

# Genetic structure of lake whitefish (*Coregonus clupeaformis*) in Lake Michigan

Justin A. VanDeHey, Brian L. Sloss, Paul J. Peeters, and Trent M. Sutton

**Abstract:** Genetic relationships among lake whitefish (*Coregonus clupeaformis*) spawning aggregates in Lake Michigan were assessed and used to predict a stock or management unit (MU) model for the resource. We hypothesized that distinct spawning aggregates represented potential MUs and that differences at molecular markers underlie population differentiation. Genetic stock identification using 11 microsatellite loci indicated the presence of six genetic MUs. Resolved MUs corresponded to geographically proximate spawning aggregates clustering into genetic groups. Within MUs, analyses suggested that all but one delineated MU was a stable grouping (i.e., no between-population differences), with the exception being the Hog Island – Traverse Bay grouping. Elk Rapids was the most genetically divergent population within Lake Michigan. However, low  $F_{st}$  values suggested that moderate to high levels of gene flow occur or have occurred in the past between MUs. Significant tests of isolation by distance and low pairwise  $F_{st}$  values potentially led to conflicting results between traditional analyses and a Bayesian approach. This data set could provide baseline data from which a comprehensive mixed-stock analysis could be performed, allowing for more efficient and effective management of this economically and socially important resource.

**Résumé :** Nous avons évalué les relations génétiques entre des rassemblements de fraie de grands corégones (*Coregonus clupeaformis*) au lac Michigan et les avons utilisées pour prédire un modèle de stocks ou d'unités de gestion (MU) pour cette ressource. Nous utilisons l'hypothèse selon laquelle, d'une part, les rassemblements de fraie distincts représentent des MUs potentielles et, d'autre part, des différences au niveau des marqueurs génétiques sous-tendent la différenciation des populations. L'identification des stocks génétiques d'après 11 locus microsatellites indique la présence de six MUs génétiques. Les MUs identifiées correspondent à des rassemblements de fraie rapprochés géographiquement qui se fusionnent en groupes génétiques. Les analyses faites au sein des MUs indiquent que toutes les MU décrites, sauf une, forment des groupes stables (c'est-à-dire sans différences entre les populations), l'exception étant le regroupement Hog Island – Traverse Bay. La population d'Elk Rapids possède la plus grande divergence génétique au lac Michigan. Cependant, les valeurs faibles de  $F_{st}$  laissent croire qu'il y a, ou il y a eu dans le passé, des niveaux modérés à élevés de flux génique entre les MUs. Des tests significatifs d'isolement par la distance et des valeurs faibles de  $F_{st}$  appariés ont conduit à des résultats contradictoires entre les analyses traditionnelles et une approche bayésienne. Cet ensemble de données pourrait représenter des informations de base à partir desquelles il serait possible de faire une analyse complète des stocks mixtes; cela permettrait une gestion plus concrète et efficace de cette ressource qui présente un intérêt économique et social.

[Traduit par la Rédaction]

## Introduction

The utility of the stock concept in natural resource management is based on scientific evidence that the overall productivity and evolutionary potential of a species is dependent on maintaining the abundance and diversity of its component stocks (Shaklee and Currens 2003). Contemporary fisheries management has relied on the stock concept

(a variant of the population concept; Booke 1981; Waples and Gaggiotti 2006) and the operational definition of a stock as "local populations that maintain recognizable genetic differentiation by separation of their spawning time or place" (Bailey and Smith 1981). As such, stocks are usually composed of a population(s) of fish spawning in the same location and time and exhibiting similar growth and mortality rates, morphological–meristic features, and age structure

Received 18 March 2008. Accepted 18 December 2008. Published on the NRC Research Press Web site at [cjfas.nrc.ca](http://cjfas.nrc.ca) on 20 February 2009.  
J20470

**J.A. VanDeHey**<sup>1,2</sup> Wisconsin Cooperative Fishery Research Unit, University of Wisconsin – Stevens Point, College of Natural Resources, 800 Reserve Street, Stevens Point, WI 54881, USA.

**B.L. Sloss.** US Geological Survey, Wisconsin Cooperative Fishery Research Unit, University of Wisconsin – Stevens Point, College of Natural Resources, 800 Reserve Street, Stevens Point, WI 54481, USA.

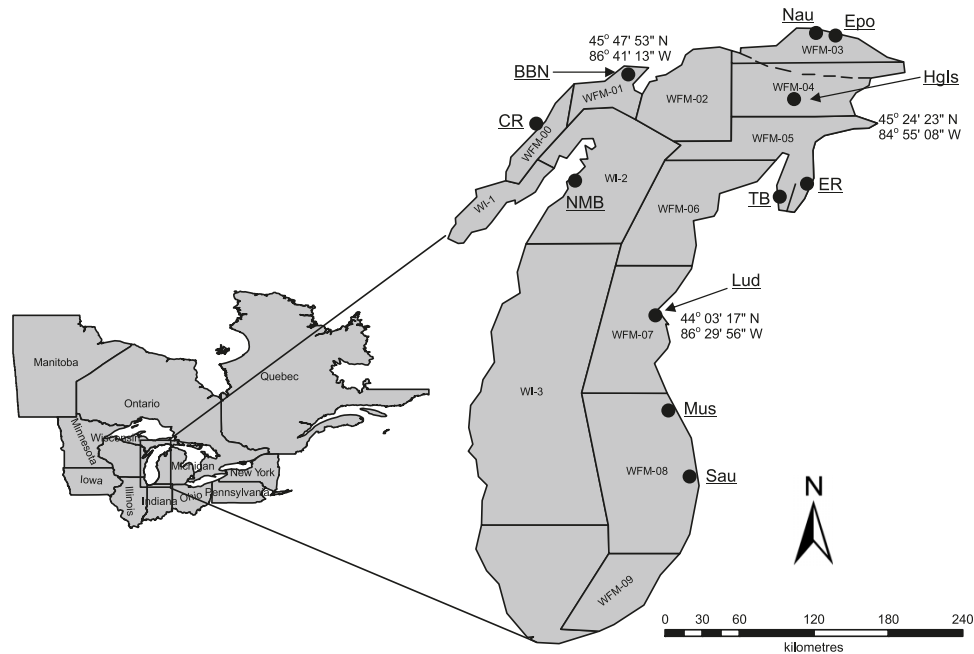
**P.J. Peeters.** Wisconsin Department of Natural Resources, 110 S. Neenah Avenue, Sturgeon Bay, WI 54235, USA.

**T.M. Sutton.** University of Alaska Fairbanks, School of Fisheries and Ocean Sciences, 245 O'Neill Building, Fairbanks, AK 99775, USA.

<sup>1</sup>Corresponding author (e-mail: [justin.vandehey@sdstate.edu](mailto:justin.vandehey@sdstate.edu)).

<sup>2</sup>Present address: Department of Wildlife and Fisheries Sciences, South Dakota State University, Northern Plains Biostress Laboratory, Box 2140 B, Brookings, SD 57007, USA.

**Fig. 1.** Lake whitefish commercial management zones in Lake Michigan with sample site locations (●). Primary sample sites included the lake side of the Door County Peninsula (NMB), Wisconsin, and Big Bay de Noc (BBN), Michigan. Secondary study sites include Cedar River (CR), Naubinway (Nau), Epoufette (Epo), Hog Island (Hgl), Traverse Bay (TB), Elk Rapids (ER), Ludington (Lud), Muskegon (Mus), and Saugatuck (Sau). Zones in Wisconsin waters include WI-1, WI-2, and WI-3. All other zones are in Michigan waters (WFM) and were originally established by the 1836 Consent Decree. The broken line runs from Waugoshance Point west to Seul Choix Point.



(Van Den Avyle 1993). Moritz (1994) suggested a more contemporary definition of the term “stock” as being synonymous with management units (MUs), where MUs are recognized as groups with significant divergence of allele frequencies at nuclear loci, regardless of the phylogenetic distinctiveness of the alleles. Management units are fundamental to proper short-term management and should be the logical unit for population and demographic monitoring (Moritz 1994).

Lake whitefish (*Coregonus clupeaformis*) have supported an important commercial fishery in the Laurentian Great Lakes since the 1800s. Presently, lake whitefish comprise the primary commercial fishery in the Great Lakes in terms of total harvest and dockside value. In Lake Michigan, lake whitefish support both state-licensed and tribal commercial fishers in Michigan and state-licensed commercial fishers in Wisconsin. The 2000 harvest season was the highest on record, producing 2.174 million kg of fish with a dockside value of over US\$5 million (Kinnunen 2003). The Lake Michigan lake whitefish commercial fishery is an interjurisdictional fishery with the Wisconsin (WDNR) and Michigan Department of Natural Resources (MDNR) managing state-licensed fishers and the management of tribal resources being handled by the Chippewa Ottawa Resource Authority (CORA), which also supervises the activities of individual tribes (e.g., Grand Traverse Band of Indians (GTBNI)). Presently, quotas are set for this fishery based on statistical catch-at-age models for 13 commercial management zones rather than on a stock basis (Fig. 1).

