

GENETIC ORIGINS OF COASTER BROOK TROUT FROM THREE NATIONAL PARKS,  
ONE NATIONAL WILDLIFE REFUGE, AND FOUR INDIAN RESERVATIONS IN  
MINNESOTA, WISCONSIN, AND MICHIGAN

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## *Summary*

Restoration of coaster brook trout to Lake Superior has been identified as a research priority. Restoration efforts include habitat restoration, regulation changes, assessments, and stocking of hatchery fish. Stocking programs in Wisconsin and Michigan tributaries of Lake Superior have provided an opportunity to study the relative success of different stocking strategies for various hatchery strains and the use of different life-history stages. Genetic markers provide a method to identify brook trout that were stocked at sizes that were too small to mark. Analysis of microsatellite DNA loci permits accurate discrimination among four brook trout hatchery strains (Tobin Harbor, Siskiwit Bay, Lake Nipigon-Red Cliff, and Jumbo River) and brook trout that were collected in streams before recent stocking events. Among stocked streams in Michigan, Minnesota, and Wisconsin, the largest numbers of fish were captured from Whittlesey Creek which was stocked with similar numbers of the Tobin Harbor and Siskiwit Bay strains. Similar numbers of adults and yearlings captured in Whittlesey Creek were assigned to the Siskiwit Bay and Tobin Harbor strain, while a larger number of Tobin Harbor young-of-year were observed. Many brook trout could not be assigned to any of the strains or wild collections used in the analysis; adding data from other genetic studies of brook trout may reduce the number of unassigned fish. In addition, further investigation is needed to examine how the classification approach we used is affected by missing baseline data and potentially small genetic diversity among collections. Microsatellite DNA loci and the assignment test method we used have potential to monitor coaster brook trout rehabilitation efforts in Michigan, Minnesota, and Wisconsin tributaries of Lake Superior. Future work should build upon genetic studies done to date and genetic sampling should be included in the design of any future efforts.

## ***Introduction***

Lake dwelling ‘coaster’ brook trout (*Salvelinus fontinalis*) are rare in Lake Superior. Prior to European settlement coasters were relatively abundant along coastal areas of Lake Superior, but are now found in a few areas of the lake (Newman and Dubois 1997). Restoration of coaster brook trout has been identified as a research priority of the Great Lakes Fishery Commission and other Lake Superior fishery management agencies through the Lake Superior Binational Program and Lakewide Management Plan (U.S. EPA 2000; Horns et al. 2003). Restoration efforts include habitat restoration, regulation changes, assessments, and stocking of hatchery fish.

University and government researchers are using genetic methods to study the evolutionary status and genetic population structure of brook trout from Lake Superior and the genetic relationship of hatchery strains to wild stocks (Burnham-Curtis 2001; D’Amelio 2002; Cooper 2004; Wilson et al. 2008; Stott et al. In Review). These analyses have detected differences among many of the sample sites, but have found no evidence of a unique genotype or evolutionary lineage associated with coasters. However, a role for genetics cannot be dismissed, because a gene or gene complex unlinked to the neutral genetic markers used to date may be found in the future (Behnke 2004).

Genetic studies contribute to other aspects of brook trout research and management. Areas of Lake Superior that once supported coaster brook trout are being stocked with brook trout hatchery strains that have their origins in the Lake Superior basin. Follow-up assessment can track the survival of the hatchery strains, monitor interactions with wild brook trout, track the development of the lake-run phenotype, and quantify the reproductive success. Molecular tools such as microsatellite DNA loci can be used to identify the origins of hatchery fish stocked into the wild without physical marks (Hansen et al. 2000; Berejikian et al. 2001; Hansen et al. 2001; Eldridge et al. 2002; Page et al. 2003).

Over the last 125 years, brook trout have been stocked into Lake Superior tributaries to create, improve, and maintain fishing opportunities and, more recently, to restore brook trout populations. However, only in the last 30 years have stocked fish have originated from the Lake Superior basin, and only since 1997 have the fish originated from Lake Superior proper. The use of Lake Superior basin hatchery strains, genetically guided mating schemes, and stocking of early life stages are expected to increase the potential for survival and reproduction and reduce negative genetic effects that can be associated with stocking (Krueger and May 1991).

Hatchery strains from Isle Royale (Michigan) and Lake Nipigon (Ontario) brook trout were developed because these fish exhibit a coaster-like phenotype. The Lake Nipigon strain was derived from a shoal spawning population in Lake Nipigon and is reared at the Ontario Ministry of Natural Resource’s Dorion Fish Culture Station (Newman et al. 2003). The Lake Nipigon broodstock, maintained at the Red Cliff Tribal Hatchery, Wisconsin, was founded from a transfer of eggs of the Lake Nipigon strain from the Dorion Fish Culture Station (Newman et al. 2003). The Lake Nipigon strain has been stocked in Michigan, Minnesota, Wisconsin, and Ontario waters of Lake Superior since the 1970s. Two Isle Royale strains (Tobin Harbor and Siskiwit Bay) are reared by the U. S. Fish and Wildlife Service at the Iron River and Genoa

National Fish Hatcheries in Wisconsin. The Siskiwit Bay area strain was founded with brook trout captured primarily from the Big Siskiwit River and the Tobin Harbor strain was derived from a shoreline spawning population (Newman et al. 2003). The Tobin Harbor and Siskiwit Bay strains have been stocked in streams at Grand Portage, Minnesota, in Whittlesey Creek, Wisconsin, in Michigan waters east of the Keweenaw Peninsula, and at Isle Royale (Table 1).

Stocking experiments in Wisconsin and Michigan waters are attempting to quantify some of the factors involved in producing and maintaining healthy populations of coaster brook trout using the Isle Royale hatchery strains. In some areas, eyed-eggs and spring fingerlings have been stocked. Stocking these early life stages precluded the ability to mark (e.g., fin-clip) fish externally, therefore genetic markers were used to identify the hatchery strain of recaptured brook trout. In particular, we were interested in the origins of unmarked brook trout captured in assessment surveys in Wisconsin and Michigan tributaries of Lake Superior from 2004 and 2005.

