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Genetic variation over space and time: analyses of extinct and remnant lake trout populations in the Upper Great Lakes

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Lake trout (*Salvelinus namaycush*) in the upper Laurentian Great Lakes of North America experienced striking reductions in abundance and distribution during the mid-twentieth century. Complete collapse of populations was documented for Lake Michigan, and a few remnant populations remained only in lakes Huron and Superior. Using DNA obtained from historical scale collections, we analysed patterns of genetic diversity at five microsatellite loci from archived historical samples representing 15 populations (range 1940–1959) and from three contemporary remnant populations across lakes Huron and Superior (total $n = 893$). Demographic declines in abundance and the extirpation of native lake trout populations during the past 40 years have resulted in the loss of genetic diversity between lakes owing to extirpation of Lake Michigan populations and a temporal trend for reduction in allelic richness in the populations of lakes Superior and Huron. Naturally reproducing populations in Lake Superior, which had been considered to be remnants of historical populations, and which were believed to be responsible for the resurgence of lake trout numbers and distribution, have probably been affected by hatchery supplementation.

Keywords: historical DNA; lake trout; Laurentian Great Lakes; genetic impact; microsatellites

1. INTRODUCTION

Many species are experiencing declines in abundance or extirpation of populations, which could lead to species-wide declines in morphological, life history and genetic diversity (Avise & Hamrick 1996; Meffe & Carroll 1997). Among vertebrates, fishes are often used to study the impacts of extirpation on biological diversity because of their economical value and high incidence of overexploitation (e.g. Schramm & Piper 1995; Laikre & Ryman 1996). Fishes are particularly susceptible to extirpation because of invasions by non-native species and human activities (e.g. pollution, habitat destruction). Evidence is accumulating of large-scale losses in abundance and diversity, and of changes in species distribution and fish population and community structure caused by natural or human-mediated environmental change (Moyle & Leidy 1992; Rahel 2000). However, it is more difficult to ascertain how decreased abundance and loss of entire populations or metapopulations affect regional or species-wide levels of genetic diversity (Amos & Harwood 1998).

Demographic bottlenecks are expected to reduce population levels of genetic variation (Nei *et al.* 1975; Hedrick & Miller 1992), and theory predicts lower fitness as a consequence of reduced genetic variation along with decreased effective population size or inbreeding (Keller & Waller 2002). Often, empirical demonstration of relationships between low effective population size and low levels of genetic diversity is difficult (Whitler *et al.* 2000). Assessments of the magnitude of loss of genetic variability would profit from knowledge of historical patterns of genetic characteristics prior to a bottleneck (Bouzat *et al.* 1998).

Historical samples can serve as a valuable source of reference with which to interpret contemporary levels of genetic diversity (Bouzat 2000; Matocq & Villablanca 2001). Accordingly, population-level studies using DNA from 'ancient' or historical samples have become increasingly common (Taylor *et al.* 1994; Groombridge *et al.* 2000; Pertoldi *et al.* 2001). The use of archival DNA samples is widespread in time-series analyses of fishes to investigate changes in genetic structure and genetic diversity across generations (Miller & Kapucinski 1997; Nielsen *et al.* 1997, 1999*a*; Ruzzante *et al.* 2001; Hansen 2002; Hauser *et al.* 2002; Koskinen *et al.* 2002).

Lake trout (*Salvelinus namaycush*) in the upper Laurentian Great Lakes of North America are a striking and highly visible example of a species that has experienced considerable reductions in population numbers and distribution (Hansen 1999). Historically, lake trout were abundant and were important to human settlement of each of the Great Lakes. Natural stocks declined owing to overexploitation and sea lamprey (*Petromyzon marinus*) invasion during the twentieth century in each lake (review in Hansen 1999). Management actions were undertaken to restore lake trout populations. However, after 35 years of considerable effort, lake trout restoration in the Great Lakes has still not been realized (Hansen 1999). Only recently have questions relating to lake trout genetic diversity been considered as part of a comprehensive restoration strategy (Burnham-Curtis *et al.* 1995; Krueger & Ihssen 1995).

