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ARTICLE Genetic Divergence of Nearby Walleye Spawning Groups in Central Lake Erie: Implications for Management

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Abstract

Spawning site philopatry may lead to genetic differentiation among reproductive populations, despite their locations being in close proximity in single bodies of water. Identifying and maintaining locally differentiated spawning groups of Walleye *Sander vitreus* constitute an agency management priority of the multi-agency Walleye Task Group advisory for the Great Lakes Fishery Commission. Although genetically separable spawning groups of Walleye have been identified from several areas in the Great Lakes, those in central Lake Erie were previously unknown. The Ohio Division of Wildlife (ODW) collected Walleye for the present analysis from two spawning groups in Lake Erie's Central Basin, located just 2 km apart – one in the Grand River, Ohio, and the other at the nearby Central Basin Reef. The hypothesis of whether the two spawning

groups (*N*=147) genetically differ was tested by analyzing variation at 14 nuclear DNA microsatellite loci from Walleye reproducing in 2012 at the two sites, and among three separate years (1996, 2003, and 2012) in the Grand River (to evaluate temporal trends). Results revealed relatively high genetic diversity in both spawning groups, with the reproductive population in the Grand River having significantly greater allelic richness and representation of private alleles. The Grand River runs showed slight temporal decline in allelic richness from 1996-2003, coinciding with ODW reports of declining numbers of Walleye reproducing there. The two spawning groups differed in genetic composition, suggesting that they are closely related yet separable reproductive subpopulations, with both contributing to the overall diversity of Lake Erie Walleye. Their maintenance and integrity thus may bear management attention and further monitoring.

Running Head: Central Lake Erie Walleye Genetics

The Walleye *Sander vitreus* supports ecologically and economically valuable North American commercial and recreational fisheries (Locke et al. 2005). In the Laurentian Great Lakes, Lake Erie contains the largest population numbers and is known as the "Walleye capital of the world" (Scott and Crossman 1973; Hartig et al. 2009). The combined commercial and recreational Walleye fisheries in Lake Erie are worth ~$2 billion U.S. per year (Gentner and Bur 2009), with an estimated harvest of 3.078 million Walleye in 2016, according to the Lake Erie Walleye Task Group of the Great Lakes Fishery Commission (WTG 2017). The Lake Erie Walleye fishery is managed by binational inter-agency cooperation and advised under the recommendations of the Interagency Walleye Task Group (WTG) to the Lake Erie Committee of the Great Lakes Fishery Commission (WTG 2017). The WTG is guided by the Joint Strategic Plan (JSP) for Management of Great Lakes Fisheries to cooperatively analyze fish communities and fisheries in Lake Erie (Kayle et al. 2015).

A key objective of the Lake Erie Walleye Management Plan is to "Maintain and promote genetic diversity by identifying, rehabilitating, conserving, and/or protecting locally adapted stocks" (Kayle et al. 2015). Likewise, across the entire Great Lakes, "Identifying and maintaining healthy Walleye genetic stocks" constitutes a Great Lakes Fishery Commission management priority (Ryan et al. 2003). Walleye populations from Lake Erie have been found to possess the greatest overall degree of genetic diversity among all populations analyzed across the species' North American range using nuclear DNA microsatellite loci, containing several genetically differentiated spawning groups (Stepien et al. 2009, 2015).

Spatial genetic differentiation and population structure characterize Walleye at their spring spawning locations, with their returns to natal sites believed to be genetically based (summarized by Stepien et al. 2009, 2015). Otherwise, individual Walleye often travel widely during most of the year and stocks show

considerable admixture (Vandergoot et al. 2010). For example, Brenden et al. (2015) analyzed microsatellite loci to determine origins of the Walleye recreational harvest in Saginaw Bay, Lake Huron, estimating that 25% were individuals born in the Lake St. Clair/western Lake Erie system.

Walleye reproductive populations across the Great Lakes and throughout other parts of their native range (encompassing much of northeastern and central North America) exhibit both broad-scale and fine-scale genetic differences, which appear to trace to their post-glacial recolonization patterns, physical and behavioral barriers to migration, and natal homing (Stepien et al. 2012, 2015; Haponski and Stepien 2014a,b). For example, some spawning groups located less than 20 km apart in the Huron–Erie Corridor (spanning the Lake Huron–St. Clair River–Lake St. Clair–Detroit River–western Lake Erie system) significantly differed from each other in allelic composition, whereas others did not (Haponski and Stepien 2014a). Genetic analysis of single nucleotide polymorphism (SNP) variation of Walleye from several lakes and watersheds in Alberta Canada likewise found both broad– and fine–scale stock differentiation among watersheds and lakes, with two nearby lakes housing a single interbreeding population, and others showing genetic demarcation that did not reflect a genetic isolation by geographic distance pattern (Allen et al. 2017). These patterns are very like those discerned for Walleye populations across Lake Erie and the Great Lakes overall using microsatellite loci (summarized by Stepien et al. 2015), and as analyzed here to assess two spawning groups in Central Lake Erie for the present study.

