Ohio Sea Grant publication OHSU-TD-136-2013 results of project # R/LR-013

A Dissertation

Entitled

Evolutionary, Biogeographic, and Population Genetic Patterns of Walleye and other *Sander*: Relationships across Continents, Corridors, and Spawning Sites

by

Amanda E. Haponski

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the

Doctor of Philosophy Degree in Biology (Ecology)

_____

Dr. Carol A. Stepien, Committee Chair

_____

Dr. Timothy G. Fisher, Committee Member

_____

Dr. Johan F. Gottgens, Committee Member

_____

Dr. Patrick M. Kocovsky, Committee Member

_____

Dr. Wendylee Stott, Committee Member

_____

Dr. Patricia R. Komuniecki, Dean
College of Graduate Studies

The University of Toledo

August 2013

An Abstract of

Evolutionary, Biogeographic, and Population Genetic Patterns of Walleye and other *Sander*: Relationships across Continents, Corridors, and Spawning Sites

by

Amanda E. Haponski

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Doctor of Philosophy Degree in Biology (Ecology)

The University of Toledo

August 2013

Understanding a species' hierarchical genetic variation may provide insights into its ability to cope with current and future stressors, such as climate change, exploitation, and degradation of habitat. Analyzing the events that led to speciation and population diversification may give a framework for predicting responses and adaptations to future changes, imparting critical information for conservation managers. I resolve the evolutionary history and population genetic patterns of the walleye *Sander vitreus* using molecular and morphological characters and a variety of analyses, including sequences from three nuclear and three mitochondrial (mt) DNA regions and nine nuclear DNA microsatellite (μsat) loci. Results show that walleye and its sister species the sauger *S. canadensis* diverged ~15.4 Mya (5.2-27.1 Mya = 95% highest posterior density (HPD)), with modern walleye haplotypes differentiating ~10.6 Mya (6.9-14.3 HPD), when climate changes were occurring across North America. Walleye exhibit significant genetic structure (mean F_{ST} mtDNA control region=0.24, μsat=0.10) and substantial genetic diversity (mean control region=0.53, μsat=0.68) across its range, at broad- and fine-scales. Contemporary patterns correspond to a genetic isolation by geographic distance

hypothesis, with northern populations reflecting differential contribution from three distinct Pleistocene glacial refugia. Comparisons of historic versus modern samples from Lake Erie reveal that allelic frequencies have changed over ~70 years (mean F_{ST} control region=0.26, μsat=0.17), with modern populations having higher diversity (mean control region=0.67, μsat=0.72 vs. control region=0.05, μsat=0.47). Today's higher diversity may be due to population rebounds following closure of the walleye fishery in 1970. Paratypes of the historic blue pike did not differ morphologically or genetically from walleye, indicating that the blue pike was not a separate taxon. Additional fine-scale results within the highly degraded Huron-Erie Corridor reveal the seven spawning groups show a mixture of connectivity and divergence. This study also discerns the effects of habitat augmentation on the genetic structure of walleye spawning groups, finding that populations are distinct and have similar levels of genetic diversity (mean control region=0.73, μsat=0.72). Some genetic exchange occurred at an augmentation site, suggesting that walleye from other locations arrived to spawn on the newly available habitat. Such migration could change the genetic composition of the original spawning group. The results of this dissertation provide insight to the processes that shaped the evolution and population genetic patterns of today's walleye, including past climate change, Pleistocene glaciations, and anthropogenic stressors. Future research identifying the adaptations underlying the genetic diversity and divergence patterns discerned here and their respective genetic contributions to fitness will aid efforts to sustain natural populations in the face of ongoing climate change and other anthropogenic stressors.

To my husband, family, and friends for all of their love and support

"Alive without breath,

As cold as death;

Never thirsty, ever drinking,

All in mail never clinking."

-Gollum, J.R.R. Tolkien's The Hobbit

Acknowledgements

This degree was a long worthwhile journey, with numerous people to thank for their help, support, and guidance through it. First and foremost, I am grateful to my advisor, Dr. Carol Stepien, for her support and mentoring throughout the course of my graduate career. Thank you for taking the time to help me grow as a scientist through all of your guidance and encouragement throughout this process. Thanks to the members of my graduate committee, Drs. Timothy Fisher, Johan Gottgens, Patrick Kocovsky, and Wendylee Stott for their valuable input and guidance throughout this journey. To my colleagues in the Great Lakes Genetics/Genomics Laboratory and the Lake Erie Center: Carson, Doug, Jhonatan, Joshua, Lindsey, Matt, Shane, and Tim, thank you for all of your help and being a valuable sounding board. To Matt, thank you for all that you have taught me. I also am very grateful to other members of the Lake Erie Center: Betsy, Jenn, Kristen, Nate, Rachel L., Rachel K., Meredith, Pat (a.k.a. Mom) for their help.

I thank my husband, Michael Bagley, for his patience, understanding, and support he has provided me through this degree. I know this was not an easy path for us to take, but it was worth it in the end ☺. To my family, Arthur, Sheila, and Alexis Haponski, for always pushing me to do my best. Last but not least I thank my in-laws David, Mary, Alex, and Chelsea Bagley for all of their support.

Table of Contents

List of Tables

List of Figures

Preface

The chapters of this dissertation are organized in order of their scope for understanding the evolutionary history and population genetic patterns of the walleye. Each chapter is largely identical to the published/submitted manuscript version, with only slight re-wordings.

Chapter 2 has been previously published as:

> Haponski, A.E. & Stepien, C.A. (2013) Phylogenetic and biogeographic relationships of the *Sander* pikeperches (Perciformes: Percidae): Patterns across North America and Eurasia. *Biological Journal of the Linnean Society.*

Chapter 3 is in review for potential publication in *Diversity and Distributions*:

> Haponski, A.E. & Stepien, C.A. (in review) A population genetic window into the past and future of the walleye *Sander vitreus*: Relation to historic walleye and the extinct blue pike variant.

Chapter 4 has been previously published as:

> Haponski, A.E. & Stepien, C.A. (2013) Genetic connectivity and diversity of walleye (*Sander vitreus*) spawning groups in the Huron–Erie Corridor. *Journal of Great Lakes Research.*

This is publication #2013-16 from the Lake Erie Research Center.

This work was funded by grant awards to CAS from NOAA Ohio Sea Grant Program R/LR-013, USEPA CR-83281401-0, and to E. Roseman from the Great Lakes Restoration Initiative Project #70. Additional funds for travel by AEH were provided to her by NSF Deepfin #RCN-0443470 (P.I.= G. Orti), a Smithsonian Fellowship visiting summer appointment, and a Sigma Xi Grant In Aid of Research. AEH was supported by a NSF GK-12 DGE#0742395 fellowship (P.I. = CAS), University of Toledo teaching and research assistantships, the International Association for Great Lakes Research (IAGLR) Norman S. Baldwin Fishery Science Scholarship (2010), and the IAGLR Scholarship award (2009). This work would not have been possible without the aid of collectors from the U.S. Fish and Wildlife Service, U.S. Geological Survey, Ohio and Michigan Departments of Natural Resources, Ontario Ministry of Natural Resources, and Keweenaw Bay Indian Community Natural Resources Department, and Great Lakes Fishery Commission Lake Erie Walleye Task Group for contributing samples. I also thank M. Bagley, L. Bernatchez, N. Billington, J. Boase, V. Boldyrev, A. Cook, I. Grigorovich, D. Grul'a, E. Hallerman, S. Ibrahimov, W. Jansen, T. Johnson, K. Kayle, V. Kovac, Y. Kvach, B. Manny, J. McFee, G. Mensch, M. Neilson, D. Nelson, E. Roseman, M. Sapota, W. Schaefer, P. Simonovic, M. Thomas, M. White, and C. Wilson for additional collection assistance.

Chapter 1

Introduction

Species today are at an increased risk of extinction (~1000x greater than past decades; Brooks *et al.,* 2006) due to anthropogenic stressors, such as climate change, habitat alteration and loss, invasive species, and exploitation (see Dawson *et al.,* 2011; Taubmann *et al.,* 2011; Hutchings *et al.,* 2012). As species richness declines, populations also tend to lose variability and unique genetic resources. Genetic variation constitutes the raw material underlying evolutionary patterns, population adaptation, and possible resilience to these anthropogenic stressors. Losses of genetic variability may undermine the ability of a species and their component populations to persist and adapt to environmental and ecological changes (Reed & Frankham, 2003; Väli *et al.*, 2008). In light of this, understanding a species' evolutionary history and population genetic patterns is critically important to develop and prioritize appropriate conservation management strategies (see Reed & Frankham, 2003; Allendorf & Luikart, 2007; Taubmann *et al.,* 2011), which hopefully will aid their survival in this ever changing environment.

Identifying the evolutionary history and genetic patterns of species and the processes that change these patterns has become easier with advances in molecular

techniques. Modern molecular genetics provide essential tools to evaluate the hierarchical levels of genetic variation within and among taxa through direct analysis of the composition and frequency of alleles. Notably, the field of systematics examines the evolutionary relationships among species, including estimating their origins and identifying the events that led to their divergence (see Moritz & Hillis, 1996; Avise, 2004). Once taxa have been defined, population genetics evaluates the intra-specific structure and organization among their populations and sub-populations (Hartl, 2000) and can be used to discern those that possess unique genetic material and warrant recognition as distinct evolutionary significant units (ESU; Ryder 1986).

Due to variations in evolutionary rates among different genetic marker systems, a variety of markers are necessary to discern the hierarchical genetic structure of taxa (see Avise, 2004; Wang, 2010, 2011). Nuclear and mitochondrial (mt) DNA sequences and nuclear microsatellite (μsat) loci have been utilized to understand both macro- and micro-evolutionary patterns. This combined approach of DNA sequencing and nuclear μsat loci DNA is especially powerful as it allows comparisons of patterns at multiple evolutionary and temporal scales (see Avise, 2004; Wang, 2010, 2011). Nuclear DNA sequences are best for resolving deeper phylogenetic relationships (e.g., among species, genera, etc.) since they evolve more slowly, i.e., ~5–10x slower than mtDNA sequences (summarized by Simon *et al.*, 1994; Hewitt, 2001). In comparison, mtDNA is best suited to intra-specific resolution due to its more rapid evolutionary rate from having ¼ the effective population size of nuclear DNA sequences, lack of proofreading by DNA polymerase I, and no recombination (see Stepien & Kocher, 1997; Avise, 2004; Marshall *et al.*, 2009). MtDNA sequences often have been used to discern historical context, such as speciation

events, origins from glacial refugia, and population genetic patterns (see Hewitt, 2001; Wang, 2010). Lastly, highly polymorphic nuclear μsat loci evolve faster, ~1 mutation every 10,000 generations, making them ideal to address contemporary processes such as gene flow and genetic drift (see Ellegren, 2004).

This dissertation study analyzes the evolutionary and population genetic patterns of the walleye *Sander vitreus* (Mitchill, 1818; Percidae: Teleostei) using a combination of phylogenetic systematic and population genetic approaches, along with selected morphological comparisons. Notably, sequences from three nuclear and three mtDNA gene regions and nine nuclear μsat loci are utilized to comprehensively assess the historical and contemporary processes that shaped the current genetic structure of walleye.

Walleye is one of five species in the genus *Sander*. Two putative subspecies of walleye are currently recognized, the yellow variant *S. v. vitreus* and the historic blue pike *S. v. glaucus*. Hubbs (1926) described the blue pike as a species, which later was demoted to a subspecies due to large numbers of morphological intergrades with walleye (Trautman, 1981). The blue pike possibly was a unique fish taxon, which was reported as endemic to Lakes Erie and Ontario (Bailey & Smith, 1981). It comprised a popular commercial fishery until its collapse in 1959, attributed to exploitation, pollution, and/or habitat alteration (Trautman, 1981). In 1983, the blue pike officially was declared extinct by the US Fish and Wildlife Service (Noecker, 1998), after having not been seen since the early 1960s (Hubbs & Lagler, 2004). The blue pike was hypothesized to have evolved in Lakes Erie and Ontario post-glacially or to have co-existed with walleye in the

Mississippian Refugium (Bailey & Smith, 1981). However, its taxonomic relationship to walleye has long been questioned.

Walleye is one of the most ecologically and economically valuable fishes in the Great Lakes, constituting a keystone species as a primary predator (Locke *et al*., 2005; Roseman *et al*., 2010; Nate *et al*., 2011) and supporting large sport and commercial fisheries (Schmalz *et al*., 2011). Walleye populations have been affected by over a century of exploitation, habitat changes, pollution, and non-indigenous species introductions. All of these may have changed its genetic variation and adaptability.

The walleye lives in a variety of habitats, from slow turbid lake environments to fast flowing clear streams (Collette & Bănărescu, 1977; Billington *et al.,* 2011). It has a wide native distribution across North America (Page & Burr, 2011) that, over the past century, has been altered by stocking walleye throughout most of the continental US and southern Canada (summarized by Billington *et al*., 2011). Many of those originated from western Lake Erie in the Great Lakes, where walleye are most abundant (USFWS/GLFC, 2010).

Maturity occurs around age three, at which time walleye begin annual migrations during the spring to early summer to reproduce at natal spawning grounds (Collette *et al.,* 1977; Barton & Barry, 2011). Walleye exhibit fidelity to these spawning sites (Jennings *et al.*, 1996), with the genetic structure of spawning groups remaining similar from year to year, among age cohorts, and from generation to generation (Stepien *et al.*, 2012). Adults leave the spawning grounds following external fertilization. Walleye typically range widely to feed at non-reproductive times of the year, travelling distances from 50–300 km in a year (see Colby *et al.,* 1979; Bozek *et al.,* 2011). They typically reach ~17–

19 years of age (Carey & Judge, 2000), with some reported as old as 30 years (Bozek *et al.,* 2011).

The general spatial genetic patterns of walleye spawning groups have been elucidated across their range using mtDNA (Billington *et al.*, 1992; Stepien & Faber, 1998; Gatt *et al.*, 2000, 2002) and nuclear μsat loci (Strange & Stepien, 2007; Stepien *et al.,* 2009, 2010, 2012). The largest genetic divisions across their native range separate populations outside of the Great Lakes region from those within (Stepien *et al*., 2009) likely due to their origins in separate glacial refugia. Primary population demarcations occur between most of the lakes and some within them, with most spawning groups significantly diverging in genetic composition (Strange & Stepien, 2007; Stepien *et al*., 2009, 2010).

The central objective of this dissertation study is to analyze the hierarchical genetic structure of walleye. This work builds upon past studies to increase the understanding of the evolutionary history of walleye and the processes that shape contemporary genetic diversity and population structure. This study uniquely includes all members of the genus *Sander,* additional sites across the range, historic (1923-1949) vs. contemporary (1990s-present) patterns within Lake Erie, and identification of the relationship to the extinct blue pike *S. v. glaucus* morphotype. The specific objectives include:

(1) Evaluate the evolutionary and biogeographic relationships of *Sander* and determine the events that led to the divergence of walleye from other members of the genus. (Chapter 2)

(2) Resolve the historic and contemporary genetic patterns of walleye across its range, including its relationship to the extinct blue pike morphotype. (Chapter 3)

(3) Discern the genetic connectivity, diversity, and divergence patterns of walleye spawning groups along the highly degraded Huron-Erie Corridor and how recent habitat augmentation affected walleye genetic structure. (Chapter 4)

Chapter 2

Phylogenetic and biogeographical relationships of the *Sander* pikeperches (Percidae: Perciformes): Patterns across North America and Eurasia

Previously published as Haponski, A.E. & Stepien, C.A. (2013) Phylogenetic and biogeographic relationships of the *Sander* pikeperches (Perciformes: Percidae): Patterns across North America and Eurasia. *Biological Journal of the Linnean Society.*

2.1 Abstract

North America and Eurasia share several closely related taxa that diverged either from the breakup of the Laurasian supercontinent or later closures of land bridges. Their modern population structures were shaped in Pleistocene glacial refugia and via later expansion patterns, which are continuing. The pikeperch genus *Sander* contains five species – two in North America (*S. canadensis* and *S. vitreus*) and three in Eurasia (*S. lucioperca, S. marinus,* and *S. volgensis*) – whose evolutionary relationships and relative genetic diversities were previously unresolved, despite their fishery importance. This is

the first analysis to include the enigmatic and rare sea pikeperch *S. marinus*, nuclear DNA sequences, and multiple mitochondrial DNA regions. Bayesian and Maximum Likelihood trees from three mitochondrial and three nuclear gene regions support the hypothesis that *Sander* diverged from its sister group *Romanichthys/Zingel* ~24.6 Mya. North American and Eurasian *Sander* then differentiated ~20.8 Mya, with the former diverging ~15.4 Mya, congruent with North American fossils dating to ~16.3–13.6 Mya. Modern Eurasian species date to ~13.8 Mya, with *S. volgensis* being basal and comprising the sister group to *S. lucioperca* and *S. marinus*, which diverged ~9.1 Mya. Genetic diversities of the North American species are higher than those in Eurasia, suggesting fewer Pleistocene glaciation bottlenecks.

2.2 Introduction

Many closely–related temperate taxa share a common native distribution across North America and Eurasia, including some plants (Axelrod, 1975; Milne, 2006; Wen & Ickert-Bond, 2009), mammals (Osborn, 1910; Pielou, 1991; Miller *et al.*, 2006), and freshwater fishes (Collette & Bănărescu, 1977; Cavender, 1998; Leveque *et al.*, 2008). Sister groups of taxa became differentiated between the two continents, either dating to the final breakup of the Laurasian supercontinent 66–58 million years ago (Mya) (Briggs, 1986; Wicander & Monroe, 1993) or via the closure or ecological inhospitability of land bridge connections (Tiffney & Manchester, 2001; Milne, 2006). Two land bridges have connected and allowed migration between the North American and Eurasian fauna at various geological times: the Bering Land Bridge (BLB) across the North Pacific Ocean

and the North Atlantic Land Bridge (NALB) across the North Atlantic Ocean. These each intermittently were available from the beginning of the Paleocene Epoch (~65 Mya) with final subsidence of the NALB during the late Miocene Epoch ~10 Mya (Tiffney, 1985; Denk *et al.*, 2011) and loss of the BLB near the end of the Pleistocene Epoch ~0.01 Mya (Gladenkov *et al.*, 2002).

Following the older continental and land bridge divergences between the pairs of taxa, their respective distributions and population genetic patterns further were modified by loss and alterations of habitats during the Pleistocene glaciations ~2.6–0.01 Mya (Hewitt, 1996; Bernatchez & Wilson, 1998; Hewitt, 2000). The North American Laurentide Ice Sheet advanced further south than the Eurasian Scandinavian Ice Sheet (Hewitt, 1996). However, North America had larger areas of glacial refugia due to its plains being oriented east–west in between north–south mountain ranges. In contrast, dispersal of Eurasian taxa was limited by boundaries of saline seas and mountain ranges that are oriented east–west (Hewitt, 1996). Following the Pleistocene Ice Ages, most north temperate taxa moved northwards to expand into old and new habitats, and these patterns now are accelerating due to anthropogenic climate change (Chu *et al.*, 2005; Sharma *et al.*, 2007). These biogeographic scenarios underlie the contemporary distributions of freshwater fishes, including the percid genera *Sander* (=*Stizostedion*; Bruner, 2011) and *Perca* (Perciformes: Percidae).

Morphological characters and fossil dates have suggested that the family Percidae diverged ~66–58 Mya during the Paleocene Epoch (Collette & Bănărescu, 1977) from an ancestor shared with the widely distributed marine Serranidae (seabasses; Collette & Bănărescu, 1977; Bruner, 2011) or with the North American Centrarchidae (sunfishes;

Collette & Bănărescu, 1977). *Sander* and *Perca* today share similar distributions across North America and Eurasia. Several hypotheses may explain their species divergences between the two continents, including that they: (1) originated in Eurasia and then dispersed to North America via the NALB at the beginning of the Eocene Epoch ~58 Mya (Svetovidov & Dorofeeva, 1963; Balon *et al.*, 1977), (2) migrated from Eurasia to North America across the BLB during the Oligocene Epoch ~37–24 Mya (Collette & Bănărescu, 1977), (3) originally shared a wide Holarctic distribution (spanning both continents) dating to ~24 Mya (Late Tertiary) and their taxa later moved south during the Pleistocene glaciations ~2.6–0.01 Mya (Cavender, 1998), (4) moved via the BLB during the Miocene/Pliocene Epoch ~10–4 Mya (Billington *et al.*, 1990, 1991; Faber & Stepien, 1998), or (5) crossed the Atlantic Ocean from Eurasia to North America in brackish water along the ice sheets during the late Pleistocene Epoch ~15 kya (Fig. 2-1; Cihar, 1975). However, recent fossil discoveries have revealed two extinct *Sander* spp. in North America (see Fig. 2-1c), one dating to ~16.3–13.6 Mya in southern Saskatchewan, CAN (Murray & Divay, 2011) and the other (*S. teneri*) ~5 Mya off Greenland (Murray *et al.*, 2009), providing new data for differentiating among these hypotheses and for calibrating a phylogeny.

Recently, the name of this genus was changed from *Stizostedion* Rafinesque 1820 to *Sander* (Cuvier, 1817) per Kottelat (1997), which was supported by the Committee on Names of Fishes (a joint committee of the American Fisheries Society and the American Society of Ichthyologists and Herpetologists; see Nelson *et al.*, 2003, 2004). This change has been controversial and is under debate, as summarized by Bruner (2011). The genus comprises five extant species – two in North America: the sauger *S. canadensis* (Griffith

& Smith, 1834) and walleye *S. vitreus* (Mitchill, 1818) and three in Eurasia: the pikeperch *S. lucioperca* (Linnaeus, 1758), Volga pikeperch *S. volgensis* (Gmelin, 1789) and the extremely rare sea pikeperch *S. marinus* (Cuvier, 1828). The latter is almost unknown from museum specimens and lacks phylogenetic information, although it has been caught by commercial fishermen in the Caspian Sea from whom we obtained two individuals (after several years of seeking them). *Sander marinus* was hypothesized by Svetidov & Dorofeeva (1963) to be morphologically intermediate between the North American and Eurasian species, rendering it important for assessing speciation and biogeographic patterns of the genus.

Sander are ecologically important as top piscivores and support economically valuable fisheries across both continents (Larsen & Berg, 2006; Kuznetsov, 2010; Schmalz *et al.*, 2011). Their populations today face challenges due to habitat loss and degradation, exploitation, competition with invasive species, and climate change (e.g., Barraclough & Nee, 2001; Olden *et al.*, 2010; Willis *et al.*, 2012). We analyze their evolutionary diversification, genetic diversity, and distribution patterns, laying a foundation for understanding their ability to adapt to future anthropogenic stressors.

2.2.1 Morphological differentiation

Sander spp. have laterally compressed elongate bodies and maximum total lengths that range from ~450 mm in *S. volgensis* to ~1,300 mm in *S. lucioperca*. They are differentiated from other percids by the following morphological characters: 1) pronounced canine teeth, 2) narrow rows of teeth on their jaws, vomer, and palatines, 3) a strongly serrated pre-opercle, 4) a continuous lateral line that extends from the head to

the caudal fin, 5) accessory lateral lines on the upper and lower portions of the caudal fin, 6) strongly forked caudal fin, 7) lack of genital papilla, 8) 7–8 branchiostegal rays, 9) 12–13 anal fin rays (Berg, 1965; Trautman, 1981; Hubbs & Lagler, 2004), and 10) a tapetum lucidum (reflective layer behind the retina) that aids in nocturnal predation (Moore, 1944; Collette *et al.*, 1977; Trautman, 1981).

Several morphological characters distinguish among *Sander* spp., including color, scale patterns, number of fin rays, and pyloric caeca (small blind sacs in the stomach that may aid in the breakdown of proteins; Moyle & Cech, 2000). These morphological characters are summarized in Table 2.1 (based on data from Berg, 1965; Trautman, 1981; Hubbs & Lagler, 2004). Coloration patterns vary greatly among *Sander,* with *S. marinus* being the most variable (Table 2.1; Fig. 2-2). Scale patterns differ among the species; *S. volgensis* has cheeks that are fully scaled, *S. canadensis* has intermediate scalation, and *S. vitreus*, *S. lucioperca,* and *S. marinus* have reduced numbers of scales or entirely lack them. The canine teeth unite the genus, but are most pronounced in *S. lucioperca,* whereas adult *S. volgensis* lose them, but have them as juveniles (Berg, 1965).

2.2.2 Species distributions

Sander spp. live in a variety of habitats, inhabiting slow turbid lake environments to fast flowing clear streams (Collette & Bănărescu, 1977; Billington *et al.*, 2011). *Sander canadensis* occurs in the Mississippi River basin, Hudson Bay, Great Lakes, and St. Lawrence River drainages, ranging from Quebec to Alberta and south to Louisiana and Alabama (Fig. 2-1a; Billington *et al.*, 2011; Page & Burr, 2011). *Sander vitreus* is more widely distributed, ranging from the Mackenzie River in the Northwest Territories

of Canada, south to the U.S. Gulf Coast, and northeastward to New Hampshire and Quebec (Fig. 2-1a; Billington *et al.*, 2011; Page & Burr, 2011). *Sander lucioperca* is the most widespread of the three Eurasian species, occurring from the Elbe River in Germany eastward to China and north into the Russian Federation, Sweden, and Finland and inhabiting the Aral, Azov, Baltic, Black, and Caspian Seas (Fig. 2-1b; Berg, 1965; Collette & Bănărescu, 1977; Freyhof & Kottelat, 2008). In contrast, *S. volgensis* has a smaller range, extending from the Danube, Dnieper, and Don Rivers in the Black and Azov Sea basins and the Volga and Ural Rivers in the Caspian Sea basin (Fig. 2-1b; Berg, 1965; Collette & Bănărescu, 1977; Freyhof, 2011). *Sander marinus* is reported from marine/estuarine waters of the Black and Caspian Sea basins (Fig. 2-1b; Berg, 1965; Collette & Bănărescu, 1977), however, museum specimens and modern records in the Black Sea are lacking. This is the first study to address genetic variation and diversity patterns of *S. volgensis* and *S. marinus*. The phylogenetic relationships of *S. marinus* are unknown, presumably because the species is exceptionally rare. The IUCN (International Union for the Conservation of Nature and Natural Resources) Red List notes it as "data deficient" (IUCN, 2012), and Maitland (2001) lists it as "vulnerable".

Due to their fishery popularity, some species of *Sander* have been transplanted outside of their native ranges (Fig. 2-1; see Billington *et al.*, 2011; Freyhof & Kottelat, 2008). Notably, *S. canadensis* individuals were introduced to the upper Savannah River in Georgia, Lake Texoma in Texas, the Apalachicola River in Florida, and the lower Bear River in Idaho (see Fig. 2-1a). *Sander vitreus* has been the most widely introduced, as far northeast as the St. Croix River in Maine, the Lower Oconee River in Georgia, west to rivers in Washington and Oregon that drain to the Pacific Ocean, and south to the

Chattahoochee River, Casablanca Reservoir, and Guadalupe River in Texas (Fig. 2-1a; Billington *et al.* 2011; Fuller & Neilson, 2012). In Eurasia, *S. lucioperca* was transplanted to Spain, the United Kingdom, France, the Netherlands, western Germany, Denmark, Italy, Lithuania, Latvia, and Turkey (Larsen & Berg, 2006). In 1989, *S. lucioperca* was moved from Europe to North America into Spiritwood Lake, North Dakota in the hopes that it would become a valuable fishery. There since has been natural reproduction, but the population remains very small and does not support a fishery (Fuller, 2012). *Sander volgensis* has commercial importance mainly in the Volga, Don, and Dnieper Rivers, and is not reported to have been transplanted (Kuznetsov, 2010). The value of the *S. marinus* fishery in the Caspian Sea is unknown.

2.2.3 Comparative life histories

Sander species mature around three years of age and reproduce during the spring to early summer, migrating to their natal spawning grounds (Berg, 1965; Collette *et al.*, 1977; Craig, 2000; Kuznetsov, 2010; Barton & Barry, 2011). *Sander vitreus* exhibits fidelity to spawning sites (Jennings *et al.*, 1996) with the genetic structure of its spawning groups remaining similar from year to year, among age cohorts, and from generation to generation (Stepien *et al.*, 2012). *Sander* spp. spawn in rivers and shallow lake waters at temperatures ranging from 5–11°C in *S. vitreus* to 10–17°C in *S. marinus* (Scott & Crossman, 1973; Craig, 2000; Kuznetsov, 2010). *Sander canadensis, S. vitreus*, and *S. volgensis* spawn in small groups and broadcast their eggs into the water column. Female *S. canadensis* and *S. vitreus* release multiple clutches of eggs for fertilization over several days, whereas *S. volgensis* and *S. lucioperca* release them in a single batch. There is no

parental care or nest guarding in *S. canadensis*, *S. vitreus*, and *S. volgensis*. In contrast, *S. lucioperca* is believed to be monogamous, with a single female spawning with a single male. Male *S. lucioperca* build a nest in sand or stone substrates, exposing plant roots to which the eggs adhere, and then remain to guard the eggs and fry (Collette *et al.*, 1977; Craig, 2000). The reproductive behavior of *S. marinus* is believed to resemble *S. lucioperca,* with the male building a nest in sandy substrate and a single female laying all of her eggs in the nest (Guseva, 1974; Craig, 2000).

The eggs have a sticky outer coating that allows them to attach to the substrate (Collette *et al.*, 1977; Craig, 2000; Barton & Barry, 2011) and hatch in about two weeks (Barton & Barry, 2011). The young have a small yolk sac and begin to feed soon after hatching, consuming phytoplankton, small zooplankton, and macroinvertebrates (Collette *et al.,* 1977; Craig, 2000; Specziar & Biro, 2003; Bozek *et al.*, 2011). *Sander* spp. rarely consume prey that exceed half of their length at any life history stage. Larger juveniles (~30–100 mm) become piscivorous, eating a variety of fish species (including conspecifics), as well as zooplankton– especially when forage fish are low in abundance (Collette *et al.*, 1977; Craig, 2000; Bozek *et al.*, 2011). *Sander vitreus* has been reported to exhibit piscivory two weeks after hatching (Bozek *et al.,* 2011).

Sander spp. characteristically range widely to feed at non–reproductive times of the year. Their migration distances vary, with *S. marinus* reported to migrate little (Berg, 1965) and *S. canadensis* the most – to ~380 km in a single season (Collette *et al.,* 1977; Bozek *et al.,* 2011). *Sander* spp. reach ~17–19 years of age (Berg, 1965; Carey & Judge, 2000), with some *S. vitreus* reported as 30 years (Bozek *et al.*, 2011).

2.2.4 Objectives and questions

The central objective is to evaluate the evolutionary and biogeographic relationships of *Sander*, including the rare and enigmatic *S. marinus*, using DNA sequences from three mitochondrial (mt; control region, cytochrome (cyt) *b*, cytochrome oxidase I (COI)) and three nuclear gene regions (recombination activating gene intron 1 (RAG1), S7 intron 1 (S7), and lactate dehydrogenase A intron 6 (LdhA6)). We compare the evolutionary divergence and diversification patterns across the range of the genus, asking: (1) Is *Sander* monophyletic?, (2) What are the phylogenetic relationships among its species?, (3) Which biogeographic factors and events explain their speciation and divergence patterns?, and (4) How are their relative patterns of genetic diversity similar or different?

This is the first study to analyze nuclear DNA sequences and multiple mtDNA regions of *Sander* and the only to include *S. marinus*. We analyze six gene regions based on background data from selected percids and other fishes. The control region of mtDNA is non-coding, houses its replication origin, and is less conserved than the other two mtDNA regions analyzed here (summarized by Simon *et al.,* 1994). Cytochrome *b* and COI encode proteins that form part of the electron transport in cellular respiration (summarized by Lunt *et al.,* 1996; Chen *et al.,* 2009). COI additionally is involved in translocating proteins across the mitochondrial membrane (summarized by Lunt *et al.,* 1996). Nuclear RAG1 is a coding gene that aids activation of recombination (Oettinger *et al*., 1990). S7 and LdhA6 are non-coding introns (Gillespie, 1991; Chow & Hazama, 1998). S7 is a ribosomal protein gene (Nomura *et al.,* 1980; Chow & Hazama, 1998), whereas LdhA6 is part of the lactate dehydrogenase enzyme that catalyzes the production of sugars used during respiration (Gillespie, 1991).

2.3 Materials and methods

2.3.1 Sampling and DNA extraction

Fin clips (1–2 cm^2 of pectoral or caudal fin) from the five *Sander* spp. were collected across their respective ranges, totaling 45 sites and 367 individuals (Fig. 2-1, Appendix 2.1). These included the North American *S. canadensis* (7 sites, 25 individuals, 2–4 per site) and *S. vitreus* (18, 232, 5–41), and the Eurasian *S. lucioperca* (13, 75, 1–10), *S. marinus* (1, 2; all that was available for this rare never–before analyzed species), and *S. volgensis* (6, 33, 1–12). We also analyzed all available sequences for the targeted genes from the National Institute of Health's GenBank database (http://www.ncbi.nlm.nih.gov/genbank), which were compared to our data. Homologous sequences were recorded and pruned. We then used the unique haplotypes (Appendix 2.2) for our phylogenetic analyses.

Outgroup taxa encompassed the other Percidae subfamilies, including the greenside darter *Etheostoma blennioides* Rafinesque, 1819, ruffe *Gymnocephalus cernua* (Linnaeus, 1758), Danube ruffe *G. baloni* (Linnaeus, 1758), yellow perch *Perca flavescens* (Mitchill, 1814), European perch *P. fluviatilis* Linnaeus, 1758, logperch darter *Percina caprodes* (Rafinesque, 1818), asprete *Romanichthys valsanicola* Dumitrescu, Bănărescu, & Stoica, 1957, and zingel *Zingel zingel* (Linnaeus, 1766), along with representatives of the family Centrarchidae, including *Micropterus dolomieu* Lacepède, 1802 and *M. salmoides* (Lacepède, 1802). Tissue samples of *R. valsanicola* (#657) and *Z. zingel* (#656) were obtained from the Kansas University Biodiversity Institute

Ichthyology collection, whereas samples of *Sander, E. blennioides, G. cernua, P. flavescens, P. fluviatilis, P. caprodes, M. dolomieu,* and *M. salmoides* were collected by us and colleagues. All tissues and specimens are preserved in 95% EtOH, stored at room temperature, and are archived in the Great Lakes Genetics/Genomics Laboratory (GLGL) at the University of Toledo's Lake Erie Center (Oregon, OH; available upon request; see Appendix 2.3). Sequences of the outgroup taxon *G. baloni* were obtained from GenBank, including the control region (AF025360; Faber & Stepien, 1997), cyt *b* (AY374279; Sloss *et al.*, 2004), COI (HQ960459; International Barcode of Life, unpub.), and LdhA6 (AY034783; Stepien *et al.*, 2005). We used a control region sequence from GenBank to represent *M. salmoides,* as our amplification did not yield sequence data (JN979719; Ray *et al.*, 2012).

DNA was extracted from fin clips using QIAGEN DNEASY extraction kits (Valencia, CA, USA), following manufacturer's directions. Extractions were assayed for quality and quantity on 1% agarose mini-gels stained with ethidium bromide and DNA quantities were verified using a Thermo Scientific (Waltham, MA, USA) Nanodrop 2000 spectrophotometer.

2.3.2 Gene amplification and DNA sequencing

Genetic diversity, phylogenetic, and biogeographic patterns were analyzed from DNA sequences of three mitochondrial (control region, cyt *b*, and COI) and three nuclear gene regions (S7 intron 1, RAG1, and LdhA6). Targeted sequence regions were amplified using the polymerase chain reaction (PCR). Primers included LW1-F (Gatt *et al.*, 2000) and HN20 (Bernatchez & Danzmann, 1993) for the control region, L14724 and

H15915 (Schmidt & Gold, 1993) for cyt *b*, FF2d and FR1d for COI (Ivanova *et al.*, 2007), RAG1F1 and RAG1R2 for RAG1 (Lopez *et al.*, 2004), S7RPEX1F and S7RPEX2R for S7 (Chow & Hazama, 1998), and LdhA6F1 and LdhA6R1 for LdhA6 (Quattro & Jones, 1999). Amplification trials of *Micropterus dolomieu* and *M. salmoides* were unsuccessful with the LW1-F primer, which was substituted with Pro-L (Palumbi, 1996). Nuclear DNA reactions for *Z. zingel* failed to amplify, but its mtDNA sequences were successful, thus *Z. zingel* was included in the mtDNA analyses alone. PCR reactions contained 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 µM of each dNTP, 0.5 µM each of the forward and reverse primers, at least 30 ng DNA template, and 1 unit of *Taq* polymerase in a 25 µl reaction. Amplifications using the LdhA6 primer set were conducted in 50 µl reactions in order to obtain sufficient product. Reactions included an initial denaturation of 2 min at 94^{o}C, followed by 42 cycles of 40 sec at 94^{o}C, 40 sec at primer and species-specific annealing temperature, and 1.5 min at 72^{o}C, with a final extension of 5 min at 72^{o}C. Annealing temperatures were 48 ^{o}C for the control region (56 ^{o}C for *M. dolomieu*), 50 ^{o}C for cyt *b*, 52 ^{o}C for COI and LdhA6, and 56 ^{o}C for RAG1 and S7 (50 ^{o}C was used for S7 of *S. marinus*).

A 4 µl aliquot of each PCR product was visualized on a 1% agarose mini-gel stained with ethidium bromide and successful reactions were purified using a QIAGEN PCR Purification Kit. Purification results then were assessed on a mini-gel and Nanodrop. DNA sequencing was outsourced to the Cornell University Life Sciences Core Laboratories Center (http://cores.lifesciences.cornell.edu/ brcinfo/), which used Applied Biosystems (ABI) Automated 3730 DNA Analyzers (Fullerton, CA, USA).

Sequences were checked, identified, and aligned by us with BIOEDIT v7.05 (Hall, 1999), and then deposited in GenBank (Accession numbers are given in Appendix 2.4). Aligned sequences, including the outgroups were: 743 bp for control region, 1,121 bp for cyt *b*, 652 for COI, 1,377 for RAG1, 575 for S7, and 259 for LdhA6, totaling 4,727 bp. All individuals and taxa first were analyzed for the mt control region, which served as a benchmark for genetic diversity (totaling 377 *Sander* + outgroups). *Sander vitreus* represented the greatest depth of coverage since it has long been a focus of our laboratory's work (Stepien, 1995; Faber & Stepien, 1998; Stepien & Faber, 1998; Stepien *et al.*, 2004), and is being used for a new population genetic study (Haponski & Stepien, in review). A subset of all *Sander* taxa, including all clades on the control region tree, then were analyzed for the other gene regions, including cyt *b* (*N*=62), COI (*N*=84), RAG1 (*N*=37), S7 (*N*=43), and LdhA6 (*N*=44). These selections thus represented the phylogenetic and geographic variability of *Sander*, using the control region as the standard.

2.3.3 Data analyses

Numbers of transitional and transversional substitutions and uncorrected pairwise (*p*-) distances among haplotypes (with both types of substitutions combined) were calculated for each of the six gene regions using MEGA v5.0 (Tamura *et al.*, 2011) and graphed using EXCEL (Microsoft Corp.). Correspondences to linear models were evaluated and analyses of covariance (ANCOVA) were used to assess possible nucleotide saturation with R v2.15.2 (R Development Core Team, 2012).

Phylogenetic relationships were evaluated for each gene region separately using maximum likelihood (ML) in PHYML v3.0 (Guindon *et al.*, 2010) and Bayesian analyses in MRBAYES v3.2.1 (Ronquist & Huelsenbeck, 2003). Corrected Akaike information criteria (AICc) from JMODELTEST V2 (Darriba *et al.*, 2012) were employed to determine the most appropriate nucleotide substitution models. For the mtDNA sequence data sets, JMODELTEST selected the TPM3uf model (Posada, 2008) with a gamma (α) distribution (α=0.3040) for control region, TPM2uf (Posada, 2008) with invariant sites (I=0.5370) and gamma (α=1.2500) for cyt *b*, and TPM2uf plus a gamma distribution (α=0.1350) for COI. Models for the nuclear data sets were TIM2ef (Posada, 2008) plus invariant sites (I= 0.6620) for RAG1, Hasegawa, Kishino, and Yano (Hasegawa *et al.*, 1985) with a gamma distribution (α=2.1880) for S7, and Kimura 80 (Kimura, 1980) plus gamma (α=1.1920) for LdhA6.

Maximum likelihood analyses in PHYML were begun with five random trees, from which the best was selected using nearest neighbor interchange (NNI) and subtree pruning and regrafting (SPR). Support for the nodes was determined from 2,000 bootstrap pseudo-replications (Felsenstein, 1985). Bayesian analyses in MRBAYES used a Metropolis-coupled Markov chain Monte Carlo (MC^3) approach and ran for 5,000,000 generations, with sampling every 100 generations. Four separate chains were run simultaneously for each analysis, and two analyses ran simultaneously. The burn-in period for the MC^3 was determined by plotting log likelihood values for each generation to identify when stationarity was reached. As burn-in, 25% of the generations were discarded, along with the trees and parameter values sampled prior to the burn-in. A 50% majority rule consensus tree was based on the remaining generations, whose branch

support was determined from the posterior probability distribution (Holder & Lewis, 2003) in MRBAYES.

To discern the overall relationships, the six gene regions were concatenated in a partitioned MRBAYES analysis following the approach of Near *et al.* (2011). The six gene region JMODELTEST models were assigned to each partition using the APPLYTO command and the model parameters (invariable sites or gamma distribution) were set using the UNLINK command. Number of generations and burn-in were the same as analyses for the individual gene regions.

Lastly, intraspecific haplotypic diversities for each species per the six different gene regions were calculated using ARLEQUIN v3.5.1.3 (Excoffier & Lischer, 2010). To determine the pairwise divergence among taxa, we calculated uncorrected *p*-distances in MEGA considering both transitions and transversions with 2,000 bootstrap replications. We also counted the number of fixed differences among the taxa.

2.3.4 Divergence time estimates

Comparative divergence time estimates among *Sander* spp. lineages were evaluated for the six gene regions and the concatenated data set using BEAST v.1.71 (Drummond *et al.*, 2012), with the general time reversible nucleotide substitution model (GTR; Lanave *et al.*, 1984). A gamma distribution and invariant sites were incorporated for those mtDNA and nuclear DNA regions identified by JMODELTEST. BEAST analyses used a relaxed molecular clock that assumed a lognormal distribution with the Yule speciation process (Gernhard, 2008) as a tree prior. Two separate runs were conducted, each with a chain length of 50,000,000 generations, and parameters sampled each 100

generations. We used fossil dates as calibration points for outgroup taxa, representing the Oligocene Epoch (~26 Mya) for *Perca* (Lebedev, 1952), ~12 Mya for the genus *Micropterus* (Wilson, 1968; Tedford *et al.*, 1987; Near *et al.*, 2005), and ~1.8 Mya for *Gymnocephalus* (Holčík & Hensel, 1974). For the RAG1 and S7 gene regions and the concatenated data set, the *Gymnocephalus* date was excluded since *Gymnocephalus* had only a single sequence represented (*G. cernua*) and multiple sequences are required to date the node (Drummond *et al.*, 2012).

2.4 Results

2.4.1 Phylogenetic relationships

The genus *Sander* is monophyletic in the Bayesian tree based on the concatenated data set of all six gene regions (Fig. 2-3), with all relationships supported by 1.00 posterior probabilities (p.p.). *Romanichthys* represents the sister group to *Sander* (p.p.=1.00; Fig. 2-3), which are separated by uncorrected *p*-distances of 0.012–0.118 and 3–70 fixed substitutions (in the six gene regions; Table 2.2). *Sander* is divided into two distinct clades, one in North America and the other in Eurasia (Fig. 2-3), with *p*-distances of 0.012–0.094 and 4–54 fixed nucleotide differences (Table 2.2). Their separation is supported by all six individual gene trees (0.83–1.00 posterior p.p./62–100% bootstrap pseudoreplications; Appendix 2-1). Within the North American clade, *S. canadensis* is the sister species to *S. vitreus* (Fig. 2-3). Within the Eurasian clade, *S. volgensis* is the basal taxon to a clade containing *S. lucioperca* and *S. marinus* as sister taxa.

Individual gene trees only partially resolve the phylogenetic relationships (see Appendix 2-1). *Sander* is monophyletic and is the sister group to a clade comprising *Romanichthys/Zingel* on the mtDNA cyt *b* and nuclear RAG1 trees (0.94–0.96 p.p./63–81%), but is paraphyletic on the other trees (control region, COI, S7, and LdhA6). The mtDNA trees differentiate among all *Sander* species, whereas all nuclear DNA trees lack sufficient resolution to distinguish between the North American species, *S. canadensis* and *S. vitreus*. Those species diverge by *p*-distances of 0.049–0.070 and by 34–63 fixed differences in the three mtDNA regions (Table 2.2). The relationships among the three Eurasian taxa are supported in four of the gene region phylogenies (control region, COI, RAG1, and S7; 0.54–1.00 p.p./73–85%), and are unresolved in the cyt *b* and LdhA6 trees (Appendix 2-1). *Sander volgensis* diverges from the clade comprising *S. lucioperca* and *S. marinus* by 0.002–0.051 uncorrected *p*-distances and 1–36 fixed differences (among the six gene regions). *Sander lucioperca* and *S. marinus* are differentiated by *p*-distances of 0.001–0.060 and by 1–68 fixed differences (Table 2.2).

2.4.2 Biogeographic relationships

According to the BEAST calculations and fossil calibrations for the concatenated data set, the genus *Sander* appears to have diverged from its most recent common ancestor shared with *Romanichthys* ~24.6 Mya (11.5–39.6 Mya = 95% highest posterior density (HPD)) during the late Oligocene Epoch (Fig. 2-3b). The North American and Eurasian clades then separated ~20.8 Mya (9.5–34.3 HPD) during the early Miocene Epoch. The common ancestor of the modern North American *Sander* originated ~15.4 Mya (5.2–27.1 HPD) during the middle Miocene Epoch, with *S. vitreus* diverging slightly

earlier ~10.8 Mya (2.5–20.7 HPD) than its sister species *S. canadensis* ~7.3 Mya (0.24–17.1 HPD), both during the late Miocene Epoch. The common ancestor of today's Eurasian taxa diverged ~13.8 Mya (4.4–25.9 HPD), leading to the *S. volgensis* and the *S. lucioperca/S. marinus* clades. The latter sister species separated ~9.1 Mya (1.8–17.6 HPD). Origins of the individual Eurasian species dated to the Pliocene and Pleistocene Epochs, *S. lucioperca* ~5.0 Mya (0.2–11.4 HPD), *S. marinus* ~2.1 Mya (0.001–7.9 HPD), and *S. volgensis* ~2.4 Mya (0.0004–9.0 HPD; Fig. 2-3b). Dates recovered from the individual gene analyses have a high degree of variation, despite their common calibration points (see Appendix 2.5). This appears to mirror differences in their relative substitution rates (see Fig. 2-4).

2.4.3 Phylogenetic signal of mitochondrial and nuclear gene regions

We evaluated each of the six gene regions for saturation of the phylogenetic signal. Overall, there is little evidence for saturation (F=133.4–24,970, df=38–1,186, p<0.0001), with transitions outnumbering transversions in five of the gene regions – all but mtDNA control region (Fig. 2-4). In the control region, transversions outnumber transitions after ~the halfway point (p-distance ~0.06), which may denote saturation since the transitions cross the line of transversions and decline in trajectory. However, its regression relationship is significant, indicating that overall substitutions continue to accumulate with increasing divergence among the taxa. Across all six gene regions, numbers of transitions and transversions correspond to linear relationships, with R^2 values ranging from 0.92–0.99 for transitions and 0.72–0.98 for transversions.

Overall, the mtDNA gene regions evolve at faster rates than the nuclear DNA introns (Table 2.2; Fig. 2-4). The mtDNA sequence divergences range from 0.00–0.14 across the sequence comparisons (Fig. 2-4a–c), whereas the nuclear DNA sequences are 0.00–0.04, about 1/3 the rate (Fig. 2-4d–f). Among the mtDNA gene regions, the relative rate of evolution appears fastest in cyt *b*, followed by COI, and then the control region. Among the nuclear gene regions, the S7 intron has the greater overall divergence, followed by LdhA6, and then RAG1.

2.4.4 Genetic diversity of Sander *spp.*

We identify a total of 107 different haplotypes across all six gene regions (Table 2.3, Appendix 2.4). Overall, the three mtDNA gene regions have more haplotypes (16–35) than the nuclear DNA regions (7–14). Among the three mtDNA gene regions, the control region has the most haplotypes (35), followed by cyt *b* (22), and then COI (16). The control region thus serves as our benchmark. Among the nuclear DNA introns, S7 has the most haplotypes (14), followed by RAG1 (13), and LdhA6 (7; Table 2.3).

The five species of the genus *Sander* share no haplotypes, with the sole exception of *S. canadensis* and *S. vitreus* sharing a single nuclear S7 intron 1 sequence (SviS71; Appendix 2.4). All *S. canadensis* samples possess that haplotype whereas *S. vitreus* has four additional S7 haplotypes represented by 13 individuals, of which four are heterozygous. The North American species (*S. canadensis* and *S. vitreus*) tended to have more haplotypes in all gene regions and higher haplotypic diversity than do the Eurasian species (*S. lucioperca, S. marinus,* and *S. volgensis*; Table 2.3). More haplotypes are found for *S. vitreus,* ranging from two for LdhA6 to 23 in the control region, with its

haplotypic diversity ranging from 0.31±0.02 for COI to 0.80±0.03 for S7. Numbers of haplotypes for *S. canadensis* range from a single S7 haplotype to seven for cyt *b*, and its diversity varies from 0.00 in S7 to 0.96±0.03 for cyt *b*.

Among the Eurasian species, *S. lucioperca* contains more haplotypes and has higher haplotypic diversity than *S. volgensis*; both species have single RAG1 and single LdhA6 haplotypes, ranging to five cyt *b* and five COI haplotypes in *S. lucioperca* (versus two each in *S. volgensis*). Haplotypic diversity of *S. lucioperca* varies from 0.00 with RAG1 and LdhA6 to 0.65±0.02 in cyt *b*. Overall, *S. volgensis* possesses the lowest number of haplotypes and the least diversity of all *Sander*, ranging from 0.00 for the control region, RAG1, S7, and LdhA6 to 0.48±0.06 in COI (Table 2.3). The rare and enigmatic *S. marinus* thus appears to have comparably more haplotypes and greater diversity than the more common *S. volgensis*. The two samples of *S. marinus* analyzed here each possess a unique haplotype for the control region, cyt *b*, and RAG1. Haplotypic diversity of *S. marinus* ranges from 0.00 with COI, S7, and LdhA6 to 1.00±0.35–0.36 in the control region and cyt *b* (Table 2.3).

2.5 Discussion

2.5.1 Evolution and divergence of Sander

We conclude that the genus *Sander* is monophyletic and is the sister group of *Romanichthys/Zingel*. Together the three genera comprise the subfamily Luciopercinae, congruent with its morphological definition by Collette & Bănărescu (1977). Other molecular evolutionary analyses using allozymes, mtDNA restriction fragment length

polymorphisms (RFLPs; Billington *et al.*, 1990, 1991), and mtDNA control region sequence data (Faber & Stepien, 1998) likewise recovered *Sander* as monophyletic, using four of the five species (*S. canadensis, S. vitreus, S. lucioperca,* and *S. volgensis*). Ours is the first investigation to analyze all five species of the genus *Sander*, to use nuclear DNA sequences, and to compare results from multiple DNA regions.

Ancestors of the genus *Sander* are estimated to have diverged ~24.6 Mya during the late Oligocene Epoch from the *Romanichthys/Zingel* clade, based on our Bayesian analyses and fossil calibration points. *Romanichthys* and *Zingel* are endemic to the Danube River watershed, surrounded by the Balkan, Carpathian, and Alps mountain ranges, suggesting that the shared ancestor of the Luciopercinae was European, as hypothesized by Collette & Bănărescu (1977) from morphological characters. During the Oligocene Epoch, tectonic activity in the Periadriatic fault system of the southern Alps (Viola *et al.*, 2001) may have led to divergence of ancestral *Romanichthys/Zingel* from the *Sander* lineage.

Our molecular results and fossil evidence corroborate that the common ancestor of *Sander* was widely distributed across both continents, as suggested by Cavender (1998). *Sander* then diverged ~20.8 Mya during the Miocene Epoch into two lineages, likely due to subsidence of the NALB that interrupted connectivity across the Atlantic Ocean (Denk *et al.*, 2010; see Fig. 2-3b), refuting hypotheses 1, 2, 4, and 5 (Introduction). It is unlikely that this divergence was due to loss of connection across the BLB, since *Sander* are not located near the BLB on either side of the Pacific Ocean, and instead are distributed in eastern–central North America, Europe, and western Asia (see Fig. 2-1; Collette & Bănărescu, 1977; Billington *et al.*, 2011). In addition, recently

discovered fossils representing *Sander* (Fig. 2-1c) are found in central (southern Saskatchewan) and eastern Canada (Ellesmere Island), respectively dating to ~16–13 Mya during the Miocene Epoch (Murray & Divay, 2011), and ~5 Mya during the Pliocene Epoch (Murray *et al.,* 2009). Today's North American *Sander* include *S. canadensis* and *S. vitreus* and the Eurasian species are *S. lucioperca, S. marinus,* and *S. volgensis*; both clades are monophyletic and well supported by our phylogeny.

Earlier DNA studies (Billington *et al.,* 1990, 1991; Faber & Stepien, 1998) also obtained phylogenetic results with high support, which were congruent to ours, but hypothesized later divergences between the North American and Eurasian clades (~10–4 Mya; Miocene/Pliocene Epoch): those investigations pre-dated the discovery of the fossil evidence used here (e.g., Murray & Divay (2011). Our results estimate evolutionary rates that are much slower - about 1/2–1/5 the previous rate estimates (which was 2% per My for the mtDNA data (Billington *et al.,* 1990; Faber & Stepien, 1998)).

In contrast to our findings, a much older separation of ~37.1 Mya during the Eocene Epoch was estimated by Imoto *et al.* (2013) between North American and Eurasian sister species of cyprinids (Cypriniformes: Cyprinidae: Leuciscinae). They used mitogenome data and a relaxed molecular clock adapted from fossil calibrations employed by Saitoh *et al.* (2011). Thus, North American and Eurasian sister taxa may have diverged at various times due to different interruptions in the NALB across a span of ~12.5 million years.

Other fishes share a common history across North America–Eurasia, but diverged across the BLB. For example, Hai *et al.* (2008) analyzed mtDNA cyt *b* sequences of *Perca,* finding that the European perch *P. fluviatilis* was basal to the clade containing the

North American yellow perch *P. flavescens* and the Eurasian Balkash perch *P. schrenkii* as sister species. In that case, the divergence between the two likely was across the BLB, since *P. schrenkii* is distributed further east in Asia. Using mtDNA cyt *b* and nuclear RAG1 intron 2 sequences, Grande *et al.* (2004) recovered a sister relationship between the northern pike *Esox lucius* (Esociformes: Esocidae), which is distributed across both North America and Eurasia, and the Amur pike *E. reichertii*, which is endemic to Eurasia; both of those species occur more widely across Asia (closer to the BLB) than does *Sander*. It appears likely that *E. lucius* continues to share a recent connectivity across the BLB, since its populations are little diverged today according to microsatellite loci (Senanan & Kapuscinski, 2000). *Esox lucius* thus has retained the ancestral pattern of wide distribution and connectivity (versus the former bi-continental history of *Sander* and *Romanichthys/Zingel*), whereas *Sander* later diverged across the NALB. Yokoyama & Goto (2005) discerned a lineage of sculpins (Scorpaeniformes: Cottidae) that today is widely distributed across Siberia, Russia, and North America. According to mtDNA control region and 12S sequences, the lineage (containing the Eurasian Kessler's sculpin *Leocottus kesslerii* and the bullhead *Cottus gobio*, the North American coastrange sculpin *Cottus aleuticus* and the widespread North American–Asian slimy sculpin *C. cognatus*) diverged ~6.2–2.5 Mya during the Late Miocene–early Pleistocene Epochs likely over the BLB, in contrast to *Sander* diverging ~20.8 Mya over the NALB. Thus it appears that taxa sharing a common ancestry across the two continents have diverged over a long temporal scale with loss of connectivity over the BLB and the NALB.

2.5.2 Phylogenetic and biogeographic patterns on each continent

The present study estimates that the North American *S. canadensis* and *S. vitreus* diverged ~15.4 Mya during the Mid-Miocene Epoch, during a period marked by climate and topographic changes. At this time, mean annual temperatures in temperate regions reached ~22°C (Böhme, 2003) and final uplift occurred of mountain ranges, including the Rockies and Cascades in the west (Wolfe *et al.,* 1998) and the Appalachians in the east (White, 2009), which may have led to differentiation of the two species. Earlier DNA work on the North American species by Billington *et al.* (1990, 1991) and Faber & Stepien (1998) likewise resolved both species as well-supported sister taxa. In our study, the individual nuclear data trees failed to distinguish *S. canadensis* from *S. vitreus* due to lack of sufficient nucleotide divergence (i.e., slow evolutionary rates), in comparison to the higher resolution evidenced in the concatenated tree and the mtDNA trees. Similar to our findings, species of North American gars (Lepisosteiformes: Lepisosteidae) were indistinguishable using sequences from several nuclear genes (ENC1, myh6, plagl2, sreb2, tbr1, and zic1), but were resolved using the mtDNA COI gene and the nuclear S7 intron 1 (Wright *et al.,* 2012).

Our results suggest that modern *S. vitreus* haplotypes date to ~10.8 Mya during the Mid-Miocene Epoch, when rapid cooling was beginning and the ice sheets were forming (Wolfe, 1994; Hewitt, 1996; Bruch *et al.,* 2007). Modern haplotypes of *S. canadensis* trace to ~7.3 Mya during the Late Miocene Epoch, when temperate regions further cooled (Zachos *et al.,* 2001; Bruch *et al.,* 2007).

Within the Eurasian clade, *S. volgensis* is the basal taxon to the clade of *S. lucioperca* and *S. marinus*, diverging ~13.8 Mya during the Mid-Miocene Epoch, during

cooling temperatures. *Sander volgensis* has a more limited geographic range than does *S. lucioperca*; the latter appears to be adapted to a wider diversity of environmental temperatures and conditions. During the Mid-Miocene Epoch, many Eurasian taxa, including crocodiles and some turtles, became extinct due to the increasingly high seasonality of precipitation (as much as six dry months annually; Böhme, 2003). The dry conditions were accompanied by the final uplifts of the Alps, Carpathian, Balkan, and Caucasus Mountains, isolating water bodies and forming the Sarmatian Sea (the region of today's Black and Caspian Seas; Reid & Orlova, 2002). These events may have led to the vicariant divergence of *S. volgensis* from the *S. lucioperca/S. marinus* lineage.

