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[Article]

Genetic Variation of 17 Wild Yellow Perch Populations from the Midwest and East Coast Analyzed via Microsatellites

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Abstract.—We used microsatellite loci, including seven newly developed by us, to analyze the population genetic structure of wild yellow perch *Perca flavescens* from 17 sampling areas in the upper Midwest and East Coast of the United States. Our results reveal greater genetic differentiation and finer-scale geographic structure than were found in previous studies of yellow perch population structure. These findings show pronounced genetic divergence between Midwest and East Coast samples. Additional genetic partitioning was noted between Chesapeake Bay and Albemarle Sound populations, between inland lakes in the upper Mississippi River system, and among sites in Lake Michigan and Lake Ontario. Further, the structuring of yellow perch populations within the Chesapeake Bay may be significantly influenced by salinity. These findings are being used to help delineate wild populations for the development of captive yellow perch broodstocks for the aquaculture industry.

The yellow perch *Perca flavescens* is an ecologically and economically important freshwater species that is prized as a food and sport fish. Its native distribution ranges throughout the Midwest and East Coast of the United States (from Maine to Georgia), as well as large portions of Canada (Nova Scotia, New Brunswick, Quebec, Ontario, Manitoba, Saskatchewan, and Alberta and some of British Columbia; Figure 1). In the 1950s, virtually the entire yellow perch market was supplied by commercial fisheries in the Great Lakes region. Yellow perch peak harvests exceeding 14,900 metric tons/year in the 1950s and 1960s were insufficient to meet market demands, and Great Lakes populations underwent a dramatic decline during the 1960s and early 1970s (Lesser and Vilstrup 1979). This decline has been attributed to predation, competition with other organisms that feed on plankton, and interferences, such as dreissenid mussels in the Great Lakes (Marsden and Robillard 2004).

Wild harvests of yellow perch continued to decrease to 5,000–8,200 metric tons/year during the 1980s and 1990s (Malison 2000). Despite this severe decline, yellow perch commercial demand has remained high for more than 30 years (Riepe 1998). With the exception of Lake Erie and Green Bay in Lake Michigan, the Great Lakes commercial yellow perch fishing was terminated and quotas for sport fishing were greatly reduced in an effort to allow the natural populations to rebound. The decline in wild yellow perch and decrease in commercial harvest have been an impetus for the development of yellow perch aquaculture to meet consumer demands (Malison 2000). However, there currently are no commercial broodstocks for the aquaculture industry, and wild stocks are an important source for their development.

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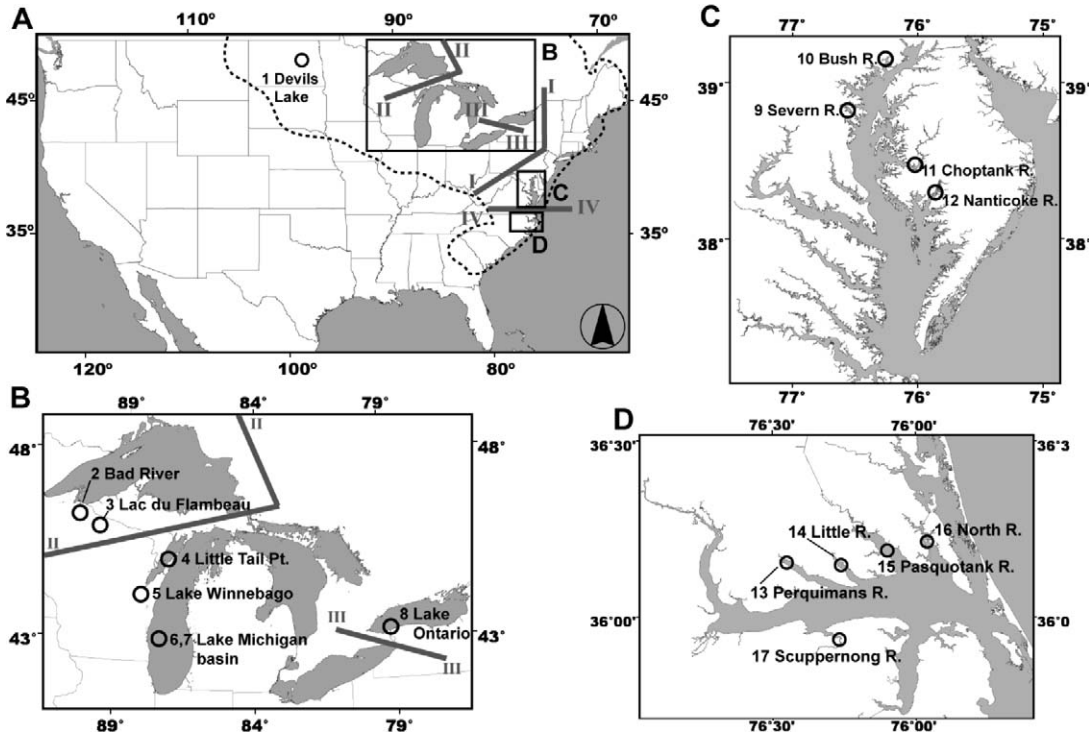

FIGURE 1.—Panel **(A)** shows the four general regions in which yellow perch were sampled for microsatellite analysis, along with the species' native range (dashed line) and the primary barriers to gene flow (solid lines labeled I–IV; Manni et al. 2004). Panels **(B)–(D)** show the sampling locations within the Great Lakes, Chesapeake Bay, and Albemarle Sound, respectively.

Using allozymes, Todd and Hatcher (1993) showed that yellow perch populations from the East Coast of the United States could be differentiated from Midwest populations; however, allelic variation was low in all of the populations sampled. Although mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) analysis of yellow perch populations from Wisconsin to Maryland identified 13 haplotypes, all populations were predominated by only a single haplotype and were not differentiable (Billington 1996). A recent study by Sepulveda-Villet et al. (2009) used mtDNA control region sequences to successfully resolve primary patterns of population structure across North America. Using microsatellites derived from walleyes *Sander vitreus*, Miller (2003) showed that samples taken from Lake Michigan and the surrounding Midwest region were very distinct from a sample taken from Vermont. In addition, whereas prior studies using allozymes were unable to demonstrate differences between samples from Green Bay and Lake Michigan (Leary and Booke 1982), Miller was able to do so using microsatellites. Several groups have developed microsatellites specifically for yellow perch that exhibit high genetic variability (Leclerc et al. 2000; Li et al. 2007). Using 16 of these microsatellites, genetic variation was found to be significantly different between yellow perch from four wild (North Carolina, Maine, Pennsylvania, New York) and two captive (Michigan, Ohio) populations (Brown et al. 2007).

As part of a research program to produce yellow perch broodstocks for the aquaculture industry from wild fish, we analyzed the genetic diversity and relatedness of yellow perch populations from two regions in North America. Our objective was to delineate the genetic structure of these wild populations for purposes of future broodstock development (i.e., use selected populations or sites to obtain fertilized gametes for the generation of parental stocks). Assessing the genetic variation and differentiation of stocks before the initiation of breeding programs for genetic improvement is considered an essential prerequisite (Gjedrem 2000).

During the process of collecting tissue samples from East Coast populations for genetic analysis, we responded to requests from state fisheries biologists on the East Coast to analyze yellow perch from several rivers entering Chesapeake Bay (Maryland portion) and

TABLE 1.—Sampling locations for yellow perch tested, along with the sample size (*N*), the numbers of males and females, and mean genetic variability values for seven microsatellite loci meeting Hardy–Weinberg equilibrium after Bonferroni correction (Sokal and Rohlf 1995). The latter include the mean number of alleles per locus (*A*), allelic richness (*R*), observed (H_O) and expected (H_E) heterozygosity, and the inbreeding coefficient (F_{IS}).

