Assessing the Contributions of Two Stocked Strains on a Minnesota-Wisconsin Boarder Water

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Stocking has long been used as a management tool in the United States even though many of its effects remain cryptic. Past comparisons between stocked strains were generally based on the survival and growth rates of physically marked individuals. More recently the use of genetic markers to assess contributions of stocked individuals has been employed by fisheries managers. Using 13 microsatellite markers we assessed the contributions of two muskellunge (*Esox masquinongy*) strains (Wisconsin and Leech) stocked into the St. Louis River Estuary. Ancestry in the adult samples remained relatively constant with differences only observed between the 2008 and 2013 samples. Samples for young of year individuals told a different story with no significant differences observed between any sample years. Due to these differences more information from additional sample years will be needed to determine if we are really witnessing a change in the genetics of fish in the system. Regardless, both strains appear to be contributing to a healthy muskellunge population; the St. Louis River Estuary shows us one of the benefits of interstate management.

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Introduction

Stocking has long been used as a management tool in the United States by fisheries biologists (Halverson 2008). It has been, and still is, used in regions with a natural abundance of the stocked species (Welcomme and Bartley 1998). The exact contributions of different strains of stocked fish remain cryptic in many systems where it is implemented (Schramm and Piper 1995, Nickum et al. 2005). In the past, comparisons between the success of different stocked strains were generally based on the survival and growth rates of physically marked individuals during recapture events (Crozier et al. 1997, Bronte et al. 2007). Over the years the use of genetic markers has gained prevalence in the fisheries community when assessing stocked strains; particularly in situations where it is not feasible/efficient to physically mark stocked fish (Eldridge et al. 2002). Genetic markers also allow for analysis of the reproductive contributions of different strains (DeKoning et al. 2006). The analysis of reproductive contributions has been conducted on several populations of muskellunge (*Esox masquinongy*) throughout the United States (Miller et al. 2012).

The stocking of muskellunge remains a popular management tool and coupled with other management practices (i.e., harvest restrictions, catch and release, etc.) has been used to establish successful fisheries (Margenau 1999, Kerr 2007, Wingate and Younk 2007). Minnesota and Wisconsin continue to have premier muskellunge fisheries through the implementation of these tools to both maintain and expand muskellunge populations (Simonson and Hewett 1999, Wingate and Younk 2007).

The St. Louis River is the largest United States tributary to Lake Superior. The St. Louis River Estuary is a 4,856 ha body of water situated between the cities of Duluth, Minnesota and Superior, Wisconsin. Like many border waters the St. Louis River is managed jointly by two separate state agencies, the Minnesota Department of Natural Resources (MNDNR) and the Wisconsin Department of Natural Resources (WDNR). From 1983-2005 the estuary was stocked with muskellunge fry, fingerlings, and yearlings from Wisconsin (Chippewa and Wisconsin Rivers) and Leech Lake (Table 1, Table 2). Past stocking by these agencies has contributed to the unique genetic makeup of the population.

Objectives- Prior muskellunge research has shown that the observable genetic makeup of a population can be attained and categorized using samples from a source population (Miller et al. 2009, Miller et al. 2012). This study will (1) determine the current ancestry of the muskellunge population in the

St. Louis River Estuary based on sampling conducted in 2013, (2) estimate the change in the genetic makeup of the population from prior sampling conducted in 2007 and 2008, and (3) determine the genetic makeup of young of year (YOY) muskellunge based on four samples collected from 2008-2013.

Table 1- Muskellunge stocked in the St. Louis River Estuary, MN by the Minnesota Department of Natural Resources (MNDNR). Included are year, size (FLG = fingerling), number and strain (WIS = Wisconsin River, LCH = Leech Lake, LCB = Leech Lake Brood).

