

Conservation Genetics of Inland Lake Trout in the Upper Mississippi River Basin: Stocked or Native Ancestry?

KYLE R. PILLER*¹

Wisconsin Department of Natural Resources, 1350 Femrite Drive,
Monona, Wisconsin 53716, USA; and University of Wisconsin, Center for Limnology,
680 North Park Street, Madison, Wisconsin 53706, USA

CHRIS C. WILSON

Ontario Ministry of Natural Resources, Aquatic Biodiversity and Conservation Unit,
Trent University, 1600 West Bank Drive, Peterborough, Ontario K9J 8N8, Canada

CAROL EUNMI LEE

University of Wisconsin, Department of Zoology, 430 Lincoln Drive,
Madison, Wisconsin 53706, USA

JOHN LYONS

Wisconsin Department of Natural Resources, 1350 Femrite Drive,
Monona, Wisconsin 53716, USA

Abstract.—Although stocking for sport fishery enhancement has been practiced by resource managers for decades, the potential genetic effects of these stocking practices have remained largely unknown. We investigated the genetic contributions of stocking lake trout *Salvelinus namaycush* in two inland lakes in Wisconsin (Trout and Black Oak lakes in Vilas County), which represent the only known indigenous lake trout populations in the upper Mississippi River basin. Exogenous sources of lake trout (Lake Michigan and Lake Superior strains) have been stocked into each of these lakes for decades, although the long-term effects of past stocking events on these populations are unknown. We used nine microsatellite loci and polymerase chain reaction–restriction fragment length polymorphism analysis of mitochondrial DNA to determine the distinctiveness and genetic ancestry of lake trout in Trout and Black Oak lakes. Measures of allelic variance indicated that Trout and Black Oak lakes were significantly different ($P < 0.05$) from each other ($F_{ST} = 0.162$) and all other populations evaluated in this study ($F_{ST} = 0.101 - 0.164$). The combined microsatellite and mitochondrial DNA data indicate that upper Mississippi River basin lake trout have been minimally affected by past stocking practices. These populations should be managed as native gene pools, and interlake and interbasin stocking should be avoided.

The conservation and management of exploited or declining fishery stocks requires a fundamental understanding of the genetic population structure of the species involved. For many years, stocking hatchery-reared fish was the most common way to restore declining fishery stocks, with little regard to the ecological or genetic consequences for native stocks (Nielsen 1993). Although a growing number of empirical studies have shown that the introduction of exogenous strains may have negative genetic impacts on native fish stocks through

hybridization and introgression (Allendorf 1991; Hindar et al. 1991; Krueger and May 1991; Philipp 1991), other studies have been unsuccessful in documenting considerable stocking effects on native fish populations (Wishard et al. 1984; Vuorinen and Berg 1989; Hansen et al. 2000).

In the last half century, the lake trout *Salvelinus namaycush* has been one of the most heavily cultured and stocked species in North America. Catastrophic declines of lake trout in the Laurentian Great Lakes during the mid-20th century triggered massive stocking and restoration efforts (Selgeby et al. 1995 and references therein). Although most management efforts have focused on rehabilitating Great Lakes populations, introductions and stocking of lake trout have also been widespread in inland lakes (Crossman 1995; Powell and Carl 2003). Only recently has significant attention been

* Corresponding author: kyle.piller@selu.edu

¹ Present address: Department of Biological Sciences, Southeastern Louisiana University, Box 10736, Hammond, Louisiana 70402, USA.

Received March 2, 2004; accepted November 16, 2004

Published online June 13, 2005

TABLE 1.—Characteristics of lake trout populations and strains sampled, with additional sampling information.

Population or strain	Abbreviation	N	Sampling methodology	Size (cm)	Date of sampling	Basin	Lake size (km ²)
Trout Lake, Wisconsin	TL	48	Fyke netting	51.6–77.5	Fall 2001 (wild)	Mississippi River	15.2
Black Oak Lake, Wisconsin	BO	40	Fyke netting	41.1–85.9	Fall 2001 (wild)	Mississippi River	2.3
Big Green Lake ^a , Wisconsin	BG	43	Fyke netting	39.6–58.9	Fall 2001 (wild)	Lake Michigan	29.3
Lewis Lake ^b , Wyoming	LL	37	Hatchery	Unknown	Fall 2001 (hatchery)	Lake Michigan	11.4
Gull Island Shoal, Wisconsin	GIS	36	Fyke netting	58.2–91.2	Fall 2002 (wild)	Lake Superior	82,100
Marquette ^c , Michigan	MQ	50	Hatchery	Unknown	Fall 2001 (hatchery)	Lake Superior	82,100

^a Original source in Lake Michigan (Hacker 1957).

^b Sampled population was derived from F₁ fish from the Saratoga National Fish Hatchery; the original stocking source was Lake Michigan.

^c Broodstock hatchery source originally derived from Lake Superior.

focused on issues relating to the sustainability of inland lake trout populations in their own right (Evans and Olver 1995; Gunn et al. 2003; Powell and Carl 2003).

Genetic research and monitoring of lake trout has similarly had a strong Great Lakes focus. Genetic studies of lake trout in the Great Lakes over the last quarter century have employed a range of molecular markers, from allozymes and mitochondrial DNA (mtDNA) to nuclear sequences and microsatellite DNA (Dehring et al. 1981; Grewe and Hebert 1988; Krueger et al. 1989; Phillips et al. 1989; Grewe et al. 1993; Kincaid et al. 1993; Guinand et al. 2003; Page et al. 2003). By contrast, comparatively few studies have assessed the genetic structure and variation among inland populations. Although Ihssen et al. (1988) and Wilson and Hebert (1996, 1998) used allozymes and whole-molecule mtDNA, respectively, to identify the genetic structure of inland lake trout populations, these markers do not offer sufficient resolution for investigating fine-scale population genetic differentiation. Hypervariable microsatellite DNA markers, by contrast, have been used extensively in salmonids to examine genetic variation over both temporal and spatial scales (Nielsen et al. 1997; Hansen 2002; Guinand et al. 2003). In particular, microsatellites have proven useful in identifying the structure and admixture of genetic stocks (Olsen et al. 2000; Beacham et al. 2001) and in providing genetic information to monitor unique stocks or strains (Nielsen et al. 1999). Comparatively few studies have used microsatellite markers to study lake trout, although recent work has demonstrated their utility for delineating population structure in this species (Guinand et al. 2003; Page et al. 2003).

Wisconsin harbors two native inland lake trout populations, those in Trout and Black Oak lakes in Vilas County. These populations are potentially unique, as they represent the only two extant populations of lake trout indigenous to the upper Mississippi River basin (Greene 1935; Becker 1983; Lyons 1984). Trout Lake is relatively small, with a surface area of 15.2 km² (Table 1), and harbors one of the most diverse deepwater assemblages in the Mississippi River basin, which includes cisco *Coregonus artedii*, burbot *Lota lota*, slimy sculpin *Cottus cognatus*, opossum shrimp *Mysis relicta*, and possibly ninespine stickleback *Pungitius pungitius*. Black Oak Lake is much smaller (2.3 km²) and less speciose than Trout Lake but contains cisco and opossum shrimp in addition to lake trout (Lyons 1984).

