

## FINAL REPORT

LEPF 00-15

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### **Genetic Markers for Defining and Evaluating Stock Structure of Yellow Perch in Lake Erie**

**Submitted by Carol A. Stepien, Ph.D.**

#### **Overview**

The purpose of the investigation was to assess and analyze the population genetic stock structure of the yellow perch *Perca flavescens* across Lake Erie, in context of genetic variability patterns across its native range - including the other Great Lakes and isolated areas along the Atlantic coast. Yellow perch is a primary sport fish in Lake Erie and is commercially fished in its Canadian waters. Fishery managers increasingly have outlined the necessity to identify differences among yellow perch stocks and to conserve local adaptations in effective management practices. Yellow perch have supported a strong Lake Erie fishery since 1900 or earlier. Peak harvests occurred from 1928-35 and the mid-1950s to the mid-1970s, coinciding with low numbers of walleye. Yellow perch were abundant during the 1980s and declined markedly in the 1990s, which has been attributed to fluctuating nutrient (phosphorous) levels, recruitment failures, overexploitation, and/or competition with the exotic white perch (not a close relative). Interagency cooperation has since reduced exploitation and sought to protect spawning groups, which has increased stocks and harvests. We developed this genetic stock structure project in conjunction with the Lake Erie Yellow Perch Task Force, and have regularly met with them to share the results and to apply use of these genetic data in their stock structure models.

The present investigation analyzed genetic variation patterns of yellow perch within and among spawning locations around Lake Erie, including the western, central, and eastern basins. Sampling was done by agencies in Ohio, Pennsylvania, New York, Michigan, and Ontario in the context of their fishery monitoring programs. Outgroup populations were analyzed in order to place the genetic variation patterns in the context of phylogeographic structure originating from two primary glacial refugia. The two outgroup areas included representatives from the Mississippi refugium group from Wisconsin Lakes, Lake Michigan, and Lake Ontario, in addition to Lake Erie; and samples from Atlantic coastal refugium group, including Maine, North Carolina, and Alabama. The entire mitochondrial DNA control region was PCR amplified and sequenced, comprising 912 bp.

Our mtDNA control region sequence data revealed greater genetic diversity in Lake Erie than was found in other studies of allozymes and mtDNA RFLPs. We found that Lake Erie and other Great Lakes populations of yellow perch have lower levels of genetic diversity overall than do walleye, which may be due to their "boom and bust" history. Eleven mtDNA control region haplotypes have been identified to date in Lake Erie, with one haplotype dominating across the Lake (comprising 80% of the Lake samples). Results showed greater genetic variation within

the samples from the Minnesota Lakes, as well as for the Atlantic coastal samples from Maine and North Carolina. Significant genetic divergences among samples were identified for some spawning sites in Lake Erie, as well as between the Great Lakes region (Lakes Erie and Ontario and Minnesota) versus the eastern population sites (Maine, North Carolina, and Georgia). The primary genetic divergence between the Great Lakes region and the Atlantic region is equivalent to about 300,000 years, and appears to correspond to historic separation patterns in Mississippi and Atlantic glacial refugia during the mid-Pleistocene Epoch. We are continuing this project with new Sea Grant funding at our new laboratory in the Lake Erie Center at the University of Toledo; using nuclear microsatellite DNA analyses to compare with these mtDNA control region sequence data results.

### **Benefits to Lake Erie**

This research specifically addresses the Fishery Research Priorities for the Great Lakes “Basinwide Priorities” specified by the Great Lakes Fishery Commission for Native Species Rehabilitation, Fish Habitat Assessment, and Development of New Fish Sampling Tools. This project meets objectives of the Lake Erie Yellow Perch Task Group, was specifically developed with them, and they have continually used our results in their management plan for the species. We worked extensively with the Ohio Division of Wildlife throughout the project. We gave a workshop that was attended by the Ohio Fishery managers, students, and professors, during which we discussed this Yellow Perch project and made plans for the future. In addition, the research meets the “Great Lakes Fishery Resources Restoration Study Recommendations” for Establishing a comprehensive fishery database, Identifying significant habitats, and Fish genetics. The results of this research are important to the Ohio and Great Lakes public in helping to preserve the native fishes of Lake Erie for the future.

Results were published by Ph.D. student Alex Ford and Dr. Carol Stepien, as "Genetic variation and spawning population structure in Lake Erie yellow perch, *Perca flavescens*: A comparison with a Maine population" In *Proceedings of Percis III, the 3rd International Symposium on Percid Fishes*, edited by T.P. Barry and J.A. Malison, University of Wisconsin Sea Grant Institute, Madison, Wisconsin in 2004. We currently are augmenting and revising another paper on the overall final results, to be submitted to the journal *Copeia* in fall 2004, “Population Genetic Structure of Yellow Perch (*Perca flavescens*) in the Great Lakes: A comparison with eastern North American populations”. This research project formed part of the Ph.D. dissertation work of Mr. Alex Ford, who is presently completing the sample sizes necessary for stock discrimination and comprehensive data analysis; increasing the same size in the *Copeia* paper draft by about 140 fish.

Dr. Stepien is continuing the Yellow Perch study at the Lake Erie Center of the University of Toledo, with postdoctoral research associate Dr. Rex Strange. Drs. Stepien and Strange will compare the results of the mtDNA control region sequencing study funded by the Lake Erie Protection Fund with new results from analysis of 15 microsatellite loci (using the same samples and more). The comparative study will yield a very powerful view of stock structure and population genetic patterns of yellow perch in Lake Erie and across the other Great Lakes.

### **Publications resulting from and crediting the Lake Erie Protection Fund study**

Ford, A.M. and C.A. Stepien. 2004. Genetic variation and spawning population structure in Lake Erie yellow perch, *Perca flavescens*: A comparison with a Maine population. In *Proceedings of Percis III, the 3rd International Symposium on Percid Fishes*, T.P. Barry and J.A. Malison, eds. University of Wisconsin Sea Grant Institute, Madison, WI. 131-2. (anonymously peer-reviewed)

Ford, A.M. and C.A. Stepien. 2005? Population Genetic Structure of Yellow Perch (*Perca flavescens*) in the Great Lakes: A comparison with eastern North American populations. In manuscript, To be submitted to *Copeia*, fall 2004.

### **New Funding to continue the genetic study begun through the Lake Erie Protection Fund study (in the context of a companion nuclear microsatellite DNA study):**

“Walleye and yellow perch stock structure in the Great Lakes: A high-resolution DNA data base for fishery management” funded by the NOAA Ohio Sea Grant Program to C.A. Stepien, 3 years, 2004-2007, \$180,000

### **Student Supported by the Lake Erie Protection Fund grant:**

Mr. Alex M. Ford, Ph.D. Candidate, Cleveland State University

### **Presentations from the Lake Erie Protection Fund results and crediting the Lake Erie Protection Fund (N=22):**

1. Ford, A.M. and C.A. Stepien. “Preliminary data for studying population genetics of yellow perch in Lake Erie”. Poster presentation at the *American Society of Ichthyologists and Herpetologists* annual meeting, June 2001, held at Pennsylvania State University, State College, PA
2. Above poster was again presented at the annual *Department of Biological, Geological, and Environmental Sciences, Cleveland State University* graduate student poster show in October 2001.
3. Above poster was again presented at the annual *Woodlake Field Station Conference*, in the Cuyahoga Valley National Park, October 2001.
4. Stepien, C.A. “Investigations of population genetic and systematic relationships among percid fishes using DNA sequences”, invited seminar presentation at *John Carroll University*. October 29, 2001.

