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Spatial and temporal genetic diversity of lake whitefish (*Coregonus clupeaformis* (Mitchill)) from Lake Huron and Lake Erie

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with 3 figures and 3 tables

Abstract: Lake whitefish (*Coregonus clupeaformis* (Mitchill)) are important commercially, culturally, and ecologically in the Laurentian Great Lakes. Stocks of lake whitefish in the Great Lakes have recovered from low levels of abundance in the 1960s. Reductions in abundance, loss of habitat and environmental degradation can be accompanied by losses of genetic diversity and overall fitness that may persist even as populations recover demographically. Therefore, it is important to be able to identify stocks that have reduced levels of genetic diversity. In this study, we investigated patterns of genetic diversity at microsatellite DNA loci in lake whitefish collected between 1927 and 1929 (historical period) and between 1997 and 2005 (contemporary period) from Lake Huron and Lake Erie. Genetic analysis of lake whitefish from Lakes Huron and Erie shows that the amount of population structuring varies from lake to lake. Greater genetic divergences among collections from Lake Huron may be the result of sampling scale, migration patterns and demographic processes. Fluctuations in abundance of lake whitefish populations may have resulted in periods of increased genetic drift that have resulted in changes in allele frequencies over time, but periodic genetic drift was not severe enough to result in a significant loss of genetic diversity. Migration among stocks may have decreased levels of genetic differentiation while not completely obscuring stock boundaries. Recent changes in spatial boundaries to stocks, the number of stocks and life history characteristics of stocks further demonstrate the potential of coregonids for a swift and varied response to environmental change and emphasise the importance of incorporating both spatial and temporal considerations into management plans to ensure that diversity is preserved.

Keywords: Allelic richness, Archival samples, Genetic bottleneck, Genetic diversity, Laurentian Great Lakes, Microsatellite DNA, Population structure, Temporal changes.

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Introduction

The original coregonine fauna of the Great Lakes occupied a variety of niches differentiated by depth preference, feeding habits and spawning time (Smith & Todd 1984). Two cisco species groups, the *Coregonus hoyi-kiyi-nigripinnis* group and the *C. zenithicus-johannae-reighardi* group (Smith & Todd 1984), were unique to the Laurentian Great Lakes region. Lake herring (*C. artedi* Leseur), lake whitefish (*C. clupeaformis* (Mitchill)), pygmy whitefish (*Prosopium coulteri* Eigenmann & Eigenmann), round whitefish (*P. cylindraceum* (Pennant)), and the two cisco groups were part of a complex and diverse species community (Smith & Todd 1984, Eshenroder & Burnham-Curtis 1999). Habitat alterations, species introductions and overfishing have resulted in the loss of some taxa (e.g. *C. nigripinnis* (Milner), *C. reighardi* (Koelz)) (Christie 1972, Smith & Todd 1984) and decline of others (e.g. *C. kiyi* (Koelz), *C. hoyi* (Milner)) (Christie 1972). The decline and/or loss of coregonine taxa have altered the flow of energy within and among trophic levels in the food webs of the Great Lakes (Eshenroder & Burnham-Curtis 1999).

Lake whitefish are an important commercial species in the Great Lakes and have been monitored by management agencies for many years. Lake Huron currently has 23 lake whitefish management units that are based on the spatial distribution of stocks and the fishery (Ebener et al. 2008), while Lake Erie lake whitefish are managed as a single unit. Lake whitefish have accounted for a large portion of commercial landings in the last half century (Todd 1986, Brown et al. 1999, Baldwin et al. 2009). For example, 58.3% of the total commercial harvest in the Great Lakes was comprised of lake whitefish in 2006 (Baldwin et al. 2009). Almost half (48.2%) of the commercial harvest of lake whitefish came from Lake Huron, while less than 1% came from Lake Erie (Baldwin et al. 2009). However, at one time Lake Erie was the source of most of the commercial production of fish in the Great Lakes (Brown et al. 1999). Lake whitefish made significant contributions to commercial harvests after the collapse of the lake herring fishery (Todd 1986), but by the 1950s lake whitefish stocks also began to decline, possibly due to loss of spawning habitat in areas like the Maumee River (Leach & Nepszy 1976), overharvest and predation by introduced species (Christie 1972). Lake whitefish abundance has since increased in Lake Erie, starting in the late 1970s and early 1980s as a result of a series of management efforts (e.g., Casselman et al. 1996, Ludsin et al. 2001), but commercial catches remain low. Similar to Lake Erie, lake whitefish harvest in Lake Huron experienced a long period of decline, starting in the 1930s, followed by a period of increase starting in the 1970s (Mohr & Ebener 2005). Loss of spawning habitat in areas such as Saginaw Bay (Beeton 1969), accompanied by over-harvest and impacts of invasive species, such as sea lamprey (*Petromyzon marinus* L.), had a negative impact on lake whitefish abundance (Mohr & Ebener 2005).

Reductions in abundance, such as those documented in Lake Huron and Lake Erie lake whitefish (e.g. Christie 1972, Regier & Hartman 1973, Leach & Nepszy 1976, Casselman et al. 1996, Ludsin et al. 2001), can be accompanied by a loss of genetic variation leading to a loss of overall fitness that may persist even after populations recover demographically (Frankham 1996, Hedrick & Kalinowski 2000). Affected populations may be more vulnerable to over-harvest in a mixed fishery, therefore it is important to be able to identify stocks that have reduced levels of genetic diversity. While substantial harvests of lake whitefish populations in Lake Huron continue, there have been recent concerns about declines in growth and

condition of stocks in the past decade, possibly related to declines in *Diporeia* spp. (Mohr & Ebener 2005). In contrast, abundance, growth and age-at-maturity have remained stable in recent years in Lake Erie, and when condition factors and maturity were compared among samples from the late twentieth century to those from the late 1920s there was little evidence that they had changed (Cook et al. 2005).

