Journal of Great Lakes Research xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Great Lakes Research

journal homepage: www.elsevier.com/locate/jglr

Genetic structure of muskellunge in the Great Lakes region and the effects of supplementation on genetic integrity of wild populations

Keith N. Turnquist [a], Wesley A. Larson [b,*], John M. Farrell [c], Patrick A. Hanchin [d], Kevin L. Kapuscinski [e], Loren M. Miller [f], Kim T. Scribner [g], Chris C. Wilson [h], Brian L. Sloss [i]

[a] Wisconsin Cooperative Fishery Research Unit, University of Wisconsin-Stevens Point, 800 Reserve St., Stevens Point, WI 54481, United States
[b] U. S. Geological Survey, Wisconsin Cooperative Fishery Research Unit, University of Wisconsin-Stevens Point, 800 Reserve St., Stevens Point, WI 54481, United States
[c] State University of New York, College of Environmental Science and Forestry, 250 Illick Hall, 1 Forestry Drive, Syracuse, NY 13212, United States
[d] Michigan Department of Natural Resources, 96 Grant Street, Charlevoix, MI 49720, United States
[e] School of Biological Sciences, Lake Superior State University, 650 W. Easterday Avenue Sault Ste. Marie, MI 49783, United States
[f] Minnesota Department of Natural Resources, University of Minnesota, 135 Skok Hall, 2003 Upper Buford Circle, St. Paul, MN 55108, United States
[g] Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824, United States
[h] Ontario Ministry of Natural Resources and Forestry, Trent University, Peterborough, ON K9J 7B8, Canada
[i] College of Natural Resources, University of Wisconsin-Stevens Point, 800 Reserve St., Stevens Point, WI 54481, United States

ARTICLE INFO

Article history:
Received 30 December 2016
Accepted 22 August 2017
Available online xxxx

Associate Editor: Stephen Charles Riley

Keywords:
Muskellunge
Genetics
Population structure
Supplementation
Hybridization
Fisheries management

ABSTRACT

Muskellunge (*Esox masquinongy*) are important apex predators that support numerous recreational fisheries throughout the Great Lakes region. Declines in muskellunge abundance from historical overharvest and environmental degradation have threatened the viability of many populations and prompted significant restoration efforts that often include stocking. The goal of our study was to investigate contemporary population structure and genetic diversity in 42 populations of muskellunge sampled across the Great Lakes region to inform future management and supplementation practices. We genotyped 1896 muskellunge ($N = 10$–123/population) at 13 microsatellite loci. The greatest genetic variation was between populations of Great Lakes origin and populations of Northern (inland) origin, with both groups also exhibiting significant substructure (overall $F_{ST} = 0.23$). Genetic structure was generally correlated with geography; however, we only found marginal evidence of isolation by distance, likely due to high genetic differentiation among proximate populations. Measures of genetic diversity were moderate across most populations, but some populations displayed low diversity consistent with small population sizes or historical bottlenecks. Many of the populations studied displayed evidence of historic introductions and supplemental stocking, including the presence of individuals with primarily non-native ancestry as well as interlineage hybrids. Our results suggest that the historic population structure of muskellunge is largely intact across the Great Lakes region, but also that stocking practices have altered this structure to some degree. We suggest that future supplementation practices use local sources where possible, and incorporate genetic tools including broodstock screening to ensure that non-native muskellunge are not used to supplement wild populations.

Published by Elsevier B.V. on behalf of International Association for Great Lakes Research.

Introduction

The muskellunge *Esox masquinongy* is an ecologically important apex predator that supports numerous recreational fisheries throughout the Great Lakes region (Crane et al., 2015; Margenau and Petchenik, 2004). Several lineages of muskellunge have been described in this region including the Great Lakes lineage, which is hypothesized to be found in the five Great Lakes and the St. Lawrence River, and the Northern lineage, which is hypothesized to be found in the Mississippi River, its tributaries, and other inland waters (Becker, 1983; Koppelman and Philipp, 1986). Declines in muskellunge abundance due to overharvest and environmental degradation have threatened the viability of many populations, prompting significant restoration efforts that often include stocking (Crossman, 1978; Farrell et al., 2007; Whillans, 1979).