Despite the commercial importance of lake whitefish, questions exist regarding the source (i.e., stock or MU) of fish being harvested. In particular, there are concerns regarding potential overharvest of shared stocks by Michigan and

Wisconsin state-licensed and Tribal commercial fishing operations. Previous tagging (Rowe 1984; Scheerer and Taylor 1985), population dynamics (Ebener and Copes 1985; Walker et al. 1993), and genetic studies (Imhoff 1977; Leary 1979; Imhoff et al. 1980) have indicated potential stock structure; however no genetic management zones have been established. To better manage this important resource, delineation of lake whitefish genetic structure in Lake Michigan is necessary. Optimal long-term management and conservation depend on knowing the number, distribution, and characteristics of all component stocks and maintaining their integrity, diversity, and abundance (Shaklee and Currens 2003), while optimal short-term management requires delineation of MUs for proper demographic management (Moritz 1994). Therefore, the primary objective of this study was to delineate genetic structure among the Lake Michigan’s lake whitefish spawning aggregates.

## Materials and methods

### Study design

Throughout the course of this research, we had one primary assumption: fish sampled at a specific spawning location belonged to that spawning aggregate and were not migrant fish from another spawning aggregate. Based on the philopatric nature of salmonids and evidence from tagging studies that Lake Michigan lake whitefish home to natal spawning grounds (Ebener and Copes 1985; Walker et al. 1993), we hypothesized that separate spawning aggregates represented separate potential genepools (i.e., MUs). We attempted to identify and sample the known spawning reefs of lake whitefish throughout Lake Michigan. Where possible, multiyear samples were included to minimize any bias asso-

**Table 1.** Locus names, locus abbreviation (code), single and multiplex PCR conditions, fluorescent labels, observed allele size range (in bp), number of alleles observed (A), and references for loci used in this study.

Locus	Code	Multiplex	PCR buffer (concentration)	MgCl <sub>2</sub> (μmol·L <sup>-1</sup> )	Primer F (μmol·L <sup>-1</sup> )	Primer R (μmol·L <sup>-1</sup> )	Label	Range	A	Reference
Bwf-1	B1	1 <sup>a</sup>	1.4×	1.70	0.250	0.250	Ned	169–243	23	Patton et al. 1997
Cocl-lav 6	C6	1 <sup>a</sup>			0.080	0.080	Ned	148–158	19	Rogers et al. 2004
Cocl-23	C23	2 <sup>a</sup>	1.0×	2.00	0.025	0.025	6Fam	250–278	15	Lu et al. 2001
Bwf-2	B2	2 <sup>a</sup>			0.100	0.100	6Fam	140–166	13	Patton et al. 1997
Cocl-lav 18	C18	3 <sup>b</sup>	2×	2.10	0.320	0.320	Hex	148–160	7	Rogers et al. 2004
Cocl-lav 68	C68	3 <sup>b</sup>			0.250	0.250	Ned	172–184	7	Rogers et al. 2004
Cocl-lav 4	C4	3 <sup>b</sup>			0.075	0.075	6Fam	148–158	6	Rogers et al. 2004
Cocl-lav 45	C45	4 <sup>b</sup>			0.180	0.180	Ned	239–259	9	Rogers et al. 2004
Cocl-lav 28	C28	4 <sup>b</sup>			0.200	0.200	Hex	168–182	8	Rogers et al. 2004
Cocl-lav 41	C41	5 <sup>c</sup>	1.5×	1.50	0.200	0.200	Ned	181–233	21	Rogers et al. 2004
Cocl-lav 52	C52	6 <sup>d</sup>	1.5×	1.75	0.040	0.040	6Fam	90–164	31	Rogers et al. 2004

<sup>a</sup>94 °C for 3 min; six series of five cycles each at 94 °C for 30 s, then 60, 59, 58, 57, 56, and 55 °C annealing for 30 s; 72 °C for 30 s, then a final elongation of 72 °C for 7 min.

<sup>b</sup>94 °C for 3 min; seven series of five cycles each at 94 °C for 30 s, then 62.5, 62, 61.5, 61, 60.5, 60, and 59 °C annealing for 30 s; 72 °C for 30 s, then a final elongation of 72 °C for 7 min.

<sup>c</sup>94 °C for 3 min; seven series of five cycles each at 94 °C for 30 s, then 60, 59.5, 59, 58.5, 58, 57.5, and 57 °C annealing for 30 s; 72 °C for 30 s, then a final elongation of 72 °C for 7 min.

<sup>d</sup>94 °C for 3 min; two series of five cycles each at 94 °C for 30 s, then 63 and 62 °C annealing for 30 s, then 72 °C for 30 s. Then two series of eight cycles each at 94 °C for 30 s, then 61 and 60.5 °C annealing for 30 s, then 72 °C for 30 s. Then a final series of five cycles of 94 °C for 30 s, then 60 °C annealing for 30 s, then 72 °C for 30 s, and a final elongation of 72 °C for 7 min.

ciated with a single-year estimate. Temporal genetic differences exist within some spawning runs of salmonid fish (Wilmot and Burger 1985; Burger et al. 1997; Waples et al. 2004). To assess the impact of potential temporal genetic differences within a single lake whitefish spawning run, two temporal samples ( $n = 75$ ) were obtained annually from North and Moonlight bays (NMB) and Big Bay de Noc (BBN). The first sample was obtained in late October and the second in mid-November to determine if genetic differences were apparent within a single year. If there were genetic differences between the sampling periods within a sample site, multiple runs of lake whitefish could be attributed to the spawning site and would be treated as separate units for stock delineation.

The second hypothesis tested was that differences in genetic diversity found within or among the spawning aggregates indicated separate gene pools (i.e., MUs). Therefore, if spawning aggregates represent unique and discernible gene pools, they could be considered and tested as genetic stocks or MUs.

### Research area

The primary research areas were the BBN (north end of Green Bay, Michigan) and NMB (lake side of the Door County Peninsula, Wisconsin) spawning reef complexes (Fig. 1). These areas are the center of Lake Michigan's commercial lake whitefish fishery (both tribal and state-licensed) and are thought to represent the two primary spawning areas for Lake Michigan lake whitefish (Ebener and Copes 1985). Secondary study sites, comprising smaller lake whitefish spawning aggregates, included Naubinway (Nau), Epoufette (Epo), Hog Island (Hgl), Traverse Bay (TB), Elk Rapids (ER), Ludington (Lud), Muskegon (Mus), Saugatuck (Sau), and the Cedar River (CR) in Michigan (Fig. 1).

### Sample collection

Sample collection of commercially harvested lake whitefish occurred from mid-October through early November (the time period in which lake whitefish spawn) of the 2005 and 2006 commercial fishing seasons. Primary study sites (BBN and NMB) and some secondary study sites (CR, Nau, Epo, Lud, Mus, and Sau) were sampled during both 2005 and 2006. Secondary spawning aggregates were sampled more opportunistically based on the availability of cooperating commercial fishers. As such, some sites were not sampled in both study years. Only fish that could be positively identified as sexually mature (i.e., presence of gametes) were included in the sample to maximize the probability that samples were from the spawning aggregate associated with that location (Ebener and Copes 1985). Primary study sites (BBN and NMB) were sampled twice during the spawn to determine if any genetic differences existed between early- and late-run fish. Samples were acquired from commercial fishers, in conjunction with WDNR and MDNR state agency sampling and commercial monitors, and from tribal harvest and CORA commercial monitors. The majority of sampled fish were obtained from trap nets, and a small proportion of samples were obtained from gill nets. A single sample was obtained from the Muskegon (2006 sample) reef by angling.

All commercially caught samples consisted of unsorted

(i.e., no intentional size discrimination beyond that induced by the commercial gear) commercial catch sampled dockside to reduce potential size-related bias in genetic diversity estimates. Lake-wide samples from known spawning aggregates in the secondary study sites were obtained through the cooperation of the MDNR, CORA, GTBNR, the Little Traverse Bay Band of Odawa Indians, and the Little River Band of Ottawa Indians. Samples were also collected during Wisconsin's closed spawning season (26 October – 30 November) through the WDNR fall, graded-mesh gill net assessment. Pelvic fin clips were placed in individually labeled tubes with 95% EtOH.

### Microsatellite DNA

Total genomic DNA was isolated from the pelvic fin tissue using the Promega Wizard Genomic DNA purification kit (Promega Corp., Madison, Wisconsin). DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware) and normalized to a standardized concentration of 20 ng· $\mu$ L<sup>-1</sup>. Eleven microsatellite loci previously used for salmonid studies (Table 1) were PCR amplified using multiplex reactions developed in-house based on the suggested protocol of Hene-gariu et al. (1997). Specific PCR reaction conditions and thermocycler profiles are described in Table 1. Genotyping was conducted on an ABI Prism 377XL DNA sequencer (Applied Biosystems Inc., Foster City, California). Allele sizes were determined by comparison to an internal size standard (GeneFlo 625; Chimerx Inc., Milwaukee, Wisconsin) and estimated using GeneScan software (Applied Biosystems Inc., Foster City, California). Allele calls were confirmed manually and the resulting values represented multilocus genotype data.

