Genetic Profiles of Selected Brook Trout *Salvelinus fontinalis* Populations from Lake Superior, Lake Huron and Selected Hatcheries.

Research Completion Report Prepared for
U. S. Department of the Interior
Fish and Wildlife Service
Ashland Fisheries Resource Office

Mary K. Burnham-Curtis
U. S. Geological Survey
Great Lakes Science Center
1451 Green Road
Ann Arbor, Michigan 48105

March 5, 2001

Abstract

In the Lake Superior drainage basin, brook trout are the only extant indigenous stream salmonid since the loss of several populations of river-run lake trout in the last century. Recent field investigations have shown evidence that brook trout populations in some Lake Superior tributaries are self-sustaining, and in some populations significant genetic diversity is still present. Some Lake Superior tributaries contained populations of "coaster" brook trout, a form of brook trout which spends a majority of its adult life in the open water of Lake Superior and returns to shoreline reefs or some upstream sites to spawn. Populations of coasters throughout the Lake Superior basin have suffered severe declines, and only a few streams are thought to still harbor remnant stocks. From throughout the Lake Superior basin, brook trout populations were sampled that represented suspected remnant wild populations, historical "coaster" populations, and populations with counterparts in above-barrier sections of streams. Much of the genetic diversity present among Lake Superior brook trout is partitioned among individual populations. Populations of brook trout in the Lake Superior basin show levels of genetic variability in the mitochondrial DNA genome that suggest that some populations are still sustained by wild fish. The combined effects of population declines and hatchery supplementation are reflected in the mtDNA profiles of several populations in this survey for which only the most common mtDNA haplotype was detected. No unique genetic markers were found in the mitochondrial DNA to correlate with samples identified as coasters, but sample sizes of known coasters were small. Further work with more known coasters and nuclear markers such as microsatellite DNA loci may shed more light on how the coaster phenotype is produced.

Introduction

Brook trout *Salvelinus fontinalis* are indigenous to North America, and since about 1872 have had their range extended by man to include all major continents (MacCrimmon and Campbell 1969, Behnke 1972). In the Lake Superior drainage basin, brook trout are the only extant indigenous stream salmonid since the loss of several populations of river-run lake trout in the last century. Recent field investigations have shown evidence that brook trout populations in some Lake Superior tributaries are self-sustaining (Newman and DuBois 1996), and in some populations significant genetic diversity is still present (Danzmann et al. 1991, Burnham-Curtis 1996). In addition, some Lake Superior tributaries contained populations of "coaster" brook trout, a form of brook trout which spends a majority of its adult life in the open waters of Lake Superior and returns to shoreline reefs or some upstream sites to spawn. Populations of coasters throughout the Lake Superior basin have suffered severe declines, and only a few streams are thought to still harbor remnant stocks.

The reliable identification of "native" brook trout is complicated by a diverse stocking history in many rivers and streams tributary to Lake Superior. Several different non-native strains have been stocked at various times during the last century, and local lore holds accounts of numerous unofficial downstream-upstream stock transfers that occurred prior to and during European settlement of the region (Eddy and Underhill 1974, Waters 1977). Despite past stocking events, some tributaries to Lake Superior still harbor native populations of brook trout capable of exhibiting migratory behavior. In Minnesota waters, an average of about 250 brook trout are caught in lower reaches of tributary streams each spring, but it is not known whether those are coasters or river-resident fish (Morse 1999). Other reports of returning lake-run brook trout continue to surface from several Michigan tributaries known to be historical coaster streams (Newman and DuBois 1996).

Modern management of brook trout populations has begun to focus on the importance of native fish populations to the health of river and lake ecosystems. Identification of existing levels of genetic diversity and the relationship between wild and hatchery-derived brook trout is an important component of the management equation for this species. In the Lake Superior basin, characterization of different life history morphs adds complexity to research and management activities. Interest in restoration of brook trout populations, particularly the "coaster" brook trout, has been identified by the U. S. Fish and Wildlife Service in their Great Lakes Fisheries Management Program (Newman and DuBois 1996), and Isle Royale National Park in their General Management Plan (Isle Royale National Park, Final General Management Plan, p.74). Individual agencies have begun investing more resources into protecting and restoring such wild fish populations. To determine the status of particular species as threatened or endangered managers will need more accurate descriptions of population identities. Concerns about the genetic integrity of wild populations, low genetic diversity, and the potential for gene flow between hatchery and wild populations, have prompted regional and federal management agencies to reevaluate hatchery stocking programs for many native fish species in the U.S., including brook trout in the Great Lakes basin.

Genetic diversity among wild brook trout populations was undoubtedly reduced by bottlenecks from recent declines in population abundance. These populations were further

impacted by interactions with hatchery-raised fish in almost every region where brook trout are native (Kreuger and Menzel 1979, Webster and Flick 1981, Danzmann et al. 1991, Perkins et al. 1993). Allozymes (Kreuger and Menzel 1979, Webster and Flick 1981) and mtDNA (Quattro et al. 1990, Perkins et al. 1993, McCracken et al. 1993) have been useful in distinguishing wild brook trout populations which inhabit discrete segments of inland streams. However, recognition of hatchery versus wild brook trout has been limited to documenting lower levels of heterozygosity in hatchery populations as opposed to identifying population-specific markers (Quattro et al 1990, Perkins et al. 1993). Danzmann et al. (1998) demonstrated that significant levels of genetic diversity can be detected using characters derived from maternally-inherited mitochondrial DNA. Numerous closely related mtDNA haplotypes derived from post-glacial colonists could be evidence of the survival of wild extant brook trout populations despite 19th and 20th-century stocking efforts. These studies have demonstrated that considerable variation still exists among extant inland brook trout populations, and have identified levels of genetic variation that support the existence of barriers to gene flow among populations within and among regional river systems (Quattro et al. 1990, Danzmann et al. 1991, Bernatchez and Danzmann 1993). Recent advances in molecular technology have promoted the development of genetic markers with the potential to provide population- and individual-specific profiles for use in parentage analysis (Angers et al. 1995, Letcher and King 1999).

The Use of Mitochondrial DNA for Population Studies of Brook Trout

Prudent management decisions for native fish species should be based on an understanding of the historical and contemporary stock structures of the resource of interest. Genetic techniques generate information about stock structure, provide a means by which populations can be compared over time, space, and life stage, and provide stable repeatable techniques that complement traditional biological surveys. Mitochondrial DNA (mtDNA) provides a good baseline from which to evaluate both the contemporary and historical genetic characteristics of brook trout populations in the Lake Superior drainage.

Widespread stocking is recognized as a factor that impedes the identification of contemporary wild brook trout populations as "remnant native" stocks (Krueger and Menzel 1986). Knowledge of historical stock structure and contemporary patterns of diversity can provide valuable information for evaluating the status of native and introduced populations. Genetic diversity is a type of biological diversity that should be measured and evaluated, like other measurable parameters such as mortality, fecundity, and abundance which guide management decisions on the basis of sustainability and long-term persistence. Changes in the partitioning of genetic variability among populations over time can indicate significant risks to populations or species that are otherwise difficult to infer from ecological and demographic analyses alone (Nielsen 1998).

Genetic material (DNA, RNA) provides all living organisms with the blueprint for form (morphology, phenotype), function (physiology), behavior, and reproductive success. Genetic markers contain information coded in the DNA that is inherited across generations, so information gathered in one year can be directly applied to subsequent and previous generations. Moreover, the same genetic information can be obtained at any lifestage and often by using non-

lethal methods. Assessment of status and trends of populations with a genetic component could prevent significant errors in judgement or policy based on a lack of knowledge of the evolutionary history or contemporary reproductive health of the populations or species of interest.

Mitochondrial DNA is one genetic marker that can be used to make inferences about historical population genetic structure and contemporary patterns of gene flow among populations. The relatedness among different genetic types ("haplotypes" or, as with mtDNA, the composite genetic characteristics inherited through a single parent) and the distribution of haplotypes detected across the range of a species provides information about the historical structure of the species, including patterns of colonization and radiation (e.g. Danzmann et al. 1999; Wilson and Hebert 1998). The diversity of haplotypes present among contemporary populations provides information about existing population structure and the extent of gene flow (Jones et al. 1998). Knowledge of these population characteristics helps to put the population structure of a species in the proper context, especially when questions exist about the presence of remnant native populations in the wild and the presence or extent of interbreeding with hatchery-raised stocked fish.

The molecular structure of mtDNA makes it very useful for studies of population structuring and gene flow. MtDNA is a closed circular molecule of double stranded DNA, about 16,700 base pairs long in teleost fish that is found in the cytoplasm of individual cells. MtDNA has a compact structure; codes mainly for proteins associated with metabolic processes (13 mRNAs, 22 tRNAs, and 2 rRNAs) and contains no repetitive spacers or introns which are widely distributed in nuclear DNA (Brown et al. 1981). MtDNA contains one highly variable region, the "control region" or "D-loop," which is involved with the origin of replication for the mtDNA molecule. In contrast to nuclear DNA, which is present in 2-4 copies per cell, mtDNA is present in about 500-1000 copies per cell (Moritz et al. 1987). High copy number makes mtDNA easy to extract and isolate, even from old or degraded samples, and tissues for analysis can often be collected non-lethally or non-invasively. In most vertebrates, mtDNA is maternally inherited and thus represents a "clonal" or haploid lineage. Nuclear DNA is subjected to recombination through every cycle of germ cell production (meiosis); in contrast, mtDNA is inherited without recombination and changes in the sequence arise as random mutations or copying errors that are easily traced through the maternal lineage (Brown 1983). Copies of mtDNA among fishes tend to be identical within an individual and a single family, but may be diverse among families in a population.

Evolutionary relationships among mtDNA haplotypes are inferred from changes in the DNA sequence. Contemporary relationships among populations are inferred from the frequency and distribution of the different mtDNA haplotypes. Populations that have undergone rapid expansion (e.g. after a colonization event) may contain a large number of different but closely related mtDNA haplotypes (Brown 1983, Moritz et al. 1987). If a population maintains a large number of effective breeders, that population will likely maintain a large amount of genetic diversity. In contrast, if the population experiences a rapid and sustained decline in abundance, subsequent generations may show more restricted genetic diversity due to genetic bottlenecking as fewer haplotypes are contributed into future generations (Thomas and Beckenbach 1989).

Risks posed by introduction of non-native or domesticated populations can have direct or indirect genetic impacts on resident populations. Direct genetic impacts refer to consequences of interbreeding and introgression, in which the introduced population successfully interbreeds with the resident population and the genetic makeup of future populations becomes homogenized (Philipp et al. 1983, 1985). Depending on the genetic relatedness between the resident and introduced populations, short term consequences may resemble "hybrid vigor," in which hybrid offspring appear to be more successful, or more genetically diverse (Philipp et al. 1983). However, the long-term consequence may be outbreeding depression, in which the adaptive characteristics of the resident population that have evolved over time are compromised by non-native genetic types (Philipp et al. 1983, 1985). Indirect genetic impacts of introduced populations are less invasive, but equally disadvantageous. Introduced populations, especially domesticated strains, are often much less genetically diverse than wild populations (Kreuger and Menzel 1979, Kreuger and May 1991, Ryman 1991. Sufficient numbers of stocked juvenile fish could out-compete resident fish for resources (habitat, food) and lead to decreased survival and recruitment of resident juveniles to the adult population. If sufficient numbers of stocked fish survive to successfully reproduce, they could swamp or outcompete resident fish for spawning redds or spawning opportunities.

Objective

In this study we surveyed the genetic diversity in the mitochondrial DNA of wild brook trout from Lake Superior and Lake Huron tributaries and from hatchery stocks that have been used to stock Lake Superior. The null hypothesis was that there were no significant differences in genetic profiles among wild brook trout populations in the Lake Superior basin. The working hypothesis is that life history differences, geographic proximity of populations, environmental heterogeneity, and variation in hatchery input will influence how genetic variation is partitioned among wild brook trout populations. Specific goals for this study were to 1) determine if remnant brook trout stocks still exist in Lake Superior tributaries below natural barriers, and 2) investigate partitioning of genetic variation among wild and hatchery populations. In order to effectively manage fish populations, it is necessary to know the genetic characteristics of the existing populations, how the genetic variation is distributed, and whether there are discrete population-specific genetic markers available to identify populations of interest.

Methods

Brook trout were collected by the U. S. Fish and Wildlife Service (USFWS) – Ashland Fisheries Resources Office, USFWS Iron River National Fish Hatchery, Minnesota Department of Natural Resources (DNR), Wisconsin DNR, Michigan DNR, Grand Portage Tribe, Fon du Lac Tribe, Keweenaw Bay Indian Community, Red Cliff Band of Lake Superior Chippewa, Bad River Band of Lake Superior Chippewa, Bay Mills Indian Community, Isle Royale National Park, Ontario Ministry of Natural Resources, and Trout Unlimited volunteers between 1994 and 1999 from 30 streams tributary to Lake Superior, 6 open water locations, 9 hatchery populations, and Lake Nipigon and the Nipigon River (Figure 1, Table 1). The majority of brook trout were not classified as resident or coaster brook trout. Fish were collected by electrofishing with a

Smith Root[1] model 11-A backpack electrofishing unit (400-600V, 60Hz), by hook and line, or from private sportfishers. Some coaster brook trout are thought to migrate up tributary streams to spawn in the fall, so some collections in areas downstream from barrier dams were conducted during the fall spawning season (September through mid-November); collections in above-barrier streams in Minnesota were made in July and August 1998. Streams with above- and below-barrier dam collections include Cross River, Devil Track River, Kadunce Creek, Kimball Creek, Onion River, and Spruce Creek in Minnesota, and the Salmon Trout River in Michigan. Other tributaries included in the analysis were sampled below natural barrier structures. Additional samples were collected from hatchery populations to represent strains that were stocked in inland or tributary streams of the Lake Superior drainage during the last century. These include the Assinica strain (Marquette State Fish Hatchery), St. Croix Falls strain (Spire Valley Fish Hatchery), Owhi strain (Egan State Fish Hatchery; stocked 1986-1992), Phillips Maine strain (Phillips State Fish Hatchery; stocked until1988), Rome strain (Rome New York Hatchery, stocked until 1983), Dorion strain (Dorion Fish Hatchery, Ontario and Red Cliff Fish Hatchery, WI), Nashua strain (Bayfield Fish Hatchery, WI), and the Minnesota Wild strain (Crystal Springs State Fish Hatchery, MN; currently stocked in inland MN). Adipose fin clips were taken in the field and preserved in either a modified Queen's buffer (Seutin et al. 1981) or 95% ethanol and sent to the Great Lakes Science Center (GLSC) for genetic analysis.