5. Stepien, C. A. “Unlocking the mysteries of Lake Erie fishes and invasive species using DNA clues” hour-long research seminar presentation at *Stone Laboratory, Ohio State University*, August 1, 2002.
6. Stepien, C.A. “Unlocking the mysteries of Great Lakes Fishes and Exotic Invasions: DNA Clues”. *Beckman Conference at the University of Pittsburgh*. 45 –minute oral Presentation, August 28, 2002, Pittsburgh, PA
7. Ford, A.M. and C.A. Stepien. “MtDNA control region stock structure study of yellow perch in Lake Erie”. Poster presentation at the annual *Department of Biological, Geological, and Environmental Sciences, Cleveland State University* graduate student poster show in October 2002.
8. Above poster also was presented at the annual *Woodlake Field Station Conference*, in the Cuyahoga Valley National Park, October 2002.
9. Stepien, C.A. “Using DNA Data to Understand Fish Stock Structure in Lake Erie”, oral research presentation at Great Lakes Environmental Genetics Laboratory workshop to the *Ohio Division of Wildlife*, November 20, 2002.
10. Ford, A.M. and C.A. Stepien. “Population genetic stock structure of Yellow Perch in Lake Erie: preliminary data ”, oral research presentation at Great Lakes Environmental Genetics Laboratory workshop to the *Ohio Division of Wildlife*, November 20, 2002. presented by Alex Ford.
11. An updated version of the poster from #7 was presented at the annual Ohio Fish and Wildlife conference held in Columbus, Ohio on February 7, 2003.
12. Ford, A.M. and C.A. Stepien. “Status of population stock structure of yellow perch in Lake Erie. Presented to the *Lake Erie Yellow Perch Task Force* at their annual meeting on February 12, 2003.
13. Ford, A.M. and C.A. Stepien. “Status of population stock structure of yellow perch in Lake Erie. Presented by Alex Ford at the March 25, 2003 *Great Lakes Management* conference.
14. Ford, A.M. “Population genetics and stock management of yellow perch in Lake Erie” Oral presentation at *the Midwest Ecology and Evolution Conference* at the University of Akron, March 28-30, 2003.
15. Ford, A.M. and C.A. Stepien. “Genetic variation and spawning population structure in Lake Erie yellow perch, *Perca flavescens*: A comparison with a Maine population”. Oral research presentation given by Alex Ford at the *The 3rd International Symposium on Percid Fishes* held in Madison, Wisconsin in July 2003.

16. Ford, A.M. and C.A. Stepien. "Population genetic and phylogeographic relationships of yellow perch (*Perca flavescens*) in the Great Lakes". Poster Presentation defended by C. Stepien at the *American Fisheries Society* annual meeting in the invited symposium on Fishery genetics. Quebec City, Canada, August. 2003.
17. Above poster was then presented and defended by Alex Ford at the annual Department of *Biological, Geological, and Environmental Sciences, Cleveland State University* graduate student poster show in October 2003.
18. Ford, A.M. and C.A. Stepien. "Population genetic divergence of yellow perch in Lake Erie." Oral research presentation by Alex Ford at the annual *Woodlake Field Station Conference*, Cuyahoga Valley National Park held in October 2003.
19. Stepien, C.A. "Molecular genetics and systematic investigations of fishes, with comments on mentorship in science". Bowling Green University Marine Laboratory Alumni Symposium, October 2003. Oral presentation.
20. An updated version of the poster from #15 was presented at the annual Ohio Fish and Wildlife conference held in Columbus, Ohio on February 8, 2004.
21. Ford, A.M. and C.A. Stepien. "Update on Population Genetic Status of Yellow Perch in Lake Erie" *The Lake Erie Yellow Perch Task Force* meeting February. 2004.
22. Ford, A.M. and C.A. Stepien. "Population Genetic Structure of Yellow Perch (*Perca flavescens*) in the Great Lakes" oral research presentation by Alex Ford at the *American Society of Ichthyologists and Herpetologists* annual meeting held in Norman, Oklahoma, June 2004.

Appendix 1. Yellow Perch samples being completed summer-fall 2004 by Ph.D. student Alex Ford (to complete the LEPF study\*this work is being completed with discretionary funds to Dr. Carol Stepien) These will be added to the *Copeia* paper in progress

<u>Spawning site</u>			
<u>Location</u>	<u>Done</u>	<u>N total</u>	<u>Left</u>
Port Clinton (1-#2, 4-#12, 1-#17)	6	15	9
	(9 Already in progress)		
South Bass (1-#2, 1-#12)	2	15	13
	(10 Already in progress)		
Cedar Point (1-#2, 1-#4, 17-#12)	19	40	21
	(20 Already in progress)		
Sandusky (4-#12)	4	15	11
	(4 Already in progress)		
West Basin (all #12)	8	10	2

Total Western 82/70 (need 31)

Bring Cedar Point numbers up to 40.

Vermillion (3-#2, 7-#12)	10	15	5
Lorain (1-#11, 10-#12)	11	15	4
Cleveland (1-#2, 13-#12, 1-#16)	15	40	25
	(20 Already in progress)		
Fairport/Geneva (1-#2, 6 #12)	7	15	8
	(8 Already in progress)		
Ashtabula (2-#2, 1-#9, 7-#12)	10	10	0
Central Basin (all #12)	8	10	2

Total Central 89/85 (need 24)

Bring Cleveland numbers up to 40.

Presque Isle (1-#2, 2-#12)	3	6	3
(may need more)			
Erie (10-#12, 2-#13, 1-#14)	13	15	2
Dunkirk (1-#2, 1-#3, 37-#12, 1-#15)	40	40	0
Long Point Bay, ONT (1-#12)	1	15	14

(11 Already in progress)  
Total Eastern 68/62 (need 5)

<u>Outgroups</u>	<u>Done</u>	<u>N Total</u>	<u>Left</u>
Maine (5-#10, 3-#10a, 2-#10b)	10	15	5
Georgia (only have 12 individuals)	2	12	10
North Carolina	4	4	0
Lake Ontario	15	15	0
Minnesota	15	15	0
Lake Michigan	0	15	15
		(15 Already in progress)	

Bring Maine, Georgia, Minnesota, and Lake Ontario numbers up to 12-15 each.

**Appendix 2. Publications Resulting from the Yellow Perch Genetic Study funded by the Lake Erie Protection Fund.**

August 10, 2004 draft copy in preparation for the journal *Copeia*. Sample sizes are being increased significantly and are in process now. Submission planned for September 2004. We have added about 140 samples this summer to the study, and the results have not yet been analyzed. They will be added to this paper.

**Population Genetic Structure of Yellow Perch (*Perca flavescens*) in the Great Lakes:**

**A comparison with eastern North American populations**

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## ABSTRACT

Great Lakes populations of Yellow Perch, *Perca flavescens*, have fluctuated in recent years due to unstable recruitment patterns and exploitation. Analysis of Yellow Perch population genetic structure and variation across the native range may provide an important management tool for conserving genetic diversity, as well as help to interpret their phylogeographic history. The present study analyzed the population genetic structure of Yellow Perch using DNA sequence data from the entire mitochondrial DNA control region (912 base pairs) of spawning individuals from Lake Erie – including the western, central and eastern basins (N=30-60 per basin, totaling 157). Comparisons were made with population samples from Minnesota (N=15), Lake Ontario (N=15), Maine (N=10), North Carolina (N=4), and Georgia (N=2) in order to evaluate to compare their genetic diversity and phylogeographic divergence patterns. The data reveal a widespread haplotype of Yellow Perch in the Great Lakes region, comprising 80% of the individuals and indicating relatively low genetic diversity. A total of eleven different mtDNA haplotypes were identified from Lake Erie, in comparison with 2 in Minnesota, 2 in Lake Ontario, 3 in Maine, 2 in North Carolina, and a single haplotype in Georgia. Significant genetic divergences among samples were identified for some spawning sites in Lake Erie, as well as between the Great Lakes region (Lakes Erie and Ontario and Minnesota) versus the eastern population sites (Maine, North Carolina, and Georgia). The primary genetic divergence between the Great Lakes region and the Atlantic region is equivalent to about 300,000 years, and appears to correspond to historic separation patterns in Mississippi and Atlantic glacial refugia during the mid-Pleistocene Epoch.

