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GENETIC VARIATION WITHIN AND AMONG ONTARIO HATCHERY STOCKS

OF LAKE TROUT (*Salvelinus namaycush*) AS MEASURED BY THREE

MOLECULAR MARKER SYSTEMS: APPLICATIONS TO REHABILITATION

AND HATCHERY MANAGEMENT

By

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Abstract

Increases in the use of hatchery fish have raised interest in the genetics of hatchery-reared fish. Four types of genetic concerns have been identified for hatchery stock management: loss of stocks, domestication, loss of within and among-stock genetic variability. Each has an impact on the quality of fish available. To address these concerns, mitochondrial DNA, randomly amplified polymorphic DNA, and microsatellite DNA loci were considered for their suitability for stock discrimination, hatchery monitoring, and mixed-stock analysis of rehabilitated populations of lake trout (*Salvelinus namaycush*).

Mitochondrial DNA variation was analyzed to determine if it could be used in a mixed-stock analysis. The accuracy of the estimates was low because one clone is found at high frequencies in all lake trout stocks studied. Mitochondrial DNA variation may have limited application in mixed stock analysis, but is well suited for further studies of variation in the wild.

Randomly amplified polymorphic DNA (RAPD) loci were tested for Mendelian inheritance in lake trout and the 13 variable loci amplified in the Killala Lake hatchery stock were inherited in a Mendelian fashion. Individual variation of RAPD markers in lake trout hatchery stocks was large which made genetic stock identification and mixed-stock analysis difficult.

Genetic relationships among hatchery stocks in Ontario were examined using

microsatellite DNA loci. Heterologous primer sets designed for other salmonid species could be used to amplify microsatellite loci in lake trout. The genetic relationships observed among the hatchery stocks were similar to those observed in previous genetic analyses using allozyme and mitochondrial DNA markers.

The results of a simulation study based on microsatellite DNA and allozyme loci indicate that microsatellite DNA loci provide more accurate estimates than allozyme loci in mixed stock analyses. Further comparisons of microsatellite DNA loci to mitochondrial DNA and RAPD loci indicate that microsatellite loci are the most useful for mixed-stock analysis. Nuclear markers in general are more useful than mitochondrial markers because they provide data on the contributions of both sexes, whereas mitochondrial DNA data only describes the contribution of females.

Comparisons of genetic variation in Ontario hatchery stocks of lake trout using mitochondrial DNA, RAPD loci, and microsatellite DNA loci indicate that within-stock variation is being maintained in most stocks. Small numbers of founders and unequal sex ratios may be responsible for any losses that have occurred. Among-stock variation is similar to that observed in wild populations. The few changes observed may be due to sampling errors of gametes used to found broodstocks or to provide yearling lake trout for hatchery supplemented lakes such as Lake Simcoe.

Preface

There are seven chapters in the thesis. The first chapter is a General

Introduction to the project and the seventh is a synthesis and set of recommendations.

The remaining chapters have been written as manuscripts for technical reports or for

submission to peer-reviewed journals. At the present time, chapter 3 has been

published. Information about the title, authors, and individual contribution to each of

the chapters is as follows:

Chapter 2: "Mitochondrial DNA variation in Ontario hatchery stocks of lake trout, *Salvelinus namaycush*, and assessment of its use in a mixed-stock analysis."

> Authors: W. Stott, Peter E. Ihssen, and Bradley N. White.
> Contribution: Field work was performed in conjunction with the Ontario Ministry of Natural Resources (OMNR). All laboratory and statistical analyses were performed by the candidate. The research was conducted under the guidance of P.E.I. and B.N.W.

Chapter 3: "Inheritance of RAPD molecular markers in lake trout, *Salvelinus namaycush*."

> Authors: W. Stott, Peter E. Ihssen, and Bradley N. White.
> Reference: Molecular Ecology. 6:609-613.
> Contribution: Experimental crosses were made by OMNR staff. Laboratory and statistical analyses were performed by the candidate. The research was conducted under the guidance of P.E.I. and B.N.W.

Chapter 4: "Variability of RAPD loci in eight hatchery and one wild stock of lake trout, *Salvelinus namaycush* from Ontario and Manitoba."

> Authors: W. Stott, Peter E. Ihssen, and Bradley N. White.
> Contribution: Field work was performed in conjunction with the OMNR. All laboratory and statistical analyses were performed by the candidate. The

research was conducted under the guidance of P.E.I. and B.N.W.

Chapter 5: "Microsatellite DNA variation in hatchery stocks of lake trout *(Salvelinus namaycush)*."

Chapter 6: "Estimation of origins of mixed-stock samples of lake trout *(Salvelinus namaycush)* using allozyme and microsatellite DNA data: a simulation analysis.

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Chapter 1
General Introduction

Genetics and Hatcheries

Fish reared in a hatchery are being employed in rehabilitation and management more frequently. More restoration programs requiring planted fish are funded as commercial and recreational demands for the resource increase. For example, in the Columbia River basin, more than 90 state, provincial and federal hatcheries raise and release over 190 million juvenile Pacific salmon (*Oncorhynchus* spp.) each year, and the number will most likely increase (Busack and Currens 1995). Hatchery-reared fish have been used in management programs since the 1800's (Moring *et al.* 1995), and while salmonids account for a large proportion of hatchery stocks, many other species are also cultured. For example, a recent conference (Schramm and Piper 1995) on the uses of cultured fish stocks in North America featured papers that mentioned more than 40 different cultured species and hybrids (Table 1.1).

Stocking programs that employ hatchery fish have three main goals: restoration, rehabilitation, and enhancement of fish stocks. As part of restoration programs, self-sustaining populations are reestablished in waters where they have been lost. Rehabilitation programs focus on the recovery of depressed populations (Moring *et al.* 1995; Cuenco *et al.* 1993) and enhancement programs attempt to increase the production of a fishery or establish a fishery where it did not exist previously (eg. a put-grow-take

1

Table 1.1 Cultured fish species used in stocking programs discussed at American Fisheries Society Symposium 15.

Common name	Scientific name	Reference
chinook salmon	*Oncorhynchus tshawytscha*	Heard *et al.* 1995
rainbow trout	*O. mykiss*	Stone 1995
coho salmon	*O. kisutch*	Lange *et al.* 1995
sockeye salmon	*O. neika*	Flagg *et al.* 1995
Apache trout	*O. apache*	Carmichael *et al.* 1995
cutthroat trout	*O. clarki*	Ludwig 1995
brook charr	*Salvelinus fontinalis*	Stone 1995
lake trout	*S. namaycush*	Lange *et al.* 1995
bull trout	*S. confluentus*	Ludwig 1995
Atlantic salmon	*Salmo salar*	Moring *et al.* 1995
brown trout	*S. trutta*	Lange *et al.* 1995
Arctic grayling	*Thymallus arcticus*	Stone 1995
whitefish	*Coregonus spp.*	Bartley *et al.* 1995
American shad	*Alosa sapidissima*	Hendricks 1995
razorback sucker	*Xyrauchen texanus*	Modde *et al.* 1995
esocids	*Esox spp.*	Wahl *et al.* 1995

Table 1.1 continued.

Common name	Scientific name	Reference
walleye	*Stizostedion vitreum*	Wahl *et al.* 1995
bass	*Micropterus spp.*	Forshage and Fries 1995
channel catfish	*Ictalurus punctatus*	Wahl *et al.* 1995
sturgeon	*Acipenser spp., Huso huso*	Bartley *et al.* 1995
red drum	*Sciaenops ocellatus*	McEachron *et al.* 1995
Atlantic cod	*Gadus morhua*	Blankenship and Leber 1995
haddock	*Melanogrammus aeglefinus*	Blankenship and Leber 1995
pollock	*Pollachius virens*	Blankenship and Leber 1995
Atlantic mackerel	*Scomber scombrus*	Blankenship and Leber 1995
winter flounder	*Pleuronectes americanus*	Blankenship and Leber 1995
white seabass	*Atracscion nobilis*	Bartley *et al.* 1995
madai	*Pagrus major*	Blankenship and Leber 1995
striped mullet	*Mugil cephalus*	Blankenship and Leber 1995

fishery). Several factors can affect the survival and performance of stocked fish in a new environment. The ability of fish to adapt to new surroundings is influenced by genetic variation within the population because particular alleles and allele combinations affect chances of survival. The success of stocking programs is variable because it is sometimes difficult to choose donor stocks that are well matched to a new environment (Ludwig 1995) although some programs are successful. For example, hatchery chinook salmon (*O. tshawytscha*) stocked to enhance the fishery in southeast Alaska have provided an increased number of fish available for harvest. The larger fishery may provide some protection for wild stocks (Heard *et al.* 1995). Analysis of trout stocking programs in Wyoming indicates return rates are low overall. The most success occurs in heavily fished areas located on smaller water bodies (Stone 1995). Atlantic salmon (*Salmo salar*) stocks are slowly being rehabilitated in rivers in southern Maine using a non-native hatchery stock (Moring *et al.* 1995). A program to restore lake trout (*Salvelinus namaycush*) to Lake Ontario is starting to produce results after over 20 years of effort (Marsden *et al.* 1993), while in other Ontario lakes the rates of reproduction and recruitment among stocked lake trout are very low (MacLean *et al.* 1981; Evans and Willox 1991; Ludwig 1995).

The genetic variation that characterizes a wild stock can be altered in a hatchery by the methods used to establish and maintain stocks used in rehabilitation programs (Wahl *et al.* 1995). The genetic effects of hatchery practices or the hatchery environment can be grouped into four categories: loss of a stock, loss of within-population genetic

variation, loss of among-population genetic variation, and domestication (Busack and Currens 1995; Hard 1995). The first, the loss of a genetic stock, may be the result of an accident in the hatchery (eg. a water flow problem) that causes the destruction of most or all of a stock. Once the alleles that characterize a population are lost it is virtually impossible to recreate them, therefore allelic combinations that may be adapted to a particular environment are gone.

The loss of within and/or among-population genetic variation can have several effects. As within-population variability decreases there is a reduction in the number of alleles and in the number of different allelic combinations. Two types of genetic change can affect within-population diversity in a hatchery, inbreeding random genetic drift. Inbreeding, or mating of closely related individuals, increases individual and overall homozygosity because related fish are more likely to have the same alleles and allele combinations than unrelated fish (reviewed in Wright 1969). Inbreeding is associated with a decrease in fitness due to increased expression of deleterious recessive alleles or due to a decrease in the performance of phenotypes that are influenced by heterozygosity (reviewed in Gall 1987). The second type of genetic change, genetic drift, is a loss of genetic variation due to sampling errors of gametes and can result in the loss of alleles and a reduction in heterozygosity. Genetic drift occurs in all natural populations during spawning, because not all gametes may be used. The rate of genetic drift is larger in smaller populations (Kimura 1955), and is quantified using the concept of effective population size. Effective population size (N_e) is defined as the number of individuals

in an idealized panmictic population that would result in the same sampling variance as found in the population under consideration. Genetic drift has been identified as a concern in hatcheries, and as the major force behind losses of variation or changes in allele frequencies in hatchery populations of salmonids, and in particular, small parental sample size is identified as the leading factor contributing to increased rates of genetic drift (Allendorf and Phelps 1980; Ryman and Ståhl 1980; Cross and King 1983; Ståhl 1983; Vuorinen 1984; Waples and Teel 1990). Other factors that could reduce N_e include: unequal sex ratio, unequal family sizes (Crow and Kimura 1970), or spawning practises, such as mixing sperm from several males (Withler 1988; Gile and Ferguson 1990). Recommendations for an appropriate sample size vary somewhat from 50 males and 50 females (N_e=100) to 100 males and 100 females (N_e=200; Kincaid 1995).

Loss of genetic variation among hatchery populations can cause the differences between populations to decrease. Among-population diversity is most affected by gene flow. High levels of gene flow may result in a loss of adaptability because new alleles may be introduced that do not allow a population to perform as well in its native environment. High levels of gene flow may also result in the disruption of co-adapted allele complexes that may increase individual performance (Busack and Currens 1995). In a hatchery, high rates of gene flow may be produced by mingling different stocks that have different geographic origins. Hatchery stocks may be affected in different ways by gene flow. Differences between wild populations may be magnified or, if stocks are mixed, hybrid stocks may be created. However, there is little evidence to date to

demonstrate changes in fitness due to hatchery x wild interactions due to difficulties in measuring performance and obtaining appropriate sample sizes (Campton 1995). Hatchery-related selection pressures may also decrease differences among stocks of different geographic origins that are maintained within the same hatchery.

The fourth genetic hazard, domestication, can be defined as changes in the quantity, variety, or combinations of alleles within a captive population compared to its source population as a result of selection in an artificial environment (Busack and Currens 1995). Wild and hatchery stocks begin to diverge at a rate dependent on the rates of selection in the two different environments and on the demographic characters of the populations (Doyle *et al.* 1995). The traits most often affected relate to physiology, morphology, or behaviour (Hynes *et al.* 1981). Domestication is the result of hatchery practises or of the hatchery environment itself. Therefore, it is difficult to isolate and control the selective forces behind domestication (Busack and Currens 1995). In many stocking programs, the offspring from yearly collections of wild parents are released at an early life history stage in order to minimize the time spent in a hatchery. However, an experiment by Doyle *et al.* (1995) indicates that domestication can occur very soon after the transfer of stock to a hatchery environment. Doyle *et al.* (1995) used genetic data to demonstrate first generation domestication in a hatchery stock of Atlantic cod (*Gadus morhua*). Differences in the survival to yolk sac adsorption of seven groups of maternal half-sibs indicated strong selective forces were acting at an early life history stage.

Genetic changes that occur in a hatchery, especially those that affect within and

among-population variation, may apply most strongly to salmonid fishes because of their biology (Hard 1995). The homing ability of some salmonids may result in more local adaptation, their relatively limited capacity for larval and egg dispersal encourages more population structuring. In addition, salmonids have a fairly complex life history that is composed of several stages and each stage has a significant genetic component in some species (Beacham 1988; Beacham and Murray 1988). Therefore it may be more important to consider the genetic profiles of both donor and recipient stocks for stocking programs that involve salmonids. In contrast, studies of marine fish like the white seabass (*Atractoscion nobilis*) reveal less among-population variation than salmonid populations (Gyllensten 1985; Bartley *et al.* 1995). White seabass also have a greater potential for dispersal during all life history stages, therefore it is felt that the existing populations would not be adversely affected by enhancement programs.

It has been recommended that hatchery broodstock and samples of planted fish be screened genetically. In addition, programs to monitor wild and supplemented populations are advised. Combining data sets from hatchery and wild samples would provide information that may be used to determine the genetic changes due to hatchery practices on, for example, disease resistance, life-stage specific survival and fecundity. Research to address the above issues is strongly recommended (Busack and Currens 1995), especially on the effects of domestication. Such research may now be possible using molecular techniques that allow a variety of areas of the genome to be examined. The first step is to describe variation in hatchery stocks. Descriptive data can then be

used for several purposes. The data will give managers insight on how well hatchery practices maintain genetic diversity, will provide markers for studies of performance of hatchery stocks once they are released, and will allow the effects of domestication to be addressed.

Data on genetic variation in hatchery and wild stocks of fish species has been gathered using a variety of genetic techniques. A short review of the more commonly used methods is given below.

Cytogenetic analysis

Karyotype analyses have proven somewhat useful for stock and species identification. Variation in both chromosome number and banding patterns have been used to analyze fish species and populations. Variation in chromosome number in fish usually results from Robertsonian translocations. Studies of salmonids in particular have revealed Robertsonian translocations (Thorgaard 1983; reviewed in Phillips and Ihssen 1990) that have definite geographic patterns.

Chromosome banding techniques involve the preparation and staining of metaphase stage chromosomes. The four most common methods for banding chromosomes are Q-banding, G-banding, R-banding, and C-banding. It is possible to detect structural changes such as deletions in the banded chromosomes. For example, Q-banded chromosomes are stained with quinacrine hydrochloride, which stains AT-rich regions most strongly and chromomycin A_3 staining can be used to stain the nucleolar organizer region (Dowling

et al. 1996). Population studies have been performed on a variety of salmonid species including the iwana (*Salvelinus leucomaenis*), the lake trout (*S. namaycush*), and the Arctic charr (*S. alpinus*; Phillips and Ihssen 1990).

Allozyme electrophoresis

Electrophoretic identification of allozymes has been used successfully for many years in fish stock analysis. Electrophoretically detectable differences in the amino acid compositions of enzymes with a common substrate are compared. Proteins from tissues such as muscle or liver are separated on starch or cellulose acetate gels and a variety of histochemical stains are used to visualize activity of the enzymes. Well over 100 enzymes can be analyzed in some species with reasonable effort and cost. In fish, blood groups were analyzed initially, and then other proteins were analyzed as staining systems and buffers were developed (see review by deLigny 1969).

In the past, allozyme variation has been the main tool used to analyze population structure of fish species in many taxa (Avise and Smith 1974; Winans 1980; Andersson *et al.* 1981; Grant *et al.* 1984; Gyllensten 1985; Grant *et al.* 1987; Ståhl 1987; Ropson *et al.* 1990; Ramon and Castro 1997), and has been especially useful for identifying stocks of salmonid species (Allendorf *et al.* 1976; Grant *et al.* 1980; Ihssen *et al.* 1988; Ferguson *et al.* 1995). Allozyme variants have also been used for species identification (Crabtree and Buth 1987; Van der Bank *et al.* 1989), and for gene mapping (Allendorf *et al.* 1994).

Mitochondrial DNA variation

While analysis of allozyme variation remains a highly useful technique to analyze stock structure in many fish species, some drawbacks exist that make the marker less useful in some applications. Allozyme variation is low in some species (eg. pike; Healy 1980), which makes analysis of population structure difficult. As well, the technique can only examine coding regions of the genome, and since protein-coding loci evolve more slowly than non-coding regions, recently developed genetic differentiation (less than 10,000 years) may not be detected. As an alternative method, or as a supplement to allozyme data, analysis of mitochondrial DNA (mtDNA) has also become a widely used technique. The mitochondrial genome in fish species is about 17,000 base pairs in length (Ferris and Berg 1987) and evolves at a faster rate than portions of the nuclear genome (Brown et al. 1979). There is no recombination in the mitochondrial genome, and in vertebrates, the molecule is inherited through the maternal lineage as a single circular unit (Ferris and Berg 1987). The mitochondrial DNA molecule has an effective population size that is one quarter that of nuclear loci because it is inherited clonally. Therefore population differences may accumulate at a faster rate (Nei and Li 1979).

MtDNA variation can be studied directly by sequencing portions of the molecule or by using restriction endonucleases. Restriction endonuclease analysis has been used widely for population studies of fish (Ward and Grewe 1995). Restriction endonucleases are enzymes that cut DNA at specific sequences. The resulting DNA fragments are separated electrophoretically and then visualized. Restriction fragment length

polymorphism analysis (RFLP) reduces costs compared to sequence analysis, handling time, and allows larger sample sizes to be examined, thus increasing statistical power. As well, mtDNA can be extracted from muscle, blood, or eggs which can allow non-lethal sampling techniques to be employed.

The properties of mtDNA make it suitable for both phylogenetic (Avise *et al.* 1987; Grewe *et al.* 1990) and population studies (Gyllensten and Wilson 1987; Smith *et al.* 1989; Beckenbach 1991; Ovenden 1990; Vitic and Strobeck 1996). The results of population studies using both allozymes and mtDNA variation often complement each other (Avise 1985), however in some cases (Ferguson *et al.* 1991) different estimates of population differentiation may be obtained. Differences in the inheritance of the molecule and in its rate of evolution combined with the demographics of the population can produce such dissimilar results.

Ribosomal DNA

The multi-gene family coding for 28S and 18S ribosomal RNA (rRNA) has been analyzed in few fish species, mostly for phylogenetic analyses (Popodi *et al.* 1985; Phillips and Pleyte 1991; Zhuo *et al.* 1994). An individual rRNA unit has one copy of each of the major genes (28S and 18S) which are separated from the next unit by an intergenic spacer. The whole unit is tandemly repeated on several chromosomes. Both the genes and the spacer unit can be analyzed for variation using DNA sequencing and RFLP analysis.

Microsatellite DNA

Microsatellite DNA loci are short (one to four nucleotides) repeats 100-300 base pairs in length (Tautz 1989). The polymerase chain reaction (PCR) is used to analyze microsatellite variation. PCR involves a series of denature-anneal-extension cycles to amplify the DNA that lies between two-primer sequences (Saiki et al. 1988). Primer sequences for the PCR reaction are chosen from single-copy regions flanking the microsatellite loci. PCR products are separated electrophoretically on a standard DNA sequencing gel and then visualized. Initially the procedure may be complex and time consuming, but improvements in the techniques, such as multiplexing, reduce the effort involved (O'Reilly et al. 1996). A potential drawback is that new primers may need to be developed for each species, which is technically demanding and time-consuming. However, as more species are studied, more primers become available that can be used in closely related species (eg. Angers and Bernatchez 1996). Preliminary analyses of microsatellite DNA loci of fish species have been performed to document the loci (Morris et al. 1996; Scribner et al. 1996), their inheritance patterns (Estoup et al. 1993; Colbourne et al. 1996), and their organization within the genome (Brooker et al. 1994). Substantial variation has been observed in species that do not display much protein or allozyme variation (eg. Miller and Kapuscinski 1996). The markers are now being used to analyze population structure in several fish species (Angers et al. 1995; McConnell et al. 1995; O'Reilly et al. 1996; Ruzzante et al. 1996).

Randomly amplified polymorphic DNA

The development of PCR has allowed other genetic loci to be examined. The PCR amplification of randomly amplified polymorphic DNA (RAPD) uses a randomly designed primer pair to amplify a variety of sequences. Therefore, no prior knowledge of the genome is required and a large number of fragments and samples may be analyzed (Williams *et al.* 1990). The technique has been most useful in studies of commercial plant species, but has had limited use in fish species so far. RAPD variation has been used most often for identification of species (eg. Bardakci and Skibinski 1994), but some population studies have been published (Bielawski and Pumo 1997) and several labs are currently examining the RAPD variation in other species. Inheritance studies of RAPD polymorphisms have produced inconsistent results; some studies find that RAPD loci can be treated as Mendelian markers while others report inconclusive or mixed results (Williams *et al.* 1990; Riedy *et al.* 1992; Bucci and Menozzi 1993). In addition, RAPD markers are usually inherited as dominant rather than co-dominant markers and as a result heterozygotes cannot be identified. The effect of inheritance patterns on stock analysis has not yet been investigated in any detail.

Lake trout biology and genetic diversity

The lake trout is an important commercial and sport fish in Ontario and has been analyzed for genetic variation using several genetic markers (Krueger and Ihssen 1995). Lake trout have been stocked in North America for many years, and have been introduced

in the southern United States, South America, New Zealand, and Europe (Martin and Olver 1980). As with other fish species, the success of lake trout stocking programs has been variable (MacLean et al. 1981; Evans and Willox 1991; Marsden et al. 1993; Krueger and Ihssen 1995; Lange et al. 1995).

Lake trout are freshwater teleosts found from Alaska to the northern United States and their east-west distribution extends from British Columbia to Labrador (Martin and Olver 1980). Lake trout are usually the top predator in oligotrophic ecosystems. However, their habitat and forage base varies. For example, planktivorous lake trout are found in Lake Louisa, Algonquin Park, Canada and river-dwelling stocks are found in British Columbia (Martin and Olver 1980). Different forms (usually called morphotypes or morphs) of lake trout have been identified based on variation of physical traits such as pigmentation, morphology and life history traits. Differences are great enough that some morphs, like the siscowet, were once given species or sub-species status. The siscowet dwells in large lake systems and is characterized by a higher body fat content than the more common lean morph (Krueger and Ihssen 1995).

At one time lake trout were present in all five Great Lakes, but now they are limited to two Lakes Huron and Superior) with small rehabilitated populations in two others (Lakes Ontario and Michigan; Krueger and Ihssen 1995). The disappearance of lake trout from the Great Lakes can be attributed to: lamprey predation (Holey et al. 1995), environmental degradation, pollution (Dorr et al. 1981), overfishing, modification of water levels for production of hydroelectric power, habitat alteration, and perhaps

competition from the introduction of exotic species. The remaining forms (mostly lean and some siscowet) may have been the most tolerant to the various pressures. Inland populations have been affected to a lesser extent. The greatest pressure on inland populations has been the loss of spawning habitat (Krueger and Ihssen 1995).

Rehabilitation or restoration efforts in the Great Lakes have included improvement of spawning habitat, lamprey control, and stocking programs. The success of stocking programs in particular has been variable, perhaps due to loss of too much habitat or the loss of appropriately adapted gene pools. For example, lake trout from Clearwater Lake (Manitoba) stocked into Lake Ontario may not have been suitable for a deep water environment. The ability to retain gases in the swimbladder (a character associated with diving ability) is a heritable trait that differed between Manitoba lake trout and other Great Lakes stocks (Ihssen and Tait 1974; Krueger and Ihssen 1995).

In order to supply lake trout for management and rehabilitation programs in Ontario, the Ontario Ministry of Natural Resources (OMNR) has created hatchery stocks originating from a number of geographic areas. Hatchery stocks are maintained in two ways. Gametes from lake trout caught in the wild are crossed in a hatchery, the offspring are reared in a hatchery for up to a year, and are returned to the lake of origin or are used as a donor stock for planting. Alternatively, a broodstock is maintained in a hatchery and offspring are used in stocking programs. Broodstock fish spend all of their lives in the hatchery environment. The most recent OMNR stocks catalogue lists eleven hatchery stocks of lake trout and one more (Iroquois Bay, Lake Huron) is in the process of being

developed (Table 1.2; Anonymous 1992).

The hatchery stocks represent lake trout adapted to a variety of different environments and have different recommended uses. For example, the manager of an inland system would be advised to choose Killala Lake or Lake Manitou over the Slate Islands lake trout stocks, and then to select either Killala Lake or Lake Manitou based on environmental factors such as water hardness, spawning season and substrate. The types of fishing pressures and goals of the rehabilitation program are also part of the decision process. Of the twelve hatchery stocks, eight are recommended for places other than their area of origin, and only five of those (Killala Lake, Lake Manitou, the Slate Islands, Michipicoten Island, and Seneca Lake) are available to managers in large numbers. The previous five stocks in particular have been used for many years with variable success (Grewe *et al.* 1994; MacLean *et al.* 1981; Evans and Willox 1991).

Genetic analyses of lake trout have been performed using four types of markers to date. The genetic variation identified has been used for phylogenetic studies of the genus *Salvelinus*, to determine the post glacial colonization routes of the species, and to analyze stock structure of hatchery and wild stocks of lake trout. Overall geographic patterns, based on chromosome data, indicate that lake trout populations form three clusters; one from the northern shores of Lakes Superior and Huron and inland lakes north of Superior,

Table 1.2 Ontario lake trout hatchery stocks.

Hatchery stock	Year created	Suggested use
Big Rideau[3]	1992	Created for rehabilitation of Big Rideau Lake, limited to assessment of supplemental stocking in Big Rideau.
Big Sound/Parry Sound	1979	Recommended for use in Big Sound and Great Lakes with habitats similar to Big Sound.
Charleston Lake[3]	1987	Created as part of study to assess supplemental stocking, and therefore are not available for use.
Devil Lake[3]	1989	Created as part of study to assess supplemental stocking, and therefore are not available for use elsewhere.
Killala Lake	1981[1]	Recommended for put-grow-take in inland lakes similar to Killala Lake, but is not recommended for the Great Lakes.
Lake Manitou[3]	1959	Recommended for the rehabilitation of Georgian Bay, Lake Huron, but not other Great Lakes. Also recommended for rehabilitation or put-grow-take in hard water inland lakes similar to Lake Manitou.
Lake Simcoe[3]	late 1970's	Recommended for Lake Simcoe only, no broodstock is maintained. Each year recaptured adults from Lake Simcoe are used to produce young lake trout that are returned to Lake Simcoe.

Table 1.2 continued.

Hatchery stock	Year created	Suggested use
Michipicoten Island	1982	Recommended for area around Michipicoten Island and eastern end of Lake Superior.
Mishibishu Lake	1988	This stock is currently being developed for the upper Great Lakes (*ie.* Lakes Superior and Huron).
Seneca Lake	1990[2]	Recommended for Lake Ontario.
Slate Islands	1980	Recommended for the rehabilitation of the west end of Lake Superior, and may be available for Lakes Huron and Ontario.
Iroquois Bay	1992	This stock is currently being developed for Lake Huron and perhaps Killarney Provincial Park.

1 This is the second broodstock developed from Killala Lake. In 1981 a broodstock was established from fish from the north basin of the lake. The first broodstock was established from the south basin in 1977 and is believed to be contaminated with lake trout from Lake Superior.

2 Lake trout for this stock came from New York State Bath Fish Hatchery.

3 No broodstocks maintained.

another cluster from the southern shore of Lake Superior, and a third from Lake Michigan (Phillips *et al.* 1989). Using variable chromomycin A₃ bands, it was possible to differentiate between two of the source populations for Ontario hatchery stocks (Michipicoten Island and Lake Manitou; Phillips *et al.* 1989). In addition, the Q-bands of seven wild and two hatchery (Jenny Lake and Marquette) stocks were examined (Phillips and Zajicek 1982). The hatchery stocks are not maintained in Ontario, but four of the wild stocks (Killala Lake, Michipicoten Island, Lake Manitou, and Seneca Lake) are sources for Ontario hatchery stocks.

The chromosomes of lake trout from other hatchery stocks have not been analyzed, and while stock-specific markers have been detected for the stocks studied so far, the technique is not routinely used. In order to analyze chromosomes, actively dividing cells are required. Living tissue is not always available for many population or management studies and considerable time and expertise are required to score the sample sizes necessary for population studies. As a result the technique may have limited use for management purposes.

An allozyme study of lake trout from Lake Superior revealed few differences among samples (Dehring *et al.* 1981). However, the sample sizes were low and samples were taken from lake trout outside their spawning aggregations, perhaps accounting for the lack of differentiation (Krueger and Ihssen 1995). Differences did exist however, between different lake trout forms (leans and siscowet) from Lake Superior (Dehring *et al.* 1981), and between leans and siscowet (Krueger *et al.* 1989) from Lake Superior.

More recent allozyme studies based on larger sample sizes collected from hatcheries or spawning populations revealed allele frequency differences at four out of five stocks in Lake Superior. The Lake Superior samples represented the western end (Gull Island), the northwest shore (Keshkabuon Island and Hare Island), the centre of the lake (the Slate Islands), and the northeast area (Michipicoten Island) of the lake. Differences existed among all samples except those on the northwest shore which were geographically more proximate to each other (<25km). On a larger geographic scale, three genetic groups appear: the upper/northwest Great Lakes and Manitoba, the southeast section of Ontario, and samples from the Haliburton area. Five alleles at five different loci were found only in the northwest group and a phosphogluconate dehydrogenase (PGDH*) allele was found only in the Haliburton ($Pgd*^{85}$) lake trout (Ihssen et al. 1988), but other stocks were not distinguished by private alleles. Genetic distances calculated using allozyme data are similar to those calculated using karyotype data (Phillips and Ihssen 1990). Hatchery and transplanted stocks of lake trout retained much of their original genetic identity in comparison to their wild donor stocks. In addition, among-stock variability was similar if wild and hatchery stocks are considered (Ihssen et al. 1988).

Lake trout stocks used to rehabilitate Lake Ontario were also analyzed using allozyme variation (Krueger et al. 1989). The dendrogram calculated from the data is similar to that produced by Ihssen et al. (1988), but the genetic distances are 100-fold larger, probably due to the fact that only polymorphic loci were analyzed, whereas Ihssen

et al. (1988) scored both polymorphic and monomorphic loci. Values of heterozygosity are slightly higher in the study by Ihssen *et al.* (1988; Table 1.3) probably due to the fact that several small inland lake populations with high levels of heterozygosity (the Haliburton Highlands, for example) were included by Ihssen *et al.* (1988), but not by Krueger *et al.* (1989). In addition, because the average (N_{Av}) sample sizes were larger ($N_{AV}=171$ versus $N_{AV}=80$ in Krueger *et al.* (1989)), rare heterozygotes were more likely to be sampled.

Three of the Ontario hatchery populations, (Killala Lake, Manitoba and Lake Manitou) were analyzed in both studies. Six polymorphic loci were scored in both studies (sAAT-1,2*, GPI-1*, sMDH-3,4*, sMEP-1*, MUP*, and SOD-2*) and can be compared. Significant (P<0.05) allele frequency differences in the samples were found at three loci for the Killala Lake samples (sMDH-3,4*, sMEP-1*, and MUP*) and at two loci for the Lake Manitou samples (sAAT-1,2* and GPI-1*). The differences observed at the three loci between the two studies are small overall. Sampling errors may account for the dissimilarity between stocks such as Lake Manitou and Killala Lake. Although the samples for both studies were acquired from the same lake, they came from different year classes and different spawning shoals.

It is possible that genetic drift in the hatchery may have resulted in a divergence from the wild samples if broodstock samples are compared to samples from the source population. For example, the Killala Lake samples in Krueger *et al.* (1989) were derived from hatchery

Table 1.3 Comparison of estimates of overall heterozygosity at allozyme loci in two studies of lake trout stocks.

| Reference | Stock | | |
	Manitoba	Killala L.	L. Manitou
Krueger *et al.* 1989	3.4%	3.2%	2.7%
Ihssen *et al.* 1988	7.4%	5.3%	4.0%

samples, whereas Ihssen *et al.* (1988) obtained samples from the wild. Differences between allelic frequencies were most pronounced in the Killala Lake fish when the two studies were compared.

Analysis of mtDNA variation in lake trout, primarily through the use of RFLP's had revealed similar geographic structuring as the allozyme data (Grewe *et al.* 1993; Wilson and Hebert 1996). Geographic variation of mtDNA reveals a western Great Lakes group, a central Great Lakes group, and an eastern Great Lakes/Ontario group. Haliburton lake trout do not have as distinctive mtDNA as allozyme profiles, and have low levels (nucleon diversity, h=0) of variation. MtDNA variation is more sensitive to reductions in population size (Bernatchez *et al.* 1989; Gyllensten and Wilson 1987). Therefore, lower mtDNA variation in the Haliburton region may be the result of a past restriction in population size during the period of time the ancestors of the present day lake trout spent in the hypothesized glacial refuge or due to the fact that the Haliburton lakes may have always supported small populations.

