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Genetic diversity among walleye (Sander vitreus) populations in inland Ontario: Relationships with life history traits.

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Genetic diversity among walleye (*Sander vitreus*) populations in inland Ontario: Relationships with life history traits.

by

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Abstract

This thesis focuses on the relationships between genetic diversity, environmental parameters, and fitness in walleye (*Sander vitreus*: Percidae), as well as the influences of natural and anthropogenic factors on walleye population structure across the province of Ontario. 11 selectively neutral microsatellite DNA loci were used to characterize the genetic diversity and population structure of 46 populations of walleye. Genetic diversity estimates (observed heterozygosity (H_O), mean square allelic distance (d^2), and Wright's inbreeding coefficient (F_{IS})) revealed high levels of genetic diversity. The relationships observed between lake parameters and population genetic diversity revealed positive relationships between lake surface area, growing degree days, number of species, and hatchery supplementation versus H_O and d^2. The only significant positive correlation observed between life history traits and genetic diversity was early growth rate versus H_O for male and female walleye. The relationship between F_{IS} and male early growth rate was significant ($p < 0.01$) and marginally non – significant for females ($p= 0.06$), and both relationships had negative slopes, indicative of inbreeding effects. No significant relationships were observed for d^2 with any life history trait.

A moderate level of population differentiation ($F_{ST} = 0.155$, 95% CI 0.125 - 0.185) exists among the 46 walleye populations. AMOVA revealed that among populations, the variance explained by within primary drainage basins (14.3 %) was more than 10 times of the variance explained by among drainage basins (1.2 %). Neighbour-joining cluster analysis did not show strong clustering of populations within drainage basins, but it did show a distinct pattern of population division into northern vs. southern clades. Geographic patterning of differentiation among four of the five primary drainage basins could not be

explained by the isolation-by-distance model. Results of genotype assignments indicated that gene flow was likely occurring through natural as well as anthropogenic means. The genetic structure of Ontario's walleye populations is being influenced by hatchery supplementation; overall population differentiation for walleye among the 23 sampled hatchery lakes (F_{ST} =0.142; 95% CI = 0.117 – 0.166) and 23 non-hatchery supplemented lakes (F_{ST} = 0.167; 95% CI = 0.131 – 0.202) were significantly different ($t = -5.2$, $df = 504$, $p < 0.001$). The results of this study provides evidence for gene flow among populations and that Ontario walleye are not exhibiting equilibrium conditions between drift and migration. These results have implications for understanding the relative importance of natural and anthropogenic factors in defining genetic diversity among populations.

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STATEMENT OF ORIGINALITY

This is to certify that, except where specific reference is made, the work contained in this project is my own work.

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Chapter 1

General Introduction

Genetic diversity is the raw material that evolution acts upon to define a species over time; the greater the genetic diversity within a species the greater the potential for a species to evolve in a changing environment (Hartl and Clarke, 1997). Genetic diversity is deemed critical for the conservation and long-term survival of a population because it provides the resources to maintain high levels of fitness and long-term potential to adapt to ongoing environmental change (Frankel and Soulé, 1981). Variation in genetic diversity among populations results from a combination of ecological factors, environmental stochastic events, mating systems, and various genetic processes (i.e. gene flow, genetic drift, and natural selection) that influence the genetic composition and structure of a population (Roff and DeRose, 2001). Such environmental and genetic processes change the allelic frequencies within populations, such that gene flow tends to make populations more similar whereas random genetic drift typically results in differentiation among populations. On the other hand, natural selection changes allelic frequencies by acting on phenotypes within a population; allele frequencies change as the best suited genotypes survive preferentially and those of less well suited genotypes will decrease from generation to generation. Populations that exhibit low levels of genetic diversity are thought to be potentially limited in their ability to respond evolutionarily to changing environmental conditions, hence resulting in reduced long-term population viability (Amos and Harwood, 1998). Correlations between genetic diversity and fitness are interpreted as evidence for the direct effect of genetic diversity on fitness, where individuals / populations with low levels of genetic diversity (increased homozygosity) will be relatively unfit compared to individuals / populations with higher

levels of genetic diversity. Therefore, it is essential to quantify the amount and distribution of genetic diversity across the geographical range of a species and to identify possible factors that may influence genetic diversity in populations so as to facilitate effective conservation and management of species at risk.

It is not always clear what effect genetic drift has on the genetic structure of a species over time, and studies are generally designed to describe population genetic structure among spatially separated populations. Environmental processes can impact the genetic diversity of populations through its effects on gene flow, genetic drift, and natural selection, where environmental heterogeneity and differences in selection pressures among habitats will result in genetic differentiation among populations. For example, spatially limited habitats should be occupied by smaller populations and thus exhibit lower genetic diversity due to drift and inbreeding (see Yamamoto *et al.*, 2004). Likewise, the productivity of a habitat is expected to have an effect on population size since higher productivity can support a greater numbers of individuals (Rigler, 1977), and hence higher levels of genetic diversity. Since environmental factors may modify genetic diversity correlations, they must have a role in shaping the overall population genetic structure within a species. Thus, population size is likely an important factor for short term survival since small populations are likely more susceptible to stochastic events (i.e. environmental, catastrophic; Frankham, 1995). Stochastic events reducing a population's size would have a greater effect on the genetic diversity of smaller populations since they would be more vulnerable to genetic bottleneck effects that would rapidly reduce genetic diversity, which would result in the fixation of deleterious alleles due to genetic drift and increased inbreeding events (Luikart *et al.*, 1998).

All things being equal, smaller populations generally exhibit lower levels of genetic diversity than larger populations (Amos and Harwood, 1998). Small populations that are reproductively isolated are more likely to be subject to increased inbreeding events, which will result in increased homozygosity and loss of alleles (Hartl and Clarke, 1997). Increased homozygosity will lead to increased expression of deleterious alleles and negatively affect fitness (i.e. inbreeding depression) since related individuals are more likely to share the same recessive allele than would unrelated individuals. Whereas large randomly mating populations exhibit higher levels of genetic diversity as a result of mating between unrelated individuals within a population (outbreeding), however progeny from outbreeding between genetically distant parents may experience a loss of fitness ("outbreeding depression"; Frankham, 1995). Outbreeding depression is attributed to the loss of local adaptation due to the mixing of genomes that evolved within differing environments and / or the disruption of co-evolved gene interactions (Templeton, 1986).

Numerous studies have attempted to correlate genetic diversity with measures of evolutionary fitness (see references in Chapter 2). Fitness is defined as the ability to survive to reproductive age and produce viable offspring. Generally, fitness is very difficult to directly measure (Stearns, 1992); hence surrogate life history traits that are closely related to fitness are often used. For example, fitness traits related to reproduction (fecundity), survival (mortality, hatching rates), and growth (length at age, early growth rate) can be measured at the individual or population level. The relationships between life history trait values and genetic diversity measures can be examined at the individual level (the number of heterozygous loci per individual) and at the population level using comparisons of mean population genetic diversity and life history traits among two or more populations (Wang *et*

al., 2002). Correlations between measures of fitness and genetic diversity measures (i.e. heterozygosity, mean squared allelic distance, Wright's F-statistics) can be useful to infer inbreeding or outbreeding depression effects (Charlesworth and Charlesworth, 1999). If these relationships are common, the reduction of genetic diversity and the loss of fitness has significant implications for conservation and long-term persistence of species at risk.

Across the geographical range of many species, spatial, temporal, and behavioral reproductive isolation contribute to the observed genetic diversity and structure among individual populations. The consequence of reproductive isolation is that genetic divergence among populations is dependent upon the rate of migration (gene flow) between populations (Amos and Harwood, 1998). When gene flow among populations is sufficiently high, the genetic differentiation between populations is low, however when gene flow among populations is restricted, isolated populations exhibit greater genetic differentiation due genetic drift and natural selection (Slatkin, 1993). Anthropogenic impacts can also contribute to the population structure through artificial gene flow (e.g. hatchery supplementation or unauthorized introductions) or by restricting gene flow (e.g. habitat fragmentation or local population extirpation). Ultimately, environmental and anthropogenic factors are both important contributors to observed genetic diversity and structure, although few studies have specifically examined either for impacts on multiple populations (see Brunner *et al.*, 1998; Gatt *et al.*, 2002; Castric and Bernatchez, 2003).

Among-population genetic divergence is usually described in terms of population structure, based on genetic distances and differentiation indexes (i.e. the fixation index, F_{ST}). These measures are used to describe genetic relationships among populations by comparing the frequency of nuclear alleles or mtDNA haplotypes among populations. As populations

become isolated from one another, the more differentiated they will likely become due to drift and/or selection, hence we can group and compare populations based on relative genetic distances to form clusters of populations that are closely related (i.e. sharing of more recent common ancestors). Estimating genetic divergence and phylogeographic relationships among populations is useful to describe relationships among populations and provide insight into the ecology and conservation priorities of a species.

Genetic structure can be partitioned into within- and among-population components. The driving factors behind the observed population genetic divergence within a species are not always clear, but phylogeographic patterns can be used to infer the differing effects of natural, historical, and anthropogenic effects on the current geographic and population genetic structure of a species (Bernatchez and Wilson, 1998). For example, post glacial colonization by aquatic species would have been limited to the habitats made available as the glaciers retreated, and landscape features would impose restrictive barriers to gene flow between the new populations. Over time, population differentiation would occur as opportunities for gene flow are constrained by the limited connectivity between aquatic habitats. Such processes are particularly important to consider when evaluating the current population genetic structure of aquatic species in the Northern Hemisphere (Castric *et al.*, 2001). On the other hand, the population genetic structure of a species may also be influenced by anthropogenic effects, such as hatchery supplementation (i.e. stocking of artificially reared fish) events, which may reduce population differentiation by genetically homogenizing the genetic structure among populations (Miller and Senanan, 2003). As isolated populations experience drift and genetic bottlenecks over time, they will become fixed for alternate alleles at some loci, and artificial gene flow, through the introduction of

non-native fish, will lead to an artificial increase in the level of genetic diversity within the target population. To ensure both the short- and long-term genetic adaptation within a species (i.e. local adaptations, maintaining genetic diversity), both within- and among-population genetic diversity needs to be maintained.

This thesis focuses on the relationships between genetic diversity, environmental parameters, and fitness in walleye (*Sander vitreus*: Percidae), as well as the influences of natural and anthropogenic factors on walleye population structure across the province of Ontario. Walleye are commonly found in freshwater and sometimes brackish waters. In North America, walleye occupy the St. Lawrence-Great Lakes, Arctic, and Mississippi River basins and range from Quebec to Northwest Territories, and south to Alabama, Mississippi, and Arkansas, in addition to introductions elsewhere in the Atlantic, Gulf, and Pacific drainages (Scott and Crossman, 1998). Spawning occurs in the spring or early summer and takes place in a variety of lake habitats (i.e. rocky reefs, gravel beds, submerged vegetation) and stream headwaters or riffle areas (Jennings *et al.*, 1996; Scott and Crossman, 1998). Larvae emerge within 1-3 weeks, depending on latitude and water temperature, and disperse to lentic areas until moving into deeper water habitats (Stepien and Faber, 1998); adult walleye have been observed to exhibit a migratory behaviour and may migrate as far as 160 kilometres between habitats (Becker, 1983; Jennings *et al.*, 1996). Males sexually mature after 2-4 years while females mature after 3-8 years (Colby and Nepszy, 1981; Scott and Crossman 1998).

This thesis incorporates two separate investigations regarding genetic diversity and structure of inland lake populations of walleye in Ontario. Chapter 2 addresses the relationships between selected physical habitat parameters and three selected measures of

population genetic diversity. Chapter 2 also examines the relationships between the population genetic diversity estimates and life history traits closely associated with fitness as a means to investigate inbreeding and outbreeding effects across the sampled populations. Chapter 3 examines the population genetic structure of walleye in Ontario, and tests for potential natural and anthropogenic gene flow that would influence the population structure among the sampled populations. The approach of this study is novel in that it utilizes eleven microsatellite loci to conduct a detailed analysis of the fine scale genetic diversity and structure within and among 46 walleye populations across a broad spatial range. This study provides information critical for the effective conservation and management of not only Ontario walleye, but for all northern freshwater species of fish, since environmental and anthropogenic effects on genetic diversity and potential inbreeding or outbreeding depression across populations are demonstrated.

References Cited:

Amos W, Harwood J (1998) Factors affecting levels of genetic diversity in natural populations. *Philosophical Transactions of the Royal Society of London* (B), **353**, 177 – 186.

Becker, G. C. (1983) *Fishes of Wisconsin.* University of Wisconsin Press, Madison, MI.

Bernatchez L, Wilson CC (1998) Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology*, **7**, 431 – 452.

Brunner PC, Douglas MR, Bernatchez L (1998) Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr *Salvelinus alpinus* (Teleostei: Salmonidae) from central Alpine lakes. *Molecular Ecology*, **7**, 209 – 223.

Castric V, Bonney F, Bernatchez L (2001) Landscape structure and hierarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. *Evolution*, **55**, 1016 – 1028.

Castric V, Bernatchez L (2003) The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). *Genetics*, **163**, 983 – 996.

Charlesworth B, Charlesworth D (1999) The genetic basis of inbreeding depression. *Genetical Research*, **74**, 329 – 340.

Colby PJ, Nepszy SJ (1981) Variation among stocks of walleye (*Stizostedion vitreum vitreum*): management implications. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1814 – 1831.

Frankel OH, Soulé ME (1981) *Conservation and Evolution.* Cambridge University Press, New York, USA.

Frankham R (1995) Conservation genetics. *Annual Review of Genetics*, **29**, 305 – 327.

Gatt MH, Fraser DJ, Liskauskas AP, Ferguson MM (2002) Mitochondrial DNA variation and stock structure of walleyes from eastern Lake Huron: an analysis of contemporary and historical samples. *Transactions of the American Fisheries Society*, **131**, 99 – 108.

Hartl D, Clarke AG (1997) *Principles of Population Genetics*, 3rd ed., Sinauer Associates, Sunderland, MA, 1997.

Jennings MJ, Claussen JE, Phillip DP (1996) Evidence for heritable preferences for spawning habitat between two walleye populations. *Transactions of the American Fisheries Society*, **125**, 978 – 982.

Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. *Molecular Ecology*, **7**, 963 – 974.

Rigler FH (1977) Lakes. 1. North America. Limnology in the high Arctic: a case study of Char Lake. *International Association of Theoretical and Applied Limnology*, **20,** 127 – 140.

Roff DA, DeRose MA (2001) The evolution of trade – offs: effects of inbreeding on fecundity relationships in the cricket *Gryllus firmus*. *Evolution*, **55**, 111 – 121.

Scott WB, Crossman EJ (1998) Freshwater Fishes of North America. Galt House Publications, Oakville, Canada.

Slatkin M (1985) Isolation by distance in equilibrium and non-equilibrium populations. *Evolution*, 47, 264 – 279.

Stearns SC (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

Stepien CA, Faber JE (1998) Population genetic structure, phylogeography and spawning philopatry in walleye (*Stizostedion vitreum*) from mitochondrial DNA control region sequences. *Molecular Ecology*, **7**, 1757 – 1769.

Templeton AR (1986) Coadaptation and outbreeding depression. In *Conservation Biology. The science of scarcity and diversity* (ed. ME Soulé), pp. 105 – 116. Sinauer Associates, Inc., Sunderland, MA.

Yamamoto S, Morita K, Koizumi I, Maekawa K (2004) Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial-temporal changes in gene frequencies. *Conservation Genetics*, **5**, 529 – 538.

Wang S, Hard JJ, Utter F (2002) Genetic variation and fitness in salmonids. *Conservation Genetics*, **3**, 321 – 333.

Chapter 2

Inbreeding, outbreeding and environmental effects on genetic diversity in 46 walleye (*Sander vitreus*) populations.

Abstract

Genetic diversity is recognized as an important population attribute for both conservation and evolutionary purposes; however, the functional relationships between the environment, genetic diversity, and fitness-related traits are poorly understood. Few empirical studies have examined if relationships exist among those parameters at the population level, across a broad geographic range. We initially examined relationships between selected lake parameters and population genetic diversity measures in 46 walleye (*Sander vitreus*) populations across the province of Ontario, Canada, and then tested for relationships between six life history traits (relative fecundity, gonadosomatic index, early growth rate, condition factor, total length, mortality) that are closely related to fitness (growth, reproductive investment, and mortality) and genetic diversity measures (11 microsatellite loci; heterozygosity, d^2, and Wright's inbreeding coefficient). Positive relationships were observed between lake surface area, growing degree days, number of species, and hatchery supplementation versus genetic diversity. Walleye early growth rate was the only life history trait significantly correlated with population heterozygosity in both males and females. The relationship between F_{IS} and male early growth rate was significant ($p < 0.01$) and marginally non – significant for females ($p= 0.06$), and both relationships had negative slopes indicative of inbreeding depression. No significant relationships were observed for d^2 and any life history trait. Stepwise regression models showed that surface area and heterozygosity had a

significant effect on female early growth rate, while hatchery supplementation, surface area and heterozygosity had a significant effect on male early growth rate. Population heterozygosity and F_{IS} proved better estimators of mean population genetic variability than population mean d^2 to assess inbreeding effects, while population mean d^2 was a better metric to determine the effects of lake parameters and hatchery supplementation on genetic diversity. The significant positive relationship between lake parameters and hatchery supplementation versus genetic diversity suggests the presence of inbreeding effects, but no evidence of outbreeding was observed. However, the weak relationships between genetic diversity and life history traits indicate that inbreeding and outbreeding depression are not yet seriously impacting Ontario walleye populations.

Introduction

Genetic variation provides the heritable genetic resource that serves as the basis for evolutionary change, and loss of genetic diversity may, over time, negatively impact a population's viability. Variation in genetic diversity among populations results from a combination of ecological factors, environmental stochastic events, mating systems, and various genetic processes (i.e. gene flow, population bottlenecks, and genetic drift) that influences the genetic composition and population structure of a species (Roff and DeRose, 2001). Alternatively, changes in allele frequencies within a population resulting from selection can alter the genetic characteristics of a population and serves as a basis for local adaptation (Hartl and Clark, 1997). Genetic variation has a critical role in the long-term viability of a population that manifests in its ability to persist and adapt to a stochastic environment (Lande and Shannon, 1996). Where populations are relatively small and isolated, long – term viability and the ability to adapt to stochastic environmental events may be compromised since genetic variation decreases as the likelihood of inbreeding events increase (Frankham, 1995b). On the other hand, outbreeding occurring between unrelated individuals is expected to increase genetic variation and ultimately, increase the fitness of offspring (heterosis) or result in lower offspring fitness (outbreeding depression) (Westemeier *et al.*, 1998). Still, specific changes in fitness resulting from inbreeding or outbreeding may vary since progeny fitness is dependent upon both the genetic architecture of a population as well as environmental influences on particular genotypes (Hedrick and Kalinkowski, 2000). Therefore, the long-term maintenance of genetic diversity, and possibly long-term population persistence, requires an understanding of environmental and mating system effects.

Various environmental factors, both natural and anthropogenic, have been shown to affect genetic diversity. Habitat size has been used as a surrogate for population size and, all things being equal, the genetic diversity of a population in a relatively smaller habitat should be lower than in populations occupying larger habitats (Heath *et al.*, 2002; Ihssen *et al.*, 1988; Yamamoto *et al.*, 2004). In addition to habitat size, habitat quality may also impact genetic diversity among populations; the carrying capacity of northern lake habitats is relatively low and characterized by a shorter growing season and lower aquatic productivity relative to southern lakes (Rigler, 1977). The lower carrying capacity of northern lakes is therefore expected to result in smaller populations, and ultimately lead to an expectation of lower genetic diversity. In addition, supplementing a population with hatchery-reared fish can inflate genetic diversity relative to unstocked lakes. Reproduction between wild and hatchery individuals in a population can, depending on the genetic background of the introduced fish, alter the genetic architecture of the population through the introduction of novel alleles, and the genetic homogenization of stocked populations (Largiadèr and Scholl, 1995). Thus, the combination of natural and anthropogenic effects can impact genetic diversity through changes in the likelihood of inbreeding (i.e. small population size or genetic bottlenecks) or outbreeding (i.e. hatchery supplementation).

Regardless of the cause, inbreeding results in a loss of genetic variation through matings between related individuals (Wang *et al.*, 2002) and any resulting loss of fitness due to inbreeding is commonly referred to as inbreeding depression. Numerous studies provide empirical evidence of inbreeding depression within small populations of laboratory and captive organisms (see review in Charlesworth and Charlesworth, 1987) as well as in wild populations (Coltman *et al.*, 1998; Marshall and Spalton, 2000; see reviews in DeRose and

Roff, 1999; Crnokrak and Roff, 1999). Two genetic mechanisms for inbreeding depression have been proposed: 1) an increased frequency of the expression of homozygous deleterious recessive alleles reduces fitness (i.e. dominance hypothesis; Charlesworth and Charlesworth, 1987), and 2) heterozygosity at specific gene loci is intrinsically beneficial (heterozygote superiority), hence the loss of genome-wide heterozygosity due to inbreeding reduces fitness (i.e. overdominance hypothesis; Charlesworth and Charlesworth, 1987). Regardless of the actual mechanism, the impact of inbreeding depression on life history traits that are closely related to fitness can be dramatic (see DeRose and Roff, 1999).

Outbreeding, the mating of genetically distant related individuals, has been used to artificially increase genetic variation and fitness for species exhibiting obvious inbreeding depression (e.g. Roelke *et al.*, 1993; Westemeier *et al.*, 1998). Although outbreeding increases the overall genetic diversity of offspring in genetically depauperate populations, progeny from outbreeding events may alternatively experience a loss of fitness ("outbreeding depression"; Frankham, 1995a). Outbreeding depression is suspected to result from: 1) a loss of local adaptation due to the mixing of genomes that evolved independently in accordance to different native environments (Phillip and Claussen, 1995), and/or 2) the disruption of beneficial gene interactions that have co-evolved (i.e. coadapted gene complexes; Lynch, 1991). Evidence for outbreeding depression in natural populations is limited, but indirect evidence has been reported in mammals (Marshall and Spalton, 2000) and fish (Phillip and Whitt, 1991; Neff, 2004), while direct evidence for outbreeding depression has been reported in plants (Waser and Price, 1989) and fish (Gharrett *et al.*, 1999). Most of those studies showed that offspring displaying extreme levels of genetic diversity exhibited lower fitness-related life history traits (e.g. survival, fecundity) than individuals with intermediate levels of

genetic diversity. Therefore, significant correlations (either positive or negative) between genetic diversity and individual fitness may result from either inbreeding or outbreeding within a population (Neff, 2004).

Fitness is often defined as the ability to survive to reproductive age and produce viable offspring, but is generally very difficult to measure directly (Stearns, 1992). Thus, life history traits that are directly related to fitness (i.e. reproduction, survival, and growth) are often used as surrogate measures of fitness. Since inbreeding is expected to result in a loss of heterozygosity and reduced fitness, it is expected to generate correlations between heterozygosity and fitness trait measures (Tsitrone *et al.*, 2001). Numerous studies have reported heterozygosity-fitness correlation for a variety of organisms including marine molluscs (e.g. Pogson and Zouros, 1994), fish (e.g. Heath *et al.*, 2002; Knaepkens *et al.*, 2002; Borrell *et al.*, 2004), mammals (e.g. Coulson *et al.*, 1998), and plants (e.g. Fischer and Matthies, 1998). However, several studies have provided evidence that these relationships are not universal (e.g.: Rowe *et al.*, 1999; for review see Wang *et al.*, 2002). In addition, a review by David (1998) indicated that correlations between heterozygosity and surrogate fitness measures can vary among species as well as among studies of the same species. A meta-analysis concluded that correlations between heterozygosity and fitness measures generally exhibited weak or non-significant relationships (Britten, 1996). Nevertheless, a detailed analysis of the correlations between measures of fitness and genetic diversity can be useful to infer inbreeding or outbreeding depression effects (Charlesworth and Charlesworth, 1999).

Although numerous analyses of heterozygosity-fitness correlations have been conducted at the individual fish level (e.g.: Thelen and Allendorf, 2001; Heath *et al.*, 2002;

Borrell *et al.*, 2004), few investigations comparing genetic diversity and fitness related life history traits among populations have been reported (i.e.: Knaepkens *et al.*, 2002; Shikano and Taniguchi, 2002). Since inbreeding is typically a population level effect, a comparison of population level genetic diversity and/or inbreeding measures should provide a powerful test for inbreeding or outbreeding depression. No analysis of this type has yet been conducted among multiple populations across a broad geographic range of natural fish populations.