Using otolith chemical signature assignments, Chen et al. (2017) found that strontium levels in otolith cores significantly differed between Walleye spawning in the Sandusky and Maumee Rivers of western Lake Erie, supporting natal homing. Stepien et al. (2012) identified lower genetic self-assignments for Walleye spawning in the Maumee River than for those reproducing in the nearby Sandusky River (or in Van Buren Bay, which is in the Eastern Basin); this was attributed to large number of spawners and the geographic proximity of nearly continuous reef and river spawning locations in Western Lake Erie. This continuity likewise explained close genetic relationships among western Lake Erie spawning groups (Maumee and Sandusky rivers and Western Basin Reefs) found with SNP analysis (Chen 2016) and microsatellite results of Brenden et al. (2015) for the Lake St. Clair/Western Lake Erie system. An acoustic telemetry study by Hayden et al. (2017) uncovered less fidelity (70%) of Walleye tagged in the Maumee River (as opposed to 95% in the Tittabawassee River off Saginaw Bay, Lake Huron), which they postulated is related to the close proximity of other spawning aggregations and sites in Western Lake Erie. Walleye spawning in the Lake St. Clair/Western Lake Erie system significantly differed from those spawning in the Tittabawassee River (Brenden et al. 2015), whose findings were similar to the differentiation elucidated by Haponski and Stepien (2014a) for Walleye spawning in the nearby Flint River, which both flow together through the Saginaw River into Saginaw Bay, Lake Huron. Overall genetic relationships of Walleye spawning groups in Lake Erie showed greater site-specific

differentiation in the Eastern Basin than in the Western Basin (summarized by Stepien et al. 2015), with the Central Basin spawning groups evaluated here.

Analyses of historic specimens of Lake Erie Walleye from the early to mid-20th century revealed lower levels of genetic diversity than found at present, which likely stemmed from habitat loss, pollution, and overexploitation (Haponski and Stepien 2014b). The lakewide Walleye population appears to have largely recovered in genetic diversity during the past several decades (Haponski and Stepien 2014b; Stepien et al. 2015). From the mid-1990s to the present, Walleye spawning groups in the Western and Eastern Basins of Lake Erie were found to have each maintained their respective genetic consistency, composition, and diversity (Stepien et al. 2012, 2015; Haponski and Stepien 2016). Spawning groups from Lake Erie's Central Basin have not previously been investigated for fine-scale differences or genetic continuity over time, largely due to the smaller size of the stocks, stochasticity in the runs (WTG 2017), and corresponding lack of samples. Notably, electrofishing surveys suggest that the Grand River OH spawning stock in the Central Basin now comprises fewer than 3,000 Walleye (C.T. Knight, Ohio Division of Wildlife, unpublished data). This is the primary spawning group in the Central Basin, which has been declining in numbers in comparison to those in the Western and Eastern Basins over the past two decades (Isermann and Knight 2005; C. T. Knight, unpublished data), rendering the current investigation of management interest. Such smaller populations and subpopulations are more likely to be negatively affected by the loss of genetic variation due to harvest, meriting monitoring of genetic variation trends over time (see Allendorf et al. 2013). Two key objectives set by the Great Lakes Fishery Commission (GLFC 2015) in the current "Fishery Research Priorities for the Great Lakes" are: "What are the stock structures of Walleye?" and "How can we identify, rehabilitate, conserve, or protect locally adapted stocks?", indicating the importance of understanding the genetic diversity and relationship of this spawning group to others across Lake Erie. It is essential to weigh genetic data carefully for management decisions on stock designation (see Waples 1999; Allendorf et al. 2013), together with behavioral data from tagging and telemetry studies, and results from ecological habitat and life history analyses.

The Ohio Division of Wildlife Fairport Harbor Fisheries Research Station thus requested an evaluation by the Stepien laboratory to determine whether Lake Erie Central Basin Walleye that spawn in the Grand River of Ohio and on the nearby Central Basin Reef (Figure 1) comprise genetically separable stocks. If they differ, conservation of both spawning groups might be subject to the Walleye Task Group's management priorities. Due to available samples and sample sizes, three Walleye spawning runs from three years over a span of 16 years were compared from the Grand River, in order to assess their temporal genetic compositions and relative diversity levels.

METHODS

Samples for the present study comprised Walleye fin clips (~2 cm^2 of pectoral or caudal fins) collected via electrofishing by the Fairport Harbor Fisheries Research Station during spring spawning runs at the Grand River (Ashtabula, OH at 41.85117^o latitude, -81.23746^o longitude) in 1996, 2003, and 2012, and by gill nets at the Central Basin Reef (just offshore at 41.76960^o latitude, -81.22623^o longitude) in 2012, totaling 147 individuals (Table 1). The Grand River, Ohio, drains a watershed of 1,844 km^2, where Walleye can utilize the lower 55 km up to Harpersfield Dam. The sampling location in this study ranged from 2.2 to 7.2 km from the mouth of Lake Erie. Due to the low numbers of spawning Walleye caught in the Grand River in 2012, we analyzed this spawning group both separately and together in comparison to stored samples from two other years of Grand River spawning runs (1996 and 2003), in order to evaluate its relationship to the Central Basin Reef run of 2012. Samples from other time points for the Central Basin Reef run were unavailable. Hardy Point Reef (here termed the Central Basin Reef) is located 4.6 km east of the Grand River, ~80m from shore, and is approximately 1.5 ha, characterized by a mixture of boulder, rock, gravel, and sand with interstitial spaces for egg deposition. All Walleye individuals analyzed were verified as being in spawning condition and when possible, were released after fin-clipping. Fin clips were placed in 95% ethanol in labeled vials by ODW, and stored in the Stepien laboratory.