Congruent with our DNA sequence phylogeny, *S. volgensis* has retained the plesiomorphic life history condition of being a broadcast spawner without parental care. This life history is shared with the North American taxa. In contrast, *S. lucioperca* and *S. marinus* share the derived life history characters of the males building nests and then guarding the eggs and fry (Guseva, 1974; Collette *et al.*, 1977; Craig, 2000). These characters are synapomorphies that unite the two species, and likely originated in their common ancestor ~13.8 Mya. This life history strategy of parental care likely increases survival of the young (see Shine, 1978; Blumer, 1982; Sargent *et al.*, 1987), as hypothesized for other fishes, such as the fathead minnow *Pimephales promelas* (Cypriniformes: Cyprinidae; Sargent, 1988), cichlids (Perciformes: Cichlidae; summarized by Smith & Wooton, 1994), and smallmouth bass *Micropterus dolomieu* (Perciformes: Centrachidae; Gillooly & Baylis, 1999).

Sander lucioperca and *S. marinus* then diverged ~9.1 Mya likely due to increases in salinity in the Ponto-Caspian Sea basin (including both the Black and Caspian Seas),

which lead to the *S. marinus* lineage in saline waters (see Reid & Orlova, 2002). *Sander lucioperca* today is reported to briefly tolerate salinities to ~12 ppt (Craig, 2000; Brown *et al.*, 2001), whereas ~9.1 Mya the Ponto-Caspian basin is believed to have been ~17–20 ppt (Reid & Orlova, 2002). *Sander marinus*, in contrast, exclusively inhabits saline waters to ~13 ppt in today's Caspian Sea, where *S. lucioperca* is absent (restricted to rivers; S. Ibramihov, pers. commun.).

Modern *S. lucioperca* haplotypes diversified ~5 Mya during the Pliocene Epoch, based on fossil calibrations and our BEAST analyses, during which time the Ponto-Caspian basins experienced many geologic and climatic changes, including changes in sea levels and salinity (Reid & Orlova, 2002), which likely isolated populations in different areas. Today's *S. volgensis* and *S. marinus* haplotypes differentiated during the Pleistocene Epoch ~2.6–2.1 Mya, when the Ponto-Caspian region experienced more fluctuations in water levels and salinities (Reid & Orlova, 2002). We analyzed two samples of the rare and enigmatic *S. marinus* from the Caspian Sea, and further sampling and analyses would be beneficial (we analyzed two of the three individuals that have been documented, the other is referred to by Lang & Mayden, 2007). Samples representing its putative Black Sea distribution would be helpful (see Berg, 1965; Collette & Bănărescu, 1977), however, it is unclear whether the *S. marinus* is extant there since we were unable to locate records in museums or the literature.

2.5.3 Phylogenetic signal of gene regions

Overall, the six gene regions we examined exhibit different rates of evolutionary divergence, with the mtDNA regions being ~3.5x faster (uncorrected *p*-distances to 0.14)

than the nuclear DNA introns (to 0.04). MtDNA sequences have been described to evolve much faster (to 5–10x) than most nuclear DNA regions (summarized by Simon *et al.*, 1994; Hewitt, 2001) due to mtDNA having ¼ the effective population size, lack of proofreading by DNA polymerase I, and no recombination (see Stepien & Kocher, 1997; Avise, 2004; Marshall *et al.*, 2009).

Similar to our findings, divergences among species of acanthomorph fishes (Acanthomorpha) were higher (~5.7x) in mtDNA 12S rDNA (Tamura-Nei (1993) distances ranging to ~0.4), than in nuclear 28S rDNA sequences (to ~0.07; Wiley *et al.*, 2000). The mtDNA cyt *b* and ATPase 8/6 genes of swamp eels *Synbranchus* and *Ophisternon* (Synbranchiformes: Synbranchidae) evolved ~7–8 times faster than the nuclear RAG1 intron, with Tamura-Nei (1993) genetic distances ranging to 0.871 for the mtDNA genes versus 0.004 in RAG1 (Perdices *et al.*, 2005). Guo & Chen (2010) similarly found much greater divergences in mtDNA cyt *b* (maximum likelihood distance to 4) than in nuclear S7 intron sequences (to 1.5) for the temperate perches *Siniperca* and *Coreoperca* (Perciformes: Sinipercidae). Similar to our results (and using four of the same gene regions), Neilson & Stepien's (2009) analysis of Ponto-Caspian gobies (Perciformes: Gobiidae: Benthophilinae) revealed mtDNA evolutionary rates for the COI and cyt *b* genes (uncorrected *p*-distances ranging to 0.22) ~4–5x greater than the nuclear RAG1 and S7 introns (*p*-distances to 0.056).

The three mtDNA regions used in our investigation differentiate the five *Sander* species. However, the mtDNA control region and COI sequence data do not resolve the genus *Sander* as monophyletic, and do not distinguish the higher-level relationships. Evolutionary rate of the control region may be affected by multiple substitutions per site,

resulting in convergence, parallelism, or character state reversals (i.e., phylogenetic noise; see Avise, 2004; McCracken & Sorenson, 2005), as indicated by Fig. 2-4a, in which transversions outnumber transitions and the latter appear to level off. Similarly, a study of control region and cyt *b* sequences among species of rainbowfishes *Melanotaenia* (Atheriniformes: Melanotaeniidae) discerned saturation in the control region (Zhu *et al.*, 1994), with its transversions outnumbering transitions, and the latter plateauing at ~0.15 pairwise sequence divergence, versus our value of an earlier plateau at 0.06. Studies of cichlid fishes found that the control region evolved slightly faster than did cyt *b* in the genera *Tropheus* (Sturmbauer & Meyer, 1992) and *Melanotaenia* (Zhu *et al.*, 1994). Notably, since we did not study the more rapidly evolving repeated regions of the control region at the 5' end, we did not analyze the realm of its highest variability, which was detailed for *Sander* by Faber & Stepien (1998) and Stepien & Faber (1998). The control region may be better suited to resolve intraspecific relationships (Simon *et al.*, 1994; Faber & Stepien, 1998), as our results indicate that its signal declines at higher levels within the genus due to saturation.

The nuclear gene regions examined here resolve the deeper divergences and some of the species relationships, but are unable to distinguish between the North American *S. canadensis* and *S. vitreus.* In our data sets, the LdhA6 intron sequences evolve the slowest, differentiating the clades between the two continents, but not among their respective species. Similarly, Quattro *et al.* (2006) analyzed mtDNA control region and COI sequences, in comparison to nuclear LdhA6 intron sequences, from hammerhead sharks *Sphyrna* (Carcharhiniformes: Sphyrnidae) and were unable to resolve species differences with the latter. Wright *et al.* (2012) distinguished among species of gars

(Lepisosteiformes: Lepisosteidae) using mtDNA COI and the nuclear S7 intron 1, but six other nuclear gene regions yielded conflicting tree topologies and differential resolution. Two of those nuclear genes (tbr1 and zic1) were restricted to distinguishing between the two genera alone (Wright *et al.,* 2012), similar to our results for the LdhA6 intron. Neilson & Stepien (2009) likewise were unable to differentiate among some goby species with nuclear RAG1 and S7 intron sequences. Similarly, Unmack *et al.* (2011) discerned incongruence among trees from mtDNA cyt *b* and nuclear S7, RAG1, and RAG2; cyt *b* performed best at tip nodes, S7 at intermediate nodes, and RAG1 and 2 at deeper divergences.

Our study found that trees from the mtDNA cyt *b* gene and the nuclear RAG1 intron yield the most robust topologies for discerning *Sander* relationships. Using a similar multi-gene approach, Neilson & Stepien (2009) analyzed the mtDNA cyt *b* and COI genes and the nuclear RAG1 and S7 introns for Ponto-Caspian gobies, finding that cyt *b* had superior resolution. Bohlen *et al.* (2011) obtained congruent and highly supported phylogenies for species of loaches *Pangio* (Cypriniformes: Cobitidae) using cyt *b* and RAG1, similar to our findings. Our pairwise divergences among *Sander* species range to 0.12 with cyt *b* gene sequences versus 0.012 for RAG1. Neilson & Stepien (2009) likewise recovered their highest pairwise divergences to 0.18 with cyt *b*, compared to just 0.04 for nuclear RAG1 and 0.03 for S7. Other studies found similar divergence levels for cyt *b*, ranging to 0.13 among *Esox* species (Esociformes: Esocidae) (López *et al.,* 2000), 0.13 among sand darters *Ammocrypta* (Perciformes: Percidae; Near *et al.,* 2000), and to 0.08 for populations of the greenside darter *Etheostoma blennioides* (Perciformes: Percidae; Haponski & Stepien, 2008).

2.5.4 Genetic diversity patterns

The mtDNA regions have more haplotypes and higher haplotypic (gene) diversities than the nuclear DNA introns. Genetic diversity is much greater for the North American species (mean=0.56, range=0.25–0.96) than in the Eurasian species (mean=0.29, range=0.25–1.00). This likely is due to their greater population sizes throughout their histories, experiencing fewer bottlenecks and founder effects. During the Pleistocene glaciations, the North American refugia were larger and more extensive in geographic area compared to the Eurasian refugia (Hewitt, 1996; Bernatchez & Wilson, 1998; Stewart & Lister, 2001). The primary North American mountain ranges are oriented north to south, which provided large refugia in the interior of the continent where the distributions of *S. canadensis* and *S. vitreus* are centered (see Fig. 2-1). Primary refugia used by *S. vitreus* and other aquatic taxa included the Atlantic refugium that existed to the east of the Appalachian mountains, the Mississippian refugium near the lower Mississippi River, and the Missourian refugium to the west of the Missouri River (see Fig. 2-1a; Ward *et al.*, 1989; Billington *et al.*, 1992; Bernatchez & Wilson, 1998).

In contrast, Eurasian refugia were limited in area by several east–west mountain ranges (the Carpathians, Balkans, Alps, etc.) along the southern portion of the continent, circumventing the retreat of taxa from the advancing ice sheets (see Fig. 2-1b). The main refugia were located on the Iberian, Italian, and Balkan peninsulas (Hewitt, 1996, 2000) and in the Ponto-Caspian region (Bănărescu, 1991), with the latter refugium likely housing ancestral Eurasian *S. lucioperca* and *S. volgensis* based on their current distributions. This Ponto-Caspian region experienced many water level and salinity

fluctuations during the Pleistocene Epoch (see Reid & Orlova, 2002), likely isolating populations into small areas. These led to genetic bottlenecks and later founder effects during re-colonizations. Thus, North American *Sander* retained greater genetic diversity whereas the Eurasian *S. lucioperca* and *S. volgensis* are relatively genetically depauperate. In contrast, *S. marinus* appears to have more genetic diversity in the Caspian Sea, despite its apparent scarcity. This may reflect the stability of its habitats during the glaciations.

Today's relatively modest genetic diversity of *S. lucioperca* and *S. volgensis* and their current native distribution patterns suggest that they likely once were isolated in the Ponto-Caspian glacial refugium that housed the modern Black and Caspian Seas. Neither species has a present-day native distribution near the locations of the other possible refugia (Iberian, Italian, and Balkan Peninsulas; see Fig. 2-1; However, *S. lucioperca* later was introduced to these areas). In addition, both species contain a small number of haplotypes (two in *S. lucioperca* and one in *S. volgensis*) that are widely distributed across their ranges, which appears to fit the hypothesis of rapid post-glacial recolonization from a single refugium (per Hewitt, 1996). In contrast, *S. vitreus* shows evidence of contributions from multiple glacial refugia, which led to higher genetic diversity and considerably more genetic structure across its range (Ward *et al.,* 1989; Stepien & Faber, 1998; Stepien *et al.,* 2009). In comparison, *S. canadensis* may have re-colonized from a single refugium – the Mississippian refugium (see Billington, 1996; White, 2012) – but also has higher haplotypic diversity than the Eurasian taxa.

Sander vitreus had the highest haplotypic diversity values (mean H_D=0.57 for the six gene regions, range 0.31–0.80) among all five species of *Sander*. Likewise, other

studies confirmed relatively high mtDNA control region diversities of *S. vitreus* populations across Lakes Erie and St. Clair (mean H_D=0.69, range=0.36–0.79; Stepien & Faber, 1998), Lakes Huron, St. Clair, and Erie (mean H_D=0.73, range=0.58–0.78; Haponski & Stepien, 2013), and in Lake Huron's Georgian Bay (mean H_D=0.49, range=0.28–0.53; Gatt *et al.,* 2002). Similarly, Stepien *et al.* (2009) discerned high genetic diversities with nine nuclear DNA microsatellite loci among spawning populations of *S. vitreus* across its native range (mean H_O=0.68, range=0.51–0.78). The present analysis also found appreciably high mtDNA control region diversity for *S. canadensis* (mean H_D=0.55, range 0.25–0.96), similar to values for walleye. White (2012) sampled mtDNA control region sequences of *S. canadensis* across its range and likewise found fairly high diversity (recovering 19 haplotypes among 60 samples, compared to our six haplotypes among 25 samples). In contrast, Billington (1996) reported lower diversity in *S. canadensis* samples using mtDNA RFLP analyses (detecting four mtDNA haplotypes among 114 samples), attributable to the relatively low resolution of the RFLP method compared to a sequencing approach (see Chubb *et al.,* 1998).

Lower haplotypic diversity levels characterized *S. lucioperca* (mean H_D=0.30, range 0.33–0.65), in comparison to the higher haplotypic diversity of the North American species. Other studies similarly found low genetic variability in *S. lucioperca* using allozyme loci (mean H_O=0.04, range=0.013–0.042; Poulet *et al.,* 2004), but more for six nuclear microsatellite loci from populations in Sweden, Finland, and Russia (mean H_O=0.53, range=0.32–0.74; Björklund *et al.,* 2007). The latter's higher values illustrated the larger effective population size of nuclear DNA (4x) in comparison with mtDNA;

mtDNA is much more affected by bottlenecks (see Hewitt, 2001; Ellegren, 2004). Moreover, the genetic diversity values of *S. vitreus* are similar for both mtDNA and nuclear microsatellite loci, providing further evidence for the lower influence of bottlenecks and founder effects in North America.

Ours is the first study to characterize the genetic diversity of *S. marinus*, discerning relatively high levels (apparently higher than the more widely distributed *S. lucioperca* and *S. volgensis*). Further sampling is recommended to determine the overall diversity and population structure of this rare and enigmatic species. In comparison, *S. volgensis* has the lowest diversity of the Eurasian species; no other studies have analyzed its population genetic patterns.

Other Eurasian fishes also had low genetic diversity for taxa that descended from a single refugium versus those originating from multiple refugia (Durand *et al.,* 1999; Nicod *et al.,* 2004; Hänfling *et al.,* 2009). For example, chub *Leuciscus cephalus* (Cyprinidae: Cypriniformes) from the Ponto-Caspian refugium had much lower cyt *b* sequence diversity than populations in the Adriatic and Aegean regions that colonized from multiple refugia (Durand *et al.,* 1999), similar to the low diversity recovered here for *S. lucioperca* and *S. volgensis.* Likewise, Nicod *et al.* (2004) found low mtDNA control region and cyt *b* diversity in *E. lucius* populations from major European watersheds, with a single widespread haplotype dominating most; that low diversity was attributed to re-colonization from only 1–2 refugia.

In contrast, European populations of *P. fluviatilis* had mean mtDNA control region diversities (mean H_D=0.33, range=0–0.87; Nesbø *et al.,* 1999), which were similar to those of North American *P. flavescens* (mean H_D=0.31, range=0–0.82; Sepulveda-

Villet & Stepien, 2012). Each of those studies pointed to multiple refugia, with *P. fluviatilis* re-colonizing Eurasia from four refugia located near the Baltic Sea, Danube River, and Ponto-Caspian region (Nesbø *et al.*, 1999), and *P. flavescens* from the Missourian, Mississippian, and Atlantic coastal refugia in North America (Sepulveda-Villet & Stepien, 2012). Those same three refugia led to today's northern populations of *S. vitreus* (Ward *et al.*, 1989; Billington *et al.*, 1992; Stepien *et al.*, 2009). Thus the relatively low diversity discerned for modern Eurasian *S. lucioperca* and *S. volgensis* may be due to their confinement and re-colonization from a single small glacial refugium, whose population experienced bottlenecks and founder effects.

2.5.5 Conclusions

The genus *Sander* is monophyletic, sharing a common ancestry with *Romanichthys/Zingel* until ~24.6 Mya during the Oligocene Epoch. *Sander* originally was widely distributed across the Holarctic, extending from the Atlantic coasts to the central regions of both continents. The genus then diverged into two clades~20.8 Mya with subsidence of the NALB: one lineage in North America (today's *S. canadensis* and *S. vitreus*) and the other in Eurasia (today's *S. lucioperca, S. marinus,* and *S. volgensis*). Fossil evidence showed that extinct North American *Sander* date to ~16–13 Mya, overlapping the estimated divergence of *S. canadensis* and *S. vitreus* ~15.4 Mya during the Miocene Epoch. The three Eurasian species diverged ~13.8 Mya into two lineages – with *S. volgensis* as the basal taxon, and *S. lucioperca* and *S. marinus* later differentiating from each other ~9.1 Mya. We hypothesize that *S. marinus* originated in high salinity seas in the Ponto-Caspian region. Today's haplotypes of *S. vitreus* date to ~10.8 Mya and

S. canadensis to ~7.3 Mya, whereas *S. lucioperca* haplotypes diversified ~5.0 Mya. Contemporary haplotypes of *S. volgensis* and *S. marinus* differentiated during the Pleistocene Epoch ~2.4–2.1 Mya. Overall, the North American species have higher genetic diversity today, attributable to their larger and more consistent population sizes, and the greater number and geographic extent of their Pleistocene glacial refugia. In comparison, Eurasian *S. lucioperca* and *S. volgensis* likely re-colonized from a single small refugium near the Caspian Sea where they experienced more genetic bottlenecks and founder effects, lowering their genetic diversity.

2.6 Acknowledgements

This is publication #2013-14 from the University of Toledo's Lake Erie Center. The project was funded by grant awards to CAS from NOAA Ohio Sea Grant Program R/LR-013 and USEPA CR-83281401-0. Additional funds for travel by AEH were provided to her by NSF Deepfin #RCN-0443470 (P.I.= G. Orti), a Smithsonian Fellowship visiting summer appointment, and a Sigma Xi Grant In Aid of Research. AEH was supported by a NSF GK-12 DGE#0742395 fellowship (P.I.= CAS), University of Toledo teaching and research assistantships, a Norman S. Baldwin Fishery Science Scholarship (2010) from the International Association for Great Lakes Research (IAGLR), and the IAGLR Scholarship award (2009). We thank the U.S. Fish and Wildlife Service, U.S. Geological Survey, Ohio and Michigan Departments of Natural Resources, and Ontario Ministry of Natural Resources for contributing samples. We also thank L. Bernatchez, N. Billington, V. Boldyrev, I. Grigorovich, D. Grul'a, E. Hallerman, S. Ibrahimov, W. Jansen, V. Kovac, Y. Kvach, M. Neilson, M. Sapota, W. Schaefer, P.

Simonovic, and M. White for additional collection assistance. Great Lakes Genetics/Genomics Laboratory members S. Karsiotis, M. Neilson, V. Palsule, L. Pierce, T. Sullivan, and S. Yerga-Woolwine assisted in the laboratory and commented on various versions of the manuscript. P. Maitland, K. Linsell, and J. Tomelleri provided their fish illustrations. Lake Erie Center staff P. Uzmann, M. Gray, and R. Lohner gave logistic support. T. Fisher offered valuable biogeographic insights on geological time periods and events. We also thank J. Bruner and two anonymous reviewers for their manuscript comments.

Table 2.1

Morphological characters differentiating *Sander* spp. +=presence of a character, –=absence, and ++=fully scaled.

Species	Coloration	Banding/Barring patterns	*N* soft dorsal fin rays	*N* pyloric caeca	Cheek scaling	Canine teeth
S. canadensis	Sandy/dull brown, saddle bands in 3 oblong blotches, rows of dusky spots on dorsal fin	3–4 dorsal saddle bands	17–21	5–8	+	+
S. vitreus	Yellow body and fins, spots on dorsal fins not in rows, black blotches on last 3 dorsal spine membranes	4–14 dorsal saddle bands	19–23	3	+, –	+
S. lucioperca	Greenish gray back, rows of dark spots on dorsal and caudal fin membranes	8–12 transverse bars	19–24	4–9	+, –	+, pronounced
S. marinus	Solid black, irregular dark speckles, first dorsal dark gray/black or dark fringe with dark spot at tip, other fins gray	0–16 transverse bars	15–18	5–7	+, – gill cover = +	+
S. volgensis	Greenish gray back, rows of dark spots on dorsal and caudal fin membranes	8–12 transverse bars	20–22	3	++	+ juveniles – adults

Table 2.2

Divergence comparisons among *Sander* spp. using (a) uncorrected *p*–distances from MEGA v5.0 and (b) number of fixed differences

(a)

	mtDNA			Nuclear		
	Control region	Cyt *b*	COI	RAG1	S7	LdhA6
Sander–R. valsanicola	0.099±0.010	0.118±0.009	0.083±0.009	0.012±0.003	0.035±0.004	0.018±0.008
North American–Eurasian taxa	0.068±0.008	0.094±0.009	0.074±0.009	0.012±0.003	0.033±0.008	0.016±0.008
S. canadensis–S. vitreus	0.049±0.007	0.070±0.007	0.068±0.009	0.004±0.001	0.000	0.000
S. lucioperca & *S. marinus–S. volgensis*	0.023±0.005	0.051±0.007	0.036±0.007	0.002±0.001	0.005±0.003	0.004±0.004
S. lucioperca–S. marinus	0.033±0.006	0.060±0.007	0.033±0.007	0.001±0.001	0.004±0.003	0.008±0.006

(b)

	mtDNA			Nuclear		
	Control region	Cyt *b*	COI	RAG1	S7	LdhA6
Sander–R. valsanicola	45	70	30	12	19	3
North American–Eurasian taxa	20	54	33	14	16	4
S. canadensis–S. vitreus	34	63	40	4	0	0
S. lucioperca & *S. marinus–S. volgensis*	14	36	19	3	2	1
S. lucioperca–S. marinus	24	68	21	1	3	2

Table 2.3

Genetic diversity values of *Sander* for six gene regions: the mtDNA control region, cytochrome (cyt) *b* gene, and cytochrome oxidase I (COI) gene and the nuclear DNA recombination activation gene intron 1 (RAG1), S7 intron 1 (S7), and lactase dehydrogenase A intron 6 (LdhA6). *N*, number of individuals, H_D, haplotypic diversity, N_H, number of haplotypes

	MtDNA								
	Control region			Cyt *b*			COI		
Species	*N*	H_D	N_H	*N*	H_D	N_H	*N*	H_D	N_H
S. canadensis	25	0.69±0.02	6	8	0.96±0.03	7	13	0.59±0.03	3
S. vitreus	232	0.76±0.01	23	15	0.76±0.03	6	24	0.31±0.02	5
S. lucioperca	75	0.45±0.01	3	19	0.65±0.02	5	28	0.33±0.02	5
S. marinus	2	1.00±0.35	2	2	1.00±0.36	2	2	0.00	1
S. volgensis	33	0.00	1	8	0.25±0.06	2	7	0.48±0.06	2

	Nuclear DNA								
	RAG1			S7			LdhA6		
Species	*N*	H_D	N_H	*N*	H_D	N_H	*N*	H_D	N_H
S. canadensis	6	0.81±0.05	5	6	0.00	1	6	0.25±0.07	2
S. vitreus	11	0.42±0.05	4	13	0.80±0.03	9	13	0.34±0.04	2
S. lucioperca	6	0.00	1	8	0.36±0.06	2	8	0.00	1
S. marinus	2	0.67±0.22	2	3	0.00	1	2	0.00	1
S. volgensis	4	0.00	1	5	0.00	1	6	0.00	1

Figure 2-1. Map of sampling sites for *Sander* from (a) North America (*S. canadensis* and *S. vitreus*), (b) Eurasia (*S. lucioperca, S. marinus,* and *S. volgensis*), and (c) the two *Sander* fossil locations. In C, site QQ dates to ~16.3–13.6 Mya (Murray & Divay, 2011) and site RR ~5–4 Mya (Murray *et al.*, 2009). Species distributions are modified from Billington *et al.* (2011) for the North American taxa, the IUCN (International Union for Conservation of Nature and Natural Resources) Red List (http://www.iucnredlist.org) for *S. lucioperca* and *S. volgensis*, and Berg (1965) for *S. marinus*.

Figure 2-2. Photograph of one of the two rare and enigmatic sea pikeperch *Sander marinus* specimens from the Caspian Sea that we analyzed (~350 mm TL, 5 pyloric caeca). The specimens are preserved in EtOH and archived at the University of Toledo's Great Lakes Genetics/Genomics Laboratory.

Figure 2-3. (a) Phylogenetic relationships of the genus *Sander* and members of the family Percidae based on the concatenated data set of six gene regions and a Bayesian analysis. Values on the nodes are posterior probabilities. Tree was rooted to *Micropterus* based on its close relationship to Percidae, according to Song *et al.* (1998) and Sloss *et al.* (2004). (b) Time-calibrated phylogeny for *Sander* and its sister taxon *Romanichthys* from BEAST analyses using two fossil calibration points, 26.0 Mya for the genus *Perca* and 12.0 Mya for the genus *Micropterus*. Values above the branches are Bayesian posterior probabilities and those below in italics are divergence estimates. Illustrations are used with permissions from P. Maitland (Eurasian taxa) and J. Tomelleri (North American taxa). Dates for the availability of the North Atlantic Land Bridge (NALB) are from

Tiffney (1985) and Denk *et al.* (2011), and for the Bering Land Bridge (BLB) from Gladenkov *et al.* (2002). PI=Pliocene, PS=Pleistocene.

Figure 2-4. Numbers of transitions and transversions versus uncorrected *p*-distances for the six gene regions A, control region, B, cyt *b*, C, COI, D, RAG1, E, S7, and F, LdhA6.

Figure 2-1(a)

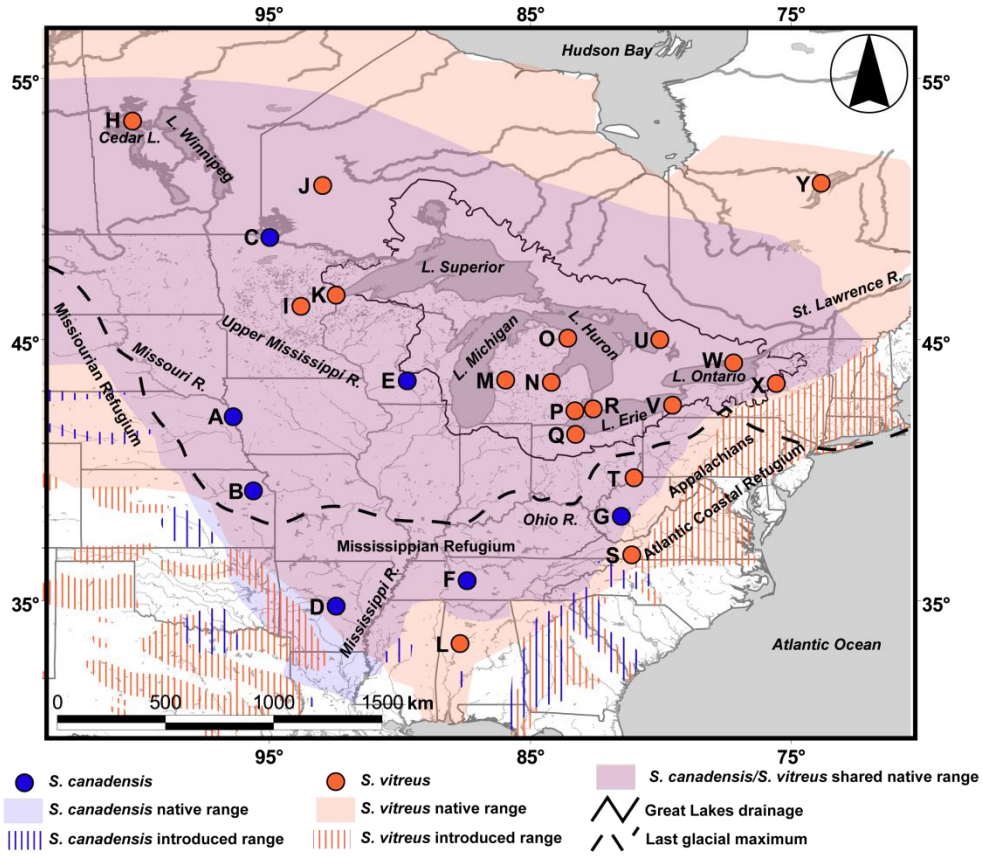

95°
85°
75°
55°
45°
35°
Hudson Bay
H
Cedar L.
L. Winnipeg
J
C
Y
L. Superior
I
K
St. Lawrence R.
Missourian Refugium
Missouri R.
Upper Mississippi R.
L. Michigan
L. Huron
O
U
W
L. Ontario
X
E
M
N
P
R
V
L. Erie
Q
A
Appalachians
Atlantic Coastal Refugium
T
B
Ohio R.
G
Mississippian Refugium
S
F
D
Mississippi R.
L
Atlantic Ocean
0
500
1000
1500 km
S. canadensis
S. vitreus
S. canadensis/S. vitreus shared native range
S. canadensis native range
S. vitreus native range
Great Lakes drainage
S. canadensis introduced range
S. vitreus introduced range
Last glacial maximum

Figure 2-1(b)

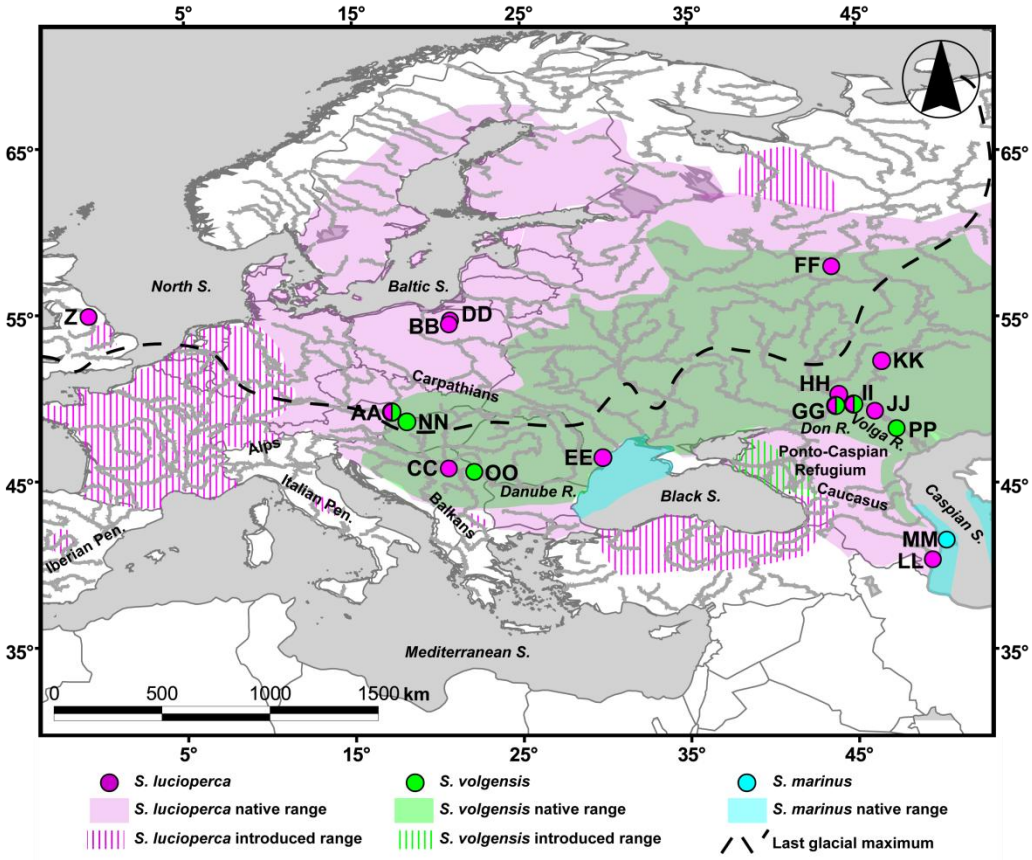

5°
15°
25°
35°
45°
65°
55°
45°
35°
North S.
Baltic S.
Carpathians
Alps
Italian Pen.
Iberian Pen.
Balkans
Danube R.
Black S.
Mediterranean S.
Don R.
Volga R.
Ponto-Caspian Refugium
Caucasus
Caspian S.
Z
BB
DD
FF
KK
HH
II
JJ
GG
PP
AA
NN
CC
OO
EE
MM
LL
0
500
1000
1500 km
S. lucioperca
S. volgensis
S. marinus
S. lucioperca native range
S. volgensis native range
S. marinus native range
S. lucioperca introduced range
S. volgensis introduced range
Last glacial maximum

Figure 2-1(c)

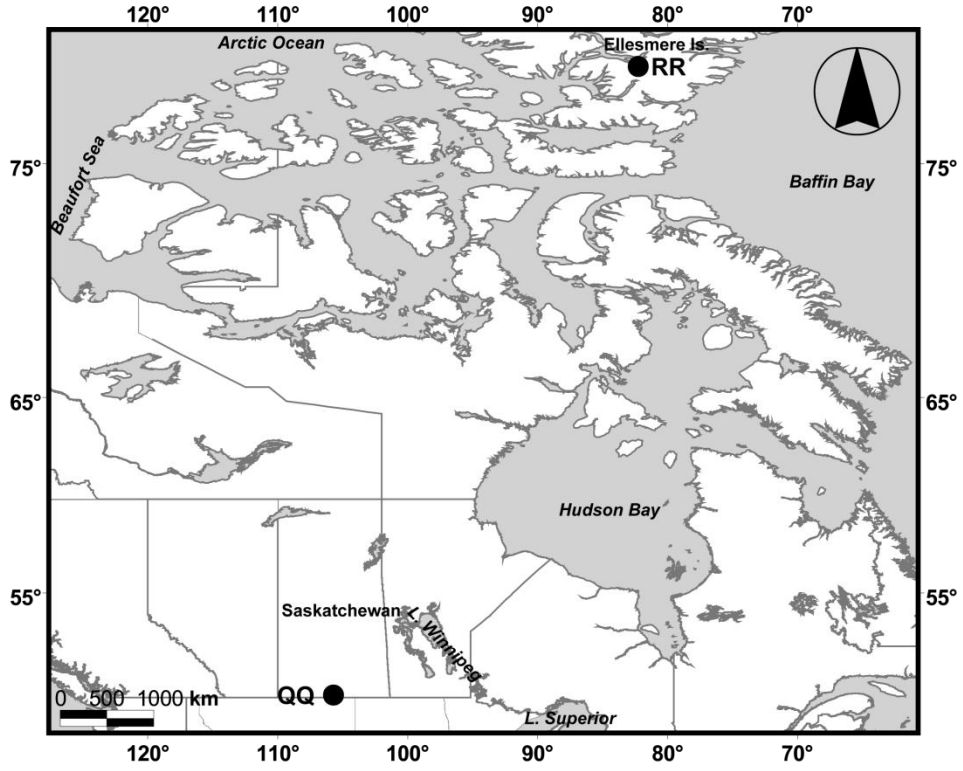

120°
110°
100°
90°
80°
70°
Arctic Ocean
Ellesmere Is.
RR
Beaufort Sea
Baffin Bay
75°
65°
55°
Hudson Bay
Saskatchewan
L. Winnipeg
0 500 1000 km
QQ
L. Superior

Figure 2-2

Figure 2-3

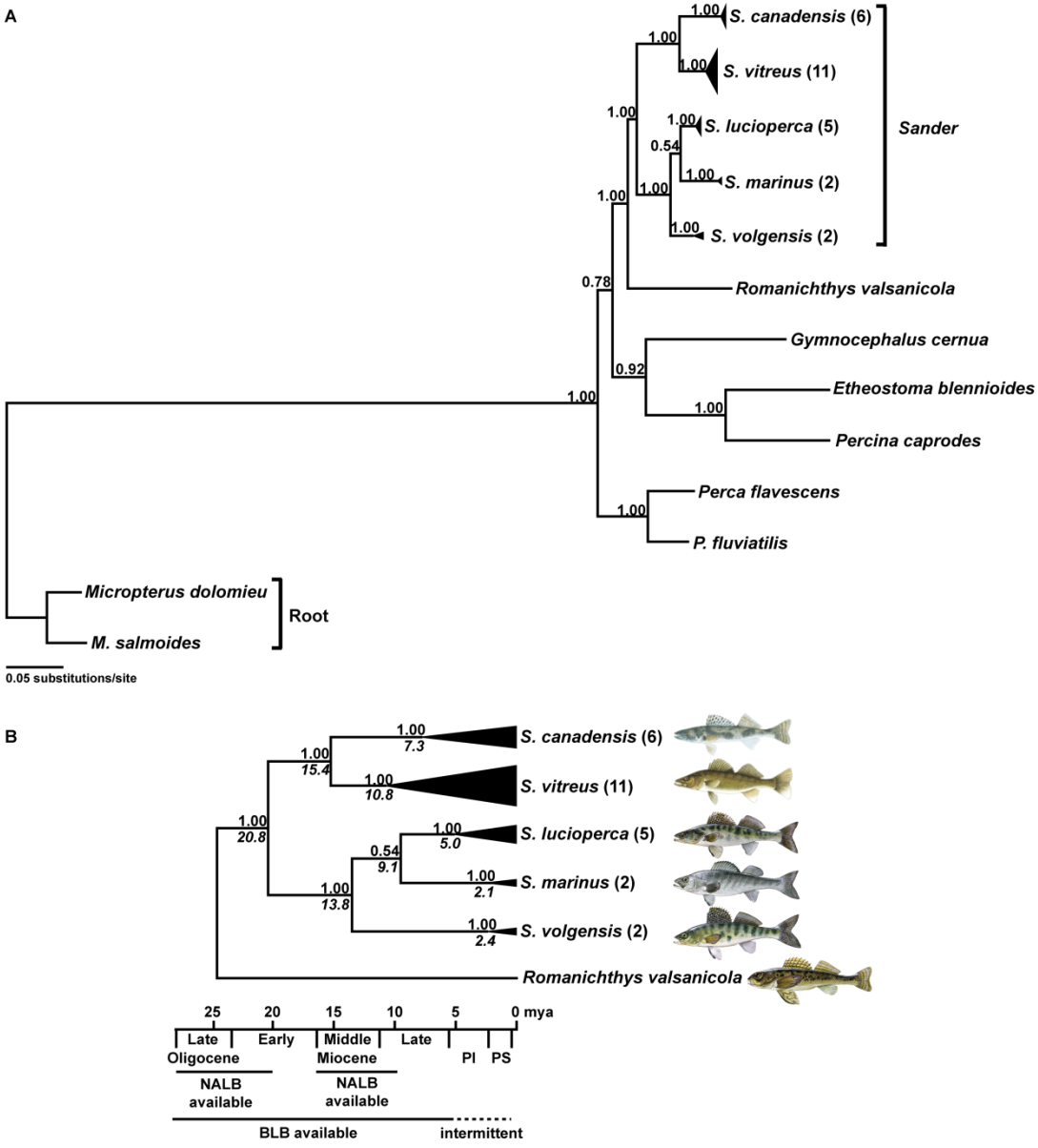
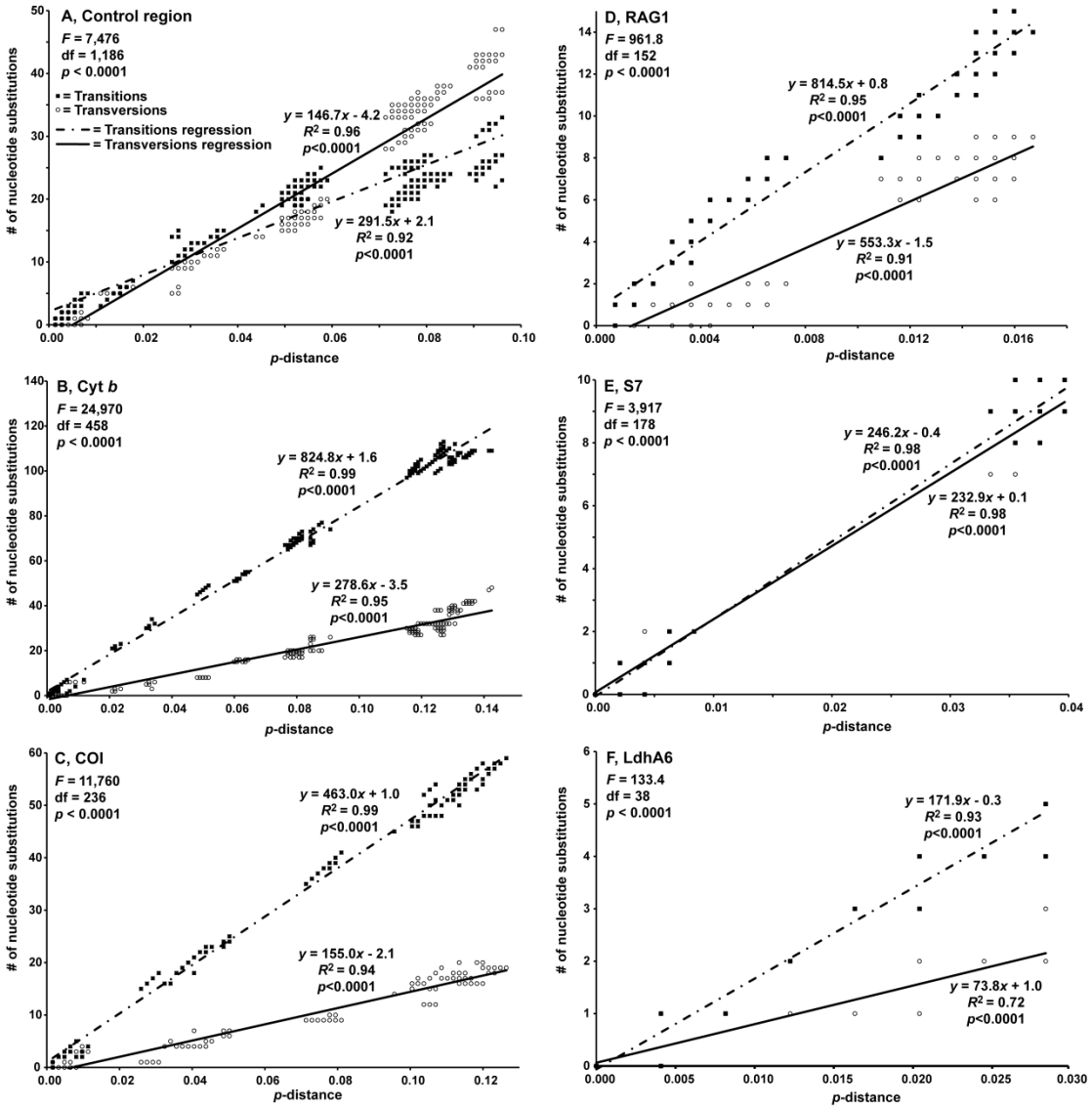

A
1.00
S. canadensis (6)
1.00
1.00
S. vitreus (11)
1.00
1.00
S. lucioperca (5)
Sander
0.54
1.00
S. marinus (2)
1.00
1.00
1.00
S. volgensis (2)
0.78
Romanichthys valsanicola
Gymnocephalus cernua
0.92
1.00
Etheostoma blennioides
1.00
Percina caprodes
Perca flavescens
1.00
P. fluviatilis
Micropterus dolomieu
Root
M. salmoides
0.05 substitutions/site
B
1.00
7.3
S. canadensis (6)
1.00
15.4
1.00
10.8
S. vitreus (11)
1.00
20.8
1.00
5.0
S. lucioperca (5)
0.54
9.1
1.00
2.1
S. marinus (2)
1.00
13.8
1.00
2.4
S. volgensis (2)
Romanichthys valsanicola
25
20
15
10
5
0 mya
Late
Early
Middle
Late
Oligocene
Miocene
Pl
PS
NALB available
NALB available
BLB available
intermittent

Figure 2-4


A, Control region
F = 7,476
df = 1,186
p < 0.0001
▪ = Transitions
○ = Transversions
= Transitions regression
= Transversions regression
y = 146.7x - 4.2
R² = 0.96
p<0.0001
y = 291.5x + 2.1
R² = 0.92
p<0.0001
# of nucleotide substitutions
p-distance
D, RAG1
F = 961.8
df = 152
p < 0.0001
y = 814.5x + 0.8
R² = 0.95
p<0.0001
y = 553.3x - 1.5
R² = 0.91
p<0.0001
B, Cyt b
F = 24,970
df = 458
p < 0.0001
y = 824.8x + 1.6
R² = 0.99
p<0.0001
y = 278.6x - 3.5
R² = 0.95
p<0.0001
E, S7
F = 3,917
df = 178
p < 0.0001
y = 246.2x - 0.4
R² = 0.98
p<0.0001
y = 232.9x + 0.1
R² = 0.98
p<0.0001
C, COI
F = 11,760
df = 236
p < 0.0001
y = 463.0x + 1.0
R² = 0.99
p<0.0001
y = 155.0x - 2.1
R² = 0.94
p<0.0001
F, LdhA6
F = 133.4
df = 38
p < 0.0001
y = 171.9x - 0.3
R² = 0.93
p<0.0001
y = 73.8x + 1.0
R² = 0.72
p<0.0001

Appendix 2.1

Locations sampled for the five species of *Sander,* including latitude (lat) and longitude (long), number of individuals (*N*), haplotypic diversity (H_D), and number of haplotypes (N_H) for the mtDNA control region data set. Letters correspond to the locations on Fig. 2 maps. * = coordinates approximated.

Species	Location	Lat.	Long.	*N*	H_D	N_H
S. canadensis	North America	—	—	25	0.69±0.02	6
	A. Missouri R. NB USA*	42.0077	–96.2416	4	0.83±0.11	3
	B. Perry L. KS USA*	39.1674	–95.4516	4	0.50±0.13	2
	C. L. of the Woods MN USA*	48.8824	–94.8320	4	0.50±0.13	2
	D. Arkansas R. AR USA*	34.7577	–92.2902	2	0.00	1
	E. L. Wisconsin WI USA*	43.3836	–89.5749	4	1.00±0.09	4
	F. Duck R. TN USA*	35.7271	–87.2679	3	0.00	1
	G. Kanawha R. WV USA*	38.1914	–81.3673	4	0.00	1
S. vitreus	North America	—	—	232	0.76±0.01	23
	H. Cedar L. MB CAN	53.3300	–100.1000	10	0.80±0.03	5
	I. Mille Lacs MN USA	46.2326	–93.6477	10	0.20±0.05	2
	J. McKim L. ON CAN	50.8669	–92.8031	10	0.36±0.05	2
	K. St. Louis R. MN USA	46.6679	–92.2889	10	0.53±0.03	2
	L. North R. AL USA	33.3264	–87.5333	5	0.40±0.11	2
	M. Muskegon R. MI USA	43.4158	–85.8087	10	0.38±0.06	3
	N. Flint R. MI USA	43.3300	–84.0543	10	0.47±0.04	3
	O. Thunder Bay MI USA	45.0200	–83.4300	10	0.51±0.05	3
	P. Detroit R.	—	—	30	0.75±0.01	6
	Grosse Ile MI USA	42.1177	–83.1781	10	0.62±0.04	3
	Fighting Is. MI USA	42.2378	–83.1295	10	0.80±0.03	5
	Belle Isle MI USA	42.3469	–82.9535	10	0.82±0.01	5
	Q. Western L. Erie	—	—	41	0.78±0.01	8
	Maumee R. OH USA	41.5594	–83.6492	10	0.71±0.04	4
	Huron R. MI USA	42.0899	–83.2902	11	0.71±0.04	5
	Sandusky R. OH USA	41.3421	–83.1091	10	0.87±0.03	6
	Hen Is. ON CAN	41.8024	–82.7804	10	0.80±0.03	4
	R. Thames R. ON CAN	42.3171	–82.4363	10	0.69±0.03	3

	S. New R. VA USA	36.7109	–80.9589	10	0.51±0.05	3
	T. Ohio R. OH USA	39.6675	–80.8641	5	1.00±0.06	5
	U. Moon/Musquash R. ON CAN	44.9594	–79.8811	10	0.20±0.05	2
	V. Eastern L. Erie	—	—	20	0.90±0.01	11
	Van Buren Bay NY USA	42.4600	–79.4100	10	0.83±0.03	5
	Cattaraugus Ck. NY USA	42.5684	–79.1041	10	0.98±0.02	9
	W. Bay of Quinte ON CAN	44.0671	–77.0719	10	0.64±0.05	4
	X. Oneida L. NY USA	43.2800	–75.4400	11	0.76±0.03	5
	Y. Lac Misstassini QC CAN	50.9500	–73.7000	10	0.36±0.05	2
S. lucioperca	Eurasia	—	—	75	0.45±0.01	3
	Z. Ouse R. GBR*	53.9577	–1.0822	1	1.00±0.00	1
	AA. Danube R. Bratislava SVK	48.1444	17.0703	10	0.36±0.05	2
	BB. L. Maróz Maróz POL	53.5052	20.3951	9	0.00	1
	CC. Danube R. Zemun SRB	44.8391	20.4005	10	0.00	1
	DD. L. Kortowskie Olsztyn POL	53.7571	20.4514	9	0.22±0.06	2
	EE. Sasyk Reservoir UKR*	45.6436	29.6575	1	1.00±0.00	1
	FF. Gorky Reservoir Puchezh RUS*	57.0000	43.1666	1	1.00±0.00	1
	GG. Don R. Kalach–na–donu RUS	48.6808	43.5054	10	0.20±0.05	2
	HH. Karpovska Reservoir Il’evka RUS	49.1432	43.6170	2	0.00	1
	II. Volga R. Volgograd RUS	48.7077	44.5405	10	0.20±0.05	2
	JJ. Volga R. Stupino RUS	48.3103	45.7973	1	1.00±0.00	1
	KK. Volga R. Saratov RUS	51.5278	46.0939	1	1.00±0.00	1
	LL. Kura R.Neftchala AZE*	39.3707	49.2536	10	0.00	1
S. marinus	MM. Caspian S. Nardaran AZE*	40.5550	50.0542	2	1.00±0.35	2
S. volgensis	Eurasia	—	—	33	0.00	1
	AA. Danube R. Bratislava SVK	48.1444	17.0703	10	0.00	1
	GG. Don R. Kalach–na–donu RUS	48.6808	43.5054	1	1.00±0.00	1
	II. Volga R. Volgograd RUS	48.7077	44.5405	12	0.00	1
	NN. Danube R.	47.7508	18.1200	1	1.00±0.00	1

	Radvan nad Dunajom SVK*					
	OO. Danube R. Dobra SRB	44.6461	21.9048	7	0.00	1
	PP. Volga R. Enotaevka RUS	47.2542	47.0845	2	0.00	1

Appendix 2.2

Comparison of *Sander* sequences from GenBank to those found in this study. NR=not recovered by us.

Gene/Region	Species	Ours	GenBank Accession #
mtDNA control region	*S. canadensis*	Scacr5	U90618 (Faber & Stepien, 1998)
	S. vitreus	SvicrA1	U90617 (Faber & Stepien, 1998), JX442946 (Haponski & Stepien, 2013)
		SvicrA3	AF162272 (Gatt *et al.*, 2000), JX442948 (Faber & Stepien, 1998; Haponski & Stepien, 2013)
	S. lucioperca	Sluccr1	U90624 (Faber & Stepien, 1998)
	S. volgensis	Svocr1	AF007824 (Faber & Stepien, 1998)
mtDNA cyt *b*	*S. canadensis*	NR	AF386603 (Near, 2002)
		NR	AY374290 (Sloss *et al.*, 2004)
	S. vitreus	NR	AF045359 (Song *et al.*, 1998)
		NR	AF386602 (Near, 2002)
	S. lucioperca	Slucb2	GU936790 (Kahilainen *et al.*, unpublished) GQ214533 (Gharibkhani *et al.*, unpublished) FJ788390, 394–99 (Kalous *et al.*, unpublished) HM049965 (Matschiner *et al.*, 2011)
		NR	AF546122 (Moyer *et al.*, unpublished) AY374291 (Sloss *et al.*, 2004)
	S. volgensis	NR	AY374292 (Sloss *et al.*, 2004)
mtDNA COI	*S. canadensis*	Scacoi2	EU524368–73 (Hubert *et al.*, 2008)
	S. vitreus	Svicoi1	EU524374–80 (Hubert *et al.*, 2008) JN028402–5 (April *et al.*, 2011)
	S. lucioperca	Slucoi1	HQ960460, 536, 621–2, 646–7, 672–4, 758, 867, 887, 991–2 (International Barcode of Life Project, unpublished) HQ557302, JN028400–1 (April *et al.*, 2011)
		NR	JN028399 (April *et al.*, 2011)
		NR	JQ623977 (Keskin, unpublished)
Nuclear RAG 1	*S. vitreus*	Svirag1	FJ381300 (Bossu & Near, 2009)
Nuclear S7 intron 1	*S. vitreus*	SviS71	EU094723 (Keck & Near, 2008)
	S. marinus	SmaS71	EF035490 (Lang & Mayden, 2007)

Appendix 2.3

Outgroup taxa used in this study and their GenBank accession numbers. All outgroup taxa were sequenced by us unless otherwise noted.

	mtDNA		
Species	Control region	Cyt *b*	COI
Zingel zingel	KC819861	KC819832	KC819882
Romanichthys valsanicola	KC819862	KC819831	KC819883
Gymnocephalus cernua	KC819863	KC819833	KC819885
Gymnocephalus baloni	AF025360 (Faber & Stepien, 1997)	AY374279 (Sloss *et al.*, 2004)	HQ960459 (IBL, unpub.)
Perca flavescens	KC819864	KC819830	KC819884
Perca fluviatilis	KC819865	KC819836	KC819887
Etheostoma blennioides	EF587849 (Haponski *et al.*, 2007; Haponski & Stepien, 2008)	EF587846 (Haponski *et al.*, 2007; Haponski & Stepien, 2008)	KC819889
Percina caprodes	EF587842 (Haponski *et al.*, 2007)	EF587838 (Haponski *et al.*, 2007)	KC819890
Micropterus dolomieu	KC819866	KC819834	KC819888
Micropterus salmoides	JN979719 (Ray *et al.*, 2012)	KC819835	KC819886

	Nuclear DNA		
Species	RAG1	S7	LdhA6
Zingel zingel	--	--	--
Romanichthys valsanicola	KC819907	KC819926	KC819947
Gymnocephalus cernua	KC819905	KC819931	KC819946
Gymnocephalus baloni	--	--	AY034783 (Stepien *et al.*, 2005)
Perca flavescens	KC819906	KC819929	KC819945
Perca fluviatilis	KC819904	KC819930	KC819948
Etheostoma blennioides	KC819908	KC819927	KC819944
Percina caprodes	KC819909	KC819928	KC819943
Micropterus dolomieu	KC819910	KC819932	KC819941
Micropterus salmoides	KC819911	KC819933	KC819942

Appendix 2.4

Haplotypes recovered by us from the six gene regions for *Sander*, with GenBank accession numbers, sampling locations, and number of individuals in parentheses. Letters correspond to locations on Fig. 2.

