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Genetic Population Structure of Muskellunge in the Great Lakes

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ARTICLE

Genetic Population Structure of Muskellunge in the Great Lakes

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Abstract

We quantified genetic relationships among Muskellunge *Esox masquinongy* from 15 locations in the Great Lakes to determine the extent and distribution of measurable population structure and to identify appropriate spatial scales for fishery management and genetic conservation. We hypothesized that Muskellunge from each area represented genetically distinct populations, which would be evident from analyses of genotype data. A total of 691 Muskellunge were sampled (*n* = 10–127/site) and genetic data were collected at 13 microsatellite loci. Results from a suite of analyses (including pairwise genetic differentiation, Bayesian admixture prediction, analysis of molecular variance, and tests of isolation by distance) indicated the presence of nine distinct genetic groups, including two that were approximately 50 km apart. Geographic proximity and low habitat complexity seemed to facilitate genetic similarity among areas, whereas Muskellunge from areas of greater habitat heterogeneity exhibited high differentiation. Muskellunge from most areas contained private alleles, and mean within-area genetic variation was similar to that reported for other freshwater fishes. Management programs aimed at conserving the broader diversity and long-term sustainability of Muskellunge could benefit by considering the genetically distinct groups as independent fisheries, and individual spawning and nursery habitats could subsequently be protected to conserve the evolutionary potential of Muskellunge.

Conservation of global biodiversity requires the preservation of ecosystems, species, and their genes (IUCN 2011), so information on within-species genetic diversity and spatial structure is essential to conservation practitioners. The Esocidae, which are fishes native to the freshwaters of Asia, Europe, and North America, have been considered to contain lower levels of within-population diversity than other freshwater fishes based on studies of Northern Pike *Esox lucius* (Hansen et al. 1999; Senanan and Kapuscinski 2000; Miller and Senanan 2003). The genetic diversity and structure of the Muskellunge *E. masquinongy*, the largest member of Esocidae, has not been described across its native range. The Muskellunge is a long-lived species (up to 30 years; Casselman et al. 1999) with overlapping generations (Scott and Crossman 1998) and is the apex aquatic predator throughout its native range in eastern North America, which includes nearshore habitats of the Great Lakes. Unfortunately, the Muskellunge has suffered significant population declines and local extinctions due in part to overexploitation and the
ongoing degradation and loss of spawning and nursery habitats (Whillans 1979; Trautman 1982; Farrell et al. 2007; Kapuscinski et al. 2007). Despite these population declines, Muskellunge are still economically important as sport fish (Menz and Wilton 1983; New York Power Authority 2005; Simonson 2008) and are managed by multiple environmental agencies in Canada and the USA.

Contemporary Muskellunge populations typically occur at low densities and are difficult to sample, so substantial information on Muskellunge reproductive biology, genetic diversity, and stock structure are lacking. The Muskellunge is a spring spawner that broadcasts eggs near shore (<1.5-m depth) among submerged aquatic vegetation and provides no parental care (Farrell et al. 1996; Scott and Crossman 1998; Zorn et al. 1998; Farrell 2001). Adult Muskellunge implanted with radio transmitters have been shown to move up to 98 km away from spawning sites postspawn (LaPan et al. 1996), and several researchers have captured tagged Muskellunge at the same spawning location in multiple years (Crossman 1990; Farrell et al. 2007; Jennings et al. 2011). Natal philopatry is assumed to be the mechanism that maintains genetic spatial structure of other vagile fishes in the Great Lakes such as the Lake Sturgeon *Acipenser fulvescens* (DeHaan et al. 2006) and Walleye *Sander vitreus* (Stepien et al. 2009), but it is unknown if spawning-site fidelity by Muskellunge is natal philopatry that influences genetic spatial structure. Although many advances in molecular techniques have recently been made, only a single published study has attempted to examine genetic diversity among several Muskellunge populations from a wide geographic range (Koppelman and Philipp 1986). Koppelman and Philipp (1986) assessed genetic diversity of Muskellunge from nine potential populations (five wild and four hatchery populations) using 10 polymorphic isozyme loci. While their study was limited by small sample sizes (*n* = 2–26 per potential population) and the use of isozyme loci that have largely been replaced by microsatellite loci in studies of genetic population structure, Koppelman and Philipp (1986) were able to demonstrate the existence of genetic variation among populations. However, they considered their study preliminary because of these aforementioned limitations and called for a more extensive genetic analysis that has not yet been conducted. As a consequence of the lack of information on the genetic structure of Muskellunge, fish have been stocked across drainage boundaries and Muskellunge in large bodies of water are typically managed as single stocks, even where demographic evidence for intrasystem stock structure exists (Haas 1978; Moordadian et al. 1986; Strand 1986; Bryant and Smith 1988; Crossman 1990; Farrell et al. 2007).

Stocking Muskellunge has been a common management action in many areas of the Great Lakes to supplement or reintroduce populations. For example, the upper Niagara River was stocked with 408,000 Muskellunge fry during 1941–1955 that were progeny of Chautauqua Lake (New York) Muskellunge and 18,425 fingerlings during 1960–1974 that were progeny of Stony Lake (Ontario) Muskellunge (M. Wilkinson, New York State Department of Environmental Conservation, personal communication). A total of 553,800 Muskellunge fry (<50 mm), 682,081 fingerlings (50–270 mm), and 195 juveniles (>270 mm) also were stocked in Lac des Deux Montagnes and the Montreal region of the St. Lawrence River during 1950–1997; fish of known origin were progeny of Chautauqua Lake (New York) Muskellunge (Y. de Lafontaine, Environment Canada, personal communication). In addition, 119,000 Muskellunge fry (mean length = 24 mm) and 763 fingerlings (mean length = 76 mm) that were progeny of locally caught, wild fish were stocked into a total of 10 sites on the St. Lawrence River during 1990–1992, 1994, and 1996 for an experiment (Farrell and Werner 1999). Finally, the Fox River (a tributary to Green Bay, Lake Michigan) was stocked with progeny of Muskellunge captured from the Indian River Spreads (northcentral Michigan) and Lake St. Clair in most years since 1989 as part of a reintroduction program (Kapuscinski et al. 2007). Although stocking has been widespread, effects on the integrity of the Muskellunge genome have not been quantified. Assessment of the spatial genetic structure of Muskellunge populations is an important first step toward genetic conservation and understanding what effects, if any, stocking has had on the genome (Koppelman and Philipp 1986).