This study examines the relationships between genetic diversity (estimated using 11 microsatellite loci) and selected lake parameters chosen to reflect habitat size (lake surface area), habitat productivity (growing degree days and number of species), and hatchery supplementation among Ontario, Canada walleye (*Sander vitreus*) populations. Walleye inhabit a wide variety of freshwater habitats across North America and exhibit a high degree of variability in life history traits (Colby and Nepszy, 1981; Scott and Crossman, 1998; Henderson and Morgan, 2002). Although few population genetic studies have been published for walleye, walleye populations that are geographically separated have been shown to be genetically distinct (Ihssen and Martin, 1995; Billington and Strange, 1995) hence individual populations likely represent independent genetic groups. We further examine the relationship between genetic diversity and life history traits reflecting growth, reproduction and mortality, all critical components of lifetime fitness. Simple and multiple regression analyses are used to examine the relationships between lake parameters and genetic diversity as well as the relationships between genetic diversity and life history traits among the 46 Ontario walleye populations. Such an approach will allow us to measure environmental effects on genetic diversity, and in turn, determine if the variation in genetic

diversity associated with environmental variation is translated into inbreeding or outbreeding depression.

Materials & Methods

Sample Collection: Forty six inland lakes, relative to the Laurentian Great Lakes, across Ontario (Figure 1; see Table 1 for corresponding lake names) were sampled for walleye; the numbers of individuals collected varied across populations (min. n = 82, max. n = 1462; mean n = 316). All sampling was conducted by the Ontario Ministry of Natural Resources (OMNR) utilizing the Fall Walleye Index Netting (FWIN) sampling protocols described by Morgan (2002). FWIN protocols follow a standardized stratified random sampling design that is used to assess the relative abundance of walleye and to provide estimates of various life history parameters of the target populations (Morgan, 2002). Each fish collected was assigned a unique serial number and identified by sex and gonad development; total length and round weight data was recorded. Scales, dorsal spines, and / or otoliths were collected (for aging) and a subset of the sampled specimens was shipped to the laboratory for DNA extraction and genetic analysis.

Lake Parameter Analysis: We selected four lake parameters to characterize local lake habitats. The selected parameters exhibit a broad degree of variability across lakes (Figure 2; Appendix 1). As a measure of potential available habitat, lake surface area (ha) was selected as an indicator of potential available habitat (e.g. see Heath *et al.*, 2001), and the lake surface area values were log transformed to reduce the distribution skew. Since fish activity and metabolism in temperate zones can be limited by water temperature (Shuter and Post, 1990), we used growing degree days (GDD) above 5°C, based on the Ontario climate model (Mackey *et al.*, 1996), as an index of lake temperature. Species richness of the fish community in each lake was estimated using the total number of species caught during FWIN sampling.

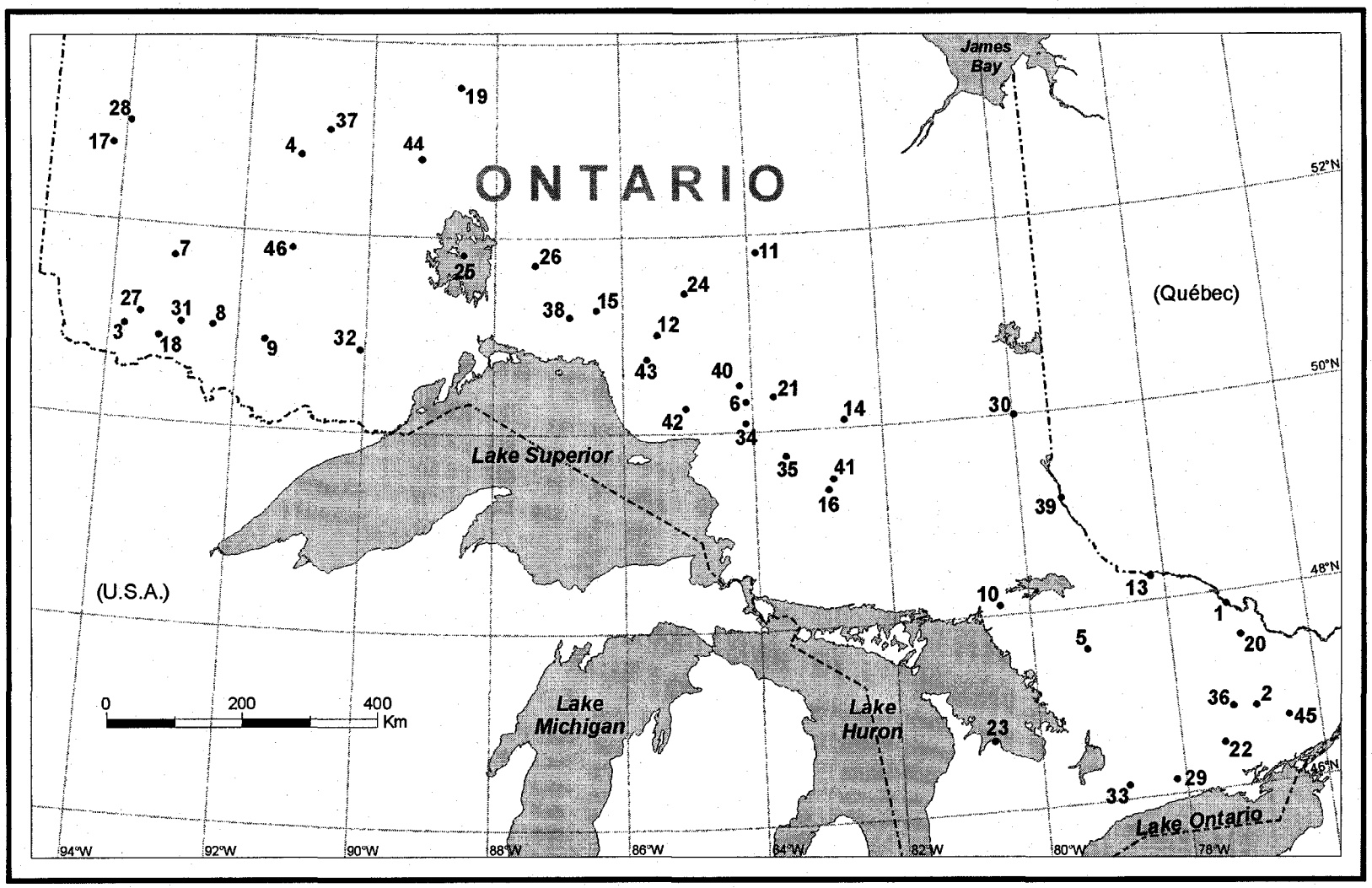

Figure 1: Map showing the lakes sampled for walleye in the province of Ontario, Canada. Numbers correspond with lake names are provided in Table 1.

Table 1: Genetic diversity and marker information for 11 micosatellite loci in 46 Ontario walleye populations (ID #'s refer to code numbers in Figure 1). Estimates of observed heterozygosity (H_o), squared allelic distance (d^2), and Wright's inbreeding coefficient (F_{IS}) are given for each population and locus. The number of alleles per locus (A), and number individuals amplified per locus (*n*) is shown for each population and locus. Tests for departure from Hardy - Weinberg at all loci across all lakes after Bonferroni correction (n = 506 tests) are denoted in boldface as significantly different from H_E (* $P < 0.001$).

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Allumette	1	**H_o**	0.83	0.88	0.72	0.60	0.81	0.79	0.84	0.67	0.79	0.80	0.60
		d^2	13.1	12.9	3.9	5.2	8.1	12.9	15.9	10.7	10.4	24.2	7.1
		F_{IS}	0.05	0.06	0.05	0.21	-0.05	-0.14	0.06	0.13	0.08	0.05	-0.17
		A	16	18	7	9	10	14	14	8	18	10	5
		n	47	43	47	43	43	43	43	42	43	44	43
Big Gull	2	**H_o**	0.79	0.88	0.67	0.79	0.80	0.79	0.73	0.62	0.80	0.91	0.72
		d^2	12.1	11.3	4.3	8.7	8.5	7.1	8.4	12.4	13.7	20.3	6.7
		F_{IS}	0.11	0.03	0.15	0.03	0.10	0.11	0.13	0.13	0.15	-0.01	0.12
		A	15	19	8	11	13	14	12	8	21	16	9
		n	42	42	46	47	46	47	48	37	45	43	47
Burditt	3	**H_o**	0.74	0.86	0.65	0.64	0.69	0.83	0.83	0.71	0.88	0.60	0.72
		d^2	9.9	10.6	4.5	5.9	7.7	13.2	9.4	13.2	16.6	20.6	7.2
		F_{IS}	0.14	0.06	0.10	0.11	0.16	0.07	0.03	0.03	0.07	0.24	-0.14
		A	15	16	8	10	9	13	13	14	25	11	8
		n	43	42	43	45	45	41	46	45	42	42	46

Table 1 cont'd

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Churchill	4	H_o	0.72	0.86	0.75	0.85	0.67	0.76	0.85	0.52	0.87	0.79	0.84
		d^2	7.6	9.6	4.1	8.4	7.0	8.8	6.6	10.9	14.4	28.9	7.9
		F_{IS}	0.14	0.03	-0.03	-0.04	0.16	-0.001	-0.03	0.01	0.05	0.08	-0.12
		A	13	16	7	12	9	12	9	7	20	21	7
		n	47	43	48	48	48	46	46	48	47	48	45
Deer	5	H_o	0.52	0.87	0.13	0.76	0.79	0.90	0.82	0.74	0.73	0.69	0.67
		d^2	5.1	8.1	4.3	6.3	6.1	7.3	9.8	4.8	18.9	9.8	7.6
		F_{IS}	0.15	-0.003	-0.05	0.05	-0.13	-0.08	-0.03	-0.07	0.05	0.05	-0.09
		A	7	16	3	9	7	10	8	9	9	8	5
		n	42	47	47	46	48	48	45	46	48	48	48
Dog	6	H_o	0.72	0.80	0.65	0.46	0.43	0.85	0.78	0.30	0.87	0.59	0.61
		d^2	5.2	15.2	4.2	8.3	8.5	12.7	10.4	6.3	17.7	25.2	6.4
		F_{IS}	0.09	0.12	0.07	0.27	0.06	0.02	-0.01	0.03	0.03	-0.17	0.07
		A	8	13	4	7	6	13	10	8	13	12	5
		n	46	46	46	46	46	46	46	46	46	46	46
Eagle	7	H_o	0.88	0.86	0.71	0.90	0.83	0.59	0.80	0.59	0.85	0.74	0.50
		d^2	9.19	8.63	4.94	5.81	7.00	7.69	5.24	8.33	18.44	27.06	5.57
		F_{IS}	0.02	0.07	0.01	-0.11	-0.001	0.09	0.02	0.13	0.10	0.00	0.18
		A	19	19	8	16	10	16	11	9	32	14	8
		n	48	44	45	48	46	44	46	41	48	46	46
Eltrut	8	H_o	0.76	0.85	0.56	0.68	0.58	0.77	0.73	0.82	0.81	0.62	0.54
		d^2	9.17	11.53	3.70	4.29	5.39	11.00	9.04	12.93	18.91	13.24	6.89
		F_{IS}	0.08	0.01	-0.01	0.11	-0.03	0.09	0.05	-0.08	0.12	0.01	0.18
		A	13	14	6	6	8	14	10	9	18	10	7
		n	38	40	48	50	40	39	37	34	43	47	50

Table 1 cont'd

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Finlayson	9	H_o	0.74	0.80	0.62	0.62	0.50	0.71	0.69	0.60	0.80	0.85	0.55
		d^2	9.74	10.24	4.00	14.62	3.73	6.00	7.17	10.24	17.09	31.14	6.25
		F_{IS}	0.14	0.13	-0.21	0.24	0.17	0.14	0.15	0.17	0.16	-0.05	0.14
		A	14	16	6	9	6	14	10	13	25	14	6
		n	42	41	42	42	44	41	42	42	44	41	44
French	10	H_o	0.76	0.89	0.79	0.70	0.76	0.86	0.79	0.72	0.86	0.90	0.70
		d^2	12.06	14.31	4.59	7.33	8.44	9.68	11.29	15.23	21.89	30.11	6.65
		F_{IS}	0.13	0.004	0.01	-0.03	0.10	0.05	0.07	-0.01	0.07	-0.05	0.13
		A	15	14	6	11	13	15	13	11	24	19	8
		n	41	44	43	43	42	43	43	43	44	41	44
Fushimi	11	H_o	0.78	0.85	0.72	0.77	0.64	0.78	0.78	0.51	0.89	0.74	0.65
		d^2	5.55	11.33	4.06	8.79	10.21	11.81	12.90	7.24	17.49	21.16	5.67
		F_{IS}	0.12	0.03	0.03	0.02	0.17	0.10	-0.10	0.06	0.04	0.003	0.05
		A	14	17	6	11	12	14	7	7	23	12	5
		n	40	46	43	43	45	41	40	41	44	42	46
Garnham	12	H_o	0.87	0.86	0.70	0.57	0.43	0.66	0.61	0.67	0.57	0.62	0.75
		d^2	6.10	6.59	2.13	4.92	9.10	6.48	11.00	10.75	16.32	12.14	7.64
		F_{IS}	-0.08	-0.001	-0.27	0.02	0.28	0.003	0.19	-0.06	0.22	0.13	-0.11
		A	11	14	4	6	5	10	8	9	18	6	9
		n	47	43	43	46	46	44	46	36	44	45	44
Holden	13	H_o	0.75	0.85	0.83	0.58	0.75	0.74	0.70	0.78	0.92	0.87	0.50
		d^2	11.9	13.2	3.4	5.8	12.0	11.2	16.6	10.4	13.4	25.7	7.6
		F_{IS}	0.13	0.09	-0.08	0.23	0.05	0.03	0.15	0.03	0.001	-0.02	0.21
		A	11	22	6	10	12	18	12	12	19	16	10
		n	48	48	48	48	48	47	47	46	48	47	48

Table 1 cont'd

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Ivanhoe	14	H_o	0.81	0.79	0.73	0.66	0.73	0.82	0.67	0.86	0.84	0.43	0.81
		d^2	10.2	10.9	3.4	5.2	7.6	6.3	9.7	10.9	15.3	24.1	5.9
		F_{IS}	0.01	0.10	-0.05	0.08	-0.03	-0.05	-0.11	-0.13	0.03	0.21	-0.18
		A	11	15	6	6	5	9	8	8	11	11	4
		n	42	43	44	47	45	45	46	43	44	44	47
Kagiano	15	H_o	0.89	0.85	0.60	0.72	0.57	0.74	0.66	0.74	**0.81***	0.81	0.62
		d^2	10.0	8.7	3.3	9.2	8.1	9.7	8.3	10.0	14.6	37.7	7.5
		F_{IS}	0.01	0.04	0.10	0.08	0.23	0.08	0.10	0.01	0.09	-0.01	0.03
		A	16	16	5	7	7	12	5	8	17	19	6
		n	47	47	47	47	47	46	47	46	47	47	47
Kebskwasheshi	16	H_o	0.85	0.85	0.71	0.43	0.64	0.74	0.65	0.70	0.58	0.48	0.40
		d^2	12.8	4.9	2.7	4.6	4.4	9.6	10.9	11.9	6.5	19.3	7.9
		F_{IS}	-0.03	0.003	0.01	0.31	0.04	-0.03	0.08	-0.15	0.20	0.12	0.10
		A	13	13	7	8	6	13	6	7	10	10	7
		n	46	46	45	44	44	43	43	47	43	46	45
Longlegged	17	H_o	0.67	0.85	0.60	0.56	0.56	0.86	0.84	0.44	0.84	0.82	0.65
		d^2	8.4	7.5	4.5	5.7	6.2	12.3	7.6	9.4	16.4	42.4	6.1
		F_{IS}	0.21	0.05	0.15	0.18	0.24	0.02	-0.05	0.23	0.10	-0.03	0.09
		A	9	14	6	8	8	14	13	6	19	18	7
		n	42	46	43	45	43	42	45	45	43	45	46
Mainville	18	H_o	0.85	0.88	0.64	0.73	0.77	0.86	0.85	0.77	0.83	0.88	0.57
		d^2	9.9	8.9	3.9	5.6	6.4	10.7	7.2	11.7	15.7	27.8	6.4
		F_{IS}	0.04	-0.01	0.08	0.002	0.05	0.03	-0.12	-0.12	0.12	-0.12	0.02
		A	15	12	7	9	8	13	7	12	30	10	6
		n	47	48	50	49	48	49	48	47	48	48	47

Table 1 cont'd

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Miminiska	19	H_o	0.78	0.95	0.79	0.80	0.65	0.78	0.80	0.66	0.84	0.88	0.45
		d^2	7.0	13.0	4.2	7.4	8.1	7.1	7.7	10.9	17.4	30.0	6.3
		F_{IS}	0.12	-0.04	0.02	0.04	0.17	0.03	0.06	-0.02	0.10	-0.02	0.26
		A	14	19	6	12	10	13	11	8	25	16	7
		n	41	44	42	44	43	40	44	41	43	41	44
Mink	20	H_o	0.92	0.87	0.82	0.78	0.96	0.71	0.81	0.54	0.98	0.93	0.91
		d^2	11.2	11.5	3.7	6.9	8.2	7.5	13.8	10.0	17.6	19.0	8.3
		F_{IS}	-0.01	0.02	-0.13	0.04	-0.08	0.14	0.05	0.08	-0.03	-0.05	-0.21
		A	14	17	7	13	14	13	11	12	24	14	10
		n	51	38	44	51	47	49	48	41	47	41	43
Missinaibi	21	H_o	0.64	0.84	0.73	0.58	0.44	0.79	0.63	0.53	0.81	0.57	0.60
		d^2	3.4	13.3	3.3	5.8	4.7	12.2	9.6	8.4	16.7	6.9	6.1
		F_{IS}	0.08	0.09	0.10	0.26	-0.07	0.10	0.18	0.08	0.10	0.11	0.03
		A	10	17	7	10	5	15	11	6	17	9	6
		n	42	38	41	43	43	43	43	38	42	42	43
Moira	22	H_o	0.81	0.64	0.70	0.64	0.74	0.76	0.83	0.56	0.84	0.76	0.61
		d^2	11.5	11.9	3.0	4.2	6.7	6.6	12.7	11.4	25.2	19.0	5.5
		F_{IS}	0.07	0.19	0.06	-0.01	0.07	-0.02	-0.07	0.05	0.10	-0.04	0.03
		A	13	12	8	7	8	9	9	8	21	12	6
		n	42	47	43	47	46	46	47	45	43	50	46
Mountain	23	H_o	0.90	0.87	0.61	0.72	0.78	0.84	0.79	0.54	0.82	0.74	0.80
		d^2	10.22	15.38	3.41	9.33	8.80	7.89	7.58	13.62	16.06	25.00	6.91
		F_{IS}	0.01	0.07	0.20	0.14	0.10	0.04	0.04	0.15	0.15	0.10	0.04
		A	13	20	8	8	12	12	13	9	28	12	9
		n	40	45	44	46	45	45	42	39	44	43	44

Table 1 cont'd

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Nagagami	24	H_o	0.69	0.78	0.57	0.74	0.54	0.85	0.87	0.78	0.87	0.80	0.52
		d^2	6.32	10.11	3.44	5.65	5.68	10.51	9.50	10.40	16.35	26.32	5.83
		F_{IS}	0.17	0.04	0.10	0.05	-0.10	0.02	-0.07	-0.11	0.003	0.06	0.25
		A	10	13	5	8	6	14	10	6	16	17	6
		n	45	45	44	46	46	46	46	45	46	46	46
Nipigon	25	H_o	0.87	0.83	0.64	0.70	0.57	0.79	0.76	0.46	0.78	0.91	0.40
		d^2	11.06	12.97	4.30	10.45	7.11	10.52	6.19	11.88	17.31	41.86	7.67
		F_{IS}	0.04	0.10	0.09	0.17	0.12	0.07	0.12	0.16	0.15	0.01	0.15
		A	18	17	5	13	10	14	10	8	22	27	8
		n	39	40	42	44	47	39	41	37	45	47	45
Partridge	26	H_o	0.74	0.77	0.78	0.68	0.75	0.79	0.64	0.75	0.48	0.75	0.50
		D^2	8.45	8.88	5.88	3.53	7.82	13.82	6.74	7.20	7.43	18.79	5.64
		F_{IS}	0.001	0.04	-0.01	-0.02	-0.01	0.09	0.04	0.01	0.09	-0.05	-0.01
		A	10	12	7	6	5	10	5	6	5	9	6
		n	42	44	41	44	44	42	42	40	44	44	44
Pipestone	27	H_o	0.85	0.83	0.57	0.71	0.72	0.89	0.79	0.83	0.83	0.81	0.55
		d^2	9.74	12.21	4.62	5.41	6.53	9.27	8.32	16.32	19.44	24.92	7.54
		F_{IS}	-0.02	0.09	0.05	-0.03	0.07	-0.04	0.01	-0.15	0.11	-0.01	-0.08
		A	12	16	6	8	7	14	8	10	19	12	6
		n	46	47	46	48	47	46	47	46	47	48	47
Red	28	H_o	0.81	0.78	0.68	0.71	0.85	0.81	0.79	0.61	0.88	0.80	0.69
		d^2	10.47	9.55	3.38	7.71	8.29	8.92	5.89	12.43	20.43	27.35	6.18
		F_{IS}	0.05	0.13	-0.11	0.16	-0.03	0.07	0.04	0.09	0.04	0.10	-0.19
		A	16	13	7	12	10	16	12	9	26	15	6
		n	47	40	47	48	48	48	48	46	48	46	48

Table 1 cont'd

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Shikwamkwa	34	H_o	0.93	0.87	0.83	0.66	0.64	0.91	0.83	0.64	0.79	0.67	0.72
		d^2	9.19	11.30	4.62	6.39	7.33	8.76	22.31	15.21	14.29	18.93	5.65
		F_{IS}	-0.10	0.03	-0.05	0.18	0.14	-0.02	-0.05	0.09	0.13	0.07	-0.02
		A	15	15	7	9	11	16	12	14	15	8	8
		n	45	46	47	47	47	46	47	44	43	42	47
Sideburned	35	H_o	**0.58***	0.98	0.90	0.71	0.52	0.87	0.65	0.77	0.96	0.79	0.75
		d^2	5.86	17.57	4.28	8.76	6.24	9.70	7.35	8.80	14.17	12.58	6.61
		F_{IS}	0.24	-0.16	-0.10	0.10	-0.15	-0.03	-0.04	-0.10	-0.09	-0.09	-0.14
		A	10	17	6	9	6	12	6	9	14	6	7
		n	48	48	48	48	48	46	48	39	48	48	48
Skootamatta	36	H_o	0.77	0.94	0.80	0.73	0.86	0.78	0.88	0.60	0.83	0.87	0.81
		d^2	11.65	12.40	4.33	5.54	9.00	5.64	7.92	9.85	16.41	18.50	6.21
		F_{IS}	0.16	-0.01	0.02	0.08	0.02	0.10	-0.10	-0.02	0.12	0.004	-0.03
		A	15	19	8	11	13	14	12	8	21	16	9
		n	44	48	45	48	58	58	58	43	47	46	58
St. Joseph	37	H_o	0.81	0.87	0.68	0.67	0.78	0.87	0.88	**0.87***	0.79	0.79	0.76
		d^2	9.43	7.74	5.53	7.07	7.26	7.64	7.06	9.49	21.94	40.29	8.06
		F_{IS}	0.06	0.06	0.05	0.11	-0.04	-0.07	-0.05	-0.22	0.09	0.06	-0.02
		A	16	18	9	11	9	13	11	6	23	26	7
		n	43	45	44	45	45	45	41	45	42	43	45
Steel	38	H_o	0.77	0.77	0.61	0.70	0.67	0.84	0.78	0.87	0.89	0.79	**0.47***
		d^2	17.8	16.2	7.5	4.7	7.7	5.1	9.0	14.6	13.5	24.1	7.2
		F_{IS}	0.10	0.13	-0.03	0.11	0.11	-0.002	-0.05	-0.09	0.01	-0.11	0.39
		A	14	17	7	7	8	10	10	12	18	8	6
		n	44	47	46	46	43	45	45	46	45	47	47