### Spawning aggregate genetic analyses

Spawning aggregate samples were tested for departure from Hardy–Weinberg equilibrium (HWE) using GENEPOP 3.4 (Raymond and Rousset 1995). Significance values were computed using Fisher's exact test implemented in GENEPOP 3.4 with a Markov chain method using 1000 batches of 1000 iterations each (Guo and Thompson 1992; Raymond and Rousset 1995). Values of  $p$  were adjusted for multiple comparisons using a sequential Bonferroni method (Rice 1989). Any locus or population not conforming to HWE expectations was specifically tested for heterozygote excess or deficiency. Departures from HWE expectations due to heterozygote excess or deficiency were determined using a  $U$  test implemented in GENEPOP 3.4 (Raymond and Rousset 1995). The small expected frequencies for rare genotypes associated with microsatellite data can lead to significant deviations from HWE using exact tests because of the cumulative effect of the rare but nonzero expected genotypes (Pamilo and Varvio-Aho 1984). Therefore, all exact tests resulting in significant deviations from HWE expectations were retested following a modification of Hedrick (2000), where the genotypes with an expected frequency of less than 1% were pooled and the locus retested using a  $\chi^2$  goodness-of-fit test in Minitab v.14.20 (Minitab Inc., State College, Pennsylvania).

Microsatellite diversity within and between samples was compared using allele frequency distributions and observed

( $H_o$ ) and expected ( $H_e$ ) heterozygosities. Additionally, allelic richness estimates ( $A_r$ ) and private allele estimates were made using the rarefaction method described by Goudet (1995) and Petit et al. (1998), as recommended by Leberg (2002), to account for unequal sample sizes. Allelic richness and private allelic richness were calculated using HP-RARE (Kalinowski 2005). Gametic disequilibrium between all pairs of loci was assessed using a likelihood ratio test of the data implemented in ARLEQUIN (version 3.0; Excoffier et al. 2005) using 10 000 permutations.

### Genetic stock identification (GSI)

Traditional genetic stock identification involves a series of hierarchical statistical tests in which the results from one test are used to group populations for subsequent analyses. We compared allele frequency distributions between all pairs of populations using the genic differentiation option in GENEPOP 3.4 with 1000 dememorization steps and 100 batches of 1000 iterations (Raymond and Rousset 1995) to assess the null hypothesis of panmixia among Lake Michigan's lake whitefish populations. The ability to combine samples from early and late runs from the NMB and BBN sites was also tested using the genic differentiation test of allele frequency distributions in GENEPOP 3.4 with the same options as previously stated (Raymond and Rousset 1995). To determine relevant groupings of populations for further analyses, we constructed a distance-based, neighbor-joining tree (NJ tree; Saitou and Nei 1987). Chord distance ( $D_c$ ; Cavalli-Sforza and Edwards 1967) was calculated for all population pairs, and a NJ tree was constructed using PowerMarker (version 3.25; Liu and Muse 2005). Groupings were considered relevant if the confidence in topology was  $\geq 70\%$ . Confidence in topology was determined by conducting 5000 bootstrap pseudoreplicates and constructing a majority-rule consensus tree using CONSENSE in the PHYLIP package (Felsenstein 1993). All trees were viewed using TREEVIEW (Page 1996).

To determine if resolved spawning aggregate clusters were biologically relevant (i.e., putative MUs), a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) was performed in ARLEQUIN (version 3.0; Excoffier et al. 2005) to compare the molecular variance within and between the putative aggregates with that which would be expected in one panmictic population. Significance levels for AMOVA were computed using 1000 nonparametric permutations of the data set (Excoffier et al. 1992). A stable group (i.e., potential MU) was inferred when significant variance existed among groups but not within groups. The degree of fine-scale, intragroup differentiation was examined by estimating population pairwise values of Weir and Cockerham's (1984)  $\theta$ , an analog of Wright's (1931)  $F_{st}$ , and testing the values for deviation from zero. All tests of  $\theta$  were conducted in ARLEQUIN (version 3.0; Excoffier et al. 2005) with significance determined by 1000 permutations of the data.

### Structure

As an alternative means of delineating genetic structure, we used a Bayesian approach as implemented in the program STRUCTURE (Pritchard et al. 2000). To determine if structure existed within spawning aggregates, we evaluated

**Table 2.** Summary genetic statistics for all 12 sampled populations including sample size ( $n$ ), expected heterozygosity ( $H_e$ ), observed heterozygosity ( $H_o$ ), the mean number of observed alleles/locus ( $A$ ), mean allelic richness based on rarefaction ( $A_r$ ; Kalinowski 2005), and the rarefacted private alleles ( $PA_r$ ).

Population*	$n$	$H_e$	$H_o$	$A$	$A_r$	$PA_r$
Epo	137	0.6305	0.6016	9.18	8.27	2.20
Nau	132	0.6391	0.6116	9.09	8.30	2.23
TB	74	0.6396	0.6432	7.64	7.59	2.74
ER	72	0.6349	0.5735	7.55	7.55	6.41
HgIs	70	0.6384	0.6091	7.82	7.82	2.02
Sau	132	0.6319	0.6051	7.91	7.15	0.09
Mus	133	0.6372	0.5927	8.45	7.74	1.07
Lud	124	0.6479	0.6397	8.64	7.97	1.11
CR	149	0.6527	0.6187	9.73	8.69	5.67
NMB	286	0.6268	0.6158	10.91	8.19	2.64
BBN	280	0.6381	0.6233	9.64	7.97	1.29

\*Population abbreviations: Epo, Epoufette; Nau, Naubinway; TB, Traverse Bay; ER, Elk Rapids; HgIs, Hog Island; Sau, Saugatuck; Mus, Muskegon; Lud, Ludington; CR, Cedar River; NMB, North and Moonlight bays; BBN, Big Bay de Noc.

our data for  $K = 1-15$  (three runs per  $K$  value), where  $K$  is the number of potential genetic units occurring within the data set. We used a 100 000 replicate burn-in followed by 100 000 replicates. We used the admixture model with a uniform prior on the degree of admixture,  $\alpha$  (initial value, 1.0; maximum, 10.0; standard deviation (SD), 0.025), and allele frequencies were considered correlated among subpopulations (prior mean, 0.01; prior SD, 0.05;  $\lambda$ , 1.0).

### Isolation by distance

To determine if migration, or a lack thereof, between spawning aggregates was a driving force behind the observed genetic structure, we conducted a test of isolation by distance (IBD) by regressing the pairwise genetic distances against a matrix of pairwise geographical distances using the program IBDWS (Jensen et al. 2005). Genetic distances,  $F_{st}(\theta)$ , between population pairs were calculated as previously described. Geographic distances were calculated in ArcMap (version 9.2; Environmental Systems Research Institute, Inc., Redlands, California) and were based on the shortest distance (in kilometres) across water from the center of where one population was sampled to the center of where the other population was sampled. Significance in the IBD test was determined using a Mantel test (Mantel 1967).

### Results

A total of 1589 fish were collected from 11 spawning aggregates, including 280 and 286 samples collected from the BBN and NMB primary study sites, respectively. Samples were collected from all but three lake whitefish commercial management zones (WI-1, WFM-06, and WFM-09; Fig. 1) known to contain spawning aggregates. Sample sizes for all spawning aggregates were  $\geq 70$  fish. Eight of the 11 spawning sites were sampled during 2005 and 2006 resulting in  $\geq 124$  samples for these eight sites (Table 2). Samples from ER were collected only in 2005, and samples from TB and HgIs were collected only in 2006.

### Genetic diversity

The overall data set had 4.97% of the possible alleles

( $N = 34\,958$  alleles) missing across all loci. Only five of 1589 individuals were missing data from four or five loci, with no individuals missing data from more than five of 11 loci. The number of alleles per locus varied from six to 31 across all spawning aggregates (Table 1), with allele frequencies varying considerably across aggregates. For example, the distribution of allele frequencies at Cocl-lav 18 (seven total alleles) showed a common allele (156) that varied by nearly 20% between the Mus sample (39.5%) and the HgIs sample (57.9%). Observed heterozygosity for each population ranged from 0.5735 (ER) to 0.6432 (TB), with a mean heterozygosity of 0.6111 (Table 2). Unbiased heterozygosity values for the microsatellite loci ranged from 0.6268 (NMB) to 0.6527 (CR), with a mean for all aggregates of 0.6379 (Table 2). Mean allelic richness estimates across all loci ranged from 7.15 (Sau) to 8.69 (CR) (Table 2). Private allelic richness estimates varied dramatically, with the overall aggregate totals ranging from 0.09 (Sau) to 6.41 (ER) across loci (Table 2).