Genomic DNA was extracted, purified, and amplified using the polymerase chain reaction (PCR) and allelic variation was assessed at 14 nuclear DNA microsatellite loci (See Table 2), following previously published procedures (Stepien et al. 2009, 2012; Haponski and Stepien 2014a, 2016). Amplification products were diluted 1:50, of which 1µl was added to 13µl of formamide and Applied Biosystems (ABI, Fullerton, CA) Gene Scan 500liz 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. Output profiles were checked manually to confirm allelic size variants.

All loci were evaluated for conformance to Hardy-Weinberg equilibrium expectations and linkage disequilibrium, using the Markov Chain Monte Carlo (MCMC) procedure with 10,000 dememorizations, 1,000 batches, and 10,000 iterations per batch in GENEPOP v4.0 (Rousset 2008). Levels of significance were adjusted with standard Bonferroni correction (Zar 1999). MICRO-CHECKER v2.2.3 (van Oosterhout et al. 2004) was used to examine results for possible scoring errors, large allele dropout, stuttering, and/or null alleles at each locus.

Per-locus calculations (Table 2) included: number of alleles (N_A), inbreeding (F_{IS}), overall genetic deviation across all samples (F_{IT}), and divergence among samples (F_{ST}) in *F*STAT v2.9.3.2 (Goudet 2002). Genetic diversity comparisons between the spawning sites and sampling years (Table 3) included observed (H_O) heterozygosity +/- standard error (SE) and expected (H_E) heterozygosity (GENEPOP), F_{IS}, N_A, and allelic richness (A_R) +/- SE, which was adjusted for sample size with rarefaction (*F*STAT). Paired

t-tests in R v3.0.1 (R Core Team 2015) were used to identify whether allelic richness and observed heterozygosity values significantly differed between the spawning groups and sampling years. Number of private alleles (N_{PA}), i.e., those appearing unique to a given spawning group or sampling year in the present study were identified with CONVERT v1.31 (Glaubitz 2004). Percentage of private alleles (P_{PA}) was determined by dividing the number of private alleles for a given sample by its total number of alleles. Due to disparity in sample size, the rarefaction representation of private alleles was evaluated with the program ADZEv1.0 (Szpiech et al. 2008a,b).

Pairwise genetic divergences among the spawning groups and sampling years were determined using the F_{ST} analog θ_{ST} (Weir and Cockerham 1984) in *F*STAT (Table 4), which is regarded as appropriate for analyzing high gene flow species, small sample sizes, and unknown number of subpopulations (Cockerham and Weir 1993; Waples 1999; Meirmans and Hedrick 2011), and to facilitate comparisons with other studies. Since *F*-statistic estimates assume a normally distributed data set (Weir and Cockerham 1984) and may be influenced by sample sizes (Raymond and Rousset 1995), we additionally conducted pairwise exact tests of differentiation (χ^2) in GENEPOP, using MCMC chains of 10,000, 1000 batches, and 10,000 iterations. Probability values for both types of pairwise comparisons were adjusted using sequential Bonferroni corrections (Rice 1989). This correction is regarded as a very conservative approach that may preclude elucidation of significance when sample sizes are low, leading to type II error (i.e., falsely rejecting the null hypothesis of no significant difference between samples; Cabin and Mitchill 2000; Moran 2003; Narum 2006). Thus, we reported significance values after (**) as well as prior to (*) sequential Bonferroni correction, so that results on the borderline could be visualized (which may have been influenced by sample size limitations), in order to aid the design of future studies (see Moran 2003).

Relationships among sampling years in the Grand River, combined years, and the Central Basin Reef samples further were examined using three-dimensional factorial correspondence analysis (3d-FCA) (Benzecri 1973) in GENETIX v4.05 (Belkhir et al. 2004), to facilitate visualization of spatial and temporal trends.

In addition to the above frequency-based methods that used the sample as the unit of comparison, we employed a Bayesian approach in STRUCTURE v2.3.3 (Evanno et al. 2005), which calculated likelihood assignments of Walleye individuals to K=1–5 hypothetical population groups (the number of sampling events +1) to determine the number of genetic stocks. Each K was run with 10 independent analyses, burn-ins of 50,000 and 100,000 replicates, with and without the LOCPRIOR function. The Evanno et al. (2005) ΔK method in STRUCTURE HARVESTER (Earl and vonHoldt 2012) was used to determine the best-supported K. Individual assignments to each of the spawning groups and year samples additionally were calculated using Bayesian analysis in GENECLASS2 (Piry et al. 2004), with the compute likelihood and enable probability functions, 100,000 simulated individuals, Rannala and Mountain's (1997)

criterion, and Paetkau et al.'s (2004) simulation algorithm. GENECLASS was run in three separate analyses: (A) for the 2012 spawning runs alone, (B) with combined years from the Grand River runs, and (C) for the separate years of Grand River, with all being compared to the Central Basin Reef (2012) sample.

