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ARTICLE

Spatial and Temporal Genetic Analysis of Walleyes in the Ohio River

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Abstract

Previous genetic analyses have shown that Walleyes *Sander vitreus* in the upper Ohio River comprise two distinct genetic strains: (1) fish of Great Lakes origin that were stocked into the Ohio River basin and (2) a remnant native strain (Highlands strain). Resource agencies are developing management strategies to conserve and restore the native strain within the upper reaches of the Ohio River. Hybridization between strains has impacted the genetic integrity of the native strain. To better understand the extent and effects of hybridization on the native strain, we used mitochondrial DNA and microsatellite markers to evaluate the spatial (river sections) and temporal (pre- and poststocking) genetic diversity of Ohio River Walleyes. Contemporary Lake Erie Walleyes and archival museum specimens collected from the Ohio River basin were used for comparison to contemporary Ohio River samples. Although there was evidence of hybridization between strains, most of the genetic diversity within the Ohio River was partitioned by basin of origin (Great Lakes versus the Ohio River), with greater similarity among river sections than between strains within the same section. Results also suggested that the native strain has diverged from historical populations. Furthermore, notable decreases in measures of genetic diversity and increased relatedness among native-strain Walleyes within two sections of the Ohio River may be related to stocking aimed at restoration of the Highlands strain. Our results suggest that although the Highlands strain persists within the Ohio River, it has diverged over time, and managers should consider the potential impacts of future management practices on the genetic diversity of this native strain.

The Walleye *Sander vitreus* is native to the Ohio River. Historically abundant, Walleyes declined throughout the Ohio River watershed during the mid-1800s through the mid-1900s due to habitat loss and degradation related to the construction of navigational dams within the Ohio River and its major tributaries (Trautman 1981). In response, state agencies stocked Walleyes from nonnative sources into the Ohio River watershed to mitigate losses in Walleye populations and to increase recreational fishing opportunities (Murphy and Nielsen 1983; White and Schell 1995). Today, the Walleye is a popular sport fish in the Ohio River, and together with Saugers *Sander canadensis*, Walleyes comprise a majority of the recreational harvest at some locales (Schell et al. 1996; Sindt 2012).

Current management of Walleyes within the Ohio River is conducted through the Ohio River Fisheries Management Team (ORFMT), a partnership among states bordering the Ohio River (Illinois, Indiana, Kentucky, Ohio, Pennsylvania, and West Virginia). The ORFMT is responsible for cooperatively managing Ohio River fisheries. Achieving an understanding of the genetic structure of sport fish populations within the Ohio River has been recognized as a priority for the ORFMT (Schell et al. 2004). In particular, resolving the genetic stock structure and interactions among Walleyes within the Ohio River would be useful for coordinating management actions (e.g., stocking) and aligning harvest regulations to create consistency among jurisdictions (Shaklee and Currens 2003). Accordingly, previous genetic investigations

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evaluating the genetics of Walleyes within the Ohio River and its tributaries (White and Schell 1995; Palmer 1999) have led to a number of actionable management outcomes (Schell et al. 2004; WVDNR 2005).

Extensive genetic analyses have been instrumental in resolving the phylogeographic origins and stock structure of the Walleye throughout its range (Billington et al. 2011). Previous studies using mitochondrial DNA suggest that Walleyes within the Ohio River consist of two genetically distinct ancestral lineages (haplotypes) or "strains" (Billington 1996; Stepien and Faber 1998; White et al. 2005). It has been posited that these strains represent (1) a remnant Walleye population native to the Ohio River (hereafter, "Highlands strain" as per White et al. 2012) and (2) nonnative Walleyes of Great Lakes origin (hereafter, Great Lakes strain). The Highlands strain is believed to have originated within the Teays River watershed (New and Kanawha rivers), the precursor to the upper Ohio River (Ver Steeg 1946; Flint 1971), whereas the Great Lakes strain may have been introduced via historical stocking of Walleyes from the Great Lakes into the Ohio River watershed (Murphy and Nielsen 1983; White and Schell 1995; Palmer 1999). Other studies have found that the Highlands and Great Lakes strains of Walleyes coexist within multiple major watersheds of the Ohio River, including the Kanawha, Cumberland, and Monongahela River systems (Palmer 1999; Zipfel 2006; White et al. 2012). Hybridization between strains is a concern, as it can result in the introgression of nonnative genes, potentially reducing the genetic fitness and long-term sustainability of native Walleye populations by disrupting gene complexes that govern physical and behavioral traits adaptive to stream environments (Campton 1995; Scribner et al. 2001). Evaluation of contemporary genetic structure and interactions between strains by using microsatellite DNA analysis has revealed no significant genetic differences between the native and Great Lakes strains within the Ohio River, suggesting extensive recent introgression and homogenization of genetic diversity (White et al. 2005, 2012). Comparing Walleyes sampled directly from Lake Erie and the Ohio River, Stepien et al. (2009) found genetic differences between Great Lakes-strain and Highlands-strain Walleyes.

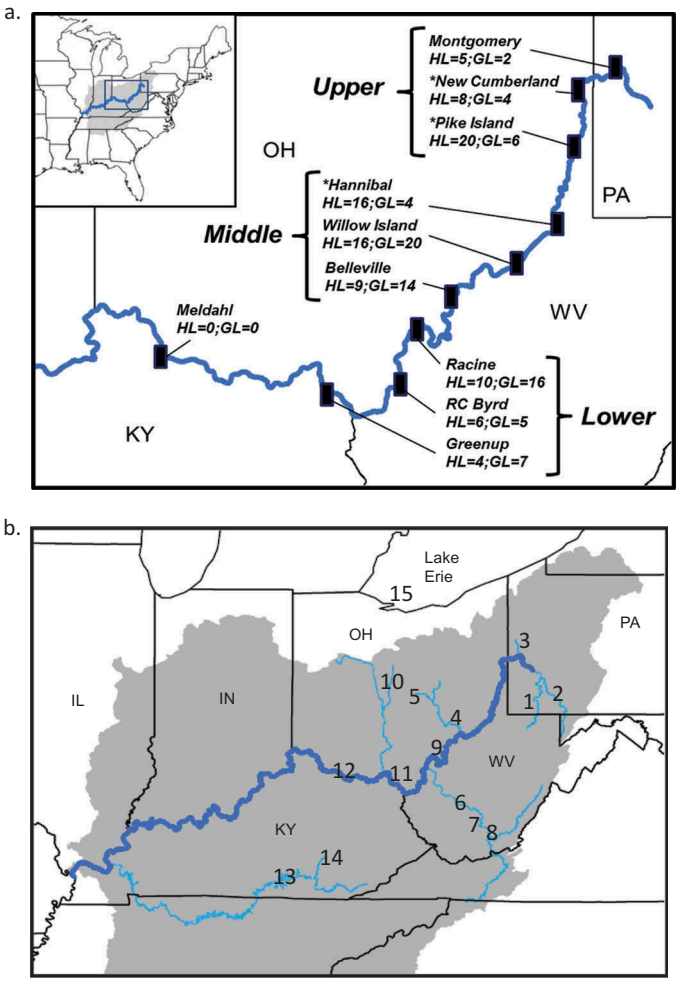
The existence of the Highlands strain of Walleyes has prompted state fisheries management agencies to adopt strategies for conserving and restoring this native strain (WVDNR 2005). Stocking programs have been initiated to reintroduce and supplement the Highlands-strain Walleye populations. Since 2005, native Walleye fingerlings have been stocked by the West Virginia Department of Natural Resources (WVDNR) within two upper Ohio River navigation pools (Hannibal and Pike Island pools; WVDNR 2005), and stocking in an additional pool was initiated in 2010 (Willow Island pool; K. Zipfel, WVDNR, personal communication). Known as the Eastern Highlands hatchery strain, these fingerlings are derived from crosses of Highlands-strain Walleyes collected annually from wild endemic sources (e.g., the Ohio and New rivers). Broodstock fish are screened by using genetic markers that are considered diagnostic for the native Highlands strain. Kentucky has focused on the stocking of native Walleyes within major tributaries (e.g., Cumberland River), while the state of Ohio has supplanted most stocking of Great Lakes Walleyes within the Ohio River watershed with presumably infertile saugeyes (Walleye × Sauger hybrids), and saugeyes are no longer stocked directly into the Ohio River or its tributaries (i.e., they are stocked in impoundments). In addition, restrictive harvest regulations have been implemented to protect remnant Highlands-strain populations. Although implemented under the auspices of conservation, restoration efforts may also provide the additional benefit of improved fishing opportunities, as anecdotal evidence suggests that Highlands-strain Walleyes grow to larger sizes than their conspecifics (McCoy 2012; Dreves 2015; Garth 2015), and a native riverine Walleye would be expected to perform better than lacustrine strains (Zipfel, personal communication).