Archival data and information about early fisheries on the Great Lakes can provide insights into changes in life history, spatial distribution and abundance (e.g., Cook et al. 2005, Bunnell et al. 2006). Advances in molecular biology in the last 30 years have provided an additional source of information; genetic material extracted from scales and otoliths can be used to examine temporal changes in genetic diversity and population structure when both temporal and spatial replicates are available (e.g., Miller & Kapuscinski 1997, Nielsen et al. 1997, Ruzzante et al. 2001). Genetic analysis of historical scale samples can also help predict what impact future perturbations may have (Miller & Kapuscinski 1997), determine what management strategies may be best suited for restoration efforts (Favé & Turgeon 2008) and increase our understanding of the interactions of genetic diversity and life history variation on population sustainability (Ruzzante et al. 2001). In this study, we used archival scale samples and contemporary collections of lake whitefish to examine temporal changes in genetic diversity in this species from Lake Huron and Lake Erie by comparing samples collected in the early 1900s to those collected in the late 1900s and early 2000s. Specifically, we compared samples from two time periods to determine if genetic relationships among sites had changed over time and if there was evidence of a reduction in genetic diversity due to a population bottleneck.

Methods

Contemporary lake whitefish samples from 622 fish were collected from four sites in Lake Huron and three sites in Lake Erie (N = 89 fish per site on average, Table 1, Fig. 1). Contemporary samples from Lake Huron were collected as part of a tagging study (Ebener et al. 2010) and those from Lake Erie were collected as part of annual assessment surveys (e.g., Ohio Division of Wildlife 2011). The tagging study and contemporary harvest assessments were carried out during November and December and samples were taken on or proximate to known spawning grounds. Scale samples (N = 295 fish) from lake whitefish sampled during assessments of commercial fisheries in the late 1920s (e.g., O'Malley 1928, O'Malley 1930) were retrieved from the scale archive at the Great Lakes Science Center (U. S. Geological Survey, Ann Arbor, MI, U.S.A.). Collection sites for archival samples were named for the port at which commercial fishermen reported their landings (Table 1). Sites sampled in November and December were used whenever possible to be consistent with the sampling period of the contemporary samples and to coincide with the lake whitefish spawning period, based on the assumption that individuals collected during spawning will belong to separate populations (Ihssen et al. 1981, Ebener & Copes 1985, VanDeHey et al. 2009). All samples except those from St. Ignace (Table 1) were sampled in either November or December; lake whitefish from St. Ignace were collected in August. The historical reporting ports and contemporary collection sites were geographically proximate, with the exception of Saginaw Bay and Bay City. An extra site was also included for comparison of the historical samples

Table 1. Collection site, year, sample size, summarised diversity statistics and the results of the demography tests (M-ratio, H deficit, H excess) for lake whitefish collected from Lakes Erie and Lake Huron between 1927 and 2005.

Collection Site	Lake	Collection Year	n	He	Ho	A	Ar	M Ratio	H deficit P-value	H excess P-value
St. Ignace	Huron	1929	54	0.653	0.657	10.4	8.1	0.47	0.188	0.852
Duncan Bay	Huron	2004	138	0.651	0.699	11.4	7.5	0.42	0.027	0.980
La Salle Island	Huron	2004	60	0.686	0.641	10.1	7.7	0.42	0.289	0.766
Alpena	Huron	1927	49	0.626	0.546	10.0	8.0	0.43	0.020	0.988
Thunder Bay	Huron	2005	143	0.662	0.630	12.3	7.6	0.44	0.012	0.992
Bay City	Huron	1929	41	0.661	0.699	8.6	7.1	0.39	0.188	0.852
Saginaw Bay	Huron	2004	95	0.674	0.636	10.7	7.6	0.47	0.055	0.961
Put-In-Bay	Erie	1927	68	0.656	0.523	11.4	8.4	0.51	0.012	0.992
Niagara	Erie	2001	44	0.656	0.647	9.6	7.9	0.44	0.039	0.973
Kelleys Island	Erie	1997	90	0.656	0.568	10.9	7.4	0.46	0.027	0.980
Kelleys Island	Erie	1927	33	0.640	0.629	9.3	8.4	0.47	0.055	0.961
Sandusky	Erie	1927	50	0.679	0.607	9.4	7.7	0.49	0.148	0.945
Cedar Point	Erie	1998	52	0.670	0.635	9.3	7.6	0.41	0.656	0.406

n: sample size, He: expected heterozygosity, Ho: observed heterozygosity, A: mean number of alleles over all loci, Ar: mean allelic richness over all loci, H: heterozygosity.

from St. Ignace. Although Duncan Bay and St. Ignace are in the same lake whitefish management unit, recent genetic analysis of lake whitefish from northwestern Lake Huron has shown that fish from an adjacent management unit might also be from the same genetic population (Stott et al. 2011). Consequently, samples from La Salle Island, which is in the adjacent management unit, were also included (Fig. 1). Contemporary samples were collected using trap nets and the archival samples were collected using either gill nets or pound nets.