Total genomic DNA was extracted from tissue subsamples using a commercial extraction kit (PureGene[1], Gentra Systems, Minneapolis, MN). Three mitochondrial regions that have been used in a previous study of brook trout from Lake Superior (Burnham-Curtis 1996) were analyzed. Mitochondrial DNA was replicated and amplified using the polymerase chain reaction (PCR, Mullis and Faloona 1987). PCR amplifications were performed using Ampli-*Taq* DNA polymerase and PCR buffer II supplied by the manufacturer (PE-Applied Biosystems[1], Foster City , CA), 2.0 to 6.0 mM $MgCl_2$, 200µM dinucleotides (dATP, dTTP, dGTP, dCTP), and 0.1 to 0.3µM of each oligonucleotide primer flanking the mtDNA genes for the control region, NADH 5-6, and NADH 2 (Table 2). PCR products were electrophoresed in 1% agarose in 1 X TAE, post-stained in ethidium bromide or Gel-Star stain (FMC BioProducts[1], Rockville, MD), and visualized under long wave ultraviolet light to determine accuracy and quality of the PCR reaction.

PCR amplicons were digested with six Type II restriction endonucleases specific for the locus of interest (D loop-*Alu*I, *Ase*I, NADH5-6-*Ban*II, *Pst*I, and NADH2-*Ban*I, *Sph*I). Restriction digestion products were electrophoresed in 2 to 4% agarose in 1 X TAE, post-stained with ethidium bromide and visualized under long wave UV light. MtDNA haplotypes were determined based on the composite pattern of presence or absence of restriction sites inferred from restriction fragment profiles. The combination of 3 gene loci and 6 restriction enzymes produced 14 distinct mtDNA haplotypes among brook trout sampled from throughout the Great Lakes drainage (Burnham-Curtis 1996).

Genetic diversity estimates were calculated from the comparison of restriction site presence or absence and from frequency of haplotype presence within and among populations and groups. Gene diversity for mitochondrial DNA data is equivalent to the estimate of expected heterozygosity (He) for nuclear DNA (diploid) data. The values of diversity (e.g. gene diversity,

[1] Mention of tradenames does not imply U. S. Government endorsement of commercial products.

nucleotide diversity, population pairwise distance) provide an estimate of the overall genetic variation among brook trout populations as well as means to evaluate the relationship between genetic distance and geographic distance among the sampled populations.

Population genetic structure was estimated using an analysis of molecular variance (AMOVA) in Arlequin Ver. 1.1 (Schneider et al. 1997). The ARLEQUIN program was used to calculate population diversity estimates and to estimate partitioning of genetic diversity within and among populations and groups in a hierarchichal manner. Groups tested included populations across all sampling sites, above versus below barrier dams, and wild versus hatchery. Cavalli-Sforza-Edwards chord distance estimates (Cavalli-Sforza and Edwards 1967) were input into the NEIGHBOR program of PHYLIP 3.5 (Felsenstein 1985) to generate a neighbor-joining network among populations. Gene frequency estimates from molecular data were used to generate 5000 replicate genetic distance matrices for a bootstrap analysis to generate confidence estimates in the neighbor-joining relationships using the PHYILP programs SEQBOOT, GENDIST, NEIGHBOR, and CONSENSE. Significance of differences in haplotype frequency distribution among populations was tested using a Monte Carlo method to generate a χ^2 statistic (Roff and Bentzen 1989).

Results

A total of 1,656 fish were included in the genetic analysis; 1261 were from wild populations and 395 were from 9 hatchery strains (Table 1). Among the wild populations, 442 were sampled from 8 populations above barrier dams, 786 were sampled from 33 locations below barrier dams; 73 fish from Lake Nipigon, Nipigon River and Nipigon Bay; 74 fish from 5 inland streams (Spring Brook and Reservation River, MN, Towes Creek, DeMull Creek, and Fox River, MI) were included for comparison. Several populations had sample sizes of less than 8 fish, and these were excluded from genetic diversity analyses.

Genetic Diversity

In Lake Superior, 11 brook trout haplotypes were detected from 14 possible haplotypes identified from the Great Lakes Basin (as identified by a similar 3 locus/6 enzyme survey, Burnham-Curtis 1996) (Table 2). Of these 11, all but 2 (BT9 and BT13) appeared to fall within a single lineage of closely related haplotypes. The frequency distribution of haplotypes is listed in Table 3. One haplotype, designated BT1, dominated the sample. BT1 was the most common haplotype in all populations, and the only haplotype detected in 24 of 55 populations. Three other haplotypes, BT10, BT12, and BT14, were found only in southern Great Lakes populations (Lake Erie and Ohio River drainages) and in the Minnesota Wild (BT10) and Assinica (BT10) hatchery strains. Haplotypes BT2, BT4, and BT5 were the next most common haplotypes distributed among the sampled populations, and were most common in populations sampled above barrier dams. Two lake-access populations, Grace Creek and Grand Marais Harbor also contained individuals typed as BT2, BT4, and BT5.

Above- and below-barrier dam populations were obtained from 8 of the tributaries sampled for this study. These included Kimball Creek, Kadunce Creek, Devil Track River, Spruce Creek, Onion River, Cross River, and Knife River in Minnesota, and the Salmon Trout River in Michigan. There were not enough individuals in the below-barrier population from the Knife River (N=3) to make reliable statistical comparisons, so the below-barrier site was not included in statistical analyses. With the exception of Spruce Creek, all above-barrier populations had higher diversity estimates than their below barrier counterparts (Table 4). Gene diversity estimates ranged from 4% to 72% above the barrier dams, and from 6% to 34% in their below-barrier counterparts. Significance estimates of the differences among the above and below-barrier samples tested in a Monte Carlo simulation with 5000 bootstrap replicates varied among locations. The distribution of haplotypes between above and below-barrier populations was significantly different in Kimball Creek, Kadunce Creek, Devil Track River, and Cross River. Spruce Creek, Onion River, and Salmon Trout River populations showed no significant difference between above and below-barrier populations (Table 5).

The Onion River population was the only population sampled multiple years both above (1995 and 1998) and below the barrier (1995 and 1997). Within a single year, there was no significant difference in haplotype frequency distribution between the above and below barrier populations. There also was no significant variation in haplotype distribution between sampling years above the barrier dam. However, there were significant differences in haplotype frequencies detected in the below-barrier population across sampling years (Table 3). In the 1995 collection, the BT5 haplotype was detected in 8 of 30 individuals (~24%) below the barrier, but it was not present in the 1998 below-barrier sample. Samples above and below the barrier in 1995 were not significantly different from each other because BT5 did appear in more than one individual above the barrier (Table 3).

The range of diversity among hatchery strains tested in this study (0.000 to 0.625) fell within the range of the wild populations, but varied widely among the hatchery groups (Table 4). The MN Wild strain had the highest estimated genetic diversity of all hatchery strains. The Assinica hatchery strain is most commonly planted in Michigan waters of Lake Superior, and showed moderate levels of heterozygosity. Pairwise comparisons of haplotype distribution between the Assinica sample and populations from locations with lake access (below-barrier dams or in tributaries with no barriers) showed that the Assinica sample was significantly different from all Minnesota tributaries with the exception of Cross River and Split Rock River (Table 5). Along the southern Lake Superior shore, the mtDNA genetic profile of the Assinica sample was significantly different from populations in the lower Salmon Trout and Kallio Creek. The Assinica mtDNA profile was also significantly different from Grace Creek, Washington Harbor, and Tobin Harbor populations sampled from Isle Royale. Genetic distance estimates based on molecular data were significant between the Dorion sample and all above-barrier populations (Table 5). Among pairwise comparisons between below-barrier populations and the Dorion strain, genetic distances were significant for comparisons with Black Bay, Tobins Harbor, Little Siskiwit River, Big Siskiwit River, Spruce Creek, Onion River, Cross River, Oak Island, Little Onion River, Little Sioux River, Keweenaw Bay, Cliff Creek, Mosquito Creek, Sucker River and Sable Creek.

Population Genetic Structure

An analysis of molecular variance (AMOVA) indicated that a moderate but significant proportion of genetic variation occurs among individual populations (17.8%, Φ_{ST}= 0.178, P < 0.001; Table 6[2]) when the data set is pooled. However, a large proportion (83%) of the genetic variation was allocated within populations (Table 6). Among only below-barrier wild populations, the results were similar and most of the variation was attributed to the within-population component. Variability among populations grouped as above- or below-barrier accounted for about 7% of the total variation and was moderately significant overall (Φ_{CT} = 0.066, P = 0.002); most of the variation was distributed among populations (10%) and among individuals within populations (83%). The importance of individual population components was further supported when population structure by geographic region was tested. For populations grouped by location as Canadian shore, Minnesota shore, Isle Royale, Wisconsin shore, Michigan shore, or Lake Huron, there was no measurable effect of region on the partitioning of molecular variation was observed. However, among population variation still reflected about 14% of the overall genetic variability. Differences among populations were generally due to frequency differences among closely related haplotypes. A test of the significance of partitioning of genetic variation between groups composed of hatchery-origin or wild fish showed no difference (Φ_{CT} = -0.005, P = 0.489).

Genetic relationships among the sampled brook trout populations were summarized in a neighbor-joining diagram (Saitou and Nei 1979) based on Cavalli-Sforza -Edwards chord distances derived from haplotype frequencies and molecular data (Cavalli-Sforza and Edwards 1967). Comparisons were made among 5000 bootstrap replicates of the original frequency distributions, from which a consensus tree was estimated (Figure 2). Above-barrier populations tended to fall within one well-supported cluster. No obvious regional associations in the clustering pattern were observed. The hatchery populations did not cluster together in the consensus tree, but were widely distributed among the other populations.

A similar analysis was performed using only the below-barrier populations as the input taxa (Figure 3). Populations with low genetic diversity (only the BT1 haplotype detected) fell into one cluster with little structure. As in the overall sample neighbor-joining diagram, suspected coaster population were not concentrated together, but scattered among the other populations. The arrangement of populations below the barrier dams can generally be defined by a predominant haplotype. The Nipigon branch (Lake Nipigon, Nipigon Bay, Dorion strain, Red Cliff hatchery) were the only populations that contained individuals with the BT9 haplotype. A well-supported cluster including Washington Harbor, Black Bay, Grand Marais Harbor, Grace Creek, and Kallio Creek share a high frequency of BT5 individuals, while the base of this cluster appeared to be defined by the presence of BT2 and BT4 in the populations. Invariant populations were all fixed for BT1, which explained the lack of structural definition among these populations relative to the rest of the sample.

Clustering algorithms were also applied to a dataset that included only those populations that were represented by more than 20 individuals. The neighbor-joining dendrogram, based on

[2] The parameter Φ_{ST} is a measure of the differentiation among populations on a scale of 0 to 1 where 0 = identical and 1 = no genetic correlation.

Cavalli-Sforza-Edwards chord distances, grouped all above-barrier populations into a well-supported cluster that also included 4 hatchery strains (St. Croix, Nashua, Owhi, and MN Wild; Fig. 4). As with the more inclusive dendrogram (Fig. 2), the Nipigon strains (Lake Nipigon, Nipigon River, Dorion Hatchery, Red Cliff Hatchery) fell into one well-supported cluster with Washington Creek. Above-barrier populations also grouped into a well-supported cluster.

Genetic relationships among the brook trout haplotypes were compared to previous studies to infer evolutionary history of the Lake Superior brook trout populations. The majority of haplotypes detected appeared to fall within one evolutionary clade which is assumed to have its origin in an Atlantic refugium during the last glacial event (Danzmann et al. 1998), based on a comparison of frequencies of haplotypes observed by Danzmann et al. (1998) to those observed in this study. Of the 14 possible brook trout haplotypes detected to date in the Great Lakes drainage basin, 11 were present in the Minnesota populations sampled. All haplotypes except BT9, BT13, and BT14 fall within the B clade; BT9 and BT13 fall within the A clade and BT14 falls within the C clade, assumed to be of southern Atlantic origin. Of all samples tested from the Great Lakes basin, BT6, BT10, and BT11 are significantly more common in the southern Great Lakes than in the upper Great Lakes basin. However since Danzmann et al. (1998) used different restriction enzymes a more thorough comparison should be performed by sequencing the haplotypes observed in this study so they can be directly compared to sequences collected in other studies of brook trout in North America (Bernatchez and Danzmann 1993).

Discussion

Genetic diversity is the substrate for natural selection and adaptation. The protection of this aspect of biodiversity is important to species conservation, and also to species restoration. The diversity of mitochondrial DNA haplotypes present among brook trout populations sampled from Lake Superior and Lake Huron tributary streams and hatchery strains indicated that a significant amount of genetic variation is still present among populations in the wild. One haplotype, BT1, dominated the sample and made up 40 to 100% of the haplotype distribution in a given population and 79.5% of the wild collections as a whole. The haplotypes identified in this study may fall into evolutionary clades with origins in Atlantic glacial refugia. Our findings corroborate results of Danzmann et al. (1998) which reported the predominance of two brook trout lineages in the upper Great Lakes drainage basin. Differences in the distribution of mtDNA haplotypes among the streams sampled in this study suggested that gene flow among tributaries, especially those geographically distant, is minimal. Despite extensive stocking of brook trout over many decades from strains outside the Great Lakes Basin, there appears to be minimal direct genetic impact (in terms of introgression) of hatchery fish on wild self-sustaining populations. If however, some of these wild populations are in low abundance, indiscriminate stocking of large numbers of brook trout from different unrelated populations could negatively impact the long term sustainability of the resident population.

Hierarchical analysis of brook trout from Lake Superior tributaries highlights the importance of individual populations to the overall genetic profile of Lake Superior brook trout. The majority of genetic variation was allocated among individuals, a common feature of closely related populations with are recently diverged. However, the estimate of the among-population

component of genetic variation was consistent across all hierarchical comparisons, comprising about 17% of the observed variation. There were no significant regional associations among the patterns of genetic variation, except for the samples of Nipigon origin. There was no significant partitioning of genetic variation between hatchery and wild fish; however this does not confirm nor refute the existence of introgression of hatchery brook trout into wild populations. It does suggest that mitochondrial DNA markers are not sufficient to resolve this particular question. MtDNA markers only detect inheritance through the maternal lineage and are not adequate to detect introgression mediated through the male parent. Additional analyses are necessary using nuclear DNA markers to specifically address this question. The main objective of this study is to summarize the overall distribution of genetic variability among brook trout sampled in Lake Superior. Specific tests of hatchery introgression are beyond the resolution of the marker system used in this study.

