

UNIVERSITY OF CENTRAL OKLAHOMA
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Jackson College of Graduate Studies

**Hybrid Swarming of Neosho Bass with non-native Smallmouth Bass in
the upper Illinois River basin of Oklahoma, and implications for
individual growth**

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By

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Edmond, Oklahoma

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**Hybrid Swarming of Neosho Bass with non-native Smallmouth Bass in
the upper Illinois River basin of Oklahoma, and implications for
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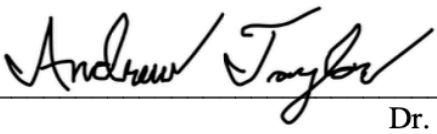
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Hybrid Swarming of Neosho Bass with non-native Smallmouth Bass in the upper Illinois River basin of Oklahoma, and implications for individual growth

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Abstract

Recent molecular investigations support the distinctiveness of the Neosho Bass (*Micropterus velox*) of the Ozark Highlands from Smallmouth Bass (*M. dolomieu*). Like other endemic black basses, Neosho Bass are threatened by hybridization with non-native *Micropterus* forms. Non-native Smallmouth Bass were stocked in Lake Tenkiller in 1991 and 1992, resulting in introgressive hybridization with Neosho Bass in areas upstream of the impoundment. To inform management and conservation efforts, the main objectives of this thesis were to 1) characterize the spatial extent and directionality of introgressive hybridization, and 2) examine the influences of genetic identity on individual growth. From 2019-2021, specimens were collected longitudinally from localities along the Illinois River, Baron Fork, Caney Creek, Flint Creek, and Lake Tenkiller. We genotyped 1,272 fish with a SNP panel designed to identify Smallmouth Bass, Neosho Bass, and interspecific hybrids. Assignment of genotypes with STRUCTURE, NewHybrids, and *introgress* revealed an ongoing hybrid swarm in the Illinois River mainstem, with some areas of non-hybrid Neosho Bass remaining in far upstream reaches of smaller tributaries. For the second objective, three independent readers estimated the age of 650 fish using transversely sectioned sagittal otoliths. Fish ranged 67-536 mm in total length and estimated ages of these fish 0-10 years. Length-at-age data was then coupled with genetic results to parameterize von Bertalanffy growth functions. Analyses of von Bertalanffy growth functions

revealed that estimated total length of non-hybrid Smallmouth Bass is noticeably larger than that of non-hybrid Neosho Bass beginning at age-1 and becomes increasingly divergent with age. The results of this thesis can be used to guide conservation plans for Neosho Bass across their narrow native range and to inform future management decisions regarding population dynamics of the sport fishery. In the future, we plan to couple our results with mitochondrial DNA (mtDNA) sequencing to provide additional insights into maternal ancestry of hybrids and the potential for directional introgression.

Chapter 1

Hybrid swarming of Neosho Bass with non-native Smallmouth Bass in the upper Illinois River basin, Oklahoma

Introduction

Smallmouth Bass (*Micropterus dolomieu*) support numerous fisheries in man-made impoundments, streams, and lakes due to their popularity among sport fishers (Martin and Fisher 2009; Brewer and Orth 2015). Trends in management of Smallmouth Bass mirror that of other popular North American sportfish, including historical widespread stocking to create angling opportunities and a recent focus on anglers pursuing trophy-sized fish (Long et al. 2015; Taylor et al. 2019). A recent paradigm shift in black bass management has emerged that prioritizes conservation of diversity, with some anglers becoming increasingly interested in catching native black bass species from their native habitats (Taylor et al. 2019). In human altered systems, this could create potential conflicts between management practices aimed at creating trophy fisheries with non-native black basses versus conserving native black basses.

In 1940, Hubbs and Bailey described the Neosho Smallmouth Bass subspecies (*M. d. velox*) which occur in the northeastern region of Oklahoma, northwestern Arkansas, and southwestern Missouri, while designating the remainder of the broad range as the Northern subspecies (*M. d. dolomieu*; Figure 1). Upon examining specimens, Hubbs and Bailey (1940) described phenotypic differences between the two subspecies, stating that compared to Northern Smallmouth Bass, Neosho Smallmouth Bass have an elongated “streamline” body and a uniform color that fades to a white underside, while also noting differences in scale and fin ray counts. Various studies have reaffirmed the genetic distinctiveness between the subspecies, while also discovering a third distinct genetic lineage, the Ouachita Smallmouth Bass (Stark and Echelle

1998; Taylor et al. 2018; Long et al. 2020). Recently, Kim et al. (2022) analyzed ddRAD data to investigate the phylogeny and species delimitation of all known black bass lineages, wherein the authors elevated the Neosho Bass (*M. velox*) to a distinct species.

Stocking of Smallmouth Bass can be traced back to the late 1800s, with some including translocations across native drainages. These early stockings were poorly documented before diversity within the black basses was appreciated (Snow 1975; Brewer and Long 2015). More recently, the state of Oklahoma stocked “Tennessee lake-strain” Smallmouth Bass from the Tennessee and Cumberland River systems into the native range of Neosho Bass. For example, in 1991 and 1992 “Tennessee lake-strain” Smallmouth Bass were stocked into Lake Tenkiller near the dam (Boxrucker et al. 2004). Prior to stocking, native Neosho Bass were present in Lake Tenkiller in low numbers (Boxrucker et al. 2004). Stocking non-native Smallmouth Bass created better angling opportunities within the reservoir, perhaps at a cost to the native Neosho Bass populations. However, the genetic distinctiveness of Neosho Bass was not reported until later that decade (Stark and Echelle 1998).

Stocking of Lake Tenkiller with non-native Smallmouth Bass eventually resulted in introgressive hybridization with Neosho Bass in the upstream tributaries of the upper Illinois River Basin (Boxrucker et al. 2004; Taylor et al. 2018). Neosho Bass are native to this basin, occurring within the Illinois River and its tributaries, including Baron Fork and Caney Creek. In 1999, Malloy (2001) conducted a post-stocking genetic survey in Lake Tenkiller that revealed Smallmouth Bass within the lake had 85-90% non-native alleles; however, no non-native alleles were detected in the riverine habitats of Baron Fork. A second survey conducted in 2014-2015 found non-hybrid “Tennessee lake-strain” Smallmouth Bass as far as 50 km upstream from Lake Tenkiller in the Illinois River (Taylor et al. 2016). A pooled sample of the Illinois River revealed

extensive introgressive hybridization with 42% of individuals classified as non-hybrid Neosho Bass, 11% non-hybrid Smallmouth Bass, 18% F1 or F2 hybrids, and the remaining 22% were backcrosses to Neosho Bass (Taylor et al. 2016, 2018). Two interesting spatial trends were revealed in the 2014-2015 survey. First, smaller streams (Caney Creek and Baron Fork) had fewer non-native alleles compared with the largest tributary (Illinois River). Second, higher amounts of hybridization occurred near the impoundment (Taylor et al. 2016, 2018).

Previous studies have found that there are landscape-level factors influencing hybridization rates between native and invasive fishes, but few have investigated this in black bass species. Peoples et al. (2021) found that endemic Bartram's Bass (*M. sp. cf. coosae*) closest to reservoirs had a higher probability of hybridization with invasive Alabama Bass (*M. henshalli*). Additionally, pure Bartram's Bass were positively associated with forest cover and stream gradient, whereas they were negatively associated with local land development (Peoples et al. 2021). Beyond the black basses, Muhlfeld et al. (2009) explained that rates of hybridization between native Rainbow Trout (*Oncorhynchus mykiss*) and non-native Westslope Cutthroat Trout (*O. clarkii lewisi*) were related to various abiotic factors, such as distance to the introduction source, increased disturbance from land use, and increased water temperature. Similar landscape-level factors could be influencing the hybridization of Neosho Bass with non-native Smallmouth Bass in the Ozarks.

The objective of this study was to provide an updated, holistic assessment of introgressive hybridization between native Neosho Bass and non-native Smallmouth Bass in the upstream tributaries of Lake Tenkiller. Using a single nucleotide polymorphism (SNP) panel to genotype specimens, we first assessed genetic identities with several complementary algorithms. Next, we mapped the severity and spatial extent of introgression with non-native Smallmouth

Bass. Lastly, we identified landscape correlates with hybridization rates. Results of this study can inform conservation efforts by identifying areas where non-native alleles have yet to infiltrate the Neosho Bass gene pool, along with identifying landscape factors associated with increased hybridization rates.

Materials and Methods

Data Collection

From the spring of 2019 to the fall of 2021, the Oklahoma Department of Wildlife Conservation (ODWC) Stream Team collected 1,272 fin-clips from field-identified “Smallmouth Bass,” which included Neosho Bass, Smallmouth Bass, and their hybrids. In April and May of 2019, 622 fin-clips were collected longitudinally from localities along Illinois River, Baron Fork, and Caney Creek (Figure 2). In the summer and fall of 2020 and 2021, an additional 650 fin-clips were taken via electrofishing (492 specimens) and angling (158 specimens) from the same three tributaries, plus Lake Tenkiller and Flint Creek (Figure 2). In general, angling was used to supplement electrofishing across the study area; however, in Flint Creek angling was the only viable sampling method. Additionally, angling was used to target larger individuals that were difficult to capture via electrofishing in the Illinois River in the fall of 2021.

Genetic Analyses

Fin-clip samples were genotyped using a SNP panel designed to identify Smallmouth Bass and Neosho Bass individuals along with determining introgressive hybridization (Long et al. 2020). A total of 46 SNPs were used in our genetic analysis, and we proceeded in a stepwise fashion, first removing genetic influences of sympatric Spotted Bass (*M. punctulatus*), which occasionally hybridizes with congeners in the study area, prior to focusing on Neosho Bass and

non-native Smallmouth Bass hybridization. First, we used 18 SNP loci to identify parental Spotted Bass and their hybrids with either Neosho Bass or Smallmouth Bass. Next, we used 28 SNP loci to distinguish parental Neosho Bass, non-native Smallmouth Bass, and their hybrids. Genotyping was performed by the Center for Aquaculture Technologies (CAT) in San Diego, California where they performed DNA extractions on pieces of tissue around 10-15 mg using the standardized magnetic bead-based extraction protocol (CAT) then arranging eluted samples randomly on 96-well plates. From each plate 10-12 samples were selected, quantified on a Nanodrop spectrophotometer, and screened for low yield and gDNA degradation on a 2% agarose gel (50-100 μ L at \sim 30ng/ μ L) for quality control. Following established guidelines, DNA samples were amplified using Kompetitive Allele Specific Polymerase Chain Reaction (KASP) technology (LGC Genomics, Beverly, MA, USA).

We used a series of three complementary algorithms to estimate individual genetic proportions, rates of introgression, and assign individuals to hybrid classes. We estimated individual genetic proportions using STRUCTURE version 2.3.4 (Pritchard et al. 2000). We adopted the admixture model with correlated allele frequencies, and used a 50,000 burn in length and 500,000 Markov chain-Monte Carlo (MCMC) iterations for each run. We conducted 5 runs each of $K=1-5$ and estimated the optimal K among reference groups. Optimal K was inferred from the best fitting run using the ΔK value (Evanno et al. 2005). STRUCTURE runs included two reference groups each based on the SNP panel being used (Spotted Bass and Smallmouth Bass; Neosho Bass and Smallmouth Bass), and unknown individuals were proportionally assigned to their genotypes based on the allele frequencies of the reference specimens using the PopFlag option.

We assigned individuals to hybrid classes using the *hybriddetective* and *parrallelnewhybrid* packages in R version 4.2.1 to implement NewHybrids (Anderson and Thompson 2002; Wringe et al. 2017). We considered six possible classifications: non-hybrid parental 1, non-hybrid parental 2, F₁, F₂, first-generation backcross towards parental 1, and first-generation backcross towards parental 2. We completed three independent replicates of analysis specifying a 500,000 burn-in length and 1,000,000 MCMC iterations. Final posterior probabilities were calculated by averaging the three replicates. Individuals with posterior probabilities ≥ 0.50 for a specific hybrid class were assigned to that class; otherwise, no classification was made (not assigned, hereafter “NA”).

To corroborate patterns of introgression estimated by STRUCTURE and NewHybrids, we used the *introgress* package (Gompert and Burke 2010) in R version 4.2.1 (R Core Team 2022). When performing this analysis, the reference individuals used in STRUCTURE were used to inform the maximum-likelihood calculation of hybrid index. The hybrid index is calculated from the proportion of alleles inherited from both parent species (Gompert and Burke 2009). This package also calculated the interspecific heterozygosity or the proportion of an individual’s genome based on alleles from both parent species (Gompert and Burke 2009). The hybrid index and interspecific heterozygosity values are then used to visualize patterns of introgression within a triangle plot.

We first analyzed the Spotted Bass x Smallmouth Bass portion of the SNP panel to determine if any individuals had Spotted Bass alleles present. Using the NewHybrids results, we removed any individuals assigned as F₁, F₂, or backcrosses either direction. Once individuals with Spotted Bass alleles were identified and removed, we then ran all analyses listed above for the Smallmouth Bass x Neosho Bass portion of the SNP panel.

