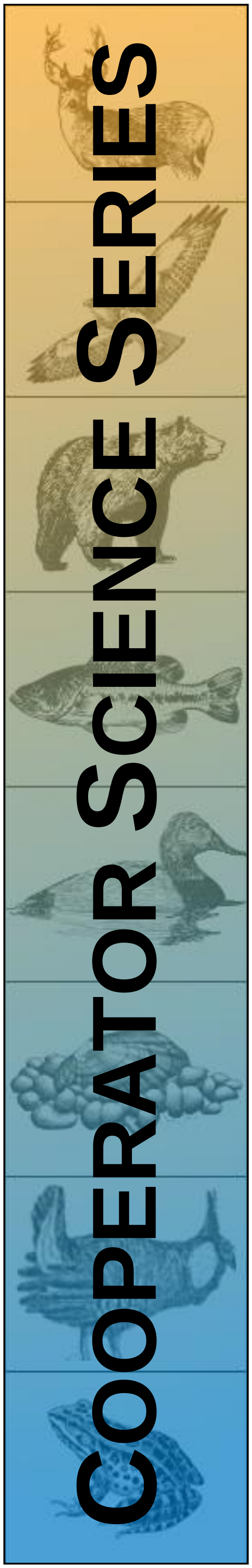

U.S. Fish and Wildlife Service

Identification of Neosho Smallmouth Bass (Micropterus dolomieu velox) Stocks for Possible Introduction into Grand Lake, Oklahoma

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IDENTIFICATION OF NEOSHO SMALLMOUTH BASS (*Micropterus dolomieu velox*) STOCKS FOR POSSIBLE INTRODUCTION INTO GRAND LAKE, OKLAHOMA

Final report to the Environmental Department of the Peoria Tribe of Indians of Oklahoma

August 2016

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EXECUTIVE SUMMARY

Stocking black basses (*Micropterus* spp.) is a common practice used to increase angling opportunities in impoundments; however, when non-native black basses are introduced they often invade riverine habitats where they threaten the persistence of other fishes, including native black basses. Neosho Smallmouth Bass (*M. dolomieu velox*) is endemic to portions of the Ozark Highlands and Boston Mountains ecoregions and is threatened by introductions of non-native Smallmouth Bass ("SMB") forms. Because of recent interest in stocking SMB into Grand Lake o' the Cherokees, we assessed the suitability of local Neosho SMB populations as potential broodstock sources by assessing introgression with non-native SMB forms, as well as characterizing population structure and genetic diversity. The majority of Neosho SMB populations contained low, but non-negligible, genomic proportions of two genetically distinct non-native SMB forms. Introgression was highest in the Illinois River upstream of Lake Tenkiller, where Tennessee 'lake strain' SMB were stocked in the early 1990's. We recovered three genetically distinct clusters of Neosho SMB at the uppermost hierarchical level of population structure: a distinct Illinois River cluster and two Grand River clusters that appear to naturally mix at some sites. Genetic diversity measures generally increased with stream size, and smaller populations with low diversity measures may benefit from immigration of novel genetic material. Overall, introgression with non-native SMB forms appears to pose a prominent threat to Neosho SMB; however, relatively intact populations of Neosho SMB exist in some Grand Lake o' the Cherokees tributaries. Results could be used in developing a stocking program that promotes and sustains existing genetic diversity within and among Neosho SMB populations.

INTRODUCTION

Black basses (*Micropterus* spp.) comprise one of the most popular sport fisheries in the U.S. and support a multi-billion dollar industry (USFWS 2006; Long et al. 2015), yet many forms are of conservation concern. Ten of thirteen described black bass species and five additional recognized forms are endemic to southeastern drainages (Birdsong et al. 2010; Baker et al. 2013; Tringali et al. 2015). Many of these species occupy relatively limited native ranges and usually occur in lotic habitats (Birdsong et al. 2010). However, impoundments have fragmented these free-flowing habitats and converted them into still bodies of water. Wide-ranging species like Largemouth Bass (*M. salmoides*), Spotted Bass (*M. punctulatus*), and Smallmouth Bass (*M. dolomieu*) that tolerate lentic systems are often stocked or introduced into impoundments outside their native ranges to increase angling opportunities. In many instances, the introduction of non-native black basses in impoundments has led to the invasion of these species into native fluvial fish communities (Marchetti et al. 2004; Guenther and Spacie 2006). Non-native black basses pose a threat to native congeners, as hybridization and subsequent backcrossing leads to introgression of non-native alleles into native gene pools (Barwick et al. 2006; Littrell et al. 2007). In extreme cases, non-native black basses have replaced native congeners altogether (Barwick et al. 2006; Stormer and Maceina 2008; Leitner et al. 2015). Conservation efforts for native black basses have increased in the last decade (Tringali et al. 2015), prompting resource managers to be more cognizant of the potential ramifications of stocking non-native black basses.

Smallmouth Bass is a wide-ranging species occurring in lakes, impoundments, and streams, but genetic and ecological variation exists across its geographic range. Two described subspecies of Smallmouth Bass ("SMB") occur; the widely-distributed Northern SMB (*M. d.*

dolomieu) and the narrow-ranging Neosho SMB (*M. d. velox*). The Neosho subspecies is endemic to tributaries of the Arkansas River that drain the Ozark Highlands and Boston Mountains ecoregions of Oklahoma, Missouri, Arkansas, and Kansas (Hubbs and Bailey 1940), and exhibits ecological and life-history aspects different from other SMB forms (Brewer and Long 2015). The Neosho subspecies is not only genetically distinct from Northern SMB inclusive of the remainder of the Ozark Highlands (Stark and Echelle 1998), but also different from an additional, undescribed endemic species of the Ouachita Highlands of Oklahoma and Arkansas (Ouachita SMB). The diversity within Smallmouth Bass native to the Interior Highlands is therefore unmatched throughout the species' range (Stark and Echelle 1998).

Previous stocking activities have likely affected the genetic composition of native SMB forms, but some have been discontinued given the recent understanding of diversity in the region. In the late 1980's, Oklahoma Department of Wildlife Conservation (ODWC) began stocking Northern SMB from Percy Priest Lake, Tennessee – the TN 'lake strain'– into various Oklahoma impoundments (Boxrucker et al. 2004). Among these impoundments included Lake Tenkiller (stocked in 1991-1992) within the Neosho SMB's native range and Broken Bow Lake (stocked in 1993) within the Ouachita SMB's native range (Boxrucker et al. 2004). In response to findings of Stark and Echelle (1998), ODWC discontinued stocking within the native ranges of both native SMB forms (Boxrucker et al. 2004). Subsequent assessment of the Broken Bow lake-river complex showed introgression of Ouachita SMB with TN 'lake strain', and non-native alleles have steadily moved upstream from stocking sites within the lake (Malloy 2001; Boxrucker et al. 2004). A similar assessment of the Lake Tenkiller region within the range of Neosho SMB has yet to be conducted. This is particularly relevant in light of recent interest by some angling groups to stock TN ‘lake strain’ SMB into Grand Lake O’ the Cherokees, which is within the range of Neosho SMB.

Grand Lake O’ the Cherokees (“Grand Lake”) is an 18,800 ha impoundment in the Ozark Highlands of northeast Oklahoma. Grand Lake supports a renowned black bass fishery comprised of native Largemouth Bass and Spotted Bass, but SMB are rare in the impoundment. Although Neosho SMB are common in tributaries of Grand Lake, such as the Elk River, the relative lack of SMB in the impoundment has led some angling groups to express desire to stock TN ‘lake strain’ SMB in Grand Lake (Boxrucker et al. 2004). Whether native Neosho SMB could serve as a source for stocking and provide a successful reservoir fishery is unknown, but their use could alleviate concerns of genetic introgression with riverine residents associated with stocking the TN ‘lake strain’.

Informing hatchery programs through the management of genetically distinct populations or ‘stocks’ is increasingly common (Hallerman 2003). Maintaining genetic diversity within and among populations can safeguard against the loss of local adaptations and maintain the evolutionary capacity of a population (or group of populations) to respond to changing environments. The population-genetic paradigm also discourages unnatural movement and stocking across natural population boundaries because the mixing of dissimilar populations can have unpredictable population-level effects. Among these unpredictable effects is outbreeding depression, which is caused by the introgression of maladapted genes or the disruption of coadapted genomes (Lynch 1991) resulting in a loss of fitness and increased susceptibility to disease (Hallerman 2003; Goldberg et al. 2005). Traditional hatchery operations – broodstock collection, spawning, rearing, and release – can impose genetic hazards to native populations (Hallerman 2003). Background knowledge about introgression rates, population boundaries, and existing genetic diversity of source and target populations can therefore guide hatchery programs towards ameliorating negative repercussions.

OBJECTIVES

To inform the identification of potentially suitable brood sources for the possible introduction of Neosho Smallmouth Bass into Grand Lake, we addressed the following two objectives:

1) Identify sources of non-native Smallmouth Bass and assess introgression into native Neosho Smallmouth Bass populations in tributaries to Grand Lake and several neighboring drainages; and
2) Identify sources of native Neosho Smallmouth Bass potentially suitable for hatchery propagation by characterizing existing population structure (i.e., boundaries) and genetic diversity.

METHODS

Sample Collection – We targeted putative Neosho SMB populations along with several other relevant black bass forms. To investigate Neosho SMB stocks in tributaries to Grand Lake, we sampled Shoal Creek, Sycamore Creek, Honey Creek, Big Sugar Creek, Indian Creek, Buffalo Creek, and Elk River (**Figure 1**). Several areas downstream of Grand Lake were also sampled to assess native Neosho SMB stocks that may have been interconnected prior to impoundment construction: Spavinaw Creek, Lake Hudson, Illinois River, Baron Fork, and Caney Creek. Reference specimens of other SMB forms included TN ‘lake strain’ (Skiatook Lake, Lake Tenkiller, and a Grand River Dam Authority [GRDA] cooling pond) and fish from an unknown origin propagated by a private hatchery in Missouri (“MO hatchery”). Spotted Bass from several localities within the study area were also included as reference specimens, as they occur in natural sympatry with Smallmouth Bass and are known to hybridize (Koppelman 2015). Because the origin of MO hatchery fish was unknown, we included SMB samples from potential source locations in the Interior Highlands in our assignment of putative Neosho SMB genotypes. These locations in the White River system (White River and Crooked Creek, Arkansas) occur outside the present range of Neosho SMB, but fish in this system are considered intergrades between Neosho SMB and an Interior Highlands form of Northern SMB (Stark and Echelle 1998).