Gene/Region	Species	Haplotype	GenBank Accession #	Location (*N*)
mtDNA control region	*S. canadensis*	Scacr1	KC819837	A. Missouri R. (1), B. Perry L. (3), C. L. of the Woods (3), D. Arkansas R. (2), E. L. Wisconsin (1), F. Duck R. (3)
		Scacr2	KC819838	C. L. of the Woods (1), G. Kanawha R. (4)
		Scacr3	KC819839	A. Missouri R. (2), E. L. Wisconsin (1)
		Scacr4	KC819840	A. Missouri R. (1), E. L. Wisconsin (1)
		Scacr5	KC819841	E. L. Wisconsin (1)
		Scacr6	KC819842	B. Perry L. (1)
	S. vitreus	SvicrA1	U90617 JX442946	H. Cedar L. (4), I. Mille Lacs (9), J. McKim L. (8), K. St. Louis R. (4), M. Muskegon R. (8), N. Flint R. (6), O. Thunder Bay (7), P. Detroit R. (11), Q. Western L. Erie (14), R. Thames R. (5), T. Ohio R. (1), U. Moon/Musquash R. (9), V. Eastern L. Erie (5), W. Bay of Quinte (2), Y. Lac Misstassini (8)
		SvicrA2	JX442947	O. Thunder Bay (1), P. Detroit R. (4), Q. Western L. Erie (10), R. Thames R. (3), T. Ohio R. (1), V. Eastern L. Erie (5), W. Bay of Quinte (1)
		SvicrA3	JX442948	M. Muskegon R. (1), N. Flint R. (3), O. Thunder Bay (2), P. Detroit R. (10), Q. Western L. Erie (8), R. Thames R. (2), S. New R. (2), T. Ohio R. (1), U. Moon/Musquash R. (1), V. Eastern L. Erie (2), W. Bay of Quinte (6), X. Oneida L. (3)
		SvicrA4	JX442949	H. Cedar L. (1), I. Mille Lacs (1), L. North R. (1), M. Muskegon R. (1), P. Detroit R. (2), Q. Western L. Erie (4), S. New R. (1), T. Ohio R. (1), V. Eastern L. Erie (1), X. Oneida L. (5)

		SvicrA5	JX442950	Q. Western L. Erie (1), V. Eastern L. Erie (1)
		SvicrA6	JX442951	Q. Western L. Erie (1)
		SvicrA7	JX442952	Q. Western L. Erie (1)
		SvicrB8	JX442953	P. Detroit R. (1), V. Eastern L. Erie (1), W. Bay of Quinte (1)
		SvicrB9	JX442954	P. Detroit R. (2)
		SvicrB10	JX442955	Q. Western L. Erie (1)
		SvicrB11	JX442956	H. Cedar L. (3), J. McKim L. (2), K. St. Louis R. (6), Y. Lac Misstassini (2)
		SvicrB12	KC819843	V. Eastern L. Erie (1)
		SvicrB13	KC819844	V. Eastern L. Erie (1)
		SvicrB14	KC819845	V. Eastern L. Erie (1)
		SvicrB15	KC819846	V. Eastern L. Erie (1)
		SvicrB16	KC819847	H. Cedar L. (1)
		SvicrB17	KC819848	V. Eastern L. Erie (1)
		SvicrB18	KC819849	H. Cedar L. (1)
		SvicrB19	KC819850	S. New R. (7), T. Ohio R. (1)
		SvicrB20	KC819851	L. North R. (4)
		SvicrB21	KC819852	X. Oneida L. (1)
		SvicrB22	KC819853	X. Oneida L. (1)
		SvicrB23	KC819854	X. Oneida L. (1)
	S. lucioperca	Slucr1	KC819855	Z. Ouse R. (1), AA. Danube R., BB. L. Maróz (9), DD. L. Kortowskie (8), GG. Don R. (9), HH. Karpovska R. (2), II. Volga R. (9), JJ. Volga R. (1), KK. Volga R (1), LL. Kura R. (10)
		Slucr2	KC819856	AA. Danube R., CC. Danube R. (10), DD. L. Kortowskie (1), EE. Sasyk Reservoir (1), GG. Don R. (1)
		Slucr3	KC819857	FF. Gorky Reservoir (1), II. Volga R. (1)
	S. marinus	Smacr1	KC819858	MM. Caspian S. (1)
		Smacr2	KC819859	MM. Caspian S. (1)
	S. volgensis	Svocr1	KC819860	GG. Don R. (1), II. Volga R. (12), NN. Danube R. (1), OO. Danube R. (7), PP. Volga R. (2)
mtDNA cyt *b*	*S. canadensis*	Scacb1	KC819814	D. Arkansas R. (1), E. L. Wisconsin (1)
		Scacb2	KC819815	E. L. Wisconsin (1)

		Scacb3	KC819816	C. L. of the Woods (1)
		Scacb4	KC819817	B. Perry L. (1)
		Scacb5	KC819818	E. L. Wisconsin (1)
	S. vitreus	Svicb1	KC819819	H. Cedar L. (2), N. Flint R. (2), O. Thunder Bay (1), V. Eastern L. Erie (1), X. Oneida L. (1)
		Svicb2	KC819820	P. Detroit R. (1), V. Eastern L. Erie (1), X. Oneida L. (1)
		Svicb3	KC819821	S. New R. (1)
		Svicb4	KC819822	L. North R. (1)
	S. lucioperca	Slucb1	KC819823	FF. Gorky Reservoir (1), II. Volga R. (1)
		Slucb2	KC819824	HH. Karpovska Reservoir (1), LL. Kura R. (1)
		Slucb3	KC819825	DD. L. Kortowskie (1), GG. Don R. (1)
		Slucb4	KC819826	CC. Danube R. (1)
	S. marinus	Smacb1	KC819827	MM. Caspian S. (1)
		Smacb2	KC819828	MM. Caspian S. (1)
	S. volgensis	Svocb1	KC819829	GG. Don R. (1), II. Volga R. (2), NN. Danube R. (1), OO. Danube R. (1), PP. Volga R. (1)
mtDNA COI	*S. canadensis*	Scacoi1	KC819867	D. Arkansas R. (1), E. L. Wisconsin (2)
		Scacoi2	KC819868	C. L. of the Woods (1)
		Scacoi3	KC819869	B. Perry L. (1), E. L. Wisconsin (1)
	S. vitreus	Svicoi1	KC819870	H. Cedar L. (2), N. Flint R. (2), O. Thunder Bay (1), P. Detroit R. (1), V. Eastern L. Erie (1)
		Svicoi2	KC819871	S. New R. (1)
		Svicoi3	KC819872	L. North R. (1)
		Svicoi4	KC819873	V. Eastern L. Erie (1)
	S. lucioperca	Slucoi1	KC819874	FF. Gorky Reservoir (1), HH. Karpovska Reservoir (1), II. Volga R. (1), LL. Kura R. (1)
		Slucoi2	KC819875	DD. L. Kortowskie (1), GG. Don R. (1)
		Slucoi3	KC819876	CC. Danube R. (1)
	S. marinus	Smacoi1	KC819877	MM. Caspian S. (2)
	S. volgensis	Svocoi1	KC819878	GG. Don R. (1), NN. Danube R. (1), OO. Danube R. (1), PP. Volga R. (1)
		Svocoi2	KC819879	II. Volga R. (2)
Nuclear RAG1	*S. canadensis*	Scarag1	KC819891	B. Perry L. (1), D. Arkansas R. (1), E. L. Wisconsin (2)
		Scarag2	KC819892	E. L. Wisconsin (1)

		Scarag3	KC819893	B. Perry L. (1)
		Scarag4	KC819894	E. L. Wisconsin (1)
		Scarag5	KC819895	E. L. Wisconsin (1)
	S. vitreus	Svirag1	KC819896	H. Cedar L. (2), N. Flint R. (2), O. Thunder Bay (1), P. Detroit R. (1), V. Eastern L. Erie (2), X. Oneida L. (1)
		Svirag2	KC819897	N. Flint R. (1)
		Svirag3	KC819898	S. New R. (1)
		Svirag4	KC819899	L. North R. (1)
	S. lucioperca	Slurag1	KC819900	CC. Danube R. (1), DD. L. Kortowskie (1), EE. Don R. (1), HH. Karpovska Reservoir (1), II. Volga R. (1), LL. Kura R. (1)
	S. marinus	Smarag1	KC819901	MM. Caspian S. (2)
		Smarag2	KC819902	MM. Caspian S. (1)
	S. volgensis	Svorag1	KC819903	GG. Don R. (1), II. Volga R. (2), OO. Danube (1), PP. Volga R. (1)
Nuclear S7 intron 1	*S. canadensis*	SviS71	KC819913	B. Perry L. (1), C. L. of the Woods (1), D. Arkansas R. (1), E. L. Wisconsin (3)
	S. vitreus	SviS71	KC819913	H. Cedar L. (2), L. North R. (1), V. Eastern L. Erie (1), X. Oneida L. (1)
		SviS72	KC819914	S. New R. (1)
		SviS73	KC819915	V. Eastern L. Erie (1)
		SviS74	KC819916	H. Cedar L. (1)
		SviS75	KC819917	P. Detroit R. (1)
	S. lucioperca	SluS71	KC819922	DD. L. Kortowskie (1), FF. Gorky Reservoir (1), GG. Don R. (1), HH. Karpovska Reservoir (1), II. Volga R. (1), LL. Kura R. (1)
		SluS72	KC819923	CC. Danube R. (1), HH. Karpovska Reservoir (1)
	S. marinus	SmaS71	KC819924	MM. Caspian S. (2)
	S. volgensis	SvoS71	KC819925	GG. Don R. (1), II. Volga R. (2), OO. Danube R. (1), PP. Volga R. (1)
Nuclear LdhA6	*S. canadensis*	Scaldh1	KC819934	B. Perry L. (1), C. L. of the Woods (1), D. Arkansas R. (1), E. L. Wisconsin (3)
		Scaldh2	KC819935	D. Arkansas R. (1)
	S. vitreus	Svildh1	KC819936	H. Cedar L. (2), L. North R. (1), N. Flint R. (2), O.

			Thunder Bay (1), P. Detroit R. (1), S. New R. (1), V. Eastern L. Erie (2), X. Oneida L. (2)
	Svildh2	KC819937	L. North R. (1), S. New R. (1)
S. lucioperca	Sluldh1	KC819938	CC. Danube R. (1), DD. L. Kortowskie (1), FF. Gorky Reservoir (1), GG. Don R. (1), HH. Karpovska Reservoir (1), II. Volga R. (1), LL. Kura R. (1)
S. marinus	Smaldh1	KC819939	MM. Caspian S. (2)
S. volgensis	Svoldh1	KC819940	GG. Don R. (1), II. Volga R. (2), OO. Danube R. (1), PP. Volga R. (1)

Appendix 2.5

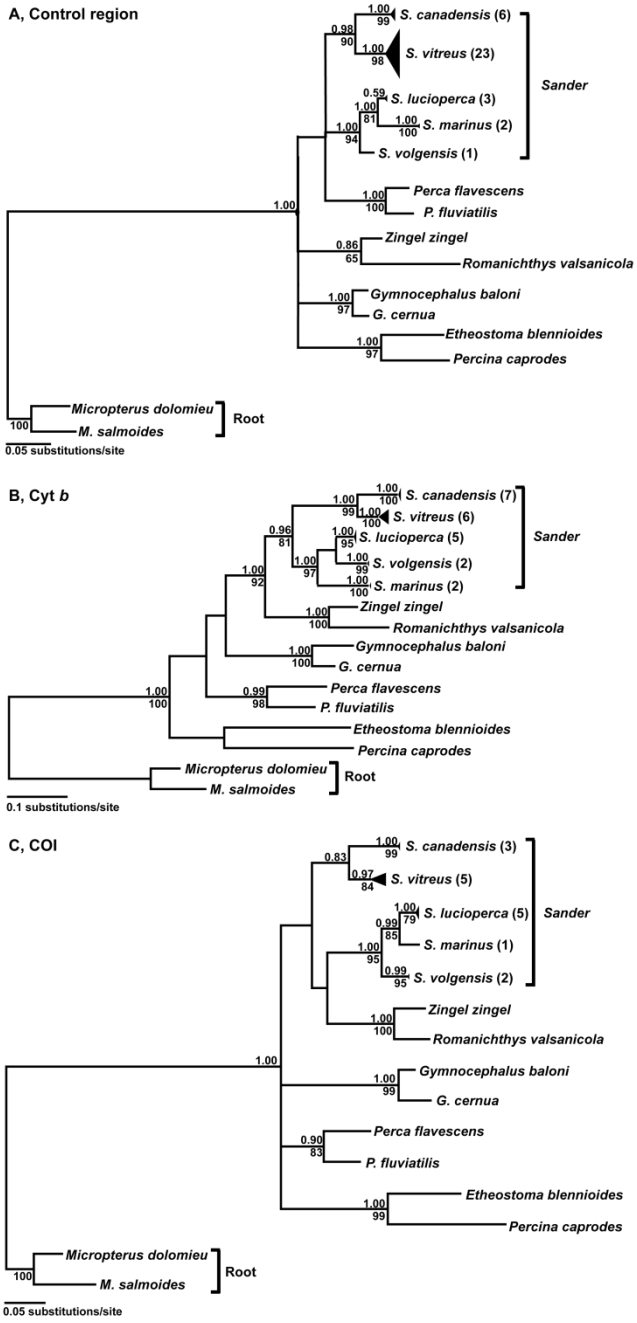
Mean divergences (and highest posterior densities) in millions of years ago of *Sander* based on the BEAST analysis and fossil record calibration. —— = nodes that were not recovered for that gene region or taxa with only a single sequence.

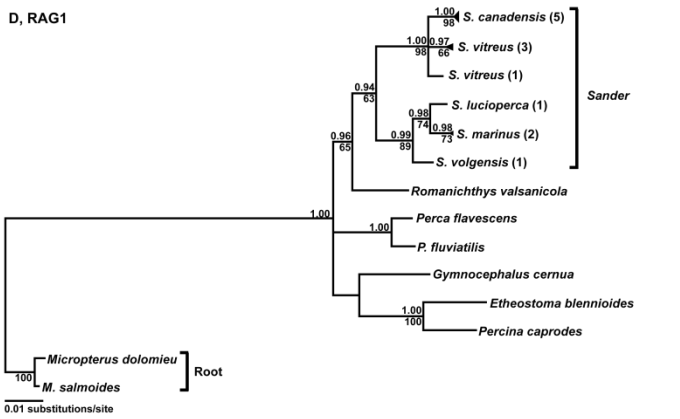
		mtDNA		
Clade	Overall	Control region	Cyt *b*	COI
Genus *Sander*	20.8 (9.5–34.3)	——	16.1 (5.9–27.9)	——
Sander + nearest outgroup	24.6 (11.5–39.6)	35.0 (25.5–47.5)	20.1 (7.6–34.9)	21.6 (11.5–33.1)
North American taxa	15.4 (5.2–27.1)	21.9 (10.5–34.3)	8.8 (2.6–16.6)	10.9 (4.3–18.4)
Eurasian taxa	13.8 (4.4–25.9)	17.1 (5.6–30.0)	8.5 (2.3–16.6)	7.3 (2.9–12.5)
S. canadensis	7.3 (0.2–17.1)	6.8 (0.9–16.0)	1.7 (0.2–5.0)	0.6 (0.04–1.3)
S. vitreus	10.8 (2.5–20.7)	14.0 (5.5–24.3)	3.9 (0.8–8.7)	4.2 (1.3–7.8)
S. lucioperca	5.0 (0.2–11.4)	3.4 (0.1–8.7)	1.3 (0.1–3.9)	1.3 (0.3–2.4)
S. marinus	2.1 (0.001–7.9)	1.7 (0.01–5.3)	0.4 (0.001–1.3)	——
S. volgensis	2.4 (0.0004–9.0)	——	0.8 (0.02–2.7)	0.4 (0.004–1.1)

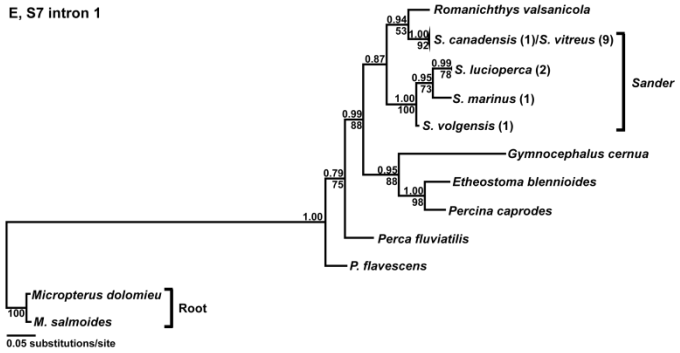
	Nuclear DNA		
Clade	RAG1	S7	LdhA6
Genus *Sander*	37.7 (4.4–100.0)	——	——
Sander + nearest outgroup	44.9 (6.4–111.3)	24.4 (1.8–64.7)	34.8 (24.3–52.3)
North American taxa	24.8 (2.2–66.2)	11.3 (0.5–31.3)	15.0 (1.0–31.5)
Eurasian taxa	18.4 (0.6–50.8)	11.0 (0.3–31.5)	14.5 (0.6–31.5)
S. canadensis	13.1 (0.6–35.8)	——	——
S. vitreus	10.1(0.1–29.7)	——	——
S. lucioperca	——	2.5 (0.0003–8.7)	——
S. marinus	3.8 (0.001–13.1)	——	——
S. volgensis	——	——	——

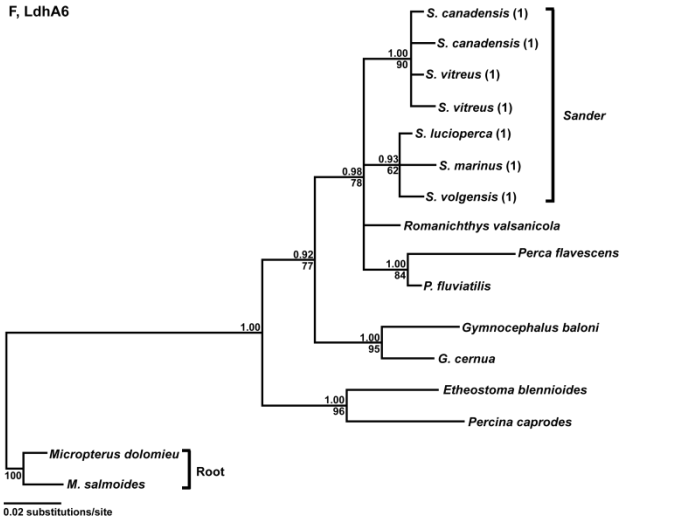
Appendix 2-1

Bayesian phylogenetic trees for the six DNA regions A, control region, B, cyt *b*, and C, COI, D, RAG1, E, S7, and F, LdhA6. Support values on nodes of the trees are posterior probabilities above the branch and % bootstrap pseudo-replicates below the branch.




D, RAG1
S. canadensis (5)
S. vitreus (3)
S. vitreus (1)
S. lucioperca (1)
S. marinus (2)
S. volgensis (1)
Sander
Romanichthys valsanicola
Perca flavescens
P. fluviatilis
Gymnocephalus cernua
Etheostoma blennioides
Percina caprodes
Micropterus dolomieu
M. salmoides
Root
0.01 substitutions/site


E, S7 intron 1
Romanichthys valsanicola
S. canadensis (1)/S. vitreus (9)
S. lucioperca (2)
S. marinus (1)
S. volgensis (1)
Sander
Gymnocephalus cernua
Etheostoma blennioides
Percina caprodes
Perca fluviatilis
P. flavescens
Micropterus dolomieu
M. salmoides
Root
0.05 substitutions/site


F, LdhA6
S. canadensis (1)
S. canadensis (1)
S. vitreus (1)
S. vitreus (1)
S. lucioperca (1)
S. marinus (1)
S. volgensis (1)
Sander
Romanichthys valsanicola
Perca flavescens
P. fluviatilis
Gymnocephalus baloni
G. cernua
Etheostoma blennioides
Percina caprodes
Micropterus dolomieu
M. salmoides
Root
0.02 substitutions/site

Chapter 3

A population genetic window into the past and future of the walleye *Sander vitreus*: Relation to historic walleye and the extinct blue pike

Currently in review for publication as Haponski, A.E. & Stepien, C.A. (2013) A population genetic window into the past and future of the walleye *Sander vitreus*: Relation to historic walleye and the extinct blue pike. *Diversity and Distributions*.

3.1 Abstract

Aim The walleye *Sander vitreus* is an ecologically and economically valuable fish. We employ a genetic "window" approach to evaluate patterns of population genetic variability, isolation, and continuity over space and time over its range, in relation to historic samples and the extinct blue pike *S. v. "glaucus*".

Location North America: Laurentian Great Lakes, lakes, Mississippi, Atlantic and Gulf coastal river systems, from today and the early 1900s

Methods Mitochondrial DNA control region sequences and allelic variants at nine nuclear DNA microsatellite loci test levels of population genetic diversity and divergence patterns across the contemporary range of walleye. These are compared to historic samples from Lake Erie, as well as the once endemic and now extinct blue pike putative variant. Morphological variation also is examined.

Results Marked population genetic differences occur across the range of walleye, distinguishing all waterbodies and most spawning groups within them. Those from Lake Erie have more mtDNA haplotypes (14) and μsat alleles (85). Historic walleye and blue pike samples were morphologically and genetically indistinguishable. Together they differed from contemporary Lake Erie walleye with the latter having greater genetic diversity (mean mtDNA=0.79, μsat=0.72) than the historic samples (mtDNA=0.05, μsat=0.47). Moreover, contemporary turquoise-mucus-colored walleye do not differ genetically from standard yellow-colored walleye, and do not correspond to extinct blue pike samples.

Main conclusions Walleye exhibit significant genetic structure and substantial genetic diversity across the range, at broad- and fine-scales. Contemporary patterns correspond to a genetic isolation by geographic distance hypothesis, with northern populations reflecting differential contribution from three Pleistocene glacial refugia. Allelic frequencies of Lake Erie walleye from ~70 years ago changed from those today, at a level typical among populations. Paratype samples of the historic blue pike were not

morphologically or genetically distinguishable from walleye, indicating that the blue pike was not a separate taxon.

3.2 Introduction

Species today are facing a variety of challenges that influence their genetic diversity and divergence patterns. Genetic variation constitutes the raw material underlying evolutionary patterns, population adaptation, and resilience to anthropogenic stressors, such as climate change, habitat alteration and loss, invasive species, and exploitation (see Dawson *et al.,* 2011; Taubmann *et al.,* 2011; Hutchings *et al.,* 2012). Losses of genetic diversity may result in decreased fitness of populations, undermining their ability to adapt to environmental and ecological changes (Reed & Frankham, 2003; Väli *et al.,* 2008). Monitoring genetic diversity and identifying populations that possess unique genetic variability thus are critically important to develop and prioritize appropriate conservation management strategies (see Reed & Frankham, 2003; Allendorf & Luikart, 2007; Taubmann *et al.,* 2011).

Genetic patterns of today's temperate taxa are the product of historic and contemporary processes, which have been influenced by the history of climate change (see Davis & Shaw, 2001; Taylor *et al.,* 2011) and habitat connectivity (Manel *et al.,* 2003; LeClerc *et al.,* 2008; Sork & Waits, 2010). Notably, during the span of the Pleistocene glaciations ~2.6–0.01 million years ago (Mya), the North American Laurentide Ice Sheet advanced as far south as the Ohio River system (Fig. 3-1), drastically altering the distributions of taxa and influencing their population genetic

patterns (Hewitt, 1996, 2000; Bernatchez & Wilson, 1998). Populations were sequestered beyond the ice sheets in three major glacial refugia: the Atlantic Refugium to the east of the Appalachian mountains, the Mississippian Refugium along the lower Mississippi River, and the Missourian Refugium to the west of today's Missouri River (see Fig. 3-1; Ward *et al.*, 1989; Billington *et al.,* 1992; Bernatchez & Wilson, 1998). Following the glacial meltwaters, most taxa moved northwards to recolonize old and expand to new habitats, whose patterns now are accelerating due to anthropogenic climate warming (Chu *et al.,* 2005; Sharma *et al.,* 2007).

The genetic diversity and divergence patterns of a given species are regulated by the distribution of its populations, habitat connectivity, and dispersal capability (Carvalho, 1993; Athrey *et al.,* 2011; Ozerov *et al.,* 2012). Populations of broadly distributed species that span a variety of latitudes have adapted to a breadth of environmental and ecological conditions, resulting in heterogeneous patterns of genetic diversity and divergence (see Slatkin, 1985; Wofford *et al.,* 2005; Ozerov *et al.,* 2012). Some of these populations may become relatively isolated from others, likely experiencing low levels of genetic diversity from drift and possibly having some unique allelic variants (Moran & Hopper, 1983; Coulon *et al.,* 2012). Other populations that are large and inter-connected would be predicted to have high gene flow and low genetic distinctiveness (Poissant *et al.,* 2005; Huey *et al.,* 2010). Migration of populations from region to region also directly affects their genetic patterns, with those having low dispersal capacity and behavior showing higher divergences, and those with greater mobility having higher gene flow and more homogeneity (see Waples, 1987; Palumbi, 1994; Pringle & Wares, 2007).

Anthropogenic factors, including alteration and loss of habitat, climate change, competition with invasive species, and exploitation, may further modify genetic patterns. These factors may force species to occupy sub-optimal habitats leading to population level fluctuations, declines in genetic diversity, loss of local adaptations, and/or extirpation (Stepien, 1995; Williams *et al.,* 2009; Faulks *et al.,* 2011). For example, in response to increasing temperatures, temperate coldwater fish species (e.g., lake trout *Salvelinus namaycush* and cutthroat trout *Oncorhynchus clarkii*) may experience marked population reductions along with range contractions (see Chu *et al.,* 2005; Williams *et al.,* 2009). These reductions may be accompanied by loss of genetic diversity from genetic drift and inbreeding, as has been shown in a variety of species, including Atlantic salmon *Salmo salar,* brown trout *Salmo trutta,* and the golden-cheeked warbler *Dendroica chrysoparia* (see Jonsson & Jonsson, 2009; Athrey *et al.,* 2011). In contrast, a more mesotrophic species, such as the walleye *Sander vitreus vitreus* (Percidae: Teleostei), may broaden its range in response, with its center of distribution shifting north, corresponding to newly available habitat (see Chu *et al.,* 2005). However, outlying populations at extremities of the range may be unable to adapt, leading to declines in genetic variability and possible extirpation (see McMahon *et al.,* 2009; Williams *et al.,* 2009), which could occur in the walleye. Exploitation also may accelerate losses of population diversity by limiting ability to respond to other anthropogenic stressors (Smith *et al.,* 1991; Hauser *et al.,* 2002; Hutchings *et al.,* 2012). Here, we analyze the contemporary genetic patterns of the walleye *S. v. vitreus,* in light of its past and potential future, employing a genetic "window" approach to examine the ebb and flow of variability, isolation, and continuity over space and time.

3.2.1 Walleye distribution, fishery, life history, and genetic patterns

The walleye is one of the most ecologically and economically valuable fishes in the Great Lakes, constituting a keystone species as a primary predator (Locke *et al*., 2005; Roseman *et al*., 2010; Nate *et al*., 2011) and supporting large sport and commercial fisheries (Schmalz *et al*., 2011). It lives in a variety of habitats from slow turbid lake environments to fast flowing clear streams (Collette & Bănărescu, 1977; Billington *et al.*, 2011). The species is widely distributed throughout much of North America (Fig. 3-1a; Page & Burr, 2011) and has been widely transplanted outside of the native range to provide recreational fishing. Maturity occurs around age three, at which time walleye migrate annually to natal spawning grounds during the spring to early summer to reproduce (Collette *et al.,* 1977; Barton & Barry, 2011). Walleye exhibit fidelity to spawning sites (Jennings *et al.,* 1996) with the genetic structure of spawning groups remaining similar from year to year, among age cohorts, and from generation to generation (Stepien *et al.,* 2012). Adults leave the spawning grounds following external fertilization, providing no parental care or nest guarding. Walleye typically range widely to feed at non-reproductive times of the year, travelling distances from 50–300 km (see Colby *et al.,* 1979; Bozek *et al.,* 2011). They often reach ~17–19 years of age (Carey & Judge, 2000), with some reported as 30 years (Bozek *et al.,* 2011).

Recent phylogenetic analyses, based on nuclear and mitochondrial genes, indicate that the North American walleye dates to ~15.4 Mya during the Mid-Miocene Epoch, when it diverged from a common ancestor shared with its sister species the sauger *S. canadensis* (Haponski and Stepien, 2013a). Following this split, contemporary haplotypes

of walleye appeared ~10.8 Mya during the late Miocene Epoch. Earlier genetic studies have described broad-scale spatial patterns of walleye across North America using mtDNA (Billington *et al.*, 1992; Stepien & Faber, 1998; Gatt *et al.*, 2000, 2002), which traced their modern populations to origins from three Pleistocene glacial refugia: the Atlantic coastal, Mississippian, and Missourian. Fine-scale patterns were discerned using nine nuclear DNA microsatellite (μsat) loci, showing that walleye spawning groups often markedly differ from one another, even within lake basins and between proximate sites (Strange & Stepien, 2007; Stepien *et al.*, 2009, 2010, 2012). The present study uniquely employs both approaches, mtDNA control region sequences and nine nuclear DNA μsat loci, to test for: (1) concordance of genetic patterns across the range, (2) the relationship of historic samples from the early 1900s in relation to today's genotypes in Lake Erie, and (3) to resolve the identity of the extinct blue pike *S. v. "glaucus"*.

3.2.2 The blue pike and its relation to walleye

The blue pike *S. v. "glaucus"* (Hubbs 1926) was an enigmatic, abundant, and possibly unique fish taxon, which was reported as endemic to Lakes Erie and Ontario (see Fig. 1b; Bailey & Smith, 1981). It comprised a popular commercial fishery until its collapse in 1959, attributed to exploitation, pollution, and/or habitat alteration (Trautman, 1981). The blue pike officially was declared extinct in 1983 by the US Fish and Wildlife Service (Noecker, 1998), after having not been seen since the early 1960s (Hubbs & Lagler, 2004). The blue pike was hypothesized to have evolved in Lakes Erie and Ontario post-glacially or to have co-existed with walleye in the Mississippian Refugium (Bailey & Smith, 1981). It has long been questioned whether the blue pike was a distinct species,

subspecies, or an ecophenotypic variant of the “yellow” walleye (see Stone, 1948; Scott & Crossman, 1973; Trautman, 1981). Hubbs (1926) described the blue pike as a species, which later was demoted to a subspecies due to large numbers of morphological intergrades with walleye (Trautman, 1981).

The blue pike reportedly differed from walleye by its steel grey-blue color, larger eyes that were located higher on the head, and smaller interorbital distance (Scott & Crossman, 1973; Trautman, 1981). Ecologically, the blue pike occurred in the deeper cooler waters of Lake Erie’s eastern basin. Little is known of its migration patterns, but individuals also were collected in Lake Erie’s western basin (Trautman, 1981). The blue pike is reported to have spawned later in the spring (typically May–June) and in deeper waters, whereas the yellow walleye moves into shallower waters of Lake Erie’s western and eastern basins earlier (on average March–April, but is temperature dependent; Stone, 1948).

The blue pike’s popular saga often has been confused by the facts that: (1) it is not a member of the pike family (i.e., is not an Esocidae) and (2) some walleye in northern waters are turquoise-blue in color (a bright turquoise-blue due to protein in the mucus; see Yu *et al.*, 2008). This coloring also occurs sympatrically with the yellow form in the same waters and additionally characterizes yellow perch *Perca flavescens* and other unrelated fishes in those northern waters. Some of this color typically "rubs off" when the fish is collected (CAS, pers. obs.). Those northern turquoise-blue-mucus walleye, however, do not match the grey-blue color of the original blue pike and do not match its morphological and genetic characters (see Stepien & Faber, 1998). We further examine their respective population genetic characters here.

3.2.3 Objectives and questions

The aim of the present study is to resolve the historic and contemporary genetic patterns of the walleye, providing a baseline for evaluating future anthropogenic population pressures, including climate change, continued exploitation, and habitat losses and alterations. We explore the relationship of the historic blue pike *S. v. "glaucus"* to yellow walleye, as well as test whether walleye genotypes have changed over time in Lake Erie. We evaluate the relationship between yellow and turquoise-blue-mucus-colored walleye that occur sympatrically, as well as to the extinct blue pike. We also relate our findings to the biogeographic patterns reported for other North American taxa. Specific questions are: (1) How does walleye genetic diversity and composition vary across its native distribution for mtDNA and nuclear DNA loci?, (2) How does this compare with historic patterns from Lake Erie?, and (3) What is the relationship of the extinct blue pike to walleye?

To address these questions we analyze modern walleye spawning groups across their range, typical yellow-colored and turquoise-mucus-colored walleye, historic Lake Erie yellow walleye, historic blue pike, and sauger samples, totaling 1206 individuals, using a dual genome approach of mtDNA control region sequences and nuclear μsat loci DNA data. This allows us to compare patterns at multiple evolutionary and temporal scales (see Avise, 2004; Wang, 2010, 2011), since the mtDNA control region sequences reveal historical context, such as origins from glacial refugia whereas μsat loci address contemporary microevolutionary processes, including migration, gene flow, and genetic drift.

3.3 Methods

3.3.1 Sample collection and DNA extraction

Fin clips of spawning adult walleye (1–2 cm^2 of pectoral or caudal fins) were collected by agency scientists, colleagues, and members of our laboratory from 23 spawning locations, totaling 1125 individuals and representing major and minor spawning runs across the walleye's range (Fig. 3-1, Table 3.1). We avoided areas of reported stocking and anthropogenic introductions, concentrating on native populations. Tissue samples immediately were placed in 95% ethanol, and then were stored at room temperature and archived in the Great Lakes Genetics/Genomics Laboratory at the University of Toledo's Lake Erie Center (Oregon, OH). To compare the taxonomic relationship of the extinct blue pike to walleye, formalin-fixed individuals were obtained from the University of Michigan's Museum of Zoology (UMMZ; lot numbers 55200−218003), the U.S. National Museum of Natural History (USNM; lot numbers 9391−231385), and The Ohio State University's Museum of Biological Diversity (OSUM; lot numbers 1449−16467; see Appendix 3.1 for material examined). Sampled blue pike individuals included paratypes collected and identified by Clark Hubbs, who described the blue pike as a separate species in 1926 (Hubbs, 1926). We additionally tested contemporary samples of modern yellow-colored walleye and turquoise-mucus-colored walleye that were sympatric in McKim Lake (site D on Fig. 3-1) to determine their relationship to the blue pike paratypes.

To assess whether the blue pike differed morphologically from walleye, museum specimens of blue pike (*N*=53) including individuals designated by C. Hubbs as the paratypes, historic Lake Erie walleye from the same time period (*N*=52), and sauger *S. canadensis* (*N*=56) were compared (*N*=161 total; see Appendix 3.1). The sauger was used as the outgroup for the for the population genetic analyses of walleye. Our phylogenetic analyses additionally included the other members of the genus *Sander*: the North American sauger and the Eurasian pikeperch *S. lucioperca,* sea pikeperch *S. marinus,* and Volga pikeperch *S. volgensis,* with the Eurasian species used as the outgroup.

DNA from fin clips was extracted using Qiagen DNEASY extraction kits (Qiagen Inc., Valencia, CA), then assessed for quality and quantity on 1% agarose mini-gels stained with ethidium bromide. To circumvent contamination, formalin fixed DNA extractions were conducted in a separate clean laboratory where modern DNA extractions have not taken place. Formalin extractions used separate sterile materials, including pipettors, pipette tips, 1.5 ml tubes, beakers, and tweezers, which were autoclaved prior to each extraction. Blue pike and historic walleye samples were given separate extraction identifiers and were extracted at separate times. To additionally ensure sterile conditions and accuracy in genotyping of extinct blue pike samples, all formalin fixed tissue extractions were conducted with a positive control (designated Lake Erie walleye tissue, sample AZE31 from the Maumee River 2009 spawning run) and a negative control (no tissue). The positive control was handled after all solutions were added and the tubes were closed for the historic formalin samples. Gloves were changed and disposed of after each handling. Prior to DNA extraction, formalin fixed tissues and controls were soaked in 10 mL of phosphate buffered saline (PBS) and placed on an orbital shaker for 48 hours

at room temperature. The buffer was changed after the first 24 hours. DNA then was extracted following the standard Qiagen kit protocol. Extractions were assayed for quality and quantity on a 1% agarose mini-gel stained with ethidium bromide and on a Thermo Scientific (Waltham, MA) Nanodrop 2000 spectrophotometer. Only the Nanodrop was used to determine DNA quantity of the formalin fixed samples due to low concentrations of DNA. We additionally attempted to extract DNA from archived scale samples collected by fisheries biologists, which failed to yield measurable quantities of DNA.

3.3.2 Mitochondrial sequence data collection

To characterize the genetic diversity and divergence patterns across the range of walleye, 711 individuals (a representative subset of the 1125 individuals used for the μsat loci) from the 23 sites were amplified and sequenced for the mtDNA control region (*N*=5–100 per site; Table 3.1). The Detroit River (site N, 95 individuals) and Lake Erie's western basin (O, 100) and eastern basin (P, 50) had larger sample sizes due to our pooling of multiple spawning groups (2–4 groups; see Table 3.1). These spawning groups were pooled to better represent the genetic variation of walleye in this area of the Great Lakes, where it reaches its highest abundances (Hubbs & Lagler, 2004). Twenty-five individuals represented most of the samples available for the remaining spawning groups. This sample size has been shown to adequately capture the genetic variation of a given population for mtDNA control region sequences (see Haponski & Stepien, 2013b). Two sites, the Ohio River (W) and North River (Y) had only 11 and five individuals available. The Ohio River (W) had a higher number of samples (11) for the mtDNA data than the μsat data (4) due to our inclusion of sequences previously analyzed by Stepien & Faber

(1998). Seven of those tissues and DNA aliquots were no longer available to the present study. We also assess the relationships of the historic blue pike (*N*=20) and historic walleye (*N*=20) from Lake Erie to modern walleye from Lakes Erie and Ontario (*N*=200).

Sequences were amplified using the polymerase chain reaction (PCR), and primers included LW1-F (Gatt *et al.*, 2000) and HN20 (Bernatchez & Danzmann 1993) for the modern samples. Since formalin fixation tends to shear DNA into smaller fragments (Shedlock *et al.*, 1997), we designed additional primer pairs for the historic samples using the most common walleye haplotype as a template to amplify the control region in three overlapping sections. Sequence overlap was sufficient to allow the primer sequences to be pruned. These new pairings included: LW1-F with SandercrIR (5'−CATTCATACTATTTTCTTGC−3'; Haponski & Stepien, this paper), SandercrIF (5'−AGTACATACTCTGTTACC−3'; Haponski & Stepien, this paper) with HN20, SandercrIF with *Svic*rIR2 (5'−GTGATTTCCACTATTTATGC−3'; Haponski & Stepien, this paper), and *Svic*rIF (5'−GCAAGAAAATAGTATGAATG−3'; Haponski & Stepien, this paper) with HN20. PCR reactions contained 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 µM of each dNTP, 0.5 µM each of the forward and reverse primers, ~30 ng DNA template, and 1 unit of *Taq* polymerase per 25 µl. Each reaction included the positive and negative controls.

Amplification procedure included an initial denaturation of 2 min at 94^{o}C, followed by 42 cycles of 40 s at 94^{o}C, 40 s at 48^{o}C, and 1.5 min at 72^{o}C, with a final extension of 5 min at 72^{o}C. A 4µl aliquot of each PCR product was visualized on a 1% agarose mini gel stained with ethidium bromide, and successful reactions were purified using a QIAGEN PCR Purification Kit. DNA sequencing was outsourced to the Cornell

University Life Sciences Core Laboratories Center, which used ABI Automated 3730 DNA Analyzers. The sequences then were checked, identified, and aligned with BIOEDIT v7.05 (Hall, 1999) in our laboratory, totaling 733 bp for control region. We related the walleye spawning group haplotypes to those of Stepien & Faber (1998), who sequenced the entire mtDNA control region (totaling ~1,086 bp) for 179 walleye across the Great Lakes and recovered 14 haplotypes (the most common haplotype was deposited in GenBank as accession #U90617). We trimmed the original sequences of Stepien & Faber (1998) to match our 733 bp, omitting the 5' repeat section, which retained seven of their original haplotypes (designated here as A1–7, National Institutes of Health (N. I. H.) GenBank Numbers U90617 and JX442946–52; http://www.ncbi.nlm.nih.gov/).

3.3.3 Nuclear microsatellite data collection

Allelic variation at nine μsat loci (*Svi*2, 4, 6, 7, 17, 18, 33, L6, and L7) was analyzed to test for population genetic structure among the 23 modern walleye spawning groups (Appendix S2). We used the data sets of Stepien *et al.* (2009, 2012) of 866 walleye individuals, and increased sample sizes for Cedar Lake (site A by 9 individuals), McKim Lake (D by 5), Thames River (M by 1), Detroit River (N by 83), Huron River (O1 by 20), and Oneida Lake (U by 5). We also added 181 individuals from six new spawning sites: Lake Winnipeg (B), Lake of the Woods (C), Lake Nipigon (G), Portage Lake (H), Pigeon Lake (S), and Lac Mistassini (V; see Fig. 3-1, Table 3.1). Samples from the Ohio River (W) included just four individuals for the μsat data, as these were all that remained from the sequencing analyses of Stepien & Faber (1998; see above). We

additionally analyzed museum samples (described earlier) of historic Lake Erie walleye (*N*=31) and the extinct blue pike (*N*=25).

Polymerase chain reaction (PCR) amplifications were conducted in 48 well plates with 10 µl reactions containing 0.6 units *Taq* polymerase, 50 µM dNTPs, 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 0.5 µM of each primer, and ~80 ng of template. A positive control (sample AZE31) and a negative control (no template) were included in all reaction runs. PCR cycling parameters consisted of 2 min at 94^{o}C for initial denaturation, followed by 35 cycles of denaturation (94^{o}C, 30 s), primer annealing (1 min) at specific temperatures (given in Appendix 3.2), and polymerase extension (72^{o}C, 30 s), followed by a final extension at 72^{o}C for 5 min.

Three sets of loci were multi-plexed as single PCR reactions for modern fin clip samples: *Svi*4 and 33, *Svi*2, 6, and 7, and *Svi*L6 and L7. *Svi*17 and 18 were run individually. All loci were run individually for the historical formalin fixed samples. Amplification products were diluted 1:50, of which 1 µl was added to 13 µl of formamide and Applied Biosystems (ABI, Fullerton, CA) Gene Scan 500 size standard in 96-well plates, denatured for 2 min at 95^{o}C, and analyzed on an ABI 3130xl Genetic Analyzer with GENEMAPPER v3.7. To minimize analyzer runs, *Svi*17 and 18 were pooled for all types of samples and visualized with different dye colors. For the formalin fixed reactions, three sets of loci were pooled: *Svi*4 and 33, *Svi*2, 6, and 7, and *Svi*L6 and L7. Output profiles were checked manually to confirm allelic size variants. Repeat number and size, and number of alleles per locus are reported in Appendix 3.2. For the historic samples, *Svi*17 and L7 failed to amplify and thus were dropped from analyses for the

comparisons of historic versus modern walleye. The total number of μsat loci used thus was seven.

3.3.4 Mitochondrial DNA data analyses

TCS V1.21 (Clement *et al.*, 2000) was used to construct a statistical parsimony haplotype network to further analyze relationships among drainages, spawning sites across the range of walleye, and the historic samples. Evolutionary trees among the mtDNA control region sequence haplotypes for the modern and historic walleye samples, extinct blue pike, sauger, and the Eurasian *Sander* (pikeperch, sea pikeperch, and Volga pikeperch) were constructed using maximum likelihood (ML) in PHYML v3.0 (Guindon *et al.*, 2010) and Bayesian analyses in MRBAYES v3.2.1 (Ronquist & Huelsenbeck, 2003). Corrected Akaike information criteria (AICc) from JMODELTEST V2 (Darriba *et al.*, 2012) were used to determine the most appropriate nucleotide substitution model, which selected the TPM3uf model (Posada, 2008) with invariant sites (I=0.3910) and a gamma distribution (α=0.2750). Maximum likelihood analyses were begun with five random trees, from which the best was selected using nearest neighbor interchange (NNI) and subtree pruning, and regrafting (SPR).

Support for nodes was determined from 2000 bootstrap pseudo-replications (Felsenstein, 1985). Bayesian analyses used a Metropolis-coupled Markov chain Monte Carlo (MC^3) approach and ran for 5000000 generations, with sampling every 100 generations. Four separate chains were run simultaneously for each analysis, and two analyses ran simultaneously. The burn-in period for the MC^3 was determined by plotting log likelihood values for each generation to identify when stationarity was reached.

Twenty-five percent of the generations were discarded as burn-in, and the trees and parameter values sampled prior to the burn-in also were discarded. A 50% majority rule consensus tree was calculated based on the remaining generations, and branch support was determined from the posterior probability distribution (Holder & Lewis, 2003) in MRBAYES.

Comparative divergence time estimates among modern walleye haplotypes and the historic samples were evaluated with the mtDNA control region sequence data using BEAST V.1.71 (Drummond *et al.*, 2012), with the general time reversible nucleotide substitution model (GTR; Lanave *et al.*, 1984). A gamma distribution and invariant sites were incorporated into the model for the control region as identified by JMODELTEST. BEAST analyses used a relaxed molecular clock that assumed a lognormal distribution, with the Yule speciation process (Gernhard, 2008) as a tree prior. Two separate runs were conducted, each with a chain length of 50000000 generations, and parameters sampled each 100 generations. To date the diversification of modern and historic walleye haplotypes and the extinct blue pike, we used four previously calibrated time points from Haponski & Stepien (2013a): 15.4 Mya as the divergence between walleye and sauger, 13.8 Mya for Eurasian *Sander*, 9.1 Mya as the divergence of the Eurasian pikeperch and sea pikeperch, and 7.3 Mya as the origin of modern sauger haplotypes (Haponski & Stepien, 2013a). Analyses that are in common between the mtDNA and nuclear μsat data sets are described below in "Population genetic data analyses".

3.3.5 Nuclear microsatellite DNA data analyses

The nine μsat loci and 26 population samples each were tested for conformance to Hardy-Weinberg equilibrium (HWE) expectations and linkage disequilibrium (LD), using a Markov Chain Monte Carlo (MCMC) chain of 10000, 1000 batches, and 10000 iterations in GENEPOP V4.0 (Rousset, 2008). Levels of significance were adjusted with the sequential Bonferroni correction (Rice, 1989). MICRO-CHECKER V2.2.3 (van Oosterhout *et al.*, 2004) was used to evaluate loci for null alleles, scoring errors, or large allele dropout. The program FREENA (Chapuis & Estoup, 2007) estimated the frequency of null alleles in each population and locus and the potential influence on F_{ST} values. Per-locus calculations also included: number of alleles (N_A), inbreeding (F_{IS}), overall genetic deviation across all samples (F_{IT}), and divergences (F_{ST}) in *F*STAT v2.9.3.2 (Goudet, 1995, 2002). Each locus was tested for the possible influence of selection through the identification of F_{ST} outlier comparisons using LOSITAN (Beaumont & Nichols, 1996; Antao *et al.*, 2008).

To examine population sub-structuring, the Bayesian-based programs STRUCTURE V2.3.3 (Pritchard *et al.*, 2000) was employed to evaluate assignments of individuals to glacial refugial sources, watersheds, and spawning locations. STRUCTURE assigned individuals to population groups of K=1 (the null hypothesis of panmixia) to K=26 (the total number, including modern sampling sites, historic Lake Erie walleye, extinct blue pike, and sauger), with the relative frequency of each individual in a population group totaling 1.00. STRUCTURE runs included 10 replicates for each K, a burn-in of 250000 generations, and run lengths of 1000000 generations. Posterior probabilities (Pritchard *et al.*, 2000) from each run were used to select the best K, i.e., the number of true population

groups. GENECLASS2 (Piry *et al.*, 2004) assigned individuals to putative populations of origin on the basis of multilocus genotype data, using a simulated dataset (N=1000000 simulations) to test self-assignment of the historic Lake Erie walleye, extinct blue pike, and modern Lakes Erie and Ontario samples. We also tested the influence of kin relationships on population sub-structuring with COLONY V2.0.4.2 (Jones & Wang, 2009), which uses a maximum likelihood approach to assign full sib-ships from multi-locus genotype data.

3.3.6 Population genetic data analyses using both data sets

Genetic diversity comparisons among the samples included haplotype diversity (H_D) and number of haplotypes (N_H) for the mtDNA control region sequence data determined in ARLEQUIN V3.5.1.3 (Excoffier & Lischer, 2010). Diversity metrics for the nine nuclear µsat loci included observed (H_O) and expected (H_E) heterozygosities in GENEPOP, and F_{IS}, N_A, and allelic richness (A_R) in *F*STAT. We tested for significant differences in diversity and A_R values using Analysis of Variance (ANOVA) in the R statistical analysis software suite v2.15.2 (R Development Core Team, 2012) followed by Tukey's *post hoc* tests (Zar, 1999). Number and proportion of private haplotypes (N_{PH}) and private alleles (N_{PA}), i.e., those that were unique to a given spawning group or system, were determined from CONVERT V1.31 (Glaubitz, 2004). Standard errors were calculated with MICROSOFT OFFICE EXCEL 2008 (Redmond, WA).

Patterns of genetic divergence were evaluated using unbiased F_{ST} estimates (Weir & Cockerham, 1984) in *F*STAT, which facilitated comparisons with other studies. Since *F*-statistic estimates assume a normally distributed data set (Weir & Cockerham, 1984)

and may be affected by small sample sizes and rare alleles (Raymond & Rousset, 1995), pairwise exact tests of differentiation (χ^2) also were evaluated in GENEPOP. Those used a MCMC procedure with a chain of 10000, 1000 batches, and 10000 iterations to randomly sample allele frequencies, a procedure that was not dependent upon sample size or a normal distribution (Raymond & Rousset, 1995). Probability values for both types of pairwise comparisons were adjusted using the sequential Bonferroni correction (Rice, 1989).

We tested partitioning of genetic variation among the 23 contemporary spawning groups using two approaches: (1) isolation by distance via Mantel's (1967) test, and (2) the BARRIER V2.2 landscape genetics approach (Manni *et al.*, 2004). For (1), fit to a genetic isolation (F_{ST} /(1– F_{ST})) by geographic distance model (shortest connected waterway using the path option in GOOGLE EARTH® (Google, 2010)) was tested with ISOLDE in GENEPOP for 10000 MCMC permutations, which predicted a linear relationship (Rousset, 1997). The BARRIER analysis (2) determined significant barriers to gene flow by identifying which neighboring sample locations were distinguished by large genetic (F_{ST}) distances in relation to their geographical separation (latitude and longitude) based on Monmonier's (1973) maximum-difference algorithm. BARRIER analyses followed Strange & Stepien (2007) and LeClerc *et al.* (2008), and were run using the entire μsat data set (all loci), separately for each individual locus (to calculate the relative number of loci that supported each barrier), and for mtDNA control region. Relative support for each genetic barrier also was evaluated from bootstrap analysis of the multilocus F_{ST} matrix, with 2000 iterations using GENELAND V3.3.0 (Guillot *et al.*, 2005; Guillot & Santos, 2009) and R. Barriers with bootstrap values higher than 50% are reported here.

We additionally tested the hierarchical partitioning of genetic variation among various possible groupings with an analysis of molecular variance (AMOVA; Excoffier *et al.*, 1992) in ARLEQUIN. Scenarios that were evaluated included comparisons of the amount of variation between historic and modern samples, among drainages, among STRUCTURE groupings, and between lake and river spawners.

Relationships among the sampling locations additionally were analyzed with neighbor-joining trees (Saitou & Nei, 1987) based on Nei's *D* genetic distances (Nei, 1972) in PHYLIP V3.68 (Felsenstein, 2005) for the mtDNA control region haplotype and μsat data sets. We also ran a combined tree that included both the mtDNA control region sequences and μsat data. Support values for nodes of the trees were evaluated using 2000 bootstrap pseudo-replications (Felsenstein, 1985) in PHYLIP.

3.3.7 Morphological comparisons

To test the morphological differentiation of historic walleye, blue pike, and sauger samples, we analyzed nine morphometric measurements and four meristic characters based on Hubbs & Lagler (2004). Measurements (to the nearest 0.1 mm) were made using MITUTOYO vernier calipers (Aurora, IL) and meristic characters were tallied using a LEICA MICROSYSTEMS dissecting microscope (Buffalo Grove, IL). Morphometric characters included: standard, head, cheek, upper jaw, and lower jaw lengths, body depth, head and interorbital widths, and orbit diameter. Meristic characters included numbers of anal, dorsal, pectoral, and pelvic fin rays. We also calculated two ratios, orbit: interorbital and upper: lower jaw, which were reported by Trautman (1981) to show a difference between the walleye and blue pike. Data are provided in Table 3.2.

Prior to analysis, morphometric measurements were standardized by removing size-dependent variation using the formula of Elliot *et al.* (1995), followed by a log transformation. Meristic data were not transformed. Log-transformed, size-free morphometric and non-transformed meristic data were analyzed separately using both univariate and multivariate analyses in the R statistical analysis software suite. Wilks' lambda (Λ; Zar 1999) test statistic of multivariate analysis of variance (MANOVA) compared whether the historic walleye, blue pike, and sauger significantly differed based on the two datasets. The means of transformed morphometric and non-transformed meristic characters were compared with ANOVA, followed by a Tukey's *post-hoc* test (Zar, 1999) to evaluate which taxa significantly differed for each character. We further explored the overall morphological variation among the three taxa with a principal components analysis (PCA), using the covariance matrix for each of the two morphological datasets. We compared whether principal components (PC) differed with a MANOVA and the Wilks' Λ test statistic, followed by individual ANOVAs and Tukey's tests.

We additionally tested whether the morphometric and meristic characters correctly distinguished historic walleye, blue pike, and sauger specimens with discriminant function analysis (DFA; dos Reis *et al.*, 1990). We first tested whether linear or quadratic DFA should be used to compare the three taxa, with a χ^2 test in SAS v9.2 (SAS Institute Inc., Cary, NC). Both the morphometric and meristic data sets showed significant heterogeneity among covariance matrices, rejecting linear DFA (morphometric = χ^2=209.406, df=90, p<0.001; meristic= χ^2=65.887, df=30, p<0.001). Thus, we employed a quadratic DFA, which based each morphological character on its

own variance-covariance matrix and used a jackknife re-sampling approach to validate taxon assignment (see McGarigal *et al.,* 2000).

3.4 Results

3.4.1 Nuclear microsatellite loci equilibrium, linkage, and null alleles

All μsat loci conform to HWE expectations and show no linkage disequilibrium following sequential Bonferroni correction. Locus *Svi*17 has the highest F_{ST} value (0.150), likely reflecting some positive selection, whereas *Svi*L7 has the lowest value (0.031), suggesting balancing selection (Appendix 3.2). The remaining seven loci conform to expectations of neutrality, having intermediate F_{ST} values (mean=0.090, 0.067-0.124). Locus *Svi*18 has the fewest number of alleles (N_A=8) and *Svi*6 possesses the most (N_A=31; Appendix 3.2). Seven of nine nuclear μsat loci successfully amplified for the historic walleye and blue pike samples, with *Svi*17 and L7 failing to amplify. We compare overall population results from the remaining seven versus nine μsat loci across the 23 modern walleye spawning groups and find them to be identical; thus to facilitate comparisons the seven are presented here. Specific results on the nine loci data set were detailed by Stepien *et al.* (2009, 2010).

Most population samples (88%, 161/182 comparisons) are free of null alleles. MICRO-CHECKER discerns no evidence of null alleles at locus *Svi*2, with some variation attributed to slight excess of homozygotes at *Svi*33 (for populations C, L, V), *Svi*4 (C, P), *Svi*18 (O, Q, R, V), *Svi*L6 (O, Q, T), *Svi*6 (F, Q, U, X, sauger), and *Svi*7 (L, M, Q, sauger) for various samples. However, since null alleles are not indicated for those loci at the

other spawning groups, and since all populations are in HWE, and are free of heterozygote deficiency, scoring error, and stuttering (see van Oosterhout *et al.,* 2004), all loci are included in our analyses. The historic walleye (Q) sample shows the highest number of loci with null alleles (57% of the loci, including *Svi*18, L6, 6, and 7). Null alleles appear to be manifested at larger allelic lengths in the historic samples, including >124 for *Svi*18, >130 for *Svi*L6, >158 for *Svi*6, and >166 for *Svi*7 (Fig. 3-2, Appendix 3.3). For example, locus *Svi*L6 ranges from 106–140 base pairs (bp) for modern walleye samples, whereas the historic samples range from 92–130 bp. *Svi*6 allele lengths range from 126–168 bp in modern samples and 132–156 bp for the historic samples. The respective estimated frequency of null alleles is 0.00–0.20 for modern walleye spawning groups, 0.02–0.22 for the historic walleye sample (site Q), 0.00–0.27 for the historic blue pike (R), and 0.00–0.19 for modern sauger. These ranges thus are similar for all.

A FREENA analysis, which tests for difference in F_{ST} values due to null alleles, discerned none; thus our F_{ST} values appear unaffected. With FREENA corrections, pairwise comparisons between the historic (Q–R) and modern population groups (O–P, S–T) slightly changed (e.g., from 0.141 before correction to 0.120 after correction), retaining a similarly large magnitude difference between the historic and modern samples. For the modern walleye spawning group comparisons, F_{ST} values changed only at the thousandth decimal point after correction (e.g., from 0.058 to 0.054). Thus, allele frequencies were not adjusted against possible influence of null alleles, as any variation was negligible.

3.4.2 Control region sequence data genetic diversity and phylogenetic patterns

Mitochondrial DNA control region haplotypes of walleye are not shared with sauger, differing by 34 fixed base pairs between the species. The phylogenetic tree of haplotypes recovers sauger as the sister species to walleye with high support (1.00 posterior probability (pp)/100% bootstrap pseudoreplications; Fig. 3-3a). Walleye diverged from sauger ~15.4 Mya (5.2-27.1 Mya = 95% highest posterior density (HPD)), according to phylogenetic analyses based fossil calibrations by Haponski & Stepien (2013a). Our sauger samples have six unique haplotypes (GenBank #KC819837–42), which differ from each other by single nucleotides. All modern and historic walleye, including the blue pike, show close genetic relationship to each other, varying by an average of two nucleotides and a range of 1-19 bp.

Modern-day walleye are characterized by 27 unique control region haplotypes (GenBank accession #s U90617, JX442946–56, KC819843–54, XX–XX) among 711 samples across the range (Fig. 3-3). Haplotypes A1–A7 and B19 (GenBank #U90617, JX442946–52) match those previously identified by Stepien & Faber (1998) and later also found by Haponski & Stepien (2013b). Haplotypes B8–11 (GenBank #JX442953–56) were identified by Haponski & Stepien (2013b) from walleye spawning in Lakes Huron, St. Clair, and Erie. Here we describe an additional 15 novel haplotypes (B12–18, B20–27; GenBank #KC819843–54, XX–XX) from contemporary walleye, as well as a unique haplotype (B28; GenBank #XX) from a historic walleye from Lake Erie, totaling 28 haplotypes. All but two haplotypes are a single mutational step from one another, excepting B19 from the Ohio and New Rivers (W, X, GenBank #KC819850), which is 19 steps away, and B20 from the North River (Y, GenBank #KC819851), which is eight

steps (Fig. 3-3b). The historic B28 haplotype differs by only a single mutational step from most other walleye (Fig. 3-3b).

Divergence estimates indicate that the 28 walleye haplotypes differentiated ~10.6 Mya (6.9-14.3 HPD), with haplotype B19 from the Ohio River system (W–X, 1.00pp/98%) basal on the phylogenetic tree (Fig. 3-4a). The remaining haplotypes separated ~7.2 Mya (3.9-10.6 HPD, 0.82pp). Three groups of the remaining haplotypes are distinguished: (1) A4, B9, B14, B21, and B23 (0.56pp), (2) B12 and B22 (0.65pp), and (3) B8, B11, B16, B26–27 (0.53pp); these appear to have diverged ~2.6–1.0 Mya (0.01-5.0 HPD) during the Pleistocene glaciations. There is no distinction of the historic samples from contemporary ones. Haplotype B28 groups with the modern haplotypes (0.82 posterior probability support; Fig. 3-3a) and haplotype A3 is shared between samples from both time points (see Fig. 3-4a).

Five common haplotypes (A1–4, B11; GenBank #U90617 and JX442946–49, 56) represent 90% of the individuals. Haplotype A1 (GenBank #U90617, JX442946) is the most abundant, occurring in 41% of the individuals and 87% of the sites (20 of 23). Haplotype A1 is absent from Oneida Lake (site U), the New River (X), and the North River (Y). A3 (GenBank #JX442948) is the next most abundant (19% of the individuals and 61% of the sites), distributed from the upper Great Lakes through the southerly locations (H–X). A3 is the sole haplotype shared between the modern and historic samples, predominating in the historic walleye (95%) and blue pike (100%) from Lake Erie, in contrast to its frequency of 22% today in Lake Erie (see Fig. 3-3b, 3-4a, Appendix S4). Our results indicate that the historic blue pike was not a genetically distinct taxon from walleye.

Haplotype A2 (GenBank #JX442947) occurs in 11% of the individuals and 43% of the sites (10/23) and also is widely distributed (sites H–W), ranging from Portage Lake through the Ohio River system. Haplotype A4 (GenBank #JX442949) characterizes 8% of the individuals from 52% (12/23) of the locations, with a broad distribution that extends from Cedar Lake to the North River (A–Y) but appears absent from some intermediate locations (Fig. 3-3b, Appendix 3.4). Haplotype B11 (GenBank #JX442956) occurs in 11% of the samples from the northern spawning sites (A–G, K, V), but largely is absent from the Great Lakes (H–J, L–P, S–U), and the Ohio (W–X) and North River (Y) systems (Fig. 3-3b, 3-4a, Appendix 3.4). Three other haplotypes appear to uniquely characterize walleye from separate drainages. Haplotype B25 (GenBank #XX) appears endemic to the Lake Winnipeg and Upper Mississippi River drainages (sites A, D, E). Two haplotypes are recovered only in the southeast: haplotype B19 (GenBank #KC819850) from the Ohio River system (27–72%, W–X) and B20 (GenBank #KC819851) in the North River (80%).

Numbers of haplotypes range from 2–11 in each of the 23 spawning groups (mean = 4; Table 3.1, Fig. 3-4a), with the eastern (P) and western (O) basins of Lake Erie having the most (11, 9) and Lake of the Woods (C), St. Louis River (F), Moon/Musquash River (L), Lac Mistassini (V), and the North River (Y) having the least (each with 2; Table 1). Historic walleye and blue pike samples possess far fewer haplotypes (N_H=1–2) compared to modern Lakes Erie and Ontario spawning groups (N_H=9–11; Table 3.1, Fig. 3-4a). Modest numbers of private haplotypes characterize the modern walleye spawning groups, ranging from 0–4 (P_{PH}=0.00–0.50; Table 3.1). Samples from Oneida Lake (U) and the North River (Y) have the highest proportions of private haplotypes (0.50).

