigned fish could be offspring from previously stocked walleye the perceived difference becomes negligible. Although, if the observed trend continued for a larger sample size then the contribution of the Primrose Lake origin individuals to the Lac La Biche subpopulation would be significantly greater than that of the Bistcho fish stocked at the same time. For example, if I simply double the current sample size and retain the same proportions, the two-tailed Chi-squared test works out to be statistically significant at X^2=0.0306 (p=0.05). Eldridge et al. (2002) noted significant year-to-year variation in which stock source was the main contributor to the population for 3 years post-stocking. It would be interesting to reassess the genetic contributions to the 2007 Lac La Biche year class in subsequent years to see if there is a difference in survival rates over time and if the trend of the Primrose stock having greater survival becomes statistically significant.

While the greater numbers of Primrose Lake origin walleye in Lac La Biche can be attributed to a greater number of fish stocked from this source and possibly better environmental conditions in some years there is also the possibility of genetic or adaptive advantage. The markers used in this study are unlikely to have any adaptive significance, but the difference in allele distribution and frequencies between subpopulations represents historical change and as suggested by Billington et al. (1992) one could infer local adaptation of the genome. It is

possible that there are adaptive differences between walleye in Primrose Lake and Bistcho Lake that would allow the Primrose stock to have a higher survival rate in Lac La Biche. Primrose Lake and Lac La Biche share similar latitude and likely similar water temperatures and growing degree days. Bistcho Lake is much farther north and walleye from that lake are likely to spawn later in the season. It would not be unusual for these environmental differences to have an impact on genetic variation and adaptability of the walleye (Taylor 1991; McKay and Latta 2002). Fox (1993) suggests local stocks are much more likely to survive than those imported from geographically distant areas and evidence of this has been shown to some extent in the field (Terre et al. 1995; Eldridge et al. 2002).

The presence of fish with admixed genetic signatures is another indicator of natural reproduction occurring in Lac La Biche, although the lack of Hardy-Weinberg equilibrium and small number of admixed samples suggests that this may be at a minimal level. Natural reproduction of stocked fish is important to the establishment of self-sustaining populations and indicates that the stocked fish are surviving to reproductive age and contributing to the subpopulation. As walleye do not reach reproductive maturity until approximately 5 to 7 years for males and 6 to 9 years for females (Joynt and Sullivan 2003), there is a significant time lag until the stocked walleye are capable of contributing new offspring to the subpopulation. Most of the admixed individuals identified in this analysis had a genetic signature that indicated partial or full parentage from one of the brood stock subpopulations. This indicates that some fish that were stocked five or more years ago in Lac La Biche may have survived to reproductive age. Evidence of natural reproduction can be used as an indicator of successful stocking events.

Significance and Recommendations

The genetic analysis suggests that the current walleye subpopulation in Lac La Biche is genetically similar to a walleye subpopulation outside of the Athabasca River basin (Primrose Lake) rather than to the geographically closest walleye subpopulation (Heart Lake). The predominantly Primrose Lake

subpopulation in Lac La Biche and the potential success of these fish to create and maintain a healthy walleye subpopulation could have large impacts on the genetic diversity of the Athabasca River basin. There is potential for walleye in Lac La Biche to move throughout the Athabasca River basin in a year of high rainfall or water flow and then not be able to return to Lac La Biche for spawning and subsequently contribute to other breeding subpopulations. This could potentially alter the natural genetic diversity in this river basin. Knowing that each sample site investigated in this study has its own genetically distinct subpopulation of walleye (Chapter 2), matching the genetics of the brood stock to the lake, or at least the river basin, is a factor that should be taken into consideration when planning a walleye stocking program. In order to preserve the natural genetic diversity in Alberta, walleye should not be moved across watershed boundaries and stock sources should be as local as possible. It has long been recommended that stocking programs should avoid mixture of stocks from different regions in order to conserve the genetic integrity of populations (Jennings and Philipp 1992). An overall reduction in population fitness has also been noted in mixed stocks (Philipp 1991). Evolutionary Significant Units (ESU's) have not been officially defined for walleye in Alberta, but it has been recommended that, at a minimum, each watershed should be considered a single unit and that fish should not be transferred across these boundaries (Johnston and Paul 2006; Sullivan 2008b). As further research elucidates the genetic diversity of walleye in Alberta the geographic boundaries of the proposed ESU's may need to be refined.

Effective evaluation of stocking events relies on identification of the hatchery raised individuals (Sullivan 2008a). Individual assignment based on population genetic differentiation may not be an appropriate tool in all situations where there is a desire for quantitative assessment of stocking activities; such as when the stock strain and natural population are not sufficiently genetically differentiated or when the genetic baseline data has not been collected for a specific population. In that case, knowing the genetic signatures of the fish used as brood stock in the hatchery program would allow for parentage assignment as

demonstrated by Eldridge et al. (2002). This technique would also allow for the identification of individuals arising from natural reproduction of previously stocked fish from newly stocked individuals of the same year class. If the parent sources for the hatchery brood stock of the walleye stocking program were genetically characterized, it would further improve the ability to genetically monitor the success of walleye stocking events. Incorporating a genetic tagging program with the current walleye hatchery program in Alberta would allow for more informative assessment and monitoring of the walleye hatchery program.

Lac La Biche is a large lake within Alberta that previously supported a large walleye subpopulation. Sullivan (2008b) has indicated that it should be a lake within the province on which to focus walleye stocking efforts in the next decade. Given the possibility that the recovery of the walleye subpopulation in Lac La Biche could potentially reverse some of the ecological changes observed since the population's collapse; it is important that stocking efforts be as effective as possible. In light of the current genetic information and previous studies that indicate local stock strains have a higher likelihood of survival; it would be prudent to choose a brood stock for Lac La Biche from a genetically similar and therefore, a geographically proximate lake within the Athabasca River basin. Heart Lake seems to be the obvious choice, but it may not be possible to collect enough eggs from this subpopulation for the hatchery. A second choice would be Lesser Slave Lake. Sullivan (2008b) recommends this lake as a hatchery brood source due to its healthy walleye subpopulation and location within the Athabasca River basin. Recovery of the Lac La Biche walleye subpopulation through supplementary stocking could increase the lake value for many users by improving the aesthetic quality of the lake and recreating a sustainable fishery. This would be a great success for Alberta walleye management.

