Genetic connectivity and diversity of walleye (Sander vitreus) spawning groups in the Huron–Erie Corridor

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ABSTRACT

The Huron–Erie Corridor (HEC) connects the upper and lower Great Lakes, providing key fish passage. A century of channelization, dredging, and pollution has led to habitat loss and declining fish numbers. Since 2004, the multi-agency HEC initiative augmented fish spawning habitat at Belle Isle and Fighting Island in the Detroit River, whose populations are examined here. We analyze genetic patterns among seven spawning groups (N = 311) of walleye Sander vitreus, a key fishery species, using nine nuclear DNA microsatellite loci and mitochondrial DNA control region sequences. Results reveal that all spawning groups contained appreciable genetic diversity (microsatellites: $H_D = 0.72$; mtDNA: $H_D = 0.73$) and showed a mixture of connectivity and divergence. Genetic relationships did not fit an isolation by geographic distance hypothesis, with some closely spaced populations being very different. Notably, the Flint River–Lake Huron spawning group was the most divergent, showing no genetic exchange. The Belle Isle and Fighting Island populations markedly differed, with the latter showing some genetic exchange with the Grosse Ile (Detroit River) and the Huron River (northwest Lake Erie) populations to the south. Walleye spawning at Fighting Island experienced no significant change in overall genetic diversity pre- versus post-habitat augmentation, but the allelic frequency changed. Our results comprise an important baseline for future population analyses and habitat assessment of these habitat augmentation areas. Despite habitat degradation and pollution, it appears that historic walleye spawning groups have persisted along the HEC, meriting continued genetic monitoring and further restoration efforts to conserve and enhance this important and diverse fishery.

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Introduction

Understanding the genetic connectivity (i.e., gene flow) and divergence of populations is fundamental to develop appropriate management strategies for ecologically and economically valuable species. Notably, identifying barriers to gene flow reveals important ecological information on species movement, dispersal, behavior, survival, and reproduction patterns that may be used to identify evolutionary significant units or other conservation management designations (see Sork and Waits, 2010; Waples, 1995; Wofford et al., 2005). Aquatic populations may maintain gene flow through connecting channels that serve as migration corridors among watersheds (LeClerc et al., 2008; Robinson et al., 2002). Vagile fishes use such avenues to disperse to spawning sites, nursery habitats, and feeding grounds (Meeuwig et al., 2010; Sheer and Steel, 2006). Some widely distributed species may exhibit high gene flow across their connected range, with low overall population structure and little specialization (Boulet et al., 2007; Hughes, 2007). On the other hand, species having spawning site fidelity may show marked genetic structure and local adaptedness, despite apparent ample opportunity for migration and gene flow among adjacent locations. Notably, populations of salmonid fishes and other species, including walleye Sander vitreus (Percidae: Teleostei) are genetically structured due to spawning site philopatry and natal homing (Banks et al., 2000; Jennings et al., 1996; Nielsen and Fountain, 1999; Stepien and Faber, 1998; Utter et al., 1989). Throughout most of the year, walleye move widely and intermingle within and among bodies of water, with some individuals traveling 50–300 km (Colby et al., 1979). In the spring walleye return to spawn at rocky shoals believed to be their natal sites (Jennings et al., 1996; Stepien and Faber, 1998; Wang et al., 2007). Anthropogenic activities, such as exploitation, stocking, and habitat fragmentation and channelization may disrupt or increase genetic exchange across migration corridors, changing relationships among sub-populations. Such factors may lower genetic diversity and increase genetic drift, or may act to homogenize formerly different groups (Laroche and Durand, 2004; Wofford et al., 2005) and lead to declines in adaptedness and fitness (Leberg, 1992; Schindler et al., 2010).

Walleye distribution and genetic patterns

The walleye is one of the most ecologically and economically valuable fishes in the Great Lakes, constituting a keystone species as a primary predator (Locke et al., 2005; Nate et al., 2011; Roseman et al., 2010)
and supporting large sport and commercial fisheries (Schmalz et al., 2011). Its native distribution ranges from the Mackenzie River in the Northwest Territories of Canada, south to the US Gulf Coast, and north-eastward to New Hampshire and Quebec (Page and Burr, 2011). Over the past century, stocking transplants – many originating from western Lake Erie in the Great Lakes – introduced walleye throughout most of the continental US and southern Canada (summarized by Billington et al., 2011).

Broad and fine-scale spatial genetic patterns of walleye spawning groups have been defined across North America using mitochondrial (mt) DNA (Billington et al., 1992; Gatt et al., 2000, 2002; Stepien and Faber, 1998) and nuclear DNA microsatellite (ssat) loci (Stepien et al., 2009, 2010, 2012; Strange and Stepien, 2007). Results have shown that many walleye spawning groups exhibited little genetic connectivity (e.g., gene flow) and significantly diverged in genetic composition, including between and within lakes, their basins, and connected tributaries (Stepien et al., 2009, 2010). The largest genetic divisions across their native range separated populations outside of the Great Lakes region from those within (Stepien et al., 2009). The Great Lakes region was colonized by walleye originating in three Pleistocene glacial refugia: the Atlantic coastal, Mississippian, and Missourian (Billington et al., 1992; Gatt et al., 2000; Stepien and Faber, 1998; Ward et al., 1989). Primary population demarcations within the Great Lakes separate the upper Lakes (Lakes Superior, Michigan, and Huron) from the lower Lakes (Lakes Erie and Ontario), with significant genetic barriers between most of the lakes and some within them (Stepien et al., 2009, 2010; Strange and Stepien, 2007). The genetic patterns of the upper Great Lakes are likely a result of fish colonizing from the Mississippian and Missourian glacial refugia. The lower Great Lakes populations also were largely founded by the Mississippian refugium, with some contribution from the Atlantic Coastal refugium (Billington et al., 1992; Gatt et al., 2000; Stepien and Faber, 1998; Ward et al., 1989). A recent investigation evaluated three closely-related Lake Erie spawning runs over 15 years, showing overall within-site genetic consistency, and some genetic connectivity and divergence among them (Stepien et al., 2012).