Introgression between the Highlands and Great Lakes strains of Walleyes within the Ohio River appears prevalent, but the extent of introgression relative to historical genetic differentiation between strains is unclear—that is, the extent of genetic differentiation that previously existed between the strains prior to the stocking of Walleyes from the Great Lakes is uncertain. Use of different genetic markers (i.e., allozymes and microsatellite DNA; White et al. 2005) has generated inconsistencies in estimates of introgression and genetic stock structure. Furthermore, current stocking practices add a level of complexity to the management of Highlands-strain Walleyes, as factors inherent in hatchery practices have the potential for altering the genetic composition of fish populations (Campton 1995; Miller and Kapuscinski 2003; Page et al. 2005). It has been advocated that the analysis of genetic data from samples collected prior to the stocking of Great Lakes Walleyes could be valuable for elucidating the current extent of introgression and providing a temporal genetic baseline as a source of inference for management (White et al. 2005).

For this study, we re-examined the genetic structure of Ohio River Walleyes within both a contemporary and historical context by using an expanded panel of genetic markers and samples. Genetic data were collected using mitochondrial DNA (restriction fragment length polymorphisms) and nine microsatellite markers. Mitochondrial DNA analyses (White et al. 2005) were used to classify Ohio River Walleyes into strains (i.e., historical lineages: Great Lakes and Highlands), and microsatellite markers were used to (1) evaluate the contemporary genetic composition, structure, and diversity within and among strains in the Ohio River; (2) compare contemporary Ohio River Walleyes to pre-stocking populations by using historical genetic data; and (3) evaluate the assertion that Highlands-strain Walleyes grow larger than Great Lakes-strain Walleyes within the Ohio River.

METHODS

Study site.—This study focused on a 696-km reach of the Ohio River (Figure 1) from the tailwaters of Montgomery Dam (river kilometer [rkm] 58) to the tailwaters of Meldahl Dam (rkm 787). Here, the Ohio River borders Kentucky, Ohio, and West Virginia, with the upstream 12.8 km completely situated within the state of Pennsylvania (Figure 1). Eight additional navigation lock-and-dam structures exist within this reach: New Cumberland (rkm 97); Pike Island (rkm 151); Hannibal (rkm 229); Willow Island (rkm 293); Belleville (rkm 367); Racine (rkm 427); R. C. Byrd (rkm 502); and Greenup (rkm 614). *Sander* spp. population assessments are conducted annually by the ORFMT within the tailwater habitats of these dams. In general, Walleyes tend to be more prevalent upstream, where the river is characterized by higher gradient flows, less-turbid water, and coarser substrates. Our study reach overlapped or encompassed regions that have been evaluated during previous studies (e.g., White et al. 2005, 2012).



1. Monongahela R., 1885, N=1; 2. Youghiogheny R., pre-1898, N=1; 3. Beaver R., pre-1898, N=8; 4. Muskingum R., 1940, N=2; 5. Licking R., 1939, N=1; 6. Kanawha R., 1935, N=1; 7. New R., 1935, N=1; 8. Hungirts C., 1933, N=1; 9. Shade C., 1939, N=1; 10. Big Walnut C., 1959, N=1; 11. and 12. Ohio R., 1935 and 1939, N=5; 13. Cumberland R., 1956, N=1; 14. Rockcastle R., 1936, N=1; 15. Lake Erie (Maumee and Sandusky R.) 2012, N=88

FIGURE 1. Locations and numbers of Walleyes sampled for genetic analysis: **(a)** dam tailwaters (black squares) sampled within the Ohio River for contemporary genetic evaluation of Highlands-strain (HL) and Great Lakes-strain (GL) Walleyes; and **(b)** locations of samples used for developing historical genetic baselines for the Ohio River strain (museum specimens) and the GL strain (contemporary Lake Erie samples). Contemporary samples from the Ohio River were pooled into lower, middle, and upper river sections for analyses. Map inset shows the Ohio River watershed (gray-shaded region) and the location of the study reach (delineated with a black box).

Contemporary genetic samples.—To evaluate the contemporary genetic structure, diversity, and strain composition of Ohio River Walleyes, genetic samples were collected during 2010–2011 via electrofishing as part of *Sander* population assessments. The tailwater habitats of all 10 dams within the study reach were surveyed during this period. Each collected Walleye was given an individual identification number, measured (TL, mm), and weighed (g). A subset of Walleyes was sacrificed to evaluate age (otolith) and growth metrics. The number of Walleyes collected from dam tailwater surveys for genetic analysis (total $N = 210$) varied among locations ($N = 0$–52 per site), with no Walleye being encountered within the tailwaters of Meldahl Dam (Figure 1a). Tissue samples (fin clips) were collected from each Walleye and were stored individually in 1.5-mL vials containing 95% nondenatured ethanol. Given the disproportionate numbers of Walleyes collected among sample sites, samples were grouped into lower (Greenup, R. C. Byrd, and Racine Dam tailwaters), middle (Belleville, Willow Island, and Hannibal Dam tailwaters), and upper (Pike Island, New Cumberland, and Montgomery Dam tailwaters) river sections and were analyzed accordingly. The upper river section encompassed locations that have been traditionally stocked (since 2005) with Eastern Highlands-strain Walleyes (New Cumberland and Pike Island Dam tailwaters). Stocking of Eastern Highlands-strain Walleyes into the Hannibal Dam tailwaters was initiated in 2010 (Zipfel, personal communication).

Historical baseline samples.—Museum specimens were used to derive a historical genetic baseline of Highlands-strain Walleyes. Museum collections were screened for candidate samples through personal communications with curators or through museum collection databases (accessed in 2010 through the Fishnet2 Portal: http://www.fishnet2.net/). Only specimens collected within the Ohio River watershed prior to extensive stocking of Walleyes from nonnative sources, as corroborated with stocking records, were considered. Overall, 30 Walleye specimens were obtained from museum collections at four institutions (Appendix Table A.1), including the Academy of Natural Sciences at Drexel University; the Museum of Biological Diversity at Ohio State University; the Museum of Zoology at the University of Michigan; and the Museum of Comparative Zoology at Harvard University. Collection years for museum specimens ranged from 1854 to 1959. Nearly all specimens were collected from either the Ohio River or primary tributaries within the study reach. Two

specimens were collected from the Cumberland River watershed of Kentucky. A recent genetic analysis suggested that Walleyes from the Cumberland River were genetically similar to Walleyes from locales within our study reach (White et al. 2012). Tissue samples from museum specimens represented a variety of tissue types, including scales, fin, muscle, and bone and were either preserved in formalin or desiccated (e.g., skeletons).

We used Walleyes from Lake Erie as the historical baseline for the Great Lakes strain. Records indicate that the origin of historical stockings within the Ohio River basin was from fish principally derived directly or indirectly from Lake Erie stocks (Murphy and Nielsen 1983; White et al. 2005), although fish originating from the Hudson Bay drainage may have been historically stocked within the New River, Virginia (Kanawha River tributary; Murphy and Nielsen 1983). During spring 2012, Walleyes were collected during the spawning run within the Maumee and Sandusky rivers (major tributaries to Lake Erie) via electrofishing. Tissue samples (fin clips) were collected from 89 Walleyes and stored individually either in 1.5-mL vials containing 95% nondenatured ethanol or in scale envelopes. Walleyes from Lake Erie were considered a reliable surrogate for the poststocking genetic baseline of Great Lakes-strain Walleyes in the Ohio River, as any genetic differences that accrued over time (e.g., genetic drift) in Lake Erie would have occurred independently of intrastrain interactions.

Extraction of DNA.—All samples were extracted by using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, California). The DNA was extracted from fin clips via the standard protocol for animal tissues. Extraction of DNA from the historical samples was achieved by using a modification of the protocol for formalin-fixed tissues; instead of rinsing the samples twice, we held the samples in potassium phosphate for 48 h (with a rinse after 24 h). Extractions from formalin-preserved samples were performed using a dedicated set of pipettes in a separate work area. The amount of DNA extracted from each sample was quantified with a Cary Win-UV spectrophotometer (Agilent Technologies, Santa Clara, California).

Mitochondrial DNA screening.—Mitochondrial DNA haplotypes were assessed by digesting a segment of the left domain of the mitochondrial control region from the proline transfer RNA gene to the central conserved section with two restriction enzymes (*Ase*I and *Bsu*36I). *Ase*I and *Bsu*36I can identify mitochondrial DNA haplotypes that are characteristic of Ohio River and Great Lakes Walleyes (Stepien and Faber 1998; White et al. 2005). The Ohio River haplotype contains a diagnostic *Ase*I restriction site that is not observed in Great Lakes Walleyes, whereas the Great Lakes haplotype is characterized by a *Bsu*36I restriction site that is not observed in Ohio River Walleyes. The primers L15926 (Kocher et al. 1989) and H16498 (Meyer et al. 1990) were used to amplify mitochondrial DNA. Polymerase chain reactions were performed in 30-μL volumes using 150 ng of DNA, 0.2 μM of each primer, 0.35-mM deoxynucleotide triphosphates (dNTPs), 2.5-mM $MgCl_2$, 0.8 units of *Taq* DNA polymerase (Promega, Madison, Wisconsin), and the manufacturer's buffer at the recommended concentration. The thermal cycler profile consisted of an initial heating step at 95°C for 2 min, followed by 35 cycles of 45 s at 95°C, 45 s at 50°C, and 1.5 min at 72°C. The reaction was completed with a final incubation at 72°C for 5 min. Five microliters of amplicon were digested for 6 h in 15 μL with 1 unit of each restriction enzyme and the manufacturer's recommended buffers (New England Biolabs, Ipswich, Massachusetts). After digestion samples were run on 2% agarose gels to separate mitochondrial DNA fragments, the gels were stained with ethidium bromide, and the mitochondrial DNA was visualized under UV light. Two size standards were used to determine the size fragments, which were compared to those of White et al. (2005), and a composite haplotype based on the two restriction enzymes was generated. A sample was classified only if DNA fragments were observed for both restriction digests.