Rearing and stocking of walleye is an expensive and time-consuming activity, but may be the most effective tool for restoring lost or damaged walleye populations. Using genetic differentiation of populations to track survival of

different stock strains and native fish in a waterbody is an effective way to monitor the success of stocking events. This study does not have a definitive answer as to the overall success of walleye stocking in Lac La Biche, as it concentrated on the most recent events, but it can act as a starting point for monitoring the genetic composition of the walleye subpopulation. A baseline of genetic data has been established for the two most recent stocking events in the lake and continued monitoring of these year classes will allow for an estimate of survival and success of two different stock strains. Annual sampling of a wide range of year classes will also allow for assessment of breeding patterns and genetic contributions from other walleye subpopulations. Establishing annual or bi-annual sampling would expand the data from a one-time assessment event to a long-term monitoring program. Incorporating parentage assignment would also allow for more detailed assessment of the effectiveness of the stocking program. The knowledge of whether one stock source has a better survival rate in some lakes than others will be valuable information to fisheries management. It could greatly increase the effectiveness of the walleye stocking program.

The recognition and preservation of natural genetic diversity within a species is an important consideration in wildlife management. With data now available on the local population genetic structure of walleye (Chapter 2), the province of Alberta has an opportunity to integrate genetic data into their walleye management plan.

Table 3-1 Walleye stocking history of Lac La Biche. Data is from the Alberta Fisheries Management Information System (FMIS).

Year	Number	Stage	Source
1968	150,000	eggs/fry	Unknown
1969	560,000	eggs/fry	Unknown
1970	500,000	eggs/fry	Unknown
1985	3,680	Summer Fingerling	Unknown
1986	522,745	Summer Fingerling	Unknown
1987	511,885	Summer Fingerling	Unknown
1988	1,132,000	Summer Fingerling	Unknown
1990	466,227	Summer Fingerling	Lesser Slave Lake
1991	60,569	Summer Fingerling	Unknown
1992	1,198,139	Summer Fingerling	Bistcho Lake
1994	1,080	Summer Fingerling	Bistcho Lake
1995	14,627,000	Fry	Primrose Lake
1996	14,005,092	Fry	Primrose Lake
1997	18,567,259	Fry	Primrose Lake
1998	17,255,327	Fry	Primrose Lake
1999	500,000	Fry	Primrose Lake
2006	21,928,475	Fry	Mixed
	256,463	*Fry*	*Lesser Slave Lake*
	21,672,012	*Fry*	*Primrose Lake*
2007	45,103,128	Fry/Fall Fingerling	Mixed
	23,985,269	*Fry*	*Bistcho Lake*
	20,993,286	*Fry*	*Primrose Lake*
	99,705	*Fall Fingerling*	*Bistcho Lake*
	24,868	*Fall Fingerling*	*Primrose Lake*

Table 3-2 Summary statistics for walleye subpopulations based on 15 microsatellite loci. N, sample size, H_o observed and H_e, expected heterozygosities, MNA, mean number of alleles per locus, A^R, allelic richness , N_{PA}, number of private alleles with a frequency ≥ 0.01, and F_{IS}, deviation from Hardy-Weinberg expectations.

Population	N	H_O	He	MNA	A_R	N_{PA}	F_{IS}
Bistcho Lake	88	0.489	0.498	7.07	6.16	0	0.019
Lesser Slave Lake	102	0.623	0.606	10.13	8.34	5	-0.029
Primrose Lake	96	0.667	0.664	10.47	8.73	0	-0.003
Heart Lake	81	0.613	0.646	7.20	6.61	0	0.049
Lac La Biche	300	0.652	0.668	13.33	9.03	0	0.024*

* indicates significance at $p<0.05$

Table 3-3 Pairwise F_{ST} comparison among subpopulations. F_{ST} values (Weir & Cockerham, 1984) are on the lower half of the matrix. Significance is indicated on the upper half of the matrix where **, significant at $p<0.01$; n.s., not significant.

	Bistcho Lake	Lesser Slave Lake	Primrose Lake	Heart Lake	Lac La Biche
Bistcho Lake		**	**	**	**
Lesser Slave Lake	0.111		**	**	**
Primrose Lake	0.144	0.114		**	n.s.
Heart Lake	0.144	0.083	0.064		**
Lac La Biche	0.120	0.102	-0.001	0.057	

Table 3-4 Individual partitioning among genetic clusters at K=4. Each subpopulation of origin has individuals assigned to the most likely genetic cluster.

Subpopulation	Cluster 1	Cluster 2	Cluster 3	Cluster 4
Bistcho Lake	0.019	0.007	**0.950**	0.024
Lesser Slave Lake	**0.921**	0.020	0.025	0.034
Primrose Lake	0.015	0.021	0.017	**0.947**
Heart Lake	0.029	**0.907**	0.012	0.052
Lac La Biche	0.026	0.039	0.070	**0.865**

Table 3-5 Assignment of Lac La Biche samples summarized by year class. Individuals were assigned to a putative subpopulation of origin based on Bayesian clustering of genotypes. One sample was excluded from originating from any of the reference subpopulations and given an unknown origin designation. A mixture designation was given to samples that were not excluded from the reference subpopulations, but showed greater than 20% belonging to more than one cluster.

Age (Year Class)	Bistcho	Lesser Slave	Primrose	Heart	Mixture	Unknown
0 (2007)	18	0	25	0	0	0
1 (2006)	0	1	213	0	6	1
2 (2005)	0	0	7	0	0	0
3 (2004)	0	0	1	0	0	0
4 (2003)	0	0	0	1	0	0
5 (2002)	0	0	2	0	0	0
6 (2001)	0	0	2	0	1	0
9 (1998)	0	0	2	0	0	0
10 (1997)	0	0	1	1	0	0
11 (1996)	0	1	1	0	0	0
pre-1985	0	0	0	3	1	0
unknown	0	1	8	0	3	0

Table 3-6 Chi-squared (X^2) test for relative survival of different stock sources introduced into Lac La Biche in a single year. The proportion of stocked individuals in terms of the actual number samples was used to create the expected values. Observed values are the individuals genetically assigned to each source subpopulation.

