Impacts of habitat loss on genetic diversity of *Etheostoma lemniscatum*, the Tuxedo Darter

By

Jacob F. Brumley

A Thesis Submitted in Partial Fulfillment of the

Requirements for the Degree of Master of Science in Biology

Austin Peay State University

August 2021

Thesis Committee:

Dr. Rebecca Johansen, Committee Chair

Dr. Mollie Cashner

Dr. Chris Gienger

August 2021

To the College of Graduate Studies:

We are submitting a thesis written by Jacob F. Brumley entitled “Impacts of habitat loss on genetic diversity of *Etheostoma lemniscatum*, the Tuxedo Darter.” We have examined the final copy of the thesis for form and content. We recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science in Biology.

Thesis Approved:

Dr. Rebecca Johansen, Committee Chair/Research Advisor

Dr. Mollie Cashner, Committee Member

Dr. Chris Gienger, Committee Member

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Jacob F. Brumley ___4 August 2021_____

Dedicated to Zachary Kammer

My brother and fellow fish fanatic.

I love and miss you.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Rebecca Johansen, for her *endless* work in making me a better researcher. Through all of the mistakes, stupid questions, and looks of bewilderment, Dr. Jo has been very patient over the last three years. She has always been faithful to her students, keeping an open door for us to stop by to discuss our projects, concerns, anxieties, and everyday lives. I want to thank her for all of the guidance and feedback on my project and constantly ripping my writing apart to make it actually understandable. Lastly, I want to thank her for being so good to Tori (and Winston) by taking care of us the past few years.

I would also like to thank Dr. Mollie Cashner for always being available to answer questions and help with anything that I needed. Her teaching and guidance within the molecular lab were always a great help. I would like to thank Dr. Chris Gienger for serving on my committee, taking precious time out of his day to provide helpful feedback on my thesis.

A special thank you to my lab mates, Nastasia Disotell, Brooke Grubb, Jessica Grady, River Watson, Alexis Culley, Abbi Etherton, and Matthew Scott, for constantly having my back, being available to talk, and helping whenever I needed it. I would especially like to thank Nastasia Disotell for constantly battling the "research dark place" with me. We went through a lot of setbacks in our three years working together, but we have found the light at the end of the tunnel. Thank you Nastasia for always being encouraging, even when we were searching for hours for a fish that was not found or when we were told that the University was shutting down because of COVID-19. You have been a great partner through and through.

For the endless help, I would like to thank Jeff Simmons, who graciously gave of his time and energy to help with fieldwork and talk about the conservation of the Tuxedo Darter. I would

like to thank him for collecting and providing data on the Tuxedo Darter for the past seven years and giving me access to it. I would like to thank Stephanie Brandt and Matt Thomas for constantly being willing at the drop of a hat to run to the Big South Fork to help collect tissue samples. They were instrumental in capturing Tuxedo Darters. I would also like to thank Crystal Ruble and Rebecca Xiques for embarking on our over-night canoe trip through the Big South Fork. They are experts at capturing these rare fish.

Thank you to Rebecca Schapansky, Etta Spradlin, and Tom Blount for coordinating with me to reach sites throughout the Big South Fork National Recreation Area and facilitating permits. I would also like to thank US Fish and Wildlife Services for funding this project and to the Austin Peay Center of Excellence for Field Biology for providing equipment needed to make this project possible.

I would also like to make a special thank you to my family, who have been so gracious with me by encouraging and supporting me through this time. Of course, I would like to thank my beautiful bride, Tori Brumley, for never giving up on me, even when I wanted to give up on myself. For her overwhelming and constant love that lifts me up even in my lowest moments. I would like to thank her for all of the support that she has given me and all of the fun we have had over the past three years. I want to thank my dad, John Brumley, for instilling in me a love for nature and, more specifically, fish. He has been a constant ear when I am frustrated or excited and a source of encouragement when I was in need. I want to thank him for always running out to the river to help and spend time with me. Best bucket boy I could ever ask for in the field. I want to thank my mom, Juliah Brumley, even though she does not understand my “shop talk” with Dad, she has always been loving and encouraging. I want to thank my amazing sister and

brother-in-law, Jessica and Zachary Kammer, for being the best siblings that I could ask for. Thank you for the constant laughs, hugs, and water bottles to take into the field.

Most importantly to me, I want to thank God, for grace, mercy, and love. I want to thank God for allowing me the opportunities to fall in love with His creation and stand to protect it. I would like to thank Him for allowing me to come to Austin Peay State University for a great education, life-altering experiences, and life-long friends. Lastly, I want to thank Him for the faith and hope during the COVID-19 pandemic; when at times it felt as though the world was crashing down around me, there was always hope.

ABSTRACT

JACOB F. BRUMLEY. Impacts of habitat loss on genetic diversity of *Etheostoma lemniscatum,* the Tuxedo Darter. (Under the direction of DR. REBECCA BLANTON JOHANSEN)

Habitat loss and alteration is often detrimental to the genetic diversity of species within the impacted area, especially those species considered imperiled due to other factors, such as a naturally small native range. For imperiled fish species in the southeastern United States, damming of rivers is a leading form of habitat alteration that poses a major conservation issue for many riverine adapted species. Dams homogenize habitat and alter natural riverine flow regimes. The Tuxedo Darter, *Etheostoma lemniscatum,* is a federally endangered fish species that inhabits the mainstem of the Big South Fork Cumberland River in Kentucky and Tennessee. The species is considered a habitat-specialist, adapted to survive and reproduce in shallow pools with clean, cobble substrate. It is threatened by damming of the Cumberland River to create Lake Cumberland, which during summer pool, inundates the lower 8 rkm of the species' range. To determine the impact of inundation on *E. lemniscatum,* we compared occurrence, abundance, and genetic diversity metrics estimated from pre- (2015) and post-inundation (2019/2020; four years after inundation) samples from eleven sites spanning the species' range. We expected to see a reduction in occurrence, abundance and genetic diversity and increased inbreeding as a response to inundation. Declines in occurrence and abundance were detected within the impacted reach. Standard and temporal comparisons of genetic diversity metrics stemming from our genotypic data from 20 microsatellite loci for the 92 individuals collected in 2019 and 2020 and data from the 107 individuals collected in 2015 (provided by Washburn et al. 2020) revealed low genetic diversity for the species. No significant changes in genetic diversity between years was detected. However, in addition to observations of local extirpation and declines in abundance in the

impacted reach, early warning signs of genetic diversity degradation, such as lowered allelic richness and an increase in the proportion of private alleles, were also observed in the impacted reach. These early warning signs indicate a likelihood of impacts to genetic diversity in future generations. Our results warrant further monitoring of the species to determine any time delayed responses to inundation that were not detected in this study.

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Introduction

Globally, habitat loss and alteration are leading contributors to high extinction and extirpation rates of animals (Tilman et al. 1994; Etnier 1997; Farhig 1997; Riccardi and Rasmussen 1999; Pimm and Raven 2000; Warren et al. 2000). In river environments, impoundments (through damming) represent one of the most common and destructive forms of habitat alteration and are regarded as the second leading factor for aquatic species declines in North America (Riccardi and Rasmussen 1999; Warren et al. 2000; Dudgeon et al. 2006; Barletta et al. 2010; Poff and Zimmerman 2010; Reis et al. 2016). River impoundments alter habitats through large-scale environmental disruption, through shifts in natural flow regimes (Graf 1999; Bunn and Arthington 2002; McCartney 2009). Upstream of dams, habitat is transformed from free-flowing lotic habitat to lentic or semi-lentic habitat (Bunn and Arthington 2002; Agostinho et al. 2008). Impoundment dams increase water depth in the catchment area, inundating tributaries and upstream reaches. Changes in flow, in turn, produce shifts in physical and chemical habitat factors, including increased rates of sedimentation of inundated areas (Graf 1999; McCartney 2009). Sedimentation and inundation reduce disturbance frequencies, thus decreasing the dynamic habitat in free-flowing rivers and creating a homogenized environment within the newly lentic (or semi-lentic) system (McCartney 2009; Haghkerdar et al. 2019). These changes stress the adaptive potential of fish species that are highly adapted to lotic habitat, resulting in population declines or loss (Hubbs and Pigg 1976; Stanford and Ward 1986; Humphries and Lake 2000; Agostinho et al. 2008; Nobile et al. 2019).

By contributing to population decline, habitat alteration and loss from impoundments impacts biodiversity at both the species and genetic levels (Farhig 1997; Hitchings and Beebee 1998; Johannesson and Andre 2006; Trush et al. 2008). Reduction in population size increases

potential for genetic drift and reduces overall genetic diversity (Frankham 1996). Inbreeding, where small populations are genetically homogenized due to few reproducing individuals, can also contribute to further loss of genetic diversity (Frankham 1996; Johannesson and Andre 2006; Thrush et al. 2008). Populations with reduced genetic diversity may have reduced fitness in individuals, making species more susceptible to environmental change that could lead to extinction or extirpation (Hitchings and Beebee 1998; Reed and Frankham 2003). These events lead to a cyclic system of genetic and demographic degradation known as the extinction vortex (Frankham et al. 2010).

The southeastern United States is both an aquatic biodiversity hotspot and region of high species imperilment (Warren et al. 2000; Jelks et al. 2008; Collen et al. 2014; Elkins et al. 2019). Leading factors contributing to imperilment are sedimentation, damming, and channelization (Warren et al. 2000). Many imperiled, endemic species of this region are habitat specialists, have naturally small native ranges, or persist as small, fragmented populations (Warren and Burr 1994; Angermeier 1995; Etnier 1997; Warren et al. 2000), and are thus, at high risk of entering the extinction vortex. One species of concern, the Tuxedo Darter, *Etheostoma lemniscatum* Blanton (2008), is a federally endangered fish species endemic to a 40 rkm stretch of the mainstem of the Big South Fork Cumberland River (Figure 2; Biggins 1993; Blanton and Jenkins 2008; Washburn et al. 2020). It is considered a habitat specialist due to its strict use of shallow pools with cobble substrate in all months of the year (Eisenhour and Burr 2000; Davis and Cook 2010). *E. lemniscatum*'s habitat specialization is related to its reproductive mode and ecology: cobble is required for both cover and egg deposition on the underside of rocks (Layman 1991). These shallow pools with cobble substrates are patchily distributed, comprising less than 25% of available habitat within the mainstem Big South Fork, (Eisenhour and Burr 2000; Davis and

Cook 2010; McConkey 2010). Thus, limited available habitat makes *E. lemniscatum* highly susceptible to habitat alteration.

A major threat to the survival of *E. lemniscatum* is the inundation of the Big South Fork by impoundment of the Cumberland River. In 1951, Wolf Creek Dam was built on the Cumberland River as part of the Flood Control Act of 1938. This large dam creates the Lake Cumberland reservoir, which inundates the lower 70 rkm of the Big South Fork at summer pool (Figure 1). This inundation fluctuates within the Big South Fork as lake levels are manipulated throughout the year. As lake levels rise in the spring for summer pool, the river is inundated, converting to semi-lentic habitat. As lake levels decline in the fall for winter pool, the river is reverted back to flowing, lotic habitat. In the late 1990's, an extensive survey was conducted for *E. lemniscatum*, which established the range of the species within a 19 rkm stretch of river from Angel Falls to the confluence of Oil Well Branch (Figure 2; Eisenhour and Burr 2000), which is upstream of the reaches impacted by summer pool inundation events. Eisenhour and Burr (2000) also surveyed a site within the inundated area and found no *E. lemniscatum* individuals. An additional locality was added to the species' range just upstream of lake inundation near Devil's Jump in 2005, where low numbers of *E. lemniscatum* were found (Simmons 2019). Until more recently, *E. lemniscatum* was not considered to be directly impacted by inundation of Lake Cumberland, as all known occurrences were upstream of impacted reaches. However, in 2007, the US Army Corps. of Engineers began repairing the faulty foundational structure of Wolf Creek Dam which required lowering lake levels. This allowed previously inundated areas of the Big South Fork to revert back to free-flowing river year-round for approximately eight years. During the time of dam repair, surveys were conducted for rare fish and mussel species occupying previously inundated stream reaches; *E. lemniscatum* had expanded its range 8 rkm

downstream from its former most downstream location, extending into the previously inundated areas (Davis 2010; USFWS 2012; Simmons 2019). Despite the range expansion of *E. lemniscatum* downstream, lake levels were returned following the conclusion of dam repairs in 2013. This re-inundated the lower section of the Big South Fork, including the 8 rkm of expanded *E. lemniscatum* range. Restoration of lake levels to pre-repair conditions was completed by 2015.

Washburn et al. (2020) conducted surveys for *E. lemniscatum* in 2015 throughout the species' range. They found that individuals were persisting within the re-inundated, expanded range. Washburn et al. (2020) also established a genetic diversity baseline for *E. lemniscatum* from tissue samples collected during these surveys. They concluded that *E. lemniscatum* had low genetic diversity despite gene flow throughout the species' range. They also presented evidence of recent bottleneck events and low effective population size. However, the impacts of re-inundation into the range of *E. lemniscatum* are largely unknown, since the data generated by Washburn et al. was taken from samples that represent the genetic diversity of the darter prior to the event.

The re-inundation event has likely reduced available habitat of the darter by deepening pools and increasing sediment deposition within the impacted area. Annual surveys have shown decreases in occurrence and abundance of *E. lemniscatum* at the lower extent of the species' expanded range, resulting from habitat changes and degradation (Simmons 2019). In this study, we examined potential impacts of the re-inundation of the lower Big South Fork on *E. lemniscatum*. We hypothesized that the re-inundation event has impacted *E. lemniscatum* and predicted changes in the darter's demographics and genetics. We examined occurrence and abundance of *E. lemniscatum* throughout the species' range and expected to see declines in both

measures within the impacted area. We also examined trends in genetic diversity of *E. lemniscatum* by comparing samples collected in 2015 (representing the time before the re-inundation event; Washburn et al. 2020) to those collected four years after re-inundation (in 2019). We predicted that habitat degradation of the impacted reach has led to declines in genetic diversity both within the impacted reach and for the and species overall. We also expected to see an increase in inbreeding within the impacted reach associated with declining abundance and occurrence of *E. lemniscatum*. This study is also the second installment of a greater bank of genetic diversity information for *E. lemniscatum* and another important step in establishing a genetic monitoring program for the species.

Methods

Sampling Collection

We conducted snorkel and seine surveys for *Etheostoma lemniscatum* from September 2019 to September 2020 at 11 localities, spanning the species' range (Figure 2). These 11 sites were a subset of the 18 sites sampled by Washburn et al. (2020). To examine impacts of re-inundation on the abundance and occurrence of *E. lemniscatum,* we recorded the total number of individuals observed at each site for both data sets. These counts were standardized using units of time spent surveying for the species at each site. For captured individuals, we recorded total length, and non-lethal fin clips were taken from the upper lobe of the caudal fin for each individual captured; clips were taken from the upper-posterior end of the right pectoral fin for individuals with a damaged caudal fin. Fin clips were preserved in 95% ethanol for DNA preservation. We placed individuals in an aerated bucket for recovery, and once all individuals recovered, and sample efforts were complete at a site, we released them back to the site of

capture. Geographic coordinates were taken at each locality using a Garmin GPSmap 60CSx handheld GPS navigator.