Yellow Perch, *Perca flavescens* (Teleostei; Percidae), are believed to have recolonized the newly formed Great Lakes system about 10,000 years ago from two primary glacial refugia, the Mississippi River system and the Atlantic coastal drainage (Bailey and Smith 1981). The geographic center of the range of yellow perch and its largest population sizes occur in the lower Great Lakes, especially Lake Erie (Figure 1). Previous genetic investigations discerned divergence patterns within Great Lakes fish species that are believed to have resulted from colonization from the glacial refugia – with Lake Erie populations largely comprising Mississippi refugium descendants (especially in the western and central basins) with input from the Atlantic refugium in the east (Seeb et al. 1987, Billington and Hebert 1988, Bernatchez and Dodson 1991, Mandrak and Crossman 1992, Murdoch and Hebert 1997, Stepien and Faber 1998, Stepien et al. 2004a). Past allozyme and mtDNA restriction fragment length polymorphism genetic studies of Yellow Perch in the Great Lakes discerned low genetic diversity levels, which may be related to historically low variability in the Mississippi refugium populations (Billington 1993, Todd and Hatcher 1993) and/or to recent bottleneck losses due to exploitation (Strittholt et al. 1988). Further analysis of Yellow Perch from other areas of the Great Lakes and throughout its historic range (Fig. 1) - as begun here - will resolve postglacial colonization patterns and biogeographic relationships, as well as help to interpret (and hopefully, preserve) what remains of its genetic diversity.

Genetic diversity has long been considered important to the ability of a population to withstand environmental changes (Moritz and Faith 1998). These alterations include natural and anthropogenic forces such as climate change, pollution, biological invasions, and exploitation. The Yellow Perch in the lower Great Lakes has endured most, if not all, of these types of changes to their environment. Fisheries exploitation since the early 1900's, severe pollution in the 1950's and 1960's, biological invasions by the round goby (*Neogobius melanostomus*) (Dillon and Stepien 1998), and possibly soon the Eurasian ruffe (*Gymnocephalus cernuus*) (Stepien et al. 1998, 2004b; Fullerton et al. 2000), as well as contemporary global climate shifts have all been factors in the sustainability of Yellow Perch for the past century alone.

Originally thought of as a second rate pan fish (Trautman 1981), the Yellow Perch plays an important role in not only the ecosystem of the Great Lakes, but also in the economy of the region. It supports both commercial and sport fisheries in the U.S. and Canada, bringing millions of dollars into the region every year (Morencie 2003). For example, in 2003, 4500 metric tonnes of Yellow Perch were harvested from the waters of Lake Erie by four U.S. states (Michigan, Ohio, Pennsylvania, and New York) and the Canadian province of Ontario (Lake Erie Commission news release April 5, 2004 via the Great Lakes Information Network: <http://www.great-lakes.net>). Fisheries management has contributed to the perseverance of this species in Lake Erie, as well as the other Great Lakes (Ryan et al. 2003). Notably, populations of Yellow Perch in Lake Erie have been rebounding after declining in the late 1980s, due to fishing regulations enacted to protect yearly recruitment (Ryan et al. 2003). However, the Lake

Erie population has yet to maintain a stable level and regain the numbers that were in existence prior to the 1980's.

At the request of the Lake Erie Yellow Perch Task Force (Departments of Natural Resources in Michigan and Ohio, Pennsylvania Fish and Boat Commission, New York State Department of Environmental Conservation, and the Ontario Ministry of Natural Resources), the objective of our present study was to analyze the current genetic stock structure and variability of Yellow Perch in Lake Erie and compare the historic genetic variation across their native North American range (Fig. 1A). *P. flavescens* is native to North America and its original range extended east of the Continental Divide from Great Slave Lake in the Northwest Territories of Canada south throughout Alberta, Saskatchewan, Ontario and to the Atlantic coast of Canada, into the northern and eastern states of the U.S. as far south as Georgia, Alabama and Florida (Scott and Crossman 1973, Boschung and Mayden 2004; see Fig 1). The Yellow Perch has been stocked extensively, and is now widely distributed west of the Continental Divide (Scott and Crossman 1973, Trautman 1981).

## METHODS

Tissue samples from 30-60 spawning individuals from each basin of Lake Erie (N=157 overall) (Fig. 1B) were collected by the Departments of Natural Resources in Ohio, Pennsylvania, and New York, and the Ontario Ministry of Natural Resources as part of their fishery monitoring programs. Fifteen samples from the two other primary Great Lakes region population areas – upper (Minnesota) and lower (Lake Ontario) were analyzed for comparison. In addition, samples from three Atlantic coastal region populations were compared, including northern (Maine, N=10), central (North Carolina, N=4), and southern sites (Georgia, N=2). Tissue samples were either preserved in 95% EtOH in the field or frozen after capture.

Latitude and longitude coordinates were recorded for each sampling location as well as length and sex data when available. Spawning sites sampled by agencies within the basins of Lake Erie included the following: West Basin; Port Clinton, OH (N 41° 31'10", W 82° 55'15"; 4-24-02, N = 6), South Bass Island, OH (N 41° 39'27", W 82° 49'20"; 5-1-93, N = 2; 4-20-01 N = 8; Total N = 10), Cedar Point, OH (N 41° 28'20", W 82° 39'30"; 4-15-02 N = 19), Sandusky, OH (N 41° 27'05", W 83° 02'54"; 9-30-93, N = 4), Central Basin; Vermilion, OH (N 41° 29'48", W 82° 22'02"; 5-1-02, N = 10), Lorain, OH (N 41° 32'17", W 82° 05'10"; 5-7-02, N = 11), Cleveland, OH (N 41° 30'02", W 81° 49'41"; 5-1-02, N = 15), Fairport, OH (N 41° 48'21", W 81° 25'04"; 5-19-03, N = 8), Geneva, OH (N 41° 57'2", W 80° 50'19"; 5-7-01, N = 7), Ashtabula, OH (N 41° 59'51", W 80° 35'23"; 6-25-02, N = 10), East Basin; Presque Isle, PA (N 42° 10'31", W 80° 06'44"; 7-10-97, N = 3), Erie, PA (N 42° 07'40", W 80° 16'10"; 6-1-97, N = 4, 6-5-01 N = 9; Total N = 13), Dunkirk, NY (N 42° 30'17", W 79° 20'2"; 7-7-97, N = 3; 4-27-01 N = 6, 4-30-01 N = 1; 5-2-01, N = 18; 5-8-01, N = 12; Total N = 40), and Long Point Bay, ONT (N 42° 38'40", W 80° 13'21"; 7-10-97, N = 1) See Fig. 1B.

The Minnesota sample group consisted of 15 individuals collected from various lakes in the central region of the state including: Green Lake (N 45° 14'13", W 94° 56'15"; 8-16-02, N=1), Florida Lake (N 45° 14'55", W 95° 02'45"; 8-16-02, N=3), Scandinavia Lake (N 45° 35'24", W 95° 58'13"; 9-6-02, N=4), Leaven Lake (N 45° 43'25", W 95° 17'07"; 8-30-02,

N=4), and Lake Mineworker (N 45° 35'24", W 95° 48'27"; 8-30-02, N=3). Lake Ontario samples were collected from the Rochester, NY area (N 43° 17'17", W 77° 08'28"; 7-16-02 N=15). In Maine, samples were collected from the Sebasticook River (N 44° 47'14", W 69° 22'53"; 5-27-98 N = 9) and Quantabacook Lake (N 44° 23'44", W 69° 11'05"; 4-7-97 N = 1; Total N = 10). The North Carolina samples were collected from Morgan Creek (N 35° 25'38", W 78° 58'29"; 3-14-03, N=4). Georgia samples were taken from the Chattahoochee River (N 31° 25'56", W 85° 03'39"; 4-5-02, N=2) (Fig. 1A).

The entire mtDNA control region (or D-loop), a non-coding portion of the mtDNA genome comprising 912 bp, was used to analyze the population structure of Yellow Perch following Faber and Stepien (1997). DNA was extracted from 25 mg of muscle or liver tissue using a Qiagen DNeasy Tissue Kit (69506 Qiagen Sciences Inc., Germantown, MD). Polymerase Chain Reaction (PCR) DNA amplification was performed using an Applied Biosystems GeneAmp PCR System 9700 thermalcycler (Applied Biosystems, Foster City, CA) with 40 cycles of 45 sec. denaturation at 92°C, 45 sec. anneal at 53°C, and 1.5 min. polymerization at 72°C; followed by a 5 min. extension step at 72°C. MtDNA primers used for PCR were: LW1-f (ACA CCA TAC ATC TAT ATT AAC C) (Gatt et al. 2000), 12Sar-h (ATA GTG GGG TAT CTA ATC CCA GTT) (Martin et al. 1992), and TI-f (TCA AAG CTT ACA CCA GTC TTG TAA ACC) (Kocher et al. 1989).

