Genetic Stock Assessment and Hatchery Contributions of Sauger Stocked into Old Hickory Lake, Tennessee

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Abstract - *Sander canadensis* (Sauger) once supported a viable fishery in many of the reservoirs throughout Tennessee; however, these populations have experienced widespread declines. To improve population numbers, the Tennessee Wildlife Resources Agency began stocking Sauger in 1992 in Tennessee and Cumberland river impoundments. Here we examine the percent contribution of hatchery-stocked Sauger to the wild population in Old Hickory Lake, a mainstem impoundment on the Cumberland River. We determined the contribution of hatchery-stocked Sauger using microsatellite markers and a categorical allocation-based parentage analysis. We also evaluated measures of genetic diversity, including estimates of heterozygosity and effective population size. Genetic variation was comparable to other stocked populations of percids. However, estimates of effective population size were low and the contribution of hatchery-reared Sauger to natural populations was moderate, averaging 25.8% across sampled year classes. Despite high genetic diversity, the Sauger population in Old Hickory Lake may be declining, and hatchery efforts to supplement Sauger numbers are contributing little to recovery of the population.

Introduction

Sander canadensis (Griffith and Smith) (Sauger) provide popular native sport fisheries in the southeastern US; however, the sustainability of this resource is of increasing concern. Sauger populations in Tennessee have been declining since the 1980s (Pegg et al. 1997). Poor recruitment and high exploitation of adults appear to be contributing to this decline (Fischbach 1998, Thomas 1994). Recruitment of percids is dependent on many factors, which vary from system to system and include dam discharges (Benson 1973), prey abundance (Madenjian et al. 1996), and variations in water temperature (Fischbach 1998). A correlation between reservoir discharges and Sauger recruitment was observed in Lewis and Clarke Lake, SD (Walburg 1972). Annual mortality rates of age-1 and older Sauger exceeded 80% in the lower Tennessee River and 60% in the upper Tennessee River (Thomas 1994). A 36% exploitation rate, unadjusted for non-reporting, was observed in Kentucky Lake, but actual values may have been closer to 50% (Pegg et al. 1996). Lack of steady recruitment to reproductive age coupled with high exploitation concerns fish biologists because both factors lead to smaller population sizes.

Poor recruitment and overfishing may be mitigated by implementing a stocking program to improve recreational fisheries by restoring or enhancing natural populations (Kerr 2011). Collectively, these programs stock millions of Sauger; for instance, in 2004 nearly 28 million Sauger were stocked in the US (Halverson

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2008). However, due to the high costs associated with any stocking program, it is imperative to assess stocking success by observing the contribution of hatchery-raised fish to the wild population. Molecular-based methods offer a non-invasive tool for identifying hatchery-raised Sauger in the wild and evaluating the contribution of stocked fish to targeted populations. With the introduction of PCR-based detection of highly variable microsatellite loci, genetic analyses have become increasingly popular for monitoring fisheries management programs (Kerr 2011). The use of molecular markers is preferable to traditional marking methods because researchers can monitor stocking by identifying individual fish that originated from the hatchery without sacrificing the fish.

In addition to monitoring numbers of hatchery-reared fish, molecular tools can provide valuable information regarding the genetic health of hatchery-supplemented populations. Many hatchery programs are successful at increasing the total numbers of fish; however, these programs often have negative consequences on genetic diversity. A meta-analysis conducted in 2010 found that fish populations supplemented by hatchery programs tend to display negative effects (Araki and Schmid 2010). Issues such as lower reproductive fitness, lower survival, lower heterozygosity, and lower effective population sizes are found in hatchery programs for many fish taxa. In 70 studies reviewed by Araki and Schmid (2010), 28 reported reduced genetic variation in hatchery populations, and 23 showed significant negative effects on the fitness of stocked individuals. Parameters such as allelic richness and heterozygosity can give insight on the genetic diversity of a population, and tests for Hardy–Weinberg equilibrium may give indications of population genetic structure and inbreeding. Additionally, patterns of genetic variation can be used to infer important demographic factors such as a past population bottleneck and effective population size.