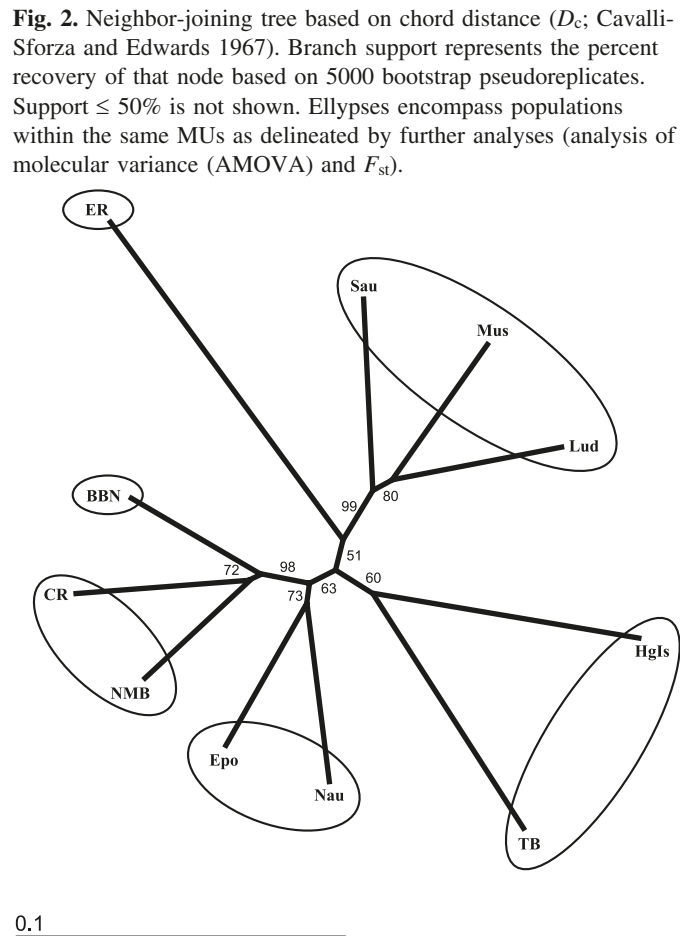
Comparisons of early- and late-run genetic samples from NMB and BBN using the genic differentiation option in GENEPOP 3.4 (Raymond and Rousset 1995) showed no differences in allele frequency distributions (data not shown), indicating temporal homogeneity within each spawning aggregate in a single year. The samples for each site were subsequently combined for all further analyses. The combination of the sites was further supported, as the conglomerate sample did not deviate significantly from HWE.

Initially, 37 of 121 exact tests significantly deviated from HWE at a nominal  $\alpha$  of 0.05. After pooling rare genotypes, 10 of 121 total comparisons (8.26%) were significant following sequential Bonferroni adjustment. Six of 11 loci had at least one significant departure from HWE, with no locus significantly out of HWE for more than two populations and no population significantly out of HWE for more than three loci. All deviations from HWE were consistent with heterozygote excess, suggesting no inadvertent sampling of multiple gene pools and (or) issues with systemic errors (i.e., null alleles or allele dropout; Navidi et al. 1992; Miller et al. 2002). Given the low number of significant HWE tests and the finding that the distribution of significant results did not

**Table 3.** Pairwise genetic distance matrix (below diagonal) based on chord distance ( $D_c$ ; Cavalli-Sforza and Edwards 1967).

	Epo	Nau	TB	ER	Hgls	Sau	Mus	Lud	CR	NMB	BBN
Epo											
Nau	0.1471										
TB	0.1044	0.1717									
ER	0.1583	0.2073	0.2046								
Hgls	0.1499	0.1597	0.1674	0.2230							
Sau	0.1573	0.1512	0.1917	0.1926	0.2013						
Mus	0.1591	0.1448	0.1936	0.2054	0.2018	0.1183					
Lud	0.1604	0.1480	0.1889	0.1953	0.2029	0.1148	0.1027				
CR	0.1258	0.1255	0.1841	0.2095	0.1817	0.1634	0.1497	0.1672			
NMB	0.1208	0.1294	0.1749	0.1943	0.1661	0.1501	0.1449	0.1515	0.0963		
BBN	0.1133	0.1220	0.1762	0.2051	0.1596	0.1540	0.1472	0.0918	0.1028	0.0002	
											0.0918

**Note:**  $p$  values for pairwise tests of genetic differentiation (above diagonal). Population abbreviations are defined for Table 2.



suggest a population- or locus-specific problem, all sites were considered to conform to HWE for subsequent analyses. Gametic disequilibrium tests between all pairs of loci showed no significant disequilibrium, and all loci were considered to be independently segregated.

**Genetic stock identification**

Tests of genic differentiation showed that the lake whitefish spawning aggregates included in this study did not represent one panmictic unit. The overall (global) test had a chi-square value of infinity (indicating that the number exceeded the maximum capacity of the GENEPOP 3.4 program to calculate), with 24 degrees of freedom and  $p \leq 0.00001$ . Significant differences in allele frequency distributions were observed among all populations at all 11 loci ( $p < 0.0001$ ).

Divergence among populations varied widely, with pairwise  $D_c$  (Cavalli-Sforza and Edwards 1967) ranging from 0.0918 (BBN and NMB) to 0.2230 (ER and Hgls) (Table 3).

3). The NJ tree resolved five to six aggregate groups (Fig. 2), including group A (BBN, NMB, CR), group B (Nau, Epo), group C (Lud, Mus, Sau), group D (ER), and group E (TB, Hgls). The six-group model would consist of BBN grouping separately from NMB and CR and all other groups remaining stable.

Three sequential AMOVA analyses were conducted (based on NJ tree groups), with the number of groups ranging from four to six (Table 4). In all tests, the majority of

**Table 4.** Analysis of molecular variance (AMOVA), including sum of squares (SS), percent of variance explained by source, and *p* values for three putative groupings of lake whitefish spawning aggregates.

		Source of variation	SS	Variance (%)	<i>p</i> value
<b>(a) Four-group AMOVA</b>					
Group 1	Epo, Nau	Among groups	67.483	0.79	<0.00001
Group 2	TB, HgIs				
Group 3	ER, Lud, Mus, Sau	Among populations within groups	32.860	0.17	<0.00001
Group 4	NMB, BBN, CR	Within populations	9976.859	99.05	<0.00001
<b>(b) Five-group AMOVA</b>					
Group 1	Epo, Nau	Among groups	76.423	0.87	<0.00001
Group 2	TB, HgIs				
Group 3	Lud, Mus, Sau	Among populations within groups	23.921	0.09	0.01383
Group 4	NMB, BBN, CR				
Group 5	ER	Within populations	9976.859	99.05	<0.00001
<b>(c) Six-group AMOVA</b>					
Group 1	Epo, Nau	Among groups	82.712	0.81	<0.00001
Group 2	TB, HgIs				
Group 3	Lud, Mus, Sau	Among populations within groups	17.631	0.04	0.17579
Group 4	NMB, CR				
Group 5	ER	Within populations	9973.859	99.15	<0.00001
Group 6	BBN				

genetic variance (97.86%–98.05%) was attributed to the differences among individuals within populations. The four- and five-group AMOVAs indicated that a significant portion of genetic variation was found within groups, indicating that further partitioning was needed. The six-group AMOVA exhibited nonsignificant within-group genetic variance (0.04% of variance,  $p = 0.17579$ ; Table 4c), resulting in the recognition of six putative MUs of lake whitefish in Lake Michigan: the North and Moonlight bays MU (NMB; NMB and Cedar River); the Big Bay de Noc MU (BBN; comprised solely of the Big Bay de Noc population); the Northern MU (NOR; including Naubinway and Epoufette); the Northeast MU (NOE; including Traverse Bay and Hog Island); the Elk Rapids MU (EKR; comprised solely of the Elk Rapids population); and the Southeastern MU (SOE; including Ludington, Muskegon, and Saugatuck). Pairwise  $F_{st}$  comparisons of within-MUs population pairs resulted in only the grouping of Hog Island and Traverse Bay (NOE) showing significant differences ( $p \leq 0.0001$ ). All other within-MUs  $F_{st}$  comparisons yielded nonsignificant  $p$  values (Table 5).

### Structure

No apparent structure was detected using the Bayesian clustering algorithm within the program STRUCTURE (Pritchard et al. 2000). All simulations indicated that the most likely number of genetic units in our sample was one. The estimated log probability of the data,  $\ln P(D)$ , decreased with every increase in  $K$  from 1 to 15, ranging from  $-46\,564$  ( $K = 1$ ) to  $-49\,704$  ( $K = 15$ ). We used the  $\Delta K$  of Evanno et al. (2005) and still failed to detect a  $K > 1$  in all analyses. Examination of individual simulation  $Q$ -value plots showed no apparent trend or consistent pattern of resolution among all simulations.