Source	Year	Stocked	% of total	Sampled	Expected
Primrose	2007	21018154	46.6	25	20
Bistcho		24057973	53.4	18	23
two-tailed $X^2 = 0.1263$					
Primrose	2006	21672012	98.8	213	211
Lesser Slave		256463	1.2	1	3
two-tailed $X^2 = 0.2449$					

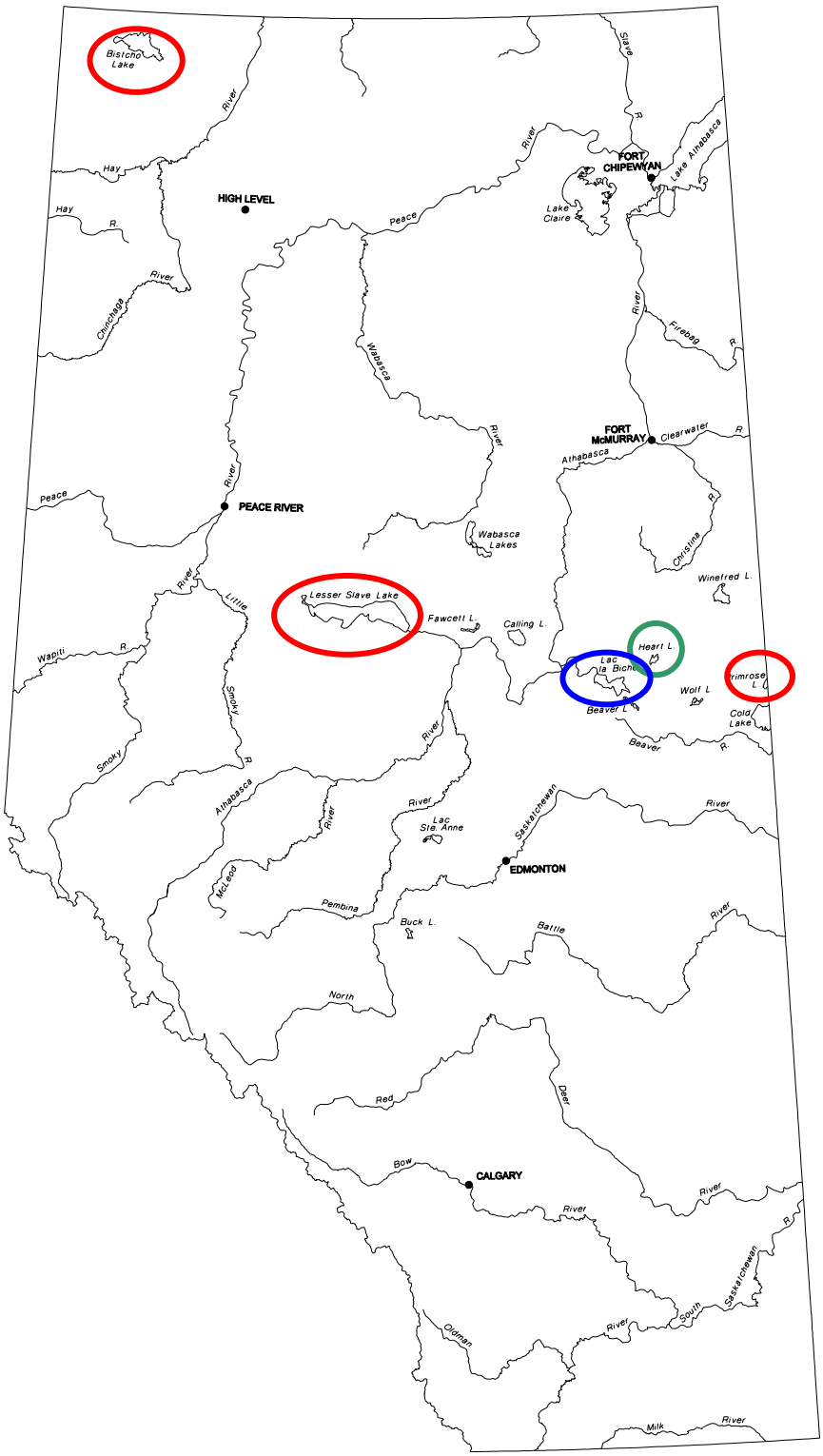

Figure 3-1 Sample collection locations. Walleye hatchery source lakes are indicated by red circles. Heart Lake is indicated by a green circle. Lac La Biche is indicated by a blue circle.

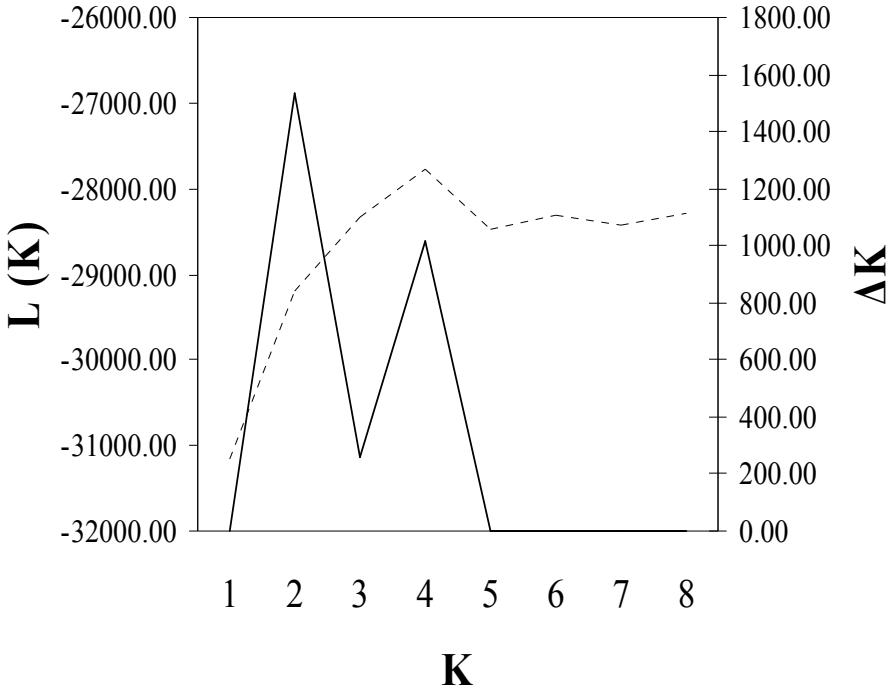

Figure 3-2 Determination of the most likely number of subpopulations (K) for the Lac La Biche, Primrose, Bistcho and Heart Lake grouping from 10 STRUCUTRE runs at each value of K. The solid line plots the change in ΔK and the dotted line is the likelihood of K.