Losses of genetically distinct Muskellunge populations, whether caused by introgression with stocked fish or failures to protect populations and their critical habitats, could ultimately reduce the species’ ability to adapt to environmental changes. Therefore, sound management plans for Muskellunge could usefully consider genetic issues such as differentiation among spawning groups, conservation of rare alleles, and inbreeding depression. The objective of our study was to determine if inter-population diversity and genetic spatial structure exists among the putative Muskellunge populations in the Great Lakes and to quantify this diversity and structure to inform management plans that will be effective at conserving the species’ evolutionary potential. In addition, we discuss how patterns in genetic structure relate to the results of Muskellunge movement studies that indicated reproductive homing and how within-population diversity of Muskellunge compares to other freshwater fishes. The genetic variation of potential Muskellunge populations was compared at 13 microsatellite loci; microsatellite loci were chosen because (1) they are highly polymorphic and therefore ideal for analyses of genetic variation among small potential populations (Allendorf and Luikart 2007), (2) they allowed for nonlethal sampling, and (3) developed markers being used in multiple locations across the Muskellunge native range were available (Sloss et al. 2008a). It was our aim that the results of this study would benefit resource managers by identifying appropriate scales for fishery management and protection of spawning and nursery habitats and by determining population boundaries, across which stocking could risk disrupting potential locally adapted gene complexes.
METHODS

Sampling locations.—Muskellunge were collected from 15 areas throughout the Great Lakes drainage, including (1) the Fox River, a tributary to Green Bay, Lake Michigan; (2) Pointe Au Baril, Georgian Bay, Lake Huron; (3) Moon River, Georgian Bay, Lake Huron; (4) Severn Sound, Georgian Bay, Lake Huron; (5) Lake St. Clair; (6) Buffalo Harbor, Lake Erie; (7) the upper Niagara River; (8) the lower Niagara River; (9) the Thousand Islands region of the St. Lawrence River; (10) Blind Bay, a known spawning location in the eastern Thousand Islands region of the St. Lawrence River; (11) Garlock Bay, a known spawning location at the eastern edge of the Thousand Islands region of the St. Lawrence River; (12) the St. Lawrence River below the dam at Massena, New York (hereafter Massena); (13) the Grasse River, a tributary to the St. Lawrence River; (14) Lac des Deux Montagnes, Quebec; and (15) the St. Lawrence River near Montreal, Quebec (Table 1; Figure 1). The geographic area of sampling localities ranged from point locations (e.g., trap nets set within Blind Bay and Garlock Bay) to an approximately 30-km-long stretch of the St. Lawrence River within the Thousand Islands region. We hypothesized that each area contained at least one spawning group that was distinct from all others, and we refer to these groups as populations.

Sample collection and storage.—Muskellunge were captured throughout the open-water season by angling, electrofishing, and trap-netting. Tissue samples, collected as scales or fin clips, were either allowed to dry in individually labeled coin envelopes or immediately placed in vials containing non-denatured 100% ethanol. Attempts were made to collect ≥50 Muskellunge from each area, but low capture numbers often made this impractical. Therefore, sample sizes ranged from 10 (Massena) to 127 (Lake St. Clair) individuals.

DNA extraction and microsatellite genotyping.—All DNA extractions and microsatellite genotyping were conducted by the Molecular Conservation Genetics Laboratory, College of Natural Resources, University of Wisconsin–Stevens Point. Genomic DNA from individual tissue samples was extracted using the Promega Wizard Genomic DNA purification kit (Promega, Madison, Wisconsin) modified for 96-well extractions with resuspension of the DNA in 100 μl of tris-low-EDTA buffer solution (TLE; 10mM NaCl, 0.1 mM EDTA, pH 8.0). Extracted DNA was quantified using a Nanodrop ND-1000 spectrophotometer (Nanodrop Technologies, Wilmington, Delaware). All DNA samples were normalized to a final concentration of 20 ng/μL prior to genotyping. Thirteen of the 14 microsatellite loci described by Sloss et al. (2008a) were used to genotype individual