DNA was extracted using the DNeasy® kit (Qiagen Inc., Valencia CA, U.S.A.) (Use of trade names does not signify endorsement by the US government) and was quantified using fluorometry. A working solution with a concentration of 50 ng μl^{-1} (historical samples) or 100 ng μl^{-1} (contemporary samples) was prepared for genotyping. Seven microsatellite DNA loci designed for *Coregonus* and *Salvelinus* species were used for analysis; *Bwf*2 (Patton et al. 1997), *Cocl*22 (Bernatchez et al. 1996), *C2*-157 (Turgeon et al. 1999), *CoclLav*4, *CoclLav*52, *CoclLav*68 (Rogers et al. 2004) and *Sfo*23 (Angers et al. 1995). The final volume of the polymerase chain reaction (PCR) was 15 µl, including 0.20 mM each dNTP, 0.10 to 0.25 µM of each primer, 1.5 to 2.0 mM $MgCl_2$ and 0.45 to 0.75 Units *Taq* DNA polymerase. One of each primer pair was labeled with a fluorescent dye (*Bwf*2-6Fam, *Cocl*22-6Fam,

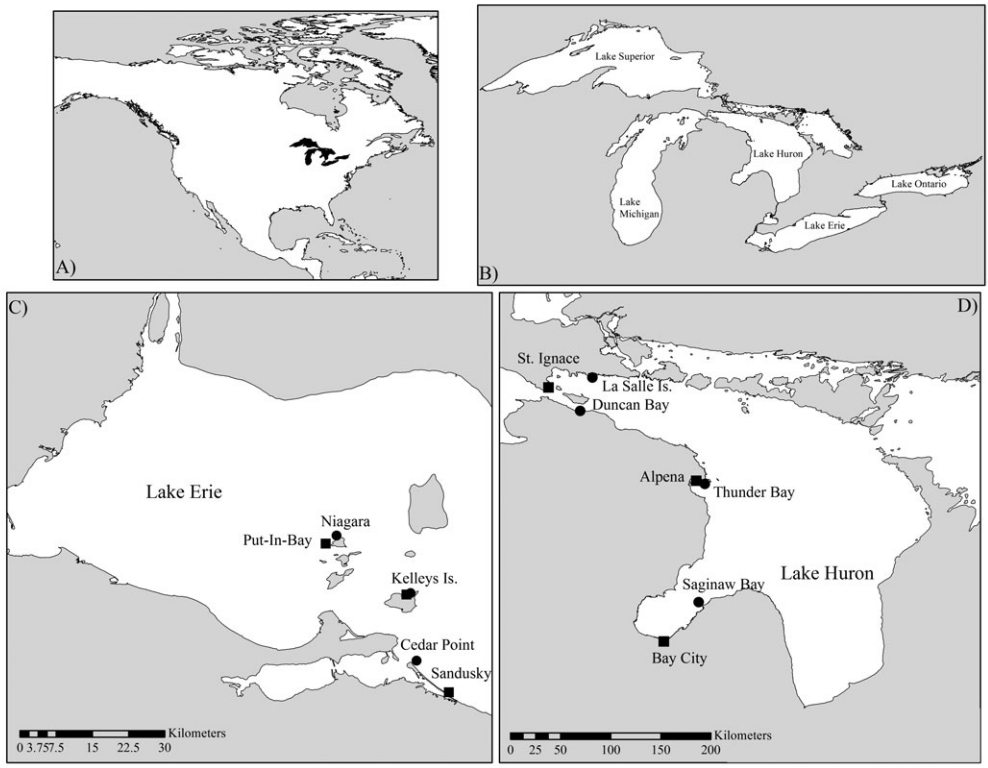

Fig. 1. A) Location of the Laurentian Great Lakes in North America. B) The five Laurentian Great Lakes. Collection sites on C) Lake Erie and D) Lake Huron for lake whitefish historical and contemporary samples. Historical samples are denoted by (■) and contemporary samples by (●).

C2-157-Hex, *CoclLav*4-Hex, *CoclLav*52-Hex, *CoclLav*68-Ned and *Sfo*23-6Fam). Temperature profiles for the PCRs were 1 min at 95 ºC, then 35 cycles of: 1 min at 94 ºC, 1 min at the annealing temperature (*Bwf*2-55 ºC, *Cocl*22-52 ºC, *C2*-157-60 ºC, *CoclLav*4-60 ºC, *CoclLav*52-62 ºC, *CoclLav*68-60 ºC and *Sfo*23-56 ºC) and 50 sec at 72 ºC. A final elongation at 72 ºC for 5 min completed the cycle. Each historical sample was amplified twice using the same reaction conditions as suggested by Nielsen et al. (1997); 1 to 2 µl of DNA from the first amplification was used as a template for the second amplification. Working solutions for genotyping were made by diluting 1 µl of each PCR product in 9 µl of water and further diluting 1 µl of this solution in 9 µl of formamide and a 400 bp size standard labelled with a fluorescent dye (400HD Rox by Applied Biosystems, Foster City CA, U.S.A.). This mixture was heated to 95 ºC for 4 min and then chilled on ice for 3 min. Electrophoresis was performed and fragment size data were collected using the ABI 3130 Genetic Analyzer (Applied Biosystems, Foster City CA, U.S.A.). The Genetic Analyzer (ver. 3.0 Applied Biosystems, Foster City CA, U.S.A.) software was used to collect data and the GeneMapper (ver. 4.0 Applied Biosystems, Foster City CA, U.S.A.) software package was used to calculate fragment sizes and assign genotypes.

We used the programme GENEPOP 4.0.7 (Raymond & Rousset 1995) to test for departures from Hardy-Weinberg equilibrium (HWE). Departures from HWE may occur if a distinct gene pool is incompletely sampled, if multiple gene pools are included in a sample or if the locus is experiencing selective pressure. A deficiency of heterozygotes across all loci in a collection may indicate that multiple gene pools were sampled and a deficiency at a locus across all collections may indicate an error in genotyping due to allelic drop out, the presence of null alleles or operator error. Allelic drop out may be observed when using low concentration or degraded DNA (Nielsen et al. 1997). The Markov chain (Guo & Thompson 1992) was run with 10,000 batches of 1,000 iterations to ensure that standard error values were minimised for the significance estimator that was calculated using Fisher's exact test. Probability values were adjusted using a sequential Bonferroni method (Rice 1989). Locus-collection combinations that had significant departures from HWE were further tested for heterozygote excess and deficiency using GENEPOP. GENEPOP 4.0.7 was also used to test for linkage disequilibrium among all loci to confirm that all loci were segregating independently.