Trout and Black Oak lakes were stocked with lake trout from Lake Michigan and Lake Superior sources at various times in an attempt to augment their populations (Table 2) (McKnight 1977; Jahns and Bozek 2000). Trout Lake has been periodically stocked with lake trout since the 1920s, the heaviest period of stocking occurring from 1956 to the present. Stocking records from the Wisconsin Department of Natural Resources (WDNR) indicate that during this period most stocked fish (55.5%) were derived from Lake Superior sources (Marquette and possibly Gull Island Shoal strains), while lake trout derived from Big Green Lake (also known as Green Lake) in central Wisconsin comprised only 2.1% of the total stockings. Although the Big Green Lake lake trout population was originally derived from Lake Michigan populations (Hacker 1957), Lake Superior strains have been stocked in Big Green Lake sporadically from 1951 to the present. Lake Superior strains were last

TABLE 2.—Stocking history for Trout and Black Oak lakes, including the number of lake trout stocked, size of stocked fish, and source population; F refers to fry, f to fingerlings, and y to yearlings.

Year	Trout Lake			Black Oak Lake		
	Number stocked	Size	Source	Number stocked	Size	Source
Pre-1950s	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
1951				31,000	F	Unknown
1959	7,122	f	Trout Lake			
1960	6,448	f	Big Green Lake			
1961	26,250	f	Trout and Big Green lakes			
1962	31,200	f	Big Green Lake			
1963	20,867	y	Lake Superior			
1964	20,000	y	Trout Lake			
1966	7,640	y	Lake Superior			
1967	9,500	y	Lake Superior			
1968	7,936	y	Lake Superior			
1969	25,000	y	Lake Superior			
1970	28,670	y	Trout Lake			
1971	42,650	y	Trout Lake			
1972	6,100	y	Trout Lake	11,120	y	Lake Superior
1973				25,349	Unknown	Lake Superior
1974				12,500	y	Lake Superior
1976	80,000	f	Lake Superior			
1977	95,000	f	Lake Superior	70,000	f	Lake Superior
1978	64,000	f	Lake Superior	80,000	f	Lake Superior
1979	45,000	f	Lake Superior	25,000	f	Lake Superior
1980	40,000	f	Lake Superior	15,000	f	Lake Superior
1983	18,994	y	Trout Lake			
1984	20,800	y	Trout Lake			
1985	15,625	y	Trout Lake			
1986	4,745	y	Trout Lake			
1987	32,000	y	Trout Lake			
1998	88,823	f	Trout Lake			

planted in Trout Lake in 1980, and the Big Green Lake strain was last stocked in 1962. In the 1980s, fisheries managers began supplemental stocking of the Trout Lake strain (42.3% of total stockings). However, the genetic composition or uniqueness of this strain was not investigated prior to culture, rearing, and stocking. Subsequent phylogeographic research on lake trout mtDNA variation based on whole-molecule restriction digests (Wilson and Hebert 1998) revealed that Trout Lake was comprised solely of fish of Mississippian ancestry. By contrast, Great Lakes stocks and hatchery strains showed mixed phylogenetic ancestry, with representation from all three major phylogenetic mtDNA lineages (Grewe and Hebert 1988; Wilson and Hebert 1996, 1998).

Black Oak Lake has also been stocked to a lesser degree with nonnative strains of lake trout from Lake Superior. From 1951 to 1980, approximately 270,000 progeny from were planted in Black Oak Lake from Lake Superior (WDNR, unpublished data). The most intensive period of stocking was from 1972 to 1980, and the last stocking event occurred in 1980 (Table 2). Undocumented stockings may also have occurred in the late-19th and

early-20th centuries (Becker 1983). Recent investigations have indicated that although the population is small, natural lake trout reproduction occurs in the lake (Wes Jahns, WDNR, personal communication). Although management programs aimed at restoring inland populations of lake trout in Wisconsin to self-sustaining levels have been implemented, detailed information regarding the genetic ancestry and population structure for upper Mississippi River basin populations is lacking. If Trout and Black Oak lakes represent genetically unique or remnant native populations despite multiple exogenous stocking events, a more aggressive restoration and protection program may be appropriate.

We used complementary genetic marker systems (microsatellite DNA and mtDNA) to determine whether lake trout in Trout and Black Oak lakes were comprised of mixed-ancestry stocks (i.e., admixed indigenous and exogenous gene pools) or were still native, indigenous populations. The study presented several challenges owing to the lakes' complex stocking history and lack of historical (prestocking) tissue samples. Rather than directly comparing current samples with historical

or archival ones to assess the contribution of past stocking events, we had to resolve the genetic ancestry and integrity of the inland populations through indirect evidence. We therefore characterized the genetic variation for the most likely source populations and applied several analytical methods to determine the genetic ancestry of the inland populations. The combined results support the hypothesis of largely intact native gene pools and provide baseline genetic data for the restoration and sustainable management of these potentially unique inland lake trout populations.

Methods

Genetic data collection.—Lake trout were captured in Trout Lake (TL) and Black Oak Lake (BO), nonlethally sampled (adipose or caudal fin clip), and released. In an effort to determine the strain composition of the inland lakes, tissue samples were also collected from all potential source populations, including the Gull Island Shoal (GIS) and Marquette (MQ) strains from the Lake Superior basin and from the Big Green Lake (BG; Green County, Wisconsin) and Lewis Lake (LL; Saratoga National Fish Hatchery, Wyoming) strains, which were originally derived from Lake Michigan stocks. All populations of wild lake trout were collected with fyke nets during fall 2001 sampling; total length ranged from approximately 40 to 91 cm. The Lewis Lake samples in this study were taken from first generation (F_1) hatchery fish that were created by crossing 68 wild females with 85 males collected from Lewis Lake in Yellowstone National Park in 2001. The Marquette strain sample was obtained from Michigan state broodstocks. The stocking history of other strains of lake trout used in this study has been discussed elsewhere in greater detail (Krueger et al. 1983; Krueger and Ihssen 1995; Page et al. 2003). All tissue samples were preserved and stored in 95% solutions of ethanol until analysis.

Genomic DNA was extracted from fin clips using the DNeasy tissue extraction kit (Qiagen). Two mtDNA segments, cytochrome *b* (cyt *b*) and subunits 3 and 4 of the NADH dehydrogenase gene (ND 3/4), were amplified for discrimination of phylogeographic ancestry. All mtDNA and microsatellite loci were amplified on an M-J Research PTC-100 Thermal Cycler. A 505-base-pair (bp) segment of the cyt *b* gene was amplified with a conserved teleost primer (5'-GTGACTTGAAAAACCACCGTTG-3') from Song et al. (1998) and an unpublished internal degenerate primer (5'-GARAABCCNCCYCARATTCATTG-3'). Unpublished ND 3/4 prim-

ers provided by R. Phillips (Washington State University) provided a second informative 724-bp amplicon. Both mtDNA segments were amplified via the polymerase chain reaction (PCR) in 10- μ L reaction volumes containing 8 ng of template DNA, 1 μ L of 10 \times PCR buffer, 2 mM of $MgCl_2$, 0.6–1.5 pmol of primers (cyt *b* and ND3/4, respectively), 1 mM of deoxynucleotide triphosphates (dNTPs), and 1 U of *Taq* polymerase. The PCR profiles for both amplicons involved a single denaturation step of 94°C for 2 min, followed by 30 cycles at 92°C for 45 s, 50°C for 1 min, and 72°C for 1 min, followed by a single extension cycle of 72°C for 6 min. The PCR amplicons for each gene were digested with 1 U *Bam* HI restriction enzyme for 1 h at 37°C in separate reactions. The digested fragments were photographed and sized in comparison with a known size standard. The fragment patterns generated by the restriction digests were assigned letter codes following the designations in Wilson and Hebert (1998).