Little is known of the genetic connectivity or divergence among walleye spawning groups in connecting channels, such as the HEC. Those spawning groups may be locally adapted, with unique ecological and physiological variations that may aid their response to external pressures such as spawning habitat loss, exploitation, invasive species, and climate change (Kerr et al., 2010; Stepien and Faber, 1998). Such perturbations likely have impacted walleye populations across the Great Lakes for more than a century, especially along fragmenting routes, supporting over $80 billion USD in annual trade (USGS, 2010). Two artificial reefs were installed in the Detroit River in waters ≥6 m deep: one in 2004 off the northeastern corner of Belle Isle (site C in Fig. 1) and another in 2008 at Fighting Island offshore from LaSalle, Ontario (site D in Fig. 1) (HTG, 2009, 2011; Habitat Task Group of the Lake Erie Committee, Great Lakes Fishery Commission). Pre-construction assessment of spawning habitat revealed that walleye spawned at the Belle Isle site (Manny et al., 2007) and walleye and lake whitefish Coregonus clupeaformis spawned on suboptimal substrates at Fighting Island (HTG, 2009; Roseman et al., 2011). Prior to installation of these artificial reefs, the Belle Isle and Fighting Island sites contained suboptimal habitat for walleye spawning with thin patches (≤8 cm thick) of sand and small-diameter gravel on hardpan clay, lacking interstitial spaces to protect fish eggs from predation or dislodgement (Manny, 2006; Roseman et al., 2011). In 2004, 1080 m² of broken limestone (41–61 cm diameter), metamorphic cobble and gravel (20–30 cm), and coal cinders (2–8 cm) were deposited at the Belle Isle reef site to augment the spawning substrate (Manny et al., 2005). In 2008, 3300 m² of four different bed materials were deposited at the Fighting Island site, including a wide size range of broken limestone (5–50 cm) and rounded rock (10–25 cm; HTG, 2009, 2011) to provide an interstitial space gradient so that fish eggs would not be swept away by the current (Roseman et al., 2011). Prior to our study, it was unknown if walleye spawning at Belle Isle and Fighting Island belonged to historical spawning groups or were migrants from other locations.

Use of the Huron–Erie Corridor by walleye

Ripe walleye have been tagged and recorded to travel through the HEC in the spring to reach their spawning grounds (Ferguson and Derksen, 1971; Wang et al., 2007). Historically, walleye were known to spawn at sites along the HEC, most of which were sampled in the present study, with major runs occurring in Lake Huron’s Saginaw Bay, the Thames River of Lake St. Clair (site B; Fig. 1), and the Hen Island shoals in northwestern Lake Erie (site G; Goodyear et al., 1982; Wolffert, 1963; known spawning sites are marked with Xs in Fig. 1). Along the remainder of the HEC, smaller walleye spawning runs were located in the Flint River (site A), St. Clair River (including at its connection to Lake Huron), Detroit River (sites C-E), including its lower reaches and mouth, and the Huron River (site F; Fielder et al., 2006; Goodyear et al., 1982). Historical walleye spawning runs likely occurred at Belle Isle (site C) and Fighting Island (site D), where the artificial reefs were constructed (HTG, 2009; Manny et al., 2007).

Walleye spawning in the HEC have experienced varying degrees of habitat degradation, exploitation, and stocking (Thomas and Haas, 1994). Saginaw Bay comprises the largest commercial walleye fishery in Lake Huron (Fielder and Baker, 2004). This population experienced spawning habitat loss in the Saginaw River and its tributaries, including the Flint River (site A), due to construction of several dams. The walleye run in the Flint River is relatively small and provides one of the sole sources of natural recruitment to Saginaw Bay (Leonardi and Gruhn, 2001). The lower reaches of the Flint River were stocked with walleye in 1976 (Leonardi and Gruhn, 2001) and the Saginaw River and Bay have been stocked on a regular basis since 1989 (USFWS/GLFC, 2010) from a western Lake Erie source. There thus is
the potential that some stocked individuals may have migrated into the Flint River and affected the genetic composition, which is evaluated here. Walleye populations from the Thames River (site B), Detroit River (sites C–E), and Hen Island (site G) are reported to be self-sustaining and have not been stocked despite anthropogenic pressures (WTG, 2005; Walleye Task Group of the Lake Erie Committee, Great Lakes Fishery Commission; USFWS/GLFC, 2010; Thomas and Towns, 2011). The Huron River (site F) of northwestern Lake Erie was dammed, reducing habitat, and has a smaller native spawning run near its mouth that has experienced low levels of exploitation (Leonardi and Thomas, 2000). In the past, some of its impoundments upstream from that spawning site were sporadically stocked, however, the spawning site itself was not stocked (Leonardi and Thomas, 2000; USFWS/GLFC, 2010).

The HEC serves as an important dispersal route for post-spawn walleye, indicated by mark-and-recapture study results. Tagged walleye have been reported to move from (1) Lake Huron down into the St. Clair River, (2) the Thames River up into Lake Huron (Fergusson and Derkson, 1971), and (3) the western basin of Lake Erie up into Lakes St. Clair and Huron (Haas et al., 1985; Todd and Haas, 1993; Wang et al., 2007). Notably, ~68% of spent walleye captured in southern Lake Huron originated from Lake Erie spawning sites where they were tagged during the spawning run (Belore et al., 2010; McParland et al., 1999). Belore et al. (2010) found that walleye in the western basin of Lake Erie moved northward along the HEC after spawning and were unlikely to travel eastward. Post-reproductive walleye from particular spawning groups thus appear to move in consistent patterns to mix among lake systems throughout most of the year, and likely then return to their natal locations during spring spawning runs (Belore et al., 2010; Jennings et al., 1996; Todd and Haas, 1993).

The objective of our study was to evaluate the genetic connectivity, diversity, and divergence patterns of walleye spawning groups in the HEC. We analyzed 311 walleye from seven spawning sites in the HEC and outlying populations (A–G; Fig. 1), with a dual approach of nine nuclear DNA μsat loci and mtDNA control region sequences. This approach allowed us to compare patterns at multiple evolutionary and temporal scales (Avise, 2004; Wang, 2010, 2011), since the μsat loci addressed contemporary microevolutionary processes, such as migration, gene flow, and genetic drift, whereas the mtDNA control region sequences revealed historical context, such as origins from Pleistocene glaciation refugia. Specific hypotheses (stated as null/alternative) tested in the present study included:

H1. Walleye spawning groups across the HEC had similar/different levels of genetic diversity.

H2. Their relationships reflected genetic connectivity/divergence among spawning groups and between the sexes.

H3. Genetic composition at the HEC Detroit River Fighting Island reef site remained similar/changed after habitat augmentation.