Genetic diversity estimates were higher for most of the above-barrier dam populations than for their below-barrier counterparts. The results of the hierarchical analysis (AMOVA) suggested significant partitioning between above and below barrier populations. The amount of variation attributed to this comparison was much larger than the partitioning detected between wild and hatchery populations, suggesting that the physical separation of these populations is acting as a reproductive isolating mechanism. The higher diversity estimates in above-barrier populations reflected similar frequencies of several different haplotypes in each of the six above-barrier populations. In contrast, the below-barrier populations had fewer individuals representing additional different haplotypes. Maintenance of diversity above the barrier dams was likely facilitated by the lack of connection among those streams and little opportunity for mixing. In contrast, access to Lake Superior suggests that mixing could occur among the below-barrier populations assuming lake-migratory components of the populations.

Hatchery vs. Wild Brook Trout

Genetic diversity of the hatchery populations varied from zero in the Phillips strain to a relatively high 62.7% in the MN Wild strain. As in the wild populations, BT1 predominated in the hatchery samples, but 6 additional haplotypes had significantly different distributions among the hatchery strains ($P < 0.001$) than among the wild populations. The MN Wild strain and the St. Croix strains were the most diverse of the hatchery strains tested; the MN Wild and Assinica strains were the only populations sampled that contained the BT10 haplotype. No partitioning of genetic variance was detected between pooled hatchery populations and wild populations in the AMOVA, most likely because both groups shared a similar overall complement of mtDNA haplotypes.

There were significant differences in mtDNA haplotype distributions between the hatchery strains and the wild populations, suggesting that the stocked fish had no detectable genetic impact on the resident populations. It is possible that hatchery fish could establish naturalized populations in streams where resident brook trout no longer exist or support dwindling populations. It is just as likely to speculate that similarities between hatchery and wild fish are due to the fact that some hatchery fish were derived from wild source populations. Neither of these hypotheses was tested in this study, but will become objectives in research

planned to survey microsatellite (nuclear) DNA marker systems (L. Miller, University of Minnesota, personal communication).

The greatest similarity between hatchery and wild populations was between the Lake Nipigon sample and the Dorion Hatchery sample. This is not surprising since the Dorion broodstock are maintained with cyclical input of randomly sampled wild fish from Lake Nipigon. Other studies of brook trout behavior and population genetics using mtDNA markers have also suggested that there is a generally low level of introgression between hatchery and wild genomes (Lachance and Mangan 1990, Danzmann et al. 1991). In contrast, recent observations of the presence of hatchery introgression in brook trout populations in Algonquin Parks have provided additional support for the use of nuclear DNA genetic markers in concert with physical studies of spawning behavior to detect the presence of introgression between hatchery and wild fish (P. Ihssen unpublished data and W. Stott, Ontario Ministry of Natural Resources, Peterborough, Ontario, personal communication). In this study, a lack of evidence for hatchery introgression with mtDNA markers contrasted with the presence of significant introgression among nuclear genes. The underlying cause for the discrepancy was a significant difference in reproductive success between hatchery females and hatchery males.

The input of hatchery fish may be having a more substantial impact on local brook trout populations through competition with resident fish. Creel surveys along the Minnesota shore showed an increase in brook trout abundance in tributaries adjacent to known stocking locations that coincided with years when brook trout were stocked (Morse 1999, Newman and Johnson 1996). It is unlikely that noticeable increases in abundance that do not correlate to expected year-class strength would be caused by a sudden influx of a naturally-produced brook trout from another location. Later surveys of this same area showed no noticeable return of hatchery-origin spawning fish to the stocked site, and brook trout showed a moderate level of fidelity to natal spawning site, based on returns of tagged fish to the same redds in multiple years (Blanchfield and Ridgway 1997, Bourke et al. 1997, and R. Swainson, Ontario Ministry of Natural Resources, personal communication).

Our interest in the interaction of wild and hatchery fish is not only limited to concerns about bottleneck effects and reduced genetic diversity. We are also concerned about changes in the genetic profiles of populations of interest that are induced by sampling error. The effect of sampling error in the process of hatchery broodstock development was evident from the data presented in this study. Dorion Hatchery broodstock use Lake Nipigon as the source population on a rotating basis which appeared to adequately capture the available wild genetic diversity. The Red Cliff Hatchery strain was developed from the Dorion broodstock and thus shows a similar suite of genetic diversity, however some haplotypes are in substantially different frequencies. These results were likely due to sampling error during the mtDNA survey, as only 48 of 200 available individuals were screened from the Red Cliff Hatchery. An additional example of the effects of sampling error can also be seen with the St. Croix and Nashua Hatchery strains screened for this survey. The Nashua strain fish tested in this study were derived from broodstock that originated at the Nashua, NH NFH and developed into the St. Croix (WI) strain. The St. Croix strain (from Spire Valley SFH, MN) was derived from broodstock developed at the St. Croix, WI SFH. Table 3 shows the disparity between the haplotype profiles of these two strains. The combination of physical sampling error and genetic sampling error during the

process of reproduction can have significant long-term impacts on the genetic characteristics of populations which originate from the same lineage.

Identification of "Coaster" Brook Trout

Behavioral and trophic polymorphisms are common among salmonid species, including anadromous and landlocked Pacific salmon (Behnke 1972), benthic and limnetic forms of arctic char (Skulason et al. 1996 and references therein), and shallow, deep, and reef-dwelling forms of lake trout (Goodier et al. 1981 and references therein). Investigations of genetic differences among populations that show morphological or behavioral variation within these species have repeatedly demonstrated that the relative importance of genetic or environmental factors in determining character variation differs among systems and among species (Skulason et al. 1996, Dynes et al. 1999). The presence of a lacustrine brook trout is not foreign to the oligotrophic lakes that were produced in the wake of Pleistocene glacial recession. Several studies of Canadian lakes have extensively documented the spawning behavior of lacustrine brook trout (Blanchfield and Ridgway 1997, Bourke et al. 1997, Ridgway and Blanchfield 1998) where redds were built along the shoreline. Bourke et al. (1997) investigated trophic polymorphisms among pelagic and benthic brook trout in two Quebec lakes and documented movement of lacustrine fish into stream inlets for spawning. Both of these spawning behaviors (shoreline and upstream) are found among suspected coaster brook trout from Lake Superior as well (Newman and DuBois 1996). The question remains regarding the role that genetic diversity plays in these behavioral and trophic polymorphisms. Dynes et al. (1999) recently investigated genetic diversity among littoral and pelagic forms of brook trout that also showed morphological variation and partial reproductive isolation. They found that some heritable genetic differences were present between the different trophic forms in one study lake, but not in the other study lake (Dynes et al. 1999). This study was not designed to look for differences between coaster and resident brook trout. We observed associations of fish that had a geographic not molecular basis which suggests that coasters are associated with streams and not a specific haplotype. Further study using nuclear markers such as microsatellite DNA and known coasters is required.

Most of the below-barrier tributaries sampled in this study are suspected to contain migratory brook trout. In Flute Reed Creek and Cascade River, no brook trout were found in extensive surveys during spring. However, spawning brook trout were encountered in fall surveys (Morse 1999). These fish were suspected to have migrated up the tributaries (reaches in these tributaries are less than one mile long) from Lake Superior. The lower levels of genetic diversity detected in the below-barrier populations may have resulted from past genetic bottlenecks. Substantial population declines of "coaster" brook trout have occurred throughout the Lake Superior drainage, and could be responsible for declines in overall genetic diversity. Equally likely may be an increased level of homogenization among below-barrier streams if lake migrants show less than 100% site fidelity. Although the below-barrier populations had lower genetic diversity estimates, they still contained a greater number of different mtDNA types, though not in high frequencies.

Management implications

Differences in mtDNA haplotype distribution among the populations sampled in this study suggest that there are more than one distinct brook trout populations in Lake Superior. Significant differences in genetic composition between hatchery and several below-barrier populations supported the presence of wild brook trout in these Lake Superior tributaries. While some mixing among adult coaster brook trout occurs in Lake Superior, there is evidence that populations have strong natal homing fidelity (Rob Swainson, 1999, Ontario Ministry of Natural Resources, personal communication). In addition, there were significant differences in the genetic composition of populations sampled in different years, supporting the presence of a heterogeneous lake-run population. Despite the high frequency of the most common mtDNA haplotype, the wild brook trout populations sampled should certainly be considered as independent entities (management units) for purposes of conservation and restoration.

With respect to the interaction between hatchery and wild fish in the wild, in *most* cases, it appeared that hatchery fish did not contribute significantly to the genetic composition of a wild population that is self-sustaining. Anecdotal explanations are that the hatchery fish are less-effective breeders, but on the other hand they tend to be more likely to be caught by anglers. An unpublished study of hatchery brook trout in Algonquin lakes suggested that hatchery females were less successful at spawning because they did not build suitable redds (W. Stott, Ontario Ministry of Natural Resources, personal communication). However, in a compromised system (low abundance of resident fish, but still self-sustaining) there may be an increased risk of physical swamping where the resident population will be negatively affected through predation by and competition with the planted fish. As in most cases, the management goal should dictate the restoration strategy -- if the goal is restoration, then it is prudent to protect native resident populations.

Despite the presence of genetic diversity, there is no evidence that coasters evolved from a unique population or colonies of brook trout. We found no direct genetic evidence from mtDNA markers that the life history pattern or behavior that managers use to define a coaster is unique to those individuals or populations that are the putative source of coasters. This does not preclude the existence of coasters, but does suggest that the cue that elicits anadromy is not singularly genetic. Future efforts to uncover commonalities in biological and behavioral characteristics among putative coaster populations may provide additional insight.

Research Needs

Similarities in the distribution of mtDNA haplotypes among unconnected river systems could be due to retained genetic diversity or hatchery influence. Preliminary information from telemetry studies suggests that some brook trout travel among different streams connected by Lake Superior, and others remain within specific river systems (C. Wilson, Ontario Ministry of Natural Resources, personal communication). Additional analyses of these populations with more sophisticated genetic markers in concert with telemetry studies and observation of spawning behavior is helping to uncover the source of the similarities. Contemporary molecular genetic technology has allowed the noninvasive assessment of genetic diversity in brook trout and other native salmonids. In a recent study, nuclear DNA variation among brook trout populations at 8 microsatellite loci was surveyed in 425 individuals among 32 sites from across

the range of brook trout in the northeastern U.S. The microsatellite DNA provided heightened resolution of stock structure among regional brook trout populations. Levels of heterozygosity ranging from 40 to 100% have the potential to identify specific populations, as well as perform parentage analyses and progeny tracking (T. L .King and M. K. Burnham-Curtis, unpublished data). These markers show promise for addressing the question of the genetic identity of coaster brook trout.

Our preliminary study of microsatellite DNA variation among Atlantic slope, Appalachian, and Great Lakes drainage populations demonstrated that there is significant genetic structuring among brook trout populations on a regional scale. Populations were compared in a phylogenetic framework and populations clustered into regional groups that were supported in 75 to100% of comparisons; within specific regional groups, genetic diversity estimates ranged from 30 to 70%. Although diversity estimates were slightly lower among Great Lakes populations, these estimates were substantially higher than the diversity estimates calculated from mtDNA data alone. For example, the Tobin Harbor population showed little diversity in the mtDNA genome, but the microsatellite DNA heterozygosity estimate for 4 loci was about 30%. No discrete markers, however, have been uncovered that uniquely correspond to the coaster morphotype.

Restoration of brook trout, especially those with a lake-migratory component, depends greatly upon a better understanding of the breeding characteristics of wild brook trout populations. Current attempts to define habitat characteristics and movement of Lake Superior brook trout populations are integral to our ability to understand population identity and gene flow among these wild stocks. Primarily, we need to answer questions about spawning site fidelity, correlation between environmental characteristics and patterns of genetic diversity, and population abundance and suitability of particular populations as potential sources for restoration activities. The genetic data alone will not be sufficient to define population structure of coaster and non-migratory brook trout in the Lake Superior basin. A coordinated effort to define correlated behavioral characteristics (migration, spawning), environmental conditions (groundwater upwellings, forage availability), and heritable characteristics will be the most profitable approach to future research on coaster brook trout.

Literature Cited

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

Behnke, R. J. 1972. The systematics of salmonid fishes of recently glaciated lakes. *Journal of the Fisheries Research Board Canada*. 29: 639-671.

Bernatchez, L. and R. G. Danzmann. 1993. Congruence in control-region sequence and restriction site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchill). *Molecular Biology and Evolution*. 10(5):1002-1014.

Blanchfield, P. J., and M. A. Ridgway. 1997. Reproductive timing and use of redd sites by lake-spawning brook trout (*Salvelinus fontinalis*). *Canadian Journal of Fisheries and Aquatic Sciences*. 54: 747-756.

Bourke, P., P. Magnan, and M. A. Rodriguez. 1997. Individual variations in habitat use and morphology in brook charr. *Journal of Fish Biology*. 51: 783-794.

Brown, W. M. 1983. Evolution of animal mitochondrial DNA. Pages 62-88 in [M. Nei and R. K. Koehn, eds.] *Evolution of Genes and Proteins*. Sinauer, Sunderland, Massachusetts.

Burnham-Curtis, M. K. 1996. Mitochondrial DNA Variation among Lake Superior Brook Trout Populations: Summary of genetic analyses. Research Completion Report, US Fish and Wildlife Service, Ashland Fisheries Resource Office, Ashland, Wisconsin.

Burnham-Curtis, M. K. 1998. Mitochondrial DNA variation among populations of brook trout (*Salvelinus fontinalis*) in the Chagrin River Drainage, Ohio and upper Ohio River drainage, Pennsylvania. Research Completion Report for Cooperative Agreement 14-48-0003-97-1080. U.S. Fish and Wildlife Service, Ohio Department of Natural Resources, U. S. Geological Survey- Biological Resources Division.

Cavalli-Sforza, L.L. and A.W.F. Edwards. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution* 21:550-570.