Location	*N* (male, female)	*A*	*R*	H_O	H_E	F_{IS}
Midwest	365 (178, 187)	7.68	6.65	0.58	0.59	0.00
1. Devils Lake, North Dakota	48 (24, 24)	7.00	6.24	0.55	0.57	0.04
2. Bad River, Wisconsin	48 (14, 34)	8.29	7.69	0.69	0.72	0.05
3. Lac du Flambeau, Wisconsin	48 (24, 24)	7.71	6.59	0.55	0.55	0.01
4. Little Tail Point, Wisconsin	48 (24, 24)	8.29	5.62	0.58	0.57	−0.09
5. Lake Winnebago, Wisconsin	48 (27, 21)	7.43	6.40	0.55	0.54	0.02
6. Lake Michigan (1998)	48 (24, 24)	7.00	6.96	0.58	0.53	−0.02
7. Lake Michigan (2002)	35 (24, 11)	7.14	6.30	0.54	0.55	−0.01
8. Lake Ontario	42 (17, 25)	8.57	7.39	0.65	0.65	0.00
Chesapeake Bay, Maryland	192 (96, 96)	7.89	6.95	0.65	0.66	0.02
9. Severn River	48 (24, 24)	8.29	7.22	0.53	0.62	0.14
10. Bush River	48 (24, 24)	8.14	7.17	0.73	0.72	−0.02
11. Choptank River	48 (24, 24)	7.29	6.39	0.66	0.64	−0.03
12. Nanticoke River	48 (24, 24)	7.86	7.02	0.69	0.66	−0.04
Albemarle Sound, North Carolina	200 (115, 85)	7.89	7.13	0.54	0.56	0.04
13. Perquimans River	48 (25, 23)	8.57	7.11	0.60	0.56	−0.08
14. Little River	29 (25, 4)	8.00	7.83	0.52	0.59	0.13
15. Pasquotank River	34 (25, 9)	8.29	7.63	0.52	0.57	0.08
16. North River	41 (16, 25)	7.29	6.69	0.55	0.58	0.06
17. Scuppernong River	48 (24, 24)	7.29	6.38	0.51	0.52	0.03
Total	757 (389, 368)	7.80	11.72	0.59	0.60	0.02

Albemarle Sound (North Carolina) to help delineate their genetic structure. Chesapeake Bay and Albemarle Sound are two extensive estuarine systems on the Atlantic Coast that support significant yellow perch fisheries. These populations in Chesapeake Bay experienced declines in the last 2 decades (Yellow Perch Work Group 2002) that were attributed to various environmental perturbations, such as acidification in the Chesapeake watershed (Klauda 1989) and hypoxic conditions created by urbanization in rivers such as the Severn (Yellow Perch Work Group 2002). Management responded to those declines with harvest limitations and stocking of rivers with adults and fry (Uphoff 1991). Yellow perch in some rivers appear to be rebounding according to assessments conducted in 2002 (Yellow Perch Work Group 2002). Past stocking of some of the rivers entering Chesapeake Bay might have affected their population genetic structure and, thus, an analysis of their current genetic structure may prove important for future management decisions. Hurricane Floyd heavily flooded Albemarle Sound in 1999 (Bales 2003), and although no significant fish kills were observed (Mallin et al. 2002), mixing of riverine populations was likely. Thus, the perturbations of Albemarle Sound may have impacted the genetic structure of these yellow perch populations, supporting a need for analysis of their current genetic structure as well.

Methods

Tissue sampling and genomic DNA isolation.—Fin clips were obtained from adult male and female yellow perch during the 2006 spawning season from eight Midwest sites, four rivers entering the Maryland portion of the Chesapeake Bay, and five rivers with outflows on Albemarle Sound (Table 1; Figure 1). In most cases, 24 males and 24 females were sampled per site. However, in a few samples, there were less than 48 total individuals, and a few had unequal numbers of males and females (Table 1). Fin clips were stored individually in 95% ethanol. We extracted DNA from all samples using Chelex buffer (Walsh et al. 1991), and samples were genotyped at 14 microsatellite loci. Of the 14 loci used, 7 microsatellites were derived from published yellow perch sequences (Leclerc et al. 2000) and 7 were newly isolated at the Great Lakes WATER Institute (*MPf*-1 to *MPf*-7; Table 2).

Identification of new yellow perch microsatellite sequences.—Genomic DNA was isolated from yellow perch livers via DNAzol (Molecular Research Center, Cincinnati, Ohio) digested with the restriction enzyme, TSP509I. Reactions were separated on a 1.3% agarose gel with ethidium bromide and a 500–1,000-base-pair size fraction was isolated from the gel and purified. Using a magnetic bead capture approach (McCauley et al. 2004), 785 potentially new yellow perch microsatellites were isolated. The probes that were used to obtain microsatellites were $(CA)_{15}$ and $(GA)_{15}$ oligonucleotides bound to streptavidin-coated magnetic beads (Dynabeads M-280 Streptavin; Dynal Biotech, Oslo, Norway). The purified products that hybridized to the oligonucleotides were eluted using water and amplified via a 15-cycle polymerase chain reaction

TABLE 2.—Microsatellite primer sequences for seven previously published yellow perch loci (*Pfla*-L2 through *Pfla*-L9; Leclerc et al. 2000) and seven new loci (*MPf*-1 through *MPf*-7) developed for this study, with their annealing temperature (T_A), size range of observed alleles, repeat type, total number of alleles detected (A), and average observed (H_O) and expected (H_E) heterozygosities totaled for all populations. Because null alleles were present at *MPf*-1, *MPf*-2, *MPf*-4, *Pfla*-L3, *Pfla*-L4, *Pfla*-L8, and *Pfla*-L9, these loci were not utilized in the analyses.

Locus[a]	Primer sequence[b]	T_A (°C)	Size range (base pairs)	Repeat type	A	H_O	H_E
Pfla-L2	F: GTAAAGGAGAAAGCCTTAAC R: TAGCATGACTGGCAAATG	52	203–259	$(CA)_{23}$	28	0.67	0.68
Pfla-L3	F: GCCGAATGTGATTGAATG R: CGCTAAAGCCAACTTAATG	52	107–195	$(TG)_{18}$	35	0.68	0.82
Pfla-L4	F: AAAGGGAAAAGGCTACGGTG R: ATCAGCAGTGCTTATGTTTG	52	101–195	$(TC)_{37}$	30	0.48	0.90
Pfla-L5	F: TGAGAGCCCATGAATTAC R: GCAAACACAGCCAATTTAG	52	129–175	$(GT)_{27}$	17	0.54	0.55
Pfla-L6	F: GCATACATATAAGTAGAGCC R: CAGGGTCTTCACTATACTGG	52	140–186	$(TG)_{18}$	20	0.62	0.66
Pfla-L8	F: GCCTTATTGTGTGACTTATCG R: GGATCTTTCACTTTTTCTTTCAG	52	131–213	$(TG)_{39}$	29	0.42	0.50
Pfla-L9	F: GTTAGTGTGAAAGAAGCATCTGC R: TGGGAAATGTGGTCAGCGGC	52	197–313	$(TG)_{24}$	42	0.56	0.64
MPf-1	F: ATGGTAGACGCAGAGGCAAGC R: GTTGAGCTGCCTCACGTCTCC	56	239–349	$(CA)_{34}$	43	0.69	0.90
MPf-2	F: TTCTTTCCATCCGTCTCCTC R: CCCCTCATACCCATTTGTGA	56	211–327	$(CA)_{39}$	52	0.74	0.93
MPf-3	F: TTGCATAATGTGGAATACAC R: CACATTCACTCACAGAAC	48	105–161	$(TG)_{27}$	23	0.53	0.54
MPf-4	F: AATGTCGCAGCTTCACTATC R: CAGGTGGTAGTATTGCCAA	52	179–241	$(AG)_{35}$	28	0.62	0.80
MPf-5	F: ATGTAAACGTGCCTGATCGC R: CCCACTCATCCTGTCTATCT	54	133–173	$(CT)_{26}$	21	0.61	0.62
MPf-6	F: GACCCTTAGAACCTGTAGTCC R: CCGTCTGAGTAACATGGTCA	54	100–180	$(GACA)_{10}$	19	0.58	0.51
MPf-7	F: CCAGCAGTCATTACTCCAAGC R: GCCTTGATCCTCCACTTCATT	55	135–191	$(TC)_{26}$	23	0.57	0.62

[a] GenBank accession numbers are as follows: *Pfla*-L2 (AF211827), *Pfla*-L3 (AF211828), *Pfla*-L4 (AF211829), *Pfla*-L5 (AF211830), *Pfla*-L6 (AF211831), *Pfla*-L8 (AF211833), *Pfla*-L9 (AF211834), *MPf*-1 (EU153815), *MPf*-2 (EU153816), *MPf*-3 (EU153817), *MPf*-4 (EU153818), *MPf*-5 (EU153819), *MPf*-6 (EU153820), *MPf*-7 (EU153821).

[b] F = forward, R = reverse.

(PCR). The final PCR products were cloned using a TOPO TA Cloning Kit (Invitrogen, Carlsbad, California). Positive colonies were selected, and plasmid preparations were prepared in a 96-well format. Plasmid cDNA was sequenced using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Inc. [ABI], Foster City, California) with a vector primer (M13-R). The reactions were precipitated in ethanol, resuspended in Hi-Di formamide (ABI) and sequenced from 96-well plates using the ABI 3730 automated sequencing system.

From the 785 microsatellites that were sequenced, primers were designed and tested for 54 sequences to evaluate locus amplification. If a product was amplified, the microsatellite locus then was screened for polymorphism using individuals from four sampling locations. The PCR products for 28 amplifying loci were visualized on high resolution MetaPhor agarose gels (Cambrex, Rockland, Maine). From this analysis, seven microsatellites were tested for inclusion in the present analysis of yellow perch populations (*MPf*-1 to *MPf*-7; Table 2).