Five OMNR hatchery stocks (Lake Manitou, Parry Sound, Killala Lake, Lake Simcoe, and Seneca Lake) have been analyzed previously for mtDNA variation (Grewe and Hebert 1988; Grewe *et al.* 1993). Clonal lines unique to Lake Manitou and Seneca Lake were observed (Grewe and Hebert 1988), and there were shifts in the frequencies of other clonal lines in all the stocks. However, the sample sizes collected from some stocks like Lake Simcoe and Parry Sound were small (10 or less). In the study by Grewe *et al.* (1993) additional endonucleases were used which allowed discrimination between

Killala Lake and other stocks, and significant differences were observed in the clonal frequencies among the stocks. Big Rideau, Charleston Lake, Devil Lake, Lake Mishibishu, the Slate.Islands, Michipicoten Island, and Iroquois Bay stocks have not been analyzed.

Variation at rDNA loci has been examined in detail in only five sample stocks (Gull Island, Lake Manitou, Seneca Lake, Lake Superior/Marquette and Jenny Lake; Zhuo *et al.* 1994) which makes it difficult to compare to other data sets. Of the transcribed spacer regions, only the intergenic spacer (IGS) region had any significant variation. Restriction fragment analysis of the region revealed differences in band frequencies and total number of bands per individual among the samples. The variation is in fact quite high, making it difficult to perform population studies (Krueger and Ihssen 1995). However, if the IGS region is restricted with the endonuclease *Dra*I, it is cut close to the 28S coding region and small bands that can be used to classify individuals to a specific stock are produced (Phillips 1990). Four stocks (Jenny Lake, Seneca Lake, Lake Superior/Marquette, and Gull Island) were classified using restriction fragment analysis. The Lake Superior lake trout are characterized by a 0.4 to 0.8 kilobase difference between fragments, and the other three stocks are distinguished by the number of bands present. To date, not enough samples have been analyzed to discern patterns from the Great Lakes region or to make comparisons of hatchery stocks from Ontario.

Thesis Objectives

DNA variation analysis will be more useful for the management of rehabilitated and hatchery populations because it should improve our ability to identify the origin of lake trout using a mixed-stock analysis and will provide more variation for hatchery stock monitoring programs. The use of DNA-markers will be examined in lake trout in two ways:

> 1) to further assess genetic diversity of hatchery stocks of lake trout in Ontario using mitochondrial and nuclear DNA markers,
>
> 2) to determine which of four different genetic markers provides the most accurate estimates in mixed-stock analyses of lake trout stocks.

Hatchery stocks from Ontario and one from Manitoba will be analyzed. The geographic origins of the stocks are: Killala Lake, Michipicoten Island (Lake Superior), the Slate Islands (Lake Superior), Lake Manitou (Lake Huron), Parry Sound/Big Bay, Iroquois Bay (Lake Huron), Lake Simcoe, and Clearwater Lake (Manitoba). In addition, lake trout from the Haliburton Highlands will be analyzed.

Three types of DNA markers will be used to generate the genetic data used to meet the above objectives. MtDNA variation will be described in the lake trout stocks. The existing mtDNA database for hatchery stocks (Grewe and Hebert 1988; Grewe et al. 1993) will be broadened by analyzing additional stocks, and mtDNA data will be used in a mixed-stock analysis of hatchery lake trout. The inheritance patterns of RAPD markers will be examined to determine if they are inherited in a Mendelian fashion, and

then the markers will be used to describe RAPD variation in hatchery stocks. Microsatellite DNA variation in hatchery stocks will also be described at six polymorphic loci. The genetic data for microsatellite and allozyme loci will be used in a simulation study to determine whether these techniques are useful in accurately determining the proportional contribution of the stocks using a mixed-stock analysis.

Chapter 2
Mitochondrial DNA variation in Ontario hatchery stocks of lake trout, *Salvelinus namaycush*, and assessment of its use in a mixed-stock analysis.

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Abstract

Variation in mitochondrial DNA (mtDNA) of Ontario lake trout stocks was used to produce genetic profiles of eight hatchery stocks and one wild stock of interest to managers. The nucleon diversity was similar to that observed in other studies of mitochondrial DNA variation in lake trout, and in other cold water species. The geographic distribution of the clonal types in the nine stocks is similar to that observed in other studies of mtDNA and allozyme variation in wild populations of lake trout in Ontario.

The profiles of the Killala Lake, Lake Manitou, Slate Islands, and Haliburton stocks were used to determine whether the origins of lake trout planted in two Ontario lakes as sac fry could be identified using a mixed-stock analysis of mtDNA data alone. Samples from two Ontario lakes (Kearney and Spring Lakes) stocked by the Stocks Assessment and Genetics Unit of the Ontario Ministry of Natural Resources were analyzed. The overall difference between the actual and estimated values of the stock contribution estimates was 14% in Spring Lake and 34% in Kearney Lake. Error is introduced into the estimate by: a small sample size from Kearney Lake, one haplotype is found in 68% of the baseline samples, no variation in Haliburton samples, and haplotypes that characterize the stocks are found at low frequencies and therefore provide little data for the estimator. Other stock combinations may have lower errors, and larger mixed-samples will also help to decrease the error. Only the contribution of the maternal line can be estimated because mtDNA is a haploid marker inherited

through the maternal lineage. However, a combination of mtDNA and nuclear markers may make it possible to identify the paternity of future generations derived from stocked fish.

Introduction

Fisheries managers use fish stocking as part of restoration programs. A lake or stream may be stocked to reestablish a wild fish population or to supplement a remnant population. Measures of genetic variation can be used to assess the relative survival of stocks planted into a lake or stream or to determine the reproductive success of stocked fish. Allozyme markers have been used to track survival and introgression of donor walleye (*Stizostedion vitreum*) into recipient walleye populations (Schweigert *et al.* 1977; Murphy *et al.* 1983) and a combination of allozyme and mitochondrial DNA markers have been used to monitor the survival and reproduction of lake trout (*Salvelinus namaycush*) stocks planted into Lake Ontario (Grewe *et al.* 1994).

Analysis of restriction fragment length polymorphisms (RFLP's) in mitochondrial DNA (mtDNA) has been used to measure stock differentiation and to genetically mark fish species (Allendorf and Utter 1979; Avise and Felley 1979; Pella and Milner 1987; Ferris and Berg 1987; Ovenden 1990). MtDNA variation has characteristics which make it a useful marker for population analyses: mtDNA evolves at ten times the rate of the nuclear genome (Brown *et al.* 1979) and it is inherited clonally, through the maternal line. Therefore the mtDNA molecule has an effective population size that is one quarter that of nuclear loci, and as a result, population differences may accumulate at a faster rate, increasing the genetic divergence among stocks (Ferris and Berg 1987).

Genetic data can be used to monitor whether hatchery practices maintain levels of variation close to those observed in the wild. In most stocking programs fish are released after spending at least a year in the hatchery and are large enough to be identified by physical marks such as fin clips or tags. However, no simple marking system is available if fish are stocked as sac fry. Therefore, we wished to determine whether or not mtDNA data could be used to accurately identify the stocks of origin of lake trout used to plant lakes in Ontario as sac fry. Two study lakes near North Bay, Ontario (Spring and Kearney Lake), were stocked with different hatchery stocks of lake trout. Before mtDNA markers could be used to identify recaptured lake trout, it was necessary to first describe the genetic profiles of donor stocks. Therefore, we analyzed the mtDNA variation in one wild and eight hatchery lake trout stocks using restriction length polymorphisms (RFLP).

The mtDNA variation of some Ontario lake trout stocks has been analyzed previously. Killala Lake, Lake Manitou, and Clearwater Lake/Manitoba stocks were sampled by Grewe and Hebert (1988) and by Grewe et al. (1993). Grewe and Hebert (1988) also sampled lake trout from Parry Sound and Lake Simcoe, and Haliburton lake trout were analyzed by Wilson and Hebert (1996). However, the number of lake trout analyzed from Parry Sound and Lake Simcoe may not have been sufficient to detect stock-specific markers. Therefore, six (Lake Manitou, Lake Killala, Parry Sound, the Slate Islands, and Michipicoten Island) of the eight hatchery stocks used for the majority of lake trout stocking programs throughout Ontario were analyzed for

mtDNA variation (Table 2.1). In addition, other stocks of interest (Manitoba, IroquoisBay, and Haliburton) were also examined.

The Iroquois Bay hatchery stock has only been recently established. Iroquois Bay lake trout are one of two remnant native Lake Huron stocks and are currently threatened. Therefore, a broodstock was established to preserve the spawning group and to provide another ecotype for the rehabilitation of other waters (eg. Killarney Park lakes). MtDNA profiles of Iroquois Bay along with life history data, can be used to determine the compatibility of the stock with other stocks in other environments. Habitat parameters, life history traits, and genetic data are catalogued for each hatchery stock maintained by the Ontario Ministry of Natural Resources' (OMNR) Fish Culture Section. The data listed in the catalogue are used by managers to guide them in their choice of stock most suitable for stocking in a particular ecosystem.

The ninth stock is a sample of wild lake trout from three lakes in the Haliburton Highlands region of Ontario (Table 2.1). The Haliburton stock was used in a matched plant study as one of the test stocks, therefore background genetic information is required in order to assess the results of the study. Haliburton lake trout are also of interest from the perspective of conservation biology. The Haliburton Highlands may have provided an interglacial refuge for lake trout and other fish species during the last glaciation. The descendants of the lake trout that used the refuge may have unique genetic characters and may therefore require special consideration for their management (Krueger and Ihssen 1995).

Table 2.1 Hatchery and wild origins of lake trout samples used in mitochondrial DNA analysis.

No.	Stock	Sample Size	Hatchery or Lake of Origin
1	Haliburton	12	Clean, Redstone, or MacDonald Lakes via STAG Unit, OMNR, Maple, Ont.
2	Iroquois Bay	19	Sandfield Fish Culture Station
3	Killala Lake	24	Hill's Lake Fish Culture Station
4	Lake Manitou	18	Lake Manitou, reared at STAG Unit, OMNR, Maple, Ont.
5	Michipicoten Is.	2	Lake Michipicoten
		14	Tarentorus Fish Culture Station
6	Parry Sound	13	Big Sound, Parry Sound, reared at STAG Unit, OMNR, Ont.
7	Lake Simcoe	20	Chatsworth Fish Culture Station
8	Slate Islands	20	Dorion Fish Culture Station
9	Manitoba	12	Clearwater Lake Fish Culture Station via Lake Simcoe
10	Kearney Lake	13	Kearney Lake, Ont.
11	Spring Lake	27	Spring Lake, Ont.

STAG- Stocks Assessment and Genetics Unit.

The genetic profiles developed for four of the stocks (Haliburton, Killala Lake,

Lake Manitou, and the Slate Islands) were compared to those from previous studies of

lake trout and then used to perform a mixed-stock analysis of lake trout recaptured

from Spring and Kearney Lakes. The stock of origin of the recaptured fish could be

determined using allozyme markers that had been bred into the lake trout stocks. The

system allowed us to test whether mtDNA alone can be used to give accurate

estimates in a mixed-stock analysis. Three stocks (Haliburton, Killala Lake, and Lake

Manitou) were used by the Stocks Assessment and Genetics (STAG) Unit to stock

both lakes as part of an experiment to assess the relative survival from the sac-fry

stage of the three stocks in a study of matched-stock plantings conducted by the

STAG Unit. Since the lake trout were stocked at an early stage of their life history

they could not be supplied with a physical mark, such as a fin clip, that would permit

their identification. Instead, combinations of allelic variants at several loci unique to

each stock had been bred to different frequencies in the stocks over several

generations at the STAG Unit research hatchery. For example, the $Pgd*^{85}$ allele has

only been found in the wild in the Haliburton lake trout at a frequency of about 12%

(Ihssen et al. 1988) and other alleles at the loci sMDH-1,2* (malate dehydrogenase,

E.C. number 1.1.1.37) and MUP* (4-methylumbelliferyl phosphatase, E.C. number

3.1.3.2) were bred into the stock to allow the identification of each individual. Large

numbers of parents were used to maintain overall genetic variation at levels similar to

that observed in the hatchery stocks. The three lake trout stocks were planted onto the

spawning shoals of each lake as sac fry.

The two lakes have different stocking histories. Spring Lake is believed to be populated solely by lake trout stocked by the STAG Unit. Kearney Lake was also stocked with the same stocks of lake trout from the STAG unit as Spring Lake, but was also previously stocked with Killala Lake, Lake Manitou, and Hill's Lake lake trout (D. Maraldo, North Bay District Biologist, OMNR, personal communication). The allozyme markers and the age of the lake trout were used to determine to which stock the recaptured lake trout belonged.

Materials and Methods

The origins of the lake trout used to develop stock profiles are outlined in Table 2.1. Spring and Kearney Lakes were sampled with gill nets in August 1992 and 1993. Samples were either processed immediately after sampling, or were preserved in TEKS buffer (50 mM Tris-HCl, 10 mM EDTA, 200 mM KCl and 250 mM sucrose, pH 7.5) and then shipped to OMNR, Maple, Ontario, for mtDNA extraction. MtDNA was isolated from lake trout for RFLP analysis using the protocol described by Chapman and Powers (1984), except that the initial grinding buffer (TEK) contained 250 mM sucrose.

The lake trout mtDNA samples were digested with restriction endonucleases (RE). The manufacturers' instructions were followed to determine the amounts of enzyme and buffer needed. The reaction was terminated with a stop buffer (20%

sucrose, 0.5% SDS, 0.2% bromophenol blue). MtDNA fragments were separated on 1% horizontal agarose gels in a 0.5X TBE running buffer (89 mM Tris-HCl, 89 mM boric acid, and 2 mM EDTA, pH 8.0). Gels were electrophoresed at 35 V for 12-16 hours and then placed in a solution of ethidium bromide (0.5 µg/mL) for 8 minutes, and the mtDNA fragments visualized with ultra-violet light. Lambda DNA digested with *Hind*III and with *Hind*III and *Eco*RI was included on each gel to act as size standards.

The findings of previous studies (Grewe and Hebert 1988; M. Burnham-Curtis, US Fish and Wildlife Service, personal communication) were used to choose sixteen different restriction endonucleases for the analysis. The polymorphic restriction patterns were labelled using the name of the RE and a letter for each variant (eg. *Bam*HIA vs. *Bam*HIB) and monomorphic patterns were scored as having an A type. Each lake trout was therefore assigned a series of letters for the mtDNA fragment patterns observed for each RE. Lake trout with the same set of letters have the same haplotype. The letters assigned to each pattern of restriction fragments for each enzyme were the same as those used by Grewe and Hebert (1988).

Measures of genetic diversity were calculated in order to compare genetic relationships among and within the stocks. Genetic distances among different haplotypes were calculated according to Nei and Tajima (1983) and then used in the AMOVA software package (Excoffier *et al.* 1992) to calculate genetic distances among the lake trout samples and to partition variance into within and among-stock

components. The genetic distances between pairs of hatchery stocks were used to construct a dendrogram using the Unweighted Pair Group of Arithmetic Means (UPGMA) method.

Nucleon diversity was calculated in order to compare the amount of diversity in the hatchery stocks to another study of hatchery stocks, to studies of wild lake trout, and to other coldwater fish species. The value of h, nucleon diversity was calculated using:

$$h=1/n-1[n(1-\Sigma x^2_i)]$$

(Nei and Tajima 1981), where n is the number of samples and x_i is the frequency of the ith haplotype.

The donor stock profiles were used in a mixed-stock analysis of the lake trout recaptured from Spring and Kearney Lakes. Each recaptured lake trout was assigned to a stock based on allozyme analysis of the genetic marks that were bred into each stock. Then the results of the mixed-stock analysis were compared to the actual proportions of the stocks planted in each lake which were calculated by determining what proportion of the total number of lake trout recaptured were from each of the donor stocks. Therefore the actual value reflects the number of lake trout planted from each stock that have survived to be recaptured. The estimator used for the mixed-stock analysis was developed by Dempster *et al.* (1977), and has been used previously (Grewe *et al.* 1994) to determine the origins of a mixed sample of fish. For the analysis, the mtDNA data are treated as a single locus and each haplotype as

an allele. The estimator uses a maximum likelihood function to calculate the joint probability of genotypes occurring in both the baseline and mixed-stock samples. The 95% confidence interval was also calculated for each estimate.

Results

Six of the restriction endonucleases (*Bam*HI, *Hind*III, *Sma*I, *Ava*I, *Ase*I, and *Eco*0109I) produced variable restriction patterns (Figure 2.1). In total 20 (Table 2.2) haplotypes were observed among 142 lake trout. Haplotype 1 was found in over half (59.6%; Table 2.3) of the lake trout sampled. The next most common haplotype was 9 (19.1%). The remaining haplotypes are rarer; each was represented by fewer than 5% of the lake trout. In fact, twelve haplotypes were found in only one stock. For example, four haplotypes (6, 13, 16, and 20) were seen in only the Slate Islands lake trout (Table 2.). Only one sample, Haliburton, was fixed for one haplotype (all type 1). Seventy-eight percent of the genetic variation occurs within a stock and 22% is the result of among-stock variation. The dendrogram (Figure 2.2) depicting the genetic distance among hatchery stocks shows that all but one of the six hatchery stock originating from the Great Lakes region (Killala Lake, Lake Manitou, Iroquois Bay, Parry Sound, Michipicoten Island, and the Slate Islands) group together, and the samples from Manitoba and the samples from inland populations (Haliburton lakes and Lake Simcoe) are more distinct. Values of nucleon diversity range widely from 0 to 0.788 (Table 2.4).

Figure 2.1 Restriction fragment length polymorphisms of mitochondrial DNA observed in lake trout using the restriction enzyme *Bam*HI. Three *Bam*HI variants are labelled A, B, and C. Lambda DNA digested with *Hind*III and with *Hind*III and *Eco*RI is labelled EHHλ, and the fragment sizes (in kilobase pairs; kbp) of two of the EHHλ fragments are shown.
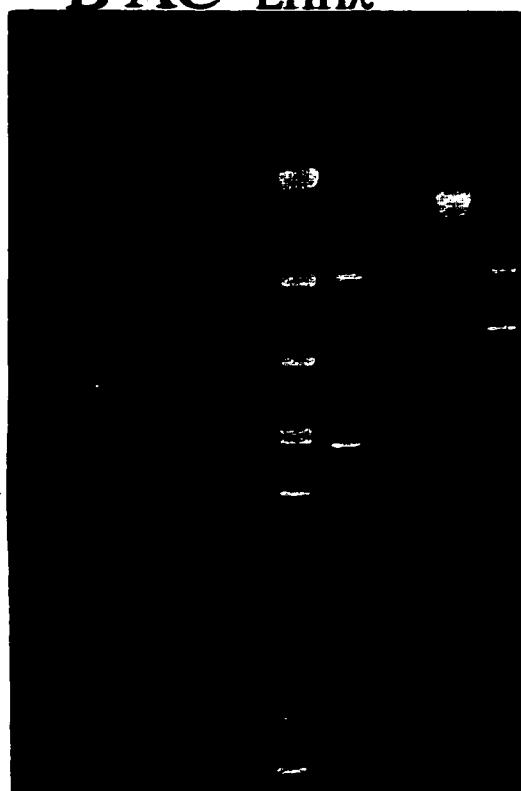
Table 2.2 Mitochondrial DNA haplotypes found in lake trout from Ontario and Manitoba.

			Restriction enzyme			
Clonal Line	*BamHI*	*AvaI*	*SmaI*	*HindIII*	*AseI*	*EcoO109I*
1	A	A	A	A	A	A
2	A	B	A	A	A	A
3	A	E	A	A	A	A
4	A	A	C	A	A	A
5	A	A	A	B	A	A
6	A	A	A	A	B	A
7	A	A	A	A	A	B
8	B	A	A	A	A	A
9	B	B	A	A	A	A
10	B	B	A	A	A	A
11	B	B	A	B	B	B
12	B	A	A	A	A	A
13	C	B	A	A	B	A
14	C	C	A	A	A	A
15	C	C	A	A	B	A
16	C	C	A	A	A	A
17	C	C	A	A	A	B
18	A	A	A	A	B	B
19	C	B	A	A	A	B
20	C	A	A	A	A	A

Table 2.3 Frequency of mtDNA haplotypes observed in nine lake trout stocks, using six restriction endonucleases.

								Haplotype										
Stock	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	20
Hali	1.000	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Iroquois	0.684	-	-	-	0.105	-	0.053	-	0.158	-	-	-	-	-	-	-	-	-
Killala	0.584	-	-	-	-	-	-	0.083	0.250	-	0.083	-	-	-	-	-	-	-
Manitob	0.417	-	0.083	-	-	-	-	-	0.167	-	-	-	-	0.083	-	-	0.250	-
Manitou	0.610	0.056	-	0.056	0.056	-	-	-	0.166	-	-	0.056	-	-	-	-	-	-
Michi	0.874	0.063	-	-	-	-	-	-	-	-	-	-	-	-	0.063	-	-	-
Parry S.	0.769	-	-	-	-	-	-	-	0.231	-	-	-	-	-	-	-	-	-
Simcoe	0.050	0.050	-	-	-	-	-	0.150	0.650	0.100	-	-	-	-	-	-	-	-
Slate Is.	0.550	-	-	-	-	0.050	-	0.150	-	-	-	-	0.050	-	-	0.100	0.050	0.050
Total	0.591	0.021	0.007	0.007	0.021	0.007	0.007	0.050	0.191	0.014	0.007	0.007	0.007	0.007	0.007	0.014	0.028	0.007

Hali=Haliburton, Michi=Michipicoten Island, Manitob=Manitoba

Figure 2.2 UPGMA clustering of lake trout haplotypes. Genetic distances were calculated according to Nei and Tajima (1983). The numbers on the dendrogram arms represent the percent of 1000 permutations which produced the observed branch point.
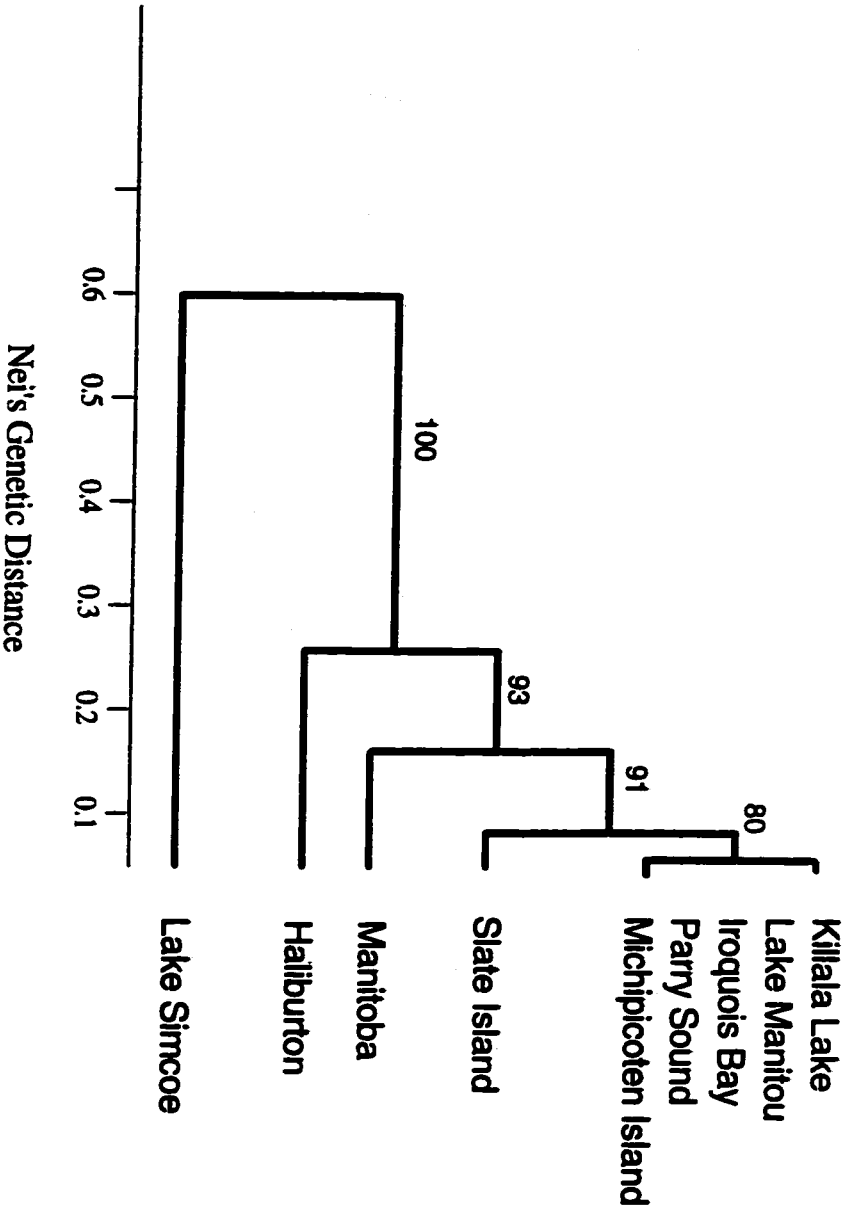
Table 2.4 Analysis of nucleon diversity and results of AMOVA analysis of genetic diversity for lake trout stocks.

Stock	Nucleon diversity (h)
Haliburton	0.000
Killala L.	0.637
L. Manitou	0.621
Slate Is.	0.689
Manitoba	0.788
Iroquois Bay	0.521
Parry Sound	0.698
Michipicoten Is.	0.242
L. Simcoe	0.568
Total	0.601

Variance component	Variance	% total	P^*
Among stocks	0.00118	22.11	<0.0099
Within stocks	0.00418	77.89	<0.0099

*Probability of more extreme random value from 1000 permutations.

Forty lake trout recaptured from Spring and Kearney Lake were analyzed (Table 2.5). Twenty-seven lake trout were recaptured from Spring Lake. Thirteen lake trout were recaptured from Kearney Lake.

Five of the Kearney Lake samples had fin clips and ages identifying them as lake trout stocked by the North Bay District of the OMNR, not the STAG Unit. The clipped lake trout samples were identified as Lake Manitou and Hill's Lake lake trout using stocking records. Hill's Lake hatchery lake trout were used to stock Kearney Lake in 1985 (D. Maraldo, OMNR, North Bay, personal communication), but the stock has since been discontinued, therefore a larger sample could not be obtained for mtDNA analysis. However, hatchery records indicate that the geographic origin of the Hill's Lake stock and the Slate Islands broodstocks from Dorion Fish Culture Station may be similar. Both came from the northern portion of Lake Superior, near the Slate Islands. Therefore, the profile generated for the Slate Islands was also included in the mixed-stock analysis of Kearney Lake. The remaining eight fish were unclipped and too young to be from the District's stocking, therefore they were naturally produced or stocked by the STAG Unit as sac fry.

The estimates of the contribution of each stock to the mixed-sample differed from the actual value. The estimates varied from the actual percent composition by up to 50% (Table 2.6). The contribution of the Lake Manitou stock is over-estimated by over 20% (22% and 50%) in both lakes, and the estimates of the Haliburton stock are also quite different than the actual values. The estimates generated for Killala Lake

Table 2.5 Frequency of mtDNA haplotypes observed in lake trout recaptured from Spring and Kearney Lakes.

Lake	Sample size	Haplotype							
		1	2	8	9	11	13	18	19
Kearney Lake									
FCS	5	0.625	-	-	-	-	0.125	0.125	0.125
STAG	8	0.600	-	-	-	0.200	-	-	0.200
Spring Lake	27	0.667	0.074	0.111	0.111	0.037	-	-	-

FCS-lake trout stocked by the OMNR's Fish Culture Section
STAG-lake trout stocked by the STAG Unit, OMNR, Peterborough, Ont.

and the Slate Islands tended to be closer to the actual value, each differed by 20% or less. On average, the actual and estimated value differed by 14% for Spring Lake and by 34% for Kearney Lake.

Discussion

Levels of genetic diversity in the hatchery stocks of lake trout are similar to those observed in wild stocks. One concern of hatchery managers is the maintenance of genetic variation in hatchery broodstocks. A loss of variation due to a small number of parents or unequal family sizes may lead to, for example, a loss of adaptability to a new environment (Ryman 1991). The amount of genetic variation due to among-stock differences (22%) is similar to that reported in an allozyme study of wild and hatchery lake trout (21% and 18% respectively; Ihssen *et al.* 1988). The overall nucleon diversity (h_T=0.601) is similar to that reported in a study of wild lake trout (h_T=0.594; Vitic and Strobeck 1996), and slightly higher than that observed by Grewe and Hebert (1988) in their study of lake trout brood stocks. However, sample sizes analyzed in the current study are larger for some stocks (Parry Sound and Lake Simcoe), therefore more variation was detected. Values of nucleon diversity are also comparable with other wild stocks of cold water species such as lake whitefish, *Coregonus clupeaformis* (h_T=0.733; Bernatchez and Dodson 1990), and walleye, *Stizostedion vitreum* (h_T=0.594; Billington and Hebert 1988).

The geographic distribution of mtDNA variation observed in the present study

is similar to that observed by Grewe and Hebert (1988) and is further supported by the addition of the Slate Islands, Michipicoten Island, and Iroquois Bay samples (Figure 2.3). Grewe and Hebert (1988) identified three clonal groups based on the restriction patterns of the enzymes *BamHI* and *AvaI*. The type A (*BamHI*A or D and *AvaI*A) group was found in all areas, but was most common in the south-eastern portion of the Great Lakes, whereas type C (*BamHI*C and *AvaI*C or D) was limited to the western portion of Ontario and in Manitoba. Type B (*BamHI*B and *AvaI*B) lake trout were most common in the eastern portion of the Great Lakes, and inland lakes of eastern Ontario, and New York. The geographical distributions of the A, B, and C clonal groups of Grewe and Hebert (1988) and the present study are also similar to geographical shifts of allele frequency and chromosome banding patterns (Ihssen *et al.* 1988; Phillips and Ihssen 1986). Lake trout populations in the Great Lakes region may have derived from three separate glacial refugia. The dominant clonal type A may have originated from lake trout which spent the Pleistocene south of the ice sheets. It is postulated that the B and C groups were derived from lake trout restricted to an eastern and perhaps western refuge respectively. An east-west split in fish from the Great Lakes region was also observed in a study of walleye (*Stizostedion vitreum*) mtDNA (Billington and Hebert 1988) suggesting that Great Lakes aquatic species persisted in at least two refugia during the last glaciation.

The dendrogram topology also indicates that samples from southern Ontario (Haliburton and Lake Simcoe) are separate from most of the Great Lakes samples. In

Figure 2.3 Geographic distribution of lake trout mtDNA clonal groups in Ontario. The number on each pie refers to the stock numbers listed in Table 2.1.

MANITOBA

Clonal Groups

C ● B ○ A ●

LAKE SUPERIOR

KILLALA

SLATE Is.

MICHIPICOTEN Is.

LAKE MICHIGAN

MANITOU

IROQUOIS BAY

PARRY SOUND

LAKE HURON

LAKE ERIE

SIMCOE

HALIBURTON

LAKE ONTARIO
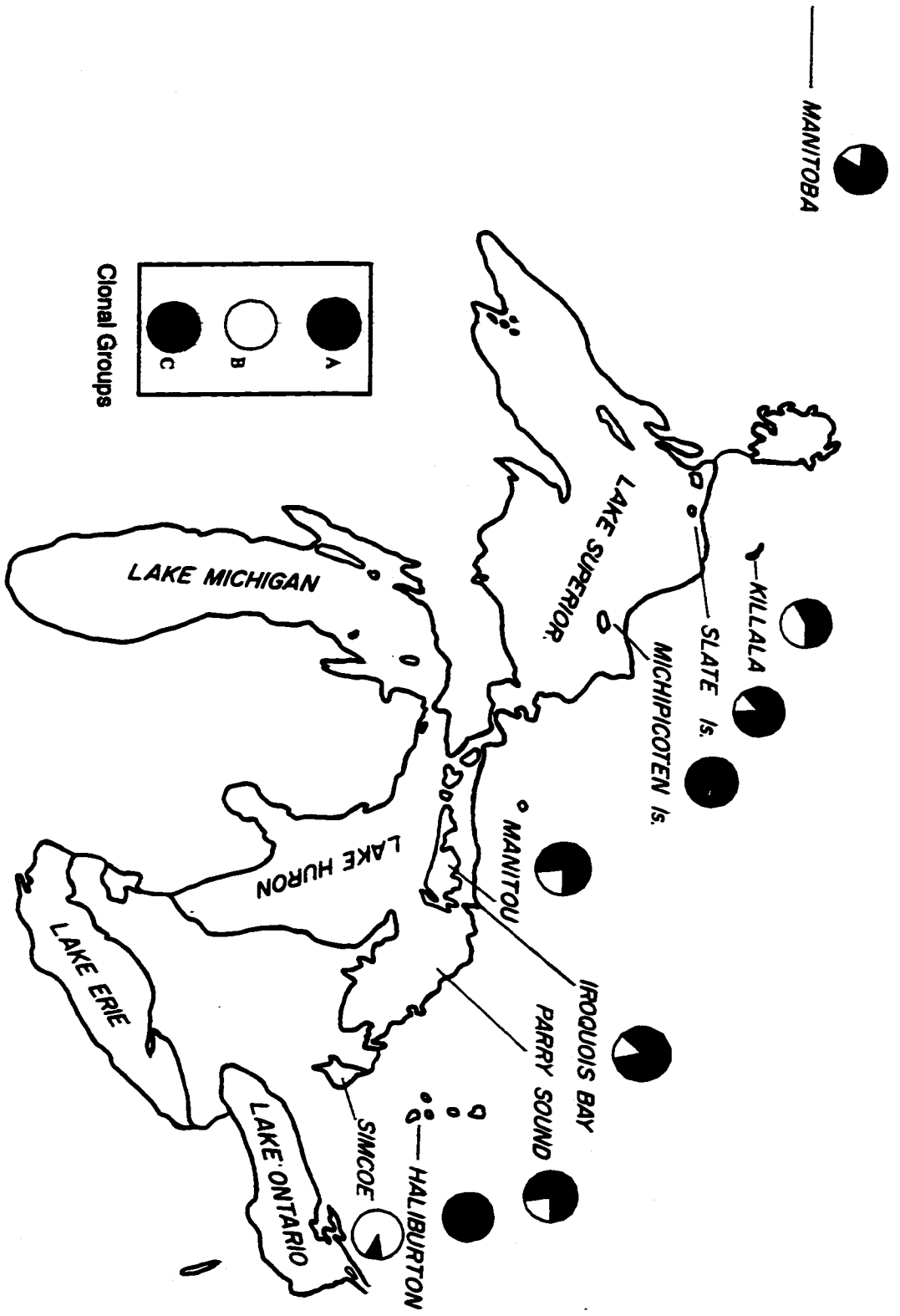
the Great Lakes the group of samples originating from the north-central shores of Lakes Huron and Superior is separate from the Manitoba samples and the western section of Lake Superior (Slate Islands).