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Table 1. Lake trout samples considered in this study (n represents the individual sample size), and labels used in the figures.

		location	date of sampling	label	n
archival	Lake Superior				
		Copper Harbor	1948	CHA48	16
		Whitefish Point	1948	WP48	15
		Isle Royale	1959	IR59	12
		Gull Island	1959	GI59	65
	Lake Huron				
		Parry Sound	1958	PS58	48
	Lake Michigan				
		Grand Traverse Bay	1940	GT40	52
		Charlevoix	1948	CH48	60
		Northport	1948	NP48	48
		Sand Bay	1948	SB48	60
		South Manitou Island	1949	SMI49	50
		Leland	1948	LE48	60
		Frankfort	1949	FR49	48
		Montague	1948	MO48	54
		Saint Joseph	1948	SJ48	69
		Waukegan	1948	WA48	48
				subtotal	$N_1 = 705$
contemporary	Lake Superior				
		Isle Royale	1995	IR95	70
		Gull Island	1995	GI95	68
	Lake Huron				
		Parry Sound	1999	PS99	50
				subtotal	$N_2 = 188$
				total	$N = 893$

Using DNA obtained from collections of scales from lake trout in Lakes Michigan, Huron and Superior, we investigate how genetic diversity was partitioned within and between historical lake trout populations. We seek to understand how levels of genetic diversity were affected by extirpation of populations across an entire lake basin. Genetic affinities among historical and contemporary populations and hatchery strains were also used to investigate whether restoration of near-shore populations was achieved from natural recruitment or from hatchery supplementation.

2. MATERIAL AND METHODS

(a) *Scale collections, lake trout collapse in the Great Lakes and reliance on hatchery fishes*

Samples from historical and contemporary lake trout populations were collected from three out of the five Great Lakes (Michigan, Huron and Superior). We analysed 18 populations (fifteen archival and three contemporary populations) by examining a total of 893 individuals (table 1; figure 1*a*). Samples from historical populations were based on archival scale samples retained by management agencies for demographic studies. Individual samples were used if sufficient records were available regarding species, morphotype (for Lake Superior populations, where distinct morphotypes exist; Moore & Bronte 2001), sampling date and location and a sufficient number of scales were available for DNA extraction. Only populations with at least 10 individuals were considered. Sampling locations for historical populations from Lake Michigan were referenced by ports where commercial fishermen were registered (figure 1*a*). For the Michigan waters of Lake Michigan the fish locations are well known (Dawson *et al.* 1995), and commercial fishery records show that fishermen originating from different ports typically exploited different stocks (table 1; figure 1*a*). Most historical samples were from fishes collected during the late summer or during the autumn. There is no evidence that individuals belonged to well-defined spawning aggregations (R. Eshenroder, personal communication). As such, although lake trout are quite sedentary (Rayrer 1968) and do not disperse widely from spawning areas, spatial variation in allele frequency across the Lake Michigan samples may be underestimated because of some level of population admixture.

The lake trout populations of lakes Huron, Michigan and Superior crashed at different times during the mid-twentieth century (Hansen 1999; figure 1*b*). Significant declines in population abundance, as measured by catch yields, occurred in the mid-1940s for Lake Michigan, and these populations were totally extirpated by 1954. The Lake Huron populations first significantly declined in 1952–1953 when populations from the main basin collapsed (figure 1*b*). The populations from Georgian Bay were severely depressed by the beginning of the 1960s (Hansen *et al.* 1995). The populations from Lake Superior were nearly completely extirpated by 1960–1961, with the exception of several remnant populations. In each basin, the collapse of populations took place within one lake trout generation (6–8 years; Hansen 1999). The lakes had sustained average harvests of *ca.* 15, 40 and 55 kg km^{-2} for lakes Superior, Huron and Michigan, respectively, just decades before complete extirpation occurred (Baldwin *et al.* 1979; Wilberg *et al.* 2003; figure 1*b*).