Microsatellite data collection.—To examine microsatellite variability within and among the populations sampled, microsatellites isolated from the bead hybridization protocol and from Leclerc et al. (2000) were used in PCR reactions with fluorescently labeled forward primers (ABI) to amplify repeats (Table 2). The PCR was performed in a 25-μL volume with 10–20 ng of template DNA, forward and reverse primers (0.16 μM final concentration), and 12.5 μL of Amplitaq Gold master mix (ABI). The PCR reactions were carried out in a MJ Research PTC-200 thermal cycler (Bio-Rad Laboratories, Hercules, California) with cycling conditions as follows: initial denaturation at 96°C for 5 min, followed by 34 cycles at 95°C for 45 s, 45 s at the locus-specific annealing temperature (see Table 2), 1 min at 65°C, and a final 10 min of elongation at 65°C. The products were held at −20°C before loading on the ABI Prism 3730 for analysis. To analyze PCR products, 1 μL of the product was

TABLE 3.—Summary statistics for pairwise yellow perch population (see Table 1) heterogeneity tests using seven loci, including *F*-statistic analogs ρ_{ST} (Michalakis and Excoffier 1996) above diagonal and θ_{ST} (Weir and Cockerham 1984) below diagonal. Values were not significant (NS), significant at $P \leq 0.05$ (*) or remained significant following sequential Bonferroni correction (**; Rice 1989). Pairwise tests that also were not significant using the exact nonparametric procedure (Goudet et al. 1996) are given in bold italics; all others were significant.

Location	1	2	3	4	5	6	7	8
1. Devils Lake		0.172**	0.111**	0.242**	0.198**	0.342**	0.337**	0.460**
2. Bad River	0.091**		0.152**	0.137**	0.131**	0.165**	0.144**	0.113**
3. Lac du Flambeau	0.151**	0.163**		0.290**	0.250**	0.386**	0.384**	0.442**
4. Little Tail Point	0.180**	0.111**	0.284**		0.072**	0.096**	0.065**	0.338**
5. Lake Winnebago	0.117**	0.152**	0.238**	0.131**		0.276**	0.249**	0.399**
6. Lake Michigan (1998)	0.157**	0.147**	0.284**	0.100**	0.126**		***0.000NS***	0.298**
7. Lake Michigan (2002)	0.146**	0.135**	0.275**	0.083**	0.089**	***0.009****		0.274**
8. Lake Ontario	0.264**	0.142**	0.287**	0.256**	0.298**	0.211**	0.217**	
9. Severn River	0.399**	0.294**	0.407**	0.399**	0.413**	0.418**	0.405**	0.322**
10. Bush River	0.347**	0.244**	0.357**	0.346**	0.361**	0.365**	0.348**	0.269**
11. Choptank River	0.392**	0.282**	0.398**	0.382**	0.397**	0.402**	0.389**	0.314**
12. Nanticoke River	0.365**	0.261**	0.374**	0.359**	0.375**	0.384**	0.366**	0.300**
13. Perquimans River	0.431**	0.341**	0.437**	0.434**	0.447**	0.452**	0.439**	0.362**
14. Little River	0.412**	0.314**	0.422**	0.415**	0.432**	0.437**	0.421**	0.338**
15. Pasquotank River	0.427**	0.332**	0.432**	0.429**	0.443**	0.449**	0.434**	0.355**
16. North River	0.417**	0.321**	0.425**	0.418**	0.434**	0.436**	0.422**	0.339**
17. Scuppernong River	0.448**	0.357**	0.455**	0.450**	0.464**	0.469**	0.457**	0.382**

combined with 9.75 μL of Hi-Di formamide and 0.25 μL of Genescan −500 LIZ size standard (ABI). This mix was incubated at 96°C for 3 min for thermal denaturation and then immediately placed into a −20°C freezer for 5–10 min to bring them approximately to room temperature. Evaluating reactions at room temperature helped to reduce stutter bands in the electrophoresed products. The PCR products were analyzed (Genemapper version 3.5) to determine allele sizes and genotypes for each individual.

Statistical analysis.—The program Fstat version 2.9.3.2 (Goudet 1995, 2002) was used to calculate allele frequencies, inbreeding coefficients (F_{IS}), and allelic richness (mean number of alleles per locus, which takes sample size into account). Significance levels were determined with two-sample *t*-tests (Sokal and Rohlf 1995), as implemented in the package XLSTAT 2008 version 6.03 (http://xlstat.com). Arlequin version 3.11 (Excoffier et al. 2005; Excoffier 2007) was used to calculate observed (H_O) and expected (H_E) heterozygosities for each population sample, along with their respective *P*-values, via a Markov chain method with 1,000 dememorization steps (Guo and Thompson 1992). Population samples were tested for conformance to Hardy–Weinberg (HW) equilibrium expectations at each locus, and the Markov chain Monte Carlo (MCMC) method and 1,000 randomization procedures were used to estimate significance (following Guo and Thompson 1992), as implemented in Genepop version 4.0 (Rousset 2008). The HW deviations were tested for heterozygosity deficiency or excess and for the presence of null alleles (Micro-Checker version 2.2.3; http://www.microchecker.hull.ac.uk; van Oosterhout et al. 2004). Each locus was tested for linkage disequilibrium (LD) in Arlequin. Levels of significance for HW and LD tests were adjusted using nonsequential Bonferroni corrections (Sokal and Rohlf 1995).

Pairwise genetic differentiation tests were used to identify samples with significantly divergent gene pools via the *F*-statistic analogs θ_{ST} (Weir and Cockerham 1984) and ρ_{ST} (Michalakis and Excoffier 1996) in Arlequin. A third pairwise comparison used an exact nonparametric procedure (chi-square contingency test; Goudet et al. 1996) was used with probabilities estimated via MCMC simulations in Genepop, which is not affected by sample size or dependent on a normal distribution (Raymond and Rousset 1995; Rousset 2008). Probability values were adjusted using sequential Bonferroni correction (Rice 1989).

A hypothesis—that genetic distance, as measured by $\theta_{ST}/(1 - \theta_{ST})$, corresponded to geographic distance, as measured as the shortest waterway distance between pairs of spawning sites in connected systems or by shortest geographic distance between unconnected locations (Rousset 1997)—was tested using Mantel's (1967) procedure with 1,000 permutations in Genepop. Separate tests were run for broadscale relationships (across all samples) and then within the respective water bodies of Lake Michigan, Chesapeake Bay, and Albemarle Sound.

Analysis of molecular variance (AMOVA; Excoffier 1992), as implemented in Arlequin, was used to test for hierarchical population structure among geographical groups. In addition, three-dimensional factorial corre-

TABLE 3.—Extended.

Location	9	10	11	12	13	14	15	16	17
1. Devils Lake	0.841**	0.817**	0.852**	0.819**	0.870**	0.889**	0.867**	0.882**	0.902**
2. Bad River	0.633**	0.613**	0.653**	0.615**	0.685**	0.668**	0.657**	0.693**	0.718**
3. Lac du Flambeau	0.835**	0.809**	0.848**	0.817**	0.867**	0.888**	0.866**	0.880**	0.899**
4. Little Tail Point	0.810**	0.778**	0.821**	0.777**	0.849**	0.863**	0.842**	0.861**	0.881**
5. Lake Winnebago	0.835**	0.806**	0.845**	0.804**	0.867**	0.885**	0.864**	0.880**	0.899**
6. Lake Michigan (1998)	0.786**	0.750**	0.800**	0.752**	0.831**	0.846**	0.823**	0.844**	0.867**
7. Lake Michigan (2002)	0.775**	0.736**	0.789**	0.737**	0.823**	0.836**	0.811**	0.836**	0.862**
8. Lake Ontario	0.533**	0.501**	0.564**	0.503**	0.615**	0.588**	0.575**	0.624**	0.655**
9. Severn River		0.051**	0.043**	0.226**	0.113**	0.053*	0.088**	0.111**	0.121**
10. Bush River	0.036**		0.112**	0.220**	0.234**	0.166**	0.193**	0.224**	0.251**
11. Choptank River	0.045**	0.040**		0.166**	0.159**	0.102**	0.152**	0.189**	0.173**
12. Nanticoke River	0.142**	0.086**	0.086**		0.304**	0.284**	0.267**	0.366**	0.368**
13. Perquimans River	0.189**	0.165**	0.182**	0.168**		***0.024****	***0.000NS***	***0.020****	0.051*
14. Little River	0.165**	0.132**	0.157**	0.144**	***0.012****		***0.032****	***0.034****	***0.000NS***
15. Pasquotank River	0.191**	0.161**	0.183**	0.169**	***0.005NS***	***0.004NS***		***0.019****	***0.077****
16. North River	0.166**	0.145**	0.170**	0.178**	***0.015****	***0.011****	***0.015****		***0.070****
17. Scuppernong River	0.203**	0.174**	0.194**	0.187**	0.012*	***0.000NS***	***0.001NS***	***0.014****	

spondence analysis (3DFCA; Benzecri 1973) in Genetix version 4.05 (Belkhir et al. 2004) was used to explore further population divisions, which makes no a priori assumptions about population relationships and evaluates variation within and among geographic sites. Nei's (1972) genetic distances (D_S) were used to construct a neighbor-joining tree (Saitou and Nei 1987), showing the relationships among the sampling sites (PHYLIP version 3.68; Felsenstein 2008). Relative support values for the nodes of the trees were estimated using 2,000 bootstrap pseudoreplicates (Felsenstein 1985) in PHYLIP.