From May 2014 to March 2016, a multi-agency sampling effort by the Peoria Tribe, ODWC, and Oklahoma State University (OSU) targeted tissue collections for genetic analysis. We sampled using boat electrofishing, barge electrofishing, backpack electrofishing, and hook-and-line angling, with gear choice dependent on accessibility and habitat. Where practical, we sampled multiple locations within a given system to best characterize the genetic composition of the entire population. Individual sampling locations typically varied from approximately 100 m to 300 m in length, with geographic coordinates taken at each location. Fin clips were taken from the posterior edge of the caudal fin and stored at room temperature (25°C) in individually labeled vials of 95% non-denatured ethanol.

Molecular analyses – Genetic diversity and hybridization was assessed using seven di-nucleotide microsatellite DNA markers previously developed to amplify *Micropterus* (*Mdo03*, Malloy et al. 2000; *Msaf01*, *Msaf05*, *Msaf06*, *Msaf14*, *Msaf17*, and *Msaf29*, Seyoum et al. 2013). *Mdo03* has been used alongside other markers as an indicator of hybridization between TN ‘lake strain’ SMB and native Neosho and Ouachita SMB forms (Malloy 2001; Boxrucker et al. 2004), and *Msaf* makers have shown utility in assessing hybridization among *Micropterus* species (Seyoum et al. 2013; *see* Alvarez et al. 2015). Genomic DNA was isolated from fin clips using the DNeasy Blood and Tissue Kit (Qiagen Corp.) Samples were multiplexed in two reactions, one with four loci (*Msaf01*, *Msaf05*, *Msaf14*, and *Msaf17*) and the other with three loci (*Mdo03*, *Msaf06*, and *Msaf29*). The following PCR amplification parameters were used for all loci: 95°C for 15 min, 35 cycles of 94°C for 30 s, 58°C for 90 s, 72°C for 90 s, and 72°C for 10 min. The multiplex reaction mix (10 μL total volume) contained 1-3 ng of template DNA in 1 μL ddH_20, 0.122 μL of each primer (10 μM), 4.025 μL ddH_2O and 4 μL Qiagen Multiplex PCR mix. Capillary electrophoresis using an ABI 3730 Genetic Analyzer was performed on solutions containing 1 μL post-amplification reaction mix (diluted 1:100), 0.2 μL Genescan ROX 500 size standard (Applied Biosystems, Inc.), and 9 μL formamide (Applied Biosystems, Inc.). Length variants were visualized and genotyped using GeneMapper v. 5 (Applied Biosystems, Inc.). Genotyping errors were evaluated by rescoring 10% of individuals. Individuals missing data at more than one locus were removed prior to analysis. In streams with multiple sampling locations, genotypes were arranged from upstream to downstream within a given creek or river system (i.e., “site”).

Objective 1 – We screened all putative Neosho SMB genotypes against reference taxa that included Spotted Bass, TN ‘lake strain’ SMB, and MO hatchery SMB. For reference Neosho SMB genotypes, we used a preliminary population assignment (see detailed methods that follow, but without the ‘PopFlag’ option) to identify 40 individuals across eight putative Neosho SMB sites that were assigned > 10% to clusters affiliated with known non-native SMB forms. We used a Bayesian clustering approach implemented in programs STRUCTURE v. 2.3.4 (Pritchard et al. 2000), STRUCTURE HARVESTER web v. 0.6.94 (Earl and vonHoldt 2012), and

CLUMPP v. 1.1.2 (Jakobsson and Rosenberg 2007) to estimate the taxonomic composition of putative Neosho SMB genotypes. Program STRUCTURE proportionally assigns individual genotypes to a given number of genetic clusters (*K*) based on non-random associations between alleles (i.e., linkage equilibrium) and conformance to Hardy-Weinberg equilibrium (Pritchard et al. 2000). Individual genotypes are thus assigned probabilistically to populations with some degree of uncertainty surrounding assignments (Pritchard et al. 2000).

In STRUCTURE, we used the admixture ancestry model and assumed allele frequencies were independent, with a 20,000 burn-in length and 200,000 Markov chain Monte Carlo (MCMC) iterations for each run. The 'PopFlag' option was employed so that genomic proportions for putative Neosho SMB genotypes were estimated based solely on the allele frequencies from reference genotypes. To determine the proper *K* value for taxonomic assignment of putative Neosho SMB samples, we ran five iterations each of $K = 1\text{-}10$ with only the reference genotypes. This exploratory analysis supported up to $K = 5$ distinct groups in the reference samples, but included two clusters within the reference Neosho SMB individuals. To avoid including substructure-level differences within Neosho SMB in the taxonomic assignment, we used $K = 4$, which mirrored the *a priori* reference groups (Spotted Bass, TN 'lake strain' SMB, MO hatchery SMB, and Neosho SMB), and ran 10 independent, randomly seeded runs for taxonomic assignment. Results were uploaded into STRUCTURE HARVESTER to obtain input files for CLUMPP, which provided an optimal alignment from the 10 independent STRUCTURE runs using cluster matching and permutation (Jakobsson and Rosenberg 2007). Within CLUMPP, we used the *G*' pairwise matrix similarity statistic and the 'Greedy' algorithm for 1,000 randomly sequenced runs. Final results from CLUMPP were used to estimate individual genomic proportion assignments, classify individuals into hybridization categories, and estimate the overall genomic proportions of each taxon by sample site. Because uncertainty in STRUCTURE's taxonomic assignments can result in small amounts of false signals (low proportional assignments to a given population), we employed the following classification of individuals into hybridization categories (Dakin et al. 2015): 'pure' species were ≥ 90% assignment to one respective group, 'backcrosses' were 75-90% assignment to one respective group, and all remaining individuals were considered first filial generation (F1) or later-generation hybrids.

Objective 2 – We characterized population structure and genetic diversity within Neosho Smallmouth Bass using individual genotypes classified as "pure' Neosho Smallmouth Bass in Objective 1. To assess population structure, we again used a Bayesian clustering approach in STRUCTURE using the same program settings but without the 'PopFlag' option. Because uneven sampling can influence results (Puechmaille 2016), we analyzed two datasets: one that contained all 'pure' Neosho SMB and one that contained ≤ 25 randomly selected individuals per site. We ran 10 independent, randomly seeded iterations of $K = 1\text{-}5$ for both datasets. We then estimated the number of genetic clusters (K) at the uppermost level of hierarchical genetic structuring within both datasets using a suite of four supervised estimators (MedMeaK, MaxMeaK, MedMedK, and MaxMedK) developed by Puechmaille (2016). These supervised estimators disregard 'spurious clusters' that fail to obtain a mean or median membership coefficient threshold of 0.5, and were found to outperform existing methods that produce downward-biased estimates of K (Puechmaille 2016). If differences in K occurred among datasets, we used the subsampled dataset results to produce final estimates of K. Final genomic proportion assignments based on the final K value were obtained in CLUMPP. Results were used to estimate individual proportional membership to K clusters and overall genomic proportions of each cluster by study site.

To characterize the genetic diversity within 'pure' Neosho SMB, we calculated a number of genetic diversity measures by site. We calculated the mean and SE over seven loci for each site using programs GENALEX v. 6.502 (Peakall and Smouse 2006) and FSTAT v. 2.9.3 (Goudet 2001) for the following measures: number of alleles (A), effective number of alleles (A_e), allelic richness (A_R), expected heterozygosity (H_e), observed heterozygosity (H_o), and the inbreeding coefficient (F). Measures A, A_e, and A_R are slightly different characterizations of allelic diversity; A_e is less sensitive to the inclusion of rare alleles (Kimura and Crow 1964) and A_R accounts for variation in sample sizes among sites to represent the number of alleles that would be expected from equal sample sizes in all sites. Heterozygosity is the state of an individual containing two different alleles at a given locus. When averaged across individuals and loci, heterozygosity gives an overall indication of relative diversity. Large, randomly-mating populations generally conform to Hardy-Weinberg expectations for heterozygosity unless influenced by other forces; thus, comparisons of H_e and H_o can indicate inbreeding. To directly characterize this phenomenon, F ($= [H_e - H_o]/ H_e$) values close to zero are indicative of random mating, whereas large positive values indicate inbreeding and negative values indicate excess heterozygosity (Peakall and Smouse 2006). The total number of private alleles ($A_{private}$) across all seven loci was also reported, which can be used to identify sites that may harbor unique diversity as well as a way to measure connectivity among sites.

Finally, we estimated the effective population size (N_e) for each site using the single-sample, linkage-disequilibrium estimator with Burrows' modification as implemented in NEESTIMATOR v. 2.01 (Waples and Do 2008; Do et al. 2014). Because low-frequency alleles can upwardly bias estimates of N_e using the linkage disequilibrium method, we computed estimates and their associated 95% confidence intervals after we removed alleles at threshold frequencies of: < 5%, < 2%, < 1%, and 0% (Waples and Do 2008). The resulting N_e estimates represent the number of reproducing adults in an 'ideal' population that would lose genetic variation at the same rate as the number of reproducing adults in the sampled population (Hallerman 2003). Because migration among sites may violate the assumption that only genetic drift is operating within each site for site-specific N_e estimates (Waples and Do 2008), we also estimated N_e for demographically connected units by removing migrants (*sensu* Neel et al. 2013); those fish with genomic proportions ≥ 90% assignment to a cluster other than the locally predominant cluster. We considered demographically connected units as those sites that shared a common genetic cluster and were not separated by dams. General rules of thumb for interpreting N_e estimates are: populations with $N_e > 500$ have low demographic and genetic risks to viability; populations with $500 > N_e > 50$ have some risk from demographic stochasticity and may be vulnerable to loss of genetic variability via random genetic drift and inbreeding depression if immigration is low; and populations with $N_e < 50$ are likely at risk from demographic stochasticity and may be losing genetic variation via random genetic drift and inbreeding depression if immigration is low (Franklin 1980; Soulé 1980).

RESULTS

Multilocus genotypes from 873 individuals were used for taxonomic assignment, of which 152 were reference specimens and 721 were putative Neosho SMB sampled from 14 sites. Samples from White River and Crooked Creek were pooled into a White River system group, resulting in 13 sites in our analyses. Genotype totals were reported for each site along with sampling locality information for reference genotypes (**Table 1)** and putative Neosho SMB genotypes **(Table 2).** For ease of interpretation, site-specific summaries of results were organized into four general geographic areas (Grand Lake, Below Grand Lake, Lake Tenkiller, and White River system) and reported in **Appendix I**.