### Isolation by distance

Results from the test of IBD indicated that lake whitefish in Lake Michigan exhibit IBD. This IBD was deemed sig-

nificant by a Mantel test ( $p = 0.001$ ,  $z = 169.8$ ,  $r = 0.4237$ ,  $R^2 = 0.179$ ).

### Discussion

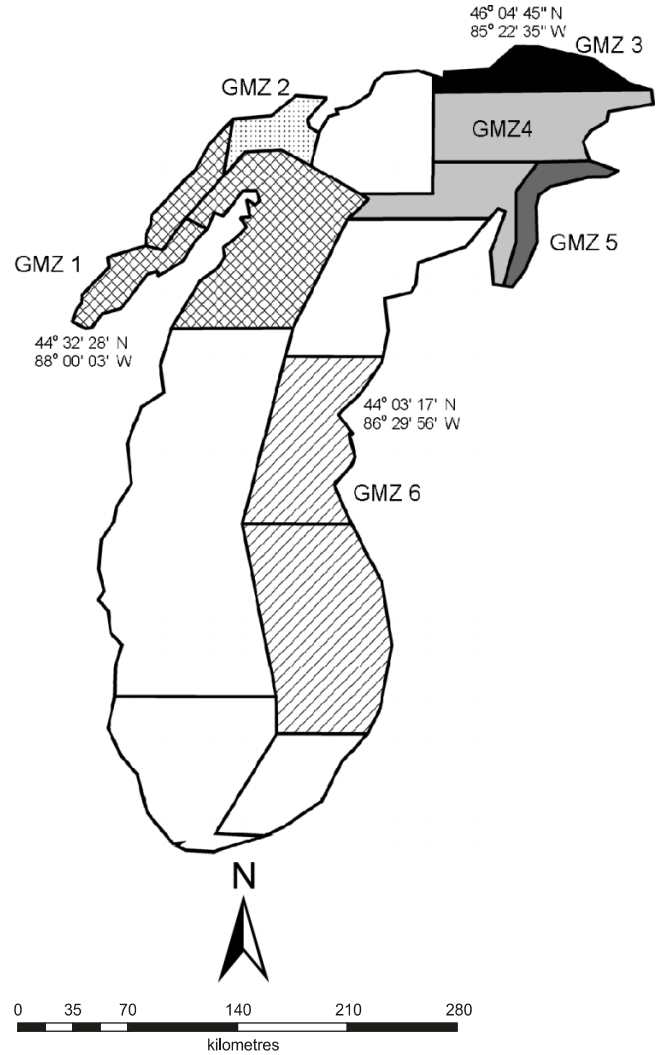
The initial phase of the genetic stock identification process indicated that Lake Michigan's lake whitefish spawning aggregates did not constitute one panmictic group and that population structuring existed. These findings were anticipated based on the tendency of philopatric fish species to show intraspecific population differentiation (Stepien and Faber 1998; Markert et al. 1999; Duftner et al. 2006), previous population dynamics studies (Rowe 1984; Walker et al. 1993), tagging studies (Ebener and Copes 1985; Scheerer and Taylor 1985), and genetic studies (Imhoff 1977; Leary 1979; Imhoff et al. 1980) conducted on lake whitefish throughout Lake Michigan. For comparison, Scheerer and Taylor (1985) used tag returns and vital statistics (e.g., mortality rates) to suggest at least three discrete stocks of lake whitefish in northeastern Lake Michigan. Additionally, Imhoff (1977) suggested that two distinct lake whitefish populations existed in the Green Bay region of Lake Michigan, one in the Big Bay de Noc area of northern Green Bay and one on the lake side of the Door County Peninsula (Wisconsin) encompassing the spawning samples from North and Moonlight bays. Furthermore, the grouping of NMB and CR fish into one MU was supported by tagging data indicating that NMB fish comprised a large majority of the spawning fish at CR (Rowe 1984). However, further research should be done to evaluate why the CR aggregate had such a large number of private alleles.

Cluster analyses based on  $D_c$  showed consistent groupings of geographically proximal aggregates. These geographic clusters were supported by data from several tagging studies conducted within Lake Michigan (Ebener and Copes 1985; Scheerer and Taylor 1985; Walker et al. 1993). The six recovered MUs showed no significant within-group molecular

**Table 5.** Pairwise  $F_{st}$  values (above diagonal) and their corresponding  $p$  values (below diagonal).

	Epo	Nau	TB	ER	HgIs	Sau	Mus	Lud	CR	NMB	BBN
Epo											
Nau	0.9990										
TB	0.0010	0.0039									
ER	<0.0001	<0.0001	0.0537								
HgIs	0.0537	0.8193	<0.0001	<0.0001							
Sau	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001						
Mus	<0.0001	<0.0001	<0.0001	<0.0001	0.0645	0.0020					
Lud	<0.0001	0.0098	<0.0001	0.2734	0.9990	0.7100					
CR	<0.0001	0.0195	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001				
NMB	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0889			
BBN	<0.0001	0.0205	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.4336	0.0010		

**Fig. 3.** Six genetic management zones (GMZs) based on genetic stock identification and spawning site locations, overlaid on the statistical and commercial management zones currently in place for Lake Michigan: GMZ 1, NMB MU; GMZ 2, BBN MU; GMZ 3, NOR MU; GMZ 4, NOE MU; GMZ 5, EKR MU; and GMZ 6, SOE MU.



variance, according to AMOVA, contained geographically proximate aggregates, and were supported by previous tagging, population dynamics, and genetic studies. Only the Northeast MU (Hog Island and Traverse Bay) exhibited genetic heterogeneity between its two component aggregates, despite their consistent resolution as a MU. These data ( $F_{st}$ ) suggested that gene flow occurred more readily between the Hog Island population and the NOR MU than between TB and the NOR MU. Scheerer and Taylor (1985) hypothesized that a shallow reef extending westward from the Waughoshance Point through the northern Beaver Islands (Hog Island) acted as a barrier to the southward movement of the NOR populations. The AMOVA results supported their findings of restricted gene flow (i.e., NOR and NOE populations were separate), but the  $F_{st}$  results suggested that significant gene flow exists across this potential barrier because Hog Island was not significantly different than the two NOR populations. If this reef is an impediment but not a barrier to



migration, significant gene flow between the NOR populations and Hog Island would be plausible. The rarity of this migration could be sufficient to explain no observed connectivity between the Traverse Bay and the NOR populations based on a stepping-stone migration model (Kimura and Weiss 1964). Despite the apparent gene flow between Hog Island and the NOR populations and the significant  $F_{st}$  value between Hog Island and Traverse Bay, the AMOVA and NJ ( $D_c$ ) results supported the grouping of Hog Island and Traverse Bay populations into one MU. Regardless of the MU into which Hog Island was placed, this was the only observable conflict between the various analyses used to assess genetic structure. Subsequent work should aim to increase the sample size of both Hog Island ( $N = 70$ ) and Traverse Bay ( $N = 74$ ) to include a minimum of 100 samples (consistent with other sample sizes) and to ensure no significant impact from only a single year sample being included in the study.

The identity of six genetic MUs of lake whitefish in Lake Michigan does not preclude gene flow among the groups. Several analyses indicated that gene flow was predominantly between neighboring populations, with less gene flow occurring between more distant populations. Wright (1943) theorized that isolation by distance results from less mixing among individuals, or pairs of populations, situated further apart than among those separated by shorter distances, a phenomenon exhibited by many species world-wide (Pogson et al. 2001; Turgeon and Bernatchez 2001; Planes and Fauvelot 2002). Thus, migrant lake whitefish are more likely to spawn on more geographically proximate spawning grounds than on spawning grounds on the other side of the lake. The clustering of geographically proximate aggregates into MUs based on genic differentiation, genetic distance, AMOVA,  $F_{st}$ , and a significant test of IBD (Mantel test) all supported Wright's (1943) theory of isolation by distance.