RESULTS

All 14 nuclear DNA microsatellite loci conformed to Hardy-Weinberg equilibrium expectations, except for a single sample at a single locus – *Svi*14 from the Central Basin Reef, which was attributed to sampling and/or stochastic error. None of the loci exhibited linkage disequilibrium. MICRO-CHECKER findings suggested slight homozygote excess at a few disparate loci in select samples, i.e., *Svi*6 (Grand River, OH 1996), *Svi*18 (Grand River 2003 and 2012), *Svi*7 (Central Basin Reef), *Svi*14 (Central Basin Reef), and *Svi*L2 (Grand River 2012). However, there were no indications of null alleles or excess homozygotes in other samples at these loci, or across the entire data set. All samples and all loci thus were included in all analyses.

Overall, 185 alleles were recovered from 147 Walleye individuals at the 14 microsatellite loci, ranging from 6 (*Svi*18) to 25 (*Svi*14) alleles per locus (Table 2). Loci showing the greatest F_{ST} divergences among the samples were *Svi*33 (0.016) and *Svi*L4 (0.018). Observed heterozygosity (Table 3) appeared slightly higher for the Grand River spawning samples overall (0.76+/-0.03) than in the Central Basin Reef spawning population (0.73+/-0.03); these values did not significantly differ. For individual years sampled in the Grand River, the earliest sample from 1996 possessed the greatest heterozygosity (0.78+/-0.04), which decreased to 0.76+/-0.03 in 2003 and 0.73+/-0.05 in 2012 (Table 3; these values were not statistically different). Heterozygosity also did not differ between the 2012 samples in the Grand River versus the Central Basin Reef. Overall number of alleles appeared greater in the Grand River (171) than in the Central Basin Reef (155), with the earlier to later samples from the Grand River appearing to decline over time. Allelic richness ($A_{R;}$ which was adjusted for sample size) was significantly higher for the Grand River spawning group overall (8.38) than in the Central Basin Reef sample (7.69; p=0.003**). Allelic richness comparisons showed some borderline significance (before sequential Bonferroni correction) between the Central Basin Reef (sample from 2012) and the Grand River spawning population in 1996 (p=0.009*) and 2003 (p=0.008*), but no difference in 2012. No significant differences in allelic richness occurred between sampling years for the Grand River population. The percentage of private alleles was 18% for the Grand River overall and 9% for the Central Basin Reef; when adjusted for rarefaction (Szpiech et al. 2008), the number of private alleles per locus significantly differed (Grand River population overall =2.18+/-0.32, Central Basin Reef=1.37+/-0.29; p=0.022*). Comparisons of private alleles between sampling years within the Grand River population were not significant, and those

for individual sampling years versus the Central Basin Reef also did not significantly differ. Estimates of F_{IS} suggested slight inbreeding depression in all samples (F_{IS}=0.013–0.066), which were significant except for the Grand River in 1996.

Pairwise F_{ST} analog and exact tests showed significant genetic divergence between Walleye spawning groups in the Grand River overall versus the Central Basin Reef, as well as between the two groups in 2012 (Table 4). Exact tests revealed significant differences between those reproducing at the Central Basin Reef in 2012 versus those from the Grand River for the 1996 and 2003 individual sampling years; the 1996 spawning group also was significant in the F_{ST} comparison. Between Grand River sampling years, the middle (2003) versus the latest (2012) samples showed slight yet insignificant variation with both F_{ST} analog and exact tests, and the 1996 and 2003 samples differed using the exact test alone (Table 4).

Genetic differences between the Walleye spawning in the Grand River and the Central Basin Reef were further depicted by 3d-FCA (Figure 2), with the temporal Grand River samples all clustering closer to one another and separately from the Central Basin Reef sample. Among the Grand River samples, those from 2003 and 2012 diverged the most, as also indicated by the exact tests of differentiation. The three axes of the 3d-FCA explained 100% of the data (Figure 2).

STRUCTURE and STRUCTURE HARVESTER analyses indicated that the number of genetic population groups (stocks) was K=2 (Figure 3), supported by delta K results, and other K alternatives were not supported. Results showed genetic difference between individuals spawning in the Grand River (colored dark grey) and at the Central Basin Reef (colored light grey). All of the Grand River individuals showed strongest assignments to the Grand River (dark grey). Self-assignments to the Grand River appeared greatest for the individuals spawning in 1996 (averaging ~97%), followed by those in 2003 (averaging ~92%), and then 2012 (averaging ~90%). Individuals spawning on the Central Basin Reef averaged about 60% assignment to the Central Basin Reef (light grey) and about 40% assignment to the Grand River (dark grey).

GENECLASS assignment tests for the two spawning groups in 2012 discerned 100% self-assignment of individuals from the Grand River to the Grand River, with none assigning to the Central Basin Reef (Table 5A). All but two of the 56 individual Walleye sampled in 2012 that spawned at the Central Basin Reef self-assigned to the Central Basin Reef (totaling 97%), with just 3% mis-assigning to the Grand River (Table 5A). When all three spawning run years for the Grand River samples were combined, 100% of the individuals spawning in the Grand River overall self-assigned to the Grand River, with none mis-assigning to the Central Basin Reef (Table 5B). However, when including multiple sampling years for the Grand River (Table 5C), 47% of the Central Basin Reef samples self-assigned to

the Central Basin Reef and 53% mis-assigned to the Grand River (including 3% to the Grand River sample from 2012, 17% to the sample from 2003, and 33% to the sample from 1996).