Microsatellite DNA screening.—Fourteen microsatellite DNA loci designed for Walleyes or other percids were genotyped: *Svi4, Svi6, Svi17, Svi18*, and *Svi33* (Borer et al. 1999); *SviL6, SviL7*, and *SviL8* (Wirth et al. 1999); *Svi2, Svi7, Svi20*, and *Svi26* (Eldridge et al. 2002); and *MSL-1* and *MSL-2* (Kohlmann and Kersten 2008). Polymerase chain reactions were performed in 15-μL volumes containing 100 ng of DNA, 0.8–0.9 units of *Taq* DNA polymerase and the recommended amount of the manufacturer's buffer (Promega), 0.25-mM dNTPs, 0.12–0.30 μM of a fluorescently labeled forward primer and an unlabeled reverse primer (*Svi2*: 0.12 μM; *Svi7, Svi20*, and *MSL-2*: 0.15 μM; *Svi18*: 0.18 μM; *SviL6, SviL7, SviL8, Svi26*, and *MSL-1*: 0.20 μM; *Svi4*: 0.25 μM; *Svi6, Svi17*, and *Svi33*: 0.30 μM), and 1.0–1.4-mM $MgCl_2$. Two separate multiplex reactions were used to combine *Svi2* with *Svi7* and *Svi26* with *MSL-2*. Polymerase chain reactions for the historical samples were performed as described above in a laminar-flow hood using a dedicated set of pipettes and were amplified a second time using diluted (1:10) PCR product to improve the amplification success. For the second amplification, 0.05 μM of each primer, 0.10-mM dNTPs, and 1.0-mM $MgCl_2$ were included in the reaction. Of the 14 microsatellite DNA markers we examined, 9 markers (*Svi2, Svi4, Svi6, Svi17, Svi18, Svi20, Svi26, Svi33*, and *SviL6*) were selected for further analyses (Tables A.1, A.2) because they provided a minimum number of historical individuals (at least 6 fish) to analyze. Only samples that were genotyped for at least five of the nine loci were used in the analyses.

Analyses.—Genetic diversity was evaluated between Walleye strains, as defined by mitochondrial DNA haplotypes, within and among river sections (lower, middle, and upper). Descriptive genetic diversity metrics were calculated using the program GenAlEx (Peakall and Smouse

2006), including observed heterozygosity (H_o), expected heterozygosity (H_e), number of alleles (N_a), and allelic frequency. Allelic richness (R_a) and private allele richness (P_a) were calculated using the programs FSTAT (Goudet 1995) and HP-Rare (Kalinowski 2005), and genetic relatedness (mean coefficient of relatedness among individuals [r_{xy}]; Queller and Goodnight 1989) was calculated using the program KINGROUP (Konovalov et al. 2004). The proportions of significant ($P < 0.05$) individual pairwise estimates of r_{xy} that were consistent with relatedness at the half-sibling ($r_{xy} = 0.25$) and full-sibling ($r_{xy} = 0.50$) levels were determined. Tests for deviations from Hardy–Weinberg equilibrium (HWE) expectations were conducted among microsatellite loci for each Walleye population by using FSTAT. The sequential Bonferroni method was used to adjust significance values for type I error (Rice 1989).

The strain composition (i.e., proportions of Great Lakes-strain Walleyes, Highlands-strain Walleyes, and interstrain hybrids) was evaluated among river sections (lower, middle, and upper) and compared to the composition reported by White et al. (2005). Results from tailwaters surveyed by White et al. (2005) were grouped according to the river sections delineated in this study; however, not all tailwaters surveyed in this study were surveyed by White et al. (2005). We also evaluated the genetic structure of Walleyes by using Wright's pairwise F_{ST} (i.e., θ_{ST} analog; Weir and Cockerham 1984), a measure of genetic differentiation among populations (allelic frequencies). To account for type I error, the nominal significance value ($P < 0.05$) was adjusted using the sequential Bonferroni method (Rice 1989).

Walleye strains were compared to historical genetic baselines (Highlands strain versus museum specimens; Great Lakes strain versus contemporary Lake Erie samples). Temporal genetic baselines can be useful for measuring genetic divergence and for identifying factors that influence genetic change (e.g., introgression). We used the program Populations version 1.2.32 (Langella 1999) to calculate Nei's genetic distance (Nei et al. 1983) among the strains, and we visualized relationships among Walleye populations by using a neighbor-joining tree (Saitou and Nei 1987) constructed in TreeView version 1.6.6 (Page 1996). Bootstrap support for nodes was evaluated using 1,000 pseudoreplicates.

We also used a Bayesian approach to investigate population structure and the origins of Walleyes from the Ohio River since samples were collected over multiple years and outside of the spawning season. The program STRUCTURE (Pritchard et al. 2000) was used to determine the most likely number of genetic clusters and then to determine the proportional contributions of Ohio River and Lake Erie Walleyes to the genetic composition of each sample. To determine the number of genetic groups, we ran the analysis using two to six clusters ($K = 2$–6) with 100,000 iterations following a burn-in period of 100,000 runs and assuming correlated allele frequencies, and we performed 10 runs for each value of K. We did not use information about the geographic origins of the samples. STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to collate the results of each run and to determine the most likely number of populations by using the K-value with the highest probability, the lowest amount of variation among simulations, and the highest value of ΔK (Evanno et al. 2005). The q-estimates (proportion of each individual's ancestry from each genetic group) were used to assign individuals to a waterbody. The assignments from the Bayesian analysis were then combined with the mitochondrial DNA results to infer the directionality of any Ohio River × Great Lakes hybrids.

Total length (TL; mm) and weight (W; g) were compared among Walleye strains and hybrids. A single TL–W relationship and a single length-at-age relationship were developed by pooling all data across each strain and hybrids (as assigned above). We used ANOVA to test whether strains and hybrids ($P < 0.05$) differed in deviations of the observed TL–W and length-at-age relationships from those expected based on their linear relationships (residuals).

RESULTS

Overall, 338 Walleye samples were collected. For mitochondrial analysis, all of the contemporary samples collected from the Ohio River ($N = 210$) were amplified; all but one of the Lake Erie samples ($N = 89$; Maumee and Sandusky rivers) were amplified; and none of the 30 museum specimens collected was amplified for mitochondrial DNA. In terms of the panel of microsatellite loci used in this study (Table A.1), 172 of the contemporary Walleye samples collected from the Ohio River were used in genetic analyses (range = 139–152 individuals per locus); 88 of the Walleyes from Lake Erie were utilized (range = 83–88 individuals per locus); and 25 of the museum specimens were utilized (range = 6–23 individuals per locus).

Mitochondrial DNA analysis of contemporary Walleyes sampled from the Ohio River revealed that 45% ($N = 78$) exhibited a genetic signature consistent with the Great Lakes strain, and 55% ($N = 94$) exhibited a signature consistent with the Highlands strain (Figure 2a). Nearly all Walleyes from Lake Erie (97%) exhibited the Great Lakes haplotype, and 3% exhibited the Highlands haplotype. It is unclear whether this represents evidence of previously undetected low levels of the Highlands haplotype in Lake Erie or whether there has been a recent infusion of this haplotype from outside the Lake Erie basin. The composition of Walleyes differed among river sections, with Highlands-strain Walleyes increasing from downstream to upstream (Figure 2a, b). Age and preservation methods (e.g., formalin preservation) inhibited mitochondrial DNA analysis of museum specimens, and consequently all specimens were assumed to be Highlands-strain fish.