Year	Size	Number	Strain
1986	FGL	800	WIS
1989	FGL	346	LCH
1991	FGL	1608	LCH
1991	FGL	3394	LLB
1992	FGL	5000	LLB
1994	FGL	5000	LLB
1997	FGL	5500	LLB
2000	FGL	5000	LLB
2001	FGL	5000	LLB
2003	FGL	5001	LLB
2005	FGL	5005	LLB

Methods

Sample Collection- Scales processed during the course of the study were collected by MNDNR biologists from fish captured during the 2013 spring assessment of muskellunge spawning in the St. Louis River Estuary using modified Fyke nets with 152 x 183 cm frame and 30.5 m leads. Young of year muskellunge from all years were obtained via electrofishing though fin clips were collected due to size. Procedures for the capture and genotyping of adult muskellunge from the 2007 and 2008 samples can be found in Miller et al. (2012). These include all source samples from Leech Lake (Leech-strain source) and Tomahawk Lake (Wisconsin-strain source). Scale availability and budget determined sample sizes for the study. Though sample sizes vary for both adult (2007 n = 45, 2008 n = 102, 2013 n = 87) and YOY (2008 n = 17, 2010 n = 16, 2011 n = 16, 2013 n = 4) muskellunge comparisons can be made based on ancestry proportions (q) among sample years.

Genotyping- All 87 scales and 53 fin clips genotyped during the study were prepared the following way. A 5% (weight/volume) solution of chelating resin (Chelex, Sigma Chemical, St. Louis, Missouri) was prepared. Scales were then cut in half (due to size) and small sections of fin clips were taken and placed in a 1.5 mL tube with 250 µL of the solution. Samples were placed in a 56 °C water bath overnight and boiled for eight minutes before preparation for polymerase chain reaction (PCR). The 14 loci described Sloss et al. (2008) were used with the following changes. The microsatellite locus EmaA5 was not use in order for the remaining loci to combine together in a single electrophoresis run as described in Miller et al. (2012). The primer EmaD126 was use in place of EmaD4 as described in Miller et al. (2009). PCR preparation was done in accordance with Miller et al (2009) with microsatellite amplification performed in 15 µL reactions containing 1 × polymerase buffer (10 mM tris-HCl, 50 mM KCl, 0.1% Triton X-100), 1.5 mM MgCl2, 0.2 mM of each deoxynucleotide triphosphate, 0.5 µM of the forward and reverse primers, and 0.5 units of Taq DNA polymerase (Promega, Madison, Wisconsin). A water blank was included in each set of samples to detect possible contamination of PCR solutions. A thermocycler (Hybaid Omn-E; Thermo-Hybaid U.S., Franklin, Massachusetts) was then used to carry out the amplification process. For each PCR plate 35 cycles were ran at the following temperature profile: 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min, followed by a 20 min extension at 72 °C. PCR plates were then screened via gel electrophoresis for success by randomly picking wells to test before pooling. Plates were then pooled into a single plate for analysis in the following amounts: 2 µL of EmaA10/EmaD12a, 2 µL of EmaC1/EmaD126, 4 µL of EmaA11/EmaB110, 3 µL of EmaD5/D116, 4 µL of EmaD6, 2 µL of EmaA102, 2 µL of EmaA104, 2 µL of EmaD114, and 2 µL of EmaB120. The pooled plate was then submitted to the University of Minnesota Genomics Center (St. Paul, MN) for electrophoresis on an ABI Prism 3130xl genetic analyzer (Applied Biosystems, Foster City, California). The core facility then returned data files and the program GENEMAPPER V4.1 (Applied Biosystems) was used to score alleles.

Population Ancestry- To determine the number of genetically distinct populations within our samples the Bayesian clustering algorithm program STRUCTURE (version 2.3.4; Pritchard et al. 2000; also refer to: pritch.bsd.uchicago.edu) was used. In order to determine unique populations (K) within our data set we ran five independent replicates for each K. Replicates were ran from K = 1(indicating a single population) to K = 6 (greater than the known number of populations stocked in the St. Louis River Estuary). The burn-in period for each replicate was 50,000 replications. This was followed by 250,000 Markov-chain Monte Carlo simulations. The simulations were run under a model that assumed admixture and correlated allele frequencies. Following this, STRUCTURE HARVESTER (version 0.6.94; also refer to: taylor0.biology.ucla.edu/struct_harvest/) was used to estimate K using the Evanno method (Dent and vonHoldt 2012). For each value of K the mean and standard deviation of likelihood estimates were used (Pr[X][K]=the posterior probability of the data given K populations). Tempered with the knowledge of stocking history on the St. Lewis River Estuary, we were able to determine the most likely value of K.