Spatial Analyses

Individual genetic proportions and hybrid class assignments were aggregated by sample location and mapped in ESRI ArcMap 10.8.1 to examine spatial trends in extent and severity of introgression. Stream segment and lake attributes were obtained from the National Hydrography Database (NHD) Plus version 2 (USEPA 2012; Table 1). Distance from the reservoir to each sample location was estimated with aerial imagery in Google Earth. We obtained land cover proportions at the local (catchment) level and watershed-levels from the United States Environmental Protection Agency's (USEPA) StreamCat dataset (Hill et al. 2016). We summarized genetic proportions at a site to their landscape and watershed characteristics in ArcMap 10.8.1. We then performed a principal component analysis (PCA) to examine the patterns of variation among landscape and watershed characteristics that could be driving hybridization outcomes. PCA techniques have been widely used due to its ability to aid in interpretation of large datasets by reducing dimensionality to increase readability (Jolliffe and Cadima 2016). In our PCA, we included distance from reservoir (km; catchment-level), watershed area (km²; watershed-level), percent deciduous forest (catchment-level), and percent hay (catchment-level).

Results

Genetic Analyses

Spotted Bass and hybrids. - The Evanno method estimated an optimal $K = 2$ for the Spotted Bass x Smallmouth Bass complex SNP panel with a clear distinction of reference Spotted Bass and individuals in the Smallmouth Bass complex (Figure 3). STRUCTURE assignments indicated that there was minimal hybridization between sympatric Spotted Bass and

the Smallmouth Bass complex species (Neosho Bass or Smallmouth Bass). Of the 1,272 captured individuals, the proportion of Spotted Bass alleles overall was 0.0085 while the proportion of Smallmouth Bass complex alleles was 0.9915. The highest estimated genomic proportion of Spotted Bass found was 0.45 from an individual captured in Caney Creek. Results from *introgress* corroborated those from STRUCTURE, indicating a minimal amount of Spotted Bass alleles. Hybrid class assignment resulted in 13 backcrosses towards Smallmouth Bass (Figure 4). These individuals were removed prior to our genomic analysis of the Neosho Bass x Smallmouth Bass portion of the SNP panel, which left us with 1,259 individuals.

Neosho Bass and Smallmouth Bass. - For the Neosho Bass x Smallmouth Bass SNP panel STRUCTURE suggested that $K = 2$. STRUCTURE assignments revealed that nonnative Smallmouth Bass alleles are widespread across the area with variation among sites (Figure 5). In the Illinois River, over half of the individuals had a high proportion of Smallmouth Bass alleles while they were not nearly as prevalent in Caney Creek, Baron Fork, or Flint Creek (Figure 5). Overall, the average proportion of native Neosho Bass alleles found across our sample area was 0.63. Within stream sites, Caney Creek had the highest average proportion of native alleles (0.86), followed by Baron Fork and Flint Creek (0.83), and a sharp decrease in the Illinois River (0.51; Figure 6). The lowest occurrence of native alleles was found in Lake Tenkiller, which had an average native proportion of 0.16, which was expected given the history of stocking non-native Smallmouth Bass into the lake.

Hybrid class assignments from NewHybrids aligned with genomic proportion estimates from STRUCTURE. Of the 1,259 individuals, 33% were non-hybrid Neosho Bass, 16% were non-hybrid Smallmouth Bass, 4% were F_1 , 10% were F_2 , 20% were backcross Neosho Bass, 15% were backcross Smallmouth Bass, and 2% were not consistently assigned (NA; Table 2).

Lake Tenkiller had 38 individuals assigned as non-hybrid Smallmouth Bass and 6 assigned as backcross Smallmouth Bass (Figure 7). Out of all sampled streams, the Illinois River had the highest percent of non-hybrid Smallmouth Bass, hybrids, and backcrosses (86%; Table 2). Conversely, Caney Creek had the highest percent of non-hybrid Neosho Bass (73%), with Baron Fork the second highest (65%). Lastly, Flint Creek was the only stream site where there were no F₁ individuals captured. Each of the stream sites except Baron Fork had more F₂ individuals than F₁. Additionally, at every stream system but Illinois River, the amount of backcross Neosho Bass were substantially higher than that of backcross Smallmouth Bass.

Results from the *introgress* package aligned with the results of STRUCTURE and NewHybrids. The hybrid index values which are similar, in a way, to STRUCTURE proportional assignments where individuals with a 0 hybrid index are non-hybrid Neosho Bass and those with a value of 1 being non-hybrid Smallmouth Bass. The individuals with the highest proportion of Neosho Bass alleles from STRUCTURE matched those given a 0 hybrid index value in *introgress*. Individuals assigned as hybrids in NewHybrids were well aligned with the heterozygosity index values gathered in *introgress* with F₁ individuals having a near 1 heterozygosity index value (Figure 8).

Spatial Analyses

There were noticeable differences in landscape and watershed values within our study area across sampled streams. Watershed area varied drastically by stream system, from the large Illinois River (mean = 2,271 km²) to the much smaller Caney Creek (mean = 216 km²; Table 3). Caney Creek had the highest average percent deciduous forest cover (68%) while the lowest was in the Illinois River (38%). Average percent hay cover was highest in Baron Fork (32%) while Caney Creek had the lowest (23%).

PCA results showed that PC1 accounted for 54% of the variation, PC2 accounted for 27%, PC3 accounted for 15%, and PC4 accounted for 4%. Sites with positive PC1 values were generally those with higher watershed areas and percent hay, whereas negative PC1 values indicate a higher percent deciduous forest land cover (Figure 9; Table 4). In PC2, positive values were associated with distance from introduction site and watershed area, whereas negative values are associated with higher percent hay land cover. We visualized the proportion of Neosho Bass alleles by site to illustrate spatial trends seen between native alleles, watershed, and landscape values (Figure 9). The largest stream site by watershed area with the lowest average percent deciduous forest was Illinois River. The Illinois River also had the lowest average proportion of native Neosho Bass alleles (0.51) and the highest number of F_1 , F_2 , and backcrossed individuals. In Baron Fork and Caney Creek, the sites closest to the reservoir had the lowest proportion of Neosho Bass alleles, 0.75 and 0.48 respectively (Figure 6; Table 5). In the Illinois River there was slight variation among sites, but not in relation to distance from reservoir. Because Flint Creek is far upstream of Lake Tenkiller (Figure 6; Table 3), distance from reservoir was not as relevant, but the site closest in proximity to the Illinois River had the lowest proportion of native alleles.

Discussion

Of the literature outlining the effects of stocking non-native Smallmouth Bass into Lake Tenkiller, this is the most thorough study within the system to date. This study takes place 30 years post-stocking of Smallmouth Bass into Lake Tenkiller and found that of the 1,259 individuals sampled, only 33% were non-hybrid Neosho Bass. Introgression and hybridization were most noticeable within the Illinois River sites, where 65% of the individuals captured were

classified as hybrids or backcrosses. Highest proportions of Neosho Bass alleles were found in the smallest watershed areas, with the lowest rates of hybridization and introgression within the upper reaches of Baron Fork and Caney Creek. These results add to the previous literature in the study area (Malloy 2001; Taylor et al. 2018), confirming that severity of introgression has increased with time between native Neosho Bass and non-native Smallmouth Bass in the area, to the point of hybrid swarming and near-complete loss of non-hybrid Neosho Bass in the mainstem Illinois River in Oklahoma.

The results of this study also provide useful insight into the abiotic factors aiding the invasion of non-native alleles. The spatial trends observed in this study were similar to those found in Peoples et al. (2021) and Muhlfeld (2009), wherein distance from introduction site (i.e., a reservoir) and abiotic landscape factors influenced the amount of non-hybrid Neosho Bass. Smallmouth Bass are stocked in many reservoirs because they do well in those systems (Brewer and Orth 2015), which explains why they can be found in the lower reaches of streams closest to the introduction site (Lake Tenkiller). Non-hybrid Neosho Bass were found in higher numbers within streams with lower watershed areas, higher percent deciduous forest cover, and farther from Lake Tenkiller. Only non-hybrid Smallmouth Bass and those that were backcrosses to Smallmouth Bass were found within Lake Tenkiller. Boxrucker et al. (2004) stated that if non-native Smallmouth Bass were introduced to Grand Lake (an Oklahoma reservoir within the Neosho Bass range), it would not be long before there are non-native alleles in the river systems. Similar to this statement, recent hybrids were found in the highest numbers at sites closest to Lake Tenkiller and in streams with larger watershed areas. Boxrucker et al. (2004) also predicted that if those non-native Smallmouth Bass were introduced to that reservoir, it would cause a scenario similar to Lake Tenkiller where the level of hybridization and introgression would be

high. These statements are still relevant today, and our results may shed light on what would happen if non-native Smallmouth Bass were introduced to similar systems within the Neosho Bass range.

The life history differences between the Neosho Bass and Smallmouth Bass contextualizes the spatial trends observed with non-native invasion and introgression. Neosho Bass inhabit stream systems at the southern extent of the Smallmouth Bass range that are commonly associated with warmer climate (Brewer and Long 2015). While Smallmouth Bass inhabit larger run and pool areas of streams, they are known to travel into smaller streams, which are areas Neosho Bass are most likely to inhabit and reproduce (Brewer and Orth 2015). Smallmouth Bass prefer clear run and pool areas of streams while Neosho Bass have been documented occupying intermittent streams with some having a high sediment load (Brewer and Orth 2015; Brewer and Long 2015). Within this study area, Neosho Bass are found in the stream systems, but are not currently known from Lake Tenkiller, whereas Smallmouth Bass are known to inhabit and thrive in impoundments (Malloy 2001; Boxrucker et al. 2004). These differences in preferred habitat may explain why our study did not recover non-hybrid Smallmouth Bass in the upper reaches of smaller watersheds (Baron Fork and Caney Creek). While these two species are closely related, there are some observed differences in their ecology, but more work needs to be done to fully understand the ecology and life history of Neosho Bass.

When hybridization events such as this go unchecked, it can lead to population level threats with impacts on fitness and potential loss of native black bass species (Koppelman 2015). Hybridization between endemic fluvial black bass species and introduced or non-native species has been well documented in other systems across the United States (e.g., Bartram's Bass: Leitner et al. 2015; Peoples et al. 2021; and Shoal Bass *M. cataractae*: Alvarez et al. 2015;

Taylor et al. 2018). Muhlfeld et al. 2009 found that small amounts of hybridization between native Westslope Cutthroat Trout and nonnative Rainbow trout caused a sharp decline in reproductive success. This repeated hybridization and introgression could lead to outbreeding depression which has been shown to result in reduced fitness. Goldberg et al. (2005) found that outbred Largemouth Bass (*M. nigricans*) had reduced fitness compared to parental individuals and the F₂ generations were more susceptible to Largemouth Bass virus than the parental and F₁ individuals. The long-term effects of this ongoing hybridization and introgression are unknown and could possibly impact Neosho Bass in the future.

The recent designation of Neosho Bass as a distinct species (Kim et al. 2022) has possible impacts on how best to manage this species. As shown in this study, the hybridization and introgression between Neosho Bass and the introduced Smallmouth Bass is ongoing and poses a threat to the conservation of Neosho Bass. In general, we found that Neosho Bass populations in upper reaches of smaller watersheds were least vulnerable to introgression, whereas those in larger watersheds and closest to the reservoir were most vulnerable. As hybridization and introgression increases, those individuals in the lower reaches with non-native alleles could continue to move into the upper reaches causing further erosion of the native genome. In other areas of Oklahoma where the Neosho Bass inhabits, the native genetic diversity is highest in the largest streams – the very streams that are most vulnerable to invasion and introgression with non-native Smallmouth Bass (Taylor et al. 2016). If Smallmouth Bass are stocked into other reservoirs near the Neosho Bass range, it could cause a continued loss of native genetic diversity, which would eventually result in the depletion of that diversity across its range. There is speculation that anglers have potentially introduced non-native Smallmouth Bass into additional Oklahoma reservoirs, which could further hinder conservation efforts of Neosho Bass

(Boxrucker et al. 2004; Taylor et al. 2016). Potential impacts on fitness of Neosho Bass and hybrids due to repeated hybridization and introgression are unknown, but differences in growth and diet may allow managers to infer this potential.

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Tables

Table 1. Landscape variables used to pair with proportion of Neosho Bass alleles to model the influence on hybridization rates within our study area downloaded from the USEPA StreamCat dataset.

Variable	Spatial Scale	Units
Watershed area	Watershed	km ²
Distance to reservoir	Catchment	km
Deciduous Forest	Catchment	Percent
Hay	Catchment	Percent

Table 2. Summarization of the number of each hybrid class found at each sample site.

Site	Neosho Bass	Smallmouth Bass	F₁	F₂	BC Neosho Bass	BC Smallmouth Bass	NA	Total
Baron Fork	216	8	12	12	68	9	6	331
Caney Creek	89	3	2	3	19	3	3	122
Flint Creek	29	1	0	1	7	1	1	40
Illinois River Lake	82	149	34	109	158	170	20	722
Tenkiller	0	38	0	0	0	6	0	44
Total	416	199	48	125	252	189	30	1,259

Table 3. Range and mean of watershed variables, landscape variables, and the average proportion of Neosho Bass alleles by sample stream.

Variables	Baron Fork		Caney Creek		Flint Creek		Illinois River	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Watershed area (km)	368-896	798	198-238	216	238-300	262	1615-2629	2271
Distance from reservoir (km)	16-65	28	3.95-8	6	92-102	97	15-111	57
Percent Deciduous Forest	28-57	45	34-79	68	46-66	53	14-68	38
Percent Hay	20-53	32	14-56	23	21-29	25	3.6-63	30
Proportion of Neosho Bass alleles	0.048-0.983	0.83	0.126-0.983	0.86	0.218-0.959	0.83	0.041-0.983	0.51

Table 4. Contribution of each model parameter to principal component axes 1-4.

Parameters	PC1	PC2	PC3	PC4
Watershed Area km ²	0.528	0.293	-0.693	-0.393
Percent Deciduous	-0.637	0.161	0.011	-0.754
Percent Hay	0.454	-0.629	0.370	-0.512
Distance from Reservoir km	0.332	0.702	0.619	-0.121

Table 5. Closest and furthest site from Lake Tenkiller at each sampled stream along with the proportion of Neosho Bass alleles at those sites.