In an attempt to restore Sauger populations to a healthy level, the Tennessee Wildlife Resources Agency (TWRA) initiated a Sauger stocking program in 1990 in Old Hickory Lake, TN. Hatchery production between 1990 and 2015 fluctuated widely; no fish were stocked in 8 of those 26 years and the numbers stocked annually into Old Hickory Lake in other years ranged from 3000 to 408,462 fingerlings (D. Roddy, Tennessee Wildlife Resources Agency [TWRA], Nashville, TN; pers. comm.). In order to assess the efficacy of this program, the TWRA and the US Fish and Wildlife Service (USFWS) implemented a pilot study in 2012 in which microsatellite-based genotypes of broodstock Sauger were compared to the genotypes of age-1 Sauger sampled a year later. Surprisingly, only 8% of the age-1 Sauger collected in 2013 were of hatchery origin (G. Moyer, USFWS, Warm Springs, GA; pers. comm.). Low percent contribution by hatchery fish indicated that TWRA's Sauger stocking program in 2012 was not very successful at augmenting the wild population. Several hypotheses could explain why the hatchery contribution was low that year. One explanation is that stocked fingerlings experienced poor survival relative to wild-born fish. A number of factors can contribute to poor survival of stocked percids, including lack of suitable zooplankton prey at the time and place of stocking, unsuitable water temperatures, and poor release methods (Kerr 2011).

Alternatively, wild Sauger could have produced a large year class in 2012, which increased the likelihood of catching more wild than hatchery-reared age-1 Sauger in 2013. Finally, the low percent contribution may have reflected the fact that relatively few Sauger (n = 92,783) were stocked in 2012.

In this study, we utilized a microsatellite-based approach to further examine the efficacy of the Sauger hatchery program at Old Hickory Lake, TN, and to evaluate patterns of genetic variability of this population. The specific objectives of our research were to: (1) examine levels of genetic diversity, (2) estimate effective population size, and (3) evaluate the percent contribution of hatchery fish to Sauger year classes in 2014 and 2015. Results from this study are discussed in the context of current and future management strategies for this population.

Study Area

We collected Sauger from the headwaters of Old Hickory Lake below the Cordell Hull Dam where spawning is thought to occur (Fig. 1; Fischbach 1998). Old Hickory Lake is a mainstem reservoir on the Cumberland River (CR km 347.9) in northern middle Tennessee, formed by the Old Hickory Lock and Dam in Sumner and Davidson counties and managed by the US Army Corps of Engineers. At full pool (135.6 msl), Old Hickory Lake has a surface area of 9105 ha. Cordell Hull Dam (CR km 504.5), also managed by the US Army Corps of Engineers, forms the upper boarder of Old Hickory Lake.

Methods

Tissue samples

TWRA biologists used gillnets to collect Sauger broodstock in winter 2013 and winter 2014 below Cordell Hull Dam. In Tennessee reservoirs, Sauger move upstream in winter and frequently congregate below headwater dams before dispersing downriver to spawn (Pegg et al. 1997). Broodstock were transported to Normandy Fish Hatchery and Springfield Fish Hatchery, where they were externally tagged with a sequentially numbered Floy tag, and a caudal-fin clip was removed and preserved in 90% ethanol; each vial containing a fin clip was matched with the Floy tag number of the fish from which the clip was taken. Collectors administered injections of human chorionic gonadotropin (HCG) to brood fish to stimulate ovulation and milt release. We stripped eggs from 1 or more females and milt from 2 or more males into a hatchery pan and placed the fertilized eggs into McDonald hatching jars. We repeated this process of stripping gametes and fertilizing the eggs in separate batches until all hatching jars (16–22 each year) were filled with fertilized eggs. We recorded the ID number of each parent that contributed to a fertilization event. Eggs hatched within several days and the fry were placed in hatchery ponds to grow to stocking size (~50 mm total length). Biologists subsequently stocked fingerlings at 3 locations in Old Hickory Lake (14 km, 24 km, and 100 km upstream from Old Hickory Dam; Fig. 1). Biologists used gill nets to sample Sauger representing the 2013 and 2014 year-classes below Cordell Hull Dam during the winter

and early spring of 2014 and 2015. Field-caught Sauger were assigned to a specific year-class by aging of sagittal otoliths following the methods of Churchill (1992).