Muskellunge populations were historically abundant in near-shore areas and tributaries across the Great Lakes region and even supported limited commercial fisheries (Schrouder, 1973; Smith and Snell, 1891). During the early to mid-1900s, many populations were adversely

---

* Corresponding author.

E-mail addresses: keith.turnquist@uwsp.edu (K.N. Turnquist), wes.larson@uwsp.edu (W.A. Larson), jmfarrell@esf.edu (J.M. Farrell), hanchinp@michigan.gov (P.A. Hanchin), kkapuscinski@lssu.edu (K.L. Kapuscinski), lmm@umn.edu (L.M. Miller), scribne3@msu.edu (K.T. Scribner), chris.wilson@ontario.ca (C.C. Wilson), brian.sloss@uwsp.edu (B.L. Sloss).

https://doi.org/10.1016/j.jglr.2017.09.005
0380-1330/Published by Elsevier B.V. on behalf of International Association for Great Lakes Research.

Please cite this article as: Turnquist, K.N., et al., Genetic structure of muskellunge in the Great Lakes region and the effects of supplementation on genetic integrity of wild populat..., J. Great Lakes Res. (2017), https://doi.org/10.1016/j.jglr.2017.09.005

affected and some were completely extirpated by habitat destruction, pollution, and over-exploitation (Farrell et al., 2003; Graff, 1986). Over the past 50 years, habitat conditions have improved in many locations and regulations have limited exploitation to sustainable levels, but muskellunge populations have generally been unable to re-establish themselves through natural dispersal (Crane et al., 2015). Strong spawning site fidelity by muskellunge has likely prevented recolonization of areas where populations were extirpated (Crossman, 1990; Diana et al., 2015; Jennings et al., 2011). Thus, restoration efforts have utilized stocking as the primary tool for rehabilitation (Jennings et al., 2010; Kapuscinski et al., 2007). However, in most cases, muskellunge were stocked without an understanding of existing genetic structure, potentially resulting in interbreeding between native and stocked fish. Although the fitness effects on these populations is not known, the disruption of native gene pools by supplemental stocking has been shown to have negative effects from outbreeding depression elsewhere (McClure et al., 2008).

Stocking of muskellunge has occurred in the Great Lakes region for over 80 years (Kerr and Lasenby, 2001). Historical stocking was often conducted without considering the genetic consequences associated with mixing fish of different ancestry. For example, many tributaries in inland Michigan that connect to the Great Lakes were historically stocked with muskellunge from the non-native Northern lineage, rather than fish from the native Great Lakes lineage (Scribner et al., 2015). This stocking history has created population admixture and eroded the natural genetic structure that previously existed in muskellunge from inland Michigan (Scribner et al., 2015). Additionally, stocking of non-native lineages has facilitated interlineage hybridization (Scribner et al., 2015). In some cases, however, stocking has been a highly successful tool for population restoration. The current world-class muskellunge fishery in Green Bay, Wisconsin, was restored over the course of the past 25 years with fish sourced from Lake St. Clair and the Indian River Chain (Michigan) (Kapuscinski et al., 2007). Although this population does not yet produce enough natural recruits to sustain itself, the economic impact and popularity of the fishery is substantial.

Currently, numerous management prescriptions in Great Lakes states and provinces call for stocking native muskellunge to re-establish sustainable populations (Crane et al., 2015). Accordingly, Great Lakes muskellunge brood sources have been or are in the process of being established to meet that need. Managers seeking to identify and develop future muskellunge brood stocks would benefit from knowledge of stock structure to better match genotypes of hatchery fish to the locations in which they will be stocked. If broodstock sources are better matched to the environments where they are being stocked, greater numbers of hatchery fish should survive and grow to sexual maturity, thereby increasing levels of natural recruitment in habitats at or near release sites; in essence, maximizing the probability of successful restoration.