Notably, walleye from Lake Erie have some of the higher values (mean=0.35, range=0.33-0.36) compared to other Great Lakes' locations (mean=0.10, range=0.00–0.50). A single rare private haplotype (B28; GenBank #XX) occurred in one of the historic walleye, which appears to have been lost from today's populations (Table 3.1). This haplotype differs by a single mutational step from the abundant walleye haplotype A3 (see Fig. 3-3b). The historic blue pike samples lack private haplotypes.

Haplotypic diversity (H_D) varies greatly among the population samples, with the Moon/Musquash Rivers (L) and Mille Lacs (E) having the lowest (H_D=0.15–0.16) and those from the Ohio River (W, H_D=0.85), eastern Lake Erie (P, H_D=0.82), Cedar Lake (A, H_D=0.77), and western Lake Erie (O, H_D=0.76) having the highest values (Table 3.1). Historic walleye and blue pike samples show much lower haplotypic diversity values (H_D=0.00–0.10) compared to modern walleye spawning in Lakes Erie and Ontario (H_D=0.47–0.82). ANOVA indicates that the diversity of mtDNA control region sequence data differs between the historic and modern walleye samples (F=47.24, df=2, p=0.005)

3.4.3 Nuclear microsatellite loci genetic diversity patterns

We recover a total of 208 alleles among seven nuclear μsat loci, across 23 contemporary spawning groups of walleye (111 alleles), historic walleye (44), blue pike (20), and sauger (79) (Table 3.1, Appendix 3.2). Many common alleles are shared among walleye spawning groups and are distributed in high frequency across the populations (example in Fig. 3-2). Numbers of alleles range from 27–78 per sampling location (mean=39; Table 3.1). More alleles characterize populations in the Great Lakes (N_A=43–78, mean=59) versus other locations (N_A=27–45, mean=39; Table 3.1). Lake Erie

spawning groups tend to have more alleles (74–78) than elsewhere in the Great Lakes (48–72), similar to the control region sequence data. Allelic richness values range from 3.00–4.71, with populations in the Great Lakes having moderate values (A_R=3.76–4.20) and lower values present in the northern sites, including Lake Winnipeg (sites A–D), the Upper Mississippi River (E), and Lac Mistassini (V; A_R=3.00–3.68). Higher values characterize the Ohio River (A_R=4.71) and North River populations (A_R=4.29; Table 3.1). An ANOVA indicates significant variation of allelic richness values among the 23 population samples (F=2.50, df=22, p<0.001). A Tukey's *post hoc* test reveals no overall pattern among them.

Samples of historic walleye (Q, N_A=44) and blue pike (R, N_A=20) have fewer alleles than characterize modern walleye from Lake Erie (sites O–P, N_A=74–78) and Lake Ontario (S–T, N_A=55–65; Table 3.1, Fig. 3-2, Appendix 3.3). Likewise, allelic richness values are lower for the historic samples (A_R=2.06–2.83) compared to the contemporary ones (A_R=4.00–4.30). The difference in their allelic richness values is significant in ANOVA (F=11.129, df=5, p<0.001). The historic samples reveal evidence of inbreeding depression (F_{IS}=0.258) for the walleye and outbreeding depression (F_{IS}=-0.191) for the blue pike. Values for the two historic samples are the highest we observed, however, the samples are in HWE. In comparison, the contemporary spawning groups from Lakes Erie and Ontario have much lower values (F_{IS}=-0.017–0.046; Table 3.1). Results from the COLONY analyses indicate some inbreeding of spawning groups, with full siblings averaging 8% in the contemporary walleye samples. Higher values occur for McKim Lake (28%, D) and Lake Nipigon (40%, G). The highest values characterize the historic blue pike samples (76%, R; Table 3.1).

The μsat alleles of the historic samples are identical to those most abundant across the range of modern walleye, similar to the homology of their control region sequence haplotypes. These same μsat alleles are common, but in lower frequencies, in the modern Lake Erie samples (Fig. 3-2, Appendix 3.3). Lengths of alleles tend to be smaller in the historic samples compared to the modern ones (Fig. 3-2, Appendix 3.3). Allele sizes range from 72–202 bp for the historic walleye (Q) and 86–202 for blue pike (R) samples whereas modern samples from Lakes Erie (O–P) and Ontario (S–T) range from 82–222 bp. Overall allele sizes are 76–222 bp across all contemporary walleye.

Modest proportions of private μsat alleles characterize the 23 contemporary samples (0–3, P_{PA}=0.00–0.11), with the North River walleye having the highest proportion (3 private alleles, P_{PA}=0.11). The historic walleye samples possess six private μsat alleles (P_{PA}=0.14) that are not recovered from the modern walleye samples. However, the historic blue pike samples all have common walleye alleles, with no unique ones, further supporting that it was not a distinct taxon from walleye. In comparison, the modern Lakes Erie (O–P) and Ontario (S–T) walleye samples each have 0–1 private alleles (P_{PA}=0.00–0.01; Table 3.1).

Genetic diversity values from the μsat loci generally appear somewhat higher (mean=0.68) than those from the control region sequence data (mean=0.53). Diversity values (H_O) from the μsat data range from 0.52 at Lac Mistassini (V) to 0.77 in the Flint River (K; Table 3.1). An ANOVA followed by a Tukey's test discerns no significant difference in μsat diversity values among the 23 spawning groups (F=1.79, df=22, p=0.02). Diversity values for the historic walleye (H_O=0.40) and blue pike (H_O=0.54) samples are relatively low. However, this difference is not significant based on an

ANOVA (F=3.83, df=5, p=0.007) followed by Tukey's *post hoc* test. Thus, the genetic diversity of all samples are comparable.

3.4.4 Spatial genetic structure across modern walleye spawning groups

Our analyses discern that most walleye spawning groups are genetically distinctive for the control region (F_{ST}=227/263 comparisons, χ^2=235/263) and μsat data sets (F_{ST}=241/263, χ^2=263/263; Table 3.3, Appendix S5), indicating a high degree of genetic structure. The most pronounced divergences occur among respective population groups from three geographic regions: the northern (A–E), Great Lakes (F–U), and southeastern (X–Y) drainages, which are distinguished by mean F_{ST} values of 0.318 for control region (range=0.000-0.801) and 0.129 for the μsat data (range=0.003-0.334). Notably, the New River (X) and North River (Y) populations are distinguished by some of the highest F_{ST} values (control region: 0.342–0.798, μsat: 0.011–0.324; Table 3.3).

Results from the two genetic markers and the two pairwise comparison methods largely are congruent, with few exceptions. For the control region sequences, walleye from Lake of the Woods (C) appear similar in haplotype composition to those in nearby McKim Lake (D), Mille Lacs (E) in the Upper Mississippi River, Muskegon River (I) in Lake Michigan, and Lake Huron's Flint (K) and Moon/Musquash (L) Rivers. Portage Lake (H; Lake Superior drainage) show genetic similarity to Lakes Huron (J–K), St. Clair (M–N) and Erie (O–P; Table 3.3). However, these same populations are significantly divergent with the μsat loci. This likely reflects the predominance of common control region haplotypes that are widely distributed (see Figs. 3-3–3-4). The sole instance of

incongruence between results of the F_{ST} and exact tests of differentiation involves Ohio River (W) walleye. This likely is due to its small sample size (N=4 individuals).

Analysis with both genetic marker systems correspond to a pattern of genetic isolation with geographic distance, with closely spaced populations being more similar than those that are distant (control region: R^2=0.10, p<0.001, μsat: R^2=0.23, p<0.001; Appendix 3-1). For example, walleye spawning within Lakes St. Clair (M–N) and Lake Erie (O–P) are genetically similar in both data sets (Table 3.3, Appendix 3.5).

BARRIER analyses likewise identify significant genetic discontinuities across walleye populations (Fig. 1c), similar to the pairwise divergence patterns. The first mtDNA barrier (1) separates the southern populations (W–Y) from the northern ones (A–P,S–V). The second (2) isolates the northernmost sites (A–G,V) and the third (3) distinguishes Lake Nipigon (G). In contrast, barriers for the μsat loci denote finer-scale discontinuities among spawning groups, rather than overall regions. The primary μsat barriers first separate the Lake Nipigon population (G, barrier I; 49% bootstrap support, 7/7 loci), then the North River (Y, II; 57%, 7/7), followed by Lac Mistassini (V, III; 43%, 6/7). The remaining barriers for both data sets reflect fine-scale divergences of walleye spawning groups, with most delineations occurring among the northern drainages (Fig. 3-1c).

The combined genetic distance tree among populations (Fig. 3-5) places walleye from the North River (Y) as basal to the other spawning groups, which matches its high pairwise divergence from other locations (Table 3.3). The tree also clusters northern walleye and those spawning in Lake Superior (sites A–G, V; 62%), similar to the

BARRIER analysis results. Populations from Lakes St. Clair (M–N) and Erie (O–P) likewise display closer genetic relationship, similar to their pairwise comparison values.

Hierarchical analysis of genetic variation in AMOVA identifies significant partitioning of variation for two geographic scenarios (Table 3.4). The first supports division of walleye populations into three primary geographic regions: northern (A–E, V), Great Lakes (F–U), and southeast (W–Y). This scenario explains the most overall variation (among the three groups = 12.06% control region/ 5.07% μsat; among their component spawning populations = 17.64%/4.97%; total=29.70%/10.04%; Table 3.5). This hierarchical structure likewise is indicated by the highest mean F_{ST} values (control region=0.318, μsat=0.129). The first scenario thus explains the data best. The second AMOVA scenario distinguishes walleye among each of the 11 drainage systems sampled (variation among 11 groups=14.38%/4.26%; variation among populations within the 11 systems=11.07%/3.13%; total=25.45%/7.39%), which also is accompanied by high mean F_{ST} values (control region=0.286, μsat=0.132; Table 3.4). We find no evidence supporting the scenario for potential partitioning of variation between samples from rivers versus lakes (0.00%/0.58%), with all of the component variation indicated among the component spawning populations (24.92%/6.59%). Thus, division among three geographic regions best explains partitioning of genetic variation for walleye across its native range

The occurrence and frequencies of control region haplotypes reveal significant genetic patterning running from east to west and north to south (Fig. 3-4a). All populations from the Lake Winnipeg region (A–D) are dominated by haplotypes A1, B11, and B25 (Fig. 3-4a). Samples from the upper Great Lakes (H–L) are characterized

by haplotypes A1–4. The Lakes St. Clair and Erie populations (M–P) have more even proportions of A1–4, with greater variety of haplotypes to the east. Lake Ontario sites are dominated by haplotype A3, having lower numbers of A1–2, along with B8. Samples to the east and south also appear distinctive.

Bayesian STRUCTURE analysis based on the μsat data set identifies K=9 modern walleye population groups with 1.00 pp (Fig. 3-4b). Northern walleye populations from the Lake Winnipeg drainage (A–D, colored ivory), Mille Lacs (E, navy), and Lac Mistassini (V, pink), each are distinguished from those in the Great Lakes region and southeast with high assignment values. Walleye populations from the southeast, particularly the North River (Y), also are strongly differentiated. Within the Great Lakes region, most walleye cluster by lake. Spawning groups from Lake Superior (F–G) mostly self-assign, whereas Portage Lake (H) shows evidence of a mixed genetic signature between the upper and lower Great Lakes. Walleye from Lakes Michigan (I) and Huron (J–K) group together, whereas those spawning in the Moon/Musquash Rivers of Georgian Bay Lake Huron (L) are different. Walleye from Lakes St. Clair and Erie cluster together, as indicated by their pairwise values (Table 3.3) and the population genetic distance tree (Fig. 3-4). An AMOVA scenario (#5) that is partitioned according to the STRUCTURE groups, supports these groupings (among groups=4.10%, p<0.001; among populations=3.32%, p<0.001; Total=7.42%). However, this explains less of the overall variation than the best two AMOVA scenarios and there is no support for this pattern with the mtDNA data (among groups=3.75%, p=0.078; Table 3.4).

3.4.5 Historic vs. modern population genetic structure

The historic walleye and blue pike samples are indistinguishable from each other in control region sequences (F_{ST}=0.000, χ^2=0.00), and differ in μsat allele frequencies alone (F_{ST}=0.050, χ^2=63.03; Table 3.5a–b). The latter difference is within the realm of values distinguishing among modern walleye spawning groups within Lakes Erie (O–P) and Ontario (S–T) (μsat: F_{ST}=0.006–0.049, χ^2=22.62–Inf.). Moreover, it is on the low end for comparisons among the 23 contemporary spawning groups (control region: F_{ST}=0.000–0.801, χ^2=0.00–Inf., μsat: F_{ST}=0.000–0.334, χ^2=14.22–Inf.; Table 3.5, Appendix 3.5). Thus, there is no genetic evidence for the hypothesis that the blue pike is a distinct taxon from walleye.

Both sets of historic samples differ in haplotypic and allelic frequencies from contemporary walleye spawning in Lakes Erie and Ontario, according to F_{ST} (control region=0.336–0.399, μsat=0.132–0.221; Table 3.5a) and χ^2 analyses (control region=5.60–Inf., μsat=Inf.; Table 3.5b). In addition, the genetic composition of the historic samples differs from all modern walleye spawning groups, with one exception (Table 3.3, Appendix 3.5). For the control region sequences, the historic samples show genetic similarity to the contemporary walleye population from Pigeon Lake (S) in the Lake Ontario drainage (F_{ST}=0.092–0.145, χ^2=5.60–6.12; Table 3.5a–b). This appears due to the high frequency of haplotype A3. Additionally, we examine the divergence of modern walleye with bright turquoise colored mucus versus standard yellow-colored walleye from McKim Lake (D); there was no genetic distinction between them (control region: F_{ST}=0.000, χ^2=0.00, μsat: F_{ST}=0.000, χ^2=14.05). In contrast, the historic walleye and blue pike samples significantly differ from both the modern yellow and turquoise

mucus walleye (control region: F_{ST}=0.627-0.672, χ^2=Inf., μsat: F_{ST}=0.384-0.440, χ^2=Inf.).

AMOVA analyses show no overall significant difference between historic and modern samples for the control region sequences, but differ with the μsat loci (Table 3.4). The overall difference between the historic versus the modern samples is about 2–5 times greater than the F_{ST} values that differentiate among modern walleye spawning groups for the μsat data. Overall, these analyses indicate that μsat allelic frequencies have changed from the historic samplings to the present.

The historic samples genetically differ from modern walleye spawning groups, according to GENECLASS assignment tests (Table 3.5c), STRUCTURE (Fig. 3-4b), and the combined genetic distance tree (Fig. 3-5). Overall, 89% of the historic samples self-assign in GENECLASS. The historic walleye self-assign frequently (77%, *N*=24/31), with just 10% mis-assigning to modern eastern Lake Erie basin walleye (P), followed by 6% to the historic blue pike (R), and 6% to samples from the modern western basin of Lake Erie (O; Table 3.5c). Historic blue pike individuals self-assign 48% of the time (*N*=12), with 48% mis-assigning to the historic walleye. A single blue pike individual assigns to modern eastern basin walleye (4%). Likewise, STRUCTURE and the genetic distance tree separate the historic samples together into a distinct group that has 93% bootstrap support (Fig. 3-4b, 3-5).

3.4.6 Morphological variation among historic samples

Historic walleye, blue pike, and sauger samples broadly overlap for the characters analyzed, but overall significantly differ in the morphometric (Wilks' Λ=0.368,

F=10.534, df=2, p<0.001) and meristic data sets (Wilks' Λ=0.563, F=9.933, df=2, p<0.001; Table 3.2); this difference however, is primarily due to sauger versus the walleye and blue pike, and not between the latter. Sauger individuals differ from walleye and blue pike in 11 of 16 total characters, including body depth, head and upper and lower jaw lengths, head and interorbital widths, and numbers of second dorsal and anal fin rays. The sauger also differs from the walleye and blue pike for the two morphometric ratios, upper:lower jaw and orbit:interorbital. Four characters (standard and cheek lengths, and numbers of first dorsal and pectoral fin rays) show no difference among sauger, historic walleye, and blue pike (Table 3.2).

Slight morphological differences between walleye and blue pike are found for only three of the 16 characters: head and interorbital widths, and numbers of second dorsal fin rays (Table 3.2). The blue pike tends to have the smallest head and interorbital widths and the highest number of second dorsal fin rays. However, the ranges of these characters overlap, making them not clearly diagnostic. The walleye and blue pike also differ in orbit:interorbital ratio, with blue pike having the largest ratio (mean=1.6, range=1.0-2.2 vs. 1.4, range =1.1-2.0 for walleye) The blue pike also tends to have more pelvic fin rays than sauger or walleye. The ranges of all of these characters extensively overlap. None of these characters permit reliable distinction of a blue pike from a walleye specimen.

PCA further explores the extent of morphological variation among the historic walleye, blue pike, and sauger specimens, with the first four morphometric PCs explaining 88% of the overall variation and the first three meristic PCs explaining 80%. A MANOVA shows that the three taxa significantly differ for the morphometric (Wilks'

Λ=0.438, F=19.268, df=2, p<0.001) and meristic (Wilks' Λ=0.645, F=12.354, df=2, p<0.001) PCAs, which is readily discernable by the differences among their mean component scores on Fig. 3-6. Morphometric PC1 is the sole component to distinguish among the three taxa (ANOVA, F=85.172, df=2, p<0.001). Morphometric PC1 is highly correlated with head width (r=0.794), body depth (r=-0.342), and interorbital width (r=0.312), with sauger having the greatest measurements for head width (mean=48.6, range=33.8-67.8) and interorbital width (mean=16.1, range=12.5-19.1) and the smallest body depth (mean=18.1, range=13.5-24.1). Walleye have intermediate head width (mean=41.6, range=34.3-55.0), interorbital width (mean=14.9, range=12.8-17.3), and body depth (mean=20.1, range=15.8-24.2) and blue pike have the smallest head (mean=38.6, range=33.6-43.9) and interorbital widths (mean=14.1, range=11.2-17.2) and largest body depth (mean=20.4, range=16.3-24.9).

Meristic PC1 also differentiates sauger from the other two groups (ANOVA, F=29.340, df=2, p<0.001), for numbers of second dorsal (r=-0.700) and anal fin rays (r=-0.600). Sauger has the fewest second dorsal (mode=19, range=18-21) and anal fin rays (mode=12, range=10-16). Walleye and blue pike are not distinguishable based on meristic PC1. However, meristic PC3, distinguishes blue pike specimens from walleye and sauger samples PC3 (ANOVA, F=9.024, df=2, p<0.001), in numbers of anal (r=0.721) and second dorsal fin rays (r=-0.665; see Fig. 3-6), with walleye and sauger showing more similarity. Meristic PC2 does not distinguish among the three taxa.

The quadratic DFA correctly assigns just 68% of the samples with the morphometric data set and 60% with the meristic characters. Each of the groups shows higher self-assignment, suggesting slight morphological separation (Table 3.6), with

sauger having higher self-assignment in the morphometric and meristic data sets (73 and 78% respective self-assignment). Sauger have some mis-assignment to the historic walleye or blue pike samples (11-16%, 6-9 individuals). The historic walleye and blue pike most often self-assign, but show high mis-assignment to the other groups. Historic walleye self-assign 65 and 45% for the two morphological datasets, and blue pike self-assign 66 and 53%. The historic walleye and blue pike both tend to mis-assign to sauger (12-31% of the samples) with walleye having the highest value in the meristic data set (31%). The historic walleye and blue pike also mis-assign to each other 22–33%. This pattern of mis-assignment between walleye and blue pike is similar to the GENECLASS results for these two groups, with 6% of walleye individuals mis-assigning to blue pike and 48% of blue pike samples to walleye (Table 3.3c). Thus our results do not support taxonomic designation of the blue pike as a subspecies or species status, since there is no reliable morphological differentiation and they appear genetically congruent (with no unique haplotypes or alleles) to historic and modern walleye.

3.5 Discussion

Our findings reveal significant genetic structure and substantial genetic diversity across the contemporary range of walleye. Patterns of walleye today correspond to a genetic isolation by geographic distance hypothesis across its range. Divergences across the northern range are explained by differential contributions from three glacial refugia. Microsatellite DNA frequencies of walleye from ~70 years ago in Lake Erie significantly vary from those today; this difference corresponds to a level characteristic among closely

located populations. The blue pike from that time, which became extinct by the 1960s, is only slightly different from the typical walleye in Lake Erie both genetically and morphologically, on the order of a nearby population. We explain these patterns below, in relation to the major questions raised in the introduction.

3.5.1 Genetic divergence among contemporary walleye populations, and their relationships to glacial refugia (Question 1)

Ancestral walleye diverged ~15.4 Mya during the late Miocene Epoch from a common ancestor shared with its sister species, the sauger (see Haponski & Stepien 2013a). Modern walleye haplotypes later differentiated ~10.6 Mya, with the earliest and most divergent walleye haplotype, B19, originating in the southeast Ohio and New Rivers (W–X). This coincided with rapid climate changes and transitions from grasslands to forests (see Cerling *et al.,* 1997; Fox, 2000). This Ohio River group has remained relatively genetically distinct from all other walleye (see Stepien & Faber, 1998; Palmer *et al.,* 2006; Stepien *et al.,* 2009) Yellow perch populations along the Atlantic coast likewise show high differentiation from other populations (Sepulveda-Villet & Stepien, 2012).

The remaining walleye contain 27 haplotypes, whose divergences trace to ~7.2 Mya during the late Miocene Epoch. The oldest and most widely distributed haplotypes are A1–3, which are absent only from the southern relict site in the North River of Alabama. Previous studies, which lacked the dating of recently discovered *Sander* fossils in North America, mistakenly dated the origin of those walleye haplotypes during the Pleistocene glaciations ~2.6–0.1 Mya (see Billington *et al.,* 1992; Stepien & Faber,

1998), much later than the calibrations here. This present study has corrected the dates for their origins, by ground-truthing to recently discovered fossils described by Murray *et al.* (2009) and Murray & Divay (2011), as outlined in our phylogenetic analysis (Haponski & Stepien, 2013a). Haplotypes A1–3 likely once were widespread across the range, with haplotype A3 originating to the east and being less prevalent to the west. Haplotype A3 was most abundant in the Atlantic Refugium, and was absent from the Missourian Refugium. In contrast, haplotype A1 originated in the west, likely in the Mississippi River watershed, and is less common to the east. Haplotype A1 dominated both the Missourian and Mississippian Refugia. Haplotype A2 is most common in the central portion of the range, and is prevalent in the Mississippi River watershed and likely was common in the Mississippian glacial Refugium.

The North River is dominated by endemic haplotype B20, which also differentiated ~7.2 Mya during the late Miocene Epoch. This river system has remained vicariantly separated from the other walleye sites we sampled, as it drains south into the Gulf of Mexico. That population is relatively small, very isolated, and relatively low in diversity. Other studies also have discerned the uniqueness of walleye populations along the Gulf Coast drainage, including Stepien *et al.* (2009) for this μsat data base, and Billington *et al.* (1992); the latter used mtDNA RFLPs. Boschung & Mayden (2004) noted that the Tombigbee River drainage, which includes the North River, is at risk of colonization from more northern spawning groups due to connections through the Tombigbee-Tennessee River waterway. In our study, the North River is the sole location to possess haplotype B20. Thus, if walleye from other areas were introduced, then this unique haplotype and diversity might be lost. Likewise, yellow perch from the Gulf

Coastal drainage show a high degree of endemism possessing unique haplotypes and diversity, and high divergence from other populations (Sepulveda-Villet & Stepien, 2012). These southerly populations of walleye and yellow perch represent unique genetic sources that may be valuable for conservation.

A few additional haplotypes form distinct groups, whose divergences range from ~2.6–1.0 Mya, tracing to the Pleistocene Epoch. The abundant haplotype A4 originated ~2.5 Mya during the early Pleistocene Epoch, likely in the Atlantic coastal Refugium (see Billington *et al.*, 1992; Stepien & Faber, 1998). This haplotype later spread across the Great Lakes from the east after the glaciations, remaining most prevalent in the east. This haplotype appears to have decreased in abundance in Lake Erie over recent decades, according to comparisons of historic with present-day data. This may be due to climatic warming. Haplotypes B11, 16, and 26–27 originated ~1.6 Mya in the Missourian Refugium, and today occur exclusively in the north. B11 also is found in Lac Mistassini to the northeast.

Populations from the northern lakes retain close genetic relationships today. Glacial Lake Agassiz formed ~14 thousand years ago (kya) as the ice sheets retreated, encompassing the Lake Winnipeg drainage as well as part of northern Lake Superior (see Larson & Schaetzl, 2001; Lepper *et al.*, 2013), including Lake Nipigon. Those walleye had inhabited the Missourian glacial Refugium, and then recolonized the northwest as glacial Lake Agassiz formed. Lake Agassiz later (~8.4 kya) joined with glacial Lake Ojibway to cover most of the Canadian Shield lakes and extended over to Hudson Bay, encompassing Lac Mistassini (see Barber *et al.*, 1999; Teller *et al.*, 2002). Walleye likely used Lake Agassiz's outlets to disperse across the north throughout much of north-central

Canada, as indicated by the relationship among walleye populations in the Lake Winnipeg (A–D), Upper Mississippi River (E), and Lac Mistassini (V) drainages (separated by barrier 2 on Fig. 1c). Once the ice sheets and proglacial lakes were gone, individual walleye populations then differentiated, as demonstrated by their high nuclear μsat loci F_{ST} values. These high values support natal homing and a tendency to return to given sites to spawn, as shown by tagging data for walleye today (see Belore *et al.*, 2010).

The genetic structure of contemporary walleye populations in the Great Lakes region retain evidence of differential contributions and pathways from multiple glacial refugia, including the Missourian, Mississippian, and Atlantic coastal. Our results correspond to findings for walleye populations using allozymes (Ward *et al.*, 1989), mtDNA RFLPs (Billington *et al.*, 1992), mtDNA control region sequence data (Stepien & Faber, 1998), and nuclear μsat loci (Stepien *et al.*, 2009, 2010; Walter *et al.*, 2012).

Walleye in Lake Superior (F–H) show a mixed signature, with ties to the Missourian and Mississippian refugia. Populations spawning in the St. Louis River (F) and Lake Nipigon (G) show predominant ties to the Missourian refugium. In contrast, the population spawning in Portage Lake (H) groups with the other Great Lakes in our analyses, tracing its ancestry to the Mississippian refugium. Likewise, the ancestors of most walleye spawning in Lakes Michigan, Huron, St. Clair, Erie, and Ontario recolonized from the Mississippian refugium. The eastern basin of Lake Erie and all of Lake Ontario additionally were founded by walleye from the Atlantic coastal glacial refugia; this is similar to patterns described by Billington & Hebert (1988), Stepien & Faber (1998), and Stepien *et al.* (2009). Notably, control region haplotypes B12 and 22 in

eastern Lake Erie and Oneida Lake, diverged ~1.0 Mya in the Atlantic refugium (see Fig. 3-1). Today they are endemic to that region, reflecting their relationship. The founding populations later differentiated due to spawning site fidelity and natal homing; many of these distinctions remain today.

Other fishes show this mixed genetic signature from the three refugia across their range. Notably, Sepulveda-Villet & Stepien (2012) discerned contribution of the Missourian refugium to northwestern groups of yellow perch using mtDNA control region sequences and 15 μsat loci. They found that spawning groups of yellow perch in Lake Superior originated from both the Missourian and Mississippian refugia, those in Lakes Michigan through the western basin of Lake Erie to the Mississippian refugium, and those from the eastern basin of Lake Erie and Lake Ontario to a mixture from the Mississippian and Atlantic coastal refugia. This pattern for yellow perch thus matches that of walleye. Smallmouth bass likewise show a similar pattern to yellow perch and walleye, based on mtDNA cytochrome *b* sequences and eight μsat loci (see Borden & Stepien, 2006; Stepien *et al.,* 2007; Karsiotis *et al.*, in review). Like walleye and yellow perch (Sepulveda-Villet & Stepien, 2012), smallmouth bass (Stepien *et al.,* 2007) show high divergence among spawning groups across their range, indicating pronounced differentiation following the glaciations when populations became isolated in separate drainages and spawning groups.

Yellow perch (control region mean F_{ST}=0.469, μsat=0.236; Sepulveda-Villet & Stepien (2012) and smallmouth bass (control region mean F_{ST}=0.412, μsat=0.232; Stepien *et al*. (2007), Karsiotis *et al*. in review) show much higher divergence among spawning groups across their ranges in comparison to walleye (control region mean

F_{ST}=0.236, μsat=0.100). This may be due to greater degree of spawning group fidelity by yellow perch and smallmouth bass, and their more limited migration (see Rawson, 1980; Lyons & Kanehl, 2002). Walleye are capable of dispersing the greatest distances from 50–300 km (see Colby *et al.*, 1979), followed by yellow perch up to 48 km with occasional individuals travelling 200 km (see Rawson, 1980), and smallmouth bass moving the least - up to 10 km (Lyons & Kanehl, 2002).

Studies also have shown that the European perch *Perca fluviatilis* (in the same genus as yellow perch and the same family as walleye) discriminates kin from non–kin via olfactory cues, and schools in full and half–sib groups maintained throughout their lives (Gerlach *et al.*, 2001; Behrmann-Godel & Gerlach, 2008). Thus, family groups of the European perch appear to move and reproduce together, genetically diverging from non-kin groups (Gerlach *et al*., 2001). This life history pattern remains to be tested for yellow perch, walleye, or smallmouth bass. However, kinship tests by our laboratory for the three species show high proportions of full siblings in spawning groups of yellow perch (mean=0.20 ranging to 38%; Sullivan & Stepien, in review) and smallmouth bass (0.15 ranging to 67%; Karsiotis *et al.*, in review), which are nearly twice those discerned here for walleye (0.08 ranging to 40%). This limited lifetime migration and apparent close association among kin for yellow perch and smallmouth bass, likely results in their higher divergences among proximate populations. Walleye seem to exhibit an intermediate level of divergence, but a higher degree of diversity due to more outbreeding. Thus it appears that the biogeographic patterns of today's widely distributed North American species largely result from a combination of extrinsic (i.e., changes in ancient and contemporary climate and drainage patterns) and intrinsic factors (dispersal

capabilities, degree of natal homing, population size, and inbreeding), which vary in degree according to behavior of the taxon.

3.5.2 Contemporary walleye genetic diversity patterns (Question 1)

Our data discern relatively high genetic diversity levels for most spawning groups mtDNA control region sequences (mean H_D=0.53, range=0.15-0.82) and nuclear μsat loci (mean H_O=0.68, range=0.52-0.77). These values are similar to those reported by Stepien *et al.* (2009) for walleye populations across their range using nine μsat loci (mean H_O=0.68, range=0.51–0.78). The difference between the mtDNA and nuclear μsat loci is attributable to the slower evolutionary rate of mtDNA control region sequences compared to nuclear μsat loci (Hewitt, 2001; Wang, 2010, 2011). Mitochondrial DNA sequences have ¼ the effective population size of nuclear DNA, rendering mtDNA more subject to declines in variability from population bottlenecks (Avise, 2004).

Walleye reproducing in the lower Great Lakes (Lakes St. Clair and Erie) have the highest diversity values we observed (mean H_D=0.76, range=0.72–0.82, H_O=0.73, range=0.70–0.75), likely due to the admixture descending from two glacial refugia (the Mississippian and Atlantic) and larger population sizes, both historic and present. In contrast, some small populations in the upper Great Lakes have the lowest mtDNA control region diversity values (mean H_D=0.20, range=0.15–0.23), but are higher with the μsat loci (mean H_O=0.72, range=0.68–0.77). Notably, our sample from the Moon/Musquash River in Georgian Bay of Lake Huron (site L) has the lowest mtDNA control region diversity value (H_D=0.15) across the range of walleye. However, we find much higher diversity at this location using the nuclear μsat loci (H_O=0.71), similar to

levels characterizing other sites across the range. Gatt *et al.* (2002) also recovered low mtDNA diversity at this location using RFLPs and control region sequence data, and attributed this to stocking and overexploitation. Our study finds that this pattern is restricted to mtDNA alone and likely is attributable to the evolutionary properties of mtDNA. This also may reflect bottlenecking and founder effects, as well as small population sizes at this location (see Gatt *et al.,* 2002).

Yellow perch populations across their range show much lower diversity levels compared to walleye using mtDNA control region sequences and 15 nuclear μsat loci (mean H_D=0.31, range=0.00-0.82, H_O=0.53, range=0.33-0.67; Sepulveda-Villet & Stepien, 2012). This is true of both genomes, and also is true of the related European perch *P. fluviatilis* (Nesbø *et al.,* 1999), and another member of the family Percidae, the ruffe *Gymnocephalus cernua* across their respective Eurasian ranges (Stepien *et al.,* 1998, 2005). This difference among percid groups thus may reflect their respective evolutionary history and behavior. Notably, the strong association of Eurasian perch in kin groups (Gerlach *et al.,* 2001; Behrmann-Godel & Gerlach, 2008) and high proportions of full siblings of yellow perch in spawning groups (Sullivan & Stepien, in re-review) may lead to lower diversity from inbreeding.

Smallmouth bass spawning groups show relatively lower genetic than found in walleye, as described from mtDNA cytochrome *b* sequences (mean H_D=0.50, range=0.00-0.85; Karsiotis *et al.,* in review) and eight nuclear μsat loci (H_O=0.46, range=0.15-0.59; Stepien *et al.,* 2007). The modest diversity of smallmouth bass, along with their high divergence among sites, may reflect association of kin groups and limited lifetime migration (see Karsiotis *et al.,* in review). Thus, genetic diversity and divergence

among spawning populations in smallmouth bass (Stepien *et al.*, 2007; Karsiotis *et al.*, in review) and yellow perch (Sullivan & Stepien, in re-review) may differ from walleye due to reproductive behavior and life history characters.

Small isolated walleye populations in the southeast have lower genetic diversity values (mean H_D=0.43, H_O=0.62) than those in northerly, formerly glaciated regions. They also contain the oldest and most unique haplotypes and μsat alleles compared to other groups. This pattern of low diversity with unique haplotypes and μsat alleles likely is due to long-term isolation of these small groups, accompanied by genetic drift and population bottlenecks (see Stepien *et al.*, 2009). Gulf Coastal populations of yellow perch also have the lowest diversity values across their range, with many unique haplotypes and alleles (Sepulveda-Villet & Stepien, 2012), similar to walleye. Due to their uniqueness, these populations represent a historic source of diversity and an important genetic resource for both species. These populations have adapted to these warmer environments and may provide a critical genetic reservoir in the face of climate change.

3.5.3 Historic vs. contemporary genetic patterns of walleye and blue pike (Question 2)

Apparently, lower genetic diversity characterized the historic Lake Erie walleye (control region=0.10, μsat=0.40) and blue pike samples (control region=0.00, μsat=0.54) from 1923-1949 compared with contemporary samples from Lakes Erie (control region=0.76–0.82, μsat=0.73–0.74) and Ontario (control region=0.47–0.62, μsat=0.69–0.73). This suggests an increase in genetic diversity over the past 70+ years, which may reflect population recovery.

Lake Erie was settled by Europeans during the 1700-1800s, who cut down forests and drained the marshlands, which both were gone by 1900 (summarized by Hartman, 1973; Trautman, 1981). As the region was developed and industrialized, untreated wastes were released into the Lake from saw mills, slaughter houses, and steel factories. By 1830, Lake Erie had a strong and important commercial fishery. In 1874, a major shipping channel was constructed that drastically modified the Lakes Huron-Erie Corridor (HEC) that ran through the St. Clair River, Lake St. Clair, and the Detroit River. Lake Erie steadily lost much of its fish habitat from 1900 through the 1970s, due to draining of wetlands, armoring of shorelines, channelization, dredging, and increased industrialization (Hartig *et al*., 2009; USGS, 2010; Bennion & Manny, 2011). High levels of phosphorus during the 1960s caused massive algal blooms, accompanied by oxygen depletion and marked fish die offs (Hartig *et al.,* 2009).

As a result of industrialization, overfishing, and pollution, native fish populations in Lake Erie experienced a steady decline from 1900 through the 1970s, including lake trout, lake sturgeon *Ascipenser fulvescens*, blue pike, and walleye (summarized by Hartman, 1973; Ryan *et al.,* 2003). The blue pike disappeared by the 1960s (Parsons, 1967; see Ryan *et al.,* 2003). Additionally, industrial outputs resulted in heavy metal contamination and declining fish health, including neoplasms, tumors, and lesions on walleye and other species (Manny & Kenaga, 1991). In 1970, the walleye fishery from Lakes Huron through Lake Erie was closed due to high levels of mercury in the tissues. By 1978, fisheries managers declared Lake Erie walleye as being at a crisis level directly related to overfishing and pollution (see Hartig *et al.,* 2009).

The lower number of alleles and diversity seen here for the historic walleye and blue pike from 1923-1949 may reflect these population declines. Rare haplotypes and alleles identified here for the historic walleye (N_{PH}=1, N_{PA}=6) may have disappeared. Other than those haplotypes and alleles, the others we found in the historic samples from Lake Erie are those that are common and widespread. The historic samples appeared to have more haplotypes that descended from the Atlantic refugium, whereas today's walleye populations have more that descended from the Mississippian refugium. This merits further testing.

Likewise, other Great Lakes fishes showed a similar pattern of lower genetic diversity from 1927-1959 and higher diversity in samples from 1995-2005 (see Guinand *et al.,* 2003; Stott *et al.,* 2013). This was attributed to population declines resulting from environmental conditions and overexploitation. Notably, seven μsat loci showed that lake whitefish *Coregonus clupeaformis* samples from Lakes Huron and Erie in 1927 had lower diversity (0.60) compared to those taken from 1997-2005 (0.65; Stott *et al.,* 2013). One site within Lake Erie's western basin (near Put-In-Bay, South Bass Island, OH) had the lowest historic diversity level (0.52), similar to the low values found here. Lake trout sampled from Lakes Superior, Michigan, and Huron in 1940-1959 also had lower diversity (0.47) compared to those from 1995-1999 (0.51) using five μsat loci (Guinand *et al.,* 2003).

In a similar case, the Atlantic cod *Gadus morhua* experienced a decline in diversity and number of alleles from 1954 to the 1980s, followed by an increase from 1980–98 according to archived otolith samples and three μsat loci (Hutchinson *et al.,* 2003). That research study documented a significant difference in genetic composition

between samples from 1954 and 1998, similar to the pattern shown here for historic samples versus modern walleye in Lake Erie. Hutchinson *et al.* (2003) attributed these patterns in Atlantic cod to the effects of genetic drift on reduced population sizes caused by intense overexploitation. The Atlantic cod recovered from these marked declines in diversity due to immigration from a nearby spawning group, which introduced new genetic material (see Hutchinson *et al.*, 2003).

The apparent increase in genetic diversity and number of alleles for walleye from contemporary sampling may have resulted from migration and recruitment of individuals from other populations, such as other areas of Lake Erie or other lakes, such as Lake St. Clair. Walleye display natal homing, and chemical cues are presumed to facilitate recognition of reproductive grounds (Olson & Scidmore, 1962; Colby & Nepszy, 1981; Backhouse-James & Docker, 2012). A study by Olson & Scidmore (1962) found that walleye homing was less prevalent in areas where habitat degradation had occurred. Our modern western Lake Erie spawning group samples show similarity to those in Lake St. Clair, indicating a potential source of individuals. The movement of walleye between Lakes St. Clair and Erie during non-spawning times has been well documented with tagging studies (Haas *et al*., 1985; Todd & Haas, 1993; Wang *et al*., 2007). Moreover, Haponski & Stepien (2013b) found that walleye from Lake Erie may have migrated into the highly degraded Detroit River following habitat augmentation to spawn at a newly constructed reef.

It also is possible that Lake Erie walleye were able recover from the declines in diversity due to rapid increases in population abundances following the closure of their fishery in the 1970s, at their low point of ~10 million fish in 1978, and implementation of

the U.S. Clean Water Act, the Canada Water Act, and the Canada-U.S. Great Lakes Water Quality Agreement (summarized by Hartig *et al.,* 2009). Over the past few decades, water temperatures have increased, especially in the shallow western basin, with the Lake Erie fish community changing from being dominated by cold water species, such as lake trout, to having greater abundances of warmer water species, including walleye and yellow perch. The decline of colder water competitors may have aided walleye abundance (see Ryan *et al.,* 2003). By 1988, walleye numbers in Lake Erie had rebounded to ~80 million fish (8x the level from 1978; WTG (Walleye Task Group of the Lake Erie Committee), 2013). Since that time, walleye numbers have fluctuated in Lake Erie, with numbers declining in recent years, to ~18 million in 2013. Recent genetic analyses show temporal consistency in genetic diversity within Lake Erie walleye spawning groups over the past 20 years (Stepien *et al*., 2012). Notably, three Lake Erie spawning groups have maintained genetic consistency within specific spawning runs from 1995–2008, including the Maumee River (0.71, N=250), Sandusky River (0.74, N=227), and Van Buren Bay Reefs (0.76, N=249) using nine μsat loci (Stepien *et al*. 2012; seven of the ones used here).

In comparison to walleye spawning in Lake Erie, populations from other areas have shown a mixture of temporal stability and decline. Franckowiak *et al.* (2009) discerned temporal genetic consistency over 50 years (1952–2002) for walleye spawning in Escanaba Lake, Wisconsin (mean H_O=0.76) using eight μsat loci (six of those used here). However, that population was stocked and monitored by fisheries biologists over this time period, likely circumventing fluctuations in abundance that could have resulted in declines due to drift and inbreeding. Haplotypic diversity of walleye spawning in Lake

Huron's Georgian Bay declined over three decades (from 0.50 in the 1960s to 0.15 in the 1990s), attributed to exploitation and stocking (according to mtDNA RFLPs by Gatt *et al.* (2002)). We here recover a similarly low value (0.15) for walleye spawning in the Moon/Musquash Rivers of Georgian Bay Lake Huron from mtDNA control region sequences, indicating that the population's mtDNA diversity remains low. This effect appears to characterize Georgian Bay Lake Huron walleye spawning groups, but appears restricted to mtDNA along, with nuclear DNA variability being average.

Other alternatives may explain the lower diversity levels we observed in the historic samples. The first is that walleye reproduce in the spring, returning with their respective groups to their natal sites (see Colby & Nepszy, 1981; Jennings *et al.*, 1996; Stepien & Faber, 1998). Our modern samples were adult walleye collected from spring spawning runs at specific spawning sites. In contrast, the historic samples we analyzed were sampled from July–November, when walleye intermix in deeper portions of the lake to feed. Thus, the lower genetic diversity seen here may have resulted from the admixture of historic spawning groups (i.e., a Wahlund effect; see Waples, 1990).

Secondly, several authors have documented the difficulty of working with historically archived samples. For example, Nielsen & Hansen (2008) provided recommendations for validating genetic data from historic samples. They recommended running positive and negative controls, using a separate laboratory space and separate chemicals, running the program Micro-Checker to test for null alleles, using samples with complete documentation of biological information, testing for HWE, and applying more than one statistical test to ensure patterns match. We followed all of these precautions to ensure the reliability of data from our formalin fixed historic samples. In addition, other

studies have documented issues with historic samples having biased amplification of alleles with shorter lengths (see Taberlet *et al.,* 1996; DeWoody *et al.,* 2006; Chapuluis & Estoup, 2007; Ugelvig *et al.,* 2011). We found evidence for a somewhat higher number of null alleles in the historic samples, with shorter allele lengths being more prevalent than larger ones. This could have been the result of template quality issues where only partial repeats were amplified due to primer sites not being available for binding (Ugelvig *et al.,* 2011), which may have been induced by shearing of DNA during formalin fixation (Shedlock *et al.,* 1997). However, our Micro-Checker tests and other analyses point to lack of statistical support for that scenario of inferior amplification. Also, our mtDNA sequences showed the same pattern as the μsat analyses. Further analysis of historic walleye and blue pike samples is warranted, perhaps using archived scale samples, instead of the formalin fixed individuals. Here we analyzed tissues from the preserved whole-fish paratypes from which the blue pike was described, avoiding morphological identification questions (as would be case with scales). It would be helpful to increase sample sizes and to examine the intermediate decades of variation in walleye to further understand their population genetic patterns.

3.5.4 Taxonomic status of the historic blue pike (Question 3)

We discern that the historic blue pike was not genetically distinctive from historic and modern walleye populations. Thus the name *S. v.* "*glaucus*" is invalid. There are some slight morphological variations, in head width, interorbital width, and orbit:interorbital ratios between the historic walleye and the blue pike samples. These suggest some slight population-level variation, but no overall reliable distinctiveness. The

turquoise-mucus walleye from McKim Lake have no genetic difference from co-occurring modern yellow walleye. The color difference is due to sandercyanin, a pigment characterizing the turquoise-mucus (see Yu *et al.,* 2008). The turquoise, as well as yellow walleye from McKim Lake have none of the morphological features of blue pike. Moreover, yellow perch and other fishes from these northern waters also produce the turquoise sandercyanin mucus.

Several fishes including lake trout, whitefish *Coregonus* spp., and arctic charr *Salvelinus alpinus*, have multiple morphological races that developed as a result of adaptation to unique environments in northern proglacial lakes (summarized by Bernatchez & Wilson, 1998; Robinson & Parsons, 2002). These morphological races are often accompanied by little genetic divergence among them (see Bernatchez & Wilson, 1998), resembling the pattern here for historic walleye and blue pike. In contrast, yellow perch from the northern and southern shore of Lake Erie show distinct morphometric and genetic differentiation (Kocovsky *et al.,* 2013). This difference for yellow perch likely is attributable to its more limited migration and closer association of kin groups, resulting in increased differentiation. The blue pike was reported to inhabit the deeper waters of Lake Erie's eastern basin, exhibit a slower growth rate (Parsons, 1967), and had a larger eye (our results) compared to walleye. However, such a slower growth rate also characterizes walleye that spend their lives in the eastern basin (summarized by Einhouse & MacDougall, 2010). Thus it appears that although the extinct blue pike had some slight morphological difference from other walleye, this was rather negligible, and unaccompanied by genetic distinction. Blue pike were walleye, and fell within the range of normal population variation of walleye.

3.5.5 Effects of climate change on walleye populations

In the next 50 years temperatures across the globe are predicted to increase, with the Great Lakes region rising by 5–5.5 °C, becoming more like today's Gulf Coast (Hayhoe *et al.*, 2010). The lower Great Lakes today house the greatest abundance of walleye (Hubbs & Lagler, 2004; see Cichosz, 2009), as well their greatest genetic diversity (shown here). Increased temperatures are predicted to shift the distribution of walleye northward (see Chu *et al.* 2005). Populations currently located at the southern fringes of their range may experience increased isolation, unless new waterway connections occur. If their respective isolation increases, they likely will be at greater risk of genetic bottlenecks and drift. This may cause an overall decline in variation, accompanied by loss of unique haplotypes and alleles. Fringe locations to the north, such as Lac Mistassini, may also experience such phenomena and diversity decline. Thus, valuable genetic resources may be lost as temperatures continue to climb and populations become further isolated.

The high connectivity of the Great Lakes region allows ample dispersal opportunities, which may lead to homogenization of genetic diversity and divergence, as distinct spawning groups move northward and mix. This might lead to a Wahlund effect, when genetic diversity is reduced due to mixing of divergent populations (see Waples, 1990). Thus, climate change may result in a decline of divergence patterns among the walleye spawning groups of today. Walleye likely will remain abundant and adapt, but unique allelic variants may be lost. Common alleles likely will increase in frequency, raising concerns for retaining adaptive genetic potential. Conservation of genetic

diversity and divergence patterns likely should increase as a management priority. It might be possible to utilize genes from the unique warm-adapted variants to the south (i.e., as in the North River) and the southeast (the New and Ohio Rivers) to aid walleye populations of the future.

The present study increases our understanding of historic and contemporary walleye genetic diversity and divergence patterns through this genetic window approach. Population patterns were shaped by climate change and drainage connections, tracing to recolonization after the Pleistocene glaciations. Genetic diversity and population sizes of walleye appear to have rebounded after drastic habitat changes and industrialization of the early 20th century. The next step is to discern the adaptations that underlie the genetic diversity and divergence patterns discerned here, via genomics (see Allendorf *et al.*, 2010; Ouborg *et al.,* 2010; Bradbury *et al.,* 2013). Such applied knowledge will aid efforts to sustain natural populations in the face of ongoing climate change and new anthropogenic stressors.

3.6 Acknowledgements

This is publication #20XX–XX from the University of Toledo's Lake Erie Center. This work was funded by grant awards to CAS from NOAA Ohio Sea Grant Program R/LR-013 and USEPA CR-83281401-0, and to AEH from Sigma Xi Grants In Aid of Research. AEH was supported by a NSF GK-12 DGE#0742395 fellowship (P.I. = CAS), University of Toledo teaching and research assistantships, the International Association for Great Lakes Research (IAGLR) Norman S. Baldwin Fishery Science Scholarship (2010), and the IAGLR Scholarship award (2009). We thank the U.S. Fish and Wildlife

Service, U.S. Geological Survey, Ohio and Michigan Departments of Natural Resources, Ontario Ministry of Natural Resources, and Keweenaw Bay Indian Community Natural Resources Department for contributing samples. We also thank M. Bagley, L. Bernatchez, N. Billington, E. Hallerman, W. Jansen, G. Mensch, D. Nelson, W. Schaefer, M. White, and C. Wilson for additional collection assistance. We are grateful to J. Bossenbroek, T. Fisher, P. Kocovsky, and Great Lakes Genetics/Genomics Laboratory members L. Pierce, C. Prichard, T. Sullivan, and S. Yerga-Woolwine for assistance in the laboratory and valuable comments on various versions of the manuscript. Lake Erie Center staff P. Uzmann, M. Gray, and R. Lohner provided logistic support.

Table 3.1

Genetic diversity values of 23 modern walleye spawning groups, historic yellow walleye, historic blue pike, and sauger samples based on mtDNA control region sequence data and seven nuclear μsat loci. Results based on the seven loci are identical to those for the nine loci (data not shown; also see Stepien *et al.*, 2009, 2010).

				Control region			
Drainage	Location	Lat.	Long.	*N*	H_D±S.E.	N_H	P_{PH}
L. Winnipeg	---	---	---	100	0.64±0.00	8	0.38
	A. Cedar L.	53.3300	-100.1000	25	0.77±0.01	6	0.33
	B. L. Winnipeg	52.7388	-97.8628	25	0.53±0.01	3	0.33
	C. L. of the Woods	49.0367	-94.9272	25	0.33±0.02	2	0.00
	D. McKim L.	50.8669	-92.8031	25	0.61±0.02	4	0.00
Up. Mississippi R.	E. Mille Lacs	46.2326	-93.6477	25	0.16±0.02	3	0.00
L. Superior	---	---	---	75	0.65±0.00	5	0.20
	F. St. Louis R.	46.6679	-92.2889	25	0.50±0.01	2	0.00
	G. L. Nipigon	49.7237	-88.6145	25	0.23±0.02	3	0.33
	H. Portage L.	47.0225	-88.5097	25	0.64±0.01	3	0.00
L. Michigan	I. Muskegon R.	43.4158	-85.8087	25	0.23±0.02	3	0.00
L. Huron	---	---	---	75	0.45±0.00	5	0.00
	J. Thunder Bay	45.0200	-83.4300	25	0.55±0.02	3	0.00
	K. Flint R.	43.3300	-84.0543	25	0.58±0.02	5	0.00
	L. Moon/Musquash R.	44.9594	-79.8811	25	0.15±0.02	2	0.00
L. St. Clair	---	---	---	120	0.73±0.01	6	0.17
	M. Thames R.	42.3171	-82.4363	25	0.72±0.01	4	0.00
	N. Detroit R.	---	---	95	0.74±0.00	6	0.17
	Belle Isle	42.3469	-82.9535	25	0.78±0.01	5	0.00
	Fighting Is.	42.2378	-83.1295	45	0.73±0.01	6	0.00
	Grosse Ile	42.1177	-83.1781	25	0.77±0.01	4	0.00
L. Erie	---	---	---	150	0.78±0.00	14	0.50
	O. Western basin L. Erie	---	---	100	0.76±0.00	9	0.33
	O1. Huron R.	42.0899	-83.2902	25	0.78±0.01	5	0.20

	O2. Hen Is.	41.8024	-82.7804	25	0.78±0.01	5	0.00
	O3. Maumee R.	41.5594	-83.6492	25	0.72±0.01	4	0.00
	O4. Sandusky R.	41.3421	-83.1091	25	0.76±0.01	7	0.29
	P. Eastern basin L. Erie	---	---	50	0.82±0.00	11	0.36
	P1. Van Buren Bay	42.4600	-79.4100	25	0.76±0.01	5	0.20
	P2. Cattaraugus Ck.	42.5684	-79.1041	25	0.88±0.01	9	0.33
	Q. Historic yellow walleye	---	---	20	0.10±0.02	2	0.50
	R. Historic blue pike	---	---	20	0.00±0.00	1	0.00
L. Ontario	---	---	---	75	0.54±0.00	9	0.44
	S. Pigeon L.	44.4703	-78.4942	25	0.47±0.02	4	0.00
	T. Bay of Quinte	44.0671	-77.0719	25	0.62±0.02	5	0.20
	U. Oneida L.	43.2800	-75.4400	25	0.66±0.01	6	0.50
L. Mistassini	V. Lac Mistassini	50.9500	-73.7000	25	0.48±0.01	2	0.00
Ohio R.	---	---	---	36	0.62±0.00	5	0.20
	W. Ohio R.	39.6675	-80.8641	11	0.85±0.02	5	0.00
	X. New R.	36.7109	-80.9589	25	0.45±0.05	3	0.00
Tombigbee R.	Y. North R.	33.3264	-87.5333	5	0.40±0.11	2	0.50
	Mean modern walleye (23 sites)	---	---	31	0.53±0.02	4	0.25
	Total modern walleye (23 sites)	---	---	711	0.77±0.00	27	---
	Sauger	---	---	25	0.69±0.02	6	---

		7 μsat loci						
Drainage	Location	*N*	H_O±S.E.	F_{IS}	N_A	A_R	P_{PA}	Sib
L. Winnipeg	---	105	0.63±0.01	0.104	51	3.64	0.00	0.11
	A. Cedar L.	25	0.63±0.04	0.025	36	3.35	0.00	0.12
	B. L. Winnipeg	25	0.67±0.02	0.003	38	3.44	0.00	0.08
	C. L. of the Woods	30	0.64±0.03	0.099	39	3.68	0.00	0.00
	D. McKim L.	25	0.57±0.04	-0.021	34	3.00	0.00	0.28
Up. Mississippi R.	E. Mille Lacs	39	0.62±0.02	0.010	38	3.22	0.05	0.15
L. Superior	---	114	0.72±0.01	0.059	67	4.23	0.00	0.18
	F. St. Louis R.	28	0.68±0.02	0.116	56	4.20	0.00	0.14
	G. L. Nipigon	30	0.74±0.03	-0.047	43	3.76	0.00	0.40
	H. Portage L.	56	0.73±0.02	0.001	52	3.97	0.00	0.07
L. Michigan	I. Muskegon R.	50	0.73±0.01	0.057	57	4.20	0.00	0.04
L. Huron	---	119	0.73±0.01	0.031	69	4.16	0.03	0.13
	J. Thunder Bay	40	0.70±0.01	0.015	55	3.88	0.04	0.15
	K. Flint R.	44	0.77±0.01	-0.023	55	4.15	0.00	0.09
	L. Moon/Musquash R.	35	0.71±0.03	0.024	49	3.87	0.00	0.14
L. St. Clair	---	162	0.72±0.01	0.019	77	4.09	0.03	0.06
	M. Thames R.	39	0.75±0.02	0.012	63	4.19	0.00	0.05
	N. Detroit R.	123	0.71±0.01	0.022	72	4.06	0.03	0.07
	Belle Isle	40	0.72±0.02	0.010	62	4.06	0.00	0.00
	Fighting Is.	48	0.68±0.01	0.044	57	3.92	0.00	0.08
	Grosse Ile	35	0.74±0.02	0.001	60	4.20	0.00	0.11
L. Erie	---	348	0.72±0.01	0.037	85	4.14	0.01	0.08
	O. Western basin L. Erie	211	0.70±0.01	0.035	78	4.02	0.00	0.07
	O1. Huron R.	40	0.73±0.02	0.021	64	4.18	0.00	0.00
	O2. Hen Is.	65	0.67±0.01	0.039	63	3.84	0.00	0.09
	O3. Maumee R.	76	0.69±0.01	0.042	65	4.01	0.00	0.11
	O4. Sandusky R.	30	0.75±0.02	0.006	54	4.15	0.00	0.00
	P. Eastern basin L. Erie	137	0.74±0.01	0.034	74	4.30	0.01	0.09
	P1. Van Buren Bay	87	0.76±0.01	0.021	64	4.33	0.02	0.13
	P2. Cattaraugus Ck.	50	0.71±0.02	0.053	66	4.18	0.00	0.04
	Q. Historic yellow walleye	31	0.40±0.04	0.258	44	2.83	0.14	0.23
	R. Historic blue pike	25	0.54±0.07	-0.191	20	2.06	0.00	0.76
L. Ontario	---	104	0.70±0.01	0.068	76	4.13	0.00	0.06
	S. Pigeon L.	29	0.73±0.02	-0.017	55	4.01	0.00	0.00

	T. Bay of Quinte	50	0.69±0.02	0.046	65	4.00	0.00	0.08
	U. Oneida L.	25	0.66±0.03	0.103	48	3.98	0.00	0.08
L. Mistassini	V. Lac Mistassini	40	0.52±0.03	0.137	45	3.09	0.04	0.05
Ohio R.	---	39	0.68±0.01	0.141	63	4.36	0.03	0.05
	W. Ohio R.	4	0.61±0.07	0.306	33	4.71	0.00	0.00
	X. New R.	35	0.68±0.01	0.121	60	4.29	0.02	0.06
Tombigbee R.	Y. North R.	5	0.56±0.11	0.197	27	3.53	0.11	0.00
	Mean modern walleye (23 sites)	49	0.68±0.03	0.055	39	3.86	0.03	0.08
	Total modern walleye (23 sites)	1125	0.73±0.00	0.029	111	15.85	---	0.09
	Sauger	25	0.72±0.03	0.103	79	4.98	---	0.00

Table 3.2

Morphological comparisons among historic walleye *Sander vitreus vitreus*, blue pike *S. v. "glaucus"* and sauger *S. canadensis.* See Appendix S1 for individuals examined. Body and head measurements are given as percentage of standard or head lengths, respectively. Ratios are given as comparisons of the original measurement values.