Stream	Site	Proportion Neosho Bass	Distance (km)
Caney Creek	Welling Bridge	0.48	3.95
Caney Creek	Middle 1	0.89	7.61
Illinois River	Horseshoe Bend	0.68	10.60
Baron Fork	Creel_010	0.75	15.80
Baron Fork	Baron Fork WMA	0.98	65.20
Flint Creek	412 Dam	0.80	91.59
Flint Creek	New Life Ranch	0.85	101.8
Illinois River	Frances Dam	0.70	111

Figures

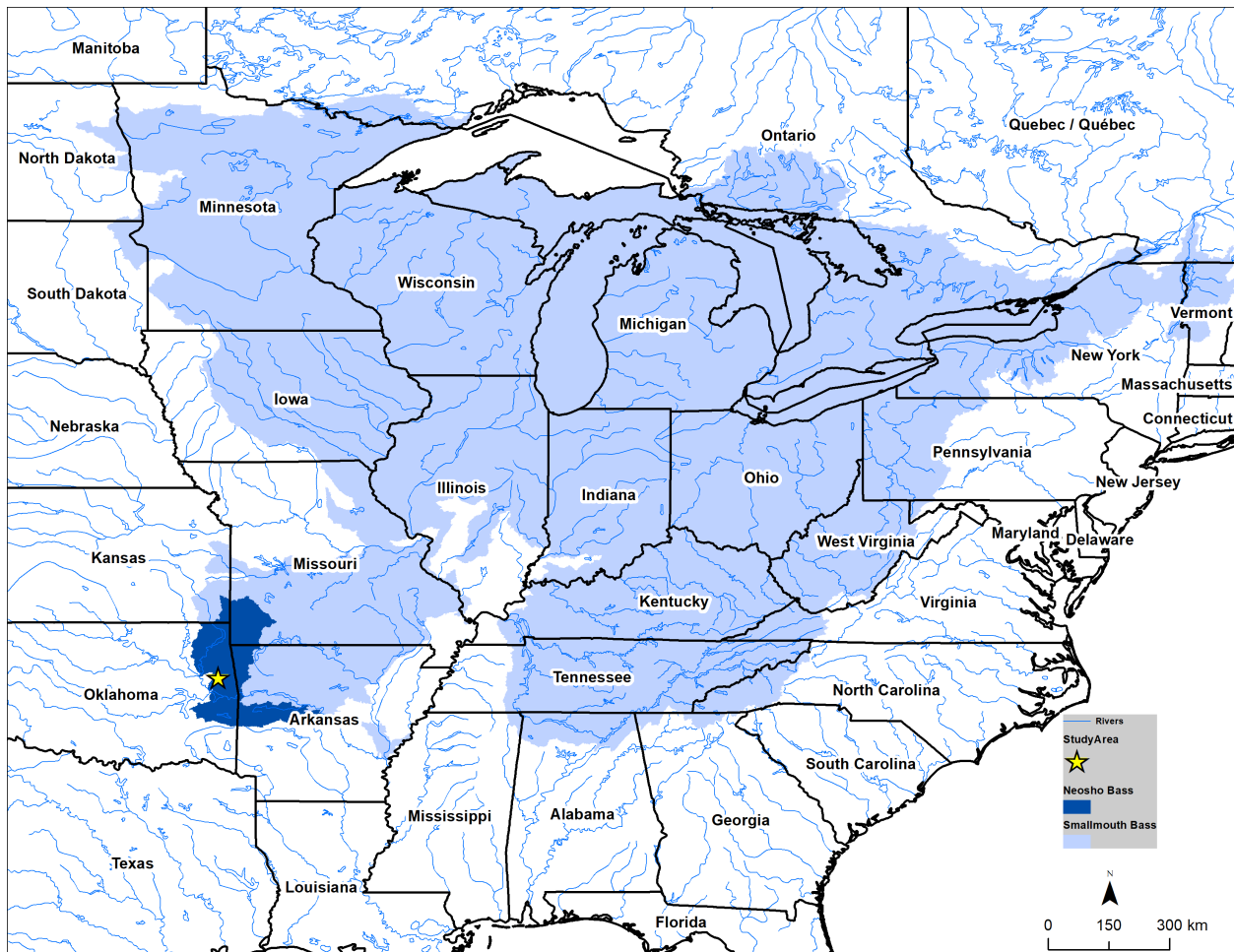


Figure 1. Native distribution map of Neosho Bass and Smallmouth Bass as adapted from Taylor et al. (2019) and Kim et al. (2022).

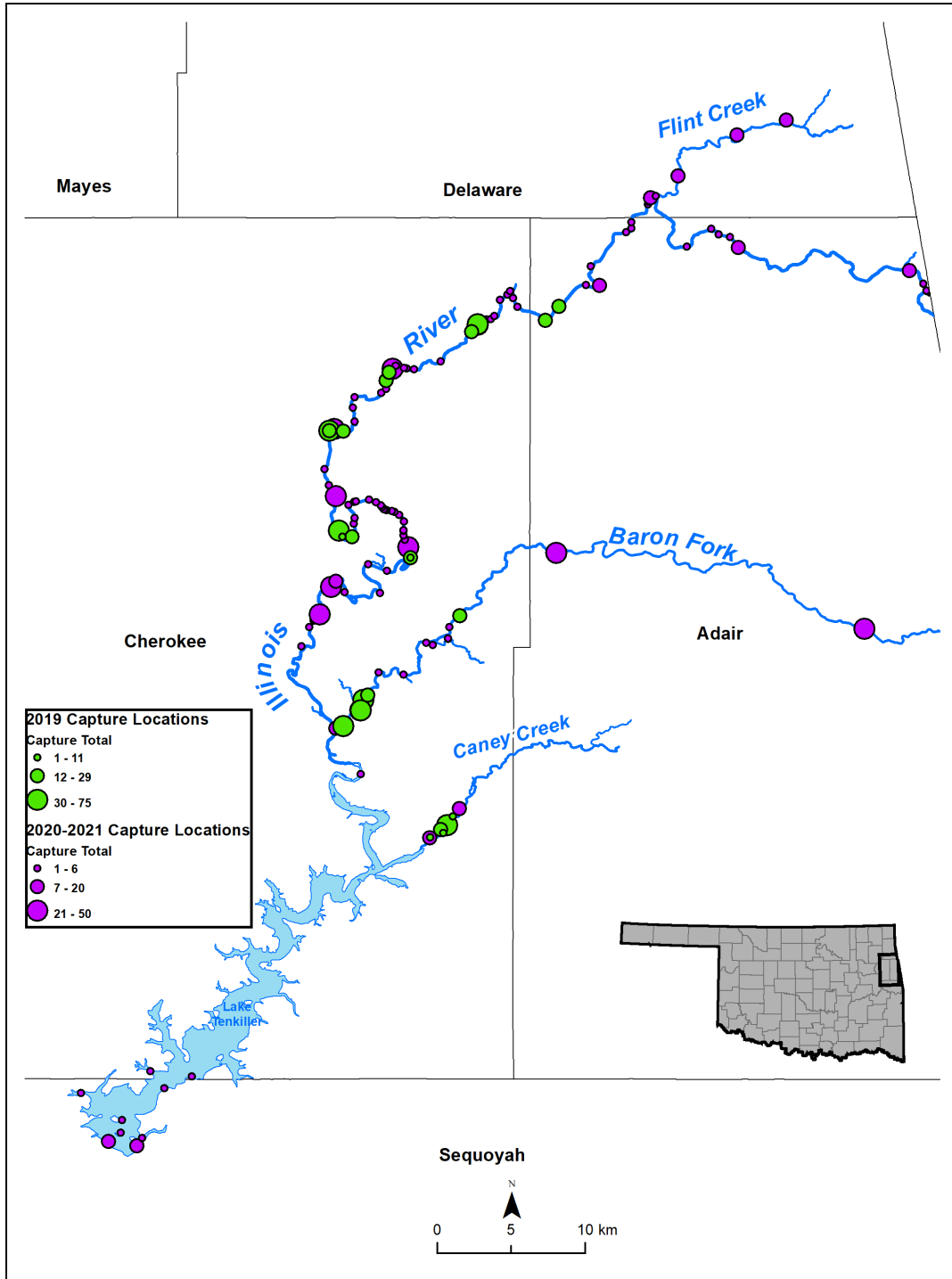


Figure 2. Capture locations by year in Lake Tenkiller, Oklahoma, and its tributaries within the upper Illinois River basin. Sample sites are indicated by the points on the map and are colored based on sample year (green= 2019, purple= 2020-2021). The size of dots represents the number of individuals captured at each sample site with increasing size indicating that more individuals were taken in those areas than the areas with smaller dots.

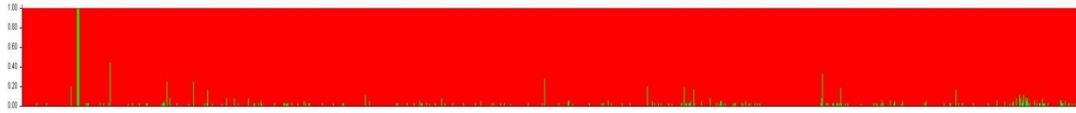


Figure 3. STRUCTURE results depicting individual proportional assignments for individuals using the Spotted Bass (green) x Smallmouth Bass complex (red) SNP panel at estimated $K=2$.

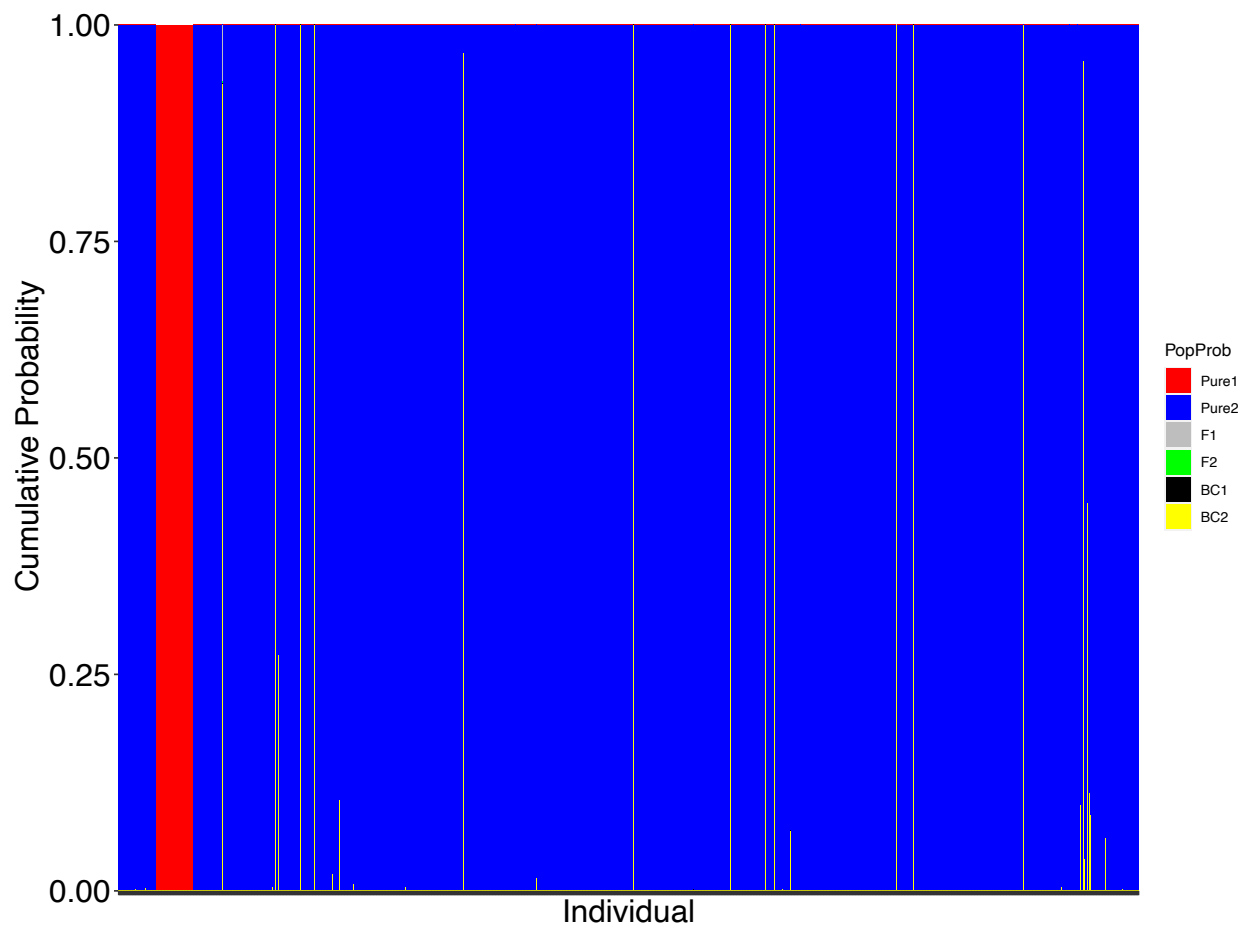


Figure 4. Individual hybrid class proportions of 1,272 sampled individuals based on *parallelnewhybrids* and *hybriddetective* assignments where Pure 1 are Spotted Bass and Pure 2 are Smallmouth Bass.