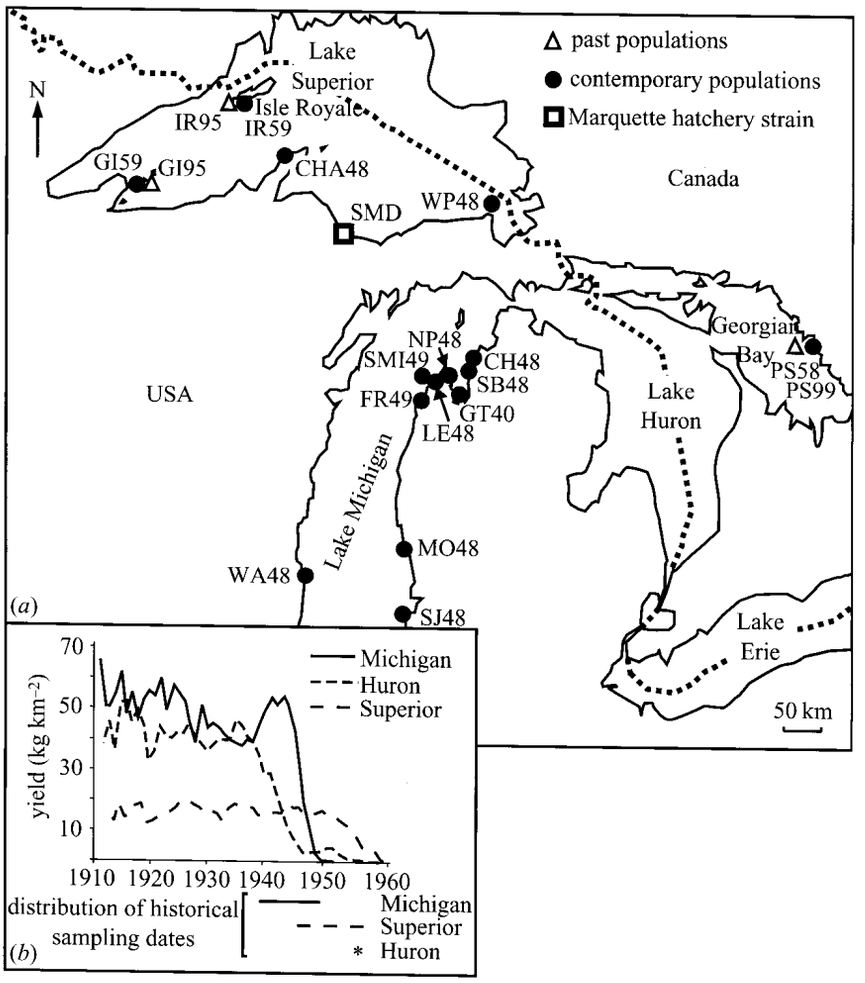

Figure 1. (*a*) Sampling locations of the historical and contemporary lake trout populations in the upper Great Lakes. Circles, past populations; triangles, contemporary populations; square, Marquette hatchery strain. Bar, 50 km. (*b*) Lake trout declines in lakes Michigan (dotted line), Superior (large dashed line) and Huron (small dashed line) (adapted from Baldwin *et al.* (1979) and Hansen *et al.* (1995)). Ranges of historical sampling dates are given for each lake (asterisk indicates one unique archival sample for Lake Huron; see table 1).

Demographic changes of populations under study before or after the population crash are generally not available except for Gull Island and Parry Sound populations (Swanson & Swedberg (1980) and Reid *et al.* (2001), respectively). However, records indicate that samples from the historical populations we surveyed were obtained at times when the population declines were already well advanced across each lake basin (figure 1*b*). Potential exceptions are samples from 1940 and 1948 in lakes Michigan and Superior, respectively (table 1), which were sampled before the general declines.

As lake trout abundance and distribution declined, representative populations of lake trout were chosen for large-scale supplementation efforts from each of the Great Lakes (reviewed in Krueger & Ihssen 1995). The Marquette strain (SMD strain) was initially founded in the late 1940s with samples from nearshore populations of lake trout from southern Lake Superior. This strain represents the most notable input of hatchery fishes in Lake Superior since stocking began and has a complex history (Page 2001). The strain originated primarily from individuals collected in waters near Marquette, MI, but originally included a small number of Gull Island individuals (Swanson & Swedberg 1980). Contemporary populations considered in this study represent all (except for one population of Lake Superior) remaining wild lake trout populations (table 1) that were not believed to be derived directly from hatchery fishes. Contemporary populations were sampled during the spawning season and were close spatial replicates of their historical counterparts (figure 1*a*).