Sequencing was performed at the DNA core facility at Cleveland State University using a Beckman CEQ-8000 capillary sequencer (Beckman-Coulter, Inc., Fullerton, CA). DNA strands were sequenced separately in both directions (for independent verification) using the PCR primers LW1-f, HW1-r (GTC CCT CAC CTT CAA TAA CCG) (Gatt et al. 2000), and HN20-r (GTG TTA TGC TTT AGT TAA GC) (Bernatchez et al. 1992) and manufacturer's directions for cycle sequencing. MtDNA sequences were aligned and read using the Beckman-Coulter CEQ 8000 software package. Sequences were read in forward and reverse directions in overlapping sections, in order to lower the possibility of a polymerization-induced mutation and to corroborate substitutions. Haplotypes were determined by base pair changes in the sequence of the region and compared to previous results from prior studies by our laboratory (Faber and Stepien 1997).

Population genetic data were analyzed using Arlequin 2.2 (Schneider et al. 2003) and Mega 2.1 (Kumar et al. 2001) software packages in order to compare patterns of genetic divergence and diversity within and among sampling locations. Neighbor joining trees using Kimura (1980) 2-parameter genetic distances (to correct for unequal rates of transitions and transversions in mtDNA) and 1000 bootstrap replications, and maximum parsimony trees were constructed with the MEGA 2.1 software package (Kumar et al. 2001). In addition, a phylogeographic network diagram was constructed following Avise (2000). Genetic variability measures (calculated using Arlequin 2.2; Schneider et al. 2003), included haplotypic diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) and their standard errors (following Nei 1987). Analysis of Molecular Variance (AMOVA) and Wright's (1965)  $F$ -statistics in Arlequin 2.2 (Schneider et al. 2003) were used to analyze hierarchal genetic variation structure.

## RESULTS

Eleven mtDNA control region haplotypes of yellow perch (Table 1) were identified in Lake Erie (Table 2); as compared with only two in Minnesota, two in Lake Ontario, three in Maine, three in North Carolina, and a single type in Georgia (Table 3). In Lake Erie, some less-common haplotypes were exclusively found in each basin; including two in the western basin, two in the central basin, and five in the eastern basin (Table 2). The two most-common haplotypes (numbered 1 and 2 in Table 1) were shared among all three basins and were the most abundant types in Lake Erie - respectively numbering 135 (86%) and 12 (7%) of the total number of individuals sampled (Table 2). Both of these haplotypes also were found in the Minnesota lakes and haplotype 1 was very prevalent in Lake Ontario (Table 3).

None of the haplotypes found in the Great Lakes region were found in the Atlantic coastal sites (Maine, North Carolina or Georgia; Table 3). Haplotypes from Maine, North Carolina, and Georgia formed a clade distinguished from the Great Lakes types by 10 synapomorphies in the neighbor joining and maximum parsimony consensus trees (Fig. 2). The genetic network diagram (sensu Avise 2001) also revealed marked divergence among the Great Lakes and Atlantic coastal clades (Fig. 3). The data revealed a significant difference between the Great Lakes and Atlantic samples of yellow perch, equivalent to 69.5% of the variation in AMOVA (Analysis of Molecular Variance) and an  $F_{ST} = 0.67$ ,  $p < 0.0001$  indicating a very great genetic divergence level (Hartl 1988; Table 4 and Fig. 3).

The western basin samples of Lake Erie were dominated by haplotype 1 (comprising 87% of the sample; NCBI GenBank Accession number U90619 by Faber and Stepien 1997) and shared haplotype 2 (7%; NC BI GenBank Accession number XXXXX by Ford and Stepien 2004) (Table 2). Haplotype 1 also was predominant in the central and eastern basins of Lake Erie (83% and 88% respectively). However, haplotype 2 comprised a larger proportion of the central basin samples (11%), and the eastern basin contained a greater number of unique haplotypes (numbering 5) (Table 2). Only two haplotypes were found in the lakes of Minnesota, 1 (54%) and 2 (46%); with the latter comprising a greater proportion than in the lower Great Lakes. The two haplotypes were evenly spread throughout the Minnesota lakes; therefore the individual lake sites were combined for the purposes of this study. Lake Ontario samples also contained the same two haplotypes, with haplotype 1 comprising 93% of the data set.

In contrast, the Atlantic coastal haplotypes were markedly divergent from those in the Great Lakes and the two regions shared no common haplotypes. Maine shared a haplotype (13) with North Carolina, which dominated both locations, representing 62% and 50% of the samples, respectively (Table 3). The two individuals from Georgia were both haplotype 18, which each diverged from Maine and North Carolina samples by a unique nucleotide mutation (constituting a possible synapomorphy; see Fig. 3).

Genetic distance analysis calibration suggested that the separation between Yellow Perch in the Great Lakes versus the Atlantic coastal sites was about 300,000 years (using a molecular clock calibration of 2% per million years for percid mtDNA control region used by Faber and Stepien (1997) and Stepien and Faber (1998)). This places their divergence during the mid-Pleistocene epoch, prior to the last glaciations. Haplotype 3 (Table 1), which was exclusively

found in the eastern basin of Lake Erie in our study, shared a single nucleotide substitution with the European Yellow Perch *Perca fluviatilis* (sequence from Nesbo et al. 2003; GenBank Accession # Y14724; see Table 1) and thus was placed basally in the neighbor joining (NJ) and consensus maximum parsimony (MP) tree to all other *P. flavescens* haplotypes (Fig. 2). The genetic divergence level separating the *P. flavescens* and *P. fluviatilis* species appears to be about 1 million years before present, corresponding to a late Pliocene-early Pleistocene divergence.

AMOVA hierarchical  $F_{ST}$  analyses showed significant pairwise divergences between only two Lake Erie spawning site populations, Vermilion, OH (central basin) and Dunkirk, NY (eastern basin). This difference accounted for only 0.98% of the overall AMOVA variation. No significant differences were found for any western basin sample sites, either among the individual sites or in comparisons with the central and eastern basins.

The overall gene diversity level of yellow perch in Lake Erie appeared relatively low, 0.23 (+/- 0.04 s.e.), as compared to the Minnesota and most Atlantic coastal locations (Table 5). These levels were higher in the eastern basin than in the western basin of Lake Erie. However, the samples from Lake Ontario had an even lower genetic diversity, equivalent to 0.13 (+/- 0.11), than those found in Lake Erie. In contrast, the Minnesota, Maine, and North Carolina population samples had much higher genetic diversities equaling 0.60 (+/- 0.16), 0.53 (+/- 0.05), and 0.833 (+/- 0.22) respectively. We discerned no genetic diversity in the sample from Georgia, which was likely due to the small sample size and/or historic low population size (personal communication, James D. Williams, USGS). Nucleotide diversity levels in the Atlantic coastal samples from Maine and North Carolina were an order of magnitude higher than those in the Great Lakes sites (Table 5).

## DISCUSSION

The overall population of Yellow Perch from Lake Erie was characterized by relatively low genetic diversity (in contrast with walleye; Stepien and Faber 1998; Stepien et al. 2004a) and was dominated by a single mtDNA haplotype (comprising 86% of the individuals). Two haplotypes were shared among all three basins, and together comprised 88% of the entire sample set from Lake Erie. However, two unique haplotypes were found in the western basin (comprising 6% of the sample), three in the central basin (6%), and three in the eastern basin (9%) – suggesting some population differences. These results suggest that that our present sample size was likely insufficient to resolve appreciable genetic divergence among most sampling locations, given the predominance of a single haplotype.