The patterns observed at *Bam*HI and *Ava*I seem to account for most of the variation among clonal groups. The use of several four-base cutters in Grewe and Hebert (1988) did not improve the ability to distinguish among all major hatchery stocks, when the results were compared to Grewe *et al.* (1993) who did not use as many restriction enzymes (Table 2.7). However, the use of two endonucleases, *Hinf*I and *Taq*I, did improve the ability to distinguish between two of the hatchery stocks that had been stocked into Lake Ontario (Killala Lake and Lake Manitou).

Lake trout from the Haliburton region are genetically distinct. The Pgd^{*85} allele is found at a high frequency only in Haliburton lake trout (Ihssen *et al.* 1988), and they are morphologically and physiologically different from southeastern Ontario lake trout stocks (Krueger and Ihssen 1995). However, no haplotypes unique to the Haliburton fish were observed using five and six-base cutters, and a more detailed analysis with four-base cutters did not reveal more variation (Wilson and Hebert 1996). In previous studies, Haliburton lake trout were fixed for the most common haplotype. Mitochondrial DNA variation is more sensitive to shifts in population size because it is inherited in an essentially haploid manner from only females (Nei and Li 1979). Allozyme data suggest that Haliburton lake trout are relics of the interglacial period that may have survived in an interglacial river (Ihssen *et al.* 1988). A

Table 2.7 A comparison of the restriction endonucleases used to examine mtDNA variation in lake trout in three separate studies.

Restriction endonuclease	Study		
	Grewe & Hebert 1988	Grewe *et al.* 1993	present study
*Ava*I	+	+	+
*Ase*I	-	-	+
*Bam*HI	+	+	+
*Bst*EII	+	-	-
*Hind*III	+	-	+
*Eco*109I	-	-	+
*Nco*I	+	-	-
*Hinf*I	+	+	-
*Sma*I	+	-	+
*Nci*I	+	-	-
*Hpa*II	+	-	-
*Taq*I	+	+	-

+restriction endonuclease used
-restriction endonuclease not used

reduction in population size during the interglacial period may account for the low variation in the Haliburton stock versus all others which have on average four different haplotypes. An alternative explanation is many small inland lakes in Ontario and Quebec were founded by small numbers of individuals (Wilson and Hebert 1996). Therefore, differences between the Haliburton region and other areas may be the result of the genetic bottleneck created during colonization. In addition the Haliburton lakes support smaller populations as compared to the Great Lakes stocks and the smaller population size may have resulted in less mitochondrial DNA diversity.

MtDNA variation has the potential for monitoring lake trout broodstocks in Ontario. Since egg samples can be analyzed to determine mtDNA profiles of mature lake trout, valuable reproductive-age adults do not have to be sacrificed. The technique of Chapman and Powers (1984) requires less sophisticated lab equipment and although the mtDNA is not completely purified the results could still be scored reliably. Additional mtDNA variation may be detected by amplifying specific regions of the mitochondrial genome using lake trout specific primers for the polymerase chain reaction (Vitic and Strobeck 1996). PCR analyses would also allow non-lethal sampling techniques to be employed.

The presence of the Slate Islands haplotypes in young, unclipped lake trout may be evidence of limited natural reproduction in Kearney Lake. Two of the eight lake trout from Kearney Lake initially identified as STAG Unit fish had haplotypes that were not observed in any of the lake trout stocks planted by the STAG Unit. The two

unclipped lake trout could only be from the STAG Unit stocking, or from natural reproduction because they were too young to have been among those stocked previously. One of the two was a clone 13 fish, a haplotype that was only seen in the Slate Islands which are probably similar to Hill's Lake.

Sac fry plants may be used as a more cost effective technique to stock lakes in Ontario (P. Ihssen, STAG Unit, OMNR, unpublished data), but a genetic marker system will be required to distinguish among recaptured fish. In the current study it was possible to create three stocks with allozyme markers for research purposes. However, it may not be feasible for stocking operations that require larger production rates, (such as the OMNR) because the space required to maintain the broodstock necessary to create and maintain marked lines may not be available. In addition, there are concerns about the effects selecting specific alleles may have on linked loci, or chromosome segments.

Using mtDNA variation in a mixed-stock analysis it was not possible to accurately assign the recaptured lake trout from Spring and Kearney Lakes to the three donor stocks used in the present study. Estimates were especially poor for Haliburton and Lake Manitou. The mtDNA profiles of Haliburton fish may reduce the accuracy of estimates because the stock is fixed for the most common haplotype, and therefore has no haplotypes that can be used to distinguish it from other stocks. As a result Haliburton lake trout were assigned to other stocks in the mixed-stock analysis. However, RFLP variation in mtDNA may be used alone to distinguish among certain

stocks that are characterized by unique clonal lines, the Slate Islands and Killala Lake for example.

There is another limitation to using mtDNA to determine the contributions of stocked fish to future generations. MtDNA is inherited through the female line as a haploid marker, therefore if mating occurs among different planted stocks it will be difficult to detect and it will also be difficult to detect differences in the survival of males and females. Instead, it may be necessary to use other genetic markers to improve the accuracy of mixed-stock analysis. For example, a simulation study of walleye showed that the combination of three types of genetic markers; allozyme, mtDNA, and randomly amplified polymorphic DNA variation greatly improved the ability to distinguish among three donor stocks of walleye (Ihssen *et al.* 1993). Therefore, in order to determine the contributions of stocked fish to future generations or to the fishery in a lake, other nuclear markers (such as microsatellite DNA loci) should be investigated that could be used alone or in conjunction with mtDNA variation.

Acknowledgements

References

Allendorf, F. W., and F. M. Utter. 1979. Population genetics. In Fish Physiology. W. S. Hoar, D. J. Randall and J. R. Brett (eds.), vol. 8. p. 407-454. Academic Press, New York.

Avise, J. C., and J. Felley. 1979. Population structure of freshwater fishes I. Genetic variation of bluegill (*Lepomis macrochirus*) populations in man-made reserves. Evolution 33:15-26.

Bernatchez, L. and J. J. Dodson. 1990. Phylogenetic structure in mitochondrial DNA of lake whitefish (*Coregonus clupeaformis*) and its relation to Pleistocene glaciations. Evolution. 44:1263-1271.

Billington, N. and P. D. N. Hebert. 1988. Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. Can. J. Fish. Aquat. Sci. 45:643-654.

Brown, M. W., M. George Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA. 76:1967-1971.

Chapman, R. W., and D. A. Powers. 1984. A method for the rapid isolation of mitochondrial DNA from fishes. University of Maryland Sea Grant Program Technical Report No. UM-SG-TS-84-05.

Dempster, A. P., N. M. Laird, and D. B. Rubin. 1977. Maximum likelihood from incomplete data via the EM method. J. R. Stat. Soc. B39:1-38.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 131:479-491.

Ferris, S. D., and W. J. Berg. 1987. The utility of mitochondrial DNA in fish genetics and fishery management. In Population Genetics and Fishery Management. N. Ryman and F. Utter (eds.). p. 277-299. University of Washington Press, Seattle and London.

Grewe, P. M., and P. D. N. Hebert. 1988. Mitochondrial DNA diversity among brood stocks of the lake trout *Salvelinus namaycush*. Can. J. Fish. Aquat. Sci. 45:2114-2122.

Grewe, P. M., C. C. Krueger, C. F. Aquadro, E. Bermingham, H. L. Kincaid, and B. May. 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario. Can. J. Fish. Aquat. Sci. 50:2397-2403.

Grewe, P. M., C. C. Krueger, J. E. Marsden, C. F. Aquadro, and B. May. 1994. Hatchery origins of naturally produced lake trout fry captured in Lake Ontario: temporal and spatial variability based on allozyme and mitochondrial DNA data. Trans. Amer. Fish. Soc. 123:309-320.

Ihssen, P. E., J. M. Casselman, G. W. Martin, and R. B. Phillips. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. Can. J. Fish. Aquat. Sci. 45:1018-1029.

Ihssen, P. E., W. Stott, and G. W. Martin. 1993. Allozyme and mitochondrial DNA markers for the determination of the genetic structure of a walleye (*Stizostedion vitreum*) population established from adult transplants of three distinct stocks. A report to the North Shore of Lake Superior Great Lakes Cleanup Fund. Technical Report Series #14.

Krueger, C. C. and P. E. Ihssen. 1995. Review of genetics of lake trout in the Great Lakes: history, molecular genetics, physiology, strain comparisons, and restoration management. J. Great Lakes Res. 21(Supp. 1):348-363.

Murphy, B. R., L. A. Nielsen, and B. J. Turner. 1983. Use of genetic tags to evaluate stocking success for reservoir walleyes. Trans. Amer. Fish. Soc. 112:457-463.

Nei, M. and Li, W.-H.. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 76:5269-5273.

Nei, M. and F. Tajima. 1981. DNA polymorphism detectable by restriction endonucleases. Genetics. 97:145-163.

Nei, M. and F. Tajima. 1983. Maximum likelihood estimation of the number of nucleotide substitutions from restriction sites data. Genetics. 105:207-217.

Ovenden, J. R. 1990. Mitochondrial DNA and marine stock assessment: A review. Aust. J. Mar. Freshwater Res. 41:835-953.

Phillips, R. B. and P. E. Ihssen. 1986. Stock structure of lake trout from the Great Lakes region as determined by chromosome and isozyme polymorphisms.

Great Lakes Fishery Commission, Research Completion Report, Ann Arbor, Michigan.

Pella, J. J. and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. In Population Genetics and Fishery Management. N. Ryman and F. Utter (eds.) p.247-276. University of Washington Press, Seattle and London.

Ryman, N. 1991. Conservation genetics considerations in fishery management. J. Fish Biol. 39(suppl. ₹.):211-224.

Schweigert, J. F. , F. J. Ward, and J. W. Clayton. 1977. Effects of fry and fingerling introductions on walleye (*Stizostedion vitreum vitreum*) production in West Blue Lake, Manitoba. J. Fish. Res. Board Can. 34:2142-2150.

Vitic, R. and C. Strobeck. 1996. Mitochondrial DNA analysis of populations of lake trout (*Salvelinus namaycush*) in west-central Canada: implications for stock identification. Can. J. Fish. Aquat. Sci. 53:1038-1047.

Wilson, C. C. and P. D. N. Hebert. 1996. Phylogenetic origins of lake trout (*Salvelinus namaycush*) in eastern North America. Can. J. Fish. Aquat. Sci. 53:2764-2775.

Chapter 3
Inheritance of RAPD Molecular Markers in Lake Trout (*Salvelinus namaycush*).

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Abstract

Inheritance patterns of randomly amplified polymorphic DNA (RAPD) phenotypes were tested in thirteen full-sib families of lake trout (*Salvelinus namaycush*). Single-pair matings of parents with known phenotypes were made, and up to twenty progeny of each mating were used to test inheritance patterns. Seven RAPD primers amplified thirteen polymorphic bands. With the exception of one family, expected segregation ratios for dominant Mendelian genetic traits were observed. Our results support previously reported findings that RAPD markers can be considered Mendelian traits and therefore could be used for analysis of genetic population structure.

Introduction

Randomly amplified polymorphic DNA (RAPD) has been used to examine a variety of problems such as: determining parentage (Levitan and Grosberg 1993), typing strains (Dweikat *et al.* 1993, Koller *et al.* 1993; Yang and Quiros 1993), and examining intraspecific population structure (Russell *et al.* 1993; Liu and Furnier 1993; Gibbs *et al.* 1994). There are concerns, however, about the reproducibility of RAPD phenotypes (Hadrys *et al.* 1992), and evidence that fragments generated by RAPD primers are not always inherited in a Mendelian fashion (Riedy *et al.* 1992; Reiter *et al.* 1992; Carlson *et al.* 1991). Breeding experiments are reported, but the number of diploid offspring or families tested has been small (eg. Riedy *et al.* 1992; Levitan and Grosberg 1993), or exact parentage of the progeny was not known (eg. Hadrys *et al.* 1993; Scott *et al.* 1992). Mendelian inheritance of RAPD loci has been confirmed using DNA from haploid tissues (Bucci and Menozzi 1993) or organisms (Hunt and Page 1992) and in plant species (Roy *et al.* 1992).

RAPD markers may be used to examine certain fisheries management problems. Fewer allozyme polymorphisms have been reported in lake trout (*Salvelinus namaycush*) than in other important freshwater game fish such as rainbow trout (*Oncorhynchus mykiss*; Ihssen *et al.* 1988). Therefore, additional genetic markers are needed to address questions related to the impact of stocking on wild populations. Before RAPD markers can be applied however, their consistent amplification in repeated reactions and their Mendelian inheritance need to be confirmed. In the

present study, parents of known RAPD phenotypes of lake trout, were selected to produce progeny for several families. The observed phenotypes of the progeny and the known phenotypes of their parents were used to examine the inheritance of RAPD markers.

Materials and Methods

The lake trout used in the inheritance study originated from Killala Lake, Ontario, via Hill's Lake Fish Culture Station (Ontario Ministry of Natural Resources, Englehart, Ontario, Canada). Liver or muscle samples were obtained from lake trout used as parents. Families of lake trout were created by single pair artificial fertilization. Eggs were incubated in Heath® incubation trays at 8°C, and after yolk sac adsorption, each family was moved into a separate tank section. The fry were reared to approximately 10 cm in length, and then 20 per family were sacrificed. The caudal fin and the tip of the caudal peduncle were used for DNA extraction.

DNA was extracted using the method described by Saghai-Maroof et al. (1984). DNA quality was checked by running 1μg of each sample on a 1.4% agarose gel. Gels were electrophoresed at 40 V for 6 hours. To visualize DNA fragments, the gel was placed in a solution of ethidium bromide (0.5 μg/mL) for 8 minutes and then viewed on an ultra violet (UV) light source. Samples with a smear of DNA along the entire gel lane were discarded and a new sample was prepared. Low weight template DNA used with RAPD primers may produce variable RAPD phenotypes (Williams *et*

al. 1990).

Amplification reactions were performed in a volume of 10 μL containing 12.5

ng template DNA, 15 ng primer, 100 μM each of dATP, dCTP, dGTP, and dTTP, 0.25

U Taq polymerase, and the recommended amount of reaction buffer (Boehringer-

Mannheim). A layer of mineral oil was added to prevent evaporation. The thermal

cycler (Precision Instruments) was programmed for 20 cycles of 1 minute at 95°C, 1

minute at 36°C, and 2 minutes at 72°C and 20 cycles of 1 minute at 92°C, 1 minute at

36°C, and 2 minutes at 72°C. Amplification products were separated

electrophoretically in 1.4% agarose gels and were run at 70 V for 5-6 hours. Lambda

DNA digested with *Hind*III was included as a size standard. Two control samples

were also included; one without template DNA as a negative control, and the DNA

from brown trout (*Salmo trutta*) as a positive control. To identify different

amplification products, each band was labelled with letters representing the

manufacturer and primer set number (eg. OPW), followed by the size of the DNA

fragment in base pairs (eg. OPW3*2300).

Parental samples were screened for variability using 84 ten base pair primers.

Primers were obtained from Operon Technologies (Alameda, CA., kit W and kit B), or

from the University of Guelph (UBC group, courtesy of R. Danzmann). The offspring

of parents that had different RAPD phenotypes were scored, and offspring from

several parents with identical RAPD phenotypes were also analyzed. Mendelian

inheritance of RAPD markers was tested using the Chi-square statistic with Yate's

correction for small samples to compare observed and expected RAPD phenotypes for
each family (Sokal and Rohlf 1969). In total, thirteen families of lake trout were
chosen to examine all three potential parental phenotypic combinations that occur with
a dominant marker system.

Results

No product was amplified with 31 of the 84 primers or the amplification was
not repeatable. Of the 53 primers that yielded repeatable results, polymorphism was
observed using OPW3, OPW4 (4 loci), OPW7, OPW17 (2 loci), UBCp43 (2 loci),
UBCp72 (2 loci), and UBCp97. A total of thirteen polymorphic loci were amplified
with the seven primers (Tables 3.1 and 3.2). Figure 3.1 is an example of observed
phenotypes for parents and offspring. The male has the dominant phenotype, the
female the recessive phenotype, and 10 of the 18 offspring have the dominant
phenotype. Hence we conclude that the male is heterozygous and the progeny have
the expected one-to-one phenotypic distribution (Table 3.2, Cross #61, P=0.637).

Table 3.1 Comparisons of observed and expected offspring phenotypic frequencies for families with both parents of the same phenotype.

Primer	Cross #	Assumed parental genotypes	Offspring phenotypes observed(expected)		X^2 Probability	Probability
			Dominant	Recessive		
OPW3*2300	95	+- X +-	16(15)	4(5)	0.796	
OPW4*2100	119	+- X +-	14(15)	6(5)	0.606	
OPW7*1900	119	+- X +-	15(13.5)	3(4.5)	0.586	
UBCp72*2000	119	+- X +-	15(14.25)	4(4.75)	0.895	
UBCp97*1400	146	+- X +-	13(14.25)	6(4.75)	0.691	
OPW4*2000	95	++ X +- or ++	11	0		0.04
UBCp97*1400	95	++ X +- or ++	11	0		0.04
OPW*1900	23	-- X --	0	12		
OPW4*2100	23	-- X --	0	12		
OPW4*2100	146	-- X --	0	12		
OPW17*1700	146	-- X --	0	12		
UBCp43*1500	62	-- X --	4	12		
UBCp43*1700	62	-- X --	0	16		

Table 3.2 Comparisons of observed and expected offspring phenotypes for families with one parent of the dominant phenotype.

Primer	Cross #	Assumed parental genotypes	Offspring phenotypes observed(expected)		X^2 Probability	Probability
			Dominant	Recessive		
OPW3*2300	64	+ X --	6(7)	8(7)	0.593	
OPW3*2300	23	+ X --	7(6)	5(6)	0.564	
OPW3*2300	61	+ X --	10(9)	9(9)	0.819	
OPW4*1700	23	+ X --	5(6)	7(6)	0.564	
OPW4*2100	61	+ X --	11(9.5)	8(9.5)	0.491	
OPW7*1900	64	+ X --	8(7)	6(7)	0.593	
OPW7*1900	62	+ X --	9(8.5)	8(8.5)	0.808	
OPW17*1700	61	+ X --	10(9)	9(9)	0.819	
UBCp43*1500	119	+ X --	12(10)	8(10)	0.371	
UBCp43*1500	61	+ X --	10(9)	8(9)	0.637	
UBCp43*1700	119	+ X --	8(10)	12(10)	0.371	
UBCp97*1400	61	+ X --	12(10)	8(10)	0.371	
OPW4*1700	119	++ X --	20	0		<0.001
OPW4*2000	61	++ X --	19	0		<0.001
OPW4*2000	146	++ X --	12	0		<0.001
OPW17*1700	95	++ X --	11	0		<0.001
OPW17*1700	64	++ X --	13	0		<0.001
OPW17*1700	119	++ X --	11	0		<0.001
OPW17*2100	146	++ X --	12	0		<0.001
UBCp43*1700	61	++ X --	10	0		0.001
UBCp72*2500	146	++ X --	12	0		<0.001

Figure 3.1 Phenotypes of parents and offspring from a cross using the primer UBC43. The parents of each cross are in the lanes marked m and f. Another species, brown trout (BN), and a size standard are also included.
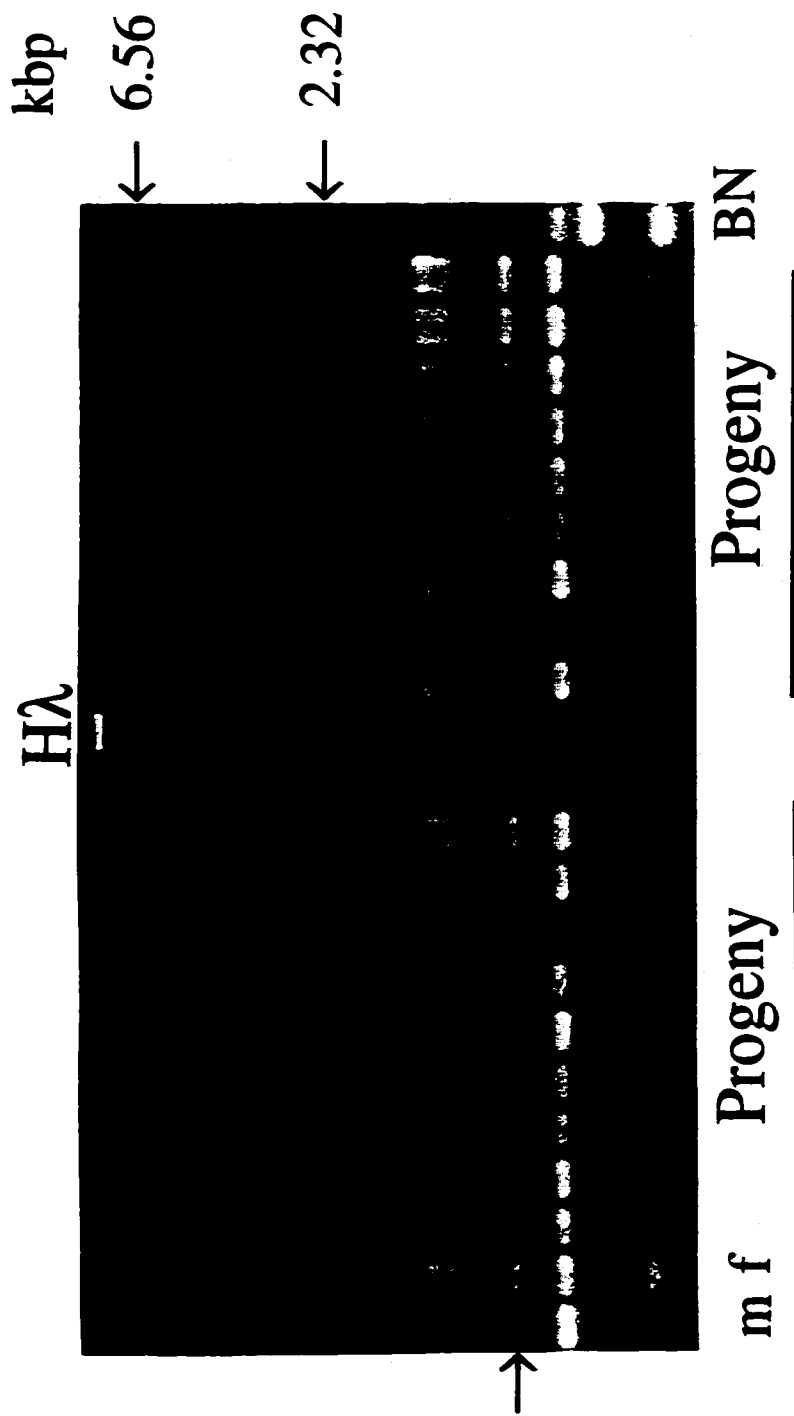
Three types of crosses were examined to analyze the inheritance of RAPD markers. For the first type, (both parents possess the dominant phenotype) five families were observed with progeny of both phenotypes (Table 3.1). Under the assumptions of the genetic model both parents need to be heterozygous (*ie.* their genotypes could be represented as +- X +-, where "+" is the dominant allele, and "-" the recessive allele). No significant deviation from the 3:1 expected segregation ratio was observed. Two families had progeny with only the dominant phenotype. The probability of both parents being heterozygous (ie. +- X +-) and all progeny having the dominant phenotype was calculated. For both families the probability is low ($P<=0.001$), therefore it is unlikely that both parents were heterozygous, and the model with at most one heterozygous parent best fits the data.

For the second cross type, only one parent of the two had the dominant phenotype. In 12 of 21 families both phenotypes were observed among the progeny (Table 3.2). Under the assumptions of the genetic model, the appearance of the recessive genotype (--) among progeny implies that the parent with the dominant phenotype is heterozygous (+- genotype) and the expected phenotypic ratio in the progeny should be 1:1. No significant deviation from the 1:1 ratio was observed for all twelve families. Nine families had progeny with only the dominant phenotype. The probability of one parent being +- and not seeing the recessive phenotype is very low for all the crosses.

The final cross type examined, in which neither parent had the dominant

phenotype (*ie.* -- X --) was less common. Only six families were produced from crosses of two recessive parents. If these markers segregate according to the genetic model, none of the offspring should exhibit the dominant phenotype. Five of the six families had the expected phenotypes, and in one family (cross #62, UBCp43, Table 3.1), four unexpected dominant phenotypes were observed.

Discussion

Not all of the DNA fragments generated by RAPD primers could be amplified consistently. Of the 31 primers that were not used in the inheritance analysis four (OPW9, OPW10, OPB13, and UBCp71) did not amplify the same phenotypes from one run to the next. Reproducibility has been a concern in other studies (eg. Gibbs *et al.* 1994), who found a wide range (33 to 100%) of phenotype reproducibility. Inconsistencies in product amplification can be eliminated by not using unreliable primers, or allowing for the error introduced into genetic diversity indices when population surveys are performed. However, Gibbs *et al.* (1994) found that the introduced error is actually very small. In the current study, only the most reliable fragments amplified by RAPD primers were considered because direct inheritance was studied.

In all the cases analyzed (except cross #62, UBCp43, Table 3.1) the RAPD markers exhibited Mendelian inheritance patterns for a dominant marker system. Four fish from cross #62 had unexpected phenotypes and did not fit the genetic model.

DNA from fish amplified with primer UBCp43 displayed a dominant phenotype that should not have been present since both parents were homozygous recessive. Two other non-parental bands were observed in family #119 using UBCp97 as a primer. The extra fragment was not observed in any of the parental fish, but has been observed in two of eleven individuals from another lake trout stock that we have examined (unpublished data), and therefore it appears that the fragment may not be an artifact. In total, six of the 530 amplification reactions using offspring DNA produced non-parental bands. The proportion of non-parental DNA fragments (0.011) amplified in the offspring is similar to that observed in a breeding study of beetles (0.017) and strawberries (0.002) (Scott *et al.* 1992), and much lower than that reported by Riedy *et al.* (1992). Riedy *et al.* (1992) reported an average of 4.4 and 2.7 non-parental bands per generation amplified by five primers in baboons (N=10) and humans (N=14) respectively, and another study, Reiter *et al.* (1992), reported that only 57% of the 397 polymorphic DNA fragments segregated in a Mendelian fashion in *Arabidopsis.*

Possible explanations for unexpected patterns are: mutation in the offspring caused a conversion to the dominant phenotype, some offspring from another family 'strayed' into either the tank or Heath tray in which the fish were raised, or RAPD bands are inherited as something other than a dominant genetic marker. A high mutation rate would be necessary to generate the number of non-parental bands observed. High mutation rates are seen in some loci, a rate of 5% per locus per gamete generation has been reported for the human microsatellite locus D1S7 (Jeffreys

et al. 1988) for example, and may partially explain the results in the case where the new amplification product was not present in any of the parents. Straying of fish is also possible; embryos may be washed from one Heath® tray compartment to another as they are moved. Straying does not occur at a very high rate, but given the large number of fish reared and sampled in the study, it is possible, and has been observed in family studies using allozymes when the fish are reared in a similar fashion (B. Martin, personal communication, Ontario Ministry of Natural Resources).

Based on the results we conclude that the primers used in this study amplify alleles that are inherited and could therefore be used to examine population structure of lake trout.

Acknowledgments

References

Bucci, G. and P. Menozzi. 1993. Segregation analysis of random amplified polymorphic DNA (RAPD) markers in *Picea abies* Karst. Molecular Ecology 2:227-232.

Carlson, J. E., L. K. Tulsieram, J. C. Glaubitz, V. W. K. Luk, C. Kauffeldt, and R. Rutledge. 1991. Segregation of random amplified DNA markers in F_1 progeny of conifers. Theoretical and Applied Genetics. 83:194-200.

Dweikat, I., S. Mackenzie, M. Levy, and H. Ohm. 1993. Pedigree assessment using RAPD-DGGE in cereal crop species. Theoretical and Applied Genetics. 85:497-505.

Gibbs, H.L., K. A. Prior, and P. J. Weatherhead. 1994. Genetic analysis of populations of threatened snake species using RAPD markers. Molecular Ecology 3:329-337.

Hadrys, H., M. Balick, and B. Schierwater. 1992. Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology. 1:55-63.

Hadrys, H., B. Schierwater, S. L. Dellaporta, R. Desalle, and L. W. Buss. 1993. Determination of paternity in dragonflies by randomly amplified DNA fingerprinting. Molecular Ecology. 2: 79-87.

Hunt, G. J., and R. E. Page Jr. 1992. Patterns of inheritance with RAPD molecular markers reveal novel types of polymorphism in the honey bee. Theoretical and Applied Genetics. 85:15-20.

Ihssen, P. E., J. M. Casselman, G. W. Martin, and R. B. Phillips. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. Can. J. Fish. Aquat. Sci. 45:1018-1029.

Jeffreys, A.J., N. J. Royle, V. Wilson, and Z. Wong. 1988. Spontaneous mutation rates to new length alleles at tandem-repetitive hypervariable loci in human DNA. Nature. 332:278-281.

Koller, B., A. Lehmann, J. M. McDermott, and C. Gessler. 1993. Identification of apple cultivars using RAPD markers. Theoretical and Applied Genetics. 85:901-904.

Levitan, D.R. and R. K. Grosberg. 1993. The analysis of paternity and maternity in the marine hydrozoan *Hydractinia symbiolongicarpus* using randomly amplified polymorphic DNA (RAPD) markers. Molecular Ecology. 2:315-326.

Liu, Z., G. R. Furnier. 1993. Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. Theoretical and Applied Genetics. 87:97-105.

Reiter, R. S., J. G. K. Williams, K. A. Feldmann, J. A. Rafalski, S. V. Tingey, and P. A. Scolnik. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc. Natl. Acad. Sci. USA. 89:1477-1481.

Riedy, M. F., W. J. Hamilton III, and C. F. Aquadro. 1992. Excess of non-parental bands in offspring from known primate pedigrees assayed using RAPD PCR. Nucleic Acids Research. 20:918.

Roy, A., N. Frascaria, J. MacKay, J. Bousquet. 1992. Segregating random amplified polymorphic DNAs (RAPDs) in *Betula alleghaniensis*. Theoretical and Applied Genetics. 85:173-180.

Russell, L. R., F. Hosein, E. Johnson, R. Waugh, and W. Powell. 1993. Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. Molecular Ecology. 2:89-97.

Saghai-Maroof, M. A. K., M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA. 81:8014-8018.

Scott, M. P., K. M. Haymes, and S. M. Williams. 1992. Parentage analysis using RAPD PCR. Nucleic Acids Research. 20:5493.

Sokal, R. R. and F. J. Rohlf. 1969. Biometry. W. H. Freeman and Co. San Francisco.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18:6531-6535.

Yang, X. and C. Quiros. 1993. Identification and classification of celery cultivars with RAPD markers. Theoretical and Applied Genetics. 86:205-212.

Chapter 4
Variability of RAPD loci in eight hatchery and one wild stock of lake trout, *Salvelinus namaycush* from Ontario and Manitoba.

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Abstract

Randomly amplified polymorphic DNA (RAPD) markers were used to generate genetic profiles of nine stocks of lake trout. Thirteen primers were used to amplify 74 DNA fragments. Each primer amplified four to seven polymerase chain reaction products which produced a range of four to 42 phenotypes. No two profiles were the same for any of the 174 lake trout examined. The observed genetic variation is partitioned within and among stocks in similar proportions to the variation detected with allozymes and mitochondrial DNA. Differences in frequencies of some fragments could be used to identify certain stocks such as Killala Lake and Lake Simcoe, but no stock-specific markers were found.

The genetic variation observed indicates that, in general, hatchery practices are maintaining variation, but some loss may have occurred over time in stocks that have been in the hatchery system for longer periods of time. RAPD profiles were used to determine if they could be used to give accurate estimates of stock composition in a mixed-stock analysis. Estimates were more accurate than those obtained with mitochondrial DNA markers, but were still low. On average the estimates of proportional contributions differed from actual values by 67%. High individual variation may account for the low accuracy. In addition, it would be difficult to use RAPD markers to test naturalized populations of lake trout because of their dominant inheritance. However, the technique could be used for mapping commercially important quantitative traits.

Introduction

The number of naturally reproducing stocks of lake trout (*Salvelinus*

namaycush) has been greatly reduced in Ontario, especially in the Great Lakes. Only

two of the five Great Lakes, Huron and Superior, have self-sustaining but threatened

populations (Krueger and Ihssen 1995). The decline of lake trout populations has been

attributed to predation by the sea lamprey and overfishing (Pycha and King 1975).

Loss of trout spawning habitat and food sources due to environmental degradation has

also contributed to the loss of native stocks (Dorr *et al.* 1981).

Current management strategies are designed to preserve the remaining lake trout

stocks and to rehabilitate lake trout habitat. In order to identify stocks that are most

appropriate for rehabilitation, information is required on possible genetic differences

among lake trout from different geographic areas. Physiological, morphological, and

behavioral differences have some genetic basis and are related to adaptation of lake

trout stocks to the local environment (STOCS 1981). Therefore, it is important to

match characteristics of a donor stock to those of the lake which is to be rehabilitated.

Previous studies using allozyme electrophoresis (Ihssen *et al.* 1988) and analysis

of restriction fragment length polymorphisms in mitochondrial DNA (Grewe and

Hebert 1988) identified genetic differences on a large geographic scale, but the ability

to clearly distinguish among all Ontario hatchery stocks is limited. Genetic markers

have also been used to determine parentage of naturally produced lake trout in

rehabilitated areas (Grewe *et al.* 1994), but the accuracy of the analysis is affected by

the limited amount of genetic variation that can be detected with allozyme

electrophoresis.

Randomly amplified polymorphic DNA (RAPD) variation is a relatively new

method for the analysis of population structure. The polymerase chain reaction (PCR)

is used to amplify short sequences that lie between an inverted single synthetic

oligonucleotide primer sequence. The sequence of the single primer is randomly

chosen, therefore no prior knowledge about the genome is needed (Williams *et al.*

1990). The RAPD system allows researchers to analyze many loci using small

amounts of DNA. To date, RAPD analysis has been used for genetic mapping (Torres

et al. 1993), for parentage studies (Levitan and Grosberg 1993; Hadrys *et al.* 1993),

for phylogenetic studies (Stammers *et al.* 1995), for species identification (Bardakci

and Skibinski 1994; Marsolais *et al.* 1993), as well as population structure analysis

(Russell *et al.* 1993; Huff *et al.* 1993; Gibbs *et al.* 1994). The RAPD technique may

provide more markers for the analysis of mixed stocks of lake trout and other fish

species. RAPD markers have had limited use in fisheries management to date, but

several laboratories are currently exploring the technique.

In the present study, RAPD variation in Ontario lake trout hatchery broodstocks

is examined as part of a study to compare the genetic variation observed using

different molecular markers. Data on the hatchery stocks is used to construct

population mixtures in order to test the accuracy of estimates obtained in a mixed-

stock analysis (MSA). MSA can be used to determine contributions of donor stocks to

a recently stocked lake, a naturalized population, or to a commercial fishery (Pella and Milner 1987).