Hypothesis 3 was limited to early findings; additional samples will be needed to evaluate long-term effects and trends in these habitat augmentation areas.
Materials and methods

Sample collection and DNA extraction

Walleye fin clips (1–2 cm² of pectoral or caudal fins) were collected by federal and state fish biologists during spring spawning runs at seven sites in the HEC, totaling 311 individuals and representing the major and minor spawning runs along the HEC (Fig. 1, Table 1; all available samples were analyzed). Sampled sites included: the Flint River–Lake Huron (site A, coordinates 43.3300 N, −84.0543 W), Thomas River (B, 42.3171 N, −82.4363), Belle Isle (C, 42.3469 N, −82.9533 W), Fighting Island (D, 42.2378 N, −83.1295 W), Grosse Ile (E, 42.1177 N, −83.1781 W), Huron River (F, 42.0899 N, −83.2902 W), and Hen Island (G, 41.8024 N, −82.7804 W). All individuals were verified as spawning condition, and most were released after fin-clipping, measurement of standard length (SL, mm) and sex determination. A total of 51 spawning females (1–23 per site), 146 spawning males (12–40 per site), and 10 unsexed individuals (3–7 per site) were recorded from samples for which sex data were available. Sex and length data were not taken for walleye spawning in the Thomas River (B) and Hen Island (G). To test effects of habitat augmentation on genetic diversity and composition (Hypothesis 3), spawning walleye were collected from the Detroit River Fighting Island reef pre- (2008–2010; Table 2), and post- (2010 N = 28) habitat augmentation (Table 1). Tissue samples were immediately placed in 95% ethanol, stored at room temperature, and archived in the Great Lakes Genetics Laboratory at the University of Toledo’s Lake Erie Center (Oregon, OH). DNA was extracted using Qiagen DNEASY extraction kits (Qiagen Inc., Valencia, CA), then assessed for quality and quantity on 1% agarose mini-gels stained with ethidium bromide.

Nuclear microsatellite data collection

Allelic variation at nine psat loci (Sv24, 4, 6, 7, 17, 18, 33, L6, and L7) was analyzed to test for population genetic structure (e.g., Stepien et al., 2009, 2010; Strange and Stepien, 2007; Table 2). Polymerase chain reaction (PCR) amplifications were conducted in 48 well plates with 10 µl reactions containing 0.6 units Taq polymerase, 50.0 µM dNTPs, 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris–HCl, 0.5 mM of each primer, and ~80 ng of template. A positive control (designated Lake Erie walleye) and a negative control (no template) were included in all reaction mixtures. PCR cycling parameters consisted of 2 min at 94 °C for initial denaturation, followed by 42 cycles of 40 s at 94 °C, 40 s at 48 °C, and 1.5 min at 72 °C, with a final extension of 72 °C, 30 s, followed by a final extension at 72 °C for 5 min. Three sets of loci were multi-plexed as single PCR reactions: Sv4 and 33, Sv2, 6, and 7, and Sv4L and S7. Sv17 and 18 were run individually. Amplification products were diluted 1:50, of which 1 µl was added to 13 µl of formamide and Applied Biosystems (ABI, Fullerton, CA) Gene Scan 500 size standard in 96-well plates, denatured for 2 min at 95 °C, and analyzed on an ABI 3130xl Genetic Analyzer with Genemapper v3.7. To minimize analyzer runs, Sv17 and 18 were pooled and visualized with different dye colors. Output profiles were checked manually to confirm allelic size variants. Repeat number and size, and number of alleles per locus, are reported in Table 2.

MtDNA control region sequence data

A subset of the 311 individuals representing the seven HEC walleye spawning groups was amplified and sequenced for the mtDNA control region (N = 195, 20–25 per site; Table 1), with the primers LW1-F (Gatt et al., 2000) and HN20 (Bernatchez and Danzmann, 1993). PCR reactions contained 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris–HCl, 50 µM of each dNTP, 0.5 µM each of the forward and reverse primers, 30 ng DNA template, and 1 unit of Taq polymerase in a 25 µl reaction. Amplification procedure was an initial denaturation for 2 min at 94 °C, followed by 42 cycles of 40 s at 94 °C, 40 s at 48 °C, and 1.5 min at 72 °C, with a final extension of 5 min at 72 °C. A 4 µl aliquot of each PCR product was visualized on a 1% agarose mini gel stained with ethidium bromide, and successful reactions were purified using a Qiagen PCR Purification kit.

Table 1

<table>
<thead>
<tr>
<th>Site</th>
<th>Microsatellites</th>
<th>Control region</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>H0</td>
</tr>
<tr>
<td>A, Flint R–L Huron (1998)</td>
<td>44</td>
<td>0.76 ± 0.05</td>
</tr>
<tr>
<td>B, Thames R (2004)</td>
<td>39</td>
<td>0.74 ± 0.04</td>
</tr>
<tr>
<td>C, Belle Isle (2006)</td>
<td>40</td>
<td>0.71 ± 0.03</td>
</tr>
<tr>
<td>D1, Fighting Is. (2008)</td>
<td>20</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>D2, Fighting Is. (2010)</td>
<td>28</td>
<td>0.69 ± 0.04</td>
</tr>
<tr>
<td>Mean Fighting Is. (pre and post)</td>
<td>24</td>
<td>0.71 ± 0.04</td>
</tr>
<tr>
<td>E, Grosse Ile (2001)</td>
<td>35</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td>Detroit R – Mean (C, D2, and E)</td>
<td>34</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>Mean L. St. Clair (B, C, D2, and E)</td>
<td>36</td>
<td>0.72 ± 0.04</td>
</tr>
<tr>
<td>F, Huron R. (2003 N=20, 2010 N=20)</td>
<td>40</td>
<td>0.73 ± 0.03</td>
</tr>
<tr>
<td>G, Hen Is. (2003)</td>
<td>65</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>Mean Northwest L. Erie (E and F)</td>
<td>53</td>
<td>0.71 ± 0.04</td>
</tr>
<tr>
<td>Total (all sites A–G)</td>
<td>311</td>
<td>0.72 ± 0.03</td>
</tr>
</tbody>
</table>
Kit. DNA sequencing was outsourced to the Cornell University Life Sciences Core Laboratories Center, which used ABI Automated 3730 DNA Analyzers. HEC walleye spawning group mtDNA control region sequences totaled 733 bp and were checked, identified, and aligned with BioEdit v7.05 (Hall, 1999). We related the haplotypes to those of Stepien and Faber (1998), who sequenced the entire mtDNA control region (totaling – 1086 bp) for 179 walleye across the Great Lakes and recovered 14 haplotypes (GenBank accession # U90617). We trimmed the original sequences of Stepien and Faber (1998) to match our 733 bp, omitting their 5’ repeat sequence, which left seven of their original haplotypes (designated here as A1–7, National Institutes of Health (N. I. H.) GenBank numbers U90617 and JX442946-52: http://www.ncbi.nlm.nih.gov/).

**Genetic data analyses**

The nine υsat loci were tested for conformance to Hardy–Weinberg equilibrium (HWE) expectations and linkage disequilibrium (LD), using a Markov Chain Monte Carlo (MCMC) chain of 10,000, 1000 batches, and 10,000 iterations in Genepop v4.0 (Rousset, 2008). Levels of significance were adjusted with the standard Bonferroni correction (Zar, 1999). The program Micro-checker v2.2.3 (van Oosterhout et al., 2004) was used to evaluate loci for null alleles, scoring errors, or large allele dropout. Per-locus calculations included: number of alleles (N_a), inbreeding (F_is), overall genetic diversity across all samples (F_STA), and divergences (F_ST) in Fst v2.9.3.2 (Goudet, 1995, 2002).