<table>
<thead>
<tr>
<th>Population</th>
<th>Latitude</th>
<th>Longitude</th>
<th>N</th>
<th>$H_e$</th>
<th>$H_e$ SD</th>
<th>$H_o$</th>
<th>$H_o$ SD</th>
<th>A</th>
<th>A SD</th>
<th>A_r</th>
<th>PA</th>
<th>PA_r</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fox River (Lake Michigan)</td>
<td>44°28′36.07″N</td>
<td>88°02′35.73″W</td>
<td>108</td>
<td>0.5242</td>
<td>0.0865</td>
<td>0.5114</td>
<td>0.0135</td>
<td>5.85</td>
<td>4.10</td>
<td>3.94</td>
<td>5</td>
<td>0.44</td>
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<tr>
<td>Pointe Au Baril (Lake Huron)</td>
<td>45°33′45.92″N</td>
<td>80°30′18.06″W</td>
<td>27</td>
<td>0.4900</td>
<td>0.0727</td>
<td>0.4843</td>
<td>0.0267</td>
<td>4.23</td>
<td>3.11</td>
<td>3.46</td>
<td>1</td>
<td>0.08</td>
</tr>
<tr>
<td>Moon River (Lake Huron)</td>
<td>45°07′00.85″N</td>
<td>80°02′47.78″W</td>
<td>34</td>
<td>0.5099</td>
<td>0.0781</td>
<td>0.4627</td>
<td>0.0240</td>
<td>5.46</td>
<td>3.86</td>
<td>4.18</td>
<td>3</td>
<td>0.17</td>
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<tr>
<td>Severn Sound (Lake Huron)</td>
<td>44°48′21.84″N</td>
<td>79°47′20.54″W</td>
<td>16</td>
<td>0.5656</td>
<td>0.0785</td>
<td>0.5240</td>
<td>0.0346</td>
<td>5.15</td>
<td>3.69</td>
<td>4.49</td>
<td>4</td>
<td>0.31</td>
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<td>42°22′33.29″N</td>
<td>82°27′43.03″W</td>
<td>127</td>
<td>0.5422</td>
<td>0.0775</td>
<td>0.5253</td>
<td>0.0126</td>
<td>7.31</td>
<td>6.42</td>
<td>4.53</td>
<td>3</td>
<td>0.12</td>
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<tr>
<td>Buffalo Harbor (Lake Erie)</td>
<td>42°51′37.25″N</td>
<td>78°53′03.59″W</td>
<td>18</td>
<td>0.5198</td>
<td>0.0618</td>
<td>0.4530</td>
<td>0.0325</td>
<td>5.00</td>
<td>3.16</td>
<td>4.06</td>
<td>1</td>
<td>0.14</td>
</tr>
<tr>
<td>Upper Niagara River</td>
<td>42°57′30.04″N</td>
<td>78°56′12.77″W</td>
<td>113</td>
<td>0.4564</td>
<td>0.0643</td>
<td>0.4498</td>
<td>0.0130</td>
<td>5.77</td>
<td>4.17</td>
<td>3.39</td>
<td>2</td>
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<tr>
<td>Lower Niagara River</td>
<td>43°14′06.07″N</td>
<td>79°03′17.78″W</td>
<td>12</td>
<td>0.4911</td>
<td>0.0686</td>
<td>0.4872</td>
<td>0.0400</td>
<td>4.00</td>
<td>2.77</td>
<td>3.76</td>
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<td>0.03</td>
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<tr>
<td>St. Lawrence River,</td>
<td>44°15′02.61″N</td>
<td>76°06′03.40″W</td>
<td>80</td>
<td>0.5274</td>
<td>0.0782</td>
<td>0.5079</td>
<td>0.0155</td>
<td>6.23</td>
<td>5.33</td>
<td>4.48</td>
<td>1</td>
<td>0.03</td>
</tr>
<tr>
<td>Thousand Islands region</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blind Bay (St. Lawrence River)</td>
<td>44°16′02.47″N</td>
<td>76°00′57.42″W</td>
<td>14</td>
<td>0.5226</td>
<td>0.0796</td>
<td>0.4911</td>
<td>0.0389</td>
<td>4.31</td>
<td>2.90</td>
<td>4.12</td>
<td>0</td>
<td>0.10</td>
</tr>
<tr>
<td>Garlock Bay (St. Lawrence River)</td>
<td>44°19′07.37″N</td>
<td>75°56′43.88″W</td>
<td>16</td>
<td>0.5029</td>
<td>0.0715</td>
<td>0.4924</td>
<td>0.0356</td>
<td>4.23</td>
<td>3.03</td>
<td>3.81</td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Massena (St. Lawrence River)</td>
<td>45°00′06.14″N</td>
<td>74°47′40.85″W</td>
<td>10</td>
<td>0.5482</td>
<td>0.0691</td>
<td>0.5154</td>
<td>0.0438</td>
<td>4.38</td>
<td>2.96</td>
<td>4.38</td>
<td>0</td>
<td>0.11</td>
</tr>
<tr>
<td>Grasse River (St. Lawrence River tributary)</td>
<td>44°44′53.01″N</td>
<td>75°07′47.18″W</td>
<td>15</td>
<td>0.5034</td>
<td>0.0593</td>
<td>0.4769</td>
<td>0.0358</td>
<td>3.46</td>
<td>1.66</td>
<td>3.25</td>
<td>0</td>
<td>0.09</td>
</tr>
<tr>
<td>Lac des Deux Montagnes</td>
<td>45°30′03.72″N</td>
<td>73°56′17.06″W</td>
<td>37</td>
<td>0.6103</td>
<td>0.0578</td>
<td>0.6119</td>
<td>0.0226</td>
<td>5.85</td>
<td>4.45</td>
<td>4.45</td>
<td>2</td>
<td>0.08</td>
</tr>
<tr>
<td>St. Lawrence River,</td>
<td>45°30′08.63″N</td>
<td>73°32′03.72″W</td>
<td>64</td>
<td>0.5974</td>
<td>0.0595</td>
<td>0.6072</td>
<td>0.0172</td>
<td>6.77</td>
<td>5.53</td>
<td>4.62</td>
<td>0</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Muskellunge and determine genetic variation among the 15 potential populations; locus EmaD12a failed in a subset of samples and was therefore removed from all records prior to analysis. Five multiplex PCR reactions were used for each sample to amplify individual loci with fluorescently labeled primers (Sloss et al. 2008a). Microsatellite variation was visualized on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, California). An in-lane standard (GeneFlo 625; Chimerx, Milwaukee, Wisconsin) was included with all samples and GeneMapper 4.0 genetic analysis software (Applied Biosystems) identified individual genotypes that were visually verified and entered into a master database.

Data analysis.—Population (sample) conformance to Hardy–Weinberg equilibrium (HWE) expectations was tested using the chi-square test implemented in GENALEX 6.3 (Peakall and Smouse 2006). Because of known biases with highly polymorphic loci and HWE tests, all locus and population comparisons with statistically significant chi-square tests were retested for deviations from HWE after rare genotypes (expected frequency <1; Hedrick 2000) were pooled into one expected and one observed frequency value. A sequential Bonferroni correction (initial $\alpha = 0.05$ for all tests herein; Rice 1989) was applied.

Next, gametic disequilibrium between each locus pair was tested to determine if loci were linked or independently assorted. Fisher’s exact tests for each locus pair were performed in GENEPOP 4.0 (Raymond and Rousset 1995a, 1995b) using a Markov chain method (10,000 dememorization steps, 100 batches, and 5,000 iterations/batch; Guo and Thompson 1992), and a sequential Bonferroni correction was applied to the resulting $P$-values.