Once conformation to HWE was confirmed, descriptive diversity statistics were calculated for the microsatellite DNA loci, including allele frequencies, observed and expected heterozygosity, average number of alleles per locus and allelic richness. Estimates of allelic richness were calculated using HP-RARE 1.0 (Kalinowski 2005), a programme that uses rarefaction to account for the effect of varying sample size on the estimates of allelic richness. The sample size for the analysis was set to the smallest sample size observed among the lake whitefish collections (N=33, Kelleys Island 1927). Statistical significance among estimates of allelic richness between contemporary and historical samples was tested using a sign test across loci as suggested by Kalinowski (2004). Genic differentiation among the collections was tested using GENPOP 4.0.7, using 10,000 dememorisation steps and 100 batches of 5,000 iterations per batch (Raymond & Rousset 1995). A second statistic, θ of Weir & Cockerham (1984) was calculated to estimate allelic variance among collections. An analog of F_{st} (Wright 1931), θ (hereafter F_{st} (θ)) can be used to test for the degree of genetic differentiation among sample collections. F_{st} (θ) was calculated and the values were tested for deviation from zero over 1,000 permutations of the data set using the programme ARLEQUIN 3.1 (Excoffier et al. 2005). Chord distances (Cavalli-Sforza & Edwards 1967) among collections were calculated using the programme POPULATIONS 1.2.3 (Langella 2002) and a neighbour-joining tree (Saitou & Nei 1987) was plotted with bootstrap support calculated over 1,000 pseudoreplicates and visualised using TREEVIEW 1.6.6 (Page 1996). Potential stocks and groups of stocks were tested using an analysis of molecular variance (AMOVA; Excoffier et al. 1992) in ARLEQUIN 3.1. Well supported groupings that may correspond to genetic stocks or groups of stocks are characterised by the lowest within group variance and the highest among group variance.

Two methods were used to test for the presence of genetic bottlenecks in historical and contemporary collections. The M statistic of Garza & Williamson (2001) is the ratio of the number of alleles and the range in allele size. Decreased values (less than 0.68) of M may signal a reduction in population size and the statistic may be useful for distinguishing between populations that have been recently reduced in size from those that have been reduced for some time. To provide a contrasting approach to that taken with the M statistic, we tested for significant excess of heterozygosity across all loci using the programme BOTTLENECK 1.2.02 (Cornuet & Luikart 1996). As suggested by the authors, we assumed a two-phase,

step-wise mutation model (variance = 30%). Under these conditions, a substantial reduction in effective population size should be reflected by a significant excess of heterozygosity.

Results

Before the Bonferroni adjustment was applied, 24 of 91 (26.3%) exact tests deviated from HWE (initial $\alpha = 0.05$) and after the adjustment eight (9%) of the tests still indicated a deviation (adjusted $\alpha = 0.0005$). The eight tests were all consistent with heterozygote deficiency and were distributed equally among the loci and sample collections, therefore no corrections were made to allele frequencies. No significant departure from linkage disequilibrium was observed when all pairs of loci were compared.

The sample sizes of the 13 collections ranged from 33 to 143 (mean = 71). The average observed heterozygosity over the loci ranged from 0.523 to 0.699 (Table 1). The lowest value was observed in the Put-In-Bay (1927) collection and the highest value was observed in the Duncan Bay (2004) collection. The mean heterozygosity for the historical samples was 0.600 (95% CI = 0.592 to 0.608) and the mean over the contemporary samples was 0.645 (95% CI = 0.642 to 0.648). The average heterozygosity for all the Lake Erie samples was 0.644 (95% CI = 0.639 to 0.649) and for all the Lake Huron samples was 0.602 (95% CI = 0.597 to 0.606). The average number of alleles per locus ranged from 8.6 (Bay City 1929) to 12.3 (Thunder Bay 2005) and the mean number of alleles was 10.3. When the number of alleles per locus was standardised for sample size (allelic richness), the range of values was 7.1 (Bay City 1929) to 8.4 (Put-In-Bay 1927) and the mean number was 7.8 (Table 1). No significant differences in allelic richness were observed among the comparisons of historical and contemporary samples collected from the same locale.

The tests of genic differentiation, comparisons of $F_{st}(\theta)$ values and the NJ tree suggested that significant differences existed among several of the collections. Significant differences in allele frequency distribution were observed in 68 of 78 tests (adjusted $\alpha = 0.00064$). Significant shifts in allele frequencies between collections from the same locale in different time periods at some loci (e.g., *CoclLav*68, Fig. 2) were observed and small but significant allele frequency variance was observed between some collections, especially those from different lakes (Table 2). Values of $F_{st}(\theta)$ ranged from 0.0003 to 0.0430 (Table 2); the average value within a single lake was 0.0190 (95% CI = 0.0176 to 0.0204) for Lake Erie and 0.0160 (95% CI = 0.0153 to 0.0168) for Lake Huron. Nodes on the NJ tree were generally not well supported; the strongest support corresponded to the node grouping La Salle Island and Duncan Bay (Fig. 3).

A series of AMOVAs was performed using groups based on (A) the time period in which the samples were collected, (B) the lake of origin of the samples, (C) the results of the genic differentiation tests, and (D and E) combinations of the genetic analyses (Table 3). The majority of genetic variance was observed within collections in all tests. The AMOVA based on a combination of genetic analyses (D) minimised within-group variance and had an among-group variance component that was significantly different from zero (Table 3).