The project had three objectives:

- 1) To generate genetic profiles of brook trout hatchery strains stocked into Lake Superior (Table 1) by collecting microsatellite DNA data for the Siskiwit Bay, Tobin Harbor, and Lake Nipigon (Red Cliff Tribal hatchery and Dorion hatchery), Jumbo River, Michigan, Assinica (from Marquette and Phillips hatcheries), St. Croix, Owhi, and Rome hatchery strains. We will determine the degree of divergence among the strains and our ability to correctly assign individuals to their sources.
- 2) To determine if genetic identification of hatchery brook trout is possible by determining the relative survival and success of hatchery strains at nine locations (Whittlesey Creek, Kelsey Creek, and Zeba Creek, Oak Island Creek, Frog Creek, Raspberry Bay, Raspberry River, and Chicago Creek) which have been stocked in the last decade.
- 3) To describe genetic variation of brook trout from three National Parks (Isle Royale, Pictured Rocks, and Apostle Islands), one National Wildlife Refuge (Whittlesey Creek) and four Indian Reservations (Keweenaw Bay, Red Cliff, Bad River, and Grand Portage). Each of these locations contains stream and nearshore waters that currently support, or historically supported, populations of coaster brook trout.

### ***Materials and Methods***

Brook trout were sampled from streams and shoreline waters of Lake Superior using backpack, towed barge, and boat electrofishing gear. A piece of anal or caudal fin was removed, dried and stored in a standard scale sample envelope, or in a vial with 95% ethanol. Hatchery samples were provided by the Iron River and Genoa National Fish Hatcheries, the Red Cliff Tribal Hatchery, and the Dorion Hatchery or were taken from existing collections at the Great Lakes Science Center-USGS (GLSC).

Brook trout were assigned to an age class based on collection date and size at capture. The size at capture and year of capture were used to determine when a fish was stocked. A fish that was less than 100 mm was called a young-of-year (YOY), a fish between 100 mm and 180 mm was called a yearling, and a fish > 180 mm was called an adult.

Tissue samples were sent to the Great Lakes Science Center-USGS for genetic analysis. DNA was extracted using the DNeasy kit (Qiagen, Valencia CA)<sup>1</sup> protocol and reagents. The extracted DNA was examined for quality, quantified, and then microsatellite DNA loci were amplified using the polymerase chain reaction (PCR). Sixteen microsatellite DNA loci designed for brook trout were used in the survey: *SfoB52*, *SfoC24*, *SfoC28*, *SfoC38*, *SfoC79*, *SfoC86*, *SfoC88*, *SfoC113*, *SfoC115*, *SfoC129*, *SfoD105*, *SfoD75* (Tim King, Leetown Science Center, unpublished data), *Sfo8*, *Sfo12*, *Sfo18*, and *Sfo23* (Angers et al. 1995). PCRs were carried out in a 15 ul volume using 1 U *Taq* DNA polymerase (Promega Co., Madison WI), the manufacturer's buffer, 0.3 mM each dNTP, 0.4 uM of each primer 1.5 mM MgCl<sub>2</sub>, and 90 to 100 ng template DNA. The PCR thermal profile was similar for all loci; only the annealing temperature was altered (Table 2). An initial denaturation step of 2 min at 94°C was performed, followed by 35 cycles of one min at 94°C, one min at the annealing temperature, and a one min extension at 72°C.

The PCR products were prepared according to manufacturer's guidelines, Applied Biosystems, Foster City CA, for capillary electrophoresis. Each PCR product was diluted with 9 uL of water, and then 10uL formamide and a 400 base pair size standard (labeled with ROX) were added to 1 uL of the diluted PCR product. Samples were denatured at 95 °C for 4 min and chilled on ice for 3 min before they were loaded on the ABI 3100-AVANT Genetic Analyzer. Fragment size data were collected using the ABI 3100-AVANT Genetic Analyzer. GeneScan (Applied Biosystems, Foster City CA) software was used to generate genotype data and the Genotyper (Applied Biosystems, Foster City CA) software package was used to score and bin genotypes.

Observed and expected heterozygosity and number of alleles per locus were calculated for the hatchery strains. The Microsoft Excel add-in program Microsatellite Toolkit (Park 2001) was used to calculate the diversity statistics. We calculated genetic differentiation among all the hatchery strains and the two pre-stocking assessment samples using two different metrics. Cavalli-Edwards chord distances (Cavalli-Sforza and Edwards 1967) and Weir and Cockerham's (1984)  $F_{st}$  analog ( $\Theta$ ) were calculated using the programs Genetix (version 4.03; Belkhir et al. 2003) and FSTAT (version 2.9.3.2; Goudet 1995), respectively. The significance of the  $F_{st}$  statistic for pair-wise tests over all loci was adjusted using the Bonferroni correction for multiple comparisons (Rice 1989).

We used assignment tests to determine whether or not brook trout captured during assessments were stocked fish or remnants of the pre-stocking population. Assignment tests determine the probability that one or more individuals come from a particular population using their multi-locus genotype and the expected probabilities of that genotype occurring in the potential source populations (Manel et al. 2002). Assignment tests can be used to include or exclude samples from a potential source population or to determine how many genetic clusters exist in a sample of fish depending on how probabilities are calculated (Manel et al. 2005). A potential source population can be a hatchery strain or a collection of wild fish. We performed three different sets of assignment tests using samples collected from different geographic regions with different stocking histories:

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<sup>1</sup> Use of trade names does not signify endorsement by the US government.