	Historic walleye (*N*=51)		Blue pike (*N*=52)		Historic sauger (*N*=56)	
Measurement	Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
Standard length (mm)	264.3 ± 49.9	187−385	248.8 ± 37.7	202−362	263.8 ± 48.1	172−359
% Standard length						
Body depth	20.1 ± 1.9	15.8−24.2	20.4 ± 2.1	16.3−24.9	18.1 ± 2.1	13.5−24.1
Head length	30.7 ± 0.8	28.9−32.2	30.6 ± 1.8	27.9−40.7	30.0 ± 1.1	27.7−33.2
% Head length						
Cheek length	48.3 ± 1.8	44.3−52.5	48.4 ± 1.6	46.3−55.7	49.4 ± 3.7	45.3−73.2
Upper jaw length	38.6 ± 3.1	33.6−43.9	37.9 ± 3.7	31.0−44.9	41.4 ± 3.5	33.3−48.3
Lower jaw length	38.8 ± 4.1	30.1−43.7	38.2 ± 4.3	31.6−44.1	37.2 ± 3.9	24.9−46.5
Head width	41.6 ± 4.5	34.3−55.0	38.6 ± 2.5	32.1−44.0	48.6 ± 7.4	33.8−67.8
Interorbital width	14.9 ± 1.1	12.8−17.3	14.1 ± 1.2	11.2−17.2	16.1 ± 1.4	12.5−19.1
Orbit diameter	20.7 ± 1.8	17.4−26.4	21.9 ± 2.1	16.6−25.9	20.4 ± 1.9	16.4−24.7
Overall MANOVA						
Orbit:interorbital	1.4 ± 0.2	1.1−2.0	1.6 ± 0.2	1.0−2.2	1.3 ± 0.2	0.9−1.8
Upper:lower jaw	1.0 ± 0.2	0.8−1.3	1.0 ± 0.2	0.7−1.3	1.1 ± 0.2	0.8−1.7
Meristic	Mode	Range	Mode	Range	Mode	Range
First dorsal fin rays	14	11−16	12	11−15	13	11−14
Second dorsal fin rays	21	19−22	20	13−22	19	18−21
Pectoral fin rays	15	11−17	14	10−16	14	10−16
Pelvic fin rays	6	5−8	7	5−8	6	5−7
Anal fin rays	14	11−16	14	11–16	12	10−16
Overall MANOVA						

Measurement	Comparisons among groups	Tukey's Test
Standard length (mm)	F=1.320, df=2, p=0.270	
% Standard length		
Body depth	F=22.517, df=2, p<0.001*	S
Head length	F=5.868, df=2, p=0.004*	S
% Head length		
Cheek length	F=0.364, df=2, p=0.695	
Upper jaw length	F=8.687, df=2, p<0.001*	S
Lower jaw length	F=5.200, df=2, p=0.007*	S
Head width	F=41.216, df=2, p<0.001*	S,W,B
Interorbital width	F=22.212, df=2, p<0.001*	S,W,B
Orbit diameter	F=11.179, df=2, p<0.001*	S
Overall MANOVA	F=10.534, λ=0.368, df=2, p<0.001*	
Orbit:interorbital	F=22.666, df=2, p<0.001*	S,W,B
Upper:lower jaw	F=6.854, df=2, p=0.001*	S
Meristic		
First dorsal fin rays	F=8.840, df=2, p<0.001*	
Second dorsal fin rays	F=18.799, df=2, p<0.001*	S,W,B
Pectoral fin rays	F=2.789, df=2, p=0.065	
Pelvic fin rays	F=17.707, df=2, p<0.001*	B
Anal fin rays	F=18.218, df=2, p<0.001*	S
Overall MANOVA	F=9.933, λ=0.563, df=2, p<0.001*	

Table 3.3

F_{ST} (Weir & Cockerham, 1984) pairwise comparisons for control region sequence data (below diagonal) and seven nuclear μsat loci (above diagonal) among the 25 sampled sites, including: 23 modern walleye spawning groups, historic yellow walleye from Lake Erie, and historic blue pike from Lake Erie. Results are congruent to those from the exact tests of differentiation comparisons. Results based on the seven nuclear μsat loci data are almost identical to those based on nine loci, differing only at the thousandth decimal place (see Stepien *et al.*, 2009, 2010 for the nine locus dataset).

Location	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.	M.
A. Cedar L.	---	0.059	0.054	0.136	0.152	0.084	0.080	0.112	0.102	0.095	0.087	0.144	0.111
B. L. Winnipeg	0.164	---	0.028	0.150	0.144	0.080	0.040	0.111	0.119	0.144	0.122	0.155	0.127
C. L. of the Woods	0.248	0.271	---	0.147	0.090	0.064	0.031	0.081	0.093	0.108	0.093	0.124	0.100
D. McKim L.	0.071	0.174	0.037	---	0.222	0.168	0.216	0.181	0.212	0.238	0.216	0.234	0.221
E. Mille Lacs	0.360	0.487	0.072	0.136	---	0.115	0.069	0.149	0.154	0.155	0.162	0.177	0.115
F. St. Louis R.	0.171	0.000	0.256	0.167	0.480	---	0.053	0.035	0.044	0.055	0.046	0.061	0.034
G. L. Nipigon	0.385	0.173	0.646	0.501	0.801	0.203	---	0.086	0.088	0.094	0.088	0.119	0.073
H. Portage L.	0.173	0.280	0.167	0.094	0.236	0.280	0.557	---	0.044	0.066	0.047	0.036	0.043
I. Muskegon R.	0.334	0.446	0.054	0.117	0.000	0.439	0.765	0.187	---	0.028	0.007	0.052	0.031
J. Thunder Bay	0.196	0.301	0.098	0.064	0.145	0.297	0.603	0.000	0.098	---	0.011	0.073	0.027
K. Flint R.	0.177	0.270	0.108	0.064	0.175	0.266	0.571	0.002	0.119	0.000	---	0.046	0.020
L. Moon/Musquash R.	0.376	0.489	0.078	0.152	0.000	0.483	0.803	0.210	0.000	0.114	0.138	---	0.045
M. Thames R.	0.165	0.280	0.258	0.152	0.339	0.285	0.518	0.000	0.283	0.046	0.030	0.315	---
N. Detroit R.	0.158	0.253	0.213	0.138	0.259	0.255	0.437	0.011	0.221	0.053	0.041	0.243	0.000
O. W. basin L. Erie	0.148	0.242	0.200	0.126	0.244	0.243	0.426	0.007	0.208	0.048	0.049	0.233	0.000
P. E. basin L. Erie	0.137	0.243	0.253	0.154	0.314	0.249	0.422	0.039	0.275	0.095	0.102	0.308	0.008
Q. Historic walleye	0.541	0.668	0.773	0.627	0.869	0.683	0.830	0.499	0.824	0.581	0.516	0.860	0.352
R. Historic blue pike	0.585	0.712	0.817	0.672	0.913	0.728	0.875	0.550	0.870	0.632	0.567	0.908	0.405
S. Pigeon L.	0.351	0.471	0.541	0.402	0.635	0.484	0.651	0.247	0.583	0.322	0.253	0.610	0.119
T. Bay of Quinte	0.272	0.392	0.455	0.319	0.547	0.403	0.576	0.155	0.495	0.233	0.182	0.522	0.046

U. Oneida L.	0.269	0.405	0.503	0.367	0.584	0.420	0.557	0.289	0.532	0.350	0.295	0.581	0.169
V. L. Mistassini	0.163	0.088	0.022	0.027	0.226	0.072	0.463	0.161	0.191	0.131	0.119	0.230	0.218
W. Ohio R.	0.116	0.259	0.293	0.142	0.413	0.269	0.531	0.068	0.346	0.118	0.116	0.416	0.041
X. New R.	0.382	0.508	0.607	0.470	0.693	0.523	0.660	0.443	0.653	0.492	0.467	0.695	0.384
Y. North R.	0.342	0.506	0.651	0.454	0.793	0.527	0.736	0.432	0.732	0.495	0.468	0.798	0.369

Location	N.	O.	P.	Q.	R.	S.	T.	U.	V.	W.	X.	Y.
A. Cedar L.	0.129	0.123	0.115	0.252	0.318	0.123	0.124	0.122	0.188	0.076	0.109	0.249
B. L. Winnipeg	0.141	0.131	0.113	0.240	0.281	0.155	0.143	0.136	0.189	0.039	0.128	0.252
C. L. of the Woods	0.113	0.109	0.091	0.222	0.267	0.128	0.121	0.125	0.162	0.034	0.104	0.223
D. McKim L.	0.230	0.222	0.195	0.384	0.440	0.253	0.244	0.203	0.239	0.213	0.211	0.334
E. Mille Lacs	0.115	0.111	0.097	0.274	0.330	0.184	0.139	0.150	0.183	0.155	0.117	0.288
F. St. Louis R.	0.045	0.040	0.029	0.179	0.211	0.087	0.067	0.050	0.138	0.003	0.044	0.164
G. L. Nipigon	0.082	0.077	0.069	0.190	0.242	0.110	0.093	0.108	0.168	0.031	0.085	0.215
H. Portage L.	0.055	0.054	0.044	0.169	0.200	0.090	0.074	0.044	0.130	0.014	0.051	0.212
I. Muskegon R.	0.046	0.042	0.041	0.095	0.135	0.047	0.041	0.042	0.167	0.008	0.031	0.156
J. Thunder Bay	0.036	0.035	0.040	0.119	0.184	0.036	0.031	0.066	0.196	0.035	0.042	0.188
K. Flint R.	0.039	0.037	0.037	0.122	0.171	0.030	0.033	0.053	0.177	0.010	0.034	0.180
L. Moon/Musquash R.	0.062	0.059	0.052	0.209	0.249	0.083	0.064	0.063	0.160	0.041	0.061	0.220
M. Thames R.	0.001	0.000	0.002	0.150	0.199	0.040	0.013	0.035	0.157	0.011	0.008	0.184
N. Detroit R.	---	0.001	0.007	0.142	0.191	0.045	0.020	0.058	0.166	0.035	0.021	0.198
O. W. basin L. Erie	0.000	---	0.006	0.136	0.182	0.048	0.017	0.048	0.160	0.024	0.018	0.198
P. E. basin L. Erie	0.010	0.000	---	0.144	0.185	0.050	0.026	0.040	0.136	0.019	0.011	0.173
Q. Historic walleye	0.304	0.336	0.364	---	0.050	0.140	0.132	0.155	0.343	0.139	0.149	0.276
R. Historic blue pike	0.334	0.366	0.399	0.000	---	0.222	0.196	0.196	0.384	0.203	0.190	0.367
S. Pigeon L.	0.136	0.171	0.194	0.092	0.145	---	0.010	0.082	0.225	0.031	0.055	0.196
T. Bay of Quinte	0.073	0.102	0.112	0.151	0.205	0.000	---	0.056	0.198	0.030	0.032	0.198
U. Oneida L.	0.150	0.161	0.138	0.400	0.454	0.240	0.186	---	0.126	0.020	0.018	0.188
V. L. Mistassini	0.191	0.179	0.211	0.694	0.739	0.473	0.389	0.430	---	0.177	0.136	0.324
W. Ohio R.	0.034	0.020	0.004	0.557	0.629	0.287	0.180	0.151	0.222	---	0.010	0.122
X. New R.	0.338	0.331	0.310	0.685	0.732	0.512	0.438	0.364	0.533	0.169	---	0.171
Y. North R.	0.338	0.327	0.288	0.836	0.923	0.551	0.447	0.361	0.541	0.299	0.543	---

Table 3.4

Relative distribution of variation among modern and historic walleye samples using analysis of molecular variance (AMOVA), based on mtDNA control region sequence and seven nuclear μsat loci data**.**

	Control region			
Source of variation	% variation	Φ value	*p*–value	Mean F_{ST}
1. Modern (O–P,S–T) vs. historic samples (Q–R)	26.01	0.260	0.067	0.260
Among sampling sites within groups	0.00	0.000	0.601	0.176
Within sampling sites	73.98	0.252	<0.001**	---
2. Among northern (A–E,V), Great Lakes (F–U), and southeast (W–Y) regions	12.06	0.121	<0.001**	0.318
Among sampling sites within groups	17.64	0.201	<0.001**	0.238
Within sampling sites	70.30	0.297	<0.001**	---
3. Among the modern 11 drainages (A–P,S–Y)	14.38	0.144	<0.001**	0.286
Among sampling sites within drainages	11.07	0.129	<0.001**	0.158
Within the sampling sites	74.05	0.254	<0.001**	---
4. Between lake (A–E,G–H,J,O2,P1,S–V) and river (F,I,K–O1,O3–4,P2,W–Y) spawners	0.00	0.000	0.482	0.306
Among sampling sites within groups	24.92	0.247	<0.001**	0.298
Within sampling sites	75.08	0.241	<0.001**	---
5. Among 9 STRUCTURE groups	3.75	0.037	0.078	0.288
Among sampling sites within groups	21.17	0.220	<0.001**	0.276
Within sampling sites	75.09	0.297	<0.001**	---

Source of variation	7 μsat loci			
	% variation	Φ value	*p*–value	Mean F_{ST}
1. Modern (O–P,S–T) vs. historic samples (Q–R)	6.99	0.070	0.066	0.167
Among sampling sites within groups	0.87	0.009	<0.001**	0.030
Within sampling sites	92.14	0.079	<0.001**	---
2. Among northern (A–E,V), Great Lakes (F–U), and southeast (W–Y) regions	5.07	0.051	<0.001**	0.129
Among sampling sites within groups	4.97	0.052	<0.001**	0.063
Within sampling sites	89.96	0.100	<0.001**	---
3. Among the modern 11 drainages (A–P,S–Y)	4.26	0.043	<0.001**	0.132
Among sampling sites within drainages	3.13	0.033	<0.001**	0.058
Within the sampling sites	92.61	0.074	<0.001**	---
4. Between lake (A–E,G–H,J,O2,P1,S–V) and river (F,I,K–O1,O3–4,P2,W–Y) spawners	0.58	0.006	0.030**	0.107
Among sampling sites within groups	6.59	0.066	<0.001**	0.100
Within sampling sites	92.83	0.071	<0.001**	---
5. Among 9 STRUCTURE groups	4.10	0.041	<0.001**	0.104
Among sampling sites within groups	3.32	0.035	<0.001**	0.071
Within sampling sites	92.58	0.074	<0.001**	---

Table 3.5

Genetic comparisons among modern Lake Erie yellow walleye, historic Lake Erie yellow walleye, historic Lake Erie blue pike, and modern Lake Ontario yellow walleye samples, using (a) pairwise F_{ST} tests (Weir & Cockerham, 1984), (b) exact tests of differentiation (Raymond & Rousset, 1995), and (c) GENECLASS (Piry *et al.*, 2002) assignment tests. Site labels follow Table 1. For (a) and (b): control region sequence results are below diagonal and nuclear μsat loci above the diagonal. ** = significant following sequential Bonferroni correction (Rice, 1989); *= *p*–values <0.05 prior to correction. (c) GENECLASS values are percentage of assignment; numbers in parentheses = number of individuals assigning to that location. Bold=greatest assignment, Italics = self–assignment.

(a)

Site	O.	P.	Q.	R.	S.	T.
O. Western Lake Erie modern walleye	---	0.006**	0.136**	0.182**	0.047**	0.017**
P. Eastern Lake Erie modern walleye	0.000	---	0.144**	0.185**	0.049**	0.026**
Q. Historic Lake Erie walleye	0.336**	0.364**	---	0.050**	0.139**	0.132**
R. Historic Lake Erie blue pike	0.366**	0.399**	0.000	---	0.221**	0.196**
S. Pigeon L. modern walleye	0.171**	0.194**	0.092*	0.145*	---	0.009**
T. Bay of Quinte modern walleye	0.102**	0.112**	0.151**	0.205**	0.000	---

(b)

Site	O.	P.	Q.	R.	S.	T.
O. Western Lake Erie modern walleye	---	64.88**	Inf.**	Inf.**	Inf.**	86.70**
P. Eastern Lake Erie modern walleye	2.85	---	Inf.**	Inf.**	Inf.**	Inf.**
Q. Historic Lake Erie walleye	Inf.**	Inf.**	---	63.03**	Inf.**	Inf.**
R. Historic Lake Erie blue pike	Inf.**	Inf.**	0.00	---	Inf.**	Inf.**
S. Pigeon L. modern walleye	19.01**	17.58**	5.60	6.12*	---	22.62
T. Bay of Quinte modern walleye	13.04**	10.87**	9.39*	9.88**	0.39	---

(c)

Site	O.	P.	Q.	R.	S.	T.
O. Western Lake Erie modern walleye	*0.43 (90)*	**0.49 (104)**	<0.01 (1)	0.00	0.03 (6)	0.05 (10)
P. Eastern Lake Erie modern walleye	0.27 (37)	***0.69 (95)***	0.01 (1)	0.00	0.02 (3)	0.00
Q. Historic Lake Erie walleye	0.06 (2)	0.10 (3)	***0.77 (24)***	0.06 (2)	0.00	0.00
R. Historic Lake Erie blue pike	0.00	0.04 (1)	**0.48 (12)**	***0.48 (12)***	0.00	0.00
S. Pigeon L. modern walleye	0.03 (1)	0.14 (4)	0.00	0.00	***0.45 (13)***	0.39 (11)
T. Bay of Quinte modern walleye	0.20 (10)	0.28 (14)	0.00	0.00	0.20 (10)	***0.32 (16)***

Table 3.6

Quadratic discriminant function analysis classification for (a) morphometric and (b) meristic characters among historic walleye, blue pike, and sauger samples. Values are represented as proportions, in parentheses = number of individuals assigned, and **Bold**=highest assignment.

(a)

	Historic yellow walleye	Historic blue pike	Historic sauger
Historic yellow walleye	**0.65 (33)**	0.22 (11)	0.13 (7)
Historic blue pike	0.22 (11)	**0.66 (33)**	0.12 (6)
Historic sauger	0.11 (6)	0.16 (9)	**0.73 (41)**

(b)

	Historic yellow walleye	Historic blue pike	Historic sauger
Historic yellow walleye	**0.45 (22)**	0.24 (12)	0.31 (15)
Historic blue pike	0.33 (17)	**0.53 (27)**	0.14 (7)
Historic sauger	0.11 (6)	0.11 (6)	**0.78 (44)**

Figure 3-1 Map of walleye (*Sander vitreus*) sampling sites and their distribution for (a) across their North American range, (b) close–up of Lake Erie, including the historic walleye and blue pike samples, and (c) BARRIER (Manni *et al.,* 2004) analysis map showing genetic discontinuities determine from the mtDNA control region sequence data and for seven nuclear μsat loci. Letter codes follow those listed in Table 1. Distribution for *S. vitreus* is modified from Billington *et al.* (2011).

Figure 3-2 Allele frequency bar graphs based on seven nuclear μsat loci for modern Lake Erie walleye, historic Lake Erie yellow walleye, historic Lake Erie blue pike, and modern Lake Ontario walleye samples.

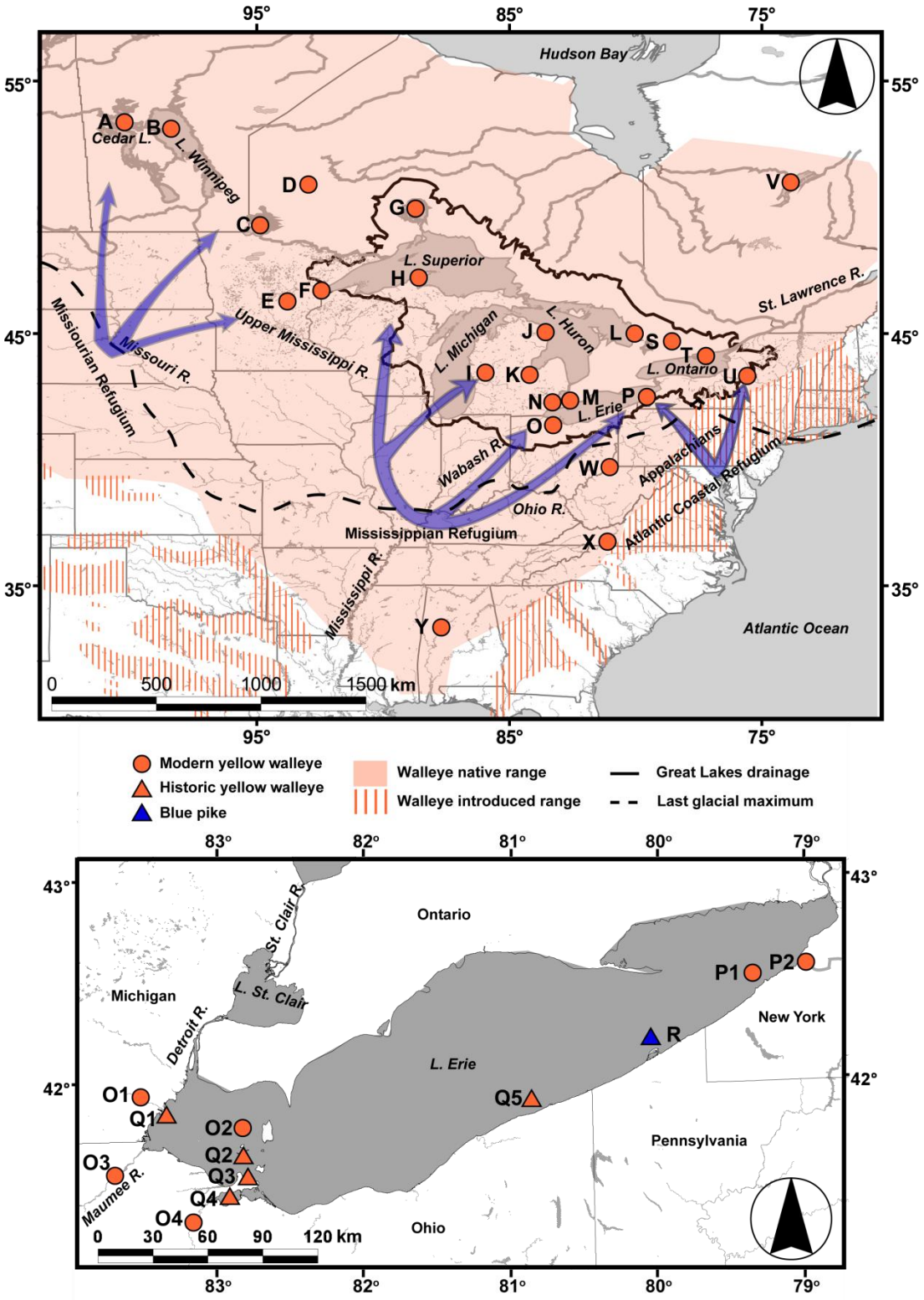
Figure 3-3 (a) Bayesian phylogenetic tree of mtDNA control region sequence haplotypes. Values above the branches are Bayesian posterior probabilities (p.p.) and bootstrap pseudoreplications (Felsenstein, 1985) and those below in italics are divergence estimates. Nodes having >0.50 pp and > 50% bootstrap support are reported. (b) mtDNA control region haplotype network constructed in the program TCS v1.21 (Clement *et al.,* 2000) including the modern (A1–7, B8–27) and historic walleye (B28) haplotypes.

Figure 3-4 (a) mtDNA control region haplotype frequencies for the 23 modern walleye spawning groups, historic walleye, historic blue pike, and sauger samples (b) Bayesian STRUCTURE analysis (Pritchard *et al.,* 2000) for K=11 population groups (p.p. = 1.00) using seven μsat loci. Estimated population clustering is shown by individuals represented as thin vertical lines, partitioned into K colored segments.

Figure 3-5 Population genetic distance neighbor joining tree using Nei's *D* in PHYLIP V3.68 (Felsenstein, 2005) for the 23 walleye spawning groups, historic yellow walleye, historic blue pike, and sauger, based on the combined frequencies of mtDNA control region sequence haplotypes and alleles for the seven nuclear μsat loci.

Figure 3-6 Principal components analysis for (a) morphometric and (b) meristic characters for historic walleye, blue pike, and sauger samples.

Fig. 3-1


95°
85°
75°
55°
45°
35°
Hudson Bay
A
B
Cedar L.
L. Winnipeg
C
D
G
V
L. Superior
H
E
F
St. Lawrence R.
Missouri R.
Upper Mississippi R.
Missourian Refugium
L. Michigan
J
L. Huron
L
S
T
L. Ontario
U
I
K
N
M
L. Erie
P
O
Wabash R.
W
Appalachians
Atlantic Coastal Refugium
Ohio R.
Mississippian Refugium
X
Mississippi R.
Y
Atlantic Ocean
0
500
1000
1500 km
Modern yellow walleye
Historic yellow walleye
Blue pike
Walleye native range
Walleye introduced range
Great Lakes drainage
Last glacial maximum
83°
82°
81°
80°
79°
43°
42°
St. Clair R.
Ontario
Michigan
L. St. Clair
Detroit R.
P1
P2
New York
R
L. Erie
Q5
O1
Q1
O2
Q2
Q3
O3
Maumee R.
Q4
O4
Pennsylvania
Ohio
0
30
60
90
120 km

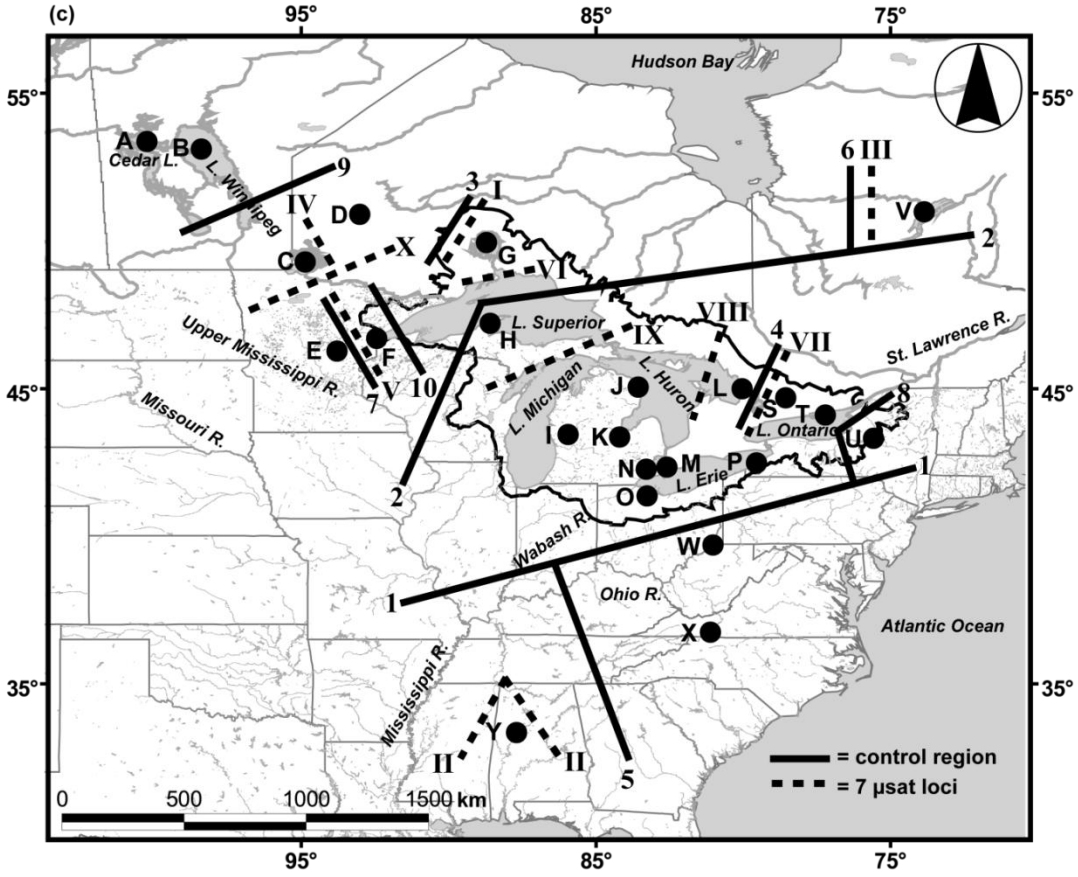

(c)
95°
85°
75°
55°
45°
35°
Hudson Bay
Cedar L.
L. Winnipeg
Upper Mississippi R.
Missouri R.
L. Superior
L. Michigan
L. Huron
L. Ontario
L. Erie
St. Lawrence R.
Wabash R.
Ohio R.
Mississippi R.
Atlantic Ocean
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
S
T
U
V
W
X
Y
I
II
III
IV
V
VI
VII
VIII
IX
X
1
2
3
4
5
6
7
8
9
10
0
500
1000
1500 km
= control region
= 7 μsat loci

Fig. 3-2

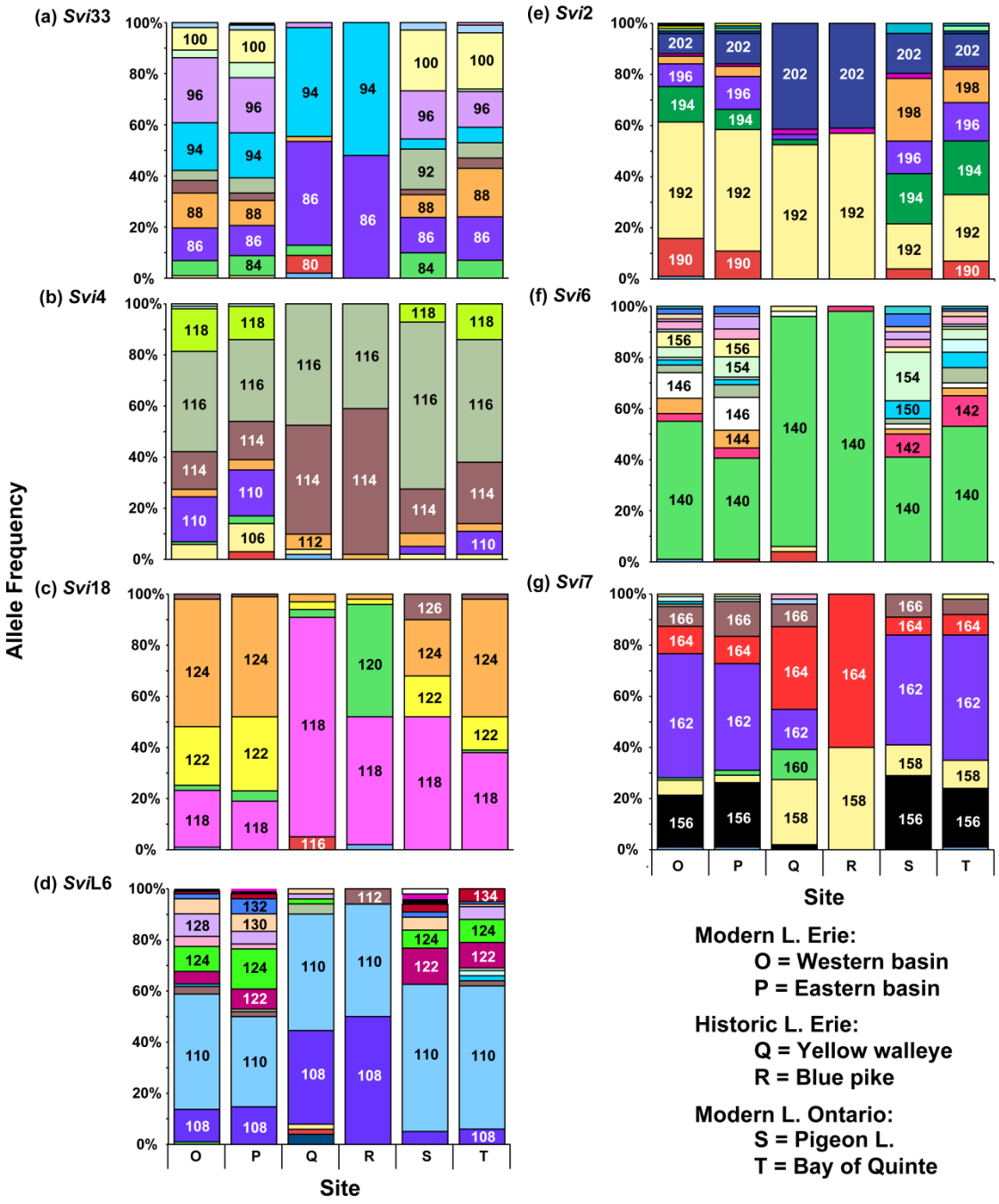

(a) Svi33
(b) Svi4
(c) Svi18
(d) SviL6
(e) Svi2
(f) Svi6
(g) Svi7
Allele Frequency
100%
80%
60%
40%
20%
0%
O
P
Q
R
S
T
Site
Modern L. Erie:
O = Western basin
P = Eastern basin
Historic L. Erie:
Q = Yellow walleye
R = Blue pike
Modern L. Ontario:
S = Pigeon L.
T = Bay of Quinte

Fig. 3-3 (a)


A2 (H, J-K, M-P, S-T, W)
A7 (O)
A5 (O, P)
A4 (A, E, I, K, M-P, U, W-Y)
B23 (U)
0.56
2.5
B14 (P)
B21 (U)
B9 (N)
B15 (P, U)
A3* (H-U, W-X)
B28* (Q)
B10 (O)
0.82
7.2
0.65
1.0
B12 (P)
B22 (U)
B16 (A)
0.82
1.6
B11 (A-D, F-G, K, V)
0.53
2.6
B27 (G)
B26 (B)
B8 (D, N-P, S-T)
S. vitreus (28)
1.00/98
10.6
A1 (A-P, S-T, V-W)
B17 (P)
B18 (A)
B24 (T)
1.00/100
A6 (O)
B25 (A, D-E)
B13 (P)
B20 (Y)
B19 (W-X)
1.00/100
S. canadensis (6)
0.69
S. lucioperca (3)
1.00/89
S. marinus (2)
S. volgensis (1)
0.05 nucleotide substitutions/site

Fig. 3-3 (b)

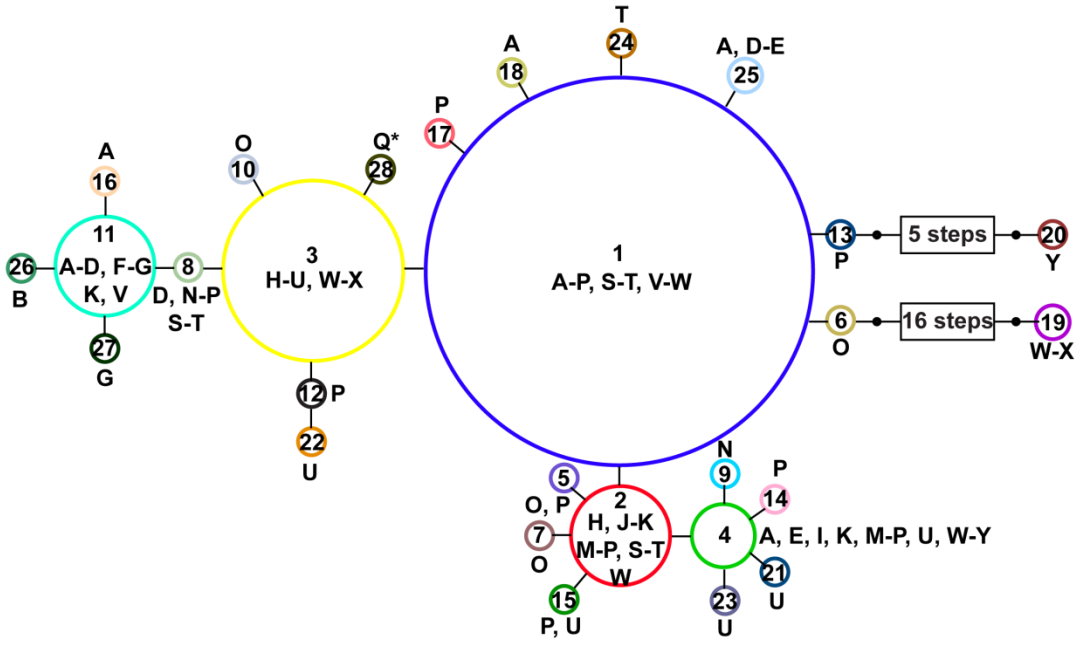

1
A-P, S-T, V-W
2
H, J-K
M-P, S-T
W
3
H-U, W-X
4
A, E, I, K, M-P, U, W-Y
5 steps
16 steps
11
A-D, F-G
K, V
A
16
26
B
27
G
8
D, N-P
S-T
O
10
Q*
28
12 P
22
U
A
18
T
24
A, D-E
25
P
17
13
P
20
Y
6
O
19
W-X
5
O, P
7
O
15
P, U
N
9
P
14
21
U
23
U

Fig. 3-4

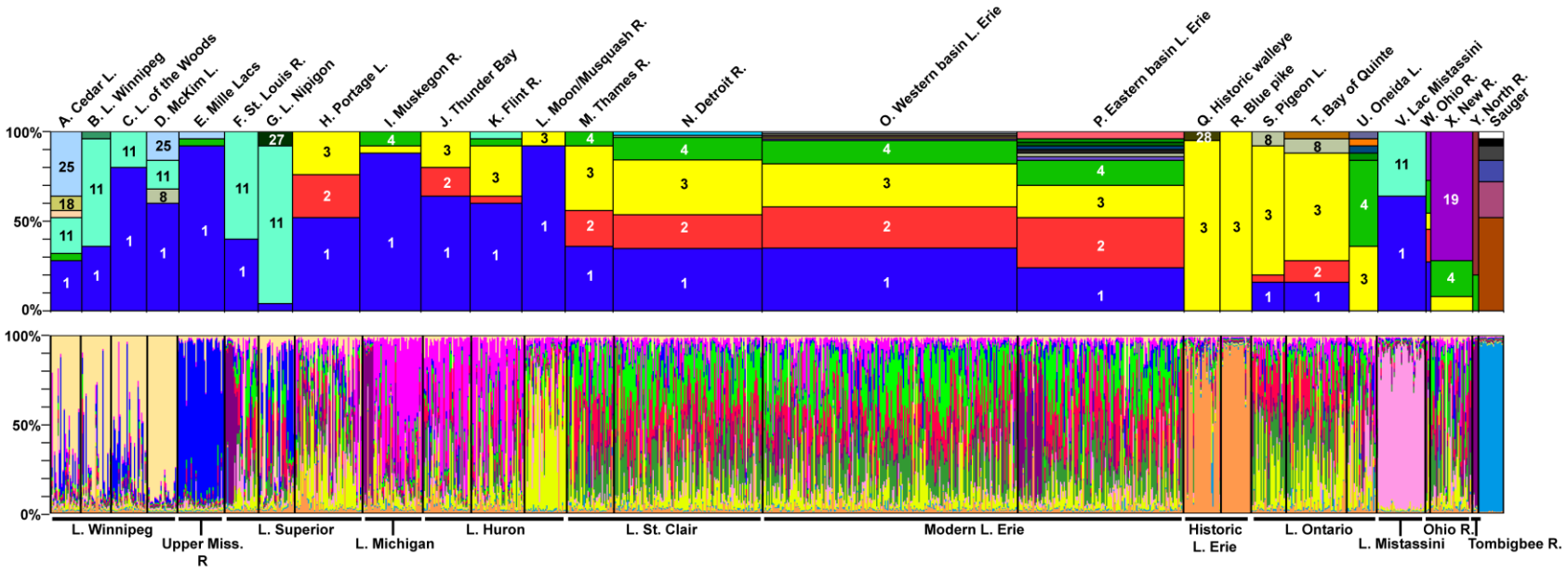

A. Cedar L.
B. L. Winnipeg
C. L. of the Woods
D. McKim L.
E. Mille Lacs
F. St. Louis R.
G. L. Nipigon
H. Portage L.
I. Muskegon R.
J. Thunder Bay
K. Flint R.
L. Moon/Musquash R.
M. Thames R.
N. Detroit R.
O. Western basin L. Erie
P. Eastern basin L. Erie
Q. Historic walleye
R. Blue pike
S. Pigeon L.
T. Bay of Quinte
U. Oneida L.
V. Lac Mistassini
W. Ohio R.
X. New R.
Y. North R.
Sauger
100%
50%
0%
L. Winnipeg
Upper Miss. R
L. Superior
L. Michigan
L. Huron
L. St. Clair
Modern L. Erie
Historic L. Erie
L. Ontario
L. Mistassini
Ohio R.
Tombigbee R.

Fig. 3-5

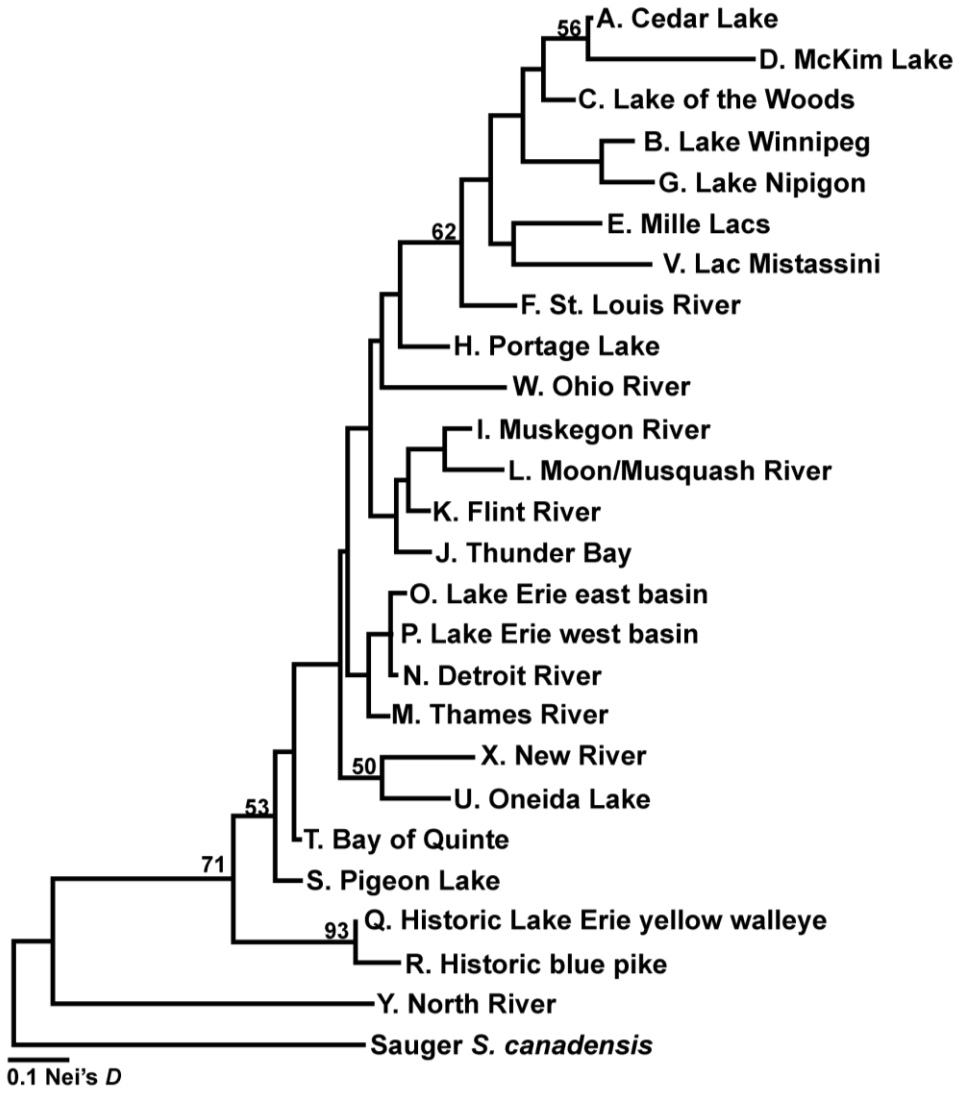
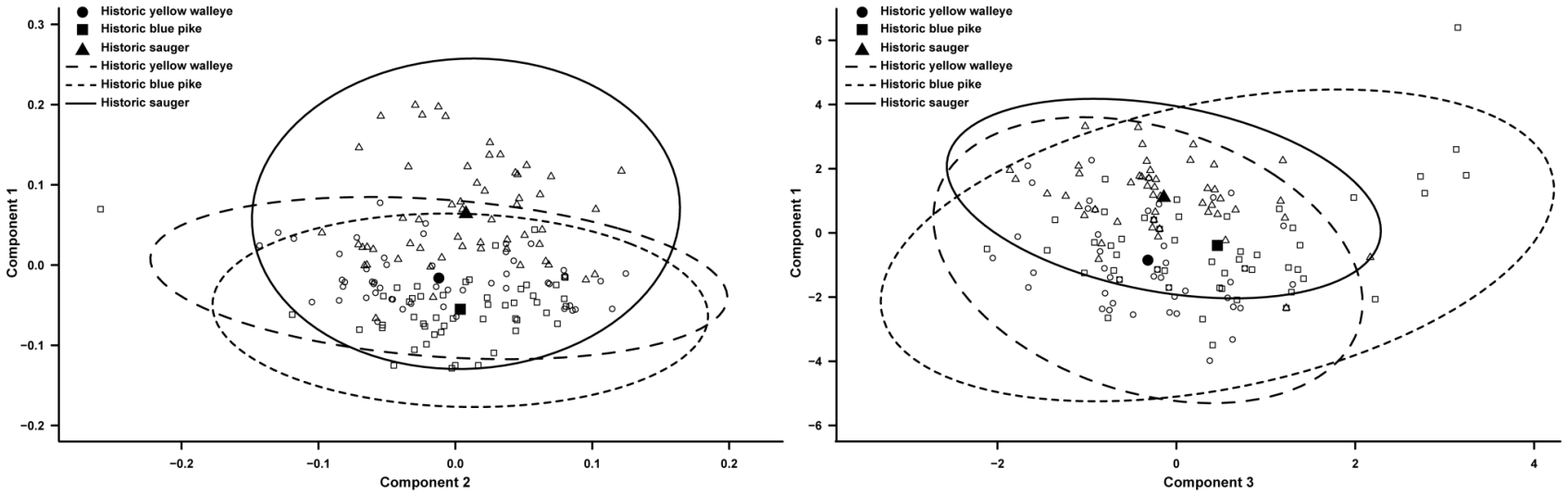

A. Cedar Lake
D. McKim Lake
C. Lake of the Woods
B. Lake Winnipeg
G. Lake Nipigon
E. Mille Lacs
V. Lac Mistassini
F. St. Louis River
H. Portage Lake
W. Ohio River
I. Muskegon River
L. Moon/Musquash River
K. Flint River
J. Thunder Bay
O. Lake Erie east basin
P. Lake Erie west basin
N. Detroit River
M. Thames River
X. New River
U. Oneida Lake
T. Bay of Quinte
S. Pigeon Lake
Q. Historic Lake Erie yellow walleye
R. Historic blue pike
Y. North River
Sauger S. canadensis
56
62
50
53
71
93
0.1 Nei's D

Fig. 3-6


Historic yellow walleye
Historic blue pike
Historic sauger
Historic yellow walleye
Historic blue pike
Historic sauger
Component 1
Component 2
Component 3

Appendix 3.1

Material examined. Institutional abbreviations follow Leviton *et al.* (1985). Sample information is listed as follows: museum – lot number, number of samples, size range (SL, mm), locality, sampling date, collector. Individuals that also were analyzed with molecular data are listed in a separate section with their respective lot numbers and GenBank accession numbers. Any individuals that were analyzed solely for molecular analyses are listed under molecular samples with lot number, number of samples, locality, sampling date, collector, GenBank accession number.

Sander canadensis (Griffith & Smith, 1834)

Morphological Samples:

UMMZ – 55200, 5, 265–288 mm SL, Saginaw Bay, 26 Nov. 1921, C.L. Hubbs; 55618, 1, 180 mm SL, Lake Erie – near Kelly's Island, 29 Nov. 1920, W. Koelz; 55651, 2, 257–263 mm SL, Saginaw Bay – near Coregon Reef, 25 Oct. 1917, W. Koelz; 55675, 2, 203–221 mm SL, Lake Erie – near Ashtabula OH, 20 Oct. 1920, W. Koelz; 57350, 2, 302–318 mm SL, Lake Superior – near Demar Point ON CAN, 20 Jul. 1922, W. Koelz; 59461, 1, 283 mm SL, Lake Huron – near Thunder Bay Island MI, 2 July 1923, W. Koelz; 59476, 1, 265 mm SL, Lake Huron – near Thunder Bay Island MI, 2 July 1923, W. Koelz; 59488, 1, 281 mm SL, Lake Huron – near Thunder Bay Island MI, 2 July 1923, W. Koelz; 72267, 1, 343 mm SL, Saginaw Bay – near Linwood MI, 4 May 1926, T.H. Langlois; 87237, 1, 308 mm SL, Lake Erie – near Kelly's Island, 31 Aug. 1865, F.W. Putnam; 87238, 1, 261 mm SL, Lake Erie – near Kelly's Island, 31 Aug. 1865, F.W. Putnam; 87239, 1, 230 mm SL, Lake Erie – near Kelly's Island, 31 Aug. 1865,

F.W. Putnam; 87733, 1, 243 mm SL, Scioto River – near Scioto Township, 22 Sep. 1929, M.B. Trautman; 87734, 1, 317 mm SL, Scioto River – near Scioto Township, 22 Sep. 1929, M.B. Trautman; 94964, 1, 251 mm SL, Muskegon River – near Newago MI, 24 Mar. 1932, I. Bullis; 101640, 1, 193 mm SL, Lake Erie – near Vermilion OH, 21 Aug. 1927, J. Van Oosten; 101647, 1, 187 mm SL, Lake Erie – near Vermilion OH, 19 Aug. 1927, J. Van Oosten; 101701, 1, 335 mm SL, Mississippi River – near Lansing IA, 5 Aug. 1932, C.L. Hubbs; 101763, 1, 241 mm SL, Saginaw Bay, 31 Oct. 1928, T.H. Langlois; 101823, 1, 292 mm SL, Saginaw Bay, 30 Nov. 1929, C.G. Manuel; 103301, 11, 172–299 mm SL, Ohio River – near Dam 32, 31 Oct. 1935, A. Poole; 114938, 1, 209 mm SL, Ohio River – near Higginsport OH, 27 Mar. 1937, M.B. Trautman; 157231, 1, 216 mm SL, Lake Erie – near Sandusky OH, 8 Oct. 1946, R. Hile.

USNM – 10072, 1, 359 mm SL, Lake Ontario – near Rochester NY, 1873, J.W. Milner; 10251, 1, 292 mm SL, Lake Erie – near Buffalo NY, 1874, J.W. Milner; 22238, 1, 356 mm SL, near Memphis TN, 1879, McDonald; 46172, 1, 245 mm SL, Smith Creek – near Lancaster TN, 1891, P.H. Kirsch; 66636, 1, 232 mm SL, Wabash River – near Bonebank IN, 1890, B.W. Evermann; 73617, 1, 309 mm SL, Lake Huron – near Huron MI, 9 June 1894, J.T. Scovell; 88460, 1, 336 mm SL, Lake Erie – near Erie PA, 1910, W.C. Kendall; 125065, 1, 203 mm SL, Lake Erie – near Toledo OH, 12 Aug. 1894, United States Bureau of Fisheries; 131333, 7, 267–311 mm SL, Lake of the Woods ON CAN, 1908, S. Meek; 231385, 1, 309 mm SL, Cosby Creek, 29 Dec. 1969, W. Starnes;

Sander vitreus "glaucus" (Hubbs, 1926)

Morphological Samples:

UMMZ – 72090, 26, 202–270 mm SL, Lake Erie – near Erie PA, 30 Nov. 1923, Lay Bros. Fishing Co., paratypes identified by C.L. Hubbs.

USNM – 88458, 17, 204–345 mm SL, Lake Erie – near Erie PA, 1910, W.H. Kendall; 88459, 5, 265–330 mm SL, 1910, W.H. Kendall; 117481, 5, 230–270 mm SL, Lake Erie – near Erie PA, 1924, Keystone Fish Co., paratypes identified by C.L. Hubbs.

Molecular samples:

For unique samples all information is given. For samples that also were done for morphology see morphological sample section for complete information.

UMMZ – 72091, 22, Lake Erie – near Erie PA, 30 Nov. 1923, Lay Bros. Fishing Co., paratypes identified by C.L. Hubbs, GenBank Accession #XXX.

Sander vitreus vitreus (Mitchill, 1818).

Morphological Samples:

UMMZ – 55299, 1, 277 mm SL, Lake Erie – near Monroe MI, 30 Nov. 1919, C.L. Hubbs; 55456, 1, 214 mm SL, Lake Erie – near Monroe MI, 31 Oct. 1920, C.L. Hubbs; 72078, 1, 270 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72079, 1, 277 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72080, 1, 252 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72081; 1, 273 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72082, 1, 262 mm SL, Lake Erie –

near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72083, 1, 283 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72084, 1, 249 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72085, 1, 292 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72086, 1, 266 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72087, 1, 270 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72088, 1, 255 mm SL, Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72089, 1, 324 mm SL Lake Erie – near Port Clinton OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72095, 1, 242 mm SL, Lake Erie – near Sandusky OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 72096, 1, 198 mm SL, Lake Erie – near Sandusky OH, 30 Nov. 1923, Lay Bros. Fishing Co.; 133669, 3, 187–247 mm SL, Lake Erie – near Erie PA, 5 Jun. 1941, F.C. Ralph; 218003, 2, 203–236 mm SL, Maumee River – near Toledo OH, 25 Aug. 1893, P.H. Kirsch.

USNM – 9391, 1, 315 mm SL, Ecorse MI, 1872, G. Clarke; 12274, 1, 257 mm SL, Ohio River – near Cincinnati OH, 1890s, Milner; 12276, 1, 312 mm SL, Ohio River – near Cincinnati OH, 1890s, Milner; 22494, 1, 303 mm SL, Susquehanna River – near Port Deposit MD, 7 Apr. 1879, R.E. McClenahan; 23226, 1, 245 mm SL, Lake Champlain, unknown, unknown; 26261, 1, 194 mm SL, Wetumpka AL, 1881, J. Skinner; 34716, 1, 279 mm SL, Lake Erie – near Washington Market OH, 1884, H.L. Todd; 34828, 1, 202 mm SL, Hudson Bay CAN, 1884, W. Haydon; 43910, 1, 302 mm SL, Fort Snelling MN, 1890, E.A. Mearns; 63801, 1, 200 mm SL, Pitmans Creek – near Greenburg KY, 1909, A.J. Woolman; 68935, 1, 250 mm SL, Lake Huron – near Point Sanilac MI, 20 June 1894, J.T. Scovell; 88446, 1, 340 mm SL, Fairport IA, early 1900s,

T. Surber; 88454, 1, 348 mm SL, Fairport IA, early 1900s, T. Surber; 88456, 1, 385 mm SL, Fairport IA, early 1900s, T. Surber; 91473, 1, 198 mm SL, Newman's Pond MD, 26 Nov. 1915, United States Bureau of Fisheries; 91474, 1, 209 mm SL, Newman's Pond MD, 26 Nov. 1915, United States Bureau of Fisheries; 91475, 1, 199 mm SL, Newman's Pond MD, 26 Nov. 1915, United States Bureau of Fisheries; 91476, 1, 209 mm SL, Newman's Pond MD, 26 Nov. 1915, United States Bureau of Fisheries; 131332, 9, Lake of the Woods ON CAN, 1908, S. Meek; 125067, 1, 197 mm SL, Lake Erie – near Toledo OH, 12 Aug. 1894, United States Bureau of Fisheries; 154817, 1, 240 mm SL, Cromwell CN, 1952, H. Woodward; 154823, 1, 222 mm SL, Cromwell CN, 1952, L. Taylor.

Molecular samples:

UMMZ – 55299, 72078–83, 72085–89, 72095, GenBank Accession #XXX; 69687, 1, 86136, 1, Lake Erie – near MI, 31 May 1926, Michigan Department of Natural Resources, GenBank #XXX; 86137, 1, Lake Erie – near MI, 31 May 1926, Michigan Department of Natural Resources, GenBank #XXX; 218002, 2, Lake Erie, unknown, unknown, GenBank #XXX; 243215, 1, Lake Erie – near Chelerna Isl. MI, 16 Aug. 1935, Rodeheffer, GenBank #XXX; 243259, 4, Lake Erie – near Sturgeon Bar, MI, 16 Aug. 1935, Rodeheffer, GenBank #XXX; 243620, 1, Lake Erie – near Blue Wig Shooting Club, 16 Aug. 1935, Rodeheffer, GenBank #XXX.

USNM – 34716, GenBank #XXX; 69687, 1, Red Brook – near Harbor OH, 1 Aug. 1893, Woolman, GenBank # XXX; 125067, GenBank #XXX.

OSUM – 431, 3, Sandusky Bay Lake Erie, 17 Aug. 1939, M.B. Trautman; 467, 6, Sandusky Bay Lake Erie, 17 Aug. 1939, M.B. Trautman; 1113, 1, Sandusky Bay Lake

Erie, 17 Aug. 1939, M.B. Trautman; 1449, 1, Lake Erie – near Stone's Cove, Jun. 1939, M.B. Trautman; 1461, 1, Lake Erie – near South Bass Island, 28 Jan. 1940, M.B. Trautman; 3045, 1, Lake Erie – near Stone's Cove, 11 Aug. 1939, M.B. Trautman; 4229, 6, Lake Erie – near Peach Point, 7 Feb. 1941, K.H. Doan; 4232, 2, Lake Erie – near Put–in–Bay OH, 12 Feb. 1941, K.H. Doan; 4235, 1, Lake Erie – near Put–in–Bay OH, 25 Feb. 1941, E. Traverso; 5715, 1, Lake Erie – near Put–in–Bay OH, 8 Aug. 1942, M.B. Trautman; 5719, 2, Lake Erie – near Put–in–Bay OH, 3 Oct. 1942, M.B. Trautman; 5721, 1, Lake Erie – near Put–in–Bay OH, 30 Oct. 1942, K.H. Doan; 5722, 1, Lake Erie – near Hatchery Bay, 4 Nov., 1942, K.H. Doan; 6071, 7, Lake Erie – near Put–in–Bay OH, 8 Jan. 1943, K.H. Doan; 6073, 2, Lake Erie – near Put–in–Bay OH, 5 Oct. 1943, K.H. Doan; 6686, 1, Lake Erie – near Rattlesnake Island, 2 May 1943, K.H. Doan; 7117, 1, Lake Erie – near South Bass Island, 29 Mar. 1947, Miller; 10637, 1, Lake Erie – near Ashtabula County, 1 Jun. 1929, M.B. Trautman; 10638, 1, Lake Erie – near Monroe MI, 10 Sep. 1928, E.L. Wickliff; 10639, 1, Lake Erie – near mouth of Detroit River, 10 Sep. 1928, E.L. Wickliff.

Appendix 3.2

Summary of genetic variation per microsatellite locus across 23 walleye spawning groups, historic yellow walleye, historic blue pike, and sauger samples, totaling 1206 individuals, showing annealing temperature (T_A), number of alleles (N_A), allelic size range (base pairs, bp), genetic deviation across all combined samples (F_{IT}), mean genetic divergence among loci (F_{ST}), inbreeding coefficient (F_{IS}, average divergence within a spawning group), and locus neutrality test from the program LOSITAN (Beaumont & Nichols, 1996; Antao *et al.,* 2008).