M ratios for the 13 collections ranged from 0.39 to 0.51 (Table 1); all of the values were below the 0.68 threshold value. The ratios for individual loci were below the threshold with the exception of *CoclLav*4 (data not shown). Estimates of the M ratios at the locus *CoclLav*4

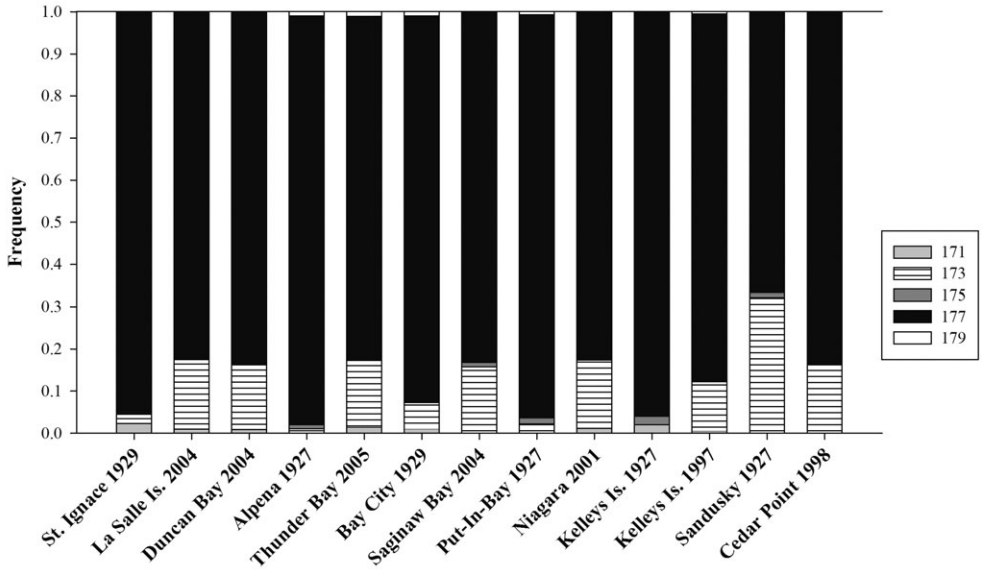

Fig. 2. Allele frequencies at the microsatellite DNA locus *CoclLav*68 for lake whitefish from Lake Erie and Lake Huron sampled in two different time periods.

for collections from St. Ignace, Alpena, Niagara, Kelleys Island (1927 and 1997 collections) and Sandusky were greater than 0.68, but none exceeded 0.82 which is indicative of a population that has not experienced a decrease in population size (Garza & Williamson 2001). In contrast, the heterozygosity excess test indicated that none of the collections had a significant excess of heterozygotes (Table 1), as might be expected in a population that experienced a population bottleneck.

Discussion

Archived collections of fish scales have the potential to provide insight into temporal changes in genetic variation and population structure. We compared lake whitefish collected up to 78 years apart (approximately 16 generations) from sites on Lakes Erie and Lake Huron to determine if genetic relationships had changed and if there was evidence of a reduction in genetic diversity due to a population bottleneck in the two time periods. The results of the genetic distance analysis, genic differences, $F_{st}(\theta)$ estimates, NJ tree and AMOVA indicated that lake whitefish collections were distinct spatially and temporally. The grouping best supported by the AMOVA suggested that collections from Duncan Bay and La Salle Island were not significantly different, but were distinct from all other sites in Lake Huron (Thunder Bay, Saginaw Bay, Alpena, St. Ignace and Bay City) and Lake Erie, and all the collections from Lake Erie (contemporary and historical) could be considered one genetic unit. $F_{st}(\theta)$ values observed among the collections ranged from low to moderate (0.0003 to 0.0430) and were similar to the ranges observed in recent analyses of lake whitefish from Lake Michigan

Table 2. Genetic differentiation among historical and contemporary lake whitefish collections from Lakes Huron (H) and Erie (E) as measured by F_{st} (θ) (below the diagonal) and by genic differences (p values for pairwise tests of genic differentiation are above the diagonal). Genic differences and allelic variances that are significant after sequential Bonferroni corrections are in bold type face.

	St. Ignace (H) 1929	**La Salle Is. (H) 2004**	**Duncan Bay (H) 2004**	**Alpena (H) 1927**	**Thunder Bay (H) 2005**	**Bay City (H) 1929**	**Saginaw Bay (H) 2004**	**Put-In-Bay (E) 1927**	**Niagara (E) 2001**	**Kelleys Is. (E) 1927**	**Kelleys Is. (E) 1997**	**Sandusky (E) 1927**	**Cedar Point (E) 1998**
St. Ignace	–	0.0000	<0.0001	**0.0016**	0.0000	0.0000	<0.00001	<0.00001	<0.00001	**0.0044**	<0.00001	<0.00001	<0.0001
La Salle Is.	0.0138	–	**0.0010**	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	**0.0024**	0.0000	<0.00001	<0.00001	0.0000
Duncan Bay	0.0174	**0.0067**	–	<0.0001	0.0000	0.0000	<0.0001	<0.0001	**0.0021**	0.0000	<0.0001	<0.0001	<0.0001
Alpena	**0.0047**	0.0230	0.0282	–	<0.0001	0.0000	<0.0001	0.0000	<0.0001	**0.1176**	<0.0001	<0.0001	<0.0001
Thunder Bay	0.0167	0.0115	0.0063	0.0251	–	0.0000	<0.0001	<0.0001	0.0002	0.0000	<0.0001	<0.0001	<0.0001
Bay City	0.0112	0.0082	0.0086	0.0202	0.0052	–	<0.0001	<0.0001	0.0000	0.0004	<0.0001	0.0000	<0.0001
Saginaw Bay	0.0244	0.0225	0.0247	0.0334	0.0090	0.0161	–	<0.0001	<0.0001	0.0000	<0.0001	<0.0001	<0.0001
Put-In-Bay	0.0104	0.0262	0.0331	**0.0076**	0.0290	0.0285	0.0430	–	<0.0001	**0.1407**	<0.0001	<0.0001	<0.0001
Niagara	0.0310	0.0140	**0.0068**	0.0327	0.0085	0.0153	0.0269	0.0328	–	0.0002	**0.2133**	0.0000	**0.3642**
Kelleys Is. 1927	**0.0060**	0.0143	0.0171	**0.0007**	0.0105	0.0114	0.0196	**0.0025**	**0.0176**	–	0.0000	<0.0001	0.0000
Kelleys Is. 1997	0.0288	0.0177	0.0110	0.0356	0.0083	0.0125	0.0245	0.0317	**0.0005**	0.0170	–	<0.0001	**0.2518**
Sandusky	0.0285	0.0255	0.0274	0.0292	0.0252	0.0264	0.0385	0.0229	0.0245	**0.0121**	0.0309	–	<0.0001
Cedar Point	0.0347	0.0179	0.0160	0.0391	0.0086	0.0138	0.0214	0.0368	**0.0008**	**0.0222**	**0.0003**	0.033	–