Nine microsatellite loci were resolved for lake trout using primers originally developed for use in other salmonid species. These loci were *Sfo8*, *Sfo12*, *Sfo18*, *Sfo23* (brook trout *S. fontinalis*; Angers et al. 1995), *SfoC24*, *SfoC28*, *SfoD75* (brook trout; T. King and M. Burnham-Curtis, U.S. Geological Survey, unpublished data), *Sco19* (bull trout *S. confluentus*; Taylor et al. 2001), and *Ots-1* (Chinook salmon *Oncorhynchus tshawytscha*; Banks et al. 1999). Microsatellite loci were coamplified using fluorescently labeled primers in three 25- μ L multiplex reactions (the particular dye labels are given in parentheses): (1) *Sfo23* (NED), *SfoD75* (6-FAM), and *SfoC24* (6-FAM); (2) *Sfo8* (NED), *Sfo12* (HEX), and *SfoC28* (6-FAM); and (3) *Ots-1* (6-FAM), *Sfo18* (6-FAM), and *Sco19* (HEX). Each 25- μ L multiplex reaction contained approximately 8 ng of template DNA, 1.5 mM of $MgCl_2$, 200 μ M dNTPs, 0.2–0.4 pmol of each primer pair, and 0.4 U of *Taq*. The PCR profiles for all multiplexes were identical and consisted of an initial denaturation step of 95°C for 11 min, followed by 27 cycles of 94°C for 1 min, 53°C for 1 min, and 72°C for 1 min, followed by a single extension step of 60°C for 45 min. The resultant amplicons were electrophoresed and visualized by means of an ABI 377 (Applied Biosystems) or MegaBace 1000 (Amersham-Bioscience) DNA sequencer. To test for ambiguities between sequencers, a subset of the amplicons were run on both sequencers and compared visually and with MicroChecker (van Oosterhout et al. 2004). Gels were scored using Genotyper (Applied Biosys-

tems) or MegaBace Genetic Profiler (Amersham-Bioscience) software but were verified manually for accuracy.

Genetic data analyses.—The frequencies of the mtDNA polymerase chain reaction–restriction fragment length polymorphism digest haplotypes were summarized for each population and compared with published data. Mitochondrial divergence among populations was estimated with FSTAT (Goudet 2001) using the author's recommendations for F_{ST} estimation with haploid data.

Statistical analysis of population parameters, including allele frequencies, observed and expected heterozygosities, and the number of alleles per locus, was performed for each population and microsatellite locus with version 3.3 of GenePop (Raymond and Rousset 1995). Comparative allelic richness within populations was calculated with FSTAT (Goudet 2001) to correct for unequal sample sizes. The genotypes at each locus for each population were tested for conformance to Hardy–Weinberg equilibrium (HWE) using the heterozygote deficiency module in GenePop. Significance levels for the HWE tests and all other multiple comparison tests were adjusted using sequential Bonferroni methods (Rice 1989) with an initial α value of $0.05/k$, k being the number of tests.

In this study, null (nonamplifying) homozygotes were detected for one locus (*SfoC28*) and all populations exhibited some null homozygotes and fewer heterozygotes than expected at this locus. The occurrence of null alleles can confound the analysis of microsatellite data (Brookfield 1996), and several methodologies have been proposed to adjust allele frequencies in the presence of null alleles (Chakraborty et al. 1992; Brookfield 1996). Null allele frequencies for *SfoC28* were estimated using equation (4) of Brookfield (1996), which treats null homozygotes as observations rather than as missing data. The adjusted allele frequencies were re-input into GenePop and the population genetic statistics were regenerated for each locus and population. Except for individual assignment tests (see below), all subsequent analyses included adjusted values for the *SfoC28* locus. To test for differentiation among lake trout populations, pairwise F_{ST} values based on adjusted allele frequencies were obtained with FSTAT (Goudet 2001).

Individual assignment tests.—Individual assignment tests of the microsatellite data were conducted to estimate the probable origins of the lake trout in Trout and Black Oak lakes. Source populations for each fish from Trout and Black Oak

lakes were inferred from similarities in multilocus genotypes using version 1.0.02 of GeneClass (Cornuet et al. 1999). The *SfoC28* locus was not included in the assignment tests owing to the existence of null alleles. We employed a Bayesian approach (Rannala and Mountain 1997) with the leave-one-out procedure to assign individuals. We incorporated the simulation procedure in independent assignments to provide a measure of assignment confidence (10,000 simulations with a rejection level of $P < 0.01$). Assignment methods incorporating distance or frequency measures result in the classification of unknowns to a single probable source population, whereas assignment methods that use a Bayesian approach allow for one of several assignment possibilities (Cornuet et al. 1999). The simulation analysis allowed for several possible outcomes, including (1) the assignment of unknowns to a single population of origin, (2) the assignment of unknowns to an unresolved Great Lakes group (LL, BG, GIS, and/or MQ), and (3) the assignment of unknowns to a mixed-ancestry group ([LL, BG, GIS, and/or MQ] and [TL or BO]).

Results

Three mitochondrial lineages observed among populations of lake trout corresponded to the major lineages identified by Wilson and Hebert (1996, 1998). Digestion of the *cyt b* and ND 3/4 amplicons with *Bam* HI identified lake trout as having Mississippian (haplotype A), Atlantic (haplotype B), or Mississippian–Missourian–Beringian ancestry (haplotype C) (Figure 1). All specimens from Trout Lake possessed mtDNA characteristic of lake trout of Mississippian lineage. With the exception of one specimen that possessed haplotype C, all individuals in Black Oak Lake were characterized by the Mississippian (A) mtDNA. By contrast, samples from the potential source populations contained all three mitochondrial lineages with frequencies similar to those observed by Grewe and Hebert (1988).