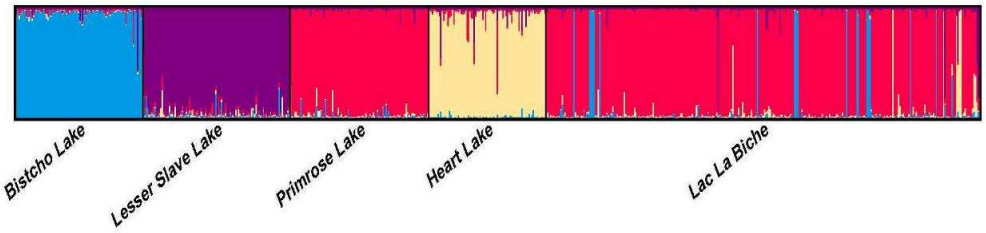

Figure 3-3 Partitioning of individuals between clusters at K=4. Each genetic cluster is represented by a single colour. Each individual is represented by a single vertical line which is partitioned into coloured segments representing the individuals cluster membership.

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Chapter 4: Forensic DNA analysis identifies illegal harvest of walleye (*Sander vitreus*) in Alberta

Introduction

Since the first acceptance of DNA evidence in a wildlife case in a North American court in 1991 (Guglich et al. 1993), the application of microsatellite DNA analysis to cases of wildlife crime has become more commonplace. DNA has provided crucial evidence in a number of wildlife offences including illegal killing of animals (Caniglia et al. 2009), trafficking (Wasser et al. 2004) and illegal possession of wildlife (Jobin et al. 2008). Terrestrial animals have been the focus of much of the use of DNA in wildlife forensic cases, but this technology is also applicable to marine and freshwater fishes (Hansen et al. 2001). The Alberta Fish and Wildlife Forensic Unit (AFWFU) has developed and used forensic DNA tests and databases for elk, moose, bighorn sheep, cougar, bear and deer (Jobin et al. 2008). These species are the highest profile targets for wildlife crime in Western Canada, but there is also a large amount of illegal harvest of freshwater fish species. Currently, fisheries enforcement in Alberta relies heavily on traditional patrols, spot checks and road side searches. These techniques are labour intensive and limited in the types of offences that they can detect. A powerful and probative DNA test for popular game fish species in Alberta would be valuable for enforcing fishing regulations and apprehending poachers.

Fishing restrictions in Alberta are usually species specific and directly linked to a specific body of water. Therefore, the ability to identify the body of water from which a fish was harvested would be useful for enforcement of fishing regulations. Identification of the population of origin for an individual fish can be accomplished through genetic assignment tests. Assignment tests determine the probability or likelihood that an individual originated from a specific population by comparing the individual's DNA profile to a genetic database of reference populations (Hansen et al. 2001). Assignment tests coupled with Monte Carlo

simulations can also be used to exclude populations as the origin of an individual. The probative power of these tests in a fisheries forensic context has already been shown by Primmer et al. (2000), Withler et al. (2004) and McCusker et al. (2008). There are a number of statistical methods available for assignment of individuals using genetic data and it is important to choose a method that that is appropriate for the hypothesis being tested and the biology of the species being investigated (Manel et al. 2005).

Walleye are one of the most popular sportfish in Alberta and contribute millions of dollars to the economy annually (Park 2007). There is also a small domestic and commercial walleye fishery operating in Alberta. Many of the Alberta walleye subpopulations are in a collapsed or vulnerable state (Berry 1995) and illegal harvest can be extremely damaging to the recovery and sustainability of these subpopulations. Sullivan (2002) estimates that approximately 18% of the protected-length walleyes caught by anglers in Alberta are illegally kept. This is likely an underestimate and does not include illegal harvest from catch and release fisheries or the commercial fishery. Once a walleye is harvested, one cannot use morphology to determine the lake of origin, therefore; fish can be caught illegally in a closed or restricted lake and then retained and/or marketed as a legal catch. Recent microsatellite DNA analysis of a number of walleye populations in Alberta showed that genetic population structure parallels the geographic structure resulting in each lake having a genetically distinct walleye subpopulation (Chapter 2). This baseline molecular data for Alberta walleye populations can be forensically applied to enforce harvest regulations.

While genetic data is available for Alberta walleye populations and has been used academically to investigate population structure, it is important that the databases are evaluated for forensic applications before being applied to active cases. There is no specific set of standards for fish and wildlife forensic DNA analysis, although best practice guidelines for animal forensic testing have been proposed (Budowle et al. 2005). In this case the genetic databases were evaluated

according to these recommendations and the established human forensic criteria insofar as possible. There are a number of quality assurance and methodology standards for DNA testing and databasing laboratories (O'Dell 2003). While it is often not possible to meet all the criteria set out for human forensic DNA analysis, every effort should be made to align locally developed wildlife DNA forensic procedures with recommended human forensic DNA testing quality control and quality assurance procedures. The forensic testing employed needs to be reproducible, conservative and transparent. This is true for both the laboratory methods and statistical calculations. The molecular data generated for Alberta walleye populations (Chapter 2) was treated as potential forensic databases and developed and validated accordingly.

Here I describe how molecular genetic assignment methods can be use to identify the subpopulation of origin of harvested walleye. In February of 2008, during a routine patrol on Lesser Slave Lake, Alberta Fish and Wildlife Officers seized two buckets of walleye pieces that were found hidden under the floorboards of an ice fishing hut (Figure 4-1). The number of fillets present would have put the angler over his daily catch limit. The angler claimed that the fish had been purchased commercially and produced a receipt. The only walleye commercial fishery that had been operating in the province during the time of the receipt was on North Wabasca Lake (Figure 4-1). The seized walleye samples were submitted to the Alberta Fish and Wildlife Forensic Unit for analysis with a request to determine the number of individual walleye present and whether they were more likely to have originated in Lesser Slave or North Wabasca Lake.

Methods and Analysis

Sample Collection and Preparation

The seized walleye parts were examined and an attempt to determine the minimum number of individuals was made by matching pairs of fillets. The majority of the fillets were incomplete or in multiple pieces and physical matching was not possible. A total of 47 complete and 2 partial fish cheeks were

identified among the seized samples. As the fish cheeks were intact and of a lower number than the various fillet pieces, a sample from the inner tissue of each cheek was taken for DNA analysis.