Locus	Source	T_A (ºC)	N_A	Size range	F_{IT}	F_{ST}	F_{IS}	Selection
*Svi*4	Borer *et al.* (1999)	60	19	98−140	0.139	0.110	0.033	Neutral
*Svi*6	"	60	31	126−246	0.131	0.071	0.065	Neutral
*Svi*17	"	54	15	92−120	0.141	0.150	–0.011	Positive
*Svi*18	"	65	8	114−128	0.206	0.124	0.094	Neutral
*Svi*33	"	60	26	72−128	0.085	0.067	0.020	Neutral
*Svi*L6	Wirth *et al.* (1999)	54	22	92−140	0.067	0.072	–0.006	Neutral
*Svi*L7	"	54	29	160−238	0.076	0.031	0.046	Balancing
*Svi*2	Eldridge *et al.* (2002)	60	30	178−258	0.076	0.071	0.006	Neutral
*Svi*7	"	60	28	140−208	0.174	0.113	0.068	Neutral
Total	---	---	208	---	0.108	0.079	0.034	---

Appendix 3.3

Microsatellite allele frequencies for the seven nuclear μsat loci comprising modern Lake Erie walleye, historic yellow walleye, historic blue pike, and modern Lake Ontario samples. Site labels follow those listed in Table 1.

a) *Svi*33

Allele length	O. Western basin	P. Eastern basin	Q. Historic yellow walleye	R. Historic blue pike	S. Pigeon L.	T. Bay of Quinte
72	–	–	0.02	–	–	–
80	–	–	0.07	–	–	–
82	0.01	0.01	–	–	–	–
84	0.06	0.08	0.04	–	0.10	0.07
86	0.13	0.12	0.41	0.48	0.14	0.17
88	0.14	0.10	0.02	–	0.09	0.19
90	0.05	0.03	–	–	0.02	0.04
92	0.04	0.06	–	–	0.16	0.06
94	0.19	0.18	0.43	0.52	0.04	0.06
96	0.26	0.22	0.02	–	0.19	0.14
98	0.03	0.06	–	–	–	0.01
100	0.09	0.13	–	–	0.24	0.22
102	0.02	0.02	–	–	0.03	0.03
104	–	–	–	–	–	0.01
106	–	0.01	–	–	–	–

b) *Svi*4

Allele length	O. Western basin	P. Eastern basin	Q. Historic yellow walleye	R. Historic blue pike	S. Pigeon L.	T. Bay of Quinte
98	–	–	0.02	–	–	–
104	–	0.03	–	–	–	–
106	0.06	0.11	0.02	–	0.02	0.02
108	0.01	0.03	–	–	–	–
110	0.18	0.18	–	–	0.03	0.09
112	0.03	0.04	0.06	0.02	0.05	0.03
114	0.15	0.15	0.43	0.57	0.17	0.24
116	0.40	0.32	0.48	0.41	0.66	0.48
118	0.17	0.13	–	–	0.07	0.14
120	0.01	0.01	–	–	–	–
122	0.01	–	–	–	–	–

c) *Svi*18

Allele length	O. Western basin	P. Eastern basin	Q. Historic yellow walleye	R. Historic blue pike	S. Pigeon L.	T. Bay of Quinte
114	0.01	–	–	0.02	–	–
116	–	–	0.05	–	–	–
118	0.223	0.19	0.85	0.50	0.52	0.38
120	0.02	0.04	0.03	0.44	–	0.01
122	0.23	0.29	0.03	0.02	0.16	0.13
124	0.50	0.47	0.03	0.02	0.22	0.46
126	0.02	0.01	–	–	0.10	0.02

d) *Svi*L6

Allele length	O. Western basin	P. Eastern basin	Q. Historic yellow walleye	R. Historic blue pike	S. Pigeon L.	T. Bay of Quinte
92	–	–	0.04	–	–	–
98	–	–	0.02	–	–	–
102	–	–	0.02	–	–	–
106	0.01	–	–	–	–	–
108	0.13	0.15	0.37	0.50	0.05	0.06
110	0.46	0.36	0.46	0.44	0.57	0.56
112	0.03	0.02	–	0.06	–	0.02
114	–	0.01	0.04	–	–	–
116	0.01	–	–	–	–	0.02
118	–	–	–	–	–	0.02
120	–	–	–	–	–	0.01
122	0.05	0.08	–	–	0.14	0.10
124	0.10	0.16	0.02	–	0.07	0.09
126	0.04	0.02	–	–	–	–
128	0.09	0.05	0.02	–	–	0.05
130	0.06	0.07	0.02	–	0.05	0.01
132	0.02	0.06	–	–	0.02	0.01
134	0.01	0.02	–	–	0.03	0.05
136	0.01	0.01	–	–	0.02	–
138	–	0.01	–	–	0.02	–
140	–	–	–	–	0.02	–

e) *Svi2*

Allele length	O. Western basin	P. Eastern basin	Q. Historic yellow walleye	R. Historic blue pike	S. Pigeon L.	T. Bay of Quinte
188	0.01	–	–	–	–	–
190	0.15	0.11	–	–	0.04	0.07
192	0.46	0.48	0.52	0.57	0.18	0.26
194	0.14	0.08	0.02	–	0.20	0.21
196	0.09	0.13	0.02	–	0.13	0.15
198	0.03	0.04	–	–	0.25	0.13
200	0.01	0.01	0.02	0.02	0.02	0.01
202	0.08	0.12	0.41	0.41	0.16	0.13
204	0.01	0.01	–	–	–	0.01
216	0.01	0.01	–	–	–	0.02
218	–	0.01	–	–	0.04	0.01
220	0.01	0.01	–	–	–	–
222	0.01	–	–	–	–	–

f) *Svi6*

Allele length	O. Western basin	P. Eastern basin	Q. Historic yellow walleye	R. Historic blue pike	S. Pigeon L.	T. Bay of Quinte
126	0.01	–	–	–	–	–
132	–	0.01	0.04	–	–	–
138	–	–	0.02	–	–	–
140	0.54	0.40	0.90	0.98	0.41	0.53
142	0.03	0.04	–	0.02	0.09	0.12
144	0.06	0.07	–	–	0.02	0.03
146	0.10	0.13	0.02	–	0.02	0.02
148	0.03	0.05	–	–	0.02	0.06
150	0.02	0.02	–	–	0.07	0.06
152	0.01	0.01	–	–	–	0.05
154	0.04	0.08	–	–	0.19	0.04
156	0.06	0.07	0.02	–	0.02	0.01
158	0.01	–	–	–	–	0.01
160	0.03	0.04	–	–	0.03	0.03
162	0.01	0.05	–	–	0.03	–
164	0.02	0.01	–	–	0.02	0.02
166	0.02	0.03	–	–	0.05	0.01
168	0.01	–	–	–	0.03	0.01

g) *Svi7*

Allele length	O. Western basin	P. Eastern basin	Q. Historic yellow walleye	R. Historic blue pike	S. Pigeon L.	T. Bay of Quinte
154	0.01	0.01	–	–	–	0.01
156	0.21	0.26	0.02	–	0.29	0.23
158	0.06	0.03	0.26	0.40	0.12	0.11
160	0.01	0.02	0.12	–	–	–
162	0.50	0.43	0.16	–	0.43	0.49
164	0.11	0.11	0.33	0.60	0.07	0.08
166	0.08	0.14	0.09	–	0.09	0.06
168	0.01	–	–	–	–	–
170	0.01	–	–	–	–	–
172	0.02	0.01	–	–	–	–
174	–	0.01	–	–	–	–
176	0.01	0.01	–	–	–	0.02
190	–	–	0.02	–	–	–
192	–	–	0.02	–	–	–

Appendix 3.4

Haplotype frequency table for mtDNA of walleye, blue pike, and sauger. Note: – = haplotype frequencies of 0.00

Location	1	2	3	4	5	6	7	8	9	10	11	12	13	14
A. Cedar L.	0.28	–	–	0.04	–	–	–	–	–	–	0.20	–	–	–
B. L. Winnipeg	0.36	–	–	–	–	–	–	–	–	–	0.60	–	–	–
C. L. of the Woods	0.80	–	–	–	–	–	–	–	–	–	0.20	–	–	–
D. McKim L.	0.60	–	–	–	–	–	–	0.08	–	–	0.16	–	–	–
E. Mille Lacs	0.92	–	–	0.04	–	–	–	–	–	–	–	–	–	–
F. St. Louis R.	0.40	–	–	–	–	–	–	–	–	–	0.60	–	–	–
G. L. Nipigon	0.04	–	–	–	–	–	–	–	–	–	0.88	–	–	–
H. Portage L.	0.52	0.24	0.24	–	–	–	–	–	–	–	–	–	–	–
I. Muskegon R.	0.88	–	0.04	0.08	–	–	–	–	–	–	–	–	–	–
J. Thunder Bay	0.64	0.16	0.20	–	–	–	–	–	–	–	–	–	–	–
K. Flint R.	0.60	0.04	028	0.04	–	–	–	–	–	–	0.04	–	–	–
L. Moon/Musquash R.	0.92	–	0.08	–	–	–	–	–	–	–	–	–	–	–
M. Thames R.	0.36	0.20	0.36	0.08	–	–	–	–	–	–	–	–	–	–
N. Detroit R.	0.35	0.19	0.31	0.13	–	–	–	0.01	0.02	–	–	–	–	–
O. Western basin L. Erie	0.35	0.23	0.24	0.13	0.01	0.01	0.01	0.01	–	0.01	–	–	–	–
P. Eastern basin L. Erie	0.24	0.28	0.18	0.14	0.02	–	–	0.02	–	–	–	0.02	0.02	0.02
Q. Historic walleye	–	–	0.95	–	–	–	–	–	–	–	–	–	–	–
R. Historic blue pike	–	–	1.00	–	–	–	–	–	–	–	–	–	–	–
S. Pigeon L.	0.16	0.04	0.72	–	–	–	–	0.08	–	–	–	–	–	–
T. Bay of Quinte	0.16	0.12	0.60	–	–	–	–	0.08	–	–	–	–	–	–
U. Oneida L.	–	–	0.36	0.48	–	–	–	–	–	–	–	–	–	–
V. Lac Mistassini	0.64	–	–	–	–	–	–	–	–	–	0.36	–	–	–
W. Ohio R.	0.27	0.18	0.09	0.18	–	–	–	–	–	–	–	–	–	–
X. New R.	–	–	0.08	0.20	–	–	–	–	–	–	–	–	–	–
Y. North R.	–	–	–	0.20	–	–	–	–	–	–	–	–	–	–

Location	15	16	17	18	19	20	21	22	23	24	25	26	27	28
A. Cedar L.	–	0.04	–	0.08	–	–	–	–	–	–	0.36	–	–	–
B. L. Winnipeg	–	–	–	–	–	–	–	–	–	–	–	0.04	–	–
C. L. of the Woods	–	–	–	–	–	–	–	–	–	–	–	–	–	–
D. McKim L.	–	–	–	–	–	–	–	–	–	–	0.16	–	–	–
E. Mille Lacs	–	–	–	–	–	–	–	–	–	–	0.04	–	–	–
F. St. Louis R.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
G. L. Nipigon	–	–	–	–	–	–	–	–	–	–	–	–	0.08	–
H. Portage L.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
I. Muskegon R.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
J. Thunder Bay	–	–	–	–	–	–	–	–	–	–	–	–	–	–
K. Flint R.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
L. Moon/Musquash R.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
M. Thames R.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
N. Detroit R.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
O. Western basin L. Erie	–	–	–	–	–	–	–	–	–	–	–	–	–	–
P. Eastern basin L. Erie	0.02	–	0.04	–	–	–	–	–	–	–	–	–	–	–
Q. Historic walleye	–	–	–	–	–	–	–	–	–	–	–	–	–	0.05
R. Historic blue pike	–	–	–	–	–	–	–	–	–	–	–	–	–	–
S. Pigeon L.	–	–	–	–	–	–	–	–	–	–	–	–	–	–
T. Bay of Quinte	–	–	–	–	–	–	–	–	–	0.04	–	–	–	–
U. Oneida L.	0.04	–	–	–	–	–	0.04	0.04	0.04	–	–	–	–	–
V. Lac Mistassini	–	–	–	–	–	–	–	–	–	–	–	–	–	–
W. Ohio R.	–	–	–	–	0.27	–	–	–	–	–	–	–	–	–
X. New R.	–	–	–	–	0.72	–	–	–	–	–	–	–	–	–
Y. North R.	–	–	–	–	–	0.80	–	–	–	–	–	–	–	–

Appendix 3.5

Exact tests of differentiation (Raymond & Rousset, 1995) for control region sequence data (below diagonal) and seven nuclear μsat loci (above diagonal) among the 25 sampled sites including, 23 modern walleye spawning groups, historic yellow walleye and historic blue pike. Results were congruent to F_{ST} comparisons and those from the seven nuclear μsat loci data were identical to values calculated based on nine loci, with values changing at the thousandth decimal place (data not shown; also see Stepien *et al.,* 2009, 2010).

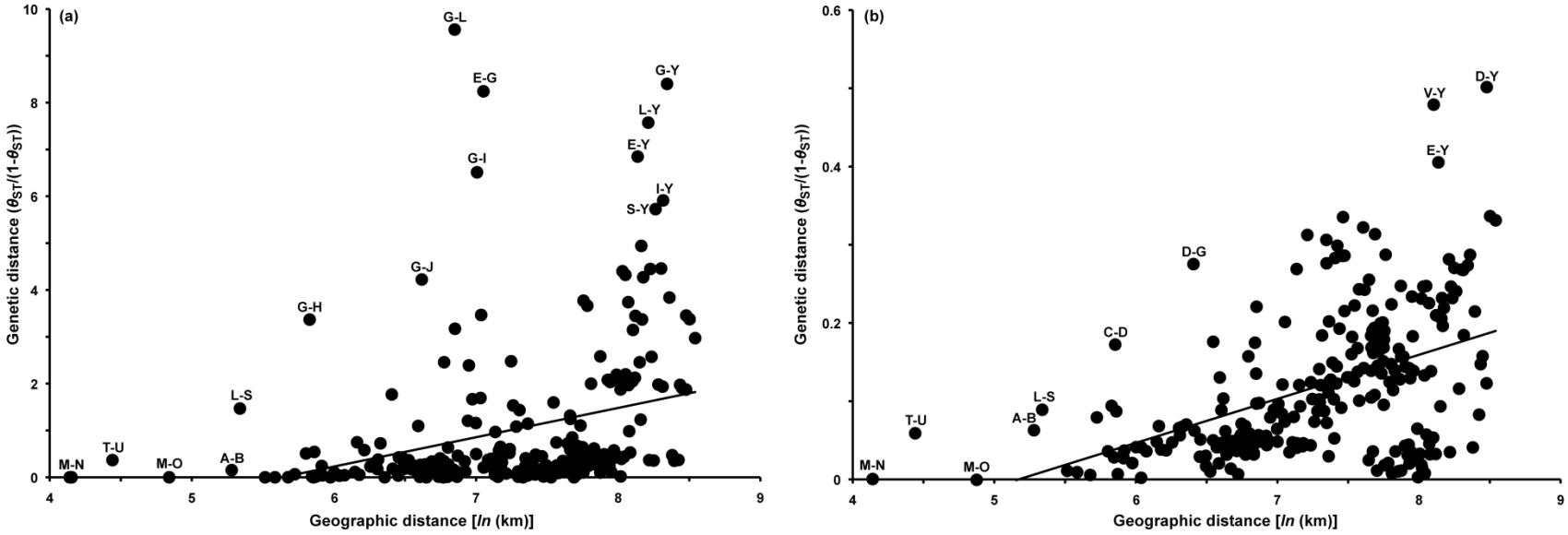
Location	A.	B.	C.	D.	E.	F.	G.	H.	I.	J.	K.	L.	M.
A. Cedar L.	---	Inf.	70.05	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
B. L. Winnipeg	17.20	---	36.31	Inf.	Inf.	Inf.	53.29	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
C. L. of the Woods	18.47	11.20	---	Inf.	Inf.	Inf.	59.57	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
D. McKim L.	6.37	14.61	5.88	---	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
E. Mille Lacs	21.55	Inf.	5.99	9.29	---	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
F. St. Louis R.	17.78	0.00	9.50	14.10	26.05	---	116.45	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
G. L. Nipigon	Inf.	11.21	Inf.	Inf.	Inf.	11.97	---	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
H. Portage L.	Inf.	Inf.	22.09	23.42	18.54	30.04	Inf.	---	Inf.	Inf.	Inf.	Inf.	132.66
I. Muskegon R.	27.44	Inf.	7.38	14.85	0.00	26.15	Inf.	14.48	---	Inf.	54.23	Inf.	Inf.
J. Thunder Bay	Inf.	Inf.	16.05	19.56	12.90	Inf.	Inf.	0.61	8.74	---	55.02	Inf.	Inf.
K. Flint R.	23.66	26.15	12.97	14.27	9.99	25.64	Inf.	3.60	6.21	1.80	---	Inf.	65.64
L. Moon/Musquash R.	Inf.	Inf.	7.40	14.79	1.43	29.02	Inf.	11.12	0.99	6.84	6.87	---	Inf.
M. Thames R.	Inf.	Inf.	27.27	27.11	24.19	Inf.	Inf.	2.19	16.62	3.74	2.69	17.76	---
N. Detroit R.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	4.18	21.84	6.31	6.12	25.88	0.15
O. W. basin L. Erie	Inf.	Inf.	Inf.	Inf.	27.44	Inf.	Inf.	2.24	19.25	4.09	5.40	24.01	0.21
P. E. basin L. Erie	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	4.19	20.64	7.22	8.77	25.72	0.33
Q. Historic walleye	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	25.80	Inf.	23.26
R. Historic blue pike	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	32.24	Inf.	Inf.	24.49	Inf.	23.26
S. Pigeon L.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	14.91	Inf.	17.95	13.16	Inf.	8.70

T. Bay of Quinte	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	10.25	Inf.	12.80	11.49	Inf.	5.40
U. Oneida L.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	30.04	Inf.	24.62
V. L. Mistassini	16.41	4.84	2.13	7.84	12.81	3.71	23.71	26.96	14.10	21.21	15.08	14.11	30.85
W. Ohio R.	16.88	25.50	21.60	18.21	17.13	25.50	Inf.	9.35	14.14	10.10	8.16	18.56	5.73
X. New R.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
Y. North R.	20.34	22.14	22.47	21.01	21.71	23.68	22.43	23.37	21.18	21.48	20.81	25.01	20.91

Location	N.	O.	P.	Q.	R.	S.	T.	U.	V.	W.	X.	Y.
A. Cedar L.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	57.66	Inf.	Inf.
B. L. Winnipeg	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	44.06	Inf.	Inf.
C. L. of the Woods	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	47.65	Inf.	Inf.
D. McKim L.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.
E. Mille Lacs	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	96.61	Inf.	Inf.
F. St. Louis R.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	28.24	Inf.	Inf.
G. L. Nipigon	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	42.29	Inf.	Inf.
H. Portage L.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	40.13	Inf.	Inf.
I. Muskegon R.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	36.44	98.92	Inf.
J. Thunder Bay	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	97.06	Inf.	Inf.	45.22	Inf.	Inf.
K. Flint R.	Inf.	Inf.	Inf.	Inf.	Inf.	77.55	88.53	121.78	Inf.	33.72	94.90	Inf.
L. Moon/Musquash R.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	54.72	Inf.	Inf.
M. Thames R.	21.34	14.22	30.93	Inf.	Inf.	85.15	46.62	71.77	Inf.	26.11	52.83	Inf.
N. Detroit R.	---	25.07	69.76	Inf.	Inf.	Inf.	86.18	Inf.	Inf.	33.81	Inf.	Inf.
O. W. basin L. Erie	0.64	---	64.88	Inf.	Inf.	Inf.	86.70	129.17	Inf.	33.64	Inf.	Inf.
P. E. basin L. Erie	7.12	2.85	---	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	33.25	Inf.	Inf.
Q. Historic walleye	32.24	Inf.	Inf.	---	63.03	Inf.	Inf.	Inf.	Inf.	82.92	Inf.	Inf.
R. Historic blue pike	Inf.	Inf.	Inf.	0.00	---	Inf.	Inf.	Inf.	Inf.	123.09	Inf.	Inf.
S. Pigeon L.	16.33	19.01	17.58	5.60	6.12	---	22.62	122.84	Inf.	29.05	99.45	Inf.
T. Bay of Quinte	12.00	13.04	10.87	9.39	9.88	0.39	---	104.12	Inf.	31.10	69.83	Inf.
U. Oneida L.	Inf.	Inf.	25.13	22.69	23.92	25.43	26.82	---	Inf.	37.85	46.14	127.57
V. L. Mistassini	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	---	113.79	Inf.	Inf.
W. Ohio R.	9.76	6.92	2.98	29.02	27.44	17.11	12.56	15.70	23.11	---	25.33	49.91
X. New R.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	Inf.	10.11	---	Inf.
Y. North R.	27.84	26.35	20.46	22.00	17.20	20.59	20.04	14.49	24.01	8.37	18.28	---

Appendix 3-1

Isolation by distance comparison among 23 modern walleye spawning groups for (a) mtDNA control region (y=0.64x–3.67, R^2=0.10, p<0.001) and (b) seven nuclear μsat loci (y=0.06x–0.29, R^2=0.23, p=0.005). Results from the seven loci were identical to those for nine loci (data not shown; also see Stepien *et al.*, 2009, 2010).



Chapter 4

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

Previously published as Haponski, A.E. & Stepien, C.A. (2013) Genetic connectivity and diversity of walleye (*Sander vitreus*) spawning groups in the Huron-Erie Corridor. *Journal of Great Lakes Research.*

4.1 Abstract

The Huron-Erie Corridor (HEC) connects the upper and lower Great Lakes, providing key fish passage. A century of channelization, dredging, and pollution have led to habitat loss and declining fish numbers. Since 2004, the multi-agency HEC initiative augmented fish spawning habitat at Belle Isle and Fighting Island in the Detroit River, whose populations are examined here. We analyze genetic patterns among seven spawning groups (N=311) of walleye *Sander vitreus,* a key fishery species, using nine nuclear DNA microsatellite loci and mitochondrial DNA control region sequences. Results reveal that all spawning groups contained appreciable genetic diversity (microsatellites: H_O=0.72; mtDNA: H_D=0.73) and showed a mixture of connectivity and

divergence. Genetic relationships did not fit an isolation by geographic distance hypothesis, with some closely spaced populations being very different. Notably, the Flint River-Lake Huron spawning group was the most divergent, showing no genetic exchange. The Belle Isle and Fighting Island populations markedly differed, with the latter showing some genetic exchange with the Grosse Ile (Detroit River) and the Huron River (northwest Lake Erie) populations to the south. Walleye spawning at Fighting Island experienced no significant change in overall genetic diversity pre- versus post-habitat augmentation, but the allelic frequency changed. Our results comprise an important baseline for future population analyses and habitat assessment of these habitat augmentation areas. Despite habitat degradation and pollution, it appears that historic walleye spawning groups have persisted along the HEC, meriting continued genetic monitoring and further restoration efforts to conserve and enhance this important and diverse fishery.

4.2 Introduction

Understanding the genetic connectivity (i.e., gene flow) and divergence of populations is fundamental to develop appropriate management strategies for ecologically and economically valuable species. Notably, identifying barriers to gene flow reveals important ecological information on species movement, dispersal, behavior, survival, and reproduction patterns that may be used to identify evolutionary significant units or other conservation management designations (see Waples, 1995; Wofford *et al*., 2005; Sork & Waits, 2010).

Aquatic populations may maintain gene flow through connecting channels that serve as migration corridors among watersheds (Robinson *et al*., 2002; LeClerc *et al*., 2008). Vagile fishes use such avenues to disperse to spawning sites, nursery habitats, and feeding grounds (Sheer & Steel, 2006; Meeuwig *et al*., 2010). Some widely distributed species may exhibit high gene flow across their connected range, with low overall population structure and little specialization (Boulet *et al*., 2007; Hughes, 2007). On the other hand, species having spawning site fidelity may show marked genetic structure and local adaptedness, despite apparent ample opportunity for migration and gene flow among adjacent locations. Notably, populations of salmonid fishes and other species, including walleye *Sander vitreus* (Percidae: Teleostei) are genetically structured due to spawning site philopatry and natal homing (Utter *et al.*, 1989; Jennings *et al*., 1996; Stepien & Faber, 1998; Nielsen & Fountain, 1999; Banks *et al*., 2000). Throughout most of the year, walleye move widely and intermingle within and among bodies of water, with some individuals travelling 50-300 km (Colby *et al*., 1979). In the spring walleye return to spawn at rocky shoals believed to be their natal sites (Jennings *et al*., 1996; Stepien & Faber, 1998; Wang *et al*., 2007).

Anthropogenic activities, such as exploitation, stocking, and habitat fragmentation and channelization may disrupt or increase genetic exchange across migration corridors, changing relationships among sub-populations. Such factors may lower genetic diversity and increase genetic drift, or may act to homogenize formerly different groups (Laroche & Durand, 2004; Wofford *et al*., 2005) and lead to declines in adaptedness and fitness (Leberg, 1992; Schindler *et al*., 2010).

4.2.1 Walleye distribution and genetic patterns

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

Broad and fine-scale spatial genetic patterns of walleye spawning groups have been defined across North America using mitochondrial (mt) DNA (Billington *et al*., 1992; Stepien & Faber, 1998; Gatt *et al.*, 2000, 2002) and nuclear DNA microsatellite (μsat) loci (Strange & Stepien, 2007; Stepien *et al*., 2009, 2010, 2012). Results have shown that many walleye spawning groups exhibited little genetic connectivity (e.g., gene flow) and significantly diverged in genetic composition, including between and within lakes, their basins, and connected tributaries (Stepien *et al*., 2009, 2010). The largest genetic divisions across their native range separated populations outside of the Great Lakes region from those within (Stepien *et al.*, 2009). The Great Lakes region was colonized by walleye originating in three Pleistocene glacial refugia: the Atlantic coastal, Mississippian, and Missourian (Ward *et al.*, 1989; Billington *et al*., 1992; Stepien & Faber, 1998; Gatt *et al*., 2000). Primary population demarcations within the Great Lakes separate the upper Lakes (Lakes Superior, Michigan, and Huron) from the lower Lakes

(Lakes Erie and Ontario), with significant genetic barriers between most of the lakes and some within them (Strange & Stepien, 2007; Stepien *et al*., 2009, 2010). The genetic patterns of the upper Great Lakes are likely a result of fish colonizing from the Mississippian and Missourian glacial refugia. The lower Great Lakes populations also were largely founded by the Mississippian refugium, with some contribution from the Atlantic Coastal refugium (Ward *et al*., 1989; Billington *et al*., 1992; Stepien & Faber, 1998; Gatt *et al*., 2000). A recent investigation evaluated three closely-related Lake Erie spawning runs over 15 years, showing overall within-site genetic consistency, and some genetic connectivity and divergence among them (Stepien *et al*., 2012).

Little is known of the genetic connectivity or divergence among walleye spawning groups in connecting channels, such as the HEC. Those spawning groups may be locally adapted, with unique ecological and physiological variations that may aid their response to external pressures such as spawning habitat loss, exploitation, invasive species, and climate change (Stepien & Faber, 1998; Kerr *et al*., 2010). Such perturbations likely have impacted walleye populations across the Great Lakes for more than a century, especially along fragile and degraded connecting channels, including the HEC. Defining the patterns of genetic connectivity and divergence of HEC walleye spawning groups may aid managers to maintain and enhance the fishery across this highly impacted system.

4.2.2 Degradation and augmentation of fish habitat along the Huron-Erie Corridor

The HEC is one of four connecting channels within the Great Lakes; it links Lakes Huron and Erie via the St. Clair River, Lake St. Clair, and the Detroit River (Fig.

4-1). The HEC constitutes a major international shipping route, supporting over $80 billion USD in annual trade (USGS, 2010). It once housed productive spawning and nursery habitats for many ecologically and economically important fish species, including lake trout *Salvelinus namaycush*, lake sturgeon *Acipenser fulvescens*, and walleye (Manny *et al*., 2010). The first reported habitat modifications began in 1874 with the construction of a shipping channel (914m long, 91m wide, and 6m deep) near Bois Blanc Island in the Detroit River, which eliminated fish spawning habitat in that area. Since that time, the HEC underwent a series of detrimental habitat modifications and fragmentation, including loss of coastal wetlands, armoring of shorelines, channelization, dredging, and industrialization (Hartig *et al*., 2009; USGS, 2010; Bennion & Manny, 2011).

In addition to habitat loss, industrial outputs along the HEC resulted in heavy metal contamination and declining fish health and numbers throughout the mid to late 20^{th} century (Hartig *et al*., 2009). Fish health problems included neoplasms, tumors, and lesions on walleye, brown bullhead *Ameirus nebulosis*, white sucker *Catostomus commersonii*, and other species (Manny & Kenaga, 1991). During the 1970s, walleye populations crashed and the entire fishery (commercial and recreational) was closed along the HEC due to high mercury levels in fish tissues.

In 2004, the HEC Initiative partnered 27 federal, state, and provincial agencies and local groups with the goal of restoring aquatic habitat (USGS, 2010). Two artificial reefs were installed in the Detroit River in waters ≥6 m deep: one in 2004 off the northeastern corner of Belle Isle (site C on Fig. 4-1) and another in 2008 at Fighting Island offshore from LaSalle, Ontario (site D on Fig. 4-1) (HTG, 2009, 2011; Habitat

Task Group of the Lake Erie Committee, Great Lakes Fishery Commission). Pre-construction assessment of spawning habitat revealed that walleye spawned at the Belle Isle site (Manny *et al*., 2007) and walleye and lake whitefish *Coregonus clupeaformis* spawned on suboptimal substrates at Fighting Island (HTG, 2009; Roseman *et al*., 2011). Prior to installation of these artificial reefs, the Belle Isle and Fighting Island sites contained suboptimal habitat for walleye spawning with thin patches (<8 cm thick) of sand and small-diameter gravel on hardpan clay, lacking interstitial spaces to protect fish eggs from predation or dislodgement (Manny, 2006; Roseman *et al*., 2011). In 2004, 1,080 m^2 of broken limestone (41-61 cm diameter), metamorphic cobble and gravel (20-30 cm), and coal cinders (2-8 cm) were deposited at the Belle Isle reef site to augment the spawning substrate (Manny *et al*., 2005). In 2008, 3300 m^2 of four different bed materials were deposited at the Fighting Island site, including a wide size range of broken limestone (5-50 cm) and rounded rock (10-25 cm; HTG, 2009, 2011) to provide an interstitial space gradient so that fish eggs would not be swept away by the current (Roseman *et al*., 2011). Prior to our study, it was unknown if walleye spawning at Belle Isle and Fighting Island belonged to historical spawning groups or were migrants from other locations.

4.2.3 Use of the Huron-Erie Corridor by walleye

Ripe walleye have been tagged and recorded to travel through the HEC in the spring to reach their spawning grounds (Ferguson & Derkson, 1971; Wang *et al*., 2007). Historically, walleye were known to spawn at sites along the HEC, most of which were sampled in the present study, with major runs occurring in Lake Huron's Saginaw Bay,

the Thames River of Lake St. Clair (site B; Fig. 4-1), and the Hen Island shoals in northwestern Lake Erie (site G; Wolfert, 1963; Goodyear *et al*., 1982; known spawning sites are marked with Xs on Fig. 4-1). Along the remainder of the HEC, smaller walleye spawning runs were located in the Flint River (site A), St. Clair River (including at its connection to Lake Huron), Detroit River (sites C-E), including its lower reaches and mouth, and the Huron River (site F; Goodyear *et al*., 1982; Fielder *et al*., 2006). Historical walleye spawning runs likely occurred at Belle Isle (site C) and Fighting Island (site D), where the artificial reefs were constructed (Manny *et al*., 2007; HTG, 2009).

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

& Towns, 2011). The Huron River (site F) of northwestern Lake Erie was dammed, reducing habitat, and has a smaller native spawning run near its mouth that has experienced low levels of exploitation (Leonardi & Thomas, 2000). In the past, some of its impoundments upstream from that spawning site were sporadically stocked, however, the spawning site itself was not stocked (Leonardi & Thomas, 2000; USFWS/GLFC, 2010).

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

The objective of our study was to evaluate the genetic connectivity, diversity, and divergence patterns of walleye spawning groups in the HEC. We analyzed 311 walleye from seven spawning sites in the HEC and outlying populations (A-G; Fig. 4-1), with a dual approach of nine nuclear DNA μsat loci and mtDNA control region sequences. This

approach allowed us to compare patterns at multiple evolutionary and temporal scales (Avise, 2004; Wang, 2010, 2011), since the μsat loci addressed contemporary microevolutionary processes, such as migration, gene flow, and genetic drift, whereas the mtDNA control region sequences revealed historical context, such as origins from Pleistocene glaciation refugia. Specific hypotheses (stated as null/alternative) tested in the present study included: (H1) walleye spawning groups across the HEC had similar/different levels of genetic diversity, (H2) their relationships reflected genetic connectivity/divergence among spawning groups and between the sexes, and (H3) genetic composition at the HEC Detroit River Fighting Island reef site remained similar/changed after habitat augmentation. Hypothesis 3 was limited to early findings; additional samples will be needed to evaluate long-term effects and trends in these habitat augmentation areas.

4.3 Materials and methods

4.3.1 Sample collection and DNA extraction

Walleye fin clips (1-2 cm^2 of pectoral or caudal fins) were collected by federal and state fishery biologists during spring spawning runs at seven sites in the HEC, totaling 311 individuals and representing the major and minor spawning runs along the HEC (Fig. 4-1, Table 4.1; all available samples were analyzed). Sampled sites included: the Flint River-Lake Huron (site A, coordinates 43.3300 N, -84.0543 W), Thames River (B, 42.3171 N, -82.4363), Belle Isle (C, 42.3469 N, -82.9533 W), Fighting Island (D, 42.2378 N, -83.1295 W), Grosse Ile (E, 42.1177 N, -83.1781 W), Huron River (F,

42.0899 N, -83.2902 W), and Hen Island (G, 41.8024 N, -82.7804 W). All individuals were verified as in spawning condition, and most were released after fin-clipping, measurement of standard length (SL, mm) and sex determination. A total of 51 spawning females (1-23 per site), 146 spawning males (12-40 per site), and 10 unsexed individuals (3-7 per site) were recorded from samples for which sex data were available. Sex and length data were not taken for walleye spawning in the Thames River (B) and Hen Island (G). To test effects of habitat augmentation on genetic diversity and composition (hypothesis 3), spawning walleye were collected from the Detroit River Fighting Island reef pre- (2008 *N*=20), and post- (2010 *N*=28) habitat augmentation (Table 4.1). Tissue samples were immediately placed in 95% ethanol, stored at room temperature, and archived in the Great Lakes Genetics Laboratory at the University of Toledo's Lake Erie Center (Oregon, OH). DNA was extracted using Qiagen DNEASY extraction kits (Qiagen Inc., Valencia, CA), then assessed for quality and quantity on 1% agarose mini-gels stained with ethidium bromide.

4.3.2 Nuclear microsatellite data collection

Allelic variation at nine µsat loci (*Svi*2, 4, 6, 7, 17, 18, 33, L6, and L7) was analyzed to test for population genetic structure (e.g., Strange & Stepien, 2007; Stepien *et al.*, 2009, 2010; Table 4.2). Polymerase chain reaction (PCR) amplifications were conducted in 48 well plates with 10µl reactions containing 0.6 units *Taq* polymerase, 50µM dNTPs, 50mM KCl, 1.5mM $MgCl_2$, 10mM Tris-HCl, 0.5µM of each primer, and ~80ng of template. A positive control (designated Lake Erie walleye tissue, sample AYD03 from the Maumee River 2006 spawning run) and a negative control (no

template) were included in all reaction runs. PCR cycling parameters consisted of 2 min at 94^{o}C for initial denaturation, followed by 35 cycles of denaturation (94^{o}C, 30 s), primer annealing (1 min) at specific temperatures (given in Table 2), and polymerase extension (72^{o}C, 30 s), followed by a final extension at 72^{o}C for 5 min. Three sets of loci were multi-plexed as single PCR reactions: *Svi*4 and 33, *Svi*2, 6, and 7, and *Svi*L6 and L7. *Svi*17 and 18 were run individually. Amplification products were diluted 1:50, of which 1µl was added to 13µl of formamide and Applied Biosystems (ABI, Fullerton, CA) Gene Scan 500 size standard in 96-well plates, denatured for 2 min at 95^{o}C, and analyzed on an ABI 3130xl Genetic Analyzer with Genemapper v3.7. To minimize analyzer runs, *Svi*17 and 18 were pooled and visualized with different dye colors. Output profiles were checked manually to confirm allelic size variants. Repeat number and size, and number of alleles per locus, are reported in Table 4.2.

4.3.3 MtDNA control region sequence data

A subset of the 311 individuals representing the seven HEC walleye spawning groups was amplified and sequenced for the mtDNA control region (*N*=195, 20-25 per site; Table 4.1), with the primers LW1-F (Gatt *et al*., 2000) and HN20 (Bernatchez & Danzmann, 1993). PCR reactions contained 50mM KCl, 1.5mM $MgCl_2$, 10mM Tris-HCl, 50µM of each dNTP, 0.5µM each of the forward and reverse primers, 30ng DNA template, and 1 unit of *Taq* polymerase in a 25µl reaction. Amplification procedure was an initial denaturation for 2 min at 94^{o}C, followed by 42 cycles of 40 sec at 94^{o}C, 40 sec at 48^{o}C, and 1.5 min at 72^{o}C, with a final extension of 5 min at 72^{o}C. A 4µl aliquot of each PCR product was visualized on a 1% agarose mini gel stained with ethidium

bromide, and successful reactions were purified using a QIAGEN PCR Purification Kit. DNA sequencing was outsourced to the Cornell University Life Sciences Core Laboratories Center, which used ABI Automated 3730 DNA Analyzers. HEC walleye spawning group mtDNA control region sequences totaled 733 bp and were checked, identified, and aligned with BioEdit v7.05 (Hall, 1999). We related the haplotypes to those of Stepien & Faber (1998), who sequenced the entire mtDNA control region (totaling ~1,086 bp) for 179 walleye across the Great Lakes and recovered 14 haplotypes (GenBank accession #U90617). We trimmed the original sequences of Stepien & Faber (1998) to match our 733 bp, omitting their 5' repeat section, which left seven of their original haplotypes (designated here as A1-7, National Institutes of Health (N. I. H.) GenBank Numbers U90617 and JX442946-52; http://www.ncbi.nlm.nih.gov/).

4.3.4 Genetic data analyses

The nine µsat loci were tested for conformance to Hardy-Weinberg equilibrium (HWE) expectations and linkage disequilibrium (LD), using a Markov Chain Monte Carlo (MCMC) chain of 10,000, 1,000 batches, and 10,000 iterations in Genepop v4.0 (Rousset, 2008). Levels of significance were adjusted with the standard Bonferroni correction (Zar, 1999). The program Micro-checker v2.2.3 (van Oosterhout *et al.*, 2004) was used to evaluate loci for null alleles, scoring errors, or large allele dropout. Per-locus calculations included: number of alleles (N_A), inbreeding (F_{IS}), overall genetic deviation across all samples (F_{IT}), and divergences (F_{ST}) in *F*stat v2.9.3.2 (Goudet, 1995, 2002).

Genetic diversity comparisons (hypothesis 1) among the spawning groups and sampling years included: observed (H_O) and expected (H_E) heterozygosities in Genepop,

and F_{IS}, N_A, and allelic richness (A_R) in *F*stat for the nine μsat loci and haplotype diversity (H_D) and number of haplotypes (N_H) calculated in Arlequin v3.5.1.3 (Excoffier & Lischer, 2010) for the mtDNA control region sequence data. Number and proportion of private alleles (N_{PA}) and private haplotypes (N_{PH}), i.e., those unique to a given spawning group or system, were calculated with Convert v1.31 (Glaubitz, 2004). Proportion of private alleles (P_{PA}) and proportion of private haplotypes (P_{PH}) were determined by dividing the number of private alleles/haplotypes for a given population sample by its total number of alleles/haplotypes. Standard errors were calculated in Microsoft Office Excel 2003 (Redmond, WA). To test for significant differences in H_O and A_R, a Friedman rank sum test in R v2.14.2 (R Development Core Team, 2012) was employed, with the loci treated as blocks. To test hypotheses of genetic diversity patterns (hypothesis 1) and genetic connectivity/divergence among walleye spawning groups along the HEC (hypothesis 2), just the samples from Fighting Island post-habitat augmentation were used.

To examine whether the relationships reflected genetic connectivity (gene flow) or divergence among spawning groups (hypothesis 2), females versus males, or a change in genetic composition pre- versus post-habitat augmentation at the Fighting Island Reef site (hypothesis 3), exact tests of differentiation (χ^2) were used to test for differences in genetic composition among pairs of samples (Raymond & Rousset, 1995), using a MCMC chain of 10,000, 1,000 batches, and 10,000 iterations in Genepop. These tested whether the seven spawning groups represented a single panmictic group or multiple sub-populations. Two different analyses tested for differences between the sexes, one included all available data (μsats: 51 females, 146 males; mtDNA: 29 females, 110

males) and the other evaluated samples from Grosse Ile (site E) that had a more even distribution of females and males (μsats: 23 females, 12 males; mtDNA: 13 females, 12 males). Probability values were adjusted using the sequential Bonferroni correction (Rice, 1989). Number of genetic migrants (N_M) between spawning groups was calculated in Arlequin, following Slatkin (1991) to evaluate how much genetic exchange may have occurred.

Three additional approaches further evaluated genetic connectivity and divergence patterns (hypothesis 2): (1) Analysis of Molecular Variance (AMOVA; Excoffier *et al*., 1992), (2) Barrier v2.2 (Manni *et al*., 2004), and (3) isolation by distance via Mantel's (1967) test. AMOVA tests assessed hierarchical relationships among samples (i.e., lakes versus spawning groups) in Arlequin. Barrier tested for significant discontinuities in gene flow (connectivity) by identifying which neighboring samples were distinguished by higher than expected genetic divergence (measured as θ_{ST} (Weir & Cockerham, 1984) in *F*stat) in relation to geographical coordinates (latitude and longitude). Support for the barriers was assessed in two ways: (1) relative number of supporting loci (per Strange & Stepien, 2007; LeClerc *et al*., 2008), and (2) bootstrap analysis of 2,000 multilocus matrices in Geneland v3.3.0 (Guillot *et al*., 2005; Guillot & Santos, 2009). Those with locus and bootstrap support values higher than 50% were reported here. Fit to a genetic isolation (θ_{ST} /(1- θ_{ST})) by geographic distance model (shortest connected waterway using the path option in Google Earth® (Google, 2010)) was tested with Isolde in Genepop, which predicted a linear relationship (Rousset, 1997), using Mantel's (1967) procedure and 10,000 MCMC permutations. Origins of individuals spawning at Fighting Island pre- and post-habitat augmentation (hypothesis 3) were

compared using a Bayesian approach in Geneclass2 (Piry *et al.*, 2004), which assigned individual fish to one of the seven HEC walleye spawning samples via 10,000 simulations per Rannala & Mountain (1997) and Cornuet *et al*. (1999). A χ^2 contingency was used to test for significant difference between individuals that self- or misassigned (Zar, 1999).

4.4 Results

4.4.1 Genetic diversity of walleye spawning groups along the HEC (hypothesis 1)

The nine μsat loci were unlinked, and the samples and the loci conformed to HWE expectations following Bonferroni correction. Only two possible cases of null alleles were detected: locus *Svi*7 from the Thames River (B) spawning group and *Svi*18 in the Huron River (F). Since null alleles were not detected at those loci in the five other HEC spawning groups, the populations were in HWE, and there were no signs of heterozygote deficiency, scoring error, or stuttering, all loci were included in our analyses (see vanOosterhout et al., 2004). Loci *Svi*2, 7, and 18 had the highest F_{ST} values (0.024, 0.028, and 0.016) and thus contributed more to divergence among the spawning groups (Table 4.2).

Overall, 119 alleles were recovered from 311 walleye at the nine μsat loci, with 74-88 alleles per spawning group (mean=80) and allelic richness (A_R) values of 7.1-8.1 (mean=7.5±1.0; Table 4.1). Walleye spawning at the Detroit River Belle Isle (C) augmentation site had the most alleles (88, A_R=7.8±1.0), followed by Hen Island (G, 85, A_R=7.1±0.8), the Thames River (B, 84, A_R=7.7±0.9), Grosse Ile (E, 84, A_R=8.1±1.1), and

the Huron River (F, 84, A_R=7.8±1.0). The population spawning at Grosse Ile (E) had the highest μsat allelic richness. Allelic richness did not significantly differ among the seven spawning groups, based on the Friedman rank sum test (χ^2=8.90, df=6, p=0.1800).

For the mtDNA control region sequence data (733 bp), eight haplotypes (GenBank accession #s JX442946-49 and JX44953-56) were identified among 195 HEC spawning walleye (Fig. 4-2; Table 4.1). Four haplotypes were common throughout the data set (these matched haplotype #s A1-4 of Stepien & Faber (1998); GenBank #U90617 and #JX442946-49). We discerned four new haplotypes that were unique from those of Stepien & Faber (1998), which here are designated as B8-11, GenBank #JX442953-56. Haplotype A1 (GenBank #U90617, JX442946) was the most abundant overall, characterized 37% of the samples, and reached its highest proportion (60%) in the Flint River (A). Haplotype A3 (GenBank #JX442948) was the next most abundant and occurred in 31% of the samples, whereas haplotypes A2 (GenBank #JX442947) and A4 (GenBank #JX442949) represented 17 and 12%, respectively. Similar number of haplotypes were found in all spawning groups, with Flint River (A), Belle Isle (C), Fighting Island (D2), Huron River (F), and Hen Island (G) having five each and the Thames River and Grosse Ile with four (Fig. 4-2; Table 4.1). The newly discovered haplotypes were: B8 (GenBank #JX442953) from Fighting Island (D1) and Hen Island (G), B9 (GenBank #JX442954) from Belle Isle and Fighting Island (D2), B10 (GenBank #JX442955) from the Huron River (F), and B11 (GenBank #JX442956) from the Flint River (A).

Numbers of private μsat alleles ranged from 1-6 per spawning group (mean=2), with the most at Belle Isle (C, 6 alleles, proportion (P_{PA})=0.07) and Hen Island (G, 5,

0.06), a moderate number at Thames River (B, 2, 0.02) and Huron River (F, 2, 0.02), and the least in the Flint River (A, 1, 0.01), Fighting Island (D2, 1, 0.01), and Grosse Ile (E, 1, 0.01; Table 4.1). Two private haplotypes were recovered in the mtDNA control region dataset, one in the Flint River (A) and one in the Huron River (F).

The spawning groups had similar μsat heterozygosities (mean H_O=0.72±0.03), ranging from 0.68±0.03 at Hen Island (site G) in Lake Erie to 0.76±0.05 at Flint River (A). Heterozygosity values at the habitat augmentation sites were relatively high: 0.73±0.03 at Belle Isle (C) and 0.69±0.04 at Fighting Island (D2). The Friedman rank sum test results showed no significant differences in observed heterozygosity values of walleye spawning groups across the HEC (χ^2=3.17, df=6, p=0.7900). The Flint River (A) sample in Lake Huron suggested some slight heterozygote excess or outbreeding (F_{IS}=-0.018±0.024), which was not significant. The other six samples (B-G) indicated slight inbreeding depression (F_{IS}=0.008±0.034-0.056±0.025; Table 4.1), which also was not significant. The Flint River-Lake Huron (A) had the lowest mtDNA haplotypic diversity (0.58±0.02), whereas the other spawning groups had similar diversity levels (0.72±0.01-0.78±0.01).

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

Several HEC walleye spawning groups significantly differed in genetic composition according to the μsat data (Table 4.3). The Flint River (A) spawning group was the most divergent (χ^2=63.5-Inf, $p\leq$0.0001), followed by the Fighting Island sample post-habitat augmentation (D2), which significantly differed from Belle Isle (C, χ^2=46.1,

p=0.0003) and Hen Island (G, χ^2=43.8, *p*=0.0006), but was less divergent from the Thames River (B, χ^2=35.0, *p*=0.0100), Grosse Ile (E, χ^2=35.3, *p*=0.0090), and Huron River samples (F, χ^2=31.5, *p*=0.0300). Walleye spawning at Belle Isle (C) also significantly differed from the Hen Island spawning group (G, χ^2=46.8, *p*=0.0002) and slightly differed from the Thames (B, χ^2=31.2, *p*=0.0300) and Huron (F) river samples (χ^2=34.3, *p*=0.0100). Appreciable genetic connectivity (Table 4.3) was evident among walleye spawning in the Thames River (B), Grosse Ile (E), Huron River (F), and Hen Island (G, χ^2=18.2-27.7, *p*=0.0700-0.4500). Estimated migration values among those four spawning groups (B, E-G) were high; values for Thames River (B) were 65 individuals exchanged with Hen Island (G), 307 with Grosse Ile (E), and calculated as infinite with the Huron River (F; Table 4.3). The Grosse Ile sample additionally showed high gene flow, with migration estimated from 76 individuals with Hen Island (G) to 114 with the Huron River (F; Table 4.3). In contrast to the higher-resolution μsat data, no significant differences were recovered from the mtDNA control region sequence data (χ^2=0.0-5.2, *p*=0.0700-1.0000). Thus, the mtDNA data were not used for Barrier, AMOVA, or isolation by distance analyses.

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

Barrier analysis recovered four primary barriers to gene flow (Fig. 4-1), in which genetic divergence was significantly greater than expected. The primary division (barrier

I; 98% bootstrap support, 100% of the loci) distinguished the Lake Huron (Flint River, site A) spawning group from all other samples. The second (II; 96%, 100% loci) separated the walleye spawning groups in the Thames River (B) and Belle Isle (C). The third (III; 87%, 100% loci) barrier separated walleye spawning at Hen Island (G), and the next (IV; 72%, 89% loci) denoted the Detroit River Fighting Island group (D2).

Hierarchical relationships among population groups analyzed with AMOVA showed significant delineation among the three Lakes (1.07%, $p<0.0001$) and among spawning groups within them (0.42%, $p=0.0100$). Relationships among the spawning sites (Fig. 4-3) did not fit a genetic isolation by geographic distance model ($p=0.0800$), with the best-fit regression line explaining 69% of the variation ($y = 0.010x - 0.03$, $R^2=0.69$). Comparisons of the Flint River (A) group with all other samples showed much greater difference than would be predicted by geographic isolation. This result was similar to the Barrier analysis and χ^2 findings, indicating that the Flint River-Lake Huron spawning group is very genetically distinct. When the Flint River (A) comparisons were excluded from analysis, the remaining HEC samples likewise did not follow an isolation by distance model ($y=-0.001x + 0.006$, $R^2=0.001$, $p=0.5800$). Thus, the relationships among spawning groups across the HEC appeared independent of geographic distance.

4.4.3 Genetic composition pre- and post-habitat augmentation (hypothesis 3)

Genetic comparisons of walleye spawning at the Fighting Island reef (D) pre- and post-habitat augmentation (Fig. 4-2, Table 4.1) showed a slight decrease in observed μsat heterozygosity (0.72±0.04 to 0.69±0.04), an increase in mtDNA haplotypic diversity (0.70±0.02 to 0.74±0.01), and a greater number of μsat alleles (67-70). Friedman rank

sum test results showed no significant differences in observed heterozygosity values of walleye spawning at Fighting Island pre- and post-habitat augmentation (χ^2=0.11, df=1, p=0.7400). Pairwise comparison tests showed a significant difference in genetic composition pre- versus post-habitat augmentation (χ^2=32.7, p=0.0200) in the μsat data (the mtDNA control region sequence data did not differ; χ^2=2.5, p=0.2800). Pre- and post-habitat augmentation samples each contained a different private allele and a unique haplotype (Fig. 4-2; Table 4.1). These results may be due to sample size effects. Apparent inbreeding (heterozygote deficiency) increased pre- to post-habitat augmentation from -0.009-0.056; the latter value was the highest in our dataset (Table 4.1).

Divergence values for the Fighting Island spawning group before habitat augmentation (D1) indicated more connectivity, suggesting more exchange of reproductive individuals with other spawning populations, than after augmentation (D2). Both samples from Fighting Island significantly diverged from the Flint River (A, χ^2=72.2-96.1, $p\leq$0.0001) and Belle Isle populations (C, χ^2=29.4-46.1, p=0.0003-0.0400; Table 4.3). However, the earlier sample was genetically similar to other HEC spawning groups (χ^2=19.0-25.8, p=0.1000-0.4000). Following the habitat augmentation, divergence increased, with the Fighting Island walleye appearing more genetically distinct (χ^2=31.5-46.1, p=0.0003-0.0300; Table 4.3). Walleye from Fighting Island in both samples most closely resembled those spawning at Grosse Ile (E) just to the south (Fig. 4-1), with N_M estimates (representing possible reproductive migrants) of 150 and 187 individuals, respectively (Table 4.3). Overall estimated N_M values ranged from 30-187 in the earlier sample versus 33-150 post-habitat augmentation. Likely returns numbered 37 individuals between the two sampling dates (88 according to the mtDNA data; Table 4.3).

Congruently, both samples from Fighting Island showed low self-assignment values (Table 4.4), with no significant difference in those self-assigning and assigning to other samples between the two dates (χ^2 contingency test=3.12, p=0.0800, 1 df). Many misassigning individuals traced to Grosse Ile (E, 40% pre- and 25% post-augmentation), similar to the N_M results. Others that misassigned traced to the Thames River (B, 20% pre- and 7% post-habitat augmentation), Belle Isle (C, 20% and 14%), Huron River (F, 20% and 29%), and Hen Island (G, 0% and 11%). Before spawning habitat augmentation, 40% of walleye spawning at Fighting Island misassigned to the north (sites B-C) and 60% to the south (E-G). Following augmentation, 21% misassigned to the north and 65% to the south (Table 4.4). This trend, however, was not significant (χ^2 contingency test=1.13, p=0.2900, 1 df).

4.5 Discussion

4.5.1 Genetic trends in relation to our hypotheses

Our results reveal relatively similar levels of genetic diversity among samples across the HEC, supporting null hypothesis 1. Walleye spawning in the HEC thus have unique variability despite over a century of habitat degradation. The population reproducing at the Belle Isle habitat augmentation site in the Lake St. Clair system had the most alleles, high allelic richness, and the greatest number and proportion of private alleles. The Fighting Island spawning group also showed appreciable genetic diversity. Walleye from the Flint River-Lake Huron site did not reproduce with those from other HEC locations (rejecting null hypothesis 2). Walleye spawning at the Belle Isle and

Fighting Island habitat augmentation sites housed unique variability and diverged from most other groups (also rejecting null hypothesis 2). Both augmentation sites thus appear to house different and potentially native spawning groups of walleye. More genetic connectivity and gene flow characterized most other groups spawning in Lake St. Clair and northwestern Lake Erie (supporting null hypothesis 2). Overall patterns among spawning populations did not fit a hypothesis of genetic isolation with geographic distance, with some HEC spawning groups located in close proximity being very divergent.

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

4.5.2 Walleye genetic diversity patterns (hypothesis 1)

The genetic diversity levels for spawning groups along the HEC appear typical for walleye populations, suggesting that despite over a century of exploitation, stocking, habitat loss, and degradation, genetic integrity likely has been maintained. In our study,

walleye spawning in the Lake St. Clair basin displayed intermediate diversity levels, having high numbers of μsat alleles and mtDNA haplotypes, greatest allelic richness, and high number and proportion of private alleles. The Belle Isle habitat augmentation site had the most μsat alleles, one of the highest allelic richness values, and the most private alleles, reflecting a diverse genetic history. Walleye spawning at the seven sites along the HEC had mean genetic diversity (observed heterozygosity) values (0.72±0.04) similar to those reported across the Great Lakes (0.71±0.01) and across their native range (0.68±0.01) by Stepien *et al.* (2009, 2010) using the same nine μsat loci.

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

despite a history of exploitation and habitat loss along the HEC, its walleye diversities are relatively high, likely due to the prevalence of large connected populations across this region.

4.5.3 Genetic divergence and connectivity of walleye spawning groups along the HEC (hypothesis 2)

Comparisons among the HEC walleye spawning groups using mtDNA control region sequence data showed no differences among them, whereas the nuclear μsat loci discerned significant differences. This difference is attributable to the slower evolutionary rate of mtDNA control region sequences compared to nuclear μsat loci (Hewitt, 2001; Wang, 2010, 2011). Mitochondrial DNA sequences have ¼ the effective population size of nuclear DNA, rendering mtDNA more subject to declines in variability from population bottlenecks. We sampled many more μsat alleles per population (here 70-88 alleles per spawning group) and many more loci with the μsat data set compared to the mtDNA control region sequence data (4-5 haplotypes with 1-2 base pair differences). The seven walleye spawning groups along the HEC are believed to trace to colonists from the Mississippian and Atlantic coastal glacial refugia (Ward *et al*., 1989; Billington *et al*., 1992; Stepien & Faber, 1998; Gatt *et al*., 2000; Stepien *et al*., 2009). Our study recovered four common mtDNA control region haplotypes (A1-4) that characterized all of our HEC sites. Common haplotypes A1 and A3 were identified as originating from the Mississippian glacial refugium, whereas common haplotype A4 came from the Atlantic coastal refugium (Billington *et al*., 1992; Stepien & Faber, 1998). We also found four rarer haplotypes that differed by 1-2 base pairs from the more common ones.

Our analyses using the nine nuclear μsat loci showed pronounced genetic differences between walleye spawning in the Flint River-Lake Huron from other sites in the HEC, which were much greater than those predicted by geographic distance. Walleye spawning in different Great Lakes do not appear to exchange genes (Stepien *et al.* 2009), although they move among systems during non-spawning times (Wang *et al.*, 2007; Vandergoot *et al*., 2010). Studies of other Great Lakes fishes likewise showed marked difference of spawning groups in Lake Huron from those in Lakes St. Clair and Erie, including yellow perch *Perca flavescens* (Sepulveda-Villet & Stepien, 2012; Sullivan & Stepien, 2013, this issue) and smallmouth bass *Micropterus dolomieu* (Stepien *et al*., 2007).

Divergence of the Flint River-Lake Huron walleye gene pool from those in Lakes St. Clair and Erie may have occurred more recently than the Pleistocene glaciations, reinforced by behavioral isolation and spawning site philopatry, since we recovered this pattern with the μsat data alone. Tagging studies showed that Flint River post-spawn walleye had limited migration, travelling only to inner Saginaw Bay (~50 km) and remaining there until the spring, when they migrated back into the river to spawn (Leonardi & Gruhn, 2001). In comparison, some individuals from other walleye spawning groups traveled longer distances (e.g., ~165 km from the western basin of Lake Erie to Lake Huron; Ferguson & Derkson, 1971; Haas *et al*., 1985; Todd & Haas, 1993; Wang *et al*., 2007). Moreover, the Saginaw River and Bay system connected with the Flint River has been stocked with individuals from the western basin of Lake Erie since 1989 (USFWS/GLFC, 2010), which may have obscured the mtDNA signal. However, our nuclear DNA data and the relatively high and unique diversity of the Flint River

spawning group supports its historical genetic signature. This appears congruent with the observation of behavioral isolation by migration patterns (Leonardi & Gruhn, 2001).