Microsatellite Genotyping

We extracted whole genome DNA from all fin clips using a Qiagen DNeasy Blood and Tissue Kit (QIAGEN, Inc.) following the manufacturer's instructions with modifications to the elution step – an initial elution of 180 µl was followed by a second elution of 80 µl in a separately labeled tube. The extracted DNA samples were quantified using a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Inc.), and all samples with concentrations exceeding 20 ng/µl were diluted to a concentration of 20 ng/µl. We assessed measures of genetic diversity and population structure using twenty species-specific microsatellite loci (Washburn et al. 2020). These loci were grouped into 5 multiplex reactions, consisting of four loci each, and were given an M13 ABI dye-label that was used throughout the study. PCR reactions were 10 µl total volume, consisting of 5.65 µl PCR pure water, 1.00 µl 10X standard Taq reaction buffer (Mg free) (New England Biolabs, Inc.), 1.20 µl 25 mM $MgCl_2$ (New England Biolabs, Inc.), 0.20 µl 10 mM dNTPs (New England Biolabs, Inc.), 0.25 µl 10 pM forward primer, 0.50 µl 10 pM reverse primer, 0.10 µl 5000 U/ml Taq Polymerase (New England Biolabs, Inc.), 0.10 µl M13 ABI dye-labeled primer (Applied Biosystems, Inc.), and 1.00 µl DNA. PCR cycle conditions were as follows: 1x cycle of initial denaturation at 94°C for 1 min; 30x cycles of denaturation at 94°C for 30 sec, annealing at primer-specific temperatures (ranging from 59-65°C) for 30 sec, and extension at 72°C for 30 sec; 1x cycle of final extension at 72°C for 5 min. PCR products were either sent to the University of Florida Interdisciplinary Center for Biotechnology Research (UF-ICBR) Genotyping and Gene Expression Core or the DNA Core Sequencing Facility at the University of Illinois, Urbana-Champagne for the collection of genotypic data, using an ABI

3730 sequencer with a LIZ600 size standard. For consistency of allele scoring, positive controls were added to each plate. We scored allele sizes using GeneMarker v2.7.0 (SoftGenetics, LLC.).

Comparisons to samples collected prior to re-inundation from Lake Cumberland

To determine the impact of the re-inundation of the lower Big South Fork on the genetic diversity of *E. lemniscatum*, we generated two data sets: a pre-inundation data set of genotypes collected from individuals captured in 2015 (labeled as the "2015" dataset in results and discussion; Washburn et al. 2020) and a post-inundation data set of genotypes collected from individuals captured in 2019 and 2020 (labeled as the "2019" data set in results and discussion). For direct comparison between the two data sets, we collected genotype data only for individuals captured at the 11 sites sampled in this study, and we used the same 20 microsatellite loci for data collection. To examine changes in genetic diversity across time, we conducted genetic diversity analyses for both data sets, and because we expected sites directly impacted by the re-inundation event to show genetic declines first, sites were grouped into two reaches for each data set: sites impacted by re-inundation (referred to as "impacted") and sites not impacted by re-inundation ("unimpacted"; Figure 2). Therefore, we were able to examine genetic diversity on both a species and reach scale. For temporal analyses, the 2015 data set was used as generation 0, while the 2019 data set was used as generation 2.

Marker Validation and Genetic Diversity

We used MICRO-CHECKER v2.2.3 (Van Oosterhout et al. 2004) to evaluate allelic data for evidence of scoring errors due to null alleles, large allele dropout, and stutter with 1,000 simulations; 95% confidence intervals (CI) were used to assess significance. We tested for departures from Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD), using the

respective functions `test_HW()` and `test_LD()` in the R package *genepop* (R Core Team 2019; Rousset 2008). Deviation from HWE was tested using exact tests per locus, per reach, and for the species overall. Both HWE and LD were conducted using Markov chain parameters with 10,000 dememorization steps, 1,000 batches, and 10,000 iterations per batch. To account for multiple comparison errors, we applied Bonferroni correction to the p-values using the `p.adjust()` function in *stats* (R Core Team 2019).

We measured genetic diversity via a standard suite of population-genetic metrics, using the `divBasic()` function of the package *diveRsity* (R Core Team 2019; Keenan et al. 2013). Metrics were generated for the species as a whole and each reach (impacted vs. unimpacted) for each data set (2015 vs. 2019) and included: mean number of alleles (N_a), observed heterozygosity (H_o), expected heterozygosity (H_e), allelic richness (AR), and inbreeding coefficient (F_{IS}). AR was conducted using rarefaction to adjust for uneven sample sizes across reaches. To assess significance of F_{IS} values, 95% confidence intervals were generated using 10,000 bootstrap iterations. We examined private alleles and measured private allele frequencies, using the `popgenreport()` function of the package *PopGenReport* (R Core Team 2019; Adamack and Gruber 2014). To identify evidence of recent population decline, we used BOTTLENECK v1.2.02 (Cornuet and Luikart 1996; Piry et al. 1999) to examine excess heterozygosity that occurs after bottleneck events. A two-phase model was used with 0%, 10%, and 20% multistep mutations, as recommended by Peery et al. (2012), and a variance set at 36% (Di Rienzo et al. 1994). A one-tailed Wilcoxon signed-rank test with 1,000 replications was used to determine significant deviation from the null hypothesis of drift-mutation equilibrium (Luikart and Cornuet 1998). We conducted effective population size (N_e) estimates, using the linkage disequilibrium (LD) method (Waples and Do 2008) in the program NeEstimator v2.01 (Do et al.

2014) for each reach and the species as a whole for each data set. Estimates were calculated with the exclusion of alleles with frequency < 0.02 to account for any bias from rare alleles (Waples and Do 2010) and with estimation of 95% CI (Waples 2006). We also conducted N_e estimates in the R package *NB*, using the function `NB.estimator()`, which uses a maximum-likelihood approach with continuous approximation (R Core Team 2019; Hui 2014). This method looked at allele frequencies in the 2015 and 2019 data sets simultaneously to give a robust estimate of effective population size. The bounds were set at 10 and 10,000,000, and approximate 95% CI were estimated, using log-likelihood. Estimates were conducted for the species overall and the two reaches. We chose this method for its ability to detect small N_e estimates and handle small, uneven sample sizes (Hui and Burt 2015; Wang et al. 2016).

To determine if the species showed continued evidence of gene flow throughout its range, as observed by Washburn et al. (2020), we evaluated population fixation and isolation between the impacted and unimpacted reaches for both data sets. We calculated pairwise F_{ST} values, using the function `pairwise.wcfst()` in the R package *hierfstat* (R Core Team 2019; Goudet 2005; Goudet et al. 2020), which applies the method described by Weir and Cockerham (1984). The function `boot.ppfst()` was used to calculate 95% confidence intervals for each value, using 10,000 bootstraps. We used the program STRUCTURE v2.3.4 (Pritchard et al. 2000) to examine population structure, using a genetic admixture model with our reaches as priors for the LOCPRIOR model (Hubisz et al. 2009). Other parameters for the analyses included: correlated allele frequencies, 10,000 burn-in Markov Chain Monte Carlo (MCMC) steps followed by an additional 100,000 MCMC steps, and 5 iterations for each K value tested (K = 1-20). We used the online program STRUCTURE HARVESTER v0.6.94 (Earl and VonHoldt 2012) to assess

the optimal number of population clusters, using the mean-likelihood (Pritchard et al. 2000) and ΔK (Evanno et al. 2015) methods.

Temporal Analyses of Genetic Diversity

We examined changes in genetic variation between the 2015 and 2019 data sets, using temporal analyses of genetic diversity. We estimated temporal pairwise F_{ST} values to determine genetic fixation between the data sets, using the function `pairwise.wcfst()` in the R package *hierfstat* and calculated 95% confidence intervals using the function `boot.ppfst()` at 10,000 bootstraps. We also examined temporal genetic variation using a spatio-temporal AMOVA, with the function `poppr.amova()` in the package *poppr* (R Core Team 2019; Kamvar et al. 2014). The analysis used the *ade4* method of AMOVA and default settings of the function (Dray and Dufour 2007; Thioulouse et al. 2018). We used the function `randtest()` in the *ade4* package to calculate significance, using 1,000 permutations in a random permutation test. Our AMOVA was structured to examine variation at 3 hierarchical levels. At the first hierarchical level, we examined variation between each data set, representing the year of collection (2015 vs. 2019). At the second level, we examined variation between reaches within each data set (impacted vs. unimpacted); while at the third level, we examined the variation among individuals within the context of each reach, assigned to a data set. The AMOVA model describes the best explanation of variance between the groupings, using phi (φ) statistics. Phi statistics are analogs of F statistics and are used to summarize variation between the two hierarchical levels of each test. We conducted a temporal Discriminant Analysis of Principal Components (DAPC; Jombart et al. 2010), using the function `dapc()` in the R package *adegenet* (Jombart 2008). This function assigns each individual into a given number of clusters based on allele frequency and composition data and provides percent of probability of correct assignment. We selected 2

clusters to be used in assignment of individuals: 2015 cluster and 2019 cluster. We then visualized allelic variation between year in a Principal Components Analysis (PCA). After replacing the missing data points with the mean allele frequency using the function `scaleGen()` in the package *adegenet*, we used the function `dudi.pca` in the package *ade4* to perform a PCA.

Results

Data Collection, Occurrence, and Abundance

We sampled 11 sites and collected fin clips from 92 individuals of *Etheostoma lemniscatum* from 150 total individuals observed during our snorkel and seine surveys in 2019 and 2020 (Figure 2; Table 1). In 2019, we failed to detect *E. lemniscatum* at the site 400m upstream of Stover Branch (site 9; Figure 2, Table 1) from the impacted stream reach. After observing 6 individuals and capturing 4, collectively, at the Rock Creek (site 10; Figure 2) and KY-92 Bridge (site 11; Figure 2) sites in the impacted reach in 2019, we returned to these sites in 2020 in an attempt to obtain more tissue samples but failed to detect *E. lemniscatum* at either site (Table 1). Along with reductions in occurrence of individuals at these sites, we also see reductions in abundance of individuals from 2015 to 2019/2020 (Table 2). We observed large declines in observed individuals per hour at sites 2, 4, 7, 9, 10, and 11 (Figure 2; Table 2). These declines were greater than 3 individuals per hour. The largest decline was at site 11, where 10 less individuals were observed per hour in 2019/2020 than 2015. However, we also observed increases in observed individuals at sites 6 and 8 within the impacted reach (Figure 2; Table 2). Overall, observations of *E. lemniscatum* individuals were lower in the 2019 and 2020 samples than in the 2015 samples for the species and within the two reaches (Table 2).

We used all 92 individuals captured in 2019-2020 for the analyses of genetic diversity. We averaged a collection of 8.36 individuals per site with a range of 0 to 19 individuals. Tissues from 59 individuals were collected at sites within the unimpacted reach and 33 from sites within the impacted reach. The 2015 data set (from Washburn et al. 2020) had a total of 107 individuals, averaging 9.72 individuals per site, and ranging from 2 to 20 individuals (Table 1; Figure 2). Sixty-two individuals were examined at sites within the unimpacted reach, and 45 from sites within the impacted reach (Figure 2; Table 1).

Microsatellite Marker Validation

For the 2019 dataset, we successfully genotyped all 92 individuals at 20 microsatellite loci with less than 0.5% missing genotypes (8/1840 total missing genotypes; all individuals were successfully genotyped for at least 17 of the 20 loci). There was no evidence of scoring errors within the data as a result of null alleles, large allele dropout, or stutter. After Bonferroni correction, there was no consistent evidence of significant LD among loci pairs, but locus Elem093 deviated from HWE; however, analyses included Elem093, as its exclusion did not change genetic diversity metrics. For the 2015 dataset, all 107 individuals were successfully genotyped for at least 16 of 20 loci with less than 1.0% missing genotypes (18/2140 total missing genotypes). There was no evidence of scoring errors, and after Bonferroni correction, there was no evidence of loci deviating from HWE. However, there was significant LD detected between loci Elem035 and Elem092. This is commonly seen in imperiled species because of the low population size (Frankham et al. 2010).

Genetic Diversity

A total of 115 alleles were amplified across the 92 individuals examined for the 2019 dataset, averaging 5.75 alleles per locus (range: 2-10 alleles per locus). The impacted reach averaged 4.85 alleles per locus, while the unimpacted reach averaged 5.20 alleles per locus (Table 3). The 2015 data had a total of 115 amplified alleles but ranged from 2 to 12 alleles per locus (N_a = 5.75). N_a of the impacted reach in 2015 was higher than our 2019 measurement (N_a = 5.00), while the unimpacted reach average was lower in 2015 (N_a = 5.05; Table 3). Allelic richness (AR) in the impacted reach was 4.48 for 2019 compared to 4.66 in 2015. AR was higher in 2015 for the unimpacted reach and the same for the species over all between data sets (AR = 4.62; Table 3; Figure 3). Private allele frequencies increased from 2015 to 2019 for both the impacted (2015 = 0.011; 2019 = 0.014) and unimpacted reaches (2015 = 0.009; 2019 = 0.012). Mean observed heterozygosity (H_o) was similar across all reaches and years sampled ranging from 0.58 to 0.63 (Table 3). H_o was greater than expected (H_e) for both data sets in the impacted reaches; it was lower than expected for the unimpacted reach in the 2019 dataset (Table 3). Evidence of bottleneck events were observed for the species overall and all reaches in both data sets (Table 3). Significant deviation from HWE was also detected for the species overall and the unimpacted reach in 2019, providing evidence for recent population declines (Table 3). No deviation from HWE was detected in the 2015 data. The overall inbreeding coefficient (F_{IS}) for 2019 was low and not significant based on the 95% confidence interval (F_{IS} = 0.002; 95% CI: -0.027-0.030); however, a significant negative F_{IS} value was observed for the impacted reach, suggesting heterozygote excess and outbreeding (Figure 4). A significant negative F_{IS} value was also observed for the impacted reach in 2015 dataset (Figure 4). Effective population size (N_e) for the species overall using the LD method was 475.9 (95% CI: 245.6-3410.0) effective

individuals for the 2019 data, compared to an estimate of 407.8 (95% CI: 238.7-1168.5) effective individuals for the 2015 data. However, we did not have confidence in the estimates for the impacted and unimpacted reaches using this method, since 3 out of 4 estimates contained infinite upper bounds for their 95% confidence intervals. We calculated more robust temporal N_e estimates with the maximum-likelihood model that examined both the 2015 and 2019 datasets simultaneously. Estimates from this model were small with high precision within the 95% CI bounds (Figure 4). The maximum likelihood estimate for *E. lemniscatum* was 92.6 (95% CI: 54.5-181.4) effective individuals. For the impacted and unimpacted reaches, the maximum likelihood estimates were 29.0 (95% CI: 17.3-54.9) and 39.8 (95% CI: 24.5-70.4) effective individuals, respectfully (Figure 4).