(b) *Genetic analysis*

We examined the patterns of genetic variation at five microsatellite loci developed for other salmonids (*Ogo1a*: Olsen *et al.* 1998; *Oneμ10*: Scribner *et al.* 1996; *Scoμ19*: Taylor *et al.* 2001; *Sfo1*: Angers *et al.* 1995; and *Ssa85*: O'Reilly *et al.* 1996). The microsatellites were selected for analysis because of their ease in scoring in past and present populations (Nielsen *et al.* 1999*b*). DNA was extracted from fish scales using a Chelex resin protocol (Yue & Orban 2001). Up to eight scales (mean three to four scales) were used for each individual from historical populations, depending on scale size and age. Polymerase chain reactions (PCRs) were performed in 25 μl volumes according to the general specifications outlined by each author. Thirty PCR cycles were performed for contemporary and historical samples. A few historical samples necessitated up to 45 PCR cycles at some loci for complete results. Microsatellite polymorphisms were screened on 6% denaturing acrylamide gels and visualized using a Hitachi FMBIO II Multi-View scanner and appropriate software (Hitachi Software). Reference samples of known allele size were run on each gel to standardize the scoring.

The general guidelines provided by Nielsen *et al.* (1999*b*) were followed to ensure the reliability of the PCR results. We

assumed that the initial scoring of the heterozygotes was correct, but that the homozygotes may be incorrectly scored with some probability, which may affect the genetic parameter estimates (Chakraborty *et al.* 1992; Taberlet *et al.* 1996). To avoid scoring errors associated with non-amplification (i.e. allelic dropout) when analysing the historical samples, we systematically scored individual genotypes two to four times at each locus when the population sample sizes were low ($n < 20$; table 1). For historical populations characterized using larger sample sizes, 12 homozygotes were randomly chosen at each locus and PCR was performed an additional four times per sample. Overall, less than 2% of the initial scorings of past populations were changed, and changes that were observed were corrected through almost complete resampling of the database for homozygous genotypes.

(c) *Data analysis*

Historical and contemporary samples were used together to construct a population tree to examine the genetic relationships between populations. Individuals originating from the SMD strain (sampled in 1999; figure 1*a*) were also included because of its widespread use in stocking events across Lake Superior. The genetic relationships of the SMD strain in the population tree could be useful in reconstructing the origins of contemporary populations in Lake Superior (i.e. via natural recruitment from remnant populations or from hatchery supplementation). No hatchery strain from Lake Huron was included owing to the particular history of the Parry Sound population (figure 1*a*). Contemporary Parry Sound individuals are derived from the past population, and foreign allele introduction is likely to be limited (Reid *et al.* 2001). The chord distance of Cavalli-Sforza & Edwards (1967) was used to construct the population phenogram using the neighbour-joining algorithm (Saitou & Nei 1987) implemented in PHYLIP v. 3.5 (Felsenstein 1993). Confidence in tree topology was assessed by bootstrapping data (3000 iterations). The unrooted tree was displayed using TREEVIEW (Page 1996).

An assignment test using Bayesian methods was performed using the program STRUCTURE as described in Pritchard *et al.* (2000) for selected contemporary populations to investigate the influences of hatchery-reared fishes (SMD strain; figure 1*a*). We estimated probabilities of individuals from contemporary populations (IR95 and GI95) originating from historical (and putative ancestral) populations (IR59 and GI59) or from hatchery fishes widely stocked across Lake Superior (SMD).

Deviations from the Hardy–Weinberg equilibrium (HWE) in each population and at each locus were investigated using an exact test approximation (Guo & Thompson 1992) implemented in GENEPOP v. 3.1 (Raymond & Rousset 1995). Multilocus estimates of significance for HWE tests were obtained following Fisher's exact tests implemented in GENEPOP. Critical significance levels for multiple testing were corrected using sequential Bonferroni procedures (Rice 1989). We performed hierarchical analyses of genetic variation using the program ARLEQUIN (Schneider *et al.* 2000) to quantify the amount of genetic variance for past and contemporary samples, respectively, and to determine whether levels and partitioning of total genetic variance changed over time.

The contributions of each population to overall gene diversity (CT%) were computed using the CONTRIBUTE program (Petit *et al.* 1998). The estimates of CT% were used to identify populations contributing less or more than average to overall levels of gene diversity. CONTRIBUTE was also used to compute the allelic richness for each population, standardized for population differences in sample size ('rarefaction method' where the sample sizes for all populations were standardized to that of the smallest sample; details in Petit *et al.* (1998)). For each locus, the levels of total gene diversity H_T and the levels of population differentiation G_{st} (Nei 1987) were also computed using the program CONTRIBUTE.