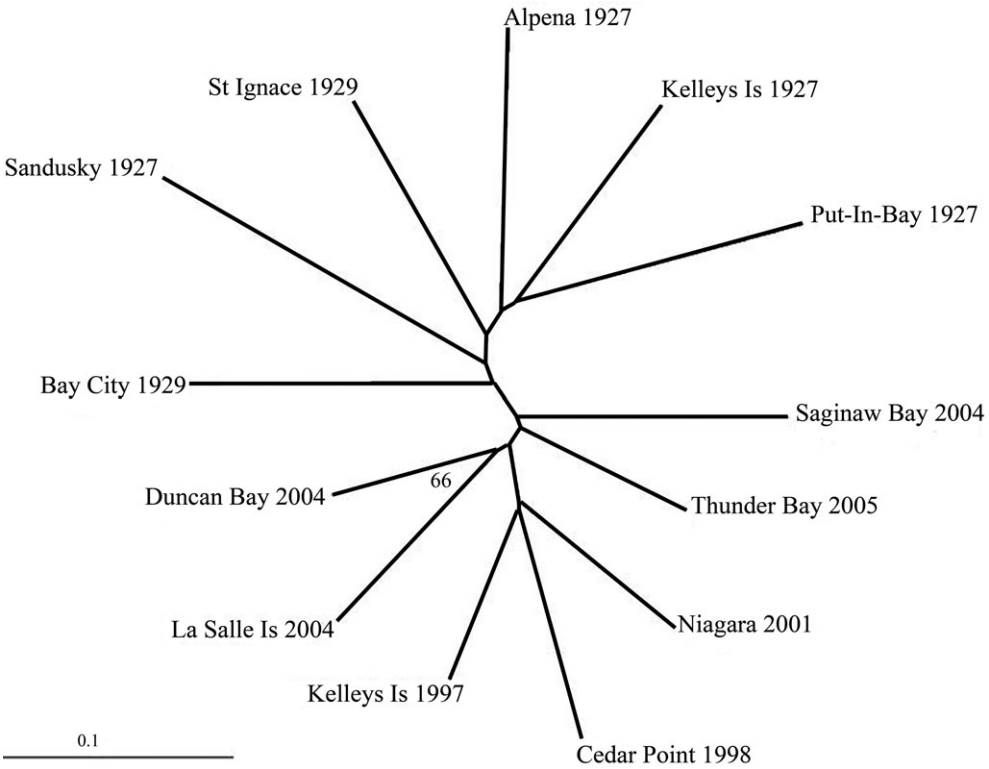

Fig. 3. Unrooted Neighbour-Joining tree of Cavalli-Sforza & Edwards (1967) chord distance among collections of lake whitefish from sites on Lake Huron and Lake Erie in two time periods. Node support represents 10,000 bootstrap pseudoreplicates. Only sites with greater than 60% support are shown.

(0.0001 to 0.0231) (VanDeHey et al. 2009), Lake Ontario (0.000 to 0.020) (Bernard et al. 2009) and Lakes Huron, Michigan, and Superior (-0.0045 to 0.0573) (Stott et al. 2011), and Isle Royale (0.031 to 0.188) (Stott et al. 2004). Another *Coregonus* species from the Great Lakes, the bloater (*C. hoyi*) also had low to moderate $F_{st}(\theta)$ values (-0.016 to 0.044) that were used to distinguish among bloater from Lakes Huron, Michigan, and Superior (Favé & Turgeon 2008). Although there is evidence of changes in allele frequencies in both lakes between the two sampling periods, overall genetic diversity appears similar. Diversity, as measured by observed heterozygosity and allelic richness, is similar among contemporary and historical collections. Observed heterozygosity levels were also similar to those calculated for lake whitefish in contemporary collections from Lake Michigan (0.574 to 0.640) (VanDeHey et al. 2009), Lake Ontario (0.54 to 0.60) (Bernard et al. 2009), and outside the Great Lakes (Lu & Bernatchez 1999).

More population structuring was evident among collections from Lake Huron as compared to Lake Erie. Three genetic groups were identified in Lake Huron in both time periods; La Salle Island/Duncan Bay, Thunder Bay and Saginaw Bay in the contemporary collections and Alpena, St. Ignace and Bay City in the historical collections. Although a relatively

Table 3. Analysis of molecular variance (AMOVA) results for five different potential groups of historical and contemporary lake whitefish collections from Lakes Huron and Erie.