Molecular methods

DNA from fin clips of 333 Sauger were extracted using the protocol described by Wang and Storm (2006) and stored at -20 °C. Polymerase chain reaction (PCR) was used to amplify DNA from broodstock and field-caught samples for a suite of 8 microsatellite loci including Svi2, Svi4, Svi7, Svi17, Svi26, Svi33, Svi18, and Svi20 (Borer et al.1999). We performed the PCR amplifications in 20-µL reactions using the following reaction components: 5x *Taq* reaction buffer, 2.00 mM $MgCl_2$, 0.375 mM of each dNTP, 0.5 µM of each primer, and 0.175 U *Taq*

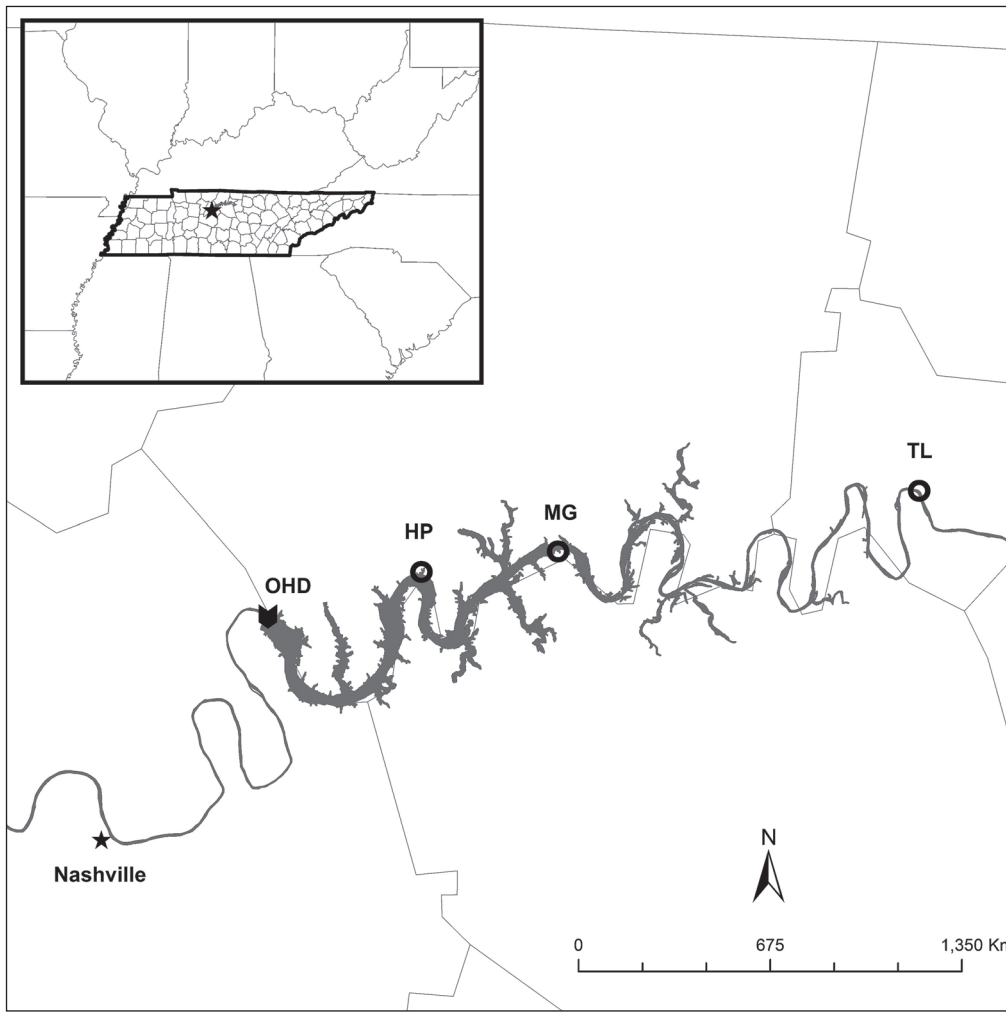

Figure 1. Map of Old Hickory Lake, TN. Sauger for parentage analysis were collected below Cordell Hull Dam (CHD). Circles indicate the location of the 3 ramps where biologists stocked fingerling Sauger between 2010 and 2015 (HP = Hunters Point; MG = Martha Gallatin; TL = Taylors Landing).

polymerase. Touchdown PCR was performed as follows: initial denaturation at 94 ºC for 10 min, 33 cycles of denaturing at 94 ºC, annealing, and extension at 74 ºC. The initial annealing temperature of 56 ºC was decreased 0.2 ºC with each cycle. We combined PCR products after amplification and prior to loading on an ABI3730 genetic analyzer (Applied Biosystems, Waltham, MA). We employed the software Peak Scanner® version 1.0 (Applied Biosystems) to score alleles manually from electropherograms.

Statistical analysis

We performed tests for genotyping error including stuttering, large allele drop-out, and the presence of null alleles in the software Micro-Checker (Oosterhout et al. 2004). Chi-squared tests for Hardy–Weinberg equilibrium were performed using the program GeneAlEx (Peakall and Smouse 2006). We also calculated basic summary statistics including allele frequencies, number of alleles, the effective number of alleles, observed and expected heterozygosity, and fixation indices in the program GeneAlEx.

We estimated contemporary effective population-size (N_e) using both a temporal method (Do et al. 2014) and a linkage disequilibrium method (Hill 1981). The temporal method measures the rate of change in allele frequencies over time and requires acquisition of data from 2 or more sampling events. Calculations using the temporal approach were estimated using the software NeEstimator version 2.01 (Do et al. 2014). The linkage disequilibrium method requires only 1 sampling event and is based on the degree of linkage disequilibrium between physically unlinked markers. We calculated estimates of effective population size based on linkage disequilibrium using Burrow's Δ in the software LDNe version 1.31 (Waples and Do 2008). Both methods for calculating contemporary effective population size employed a jackknife method to obtain confidence intervals.

