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MANAGEMENT BRIEF

Using Genetic Data to Estimate Capture Rate of Wisconsin and Leech Lake Strains of Muskellunge Stocked in Four Wisconsin Lakes

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Abstract

Many inland fisheries are supported by stocking hatchery-produced fish, and fisheries managers often face difficult decisions regarding strain selection. Stocking evaluations that are designed to quantify differences in strain performance provide valuable data for designing stocking programs. Here, we used genetic tools to investigate the capture rate of two strains of Muskellunge that were stocked in Wisconsin lakes. We genotyped a total of 1,011 Muskellunge at 13 microsatellites and used the data from five reference populations to assign fish that were stocked in four Wisconsin lakes to their strain of origin. The strains that were stocked in these lakes were derived from Wisconsin populations in the upper Chippewa River and Wisconsin River drainages and from Leech Lake, Minnesota. The Muskellunge from Leech Lake demonstrated much lower capture rates than the Wisconsin strain, but the results were variable, with a 10% capture rate of fish from the Leech Lake strain in Lake Monona and a 2% capture rate in Lake Wissota, despite similar stocking rates (~25%) in both lakes. We hypothesized that the higher capture rates of Muskellunge from the Wisconsin strain could be due the adaptative advantages of the Wisconsin strain in these waters and suggest that managers continue to stock the nearest native (i.e., Wisconsin) strain to achieve the highest return on investment.

Stocking hatchery-produced fish is one of the most common tools employed by fisheries managers to rebuild and sustain inland fisheries (Halverson 2008). One important consideration when building a propagation program is that of choosing appropriate strains that display high survival and growth to maximize the return on stocking investment while also preserving the genetic integrity of

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wild populations (if they are stocked in the same waters; Miller and Kapuscinski 2003; Jennings et al. 2010). Information on the growth and survival of different strains to inform these choices is often provided through stocking evaluations, which generally employ physical tagging (e.g., fin clips, coded wire or passive integrated transponder tags) to track hatchery outplants (e.g., Younk and Strand 1992; McDougall et al. 2014; Wagner et al. 2017; Kornis et al. 2019). However, genetic methods can be used to track different hatchery strains if the strains are sufficiently genetically diverged from each other (e.g., Homola et al. 2019).

In the current study, we investigated the capture rates of two strains of Muskellunge *Esox masquinongy* that were stocked into four lakes in Wisconsin, USA, using genetic analysis. Muskellunge are a prized gamefish in Midwestern North America that are highly sought after by anglers that are motivated to catch large (i.e., trophy) fish (Casselman et al. 1999). Many Muskellunge fisheries in Wisconsin are partially or fully supported by stocking, with most of the stocked fish being produced by state-run hatcheries (Simonson and Hewett 1999). The propagation practices at these hatcheries are guided by conservation genetic principals that include using broodstock from lakes in the upper Wisconsin River and upper Chippewa River drainages that have been found to contain adequate diversity and putatively native genetic ancestry (Jennings et al. 2010; Turnquist et al. 2017). However, the stocking of fish from private sources does occur in a subset of Wisconsin waters to provide Muskellunge fisheries in areas where Muskellunge survive but were not naturally colonized and are not thought to reproduce (Rowe et al. 2017). Funding for private Muskellunge stocking is typically provided by angling clubs, occasionally with the goal of stocking genetic strains with potentially higher growth rates and size potential compared with Wisconsin strains (Gerbyshak et al. 2017).

In addition to growth, survival is a major factor to be considered when choosing strains to stock. Differences in strain-specific survival have been demonstrated in many fish species (e.g., Eldridge et al. 2002; Negus et al. 2012; Kornis et al. 2019), with strains derived from local genetic backgrounds typically outperforming more divergent strains (Miller and Kapuscinski 2003). Differences in survival as a consequence of genetic strain have also been relatively well documented in Muskellunge. Andree et al. (2018) conducted pond experiments and found that strain survival differed between two strains that potentially were adapted to different temperatures. Additionally, Miller et al. (2012) used genetics to investigate the ancestry of multiple stocked populations of Muskellunge in Minnesota lakes and found high variation in strain contributions but no significant relationship between ancestry and stocking rates. Finally, Wagner et al. (2017) investigated the survival rates of two strains that were stocked in Illinois reservoirs and found differences in survival one year after stocking but no differences between one year and two years poststocking and concluded that the best course of action was to stock the nearest native strain.

The primary goal of the current study was to evaluate strain-specific differences in the capture rates of Muskellunge from the Leech Lake and Wisconsin strains that were stocked in four Wisconsin lakes. The four stocked lakes in this study were Lake Monona, a 1,300 ha natural lake that is located in southern Wisconsin, Petenwell and Castle Rock lakes, which are large (>4,000 ha) reservoirs of the Wisconsin River that are located in central Wisconsin, and Lake Wissota, a 2,500 ha reservoir of the Chippewa River in northwestern Wisconsin (Figure 1). All four of these systems show little to no natural recruitment and have historically been classified by the Wisconsin Department of Natural Resources (WDNR) as universal receptor lakes, where stocking of out-of-basin strains is permitted. All of these systems (with the exception of Castle Rock Lake, which is downstream of Petenwell Lake) have been stocked with Muskellunge from the Wisconsin strain (i.e., derived from the upper Chippewa or Wisconsin River populations) by WDNR and with Muskellunge from the Leech Lake strain that was purchased by private angling clubs. The Leech Lake strain, which is derived from Leech Lake, a large (>40,000 ha) lake in northern Minnesota, is revered by anglers because fish from Leech Lake are thought to grow larger than those from the Wisconsin strain. Gerbyshak et al. (2017) investigated the survival of fish from the Leech Lake strain in Lake Wissota and found lower survival compared with those from the Wisconsin strain, and Rowe et al. (2017) investigated the growth of Leech Lake and Wisconsin strains in Lake Monona and found no significant differences between the strains. However, these studies were limited in scope, as they were published as extended abstracts and each only investigated a single lake. In this study, we conducted an evaluation using microsatellite DNA markers to assign fish of unknown origin that were recovered from four stocked lakes to the Wisconsin or Leech Lake strains. These data allowed us to compare recoveries as inferred from genetic data with strain-specific stocking rates to investigate differences between the two strains.