Genetic diversity comparisons (Hypothesis 1) among the spawning groups and sampling years included: observed (H_o) and expected (H_e) heterozygosities in Genepop, and FST, N_a, and allelic richness (A_r) in Fst for the nine υsat loci, and haplotype diversity (H_T) and number of haplotypes (N_H) calculated in Arlequin v3.5.1.3 (Excoffier and Lischier, 2010) for the mtDNA control region sequence data. Number and proportion of private alleles (N_Pr) and private haplotypes (N_PH), i.e., those unique to a given spawning group or system, were calculated with Convert v1.31 (Glaubitz, 2004). Proportion of private alleles (P_PR) and proportion of private haplotypes (P_PH) were determined by dividing the number of private alleles/haplotypes for a given population sample by its total number of alleles/haplotypes. Standard errors were calculated in Microsoft Office Excel 2003 (Redmond, WA). To test for significant differences in H_o and A_r, a Friedman rank sum test in R v2.14.2 (R Development Core Team, 2011) was employed, with the loci treated as blocks. To test hypotheses of genetic diversity patterns (Hypothesis 1) and genetic connectivity/divergence among walleye spawning groups along the HEC (Hypothesis 2), just the samples from Fighting Island post-habitat augmentation were used.

To examine whether the relationships reflected genetic connectivity (gene flow) or divergence among spawning groups (Hypothesis 2), females versus males, or a change in genetic composition pre- versus post-habitat augmentation at the Fighting Island Reef site (Hypothesis 3), exact tests of differentiation (χ^2) were used to test for differences in genetic composition among pairs of samples (Raymond and Rousset, 1995), using a MCMC chain of 10,000, 1000 batches, and 10,000 iterations in Genepop. These tested whether the seven spawning groups represented a single panmictic group or multiple sub-populations. Two different analyses tested for differences between the sexes, one included all available data (usats: 51 females, 146 males; mtDNA: 29 females, 110 males) and the other evaluated samples from Grosse Ile (site E) that had a more even distribution of females and males (usats: 23 females, 12 males; mtDNA: 13 females, 12 males). Probability values were adjusted using the sequential Bonferroni correction (Rice, 1989). Number of genetic migrants (N_M) between spawning groups was calculated in Arlequin, following Slatkin (1991) to evaluate how much genetic exchange may have occurred.

Three additional approaches further evaluated genetic connectivity and divergence patterns (Hypothesis 2): (1) Analysis of Molecular Variance (AMOVA; Excoffier et al., 1992), (2) Barrier v2.2 (Manni et al., 2004), and (3) isolation by distance via Mantel’s (1967) test. AMOVA tests assessed hierarchical relationships among samples (i.e., lakes versus spawning groups) in Arlequin. Barrier tested for significant discontinuities in gene flow (connectivity) by identifying which neighboring samples were distinguished by higher than expected genetic divergence (measured as θ_ST (Weir and Cockerman, 1984) in Fst) in relation to geographical coordinates (latitude and longitude). Support for the barriers was assessed in two ways: (1) relative number of supporting loci (per LeClerc et al., 2008; Strange and Stepien, 2007), and (2) bootstrap analysis of 2000 multilocus matrices in Geneland v3.30 (Guillot and Santos, 2009; Guillot et al., 2005). Those with low and bootstrap support values higher than 5% were reported here. Fit to a genetic isolation (θ_ST/(1–θ_ST)) by geographic distance model (shortest connected waterway using the path option in Google Earth (Google, 2010)) was tested with Isolde in Genepop, which predicted a linear relationship (Rousset, 1997), using Mantel’s (1967) procedure and 10,000 MCMC permutations. Origins of individuals spawning at Fighting Island pre- and post-habitat augmentation (Hypothesis 3) were compared using a Bayesian approach in Genecllass2 (Piry et al., 2004), which assigned individual fish to one of the seven HEC walleye spawning samples via 10,000 simulations per Rannala and Mountain (1997) and Cornuet et al. (1999). A χ^2 contingency was used to test for significant difference between individuals that self- or misassigned (Zar, 1999).

**Results**

**Genetic diversity of walleye spawning groups along the HEC (Hypothesis 1)**

The nine υsat loci were unlinked, and the samples and the loci conformed to HWE expectations following Bonferroni correction. Only two possible cases of null alleles were detected: locus Svl7 from the Thames River (B) spawning group and Svl18 in the Huron River (F). Since null alleles were not detected at those loci in the five other HEC spawning groups, the populations were in HWE, and there were no signs of heterozygote deficiency, scoring error, or stuttering, all loci were included in our analyses (see van Oosterhout et al., 2004). Loci Svl2, 7, and 18 had the highest F_C values (0.024, 0.028, and 0.016) and thus contributed more to divergence among the spawning groups (Table 2).

Overall, 119 alleles were recovered from 311 walleye at the nine υsat loci, with 74–88 alleles per spawning group (mean = 80) and allelic richness (A_r) values of 7.1–8.1 (mean = 7.5 ± 1.0). Table 1). Walleye spawning at the Detroit River Belle Isle (C) augmentation site had the most alleles (88, A_r = 7.8 ± 1.0), followed by Huron Island (G, 85, A_r = 7.1 ± 0.8), the Thames River (B, 84, A_r = 7.7 ± 0.9), Grosse Ile (E, 84, A_r = 8.1 ± 1.1), and the Huron River (F, 84, A_r = 7.8 ± 1.0). The population spawning at Grosse Ile (E) had the highest υsat allelic richness. Allelic richness did not significantly differ among the seven spawning groups, based on the Friedman rank sum test (χ^2 = 8.90, df = 6, p = 0.1800).

For the mtDNA control region sequence data (733 bp), eight haplotypes (GenBank accession #s JX442946-49 and JX44953-56) were identified among 195 HEC spawning walleye (Fig. 2; Table 1). Four haplotypes were common throughout the data set (these matched haplotype #s A1–4 of Stepien and Faber (1998); GenBank # U90617 and # JX442946-49). We discerned four new haplotypes that were unique from those of Stepien and Faber (1998), which here are designated as B8-11, GenBank # JX442953-56. Haplotype A1 (GenBank # U90617, JX442946) was the most abundant overall, characterized 37% of the samples, and reached its highest proportion (60%) in the Flint River (A). Haplotype A3 (GenBank # JX442948) was the next most abundant and occurred in 31% of the samples, whereas haplotypes A2 (GenBank # JX442947) and A4 (GenBank # JX442949) represented 17 and 12%, respectively. Similar number of haplotypes were found in all spawning groups, with Flint River (A), Belle Isle (C), Fighting Island (D2), Huron River (F), and Hen Island (G) having five each and the
Thames River and Grosse Ile with four (Fig. 2; Table 1). The newly discovered haplotypes were: B8 (GenBank # JX442953) from Fighting Island (D1) and Hen Island (G), B9 (GenBank # JX442954) from Belle Isle and Fighting Island (D2), B10 (GenBank # JX442955) from the Huron River (F), and B11 (GenBank # JX442956) from the Flint River (A).