Table 1 cont'd

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Rice	29	H_o	0.92	0.88	0.80	0.78	0.86	0.85	0.97	0.64	0.81	0.87	0.71
		d^2	13.89	12.83	4.31	8.38	7.00	8.29	7.14	11.20	14.13	20.00	6.93
		F_{IS}	0.004	0.05	0.004	0.09	-0.03	0.02	-0.13	0.06	0.14	0	0.12
		A	18	18	8	10	11	12	13	11	22	11	9
		n	38	41	40	41	37	41	36	39	37	39	42
Round	30	H_o	0.80	0.82	0.67	0.66	0.62	0.68	0.81	0.93	0.78	0.53	0.76
		d^2	6.06	6.50	2.71	7.68	4.69	5.27	9.74	10.31	22.78	21.57	8.23
		F_{IS}	0.01	-0.02	-0.01	0.15	0.05	0.10	0.05	-0.07	0.10	-0.04	-0.10
		A	14	14	4	8	5	8	15	12	17	10	9
		n	44	44	46	47	42	44	47	28	46	43	46
Sakwite	31	H_o	0.81	0.78	0.80	**0.63***	0.81	0.89	0.84	0.76	0.89	0.67	0.71
		d^2	7.43	8.78	5.73	6.41	6.82	9.23	5.00	10.55	18.93	28.27	7.82
		F_{IS}	0.08	0.07	0.04	0.12	-0.06	-0.13	-0.07	-0.10	0.06	0.01	-0.03
		A	15	14	7	7	9	14	8	8	31	13	7
		n	43	46	46	46	48	44	45	38	46	45	48
Savanne	32	H_o	0.65	0.71	0.59	0.66	0.49	0.77	0.81	0.54	0.86	0.33	**0.24***
		d^2	8.69	5.24	3.15	10.24	2.64	3.83	6.29	13.47	5.32	30.53	2.67
		F_{IS}	0.13	0.17	-0.23	0.19	-0.10	-0.18	-0.15	0.02	-0.01	0.15	0.37
		A	12	10	4	10	4	9	7	6	15	10	7
		n	40	41	44	50	45	47	42	28	44	45	50
Scogogg	33	H_o	0.89	0.86	0.73	0.80	0.85	0.86	0.78	0.80	0.87	0.76	0.85
		d^2	12.87	13.41	3.45	7.84	7.08	6.11	7.61	10.00	11.79	24.29	7.28
		F_{IS}	0.01	0.05	0	0.01	0.01	-0.03	0.04	-0.03	0.09	0.08	-0.02
		A	15	18	6	11	12	13	10	12	23	17	9
		n	44	43	45	46	46	43	46	40	45	45	46

Table 1 cont'd

Lake	ID #		Pfla 2	Svi 1	Svi 2	Svi 4	Svi 5	Svi 7	Svi 8	Svi 9	Svi 10	Svi 11	Svi 17
Timiskaming	39	H_o	0.68	0.83	0.66	0.53	0.62	0.87	0.60	0.78	0.91	0.70	0.67
		d^2	8.3	7.5	3.9	8.3	9.1	8.7	22.2	12.4	16.2	22.1	6.4
		F_{IS}	0.23	0.10	0.02	0.23	0.11	0.01	0.21	0.02	0.02	0.05	0.12
		A	10	18	6	11	9	14	11	11	20	11	6
		n	40	41	44	45	45	45	45	41	44	43	45
W. Kabenung	40	H_o	0.24	0.80	0.18	0.02	0.60	0.51	0.31	0.63	0.74	0.88	0.45
		d^2	3.6	3.8	2.8	2.0	3.0	6.6	6.2	5.9	12.8	9.1	3.9
		F_{IS}	0.09	-0.03	0.05	0.66	-0.05	0.18	0.13	-0.15	0.02	0.04	0.26
		A	5	8	3	3	4	7	7	3	9	18	5
		n	42	41	44	43	43	43	42	41	43	41	44
Wabatongushi	41	H_o	0.70	0.89	0.50	0.47	0.43	0.84	0.53	0.50	0.85	0.47	0.66
		d^2	3.8	10.6	4.3	3.6	3.0	6.6	12.8	4.6	20.4	2.9	6.5
		F_{IS}	-0.08	0.003	0.22	0.25	0.04	-0.01	0.06	-0.11	-0.04	0.04	0.14
		A	6	17	6	6	6	13	6	5	17	7	7
		n	47	47	46	47	47	45	47	38	47	47	47
Wakami	42	H_o	0.66	0.82	0.43	**0.23***	0.69	0.81	0.65	0.47	0.72	0.40	0.77
		d^2	3.0	8.6	2.4	3.6	4.5	7.8	11.7	11.3	14.1	18.7	7.9
		F_{IS}	0.11	0.07	-0.04	0.62	-0.02	-0.03	0.05	0.02	0.06	0.06	-0.20
		A	7	12	3	4	4	11	6	5	8	5	5
		n	47	44	47	48	48	48	48	45	47	48	48
White	43	H_o	0.83	0.91	0.86	0.86	0.61	0.93	0.81	0.74	0.87	0.84	0.67
		d^2	7.1	10.7	4.1	6.7	8.9	10.1	7.6	9.5	16.7	23.3	7.2
		F_{IS}	0.06	0.03	-0.10	-0.06	0.03	-0.004	0.04	0.01	0.08	0.06	0.06
		A	14	15	7	6	5	17	10	9	20	11	6
		n	54	56	57	57	57	57	57	54	55	56	57

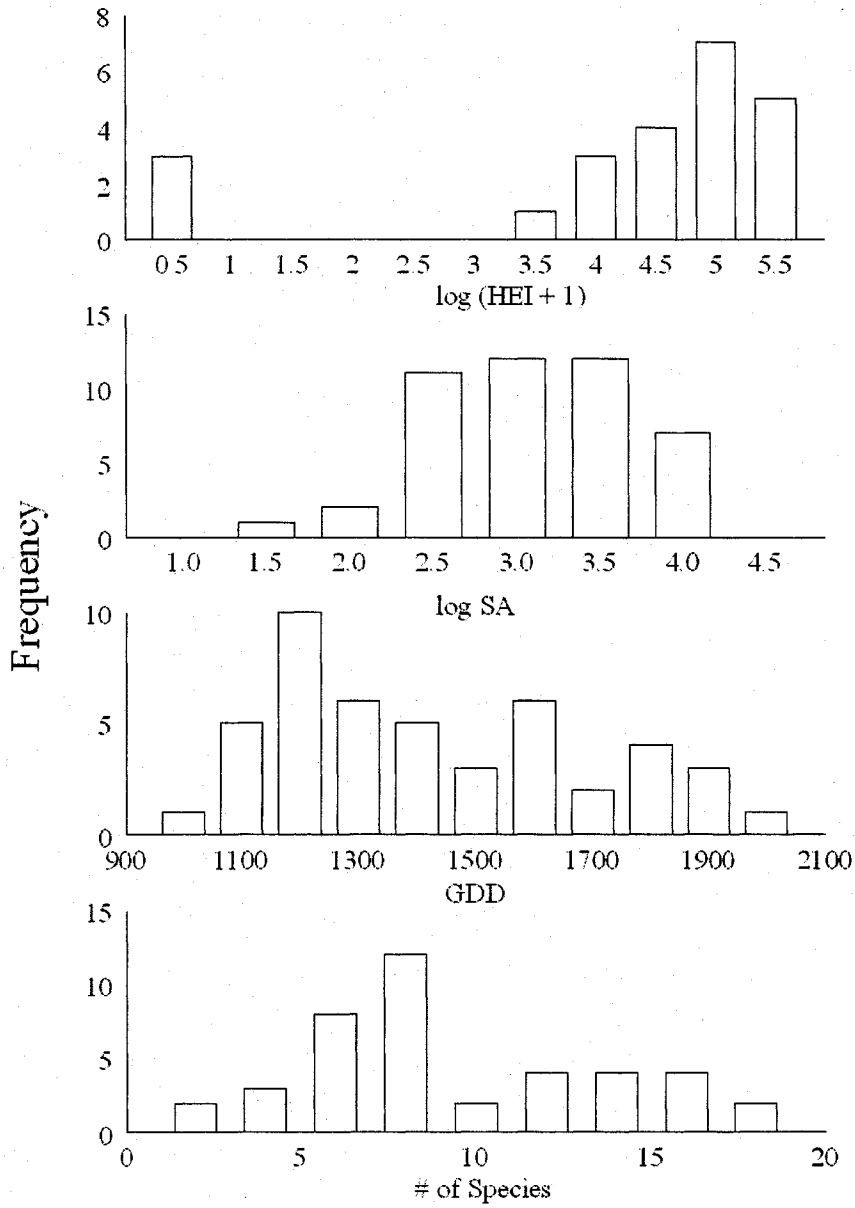

Figure 2: Frequency distribution of selected lake parameters that characterize the 46 walleye lakes sampled across Ontario, Canada. Walleye hatchery supplementation effort (log (HEI+1) was estimated for the 23 lakes that received supplementation while surface area (log SA), growing degree days (GDD), number of species sampled within each lake (# of species) were estimated for all 46 lakes.

Supplementation of populations with artificially propagated (i.e. hatchery) fish is usually implemented to restore or augment existing populations. Twenty-three of the 46 lakes examined were subject to various levels of hatchery supplementation and stocking records of each lake were obtained from the Ontario Community Fisheries Involvement Program (Kerr, 2002) and the OMNR provincial fish hatchery program (Dimond and Potter, 1996). A hatchery effort index (HEI) was developed to estimate the magnitude of supplementation effort for each lake. HEI is based on the assumption that introduced hatchery fish contribute to the mating population, and was calculated for the period for which stocking data was available (1946 – 2001) as:

$$\text{HEI} = \frac{\text{total \# of fish stocked}}{\text{log surface area x time}}$$

where t is the time period in years from the first record of stocking to the year of last stocking. HEI data was log transformed to reduce distribution skew (Appendix I).

***Life History Traits*:** We selected six life history traits (LHTs) that are closely associated with fitness (i.e. growth, mortality, and reproduction). Life history trait measures were based on data collected by the OMNR (Morgan *et al.*, 2003; Appendix II)). We selected the age-3 walleye cohort for analysis of LHTs that are age-dependent, since this age class had the largest sample size compared to other age classes. The specific LHTs selected were: gonadosomatic ratio; relative fecundity; adult mortality; total length at age three; early growth rate; and condition factor at age three. All LHTs exhibited a considerable range of variation among lakes (Figure 3).

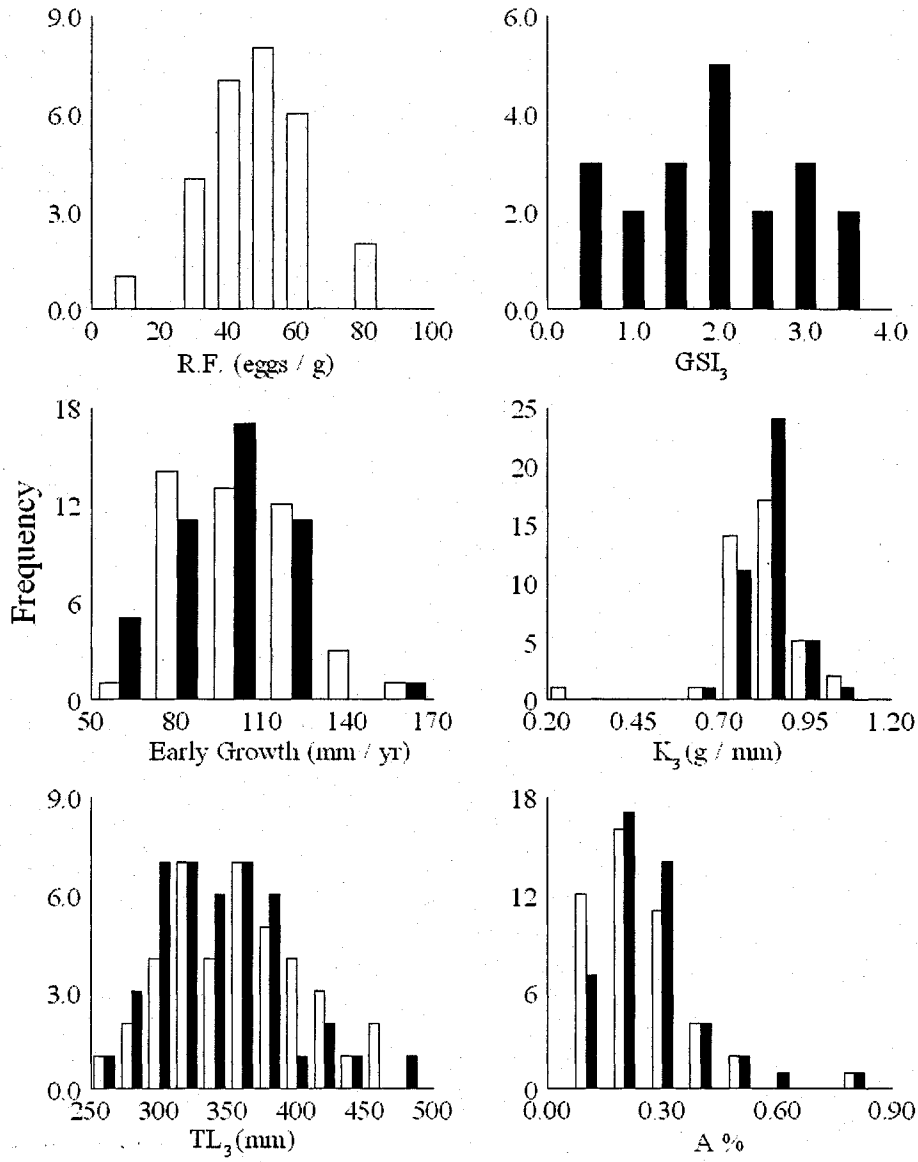

Figure 3: Distribution of mean population walleye life history traits for 46 lakes across the province of Ontario, Canada. Life history traits include: mean population relative fecundity (R.F., eggs / g); mean population gonadosomatic index at age 3 (GSI_3); early growth rate (mm / yr); mean condition factor (K_3; g/mm^3) at age 3; mean population total length (TL_3; mm) at age three; annual mortality (A %) by sex for 46 Ontario walleye populations at age 3 where available. Open bars = female, filled bars = male.

Life history trait estimates were analyzed separately for males and females within each population since significant differences between sexes are expected (Colby and Nepszy, 1981; Morgan *et al.*, 2003). FWIN sampling is conducted in the fall, which allows for quantitative comparisons of life history traits among populations since it standardizes seasonal effects. The LHTs were calculated as follows:

Reproduction: GSI was selected as a measure of reproductive investment of age-3 males. Individual GSI_3 was calculated for all populations having 10 or more age-3 mature males (N = 20) as the ratio of testis wet weight to total somatic wet weight expressed as a percentage. Mean population GSI_3 was then calculated as the average over all individuals within a population.

Relative fecundity was estimated for individual female walleye in 29 populations where a minimum of six or more female egg counts were available as the number of eggs divided by the total wet weight. Mean population relative fecundity was then calculated as the average over all individuals within a population.

Mortality: Mortality is difficult to measure for wild populations of fish, however indirect methods exist that are based on changes in the relative proportion of animals in successive year classes. One such method is the Robson – Chapman's estimator (Robson and Chapman, 1961; Ricker, 1975). For our data, we used a modified Robson Chapman's estimator of mortality at age 5 and older since younger fish were subject to sampling bias resulting from gear selectivity. The modified Robson – Chapman's estimator allows for separate estimates of male and female walleye mortality and is expressed as a percent (Morgan *et al.*, 2003).

Growth: Total length (mm) at age 3 was used as a measure of growth; however fish size at age is often used as an indicator of overall performance (Liskauskas and Ferguson, 1991). Following Gallucci and Quin (1979), a reparameterized von Bertalanffy model was used to describe population early life growth rate (mm/yr) that includes the time from hatching to maturation. Early growth rate (ω) was calculated as the product of k and L_∞, where k is the Brody growth coefficient (i.e.: relative growth rate) and L_∞ is the asymptotic length (mm).

As an additional growth parameter, Fulton's condition factor (K) was calculated as an indicator of metabolic efficiency and overall performance (Ricker, 1975). Fish with high K values generally demonstrate higher growth rates, greater reproductive potential, and higher survival rates than individuals with lower K values occupying a similar environment (Lambert and Dutil, 1997). K values were calculated from field measurements as wet weight divided by the cube of total length, expressed as a percentage. Mean population K values were calculated as the average over all individuals within a population.

Genetic Analyses: DNA was extracted from 2182 walleye (mean number of individuals per lake n = 47; min. n = 42, max. n = 58; see Table 1) from scales or dorsal spines using a Wizard® Genomic DNA Purification Kit (Promega Corp. Madison, WI). Extracted DNA was visualized on 1.5% ethidium bromide stained agarose gels on a UV transilluminator. All DNA samples that were severely degraded were re-extracted and individuals that failed DNA extraction after the second attempt were eliminated from the sample.

Eleven published microsatellite DNA loci were amplified using a polymerase chain reaction (PCR). One tri-nucleotide (Svi 2) and 10 di-nucleotide microsatellite loci markers

were analyzed in this study (Table 2). Amplifications were performed on a PTC-255 thermocycler (MJ Research) using 25 μL reactions. Each 25-μl reaction contained 2.5 μL of 10x reaction buffer (Invitrogen), 2.1 μL of 25 mM $MgCl_2$, 0.2 μL dNTPS (0.1mM each dNTP), 0.22 μL of 20 μM of each primer, 0.1 μL of *Taq* DNA polymerase (Invitrogen), 1.3 μL of the extracted DNA solution, and ddH_2O to complete the 25 μL reaction. The optimized PCR conditions determined for walleye included a 'hot-start' (Löffert *et al.*, 1999) and a 1.5-minute initial denature cycle (94°C) followed by 35 cycles of a 1-minute denature step (94°C), a 1-minute annealing step (variable temperatures - see Table 2), a 1.5 minute extension cycle (72°C), ending with a final 5-minute extension cycle (72°C).

Due to overlap in the size range of the amplified fragments, primers were redesigned using the flanking sequences of the repeat regions for six of the markers. Forward and reverse primers were redesigned for Svi 2, Svi 4, Svi 9, and Svi 11 and the reverse primer was redesigned for Pfla 2 (see Table 2 for redesigned primer sequences). The forward primers for all loci were dye labeled, and amplified products were run on ethidium bromide stained 1.5% agarose gels and visualized with UV transillumination to confirm successful amplification. Microsatellite allele lengths were estimated using a CEQ™ 8000 DNA Fragment Analysis System (Beckman Coulter, Inc., Fullerton, CA) using CEQ™ DNA 60 – 400 bp size standard. All microsatellite fragment sizes were rounded to the nearest whole repeat number. To confirm repeatability of allele size determination, additional PCR amplifications were conducted and compared with previous allele scoring of individuals (between 6 – 10 % were re-analyzed for each locus). Repeatability of alleles scoring had a mean value of 97 % across all loci. The number of walleye genotyped per lake ranged from 42 to 58 (Table 1).

Table 2: Walleye (*Sander vitreus*) microsatellite primer sequences, allele size range (bp), number of alleles (A), annealing temperature (T), and observed (H_o) heterozygosity of sampled walleye across all lakes (n = 2182). Bold text denotes primer sequences redesigned from original published sequences.

Locus	Primer sequences (5' - 3')	bp	A	T	H_O
Pfla 2[1]	F – GTA AAG GAG AAA GCC TTA AC **R – TTA GAA GTG GTC TTG CAG TAG C**	240-302	29	48	0.77
Svi 1[2]	F – AAA GGT CGG AGA GCC ACT GT R – TGT ATT TGG ATT TCA GCC CTT C	244-304	30	55	0.84
Svi 2[2]	**F – GTT TTA AGA CAT AAA CAT ACT CTG TA** **R – ACA AGT GTG TTA GCC AAT CAT**	242-290	12	54	0.68
Svi 4[2]	**F – TTT TGA TGT TTT TCT GAT TAT CG** **R – AAA GAC CCC TGC TGT AGA ATG**	105-159	24	54	0.66
Svi 5[2]	F – CAT ATC CTA CTG TAG TAT GG R – CAA ATC CCA TTT ACA CCC AC	177-225	22	54	0.68
Svi 7[2]	F – GAT GTG CAT ACA TTT ACT CC R – GCT TTA ATC TGC TGA GAA C	174-234	26	53	0.80
Svi 8[2]	F – GCT TAT ACG TCG TTC TTA TG R – ATG GAG AAG CAA GTT GAG	107-151	20	53	0.76
Svi 9[2]	**F – GGA TCT GTA AAC TTG TCA AAT GGA** **R – ACG GAT TGG TAA AAC TAC AGA A**	330-370	21	53	0.66
Svi 10[2]	F – GGT AAT GTA TTT TCA GTT ATT GC R – GCT GTT CTC CAA GTA AAG CC	163-253	43	54	0.82
Svi 11[2]	**F – TGG TGA AGT CTT GAT GCT GA** **R – ATT GGG TCA GCC ACT TCA AA**	308-451	64	55	0.73
Svi 17[3]	F – GCG CAC TCT CGA ATA GGC CCT G R – CGT TAA AGT CCT TGG AAA CC	93-139	15	54	0.64

Note : [1]LeClerc et al., 2000; [2]Wirth *et al.*, 1999; [3]Borer *et al.*, 1999

***Measures of Genetic Diversity*:** Heterozygosity is widely used as a measure of genetic diversity and reflects recent inbreeding since it is expected to be inversely correlated with the recent history of inbreeding (Coulson *et al.*, 1998). Heterozygosity was averaged over all loci to yield mean individual heterozygosity. Mean population heterozygosity was then calculated as the average over all individuals within a population, hereafter referred to as population heterozygosity. We also calculated separate male and female mean heterozygosity within each population.

Mean d^2 is also a measure of genetic diversity, but in contrast to mean heterozygosity, mean d^2 is a more sensitive measure of distant inbreeding events, and higher values indicate a longer time since coalescence due to a greater probability that allele lengths have diverged through multiple, independent stepwise mutations (Coulson *et al*, 1998). While mean d^2 incorporates components of heterozygosity, Coulson et al. (1998) suggests that, provided populations have been separated long enough for allele lengths to diverge, mean d^2 is also a more sensitive measure of outbreeding. Individual mean d^2 was calculated as the squared difference in the number of repeats between the two alleles at each locus, averaged across all loci. Mean population d^2 was calculated as the average over all individuals within a population. Separate male and female mean d^2 values were also calculated.

Wright's inbreeding coefficient (F_{IS}) reflects the deviation from Hardy-Weinberg equilibrium (HWE) expectations for the frequency of heterozygotes. F_{IS} values will be positive when there are fewer heterozygotes than expected, whereas negative values indicate there are more heterozygotes than expected. F_{IS} values were calculated for each population at all loci (Table 1) using the program FSTAT 2.9.3 (Goudet, 2001).

Statistical Analyses: We tested for deviations from HWE at all loci for each population using *Tools for Populations Genetic Analyses* (TFPGA) software (v. 1.3) by Mark Miller (Department of Biological Sciences; Northern Arizona University, Flagstaff AZ 86011 – 5640) (Table 1). We used an exact test (10 batches, 2000 permutations per batch) for each locus, followed by a sequential Bonferroni correction to account for multiple simultaneous tests (11 loci x 46 populations = 506 tests).

We tested for differences between male and female walleye life history traits within the study lakes using paired sample t – tests with STATISTICA (v. 6.0; StatSoft, Inc.; Tulsa OK). Paired sample t – tests were used for age-3 length and condition factor, as well as male and female population mean adult mortality and early growth rate across all sample lakes.

We tested for relationships between total population mean heterozygosity, mean d^2, and F_{IS} versus the various lake parameters utilizing linear regression analyses, and scatter plots of the relationships were visually inspected for possible nonlinear relationships. To evaluate the functional relationships between LHTs and genetic diversity, we also used linear regression analyses. The relationship between both measures of female and male population mean genetic diversity (mean heterozygosity and mean d^2) and Wright's inbreeding coefficient (F_{IS}) versus the various life history measures were analyzed using STATISTICA (v. 6.0). Scatter plots of all relationships were visually inspected for possible nonlinear relationships.

Additionally, forward and backward stepwise regression models were used to examine complex relationships between genetic diversity and the selected lake parameters. Also, genetic diversity and lake parameters were used as independent variables in a stepwise

regression analysis to explain variation in the individual LHTs. All stepwise regression models were evaluated using SYSTAT (v. 7.1) software.

Results

Genetic Variability: The eleven assayed microsatellite loci exhibited a broad range of mean locus heterozygosity (min. $H_o = 0.49$, max. $H_o = 0.85$, mean H_o across all lakes = 0.73), mean d^2 (min. $d^2 = 3.14$, max. $d^2 = 10.5$, mean d^2 across all lakes = 7.7), and F_{IS} (min. $F_{IS} = -0.05$, max. $F_{IS} = 0.12$, mean F_{IS} across all lakes = 0.04) values across populations (Figure 4). The number of alleles amplified per locus ranged from 3 to 32 (Table 2). A table-wide sequential Bonferroni test (corrected for $\alpha = 0.00002$; $p = 0.01$) resulted in seven walleye populations showing single locus-specific significant deviations from HWE (Table 2). The seven lakes with loci deviating from HWE were Sideburned at locus Pfla 2 ($F_{IS} = + 0.236$, $p < 0.001$), Sakwite at Svi 4 ($F_{IS} = + 0.116$, $p < 0.001$), Wakami at locus Svi 4 ($F_{IS} = + 0.617$, $p < 0.001$), St. Joseph at locus Svi 9 ($F_{IS} = - 0.218$, $p < 0.001$), Kagiano at locus Svi 10 ($F_{IS} = + 0.087$, $p < 0.001$), Savanne at locus Svi 17 ($F_{IS} = + 0.374$, $p < 0.001$), and Steel at locus Svi 17 ($F_{IS} = +0.391$, $p < 0.001$) (Table 2).