Materials and Methods

Lake trout were sampled from hatchery and wild stocks. The samples represent fish from eight Ontario Ministry of Natural Resources' (OMNR) hatchery supplemented stocks, hatchery broodstocks, and one wild lake population of interest to OMNR managers (Table 4.1). The hatchery broodstocks used were: Killala Lake, Parry Sound (also called Big Sound, Lake Huron), Michipicoten Island (Lake Superior), and Slate Islands (Lake Superior). In order to maintain hatchery supplemented stocks, gametes are taken from the wild, crossed and the progeny reared to the yearling stage before they are released into Lake Manitou (on Manitoulin Island, Lake Huron) and Lake Simcoe. Hatchery-reared lake trout supplement natural recruitment in Lake Manitou but are the only source of lake trout in Lake Simcoe. Lake trout from Iroquois Bay (Lake Huron) were also examined. Iroquois Bay lake trout are currently being added to the Ontario Ministry Natural Resources' pool of broodstocks. A collection was also made from three lakes in the Haliburton region (Clean, Redstone and MacDonald Lakes). The ninth sample came from Clearwater Lake, Manitoba. Lake trout from Clearwater Lake have been used to stock Ontario waters in the past.

DNA was extracted from muscle, liver, or fin samples using a method similar to

Table 4.1 Origins of samples for RAPD-PCR analysis.

Stock	Sample Size	Hatchery or Lake of Origin
Haliburton	20	Clean, Redstone, or MacDonald Lakes via STAG Unit, OMNR, Maple, Ont.
Iroquois Bay	20	Sandfield Fish Culture Station
Killala Lake	20	Hill's Lake Fish Culture Station
Lake Manitou	21	Lake Manitou, reared at STAG Unit, OMNR, Maple, Ont.
Michipicoten Is.	2	Lake Michipicoten
	17	Tarentorus Fish Culture Station
Parry Sound	20	Big Sound, Parry Sound, reared at STAG Unit, OMNR, Ont.
Lake Simcoe	20	Chatsworth Fish Culture Station
Slate Is.	20	Dorion Fish Culture Station
Manitoba	14	Clearwater Lake Fish Culture Station via Lake Simcoe

STAG-Stocks Assessment and Genetics Unit

that of Saghai-Maroof *et al.* (1984); the lysis buffer used was different in the present study. Tissues were incubated at 37°C in lysis buffer (1X buffer is 4 M urea, 0.2 M NaCl, 0.5% n-laurylsarcosine, 10 mM CDTA (1,2-cyclohexanediamine), 0.1 M Tris-HCl, pH 8.0), DNA was extracted twice with phenol and once with a chloroform and isoamyl alcohol mixture, and precipitated using ethanol. A pellet of DNA was obtained by centrifuging and then drying the samples. The isolated DNA was dissolved in TE buffer (10 mM Tris-HCl, 100 mM EDTA, pH 7.5). The concentration of DNA was determined using a fluorometer (Hoefer TKO100). DNA was checked by running 1μg of each sample on an agarose gel. Low weight template DNA used with RAPD primers may produce variable RAPD phenotypes (Williams *et al.* 1990). If large smears appeared along the length of the gel, the sample was replaced and tested again.

Amplification reactions were performed in a volume of 10 μL containing 12.5 ng template DNA, 0.20 to 0.36 μmol primer, 100 μM each of dATP, dCTP, dGTP, and dTTP, 0.5 U Taq polymerase (Boehringer-Mannheim), buffered according to the supplier's instructions. A layer of mineral oil was added to prevent evaporation. The thermal cycler (Precision Instruments) was programmed for 20 cycles of 1 minute at 95°C, 1 minute at 36°C, and 2 minutes at 72°C, followed by 20 cycles of 1 minute at 92°C, 1 minute at 36°C, and 2 minutes at 72°C. Amplification products were separated electrophoretically in 1.4% agarose gels. Gels were electrophoresed at 70 V for 5 to 6 hours. DNA fragments were stained in a solution of ethidium bromide (0.5

g/mL) and were visualized under an ultra violet (UV) light source. Lambda DNA

digested with *Hind*III was included on each gel to serve as a size standard. One

sample without template DNA was run as a negative control on each gel.

Fifty-three RAPD primers from Operon Technologies (Alameda, CA) and the

University of British Columbia (courtesy of R. Danzmann, University of Guelph) were

used. Seven of the 53 primers (OPW3, OPW4, OPW7, OPW17, UBCp43, UBCp72,

and UBCp97) were previously used to conduct an inheritance study on fish from the

Killala stock (Stott *et al.* 1997; Chapter 3).

Shannon's index of diversity (Ho=-$\Sigma\pi$logπ, where π is the phenotypic

frequency) was used to estimate the amount of phenotypic diversity detected, and to

partition the variation in within and among-stock components. Genotypic variance

components were estimated with an Analysis of Molecular Variance (AMOVA;

Excoffier *et al.* 1992). The Euclidean metric is recommended to perform an AMOVA

of RAPD data (Huff *et al.* 1993) using only polymorphic RAPD markers. Therefore,

RAPD bands found in all lake trout analyzed were excluded and the number of band

differences between individuals was calculated. The AMOVA was performed on

various portions of the data set to compare how different primer and fragment

combinations affect estimates of genetic variation. Each primer was analyzed

separately using all the polymorphic fragments. Then the analysis was performed

using all primers but only the 13 polymorphic bands whose inheritance had been

confirmed were used and finally the analysis was rerun with all polymorphic bands

generated by all the primers. The AMOVA was repeated in order to determine if analyzing the 61 untested fragments would change the results of a genetic analysis if they were not inherited in a Mendelian fashion. A cluster analysis of the distance measures was performed using UPGMA (Unweighted Pair Group of Arithmetic Means) to create a dendrogram of relationships among the stocks.

The frequency of the recessive (ie. no band produced) was not used to estimate allele frequencies since it can result in an underestimate of heterozygosity and an overestimate of genetic differences among populations. Other methods to obtain allele frequency estimates do exist (eg. Lynch and Milligan 1994), but they require a large number of assumptions about the data and sample sizes of at least 100. However, in the current study and in studies of freshwater fish populations it is not always possible to gather the required sample sizes.

The polymorphic RAPD fragments were also used in a MSA analysis. Four different mixtures of lake trout were created. Two mixtures involved lake trout from three stocks (Killala Lake, Lake Manitou, and Manitoba) and two involved lake trout from five stocks (Killala Lake, Lake Manitou, Manitoba, the Slate Islands, and Michipicoten Island). Therefore, the majority of the stocks that have been recommended for inland and Great Lakes stocking programs were included (Table 4.2).

Mixed-samples of known proportions were constructed by randomly selecting different numbers of individuals from the hatchery samples. The remaining lake trout

Table 4.2 Stock mixtures used to test accuracy of RAPD data in a mixed-stock analysis. Contributions of each donor stock to the mixed sample are given as a proportion.

Mixture	Killala L.	L. Manitou	Stock Manitoba	Slate Is.	Michipicoten Is.
1	0.334	0.333	0.333	0.00	0.00
2	0.400	0.400	0.200	0.00	0.00
3	0.200	0.200	0.200	0.200	0.200
4	0.350	0.350	0.100	0.100	0.100

were used to construct genetic profiles for baseline populations. A mixed sample of 20 lake trout was used to reflect the sample size often obtained in a single survey of an inland lake. An estimator described by Grant *et al.* 1980 was used to estimate the contribution of each baseline stock to the mixed-sample. Calculations were performed for each primer and then averaged over all primers. The phenotypic frequencies for each primer were used since heterozygotes cannot be scored in a RAPD analysis. The calculations were performed in the manner described by Grewe *et al.* 1994 in a similar analysis of mitochondrial DNA. The accuracy of the results was examined by comparing the estimated percentage contribution to the actual percentage contribution.

Results

Thirteen RAPD primers consistently amplified a total of 74 DNA fragments or presumed loci in nine lake trout stocks (Table 4.3). Nine of the fragments were present in all lake trout examined. Each primer generated 4 to 7 amplification products and a range of 4 to 42 different phenotypes per primer. An example of the phenotypes generated by one primer, OPW15 is presented in Figure 4.1. As in previous studies of lake trout genetic variation, no fixed differences were observed that could be used to identify the stocks. Several RAPD fragments appear at low frequencies in some stocks, but not others. For example, OPW3, OPB7 and UBCp72 amplify bands that are not seen often in the Michipicoten Island lake trout, the Killala Lake fish have a low frequency of an UBCp43 product, and a band amplified by

Table 4.3 The number of amplification products and phenotypes generated with thirteen primers for the nine lake trout stocks.

Primer	No. of amplification products	No. of phenotypes
OPW3	6	6
OPW4	5	15
OPW5	5	15
OPW6	7	21
OPW7	5	4
OPW15	7	42
OPW16	5	11
OPW17	7	36
OPB7	4	8
UBCp43	6	10
UBCp72	6	16
UBCp75	6	30
UBCp97	5	4

Figure 4.1 PCR products amplified in lake trout using the RAPD primer OPW6.
Lambda DNA digested with *Hind*III is included as a size standard (HL).

kbp

H λ

← 6.56

← 2.32
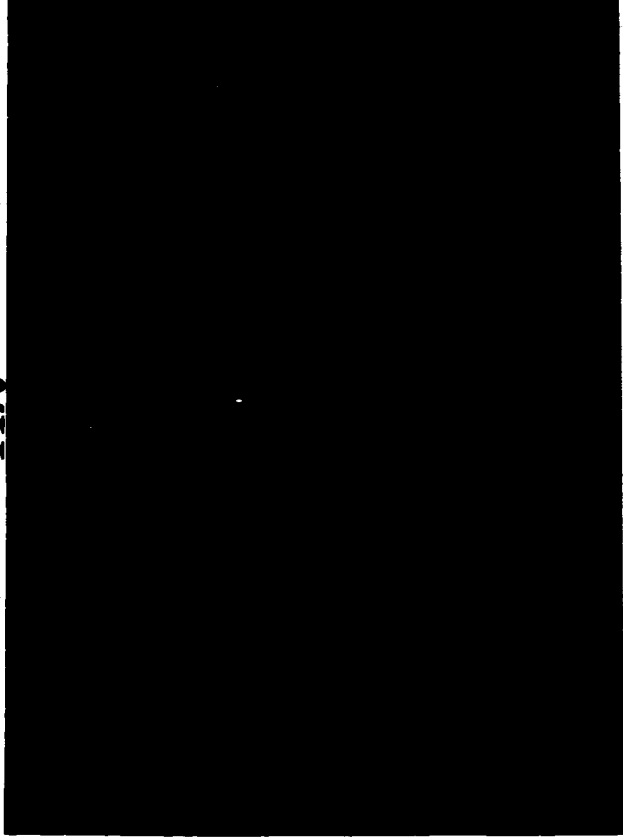
UBCp75 occurs at high frequencies in all stocks but Lake Manitou. The Haliburton, Michipicoten Island, and Lake Manitou samples have the largest number of rarely observed fragments and the greatest variety of phenotypes.

The AMOVA, based on the Euclidian distance matrix, indicates that 86% of the variation occurs within stocks. Similar results are observed for the data set consisting only of the inheritance tested fragments and for the entire set of 63 polymorphic fragments (Table 4.4). Ten of the 13 primers produced similar variance partitions to the total data set (Figure 4.2). The AMOVA analysis detected very high within-stock diversity for three of the RAPD primers (OPW3, OPW15 and UBCp43). The dendrogram of relationships among the stocks (Figure 4.3) is not consistent with the geographic relationships among the source populations for the hatchery stocks. For example, Great Lakes stocks do not group together by lake of origin.

The results of an analysis using Shannon's diversity index, a measure of phenotypic variation are presented in Table 4.5. The most diversity was detected in the samples from Michipicoten Island. If individual primers are considered, OPW15 reveals the most variation in the Lake Manitou samples, and OPW17 detects the most in the Slate Islands rather than the Michipicoten Island samples. H_{pop} measures the average phenotypic diversity within stocks. The most diversity is detected with OPW15, and the least with OPW7. The relative proportion of diversity present within (H_{pop}/H_{tot}) and among $(H_{tot}-H_{pop}/H_{tot})$ stocks indicates that a higher level of diversity is observed within stocks for all primers.

Table 4.4 Molecular analyses of variance for lake trout using RAPD primers, for all polymorphic fragments and for 13 inheritance tested fragments. Statistics include mean squared deviations (MSD), the percentage of the total variance (% Total) contributed by each component

Source of variation	df	MSD	F ratio	P	% Total
Inheritance tested fragments					
Among samples	8	9.00	4.15	<0.005	14.01
Within samples	165	2.17			85.99
All polymorphic fragments					
Among samples	8	34.91	4.50	<0.005	15.4
Within samples	165	7.76			84.6

Figure 4.2 Pie charts for individual RAPD primers showing partitioning of genetic variation within and among stocks. The pie in the centre is the result for all 13 primers. Within stock values are black and among stock values are white.

UBC72

UBC43

UBC75

OPB7

UBC97

OPW17

All primers

OPW3

OPW16

OPW4

OPW15

OPW5

OPW7

OPW6
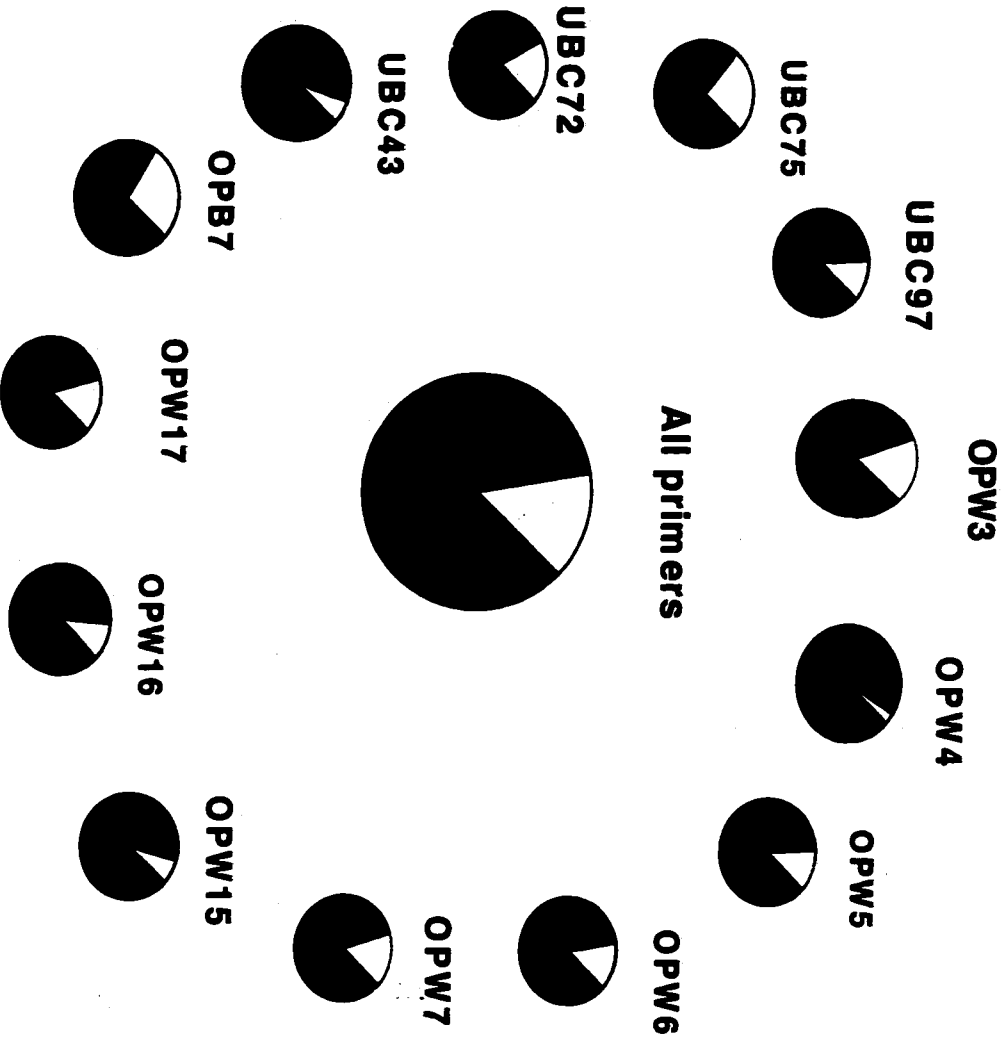
Figure 4.3 Dendrogram showing UPGMA clustering of lake trout stocks. Distances were calculated using the Euclidean distance metric (Huff *et al.* 1993). The numbers on the dendrogram arms represent the percent of 1000 permutations which produced the observed branch point.

Euclidian Distance X100
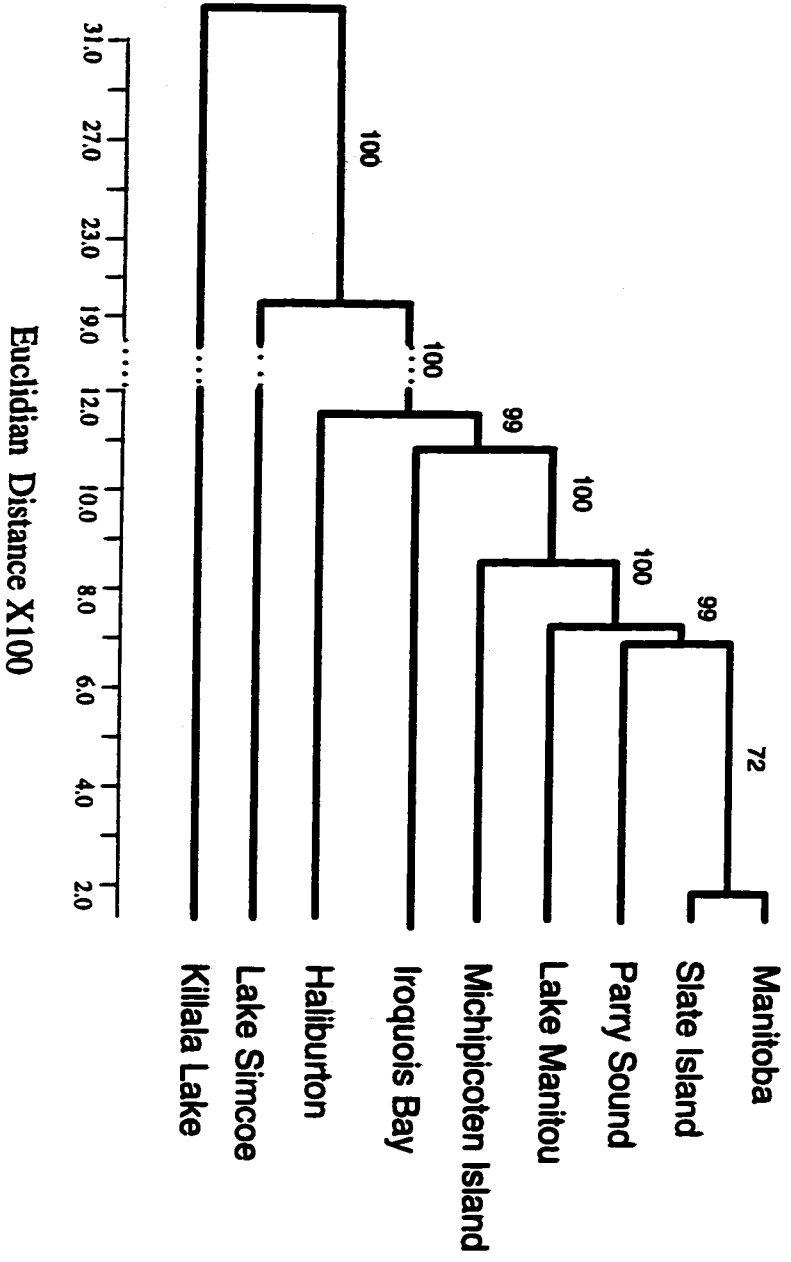
Table 4.5 Partitioning of genetic diversity revealed by thirteen primers into within and among-stock components using Shannon's diversity index.

Primer	Haliburton	Iroquois	Killala	Manitou	Manitoba	Michipicoten	Parry S.	Simcoe	Slate	H_{pop}	H_{tot}	H_{pop}/H_{tot}	$(H_{tot}-H_{pop})/H_{tot}$
OPW3	0.504	0.467	0.299	0.499	0.124	0.441	0.347	0.347	0.219	0.360	0.564	0.638	0.362
OPW4	0.646	0.701	0.770	0.824	0.576	0.809	0.596	0.712	0.593	0.692	0.829	0.835	0.165
OPW5	0.729	0.439	0.000	0.439	0.868	0.664	0.669	0.675	0.547	0.559	0.764	0.732	0.269
OPW6	0.086	0.442	0.308	0.867	0.361	0.677	0.493	0.217	0.495	0.439	0.670	0.655	0.345
OPW7	0.171	0.265	0.281	0.310	0.176	0.234	0.086	0.141	0.184	0.205	0.277	0.742	0.258
OPW15	0.816	0.977	0.299	1.160	1.002	0.911	1.090	0.629	1.189	0.886	1.194	0.742	0.258
OPW16	0.584	0.504	0.086	0.662	0.597	0.637	0.590	0.628	0.646	0.548	0.735	0.746	0.254
OPW17	0.800	0.976	0.763	0.441	0.867	0.921	0.906	0.771	1.068	0.835	1.285	0.650	0.350
OPB7	0.378	0.410	0.259	0.620	0.281	0.582	0.445	0.234	0.385	0.399	0.621	0.643	0.357
UBCp43	0.433	0.225	0.465	0.564	0.284	0.705	0.772	0.803	0.506	0.528	0.589	0.896	0.104
UBCp72	0.844	0.567	0.299	0.552	0.755	0.862	0.733	0.940	0.615	0.685	0.950	0.721	0.279
UBCp75	1.049	0.702	0.000	0.926	0.999	0.934	0.669	0.626	0.960	0.762	1.255	0.607	0.393
UBCp97	0.266	0.469	0.281	0.435	0.390	0.491	0.299	0.299	0.538	0.385	0.488	0.790	0.210
Mean	0.562	0.550	0.316	0.638	0.560	0.682	0.592	0.540	0.603	0.560	0.786	0.712	0.288

Mixed-stock analyses were performed on four different stock mixtures. The estimated values and percent accuracy for the mixed stock analysis are presented in Table 4.6. Values of accuracy ranged from 57% to 73% and were on average, 67%. Eleven of the calculations for individual primers did not converge on an estimate even after 500 iterations of the calculation. The eleven values were not included in the accuracy analysis, but would reduce the accuracy since a lack of convergence means that any mixture of baseline stocks is equally likely.

Discussion

The findings presented here represent one of the few population studies using RAPD loci on a fish species. If each band amplified by a primer can be considered a separate locus then sixty-three variable loci were observed in the lake trout samples, or about 85% of the loci analyzed were polymorphic. A higher percentage of the loci analyzed were polymorphic as compared to an allozyme study of lake trout in Ontario (46% polymorphic; Ihssen et al. 1988). An increased number of polymorphisms is also observed in other studies that compared RAPD loci and allozyme variation (Liu and Furnier 1993; Torres et al. 1993; Yang and Quiros 1993). It is not unexpected that more polymorphic characters are observed because the RAPD technique measures variation at the DNA-level, whereas allozyme analysis of protein-coding loci is one step removed from the DNA-level.

Results from the AMOVA analysis were similar for the entire data set and for a

Table 4.6 Estimated values and per cent accuracy for mixed-stock analysis of lake trout in four mixtures.

			Stock			
	Killala L.	L. Manitou	Manitoba	Slate Is.	Michipicoten Is.	% accuracy
Mixture						
1	0.266	0.266	0.468	0.000	0.000	73
2	0.326	0.261	0.413	0.000	0.000	57
3	0.218	0.297	0.221	0.169	0.095	73
4	0.375	0.180	0.173	0.128	0.144	66

smaller data set based only on fragments that have been tested for Mendelian

inheritance (Stott *et al.* 1997; Chapter 3). One concern about the use of the RAPD

technique is the heritability of the amplified fragments and it has been suggested that

the inheritance of fragments should be tested whenever possible (Dowling *et al.* 1996).

Although not all the fragments used in the current study have been tested, the

comparison of AMOVA data suggests that the untested fragments are segregating

within the populations in the same way as the 13 confirmed loci. The single primer

analysis showed that the majority of the primers have a similar distribution of genetic

variation as the entire data set. Of the five primers that were different, three have

very high levels of within stock diversity (over 90%). Huff *et al.* 1993 suggest that

primers which reveal different partitions of genetic variation may be used for different

purposes. For example, a culturist interested in pedigree analysis may prefer primers

that are very polymorphic in individuals, whereas stock identification requires primers

that have less variation within stocks and more among them. No stock-specific

markers were observed in the current study, but some have been observed in studies of

plant species (Huff *et al.* 1993).

If three different genetic markers located in different areas of the genome are

compared, levels of genetic diversity are similar. The markers are: RAPD loci, which

analyze variation at the DNA level, allozyme variation or the variation in protein-

coding genetic loci, and mitochondrial DNA variation (mtDNA), which is inherited

maternally. Within-stock genetic variation observed in a study of protein coding loci

of hatchery stocks in Ontario was 82% (Ihssen *et al.* 1988). About 85% of the total

genetic variation observed is due to variation among the sample stocks for RAPD loci,

which measure variation at the DNA level of coding and non-coding regions. Most of

the phenotypic diversity in RAPD data, measured by Shannon's Index, was also

detected within the samples (74%). A similar result is also observed in a study of

restriction fragment length polymorphisms of mtDNA using the same samples as were

analyzed in the current study (78%; Chapter 2). The results may indicate that

demographic processes have acted on the mitochondrial and nuclear genomes in a

similar fashion. The processes may include genetic drift, migration, inbreeding, and

selection. The demographic processes may be a result of hatchery practices or may

reflect the variation in the wild if hatchery practises have maintained genetic variation.

In contrast to the present study, extremely divergent estimates of genetic diversity

were obtained for populations of brook charr (*S. fontinalis*) from the south eastern

coast of Newfoundland (Ferguson *et al.* 1991).

The RAPD profiles of each individual lake trout are unique, as might be

expected in an outbred population, or a hatchery population with a good genetic base

and a breeding program design to maintain levels of genetic variation. In contrast,

low levels of within-stock variation were observed in hatchery stocks of tilapia

(*Oreochromis* spp.) that were founded from small numbers of individuals (Bardakci

and Skibinski 1994). High within-stock variation is observed in other studies of

natural populations (eg. Chapco *et al.* 1992), while studies of some plant species report

lower within-stock variation (Russell *et al.* 1993; Koller *et al.* 1993; Yang and Quiros 1993). Several of the RAPD analyses of plants have been performed on species of commercial interest that have been part of breeding programs that may have involved selection within lines. Any breeding program that is designed to produce a consistent product will usually reduce individual variation, therefore any differences detected at RAPD loci will be magnified. For example, cultivar-specific markers were observed in celery (Yang and Quiros 1993) and apples (Koller *et al.* 1993).

Genetic variation within the hatchery stocks is being maintained at levels that might be expected of a captive population with an adequate effective population size. Stocks with lower levels of diversity have been founded with small numbers of individuals (eg. Iroquois Bay; founder size was less than 50 (Anonymous 1992); Table 4.5). In contrast, the Michipicoten Island stock was created using larger samples of lake trout, has been maintained using rotational line crossing, and has relatively larger values of diversity. One exception is the Killala Lake stock broodstock which was founded with relatively large numbers of fish, but the stock has a low value of genetic diversity. The low levels of diversity may be a reflection of diversity that existed in the wild. Small inland populations of fish such as the Killala Lake stock will have less variation when compared to stocks from larger areas such as Michipicoten Island.

The dendrogram (Figure 4.3) produced using RAPD data has a different branching pattern compared to that observed using allozyme or mitochondrial DNA data (Ihssen *et al.* 1988; Grewe and Hebert 1988; Chapter 2). Allozyme and

mitochondrial DNA data reveal clusters of lake trout from north-western Ontario and Manitoba, from the lower Great Lakes, and from south-eastern Ontario. Also, the allozyme data indicate that Haliburton lake trout are quite distinct. In contrast, the RAPD analysis did not group the stocks by lake of origin, or by geographic location. Little geographic clustering of any type is observed. The discrepancy could be due to the different inheritance mode of allozymes and mitochondrial DNA versus RAPD's, high within-stock variation observed at RAPD loci, or the method of analysis. The high within-stock variation observed at RAPD loci may obscure among-stock structure patterns. In order to generate genetic distances among samples, correlations between random RAPD profiles within a group of populations are calculated (called PHI_{ST}). Some of the values of PHI_{ST} generated with the current data are negative. Small negative values of PHI_{ST} would suggest that lake trout from different stocks are more closely related to each other than to lake trout in the same stock (Excoffier *et al.* 1992). The majority of negative values occur among stocks that originated from Lake Superior, Manitoba and Lake Simcoe, indicating that the lake trout from these stocks in particular are as closely related, or more similar to lake trout from another stock than to other lake trout in the same stock. Lake Simcoe has been stocked with Manitoba lake trout in the past (Anonymous 1992). If Manitoba lake trout from Lake Simcoe were recaptured and accidently classified as Lake Simcoe lake trout they would appear to be more similar to each other than might be expected. If stocks with negative correlations are grouped together, then the analysis shows that the set of Lake

Superior stocks, Manitoba, and Lake Simcoe groups with Lake Manitou, Parry Sound and Iroquois Bay. Consequently, the inland Killala Lake and Haliburton samples are on distinct branches. This arrangement is more similar to that of previous genetic studies of lake trout.

Although differences existed in the frequencies of the RAPD markers, no stock specific markers were observed. In a study of 32 polymorphic RAPD markers in striped bass, one stock specific marker was observed (Bielawski and Pumo 1997). However, the marker could only be used to identify some samples since it was observed at a low frequency (19%) in one of five sample sites. Killala Lake and Lake Simcoe lake trout could be identified in a similar fashion with markers amplified by OPW3, OPW5, and UBCp43.

RAPD loci may not be an effective marker system for mixed-stock analysis. In the mixed-stock analysis the average percent accuracy was under 70% for RAPD data which is lower than the values obtained in a simulation of chinook salmon (*Oncorhynchus tshawytscha*) mixed-stock data (Pella and Milner 1987), and in a study of sockeye salmon (*O. nerka*) data (Grant *et al.* 1980) using allozyme variation. It would also be difficult to use RAPD loci to monitor relative reproduction success of different donor stocks since the algorithm most often used requires data on frequency of heterozygotes (Marsden *et al.* 1989) and heterozygotes cannot be scored directly with RAPD loci since they are inherited in a dominant fashion.

Acknowledgments

References

Anonymous. 1992. Stocks Catalogue: handbook of stocks available in the Provincial Fish Culture System. Fish Culture Section, Great Lakes Branch, Operations Division, Ontario Ministry of Natural Resources.

Bardakci, F. and D. O. F. Skibinski. 1994. Application of the RAPD techniques in tilapia fish: species and subspecies identification. Heredity 73:117-123.

Bielawski, J. P. and D. E. Pumo. 1997. Randomly amplified polymorphic DNA (RAPD) analysis of Atlantic coast striped bass. Heredity. 78:32-40.

Chapco, W., N. W. Ashton, R. K. B. Martel, N. Antonishyn, and W. L. Crosby. 1992. A feasibility study of the use of random amplified polymorphic DNA in the population genetics and systematics of grasshoppers. Genome 35:569-574.

Dorr, J. A. III; D. V. O'Connor, N. R. Foster, and D. J. Dude. 1981. Substrate conditions and abundance of lake trout eggs in a traditional spawning area in southeastern Lake Michigan. N. Am. J. Fish. Manage. 1:165-172.

Dowling, T. E., C. Moritz, J. D. Palmer, and L. H. Rieseberg. 1996. Nucleic acids III: analysis of fragments and restriction sites. In Molecular Systematics. D. M. Hillis, C. Moritz, and B. K. Mable (eds.) . Second Edition. pg. 249-320. Sunderland Associates, Inc. Sunderland, Massachusetts USA.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 131:479-491.

Ferguson, M. M., R. G. Danzmann, and J. A Hutchings. 1991. Incongruent estimates of population differentiation among brook charr, Salvelinus fontinalis, from Cape Race, Newfoundland, Canada, based upon allozyme and mitochondrial DNA variation. J. Fish Biology 39(Supplement A):79-85.

Gibbs, H. L., K. A. Prior, and J. P Weatherhead. 1994. Genetic analysis of populations of threatened snake populations using RAPD markers. Molecular Ecology 3:329-337.

Grant, W. S., G. B. Milner, P. Krasnowski, and F. M. Utter. 1980. Use of biochemical variants for identification of sockeye salmon (Oncorhynchus nerka) stocks in

Cook Inlet, Alaska. Can. J. Fish. Aquat. Sci. 37:1236-1247.

Grewe, P. M. and P. D. N. Hebert. 1988. Mitochondrial DNA diversity among brood
stocks of the lake trout, *Salvelinus namaycush*. Great Lakes Fishery
Commission Research Completion Report.

Grewe, P. M., C. C. Krueger, J. E. Marsden, C. F. Aquadro, and B. May. 1994.
Hatchery origins of naturally produced lake trout fry captured in Lake Ontario:
temporal and spatial variability based on allozyme and mitochondrial DNA
data. Trans. Amer. Fish. Soc. 123:309-320.

Hadrys, H., B. Schierwater, S. L. Dellaporta, R. Desalle, and L. W. Buss. 1993.
Determination of paternity in dragonflies by random amplified polymorphic
DNA fingerprinting. Molecular Ecology. 2:79-87.

Huff, D. R., R. Peakall, and P. E. Smouse. 1993. RAPD variation within and among
natural populations of outcrossing buffalograss [*Buchloe dactyloides* (Nutt.)
Engelm.]. Theoretical and Applied Genetics. 86:927-934.

Ihssen P.E., J. M. Casselman, G. W. Martin, and R. B. Phillips. 1988. Biochemical
genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great
Lakes region. Can. J. Fish. Aquat. Sci. 45:1018-1029.

Koller, B., A. Lehmann, J. M. McDermott, and C. Gessler. 1993. Identification of
apple cultivars using RAPD markers. Theoretical and Applied Genetics.
85:901-904.

Krueger, C. C. and P. E. Ihssen. 1995. Review of genetics of lake trout in the Great
Lakes: history, molecular genetics, physiology, strain comparisons, and
restoration management. J. Great Lakes Res. 21(suppl. 1):348-363.

Levitan, D. R. and R. K. Grosberg. 1993. The analysis of paternity and maternity in
the marine hydrozoan *Hydractinia symbiolongicarpus* using randomly amplified
polymorphic DNA (RAPD) markers Molecular Ecology. 2:315-326.