METHODS

Sampling locations.— We obtained tissue samples (either dried fin rays or fin clips that were preserved in >95% ethanol) from nine sample sites (see Table 1 for the sample sizes and Figure 1 for a map of the sampling locations). Five of these sites were used as references to assign fish of unknown origins, and four sites contained fish of unknown origin. The reference sites included Leech Lake

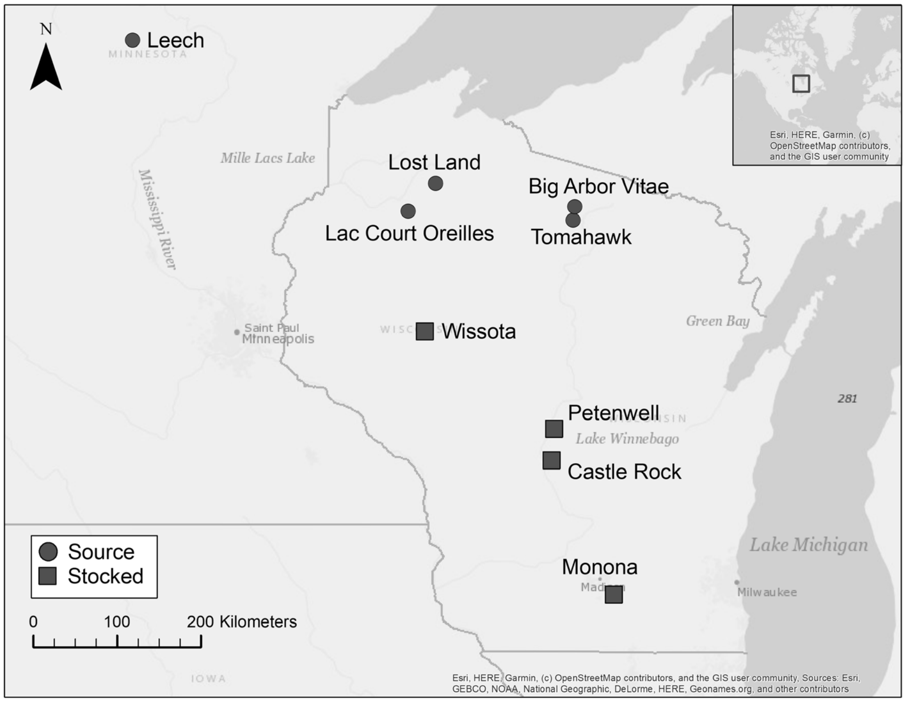

FIGURE 1. Map of sample locations for Muskellunge in Wisconsin and Minnesota. The source sites were used as a reference to assign fish of unknown origin that were stocked in the stocked sites. See Table 1 for more information on these sites.

and four populations from northern Wisconsin that have been shown to be genetically similar (Turnquist et al. 2017), are likely representative of the native genetics in the region (Jennings et al. 2010), and are either used for broodstock collection for Wisconsin hatcheries or are genetically similar to populations that are used for this purpose. The four sites containing fish of unknown origin have each been stocked with Muskellunge from the Wisconsin strain (either the upper Chippewa River or upper Wisconsin River), and three of the four have been stocked with fish from the Leech Lake strain, with the fourth (Castle Rock) found directly downstream of a system that has been stocked with fish from Leech Lake (Table 2). The samples from the reference lakes were collected using fyke nets, electrofishing, and hook and line, and fish from the four stocked lakes were collected during routine WDNR surveys. These routine WDNR surveys target adult fish primarily by using fyke nets in the spring and some electrofishing effort in the fall, and they should provide a representative sample of the fish that are available to anglers. The samples from the reference populations were analyzed by Turnquist et al. (2017), and approximately 50% of the samples that were taken from Lake Wissota were analyzed by Gerbyshak et al. (2017).

Genetic analysis.— All tissue of the samples were genotyped at 13 of the 14 microsatellites that are described in Sloss et al. (2008)—all but D4, which produced inconsistent amplification—using methods similar to those of Turnquist et al. (2017). Genomic DNA was extracted from the tissue samples with either Promega Wizard Genomic DNA purification kits or Qiagen DNeasy Blood and Tissue Kits. The microsatellite loci were amplified with polymerase chain reaction, and the microsatellites were genotyped with an ABI 3730 DNA Analyzer. Individuals that were missing >50% of the genotypes were removed from the subsequent analyses. We then used GenAlEx (Peakall and Smouse 2012) to calculate basic summary statistics including observed and expected heterozygosity (H_{O}, H_{E}), number of alleles (N_{A}), and genetic differentiation (pairwise F_{ST}). Allelic richness (A_{R}), calculated with rarefaction, was estimated in the R package diveRsity (Keenan et al. 2013). There was very little divergence among the reference populations that were sampled from Wisconsin (see the results and Turnquist et al. 2017); therefore, we classified all of the reference populations from Wisconsin in our study as Wisconsin strain rather than separating them into multiple strains.