Regression Analyses: Regression analyses between lake physical parameters and mean heterozygosity and mean d^2 yielded several significant relationships (Table 3). Positive associations were found between mean heterozygosity with log (HEI + 1), GDD, and number of species while mean d^2 was positively associated with log (HEI + 1), log SA, GDD, and number of species. No significant relationships were found between F_{IS} and lake physical parameters. Visual inspection of the scatter plots provided no indication of non-linear relationships.

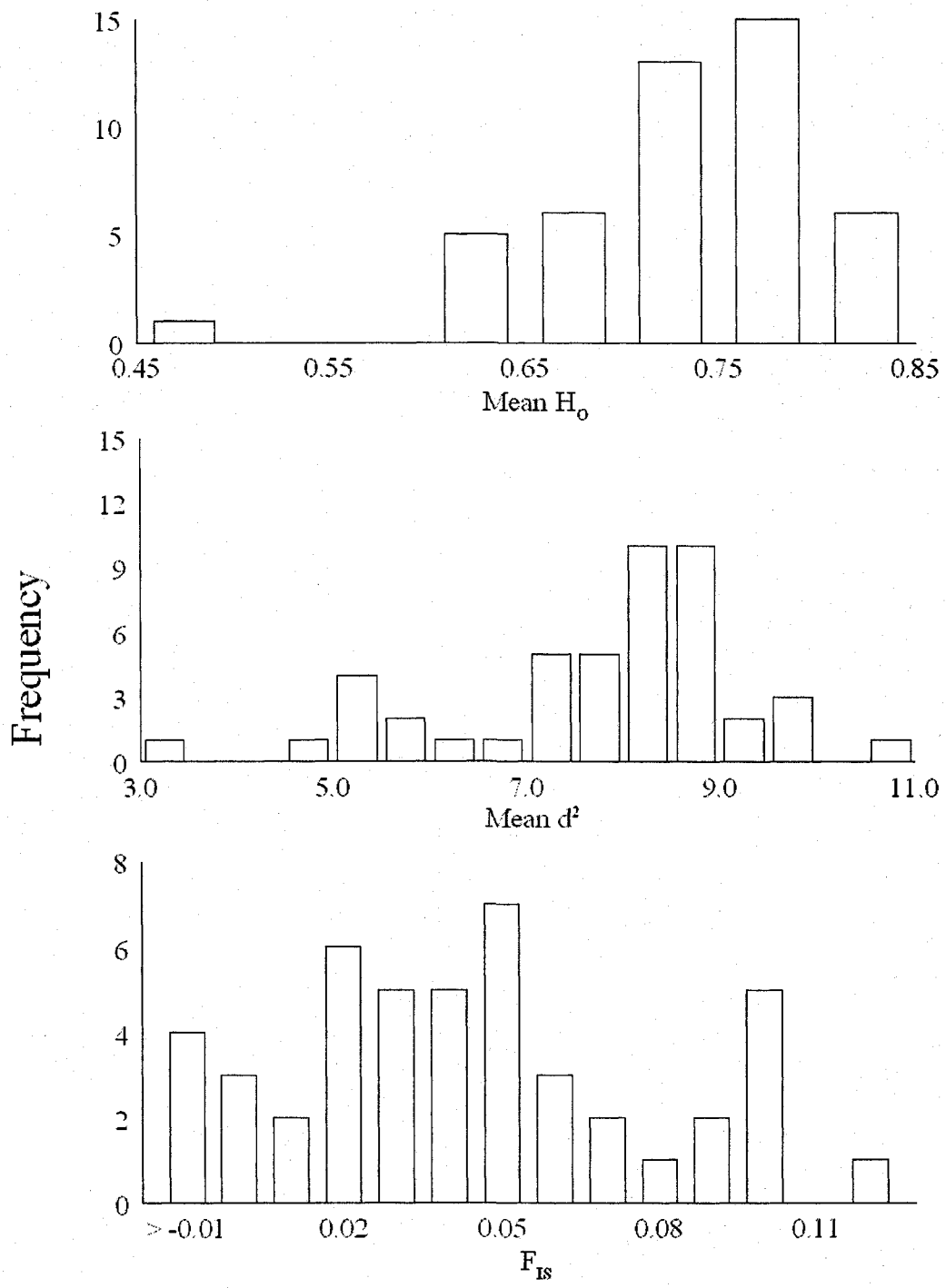

Figure 4: Frequency distribution of measures of genetic variation of walleye lakes (n = 46) across Ontario, Canada. Mean population heterozygosity (H_O), mean population squared allelic distance (d^2), and Wright's inbreeding coefficient (F_{IS}) are presented.

Table 3: Results of the regression analysis between mean heterozygosity, mean d^2, and F_{IS} and lake physical parameters: hatchery supplementation effort (log (HEI + 1)); lake surface area (log SA); growing degree days (GDD); number of species caught within each lake (No. Species) for Ontario walleye lake populations (n = 46). Boldfaced probability (p) values are significant.

Parameter	Heterozygosity			d^2			F_{IS}		
	Slope	r^2	p	Slope	r^2	P	Slope	r^2	p
log (HEI +1)	0.12	0.25	**0.015**	3.18	0.36	**0.002**	0.03	0.03	0.42
log SA	0.009	0.01	0.57	0.72	0.10	**0.037**	0.02	0.07	0.08
GDD	0.0001	0.25	**0.001**	0.002	0.09	**0.042**	0.000	0.02	0.39
No. Species	0.006	0.15	**0.011**	0.18	0.24	**0.001**	0.001	0.04	0.21

Multivariate stepwise regression analyses ($\alpha = 0.15$) showed a limited number of significant lake parameters affecting genetic diversity. GDD was the only lake parameter to emerge from the forward and backward stepwise regressions as being significantly correlated with population heterozygosity ($p < 0.001$, $r^2 = 0.26$). Number of species was the only lake parameter in the final model predicting population mean d^2 ($p < 0.001$, $r^2 = 0.24$). The two stepwise algorithms did not produce consistent models, nor were the individual parameters significant for lake parameter effects on F_{IS}.

Paired sample t-tests for male versus female walleye LHT showed significant sex-related differences in LHTs. Across all lakes, K for males at age-3 was significantly greater than for age-3 females ($p < 0.001$) while female total lengths at age-3 were significantly

greater than age-3 males ($p < 0.05$). Population mortality estimates for males and females differed significantly ($p < 0.01$) as did male and female population ω ($p < 0.01$). A positive significant relationship across lakes was found between relative fecundity and female length at age ($r = 0.3948$; $p < 0.01$).

Male and female walleye regression analyses of LHTs versus genetic diversity produced a limited number of significant relationships (Table 4). Significant linear relationships were observed between male and female walleye mean heterozygosity versus population ω (Fig. 5a, b). After reviewing the plotted data, one outlying population (West Kabenung Lake: $H_O = 0.49$) was removed to determine if this one population was unduly influencing the regression analysis. Reanalysis indicated that the positive relationship remained between mean population heterozygosity and ω, which was still significant among females ($p = 0.013$) and marginally non-significant for males ($p = 0.058$). A marginally significant negative regression was observed between male F_{IS} and ω (Fig. 5c), and a positive association between female mean d^2 with ω, but after reanalysis with the removal of the outlier, those relationships were no longer significant ($p > 0.2$). Regression between Female F_{IS} and ω was marginally non-significant ($p = 0.06$) with a negative slope (Fig. 5d). Visual inspection of the univariate scatter plots revealed no evidence of nonlinear relationships for any of the fitness traits examined.

Table 4: Regression analysis between male and female walleye population heterozygosity, mean population squared allelic distance (d^2), and the inbreeding coefficient (F_{IS}) and mean population fitness trait values: total length at age 3 (TL_3); ω (early growth); condition factor (K_3); gonaosomatic index at age 3 (GSI_3); R.F. (relative fecundity); A% (annual adult mortality). Boldfaced probability (p) values are significant.

		Heterozygosity			d^2			F_{IS}		
Trait	**n**	**Slope**	**r^2**	**p**	**Slope**	**r^2**	**p**	**Slope**	**r^2**	**p**
Female TL_3	40	144.6	0.046	0.18	7.67	0.062	0.12	-106.5	0.007	0.62
Male TL_3	42	197.4	0.091	0.05	4.31	0.021	0.36	-269.3	0.048	0.16
Female K_3	40	-0.07	0.002	0.81	0.001	0.0002	0.94	0.3	0.006	0.64
Male K_3	42	0.12	0.013	0.48	0.001	0.001	0.84	-0.3	0.024	0.32
Male GSI_3	20	1.27	0.009	0.69	0.002	0.001	0.99	-4.5	0.024	0.52
Female A%	46	0.26	0.018	0.37	-0.01	0.004	067	-0.9	0.07	0.08
Male A%	46	0.24	0.013	039	-0.01	0.004	0.66	-0.9	0.075	0.06
Female ω	44	125.4	0.211	**0.002**	4.12	0.093	**0.04**	-159.4	0.079	0.06
Male ω	45	102.7	0.13	**0.01**	2.32	0.029	0.26	-207.0	0.14	**0.01**
R.F.	28	10.8	0.003	0.78	-0.32	0.0013	0.86	32.6	0.007	0.68

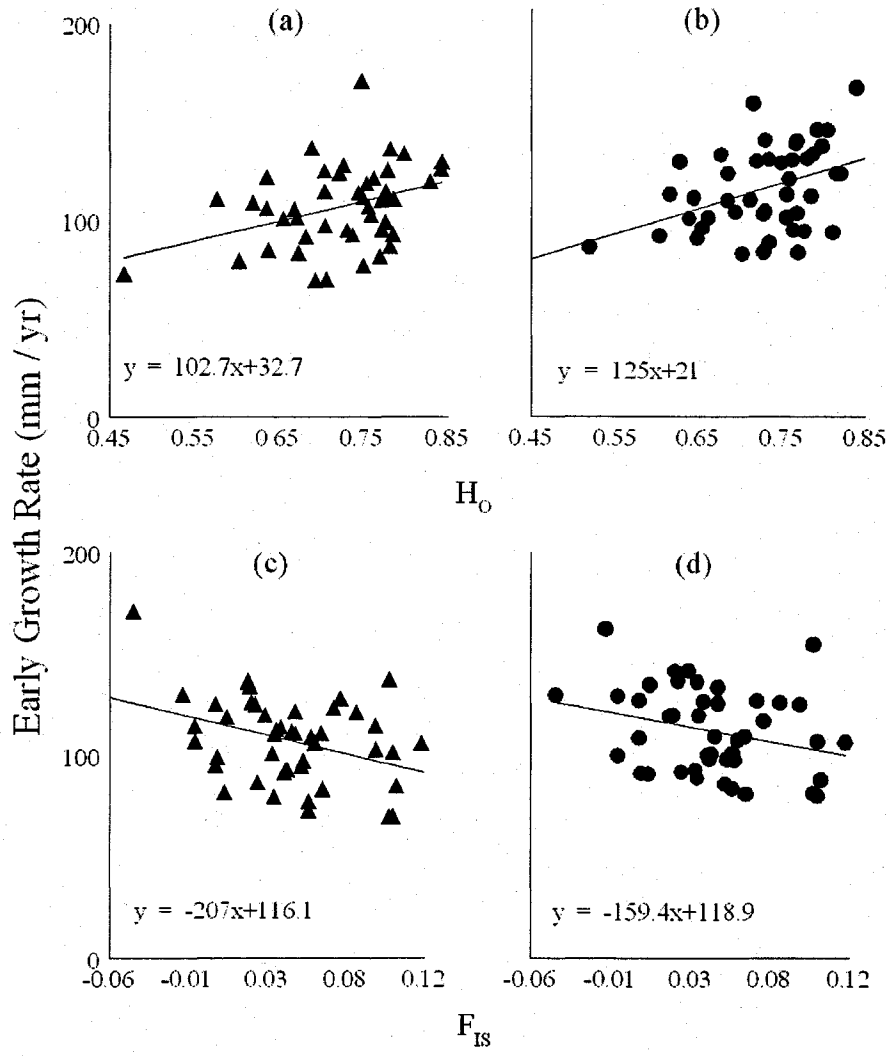

Figure 5: Regression analysis for early growth rate (ω) versus mean heterozygosity (H_O) (a and b) and the inbreeding coefficient (F_{IS}) (c and d). Panel (a): regression of mean male H_O and early growth for 45 lake populations ($r^2 = 0.13$, $P < 0.05$). Panel (b): regression of mean female H_O and early growth for 44 lake populations ($r^2 = 0.21$, $P < 0.005$). Panel (c): regression of mean male F_{IS} and early growth for 45 lake populations ($r^2 = 0.14$, $P < 0.05$). Panel (d): regression of mean female F_{IS} and early growth for 44 lake populations ($r^2 = 0.08$, $P = 0.06$).

The multivariate forward and backward stepwise regression analyses ($\alpha = 0.15$) that included genetic diversity measures and lake parameters, produced consistent and significant models only for early growth rate (ω). Both mean heterozygosity and log SA showed significant effects on female ω ($y = 188.5\ (H_O) - 17.2\ (\log SA)$; all variables $p < 0.05$, $r^2 = 0.43$). Mean heterozygosity, log SA, and log (HEI + 1) showed significant effects on male ω ($y = 186.5(H_O) - 15.8\ (\log SA) - 6.8\ (\log (HEI + 1))$; all variables $p < 0.05$, $r^2 = 0.50$).

Discussion

We found strong correlations between lake parameters (and hatchery supplementation) versus genetic diversity that suggests inbreeding and/or outbreeding is occurring in some of the sampled walleye populations. Lake parameters were observed to have a significant effect on population genetic diversity, possibly resulting from variation in habitat stability and population size. Both of those factors are expected to not only reduce genetic diversity, but also to lead to inbreeding, with the possibility of inbreeding depression. However, we observed evidence for inbreeding depression only in early growth rate. Otherwise, it appears that population heterozygosity, mean d^2 and F_{IS} have weak and non-significant relationships with our 6 selected life history traits (LHTs), despite the fact that the selected LHTs are closely related to lifetime fitness. Since inbred populations may express inbreeding depression in one environment and not in another, due to different environmental stress response buffers (Pray *et al.*, 1994), the presence of inbreeding does not imply inbreeding depression.

Theoretical expectations predict a positive relationship between population size and genetic diversity (Avise, 1994); furthermore population size is likely correlated with habitat size (Heath *et al.*, 2002; Morita and Yamamoto, 2002). Thus, a relationship between lake size and genetic diversity may be expected, driven by habitat availability. However, anthropogenic and stochastic environmental events may also affect genetic diversity, as smaller habitats are inherently more variable than larger systems (Jackson *et al.*, 2001), hence resulting in recurring population bottlenecks. Other studies have shown a positive relationship between habitat size, as an indirect measure of population size, and heterozygosity (e.g.: Fischer and Matthies, 1998; Heath *et al.*, 2002; Yamamoto *et al.*, 2004).

In this study, there was no significant association between lake surface area and heterozygosity, but mean d^2 did show a significant relationship with lake surface area. Mean d^2 is a sensitive measure of historic population size fluctuations and inbreeding events (Marshall and Spalton, 2000). Thus the relationship between population mean d^2 and lake surface area may be due to founder effects, weak inbreeding effects, or the mixing of divergent populations that are not reflected in heterozygosity. Since lake size is correlated with genetic diversity, management of walleye populations in small lakes may require different strategies since evolutionary potential may be reduced and the likelihood of inbreeding is higher.

As indicators of productivity and habitat diversity, growing degree days (GDD) and species diversity were found to have a significant positive relationship with walleye population heterozygosity and mean d^2. The effects of climate have been shown to explain a significant amount of the variation in walleye life history traits, including growth rate, age at maturity, and maximum age (Colby and Nepszy, 1981). Thus, our GDD – genetic diversity relationship may be driven by reduced effective population sizes that are associated with colder, northern lakes and associated life history trait patterns. Similarly, since the northern lakes in this study are beyond the range of many of the other freshwater lake fish species (Scott and Crossman, 1998), our measure of species richness may indirectly reflect climate effects as well. In fact, the dominant link between species richness and distribution in Ontario has been shown to be climate related variables (Mandrak, 1995). Nevertheless, the strong association we observed between genetic diversity and species richness was surprising and the mechanism behind this relationship is not clear. One possible explanation may be that northern lakes are relatively environmentally unstable and less productive than southern

lakes. Fish populations in northern lakes are generally limited by lower carrying capacities (Rigler, 1977), and hence a stochastic environmental event in a northern lake would be likely to cause a severe population genetic bottleneck. Independent of the mechanisms behind the genetic diversity-species diversity relationship, the relationship should prove useful in managing and conserving genetic diversity across broad geographical ranges.

The introduction of cultured fish overrides existing barriers to gene flow among populations, especially since hatchery stocks are generally quite genetically divergent from the target supplemented population (i.e. Largiadèr and Scholl, 1995; Hansen *et al.*, 2000). Hence, hatchery supplementation often alters the genetic structure of potentially locally adapted gene pools. These changes could have a detrimental affect on a population's viability and future evolutionary potential (Phillip and Whitt, 1991). We found that the level of hatchery supplementation (i.e. HEI) was strongly correlated with both population heterozygosity and mean d^2. Even though wild fish generally have a higher survival than artificially reared individuals (Hansen *et al.*, 2000), our data indicate that hatchery walleye are surviving. Despite the evidence for hatchery walleye in some populations, the question remains whether or not there exists some level of reproductive isolation between wild and hatchery walleye. Interbreeding between wild and introduced domesticated fish has been documented elsewhere (Largiadèr and Scholl, 1995; Hansen *et al.*, 2000). Since d^2 measures the size distance between alleles within individuals, mating between wild and hatchery fish should inflate the d^2 if the hatchery and target populations are genetically differentiated. Thus, the strong positive linear relationship between d^2 and HEI indicates that genetic introgression is likely occurring, and hence inflating d^2, between the introduced hatchery and native walleye.

We demonstrate significant correlations between lake parameters and hatchery supplementation with genetic diversity, and the likely mechanisms driving these relationships involve either population size/bottlenecks or breeding between distantly related individuals. Therefore, we predicted that the study populations are likely subject to some degree of inbreeding or outbreeding. We tested this prediction by regression of various LHTs closely associated with fitness with measures of genetic diversity, and found evidence for inbreeding depression in early growth, but no evidence for outbreeding depression. The positive linear relationship between early growth rate and observed population heterozygosity suggests that genetic diversity has an important role during the early life stages of juvenile walleye. In a similar study examining genetic diversity and physiological trait correlations, Shikano and Taniguchi (2002) found a positive association between salinity tolerance and genetic diversity among 17 populations of guppies *(Poecilia reticulata).* Although it is not possible to discriminate between inbreeding depression or heterosis effects from our data, the positive association between population genetic diversity and early growth rate, coupled with the negative associations observed with F_{IS}, suggest that the effects on early growth rate is a consequence of inbreeding due to small effective population sizes. Our results indicate that early growth rate is a sensitive life history indicator of inbreeding depression in walleye. Early growth rate is a critical LHT for northern freshwater fish populations since individuals with higher rates of growth early in life (i.e.: first year after hatching) would likely have a higher probability for survival through the first winter. Larger body size prior to the winter season reflects higher energy content (Hurst and Connover, 1998), and hence over-wintering survival would be higher as fat, protein, and specific energy reserves have been shown to decrease critically during the winter period (Berg and Bremset, 1998). Since the amount of

energy available is related to annual variation (Henderson and Morgan, 2002) and the time available for juvenile fish to achieve a minimum size prior to over-wintering is limited for freshwater temperate zone fish (Shuter and Post, 1990), fast early growth is a critical survival parameter. Furthermore, we suggest that future work involving fitness-related research in northern freshwater fish species should include early life traits in preference over later-life traits.

In addition to the regression analyses at the population level, we analyzed genetic diversity-LHT relationships within each of our 46 sampled populations using individual fish life history trait measures: total length at age three, GSI, relative fecundity, and condition factor. We found no statistically significant genetic diversity-fitness relationships for LHTs in any of the populations after Bonferroni correction (results not shown). This is despite the expectation for inbreeding or outbreeding effects within lakes, given the wide variation in population size and levels of hatchery supplementation. While these results do not support other studies that have reported significant heterozygosity-LHT relationships within populations for fish, such as Heath *et al.* (2002) - relative fecundity, Knaepkens *et al.* (2002) - condition factor, and Neff (2004) - reproductive success, it does lend support to other studies that have not found evidence of this type of relationship among individuals within a population (e.g.: Thelen and Allendorf, 2001; Borrell *et al.*, 2004). Among-population analysis appears to be a more powerful approach for detecting genetic diversity-fitness correlations than the more traditional within-population approach. Such a conclusion is not surprising since an among-population analysis incorporates a broad geographical distribution of populations subjected to variety of environmental conditions.

In summary, our results indicate that genetic diversity of walleye is strongly correlated with lake parameters and hatchery supplementation. We cannot provide a conclusive explanation for those relationships; however, population bottlenecks, small population size and divergent genotype introductions are likely involved. Although we found evidence for inbreeding depression in only one of 6 selected life history traits (early growth), the strength and consistency of the associations between early growth and genetic diversity indicates that inbreeding depression effects are detectable at the among-population level in Ontario inland lake populations of walleye. Our results further suggest that hatchery supplemented walleye are genetically affecting wild walleye stocks, but we found no evidence for outbreeding depression. As a cautionary note, the fact that we found evidence for inbreeding depression effects as well as for genetic effects resulting from hatchery supplementation may represent early evidence for an ongoing change in the genetic architecture within and among Ontario walleye populations. The potential for increasing levels of inbreeding and outbreeding in Ontario walleye may lead to measurable inbreeding and outbreeding depression effects and should not be ignored until they become a painful reality. Our results are clearly important for the maintenance and conservation of genetic diversity as a goal of fisheries management, but also represent a contribution to our understanding of the environmental factors that contribute to the observed variation in genetic diversity, and hence population genetics of natural populations.

References Cited

Avise JC (1994) *Molecular markers, natural history and evolution.* Kluwer Academic Publishers, Massachusetts, USA.

Berg OK, Bremset G (1998) Seasonal changes in the body composition of young riverine Atlantic salmon and brown trout. *Journal of Fish Biology*, **52**, 1272 – 1288.

Billington N, Strange RM (1995) Mitochondrial DNA analysis confirms the existence of a genetically divergent walleye population in northeastern Mississippi. *Transactions of the American Fisheries Society*, **124**, 770 – 776.

Borer SO, Miller LM, Kapuscinski A R (1999) Microsatellites in walleye *Stizostedion vitreum*. *Molecular Ecology*, **8**. 336-338.

Borrell YJ, Pineda H, McCarthy I, Vázquez E, Sánchez JA, Lizana GB (2004) Correlations between fitness and heterozygosity at allozyme and microsatellite loci in the Atlantic salmon, *Salmo salar* L. *Heredity*, **92**, 585 – 593.

Britten HB (1996) Meta – analyses of the association between multilocus heterozygosity and fitness. *Evolution*, **50**, 2158 – 2164.

Charlesworth B, Charlesworth D (1987) Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, **18**, 237 – 268.

Charlesworth B, Charlesworth D (1999) The genetic basis of inbreeding depression. *Genetical Research*, **74**, 329 – 340.

Colby PJ, Nepszy SJ (1981) Variation among stocks of walleye (*Stizostedion vitreum vitreum*): management implications. *Canadian Journal of Fisheries and Aquatic Sciences*, **38**, 1814 – 1831.

Coltman DW, Bowen WD, Wright JM (1998) Birth weight and neonatal survival of harbour seal pups are positively correlated with genetic variation measured by microsatellites. *Proceedings of the Royal Society of London (B)*, **265**, 489 – 495.

Coulson TN, Pemberton JM, Albon SD, *et al.* (1998) Microsatellites reveal heterosis in red deer. *Proceedings of the Royal Society of London (B)*, **265**, 489-495.

Crnokrak P, Roff DA (1999) Inbreeding depression in the wild. *Heredity*, **83**, 260 – 270.

David P (1998) Modeling the genetic basis of heterosis: Tests of alternative hypothesis. *Evolution*, **51**, 1049 – 1057.

DeRose MA, Roff DA (1999) A comparison of inbreeding depression in life – history and morphological traits in animals. *Evolution*, **54**, 1288 – 1292.

Dimond PE, Potter BA (1996) Documentation of walleye stocking in Ontario. Percid Community Synthesis, Walleye Stocking Working Group. Fish & Wildlife Branch, Ministry of Natural Resources. Peterborough, Ontario.

Fischer M, Matthies D (1998) RAPD variation in relation to population size and plant fitness in the rare *Gentianella germanica* (Gentianaceae). *Americn Journal of Botany*, **85**, 811 – 819.

Frankham R (1995a) Conservation genetics. *Annual Review of Genetics*, **29**, 305 – 327.