Pairwise F_{ST} values between the impacted and unimpacted reaches were low and not significant in either data set (Table S1). The STRUCTURE analysis using our 2-reach LOCPRIOR parameters identified K=1 as the most likely number of clusters for *E. lemniscatum*, using the mean log-likelihood model for both data sets (Figure S2). However, the ΔK method cannot detect K=1 (Evanno et al. 2005). ΔK results indicated K>1 as the most likely number of clusters but associated STRUCTURE plots assigned individuals equally to the suggested K >1 clusters, concluding that K=1 is the optimal number of clusters for both data sets (Figure S3).

Temporal Analyses of Genetic Diversity

Temporal pairwise F_{ST} values were determined between each year for the species overall and at each reach. All F_{ST} estimates were low, and not significant (between years for species overall: $F_{ST} = 0.002$, $p = 0.20$; impacted reach: $F_{ST} = 0.001$, $p = 0.43$; and unimpacted reach: $F_{ST} = 0.001$, $p = 0.39$). Analysis of molecular variance (AMOVA) found no evidence of genetic differentiation between the two years of collection (2015 vs. 2019). Most genetic variation and

fixation were expressed between reaches within each study year as compared to variation between each year, but variation was not significant (Table 4). For the DAPC parameters, we set K equal to 2 coinciding with sample year. DAPC plots were produced for both reaches and the species overall. Individuals were not consistently assigned to the group representing the year in which they were collected for the species overall (Figure 6a). However, individuals are assigned into the correct clusters with greater percent probability of correct assignment at the reach scale (Figure 6b,c). Percent probability of correct assignment was higher within the impacted reach than the unimpacted reach (Figure 6b,c). Our PCA also showed, little to no variation between the 2015 and 2019 data sets (Figure 7).

Discussion

River impoundment is a major global contributor to freshwater habitat alteration and loss (Dudgeon et al. 2006; Barletta et al. 2010; Poff and Zimmerman 2010; Reis et al. 2016). Such habitat alterations are typically detrimental to the survival and adaptive potential of stream-adapted fish species (Gorman and Karr 1978; Pandit et al. 2009). Habitat alterations contribute to population declines and may lead to extirpations of vulnerable populations or species extinction (Bender et al. 1998; Riccardi and Rasmussen 1999; Warren et al. 2000). Population declines, in turn, can contribute to adverse effects in genetic diversity (Frankham 1996; Reed and Frankham 2003; Johannesson and Andre 2006; Trush et al. 2008; Frankham et al. 2010). Our study examined the impacts of habitat alteration and loss stemming from river inundation for *Etheostoma lemniscatum*, an endangered, habitat-specialist darter species. Although we expected a reduction in allelic diversity and an increase in inbreeding in the impacted reach, we did not find significant differences in estimates of genetic diversity between our data (post-inundation,

2019) and the baseline pre-inundation estimates (2015 data set). Our results were consistent with those of Washburn et al. (2020) in that we found *E. lemniscatum* has low genetic diversity and maintains gene flow throughout its range. However, we observed several early warning signs of likely future adverse effects of habitat degradation that suggest future genetic monitoring is warranted, including decreases in abundance and extirpation from a few inundated sites, and declines in allelic richness.

Early Warnings

Occurrence and Abundance

Declines in occurrence and abundance of *E. lemniscatum* were observed within the impacted reach. We failed to detect *E. lemniscatum* individuals at impacted reach, site 9 (Figure 2) in our initial sampling in 2019 (compared to 6 individuals observed in 2015). After detecting a combined 6 individuals at sites 10 and 11 (Figure 2) in 2019, from the impacted reach, additional sampling was conducted in 2020. However, we failed to detect *E. lemniscatum* at these sites in this second sampling attempt (compared to 25 individuals observed in 2015). These observations are confirmed by large declines in observed individuals per hour between 2015 and 2019 (Site 9 $\Delta = 5.1$; Site 10 $\Delta = 5.7$; Site 11 $\Delta = 10.0$). These are the three most downstream sites surveyed in this study and most heavily impacted by inundation events (Simmons 2018). The major declines in occurrence and abundance of *E. lemniscatum* at sites 9-11 began following a prolonged, intense inundation event in 2018 (Simmons 2018; Figure 8). Simmons (2018) also describes declines in a downstream historic site (at the mouth of Lick Creek) not sampled for this study, in which *E. lemniscatum* is no longer found.

The observed reductions in abundance and occurrence at sites within the impacted reach (Figure 8) are attributed to a decline in habitat availability (Simmons 2018). Inundation within the impacted reach has led to higher-than-normal rates of sediment deposition, imbedding the cobble substrate with layers of silt 2-4 cm deep in typical habitat of *E. lemniscatum* (pers. obs.). This lowers the amount of available habitat and increases the amount of energy that individuals have to use to clear sediments from cover and nesting areas. Survivorship of eggs is also reduced with the increase of fine sediments, due to clogging of micropores which prevent oxygen from entering the eggs (Carling 1984; Magee et al. 1996; Ingendahl 2001; Greig et al. 2007; Jensen et al. 2009; Kemp et al. 2011; Gatch et al. 2020). In general, increased sedimentation within riverine systems is a leading cause of species declines in North American fishes (Riccardi and Rasmussen 1999; Warren et al. 2000).

Allelic Diversity

Genotype data from 20 microsatellite loci uncovered low genetic diversity throughout the range of *E. lemniscatum*. Although no significant variation in genetic diversity was detected between pre- and post-inundation data sets (2015 vs. 2019), we observed a reduction in allelic diversity within the impacted reach. We observed this decline in mean allele per locus (N_a) from pre-inundation samples (2015) to post-inundation samples (2019; Impacted reach: $N_{a2015} = 5.00$, $N_{a2019} = 4.85$, $\Delta_{Na} = 0.15$; Table 3). This is further confirmed by a reduction in allelic richness within the impacted reach (Impacted reach: $AR_{2015} = 4.66$, $AR_{2019} = 4.48$, $\Delta_{AR} = 0.18$; Figure 3). Allelic richness is considered a more sensitive measure of genetic diversity than heterozygosity-based measures, with respect to impacts of population loss on genetic diversity (Allendorf and Luikart 2007; Schlaepfer et al. 2018; Barrandeguy and Garcia 2021). After population decline, allelic diversity is typically reduced faster than heterozygosity (Cornuet and Luikart 1996). For

example, in a temporal study of the impacts of habitat fragmentation and population declines in capercaillie grouse in Germany, Segelbacher et al. (2008) saw no significant reduction in overall genetic diversity of the species but noted a reduction in allelic richness. Dures et al. (2019) also found evidence of declines in allelic diversity, particularly allelic richness, prior to declines in heterozygosity within the African lion.

We also detected a change in allele frequency and composition between the 2015 and 2019 data sets within the impacted reach using a DAPC. Our DAPC results for the impacted reach correctly assigned individuals to their data set (represented by year of collection) at higher probabilities than for the unimpacted reach and the species as a whole. Within the 2015 data set, 52 of 59 individuals were assigned correctly to the 2015 cluster with 75% or greater membership probability; in the 2019 data set, 25 of 33 individuals were assigned correctly to the 2019 cluster with 75% or greater membership probability. The DAPC clusters individuals into groups of similar allelic diversity, particularly allele frequency and composition. Our results suggest that there is evidence of differentiation in allelic composition between pre-inundation and post-inundation individuals within the impacted reach. This is supported by a slight increase in private allele frequency within the impacted reach from 2015 to 2019 ($\Delta = 0.003$), suggesting a shift in allelic composition. A shift in allelic composition between years, likely reflects the random loss of alleles in this area that is also reflected by the observed trend of declines allelic richness in the impacted reach.

Temporal Data – The Time-Lag Effect

Although we did not observe significant declines in genetic diversity associated with the recent inundation of the impacted reach of the Big South Fork, early warning signs indicate the likelihood of impacts in later generations. The temporal spread of our samples for *E.*

lemniscatum was four years between sample collections, with the most recent data set also collected four years after the re-inundation of the impacted reach. Based on life history studies, we estimate this to be approximately 2 generations for *E. lemniscatum* (Layman 1991; Eisenhour and Burr 2000). All levels of biodiversity show time-lags in response to habitat alteration and reduction (sometimes called extinction debt or relaxation time; Diamond 1972; Tilman et al. 1994; Helm et al. 2006; Sousa et al. 2010; Essl et al. 2015; Aavik et al. 2019; Liu et al. 2020). These delays are important considerations when looking at temporal genetic variation (Nei et al. 1975; Cornuet and Luikart 1996; Epps and Keyghobadi 2015; Essl et al. 2015; Carvalho et al. 2019). For example, Richmond et al. (2009) and McCoy et al. (2010) examined the genetic response of the Florida Sand Skink to historical and current habitat fragmentation. The delay in response time of these skinks to habitat change was over 15 generations before genetic metrics began to reveal significant changes in diversity. Other studies that found little changes in genetic diversity after known population declines or isolation events, similar to the results herein, have advocated for the use of temporal genetic diversity comparisons to account for time-lag effects on their results (Tessier and Bernatchez 1999; Segelbacher et al. 2008; Riccioni et al. 2010; Valtonen et al. 2012; Sonsthagen et al. 2020). However, some studies have detected a relatively quick genetic response to habitat alteration, such as within a decade after the event (Barcia et al. 2005; Angeletti et al. 2010; Perez-Portela et al. 2012).

Time-lags in genetic responses to habitat alteration differ among species. Life history, gene flow, and population size are strong drivers of time-lag lengths (Ewers and Didham 2006; Richmond et al. 2009; Epps and Keyghobadi 2015; Essl et al. 2015; Schlaepfer et al. 2018). For example, long-lived species with high dispersal ability and migration have longer time-lag genetic responses to habitat alteration, due to the reduced potential for inbreeding (high gene

flow) and long generation times (Ewers and Didham 2006; Richmond et al. 2009; Essl et al. 2015). Species that are adapted to patchy habitats and small population sizes, similar to *E. lemniscatum,* are also expected to have longer time-lag responses (Richmond et al. 2009); these species are suited to handle fragmentation and isolation that come with habitat destruction. Based on these studies, several traits of *E. lemniscatum* may lengthen the genetic time-lag response to habitat alteration and loss from inundation by Lake Cumberland, particularly the occurrence of gene flow across its range and an adaptation to a patchy habitat distribution. Thus, it is likely that two generations are not sufficient to detect a genetic response.

Conservation Implications and Recommendations

We conclude that the overall genetic diversity of *E. lemniscatum* remains low. This is observed in the population genetic diversity metrics for both pre- and post-inundation (Table 3), suggesting the continued imperilment of the species from legacy effects of historical anthropogenic disturbance events. Another continued concern for *E. lemniscatum* is its low effective population size. Our most robust estimates of N_e were calculated by examining both the 2015 and 2019 data sets simultaneously and were low for the species overall and the impacted and unimpacted reaches (Figure 5). Frankham et al. (2014) determined that N_e of 100 individuals is minimally necessary to avoid severe inbreeding within a population and N_e of 1000 individuals is minimally necessary to retain evolutionary potential. Our estimates of N_e are well below these marks, suggesting that *E. lemniscatum* is likely at risk of adverse genetic effects of environmental changes and population decline. We also we found evidence for a recent bottleneck event in both data sets for the species overall and within the impacted and unimpacted reaches. As suggested by Washburn et al. (2020), this is likely due to low genetic variation that is most likely a result of a combination of historic population decline and contemporary population

loss. *E. lemniscatum* has been impacted by many anthropogenic activities, including mining and logging (O'Bara et al. 1982; Rikard et al. 1986; USFWS 2012) and likely had a larger historical range (Jenkins and Burkhead 1994; Blanton and Jenkins 2008). Many imperiled darter species have experienced bottleneck events (Moyer and Williams 2012; Robinson et al. 2013; Fluker et al. 2014; Olsen et al. 2016; Blanton et al. 2019; Fluker et al. 2019) due to a myriad of anthropogenic activities that have led to habitat loss or alteration. Population declines that contribute to bottleneck events are frequently associated with increased inbreeding and genetic drift that continue to reduce genetic diversity (Frankham 1995; Frankham 1996; Hendrick and Kalinowski 2000; England et al. 2003; Reed and Frankham 2003; Spielman et al. 2004; Frankham et al. 2010). However, we did not detect evidence of inbreeding in *E. lemniscatum*. Instead, we found evidence of outbreeding within the impacted reach (F_{IS} = -0.067; 95% CI: -0.1041--0.0307). This phenomenon can be explained by an excess of heterozygotes in the impacted reach due to random mating within a small effective population (Allendorf and Luikart 2007). This is supported by higher observed than expected heterozygosity (Table 3) and low estimates of effective population size within the impacted reach. Heterozygosity excess is also indicative of a population that has undergone a recent bottleneck (Cornuet and Luikart 1996). Because both data sets reveal evidence for low genetic diversity, low effective population size, and evidence of recent bottlenecks in the species, we do not attribute these results to the recent re-inundation of the lower 8 rkm of the darter's range. However, the loss of habitat from inundation that has resulted in population loss and declines in abundance will certainly be an added confounding factor along with the noted historical events to the ongoing imperilment of the species.

As noted, our observations of habitat loss and declines in occurrence and abundance within impacted sites suggest that *E. lemniscatum* is directly threatened by inundation from Lake Cumberland. The extinction vortex model suggests a logical flow of compounding events that lead to extinction or extirpation (Frankham et al. 2010). Loss of habitat leads to reduced population sizes. We observed habitat loss and extirpation in the impacted reach. The species already persists as a relatively small, isolated population; such populations (or species) are more susceptible to the negative effects of inbreeding and genetic drift, which reduce genetic diversity. Although we did not observe evidence of inbreeding, we did see a reduction in allelic richness. Allelic diversity measures, including allelic richness, are considered good indicators of evolutionary potential (Allendorf 1986; Allendorf and Luikart 2007; Caballero and Garcia-Dorado 2013) and is an important factor in population viability and adaptability (Spielman et al. 2004). The next step in the extinction vortex after reduced genetic diversity is reduced adaptive potential and survival. Based on our observations for reduced allelic richness, and the potential of a time-lag response in genetic diversity, it is likely that *E. lemniscatum* will exhibit reduced adaptability to further habitat alteration and stochastic events. This could lead the species further into the extinction vortex that compounds over time.

Our findings indicate the species warrants future genetic monitoring to promote persistence of *E. lemniscatum* individuals within the impacted reaches and to ultimately capture the genetic impacts of habitat loss. Genetic monitoring is the temporal collection of population genetic measures, typically using neutral genetic markers, and is an effective approach for examining the impacts of habitat loss and fragmentation on a population (Luikart et al. 1998; Schwartz et al. 2006; Antao et al. 2011; Hansen et al. 2012). Such time-series data allows researchers and government agencies to specifically examine the impacts of habitat alteration

events or management actions to the genetic diversity of the species. Although we did not observe significant genetic variation between the pre-inundation data baseline (2015; Washburn et al. 2020) and our post-inundation data (2019), early warning signs of population decline and reduced allelic richness suggest this may be due to a lag effect between events and genetic response as found in other studies. A regular genetic monitoring program, including re-examination of genetic diversity metrics examined herein, in the future (say 10 years post inundation) would provide another important update to the status of the species and improve the foundation for management decisions regarding impacts from Lake Cumberland. A genetic monitoring program set in an adaptive management framework, allows managers to adjust strategies to those that promote increases in genetic diversity (Luikart et al. 1998; Schwartz et al. 2006; Antao et al. 2011; Hansen et al. 2012).