Liu, Z. and G. R. Furnier. 1993. Comparison of allozyme, RFLP, and RAPD markers
for revealing genetic variation within and between trembling aspen and
bigtooth aspen. Theoretical and Applied Genetics. 87:97-105.

Lynch, M., and B. G. Milligan. 1994. Analysis of population genetic structure with
RAPD markers. Molecular Ecology 3:91-99.

Marsden, J. E., C. C. Krueger, and B. May. 1989. Identification of parental origins of naturally produced lake trout in Lake Ontario: application of mixed-stock analysis to a second generation. N. Amer. J. Fish. Manage. 9:257-268.

Marsolais J.V., J. S. Pringle, and B. N. White. 1993. Assessment of randomly amplified polymorphic DNA (RAPD) as a genetic marker for determining the origin of interspecific lilac hybrids. Taxon. 42:531-537.

Pella, J. J. and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. In Population Genetics and Fishery Management. N. Ryman and F. Utter (eds.) pg. 247-276. University of Washington Press, Seattle and London.

Pycha, R. L., and G. R. King. 1975. Changes in the lake trout population of southern Lake Superior in relation to the fishery, the sea lamprey and stocking, 1950-1970. Great Lakes Fish. Comm. Tech. Rep. 28:34p.

Russell, J. R., F. Hosein, E. Johnson, R. Waugh, and W. Powell. 1993. Genetic differentiation of cocoa (*Theobroma cacao* L.) populations revealed by RAPD analysis. Molecular Ecology 2:89-97.

Saghai-Maroof, M. A. K., M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA 81:8014-8018.

Stammers, M., J. Harris, G. M. Evans, M. D. Hayward, and J. W. Forster. 1995. Use of PCR (RAPD) technology to analyze phylogenetic relationships in the *Lolium/Festuca* complex. Heredity 74:19-27.

STOCS. 1981. Stock concept international symposium. Can. J. Fish. Aquat Sci. 38:1457-1921.

Stott, W., P. E. Ihssen, and B. N. White. 1997. Inheritance of RAPD molecular markers in lake trout, *Salvelinus namaycush*. Molecular Ecology. 6:609-613.

Torres, A. M., N. F. Weeden , and A. Martin. 1993. Linkage among isozyme, RFLP and RAPD markers in *Vicia faba*. Theoretical and Applied Genetics. 85:937-945.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic

markers. Nucleic Acids Research. 18:6531-6535.

Yang, X. and C. Quiros. 1993. Identification and classification of celery cultivars with RAPD markers. Theoretical and Applied Genetics 86:205-212.

Chapter 5
Microsatellite DNA variation in hatchery stocks of lake trout (*Salvelinus namaycush*)

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Abstract

Microsatellite DNA variation was assessed in nine stocks of lake trout

(*Salvelinus namaycush*) using eight heterologous primer pairs. Six of the primers

amplified polymorphic loci. No departures from Hardy-Weinberg expectations were

observed at any of the loci in any of the stocks. The number of alleles per locus

ranged from four to 27, which is larger than the number observed in studies of

allozyme variation. The observed heterozygosity ranged from 30% to 88% which is

similar to that observed at microsatellite loci in other salmonid species and higher than

that observed in studies of allozymes. Comparisons of allozyme and microsatellite

DNA data reveal that stocks with higher levels of allozyme heterozygosity also have

higher levels of microsatellite DNA heterozygosity. A comparison of levels of

heterozygosity among stocks indicates differences that may be the result of hatchery

practices and the age of the hatchery stocks. Analyses of patterns of geographic

variation show that samples group by lake of origin, with the exception of the Lake

Simcoe and Manitoba samples. Since Manitoba lake trout have been introduced into

Lake Simcoe, similarity between Lake Simcoe and Manitoba samples may be the

result of an accidental introgression of Manitoba alleles into Lake Simcoe broodstock

over time. Samples from the Haliburton Highlands were also quite distinct, supporting

the idea that lake trout from the Haliburton region may be descendants of lake trout

from a glacial refuge. The results indicate that microsatellite loci will provide

sufficient genetic markers to monitor hatchery stocks and to accurately analyze the

contribution of stocks in mixed stock analysis.

Introduction

Analysis of microsatellite DNA variation is a relatively new approach for the investigation of genetic differences among populations. Microsatellite DNA loci have been used to examine the genetic diversity of a variety of species (eg. Atlantic salmon, *Salmo salar*; O'Reilly *et al.* 1996; birds, Ellegren 1992; black bears, *Ursus americanus*; Paetkau and Strobeck 1994; brook charr, *Salvelinus fontinalis*; Angers *et al.* 1995; cattle, *Bos taurus* and goats, *Capra hircus*; Pepin *et al.* 1995; white-tailed deer, *Odocoileus virginianus*; DeWoody *et al.* 1995; and wombats, *Lasiorhinus krefftii*; Taylor *et al.* 1994). Variable microsatellite DNA loci have also been observed in species which displayed little variation at other loci, such as allozyme loci (Hughes and Queller 1993; Miller and Kapuscinski 1996).

Microsatellite loci are characterized by DNA sequences that consist of short, tandemly repeated motifs one to five nucleotides in length. Polymorphism occurs as the result of variation in the number of times the motif is repeated (reviewed in Dowling *et al.* 1996). Pairs of primers flanking microsatellite DNA loci are used in the polymerase chain reaction (PCR), and the PCR product is separated on a standard acrylamide sequencing gel. A potential drawback of the technique is that microsatellite loci are identified by primers whose sequence is determined from a cloned region isolated from a genomic library. Therefore, if the species of interest has not been previously studied, time, technical expertise, and a considerable budget may be required. However, studies have shown that primers produced for one species may

also be applied to another, closely related species (Moore *et al.* 1991; Angers and

Bernatchez 1996; Pepin *et al* 1995).

The purpose of this paper is to investigate the use of heterologous primers to

describe microsatellite DNA variation in Ontario hatchery stocks of lake trout (*S.*

namaycush). Primers from two other salmonid species, brook charr (Angers *et al.*

1995) and Atlantic salmon (McConnell *et al.* 1995; O'Reilly *et al.* 1996) were used to

assess microsatellite DNA variation in nine stocks of lake trout. Genetic markers that

distinguish among the various Ontario hatchery stocks would be useful for

rehabilitation programs. Markers could be used to monitor the relative survival of

various stocks planted into a lake, especially the origin of naturally produced lake

trout. Such information would be useful to provincial resource managers since close

to 20% of the lake trout lakes in Ontario have been stocked with lake trout of non-

native origin (Evans and Willox 1991). The necessary variation required to perform

accurate mixed stock analyses has not been detected in all Ontario hatchery stocks

using allozymes or molecular markers such as mitochondrial DNA or RAPD loci

(Chapters 2 and 4).

Materials and Methods

Nine stocks of lake trout were sampled for the study, including six Ontario

hatchery stocks and a sample of lake trout from Clearwater Lake, Manitoba. The

Manitoba lake trout were introduced into Lake Simcoe and recaptured as adults. The

Iroquois Bay (Lake Huron) sample represents a broodstock that is currently under development. The ninth sample was from two lakes in the Haliburton region (Table 5.1, Figure 5.1). Lake trout from the Haliburton region are believed to represent fish from an interglacial refuge (Ihssen *et al.* 1988). Previous studies indicate that Haliburton lake trout have a unique genetic profile, and may require special consideration by Ontario Ministry of Natural Resources (OMNR) managers.

DNA was extracted from liver or muscle samples using the technique outlined in Saghai-Maroof *et al.* (1984), except that a different lysis buffer (1X buffer is 4 M urea, 0.2 M NaCl, 0.5% n-laurylsarcosine, 10 mM CDTA (1,2-cyclohexanediamine), 0.1 M Tris-HCl, pH 8.0) was used to incubate the tissues. Tissues were incubated for 2 hrs at 37°C, then the lysate was extracted twice with phenol and once with a chloroform and isoamyl alcohol mixture, and DNA was precipitated out of solution using ethanol. A pellet of DNA was obtained by centrifuging and then drying the samples. The isolated DNA was dissolved in TE buffer (10 mM Tris-HCl, 100 mM EDTA, pH 7.5). The concentration of DNA was determined using a fluorometer (Hoefer TKO100).

Eight primers were tested on lake trout samples; five designed for brook charr (Sfo8, 11, 12, 18, 23; Angers *et al.* 1995; Angers and Bernatchez 1996) and three designed for Atlantic salmon (Ssa289; McConnell *et al.* 1995 and Ssa85, 197; O'Reilly *et al.* 1996; Table 5.2). One primer (20 pmol) from each pair was end-labelled with [γ-^{33}P] ATP using T4 polynucleotide kinase according to manufacturer's

Table 5.1 Origins of samples for microsatellite DNA analysis.

Stock	Sample Size	Hatchery or Lake of Origin
Haliburton	20	Redstone and MacDonald Lakes via Stocks Assessment and Genetics Unit, OMNR, Maple, Ont.
Iroquois Bay	19	Sandfield Fish Culture Station
Killala Lake	24	Hill's Lake Fish Culture Station
Lake Manitou	21	Lake Manitou, reared at STAG Unit, OMNR, Maple, Ont.
Michipicoten Is.	2	Lake Michipicoten
	17	Tarentorus Fish Culture Station
Parry Sound	20	Big Sound, Parry Sound, reared at STAG Unit, OMNR, Ont.
Lake Simcoe	20	Chatsworth Fish Culture Station
Slate Is.	20	Dorion Fish Culture Station
Manitoba	14	Clearwater Lake, Manitoba via Lake Simcoe

STAG-Stocks Assessment and Genetics Unit

Figure 5.1 Geographic origins of lake trout analyzed in the study.

MANITOBA

LAKE SUPERIOR

KILLALA

SLATE Is.

MICHIPICOTEN Is.

LAKE MICHIGAN

MANITOU

IROQUOIS BAY

PARRY SOUND

LAKE HURON

HALIBURTON

SIMCOE

LAKE ERIE

LAKE ONTARIO
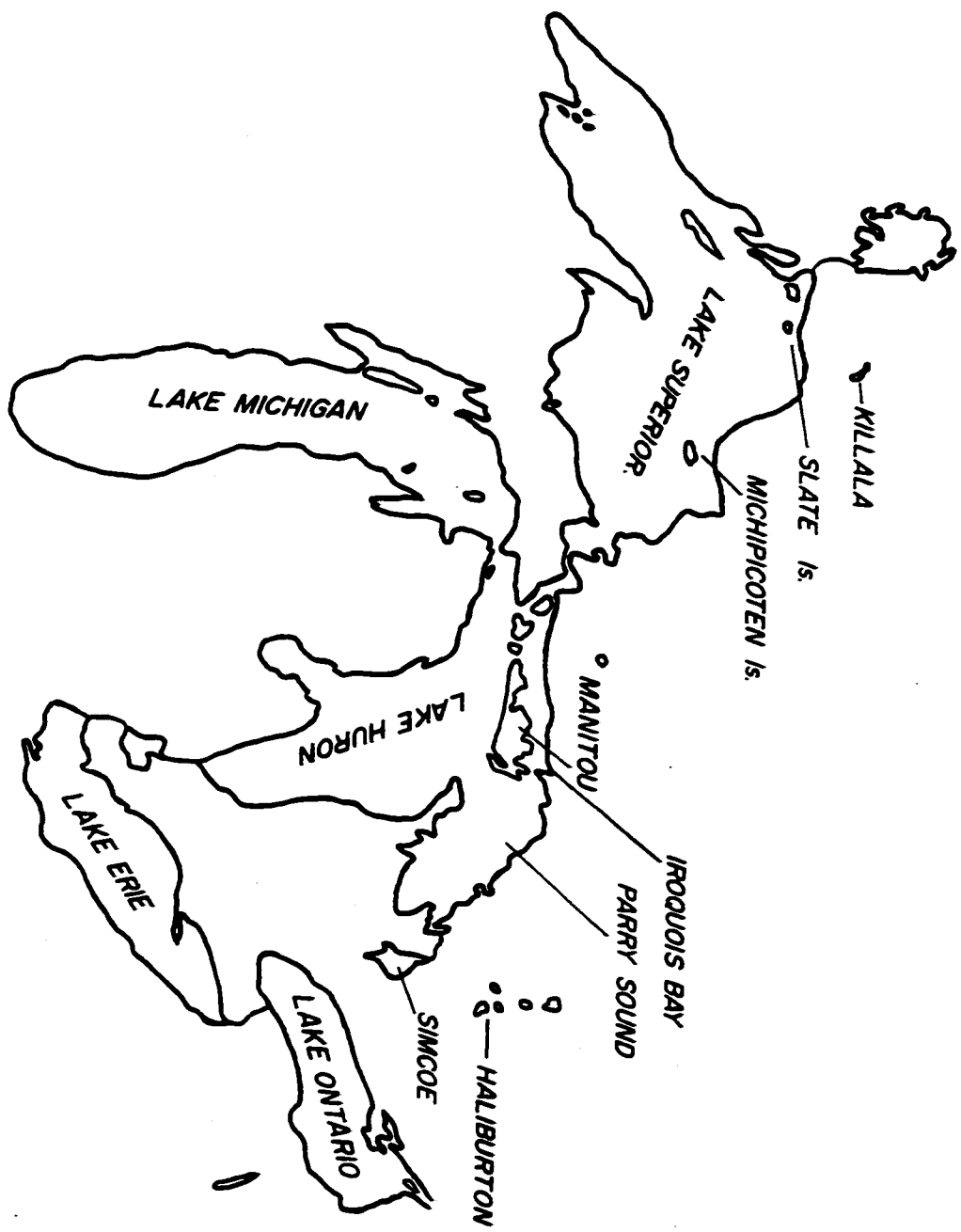
Table 5.2 Locus names, primer sequences, annealing temperatures, and range of allele sizes for eight microsatellite primers used to amplify lake trout DNA.

Locus	Primer sequence (5'-3')	Annealing temperature (°C)	Range of allelic sizes in base pairs
Sfo8	CAA CGA GCA CAG AAC AGG* CTT CCC CTG GAG AGG AAA	58	266-298
Sfo11	GGT TTC ATG CAT TTG TCT TGT* CTC GAA AAA ATC ACC AAT TAC	58	not scorable
Sfo12	GGT TTT GAA GAG TGA CAG CCC GTT TCA CAA TCA GAG*	56	254-260
Sfo18	TGG TGT ATC CTG CTC CTG* TGG AAT GTG TGT CTG TTT TCT	60	170-194
Sfo23	GTG TTC TTT TCT CAG CCC AAT GAG CGT TAC GAG AGG*	58	186-242
Ssa85	AGG TGG GTC CTC CAA GCT AC* ACC CGC TCC TCA CTT AAT C	58	not scorable
Ssa197	GGG TTG AGT AGG GAG GCT TG* TGG CAG GGA TTT GAC ATA AC	58	174-270
Ssa289	CTT TAC AAA TAG ACA GAC T TCA TAC AGT CAC TAT CAT C*	46	172-224

*End-labelled primer used for PCR reactions.

instructions (New England Biolabs). The PCR reaction took place in a volume of 10

μL containing 10 pmol of each primer, 2 μM of each dNTP, 0.25 U *Taq* polymerase

(Boehringer-Mannheim) and the manufacturer's buffer. Thirty-five cycles of DNA

amplification were performed, consisting of: 50 seconds at 94°C, 60 seconds at the

annealing temperature (Table 5.2) and 50 seconds at 72°C. After a formamide loading

buffer was added, PCR products were heated at 92°C for 10 minutes, separated on 8%

acrylamide sequencing gels, and visualized by autoradiography (Figure 5.2). An M13

sequencing reaction (Boehringer Mannheim) was also run to provide a size standard.

Tests for conformation to Hardy-Weinberg expectations were performed using

the method of Guo and Thompson (1992). Tests of allele frequency heterogeneity

were performed for all the microsatellite loci using the computer package Genpop

(version 1.2; Raymond and Rousset 1995). Probability values were adjusted for

multiple tests using the Bonferroni correction (Rice 1989). Genetic distances among

samples were calculated using the method of Nei (1972) and the Unweighted Pair

Group of Arithmetic Means (UPGMA) method was used to create a dendrogram

showing the relationships among the lake trout samples.

Results

Primer sequences developed for brook charr and Atlantic salmon could be used

to amplify microsatellite loci in lake trout. Repeatable DNA amplification of

microsatellite loci was obtained with six of the primers (Sfo8, 12, 18, 23, Ssa197, and

Figure 5.2 Autoradiograph of lake trout DNA samples amplified with the primer set for the microsatellite locus Sfo18. An M13 sequencing reaction is also shown as a size standard (lanes 7 to 10, labelled ACGT), and two alleles measuring 182 and 172 base pairs are labelled.
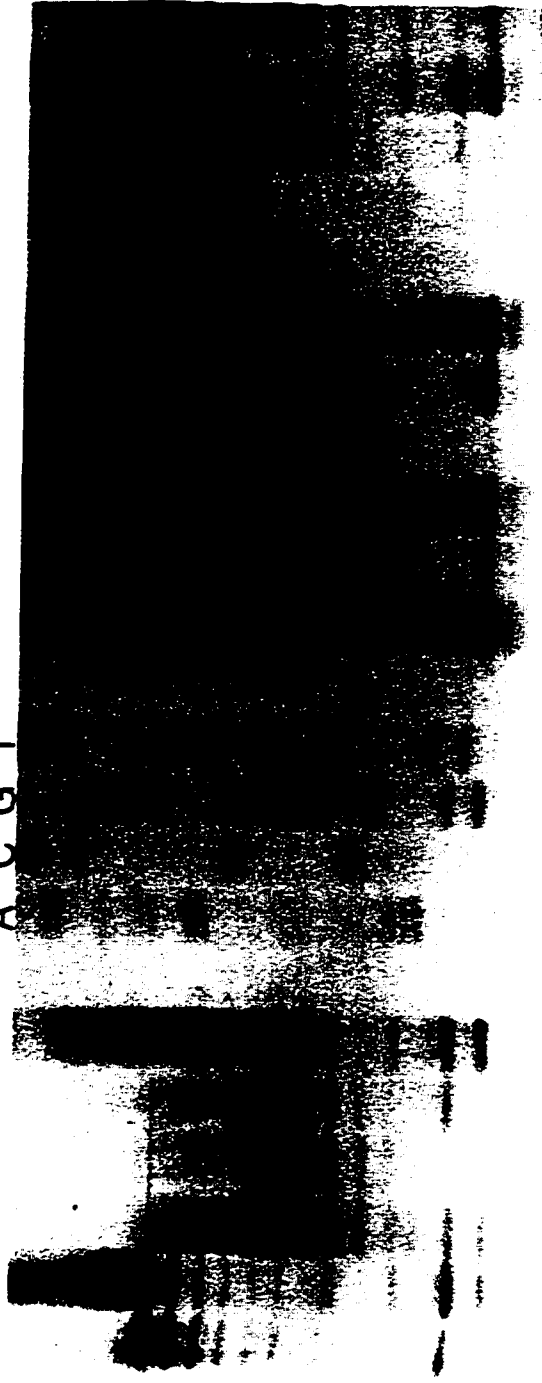
289), and all six were polymorphic. Two of the primers Sfo11 and Ssa85 could not

be scored (Table 5.2), either because no product was amplified (Ssa85) or the

amplifications were not repeatable (Sfo11). The number of alleles per locus ranged

from 4 to 27. Six of the alleles observed at Sfo18 are shown in Figure 5.2. Values of

heterozygosity varied for each locus scored. The observed heterozygosity at each

locus varied from 30% (Sfo12) to 88% (Ssa197; Table 5.3). The observed

heterozygosity over all loci in each stock ranged from a low of 60% in Killala Lake to

a high of 71% in Iroquois Bay and Manitoba, and was 66% on average (Table 5.3).

No significant departures from Hardy-Weinberg expectations were observed at $\alpha=0.05$

(with the Bonferroni correction for multiple tests).

The UPGMA dendrogram depicting genetic distances among the stocks (Figure

5.3) shows that the samples from the Great Lakes group by lake of origin. The Killala

Lake samples are closer to Great Lakes samples than the other inland lake samples.

Manitoba and Lake Simcoe samples are grouped, and the samples from the Haliburton

region are quite distinct. Comparisons over all stocks revealed significant differences

in allelic frequencies. Comparisons between pairs of populations for individual loci

were also made (Table 5.4). The most significant differences were observed at Sfo23

(31; Table 5.4) and the fewest at Sfo8 and Sfo12 (both with 6; Table 5.4). Only one

pair of populations (Parry Sound and Iroquois Bay) had no differences between them

at any locus.

Table 5.3 Number of alleles and observed heterozygosity at six microsatellite loci amplified in lake trout populations.

Stock	Sfo8		Sfo12		Sfo18		Sfo23		Ssa197		Ssa289		Total
	A	H_{obs}	A	H_{obs}	A	H_{obs}	A	H_{obs}	A	H_{obs}	A	H_{obs}	H_{obs}
Haliburton	11	0.80	3	0.50	6	0.70	11	0.80	11	0.75	11	0.84	0.73
Iroquois B.	10	0.71	2	0.19	8	0.75	15	0.95	11	0.94	10	0.72	0.71
Killala L.	12	0.82	3	0.10	2	0.10	16	1.00	11	0.87	12	0.78	0.60
Manitoba	9	0.77	3	0.57	3	0.54	8	0.77	13	0.82	10	0.79	0.71
L. Manitou	12	0.81	3	0.10	6	0.62	14	0.67	12	0.94	14	0.71	0.63
Michipicoten	11	0.67	3	0.16	6	0.63	10	0.71	14	0.84	10	0.74	0.62
Parry Sound	9	0.75	2	0.10	7	0.55	12	0.90	14	0.89	10	0.70	0.66
L. Simcoe	10	0.67	3	0.48	5	0.40	12	0.89	10	0.92	11	0.80	0.63
Slate Is.	12	0.75	3	0.50	6	0.70	13	0.80	17	0.94	11	0.80	0.66
Total	16	0.75	4	0.30	12	0.55	27	0.83	22	0.88	25	0.76	0.66

A=number of alleles H_{obs}=observed heterozygosity

Figure 5.3 Dendrogram showing the genetic similarity among the lake trout stocks analyzed for microsatellite DNA variation. The dendrogram is based on Nei's (1972) genetic distance and obtained by UPGMA cluster analysis, the standard error of the distance at each branch point is included on the dendrogram arms.

Nei's Genetic Distance X10

5.0 4.0 3.0 2.0 1.0

0.240

0.122

0.097

0.102

0.098

0.110

0.124

Haliburton

Killala Lake

Slate Island

Michipicoten Island

Iroquois Bay

Parry Sound

Lake Manitou

Lake Simcoe

Manitoba
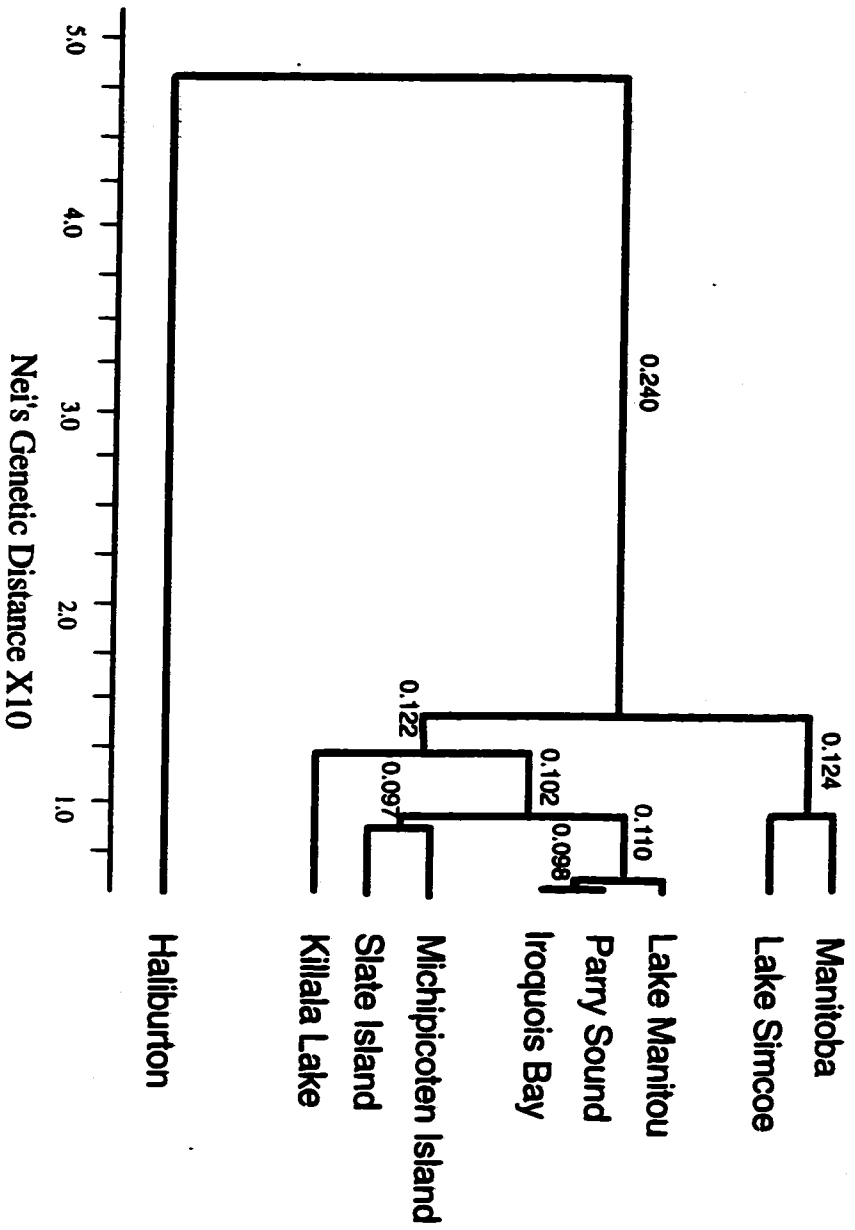
Table 5.4 Level of significance (α) of pairwise tests for heterogeneity between all pairs of nine stocks of lake trout, using Bonferroni (Rice 1989) correction. Values above the diagonal are for Sfo8 and the values below the diagonal are for Sfo12.

Stock	Manitoba	Simcoe	Manitou	Parry	Iroquois	Michi	Slate	Killala	Haliburton
Manitoba	-	nsd	nsd	+++	+	nsd	nsd	nsd	nsd
Simcoe	nsd	-	nsd	nsd	nsd	nsd	nsd	nsd	nsd
Manitou	nsd	nsd	-	nsd	+	nsd	nsd	nsd	nsd
Parry	+	nsd	nsd	-	nsd	++	nsd	nsd	+++
Iroquois	+	nsd	nsd	nsd	-	nsd	nsd	+++	nsd
Michi	nsd	nsd	nsd	nsd	nsd	-	nsd	nsd	nsd
Slate	nsd	nsd	nsd	+	nsd	nsd	-	nsd	nsd
Killala	nsd	nsd	nsd	nsd	nsd	nsd	+	-	+
Haliburton	nsd	nsd	+++	++	+	nsd	nsd	+++	-

nsd=no significant difference, α=0.05 +, α=0.01 ++, α=0.001 +++

Table 5.4 continued. Values above the diagonal are for Ssa197 and the values below the diagonal are for Ssa289.

					Stock				
Stock	Manitoba	Simcoe	Manitou	Parry	Iroquois	Michi	Slate	Killala	Haliburton
Manitoba	-	nsd	nsd	nsd	nsd	nsd	nsd	+	+++
Simcoe	nsd	-	nsd	nsd	nsd	+	+	+++	+++
Manitou	nsd	nsd	-	nsd	nsd	nsd	nsd	nsd	+++
Parry	nsd	nsd	nsd	-	nsd	nsd	nsd	nsd	++
Iroquois	nsd	nsd	nsd	nsd	-	nsd	nsd	nsd	+++
Michi	+	nsd	nsd	nsd	nsd	-	nsd	nsd	+++
Slate	nsd	nsd	nsd	nsd	nsd	nsd	-	++	+
Killala	+++	+++	nsd	++	+++	nsd	nsd	-	+++
Haliburton	++	nsd	++	nsd	++	+++	+	+++	-

nsd=no significant difference, α=0.05 +, α=0.01 ++, α=0.001 +++

Discussion

The results show that primer sets created for other salmonid species can be used to amplify microsatellite DNA loci in lake trout. Six of the primer sets tested amplified polymorphic loci with only slight changes in the annealing temperature (Table 5.2). The Sfo-series primers have also been used on other *Salvelinus* species (Angers and Bernatchez 1996). The allele sizes for lake trout observed in the eight fish examined by Angers and Bernatchez (1996) overlap with those observed in the present study (Table 5.2). The allele sizes reported by Angers and Bernatchez (1996) were similar to those reported in the current study. For example, a difference of one base pair between allele sizes at the locus Sfo12 was observed. The difference is probably due to slight differences in the mobility of the M13 sequencing ladder under different laboratory conditions.

The amount of genetic variation detected using microsatellite loci is greater than that detected using allozyme variation. A relatively small number of loci (six) were analyzed, but all were polymorphic. Average heterozygosity (H=66%, over all loci), is higher than that observed in studies of allozyme variation of lake trout (H=4.7%; Ihssen *et al.* 1988). The observed heterozygosity is similar to that reported in analyses of microsatellite DNA variation of other salmonid species (Table 5.5). A study of Atlantic cod (*Gadus morhua*) also revealed high levels of variation (H=86%; Bentzen *et al.* 1996). Lower levels of variation are often reported in marine species as compared to freshwater species (Gyllenste 1985) due to larger population sizes and

Table 5.5 Values of overall observed heterozygosity at microsatellite loci for various fish species.

Species	H_{obs}	Reference
Atlantic salmon	86%	O'Reilly *et al.* 1996
Atlantic salmon	53%	McConnell *et al.* 1995
brook charr	41%	Angers *et al.* 1995
brown trout	48%	Estoup *et al.* 1993
bluegill sunfish (*Lepomis macrochirus*)	53%	Colbourne *et al.* 1996
northern pike (*Esox lucius*)	21%	Miller and Kapuscinski 1996
Atlantic cod	86%	Bentzen *et al.* 1996
lake trout	66%	present study

the lack of geographic barriers to migration observed in a marine environment.

The levels of average heterozygosity per locus for each stock are correlated in studies of both allozyme and microsatellite DNA variation of lake trout. That is, stocks with higher levels of allozyme heterozygosity, also have higher levels of microsatellite heterozygosity. Manitoba and Haliburton lake trout have the highest levels of heterozygosity for both allozyme (7.4% and 6.7% respectively; Ihssen *et al.* 1988) and microsatellite DNA data (Table 5.3), and Lake Manitou and Lake Simcoe are among stocks with the lowest levels (4.0% and 2.7% respectively; Ihssen *et al.* 1988). In both studies the highest levels of heterozygosity are observed in stocks that come directly from the wild, while the stocks with the lowest levels come from hatchery stocks. Some erosion of genetic variation may have resulted due to a reduction of population size or as a result of hatchery practices. The rate of loss of heterozygosity per generation depends on the size and average age of the breeding population (Lande and Barrowclough 1987). The Lake Manitou stock is one of the oldest stocks of lake trout maintained by the OMNR. Since 1947, the native population in Lake Manitou has been supplemented by annual stocking of hatchery-reared lake trout (Anonymous 1992). One million eggs have been collected each year since 1959 to produce lake trout for the OMNR's stocking program, and although lake trout have been stocked back into Lake Manitou the number of individuals used to found each new generation may not have been sufficient to maintain levels of heterozygosity. A similar situation is observed in Lake Simcoe, which has also been

supplemented with hatchery-reared lake trout since the 1950's (Anonymous 1992). In contrast, the hatchery stock from the Slate Islands has higher levels of heterozygosity. The Slate Islands stock was founded more recently (1980) and is maintained within two hatcheries as a broodstock, rather than in the wild, and is periodically supplemented by wild stock. Higher levels of heterozygosity may be observed currently because not enough time has elapsed for any reduction in population size to have affected heterozygosity, or current hatchery practices used to maintain the Slate Islands stock (*ie.* a hatchery-maintained broodstock) may be more effective in maintaining levels of heterozygosity. Alternatively, lower levels of variation may have always existed in Lake Simcoe and Lake Manitou compared to the Slate Islands. Less genetic variability was observed in stocks from small lakes (eg. Lake Manitou and Lake Simcoe) compared with stocks from larger lakes (the Slate Islands are in Lake Superior) in a study of allozyme variation (Ihssen *et al.* 1988). Larger lakes may have larger populations of lake trout than smaller lakes, and may have larger gene pools which results in a higher level of heterozygosity. A periodic assessment of hatchery stocks may help to determine if indeed genetic variation is lost in the future.

Genetic distances observed among hatchery stocks at microsatellite loci are closer to those reported in an allozyme study by Krueger *et al.* (1989) than to those reported by Ihssen *et al.* (1988). The calculations for genetic distance for microsatellite loci and in Krueger *et al.* (1989) were based only on data for polymorphic loci, while in Ihssen *et al.* (1988) 11 monomorphic loci were also

considered, which would decrease the distances.

Allozyme and mtDNA variation have been used to examine the phylogenetic origins of lake trout. Changes in allele and haplotype frequencies from the northwest to the southeast of Ontario may be a reflection of the colonization history of lake trout in the Great Lakes region. Lake trout sampled in previous studies (Ihssen *et al.* 1988; Grewe and Hebert 1988; Wilson and Hebert 1996) fall into three geographic groupings, northwestern Ontario and Manitoba, the upper Great Lakes, and southeastern Ontario. Microsatellite variation indicates that samples group by lake of origin (Figure 5.2); sympatric stocks are more similar than allopatric stocks. The groups generated using microsatellite data may be a reflection of more recent demographic events (*ie.* sampling of wild stocks in order to establish a hatchery broodstock) since microsatellite loci evolve at a faster rate than both allozyme loci and mtDNA (Wright and Bentzen 1995).

Lake trout from the Haliburton region are distinct for allozyme (Ihssen *et al.* 1988) and microsatellite DNA variation. Haliburton lake trout also display differences in morphology and life history traits (Krueger and Ihssen 1995) making them a unique stock that may require special considerations for their management. Haliburton lake trout are believed to be the descendants of lake trout that survived in the Haliburton Highlands during an interglacial period, and genetic differences may have been magnified by more recent demographic processes, like genetic drift.