Danzmann, R. G., P. E. Ihssen, and P. D. N. Hebert. 1991. Genetic discrimination of wild and hatchery populations of brook charr, *Salvelinus fontinalis* (Mitchill), in Ontario using mitochondrial DNA analysis. *Journal of Fish Biology*. 39(Suppl. A): 69-78.

Danzmann, R. G., R. P. Morgan, II, M. W. Jones, L. Bernatchez, and P. E. Ihssen. 1998. A major sextet of mitochondrial DNA phylogenetic assemblages extant in eastern North American brook trout (*Salvelinus fontinalis*): distribution and postglacial dispersal patterns. *Can. J. Zoology*. 76: 1300-1318.

Dynes, J., P. Magnan, L. Bernatchez, and M. A. Rodriguez. 1999. Genetic and morphological variation between two forms of lacustrine brook charr. *Journal of Fish Biology*. 54: 955-972.

Eddy, S., and J. C. Underhill. 1974. *Northern Fishes*. University of Minnesota Press, Minneapolis, MN. 414 p.

Felsenstein, J. 1986-1999. PHYLIP 3.5 for Windows 95. University of Washington.

Halpern, T. 1995. Brook trout in Minnesota waters of Lake Superior. Minnesota Department of Natural Resources, Duluth, MN. 4pp.

Hendy, M. D., and D. Penny. 1982. Branch and bound algorithms to determine minimal evolutionary trees. *Mathematics and Bioscience*. 59: 277-290.

Kreuger, C. C., and B. Menzel. 1979. Effects of stocking on genetics of wild brook trout populations. *Transactions of the American Fisheries Society.* 108: 277-287.

Kreuger, C.C., and B. May. 1991. Ecological and genetic effects of salmonid introductions in North America. *Canadian Journal of Fisheries and Aquatic Sciences*. 48(Suppl.1): 66-77.

Lachance, S. and P. Magnan. 1990. Performance of domestic, hybrid, and wild strains of brook trout, Salvelinus fontinalis, after stocking: the impact of intra- and inter-specific competition. *Canadian Journal of Fisheries and Aquatic Sciences*. 47: 2278-2284.

Lande, R. 1995. Breeding plans for small populations, based on the dynamics of quantitative genetic variance. Pp. 318-340. In: *Population management for survival and recovery*, eds. J.D. Ballou, M. Gilpin, and T.J. Foose. Columbia University Press, New York, NY.

Leberg, P.L. 1993. Strategies for population reintroduction: effects of genetic variability on population growth and size. *Conservation Biology* 7:194-199.

Letcher, B. H., and T. L. King. 1999. Targeted stock identification using multilocus genotype `familyprinting.' *Fisheries Research*. 43: 99-111.

MacCrimmon, H. R., and J. S. Campbell. 1969. World distribution of brook trout, *Salvelinus fontinalis. Journal of the Fisheries Research Board Canada*. 26(7): 1699-1725.

McCracken, G. F., C. R. Parker, and S. Z. Guffey. 1993. Genetic differentiation and hybridization between stocked hatchery and native brook trout in Great Smoky Mountains National Park. *Transactions of the American Fisheries Society*. 108: 277-287.

Moritz, C., T. E. Dowling, and W. M. Brown. 1987. Evolution of animal mitochondrial DNA: Relevance for population biology and systematics. *Annual Review of Ecology and Systematics*. 18: 269-292.

Morse, S. D. 1999. Completion report: Lake Superior spring anadromous creek survey, 1998. Minnesota Department of Natural Resources, Division of Fish and Wildlife, Section of Fisheries. Study 4, Job 457.

Mullis, K. B., and F. A. Faloona. 1987. Specific synthesis of DNA *in vitro* via a polymerase catalyzed chain reaction. *Methods in Enzymology*. 155: 335-350.

Newman, L., and R. DuBois. 1996. Status of brook trout in Lake Superior. Prepared for the Lake Superior Technical Committee by the Brook Trout Subcommittee. Great Lakes Fishery Commission, 27 p.

Newman, L., R. DuBois, and T. Halpern, (eds.). 1998. A brook trout rehabilitation plan for Lake Superior. Great Lakes Fishery Commission Report. 25 pp.

Newman, L. and J. Johnson. 1996. Development of a reintroduced, anadromous brook trout population at Grand Portage, Minnesota, 1991-1996. Report to the Grand Portage Natural resources Department and the U. S. Fish and Wildlife Service Ashland Fisheries Resources Office. 24 p.

Nielsen, J. L. 1998. Population genetics and the conservation and management of Atlantic salmon (S*almo salar*). Canadian Journal of Fisheries and Aquatic Sciences. 55(Suppl. 1): 145-152.

Ostazeski, J., and S. Morse. 1999. Progress report: Coaster brook trout study in Lake Superior and its north shore tributaries above barriers. Minnesota Department of Natural Resources, Division of Fish and Wildlife, Fisheries Section.

Park, L. K., M. A. Brainard, D. A. Dightman, and G. A. Winans. 1993. Low levels of intraspecific variation in the mitochondrial DNA of chum salmon (*Oncorhynchus keta*). *Molecular Marine Biology and Biotechnology*. 2(6): 362-370.

Perkins, D. L., C. C. Kreuger, and B. May. 1993. Heritage brook trout in northeastern USA: Genetic variability within and among populations. *Transactions of the American Fisheries Society.* 122(4): 515-532.

Philipp, D. P., W. F. Childers, and G. S. Whitt. 1983. A biochemical genetic evaluation of the northern and Florida species of largemouth bass. *Transactions of the American Fisheries Society*. 112(1): 1-20.

Philipp, D. S., W. F. Childers, and G. S. Whitt. 1985. Correlations of allele frequencies with physical and environmental variables for populations of largemouth bass, *Micropterus salmoides* (Lacepede). *Journal of Fish Biology*. 27: 347-365.

Quattro, J. M., R. P. Morgan, II, and R. W. Chapman. 1990. Mitochondrial DNA variability in brook trout populations from Western Maryland. *American Fisheries Society Symposium.* 7:470-474.

Ridgway, M. A., and P. J. Blanchfield. 1998. Brook trout spawning areas in lakes. *Ecology of Freshwater Fish*. 7: 140-145.

Roff, D. A. and J. Bentzen. 1989. The statistical analysis of mitochondrial DNA polymorphisms: χ^2 and the problem of small samples. *Molecular Biology and Evolution*. 6:539-545.

Ryman, N. 1991. Conservation genetics considerations in fishery management. *Journal of Fish Biology*. 39(Suppl. A):211-224.

Seutin, G., B. N. White, and P. T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. *Canadian Journal of Zoology*. 69: 82-90.

Schneider, S., J.-M. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin ver. 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schreiner, D. R. 1995. Fisheries management plan for the Minnesota waters of Lake Superior. Minnesota Department of Natural Resources, Special Publication No. 149.

Skulason, S., S. S. Snorrason, D. L. G. Noakes, and M. M. Ferguson. 1996. Genetic basis of life history variations among sympatric morphs of Arctic char, *Salvelinus alpinus. Canadian Journal of Fisheries and Aquatic Science*. 53: 1807-1813

Slade, J. W. 1994. A pilot study on the status of coaster brook trout in the waters of Isle Royale National Park, Lake Superior. U. S. Fish and Wildlife Service, Ashland, WI. 19 pages.

Templeton, A.R. 1986. Coadaptation and outbreeding depression. Pp. 105-116. In: *Conservation Biology-The Science of Scarcity and Diversity*. M. Soule (ed.) Sinauer Associates, Inc., Sunderland, MA.

Thomas, W. K., and A. T. Beckenbach. 1989. Variation in salmonid mitochondrial DNA: Evolutionary constraints and mechanisms of substitution. *Journal of Molecular Evolution*. 29:233-245.

Tillma, J. S. 1997. Progress report: Coaster brook trout study in Lake Superior and its north shore tributaries. Minnesota Department of Natural Resources, Division of Fish and Wildlife, Fisheries Section.

Waters, T. F. 1977. Streams and Rivers of Minnesota. University of Minnesota Press, Minneapolis, MN. 393 p.

Webster, D. A. and W. A. Flick. 1981. Performance of indigenous, exotic, and hybrid strains of brook trout (Salvelinus fontinalis) in waters of the Adirondack Mountains, New York. *Canadian Journal of Fisheries and Aquatic Sciences*. 38: 1701-1707.

Appendix A. Glossary of genetics terms. (from Lewin 1987, Roff 1997)

Allele: basic element of inheritance. Each character is composed of a series of loci, and at each locus there may be one to several alleles, each allele contributing a different amount to the genotype and, hence, the phenotype.
Amplification: the production of multiple copies of a nucleic acid sequence.
Bootstrap: a robust statistical method by which a population is resampled and a distribution of statistical parameters is obtained. This is one method by which confidence estimates can be made for genetic characters for which the "whole" population parameter cannot be measured.
Divergence: the percent difference in nucleotide sequence between two related DNA sequences.
Genotype: genetic constitution of an organism.
Haplotype: genetic constitution of an organism defined by heritable units derived from a single parent in diploid organisms. For example, the genetic type assigned from mitochondrial DNA data is derived from the maternal parent, and so is representative of only the maternal lineage.
Heterozygosity: the presence of alternate alleles at a given locus in a diploid organism. For haploid data, alleles are considered to be alternate forms of the locus in question.
Inbreeding: the mating of related individuals.
Inbreeding depression: the decline in a trait value (fitness, genetic diversity) due to inbreeding.
Locus: the unit of inheritance. Each character is made up of one to many loci, at which there are one to many alleles. The mitochondrial DNA molecule is considered to be a single locus because it is inherited as a unit. Alternative mtDNA haplotypes represent differences in the sequences among the genes coded for in the mtDNA.
Microsatellite DNA: refers to DNA sequences defined by the presence of repetitive units of 2, 3, or 4 nucleotides. Microsatellite DNA is considered to evolve at random by the gain and loss of unit sequences and is not thought to have any coding genes. Variability in microsatellite DNA ranges from genus and species level specificity to variation at the individual level (DNA fingerprint).
Mitochondrial DNA (mtDNA): circular molecule of double-stranded DNA about 16,700 base pairs in length in teleost fishes. MtDNA is comprised of genes which code for metabolic proteins used for energy metabolism. Multiple copies of mtDNA are located within the mitochondria of each cell. Also called "organellar DNA." MtDNA is useful for genetic studies of closely related populations because it is transmitted maternally with no recombination, it has a conserved gene order, a rapid rate of divergence (compared to nuclear DNA), small genome size, and is relatively easy to extract and amplify due to a large copy number per cell.
Nuclear DNA (nucDNA): sequences of double-stranded DNA which are located within the nucleus of a cell. This DNA makes up the chromosomes. Nuclear DNA contains both coding and non-coding regions.
Outbreeding: the mating of unrelated individuals
Outbreeding depression: the depression in fitness obtained by the crossing of unrelated individuals. This applies most specifically to individuals drawn from different habitats.
Phenotype: the physical appearance of an organism resulting from the expression of a genotype and the interaction of the organism with its environment.
PCR: polymerase chain reaction; an *in vitro* method of producing additional copies of a nucleic acid sequence.
Polymorphism: refers to the simultaneous occurrence in the population of genomes showing allelic variations. The variations may show up as different phenotypes, different restriction fragment patterns, different sizes of microsatellite DNA repeat units, or different DNA sequences.
Total genomic DNA: refers to the total complement of DNA that can be extracted from cells. Includes nuclear as well as organellar DNA.

Table 1. Sampling locations for wild and hatchery brook trout 1994 to1999. Map # refers to numbered location on Figure 1.

Map#	Location	Drainage	Source	N	Year sampled
Wild Samples					
1.	Lake Nipigon (ON)	Nipigon	Above barrier	33	1998
2.	Nipigon River (ON)	Superior	Below barrier	15	1995, 1997
3.	Nipigon Bay (ON)	Superior	Below barrier	23	1995, 1998
3a.	Cypress River (ON)	Superior	Below barrier	4	1998
4.	Black Bay (ON)	Superior	Below barrier	5	1995
5.	Tobin Harbor (MI)	Superior	Below barrier	91	1995, 1998
6.	Big Siskiwit River (MI)	Superior	Below barrier	21	1994, 1995
7.	Little Siskiwit River (MI)	Superior	Below barrier	5	1994
8.	Grace Creek (MI)	Superior	Below barrier	29	1994
9.	Washington Creek (MI)	Superior	Below barrier	37	1994
10.	Washington Harbor (MI)	Superior	Lake Superior	5	1997
11.	Grand Portage Creek (MI)	Superior	Below barrier	27	1995, 1998
12.	Reservation River (MN)	Superior	Above barrier	15	1998
13.	Flute Reed Creek (MN)	Superior	Below barrier	2	1997
14.	Kimball Creek (MN)	Superior	Above barrier	43	1998
			Below barrier	24	1997
15.	Kadunce Creek (MN)	Superior	Above barrier	50	1998
			Below barrier	35	1997
16.	Devil Track River (MN)	Superior	Above barrier	50	1998
			Below barrier	55	1997
17.	Grand Marais Harbor (MN)	Superior	Lake Superior	30	1998
18.	Cascade River (MN)	Superior	Below barrier	8	1997
19.	Spruce Creek (MN)	Superior	Above barrier	50	1998
			Below barrier	47	1997
20.	Onion River (MN)	Superior	Above barrier	79	1995,1998
			Below barrier	87	1995,1997
21.	Cross River (MN)	Superior	Above barrier	68	1998
			Below barrier	35	1997
22.	Little Marais Creek (MN)	Superior	Below barrier	5	1997
23.	Baptism River (MN)	Superior	Below barrier	3	1997
24.	Split Rock River (MN)	Superior	Below barrier	11	1997
25.	Encampment River (MN)	Superior	Below barrier	1	1997
26.	Silver Creek (MN)	Superior	Below barrier	2	1997
27.	Stewart River (MN)	Superior	Below barrier	2	1997
28.	Knife River (MN)	Superior	Above barrier	28	1998
			Below barrier	3	1997
29.	French River (MN)	Superior	Below barrier	1	1997
30.	Oak Island (WI)	Superior	Below barrier	30	1995
31.	Little Onion River (WI)	Superior	Below barrier	16	1995
32.	Little Sioux River (WI)	Superior	Below barrier	15	1995
33.	Graveyard Creek (WI)	Superior	Below barrier	8	1998
34.	Keweenaw Bay (MI)	Superior	Lake Superior	4	1997
35.	Salmon Trout River (MI)	Superior	Above barrier	15	1995
			Below barrier	24	1995,1999
36.	Cliff River (MI)	Superior	Below barrier	15	1995

Table 1. Continued.