For each population, the tests for genetic bottlenecks were performed using the M ratio method (Garza & Williamson 2001). The M ratio is the ratio of the number of alleles to the total range in allele size (in base pairs). Computations were made for all loci, and an average M ratio was computed across loci for each population. M ratio values of less than 0.7 provide evidence of a bottleneck, whereas values of more than 0.8 indicate species with no bottleneck history (Garza & Williamson 2001).

3. RESULTS

(a) *Population structure and differentiation*

Four out of 18 populations, all from historical Lake Michigan locales (GT40, NP48, SB48 and SMI49; figure 1*a*), were not in HWE. Samples from these locales probably represent admixtures of multiple genetically differentiated spawning populations, as historical fisheries exploiting these areas harvested from several reefs and intervening habitats during the non-reproductive season (R. Eshenroder, personal communication). Given the extensive levels of resampling performed to confirm genotypic assignments (see § 2b above), sampling effects associated with the fishery and not PCR artefacts (allelic dropout) were likely to have produced the observed heterozygote deficiencies.

Hierarchical F statistics computed for historical populations indicated that components of total genetic variance were spatially apportioned among lakes ($F_{ct} = 0.012$; $p = 0.05$) and among populations within lakes ($F_{sc} = 0.015$; $p < 0.001$). Relative levels of genetic variance within historical and contemporary populations were concordant (mean G_{st} over five loci for 15 historical populations 0.043 ± 0.015 versus 0.041 ± 0.011 for three contemporary populations).

The population tree also revealed geographical genetic structuring (figure 2). The clustering of populations from Lake Superior (figure 2) was based both on the location within the basin and on the sampling period (CHA48 and WP48 from the eastern portion of the basin collected in 1948, IR59 and GI59 from the western portion of the basin collected in 1959, and GI95 and IR95 collected during 1995). Lake Huron samples PS99 and PS58 were genetically similar in allele frequency and clustered together with historical samples from Lake Michigan. The high bootstrap value (figure 2) indicates that the samples were temporal replicates of each other. In Lake Superior, samples GI95 and IR95 did not directly cluster with their respective historical counterparts. Accordingly, they cannot be considered contemporary replicates of the IR59 and GI59 Lake Superior populations (figure 2). Few nodes of the tree received high bootstrap support as only five loci were used. However, the tree topology remained unchanged when other distance metrics were employed (results not shown).

The genetic affinities of the SMD hatchery strain relative to the historical and contemporary populations in the

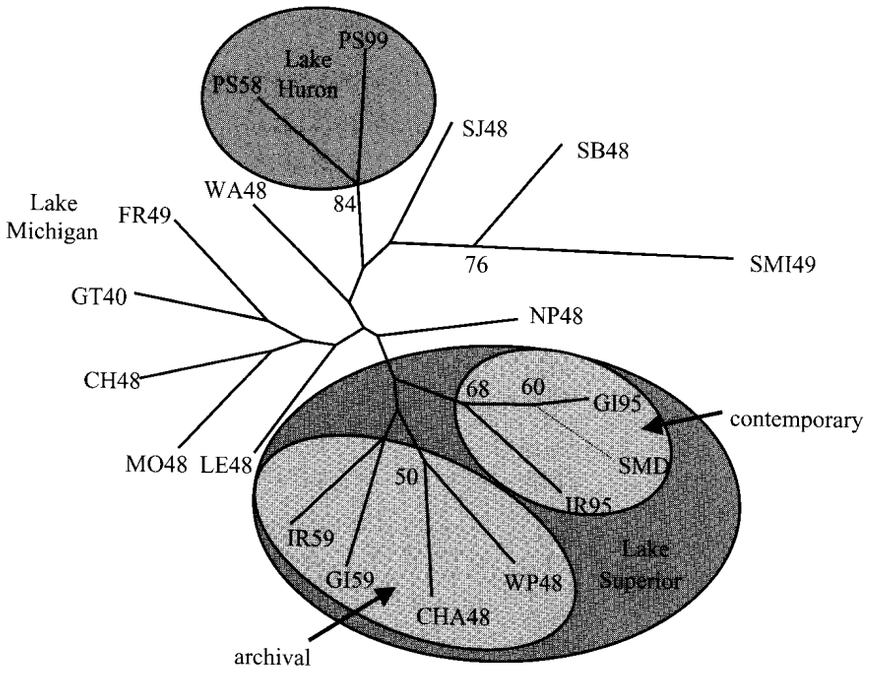

Figure 2. Neighbour-joining tree of the 18 lake trout samples and the Marquette hatchery strain (SMD; figure 1) based on the chord distance of Cavalli-Sforza & Edwards (1967). Bootstrap values of more than 50 are given.