DNA Profile Development

Genotypes were developed for all individual samples using the same methodology as described in Chapter 2. All allele calls were confirmed by a second analyst.

Reference Lakes

DNA databases for the walleye subpopulations in Lesser Slave Lake (N=104) and North Wabasca Lake (N=43) have been previously compiled and analyzed. Genetic diversity, differentiation and structure have been characterized for both walleye subpopulations (Chapter 2).

Number of Individuals

I used the Excel Microsatellite Toolkit (Park 2001) to identify the number of unique genotypes among the seized walleye samples for which DNA profiles were developed.

Assignment

I assumed that all of the seized fish originated from a single source subpopulation and calculated pairwise F_{ST} comparisons (Weir and Cockerham 1984) between the seized group of fish and the two reference subpopulations. Significance of the pairwise comparisons was estimated with 1000 MCMC permutations in FSTAT (Goudet 1995, 2002).

In order to determine a potential subpopulation of origin, genotypes for the seized walleye samples were compared against the walleye genetic databases for the two potential source lakes. I used both the classical allele frequency based (Paetkau et al. 1995) and Bayesian (Rannala and Mountain 1997) assignment methods as implemented in GENECLASS2 (Piry et al. 2004). Alleles not found in

the database samples were given a frequency of 0.01. The likelihoods of the seized walleye genotypes arising in each reference subpopulation were compared as a likelihood ratio.

I also performed an exclusion calculation to confirm the assumption that one of my two reference subpopulations is in fact the true source subpopulation of the seized walleye samples. I simulated 10,000 individual genotypes using the Monte Carlo resampling approach of Paetkau et al. (2004) for both the frequency and the Bayesian methods within GENECLASS2 with the probability threshold set to 0.01.

Results

Of the 49 samples genotyped, all but five gave complete genotypes at the 15 loci. Of those five samples, one was missing information for three loci, one was missing information for two loci and three were missing information for one locus. All 49 genotypes were included in subsequent analyses.

Reference Lakes

Previous study (Chapter 2) has shown that Lesser Slave Lake and North Wabasca Lake walleye subpopulations were moderately genetically differentiated (pairwise $F_{ST} = 0.121$, $p<0.01$) and that each lake had a genetically distinct walleye subpopulation. Neither subpopulation exhibited significant departures from Hardy-Weinberg expectations.

Number of Individuals

I identified 24 individual genotypes from the 49 samples analyzed. The two partial fish cheeks shared the same genotype and matched another whole fish cheek. Each individual genotype identified was represented by a pair of fish cheeks that matched at all loci for which there was information.

Assignment

There was no significant genetic differentiation observed between the Lesser Slave Lake walleye subpopulation and the group of seized walleye samples (pairwise F_{ST}= 0.001, p>0.05) (Table 4-1). The pairwise F_{ST} estimate between the North Wabasca Lake walleye subpopulation and the group of seized samples (F_{ST}=0.142, p<0.01) was similar to that between the Lesser Slave Lake and North Wabasca Lake walleye subpopulations.

All seized walleye samples were assigned to the Lesser Slave Lake walleye subpopulation with both the classical and Bayesian assignment methods (Figure 4-2). The likelihood ratios for each individual genotype ranged from 39.0 to 2.92X10^4 for the classical assignment calculation and 6.46X10^3 to 1.51X10^20 for the Bayesian method (Table 4-2). The likelihood ratios calculated with the Bayesian method were higher for all individual genotypes. In both the classical and Bayesian assignment calculations, one sample from the Lesser Slave Lake reference database assigned to the North Wabasca walleye subpopulation (Figure 4-2). This was not the same individual in both calculations.

None of the seized walleye genotypes were excluded from the Lesser Slave Lake reference subpopulation (p<0.01). All but one genotype was excluded from the North Wabasca subpopulation (p<0.01) and that genotype was assigned a greater probability of originating from the Lesser Slave Lake subpopulation in both the frequency (P(LSL)=0.76, P(WAB)=0.06) and Bayesian (P(LSL)=0.81, P(WAB)=0.03) calculation methods.

Discussion

The genetic differentiation between the North Wabasca and Lesser Slave Lake walleye subpopulations was sufficient for genetic assignment of individual fish. All 24 individual genotypes from the seized walleye samples were more likely to have arisen from the Lesser Slave Lake walleye subpopulation than the

North Wabascsa Lake subpopulation. The Bayesian method resulted in larger likelihood ratios than the classical frequency based method. Taken alone, some of the likelihood ratios may not seem large enough to draw a firm conclusion regarding the origin of the individual fish. When viewed as a whole, it becomes significantly more likely that all 24 fish originated from Lesser Slave Lake as compared to North Wabasca Lake. An increase in the number of individuals for assignment can add power to the test.

The power of the assignment test also varies with the number of markers used (Bernatchez and Duchesne 2000), the differentiation between reference populations and the genetic structure of those populations (Hansen et al. 2001). It has been shown previously (Cornuet et al. 1999; Hansen et al. 2001) that F_{ST} between populations has a significant correlation to the accuracy of assignment tests and can be used as an initial indicator of population similarity. Cornuet et al. (1999) estimated that using 10 microsatellite loci and 30-50 individuals from each population will give a 100% correct assignment rate of individuals to populations if the genetic diversity between the populations is significant (F_{ST} >0.1). The number of samples, loci and the amount of genetic differentiation between the two potential source subpopulations in this case study meet these estimated criteria for 100% correct assignment. The ten other walleye subpopulations that have been genetically characterized (Chapter 2) meet these general criteria for number of loci and samples, but some pairwise comparisons have F_{ST} values less than 0.1. Individual assignment between subpopulations with lower pairwise F_{ST} comparisons may not be as significant.