1. We analyzed fish captured in or near Wisconsin waters stocked with Tobin Harbor, Siskiwit Bay, and Lake Nipigon-Red Cliff Hatchery fish (Buffalo Bay, Chicago Creek, Frog Creek, two Oak Island streams, Raspberry Bay, and Raspberry River, and Whittlesey Creek). Brook trout collected from Oak Island streams in 1995 and Whittlesey Creek between 2001 and 2003 (previous to stocking Isle Royale strains) were included as source populations. A resident brook trout population was purported to be present at low abundance in Whittlesey Creek prior to stocking of Lake Nipigon brook trout in the 1980s, so brook trout from Whittlesey Creek were analyzed for microsatellite DNA variation by the USGS Wisconsin Cooperative Fishery Research Unit (University of Wisconsin-Stevens Point; Sloss et al. 2008). We acquired the genotype data for these fish and additional hatchery fish (Tobin Harbor and Siskiwit Bay). The genotypes from Sloss et al. (2008) were adjusted using offset values calculated in a previous project (Wilson et al. 2006). We used a subset of the 16 loci listed above (*Sfo18*, *SfoB52*, *SfoC113*, *SfoC24*, *SfoC38*, *SfoC86*, *SfoC88*, and *SfoD75*) to analyze these data.
2. We tested fish captured in Michigan waters, near areas stocked with Tobin Harbor, Siskiwit Bay, and Jumbo River fish (Kelsey Creek and Zeba Creek/Little Silver Creek).
3. We tested brook trout captured from Mosquito River in the Pictured Rocks National Lakeshore (PRNL), Michigan. Rivers in the park were stocked with the Tobin Harbor strain between 1997 and 2005. The Lake Nipigon-Red Cliff Hatchery strain was stocked into the Anna River, located just to the west of PRNL so it was also included in the analysis. Samples identified as Mosquito Creek brook trout were used as a source population. Mosquito River samples were provided by Dr. Jill Leonard, Northern Michigan University.

To assess the origins of brook trout described above, we employed a Bayesian-based approach using the program GeneClass 2.0.h (Piry et al. 2004). Before we tested the wild caught fish, we performed a self-classification test of the hatchery strains (or the hatchery strains and the pre-stocking Oak Island and Whittlesey Creek data) to determine the accuracy of the assignment test using the ‘leave-one-out’ approach in GeneClass (Efron 1983). In this approach each fish from the potential source populations is treated as an unknown and then tested to see if it is assigned back to the correct source. The number of fish assigned correctly was tabulated to determine how much (or how little) overlap there is in the genotypic profiles of the hatchery strains. An assignment was considered correct if a fish was assigned to its source with an assignment score of 80 or more. This assignment score was calculated

as  $AssignmentScore_{i,t} = 100 * \left( \frac{L_{i,t}}{\sum_{j=1}^p L_{i,j}} \right)$ .  $L_{i,t}$  is the likelihood individual  $i$  belongs to

population  $t$  of  $p$  populations. We used log of odds ratios (LOD) to assess confidence in a sample’s assignment (Banks and Eichert 2000). If a LOD score  $\geq 1$  then the population of assignment is at least 10 times as likely as any other population and a LOD  $\geq 2$  the population of assignment is at least 100 times more likely.

After performing self-classification tests, we used GeneClass to determine the origins of the wild caught brook trout. If a fish had an assignment score of 80 or more then it was assigned

to the source population with the highest likelihood value. If the assignment score was less than 80, then the brook trout was classified as 'unassigned'. LOD values were calculated for all brook trout.

A second analysis using a different approach was used to determine the origins of brook trout from Mosquito River. We decided to explore the genetic structure of brook trout from the three rivers in PRNL (Hurricane River, Mosquito River, and Sevenmile Creek) first to determine the number of populations and second to identify fish that are migrants among rivers and/or hybrids. Researchers believe there is significant migration of brook trout among rivers in PRNL (J. Leonard, Northern Michigan University, personal communication) which may have an impact on the number of genetic populations in the park. Genotype data for the three rivers were collected previously as part of a basin-wide survey of brook trout (W. Stott, unpublished data). Since samples were collected over several years and at different times of the year, we used a model-based Bayesian procedure, implemented in the program STRUCTURE (ver. 2.2; Pritchard et al. 2000) to determine the number of populations we had actually sampled. First, we analyzed all the brook trout caught in the park. Five independent runs of  $K=1$  to 7 (where  $K$  is the number of populations) were performed using 100,000 iterations, following a burn-in of 100,000 iterations, assuming correlated allele frequencies and admixture among populations. The posterior probability was then calculated for each value of  $K$  using the estimated log-likelihood of  $K$ . We tested  $K=1$  to 7 to account for the combination of rivers and sampling years. We ran this analysis a second time, including the hatchery fish and testing  $K=3$  to 12. For each simulation we determined the average membership coefficient ( $Q$ ) for the most likely value of  $K$ . A river or hatchery strain belonged to a single cluster if  $Q \geq 0.9$  and more than one if  $Q < 0.9$ . The choice of 0.9 was arbitrary, but is within the limits of values that have been suggested for wildlife forensic analyses (Manel et al. 2002). We assessed each fish's membership in each cluster using  $q$ , the average proportion of each fish's genotype originating from each cluster. A fish was assigned to a single cluster if  $q \geq 0.9$  or to more than one if  $q < 0.9$ . Brook trout with  $q < 0.9$  are fish that could have a mixed ancestry. Fish that had a  $q < 0.9$  in both tests were considered to be of mixed ancestry. These fish were further investigated by examining the origins of their genotypes at three different values of  $K$ . Specifically, we looked at the most likely value of  $K$ , hatchery fish versus all fish from PRNL ( $K=3$ ), and all collection sites ( $K=5$ ). For this simulation we did not use the information on the capture site of these fish, but did use the information on the remaining fish. The results from both analyses of Mosquito River brook trout were then compared.

## ***Results***

We obtained samples from each of the three National Parks, two of the four Indian Reservations, and one National Wildlife Refuge (Figure 1). Collections were obtained from the Tobin Harbor, Siskiwit Bay, Jumbo River, and Lake Nipigon-Red Cliff hatchery strains. We did not obtain samples from waters in or adjacent to the Grand Portage Band and Bad River Band Reservations as anticipated. Because we obtained small sample sizes or no samples from several sites (e.g., Pictured Rocks and Apostle Islands National Parks, Bad River and Grand Portage Reservations) we did not complete the detailed population structure analysis described in objective #3.

Due to funding limitations, we had to prioritize samples caught during assessment surveys made between 2002 and 2005 for genotyping. High priority was assigned to fish lacking an external mark captured in or near a stocked location and to yearling fish captured in Whittlesey Creek marked with an adipose fin-clip (an adipose clip and either a coded wire tag or an elastomer dye mark were applied to Tobin Harbor and Siskiwit Bay strains stocked into Whittlesey Creek in 2004). Brook trout marked with a fin-clip that did not correspond to the clips used on fish in the project area were also assigned a high priority. Moderate priority was given to unmarked fish from streams where there is no stocking and where we have no previous microsatellite DNA data (Benson, Washington, and Grace creeks and Tobin Harbor at Isle Royale, and Hurricane River at PRNL). Low priority was given to fish with a fin-clip or other external mark captured in or near the project area because their origins could be determined using the clip information.