Frankham R (1995b) Inbreeding and extinction – a threshold effect. *Conservation Biology*, **9**, 792 – 799.

Gallucci VF, Quin TJ (1979) Reparameterizing, fitting and testing a simple growth model. *Transactions of the American Fisheries Society*, **108**, 14 – 25.

Gharrett AJ, Smoker WW, Reisenbichler RR, Taylor SG (1999) Outbreeding depression in hybrids between odd- and even-broodyear pink salmon. *Aquaculture*, **173**, 117 – 129.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html.

Hansen MM, Ruzzante DE, Nielsen EE, Mensberg K-LD (2000) Microsatellite and mitochondrial DNA polymorphism reveals life-history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). *Molecular Ecology*, **9**, 583 – 594.

Hartl D, Clark AG (1997) *Principles of Population Genetics*, 3rd ed., Sinauer Associates, Sunderland, MA, 1997.

Heath DD, Pollard S, Herbinger C (2001) Genetic structure and relationships among steelhead trout (*Oncorhychus mykiss*) populations in British Columbia. *Heredity*, **86**, 618 – 627.

Heath DD, Bryden CA, Shrimpton JM, Iwama GK, Kelly J, Heath JW (2002) Relationships between heterozygosity, genetic distance (d^2), and reproductive traits in Chinook salmon, *Oncorhynchus tshawytscha*. *Canadian Journal of Fisheries and Aquatic Sciences*, **59**, 77 – 84.

Hedrick PW, Kalinkowski ST (2000) Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, ***31***, *139 – 162.*

Henderson BA, Morgan GE (2002) Maturation of walleye by age, size and surplus energy. *Journal of Fish Biology*, **61**, 999 – 1011.

Hurst TP, Conover DO (1998) Winter mortality of young – of – the – year Hudson River striped bass (*Morone saxatilis*): size – dependent patterns and effects on recruitment. *Canadian Journal of Fisheries and Aquatic Sciences*, **55**, 1122 – 1130.

Ihssen PE, Casselman JM, Martin GW, Phillips RB (1988) Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. *Canadian Journal of Fisheries and Aquatic Sciences*, **45**, 1018 – 1029.

Ihssen PE, Martin GW (1995) Biochemical genetic diversity of Ontario walleye (*Stizostedion vitreum vitreum*) populations: implications for stocking. Aquatic Ecosystems Research Section in Contribution to Percid Community Synthesis, Ontario Minsitry of Natural Resources, Peterborough, Ontario.

Jackson DA ,Peres – Neto PR, Olden JD (2001) What controls who is where in freshwater fish communities – the roles of biotic, abiotic, and spatial factors. *Canadian Journal of Fisheries and Aquatic Sciences*, **58**, 157 – 170.

Kerr SJ (2002) Fish stocking activities under the Community Fisheries Involvement Program (CFIP), 1982-2001. Fish & Wildlife Branch, Ministry of Natural Resources. Peterborough, Ontario. ISBN 0-7794-3288-6

Knaepkens G, Knapen D, Bervoets L, Hanfling B, Verheyen E, Eens M. (2002). Genetic diversity and condition factor: a significant relationship in Flemish but not in German populations of the European bullhead (*Cottus gobio* L.). *Heredity*, **89**, 280-287.

Lande R, Shannon S (1996) The role of genetic variation in adaptation and population persistence in a changing environment. *Evolution*, **50**, 434 – 437.

Largiadèr CR, Scholl A (1995) Effects of stocking on the genetic diversity of brown trout populations of the Adriatic and Danubian drainages in Switzerland. *Journal of Fish Biology*, **47** (Suppl. A), 220 – 225.

Lambert Y., Dutil J.-D. (1997). Condition and energy reserves of Atlantic cod (*Gadus morhua*) during the collapse of the northern Gulf of St. Lawrence stock. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 2388-2400.

LeClerc D, Wirth T, Bernatchez L (2000) Isolation and characterization of microsatellite loci in the yellow perch (*Perca flavescens*), and cross-species amplification within the family Percidae. *Molecular Ecology*, **9**, 995-997.

Liskauskas AP, Ferguson MM (1991) Genetic variation and fitness: a test in a naturalized population of brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences*, **48**, 2152 – 2162.

Löffert D, Karger S, Twieling G, Ulber V, Kang J (1999) Otimization of multiplex PCR. *Qiagen News*, **2**, 5-7.

Lynch M (1991) The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution*, **45**, 622 – 629.

Mackey BD, McKenny D, Yang Y-Q, McMahon J, Hutchinson M (1996) Site regions revisited: a climatic analysis of Hills' site regions for the province of Ontario using a parametric method. *Canadian Journal of Forest Research*, **26**, 333 – 354.

Mandrak NE (1995) Biogeographic patterns of fish species richness in Ontario lakes in relation to historical and environmental factors. *Canadian Journal of* Fisheries and Aquatic Sciences, **52,** 1462 – 1474.

Marshall TC, Spalton JA (2000) Simultaneous inbreeding and outbreeding depression in reintroduced Arabian oryx. *Animal Conservation*, **3**: 241-248.

Morgan GE, Malette MD, Kushneriuk RS, Mann SE (2003) Regional summaries of walleye life history characteristics based on Ontario's fall walleye index netting (FWIN) program 1993 to 2001. Diagnostics and Sampling Standards Working Group, Percid Community Synthesis, Ontario Minsitry of Natural Resources, Peterborough, Ontario.

Morgan GE (2002) Manual of Instructions – Fall Walleye Index Netting (FWIN). Diagnostics and Sampling Standards Working Group, Percid Community Synthesis, Ontario Minsitry of Natural Resources, Peterborough, Ontario.

Morita K, Yamamoto S (2002) Effects of habitat fragmentation by damming on the persistence of stream – dwelling charr populations. *Conservation Biology*, **16**, 1318 – 1323.

Neff BD (2004) Stabilizing selection on genomic divergence in a wild fish population. *Proceedings of the National Academy of Science of the United States of America*, **101**, 2381 – 2385.

Pray LA, Schwartz JM, Goodnight CJ, Stevens L (1994) Environmental dependency of inbreeding depression: implications for conservation biology. *Conservation Biology*, **8**, 562 – 568.

Phillip DP, Whitt GS (1991) Survival and growth of northern, Florida, and reciprocal F_1 hybrid largemouth bass in central Illinois. *Transaction of the American Fisheries Society*, **120**, 58 – 64.

Phillip DP, Claussen JE (1995) Fitness and performance differences between two stocks of largemouth bass from different river drainages within Illinois. Pages 236 – 243 *in* H.L. Schramm, Jr., and R.G. Piper. *Uses and effects of cultured fishes in aquatic ecosystems*. American Fisheries Society Symposium 15. Bethesda, Maryland.

Pogson GH, Zouros E (1994) Allozyme and RFLP heterozygosities as correlates of growth rate in the scallop *Pacopecten magellanicus*: a test of the associative overdominance hypothesis. *Genetics*, **137**, 221 – 231.

Ricker WE (1975) Computation and interpretation of biological statistics of fish populations. *Bulletin of the Fisheries Research Board of Canada*, 191. Department of the Environment, Fisheries and Marine Service, Ottawa. 382 p.

Rigler FH (1977) Lakes. 1. North America. Limnology in the high Arctic: a case study of Char Lake. *International Association of Theoretical and Applied Limnology*, **20,** 127 – 140.

Robson DS, Chapman DG (1961) Catch curves and mortality rates. *Transactions of the American Fisheries Society*, **90**, 181 – 189.

Roelke ME, Martenson JS, O'Brien SJ (1993) The consequences of demographic reduction and genetic depletion in the endangered Florida panther. *Current Biology*, **3**, 340 – 350.

Roff DA, DeRose MA (2001) The evolution of trade – offs: effects of inbreeding on fecundity relationships in the cricket *Gryllus firmus*. *Evolution*, **55**, 111 – 121.

Rowe G, Beebee TJC, Burke T (1999) Microsatellite heterozygosity, fitness and demography in natterjack toads, *Bufo calamita*. *Animal Conservation*, **2**, 85 – 92.

Scott WB, Crossman EJ (1998) *Freshwater Fishes of North America*. Galt House Publications, Oakville, Canada.

Shikano T, Taniguchi N (2002) Relationships between genetic variation measured by microsatellite DNA markers and a fitness – related trait in the guppy (*Poecilia reticulate*). *Aquaculture* **209**: 77 – 90.

Shuter BJ, Post JR (1990) Climate, population viability, and the zoogeography of temperate fishes. *Transactions of the American Fisheries Society*, **119**: 314 – 336.

Stearns SC (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

Thelen GC, Allendof FW (2001) Heterozygosity – fitness correlations in rainbow trout: Effects of allozyme loci or associative overdominance? *Evolution*, **55**, 1180 – 1187.

Tsitrone A, Rousset F, David P (2001) Heterosis, marker mutational processes and population inbreeding history. *Genetics*, 159, 1845 – 1859.

Wang S, Hard JJ, Utter F (2002) Genetic variation and fitness in salmonids. *Conservation Genetics*, **3**, 321 – 333.

Waser NM, Price MV (1989) Optimal outcrossing in *Ipomopsis aggregate*. Seed set and offspring fitness. *Evolution*, **43**, 1097 – 1109.

Westemeier R.J., Brown J.D., Simpson S.A., Esker T.L., Jansen R.W., *et al.* (1998). Tracking the long – term decline and recovery of an isolated population. *Science*, **282**, 1695 – 1698.

Wirth T, Saint-Laurent R, Bernatchez L (1999) Isolation and characterization of microsatellite loci in the walleye (*Stizostedion vitreum*), and cross-species amplification within the family Percidae. *Molecular Ecology*, **8**, 1960-1962.

Yamamoto S, Morita K, Koizumi I, Maekawa K (2004) Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial-temporal changes in gene frequencies. *Conservation Genetics*, **5**, 529 – 538.

Chapter 3

Intra-population genetic structure of Ontario walleye: Evidence of natural and anthropogenic mediated gene flow.

Abstract

Past studies have used mitochondrial DNA to examine the genetic and phylogeographic structure of walleye (*Sanders vitreus*) populations at various geographical scales throughout their North American range. In this study, we used 11 microsatellite loci to genotype fish from 46 walleye populations taken from the five primary drainages of the province of Ontario, Canada. The primary objectives were 1) to determine the spatial distribution of genetic variation within and among 46 Ontario walleye populations; and 2) to determine if population divergence is primarily due to natural / historic processes or recent anthropogenic events. Genetic analyses provide evidence that significant population differentiation exists among the 46 sampled walleye populations (F_{ST} = 0.155, 95% CI 0.125 – 0.185) across all drainage basins. AMOVA partitioned most of the genetic variation to the individual level (84.5%), but with significant variation within drainage basins (14.3%) and among the five primary drainage basins (1.2%). Neighbour-joining cluster analysis of populations shows a division on a north-south axis but also suggests that gene flow is occurring among watersheds within the two major clades. Mantel tests of correlation between pair-wise genetic distances and geographical distances yielded one significant within-drainage basin correlation ($p < 0.05$), but no significant relationships were found using all populations combined or within the other four drainage basins, indicating that most populations are not likely at genetic drift - migration equilibrium. Genotype assignment analyses assigned a high proportion of individuals to their population of sampling origin (85%), but 15% of the

individuals were either assigned to populations other than the individual's lake of origin, or were not assigned at all, in a pattern suggestive of both natural and anthropogenic mitigated transfers of walleye among populations. Ontario walleye are not a panmictic population and their population structure has resulted from historical, natural and anthropogenic processes.

Introduction

Molecular genetic methods are commonly used to determine genetic diversity and structure within and among populations (e.g. Billington and Strange, 1995; Stepien and Faber, 1998; Heath *et al.*, 2001; among others). Based on the principle that genetic differences exist among species and populations, such methods can be employed to differentiate between various "stocks", or populations (Shaklee and Currens, 2003). To infer biological significance to genetic structuring among populations, knowledge of the relative effects of mutation, random genetic drift, migration, and selection (e.g. Slatkin, 1985) as well as geological and geographical factors, (e.g. Castric *et* al., 2001) is required. Unlike unfettered panmictic populations, geographic, temporal and behavioral reproductive isolation (the primary causes of gene flow disruption) allows, over time, genetic divergence among populations. Even on a small geographical scale, intra-population genetic differentiation can occur, especially if conspecifics inhabit alternative environments such as those found within river versus lake habitats (Carvalho, 1993). A significant amount of the variation in genetic diversity found among present day North American fish populations can be traced to dispersal effects after the Pleistocene Ice Age (approximately 12 000 years ago). The manner in which the genes of a species disperse over its range has implications regarding the demographics, potential for local adaptations to evolve, and the development of genetic structure within a species (Garant *et al.*, 2001; Cox and Hebert, 2001). Post-glacial dispersal and re-colonization was largely responsible for the current distribution of fish populations across North America (Scott and Crossman, 1998; Bernatchez and Wilson, 1998). Population genetic theory predicts that processes associated with range shifts during the Pleistocene, such as fragmentation, range expansion and reduced population size, will impact

the genetic variation in present day populations (Hänfling *et al.*, 2002). By estimating genetic diversity and the phylogeographic relationships among populations, questions regarding the ecology and conservation of a species can be addressed.

Conservation of genetic variation within and among fish populations is important when considering population viability over the short term (i.e. prevention of inbreeding or outbreeding depression) and the maintenance of genetic variation is critical to prevent the loss of evolutionary potential over the long term (Allendorf *et al.*, 1987). In light of this, the effective management of fish populations is facilitated by knowledge of population structure and genetic diversity of the target species. Confounding factors, such as documented and undocumented introductions of fish, can compromise population structure (Shaklee and Currens, 2003). Introgression between native fish and supplemented hatchery fish can drive changes in genetic diversity, which in turn, can modify genetic divergence among populations (Hansen *et al.*, 2000). Determination of population structure is becoming a critical issue for effective fisheries management, and molecular genetic techniques are now widely used for identifying populations and stocks for both conservation and evolutionary purposes (Avise, 2004).

Anthropogenic factors also have the potential to contribute to the genetic architecture of a species by altering genetic structure within and among populations (e.g. Nerass and Spruell, 2001; Hansen *et al.*, 2001; Yamamoto *et al.*, 2004). Habitat alterations can interrupt gene flow and spatially isolate groups of individuals which can then lead to a reduction in the genetic diversity resulting in a rapid increase in the rate of genetic drift (Nielsen *et al.*, 1997; Yamamoto *et al.*, 2004). Likewise, artificial gene flow (population supplementation) has the potential to alter the genetic structure among fish populations. Reductions in genetic

divergence among supplemented and source populations are expected as allele frequencies are homogenized (Miller and Senanan, 2003). On the other hand, population supplementation might not necessarily impact the genetic structure of a population over the long term (see Ruzzante *et al.*, 2001), which is consistent with the hypothesis that hatchery fish exhibit lower fitness in the wild than do wild, native fish (Fleming *et al.* 2000). This implies that native populations have developed beneficial genetic adaptations to the local environment, and that introduced individuals are selected against in the same environment. Alternatively, if small numbers of fish are introduced relative to the size of the native population, their contribution to the gene pool might be small and have undetectable effects on the overall population genetic structure in the short term (Brunner *et al.*, 1998). Furthermore, detecting the genetic effects of long stocking histories is difficult due to factors such as incomplete records regarding the number of individuals introduced and often undocumented origins of the stocked fish. Even though hatchery supplementation can serve a valuable conservation role, the possible negative effects involving changes to the gene pool that result in reduced population fitness must be considered

The source of the Ontario populations of walleye (*Sanders vitreus*) is attributed to colonization events by Missouri, Mississippi and Atlantic glacial refugia populations (Billington *et al.*, 1992; Murdoch and Hebert, 1997). Analyses of mitochondrial (mt) DNA have revealed phylogeographic structure and significant population differentiation in walleye within the Great Lakes region (e.g. Billington *et al*, 1992; Stepien and Faber, 1998; Gatt *et al.*, 2002; among others). The walleye range in North America includes Quebec and south to the west of the Appalachian Mountains to the Gulf coast, northwest to the eastern half of Nebraska to North Dakota, north to near the Arctic coast, across the southeast to east coast of

upper James Bay (Scott and Crossman, 1998). In Ontario, walleye are found in all of the major drainage basins from the Lake Superior / Lake Huron basin in the south to the Hudson's Bay / James Bay basin in the north and Nelson River basin in the northwest to the Lake Erie / Lake Ontario and Ottawa River basins in the east. Spawning takes place during the spring months when lake resident walleye move to rocky reefs, gravel areas or submerged vegetation, or migrate upstream to headwaters or riffle areas to spawn (Jennings et al. 1996; Scott and Crossman, 1998). Walleye eggs hatch within approximately two weeks and the larvae disperse to open water areas until they move into deeper water habitats as juveniles (Stepien and Faber, 1998; Scott and Crossman, 1998). Adult walleye have been observed to exhibit migratory behaviour and may migrate as far as 160 kilometres between habitats (Becker, 1983; Nepszy *et al.*, 1991).

Migratory behaviour in walleye may act as a reproductive isolating mechanism if individual populations display fidelity to natal spawning sites. The return of offspring to natal spawning sites has been well documented in salmonids (for review see Quinn, 1993) and this homing limits gene flow resulting in the genetic differentiation among populations. Jennings et al.'s (1996) experimental results supports the possibility of natal homing by walleye as laboratory reared progeny of river- and reef-spawning walleye populations were observed to return to the spawning site of origin. Natal homing may be an important evolved behaviour that allows local adaptation and hence contributes to spatial and genetic patterns among walleye populations.

In this study we used allele size data from 11 microsatellite loci to determine the genetic structure of 46 geographically dispersed walleye populations. The objectives of this study are 1) to determine if population divergence among 46 sampled walleye populations is

present; and 2) to determine if population divergence is primarily due to natural / historic processes or recent anthropogenic events. To test for population structure among the 46 walleye populations, we calculated the degree of population divergence based F_{ST}, across all loci. To determine the likely mechanisms driving the population structure we tested for isolation-by-distance to determine if population genetic divergence is a result of reproductive isolation as a function of increasing geographical distance (Slatkin, 1993). In addition, neighbour-joining cluster analysis was used to determine the geographic distribution of the population clusters and we performed a hierarchical analysis of molecular variance (AMOVA). To attribute the observed unusual pattern of genetic structure to anthropogenic or natural processes we evaluated drainage basin level gene flow using genotype assignment analyses. We expect that the population structure of Ontario walleye is likely to be a result of combined interactions between historical recolonization patterns and ongoing natural gene flow, confounded by artificial gene flow due to anthropogenic supplementation and / or introductions.

Materials and Methods

Sample collection and genetic methods **:** Detailed descriptions of the data collection is provided elsewhere (Chapter 1) and the present study is part of a larger project designed to examine the genetic attributes of inland walleye populations. In brief, scales and dorsal spines from 2182 walleye from 46 populations were analyzed for microsatellite DNA allele variation (Figure 6; for corresponding lake name identifiers see Table 5). The mean number of individuals analyzed per lake was 47 (min. n = 42 max. n = 58). All samples were collected by the Ontario Ministry of Natural Resources (OMNR) during the fall using the Fall Walleye Index Netting sampling protocols as described by Morgan (2002). Scales and / or dorsal spines were collected and shipped to the laboratory for DNA extraction and genetic analysis. Twenty-three of the 46 populations sampled have been supplemented with hatchery reared fish, based on available stocking records (Dimond and Potter, 1996; Kerr, 2002).

Eleven published walleye microsatellite DNA loci were used in this study; however we modified five of the primer sets to facilitate the pooling of markers for automated sequencing (see Chapter 1 for marker information). DNA was extracted from scale or dorsal spines using a Wizard® Genomic DNA Purification Kit (Promega Corp. Madison, WI). Optimized PCR amplification conditions, analysis of microsatellite fragment size scoring, and verification of replicate repeatability are described in detail in Chapter 1.

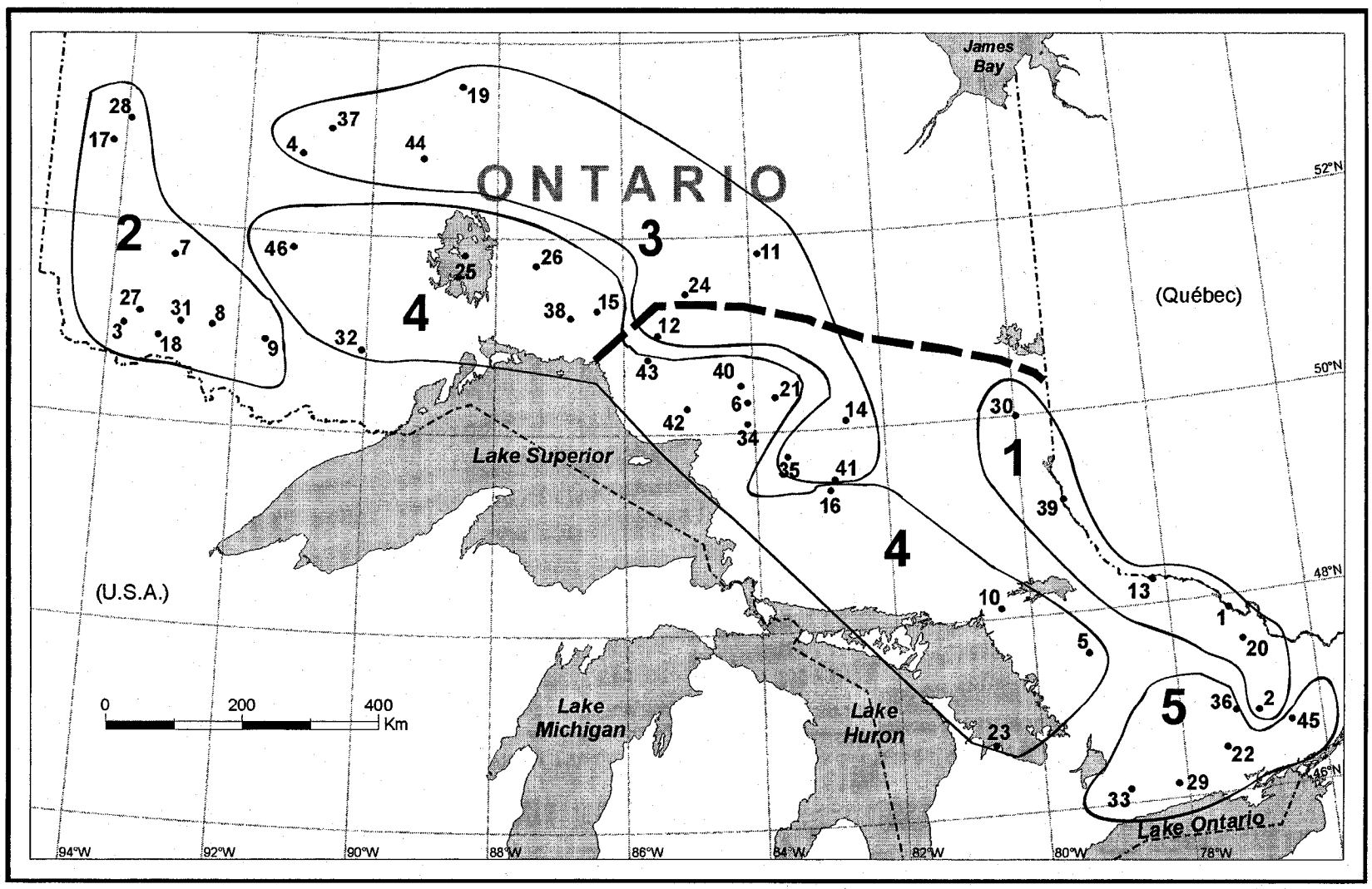

Figure 6: Map showing the lakes sampled for walleye in the province of Ontario, Canada. Bold dashed line indicates separation of walleye populations into north and south clades base on D_C genetic distance measures. Numbers correspond with lake names provided in Table 5. Large bold face numbers correspond with drainage basin identifiers in Table 6.

Table 5: Genetic diversity estimates and individual genotype assignment results (n = 2182 fish) for 46 walleye populations with 11 microsatellite DNA loci (ID # refer to lake identifier # in Figure 6; basin ID refers to a primary drainage basin in Figure 6) across the province of Ontario, Canada. The number of individuals sampled per population (n), stocking record available (Yes / No), estimates of mean locus direct count (observed) heterozygosity (H_o), expected heterozygosity (H_E), allelic richness (A_R), and hatchery supplementation effort (log(HEI+1); Appendix I) are provided. Results of genotype assignment analyses are shown as the proportion of individuals assigned to lake of origin (Self), not assigned to a population (Not), assigned to a lake other than that of origin (Other), the name of the other population assigned is also given (numbers in parentheses indicate multiple assignments).