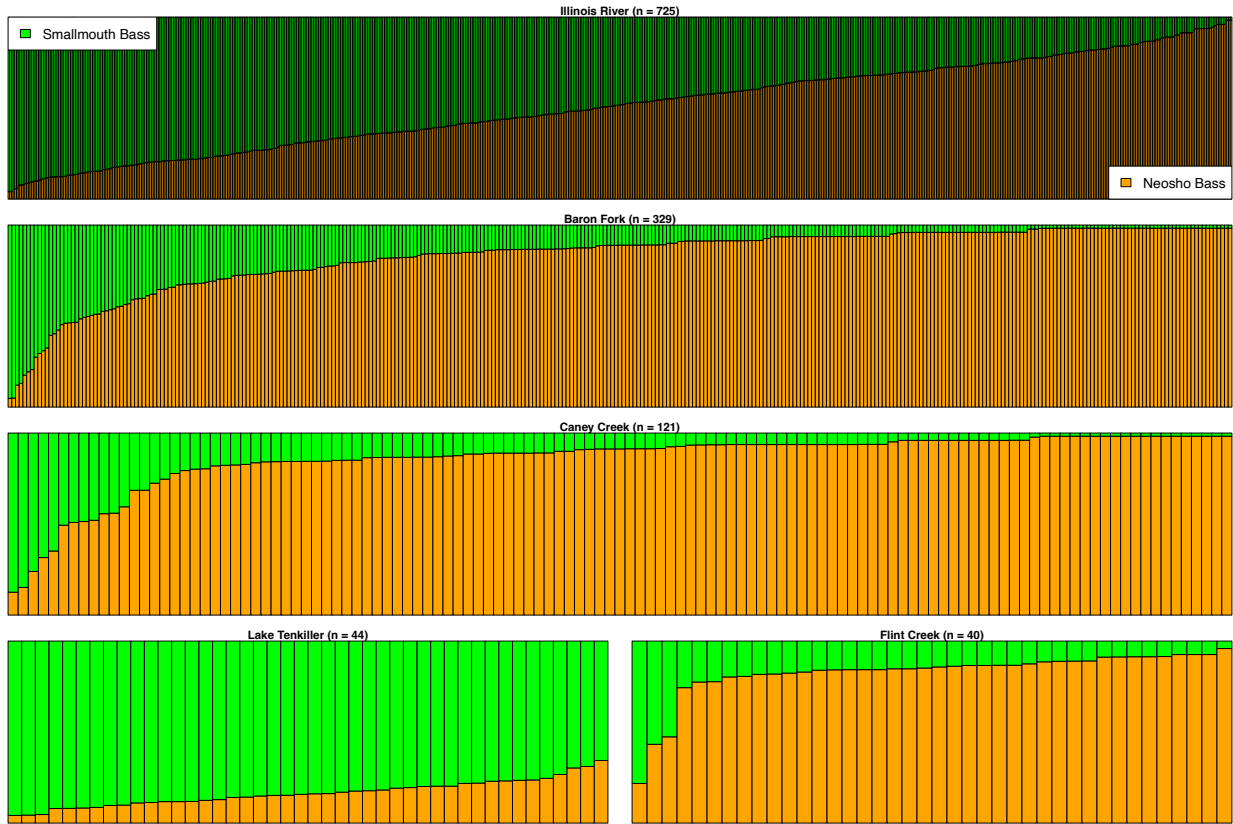


Figure 5. STRUCTURE results depicting individual proportional assignments (orange = Neosho Bass, green = Smallmouth Bass) by stream systems and Lake Tenkiller. Individuals are organized by their assignment proportions (left to right, low to high Neosho Bass proportions).

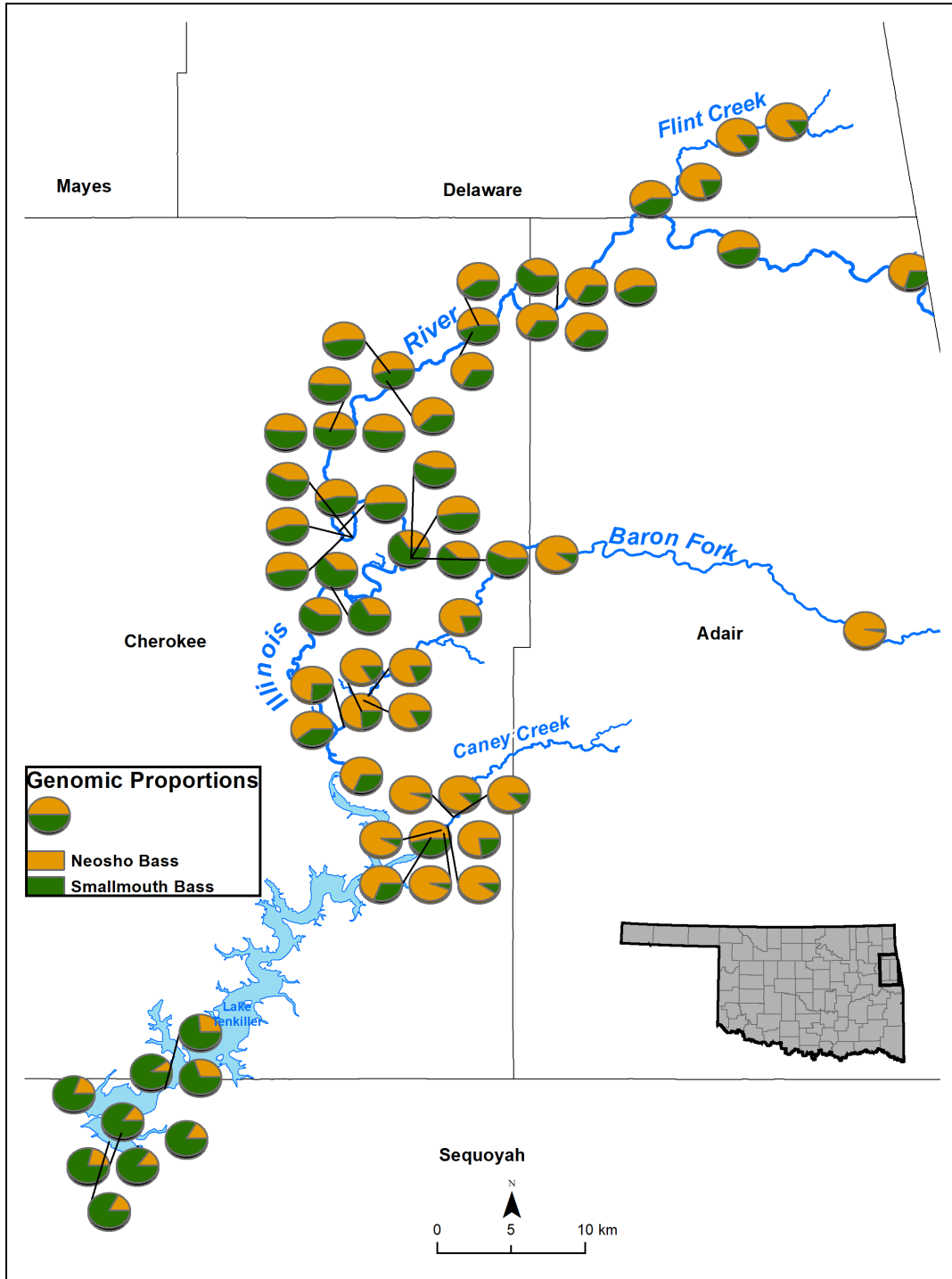


Figure 6. Average genomic class proportions by sample site with pie chart values based on STRUCTURE assignments (orange = Neosho Bass, green = Smallmouth Bass). Locations where fish were captured via angling were excluded from figure all sites except those in Flint Creek to increase readability of pie charts.

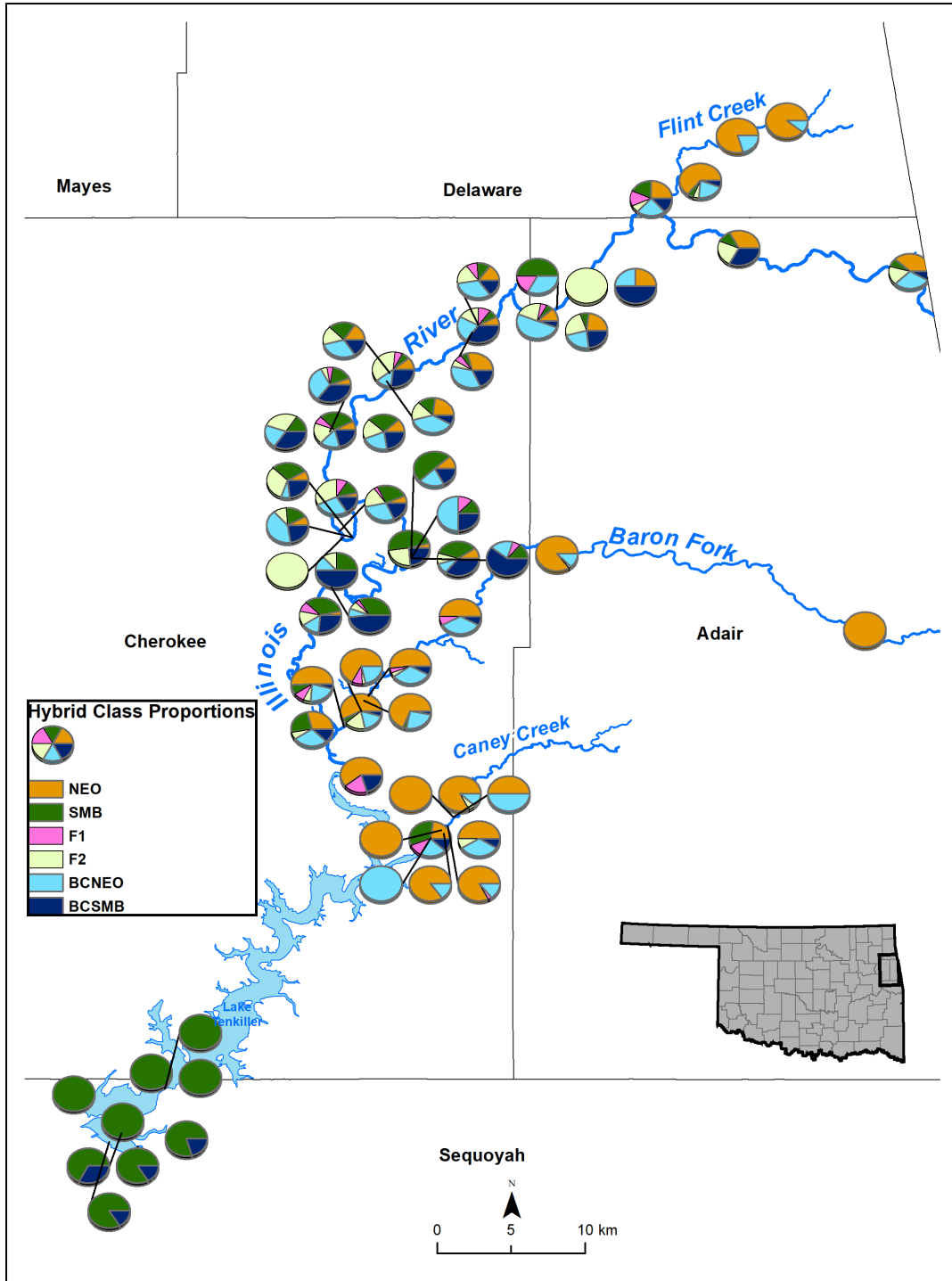


Figure 7. Average hybrid class proportions (NEO = Neosho Bass, SMB= Smallmouth Bass, BCNEO= backcross Neosho Bass, and BCSMB= backcross Smallmouth Bass) by sample site with pie chart values based on *parallelnewhybrids* and *hybriddetective* assignments. Locations where fish were captured via angling were excluded from figure all sites except those in Flint Creek to increase readability of pie charts.

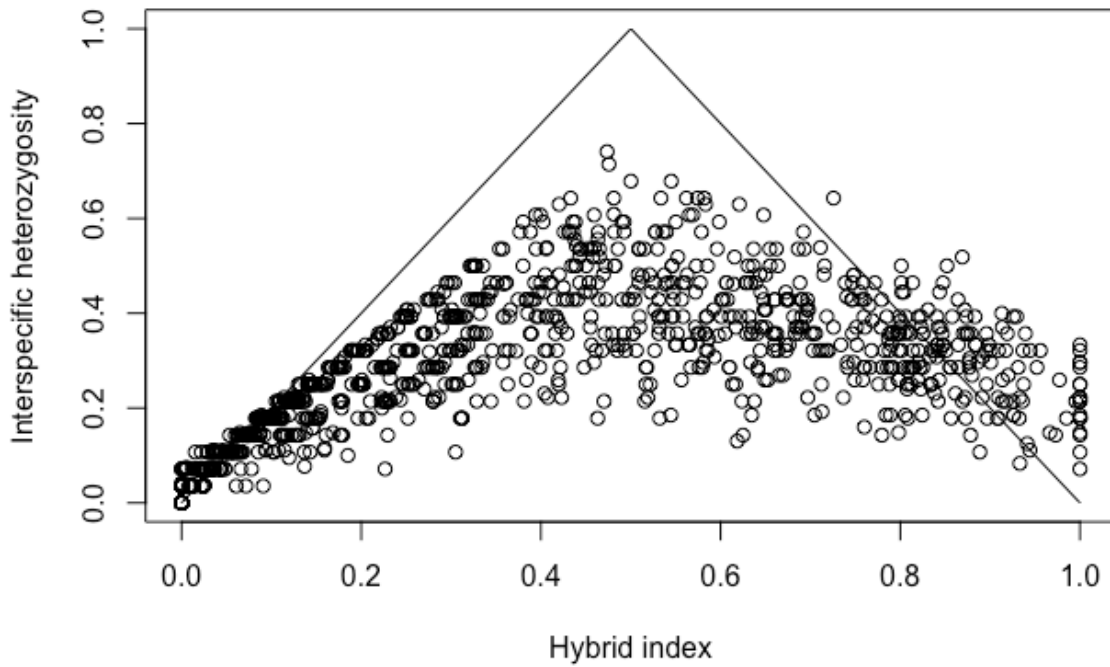


Figure 8. Triangle plot created in *introgress* with each dot representing individuals in our dataset. The value 0.0 on the x axis represents non-hybrid Neosho Bass and 1.0 represents non-hybrid Smallmouth Bass while on the y axis individuals close to 0.0 would again be non-hybrid individuals whereas those close to 1.0 would be F1 hybrids.