The Monmonier method in Barrier version 2.2 (Manni et al. 2004) was used to identify geographically continuous and discontinuous assemblages of sampling sites, independent from a priori knowledge of geographical population structure (e.g., lakes or river drainages). This procedure ranked each identified barrier in relative magnitude, according to respective support from individual locus θ_{ST} values. The spatial organization of the spawning aggregations was simulated by Voronoi tessellation modeling, and a Monmonier (1973) maximum-difference algorithm identified the borders between neighboring aggregations that exhibited the highest levels of genetic differences (Manni et al. 2004a, 2004b).

To evaluate distinctive population groups, a Bayesian-based clustering algorithm was used, as implemented in the program Structure version 2.3 (Pritchard et al. 2000; Pritchard and Wen 2004). This analysis assigned individual fish to one or more population groups, without prior knowledge of true sample identity. We tested the hypotheses of all samples composing a single population group of $K = 1$ (the null hypothesis of panmixia) to $K = 17$ (the hypothesis of each sampling location being an independent population group, thus equaling the total N of sampling sites), using 5 independent runs for each K, 100,000 burn-ins, and 500,000 replicates. We then examined consistency among runs, comparative probabilities of individuals assigning to one or more groups, and their respective grouping patterns. Optimal K values were determined with log likelihood of posterior probability values (Pritchard et al. 2000), and ΔK evaluations (Evanno et al. 2005), based on the rate of change in the log probability of data between successive K-values. The magnitude of ΔK over the replicate runs was graphed against K for $K = 2$–17, and the heights of the modal value of the distribution were used to additionally verify the correct K-value. Results of the Structure analyses then were compared with population relationships derived from the genetic divergence, 3DFCA, AMOVA, and Barrier analyses.

Results

Population Genetic Variability

Across all 17 populations sampled, a total of 404 alleles were detected at the 14 loci, whose total per population site ranged from a single allele at locus *Pfla*-L8 (mean = 8.9 alleles/population sample) to 31 alleles at locus *MPf*-2 (mean = 23.5 alleles/population sample); the overall mean was 28.9 alleles per locus (mean = 11.6 alleles/population sample; Table A.1 in the appendix). No significant gametic associations among loci were observed. The HW deviations (after Bonferroni correction) and heterozygote deficiencies

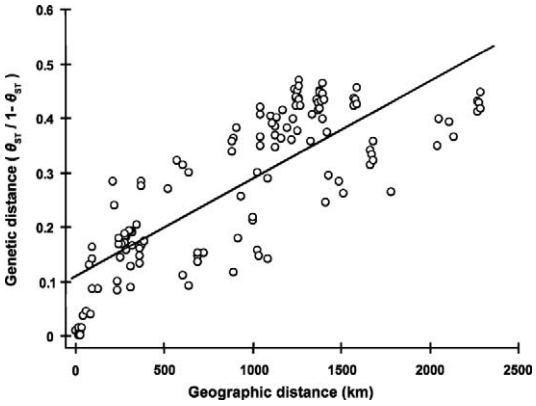

FIGURE 2.—Relationship between genetic distance and geographic distance among yellow perch sampling sites across North America ($y = 0.0001x + 0.0917$). The Mantel (1967) pairwise test showed significant differences ($R^2 = 0.795$, $P = 0.0001$).

indicated the presence of null alleles in loci *MPf*-1, *MPf*-2, *MPf*-4, *Pfla*-L3, *Pfla*-L4, *Pfla*-L8, and *Pfla*-L9, and thus those loci were excluded from further analyses.

The average expected heterozygosities (for the seven remaining loci) were similar in samples from the Midwest (range = 0.53–0.72, mean = 0.59) and the East Coast (range = 0.52–0.72, mean = 0.61; Table 1), and no significant differences were detected after a two-sample *t*-test ($P = 0.62$). Their mean numbers of alleles also were not significantly different ($P = 0.82$), the Midwest samples averaging 7.7 (range = 7.0–8.6) and the East Coast averaging 7.9 (7.3–8.6). Mean allelic richness was somewhat greater in the East Coast group (mean = 7.04, range = 6.4–7.8) than in the Midwest (mean = 6.7, range = 6.3–7.7; Table 1), but not significantly so ($P = 0.33$).

No significant differences were observed in the number of alleles or expected heterozygosities between males and females in each population, indicating that there were no sex-linked loci. Fisher's (1922) exact test of population differentiation as implemented by Rousset (2008) showed that no significant differences occurred in allelic compositions between the sexes; thus, males and females were analyzed together in all analyses.

Genetic Structure of Midwest and East Coast Yellow Perch

All yellow perch population samples significantly differed in pairwise tests, except for some θ_{ST} comparisons from Albemarle Sound (Table 3, below diagonal). All pairwise comparison methods between all samples yielded congruent results (Table 3, upper diagonal and shaded cells, respectively). Pairwise comparisons did not significantly differentiate between samples from the Little, Pasquotank, and Scuppernong rivers. One of the lowest θ_{ST} values observed occurred between the two year-classes in Lake Michigan (0.009), which were sampled on the same spawning reef. Yellow perch populations that were most diverged from all other sites tested were located in the Midwest, including Lac du Flambeau (average $\theta_{ST} = 0.337$), Lake Michigan 2002 (0.307), and Little Tail Point (0.303).

Pairwise comparisons between samples ranged from $\theta_{ST} = 0.469$ between Little Tail Point and the Scuppernong River, to less than 0.001 between the Little and Scuppernong rivers in Albemarle Sound. The mean divergence between the Midwest and East Coast population samples ($\theta_{ST} = 0.388$) was over twice that separating the Chesapeake Bay and Albemarle Sound samples (0.171). Samples from the Midwest diverged from one another by mean $\theta_{ST} = 0.138$, whereas those in the Chesapeake Bay differed by only 0.048, and those in Albemarle Sound by only 0.007. The hypothesis of genetic relatedness according to geographic distance was supported across the broad scale range of yellow perch (Figure 2), but was not supported for any fine-scale analyses, including separate tests of relatedness across Lake Michigan, Chesapeake Bay, and Albemarle Sound (regressions not shown).

The AMOVA analysis (Table 4) revealed two primary clusters of yellow perch populations, from the Midwest (Wisconsin, North Dakota, and Lake Michigan) and the East Coast (North Carolina and Maryland). These two primary clusters were further divided into three groups: Midwest, Chesapeake Bay, and Albemarle Sound, which were supported by the AMOVA (Table 4), 3DFCA analyses (Table 4, Figure

TABLE 4.—Distribution of genetic variability among and within yellow perch samples using Analysis of MOlecular VAriance for two main population groups (Midwest and East Coast) and three main population groups (Midwest, Chesapeake Bay, and Albemarle Sound). All comparisons were significant ($P < 0.001$).

Source of variation	Percent variation	Fixation index
Midwest and East Coast		
Between regions	73.80	$F_{CT} = 0.738$
Among sampling sites within regions	5.17	$F_{SC} = 0.197$
Within sampling sites	21.03	$F_{ST} = 0.790$
Midwest, Chesapeake Bay, and Albemarle Sound		
Among the three regions	70.61	$F_{CT} = 0.706$
Among sampling sites within regions	4.65	$F_{SC} = 0.158$
Within sampling sites	24.75	$F_{ST} = 0.753$