Table 2- Muskellunge stocked in the St. Louis River Estuary, WI by the Wisconsin Department of Natural Resources (WDNR). Included are year, size (N/A=no sizes were available), number, and strain (WIS = Wisconsin River).

Year	Size	Number	Strain
1983	N/A	500	WIS
1984	N/A	500	WIS
1986	N/A	2000	WIS
1987	N/A	3039	WIS*
1988	N/A	2500	WIS
1989	N/A	5000	WIS
1990	N/A	5000	WIS
1991	N/A	4658	WIS
1992	N/A	2500	WIS
1993	N/A	2500	WIS*
1997	N/A	2500	WIS
2000	N/A	2500	WIS
2001	N/A	3500	WIS
2002	N/A	2500	WIS
2004	N/A	2500	WIS

*May have included fish from the Chippewa River, WI.

Individual Ancestry- For each given K STRUCTURE runs algorithms to estimate the proportion of ancestry both to compare individuals and ancestry among populations by averaging q among individuals within each sample year. One of five replicates from the most likely K was used to evaluate the ancestry of individual fish within the samples. Due to the fact that STRUCTURE typically produces nonzero estimates for ancestry from all populations, we assigned ancestry based on a 90% rule. This was determined by calculating the greatest percentage at which known source population samples would assign to their respective population with <10% error.

A Shapiro Wilks normality test was run to determine if the data was normally distributed (Zar 1999). These finding were confirmed by constructing Q-Q plots for adult and YOY muskellunge in Program R (http://www.R-project.org/). Due to the data's non-normal distribution a Kruskal-Wallis test was used to test for differences in ancestry proportion (q) among years for both adults and YOY individually. As needed a multiple comparison test was conducted between each of the years (Siegel and Castellan 1988). Post hoc multiple comparison tests were run in Program R using the package pgirmess (http://CRAN.R-project.org/package=pgirmess) and the kruskalmc function (Giraudoux 2014).

Results

Number of Observed Populations- Based on STRUCTURE output tempered with the knowledge of muskellunge stocking into the St. Louis River Estuary, the conclusion was made that two distinct populations contributed to the ancestry of the sample. When run through STRUCTURE HAVESTER for five repeated simulations with values of K ranging from 1-6, the highest average likelihood estimate (logePr[X|K]) was observed at K=6 (Figure 1). Examination of the data revealed a dramatic increase in likelihood from K=1 to K=2. It is suggested by Pritchard et al. (2010) that the point of this inflection is the true K of the population. Coupled with the higher standard deviations observed for each K after K=2 it was deemed the most likely fit. Though a native strain of muskellunge may have existed in the estuary at some point they appear to have been extirpated prior to any genetic analysis.

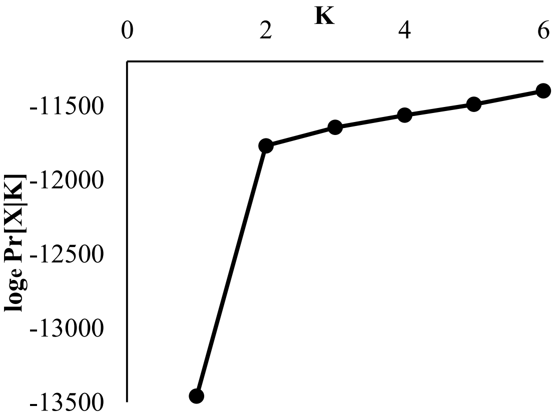

Figure 1- Mean (±SD) of the posterior probability of the data given K clusters (log$_e$Pr[X|K]), across five replicate simulations with K-values of 1-6, for muskellunge from the St. Louis River Estuary captured between 2007 and 2013.