The genetic variation of populations was compared using the following measures: (1) expected heterozygosity $H_e$, ...
(2) observed heterozygosity \( H_o \), (3) mean number of alleles per locus \( A \), (4) mean allelic richness per locus after rarefaction \( A_r \), (5) observed number of private alleles \( PA \), and (6) mean number of private alleles per locus after rarefaction \( PA_r \). We used the Excel Microsatellite Toolkit (Park 2001) to calculate \( A \), \( H_o \), and \( H_r \), and Hp-RARE 1.1 (Kalinowski 2005) to calculate \( A_r \) and \( PA_r \), based on a minimum sample size of 10 individuals (20 genes).

Genetic differentiation among potential populations was quantified with pairwise comparisons of (1) Weir and Cockerham’s (1984) \( \theta \), an analog of Wright’s (1931) \( F_{ST} \) that uses a weighted analysis of variance (ANOVA) to quantify the amount of allelic variation in the entire sample that is attributable to differences among potential populations, and (2) harmonic means (across all loci) of Jost’s \( D \) (Jost 2008), which provides the proportion of each potential population’s alleles that are unique to that group. Tests of \( \theta \) and significant deviations from zero were conducted in ARLEQUIN 3.5.1.2 (1,000 permutations; Excoffier and Lischer 2010), and harmonic means of \( D \) were calculated in SMOGD 1.2.5 (1,000 bootstrap replicates; Crawford 2010). We then used the values of \( \theta \) and \( D \) from each potential population pair to generate two similarity matrices (similarity = 1–\( \theta \) and 1–\( D \)), and created a nonmetric multidimensional scaling model (NMDS; SAS 9.2, SAS Institute, Cary, North Carolina) based on each to visually examine the genetic similarity among populations.

We further examined the spatial genetic structure of Muskellunge populations with the Bayesian algorithm implemented in the program STRUCTURE 2.3.3 (Pritchard et al. 2000), using the following hierarchical approach. First, we excluded the Fox River population because it was introduced, and then evaluated the remaining data set (583 Muskellunge from 14 populations) for \( K \) = 1–12 potential discrete groups (five runs per \( K \) value) with the following conditions: (1) 500,000 replicate burn-ins followed by 500,000 replicates, (2) the admixture model with a uniform prior on the degree of admixture \( \alpha \) (initial value = 1, maximum = 10, SD = 0.025), and (3) allele frequencies were considered correlated among populations (prior mean = 0.01, prior SD = 0.05, \( \lambda = 1 \)). The best estimate of \( K \) groups was chosen following the methods of Evanno et al. (2005), using STRUCTURE HARVESTER 0.56.4 (Earl 2010). Each group was then evaluated for \( K = n + 2 \) (where \( n \) = the number of populations within a group) potential discrete subgroups using the conditions outlined above to determine whether spatial genetic structure (multiple spawning groups) existed within the group. Following the suggestions of Pritchard et al. (2000), we used iterative STRUCTURE analysis to determine if further hierarchical structure existed in the data. After \( K \) was determined as described above, each population sample was categorically put into discrete subgroups for testing based on its majority \( Q \)-value score. The composite subgroups were then tested using STRUCTURE as described.

Next, we performed a hierarchical analysis of molecular variance (AMOVA; Excoffier et al. 1992) in ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) to compare the molecular variance within and between population groups. The population groups tested were those suggested during three main steps of the STRUCTURE analysis (\( K = 2, 5, \) and \( 8 \)). Significance levels for each AMOVA were calculated using 50,175 permutations of the data (Excoffier et al. 1992), and the three different grouping strategies were evaluated based on which maximized molecular variance among groups and minimized molecular variance among populations within groups.

Finally, tests of isolation by distance (IBD) were conducted using the ISOLATION BY DISTANCE WEB SERVICE 3.21 (Jensen et al. 2005) to determine if migration between populations influenced spatial genetic structure. The IBD tests consisted of a reduced major axis regression of pairwise \( \theta \) and \( D \) values against pairwise geographical distance measurements (distance in kilometers between each population pair measured across water), followed by a Mantel test (Mantel 1967) with 999 randomizations to determine the statistical significance of the relation between genetic and geographic distance measures. In initial IBD tests, the relations of \( \theta \) versus geographic distance and \( D \) versus geographic distance were nonlinear, with an inflection at Niagara Falls. Therefore, prior to the final IBD tests reported here, the \( \theta \) versus geographic distance and \( D \) versus geographic distance matrices were each divided into two matrices based on whether potential populations were located upstream or downstream of Niagara Falls. The Fox River population was excluded from IBD tests because it was established by stocking, with one of the sources having been the Lake St. Clair population.

RESULTS

A total of 691 individual Muskellunge samples remained for analyses after duplicates (e.g., recaptured Muskellunge) were removed and records with genotype data missing from five or more loci were excluded. The number of alleles per locus (across all populations) ranged from 2 to 25 and averaged 10.4. All populations were considered to conform to HWE expectations for subsequent analyses because only one of 184 (0.5%) exact tests deviated from HWE following sequential Bonferroni correction (locus EmaA11 for the FOX population). Linkage analysis, excluding the FOX population because it resulted from a small number of founding events, showed 12 significant locus-by-locus comparisons following sequential Bonferroni correction within populations (66 comparisons per population); the maximum number of significant locus pair comparisons was three. Given a total of 924 locus pair comparisons, we concluded the data conformed to overall linkage equilibrium expectations with the 12 significant comparisons being the result of genetic drift within select populations.

Each locus was polymorphic for each population, with the following exceptions: (1) locus Ema-A10 was monomorphic for the Moon River population, (2) Ema-D4 was monomorphic for the Blind Bay and Garlock Bay populations, and (3) Ema-A104 was monomorphic for the Fox River, Pointe Au Baril, Severn Sound, Lake St. Clair, Buffalo Harbor, the upper
Niagara River, the lower Niagara River, and the Grasse River populations. Expected heterozygosity values ranged from 0.4564 (upper Niagara River) to 0.6103 (Lac des Deux Montagnes), with a mean across all populations of 0.5274 (Table 1). Observed heterozygosity values ranged from 0.4498 (upper Niagara River) to 0.6119 (Lac des Deux Montagnes), with a mean of 0.5067. The mean number of alleles per locus ranged from 3.46 (Grasse River) to 7.31 (Lake St. Clair), with a mean across all populations of 5.20, whereas the mean allelic richness following rarefaction ranged from 3.25 (Grasse River) to 4.62 (Montreal) and averaged 4.06. The number of private alleles per population ranged from 0 (six population pairs) to 5 (Fox River), averaged 1.5, and was positively related to sample size (Pearson’s correlation coefficient = 0.524, *p* = 0.022 for a one-tailed test). The mean number of private alleles per locus following rarefaction ranged from 0.01 (Garlock Bay) to 0.44 (Fox River) and averaged 0.12.