Numbers of private sats alleles ranged from 1–6 per spawning group (mean = 2), with the most at Belle Isle (C, 6 alleles, proportion (PN) = 0.07) and Hen Island (G, 5, 0.06), a moderate number at Thames River (B, 2, 0.02) and Huron River (F, 2, 0.02), and the least in the Flint River (A, 1, 0.01), Fighting Island (D2, 1, 0.01), and Grosse Ile (E, 1, 0.01; Table 1). Two private haplotypes were recovered in the mtDNA control region dataset, one in the Flint River (A) and one in the Huron River (F).

The spawning groups had similar sats heterozygositys (mean $H_0 = 0.72 \pm 0.03$), ranging from 0.68 ± 0.03 at Hen Island (site G) in Lake Erie to 0.76 ± 0.05 at Flint River (A). Heterozygosity values at the habitat augmentation sites were relatively high: 0.73 ± 0.03 at Belle Isle (C) and 0.69 ± 0.04 at Fighting Island (D2). The Friedman rank sum test results showed no significant differences in observed heterozygosity values of walleye spawning groups across the HEC ($\chi^2 = 3.17$, df = 6, $p = 0.7900$). The Flint River (A) sample in Lake Huron suggested some slight heterozygote excess or outbreeding ($F_{Is} = -0.018 \pm 0.024$), which was not significant. The other six samples (B–G) indicated slight inbreeding depression ($F_{Is} = 0.008 \pm 0.034-0.056 \pm 0.025$; Table 1), which also was not significant. The Flint River–Lake Huron (A) had the lowest mtDNA haplotypic diversity (0.58 ± 0.02), whereas the other spawning groups had similar diversity levels (0.72 ± 0.01–0.78 ± 0.01).

Genetic divergence and connectivity among walleye spawning groups along the HEC (Hypothesis 2)

Several HEC walleye spawning groups significantly differed in genetic composition according to the sats data (Table 3). The Flint River (A) spawning group was the most divergent ($\chi^2 = 63.5$–Inf, $p \leq 0.0001$), followed by the Fighting Island sample post-habitat augmentation (D2), which significantly differed from Belle Isle (C, $\chi^2 = 46.1$, $p = 0.0003$) and Hen Island (G, $\chi^2 = 43.8$, $p = 0.0006$), but was less divergent from the Thames River (B, $\chi^2 = 35.0$, $p = 0.0100$), Grosse Ile (E, $\chi^2 = 35.3$, $p = 0.0090$), and Huron River samples (F, $\chi^2 = 31.5$, $p = 0.00$). Walleye spawning at Belle Isle (C) also significantly differed from the Hen Island spawning group (G, $\chi^2 = 46.8$, $p = 0.0002$) and slightly differed from the Thames (B, $\chi^2 = 31.2$, $p = 0.00$) and Huron (F) river samples ($\chi^2 = 34.3$, $p = 0.010$). Appreciable genetic connectivity (Table 3) was evident among walleye spawning in the Thames River (B), Grosse Ile (E), Huron River (F), and Hen Island (G). Estimated migration values among those four spawning groups (B, E–G) were high; values for Thames River (B) were 65 individuals exchanged with Hen Island (G), 307 with Grosse Ile (E), and calculated as infinite with the Huron River (F). The Grosse Ile sample additionally showed high gene flow, with migration estimated from 76 individuals with Hen Island (G) to 114 with the Huron River (F). In contrast to the higher-resolution sats data, no significant differences were recovered from the mtDNA control region sequence data ($\chi^2 = 0.00–5.2$, $p = 0.0700–1.0000$). Thus, the mtDNA data were not used for Barrier, AMOVA, or isolation by distance analyses.

The overall genetic composition of females (N = 51 sats; N = 51; mtDNA: N = 29) and males (sats: N = 146; mtDNA: N = 110) did not significantly differ (sats: $\chi^2 = 16.50, p = 0.5600$; mtDNA: $\chi^2 = 0.24, p = 0.8900$). The genetic composition of females and males spawning at a single site likewise did not significantly differ (sats: $N = 146$: $\chi^2 = 20.18, p = 0.3200$; mtDNA: $\chi^2 = 4.41, p = 0.1100$).

Barrier analysis recovered four primary barriers to gene flow (Fig. 1), in which genetic divergence was significantly greater than expected. The primary division (barrier I; 98% bootstrap support, 100% of the loci) distinguished the Lake Huron (Flint River, site A) spawning group from all other samples. The second (II; 96%, 100% loci) separated walleye spawning at the Detroit River (B) and Belle Isle (C). The third (III; 87%, 100% loci) barrier separated walleye spawning in the Thames River (B) and Belle Isle (C). The next (IV; 72%, 89% loci) denoted the Detroit River–Flint River–Grosse Ile–Huron River (F) variation among walleye spawning in the Thames (B), Grosse Ile (E), Huron River (F), and Hen Island (G).

Fig. 2. mtDNA control region haplotype frequency distribution in the seven Huron–Erie Corridor walleye spawning groups, including pre- and post-habitat augmentation comparisons. Each haplotype is represented by a single color. Haplotype numbering follows Stepien and Faber (1998), for A1–4 (GenBank # JU06617, and JX442946–49). Haplotypes B8–11 are new haplotypes recovered in this study, which are GenBank # JX442953–56. Note: Haplotypes A5–7 of Stepien and Faber (1998) were not recovered in the HEC in our study; thus those numbers are not used here.
Hierarchical relationships among population groups analyzed with AMOVA showed significant delineation among the three Lakes (1.07%, \( p = 0.0001 \)) and among spawning groups within them (0.42%, \( p = 0.0100 \)). Relationships among the spawning sites (Fig. 3) did not fit a genetic isolation by geographic distance model (\( p = 0.0800 \)), with the best-fit regression line explaining 69% of the variation (\( r^2 = 0.69 \)). Comparisons of the Flint River (A) group with all other samples showed much greater difference than would be predicted by geographic isolation. This result was similar to the Barrier analysis and \( \chi^2 \) findings, indicating that the Flint River–Lake Huron spawning group is very genetically distinct. When the Flint River (A) comparisons were excluded from analysis, the remaining HEC samples likewise did not follow an isolation by distance model (\( y = -0.001x + 0.006, \quad R^2 = 0.001, \quad p = 0.5800 \)). Thus, the relationships among spawning groups across the HEC appeared independent of geographic distance.