Genetic diversity measures of contemporary Walleyes from the Ohio River were generally greater for the Great Lakes

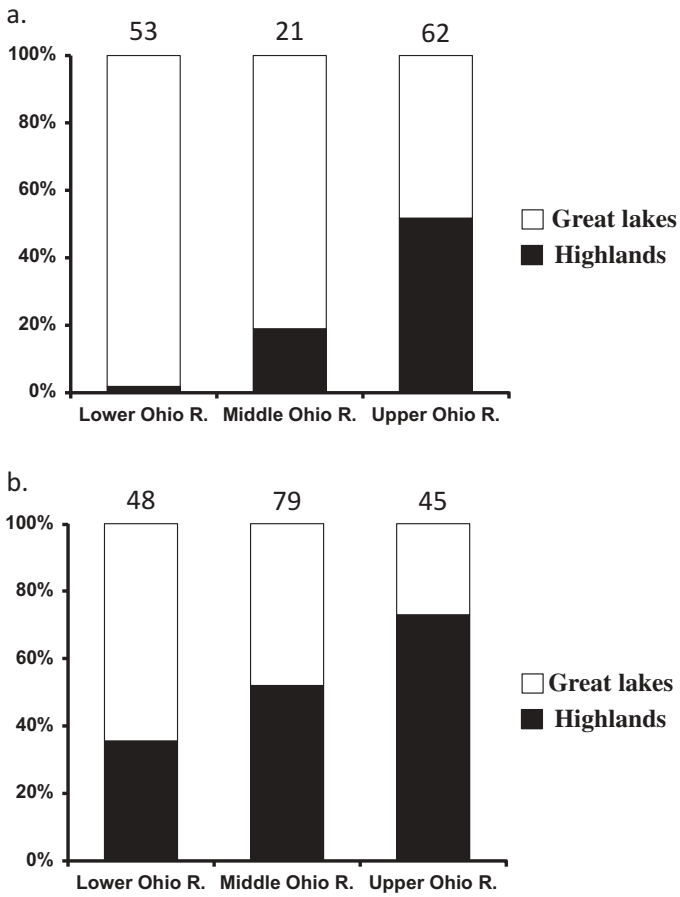


FIGURE 2. Proportions of Walleyes sampled from the lower, middle, or upper section of the Ohio River that were identified as belonging to either the Great Lakes strain or the Highlands strain based on mitochondrial DNA analyses conducted **(a)** by White et al. (2005) and **(b)** during the present study. Note that some of the tailwaters sampled in this study were not sampled by White et al. (2005). Total sample sizes are provided above each bar.

strain among river sections (Tables 1, A.2). For the Great Lakes strain, the mean (among-loci) H_o ranged from 0.69 to 0.78, mean N_a ranged from 7.6 to 11.1, mean R_a ranged from 6.3 to 6.9, and mean P_a ranged from 0.31 to 0.40. In contrast, for the Highlands strain, mean H_o ranged from 0.59 to 0.72, N_a ranged from 8.2 to 9.8, R_a ranged from 5.5 to 6.5, and P_a ranged from 0.14 to 0.30. Relatedness metrics also differed between strains: r_{xy} values ranged from 0.038 to 0.067 for the Great Lake strain and from 0.068 to 0.221 for the Highlands strain. The proportion of coefficients at the level of half-sibling or greater (r_{xy},0.25) ranged from 0.08 to 0.14 for the Great Lakes strain and from 0.16 to 0.41 for the Highlands strain. The proportion of r_{xy} values at the level of full-sibling or greater (r_{xy},0.50) ranged from 0.00 to 0.02 for the Great Lakes strain and from 0.02 to 0.05 for the Highlands strain. In general, Highlands-strain Walleyes from the upper section of the Ohio River exhibited lower levels of genetic diversity and increased relatedness. Significant departures from HWE (heterozygote deficit) were found for both the contemporary Highlands-strain Walleyes (lower section: *Svi6, Svi33*, and *SviL6*; middle section: *Svi4, Svi6*, and *Svi33*; upper section: *Svi6, Svi33*, and *SviL6*) and the Highlands-strain baseline samples (*Svi4, Svi6, Svi17, Svi1*8, and *SviL6*). No deviations from HWE were observed for the Great Lakes-strain Walleyes.

Pairwise estimates of F_{ST} ranged from 0.001 to 0.138 (Table 2). Significant F_{ST} values (adjusted for type I error) were principally found between strains within and among river sections. Only within the lower section were the strains not significantly different. None of the intrastrain comparisons among river sections were significantly different. The neighbor-joining tree (Figure 3) revealed a similar pattern, with samples clustered by strain type and with the Great Lakes and Highlands strains showing the least divergence within the lower river section. The Great Lakes strain from the Ohio River clustered closely with Lake Erie Walleyes (the Great Lakes-strain temporal genetic baseline), while the Highlands-strain Walleyes appeared to be genetically diverged from their historical counterparts.

Based on the Bayesian analysis, the most likely number of genetic clusters identified in the entire data set was two. Waterbody-specific patterns were apparent when the membership probability (q) values were plotted for $K = 2$ (Figure 4). Values of q are used to assign an individual to one or more clusters (Pritchard et al. 2000; Manel et al. 2002); therefore, the lowest q score (0.86) for the cluster associated at $K = 2$ was used to determine a cutoff value for classifying an individual as a "pure Lake Erie-strain" fish. Any Walleye with a score below this value was classified as belonging to the Highlands strain.

Since it is possible that a fish assigned to a given strain based on mitochondrial DNA could be an interstrain hybrid, we compared the mitochondrial DNA data for Ohio River Walleyes to the microsatellite data to determine whether individuals with the Great Lakes mitochondrial haplotype also had a microsatellite DNA profile that was consistent with the Lake Erie genetic cluster. Sixty-three Walleyes from the Ohio River had microsatellite DNA profiles that were consistent with the Lake Erie genetic cluster; 55 of those individuals had a mitochondrial DNA haplotype that was consistent with the Great Lakes haplotype. There were 109 Walleyes from the Ohio River with microsatellite DNA profiles consistent with the Highlands genetic cluster; 23 of those fish had a mitochondrial DNA haplotype that was consistent with the Great Lakes haplotype. Classifying the various types of crosses allows for a determination of how much interstrain mating may have occurred. Based on the combined mitochondrial and nuclear data sets, 50.0% ($N = 86$) of the Walleyes sampled from the Ohio River were pure Highlands-strain fish (mitochondrial and nuclear profiles were consistent with the Ohio River), 32.0% ($N = 55$) were pure Great Lakes-strain fish, and 18.0% ($N = 31$) were the result of interstrain hybridization (Figure 5). Pure

TABLE 1. Summary of descriptive genetic statistics for contemporary Ohio River Walleye strains (by river section: lower, middle, and upper) and historical baseline populations (N = mean number of samples across loci; H_o = mean observed heterozygosity; H_e = mean expected heterozygosity; N_a = mean number of alleles; R_a = mean allelic richness; P_a = mean private allele richness; r_{xy} = mean coefficient of relatedness; $r_{xy,0.25}$ = proportion of pairwise r_{xy} estimates that were consistent with relatedness at the half-sibling level or greater; $r_{xy,0.50}$ = proportion of pairwise r_{xy} estimates that were consistent with relatedness at the full-sibling level or greater).

Strain and location	N	H_o	H_e	N_a	R_a	P_a	r_{xy}	$r_{xy,0.25}$	$r_{xy,0.50}$
Contemporary Ohio River									
Great Lakes strain									
Lower	25	0.78	0.80	9.7	6.7	0.31	0.038	0.13	0.02
Middle	38	0.76	0.81	11.1	6.9	0.40	0.067	0.08	0.01
Upper	12	0.69	0.76	7.6	6.3	0.34	0.067	0.14	0.00
Highlands strain									
Lower	18	0.72	0.77	8.2	6.5	0.14	0.068	0.16	0.02
Middle	40	0.60	0.76	9.8	5.9	0.29	0.111	0.21	0.02
Upper	33	0.59	0.70	8.6	5.5	0.30	0.221	0.41	0.05
Historical baselines									
Great Lakes strain									
Lake Erie[a]	88	0.75	0.77	13.6	6.5	0.58	0.117	0.20	0.01
Highlands strain									
Ohio River basin	12	0.49	0.79	7.7	6.2	1.48	0.024	0.25	0.13

[a]Represents fish that were collected from the Maumee and Sandusky rivers.

Highlands-strain Walleyes were more common in the upper Ohio River and less common in the lower part of the river. In addition, interstrain hybrids appeared to be more common in the lower Ohio River.

For the evaluation of TL and W among strains and hybrids, the mean (±SE) values were as follows. For the Great Lakes strain, TL was 347 ± 17.5 mm, and W was 619 ± 108.8 g. For the Highlands strain, TL was 410 ± 25.4 mm, and W was 954 ± 175.1 g. The TL of hybrids was 400 ± 14.4 mm, and the W of hybrids was 830 ± 92.9 g. Comparisons of TL (ANOVA: $F = 0.79$, df = 2, $P = 0.455$) and W ($F = 1.44$, df = 2, $P = 0.239$) among strains and hybrids indicated no significant differences.

TABLE 2. Pairwise estimates of the genetic differentiation index F_{ST}, comparing native (Highlands-strain) and Great Lakes-strain Walleye populations sampled within the lower, middle, and upper sections of the Ohio River. Asterisks denote instances of significant genetic differentiation between populations (following sequential Bonferroni adjustments for multiple comparisons).