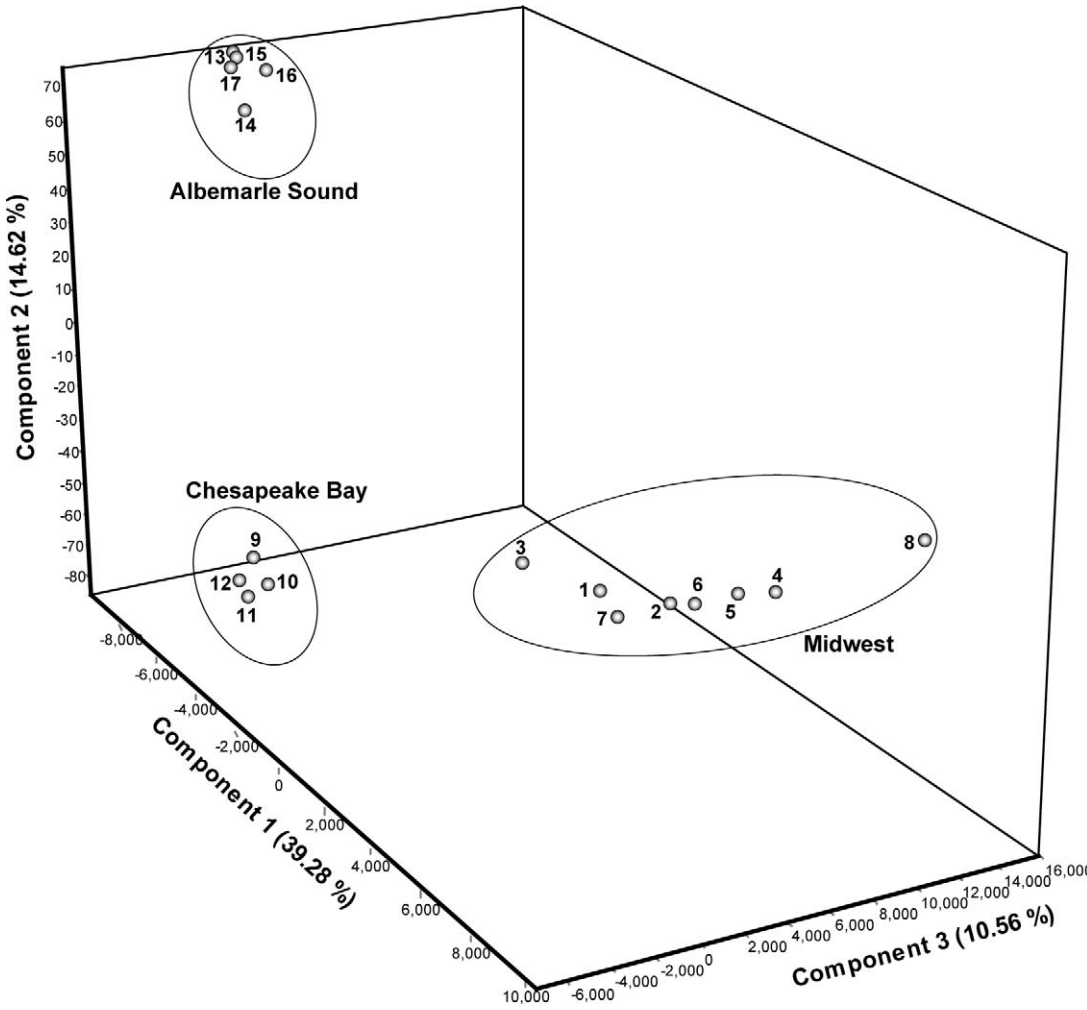

FIGURE 3.—Three-dimensional factorial correspondence analysis for yellow perch microsatellite data from seven loci showing three regional population clusters (see Figure 1 for specific locations).

3), and the neighbor-joining tree (Figure 4; primary clade separations between the Midwest group and the East Coast each had 100% bootstrap support). On the tree, divergent populations within these groups were supported for Lake Ontario (100%), the Bad River in Lake Superior (97%), and Albemarle Sound (100%).

Barrier analysis (map on Figure 1, barriers given by Roman numerals) was congruent in showing the primary population group division between the Midwest and East Coast groups (ranked I), further denoting the second largest genetic separation (ranked II) as isolating a Midwest group containing Devils Lake, Lac de Flambeau (Mississippi River drainage), and Bad River (Lake Superior drainage). The third barrier (III) then separated all samples in the Lake Michigan drainage from all others. The fourth significant genetic barrier (IV) separated the two East Coast population areas of the Chesapeake Bay and Albemarle Sound, which also is supported by the neighbor-joining tree (Figure 4).

Results from the Structure analysis (Figure 5) showed primary genetic separations, division of the Chesapeake Bay (green) and Albemarle Sound (pink) samples being evident at $K = 4$ (Figure 5A) and samples from the Midwest (red or light blue). At $K = 11$, further division occurred within the Chesapeake Bay system samples from Nanticoke River (orange) and the Choptank River (light blue), whereas samples from Albemarle Sound were undifferentiated from one another at all K-values. This close genetic relationship among all samples from Albemarle Sound samples also was supported by the neighbor-joining tree (Figure 4)

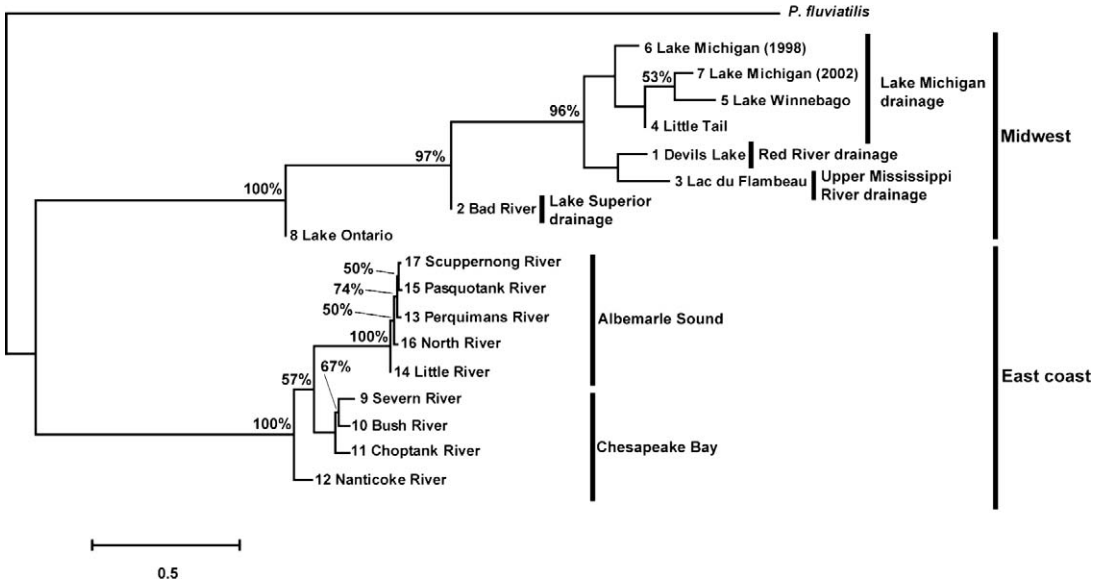

FIGURE 4.—Neighbor-joining tree of yellow perch population samples based on Nei's (1972) distance for seven microsatellite loci. Bootstrapping values indicate nodes supported by 50% or more of 1,000 resampling events. The lengths of the horizontal lines are proportional to the genetic distances.

and θ_{ST} analyses (Table 3). Within the Midwest group at $K = 11$, population samples were differentiated from Lake Ontario (purple), Bad River in the Lake Superior drainage (medium blue), and samples from the Lake Michigan drainage were linked (dark blue); those from Devils Lake and Lac du Flambeau appeared similar but were separate at $K = 15$ (Figure 5B). At $K = 15$, further divisions were apparent, samples from Lake Michigan proper being linked for the 2 years sampled (1998, 2002). The Structure analysis additionally showed that

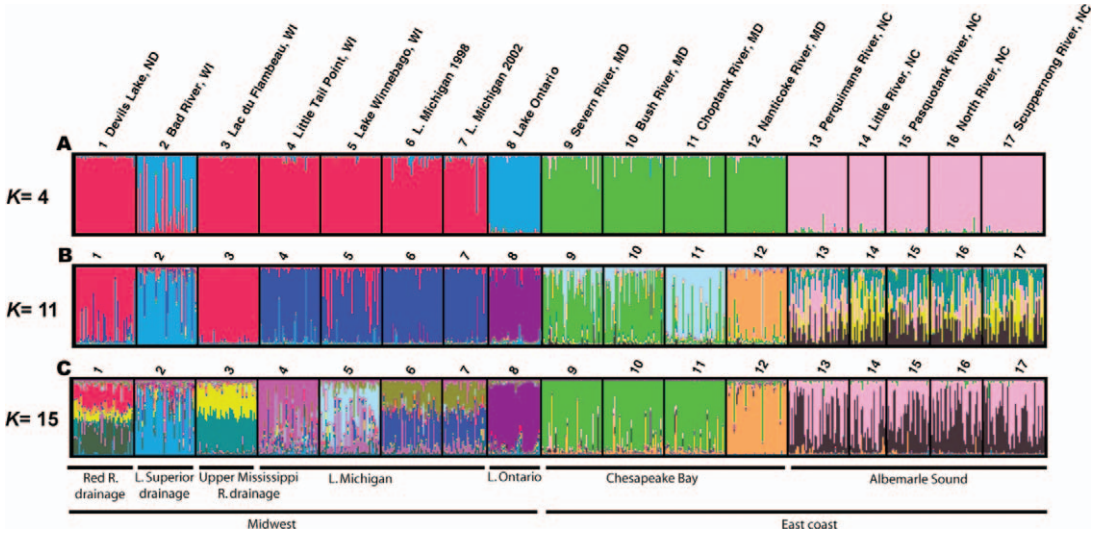

FIGURE 5.—Population groups of yellow perch determined from Bayesian Structure analysis (Pritchard et al. 2000; Pritchard and Wen 2004) for **(A)** $K = 4$ groups, **(B)** $K = 11$ groups, and **(C)** $K = 15$ groups. These were the three optimal runs of K determined from ΔK evaluations (Evanno et al. 2005), $K = 15$ having the highest mean posterior probability (0.999). Individuals are represented by the thin vertical lines, which are partitioned into K colored segments representing the individual's estimated membership fractions. The black lines separate the different samples.

within Chesapeake Bay, samples from the Choptank and Nanticoke rivers exhibited clear genetic distinctiveness, whereas those from the Severn and Bush rivers were more closely related to each other (Figure 5C). This analysis also illustrated closer genetic relationship among most samples from Albemarle Sound, except for the Perquimans River (Figure 5B, Table 3), which appeared genetically distinct.