### Bayesian estimates of the number of genetic units

Contrary to the results from the genetic stock identification portion of this research, the Bayesian clustering program STRUCTURE did not indicate that genetic structure existed among Lake Michigan's lake whitefish aggregates. We suggest that the lack of recognized structure using the Bayesian algorithm in STRUCTURE was primarily the result of two factors. First, the number of spawning aggregates and their autocorrelation poses a challenge to STRUCTURE's Bayesian algorithm for detecting the number of genetic units. Pritchard et al. (2000) showed that STRUCTURE could easily detect two to four highly differentiated populations; however, little is known about its ability to provide accurate estimates of  $L(K)$  when more than four populations are included (Evanno et al. 2005). Pritchard et al. (2007) further explained that the underlying Bayesian model is not well suited to handle data from aggregates exhibiting IBD, and that when IBD is present, the inferred  $K$  values and the corresponding allele frequencies for each group can be arbitrary. Schwartz and McKelvey (2008) recommended that prior to analyzing data for population structure, one should determine if patterns of local autocorrelation exist (i.e., isolation by distance) as these are likely to confuse programs such as STRUCTURE. The second problem with using a Bayesian clustering algorithm for detecting the number of genetic units in our data was the

low levels of  $F_{st}$  observed among populations in this study (mean = 0.009). Latch et al. (2006) used simulated data to show that  $F_{st} \leq 0.02$  among genetic units resulted in a failure of Bayesian clustering algorithms in both STRUCTURE and BAPS (Corander et al. 2006) to accurately predict the number of genetic units present. Therefore, we concluded the STRUCTURE results did not necessarily conclude that no structure is present, just that the data that we have do not lend themselves to analysis in this manner. Further, analysis of genetic structure of coregonids in large lakes with apparent moderate to high levels of gene flow would further benefit from applying additional genetic analysis that would both take into consideration geographic location of each fish and handle  $F_{st}$  estimates lower than 0.05.

### Levels of genetic differentiation

Despite moderately low  $F_{st}$  values between lake whitefish MUs in Lake Michigan (0.0001 to 0.0231), these values were similar to those found to differentiate stocks or morphs in other intralacustrine studies of coregonids. For example, Østbye et al. (2005) found  $F_{st}$  values between morphs of European whitefish (*Coregonus lavaretus*) ranging from 0.008 to 0.1530, with significant differences found at  $F_{st}$  values as low as 0.013. In another study comparing intralacustrine morphs of European whitefish, Østbye et al. (2006) also found significant differences at  $F_{st}$  values ranging from 0.010 to 0.075. Bernard (2006) suggested that  $F_{st}$  values ranging from 0.005 to 0.020 were sufficient to differentiate lake whitefish spawning aggregates in Lake Ontario. The  $F_{st}$  values found in this study were slightly lower than those of previous studies of lake whitefish with the same mtDNA lineage (0.01 to 0.084) but were substantially lower than those from different mtDNA lineages (Bodaly et al. 1992; Lu and Bernatchez 1999). These findings are corroborated by those of Bernatchez and Dodson (1991), who found that lake whitefish in the North American Great Lakes belong to the same mtDNA lineage. Levels of genetic differentiation of Lake Michigan lake whitefish stocks were similar to or greater than those found between morphologically different ciscos (*Coregonus* spp.) in Lake Nipigon (Turgeon et al. 1999). In an interlacustrine comparison of two populations from the North American Upper Great Lakes (Lakes Superior and Huron), Stott et al. (2004) found an  $F_{st}$  value of 0.031. In another interlacustrine comparison of coregonids, Douglas et al. (1999) found a wide range of  $F_{st}$  values (0.001 to 0.253).

Two MUs were formed by single spawning aggregates, with Big Bay de Noc and Elk Rapids showing genetic isolation from other surrounding populations based on all genetic analyses conducted in this study. Tagging data indicated that the BBN spawning aggregate exhibits high spawning-site fidelity (M.P. Ebener, Chippewa Ottawa Resource Authority, 179 W Three Mile Road, Sault Ste. Marie, MI 49783, USA, personal communication) that, if coupled with little immigration from neighboring populations, would result in increased genetic divergence of BBN compared with neighboring lake whitefish spawning aggregates.

The reduced levels of gene flow between neighboring spawning aggregates and the Elk Rapids aggregate agreed with the findings of Walker et al. (1993), who reported that little mixing, if any, occurred between fish from the west

side of Grand Traverse Bay (Traverse Bay population) and fish from the east side of Grand Traverse Bay (Elk Rapids) due to a potential thermal barrier to gene flow. The outer bay (area directly north of Old Mission Point, the peninsula separating west bay from east bay) is divided by a deep trough (>75 m) that may thermally separate the west bay (Traverse Bay) from the east bay (Elk Rapids; Walker et al. 1993). Thermal barriers have been suggested to potentially segregate stocks of lake whitefish in Lake Huron (Caselman et al. 1981), but further research is needed to determine the true effects of thermal barriers on population structuring. Additionally, depths on the Elk Rapids side of Grand Traverse Bay exceed 150 m compared with depths on the Traverse Bay side that reach slightly over 100 m (National Oceanic and Atmospheric Administration 2007). These differences in depth may indicate two different habitat types, leading to the potential of genetic differentiation through local adaptation of these lake whitefish populations. Theoretical and empirical evidence have indicated that vertical temperature gradients in deep lakes along with differential habitat use could contribute to ecological segregation (Chouinard and Bernatchez 1998; Parker et al. 2001; Helland et al. 2007). These findings coupled with those of other studies of philopatric fish species (Stepien and Faber 1998; Markert et al. 1999; Duftner et al. 2006) and Elk Rapids' large number of private alleles have led us to conclude that Elk Rapids represents a genetically divergent stock reinforced by migratory restrictions or life history differences.

### Management implications

Based on the genetic management zones (GMZs) established through a combination of our proposed MUs and contemporary management zones (Fig. 3), the harvest from commercial and statistical management zones may need to be adjusted to properly conserve current genetic variation. Two GMZ boundaries corresponded directly with the statistical management zones currently in place, GMZ-2 corresponded with WFM-01 and GMZ-3 corresponded with WFM-03. Several of the GMZs encompassed multiple management zones. However, one management zone (WFM-05) may need to be split because of the presence of multiple genetic MUs within a single zone (EKR and NOE). When the goal of a commercial fishery is to optimize sustainable yield without disrupting the genetic characteristics and when multiple MUs exist within a single management zone, either the zone needs to be managed for the smallest MU present or the zone needs to be split to represent individual MUs.

### Future research

Tagging data have shown that following spawning, lake whitefish are highly vagile and disperse throughout Lake Michigan (Ebener and Copes 1985; Scheerer and Taylor 1985; Schneeberger et al. 2005), and evidence exists of a mixed-stock commercial harvest of lake whitefish in Lake Michigan in the form of tagged fish from GMZ 2 being harvested in GMZ 1 (different commercial management zones; Jones et al. 2008). However, current regulations are unable to account for the harvest of MUs outside of their management zone of origin during the nonbreeding season, necessitating a comprehensive mixed-stock analysis to ensure proper management of this economically and ecologically

important native species. Utter and Ryman (1993) laid out several major requirements necessary for genetic analysis of mixed-stock fisheries, and this research allows us to meet the first four requirements: (i) to have a reasonable understanding of the genetic populations potentially contributing to the commercial harvest, (ii) to be able to determine if there are genetic differences among these groups, (iii) to have the ability to identify differences among these groups, and (iv) to have reliable, multilocus baseline genetic data. The next logical step is to evaluate the utility of our data set for mixed-stock analysis and then perform a comprehensive, lake-wide, mixed-stock analysis to truly manage this fishery on a stock basis. Management of Lake Michigan's lake whitefish populations on a genetic basis not only may benefit the commercial fishery and help to ensure its sustainability, but also may benefit fisheries managers, fishers, and local and regional economies and preserve a long-standing tradition in the Great Lakes region.

### Acknowledgements

This research was funded solely by the Great Lakes Fishery Commission. We thank M. Ebener, E. Olsen, S. Lenart, A. Martell, P. Hanchin, C. Schelb, R. Claramunt, D. Dupras, P. Schneeberger, K. Koval, R. O'Neal, T. Kroeff, K. Royseck, M. Donofrio, D. Traynor, and E. Volkman for assistance with sample collection. We also thank the following commercial fishermen for their assistance in sample collection: D. Hickey, T. King, K. King, B. Petersen, B. Peterson, Weborgs, C. Henriksen, G. Ruleau, L. Barbeau, K. Frazier, E. John, and M. Hermes. We extend a special thanks to all of the graduate students at the University of Wisconsin – Stevens Point for their assistance in sample collection and processing. We thank B.D.S. Graeb, D.W. Willis, G.R. Moyer, M.A. Bozek, and two anonymous reviewers for their insightful comments on a previous draft of this manuscript. Use of trade names throughout the manuscript does not constitute endorsement by the USA, Michigan, or Wisconsin governments.