We performed tests for the occurrence of a recent bottleneck event including the M-ratio test and the test for heterozygosity excess in the software BOTTLENECK version 1.2.02 (Cornuet and Luikart 1996). For the M-ratio test, BOTTLENECK assumes mutation-drift equilibrium, and computes the distribution of expected heterozygosity values given the number of alleles. We acquired this distribution by simulation of coalescent data under the 2-phase mutation model (TPM). Heterozygote excess was tested using a 1-tailed Wilcoxon-signed rank test which uses the observed number of alleles and sample size under the TPM model (Bellinger et al. 2003).

We also performed a categorical allocation-based parentage analysis comparing the genotypes of field-caught Sauger to broodstock in the software Cervus (Marshall et al. 1998) to determine the proportion of the field-collected Sauger that were originally spawned in a hatchery. The Cervus program assigns parentage by calculating the natural logarithm of the likelihood ratio (LOD score) for each parent–offspring possibility and matches potential offspring with the most likely parent. Parings with positive LOD scores indicate that the assigned parent has a higher probability of being the true parent than not. We used simulated data

to determine critical values for Δ (the difference between the 2 most likely candidate parents and the LOD scores) to assign confidence. We set run parameters for simulation analysis for 10,000 simulated offspring genotypes and assumed a 1% genotyping error. Computer simulations and examination of empirical datasets have shown that allowing for genotyping error increases the accuracy of paternity assignment (Kalinowski et al. 2007). Cervus simulations also require an estimate of the percent of the population that was sampled. Population-census size-estimates were not available to estimate this value; therefore, to examine the influence of this parameter we ran the analysis with 4 different values of percent population sampled (10%, 30%, 50%, and 70%). Eighty-eight out of 124 field-caught Sauger were of appropriate age to potentially be progeny of brood fish. These 88 individuals, and an additional individual, whose age was unknown, were utilized for this analysis. We designated individuals with positive LOD scores as hatchery stock.