Nine microsatellite loci were scored for at least 37 individual lake trout per population (Table 3; Appendix 1). The results indicated that there were no differences in allele size calls between DNA sequencers. All microsatellite loci were polymorphic; the total number of alleles per locus (excluding null alleles) ranged from 4 (*Sfo12*) to 34 (*Sfo23*) across all populations, and the average number of alleles per locus was 15.2. The two Great Lakes populations (Gull Island Shoal and Marquette) showed the greatest allelic diversity at

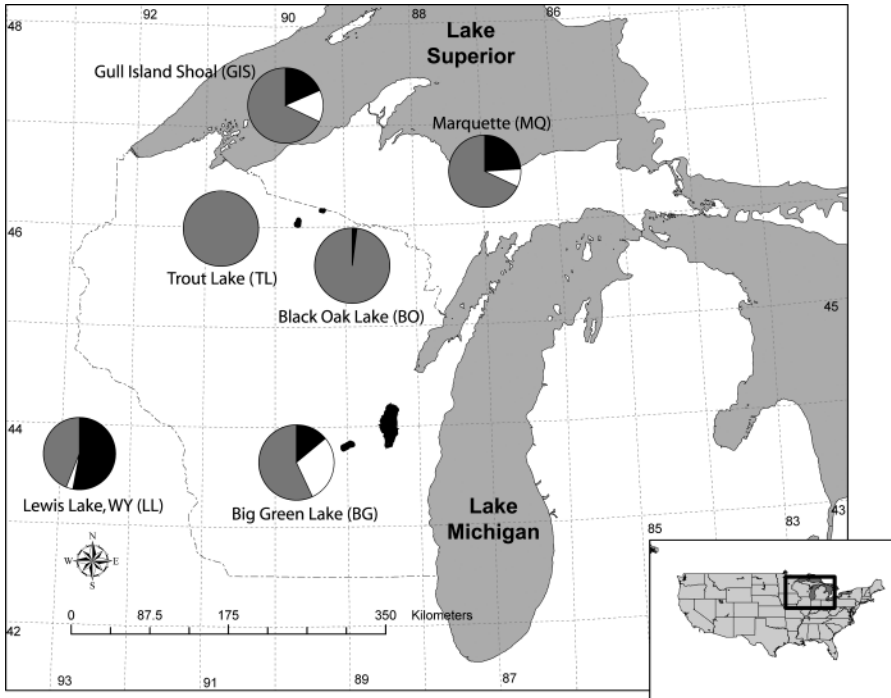


Figure 1.—Distribution of lake trout mtDNA haplotypes for six lake trout populations; sample sizes are given in Table 1. Within the pie charts, gray shading refers to the Mississippian, white to the Atlantic, and black to the Mississippi-Missourian-Beringian phylogeographic lineages (Grewe and Hebert 1988; Wilson and Hebert 1996, 1998).

five of the microsatellite loci, despite differences in sample size. Although allelic richness was lowest for Trout and Black Oak lakes independent of sample size, the differences in allelic richness among populations were not significant ($P = 0.072$).

Tests of heterozygote deficiency showed significant deviations from Hardy-Weinberg equilibri-

um in 6 of 54 tests ($P < 0.05$; $k = 54$) after adjustment for null alleles. Despite this correction, most deficits (5/6) were at the *SfoC28* locus. Null alleles (i.e., null-null homozygotes) were detected in all populations at the *SfoC28* locus and were confirmed by repeated amplification efforts alongside positive controls. The proportion of null allele homozygotes ranged from 0.075 (Black Oak Lake)

TABLE 3.—Observed genetic diversity at nine microsatellite loci for six populations of lake trout, including the total number of alleles, mean number of alleles per population (N_A), mean expected heterozygosity (H_E), mean observed heterozygosity (H_O), and allele size range.

Locus and mean	Total number of alleles	N_A	H_E	H_O	Allele size range
<i>Ots1</i>	25	14.33	0.834	0.884	219–263
<i>Sfo18</i>	7	4.17	0.546	0.545	172–188
<i>SfoC24</i>	10	4.33	0.566	0.548	81–114
<i>SfoD75</i>	19	10.67	0.841	0.741	272–350
<i>Sfo23</i>	34	19.00	0.912	0.899	169–247
<i>Sco19</i>	11	6.33	0.609	0.589	158–178
<i>SfoC28</i> ^a	11	4.83	0.578	0.193	251–293
<i>Sfo8</i>	16	8.83	0.828	0.897	263–297
<i>Sfo12</i>	4	2.83	0.218	0.210	253–261
Mean	15.22	8.40	0.659	0.612	

^a Unadjusted for the presence of null alleles.

TABLE 4.—Pairwise F_{ST} comparisons among six lake trout populations in the upper Mississippi River and Great Lakes basins for polymerase chain reaction–restriction fragment length polymorphism mtDNA (above diagonal) and microsatellite DNA analyses (below diagonal; mean values across 9 loci). Asterisks indicate estimates that were significantly different from zero ($P < 0.05$) after sequential Bonferroni correction for multiple comparisons. See Table 1 for abbreviations.

Population	TL	BO	BG	LL	GIS	MQ
TL		−0.0004	0.3476*	0.4702*	0.2223*	0.2428*
BO	0.162*		0.3098*	0.4374*	0.1837*	0.2119*
BG	0.164*	0.101*		0.1129*	0.0130*	0.0459
LL	0.138*	0.149*	0.085*		0.1043	0.0435
GIS	0.117*	0.127*	0.051*	0.023		−0.0037
MQ	0.111*	0.109*	0.067*	0.060*	0.025	

to 0.729 (Trout Lake) (mean = 0.381); the adjusted r values based on the Brookfield (1996) null allele adjustment method ranged from 0.382 (Black Oak Lake) to 0.807 (Trout Lake).

Population Differentiation

Estimates of population divergence based on mtDNA haplotype frequencies and microsatellite allelic variances (F_{ST}) showed sharply contrasting patterns for pairwise differentiation among all pairwise comparisons of the lake trout populations (Table 4). Trout Lake and Black Oak Lake were very similar based on mtDNA, but both differed significantly from all other populations, with F_{ST} estimates ranging from 0.18 to 0.47. In comparison, the Big Green Lake and Lewis Lake populations were significantly different from each other, but neither differed significantly from those of Great Lakes sources (Table 4).

By contrast, F_{ST} estimates based on microsatellite data showed significant differentiation among all population pairs (Table 4). Pairwise F_{ST} values ranged from 0.023 (LL and GIS) to 0.164 (BG and TL). The Trout Lake and Black Oak Lake populations were significantly distinct from each other ($F_{ST} = 0.162$; $P < 0.05$) and other populations ($F_{ST} = 0.101 - 0.164$; $P < 0.05$), whereas

Great Lakes (or Great Lakes–derived) populations showed much lower levels of genetic differentiation ($F_{ST} = 0.023 - 0.085$; $P < 0.05$).

Individual Assignment

Individual assignments using multilocus genotypes showed that Trout and Black Oak lakes were comprised of relatively pure populations. The greatest assignment success in simulations was found for Trout Lake, 90% (43/48 fish) being assigned back to Trout Lake as the most likely source (log-likelihood ratios ≥ 2 , equivalent to $P \leq 0.01$). Lake trout sampled from Black Oak Lake were similarly recognizable as a distinct population (85% of fish with log-likelihood ratios ≥ 2 compared with the next most probable source). Gull Island Shoal had the lowest proportion of self-assigned individuals (21%), perhaps reflecting the high genetic diversity within this population and its relatedness to the Marquette strain and introduced populations (Table 5). A single individual in Trout Lake was assigned to Gull Island Shoal (a population previously stocked in Trout Lake), and two had mixed ancestry. One fish in Black Oak Lake had mixed ancestry, and four were misassigned to Trout Lake. By contrast, lake trout

TABLE 5.—Proportional assignment of individual lake trout from each population based on individual multilocus genotypes and population allelic frequencies at nine microsatellite loci. Correct assignments are shown in bold italics. Assignment probabilities are based on Bayesian assignments using 10,000 simulations per population and a rejection probability of 0.01. The abbreviation UGL (unresolved Great Lakes) refers to Great Lakes populations or populations originally derived from Great Lakes sources that could not be identified further; the term “mixed” refers to individual genotypes that could be assigned with equal probability to more than one source. See Table 1 for other abbreviations.