Patterns of genetic differentiation among pairs of potential populations were consistent whether calculated by *θ* or *D*. All but nine population pairs were significantly different according to *θ*, and each of these nine pairs had *D* values < 0.05 (i.e., < 5% allelic difference between pairs; Table 2); an additional seven population pairs had *D* values < 0.05. Values of *θ* ranged from −0.0025 (effectively zero; Thousand Islands region of the St. Lawrence River–Garlock Bay pair) to 0.2510 (Fox River–Grasse River pair) and averaged 0.1428 across all population pairs. Values of *D* ranged from 1.3 × 10−7 (upper Niagara River–lower Niagara River pair) to 0.2712 (Fox River–Grasse River pair) and averaged 0.11 across all population pairs. Genetic similarities among populations (1−*θ*) and each of these nine pairs had *D* values (above diagonal) and *P*-values in parentheses; an asterisk indicates *P* < 0.0001 calculated across 13 microsatellite loci for 15 potential populations of Muskellunge in the Great Lakes. Population abbreviations are as follows: FOX = Fox River, PAB = Pointe Au Baril, MOR = Moon River, SEV = Severn Sound, LSC = Lake St. Clair, BUH = Buffalo Harbor, UNR = upper Niagara River, LNR = lower Niagara River, SLR = St. Lawrence River, BLB = Blind Bay, GAR = Garlock Bay, MAS = Massena, GRA = Grasse River, LDM = Lac des Deux Montagnes, and MON = St. Lawrence River near Montreal.

**Table 2.** Pairwise *D* values (above diagonal) and *θ* values (below diagonal; *P*-values in parentheses; an asterisk indicates *P* < 0.0001) calculated across 13 microsatellite loci for 15 potential populations of Muskellunge in the Great Lakes. Population abbreviations are as follows: FOX = Fox River, PAB = Pointe Au Baril, MOR = Moon River, SEV = Severn Sound, LSC = Lake St. Clair, BUH = Buffalo Harbor, UNR = upper Niagara River, LNR = lower Niagara River, SLR = St. Lawrence River, BLB = Blind Bay, GAR = Garlock Bay, MAS = Massena, GRA = Grasse River, LDM = Lac des Deux Montagnes, and MON = St. Lawrence River near Montreal.
populations. The Grasse River population was positioned closer to the St. Lawrence River populations than others, consistent with its connectivity to the St. Lawrence River.

The Bayesian clustering algorithm of the program STRUCTURE (Pritchard et al. 2000) identified two likely grouping scenarios \((K = 2 \text{ and } K = 5)\) for Muskellunge sampled from the 14 potential populations (Figure 3). The hierarchical approach further parsed the \(K = 5\) groups into eight total groups (Figure 3). Percent assignment of Muskellunge from each potential population to the most likely group averaged 89% (range 83–94%) for \(K = 2\), 78% (range 55–89%) for \(K = 5\), and 82% (range 55–95%) for \(K = 8\) (Table 3).

In the three subsequent AMOVA analyses, 82.42–87.04\% of molecular variance was attributed to differences among individual Muskellunge (Table 4). Of the three grouping scenarios tested (Table 3; Figure 3), the eight-group AMOVA maximized the amount of molecular variance among groups and minimized the amount of molecular variance among populations within groups. The assignment of 14 populations into eight groups, as suggested by the program STRUCTURE and supported by AMOVA, is consistent with patterns observed from the genetic differentiation measures \(\theta\) and \(D\). No pair of populations within a group had \(D\) values \(>0.05\), and only the Lac des Deux Montagnes–Montreal pair \((\theta = 0.0125, P = 0.0020)\) and Blind Bay–Massena pair \((\theta = 0.0416, P = 0.0147)\) had significant \(\theta\) values.

Muskellunge in the Great Lakes exhibited a strong pattern of IBD that was statistically significant whether based on \(\theta\) (linear regression, \(P < 0.001, r^2 = 0.678\) above Niagara Falls; \(P < 0.001, r^2 = 0.392\) below Niagara Falls; Figure 4) or \(D\) (linear regression, \(P < 0.001, r^2 = 0.704\) above Niagara Falls; \(P < 0.001, r^2 = 0.460\) below Niagara Falls; Figure 5). This pattern was consistent with results from the program STRUCTURE, which only assigned geographically proximate populations into common groups.

**DISCUSSION**

Significant spatial structuring of Muskellunge genetic resources in the Great Lakes was evident from multiple methods of examination, including NMDS models of genetic similarity \((1–0 \text{ and } 1–D)\), results of the Bayesian clustering algorithm generated with the program STRUCTURE, AMOVA, tests of isolation by distance, and the presence of private alleles in 9 of the 15 potential populations sampled. Most populations examined were geographically distant enough to prevent reproductive mixing at rates high enough to cause genetic homogenization. For example, Muskellunge populations from three areas in Georgian Bay that are about 50–100 km apart had significant pairwise \(\theta\) values ranging from 0.0404 to 0.1030, mean pairwise \(D\) values ranging from 4% to 8%, and contained private alleles. While Koppelmann and Philipp’s (1986) results from isozyme loci supported the hypothesis that genetically distinct stocks of Muskellunge likely existed, our work clearly shows spatial genetic structure of Muskellunge populations across the Great Lakes basin and describes how genetic resources are partitioned in the Great Lakes. These findings are also evidence that stocking of Muskellunge in the Great Lakes basin has not eradicated underlying genetic structure and likely signify locally adapted groups with genetic resources critical for conservation of Great Lakes Muskellunge.