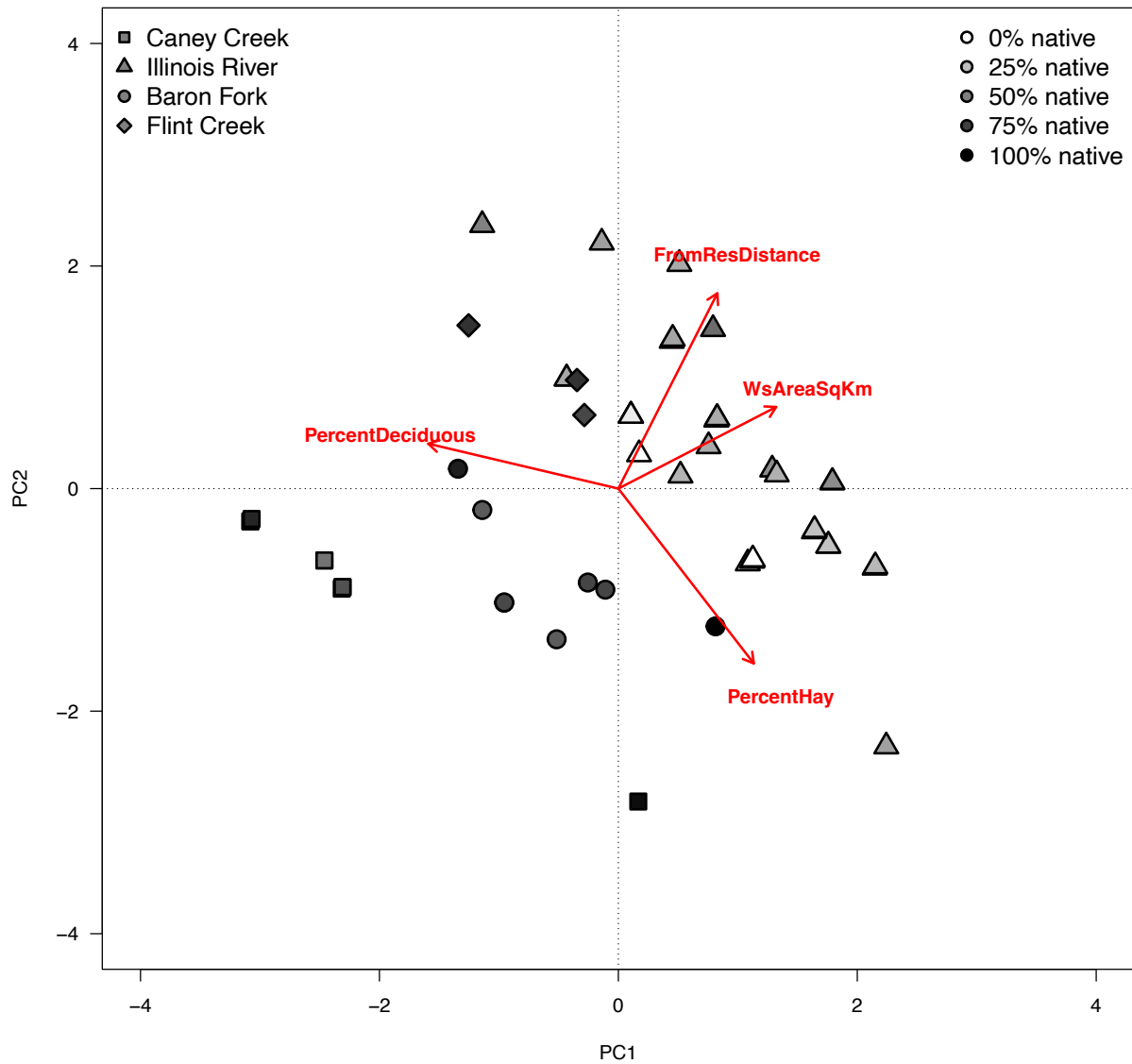


Figure 9. PCA plot with shapes indicating different sample sites within a stream system, and grayscale indicating average proportion of Neosho Bass alleles at each site.

Chapter 2

Influence of genetic identity on age and growth of native Neosho Bass and non-native Smallmouth Bass within the upper Illinois River basin, Oklahoma

Introduction

Due to their popularity among sport fishers, Smallmouth Bass (*Micropterus dolomieu*) have been introduced in many man-made impoundments, streams, and lakes (Martin and Fisher 2009; Brewer and Orth 2015). Smallmouth Bass management techniques have included historical widespread stocking to create angling opportunities where anglers are pursuing trophy-sized fish (Brewer and Orth 2015; Long et al. 2015; Taylor et al. 2019). An emerging paradigm shift in management has been seen where conservation of native black bass diversity is prioritized. As a result, some anglers have been pursuing native black bass species from their native habitats (Taylor et al. 2019). In Oklahoma however, stream anglers prefer larger sized fish regardless of their native or non-native status (Joshi et al. 2021). In human altered systems, a potential conflict could arise between management practices aimed at creating trophy fisheries with non-native black basses and efforts to conserve native black basses.

Hubbs and Bailey (1940) described the Neosho Smallmouth Bass subspecies (*M. d. velox*; hereafter Neosho Bass) which occurs in the northeastern region of Oklahoma, northwestern Arkansas, and southwestern Missouri, while designating the remainder of the broad range as the Northern subspecies (*M. d. dolomieu*; now Smallmouth Bass; Figure 1). Neosho Bass have an elongated “streamline” body and a uniform color that fades to a white underside. Gunn et al. (2020) also described morphological differences between the Northern and Neosho subspecies and found that relative to total length, Neosho Bass has a larger head length than Smallmouth Bass. Several studies that have reaffirmed the genetic distinctiveness between the

Northern and Neosho subspecies (Stark and Echelle 1998; Taylor et al. 2018; Long et al. 2020), and recently Kim et al. (2022) elevated the Neosho Bass, *M. velox*, to a distinct species.

In 1991 and 1992 Northern “Tennessee lake-strain” Smallmouth Bass, from the Tennessee and Cumberland River systems, were stocked into the lower reaches of Lake Tenkiller near the dam for angling opportunities (Boxrucker 2004). Prior to stocking, native Neosho Bass were abundant in streams and present in Lake Tenkiller in low numbers (Boxrucker et al. 2004). Stocking of Lake Tenkiller with non-native Smallmouth Bass eventually resulted in introgressive hybridization with Neosho Bass in the upstream tributaries of the upper Illinois River Basin (see Chapter 1; also Boxrucker et al. 2004; Taylor et al. 2018). Changes in genetic composition have the potential to alter life history strategies and population dynamics, both of which are relevant to fisheries management. For example, measurements of growth are used by managers to determine size limits and quality of a fishery (Starks and Rodger 2020).

There are many existing studies on Smallmouth Bass growth, but there are confounding factors when comparing existing data on Neosho Bass. Smallmouth Bass are generally thought to grow larger and faster than the native Neosho subspecies (Fiss et al. 2001; Brewer and Long 2015). Smallmouth Bass are long lived with a longevity of 12-15 years, whereas Neosho Bass rarely reach 6 years (Brewer and Orth 2015; Brewer and Long 2015). Smallmouth Bass length-at-age is highly variable across its broad distribution, but on average from years 1 to 15 they are between 92-472 mm total length (TL) with some reaching over 500 mm (Beamesderfer and North 1995). Lotic Smallmouth Bass are thought to grow relatively slower than those found in reservoirs, with those in larger streams growing faster than those in smaller streams (Fiss et al. 2001; Brewer and Orth 2015; Starks and Rodger 2020). Various studies have examined the growth of Neosho Bass within their native range, but did not include genetic surveys to confirm

the identity of captured individuals (Leonard and Jenkins 1952; Balkenbush and Fisher 1999; Starks and Rodger 2020). Leonard and Jenkins (1952) performed back-calculated TL on Smallmouth Bass (presumably Neosho Bass) within the Illinois River and reported that all fish within the river displayed growth patterns that mirrored that of a smaller tributary, Caney Creek, reaching 330 mm by age 4 and rarely exceeding 5 years of age. Following the stocking of Lake Tenkiller in 1991-1992, Smallmouth Bass collected from Baron Fork reached 282 mm TL by age 4, also rarely exceeding 5 years of age (Balkenbush and Fisher 1999). Most recently, a genetic survey of these tributaries by Taylor et al. (2016, 2018) documented that fish genotyped as non-hybrid Smallmouth Bass found in the Illinois River had a mean TL of 282 mm with fish genotyped as non-hybrid Neosho Bass having a mean TL of 240 mm; however, no age estimates were obtained for these genotyped samples. Another issue when comparing existing growth data on Neosho Bass and Smallmouth Bass is that pre-stocking studies of presumptive Neosho Bass commonly used scales to estimate age, whereas otoliths are commonly used to age southern Smallmouth Bass populations (Long and Fisher 2011; Starks and Rodger 2020). Finally, we cannot assume that Neosho Bass growth has been accurately described in the region because populations often feature high amounts of introgression (see Chapter 1; also Gunn et al. 2022).

The objective of this study was to quantify the influence of genetic identity on growth of native Neosho Bass, non-native Smallmouth Bass, and their hybrids within the upper Illinois River Basin. Using transversely sectioned sagittal otoliths, we first estimated the ages of specimens. Next, we modeled growth by fitting nonlinear growth curves that were then compared across genetic identities. Results of this study can be used to inform management decisions by addressing the speculated differences in growth between Neosho Bass and Smallmouth Bass that, as of now, remain enigmatic.

Materials and Methods

Data Collection and Genotyping

In the Summer and Fall of 2020 and 2021, the Oklahoma Department of Wildlife Conservation (ODWC) Stream Team collected 650 field-identified “Smallmouth Bass,” which included Neosho Bass, Smallmouth Bass, and their hybrids. Fin-clips and otoliths were taken from the 650 specimens to couple age estimates with genetic analyses. Specimens were collected via electrofishing and angling within the Illinois River, Flint Creek, Baron Fork, Caney Creek, and Lake Tenkiller. All specimens encountered throughout field surveys were collected regardless of size. During our electrofishing surveys, larger individuals (>355mm) were somewhat rarely encountered, but angling reports in the region suggested larger fish occurred in the Illinois River; therefore, angling was used within the Illinois River at the end of the study to ensure these larger size classes were represented in our dataset.

Genotyping was performed using a SNP panel designed to identify Neosho Bass and Smallmouth Bass individuals (Long et al. 2020). We then assigned individuals to hybrid classes using NewHybrids (Anderson and Thompson 2002) as implemented in the *hybriddetective* and *parrallelnewhybrid* packages (Wringe et al. 2017) in R version 4.2.1. We assigned individuals to six classes: non-hybrid Neosho Bass, non-hybrid Smallmouth Bass, F₁, F₂, first-generation backcross towards Neosho Bass, and first-generation backcross towards Smallmouth Bass (see Chapter 1).

Age Estimation

Age estimation (in years) was conducted by counting annuli of sectioned otoliths – a common methodology that generally provides precise and accurate estimates for black bass (Buckmeier and Howells 2003; Maceina et al. 2007; Long and Fisher 2011). To enhance annuli

visibility, we embedded whole sagittal otoliths in epoxy and cut transverse sections of the otoliths. Transverse sections were placed under a microscope for viewing and clear pictures were taken for age estimation (Figure 2). Once otoliths were sectioned, age was estimated independently by three independent readers to allow quantification of among-reader precision of age estimates. Discrepancies among readers were resolved by consensus reads first between two readers at the University of Central Oklahoma (UCO), then consensus estimates generated by UCO and estimates by ODWC biologists. To assess variation in reader annuli count precision, coefficient of variation (CV) was calculated for each reader following Chang (1982), wherein we also added one to all annuli counts to avoid division by zero. To visualize the change in precision with each number of annuli counted, overall CV estimates (UCO and ODWC) were plotted against the final consensus annuli count.

Growth Analyses

Once age estimation and genetic assignment (see Chapter 1) of each fish was completed, we modeled individual growth. To model growth, we used von Bertalanffy growth functions (VBGF) in the *FSA* package (Ogle et al. 2023) for nonlinear regression in R version 4.2.1 (R Core Team 2022). The VBGF is a commonly used function when modelling the growth of fishes (Ogle et al. 2017). A common VBGF model was used to collect the mean length-at-age of sampled individuals:

$$E[L|t] = L_{\infty}(1 - e^{-K(t-t_0)})$$

where $E[L|t]$ is the mean length-at-age, t , while K , t_0 , and L_{∞} are parameters that are being estimated. L_{∞} is the maximum mean length for a population, individuals can be above or below this value. The parameter t_0 , also the x-intercept, is used to describe when the mean length is zero and can be a positive or negative value. Brody growth coefficient (K) describes how quickly the

mean length-at-age is approaching L_{∞} (Ogle et al. 2017). Individuals that lacked an estimated age were excluded from the overall VBGF model.