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

The other HEC walleye spawning groups – Thames River, Fighting Island, Grosse Ile, Huron River, and Hen Island – displayed more inter-migration and connectivity, but significant difference between the Fighting Island and Hen Island populations. Using lower resolution allozyme and mtDNA restriction fragment length

polymorphism markers, McParland *et al.* (1999) found no differences in spawning walleye collected from our sites in Lakes St. Clair (Thames River) and Erie (Huron River), along with Chickenolee Reef in western Lake Erie. Stepien *et al*. (2012) found only a slight difference with μsat data between the Maumee and Sandusky River spawning groups (two of Lake Erie's largest spawning runs, located in close proximity; Mion *et al*., 1998), compared to a larger genetic divergence from other populations. Walleye spawning in some western Lake Erie sites thus may comprise a single connected spawning group, which may extend into the HEC.

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

The high genetic connectivity observed among some of the HEC walleye spawning groups also may be influenced by anthropogenic habitat degradation. Walleye homing behavior could be more facultative in degraded areas due to disruption of habitat and associated chemical cues (Olson & Scidmore, 1962; Colby & Nepszy, 1981; Backhouse-James & Docker, 2012). Olson & Scidmore (1962) stated that with increased

stream flow (as occurred from modification of the Detroit River), eggs and larvae would have less time to imprint, which would increase straying and lead to genetic homogenization. Optimal egg deposition for walleye in river systems occurred at velocities of 0.4-1.0 m/s (Paragamian, 1989; Bozek *et al*., 2011), whereas present-day estimates were 0.76-1.68 m/s for the Detroit River (U.S. Army Corp. of Engineers, 2006), indicating that it might be difficult for eggs to remain in place (see Manny *et al*., 2005; Roseman *et al*., 2011). Roseman *et al*. (2011) documented a decline in water velocity to 0.3-0.8 m/s at the Fighting Island site after installation of the artificial reefs. This suggests that artificial habitats may improve egg retention and imprinting of walleye to natal sites, ultimately leading to localized adaptation.

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

In contrast to our results for walleye, Sullivan and Stepien (2013, this issue) found great genetic divergence and no connectivity among yellow perch spawning groups across the HEC. This may be due to higher spawning group fidelity of yellow perch and their more limited migration (Rawson, 1980). Studies showed that the related European perch discriminates kin from non-kin via olfactory cues, and schools in full and half-sib groups maintained throughout their lives (Gerlach *et al*., 2001; Behrmann-Godel &

Gerlach, 2008). Thus, family groups of the European perch appear to move and reproduce together, genetically diverging from non-kin groups (Gerlach *et al*., 2001). This life history pattern remains to be tested for yellow perch and walleye.

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

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

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

4.5.5 Effects of habitat augmentation on genetic composition (hypothesis 3)

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

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

need for continued long-term monitoring of these spawning groups to include additional generations of walleye.

4.5.6 Summary

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

4.6 Acknowledgements

This is publication number 2013-006 from the Lake Erie Research Center. Grant awards to CAS from NOAA Ohio Sea Grant Program R/LR-013, "*Temporal and spatial*

analyses of walleye and yellow perch genetic stock structure: A high-resolution database for fishery management" and USEPA CR-83281401-0, "*High resolution delineation of Lake Erie Fish Populations: DNA databases for resource management*", and to E. Roseman from the Great Lakes Restoration Initiative Project #70, "*Habitat Enhancement Strategies for the Huron-Erie Corridor*" funded the study. AEH was supported by a NSF GK-12 DGE#0742395 fellowship "*Graduate Fellows in STEM High School Education: An Environmental Science Learning Community at the Land-Lake Ecosystem Interface*" (P.I. = CAS), University of Toledo teaching and research assistantships, the International Association for Great Lakes Research (IAGLR) Norman S. Baldwin Fishery Science Scholarship (2010), and the IAGLR Scholarship award (2009). We thank the U.S. Fish and Wildlife Service, U.S. Geological Survey, Ohio and Michigan Departments of Natural Resources and Ontario Ministry of Natural Resources for contributing samples. We also thank the Great Lakes Fishery Commission Lake Erie Walleye Task Group, J. Boase, A. Cook, T. Johnson, K. Kayle, B. Manny, J. McFee, E. Roseman, and M. Thomas for sampling and research design input. We are grateful to P. Kocovsky, three anonymous reviewers, and the Great Lakes Genetics Laboratory members D. Murphy, L. Pierce, O. Sepulveda-Villet, T. Sullivan, and S. Yerga-Woolwine for help in the laboratory and valuable comments on various drafts of the manuscript. Lake Erie Center staff P. Uzmann, M. Gray, and R. Lohner provided logistic support.

Table 4.1

Genetic variation of the seven walleye spawning groups (sites are labeled according to Fig. 1) using nine nuclear microsatellite loci and mitochondrial DNA control region sequences, including the number of individuals (N), observed heterozygosity (H_O), inbreeding coefficient (F_{IS}), total number of alleles (N_A) or haplotypes (N_H), allelic richness (A_R), number of private alleles (N_{PA}) or haplotypes (N_{PH}), i.e., those found only in that spawning group, proportion of private alleles (P_{PA}) and haplotypes (P_{PH}), and gene diversity (H_D). Bold rows indicate means of designated sites.

	Microsatellites						
Site	N	H_O	F_{IS}	N_A	A_R	N_{PA}	P_{PA}
A. Flint R. – L. Huron (1998)	44	0.76 ± 0.05	-0.018 ± 0.024	74	7.2 ± 0.9	1	0.01
B. Thames R. (2004)	39	0.74 ± 0.04	0.008 ± 0.034	84	7.7 ± 0.9	2	0.02
C. Belle Isle (2006) – Post	40	0.73 ± 0.03	0.018 ± 0.030	88	7.8 ± 1.0	6	0.07
D1. Fighting Is. (2008) – Pre	20	0.72 ± 0.04	-0.009 ± 0.045	67	7.3 ± 1.3	1	0.01
D2. Fighting Is. (2010) – Post	28	0.69 ± 0.04	0.056 ± 0.025	70	7.2 ± 1.0	1	0.01
Mean Fighting Is. (Pre and Post)	**24**	**0.71 ± 0.04**	**0.023 ± 0.035**	**69**	**7.3 ± 1.2**	**1**	**0.01**
E. Grosse Ile (2001)	35	0.73 ± 0.05	0.013 ± 0.030	84	8.1 ± 1.1	1	0.01
Detroit R. – Mean (C, D2, and E)	**34**	**0.72 ± 0.04**	**0.029 ± 0.030**	**81**	**7.7 ± 1.0**	**3**	**0.03**
Mean L. St. Clair (B, C, D2, and E)	**36**	**0.72 ± 0.04**	**0.023 ± 0.030**	**82**	**7.7 ± 1.0**	**3**	**0.03**
F. Huron R. (2003 N=20, 2010 N=20)	40	0.73 ± 0.03	0.019 ± 0.040	84	7.8 ± 1.0	2	0.02
G. Hen Is. (2003)	65	0.68 ± 0.03	0.045 ± 0.020	85	7.1 ± 0.8	5	0.06
Mean Northwest L. Erie (E and F)	**53**	**0.71 ± 0.04**	**0.032 ± 0.030**	**85**	**7.5 ± 0.9**	**4**	**0.04**
Total (all sites A-G)	311	0.72 ± 0.03	0.028 ± 0.017	119	13.2 ± 1.9	---	---
Mean (all sites A-G)	**39**	**0.72 ± 0.04**	**0.017 ± 0.031**	**80**	**7.5 ± 1.0**	**2**	**0.03**

Site	Control region				
	N	H_D	N_H	N_{PH}	P_{PH}
A. Flint R. – L. Huron (1998)	25	0.58 ± 0.02	5	1	0.20
B. Thames R. (2004)	25	0.72 ± 0.01	4	0	0.00
C. Belle Isle (2006) – Post	25	0.78 ± 0.01	5	0	0.00
D1. Fighting Is. (2008) – Pre	20	0.70 ± 0.02	5	0	0.00
D2. Fighting Is. (2010) – Post	25	0.74 ± 0.01	5	0	0.00
Mean Fighting Is. (Pre and Post)	**23**	**0.72 ± 0.02**	**5**	**0**	**0.00**
E. Grosse Ile (2001)	25	0.77 ± 0.01	4	0	0.00
Detroit R. – Mean (C, D2, and E)	**25**	**0.76 ± 0.01**	**5**	**0**	**0.00**
Mean L. St. Clair (B, C, D2, and E)	**25**	**0.75 ± 0.01**	**4**	**0**	**0.00**
F. Huron R. (2003 N=20, 2010 N=20)	25	0.78 ± 0.01	5	1	0.20
G. Hen Is. (2003)	25	0.78 ± 0.01	5	0	0.00
Mean Northwest L. Erie (E and F)	**25**	**0.78 ± 0.01**	**5**	**1**	**0.20**
Total (all sites A-G)	195	0.73 ± 0.01	8	---	---
Mean (all sites A-G)	**24**	**0.73 ± 0.01**	**5**	**1**	**0.20**

Table 4.2

Summary of genetic variation per microsatellite locus across the seven walleye spawning groups and temporal comparison at the Fighting Island reef, totaling 311 individuals in the Huron-Erie corridor, showing annealing temperature (T_A), number of alleles (N_A), allelic size range (base pairs, bp), inbreeding coefficient (F_{IS}, average divergence within a spawning group), genetic deviation across all combined samples (F_{IT}), and mean genetic divergence among loci (F_{ST}).

Locus	Source	T_A (ºC)	N_A	Size range	F_{IS}	F_{IT}	F_{ST}
*Svi*4	Borer et al. (1999)	60	8	106-122	-0.027	-0.021	0.007
*Svi*6	“	60	16	126-168	-0.017	-0.008	0.009
*Svi*17	“	54	8	102-118	0.014	0.023	0.009
*Svi*18	“	65	7	114-126	0.123	0.137	0.016
*Svi*33	“	60	13	82-106	0.016	0.019	0.004
*Svi*L6	Wirth et al. (1999)	54	16	104-140	0.003	0.002	0.001
*Svi*L7	“	54	25	174-238	0.031	0.039	0.008
*Svi*2	Eldridge et al. (2002)	60	13	188-222	-0.037	-0.012	0.024
*Svi*7	“	60	13	140-178	0.068	0.094	0.028
Total	---	---	119	---	0.017	0.030	0.013

Table 4.3

Pairwise tests of genetic divergence among the seven Huron-Erie corridor walleye spawning samples, including pre- and post-habitat augmentation at Fighting Island based on nine microsatellite loci. Exact tests of differentiation are below the diagonal with *p*-values in parentheses and genetic migration estimates (N_M) are above. *=significant at α=0.05, **=remains significant following sequential Bonferroni correction (Rice, 1989). Inf = infinite value denoted by computer programs Genepop and Arlequin. Note: no significant differences were recovered using the mtDNA control region sequence data for the exact tests of differentiation and its N_M values were mostly infinite (Inf).

Site	A.	B.	C.	D1.	D2.	E.	F.	G.
A. Flint R.	---	14	9	7	6	7	7	12
B. Thames R.	77.4** (<0.0001)	---	195	43	33	307	Inf	65
C. Belle Isle	111.6** (<0.0001)	31.2* (0.0280)	---	30	45	158	214	47
D1. Fighting Is. Pre	72.2** (<0.0001)	23.3 (0.1780)	29.4* (0.0430)	---	37	187	35	45
D2. Fighting Is. Post	96.1** (<0.0001)	35.0* (0.0100)	46.1** (0.0003)	32.7* (0.0180)	---	150	32	47
E. Grosse Ile	116.7** (<0.0001)	20.9 (0.2870)	26.7 (0.0840)	19.0 (0.3950)	35.3* (0.0090)	---	114	76
F. Huron R.	63.5** (<0.0001)	18.2 (0.4460)	34.3* (0.0120)	25.8 (0.1040)	31.5* (0.0250)	23.9 (0.1570)	---	61
G. Hen Is.	Inf** (<0.0001)	26.3 (0.0940)	46.8** (0.0002)	23.3 (0.1780)	43.8** (0.0006)	27.7 (0.0670)	27.5 (0.0700)	---

Table 4.4

Geneclass2 analysis showing the percentage of walleye spawning at the Detroit River Fighting Island habitat augmentation site that self-assigned or assigned to other HEC spawning locations. **Bold**=percentage that self assign, underlined=highest percentage assigned to a given group, and ( )=number of individuals assigning to a given location.

Site	B. Thames R.	C. Belle Isle	D. Self	E. Grosse Ile	F. Huron R.	G. Hen Is.
D1. Fighting Is. – Pre	20 (4)	20 (4)	**0**	40 (8)	20 (4)	0
D2. Fighting Is. – Post	7 (2)	14 (4)	**14 (4)**	25 (7)	29 (8)	11 (3)

Figure 4-1. Walleye spawning groups sampled in the Huron-Erie Corridor with their primary population genetic barriers (black lines; I = strongest) from Manni *et al*. (2004) Barrier analysis using nine nuclear microsatellite loci. X = approximate locations of the historical walleye spawning grounds reported by Wolfert (1963) and Goodyear *et al*. (1982). A-Flint River, B-Thames River, C-Belle Isle, D-Fighting Island, E-Grosse Ile, F-Huron River, and G-Hen Island. Open circles denote the two Detroit River habitat augmentation sites.

Figure 4-2. MtDNA control region haplotype frequency distribution in the seven Huron-Erie Corridor walleye spawning groups, including pre- and post-habitat augmentation comparisons. Each haplotype is represented by a single color. Haplotype numbering follows Stepien & Faber (1998), for A1-4 (GenBank#s U90617, and JX442946-49). Haplotypes B8-11 are new haplotypes recovered in this study, which are GenBank #JX442953-56). Note: Haplotypes A5-7 of Stepien & Faber (1998) were not recovered in the HEC in our study; thus those numbers are not used here.

Figure 4-3. Relationship between genetic divergence [$\theta_{ST}/(1-\theta_{ST})$] of walleye spawning groups and geographic distance (natural logarithm of nearest waterway distance in kilometers (km)) using the nine nuclear microsatellite loci for a) all seven sites sampled (y=0.010x – 0.03, R^2=0.69, p=0.08) and b) excluding the Flint River-Lake Huron outlier comparisons (y=-0.001x + 0.006, R^2=0.001, p=0.58). Comparisons between sites are labeled as: A-Flint River, B-Thames River, C-Belle Isle, D-Fighting Island, E-Grosse Ile, F-Huron River, and G-Hen Island.

Fig. 4-1

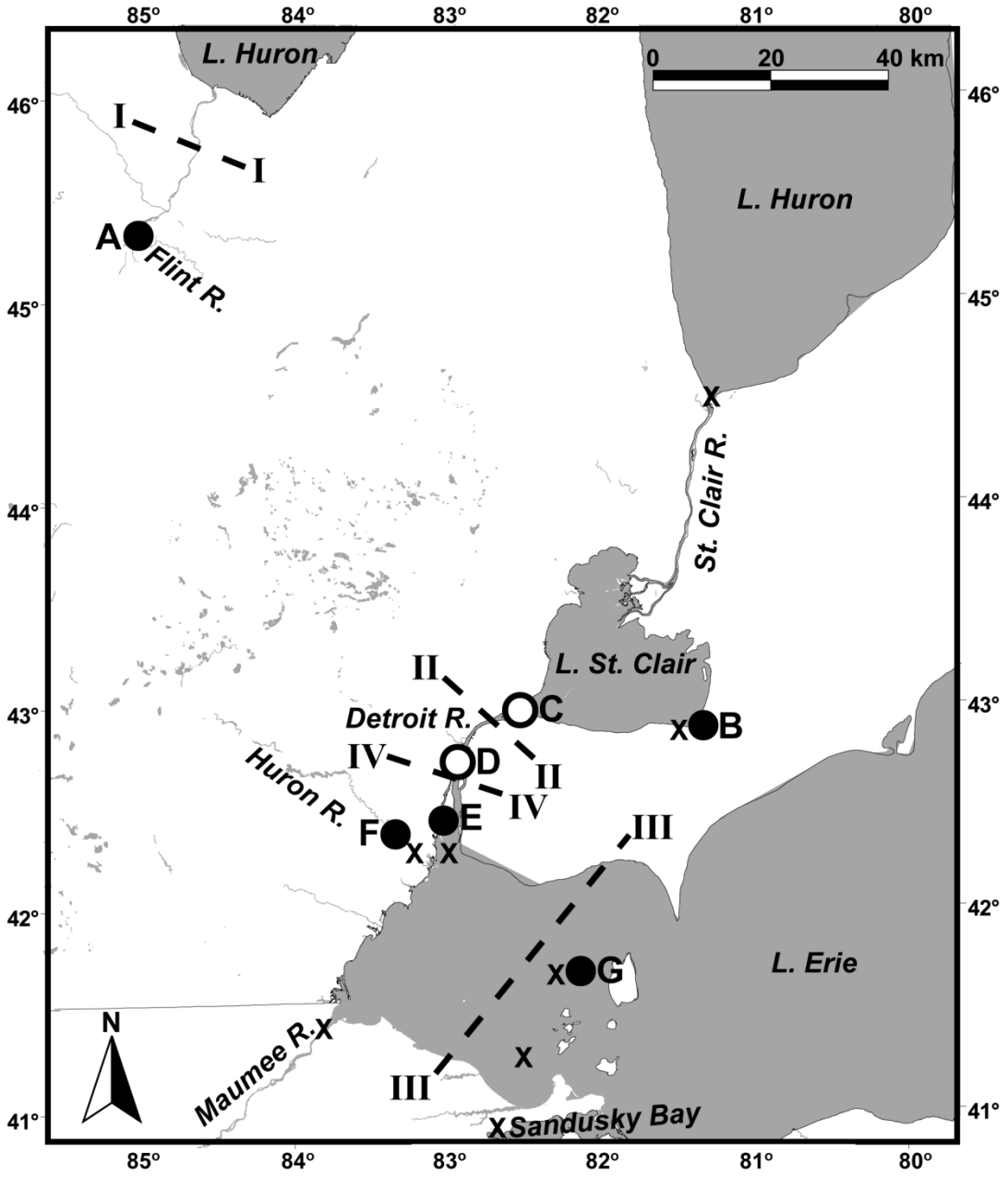

85°
84°
83°
82°
81°
80°
46°
45°
44°
43°
42°
41°
L. Huron
0
20
40 km
I
I
A
Flint R.
L. Huron
St. Clair R.
L. St. Clair
II
C
Detroit R.
B
IV
D
II
Huron R.
IV
E
F
III
L. Erie
G
N
Maumee R.
III
Sandusky Bay

Fig. 4-2

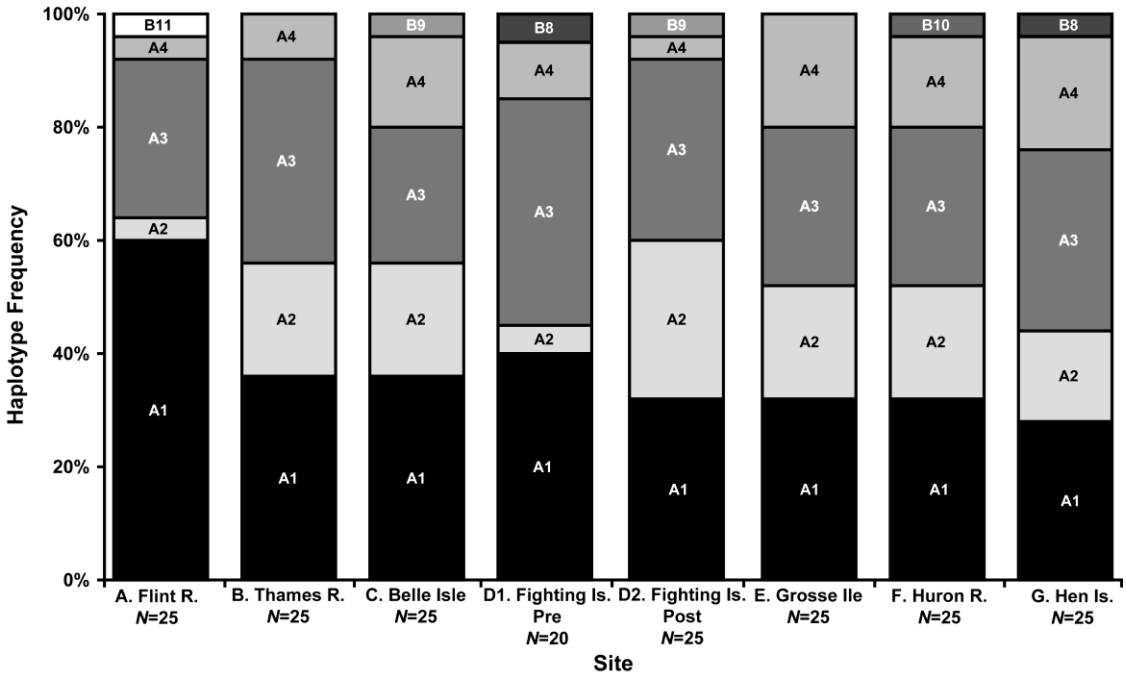
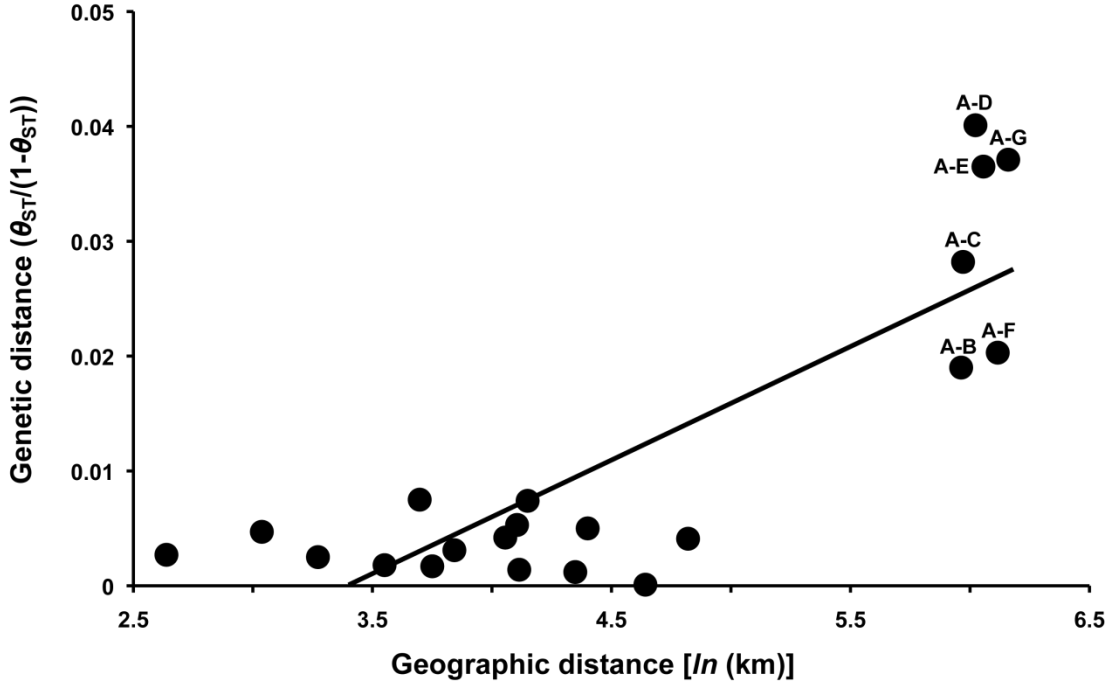
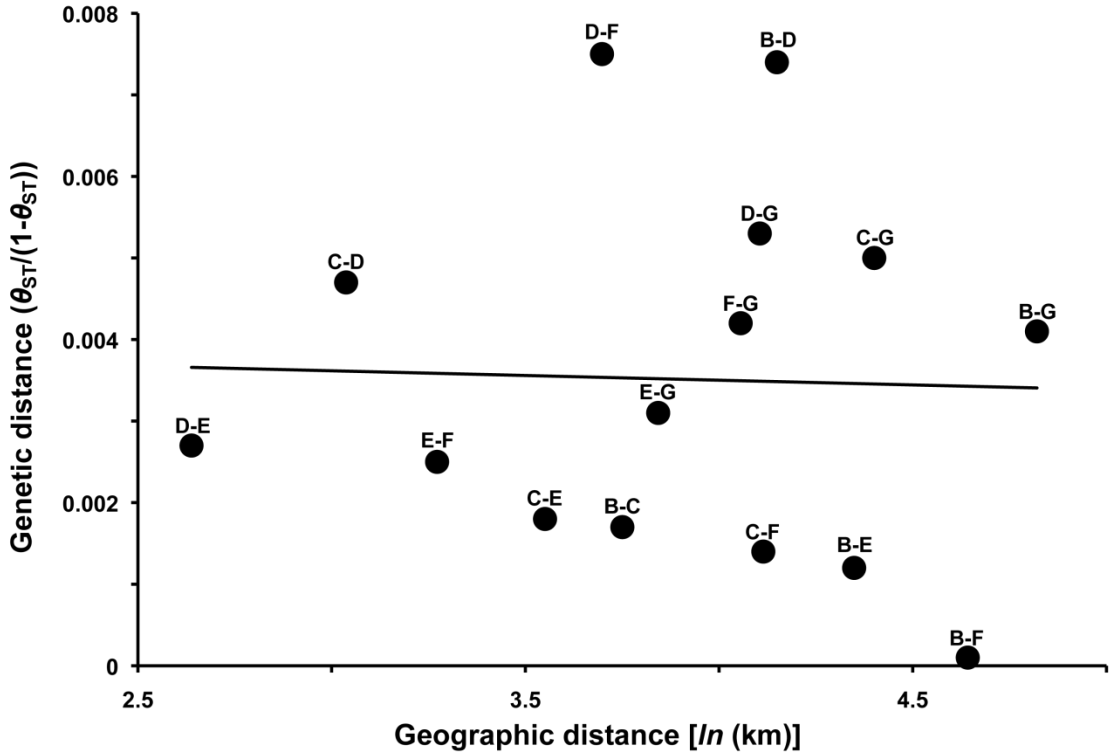

100%
80%
60%
40%
20%
0%
Haplotype Frequency
B11
A4
A3
A2
A1
A4
A3
A2
A1
B9
A4
A3
A2
A1
B8
A4
A3
A2
A1
B9
A4
A3
A2
A1
A4
A3
A2
A1
B10
A4
A3
A2
A1
B8
A4
A3
A2
A1
A. Flint R. N=25
B. Thames R. N=25
C. Belle Isle N=25
D1. Fighting Is. Pre N=20
D2. Fighting Is. Post N=25
E. Grosse Ile N=25
F. Huron R. N=25
G. Hen Is. N=25
Site

Fig. 4-3

(a)


0.05
0.04
0.03
0.02
0.01
0
Genetic distance ($\theta_{ST}/(1-\theta_{ST})$)
A-D
A-G
A-E
A-C
A-F
A-B
2.5
3.5
4.5
5.5
6.5
Geographic distance [*ln* (km)]

(b)


0.008
0.006
0.004
0.002
0
Genetic distance ($\theta_{ST}/(1-\theta_{ST})$)
D-F
B-D
D-G
C-G
C-D
F-G
B-G
E-G
D-E
E-F
C-E
B-C
C-F
B-E
B-F
2.5
3.5
4.5
Geographic distance [*ln* (km)]

Chapter 5

Discussion

5.1 General conclusions

This Ph.D. dissertation investigates the evolutionary history of walleye and provides insights as to how the processes of genetic connectivity and divergence interacted through time and space to shape contemporary genetic patterns. Chapter 2 evaluates the relationship of walleye to other members of the genus *Sander* and hypothesizes the events that led to its speciation. Additionally, this chapter analyzes intra-specific genetic diversity levels and suggests why the North American walleye and sauger possess greater diversity than their Eurasian counterparts. Chapter 3 provides a detailed examination of the population genetic patterns of spawning groups across the walleye's range, including comparisons between historic and contemporary spawning groups in Lake Erie, and addresses the taxonomic and morphological relationship of the extinct blue pike morphotype. Lastly, Chapter 4 discerns the fine-scale genetic connectivity, diversity, and divergence patterns of seven spawning groups along the highly degraded Huron-Erie Corridor and assesses how recent habitat augmentation affects walleye genetic structure. Overall, these three chapters show the broad- and fine-

scale genetic patterns of walleye and the influences that past climate change, geologic events, and anthropogenic stressors (e.g., exploitation and global warming) that resulted in the hierarchical genetic structure of walleye.

5.1.1. Evolution of walleye Sander vitreus*: Influences of ancient climate and geological processes*

Two important results of this dissertation are that (1) the walleye is much older than previously thought, and (2) its current genetic structure traces to climatological and geological events that occurred during the Miocene Epoch. Using a unique combination of fossil calibrations and sequence data representing all five members of the genus *Sander*, modern walleye was found to have diverged from a common ancestor shared with its sister species the sauger *S. canadensis* ~15.4 Mya during the Mid-Miocene Epoch.

Contemporary walleye haplotypes later differentiated ~10.6 Mya during the Late Miocene Epoch, with the earliest and most divergent walleye haplotype originating in the southeast portion of the range in the Ohio and New Rivers. During this time, temperatures were rapidly cooling, ice sheets were beginning to form at the poles (Wolfe, 1994), and habitats were transitioning from grasslands to forests (see Cerling *et al.*, 1997; Fox, 2000) likely isolating populations in this river system. The other haplotypes differentiated beginning ~7.2 Mya. One of these older haplotypes is endemic to the North River, Alabama, part of the Tombigbee River drainage that flows into the Gulf of Mexico. These unglaciated southern walleye populations in the Ohio, New, and North River systems have remained genetically different due to their longterm isolation in

separate drainage systems. Likewise, southern populations of yellow perch *Perca flavescens* show high divergence from others across its range, possessing unique haplotypes and diversity (Sepulveda-Villet & Stepien, 2012). These southerly populations of walleye and yellow perch thus represent unique genetic sources that have adapted to warm environments and may be valuable for conservation, especially in the face of global warming.

The three most widely distributed walleye haplotypes also differentiated ~7.2 Mya durin climate changes and geological events during the Mid-Late Miocene Epoch. These three haplotypes are found across the range from the northern Canadian Shield lakes to the south in the Ohio and New Rivers, and are solely absent from the Gulf Coastal North River relict population. Due to their widespread occurrence, these haplotypes appear to be well suited to a variety of environments. A similar haplotype distribution also was discerned for yellow perch, with common haplotypes found in most populations across the range (Sepulveda-Villet & Stepien, 2012). This indicates that walleye and yellow perch have persisted in and adapted to a wide range of environmental conditions.

5.1.2 Population genetic patterns of walleye across the range: Influence of Pleistocene glaciations, drainage connections, and biology

Walleye exhibits broad-scale genetic structure across the range, with populations separated into three major geographic regions: (1) Lake Winnipeg, Upper Mississippi River, and Lac Mistassini drainages, (2) the Great Lakes, and (3) Ohio, New, and North Rivers in the south. These three large divisions correspond to isolation of the spawning

groups due to drainage boundaries, and indicate their origins in distinct glacial refugia: the Missourian, Mississippian, and Atlantic Coastal. Notably, some haplotypes found in today's populations differentiated ~2.6-1.0 Mya during the Pleistocene Epoch, when walleye would have been isolated in these three refugia. Ancestors of today's walleye then re-colonized its present-day range through drainage connections from proglacial lakes and their outlets, as the glaciers receded. Walleye populations have continued to diverge in these regions due to spatial and reproductive segregation of spawning groups, according to degree of natal site fidelity (see Jennings *et al.*, 1996; Stepien & Faber, 1998). Similar patterns have been identified for yellow perch (Sepulveda-Villet & Stepien, 2011, 2012) and smallmouth bass *Micropterus dolomieu* (Stepien *et al.*, 2007; Karsiotis *et al.*, in review).

Walleye have higher diversity levels (mean mtDNA=0.53 range=0.15-0.82, μsat=0.68 range=0.52-0.77) than other North American fishes with similar ranges, such as yellow perch (mtDNA=0.31, range=0.00-0.82, μsat=0.53, range=0.33-0.67; Sepulveda-Villet & Stepien, 2012) and smallmouth bass (mtDNA=0.50, range=0.00-0.85, μsat=0.46, range=0.15-0.59; Stepien *et al.*, 2007; Karsiotis *et al.*, in review). This difference may reflect their respective life history characteristics and behavior. A close relative of the yellow perch, Eurasian perch *P. fluviatilis* also has lower diversity (H_D=0.33, range=0.00–0.87; Nesbø *et al.*, 1999), which may be attributed to the close association of kin groups (Gerlach *et al.*, 2001; Behrmann-Godel & Gerlach, 2008). Sullivan & Stepien (in re-review) found that yellow perch spawning groups had higher proportions of full siblings, averaging 20% and reaching 38% for some populations, >2x higher than values for walleye. Similarly, smallmouth bass had a higher proportion of full

siblings (~2x, mean=15%; Karsiotis *et al.*, in review) than walleye. This close association of kin in yellow perch and smallmouth bass likely led to their lower diversity levels compared to those for the walleye. This merits further investigation.

5.1.3 Historic and contemporary genetic patterns: Influences of anthropogenic habitat degradation, exploitation, and climate change

Historic Lake Erie was once believed to house two subspecies of walleye, the yellow walleye *S. v. vitreus* and the blue pike *S. v. glaucus* (Trautman, 1981). This study found that paratypes of the historic blue pike had no unique haplotypes, alleles, or diagnostic morphological characters from walleye. Results indicate that the blue pike was not a separate taxon. The blue pike may have represented a population-level variant of walleye, but further analyses are needed. This study relied on formalin fixed tissues and future investigations should consider utilizing scale samples that were archived by fisheries agencies.

Allelic frequencies of these historic Lake Erie walleye significantly differ from modern populations, with today's spawning groups having higher diversity (mtDNA=0.76–0.82, μsat=0.73–0.74) compared to those from 1923-1949 (mtDNA=0.00-0.10, μsat=0.40-0.54). At the earlier time period, Lake Erie was in an ecological crisis, marked by habitat degradation, heavy pollution, and overexploitation (see Hartman, 1973; Hartig *et al.*, 2009). The walleye fishery was closed in 1970 due to high mercury concentrations in its tissues (summarized by Hartig *et al.*, 2009). By 1978, fisheries agencies declared a crisis fishery due to low abundance (~10 million fish; WTG, 2013). Following the closure, populations rebounded and apparently increased in genetic

diversity. Lake Erie walleye today may have had a very different genetic structure without these declines.

In recent years, federal, state, and provincial agencies and local groups have partnered to mitigate some of the past habitat degradation by installing artificial reefs to supplement spawning habitat (see USGS, 2010; Roseman *et al.,* 2011). The present study shows that walleye populations spawning at these artificial reef sites markedly differ from other non-augmented sites, with comparable levels of genetic diversity. Some genetic exchange was found at the reef sites, indicating that individuals from other locations migrated to spawn on the new habitat. Thus, if foreign walleye continue to migrate and spawn at these reef locations, the original spawning group's genetic composition could be further altered, potentially resulting in the loss of unique variation. Overall, it appears that despite over a century of heavy exploitation, habitat degradation, and pollution, walleye populations have maintained relatively high genetic diversity and divergence patterns. This suggests high resiliency that should be conserved.

5.2 Future research

The present study examines the evolutionary history and population genetic patterns of the walleye establishing an important baseline for gauging future parameters. The results discerned here raise several additional questions. Future studies could include:

5.2.1 Adaptation and genetic structure of walleye and other Sander

With increasing anthropogenic stressors, including climate change and overexploitation, it is important to understand how species will adapt to aid conservation efforts. The next step is to translate genetic variation to adaptation and fitness in the organism through genomics. For example, studies of Atlantic salmon *Salmo salar* have used gene expression and functional genetic divergence to identify unique conservation units (summarized by Allendorf *et al.*, 2010). For walleye, study of the relict southern population in the North River, Alabama, may provide important data for adaptation to warmer temperatures. This site has been geographically and genetically isolated and possesses one of the oldest walleye control region haplotypes, making it an ideal case study. However, this important genetic resource may be lost due to interbreeding with non-local genotypes introduced through the Tennessee-Tombigbee waterway (see Chapter 3).

Comparing and contrasting the adaptations of the North American and Eurasian *Sander* also may provide further insights for their conservation. This dissertation discerns that walleye and its sister species, the sauger, have much higher diversity levels compared to Eurasian *Sander*. This difference may have resulted from the isolation of Eurasian species in much smaller, geographically constrained refugia (due to mountain ranges and seas) compared to the North American taxa. Alternatively, humans have existed in Eurasia for ~40 kya (Benazzi *et al.*, 2011), therefore this lower diversity may be the result of long-term exploitation. Eurasian *Sander* may be more sensitive to future anthropogenic stressors as a result of their lower diversity. Further sampling across their range would be valuable, especially for the rare sea pikeperch *S. marinus.*

5.2.2. Temporal population genetic patterns

Historic Lake Erie walleye have lower diversity levels than contemporary spawning groups, attributable to the pollution and exploitation history of Lake Erie. Similarly, Atlantic cod showed a decline in diversity due to overfishing followed by a rebound (Hutchinson *et al.,* 2003). The present study captured just the rebound of walleye following the closure of their fishery in the 1970s. Studies of recent years have discerned temporal stability of walleye in Lake Erie (1995-2008; Stepien *et al.,* 2012). Analysis of these spawning groups back to the beginning of the 20th century would further identify population and genetic fluctuations and could use archived scale samples.

5.2.3 Genetic composition of a given spawning run

This dissertation research shows that walleye spawning groups significantly diverge. Further research could determine the genetic patterns within a spawning run, including the genetic composition over a single spawning season, age cohorts, and relationship of larvae to adults. Temporal stability was observed from 1995-2008 in Lake Erie walleye spawning groups, and knowledge of the influence of age cohorts on the genetic structure would be useful. Walleye population abundances fluctuate from year to year due to variations in larval recruitment (summarized by Zhao *et al.,* 2009), leading to the questions of (1) do walleye cohorts differ in genetic composition? and (2) how does the genetic composition of larval walleye compare to adults? Sullivan & Stepien (in re-review) found age cohorts of yellow perch to vary genetically suggesting differential contributions of annual spawning groups. This remains to be tested for walleye.

The present study also shows a lower proportion of full siblings within walleye spawning groups, compared to yellow perch and smallmouth bass. This mechanism has not been experimentally tested for these North American species. Studies of the Eurasian perch found that they associate with kin throughout their lives, including during reproduction (Gerlach *et al.*, 2001; Behrmann-Godel *et al.*, 2006). Do these three North American species likewise stay with their kingroups? Do lower values for walleye lead to its higher diversity?

References

Allendorf, F.W. & Luikart, G. (2007) Conservation and the genetics of populations. Blackwell Publishing, Oxford.

Allendorf, F.W., Hohenlohe, P.A., & Luikart, G. (2010) Genomics and the future of conservation genetics. *Nature Reviews Genetics* **11,** 697–710.

Antao, T., Lopes, A., Lopes, R.J., Beja–Pereira, A., & Luikart, G. (2008) LOSITAN: A workbench to detect molecular adaptation based on a Fst–outlier method. *BMC Bioinformatics* **9,** 323.

April, J., Mayden, R.L., Hanner, R.H., & Bernatchez, L. (2011) Genetic calibration of species diversity among North America's freshwater fishes. *Proceedings of the National Academy of Sciences* **108,** 10602-10607.

Athrey, G., Lindsay, D.L., Lance, R.F., & Leberg, P.L. (2011) Crumbling diversity: Comparison of historical archived and contemporary natural populations indicate reduced genetic diversity and increasing genetic differentiation in the golden–cheeked warbler. *Conservation Genetics* **12,** 1345–1355.

Avise, J.C. (2004) *Molecular Markers, Natural History, and Evolution.* 2nd ed. Sunderland, MA: Sinauer Associates.

Axelrod, D.I. (1975) Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. *Annals of the Missouri Botanical Garden* **62,** 280–334.

Backhouse-James, S.M. & Docker, M.F. (2012) Microsatellite and mitochondrial DNA markers show no evidence of population structure in walleye (*Sander vitreus*) in Lake Winnipeg. *Journal of Great Lakes Research* **38,** 47-57.

Bailey, R.M. & Smith, G.R. (1981) Origin and geography of the fish fauna of the Laurentian Great Lakes basin. *Canadian Journal of Fisheries and Aquatic Sciences* **38**, 1539–1561.

Balon, E.K., Momot, W.T., & Regier, H.A. (1977) Reproductive guilds of percids: Results of the paleogeographical history and ecological successions. *Journal of the Fisheries Research Board of Canada* **34,** 1910–1921.

Bănărescu, P. (1991) *Zoogeography of Fresh Waters, vol. 2. Distribution and Dispersal of Freshwater Animals in North America and Eurasia.* Wiesbaden: AULA-Verlag.

Banks, M.A., Rashbrook, V.K., Calavetta, M.J., Dean, C.A., & Hedgecock, D. (2000) Analysis of microsatellite DNA resolves genetic structure and diversity of chinook salmon (*Oncorhynchus tshawytscha*) in California's Central Valley. *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 915-927.

Barber, D.C., Dyke, A., Hillaire–Marcel, C., Jennings, A.E., Andrews, J.T., Kerwin, M.W., Bilodeau, G., McNeely, R., Southon, J., Morehead, M.D., & Gagnon, J.-M. (1999) Forcing of the cold event of 8,200 years ago by catastrophic drainage of Laurentide lakes. *Nature* **400,** 344–348.

Barraclough, T.E. & Nee, S. (2001) Phylogenetics and speciation. *TRENDS in Ecology and Evolution* **16,** 391–399.

Barton, B.A. & Barry, T.P. (2011) Reproduction and environmental biology. In: *Biology, Management, and Culture of Walleye and Sauger* (ed. by B.A. Barton), pp. 199–231. American Fisheries Society, Bethesda.

Beaumont, M.A. & Nichols, R.A. (1996) Evaluating loci for use in the genetic analysis of population structure. *Proceedings of the Royal Society of London B* **263,** 1619–1626.

Behrmann–Godel, J. & Gerlach, G. (2008) First evidence for postzygotic reproductive isolation between two populations of Eurasian perch (*Perca fluviatilis* L.) within Lake Constance. *Frontiers in Zoology* **5,** 1–7.

Behrmann-Godel, J., Gerlach, G., & Eckmann, R. (2006) Kin and population recognition in sympatric Lake Constance perch (*Perca fluviatilis* L.): Can assortative shoaling drive population divergence? *Behavioral Ecology and Sociobiology* **59,** 461-468.

Belore, M., Thomas, M., & Locke, B. (2010) Status of walleye in Lake St. Clair and the Detroit and St. Clair Rivers, In: Status of walleye in the Great Lakes: Proceedings of the 2006 symposium (ed. by E. Roseman, P. Kocovsky, C. Vandergoot), pp. 91-121. Great Lakes Fishery Commission Technical Report 69, Ann Arbor, Michigan.

Benazzi, S., Douka, K., Fornai, C., Bauer, C.C., Kullmer, O., Svoboda, J., Pap, I., Mallegni, F., Bayle, P., Coquerelle, M., Condemi, S., Ronchitelli, A., Harvati, K., & Weber, G.W. (2011) Early dispersal of modern humans in Europe and implications for Neanderthal behaviour. *Nature* **479,** 525-529.

Bennion, D.H., Manny, B.A., 2011. Construction of shipping channels in the Detroit River-History and environmental consequences. U.S. Geological Survey Scientific Investigations Report 2011-5122, Reston, Virginia.

Berg, L.S. (1965) *Freshwater Fishes of the U.S.S.R. and Adjacent Countries.* Moscow-Leningrad: Academy of Sciences of the U.S.S.R. Zoological Institute.

Bernatchez, L. & Danzmann, R.G. (1993) Congruence in control-region sequence and restriction-site variation in mitochondrial DNA of brook charr (*Salvelinus fontinalis* Mitchill). *Molecular Biology and Evolution* **10,** 1002–1014.

Bernatchez, L. & Wilson, C.C. (1998) Comparative phylogeography of Nearctic and Palearctic fishes. *Molecular Ecology* **7,** 431–452.

Billington, N. (1996) Geographical distribution of mitochondrial DNA (mtDNA) variation in walleye, sauger, and yellow perch. *Annales Zoologici Fennici* **33,** 699–706.

Billington, N. & Hebert, P.D.N. (1988) Mitochondrial DNA variation in Great Lakes walleye (*Stizostedion vitreum*) populations. *Canadian Journal of Fisheries and Aquatic Sciences* **45,** 643–654.

Billington, N., Hebert, P.D.N., & Ward, R.D. (1990) Allozyme and mitochondrial DNA variation among three species of *Stizostedion* (Percidae): Phylogenetic and zoogeographic implications. *Canadian Journal of Fisheries and Aquatic Sciences* **47,** 1093–1102.

Billington, N., Barrette, R.J., & Hebert, P.D.N. (1992) Management implications of mitochondrial DNA variation in walleye stocks. *North American Journal of Fisheries Management* **12,** 276–284.

Billington, N., Wilson, C., & Sloss, B.L. (2011) Distribution and population genetics of walleye and sauger. In: *Biology, Management, and Culture of Walleye and Sauger* (ed. by B.A. Barton), pp. 105–132. American Fisheries Society, Bethesda.

Billington, N., Danzmann, R.G., Hebert, P.D.N, & Ward, R.D. (1991) Phylogenetic relationships among four members of *Stizostedion* (Percidae) determined by mitochondrial DNA and allozyme analyses. *Journal of Fish Biology* **39,** 251–258.

Björklund, M., Aho, T., & Larsson, L.C. (2007) Genetic differentiation in pikeperch (*Sander lucioperca*): The relative importance of gene flow, drift and common history. *Journal of Fish Biology* **71,** 264–278.

Blumer, L.S. (1982) A bibliography and categorization of bony fishes exhibiting parental care. *Zoological Journal of the Linnean Society* **76,** 1-22.

Bohlen, J., Slechtova, V., Tan, H.H., & Britz, R. (2011) Phylogeny of the southeast Asian freshwater fish genus *Pangio* (Cypriniformes; Cobitidae). *Molecular Phylogenetics and Evolution* **61,** 854–865.

Böhme, M. (2003) The Miocene climatic optimum: Evidence from ectothermic vertebrates of central Europe. *Palaeogeography Palaeoclimatology Palaeoecology* **195,** 389–401.

Borden, W.C. & Stepien, C.A. (2006) Discordant population genetic structure of smallmouth bass, *Micropterus dolomieu* Lacepede, in Lake Erie based on mitochondrial DNA sequences and nuclear DNA microsatellites. *Journal of Great Lakes Research* **32,** 242–257.

Borer, S., Miller, L.M., & Kapuscinski, A.R. (1999) Microsatellites in walleye *Stizostedion vitreum*. *Molecular Ecology* **8**, 336–338.

Boschung, H.T. & Mayden, R.L. (2004) *Fishes of Alabama.*Smithsonian Institution, Washington D.C.

Bossu, C.M. & Near, T.J. (2009) Gene trees reveal repeated instances of mitochondrial DNA introgression in orangethroat darters (Percidae: *Etheostoma*). *Systematic Biology* **58,** 114-129.

Boulet, M., Couturier, S., Cote, S.D., Otto, R.D., & Bernatchez, L. (2007) Integrative use of spatial, genetic, and demographic analyses for investigating genetic connectivity between migratory, montane, and sedentary caribou herds. *Molecular Ecology* **16,** 4223-4240.

Bozek, M.A., Baccante, D.A., & Lester, N.P. (2011) Walleye and sauger life history. In: *Biology, Management, and Culture of Walleye and Sauger* (ed. by B.A. Barton), pp. 233–301. American Fisheries Society, Bethesda.

Bradbury, I.R., Hubert, S., Higgins, B., Bowman, S., Borza, T., Paterson, I.G., Snelgrove, P.V.R., Morris, C.J., Gregory, R.S., Hardie, D., Hutchings, J.A., Ruzzante, D.E., Taggart, C.T., & Bentzen, P. (2013) Genomic islands of divergence and their consequences for the resolution of spatial structure in an exploited marine fish. *Evolutionary Applications* **6,** 450–461.

Briggs, J.C. (1986) Introduction to the zoogeography of North American Fishes. In: Hocutt CH, Wiley EO, eds. *The Zoogeography of North American Freshwater Fishes.* New York, NY: Wiley-Interscience, 1–16.

Brooks, T.M., Mittermeier, R.A., da Fonseca, G.A.B., Gerlach, J., Hoffman, M., Lamoreux, J.F., Mittermeier, C.G., Pilgrim, J.D., & Rodrigues, A.S.L. (2006) Global biodiversity conservation priorities. *Science* **313,** 58-61.

Brown, J.A., Moore, W.M., & Quabius, E.S. (2001) Physiological effects of saline waters on zander. *Journal of Fish Biology* **59,** 1544–1555.

Bruch, A.A., Uhl, D., & Mosbrugger, V. (2007) Micoene climate in Europe – Patterns and evolution A first synthesis of NECLIME. *Palaeogeography Palaeoclimatology Palaeoecology* **253,** 1–7.

Bruner, J.C. (2011) A phylogenetic analysis of Percidae using osteology. In: Barton BA, ed. *Biology, Management, and Culture of Walleye and Sauger*. Bethesda, MD: American Fisheries Society, 5–84.

Carey, J.R. & Judge, D.S. (2000) *Longevity records: life spans of mammals, birds, amphibians, reptiles, and fish*. Odense: Odense University Press.

Carvalho, G.R. (1993) Evolutionary aspects of fish distribution: Genetic variability and adaptation. *Journal of Fish Biology* **43,** 53–73.

Cavender, T.M. (1998) Development of the North American Tertiary freshwater fish fauna with a look at parallel trends found in the European record. *Italian Journal of Zoology* **65,** 149–161.

Cerling, T.E., Harris, J.M., MacFadden, B.J., Leakey, M.G., Quade, J., Eisenmann, V., & Ehleringer, J.R. (1997) Global vegetation change through the Miocene/Pliocene boundary. *Nature* **389,** 153–158.

Chapuis, M. & Estoup, A. (2007) Microsatellite null alleles and estimation of population differentiation. *Molecular Biology and Evolution* **24,** 621–631.

Chen, Q., Tang, X., Yao, W., & Lu, S. (2009) Bioinformatics analysis the complete sequences of cytochrome *b* of *Takydromus sylvaticus* and modeling the tertiary structure of encoded protein. *International Journal of Biological Sciences* **5,** 596–602.

Chow, S. & Hazama, K. (1998) Universal PCR primers for S7 ribosomal protein genes in fish. *Molecular Ecology* **7,** 1255–1256.

Chu, C., Mandrak, N.E., & Minns, C.K. (2005) Potential impacts of climate change on the distributions of several common and rare freshwater fishes in Canada. *Diversity and Distributions* **11,** 299–310.

Chubb, A.L., Zink, R.M., & Fitzsimons, J.M. (1998) Patterns of mtDNA variation in Hawaiian freshwater fishes: The phylogeographic consequences of amphidromy. *Journal of Heredity* **89,** 8–16.

Cichosz, T.A. (2009) Wisconsin Department of Natural Resources 2005–2006 ceded territory fishery assessment report. *Administrative Report #63 Treaty Fisheries Assessment Unit Bureau of Fisheries Management.*

Cihar, J. (1975) Geographic and ecological variability of perch (*Perca fluviatilis* (Linnaeus)) and history of its distribution from Eurasia to North America. *Acta Musei Nationalis Pragae* **31B,** 57–89.

Clement, M., Posada, D., & Crandall, K.A. (2000) TCS: A computer program to estimate gene genealogies. *Molecular Ecology* **9**, 1657–1660. Available at http://darwin.uvigo.es/software/tcs.html (Accessed on 26 June 2013)

Colby, P.J. & Nepszy, S.J. (1981) Variation among stocks of walleye (*Stizostedion vitreum vitreum*): Management implications. *Canadian Journal of Fisheries and Aquatic Sciences* **38,** 1814-1831.

Colby, P.J., McNicol, R.E., & Ryder, R.A. (1979) Synopsis of biological data on the walleye *Stizostedion v. vitreum.* FAO Fisheries Synopsis 119, Rome.

Collette, B.B. & Bănărescu, P. (1977) Systematics and zoogeography of the fishes of the family Percidae. *Journal of the Fisheries Research Board of Canada* **34,** 1450–1463.

Collette, B.B., Ali, M.A., Hokanson, K.E.F., Nagięć, M., Smirnov, S.A., Thorpe, J.E., Weatherley, A.H., & Willemsen, J. (1977) Biology of the Percids. *Journal of the Fisheries Research Board of Canada* **34,** 1890–1899.

Cornuet, J-M, Piry, S., Luikart, G., Estoup, A., & Solignac, M. (1999) New methods employing multilocus genotypes to select or exclude populations as origins of individuals. *Genetics* **153,** 1989-2000.

Coulon, A., Fitzpatrick, J.W., Bowman, R., & Lovette, I.J. (2012) Mind the gap: Genetic distance increases with habitat gap size in Florida scrub jays. *Biology Letters* **8,** 582–585.

Craig, J.F. (2000) *Percid Fishes: Systematics, Ecology, and Exploitation.* Oxford: Blackwell Science.

Cuvier, G. (1817) *La Règne Animal distribuè d' après son Organisation pour servir de base à l'Histoire Naturelle des Animaux et d'Introduction à l'Anatome comparèe. Les Reptiles, les Poissons, les Mollusques et les Annélides* **2:** 1-532**.**

Cuvier G. (1828) *Histoire Naturelle des Poissons. Tome second. Livre Troisième. Des Poissons de la Famille des Perches, ou des Percoïdes. Historie Naturelle des Poissons,* volume 2. Strasbourg, France.

Darriba, D., Taboada, G.L., Doallo, R., & Posada, D. (2012) jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods* **9,** 772. Available at http://code.google.com/p/jmodeltest2/. (Accessed on 26 March 2013).

Davis, M.B. & Shaw, R.G. (2001) Range shifts and adaptive responses to quaternary climate change. *Science* **292,** 673–679.

Dawson, T.P., Jackson, S.T., House, J.I., Prentice, I.C., & Mace, G.M. (2011) Beyond predictions: Biodiversity conservation in a changing climate. *Science* **332,** 53–58.

DeWoody, J., Nason, J.D., & Hipkins, V.D. (2006) Mitigating scoring errors in microsatellite data from wild populations. *Molecular Ecology Notes* **6,** 951–957.

Denk, T., Grimsson, F., & Zetter, R. (2010) Episodic migration of oaks to Iceland: Evidence for a North Atlantic "Land Bridge" in the latest Miocene. *American Journal of Botany* **97,** 276–287.

Denk, T., Grimsson, F., Zetter, R., & Simonarson, L.A. (2011) The biogeographic history of Iceland – The North Atlantic Land Bridge revisited. *Topics in Geobiology* **35,** 647–668.

dos Reis, S.F., Pessôa, L.M., & Strauss, R.E. (1990) Application of size–free canonical discriminant analysis to studies of geographic differentiation. *Brazilian Journal of Genetics* **13,** 509–520.

Drummond, A.J., Suchard, M.A., Xie, D., & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* **29,** 1969–1973. Available at http://beast.bio.ed.ac.uk/Main_Page#Citing_BEAST (Accessed on 26 March 2013).

Dumitrescu, M., Bănărescu, P.M., & Stoica, N. (1957) *Romanichthys valsanicola* nov. gen. nov. sp. (Pisces, Percidae). *Travaux du Museum d'Histoire Naturelle "Grigore Antipa"* **1,** 225–244.

Durand, J.D., Persat, H., & Bouvet, Y. (1999) Phylogeography and postglacial dispersion of the chub (*Leuciscus cephalus*) in Europe. *Molecular Ecology* **8,** 989–997.

Einhouse, D.W. & MacDougall, T.M. (2010) An emerging view of the mixed-stock structure of Lake Erie's eastern-basin walleye population. In: Status of walleye in the Great Lakes: Proceedings of the 2006 symposium (ed. by E. Roseman, P. Kocovsky, C. Vandergoot), pp. 151–164. Great Lakes Fishery Commission Technical Report 69, Ann Arbor.

Eldridge, W. H., Bacigalupi, M.D., Adelman, I.R., Miller, L.M., & Kapuscinski, A.R. (2002) Determination of relative survival of two stocked walleye populations and resident natural–origin fish by microsatellite DNA parentage assignment. *Canadian Journal of Fisheries and Aquatic Sciences* **59**, 282–290.

Ellegren, H. (2004) Microsatellites: Simple sequences with complex evolution. *Nature Reviews Genetics* **5,** 435–445.

Elliott, N.G., Haskard, K., & Koslow, J.A. (1995) Morphometric analysis of orange roughy (*Hoplostethus atlanticus*) off the continental slope of southern Australia. *Journal of Fish Biology* **46,** 202–220.

Excoffier, L. & Lischer, H.E. (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* **10,** 564–567. Available at http://cmpg.unibe.ch/software/arlequin35/ (Program updated 19 September 2011, Website accessed on 26 March 2013)

Excoffier, L., Smouse, P., & Quattro, J. (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**, 479–491.

Faber, J.E. & Stepien, C.A. (1997) The utility of mitochondrial DNA control region sequences for analyzing phylogenetic relationships among populations, species, and genera of the Percidae. In: Kocher TD, Stepien, CA, eds. *Molecular Systematics of Fishes.* San Diego, CA: Academic Press, 125–139.

Faber, J.E. & Stepien, C.A. (1998) Tandemly repeated sequences in the mitochondrial DNA control region and phylogeography of the pike–perches *Stizostedion. Molecular Phylogenetics and Evolution* **10,** 310–322.

Faulks, L.K., Gilligan, D.M., & Beheregaray, L.B. (2011) The role of anthropogenic vs. natural in–stream structures in determining connectivity and genetic diversity in an endangered freshwater fish, Macquarie perch (*Macquaria australasica*). *Evolutionary Applications* **4,** 589–601.

Felsenstein, J. (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution* **39,** 783–791.

Felsenstein, J. (2005) Phylip (Phylogeny Inference Package) version 3.6. *Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.* http://evolution.genetics.washington.edu/phylip.html (Accessed on 26 June 2013)

Ferguson, R.G. & Derkson, A.J. (1971) Migrations of adult and juvenile walleyes (*Stizostedion vitreum vitreum*) in southern Lake Huron, Lake St. Clair, Lake Erie, and connecting waters. *Journal of the Fisheries Research Board of Canada* **8,** 1133-1142.

Fielder, D.G. & Baker, J.P. (2004) Strategy and options for completing recovery of walleye in Saginaw Bay, Lake Huron. MIDNR Fisheries Special Report 29.