DISCUSSION

The research objective was to provide genetic assessment to the WTG and the Ohio Division of Wildlife Fairport Fiseries Research Station of the relationship between two nearby Walleye spawning groups in Lake Erie's Central Basin. Our results indicated that Walleye spawning in the Grand River appear to genetically differ from those reproducing at the nearby Central Basin Reef. All Walleye spawning in the Grand River self-assigned to the Grand River, and 97% of individuals sampled on the Central Basin Reef in 2012 self-assigned. These data indicate that the groups likely comprise separable reproductive stocks, which may merit management attention.

This research found that mis-assignments were rare for the Central Basin individuals when just the 2012 spawners were included, with only 3% mis-assigning to those spawning in the Grand River in 2012. However, when the other temporal runs in the Grand River were considered, over half of the Central Basin Reef samples then mis-assigned to the Grand River – especially to the 1996 spawners from the Grand River. There are several possible explanations for these findings, including: (1) The Central Basin Reef population may have been historically derived from the Grand River population, (2) Walleye that were born in the Grand River may occasionally spawn on the Central Basin Reef, but not vice-versa, and/or (3) Some of the Walleye caught on the Central Basin Reef in spawning condition may have been *en route* to spawning in the Grand River. It may be that some of the population that once spawned on the Grand River is now spawning on the Central Basin Reef or may reproduce at both locations. Alternatively, some individuals may travel back and forth between these locations before or after spawning (and might have been inadvertently sampled here), for which behavioral data from telemetry studies may be very useful. Further work involving tagging and telemetry, coupled with genetic and otolith signature analyses would help to resolve these questions. Given that the Ohio Division of Wildlife (ODW) has found that the Grand River spawning run has declined in numbers over the past two decades (C. T. Knight, Ohio Division of Wildlife, unpublished data), our baseline genetic information may provide an important gauge to monitor its future success.

In a previous study, some of the Walleye spawning groups across the Huron–Erie Corridor were discerned to significantly diverge from one another (with levels of genetic difference comparable to that found here between the Grand River and Central Basin Reef samples); these patterns did not correspond to genetic isolation by geographic distance (Haponski and Stepien 2014). Similarly, small yet significant divergences differentiated among closely located populations of the Walleye's congener, the European Pikeperch *S. lucioperca*, in the Baltic Sea (Björkland et al. 2007). Very significant divergences have been

discerned among spawning Lake Erie groups of another percid fishery, the related Yellow Perch *Perca flavescens*, including Central Lake Erie groups that were located near to the Walleye sampled in the present study (Sepulveda-Villet and Stepien 2011; Kocovsky et al. 2013; Sullivan and Stepien 2015). Moreover, Yellow Perch spawning adults in Central Lake Erie exhibited morphometric differences among spawning locations (Kocovsky et al. 2013). Fine-scale divergences of Walleye and Yellow Perch across their ranges do not correspond to geographic proximity; they instead appear to reflect historic and behavioral homing patterns (Stepien et al. 2015). Similar to Yellow Perch, populations of the congeneric European Perch *P. fluviatilis* showed significant divergence between spawning groups located only about one km apart in Lake Erken, Sweden (Bergek and Olsson 2009).

In the present study, Walleye spawning in the Grand River had slightly higher overall genetic diversity (allelic richness and private alleles) than did the Central Basin Reef population. Levels of genetic diversity in these Central Basin Walleye spawning groups were similar to those of other Walleye reproductive groups throughout the Great Lakes, using these same loci in studies also conducted by our laboratory (mean observed heterozygosity (H_O)=0.72+/-0.04; Haponski and Stepien (2014a, b, 2016).

Allelic richness of Walleye spawning in the Grand River declined over the timescale of this study (decreasing from 1996 to 2003) and other diversity measures (observed heterozygosity and private alleles) appeared to follow a similar trend but were not significant (likely due to sample size limitations). This decrease appears to coincide with reduced numbers of Walleye spawning in the Grand River, discerned by the Ohio Division of Wildlife Fairport Harbor Fisheries Research Station over the past two decades (C.T. Knight, Ohio Division of Wildlife, unpublished observations). Some factors that may have in the Grand River influenced walleye habitat and populations include a “500-year” flood event in July 2006, which moved mobile substrate and re-channelized some areas, along with overall warming temperatures, increasing hypoxia, and increased harmful algal blooms across Lake Erie (see WTG 2017). Influences of these latter factors on Lake Erie tributary habitats, including the Grand River, tend to be more rapid and stochastic than those in Lake Erie proper.

In comparison, temporal genetic analyses of other Walleye spawning runs in Lake Erie revealed overall genetic consistency in diversity and allelic composition over time (the last two decades) in the Western Basin (Maumee River and Sandusky Rivers; Stepien et al. 2012; Haponski and Stepien 2016) and the Eastern Basin (Van Buren Bay and Cattaraugus Creek; Stepien et al. 2012; Haponski et al. (2014). Thus, although other spawning stocks in Lake Erie have maintained consistent levels of genetic diversity over the past two decades, this may not be the case for Walleye in the Central Basin. Results of the present study may indicate need for continued monitoring and attention by the Walleye Task Group.