We constructed a series of VBGF models to compare growth across genetic identities. We first constructed a global model (all genetic identities included), followed by a comparison of non-hybrid Neosho Bass to non-hybrid Smallmouth Bass. Last, we calculated a VBGF for each hybrid identity (F_1 , F_2 , and first-generation backcrosses in either direction) to compare with those of non-hybrid parental species. For comparisons across identities, we first examined for differences in estimated VBGF parameters (K , t_0 , and L_{∞}), followed by comparisons of estimated length-at-age. Statistical significance of model parameters and length-at-age estimates was inferred from non-overlapping 95% confidence intervals. Individuals that were unable to be assigned to a genetic identity and lacked an age estimate were excluded from this part of the growth modelling.

Results

Data Collection and Genotyping

Of the 650 collected individuals, 403 were collected from the Illinois River, 122 from Baron Fork, 40 from Caney Creek, 40 from Flint Creek, and 45 collected in Lake Tenkiller (Figure 3). There were 492 specimens collected via electrofishing and 158 specimens collected via angling. The Illinois River, Baron Fork, and Flint Creek were the only streams where angling was performed ($n= 97, 21, \text{ and } 40$ respectively). The genetic identities within the sample were 194 non-hybrid Neosho Bass, 131 non-hybrid Smallmouth Bass, 25 F_1 , 73 F_2 , 97 backcross Neosho Bass, and 108 backcross Smallmouth Bass (see Chapter 1). There were also 22 non-assigned individuals that were removed prior to genetic identity growth modelling.

Age Estimation

Overall mean CV among readers was 2.45% (n = 647; SE = 0.98) with the highest mean CV occurring in fish ranging 5-8 years of age (Figure 4). Age estimation agreement among independent readers was 97%, wherein highest disagreements occurred when estimating age 8 individuals (± 2).

Of the 650 sampled fish, three individuals were not assigned an estimated age due to either damaged otoliths or unable to come to an agreement on age, leaving 647 individuals. Estimated ages ranged from 0-10 years of age with an average of 2 years of age. Ages 1 and 3 had the highest number of individuals (297 and 150, respectively) while ages 9 and 10 only had one individual each. The maximum sampled age for non-hybrid Neosho Bass was 5 years of age while for non-hybrid Smallmouth Bass it was 8 years of age. The oldest individual was estimated at 10 years of age and was an F₁ hybrid captured in Baron Fork (Figure 5).

Growth Analyses

Overall, the 647 sampled individuals had a total length (TL) range of 67-536 mm with an average of 250 mm TL (Table 1). Non-hybrid Neosho Bass had a TL range of 67-423 mm while non-hybrid Smallmouth Bass ranged 97-536 mm. Average TL also varied among non-hybrid Neosho Bass and non-hybrid Smallmouth Bass where non-hybrid Neosho Bass had an average TL of 234 mm and non-hybrid Smallmouth Bass had the largest overall at 283 mm TL. Amongst all hybrid classes, the smallest average TL was backcross Neosho Bass (230 mm TL). The largest fish captured in the study was a non-hybrid Smallmouth Bass captured in Lake Tenkiller that had a TL of 536 mm.

Our global VBGF analysis included 647 fish with an estimated age regardless of their genetic status. The following parameters were estimated for those 647 individuals: $L_{\infty}=487.77$,

$K=0.306$, and $t_0=-0.874$ (Figure 6). The estimation of the parameters by genetic identity included 625 fish total that had an assigned genetic identity and an estimated age. The VBGF analysis for non-hybrid Neosho Bass (194 individuals), estimated parameters $L_\infty=410$, $K=0.29$, and $t_0=-1.00$, whereas parameters for non-hybrid Smallmouth Bass (131 individuals) were $L_\infty=550$, $K=0.32$, and $t_0=-0.69$ (Figure 7). There were no significant differences in model parameters between non-hybrid Neosho Bass and non-hybrid Smallmouth Bass (Table 2). However, the two species show significant differences in estimated length-at-age beginning at age-1. Once these two species exceed 3 years of age, non-hybrid Smallmouth Bass have an estimated length-at-age that is over 100 mm TL larger than non-hybrid Neosho Bass (Table 3; Figure 7).

Our VBGF analysis for all genetic identities revealed that F₁ and F₂ hybrid individuals have growth curves that are intermediate of non-hybrid Neosho Bass and non-hybrid Smallmouth Bass (Figure 8). The VBGF analysis estimated that backcross Neosho Bass (97 individuals) had the highest L_∞ (680 mm TL), whereas non-hybrid Neosho Bass had the lowest (410 mm TL).

Discussion

Although many have studied age and growth of Smallmouth Bass, this is the first study to pair genetics with age and growth to describe differences in growth among Neosho Bass, Smallmouth Bass, and their hybrids. Unlike previous studies within the area, our study used a single structure to estimate age, sagittal otoliths, which have been shown to produce more accurate readings and growth estimations (Starks and Rodger 2020). Similar to previous studies that may pre-date pervasive hybridization (Leonard and Jenkins 1952; Balkenbush and Fisher

1999), there were no non-hybrid Neosho Bass over 5 years of age. We found that the estimated length-at-age of non-hybrid Smallmouth Bass becomes substantially higher than non-hybrid Neosho Bass as age increases. These results confirm marked differences in longevity and growth between Neosho Bass and Smallmouth Bass.

We found significant variation in growth and longevity between Neosho Bass and Smallmouth Bass which supports their differences as unique species with possible life history differences. As stated above, non-hybrid Smallmouth Bass had a maximum estimated age of 8 years while we found no non-hybrid Neosho Bass over 5 years of age, indicating that non-hybrid Smallmouth Bass have the advantage of increased years of reproductive activity. On average, Smallmouth Bass are sexually mature when they reach 200-250 mm TL, and maturity is not necessarily associated with age (Ridgway et al. 1991; Wiegmann et al. 1997). However, there have been no studies confirming the size at maturity of Neosho Bass. If we apply these literature-based values to our system, non-hybrid Smallmouth Bass would potentially be sexually mature by age 1, whereas non-hybrid Neosho Bass would not reach mature size until an average of 2 or 3 years (Figure 7). Female Smallmouth Bass also have increased fecundity with increased size (Ridgway et al. 1991), meaning that within this study area, non-hybrid Smallmouth Bass have another reproductive advantage over the smaller native Neosho Bass. The increased longevity and length-at-age of Smallmouth Bass gives them a concerning reproductive advantage over Neosho Bass that should be considered when making management decisions in the future.

Hybrids of other black back species, particularly Florida Bass (*M. salmoides*) and Largemouth Bass (*M. nigricans*), have been shown to display heterosis or hybrid vigor in their maximum length. Lutz-Carrillo et al. (2022) found that size-related hybrid vigor (heterosis) in Florida Bass and Largemouth Bass F₁ hybrids was positively related to levels of Florida Bass

alleles. Such observations provide justification for numerous stocking strategies for these hybrids due to their growth potential (Long et al. 2015; Taylor et al. 2019); however, we saw no evidence of size-related heterosis in Smallmouth Bass and Neosho Bass F_1 hybrids. In our study, F_1 hybrids and non-hybrid Smallmouth Bass have similar growth in early stages (0-3 years of age), but growth begins to slow at 4 years of age (Figure 8). Although the estimated L_∞ is higher in F_1 individuals (480) than non-hybrid Neosho Bass (410), it is still much smaller than non-hybrid Smallmouth Bass (550). We also see that F_2 hybrids have a slightly larger estimated L_∞ (500) than F_1 hybrids. We also did not find any evidence of growth-related outbreeding depression amongst sampled F_2 hybrids or backcrossed individuals that is typically seen in Florida Bass and Largemouth Bass F_2 hybrids and backcrosses. Back-cross Neosho Bass had the highest estimated L_∞ out of all hybrid classes, but this could be an artefact of the high variability of TL-at-age values of sampled individuals. Within our study, we did find that the two oldest fish (9 and 10 years of age) were F_1 hybrids, which could be a potential display of longevity-related heterosis. Similar longevity-related heterosis in F_1 hybrids was also seen in Lusk et al. (2023), where the oldest individuals in their study were Florida Bass and Largemouth Bass F_1 hybrids. Further study is needed to examine the occurrence of longevity-related heterosis and its potential ramifications in black bass introgression levels.

Trophy fishing for black bass species has grown in popularity over the years and has heightened the catch-and-release mindset among black bass anglers (Long et al. 2015). This increase in trophy fishing has resulted in historic and current stocking programs to promote angling opportunities, which was the initial justification for Smallmouth Bass being stocked into Lake Tenkiller. We found that of the individuals sampled above the typical legal-size limit (>356 TL mm) within our study, 40% were non-hybrid Smallmouth Bass while only 4% were non-

hybrid Neosho Bass. With Oklahoma stream anglers preferring larger-sized fish regardless of their native or non-native status (Joshi et al. 2021), non-native Smallmouth Bass have found favor among angling groups and could create potential conflicts in conserving the native Neosho Bass. Chapagain et al. (2020) found that Smallmouth Bass are the most sought-after species by anglers in eastern Oklahoma, with a related study in Oklahoma by Joshi et al. (2021) finding that anglers are most interested in catching trophy sized fish. Therefore, managers must navigate a tradeoff of potential economic benefits garnered by Smallmouth Bass fisheries with the threat that non-native Smallmouth Bass pose to the conservation of the native, endemic Neosho Bass. Conservation strategies for native Neosho Bass within our study area could also benefit from this size variation if harvest of the larger size classes (>356 mm TL) was encouraged.

As shown within our study, non-native Smallmouth Bass have a higher longevity and grow larger than their native counterpart, the Neosho Bass. While we found differences in growth attributed to genetic identity, there are also numerous environmental factors (i.e., stream size, lotic vs lentic habitats, temperature, and prey availability) that are known to influence Smallmouth Bass growth (Brewer and Orth 2015). Future research would benefit from the inclusion of environmental influences, such as variation in stream size or habitat to better understand all potential factors relating to the differences in growth between these species. Furthermore, there are other individual biological factors that may affect growth beyond genetic identity. For example, differences in growth related to sex have been documented in some black bass species; however, in Smallmouth Bass the consensus is that growth is similar between sexes (Carlander 1977). Because Neosho Bass are now considered a distinct species (Kim et al. 2022), future studies should also examine the differences in growth between the sexes in Neosho Bass to determine if variation exists.

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Tables

Table 1. Minimum, maximum, and average total lengths (TL) for each age as estimated from a von Bertalanffy growth function that included all Smallmouth Bass, Neosho Bass, and hybrids (n = 647).

Consensus Age (Years)	Minimum TL (mm)	Average TL (mm)	Maximum TL (mm)	Sample Size (n)
0	67	114	167	60
1	127	206	289	297
2	185	262	393	84
3	217	330	454	150
4	259	368	484	28
5	313	419	482	16
6	448	466	492	3
7	380	447	491	4
8	471	499	536	3
9	444	444	444	1
10	463	463	463	1
NA	161	236	350	3

Table 2. Point estimates along with lower (LCI) and upper (UCI) 95% confidence intervals, for estimated von Bertalanffy growth function parameters between individuals identified as non-hybrid Neosho Bass (n = 194) and non-hybrid Smallmouth Bass (n = 131).

Genetic Identity	L_{∞} (LCI, UCI)	K (LCI, UCI)	t_0 (LCI, UCI)
Neosho Bass	410 (357, 547)	0.29 (0.16, 0.42)	-1.00 (-1.51, -0.67)
Smallmouth Bass	550 (503, 605)	0.32 (0.25, 0.39)	-0.69 (-0.89, -0.52)

Table 3. von Bertalanffy growth function length-at-age estimates for non-hybrid Neosho Bass (n= 194; max sampled age= 5 years of age) and non-hybrid Smallmouth Bass (n= 131; max sampled age= 8 years of age) along with the upper (UCI) and lower (LCI) 95% bootstrap confidence intervals.