		Basin		Stocked				Assignment			
ID	**Lake**	**ID**	**n**	**Y / N**	**H_O**	**H_E**	**A_R**	**Self**	**Not**	**Other**	**Other Population Assigned**
1	Allumette	1	47	N	0.76	0.79	10.4	0.92	0.04	0.04	Skootamatta
2	Big Gull	1	49	Y	0.77	0.84	11.9	0.94	0.06	0	
3	Burditt	2	47	Y	0.74	0.81	11.3	0.96	0.04	0	
4	Churchill	3	48	N	0.77	0.79	10.4	0.94	0.06	0	
5	Deer	4	48	N	0.69	0.69	7.1	1.00	0	0	
6	Dog	4	46	N	0.64	0.68	7.9	0.72	0.19	0.09	Sideburned(2),Skootamatta(2)
7	Eagle	2	48	Y	0.75	0.78	12.4	0.96	0.04	0	
8	Eltrut	2	50	N	0.70	0.74	9.5	0.96	0.02	0.02	Fushimi
9	Finlayson	2	44	Y	0.68	0.77	10.7	0.91	0.07	0.02	Big Gull
10	French	4	44	Y	0.79	0.83	11.9	0.93	0.02	0.05	Mink
11	Fushimi	3	46	N	0.74	0.77	10.3	1.00	0	0	
12	Granham	3	47	N	0.66	0.69	7.6	0.94	0.04	0.02	Mink

Table 5 cont'd

		Basin		Stocked				Assignment			
ID	**Lake**	**ID**	**n**	**Y/N**	**H_O**	**H_E**	**A_R**	**Self**	**Not**	**Other**	**Other Population Assigned**
13	Holden	1	48	Y	0.75	0.81	11.6	0.83	0.15	0.02	Mink
14	Ivanhoe	3	47	Y	0.74	0.73	7.7	0.9	0.04	0.06	Big Gull, Rice, Skootamatta
15	Kagiano	4	47	N	0.73	0.78	7.7	0.89	0.09	0.02	Mink
16	Kebskewasheshi	4	47	Y	0.64	0.68	9.3	0.94	0.04	0.02	Mink
17	Longlegged	2	46	N	0.70	0.78	9.6	0.89	0.11	0	
18	Mainville	2	50	N	0.78	0.79	11.8	0.94	0.06	0	
19	Miminiska	3	44	N	0.76	0.81	11.3	0.98	0.02	0	
20	Mink	1	57	Y	0.84	0.82	10.3	1.00	0	0	
21	Missinaibi	4	43	Y	0.65	0.73	8.9	0.86	0.12	0.02	Mink
22	Moira	5	51	Y	0.72	0.75	9.1	0.96	0.04	0	
23	Mountain	4	46	N	0.76	0.84	11.7	0.96	0.04	0	
24	Nagagami	3	46	N	0.73	0.76	8.7	0.87	0.09	0.04	Mink
25	Nipigon	4	47	N	0.70	0.78	12.1	0.96	0.04	0	
26	Partridge	4	44	Y	0.69	0.71	9.4	0.84	0.11	0.05	Mink
27	Pipestone	2	48	Y	0.76	0.77	6.7	0.81	0.19	0	
28	Red	2	48	Y	0.76	0.80	11.2	0.88	0.1	0.02	Nipigon
29	Rice	5	42	Y	0.83	0.85	12.0	0.95	0.05	0	
30	Round	1	47	Y	0.73	0.75	9.3	0.96	0.04	0	
31	Sakwite	2	48	N	0.78	0.78	8.1	0	1.00	0	
32	Savanne	4	50	N	0.60	0.63	9.7	0.96	0.02	0.02	Mink

Table 5 cont'd

		Basin		Stocked				Assignment			
ID	**Lake**	**ID**	**n**	**Y/N**	**H_O**	**H_E**	**A_R**	**Self**	**Not**	**Other**	**Other Population Assigned**
33	Scugogg	5	46	Y	0.82	0.84	10.5	0.46	0.39	0.15	Big Gull(3), Mink,Skootamatta(3)
34	Shikwamkwa	4	47	N	0.77	0.80	11.7	0.89	0.11	0	
35	Sideburned	3	48	N	0.77	0.74	7.5	0.96	0.04	0	
36	Skootamatta	5	58	Y	0.81	0.84	10.4	0.88	0.09	0.03	Rice
37	St. Joseph	3	45	Y	0.80	0.80	11.4	0.84	0.09	0.07	Rice(2), Skootamatta
38	Steel	4	47	N	0.74	0.78	11.7	0.98	0.02	0	
39	Timiskaming	2	45	N	0.71	0.79	10.1	0.84	0.13	0.03	Skootamatta
40	Wabatongushi	4	47	Y	0.62	0.65	5.9	0.64	0.17	0.19	Mink
41	Wakami	3	48	Y	0.60	0.64	11.3	0.9	0.08	0.02	Skootamatta
42	W. Kabenung	4	44	N	0.49	0.52	12.2	0.96	0.02	0.02	Timiskaming
43	White	4	57	N	0.81	0.83	7.6	0.86	0.14	0	
44	Whitewater	3	45	Y	0.70	0.78	5.8	0.76	0.13	0.11	Skootamatta
45	Wolfe	5	48	Y	0.78	0.78	11.7	0	1.00	0	
46	Young	2	47	N	0.80	0.82	10.5	0.43	0.27	0.3	Skootamatta(11), Rice(2)

Genetic analysis **:** To visualize the genetic relationship among the 46 sampled walleye populations, we performed a neighbour-joining (N-J) cluster analysis with Cavalli-Sforza and Edwards (1967) chord distance (D_C) using the computer shareware program Populations, Version 1.2.26 (CNRS UPR9034; O. Langella, Centre National de la Recherche Scientifique, Laboratoire Populations, Genetique et Evolution, Gif sur Yvette; http: // www. cnrsgif. fr/pge/bioinfo/populations). D_C, which makes no assumptions regarding either constant population size or mutation rates among loci, was used as a measure of genetic distance because it has been demonstrated to create accurate representations of population tree topologies for relatively closely related populations (Angers and Bernatchez, 1998). The un-rooted neighbour-joining tree was visualized with TREEVIEW software version 1.5.2 (Page, 1996). The degree of population divergence was estimated by calculating F_{ST} over all loci across all populations within each of the drainage basins. F_{ST} provides a means to quantify the degree of population differentiation based on the differences in allele frequency and heterozygosity among populations (Hartl and Clarke, 1997). Confidence intervals (95 % CI) for F_{ST} values were generated by bootstrapping samples over loci (Weir and Cockerham, 1984). Hierarchical analysis of molecular variation (AMOVA) (Michalakis and Excoffier, 1996) was used to partition observed allele variation within- and among-basins using ARLEQUIN (v.2.0, Schneider *et al.*, 2000). Mean drainage basin allelic richness (A_R) and observed heterozygosity (H_O) were estimated for all populations separately and combined within each drainage basin using FSAT (Goudet, 2001). Allele richness was calculated to allow comparisons among populations with different sample sizes (El Mousadik and Petit, 1996).

Natural and anthropogenic effects on population structure

We tested for natural / historic and anthropogenic mechanisms that underlie the population structure of the 46 walleye populations in this study. First, regression analysis coupled with Mantel tests was used to examine the relationship among pair-wise population genetic distances and geographic distances. It is expected that an isolation-by-distance model of genetic divergence will result in a linear increase of genetic differentiation with geographic separation (Slatkin, 1993). Positive correlations are indicative of drift-migration equilibrium and that the population structure is therefore stable and undisturbed. We regressed the shortest straight line geographic distance between all 1035 pair-wise lake comparisons (distances acquired from Natural Resources Canada; www.geonames.nrcan.gc.ca/index_e.php) and pair-wise D_C genetic distances. Mantel tests (20 000 permutations) were used as unbiased tests for correlation significance for all genetic distance – geographic distance relationships. We also tested for isolation-by-distance relationships among populations within each drainage basin. Each of the sampled lakes was assigned to one of the five drainage basins (1 – Ottawa River Basin; 2 – Nelson River Basin; 3 – Hudson Bay / James Bay Basin; 4 – Lake Superior / Lake Huron Basin; 5 – Lake Erie / Lake Ontario Basin) within the province of Ontario. Each drainage basin was identified based on the drainage patterns as determined by the height of land (identified on a 1:500 000 scale map; Land and Information Branch, Ontario Ministry of Natural Resources, 1992).

To test for anthropogenic effects (i.e. artificial gene flow) on population structure we used genotype assignment analyses to identify possible immigrants in each population. This approach assigns individuals to a population based on the likelihood of

its genotype being derived from the allele distribution characterizing each of the sampled populations (Paetkau *et al.*, 1995). All assignment tests were performed using the software GeneClass2 (Piry *et al.*, 2004) with the Bayesian approach (Baudouin and Lebrun, 2001) and Monte-Carlo re-sampling (10 000 replicates; $\alpha = 0.001$) to test every individual for its status as a resident or migrant individual (Paetkau *et al.*, 2004). We considered individuals to be self-assigned to the lake of origin if the likelihood of assignment was more than 10% higher than the next most likely assigned lake. If the likelihood difference between the most likely and the next most likely assignment was less than 10% the individual was unassigned. If the most likely assigned population was more than 10% greater than all other population assignment likelihoods, but the assigned population was not the lake of origin, then the fish was identified as a probable migrant. The 10% threshold was chosen as it yielded a relatively high percentage (85%) of successful assignments.

To examine the influence of hatchery supplementation on the overall genetic population structure of the sampled populations, we separated the hatchery supplemented and the un-supplemented populations and calculated the overall F_{ST} for both groups. A difference in the F_{ST} is expected if genetic introgression between wild and hatchery supplemented individuals is altering the genetic structure by homogenizing the allele frequencies among supplemented populations (Largiadèr and Scholl, 1995). We used FSTAT to calculate F_{ST} over all loci among the hatchery supplemented lakes and separately for all of the lakes with no record of hatchery supplementation. Confidence intervals (95 % CI) were generated with 15 000 bootstraps over loci. A paired sample t-

test was used to test for a significant difference between the estimates of F_{ST} from the two groups.

Results

Among populations and drainage basin genetic diversity

Neighbour-joining cluster analysis using the Cavalli-Sforza and Edwards (1967) chord distance (D_C) provided a fine scale resolution of the population structure for the 46 walleye populations (Figure 7). None of the five drainage basins formed distinct clusters; however, the general pattern observed indicated two clades that grouped the populations in a north – south separation (Figure 7). This separation is highlighted on the map of Ontario and on the phylogram by a dashed line (Figures 6 and 7). The sampled populations above the dashed line represent the northern clade (Figure 7). Note that three of the populations were placed in clades that did not agree with the general north – south pattern. The Wolfe (lake ID# 45) and Deer Lake (ID# 5) populations, which are located in southern Ontario, clustered among populations within the northern clade, whereas Young Lake (ID# 46), which is located north-west of northern Lake Superior, clustered within the southern clade. One other distinct clustering of walleye populations consisted of Moira Lake (ID# 22), Mink Lake (ID# 20), and French River (ID# 10; Figure 7). The distinct clustering of these three populations does not appear to reflect geographic location (Figure 6), and may be the result of high levels of hatchery walleye supplementation to these populations (see Appendix I).

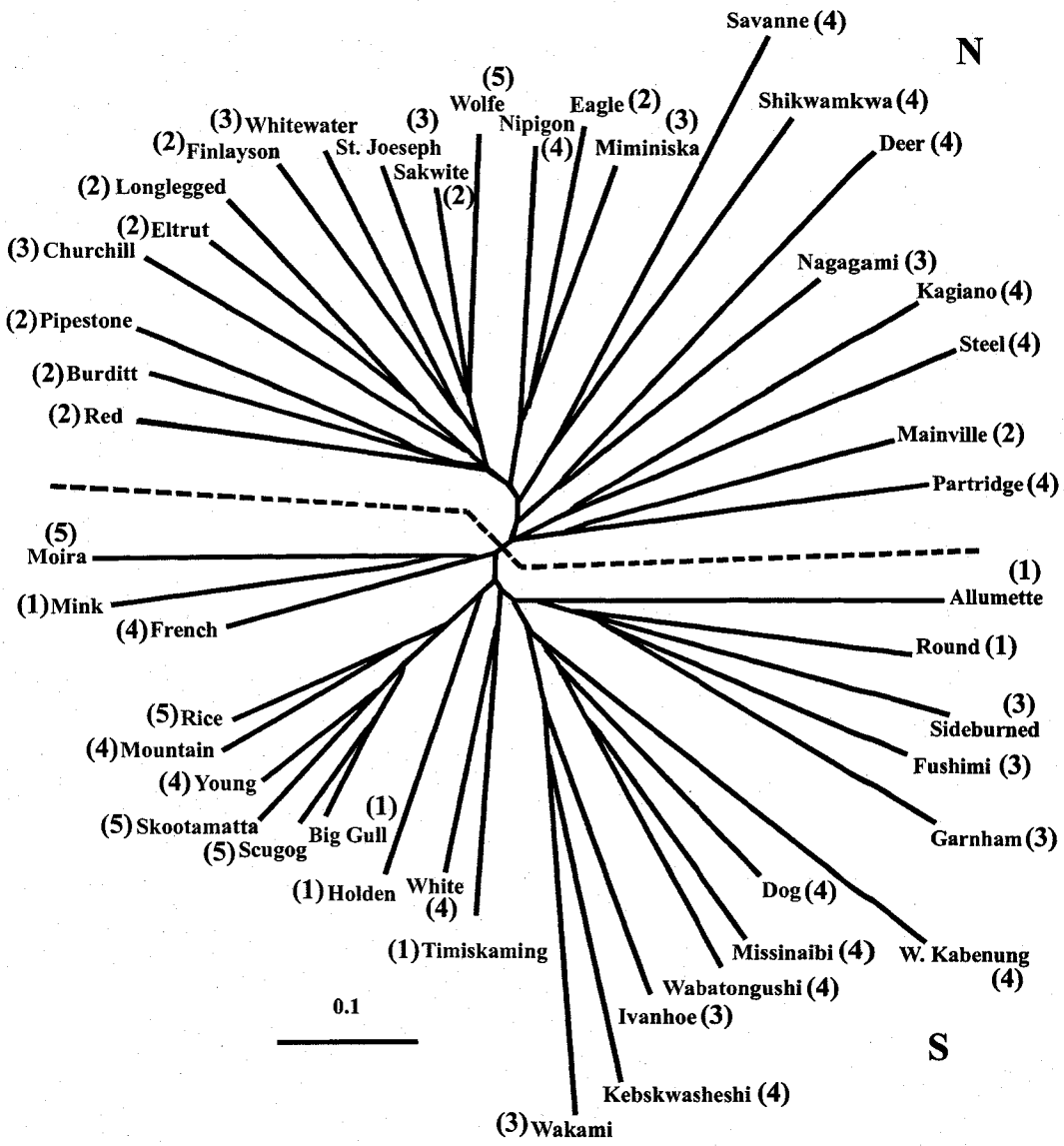

Figure 7: Unrooted neighbour – joining tree for 46 Ontario walleye populations generated from genetic chord distance matrix (Cavalli and Sforza and Edward, 1967) calculated using 11 microsatellite loci. North and south clades delineated by dashed line; numbers in parentheses correspond with primary drainage basin (see Figure 6).

Estimates of genetic variation for the sampled walleye populations were not uniform across the five major Ontario drainage basins (Table 6). Estimates of population H_O and A_R ranged form 0.69 – 0.79 and 9.1 – 10.9 respectively. The F_{ST} value across all 46 populations was 0.155 (95% CI 0.125 - 0.185; Table 6) but population pair-wise F_{ST} values exhibited a wide range of values (Figure 8). Estimates of population differentiation (F_{ST}) likewise varied among the five individual drainage basins. The two drainage basins having the lowest values of H_O and A_R showed the greatest degree of population structure (Hudson Bay / James Bay, $F_{ST} = 0.16$; Lake Superior / Lake Huron, $F_{ST} = 0.194$; Table 6). The F_{ST} values for these two drainage basins were significantly greater than the other three basins as indicated by the non-overlapping 95% confidence intervals (Table 6). The Lake Erie / Lake Ontario drainage basin was observed to have the lowest degree of population structure ($F_{ST} = 0.081$; Table 6), which is significantly lower than the other four drainage basins as indicated by the 95% confidence interval, but exhibits the highest values of H_O and A_R. The F_{ST} 95% confidence intervals for the Ottawa River Basin ($F_{ST} = 0.11$) and the Nelson River Basin ($F_{ST} = 0.11$) overlapped; however, both differed significantly from the other three basins. Mantel tests carried out within each of the five drainage basins yielded one significantly positive relationship between pair-wise D_C and geographical distance; the Nelson River Basin (Mantel test, $P < 0.05$, $r^2 = 0.09$; Figure 9). Partitioning of the molecular variance by AMOVA yielded the highest contribution from within populations (84.5 %, $p < 0.001$), although there was a significant within-basin component as well (14.3 %, $p < 0.001$) while the among-basin component was the lowest, but still significant (1.2 %, $p < 0.01$).

Table 6: Estimates of within primary drainage basin observed heterozygosity (H_O), allelic richness (A_R), and population divergence (F_{ST}) with 95% confidence intervals (CI), for the combined 46 walleye populations (All) and for *n* populations from each of the five drainage basins in Ontario, Canada (1 - Ottawa River Basin; 2 - Nelson River Basin; 3 – Hudson Bay / James Bay Basin; 4 – Lake Superior / Lake Huron Basin; 5 – Lake Erie / Lake Ontario Basin: see Figure 6).

	Basin	*n*	H_O	A_R	F_{ST}	95% CI
	All combined	46	0.76	9.9	0.16	0.125 - 0.185
1	Ottawa River	6	0.76	10.9	0.11	0.078 - 0.144
2	Nelson River	10	0.75	10.6	0.11	0.071 - 0.134
3	Hudson Bay/James Bay	10	0.73	9.3	0.16	0.131 - 0.191
4	Lake Superior/Lake Huron	15	0.69	9.1	0.19	0.156 - 0.232
5	Lake Erie/Lake Ontario	5	0.79	11	0.08	0.053 - 0.108

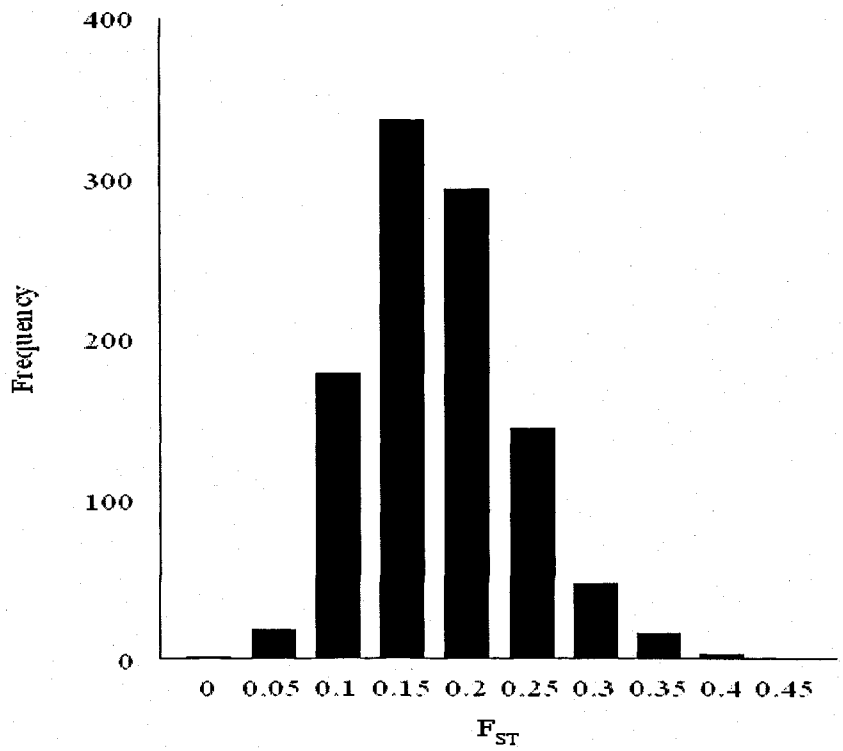

Figure 8: Frequency histogram of F_{ST} values calculated using 11 microsatellite loci for 1035 pair-wise comparisons among 46 walleye populations in Ontario.

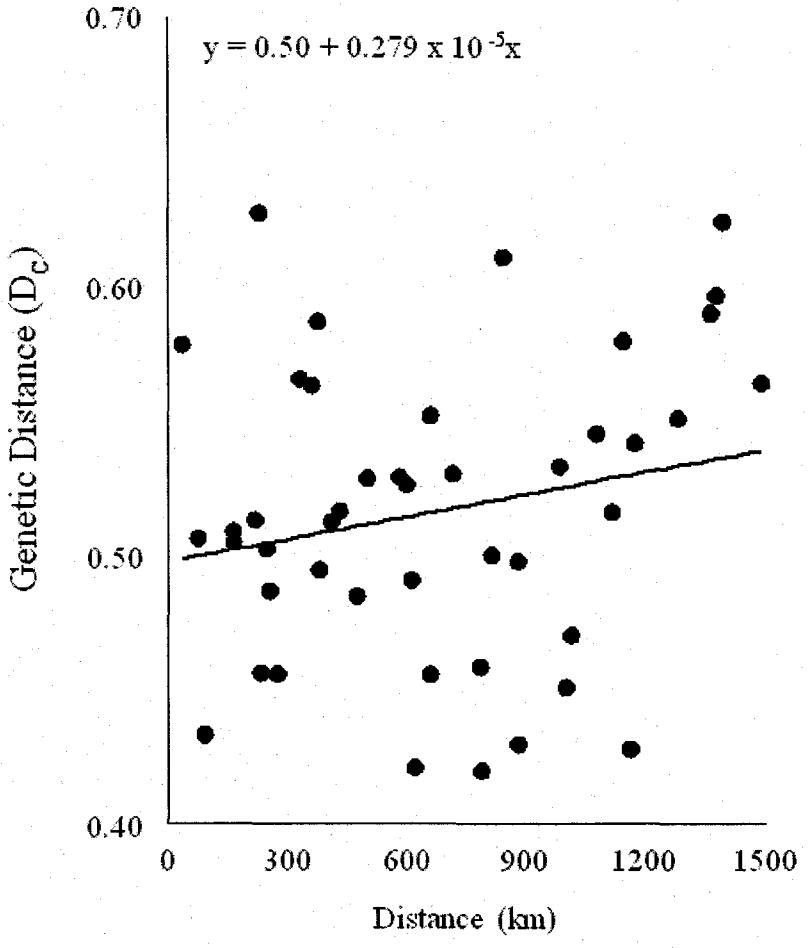

Figure 9: Isolation-by-distance relationship between Nelson River drainage basin population pairs using pair-wise genetic chord distance (D_C; Cavali-Sforza and Edwards, 1967) versus geographic distance (km) for walleye populations in the province of Ontario, Canada. The regression equation is shown ($r^2 = 0.1$, $P < 0.05$, Mantel test).

Natural and anthropogenic effects on population structure

GeneClass2 was used as an indirect method to test for admixture among populations, indicative of possible gene flow. The average proportion of individuals self-assigned to their population of origin (source population) across all lakes was 0.85.

Scugogg Lake and Young Lake had the highest proportion of individuals assigned to populations other than the lake of origin (0.15 and 0.3 respectively) indicating likely introgression. Surprisingly, all of the individuals sampled from Sakwite and Wolfe lakes were not successfully assigned to any one population, which may be indicative of admixture of populations due to migration or hatchery supplementation from a source population not included within this study. Where individuals were assigned to populations different from their lake of origin, the majority of individuals were generally assigned to one of four lakes (10% - Big Gull; 33% - Mink; 13% - Rice; 30% - Skootamatta; see Table 1). Pair-wise analysis of population differentiation (F_{ST}) among hatchery supplemented populations and among populations that have no record of being supplemented revealed a lower level of population differentiation among hatchery populations (F_{ST} = 0.142; 95% CI = 0.117 – 0.166) than non-supplemented populations (F_{ST} = 0.167; 95% CI = 0.131 – 0.202). The level of population differentiation between the two groups was significantly different as indicated by a paired sample t-test ($t = -5.2$, $df = 504$, $p < 0.001$).

Discussion

Population Structure within and among Drainage Basins

A moderate amount of genetic population structure was found to exist among inland lake walleye populations across Ontario. Population genetic differentiation in walleye populations was not unexpected since these populations are likely the result from colonization from multiple refugia and have been geographically reproductively isolated to varying degrees for up to 12 000 years. The lakes surveyed contain genetically distinct populations and the divergence exhibited a wide range of variability with a maximum observed pair-wise F_{ST} of 0.37 and minimum of 0.011 (Figure 4). The overall F_{ST} (0.155) for walleye populations in this study was comparable to the level of divergence in Lakes Erie and St. Clair walleye using a measure analogous to F_{ST} for mtDNA, φ_{ST} = 0.131 (Stepien and Faber, 1998). The close agreement between the two divergence estimates is despite differing mutation rates and very different sampling designs. The level of population differentiation for walleye is similar to the level of microsatellite DNA population differentiation observed among 35 northeastern populations of lake resident lake whitefish, *Coregonus clupeaformis,* (F_{ST} = 0.161; Lu *et al.*, 2001) on a comparable geographical scale. However, to our knowledge, no published studies have examined genetic population structure of walleye among inland lakes, although a number of studies have been published on the walleye populations of the Great Lakes (Todd and Haas, 1993; Billington *et al.*, 1992; Stepien and Faber, 1998; Gatt *et al.*, 2002).