Discussion

Our study is the first to use microsatellite DNA markers to examine the genetic variation within and among wild populations of yellow perch of the upper Midwest and eastern United States. Previous fine-scale studies of yellow perch using microsatellites revealed a lower number of alleles per locus (mean = 4.8, range = 2–16 [Li et al. 2006]; mean 8.7, range = 3.2–19.1 [Miller 2003]) than we observed (mean = 11.6, range = 6–23), which may have been due to the broader geographic range we sampled, the larger number of populations we sampled, or the loci we selected.

Midwest and East Coast Population Divergence

The greatest genetic structuring we found supported two main population groupings: the Midwest and the East Coast. This is consistent with results using allozymes by Todd and Hatcher (1993) and mtDNA control region sequences by Sepulveda-Villet et al. (2009), reflecting their historical separation in the Mississippian and Atlantic glacial refugia. The higher genetic divergences we found distinguishing Midwest populations than those found among the East Coast samples may be due to the fact that the Midwest populations are landlocked in lakes, whereas the East Coast populations may intermix via connections through the Chesapeake Bay and Albemarle Sound (Figure 1).

Midwest Yellow Perch Population Genetics

Large genetic separations apparent in our Midwest samples indicate isolation of those in the Mississippi River drainage (Bad River and Lac de Flambeau) from the Great Lakes and further distinguish the samples in Lake Michigan. The genetic distinction of the Lac du Flambeau population from other Midwest samples may be due to the fact that this lake is in the Mississippi River basin and separate from nearby populations in the Great Lakes basin. Additionally, the Lac du Flambeau sample shows similarities to that from Devils Lake in North Dakota, which drains to the Red River. Both Devils Lake and Lac du Flambeau were once contained within the glacial Lake Agassiz basin, which at one point drained to the Mississippi River system (Fisher 2003, 2004) and may have also drained towards the east, including a spillway adjacent to present-day Lake Superior (Leverington and Teller 2003). Our sample from Devils Lake had the lowest mean number of alleles per locus and allelic richness of all sites assessed, which was unexpected because it has been heavily managed from 1970 through the present and stocked with fry, fingerlings, and adult yellow perch from many other lakes (e.g., Lake Metigoshe, Gravel Lake, and Wood Lake) as well as from a hatchery whose brood source originated from several lakes in North Dakota (Randy Hiltner, North Dakota Department of Natural Resources, personal communication). However, it is also possible that there were smaller glacial refugia in the Upper Plains regions, which harbored populations that became isolated. If fish stockings were unsuccessful in Devils Lake, then its low genetic diversity may have resulted from bottlenecking of founding populations, leading to its modest genetic variation today.

The Bad River population, which is connected to Lake Superior, also appeared genetically distinct from other Wisconsin samples. In general, yellow perch populations in Lake Superior are small and restricted to specific nearshore areas and bays (Bronte et al. 1993); thus, because there are no basinwide yellow perch populations to mix with, those from the Bad River appear very isolated.

The sites sampled in Lake Michigan (two year-classes and Little Tail in Green Bay) were more similar to one other than to other Midwest populations. However, as also documented by Miller (2003), yellow perch from Green Bay are genetically distinct from those in Lake Michigan's main basin. Our Lake Michigan basin samples had relatively low expected heterozygosities (0.53 and 0.55), which may reflect the small number of founders that gave rise to the current yellow perch population after its collapse in the 1990s (Francis et al. 1996).

We found yellow perch from Lake Ontario were very distinct from both Midwest and East Coast populations, although they grouped with the former; a result that also was observed by Todd and Hatcher (1993). The fish fauna of all the Great Lakes, except Lake Ontario, are believed to have originated from the Mississippi River glacial refugium (Bailey and Smith 1981). In contrast, Lake Ontario is believed to have been a zone of secondary contact between the Mississippi and Atlantic refugia and may have been populated via the Susquehanna River drainage. Thus, the distinctness of this population may be the result of yellow perch descent from two refugia (Todd and Hatcher 1993).

East Coast Yellow Perch Population Genetics

The East Coast population samples had higher than expected allelic heterozygosities compared with those from the Midwest, which may be due to the mixing of yellow perch within the Chesapeake Bay area and Albemarle Sound with those from other locations. Yellow perch can tolerate salinities up to 10‰ (Collette et al. 1977) and, therefore, at times they could theoretically move through these bodies of water from one river to another (see below). This appears more likely for yellow perch from Albemarle Sound because the sites we sampled in Chesapeake Bay all differed significantly, yet those from the Albemarle Sound were more closely related. In contrast, the Midwest populations are landlocked in lakes, which led to pronounced divergence among them. Alternatively, this higher variation along the central East Coast region may be historical; that is, fish and other populations in areas that were unglaciated generally have higher levels of genetic diversity than populations to the north that recolonized from glacial refugia and thereby experienced bottlenecks and founder effect (see Billington and Hebert 1991; Bernatchez and Wilson 1998).

Not surprisingly, there are two main groupings within the East Coast samples: Chesapeake Bay (Severn, Bush, Nanticoke, and Choptank rivers), and Albemarle Sound (Perquimans, Scuppernong, North, Pasquotank, and Little rivers). These two systems have experienced several cycles of water level fluctuations since the last glaciations, inferred sea levels being as low as 30 m below current levels in Albemarle Sound (Parham et al. 2007). Cyclical sea level fluctuations also changed landscape features, coastal transgression characterizing most of Chesapeake Bay (Hobbs 2004); the absence of barrier islands in the northern portion of the Albemarle Sound during the late Pleistocene (Mallinson et al. 2005) probably contributed to a more saline environment than the present and restricted passage of yellow perch from one estuarine system to the next. The Chesapeake Bay area has been heavily managed since the mid-1900s (Yellow Perch Work Group 2002). From 1940–1955, yellow perch were stocked in the Severn River at an average of 68.25×10^6 fry/year. From 1940–1952 the Choptank River was stocked at much lower rates of 13.1 million/year. In addition, in 1992 juveniles and fingerlings were again stocked into the Severn River. Stocking of several rivers with adult yellow perch in spawning conditions also was attempted from 1989 to 1991, but based on the movement of tagged yellow perch, they apparently did not stay in the stocked rivers (Yellow Perch Work Group 2002) and, therefore, are unlikely to have influenced the genetics of the resident populations.

Within the Chesapeake Bay, Structure analysis suggests mixing among certain riverine populations, including the Severn and Bush rivers. In contrast, riverine samples in the lower Chesapeake Bay appear genetically distinctive from each other (i.e., Chopank and Nanticoke rivers), as well as from those in the upper bay. Even though the Severn River was heavily stocked in the past (see above), the Bush River was not the source. In addition, the Bush River was never stocked itself. Thus, how are the population samples within these rivers related? A possibility is that fish move between them, given the close proximity between their outflows (Figure 1). In the upper area of the Chesapeake Bay, movement of yellow perch would theoretically be unimpeded because both rivers are in the upper oligohaline region of the Chesapeake, where salinity is fairly low (Gibson and Najjar 2000). In contrast, even though the outflows of the Choptank and the Nanticoke rivers are also close to one another, they open into the mesohaline region of the Chesapeake (Gibson and Najjar 2000), where salinity is higher and perhaps prohibitive to yellow perch movement, except under conditions of high tributary discharge. In accordance with this, the yellow perch in the Choptank and Nanticoke rivers appear to be genetically distinct (Figure 5B).

In contrast, the Albemarle Sound populations (Pasquotank, Little, Scuppernong, and North rivers) appear to be relatively well mixed, with only small genetic distances (0.006–0.011) detected between most of them (Figure 4). They also have some of the highest F_{IS} values of 0.02–0.13, suggesting inbreeding, and are the only sites in the study without significant pairwise θ_{ST} comparisons. In contrast to the Chesapeake Bay, the Albemarle Sound is somewhat separated from the Atlantic Ocean by North Carolina's Outer Banks, and salinities within the sound are low, ranging from 0 to 7‰, about 1‰ being characteristic of the estuary (Bowden and Hobbie 1977). In addition, massive storms, such as Hurricane Floyd in 1999 (Bales 2003), periodically flood the entire estuary further decreasing the salinity and probably facilitating the mixing of fish populations. Thus, the close relatedness of yellow perch samples in the Albemarle Sound appears reasonable and suggests that there may only be a single management unit within the sound.

Summary

We found that the largest genetic divergence separated yellow perch population groups of the Midwest and East Coast, and we found further partitioning between upper Mississippi River and Great Lakes sites in the Midwest and between the Chesapeake Bay and Albemarle Sound in the East Coast.