Lake Simcoe and Manitoba samples are closely grouped in the dendrogram

based on microsatellite data (Figure 5.2). The relatively small genetic distance

between the two stocks is not observed in other studies. The results of earlier

allozyme (Ihssen et al. 1988) and mtDNA studies (Chapter 2) show that the Manitoba

lake trout are more similar to samples from the upper Great Lakes than to Lake

Simcoe samples, whereas another mtDNA study (Grewe and Hebert 1988) and the

current study observed a closer grouping of the two stocks. Since the 1950's the Lake

Simcoe lake trout population has been maintained by planting the lake with hatchery-

reared fish that are marked with fin clips before they are stocked. Each new

generation of Lake Simcoe lake trout is created from recaptured fish that are identified

as Lake Simcoe lake trout using the fin clips. Spawn is taken from the recaptured

Simcoe lake trout and used to make crosses which are reared in a hatchery and then

released into Lake Simcoe. Also, lake trout from Manitoba (Clearwater Lake) were

stocked into Lake Simcoe for a period of about ten years starting in 1974. The close

grouping of Manitoba and Lake Simcoe lake trout may be the result of an

introgression of Manitoba alleles into the Lake Simcoe stock which may have occurred

through natural reproduction. Manitoba lake trout have been observed on spawning

shoals in Lake Simcoe, but there is little direct evidence of natural reproduction

(MacLean et al. 1981; Evans and Willox 1991). Alternatively, the fin clips of wild

caught Manitoba fish in Lake Simcoe may have been misread or may have

regenerated, causing Manitoba lake trout to be mistaken for Lake Simcoe lake trout

and consequently used in the spawn collection, or as samples for genetic studies. It is

interesting to note that the dendrogram pairings have changed over time. The allozyme samples were taken in 1981, 1982, and 1983 (Ihssen *et al.* 1988) and one set of samples for the study of mtDNA variation were collected in 1981 and 1982 (Chapter 2). In both studies, the Manitoba lake trout are more similar to samples from the upper Great Lakes than to Lake Simcoe samples. Grewe and Hebert (1988) observed a closer grouping of the two stocks using samples collected in 1986, and the samples for the present study came from the Chatsworth Fish Culture Station in 1991. Contamination of the Lake Simcoe hatchery stock has also been observed in recent allozyme surveys of hatchery fish (B. Martin, STAG Unit, Ontario Ministry of Natural Resources, personal communication). Over time, the gradual addition of Manitoba lake trout to the population may have caused a change in allele frequencies.

Both mtDNA and allozyme data have been employed to identify recaptured and naturalized lake trout (Grewe *et al.* 1994). Such analyses are limited to just a few stocks however, such as the Lake Superior lake trout which are characterized by high frequencies of two mtDNA haplotypes (Grewe *et al.* 1993). MtDNA variation alone has been used to identify recaptured lake trout (Chapter 2) of Killala Lake, Lake Manitou, and Haliburton origin with a high error rate. In addition, mtDNA variation used alone will identify only the maternal contribution to the next generation, since the molecule is only passed on by females in most vertebrates. It is important to be able to track the success of both sexes in rehabilitation programs. For example, a study of Algonquin Park brook charr (P. Ihssen, OMNR, unpublished data) shows that there are

differences in survival of stocked males vs. stocked female brook charr.

The genetic markers observed in the present study may prove useful for the mixed-stock analysis of naturalized lake trout in rehabilitated areas using a mixed-stock analysis. Additional microsatellite loci could be assessed by developing lake trout-specific microsatellite primers or by testing primers designed for other salmonid species, such as rainbow trout (*Oncorhynchus mykiss*). One advantage of microsatellite DNA analysis, or any PCR-based technique is that non-lethal sampling techniques may be used to analyze recaptured samples. Microsatellite DNA markers will also be useful for further phylogenetic studies of wild lake trout in North America. Unlike RAPD loci (Chapter 4), the geographic relationships observed among lake trout stocks using microsatellite loci are similar to those calculated using mtDNA or allozymes.

Acknowledgements

References

Anonymous. 1992. Stocks Catalogue: handbook of stocks available in the Provincial Fish Culture System. Fisn Culture Section, Great Lakes Branch, Operations Division, Ontario Ministry of Natural Resources. 225pp.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. J. Fish Biology. 47A:177-185.

Angers, B. and L. Bernatchez. 1996. Usefulness of heterologous microsatellites obtained from brook charr, *Salvelinus fontinalis* Mitchill, in other *Salvelinus* species. Molecular Ecology. 5:317-319.

Bentzen, P., C. T. Taggart, D. E. Ruzzante, and D. Cook. 1996. Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 53:2706-2721.

Colbourne, J. K., B. D. Neff, J. M. Wright, and M. R. Gross. 1996. DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using $(GT)^n$ microsatellites and its potential for assessment of mating success. Can. J. Fish. Aquat. Sci. 53:342-349.

DeWoody, J. A., R. L. Honeycutt, and L. C. Skow. 1995. Microsatellite markers in white-tailed deer. J. Heredity. 86:317-319.

Dowling, T. E., C. Moritz, J. D. Palmer, and L. H. Rieseberg. 1996. Nucleic acids III: analysis of fragments and restriction sites. In Molecular Systematics. D. M. Hillis, C. Moritz, and B. K. Mable (eds.) pgs. 249-320. Sinauer Assoc. Inc. Massachusetts, USA.

Ellegren, H. 1992. Polymerase-chain reaction (PCR) analysis of microsatellites- a new approach to studies of genetic relationships in birds. The Auk. 109:886-895.

Estoup, A. P. Presa, F. Krieg, D. Vaiman, and R. Guyomard. 1993. $(CT)^n$ and $(GT)^n$ microsatellites: a new class of genetic markers for *Salmo trutta* L. (brown trout). Heredity. 71:488-496.

Evans, D. O. and C. C. Willox. 1991. Loss of exploited, indigenous populations of lake trout, *Salvelinus namaycush*, by stocking of non-native stocks. Can. J. Fish. Aquat. Sci. 48(Suppl. 1):134-147.

Grewe, P. M. and P. D. N. Hebert. 1988. Mitochondrial DNA diversity among broodstocks of the lake trout, *Salvelinus namaycush*. Can. J. Fish. Aquat. Sci. 45:2114-2122.

Grewe, P. M., C. C. Krueger, C. F. Aquadro, E. Bermingham, H. L. Kincaid, and B. May. 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush* strains stocked into Lake Ontario. Can. J. Fish. Aquat. Sci. 50:2397-2403.

Grewe, P. M., C. C. Krueger, J. E. Marsden, C. F. Aquadro, and B. May. 1994. Hatchery origins of naturally produced lake trout fry captured in Lake Ontario: temporal and spatial variability based on allozyme and mitochondrial DNA data. Trans. Amer. Fish. Soc. 123:309-320.

Guo, S. W. and E. A. Thompson. 1992. Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372.

Gyllensten, U. 1985. The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. J. Fish Biol. 26:691-699.

Hughes, C. R. and D. C. Queller. 1993. Detection of highly polymorphic microsatellite loci in a species with little allozyme variation. Molecular Ecology. 2:131-137.

Ihssen, P. E., J. M. Casselman, G. W. Martin, and R. B. Phillips. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. Can. J. Fish. Aquat. Sci. 45:1018-1029.

Krueger, C. C., J. E. Marsden, H. L. Kincaid, and B. May. 1989. Genetic differentiation among lake trout strains stocked into Lake Ontario. Trans. Amer. Fish. Soc. 118:317-330.

Krueger, C. C. and P. E. Ihssen. 1995. Review of genetics of lake trout in the Great Lakes: history, molecular genetics, physiology, strain comparisons, and restoration management. J. Great Lakes Res. 21(Supp. 1):348-363.

Lande, R. and G. F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. In Viable Populations for Conservation. M. E. Soulé (ed.) pg.87-123. Cambridge University Press, Cambridge, England.

MacLean, J. A., D. O. Evans, N. V. Martin, and R. L. DesJardine. 1981. Survival, growth, spawning distribution, and movements of introduced and native lake trout (*Salvelinus namaycush*) in two inland Ontario lakes. Can. J. Fish. Aquat. Sci. 38:1685-1700.

McConnell, S., P. O'Reilly, L. Hamilton, J. M. Wright, and P. Bentzen. 1995. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. Can. J. Fish. Aquat. Sci. 52:1863-1872.

Miller, L. M. and A. R. Kapuscinski. 1996. Microsatellite DNA markers reveal new levels of genetic variation in northern pike. Trans. Amer. Fish. Soc. 125:971-977.

Moore, S. S., L. L. Sargeant, T. J. King, J. S. Mattick, M. Georges, and D. J. S. Hetzel. 1991. The conservation of dinucleotide microsatellites among mammalian genomes allows the use of heterologous PCR primer pairs in closely related species. Genomics 10:654-660.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106:283-292.

O'Reilly, P., L. C. Hamilton, S. K. McConnell, and J. M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. 53:2292-2298.

Paetkau, D. and C. Strobeck. 1994. Microsatellite analysis of genetic variation in black bear populations. Molecular Ecology 3:489-495.

Pepin, L., Y. Amigues, A. Lepingle, J.L. Berthier, A. Bensaid, and D. Vaiman. 1995. Sequence conservation of microsatellites between *Bos taurus* (cattle), *Capra hircus* (goat) and related species. Examples of use in parentage testing and phylogeny analysis. Heredity. 74:53-61.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): a population genetics software for exact tests and ecumenicism. J. Heredity. 86:248-249.

Rice, W. R. 1989. Analyzing tables of statistical tests. Evolution. 43:223-225.

Saghai-Maroof M. A. K., M. Soliman, R. A. Jorgensen, and R. W. Allard. 1984. Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance,

chromosomal location and population dynamics. Proc. Natl. Acad. Sci. USA. 81:8014-8018.

Taylor, A. C., W. B. Sherwin, and R. K. Wayne. 1994. Genetic variation of microsatellite loci in a bottlenecked species: the northern hairy-nosed wombat *Lasiorhinus krefftii*. Molecular Ecology. 3:277-290.

Wilson, C. C. and P. D. N. Hebert. 1996. Phylogenetic origins of lake trout (*Salvelinus namaycush*) in eastern North America. Can. J. Fish. Aquat. Sci. 53:2764-2775.

Wright, J. M. and P. Bentzen. 1995. Microsatellites: genetic markers for the future. In Molecular Genetics in Fisheries. G. R. Carvalho and T. J. Pitcher (eds.) pgs. 117-121. Chapman and Hall, London.

Appendix 1: Allelic frequencies for the six microsatellite loci examined in lake trout stocks in Ontario and Manitoba.

Table 5.6 Frequencies of alleles at microsatellite locus Sfo12 in lake trout

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L. Manitou	Iroquois Bay
254	0.075	0.026	0.250	0.000	0.225	0.167	0.025	0.024	0.000
256	0.575	0.869	0.714	0.950	0.650	0.762	0.925	0.952	0.905
258	0.350	0.105	0.036	0.050	0.125	0.071	0.000	0.024	0.095
260	0.000	0.000	0.000	0.000	0.000	0.000	0.050	0.000	0.000

Table 5.7 Frequencies of alleles at microsatellite locus Sfo23 in lake trout.

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L. Manitou	Iroquois Bay
186	0.025	0.000	0.078	0.000	0.000	0.000	0.026	0.000	0.024
190	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.000
192	0.100	0.059	0.000	0.025	0.000	0.158	0.053	0.190	0.000
194	0.250	0.000	0.000	0.125	0.000	0.079	0.000	0.000	0.000
196	0.000	0.029	0.000	0.000	0.000	0.053	0.000	0.260	0.000
198	0.200	0.059	0.000	0.200	0.025	0.000	0.000	0.024	0.095
200	0.000	0.000	0.000	0.050	0.050	0.105	0.053	0.000	0.000
202	0.000	0.000	0.000	0.050	0.000	0.316	0.026	0.000	0.048
204	0.075	0.000	0.000	0.000	0.000	0.000	0.026	0.048	0.048
206	0.025	0.118	0.000	0.000	0.025	0.000	0.000	0.000	0.071
208	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.048	0.048
210	0.000	0.147	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.000	0.088	0.000	0.000	0.100	0.026	0.105	0.000	0.000
214	0.000	0.118	0.154	0.100	0.050	0.000	0.026	0.024	0.071
216	0.100	0.000	0.038	0.000	0.200	0.000	0.000	0.000	0.071
218	0.000	0.000	0.154	0.050	0.000	0.000	0.105	0.024	0.095

Table 5.7 continued.

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L. Manitou	Iroquois Bay
220	0.050	0.088	0.115	0.150	0.150	0.026	0.080	0.024	0.048
222	0.025	0.000	0.000	0.125	0.100	0.053	0.053	0.095	0.048
224	0.100	0.206	0.000	0.075	0.100	0.026	0.080	0.119	0.095
226	0.000	0.088	0.000	0.000	0.000	0.000	0.105	0.000	0.000
228	0.000	0.000	0.000	0.025	0.025	0.000	0.000	0.000	0.000
230	0.000	0.000	0.000	0.000	0.075	0.026	0.105	0.048	0.000
232	0.000	0.000	0.000	0.000	0.000	0.000	0.026	0.024	0.000
234	0.000	0.000	0.346	0.025	0.000	0.079	0.026	0.000	0.166
236	0.000	0.000	0.077	0.000	0.025	0.053	0.105	0.024	0.048
238	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024
242	0.000	0.000	0.038	0.000	0.075	0.000	0.000	0.000	0.000

Table 5.8 Frequencies of alleles at microsatellite locus Sfo18 in lake trout

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L. Manitou	Iroquois Bay
170	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.050
172	0.125	0.446	0.654	0.650	0.425	0.725	0.955	0.548	0.475
174	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.048	0.150
176	0.000	0.000	0.000	0.025	0.000	0.000	0.045	0.048	0.000
178	0.000	0.000	0.000	0.125	0.000	0.000	0.000	0.000	0.025
180	0.050	0.053	0.000	0.025	0.000	0.000	0.000	0.000	0.000
182	0.500	0.237	0.269	0.025	0.175	0.125	0.000	0.095	0.050
184	0.075	0.132	0.077	0.075	0.150	0.025	0.000	0.238	0.050
186	0.200	0.079	0.000	0.000	0.025	0.025	0.000	0.000	0.000
188	0.050	0.053	0.000	0.075	0.175	0.000	0.000	0.000	0.125
190	0.000	0.000	0.000	0.000	0.050	0.000	0.000	0.000	0.075
194	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.023	0.000

Table 5.9 Frequencies of alleles at microsatellite locus Ssa289 in lake trout

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L. Manitou	Iroquois Bay
172	0.026	0.000	0.179	0.000	0.000	0.075	0.000	0.024	0.000
176	0.026	0.000	0.071	0.000	0.075	0.075	0.000	0.071	0.000
178	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000
180	0.079	0.079	0.000	0.025	0.050	0.050	0.000	0.048	0.028
182	0.158	0.026	0.107	0.050	0.050	0.200	0.000	0.095	0.194
184	0.316	0.237	0.215	0.300	0.300	0.275	0.083	0.166	0.250
186	0.000	0.053	0.000	0.025	0.025	0.050	0.083	0.095	0.111
188	0.000	0.316	0.107	0.125	0.225	0.150	0.332	0.261	0.139
190	0.000	0.000	0.036	0.000	0.000	0.025	0.000	0.024	0.000
192	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.024	0.000
194	0.000	0.000	0.107	0.100	0.025	0.000	0.000	0.000	0.028
196	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.048	0.083
198	0.000	0.026	0.071	0.075	0.025	0.000	0.028	0.000	0.028
200	0.000	0.000	0.000	0.000	0.100	0.050	0.028	0.048	0.000
202	0.000	0.053	0.071	0.000	0.000	0.000	0.056	0.000	0.000
204	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000

Table 5.9 continued.

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L. Manitou	Iroquois Bay
206	0.000	0.000	0.036	0.000	0.025	0.025	0.083	0.000	0.056
208	0.026	0.132	0.000	0.025	0.000	0.000	0.000	0.000	0.000
210	0.054	0.025	0.000	0.000	0.000	0.000	0.000	0.000	0.000
212	0.184	0.053	0.000	0.175	0.100	0.025	0.111	0.048	0.000
214	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
216	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000	0.000
218	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.083
222	0.026	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
224	0.079	0.000	0.000	0.000	0.000	0.000	0.028	0.000	0.000

Table 5.10 Frequencies of alleles at microsatellite locus Ssa197 in lake trout

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L. Manitou	Iroquois Bay
174	0.000	0.000	0.091	0.028	0.027	0.083	0.000	0.000	0.000
182	0.000	0.000	0.091	0.000	0.027	0.000	0.000	0.000	0.000
190	0.025	0.000	0.000	0.000	0.027	0.000	0.000	0.000	0.000
198	0.025	0.053	0.000	0.000	0.000	0.042	0.000	0.000	0.000
202	0.250	0.053	0.045	0.056	0.056	0.000	0.087	0.056	0.000
206	0.300	0.105	0.045	0.056	0.139	0.125	0.022	0.167	0.111
210	0.050	0.026	0.136	0.056	0.167	0.000	0.000	0.000	0.083
214	0.025	0.000	0.000	0.056	0.056	0.042	0.065	0.056	0.056
218	0.025	0.026	0.182	0.111	0.028	0.292	0.000	0.056	0.028
222	0.000	0.105	0.000	0.000	0.083	0.042	0.043	0.139	0.083
226	0.000	0.000	0.045	0.056	0.028	0.207	0.174	0.194	0.167
230	0.000	0.026	0.000	0.139	0.028	0.000	0.130	0.056	0.222
234	0.000	0.105	0.046	0.167	0.083	0.000	0.109	0.139	0.000
238	0.000	0.053	0.046	0.027	0.056	0.000	0.000	0.028	0.083
242	0.000	0.000	0.046	0.027	0.000	0.000	0.022	0.000	0.000
246	0.000	0.079	0.046	0.000	0.028	0.042	0.000	0.000	0.028

Table 5.10 continued.

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L, Manitou	Iroquois Bay
250	0.125	0.079	0.000	0.139	0.000	0.083	0.174	0.000	0.083
254	0.025	0.237	0.136	0.027	0.000	0.042	0.152	0.028	0.056
258	0.075	0.027	0.045	0.055	0.111	0.000	0.022	0.055	0.000
262	0.075	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
266	0.000	0.026	0.000	0.000	0.028	0.000	0.000	0.028	0.000
270	0.000	0.000	0.000	0.000	0.028	0.000	0.000	0.000	0.000

Table 5.11 Frequencies of alleles at microsatellite locus Sfo8 in lake trout

Allele	Haliburton	Michipicoten Is.	Manitoba	Parry Sound	Slate Is.	L. Simcoe	Killala L.	L. Manitou	Iroquois Bay
266	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000
268	0.000	0.000	0.078	0.000	0.025	0.000	0.088	0.000	0.000
272	0.000	0.056	0.038	0.000	0.000	0.000	0.000	0.024	0.024
274	0.000	0.139	0.000	0.050	0.050	0.139	0.059	0.000	0.048
276	0.050	0.000	0.154	0.000	0.025	0.028	0.118	0.024	0.024
278	0.025	0.056	0.000	0.050	0.000	0.028	0.000	0.024	0.000
280	0.050	0.056	0.000	0.050	0.025	0.055	0.000	0.000	0.071
282	0.025	0.056	0.038	0.325	0.125	0.083	0.059	0.167	0.190
284	0.100	0.167	0.269	0.050	0.025	0.167	0.119	0.024	0.095
286	0.100	0.110	0.115	0.000	0.025	0.111	0.029	0.095	0.024
288	0.050	0.110	0.000	0.125	0.150	0.000	0.088	0.143	0.095
290	0.300	0.083	0.115	0.075	0.200	0.194	0.059	0.095	0.381
292	0.025	0.111	0.000	0.000	0.025	0.000	0.029	0.071	0.000
294	0.100	0.056	0.115	0.175	0.200	0.167	0.235	0.190	0.048
296	0.175	0.000	0.000	0.000	0.000	0.000	0.000	0.024	0.000
298	0.000	0.000	0.078	0.100	0.125	0.028	0.088	0.119	0.000

Chapter 6

Estimation of origins of mixed-stock samples of lake trout (*Salvelinus namaycush*) using allozyme and microsatellite DNA data: a simulation analysis.

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Abstract

Simulated data were used to compare estimated and actual values obtained from a maximum likelihood estimator employed in mixed-stock analysis of lake trout. Allele frequency data for allozyme and microsatellite DNA loci from five lake trout hatchery stocks were used to simulate genotype frequencies in a sample of mixed hatchery origin under five different stocking regimes. Several factors were examined for their effects on the difference between predicted and actual values. The factors were: mixed-stock sample size, genetic differentiation among stocks, number of loci, and the number of alleles (and therefore genotypes).

Larger mixed-sample sizes decreased the variance of the estimates, as might be expected, but did not always increase the accuracy. The number and frequency of genotypes and the number of baseline stocks had the greatest effect on the accuracy of the estimate. Loci with more than three alleles, like the microsatellite DNA loci, gave better estimates, since there were more genotypes to characterize a hatchery stock. If a locus in the baseline stock had rare alleles (5% or less) in one or more of the baseline stocks, the accuracy was reduced. Loci with few alleles, like the allozyme loci, gave accurate estimates if the locus was fixed for the most common allele in most of the baseline stocks. An increased number of baseline stocks reduced the accuracy, because an increase in the number of baseline stocks means that each contributes a smaller proportion and the estimator becomes less accurate as actual proportions approach zero.

The maximum likelihood estimator will be a valuable tool for analyzing the success of Ontario lake trout rehabilitation programs but loci must be chosen carefully to ensure that estimates of proportional contributions of donor stocks are as accurate as possible. The current study indicates that overall, microsatellite loci produced better estimates than mitochondrial DNA, allozymes, and RAPD loci because more alleles per locus are detected with the technique. Compared to microsatellite DNA loci, stock composition estimates generated with allozyme loci are less accurate because few alleles (three or less) are detected at the allozyme loci screened to date in lake trout hatchery stocks.

Introduction

A variety of methods are used to rehabilitate fish populations. A species may be restored to an area by stocking multiple hatchery strains in the hope that at least one will survive. For example, six different hatchery stocks of lake trout (*Salvelinus namaycush*; Clearwater Lake, Jenny Lake, Killala Lake, Lake Manitou, Seneca Lake, and Lake Superior) have been stocked into Lake Ontario since 1973 as part of a cooperative effort between Canada and the United States to restore lake trout (Marsden *et al.* 1993). The relative survival of each hatchery strain may be monitored by examining fin clips, coded wire tags, or genetic data. Genetic markers have several advantages over other marking techniques. They are not altered by environmental conditions, therefore standards do not need to be readjusted on a regular basis. In contrast to physical tags or marks, genetic markers occur naturally in both wild and hatchery samples and cannot be removed or changed. Genetic markers can be measured at all stages of a fish's life cycle, and the frequencies of the markers are fairly stable over time (Fournier *et al.* 1984). Relative reproductive success can only be evaluated using genetic characters to discriminate among strains which contribute as parents to fry production.

Mixed stock analysis (MSA) of genetic data is used to determine the hatchery origins of individuals in a sample that contains fish from two or more hatchery stocks. Some stocks may be characterized by allele and genotype frequencies, but since many genotypes are common to all stocks, not all fish can be classified with absolute

certainty. However, for many management purposes it is not necessary to identify every single individual (Pella and Milner 1987). MSA has become a standard technique to determine the proportions of fish stocks that contribute to, for example, commercial harvests of sockeye salmon (*Oncorhynchus nerka*; Millar 1990; Grant *et al.* 1980).

One statistical procedure used to determine the proportion of stocks contributing to a mixed sample is a maximum likelihood estimator (MLE) designed for genetic data (Millar 1987). The estimator maximizes an algorithm that describes the probability of a genotype occurring in both a baseline (eg. hatchery) stock and in a mixed-sample of fish that is assumed to contain individuals from each of the baseline stocks. The MLE procedure allocates genotypes from the mixed-sample to a baseline stock in proportions similar to the probability that each genotype originated from the baseline stock. In other words, the estimate of the contribution of each stock is based on the joint probability of genotypes occurring in both the mixed-sample and baseline stocks. The EM algorithm of Dempster *et al.* (1977) was used to estimate θ, the proportion of fish in a mixed-stock sample:

$$\theta^{*}_{j} = \Sigma_{i}^{g} \frac{Y_{i}X_{ij}\theta_{j}}{(\Sigma_{i}^{g}Y_{i})(\Sigma_{j}^{n}X_{ij}\theta_{j})},$$

where g is the number of genotypes, n is the number of baseline stocks (eg. spawning areas or hatchery stocks), Y_{i} is the number of fish in a mixed-stock sample having the *ith* genotype, X_{ij} is an estimate of the *ith* genotype in the *jth* baseline stock. To start

the calculation, θ_j is set equal for all baseline stocks and the θ^*_j of the result is inserted into the equation and the equation is solved again. The iteration is continued until the values of θ^*_j differ by less than some value (eg. 0.001).

Past studies have shown that the MLE performs better than other types of estimators (Millar 1990), but several factors may introduce error. The amount of genetic divergence among the potentially contributing stocks and whether or not all baseline stocks are known can bias an estimate. The actual composition of the mixture and reliability of the estimates of genotypes in the mixed-stock and the contributing hatchery stocks can also affect estimates. In a study of sockeye salmon from three rivers in Cook Inlet, Alaska the classification accuracy was 93% for the Kasilof River, 93% for the Kenai River, and 33% for the Susitna River (Grant et al. 1980). However, contributions from stocks that were rare or did not contribute were overestimated, unless sample sizes were very large. Suggestions were made to help correct bias after analyses were performed but no guidelines for selecting genetic markers a priori were given (Pella and Milner 1987).

The estimator has also been used to analyze naturalized lake trout samples captured from Lake Ontario (Marsden et al. 1989; Grewe et al. 1994a; Grewe et al. 1994b), but sample sizes were smaller (N=80 on average; Grewe et al. 1994a; Grewe et al. 1994b; Krueger et al. 1989) than those used in previous analyses using the MLE (eg. N=130 on average; Grant et al. 1980). In general, sample sizes used in studies of landlocked species tend to be lower because the samples available for baseline

frequency calculations and the sizes of the mixed samples tend to be smaller, especially if the recaptured fish are part of a recently established population. For example, three stocks of walleye were used to rehabilitate Nipigon Bay, Lake Superior, and to date very few naturally produced walleye (N<10) have been captured (Ihssen *et al.* 1993; Ihssen, unpublished data).

In addition, sometimes the loci available for a MSA analysis are not very variable. For example, eight of the loci: ACP-1* (acid phosphatase, E.C. No. 3.1.3.2), G3PDH-1* (glycerol-3-phosphate dehydrogenase, E.C. No. 1.1.1.8), LDH-4* (L-lactate dehydrogenase, E.C. No. 1.1.1.27), sMDH-3,4* (malate dehydrogenase, E.C. No. 1.1.1.37), mMEP-2* (malic enzyme, E.C. No. 1.1.1.40), PGK-1* (phosphoglycerate kinase, E.C. No. 2.7.2.3), PGM-2* (phosphoglucomutase, E.C. No. 5.4.2.2), and SOD* (superoxide dismutase, E.C. No. 1.15.1.1), used to generate estimates of θ^*_j in studies of naturalized lake trout (Marsden *et al.* 1989; Grewe *et al.* 1994a; Grewe *et al.* 1994b) are either fixed for the most common allele in one or more of the hatchery stocks, or the rare allele occurs at a very low frequency (5% or less; Grewe *et al.* 1994a; Grewe *et al.* 1994b; Krueger *et al.* 1989). The extent to which allele frequency data affect estimates of contributions of baseline stocks to a mixed sample is not clear.

It has been suggested that genetic data be tested to determine if they are sufficient to produce useful estimates before they are used in a MSA (Pella and Milner 1987). Baseline data may be tested using simulated mixed-stock samples. Therefore,

in the current study, we examine the effects of mixed-stock sample size, number of loci, number of genotypes and genetic divergence on the MLE for θ^*_j. Baseline sample size and the estimator of variance were not assessed, since they have been discussed in detail elsewhere (Pella and Milner 1987; Millar 1987). Simulation results based on allozyme data (Ihssen *et al.* 1988) and microsatellite DNA data (Chapter 4) for Ontario lake trout hatchery stocks are used to assess the estimator. In addition, data reported in studies of naturalized lake trout from Lake Ontario provide allele frequency estimates and stocking proportions (Grewe *et al.* 1994a & b; Krueger *et al.* 1989).

Materials and Methods

Five sets of data were simulated to represent the allele frequencies at allozyme and microsatellite DNA loci in a sample of lake trout of different hatchery origins. The MLE described above was used to estimate θ^*_j. Calculations were iterated until values of θ^*_j differed by less than 0.001. Convergence was verified, since it does not always occur. In test runs it took 70 to 90 iterations of the estimation procedure for the estimates to converge at one value. If the estimate did not converge at a value then the simulation was not included in the calculation of averages. Values of θ^*_j were set as equal to start the iterations.

The baseline data for the first four simulations were taken from genetic data for hatchery stocks from Ontario Ministry of Natural Resource (OMNR) hatcheries (Lake

Manitou, Killala Lake, the Slate Islands, Michipicoten Island, and Clearwater Lake,

Manitoba). Allele frequency data from seven allozyme (Ihssen *et al.* 1988; AAT-1,2*

(aspartate aminotransferase, E.C. No. 2.6.1.1), GPI-1* (glucose-6-phosphate isomerase,

E.C. No. 5.3.1.9), LDH-4*, MDH-3,4*, MEP-1*, MUP* (4-methylumbelliferyl

phosphatase, E.C. No. 3.1.3.2), and SOD*) and six microsatellite DNA loci (Chapter

5; Sfo8, Sfo12, Sfo18, Sfo23, Ssa197, and Ssa289) were used to simulate the allele

frequencies that would be observed in a mixed-sample of lake trout. The allele

frequencies in each baseline stock and the actual proportional contribution of each

stock were used as probabilities to determine how many fish of each genotype were in

the mixed-sample. The simulation program allows the proportional contribution of

each baseline stock to be altered. Three sets of proportions were used to determine

how the estimator performs as proportions move away from equality (Table 6.1). For

each simulation it was assumed that all the stocks contributing to the mixed-sample

were known.

The effect of mixed-sample size on accuracy was examined by altering the

number of fish in the mixed-stock sample. In the first three sets of simulations, three

different sample sizes were used (N=20, N=60, and N=100; Table 6.1). The three

stocks chosen as baseline stocks are actually used in rehabilitation programs. Lake

Manitou and Killala Lake hatchery lake trout are most often stocked in Ontario by

OMNR and the Clearwater Lake, Manitoba stock was used regularly in the past. The

Clearwater Lake stock was used in order to study the effect of genetic distance. The

Table 6.1 Actual values of the proportional contribution of each stock for simulations performed in the study using Ontario hatchery stocks and stocks of fish stocked into Lake Ontario.

Simulation number		Stocks used	

Simulation number	Killala L.	L. Manitou	Clearwater L.
1	0.333	0.333	0.333
2	0.425	0.425	0.150
3	0.600	0.300	0.100

	Killala L.	L. Manitou	Clearwater L.	Slate Is.	Michipicoten Is.
4	0.300	0.300	0.150	0.100	0.150

	Seneca	L. Manitou	Jenny L.	Killala L.	Clearwater L.	L. Superior
5	0.140	0.110	0.030	0.150	0.150	0.420

genetic distance between Clearwater Lake and each of the other stocks is relatively

large compared to the distances between the two other hatchery stocks (Ihssen *et al.*

1988; Chapter 5). The fourth type of simulation was performed using five stocks

(Lake Manitou, Killala Lake, Clearwater Lake, the Slate Islands, and Michipicoten

Island; N=60) to determine how the accuracy of the estimator is affected as more

baseline stocks contribute to the mixed-sample (Table 6.1). The Slate Islands and

Michipicoten Island stocks are most often used in Lake Superior stocking programs.

Therefore, the fourth simulation is based on data that represent the bulk of the lake

trout stocks found in OMNR's Fish Culture Stations. The fifth simulation was based

on allozyme data recorded in studies of naturally produced lake trout in Lake Ontario

(Grewe *et al.* 1994a; Grewe *et al.* 1994b; Krueger *et al.* 1989). The baseline allele

frequencies for 13 loci (sAAT-1,2*, ACP-1*, FH-1,2* (fumarate hydratase, E.C. No.

4.2.1.2), G3PDH-1*, GPI-1*, LDH-4*, sMDH-3,4*, mMEP-2*, PEPD-1,2* (proline

dipeptidase E.C. No. 3.4.13.9), PGK-1*, and PGM-2*) from six hatchery stocks were

used to simulate allele frequencies in a mixed-sample. The proportional contribution

of each stock was equivalent to the proportion of each stock planted into Lake Ontario

(Marsden *et al.* 1993, their Figure 4; Grewe *et al.* 1994a, their Table 1). Stocking

densities may not be a direct indicator of the relative survival of each stock, but give a

starting point for the simulation study that has a realistic base. The sample size taken

(N=80) for the mixed stock sample is similar to that used in the studies of naturally

produced lake trout in Lake Ontario.

Each of the above simulations was run 100 times for each locus, the average

and standard deviation of each set of 100 simulations were calculated for each locus,

each genetic marker, and over all loci. The difference between the actual and

estimated value for all the donor stocks was used as a measure of the accuracy of the

estimate. The standard deviation of the average of the 100 simulations is used to

measure how much variation was associated with the estimate for each set of

conditions.

Results

In total, 3900 simulations were run for each of the first three sets of simulation

conditions, 1300 for the fourth, and 1300 for the Lake Ontario data set (simulation #5,

Table 6.1). Convergence of estimates occurred at all loci except Sfo23 in simulation

#4, therefore the average estimate in that simulation is based on only the repetitions of

the simulation conditions that did produce an estimate. Differences between the actual

and estimated values vary widely for each locus and set of simulation conditions

(Tables 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, and 6.8). The standard deviations of each set of

100 simulations tend to decrease with a larger sample size, but the percent estimate

correct does not always increase as sample size increases and, in general, the

microsatellite DNA loci tend to give more accurate estimates at all sample sizes

(Tables 6.2 to 6.9). Estimates of proportional contribution averaged over all allozyme

loci, all microsatellite DNA loci, and over all loci were also calculated. The accuracy

Table 6.2 The average estimates (AV) and standard deviations (SD) of θ^*_j over 100 replicates of simulation #1 for three stocks of lake trout.