	Great Lakes strain			Highlands strain		
Strain and location	Lower	Middle	Upper	Lower	Middle	Upper
Great Lakes strain						
Lower	–					
Middle	0.006	–				
Upper	0.017	0.004	–			
Highlands strain						
Lower	0.033	0.053*	0.081*	–		
Middle	0.051*	0.064*	0.096*	0.001	–	
Upper	0.101*	0.107*	0.138*	0.022	0.014	–

DISCUSSION

Genetic analyses can enhance the management of stream fishes by elucidating ecological and anthropogenic factors influencing populations at spatial and temporal scales (Scribner et al. 2016) and can provide valuable biological and population metrics (e.g., effective population sizes, gene flow, and mating structures; Scribner and Chesser 2001). Previous genetic work has described hybridization between the native Ohio River strain and nonnative Great Lakes strain of Walleyes within the Ohio River (White et al. 2005) and the upper Ohio River (White et al. 2012). In response to these findings, management actions taken to protect and enhance the native strain included the implementation of restrictive harvest regulations and supplemental stocking of the native strain within some locations. We conducted genetic analyses that included temporal genetic data (temporal baseline), as suggested by White et al. (2005), to provide insight into the historical genetic variability of the Ohio River Walleyes prior to the introduction of Walleyes from nonnative sources. This analysis re-examined the genetic diversity of contemporary Walleyes from Lake Erie and the Ohio River to identify changes in genetic structure and variability within and among strains, particularly as such changes pertain to recent stocking activities.

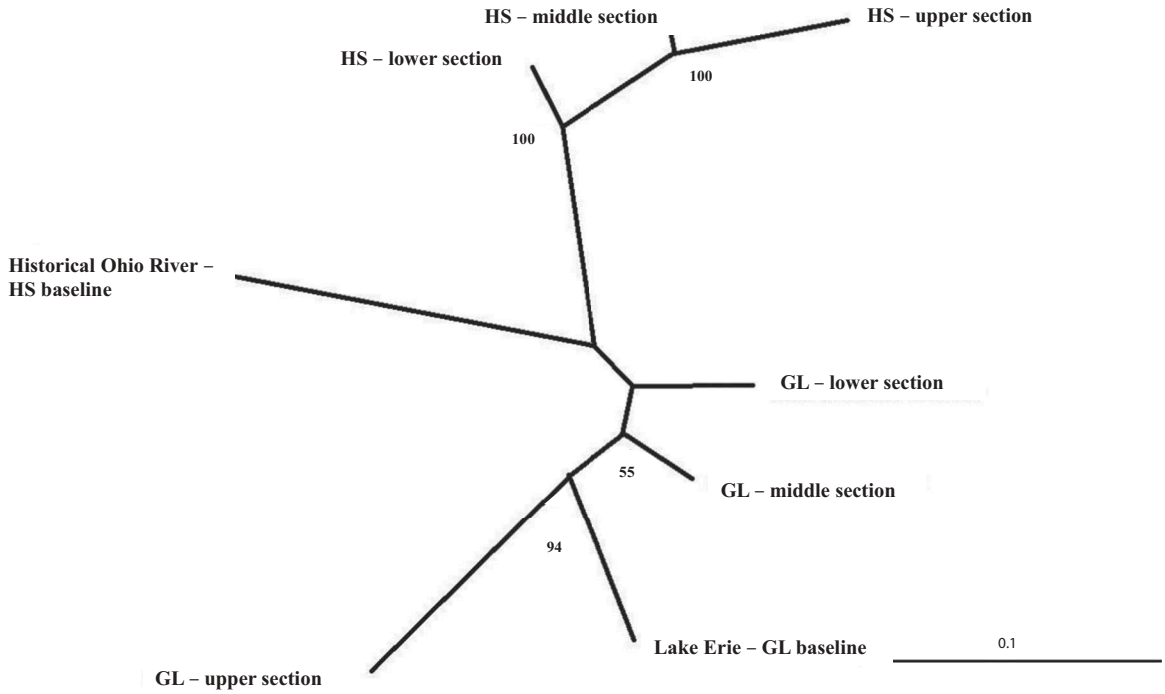


FIGURE 3. Neighbor-joining tree of Nei's genetic distance among contemporary Walleye strains (HS = Highlands strain; GL = Great Lakes strain) within lower, middle, and upper sections of the upper Ohio River and their historical genetic baselines. Only nodes receiving greater than 55% bootstrap support are labeled.

Assessment of Spatial Structure

Similar to the results of other studies (e.g., Stepien and Faber 1998; Stepien et al. 2009), we observed that despite a history of stocking, genetic differences between Lake Erie and Ohio River Walleyes remain. We evaluated the composition and genetic structure of native and nonnative Walleye strains. Results of mitochondrial DNA analyses revealed that the trends in spatial composition of Ohio River and Great Lakes-strain Walleyes among river sections were similar to those identified in previous studies, with the proportion of Ohio River-strain Walleyes increasing from downstream to upstream sections of the Ohio River. However, the proportion of each strain within river sections differed from previous research. On average, within the river sections evaluated, the proportion of Highlands-strain Walleyes increased by 30% over previous estimates (White et al. 2005). In particular,

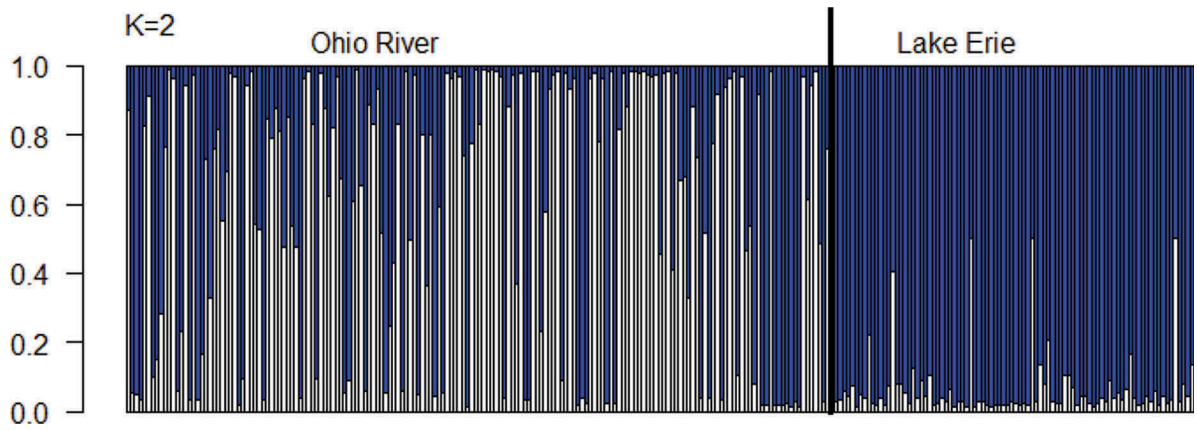


FIGURE 4. Results of the Bayesian analysis implemented in STRUCTURE, showing the proportional contribution of each genetic group (K = 2 genetic clusters) to the ancestry of each Walleye from Lake Erie and the Ohio River. Each fish is represented by a vertical bar that is colored according to the contribution of each genetic group.

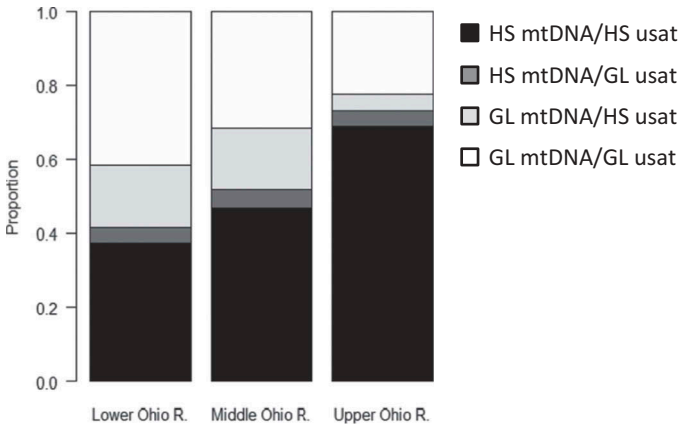


FIGURE 5. Summary of the status of Walleyes (HS = Highlands strain; GL = Great Lakes strain) from the upper, middle, and lower sections of the upper Ohio River based on classification using nuclear DNA (microsatellites [μsat]) and mitochondrial DNA (mtDNA) data.

within the upper section of the Ohio River, the proportion of Highlands-strain Walleyes appeared to have increased from 51% (White et al. 2005; New Cumberland and Hannibal Dam tailwaters: WVDNR 2008) to 73% based on our estimates. The reason for this trend is unclear, but increases in Highlands-strain Walleyes are likely a function of supplemental stocking that began after 2005. Natural reproduction, however, may also be a factor, as population assessment data (age data) indicated the presence of juvenile Highlands-strain Walleyes during years in which stocking did not occur (WVDNR 2008). For a number of Ohio River tributaries, genetic analyses have shown that Great Lakes-strain and Highlands-strain Walleyes remained genetically distinct within some locales (Cumberland, Kanawha, and New rivers), suggesting that some level of reproductive isolation is also plausible (Palmer 1999; Palmer et al. 2005; White et al. 2012). The relative contribution of hatchery or native fish to population recovery is often difficult to resolve but is an important metric for management (Murphy and Nielsen 1983; Guinand et al. 2003; Page et al. 2003). Quantifying the hatchery contribution is important to facilitate the monitoring of stocking success and native population recovery. Tagging (e.g., oxytetracycline) and monitoring of hatchery fish could be an option for elucidating the natural and hatchery contributions to the Highlands strain's recovery (Hendricks 1995).