Objective 1 – The resulting STRUCTURE plot illustrates individual genomic proportion assignments among four genetic clusters (*K* = 4; **Figure 2**). In our reference samples, some putative TN ‘lake strain’ individuals from Lake Tenkiller contained genomic contributions from MO hatchery and Neosho SMB, and one fish from MO hatchery was assigned to the TN ‘lake strain’ cluster. Only 9 of 721 (1.2%) putative Neosho SMB individuals had proportional assignments > 25% to Spotted Bass, and these individuals comprised < 5% of any population (highest rate was 4.87% in Sycamore Creek; **Figure 2**). As such, we focused on introgression between the three genetic groups of Smallmouth Bass (**Figures 3-4**). The two putative Neosho sites that appeared most impacted by non-native SMB forms were Illinois River (overall genomic proportion of TN ‘lake strain’ = 28.2%) and Shoal Creek (overall genomic proportions of MO hatchery = 13.5% and TN ‘lake strain’ = 7.2%), although the sole fish from Lake Hudson was backcrossed TN ‘lake strain’. Grand Lake area sites had overall genomic proportions of Neosho SMB ranging from 77.6% (Shoal Creek) to 97.0% (Honey Creek).

Objective 2 – The genotypic dataset of ‘pure’ Neosho SMB (482 total individuals) supported *K* = 3 as the optimal number of genetic clusters based on the supervised estimators of Puechmaille (2016), regardless of whether the complete or the subsampled dataset was examined. The resulting STRUCTURE plot for the complete dataset (**Figure 5**) illustrates individual genomic proportion assignments of Neosho SMB genotypes to the three genetic clusters that were recovered: “Grand River 1”, “Grand River 2”, and “Illinois River”. Overall genomic proportions by site (**Figure 6)** indicate that Neosho SMB from the Illinois River, Baron Fork, and Caney Creek represent a distinct population from fish inhabiting other sites examined in our study. The two Grand River clusters indicate population structuring among Grand Lake area sites, although most sites contained a mixture of both clusters. Genetic diversity measures (**Table 3**) by site showed that Elk River harbored the most native diversity of all sites considered, with Big Sugar Creek, Buffalo Creek, and Illinois River all containing higher than average diversity measures. Sites with lower than average diversity measures included smaller systems like Caney Creek, Sycamore Creek, and Honey Creek. Estimates of N_e varied across the allelic frequency thresholds examined (**Tables 4-5**), but we used estimates at the < 5% threshold to interpret results because they should be less influenced by rare alleles and, thus, less biased (Waples and Do 2008). Point estimates of N_e by site were generally greater in larger streams, as larger sites like Illinois River and Elk River had some of the highest point estimates (450 and 276, respectively) whereas smaller streams like Honey Creek and Buffalo Creek had the lowest estimates (51 and 29, respectively). Estimates of N_e with migrants removed were calculated for the following demographically-connected units: Shoal Creek; Sycamore and Honey creeks; Elk River and Big Sugar, Indian, and Buffalo creeks; Spavinaw Creek; and Illinois River, Baron Fork, and Caney Creek. Point estimates of N_e for demographically connected units without migrants were similar to site-specific estimates: lowest in Sycamore and Honey creeks (66) and highest in the Illinois River, Baron Fork, and Caney Creek and Illinois River (628). The Elk River and its tributaries had an intermediate N_e estimate of 167.

DISCUSSION

Introgression of non-native SMB forms was detected in all Neosho SMB populations surveyed; however, the severity of introgression appeared to vary with proximity to impoundment and stream size. The most introgressed populations were associated with Lake

Tenkiller, where previous stockings have occurred. There, pure TN 'lake strain' individuals were detected 55 river-kilometers upstream of the river-reservoir interface into the Illinois River (Round Hollow public access, the farthest upstream site sampled) and introgressive hybridization with native Neosho SMB appears to be prevalent throughout the sampled reach. These results mirror those found with native Ouachita SMB, whose gene pool was affected by non-native TN 'lake strain' alleles invading upstream from Broken Bow Lake (Boxrucker et al. 2004) and suggest a general pattern of native populations in upstream tributaries being impacted by non-native, yet related, forms stocked in impoundments. Although non-native SMB forms have not been stocked by the state in the Grand Lake area, a backcrossed TN 'lake strain' individual was found in Lake Hudson. Furthermore, results from Grand Lake tributaries, along with occasional reports of anglers catching SMB in the main lake, suggest that TN 'lake strain' SMB may occur at low abundance in the Grand Lake area. Anglers have historically advocated for ODWC to stock TN 'lake strain' in Grand Lake (Boxrucker et al. 2004), but how TN 'lake strain' entered these systems is unknown. Interestingly, Neosho SMB gene pools in smaller tributaries, particularly areas farther upstream from impoundment interfaces, were less altered by TN 'lake strain' genetics.

Some results are consistent with the hypothesis that MO hatchery fish represent Interior Highlands SMB. SMB from White River and Crooked Creek, a natural intergrade zone between Neosho SMB and Interior Highlands SMB (Stark and Echelle 1998), had overall genomic proportions of 23.7% MO hatchery, suggesting the MO hatchery stock may have originated from Interior Highlands SMB. Despite this evidence, a broad-scope genetic survey of Northern SMB would be necessary to definitively confirm the source of the MO hatchery SMB found in our systems. Regardless, our results suggest that MO hatchery SMB genes are not currently as widespread as TN ‘lake strain’ genes within the Neosho SMB’s range. The combination of two non-native SMB forms comingling with Neosho SMB may foster increased hybridization and disruption of native, coadapted gene complexes (*sensu* Koppelman 2015). Because of the widespread occurrence of non-native SMB alleles in the Neosho SMB’s native range, genetic screening of any potential brood fish is warranted.

The population structure and genetic diversity measures reported herein can help inform hatchery procedures, like broodstock collection, that balance the risks inherent with outbreeding and inbreeding depression. Mixing of populations with pronounced differences, such as mixing fish from the “Illinois River” cluster with either “Grand River” cluster, could disrupt coadapted gene complexes and result in outbreeding depression (Lynch 1991). The Grand River clusters each likely contain some unique adaptations, although natural mixing appears to occur in at least 6 of 8 sites (75%), with possible exceptions in Sycamore and Honey creeks. The Grand River 1 cluster consisted of populations found in small stream systems, such as Sycamore, Honey, and Spavinaw creeks, that are separated by Grand Lake and area dams. The Grand River 2 cluster was associated with larger stream systems, such as Shoal Creek, Elk River, and Elk River tributaries. Differences in the frequencies of the most common alleles in each Grand River cluster, along with a lack of rare alleles in Grand River 1 cluster, appeared to contribute to population structure signals. The three genetic clusters recovered in this study represent the uppermost level of hierarchical population structure (Puechmaille 2016), and genetic structure relevant to stocking programs could exist at finer scales. Thus, obtaining broodstock from streams in close geographic proximity to Grand Lake could help avoid artificial mixing of populations by stocking – an activity that could lead to outbreeding depression in native populations.

Although minimizing risks for outbreeding depression is warranted, measures of genetic diversity also suggest that minimizing potential inbreeding depression and alleviating low effective population sizes may also be important considerations. Diversity was generally highest in larger streams where connectivity among populations appears high, whereas smaller streams had lower measures of diversity. Lower diversity in smaller streams may result from isolating mechanisms related to habitat availability or from anthropogenic alterations. For example, Sycamore, Honey, and Caney creeks are direct tributaries to impoundments that may serve as a barrier to gene flow. Additionally, Honey Creek has a history of fish kills (Oklahoma Water Resources Board 2000), which may further account for the low genetic diversity observed there. A genetically diverse Neosho SMB broodstock for introduction into Grand Lake could support existing diversity and evolutionary potential while potentially encouraging immigration of novel genetic material into isolated populations that may be vulnerable to inbreeding depression (e.g., Honey Creek).

This study represents the first directed population genetic investigation of Neosho SMB since its genetic distinctiveness was discovered (Stark and Echelle 1998), and our findings have direct implications for potential hatchery-based introduction of Neosho SMB into Grand Lake. Because introgression of non-native SMB alleles has occurred in all Neosho SMB populations examined, genetic screening of possible brood fish is warranted. Furthermore, accidental mixing or stocking of non-native SMB could be best avoided by keeping only pure Neosho SMB on hatchery grounds while actively excluding any non-native SMB forms and their associated hybrids. Consideration of population boundaries and genetic diversity within and among Neosho SMB populations in development of broodstock collection, propagation, and release procedures can serve to complement and sustain native biodiversity, instead of diminishing it (e.g., outbreeding or inbreeding depression). Such precautionary measures should help ensure that the relatively diverse Neosho SMB populations of the Elk River and other Grand Lake tributaries remain intact.

REFERENCES

Alvarez, A., D. Peterson, A. Taylor, M. Tringali, and B. Barthel. 2015. Distribution and amount of hybridization between Shoal Bass *Micropterus cataractae* and the invasive Spotted Bass *Micropterus punctulatus* in the lower Flint River, GA. Pages 503-521 *in* Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. *Black Bass Diversity: Multidisciplinary Science for Conservation*. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Baker, W. H., R. E. Blanton, and C. E. Johnston. 2013. Diversity within the redeye bass, *Micropterus coosae* (Perciformes: Centrarchidae) species group, with descriptions of four new species. *Zootaxa* 3635(4):379-401.

Barwick, D. H., K. J. Oswald, J. M. Quattro, and R. D. Barwick. 2006. Redeye Bass (*Micropterus coosae*) and Alabama Spotted Bass (*M. punctulatus henshalli*) hybridization in Keowee Reservoir. *Southeastern Naturalist* 5(4):661-668.

Birdsong, T., D. Krause, J. Leitner, J.M. Long, S. Robinson, and S. Sammons. 2010. A business plan for the conservation of native black bass species in the southeastern US: a ten year plan. National Fish and Wildlife Foundation, Washington, D.D. Pp.1-59.

Boxrucker, J., A. A. Echelle, and R.A. Van Den Bussche. 2004. Determining the degree of hybridization in the Smallmouth Bass population of Broken Bow Reservoir and the Mountain Fork River. Oklahoma Department of Wildlife Conservation, Final Report F-50-R, Project 19, Oklahoma City, Oklahoma.

Brewer, S. K., and J. M. Long. 2015. Biology and ecology of Neosho Smallmouth Bass and the genetically-distinct Ouachita lineage. Pages 281-296 *in* Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. *Black Bass Diversity: Multidisciplinary Science for Conservation*. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Dakin, E.E., B.A. Porter, B.J. Freeman, and J.M. Long. 2015. Hybridization threatens shoal bass populations in the upper Chattahoochee River basin. Pages 491-502 *in* Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. *Black Bass Diversity: Multidisciplinary Science for Conservation*. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NEESTIMATOR v2: re-implementation of software for the estimation of contemporary effective population size (Ne) from genetic data. *Molecular Ecology Resources* 14(1):209-214.