A total of 624 wild caught fish and 231 hatchery fish were sent to the GLSC for genetic analysis. Sample sizes of wild caught brook trout ranged from one (Raspberry Bay) to 217 (Whittlesey Creek). DNA was extracted from all 855 brook trout. We genotyped all the high priority fish (N=369; Table 3) and the hatchery fish (Table 4). The high priority brook trout correspond to fish collected between 2003 and 2005. No moderate or low priority samples were analyzed. All genotype data, information about the collection site, and biological data was stored in a database maintained by the GLSC's genetics laboratory and are available for future analyses.

Observed heterozygosity of hatchery samples ranged from 0.392 to 0.670 and the average number of alleles ranged from 4.05 to 10.24 (Table 4). The Jumbo River strain had the highest value for both estimates and the Lake Nipigon (ON) and the Siskiwit Bay strains had the lowest values for observed heterozygosity and average number of alleles respectively.

Genetic differentiation statistics for the hatchery strains and the pre-stocking wild collections are summarized in Table 5. The Lake Nipigon-Red Cliff hatchery strain was most distinct for both estimators. Siskiwit Bay and Oak Island creeks were separated by the smallest genetic distances followed by Tobin Harbor and Jumbo River. All the pair-wise estimates of  $F_{st}$  were significantly different from zero. The smallest  $F_{st}$  value was observed between Tobin Harbor and Jumbo River, followed by Siskiwit Bay and Tobin Harbor.

### **GeneClass**

Assignment tests could be used to distinguish among all the combinations of strains and rivers designated as possible sources (Table 6). Three sets of simulations were performed, each corresponding to the different combinations of hatchery and/or pre-stocking data that we used.

Tobin Harbor, Siskiwit Bay, Lake Nipigon-Red Cliff Hatchery, Oak Island streams, and Whittlesey Creek strain brook trout were tested first. Sixty-four (17.4%) fish had an assignment score < 80. The majority of the fish with low assignment scores came from the Siskiwit Bay strain. The average assignment score was 91.4 (SD=15.63), the average LOD was 2.89 (SD=1.97). More than half (59.9%) of the brook trout had a  $LOD \geq 2$  and thus their estimated assignment was at least 100 times more likely than any other possible assignment. Six Siskiwit

Bay fish were incorrectly assigned as Tobin Harbor fish. All six of the brook trout had assignment scores  $< 80$ . Two Tobin Harbor fish were incorrectly assigned Whittlesey Creek fish, but only one had an assignment score  $\geq 80$ . Seven of the Oak Island brook trout were misclassified to the Siskiwit Bay strain and one to the Tobin Harbor strain. All of the misclassified Siskiwit Bay fish had assignment scores  $< 80$ . Overall 95.6% of the brook trout were classified correctly.

Among the Jumbo River, Tobin Harbor, and Siskiwit Bay strains 194 of 231 fish had an assignment score  $\geq 80$  and the average assignment score was 92.8 (SD=13.68). Three Tobin Harbor fish and three Siskiwit Bay fish were incorrectly classified as Jumbo River fish, but overall 97.4% of the fish were assigned correctly. Three of the misclassified fish had LOD  $\geq 1$  and the average LOD was 2.57 (SD=1.6).

Among the Tobin Harbor, Lake Nipigon-Red Cliff hatchery, and Mosquito River fish 131 of 154 brook trout had an assignment score  $\geq 80$  and the average assignment score was 88.3 (SD=14.18). The average LOD was 1.85 (SD=1.61) and 93.5% of the brook trout were correctly classified. Five of the misclassified fish had assignment scores  $\geq 80$ .

Next, we analyzed brook trout captured during field surveys (Table 7). Three sets of tests were performed. We performed assignment tests on brook trout from Buffalo Bay, Chicago Creek, Frog Creek, Raspberry Bay, Raspberry River, two Oak Island creeks, Whittlesey Creek, Mosquito River, Kelsey Creek and Zeba Creek. We tested 369 brook trout from these sites against the Siskiwit Bay, Tobin Harbor, Lake Nipigon-Red Cliff strains, pre-stocking Oak Island stream and Whittlesey Creek fish. Seventy-eight brook trout were not classified to any of the strains because they had assignment scores  $< 80$ ; these fish had an average assignment score of 48.0. This included all of the fish from Frog Creek and Raspberry Bay. One hundred and eighty fish had assignment scores  $\geq 80$ , 11 of these fish were assigned to the Oak Island streams (six fish from Oak Island, three from Chicago Creek, and two from Whittlesey Creek), four fish were assigned to Whittlesey Creek, five fish were assigned to the Lake Nipigon-Red Cliff hatchery strain, 44 to the Siskiwit Bay strain, and 116 to the Tobin Harbor strain. The average LOD of the fish with assignment scores  $\geq 80$  was 3.45.

Ninety-five brook trout from Kelsey Creek and Zeba Creek were tested against the Siskiwit Bay, Tobin Harbor, and Jumbo River strains (Table 7). Forty-four of these fish were classified as Jumbo River fish. The average LOD was 3.33 and the majority of fish (86.4%) had LOD  $\geq 2$ . Thirty-four fish were assigned to the Siskiwit Bay strain and their average LOD was 2.39. No fish were assigned to the Tobin Harbor strain and 17 fish were not assigned to any strain when a score of 80 was used as a cut-off for the assignment threshold. All of the 17 unassigned fish from Zeba Creek had assignment scores less than 35.

Sixteen Mosquito River samples were tested against the Tobin Harbor and Lake Nipigon-Red Cliff strains and known Mosquito River brook trout (Table 7). Thirteen of the 16 fish were classified as Mosquito River fish with an assignment score  $\geq 80$ . Two of the three fish with assignment scores  $< 80$  were also classified as Mosquito River brook trout and the other was classified as a Tobin Harbor brook trout. The average assignment score and LOD were 93.4 and 3.27, respectively.