Fielder, D.G., Liskauskas, A.P., Gonder, D.J.A., Mohr, L.C., & Thomas, M.V. (2006) Status of walleye in Lake Huron, In: Status of walleye in the Great Lakes: Proceedings of the 2006 symposium (ed. by E. Roseman, P. Kocovsky, C. Vandergoot), pp. 71-90. Great Lakes Fishery Commission Technical Report 69, Ann Arbor, Michigan.

Fox, D.L. (2000) Growth increments in *Gomphotherium* tusks and implications for late Miocene climate change in North America. *Palaeogeography, Palaeoclimatology, Palaeoecology* **156,** 327–348.

Franckowiak, R.P., Sloss, B.L., Bozek, M.A., & Newman, S.P. (2009) Temporal effective size estimates of a managed walleye *Sander vitreus* population and implications for genetic–based management. *Journal of Fish Biology* **74,** 1086–1103.

Freyhof, J. (2011) *Sander volgensis.* In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. Available at http://www.iucnredlist.org/apps/redlist/details/20862/0 (Accessed 26 March 2013).

Freyhof, J. & Kottelat, M. (2008) *Sander lucioperca.* In: IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2 Available at http://www.iucnredlist.org/apps/redlist/details/20860/0 (Accessed 26 March 2013).

Fuller, P. (2012) *Sander lucioperca.* USGS Nonindigenous Aquatic Species Database, Gainesville, FL. http://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=830 (Accessed 26 March 2013).

Fuller, P. & Neilson, M. (2012) *Sander vitreus.* USGS Nonindigenous Aquatic Species Database, Gainesville, FL. http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=831 (Accessed 26 March 2013).

Gatt, M.H., Ferguson, M.M., & Liskauskas, A.P. (2000) Comparison of control region sequencing and fragment RFLP analysis for resolving mitochondrial DNA variation and phylogenetic relationships among Great Lakes Walleye. *Transactions of the American Fisheries Society* **129,** 1288–1299.

Gatt, M.H., Fraser, D.J., Liskauskas, A.P., & Ferguson, M.M. (2002) Mitochondrial DNA variation and stock structure of walleye from eastern Lake Huron: An analysis of contemporary and historical samples. *Transactions of the American Fisheries Society* **131,** 99–108.

Gerlach, G., Schardt, U., Eckmann, R., & Meyer, A. (2001) Kin–structured subpopulations in Eurasian perch (*Perca fluviatilis* L.). *Heredity* **86,** 213–221.

Gernhard, T. (2008) New analytical results for speciation times in neutral models. *Bulletin of Mathematical Biology* **70,** 1082–1097.

Gillooly, J.F. & Baylis, J.R. (1999) Reproductive success and the energetic cost of parental care in male smallmouth bass. *Journal of Fish Biology* **54,** 573–584.

Gillespie, J.H. (1991) *The Causes of Molecular Evolution.* New York, NY: Oxford University Press.

Gladenkov, A.Y., Oleinik, A.E., Marincovich, L., & Barinov, K.B. (2002) A refined age for the earliest opening of Bering Strait. *Palaeogeography Palaeoclimatology Palaeoecology* **183,** 321–328.

Glaubitz, J. C. (2004) CONVERT: A user–friendly program to reformat diploid genotypic data for commonly used population genetic software packages. *Molecular Ecology Notes* **4**, 309–310. http://www.agriculture.purdue.edu/fnr/ html/faculty/rhodes/students%20and%20staff /glaubitz/software.htm. (Accessed on 26 June 2013).

Gmelin, J.F. (1789) *Caroli a Linné. Systema naturae pe regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus Primus.* Editio decimo tertia, aucta, reformata.

Goodyear, C.S., Edsall, T.A. Ormsby Dempsey, D.M., Moss, G.D., & Polanski, P.E. (1982) Atlas of the Spawning and Nursery Areas of Great Lakes Fishes. U.S. Fish and Wildlife Service, Washington, DC FWS/OBS-82/52.

Google (2010) Google Earth® v. 6.0. Available at http://www.google.com/earth/index.html. (Accessed 26 June 2013).

Goudet, J. (1995) *F*stat version 1.2: a computer program to calculate Fstatistics. *Journal of Heredity* **86**, 485–486.

Goudet, J. (2002) *F*stat version 2.9.3.2. Available at http://www2.unil.ch/popgen/softwares/fstat.htm. (Accessed on 26 June 2013).

Grande, T., Laten, H., & Lopez, J.A. (2004) Phylogenetic relationships of extant esocid species (Teleostei: Salmoniformes) based on morphological and molecular characters. *Copeia* **2004,** 743–757.

Griffith, E. & Smith, C.H. (1834) The class Pisces, arranged by the Baron Cuvier, with supplementary additions, by Edward Griffith, F.R.S., and Lieut.–Col. Charles Hamilton Smith, F.R., L.S.S. In: *The Animal Kingdom* (Cuvier, G.L.C.F.D.). London: Whittaker and Co., 1–62.

Guillot, G. & Santos, F. (2009) A computer program to simulate multilocus genotype data with spatially autocorrelated allele frequencies. *Molecular Ecology Resources* **9**, 1112–1120.

Guillot, G., Mortier, F., & Estoup, A. (2005) Geneland: A computer package for landscape genetics. *Molecular Ecology Notes* **5**, 712–715. Available at http://www2.imm.dtu.dk/~gigu/Geneland/ (Accessed on 26 June 2013)

Guinand, B., Scribner, K.T., Page, K.S., & Burnham-Curtis, M.K. (2003) Genetic variation over space and time: Analyses of extinct and remnant lake trout populations in the Upper Great Lakes. *Proceedings of the Royal Society of London B* **270,** 425-433.

Guindon, S., Dufayard, J.F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010) New Algorithns and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology* **59,** 307-321. Available at http://www.atgc-montpellier.fr/phyml/ (Accessed 26 March 2013).

Guo, X. & Chen, D. (2010) Comparative evolution of the mitochondrial cytochrome *b* gene and nuclear S7 ribosomal protein gene intron 1 in sinipercid fishes and their relatives. *Hydrobiologia* **649,** 139–156.

Guseva, T.V. (1974) The ecology of spawning and embryonic development of the sea zander, *Lucioperca marina*. *Izvestiya Akademii Nauk SSSR Seriya Biologicheskaya* **2,** 87–91.

Haas, R.C., Bryant, W.C., Smith, K.D., & Nuhfer, A.J. (1985) Movement and harvest of fish in Lake St. Clair, St. Clair River, and Detroit River. *Final Report Winter Navigation Study U.S. Army Corps of Engineers.*

Hai, S., Li, J., Feng, J., & Mu, L. (2008) Systematics of *Perca* species based on multivariate morphometrics and mitochondrial cytochrome *b* gene variation analysis. *Zoological Research* **29,** 113–120.

Hall, T.A. (1999) BioEdit: A user–friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* **41:** 95. Available at http://www.mbio.ncsu.edu/BioEdit/bioedit.html. (Accessed on 26 March 2013).

Hänfling, B., Dümpelmann, C., Bogutskaya, N.G., Brandl, R., & Brändle, M. (2009) Shallow phylogeographic structuring of Vimba vimba across Europe suggests two distinct refugia during the last glaciation. *Journal of Fish Biology* **75,** 2269-2286.

Haponski, A.E. & Stepien, C.A. (2008) Molecular, morphological, and biogeographic resolution of cryptic taxa in the greenside darter *Etheostoma blennioides* complex. *Molecular Phylogenetics and Evolution* **49,** 69-83.

Haponski, A.E. & Stepien, C.A. (2013a) Phylogenetic and biogeographic relationships of the *Sander* pikeperches (Perciformes: Percidae): Patterns across North America and Eurasia. *Biological Journal of the Linnean Society* In press.

Haponski, A.E. & Stepien, C.A. (2013b) Genetic connectivity and diversity of walleye (*Sander vitreus*) spawning groups in the Huron–Erie Corridor. *Journal of Great Lakes Research* In press.

Haponski, A.E., Marth, T.A., & Stepien, C.A. (2007) Genetic divergence across a low-head dam: A preliminary analysis using logperch and greenside darters. *Journal of Great Lakes Research* **33,** 117–126.

Hartig, J.H., Zarull, M.A., Ciborowski, J.J.H., Gannon, J.E., Wilke, E., Norwood, G., & Vincent, A.N. (2009) Long–term ecosystem monitoring and assessment of the Detroit River and western Lake Erie. *Environmental Monitoring and Assessment* **158,** 87–104.

Hartl, D.L. (2000) *A Primer of Population Genetics* 3rd ed. Sinauer Associates, Inc., Sunderland.

Hartman, W.L. (1973) The effects of exploitation, environmental changes, and new species on the fish habitats and resources of Lake Erie. Great Lakes Fishery Commission Technical Report 22.

Hasegawa, M., Kishino, H., & Yano, T. (1985) Dating of the human–ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22,** 160–174.

Hauser, L., Adcock, G.J., Smith, P.J., Ramírez, J.H.B., & Carvalho, G.R. (2002) Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). *PNAS* **99**, 11742–11747.

Hayhoe, K., VanDorn, J., Croley, T., Schlegal, N., & Wuebbles, D. (2010) Regional climate change projections for Chicago and the US Great Lakes. *Journal of Great Lakes Research* **36,** 7–21.

Hedrick, P. (2005) "Genetic restoration": A more comprehensive perspective than "genetic rescue". *Trends in Ecology and Evolution* **20,** 109.

Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society* **58,** 247–276.

Hewitt, G.M. (2000) The genetic legacy of the Quaternary ice ages. *Nature* **405,** 907–913.

Hewitt, G.M. (2001) Speciation, hybrid zones and phylogeography – or seeing genes in space and time. *Molecular Ecology* **10,** 537–549.

Holčík, J. & Hensel, K. (1974) A new species of *Gymnocephalus* (Pisces: Percidae) from the Danube, with remarks on the genus. *Copeia* **1974,** 481–486.

Holder, M. & Lewis, P.O. (2003) Phylogeny estimation: Traditional and Bayesian approaches. *Nature Reviews Genetics* **4,** 275–284.

HTG (Habitat Task Group of the Lake Erie Committee, Great Lakes Fishery Commission) (2009) Report of the Lake Erie Habitat Task Group. Great Lakes Fishery Commission Ann Arbor, Michigan. Available at http://www.glfc.org/lakecom/lec/HTG_docs/annual_reports/HTG_AnnualReport2009.pdf (Accessed on 8 August 2012).

HTG (2011) Report of the Lake Erie Habitat Task Group. Great Lakes Fishery Commission Ann Arbor, Michigan. Available at http://www.glfc.org/lakecom/lec/HTG_docs/annual_reports /HTG_AnnualReport2011.pdf (Accessed on 8 August 2012).

Hubbs, C.L. (1926) A check–list of the fishes of the Great Lakes and tributary waters, with nomenclatorial notes and analytical keys. University of Michigan Museum of Zoology Miscellaneous Publication 15, Ann Arbor.

Hubbs, C.L. & Lagler, K.F. (2004) *Fishes of the Great Lakes Region.* (G.R. Smith, revised). Ann Arbor, MI: University of Michigan.

Hubert, N., Hanner, R., Holm, E., Mandrak, N.E., Taylor, E., Burridge, M., Watkinson, D., Dumont, P., Curry, A., Bentzen, P., Zhang, J., April, J., & Bernatchez, L. (2008) Identifying Canadian freshwater fishes through DNA barcodes. *PLoS ONE* **3,** e2490.

Huey, J.A., Baker, A.M., & Hughes, J.M. (2010) High levels of genetic structure in the Australian freshwater fish, *Ambassis macleayi. Journal of the North American Benthological Society* **29,** 1148–1160.

Hughes, J.M. (2007) Constraints on recovery: Using molecular methods to study connectivity of aquatic biota in rivers and streams. *Freshwater Biology* **52,** 616-631.

Hutchinson, W.F., van Oosterhout, C., Rogers, S.I., & Carvalho, G.R. (2003) Temporal analysis of archived samples indicates marked genetic changes in declining North Sea Cod (*Gadus morhua*). *Proceedings of the Royal Society of London B* **270,** 2125–2132.

Hutchings, J.A., Butchart, S.H.M., Collen, B., Schwartz, M.K., & Waples, R.S. (2012) Red flags: Correlates of impaired species recovery. *Trends in Ecology and Evolution* **27**, 542–546.

Imoto, J.M., Saitoh, K., Sasaki, T., Yonezawa, T., Adachi, J., Kartavtsev, Y.P., Miya, M., Nishida, M., & Hanzawa, N. (2013) Phylogeny and biogeography of highly diverged freshwater fish species (Leusciscinae, Cyprinidae, Teleostei) inferred from mitochondrial genome analysis. *Gene* **514,** 112-124.

IUCN (2012) *Sander marinus* fact sheet. Available at http://www.iucnredlist.org/details/20861/0 (Accessed on 26 March 2013)

Ivanova, N.V., Zemlak, T.S., Hanner, R.H., & Hebert, P.D.N. (2007) Universal primer cocktails for fish DNA barcoding. *Molecular Ecology Notes* **7,** 544–548.

Jennings, M.J., Claussen, J.E., & Philipp, D.P. (1996) Evidence for heritable preferences for spawning habitat between two walleye populations. *Transactions of the American Fisheries Society* **125,** 978–986.

Jones, O. & Wang, J. (2009) COLONY: A program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* **10,** 551–555.

Jonsson, B. & Jonsson, N. (2009) A review of the likely effects of climate change on anadromous Atlantic salmon *Salmo salar* and brown trout *Salmo trutta,* with particular reference to water temperature and flow. *Journal of Fish Biology* **75,** 2381–2447.

Karsiotis, S.I., Sullivan, T.J., & Stepien, C.A. (In review) Population genetic structure and comparative diversity of smallmouth bass: Patterns from two genomes. *Diversity and Distributions.*

Keck, B.P. & Near, T.J. (2008) Assessing phylogenetic resolution among mitochondrial, nuclear, and morphological datasets in *Nothonotus* darters (Teleostei: Percidae). *Molecular Phylogenetics and Evolution* **46,** 708-720.

Kerr, L.A., Cadrin, S.X., & Secor, D.H. (2010) Simulation modeling as a tool for examining the consequences of spatial structure and connectivity on local and regional population dynamics. ICES *Journal of Marine Science* **67,** 1631-1639.

Kimura, M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* **16,** 111–120.

Kocovsky, P.M., Sullivan, T.J., Knight, C.T., & Stepien, C.A. (2013) Genetic and morphometric differences demonstrate fine–scale population substructure of the yellow perch *Perca flavescens*: Need for redefined management units. *Journal of Fish Biology* **82,** 2015–2030.

Kottelat, M. (1997) European freshwater fishes. *Biologia* **52,** 1–271.

Kuznetsov, V.A. (2010) Growth, size–age structure of catches and reproduction of the Volga zander *Sander volgensis* (Percidae) in the upper part of the Volga stretch of the Kuibyshev Reservoir. *Journal of Ichthyology* **50,** 772–777.

Lacepède, B.G.E. (1802) Histoire naturelle des poisons, volume 4. Paris.

Lanave, C., Preparata, G., Saccone, C., & Serio, G. (1984) A new method for calculating evolutionary substitution rates. *Journal of Molecular Evolution* **20,** 86–93.

Lang, N.J. & Mayden, R.L. (2007) Systematics of the subgenus *Oligocephalus* (Teleostei: Percidae: *Etheostoma*) with complete subgeneric sampling of the genus *Etheostoma*. *Molecular Phylogenetics and Evolution* **43,** 605–615.

Laroche, J. & Durand, J.D. (2004) Genetic structure of fragmented populations of a threatened endemic percid of the Rhone River: *Zingel asper*. *Heredity* **92,** 329-334.

Larsen, L.K. & Berg, S. (2006) NOBANIS – Invasive Alien Species Fact Sheet – *Sander lucioperca* – From: Online database of the North European and Baltic Network on Invasive Alien Species – NOBANIS www.nobanis.org (Accessed 26 March 2013).

Larson, G. & Schaetzl, R. (2001) Origin and evolution of the Great Lakes. *Journal of Great Lakes Research* **27,** 518–546.

LeClerc, E., Mailhot, Y., Mingelbier, M., & Bernatchez, L. (2008) The landscape genetics of yellow perch (*Perca flavescens*) in a large fluvial ecosystem. *Molecular Ecology* **17**, 1702–1717.

Lebedev, V.D. (1952) Fishes from a late–paleolithic settlement at Murzak–Koba in Crimea. *Bulletin of the Society of Natural History, Moscow* **51,** 46–51.

Leberg, P.L. (1992) Effects of population bottlenecks on genetic diversity as measured by allozyme electrophoresis. *Evolution* **46,** 477-494.

Leonardi, J.M. & Thomas, M.V. (2000) An assessment of the Huron River walleye population. MIDNR Fisheries Technical Report No. 97-2, 1997.

Leonardi, J.M. & Gruhn, W.J. (2001) Flint River assessment. MIDNR Fisheries Special Report 27.

Lepper, K., Buell, A.W., Fisher, T.G., & Lowell, T.V. (2013) A chronology for glacial Lake Agassiz shorelines along Upham's namesake transect. *Quaternary Research* In press.

Lévêque, C., Oberdorff, T., Paugy, D., Stiassny, M.L.J., & Tedesco, P.A. (2008) Global diversity of fish (Pisces) in freshwater. *Hydrobiologia* **595:** 545–567.

Linnaeus, C. (1758) *Tomus I. Systema naturae pe regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis.* Editio decimo reformata. Holmiae. Laurentii Salvii.

Linnaeus, C. (1766) *Systema naturae sive regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis.* Laurentii Salvii, Holminae. 12th edition. **1,** 1-532.

Locke, B., Belore, M., Cook, A., Einhouse, D., Kenyon, R., Knight, R., Newman, K., Ryan, P., & Wright, E. (2005) Walleye management plan. *Lake Erie Committee Great Lakes Fishery Commission*. Available at http://www.glfc.org/lakecom/lec/WTG_docs/other_reports_and_docs/ wmp20051207.pdf (Accessed on 26 June 2013).

López, J.A., Bentzen, P., & Pietsch, T.W. (2000) Phylogenetic relationships of esocoid fishes (Teleostei) based on partial cytochrome *b* and 16S mitochondrial DNA sequences. *Copeia* **2000,** 420–431.

López, J.A., Chen, W.J., & Ortí, G. (2004) Esociform phylogeny. *Copeia* **2004,** 449–464.

Lunt, D.H., Zhang, D.X., Szymura, J.M., & Hewitt, G.M. (1996) The insect cytochrome oxidase I gene: Evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology* **5,** 153–165.

Lyons, J. & Kanehl, P. (2002) Seasonal movements of smallmouth bass in streams. In: *Black Bass: Ecology, Conservation, and Management* (ed. by D.P. Philipp & M.S. Ridgway), pp. 149–160. American Fisheries Society, Bethesda.

Maitland, P.S. (2001) *Freshwater Fish of Britain and Europe.* London: Hamlyn.

Manel, S., Schwartz, M.K., Luikart, G., & Taberlet, P. (2003) Landscape genetics: Combining landscape ecology and population genetics. *Trends in Ecology and Evolution* **18,** 189–197.

Manni, F., Guérard, E., & Heyer, E. (2004) Geographic patterns of (genetic, morphologic, linguistic) variation: How barriers can be detected by using Monmonier's algorithm. *Human Biology* **76**, 173–190. Available at http://www.mnhn.fr/mnhn/ecoanthropologie/software/barrier.html. (Accessed on 26 June 2013)

Manny, B.A. (2006) Monitoring element of the Belle Isle/Detroit River sturgeon habitat restoration, monitoring, and education project. Research Completion Report to Michigan Sea Grant-University of Michigan

Manny, B.A. & Kenaga, D. (1991) The Detroit River: Effects of contaminants and human activities on aquatic plants and animals and their habitats. *Hydrobiologia* **219,** 269-279.

Manny, B.A., Kennedy, G.W., Allen, J.D., & French, R.P. (2007) First evidence of egg deposition by walleye (*Sander vitreus*) in the Detroit River. *Journal of Great Lakes Research* **33,** 512-516.

Manny, B.A., Kennedy, G.W., Boase, J.C., Allen, J.D., & Roseman, E.F. (2010) Spawning by walleye (*Sander vitreus*) and white sucker (*Catostomus commersonii*) in the Detroit River: Implications for spawning habitat enhancement. *Journal of Great Lakes Research* **36,** 490-496.

Manny, B.A., Read, J., Denison, D., Reider, R., Kennedy, G., Caswell, N., Boase, J., & McClain, J. (2005) Creation of lake sturgeon spawning habitat in the Detroit River, In: State of the Strait: Monitoring for Sound Management. (ed. by R. Eedy, J. Hartig, C. Bristol, M. Coulter, T. Mabee, & J. Ciborowski), pp. 96-99. Great Lakes Institute for Environmental Research, Occasional Publication No. 4, University of Windsor, Windsor, Ontario.

Mantel, N. (1967) The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**, 209–220.

Marshall, H.D., Coulson, M.W., & Carr, S.M. (2009) Near neutrality, rate heterogeneity, and linkage govern mitochondrial genome evolution in Atlantic cod (*Gadus morhua*) and other gadine fish. *Molecular Biology and Evolution* **26,** 579–589.

Mascarelli, A.L. (2009) Quaternary geologists win timescale vote. *Nature* **459,** 624.

Matschiner, M., Hanel, R., & Salzburger, W. (2011) On the origin and trigger of the notothenoid adaptive radiation. *PLoS ONE* **6,** e18911.

McCracken, K.G. & Sorenson, M.D. (2005) Is homoplasy or lineage sorting the source of incongruent mtDNA and nuclear gene trees in the stiff–tailed ducks (*Nomonyx–Oxyura*). *Systematic Biology* **54,** 35–55.

McGarigal, K., Cushman, S., & Stafford, S. (2000). *Multivariate Statistics for Wildlife and Ecology Research.* Springer–Verlag, NY.

McMahon, S.M., Dietze, M.C., Hersh, M.H., Moran, E.V., & Clark, J.S. (2009) A predictive framework to understand forest responses to global change. *Annals of the New York Academy of Sciences* **1162,** 221–236.

McParland, T.L., Ferguson, M.M., & Liskauskas, A.P. (1999) Genetic population structure and mixed-stock analysis of walleyes in the Lake Erie-Lake Huron Corridor using allozymes and mitochondrial DNA markers. *Transactions of the American Fisheries Society* **128,** 1055-1067.

Miller, C.R., Waits, L.P., & Joyce, P. (2006) Phylogeography and mitochondrial diversity of extirpated brown bear (*Ursus arctos*) populations in the contiguous United States and Mexico. *Molecular Ecology* **15,** 4477–4485.

Milne, R.I. (2006) Northern hemisphere plant disjunctions: A window on tertiary land bridges and climate change? *Annals of Botany* **98,** 465–472.

Mion, J.B., Stein, R.A., & Marschall, E.A. (1998) River discharge drives survival of walleye. *Ecological Applications* **8,** 88-103.

Mitchill, S.L. (1814) Report in part of Samuel L. Mitchill, M.D., on the fishes of New York. New York.

Mitchill, S.L. (1818) Memoir on ichythology. The Fishes of New York, described and arranged. *The American Monthly Magazine and Critical Review* **6,** 321–328.

Monmonier, M. (1973) Maximum–difference barriers: An alternative numerical regionalization method. *Geographical Analysis* **3**, 245–261.

Moore, G.A. (1944) The retinae of two North American teleosts, with special reference to their tapeta lucida. *The Journal of Comparative Neurology* **80,** 369–379.

Moran, G.F. & Hopper, S.D. (1983) Genetic diversity and the insular population structure of the rare granite rock species, *Eucalyptus caesia* Benth. *Australian Journal of Botany* **31,** 161–172.

Moritz, C. & Hillis, D.M. (1996) Molecular systematics: Context and controversies. In: *Molecular Systematics 2nd ed.* (ed. by D.M. Hillis, C. Moritz, & B.K. Marble), pp. 1-13. Sinauer Associates, Sunderland.

Moyle, P.B. & Cech, J.J. (2000) *Fishes. An Introduction to Ichthyology.* 4th ed. Prentice Hall, Upper Saddle.

Murray, A.M. & Divay, J.D. (2011) First evidence of percids (Teleostei: Perciformes) in the Miocene of North America. *Canadian Journal of Earth Sciences* **48,** 1419–1424.

Murray, A.M., Cumbaa, S.L., Harington, C.R., Smith, G.R., & Rybczynski, N. (2009) Early Pliocene fish remains from Arctic Canada support a pre–Pleistocene dispersal of percids (Teleostei: Perciformes). *Canadian Journal of Earth Sciences* **46,** 557–570.

Nate, N.A., Hansen, M.J., Rudstam, L.G., Knight, R.L., & Newman, S.P. (2011) Population and community dynamics of walleye. In: *Biology, Management, and Culture of Walleye and Sauger* (ed. by B.A. Barton), pp. 321–374. American Fisheries Society, Bethesda.

Near, T.J. (2002) Phylogenetic relationships of *Percina* (Percidae: Etheostomatinae). *Copeia* **2002,** 1–14.

Near, T.J., Porterfield, J.C., & Page, L.M. (2000) Evolution of the cytochrome *b* and the molecular systematics of *Ammocrypta* (Percidae: Etheostomatinae). *Copeia* **2000**, 701–711.

Near, T.J., Bolnick, D.I., & Wainwright, P.C. (2005) Fossil calibrations and molecular divergence time estimates in centrarchid fishes (Teleostei: Centrarchidae). *Evolution* **59,** 1768–1782.

Near, T.J., Bossu, C.M., Bradburd, G.S., Carlson, R.L., Harrington, R.C., Hollingsworth, P., Keck, B.P., & Etnier, D.A. (2011) Phylogeny and temporal diversification of darters (Percidae: Etheostomatinae). *Systematic Biology* **60,** 565–595.

Neilson, M.E. & Stepien, C.A. (2009) Escape from the Ponto–Caspian: Evolution and biogeography of an endemic goby species flock (Benthophilinae: Gobiidae: Teleostei). *Molecular Phylogenetics and Evolution* **52,** 84–102.

Nei, M. (1972) Genetic distance between populations. *The American Naturalist* **106,** 283–282.

Nelson, J.S., Crossman, E.J., Espinosa–Pérez, H., Findley, L.T., Gilbert, C.R., Lea, R.N., & Williams, J.D. (2003) The "names of fishes" list, including recommended changes in fish names: Chinook salmon for chinook salmon, and *Sander* to replace *Stizostedion* for the sauger and walleye. *Fisheries* **28,** 38–39.

Nelson, J.S., Crossman E.J., Espinosa–Pérez, H., Findley, L.T., Gilbert, C.R., Lea, R.N., & Williams, J.D. (2004) *Common and scientific names of fishes from the United States, Canada, and Mexico.* 6th ed. Bethesda, MD: American Fisheries Society, special publication 29.

Nesbø, C.L., Fossheim, T., Vollestad, L.A., & Jakobsen, K.S. (1999) Genetic divergence and phylogeographic relationships among European perch (*Perca fluviatilis*) populations reflect glacial refugia and postglacial colonization. *Molecular Ecology* **8,** 1387–1404.

Nicod, J.C., Wang, Y.Z., Excoffier, L., Largiader, C.R. (2004) Low levels of mitochondrial DNA variation among central and southern European *Esox lucius* populations. *Journal of Fish Biology* **64,** 1442–1449.

Nielsen, E.E. & Hansen, M.M. (2008) Waking the dead: The value of population genetic analyses of historical samples. *Fish and Fisheries* **9,** 450–461.

Nielsen, J.L. & Fountain, M.C. (1999) Microsatellite diversity in sympatric reproductive ecotypes of Pacific steelhead (*Oncorhynchus mykiss*) from the Middle Fork Eel River, California. *Ecology of Freshwater Fishes* **8,** 159-168.

Noecker, R.J. (1998) Endangered species list revisions: A summary of delisting and downlisting. *CRS Report for Congress 98–32 ENR.*

Nomura, M., Yates, J.L., Dean, D., & Post, L.E. (1980) Feedback regulation of ribosomal protein gene expression in *Escherichia coli*: Structural homology of ribosomal RNA and ribosomal protein mRNA. *Proceedings of the National Academy of Sciences* **77,** 7084–7088.

Oettinger, M.A., Schatz, D.G., Gorka, C., & Baltimore, D. (1990) RAG–1 and RAG–2, adjacent genes that synergistically activate V(D)J recombination. *Science* **248,** 1517–1523.

Olden, J.D., Kennard, M.J., Leprieur, F., Tedesco, P.A., Winemiller, K.O., & García–Berthou, E. (2010) Conservation biogeography of freshwater fishes: Recent progress and future challenges. *Diversity and Distributions* **16,** 496–513.

Olson, D.E. & Scidmore, W.J. (1962) Homing behavior of spawning walleyes. *Transactions of the American Fisheries Society* **91,** 355–361.

Osborn, H.F. (1910) *The Age of Mammals in Europe, Asia, and North America.* New York, NY: MacMillan Company.

Ouborg, N.J., Pertoldi, C., Loeschcke, V., Bijlsma, R., & Hedrick, P.W. (2010) Conservation genetics in transition to conservation genomics. *TRENDS in Genetics* **26,** 177–187.

Ozerov, M.Y., Veselov, A.E., Lumme, J., & Primmer, C.R. (2012) "Riverscape" genetics: River characteristics influence the genetic structure and diversity of anadromous and freshwater Atlantic salmon (*Salmo salar*) populations in northwest Russia. *Canadian Journal of Fisheries and Aquatic Sciences* **69**, 1947–1958.

Page, L.M. & Burr, B.M. (2011) *Field Guide to the Freshwater Fishes of North America.* 2nd edition. Boston, MA: Houghton Mifflin Harcourt.

Palmer, G.C., Culver, C., Dutton, D., Murphy, B.R., Hallerman, E.M., Billington, N., & Williams, J. (2006) Genetic distinct walleye stocks in Claytor Lake and the Upper New River, Virginia. *Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies* **60,** 125–131.

Palumbi, S.R. (1994) Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics* **25,** 547–572.

Palumbi, S.R. (1996) Nucleic acids II: the polymerase chain reaction. In: Hillis DM, Moritz C, Mable BK, eds. *Molecular systematics.* 2nd ed. Sunderland, MA: Sinauer Associates, 205–221.

Paragamian, V.L. (1989) Seasonal habitat use by walleye in a warmwater river system, as determined by radiotelemetry. *North American Journal of Fisheries Management* **9,** 392-401.

Parsons, J.W. (1967) Contributions of year–classes of blue pike to the commercial fishery of Lake Erie, 1943–59. *Fisheries Research Board of Canada* **24,** 1035–1066.

Perdices, A., Doadrio, I., & Bermingham, E. (2005) Evolutionary history of the synbranchid eels (Teleostei: Synbranchidae) in Central America and the Caribbean islands inferred from their molecular phylogeny. *Molecular Phylogenetics and Evolution* **37,** 460–473.

Pielou, E.C. (1991) *After the Ice Age: The Return of Life to Glaciated North America.* Chicago, IL: University of Chicago Press.

Piry, S., Alapetite, A., Cornuet, J.M., Paetkau, D., Baudouin, L., & Estoup, A. (2004) Geneclass2: A software for genetic assignment and first–generation migrant detection. *Journal of Heredity* **95**, 536–539. Available at http://www.ensam.inra.fr/URLB/index.html (Accessed on 26 June 2013)

Poissant, J., Knight, T.W., & Ferguson, M.M. (2005) Nonequilibrium conditions following landscape rearrangement: The relative contribution of past and current hydrological landscapes on the genetic structure of a stream–dwelling fish. *Molecular Ecology* **14**, 1321–1331.

Posada, D. (2008) jModelTest: Phylogenetic model averaging. *Molecular Biology and Evolution* **25**,1253–1256. Available at http://darwin.uvigo.es/software/ software.html (Accessed on 26 March 2013)

Poulet, N., Berrebi, P., Crivelli, A.J., Lek, S., & Argillier, C. (2004) Genetic and morphometric variations in the pikeperch (*Sander lucioperca* L.) of a fragmented delta. *Archiv für Hydrobiologie* **159,** 531–554.

Pringle, J.M. & Wares, J.P. (2007) Going against the flow: Maintenance of alongshore variation in allele frequency in a coastal ocean. *Marine Ecology Progress Series* **335,** 69–84.

Pritchard, J. K., Stephens, M. & Donnelly, P. (2000) Inference of population structure using multilocus genotype data. *Genetics* **155**, 945–959.

Quattro, J.M. & Jones, W.J. (1999) Amplification primers that target locus–specific introns in Actinopterygian fishes. *Copeia* **1999,** 191–196.

Quattro, J.M., Stoner, D.S., Driggers, W.B., Anderson, C.A., Priede, K.A., Hoppmann, E.C., Campbell, N.H., Duncan, K.M., & Grady, J.M. (2006) Genetic evidence of cryptic speciation within hammerhead sharks (Genus *Sphyrna*). *Marine Biology* **148,** 1143–1155.

R Development Core Team (2012) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. Available at http://www.r-project.org/. (Accessed on 26 March 2013).

Rafinesque, C.S. (1818) Second decade of new North American fishes. *The American Monthly Magazine and Critical Review* **2,** 204–206.

Rafinesque, C.S. (1819) Prodrome de 70 nouveau genres d'animaux découverts dans l'interieur des États–Unis d'Amerique durant l'année 1818. *Journal de Physique de Chimie et d'Histoire naturelle* **88,** 417–429.

Rafinesque, C.S. (1820) *Ichthyologia Ohiensis, or Natural History of the Fishes Inhabiting the River Ohio and its Tributary Streams, Preceeded by a Physical Description of the Ohio and its Branches.* Lexington, KY: WG Hunt.

Rannala, B. & Mountain, J.L. (1997) Detecting immigration by using multilocus genotypes. *PNAS* **94,** 9197-9201.

Rawson, M.R. (1980) Yellow perch movements. *Ohio Department of Natural Resources Job Program Report, Dingell–Johnson Project Number F–35–R–18, Study Number. 4, July 1, 1979–June 30, 1980.*

Ray, J.W., Husemann, M., King, R.S., & Danley, P.D. (2012) Genetic analysis reveals dispersal of Florida bass haplotypes from reservoirs to rivers in central Texas. *Transactions of the American Fisheries Society* **141,** 1269–1273.

Raymond, M. & Rousset, F. (1995) An exact test for population differentiation. *Evolution* **49**, 1280–1283.

Reed, D.H. & Frankham, R. (2003) Correlation between fitness and genetic diversity. *Conservation Biology* **17**, 230–237.

Reid, D.F. & Orlova, M.I. (2002) Geological and evolutionary underpinnings for the success of Ponto–Caspian species invasions in the Baltic Sea and North American Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **59,** 1144–1158.

Rice, R.M. (1989) Analyzing tables of statistical tests. *Evolution* **43**, 223–225.

Robinson, B.W. & Parsons, K.J. (2002) Changing times, spaces, and faces: Tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **59,** 1819–1833.

Robinson, C.T., Tockner, K., & Ward, J.V. (2002) The fauna of dynamic riverine landscapes. *Freshwater Biology* **47,** 661-677.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19,** 1572–1574. http://mrbayes.sourceforge.net/. (v3.1.2, 2005) (Accessed on 26 March 2013).

Roseman, E., Kocovsky, P., & Vandergoot, C. (2010) Status of walleye in the Great Lakes: Proceedings of the 2006 symposium. *Great Lakes Fishery Commission Technical Report 69.*

Roseman, E.F., Manny, B., Boase, J., Child, M., Kennedy, G., Craig, J., Soper, K., & Drouin, R. (2011) Lake sturgeon response to a spawning reef constructed in the Detroit River. *Journal of Applied Ichthyology* **27,** 66-76.

Rousset, F. (1997) Genetic differentiation and estimation of gene flow from *F*-statistics under isolation by distance. *Genetics* **145**, 1219–1228.

Rousset, F. (2008) Genepop'008: A complete re–implementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* **8**, 103–106. Available at http://kimura.univmontp2.fr/ ~rousset/Genepop.htm (Accessed on 26 June 2013)

Ryan, P.A., Knight, R., MacGregor, R., Towns, G., Hoopes, R., & Culligan, W. (2003) Fish–community goals and objectives for Lake Erie. *Great Lakes Fishery Commission Special Publication 03–02.*

Ryder, R.A. (1968) Dynamics and exploitation of mature walleyes, *Stizostedion vitreum vitreum*, in the Nipigon Bay region of Lake Superior. *Journal of the Fisheries Research Board of Canada* **25,** 1347-1376.

Ryder, O.A. (1986) Species conservation and systematics: The dilemma of subspecies. *Trends in Ecology and Evolution* **1,** 9-10.

Saitoh, K., Sado, T., Doosey, M.H., Bart, H.L., Inoue, J.G., Nishida, M., Mayden, R.L., & Miya, M. (2011) Evidence from mitochondrial genomics supports the lower Mesozoic of South Asia as the time and place of basal divergence of cypriniform fishes (Actinopterygii: Ostariophysi). *Zoological Journal of the Linnean Society* **161,** 633–662.

Saitou, N. & Nei, M. (1987) The neighbor joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* **4,** 406–425.

Sargent, R.C. (1988) Paternal care and egg survival both increase with clutch size in the fathead minnow *Pimephales promelas*. *Behavioral Ecology Sociobiology* **23,** 33–37.

Sargent, R.C., Taylor, P.D., & Gross, M.R. (1987) Parental care and the evolution of egg size in fishes. *American Naturalist* **129,** 32–46.

Schindler, D.E., Hilborn, R., Chasco, B., Boatright, C.P., Quinn, T.P., Rogers, L.A., & Webster, M.S. (2010) Population diversity and the portfolio effect in an exploited species. *Nature* **465,** 609-613.

Schmalz, P.J., Fayram, A.H., Isermann, D.A., Newman, S.P., & Edwards, C.J. (2011) Harvest and exploitation. In: Barton BA, ed. *Biology, Management, and Culture of Walleye and Sauger.* Bethesda, MD: American Fisheries Society, 375–402.

Schmidt, T.R. & Gold, J.R. (1993) Complete sequence of the mitochondrial cytochrome *b* gene in the Cherryfin Shiner, *Lythrurus roseipinnis* (Teleostei: Cyprinidae). *Copeia* **1993,** 880–883.

Scott, W.B. & Crossman, E.J. (1973) Freshwater fishes of Canada. *Journal of the Fisheries Research Board of Canada* **184,** 1–196.

Senanan, W. & Kapuscinski, A.R. (2000) Genetic relationships among populations of northern pike (*Esox lucius*). *Canadian Journal of Fisheries and Aquatic Sciences* **57,** 391–404.

Sepulveda-Villet, O.J. & Stepien, C.A. (2011) Fine-scale population genetic structure of the yellow perch *Perca flavescens* in Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* **68,** 1435-1453.

Sepulveda–Villet, O.J. & Stepien, C.A. (2012) Waterscape genetics of the yellow perch (*Perca flavescens*): Patterns across large connected ecosystems and isolated relict populations. *Molecular Ecology* **21,** 5795–5826.

Sepulveda-Villet, O.J., Ford, A.M., Williams, J.D., & Stepien, C.A. (2009) Population genetic diversity and phylogeographic divergence patterns of the yellow perch (*Perca flavescens*). *Journal of Great Lakes Research* **35,** 107-119.

Sharma, S., Jackson, D.A., Minns, C.K., & Shuter, B.J. (2007) Will northern fish populations be in hot water because of climate change? *Global Change Biology* **13,** 2052–2064.

Shedlock, A.M., Haygood, M.G., Pietsch, T.W., & Bentz, P. (1997) Enhanced DNA extraction and PCR amplification of mitochondrial genes from formalin fixed museum specimens. *Biotechniques* **22**, 394–400.

Sheer, M.B. & Steel, E.A. (2006) Lost watersheds: Barriers, aquatic habitat connectivity, and salmon persistence in the Willamette and Lower Columbia River basins. *Transactions of the American Fisheries Society* **135,** 1654-1669.

Shine, R. (1978) Propagule size and parental care: The “safe harbor” hypothesis. *Journal of Theoretical Biology* **75,** 417-424.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H., & Flook, P. (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87,** 651–701.

Slatkin, M. (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics* **16,** 393–430.

Slatkin, M. (1991) Inbreeding coefficients and coalescence times. *Genetics Research Cambridge* **58,** 167-175.

Sloss, B.L., Billington, N., & Burr, B.M. (2004) A molecular phylogeny of the Percidae (Teleostei, Perciformes) based on mitochondrial DNA sequence. *Molecular Phylogenetics and Evolution* **32,** 545–562.

Smith, C. & Wootton, R.J. (1994) The cost of parental care in *Haplochromis ‘argens’* (Cichlidae). *Environmental Biology of Fishes* **40,** 99–104.

Smith, P.J., Francis, R.I.C.C., & McVeagh, M. (1991) Loss of genetic diversity due to fishing pressure. *Fisheries Research* **10,** 309–316.

Song, C.B., Near, T.J., & Page, L.M. (1998) Phylogenetic relations among Percid fishes as inferred from mitochondrial cytochrome *b* DNA sequence data. *Molecular Phylogenetics and Evolution* **10,** 343-353.

Sork, V.L. & Waits, L. (2010) Contributions of landscape genetics – approaches, insights, and future potential. *Molecular Ecology* **19,** 3489–3495.

Specziár, A. & Bíró, P. (2003) Population structure and feeding characteristics of Volga pikeperch, *Sander volgensis* (Pisces, Percidae), in Lake Balaton. *Hydrobiologia* **506–509,** 503–510.

Stepien, C.A. (1995) Population genetic divergence and geographic patterns from DNA sequences: Examples from marine and freshwater fishes. In: Nielsen JL, ed. *Evolution and the Aquatic Ecosystem: Defining Unique Units in Population Conservation*. Bethesda, MD: American Fisheries Society Symposium 17, 263–287.

Stepien, C.A. & Kocher, T.D. (1997) Molecules and morphology in studies of fish evolution. In: Kocher TD, Stepien, CA, eds. *Molecular Systematics of Fishes.* San Diego, CA: Academic Press, 1–11.

Stepien, C.A. & Faber, J.E. (1998) Population genetic structure, phylogeography and spawning philopatry in walleye (*Stizostedion vitreum*) from mitochondrial DNA control region sequences. *Molecular Ecology* **7,** 1757–1769.

Stepien, C. A., Dillon, A. K., & Chandler, M. D. (1998). Genetic identity, phylogeography, and systematics of ruffe *Gymnocephalus* in the North American Great Lakes and Eurasia. *Journal of Great Lakes Research* **24,** 361–378.

Stepien, C.A., Taylor, C.D., & Einhouse, D.W. (2004) An analysis of genetic risk to a native spawning stock of walleye *Sander vitreus* (*Stizostedion vitreum)* due to stocking in Cattaraugus Creek. In: Barry TP, Malison JA, eds. *Proceedings of Percis III, the 3rd International Symposium on Percid Fishes*. Madison, WI: University of Wisconsin Sea Grant Institute, 93-94.

Stepien, C.A., Murphy, D.J., & Strange, R.M. (2007) Broad- to fine-scale population genetic patterning in the smallmouth bass *Micropterus dolomieu* across the Laurentian Great Lakes and beyond: An interplay of behaviour and geography. *Molecular Ecology* **16,** 1605–1624.

Stepien, C.A., Brown, J.E., Neilson, M.E., & Tumeo, M.A. (2005) Genetic diversity of invasive species in the Great Lakes versus their Eurasian source populations: Insights for risk analysis. *Risk Analysis* **25,** 1043–1060.

Stepien, C.A., Banda, J.A., Murphy, D.M., & Haponski, A.E. (2012) Temporal and spatial genetic consistency of walleye (*Sander vitreus*) spawning groups. *Transactions of the American Fisheries Society* **141,** 660–672.

Stepien, C.A., Murphy, D.M., Lohner, R.N., Sepulveda–Villet, O.J., & Haponski, A.E. (2009) Signatures of vicariance, postglacial dispersal, and spawning philopatry: Population genetics and biogeography of the walleye *Sander vitreus*. *Molecular Ecology* **18,** 3411–3428.

Stepien, C.A., Murphy, D.J., Lohner, R.N., Haponski, A.E., & Sepulveda–Villet, O.J. (2010) Status and delineation of walleye (*Sander vitreus*) genetic stock structure across the Great Lakes. In: Status of walleye in the Great Lakes: Proceedings of the 2006 symposium (ed. by E. Roseman, P. Kocovsky, C. Vandergoot), pp. 189–223. Great Lakes Fishery Commission Technical Report 69, Ann Arbor.

Stewart, J.R. & Lister, A.M. (2001) Cryptic northern refugia and the origins of the modern biota. *TRENDS in Ecology and Evolution* **16,** 608–613.

Stone, F.L. (1948) A study of the taxonomy of the blue and yellow pikeperches (*Stizostedion*) of Lake Erie and Lake Ontario. Unpublished Ph.D. dissertation University of Rochester, Rochester, NY.

Stott, W., Ebener, M.P., Mohr, L., Hartman, T., Johnson, J., & Roseman, E.F. (2013) Spatial and temporal genetic diversity of lake whitefish (*Coregonus clupeaformis* (Mitchill)) from Lake Huron and Lake Erie. *Advances in Limnology* **64,** 205-222.

Strange, R. M. & Stepien, C.A. (2007) Genetic divergence and connectivity among river and reef spawning groups of walleye (*Sander vitreus*) in Lake Erie. *Canadian Journal of Fisheries and Aquatic Sciences* **64**, 437–448.

Sturmbauer, C. & Meyer, A. (1992) Genetic divergence, speciation and morphological stasis in a lineage of African cichlid fishes. *Nature* **358,** 578–581.

Sullivan, T.J. & Stepien, C.A. (2013) Genetic diversity, divergence, and connectivity within a complex lakescape: A case study of yellow perch along the Huron-Erie Corridor. *Journal of Great Lakes Research.* In press.

Sullivan, T.J. & Stepien, C.A. (In re-review) Temporal population genetic structure of the yellow perch *Perca flavescens* (Percidae: Teleostei) within a complex lakescape. *Conservation Genetics.*

Svetovidov, A.N. & Dorofeeva, E.A. (1963) Systematics, origin and history of the distribution of the Eurasian and North American perches and pikeperches (genera *Perca*, *Lucioperca,* and *Stizostedion*). *Voprosy Ikhtiologii* **3,** 625–651.

Taberlet, P., Griffin, S., Goossens, B., Questiau, S., Manceau, V., Escaravage, N., Waits, L.P., & Bouvet, J. (1996) Reliable genotyping of samples with very low DNA quantities using PCR. *Nucleic Acids Research* **24,** 3189–3194.

Tallmon, D.A., Luikart, G., & Waples, R.S. (2004) The alluring simplicity and complex reality of genetic rescue. *Trends in Ecology and Evolution* **19,** 489-496.

Tamura, K. & Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution* **10,** 512–526.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011) MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution* **28,** 2731–2739.

Taubmann, J., Theissinger, K., Feldheim, K.A., Laube, I., Graf, W., Haase, P., Johannesen, J., & Pauls, S.U. (2011) Modelling range shifts and assessing genetic diversity distribution of the montane aquatic mayfly *Ameletus inopinatus* in Europe under climate change scenarios. *Conservation Genetics* **12**, 503–515.

Taylor, E.B., Gow, J.L., Witt, J., & Zemlak, R. (2011) Connectivity among populations of pygmy whitefish (*Prosopium coulterii*) in northwestern North America inferred from microsatellite DNA analyses. *Canadian Journal of Zoology* **89**, 255–266.

Tedford, R.H., Skinner, M.F., Fields, R.W., Rensberger, J.M., Whistler, D.P., Galusha, T., Taylor, B.E., Macdonald, J.R., & Webb, S.D. (1987) Faunal succession and the biochronology of the Arikareean through Hemphillian interval (late Oligocene through earliest Pliocene Epochs) in North America. In: Woodburne MO, ed., *Cenozoic Mammals of North America*. Berkeley, CA: University of California Press, 153–210.

Teller, J.T., Leverington, D.W., & Mann, J.D. (2002) Freshwater outbursts to the oceans from glacial Lake Agassiz and their role in climate change during the last deglaciation. *Quaternary Science Reviews* **21,** 879–887.

Thomas, M.V. & Haas, R.C. (1994) Status of yellow perch and walleye in Michigan waters of Lake Erie, 1989-93. MIDNR Fisheries Research Report No. 2011, 1994.

Thomas, M.V. & Towns, G.L. (2011) Summary of creel survey results for the St. Clair River, Lake St. Clair, and the Detroit River, 2002-05. MIDNR Fisheries Research Report No. 2011-1, 2011.

Tiffney, B.H. (1985) The Eocene North Atlantic Land Bridge: Its importance in Tertiary and modern phytogeography of the Northern Hemisphere. *Journal of the Arnold Arboretum* **66,** 243–273.

Tiffney, B.H. & Manchester, S.R. (2001) The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Sciences* **162,** S3–S17.

Todd, T.N. & Haas, R.C. (1993) Genetic and tagging evidence for movement of walleyes between Lake Erie and Lake St. Clair. *Journal of Great Lakes Research* **19,** 445–452.

Trautman, M.B. (1981) *The Fishes of Ohio.* Columbus, OH: Ohio State University Press.

Ugelvig, L.V., Nielsen, P.S., Boomsma, J.J., & Nash, D.R. (2011) Reconstructing eight decades of genetic variation in an isolated Danish population of the large blue butterfly *Maculinea arion. BMC Evolutionary Biology* **11,** 201.

Unmack, P.J., Hammer, M.P., Adams, M., & Dowling, T.E. (2011) A phylogenetic analysis of pygmy perches (Teleostei: Percichthyidae) with an assessment of the major historical influences on aquatic biogeography in southern Australia. *Systematic Biology* **60,** 797–812.

USFWS/GLFC (United States Fish and Wildlife Service/Great Lakes Fishery Commission) (2010) Great Lakes Fish Stocking database. U.S. Fish and Wildlife Service, Region 3 Fisheries Program, and Great Lakes Fishery Commission. Available at http://www.glfc.org/fishstocking/ (Accessed on 8 August 2012).

USGS (United States Geological Survey) (2010) Fish habitat enhancement strategies for the Huron-Erie Corridor. Great Lakes Restoration Initiative. Available at http://cida.usgs.gov/glri/projects/habitat_and_wildlife/fish_habitat_HEC.html (Accessed on 8 August 2012)

Utter, F., Milner, G., Stahl, G., & Teel, D. (1989) Genetic population structure of chinook salmon, *Oncorhynchus tshawytscha*, in the Pacific Northwest. *Fishery Bulletin* NOAA **87,** 239-264.

van Oosterhout, C., Hutchinson, W.F., Wills, D.P.M, & Shipley P. (2004) Micro–checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* **4**, 535–538. Available at http://www.microchecker.hull.ac.uk/ (Accessed on 26 June 2013)

Väli, U., Einarsson, A., Waits, L., & Ellegren, H. (2008) To what extent do microsatellite markers reflect genome–wide genetic diversity in natural populations? *Molecular Ecology* **17**, 3808–3817.

Vandergoot, C.S., Cook, H.A., Thomas, M.V., Einhouse, D.W., & Murray, C. (2010) Status of walleye in western Lake Erie, 1985-2006, In: Status of walleye in the Great Lakes: Proceedings of the 2006 symposium (ed. by E. Roseman, P. Kocovsky, & C. Vandergoot), pp. 123-150. Great Lakes Fishery Commission Technical Report, Ann Arbor, MI.

Viola, G., Mancktelow, N.S., & Seward, D. (2001) Late Oligocene–Neogene evolution of Europe–Adria collision: New structural and geochronological evidence from the Giudicarie fault system (Italian Eastern Alps). *Tectonics* **20,** 999–1020.

Walter, R.P., Cena, C.J., Morgan, G.E., & Heath, D.D. (2012) Historical and anthropogenic factors affecting the population genetic structure of Ontario's inland lake populations of walleye (*Sander vitreus*). *Journal of Heredity* **103,** 831-841.

Wang, H.Y., Rutherford, E.S., Cook, H.A., Einhouse, D.W., Haas, R.C., Johnson, T.B., Kenyon, R., Locke, B., & Turner, M.W. (2007) Movement of walleye in Lakes Erie and St. Clair inferred from tag return and fisheries data. *Transactions of the American Fisheries Society* **136,** 539–551.

Wang, I.J. (2010) Recognizing the temporal distinctions between landscape genetics and phylogeography. *Molecular Ecology* **19**, 2605–2608.

Wang, I.J. (2011) Choosing appropriate genetic markers and analytical methods for testing landscape genetic hypotheses. *Molecular Ecology* **20**, 2480–2482.

Waples, R.S. (1987) A multispecies approach to the analysis of gene flow in marine shore fishes. *Evolution* **41,** 385–400.

Waples, R.S. (1990) Conservation genetics of pacific salmon. III. Estimating effective population size. *Journal of Heredity* **81,** 277–289.

Waples, R.S. (1995) Evolutionarily significant units and the conservation of biological diversity under the Endangered Species Act, In: Evolution and the Aquatic Ecosystem: Defining unique units in population conservation (ed. by J.L. Nielsen), pp. 8-27. American Fisheries Society Symposium 17, Bethesda, Maryland.

Ward, R.D., Billington, N., & Hebert, P.D.N. (1989) Comparison of allozyme and mitochondrial DNA variation in populations of walleye, *Stizostedion vitreum*. *Canadian Journal of Fisheries and Aquatic Sciences* **46,** 2074–2084.

Weir, B. S. & Cockerham, C. C. (1984) Estimating F–statistics for the analysis of population structure. *Evolution* **38**, 1358–1370.

Wen, J. & Ickert–Bond, S.M. (2009) Evolution of the Madrean–Tethyan disjunctions and the North and South American amphitropical disjunctions in plants. *Journal of Systematics and Evolution* **47,** 331–348.

White, W.B. (2009) The evolution of Appalachian fluviokarst: Competition between stream erosion, cave development, surface denudation, and tectonic uplift. *Journal of Cave and Karst Studies* **71,** 159–167.

White, M.M. (2012) Mitochondrial DNA sequence variation in saugers. *Transactions of the American Fisheries Society* **141,** 795–801.

Wicander, R. & Monroe, J.S. (1993) *Historical Geology; Evolution of the Earth and Life through Time.* 2nd ed. St. Paul, MN: West Publishing Company.

Wiley, E.O., Johnson, G.D., & Dimmick, W.W. (2000) The interrelationships of Acanthomorph fishes: A total evidence approach using molecular and morphological data. *Biochemical Systematics and Ecology* **28,** 319–350.

Williams, J.E., Haak, A.L., Neville, H.M., & Colyer, W.T. (2009) Potential consequences of climate change to persistence of cutthroat trout populations. *North American Journal of Fisheries Management* **29,** 533–548.

Williamson, K.S. & May, B. (2005) Homogenization of fall-run Chinook salmon gene pools in the Central Valley of California, USA. *North American Journal of Fisheries Management* **25,** 993-1009.

Willis, S.C., López–Fernández, H., Montaña, C.G., Farias, I.P., & Ortí, G. (2012) Species–level phylogeny of 'Satan's perches' based on discordant gene trees (Teleostei: Cichlidae: *Satanoperca* Günther 1862). *Molecular Phylogenetics and Evolution* **63,** 798–808.

Wilson, R.L. (1968) Systematics and faunal analysis of a lower Pliocene vertebrate assemblage from Trego County, Kans. *Contributions from the Museum of Paleontology University of Michigan* **22,** 75–126.

Wilson, C.C., Lavender, M., & Black, J. (2007) Genetic assessment of walleye (*Sander vitreus*) restoration efforts and options in Nipigon Bay and Black Bay, Lake Superior. *Journal of Great Lakes Research* **33,** 133-144.

Wirth, T., Saint-Laurent, R., & Bernatchez, L. (1999) Isolation and characterization of microsatellite loci in the walleye (*Stizostedion vitreum*), and cross–species amplification within the family Percidae. *Molecular Ecology* **8**, 1960–1962.

Wofford, J.E.B., Gresswell, R.E., & Banks, M.A. (2005) Influence of barriers to movement on within–watershed genetic variation of coastal cutthroat trout. *Ecological Applications* **15**, 628–637.

Wolfe, J.A. (1994) Tertiary climate changes at middle latitudes of western North America. *Palaeogeography Palaeoclimatology Palaeoecology* **108,** 195–205.

Wolfe, J.A., Forest, C.E., & Molnar, P. (1998) Paleobotanical evidence of Eocene and Oligocene paleoaltitudes in midlatitude western North America. *GSA Bulletin* **110,** 664–678.

Wolfert, D.R. (1963) The movements of walleyes tagged as yearlings from the eastern and western basins of Lake Erie. *Transactions of the American Fisheries Society* **92,** 414-420.

Wright, J.J., David, S.R., & Near, T.J. (2012) Gene trees, species trees, and morphology converge on a similar phylogeny of living gars (Actinopterygii: Holostei: Lepisosteidae), an ancient clade of ray–finned fishes. *Molecular Phylogenetics and Evolution* **63,** 848–856.

WTG (Walleye Task Group of the Lake Erie Committee, Great Lakes Fishery Commission) (2005) Report for 2004 by the Lake Erie Walleye Task Group. Great Lakes Fishery Commission Ann Arbor, Michigan. Available at http://www.glfc.org/lakecom/lec/WTG_docs/annual_reports/WTG_report_2005.pdf (Accessed on 8 August 2012).

WTG (Walleye Task Group of the Lake Erie Committee, Great Lakes Fishery Commission) (2013) Report for 2012 by the Lake Erie Walleye Task Group. Great Lakes Fishery Commission Ann Arbor, Michigan. Available at http://www.glfc.org/lakecom/lec/WTG_docs/annual_reports/WTG_report_2013.pdf (Accessed on 26 June 2013).

Yokoyama, R. & Goto, A. (2005) Evolutionary history of freshwater sculpins, genus *Cottus* (Teleostei; Cottidae) and related taxa, as inferred from mitochondrial DNA phylogeny. *Molecular Phylogenetics and Evolution* **36,** 654–668.

Yu, C., Ferraro, D., Ramaswamy, S., Schmitz, M.H., Schaefer, W.F., & Gibson, D.T. (2008) Purification and properties of Sandercyanin, a blue protein secreted in the mucus of blue forms of walleye, *Sander vitreus*. *Environmental Biology of Fishes* **82**, 51–58.

Zachos, J., Pagani, M., Sloan, L., Thomas, E., & Billups, K. (2001) Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292,** 686–693.

Zar, J. H. (1999) *Biostatistical Analysis*, 4th edition. Prentice Hall, Inc., Upper Saddle River.

Zhao, Y., Jones, M.L., Shuter, B.J., & Roseman, E.F. (2009) A biophysical model of Lake Erie walleye (*Sander vitreus*) explains interannual variations in recruitment. *Canadian Journal of Fisheries and Aquatic Sciences* **66,** 114-125.

Zhu, D., Jamieson, B.G.M., Hugall, A., & Moritz, C. (1994) Sequence evolution and phylogenetic signal in control–region and cytochrome *b* sequences of rainbow fishes (Melanotaeniidae). *Molecular Biology and Evolution* **11,** 672–683.