We used STRUCTURE 2.3.4 (Pritchard et al. 2000) to assign fish of unknown origin from the stocked lakes to either the Leech Lake strain or the Wisconsin strain. First, we ran STRUCTURE on a data set containing only the reference strains for K-values from 1–10, with 10 runs per K, 100,000 burn-in iterations, and 500,000 iterations. We then assessed support for each K with likelihood values and using the ΔK method (Evanno et al. 2005), visualized the likelihood results with Structure Harvester (Earl and

TABLE 1. Information on sample locations for Muskellunge from reference and stocked lakes. Reference populations were used to assign fish from stocked lakes to their strain or origin. The abbreviations are as follows: N is the number of fish that were successfully genotyped, H_O is observed heterozygosity, H_E is expected heterozygosity, N_A is number of alleles, and A_R is the allelic richness calculated using rarefaction. See Figure 1 for a map of the sample locations.

Population	Reference or stocked	N	Watershed	Latitude	Longitude	H_O	H_E	N_A	A_R
Leech	Reference	93	Mississippi River	47.1566	−94.3993	0.43	0.43	4.38	3.97
Lac Courte Oreilles	Reference	41	Chippewa River	45.8942	−91.4388	0.54	0.56	5.23	4.93
Lost Land	Reference	72	Chippewa River	46.0995	−91.1465	0.56	0.55	5.92	5.19
Tomahawk	Reference	28	Wisconsin River	45.8254	−89.6696	0.56	0.56	5.08	5.08
Big Arbor Vitae	Reference	69	Wisconsin River	45.9278	−89.6513	0.57	0.55	5.69	5.01
Wissota	Stocked	328	Chippewa River	44.9600	−91.3232	0.56	0.58	6.96	5.55
Petenwell	Stocked	134	Wisconsin River	44.1460	−89.9631	0.58	0.58	6.54	5.49
Castle Rock	Stocked	127	Wisconsin River	43.9357	−89.9657	0.56	0.59	6.54	5.56
Monona	Stocked	119	Rock River	43.0626	−89.3651	0.54	0.6	7.31	5.85

TABLE 2. Comparison of stocking data for Wisconsin and Leech Lake strains to recoveries for each strain based on genetic assignments. The stocking data were summed from 2005 to 2015. Strain-specific data are not available before 2005, and most of the fish (>97% based on aging data) that were sampled in this study were stocked before 2015. All of the fish in these systems were stocked as large fingerlings, with the exception of 757 fish from the Leech Lake strain that were stocked as yearlings into Lake Wissota in 2010 and 300 fish from the Leech Lake strain that were stocked as yearlings into Lake Wissota in 2011. The proportion of fish from the Leech Lake strain that was captured in lakes Monona and Wissota was significantly smaller than the proportion that was stocked ($P < 0.01$); we did not assess significance in Petenwell and Castle Rock due to the low stocking and capture rates. Individuals were classified as Leech Lake strain if the Leech Lake strain was the most likely assignment group in either STRUCTURE, ONCOR, or NewHybrids. Individuals were classified as putative hybrids if the F_1 category had the highest assignment probability in NewHybrids.

Population	Wisconsin stocked (N)	Leech Lake stocked (N)	Leech Lake stocked (%)	Wisconsin recovered (N)	Leech Lake recovered (N)	Leech Lake recovered (%)	Putative F_1 hybrids (N)	Hybrids (%)
Monona	15,734	4,523	22.33	107	12	10.08	5	4
Castle Rock	37,894	0	0.00	126	1	0.79	2	2
Petenwell	45,013	1,200	2.60	134	0	0.00	4	3
Wissota	23,963	8,753	26.75	297	7	2.30	14	5
Total	122,604	14,476	10.56	664	20	2.92	25	4

vonHoldt 2012), and combined the results from multiple runs with CLUMPAK (Kopelman et al. 2015). We determined that a K-value of two best fit our data (see below). To assign individuals of unknown origin from the stocked populations to either the Leech Lake or Wisconsin strain, we ran STRUCTURE with $K = 2$ using the same parameters as above, a PopFlag = 1 for the reference populations, and a PopFlag = 0 for the stocked populations. Any individual with a q-value >0.7 for the Leech Lake population cluster was assigned to the Leech Lake strain, and any individual with a q-value >0.7 for the cluster containing Wisconsin populations was assigned to the Wisconsin strain. This cutoff value was determined based on simulations that were conducted in HYBRIDLAB (Nielsen et al. 2006). Specifically, we used our reference data to simulate 500 pure Wisconsin, 500 pure Leech Lake, and 500 F_1 Wisconsin × Leech Lake hybrids and assigned these simulated individuals to the Leech Lake and Wisconsin strains using the methods described above. We then assessed assignment accuracy (i.e., how many simulated individuals from the Leech Lake strain were assigned to the Wisconsin strain and vice versa) at three different q-value cutoffs (0.6, 0.7, and 0.8) and determined that a q-value of 0.7 minimized incorrect assignments of pure individuals, without classifying a large proportion of putative hybrids as pure.