Hierarchical analysis of the population differentiation indicated that the pattern of genetic differentiation among populations was not as expected. Gene flow within a drainage basin would be expected to produce equal or less genetic differentiation within drainage basins relative to among drainage basins. For example, observations of European populations of Arctic charr (*Salvelinus* alpinus) Brunner *et al.* (1998) note that in these lake resident populations 18 % of the microsatellite genetic variance explained was among-drainage basins, and 19.2 % of the variance explained was within-drainage basins. A similar pattern of microsatellite variance was reported for among basins vs. within basins (8.8% and 7.9% respectively) in North American lake cisco (*Coregonus artedi*) populations (Turgeon and Bernatchez, 2001). In contrast, we observed that more than 10 times of the genetic variance explained at the within drainage basin level (14.3%) relative to among drainage basins (1.2%). Similar results were reported by Castric *et al.* (2001) for brook charr (*Salvelinus fontinalis*) from five major drainage basins in Maine, where they observed that within drainage basin explained variance was approximately six times higher than among-drainage basin explained variance (0.203 and 0.037, respectively). Although the precise mechanisms for the greater explained within-drainage basin variance are unclear, these results may possibly reflect departure from migration-drift equilibrium or gene flow between drainage basins. Departure from migration-drift equilibrium would suggest that the amount of time since postglacial colonization has been insufficient for the accumulation of genetic differences among drainage basins (Castric *et al.*, 2001). In addition, natural gene flow and / or hatchery reared progeny from one population to supplement another population (anthropogenic gene flow) cannot be ruled out as a possible factor, especially since the supplementation

of walleye populations is common within Ontario. Gene flow between drainage basins is expected to reduce population genetic differentiation (Waples, 1994), and hence possibly result in the low among-drainage basin variance explained, but relatively high within drainage basin variance explained (Heath *et al.*, 2001; Miller and Kapuscinski, 2003).

Neighbour-joining cluster analysis also indicates that inter-basin gene flow is a likely factor resulting in greater levels of population structure within-drainage basins than among-drainage basins. The phenogram depicts the populations as being loosely clustered by drainage basin with populations intermingled among drainage basins instead of being grouped within their respective drainage basins; populations within the Nelson River basin (drainage basin 2; Figure 2) exhibit the strongest within basin clustering. As mentioned above, gene flow, natural or anthropogenic, could account for this pattern, as the introduction of fish for supplementation purposes among primary drainage basins could further weaken drainage basin differentiation, with weaker effects on the within basin structure. Despite the low degree of differentiation among drainage basins, population structure is evident among walleye.

The neighbour-joining cluster analysis provides evidence of anomalous gene flow between walleye populations that did not generally clustered geographically, irrespective of the primary drainage basin of origin, but gene flow generally did not occur between the north versus south clades (Figure 2). Evidence from the cluster analysis is made somewhat ambiguous by the placement of two southern lakes (Wolfe and Deer lakes) into the northern clade and one northern lake (Young Lake) being placed into the southern clade. The individual grouping patterns of the populations within the clades is somewhat less distinct and, again may be indicative of migration by individuals (i.e. gene

flow) between geographically proximate lakes within and among basins. If gene flow is occurring among geographically close populations, then the isolation-by-distance model is expected to be valid, but our neighbour-joining cluster analysis also shows a close association among some populations that are geographically distant. This observation is not readily explainable by natural gene flow between populations because even a few migrants per generation would homogenize allele frequencies among populations, nor is it explained by the divergence of allele frequencies in populations that are relatively isolated (Miller and Senanan, 2003). Although it has been shown that Ontario walleye have likely originated from three glacial refugia (Todd and Haas, 1993; Billington *et al.*, 1992; Murdoch and Hebert, 1997), our data identifies only two glacial refugia as being more likely, and such a historical genetic signature may be the explanation for the observed division into a north-south clade.

Natural and Anthropogenic Influences on Population Structure

Isolation-by-distance is expected based on a stepping-stone model of population divergence (Kimura and Weiss, 1964) and is used as a basis to predict increasing genetic differences with increasing separating distance. However this relationship may be confounded by natural and anthropogenic factors that can influence population differentiation among freshwater fish populations. Substantial genetic differentiation is expected among populations of a freshwater species as populations become isolated from one another due to past vicariance events (Senanan and Kapuscinski, 2000) in combination with species-specific dispersal limitations (Carvalho, 1993). The Nelson River Basin, the only primary drainage basin to exhibit evidence for within drainage

basin clustering as depicted by the neighbour-joining analysis, was also the only basin to provide evidence for isolation by distance, suggesting that those populations are probably approaching genetic equilibrium (Slatkin, 1993, Hutchinson and Templeton, 1999). This positive correlation may be simply due to low levels of anthropogenic disturbances among these extreme north-west populations, or that gene flow among the populations follows a stepping-stone model. There was no evidence of isolation-by distance among the 46 sampled populations or for the other four drainage basins. The failure to show an isolation-by-distance relationship at either scale for those groups suggests that they are not at migration–drift equilibrium (Hutchinson and Templeton, 1999), which may be a consequence of recent colonization of the sampled lakes or that isolation-by-distance may be weak or undetectable over large geographic scales (Castric and Bernatchez, 2003), but may be present at a smaller geographic scale between geographically close populations (Hutchinson and Templeton, 1999). It seems unlikely that the time since colonization would have been solely responsible for the isolation-by-distance pattern observed in the Nelson River Basin as it would likely have been colonized on a latitudinal cline similar to other basins as the glaciers retreated. Geographical scale and artificial gene flow appears to be the most likely explanation for the isolation-by-distance pattern for the sampled populations as this basin encompasses populations that are closer together relative to the other basins, thus we suspect that smaller basins would be better suited for a stepping-stone model describe dispersal as opposed to the larger primary drainage basins (i.e. Lake Superior / Lake Huron Basin, Hudson Bay / James Bay Basin)

Genotype assignment analyses are being used by fisheries managers primarily to determine possible population admixture due to cryptic migration, or to quantify the

possible genetic impact of artificial supplementation programs. We had expected that artificial gene flow would have an effect on the population structure because the transfer or supplementation of non-native fish can result in a reduction in genetic diversity among populations (Ryman *et al.*, 1995). For example, among 32 populations of Atlantic salmon (*Salmo salar*) in Denmark that have been exposed to variable levels of supplementation effort, there was significantly lower population differentiation among hatchery supplemented populations compared to populations with limited or no stocking (Ruzzante *et al.*, 2001). We observed that hatchery supplemented walleye populations in Ontario were significantly more similar to one another than non-stocked populations ($p < 0.001$), based on pair-wise F_{ST} comparisons between hatchery and non-hatchery supplemented walleye populations. In addition, the majority of genotype assignments identified individuals as belonging to their sampled source population (85 %) and a much lower proportion of individuals (12 %; see Table 1) were not assigned to any population. The inability of the model to assign individuals to a specific population is likely a result of the population of origin not being included in the analysis, or the individuals were the progeny of introgression, again with unknown source populations (Baudouin *et al.*, 2004). Interestingly, 3 % of the individuals were assigned to other source populations with the majority of individuals being assigned to one of four lakes. The assignment of individuals to a population other than the sampled source population (mis-assignment) provides statistical evidence of gene flow among the sampled walleye populations (Paetkau *et al.*, 2004). Where individuals were assigned to a population not of sample origin, 86% of the individuals were assigned to four populations located in south-eastern Ontario (Big Gull, Skootamatta, Mink, and Rice lakes), but not all mis-assignments were

associated with known stocking records; 24% of the sampled populations have no record of being stocked but do have individuals assigned to other populations. Some of the anomalous individual assignments (i.e. 30% of the sampled individuals from Young Lake were assigned elsewhere; Table 1) reflect probable human mediated gene flow as the identified source populations included ones that were geographically distant (e.g. > 1000km). Although genotype assignments indicated that only a small fraction of the fish appeared to be migrants (approximately 3%), the genetic structure of Ontario walleye reflects human mediated effects. Nevertheless, consideration of conservation and ecological concerns are necessary as introgression between native and introduced individuals may eventually erode the overall genetic population structure and compromise local adaptations in the populations involved (reviewed in Allendorf & Waples, 1996).

In summary, moderate to high levels of population structure were found among the 46 sampled populations, as well as at the finer geographical scales of individual drainage basins. Our results indicate that Ontario walleye populations are genetically differentiated into northern and southern clades, which is likely a result of post-glacial colonization events. Within each clade, neighbour-joining analysis suggests that gene flow is occurring among populations and drainage basins, and this gene flow is not simple dispersal, but rather likely due to anomalous fish movement since we found no evidence for genetic isolation by distance within four of the five drainage basins. Genotype assignment analysis also indicated that the population structure of walleye in upland Ontario lakes is primarily due to historic geographic isolation among the sampled populations, but with a substantial component of gene flow that is best explained by

human-mediated fish transfers. The evidence of anthropogenic contributions to the population structure of walleye, supported by hatchery lakes exhibiting lower F_{ST} relative to un-supplemented populations, has important evolutionary and conservation implications for walleye. Management activities, such as population supplementation and harvest practices, need to take into account that without some degree of consideration for the genetic structure of a species, the homogenization of the genetic structure within and among populations will reduce long-term persistence within its range in a stochastic environment.

References Cited

Angers B, Bernatchez L (1998) Combined use of SMM and non-SMM methods to infer fine structure and evolutionary history of closely related brook charr (*Salvalinus fontinalis*, Salmonidae) populations from microsatellites. *Molecular Biology and Evolution*, **15**, 143 – 159.

Allendorf F, Ryman N, Utter F (1987) Genetic and fishery management: past, present and the future. *In* Population genetics and fishery management. (ed. Ryman N and Utter FM), pp. 1 – 18. University of Washington Press, Washington.

Allendorf FW, Waples RS (1996) Conservation and genetics of salmonid fishes. *In* Conservation Genetics: Case Histories from Nature (eds Avise, J.C. & Hamrick, J.L.), pp. 238 – 280. Chapman & Hall, New York.

Avise JC (2004) *Molecular Markers, Natural History, and Evolution*, 2 edn. Sinauer Associates, Sunderland, MA.

Baudouin L, Lebrun P (2001) An operational Bayesian operational approach for the identification of sexually reproduced cross – fertilized populations using molecular markers. *Acta Horticulturae*, **546**, 81 – 93.

Baudouin L, Piry S, Cornuet JM (2004) Analytical Bayesian approach for assigning individuals to populations. *Journal of Heredity*, 217 – 224.

Becker G. C. 1983. *Fishes of Wisconsin*. University of Wisconsin Press, Madison, MI.

Bernatchez L, Wilson CC (1998) Comparative phylogeography of nearartic and palearctic fishes. *Molecular Ecology*, **7**, 431 – 452.

Billington N, Barrette RJ, Hebert PDN (1992) Management implications of mitochondrial DNA variation in walleye stocks. *North American Journal of Fisheries Management*, **12**, 276 – 284.

Billington N, Strange RM (1995) Mitochondrial DNA analysis confirms the existence of a genetically divergent walleye population in northeastern Mississippi. *Transactions of the American Fisheries Society*, **124**, 770 – 776.

Brunner PC, Douglas MR, Bernatchez L (1998) Microsatellite and mitochondrial DNA assessment of population structure and stocking effects in Arctic charr *Salvelinus alpinus* (Teleostei: Salmonidae) from central Alpine lakes. *Molecular Ecology*, **7**, 209 – 223.

Carvalho GR (1993) Evolutionary aspects of fish distribution: genetic variability and adaptation. *Journal of Fish Biology*, **43** (Supplement A), 53 – 73.

Castric V, Bonney F, Bernatchez L (2001) Landscape structure and hierarchical genetic diversity in the brook charr, *Salelinus fontinalis*. *Evolution*, **55**, 1016 – 1028.

Castric V, Bernatchez L (2003) The rise and fall of isolation by distance in the anadromous brook charr (*Salvalinus fontinalis* Mitchill). *Genetics*, **163**, 983 – 996.

Cavalli – Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. *Evolution*, **32**, 550 – 570.

Cox AJ, Hebert PDN (2001) Colonization, extinction, and phylogeographic patterning in a freshwater crusteacean. *Molecular Ecology*, **10**, 371 – 386.

Dimond PE, Potter BA (1996) Documentation of walleye stocking in Ontario. Percid Community Synthesis, Walleye Stocking Working Group. Fish & Wildlife Branch, Ministry of Natural Resources. Peterborough, Ontario.

El Mousadik A, Petit RJ (1996) High level of genetic differentiation for allelic richness among populations of the argan tree (*Argania spinosa* (L.) Skeels) endemic to Morrocco. *Theoretical and Applied Genetics*, **92**, 832 – 839.

Gatt MH, Fraser DJ, Liskauskas AP, Ferguson MM (2002) Mitochondrial DNA variation and stock structure of walleyes from eastern Lake Huron: an analysis of contemporary and historical samples. *Transactions of the American Fisheries Society*, **131**, 99 – 108.

Garant D, Dodson JJ, Bernatchez (2000) Ecological determinants and temporal stability of the within-river population structure in Atlantic salmon (*Salmo salar* L.). *Molecular Ecology*, **9**, 615 – 628.

Goudet J. (2001) FSTAT, a program to estimate and test gene diversities and fixation Indicies (v. 2.9.3). Available from http://www.Unil.ch/izea/softwares/fstat.html.

Hänfling B, Hellemans B, Volckaert FAM, Carvalho GR (2002) Late glacial history of the cold-adapted freshwater fish *Cottus gobio,* revealed by microsatellites. *Molecular Ecology*, **11**, 1717 – 1729.

Hartl DL, Clarke AG (1997) *Principles of Population Genetics*. Sinauer Associates, Inc., Sunderland, MA, USA.

Hutchinson DW, Templeton AR (1999) Correlation of pair-wise genetic and geographic distance measures: inferring the relative influence of gene flow and drift on the distribution of genetic variability. *Evolution*, **52**, 1898 – 1914.

Fleming IA, Hindar K, Mjolnerod IB, Jonsson B, Balstad T, Lamberg A (2000) Lifetime success and interactions of farm salmon invading a native population. *Proceedings of the Royal Society of London* (Series B), **267**, 1517 – 1523.

Hansen MM, Ruzzante DE, Nielsen EE, Mensberg K-LD (2000) Microsatellite and mitochondrial DNA polymorphism reveals life – history dependent interbreeding between hatchery and wild brown trout (*Salmo trutta* L.). *Molecular Ecology*, **9**, 583 – 594.

Hansen MM, Ruzzante DE, Nielsen EE, Mensberg K-LD (2001) Brown trout (*Salmo trutta*) stocking impact assessment using microsatellite DNA markers. *Ecological Applications*, **11**, 148 – 160.

Heath DD, Pollard S, Herbinger C (2001) Genetic structure and relationships among steelhead trout (*Oncorhychus mykiss*) populations in British Columbia. *Heredity*, **86**, 618 – 627.

Jennings MJ, Claussen JE, Phillip DP (1996) Evidence for heritable preferences for spawning habitat between two walleye populations. *Transactions of the American Fisheries Society*, **125**, 978 – 982.

Kerr SJ (2002) Fish stocking activities under the Community Fisheries Involvement Program (CFIP), 1982-2001. Fish & Wildlife Branch, Ministry of Natural Resources. Peterborough, Ontario. ISBN 0-7794-3288-6.

Kimura M, Weiss GH (1964) The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**, 561 – 576.

Largiadèr CR, Scholl A (1995) Effects of stocking on the genetic diversity of brown trout populations of the Adriatic and Danubian drainages in Switzerland. *Journal of Fish Biology*, **47** (Suppl. A), 220 – 225.

Lu G, Basley DJ, Bernatchez L (2001) Contrasting patterns of mitochondrial DNA and microsatellite introgressive hybridization between lineages of lake whitefish (*Corregonus clupeaformis*); relevance for speciation. *Molecular Ecology*, **10**, 965 – 985.

Michalakis Y, Excoffier L (1996) A generic estimation population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics*, **142**, 1061 – 1064.

Miller LM, Senanan W (2003) A review of northern pike population genetics research and its implications for management. *North American Journal of Fisheries Management*, **23**, 297 – 306.

Miller LM, Kapuscinski AR (2003) Genetic guidelines for hatchery supplementation programs. In: *Population Genetics: Principles and Applications for Fisheries Scientists*. (ed. Hallerman EM), pp. 329 – 355. American Fisheries Society, Bethesda, Maryland.

Morgan G.E. (2002). Manual of Instructions – Fall Walleye Index Netting (FWIN). Diagnostics and Sampling Standards Working Group, Percid Community Synthesis, Ontario Minsitry of Natural Resources, Peterborough, Ontario.

Murdoch MH, Hebert PDN (1997) Mitochondrial DNA evidence of distinct glacial refugia for brown bullhead (*Ameiurus nebulosus*) in the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, **54**, 1450 – 1460.

Nepszy SJ, Davies D, Einhouse D, et al. (1991) Walleye in Lake Erie and Lake St. Clair. In: *Status of walleye in the Great Lakes: Case studies prepared for the 1989 Workshop* (eds Colby CA, Lewis CA, Eschenroder RL), pp. 145 – 568. *Great Lakes Fishery Commision Special Publication*, **91 – 1**, Ann Arbor, MI.

Neraas LP, Spruell P (2001) Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork River system. *Molecular Ecology*, **10**, 1153 – 1164.

Nielsen JL, Carpanzano C, Fountain MC, Gan CA (1997) Mitochondrial DNA and nuclear microsatellite diversity in hatchery and wild *Oncorhynchus mykiss* from freshwater habitats in southern California. *Transactions of the American Fisheries Society*, **126**, 397 – 417.

Page RDM (1996) TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in Biosciencs*, **12**, 357 – 358.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology*, **4**, 347 – 354.

Paetkau D, Slade R, Burdens M, Estoup A. (2004) Genetic assignment methods for the direct, real time estimation of migration rate: a simulation – based exploration of accuracy and power. *Molecular Ecology*, **13**, 55 – 65.

Piry S, Alapetite A, Cornuet JM, Paetkau D, Baudouin L, Estoup A (2004) GeneClass2: a software for genetic assignment and first – generation migrant detection. *Journal of Heredity*, **95**, 536 – 539.

Quinn TP (1993) A review of homing and straying in hatchery-produced salmon. *Fisheries Research*, **18**, 29 – 44.

Ryman N, Utter F, Laikre L (1995) Protection of intraspecific biodiversity of exploited fishes. *Reviews in Fish Biology andFisheries*, **5**, 417–446.

Ruzzante DE, Hansen MM, Meldrup D (2001) Distribution of individual inbreeding coefficients, relatedness and influence of stocking on native anadromous brown trout (*Salmo trutta*) population structure. *Molecular Ecology*, **10**, 2107 – 2128.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN* version 2.0: *A software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, Dept. of Anthropology and Ecology, University of Geneva, CP 511, 1211 Geneva 24, Switzerland. Available from: http://anthropologie.unige.ch/arlequin.

Scott WB, Crossman EJ (1998) Freshwater Fishes of North America. Galt House Publications, Oakville, Canada.

Senanan W, Kapuscinski AR (2000) Genetic relationships among populations of northern pike (*Esox lucius*). *Canadian Journal of Fisheries and Aquatic Sciences*, **57**, 391 – 404.

Shaklee JB, Currens KR (2003) Genetic stock identification and risk assessment. In: *Population Genetics: Principles and Applications for Fisheries Scientists*. (ed. Hallerman EM), pp. 291 – 328. American Fisheries Society, Bethesda, Maryland.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics*, **16**, 393 – 430.

Slatkin M (1993) Isolation by distance in equilibrium and non – equilibrium populations. Evolution, 47, 264 – 279.

Stepien CA, Faber JE (1998) Population genetic structure, phlogeography and spawning philopatry in walleye (*Stizostedion vitreum*) from mitochondrial DNA control region sequences. *Molecular Ecology*, **7**, 1757 – 1769.

Todd TN, Haas RC (1993) Genetic and tagging evidence for movement of walleye between Lake Erie and Lake St. Claire. *Journal of Great Lakes Research*, **19**, 445 – 452.

Turgeon J, Bernatchez L (2001) Clinal variation at microsatellite loci reveal historical secondary intergradation between glacial races of *Coregonus artedi* (Teleostei: Coregoninae). *Evolution*, **55**, 2274 – 2286.

Waples RS (1994) Genetic considerations in recovery efforts for Pacific salmon. *Conservation Biology*, **8**, 884 – 886.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution*, **38**,1358-1370.

Yamamoto S, Morita K, Koizumi I, Maekawa K (2004) Genetic differentiation of white-spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial-temporal changes in gene frequencies. *Conservation Genetics*, **5**, 529 – 538.

Chapter 4

Discussion and Conclusion

The objectives of this study were to investigate the genetic diversity and population structure of Ontario walleye with the following specific goals: 1) to determine the relationships between lake parameters and population genetic diversity estimators and genetic diversity and life history traits, which are critical components of fitness (Chapter 2); 2) to investigate for population structure among the 46 sampled walleye populations (Chapter 3); and 3) to determine if population divergence is primarily due to natural processes or recent anthropogenic events (Chapter 3).

The genetic diversity and population structure of walleye have been considerably influenced by past glacial events and have undoubtedly resulted in changes to habitat and range. The population structure of Ontario walleye has been affected by post-glacial dispersal and reproductive isolation that has resulted in limited migration (gene flow) among populations. As we have shown, there is a well defined division between northern and southern walleye populations indicating restricted gene flow. Varying selection pressures across the walleye range has likely contributed further to the genetic diversity observed among current populations. As was observed, genetic diversity of walleye is strongly influenced by lake habitat parameters possibly reflecting the variation in habitat stability and population size. Generally, larger habitats (i.e. lakes) have the potential to support greater numbers of individuals; lake size was found to have a significant influence on genetic diversity. This observation has implications for the management of smaller lake populations since smaller systems tend to be at greater risk than larger systems to stochastic events, which could result in smaller effective population sizes and /

or recurring severe bottlenecks that could increase the incidence of inbreeding and expression of deleterious alleles. The influence of climate effects on population size can also have a significant effect on genetic diversity across populations. The variability of productivity and carrying capacity among lakes would restrict population size, and thus genetic diversity. Finally, transfers of fish among populations, either by authorized stock transfers of individuals for supplementation purposes or by "bait-bucket-biologists" to supposedly improve or create a fishery, have had an effect on the genetic diversity of walleye. Introgression between native and introduced individuals can genetically homogenize populations, and thus reducing genetic differentiation among populations. Nevertheless, natural and anthropogenic effects have impacted the genetic diversity and population structure driven by changes in the probability of inbreeding (i.e. small population size or genetic bottlenecks) or outbreeding (i.e. hatchery supplementation).

Genetic diversity has an important role in the long-term persistence of a species, which becomes evident as individual populations persistence in changing environments. Positive correlations between genetic diversity measures and lake parameters and hatchery supplementation are likely due to inbreeding as a result of small population sizes and / or recurring bottlenecks or outbreeding between distantly related individuals. It appears that inbreeding depression was limited among the sampled walleye populations and was only detectable at the early life stages of juvenile walleye across populations, but there was no evidence suggesting outbreeding depression. Positive associations between population genetic diversity and early growth rate, coupled with the negative associations with F_{IS}, suggest that the effect on early growth rate is a consequence of inbreeding due to small effective population sizes. This relationship could have critical implications for

the long term persistence of small populations as juvenile walleye may not survive to sexual maturity to contribute to future generations.

Introgression between wild and hatchery reared individuals can alter the genetic structure within and among populations through the introduction of novel alleles that may reduce beneficial genetic adaptations that have evolved within a specific habitat, and by increasing the genetic similarity among stocked populations. The removal of reproductive isolating barriers by transfers of fish stocks among populations (artificial gene flow) can drive genetic homogenization of populations. Among the walleye populations sampled, those populations supplemented with hatchery reared individuals were significantly less differentiated than populations of native origin. The positive linear relationship between genetic diversity estimates (d^2) and the magnitude of hatchery supplementation effort indicate that genetic introgression is likely occurring between the introduced hatchery and native walleye, but as mentioned above, there is no evidence of outbreeding depression in walleye populations.