Muskellunge sampled from Buffalo Harbor, the upper Niagara River, and the lower Niagara River, which are separated by about 10–60 km, were genetically very similar but collectively very different from all other populations. The small sample sizes from Buffalo Harbor \((n = 18)\) and the lower Niagara River \((n = 12)\) make conclusive statements about genetic differentiation among these areas tenuous. However, the significant amount of habitat destruction that has occurred in this region could have eliminated areas that once supported distinct spawning groups of Muskellunge and forced reproductive mixing (genetic homogenization) in the remaining suitable habitats. For example, about 60% of the upper Niagara River shoreline is armored with bulkhead, riprap, or other materials (Wooster and Matthies 2008), and more than 75% of wetlands are thought to have been destroyed (Whillans 1982; NYSDEC 1994). Furthermore, Muskellunge stocked in the Niagara River that were progeny of fish from Chautauqua Lake (New York) and Stony Lake (Ontario) may have introgressed with native Muskellunge of Buffalo Harbor and the Niagara River, contributing to the genetic similarity within these waters and dissimilarity with other populations (see Management Implications and Research Needs, below). In addition to the genetic homogenization that may have resulted from habitat destruction and stocking, downstream migration probably contributes to the genetic similarity of Muskellunge in Buffalo Harbor and the Niagara River. Recapture data from Muskellunge tagged by anglers and in agency surveys shows downstream movement among Buffalo Harbor, the upper Niagara River, and the lower Niagara River areas, whereas no upstream movement has been observed (Niagara Musky Association and K. L. Kapuscinski, unpublished data).

Of the 10 recaptures of Muskellunge tagged in Buffalo Harbor with complete tag–recapture records, eight occurred in Buffalo Harbor and two occurred downstream in the upper Niagara River. Similarly, of the 51 recaptures of Muskellunge tagged in the upper Niagara River, 50 occurred in that same stretch of river and 1 occurred in the lower river, confirming downstream passage of Niagara Falls occurs.

The observed genetic structure of Muskellunge in the Great Lakes and the presence of IBD are consistent with results of tag–recapture studies on the species. LaPan et al. (1996), who implanted St. Lawrence River Muskellunge with radio transmitters during the spawning season, tracked their postspawn movements, and recaptured them the following spawning season, concluded that Muskellunge returned to the same spawning area in successive years despite migrating about 30–80 km away after spawning. In addition, all 33 Muskellunge tagged and recaptured during the spawning season over many years in the Thousands Islands region of the St. Lawrence River were
FIGURE 2. First two dimensions for NMDS analysis of genetic similarity quantified as $1-\theta$ (top panel) and $1-D$ (bottom panel) for 15 potential populations of Great Lakes Muskellunge. Note: solid lines connect population pairs with $\theta$ values that did not differ (top) or $D$ values <0.05 (bottom), dashed ovals indicate populations assigned to common groups by the program STRUCTURE, and population abbreviations are as follows: FOX = Fox River, PAB = Pointe Au Baril, MOR = Moon River, SEV = Severn Sound, LSC = Lake St. Clair, BUH = Buffalo Harbor, UNR = upper Niagara River, LNR = lower Niagara River, SLR = St. Lawrence River, BLB = Blind Bay, GAR = Garlock Bay, MAS = Massena, GRA = Grasse River, LDM = Lac des Deux Montagnes, and MON = St. Lawrence River near Montreal.
FIGURE 3. Plot of mean $Q$-values for all sampled individuals for $K = 5$. The two-colored bar under the plot shows the majority distribution for $K = 2$ (see A in Table 3). Results of iterative STRUCTURE analysis are presented below the plot for each of the five predicted groups. Plots of mean $Q$-values for all sampled individuals for PAB–MOR–SEV (see B in Table 3) and GRA–LDM–MON (see C in Table 3) are below each group. Population abbreviations are as follows: FOX = Fox River, PAB = Pointe Au Baril, MOR = Moon River, SEV = Severn Sound, LSC = Lake St. Clair, BUH = Buffalo Harbor, UNR = upper Niagara River, LNR = lower Niagara River, SLR = St. Lawrence River, BLB = Blind Bay, GAR = Garlock Bay, MAS = Massena, GRA = Grasse River, LDM = Lac des Deux Montagnes, and MON = St. Lawrence River near Montreal. [Figure available online in color.]

Muskellunge in Stony Lake, Ontario (Crossman 1990), and four Wisconsin lakes (Jennings et al. 2011) also made postspawn movements away from spawning sites and exhibited reproductive philopatry in subsequent years. Reproductive philopatry by Muskellunge and the considerable geographic separation of Great Lakes populations has likely facilitated the creation and further strengthening of the spatial genetic structure described above. Researchers using tag–recapture and genetic data found evidence of spawning-site and natal-site fidelity by the congeneric Northern Pike in the upper Niagara River (Harrison and Hadley 1978), the St. Lawrence River (Bosworth and Farrell 2006), and the relatively large (>10,000 ha) Kabetogama Lake (Miller et al. 2001). Isolation by distance also was considered important in shaping the genetic structure of Northern Pike in the Baltic Sea (Laikre et al. 2005), and distinct spawning groups have been identified that either reproduce in fresh or brackish waters, but not both (Westin and Limburg 2002).

Esocids have typically been considered to contain low within-population genetic variation, but results from recent studies suggest this is not the case. Early studies of Northern Pike, although broad in geographic scope, found low levels of within-population genetic variation. For example, mean $H_e = 0.14$ at eight microsatellite loci for 20 populations throughout the global range of Northern Pike (Senanan and Kapuscinski 2000) and mean $H_e = 0.24$ at 13 loci for two Danish populations (Hansen et al. 1999). These findings led Miller and Senanan (2003) to conclude that Northern Pike contained lower genetic variation than many other fishes, including sympatric Walleye and Yellow Perch Perca flavescens; no comparable data were available for the congeneric Muskellunge at the time. Subsequent studies of Northern Pike found relatively high levels of within-population genetic variation. Laikre et al. (2005), using five of the eight loci used by Senanan and Kapuscinski (2000), found mean $H_e = 0.54$ for Northern Pike from nine sites in the Baltic Sea. Bosworth and Farrell (2006), who used the six most polymorphic loci from an initial suite of 14, found mean $H_e = 0.66$ for Northern Pike from four sites in the upper St. Lawrence River.