We also used two other programs, ONCOR (http://www.montana.edu/kalinowski/software/oncor.html) and NewHybrids (Anderson and Thompson 2002) to classify unknown individuals, and assignment accuracies were assessed with the simulated data set that is described above. Individual assignment in ONCOR was conducted using the default parameters and the reference populations separated into reporting groups containing (1) all of the Wisconsin populations and (2) the Leech Lake population. In NewHybrids, we assessed whether unknown and simulated individuals belonged to the Wisconsin strain, the Leech Lake strain, an F_1 hybrid group, or an F_2 hybrid group. The NewHybrids analysis was run using uniform priors, 10,000 burn-in iterations, and a run time of 100,000 iterations. For both ONCOR and NewHybrids, we assigned individuals based on the maximum assignment probability across categories. To ensure that we did not underestimate the capture rate of fish from the Leech Lake strain, any individual that was classified as belonging to the Leech Lake strain in any of the three programs was considered to be from that strain.

Investigating strain-specific capture rates.— To estimate the capture rates for fish from the Leech Lake and Wisconsin strains, we first assembled stocking records from 2005 to 2015 from all four of the stocked lakes in our study. The fish that we genotyped from the stocked lakes were collected from 2013 to 2019, and the vast majority of these fish (~87% according to the aging data, see below) were stocked between 2005 and 2015. No strain-specific stocking data is available before 2005, and very few of our fish (<3% according to the aging data) were stocked after 2015. Based on genetic data, we then calculated the proportion of fish from the Leech Lake strain that were stocked in each lake from 2005 to 2015 and compared those values with the proportion of fish from the Leech Lake strain that was recovered. Significant differences between the proportion of fish from the Leech Lake strain that was stocked and the proportion that was captured were determined by using the prop.test() function in R (R Development Core Team 2019). No known stockings of fish from the Leech Lake strain occurred in Castle Rock Lake during this period, but large numbers of Muskellunge have been shown to migrate downstream through dams (Wolter et al. 2013) and Castle Rock Lake is directly downstream of Petenwell Lake, which has been stocked with Leech Lake fish. We estimated brood-year-specific capture rates in lakes Monona and Wissota (not Petenwell and Castle Rock lakes due to low sample sizes) by combining age frequency data with stocking records. Aging was conducted by WDNR biologists by using anal fin rays for all of the lakes except Wissota, where scales were used. Aging was conducted using established WDNR protocols and comparisons to known-age fish, and age data was available for 96% of the fish from Lake Monona, 62% of the fish from Lake Wissota, and <5% of the fish from Petenwell and Castle Rock lakes.

RESULTS

Genetic Analysis

Genotyping was successful for 1,001 of 1,011 fish that we analyzed (99%). Genetic diversity (H_O, H_E, N_A, and N_E) was similar for populations from Wisconsin but was lower for the population from Leech Lake (e.g., Leech Lake: $H_O = 0.43$, $A_R = 3.97$; Wisconsin: average $H_O =$ 0.56, average $A_R = 5.33$; Table 1). It is notable that the stocked populations had slightly higher diversity metrics than the other Wisconsin populations; this may be due to mixing of brood-source populations and strains across stocking years. Genetic differentiation (pairwise F_{ST}) was high between the Wisconsin and Leech Lake populations (average = 0.13, range = 0.11–0.155) and low among the Wisconsin populations (average = 0.007, range = 0.002–0.018). The stocked populations were all genetically similar to each other, with F_{ST} values ≤ 0.008, and they were much more genetically similar to the Wisconsin strain (F_{ST} ~0.01) than the Leech Lake strain (F_{ST} ~0.10). The genotypes and metadata for this study are found in Supplementary Files S1 and S2 (available in the online version of this article).

The STRUCTURE analysis with the reference stocks revealed that $K = 2$ had the highest support based on the ΔK method ($\Delta K = 4{,}975$ for $K = 2$; the next highest $\Delta K =$ 33.77). Although $K = 2$ did have a high likelihood compared with most of the K-values, the highest likelihood value was at $K = 5$. However, clustering at K-values >2 was not well associated with the sample locations and was likely statistical noise. Additionally, poor convergence was observed at K-values >3, providing further support for $K = 2$ as the most likely number of clusters in our data set. See Supplementary Files S3 and S4 for more information on various K-values from CLUMPAK and the Structure Harvester analysis. At $K = 2$, the average q-value for the Leech Lake population cluster was 0.95 (range: 0.8–0.99) and the average q-value for the Wisconsin reference populations was 0.92 (range: 0.41–0.99); see Figure 2. It is important to note that even though reference populations from the Wisconsin River and Chippewa River drainages could not be reliably differentiated in our analysis, we do not advocate stocking these strains across drainage boundaries in areas where Muskellunge are native.