We found that the genetic variability of Walleyes within the Ohio River was principally partitioned by strain (i.e., basin of origin). Nearly all pairwise comparisons of F_{ST} between the Great Lakes and Highlands strains, both within and among river sections, were found to be significant, and the Bayesian analysis identified two genetic clusters reflecting the Ohio River and Lake Erie. This was a departure from previous research using microsatellite DNA and mitochondrial DNA data, which found no significant differences between strains within the same tailwaters (White et al. 2005, 2012). There was no evidence of spatial structuring within strains in the Ohio River; however, the level of genetic differentiation between strains did decrease from upstream to downstream, with no significant difference found between the Great Lakes and the Highlands strains within the lower section of the Ohio River. Lack of differentiation between strains in the lower section may be related to the increased occurrence of interstrain hybridization. The proportion of hybrids was greatest within the lower section of the Ohio River. Differences in levels of hybridization may be influenced by a number of factors (Scribner et al. 2001; Almodóvar et al. 2006; Harbicht et al. 2014), but in this case, the combination of relative abundance and habitat may be an important contributor. Lower sections of our study reach possess limited spawning habitat and turbid water conditions, potentially making it more difficult for individuals to locate conspecifics for mating. However, it should be noted that the stocking of large numbers of pure Highlands-strain individuals into the upper sections of the river may also mask hybridization, which could account for differences in levels of hybridization (1) among our study sections and (2) between this study and those reported in previous studies. Prior studies using microsatellite DNA and mitochondrial DNA data also found evidence for mating between the Great Lakes-strain and Highlands-strain Walleyes at the same Ohio River sites we examined (Pike Island, New Cumberland, and Willow Island; White et al. 2005, 2012). The present study also found evidence of interstrain hybridization in the lower reaches of the Ohio River, which could not be tested previously due to sample size limitations. Identifying the relative contributions of hatchery and naturally spawned fish would enhance our understanding of the factors influencing changes in Walleye strain abundance, which in turn would be beneficial for evaluating current management actions and informing future management decisions, such as determining the success and impacts of stocking.

Genetic Diversity

Highlands-strain Walleyes from the upper and middle sections of the Ohio River exhibited the lowest levels of H_o and R_a and the greatest levels of relatedness, with an r_{xy} value only slightly less than the level of half-sibling (0.221), and a substantial proportion (41%) of individual pairwise r_{xy} comparisons indicated relatedness at the half-sibling level or greater. These results may be indicative of a hatchery effect on the genetic diversity of Highlands-strain Walleyes. Hatchery supplementation can have adverse effects on natural populations, thereby hindering recovery efforts and reducing long-term population sustainability (Busack and Currens 1995; Campton 1995; Page et al. 2011). In particular, hatchery production typically exceeds natural production, causing reductions in the genetic variability and effective population sizes of native populations (genetic swamping; Ryman and Laikre 1991; Bartron and Scribner 2004) as well as altering behavioral traits (e.g., run timing; Ford et al. 2006). Effects may be compounded by using small numbers of broodstock individuals collected from nonlocal sources and spawning techniques that promote disproportionate contributions of adults to stocked progeny (e.g., unequal sex ratios and sequential spawning). The upper and middle sections of

the Ohio River are most likely to be influenced by hatchery supplementation, which may account for the reduced levels of diversity we observed. Methods for supplementing Highlands-strain Walleyes are generally consistent with those attributed to promoting reductions in genetic diversity and increased relatedness. The program stocks as many as 65,500 Highlands-strain fingerlings between the New Cumberland Dam and Hannibal Dam tailwaters annually (WVDNR 2008). Hatchery practices seek to take advantage of the current abundance and diversity of Highlands-strain populations within Ohio River tributaries. Adults used for hatchery production are collected opportunistically from the wild; before spawning, they are genetically screened to ensure that only Highlands-strain individuals are crossed. Consequently, at times, broodstocks may comprise low numbers of adults, potentially including individuals collected from Ohio River tributaries (e.g., Kanawha and New rivers) where populations are small relative to those historically found within the Ohio River. Within stream systems, including the Ohio River, fish populations from tributaries have been shown to exhibit lower genetic diversity than their main-stem counterparts (Zipfel 2006; Vähä et al. 2008; White et al. 2012). Although historical declines in the abundance of Highlands-strain Walleyes cannot be ruled out as having contributed to a reduction in genetic diversity, the greater levels of genetic diversity (i.e., H_o) found previously by White et al. (2005) within Highlands-strain samples collected from locations similar to our study sites would indicate a more recent decline. Consequently, broodstocks derived from Ohio River tributaries and concomitant hatchery practices may have negative impacts on the genetic diversity of main-stem Ohio River Walleyes, thereby inhibiting population recovery.

One option for maintaining the genetic integrity of Highlands-strain Walleyes within the Ohio River would be to adopt a "conservation hatchery" strategy that balances restoration and fishery goals (Flagg and Nash 1999; Paquet et al. 2011). For example, creating and maintaining a captive strain of native Walleyes or establishing a broodstock lake (Schell et al. 2004) would allow managers to limit the negative impacts of artificial propagation by controlling the numbers and sources of fish available for spawning. Additionally, broodstock and progeny could be closely monitored to avoid undesirable deviations in genetic diversity relative to the natural population. Furthermore, a captive broodstock would provide a consistent source of progeny for meeting hatchery production goals. Efforts to adopt a broodstock lake for native Walleyes are ongoing (Zipfel, personal communication). A broodstock lake would be helpful in providing a larger, more consistent source of known adult spawners (i.e., tagged fish) over time, thus reducing the chances for large fluctuations in genetic diversity and increased relatedness among stocked progeny. An added benefit of a broodstock lake would be that the genetic diversity of the broodstock could be effectively monitored. Ultimately, the level at which these strategies can be adopted will depend on hatchery program limitations (e.g., cost and hatchery space). However, if the goal is to restore and preserve a native population(s)—for which genetic diversity and integrity should be a key component—then it is vital to consider the cost of current hatchery practices in relation to the long-term genetic integrity and viability of the native strain of Walleyes.

Temporal Genetic Assessment

Analyses of historical genetic data can provide information useful for evaluating the impacts of anthropogenic effects and generating population genetic baselines by which to measure genetic change and define management goals (Vähä et al. 2008; Wilson et al. 2008; Metcalf et al. 2012). For this study, we developed historical genetic baselines for the Ohio River strain (museum specimens) and Great Lakes strain (contemporary Lake Erie basin) of Walleyes to compare against contemporary Ohio River populations. A neighbor-joining tree summarizing the genetic relationships among contemporary and historical Walleye strain populations revealed that although the Great Lakes-strain Walleyes within the Ohio River clustered closely with their historical source population (Lake Erie), Highlands-strain Walleyes appear to have diverged from their historical counterparts and now have greater genetic affinity with Great Lakes Walleyes. Genetic divergence of natural populations due to historical reductions in population size and due to stocking has been described within a number of fish populations, including Atlantic Cod *Gadus morhua* in the North Sea (Hutchinson et al. 2003), Brown Trout *Salmo trutta* (Hansen et al. 2009), and Lake Trout *Salvelinus namaycush* in the Great Lakes (Guinand et al. 2012). However, although notable temporal deviations in genetic diversity have been documented, these same studies also found that genetic structure (i.e., genetic distinctiveness) among individual populations has generally remained intact over time. Similarly, we found that genetic structuring among Ohio River Walleyes continues to be principally based on basin of origin, despite the temporal genetic divergence in the Highlands strain.

Interstrain Length and Weight Comparison

It is recognized that native populations will evolve physical and behavioral traits (e.g., growth, fecundity, and spawning time) adapted to their local environments. Accordingly, native fish may possess competitive advantages or desirable traits (e.g., growth) over nonnative strains or hybrids (Page et al. 2003; Wingate and Younk 2007; Fraser et al. 2008; Sloss et al. 2008). Within the Ohio River, anecdotal evidence suggests that Walleyes of the native strain tend to grow larger than Great Lakes-strain individuals. This has important management implications, as efforts to restore Highlands-strain Walleye populations would have the added benefit of increasing angling opportunities for catching larger fish, and differences in growth may necessitate the adoption of regulations within certain areas of the Ohio River to protect larger-sized native Ohio River Walleyes. By combining genetic and biological data, we compared length-at-age and *W* measurements and detected no significant differences in these metrics between Walleye strains. We also found no evidence that hybrid Walleyes differed significantly from their parental strains in terms of TL or *W*. These results concur with previous comparisons that found no significant

differences in growth between these strains (WVDNR 2008). Accordingly, there does not appear to be a genetic (adaptive) predisposition for Highlands-strain Walleyes to grow larger than the Great Lakes-strain Walleyes within the Ohio River. Therefore, interstrain growth differences should not be a factor when adopting management strategies for the restoration of native-strain Walleyes.