Map#	Location	Drainage	Source	N	Year sampled
37.	Kallio Creek (MI)	Superior	Below barrier	6	1995
38.	Fox River (MI)	Michigan	Above barrier	15	1997
39.	Mosquito Creek (MI)	Superior	Below barrier	9	1998
40.	Seven Mile Creek (MI)	Superior	Below barrier	8	1998
41.	Towes Creek (MI)	Superior	Above barrier	9	1998
42.	DeMull Creek (MI)	Superior	Above barrier	8	1998
43.	Sable Creek (MI)	Superior	Below barrier	8	1998
44.	Sucker River (MI)	Superior	Below barrier	33	1995, 1997
45.	Blue Jay Creek (ON)	Huron	Below barrier	9	1999
46.	Elliott Creek (MI)	Huron	Below barrier	7	1999
47.	Albany Creek (MI)	Huron	Below barrier	5	1999
48.	Black River (MI)	Huron	Below barrier	4	1999
49.	Crystal Creek (MI)	Huron	Below barrier	1	1999

Map#	Location	Strain	Source	N	Year sampled
Hatchery Samples					
50.	Dorion Hatchery (ONT)	Dorion	Lake Nipigon	33	1998
51.	Red Cliff Hatchery (WI)	Dorion	Lake Nipigon	48	1998
52.	Marquette State Fish Hatchery (MI)	Assinica	East coast, domestic	21	1997
53.	Crystal Springs Hatchery (MN)	MN Wild	Hemingway and Spring Brook (MN inland)	53	1998
54.	Phillips State Fish Hatchery Assinica (ME)	Phillips ME	Paradise, PA Hatchery	51	1998
55.	Spire Valley Fish Hatchery (MN)	St. Croix	St. Croix domestic	49	1998
56.	Egan State Fish Hatchery	Owhi	Crawford (NE) NFH	52	1998
57.	Rome Hatchery (NY)	Rome	Rome domestic	50	1998
58.	Bayfield Fish Hatchery (WI)	Nashua strain	St. Croix domestic	36	1997

Table 2. Specific gene loci targeted for analyses.

Gene	Location	Est. size (bp=base pairs)	Variable or Conserved	Primer source
Control region (D-loop)	mitochondria	1150 bp	variable	Bernatchez et al. 1992
NADH 2	mitochondria	1280 bp	variable	Park et al. 1993
NADH 5/6	mitochondria	1500 bp	variable	Park et al. 1993

Table 3. Frequency distribution of mitochondrial DNA haplotypes for Lake Superior brook trout populations sampled 1995-1999.

Location	N	BT1	BT2	BT3	BT4	BT5	BT6	BT7	BT8	BT9	BT10	BT11	BT12	BT13	BT14
Above barrier dams															
Reservation River	15	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Kimball Creek	43	0.51	0.28	--	0.14	0.05	--	--	--	0.02	--	--	--	--	--
Kadunce Creek	50	0.58	0.38	--	0.02	0.02	--	--	--	--	--	--	--	--	--
Devils Track River	50	0.46	0.34	--	0.06	0.10	0.02	--	0.02	--	--	--	--	--	--
Spruce Creek	50	0.62	0.38	--	--	--	--	--	--	--	--	--	--	--	--
Onion River	79	0.80	0.11	--	0.04	0.05	--	--	--	--	--	--	--	--	--
Cross River	68	0.40	0.26	--	0.16	0.18	--	--	--	--	--	--	--	--	--
Knife River	28	0.46	0.29	0.04	0.07	0.14	--	--	--	--	--	--	--	--	--
Salmon Trout River	15	0.73	0.07	--	--	0.20	--	--	--	--	--	--	--	--	--
DeMull Creek	8	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Towes Creek	9	0.78	--	--	--	0.22	--	--	--	--	--	--	--	--	--
Fox River	15	0.60	0.07	--	0.07	--	--	--	0.07	--	--	0.20	--	--	--
Lake Nipigon	33	0.88	--	--	--	--	--	--	--	0.12	--	--	--	--	--
Lake Superior Tributaries															
Nipigon River	13	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Nipigon Bay	23	0.83	0.04	--	--	--	--	--	--	0.13	--	--	--	--	--
Black Bay	5	0.80	--	--	--	0.20	--	--	--	--	--	--	--	--	--
Cypress River	4	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Tobins Harbor	91	0.98	--	--	0.01	--	--	--	--	0.01	--	--	--	--	--
Big Siskiwit	21	0.86	0.05	--	0.09	--	--	--	--	--	--	--	--	--	--
Little Siskiwit	5	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Grace Creek	29	0.52	0.17	--	0.07	0.24	--	--	--	--	--	--	--	--	--
Washington Creek	37	0.84	--	--	0.08	--	--	0.08	--	--	--	--	--	--	--
Washington Harbor	5	0.20	--	--	0.20	0.40	--	--	--	--	--	0.20	--	--	--
Grand Portage Creek	27	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Flute Reed Creek	2	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Kimball Creek	24	0.67	0.04	--	0.17	--	--	--	0.04	0.08	--	--	--	--	--
Kadunce Creek	35	0.97	--	--	--	--	--	--	--	0.03	--	--	--	--	--
Devils Track River	55	0.83	0.03	0.02	0.08	--	--	--	0.02	0.02	--	--	--	--	--
Grand Marais Harbor	30	0.43	0.20	--	0.20	0.13	0.04	--	--	--	--	--	--	--	--

Table 3. Continued.

Location	N	BT1	BT2	BT3	BT4	BT5	BT6	BT7	BT8	BT9	BT10	BT11	BT12	BT13	BT14
Cascade River	8	0.87	--	--	0.13	--	--	--	--	--	--	--	--	--	--
Spruce Creek	47	0.85	--	--	0.15	--	--	--	--	--	--	--	--	--	--
Onion River	87	0.65	0.05	0.05	0.15	0.10	--	--	--	--	--	--	--	--	--
Cross River	35	0.60	--	--	0.37	--	--	--	--	0.03	--	--	--	--	--
Little Marais Creek	5	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Baptism River	3	0.67	--	--	0.33	--	--	--	--	--	--	--	--	--	--
Split Rock River	11	0.82	--	--	0.18	--	--	--	--	--	--	--	--	--	--
Encampment River	1	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Silver Creek	2	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Stewart River	2	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Knife River	3	0.67	--	--	0.33	--	--	--	--	--	--	--	--	--	--
French River	1	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Oak Island	30	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Little Onion River	16	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Little Sioux River	15	0.66	--	--	0.20	--	0.07	--	0.07	--	--	--	--	--	--
Keweenaw Bay	4	0.75	--	--	--	--	--	--	--	--	--	--	--	0.25	--
Salmon Trout River	24	0.92	0.08	--	--	--	--	--	--	--	--	--	--	--	--
Cliff River	15	0.93	--	--	0.07	--	--	--	--	--	--	--	--	--	--
Kallio Creek	6	--	--	--	0.67	0.33	--	--	--	--	--	--	--	--	--
Mosquito Creek	9	0.89	0.11	--	--	--	--	--	--	--	--	--	--	--	--
Sucker River	33	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Sable River	8	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Graveyard Creek	8	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
SevenMile Creek	8	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Lake Huron															
Blue Jay Creek	9	0.67	--	--	0.22	--	--	0.11	--	--	--	--	--	--	--
Elliott Creek	7	0.71	--	--	0.29	--	--	--	--	--	--	--	--	--	--
Albany Creek	5	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Black River	4	0.50	0.50	--	--	--	--	--	--	--	--	--	--	--	--
Crystal Creek	1	--	--	--	1.00	--	--	--	--	--	--	--	--	--	--

Table 3. Continued.

Hatchery Strains															
Dorion Strain	33	0.94	--	--	--	--	--	--	--	0.06	--	--	--	--	--
Red Cliff Hatchery	48	0.46	--	--	--	--	--	0.08	--	0.46	--	--	--	--	--
Assinica Strain	21	0.81	--	--	0.05	--	--	--	--	--	0.14	--	--	--	--
St Croix Strain	49	0.75	0.10	--	--	0.05	0.08	--	0.02	--	--	--	--	--	--
Owhi Strain	52	0.81	--	--	--	0.19	--	--	--	--	--	--	--	--	--
Phillips ME Strain	51	1.00	--	--	--	--	--	--	--	--	--	--	--	--	--
Rome Strain	50	0.86	--	--	0.14	--	--	--	--	--	--	--	--	--	--
MN Wild Strain	53	0.19	0.60	--	0.19	--	--	--	--	--	0.02	--	--	--	--
Nashua Strain	38	0.45	0.42	--	0.11	0.03	--	--	--	--	--	--	--	--	--

Table 4. Genetic diversity estimates for brook trout populations from the Lake Superior basin. Included are diversity estimates for several hatchery strains which are, or are known to have been stocked in the Lake Superior basin. Expected heterozygosity (*H*) is an estimate of the probability that two randomly chosen haplotypes are different in the sample; nucleotide diversity is an estimate of gene diversity at the nucleotide level, e.g. diversity of the mtDNA in a population; mean number of pairwise differences (*B*) is a measure of the mean number of nucleotide differences among the haplotypes that are present within a population. Populations with sample sizes of less than 8 were excluded from the analysis of population structure and genetic diversity.

Population	N	Expected Heterozygosity (*H*)	Nucleotide Diversity	Mean No. Pairwise Differences (*B*)
Above Barrier Dams				
Reservation River	15	0.000	0.000	0.000
Kimball Creek	43	0.653 ± 0.052	0.023 ± 0.018	0.944 ± 0.659
Kadunce Creek	50	0.529 ± 0.040	0.015 ± 0.013	0.608 ± 0.491
Devil Track River	50	0.671 ± 0.043	0.026 ± 0.019	1.073 ± 0.720
Spruce Creek	50	0.480 ± 0.035	0.011 ± 0.011	0.481 ± 0.423
Onion River	79	0.351 ± 0.065	0.013 ± 0.012	0.539 ± 0.452
Cross River	68	0.725 ± 0.025	0.030 ± 0.021	1.250 ± 0.800
Knife River	28	0.701 ± 0.059	0.028 ± 0.021	1.169 ± 0.776
Salmon Trout River	15	0.447 ± 0.134	0.027 ± 0.021	1.104 ± 0.764
DeMull Creek	8	0.000	0.000	0.000
Towes Creek	9	0.389 ± 0.164	0.028 ± 0.023	1.167 ± 0.825
Fox River	15	0.629 ± 0.125	0.035 ± 0.025	1.428 ± 0.922
Springbrook	27	0.000	0.000	0.000
Lake Nipigon	33	0.219 ± 0.087	0.011 ± 0.011	0.439 ± 0.403
Below Barrier Dams				
Nipigon River	13	0.000	0.000	0.000
Nipigon Bay	23	0.312 ± 0.115	0.014 ± 0.013	0.561 ± 0.475
Black Bay	5	0.400 ± 0.237	0.029 ± 0.026	1.200 ± 0.908
Cypress River	4	0.000	0.000	0.000
Tobins Harbor	91	0.044 ± 0.030	0.001 ± 0.003	0.044 ± 0.112
Big Siskiwit	21	0.267 ± 0.119	0.007 ± 0.008	0.276 ± 0.310
Little Siskiwit River	5	0.000	0.000	0.000
Grace Creek	29	0.662 ± 0.065	0.033 ± 0.023	1.325 ± 0.849
Washington Creek	37	0.293 ± 0.092	0.007 ± 0.009	0.306 ± 0.324
Washington Harbor	6	0.867 ± 0.129	0.055 ± 0.040	2.267 ± 1.441
Grand Portage Creek	27	0.000	0.000	0.000
Kimball Creek	24	0.539 ± 0.109	0.021 ± 0.017	0.851 ± 0.624
Kadunce Creek	35	0.301 ± 0.091	0.009 ± 0.010	0.366 ± 0.361
Devil Track River	55	0.298 ± 0.079	0.010 ± 0.010	0.420 ± 0.389
Grand Marais Harbor	30	0.737 ± 0.052	0.031 ± 0.022	1.267 ± 0.820

Table 4. continued.

Population	N	Expected Heterozygosity (*H*)	Nucleotide Diversity	Mean No. Pairwise Differences (*B*)
Cascade River	8	0.250 ± 0.180	0.006 ± 0.008	0.250 ± 0.311
Spruce Creek	47	0.259 ± 0.073	0.006 ± 0.007	0.259 ± 0.293
Onion River	87	0.540 ± 0.056	0.023 ± 0.017	0.957 ± 0.660
Cross River	35	0.516 ± 0.049	0.014 ± 0.013	0.594 ± 0.487
Knife River	3	0.000	0.000	0.000
Split Rock River	11	0.327 ± 0.153	0.008 ± 0.010	0.327 ± 0.354
Oak Island	30	0.000	0.000	0.000
Little Onion River	16	0.000	0.000	0.000
Little Sioux River	15	0.542 ± 0.132	0.022 ± 0.183	0.914 ± 0.669
Keweenaw Bay	4	0.500 ± 0.265	0.061 ± 0.049	2.500 ± 1.685
Salmon Trout River	24	0.159 ± 0.094	0.004 ± 0.006	0.159 ± 0.227
Cliff River	15	0.133 ± 0.112	0.003 ± 0.006	0.133 ± 0.209
Kallio Creek	6	0.533 ± 0.172	0.026 ± 0.023	1.067 ± 0.811
Mosquito Creek	9	0.222 ± 0.166	0.005 ± 0.008	0.222 ± 0.288
Sucker River	33	0.000	0.000	0.000
Sable Creek	8	0.000	0.000	0.000
Sevenmile Creek	7	0.000	0.000	0.000
Graveyard Creek	8	0.000	0.000	0.000
Lake Huron				
Blue Jay Creek	9	0.555 ± 0.165	0.015 ± 0.015	0.611 ± 0.530
Elliott Creek	7	0.476 ± 0.171	0.011 ± 0.013	0.476 ± 0.464
Albany Creek	5	0.000	0.000	0.000
Black River	4	0.667 ± 0.204	0.016 ± 0.018	0.667 ± 0.626
Crystal Creek	1	0.000	0.000	0.000
Hatchery Strains				
Dorion Strain	33	0.117 ± 0.073	0.006 ± 0.007	0.235 ± 0.279
Red Cliff Hatchery	48	0.585 ± 0.033	0.028 ± 0.021	1.170 ± 0.767
Assinica Strain	21	0.338 ± 0.120	0.014 ± 0.013	0.581 ± 0.487
St. Croix Strain	49	0.419 ± 0.084	0.021 ± 0.017	0.864 ± 0.620
Owhi Strain	52	0.317 ± 0.068	0.023 ± 0.018	0.950 ± 0.661
Phillips Strain	51	0.000	0.000	0.000
Rome Strain	50	0.246 ± 0.071	0.006 ± 0.008	0.246 ± 0.283
Minnesota Wild Strain	53	0.574 ± 0.058	0.021 ± 0.017	0.861 ± 0.617
Nashua Strain	38	0.627 ± 0.042	0.019 ± 0.016	0.795 ± 0.589

Table 5. Matrix of population pairwise genetic distance (Fst; lower triangular matrix) and significance of differences between pairwise comparisons (upper triangular matrix). Significance values are coded as follows: n.s. = not significant; * = P < 0.05; ** = P < 0.005; *** = P < 0.001.