Additional genetic separations characterized these groups, except for those from Albemarle Sound, which probably experience more and regular gene flow. Based on the genetic information presented here, most of the Chesapeake Bay populations appear diverse enough to use in beginning a selective breeding population, because at least three populations had high observed heterozygosities (the Bush, Choptank, and Nanticoke rivers). In addition, if multiple broodstocks were derived from the Chesapeake populations, at least two genetically distinct stocks could be obtained according to the Structure analysis (e.g., the Choptank and Nanticoke rivers). Care should be taken with the Albemarle Sound population sites given the amount of inbreeding that is indicated because they had the highest F_{IS} values and low genetic differentiation. In the Midwest, the Bad River population had high heterozygosity, and populations from Lake Ontario had high observed heterozygosity and low levels of inbreeding; thus, they may be a promising source for broodstock development. In contrast, populations such as those in Lake Michigan should be avoided because of their lower heterozygosities and possible bottleneck and founder effects.

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Appendix follows

Appendix: Microsatellite Analysis of Yellow Perch

TABLE A.1.—Microsatellite analysis of the genetic variation in 17 wild populations of yellow perch from the Midwest and East Coast. The observed number of alleles (A), number and proportion of private alleles (A_P) observed (H_O) and expected (H_E) heterozygosities, and probability values (P) for Hardy–Weinberg equilibrium (HWE) are provided for each locus and population. Locations are as follows: (1) Devils Lake, (2) Bad River, (3) Lac du Flambeau, (4) Little Tail Point, (5) Lake Winnebago, (6) Lake Michigan in 1998, (7) Lake Michigan in 2002, (8) Lake Ontario, (9) Severn River, (10) Bush River, (11) Choptank River, (12) Nanticoke River, (13) Perquimans River, (14) Little River, (15) Pasquotank River, (16) North River, and (17) Scuppernong River (see Figure 1 and Table 1).

Locus and statistic	Midwest populations							
	1	2	3	4	5	6	7	8
Pfla-L2								
A	8	8	5	7	8	6	9	5
A_P	0	0	0	0	1 (0.010)	0	0	0
H_O	0.650	0.825	0.363	0.160	0.313	0.564	0.596	0.574
H_E	0.667	0.813	0.375	0.167	0.343	0.583	0.667	0.610
HWE	0.377	0.294	0.588	1.000	1.000	0.934	0.461	0.743
F_{IS}	−0.026	0.015	−0.034	−0.044	−0.097	−0.035	−0.119	−0.062
Pfla-L3								
A	11	15	14	17	15	12	14	20
A_P	0	0	0	0	0	0	0	3 (0.048)
H_O	0.681	0.521	0.463	0.529	0.724	0.791	0.667	0.896
H_E	0.827	0.649	0.767	0.800	0.871	0.851	0.780	0.844
HWE	0.193	0.093	0.000	0.000	0.003	0.000	0.000	0.577
F_{IS}	0.177	0.197	0.396	0.338	0.168	0.071	0.146	−0.062
Pfla-L4								
A	15	20	13	16	16	16	16	16
A_P	1 (0.010)	2 (0.064)	0	0	0	0	0	0
H_O	0.830	0.522	0.659	0.588	0.414	0.237	0.229	0.313
H_E	0.860	0.837	0.821	0.852	0.928	0.867	0.950	0.906
HWE	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000
F_{IS}	0.035	0.377	0.198	0.309	0.554	0.727	0.759	0.655
Pfla-L5	1	2	3	4	5	6	7	8
A	5	3	7	5	4	4	4	6
A_P	0	0	0	0	0	0	0	0
H_O	0.332	0.448	0.545	0.541	0.471	0.309	0.122	0.662
H_E	0.292	0.458	0.479	0.500	0.600	0.362	0.042	0.683
HWE	0.010	0.940	0.059	0.574	0.302	0.752	0.001	0.788
F_{IS}	0.122	−0.022	0.120	0.075	−0.273	−0.170	0.658	−0.031
Pfla-L6								
A	8	6	6	9	7	7	9	9
A_P	0	0	0	0	0	1 (0.010)	0	2 (0.024)
H_O	0.534	0.625	0.694	0.430	0.476	0.481	0.626	0.640
H_E	0.521	0.667	0.750	0.458	0.429	0.458	0.708	0.595
HWE	0.681	0.560	0.688	0.180	0.356	0.354	0.234	0.419
F_{IS}	0.025	−0.067	−0.081	−0.065	0.100	0.047	−0.131	0.070
Pfla-L8								
A	2	5	1	3	1	1	2	1
A_P	1 (0.011)	1 (0.022)	0	0	0	0	1 (0.014)	0
H_O	0.575	0.723	0.625	0.906	0.931	0.622	0.638	0.667
H_E	0.930	0.858	0.868	0.895	0.921	0.834	0.825	0.859
HWE	0.000	0.002	0.000	0.171	0.915	0.000	0.000	0.000
F_{IS}	0.381	0.157	0.280	−0.012	−0.011	0.254	0.226	0.224
Pfla-L9								
A	4	11	7	12	11	7	10	10
A_P	0	1 (0.031)	1 (0.021)	0	1 (0.011)	0	1 (0.014)	1 (0.088)
H_O	0.636	0.500	0.282	0.588	0.280	0.478	0.750	0.521
H_E	0.863	0.470	0.462	0.767	0.359	0.683	0.809	0.646
HWE	0.000	0.581	0.000	0.000	0.228	0.000	0.004	0.000
F_{IS}	0.263	−0.064	0.389	0.233	0.220	0.299	0.073	0.194
MPf-1	1	2	3	4	5	6	7	8
A	18	20	21	22	23	19	20	26
A_P	1 (0.031)	0	0	0	0	0	0	0
H_O	0.591	0.404	0.585	0.485	0.517	0.744	0.583	0.771
H_E	0.890	0.929	0.921	0.886	0.913	0.796	0.835	0.887
HWE	0.000	0.000	0.000	0.000	0.000	0.783	0.000	0.186
F_{IS}	0.336	0.565	0.364	0.453	0.434	0.065	0.301	0.131

TABLE A.1.—Extended.

Locus and statistic	East Coast populations									Mean
	9	10	11	12	13	14	15	16	17	
Pfla-L2										
A	13	11	9	13	17	12	15	10	11	9.824
A_P	0	0	0	0	1 (0.043)	0	0	0	0	
H_O	0.873	0.889	0.794	0.817	0.866	0.844	0.817	0.859	0.816	0.683
H_E	0.826	0.875	0.907	0.833	0.915	0.655	0.794	0.821	0.596	0.673
HWE	0.187	0.395	0.000	0.365	0.073	0.000	0.313	0.033	0.006	
F_{IS}	0.054	0.016	−0.143	−0.020	−0.057	0.224	0.028	0.045	0.270	0.001
Pfla-L3										
A	14	13	12	7	12	13	14	12	10	13.235
A_P	0	1 (0.010)	0	0	0	0	1 (0.029)	1 (0.012)	0	
H_O	0.604	0.792	0.813	0.771	0.500	0.723	0.857	0.604	0.595	0.678
H_E	0.888	0.802	0.859	0.870	0.785	0.856	0.885	0.888	0.754	0.822
HWE	0.000	0.905	0.063	0.064	0.000	0.053	0.213	0.000	0.000	
F_{IS}	0.320	0.013	0.054	0.114	0.363	0.155	0.031	0.320	0.210	0.177
Pfla-L4										
A	18	15	17	22	15	16	14	11	15	15.941
A_P	2 (0.066)	0	1 (0.026)	0	0	0	0	0	0	
H_O	0.733	0.778	0.894	0.146	0.771	0.192	0.257	0.458	0.171	0.482
H_E	0.911	0.927	0.937	0.934	0.880	0.924	0.911	0.887	0.880	0.895
HWE	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
F_{IS}	0.195	0.161	0.046	0.844	0.124	0.793	0.718	0.483	0.806	0.458
Pfla-L5	9	10	11	12	13	14	15	16	17	
A	7	8	8	7	9	9	8	9	8	6.529
A_P		1 (0.010)	0	0	1 (0.010)	0	0	0	0	
H_O	0.652	0.760	0.353	0.390	0.725	0.770	0.732	0.823	0.766	0.553
H_E	0.583	0.729	0.354	0.383	0.958	0.621	0.677	0.781	0.688	0.541
HWE	0.102	0.539	0.389	0.613	0.022	0.059	0.252	0.320	0.041	
F_{IS}	0.105	0.040	−0.004	0.018	−0.323	0.194	0.076	0.052	0.102	0.043
Pfla-L6										
A	13	12	8	11	10	7	8	9	9	8.706
A_P	0	0	0	0	0	0	0	0	0	
H_O	0.862	0.836	0.767	0.861	0.743	0.703	0.610	0.688	0.577	0.656
H_E	0.708	0.729	0.783	0.875	0.604	0.517	0.515	0.625	0.625	0.622
HWE	0.039	0.451	0.989	0.358	0.000	0.033	0.079	0.138	0.988	
F_{IS}	0.178	0.128	−0.021	−0.016	0.187	0.264	0.156	0.091	−0.084	0.046
Pfla-L8										
A	15	15	11	12	17	17	16	16	12	8.647
A_P	2 (0.098)	0	1 (0.056)	1 (0.011)	0	0	0	0	0	
H_O	0.913	0.129	0.422	0.000	0.021	0.000	0.029	0.000	0.000	0.424
H_E	0.865	0.124	0.392	0.000	0.021	0.000	0.029	0.000	0.000	0.495
HWE	0.000	1.000	0.593	–	–	–	–	–	–	
F_{IS}	−0.056	−0.039	−0.078	–	0.000	–	0.000	–	–	0.102
Pfla-L9										
A	12	16	14	15	20	7	14	10	11	11.235
A_P	1 (0.011)	0	0	3 (0.147)	2 (0.182)	0	0	0	0	
H_O	0.318	0.542	0.750	0.745	0.542	0.575	0.543	0.750	0.775	0.563
H_E	0.498	0.592	0.807	0.715	0.559	0.590	0.552	0.720	0.833	0.643
HWE	0.000	0.090	0.250	0.361	0.643	0.611	0.108	0.125	0.512	
F_{IS}	0.361	0.085	0.070	−0.041	0.032	0.027	0.016	−0.042	0.070	0.129
MPf-1	9	10	11	12	13	14	15	16	17	
A	14	18	8	19	20	16	20	24	21	19.353
A_P	0	1 (0.052)	0	3 (0.073)	1 (0.011)	0	0	1 (0.012)	1 (0.011)	
H_O	0.761	0.813	0.854	0.854	0.729	0.745	0.829	0.787	0.643	0.688
H_E	0.880	0.938	0.905	0.944	0.897	0.939	0.935	0.929	0.956	0.905
HWE	0.095	0.003	0.258	0.066	0.000	0.001	0.016	0.009	0.000	
F_{IS}	0.136	0.133	0.056	0.095	0.187	0.207	0.114	0.152	0.327	0.239