**Genetic composition pre- and post-habitat augmentation (Hypothesis 3)**

Genetic comparisons of walleye spawning at the Fighting Island reef (D) pre- and post-habitat augmentation (Fig. 2, Table 1) showed a slight decrease in observed \( \mu \)sat heterozygosity (0.72 ± 0.04 to 0.69 ± 0.04), an increase in mtDNA haplotypic diversity (0.70 ± 0.02 to 0.74 ± 0.01), and a greater number of \( \mu \)sat alleles (67–70). Friedman rank sum test results showed no significant differences in observed heterozygosity values of walleye spawning at Fighting Island pre- and post-habitat augmentation (\( \chi^2 = 0.11, \quad df = 1, \quad p = 0.7400 \)). Pairwise comparison tests showed a significant difference in genetic composition pre- versus post-habitat augmentation (\( \chi^2 = 32.7, \quad p = 0.0200 \)) in the \( \mu \)sat data (the mtDNA control region sequence data did not differ; \( \chi^2 = 2.5, \quad p = 0.2800 \)). Pre- and post-habitat augmentation samples each contained a different private allele and a unique haplotype (Fig. 2; Table 1). These results may be due to sample size effects. Apparent inbreeding (heterozygote deficiency) increased pre- to post-habitat augmentation from −0.09−0.056; the latter value was the highest in our dataset (Table 1).

Divergence values for the Fighting Island spawning group before habitat augmentation (D1) indicated more connectivity, suggesting more exchange of reproductive individuals with other spawning populations, than after augmentation (D2). Both samples from Fighting Island significantly diverged from the Flint River (\( \chi^2 = 72.2–96.1, \quad p \leq 0.0001 \)) and Belle Isle populations (\( \chi^2 = 29.4–46.1, \quad p = 0.0003–0.0400 \); Table 3). However, the earlier sample was genetically similar to other HEC spawning groups (\( \chi^2 = 19.0–25.8, \quad p = 0.1000–0.4000 \)). Following the habitat augmentation, divergence increased, with the Fighting Island walleye appearing more genetically distinct (\( \chi^2 = 31.5–46.1, \quad p = 0.0003–0.0300 \); Table 3). Walleye from Fighting Island
in both samples most closely resembled those spawning at Grosse Ile (E) just to the south (Fig. 1), with \( N_{HA} \) estimates (representing possible reproductive migrants) of 150 and 187 individuals, respectively (Table 3). Overall estimated \( N_{HA} \) values ranged from 30–187 in the earlier sample versus 33–150 post-habitat augmentation. Likely returns numbered 37 individuals between the two sampling dates (88 according to the mtDNA data; Table 3). Congruently, both samples from Fighting Island showed low self-assignment values (Table 4), with no significant difference in those self-assigning and assigning to other samples between the two dates (\( \chi^2 \) contingency test = 3.12, \( p = 0.0800, 1 \text{ df} \)). Many misassigning individuals traced to Grosse Ile (E, 40% pre- and 25% post-augmentation), similar to the \( N_{HA} \) results. Others that misassigned traced to the Thames River (B, 20% pre- and 7% post-habitat augmentation), Belle Isle (C, 20% and 14%), Huron River (F, 20% and 29%), and Hen Island (G, 0% and 11%). Before spawning habitat augmentation, 40% of walleye spawning at Fighting Island misassigned to the north (sites B–C) and 60% to the south (E–G). Following augmentation, 21% misassigned to the north and 65% to the south (Table 4). This trend, however, was not significant (\( \chi^2 \) contingency test = 1.13, \( p = 0.2900, 1 \text{ df} \)).

**Discussion**

**Genetic trends in relation to our hypotheses**

Our results reveal relatively similar levels of genetic diversity among samples across the HEC, supporting null Hypothesis 1. Walleye spawning in the HEC thus have unique variability despite over a century of habitat degradation. The population reproducing at the Belle Isle habitat augmentation site in the Lake St. Clair system had the most alleles, high allelic richness, and the greatest number and proportion of private alleles. The Fighting Island spawning group also showed appreciable genetic diversity. Walleye from the Flint River–Lake Huron site did not reproduce with those from other HEC locations (rejecting null Hypothesis 2). Walleye spawning at the Belle Isle and Fighting Island habitat augmentation sites housed unique variability and diverged from most other groups (also rejecting null Hypothesis 2). Both augmentation sites thus appear to house different and potentially native spawning groups of walleye. More genetic connectivity and gene flow characterized most other groups spawning in Lake St. Clair and northwestern Lake Erie (supporting null Hypothesis 2). Overall patterns among spawning populations did not fit a hypothesis of genetic isolation with geographic distance, with some HEC spawning groups located in close proximity being very divergent.

The genetic composition of walleye spawning at the Fighting Island reef habitat augmentation site in the Detroit River changed pre- versus post-habitat augmentation (rejecting null Hypothesis 3 for genetic composition). In contrast, the overall amount of genetic diversity was similar between the two (failing to reject the null hypothesis). The results likely were influenced by sample size. Fewer individuals self-assigned pre-versus post-habitat augmentation, with most individuals originating from the south in both samples. Numbers from the south increased in the later sample. This represents an important baseline and suggests that walleye spawning at the Fighting Island site originated from a variety of source populations, which should be further investigated with additional samples and years.

**Walleye genetic diversity patterns (Hypothesis 1)**

The genetic diversity levels for spawning groups along the HEC appear typical for walleye populations, suggesting that despite over a century of exploitation, stocking, habitat loss, and degradation, genetic integrity likely has been maintained. In our study, walleye spawning in the Lake St. Clair basin displayed intermediate diversity levels, having high numbers of μsat alleles and mtDNA haplotypes, greatest allelic richness, and high number and proportion of private alleles. The Belle Isle habitat augmentation site had the most μsat alleles, one of the highest allelic richness values, and the most private alleles, reflecting a diverse genetic history. Walleye spawning at the seven sites along the HEC had mean genetic diversity (observed heterozygosity) values (0.72±0.04) similar to those reported across the Great Lakes (0.71±0.01) and across their native range (0.68±0.01) by Stepien et al. (2009, 2010) using the same nine μsat loci.