Table 1. Number of *Etheostoma lemniscatum* individuals captured (N_{cap}) and observed (N_{obs}) at the 11 sites and the two reaches (unimpacted and impacted) examined. Sites are listed from upstream to downstream. Counts are provided for the current (2019-2020) and 2015 (Washburn et al. 2020) studies. Site numbers and reaches correspond with those in Figure 1. An asterisk denotes sites where we failed to detect the species in 2020, thus numbers for these sites were only from 2019 collections.

		2015		2019-2020	
Site	**Site**	**N_{cap}**	**N_{obs}**	**N_{cap}**	**N_{obs}**
1	Station Camp Creek	20	20	19	35
2	Big Island	17	31	11	25
3	Upstream Hurricane Branch	9	16	14	20
4	Upstream Oil Well Branch	16	27	15	15
5	Upstream Devils Creek, Blue Heron	5	7	3	5
6	Between Devils Creek & Roaring Paunch	2	12	8	16
7	Mouth of Roaring Paunch Creek	6	16	2	5
8	Downstream 1.3 km of Roaring Paunch Creek	14	20	16	23
9	Upstream 400m Stover Branch	4	6	0	0
10	Downstream of Rock Creek	5	14	2	4*
11	Downstream of KY-92 Bridge	9	11	2	2*
	Unimpacted Reach	62	94	59	95
	Impacted Reach	45	86	33	55
	Total	**107**	**180**	**92**	**150**

Table 2. Number of *E. lemniscatum* individuals observed and standardized per hour of surveying (N_{obs}/hour). Sites are listed from upstream to downstream. Standardized counts are provided for the current (2019-2020) and 2015 (Washburn et al. 2020) studies. Site numbers and reaches correspond with those in Figure 1.

		2015	2019-2020
Site	**Site**	**N_{obs}/hour**	**N_{obs}/hour**
1	Station Camp Creek	4.8	5.8
2	Big Island	16.9	8.3
3	Upstream Hurricane Branch	10.7	10.0
4	Upstream Oil Well Branch	19.1	15.0
5	Upstream Devils Creek, Blue Heron	4.7	3.1
6	Between Devils Creek & Roaring Paunch	4.8	8.0
7	Mouth of Roaring Paunch Creek	5.3	1.8
8	Downstream 1.3 km of Roaring Paunch Creek	4.7	7.1
9	Upstream 400m Stover Branch	5.1	0
10	Downstream of Rock Creek	7.0	1.3
11	Downstream of KY-92 Bridge	11.0	1.0
	Unimpacted Reach	10.5	7.9
	Impacted Reach	5.6	3.6
	Total	**7.4**	**5.5**

Table 3. Genetic diversity measures for *Etheostoma lemniscatum* for the unimpacted reach, impacted reach, and species overall. Measures provided for data collected in the current study (2019) and from Washburn et al. (2020). Information includes number of individuals analyzed per group (n), mean number of alleles per locus (N_a), allelic richness (AR), mean observed heterozygosity (H_o), mean expected heterozygosity (H_e), and p-values for tests of deviation from Hardy-Weinberg equilibrium (HWE) and bottleneck events. Bold values indicate significance ($p < 0.05$ for HWE and confirmed after Bonferroni correction; $p < 0.05$ for Bottleneck). Unimpacted and impacted reaches are defined in Figure 1.

		n	**N_a**	**AR**	**H_o**	**H_e**	**HWE**	**Bottleneck**
2015 Data Set	Unimpacted	62	5.05	4.62	0.59	0.59	0.787	**0.009**
	Impacted	45	5.00	4.66	0.63	0.60	0.493	**0.008**
	Species	107	5.75	-	0.61	0.60	0.455	**0.013**
2019 Data Set	Unimpacted	59	5.20	4.62	0.58	0.60	**0.006**	**0.032**
	Impacted	33	4.85	4.48	0.63	0.59	0.628	**0.022**
	Species	92	5.75	-	0.60	0.60	**0.041**	**0.045**

Table 4. Analysis of molecular variance (AMOVA) across 3 different data hierarchies. The AMOVA was conducted using the 2015 and 2019 data sets. The first hierarchy examined variance among individuals within a defined reach ("impacted" or "unimpacted") that is assigned to a data set, represented by year ("2015" or "2019"). The second hierarchy examined variance between the reaches within each data set. The third hierarchy examined the variance between the two data sets, representing variation between pre- and post-inundation. Hierarchy of each test conducted by the AMOVA is provided with the corresponding phi (φ) statistic and p-value. Significance of variation is based on $p < 0.05$.

Hierarchy of Test	Phi (φ)	P-value
Variance between individuals within each reach	-0.003	0.51
Variance between reaches within a year	0.003	0.06
Variance between year	0.0001	0.68

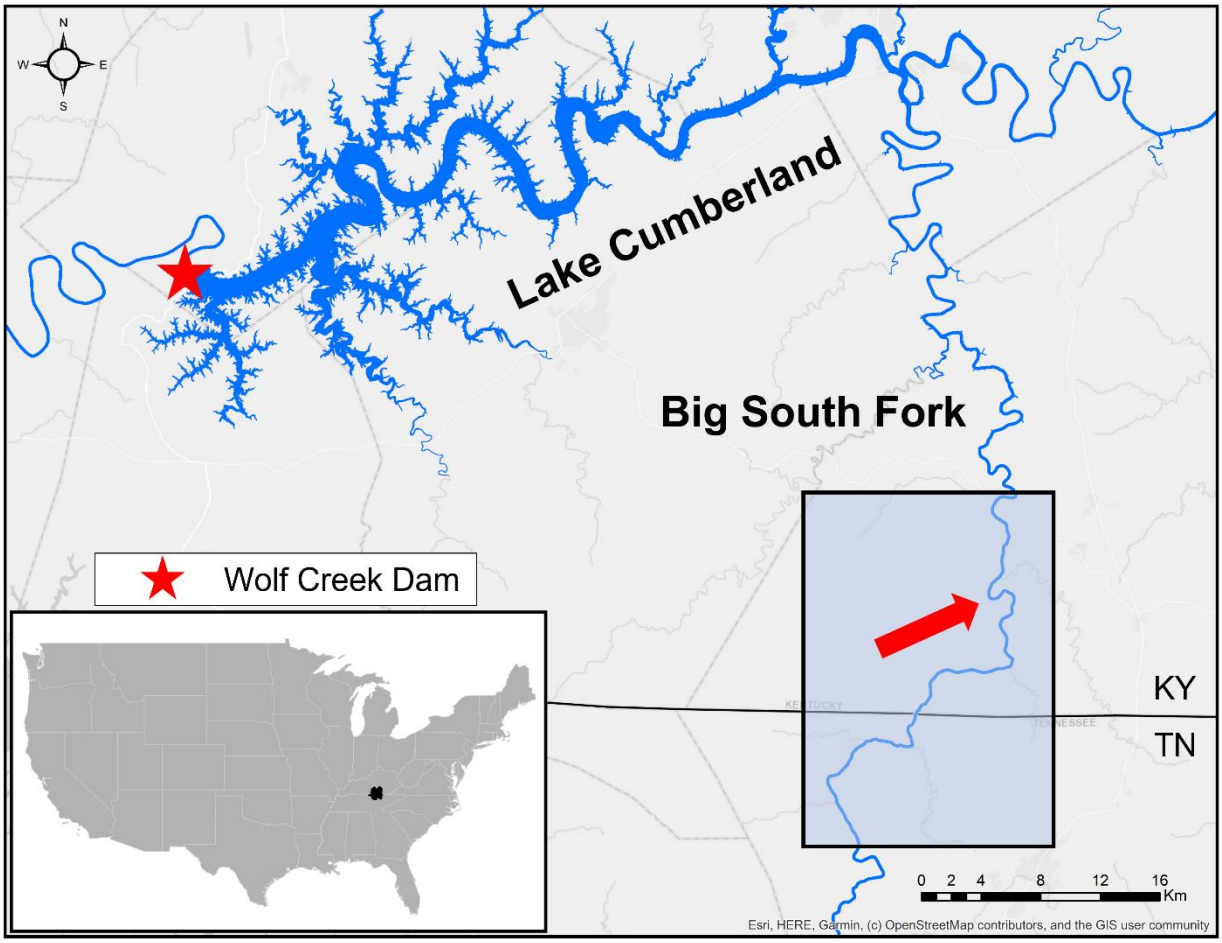
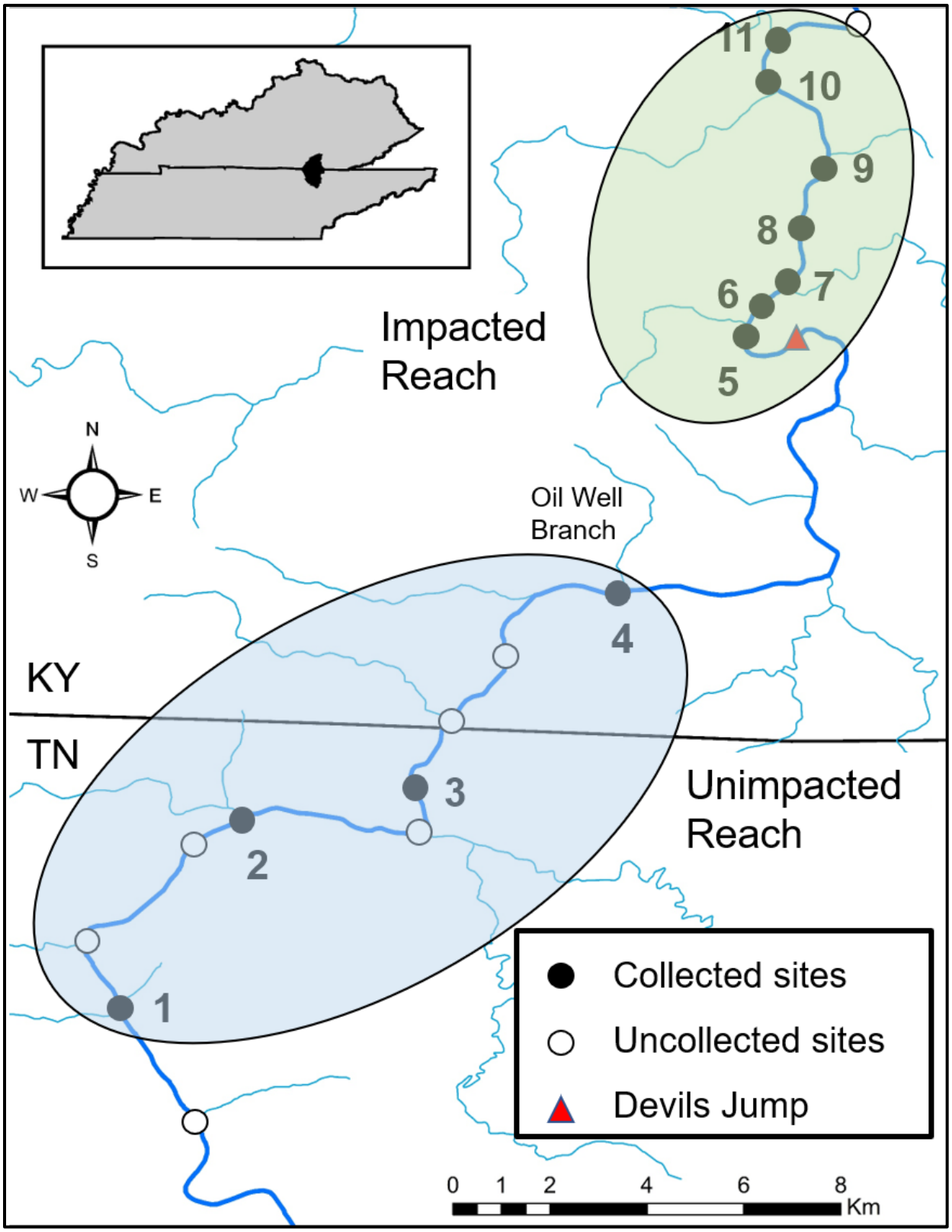


Figure 1. Map of the Cumberland River and Big South Fork Cumberland River in Kentucky and Tennessee, USA. Wolf Creek Dam on the Cumberland River [indicated by the red star] forms Lake Cumberland. This reservoir inundates the lower 70 rkm of the Big South Fork during summer pool; the red arrow represents the extent of inundation impacts upstream. The known range of *Etheostoma lemniscatum* is indicated by the shaded box.


11
10
9
8
7
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5
Impacted
Reach
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2
1
Unimpacted
Reach
Collected sites
Uncollected sites
Devils Jump
0 1 2 4 6 8
Km

Figure 2. Sites collected for *Etheostoma lemniscatum* in the Big South Fork. Solid circles indicate the subset of sites where tissue samples were collected in the current study and genetic diversity metrics were compared to those estimated from samples collected by Washburn et al. (2020). Hollow circles indicate other known localities for *E. lemniscatum* that were examined by Washburn et al. (2020) but not re-analyzed or examined in this study. Sites impacted by inundation of the Big South Fork by Lake Cumberland are indicated by the green circle at the downstream extent of the species' range [top of map]. Sites that are not directly impacted by inundation are indicated by the blue circle at the upstream extent of the species' range [bottom of map]. Devils Jump is indicated on the map by a red triangle, representing the extent of inundation within the Big South Fork. The numbers are specific to each site sampled and correspond with Tables 1 and 2.