However, mtDNA control region sequence haplotypes revealed greater genetic diversity in Lake Erie Yellow Perch than was found in analyses of allozymes in the western Great Lakes region by Todd and Hatcher (1993) and in Lake Michigan by Leary and Booke (1982). Notably, Todd and Hatcher (1993) discerned mean heterozygosity values (analogous to gene diversity ; see our Table 5) between 0.000 and 0.039 for 31 loci at locations across North America. Leary and Booke (1982) sampled Yellow Perch from Lake Michigan and Green Bay for 19 loci and

found only eight of those to be polymorphic, all of which were monomorphic by location. This may be due to the higher amount of mutations detectable with sequence data in comparison to protein analysis, since synonymous mutations in allozymic loci due to single nucleotide polymorphisms (SNPs) will be undetected. MtDNA also has a higher rate of mutation and smaller effective population size, rendering larger divergences (Awise 1994, Stepien and Kocher 1997).

Yet, Lake Erie populations of Yellow Perch still have much lower levels of genetic diversity overall than do walleye (Stepien and Faber 1998, Stepien et al. 2004a), which may be due to their “boom and bust” history in Lake Erie. However, since European Yellow Perch, *P. fluviatilis*, also display similarly low levels of genetic diversity in allozymes (Gyllensten et al. 1985, Bodaly et al. 1989) - and both *Perca* are similar in mtDNA variation levels to their sister genus *Gymnocephalus* (Stepien et al. 1998, 2004b) - some of this low genetic diversity may reflect the phylogenetic history of these genera. For example, Gyllensten et al. (1985) found only five polymorphic individuals out of 33 allozyme loci tested for 13 population samples of *P. fluviatilis* in northern Europe and Bodaly et al. (1989) identified only 2 of 52 loci to be polymorphic in perch from Windermere, England. Phylogenetically low genetic diversity of populations of *Perca* coupled with their boom-and-bust history due to exploitation appears to have resulted in relatively homogeneous populations that show only very broad-scale geographic divergences.

Nucleotide diversity (Table 5) was higher in general for populations of *P. flavescens* from the Atlantic sites than in the Great Lakes populations. Yellow perch had more haplotypes in the eastern basin of Lake Erie than in the west (Table 2). This is consistent with findings by Todd and Hatcher (1993) for yellow perch using allozymes, who also discerned greater genetic diversity in the eastern populations of Lake Erie than in the western basin. Increasing genetic diversity levels in eastern Lake Erie also was found in walleye spawning populations analyzed across Lake Erie (Stepien and Faber 1998; Stepien et al. 2004a). Stepien and Faber (1998) and Stepien et al. (2004a) overall also discerned greater genetic divergence among spawning sites than was found for yellow perch in the present study. Stepien and Faber (1998) postulated that genetic divergence patterns of walleye spawning sites across Lake Erie were the result of historic recolonization patterns from western (Mississippian) and eastern (Atlantic) glacial region, maintained by spawning site philopatry and likely natal homing to spawning sites. It may be that lesser contribution of Atlantic refugial yellow perch to Lake Erie historically may have resulted in lower genetic diversity values overall, as compared to walleye.

Genetic diversity studies of Yellow Perch by Todd and Hatcher (1993) and us reveal the highest levels of diversity in the North Carolina population. This appears to correspond to findings of greater overall genetic diversity in fishes originating from Atlantic glacial refugium than in the Mississippian glacial refugium (Todd and Hatcher 1993, Murdoch and Hebert 1997, Stepien and Faber 1998). Since North Carolina was unglaciated during the Ice Ages and is one of the most southerly regions in this study, it likely accumulated the greatest amount of variation over time.

In comparison to the other North American samples, Lake Erie Yellow Perch shared haplotypes with only the Great Lakes region, and not with the Atlantic and Gulf coastal states.

This suggests that there was a common Mississippi glacial refugium source for the lower Great Lakes region that may account for the low level of diversity found in all the Great Lakes sites studied for Yellow Perch. A genetic bottleneck occurring in the Mississippi refuge during the last glacial period or a low number of founding events in the newly formed Great Lakes may have led to the overall lack of genetic diversity in the region. This has been suggested for other Great Lakes fish species (McPhail and Lindsey 1970, Bailey and Smith 1981, Stepien and Faber 1998). Conversely, the exploitation of the fishery and subsequent reduction in numbers of Yellow Perch since the early 1900's, may have negatively impacted the genetic variation of the Lake Erie stock, as suggested by Strittholt et al. (1988). Either way, the current level of genetic diversity is relatively low, and should be accounted for in future management considerations.

Atlantic refuge fish were found to be more genetically diverse by Todd and Hatcher (1993) and this is consistent with the present findings. It is thought that the lower Great Lakes region represents the meeting point of colonizers from both the Mississippi and Atlantic glacial refugium populations (McPhail and Lindsey 1970, Bailey and Smith 1981, Todd and Hatcher 1993, Stepien and Faber 1998). The present study finds that the mtDNA control region haplotype sequences of Yellow Perch in the Great Lakes are more closely related to Mississippi refuge populations and significantly differ from those from Atlantic refugia. The commonality of lower genetic diversity among Mississippi refugia fish and an absence of shared haplotypes between the Great Lakes region populations and the Atlantic coastal region populations of Yellow Perch indicate that the Mississippi basin glacial refuge had a greater impact on the colonization of Lake Erie and perhaps the entire Great Lakes region.

Nuclear microsatellite analyses of these populations are now underway in the Stepien laboratory to discern whether further population structure can be discerned in Lake Erie. Other microsatellite studies found significant variation in Lake Michigan samples of Yellow Perch (Miller 2003) and in *P. fluviatilis* by Refseth et al. (1998) and Gerlach et al. (2001). For example, Miller (2003) discerned significant divergence, via pairwise  $R_{st}$  analysis ( $P < 0.01$ ), between Yellow Perch in southern Lake Michigan from those in Green Bay and surrounding inland lakes. European Yellow Perch in Lake Constance, Germany displayed a significant  $G_{st}$  ( $P < 0.001$ ) separation between eastern and western ends of the lake as well as possible kin preferences (Gerlach et al. 2001). It is possible that kin recognition and olfactory cues (personal communication, Gerlach) may aid recognition of natal homing sites in spawning site philopatry. Similar to our results, Refseth et al. (1998) found low genetic diversity values using mtDNA - but significantly greater differences using microsatellite analyses for *P. fluviatilis* populations of Scandinavia. In conclusion, further studies of genetic patterns in Yellow Perch may aid stock discrimination and help us to conserve what little genetic diversity remains.