Results

We genotyped 8 microsatellite loci each of 333 individual Sauger, which included 209 broodstock and 124 potential broodstock progeny. Examination of genotype frequencies for all individuals included in this study ($n = 333$) using MicroChecker revealed a deficiency of observed heterozygotes in 7 out of 8 of the loci examined (Svi2, Svi4, Svi7, Svi26, Svi33, Svi18, and Svi20; $P < 0.001$; Table 1). Results suggested the presence of scoring errors due to stuttering for 3 loci (Svi4, Svi33, and Svi7); however, reexamination of electropherograms did not reveal any miscalled peaks. We found no evidence for allele dropout for any of the loci. Results of chi-squared tests for deviations from Hardy–Weinberg equilibrium were significant for all 3 sample years due to an overall heterozygote deficiency. Heterozygote deficiencies were distributed across all 8 loci, suggesting that departures were the result of population-level dynamics and not at the locus level. Therefore, we retained all 8 loci for further analyses.

The number of alleles per locus was high, varying from 4 to 28 (average = 18.5, SE = 3.3). The size range of alleles, number of alleles and effective number of alleles per locus, observed and expected heterozygosity values per locus, and fixation

Table 1. List of 8 microsatellite loci used for Sauger population ($n = 333$) analysis, size ranges in base pairs, number of alleles (N_a), number of effective alleles (N_{ae}), observed (H_o) and expected (He) heterozygosity, and fixation-index values (F_{ST}) for each allele. *Indicates significant deficiency of H_O compared to Hardy–Weinberg expectations ($P < 0.001$).

Locus	Size range	*Na*	*Nae*	*Ho*	*He*	*FST*
Svi2	195–271	18	8.879	0.766*	0.887	0.137
Svi4	101–141	23	10.774	0.898*	0.907	0.010
Svi7	164–226	25	13.685	0.868*	0.927	0.064
Svi17	96–116	4	1.925	0.453	0.481	0.056
Svi26	151–195	25	6.892	0.835*	0.855	0.023
Svi33	87–145	28	15.520	0.883*	0.936	0.056
Svi18	120–126	5	2.640	0.544*	0.621	0.125
Svi20	160–198	20	12.568	0.853*	0.920	0.073

index values are listed in Table 1. The observed and expected heterozygosity values averaged across loci were calculated at 0.793 and 0.818 in 2013, 0.727 and 0.811 in 2014, and 0.755 and 0.797 in 2015, respectively.

The estimate of effective population size based on the temporal method was 38.9 (95% CI: 25.9–59.6). We calculated linkage-disequilibrium estimates of effective population size separately for each sample year. The upper bound for the confidence interval for the 2013 sample year was infinity (N_e = 2400, CI: 404.7–∞). Estimates for 2014 and 2015 were 273.2 (CI: 1486–1015.9) and 109.0 (CI: 67.2–218.1), respectively.

Results from the Wilcoxon sign-rank test for heterozygosity excess performed using BOTTLENECK did not suggest a recent population bottleneck. An excess of heterozygosity compared to expectations under drift-mutation equilibrium was calculated in 6 loci; however, these results were not statistically significant (P = 0.098). Additionally, the M-ratio test run on BOTTLENECK determined an uneven allele frequency distribution with a high proportion of alleles at low frequency as predicted from a demographically stable population.