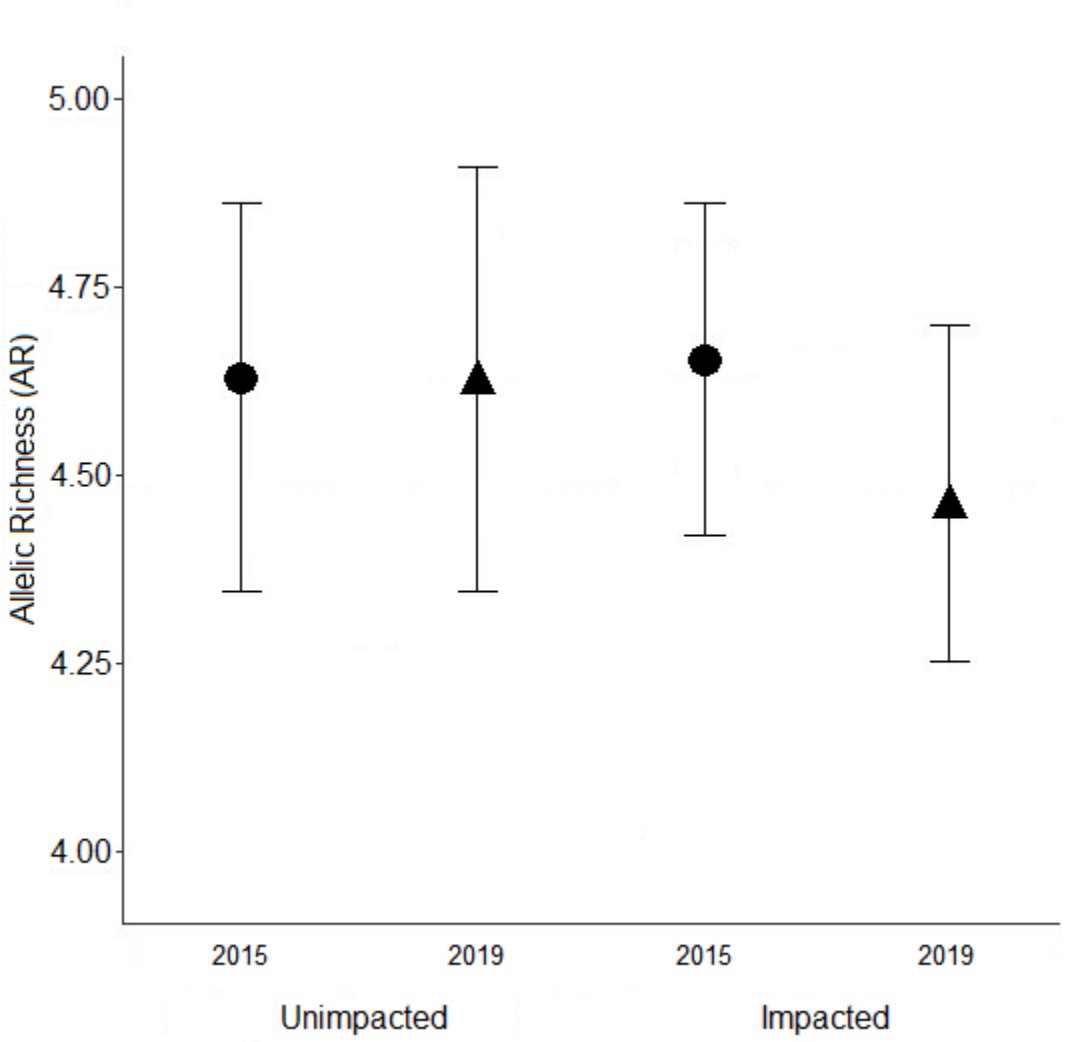


Figure 3. Allelic richness (AR) estimates for the 2015 (circles) and 2019 (triangles) data sets with 95% CI [shown as bars for each value]. AR values were estimated for the two reaches (unimpacted sites and impacted sites).

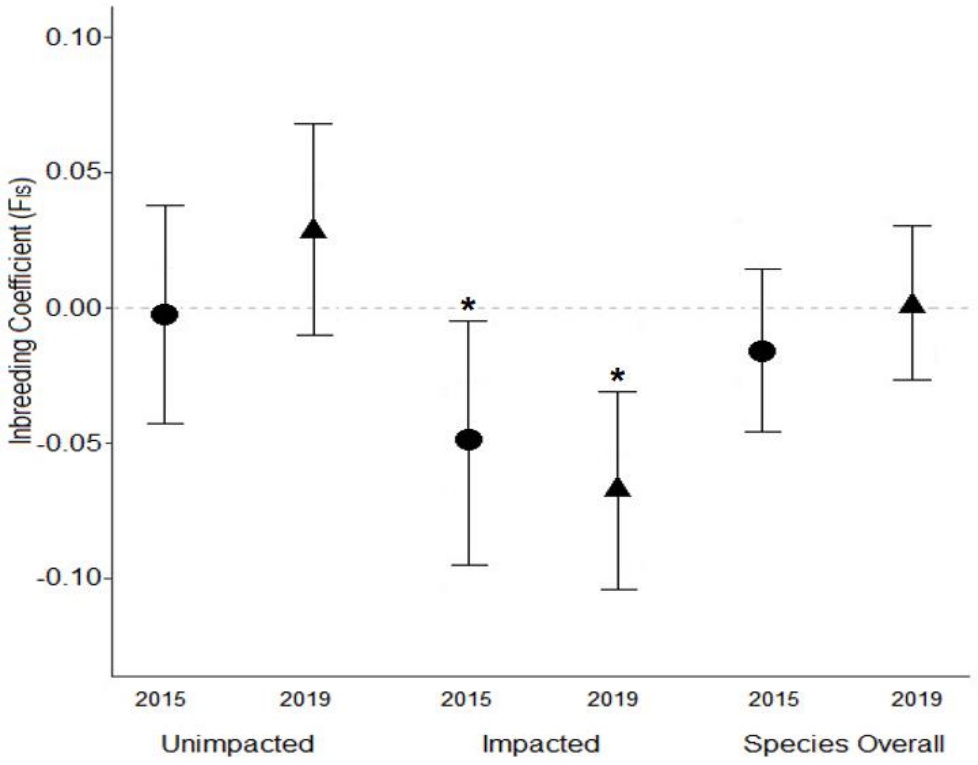


Figure 4. Inbreeding coefficients (F_{IS}) estimated for the 2015 (circles) and 2019 (triangles) data sets. F_{IS} values were estimated for the species overall and the two reaches (unimpacted sites and impacted sites) with 95% CI [shown as bars for each value]. An asterisk indicates estimates that were significant from 0 (determined by the CI of the estimates) and indicative of inbreeding (positive values) or outbreeding (negative values).

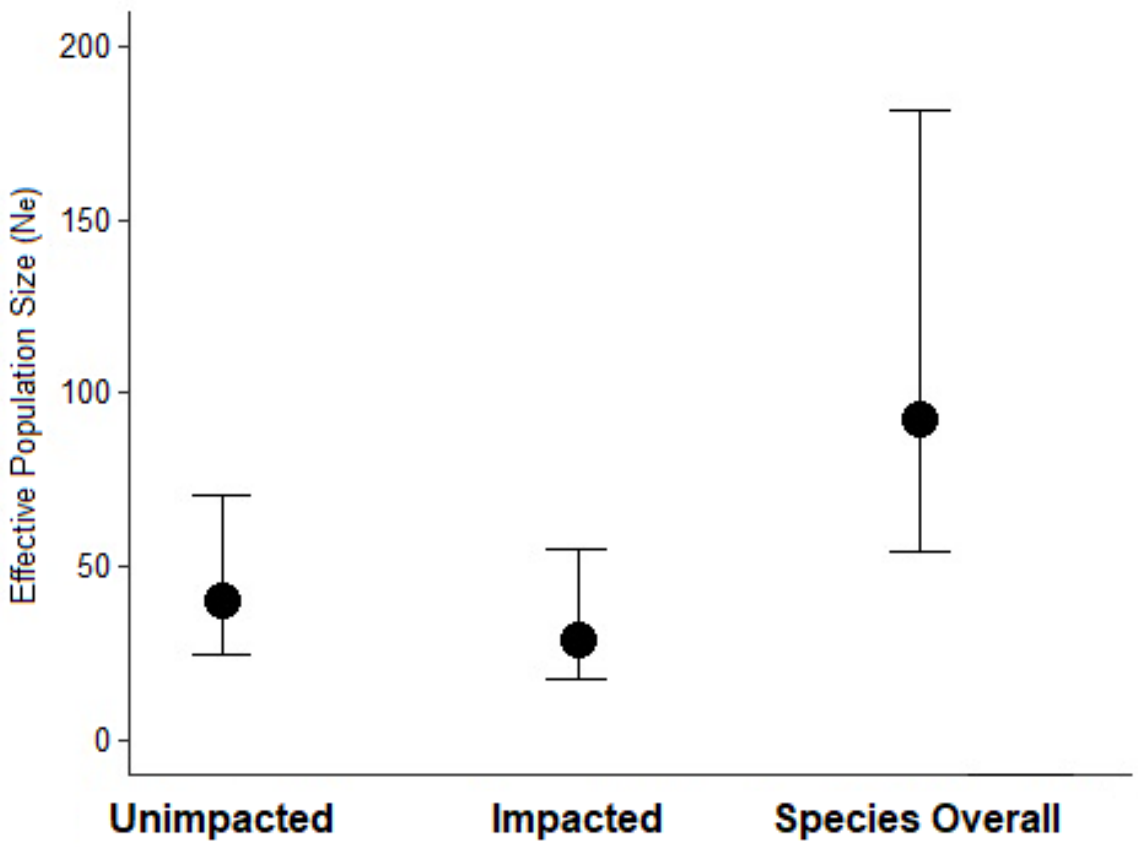
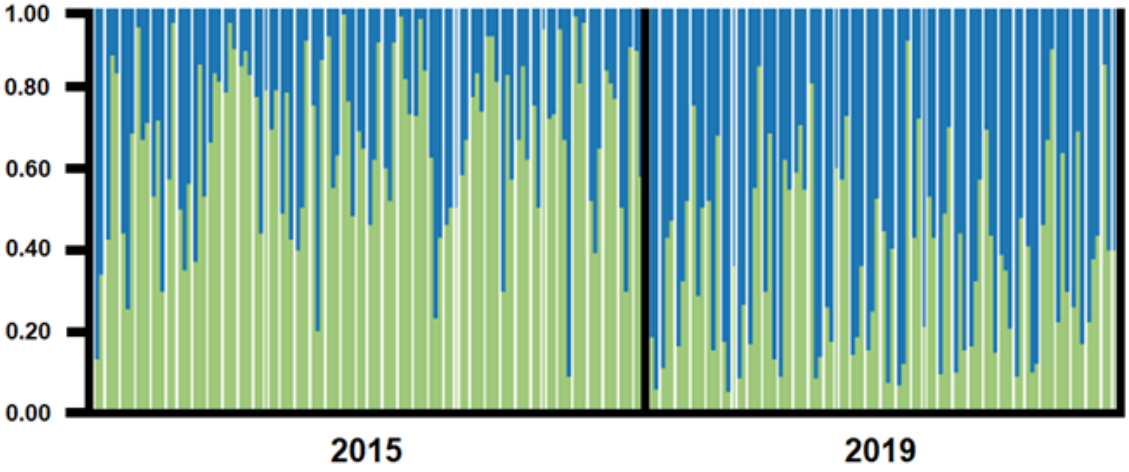
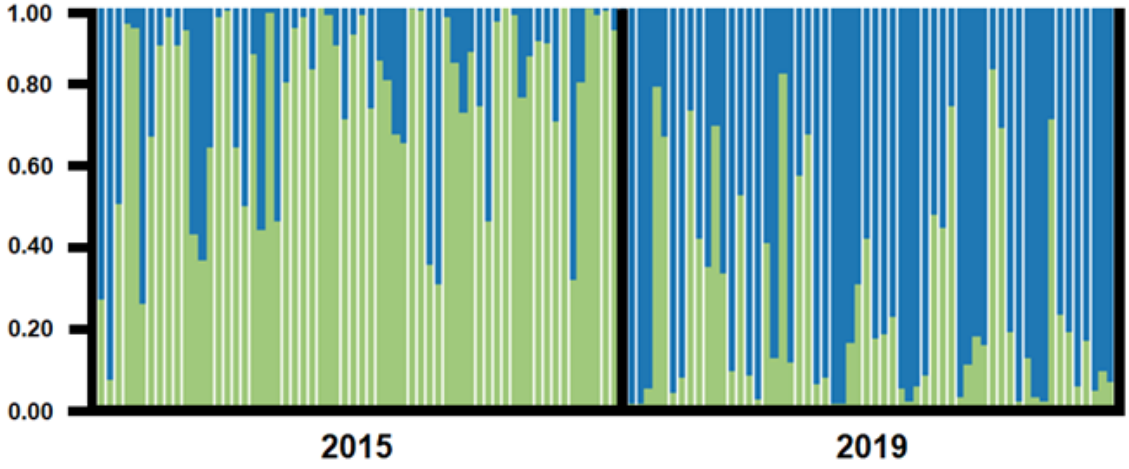


Figure 5. Temporal effective population size (N_e) estimates for the species overall and the two reaches (unimpacted sites and impacted sites), presented with 95% CI. These estimates were calculated by examining the 2015 and 2019 data sets simultaneously, using a maximum-likelihood method.

a) Species Overall



b) Unimpacted Reach



c) Impacted Reach

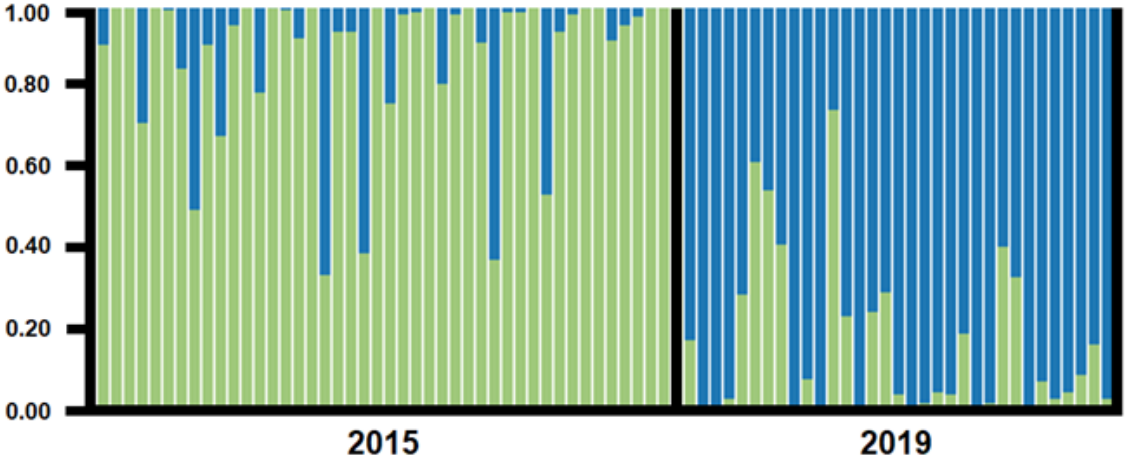


Figure 6. Temporal Discriminant Analysis of Principal Components (DAPC) plots: (a) all individuals of both data sets; (b) all individuals within the unimpacted reach of both data sets; (c) all individuals within the impacted reach of both data sets. Each vertical bar represents an individual. Individuals are placed in the year block in which they were collected, while the color of the bars represents the grouping that the DAPC assigned to each individual (2015 group = green; 2019 group = blue). The amount of color for each bar presents the percent of probability of correct assignment to a given group by the DAPC.