tree indicate that contemporary Lake Superior populations (GI95 and IR95) have been genetically affected by stock supplementation (figure 2). We tested this interpretation *a posteriori* using an assignment test (Pritchard *et al.* 2000). We used SMD, GI59 and IR59 as baselines and classified all individuals from contemporary populations GI95 and IR95 as more closely related (by ancestral descent) either to hatchery strain SMD or to historical populations IR59 and GI59. The estimated percentages of each contemporary population that were more closely related to sources SMD, GI59 and IR59 were, respectively, 70.6, 8.8 and 20.6% for GI95, and 51.4, 11.1 and 37.5% for IR95. The results supported the genetic affinities revealed by the population tree (figure 2) and illustrate the probable impact of hatchery fishes.

(b) *Spatial and temporal variation in standardized allelic richness and impact of bottlenecks*

The levels of total gene diversity were not significantly different across lakes ($\overline{H}_{obs} = 0.436$ and 0.476 for the historical samples from lakes Michigan and Superior, respectively). Estimates of the total gene diversity were lower in historical than in contemporary populations ($H_T = 0.466 \pm 0.011$ and 0.513 ± 0.036, respectively). However, the differences were not significant for individual loci or means across loci (results not shown). Within Lake Superior, the estimates of standardized allelic richness (Petit *et al.* 1998) were lower in contemporary populations than in their historical counterparts (table 2). Standardized allelic richness was also higher in the historical Lake Superior populations than in the historical populations inhabiting Lake Michigan (table 2), probably reflecting the earlier demographic collapse of populations across the Lake Michigan basin. The 1948 samples (WP48 and CHA48) had higher levels of standardized allelic richness than did samples from 1959 (IR59 and GI59) or contemporary samples (IR95 and GI95), reflecting a temporal trend of declining genetic variation. The samples from Lake Huron had the lowest estimates of allelic richness.

Analyses revealed heterogeneous contributions of populations to total gene diversity (figure 3). Historical populations WP48 and CHA48 from Lake Superior and GT40 and FR49 from Lake Michigan contributed more to total historical levels of gene diversity than did other populations (figure 3). The GT40, CHA48 and WP48 samples were collected a generation before the bottleneck event in lakes Michigan and Superior (figure 1; table 1).

Analyses of the M ratios indicated that basin-wide declines in abundance (figure 1*b*) were accompanied by concomitant changes in allele-frequency distributions indicating population bottlenecks. The heterogeneity of the M ratios for historical Lake Michigan populations indicates that, at the time of sampling, each locale was potentially differentially affected by basin-wide stock collapses in the late 1940s. The highest M ratio was reported for the oldest sample (GT40) in Lake Michigan (table 2). The M-ratio computations consistently showed that estimates were lower in contemporary Lake Superior samples than in historical samples from corresponding locales (table 2). According to the guidelines reported in Garza & Williamson (2001), only three populations (GT40, CH48 and SJ48) have values of $M > 0.8$, denoting the probable absence of bottlenecks, while the estimated M ratios for six populations were consistent with the existence of a bottleneck (M ratio < 0.7) (table 2).

4. DISCUSSION

The ability to characterize the genetic structure of historical populations brings a new dimension to animal population genetics (Bouzat *et al.* 1998). For fishes, the

Table 2. Values of the *M* ratio (Garza & Williamson 2001) and mean standardized allelic richness across loci (Petit *et al.* 1998) in each population.
(Averages over lakes or time periods of interest are also reported. Average values are not relevant for the *M*-ratio results. No significant results were observed.)

lake	population	*M* ratio[a]	mean standardized allelic richness
Lake Superior			
	CHA48	0.759	3.345
	WP48	0.688	3.692
	IR59	0.723	2.800
	IR95	0.630	2.405
	GI59	0.698	2.349
	GI95	0.667	1.922
Lake Huron			
	PS58	0.713	1.894
	PS99	0.698	1.374
Lake Michigan			
	GT40	0.915	3.284
	CH48	0.854	2.290
	NP48	0.764	2.774
	SB48	0.739	2.752
	SMI49	0.758	2.837
	LE48	0.646	2.189
	FR49	0.779	4.023
	MO48	0.713	2.036
	SJ48	0.880	2.394
	WA48	0.707	2.329
average for Lake Michigan only			2.691
average for past populations of Lake Superior			3.047
average for present populations of Lake Superior			2.164

[a] Computations of various averages are not relevant for the *M* ratio.