Numerous studies have been undertaken to characterize spatial genetic structure among muskellunge populations at local geographic scales in the Great Lakes region (e.g., Kapuscinski et al., 2013; Miller et al., 2012; Scribner et al., 2015; Wilson et al., 2016). These studies have generally demonstrated high genetic differentiation among populations, even at relatively small geographic scales. For example, Kapuscinski et al. (2013) found nine genetically distinct groups of muskellunge across the St. Lawrence River, Lake Ontario, Lake Huron, and Lake Michigan, including two groups that were separated by <50 km. However, no previous studies have concurrently analyzed Great Lakes and Northern lineage muskellunge from a large portion of the Great Lakes region.

The goal of our study was to investigate contemporary spatial population structure and genetic diversity in 42 populations of muskellunge sampled across Great Lakes spawning aggregates, associated Great Lakes tributaries, and adjacent inland populations to inform future management and supplementation practices. Our objectives were to: (1) build on previous studies to determine if significant genetic structure exists among muskellunge spawning aggregates across the Great Lakes region; (2) determine if significant admixture is present in Great Lakes populations consistent with introgression between stocked and native muskellunge; and (3) guide supplementation efforts by identifying broad-scale genetic groups and determining which populations included in our study are unsuitable broodstock sources. This study represents the most comprehensive investigation of muskellunge population structure to date and should provide a useful resource for future management efforts.

Methods

Tissue sampling

In total, 1876 genetic samples from 42 populations of muskellunge distributed across the Great Lakes, associated tributaries, and neighboring major drainages were included in this study (Fig. 1; Tables 1, S1). These samples were obtained from a combination of previous genetic studies (Kapuscinski et al., 2013; Miller et al., 2012; Scribner et al., 2015; Spude, 2010), existing collections from state management agencies and academic institutions, and directed field surveys. Muskellunge were captured by electrofishing, trap-netting, and angling in spawning aggregates at or near the time of spawning from 1991 to 2014. Tissue samples from each fish were collected as scales, spines, or fin clips, and were either dried in individually labeled coin envelopes or placed in vials containing non-denatured 95% ethanol. Attempts were made to collect a minimum of 50 tissue samples from each location, although this target sample size was not obtained in some locations due to low population densities and infrequent historical sampling. Nevertheless, simulations based on subsampling multiple datasets suggest that sample sizes of 25 to 30 per population are sufficient to accurately estimate allele frequencies from typical microsatellite data and sample sizes as low as 10 still provide relatively narrow confidence intervals for allele frequency estimates (Hale et al., 2012).

Laboratory analysis

Molecular analysis for 40 of the 42 populations included in this study was conducted at the Molecular Conservation Genetics Laboratory, College of Natural Resources, University of Wisconsin–Stevens Point using methods identical to Kapuscinski et al. (2013). Genotype data from 16 of these populations was obtained by Kapuscinski et al. (2013) and data from 24 populations was produced as part of the current study. For each population, genomic DNA was extracted from tissue samples using Promega Wizard Genomic DNA purification kit (Promega, Madison, Wisconsin) and DNA concentrations were normalized to 20 ng/μL prior to genotyping. Each individual was then genotyped at the 14 microsatellites described in Sloss et al. (2008), see Table 2 for locus information). Molecular analysis of samples from two populations collected in Minnesota (Leech Lake and the St. Louis River) was conducted at the AquaGen Laboratory, University of Minnesota according to Miller et al. (2012), and data were standardized between the two facilities by comparing the genotypes of individuals analyzed in both labs (cf., Seeb et al., 2007).

Quality control and summary statistics

Basic summary statistics were calculated for each locus and population to estimate genetic diversity indices and ensure that the loci incorporated in this study were not physically linked and did not violate Hardy-Weinberg assumptions. Exact tests for deviations from Hardy-Weinberg and linkage equilibrium were conducted in the program GENEPOP 4 (Rousset, 2008, alpha = 0.05). Diversity indices including the number of alleles at each locus, mean number of alleles per population (A), and observed and expected heterozygosity (H_O and H_E) were calculated in GenAlEx 6.5 (Peakall and Smouse, 2012). Allelic richness

Please cite this article as: Turnquist, K.N., et al., Genetic structure of muskellunge in the Great Lakes region and the effects of supplementation on genetic integrity of wild populat..., J. Great Lakes Res. (2017), https://doi.org/10.1016/j.jglr.2017.09.005