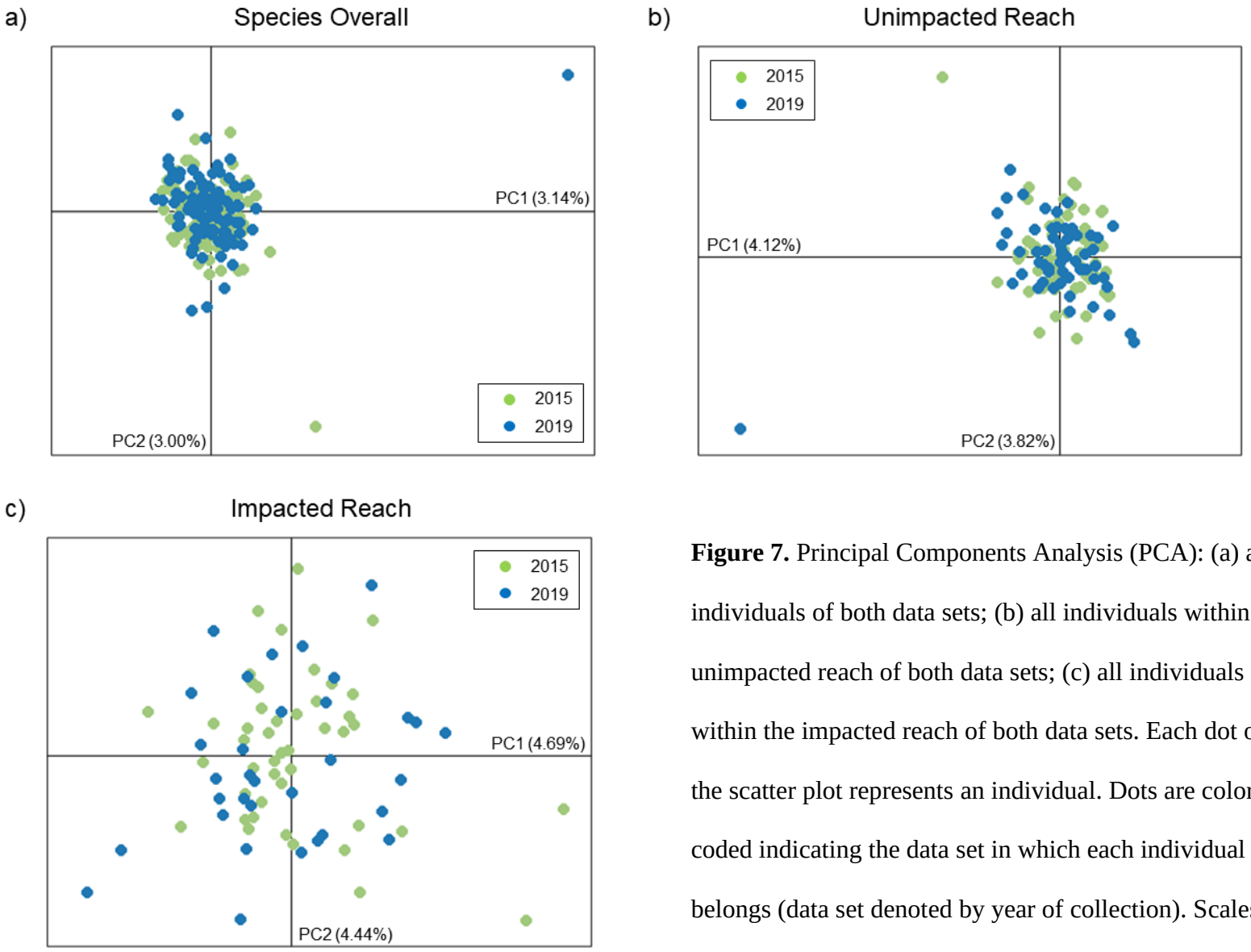


Figure 7. Principal Components Analysis (PCA): (a) all individuals of both data sets; (b) all individuals within the unimpacted reach of both data sets; (c) all individuals within the impacted reach of both data sets. Each dot on the scatter plot represents an individual. Dots are color-coded indicating the data set in which each individual belongs (data set denoted by year of collection). Scales of graphs vary between each PCA.

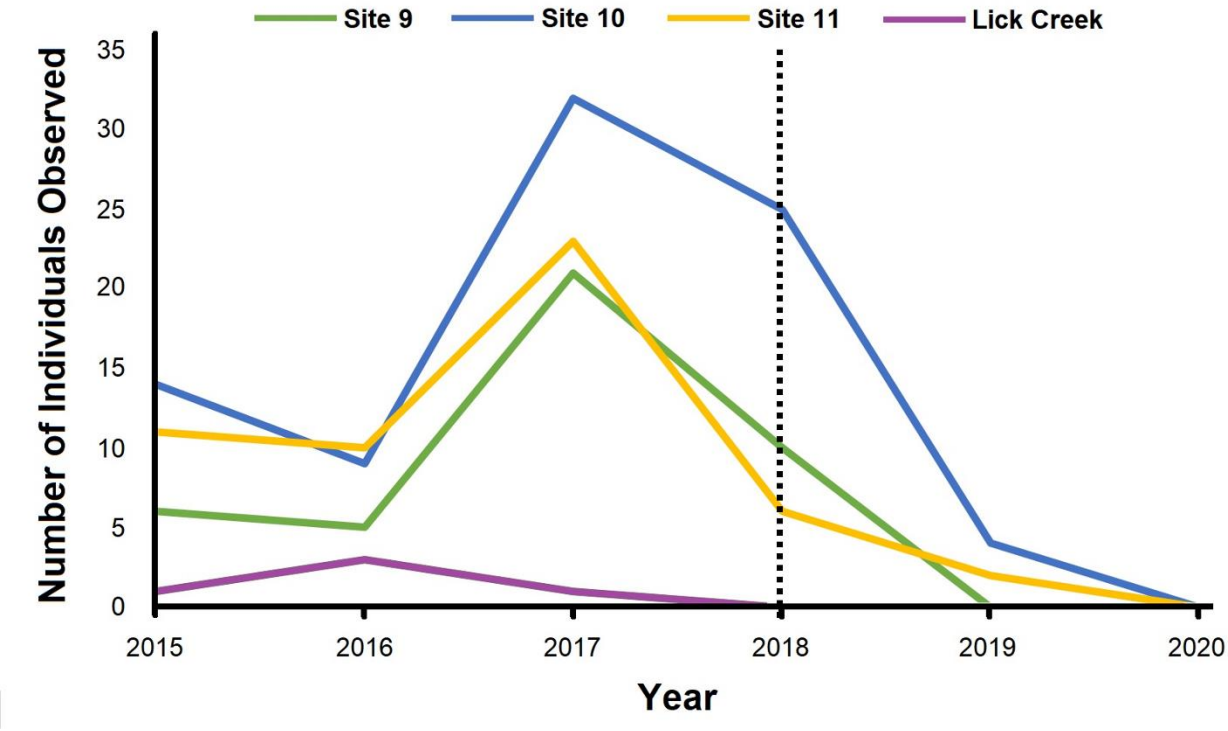


Figure 8. Occurrence and abundance of *Etheostoma lemniscatum* at sites 9-11 and at the Mouth of Lick Creek. Data presented are counts of observed individuals at each site (Simmons 2019; our study). Each line represents counts at a single site (green: Site 9; blue: Site 10; yellow: Site 11; purple: site at Mouth of Lick Creek). Site numbers correspond to those in Figure 1. Dam repairs were completed in 2013, re-inundating *E. lemniscatum*'s range by 2015. The dotted line (2018) denotes a prolonged summer pool inundation event.

Literature Cited

Aavik T, Thetloff M, Trager S, Hernandez-Agramonte IM, Reinula I, Partel M (2019) Delayed and immediate effects of habitat loss on the genetic diversity of the grassland plant *Trifolium montanum*. Biodiversity and Conservation 28:3299-3319. https://doi.org/10.1007/s10531-019-01822-8

Adamack AT, Gruber B (2014) PopGenReport: simplifying basic population genetics analyses in R. Methods in Molecular Ecology and Evolution 5:384-387. https://doi.org/10.1111/2041-210X.12158

Agostinho AA, Pelicice FM, Gomes LC (2008) Dams and the fish fauna of the Neotropical region: impacts and management related to diversity and fisheries. Brazilian Journal of Biology 68: 1119-1132. https://doi.org/10.1590/S1519-69842008000500019

Allendorf FW (1986) Genetic drift and the loss of alleles versus heterozygosity. Zoo Biology 5:181-190. https://doi.org/10.1002/zoo.1430050212

Allendorf FW, Luikart GH (2007) Conservation and the genetics of populations. Blackwell Publishing, Malden, MA, USA.

Angeletti D, Cimmaruta R, Nascetti G (2010) Genetic diversity of the killifish *Aphanius fasciatus* paralleling the environmental changes of Tarquinia salterns habitat. Genetica 138:1011-1021. https://doi.org/10.1007/s10709-010-9487-3

Angermeier PL (1995) Ecological attributes of extinction-prone species: loss of freshwater fishes of Virginia. Conservation Biology 9:143-158. https://doi.org/10.1046/j.1523-1739.1995.09010143.x

Antao T, Perez-Figueroa A, Luikart G (2011) Early detection of population declines: high power of genetic monitoring using effective population size estimators. Evolutionary Applications 4:144-154. https://doi.org/10.1111/j.1752-4571.2010.00150.x

Barcia AR, Lopez GE, Hernandez D, Garcia-Machado E (2005) Temporal variation of the population structure and genetic diversity of *Farfantepenaeus notialis* assessed by allozyme loci. Molecular Ecology 14:2933-2942. https://doi.org/10.1111/j.1365-294X.2005.02613.x

Barletta M, Jaureguizar AJ, Baigun C, Fontoura NF, Agostinho AA, Almeida-Val VMF, Val AL, Torres RA, Jimenes-Segura LF, Giarrizzo T, Fabré NN, Batista VS, Lasso C, Taphorn DC, Costa MF, Chaves PT, Vieira JP, Correa MFM (2010) Fish and aquatic habitat conservation in South America: a continental overview with emphasis on neotropical systems. Journal of Fish Biology 76:2118-2176. https://doi.org/10.1111/j.1095-8649.2010.02684.x

Barrandeguy ME, Garcia MV (2021) The sensitiveness of expected heterozygosity and allelic richness estimates for analyzing population genetic diversity. In Genetic Variation, IntechOpen. https://doi.org/10.5772/intechopen.95585

Bender DJ, Contreras TA, Farhig L (1998) Habitat loss and population decline: a meta-analysis of the patch size effect. Ecology 79:517-533. https://doi.org/10.1890/0012-9658(1998)079[0517:HLAPDA]2.0.CO;2

Biggins RE (1993) Endangered and threatened wildlife and plants: determination of endangered status for the duskytail darter, palezone shiner and pygmy madtom. Federal Register 58:25758-25763.

Blanton RE, Cashner MF, Thomas MR, Brandt SL, Floyd MA (2019) Increased habitat fragmentation leads to isolation among and low genetic diversity within populations of the imperiled Kentucky Arrow Darter (*Etheostoma sagitta spilotum*). Conservation Genetics 20:1009-1022. https://doi.org/10.1007/s10592-019-01188-y

Blanton RE, Jenkins RE (2008) Three new darter species of the *Etheostoma percnurum* species complex (Percidae, subgenus *Catonotus*) from the Tennessee and Cumberland River drainages. Zootaxa 1963:1-24. https://doi.org/10.11646/zootaxa.1963.1.1

Bunn SE, Arthington AH (2002) Basic principles and ecological consequences of altered flow regimes for aquatic biodiversity. Environmental Management 30:492-507. https://doi.org/10.1007/s00267-002-2737-0

Caballero A, Garcia-Dorado A (2013) Allelic diversity and its implications for the rate of adaptation. Genetics 195:1373-1384. https://doi.org/10.1534/genetics.113.158410

Carling PA (1984) Deposition of fine and coarse sand in an open-work gravel bed. Canadian Journal of Fisheries and Aquatic Sciences 41:263-270. https://doi.org/10.1139/f84-030

Carvalho CS, Lanes E, Silva AR, Caldeira CF, Carvalho-Filho N, Gastauer M, Imperatriz-Fonseca VL, Nascimento W Jr., Oliveria G, Siqueira JO, Viana PL, Jaffe R (2019) Habitat loss does not always entail negative genetic consequences. Frontiers in Genetics 10:1101. https://doi.org/10.3389/fgene.2019.01101

Collen, B., F. Whitton, E. E. Dyer, J. E. M. Baillie, N. Cumberlidge, W. R. T. Darwell, C. Pollock, N. I. Richman, A. Soulsby, and M. Bohm. 2014. Global patterns of freshwater species diversity, threat and endemism. Global Ecology and Biogeography 23(1):40-51. https://doi.org/10.1111/geb.12096

Converse YK, Hawkins CP, Valdez RA (1998) Habitat relationships of subadult humpback chub in the Colorado River through the Grand Canyon: spatial variability and implications of flow regulation. Regulated Rivers: Research and Management 14:267-284. https://doi.org/10.1002/(SICI)1099-1646(199805/06)14:3%3C267::AID-RRR501%3E3.0.CO;2-5

Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014. https://doi.org/10.1093/genetics/144.4.2001

Davis JG (2010) Development of long-term monitoring protocols for two rare fishes, the Tuxedo Darter and the Spotfin Chub, in Tennessee. Dissertation, Tennessee Tech University, Cookeville, Tennessee, USA.

Davis JG, Cook SB (2010) Habitat use of the Tuxedo Darter (*Etheostoma lemniscatum*) at macrohabitat and microhabitat spatial scales. Journal of Freshwater Ecology 25:321-330. https://doi.org/10.1080/02705060.2010.9664374

Diamond JM (1972) Biogeographic kinetics: estimation of relaxation times for avifaunas of southwest Pacific islands. Proceedings of the National Academy of Sciences 69:3199-3203. https://doi.org/10.1073/pnas.69.11.3199

Di Rienzo A, Peterson AC, Garza JC, Valdes AM, Slatkin M, Freimer NB (1994) Mutational processes of simple-sequence repeat loci in human populations. Proceedings of the National Academy of Sciences of the United States of America 91:3166-3170. https://doi.org/10.1073/pnas.91.8.3166

Do C, Waples RS, Peel D, Macbeth GM, Tillett BJ, Ovenden JR (2014) NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Molecular Ecology Resources 14:209-214. https://doi.org/10.1111/1755-0998.12157

Dray S, Dufour A (2007) The ade4 package: implementing the duality diagram for ecologists. Journal of Statistical Software 22:1-20. https://doi.org/10.18637/jss.v022.i04

Dudgeon D, Arthington AH, Gessner MO, Kawabata ZI, Knowler DJ, Léveque C, Naiman RJ, Prieur-Richard AH, Soto D, Stiassny ML, Sullivan CA (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. Biological Reviews 81:163-182. https://doi.org/10.1017/S1464793105006950

Dures SG, Carbone C, Loveridge AS, Maude G, Midlane N, Aschenborn O, Gotelli D (2019) A century of decline: loss of genetic diversity in a southern African lion-conservation stronghold. Diversity and Distributions 25:870-879. https://doi.org/10.1111/ddi.12905

Earl DA, VonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources 4:359-361. https://doi.org/10.1007/s12686-011-9548-7

Elkins D, Sweat SC, Kuhajda BR, George AL, Hill KS, Wenger SJ (2019) Illuminating hotspots of imperiled aquatic biodiversity in the southeastern US. Global Ecology and Conservation 19:e00654. https://doi.org/10.1016/j.gecco.2019.e00654

Eisenhour DJ, Burr BM (2000) Conservation status and nesting biology of the endangered Duskytail Darter, *Etheostoma percnurum*, in the Big South Fork of the Cumberland River, Kentucky. Journal of Kentucky Academy of Science 61:67-76.