In this study, we identified a significant level of population structure and genetic diversity among 46 widely dispersed walleye populations across the province of Ontario using microsatellite DNA. Such an approach allowed us to detect for large scale environmental effects on genetic diversity, and in turn determine if these changes in genetic diversity were associated with environmental variation and translated into inbreeding or outbreeding depression. The power of such a large genetic survey, as conducted in this study, is important because of its robustness and captures a greater range of genetic variability than a smaller scaled study could. Our results are thus

important for the maintenance and conservation of genetic diversity and evolutionary potential of a species as a goal of fisheries management, and contributes to further our understanding of the environmental and anthropogenic factors that contribute to the genetic diversity of a species, and hence population genetics of natural populations.

Management Implications

The data from this study indicate that factors influencing genetic diversity, population viability, and the overall population structure have serious evolutionary and conservation implications for walleye populations. Prior to this study, knowledge of the genetic diversity and population structure of inland walleye populations in Ontario was non-existent. Past management activities, such as hatchery supplementation, have altered the genetic architecture of some populations while others have remained relatively undisturbed. The data from this study provides evidence that Ontario walleye populations are genetically differentiated.

Even though there are numerous walleye populations within Ontario it would take an enormous amount of resources to manage each on an individual basis. I would propose that it would be more feasible to manage populations on the basis of relatively small watershed units, relative to current practices. Since walleye hatchery enhancement is common practice within Ontario, the brood stock should originate from the target population in order to eliminate the possibility of introducing new alleles or possibly weaken / eliminate local adaptations that have developed over time. By designing broodstock capture programs to simulate natural effective population sizes, or as close to it as possible, would guard against stocking too few progeny of limited genotypes.

The use of native or genetically similar stocks for supplementation programs will help preserve unique and potentially locally adapted genotypes of native Ontario upland walleye populations. Because of the numerous genetically differentiated stocks of walleye throughout Ontario, management practices may need to adjust to managing populations at a smaller scale for genetic conservation purposes. Small scale management areas would, to a large degree, increase the probability of long-term population viability and evolutionary potential instead of being managed as a few large populations inhabiting arbitrarily designated management areas.

The genetic techniques used in this study can and should continue to be used to provide information necessary for the management and enhancement of Ontario walleye populations. Sampling and collection of life history data and tissue for DNA analyses should therefore continue to allow for the monitoring of changes in genetic diversity among populations. This would be extremely useful in detecting temporal changes in genetic variation that may possibly expose reductions in fitness due to inbreeding / outbreeding effects, especially for walleye populations subject to heavy anthropogenic influences (e.g. harvest rates, habitat destruction, intense supplementation). Additional genetic surveys of walleye populations throughout Ontario are therefore recommended to further identify the population structure of walleye for improved management capabilities and to expand the understanding of walleye biology and population genetics.

Appendix I: Summary of population mean direct count (observed) heterozygosity (H_O), mean d^2, and the inbreeding coefficient (F_{IS}), lake identification number (ID #), and individual lake physical parameters: latitude, hatchery supplementation (HEI + 1); surface area (log ha); climate above 5^O (GDD); and number of species sampled (# Species).

Lake	ID #	H_O	d^2	F_{IS}	Latitude	log (HEI +1)	log ha	GDD ($< 5^O$)	# Species
Allumette	1	0.76	8.81	0.038	45.88	0	4.13	1821	17
Big Gull	2	0.77	8.15	0.092	44.83	5.12	3.37	1842	14
Burditt	3	0.74	8.05	0.081	48.95	5.40	3.15	1641	5
Churchill	4	0.77	8.06	0.025	50.79	0	3.62	1367	8
Deer	5	0.69	5.78	-0.011	45.58	0	2.08	1637	7
Dog	6	0.64	7.36	0.057	48.30	0	3.72	1255	7
Eagle	7	0.75	7.46	0.044	49.68	5.54	4.44	1579	15
Eltrut	8	0.70	7.04	0.051	49.01	0	3.38	1591	
Finlayson	9	0.68	7.96	0.118	48.91	4.62	3.16	1461	
French R.	10	0.79	10.54	0.046	45.93	5.99	3.91	1735	18
Fushimi	11	0.74	7.94	0.05	49.82	0	3.09	1229	8
Garnham	12	0.66	5.45	0.033	49.01	0	2.83	1228	8
Holden	13	0.75	9.24	0.072	46.25	5.36	3.88	1662	16
Ivanhoe	14	0.74	7.04	-0.011	48.08	5.07	3.01	1367	6
Kagiano	15	0.73	8.77	0.068	49.27	0	2.13	1169	8
Kebskwasheshi	16	0.64	5.49	0.055	47.39	0.97	2.93	1396	6
Longlegged	17	0.70	8.61	0.102	50.78	0	3.84	1481	7
Mainville	18	0.79	8.47	0.002	48.87	0	2.94	1642	3
Miminiska	19	0.76	8.63	0.062	51.55	0	3.79	1148	9
Mink	20	0.84	9.02	-0.018	45.57	5.51	2.73	1839	12
Missinaibi	21	0.65	5.68	0.104	48.36	0	3.89	1286	9
Moira	22	0.72	7.90	0.042	44.49	5.41	2.92	1932	13
Mountain	23	0.76	8.63	0.092	44.71	0	1.8	1848	4
Nagagami	24	0.73	7.74	0.04	49.42	0	3.73	1248	8
Nipigon	25	0.70	9.81	0.1	49.83	0	4.19	1205	17

Appendix I cont'd

Lake	ID #	H_O	d^2	F_{IS}	Latitude	log (HEI +1)	log ha	GDD (< 5^O)	# Species
Partridge	26	0.69	6.08	0.018	49.72	4.47	2.82	1148	6
Pipestone	27	0.76	8.95	0.007	49.09	4.62	3.59	1640	10
Red	28	0.76	8.57	0.041	51.04	5.73	4.25	1450	14
Rice	29	0.83	8.62	0.029	44.17	4.71	4.00	1960	16
Round	30	0.73	6.93	0.023	48.02	4.42	3.08	1438	12
Sakwite	31	0.78	8.04	0.001	49.03	0	2.58	1612	
Savanne	32	0.60	4.53	0.034	48.83	0	2.56	1244	5
Scugogg	33	0.82	8.34	0.021	44.17	3.99	3.80	1920	14
Shikwamkwa	34	0.77	8.67	0.034	48.09	0	2.75	1262	8
Sideburned	35	0.77	7.49	-0.046	47.74	0.94	3.06	1313	5
Skootamatta	36	0.81	7.94	0.035	44.84	5.72	3.09	1780	11
St. Joseph	37	0.80	9.52	0.006	51.08	4.78	4.71	1301	9
Steel	38	0.74	8.86	0.054	49.20	0	3.20	1097	7
Timiskaming	39	0.71	8.11	0.1	47.13	0	4.47	1587	19
Wabatongushi	40	0.49	3.14	0.054	48.47	0	3.58	1232	6
Wakami	41	0.62	5.06	0.046	47.49	4.42	3.23	1383	5
W. Kabenung	42	0.60	5.17	0.061	48.26	0.94	3.19	1126	5
White	43	0.81	8.34	0.02	48.77	0	3.77	1210	8
Whitewater	44	0.71	9.60	0.102	50.81	5.08	4.02	1154	9
Wolfe	45	0.80	8.27	0.019	44.68	5.15	2.98	2028	12
Young	46	0.78	8.06	0.001	49.85	0	2.91	1471	8

Appendix II: Summary of mean observed heterozygosity ($\bar{x}$ H_O) and mean d^2, mean total length(mm), mean condition factor (g/mm^3), mean relative fecundity (eggs / g), mean GSI (gonadosomatic index), early growth rate (mm / yr), and annual mortality (A %) by sex for 46 Ontario walleye populations at age 3 where available.

Lake	$\bar{x}$ H_o		$\bar{x}$ d^2		$\bar{x}$ mm		$\bar{x}$ g/mm^3		$\bar{x}$ eggs/g	$\bar{x}$ GSI	$\bar{x}$ mm/yr		A%	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Allumette	0.76	0.75	8.20	9.93	359.6	307.3	1.06	1.07	62.11	1.18	126.6	114.1	0.33	0.35
Big Gull	0.75	0.78	8.15	7.91	380.1	398.8	1.05	1.05	51.26	2.54	125.0	114.2	0.32	0.29
Burditt	0.72	0.76	7.98	8.41	438.5	418.0	0.88	0.88			126.0	121.3	0.21	0.24
Churchill	0.76	0.78	8.33	8.05	385.8	399.4	1.01	1.02			91.8	86.6	0.18	0.21
Deer	0.68	0.71	5.16	6.34	322.7	385.9	0.95	0.93	60.52	3.36	129.1	114.5	0.30	0.32
Dog	0.64	0.64	7.44	7.16	392.1	335.8	1.01	0.94	63.87	1.72	107.6	106.1	0.17	0.20
Eagle	0.76	0.75	7.69	6.74	430.8	385.7	1.02	1.04	39.22		109.4	111.7	0.23	0.26
Eltrut	0.66	0.71	6.10	7.11	332.9	314.1	0.86	0.89			98.2	97.2	0.58	0.61
Finlayson	0.69	0.67	7.69	8.76	330.9	310.3	0.92	0.94			106.4	105.7	0.28	0.30
French River	0.79	0.79	10.0	10.8	445.4	397.1	1.00	1.01	45.49	1.84	133.4	110.6	0.31	0.34
Fushimi	0.74	0.73	9.38	7.58	349.8	346.4	1.10	1.04	55.80	2.02	85.8	94.7	0.37	0.40
Garnham	0.65	0.66	5.67	5.32	403.3	387.0	0.95	0.86	36.61	1.41	93.0	101.1	0.29	0.32
Holden	0.76	0.73	9.42	8.89	361.5	344.4	0.99	0.98	53.72	1.74	117.0	127.7	0.31	0.34
Ivanhoe	0.73	0.76	6.73	7.30	378.9	350.8	1.03	0.99	48.93	2.10	99.9	107.0	0.30	0.32
Kagiano	0.73	0.72	8.72	8.59	341.1	319.4	1.02	0.97	43.48		126.8	123.5	0.16	0.19
Kebskewasheshi	0.64	0.62	5.65	5.18	368.0	335.4	1.04	0.97	52.82	2.69	97.7	108.9	0.14	0.17
Longlegged	0.71	0.67	8.60	8.70	471.7	415.3	1.02	1.06	50.29		106.8	101.3	0.23	0.26
Mainville	0.78	0.78	8.76	8.14	346.2	333.2	0.90	0.87			91.2	99.2	0.22	0.25
Miminiska	0.77	0.68	8.70	7.70	425.4	389.1	0.95	0.98	18.04	1.09	80.8	83.2	0.13	0.16
Mink	0.84	0.84	7.92	10.1	385.3	411.6	1.01	1.02	67.14	2.90	162.1	129.8	0.41	0.43
Missinaibi	0.65	0.64	5.58	5.58	358.0	300.1	0.97	0.92	62.50	2.96	87.9	84.6	0.23	0.25
Moira	0.69	0.74	7.72	8.22	406.2	356.6	1.02	1.05	34.01	2.88	100.9	92.7	0.22	0.25
Mountain	0.77	0.76	8.74	8.48	340.5	355.7	0.95	0.94	80.32	2.69		102.9	0.26	0.28
Nagagami	0.77	0.68	7.94	7.52	398.2	373.1	0.97	0.91	45.30	3.81	100.3	91.7	0.29	0.31

Appendix II cont'd

Lake	$\bar{x}$ H_o		$\bar{x}$ d^2		$\bar{x}$ mm		$\bar{x}$ g/mm^3		$\bar{x}$ eggs/g	$\bar{x}$ GSI	$\bar{x}$ mm/yr		A%	
	F	M	F	M	F	M	F	M	F	M	F	M	F	M
Nipigon	0.72	0.69	10.1	12.4	332.9	301.0	1.11	1.13			154.4	136.8	0.27	0.29
Partridge	0.69	0.69	6.18	5.94	376.8	332.3	1.01	1.06			119.6		0.81	0.83
Pipestone	0.77	0.76	8.79	9.02	505.8	459.2	1.08	1.12			134.7	118.7	0.15	0.18
Red	0.76	0.79	8.51	8.40	406.9	368.6	1.02	1.04			98.2	92.8	0.19	0.22
Rice	0.79	0.83	7.39	9.02	429.5	392.2	0.99	1.03	83.48		141.3	119.6	0.33	0.36
Round	0.73	0.71	6.87	6.67	365.7	377.5	1.16	1.13	42.94	1.71	136.2	125.0	0.32	0.35
Sakwite	0.79	0.77	8.14	7.81	372.6	357.3	0.84	0.87			108.7	95.1	0.41	0.44
Savanne	0.60	0.60	4.48	4.44	400.5	363.0	0.98	1.04			89.0	79.3	0.20	0.23
Scugogg	0.80	0.84	8.64	8.04	268.8	270.4	0.99	0.95			141.2	126.2	0.40	0.43
Shikwamkwa	0.77	0.77	9.04	8.11	336.8	304.8	0.97	0.91	40.07	2.63	136	110.3	0.50	0.52
Sideburned	0.79	0.75	7.61	7.30	414.0	368.6	1.10	1.03	32.73	3.71	129.5	170.6	0.30	0.33
Skootamatta	0.82	0.78	8.02	7.16	527.8	499.5	1.13	1.12	85.04	3.80	119.6	112.8	0.14	0.17
St. Joseph	0.81	0.77	9.58	9.05	398.1	408.0	0.99	1.02	50.09		90.8	81.5	0.17	0.20
Steel	0.73	0.75	8.78	9.03	357.3	349.5	0.93	0.95			101.2	76.9	0.30	0.32
Timiskaming	0.73	0.69	8.09	8.41	298.1	307.8	0.98	0.94	68.95	1.64	81.2	69.5	0.22	0.25
Wabatongushi	0.52	0.47	3.35	2.98	375.8	359.3	1.00	0.93	58.92	2.52	83.5	72.4	0.33	0.36
Wakami	0.63	0.64	5.13	3.35	422.1	361.0	1.08	1.01	54.61		125.7	121.7	0.18	0.21
W. Kabenung	0.62	0.58	5.33	4.87	390.9	395.6	0.99	0.95	48.62	2.81	109.4	110.8	0.26	0.28
Whitewater	0.82	0.71	9.48	9.62	410.1	383.0	1.02	1.03			80.0	69.7	0.55	0.58
White	0.70	0.80	8.67	7.54	340.4	335.2	0.84	0.85		0.32	119.8	133.9	0.18	0.21
Wolfe	0.84	0.78	8.74	7.80	311.9	381.0	0.98	0.95		2.28		136.2	0.21	0.24
Young	0.78	0.78	7.89	8.08	437.1	435.5	1.00	1.03			127.1	125.3	0.45	0.47

Appendix III: Results of the univariate regression analyses by population for female and male life history traits with heterozygosity and mean d^2. Numbers in parentheses indicate the number of females used for relative fecundity correlations.

			Gonadosomatic Index				Condition Factor				Relative Fecundity				Total Length			
Lake	**Sex**	**n**	**Part. Corr.**	**H_O**	**Part. Corr.**	**d^2**	**Part. Corr.**	**H_O**	**Part. Corr.**	**d^2**	**Part. Corr.**	**H_O**	**Part. Corr.**	**d^2**	**Part. Corr.**	**H_O**	**Part. Corr.**	**d^2**
Allumette	F	30 (10)					-0.22	NS	-0.05	NS	-0.14	NS	-0.41	NS	-0.21	NS	-0.06	NS
	M	17	-0.07	NS	0.10	NS	0.44	NS	0.37	NS					-0.29	NS	-0.31	NS
Big Gull	F	16					0.01	NS	0.31	NS					-0.10	NS	-0.28	NS
	M	26	0.25	NS	-0.07	NS	0.33	NS	-0.06	NS					0.17	NS	0.29	NS
Burditt	F	33					0.40	NS	0.24	NS					-0.19	NS	-0.19	NS
	M	14					0.01	NS	0.23	NS					0.42	NS	0.58	*
Churchill	F	36					-0.03	NS	-0.16	NS					0.26	NS	0.51	**
	M	20					0.26	NS	-0.36	NS					0.05	NS	-0.38	NS
Deer	F	22					0.20	NS	0.26	NS					-0.16	NS	-0.14	NS
	M	26	-0.12	NS	0.77	NS	0.12	NS	0.06	NS					-0.40	*	0.21	NS
Dog	F	33 (11)					-0.19	NS	-0.16	NS	0.51	NS	0.08	NS	-0.02	NS	-0.03	NS
	M	13	0.47	NS	-0.37	NS	0.30	NS	0.01	NS					0.23	NS	0.11	NS
Eagle	F	42 (13)					0.06	NS	0.08	NS	0.51	NS	0.35	NS	-0.01	NS	-0.07	NS
	M	6																
Eltrut	F	28					0.07	NS	0.02	NS					-0.28	NS	-0.29	NS
	M	22					-0.26	NS	-0.42	NS					-0.03	NS	0.01	NS
Finlayson	F	30					-0.19	NS	-0.13	NS					0.29	NS	-0.09	NS
	M	14					-0.50	NS	-0.45	NS					0.06	NS	-0.18	NS
French	F	22 (6)					0.003	NS	0.10	NS	0.60	NS	0.19	NS	-0.09	NS	-0.20	NS
	M	22	0.52	NS	0.17	NS	0.09	NS	-0.08	NS					-0.06	NS	0.03	NS
Fushimi	F	11					0.19	NS	-0.30	NS					0.49	NS	0.51	NS
	M	35	0.10	NS	0.10	NS	0.17	NS	-0.24	NS					-0.25	NS	-0.28	NS

Appendix III cont'd

			Gonadosomatic Index				Condition Factor				Relative Fecundity				Total Length			
Lake	Sex	n	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2
Holden	F	38 (13)					-0.13	NS	0.21	NS	0.52	NS	0.47	NS	-0.10	NS	0.07	NS
	M	10	-0.18	NS	0.33	NS	-0.34	NS	-0.11	NS					-0.28	NS	-0.37	NS
Ivanhoe	F	31					-0.11	NS	-0.34	NS	-0.45	NS	-0.11	NS	0.11	NS	-0.43	*
	M	16	0.49	NS	0.26	NS	-0.13	NS	-0.08	NS					0.24	NS	0.28	NS
Kagiano	F	25					0.04	NS	0.20	NS					-0.48	*	-0.26	NS
	M	21					-0.41	NS	-0.23	NS					-0.23	NS	0.04	NS
Kebskewasheshi	F	33 (18)					-0.13	NS	-0.03	NS	0.08	NS	0.19	NS	-0.13	NS	-0.15	NS
	M	14	-0.25	NS	-0.48	NS	-0.35	NS	-0.49	NS					-0.18	NS	-0.28	NS
Longlegged	F	29					0.21	NS	0.27	NS					-0.20	NS	-0.06	NS
	M	17					0.49	NS	-0.17	NS					0.57	*	0.32	NS
Mainville	F	24					-0.17	NS	0.09	NS					-0.22	NS	0.11	NS
	M	26					0.24	NS	0.36	NS					-0.29	NS	-0.27	NS
Miminiska	F	40 (21)					-0.07	NS	0.17	NS	0.08	NS	-0.06	NS	0.10	NS	0.03	NS
	M	4																
Mink	F	20					-0.05	NS	0.28	NS					-0.22	NS	0.06	NS
	M	35	-0.01	NS	0.06	NS	0.08	NS	-0.005	NS					-0.09	NS	0.07	NS
Missinaibi	F	24					-0.21	NS	-0.20	NS					0.19	NS	-0.07	NS
	M	19					-0.15	NS	0.24	NS					0.09	NS	0.04	NS
Moira	F	23 (10)					-0.09	NS	-0.20	NS					0.15	NS	0.11	NS
	M	21	-0.35	NS	-0.29	NS	-0.29	NS	-0.45	*					-0.38	NS	-0.08	NS
Mountain	F	18					0.13	NS	0.16	NS					-0.31	NS	-0.23	NS
	M	26	0.08	NS	0.12	NS	-0.15	NS	-0.02	NS					0.14	NS	0.25	NS
Nagagami	F	24					0.12	NS	-0.01	NS					0.06	NS	-0.03	NS
	M	22					0.25	NS	0.26	NS					0.18	NS	0.16	NS
Nipigon	F	30					-0.21	NS	0.16	NS					0.12	NS	0.04	NS
	M	13					0.04	NS	-0.25	NS					-0.46	NS	0.47	NS

Appendix III cont'd

			Gonadosomatic Index				Condition Factor				Relative Fecundity				Total Length			
Lake	Sex	n	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2
Partridge	F	24					-0.06	NS	0.16	NS					0.19	NS	0.12	NS
	M	20					-0.26	NS	0.16	NS					0.05	NS	0.35	NS
Pipestone	F	22					-0.26	NS	-0.20	NS					-0.02	NS	0.17	NS
	M	26					0.05	NS	-0.24	NS					-0.16	NS	-0.16	NS
Red	F	39					-0.15	NS	-0.10	NS					0.18	NS	0.14	NS
	M	9					-0.40	NS	-0.29	NS					-0.39	NS	-0.31	NS
Rice	F	14					-0.14	NS	-0.58	*					-0.01	NS	0.07	NS
	M	28					0.27	NS	0.09	NS					-0.12	NS	0.08	NS
Round	F	21					-0.58	**	-0.21	NS					-0.26	NS	-0.16	NS
	M	13	-0.14	NS	-0.50	NS	-0.06	NS	0.17	NS					-0.24	NS	-0.29	NS
Sakwite	F	26					0.35	NS	0.35	NS					0.09	NS	0.12	NS
	M	22					0.04	NS	-0.25	NS					0.06	NS	0.10	NS
Savanne	F	28					0.24	NS	0.14	NS					-0.24	NS	-0.18	NS
	M	20					-0.15	NS	-0.16	NS					-0.04	NS	-0.17	NS
Scugogg	F	24					-0.37	NS	-0.23	NS					0.18	NS	0.01	NS
	M	22					0.35	NS	-0.49	*					0.37	NS	-0.19	NS
Shikwamkwa	F	23					0.42	NS	0.54	*					0.28	NS	0.13	NS
	M	24	0.64	*	0.05	NS	0.25	NS	0.02	NS					0.12	NS	-0.07	NS
Sideburned	F	25(18)					0.02	NS	0.16	NS	-0.06	NS	-0.18	NS	-0.27	NS	-0.06	NS
	M	23					0.32	NS	0.24	NS					0.17	NS	0.16	NS
Skootamatta	F	26(19)					0.05	NS	0.02	NS	0.07	NS	-0.18	NS	0.21	NS	-0.04	NS
	M	31	-0.07	NS	0.25	NS	0.10	NS	0.22	NS					-0.20	NS	0.36	NS
St. Joseph	F	28					0.14	NS	-0.33	NS					-0.20	NS	-0.14	NS
	M	17					0.00	NS	-0.20	NS					-0.07	NS	0.03	NS
Steel	F	27					-0.02	NS	0.19	NS					-0.19	NS	-0.32	NS
	M	20					0.25	NS	-0.10	NS					0.16	NS	0.18	NS

Appendix III cont'd

			Gonadosomatic Index				Condition Factor				Relative Fecundity				Total Length			
Lake	Sex	n	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2	Part. Corr.	H_O	Part. Corr.	d^2
Timiskaming	F	29					0.21	NS	0.05	NS					0.33	NS	-0.04	NS
	M	12	0.15	NS	-0.16	NS	-0.38	NS	-0.14	NS					0.03	NS	-0.34	NS
West Kabenung	F	15					-0.55	*	-0.26	NS					-0.30	NS	0.24	NS
	M	29	0.29	NS	0.02	NS	0.04	NS	-0.09	NS					0.16	NS	0.002	NS
Wabatongushi	F	28 (8)					-0.19	NS	0.02	NS					0.29	NS	0.25	NS
	M	5																
Wakami	F	28 (9)					0.58	**	0.56	**	0.20	NS	0.10	NS	0.13	NS	0.05	NS
	M	20	0.12	NS	0.10	NS	-0.32	NS	-0.43	NS					0.24	NS	-0.002	NS
White	F	38					0.07	NS	0.08	NS					-0.20	NS	-0.16	NS
	M	19	-0.45	NS	0.34	NS	-0.26	NS	0.10	NS					-0.12	NS	0.02	NS
Whitewater	F	29					-0.38	*	-0.48	*					-0.08	NS	0.13	NS
	M	16					0.32	NS	-0.02	NS					0.23	NS	0.14	NS
Wolfe	F	18					0.23	NS	-0.05	NS					-0.69	**	-0.42	NS
	M	26	-0.01	NS	-0.15	NS	0.03	NS	-0.18	NS					-0.26	NS	-0.13	NS
Young	F	25					0.05	NS	0.01	NS					0.56	*	0.20	NS
	M	23					0.20	NS	0.03	NS					0.28	NS	-0.09	NS

Note: Boldfaced values are those that remain significant (* $P < 0.05$; ** $P < 0.01$) after sequential Bonferroni correction was applied to the heterozygosity and mean d^2 independently. NS (non-significant). Numbers in parentheses indicate the number of females available for relative fecundity measures.

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