Earl D. A., and B. M. vonHoldt. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* 4(2):359-361.

Franklin, R. 1980. Evolutionary change in small populations. Pages 135-149 *in* Soulé, M. E., and B. A. Wilcox, editors. *Conservation Biology: An Evolutionary-Ecological Perspective.* Sinauer, Sunderland, Massachusetts.

Goldberg, T. L., E. C. Grant, K. R. Inendino, T. W. Kassler, J. E. Claussen, and D. P. Philipp. 2005. Increased infectious disease susceptibility resulting from outbreeding depression. *Conservation Biology* 19(2):455-462.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available at http://www2.unil.ch/popgen/softwares/fstat.htm

Guenther, C. B., and A. Spacie. 2006. Changes in fish assemblage structure upstream of impoundments within the upper Wabash River basin, Indiana. *Transactions of the American Fisheries Society* 135:570-583.

Hallerman, E. M., editor. 2003. *Population Genetics: Principles and Applications for Fisheries Scientists*. American Fisheries Society, Bethesda, Maryland.

Hubbs, C. L., and R. M. Bailey. 1940. A revision of the black basses (*Micropterus* and *Huro*) with descriptions of four new forms. *Miscellaneous Publications of the Museum of Zoology, University of Michigan, No. 48.* Ann Arbor, Michigan.

Jakobsson, M., and N. A. Rosenberg. 2007. CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. *Bioinformatics* 23:1801-1806.

Kimura, M., and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. *Genetics* 49(4):725-738.

Koppelman, J. B. 2015. Black bass hybrids: a natural phenomenon in an unnatural world. Pages 467-479 *in* Tringali, M.D., J.M. Long, M.S. Allen, and T. Birdsong, editors. *Black Bass Diversity: Multidisciplinary Science for Conservation*. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Leitner, J. K., K. J. Oswald, M. Bangs, D. Rankin, and J. M. Quattro. 2015. Hybridization between native Bartram's Bass and two introduced species in Savannah Drainage streams. Pages 481-490 *in* Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. *Black Bass Diversity: Multidisciplinary Science for Conservation*. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Littrell, B. M., D. J. Lutz-Carrillo, T. H. Bonner, and L. T. Fries. 2007. Status of an introgressed Guadalupe Bass population in a central Texas stream. *North American Journal of Fisheries Management* 27(3):785-791.

Long, J.M., M.S. Allen, W.F. Porak, and C.D. Suski. 2015. A historical perspective of black bass management in the United States. Pages 99-122 *in* Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. *Black Bass Diversity: Multidisciplinary Science for Conservation*. American Fisheries Society, Symposium 82, Bethesda, Maryland.

Lynch, M. 1991. The genetic interpretation of inbreeding depression and outbreeding depression. *Evolution* 45(3):622-629.

Malloy Jr., T. P. 2001. Introgressive hybridization between native and non-native Smallmouth Bass in Oklahoma. Doctoral Dissertation, Oklahoma State University, Stillwater, Oklahoma. 48 pp.

Malloy Jr., T. P., R. A. Van Den Bussche, W. D. Coughlin, and A. A. Echelle. 2000. Isolation and characterization of microsatellite loci in Smallmouth Bass, *Micropterus dolomieu* (Teleostei: Centrarchidae), and cross-species amplification in Spotted Bass, *M. punctulatus*. *Molecular Ecology* 9(11):1946-1948.

Marchetti, M. P., P. B. Moyle, and R. Levine. 2004. Invasive species profiling? Exploring the characteristics of non-native fishes across invasion stages in California. *Freshwater Biology* 49(5):646-661.

Neel, M. C., K. McKelvey, N. Ryman, M. W. Lloyd, R. Short Bull, F. W. Allendorf, M. K. Schwartz, and R. S. Waples. 2013. Estimation of effective population size in continuously distributed populations: there goes the neighborhood. *Heredity* 111(3):189-199.

Oklahoma Water Resources Board. 2000. *Status of Water Quality Monitoring in Oklahoma*. Final Report to the Oklahoma Legislature for Year 2000. 136 pp.

Peakall, R. O. D., and P. E. Smouse. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Resources* 6(1):288-295.

Pritchard J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945-959.

Puechmaille, S. J. 2016. The program STRUCTURE does not reliably recover the correct population structure when sampling is uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources* 16(3):608-627.

Seyoum, S., B. L. Barthel, M. D. Tringali, M. C. Davis, S. L. Schmitt, P. S. Bellotti, and W. F. Porak. 2013. Isolation and characterization of eighteen microsatellite loci for the Largemouth Bass, *Micropterus salmoides*, and cross amplification in congeneric species. *Conservation Genetic Resources* 5:697-701.

Soulé, M. E. 1980. Thresholds for survival: maintaining fitness and evolutionary potential. Pages 151-169 *in* Soulé, M. E., and B. A. Wilcox, editors. *Conservation Biology: An Evolutionary-Ecological Perspective.* Sinauer, Sunderland, Massachusetts.

Stark, W. J. and A. A. Echelle. 1998. Genetic structure and systematic of smallmouth bass, with emphasis on interior highlands populations. *Transactions of the American Fisheries Society* 127:393-416.

Stormer, D. G., and M. J. Maceina. 2008. Relative abundance, distribution, and population metrics of Shoal Bass in Alabama. *Journal of Freshwater Ecology* 23(4):651-661.

Tringali, M. D., J. M. Long, T. W. Birdsong, and M. S. Allen, editors. 2015. *Black Bass Diversity: Multidisciplinary Science for Conservation*. American Fisheries Society, Symposium 82, Bethesda, Maryland.

USFWS (United States Fish and Wildlife Service). 2006. National Survey of Fishing, Hunting, and Wildlife-Associated Recreation.

Waples, R. S., and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources* 8:753-756.

TABLES

Table 1. Locality and sampling information associated with reference taxa used to assess purity of putative Neosho Smallmouth Bass samples. Abbreviations are as follows: Smallmouth Bass (SMB), upstream (US), downstream (DS), river-reservoir interface (RRI), electrofishing (EF).

Taxa	**Site**	**Sampling Location**	**Year**	**Method**	**n**
Spotted Bass	Spring River	US of Grand Lake RRI	2014	Boat EF	7
Spotted Bass	Honey Creek	US of Grand Lake RRI	2015	Boat EF	2
Spotted Bass	Elk River	Multiple sites	2015	Various	2
Spotted Bass	Lake Hudson	(Unspecified)	2016	Angling	1
Spotted Bass	Illinois River	US of Lake Tenkiller RRI	2015	Various	18
					30
TN 'lake strain' SMB	Skiatook Lake	Multiple sites	2014	Boat EF	32
TN 'lake strain' SMB	Lake Tenkiller	Multiple sites on lower end	2014	Boat EF	29
TN 'lake strain' SMB	GRDA cooling pond	(Unspecified)	2014	(Unknown)	4
					65
MO hatchery SMB	Private hatchery, MO	(Unspecified)	2014	(Unknown)	17
					17
Neosho SMB	Honey Creek	Multiple sites	2015	Various	10
Neosho SMB	Big Sugar Creek	Multiple sites	2015	Various	6
Neosho SMB	Indian Creek	Multiple sites	2015	Angling	2
Neosho SMB	Elk River	Multiple sites	2015	Various	7
Neosho SMB	Buffalo Creek	US of confluence with Elk River	2014	Barge EF	2
Neosho SMB	Illinois River	Multiple sites	2015	Various	6
Neosho SMB	Baron Fork	Multiple sites	2015	Angling	3
Neosho SMB	Caney Creek	Multiple sites	2015	Various	4
					40
				Total:	**152**

Table 2. Putative Neosho Smallmouth Bass and intergrades (*) genotyped (n=721), with locality and sampling information. Abbreviations: upstream (US), downstream (DS), river-reservoir interface (RRI), backpack (BP), electrofishing (EF), confluence (confl.).

Site	Sampling Location	Latitude	Longitude	Year(s)	Method	n
Shoal Creek	Cherry Corner to Lime Kiln access	36.920256	-94.345707	2015	Angling	44
Shoal Creek	DS Lime Kiln lowhead dam	36.896471	-94.368162	2015	Angling	1
Shoal Creek	McIndoe Park low water bridge	37.034311	-94.527889	2015	Angling	2
Sycamore Creek	Hwy 10 and N 670 Rd	36.774256	-94.684734	2015	Various	33
Sycamore Creek	Hwy 10 and N 670 Rd	36.774256	-94.684734	2016	Various	8
Honey Creek	DS of S 690 Rd bridge	36.551242	-94.650555	2015	BP EF	15
Honey Creek	DS of S 670 Rd iron bridge	36.546240	-94.687641	2015	BP EF	16
Honey Creek	Private road near S 650 Rd	36.547813	-94.722284	2015	Angling	16
Honey Creek	US of Grand Lake RRI	36.547081	-94.734297	2015	Boat EF	7
Big Sugar Creek	Multiple sites (MDC sample)	-	-	2015	Angling	15
Big Sugar Creek	Deep Ford access	36.616725	-94.350701	2015	Various	33
Big Sugar Creek	Crag-O-Lea bridge	36.611381	-94.351593	2015	Angling	14
Indian Creek	Hwy D bridge	36.793180	-94.243119	2015	Barge EF	20
Indian Creek	Canning Factory Rd bridge	36.646251	-94.447541	2015	Angling	11
Indian Creek	Lanagan City Park	36.607031	-94.446399	2015	Angling	27
Elk River	200 m DS of mill dam in Noel	36.554908	-94.497854	2015	Angling	13
Elk River	Large bluff DS of Noel	36.584350	-94.515960	2015	Angling	13
Elk River	US of Cowskin access	36.608120	-94.578340	2015	Angling	11
Elk River	Cowskin access to Buffalo Creek	36.626387	-94.613874	2014, 16	Various	39
Elk River	Buffalo Creek to Grand Lake RRI	36.633853	-94.630633	2015, 16	Boat EF	65
Buffalo Creek	100 m US confl. with Elk	36.641591	-94.624775	2014	Barge EF	38
Spavinaw Creek	Ozark Plateau NWR	36.324321	-94.699308	2015	Barge EF	15
Spavinaw Creek	DS border of Ozark Plateau NWR	36.321305	-94.713257	2015	Angling	10
Hudson Lake	Lower end of lake	-	-	2016	Angling	1
Illinois River	Round Hollow to Peavine access	36.094210	-94.830422	2015	Angling	47
Illinois River	Tahlequah Riverside Park	35.922055	-94.923975	2015	Angling	22
Illinois River	Baron Fork confl. to Tenkiller RRI	35.842261	-94.920055	2015	Various	26
Baron Fork	US Hwy 51 bridge	35.936556	-94.827673	2015	Angling	12
Baron Fork	US Wall Trip Branch confl.	35.912631	-94.846221	2015	Angling	12
Baron Fork	West of N 4580 Rd	35.894349	-94.863118	2015	Angling	12
Baron Fork	500 m US Welling Rd bridge	35.870224	-94.896924	2015	Angling	11
Caney Creek	N 6430 Rd crossing	35.841508	-94.772695	2015	Various	24
Caney Creek	Bidding Creek confl.	35.841145	-94.789427	2015	Angling	17
Caney Creek	S 581 Rd access	35.798125	-94.840462	2015	Angling	29
Caney Creek	S 581 Rd to Lake Tenkiller RRI	35.793278	-94.846425	2015	Various	24
White River*	Madison 5430 Rd access	35.873452	-93.909160	2015	Angling	5
Crooked Creek*	Harmon Rd bridge	36.233982	-92.922022	2015	Angling	13

Table 3. Mean (SE) of genetic diversity measures for ‘pure’ Neosho Smallmouth Bass, as averaged over seven microsatellite loci for each site: number of alleles (A), effective number of alleles (A_e), allelic richness (A_R), expected heterozygosity (H_e), observed heterozygosity (H_o), and the inbreeding coefficient (F). Also reported is the total number of private alleles ($A_{private}$) across all seven loci and the sample size (n) of pure Neosho Smallmouth Bass included from each site.