According to our simulations, the assignment accuracy of pure Leech Lake and pure Wisconsin populations to their strain of origin was high (≥95%) with all three of the programs that we used (Table 3). Assignment accuracy to the Leech Lake strain was >99% across all three of the STRUCTURE q-value cutoffs that we explored (0.6, 0.7, and 0.8); assignment accuracy to the Leech Lake strain was ≥99% at q-value cutoffs of 0.6 and 0.7 but only 95% at a q-value cutoff of 0.8. Based on these simulations, q-values of 0.6 and 0.7 performed similarly on pure crosses, but we chose a q-value cutoff of 0.7 for assigning unknown fish back to their strain of origin, as fewer putative hybrids were assigned as pure strains at this value (34% of the simulated hybrids assigned to Leech Lake at a q-value of 0.6 and 13% at a q-value of 0.7); see Table 3. Simulated F_1 hybrids had an average q-value of 0.46, which is close to the 50% ancestry from each strain that is expected with an F_1 cross. However, the q-values for

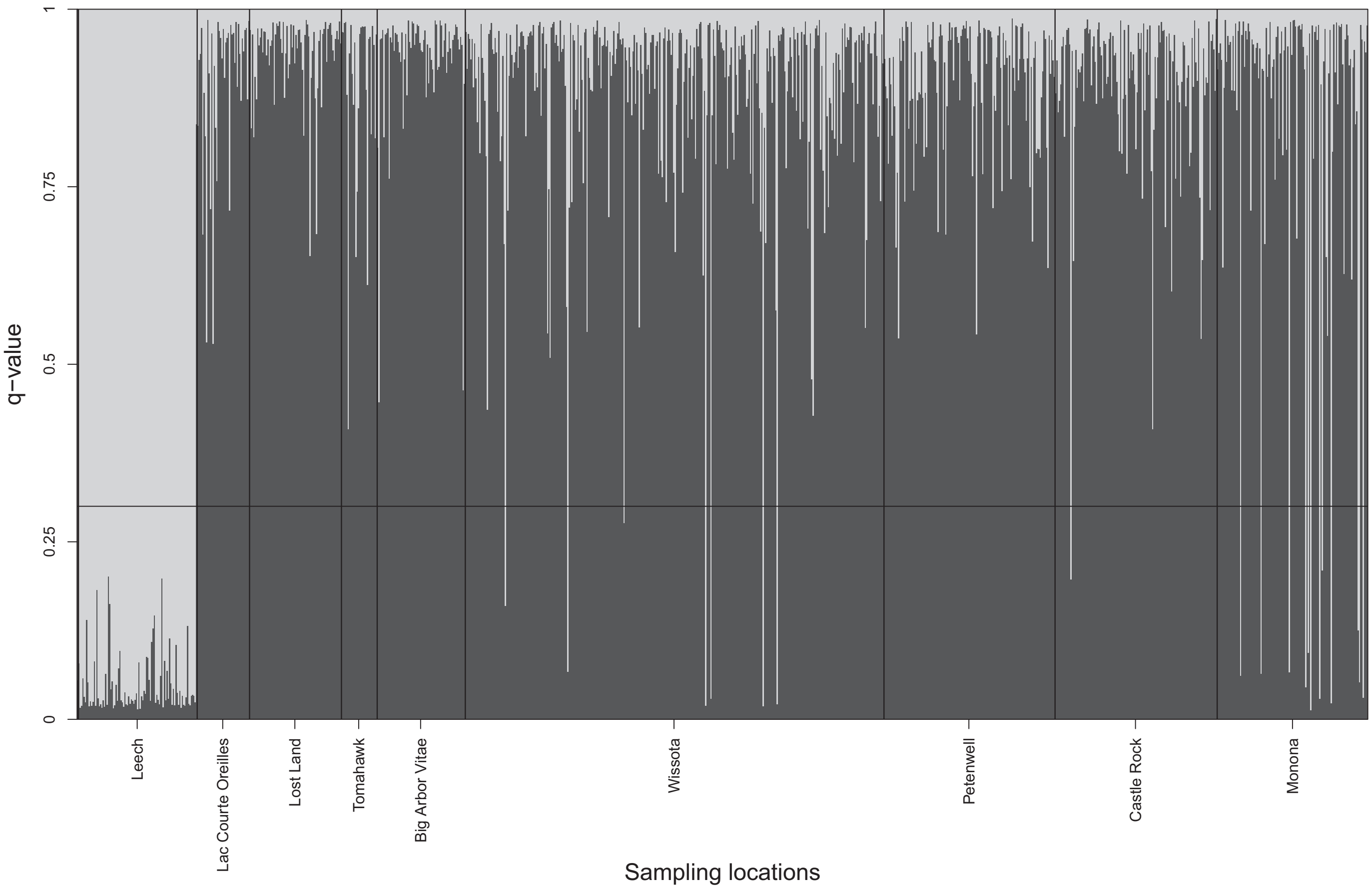

FIGURE 2. Results from the STRUCTURE clustering analysis using $K = 2$. Samples from Leech Lake, Lac Court Oreilles, Lost Land Lake, Tomahawk Lake, and Big Arbor Vitae were used as references to assign fish of unknown origin to their genetic strain. The samples with high *q*-values for the gray cluster are of Leech Lake origin, and the samples with high *q*-values for the black cluster are of Wisconsin origin. Individuals with *q*-values <0.3 (vertical line) were assigned to the Leech Lake strain, and individuals with *q*-values >0.3 were assigned to the Wisconsin strain. See Figure 1 and Table 1 for more information on the sampling locations.

TABLE 3. Results from simulations conducted in HYBRIDLAB to determine the accuracy of genetic assignment. The columns denote the results from simulations of 500 pure Wisconsin individuals, 500 Leech Lake individuals, and 500 F_1 hybrids between the two strains. The rows including *q*-values refer to the STRUCTURE analysis. In the ONCOR analysis, individuals with >50% probability of assignment to a given strain were assigned to that strain. For the NewHybrid analysis, individuals were assigned to the category with the highest assignment probability; F_2 was not the highest assignment category for any individual.