England PR, Osler GH, Woodworth LM, Montgomery ME, Briscoe DA, Frankham R (2003) Effects of intense versus diffuse population bottlenecks on microsatellite genetic diversity and evolutionary potential. Conservation Genetics 4:595-604. https://doi.org/10.1023/A:1025639811865

Epps CW, Keyghobadi N (2015) Landscape genetics in a changing world: disentangling historical and contemporary influences and inferring change. Molecular Ecology 24:6021-6040. https://doi.org/10.1111/mec.13454

Essl F, Dullinger S, Rabitsch W, Hulme PE, Pysek P, Wilson JRU, Richardson DM (2015) Historical legacies accumulate to shape future biodiversity in an era of rapid global change. Diversity and Distributions 21:534-547. https://doi.org/10.1111/ddi.12312

Etnier DA (1997) Jeopardized southeastern freshwater fishes: a search for causes. Aquatic fauna in peril: the southeastern perspective 1:87-104.

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Molecular Ecology 14:2611-2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

Ewers RM, Didham RK (2006) Confounding factors in the detection of species responses to habitat fragmentation. Biological Reviews 81:117-142. https://doi.org/10.1017/S1464793105006949

Farhig L (1997) Relative effects of habitat loss and fragmentation on population extinction. The Journal of Wildlife Management 61:603-610. https://doi.org/10.2307/3802168

Fluker BL, Kuhajda BR, Harris PM (2014) The effects of riverine impoundment on genetic structure and gene flow in two stream fishes in the Mobile River basin. Freshwater Biology 59:526-543. https://doi.org/10.1111/fwb.12283

Fluker BL, Jones KD, Kuhajda BR (2019) Genetic structure and diversity of the blueface darter *Etheostoma cyanoprosopum*, a microendemic freshwater fish in the southeastern USA. Endangered Species Research 40:133-147. https://doi.org/10.3354/esr00986

Frankham R (1995) Conservation genetics. Annual Review of Genetics 29(1):305-327. https://doi.org/10.1146/annurev.ge.29.120195.001513

Frankham R (1996) Relationship of genetic variation to population size in wildlife. Conservation Biology 10:1500-1508. https://doi.org/10.1046/j.1523-1739.1996.10061500.x

Frankham R, Ballou JD, Briscoe DA (2010) Introduction to conservation genetics. Ed. 2. Cambridge University Press, Cambridge, UK.

Frankham R, Ballou JD, Eldridge MD, Lacy RC, Ralls K, Dudash MR, Fenster CB (2011) Predicting the probability of outbreeding depression. Conservation Biology 25:465-475. https://doi.org/10.1111/j.1523-1739.2011.01662.x

Frankham R, Bradshaw CJA, Brooks BW (2014) Genetics in conservation management: revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation 170:56-63. https://doi.org/10.1016/j.biocon.2013.12.036

Gatch AJ, Koenigbauer ST, Roseman EF, Hook TO (2020) The effect of sediment cover and female characteristics on the hatching success of walleye. North American Journal of Fisheries Management 40:292-302. https://doi.org/10.1002/nafm.10407

Gorman OT, Karr JR (1978) Habitat structure and stream fish communities. Ecology 59:507-515. https://doi.org/10.2307/1936581

Goudet J (2005) HIERFSTAT, a package for R to compute and test hierarchical F-statistics. Molecular Ecology 5:184-186. https://doi.org/10.1111/j.1471-8286.2004.00828.x

Goudet J, Jombart T (2020) HIERFSTAT: estimation and tests of hierarchical F-statistics. R package version 0.5-7. https://cran.r-project.org/package=hierfstat

Graf WL (1999) Dam nation: A geographic census of American dams and their large-scale hydrologic impacts. Water Resources Research 35:1305-1311. https://doi.org/10.1029/1999WR900016

Greig SM, Sear DA, Carling PA (2007) A review of factors influencing the availability of dissolved oxygen to incubating salmonid embryos. Hydrological Processes: An International Journal 21:323-334. https://doi.org/10.1002/hyp.6188

Haghkerdar JM, McLachlan JR, Ireland A, Greig HS (2019) Repeat disturbances have cumulative impacts on stream communities. Ecology and Evolution 9: 2898-2906. https://doi.org/10.1002/ece3.4968

Hansen MM, Olivieri I, Waller DM, Nielsen EE, GeM Working Group (2012) Monitoring adaptive genetic responses to environmental change. Molecular Ecology 21:1311-1329. https://doi.org/10.1111/j.1365-294X.2011.05463.x

Helm A, Hanski I, Partel M (2006) Slow response of plant species richness to habitat loss and fragmentation. Ecology Letters 9:72-77. https://doi.org/10.1111/j.1461-0248.2005.00841.x

Hendrick PW, Kalinowski ST (2000) Inbreeding depression in conservation biology. Annual Review of Ecology and Systematics 31:139-162. https://doi.org/10.1146/annurev.ecolsys.31.1.139

Hitchings SP, Beebee TJC (1998) Loss of genetic diversity and fitness in Common Toad (*Bufo bufo*) populations isolated by inimical habitat. Journal of Evolutionary Biology 11:269-283. https://doi.org/10.1046/j.1420-9101.1998.11030269.x

Hubbs C, Pigg J (1976) The effects of impoundments on threatened fishes of Oklahoma. Annals of the Oklahoma Academy of Science 5:133-177.

Hubisz MJ, Falush D, Stephens M, Pritchard JK (2009) Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources 9:1322-1332. https://doi.org/10.1111/j.1755-0998.2009.02591.x

Hui TY (2014) NB: maximum likelihood method in estimating effective population size from genetic data. R package version 0.9. https://cran.r-project.org/package=NB

Hui TY, Burt A (2015) Estimating effective population size from temporally spaced samples with a novel, efficient maximum-likelihood algorithm. Genetics 200:285-293. https://doi.org/10.1534/genetics.115.174904

Humphries P, Lake PS (2000) Fish larvae and the management of regulated rivers. Regulated Rivers: Research and Management 16:421-432. https://doi.org/10.1002/1099-1646(200009/10)16:5%3C421::AID-RRR594%3E3.0.CO;2-4

Ingendahl D (2001) Dissolved oxygen concentration and emergence of sea trout fry from natural redds in tributaries of the River Rhine. Journal of Fish Biology 58:325-341. https://doi.org/10.1111/j.1095-8649.2001.tb02256.x

Jelks HL, Walsh SL, Burkhead NM, Contreras-Balderas S, Diaz-Pardo E, Hendrickson DA, Lyons J, Mandrak NE, McCormick F, Nelson JS, Platania SP, Porter BA, Renaud CB, Schmitter-Soto JJ, Taylor EB, Warren ML Jr (2008) Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372-407. https://doi.org/10.1577/1548-8446-33.8.372

Jenkins RE, Burkhead NM (1994) Freshwater fishes of Virginia. American Fisheries Society, Bethesda, MD, USA.

Jensen DW, Steel EA, Fullerton AH, Pess GR (2009) Impact of fine sediment on egg-to-fry survival of Pacific salmon: a meta-analysis of published studies. Reviews in Fisheries Science 17:348-359. https://doi.org/10.1080/10641260902716954

Johannesson K, Andre C (2006) Life on the margin: Genetic isolation and diversity loss in a peripheral marine ecosystem, the Baltic Sea. Molecular Ecology 15:2013-2029. https://doi.org/10.1111/j.1365-294X.2006.02919.x

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24:1403-1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:1-15. https://doi.org/10.1186/1471-2156-11-94

Jorde PE, Ryman N (2007) Unbiased estimator for genetic drift and effective population size. Genetics 177:927-935. https://doi.org/10.1534/genetics.107.075481

Kamvar ZN, Tabima JF, Grunwald NJ (2014) Poppr: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281. https://doi.org/10.7717/peerj.281

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodohl PA (2013) diveRsity: an R package for the estimation and exploration of population genetics parameters and their associated errors. Methods in Ecology and Evolution 4:782-788. https://doi.org/10.1111/2041-210X.12067

Kemp P, Sear D, Collins A, Naden P, Jones I (2011) The impacts of fine sediment on riverine fish. Hydrological Processes 25:1800-1821. https://doi.org/10.1002/hyp.7940

Layman SR (1991) Life history of the relict, Duskytail Darter, *Etheostoma (Catonotus) sp.*, in Little River, Tennessee. Copeia 1991:471-485. https://doi.org/10.2307/1446595

Liu X, Wu R, Chen X, Zhou Y, Yang L, Ouyang S, Wu X (2020) Effects of dams and their environmental impacts on the genetic diversity and connectivity of freshwater mussel populations in Poyang Lake Basin, China. Freshwater Biology 65:264-277. https://doi.org/10.1111/fwb.13419

Luikart G, Cornuet JM (1998) Empirical evaluation of a test for identifying recently bottlenecked populations from allele frequency data. Conservation Biology 12:228-237. https://doi.org/10.1111/j.1523-1739.1998.96388.x

Luikart G, Sherwin WB, Steele BM, Allendorf WF (1998) Usefulness of molecular markers for detecting population bottlenecks via monitoring genetic change. Molecular Ecology 7: 963-974. https://doi.org/10.1046/j.1365-294x.1998.00414.x

Magee JP, McMahon TE, Thurow RF (1996) Spatial variation in spawning habitat of cutthroat trout in sediment-rich stream basin. Transactions of the American Fisheries Society. 125:768-779. https://doi.org/10.1577/1548-8659(1996)125%3C0768:SVISHO%3E2.3.CO;2

McCartney M (2009) Living with dams: managing the environmental impacts. Water Policy 11(Supp. 1):121-139. https://doi.org/10.2166/wp.2009.108

McConkey JB (2010) Georeferenced riverine habitat mapping in the Big South Fork National River and Recreation Area. Thesis, University of Tennessee, Knoxville, Tennessee, USA.

McCoy ED, Richmond JQ, Mushinsky HR, Britt EJ, Godley JS (2010) Long generation time delays the genetic response to habitat fragmentation in the threatened Florida sand skink. Journal of Herpetology 44:641-644. https://doi.org/10.1670/09-089.1

Moyer GR, Williams AS (2012) Genetic assessment of Abrams Creek reintroduction program for the federally threatened yellowfin madtom (*Noturus flavipinnis*), and endangered smoky madtom (*Noturus baileyi*) and Citico darter (*Etheostoma sitikuense*). https://www.fws.gov/

Nei M, Maruyama T, Chakraborty R (1975) The bottleneck effect and genetic variability in populations. Evolution 29:1-10. https://doi.org/10.2307/2407137

Nobile AB, Freitas-Souza D, Lima FP, Queiroz J, Bayona-Perez IL, Carvalho ED, Ramos IP (2019) Damming and seasonality as modulators of fish community structure in a small tributary. Ecology of Freshwater Fish 28:563-572. https://doi.org/10.1111/eff.12475

O'Bara CJ, Pennington WL, Bonner WP (1982) A survey of water quality, benthic macroinvertebrates and fish for sixteen streams within Big South Fork National River and Recreational Area. Cookeville, TN: Tennessee Cooperative Fishery Research Unit, Tennessee Technological University.

Olsen JB, Kinziger AP, Wenburg JK, Lewis CJ, Phillips CT, Ostrand KG (2016) Genetic diversity and divergence in the fountain darter (*Etheostoma fonticola*): implications for conservation of an endangered species. Conservation Genetics 17:1393-1404. https://doi.org/10.1007/s10592-016-0869-7

Pandit SN, Kolasa J, Cottenie K (2009) Contrasts between habitat generalists and specialists: an empirical extension to the basic metacommunity framework. Ecology 90:2253-2262. https://doi.org/10.1890/08-0851.1

Peery MZ, Kirby R, Reid BN, Stoelting R, Doucet-Beer E, Robinson S, Vasquez-Carrillo C, Pauli JN, Palsboll PJ (2012) Reliability of genetic bottleneck tests for detecting recent population declines. Molecular Ecology 21:3403-3418. https://doi.org/10.1111/j.1365-294X.2012.05635.x

Perez-Portela R, Turon X, Bishop JDD (2012) Bottlenecks and loss of genetic diversity: spatio-temporal patterns of genetic structure in an ascidian recently introduced in Europe. Marine Ecology Progress Series 451:93-105. https://doi.org/10.3354/meps09560

Pimm SL, Raven P (2000) Extinction by numbers. Nature 403:843-845. https://doi.org/10.1038/35002708

Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in effective size using allele frequency data. Journal of Heredity 90:502-503. https://doi.org/10.1093/jhered/90.4.502

Poff NL, Zimmerman JKH (2010) Ecological responses to altered flow regimes: a literature review to inform the science and management of environmental flows. Freshwater Biology 55:194-205. https://doi.org/10.1111/j.1365-2427.2009.02272.x

Pritchard JK, Stephens M, Donelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959. https://doi.org/10.1093/genetics/155.2.945

R Core Team (2019) R: a language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org/

Reed DH, Frankham R (2003) Correlation between fitness and genetic diversity. Conservation Biology 17:230-237. https://doi.org/10.1046/j.1523-1739.2003.01236.x

Reis RE, Albert JS, Di Dario F, Mincarones MM, Petry P, Rocha LA (2016) Fish biodiversity and conservation in South America. Journal of Fish Biology 89:12-47. https://doi.org/10.1111/jfb.13016

Riccardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. Conservation Biology 13:1220-1222. https://doi.org/10.1046/j.1523-1739.1999.98380.x

Riccioni G, Landi M, Ferrara G, Milano I, Cariani A, Zane L, Sella M, Barbujani G, Tinti F (2010) Spatio-temporal population structuring and genetic diversity retention in depleted

Atlantic bluefin tuna of the Mediterranean Sea. Proceedings of the National Academy of Sciences 107:2102-2107. https://doi.org/10.1073/pnas.0908281107

Richmond JQ, Reid DT, Ashton KG, Zamudio KR (2009) Delayed genetic effects of habitat fragmentation on the ecologically specialized Florida sand skink (*Plestiodon reynoldsi*). Conservation Genetics 10:1281-1297. https://doi.org/10.1007/s10592-008-9707-x

Rikard M, Kunkle S, Wilson J (1986) Big South Fork National River and Recreational Area: water quality report 1982-1984.