For parentage analysis, we separated individuals by age and sample year (Table 2). The substitution of values for percent population sampled had no effect on the percentage or identity of individuals that were assigned hatchery parentage. We caught only 9 age-1 Sauger in 2014 and they represented the 2013 year class, a year when 255,144 fingerlings were stocked. Of those 9 Sauger, 3 (33.3%) were assigned parentage to hatchery broodstock. Sixteen age-2 individuals from the 2013-year class were caught in 2015 and 5 (31.3%) were assigned to hatchery parentage. Sixty-three age-1 Sauger caught in 2015 represented the 2014-year class, a year when 253,226 fingerlings were stocked. Of those 63 individuals, 8 (12.7%) were assigned hatchery parentage.

Discussion

Highly variable molecular markers are a valuable tool for assessing the efficacy of stocking programs and their impact on genetic diversity. Earlier attempts to measure the success of the Sauger hatchery program on the Old Hickory Lake population have had ambiguous results. Bettoli and Fischbach (1998) compared catch rates of adults to the number of fingerlings stocked in previous years to infer that stocking fingerlings boosted Sauger recruitment in some Tennessee reservoirs. However, Bettoli and Fischbach (1998) were not able to distinguish fish caught at

Table 2. Year class, sampling year, age, number of Sauger collected, and percent contribution of hatchery-reared Sauger stocked into Old Hickory Lake in 2013 and 2014. The number of collected Sauger represent the Sauger that were of appropriate age to be potential progeny of broodstock.

Year class	Sampling year	Age	Number collected	Percent contribution
2013	2014	1	9	33.3
2013	2015	2	16	31.3
2014	2015	1	63	12.7

age-1 or age-2 as wild or hatchery-reared. Therefore, it is unclear as to whether the increased catch rates were due to the success of stocking efforts, or if recruitment of native fish was higher during those years. Our molecular approach provides a clearer connection between stocking and recruitment and suggests that the contribution of hatchery fish to wild populations is more modest than suggested by indirect assessments. Our molecular assessment has also provided valuable insights into the impacts of hatchery supplementation on the genetic diversity of Sauger at Old Hickory Lake. The genetic impact of augmentation programs is of increasing concern because both empirical and theoretical studies have shown that large-scale hatchery supplementation can negatively impact genetic variation, population structure, and the potential for adaptation (Hansen et al. 2000a, Laikre et al. 2010).

One surprising result from our research that warrants further investigation was the observed deficiency of heterozygotes compared to Hardy–Weinberg expectations across all loci. There are several possible explanations for this pattern. First, the presence of null alleles may have contributed to reduced heterozygosity. However, it is unlikely that null alleles were present in all 8 examined loci. Furthermore, null alleles are more commonly found in populations with large effective-population sizes (Chapuis and Estoup 2006), which was not observed in the Old Hickory Lake population. A second possible explanation is that genotyping error may have led to a heterozygote deficit; however, we rescored all electropherograms and found no evidence of miscalled genotypes in the dataset. The observation of heterozygote deficiency across the majority of loci suggests that population-level factors and not locus-specific factors are influencing genotype frequencies. For example, a deficiency of heterozygosity can be a result of the Wahlund effect (Wahlund 1928). The Wahlund effect occurs when a sample from multiple sub-populations is combined as a single population during genetic-data analysis. Pooling data from multiple subpopulations results in a deficiency of observed heterozygotes, even if each sub-population is in Hardy–Weinberg equilibrium. Sauger in Old Hickory Lake may be divided into separate migratory breeding populations, which could explain the deficiency in heterozygosity. The Wahlund effect has been observed in stocked populations of *Sander vitreus* (Mitchill) (Walleye) (Carroffino et al. 2011) and can occur without obvious barriers to gene flow. Kazyak et al. (2016) found evidence of relatively little genetic exchange among *Salvelinus fontinalis Mitchill (Brook Trout*) occurring in the same spatial habitat, which lacked obvious physical boundaries. Finally, low heterozygosity can also be caused by non-random mating within a population; inbreeding results in a genome-wide deficit of observed heterozygosity compared with Hardy–Weinberg expectations. Evidence of non-random mating has been reported in other stocked populations (Cagigas et al. 1999, Marie et al. 2010), where preferential matings within native fish (or hatchery fish) may have contributed to a genome-wide deficit of observed heterozygotes.