Simulation	Pure Wisconsin	Pure Leech Lake	F_1 Wisconsin × Leech Lake
Average *q*-value	0.81	0.10	0.46
Standard deviation *q*-value	0.11	0.06	0.13
Number (%) assigned to Leech at $q = 0.6$	2/500 (0)	499/500 (100)	172/500 (34)
Number (%) assigned to Leech at $q = 0.7$	0/500 (0)	493/500 (99)	64 (13)
Number (%) assigned to Leech at $q = 0.8$	0/500 (0)	474/500 (95)	8/500 (2)
Number (%) assigned to Leech with Oncor	1/500 (0)	500/500 (100)	135/500 (27)
Number (%) assigned to Leech with NewHybrids	0/500 (0)	494/500 (100)	25/500 (5)
Number (%) assigned to Wisconsin with NewHybrids	476/500 (95)	0/500 (99)	44/500 (9)
Number (%) assigned to F_1 with NewHybrids	24/500 (5)	6/500 (1)	431/500 (86)

hybrids were highly variable (twice the standard deviation of pure crosses). The ONCOR program displayed very high assignment accuracy (>99%) for pure individuals, and most simulated hybrids (95%) were assigned to the Wisconsin strain. Assignment to hybrid categories is not possible with ONCOR. The assignment accuracy of pure individuals with NewHybrids was still high (≥95%) but was lower than for the other two programs. Assignment to the F_1 category was 5% for the simulated pure Wisconsin strain, 1% for the simulated pure Leech Lake strain, and 86% for the simulated F_1 individuals. No simulated or unknown origin individuals were assigned to the F_2 class.

We assigned a total 664 unknown fish to the Wisconsin strain and 20 unknown fish to the Leech Lake strain across the four stocked lakes (Table 2; Figure 2). Lake Monona had the most recoveries of fish from the Leech Lake strain ($n = 12$), followed by Wissota ($n = 7$), Castle Rock ($n = 1$), and Petenwell ($n = 0$) lakes for a total of 3% of the sampled fish. Of these 20 fish, all 20 were assigned to the Leech Lake strain in STRUCTURE, 19 of 20 were assigned to the Leech Lake strain in ONCOR, and 18 of 20 were assigned to the Leech Lake strain in NewHybrids. All of the Leech Lake fish from Lake Monona were identified with all three methods, six of the seven fish from Lake Wissota were identified with all three methods (with one identified in STRUCTURE and ONCOR but not NewHybrids), and the one Leech Lake fish from Castle Rock Lake was only identified with STRUCTURE. This high consistency among methods indicates that our genetic markers provided high power for identifying the strains. Additionally, 10 of the 12 Leech Lake fish that we recovered from Lake Monona were tagged at the time of stocking and we correctly assigned all 10 of these fish to the Leech Lake strain. We identified 25 putative F_1 hybrids with NewHybrids, representing ~4% of the unknown individuals (Table 2). The percentage of putative hybrids that was encountered ranged from 2% in Castle Rock Lake to 5% in Lake Wissota. It is important to note that the percentage of putative hybrids that we observed is close to the 5% of simulated pure Wisconsin fish that were classified as F_1 hybrids with NewHybrids.

Investigating Strain-Specific Capture Rates

In total 122,604 large fingerlings from the Wisconsin strain and 14,476 large fingerlings from the Leech Lake strain were stocked between 2005 and 2015 in our four lakes, representing a stocking rate of ~11% for the Leech Lake strain (Table 2). Based on genetic data, the stocking rate of 11% is much higher than the capture rate of Leech Lake fish (3%; Table 2). Stocking rates for the Leech Lake strain varied by lake, with lakes Monona and Wissota receiving ~25% Leech Lake strain, Petenwell Lake receiving 3%, and Castle Rock Lake receiving 0%. The capture rates of fish from the Leech Lake strain also varied, with 10% recovered in Lake Monona, 2% recovered in Lake Wissota, 1% recovered in Castle Rock Lake, and 0% recovered in Petenwell Lake. The proportion of fish from the Leech Lake strain that was captured in lakes Monona and Wissota was significantly smaller than the proportion that was stocked ($P < 0.01$); we did not assess significance in Petenwell and Castle Rock due to low stocking and capture rates. We investigated brood-year-specific capture rates in lakes Monona and Wissota and recovered 0–2 fish from the Leech Lake strain per year compared with ~10 fish from the Wisconsin strain (Table 4). However, we did recover seven Leech Lake fish from the 2009 year-class in Lake Monona, representing a 41% capture rate compared with a 26% stocking rate (Table 4).