Statistic	Shoal Creek	Sycamore Creek	Honey Creek	Big Sugar Creek	Indian Creek	Elk River	Buffalo Creek	Spavinaw Creek	Illinois River	Baron Fork	Caney Creek	**Mean All Sites**
A	8.71 (1.09)	8.43 (1.54)	8.29 (1.76)	13.29 (2.23)	12.00 (1.85)	17.71 (2.95)	10.29 (1.41)	8.57 (1.13)	11.57 (1.88)	10.29 (2.14)	11.27 (2.73)	10.95 (0.63)
A_e	5.03 (0.86)	3.46 (0.54)	4.09 (1.06)	5.90 (1.06)	4.86 (0.97)	6.34 (1.03)	5.31 (0.88)	4.19 (0.84)	4.70 (1.13)	3.97 (1.21)	3.70 (1.32)	4.69 (0.30)
A_R	8.54 (1.06)	6.39 (1.15)	6.11 (1.29)	9.12 (1.22)	8.28 (1.17)	9.55 (1.20)	8.76 (1.10)	7.74 (1.01)	8.47 (1.31)	6.97 (1.50)	6.23 (1.47)	7.76 (1.24)
H_e	0.76 (0.07)	0.63 (0.11)	0.62 (0.12)	0.77 (0.08)	0.72 (0.09)	0.77 (0.08)	0.77 (0.07)	0.68 (0.09)	0.69 (0.09)	0.62 (0.11)	0.56 (0.11)	0.69 (0.03)
H_o	0.77 (0.07)	0.63 (0.11)	0.58 (0.11)	0.73 (0.08)	0.68 (0.09)	0.76 (0.08)	0.74 (0.06)	0.69 (0.11)	0.66 (0.08)	0.63 (0.11)	0.55 (0.10)	0.67 (0.03)
F	-0.04 (0.03)	0.00 (0.05)	0.07 (0.03)	0.04 (0.03)	0.04 (0.03)	0.01 (0.03)	0.01 (0.03)	-0.04 (0.05)	0.03 (0.03)	-0.03 (0.04)	0.01 (0.02)	0.01 (0.01)
$A_{private}$	4	2	1	2	2	6	3	2	2	0	5	3
n	16	33	53	45	41	91	25	20	35	42	81	44

Table 4. Estimates of effective population size (N_e) and associated parametric 95% confidence intervals by site for ‘pure’ Neosho Smallmouth Bass produced by the single-sample, linkage-disequilibrium estimator of Burrows. Estimates were produced at several different allele frequency thresholds that remove low-frequency alleles (< 5%, < 2%, < 1%, and 0%), as low-frequency alleles can upwardly bias estimates. The number (*n*) of individual genotypes used to estimate N_e for each cluster is also reported.

Threshold	Shoal Creek	Sycamore Creek	Honey Creek	Big Sugar Creek	Indian Creek	Elk River	Buffalo Creek
0.05	253.4 (26.4 - ∞)	98.3 (28.8 - ∞)	51.2 (27.3 - 140.1)	142.1 (59.5 - ∞)	133.5 (45.9 - ∞)	275.9 (117.1 - ∞)	29.3 (16.4 - 75.6)
0.02	∞ (102.4 - ∞)	269.1 (53.4 - ∞)	50.6 (32.7 - 90.7)	246.7 (101.2 - ∞)	106.7 (59.9 - 331.9)	212.8 (133.0 - 459.1)	129.3 (50.5 - ∞)
0.01	∞ (102.4 - ∞)	21.1 (15.4 - 30.1)	65.7 (43.2 - 117.4)	∞ (267.8 - ∞)	614.5 (155.2 - ∞)	483.6 (254.3 - 2875.2)	129.3 (50.5 - ∞)
0.00	∞ (102.4 - ∞)	21.1 (15.4 - 30.1)	87.7 (54.6 - 182.5)	∞ (267.8 - ∞)	614.5 (155.2 - ∞)	∞ (780.5 - ∞)	129.3 (50.5 - ∞)
n	16	33	53	45	41	91	25

(Continued Below)

Threshold	Spavinaw Creek	Illinois River	Baron Fork	Caney Creek
0.05	51.9 (21.6 - ∞)	449.7 (56.9 - ∞)	∞ (63.9 - ∞)	136.0 (61.8 - 1536.9)
0.02	44.8 (23.4 - 181.4)	103.0 (55.7 - 393.0)	305.8 (84.8 - ∞)	117.1 (68.6 - 281.2)
0.01	44.8 (23.4 - 181.4)	160.7 (79.8 - 1680.7)	454.9 (126.8 - ∞)	226.7 (119.2 - 1039.5)
0.00	44.8 (23.4 - 181.4)	160.7 (79.8 - 1680.7)	454.9 (126.8 - ∞)	145.5 (101.1 - 240.6)
n	20	35	42	81

Table 5. Estimates of effective population size (N_e) and associated parametric 95% confidence intervals for demographically connected units of 'pure' Neosho Smallmouth Bass, with migrants removed. Estimates were produced by a single-sample, linkage-disequilibrium estimator at several different allele frequency thresholds that remove low-frequency alleles (< 5%, < 2%, < 1%, and 0%), as low-frequency alleles can bias estimates. The number (n) of individual genotypes used to estimate N_e for each cluster is also reported.

Threshold	Shoal Creek	Sycamore & Honey creeks	Elk River & Indian, Buffalo, Big Sugar creeks	Spavinaw Creek	Illinois River, Baron Fork, and Caney Creek
0.05	593.1 (25.5 - ∞)	66.3 (39.5 - 136.3)	166.9 (110.6 - 287.4)	107.5 (25.0 - ∞)	627.9 (187.1 - ∞)
0.02	∞ (98.6 - ∞)	98.0 (64.6 - 174.1)	283.3 (198.4 - 458.8)	47.0 (21.2 - 1682.4)	275.8 (155.1 - 793.6)
0.01	∞ (98.6 - ∞)	113.7 (77.2 - 193.0)	465.4 (319.9 - 803.0)	47.0 (21.2 - 1682.4)	276.9 (186.0 - 490.8)
0.00	∞ (98.6 - ∞)	53.9 (44.1 - 67.2)	∞ (3950.4 - ∞)	47.0 (21.2 - 1682.4)	333.9 (251.0 - 483.0)
n	15	85	188	18	158

FIGURES

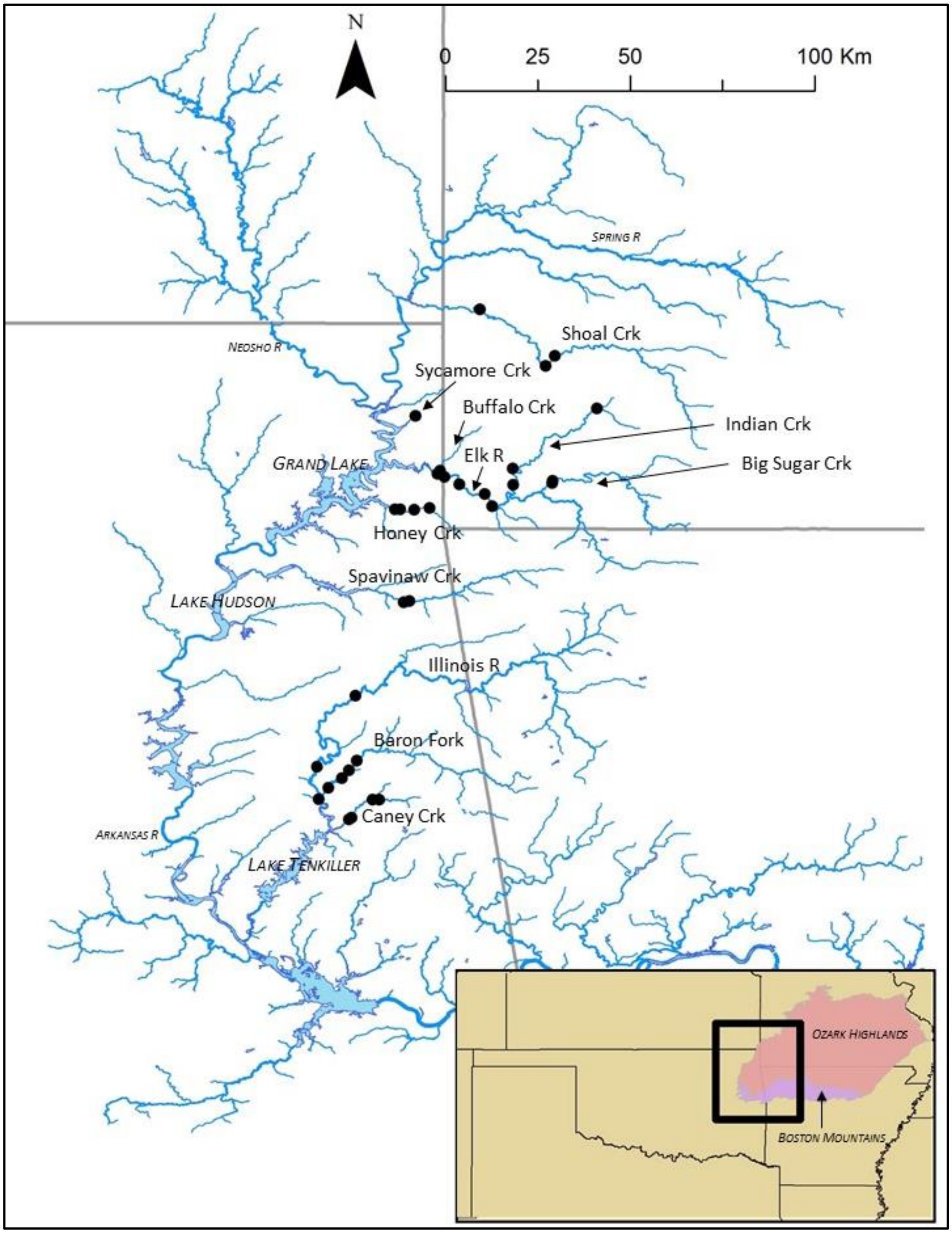

Figure 1. Study area and sample locations for putative Neosho Smallmouth Bass genotypes. Coordinates for sample locations were recorded near the center of each sample reach.