Measures of genetic variation including the number of alleles per locus and heterozygosity values were relatively high in the study population and were comparable to what has been found in other percids. Bingham et al. (2011) used microsatellites to analyze the genetic population structure of Sauger and Walleye in

the upper Missouri River drainage and found that the average expected heterozygosity values for Sauger and Walleye were 0.689 and 0.809, respectively. Eldridge et al. (2002) sought to determine relative survival of 2 stocked populations of Missouri Walleye and calculated expected heterozygosity to be 0.68 and 0.65. Finally, Cena et al. (2006) estimated expected heterozygosity in 46 Walleye populations across Ontario, Canada, using the same loci used in the present study and found the average heterozygosity across all populations to be 0.73. Expected heterozygosity averaged across all 8 loci in the present study was slightly higher than values observed in these Walleye populations at 0.817.

Despite the high levels of observed genetic variation, estimates of effective population size were generally small and are likely to be far less than the census size, though the actual population size of Sauger in Old Hickory Lake is unknown. Point estimates of effective population size did differ between the 2 applied methods; the temporal method estimated a smaller effective population size than was calculated from the linkage disequilibrium method. Temporal methods of estimating effective population size are based on the idea that allele frequencies change more rapidly in populations with small effective population size due to genetic drift. This method assumes that generations are not overlapping, which is likely to be violated in the present study. Estimates of effective population size using the temporal method in populations with non-discrete generations will cause a bias unless representatives from all ages present in the population are sampled (Waples and Yokota 2007). Calculations of effective population size based on linkage disequilibrium varied across sampling years and had broad confidence intervals. Although our inferences rely on large samples sizes (>90 individuals per sampled year) and high allelic richness, the precision of these estimates may have been limited by the small number of loci examined. Tallmon et al. (2010) showed that confidence intervals obtained from linkage disequilibrium estimates of effective population size decrease rapidly as the number of loci increases and recommend a target of 15 loci to obtain robust estimates with finite confidence intervals. Although estimates of effective population size varied, both methods estimated a low effective population size, which is similar to what has been observed in other stocked populations where unequal contributions of hatchery raised progeny results in reduced effective population size relative to census numbers (Gold et al. 2008, Hansen et al. 2000b, Karlsson et al. 2008, Romo et al. 2005,; Romo et al. 2006, Taniguchi et al.1983). Results from both methods suggest that the current effective population size is less than what is recommended to maintain long-term population viability and evolutionary potential (Franklin and Frankam 1998, Hastings et al. 2008). Changes to stocking methodology that prioritize the maintenance of genetic variation are needed to ensure long-term population persistence and adaptive potential.

A population-bottleneck event may have contributed to a reduction in the effective population size of Sauger at Old Hickory Lake. The construction of Cordell Hull Dam in 1973 had a severe effect on spawning habitats and reproductive migrations of Sauger, and likely contributed to population declines (Scholten 2014). In the event of a bottleneck, rare alleles are lost at a faster rate than heterozygosity.

Populations that have experienced a bottleneck event will possess fewer alleles at low frequencies. Results of our M-ratio test did not suggest a past bottleneck event because we found a high proportion of alleles at low frequency. Additionally, populations that have experienced bottlenecks display an excess of Hardy–Weinberg expected heterozygosity compared to what is expected under drift-mutation equilibrium (Cornuet and Luikart 1996). Results of the present study were suggestive of heterozygosity excess; an excess of expected heterozygosity was detected in 6 of the 8 loci measured. However, these results were not significant. The inclusion of additional loci could increase the power to detect a historical bottleneck using the heterozygosity-excess test (Peery et al. 2012).