We summarized the assignments by both collection year and life stage at capture because we are interested in monitoring the success of the different age classes that were stocked (Table 7). The combination of age class at capture and the capture year allows us to determine the age at stocking of the brook trout. More than half of the brook trout recaptured to date from all sites were YOY (61.5%) and most of these fish came from Whittlesey Creek. One quarter (26.4%) of the YOY could not be classified, 18.1% were assigned to the Siskiwit Bay strain, 46.7% to the Tobin Harbor strain, 6.1% to the Jumbo River strain, and no YOY were assigned to the Lake Nipigon strain. Yearlings made up 27.6% of the catch; 29.4% of the yearlings were assigned to the Siskiwit Bay strain, 22.5% of the yearlings were assigned to the Jumbo River strain and 26.5% were not assigned to any strain. Small numbers (<10) of fish were assigned to each of the Lake Nipigon and Tobin Harbor strains. Thirty-nine (10.6% of the total catch) adults were caught. One quarter (25.6%) of the adults were not assigned to any strain, 15.4% were assigned to the Siskiwit Bay strain, 17.9% to the Jumbo River strain, and small numbers (<5) of fish were assigned to the Lake Nipigon and Tobin Harbor strains.

### **STRUCTURE (PRNL)**

Genotype data were collected for wild caught brook trout from PRNL as part of a previous project (Hurricane River-26, Mosquito River-43, and Sevenmile Creek-27). Untransformed log-likelihood probabilities for the simulation were highest (i.e., had the lowest negative values) at  $K=5$  (average of 5 repetitions  $\pm$  one SE =  $-2599.48 \pm 5.87$ ). Brook trout from Hurricane River and Mosquito River were in separate clusters and had Q-values  $> 0.90$  (QI=0.939 and QII=0.936). Brook trout from Sevenmile Creek were assigned to more than one cluster. One cluster (QIV) had low values of Q ( $<0.05$ ) for all samples.

The simulation using all the data for hatchery fish (Tobin Harbor and Lake Nipigon strains) and brook trout caught in PRNL had a peak at  $K=9$  (average of 5 repetitions  $\pm$  one SE =  $-5393.68 \pm 2.96$ ). Brook trout from the Hurricane River and Mosquito River were again in separate clusters (QI=0.938 and QII=0.960). Hatchery fish were also assigned to single clusters with high values of Q (QIV=0.977 and QV=0.994). Brook trout from Sevenmile Creek were assigned to more than one cluster. There was little overlap among clusters with the exception of Sevenmile Creek; it overlapped with the cluster occupied by Tobin Harbor fish. Four clusters (QVI, QVII, QVIII, and QIX) had low values of Q for all samples.

We decided to use  $K=5$  to assign the Mosquito River brook trout because it best represented the number of clusters after clusters with low Q-values were removed. All sixteen fish were in the same cluster and had an average  $q$  of 0.987. The majority of fish in this cluster were captured in Mosquito River. This result agrees with the assignment tests run with GeneClass, i.e., most of the brook trout were Mosquito River fish. There was one disagreement; GeneClass classified one brook trout as a Tobin Harbor fish.

### ***Discussion***

The reappearance of healthy lake run brook trout at historical locations will be a major benchmark in restoration efforts. Molecular tools can supply information on the progress of rehabilitation efforts. Inventories of genetic variation have multiple uses. For example, they

help us understand how diversity is partitioned across the landscape in response to historical events such as glaciation and they can provide information on the effects of more recent events such as habitat disruption and rehabilitation. Burnham-Curtis (2001) used mitochondrial DNA variation to assess the population structure of brook trout from 49 wild collections and nine hatchery stocks. The majority of the wild collections were from the Lake Superior drainage, but some collections from Lake Huron were also included. While there was significant variation within Lake Superior, the study found little geographic structure among the collections and the observed patterns of mtDNA diversity were thought to describe patterns of postglacial recolonization by brook trout (Burnham-Curtis 2001). D'Amelio (2002) found differences among brook trout from rivers flowing into Nipigon Bay using nuclear genetic markers (microsatellite DNA). She also found genetic differences above and below barriers on individual rivers. Stott et al. (In Review) found genetic differences at large and small scales when they analyzed microsatellite DNA variation in brook trout from Isle Royale, Lake Nipigon, and three Minnesota tributaries to Lake Superior. Several laboratories are collaborating to produce a comprehensive picture of genetic population structure of brook trout around Lake Superior (Wilson et al. 2008), but some spatial gaps still remain in the dataset. As part of this project we increased sample sizes of existing collections and acquired brook trout from rivers that had not been sampled for genetics previously. Samples from Raspberry and Buffalo Bays, Raspberry, Siskiwit and Mosquito rivers, Frog, Chicago, Kelsey, Whittlesey, and Zeba creeks were analyzed during this project and additional samples were collected from the Big Siskiwit River, Little Siskiwit River, Siskiwit River, Benson Creek, Grace Creek, Mosquito River, Whittlesey Creek, two Oak Island creeks and Tobin Harbor. Funding and sample size limitations prevented complete analysis of all the samples, but DNA is available for future work.

Genetic data on the Tobin Harbor, Siskiwit Bay, and Lake Nipigon hatchery strains have been reported elsewhere and the estimates of heterozygosity and number of alleles calculated for this report are similar to those reported previously (D'Amelio 2002; Sloss et al. 2008). The results are not directly comparable since different sets of microsatellite loci were used in each study, but the relative amount of heterozygosity and number of alleles is similar. For example, in both the current study and the study by Sloss et al. (2008), Tobin Harbor had more alleles per locus and a higher heterozygosity than the Siskiwit Bay strain. This was also the case for samples of wild caught fish from the Siskiwit Bay and Tobin Harbor (Stott et al. In Review). The levels of heterozygosity reported for the surveyed wild-caught fish is also within the range of that reported previously for sites in Lake Superior (D'Amelio 2002; Sloss et al. 2008; Stott et al. In Review).