Locus	Sample size	Stock (actual contribution)					
		Killala L. (0.333)		L. Manitou (0.333)		Clearwater L. (0.333)	
		AV	SD	AV	SD	AV	SD
sAAT-1,2*	20	0.080	0.023	0.781	0.077	0.139	0.031
	60	0.030	0.003	0.904	0.017	0.066	0.009
	100	0.030	0.002	0.901	0.009	0.069	0.004
GPI-1*	20	0.529	0.005	0.427	0.001	0.044	0.011
	60	0.533	0.001	0.431	0.000	0.036	0.002
	100	0.530	0.001	0.430	0.000	0.040	0.001
LDH-4*	20	0.326	0.042	0.293	0.021	0.381	0.121
	60	0.318	0.018	0.307	0.010	0.375	0.054
	100	0.335	0.013	0.325	0.006	0.340	0.036
sMDH-3,4*	20	0.143	0.028	0.704	0.139	0.153	0.042
	60	0.117	0.012	0.778	0.050	0.105	0.012
	100	0.359	0.013	0.303	0.038	0.338	0.007
MEP-1*	20	0.320	0.025	0.315	0.138	0.365	0.046
	60	0.343	0.013	0.333	0.067	0.324	0.024
	100	0.331	0.008	0.324	0.044	0.345	0.015
MUP*	20	0.278	0.015	0.319	0.079	0.403	0.077
	60	0.329	0.005	0.237	0.030	0.434	0.032
	100	0.337	0.004	0.256	0.020	0.407	0.020
SOD*	20	0.311	0.024	0.264	0.027	0.425	0.075
	60	0.338	0.010	0.267	0.009	0.395	0.030
	100	0.342	0.006	0.262	0.005	0.396	0.017
TOTAL	20	0.287	0.040	0.436	0.105	0.277	0.079
	60	0.287	0.032	0.462	0.088	0.248	0.048
	100	0.324	0.025	0.400	0.062	0.276	0.035

Table 6.3 Average estimates (AV) and standard deviations (SD) over 100 replicates of the simulation for each of the loci scored in three stocks of lake trout. Results are for simulation #2.

Locus	Sample size	Killala L. (0.425) AV	SD	L. Manitou (0.425) AV	SD	Clearwater L. (0.425) AV	SD
sAAT-1,2*	20	0.031	0.039	0.906	0.013	0.063	0.006
	60	0.067	0.018	0.817	0.064	0.116	0.028
	100	0.030	0.002	0.912	0.006	0.058	0.002
GPI-1*	20	0.559	0.000	0.441	0.000	0.000	0.000
	60	0.559	0.000	0.441	0.000	0.000	0.000
	100	0.552	0.000	0.439	0.000	0.009	0.000
LDH-4*	20	0.467	0.027	0.387	0.012	0.146	0.075
	60	0.430	0.012	0.381	0.004	0.189	0.031
	100	0.442	0.007	0.391	0.002	0.167	0.018
sMDH-3,4*	20	0.140	0.027	0.711	0.134	0.149	0.040
	60	0.101	0.011	0.809	0.043	0.090	0.011
	100	0.324	0.018	0.371	0.053	0.305	0.010
MEP-1*	20	0.301	0.028	0.357	0.153	0.342	0.050
	60	0.28ΰ	0.011	0.425	0.059	0.289	0.019
	100	0.315	0.008	0.363	0.042	0.322	0.014
MUP*	20	0.264	0.015	0.425	0.084	0.311	0.064
	60	0.331	0.004	0.363	0.029	0.306	0.020
	100	0.340	0.003	0.383	0.018	0.277	0.010
SOD*	20	0.359	0.029	0.453	0.064	0.188	0.034
	60	0.422	0.007	0.378	0.016	0.200	0.017
	100	0.445	0.004	0.385	0.006	0.170	0.007
TOTAL	20	0.308	0.046	0.513	0.100	0.179	0.053
	60	0.314	0.036	0.521	0.066	0.165	0.026
	100	0.350	0.029	0.463	0.052	0.187	0.021

Table 6.4 The average (AV) and standard deviations (SD) of 100 runs of simulation #3 for each of the allozyme loci.

Locus	Sample size	Killala L. (0.600) AV	Killala L. (0.600) SD	L. Manitou (0.300) AV	L. Manitou (0.300) SD	Clearwater L. (0.100) AV	Clearwater L. (0.100) SD
sAAT-1,2*	20	0.077	0.024	0.790	0.073	0.133	0.028
	60	0.044	0.006	0.880	0.012	0.076	0.004
	100	0.053	0.005	0.851	0.017	0.096	0.005
GPI-1*	20	0.556	0.000	0.440	0.000	0.004	0.000
	60	0.558	0.000	0.440	0.000	0.002	0.000
	100	0.557	0.000	0.440	0.000	0.003	0.000
LDH-4*	20	0.510	0.014	0.416	0.005	0.074	0.036
	60	0.480	0.009	0.407	0.002	0.113	0.020
	100	0.481	0.005	0.410	0.001	0.109	0.010
MDH-3,4*	20	0.359	0.056	0.377	0.119	0.264	0.020
	60	0.387	0.026	0.288	0.060	0.325	0.010
	100	0.392	0.015	0.258	0.035	0.350	0.005
MEP-1*	20	0.342	0.023	0.262	0.126	0.396	0.042
	60	0.342	0.010	0.289	0.059	0.369	0.021
	100	0.339	0.007	0.305	0.038	0.356	0.013
MUP*	20	0.273	0.016	0.329	0.080	0.398	0.071
	60	0.341	0.004	0.267	0.027	0.392	0.024
	100	0.339	0.003	0.292	0.021	0.369	0.018
SOD*	20	0.360	0.029	0.446	0.066	0.194	0.036
	60	0.432	0.008	0.412	0.016	0.156	0.015
	100	0.440	0.006	0.418	0.013	0.142	0.009
TOTAL	20	0.354	0.044	0.437	0.091	0.209	0.053
	60	0.369	0.032	0.426	0.064	0.205	0.034
	100	0.371	0.028	0.425	0.052	0.204	0.028

Stock (actual contribution)

Table 6.5 Average estimates (AV) and standard deviations (SD) for θ over 100 replicates of the simulation for each of the microsatellite DNA loci and the totals for all 13 scored in three stocks of lake trout. Results are for simulation #1.

Locus	Sample size	Killala L. (0.333) AV	Killala L. (0.333) SD	L. Manitou (0.333) AV	L. Manitou (0.333) SD	Clearwater L. (0.333) AV	Clearwater L. (0.333) SD
Sfo8	20	0.376	0.058	0.255	0.026	0.369	0.033
	60	0.418	0.008	0.243	0.007	0.339	0.007
	100	0.422	0.005	0.243	0.004	0.335	0.005
Sfo12	20	0.000	0.000	0.000	0.000	1.000	0.000
	60	0.327	0.039	0.621	0.042	0.052	0.005
	100	0.308	0.026	0.657	0.027	0.035	0.002
Sfo18	20	0.630	0.022	0.138	0.018	0.232	0.027
	60	0.579	0.008	0.179	0.009	0.242	0.027
	100	0.578	0.005	0.168	0.006	0.254	0.007
Sfo23	20	0.530	0.022	0.234	0.011	0.236	0.009
	60	0.533	0.006	0.240	0.004	0.227	0.003
	100	0.539	0.003	0.239	0.027	0.222	0.001
Ssa197	20	0.280	0.022	0.419	0.026	0.301	0.013
	60	0.300	0.006	0.408	0.007	0.292	0.004
	100	0.332	0.007	0.478	0.008	0.190	0.008
Ssa289	20	0.293	0.022	0.402	0.024	0.305	0.018
	60	0.302	0.006	0.404	0.007	0.294	0.005
	100	0.312	0.004	0.382	0.004	0.306	0.003
TOTAL	20	0.352	0.066	0.241	0.039	0.407	0.094
	60	0.403	0.027	0.349	0.036	0.248	0.014
	100	0.415	0.002	0.361	0.038	0.224	0.014
ALL LOCI	20	0.320	0.053	0.339	0.086	0.341	0.089
	60	0.345	0.033	0.407	0.068	0.248	0.032
	100	0.370	0.025	0.380	0.052	0.250	0.026

Table 6.6 Average estimates (AV) and standard deviations (SD) over 100 replicates of simulation #2 for six microsatellite DNA loci and the totals (TOTAL, ALL LOCI) for all 13 scored in three stocks of lake trout.

Locus	Sample size	Stock (actual contribution)					
		Killala L. (0.425)		L. Manitou (0.425)		Clearwater L. (0.425)	
		AV	SD	AV	SD	AV	SD
Sfo8	20	0.471	0.047	0.344	0.030	0.185	0.027
	60	0.495	0.011	0.329	0.008	0.176	0.007
	100	0.497	0.007	0.335	0.004	0.168	0.004
Sfo12	20	0.000	0.000	0.000	0.000	1.000	0.000
	60	0.000	0.000	0.000	0.000	1.000	0.000
	100	0.000	0.000	0.000	0.000	1.000	0.000
Sfo18	20	0.666	0.025	0.200	0.018	0.134	0.025
	60	0.670	0.007	0.213	0.007	0.117	0.009
	100	0.672	0.004	0.214	0.004	0.114	0.005
Sfo23	20	0.599	0.014	0.350	0.011	0.051	0.003
	60	0.588	0.006	0.353	0.006	0.059	0.002
	100	0.581	0.003	0.357	0.002	0.062	0.001
Ssa197	20	0.357	0.028	0.532	0.021	0.111	0.007
	60	0.370	0.007	0.523	0.007	0.107	0.002
	100	0.380	0.006	0.513	0.005	0.107	0.001
Ssa289	20	0.428	0.017	0.431	0.016	0.141	0.001
	60	0.435	0.006	0.433	0.009	0.132	0.004
	100	0.432	0.004	0.433	0.005	0.135	0.002
TOTAL	20	0.420	0.069	0.309	0.044	0.271	0.124
	60	0.426	0.053	0.308	0.034	0.266	0.113
	100	0.427	0.051	0.308	0.031	0.265	0.112
ALL LOCI	20	0.364	0.148	0.411	0.152	0.225	0.079
	60	0.370	0.047	0.415	0.063	0.215	0.069
	100	0.389	0.040	0.386	0.049	0.225	0.065

Table 6.7 Average estimates (AV) and standard deviations (SD) for θ over 100 replicates of the simulation for each of the microsatellite DNA loci and the totals for all 13 loci used in simulation #3.

		Stock (actual contribution)					
		Killala L. (0.600)		L. Manitou (0.300)		Clearwater L. (0.100)	
Locus	Sample size	AV	SD	AV	SD	AV	SD
Sfo8	20	0.631	0.049	0.223	0.025	0.146	0.021
	60	0.657	0.015	0.228	0.008	0.115	0.006
	100	0.642	0.011	0.300	0.005	0.128	0.006
Sfo12	20	0.513	0.169	0.477	0.170	0.010	0.001
	60	0.567	0.067	0.432	0.068	0.001	0.000
	100	0.597	0.034	0.401	0.035	0.002	0.000
Sfo18	20	0.823	0.011	0.102	0.010	0.075	0.010
	60	0.821	0.003	0.115	0.004	0.064	0.004
	100	0.809	0.003	0.135	0.003	0.056	0.002
Sfo23	20	0.713	0.014	0.190	0.012	0.097	0.003
	60	0.726	0.005	0.185	0.004	0.089	0.001
	100	0.715	0.004	0.193	0.002	0.092	0.000
Ssa197	20	0.519	0.037	0.423	0.037	0.058	0.003
	60	0.517	0.010	0.417	0.009	0.066	0.001
	100	0.531	0.006	0.403	0.006	0.066	0.001
Ssa289	20	0.615	0.024	0.301	0.026	0.084	0.008
	60	0.606	0.006	0.309	0.006	0.085	0.002
	100	0.608	0.003	0.305	0.003	0.087	0.002
TOTAL	20	0.636	0.104	0.286	0.060	0.078	0.009
	60	0.649	0.030	0.281	0.030	0.070	0.004
	100	0.650	0.020	0.290	0.019	0.060	0.003
ALL LOCI	20	0.495	0.081	0.362	0.088	0.143	0.038
	60	0.509	0.049	0.354	0.054	0.137	0.025
	100	0.511	0.042	0.358	0.042	0.131	0.022

Table 6.8 Average estimates (AV) and standard deviations (SD) for θ over 100 replicates of simulation #4. Totals over all allozyme (ALLO), microsatellite DNA (MICROS), and all (ALL LOCI) loci are also included.

	Stock									
Actual θ	Killala L. 0.300		L. Manitou 0.300		Clearwater L. 0.150		Slate Is. 0.100		Michipicoten Is. 0.150	
Locus	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
sAAT-1,2*	0.049	0.015	0.683	0.104	0.096	0.019	0.065	0.019	0.107	0.027
GPI-1*	0.403	0.005	0.336	0.001	0.003	0.000	0.194	0.002	0.037	0.004
LDH-4*	0.267	0.013	0.239	0.006	0.148	0.008	0.200	0.002	0.146	0.012
MDH-3,4*	0.249	0.030	0.195	0.014	0.197	0.008	0.193	0.012	0.166	0.002
MEP-1*	0.222	0.021	0.182	0.008	0.212	0.016	0.224	0.026	0.160	0.003
MUP*	0.040	0.001	0.007	0.001	0.164	0.024	0.775	0.032	0.014	0.000
SOD*	0.244	0.002	0.218	0.003	0.131	0.007	0.173	0.004	0.234	0.004
Sfo8	0.321	0.014	0.223	0.011	0 156	0.009	0.102	0.008	0.198	0.007
Sfo12	0.338	0.037	0.326	0.057	0.014	0.001	0.010	0.001	0.312	0.027
Sfo18	0.483	0.006	0.183	0.010	_0.208	0.013	0.043	0.002	0.083	0.004
Sfo23	0.426	0.007	0.170	0.004	0.154	0.001	0.108	0.003	0.142	0.003
Ssa197	0.250	0.006	0.359	0.012	0.090	0.004	0.164	0.006	0.137	0.006
Ssa289	0.302	0.006	0.271	0.012	0.102	0.004	0.198	0.009	0.127	0.004
ALLO	0.211	0.021	0.266	0.054	0.140	0.012	0.260	0.030	0.123	0.057
MICROS	0.353	0.020	0.255	0.023	0.121	0.009	0.104	0.009	0.167	0.014
ALL LOCI	0.282	0.029	0.261	0.039	0.131	0.011	0.182	0.023	0.144	0.040

Table 6.9 Accuracy of maximum likelihood estimates for simulations 1 to 3 with three sample sizes.

Type of data	Sample size	Percent of estimate correct
allozyme data	20	69.1
	60	68.4
	100	75.3
microsatellite DNA data	20	82.7
	60	83.2
	100	81.6
All loci	20	85.4
	60	84.0
	100	83.6

of the estimate does not always increase as more loci are considered (Table 6.9). Six loci (AAT-1,2*, GPI-1*, MDH-3,4*, SOD*, Sfo12, and Sfo18) give consistently poor results in all simulation conditions (Tables 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, and 6.8). The relatively smaller genetic distances between two of the stocks, Lake Manitou and Killala Lake, do not seem to affect the accuracy of the estimates. However, the actual values of θ^*_j have some effect on the accuracy of the MLE. The accuracy is reduced the further away from equality the actual θ^*_j's are, and the loss of accuracy is more pronounced for the allozyme data than for the microsatellite DNA data (Table 6.10; simulations 1 to 3). The percent of the estimate correct over all loci is reduced slightly with the addition of two stocks (Table 6.10; simulation 4).

The results of the Lake Ontario simulations (Table 6.11) show that the error rate is quite high for each locus and high over all loci. The percent of the estimate correct was 58.6% over 13 loci, and only four had values that were over 5% (LDH-4*, 6.3%; PEPD-1,2*, 35.5%; PGK-1*, 29.0%; PGM-2*, 35.2%).

Discussion

Several factors may affect estimates of the MLE: the size of the mixed-stock sample, the accuracy of the estimates of baseline allele frequencies, the genetic differentiation among stocks at each locus, the number of loci analyzed, the number of alleles and therefore genotypes analyzed, the similarity among genotypic frequencies in baseline stocks, the number of baseline stocks, and the actual values of the estimator.

Table 6.10 Accuracy of maximum likelihood estimates over each technique and all loci for the first four simulations, for all sample sizes.

Simulation	Type of data	Sample size	Percent of estimate correct
1	allozyme	20	79.5
		60	73.7
		100	86.7
1	microsatellite	20	81.5
		60	82.9
		100	78.2
1	All loci	20	95.8
		60	82.9
		100	83.8
2	allozyme	20	76.6
		60	77.8
		100	85.0
2	microsatellite	20	75.8
		60	76.6
		100	76.6
2	All loci	20	82.5
		60	87.0
		100	85.0
3	allozyme	20	51.1
		60	53.8
		100	54.2
3	microsatellite	20	83.2
		60	90.2
		100	90.0

Table 6.10 continued.

Simulation	Type of data	Sample size	Percent of estimate correct
3	All loci	20	78.0
		60	82.2
		100	82.5
4	allozyme	60	48.0
4	microsatellite	60	85.2
4	All loci	60	83.6

Table 6.11 Average estimates (AV) and standard deviations (SD) of θ over 100 replicates of simulation #5, Sen=Seneca L., Mani=L. Manitou, Jen=Jenny L., Kill=Killala L., Clear=Clearwater L., and Sup=Superior.

	Stock (actual contribution)											
	Sen (0.14)		Mani (0.11)		Jen (0.03)		Kill (0.15)		Clear (0.15)		Sup (0.42)	
Locus	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
sAAT-1,2*	0.015	0.001	0.004	0.000	0.068	0.006	0.401	0.039	0.420	0.037	0.056	0.002
ACP-1*	0.023	0.001	0.119	0.005	0.076	0.009	0.433	0.018	0.327	0.007	0.022	0.006
FH-1,2*	0.043	0.000	0.001	0.000	0.236	0.000	0.000	0.000	0.549	0.004	0.171	0.001
G3PDH-1*	0.393	0.000	0.000	0.000	0.374	0.000	0.093	0.000	0.000	0.000	0.114	0.000
GPI-1*	0.302	0.004	0.009	0.000	0.351	0.002	0.117	0.002	0.003	0.000	0.138	0.003
LDH-4*	0.250	0.001	0.229	0.000	0.215	0.000	0.204	0.000	0.069	0.001	0.033	0.002
sMDH-3,4*	0.312	0.000	0.010	0.000	0.313	0.000	0.270	0.000	0.004	0.000	0.091	0.000
MEP-1*	0.031	0.000	0.347	0.006	0.138	0.002	0.049	0.004	0.332	0.006	0.133	0.002

Table 6.11 continued.

	Stock (actual contribution)											
	Sen (0.14)		Mani (0.11)		Jen (0.03)		Kill (0.15)		Clear (0.15)		Sup (0.42)	
Locus	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD	AV	SD
PEPD-1,2*	0.039	0.001	0.111	0.013	0.002	0.000	0.302	0.006	0.319	0.021	0.227	0.004
PGK-1*	0.207	0.000	0.199	0.000	0.192	0.000	0.187	0.000	0.008	0.000	0.207	0.000
PGM-1*	0.054	0.000	0.200	0.000	0.193	0.000	0.188	0.000	0.184	0.000	0.181	0.000
PGM-2*	0.374	0.044	0.423	0.047	0.041	0.002	0.042	0.001	0.030	0.001	0.090	0.004
SOD*	0.058	0.008	0.447	0.048	0.118	0.008	0.057	0.013	0.290	0.027	0.030	0.004
TOTAL	0.162	0.017	0.168	0.027	0.178	0.033	0.180	0.014	0.198	0.035	0.114	0.023

In the current study, the sample size of the mixed-stock sample, the number of loci, and the number of alleles and therefore genotypes were examined.

The sample size of baseline stocks, although not tested here, will also have an effect. Any loss in accuracy of estimates of baseline allele frequencies will degrade the accuracy of the MLE since estimates of θ_j are the result of the product of the probabilities of both the baseline and mixed-stock sample.

The variance of the estimate decreases as more fish are sampled, as might be expected. The reduction of the standard deviation is not linear however, it is larger between N=20 and N=60 than between N=60 and N=100 in most of the simulations and the average estimate of θ_{*j} and its standard deviation is often similar for both N=60 and N=100. Increases in accuracy with sample size were observed, but are not consistent over all simulations. It is important to balance the sample size used against the cost per sample and the information gained. In the present study, the cost and effort involved in adding 40 more fish to the sample may not add to the information content. In addition, the number of fish available for sampling may be limited if the species is rare or exists in small populations. The inconsistent effect of sample size points to other factors, such as number of genotypes per locus, as having a larger effect on the estimator. Sample size has some effect that may interact with other factors or is modified by other factors.

The microsatellite DNA loci give better estimates than allozyme data because more alleles (and genotypes) are detected at microsatellite loci (2 alleles on average

for allozyme loci vs. 14 for microsatellite loci) in the three hatchery stocks. The majority of the allozyme loci have a maximum of three genotypes which is equal to or less than the number of baseline stocks in each of the simulations. As the number of genotypes in the mixed sample and the number of baseline stocks become similar there is a higher probability of getting identical genotypic frequencies in the mixed-sample from different baseline mixtures (Pella and Milner 1987). Therefore the number of genotypes per locus should exceed the number of baseline stocks. The minimum difference between the number of genotypes and baseline stocks required to get an accurate estimate will also depend upon the distribution of genotypic frequencies among the baseline stocks. As baseline stocks become more distinct from each other the difference between the number of genotypes and number of baseline stocks will be reduced. The four microsatellite loci that consistently produced more accurate estimates were characterized by several (more than three) alleles. Over 60% of the alleles at these loci were found at frequencies of 5% or more in at least one of the baseline stocks.

In general, allozyme loci produced less accurate estimates. They tended to have fewer (usually two) alleles and observed genotypes (usually two) and the allozyme loci that produced the more accurate estimates usually did so because only one of the baseline stocks was variable (eg. LDH-4*, MEP-1*). Therefore, any genotypes other than the most common one, observed in the mixed-sample would be immediately assigned with a high probability to the correct stock. The estimates produced using

microsatellite DNA loci were also better than those produced using mitochondrial DNA variation or another nuclear marker; RAPD loci. One reason for the lower accuracy of mitochondrial DNA is that clones characterizing the stocks are found at low frequencies and therefore provide little data for the estimator (Chapter 2). Analyses of RAPD data indicate estimates of proportional contribution are less accurate (67% of estimates correct on average; Chapter 4) than those obtained with microsatellite DNA and allozyme loci, but more accurate than those obtained using mitochondrial DNA. Higher individual variation may also account for the low accuracy.

Analyzing more loci would increase the number of genotypes surveyed, but the data collected here indicate that an increase in the number of loci does not guarantee an increase in accuracy. Some loci (sAAT-1,2*, GPI-1*, MDH-3,4*, SOD*, Sfo12, and Sfo18) give consistently poor estimates of θ_j and detract, rather than add to the overall accuracy (Table 6.9). Therefore, it is important to consider the allele frequencies and number of genotypes at each locus. For example, if the estimate of θ^*_j is recalculated for simulation #1 (N=60) using only the seven loci that are consistently more accurate (LDH-4*, MEP-1*, MUP*, Sfo8, Sfo23, Ssa197, and Ssa289) then the overall accuracy of the estimate rises from 82.9% to 95.2%.

The actual contributions of each baseline stock had an effect on the accuracy of the allozyme data. Errors are less obvious in simulation #1 (in which the actual contributions are the same for each donor stock) since there is a greater probability of

assigning a fish correctly by chance. As the differences among the actual proportions

of each stock become greater (simulations #2 and #3), the allozyme data have fewer

genotypes that would increase the joint probability of the observed genotype in the

mixed sample and baseline stock, therefore the percent estimate correct declines.

Microsatellite DNA loci on the other hand are characterized by a greater number of

unique genotypes and therefore the accuracy is relatively unaffected as the actual

values of θ^*_j move away from equality (Tables 6.3, 6.5, 6.7, and 6.10).

The accuracy of the estimator is also reduced when values of θ_j are close to

zero. One condition on the estimator is that $\theta_j > 0$, and as θ^*_j approaches zero, bias is

introduced because the estimate falls near, or out of the space defined by the

likelihood function (Pella and Milner 1987). Ten percent seems to be the lowest

proportion based on the present data set. In simulation #4 and #5 the stocks with an

actual θ_j of 10% to 15% are rarely estimated accurately, even if the stock is fairly

distinct genetically from the other baseline stocks (eg. Clearwater Lake).

The number of baseline stocks involved may have an effect on the accuracy.

As more stocks are added the actual value of θ_j decreases since the total of all

estimates must sum to one. As discussed above the accuracy of the estimator is

reduced as the actual value of θ_j approaches zero and as the number of baseline

stocks becomes closer to the number of genotypes in the mixed sample.

A small genetic distance between two of the hatchery stocks (Lake Manitou and

Killala Lake, D=0.001 for allozyme data, Ihssen et al. 1988; D=0.101 for

microsatellite DNA data; Chapter 5) does not seem to affect the accuracy of the

estimate. In order to get an accurate estimate of θ^*_j, baseline stocks must have

characteristic allele frequencies or private alleles. Bias will be introduced when stocks

that are similar differ in abundance and similarities among stocks can produce errors

not affected by sample size (Beacham *et al.* 1985), but there are no generalizations to

determine how similar is too similar (Pella and Milner 1987). It has been suggested

that some bias could be removed by combining stocks that are similar using measures

of genetic distance and correlations of estimates of covariance between stocks as a

guide. Millar (1987) suggests that stocks with strong negative correlations could be

combined, but only if the combinations can still give practical information. For

example, one might combine the genetic data for a number of tributaries in a river that

may contribute to a mixed-sample or data from hatchery stocks of similar origin (eg.

the Lake Superior stocks, the Slate Islands ard Michipicoten Island). However,

combining hatchery stock data may not be practical if the contribution of individual

hatchery stocks is the parameter of interest.

The estimates generated using the Lake Ontario data set (simulation #5) had a

small variance, (standard deviation is often 0.001 or less), suggesting that sample sizes

are adequate, but they did not accurately estimate the true proportion of the baseline

stocks in the mixed sample. Estimates are especially poor for the Jenny Lake stock

which contributed 3% to the total. It has been noted previously that it is difficult to

get an accurate estimate if the actual contribution is low. Low levels of accuracy may

occur because most loci examined have only two alleles, one of which occurs at a high frequency. Therefore, very few of the stocks are characterized by unique genotypes.

Researchers studying naturalized lake trout in Lake Ontario also used mitochondrial DNA (mtDNA) variation in their analyses (Marsden *et al.* 1989; Grewe *et al.* 1994a; Marsden *et al.* 1993) which would improve the accuracy by adding one locus with seven alleles, or the equivalent of seven additional genotypes (where the genotypes are equivalent to the number of mitochondrial haplotypes). However, the results from the mtDNA data do not give complete information, because the mitochondrial molecule is inherited in a clonal fashion through the maternal lineage (Brown *et al.* 1979). The clonal inheritance means that only the contribution of females is measured. If the contribution rate is different between males and females then mtDNA markers will add another source of bias. Studies of another salmonid, brook charr (*S. fontinalis*), indicate that differences in survival and genetic contributions to future generations do exist between the sexes (P. Ihssen, OMNR, unpublished data). MtDNA data may prove useful, but perhaps should be considered separately. Comparisons of nuclear and mitochondrial markers in naturalized fish populations will provide insight on differences in reproductive rates between male and females.

MSA may also be applied to studies of naturalized fish to determine what proportion of a sample of naturally-produced fish is derived from mating among and

within the hatchery stocks. To perform the analysis, baseline genotypic frequencies for crosses among stocks are estimated by taking the product of the genotypic frequencies in the hatchery stocks. Such an approach can be used to give an idea of the mating structure of a rehabilitated population (eg. Grewe *et al.* 1994a), but the estimates will most likely have a fairly large error attached to them. To consider all possible crosses among donor stocks will increase the number of baseline samples considerably and, as pointed out above, the accuracy of the estimate decreases as more baseline stocks are considered, and as the number of baseline stocks and genotypes become more similar.

Based on the results of the current study we conclude that the MLE used currently in many management practises can provide a fairly accurate estimate (greater than 75%) of an individual stock's contribution to a mixed-stock sample for Ontario hatchery stocks. Such a conclusion is not new (Grant *et al.* 1980; Pella and Milner 1987), but this study demonstrates a need to consider sample size and especially the number and distribution of genotypes among the baseline stocks, because these two seem to have the greatest effects on the accuracy of the MLE.

Before performing MLE on a mixed-stock, the choice of genetic techniques and loci used should be considered carefully. Allelic frequencies in baseline data sets should be examined to determine whether genotype frequencies are sufficiently unique. For example, if a locus has two alleles and one occurs at a frequency of less than 5% in more than one of the baseline stocks, an accurate estimate will not be obtained.

Such a restriction may preclude the use of allozyme loci in some species. Therefore other markers, such as microsatellite DNA loci should be considered, since they often detect more alleles per locus than allozyme electrophoresis. Using Ontario lake trout hatchery data, good estimates of θ^*_j could be obtained using the microsatellite loci Sfo8, Sfo23, Ssa197, and Ssa289, and the allozyme loci LDH-4*, MEP-1*, and MUP*. All the loci can be scored using a sample of muscle which could be obtained using non-lethal sampling techniques. Therefore the newly rehabilitated population can be sampled without destroying fish.

Multi-strain stocking is one of a variety of rehabilitation strategies employed by fisheries managers. In order to optimize the chance of success of a rehabilitation program more than one strategy should be employed (Marsden et al. 1993). For example, improving the environment of the stocked lake may be as important as choosing the most appropriate stock for rehabilitation (Marsden et al. 1993). Multi-strain stocking and parental strain composition analysis can then be used to choose the most appropriate stocks. As a rehabilitation program proceeds the strains used for stocking can be altered so that only the most successful in a particular area are used. Large budgets are devoted to rehabilitation programs, therefore it is very important to evaluate mixed-stock data very carefully and to ensure that markers are used that give the best estimates possible so that well informed decisions can be made in order to most efficiently achieve rehabilitation targets

Acknowledgements

References

Beacham, T. D., R. E. Withler, and A. P. Gould. 1985. Biochemical genetic stock identification of pink salmon (*Oncorhynchus gorbuscha*) in southern British Columbia and Puget Sound. Can. J. Fish. Aquat. Sci. 42:437-448.

Brown, M. W., M. George Jr., and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA. 76:1967-1971.

Dempster, A. P., N. M. Laird, and D. B. Rubin. 1977. Maximum likelihood from incomplete data via the EM method. J. R. Stat. Soc. B39:1-38.

Fournier, D. A., T. D. Beacham, B. E. Riddell, and C. A. Busack. 1984. Estimating stock composition in mixed stock fisheries using morphometric, meristic, and electrophoretic characteristics. Can. J. Fish. Aquat. Sci. 41:400-408.

Grant, W. S., G. B. Milner, P. Krasnowski, and F. M. Utter. 1980. Use of biochemical variants for identification of sockeye salmon (*Oncorhynchus nerka*) stocks in Cook Inlet, Alaska. Can. J. Fish. Aquat. Sci. 37:1236-1247.

Grewe, P. M., C. C. Krueger, J. E. Marsden, C. F. Aquadro, and B. May. 1994a. Hatchery origins of naturally produced lake trout fry captured in Lake Ontario: temporal and spatial variability based on allozyme and mitochondrial DNA data. Trans. Amer. Fish. Soc. 123:309-320.

Grewe, P. M., C. C. Krueger, C. F. Aquadro, and B. May. 1994b. Stability of allozyme and mitochondrial DNA markers among three year-classes of lake trout propagated from Seneca Lake, New York. N. Amer. J. Fish. Manage. 14:467-474.

Ihssen, P. E., J. M. Casselman, G. W. Martin, and R. B. Phillips. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. Can. J. Fish. Aquat. Sci. 45:1018-1029.

Ihssen, P. E., W. Stott, and G. W. Martin. 1993. Allozyme and mitochondrial DNA markers for the determination of the genetic structure of a walleye (*Stizostedion vitreum*) population established from adult transplants of three distinct stocks. A report to the North Shore of Lake Superior Great Lakes Cleanup Fund. Technical Report Series #14.

Krueger, C. C., J. E. Marsden, H. L. Kincaid, and B. May. 1989. Genetic

differentiation among lake trout strains stocked into Lake Ontario. Trans. Amer. Fish. Soc. 118:317-330.

Marsden, J. E., C. C. Krueger, and B. May. 1989. Identification of parental origins of naturally produced lake trout in Lake Ontario: application of mixed-stock analysis to a second generation. N. Amer. J. Fish. Manage. 9:257-268.

Marsden, J. E., C. C. Krueger, P. M. Grewe, H. L. Kincaid, and B. May. 1993. Genetic comparison of naturally spawned and artificially propagated Lake Ontario lake trout fry: evaluation of a stocking strategy for species rehabilitation. N. Amer. J. Fish. Manage. 13:304-317.

Millar, R. B. 1987. Maximum likelihood estimation of mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 44:583-590.

Millar, R. B. 1990. Comparison of methods for estimating mixed stock fishery composition. Can. J. Fish. Aquat. Sci. 47:2235-2241.

Pella, J. J. and G. B. Milner. 1987. Use of genetic marks in stock composition analysis. In Population Genetics and Fishery Management. N. Ryman and F. Utter (ed.) pg. 247-276. University of Washington Press, Seattle and London.

Chapter 7
General Discussion

Broodstock variation and genetic concerns in the hatchery

Hatchery managers try to maintain distinctions among lake trout hatchery stocks so that gene complexes or allele combinations that improve survival or adaptability in a particular ecosystem are preserved. If all the genetic data about Ontario lake trout hatchery stocks are considered, observations and conclusions may be made about levels of genetic variation within and among the stocks.

Distinct lake trout stocks are being maintained within the OMNR's fish culture system. Dendrogram topologies derived from three of the genetic markers described in the preceding chapters are similar to each other indicating that both nuclear and mitochondrial genomes are being affected in a similar fashion in the hatchery. In addition, they are similar to dendrograms produced in earlier studies of wild and hatchery lake trout (Ihssen *et al.* 1988; Wilson and Hebert 1996; Chapters 2 and 5). For example, inland lake populations such as Haliburton are quite distinct for microsatellite DNA and allozyme loci. In general, samples from different parts of each of the Great Lakes are more similar to each other than to samples from inland populations (Chapters 2 and 5). However, exceptions to the above are worth mentioning. First, the similarity of Lake Simcoe and Manitoba lake trout observed with both microsatellite DNA data (Chapter 5) and recent allozyme data (Bill Martin,

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STAG Unit, OMNR, personal communication) indicates that among-stock differences are being lost. This change may be the result of the management practice employed to maintain the Lake Simcoe stock, and seems to have occurred recently (Chapter 5).

Based on microsatellite DNA data (Chapter 5), it appears that samples from the Great Lakes are more similar to each other than to samples from inland populations. Allozyme data on earlier hatchery samples (Ihssen *et al.* 1988; Grewe and Hebert 1988) indicate that samples from the northwest areas of the province are more similar to each other. However, the genetic distances are small (for example largest D=0.0600 in Ihssen *et al.* 1988) in all studies and could be a result of sampling errors among different year classes or sample sites. Similarly, allele frequency differences were obsei .:d between Killala Lake samples derived from the hatchery and the wild (Krueger *et al.* 1989; Ihssen *et al.* 1988). Repeating the genetic analyses with one or more year classes would help to determine if differences are the result of sampling error (due to sample size or temporal changes in allele frequency) during the studies or a result of changes in allele frequency within the hatchery.