The use of different suites of loci in these studies makes direct comparisons difficult and highlights the need for the use of standardized suites of loci. Our study used 13 of the 14 loci identified by Sloss et al. (2008a) that also were used in two other recent studies of Muskellunge, so comparisons can be made regarding within-population genetic variation across a large portion of the range of Muskellunge. The mean $H_e = 0.53$ for Muskellunge from 15 areas of the Great Lakes was similar to that observed for Muskellunge from 43 water bodies in northern Wisconsin ($H_e = 0.56$; Spude 2010), both much greater than the genetic variation of Muskellunge from Shoepack Lake in northern Minnesota ($H_e = 0.25$; Miller et al. 2009). For the 55 total Muskellunge populations sampled to date, $H_e$
TABLE 3. Mean population-specific Q-value of each predicted genetic unit for (A) K = 2 and K = 5, the two most likely number of genetic units according to the Evanno et al. (2005) method (Figure 3), (B) all individual Muskellunge sampled from PAB–MOR–SEV, and (C) all individual Muskellunge sampled from GRA–LDM–MON. Colors below group designations are from Figure 3, and population abbreviations are as follows: FOX = Fox River, PAB = Pointe Au Baril, MOR = Moon River, SEV = Severn Sound, LSC = Lake St. Clair, BUH = Buffalo Harbor, UNR = upper Niagara River, LNR = lower Niagara River, SLR = St. Lawrence River, BLB = Blind Bay, GAR = Garlock Bay, MAS = Massena, GRA = Grasse River, LDM = Lac des Deux Montagnes, and MON = St. Lawrence River near Montreal.

<table>
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<tr>
<th>Site</th>
<th>N</th>
<th>group 1 (Blue)</th>
<th>group 2 (Red)</th>
<th>group 1 (Blue)</th>
<th>group 2 (Red)</th>
<th>group 3 (Green)</th>
<th>group 4 (Purple)</th>
<th>group 5 (Light Blue)</th>
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<td>0.9703</td>
<td>0.0160</td>
<td>0.9840</td>
<td>0.0133</td>
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<td>0.8568</td>
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<td>0.9650</td>
<td>0.0650</td>
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<tr>
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<td>0.9230</td>
<td>0.0300</td>
<td>0.7890</td>
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<td>64</td>
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<td>0.0320</td>
<td>0.6433</td>
<td>0.2453</td>
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TABLE 4. Analysis of molecular variance (AMOVA), including sum of squares SS, percent variance, and fixation index (F) explained by source for three grouping strategies of Muskellunge sampled from 14 potential populations in the Great Lakes. The P-values for each variance component were < 0.003. Population abbreviations are as follows: FOX = Fox River, PAB = Pointe Au Baril, MOR = Moon River, SEV = Severn Sound, LSC = Lake St. Clair, BUH = Buffalo Harbor, UNR = upper Niagara River, LNR = lower Niagara River, SLR = St. Lawrence River, BLB = Blind Bay, GAR = Garlock Bay, MAS = Massena, GRA = Grasse River, LDM = Lac des Deux Montagnes, and MON = St. Lawrence River near Montreal.

<table>
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<th>Grouping scenario</th>
<th>Source of variation</th>
<th>SS</th>
<th>Variance (%)</th>
<th>F</th>
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<td><strong>Two-group AMOVA</strong></td>
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<tr>
<td>Group 1 (BUH, UNR, LNR)</td>
<td>Among groups</td>
<td>204.21</td>
<td>10.55</td>
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<td>Group 2 (all others)</td>
<td>Among populations within groups</td>
<td>240.37</td>
<td>7.03</td>
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<td></td>
<td>Within populations</td>
<td>3,307.46</td>
<td>82.42</td>
<td>0.176</td>
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<td><strong>Five-group AMOVA</strong></td>
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<td>Group 1 (PAB, MOR, SEV)</td>
<td>Among groups</td>
<td>369.84</td>
<td>9.79</td>
<td>0.098</td>
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<td>Group 2 (LSC)</td>
<td>Among populations within groups</td>
<td>74.74</td>
<td>3.24</td>
<td>0.036</td>
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<td>Group 3 (BUH, UNR, LNR)</td>
<td>Within populations</td>
<td>3,307.46</td>
<td>86.97</td>
<td>0.130</td>
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<td>Group 4 (SLR, BLB, GAR, MAS)</td>
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<tr>
<td>Group 5 (GRA, LDM, MON)</td>
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<td><strong>Eight-group AMOVA</strong></td>
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<td>Group 1 (PAB)</td>
<td>Among groups</td>
<td>418.24</td>
<td>12.09</td>
<td>0.121</td>
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<td>Group 2 (MOR)</td>
<td>Among populations within groups</td>
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<td>0.87</td>
<td>0.010</td>
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<td>Group 3 (SEV)</td>
<td>Within populations</td>
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<td>87.04</td>
<td>0.130</td>
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<tr>
<td>Group 4 (LSC)</td>
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<td>Group 5 (BUH, UNR, LNR)</td>
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<td>Group 6 (SLR, BLB, GAR, MAS)</td>
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<td>Group 7 (GRA)</td>
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<td>Group 8 (LDM, MON)</td>
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FIGURE 4. Pairwise $\theta$ (top panel) and $D$ (bottom panel) calculated across 13 microsatellite loci for potential populations of Muskellunge in the Great Lakes upstream of Niagara Falls versus geographic distance (km) between potential populations. Note: the lines, equations, and $r^2$ values resulted from reduced major axis regressions, $P_M$ denotes the $P$-values of Mantel tests with 999 permutations, and $r$ denotes the correlation coefficients from the Mantel tests.
FIGURE 5. Pairwise $\theta$ (top panel) and $D$ (bottom panel) calculated across 13 microsatellite loci for potential populations of Muskellunge in the Great Lakes downstream of Niagara Falls versus geographic distance (km) between potential populations. Note: the lines, equations, and $r^2$ values resulted from reduced major axis regressions, $P_M$ denotes the $P$-values of Mantel tests with 999 permutations, and $r$ denotes the correlation coefficients from the Mantel tests.
ranged from 0.25 (Shoepack Lake; Miller et al. 2009) to 0.61 (Lac des Deux Montagnes, this study; Grindstone Lake, Spude 2010) and averaged 0.54. This mean level of within-population variation is essentially equivalent to that reported for Yellow Perch in Lake Michigan ($H_e = 0.54$; Miller 2003) and Brook Trout $Salvelinus fontinalis$ in Lake Superior ($H_e = 0.55$; Sloss et al. 2008b), but less than that reported for Lake Sturgeon in the upper Great Lakes ($H_e = 0.63$; DeHaan et al. 2006), Walleyes in the Great Lakes ($H_e = 0.78$; Stepien et al. 2009), and Lake Whitefish $Coregonus clupeaformis$ in Lake Michigan ($H_e = 0.64$; VanDeHey et al. 2009). On average, the within-population variation exhibited by Muskellunge is equal to that reported by DeWoody and Avise (2009). On average, the within-population variation exhibited by Muskellunge is equal to that reported by DeWoody and Avise (2009).