The microsatellite loci used in this study allowed us to distinguish among the hatchery strains with good confidence. The ability to distinguish between Tobin Harbor and Siskiwit Bay strain fish also extends to future generations. A previous study used simulated data to show that first generation hybrids between the two hatchery strains could be accurately identified (Sloss et al. 2008). Hybrids between Whittlesey Creek remnants and the Tobin Harbor and Siskiwit Bay hatchery strains could also be identified confidently (Sloss et al. 2008). Therefore, we have a powerful tool to evaluate the relative survival of hatchery strains after stocking and the reproductive success of these strains after stocked fish begin to reproduce. Using genetic and age data of captured fish it is possible to determine the relative survival of each strain (where matched plants have occurred) and the life history stages that were stocked.

The self-assignment tests showed that it was possible to distinguish among the hatchery strains and the pre-stocking Whittlesey Creek and Oak Island creeks brook trout. However, a substantial number of fish from all the collections could not be assigned to any of the baseline samples used in this study. We were unable to determine the origins of more than half of the brook trout collected from Oak Island streams, Frog Creek, Raspberry Bay, Raspberry River, Buffalo Bay, and Chicago Creek and one quarter of the brook trout recaptured in Whittlesey Creek. If the unidentified brook trout could be assigned to another stream it may be an indication that there is movement of brook trout among streams in the area near the Apostle Islands and Chequamegon Bay. A non-classification in an assignment test can occur for several reasons: a fish may possess a common genotype and its assignment would be equally strong to all the learning samples, the actual source population was not included in the analysis, the fish may be a hybrid, or there was an error in the assignment (Cornuet et al. 1999; Manel et al. 2002; Manel et al. 2005). We do not have pre-stocking sample data from Buffalo Bay, Frog Creek, Chicago Creek, Raspberry Bay, and Raspberry River. Baseline population genetic data has been collected for other streams (Bois Brule River, Bark River and Graveyard Creek) along the Wisconsin shoreline (Sloss et al. 2008). Including data from other tributaries will help to determine why so many brook trout were unassigned. The effects of missing data on this estimator should be investigated. The statistical approach used in GeneClass is supposed to allow a sample to be excluded from a given population so that, in theory, not all the potential source populations need to be sampled. However, the utility of this method has not been tested for sample sets that have relatively low genetic differentiation ( $F_{st} < 0.05$ ; Manel et al. 2005) and the effect of missing data has not been investigated. The majority of the hatchery strains and wild collections used in our analysis were distinct (Table 5) with  $F_{st}$  values above the critical level discussed by Manel et al. (2005), but some were low and it is unknown how any missing baselines might be related to the samples we did use. In preliminary analyses of our data, the Whittlesey Creek and Oak Island creeks data were not used. Many of the samples that went unclassified (i.e., assignment score  $< 80$ ) in the preliminary analyses had weak assignments to Tobin Harbor. When the Whittlesey and Oak Island creek data were added, the assignment scores of some of these fish increased so that an assignment could be made (data not shown). Eight of the 16 loci used at the GLSC were calibrated with the USGS Wisconsin Cooperative Fishery Research Unit, if we could use additional loci this may improve our ability to assign more brook trout to a strain and identify hybrids. We did not expect to find any Isle Royale strain hybrids in Whittlesey Creek in these collections because brook trout stocked to date may not have started to spawn. However, Lake Nipigon strain fish were stocked in Whittlesey Creek in 1994 and 1995, therefore introgressed fish from that strain may be present. The Lake Nipigon strain was not considered by Sloss et al. (2008).

Whittlesey Creek was stocked with similar numbers of the Isle Royale strains to compare the performance of the two strains. The Whittlesey Creek experiment is still in its initial phases so it is too early to make definitive statements about the relative success of the hatchery strains and the different life stages that were stocked. We did observe some trends that are interesting and should be monitored. Similar numbers of adults and yearlings was assigned to both strains, while a larger number of Tobin Harbor YOY was observed (Table 7). Brook trout from wild populations at Tobin Harbor and Siskiwit Bay had different habitat associations (Quinlan 1999; Gorman et al. 2008). Different habitat preferences may have an affect on their ability to survive

and reproduce in Whittlesey Creek. Surveys will continue in Whittlesey Creek to evaluate stocked fish survival, growth, movement, and their success at reproduction.

Two yearling brook trout captured in the Oak Island streams were classified Siskiwit Bay and Tobin Harbor strain brook trout, but these streams were not stocked with the Siskiwit Bay strain. Whittlesey Creek (about 30 km to the south) is the nearest location where the Isle Royale strains were stocked. Stocking of immature Siskiwit Bay and Tobin Harbor strain fish in Whittlesey Creek commenced in 2004. Eyed-eggs were stocked in winter and yearlings marked with an adipose fin-clip and coded wire tags were released in May 2004. Field crews did not report fin-clips on brook trout captured in Oak Island streams in 2004. Either a fin-clip was not detected and these brook trout were fish stocked into Whittlesey Creek or these fish were mis-assigned during the assignment tests. During self-assignment tests there was misclassification of Oak Island stream fish as Siskiwit Bay and Tobin Harbor hatchery fish. Therefore misclassification is a likely explanation. Another possibility is that we are missing samples from our baseline that are similar enough to the Isle Royale strains that the samples would be put into that category.

Most of the brook trout from Mosquito River were classified as Mosquito River brook trout regardless of the statistical approach used. None were assigned to the Lake Nipigon strain. Tobin Harbor strain brook trout were stocked into Lake Superior tributaries at the Pictured Rocks National Lakeshore in 1997 and between 2000 and 2005. In 2001, the Lake Nipigon strain was stocked in the Anna River (MI) which is located just outside the boundary of Pictured Rocks.

The presence of adult Siskiwit Bay strain fish in Zeba Creek indicates that stocked fish have survived for several years and the assignment of YOY to the Jumbo River strain suggests that Jumbo River strain fish reproduced in Zeba Creek or fish produced in another stream migrated into Zeba Creek. Yearling Siskiwit Bay strain brook trout could be fish captured from 2004 stocking events or progeny of previously stocked fish. YOY brook trout are most likely fish stocked earlier in the year, but could also be offspring of fish stocked between 1999 and 2003. Almost half (46%) of the brook trout captured in Kelsey and Zeba creeks were assigned to the Jumbo River strain and about half of the fish from Zeba Creek were assigned to the Siskiwit Bay strain. Siskiwit Bay strain spring fingerlings were stocked in Zeba Creek between 1999 and 2005, the Silver River in Huron Bay in 2002 and 2003, Baraga in 2000, Little Silver Creek in 2001, and Pequaming Point in 2000. Not all of these brook trout were marked with fin-clips; in some years no marks were applied or oxytetracycline was used to mark otoliths, therefore recaptured adults could be from early stocking events.