A study of genetic variation for 420 homologous single nucleotide polymorphisms (SNPs) described decline in overall genetic diversity of Walleye from Smoke Lake, Alberta Canada in comparisons from

1973 (13 individuals) versus 2005 (19 individuals), which appeared to be linked to high fishery harvest exploitation and the collapse of the fishery (Allen et al. 2017). Like the present results for Walleye spawning in the Grand River, Allen et al. (2017) found a decrease in overall percentage of private alleles and observed heterozygosity. It thus appears warranted to continue monitoring the numbers, genetic diversities, and compositions of the Lake Erie Grand River and Central Basin Reef Walleye spawning groups in the future.

In contrast to Walleye, the genetic compositions of Yellow Perch spawning groups in Lake Erie (Sullivan and Stepien 2015) as well as those of European Perch in Lake Erken, Sweden, have varied as much temporally as spatially (Bergek and Olsson 2009). Yellow Perch spawning groups appear to exhibit less fidelity to specific locations from year to year than do Walleye; thus, their genetic divergence patterns fluctuate (Sullivan and Stepien 2015; Stepien et al. 2015). In comparison, large and significant temporal population genetic variation changes in Atlantic Cod *Gadus morhua* of the North Sea have been associated with extreme stock declines due to overfishing and subsequent increased immigration from other populations (Hutchinson et al. 2003). Such overall stock decline factors do not appear to be the case at present for the Lake Erie Walleye and Yellow Perch fisheries (see Stepien et al. 2015). Whether and how population genetic relationships and stock continuities coincide with management practices for Walleye and Yellow Perch, versus those for other species, is a matter for further investigation.

The present study provides new insight into the divergent genetic compositions of Walleye spawning in geographically close but physically different habitats. Overall, both spawning groups of Walleye appear genetically diverse and different; such smaller reproductive subpopulations may significantly contribute to Lake Erie's stock structure as a whole and point to a need for additional surveillance. It is important that future studies investigate these runs over multiple years with larger sample sizes (preferably with non-invasive sampling, such as environmental DNA), and interpret the data in light of Walleye tagging and telemetry studies, life history, and reproductive behavior. In relation to the Lake Erie Walleye Management Plan objective to "Maintain and promote genetic diversity by identifying, rehabilitating, conserving, and/or protecting locally adapted stocks" (Kayle et al. 2015), here we identified two apparently locally differentiated stocks, which merit continued monitoring and possible genetic conservation. Lake Erie Walleye are managed lakewide as a single population, yet improved understanding of the numbers, abundances, and diversity of stocks is critical to managers. Although managers might be hard pressed to limit harvest of an individual stock of lake caught fish, they may be able to further monitor spawning habitat and exploitation in specific areas, where warranted. Identification of stocks needs to be accomplished before management decisions can be considered. Future sampling and analyses of these spawning groups in the Grand River and at the Central Basin Reef should be undertaken to evaluate potential changes to their temporal genetic dynamics and spatial structure.

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TABLE 1. Collection information including date, location, number of individuals sampled (*N*), and sample set designation for spawning Walleye collected by the Ohio Division of Wildlife Fairport Harbor Fisheries Research Station.

Collection date	Location	*N*	Spawning group sample
03/27–04/15/1996	Grand River east of Fairport Harbor, OH 41.75117^{o}lat., -81.23746^{o}long.	30	Grand River (GR) 1996
04/02–06/12/2003	" " " "	30	Grand River (GR) 2003
03/28–04/03/2012	" " " "	16	Grand River (GR) 2012
03/20–05/02/2012	Central Lake Erie Reef, off	58	Central Basin Reef (RF) 2012

	Painesville-on-the-Lake, OH
	41.76960°lat., -81.22623°long.

562

563 TABLE 2. Summary of genetic variation per microsatellite locus for Grand River and Central Basin Reef
564 Walleye spawning groups. Table shows primer reference source, annealing temperature (T_A), number of
565 alleles (N_A), allelic size range (nucleotides), genetic deviation across all combined samples (F_{IT}), mean
566 genetic divergence (F_{ST}), and inbreeding coefficient (F_{IS}, average divergence within a spawning group),
567 calculated using *F*STAT.
568

Locus	Source	T_A (ºC)	N_A	Size range	F_{IT}	F_{ST}	F_{IS}
*Svi*4	Borer et al. (1999)	60	8	96−120	-0.052	0.005	-0.057
*Svi*6	"	60	14	140−166	0.039	0.015	0.025
*Svi*17	"	54	10	102−142	0.088	0.005	0.084
*Svi*18	"	65	6	114−126	0.203	0.000	0.211
*Svi*33	"	60	14	78−106	0.030	0.016	0.015
*Svi*L2	Wirth et al. (1999)	53	7	263−281	-0.014	0.000	-0.009
*Svi*L3	"	53	16	233−263	-0.067	0.008	-0.076
*Svi*L4	"	54	15	121−161	0.115	0.018	0.099
*Svi*L6	"	54	11	108−136	0.014	0.009	0.004
*Svi*L7	"	54	20	174−236	-0.005	0.000	-0.004
*Svi*2	Eldridge et al. (2002)	60	12	190−220	0.071	0.007	0.064
*Svi*7	"	60	9	154−172	0.137	0.000	0.139
*Svi*14	"	54	25	154−214	0.119	0.005	0.115
*Svi*20	"	50	18	152−190	0.040	0.011	0.043
Total	---	---	185	---	0.049	0.006	0.043

569

570 TABLE 3. Summary of genetic data from spawning Walleye samples in the Grand River and Central
571 Basin Reef including: number of individuals (N), observed heterozygosity (H_O) ± standard error (SE),
572 number of alleles (N_A), number of private alleles (N_{PA}), percentage of private alleles (P_{PA}), allelic
573 richness (A_R±SE; adjusted for sample size), and inbreeding coefficients (F_{IS}) calculated from the 14
574 nuclear DNA microsatellite loci using *F*STAT.