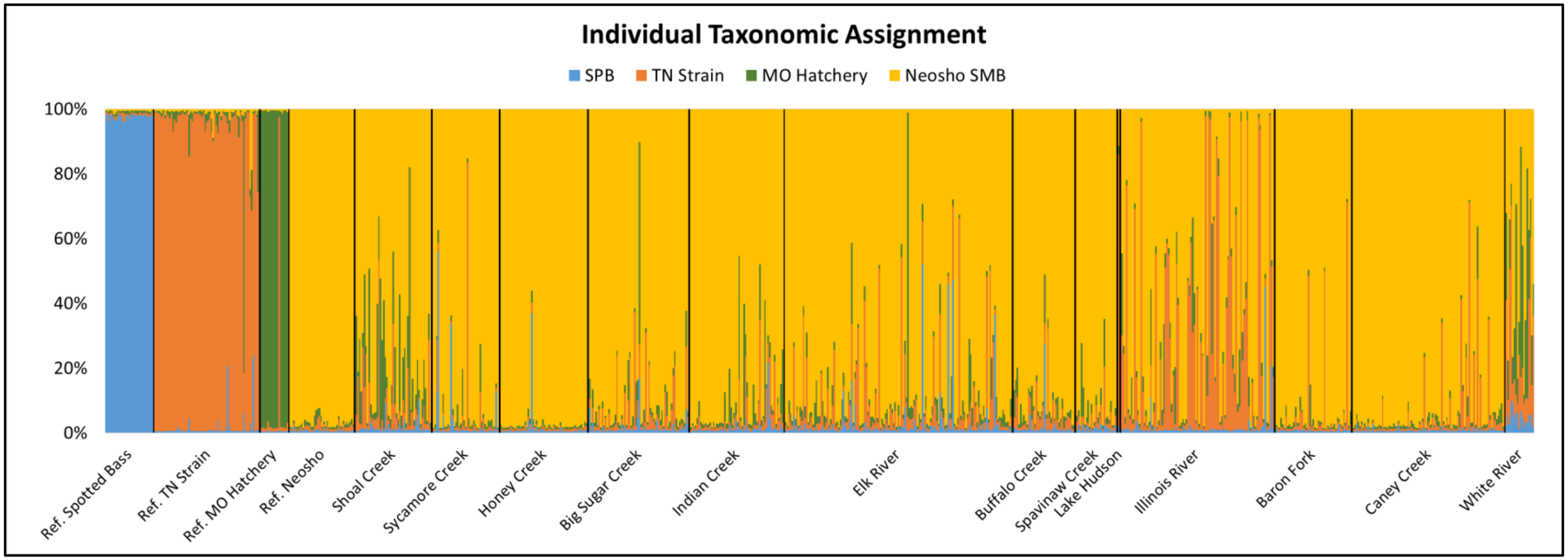

Figure 2. Taxon-level proportional assignment of 873 individual genotypes (individual vertical bars) to four genetic clusters. Assignments were estimated in Program STRUCTURE using the allele frequencies of four reference taxa groups (Spotted Bass [SPB], Tennessee 'lake strain' Smallmouth Bass [TN Strain], Smallmouth Bass stock from private hatchery in Missouri [MO hatchery], and Neosho Smallmouth Bass [Neosho SMB]) to proportionally assign all putative Neosho Smallmouth Bass genotypes, which were organized by sampling location so that left-to-right is approximately upstream-to-downstream within a given site.

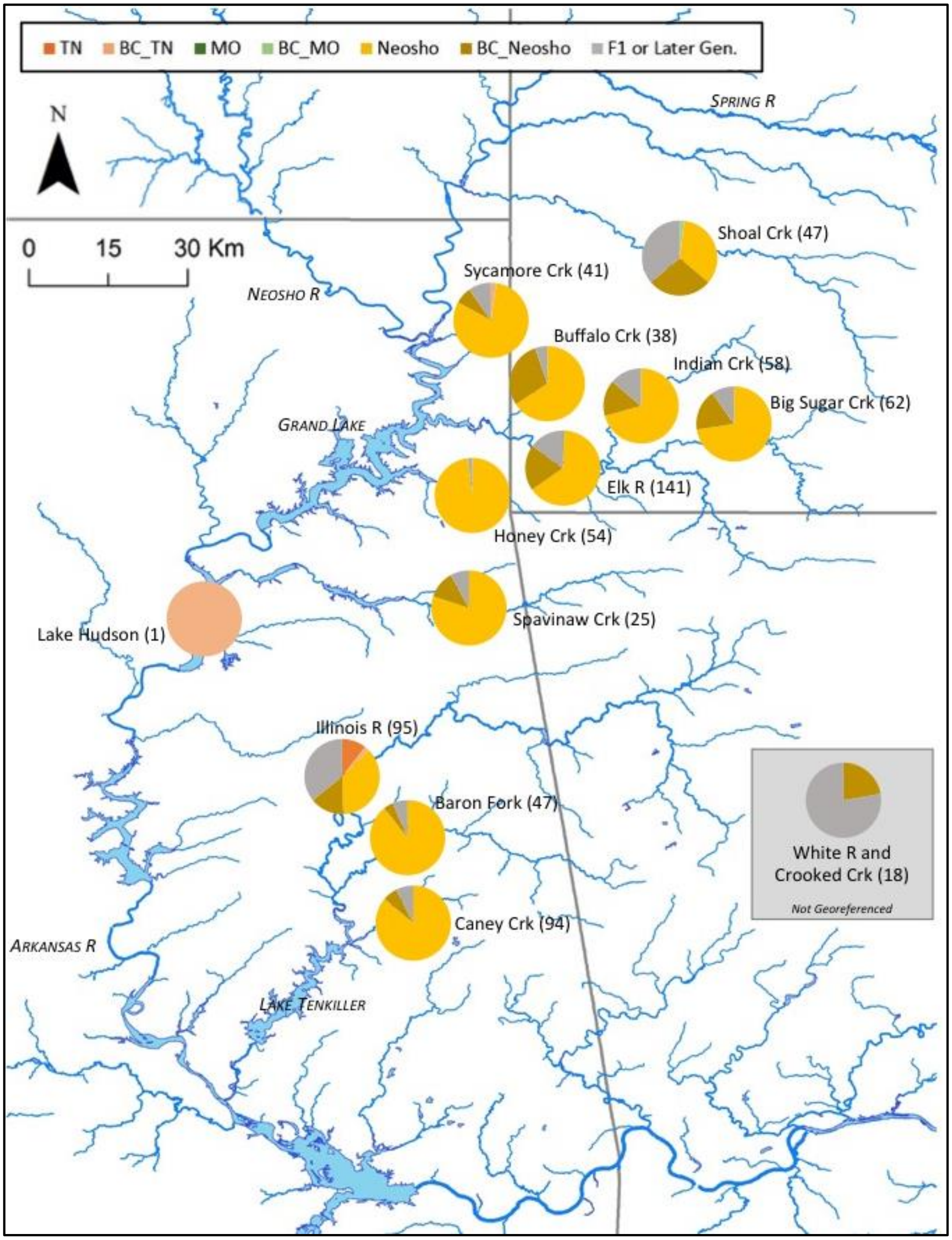

Figure 3. Proportion of each resulting taxonomic classification for putative Neosho Smallmouth Bass genotypes by site. ‘Pure’ species were ≥ 90% assignment to one respective group and were denoted by taxa as Tennessee ‘lake strain’ Smallmouth Bass (TN), Smallmouth Bass stock from private hatchery in Missouri [MO], and Neosho Smallmouth Bass (Neosho). ‘Backcrosses’ were 75-90% assignment to one respective group (taxa preceeded by “BC_”). Finally, all remaining individuals were considered first-filial (F1) generation or later-generation hybrids (“F1 or Later Gen”). Sample sizes are included in parentheses alongside each site name.