I chose to use both the classical frequency based assignment test described by Paetkau et al. (1995) and the Bayesian method of Rannala and Mountain (1997). Both methods have their strengths and weaknesses and it is important to understand these in order to choose the appropriate method for the hypothesis in question. Bayesian likelihood tests have been shown to be slightly more accurate than the classical method (Cornuet et al. 1999) but are controversial because they

depend on the validity of prior distribution and this cannot be tested statistically. Also, Bayesian calculations may be less sensitive to deviations from true allele frequencies or small sample sizes. The classical frequency based method assumes that the allele frequency data collected for the reference populations is an accurate representation of the true population values. This may not be true, depending on the sample size and distribution of the data set. The choice between the two likelihood methods may depend on how representative of the population one believes the reference datasets are and the amount of migration and admixture in those populations. In a forensic context, I recommend reporting the likelihood ratios from the classical assignment test. Forensically, statistical calculations need to be transparent and conservative. Classical likelihood calculations rely on observed data, have unambiguous assumptions and allow for a clear comparison between two hypotheses. The constant value for missing data, minimum allele frequencies and the calculations of genotype frequencies can be easily modified to make the likelihood ratio conservative in favour of the defence hypothesis. Bayesian methods of assignment can be calculated by a number of different software programs but the creation of the prior distribution and the calculations employed are not explicit, making the method less transparent to the end user. The Bayesian method could be used for additional support or to lend added weight to results, if necessary.

While exclusion may be the most universally applied assignment test in a forensic context (Glover et al. 2009) in this case it was unnecessary as I was able to directly evaluate the evidence under both the defence and prosecution hypotheses. I chose to include an exclusion test in order to confirm my assumption that the actual source for the fishes in question was included in my analysis and to add support to my conclusions. The exclusion method is potentially very useful in the forensic context for cases where the source population may not be present in the analysis or there is only genetic information available for one reference population. However, forensic scientists should be wary of using exclusion or probabilities as the sole statistical method for

assignment. There are inherent concerns regarding translating a significance value into a definitive legal statement, such as uncertainty in the genetic data and an inability to evaluate the evidence under both hypotheses (Ogden 2008). While the courts may be more familiar with the concept of likelihood ratios; the ability to assign a threshold or confidence to a result can be valuable. Combining both an assignment and exclusion test may be a preferred option for forensic casework. There may be situations where an unknown sample cannot be excluded from all but one population (i.e. when reference populations have minimal differentiation between them) and a likelihood based assignment would resolve the issue. Being able to state that the unknown individuals are most likely to originated from a specific population as well as that other populations can be excluded as potential sources lends more weight to the conclusion.

Conclusion

The case described above was taken to the provincial court of Alberta. The accused pled guilty and received a $2000 fine and a 1-year sport-fishing license suspension. Without the genetic data the Fish and Wildlife Officers would not have been able to determine that the commercial receipt alibi was false.

The combination of microsatellite data and powerful statistical software will allow for greater enforcement of walleye harvest restrictions. In the past, individuals who illegally harvested or trafficked walleye could only be convicted if they were apprehended while in the act of committing the offence. Now the individual fish can be linked to a population of origin after being removed from the body of water. The presence of a forensic capacity for walleye enforcement will not only expand the ability to detect and convict resource abusers, but it will also increase the perception of detection. This increased perception of detection could result in lower occurrences of illegal harvest as Walker et al (2007) observed a trend towards reduced illegal harvest of northern pike (*Esox lucius*) on lakes where anglers perceived high deterrence due to increased enforcement

efforts. A similar deterrent effect may occur with anglers perceiving a higher probability for detection of illegal harvest due to the availability of DNA testing.

Table 4-1 Pairwise F_{ST} comparisons between walleye reference database subpopulations and the group of seized walleye samples (unknown). Significance of the comparisons was test with 1000 permutations in FSTAT (Goudet 1995). *, significant at $p<0.01$; n.s., not significant.

	Lesser Slave	North Wabasca	unknown
Lesser Slave		*	ns
North Wabasca	0.120		*
unknown	0.001	0.142	

Table 4-2 Likelihood ratios of assignment for genotypes from seized walleye samples.

Unknown Genotype	Assigned Subpopulation	Likelihood Ratio LSL/WAB	
		frequency	Bayesian
1	LSL	1.42x10^12	1.53x10^16
2	LSL	3.54x10^5	1.21x10^8
3	LSL	2.75x10^13	1.55x10^19
4	LSL	4.20x10^5	3.08x10^9
5	LSL	1.98x10^12	3.97x10^16
6	LSL	3.46x10^7	4.48x10^10
7	LSL	5.55x10^3	4.49x10^6
8	LSL	1.01x10^6	1.10x10^7
9	LSL	2.76x10^10	1.78x10^16
10	LSL	3.68x10^13	7.67x10^17
11	LSL	1.24x10^7	2.08x10^9
12	LSL	1.04x10^5	2.34x10^9
13	LSL	1.42x10^8	1.60x10^11
14	LSL	2.92x10^14	1.51x10^20
15	LSL	3.94x10^9	1.36x10^12
16	LSL	9.38x10^6	3.52x10^7
17	LSL	3.70x10^9	5.62x10^14
18	LSL	5.18x10^11	6.03x10^16
19	LSL	3.90x10^1	6.46x10^3
20	LSL	6.62x10^8	3.18x10^14
21	LSL	2.35x10^9	2.36x10^15
22	LSL	1.37x10^9	2.43x10^12
23	LSL	5.62x10^7	1.23x10^11
24	LSL	1.11x10^13	3.46x10^19