Sample		N	H_O±SE	N_A	N_{PA}	P_{PA}	A_R±SE	F_{IS}
Grand River	1996	30	0.78±0.04	147	13	0.05	8.50±0.83	0.013

	2003	30	0.76±0.03	135	5	0.02	8.18±0.69	0.045
	2012	16	0.73±0.05	116	4	0.02	8.11±0.71	0.066
Total Grand River	----	76	0.76±0.03	171	30	0.18	8.38±0.73	0.039
Central Basin Reef	2012	58	0.73±0.03	155	14	0.09	7.69±0.68	0.051

TABLE 4. Pairwise genetic comparisons between Walleye spawning samples from Grand River (all years combined and separate years (1996, 2003, 2012)) vs. the Central Basin Reef (2012). F_{ST} analog (calculated in *F*STAT; below diagonal) and χ^2 (calculated in GENEPOP2; above diagonal). *=significant before, but not after, Bonferroni correction. **= significant difference remained after sequential Bonferroni correction. NS= not significant.

Site (*N*)	GR All (76)	GR 1996 (30)	GR 2003 (30)	GR 2012 (16)	RF 2012 (58)
Grand River (GR) All	~	NS	NS	NS	**
GR 1996	0.000	~	*	NS	**
GR 2003	0.000	0.001	~	*	**
GR 2012	0.000	0.004	0.010*	~	*
Central Basin Reef (RF)	0.006**	0.008**	0.004	0.009*	~

TABLE 5. GENECLASS assignment test results for Walleye spawning samples from Grand River vs. the Central Basin Reef in 2012 (Percentage assignments are in parentheses). A. 2012 spawning runs alone, B. All sampling years combined for the Grand River, and C. Separate Grand River spawning runs (1996, 2003, 2012). Self-assignments are *in italics.*

A. Samples from the 2012 spawning run

	Assigned to	
Sample	GR 2012	RF 2012
Grand River (GR) 2012	*16 (1.00)*	~
Reef (RF) 2012	2 (0.03)	*56 (0.97)*

B. All sampling years combined for the Grand River

	Assigned to	
Population	GR	RF

Grand River (GR)	*76 (1.00)*	~
Reef (RF) 2012	31 (0.53)	*27 (0.47)*

C. Separate Grand River spawning run samples and Central Basin Reef 2012

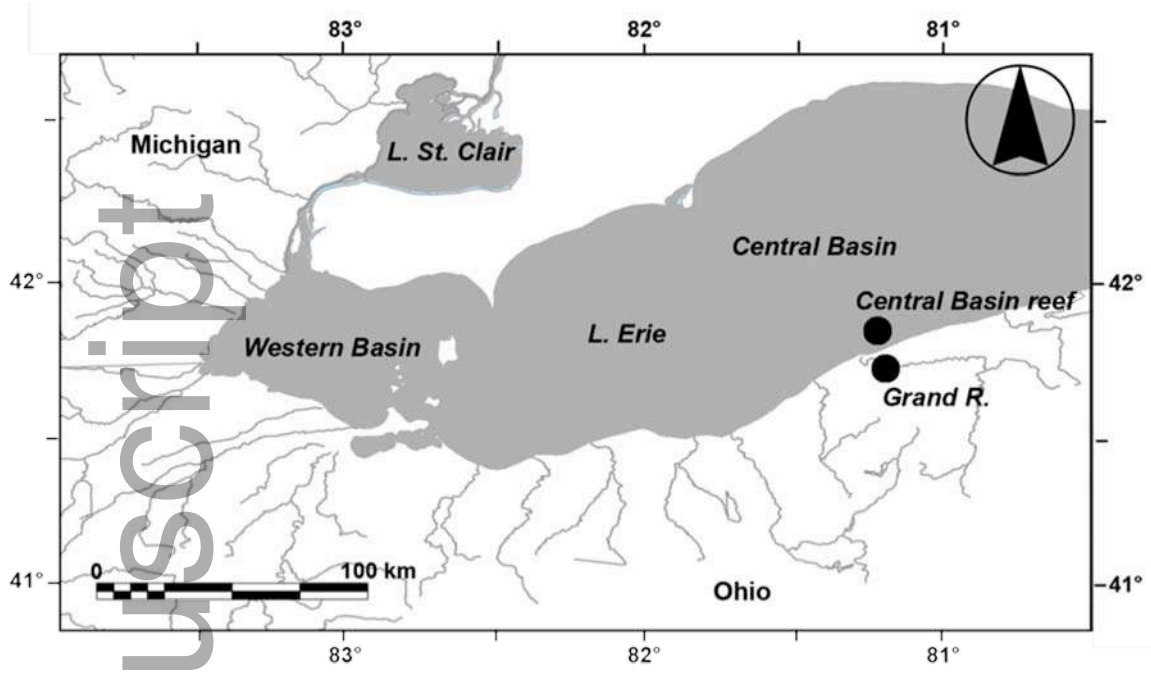
	Assigned to			
Sample	GR 1996	GR 2003	GR 2012	RF 2012
Grand River (GR) 1996	*20 (0.67)*	8 (0.27)	2 (0.07)	~
GR 2003	11 (0.37)	*17 (0.57)*	2 (0.07)	~
GR 2012	2 (0.13)	4 (0.25)	*10 (0.63)*	~
Reef (RF) 2012	19 (0.33)	10 (0.17)	2 (0.03)	*27 (0.47)*