The number of populations identified by STRUCTURE HARVESTER was compared with ancestry proportion (q) output for source populations from STRUCTURE. Source populations were obtained from Leech Lake and Tomahawk Lake (a genetically similar population of fish obtained from the Wisconsin and Chippewa Rivers). Hereafter q values will be subscripted based upon source populations (i.e., Wisconsin-strain ancestry will denoted as q_W). Individuals from Tomahawk Lake showed strong affinity with $q_W \geq 90\%$ for all but two individuals that had q_W values of 0.85 and 0.84. Leech Lake individuals showed slightly lower affinity with 91% of individuals having $q_L \geq 90\%$. The remaining 9% of individuals had q_L values between 0.72 and 0.85.

Ancestry by Sample- The genetic ancestry for all sample years was calculated with YOY muskellunge combined into a single run. Our earliest sample obtained in 2007 comprised 53% Leech-strain ancestry with the remaining fish belonging to the Wisconsin-strain. The 2008 sample saw an increase in Leech-strain ancestry to 60% with the remaining 40% belonging to the Wisconsin-strain. Our most recent sample from 2013 contained 44% Leech-strain ancestry and 56% Wisconsin-Strain. Overall the YOY sampled from 2008-2013 were composed of 82% Leech-strain ancestry and 18% Wisconsin-strain (Figure 2).

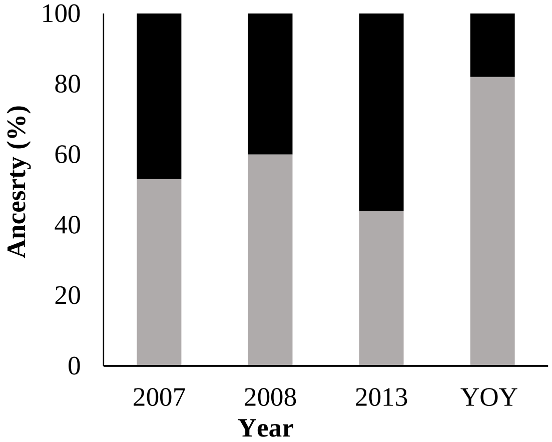

Figure 2- Mean ancestry of muskellunge from the St. Louis River Estuary, for samples from three assessments from 2007 to 2013. Also included are young of year (YOY) individuals from four samples taken between 2008 and 2013. Black denotes Wisconsin-strain ancestry and grey denotes Leech-strain ancestry.

Individual Ancestry- Results of the Kruskal-Wallis rank sum test showed that variation existed between our sample years (chi-squared = 7.92, df = 2, p = 0.02). After a multiple comparison test was conducted no significant difference in ancestry proportion (q) was observed in comparisons of adult muskellunge in the 2007 and 2008 samples or the 2007 and 2013 samples years ($p > 0.05$). Significant variation was observed when comparing the muskellunge from the 2008 and 2013 samples ($p < 0.05$). Muskellunge with Leech-strain ancestry increased from 49% in the 2007 sample year to 51% in the 2008 sample year before decreasing to 31% in the most recent sample. Inversely the Wisconsin-strain decreased 11% from 2007 to 2008 then increased 14% between the 2008 and 2013 samples. Admixed individuals increased throughout the sample years with 9% Wisconsin-strain x Leech-strain ancestry observed in 2007 and 23% observed in 2013. Throughout all three sample years q_W and q_L values ranged from 0.01 to 0.99 (Table 3, Figure 3).