TABLE A.1.—Continued.

Locus and statistic	Midwest populations							
	1	2	3	4	5	6	7	8
MPf-2								
A	22	26	20	25	26	28	25	27
A_P	0	0	0	0	0	0	0	0
H_O	0.813	0.833	0.750	0.800	0.750	0.833	0.813	0.810
H_E	0.935	0.952	0.943	0.955	0.949	0.952	0.949	0.958
HWE	0.006	0.004	0.000	0.000	0.011	0.102	0.068	0.004
F_{IS}	0.131	0.124	0.205	0.162	0.209	0.125	0.144	0.155
MPf-3								
A	8	12	11	12	9	11	7	10
A_P	0	0	1 (0.010)	2 (0.020)	0	1 (0.011)	0	0
H_O	0.734	0.872	0.660	0.771	0.729	0.625	0.498	0.605
H_E	0.688	0.854	0.604	0.783	0.600	0.583	0.489	0.643
HWE	0.235	0.582	0.498	1.000	0.005	0.346	0.653	0.954
F_{IS}	0.063	0.020	0.084	−0.016	0.177	0.067	0.016	−0.062
MPf-4								
A	12	13	14	16	13	7	9	16
A_P	0	0	0	0	0	0	0	1 (0.013)
H_O	0.702	0.646	0.537	0.735	0.577	0.604	0.646	0.617
H_E	0.857	0.829	0.841	0.869	0.825	0.890	0.780	0.741
HWE	0.000	0.000	0.000	0.055	0.000	0.000	0.033	0.073
F_{IS}	0.181	0.221	0.362	0.154	0.300	0.321	0.172	0.167
MPf-5	1	2	3	4	5	6	7	8
A	4	11	8	7	8	9	7	10
A_P	0	0	1 (0.011)	0	0	0	0	0
H_O	0.361	0.753	0.344	0.493	0.527	0.396	0.610	0.719
H_E	0.354	0.717	0.298	0.553	0.529	0.396	0.542	0.732
HWE	0.255	0.791	0.026	0.755	0.564	0.252	0.118	0.132
F_{IS}	0.018	0.048	0.134	−0.121	−0.005	0.000	0.111	−0.018
MPf-6								
A	5	5	7	6	8	4	6	6
A_P	0	0	0	1 (0.010)	0	0	3 (0.628)	0
H_O	0.583	0.625	0.543	0.569	0.585	0.712	0.517	0.481
H_E	0.617	0.604	0.583	0.688	0.514	0.813	0.583	0.452
HWE	0.299	0.721	1.000	0.413	0.027	0.043	0.846	0.763
F_{IS}	−0.058	0.034	−0.073	−0.209	0.121	−0.141	−0.129	0.059
MPf-7								
A	11	13	10	12	8	8	8	14
A_P	0	0	0	0	0	0	0	0
H_O	0.778	0.908	0.718	0.763	0.749	0.868	0.831	0.876
H_E	0.688	0.708	0.750	0.896	0.743	0.854	0.792	0.846
HWE	0.120	0.000	0.237	0.735	0.895	0.182	0.164	0.551
F_{IS}	0.116	0.220	−0.044	−0.174	0.008	0.016	0.047	0.034

TABLE A.1.—Extended. Continued.

Locus and statistic	East Coast populations									Mean
	9	10	11	12	13	14	15	16	17	
MPf-2										
A	23	31	20	23	19	22	20	19	20	23.294
A_P	0	0	0	0	0	0	0	0	0	
H_O	0.667	0.708	0.909	0.646	0.659	0.714	0.618	0.561	0.604	0.735
H_E	0.923	0.953	0.933	0.782	0.946	0.953	0.941	0.886	0.888	0.929
HWE	0.000	0.007	0.000	0.060	0.000	0.000	0.000	0.001	0.000	
F_{IS}	0.277	0.257	0.026	0.174	0.303	0.251	0.344	0.367	0.320	0.210
MPf-3										
A	3	3	4	3	4	6	5	5	5	6.941
A_P	0	0	0	0	0	1 (0.052)	0	0	0	
H_O	0.585	0.608	0.543	0.567	0.230	0.368	0.294	0.269	0.283	0.544
H_E	0.500	0.604	0.521	0.646	0.250	0.379	0.294	0.220	0.313	0.528
HWE	0.420	1.000	0.686	0.572	1.000	0.455	0.619	0.116	1.000	
F_{IS}	0.145	0.006	0.040	−0.138	−0.088	−0.032	0.000	0.184	−0.104	0.021
MPf-4										
A	18	10	15	10	11	10	12	10	9	12.059
A_P	2 (0.020)	0	1 (0.010)	0	0	0	0	0	0	
H_O	0.688	0.479	0.625	0.646	0.563	0.479	0.743	0.750	0.425	0.615
H_E	0.903	0.781	0.796	0.857	0.833	0.632	0.763	0.744	0.676	0.801
HWE	0.000	0.000	0.039	0.003	0.000	0.035	0.148	0.248	0.000	
F_{IS}	0.239	0.387	0.215	0.246	0.325	0.242	0.027	−0.008	0.371	0.231
MPf-5	9	10	11	12	13	14	15	16	17	
A	12	13	10	10	8	11	9	8	9	9.059
A_P	0	1 (0.011)	0	0	0	0	0	0	0	
H_O	0.722	0.762	0.864	0.682	0.671	0.629	0.669	0.653	0.619	0.616
H_E	0.458	0.787	0.833	0.563	0.813	0.615	0.706	0.700	0.708	0.606
HWE	0.000	0.227	0.411	0.001	0.558	0.633	0.292	0.338	0.558	
F_{IS}	0.365	−0.033	0.035	0.175	−0.212	0.021	−0.055	−0.072	−0.144	0.015
MPf-6										
A	5	5	9	6	6	6	7	4	5	5.882
A_P	0	0	2 (0.020)	0	1 (0.010)	0	0	0	0	
H_O	0.298	0.586	0.567	0.680	0.363	0.434	0.465	0.246	0.298	0.503
H_E	0.292	0.958	0.792	0.896	0.396	0.483	0.471	0.268	0.292	0.571
HWE	0.613	0.000	0.096	0.000	0.595	0.594	0.907	1.000	0.084	
F_{IS}	0.020	−0.635	−0.396	−0.317	−0.092	−0.114	−0.012	−0.092	0.020	−0.118
MPf-7										
A	5	5	3	5	6	5	6	6	4	7.588
A_P	0	1 (0.010)	0	0	0	0	1 (0.029)	0	0	
H_O	0.345	0.594	0.578	0.634	0.296	0.405	0.384	0.547	0.308	0.622
H_E	0.354	0.438	0.404	0.604	0.244	0.345	0.206	0.439	0.333	0.567
HWE	0.031	0.000	0.000	0.031	0.086	0.297	0.001	0.289	0.642	
F_{IS}	−0.027	0.264	0.301	0.047	0.175	0.149	0.463	0.198	−0.081	0.101