Comparison	Groups in Comparison	Source of Variation	Variance (%)	*p* value
A	1) Historical Collections	Between time periods	1.13	0.0020
	2) Contemporary Collections	Among populations within a time period	1.01	<0.0001
		Within population	97.86	<0.0001
B	1) Lake Erie	Between lakes	0.38	0.0479
	2) Lake Huron	Among populations within a lake	1.36	<0.0001
		Within population	98.26	<0.0001
C	1) Lake Huron Historical	Among the four groups	1.19	0.0010
	2) Lake Huron Contemporary	Among populations within groups	0.66	<0.0001
	3) Lake Erie Historical	Within population	98.15	<0.0001
	4) Lake Erie Contemporary			
D	1) St. Ignace, La Salle Is., Duncan Bay	Among the four groups	0.13	0.2239
	2) Alpena, Thunder Bay	Among populations within groups	1.45	<0.0001
	3) Bay City, Saginaw Bay	Within population	98.41	<0.0001
	4) All Lake Erie Collections			
E	1) St. Ignace	Among the seven groups	0.45	0.1500
	2) La Salle Is., Duncan Bay	Among populations within groups	1.17	<0.0001
	3) Alpena	Within population	98.38	<0.0001
	4) Thunder Bay			
	5) Bay City			
	6) Saginaw Bay			
	7) All Lake Erie Collections			

small number of collection sites were examined in this study, the results for Lake Huron are similar to what was observed in a detailed analysis of lake whitefish genetic population structure (Stott et al. 2011, Stott et al. 2012). In contrast, fewer genic differences were observed among the collections from Lake Erie and the AMOVA indicated that samples from Lake Erie could be considered one genetic group. The collections from Lake Huron covered a broader geographic range than those from Lake Erie which might account for the presence of more structuring. Isolation-by-distance effects have been shown to influence lake whitefish population structure in the Great Lakes (VanDeHey et al. 2009, Stott et al. 2011). A previous

genetic analysis of lake whitefish from the Canadian waters of Lake Huron also found that samples from the central to southern portion of the lake were distinct from those in the north (Casselman et al. 1981). Lake whitefish collected from Lake Michigan over a similar scale (more than 250 km apart) also displayed genetic differentiation among sites collected in the south-central portion of the basin as compared to those collected in the north (VanDeHey et al. 2009). However, discrete genetic populations were observed over a smaller spatial scale (approximately 120 km apart) when sites from Green Bay were compared to those collected in the northern main basin of Lake Michigan (Imhof et al. 1980, VanDeHey et al. 2009). In contrast, lake whitefish in Lake Ontario collected over a similar spatial scale displayed little or weak genetic differentiation (Bernard et al. 2009).

Temporal changes in differentiation were observed among the historical and contemporary collections based on genic differentiation, $F_{st}(\theta)$, and AMOVA. Allele frequency shifts were observed at some loci (e.g. *CoclLav*68, Fig. 3) and were especially prominent in comparisons between collections from Saginaw Bay and Bay City, Sandusky and Cedar Point, and St. Ignace, Duncan Bay and La Salle Island. Most notably, collections from Lake Erie had lower $F_{st}(\theta)$ values among the contemporary samples (average $F_{st}(\theta) = 0.0005$, 95% CI =0.0005 to 0.0006) as compared to the historical samples (average $F_{st}(\theta) = 0.0125$, 95% CI=0.0109 to 0.0141). Little change in allele frequency was observed among collections of lake whitefish from eastern Lake Ontario that were collected 11 years apart (Bernard et al. 2009). Temporal analyses of other fish species have also yielded varying results. For example, lake trout (*Salvelinus namaycush* (Walbaum)) from the upper Great Lakes in the 1940s had similar diversities to those collected in the late 1990s, but the NJ tree indicated that historical collections from Lake Superior were more similar to each other than to their contemporary counterparts (Guinand et al. 2002). Walleye (*Sander vitreus* (Mitchill)) collected from the Black Bay (Lake Superior) in the 1960s were more closely related to the contemporary population in a tributary of Black Bay than to the nearby Nipigon Bay (Wilson et al. 2007) and Atlantic salmon (*Salmo salar* L.) collected from the Skjern River in Denmark almost 60 years apart showed both temporal and spatial differences when the temporal replicates were compared to contemporary samples from two other rivers (Nielsen et al. 1997).

Spatial and temporal considerations are important in order to ensure that preservation of diversity is optimised. Changes in population structure due to migration, declines in abundance and stochastic effects may account for observed shifts in allele frequencies. Changes in migration patterns can elevate gene flow among stocks and potentially alter their spatial boundaries. Fluctuations in abundance over time can alter allele frequencies through genetic drift. Lake whitefish may use migration as a mechanism to optimise growth by moving horizontally and vertically to better quality habitat (Ebener et al. 2010). A slow recovery of lake whitefish populations in the Bay of Quinte (Lake Ontario) in the 1970s may have prompted increased immigration from lake populations and decreased genetic diversity between the main basin of the lake and the Bay of Quinte, although they remain ecologically distinct (Bernard et al. 2009). In contrast, the spatial distribution and size-at-age of the Big Bay de Noc lake whitefish stock in Lake Michigan have changed since the 1970s (Ebener et al. 2010), but the stock remains genetically distinct from others in Lake Michigan (Imhof et al. 1980, VanDeHey et al. 2009), although it is unknown if allelic characteristics of the Big Bay de Noc stock have changed over time. In the current study, similar population groupings were observed in both time periods, but the allele frequencies between some of the temporal

replicates appear to have changed. Recent genetic analyses of lake whitefish from the Great Lakes (e.g., Bernard et al. 2009, VanDeHey et al. 2009) and studies of movement and life history (e.g., Cook et al. 2005, Mohr & Ebener 2005, Ebener et al. 2010) further demonstrate the potential for a swift and varied response at multiple levels (individual, stock, community) to environmental change (Lindsey et al. 1981) and emphasise the importance of awareness of the potential for changing stock boundaries when setting management goals.