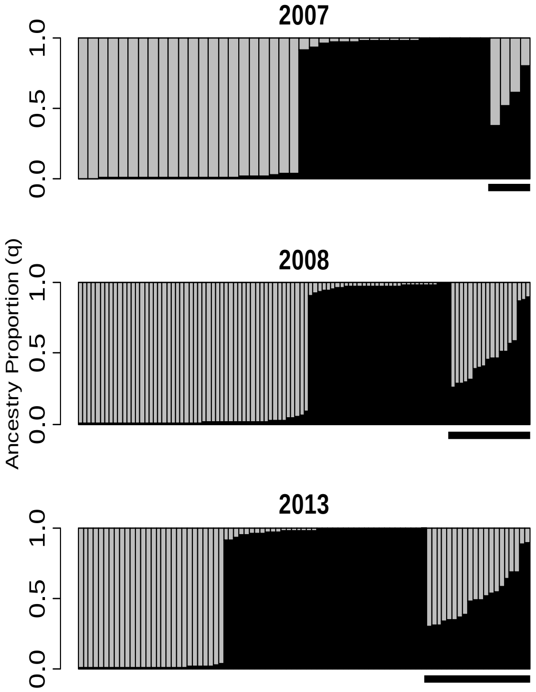

Figure 3- Individual ancestry proportion (q) of adult muskellunge in the St. Louis River Estuary in samples from three assessments conducted between 2007 to 2013. Individuals are represented vertically and ordered based on Leech source ancestry to show changes over time. Admixed individuals were put at the end and a black bar was placed under them to show their increase over time. Shading represents the q assigned to each ancestral source with Wisconsin represented as black and Leech source ancestry represented in grey.

The result of the Kruskal-Wallis rank sum test showed no variation between samples years of YOY individuals (chi-squared = 4.39, df = 3, p = 0.22). The 2008 sample contained 69% Leech-strain ancestry with the remaining 31% ancestry attributed to Wisconsin-strain x Leech-strain crosses. Values of q_W ranged from 0.01 to 0.88. In 2010 Leech-strain ancestry increased to 75% and the remaining 25% is attributed to admixed individuals, with q_W values between 0.01 and 0.40. No pure Wisconsin-strain was found in YOY samples from 2008 or 2010 (Figure 4). The 2011 sample contained 68% Leech-strain ancestry, 13% Wisconsin-strain ancestry, and 19% Wisconsin-strain x Leech-strain ancestry. Values of q_W (range = 0.01-0.98) and q_L (range = 0.02-0.99) varied greatest in this year. In the most recent sample (2013), YOY fish returned to 75% Leech-strain ancestry with the remaining 25% attributed to admixed individuals. Values of q_W in 2013 ranged from 0.01 to 0.62.

Table 3- Individual ancestry of muskellunge from St. Louis River Estuary, in samples from three assessments from 2007 to 2013. Shown are the number of individuals and the percent of each sample estimated to have pure ancestry from the Leech-strain (L) and Wisconsin-strain (W). Also included are number and percent of admixed individuals (W x L) with ancestors from both sources.

	Sample Year		
Ancestral Source(s)	2007	2008	2013
L	22 (49%)	52 (51%)	28 (32%)
W	19 (42%)	32 (31%)	39 (45%)
W x L	4 (9%)	18 (18%)	20 (23%)

Discussion

Though the difference in muskellunge ancestry proportion (q) for adults sampled from 2008 and 2013 suggests that Wisconsin-strain genetics are increasing that may not be the whole story. Differences in muskellunge ancestry proportion (q) could be attributed to two main things. First, we may still be observing the differences in stocked amounts for the strains in the system. The large amount of Leech-strain ancestry observed in 2007 and 2008 could be due to the fact that the Leech-strain was stocked at almost two times the amount as the Wisconsin-strain from 1992-2005. The second possibility being one of the strains is better suited for the environmental conditions of the St. Louis River Estuary and we are observing differences in recruitment or survival of each strain as we get further away from the last known stocking event. It is more than likely that both are having an effect on the ancestry in the system and now that stocking has been discontinued we may be beginning to observe the natural state of the system.