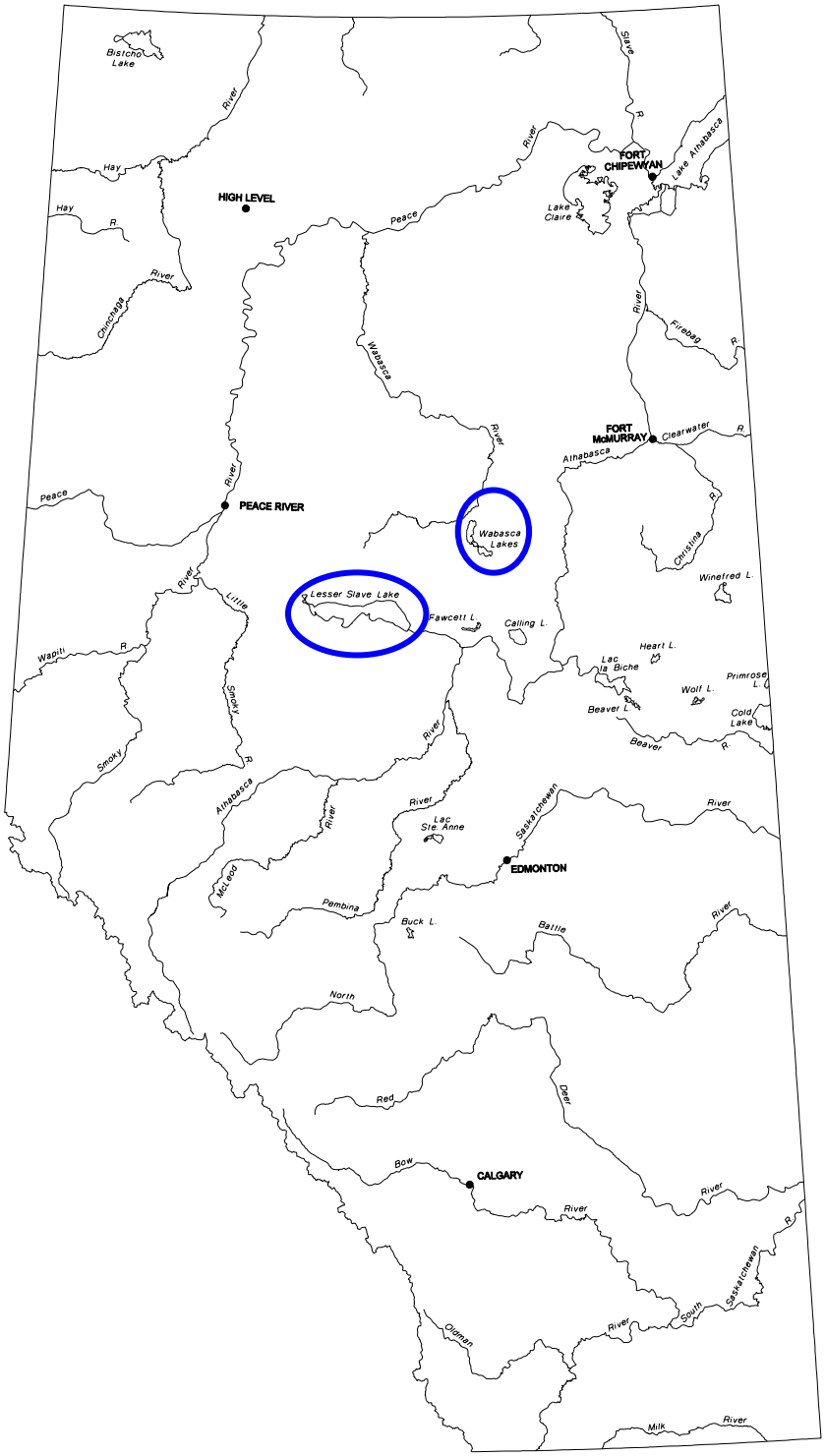

Figure 4-1 Sample collection locations for walleye reference databases. Lakes from which walleye samples were collected are indicated by blue circles.

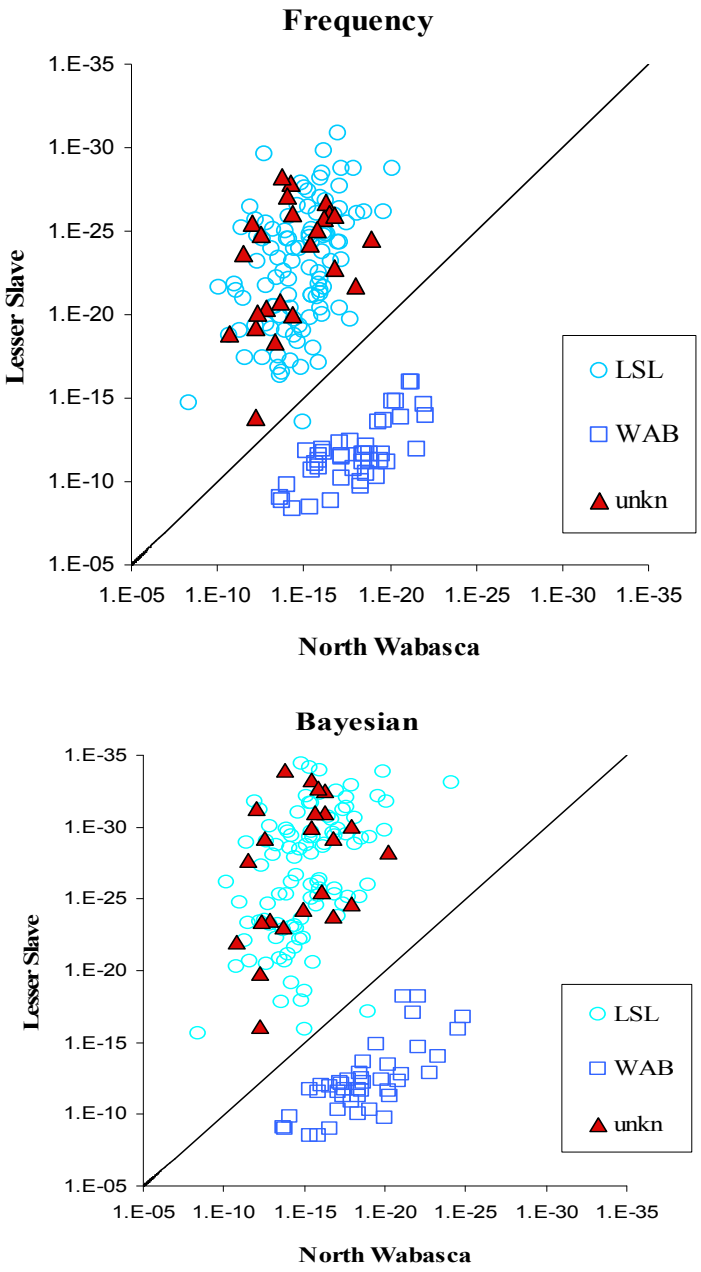

Figure 4-2 Likelihood based assignment of unknown samples to a reference subpopulation. Individuals are plotted according to the likelihood that their genotypes would arise in either North Wabasca Lake or Lesser Slave Lake. The diagonal line indicates an equal likelihood to either subpopulation. The frequency based calculation of Paetkau et al (1995) is the upper graph and the Bayesian method of Rannala & Mountain (1997) is the lower graph.