Population	<i>N</i>	TL	BO	BG	LL	GIS	MQ	UGL	Mixed
TL	48	0.90				0.02			0.08
BO	40	0.10	0.85						0.05
BG	43			0.42				0.53	0.05
LL	37			0.03	0.35	0.05		0.49	0.08
GIS	39					0.21	0.03	0.73	0.03
MQ	50						0.58	0.42	

sampled from putative source populations were never solely misassigned to inland populations.

Discussion

This study focused on determining the genetic ancestry of lake trout in Trout and Black Oak lakes, two systems that have been influenced by stocking from exogenous sources. Data from both mitochondrial and microsatellite DNA markers suggest that stocking has had relatively little influence on the genetic composition of these inland lakes. Despite the absence of historical (prestocking) samples to compare with the extant populations, all lines of evidence suggest that introgression from stocking has had no impact on the genetic characteristics of the Trout and Black Oak Lake populations of lake trout.

Mitochondrial DNA Diversity

The data from this study indicate that Great Lakes populations or those originally derived from Great Lakes sources (i.e., BG and LL) are mitochondrially more diverse than inland populations (Figure 1). Mitochondrially, three distinct groups occur among all the populations examined in this study and correspond to those identified in previous studies (Wilson and Hebert 1996, 1998). Possession of multiple haplotypic lineages within a single lake trout strain implies either secondary contact among historic lineages during postglacial re-colonization (Wilson and Hebert 1996) or human-mediated contact via stocking. Although Grewe and Hebert (1988) detected only lineage A lake trout from Big Green Lake in their study ($n = 15$ fish from the Jordan National Fish Hatchery), all three lineages were detected in the larger sample ($n = 50$) taken for this study (Figure 1).

Trout Lake was comprised solely of the Mississippian mtDNA lineage, a result that corroborated observations by Wilson and Hebert (1996). This sample lineage was similarly predominant in Black Oak Lake, which had not been previously studied. The single lake trout from Black Oak Lake that exhibited the lineage C haplotype could also indicate that Black Oak Lake has a pure native ancestry based on mtDNA, as Wilson and Hebert (1998) reported that some lake trout that exhibited the mitochondrial C haplotype dispersed from a southern (Mississippian or Missourian) refuge. Conversely, this fish could represent some genetic contribution of past stocking to Black Oak Lake, as the Lake Superior sources used to stock the lake contain moderate percentages of lake trout with haplotype C (Wilson and Hebert 1998). Even if

this individual represents a mitochondrial contribution from a stocking source, introgression from exogenous sources appears to have been minimal.

By contrast, the mitochondrial data from the introduced populations (Big Green Lake and Lewis Lake) indicated their origin from Great Lakes sources. All three major mtDNA phylogeographic lineages observed by Wilson and Hebert (1996, 1998) were present in both introduced populations, which is concordant with data from Great Lakes populations and hatchery stocks (Grewe and Hebert 1988; Wilson and Hebert 1998). Although the different frequencies of the mtDNA lineages within Big Green Lake and Lewis Lake may reflect some genetic drift since these populations were established, there has been insufficient time and large enough population sizes to obscure their stocked origins (Table 4).

Microsatellite DNA Differentiation

Microsatellite data revealed strong levels of genetic differentiation between upper Mississippi River basin populations and Great Lakes (or Great Lakes-derived) sources. Although inland populations of lake trout (Trout and Black Oak lakes) contained the lowest levels of allelic variation, they were the most differentiated populations examined in this study. Their constrained population sizes and long isolation (10,000–12,000 years; Lyons 1984) have probably enhanced their differentiation through genetic drift and local adaptation. The Great Lakes populations were somewhat differentiated from each other based on microsatellite-based F_{ST} estimates, but they were more similar to each other than to the inland lakes because of their shared ancestry, geographic proximity (GIS and MQ), and recent historical connections through stocking (BG and LL).

The apparently contradictory results for the two genetic marker systems reinforce the probable native status of the lake trout populations in Trout and Black Oak lakes. Both populations were fixed or nearly so for the Mississippian mtDNA lineage and have retained their phylogeographic (Mississippian) ancestry (Wilson and Hebert 1998). By contrast, the diverse mtDNA composition of the introduced inland populations (Big Green Lake and Lewis Lake) reflects their founding from diverse ancestral (Great Lakes) sources (Grewe and Hebert 1988). Similarly, the substantial nuclear DNA differentiation between Trout Lake and Black Oak Lake ($F_{ST} = 0.162$) reflects their historical isolation and subsequent divergence,

whereas the lower F_{ST} values among the other populations reflect substantial gene flow, either through natural or anthropogenic means. Interestingly, both sets of genetic data reflect the close affinities between the introduced inland populations (Big Green Lake and Lewis Lake) and the Great Lakes hatchery strains, indicating their recent establishment and comparative lack of divergence.

Other aspects of the genetic data similarly support the hypothesis of native gene pools and argue against introgression from stocked fish. Both Trout Lake and Black Oak Lake populations had substantially lower allelic richness than Great Lakes strains or the introduced inland populations with respect to both mtDNA haplotypes and microsatellite loci, which is consistent with genetic drift or coalescence within limited populations over long periods of time. The comparable levels of heterozygosity across populations (Appendix 1) indicate that this is not an artifact of sampling effort or inbreeding but reflects the genetic diversity present within the studied populations.

The ability to identify distinct strains is contingent on the degree of genetic differentiation among populations (Cornuet et al. 1999). In this study, the inland populations were highly differentiated from stocking source populations and each other based on the combined mitochondrial and microsatellite DNA data. As a result, the individual assignment tests based on the microsatellite loci resulted in high assignment success for highly differentiated populations, including Trout Lake and Black Oak Lake. The largest proportion of misclassifications was attributed to the GIS strain, which was poorly differentiated from the other populations. Guinand et al. (2003) provided evidence that contemporary Lake Superior populations, including GIS and Isle Royale, had closer genetic affinities with contemporary hatchery-reared fish than with historic (prestocked) wild fish, indicating that these populations may have introgressed with native stocks. Conversely, successful large-scale facilitated gene flow through the introduction or introgression of genes from donor ("source") populations would also reduce assignment power. Situations such as these can confound assignment tests through the homogenization of formerly distinctive strains, resulting in poor assignment success for individual populations.

Conservation and Management Implications

Based on both direct and indirect estimates of gene flow, our results show that there was minimal

genetic contribution of exogenous lake trout in the Trout and Black Oak Lake populations. This contrasts with the results from numerous studies that have documented introgression and negative impacts of cultured salmonids on congeneric or conspecific populations through hybridization, introgression, and competition (Allendorf and Leary 1988; Krueger and May 1991). Although it is not known why so little introgression occurred in these populations, Evans and Olver (1995) and Powell and Carl (2003) discuss several ecological hypotheses that could merit investigation with neutral genetic markers.

The results from this study have several conservation and management implications. The populations of lake trout in Trout and Black Oak lakes are small and isolated and may constitute the most representative (unmixed) remnants of the Pleistocene Mississippian refugial race of lake trout. As such, these populations may represent unique biodiversity elements for the species and should be given a high level of protection. A concerted effort should be put made to shield Trout and Black Oak lakes from additional human-mediated impacts, including overfishing, habitat loss, and genetic degradation.