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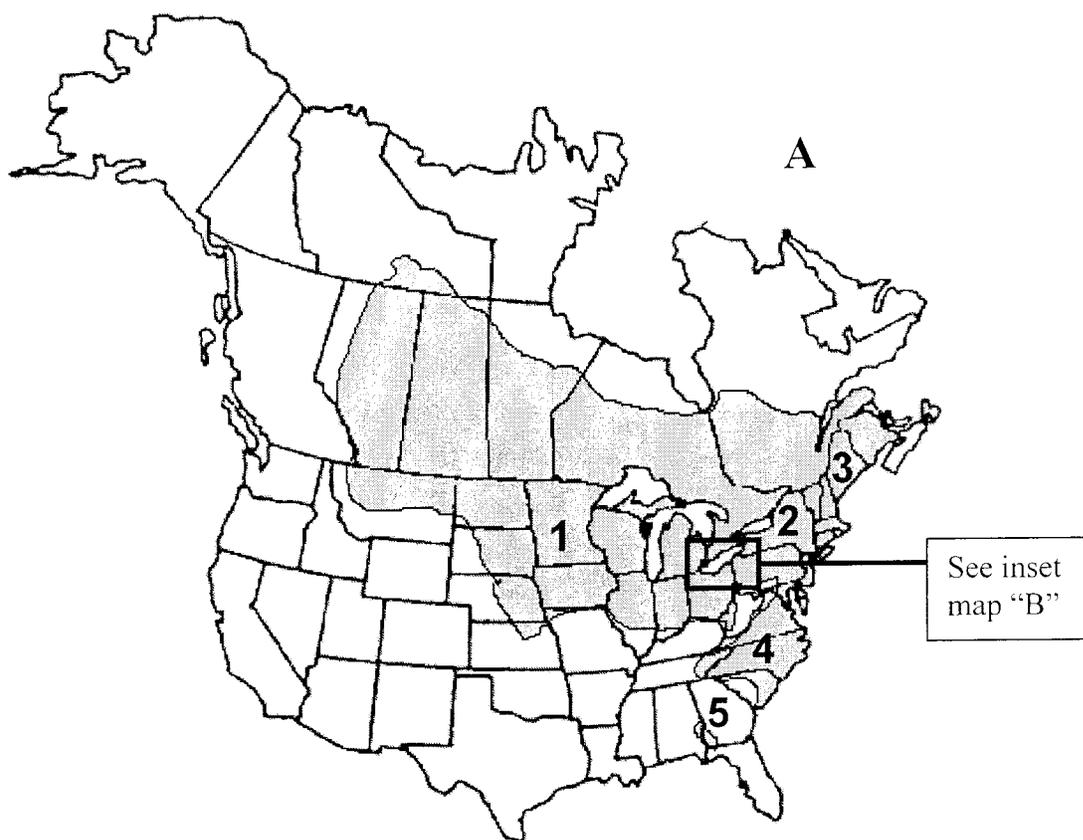
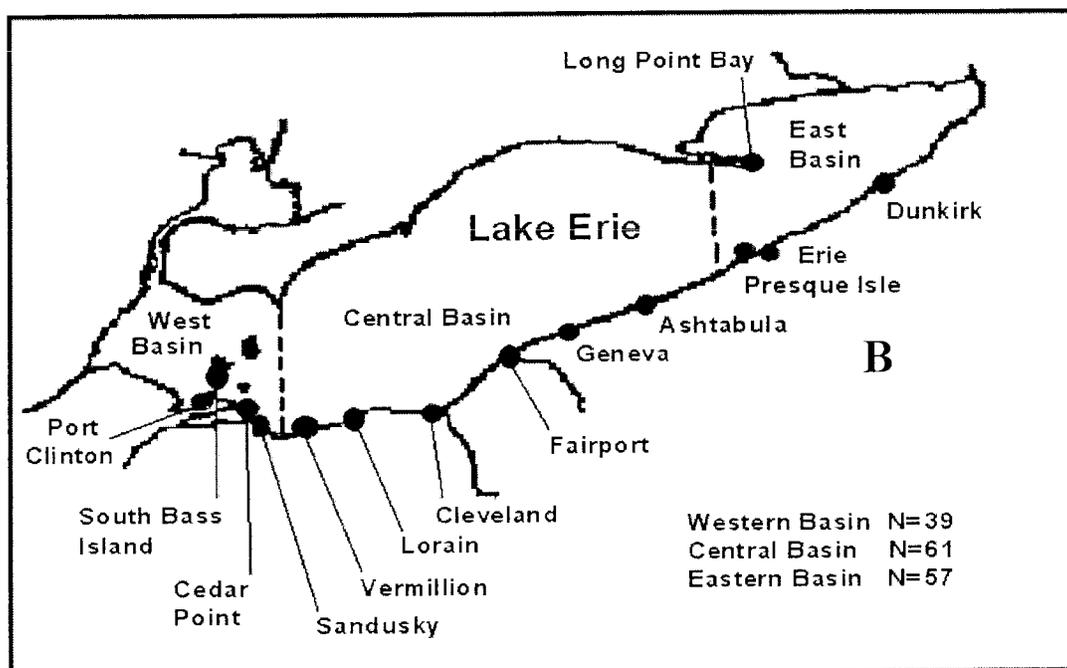


Fig. 1. Sampling sites and native distribution of Yellow Perch, *Perca flavescens* in: (A) North America. 1) Minnesota (N=15), 2) Lake Ontario (N=15), 3) Maine (N=10), 4) North Carolina (N=4), and 5) Georgia (N=2) and (B) Lake Erie. (N indicates spawning population sample size).

Table 1. MTDNA HAPLOTYPES OF NORTH AMERICAN YELLOW PERCH. Numbers across the top represent the base pair location of each change. "+" indicates a nucleotide insertion and "~" indicates a nucleotide deletion. "\*" sequence from Nesbo et al. 2003.

Haplotype Number	0	0	0	1	1	1	1	3	4	4	5	5	5	5	5	6	6	6	6	7	7	N
	0	0	8	1	2	3	6	5	4	7	2	6	7	7	8	0	5	6	7	5	5	
	5	6	0	4	5	8	8	5	7	6	3	5	0	5	0	4	8	6	2	3	4	
1	A	A	C	T	C	T	C	A	A	G	C	A	T	C	T	A	A	T	G	G	T	157
2									G													19
3				C																		1
4			T						G													1
5											T											1
6																G						1
7								+A														2
8																			C			1
9		G																				1
10													T									1
11	T																					1
12									A													1
13				C		C	T			T		A		C	G				A	A	C	15
14				C		C	T			T		A		C	G				A	A	C	3
15				C		C	T			T		A		C	G			~	A	A	C	2
16				C		C				T		A		C	G				A	A	C	1
17				C		C	T					A		C	G				A	A	C	1
18				C	T	C	T					A		C	G				A	A	C	2
<i>Perca fluviatilis</i> *	A	A	C	C	C	C	T	A	A	G	C	A	T	C	C	A	G	T	G	G	C	

Table 2. FREQUENCIES OF MTDNA HAPLOTYPES IN LAKE ERIE BY BASIN. Numbers are individuals per basin with each haplotype. Percentages are percent of each haplotype per column.

Haplotype Number	Western Basin	Central Basin	Eastern Basin	Lake Total
1	34 (87%)	51 (83%)	50 (88%)	135 (86%)
2	3 (7%)	7 (11%)	2 (3%)	12 (7%)
3	--	--	1 (2%)	1 (0.6%)
4	1 (3%)	--	--	1 (0.6%)
5	--	1 (2%)	--	1 (0.6%)
6	--	1 (2%)	--	1 (0.6%)
7	--	--	2 (3%)	2 (1.2%)
8	--	--	1 (2%)	1 (0.6%)
9	--	--	1 (2%)	1 (0.6%)
10	--	1 (2%)	--	1 (0.6%)
11	1 (3%)	--	--	1 (0.6%)
Total per site	39	61	57	157

Table 3. FREQUENCIES OF mtDNA HAPLOTYPES IN OTHER NORTH AMERICAN SAMPLING LOCATIONS. Numbers are individuals per location in each haplotype. Percentages are percent of each haplotype per column.

Haplotype Number	MN	NY (L. Ont.)	ME	NC	GA	Total
1	8 (54%)	14 (93%)	--	--	--	22 (50%)
2	7 (46%)	--	--	--	--	7 (16%)
12	--	1 (7%)	--	--	--	1 (2%)
13	--	--	5 (50%)	2 (50%)	--	7 (16%)
14	--	--	3 (30%)	--	--	3 (6%)
15	--	--	2 (20%)	--	--	2 (4%)
16	--	--	--	1 (25%)	--	1 (2%)
17	--	--	--	1 (25%)	--	1 (2%)
18	--	--	--	--	2 (100%)	2 (4%)
N	15	15	10	4	2	46

Table 4.  $F_{ST}$  AND PROBABILITY (P) VALUES FOR NORTH AMERICAN YELLOW PERCH SAMPLES. Numbers above the diagonal are  $F_{ST}$  values. Numbers below the diagonal are p values where “\*” indicates statistical significance at  $p < 0.01$  after sequential Bonferroni correction (Sokal and Rohlf 1995).

	Lake Erie	Minnesota	Lake Ontario	Maine	North Carolina	Georgia
Lake Erie	--	0.687	0.765	0.685	0.663	0.761
Minnesota	0.0001*	--	0.336	0.436	0.371	0.550
Lake Ontario	0.0001*	0.0067*	--	0.688	0.694	0.878
Maine	0.0001*	0.0001*	0.0001*	--	0.304	0.516
North Carolina	0.0001*	0.0001*	0.0003*	0.025	--	0.420
Georgia	0.0025*	0.0055*	0.0079*	0.055	0.123	--

Table 5. GENETIC DIVERSITY MEASURES AND STANDARD ERRORS FROM YELLOW PERCH SAMPLING LOCATIONS.