DISCUSSION

We were able to accurately assign Muskellunge from Leech Lake and Wisconsin strains back to their strain of origin, and our findings provide evidence that the capture rates of Muskellunge from the Leech Lake strain are consistently lower than those of the Wisconsin strain in Wisconsin waters. These findings suggest that stocking primarily the Wisconsin strain will likely maximize return on investment for the Muskellunge stocking program in Wisconsin. We also discovered differences in capture rates among waterbodies. Fish from the Leech Lake strain were recovered at much higher rates in Lake Monona (10% with a stocking rate of 22%) compared with all of the other lakes in the study. Notably, Lake Wissota had a higher stocking rate for fish from the Leech Lake strain than Lake Monona (27%) but a much lower capture rate (2%). It is possible that emigration downstream over dams may have contributed to these lower capture rates in reservoir systems such as Lake Wissota (Wolter et al. 2013). However, it is also possible that these differences in capture rates could be due to adaptive differences between the Leech Lake and Wisconsin strains. The Leech Lake strain is derived from Leech Lake, a large (>40,000 ha) and deep (maximum depth >45 m) lake in Northern Minnesota, whereas Muskellunge from the Wisconsin strain are derived from lakes such as Big Arbor Vitae, a relatively small (~400 ha) and shallower (maximum depth = 12 m) lake, or reservoirs such as the Chippewa Flowage (size = 6,200 ha, maximum depth = 28 m). Fish from the Leech Lake strain have likely evolved to thrive in large, deep lakes, whereas fish from the Wisconsin strain may be more suited to smaller lakes or more lotic habitats such as rivers and reservoirs.

Adaptive differences between fish that inhabit lotic and lentic habitats have been commonly documented in species including Stickleback *Gasterosteus aculeatus* (Marques et al. 2016), Sockeye Salmon *Oncorhynchus nerka* (Larson

TABLE 4. Year-class-specific comparisons of stocking data for Wisconsin and Leech Lake strains to recoveries for each strain based on genetic assignments. All of the fish in these systems were stocked as large fingerlings, with the exception of 757 fish from the Leech Lake strain that were stocked as yearlings into Lake Wissota in 2010 and 300 fish from the Leech Lake strain that were stocked as yearlings into Lake Wissota in 2011. The year-classes for recoveries were inferred from ages that were calculated by examining anal fin rays.

Year stocked	Lake	Wisconsin stocked (*N*)	Leech Lake stocked (*N*)	Leech Lake stocked (%)	Wisconsin recovered (*N*)	Leech Lake recovered (*N*)	Leech Lake recovered (%)
2005	Monona	700	700	50.00	5	1	16.67
2006	Monona	997	61	5.77	11	1	8.33
2007	Monona	1,470	499	25.34	6	1	14.29
2008	Monona	1,420	461	24.51	7	1	12.50
2009	Monona	1,470	506	25.61	10	7	41.18
2011	Monona	1,464	507	25.72	8	0	0.00
2012	Monona	1,970	501	20.28	7	0	0.00
2013	Monona	1,462	503	25.60	2	0	0.00
2005	Wissota	2,497	500	16.68	8	2	20.00
2007	Wissota	1,666	831	33.28	7	0	0.00
2008	Wissota	1,249	1,307	51.13	8	0	0.00
2009	Wissota	2,498	1,397	35.87	12	0	0.00
2010	Wissota	660	757	53.42	9	0	0.00
2011	Wissota	5,781	800	12.16	21	0	0.00
2013	Wissota	777	2,494	76.25	14	1	6.67
2015	Wissota	2,494	667	21.10	12	0	0.00

et al. 2017), and Rainbow Trout *Oncorhynchus mykiss* (Arostegui et al. 2019), and similar patterns of adaptive divergence may be occurring in Muskellunge. Although no studies to our knowledge have investigated potential adaptive differences in Muskellunge that inhabit lake and river habitats, adaptive divergence has been hypothesized for some traits between genetically divergent populations of Muskellunge. For example, Muskellunge from different genetic groups have demonstrated differences in metabolism (Clapp and Wahl 1996), temperature tolerance (Diana et al. 2017), and maximum size (Miller et al. 2009). It is likely that adaptive differences between the Leech Lake and Wisconsin strains at least partially explain the differences in capture rates that we observed between Lake Wissota and Lake Monona. However, it is important to note that fish from the Leech Lake strain were still recovered at much a lower rate than those from the Wisconsin strain in Lake Monona, suggesting that the Leech Lake strain may survive more poorly than the Wisconsin strain even in waters that are potentially better matched to the environmental preferences of the Leech Lake strain. Additionally, over half (7 of 12) of the fish from the Leech Lake strain that were recovered from Lake Monona were from the 2009 year-class. It is possible that the high contribution of this year-class was atypical and, if this is the case, the contribution of the Leech Lake strain in Lake Monona may be lower on average than is reported here. Our results suggest that the Leech Lake strain of Muskellunge may simply not be well matched to Wisconsin waters. Leech Lake is substantially to the north and west of all of the Wisconsin populations and is larger than any lake with a robust Muskellunge population in Wisconsin. These differences in lake size and location undoubtedly influence temperature, growing degree days, and other important variables, and they have likely led to adaptive divergence that may explain the differences in capture rates that we observed between the Leech Lake and Wisconsin strains.

Adaptive divergence may also lead to differences in size among different fish populations. In Sockeye Salmon, smaller fish have higher fitness when spawning in shallower streams and larger fish have higher fitness when spawning on lake beaches, leading to substantial adaptive differences in body size between fish spawning in these two proximate habitats (Peterson et al. 2014; Oke et al. 2019). It has been hypothesized that Muskellunge from the Leech Lake strain grow larger than those from the Wisconsin strain, as Leech Lake produces many trophy Muskellunge. However, this difference in size may be a function of the habitat of Leech Lake and the fact that larger lakes tend to produce larger fish (Griffiths 2013). To decouple the effects of local habitat from genetic differences in strains, it is necessary to conduct a common garden experiment where fish from multiple strains are exposed to the same habitat conditions. Data such as this are generally lacking for the Leech Lake strain (but see Younk and Strand 1992; Diana et al. 2017; Wagner et al. 2017; Andree et al. 2018), and unfortunately we did not obtain a sufficient sample size to investigate whether the Leech Lake strain grows differently than the Wisconsin strain. Data continue to be collected on fish from the Leech Lake strain that are stocked in lakes Monona and Wissota as well as other Wisconsin lakes; these data will facilitate an evaluation of strain-specific growth rates in the coming years.