The absence of Siskiwit Bay genotypes in Kelsey Creek is perplexing. Siskiwit Bay spring fingerlings were stocked into Kelsey Creek between 1999 and 2005. The Jumbo River strain has been stocked into Zeba Creek, Menge Creek and other streams near Kelsey Creek, but not into Kelsey Creek itself. Various life stages of Jumbo River strain fish have been stocked into Michigan waters, some were clipped, others were marked with oxytetracycline, and others have no marks. There is evidence that during low precipitation years, water level and temperature may be unsuitable for brook trout in some sections of Kelsey Creek, which might force brook trout to leave the stream during these times. However, this should affect both strains equally. Again, while our ability to distinguish among the strains we used in the analysis is

good, we do not know what the effect of missing baseline data might be. The Jumbo River strain was developed locally within the Keweenaw Bay area (see Figure 1). It is unknown how similar fish from other tributaries are to Jumbo River; therefore it is possible that remnant Kelsey or Zeba creek or other sources are being classified as Jumbo River brook trout.

This study has shown that genetic data can be used to assess the relative success of the stocking efforts. Both the survival of the different life history stages and their reproductive success can be tracked when cross-referenced with field collection and stocking information. We have also demonstrated that data calibration efforts among different laboratories are an efficient way to increase sample sizes and statistical power and improves our ability to process and report on these samples in a timely manner to fish managers and the interested public. We were able to combine our genotype data with data collected by the USGS laboratory at the University of Wisconsin-Stevens Point (USGS-WICFRU; Wilson et al. 2005). Since this study began, additional samples have been collected from many of the areas reported in this study as well as other locations in Lake Superior. Analyzing these samples to determine the likely source population is a high priority for the agencies involved.

### ***Recommendations***

1. In addition to Whittlesey Creek, baseline population genetic data has been collected by Wisconsin DNR and analyzed by Dr. B. Sloss (USGS-WICFRU) for a number of other streams along the Wisconsin shoreline. Our samples collected from waters in and around the Red Cliff Reservation and Apostle Islands should be included with baseline genetic data from other streams of the Bayfield Peninsula to improve the assignment accuracy of these fish. The GLSC genetics lab and the USGS-WICFRU laboratory should also increase the number of microsatellite DNA loci that are calibrated for data sharing. Further investigations into the detection of hybrids among all strains are also required.
2. Samples continue to be collected in Whittlesey Creek and other locations around the lake. Securing funds and timely analysis of these samples is needed to provide fishery managers with information beneficial to management actions. Spatial sampling gaps should be identified and sampled so more complete baseline data are available as other rehabilitation programs with stocking components are developed.
3. Some effort should be made to investigate the behavior of the assignment test methodology in situations where source populations are missing from the data set. Numerous simulation studies compare how different estimators perform, the effect of sample size, divergence among populations, and number of loci (e.g., Cornuet et al. 1999; Bernatchez and Duchesne 2000; Manel et al. 2005), but few have examined the impacts of missing data. While it was possible to distinguish among the hatchery strains and pre-stocking populations, missing baseline data may have had some impact on accuracy and/or our ability to assign a fish to the correct strain.
4. Few fish sampled were assigned to the Lake Nipigon hatchery strain which has been stocked along the Red Cliff shoreline (MI) for over a decade. The reasons for this poor showing are not clear but may include broodstock viability and domestication, or issues

related to stocking and/or assessment practices. Improved planning and coordination would help resolve some of these issues and facilitate stocking evaluation.

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Table 1. Brook trout hatchery stocks used to stock Michigan, Wisconsin, Minnesota, and Ontario waters of Lake Superior since 1976. From Great Lakes Fishery Commission fish stocking database: <http://www.glfsc.org/fishstocking/index.htm>; March 15, 2006.

Stock/Strain	Source	States/provinces stocking strains into Lake Superior			
		MI	MN	WI	ON
Assinica (ME)	Phillips State Hatchery	X			
Assinica (MI)	Marquette State Hatchery	X			
Jumbo River	Keweenaw Bay Natural Resources Department Hatchery	X			
Lake Nipigon (ON)	Dorion Fish Culture Station				X
Lake Nipigon (WI)	Red Cliff State Hatchery	X	X	X	
Owhi	Egan State Fish Hatchery	X			
Rome	Rome Hatchery	X			
St. Croix	Spire Valley Fish Hatchery			X	
Siskiwit Bay	Iron River and Genoa National Fish Hatcheries	X	X	X	
Tobin Harbor	Iron River and Genoa National Fish Hatcheries	X	X	X	

Table 2. Annealing temperatures of microsatellite loci used in analysis of hatchery strains and wild caught brook trout.

Locus	Annealing Temperature (°C)	Reference
<i>Sfo8</i>	58	Angers <i>et al.</i> 1995
<i>Sfo12</i>	60	Angers <i>et al.</i> 1995
<i>Sfo18</i>	56	Angers <i>et al.</i> 1995
<i>Sfo23</i>	58	Angers <i>et al.</i> 1995
<i>SfoB52</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC113</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC115</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC129</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC24</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC28</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC38</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC79</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC86</i>	58	King and Burnham-Curtis unpublished data
<i>SfoC88</i>	58	King and Burnham-Curtis unpublished data
<i>SfoD100</i>	58	King and Burnham-Curtis unpublished data
<i>SfoD105</i>	58	King and Burnham-Curtis unpublished data
<i>SfoD75</i>	58	King and Burnham-Curtis unpublished data

Table 3. Brook trout samples from Wisconsin and Michigan that could be genotyped during the course of this project.