<b>Genetic Measures</b>	<b>Lake Erie</b>	<b>Minnesota</b>	<b>Lake Ontario</b>	<b>Maine</b>	<b>North Carolina</b>	<b>Georgia</b>
<b>Haplotype Diversity</b>	<b>0.23</b> +/- 0.04	<b>0.55</b> +/- 0.05	<b>0.15</b> +/- 0.11	<b>0.59</b> +/- 0.15	<b>0.84</b> +/- 0.22	<b>0.00</b> +/- 0.00
<b>Nucleotide Diversity</b>	<b>0.0003</b> +/- 0.0003	<b>0.0006</b> +/- 0.0006	<b>0.0002</b> +/- 0.0002	<b>0.0020</b> +/- 0.0015	<b>0.0040</b> +/- 0.0032	<b>0.0000</b> +/- 0.0000

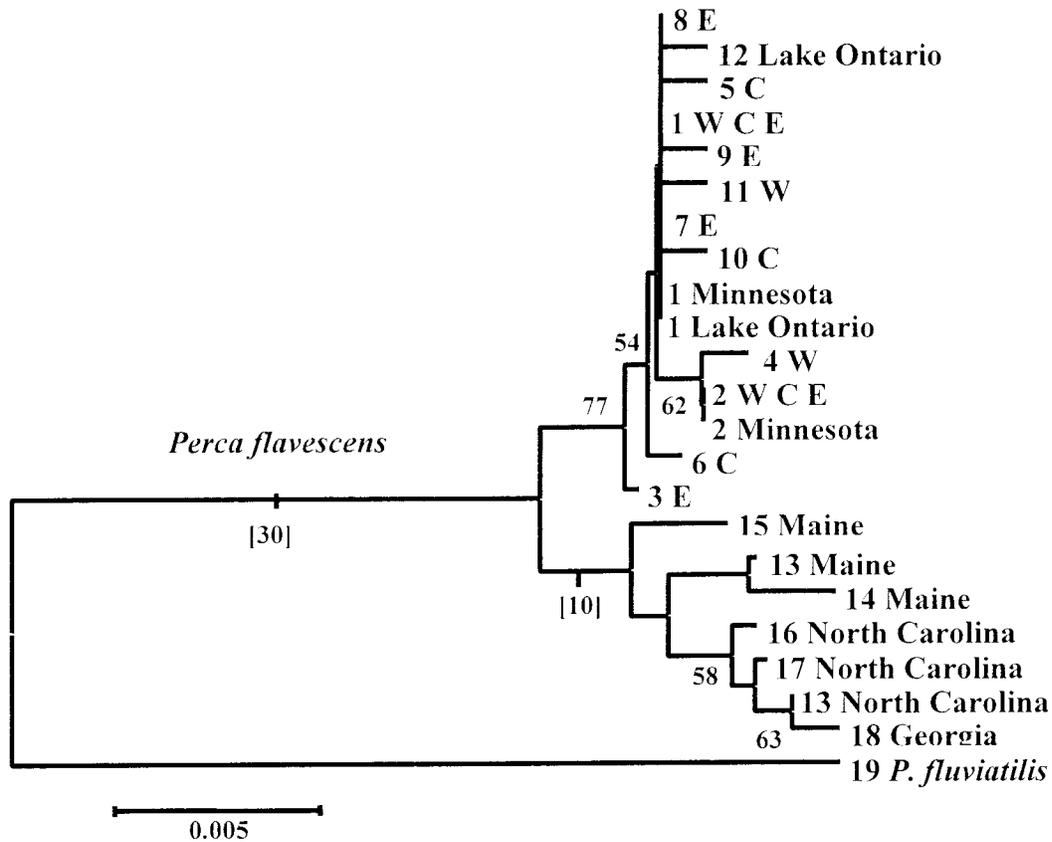


Fig. 2. Neighbor joining and maximum parsimony consensus tree. W= Western Basin of Lake Erie, C= Central Basin of Lake Erie, E= Eastern Basin of Lake Erie. Numbers at branch ends are haplotype designations. Numbers at branch points are bootstrap values (> 50%). Numbers in brackets represent the amount of synapomorphies separating the designated group.

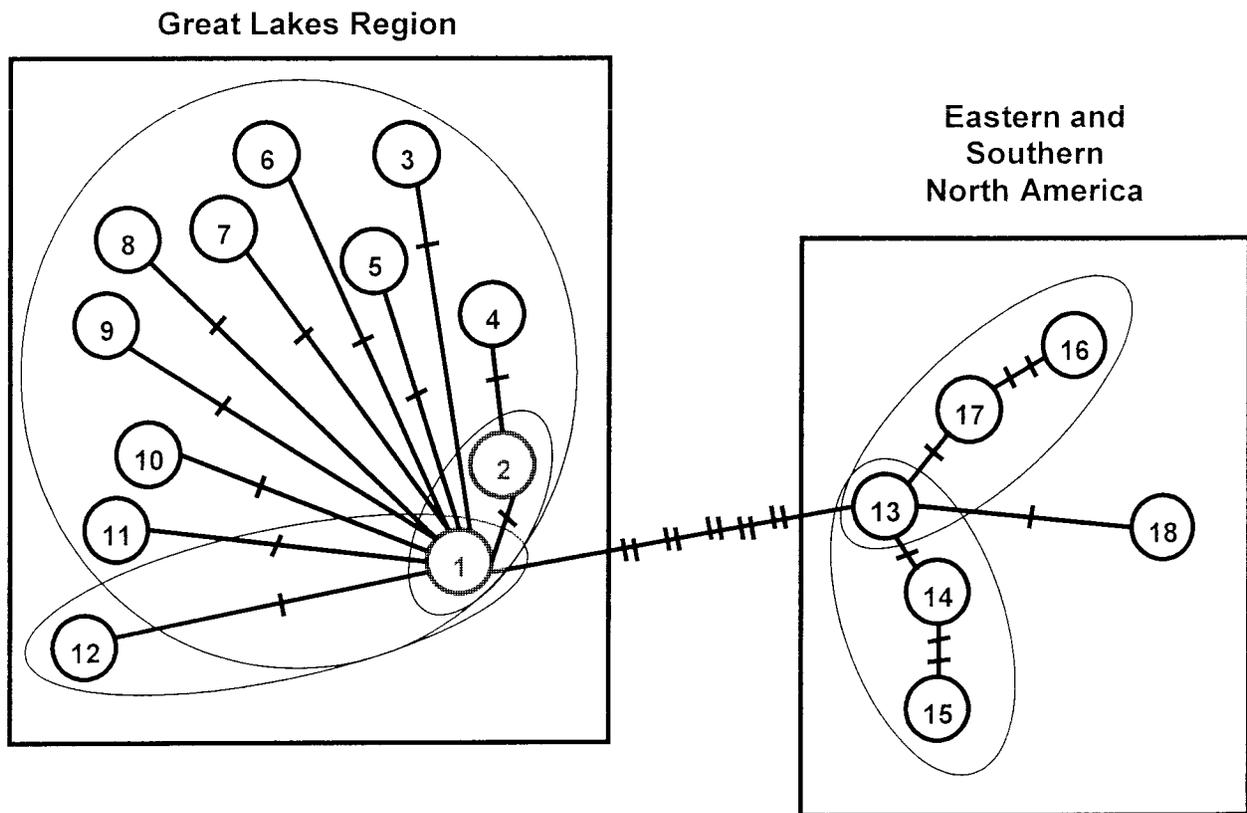


Fig. 3. Clade phylogeographic network diagram of North American Yellow Perch mtDNA control region haplotypes, modeled after Avise (2000).

## GENETIC VARIATION AND SPAWNING POPULATION STRUCTURE IN LAKE ERIE YELLOW PERCH, *Perca flavescens*: A COMPARISON WITH A MAINE POPULATION

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**Introduction.** Yellow perch, *Perca flavescens*, recolonized the newly formed Great Lakes system about 10,000 years ago from three proposed glacial refugia. Previous genetic investigations discerned differences among groups of Great Lakes fishes related to differential colonization from the glacial refugia – with Lake Erie populations largely comprising Mississippi refugium descendants (especially in the western and central basins) and some from the Atlantic refugium in the east. Allozyme and mtDNA RFLP genetic studies of yellow perch in Lake Erie found low genetic variability, which may be related to historically low variability in the Mississippi refugium populations (Todd and Hatcher 1993, Billington 1993).