### References

- Bailey, R.M., and Smith, G.R. 1981. Origin and geography of the fish fauna of the Laurentian Great Lakes basin. *Can. J. Fish. Aquat. Sci.* **38**: 1539–1561. doi:10.1139/f81-206.
- Bernard, A. 2006. Cryptic structure and diversity of lake whitefish (*Coregonus clupeaformis*) in Ontario waters. M.Sc. thesis, University of Guelph, Guelph, Ont., Canada.
- Bernatchez, L., and Dodson, J.J. 1991. Phylogeographic structure in mitochondrial DNA of the lake whitefish (*Coregonus clupeaformis*) in North America and its relationship to Pleistocene glaciations. *Evolution*, **45**: 1016–1035. doi:10.2307/2409706.
- Bodaly, R.A., Clayton, J.W., Lindsey, C.C., and Vuorinen, J. 1992. Evolution of lake whitefish (*Coregonus clupeaformis*) in North America during the Pleistocene: genetic differentiation between sympatric populations. *Can. J. Fish. Aquat. Sci.* **49**: 769–779. doi:10.1139/f92-086.
- Booke, H.E. 1981. The conundrum of the stock concept — are nature and nurture definable in fishery science? *Can. J. Fish. Aquat. Sci.* **38**: 1479–1480. doi:10.1139/f81-200.
- Burger, C.V., Spearman, W.J., and Cronin, M. 1997. Genetic differentiation of sockeye salmon subpopulations from a geologically young Alaskan lake system. *Trans. Am. Fish. Soc.* **126**: 926–938. doi:10.1577/1548-8659(1997)126<0926:GDOSSS>2.3.CO;2.

- Casselman, J.M., Collins, J.J., Crossman, E.J., Ihssen, P.E., and Spangler, G.R. 1981. Lake whitefish (*Coregonus clupeaformis*) stocks of the Ontario waters of Lake Huron. *Can. J. Fish. Aquat. Sci.* **38**: 1772–1789. doi:10.1139/f81-225.
- Cavalli-Sforza, L.L., and Edwards, A.W.F. 1967. Phylogenetic analysis: models and estimation procedures. *Evolution*, **21**: 550–570. doi:10.2307/2406616.
- Chouinard, A., and Bernatchez, L. 1998. A study of trophic niche partitioning between larval populations of reproductively isolated whitefish (*Coregonus* sp.) ecotypes. *J. Fish Biol.* **53**: 1231–1242.
- Corander, J., Marttinen, P., and Mäntyniemi, S. 2006. Bayesian identification of stock mixtures from molecular marker data. *Fish. Bull. (Washington, D.C.)*, **104**: 550–558.
- Douglas, M.P., Brunner, P.C., and Bernatchez, L. 1999. Do assemblages of *Coregonus* (Teleostei: Salmoniformes) in the Central Alpine region of Europe represent species flocks? *Mol. Ecol.* **8**: 589–603. doi:10.1046/j.1365-294x.1999.00581.x.
- Duftner, N., Sefc, K.M., Koblmüller, S., Nevado, B., Verheyen, E., Phiri, H., and Sturmhuber, C. 2006. Distinct population structure in a phenotypically homogeneous rock-dwelling cichlid fish from Lake Tanganyika. *Mol. Ecol.* **15**: 2381–2395. doi:10.1111/j.1365-294X.2006.02949.x. PMID:16842413.
- Ebener, M.P., and Copes, F.A. 1985. Population statistics, yield estimates, and management considerations for two lake whitefish stocks in Lake Michigan. *N. Am. J. Fish. Manage.* **5**: 435–448. doi:10.1577/1548-8659(1985)5<435:PSYEAM>2.0.CO;2.
- Evanno, G., Regnaut, S., and Goudet, J. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**: 2611–2620. doi:10.1111/j.1365-294X.2005.02553.x. PMID:15969739.
- Excoffier, L., Smouse, P.E., and Quattro, J.M. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics*, **131**: 479–491. PMID:1644282.
- Excoffier, L., Laval, G., and Schneider, S. 2005. ARLEQUIN, version 3.0: an integrated software package for population genetics data analysis. *Evol. Bioinform. Online*, **1**: 47–50.
- Felsenstein, J. 1993. Phylogeny inference package (PHYLIP). Ver. 3.5. University of Washington, Seattle, Washington.
- Goudet, J. 1995. Fstat version 1.2: a computer program to calculate *F*-statistics. *J. Hered.* **86**: 485–486.
- Guo, S.W., and Thompson, E.A. 1992. Performing the exact test of Hardy–Weinberg proportions for multiple alleles. *Biometrics*, **48**: 361–372. doi:10.2307/2532296. PMID:1637966.
- Hedrick, P.W. 2000. *Genetics of populations*. Jones and Bartlett Publishers, Boston, Mass.
- Helland, I.P., Freyhof, J., Kasprzak, P., and Mehner, T. 2007. Temperature sensitivity of vertical distributions of zooplankton and planktivorous fish in a stratified lake. *Oecologia*, **151**: 322–330. doi:10.1007/s00442-006-0541-x. PMID:17024386.
- Henegariu, O., Heerema, N.A., Dlouhy, S.R., Vance, G.H., and Vogt, P.H. 1997. Multiplex PCR: critical parameters and step-by-step protocol. *BioTechniques*, **23**: 504–511. PMID:9298224.
- Imhoff, M.A. 1977. Population genetic structure of lake whitefish, *Coregonus clupeaformis*, in Green Bay and northern Lake Michigan, as assessed by electrophoresis of lactate, glycerol-3-phosphate, and malate dehydrogenase isozymes. M.Sc. thesis, College of Natural Resources, University of Wisconsin – Stevens Point, Stevens Point, Wisc.
- Imhoff, M.A., Leary, R.F., and Booke, H.E. 1980. Population or stock structure of lake whitefish (*Coregonus clupeaformis*) in northern Lake Michigan as assessed by isozyme electrophoresis. *Can. J. Fish. Aquat. Sci.* **37**: 783–793. doi:10.1139/f80-105.
- Jensen, J.L., Bohonak, A.J., and Kelley, S.T. 2005. Isolation by distance, web service. Ver. 3.14. *BMC Genetics*, **6**: 13. Available from [ibdws.sdsu.edu/](http://ibdws.sdsu.edu/).
- Jones, M.L., Arts, M.T., Faisal, M., Ebener, M.P., Wagner, T., Brenden, T.O., Honeyfield, D., and Wright, G. 2008. Magnitude and potential causes of mortality in four lake whitefish populations in Lakes Michigan and Huron: a multidisciplinary approach. Great Lakes Fishery Trust Project Completion Report, Lansing, Mich. Project No. 2003-06.
- Kalinowski, S.T. 2005. HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. *Mol. Ecol.* **5**: 187–189. doi:10.1111/j.1471-8286.2004.00845.x.
- Kimura, M., and Weiss, G.H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**: 561–576. PMID:17248204.
- Kinnunen, R.E. 2003. Great Lakes Commercial Fisheries. Great Lakes Fisheries Leadership Institute Technology Report. Available from [www.miseagrant.umich.edu/downloads/fisheries/GLCommercialFinal.pdf](http://www.miseagrant.umich.edu/downloads/fisheries/GLCommercialFinal.pdf) [accessed 10 August 2005].
- Latch, E.K., Dharmarajan, G., Glaubitz, J.C., and Rhodes, O.E. 2006. Relative performance of Bayesian clustering software for inferring population substructure and individual assignment at low levels of population differentiation. *Conserv. Genet.* **7**: 295–302. doi:10.1007/s10592-005-9098-1.
- Leary, R. 1979. Population or stock structure of lake whitefish, *Coregonus clupeaformis*, in northern Lake Michigan as assessed by isozyme electrophoresis. M.Sc. thesis, College of Natural Resources, University of Wisconsin – Stevens Point, Stevens Point, Wisc.
- Leberg, P.L. 2002. Estimating allelic richness: effects of sample size and bottlenecks. *Mol. Ecol.* **11**: 2445–2449. doi:10.1046/j.1365-294X.2002.01612.x. PMID:12406254.
- Liu, K., and Muse, S.V. 2005. PowerMarker: integrated analysis environment for genetic marker data. *Bioinformatics (Oxford)*, **21**: 2128–2129. doi:10.1093/bioinformatics/bti282.
- Lu, G., and Bernatchez, L. 1999. Correlated trophic specialization and genetic divergence in sympatric lake whitefish ecotypes (*Coregonus clupeaformis*): support for the ecological speciation hypothesis. *Evolution*, **53**: 1491–1505. doi:10.2307/2640895.
- Lu, G., Basley, D.J., and Bernatchez, L. 2001. Contrasting patterns of mitochondrial DNA and microsatellite introgressive hybridization between lineages of lake whitefish (*Coregonus clupeaformis*): relevance for speciation. *Mol. Ecol.* **10**: 965–985. doi:10.1046/j.1365-294X.2001.01252.x. PMID:11348504.
- Mantel, N. 1967. The detection of disease clustering and generalized regression approach. *Cancer Res.* **27**: 209–220. PMID:6018555.
- Markert, J.A., Arnegard, M.E., Danley, P.D., and Kocher, T.D. 1999. Biogeography and population genetics of the Lake Malawi cichlid *Melanochromis auratus*: habitat transience, philopatry and speciation. *Mol. Ecol.* **8**: 1013–1026. doi:10.1046/j.1365-294x.1999.00658.x.
- Miller, C.R., Joyce, P., and Waits, L.P. 2002. Assessing allelic dropout and genotype reliability using maximum likelihood. *Genetics*, **160**: 357–366. PMID:11805071.
- Moritz, C. 1994. Defining ‘evolutionarily significant units’ for conservation. *Trends Ecol. Evol.* **9**: 373–375. doi:10.1016/0169-5347(94)90057-4.
- National Oceanic and Atmospheric Administration. 2007. Bathymetry of Lake Michigan. Available from [www.ngdc.noaa.gov/mgg/greatlakes/lakemich\\_cdrom/images/area7hi.gif](http://www.ngdc.noaa.gov/mgg/greatlakes/lakemich_cdrom/images/area7hi.gif) [accessed 10 March 2007].
- Navidi, W., Arnheim, N., and Waterman, M.S. 1992. A multiplex approach for accurate genotyping of very small DNA sam-