Though significant differences were observed between adult fish from 2008 and 2013, no significant difference was observed between fish from the 2007 and 2013 samples. This coupled with the fact that no overall increasing trend of the Wisconsin-strain was found throughout the samples provides evidence to suggest that the observed difference could be due to random sampling variation between years. As the system begins to depend solely on natural reproduction we are likely beginning to observe the natural variation of a population with unaided reproduction. The lack of significance in the observed difference between adult samples of Wisconsin-strain individuals is further reinforced by the lack of significant differences in YOY ancestry across years. Though it may be due to the low sample sizes for YOY individuals, the lack of variation between fish when compared to adults (whose ancestry is biased by stocking) points to a Leech-strain advantage for YOY individuals. This is due to the fact that Wisconsin ancestry for YOY individuals is much lower than adults and a majority of these fish are Wisconsin x Leech crosses. This suggests (although in fairly small sample sizes) that Wisconsin-strain fish have lower reproductive fitness when compared to the Leech-strain within the estuary; however, across adult samples Wisconsin-strain individuals appear to have similar (or better) survival after stocking. For this reason more sample years as well as larger sample sizes within each year are needed to determine if the most recent change is the first glimpse at a changing population or just a snapshot of the variation in the genetics of a population. The St. Louis River Estuary offers a unique chance to observe the changing genetics of a muskellunge population after stocking has ceased.

Based on admixed individuals in the adult population both strains are able to successfully reproduce and create offspring that are able to thrive in the St. Louis River Estuary. These admixed individuals show us that not only is natural reproduction occurring in the system but that strains are not just selecting for like individuals. Admixture of different strains of muskellunge has been documented in Minnesota lakes prior to this study (Miller et al. 2012). This observed increase in admixed individuals from the estuary is to be expected as natural reproduction takes the place of stocking fish. Introgression following stocking events has been documented for many species (Hindar et al. 1991).

The persistence of the Wisconsin and Leech-strains after the extirpation of any native muskellunge show the positive effects of stocking fish from within or close to the natural drainage of the system. Fish from within or close to the drainage area of the system

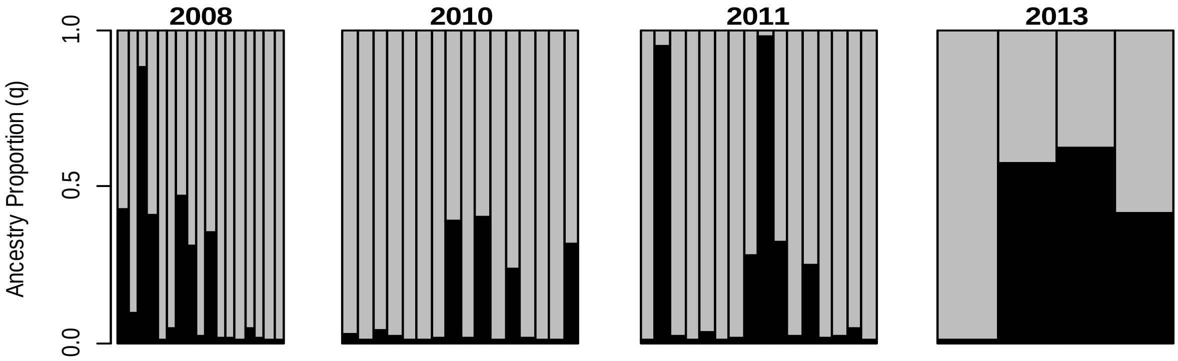

Figure 4- Ancestry Proportion (q) for young of year muskellunge from the St. Louis River Estuary from four samples taken between 2008 and 2013. Black denotes Wisconsin-strain individuals and grey denotes Leech-strain individuals.

are already living under similar environmental conditions making them predisposed as a stock source for species reintroduction. Using fish from the same or similar drainages to stock is an idea that is gaining prevalence in the fisheries community (Cowx 1994, Miller and Kapuscinski 2003).

From a managerial standpoint the St. Louis River Estuary shows us one of the benefits of interstate management. The combined stocking of muskellunge into the estuary allowed for a savings in cost to both agencies as each shared the responsibility. Stocking two separate strains also allowed for an increase in the genetic variation of muskellunge in the system. Both the Leech and Wisconsin strains appear to be contributing to a healthy muskellunge population. This study has shown that the two separate stocked populations cannot only coexist but comingle within an aquatic environment.

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