Populations of yellow perch in Lake Erie have been rebounding after declining in the late 1980s. At the request of the Lake Erie Yellow Perch Task Force, the objective of the present study is to analyze the genetic stock structure and variability of yellow perch in Lake Erie. We also are comparing the data to several outgroup population areas of their range, including the other Great Lakes. This investigation analyzes the entire mtDNA control region (912 bp) of 118 yellow perch from spawning locations spanning Lake Erie (Fig.1), and makes comparisons with a native outgroup population from south-central Maine. **Methods.** Fin clip tissue samples were collected from 16 spawning locations around Lake Erie (Fig. 1), including the western, central, and eastern basins.

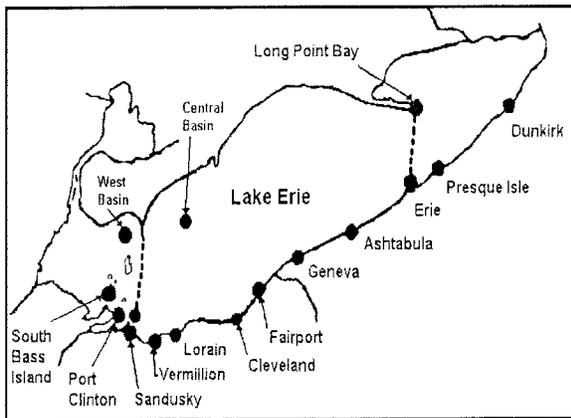


Figure 1. Lake Erie sample sites for yellow perch.

Maine samples were from Searsmont. DNA was extracted and purified with a Quiaquick kit. The entire mtDNA control region was PCR amplified following Stepien and Faber (1998). PCR products were purified using an Exosap kit and sequenced separately in both directions for verification on a Beckman CEQ 8000 capillary autosequencer.

The proportion of polymorphic nucleotides ( $p_n$ ), as well as haplotypic diversity ( $h$ ) and nucleotide diversity ( $\pi$ ) were calculated. Neighbor joining trees and maximum parsimony analysis of relationships among haplotypes, including comparison to the European yellow perch *P. fluviatilis* were constructed with MEGA2 and PAUP\*, and support for relationships was compared with 1000 bootstrap replications. Hierarchical analysis of variance using AMOVA in Arlequin examined divergence among spawning sites and population groups.

**Results.** Ten mtDNA control region haplotypes were identified to date in Lake Erie, and three others in Maine (Fig. 2). Gene diversity in Maine was 0.57 +/- 0.12 s.e. and 0.37 +/- 0.06 in Lake Erie. Haplotypic diversity was 0.71 +/- 0.20 and 0.33 +/- 0.03, respectively. Nucleotide diversity was 0.67 +/- 0.03 for Maine and 0.39 +/- 0.03 for Lake Erie.

No haplotypes were shared between Lake Erie and Maine, and those from Maine formed a clade distinguished by 5 synapomorphies in the neighbor-joining (NJ) and maximum parsimony (MP) consensus tree. The data revealed a significant difference between the Maine and Lake Erie populations, equivalent to 62.8% of the variation in AMOVA (Analysis of Molecular Variance) comparisons in Arlequin and an  $F_{ST} = 0.66$ ,  $p < 0.00001$ , and a very great genetic divergence.

Haplotype #2 was located basally in the tree (Fig. 2) to all other *P. flavescens* haplotypes, including those from Maine, and was the sister type to all other *P. flavescens*. The frequency of #2 was greatest in western Lake Erie, and decreased from west to east. Two haplotypes were unique to the central basin and 5 to the eastern basin. The western basin was dominated by 3 shared haplotypes, but no unique ones to date. Haplotype #1 was widely distributed across Lake Erie, comprising about 80% of the samples. Haplotype #3 was relatively common in the west, less common in the central basin, and absent from the eastern basin.

AMOVA hierarchical  $F_{ST}$  analyses showed that

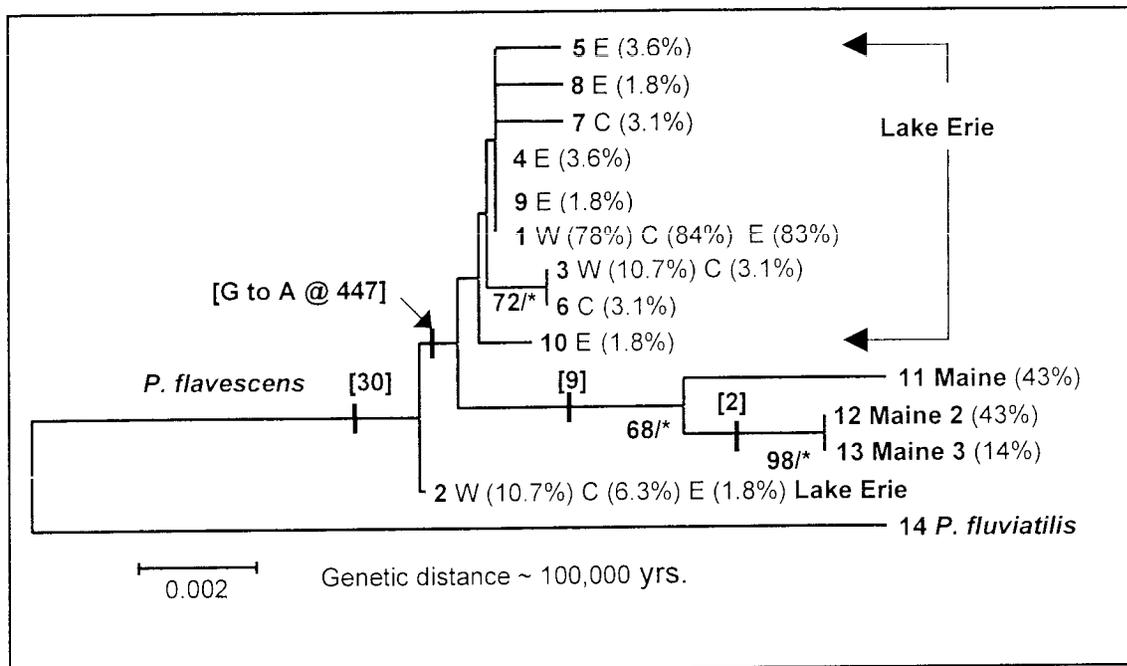


Figure 2. NJ (MEGA 2.0) tree of yellow perch mtDNA from Lake Erie and Maine with comparison to *P. fluviatilis*. Genetic distance is calibrated using 2%/my clock (Stepien and Faber 1998). Numbers in parentheses are haplotype frequencies. W = West Basin, C = Central Basin, and E = East Basin. Numbers with “/\*” are bootstrap values congruent with the most-parsimonious maximum parsimony consensus tree (PAUP\*).

pairwise divergences among some spawning site populations were significant (e.g., those with larger sample sizes at present), comprising about 3% of the variance overall.

**Discussion.** MtDNA control region sequence haplotypes revealed greater genetic diversity in Lake Erie than was found in analyses of allozymes (Todd and Hatcher 1993) and mtDNA RFLPs (Billington 1993). However, our data show that Lake Erie is dominated by a single mtDNA control region haplotype (#1), which comprises about 80% of the population in all basins. We discerned greater genetic variation within the Maine population.

Lake Erie populations of yellow perch have lower levels of genetic diversity overall than do walleye (Stepien and Faber 1998, Stepien and Taylor, in progress), which may be due to their “boom and bust” history in Lake Erie in comparison to the Maine population. However, since European yellow perch *P. fluviatilis* display similarly low levels of genetic diversity in allozymes and the mtDNA control region in comparison to *Stizostedion* - and both *Perca* are similar in levels to their sister genus *Gymnocephalus* (Stepien et al. 1998) - some of this low genetic diversity may reflect the phylogenetic history of these genera.

MtDNA control region sequences reveal higher genetic variation than was previously discerned, as well as significant population divergences among spawning sites for yellow perch in Lake Erie (e.g.,

those with larger sample sizes at present), which suggests spawning site philopatry and differential colonization patterns stemming from glacial refugia.

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