The present genetic analyses re-examined and extended our previous knowledge of Walleye genetics within the Ohio River. Results should be informative for managing Walleye populations in the Ohio River basin, as this has been identified as a priority among fish management agencies. Most notable is the evidence suggesting an increase in the proportion of the native Highlands strain within the Ohio River, accompanied by an apparent shift in the genetic structure. Evidence suggests that differences may be related to stocking, indicating a success in the hatchery supplementation program at restoring native-type Walleyes to the Ohio River. However, an evaluation of stocking success combined with genetic monitoring would be helpful for elucidating the natural and hatchery contributions to population recovery and the potential impacts of stocking on genetic diversity. Results could then be measured against hatchery program goals as a means of quality control, as reductions in genetic diversity and increases in relatedness appear to be concomitant with—but not necessarily caused by—supplementation practices. Furthermore, historical genetic data suggest that Highlands-strain Walleyes within the Ohio River have diverged appreciably from their historical counterparts, possibly due to a combination of a historical reduction in population size and introgression with Great Lakes-strain Walleyes. Accordingly, restoration of the historical genetic diversity is unlikely, and we suggest that managers weigh the cost and benefits associated with maintaining current genetic diversity of native Ohio River Walleyes, particularly given evidence of continued background interstrain hybridization and hatchery limitations.

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Appendix: Museum Specimens and Allele Frequency Data

TABLE A.1. Complete list of historical specimens screened for this study, showing the location and year of collection and the loci for which there was successful microsatellite amplification in each specimen (not all loci were used in this study). Tissue from these specimens were provided by the Academy of Natural Sciences, Drexel University, Philadelphia (ANSP); the Museum of Comparative Zoology, Harvard University (MCZ); the Museum of Biological Diversity, Ohio State University (OSUM); and the Museum of Zoology, University of Michigan (UMMZ). Additional information on these specimens is available via the Fishnet2 database (http://www.fishnet2.net/).

Body of water	State	Year	Museum collection	Reference number	Loci
Beaver River	PA	1880	ANSP	21059.1	None
		1880	ANSP	21059.2	*Svi2, Svi4, Svi17, Svi18, Svi33, SviL8*
		1880	ANSP	21059.3	*Svi2, Svi6, Svi7, Svi18, Svi20, SviL6*
		1880	ANSP	21059.4	*Svi18, SviL6*
		1880	ANSP	32613.1	*Svi2, Svi6, Svi17, Svi18, Svi20, Svi26, Svi33*
		1880	ANSP	32613.2	*Svi2, Svi4, Svi18, SviL8*
		1880	ANSP	32613.3	*Svi2, Svi4, Svi17, Svi18, Svi20*
		1880	ANSP	32613.4	*Svi6, Svi18, SviL6, SviL8*
		1880	ANSP	32618.1	*Svi18, SviL6*
		1880	ANSP	32618.2	*Svi18, SviL6*
		1880	ANSP	32618.3	*Svi2, Svi4, Svi18, Svi33, SviL6*
Big Walnut Creek	OH	1959	UMMZ	35589	*MSL-1, Svi17, Svi18, SviL6, SviL8*
Cumberland River	KY	1956	UMMZ	177882	*Svi2, Svi4, Svi7, Svi17, Svi18, Svi26, Svi33, SviL8*
Hungirts Creek	WV	1933	UMMZ	118760.1	*MSL-1, MSL-2, Svi4, Svi6, Svi7, Svi17, Svi18, Svi33, SviL6, SviL7, Svi8*
		1933	UMMZ	118760.2	None
Kanawha River	WV	1935	UMMZ	119359	*MSL-2, Svi2, Svi4, Svi6, Svi17, Svi18, Svi26, Svi33, SviL6, SviL8*
Licking River	OH	1939	OSUM	3860	*Svi18, SviL8*
Monongahela River	PA	1885	UMMZ	212943	*Svi18*
Muskingum River	OH	1940	OSUM	1839	*MSL-1, Svi18, Svi20*
		1940	OSUM	1844	*Svi4, Svi17, Svi18, SviL6, SviL8*
New River	WV	1935	UMMZ	119270	*MSL-1, Svi2, Svi4, Svi6, Svi7, Svi17, Svi18, Svi26, Svi33, SviL8*
Ohio River	KY	1939	OSUM	595	*Svi2, Svi4, Svi17, Svi18, SviL7*
		1939	OSUM	1081	*Svi17, Svi18, Svi20*
Ohio River	OH	1935	UMMZ	103302.1	*MSL-1, MSL-2, Svi2, Svi4, Svi6, Svi7, Svi17, Svi18, Svi20, Svi33, SviL6, SviL7, SviL8*
		1935	UMMZ	103302.2	*Svi2, Svi4, Svi6, Svi17, Svi18, Svi20, Svi26, Svi33, SviL6, SviL8*
Rockcastle River	WV	1936	UMMZ	139047	*Svi2, Svi4, Svi6, Svi17, Svi26, Svi33, SviL8*
Shade Creek	OH	1939	OSUM	13656.2	*Svi18, SviL6, SviL8*
Tennessee River	TN	1854	MCZ	10341	None
Unknown	PA		ANSP	13685	None
Youghiogheny River	PA	1898	ANSP	13684	*Svi18, SviL6*

TABLE A.2. Allele frequency data and sample sizes for the Ohio River Walleye populations evaluated in this study (lower, middle, and upper = contemporary Ohio River samples from three river sections; Lake Erie = contemporary Lake Erie samples that served as the historical genetic baseline for the Great Lakes strain; historical = museum specimens that served as the historical genetic baseline for the Highlands strain).

		Great Lakes strain				Highlands strain			
Locus	Allele	Lower	Middle	Upper	Lake Erie	Lower	Middle	Upper	Historical
Svi17	86	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.03
	92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13
	94	0.00	0.00	0.00	0.02	0.03	0.00	0.00	0.23
	96	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.03
	98	0.30	0.20	0.13	0.01	0.75	0.62	0.83	0.20
	100	0.03	0.08	0.00	0.01	0.03	0.10	0.06	0.00
	102	0.33	0.43	0.54	0.53	0.09	0.15	0.08	0.27
	104	0.00	0.00	0.00	0.03	0.00	0.02	0.00	0.00
	108	0.20	0.09	0.08	0.17	0.06	0.00	0.00	0.10
	110	0.03	0.04	0.17	0.03	0.00	0.07	0.03	0.00
	112	0.13	0.16	0.08	0.15	0.03	0.04	0.00	0.06
	114	0.00	0.00	0.00	0.03	0.00	0.00	0.00	0.00
	116	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	N	20	38	12	88	16	41	33	15
Svi18	114	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	116	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.00
	118	0.29	0.25	0.25	0.25	0.38	0.30	0.29	0.37
	120	0.21	0.17	0.17	0.04	0.22	0.37	0.29	0.22
	122	0.18	0.11	0.25	0.18	0.09	0.05	0.05	0.04
	124	0.21	0.34	0.25	0.49	0.03	0.07	0.11	0.28
	126	0.11	0.13	0.08	0.02	0.28	0.18	0.27	0.07
	128	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.02
	N	19	38	12	88	16	41	33	23
Svi2	187	0.18	0.12	0.21	0.11	0.06	0.06	0.02	0.04
	189	0.18	0.37	0.33	0.43	0.09	0.07	0.03	0.15
	191	0.05	0.05	0.08	0.11	0.06	0.00	0.02	0.31
	193	0.11	0.09	0.00	0.11	0.13	0.16	0.14	0.08
	195	0.11	0.13	0.33	0.03	0.22	0.23	0.36	0.12
	197	0.05	0.04	0.00	0.01	0.06	0.07	0.06	0.12
	199	0.32	0.08	0.00	0.13	0.28	0.27	0.23	0.12
	201	0.00	0.03	0.00	0.02	0.00	0.00	0.00	0.00
	203	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	207	0.00	0.00	0.00	0.00	0.00	0.02	0.02	0.00
	209	0.00	0.04	0.00	0.00	0.06	0.05	0.09	0.00
	215	0.00	0.01	0.04	0.02	0.00	0.00	0.00	0.08
	217	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	219	0.00	0.00	0.00	0.01	0.00	0.02	0.03	0.00
	225	0.00	0.03	0.00	0.00	0.03	0.02	0.02	0.00
	243	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00
	247	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	249	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	255	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	N	19	38	12	88	16	41	33	13
Svi20	147	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	149	0.04	0.01	0.08	0.02	0.10	0.12	0.20	0.00

TABLE A.2. Continued.