Figure Captions

FIGURE 1. Sampling locations of the Walleye spawning groups in Central Lake Erie.

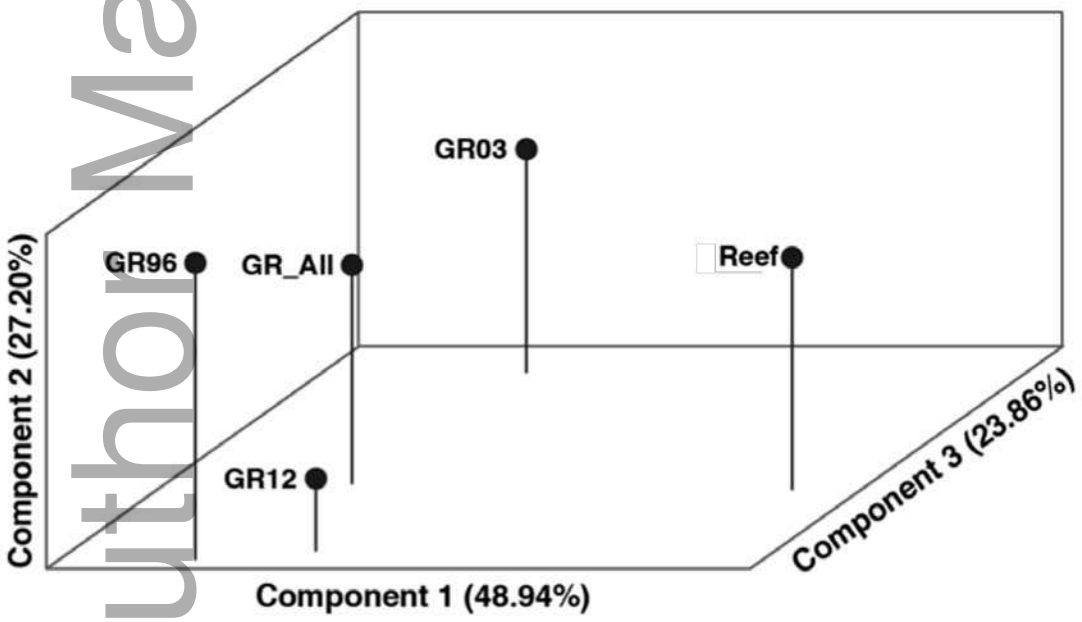
FIGURE 2. Three–Dimensional Factorial Correspondence analysis illustrating relationships among Walleye spawning groups per sampling year from the Grand River (GR96, GR03, GR12), the Central Basin Reef (Reef), and the combined Grand River samples (GR_All).

FIGURE 3. STRUCTURE Bayesian assignment results showing individual Walleye (thin vertical lines) from the two population groups, for which K=2 population groups (light and dark grey) were supported (K =1–5 were tested; ΔK=6.91).



611

612 FIGURE 1.



614 FIGURE 2.

615

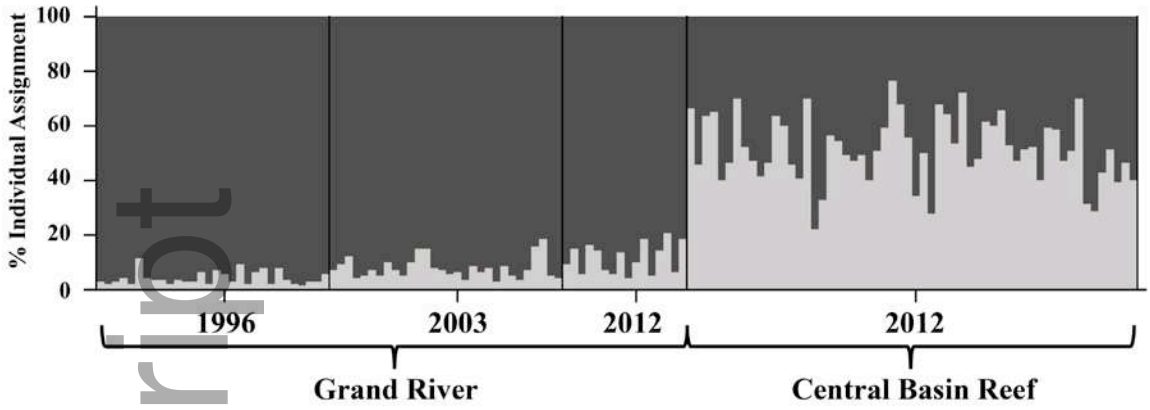


FIGURE 3.