The efficacy of the Sauger stocking program at Old Hickory Lake appears to be modest compared to what has been observed for some Walleye stocking programs. Contributions of stocked fry to adult catch in Walleye elsewhere have been reported to be nearly 100% (Logsdon et al. 2016, Lucchesi 2002). Success of Sauger stocking programs has been more unpredictable and frequently unsuccessful in increasing population size (Baker 2015, Heidlinger and Brooks 1998). When considering the results from the 2012 pilot study at Old Hickory Lake, stocking contributions at age-1 were only 8.0–12.7% in 2 of 3 years. In the third year (the 2013 year-class), stocked fish represented about a third of all fish collected. It is important to note the sample sizes representing the 2013 year-class were low. We sampled on 10 dates in 2014 and 2015, and the low catches of the 2013 year-class (compared to the much higher high catch of the 2014 year-class) indicated that the 2013 year-class was weak. Thus, more-robust conclusions regarding the effects of hatchery programs on wild populations may require larger sample sizes than what was obtained here. The small sample sizes obtained in this study may reflect the overall decline of Sauger in Old Hatchery Lake.

Several factors may have contributed to the limited success of TWRA's Sauger stocking program at Old Hickory Lake. In large, regulated riverine systems such as Old Hickory Lake, the influence of hydrology on the survival of juveniles and subsequent recruitment is well recognized. In Lewis and Clarke Lake, SD, ~700,000 Sauger were flushed following 24 h of high discharge (Benson 1973), and mortality of individuals less than 25 mm TL were related to flushing rates in the same system (Walburg 1972). A study in Brier Creek, OK, found that age-0 fishes <10 mm TL were physically damaged following displacement (Harvey 1987). As noted above, the presence or lack of suitably-sized zooplankton prey at the time and place of stocking is known to influence the success of percid stocking programs (Kerr 2011), but those aspects of TWRA's Sauger stocking program have not been studied. The TWRA conducts annual creel surveys of fishing pressure, harvest, and number of fish of each species caught each year from Old Hickory Lake; those catch and harvest statistics date back to 1998. When the number of Sauger caught and harvested each year is regressed against the number of fingerlings stocked the previous year, there is no statistical relationship (df = 1,15, $F \leq 0.15$, $r^2 \leq 0.01$; $P \geq 0.7003$). Likewise, no statistical relationship exists when the number of fingerlings stocked and catch and harvest 2 years later are compared (df = 1,14, $F \leq 1.82$, $r^2 \leq 0.11$,

$P \geq 0.1989$). The lack of relationships between the number of fingerlings stocked annually and subsequent catch and harvest statistics is further, albeit indirect, evidence that the Sauger stocking program in Old Hickory Lake is not contributing meaningfully or predictably to the wild population.

Some changes in stocking methods have been shown to improve the success of hatchery programs. Laarman et al. (1978) suggested taking measures to reduce stocking stress to improve survivorship. Increasing the number of release sites may also improve stocking success (Barton 2011). The TWRA typically releases Sauger fingerlings at only 3–5 sites in Old Hickory Lake (Todd St. John, TWRA, pers. comm.). Increasing the number of stocking sites may positively affect hatchery success. Finally, temperature and zooplankton abundance are key factors in determining the success of stocked percids (Ney and Orth 1986, Santucci and Wahl 1993). Kerr (2011) suggested stocking at times that correspond with low water temperatures and peak abundance of appropriate food items, which will increase the survival of stocked fish. Ellison and Franzin (1992) observed greater success in Walleye programs that matched time and location of release with suitable food resources. The TWRA has never associated release times or locations to potential prey items. Hatchery contribution to the Sauger population in Old Hickory Lake might increase if stocking events occurred at times and locations where sufficient, suitably-sized prey existed.

Here we have shown that microsatellite analysis of Sauger in Old Hickory Lake can be used to assess the efficacy of TWRA's hatchery program and study the effects hatcheries have on the genetic health of the population. Increasing the number of fish collected from the reservoir and analyzed each year would provide more accurate estimates of stocking contributions and effective population size. Although the population genetic parameters we studied were based on a small number of loci, there were strong indications of reduced effective population size. Results also indicated that the current stocking program may not be contributing substantially to numbers of fish in the wild. These results stress the need for routine genetic monitoring of supportive breeding programs.

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