Mean mtDNA control region haplotype diversity of the HEC walleye spawning groups (0.73±0.01) was similar to values from Stepien and Faber (1998) across Lakes St. Clair and Erie populations (0.69±0.05). Our diversity values were higher than those calculated from mtDNA restriction fragment length polymorphisms by Gatt et al. (2002) for walleye spawning in Lake Huron’s Georgian Bay (0.49±0.06). That population experienced a decline in haplotype diversity over three decades (from 0.50 in the 1960s to 0.15 in the 1990s) attributed to exploitation and stocking. In contrast, Franckowiak et al. (2009) discerned temporal genetic consistency over 50 years (1952–2002) for walleye spawning in Escanaba Lake, Wisconsin (\( H_0 = 0.76±0.01 \)) using eight μsat loci (six of those used here). Likewise, Stepien et al. (2012) found temporal consistency of three Lake Erie spawning groups from 1995 to 2008, including the Maumee River (0.71±0.01, \( N = 250 \)), Sandusky River (0.74±0.01, \( N = 227 \)), and Van Buren Bay Reefs (0.76±0.02, \( N = 249 \)), using the nine μsat loci employed here. The Maumee River is thought to be the largest Lake Erie spawning group (Mion et al., 1998) and experiences high exploitation (Schnalz et al., 2011), yet houses a genetically diverse spawning run. In conclusion, despite a history of exploitation and habitat loss along the HEC, its walleye diversities are relatively high, likely due to the prevalence of large connected populations across this region.

**Genetic divergence and connectivity of walleye spawning groups along the HEC (Hypothesis 2)**

Comparisons among the HEC walleye spawning groups using mtDNA control region sequence data showed no differences among them, whereas the nuclear μsat loci discerned significant differences. This difference is attributable to the slower evolutionary rate of mtDNA control region sequences compared to nuclear μsat loci (Hewitt, 2001; Wang, 2010, 2011). Mitochondrial DNA sequences have 1/4 the effective population size of nuclear DNA, rendering mtDNA more subject to declines in variability from population bottlenecks. We sampled many more μsat alleles per population (here 70–88 alleles per spawning group) and many more loci with the μsat data set compared to the mtDNA control region sequence data (4–5 haplotypes with 1–2 base pair differences).

The seven walleye spawning groups along the HEC are believed to trace to colonists from the Mississippian and Atlantic coastal glacial refugia (Billington et al., 1992; Gatt et al., 2000; Stepien and Faber, 1998; Stepien et al., 2009; Ward et al., 1989). Our study recovered four

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<th>Table 4</th>
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<td>Geneclasc2 analysis showing the percentage of walleye spawning at the Detroit River Fighting Island habitat augmentation site that self-assigned or assigned to other HEC spawning locations. Bold = percentage that self assign, underlined = highest percentage assigned to a given group, and () = number of individuals assigning to a given location.</td>
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<td>D1. Fighting Is. — pre</td>
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common mtDNA control region haplotypes (A1–4) that characterized all of our HEC sites. Common haplotypes A1 and A3 were identified as originating from the Mississippian glacial refugium, whereas common haplotype A4 came from the Atlantic coastal refugium (Billington et al., 1992; Stepien and Faber, 1998). We also found four rarer haplotypes that differed by 1–2 base pairs from the more common ones.

Our analyses using the nine nuclear msat loci showed pronounced genetic differences between walleye spawning in the Flint River–Lake Huron from other sites in the HEC, which were much greater than those predicted by geographic distance. Walleye spawning in different Great Lakes do not appear to exchange genes (Stepien et al., 2009), although they move among systems during non-spawning times (Vandergoot et al., 2010; Wang et al., 2007). Studies of other Great Lakes fisheries likewise showed marked difference of spawning groups in Lake Huron from those in Lakes St. Clair and Erie, including yellow perch Perca flavescens (Sepulveda-Villet and Stepien, 2012; Sullivan and Stepien, this issue) and smallmouth bass Micropterus dolomieu (Stepien et al., 2007).

Divergence of the Flint River–Lake Huron walleye gene pool from those in Lakes St. Clair and Erie may have occurred more recently than the Pleistocene glaciations, reinforced by behavioral isolation and spawning site philopatry, since we recovered this pattern with the msat data alone. Tagging studies showed that Flint River post-spawn walleye had limited migration, traveling only to inner Saginaw Bay (~50 km) and remaining there until the spring, when they migrated back into the river to spawn (Leonardi and Gruhn, 2001). In comparison, some individuals from other walleye spawning groups traveled longer distances (e.g., ~165 km from the western basin of Lake Erie to Lake Huron; Ferguson and Derksen, 1971; Haas et al., 1985; Todd and Haas, 1983; Wang et al., 2007). Moreover, the Saginaw River and Bay system connected with the Flint River has been stocked with individuals from the western basin of Lake Erie since 1989 (USFWS/GLFC, 2010), which may have obscured the mtDNA signal. However, our nuclear DNA data and the relatively high and unique diversity of the Flint River spawning group supports its historical genetic signature. This appears congruent with the observation of behavioral isolation by migration patterns (Leonardi and Gruhn, 2001).

Walleye spawning groups along the lower HEC showed a mixed pattern of genetic divergence and connectivity. The Belle Isle population significantly differed from others, including Fighting Island located only ~21 km away, indicating that Belle Isle likely houses a historical spawning group. Habitat along its north side was left relatively undisturbed by human activities during the history of the HEC (Bennion and Manny, 2011), likely providing a long-term refuge for spawning walleye that led to this genetic divergence. Similarly, Wilson et al. (2007), described a previously undocumented walleye spawning population in Lake Superior’s Nipigon Bay, where habitat degradation and loss had occurred (Ryder, 1968). Managers had stocked Nipigon Bay with walleye from other areas; however, the population genetically differed from the stocked individuals, indicating persistence of a native population (Wilson et al., 2007). Our study likewise indicates that walleye spawning at Belle Isle have high genetic diversity and are genetically distinct, supporting retention of a historical genetic signature. Manny et al. (2007) found evidence for walleye spawning at Belle Isle in the spring of 2004 – before the installation of the artificial reef – further supporting the existence of a native population. The Belle Isle spawning group thus may provide an important genetic resource in the HEC restoration project, meriting conservation.

The other HEC walleye spawning groups – Thames River, Fighting Island, Grosse Ile, Huron River, and Hen Island – displayed more inter-migration and connectivity, but significant difference between the Fighting Island and Hen Island populations. Using lower resolution allozyme and mtDNA restriction fragment length polymorphism markers, McFarland et al. (1999) found no differences in spawning walleye collected from our sites in Lakes St. Clair (Thames River) and Erie (Huron River), along with Chickenokee Reef in western Lake Erie. Stepien et al. (2012) found only a slight difference with msat data between the Maumee and Sandusky River spawning groups (two of Lake Erie’s largest spawning runs, located in close proximity; Mion et al., 1998), compared to a larger genetic divergence from other populations. Walleye spawning in some western Lake Erie sites thus may comprise a single connected spawning group, which may extend into the HEC.