Management Implications and Research Needs

Management plans for Muskellunge could focus on conserving individual populations, even those in close geographic proximity to each other within larger water bodies (e.g., Moon River and Severn Sound populations in Georgian Bay, Lake Huron), and protecting individual spawning and nursery habitats. The significant spatial structuring of Muskellunge genetic resources in the Great Lakes, presence of private alleles in most populations, and evidence of reproductive philopatry by esocids suggest that robust Muskellunge populations and individual spawning and nursery habitats could be maintained as part of an effort to preserve the evolutionary potential of the species. Knowing whether the reproductive philopatry exhibited by Muskellunge is actually natal or site fidelity following adulthood would help further refine management plans for Muskellunge and their habitat, as suggested by Farrell et al. (2003). Although evidence suggestive of natal philopatry is mounting, this hypothesis has not been adequately tested, so the appropriate spatial scales for management of spawning and nursery habitat remain unidentified. Research focused on obtaining movement data and genetic samples from multiple geographically proximate spawning groups (e.g., within Georgian Bay or the Thousand Islands region of the St. Lawrence River) should be able to provide this critical information, although obtaining adequate sample sizes will be a challenge. Until such a study is conducted, an approach that individually manages populations and fisheries and preserves all spawning and nursery habitats could be adopted to avoid losses of genetic resources.

Our study identified three additional areas of research that could enhance the management of Muskellunge in the Great Lakes and our understanding of the historical distribution of this species. First, a more extensive genetic analysis of Muskellunge from Buffalo Harbor and the upper and lower Niagara River (with larger sample sizes) is needed to determine if Muskellunge from these three areas are truly genetically similar or if the small sample sizes used in this study failed to detect more than one genetic unit. Second, an assessment is needed to determine if stocking Muskellunge across population boundaries has affected contemporary gene pools. For example, two brood sources (Indian River Spreads, north-central Michigan, and Lake St. Clair) were initially used for stocking Green Bay and its tributaries, but the effective contributions of each source to current populations are mostly unknown. Our results show genetic dissimilarity between the Fox River and Lake St. Clair populations, suggesting that most Muskellunge sampled from the Fox River were progeny of broodstock captured from the Indian River Spreads (not sampled in this study), rather than Lake St. Clair. An in-depth analysis of source populations could adequately address this issue and rule out the unlikely possibility that remnant native Muskellunge persist—this is especially important because the majority of Muskellunge stocked into Green Bay since the program began in 1989 are progeny of recaptured stocked fish. Research is also needed to determine if the progeny of Chautauqua Lake (New York) and Stony Lake (Ontario) Muskellunge that were stocked into the Niagara and St. Lawrence rivers, which contained naturally reproducing populations of native fish, successfully reproduced and contributed to contemporary populations. The Niagara River was stocked with 408,000 fry during 1941–1955 that were progeny of Chautauqua Lake (New York) Muskellunge and 18,425 fingerlings during 1960–1974 that were progeny of Stony Lake (Ontario) Muskellunge (M. Wilkinson, New York State Department of Environmental Conservation, personal communication). A total of 1,236,076 Muskellunge from three size-classes (<50 mm, n = 553,800; 50–270 mm, n = 682,081; >270 mm, n = 195) were stocked into Quebec waters of the St. Lawrence River and its tributaries during 1950–1997 (Y. de Lafontaine, Environment Canada, personal communication). Most of these stocked Muskellunge appear to have been progeny of Chautauqua Lake (New York) Muskellunge, but information on the origin of stocked fish is incomplete. Information on the presence and extent of introgression between stocked and native Muskellunge is needed so managers can mitigate negative consequences (e.g., see Miller et al. 2009) and avoid propagating nonnative Muskellunge within the Great Lakes. Finally, more extensive sampling of Muskellunge from throughout the Great Lakes and other drainages (i.e., the Ohio River, Mississippi River, Hudson Bay, and Atlantic drainages) could elucidate which areas served as glacial refugia and recolonization pathways. Understanding the historical distribution and contemporary genetic spatial structure of Muskellunge will help resource managers conserve the genetic resources of this important species.

Koppelman and Philipp (1986) stated that stocking Muskellunge into an established population may reduce fitness by irreversibly disrupting locally adapted gene complexes. This warning is repeatedly echoed in the fisheries literature (Crossman 1984; Reisenbichler and Rubin 1999; Miller and Kapuscinski 2003; Jennings et al. 2010). If one assumes that observations of neutral (microsatellite) genetic diversity and spatial structure are indicative of adaptive variation in the genome, even at a remedial level, then our results strongly support these warnings.
Stocking Muskellunge across population boundaries into areas containing native Muskellunge increases the risk of disrupting adaptive genetic diversity.

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