Sample Source	Year Collected	Sample Size
Buffalo Bay	2004	3
Chicago Creek	2004	10
Frog Creek	2004	5
Kelsey Creek	2005	28
Mosquito River	2003	5
Mosquito River	2004	11
Two Oak Island streams	2004	20
Raspberry Bay	2004	1
Raspberry River	2004	2
Whittlesey Creek	2005	217
Zeba Creek	2005	67
Total		369

Table 4. Diversity statistics for hatchery brook trout collections calculated over 17 microsatellite loci.  $H_e$  is expected heterozygosity,  $H_o$  is observed heterozygosity, and  $A$  is the average number of alleles.

Hatchery Strain	Sample size	$H_e$	$H_o$	$A$
Assinica (MI)	21	0.675	0.601	5.18
Jumbo River	59	0.747	0.670	10.24
Lake Nipigon (ON)	81	0.480	0.392	6.06
Owhi	52	0.710	0.627	6.94
Assinica (ME)	51	0.526	0.484	4.35
Lake Nipigon (WI)	45	0.465	0.425	5.29
Rome	50	0.517	0.452	4.71
Siskiwit Bay	92	0.460	0.439	4.06
St. Croix	49	0.705	0.596	7.06
Tobin Harbor	80	0.619	0.498	5.82

Table 5. Values of genetic differentiation among hatchery and wild brook trout. Cavalli-Sforza chord distances (Cavalli-Sforza and Edwards 1967) are above the diagonal and Fst (Θ; Weir and Cockerham 1984) values (in bold) are below the diagonal. All pair-wise estimates of Fst are significantly different from zero.

	Jumbo R.	L. Nipigon-Red Cliff	Siskiwit Bay	Tobin Harbor	Whittlesey Cr.	Oak Island creeks
Jumbo R.	0.000	0.154	0.131	0.101	0.136	0.118
L. Nipigon-Red Cliff	<b>0.275</b>	0.000	0.157	0.137	0.169	0.105
Siskiwit Bay	<b>0.139</b>	<b>0.374</b>	0.000	0.102	0.168	0.078
Tobin Harbor	<b>0.115</b>	<b>0.285</b>	<b>0.135</b>	0.000	0.123	0.105
Whittlesey Cr.	<b>0.173</b>	<b>0.325</b>	<b>0.266</b>	<b>0.170</b>	0.000	0.172
Oak Island creeks	<b>0.140</b>	<b>0.333</b>	<b>0.162</b>	<b>0.192</b>	<b>0.303</b>	0.000

Table 6. Percent accuracy of self-classification tests performed on hatchery stocks, the number of fish correctly classified is in parentheses. The number of fish genotyped from each hatchery stock is in parentheses. The additional hatchery fish and brook trout from Whittlesey Creek in the first comparison are from Sloss et al. (2008).

Collection	Percent Correct Assignment						
	Siskiwit Bay	Tobin Harbor	Lake Nipigon	Mosquito R.	Jumbo R.	Whittlesey Cr.	Oak Island creeks
Siskiwit Bay (142)	96 (136)	4 (6)	0	-	-	0	0
Tobin Harbor (120)	0	98 (118)	0	-	-	2 (2)	0
Lake Nipigon (46)	0	0	100 (46)	-	-	0	0
Whittlesey Cr. (36)	0	0	0	-	-	100 (36)	0
Oak Island streams (23)	30 (7)	4 (1)	0	-	-	0	66 (15)
Siskiwit Bay (92)	97 (89)	0	-	-	3 (3)	-	-
Tobin Harbor (81)	0	96 (78)	-	-	4 (3)	-	-
Jumbo River (59)	0	0	-	-	100 (59)	-	-
Tobin Harbor (81)	-	93 (75)	0	7 (6)	-	-	-

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Lake Nipigon (46)	-	0	93 (43)	7 (3)	-	-	-
Mosquito R. (27)	-	4 (1)	0	96 (26)	-	-	-

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Table 7. Summary of brook trout captured from Wisconsin and Michigan streams in 2004 and 2005 by age-class and hatchery origins.

YOY is <100mm, yearling is 100 to 180mm and adult is >180mm.

Collection Site	Year Collected	Age-Class	Assignment							
			Jumbo R.	Lake Nipigon	Siskiwit Bay	Tobin Harbor	Whittlesey Cr. prestock	Oak Island streams prestock	Mosquito R.	Not assigned
Buffalo Bay	2004	Adult	-	1	0	0	0	0	-	2
Chicago Cr.	2004	Adult	-	0	0	0	0	1	-	1
		Yearling	-	3	0	0	0	0	-	1
		YOY	-	0	0	0	0	2	-	2
		Yearling	-	0	0	0	0	0	-	5
Frog Cr.	2004	Yearling	-	0	0	0	0	-	5	
Kelsey Cr.	2005	Adult	6	-	0	0	-	-	-	0
		Yearling	20	-	0	0	-	-	-	0
		YOY	2	-	0	0	-	-	-	0
Mosquito R.	2003	Adult	-	0	0	0	-	-	1	1
		Yearling	-	0	0	0	-	-	1	2
	2004	Adult	-	0	0	0	-	-	10	0
		Yearling	-	0	0	0	-	-	1	0
Two Oak Is.	2004	Adult	-	0	0	0	1	0	-	1
		Yearling	-	1	1	1	0	5	-	7
		YOY	-	0	0	0	0	1	-	2
Raspberry Bay	2004	Adult	-	0	0	0	0	0	-	1
Raspberry R.	2004	Adult	-	0	0	0	0	0	-	1
		Yearling	-	0	0	1	0	0	-	0
		Adult	-	0	5	3	0	0	-	1
Whittlesey Cr.	2005	Yearling	-	0	3	5	2	0	-	2
		YOY	-	0	34	107	1	2	-	52
		Adult	1	-	2	0	-	-	-	1
Zeba Cr.	2005	Yearling	3	-	26	0	-	-	-	12
		YOY	12	-	-	0	-	-	-	4

Figure 1. Locations of Lake Superior tributaries where brook trout were collected for genetic analysis between 2002 and 2005. The wild source of the Jumbo River hatchery strain is indicated on the map. Three national parks are shown in dark grey: Pictured Rocks National Lakeshore (PRNL), Isle Royale National Park (ISRO), and Apostle Islands National Lakeshore (APIS).