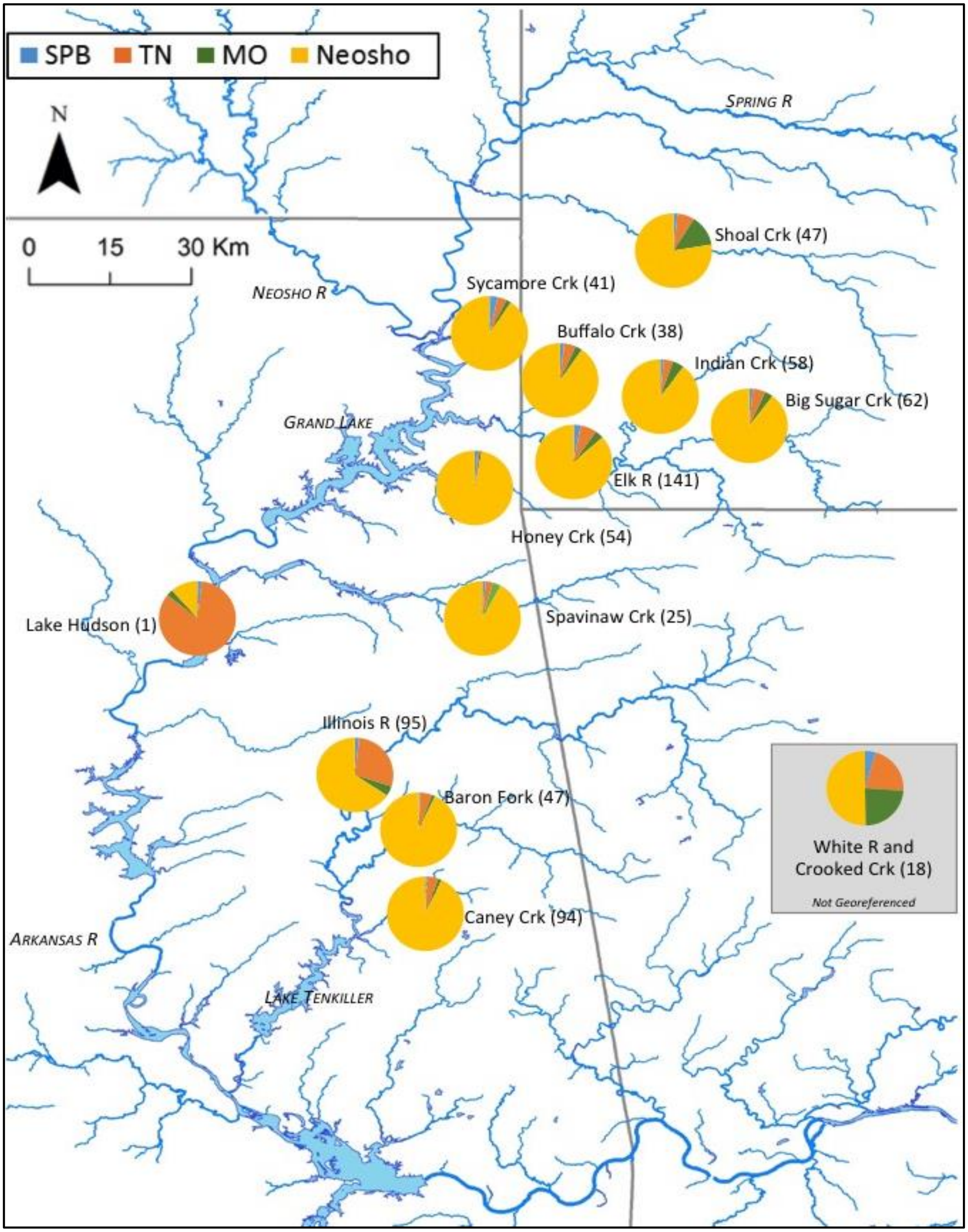

Figure 4. Spatial depiction of overall genomic proportions of four taxa (Spotted Bass [SPB], Tennessee ‘lake strain’ Smallmouth Bass [TN], Smallmouth Bass stock from private hatchery in Missouri [MO], and Neosho Smallmouth Bass [Neosho]) within putative Neosho Smallmouth Bass samples, calculated by site with sample sizes in parentheses.

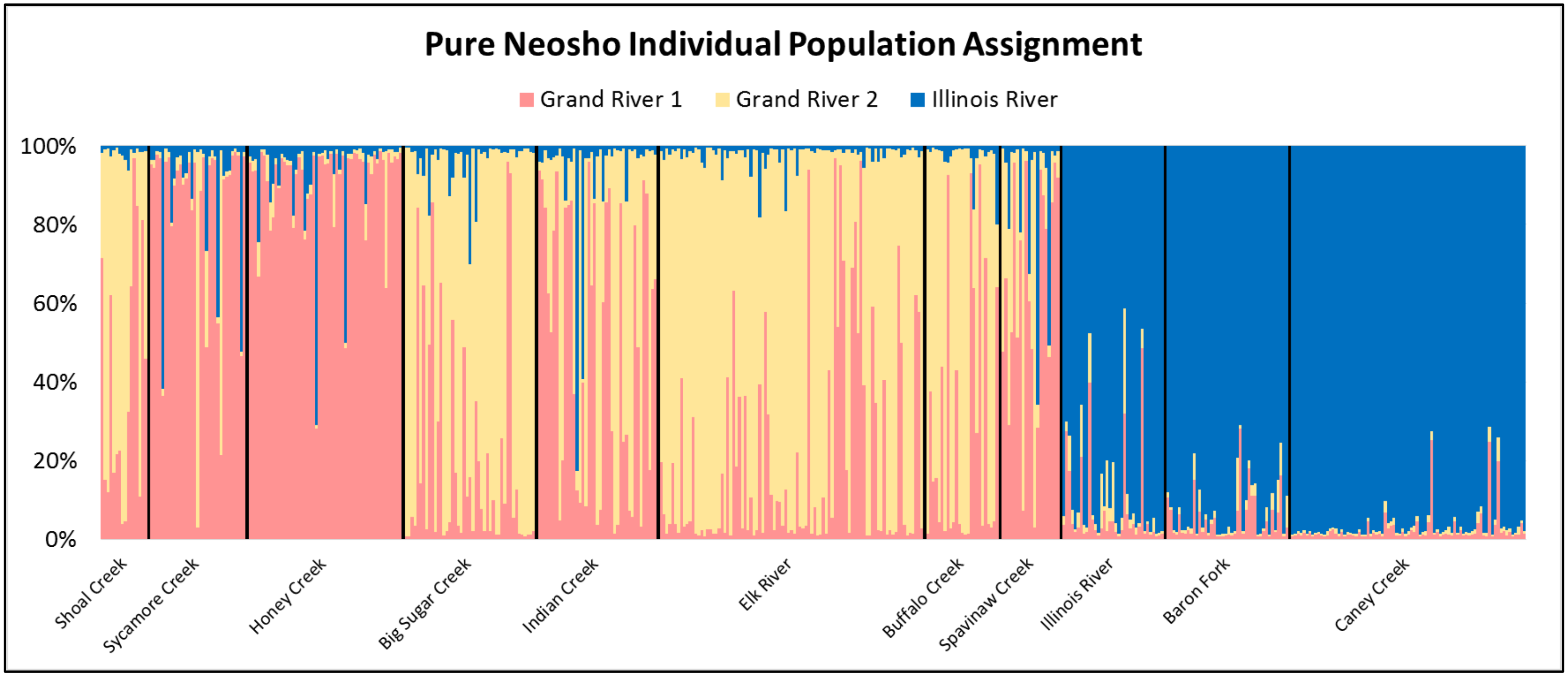

Figure 5. Proportional assignment of 482 individual Neosho Smallmouth Bass genotypes to three genetic clusters. Assignments were estimated in Program STRUCTURE, and the resulting clusters were coined "Grand River 1", "Grand River 2", and "Illinois River" based on the spatial juxtaposition of each cluster.

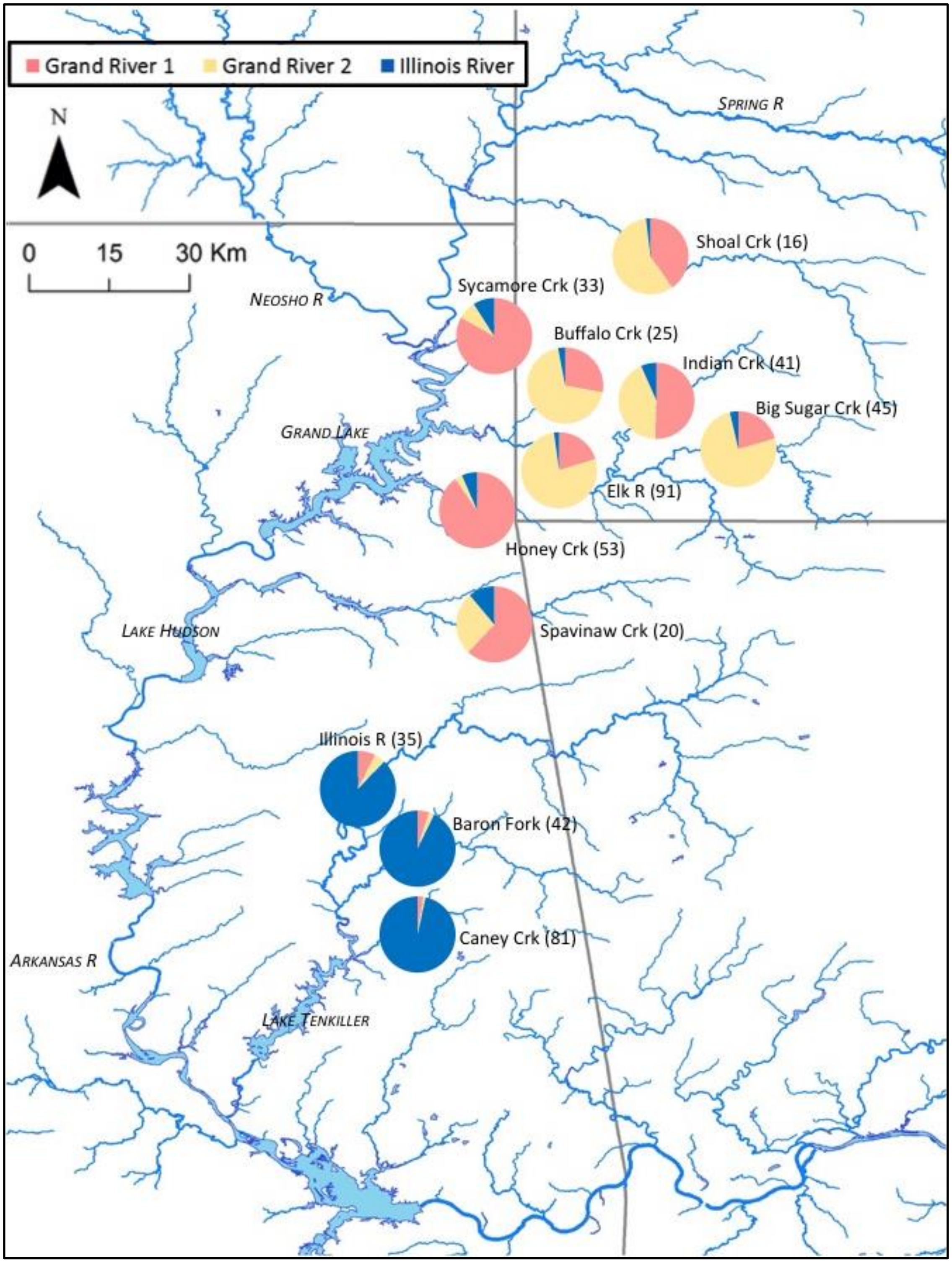

Figure 6. Spatial depiction of overall genomic proportions of three obtained genetic clusters ("Grand River 1", "Grand River 2", and "Illinois River"), by site for pure Nesoho Smallmouth Bass with sample sizes in parentheses.