The current management plan for Trout Lake includes angling harvest regulations that protect 95% of the population and closed fishing seasons (Jahns and Bozek 2000). A similar plan does not currently exist for Black Oak Lake, but one is being developed. Additional research should focus on the life history and population structure of lake trout within these lakes. Collecting data for existing life history and exploitation models for lake trout, as in Shuter et al. (1998), could provide valuable and cost-effective management information.

Future management plans should include safeguarding the genetic integrity of these populations and exclude exogenous stocking or transfers. Presently, Black Oak Lake has a self-sustaining population at the average lake trout density for a lake of its size, whereas Trout Lake is below average in density and fish numbers are sustained by supplemental (same-source) stocking. Unfortunately, supplemental stocking can have long-term negative genetic impacts through decreases in effective population size (Krueger and May 1991; Ryman et al. 1995; Laikre and Ryman 1996; but see Duchesne and Bernatchez 2002). However, continued genetic monitoring can help to detect these effects. Alternatively, an annual monitoring program addressing the differential contribution of hatchery and wild fish to future generations, through either

the incorporation of additional genetic studies or continued usage of traditional mark-recapture methodologies, should be implemented.

As lake trout populations face increasingly diverse pressures across the species' range, resolving the ancestry and status of individual-lake populations should help fisheries professionals and management agencies assess options for regional, lake-specific management strategies. Regardless of stocking or exploitation histories, management agencies should make a concerted effort to identify native populations in order to protect their long-term viability and safeguard these evolutionary and adaptive resources for the species as a whole.

Acknowledgments

We would like to thank WDNR biologists Dave Bartz, Wes Jahns, Dave Paynter, and Steve Schramm for collecting tissues or for providing stocking information. Kim Scribner (Michigan State University) and Ed Stege (U.S. Fish and Wildlife Service, Saratoga National Fish Hatchery, Wyoming) provided DNA and tissue samples from the Marquette hatchery strain and Lewis Lake population, respectively. Mike Lavender (Ontario Ministry of Natural Resources) provided invaluable assistance in the laboratory for all stages of data collection. Funding was provided in part by the Federal Aid in Sport Fish Restoration Program, Project F-95-P, study SSOT and SSDI, administered through the Fish and Habitat Research Section of the WDNR Bureau of Integrated Science Services.

References

- Allendorf, F. W. 1991. Ecological and genetic effects of fish introductions: synthesis and recommendations. *Canadian Journal of Fisheries and Aquatic Sciences* 48(Supplement 1):178–181.
- Allendorf, F. W., and R. F. Leary. 1988. Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conservation Biology* 2:170–184.
- Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook charr (*Salvelinus namaycush*) reveals strong population subdivision on a microgeographic scale. *Journal of Fish Biology* 47(Supplement A):177–185.
- Banks, M. A., M. S. Blouin, B. A. Baldwin, V. K. Rashbrook, H. A. Fitzgerald, S. M. Blankenship, and D. Hedgecock. 1999. Isolation and inheritance of microsatellites in Chinook salmon (*Oncorhynchus tshawytscha*). *Journal of Heredity* 90:281–288.
- Beacham, T. D., J. R. Candy, K. J. Supernault, T. Ming, B. Deagle, A. Schulze, D. Tuck, K. Kaukinen, J. R. Irvine, K. M. Miller, and R. E. Withler. 2001. Evaluation and application of microsatellite and major histocompatibility complex variation for stock identification of coho salmon in British Columbia. *Transactions of the American Fisheries Society* 130:116–1149.
- Becker, G. C. 1983. *Fishes of Wisconsin*. University of Wisconsin Press, Madison.
- Brookfield, J. F. Y. 1996. A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* 5:453–455.
- Chakraborty, R., M. Andrade, S. P. Daiger, and B. Budowle. 1992. Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics* 56:45–57.
- Cornuet, J. M., S. Piry, G. Luikart, A. Estoup, and M. Solignac. 1999. New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* 153:1989–2000.
- Crossman, E. J. 1995. Introduction of the lake trout (*Salvelinus namaycush*) in areas outside its native distribution: a review. *Journal of Great Lakes Research* 21(Supplement 1):17–29.
- Dehring, T. R., A. F. Brown, C. H. Daugherty, and S. R. Phelps. 1981. Survey of the genetic variation among eastern Lake Superior lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences* 38:1738–1746.
- Duchesne, P., and L. Bernatchez. 2002. An analytical investigation of the dynamics of inbreeding in multigeneration supportive breeding. *Conservation Genetics* 3:47–60.
- Evans, D. O., and C. H. Olver. 1995. Introduction of lake trout (*Salvelinus namaycush*) to inland lakes of Ontario, Canada: factors contributing to successful colonization. *Journal of Great Lakes Research* 21(Supplement 1):30–53.
- Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices, version 2.9.3. Available: <http://www.unil.ch/izea/software/fstat.html>.
- Greene, C. W. 1935. *The distribution of Wisconsin fishes*. State of Wisconsin Conservation Commission, Madison.
- Grewe, P. E., and P. D. N. Hebert. 1988. Mitochondrial DNA diversity among brood stocks of the lake trout, *Salvelinus namaycush*. *Canadian Journal of Fisheries and Aquatic Sciences* 45:2114–2122.
- Grewe, P. E., C. C. Krueger, C. F. Aquadro, E. Bermingham, H. L. Kincaid, and B. May. 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* 50:2397–2403.
- Guinand, B., K. T. Scribner, K. S. Page, and M. K. Burnham-Curtis. 2003. Genetic variation over space and time: analyses of extinct and remnant lake trout populations in the upper Great Lakes. *Proceedings of the Royal Society of London B* 270:425–433.
- Gunn, J. M., R. J. Steedman, and R. A. Ryder, editors. 2003. *Boreal Shield watersheds: lake trout ecosystems in a changing environment*. CRC Press, Boca Raton, Florida.