		Reservation	KimballA	KadunceA	DevilTrackA	SpruceA	OnionA	CrossA	KnifeRiver
Reservation River	Above barrier	----	ns	*	*	ns	ns	***	***
Kimball Creek		0.1367	-----	*	ns	***	ns	ns	ns
Kadunce Creek		0.2303	0.0157	-----	ns	***	*	ns	*
Devil Track		0.2221	0.0074	0.0200	-----	***	*	ns	ns
Spruce Creek		0.2553	0.0358	-0.0158	0.0472	----	*	***	***
Onion River		0.0406	0.0344	0.0804	0.1180	0.0856	-----	***	**
Cross River		0.2238	0.0283	0.0911	0.0114	0.1259	0.1413	----	ns
Knife River		0.2480	0.0078	0.0440	0.0199	0.0819	0.1302	-0.0124	-----
Salmon Trout		0.1714	-0.0165	0.0585	0.0098	0.0963	0.0315	0.0002	-0.0168
Towes Creek		0.2065	0.0000	0.0812	0.0124	0.1295	0.0222	-0.0119	0.0221
DeMull Creek		0.0000	0.0940	0.1883	0.1802	0.2130	0.0069	0.1875	0.1907
Fox River		0.2347	0.0028	0.1053	0.0050	0.1560	0.1158	-0.0285	-0.0229
Lake Nipigon		0.0455	0.1642	0.2471	0.2561	0.2587	0.0987	0.2601	0.2776
Nipigon River		0.0000	0.1273	0.2207	0.2126	0.2455	0.0342	0.2158	0.2343
Nipigon Bay		0.0473	0.1214	0.1955	0.2076	0.2060	0.0745	0.2210	0.2204
Black Bay		0.2405	0.0000	0.0386	-0.0272	0.0910	-0.0454	-0.0470	-0.0613
Tobin Harbor		-0.0336	0.2892	0.3936	0.3940	0.4170	0.1062	0.3684	0.4813
Little Siskiwit		0.0000	0.0525	0.1512	0.1429	0.1768	-0.0344	0.1533	0.1454
Big Siskiwit		0.0125	0.0796	0.1773	0.1739	0.1999	0.0034	0.1720	0.1914
Grace Creek		0.2414	0.0272	0.1019	0.0096	0.1456	0.1483	-0.0205	-0.0230
Washington Harbor		0.5225	0.1883	0.3865	0.1799	0.4611	0.4063	0.0571	0.1099
Washington Creek		0.0126	0.1454	0.2468	0.2435	0.2667	0.0548	0.2329	0.2704
Grand Portage Creek		0.0000	0.1781	0.2749	0.2663	0.3013	0.0635	0.2603	0.3132
Kimball Creek		0.0393	0.0427	0.1395	0.1306	0.1586	0.0229	0.1302	0.1318
Kadunce Creek		0.0360	0.1187	0.2394	0.2239	0.2643	0.0473	0.2046	0.2424
Devil Track River		-0.0068	0.0904	0.1633	0.1899	0.1707	0.0064	0.1985	0.2137
Grand Marais Harbor		0.2229	0.0125	0.1040	0.0157	0.1467	0.1291	-0.0169	-0.0051
Cascade River		0.0840	0.0563	0.1837	0.1526	0.2197	-0.0088	0.1416	0.1527
Spruce Creek		0.0634	0.1428	0.2710	0.2523	0.2985	0.0561	0.2254	0.2827
Onion River		0.1008	0.0170	0.0986	0.0695	0.1210	0.0362	0.0476	0.0480
Cross River		0.2209	0.1343	0.2991	0.2280	0.3395	0.1521	0.1669	0.2250
Split Rock River		0.1406	0.0701	0.2105	0.1669	0.2508	0.0221	0.1443	0.1671
Oak Island		0.0000	0.1866	0.2844	0.2756	0.3110	0.0675	0.2679	0.3270
Little Onion		0.0000	0.1409	0.2347	0.2265	0.2598	0.0433	0.2275	0.2544
Little Sioux		0.1429	0.0234	0.1414	0.0891	0.1777	0.0469	0.0776	0.0896
Keweenaw Bay		0.3548	0.1583	0.3327	0.2175	0.3865	0.2691	0.1927	0.1936
Salmon Trout		0.0157	0.1011	0.1591	0.1850	0.1680	0.0103	0.2023	0.2173
Cliff Creek		0.0000	0.1085	0.2170	0.2011	0.2455	0.0229	0.1953	0.2181
Kallio Creek		0.8091	0.4064	0.5969	0.3774	0.6652	0.6085	0.2264	0.3196
Mosquito Creek		0.0600	0.0667	0.1890	0.1620	0.2230	-0.0028	0.1528	0.1649
Sucker River		0.0000	0.1948	0.2934	0.2845	0.3203	0.0711	0.2725	0.3400
Sable Creek		0.0000	0.0940	0.1883	0.1802	0.2130	0.0069	0.1875	0.1907
Graveyard Creek		0.0000	0.0940	0.1883	0.1802	0.2130	0.0069	0.1875	0.1907
Seven Mile Creek		0.0000	0.0836	0.1787	0.1706	0.2036	-0.0029	0.1788	0.1785
Dorion strain		-0.0035	0.1679	0.2551	0.2618	0.2703	0.0723	0.2617	0.2962
Nashua Strain		0.2490	0.0000	0.0000	0.0000	0.0133	0.1016	0.0380	0.0008
Assinica strain		0.0981	0.1083	0.2338	0.1910	0.2650	0.0742	0.1758	0.2003

Table 5. continued

		SalmonTroutA	TowesCreek	DeMullCreek	Fox River	LakeNipigon	NipigonRiver	NipigonBay	BlackBay
Reservation River	Above barrier	ns	ns	ns	***	ns	ns	ns	ns
Kimball Creek		ns	ns	ns	ns	***	ns	ns	ns
Kadunce Creek		ns	**	ns	ns	***	*	ns	ns
Devil Track		ns	ns	ns	ns	***	*	ns	ns
Spruce Creek		**	ns	ns	***	ns	ns	ns	ns
Onion River		ns	ns	ns	ns	*	ns	ns	ns
Cross River		ns	**	*	ns	***	***	ns	ns
Knife River		ns	ns	ns	ns	***	*	ns	ns
Salmon Trout		-----	ns	ns	ns	**	ns	ns	ns
Towes Creek		-0.0944	----	ns	ns	ns	ns	ns	ns
DeMull Creek		0.0986	0.1072	----	ns	ns	ns	ns	ns
Fox River		-0.0478	-0.0714	0.1555	-----	***	ns	ns	ns
Lake Nipigon		0.1888	0.1889	0.0038	0.2600	-----	ns	ns	ns
Nipigon River		0.1541	0.1826	0.0000	0.2156	0.0369	-----	ns	ns
Nipigon Bay		0.1357	0.1309	0.0001	0.1996	-0.0338	0.0373	----	ns
Black Bay		-0.1486	-0.1824	0.1011	-0.1164	0.1453	0.2073	0.0811	-----
Tobin Harbor		0.4279	0.4993	-0.0662	0.5212	0.1525	-0.0392	0.1811	0.5323
Little Siskiwit		0.0401	0.0337	0.0000	0.0964	-0.0416	0.0000	-0.0483	0.0000
Big Siskiwit		0.0935	0.0922	-0.0319	0.1571	0.0647	0.0034	0.0528	0.0464
Grace Creek		-0.0227	-0.0395	0.1854	-0.0426	0.2775	0.2281	0.2250	-0.0773
Washington Harbor		0.0850	0.0346	0.3823	-0.0021	0.4678	0.4902	0.3854	-0.0111
Washington Creek		0.1680	0.1678	-0.0254	0.2407	0.0797	0.0052	0.0796	0.1236
Grand Portage Creek		0.2531	0.3177	0.0000	0.3250	0.0805	0.0000	0.0904	0.3871
Kimball Creek		0.0396	0.0170	-0.0062	0.0751	0.0275	0.0298	0.0075	-0.0449
Kadunce Creek		0.1318	0.1196	-0.0043	0.1926	0.0602	0.0278	0.0576	0.0677
Devil Track River		0.1025	0.0945	-0.0412	0.1879	0.0492	-0.0131	0.0372	0.0301
Grand Marais Harbor		-0.0145	-0.0325	0.1686	-0.0461	0.2587	0.2102	0.2094	-0.0731
Cascade River		0.0445	0.0276	0.0000	0.0878	0.0323	0.0643	0.0213	-0.0191
Spruce Creek		0.1703	0.1656	0.0249	0.2380	0.1143	0.0556	0.1154	0.1201
Onion River		-0.0292	-0.0573	0.0674	-0.0074	0.1434	0.0941	0.1197	-0.1113
Cross River		0.1255	0.0945	0.1709	0.1241	0.2121	0.2093	0.1936	0.0515
Split Rock River		0.0601	0.0406	0.0599	0.0953	0.0832	0.1215	0.0690	-0.0062
Oak Island		0.2701	0.3399	0.0000	0.3435	0.0873	0.0000	0.0990	0.4146
Little Onion		0.1795	0.2177	0.0000	0.2436	0.0493	0.0000	0.0518	0.2558
Little Sioux		0.0124	0.0000	0.0734	0.0091	0.1558	0.1265	0.1169	-0.0697
Keweenaw Bay		0.0998	0.0457	0.1864	0.0707	0.1790	0.3158	0.1133	-0.0371
Salmon Trout		0.1420	0.1702	-0.0272	0.2228	0.0713	0.0069	0.0544	0.1509
Cliff Creek		0.1240	0.1324	-0.0480	0.1827	0.0469	-0.0100	0.0450	0.1156
Kallio Creek		0.3281	0.2954	0.7225	0.1892	0.6960	0.7905	0.6392	0.2941
Mosquito River		0.0592	0.0466	-0.0141	0.1057	0.0335	0.0429	0.0246	0.0048
Sucker River		0.2862	0.3607	0.0000	0.3609	0.0937	0.0000	0.1073	0.4397
Sable Creek		0.0986	0.1072	0.0000	0.1555	0.0038	0.0000	0.0001	0.1011
Graveyard Creek		0.0986	0.1072	0.0000	0.1555	0.0038	0.0000	0.0001	0.1011
Seven Mile Creek		0.0831	0.870	0.0000	0.1393	-0.0072	0.0000	-0.0119	0.0728
Dorion Strain		0.2118	0.2298	-0.0411	0.2906	-0.0086	-0.0107	-0.0053	0.2045
Nashua Strain		0.0293	0.0424	0.2004	0.0427	0.2671	0.2376	0.4448	0.0019
Assinica strain		0.0975	0.0751	0.0434	0.1178	0.1192	0.0858	0.1045	0.0226

Table 5. continued

		TobinHarbor	LittleSiskiwit	BigSiskiwit	GraceCreek	Wash. Harbor	Wash. Creek	GrandPortage
Reservation River	Above barrier	ns	ns	ns	*	***	ns	ns
Kimball Creek		***	ns	*	*	**	***	*
Kadunce Creek		***	ns	***	***	***	***	**
Devil Track		***	ns	**	ns	***	***	*
Spruce Creek		ns	ns	ns	***	***	**	ns
Onion River		***	ns	ns	*	***	*	ns
Cross River		***	ns	***	ns	***	***	***
Knife River		***	ns	***	ns	***	***	***
Salmon Trout		**	ns	ns	ns	***	*	*
Towes Creek		ns	ns	ns	ns	ns	ns	ns
DeMull Creek		ns	ns	ns	ns	ns	ns	ns
Fox River		***	ns	*	ns	*	*	*
Lake Nipigon		ns	ns	ns	***	***	*	ns
Nipigon River		ns	ns	ns	*	***	ns	ns
Nipigon Bay		*	ns	ns	ns	***	ns	ns
Black Bay		ns	ns	ns	ns	*	ns	ns
Tobin Harbor		-----	ns	ns	***	***	**	ns
Little Siskiwit		-0.1108	-----	ns	ns	*	ns	ns
Big Siskiwit		0.0747	-0.0805	-----	*	***	ns	ns
Grace Creek		0.4722	0.1407	0.1854	-----	***	***	**
Washington Harbor		0.8116	0.2807	0.4270	0.0328	-----	***	***
Washington Creek		0.0598	-0.0705	-0.0068	0.2609	0.5130	-----	ns
Grand Portage Creek		-0.0172	0.0000	0.0495	0.3048	0.6520	0.0402	-----
Kimball Creek		0.1659	-0.0542	-0.0070	0.1268	0.2270	0.0347	0.0797
Kadunce Creek		0.1110	-0.0494	-0.0199	0.2264	0.4379	0.0080	0.0683
Devil Track River		0.0407	-0.0854	-0.0272	0.2189	0.4699	0.0126	0.0141
Grand Marais Harbor		0.4434	0.1242	0.1560	-0.0203	0.0318	0.2298	0.2835
Cascade River		0.1342	-0.0687	-0.0806	0.1364	0.2743	-0.0498	0.1760
Spruce Creek		0.1289	-0.0179	-0.0152	0.2636	0.5246	0.0156	0.0949
Onion River		0.1881	0.0294	0.0510	0.0327	0.1544	0.0966	0.1266
Cross River		0.3959	0.1289	0.1151	0.1820	0.2491	0.1521	0.2760
Split Rock River		0.2617	-0.0041	-0.0373	0.1444	0.2846	-0.0052	0.2306
Oak Island		-0.0151	0.0000	0.0567	0.3181	0.6739	0.0452	0.0000
Little Onion		-0.0313	0.0000	0.0165	0.2476	0.5370	0.0158	0.0000
Little Sioux		0.3704	0.0164	0.0421	0.0751	0.1568	0.1022	0.2196
Keweenaw Bay		0.7027	0.0625	0.2532	0.1622	0.0185	0.3158	0.5157
Salmon Trout		0.7424	-0.0749	0.0037	0.2259	0.5356	0.0443	0.0507
Cliff Creek		0.1836	-0.0995	-0.0449	0.2068	0.4449	-0.0210	0.0415
Kallio Creek		0.9242	0.6527	0.6896	0.2193	-0.0714	0.7127	0.8750
Mosquito Creek		0.1010	-0.0778	-0.0749	0.1500	0.3068	-0.0453	0.1394
Sucker River		-0.0134	0.0000	0.0635	0.3308	0.6932	0.0498	0.0000
Sable Creek		-0.0662	0.0000	-0.0319	0.1854	0.3823	-0.0254	0.0000
Graveyard Creek		-0.0662	0.0000	-0.0319	0.1854	0.3823	-0.0254	0.0000
Seven Mile Creek		-0.0765	0.0000	-0.0437	0.1735	0.3532	-0.0361	0.0000
Dorion Strain		0.0566	-0.0869	0.0343	0.2936	0.5564	0.0444	0.0225
Nashua Strain		0.4448	0.1603	0.1850	0.0450	0.2681	0.2605	0.3033
Assinica strain		0.2555	-0.0068	0.0258	0.1798	0.3172	0.0557	0.1540