Age (Years)	Neosho Bass (Estimated TL mm)	Neosho Bass 95% LCI, UCI	Smallmouth Bass (Estimated TL mm)	Smallmouth Bass 95% LCI, UCI
0	104	87, 122	108	92, 122
1	182	175, 187	228	221, 234
2	239	233, 245	315	307, 323
3	282	277, 288	378	370, 386
4	315	308, 325	424	414, 434
5	339	325, 357	458	445, 471
6	357	337, 386	483	465, 500
7	370	344, 411	500	479, 523
8	380	350, 432	513	489, 540
9	388	353, 449	523	495, 534
10	393	356, 464	530	500, 564

Figures

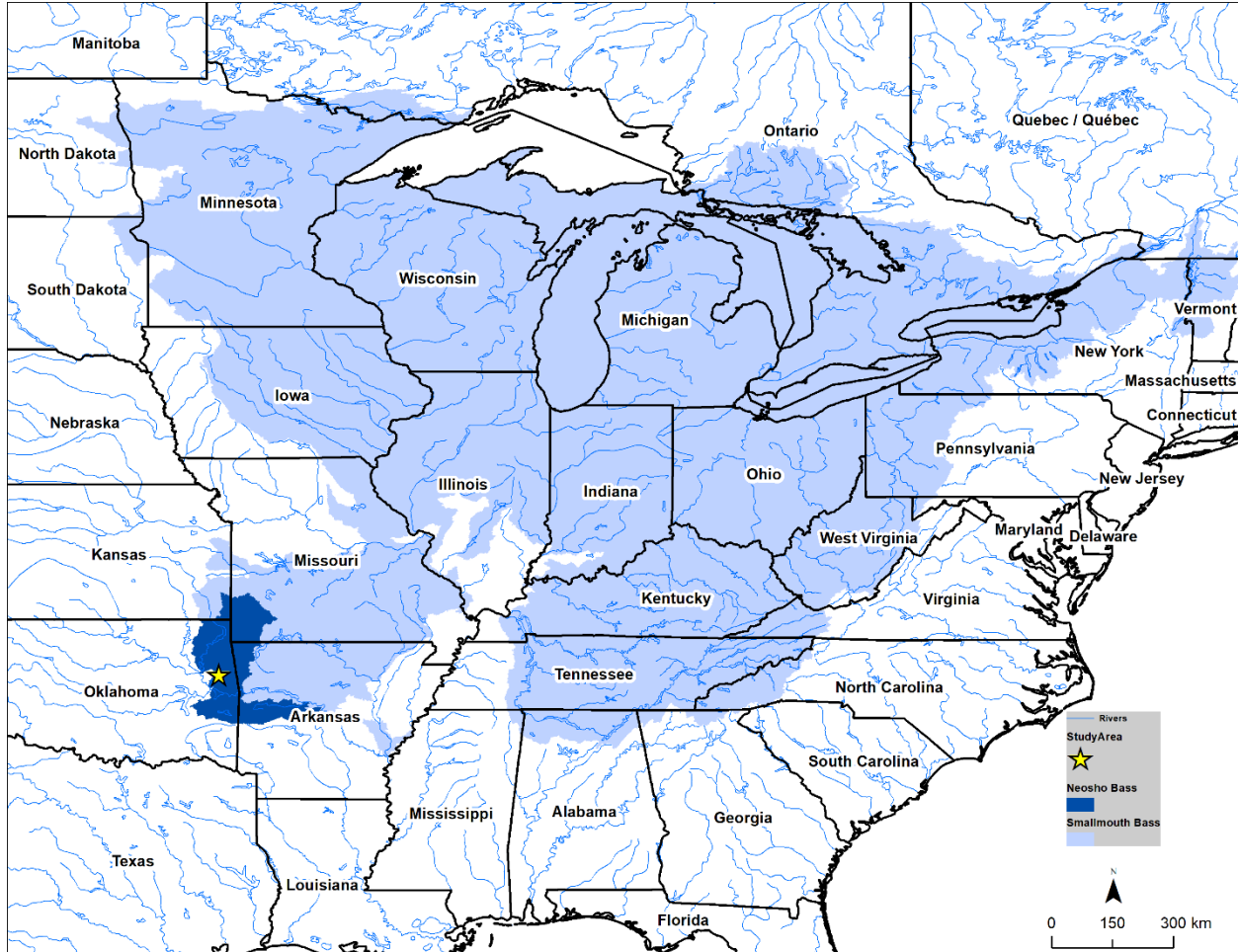


Figure 1. Native distribution map of Neosho Bass and Smallmouth Bass as adapted from Taylor et al. (2019) and Kim et al. (2022).

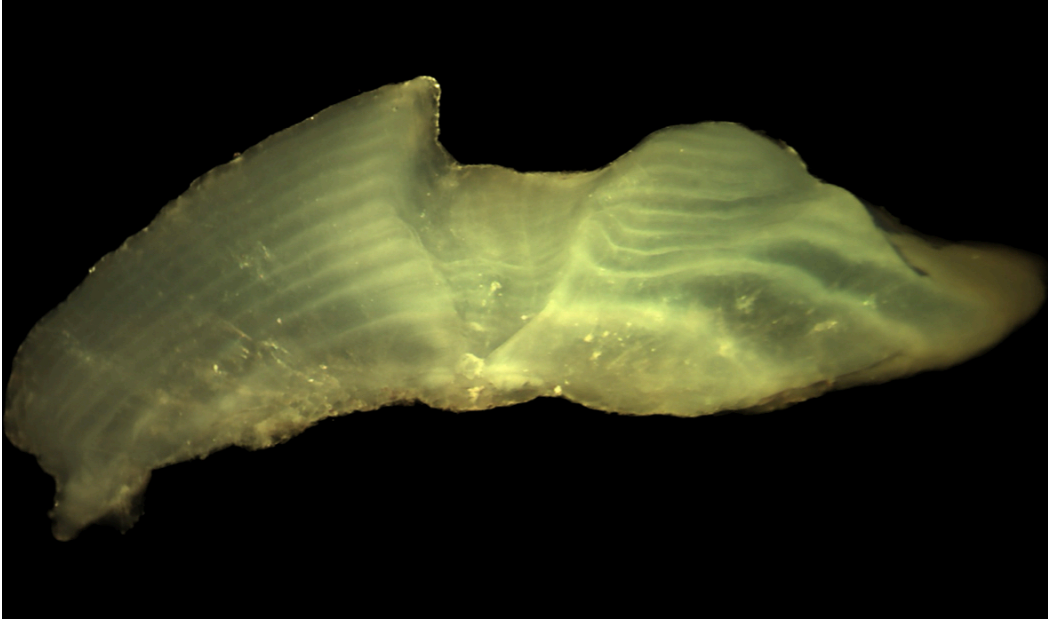


Figure 2. Example image of a transverse sectioned sagittal otolith used to estimate age in this study.

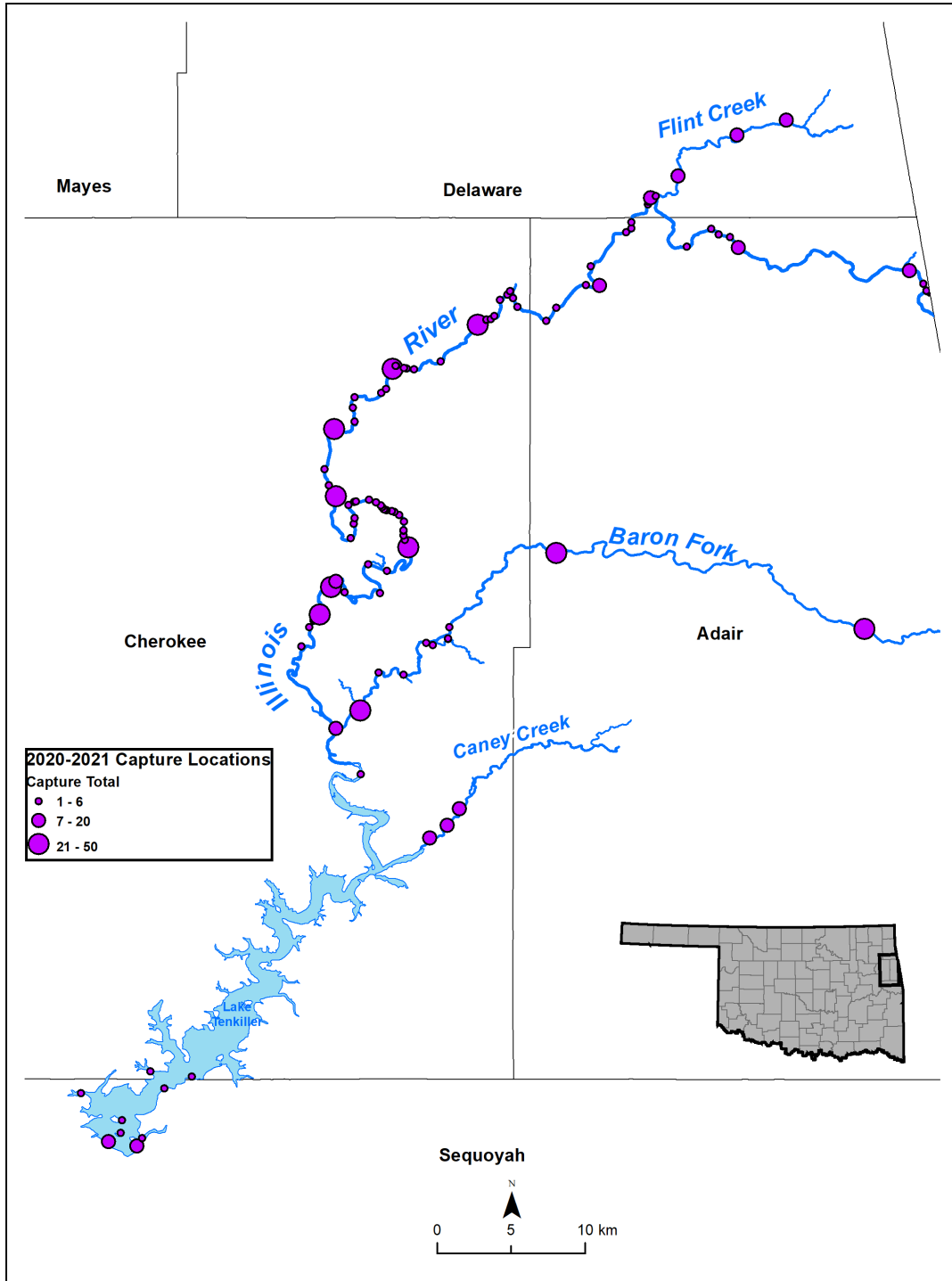


Figure 3. Map depicting the total individuals captured at each capture location in Lake Tenkiller, Oklahoma, and its tributaries within the upper Illinois River basin. Sample sites are indicated by the points with the size of points representing the number of individuals captured at each sample site with increasing size indicating that more individuals were taken in those areas than the areas with smaller points.

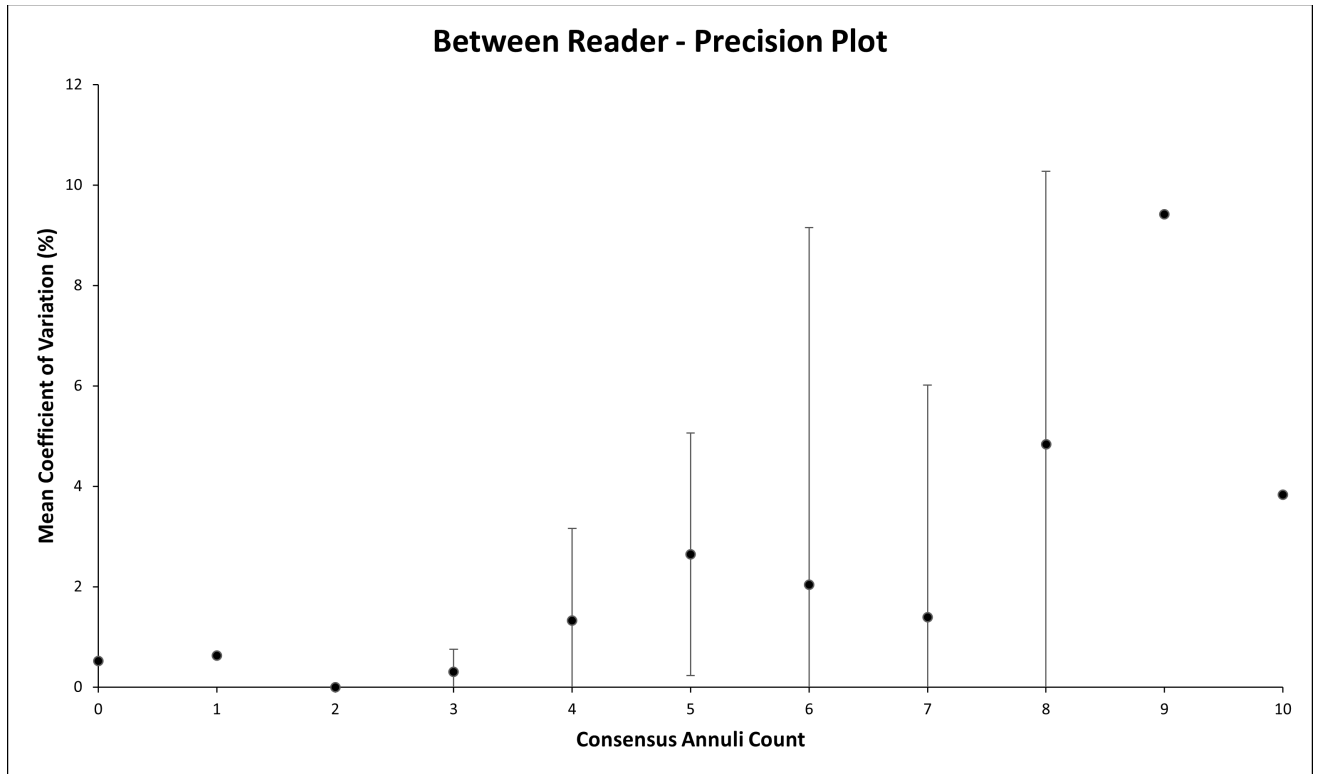


Figure 4. Mean coefficient of variation among UCO and ODWC readers of cross sectioned sagittal otolith annuli counts of the 650 sampled Neosho Bass, Smallmouth Bass, or their hybrids captured in 2020-2021. Error bars represent +/- 1 standard deviation.