- Hacker, V. A. 1957. Biology and management of lake trout in Green Lake, Wisconsin. *Transactions of the American Fisheries Society* 86:1–13.
- Hansen, M. M. 2002. Estimating the long-term effects of stocking domesticated brown trout into wild brown trout (*Salmo trutta*) populations: an approach using microsatellite DNA analysis of historical and contemporary samples. *Molecular Ecology* 11: 1003–1015.
- Hansen, M. M., D. E. Ruzzante, E. E. Nielsen, and K. L. D. Mensberg. 2000. Microsatellite and mitochondrial DNA polymorphism reveals life-history-dependent interbreeding between hatchery trout and wild brown trout (*Salmo trutta* L.). *Molecular Ecology* 9:583–594.
- Hindar, K., N. Ryman, and F. Utter. 1991. Genetic effects of cultured fish on natural populations. *Canadian Journal of Fisheries and Aquatic Sciences* 48:945–957.
- Ihssen, P. E., J. M. Casselman, G. W. Martin, and R. B. Phillips. 1988. Biochemical genetic differentiation of lake trout (*Salvelinus namaycush*) stocks of the Great Lakes region. *Canadian Journal of Fisheries and Aquatic Sciences* 46:1018–1029.
- Jahns, W., and M. Bozek. 2000. Upper Wisconsin basin inland lake trout management plan. Wisconsin Department of Natural Resources Report, Madison.
- Kincaid, H. L., C. C. Krueger, and B. May. 1993. Preservation of genetic variation in the Green Lake strain lake trout derived from remnant domestic and feral populations. *North American Journal of Fisheries Management* 13:318–325.
- Krueger, C. C., and B. May. 1991. Ecological and genetic consequences of salmonid introductions in North America. *Canadian Journal of Fisheries and Aquatic Sciences* 48(Supplement 1):66–77.
- Krueger, C. C., and P. E. Ihssen. 1995. Review of genetics of lake trout in the Great Lakes: history, molecular genetics, physiology, strain comparisons, and restoration management. *Journal of Great Lakes Research* 21(Supplement 1):348–363.
- Krueger, C. C., R. M. Horrall, and H. Gruenthal. 1983. Strategy for the use of lake trout strains in Lake Michigan. Wisconsin Department of Natural Resources, Administrative Report 17, Madison.
- Krueger, C. C., J. E. Marsden, H. L. Kincaid, and B. May. 1989. Genetic differentiation among lake trout strains stocked in Lake Ontario. *Transactions of the American Fisheries Society* 118:317–330.
- Laikre, L., and N. Ryman. 1996. Effects on intraspecific biodiversity from harvesting and enhancing natural populations. *Ambio* 25:504–509.
- Lyons, J. 1984. The distribution and zoogeography of the lake trout, lake whitefish, and ninespine stickleback in Vilas and Oneida counties, Wisconsin. *Wisconsin Academy of Science and Letters* 72:201–211.
- McKnight, T. 1977. Investigations of lake trout and whitefish management in Trout Lake, Vilas Co., WI. Wisconsin Department of Natural Resources, Madison.
- Nielsen, E. E., M. M. Hansen, and V. Loeschcke. 1997. Analysis of microsatellite DNA from old scale samples of Atlantic salmon: a comparison of genetic composition over sixty years. *Molecular Ecology* 6: 487–492.
- Nielsen, L. A. 1993. History of inland fisheries management in North America. Pages 3–31 in C. C. Kohler and W. A. Hubert, editors. *Inland fisheries management in North America*. American Fisheries Society, Bethesda, Maryland.
- Olsen, J. B., P. Bentzen, M. A. Bass, J. B. Shaklee, and S. Young. 2000. Microsatellites reveal population identity of individual pink salmon to allow supportive breeding of a population at risk of extinction. *Transactions of the American Fisheries Society* 129:233–242.
- Page, K. S., K. T. Scribner, K. R. Bennett, L. M. Garzel, and M. K. Burnham-Curtis. 2003. Genetic assessment of strain-specific sources of lake trout recruitment in the Great Lakes. *Transactions of the American Fisheries Society* 132:877–894.
- Philipp, D. L. 1991. Genetic implications of introducing Florida largemouth bass, *Micropterus salmoides floridanus*. *Canadian Journal of Fisheries and Aquatic Sciences* 48:58–65.
- Phillips, R. B., K. D. Zajicek, and P. E. Ihssen. 1989. Population differences in chromosome-banding polymorphisms in lake trout. *Transactions of the American Fisheries Society* 118:64–73.
- Powell, M. J., and L. M. Carl. 2003. Lake trout stocking in small lakes: factors affecting success. Pages 219–238 in J. M. Gunn, R. J. Steedman, and R. A. Ryder, editors. *Boreal Shield watersheds: lake trout ecosystems in a changing environment*. CRC Press, Boca Raton, Florida.
- Rannala, B., and J. L. Mountain. 1997. Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the USA* 94:9197–9221.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86:248–249.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- Ryman, N., F. Utter, and L. Laikre. 1995. Protection of intraspecific biodiversity of exploited fishes. *Reviews in Fish Biology and Fisheries* 5:417–446.
- Selgeby, J. H., R. L. Eschenroder, C. C. Krueger, J. E. Marsden, and R. L. Pycha, editors. 1995. International conference on restoration of lake trout in the Laurentian Great Lakes. *Journal of Great Lakes Research* 21(Supplement 1).
- Shuter, B. J., M. L. Jones, R. M. Korver, and N. P. Lester. 1998. A general, life-history-based model for regional management of fish stocks: the inland lake trout (*Salvelinus namaycush*) fisheries of Ontario. *Canadian Journal of Fisheries and Aquatic Sciences* 55:2161–2177.
- Song, C. B., T. J. Near, and L. M. Page. 1998. Phylogenetic relations among percid fishes as inferred from mitochondrial cytochrome *b* sequence data. *Molecular Phylogenetics and Evolution* 10:343–353.
- Taylor, E. B., Z. Redenbach, A. B. Costello, S. M. Pol-

- lard, and C. J. Pacas. 2001. Nested analysis of genetic diversity in northwestern North American Dolly Varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*). *Canadian Journal of Fisheries and Aquatic Sciences* 58:406–420.
- van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills, and P. Shipley. 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4: 535–538.
- Vuorinen, J., and O. K. Berg. 1989. Genetic divergence of anadromous and nonanadromous Atlantic salmon (*Salmo salar*) in the River Namsen, Norway. *Canadian Journal of Fisheries and Aquatic Sciences* 46:406–409.
- Wilson, C. C., and P. D. N. Hebert. 1996. Phylogeographic origins of lake trout (*Salvelinus namaycush*) in eastern North America. *Canadian Journal of Fisheries and Aquatic Sciences* 53:2764–2775.
- Wilson, C. C., and P. D. N. Hebert. 1998. Phylogeography and postglacial dispersal of lake trout (*Salvelinus namaycush*) in North America. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1010–1024.
- Wishard, L. N., J. E. Seeb, F. M. Utter, and D. Stearn. 1984. A genetic investigation of suspected redband trout populations. *Copeia* 1984:120–132.

Appendix: Genetic Variability of Lake Trout

TABLE A.1.—Estimates of allele frequencies and measures of genetic variability for lake trout populations by locality. Population abbreviations are as follows: TL, Trout Lake; BO, Black Oak Lake; BG, Big Green Lake; LL, Lewis Lake; GIS, Gull Island Shoal; and MQ, Marquette. See Table 1 in the text for more information on these populations.