		Great Lakes strain				Highlands strain			
Locus	Allele	Lower	Middle	Upper	Lake Erie	Lower	Middle	Upper	Historical
	151	0.38	0.29	0.25	0.26	0.30	0.48	0.52	0.33
	153	0.09	0.08	0.08	0.07	0.13	0.07	0.06	0.00
	155	0.04	0.01	0.00	0.02	0.05	0.04	0.06	0.00
	157	0.09	0.08	0.04	0.06	0.08	0.06	0.03	0.00
	159	0.07	0.01	0.04	0.03	0.08	0.06	0.03	0.17
	161	0.05	0.09	0.00	0.03	0.10	0.12	0.11	0.08
	163	0.00	0.04	0.04	0.02	0.03	0.01	0.00	0.00
	165	0.07	0.11	0.04	0.21	0.05	0.01	0.00	0.00
	167	0.02	0.07	0.04	0.07	0.05	0.01	0.00	0.00
	169	0.05	0.05	0.08	0.07	0.03	0.00	0.00	0.17
	171	0.02	0.01	0.04	0.06	0.00	0.00	0.00	0.17
	173	0.07	0.12	0.17	0.04	0.03	0.01	0.00	0.00
	175	0.00	0.03	0.04	0.01	0.00	0.00	0.00	0.00
	177	0.00	0.00	0.04	0.03	0.00	0.00	0.00	0.00
	183	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
	191	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	N	28	38	12	88	20	41	33	6
Svi26	143	0.02	0.00	0.00	0.00	0.05	0.00	0.00	0.00
	149	0.00	0.00	0.00	0.00	0.00	0.00	0.03	0.00
	151	0.04	0.00	0.00	0.00	0.05	0.00	0.02	0.00
	153	0.00	0.00	0.00	0.00	0.00	0.03	0.05	0.00
	155	0.06	0.01	0.00	0.01	0.05	0.09	0.02	0.00
	157	0.11	0.15	0.13	0.17	0.28	0.43	0.48	0.36
	159	0.06	0.03	0.00	0.04	0.05	0.01	0.03	0.00
	161	0.00	0.00	0.04	0.01	0.00	0.01	0.05	0.00
	163	0.07	0.14	0.04	0.05	0.25	0.25	0.26	0.14
	165	0.15	0.07	0.04	0.07	0.13	0.05	0.02	0.00
	167	0.04	0.00	0.00	0.01	0.00	0.01	0.00	0.07
	169	0.22	0.21	0.21	0.20	0.10	0.04	0.00	0.00
	171	0.00	0.04	0.04	0.07	0.03	0.00	0.00	0.00
	173	0.04	0.06	0.00	0.06	0.03	0.03	0.00	0.14
	175	0.00	0.01	0.00	0.04	0.00	0.00	0.00	0.00
	177	0.00	0.06	0.00	0.01	0.00	0.00	0.00	0.00
	179	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	181	0.00	0.03	0.00	0.04	0.00	0.00	0.00	0.00
	183	0.02	0.00	0.04	0.03	0.00	0.00	0.00	0.07
	185	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07
	187	0.04	0.01	0.00	0.02	0.00	0.00	0.00	0.14
	189	0.00	0.00	0.04	0.04	0.00	0.00	0.00	0.00
	191	0.00	0.01	0.00	0.02	0.00	0.01	0.00	0.00
	193	0.00	0.01	0.00	0.01	0.00	0.01	0.02	0.00
	195	0.06	0.11	0.04	0.06	0.00	0.00	0.02	0.00
	197	0.04	0.01	0.29	0.02	0.00	0.04	0.02	0.00
	199	0.04	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	201	0.00	0.03	0.04	0.02	0.00	0.00	0.00	0.00
	203	0.00	0.00	0.00	0.01	0.00	0.00	0.02	0.00
	207	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00

TABLE A.2. Continued.

		Great Lakes strain				Highlands strain			
Locus	Allele	Lower	Middle	Upper	Lake Erie	Lower	Middle	Upper	Historical
	209	0.00	0.00	0.04	0.00	0.00	0.00	0.00	0.00
	N	27	36	12	83	20	40	33	7
Svi33	79	0.00	0.04	0.08	0.00	0.10	0.24	0.27	0.00
	81	0.10	0.13	0.00	0.03	0.28	0.38	0.50	0.00
	83	0.04	0.08	0.04	0.09	0.03	0.01	0.00	0.00
	85	0.10	0.08	0.08	0.16	0.05	0.06	0.03	0.00
	87	0.00	0.01	0.00	0.06	0.00	0.00	0.00	0.00
	89	0.02	0.01	0.04	0.03	0.00	0.01	0.00	0.00
	91	0.19	0.22	0.25	0.13	0.23	0.12	0.08	0.05
	93	0.44	0.20	0.25	0.35	0.30	0.12	0.05	0.18
	95	0.00	0.03	0.08	0.05	0.00	0.01	0.00	0.05
	97	0.08	0.17	0.17	0.08	0.03	0.04	0.08	0.14
	99	0.00	0.01	0.00	0.02	0.00	0.00	0.00	0.05
	101	0.02	0.00	0.00	0.01	0.00	0.00	0.00	0.09
	103	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	105	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.14
	115	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
	117	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.05
	121	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09
	129	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05
	131	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05
	N	26	38	12	86	20	34	32	11
Svi4	99	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.04
	101	0.13	0.12	0.00	0.06	0.13	0.04	0.02	0.15
	103	0.07	0.08	0.00	0.00	0.20	0.24	0.05	0.12
	105	0.34	0.29	0.25	0.20	0.30	0.35	0.15	0.15
	107	0.18	0.12	0.08	0.02	0.18	0.21	0.30	0.00
	109	0.18	0.17	0.25	0.01	0.13	0.14	0.12	0.08
	111	0.05	0.03	0.17	0.17	0.00	0.03	0.30	0.04
	113	0.04	0.14	0.13	0.34	0.05	0.00	0.03	0.15
	115	0.02	0.05	0.13	0.19	0.03	0.00	0.03	0.27
	119	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	121	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	N	28	38	12	88	20	40	33	13
Svi6	133	0.00	0.04	0.00	0.00	0.00	0.01	0.00	0.00
	137	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	139	0.43	0.49	0.54	0.45	0.12	0.15	0.11	0.22
	141	0.05	0.11	0.04	0.09	0.06	0.05	0.00	0.00
	143	0.04	0.00	0.04	0.07	0.03	0.02	0.05	0.00
	145	0.07	0.05	0.08	0.06	0.03	0.02	0.02	0.00
	147	0.09	0.08	0.00	0.05	0.12	0.23	0.23	0.11
	149	0.00	0.09	0.04	0.01	0.24	0.11	0.27	0.06
	151	0.05	0.04	0.08	0.09	0.18	0.18	0.09	0.22
	153	0.05	0.03	0.08	0.07	0.12	0.12	0.05	0.11
	155	0.02	0.04	0.00	0.01	0.00	0.04	0.12	0.06
	157	0.04	0.00	0.04	0.03	0.12	0.02	0.06	0.00
	159	0.00	0.00	0.00	0.03	0.00	0.00	0.02	0.00

TABLE A.2. Continued.

		Great Lakes strain				Highlands strain			
Locus	Allele	Lower	Middle	Upper	Lake Erie	Lower	Middle	Upper	Historical
	161	0.05	0.01	0.00	0.01	0.00	0.00	0.00	0.00
	163	0.00	0.00	0.00	0.02	0.00	0.02	0.00	0.06
	165	0.09	0.03	0.04	0.01	0.00	0.00	0.00	0.06
	167	0.00	0.00	0.00	0.01	0.00	0.01	0.00	0.00
	203	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	215	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	217	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	N	28	38	12	88	17	41	33	9
SviL6	108	0.15	0.12	0.00	0.12	0.08	0.23	0.11	0.00
	110	0.37	0.34	0.54	0.48	0.29	0.24	0.24	0.57
	112	0.06	0.03	0.00	0.03	0.00	0.01	0.02	0.18
	114	0.00	0.03	0.00	0.00	0.00	0.00	0.00	0.00
	118	0.00	0.01	0.04	0.01	0.08	0.05	0.08	0.00
	120	0.00	0.01	0.00	0.01	0.00	0.00	0.00	0.00
	122	0.06	0.03	0.21	0.03	0.03	0.00	0.00	0.00
	124	0.11	0.20	0.17	0.08	0.29	0.18	0.33	0.11
	126	0.04	0.04	0.00	0.03	0.05	0.09	0.06	0.00
	128	0.09	0.05	0.00	0.06	0.00	0.02	0.00	0.00
	130	0.04	0.04	0.04	0.12	0.08	0.01	0.02	0.07
	132	0.00	0.00	0.00	0.01	0.00	0.00	0.00	0.00
	134	0.04	0.05	0.00	0.01	0.05	0.02	0.02	0.00
	136	0.02	0.00	0.00	0.01	0.00	0.02	0.02	0.00
	138	0.04	0.01	0.00	0.01	0.00	0.04	0.06	0.00
	140	0.00	0.01	0.00	0.00	0.00	0.00	0.00	0.00
	146	0.00	0.01	0.00	0.00	0.05	0.06	0.03	0.07
	152	0.00	0.00	0.00	0.00	0.00	0.01	0.02	0.00
	154	0.00	0.00	0.00	0.00	0.00	0.00	0.02	0.00
	N	27	37	12	88	19	41	33	14