As with many stocking evaluations, there are some potential caveats that could have influenced differences in the strain-specific capture rates. For example, while the original design of the paired stocking on Lake Monona included plans to raise gametes from Leech Lake in the WDNR hatchery system alongside the Wisconsin strain, the discovery of viral hemorrhagic septicemia in 2007 meant that only 1 year of paired rearing was possible and interstate transport of wild-sourced eggs was not allowed. Because Wisconsin fish were reared in WDNR hatcheries and Leech Lake fish were raised in private hatcheries, there were differences in multiple factors that could have contributed to variation in the strain-specific capture rates. First, size at stocking of the fish from the Wisconsin strain was generally larger. In Lake Monona, the average size of fish from the Wisconsin strain during 2007–2015 was 300 mm compared with 264 mm for fish from the Leech Lake strain. Even in 2006, when the fish were raised together at Governor Thompson Hatchery, there was a size difference at the time of stocking between the two strains (Wisconsin = 315 mm and Leech Lake = 279 mm) because the fish from the Leech Lake strain spawned later than those from the Wisconsin strain, giving them a shorter growing season. Several studies have shown that survival of fingerling Muskellunge is related to stocking size (Szendrey and Wahl 1996; Margenau 1999), and these size differences may have influenced the strain-specific capture rates in our study. The date of stocking was also earlier for the fish from the Wisconsin strain (despite being larger) on Lake Monona, normally occurring during the second week of September, compared with fish from the Leech Lake strain, which are normally stocked during the first week of November. This likely meant higher prey availability and a longer growth period for the Wisconsin strain, which may have led to increased survival (Szendrey and Wahl 1996). Interestingly, the highest year of contribution of fish from the Leech Lake strain in Lake Monona was 2009, when these fish were stocked slightly earlier than normal (October 28 versus a typical stocking date of the first week of November). The handling of the two strains that were stocked into Lake Monona was also markedly different. The fish from the Wisconsin strain were anesthetized at the hatchery for PIT tagging and then allowed to recover for 24 h before being transported and stocked, while the fish from the Leech Lake strain were transported from Minnesota, anesthetized, PIT-tagged, and held only until they recovered and established equilibrium before being stocked into the lake, which was colder because of the later stocking date.

It is also important to note that we measured capture rate in this study, which can be influenced by catchability, mortality, or both. For example, it is possible that fish from the Leech Lake strain spend more time in deeper water, as they are derived from a deeper lake, leading to lower capture rates than for fish from the Wisconsin strain. Alternatively, fish from the Leech Lake strain may simply have suffered higher mortality rates due to a mismatch between the environments they are adapted to and Wisconsin rivers and lakes. Finally, fish from the Leech Lake strain may have higher mortality rates than those from the Wisconsin strain but also lower catchability, leading to an underestimate of the frequency of Leech Lake fish in our study lakes. We suggest that future studies incorporate angler-captured fish with data on depth of capture to investigate potential depth preferences between the strains.

Our genetic simulations in conjunction with the comparison of genetic and tagging results indicate that the 13 microsatellite markers that were used in this study can be used to assign fish from Leech Lake and Wisconsin strains to their strain of origin with high accuracy. However, we believe that the current marker panel is not sufficient for designating fish as either pure or hybrid crosses. The NewHybrids analysis indicated that ~4% of the individuals in our data set were putative hybrids, but this percentage is close to the 5% of simulated pure Wisconsin individuals that were classified as hybrids. These results indicate that hybrids could be present in our samples but that our marker panel may have insufficient power to accurately assign hybrid status. Natural reproduction of Muskellunge rarely occurs in the southern half of Wisconsin, which is thought to be outside the native Muskellunge range (Simonson and Hewett 1999), making it unlikely that hybridization is occurring in lakes Monona, Castle Rock, and Petenwell. However, Lake Wissota, where the highest proportion of putative hybrids was observed, is found at a latitude where natural reproduction has been shown to occur, but there is limited evidence for natural reproduction in this lake. Conclusive evidence of hybridization in any of these waterbodies would provide important information for fisheries managers, as hybridization would indicate natural reproduction, which would trigger a different set of management actions that is designed for lakes with naturally reproducing populations (Simonson and Hewett 1999). We suggest that future studies use genomic approaches that leverage genotypes from thousands of markers to improve the resolution for identifying putative hybridization in Muskellunge populations (e.g., Wells et al. 2019). We also recommend developing tools for parentage-based tagging to track potential natural reproduction of stocked fish (Christie et al. 2014).

In conclusion, we documented low but variable capture rates of Muskellunge from the Leech Lake strain that were stocked in four Wisconsin lakes. We hypothesized that differences in capture rates between the Wisconsin and Leech Lake strains of Muskellunge may be due to adaptive differences that favor the nearest native strain in systems that are more similar to the environments where they have existed and locally adapted for thousands of years. We therefore suggest that anglers and fisheries managers stock Wisconsin-strain Muskellunge in Wisconsin waters to maximize survival and return on investment for stocking programs.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.