- ples by using PCR: statistical considerations. *Am. J. Hum. Genet.* **50**: 347–359. PMID:1734715.
- Østbye, K., Næsje, T.F., Bernatchez, L., Sandlund, O.T., and Hindar, K. 2005. Morphological divergence and origin of sympatric populations of European whitefish (*Coregonus lavaretus* L.) in Lake Femund, Norway. *J. Evol. Biol.* **18**: 683–702. doi:10.1111/j.1420-9101.2004.00844.x. PMID:15842498.
- Østbye, K., Amundsen, P.A., Bernatchez, L., Klemetsen, A., Knudsen, R., Kristoffersen, Næsje, T.F., and Hindar, K. 2006. Parallel evolution of ecomorphological traits in the European whitefish *Coregonus lavaretus* (L.) species complex during postglacial times. *Mol. Ecol.* **15**: 3983–4001. doi:10.1111/j.1365-294X.2006.03062.x.
- Page, R.D.M. 1996. TREEVIEW: an application to display phylogenetic trees on personal computers. *Comp. Appl. Biosci.* **12**: 357–358. PMID:8902363.
- Pamilo, P., and Varvio-Aho, S. 1984. Testing genotype frequencies and heterozygosities. *Mar. Biol. (Berl.)*, **79**: 99–100. doi:10.1007/BF00404990.
- Parker, H.H., Noonburg, E.G., and Nisbet, R.M. 2001. Models of alternative life-history strategies, population structure and potential speciation in salmonid fish stocks. *J. Anim. Ecol.* **70**: 260–272. doi:10.1046/j.1365-2656.2001.00488.x.
- Patton, J.C., Galloway, B.J., Fechhelm, R.G., and Cronin, M.A. 1997. Genetic variation of microsatellite and mitochondrial DNA genetic markers in broad whitefish (*Coregonus nasus*) in the Colville and Sagavanirktok rivers in northern Alaska. *Can. J. Fish. Aquat. Sci.* **54**: 1548–1556. doi:10.1139/cjfas-54-7-1548.
- Petit, R., El Mousadik, A., and Pons, O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conserv. Biol.* **12**: 844–855. doi:10.1046/j.1523-1739.1998.96489.x.
- Planes, S., and Fauvelot, C. 2002. Isolation by distance and vicariance drive genetic structure of a coral reef fish in the Pacific Ocean. *Evolution*, **56**: 378–399. PMID:11926506.
- Pogson, G.H., Taggart, C.T., Mesa, K.A., and Boutillier, R.G. 2001. Isolation by distance in the Atlantic cod, *Gadus morhua*, at large and small geographic scales. *Evolution*, **55**: 131–146. PMID:11263734.
- Pritchard, J.K., Stephens, P., and Donnelly, P. 2000. Inference of population structure using multilocus genotype data. *Genetics*, **155**: 945–959. PMID:10835412.
- Pritchard, J.K., Wen, X., and Falush, D. 2007. Documentation for *structure* software. Ver. 2.2. Available from pritch.bsd.uchicago.edu/software [accessed 15 July 2008].
- Raymond, M., and Rousset, F. 1995. An exact test for differentiation. *Evolution*, **49**: 1280–1283. doi:10.2307/2410454.
- Rice, W.R. 1989. Analyzing tables of statistical tests. *Evolution*, **43**: 223–225. doi:10.2307/2409177.
- Rogers, S.M., Marchand, M.H., and Bernatchez, L. 2004. Isolation, characterization and cross-salmonid amplification of 31 microsatellite loci in the lake whitefish (*Coregonus clupeaformis*, Mitchell). *Mol. Ecol.* **4**: 89–92. doi:10.1046/j.1471-8286.2003.00578.x.
- Rowe, M. 1984. Population dynamics of lake whitefish in the Big Bay de Noc, Bark and Cedar Rivers, and Portage Bay areas of Lake Michigan. M.Sc. thesis, College of Natural Resources, University of Wisconsin – Stevens Point, Stevens Point, Wisc.
- Saitou, N., and Nei, M. 1987. The neighbor-joining method: a method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425. PMID:3447015.
- Scheerer, P.D., and Taylor, W.W. 1985. Population dynamics and stock differentiation of lake whitefish in northeastern Lake Michigan with implications for their management. *N. Am. J. Fish. Manage.* **5**: 526–536. doi:10.1577/1548-8659(1985)5<526:PDASDO>2.0.CO;2.
- Schneeberger, P.J., Ebener, M.P., Toney, M., and Peeters, P.J. 2005. Status of lake whitefish in Lake Michigan. *Gt. Lakes Fish. Comm. Tech. Rep.* **66**: 67–86.
- Schwartz, M.K., and McKelvey, K.S. 2008. Why sampling scheme matters: the effect of sampling scheme on landscape genetic results. *Conserv. Genet.* doi:10.1007/s10592-008-9622-1.
- Shaklee, J.B., and Currens, K.P. 2003. Genetic stock identification and risk assessment. *In* Population genetics: principles and applications for fisheries scientists. Edited by E.M. Hallerman. American Fisheries Society, Bethesda, Md. pp. 291–328.
- Stepien, C.A., and Faber, J.E. 1998. Population genetic structure, phylogeography and spawning philopatry in walleye (*Stizostedion vitreum*) from mitochondrial DNA control region sequences. *Mol. Ecol.* **7**: 1757–1769. doi:10.1046/j.1365-294x.1998.00512.x. PMID:9859203.
- Stott, W., Todd, T.N., and Kallemeyn, L. 2004. Genetic variability among lake whitefish from Isle Royale and the upper Great Lakes. *Ann. Zool. Fenn.* **41**: 51–59.
- Turgeon, J., and Bernatchez, L. 2001. Clinal variation at microsatellite loci reveals historical secondary intergradation between glacial races of *Coregonus artedii* (Teleostei: Coregoninae). *Evolution*, **55**: 2274–2286. PMID:11794787.
- Turgeon, J., Estoup, A., and Bernatchez, L. 1999. Species flock in the North American Great Lakes: molecular ecology of Lake Nipigon ciscoes (Teleostei: Coregonidae: *Coregonus*). *Evolution*, **53**: 1857–1871. doi:10.2307/2640446.
- Utter, F., and Ryman, N. 1993. Genetic markers and mixed stock fisheries. *Fisheries*, **18**: 11–21. doi:10.1577/1548-8446(1993)018<0011:GMAMSF>2.0.CO;2.
- Van Den Avyle, M.J. 1993. Dynamics of exploited fish populations. *In* Inland fisheries management in North America. Edited by C.C. Kohler and W.A. Hubert. American Fisheries Society, Bethesda, Md. pp. 105–134.
- Walker, S.H., Prout, M.W., Taylor, W.W., and Winterstein, S.R. 1993. Population dynamics and management of lake whitefish stocks in Grand Traverse Bay, Lake Michigan. *N. Am. J. Fish. Manage.* **13**: 73–85. doi:10.1577/1548-8675(1993)013<0073:PDAMOL>2.3.CO;2.
- Waples, R.S., and Gaggiotti, O. 2006. What is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Mol. Ecol.* **15**: 1419–1439. doi:10.1111/j.1365-294X.2006.02890.x. PMID:16629801.
- Waples, R.S., Teel, D.J., Myers, J.M., and Marshall, A.R. 2004. Life-history divergence in chinook salmon: historic contingency and parallel evolution. *Evolution*, **58**: 386–403. doi:10.1554/03-323. PMID:15068355.
- Weir, B.S., and Cockerham, C.C. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution*, **38**: 1358–1370. doi:10.2307/2408641.
- Wilmot, R.L., and Burger, C.V. 1985. Genetic differences among populations of Alaskan sockeye salmon. *Trans. Am. Fish. Soc.* **114**: 236–243. doi:10.1577/1548-8659(1985)114<236:GDAPAO>2.0.CO;2.
- Wright, S. 1931. Evolution in Mendelian populations. *Genetics*, **16**: 97–159. PMID:17246615.
- Wright, S. 1943. Isolation by distance. *Genetics*, **28**: 114–138. PMID:17247074.