Table 5. continued

		KimballB	KadunceB	DevilTrackB	GrandMarais	Cascade	SpruceB	OnionB	CrossB
Reservation River	Above barrier	ns	*	ns	ns	ns	ns	ns	ns
Kimball Creek		*	***	***	ns	ns	***	ns	*
Kadunce Creek		***	***	***	*	ns	***	***	***
Devil Track		***	***	***	ns	ns	***	***	***
Spruce Creek		ns	ns	ns	***	ns	ns	ns	**
Onion River		ns	*	ns	ns	ns	*	ns	**
Cross River		***	***	***	*	*	***	***	***
Knife River		***	***	***	ns	ns	***	***	***
Salmon Trout		ns	*	*	ns	ns	**	ns	*
Towes Creek		ns	ns	ns	ns	ns	*	ns	ns
DeMull Creek		ns	ns	ns	ns	ns	ns	ns	ns
Fox River		ns	***	*	ns	ns	***	ns	*
Lake Nipigon		ns	ns	ns	***	ns	ns	*	*
Nipigon River		ns	ns	ns	ns	ns	ns	ns	ns
Nipigon Bay		ns	ns	ns	ns	ns	ns	ns	ns
Black Bay		ns	ns	ns	ns	ns	ns	ns	ns
Tobin Harbor		*	ns	*	***	ns	ns	***	***
Little Siskiwit		ns	ns	ns	ns	ns	ns	ns	ns
Big Siskiwit		ns	ns	ns	ns	ns	ns	ns	ns
Grace Creek		**	***	***	ns	ns	***	***	***
Washington Harbor		***	***	***	**	***	***	***	***
Washington Creek		*	*	*	**	ns	ns	*	ns
Grand Portage Creek		ns	ns	ns	**	ns	ns	ns	ns
Kimball Creek		-----	ns	ns	*	ns	*	ns	*
Kadunce Creek		-0.0081	-----	ns	ns	ns	ns	*	*
Devil Track River		0.0034	0.0044	-----	***	ns	ns	ns	**
Grand Marais Harbor		0.0932	0.1881	0.1905	-----	ns	**	ns	*
Cascade River		-0.0517	-0.0766	-0.0507	0.1063	-----	ns	ns	ns
Spruce Creek		0.0183	-0.0198	0.0159	0.2201	-0.0763	-----	*	ns
Onion River		0.0342	0.0689	0.0678	0.0194	0.0165	0.0770	-----	***
Cross River		0.0439	0.0742	0.1469	0.1232	0.0380	0.0928	0.0646	-----
Split Rock River		-0.0345	-0.0537	-0.0090	0.1077	-0.1074	-0.0550	0.0218	0.0105
Oak Island		0.0877	0.0745	0.0175	0.2963	0.1950	0.1010	0.1315	0.2876
Little Onion		0.0436	0.0395	-0.0042	0.2289	0.0931	0.0669	0.1037	0.2264
Little Sioux		-0.0146	0.0496	0.0601	0.0292	-0.0158	0.0706	0.0073	0.0219
Keweenaw Bay		0.0220	0.2281	0.2637	0.1288	0.0906	0.3426	0.1503	0.1511
Salmon Trout		0.0423	0.0631	-0.0104	0.2083	0.0400	0.0888	0.0898	0.2392
Cliff Creek		0.0020	-0.0232	-0.0246	0.1810	-0.0823	-0.0147	0.0689	0.1362
Kallio Creek		0.4706	0.6671	0.6745	0.2053	0.6044	0.7301	0.3528	0.4585
Mosquito Creek		-0.0407	-0.0662	-0.0462	0.1211	-0.1328	-0.0644	0.0279	0.0589
Sucker River		0.0954	0.0803	0.0204	0.3085	0.2129	0.1067	0.1360	0.2987
Sable Creek		-0.0062	-0.0043	-0.0412	0.1686	0.0000	0.0249	0.0674	0.1709
Graveyard Creek		-0.0062	-0.0043	-0.0412	0.1686	0.0000	0.0249	0.0674	0.1709
Seven Mile Creek		-0.0180	-0.0152	-0.0516	0.1569	-0.0182	0.0145	0.0583	0.1599
Dorion Strain		0.0386	0.0429	0.0177	0.2727	0.0187	0.0849	0.1321	0.2876
Nashua strain		0.1302	0.2401	0.1875	0.0425	0.1721	0.2754	0.0773	0.2561
Assinica strain		-0.0010	0.0162	0.0422	0.1360	-0.0378	0.0269	0.0620	0.0519

Table 5. continued

		Split Rock	OakIsland	LittleOnion	LittleSioux	KeweenawBay	SalmonTroutB	CliffCreek	KallioCreek
Reservation River	Above barrier	ns	ns	ns	ns	ns	ns	ns	***
Kimball Creek		*	***	ns	ns	ns	ns	ns	**
Kadunce Creek		ns	***	*	**	*	*	*	***
Devil Track		ns	***	*	*	*	*	*	***
Spruce Creek		ns	ns	ns	***	ns	ns	ns	***
Onion River		**	*	ns	ns	ns	ns	ns	***
Cross River		*	***	***	**	***	***	***	**
Knife River		ns	***	**	*	ns	**	*	**
Salmon Trout		ns	*	*	ns	ns	ns	ns	***
Towes Creek		ns	ns	ns	ns	ns	ns	ns	ns
DeMull Creek		ns	ns	ns	ns	ns	ns	ns	ns
Fox River		ns	***	*	ns	ns	ns	*	ns
Lake Nipigon		ns	ns	ns	***	ns	ns	ns	***
Nipigon River		ns	ns	ns	ns	ns	ns	ns	***
Nipigon Bay		ns	ns	ns	ns	ns	ns	ns	***
Black Bay		ns	ns	ns	ns	ns	ns	ns	ns
Tobin Harbor		ns	ns	ns	***	**	ns	ns	***
Little Siskiwit		ns	ns	ns	ns	ns	ns	ns	ns
Big Siskiwit		ns	ns	ns	*	ns	ns	ns	***
Grace Creek		*	***	*	ns	ns	ns	*	***
Washington Harbor		***	***	***	*	*	ns	***	ns
Washington Creek		ns	ns	ns	ns	ns	ns	ns	***
Grand Portage Creek		ns	ns	ns	*	ns	ns	ns	***
Kimball Creek		ns	*	ns	ns	ns	ns	ns	***
Kadunce Creek		ns	ns	ns	**	*	ns	ns	***
Devil Track River		ns	ns	ns	**	ns	ns	ns	***
Grand Marais Harbor		ns	**	ns	ns	ns	ns	ns	**
Cascade River		ns	ns	ns	ns	ns	ns	ns	***
Spruce Creek		ns	ns	ns	*	**	ns	ns	***
Onion River		ns	ns	ns	ns	ns	ns	ns	***
Cross River		ns	*	ns	ns	ns	*	ns	***
Split Rock River		-----	ns	ns	ns	ns	ns	ns	***
Oak Island		0.2491	-----	ns	***	*	ns	ns	***
Little Onion		0.1495	0.0000	-----	*	ns	ns	ns	***
Little Sioux		-0.0152	0.2357	0.1504	-----	ns	ns	ns	**
Keweenaw Bay		0.1204	0.5437	0.3725	0.0246	-----	ns	ns	**
Salmon Trout		0.1103	0.0575	0.0195	0.1313	0.3742	-----	ns	ns
Cliff Creek		-0.0171	0.0498	0.0044	0.0720	0.2628	0.0215	-----	***
Kallio Creek		0.5877	0.8849	0.8171	0.3841	0.3039	0.7801	0.7346	-----
Mosquito Creek		-0.0907	0.1559	0.0680	0.0013	0.1218	0.0324	-0.0834	0.6312
Sucker River		0.2667	0.0000	0.0000	0.2508	0.5686	0.0638	0.0575	0.8934
Sable Creek		0.0599	0.0000	0.0000	0.0734	0.1864	-0.0272	-0.0480	0.7225
Graveyard Creek		0.0599	0.0000	0.0000	0.0734	0.1864	-0.0272	-0.0480	0.7225
Seven Mile Creek		0.0428	0.0000	0.0000	0.0583	0.1515	-0.0388	-0.0606	0.7029
Dorion Strain		0.0882	0.0271	-0.0005	0.1733	0.3170	0.0332	0.0092	0.7749
Nashua Strain		0.1894	0.3148	0.2544	0.1047	0.2676	0.1929	0.2234	0.4857
Assinica strain		-0.0288	0.1657	0.1037	-0.0248	0.0866	0.1184	0.0319	0.5490

Table 5. continued

		Mosquito	Sucker River	Sable Creek	Graveyard	Seven Mile	Dorion	Nashua	Assinica
Reservation River	Above barrier	ns	ns	ns	ns	ns	*	ns	ns
Kimball Creek		ns	***	ns	ns	ns	**	*	*
Kadunce Creek		ns	***	ns	ns	ns	**	**	**
Devil Track		ns	***	ns	ns	ns	**	***	***
Spruce Creek		ns	ns	ns	ns	ns	*	*	*
Onion River		*	*	ns	ns	ns	*	ns	*
Cross River		ns	***	**	*	*	***	ns	***
Knife River		ns	***	ns	ns	ns	**	***	***
Salmon Trout		ns	*	ns	ns	ns	**	**	ns
Towes Creek		ns	ns	ns	ns	ns	*	*	ns
DeMull Creek		ns	ns	ns	ns	ns	*	ns	ns
Fox River		ns	***	ns	ns	ns	***	***	ns
Lake Nipigon		ns	ns	ns	ns	ns	*	ns	*
Nipigon River		ns	ns	ns	ns	ns	ns	ns	ns
Nipigon Bay		ns	ns	ns	ns	ns	ns	ns	ns
Black Bay		ns	ns	ns	ns	ns	*	**	ns
Tobin Harbor		ns	ns	ns	ns	ns	*	ns	*
Little Siskiwit		ns	ns	ns	ns	ns	**	***	ns
Big Siskiwit		ns	ns	ns	ns	ns	***	ns	ns
Grace Creek		ns	***	ns	ns	ns	ns	***	***
Washington Harbor		***	***	ns	ns	ns	ns	ns	***
Washington Creek		ns	ns	ns	ns	ns	ns	ns	ns
Grand Portage Creek		ns	ns	ns	ns	ns	*	*	ns
Kimball Creek		ns	*	ns	ns	ns	ns	ns	ns
Kadunce Creek		ns	ns	ns	ns	ns	ns	ns	*
Devil Track River		ns	ns	ns	ns	ns	ns	ns	*
Grand Marais Harbor		ns	***	ns	ns	ns	ns	ns	*
Cascade River		ns	ns	ns	ns	ns	ns	ns	ns
Spruce Creek		ns	ns	ns	ns	ns	*	ns	*
Onion River		ns	ns	ns	ns	ns	*	ns	*
Cross River		ns	*	ns	ns	ns	**	ns	ns
Split Rock River		ns	ns	ns	ns	ns	ns	ns	ns
Oak Island		ns	ns	ns	ns	ns	**	ns	ns
Little Onion		ns	ns	ns	ns	ns	**	ns	ns
Little Sioux		ns	**	ns	ns	ns	*	ns	ns
Keweenaw Bay		ns	ns	ns	ns	ns	**	ns	ns
Salmon Trout		ns	ns	ns	ns	ns	ns	ns	*
Cliff Creek		ns	ns	ns	ns	ns	**	ns	ns
Kallio Creek		***	***	ns	ns	ns	ns	ns	***
Mosquito Creek		-----	ns	ns	ns	ns	*	ns	ns
Sucker River		0.1715	-----	ns	ns	ns	**	ns	ns
Sable Creek		-0.0141	0.0000	-----	ns	ns	*	ns	ns
Graveyard Creek		-0.0141	0.0000	0.0000	-----	ns	ns	ns	ns
Seven Mile Creek		-0.0307	0.0000	0.0000	0.0000	-----	ns	ns	ns
Dorion Strain		0.0138	0.0312	-0.0412	-0.0412	-0.0520	----	ns	ns
Nashua Strain		0.1814	0.3258	0.2004	0.2004	0.1898	0.2821	----	ns
Assinica strain		-0.0241	0.1768	0.0434	0.0434	0.0306	0.1190	0.2158	-----

Table 6. Results of hierarchical analyses of molecular variance (AMOVA).