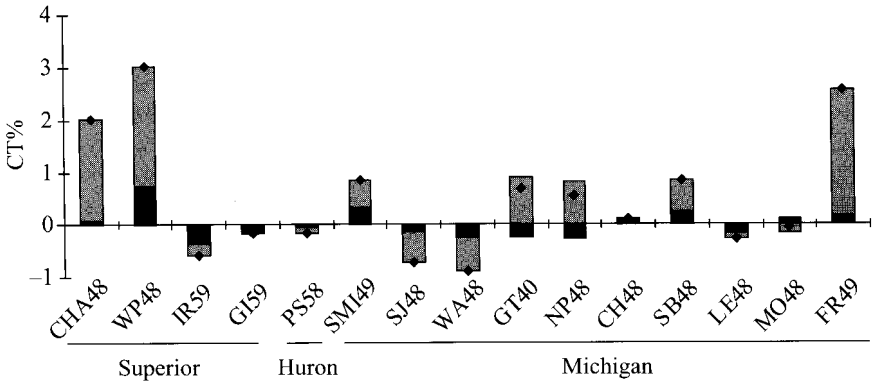

Figure 3. Relative contribution (CT%) of each past population to gene diversity H_T *sensu* Nei (1987). Values above the horizontal line indicate populations that contribute more than average to diversity; values below the line indicate populations that contribute less than average to genetic diversity. Contributions are divided into two components according to Petit *et al.* (1998). One component indicates the relative contribution to total diversity of a population as a result of its differentiation from other populations (light grey). The second component indicates the relative contribution of populations to total gene diversity as a result of their own level of diversity (dark grey). Sum indicates total contribution (diamonds). The observed variation in the contribution to genetic diversity (−1% up to 3%) is in close agreement with other reported values for lake trout (Page 2001).

analyses of historical samples from archival data have recently increased in number owing in part to the availability of microsatellite loci (e.g. Nielsen *et al.* 1997, 1999*a*, 2001). In this study, we sought a greater understanding of how populations of lake trout were structured genetically before populations were extirpated from large areas of the Great Lakes basin. We wished to ascertain how genetic diversity, allelic richness and partitioning of genetic variance changed as a result of declining abundance and distribution, and owing to hatchery supplemen-

tation. For lake trout from the Great Lakes, it is important to evaluate the effects of general declines in abundance and extinction of populations (figure 1*b*) and of the supplementation of hatchery-reared fishes on present-day genetic diversity.

(a) *Structure of historical populations*

Phylogeographical studies of lake trout performed across the species range (Wilson & Hebert 1996, 1998) have indicated that post-glacial migrations occurred into the Great Lakes Basin from three major refugia across North America. However, these analyses did not include natural populations from Lake Michigan and thus may not reflect the entire range and spatial dispersion of genetic variation that once existed across the upper Great Lakes basin. Microsatellite data revealed that historical populations of lake trout differed genetically according to their basin of origin (Superior versus Michigan; figure 2). The extirpation of Lake Michigan populations has led to the extinction of original lineages, representing unique portions of lake trout post-glacial diversity. Genetic affinities of historical Lake Huron populations were closer to Lake Michigan historical stocks than to those from Lake Superior (figure 2).

(b) *Differences between historical and contemporary populations*

Inferences about the impacts that changes in abundance and distribution have on genetic diversity benefit from the use of reference populations sampled prior to putative bottleneck events (Bouzat 2000). The analysis of genetic partitioning for both historical and contemporary populations indicated that a large portion of the total genetic variance was segregated within historical populations. In a study using both archival and contemporary samples, Ruzzante *et al.* (2001) reported similar results regarding variance partitioning for Atlantic cod (*Gadus morhua*) in light of reductions in census population size of two orders of magnitude. We observed similar estimates of inter-population differences in allele frequency (G_{st}) for lake trout between two time periods (0.043 versus 0.041 for historical and contemporary populations, respectively), which may be partly explained by differences in the size and geographical dispersion of the historical and contemporary population samples.