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Chapter 5: Discussion

Walleye Population Structure and Genetic Diversity in Alberta

Walleye in Alberta do not comprise a single panmictic population but instead exhibit hierarchical population genetic structure among lakes and river basins. I found that each of the twelve lakes included in this study have genetically distinct walleye subpopulations within the larger population of the river basin. My study agrees with the previous work of Thomas et al. (1999) that concluded distinct walleye populations exist within the Parkland region of Alberta. The broad-scale walleye population structure in Alberta aligns with the contemporary hydro-geographic landscape. My analyses support the hypothesis of hierarchical partitioning of genetic variation based on geographic patterning. In general, subpopulations from the same river basin were more similar than subpopulations from different river basins. I observed a broad-scale isolation by distance pattern where waterway distance and river basin boundaries have similar correlations to genetic distance. As the current hydro-geography of Alberta has likely been relatively stable since the retreat of glacial Lake Agassiz following the end of the Pleistocene glacial period, populations that have been separated by river basin boundaries are unlikely to have had any significant gene flow between them over the last 10,000 years. Mutation and drift would have functioned to create genetic differentiation between these populations. The higher θ_{ST} values between populations that have likely been separated for longer times suggest that the river basin boundaries have been functioning as barriers to gene flow resulting in vicariant populations. Subpopulation structure within river basins is defined by habitat connectivity and behaviour. The genetic differentiation pattern suggests that geographic separation is functioning along with natal site fidelity to maintain distinct subpopulations. Philopatry has been identified as a contemporary mechanism for maintaining historical genetic diversity in other walleye populations (Strange and Stepien 2007) and other species, such as rainbow smelt (*Osmerus mordax*; Bernatchez 1997). The genetic pattern of walleye subpopulations in Alberta is likely the result of behaviour and environmental

barriers to gene flow. This is congruent with what is previously described for other walleye populations in North America (Stepien et al. 2009).

Microsatellite DNA variation of Alberta walleye populations revealed lower diversity than that reported for Quebec (Dupont et al. 2007) and Great Lakes (Strange and Stepien 2007; Stepien et al. 2009) walleye populations. Due to inherent differences in the markers used for population characterization it is difficult to determine if the lower diversity is a result of the study design or an accurate reflection of differences between the populations. The recent study of Stepien et al. (2009) included walleye populations from the Hudson Bay drainage and found that western populations exhibited lower diversity and significant genetic differentiation from Great Lakes walleye populations. This lower diversity for walleye populations outside of the Great Lakes watershed could be attributed to populations originating from different glacial refugia, population sizes, restricted geographic patterning, or the life history of the individual populations studied. Lower diversity in Alberta walleye could also be due to environmental conditions as Alberta walleye have longer generation times and lower productivity than fish that are living in warmer, more productive climates. Despite possible lower overall diversity than other walleye populations in North America, walleye in Alberta exhibit sufficient diversity for each lake to have its own genetically distinct population.

Management Implications

The identification of genetically distinct walleye subpopulations in each of the lakes included in this study creates important considerations for fisheries managers in Alberta. The province of Alberta has an active walleye management and stocking program. While each lake is currently treated as an independent management unit the stocking program gives little consideration to genetic diversity when deciding on hatchery stock sources and recipient lakes. I illustrated the utility of using the genetic data to assess walleye stocking events in Lac La Biche (see Chapter 2). The genetic analysis suggested that the Lac La Biche

walleye subpopulation is genetically similar to a subpopulation outside of the Athabasca River basin (Primrose Lake). As it is possible for walleye to migrate out of Lac La Biche, this change in population genetics could potentially alter the natural genetic diversity in the Athabasca River basin. Knowing that each sample site investigated in this study has its own genetically distinct subpopulation of walleye, matching the genetics of the brood stock to the lake or river basin is a factor that should be taken into consideration for translocations. It has long been recommended that stocking programs should avoid mixture of stocks from different regions in order to conserve the genetic integrity of populations (Jennings and Philipp 1992) and to avoid outbreeding depression. Incorporating genetic tagging with the current walleye hatchery program in Alberta would allow for more informative assessment and monitoring of the walleye hatchery program.

Evolutionary Significant Units (ESU's) have not been officially defined for walleye in Alberta, but the general consensus is that, at a minimum, each watershed should be considered a single unit and that fish should not be transferred across these boundaries (Johnston and Paul 2006; Sullivan 2008). The genetic structure identified with this study supports this recommendation. The results of this study could be used to aide in the delineation of ESUs and to refine current management units for Alberta walleye. Expanding the genetic characterization of walleye populations in Alberta would be useful for conservation planning, monitoring population changes and assessing management activities.

Enforcement Applications

Genetic data for local walleye populations has the potential to be valuable for fisheries enforcement. There is sufficient genetic differentiation between the walleye subpopulations included in this study to assign individual fish to a putative subpopulation of origin, as illustrated by the forensic case study (Chapter 3). The combination of microsatellite data and powerful statistical software allows

for enforcement of harvest restrictions through DNA profile comparisons and population assignment. The power of the statistical test will vary depending on the number of individual fish involved, the genetic data available and the specific populations being compared (Bernatchez and Duchesne 2000; Hansen 2001). I recommend conservative frequency based likelihood comparisons for forensic DNA population assignment. While Bayesian methods may perform better academically (Cornuet et al. 1999), transparent and conservative calculations are more important forensically. There may be case scenarios where an exclusion test is the only option. In this case it is imperative that the probabilities are interpreted correctly. Incorrect interpretation or misleading statements may result in the evidence not being accepted. Forensic methodology and statistics for fish and wildlife DNA evidence should strive to meet the standards established by the human forensic DNA community.

The baseline of genetic data for walleye populations developed in the course of this study gives wildlife officers the ability to link an individual fish to a population of origin after it has been removed from a body of water. This eliminates the need for *in situ* detection of illegal harvest in order to gain a conviction. The ability to use DNA analysis as an enforcement tool may also reduce the amount of illegal harvest of walleye through a deterrent effect (Walker et al. 2007).

Conclusion

The current microsatellite genetic data for walleye populations in Alberta should aid in fish management by providing information that will guide decisions designed to conserve genetic diversity as well as to detect and assist in convicting individuals who illegally take and/or traffic in walleye. Hopefully more walleye populations in Alberta will be genetically characterized and that the molecular data will be incorporated into the provincial active management plan. The incorporation of genetic sampling with the current Fall Walleye Index Netting

program could be extremely useful for population assessment and monitoring. The techniques and application outlined in this study could potentially be useful in the management of other freshwater fish species in Alberta.

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