Variance Component	df	Variance	% of Total	P^3	Φ statistic
Among all populations	62	0.0626	17.8	<0.001	Φ_{ST} = 0.178
Among all below-barrier populations	36	0.0342	12.9	<0.001	Φ_{ST} = 0.129
Above vs. Below-barrier populations					
Among groups	1	0.0233	6.6	0.002	Φ_{CT} = 0.066
Among populations within groups	49	0.0360	10.2	<0.001	Φ_{SC} = 0.110
Within populations	1209	0.2927	83.2	<0.001	Φ_{ST} = 0.168
Hatchery vs. Wild populations					
Among groups	1	0.0000	0	0.489	Φ_{CT} = -0.005
Among populations within groups	59	0.0614	17.3	<0.001	Φ_{SC} = 0.172
Within populations	1621	0.2955	83.2	<0.001	Φ_{ST} = 0.167
Among geographic regions: Canada/Minnesota/Isle Royale/Wisconsin/Michigan/Lake Huron					
Among regions	5	0.0000	0	0.665	Φ_{CT} = -0.017
Among populations within region	31	0.0375	14.2	<0.001	Φ_{SC} = 0.139
Within populations	747	0.2316	87.5	<0.001	Φ_{ST} = 0.125
Among lake basins: Lake Superior (below barrier) vs Lake Huron (below barrier)					
Among basin	1	0.0000	0	0.963	Φ_{CT} = -0.032
Among populations within basin	35	0.0348	13.5	<0.001	Φ_{SC} = 0.130
Within populations	747	0.2316	89.7	<0.001	Φ_{ST} = 0.103

Φ_{CT} = estimate of differentiation among groups (correlation of alleles within groups)
Φ_{SC} = estimate of differentiation among populations within groups (correlation of alleles within populations relative to groups)
Φ_{ST} = estimate of differentiation among populations (correlation of alleles within a population relative to the species)

[3] Probability of more extreme variance estimates in 1000 random permutations.

Figure 1. Locations of brook trout samples taken for this study. Numbers on map correspond to numbered locations listed in Table 1.

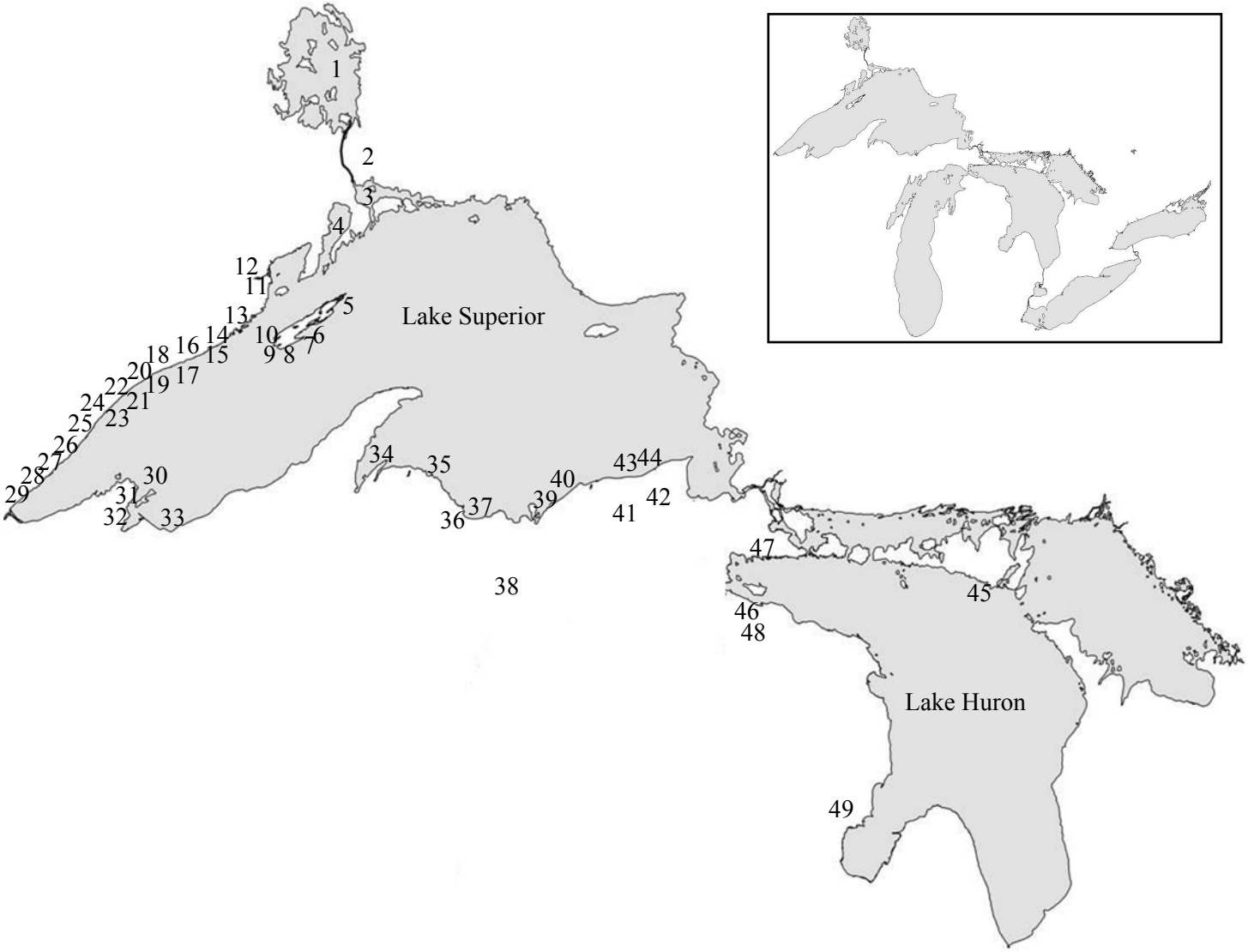

Figure 2. Neighbor-joining dendrogram among all populations based on Cavalli-Sforza-Edwards chord distances. Numbers at branching points indicate the percent of trees in 5000 bootstrap replicates that contained this arrangement of populations. Hatchery stocks are indicated in shaded italics, suspected coaster populations are underlined, Lake Huron populations are labeled with "LH," and above-barrier populations are labeled with an "A."

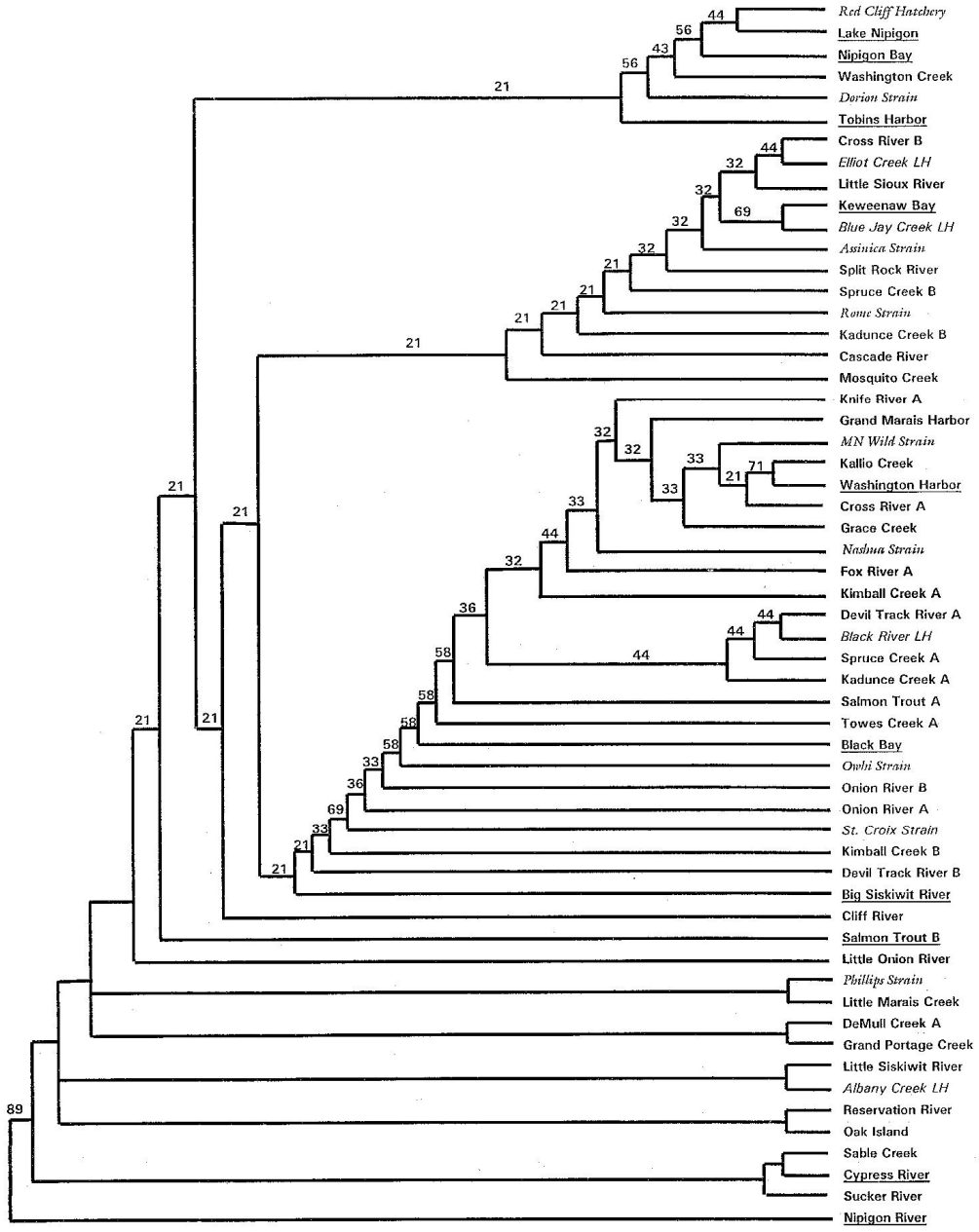

Figure 3. Neighbor-joining dendrogram among below-barrier populations based on Cavalli-Sforza-Edwards chord distances. Numbers at some branching points indicate the percent of trees in 5000 bootstrap replicates that contain this arrangement of populations (branches supported in less than 45% of bootstrap tests were not labeled). Suspected coaster populations are underlined, hatchery populations are in script italics, and Lake Huron below barrier populations are in normal italics.

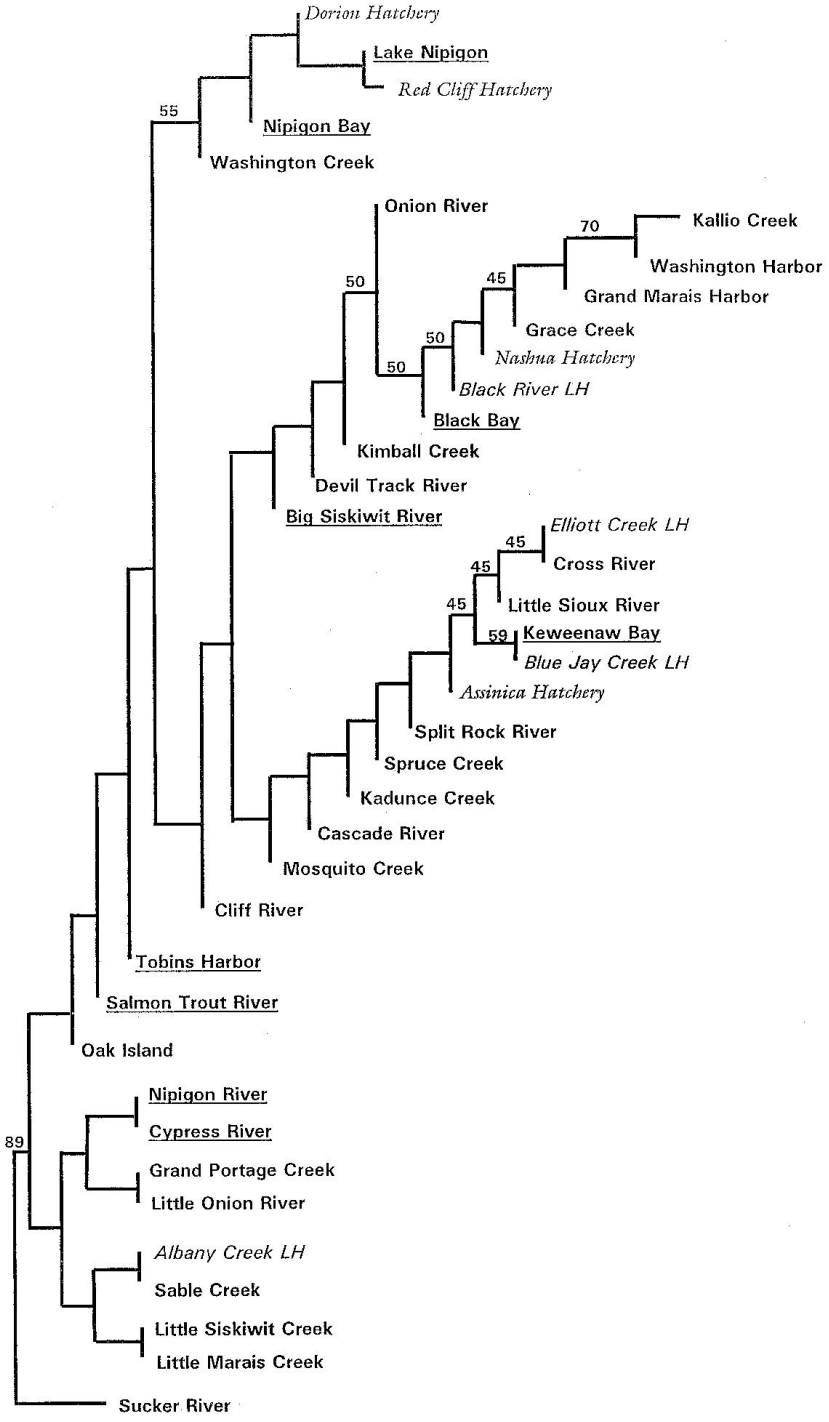

Figure 4. Neighbor-joining dendrogram among populations in which N>20, based on Cavalli-Sforza-Edwards chord distances. Numbers at some branching points indicate the percent of trees in 5000 bootstrap replicates that contain this arrangement of populations (branches supported in less than 50% of bootstrap tests were not labeled). Suspected coaster populations are underlined and hatchery populations are in script italics.