In addition to the biological causes, allele frequency differences could have resulted from the sampling of different populations in the different time periods. For example, Saginaw Bay was an historically important source of lake whitefish production on Lake Huron and numerous spawning grounds have been reported in the bay (Goodyear et al. 1982), however many of these sites were lost to habitat alterations (Beeton 1969). Therefore, it is possible that the changes observed in the collections represent both temporal and spatial differences. Significant population differentiation has been observed in lake whitefish on this spatial scale at other sites in Lake Huron and also in Lake Michigan (Casselman et al. 1981, VanDeHey et al. 2009, Stott et al. 2011). The observed genetic differences between the collections from St. Ignace and the La Salle Island/Duncan Bay group may be the result of a similar effect. The samples from St. Ignace were not collected during the spawning season, but in late summer (August). Therefore, it is possible that the fish from the sample may contain individuals from other stocks including those from Lake Michigan. Ebener et al. (2010) reported that adult lake whitefish tagged in north-eastern Lake Michigan were recaptured predominately in northern Lake Michigan, but 7% of them were recaptured in northern Lake Huron, near St. Ignace, several months after spawning between 2004 and 2008. Similarly, the collections of lake whitefish from Sandusky in 1927 may have contained fish migrating to spawning grounds further to the west and/or fish collected from other sites. Sandusky once housed a processing plant that received a substantial portion of the lake whitefish caught in Lake Erie; therefore fish reported to this port may have been collected from other sites in the western basin and even from outside Lake Erie. Details of the origins of historical lake whitefish catches reported to various ports on Lake Erie are not readily available to determine how or if the spatial extent of the fishing grounds reported to a particular port have changed. This demonstrates that care should be taken to ensure that samples collected for historical analyses are indeed true temporal and spatial replicates. This is especially important in a species such as the lake whitefish that has the potential to show genetic differentiation over a small spatial scale.

The M ratio indicated that collections from both time periods experienced a genetic bottleneck whereas the heterozygosity excess test did not. Discrepancies in the results of these two tests may be the product of the assumptions associated with each test. For example, the heterozygosity excess test detects only very severe and precipitous declines in population size (Luikart & Cornuet 1998). The decline of lake whitefish populations in the early and mid-twentieth century occurred over an extended period of time (Brown et al. 1999). Harvests reported for Lake Huron between the late 1880s and the late 1950s are characterised by a series of fluctuations before reaching historical lows in the late 1950s (Baldwin et al. 2009). In addition, declines in harvest do not necessarily mean that the populations supporting the fishery declined to a point where a significant loss of diversity occurred. Even at their lowest reported harvest levels, lake whitefish populations most likely still numbered in the thousands. The buffering effect of a slow population decline may have minimized the loss of

genetic diversity in the copper redhorse (*Moxostoma hubbsi* Legendre) (Lippé et al. 2006), the razorback sucker (*Xyrauchen texanus* (Abbott)) (Dowling et al. 2005), and the bloater (Favé & Turgeon 2008).

Other factors that could impact the results of the bottleneck analyses are the number of loci used in the study and departures from HWE (Luikart & Cornuet 1998). While we did not observe any significant departures from HWE, the number of loci examined in this study was below the minimum number of loci recommended by Luikart & Cornuet (1998) (10 to 20 loci). Other microsatellite loci have been developed for lake whitefish (e.g., *CoclLav*6, 8, 27, 45 and 72 by Rogers et al. 2004), but we could not get sufficient and consistent amplification in the historical samples at these loci.

The heterozygosity excess test is also sensitive to the degree of isolation among populations. The contributions of even a few migrants may be enough to obscure the genetic effects of population decline (Luikart & Cornuet 1998). Tagging studies of lake whitefish from Lake Huron (performed during the same time period as the contemporary collections) indicated that there is a relatively small but constant movement among management units (Ebener et al. 2010). In Lake Erie, lake whitefish appear to function as one stock based on movement patterns (Hardy 1994). The stock moves between the eastern and western basins of the lake; utilising the habitat in the western basin for spawning and over-wintering and then moving to the eastern basin during the summer and early autumn.

Migration can also affect estimates of the M ratio; new alleles may be introduced into a population through migration, but this would only impact the range of allele size (r, the denominator of the ratio) if they were outside the initial allele size range (Garza & Williamson 2001). The M ratio retains the signature of a population decline longer than the heterozygosity excess test (Garza & Williamson 2001). The time periods sampled in this study (1927 to 1929 and 1997 to 2005) both correspond to periods when harvests of lake whitefish were increasing (Baldwin et al. 2009). The samples from the late 1920s were collected during a period when harvest increased, although not as dramatically as during the contemporary period (Baldwin et al. 2009). It is possible that the low M ratios calculated for both time periods may reflect the impact of the declines while the heterozygosity excess test reflects the increases that occurred just before the sample collection dates. Additional samples from one of the periods of sustained decline in harvest (e.g., 1960 to 1970) may provide additional insight into the impacts of population fluctuation on genetic diversity.

The Great Lakes ecosystem has experienced many changes in the last 100 years and the impacts of over-harvest, invasive species, habitat alteration and possibly climate change have had a pronounced impact on the coregonine community (Smith & Todd 1984). Genetic analyses of lake whitefish from Lakes Huron and Erie (this study) and from other Great Lakes (Bernard et al. 2009, VanDeHey et al. 2009) show that the amount of population structuring can vary in different lakes. Fluctuations in abundance of lake whitefish populations may have resulted in periodic changes in genetic diversity that has resulted in changes in allele frequencies over time through stochastic processes and/or migration. The extent of the changes varies between lakes, although sampling issues may have had an impact on interpretation. Lake whitefish, like many coregonids, have a great capacity to respond quickly to environmental change that should be considered in their management to ensure their continued sustainability in the Great Lakes.

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