The genetic analysis of hatchery stocks indicates that within-stock genetic variation is being maintained. The levels of within-stock genetic diversity observed in eight hatchery and one wild stock was similar for both mtDNA variation and RAPD-PCR and similar to that reported in a study of allozyme variation (Ihssen *et al.* 1988). The three different techniques (mtDNA variation, RAPD-PCR, and allozymes) measure diversity in different parts of the genome (nuclear versus mitochondrial) and at

different levels of resolution (*ie*. DNA versus protein products). Within-stock genetic variation is affected by the number of parents, sex ratio, and variation in relative family size. It is very important to maintain within-stock variation in order to avoid, for example, the effects of inbreeding. Although levels of variation are currently being maintained some effort should be made to monitor within-stock variation in hatchery stocks because genetic variation will be lost over time within the hatchery at a rate that is affected by effective population size and original heterozygosity (Lande and Barrowclough 1987). Therefore, stocks that are based on small founding numbers of parents may experience loss of genetic variation in the future. Two of the lake trout stocks maintained within the hatchery (Parry Sound, Iroquois Bay) were founded with smaller samples than is recommended, while the Slate Islands and Killala Lake were founded with more than 200 individuals. Michipicoten Island was founded using rotational line crossing between three year classes in order to maintain or increase the amount of genetic variation. The number of males and females captured in each of the three founding years was 38, 24 and 14 respectively. It is interesting to note that if allozyme, RAPD, and microsatellite DNA data are considered, the Slate Islands are generally the most variable stock, followed by Killala Lake, Michipicoten Island, Parry Sound, and Iroquois Bay (the latter stock was started with a very small sample of wild fish).

Sex ratio may also have some effect on within-stock variation. Sex ratios were equalized in only the Michipicoten Island and Parry Sound stocks, and only the

Michipicoten Island stock had families that were reared separately (Anonymous 1992).

While levels of variation in the nuclear genome are in the middle to low range for

Killala Lake, levels of mtDNA variation are relatively high. Founding parents for the

Killala Lake stock included twice the number of females as males (600 males and

1200 females; Anonymous 1992), therefore more of the mitochondrial DNA variation

in the wild was captured as compared to the nuclear DNA variation. A similar finding

is observed for the Slate Islands stock which was founded with twice as many females

as males. In contrast, the Michipicoten Island hatchery stock was founded with

relatively fewer female fish than Killala Lake and the Slate Islands and it has less

mtDNA variation.

No data are available to indicate the number of parents, sex ratio, or relative

family sizes used in the two stocks that are maintained in the wild (Lake Manitou and

Lake Simcoe). Lake Manitou and Lake Simcoe stocks are maintained by collecting

and fertilizing eggs from the wild each year, rearing the progeny, and then releasing

them back into the lake as yearlings. The Lake Simcoe and Lake Manitou stocks are

the least variable stocks for each type of genetic analysis if they are compared to the

two other stocks that originated from smaller inland lakes (Haliburton and Clearwater

Lake, Manitoba). Lake Simcoe has no natural recruitment and the population is

maintained only by hatchery fish (MacLean et al. 1981), whereas natural reproduction

does occur in Lake Manitou.

Lake trout from Clearwater Lake, Manitoba have the highest levels of within-

population variation of all the stocks. A similar observation is made in a study of lake trout from Manitoba, Saskatchewan, and Alberta that also included samples from Clearwater Lake (Vitic and Strobeck 1996). Clearwater Lake has an endemic population of lake trout with natural recruitment. Genetically, it is characterized by variants that are found in both eastern and western Canada, and may have been successfully colonized by fish from more than one area.

The final genetic concern, domestication, cannot be fully assessed yet because records on performance traits for the individual families are not kept in the hatchery. Only one stock, Michipicoten Island, is reared in a fashion (individually-reared families) that would allow the effects of domestication to be analyzed. Yearly allozyme analysis of hatchery stocks reveal few changes in allele frequencies in the stocks (Bill Martin, STAG Unit, OMNR, personal communication). However, gene complexes that may not be linked to allozyme loci could have an affect on performance traits and may be changing if selection within the hatchery is occurring. It is possible for genetic markers in different areas of the genome to exhibit different levels of genetic variation within a stock. A continuation of the current hatchery monitoring program and more detailed record keeping for the hatchery broodstocks would provide the information necessary to address concerns about domestication. The genetic variation detected at microsatellite DNA and RAPD loci may provide sufficient markers to study domestication.

Comparison of genetic data obtained using different marker systems

A number of similarities and differences can be identified when the results from the genetic analyses of lake trout are compared. Estimates of the proportion of within versus among-population variability are similar in lake trout regardless of the technique or method of analysis used to generate the data (Table 7.1).

Values range from 73% to 82% for within-population diversity and from 15% to 21% for among-population diversity. The similarity of the estimates may mean that the nuclear markers are in the same region of the genome, or much of the nuclear genome has been affected the same way by past and/or current demographic processes. The similarity between nuclear and mitochondrial DNA data may indicate that demographic processes have affected males and females in the same manner. A similar result was observed in a study of the American horseshoe crab (*Limulus polyphemus*) populations from the east coast of North American (Avise 1985). Both the mtDNA and allozyme data indicated that populations were split into north and south groups. However, other studies of salmonids indicate that there are differences in the population structure of nuclear and mitochondrial genomes. For example, brook charr from rivers in Newfoundland were compared (Ferguson *et al.* 1991) and while the mtDNA data indicated that there was little difference between rivers, allozyme data revealed significant structuring. Similar results were observed when mtDNA and microsatellite DNA loci were analyzed in brook charr from Quebec (Angers *et al.* 1995). Allozyme and mtDNA studies of rainbow (*O. mykiss*) and cutthroat trout

Table 7.1 Comparison of within and among-stock genetic variability for lake trout stocks using different genetic marker systems.

Marker system	Source of lake trout	Analytical technique	Genetic variation		Reference
			within stock	among stock	
allozymes	wild	F_{ST}	82%	18%	Ihssen et al. 1988
allozymes	hatchery	F_{ST}	79%	21%	Ihssen et al. 1988
mtDNA	hatchery	AMOVA	78%	22%	Chapter 1
RAPD	hatchery	AMOVA	85%	15%	Chapter 3
RAPD	hatchery	Shannon's diversity index	73%	27%	Chapter 3

species (*O. clarki bouvieri*) also reported different degrees of genetic relatedness (Gyllensten and Wilson 1987). Allozyme data indicated that there was a closer relationship between Westslope cutthroat trout and rainbow trout than there was between rainbow trout and Yellowstone cutthroat trout and rainbow trout whereas the mtDNA data indicated that the cutthroat species were more similar to each other. In the wild, differences in male/female migration rates or in the survival of hybrids may account for incongruent estimates. Since the haploid inheritance of the mitochondrial DNA molecule makes it more sensitive to population bottlenecks (Nei and Li 1979), fish species living in inland freshwater lakes and streams (eg. brook charr and some lake trout) which have small effective population sizes will tend to display more differences in population structure detected by nuclear and mitochondrial DNA than a species found in larger populations. For example, lake trout from the Great Lakes or other fish species that are not physically separated such as marine species will have larger population sizes than lake trout from inland lakes or other freshwater species.

Genetic relationships among lake trout stocks observed in the current study have features that are similar to those generated in previous studies of lake trout from the Great Lakes regions (Ihssen *et al.* 1988; Phillips *et al.* 1989). The dendrograms generated using microsatellite DNA and mtDNA data show that all Great Lake samples are more similar to each other than any samples taken from inland populations such as Killala Lake. Allozyme and karyotype data show that northern Lake Superior stocks such as the Slate Islands are more similar to Killala Lake

samples than to samples from the south shores of Lakes Superior and Huron (Ihssen *et al.* 1988; Phillips *et al.* 1989). However, while geographic trends are fairly similar for chromosome, allozyme, mtDNA, and microsatellite DNA data, those observed using RAPD loci are not (Chapter 4). Incongruent results may be due to differences in the area and amount of the genome screened. For example, PCR-based markers such as RAPD loci or microsatellite DNA variation will screen a larger genomic sample size than allozymes and RFLP analyses performed with small numbers of restriction enzymes (Dowling *et al.* 1996). Allozymes reflect diversity in the nuclear coding regions as does an RFLP analysis of a large portion of the mtDNA molecule. It is not clear what proportion of the DNA amplified with RAPD or microsatellite DNA primers are located in functional or non-functional regions. Recent research suggests that some RAPD markers may be located near coding regions (reviewed in Dowling *et al.* 1996; Torres *et al.* 1993). Genetic mapping of microsatellites in brown trout (*S. trutta*; Estoup et al. 1993) indicate that microsatellite DNA loci are clustered at a high density, but no linkage groups with allozyme markers were established.

Genetic relationships estimated using different nuclear markers are not always in agreement, but no one marker system gives consistently different results. Comparisons of population differentiation observed with different nuclear markers, such as RAPD and allozyme loci are not common, often because complete data sets are not usually available. To date, the RAPD markers have been analyzed in species that have not been studied previously or in species for which preliminary investigations revealed

little variation at other loci. When comparisons have been made, they are usually

among species. For example, the genetic relationship among three tilapia species

(*Oreochromis aureus*, *O. niloticus*, and *O. mossambicus*) calculated with RAPD

markers was different from that estimated using allozyme data. In addition, mtDNA

data suggested that *O. niloticus* is more closely related to a fourth *Oreochromis*

species (Bardakci and Skibinski 1994). A phylogenetic analysis of whitefish species

of the subfamily Coregoninae revealed similar relationships among species for RAPD

and allozyme data, but they were different when compared to mtDNA, karyological,

and morphological data (Elo 1996). Liu and Furnier (1993) compared levels of

variation detected in two species of aspen using allozymes, RFLP's of nuclear DNA

loci, and RAPD's. The RFLP's and allozyme loci had similar levels of variation

(measured as percent loci polymorphic) and the RAPD variation was higher. They

had expected that the two nuclear markers would be similar, and also noted that

RAPD markers provided fewer species-specific markers than allozyme loci or RFLP's.

Finally, they expressed concerns over the utility of RAPD markers for estimates of

population differentiation because of their inheritance pattern. Similar concerns were

raised in the current study. While RAPD variation is much greater than allozyme

variation and will be useful for genetic mapping and pedigree analysis, its use for

mixed-stock analysis will be somewhat limited (Chapter 4).

Conclusions

The majority of lake trout stocks used in Ontario have now been analyzed with a variety of genetic techniques, and we have found that nuclear DNA markers detect more variability than allozyme markers, as was expected. Nine stocks of lake trout were analyzed with three different techniques and the results were compared. Analysis of mtDNA detects variation at a reasonable cost but may not be suitable for mixed-stock analysis. Instead, it may have more potential for further studies on geographic origins of the species or may be used as a genetic marking system in breeding programs (Chapter 2). RAPD-PCR markers are inherited as Mendelian genetic markers in lake trout (Chapter 3), are highly variable, and may be most useful for gene mapping, fingerprint analysis, or analyzing quantitative traits (Chapter 4). Microsatellite DNA primers from other salmonid species can be used to describe substantial variation in lake trout (Chapter 5). Microsatellite DNA loci provide the most accurate estimates in a mixed-stock analysis (Chapter 6). Microsatellite DNA loci will be a useful tool for monitoring the variation of broodstocks in a hatchery program, and should be added to the genetic analysis of lake trout in Ontario.

All techniques indicated that genetic diversity is being maintained within most stocks in the hatchery system. Genetic relationships among the stocks are also being maintained with the exception of the Lake Simcoe stock which may have been contaminated with Manitoba genotypes. Genetic data combined with improvements in record keeping will allow genetic concerns such as domestication to be examined in

the future.

Rehabilitation programs that involve hatchery stocks such as lake trout are becoming more common as improvements in habitat allow reintroductions to be attempted. Genetic marker systems should be used in more rehabilitation programs because they can provide data on the success or failure of rehabilitation efforts.

References

Allendorf, F. W., N. Ryman, A Stennek, and G. Ståhl. 1976. Genetic variation in Scandinavian brown trout (Salmo trutta L.): evidence of distinct sympatric populations. Hereditas. 83:73-82.

Allendorf, F. W. and S. R. Phelps. 1980. Loss of genetic variation in hatchery stocks of cutthroat trout. Trans. Amer. Fish. Soc. 109:537-543.

Allendorf, F. W., W. A. Gellman, and G. H. Thorgaard. 1994. Sex-linkage of two enzyme loci in *Oncorhynchus mykiss* (rainbow trout). Heredity. 72:498-507.

Andersson, L., N. Ryman, R. Rosenberg, and G. Ståhl. 1981. Genetic variability in Atlantic herring (*Clupea harengus harengus*): description of protein loci and population data. Hereditas. 95:69-78.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr reveal strong population subdivision on a microgeographic scale. J. Fish Biology. 47A:177-185.

Angers, B. and L. Bernatchez. 1996. Usefulness of heterologous microsatellites obtained from brook charr, *Salvelinus fontinalis* Mitchill, in other *Salvelinus* species. Molecular Ecology. 5:317-319.

Anonymous. 1992. Stocks Catalogue: handbook of stocks available in the Provincial Fish Culture System. Fish Culture Section, Great Lakes Branch, Operations Division, Ontario Ministry of Natural Resources.

Avise, J. C. and M. F. Smith. 1974. Biochemical genetics of sunfish. I. Geographic variation and subspecific intergradation in the bluegill, *Lepomis macrochirus*. Evolution. 28:42-56.

Avise, J. C. 1985. Identification and interpretation of mitochondrial DNA stocks in marine species. NOAA Technical Memorandum NMFS-SEFC-199:105-136.

Avise, J. C., J. Arnold, R. M. Ball, E. Bermingham, T. Lamb, J. E. Neigel, C. Reeb, and N. C. Saunders. 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Ann. Rev. Ecol. Syst. 18:489-522.

Bardakci, F. and D. O. F Skibinski. 1994. Application of the RAPD techniques in

tilapia fish: species and subspecies identification. Heredity 73:117-123.

Bartley, D. M., D. B. Kent, and M. A. Drawbridge. 1995. Conservation of genetic diversity in a white seabass hatchery enhancement program in southern California. American Fisheries Society Symposium 15:249-258.

Beacham, T. D. 1988. Genetic variation in body weight of pink salmon (*Oncorhynchus gorbuscha*). Genome. 30:89-96.

Beacham, T. D. and C. B. Murray. 1988. Genetic analysis of growth and maturity in pink salmon (*Oncorhynchus gorbuscha*). Genome. 30:529-535.

Beckenbach, A. T. 199 Rapid mtDNA sequence analysis of fish populations using the polymerase chain reaction (PCR). Can. J. Fish. Aquat. Sci. 48(Suppl. 1):95-98.

Bernatchez, L., J. J. Dodson, and S. Boivin. 1989. Population bottlenecks: influence on mitochondrial DNA diversity and its effect in coregonine stock discrimination. J. Fish Biology. 35A:233-244.

Bielawski, J. P. and D. E. Pumo. 1997. Randomly amplified polymorphic DNA (RAPD) analysis of Atlantic coast striped bass. Heredity. 78:32-40.

Blankenship, H. L. and K. M. Leber. 1995. A responsible approach to marine stock enhancement. American Fisheries Society Symposium 15:167-175.

Brooker, A., D. Cook, J. M. Wright, and k. ev. Doyle. 1994. The organization of microsatellites differs between mammals and cold-water teleost fishes. Can. J. Fish. Aquat. Sci. 51:1959-1966.

Brown, W. M., M. George, and A. C. Wilson. 1979. Rapid evolution of animal mitochondrial DNA. Proc. Natl. Acad. Sci. USA 76:1967-1971.

Bucci, G. and P. Menozzi. 1993. Segregation analysis of random amplified polymorphic DNA (RAPD) markers in *Picea abies* Karst. Molecular Ecology. 2:227-232.

Busack, C. A. and K. P Currens. 1995. Genetic risks and hazards in hatchery operations: fundamental concepts and issues. American Fisheries Society Symposium 15:71-80.

Campton, D. E. 1995. Genetic effects of hatchery fish on wild populations of Pacific salmon and steelhead: what do we really know? American Fisheries Society Symposium 15:337-353.

Carmichael, G. J., J. N. Hanson, J. R. Novy, K. J. Meyer, and D. C. Morizot. 1995. Apache trout management: cultured fish, genetics, habitat improvements, and regulations. American Fisheries Society Symposium 15:112-121.

Colbourne, J. K., B. D. Neff, J. M. Wright, and M. R. Gross. 1996. DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using $(GT)^n$ microsatellites and its potential for assessment of mating success. Can. J. Fish. Aquat. Sci. 53:342-349.

Crabtree, C. B. and D. G. Buth. 1987. Biochemical systematics of the catostomid genus *Catostomus*: assessment of *C. clarki*, *C. plebeius* and *C. discobolus* including the Zuni sucker, *C. d. yarrowi*. Copeia. 1987:843-854.

Cross, T. F. and J. King. 1983. Genetic effects of hatchery rearing in Atlantic salmon. Aquaculture. 33:33-40.

Crow, J. F. and M. Kimura. 1970. An Introduction to Population Genetics Theory. Harper and Row, New York.

Cuenco, M. L., W. H. Backman, and P. R. Mundy. 1993. The use of supplementation to aid in natural stock restoration. In Genetic Conservation of Salmonid Fishes. J. G. Cloud and G. H. Thorgaard (eds.) pg. 269-293. Plenum Press, New York.

Dehring, T. R., A. F. Brown, C. H. Daugherty, and S. R. Phelps. 1981. Survey of the genetic variation among eastern Lake Superior lake trout (*Salvelinus namaycush*). Can. J. Fish. Aquat. Sci. 38:1738-1746.

Dorr, J. A. III, D. V. O'Connor, N. R. Foster, D. J. Jude. 1981. Substrate conditions and abundance of lake trout eggs in a traditional spawning area in southeastern Lake Michigan. N. Am J. Fish. Manage. 1:165-172.

Dowling, T. E., C. Moritz, J. D. Palmer, and L. H. Rieseberg. 1996. Nucleic acids III. Analysis of fragments and restriction sites. In Molecular Systematics. D. M. Hillis, C. Moritz, and B. K. Mable (eds.) pg. 249-320. Sinauer Associates, Inc. Sunderland, Mass. USA.

Doyle, R. W., C. Herbinger, C. T. Taggart, and S. Lochmann. 1995. Use of DNA

microsatellite polymorphism to analyze genetic correlations between hatchery and natural fitness. American Fisheries Society Symposium 15:205-211.

Elo, K. 1996. Intra-and interspecific gene flow and phylogenetic relationships in the Salmonidae as revealed by protein and DNA analyses. PhD Thesis. University of Joensuu, Finland. Publications in Sciences 37:51p.

Estoup, A., P. Presa, F. Krieg, D. Vaiman, and R. Guyomard. 1993. $(CT)^n$ and $(GT)^n$ microsatellites: a new class of genetic markers for Salmo trutta L. (brown trout). Heredity. 71:488-496.

Evans, D. O. and C. C. Willox. 1991. Loss of exploited, indigenous populations of lake trout, Salvelinus namaycush, by stocking of non-native stocks. Can. J. Fish. Aquat. Sci. 48(Suppl. 1):134-147

Ferguson, A., J. B. Taggart, P. A. Prodohl, O. McMeel, C. Thompson, C. Stone, P. McGinty, and R. A. Hynes. 1995. The application of molecular markers to the study and conservation of fish populations, with special reference to Salmo. J. Fish Biology. 47A:103-126.

Ferguson, M. M., R. G. Danzmann, and J. A. Hutchings. 1991. Incongruent estimates of population differentiation among brook charr, Salvelinus fontinalis, from Cape Race, Newfoundland, Canada, based upon allozyme and mitochondrial DNA variation. J. Fish Biology. 39A:79-85.

Ferris, S. D., and W. J. Berg. 1987. The utility of mitochondrial DNA in fish genetics and fishery management. In Population Genetics and Fishery Management. N. Ryman and F. Utter (eds.) pg. 277-299. University of Washington Press, Seattle and London.

Flagg, T. A., C. V. W. Mahnken, and K. A. Johnson. 1995. Captive broodstocks for recovery of Snake River sockeye salmon. American Fisheries Society Symposium 15:81-90.

Forshage, A. A., L. T. Fries. 1995. Evaluation of the Florida largemouth bass in Texas, 1972-1993. American Fisheries Society Symposium 15:484-491.

Gall, G. A. E. 1987. Inbreeding. In Population Genetics and Fishery Management. N. Ryman and F. Utter (eds.) pgs. 47-87. University of Washington Press. Seattle and London.

Gile, S. R. and M. M. Ferguson. 1990. Crossing methodology and genotypic diversity in a hatchery strain of rainbow trout (Oncorhynchus mykiss). Can. J. Fish. Aquat. Sci. 47:719-724.

Grant, W. S., G. B. Milner, P. Krasnowski, and F. M. Utter. 1980. Use of biochemical variants for identification of sockeye salmon (Oncorhynchus nerka) stocks in Cook Inlet, Alaska. Can. J. Fish. Aquat. Sci. 37:1236-1247.

Grant, W. S., D. J. Teel, T. Kobayashi, and C. Schmitt. 1984. Biochemical genetics of Pacific halibut (Hippoglossus stenolepis) and comparison with Atlantic halibut (H. hippoglossus). Can. J. Fish. Aquat. Sci. 41:1083-1088.

Grant, W. S., C. I. Zhang, T. Kobayashi, and G. Ståhl. 1987. Lack of genetic stock structure in Pacific cod (Gadus macrocephalus). Can. J. Fish. Aquat. Sci. 44:490-498.

Grewe, P. M., and P. D. N. Hebert. 1988. Mitochondrial DNA diversity among brood stocks of the lake trout Salvelinus namaycush. Can. J. Fish. Aquat. Sci. 45:2114-2122.

Grewe, P. M., N. Billington, and P. D. N. Hebert. 1990. Phylogenetic relationships among members of Salvelinus inferred from mitochondrial DNA divergence. Can. J. Fish. Aquat. Sci. 47:984-991.

Grewe, P. M., C. C. Krueger, C. F. Aquadro, E. Bermingham, H. L. Kincaid, and B. May. 1993. Mitochondrial DNA variation among lake trout (Salvelinus namaycush) strains stocked into Lake Ontario. Can. J. Fish. Aquat. Sci. 50:2397-2403.

Grewe, P. M., C. C. Krueger, J. E. Marsden, C. F. Aquadro, and B. May. 1994. Hatchery origins of naturally produced lake trout fry captured in Lake Ontario: temporal and spatial variability based on allozyme and mitochondrial DNA data. Trans. Amer. Fish. Soc. 123:309-320.

Gyllensten, U. 1985. The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. J. Fish Biology. 26:691-699.

Gyllensten, U. and A. C. Wilson. 1987. Mitochondrial DNA of salmonids: inter- and intraspecific variability detected with restriction enzymes. In Population Genetics and Fishery Management. N. Ryman and F. Utter (eds.) pg. 301-317.

University of Washington Press, Seattle and London.

Hard, J. J. 1995. Genetic monitoring of life-history characters in salmon supplementation: problems and opportunities. American Fisheries Society Symposium 15:212-225.

Healy, J. A. and M. F Mulcahy. 1980. A biochemical genetic analysis of populations of the northern pike, *Esox lucius* L., from Europe and North America. J. Fish Biology. 17:317-324.

Heard, W., R. Burkett, F. Thrower, and S. McGee. 1995. A review of chinook salmon resources in southeast Alaska and development of an enhancement program designed for minimal hatchery-wild stock interaction. American Fisheries Society Symposium 15:21-37.

Hendricks, M. L. 1995. The contribution of hatchery fish to the restoration of American shad in the Susquehanna River. American Fisheries Society Symposium 15:329-336.

Holey, M. E., R. W. Rybicki, G. W. Eck, E. H. Brown Jr., J. E. Marsden, D. S. Lavis, M. L. Toneys, T. N. Trudeau, and R. M. Horrall. 1995. Progress toward lake trout restoration in Lake Michigan. J. Great Lakes Res. 21(Suppl. 1):128-151.

Hynes, J. D., E. H. Brown, Jr., J. H. Helle, N. Ryman, and D. A. Webster. 1981. Guidelines for the culture of fish stocks for resource management. Can. J. Fish. Aquat. Sci. 38:1867-1876

Ihssen, P. E. and J. S. Tait. 1974. Genetic differences in retention of swimbladder gas between two populations of lake trout (*Salvelinus namaycush*). J. Fish. Res. Board Can. 31.351-1354.

Ihssen, P. E., J. M. Casselman, G. W. Martin, and R. B. Phillips. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. Can. J. Fish. Aquat. Sci. 45:1018-1029.

Kimura, M. 1955. Solutions of a process of random genetic drift with a continuous model. Proc. Natl. Acad. Sci. USA 41:144-150.

Krueger, C. C., J. E. Marsden, H. L. Kincaid, and B. May. 1989. Genetic differentiation among lake trout strains stocked into Lake Ontario. Trans. Amer. Fish. Soc. 118:317-330.

Krueger, C. C. and P. E. Ihssen. 1995. Review of genetics of lake trout in the Great Lakes: history, molecular genetics, physiology, strain comparisons, and restoration management. J. Great Lakes Res. 21(Supp. 1):348-363.

Lande, R. and G F. Barrowclough. 1987. Effective population size, genetic variation, and their use in population management. In Viable Populations for Conservation. M. E. Soulé (ed.) pg. 87-123. Cambridge University Press, Cambridge, England.

Lange R. E., G. C. LeTendre, T. H. Eckert, and C. P. Schneider. 1995. Enhancement of sportfishing in New York waters of Lake Ontario with hatchery-reared salmonines. American Fisheries Society Symposium 15:7-11.

Ligny, W. de. 1969. Serological and biochemical studies in fish populations. Oceanogr. Mar. Fiol. Ann. Rev. 7:411-513.

Liu Z., G. R. Furnier. 1993. Comparison of allozyme, RFLP, and RAPD markers for revealing genetic variation within and between trembling aspen and bigtooth aspen. Theoretical and Applied Genetics. 87:97-105.

Ludwig, B. 1995. British Columbia's trout hatchery program and the stocking policies that guide it. American Fisheries Society Symposium 15:139-143.

MacLean, J. A., D. O. Evans, N. V. Martin, and R. L. DesJardine. 1981. Survival, growth, spawning distribution, and movements of introduced and native lake trout (*Salvelinus namaycush*) in two inland Ontario lakes. Can. J. Fish. Aquat. Sci. 38.1685-1700.

Marsden, J. E., C. C Krueger, P. M. Grewe, H. L. Kincaid, and B. May. 1993. Genetic comparison of naturally spawned and artificially propagated Lake Ontario lake trout fry: evaluation of a stocking strategy for species rehabilitation. N. Amer. J. Fish. Manage. 13:304-317.

Martin, N. V. and C. H. Olver. 1980. The lake charr, *Salvelinus namaycush*. In Charrs: Salmonid Fishes of the Genus *Salvelinus*. E. K. Balon (ed.) pg. 205-277. Dr. W. Junk, bvPublishers, The Hague, The Netherlands.

McConnell, S., P. O'Reilly, L. Hamilton, J. M. Wright, and P. Bentzen. 1995. Polymorphic microsatellite loci from Atlantic salmon (*Salmo salar*): genetic differentiation of North American and European populations. Can. J. Fish. Aquat. Sci. 52:1863-1872.

McEachron, L. W., C. E. McCarty, and R. R. Vega. 1995. Beneficial uses of marine fish hatcheries: enhancement of red drum in Texas coastal waters. American Fisheries Society Symposium 15:161-166.

Miller, L. and A. R. Kapuscinski. 1996. Microsatellite DNA markers reveal new levels of genetic variation in northern pike. Trans. Amer. Fish. Soc. 125:971-977.

Modde, T., A. T. Scholz, J. H. Williamson, G. B. Haines, B. D. Burdick, and F. K. Pfeifer. 1995. An augmentation plan for razorback sucker in the upper Colorado River basin. American Fisheries Society Symposium 15:102-111.

Moring, J. R., J. Marancik, and F. Griffiths. 1995. Changes in stocking strategies for Atlantic salmon restoration and rehabilitation in Maine, 1871-1993. American Fisheries Society Symposium 15:38-46.

Morris, D. B., K. R. Richard, and J. M. Wright. 1996. Microsatellites from rainbow trout (*Oncorhynchus mykiss*) and their use for genetic study of salmonids. Can. J. Fish. Aquat. Sci. 53:120-126.

Nei, M. and Li, W.-H. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 76:5269-5273.

O'Reilly, P., L. C. Hamilton, S. K. McConnell, and J. M. Wright. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci. 53:2292-2298

Ovenden, J. R. 1990. Mitochondrial DNA and marine stock assessment: A review. Aust. J. Mar. Freshwater Res. 41:835-953.

Phillips, R. B. and P. E. Ihssen. 1990. Genetic marking of fish by use of variability in chromosomes and nuclear DNA. Amer. Fish. Soc. Symposium 7:499-513.

Phillips, R. B. and K. A. Pleyte. 1991. Nuclear DNA and salmonid phylogenetics. J. Fish Biology. 39A:259-275.

Phillips, R. B., K. D. Zajicek, and P. E. Ihssen. 1989. Population differences in chromosome banding polymorphisms in lake trout. Trans. Am. Fish. Soc. 118:64-73.

Phillips, R. B. and K. D. Zajicek. 1982. Q band chromosomal banding polymorphism

in lake trout (*Salvelinus namaycush*). Genetics 101:222-234.

Popodi, E. M., D. Greve, R. B. Phillips, and P. J. Wejksnora. 1985. The ribosomal RNA genes of three salmonid species. Biochemical Genetics. 23:997-1010.

Ramon, M. M. and J. A. Castro. 1997. Genetic variation in natural stocks of *Sardina pilchardus* (sardines) from the western Mediterranean Sea. Heredity. 78:520-528.

Riedy, M. F., W. J. Hamilton III, and C. F. Aquadro. 1992. Excess of non-parental bands in offspring from known primate pedigrees assayed using RAPD PCR. Nucleic Acids Research, 20:918.

Ropson, I. J., D. C. Brown, and D. A. Powers. 1990. Biochemical genetics of *Fundulus heteroclitus* (L.) VI. Geographical variation in the gene frequencies of 15 loci. Evolution. 44:16-26.

Ruzzante, D. E., C. T. Taggart, and D. Cook. 1996. Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort contribution and genetic stability. Can. J. Fish. Aquat. Sci. 53:2695-2705.

Ryman, N. and G. Ståhl. 1980. Genetic changes in hatchery stocks of brown trout (*Salmo trutta*). Can. J. Fish. Aquat. Sci. 37:82-87.

Saiki, R. K., D. H. Gelfland, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Ehrlich. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487-491.

Scribner, K. T., J. R. Gust, and R. L. Fields. 1996. Isolation and characterization of novel salmon microsatellite loci: cross-species amplification and population genetic applications. Can. J. Fish. Aquat. Sci. 53:833-841.

Schramm, H. L. Jr. and R. G. Piper, editors. 1995. Uses and effects of cultured fishes in aquatic ecosystems. American Fisheries Society Symposium 15.

Smith, P. J., A. J. Birley, A. Jamieson, and C. A. Bishop. 1989. Mitochondrial DNA in the Atlantic cod, Gadus morhua: lack of genetic divergence between eastern and western populations. J. Fish Biology. 34:369-373.

Ståhl, G. 1983. Differences in the amount and distribution of genetic variation between natural populations and hatchery stocks of Atlantic salmon. Aquaculture. 33:23-32.

Ståhl, G. 1987. Genetic population structure of Atlantic salmon. In Population Genetics and Fishery Management. N. Ryman and F. Utter (eds.) pg. 121-140. University of Washington Press. Seattle and London.

Stone, M. D. 1995. Fish stocking programs in Wyoming: a balanced perspective. American Fisheries Society Symposium 15:47-51.

Tautz, D. 1989. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucleic Acids Research. 17:6463-6471.

Thorgaard, G. H. 1983. Chromosomal differences among rainbow trout populations. Copeia. 1983:650-662.

Torres, A. M., N. F. Weeden, and A. Martin. 1993. Linkage among isozyme, RFLP, and RAPD markers in *Vicia faba*. Theoretical and Applied Genetics. 85:937-945.

Van der Bank, F. H., W. S. Grant, and J. T. Ferreira. 1989. Electrophoretically detectable genetic data for fifteen southern African cichlids. J. Fish Biology. 34:465-483.

Vitic, R. and C. Strobeck. 1996. Mitochondrial DNA analysis of populations of lake trout (*Salvelinus namaycush*) in west central Canada: implications for stock identification. Can. J. Fish. Aquat Sc. 53:1038-1047.

Vuorinen, J. 1984. Reduction of genetic variability in a hatchery stock of brown trout, *Salmo trutta* L. J. Fish Biology. 24:339-348.

Wahl, D. H., R. A. Stein, and D. R. DeVries. 1995. An ecological framework for evaluating the success and effects of stocked fishes. American Fisheries Society Symposium 15:176-189.

Waples, R. S. and D. J. Teel. 1990. Conservation genetics of Pacific salmon, part 1. Temporal changes in allele frequency. Conservation Biology. 4:144-156.

Ward, R. D. and P. M. Grewe. 1995. Appraisal of molecular genetic techniques in

fisheries. In Molecular Genetics in Fisheries. G. R. Carvalho and T. J. Pitcher. (eds.) pg. 55-79. Chapman and Hall, London.

Williams, J. G. K., A. R. Kubelik, K. J. Livak, J. A. Rafalski, and S. V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research. 18:6531-6535.

Wilson, C. C. and P. D. N. Hebert. 1996. Phylogenetic origins of lake trout (*Salvelinus namaycush*) in eastern North America. Can. J. Fish. Aquat. Sci. 53:2764-2775.

Winans, G. A. 1980. Geographic variation in the milkfish, *Chanos chanos*. I. Biochemical evidence. Evolution. 34:558-574.

Withler, R. E. 1988. Genetic consequences of fertilizing chinook salmon (*Oncorhynchus tshawytscha*) eggs with pooled milt. Aquaculture. 68:15-25.

Wright, S. 1969. Evolution and the genetics of populations. Vol. 2: The Theory of Gene Frequencies. University of Chicago Press, Chicago.

Zhuo, L., S. L. Sajdak, and R. B. Phillips. 1994. Minimal intraspecific variation in the sequence of the transcribed spacer regions of the ribosomal DNA of lake trout (*Salvelinus namaycush*). Genome 37:664-671.