The differences in the levels of allelic richness across historical populations of Lake Superior could indicate either localized areas of higher diversity (perhaps correlated with regions of greater localized abundance), or declines in abundance and concomitant erosion of genetic diversity in Lake Superior populations during the 11 years over which the historical samples were collected in the different locales. In Lake Superior, standardized allelic richness was also lower in contemporary populations than in historical populations, owing most probably to the replacement of original pre-crash stocks with fishes descended from hatchery stocks. The estimates of standardized allelic richness were also slightly higher in historical Lake Superior populations than in those in lakes Michigan and Huron. Further, the historical populations that contributed more than average to the total level of gene diversity (especially CHA48, WP48, GT40, FR49; figure 3) are now extinct. Most of those populations were represented by the oldest samples from lakes Michigan and Superior (table 1). The older samples in Lake Superior (CHA48, WP48) contributed more to diversity than did populations sampled after an additional decade of declining abundance (figure 3).

(c) *Impacts of hatchery supplementation on Lake Superior populations*

The potential genetic effects of hatchery supplementation in the Great Lakes on extant lake trout populations have been widely debated (Burnham-Curtis *et al.* 1995; Krueger & Ihssen 1995), but not tested empirically. Our data based on population genetic affinities (figure 2) indicate that two contemporary populations (GI95 and IR95) are not likely to be true temporal replicates of ancestral populations from the same geographical areas, whereas the contemporary Lake Huron population (PS99) is. This is in accordance with the history of the Parry Sound population (Reid *et al.* 2001). Hatchery fishes probably replaced or introgressed with fishes from remnant natural populations in Lake Superior. Assignment tests indicate that hatchery fishes may have had a greater influence on the contemporary 'restored' populations than originally proposed (*ca.* 70% and 50% for GI95 and IR95, respectively). Previous reports (Swanson & Swedberg 1980; Schram *et al.* 1995) based on patterns of recruitment at Gull Island indicated that wild females made significant contributions to total recruitment. Our results do not support this conclusion, but rather indicate a significant influence of hatchery fishes on the Gull Island reef (GI95) population. Genetic affinities of the Isle Royale samples (IR95) to SMD hatchery fishes are less pronounced (figure 2 and lower proportional assignment test results compared with GI95), but are also indicative of hatchery supplementation. Natural recruitment was generally considered the primary reason for population persistence in these locales (see Hansen *et al.* 1995). Further investigations are warranted to understand the differential susceptibilities of the genomes of indigenous individuals to introgression by hatchery-reared conspecifics (Nielsen *et al.* 2001; Hansen 2002).

5. CONCLUSION

By exploiting DNA from archived historical scale samples and contemporary populations we were able to evaluate the impacts of basin-wide demographic and distributional declines on the genetic relationships of lake trout in the Great Lakes and on levels of genetic diversity, and investigate the merits of competing hypotheses concerning the success of restoration efforts. The demise of a fishery that had sustained harvests of 15–55 kg km^{-2} over all basin areas just decades before complete extirpation appears to have precipitated concurrent losses of genetic diversity. The diversity historically present in Lake Michigan populations (figure 2) has been entirely lost. Contemporary populations from Lake Superior, once believed to be relatively unaffected by hatchery supplementation, have greater affinities to hatchery fishes than to ancestral stocks, indicating that recovery of near-shore populations in this lake has been facilitated predominantly by hatchery contribution and not by the natural recruitment of remnant stocks.

Historically, lake trout of the upper Great Lakes were abundant and biologically diverse. The size of the Great Lakes basin, the heterogeneous nature of the lakes and contributions from multiple isolated Pleistocene glacial refugia (Wilson & Hebert 1996) promoted geographical and eco-phenotypic variation between lake trout populations (Page 2001). Extinction of subpopulations as documented across the basin can lead to demonstrable declines in effective population size (Whitlock & Barton 1997). The loss of diversity or allelic richness in genetically structured populations can decrease individual adaptive potential, facilitating inbreeding and lowering the long-term fitness of populations (Mills & Smouse 1994; Keller *et al.* 2001). Reliance on extensive hatchery supplementation can lead to reduced effective population size (Laikre & Ryman 1996) and to selection for non-adaptive phenotypes, which may significantly reduce a wild population's fitness (Ford 2002). Development of a fundamental understanding of the levels and partitioning of genetic diversity of historical, remnant wild and hatchery fishes should be a prerequisite for the establishment of conservation and restoration strategies for lake trout and other species subjected to anthropogenic influences.

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