The observed genetic connectivity and greater homogeneity among some HEC walleye spawning groups might be the product of population exploitation along the HEC. This would lead to loss of rare alleles and haplotypes and increased presence of common ones, similar to the pattern observed by Gatt et al. (2002) in Georgian Bay walleye spawning runs (whose populations were extensively stocked). However, the seven HEC walleye spawning groups sampled here are self-sustaining via natural reproduction and recruitment (Leonardi and Thomas, 2000; Leonardi and Gruhn, 2001; WIT, 2005; Thomas and Towns, 2011). Our results showed that these spawning groups possessed high diversity levels in both the nuclear and mtDNA data sets, typical of walleye populations within and outside of the Great Lakes region.

The high genetic connectivity observed among some of the HEC walleye spawning groups also may be influenced by anthropogenic habitat degradation. Walleye homing behavior could be more facultative in degraded areas due to disruption of habitat and associated chemical cues (Backhouse-James and Docker, 2012; Colby and Nepsy, 1981; Olson and Scidmore, 1962). Olson and Scidmore (1962) stated that with increased stream flow (as occurred from modification of the Detroit River), eggs and larvae would have less time to imprint, which would increase straying and lead to genetic homogenization. Optimal egg deposition for walleye in river systems occurred at velocities of 0.4–1.0 m/s (Bozek et al., 2011; Paragamian, 1989), whereas present-day estimates were 0.76–1.68 m/s for the Detroit River (U.S. Army Corp. of Engineers, 2006), indicating that it might be difficult for eggs to remain in place (see Manny et al., 2005; Roseman et al., 2011). Roseman et al. (2011) documented a decline in water velocity to 0.3–0.8 m/s at the Fighting Island site after installation of the artificial reefs. This suggests that artificial habitats may improve egg retention and imprinting of walleye to natal sites, ultimately leading to localized adaptation.

Spawning runs of chinook salmon Oncorhynchus tshawytscha showed greater genetic connectivity after logging and mining had decimated spawning runs in the Sacramento and San Joaquin River drainages, measured from 10 msat loci (Williamson and May, 2005). This greater genetic homogeneity was attributed to increased straying by ripe adults. A similar pattern of increased genetic connectivity might account for low divergence among walleye spawning groups along much of the HEC, whose natal sites may have been highly degraded.

In contrast to our results for walleye, Sullivan and Stepien (this issue) found great genetic divergence and no connectivity among yellow perch spawning groups across the HEC. This may be due to higher spawning group fidelity of yellow perch and their more limited migration (Rawson, 1980). Studies showed that the related European perch discriminates kin from non-kin via olfactory cues, and schools of full and half-sib groups were maintained throughout their lives (Behrmann-Godel and Gerlach, 2008; Gerlach et al., 2001). Thus, family groups of the European perch appear to move and reproduce together, genetically diverging from non-kin groups (Gerlach et al., 2001). This life history pattern remains to be tested for yellow perch and walleye.

Lack of genetic isolation by geographic distance along the HEC (Hypothesis 2)

Broad-scale genetic relationships of walleye spawning groups across North America were explained by a general pattern of genetic isolation by geographic distance, but did not follow this relationship across finer scales (e.g., within lakes or among more closely spaced
spawning samples; Stepien et al., 2009, 2010; Strange and Stepien, 2007). Spawning groups along the HEC, likewise, did not fit an isolation by distance pattern. Moreover, walleye spawning at neighboring sites along the HEC, especially at Belle Isle, significantly differed from other groups, including Fighting Island and the Thames River. Other spawning groups showed more genetic similarity to those farther away (e.g., between the Thames River in Lake St. Clair and Hen Island in northwestern Lake Erie).

Yellow perch likewise exhibited isolation by distance across its broad-scale range (Sepulveda-Villet and Stepien, 2012; Sepulveda-Villet et al., 2009), but not along the HEC (Sullivan and Stepien, 2013) or within Lake Erie (Sepulveda-Villet and Stepien, 2011). Similarly, analyses of smallmouth bass using eight microsatellite loci recovered an overall pattern of genetic isolation by geographic distance across its broad-scale range, but spawning groups in adjacent Lake Erie tributaries were more divergent than expected (Stepien et al., 2007). Thus, the genetic compositions of walleye, yellow perch, and smallmouth bass spawning groups often are much more divergent than predicted by geographic proximity.

**Effects of habitat augmentation on genetic composition (Hypothesis 3)**

We discerned a significant difference in the genetic composition of walleye spawning at Fighting Island pre- (spring 2008) and post- (2010) installation of the artificial reef in fall 2008. In the later sample, overall microsatellite heterozygosity and number of alleles were greater. Results indicated that approximately equal numbers of walleye self-assigned and misassigned to other samples pre- and post-habitat augmentation. Thus, the overall amount of straying did not appear to change. More individuals spawning at Fighting Island originated from the south (60% pre- and 65% post-habitat augmentation) compared to the north (40% pre- and 21% post-augmentation). These results may be due to sampling variability, with 20 individuals sampled pre-habitat augmentation and 28 post-habitat augmentation. Our study represents an important baseline comparison and should be investigated with more samples and additional sampling years.

Apparent declines in microsatellite diversity at Fighting Island following habitat augmentation should be further evaluated with additional samples and timepoints. This decline might be followed by an eventual increase, i.e., a genetic “restoration” or “rescue” (Hedrick, 2005; Tallmon et al., 2004), as individuals spawned at other locations may arrive to spawn at the new habitat. It will be interesting to discern whether this spawning population experiences increased reproductive migration, and to identify the origin of any new immigrants. Alternatively, migration could lead to decline of the historical genetic signature at Fighting Island via dilution of unique alleles and adaptations. The present study thus represents an important baseline and points to the need for continued long-term monitoring of these spawning groups to include additional generations of walleye.

**Summary**

Our results show that genetic connectivity and divergence patterns of walleye spawning groups varied along the HEC. The Flint River—Lake Huron spawning population was very different from the others, showing no genetic exchange, which was much greater than that predicted by isolation by distance. Across the remainder of the HEC, the Belle Isle spawning group significantly diverged, with high genetic diversity and more unique alleles, indicating persistence of this native spawning population. Likewise, the group spawning at Fighting Island differed from some nearby populations. There was greater genetic similarity and more connectivity among the other Lake St. Clair and northwestern Lake Erie samples. The Fighting Island walleye spawning population may have lost some overall genetic diversity, and appeared to exchange genes with the nearby Grosse Ile group (which appeared greater in the pre-augmentation sample). Further study is needed to evaluate these long-term population trends. In conclusion, despite habitat degradation and pollution, it appears that historical walleye spawning groups have persisted along the HEC, meriting conservation and further restoration efforts.

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