APPENDIX I: Site-Specific Result Summaries

Grand Lake Area:

- *Shoal Creek* – a fourth-order tributary to Spring River, Shoal Creek contained the lowest percentage of pure Neosho Smallmouth Bass (34%) and the highest percentage of F1 or later-generation hybrids (36.2%) among Grand Lake area sites. No pure specimens of either non-native Smallmouth Bass ("SMB") form were recovered, but one MO hatchery backcross was collected. Overall genomic proportions of Shoal Creek were elevated for MO hatchery (13.5%) and TN 'lake strain' (7.2%), whereas Neosho SMB comprised 77.6%. Regarding pure Neosho SMB samples, overall genomic proportions were 40.4% Grand River 1 and 57.7% Grand River 2. Genetic diversity measures for Shoal Creek were, in general, slightly higher than the mean for all sites and the population also harbored 4 private alleles. A site-specific point estimate of $N_e = 253$ was also relatively high among sites, as was $N_e = 593$ with migrants removed; however, low sample size of pure Neosho SMB may have influenced these estimates
- *Sycamore Creek* – a third-order direct tributary to the upper end of Grand Lake, Sycamore Creek contained a high percentage of pure Neosho SMB (80.5%). Although one TN 'lake strain' backcross was recovered, the proportions of F1 or later hybrids (9.8%) and Neosho backcrosses (7.3%) were relatively low. Overall genomic proportions in Sycamore Creek were similar to most Grand Lake area sites, with 90.6% Neosho, 3.6% TN 'lake strain', and 2.1% MO hatchery. Population assignment of pure Neosho individuals revealed that overall genomic proportions were dominated by the Grand River 1 cluster (83.2%). Sycamore

Creek had relatively low measures of genetic diversity compared to the overall mean, and the site-specific point estimate of N_e was also relatively low at 98. The combined point estimate of N_e for Sycamore and Honey creek with migrants removed was also relatively low (66).

- *Honey Creek* – a third-order direct tributary to the middle of Grand Lake, Honey Creek contained the highest percentage of pure Neosho SMB (98.1%) among all sampling sites included in this study. Overall genomic proportions indicated that Honey Creek has the lowest percentage of non-native alleles, with < 1% assignment to each non-native Smallmouth Bass form. Pure Neosho SMB overall genomic proportions were dominated by the Grand River 1 cluster (90.3%). Allelic diversity measures were among the lowest at any site, and $F = 0.07$ was the highest among sites but still does not indicate appreciable inbreeding depression. $N_e = 51$ was among the lowest point estimates for any site. The combined point estimate of N_e for Sycamore and Honey creek with migrants removed was also relatively low (66).
- *Big Sugar Creek* – a fourth-order tributary to the Elk River, Big Sugar Creek contained 72.5% pure Neosho SMB and an additional 17.7% in Neosho backcrosses. Non-native SMB alleles were present at relatively low levels, with 5.2% TN 'lake strain' and 3.5% MO hatchery in the overall genomic proportions for Big Sugar Creek, whereas Neosho SMB comprised 89.6%. Regarding pure Neosho SMB, overall genomic proportions were assigned 20.5% to Grand River 1 and 75.9% to Grand River 2 clusters. Big Sugar Creek had above average allelic diversity measures, and a point estimate of $N_e = 142$ placed near the median of

sites sampled. The point estimate for the demographically connected unit of Elk River, Big Sugar, Indian, and Buffalo creeks with migrants removed was relatively high at 167.

- *Indian Creek* – a fourth-order tributary to the Elk River, Indian Creek contained 70.7% pure Neosho SMB and had similar percentages of hybrids as those found in Big Sugar Creek, with Neosho backcrosses at 15.5% and F1 or later generation hybrids at 13.7%. Overall genomic proportions for Indian Creek comprised 89.7% Neosho SMB, whereas TN 'lake strain' comprised 3.8% and MO hatchery comprised 4.9%. A longitudinal trend in introgression was evident wherein upstream reaches near Boulder City, MO were much less influenced by non-native alleles than those collected from downstream reaches near Anderson, MO and Lanagan, MO. Pure Neosho genomic proportions were assigned 50.6% to Grand River 1 and 42.8% to Grand River 2. Genetic diversity measures were near or slightly higher than the overall mean, and a point estimate of N_e = 134 placed near the median of sites sampled. The point estimate for the demographically connected unit of Elk River, Big Sugar, Indian, and Buffalo creeks with migrants removed was relatively high at 167.
- *Elk River* – a major, fifth-order tributary to Grand Lake, Elk River had individual taxonomic classifications and overall genomic proportions similar to Big Sugar Creek and Indian Creek, with a slightly higher percentage of Neosho SMB backcrosses (19.9%) and F1 or later generation hybrids (14.9%). One pure MO hatchery fish was recovered (0.7%), but overall genomic proportions indicated that TN 'lake strain' alleles were more prominent (6.9%) than MO hatchery

(3.6%). Individuals with higher genomic proportions of TN ‘lake strain’ alleles were encountered closer to the interface with Grand Lake. Regarding pure Neosho SMB, the site was assigned the highest overall genomic proportion of Grand River 2 cluster (77.6%), with only 20.3% assigned to Grand River 1. The Elk River site had the highest allelic diversity measures among all sites, with the highest number of private alleles (6) indicating a relatively high amount of unique genetic diversity. N_e = 276 was one of the highest effective size estimates among all sites, and the highest estimate among sites in the Grand Lake area. The N_e point estimate for the demographically connected unit of Elk River, Big Sugar, Indian, and Buffalo creeks with migrants removed was relatively high at 167.

- *Buffalo Creek* – a third-order tributary to the Elk River just upstream of the Elk River’s interface with Grand Lake, Buffalo Creek had the highest percentage of Neosho SMB backcrosses (28.9%) of any sampling site considered in this study. However, overall genomic proportions were similar to the Elk River and its other tributaries, with 90.4% Neosho, 4.8% TN ‘lake strain’, and 3.6% MO hatchery. Pure Neosho SMB had overall genomic proportions of 69.0% Grand River 2 and 28.0% Grand River 2. Allelic diversity measures were relatively high; however, a point estimate of N_e = 29 was the lowest among all sites. The point estimate for the demographically connected unit of Elk River, Big Sugar, Indian, and Buffalo creeks with migrants removed was relatively high at 167.

Below Grand Lake Area:

- *Spavinaw Creek* – a fourth-order tributary to Lake Hudson, we sampled Spavinaw Creek above Lake Eucha. Pure Neosho SMB comprised 80% of sampled individuals, with the remaining 20% split nearly equally among Neosho backcrosses and F1 or later generation hybrids. Overall genomic proportions were 92.0% Neosho, 3.0% TN ‘lake strain’, and 3.6% MO hatchery. Considering pure Neosho, the overall population genomic proportions were 62.1% Grand River 1 and 26.8% Grand River 2, which were similar to other Grand Lake area sites; however, there was a slightly elevated proportion (15.6%) of the Illinois River cluster. Allelic diversity measures were near the overall mean for all sites; however, the site-specific point estimate of $N_e = 52$ was relatively low. With migrants removed, the point estimate of N_e increased slightly to 108.
- *Lake Hudson* – one genetic sample was obtained from an angler-caught SMB in Lake Hudson. The 2.49-kg fish was entered into ODWC’s lake record program and was caught on March 13, 2016 in the lower end of Lake Hudson. This fish was assigned as a TN ‘lake strain’ backcross with genomic proportions of 83.9% TN ‘lake strain’, 11.4% Neosho, and 2.8% MO hatchery. As no ‘pure’ Neosho Smallmouth Bass were recovered, no further analyses were performed for this site.

Lake Tenkiller Area:

- *Illinois River* – a sixth-order tributary to Lake Tenkiller, putative Neosho SMB in the Illinois River contained a large amount of non-native TN ‘lake strain’ alleles.

Pure TN 'lake strain' comprised 10.5% of individuals, with another 2.1% of TN 'lake strain' backcrosses. A large percentage of fish were F1 or later generation hybrids between TN 'lake strain' and Neosho SMB (35.8%), whereas pure Neosho SMB comprised 36.8%. Overall genomic proportions were 65.7% Neosho, 28.2% TN 'lake strain', and 4.4% MO hatchery. More pure TN 'lake strain' fish were encountered closer to the interface with Lake Tenkiller, but hybrids and a pure individual were captured at upstream locations as well. Of the pure Neosho individuals, overall genomic proportions were assigned 87.8% to the Illinois River cluster. Allelic diversity measures were high compared to the overall mean for all sites, and a site-specific point estimate of N_e = 450 was the highest among all sites. The N_e point estimate for Illinois River, Baron Fork, and Caney Creek combined with migrants removed was the highest among demographically connected units at 628.

- *Baron Fork* – a fifth-order tributary to the Illinois River just upstream of the Illinois River's interface with Lake Tenkiller, Baron Fork contained 89.4% pure Neosho SMB, with the remainder split nearly equally among Neosho backcrosses and F1 or later generation hybrids. The three fish that were F1 or later generation hybrids contained TN 'lake strain' alleles. Overall genomic proportions were 92.9% Neosho, 4.9% TN 'lake strain', and 1.4% MO hatchery. Regarding pure Neosho SMB population structure, overall genomic proportions were 92.8% assigned to the Illinois River cluster. Genetic diversity measures were slightly lower than the average for all sites. The N_e estimate at the 0.05 threshold did not converge on a real number, so we used the estimate of N_e = 306 provided by < 2%

allele frequency threshold for interpretation of results, which was the second-highest effective size estimate obtained among all sites. The N_e point estimate for Illinois River, Baron Fork, and Caney Creek combined with migrants removed was the highest among demographically connected units at 628.

- *Caney Creek* – a fourth-order direct tributary to Lake Tenkiller, Caney Creek contained 86.2% pure Neosho SMB, with the remainder comprised nearly equally of Neosho backcrosses and F1 or later generation hybrids. The F1 or later generation hybrids contained TN 'lake strain' genetics, and were recovered closer to the interface with Lake Tenkiller. Overall genomic proportions were 93.1% Neosho, 4.6% TN 'lake strain', and 1.5% MO hatchery. Among pure Neosho SMB, Caney Creek fish had the highest overall assignment to the Illinois River cluster (96.5%). Allelic diversity measures were lower than the average of all sites; however, five private alleles were recovered which suggests some unique genetic diversity is harbored in Caney Creek. A site-specific point estimate of $N_e = 136$ was near the median of all sites. The N_e point estimate for Illinois River, Baron Fork, and Caney Creek combined with migrants removed was the highest among demographically connected units at 628.

White River System

- *White River and Crooked Creek* – these streams lie outside the native range of the Neosho Smallmouth Bass; however, Stark and Echelle (1998) described this area as a natural intergrade zone between Neosho SMB and Northern SMB from the Interior Highlands. Individuals were assigned to Neosho backcross (22.2%) and

F1 or later generation hybrids (77.8%). Overall genomic proportions were 50.2% Neosho, 23.7% MO hatchery, and 21.5% TN ‘lake strain’. As no ‘pure’ Neosho SMB were recovered, no further analyses were performed for this site.