Locus and statistic	Allele	Population					
		TL	BO	BG	LL	GIS	MQ
<i>Ots1</i>	215	0	0	0	0.014	0	0
	217	0	0	0	0	0	0.070
	219	0	0	0.198	0.108	0.385	0.420
	221	0.010	0.013	0.279	0.014	0.026	0.100
	223	0	0.013	0.035	0	0.038	0.040
	225	0.010	0	0.186	0.230	0.115	0.040
	227	0	0	0.023	0.068	0.026	0.010
	229	0.010	0	0.012	0.027	0.064	0
	231	0.073	0.013	0.047	0.149	0.103	0.020
	233	0	0.038	0.012	0.095	0	0.040
	235	0.021	0.050	0	0.014	0.038	0
	237	0.031	0	0	0	0	0
	239	0.010	0	0.023	0.014	0.013	0.030
	241	0	0	0	0.014	0	0
	243	0.010	0	0	0	0	0.060
	245	0	0	0.047	0.054	0.051	0.050
	247	0	0	0.023	0.122	0.013	0
	249	0.010	0.075	0	0	0.026	0
	251	0.063	0.325	0	0	0.013	0.010
	253	0.302	0.088	0	0	0	0.060
	255	0.219	0.088	0.070	0.027	0.051	0.030
	257	0.125	0.238	0.035	0.054	0	0
	259	0.104	0.050	0.012	0	0.026	0.020
	261	0	0	0	0	0.013	0
	263	0	0.013	0	0	0	0
<i>Sfo18</i>	172	0.177	0.213	0.593	0.649	0.577	0.540
	174	0.188	0.063	0	0	0	0
	176	0	0	0	0	0.013	0.040
	182	0.635	0.700	0.314	0.216	0.282	0.300
	184	0	0.025	0.093	0.108	0.026	0
	186	0	0	0	0.027	0.013	0.010
	188	0	0	0	0	0.090	0.110
<i>SfoC24</i>	81	0	0	0	0	0.013	0
	84	0	0	0	0	0.013	0
	90	0	0	0	0	0	0.020
	93	0	0	0	0.027	0	0
	96	0	0	0.023	0	0	0.010
	102	0.635	0.463	0.233	0.243	0.218	0.380
	105	0.365	0.238	0.605	0.622	0.667	0.440

TABLE A.1.—Continued.

Locus and statistic	Allele	Population					
		TL	BO	BG	LL	GIS	MQ
<i>SfoD75</i>	108	0	0	0	0	0	0.010
	111	0	0.300	0.140	0.095	0.090	0.130
	114	0	0	0	0.014	0	0.010
	272	0	0	0	0	0	0.020
	274	0	0	0	0	0	0.040
	282	0	0	0	0	0	0.020
	286	0.031	0.038	0	0.041	0.038	0.020
	290	0.010	0.250	0.093	0	0.154	0.100
	294	0.094	0.125	0.128	0.027	0.051	0.130
	298	0	0.125	0.023	0.122	0.103	0.150
	302	0.146	0.038	0.105	0.351	0.167	0.050
	306	0.240	0.225	0.419	0.243	0.179	0.030
	308	0	0	0	0	0	0.020
	310	0.260	0.163	0.093	0.054	0.115	0.110
	312	0	0	0	0	0.026	0
	314	0.083	0.025	0.105	0.081	0.064	0.170
	318	0.104	0.013	0.035	0.068	0	0.060
	322	0.021	0	0	0.014	0.038	0
	326	0	0	0	0	0.026	0.060
	330	0	0	0	0	0.026	0.020
	334	0.010	0	0	0	0	0
	350	0	0	0	0	0.013	0
<i>Sfo23</i>	169	0	0	0	0	0.026	0
	175	0.010	0	0	0	0	0
	179	0	0	0	0.041	0	0
	181	0.052	0.013	0.012	0.027	0.038	0.010
	183	0.010	0	0	0	0.038	0
	187	0.125	0.213	0.151	0.189	0.090	0.010
	189	0	0	0	0.014	0.026	0.050
	191	0.219	0.025	0.023	0.041	0.051	0.020
	193	0	0	0.105	0	0.013	0
	195	0.021	0	0.023	0	0.013	0
	199	0	0	0	0.027	0.038	0
	201	0.010	0	0	0.014	0.051	0
	203	0	0	0	0.014	0.038	0
	205	0	0	0	0.041	0.026	0.030
	207	0.042	0	0.116	0.122	0.026	0.020
	209	0.146	0.038	0.070	0.068	0.051	0.050
	211	0.063	0.075	0	0.041	0.038	0.090
	213	0.063	0.038	0.023	0.027	0.038	0.120
	215	0.052	0	0.105	0.095	0.141	0.130
	217	0.031	0.025	0.081	0.068	0.103	0.030
	219	0.042	0	0.058	0.041	0.051	0.280
	221	0.031	0	0.105	0	0.051	0.070
	223	0.063	0.113	0.058	0.054	0.013	0.020
	225	0.010	0	0.035	0.054	0	0.030
	227	0	0.138	0	0.014	0	0.010
	229	0	0.025	0	0	0.013	0.010
	233	0.010	0.063	0.023	0.014	0.013	0
	235	0	0.025	0	0	0	0
	237	0	0.125	0.012	0	0.013	0
	239	0	0.038	0	0	0	0
	241	0	0.025	0	0	0	0
	243	0	0.013	0	0	0	0
	245	0	0.013	0	0	0	0.010
	247	0	0	0	0	0	0.010
<i>Sco19</i>	158	0	0	0	0	0	0.010
	160	0	0	0.174	0.095	0.179	0.050
	162	0	0.013	0	0	0	0
	164	0	0	0.047	0	0.013	0.070
	166	0.010	0	0.012	0.027	0	0

TABLE A.1.—Continued.

Locus and statistic	Allele	Population					
		TL	BO	BG	LL	GIS	MQ
<i>SfoC28</i>	168	0	0	0	0	0.026	0
	170	0.865	0.300	0.349	0.230	0.308	0.390
	172	0.094	0.125	0.012	0.027	0.026	0.020
	174	0.031	0.500	0.326	0.595	0.372	0.400
	176	0	0.063	0	0.027	0.051	0.030
	178	0	0	0.081	0	0.026	0.030
	251	0.846	0	0.100	0.750	0.603	0.387
	263	0.077	0.027	0.175	0	0.103	0.242
	266	0	0	0	0	0.034	0
	275	0	0.095	0	0	0	0
	278	0	0	0	0.067	0	0
	281	0	0.419	0.550	0	0.103	0
	284	0	0.392	0.025	0.050	0.069	0.145
	287	0	0.068	0	0.133	0.086	0.129
	290	0.077	0	0	0	0	0.097
<i>Sfo8</i>	293	0	0	0.150	0	0	0
	263	0	0	0.012	0	0	0
	265	0	0	0.058	0.014	0	0
	269	0	0	0	0	0	0.020
	271	0	0	0	0	0.013	0
	273	0	0.038	0.081	0	0.026	0
	275	0	0.338	0.047	0.135	0.013	0.050
	277	0.042	0	0.128	0.068	0.026	0.020
	279	0.104	0	0.058	0.176	0.077	0.070
	281	0.083	0.100	0.023	0.108	0.077	0.030
	283	0.188	0.075	0.105	0.081	0.090	0.320
	285	0.385	0.150	0.267	0.257	0.269	0.230
	287	0.073	0.113	0.151	0.095	0.295	0.100
	289	0.125	0.188	0	0.041	0.077	0.070
	291	0	0	0.012	0.014	0.013	0.060
<i>Sfo12</i>	293	0	0	0	0.014	0.026	0.030
	297	0	0	0.058	0	0	0
	253	0.135	0.075	0	0.108	0.077	0.100
	255	0	0.013	0.035	0.081	0.051	0.040
	257	0.865	0.913	0.965	0.797	0.872	0.860
	261	0	0	0	0.014	0	0
<i>N</i>		47	40	43	37	39	50
Observed heterozygosity		0.560	0.640	0.587	0.601	0.648	0.631
Mean number of alleles		7.0	7.2	7.9	8.7	10.3	10.0