Robinson JD, Simmons JW, Williams AS, Moyer GR (2013) Population structure and genetic diversity in the endangered bluemask darter (*Etheostoma akatulo*). Conservation Genetics 14:79-92. https://doi.org/10.1007/s10592-012-0427-x

Rousset F (2008) GENEPOP'007: a complete re-implementation of the genepop software for Windows and Linus. Molecular Ecology Resources 8:103-106. https://doi.org/10.1111/j.1471-8286.2007.01931.x

Schlaepfer DR, Braschler B, Rusterholz HP, Baur B (2018) Genetic effects of anthropogenic habitat fragmentation on remnant animal and plant populations: a meta-analysis. Ecosphere 9:e02488. https://doi.org/10.1002/ecs2.2488

Schwartz MK, Luikart G, Waples RS (2006) Genetic monitoring as a promising tool for conservation and management. TRENDS in Ecology and Evolution 22:25-33. https://doi.org/10.1016/j.tree.2006.08.009

Segelbacher G, Manel S, Tomiuk J (2008) Temporal and spatial analyses disclose consequences of habitat fragmentation on the genetic diversity in capercaillie (*Tetrao urogallus*). Molecular Ecology 17:2356-2367. https://doi.org/10.1111/j.1365-294X.2008.03767.x

Simmons JW (2018) Biological monitoring in the Big South Fork Cumberland River following return of Lake Cumberland to normal pool level operations- year five, 2018. Report to U.S. Army Corps of Engineers - Nashville District.

Sonsthagen S, Haughey C, Sexson M, Solovyeva D, Petersen M, Powell A (2020) Temporal variation in genetic structure within the threatened spectacled eider. Conservation Genetics 21:175-179. https://doi.org/10.1007/s10592-019-01234-9

Sousa V, Penha F, Pala I, Chikhi L, Coelho MM (2010) Conservation genetics of a critically endangered Iberian minnow: evidence of population decline and extirpations. Animal Conservation 13:162-171. https://doi.org/10.1111/j.1469-1795.2009.00317.x

Spielman D, Brook BW, Frankham R (2014) Most species are not driven to extinction before genetic factors impact them. Proceedings of the National Academy of Sciences 101:15261-15264. https://doi.org/10.1073/pnas.0403809101

Stanford JA, Ward JV (1986) Reservoirs of the Colorado River system. In: Davis BR, Walker KF (eds), The ecology of river systems. Dr. W. Junk, Publishers, Dordrecht, pp 375-383.

Stowell SML, Pinzone CA, Martin AP (2017) Overcoming barriers to active interventions for genetic diversity. Biodiversity and Conservation 26:1753-1765. https://doi.org/10.1007/s10531-017-1330-z

Tessier N, Bernatchez L (1999) Stability of population structure and genetic diversity across generations assessed by microsatellites among sympatric populations of landlocked Atlantic salmon (*Salmo salar* L.). Molecular Ecology 8:169-179. https://doi.org/10.1046/j.1365-294X.1999.00547.x

Thioulouse J, Dray S, Dufour A, Siberchicot A, Jombart T, Pavoine S (2018) Multivariate analysis of ecological data with ade4. Springer. https://doi.org/10.1007/978-1-4939-8850-1

Thrush SF, Halliday J, Hewitt JE, Lohrer AH (2008) The effects of habitat loss, fragmentation, and community homogenization on resilience in estuaries. Ecological Applications 18:12-21. https://doi.org/10.1890/07-0436.1

Tilman D, May RM, Lehman CL, Nowak MA (1994) Habitat destruction and the extinction debt. Nature 371:65-66. https://doi.org/10.1038/371065a0

US Fish and Wildlife Service (2012) Duskytail Darter (*Etheostoma percnurum*) 5-year review: Summary and evaluation.

Valtonen M, Palo JU, Ruokonen M, Kunnasranta M, Nyman T (2012) Spatial and temporal variation in genetic diversity of an endangered freshwater seal. Conservation Genetics 13:1231-1245. https://doi.org/10.1007/s10592-012-0367-5

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Note 4:535-538. https://doi.org/10.1111/j.1471-8286.2004.00684.x

Wang J, Santiago E, Caballero A (2016) Prediction and estimation of effective population size. Heredity 117:193-206. https//doi.org/10.1038/hdy.2016.43

Waples RS (2006) A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. Conservation Genetics 7:167-184. https://doi.org/10.1007/s10592-005-9100-y

Waples RS, Do C (2008) LDNE: a program for estimating effective population size from data on linkage disequilibrium. Molecular Ecology Resources 8:753-756. https://doi.org/10.1111/j.1755-0998.2007.02061.x

Waples RS, Do C (2010) Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. Evolutionary Applications 3:244-262. https://doi.org/10.1111/j.1752-4571.2009.00104.x

Warren ML, Burr BM (1994) Status of freshwater fishes of the United States: overview of an imperiled fauna. Fisheries 19:6-18. https://doi.org/10.1577/1548-8446(1994)019%3C0006:SOFFOT%3E2.0.CO;2

Warren ML, Burr BM, Walsh SJ, Bart HL Jr, Cashner RC, Etnier DA, Freeman BJ, Kuhajda BR, Mayden RL, Robison HW, Ross ST, Starnes WC (2000) Diversity, distribution, and conservation status of the native freshwater fishes of the southern United States. Fisheries 25:7-31. https://doi.org/10.1577/1548-8446(2000)025%3C0007:DDACSO%3E2.0.CO;2

Washburn BA, Cashner MF, Blanton RE (2020) Small fish, large river: surprisingly minimal genetic structure in a dispersal-limited, habitat specialist fish. Ecology and Evolution 10:2253-2268. https://doi.org/10.1002/ece3.6064

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370. https://doi.org/10.2307/2408641

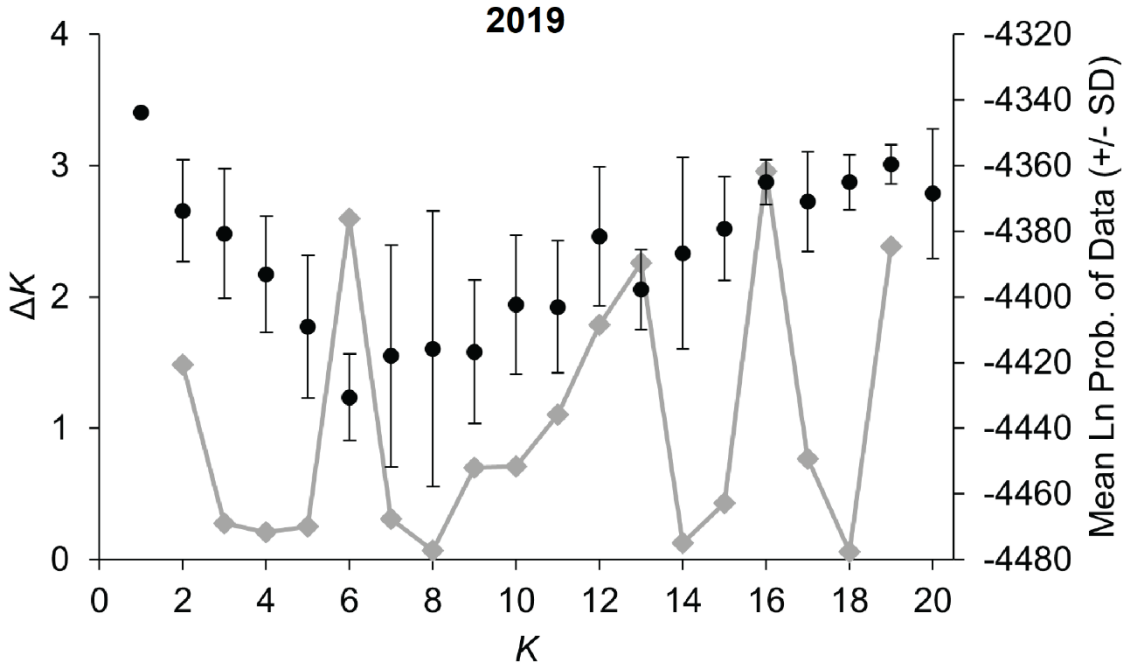
Appendix A

Supplementary Materials

Table S1. Pairwise F_{ST} values between the impacted and unimpacted reaches for the 2015 and 2019 data sets. Significance of fixation is based on $p < 0.05$.

	F_{ST} Value	**P-value**
2015 (pre-inundation)	0.003	0.20
2019 (post-inundation)	0.002	0.52

a)


2019
ΔK
K
Mean Ln Prob. of Data (+/- SD)
0
1
2
3
4
0
2
4
6
8
10
12
14
16
18
20
-4320
-4340
-4360
-4380
-4400
-4420
-4440
-4460
-4480

b)

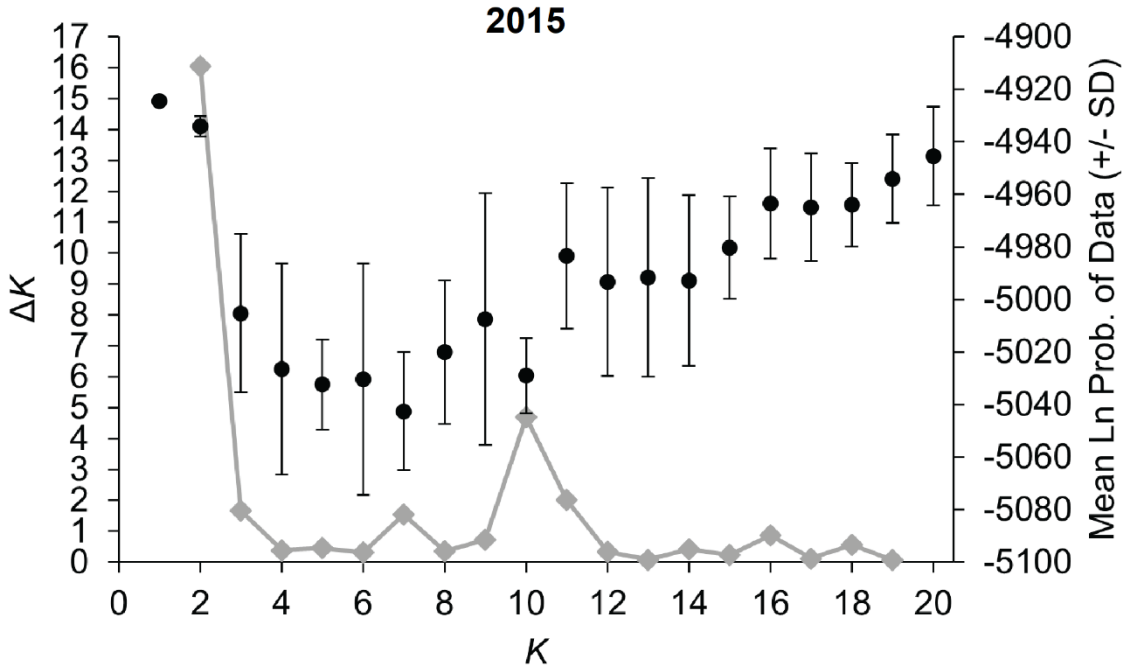

2015
ΔK
K
Mean Ln Prob. of Data (+/- SD)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
0
2
4
6
8
10
12
14
16
18
20
-4900
-4920
-4940
-4960
-4980
-5000
-5020
-5040
-5060
-5080
-5100

Figure S1. Estimates of genetic clusters (K) based on the mean log likelihood and ΔK in STRUCTURE: (a) K estimates using the LOCPRIOR model with the unimpacted and impacted reaches as priors for the current study data set (2019); (b) K estimates using the LOCPRIOR model with the unimpacted and impacted reaches as priors for the 2015 samples. Black circles with standard deviation error bars indicated the mean log likelihood estimates of K over 5 iterations for each K-estimate, while the gray diamonds indicate the ΔK values.

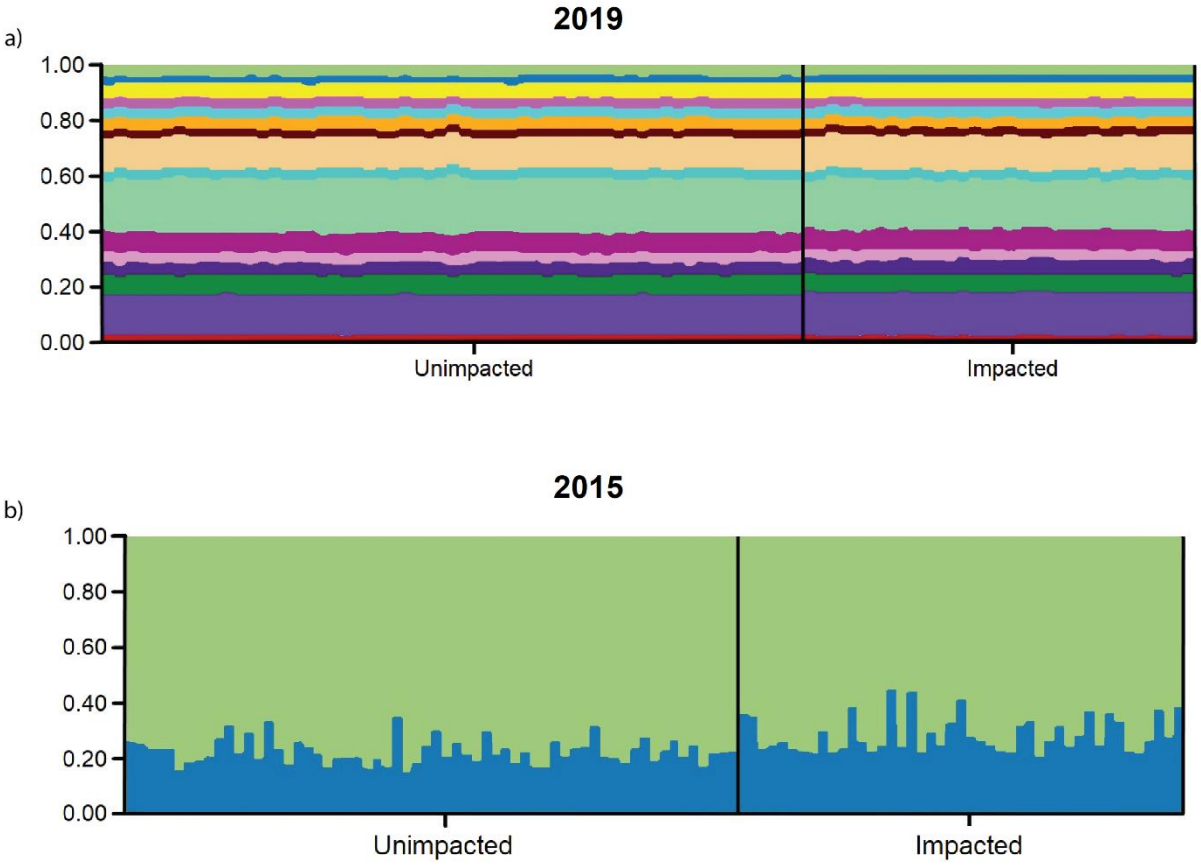


Figure S2. STRUCTURE plots depicting population structure analyses across 5 iterations for the optimal K value: (a) K=16 optimal clusters suggested by ΔK for the 2019 data set; (b) K=22 optimal clusters suggested by ΔK for the 2015 data set. Each vertical bar represents an individual's genotype, and the colors indicate the proportion of the individual's genotype that is assigned to a cluster. The reaches used as the priors for the LOCPRIOR method are indicated on each plot.