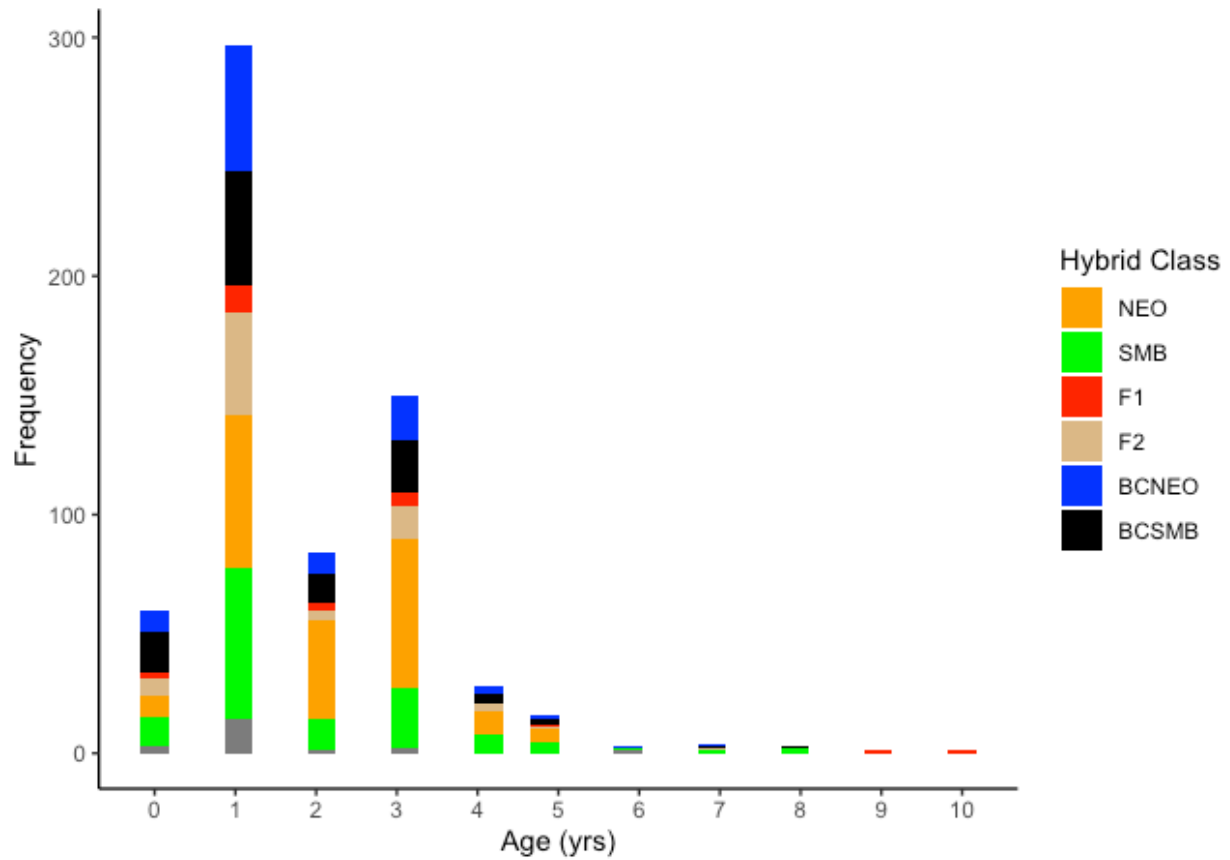


Figure 5. Age frequency histogram for all captured individuals color coded by individuals identified as non-hybrid Neosho Bass (NEO), non-hybrid Smallmouth Bass (SMB), F₁, F₂, backcross Neosho Bass (BCNEO), and backcross Smallmouth Bass (BCSMB).

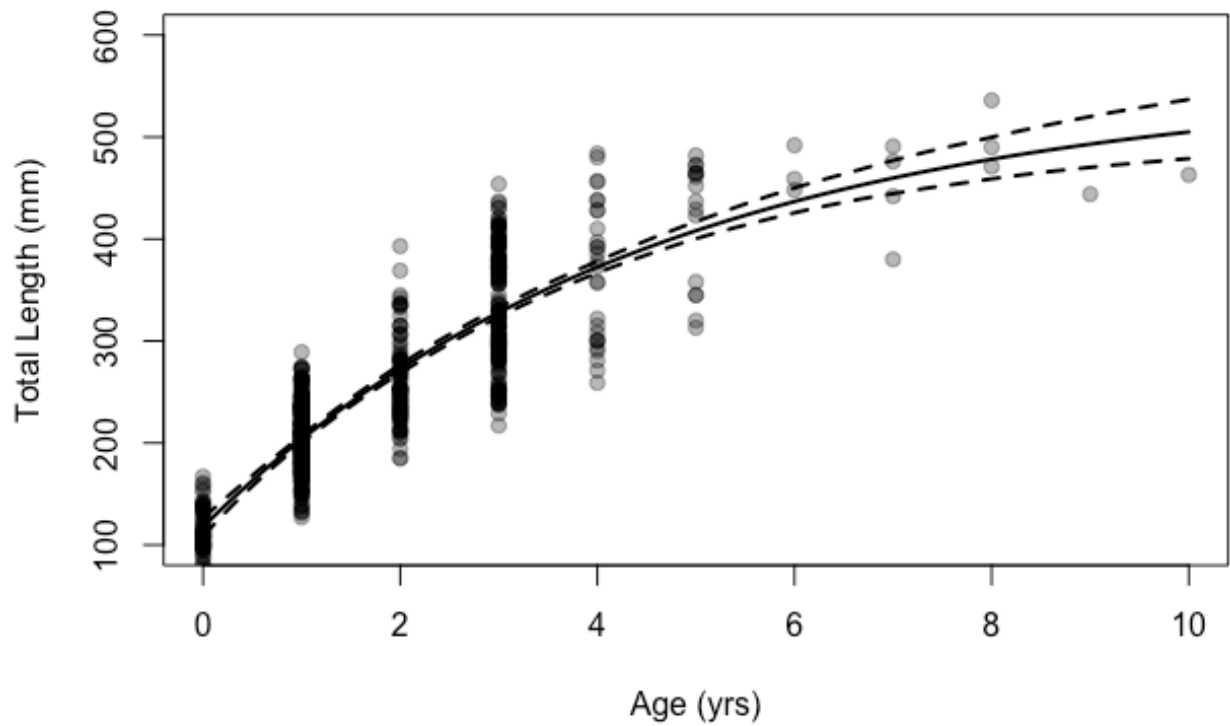


Figure 6. von Bertalanffy growth function displaying average length-at-age, lower (dashed line), and upper 95% confidence intervals (dashed line) of sampled Neosho Bass, Smallmouth Bass, or their hybrids captured in 2020-2021.

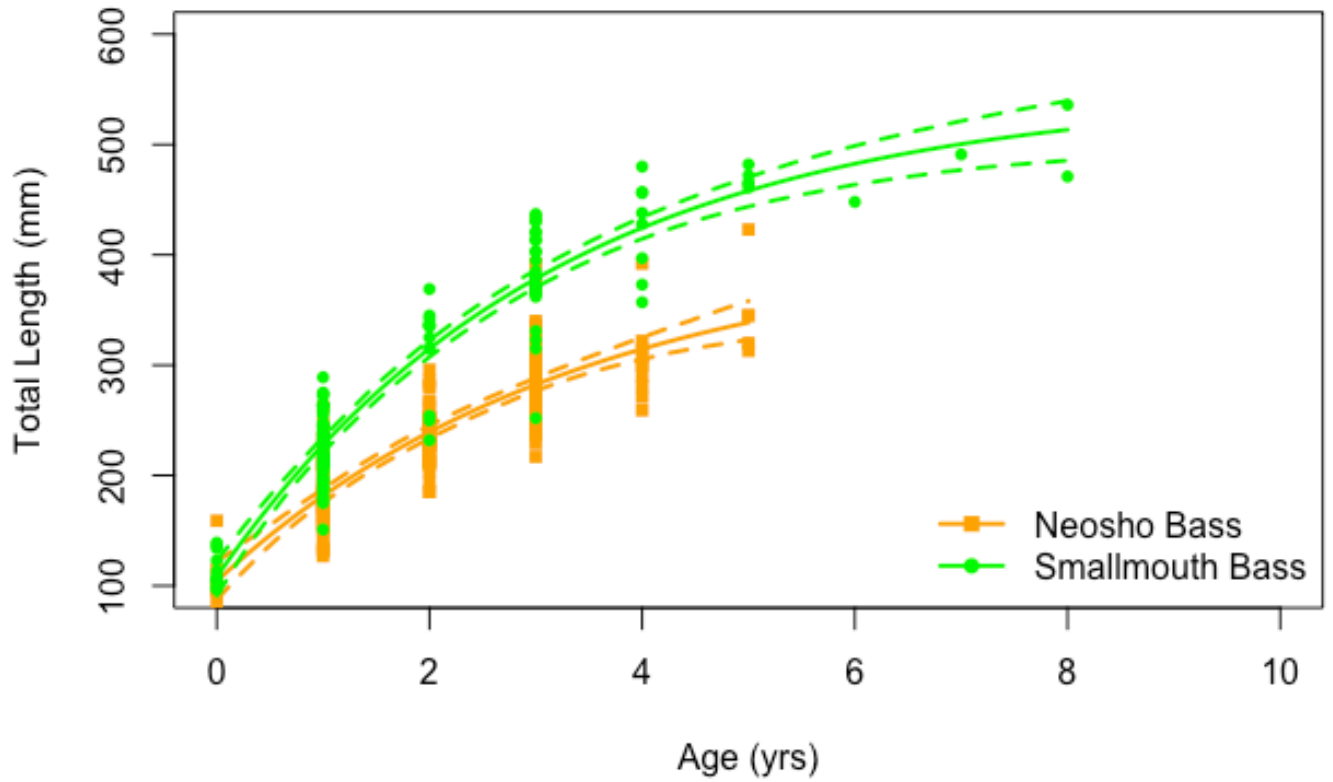


Figure 7. von Bertalanffy growth function displaying average length-at-age curve (solid lines), lower (dashed lines), and upper 95% confidence intervals (dashed lines) for non-hybrid Neosho Bass and non-hybrid Smallmouth Bass.

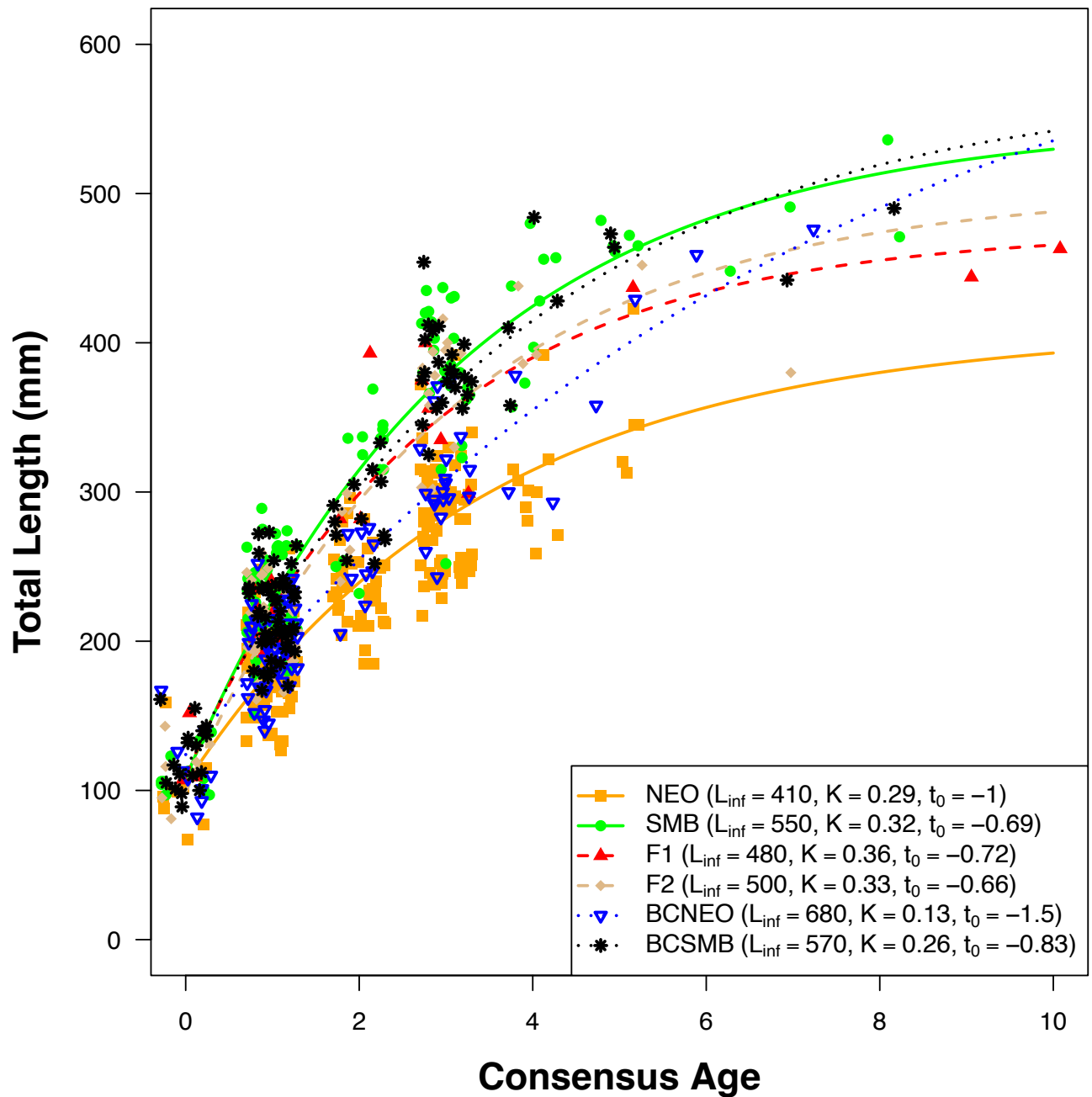


Figure 8. von Bertalanffy growth function displaying the growth curve for each genetic identity as well as the parameters estimated within the VBGF model for each genetic identity: non-hybrid Neosho Bass (NEO), non-hybrid Smallmouth Bass (SMB), F₁, F₂, backcross Neosho Bass (BCNEO), and backcross Smallmouth Bass (SMB).